(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) EP 3 037 529 B1

(12) **EUROPEAN PATENT SPECIFICATION**

(45) Date of publication and mention of the grant of the patent:
27.03.2019 Bulletin 2019/13

(21) Application number: **15200797.7**

(22) Date of filing: **09.12.2009**

(51) Int Cl.:
C12N 9/26 *(2006.01)* ***A61K 47/60*** *(2017.01)*
A61K 47/61 *(2017.01)* ***A61K 47/64*** *(2017.01)*
A61K 38/00 *(2006.01)*

(54) **EXTENDED SOLUBLE PH20 POLYPEPTIDES AND USES THEREOF**

ERWEITERTE LÖSLICHE PH20-POLYPEPTIDE UND VERWENDUNGEN DAVON

POLYPEPTIDES PH20 SOLUBLES ÉTENDUS ET LEURS UTILISATIONS

(84) Designated Contracting States:
AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HR HU IE IS IT LI LT LU LV MC MK MT NL NO PL PT RO SE SI SK SM TR
Designated Extension States:
AL BA RS

(30) Priority: **09.12.2008 US 201384 P**
13.11.2009 US 281240 P

(43) Date of publication of application:
29.06.2016 Bulletin 2016/26

(62) Document number(s) of the earlier application(s) in accordance with Art. 76 EPC:
09804345.8 / 2 367 936

(73) Proprietor: **Halozyme, Inc.**
San Diego, CA 92121 (US)

(72) Inventors:
- **Wei, Ge**
  San Diego, CA 92131 (US)
- **Panneerselvam, Krishnasamy**
  Poway, CA 92064 (US)
- **Frost, Gregory I.**
  Palm Beach, FL 33480 (US)
- **Bookbinder, Louis**
  San Diego, CA 92128 (US)

(74) Representative: **Hoffmann Eitle**
Patent- und Rechtsanwälte PartmbB
Arabellastraße 30
81925 München (DE)

(56) References cited:
WO-A1-2006/091871 **WO-A1-2009/128918**
WO-A2-2004/078140 **WO-A2-2009/128917**
WO-A2-2009/134380 **US-A- 6 057 110**

- **BOOKBINDER L H ET AL: "A recombinant human enzyme for enhanced interstitial transport of therapeutics", JOURNAL OF CONTROLLED RELEASE, ELSEVIER, AMSTERDAM, NL LNKD-DOI:10.1016/J.JCONREL.2006.05.027, vol. 114, no. 2, 28 August 2006 (2006-08-28), pages 230-241, XP024957594, ISSN: 0168-3659 [retrieved on 2006-08-28]**
- **MEYER M F ET AL: "The soluble hyaluronidase from bull testes is a fragment of the membrane-bound PH-20 enzyme", FEBS LETTERS, ELSEVIER, AMSTERDAM, NL LNKD-DOI:10.1016/S0014-5793(97)00936-8, vol. 413, no. 2, 18 August 1997 (1997-08-18), pages 385-388, XP000961262, ISSN: 0014-5793**
- **CHER G N ET AL: "The dual functions of GPI anchored PH-20: hyaluronidase and intracellular signalling", MATRIX BIOLOGY, XX, XX, vol. 20, no. 8, 1 December 2001 (2001-12-01), pages 515-525, XP002990038,**
- **LIN YING ET AL: "Molecular cloning of the human and monkey sperm surface protein PH-20", PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA. (PNAS), NATIONAL ACADEMY OF SCIENCE, WASHINGTON, DC, US LNKD- DOI:10.1073/PNAS.90.21.10071, vol. 90, no. 21, 1 January 1993 (1993-01-01), pages 10071-10075, XP002386599, ISSN: 0027-8424**
- **CHER G N ET AL: "The PH-20 protein in cynomolgus macaque spermatozoa:identification of two different forms exhibiting hyaluronidase activity", MATRIX BIOLOGY, XX, XX, vol. 175, no. 1, 1 January 1996 (1996-01-01), pages 142-153, XP002990039,**

Note: Within nine months of the publication of the mention of the grant of the European patent in the European Patent Bulletin, any person may give notice to the European Patent Office of opposition to that patent, in accordance with the Implementing Regulations. Notice of opposition shall not be deemed to have been filed until the opposition fee has been paid. (Art. 99(1) European Patent Convention).

EP 3 037 529 B1

Printed by Jouve, 75001 PARIS (FR)

HALOZYME - EXHIBIT 1006

Description

FIELD OF INVENTION

[0001] Soluble PH20 polypeptides are provided, including extended soluble PH20 polypeptides, and uses thereof. Also provided are other C-terminally truncated PH20 polypeptides and partially deglycosylated PH20 polypeptides and uses thereof.

BACKGROUND

[0002] Hyaluronan (hyaluronic acid; HA) is a polypeptide that is found in the extracellular matrix of many cells, especially in soft connective tissues. HA also is found predominantly in skin, cartilage, and in synovial fluid in mammals. Hyaluronan also is the main constituent of the vitreous of the eye. HA has a role in various physiological processes, such as in water and plasma protein homeostasis (Laurent TC et al (1992) FASEB J 6: 2397-2404). Certain diseases are associated with expression and/or production of hyaluronan. Hyaluronidases are enzymes that degrade hyaluronan. By catalyzing HA, hyaluronidases can be used to treat diseases or disorders associated with accumulation of HA or other glycosaminoglycans. Also, since HA is a major component of the interstitial barrier, hyaluronidase increases tissue permeability and therefore can be used to increase the dispersion and delivery of therapeutic agents. Various hyaluronidases have been used therapeutically (e.g. Hydase™, Vitrase™ and Wydase™), typically as dispersing and spreading agents in combination with other therapeutic agents. Many of these are ovine or bovine forms, which can be immunogenic for treatment of humans. Improved compositions of hyaluronidases that can be used for treatment are needed.

SUMMARY

[0003] The present invention is defined in the claims, and provides an extended soluble PH20 hyaluronidase (esPH20), wherein:

the esPH20 polypeptide is active at neutral pH;
the esPH20 polypeptide is soluble; and
the esPH20 polypeptide is selected from among:

a polypeptide that consists of the sequence of amino acids set forth as amino acid residues 36-495, 36-496, 36-497, 36-498, 36-499 or 36-500 of SEQ ID NO: 107; or
a polypeptide that contains amino acid substitutions in the sequence of amino acids set forth as amino acids 36-495, 36-496, 36-497, 36-498, 36-499 or 36-500 of SEQ ID NO: 107, whereby the amino acid-substituted polypeptide consists of a sequence of amino acids that has at least 95% sequence identity with the corresponding sequence of amino acids set forth as amino acids 36-495, 36-496, 36-497, 36-498, 36-499 or 36-500 of SEQ ID NO: 107.

[0004] Further aspects of the invention are defined in the claims.

[0005] The following description provides details relating to both embodiments covered by the claims and subject matter not covered by the claims. The description of subject matter not covered by the claims is provided for information only.

[0006] Provided herein are soluble PH20 polypeptides, including extended soluble PH20 (esPH20) polypeptides, and compositions. The PH20 polypeptides provided herein are soluble proteins that are truncated at the C-terminus and include those that lack all of the GPI-anchor attachment signal sequence (e.g. are truncated at amino acid positions 450 to 490). Soluble PH20 polypeptides also include extended soluble PH20 polypeptides that retain one or more residues located in the GPI-anchor attachment signal sequence of the corresponding full length wild-type PH20 polypeptide. Also provided herein are other modified PH20 polypeptides that contain C-terminal truncations. Partially deglycosylated forms of any of the polypeptides also are provided. Also provided are methods of treatment using the PH20 polypeptides provided herein.

[0007] Provided herein are isolated substantially purified extended soluble PH20 (esPH20) hyaluronidases, that can be N-glycosylated or N-partially glycosylated. In some examples, the N-partially glycosylated esPH20 polypeptide contains at least an N-acetylglucosamine moiety linked to each of at least two N-linked moieties, such as, for example, amino acid residues residues 368 and 393 of SEQ ID NO:107 or residues corresponding to amino acid residues 368 and 393 of SEQ ID NO:107. In some aspects, the N-partially glycosylated esPH20 polypeptide contains at least two N-acetylglucosamine moieties linked to each of at least two N-linked moieties. The N-partially glycosylated esPH20 polypeptide provided herein also can contain a branched sugar.

[0008] Provided herein are esPH20 polypeptides that have the sequence of amino acids set forth in any of SEQ ID NOS: 60-63 and 102-104 or an allelic or species variant thereof. Also provided are esPH20 polypeptide variants having at least 40%, 45%, 50%, 55%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% sequence identity with any of SEQ ID NOS: 60-63 and 102-104 and retaining at least 30% of the hyaluronidase activity of the corresponding unmodified form or of a polypeptide that is encoded by a nucleic acid that encodes a polypeptide with amino acids 36-482 of SEQ ID NO:107. Such esPH20 polypeptides remain soluble and neutral active. In one example, the esPH20 is a human esPH20, such as one with a sequence of amino acids set forth in any of SEQ ID NOS: 60-63 and 102-104, or a chimpanzee esPH20, such as one with a sequence of amino acids set forth as amino acids 36-491, 36-492, 36-493, 36-494, 36-495, 36-496, 36-497 or 36-498 of SEQ ID NO:197.

[0009] Also provided herein are substantially purified PH20 polypeptides. These PH20 polypeptides can have a sequence of amino acids set forth in any of SEQ ID NOS: 55-63 and 64-95 or an allelic or species variant thereof. In other examples, the PH20 polypeptides are variants having at least 40%, 45%, 50%, 55%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% sequence identity with any of SEQ ID NOS: 55-63 and 64-95, that retain at least 30% of the hyaluronidase activity of the corresponding unmodified form or of a polypeptide that is encoded by a nucleic acid that encodes a polypeptide with amino acids 36-482 of SEQ ID NO:107. Such PH20 polypeptides are neutral active.

[0010] The PH20 polypeptides provided herein can be N-glycosylated or N-partially glycosylated. In some examples, the N-partially glycosylated esPH20 polypeptide contains at least an N-acetylglucosamine moiety linked to each of at least two N-linked moieties, such as, for example, amino acid residues residues 368 and 393 of SEQ ID NO:107 or residues corresponding to amino acid residues 368 and 393 of SEQ ID NO:107. In some aspects, the N-partially glycosylated PH20 polypeptide contains at least two N-acetylglucosamine moieties linked to each of at least two N-linked moieties. The N-partially glycosylated PH20 polypeptides provided herein also can contain a branched sugar. In some aspects, the PH20 polypeptides provided herein are soluble, and can be selected from among human, chimpanzee, rhesus monkey, cynomolgus monkey, mouse, rabbit, guinea pig, cow or sheep PH20.

[0011] The esPH20 and PH20 polypeptides provided herein can be modified by, for example, sialation, albumination, farnysylation, carboxylation, hydroxylation or phosphorylation. In some aspects, the esPH20 and PH20 polypeptides are modified by a polymer, such as dextran or PEG. Also provided herein are conjugates containing the esPH20 or PH20 polypeptides. Exemplary conjugates include those in which the esPH20 or PH20 is conjugated to a multimerization domain (such as an Fc domain), toxin, detectable label or drug.

[0012] Provided herein are nucleic acids encoding the esPH20 and PH20 polypeptides described above and provided herein. These nucleic acids include those that encode an esPH20 or PH20 polypeptide with amino acids corresponding to amino acids 36-450, 36-451, 36-452, 36-453, 36-454, 36-455, 36-456, 36-457, 36-458, 36-459, 36-460, 36-461, 36-462, 36-463, 36-464, 36-465, 36-484, 36-485, 36-486, 36-487, 36-489, 36-491, 36-492, 36-493, 36-494, 36-495, 36-496 or 36-497 of SEQ ID NO:107, and those that encode an esPH20 with amino acids corresponding to amino acids 36-491, 36-492, 36-493, 36-494, 36-495, 36-496, 36-497 or 36-498 of SEQ ID NO:197. Also provided herein are vectors that contain these nucleic acids and cells that contain the vectors, such as CHO cells.

[0013] Provided herein are compositions containing any one or more of the esPH20 or PH20 polypeptides described herein. In some examples, the compositions contain a plurality of esPH20 or PH20 polypeptides. For example, the compositions can contain a plurality of esPH20 polypeptides that are encoded by a nucleic acid molecule encoding amino acids corresponding to amino acids 36-450, 36-451, 36-452, 36-453, 36-454, 36-455, 36-456, 36-457, 36-458, 36-459, 36-460, 36-461, 36-462, 36-463, 36-464, 36-465, 36-484, 36-485, 36-486, 36-487, 36-489, 36-491, 36-492, 36-493, 36-494, 36-495, 36-496 or 36-497 of SEQ ID NO:107, and those that encode an esPH20 with amino acids corresponding to amino acids 36-491, 36-492, 36-493, 36-494, 36-495, 36-496, 36-497 or 36-498 of SEQ ID NO:197. In some examples, the compositions contain esPH20 or PH20 polypeptides that are secreted from CHO cells.

[0014] The compositions provided herein can be pharmaceutical compositions. In some examples, the compositions contain an additional therapeutic agent, which can be formulated with the composition or in a separate composition. Exemplary of the therapeutic agents that can be included in the compositions provided herein are chemotherapeutic agents, analgesic agents, anti-inflammatory agents, antimicrobial agents, amoebicidal agents, trichomonocidal agents, anti-parkinson agents, anti-malarial agents, anticonvulsant agents, anti-depressant agents, anti-arthritics agents, anti-fungal agents, antihypertensive agents, antipyretic agents, anti-parasite agents, antihistamine agents, alpha-adrenergic agonist agents, alpha blocker agents, anesthetic agents, bronchial dilator agents, biocide agents, bactericide agents, bacteriostat agents, beta adrenergic blocker agents, calcium channel blocker agents, cardiovascular drug agents, contraceptive agents, decongestant agents, diuretic agents, depressant agents, diagnostic agents, electrolyte agents, hypnotic agents, hormone agents, hyperglycemic agents, muscle relaxant agents, muscle contractant agents, ophthalmic agents, parasympathomimetic agents, psychic energizer agents, sedative agents, sympathomimetic agents, tranquilizer agents, urinary agents, vaginal agents, viricide agents, vitamin agents, non-steroidal anti-inflammatory agents, angiotensin converting enzyme inhibitor agents, polypeptides, proteins, nucleic acids, drugs, organic molecules and sleep inducers. In particular examples, the therapeutic agent is an antibody, an immunoglobulin, a bisphosphonate (such as

zolentronic acid), a cytokine, a chemotherapeutic agent or an insulin (such as a fast-acting insulin).

[0015] Other therapeutic agents that can be included in the compositions provided herein include, but are not limited to, Acivicins; Aclarubicins; Acodazoles; Acronines; Adozelesins; Aldesleukins; Alemtuzumabs; Alitretinoins (9-Cis-Retinoic Acids); Allopurinols; Altretamines; Alvocidibs; Ambazones; Ambomycins; Ametantrones; Amifostines; Aminoglutethimides; Amsacrines; Anastrozoles; Anaxirones; Ancitabines; Anthramycins; Apaziquones; Argimesnas; Arsenic Trioxides; Asparaginases; Asperlins; Atrimustines; Azacitidines; Azetepas; Azotomycins; Banoxantrones; Batabulins; Batimastats; BCG Live; Benaxibines; Bendamustines; Benzodepas; Bexarotenes; Bevacizumab; Bicalutamides; Bietaserpines; Biricodars; Bisantrenes; Bisantrenes; Bisnafide Dimesylates; Bizelesins; Bleomycins; Bortezomibs; Brequinars; Bropirimines; Budotitanes; Busulfans; Cactinomycins; Calusterones; Canertinibs; Capecitabines; Caracemides; Carbetimers; Carboplatins; Carboquones; Carmofurs; Carmustines with Polifeprosans; Carmustines; Carubicins; Carzelesins; Cedefingols; Celecoxibs; Cemadotins; Chlorambucils; Cioteronels; Cirolemycins; Cisplatins; Cladribines; Clanfenurs; Clofarabines; Crisnatols; Cyclophosphamides; Cytarabine liposomals; Cytarabines; Dacarbazines; Dactinomycins; Darbepoetin Alfas; Daunorubicin liposomals; Daunorubicins/Daunomycins; Daunorubicins; Decitabines; Denileukin Diftitoxes; Dexniguldipines; Dexonnaplatins; Dexrazoxanes; Dezaguanines; Diaziquones; Dibrospidiums; Dienogests; Dinalins; Disermolides; Docetaxels; Dofequidars; Doxifluridines; Doxorubicin liposomals; Doxorubicin HCL; Docorubicin HCL liposome injection; Doxorubicins; Droloxifenes; Dromostanolone Propionates; Duazomycins; Ecomustines; Edatrexates; Edotecarins; Eflornithines; Elacridars; Elinafides; Elliott's B Solutions; Elsamitrucins; Emitefurs; Enloplatins; Enpromates; Enzastaurins; Epipropidines; Epirubicins; Epoetin alfas; Eptaloprosts; Erbulozoles; Esorubicins; Estramustines; Etanidazoles; Etoglucids; Etoposide phosphates; Etoposide VP-16s; Etoposides; Etoprines; Exemestanes; Exisulinds; Fadrozoles; Fazarabines; Fenretinides; Filgrastims; Floxuridines; Fludarabines; Fluorouracils; 5-fluorouracils; Fluoxymesterones; Flurocitabines; Fosquidones; Fostriecins; Fostriecins; Fotretamines; Fulvestrants; Galarubicins; Galocitabines; Gemcitabines; Gemtuzumabs/Ozogamicins; Geroquinols; Gimatecans; Gimeracils; Gloxazones; Glufosfamides; Goserelin acetates; Hydroxyureas; Ibritumomabs/ Tiuxetans; Idarubicins; Ifosfamides; Ilmofosines; Ilomastats; Imatinib mesylates; Imexons; Improsulfans; Indisulams; Inproquones; Interferon alfa-2as; Interferon alfa-2bs; Interferon Alfas; Interferon Betas; Interferon Gammas; Interferons; Interleukin-2s and other Interleukins (including recombinant Interleukins); Intoplicines; Iobenguanes [131-I]; Iproplatins; Irinotecans; Irsogladines; Ixabepilones; Ketotrexates; L-Alanosines; Lanreotides; Lapatinibs; Ledoxantrones; Letrozoles; Leucovorins; Leuprolides; Leuprorelins (Leuprorelides); Levamisoles; Lexacalcitols; Liarozoles; Lobaplatins; Lometrexols; Lomustines/CCNUs; Lomustines; Lonafarnibs; Losoxantrones; Lurtotecans; Mafosfamides; Mannosulfans; Marimastats; Masoprocols; Maytansines; Mechlorethamines; Mechlorethamines/Nitrogen mustards; Megestrol acetates; Megestrols; Melengestrols; Melphalans; MelphalansIL-PAMs; Menogarils; Mepitiostanes; Mercaptopurines; 6-Mecaptopurine; Mesnas; Metesinds; Methotrexates; Methoxsalens; Metomidates; Metoprines; Meturedepas; Miboplatins; Miproxifenes; Misonidazoles; Mitindomides; Mitocarcins; Mitocromins; Mitoflaxones; Mitogillins; Mitoguazones; Mitomalcins; Mitomycin Cs; Mitomycins; Mitonafides; Mitoquidones; Mitospers; Mitotanes; Mitoxantrones; Mitozolomides; Mivobulins; Mizoribines; Mofarotenes; Mopidamols; Mubritinibs; Mycophenolic Acids; Nandrolone Phenpropionates; Nedaplatins; Nelzarabines; Nemorubicins; Nitracrines; Nocodazoles; Nofetumomabs; Nogalamycins; Nolatrexeds; Nortopixantrones; Octreotides; Oprelvekins; Ormaplatins; Ortataxels; Oteracils; Oxaliplatins; Oxisurans; Oxophenarsines; Paclitaxels; Pamidronates; Patubilones; Pegademases; Pegaspargases; Pegfilgrastims; Peldesines; Peliomycins; Pelitrexols; Pemetrexeds; Pentamustines; Pentostatins; Peplomycins; Perfosfamides; Perifosines; Picoplatins; Pinafides; Pipobromans; Piposulfans; Pirfenidones; Piroxantrones; Pixantrones; Plevitrexeds; Plicamycid Mithramycins; Plicamycins; Plomestanes; Plomestanes; Porfimer sodiums; Porfimers; Porfiromycins; Prednimustines; Procarbazines; Propamidines; Prospidiums; Pumitepas; Puromycins; Pyrazofurins; Quinacrines; Ranimustines; Rasburicases; Riboprines; Ritrosulfans; Rituximabs; Rogletimides; Roquinimexs; Rufocromomycins; Sabarubicins; Safingols; Sargramostims; Satraplatins; Sebriplatins; Semustines; Simtrazenes; Sizofirans; Sobuzoxanes; Sorafenibs; Sparfosates; Sparfosic Acids; Sparsomycins; Spirogermaniums; Spiromustines; Spiroplatins; Spiroplatins; Squalamines; Streptonigrins; Streptovarycins; Streptozocins; Sufosfamides; Sulofenurs; Sunitinib Malate; 6-TG; Tacedinalines; Talcs; Talisomycins; Tallimustines; Tamoxifens; Tariquidars; Tauromustines; Tecogalans; Tegafurs; Teloxantrones; Temoporfins; Temozolomides; Teniposides/VM-26s; Teniposides; Teroxirones; Testolactones; Thiamiprines; Thioguanines; Thiotepas; Tiamiprines; Tiazofurins; Tilomisoles; Tilorones; Timcodars; Timonacics; Tirapazamines; Topixantrones; Topotecans; Toremifenes; Tositumomabs; Trabectedins (Ecteinascidin 743); Trastuzumabs; Trestolones; Tretinoins/ATRA; Triciribines; Trilostanes; Trimetrexates; Triplatin Tetranitrates; Triptorelins; Trofosfamides; Tubulozoles; Ubenimexs; Uracil Mustards; Uredepas; Valrubicins; Valspodars; Vapreotides; Verteporfins; Vinblastines; Vincristines; Vindesines; Vinepidines; Vinflunines; Vinformides; Vinglycinates; Vinleucinols; Vinleurosines; Vinorelbines; Vinrosidines; Vintriptols; Vinzolidines; Vorozoles; Xanthomycin A's (Guamecyclines); Zeniplatins; Zilascorbs [2-H]; Zinostatins; Zoledronate; Zorubicins; and Zosuquidars.

[0016] Provided herein are methods for treating a hyaluronan-associated disease or condition, wherein a subject is administered an esPH20 or PH20, or a composition containing an esPH20 or PH20, provided and described herein. Also provided are methods for treating an excess of glycosaminoglycans; for treating a tumor; for treating glycosaminoglycan accumulation in the brain; for treating a cardiovascular disorder; for treating an ophthalmic disorder; for treating

pulmonary disease; for increasing penetration of chemotherapeutic agents into solid tumors; for treating cellulite; or for increasing bioavailability of drugs and other therapeutic agents. Such methods involve administering to a subject any of the esPH20 or PH20 polypeptides or compositions described herein.

[0017] The esPH20 and PH20 polypeptides provided herein can be used in place of a PH20 hyaluronidase, alone or in combination, in any method of treatment or combination therapy for which a PH20 hyaluronidase is used in U.S. Publication Nos. US20040268425, US20050260186, and US20060104968; and U.S. Application Serial Nos. 12/381,844, 12/386,249, 12/387,225 and 12/386,222.

BRIEF DESCRIPTION OF THE FIGURES

[0018]

Figure 1 depicts an alignment of the amino acid sequence of the human (SEQ ID NO:107) and chimpanzee (SEQ ID NO:197) PH20 polypeptides (performed using the ClustalW2 alignment program). The amino acid residues of the human PH20 GPI-anchor attachment signal sequence, and the corresponding amino acids in the chimpanzee PH20 sequence, are in bold and underlined. "*" indicates that the residues above are identical in both sequences in the alignment. ":" indicates conserved substitutions, and "." indicates semi-conserved substitutions.

Figure 2 depicts the major types of N-glycans in vertebrates, including high mannose glycans, hybrid glycans and complex glycans.

Figure 3 depicts the endoglycosidase cleavage sites. Figure 3A illustrates the cleavage sites for Endoglycosidase F1 and Peptide N Glycosidase F (PNGaseF).

Figure 3B illustrates the cleavage sites for Endoglycosidase F2 and PNGaseF. Figure 3C illustrates the cleavage sites for Endoglycosidase F3 and PNGaseF. Figure 3D illustrates the cleavage sites for Endoglycosidase F4 and PNGaseF.

DETAILED DESCRIPTION

Overview

[0019]

A. Definitions
B. Overview

1. PH20

a. Glycosylation
b. GPI-Anchoring

C. Extended Soluble PH20 Polypeptides

1. Human esPH20 polypeptides
2. Other species esPH20 polypeptides

D. N-Partially glycosylated PH20 polypeptides

1. PH20 polypeptides
2. C-terminally truncated PH20 polypeptides
3. Additional Modifications
Conjugation to polymers

E. Methods of Producing Nucleic Acids Encoding Extended Soluble PH20 and other Soluble PH20 Hyaluronidases, and Polypeptides Thereof

1. Vectors and Cells
2. Expression

a. Prokaryotic Cells

b. Yeast Cells
c. Insect Cells
d. Mammalian Cells
e. Plants

3. Purification Techniques

F. Preparation, Formulation and Administration of Extended Soluble PH20 Polypeptides, and Other Soluble PH20 Polypeptides

1. Injectables, solutions and emulsions Lyophilized Powders
2. Topical Administration
3. Compositions for other routes of administration
4. Dosage and Administration
5. Packaging, Articles of Manufacture and Kits

G. Assays

1. Hyaluronidase Activity
2. Solubility

H. Methods of Treatment and Uses of Extended Soluble PH20 and other Soluble PH20 and Combination Therapy

1. Use as a Spreading Agent and Combination Therapy
2. Use to Remove Excess Glycosaminoglycanases

a. Use in cancer treatment
b. Use in treatment of glycosaminoglycan accumulation in the brain
c. Use in treatment of glycosaminoglycan accumulation in cardiovascular disease
d. Use in vitrectomy and ophthalmic disorders and conditions
e. Use in hypodermoclysis
f. Use in gene therapy
g. Cosmetic uses
h. Use in organ transplantation
i. Use in pulmonary disease

3. Other uses

I. Examples

A. Definitions

[0020] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as is commonly understood by one of skill in the art to which the invention(s) belong.

[0021] In the event that there are a plurality of definitions for terms herein, those in this section prevail. Where reference is made to a URL or other such identifier or address, it understood that such identifiers can change and particular information on the internet can come and go, but equivalent information can be found by searching the Internet. Reference thereto evidences the availability and public dissemination of such information.

[0022] As used herein, hyaluronidase refers to a class of enzymes that degrade hyaluronan. Hyaluronidases include, but are not limited to, bacterial hyaluronidases (EC 4.2.2.1 or EC 4.2.99.1), hyaluronidases from leeches, other parasites, and crustaceans (EC 3.2.1.36), and mammalian-type hyaluronidases (EC 3.2.1.35). Hyaluronidases include any of non-human origin including, but not limited to, murine, canine, feline, leporine, avian, bovine, ovine, porcine, equine, piscine, ranine, bacterial, and any from leeches, other parasites, and crustaceans. Exemplary human hyaluronidases include HYAL1, HYAL2, HYAL3, HYAL4, and PH20 (SEQ ID NO:107). Also included amongst hyaluronidases are soluble hyaluronidases, including, ovine and bovine PH20, soluble human PH20 and rHuPH20. Examples of commercially available bovine or ovine soluble hyaluronidases are Vitrase® hyaluronidase (ovine hyaluronidase) and Amphadase® hyaluronidase (bovine hyaluronidase).

[0023] As used herein, PH20 refers to a type of hyaluronidase that occurs in sperm and is neutral-active. PH-20 occurs on the sperm surface, and in the lysosome-derived acrosome, where it is bound to the inner acrosomal membrane. PH20 includes those of any origin including, but not limited to, human, chimpanzee, Cynomolgus monkey, Rhesus monkey, murine, bovine, ovine, guinea pig, rabbit and rat origin. Exemplary PH20 polypeptides include those from human (SEQ ID NO:107), chimpanzee (SEQ ID NO:197), Rhesus monkey (SEQ ID NO:198), Cynomolgus monkey (SEQ ID NO:114), cow (e.g., SEQ ID NOS:111 and 119); mouse (SEQ ID NO:117); rat (SEQ ID NOS:116); rabbit (SEQ ID NO:112); sheep (SEQ ID NOS:113, 118 and 120) and guinea pig (SEQ ID NO:115). Reference to PH20 includes precursor PH20 polypeptides and mature PH20 polypeptides (such as those in which a signal sequence has been removed), truncated forms thereof that have activity, and includes allelic variants and species variants, variants encoded by splice variants, and other variants, including polypeptides that have at least 40%, 45%, 50%, 55%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or more sequence identity to the precursor polypeptides set forth in SEQ ID NO:107 and 109, or the mature forms thereof. PH20 polypeptides also include those that contain chemical or post-translational modifications and those that do not contain chemical or posttranslational modifications. Such modifications include, but are not limited to, pegylation, albumination, glycosylation, farnysylation, carboxylation, hydroxylation, phosphorylation, and other polypeptide modifications known in the art. A truncated PH20 hyaluronidase is any C-terminal shortened form thereof, particularly forms that are truncated and neutral active when N-glycosylated.

[0024] As used herein, a soluble PH20 refers to any form of PH20 that is soluble under physiologic conditions. A soluble PH20 can be identified, for example, by its partitioning into the aqueous phase of a Triton® X-114 solution at 37 °C (Bordier et al., (1981) J. Biol. Chem., 256:1604-7). Membrane-anchored PH20, such as lipid-anchored PH20, including GPI-anchored PH20, will partition into the detergent-rich phase, but will partition into the detergent-poor or aqueous phase following treatment with Phospholipase-C. Included among soluble PH20 are membrane-anchored PH20 in which one or more regions associated with anchoring of the PH20 to the membrane has been removed or modified, where the soluble form retains hyaluronidase activity. Soluble PH20 also include recombinant soluble PH20 and those contained in or purified from natural sources, such as, for example, testes extracts from sheep or cows. Exemplary of such soluble PH20 is soluble human PH20.

[0025] As used herein, soluble human PH20 or sHuPH20 includes PH20 polypeptides lacking all or a portion of the glycosylphospatidylinositol (GPI) anchor sequence at the C-terminus such that upon expression, the polypeptides are soluble under physiological conditions. Solubility can be assessed by any suitable method that demonstrates solubility under physiologic conditions. Exemplary of such methods is the Triton® X-114 assay, that assesses partitioning into the aqueous phase and that is described above and in the examples. In addition, a soluble human PH20 polypeptide is, if produced in CHO cells, such as CHO-S cells, a polypeptide that is expressed and is secreted into the cell culture medium. Soluble human PH20 polypeptides, however, are not limited to those produced in CHO cells, but can be produced in any cell or by any method, including recombinant expression and polypeptide synthesis. Reference to secretion in CHO cells is definitional. Hence, if a polypeptide could be expressed and secreted in CHO cells and is soluble, *i.e.* partitions into the aqueous phase when extracted with Triton® X-114, it is a soluble PH20 polypeptide whether or not it is so-produced. The precursor polypeptides for sHuPH20 polypeptides can include a signal sequence, such as a heterologous or non-heterologous (i.e. native) signal sequence. Exemplary of the precursors are those that include a signal sequence, such as the native 35 amino acid signal sequence at amino acid positions 1-35 (see, e.g., amino acids 1-35 of SEQ ID NO:107).

[0026] As used herein, an "extended soluble PH20" or "esPH20" includes soluble PH20 polypeptides that contain residues up to the GPI anchor-attachment signal sequence and one or more contiguous residues from the GPI-anchor attachment signal sequence such that the esPH20 is soluble under physiological conditions. Solubility under physiological conditions can be determined by any method known to those of skill in the art. For example, it can be assessed by the Triton® X-114 assay described above and in the examples. In addition, as discussed above, a soluble PH20 is, if produced in CHO cells, such as CHO-S cells, a polypeptide that is expressed and is secreted into the cell culture medium. Soluble human PH20 polypeptides, however, are not limited to those produced in CHO cells, but can be produced in any cell or by any method, including recombinant expression and polypeptide synthesis. Reference to secretion in CHO cells is definitional. Hence, if a polypeptide could be expressed and secreted in CHO cells and is soluble, *i.e.* partitions into the aqueous phase when extracted with Triton® X-114, it is a soluble PH20 polypeptide whether or not it is so-produced. Human soluble esPH20 polypeptides include, in addition to residues 36-490, one or more contiguous amino acids from amino acid residue position 491 of SEQ ID NO:107, inclusive, such that the resulting polypeptide is soluble. Exemplary human esPH20 soluble polypeptides are those that have amino acids residues corresponding to amino acids 36-491, 36-492, 36-493, 36-494, 36-495, 36-496 and 36-497 of SEQ ID NO.107. Exemplary of these are those with an amino acid sequence set forth in any of SEQ ID NOS:60-63 and 102-104. Also included are allelic variants and other variants, such as any with 40%, 45%, 50%, 55%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or greater sequence identity with the corresponding polypeptides of SEQ ID NOS: 60-63 and 102-104 that retain neutral activity and are soluble. Reference to sequence identity refers to variants with amino acid substitutions.

[0027] As used herein, reference to esPH20s includes precursor esPH20 polypeptides and mature esPH20 polypep-

tides (such as those in which a signal sequence has been removed), truncated forms thereof that have enzymatic activity (retaining at least 1%, 10%, 20%, 30%, 40%, 50% or more of the full-length form) and are soluble, and includes allelic variants and species variants, variants encoded by splice variants, and other variants, including polypeptides that have at least 40%, 45%, 50%, 55%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to the precursor polypeptides set forth in SEQ ID NOS:107 and 109, or the mature forms thereof.

[0028] As used herein, reference to esPH20s also include those that contain chemical or posttranslational modifications and those that do not contain chemical or posttranslational modifications. Such modifications include, but are not limited to, PEGylation, albumination, glycosylation, farnysylation, carboxylation, hydroxyiation, phosphorylation, and other polypeptide modifications known in the art.

[0029] As used herein, soluble recombinant human PH20 (rHuPH20) refers to a soluble form of human PH20 that as recombinantly expressed and secreted in Chinese Hamster Ovary (CHO) cells. Soluble rHuPH20 is encoded by nucleic acid that includes the signal sequence and is set forth in SEQ ID NO:109. Also included are DNA molecules that are allelic variants thereof and other soluble variants. The nucleic acid encoding soluble rHuPH20 is expressed in CHO cells which secrete the mature polypeptide. As produced in the culture medium, there is heterogeneity at the C-terminus so that the product includes a mixture of species that can include any one or more of SEQ ID NO:122 to SEQ ID NO:127 in various abundance.

[0030] Similarly, for other forms of PH20, such as the esPH20s, recombinantly expressed polypeptides and compositions thereof can include a plurality of species whose C-terminus exhibits heterogeneity. For example, compositions of recombinantly expressed esPH20 produced by expression of the polypeptide of SEQ ID NO:8, which encodes an esPH20 that has amino acids 36-497, can include forms with fewer amino acids, such as 36-496, 36-495.

[0031] As used herein, an N-linked moiety refers to an asparagine (N) amino acid residue of a polypeptide that is capable of being glycosylated by post-translational modification of a polypeptide. Exemplary N-linked moieties of human PH20 include amino acids N82, N166, N235, N254, N368 and N393 of human PH20 set forth in SEQ ID NO:107.

[0032] As used herein, an N-glycosylated polypeptide refers to a PH20 polypeptide or truncated form thereo containing oligosaccharide linkage of at least three N-linked amino acid residues, for example, N-linked moieties corresponding to amino acid residues N235, N368 and N393 of SEQ ID NO:107. An N-glycosylated polypeptide can include a polypeptide where three, four, five and up to all of the N-linked moieties are linked to an oligosaccharide. The N-linked oligosaccharides can include oligomannose, complex, hybrid or sulfated oligosaccharides, or other oligosaccharides and monosaccharides.

[0033] As used herein, an N-partially glycosylated polypeptide refers to a polypeptide that minimally contains an N-acetylglucosamine glycan linked to at least three N-linked moieties. A partially glycosylated polypeptide can include various glycan forms, including monosaccharides, oligosaccharides, and branched sugar forms, including those formed by treatment of a polypeptide with EndoH, EndoF1, EndoF2. and/or EndoF3. The cleavage of glycans by such enzymes is depicted, for example, in Figure 2.

[0034] As used herein, a deglycosylated PH20 polypeptide refers to a PH20 polypeptide provided herein in which fewer than all possible glycosylation sites are glycosylated. Deglycosylation can be effected, for example, by removing glycosylation, by preventing it, or by modifying the polypeptide to eliminate a glycosylation site. As shown herein, particular N-glycosylation sites are not required for activity, whereas others are.

[0035] As used herein, a hyaluronan-associated disease, disorder or condition refers to any disease or condition in which hyaluronan levels are elevated as cause, consequence or otherwise observed in the disease or condition. Hyaluronan-associated diseases and conditions are associated with elevated hyaluronan expression in a tissue or cell, increased interstitial fluid pressure, decreased vascular volume, and/or increased water content in a tissue. Hyaluronan-associated diseases, disorders or conditions can be treated by administration of a composition containing a hyaluronan degrading enzyme, such as a hyaluronidase, for example, a soluble hyaluronidase, either alone or in combination with or in addition to another treatment and/or agent. Exemplary diseases and conditions, include, but are not limited to, hyaluronan-rich cancers, for example, tumors, including solid tumors such as late-stage cancers, a metastatic cancers, undifferentiated cancers, ovarian cancer, in situ carcinoma (ISC), squamous cell carcinoma (SCC), prostate cancer, pancreatic cancer, non-small cell lung cancer, breast cancer, colon cancer and other cancers. Also exemplary of hyaluronan-associated diseases and conditions are diseases that are associated with elevated interstitial fluid pressure, such as diseases associated with disc pressure, and edema, for example, edema caused by organ transplant, stroke, brain trauma or other injury. Exemplary hyaluronan-associated diseases and conditions include diseases and conditions associated with elevated interstitial fluid pressure, decreased vascular volume, and/or increased water content in a tissue, including cancers, disc pressure and edema. In one example, treatment of the hyaluronan-associated condition, disease or disorder includes amelioration, reduction, or other beneficial effect on one or more of increased interstitial fluid pressure (IFP), decreased vascular volume, and increased water content in a tissue.

[0036] As used herein, a conjugate refers to soluble PH20 polypeptides linked directly or indirectly to one or more other polypeptides or chemical moieties. Such conjugates include fusion proteins, those produced by chemical conjugates and those produced by any other method whereby at least one soluble PH20 polypeptide is linked, directly or indirectly

to another polypeptide or chemical moiety so long as the conjugate retains hyaluronidase activity. Exemplary of conjugates provided herein include PH20 polypeptides linked directly or indirectly to a multimerization domain, such as an Fc moiety, a toxin, a label or a drug.

[0037] As used herein, a fusion protein refers to a polypeptide encoded by a nucleic acid sequence containing a coding sequence from one nucleic acid molecule and the coding sequence from another nucleic acid molecule in which the coding sequences are in the same reading frame such that when the fusion construct is transcribed and translated in a host cell, the protein is produced containing the two proteins. The two molecules can be adjacent in the construct or separated by a linker polypeptide that contains, 1, 2, 3, or more, but typically fewer than 10, 9, 8, 7, or 6 amino acids. The protein product encoded by a fusion construct is referred to as a fusion polypeptide. Exemplary of fusion polypeptides include Fc fusions.

[0038] As used herein, a polymer that is conjugated to a hyaluronan degrading enzyme, such as a hyaluronidase, refers to any polymer that is covalently or otherwise stably linked, directly or via a linker, to a hyaluronan degrading enzyme. Such polymers, typically increase serum half-life, and include, but are not limited to sialic moieties, pegylation moieties, dextran, and sugar and other moieties, such as for glycosylation.

[0039] As used herein, activity refers to a functional activity or activities of a polypeptide or portion thereof associated with a full-length (complete) protein. Functional activities include, but are not limited to, biological activity, catalytic or enzymatic activity, antigenicity (ability to bind or compete with a polypeptide for binding to an anti-polypeptide antibody), immunogenicity, ability to form multimers, and the ability to specifically bind to a receptor or ligand for the polypeptide.

[0040] As used herein, hyaluronidase activity refers to the ability to enzymatically catalyze the cleavage of hyaluronic acid. The United States Pharmacopeia (USP) XXII assay for hyaluronidase determines hyaluronidase activity indirectly by measuring the amount of higher molecular weight hyaluronic acid, or hyaluronan, (HA) substrate remaining after the enzyme is allowed to react with the HA for 30 min at 37 °C (USP XXII-NF XVII (1990) 644-645 United States Pharmacopeia Convention, Inc, Rockville, MD). A Reference Standard solution can be used in an assay to ascertain the relative activity, in units, of any hyaluronidase. *In vitro* assays to determine the hyaluronidase activity of hyaluronidases, such as PH20, including soluble PH20 and esPH20, are known in the art and described herein. Exemplary assays include the micro-turbidity assay described below (see e.g. Example 12) that measures cleavage of hyaluronic acid by hyaluronidase indirectly by detecting the insoluble precipitate formed when the uncleaved hyaluronic acid binds with serum albumin. Reference Standards can be used, for example, to generate a standard curve to determine the activity in Units of the hyaluronidase being tested.

[0041] As used herein, neutral active refers to the ability of a PH20 polypeptide to enzymatically catalyze the cleavage of hyaluronic acid at neutral pH. A neutral active C-terminally truncated or N-partially glycosylated PH20 provided herein has or has about 30%, 40%, 50%, 60%, 70%, 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100%, 110%, 120%, 130%, 140%, 150%, 200%, 300%, 400%, 500%, 1000% or more activity compared to the hyaluronidase activity of a corresponding neutral active PH20 that is not C-terminally truncated or N-partially glycosylated.

[0042] As used herein, a GPI-anchor attachment signal sequence is a C-terminal sequence of amino acids that directs addition of a preformed GPI-anchor to the polypeptide within the lumen of the ER. GPI-anchor attachment signal sequences are present in the precursor polypeptides of GPI-anchored polypeptides, such as GPI-anchored PH20 polypeptides. The C-terminal GPI-anchor attachment signal sequence typically contains a predominantly hydrophobic region of 8-20 amino acids, preceded by a hydrophilic spacer region of 8-12 amino acids, immediately downstream of the ω-site, or site of GPI-anchor attachment. GPI-anchor attachment signal sequences can be identified using methods well known in the art. These include, but are not limited to, *in silico* methods and algorithms (see, e.g. Udenfriend et al. (1995) Methods Enzymol. 250:571-582, Eisenhaber et al., (1999) J. Biol. Chem. 292: 741-758, Kronegg and Buloz, (1999), "Detection/prediction of GPI cleavage site (GPI-anchor) in a protein (DGPI)", e.g., the website 129.194.185.165/dgpi/, Fankhauser et al., (2005) Bioinformatics 21:1846-1852, Omaetxebarria et al., (2007) Proteomics 7:1951-1960, Pierleoni et al., (2008) BMC Bioinformatics 9:392), including those that are readily available on bioinformatic websites, such as the ExPASy Proteomics tools site (e.g., the WorldWideWeb site expasy.ch/tools/).

[0043] As used herein, a bifucosylated polypeptide refers to a polypeptide that has two fucose residues, one with a α1,3-linkage and the other with α1,6-linkage, linked to the same core N-acetylglucosamine moiety, with the N-acetylglucosamine moiety linked to the asparagine residue in the polypeptide chain. Bifucosylated polypeptides generally are produced in insect cells.

[0044] As used herein, nucleic acids include DNA, RNA and analogs thereof, including peptide nucleic acids (PNA) and mixtures thereof. Nucleic acids can be single or double-stranded. When referring to probes or primers, which are optionally labeled, such as with a detectable label, such as a fluorescent or radiolabel, single-stranded molecules are contemplated. Such molecules are typically of a length such that their target is statistically unique or of low copy number (typically less than 5, generally less than 3) for probing or priming a library. Generally a probe or primer contains at least 14, 16 or 30 contiguous nucleotides of sequence complementary to or identical to a gene of interest. Probes and primers can be 10, 20, 30, 50, 100 or more nucleic acids long.

[0045] As used herein, a peptide refers to a polypeptide that is greater than or equal to 2 amino acids in length, and

less than or equal to 40 amino acids in length. As used herein, the amino acids which occur in the various sequences of amino acids provided herein are identified according to their known, three-letter or one-letter abbreviations (Table 1). The nucleotides which occur in the various nucleic acid fragments are designated with the standard single-letter designations used routinely in the art.

[0046] As used herein, an "amino acid" is an organic compound containing an amino group and a carboxylic acid group. A polypeptide contains two or more amino acids. For purposes herein, amino acids include the twenty naturally-occurring amino acids, non-natural amino acids and amino acid analogs (i.e., amino acids wherein the α-carbon has a side chain).

[0047] As used herein, "amino acid residue" refers to an amino acid formed upon chemical digestion (hydrolysis) of a polypeptide at its peptide linkages. The amino acid residues described herein are presumed to be in the "L" isomeric form. Residues in the "D" isomeric form, which are so designated, can be substituted for any L-amino acid residue as long as the desired functional property is retained by the polypeptide. NH2 refers to the free amino group present at the amino terminus of a polypeptide. COOH refers to the free carboxy group present at the carboxyl terminus of a polypeptide. In keeping with standard polypeptide nomenclature described in J. Biol. Chem., 243: 3557-3559 (1968), and adopted 37 C.F.R. §§ 1.821-1.822, abbreviations for amino acid residues are shown in Table 1:

Table 1 - Table of Correspondence

SYMBOL		
1-Letter	3-Letter	AMINO ACID
Y	Tyr	Tyrosine
G	Gly	Glycine
F	Phe	Phenylalanine
M	Met	Methionine
A	Ala	Alanine
S	Ser	Serine
I	Ile	Isoleucine
L	Leu	Leucine
T	Thr	Threonine
V	Val	Valine
P	Pro	Proline
K	Lys	Lysine
H	His	Histidine
Q	Gln	Glutamine
E	Glu	Glutamic acid
Z	Glx	Glu and/or Gln
W	Trp	Tryptophan
R	Arg	Arginine
D	Asp	Aspartic acid
N	Asn	Asparagine
B	Asx	Asn and/or Asp
C	Cys	Cysteine
X	Xaa	Unknown or other

[0048] All amino acid residue sequences represented herein by formulae have a left to right orientation in the conventional direction of amino-terminus to carboxyl-terminus. In addition, the phrase "amino acid residue" is defined to include the amino acids listed in the Table of Correspondence (Table 1) and modified and unusual amino acids, such as those

referred to in 37 C.F.R. §§ 1.821-1.822 .

[0049] Furthermore, it should be noted that a dash at the beginning or end of an amino acid residue sequence indicates a peptide bond to a further sequence of one or more amino acid residues, to an amino-terminal group such as NH_2 or to a carboxyl-terminal group such as COOH.

[0050] As used herein, the "naturally occurring α-amino acids" are the residues of those 20 α-amino acids found in nature which are incorporated into protein by the specific recognition of the charged tRNA molecule with its cognate mRNA codon in humans. Non-naturally occurring amino acids thus include, for example, amino acids or analogs of amino acids other than the 20 naturally-occurring amino acids and include, but are not limited to, the D-isostereomers of amino acids. Exemplary non-natural amino acids are described herein and are known to those of skill in the art.

[0051] As used herein, a DNA construct is a single- or double-stranded, linear or circular DNA molecule that contains segments of DNA combined and juxtaposed in a manner not found in nature. DNA constructs exist as a result of human manipulation, and include clones and other copies of manipulated molecules.

[0052] As used herein, a DNA segment is a portion of a larger DNA molecule having specified attributes. For example, a DNA segment encoding a specified polypeptide is a portion of a longer DNA molecule, such as a plasmid or plasmid fragment, which, when read from the 5' to 3' direction, encodes the sequence of amino acids of the specified polypeptide.

[0053] As used herein, the term polynucleotide means a single- or double-stranded polymer of deoxyribonucleotides or ribonucleotide bases read from the 5' to the 3' end. Polynucleotides include RNA and DNA, and can be isolated from natural sources, synthesized in vitro, or prepared from a combination of natural and synthetic molecules. The length of a polynucleotide molecule is given herein in terms of nucleotides (abbreviated "nt") or base pairs (abbreviated "bp"). The term nucleotides is used for single- and double-stranded molecules where the context permits. When the term is applied to double-stranded molecules it is used to denote overall length and will be understood to be equivalent to the term base pairs. It will be recognized by those skilled in the art that the two strands of a double-stranded polynucleotide can differ slightly in length and that the ends thereof can be staggered; thus all nucleotides within a double-stranded polynucleotide molecule may not be paired. Such unpaired ends will, in general, not exceed 20 nucleotides in length.

[0054] As used herein, "similarity" between two proteins or nucleic acids refers to the relatedness between the sequence of amino acids of the proteins or the nucleotide sequences of the nucleic acids. Similarity can be based on the degree of identity and/or homology of sequences of residues and the residues contained therein. Methods for assessing the degree of similarity between proteins or nucleic acids are known to those of skill in the art. For example, in one method of assessing sequence similarity, two amino acid or nucleotide sequences are aligned in a manner that yields a maximal level of identity between the sequences. "Identity" refers to the extent to which the amino acid or nucleotide sequences are invariant. Alignment of amino acid sequences, and to some extent nucleotide sequences, also can take into account conservative differences and/or frequent substitutions in amino acids (or nucleotides). Conservative differences are those that preserve the physico-chemical properties of the residues involved. Alignments can be global (alignment of the compared sequences over the entire length of the sequences and including all residues) or local (the alignment of a portion of the sequences that includes only the most similar region or regions).

[0055] "Identity" per se has an art-recognized meaning and can be calculated using published techniques. (See, e.g. Computational Molecular Biology, Lesk, A.M., ed., Oxford University Press, New York, 1988; Biocomputing: Informatics and Genome Projects, Smith, D.W., ed., Academic Press, New York, 1993; Computer Analysis of Sequence Data, Part I, Griffin, A.M., and Griffin, H.G., eds., Humana Press, New Jersey, 1994; Sequence Analysis in Molecular Biology, von Heinje, G., Academic Press, 1987; and Sequence Analysis Primer, Gribskov, M. and Devereux, J., eds., M Stockton Press, New York, 1991). While there exists a number of methods to measure identity between two polynucleotide or polypeptides, the term "identity" is well known to skilled artisans (Carillo, H. & Lipton, D., SIAM J Applied Math 48:1073 (1988)).

[0056] As used herein, homologous (with respect to nucleic acid and/or amino acid sequences) means about greater than or equal to 25% sequence homology, typically greater than or equal to 25%, 40%, 50%, 60%, 70%, 80%, 85%, 90% or 95% sequence homology; the precise percentage can be specified if necessary. For purposes herein the terms "homology" and "identity" are often used interchangeably, unless otherwise indicated. In general, for determination of the percentage homology or identity, sequences are aligned so that the highest order match is obtained (see, e.g.: Computational Molecular Biology, Lesk, A.M., ed., Oxford University Press, New York, 1988; Biocomputing: Informatics and Genome Projects, Smith, D.W., ed., Academic Press, New York, 1993; Computer Analysis of Sequence Data, Part I, Griffin, A.M., and Griffin, H.G., eds., Humana Press, New Jersey, 1994; Sequence Analysis in Molecular Biology, von Heinje, G., Academic Press, 1987; and Sequence Analysis Primer, Gribskov, M. and Devereux, J., eds., M Stockton Press, New York, 1991; Carillo et al. (1988) SIAM J Applied Math 48:1073). By sequence homology, the number of conserved amino acids is determined by standard alignment algorithms programs, and can be used with default gap penalties established by each supplier. Substantially homologous nucleic acid molecules would hybridize typically at moderate stringency or at high stringency all along the length of the nucleic acid of interest. Also contemplated are nucleic acid molecules that contain degenerate codons in place of codons in the hybridizing nucleic acid molecule.

[0057] Whether any two molecules have nucleotide sequences or amino acid sequences that are at least 60%, 70%,

80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% "identical" or "homologous" can be determined using known computer algorithms such as the "FASTA" program, using for example, the default parameters as in Pearson et al. (1988) Proc. Natl. Acad. Sci. USA 85:2444 (other programs include the GCG program package (Devereux, J., et al., Nucleic Acids Research 12(I):387 (1984)), BLASTP, BLASTN, FASTA (Atschul, S.F., et al., J Mol Biol 215:403 (1990)); Guide to Huge Computers, Martin J. Bishop, ed., Academic Press, San Diego, 1994, and Carillo et al. (1988) SIAM J Applied Math 48:1073). For example, the BLAST function of the National Center for Biotechnology Information database can be used to determine identity. Other commercially or publicly available programs include, DNAStar "MegAlign" program (Madison, WI) and the University of Wisconsin Genetics Computer Group (UWG) "Gap" program (Madison WI). Percent homology or identity of proteins and/or nucleic acid molecules can be determined, for example, by comparing sequence information using a GAP computer program (e.g., Needleman et al. (1970) J. Mol. Biol. 48:443, as revised by Smith and Waterman ((1981) Adv. Appl. Math. 2:482). Briefly, the GAP program defines similarity as the number of aligned symbols (i.e., nucleotides or amino acids), which are similar, divided by the total number of symbols in the shorter of the two sequences. Default parameters for the GAP program can include: (1) a unary comparison matrix (containing a value of 1 for identities and 0 for non-identities) and the weighted comparison matrix of Gribskov et al. (1986) Nucl. Acids Res. 14:6745, as described by Schwartz and Dayhoff, eds., ATLAS OF PROTEIN SEQUENCE AND STRUCTURE, National Biomedical Research Foundation, pp. 353-358 (1979); (2) a penalty of 3.0 for each gap and an additional 0.10 penalty for each symbol in each gap; and (3) no penalty for end gaps.

[0058] Therefore, as used herein, the term "identity" or "homology" represents a comparison between a test and a reference polypeptide or polynucleotide. As used herein, the term at least "90% identical to" refers to percent identities from 90 to 99.99 relative to the reference nucleic acid or amino acid sequence of the polypeptide. Identity at a level of 90% or more is indicative of the fact that, assuming for exemplification purposes a test and reference polypeptide length of 100 amino acids are compared. No more than 10% (i.e., 10 out of 100) of the amino acids in the test polypeptide differs from that of the reference polypeptide. Similar comparisons can be made between test and reference polynucleotides. Such differences can be represented as point mutations randomly distributed over the entire length of a polypeptide or they can be clustered in one or more locations of varying length up to the maximum allowable, e.g. 10/100 amino acid difference (approximately 90% identity). Differences are defined as nucleic acid or amino acid substitutions, insertions or deletions. At the level of homologies or identities above about 85-90%, the result should be independent of the program and gap parameters set; such high levels of identity can be assessed readily, often by manual alignment without relying on software.

[0059] As used herein, an aligned sequence refers to the use of homology (similarity and/or identity) to align corresponding positions in a sequence of nucleotides or amino acids. Typically, two or more sequences that are related by 50% or more identity are aligned. An aligned set of sequences refers to 2 or more sequences that are aligned at corresponding positions and can include aligning sequences derived from RNAs, such as ESTs and other cDNAs, aligned with genomic DNA sequence.

[0060] As used herein, "primer" refers to a nucleic acid molecule that can act as a point of initiation of template-directed DNA synthesis under appropriate conditions (e.g., in the presence of four different nucleoside triphosphates and a polymerization agent, such as DNA polymerase, RNA polymerase or reverse transcriptase) in an appropriate buffer and at a suitable temperature. It will be appreciated that a certain nucleic acid molecules can serve as a "probe" and as a "primer." A primer, however, has a 3' hydroxyl group for extension. A primer can be used in a variety of methods, including, for example, polymerase chain reaction (PCR), reverse-transcriptase (RT)-PCR, RNA PCR, LCR, multiplex PCR, panhandle PCR, capture PCR, expression PCR, 3' and 5' RACE, in situ PCR, ligation-mediated PCR and other amplification protocols.

[0061] As used herein, "primer pair" refers to a set of primers that includes a 5' (upstream) primer that hybridizes with the 5' end of a sequence to be amplified (e.g. by PCR) and a 3' (downstream) primer that hybridizes with the complement of the 3' end of the sequence to be amplified.

[0062] As used herein, "specifically hybridizes" refers to annealing, by complementary base-pairing, of a nucleic acid molecule (e.g. an oligonucleotide) to a target nucleic acid molecule. Those of skill in the art are familiar with in vitro and in vivo parameters that affect specific hybridization, such as length and composition of the particular molecule. Parameters particularly relevant to in vitro hybridization further include annealing and washing temperature, buffer composition and salt concentration. Exemplary washing conditions for removing non-specifically bound nucleic acid molecules at high stringency are 0.1 x SSPE, 0.1% SDS, 65°C, and at medium stringency are 0.2 x SSPE, 0.1% SDS, 50°C. Equivalent stringency conditions are known in the art. The skilled person can readily adjust these parameters to achieve specific hybridization of a nucleic acid molecule to a target nucleic acid molecule appropriate for a particular application. Complementary, when referring to two nucleotide sequences, means that the two sequences of nucleotides are capable of hybridizing, typically with less than 25%, 15% or 5% mismatches between opposed nucleotides. If necessary, the percentage of complementarity will be specified. Typically the two molecules are selected such that they will hybridize under conditions of high stringency.

[0063] As used herein, substantially identical to a product means sufficiently similar so that the property of interest is

sufficiently unchanged so that the substantially identical product can be used in place of the product.

[0064] As used herein, it also is understood that the terms "substantially identical" or "similar" varies with the context as understood by those skilled in the relevant art.

[0065] As used herein, an allelic variant or allelic variation references any of two or more alternative forms of a gene occupying the same chromosomal locus. Allelic variation arises naturally through mutation, and can result in phenotypic polymorphism within populations. Gene mutations can be silent (no change in the encoded polypeptide) or can encode polypeptides having altered amino acid sequence. The term "allelic variant" also is used herein to denote a protein encoded by an allelic variant of a gene. Typically the reference form of the gene encodes a wildtype form and/or predominant form of a polypeptide from a population or single reference member of a species. Typically, allelic variants, which include variants between and among species typically have at least 80%, 90% or greater amino acid identity with a wildtype and/or predominant form from the same species; the degree of identity depends upon the gene and whether comparison is interspecies or intraspecies. Generally, intraspecies allelic variants have at least about 80%, 85%, 90% or 95% identity or greater with a wildtype and/or predominant form, including 96%, 97%, 98%, 99% or greater identity with a wildtype and/or predominant form of a polypeptide. Reference to an allelic variant herein generally refers to variations n proteins among members of the same species.

[0066] As used herein, "allele," which is used interchangeably herein with "allelic variant" refers to alternative forms of a gene or portions thereof. Alleles occupy the same locus or position on homologous chromosomes. When a subject has two identical alleles of a gene, the subject is said to be homozygous for that gene or allele. When a subject has two different alleles of a gene, the subject is said to be heterozygous for the gene. Alleles of a specific gene can differ from each other in a single nucleotide or several nucleotides, and can include modifications such as substitutions, deletions and insertions of nucleotides. An allele of a gene also can be a form of a gene containing a mutation.

[0067] As used herein, species variants refer to variants in polypeptides among different species, including different mammalian species, such as mouse and human. Exemplary of species variants provided herein are primate PH20, such as, but not limited to, human, chimpanzee, macaque and cynomologus monkey. Generally, species variants have 70%, 75%. 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or sequence identity. Corresponding residues between and among species variants can be determined by comparing and aligning sequences to maximize the number of matching nucleotides or residues, for example, such that identity between the sequences is equal to or greater than 95%, equal to or greater than 96%, equal to or greater than 97%, equal to or greater than 98% or equal to greater than 99%. The position of interest is then given the number assigned in the reference nucleic acid molecule. Alignment can be effected manually or by eye, particularly, where sequence identity is greater than 80%. For example, the alignment in Figure 1 shows that amino acid residue 491 of human PH20 corresponds to amino acid residue 491 of chimpanzee PH20 and amino acid residue 497 of human PH20 corresponds to amino acid residue 498 of chimpanzee PH20.

[0068] As used herein, a splice variant refers to a variant produced by differential processing of a primary transcript of genomic DNA that results in more than one type of mRNA.

[0069] As used herein, modification is in reference to modification of a sequence of amino acids of a polypeptide or a sequence of nucleotides in a nucleic acid molecule and includes deletions, insertions, and replacements of amino acids and nucleotides, respectively. Methods of modifying a polypeptide are routine to those of skill in the art, such as by using recombinant DNA methodologies.

[0070] As used herein, the term promoter means a portion of a gene containing DNA sequences that provide for the binding of RNA polymerase and initiation of transcription. Promoter sequences are commonly, but not always, found in the 5' non-coding region of genes.

[0071] As used herein, isolated or purified polypeptide or protein or biologically-active portion thereof is substantially free of cellular material or other contaminating proteins from the cell or tissue from which the protein is derived, or substantially free from chemical precursors or other chemicals when chemically synthesized. Preparations can be determined to be substantially free if they appear free of readily detectable impurities as determined by standard methods of analysis, such as thin layer chromatography (TLC), gel electrophoresis and high performance liquid chromatography (HPLC), used by those of skill in the art to assess such purity, or sufficiently pure such that further purification would not detectably alter the physical and chemical properties, such as enzymatic and biological activities, of the substance. Methods for purification of the compounds to produce substantially chemically pure compounds are known to those of skill in the art. A substantially chemically pure compound, however, can be a mixture of stereoisomers. In such instances, further purification might increase the specific activity of the compound.

[0072] Hence, reference to a substantially purified polypeptide, such as a substantially purified extended soluble PH20 refers to preparations of PH20 proteins that are substantially free of cellular material includes preparations of proteins in which the protein is separated from cellular components of the cells from which it is isolated or recombinantly-produced. In one embodiment, the term substantially free of cellular material includes preparations of enzyme proteins having less that about 30% (by dry weight) of non-enzyme proteins (also referred to herein as a contaminating protein), generally less than about 20% of non-enzyme proteins or 10% of non-enzyme proteins or less that about 5% of non-enzyme proteins. When the enzyme protein is recombinantly produced, it also is substantially free of culture medium, i.e., culture

medium represents less than about or at 20%, 10% or 5% of the volume of the enzyme protein preparation.

[0073] As used herein, the term substantially free of chemical precursors or other chemicals includes preparations of enzyme proteins in which the protein is separated from chemical precursors or other chemicals that are involved in the synthesis of the protein. The term includes preparations of enzyme proteins having less than about 30% (by dry weight), 20%, 10%, 5% or less of chemical precursors or non-enzyme chemicals or components.

[0074] As used herein, synthetic, with reference to, for example, a synthetic nucleic acid molecule or a synthetic gene or a synthetic peptide refers to a nucleic acid molecule or polypeptide molecule that is produced by recombinant methods and/or by chemical synthesis methods.

[0075] As used herein, production by recombinant means or using recombinant DNA methods means the use of the well known methods of molecular biology for expressing proteins encoded by cloned DNA.

[0076] As used herein, vector (or plasmid) refers to discrete elements that are used to introduce a heterologous nucleic acid into cells for either expression or replication thereof. The vectors typically remain episomal, but can be designed to effect integration of a gene or portion thereof into a chromosome of the genome. Also contemplated are vectors that are artificial chromosomes, such as yeast artificial chromosomes and mammalian artificial chromosomes. Selection and use of such vehicles are well known to those of skill in the art.

[0077] As used herein, an expression vector includes vectors capable of expressing DNA that is operatively linked with regulatory sequences, such as promoter regions, that are capable of effecting expression of such DNA fragments. Such additional segments can include promoter and terminator sequences, and optionally can include one or more origins of replication, one or more selectable markers, an enhancer, a polyadenylation signal, and the like. Expression vectors are generally derived from plasmid or viral DNA, or can contain elements of both. Thus, an expression vector refers to a recombinant DNA or RNA construct, such as a plasmid, a phage, recombinant virus or other vector that, upon introduction into an appropriate host cell, results in expression of the cloned DNA. Appropriate expression vectors are well known to those of skill in the art and include those that are replicable in eukaryotic cells and/or prokaryotic cells and those that remain episomal or those which integrate into the host cell genome.

[0078] As used herein, vector also includes "virus vectors" or "viral vectors." Viral vectors are engineered viruses that are operatively linked to exogenous genes to transfer (as vehicles or shuttles) the exogenous genes into cells.

[0079] As used herein, "operably" or "operatively linked" when referring to DNA segments means that the segments are arranged so that they function in concert for their intended purposes, e.g., transcription initiates downstream of the promoter and upstream of any transcribed sequences. The promoter is usually the domain to which the transcriptional machinery binds to initiate transcription and proceeds through the coding segment to the terminator.

[0080] As used herein the term "assessing" is intended to include quantitative and qualitative determination in the sense of obtaining an absolute value for the activity of a protease, or a domain thereof, present in the sample, and also of obtaining an index, ratio, percentage, visual or other value indicative of the level of the activity. Assessment can be direct or indirect and the chemical species actually detected need not of course be the proteolysis product itself but can for example be a derivative thereof or some further substance. For example, detection of a cleavage product of a complement protein, such as by SDS-PAGE and protein staining with Coomasie blue.

[0081] As used herein, biological activity refers to the *in vivo* activities of a compound or physiological responses that result upon *in vivo* administration of a compound, composition or other mixture. Biological activity, thus, encompasses therapeutic effects and pharmaceutical activity of such compounds, compositions and mixtures. Biological activities can be observed in *in vitro* systems designed to test or use such activities. Thus, for purposes herein a biological activity of a protease is its catalytic activity in which a polypeptide is hydrolyzed.

[0082] As used herein equivalent, when referring to two sequences of nucleic acids, means that the two sequences in question encode the same sequence of amino acids or equivalent proteins. When equivalent is used in referring to two proteins or peptides, it means that the two proteins or peptides have substantially the same amino acid sequence with only amino acid substitutions that do not substantially alter the activity or function of the protein or peptide. When equivalent refers to a property, the property does not need to be present to the same extent (e.g., two peptides can exhibit different rates of the same type of enzymatic activity), but the activities are usually substantially the same.

[0083] As used herein, "modulate" and "modulation" or "alter" refer to a change of an activity of a molecule, such as a protein. Exemplary activities include, but are not limited to, biological activities, such as signal transduction. Modulation can include an increase in the activity (i.e., up-regulation or agonist activity), a decrease in activity (i.e., down-regulation or inhibition) or any other alteration in an activity (such as a change in periodicity, frequency, duration, kinetics or other parameter). Modulation can be context dependent and typically modulation is compared to a designated state, for example, the wildtype protein, the protein in a constitutive state, or the protein as expressed in a designated cell type or condition.

[0084] As used herein, a composition refers to any mixture. It can be a solution, suspension, liquid, powder, paste, aqueous, non-aqueous or any combination thereof.

[0085] As used herein, a combination refers to any association between or among two or more items. The combination can be two or more separate items, such as two compositions or two collections, can be a mixture thereof, such as a

single mixture of the two or more items, or any variation thereof. The elements of a combination are generally functionally associated or related.

[0086] As used herein, "disease or disorder" refers to a pathological condition in an organism resulting from cause or condition including, but not limited to, infections, acquired conditions, genetic conditions, and characterized by identifiable symptoms. Diseases and disorders of interest herein are those involving components of the ECM. As used herein, "treating" a subject with a disease or condition means that the subject's symptoms are partially or totally alleviated, or remain static following treatment. Hence treatment encompasses prophylaxis, therapy and/or cure. Prophylaxis refers to prevention of a potential disease and/or a prevention of worsening of symptoms or progression of a disease. Treatment also encompasses any pharmaceutical use of a modified interferon and compositions provided herein.

[0087] As used herein, a pharmaceutically effective agent, includes any therapeutic agent or bioactive agent, including, but not limited to, for example, anesthetics, vasoconstrictors, dispersing agents, conventional therapeutic drugs, including small molecule drugs, including, but not limited to, bisphosphonates, and therapeutic proteins, including, but not limited to, insulin, IgG molecules, and antibodies.

[0088] As used herein, a therapeutic agent, includes any pharmaceutically effective agent or bioactive agent, including, but not limited to, for example, anesthetics, vasoconstrictors, dispersing agents, conventional therapeutic drugs, including small molecule drugs, including, but not limited to, bisphosphonates, and therapeutic proteins, including, but not limited to, insulin, IgG molecules, and antibodies.

[0089] As used herein, treatment means any manner in which the symptoms of a condition, disorder or disease or other indication, are ameliorated or otherwise beneficially altered.

[0090] As used herein, therapeutic effect means an effect resulting from treatment of a subject that alters, typically improves or ameliorates the symptoms of a disease or condition or that cures a disease or condition. A therapeutically effective amount refers to the amount of a composition, molecule or compound which results in a therapeutic effect following administration to a subject.

[0091] As used herein, the term "subject" refers to an animal, including a mammal, such as a human being.

[0092] As used herein, a patient refers to a human subject exhibiting symptoms of a disease or disorder.

[0093] As used herein, amelioration of the symptoms of a particular disease or disorder by a treatment, such as by administration of a pharmaceutical composition or other therapeutic, refers to any lessening, whether permanent or temporary, lasting or transient, of the symptoms that can be attributed to or associated with administration of the composition or therapeutic.

[0094] As used herein, prevention or prophylaxis refers to methods in which the risk of developing disease or condition is reduced.

[0095] As used herein, a "therapeutically effective amount" or a "therapeutically effective dose" refers to the quantity of an agent, compound, material, or composition containing a compound that is at least sufficient to produce a therapeutic effect. Hence, it is the quantity necessary for preventing, curing, ameliorating, arresting or partially arresting a symptom of a disease or disorder.

[0096] As used herein, unit dose form refers to physically discrete units suitable for human and animal subjects and packaged individually as is known in the art.

[0097] As used herein, a single dosage formulation refers to a formulation for direct administration.

[0098] As used herein, an "article of manufacture" is a product that is made and sold. As used throughout this application, the term is intended to encompass a therapeutic agent with a soluble PH20, such as esPH20, or an esPH20 alone, contained in the same or separate articles of packaging.

[0099] As used herein, fluid refers to any composition that can flow. Fluids thus encompass compositions that are in the form of semi-solids, pastes, solutions, aqueous mixtures, gels, lotions, creams and other such compositions.

[0100] As used herein, a "kit" refers to a combination of compositions provided herein and another item for a purpose including, but not limited to, reconstitution, activation, and instruments/devices for delivery, administration, diagnosis, and assessment of a biological activity or property. Kits optionally include instructions for use.

[0101] As used herein, a cellular extract or lysate refers to a preparation or fraction which is made from a lysed or disrupted cell.

[0102] As used herein, animal includes any animal, such as, but are not limited to primates including humans, gorillas and monkeys; rodents, such as mice and rats; fowl, such as chickens; ruminants, such as goats, cows, deer, sheep; pigs and other animals. Non-human animals exclude humans as the contemplated animal. The enzymes provided herein are from any source, animal, plant, prokaryotic and fungal. Most enzymes are of animal origin, including mammalian origin.

[0103] As used herein, a control refers to a sample that is substantially identical to the test sample, except that it is not treated with a test parameter, or, if it is a plasma sample, it can be from a normal volunteer not affected with the condition of interest. A control also can be an internal control.

[0104] As used herein, the singular forms "a," "an" and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to a compound comprising "an extracellular domain" includes compounds with one or a plurality of extracellular domains.

[0105] As used herein, ranges and amounts can be expressed as "about" a particular value or range. About also includes the exact amount. Hence "about 5 bases" means "about 5 bases" and also "5 bases."

[0106] As used herein, "optional" or "optionally" means that the subsequently described event or circumstance does or does not occur, and that the description includes instances where said event or circumstance occurs and instances where it does not. For example, an optionally substituted group means that the group is unsubstituted or is substituted.

[0107] As used herein, the abbreviations for any protective groups, amino acids and other compounds, are, unless indicated otherwise, in accord with their common usage, recognized abbreviations, or the IUPAC-IUB Commission on Biochemical Nomenclature (see, (1972) Biochem. 11:1726).

B. Overview

[0108] Hyaluronidases are enzymes that catalyze the hydrolysis of hyaluronic acid, thereby lowering the viscosity of hyaluronic acid and increasing tissue permeability. PH20 is a neutral-active and acid-active hyaluronidase that exhibits optimal activity when glycosylated. Human PH20 is a GPI-anchored protein that is anchored to the extracellular leaflet of the plasma membrane via a glycosylphosphatidylinositol (GPI) anchor attached to the C-terminus of the protein. The addition of the GPI anchor to all GPI-anchored proteins occurs following cleavage at a specific amino acid position, called the ω-site (typically located approximately 20-30 amino acids from the C-terminus), and removal of the C-terminal portion in the ER. This C-terminal portion is the GPI-anchor attachment signal sequence. The GPI-anchor attachment signal sequence of human PH20 is located at amino acid positions 491-509 of the precursor polypeptide set forth in SEQ ID NO:107, and the ω-site is amino acid position 490. GPI-anchored PH20 polypeptides such as human PH20 are membrane-bound and, therefore, insoluble. Insoluble forms of PH20 typically are not suitable for therapeutic purposes.

[0109] PH20 polypeptides that lack a GPI anchor generally are secreted by cells upon expression because they do not contain a GPI-attachment signal sequence that locks the polypeptide to the membrane. It is found herein that soluble forms of PH20 also include those that contain residues within the GPI-anchor attachment signal sequence. Extended soluble PH20 (esPH20) polypeptides are soluble PH20 proteins that are truncated at the C-terminus but retain one or more amino acid residues located in the GPI-anchor attachment signal sequence of the corresponding wild-type PH20 polypeptide. Such esPH20 polypeptides are soluble and can be used as therapeutic polypeptides, such as to treat hyaluronan-associated diseases or conditions and/or to serve as a spreading or dispersing agent to promote, enhance or increase the dispersion and delivery of other agents, drugs and proteins thereby improving the pharmocokinetic and pharmacodynamic profile of the co-administered agent, drug or protein.

1. PH20

[0110] PH20, also known as sperm surface protein, sperm adhesion molecule 1, spam1 or HYAL3, is a hyaluronidase. Hyaluronidases are a family of enzymes that degrade hyaluronic acid (also known as hyaluronan or hyaluronate or HA), an essential component of the extracellular matrix and a major constituent of the interstitial barrier. By catalyzing the hydrolysis of hyaluronic acid, hyaluronidase lowers the viscosity of hyaluronic acid, thereby increasing tissue permeability. As such, hyaluronidases have been used, for example, as a spreading or dispersing agent in conjunction with other agents, drugs and proteins to enhance their dispersion and delivery, and to improve the pharmocokinetic and pharmacodynamic profile of the co-administered agent, drug or protein.

[0111] PH20, like other mammalian hyaluronidases, is an endo-β-N-acetyl-hexosaminidase that hydrolyzes the β1→4 glycosidic bond of hyaluronic acid into various oligosaccharide lengths such as tetrasaccharides and hexasaccharides. PH20 has both hydrolytic and transglycosidase activities and can degrade hyaluronic acid and chondroitin sulfates, such as C4-S and C6-S. PH20 is naturally involved in sperm-egg adhesion and aids penetration by sperm of the layer of cumulus cells by digesting hyaluronic acid. PH20 is located on the sperm surface, and in the lysosome-derived acrosome, where it is bound to the inner acrosomal membrane. Plasma membrane PH20 has hyaluronidase activity only at neutral pH, while inner acrosomal membrane PH20 has activity at both neutral and acidic pH. In addition to being a hyaluronidase, PH20 also appears to be a receptor for HA-induced cell signaling, and a receptor for the zona pellucida surrounding the oocyte.

[0112] Exemplary PH20 proteins include, but are not limited to, human (precursor polypeptide set forth in SEQ ID NO:107, mature polypeptide set forth in SEQ ID NO:108), bovine (SEQ ID NOS:111 and 119), rabbit (SEQ ID NO:112), ovine (SEQ ID NOS:113, 118 and 120), Cynomolgus monkey (SEQ ID NO:114), guinea pig (SEQ ID NO:115), rat (SEQ ID NO:116), mouse (SEQ ID NO:117), chimpanzee (SEQ ID NO:197) and Rhesus monkey (SEQ ID NO:198) PH20 polypeptides. The human PH20 mRNA transcript is normally translated to generate a 509 amino acid precursor protein (SEQ ID NO:107) containing a 35 amino acid signal sequence at the N-terminus (amino acid residue positions 1-35 of SEQ ID NO:107). Thus, following transport to the ER and removal of the signal peptide, a 474 amino acid mature polypeptide with an amino acid sequence set forth in SEQ ID NO:108 is produced. As discussed below, a C-terminal peptide is then cleaved in the ER to facilitate covalent attachment of a GPI anchor to the newly-formed C-terminal amino

acid at the amino acid position corresponding to position 490 of the precursor polypeptide set forth in SEQ ID NO:107.

[0113] Human PH20 is the prototypical neutral-active hyaluronidase that is generally locked to the plasma membrane via a glycosylphosphatidylinositol (GPI) anchor. As noted above, PH20 also is expressed on the inner acrosomal membrane where it has hyaluronidase activity at both neutral and acidic pH. Evidence suggests that the Peptide 1 region of PH20, which corresponds to amino acids 142-172 of the precursor polypeptide set forth in SEQ ID NO:107, is required for enzyme activity at neutral pH. The Peptide 3 region, which corresponds to amino acids 277-297 of the precursor polypeptide set forth in SEQ ID NO:107, appears to be important for enzyme activity at acidic pH (Cherr et al., (2001) Matrix Biology 20:515-525). Thus, it appears that PH20 contains two catalytic sites. In addition to the catalytic sites, PH20 also contains a hyaluronan-binding site. Experimental evidence suggests that this site is located in the Peptide 2 region, which corresponds to amino acid positions 205-235 of the precursor polypeptide set forth in SEQ ID NO:107. This region is highly conserved among hyaluronidases and is similar to the heparin binding motif.

a. Glycosylation

[0114] Glycosylation, including N- and O-linked glycosylation, of some hyaluronan degrading enzymes, including hyaluronidases, can be important for their catalytic activity and stability. N-linked oligosaccharides fall into several major types (oligomannose, complex, hybrid), all of which have $(Man)_3$-GlcNAc-GlcNAc-cores attached via the amide nitrogen of Asn residues that fall within -Asn-Xaa-Thr/Ser-sequences (where Xaa is not Pro). An additional glycosylation site at -Asn-Xaa-Cys-has been reported for coagulation protein C. In some instances, a hyaluronan degrading enzyme, such as a hyaluronidase, can contain both N-glycosidic and O-glycosidic linkages. For example, PH20 has one O-linked oligosaccharide at amino acid T475 as well as six N-linked oligosaccharides at amino acids N82, N166, N235, N254, N368, and N393 of human PH20, exemplified in SEQ ID NO: 107. Amino acid residues N82, N166 and N254 are occupied by complex type glycans whereas amino acid residues N368 and N393 are occupied by high mannose type glycans (see, e.g. Example 6 below). Amino acid residue N235 is occupied by approximately 80% high mannose type glycans and 20% complex type glycans.

[0115] While altering the type of glycan modifying a glycoprotein can have dramatic affects on a protein's antigenicity, structural folding, solubility, and stability, most enzymes are not thought to require glycosylation for optimal enzyme activity. For some hyaluronidases, removal of N-linked glycosylation can result in near complete inactivation of the hyaluronidase activity. Thus, for such hyaluronidases, the presence of N-linked glycans is required for generating an active enzyme. The presence of N-linked glycans in PH20 polypeptides is required for generating an active enzyme. For example, it is found herein, that complete deglycosylation of human PH20, by treatment with the endoglycosidase PNGaseF or the GlcNAc phosphotransferase (GPT) inhibitor tunicamycin, results in the total loss of hyaluronidase activity (see, e.g. Examples 7-8, below). In contrast, partial deglycosylation of human PH20, by treatment with endoglycosidase EndoF1, EndoF2, EndoF3 or EndoH, does not affect the hyaluronidase activity of human PH20 (see, e.g., Example 7, below).

b. GPI-anchoring

[0116] Human PH20 is a GPI-anchored protein. As such, the PH20 polypeptide is anchored to the extracellular leaflet of the plasma membrane via a glycosylphosphatidylinositol (GPI) anchor attached to the C-terminus of the protein. GPI-anchored proteins such as human PH20 are translated with a cleavable N-terminal signal peptide that directs the protein to the endoplasmic reticulum (ER). At the C-terminus of these proteins is another signal sequence that directs addition of a preformed GPI-anchor to the polypeptide within the lumen of the ER. Addition of the GPI anchor occurs following cleavage of the C-terminal portion at a specific amino acid position, called the ω-site (typically located approximately 20-30 amino acids from the C-terminus). Although there appears to be no consensus sequence to identify the location of the ω-site, GPI anchored proteins contain a C-terminal GPI-anchor attachment signal sequence or domain that typically contains a predominantly hydrophobic region of 8-20 amino acids, preceded by a hydrophilic spacer region of 8-12 amino acids immediately downstream of the ω-site. This hydrophilic spacer region often is rich in charged amino acids and proline (White et al., (2000) J. Cell Sci. 113(Pt.4):721-727). More detailed analysis suggests that there is a region of approximately 11 amino acids before the ω-1 position that is characterized by a low amount of predicted secondary structure, a region around the cleavage site (ω-site), from ω-1 to ω+2 that is characterized by the presence of small side chain residues, the spacer region between positions ω+3 and ω+9, and a hydrophobic tail from ω+10 to the C-terminal end (Pierleoni et al., (2008) BMC Bioinformatics 9:392).

[0117] Although there is no GPI-anchor attachment signal consensus sequence, various *in silico* methods and algorithms have been developed that can be used to identify such sequences in polypeptides (see, e.g. Udenfriend et al. (1995) Methods Enzymol. 250:571-582; Eisenhaber et al., (1999) J. Biol. Chem. 292: 741-758; Kronegg and Buloz, (1999), "Detection/prediction of GPI cleavage site (GPI-anchor) in a protein (DGPI)," 129.194.185.165/dgpi/; Fankhauser et al., (2005) Bioinformatics 21:1846-1852; Omaetxebarria et al., (2007) Proteomics 7:1951-1960; Pierleoni et al., (2008)

BMC Bioinformatics 9:392), including those that are readily available on bioinformatic websites, such as the ExPASy Proteomics tools site (expasy.ch/tools/). Thus, one of skill in the art can determine whether a PH20 polypeptide likely contains a GPI-anchor attachment signal sequence, and, therefore, whether the PH20 polypeptide is a GPI-anchored protein.

[0118] The GPI-anchor attachment signal sequence of human PH20 is located at amino acid positions 491-509 of the precursor polypeptide set forth in SEQ ID NO:107, and the ω-site is amino acid position 490. Thus, in this modeling of human PH20, amino acids 491-509 are cleaved following transport to the ER and a GPI anchor is covalently attached to the serine residue at position 490. The covalent attachment of a GPI-anchor to the C-terminus of human PH20 and, therefore, the membrane-bound nature of PH20, has been confirmed using phosphatidylinositol-specific phospholipase C (PI-PLC) hydrolysis studies (see, e.g., Lin et al., (1994) J. Biol. Chem. 125:1157-1163 and Example 3, below). Phosphatidylinositol-specific phospholipase C (PI-PLC) and D (PI-PLD) hydrolyze the GPI anchor, releasing the PH20 polypeptide from the cell membrane. The resulting released PH20 polypeptide is, therefore, soluble. Soluble PH20 can be detected and discriminated from insoluble, membrane-bound PH20 using methods well known in the art, including, but not limited to, those using a Triton® X-114 assay, as described below and in Example 4. In this assay, soluble PH20 hyaluronidases partition into the aqueous phase of a Triton® X-114 solution warmed to 37 °C (Bordier et al., (1981) J. Biol. Chem., 256:1604-7) while membrane-anchored PH20 hyaluronidases partition into the detergent rich phase. Thus, in addition to using algorithms to assess whether a PH20 polypeptide is naturally GPI-anchored, solubility experiments also can be performed.

C. Extended Soluble PH20 Polypeptides

[0119] Provided herein are extended soluble PH20 (esPH20) polypeptides and compositions. Exemplary of the esPH20 polypeptides provided herein are primate esPH20 polypeptides, including, but not limited to, human and chimpanzee esPH20 polypeptides. The esPH20 polypeptides provided herein are soluble, i.e. secreted, PH20 proteins that are truncated at the C-terminus but retain at least one or more amino acid residues located in the GPI-anchor attachment signal sequence of the corresponding wild-type PH20 polypeptide (e.g. are truncated at amino acid positions 491-500). EsPH20 polypeptides can be produced from any GPI-anchored PH20 polypeptide by modification of the GPI-anchored PH20 polypeptide, that is by removal of a portion of the GPI-anchor attachment signal sequence, provided that the resulting esPH20 polypeptide is soluble. Solubility, or secretion into the cell culture medium, can be determined by SDS-PAGE and western blot analysis upon expression, or alternatively, in a Triton® X-114 assay, as described below and in Example 4, when the PH20 polypeptide is produced by any method known to one of skill in the art, including recombinant expression and polypeptide synthesis. The esPH20 polypeptides provided herein can be used, for example, as therapeutic polypeptides, such as a spreading or dispersing agent in conjunction with other agents, drugs and proteins to enhance their dispersion and delivery, and to improve the pharmocokinetic and pharmacodynamic profile of the co-administered agent, drug or protein.

[0120] The esPH20 polypeptides provided herein contain 1, 2, 3, 4, 5, 6, 7 or more amino acid residues from the GPI-anchor attachment signal sequence, providing the esPH20 polypeptide is soluble, i.e., partitions into the aqueous phase of a Triton® X-114 solution, as described below. The extended soluble PH20 polypeptides provided herein can be produced by making C-terminal truncations to any naturally GPI-anchored PH20 polypeptide, wherein the resulting esPH20 polypeptide is soluble and contains 1 or more amino acid residues from the GPI-anchor attachment signal sequence. One of skill in the art can determine whether a PH20 polypeptide is GPI-anchored using methods well known in the art. Such methods include, but are not limited to, using known algorithms to predict the presence and location of the GPI-anchor attachment signal sequence and ω-site, and performing solubility analyses before and after digestion with phosphatidylinositol-specific phospholipase C (PI-PLC) or D (PI-PLD).

[0121] Exemplary esPH20 polypeptides include, but are not limited to, esPH20 polypeptides of primates, such as, for example, human and chimpanzee esPH20 polypeptides. For example, the esPH20 polypeptides provided herein can be made by C-terminal truncation of any of the mature or precursor polypeptides set forth in SEQ ID NOS:107, 108, or 197, or allelic or other variations thereof, including active fragments thereof, wherein the resulting polypeptide is soluble and retains 1 or more amino acid residues from the GPI-anchor attachment signal sequence. Allelic variants and other variants are known to one of skill in the art, and include polypeptides having 60%, 70%, 80%, 90%, 91%, 92%, 93%, 94%, 95% or more sequence identity to any of SEQ ID NOS: 107, 108 and 197. The esPH20 polypeptides provided herein can be C-terminally truncated by 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more amino acids compared to the wild type polypeptide, such as a polypeptide with a sequence set forth in SEQ ID NOS: 107, 108 and 197, provided the resulting esPH20 polypeptide is soluble and retains 1 or more amino acid residues from the GPI-anchor attachment signal sequence.

[0122] The extended soluble PH20 polypeptides provided herein retain hyaluronidase activity. Additionally, the esPH20 polypeptides are neutral active, that is, they retain hyaluronidase activity at neutral pH. The hyaluronidase activity can be increased or decreased compared to the wild-type GPI-anchored form of the PH20. For example, the esPH20 polypep-

tides provided herein can exhibit hyaluronidase activity that is 1%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 110%, 120%, 130%, 140%, 150%, 200%, 300%, 400%, 500%, 1000% or more of the hyaluronidase activity exhibited by the wildtype GPI-anchored form.

1. Human esPH20 polypeptides

[0123] Exemplary of the esPH20 polypeptides provided herein are human esPH20 polypeptides. The human esPH20 polypeptides provided herein are soluble and contain 1 or more amino acid residues from the GPI-anchor attachment signal sequence. Thus, provided herein are soluble forms of human PH20 that GPI do not completely lack the GPI-anchor attachment signal sequence.

[0124] Precursor human esPH20 polypeptides provided herein include, but are not limited to, those having C-terminal truncations to generate polypeptides containing amino acid 1 to amino acid 491, 492, 493, 494, 495, 496, 497, 498, 499 or 500 of the sequence of amino acids set forth in SEQ ID NO: 107. When expressed in mammalian cells, the 35 amino acid N-terminal signal sequence is cleaved during processing, and the mature form of the protein is secreted. Thus, the mature human esPH20 polypeptides contain amino acids 36 to 491, 492, 493, 494, 495, 496, 497, 498, 499 or 500 of SEQ ID NO:107. Hence, mature human esPH20 polypeptides provided herein include those set forth in SEQ ID NOS: 59-63 and 100-104, or allelic or other variants thereof.

[0125] The human esPH20 polypeptides provided herein can be expressed in CHO cells, or alternatively produced in any cell or by any method known to one of skill in the art, provided they are soluble and contain at least one amino acid from the GPI-anchor attachment signal sequence. Soluble human esPH20 polypeptides produced in CHO cells are those that are secreted into the cell culture medium. It is understood by one of skill in the art that a human esPH20 can be partially secreted, that is, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more of the expresssed polypeptide is secreted into the culture medium, provided that the secreted esPH20 is soluble, i.e., partitions into the aqueous phase of a Triton® X-114 solution, as described below. Human esPH20 polypeptides provided herein that contain amino acids 1-500, or 36-500, are partially secreted. Additionally, when expressed in CHO cells, the precursor human esPH20 polypeptides containing amino acids 1 to 498, 499 or 500, or the mature human esPH20 polypeptides containing amino acids 36 to 498, 499 or 500, are weakly expressed (see, e.g., Example 3 below).

[0126] Thus, exemplary precursor human esPH20 polypeptides include, but are not limited to, any having C-terminal truncations to generate polypeptides containing amino acid 1 to amino acid 491, 492, 493, 494, 495, 496 or 497 of the sequence of amino acids set forth in SEQ ID NO: 107. When expressed in mammalian cells, following cleavage of the N-terminal signal peptide during processing, mature human esPH20 polypeptides contain amino acids 36 to 491, 492, 493, 494, 495, 496 or 497 of SEQ ID NO:107. Hence, exemplary mature human esPH20 polypeptides provided herein include those that are 456, 457, 458, 459, 460, 461 or 462 amino acids in length, such as set forth in any of SEQ ID NOS: 60-63 and 102-104, or allelic or other variants thereof. Allelic variants and other variants are known to one of skill in the art, and include polypeptides having 60%, 70%, 80%, 90%, 91%, 92%, 93%, 94%, 95% or more sequence identity to any of SEQ ID NOS: 107 or 108.

[0127] Also provided herein are amino acid-substituted human esPH20 polypeptides, Amino acid substituted esPH20 polypeptides are human esPH20 polypeptides that are modified such that they contain amino acid substitutions, as compared to the human esPH20 polypeptides provided herein, for example, as set forth in SEQ ID NOS: 60-63 and 102-104. Thus, amino acid-substituted human esPH20 polypeptides are those having C-terminal truncations. In some examples, the amino acid substituted human esPH20 polypeptides provided herein have at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 87%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity with the sequence of amino acids set forth as amino acids 1 to 491, 492, 493, 494, 495, 496 or 497, or amino acids 36 to 491, 492, 493, 494, 495, 496 or 497, of the sequence of amino acids set forth in SEQ ID NO: 107. In other examples, the amino acid substituted human esPH20 polypeptides have 85%, 87%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity with the sequence of amino acids set forth as in SEQ ID NOS: 60-63 and 102-104.

[0128] The human esPH20 polypeptides provided herein can exhibit hyaluronidase activity that is increased or decreased compared to the wild-type GPI-anchored form of PH20. For example, the human esPH20 polypeptides provided herein can exhibit hyaluronidase activity that is 1%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 110%, 120%, 130%, 140%, 150%, 200%, 300%, 400%, 500%, 1000% or more of the hyaluronidase activity exhibited by the wildtype GPI-anchored form. In some examples, human esPH20 polypeptides exhibit increased hyaluronidase activity compared to the wildtype GPI-anchored form. The hyaluronidase activity of human esPH20 polypeptides can be increased by 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 110%, 120%, 130%, 140%, 150%, 200%, 300%, 400%, 500%, 1000% or more compared to the hyaluronidase activity of the wildtype GPI-anchored form.

[0129] The human esPH20 polypeptides provided herein exhibit neutral active hyaluronidase activity, or hyaluronidase activity when measured at neutral pH, that is increased or decreased compared to the the compared to the wild-type GPI-anchored form of PH20. For example, the human esPH20 polypeptides provided herein can exhibit hyaluronidase

activity that is 1%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 110%, 120%, 130%, 140%, 150%, 200%, 300%, 400%, 500%, 1000% or more of the hyaluronidase activity exhibited by the wildtype GPI-anchored form. In some examples, human esPH20 polypeptides exhibit decreased neutral active hyaluronidase activity compared to the wildtype GPI-anchored form. The neutral active hyaluronidase activity can be decreased by 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95% or more compared to the neutral active hyaluronidase activity of the wildtype GPI-anchored form. In other examples, human esPH20 polypeptides exhibit increased neutral active hyaluronidase activity compared to the wildtype GPI-anchored form. The neutral active hyaluronidase activity can be increased by 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 110%, 120%, 130%, 140%, 150%, 200%, 300%, 400%, 500%, 1000% or more compared to the neutral active hyaluronidase activity of the wildtype GPI-anchored form.

[0130] Typically, human esPH20 polypeptides are produced using protein expression systems that facilitate correct N-glycosylation to ensure the polypeptide retains activity, since glycosylation is important for the catalytic activity and stability of these polypeptides. Exemplary cells useful for recombinant expression of esPH20 polypeptides include, for example Chinese Hamster Ovary (CHO) cells (e.g. DG44 CHO or CHO-S cells).

2. Other species esPH20 polypeptides

[0131] Provided herein are non-human extended soluble PH20 polypeptides. One of skill in art can align the amino acid sequence of human PH20 with any non-human PH20 polypeptide to identify positions corresponding to positions 491-500 of the human PH20 polypeptide set forth in SEQ ID NO:107, and at which C-terminal truncations can be made to produce extended soluble PH20 polypeptides. Additionally, algorithms, such as those described elsewhere herein, can be used to predict the location of the GPI-anchor attachment signal sequence. The solubility of the C-terminally truncated polypeptides can be assessed using methods well known in the art, including the Triton® X-114 assays described below and in Example 4, to determine whether the produced C-terminally truncated polypeptides are soluble and, therefore, esPH20 polypeptides.

[0132] Provided herein are extended soluble PH20 polypeptides of non-human primate species. Exemplary non-human primate GPI-anchored PH20 polypeptides include, but are not limited to, chimpanzee PH20 (SEQ ID NO:197). Thus, provided herein are chimpanzee esPH20 polypeptides. The esPH20 polypeptides of chimpanzee provided herein contain C-terminal truncations that correspond to the C-terminal truncations described above for the human esPH20 polypeptides. Thus, the chimpanzee esPH20 polypeptides provided herein contain amino acids corresponding to amino acid residues 1 to 491, 492,493, 494, 495, 496, 497, 498, 499, 500 or 501 of the sequence of amino acids set forth in SEQ ID NO: 107.

[0133] The chimpanzee PH20 polypeptides can be aligned to the human PH20 polypeptide by any method known to those of skill in the art. Such methods typically maximize matches, and include methods such as using manual alignments and by using the numerous alignment programs available (for example, BLASTP) and others known to those of skill in the art. Figure 1 provides an alignment of the precursor polypeptides of human and chimpanzee PH20. Amino acid residues 491 to 500 of the human PH20 (at which the human esPH20 polypeptides provided herein are truncated compared to the wild-type human PH20 polypeptide) correspond to amino acid residues 491 to 501 of chimpanzee PH20. Thus, provided herein are chimpanzee esPH20 polypeptides that contain amino acid residues 1 to 491, 492, 493, 494, 495, 496, 497, 498, 499, 500 or 501 of the sequence of amino acids set forth in SEQ ID NO: 197. When expressed in a mammalian expression system, the 35 amino acid signal peptide is cleaved during processing, and the mature form of the protein is secreted. Thus, the mature chimpanzee esPH20 polypeptides contain amino acids 36 to 491, 492, 493, 494, 495, 496, 497, 498, 499, 500 or 501 of SEQ ID NO:197.

[0134] Exemplary chimpanzee esPH20 polypeptide are those that contain amino acids residues 1 to 491, 492, 493, 494, 495, 496, 497 or 498 of the sequence of amino acids set forth in SEQ ID NO:197. When expressed in a mammalian expression system, the 35 amino acid signal peptide is cleaved during processing, and the mature form of the protein is secreted. Thus, the mature chimpanzee esPH20 polypeptides contain amino acids 36 to 491, 492, 493, 494, 495, 496, 497, or 498 of SEQ ID NO:197.

D. N-Partially glycosylated PH20 polypeptides

[0135] Provided herein are N-partially glycosylated hyaluronidases, including partially deglycosylated PH20 polypeptides, that retain all or a portion of the hyaluronidase activity of an N-glycosylated hyaluronidase. Exemplary partially deglycosylated hyaluronidases include partially deglycosylated PH20 polypeptides from any species, such as any set forth in any of SEQ ID NOS:107-109, 111-120, 197 and 198, or allelic variants or other variants thereof. Allelic variants and other variants are known to one of skill in the art, and include polypeptides having 60%, 70%, 80%, 90%, 91%, 92%, 93%, 94%, 95% or more sequence identity to any of SEQ ID NOS: NOS:107-109, 111-120, 197 and 198, or truncated forms thereof. The partially deglycosylated hyaluronidases provided herein also include hybrid, fusion and chimeric

partially deglycosylated hyaluronidases, and partially deglycosylated hyaluronidase conjugates.

[0136] The N-partially glycosylated hyaluronidases provided herein can be produced by digestion with one or more glycosidases. Thus, although all N-linked glycosylation sites (such as, for example, those at amino acids N82, N166, N235, N254, N368, and N393 of human PH20, exemplified in SEQ ID NO:107) can be glycosylated, the extent of glycosylation is reduced compared to a hyaluronidase that is not digested with one or more glycosidases. The partially deglycosylated hyaluronidase polypeptides provided herein, including partially deglycosylated soluble PH20 polypeptides, can have 10%, 20%, 30%, 40%, 50%, 60%, 70% or 80% of the level of glycosylation of a fully glycosylated hyaluronidase. In one example, 1, 2, 3, 4, 5 or 6 of the N-glycosylation sites corresponding to amino acids N82, N166, N235, N254, N368, and N393 of SEQ ID NO: 107 are partially deglycosylated, such that they no longer contain high mannose or complex type glycans, but rather contain at least an N-acetylglucosamine moiety. In some examples, 1, 2 or 3 of the N-glycosylation sites corresponding to amino acids N82, N166 and N254 of SEQ ID NO: 107 are deglycosylated, that is, they do not contain a sugar moiety. In other examples, 3, 4, 5, or 6 of the N-glycosylation sites corresponding to amino acids N82, N166, N235, N254, N368, and N393 of SEQ ID NO: 107 are glycosylated. Glycosylated amino acid residues minimally contain an N-acetylglucosamine moiety.

[0137] Also provided herein are N-partially glycosylated C-terminally truncated PH20 polypeptides. The partially deglycosylated C-terminally truncated PH20 polypeptides provided herein lack one or more amino acids from the C-terminus of a full length PH20 polypeptide, such as any of those set forth in SEQ ID NOS:107-109, 111-120, 197 and 198. Thus, the N-partially glycosylated C-terminally truncated PH20 polypeptides provided herein can be C-terminally truncated by 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60 or more amino acids compared to the full length wild type polypeptide, such as a full length wild type polypeptide with a sequence set forth in SEQ ID NOS:107-109, 111-120, 197 and 198. In some examples, 3, 4, 5, or 6 of the N-glycosylation sites corresponding to amino acids N82, N166, N235, N254, N368, and N393 of SEQ ID NO: 107 are glycosylated. Glycosylated amino acid residues minimally contain an N-acetylglucosamine moiety. In other examples, 1, 2 or 3 of the N-glycosylation sites corresponding to amino acids N82, N166, N235, N254, N368, and N393 of SEQ ID NO: 107 are not glycosylated. In further examples, the extent of glycosylation can be reduced, such that, the partially glycosylated C-terminally truncated PH20 polypeptides do not contain high mannose and complex type glycans, rather they contain at least an N-acetylglucosamine moiety, so long as they retain hyaluronidase activity. Thus the partially deglycosylated C-terminally truncated PH20 polypeptides provided herein can have 10%, 20%, 30%, 40%, 50%, 60%, 70% or 80% of the level of glycosylation of a fully glycosylated C-terminally truncated PH20 polypeptide.

[0138] The partially deglycosylated PH20 polypeptides and C-terminally truncated PH20 polypeptides provided herein retain hyaluronidase activity. Additionally, the partially deglycosylated PH20 polypeptides and C-terminally truncated PH20 polypeptides are neutral active, that is, they retain hyaluronidase activity at neutral pH. The hyaluronidase activity can be increased or decreased compared to the glycosylated full length and C-terminally truncated PH20 polypeptides. For example, the partially deglycosylated PH20 polypeptides and C-terminally truncated PH20 polypeptides provided herein can exhibit hyaluronidase activity that is 1%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 110%, 120%, 130%, 140%, 150%, 200%, 300%, 400%, 500%, 1000% or more of the hyaluronidase activity exhibited by the glycosylated full length and C-terminally truncated PH20 polypeptides.

[0139] Thus, the PH20 polypeptides provided herein can be used as therapeutic polypeptides, such as to treat hyaluronan-associated diseases or conditions. The partially deglycosylated PH20 polypeptides and C-terminally truncated PH20 polypeptides also can be used, for example, in combination therapy.

1. PH20 polypeptides

[0140] Exemplary N-partially glycosylated hyaluronidases provided herein include partially deglycosylated PH20 polypeptides from any species, such as any set forth in any of SEQ ID NOS:107-109, 111-120, 197 and 198, or allelic variants or other variants thereof. Allelic variants and other variants are known to one of skill in the art, and include polypeptides having 60%, 70%, 80%, 90%, 91%, 92%, 93%, 94%, 95% or more sequence identity to any of SEQ ID NOS: NOS:107-109, 111-120, 197 and 198, or truncated forms thereof. In some examples, 3, 4, 5, or 6 of the N-glycosylation sites corresponding to amino acids N82, N166, N235, N254, N368, and N393 of SEQ ID NO: 107 are glycosylated. In other examples, 1, 2, or 3 of the N-glycosylation sites corresponding to amino acids N82, N166 and N254 of SEQ ID NO: 107 are not glycosylated. In some examples, 1, 2, 3, 4, 5, or 6 of the N-glycosylation sites corresponding to amino acids N82, N166, N235, N254, N368, and N393 of SEQ ID NO: 107 minimally contain an N-acetylglucosamine moiety.

[0141] The partially deglycosylated hyaluronidases provided herein can be produced by digestion with one or more glycosidases. Thus, although all N-linked glycosylation sites (such as, for example, those at amino acids N82, N166, N235, N254, N368, and N393 of human PH20, exemplified in SEQ ID NO:107) can be glycosylated, the extent of glycosylation is reduced compared to a hyaluronidase that is not digested with one or more glycosidases. In particular, partially glycosylated hylaruonidases retain at least an N-acetylglucosamine moiety at each of the N-linked glycosylation

sites. Partially glycosylated hyaluronidases can be glycosylated at 3, 4, 5, or 6 of the N-glycosylation sites corresponding to amino acids N82, N166, N235, N254, N368, and N393 of SEQ ID NO: 107. In some examples, the hyaluronidases are deglycosylated at 1, 2, or 3 of the N-glycosylation sites corresponding to amino acid residues N82, N166, N235, N254, N368, and N393 of SEQ ID NO: 107. The partially deglycosylated PH20 polypeptides provided herein can have 10%, 20%, 30%, 40%, 50%, 60%, 70% or 80% of the level of glycosylation of a fully glycosylated hyaluronidase.

[0142] Glycosidases, or glycoside hydrolases, are enzymes that catalyze the hydrolysis of the glycosidic linkage to generate two smaller sugars. As shown in Figure 2, the major types of N-glycans in vertebrates include high mannose glycans, hybrid glycans and complex glycans. There are several glycosidases that result in only partial protein deglycosylation, including: EndoF1, which cleaves high mannose and hybrid type glycans; EndoF2, which cleaves biantennary complex type glycans; EndoF3, which cleaves biantennary and more branched complex glycans; and EndoH, which cleaves high mannose and hybrid type glycans (Figure 3). Treatment of a hyaluronidase, such as a soluble hyaluronidase, such as a soluble PH20, with one or all of these glycosidases results can result in only partial deglycosylation and, therefore, retention of hyaluronidase activity.

[0143] For example, treatment of rHuPH20 with one or all of these glycosidases results in partial deglycosylation. These partially deglycosylated rHuPH20 polypeptides exhibit hyaluronidase enzymatic activity that is comparable to the fully glycosylated polypeptides (see e.g. Example 7). In contrast, treatment of rHuPH20 (SEQ ID NO:122) with PNGaseF, a glycosidase that cleaves all N-glycans (see Figure 3), or treatment with the GlcNAc phosphotransferase (GPT) inhibitor tunicamycin, results in complete deglcosylation of all N-glycans and thereby renders PH20 enzymatically inactive (see e.g., Examples 7-8, below).

[0144] The partially deglycosylated hyaluronidase polypeptides provided herein, including partially deglycosylated soluble PH20 polypeptides, can have 10%, 20%, 30%, 40%, 50%, 60%, 70% or 80% of the level of glycosylation of a fully glycosylated hyaluronidase. Typically, the partially deglyclosylated hyaluronidases, including partially deglycosylated soluble PH20 polypeptides, provided herein exhibit hyaluronidase activity that is 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 110%, 120%, 130%, 140%, 150%, 200%, 300%, 400%, 500%, 1000% or more of the hyaluronidase activity exhibited by the fully glycosylated hyaluronidase.

[0145] The partially deglycosylated hyaluronidases provided herein also include hybrid, fusion and chimeric partially deglycosylated hyaluronidases, and partially deglycosylated hyaluronidase conjugates.

2. C-terminally truncated PH20 polypeptides

[0146] Exemplary of the N-partially glycosylated, or partially deglycosylated, PH20 peptides provided herein are C-terminally truncated PH20 polypeptides. The partially glycosylated C-terminally truncated PH20 polypeptides provided herein lack one or more amino acids from the C-terminus of the full length PH20 polypeptide as set forth in SEQ ID NOS: 107-109, 111-120, 197 and 198. Thus, the partially glycosylated C-terminally truncated PH20 polypeptides provided herein can be C-terminally truncated by 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60 or more amino acids compared to the full length wild type polypeptide, such as a full length wild type polypeptide with a sequence set forth in SEQ ID NOS:107-109, 111-120, 197 and 198. In some examples, 3, 4, 5, or 6 of the N-glycosylation sites, corresponding to amino acids N82, N166, N235, N254, N368, and N393 of SEQ ID NO: 107, are glycosylated. In other examples, 1, 2, or 3 of the N- glycosylation sites, corresponding to amino acids N82, N166 and N254 of SEQ ID NO: 107, are not glycosylated.

[0147] The partially deglycosylated C-terminally truncated PH20 polypeptides provided herein can be produced by digestion with one or more glycosidases. Although all N-linked glycosylation sites (such as, for example, those at amino acids N82, N166, N235, N254, N368, and N393 of human PH20, exemplified in SEQ ID NO:107) can be glycosylated, the extent of glycosylation is reduced compared to a hyaluronidase that is not digested with one or more glycosidases. Thus, the partially deglycosylated C-terminally truncated PH20 polypeptides provided herein can have 10%, 20%, 30%, 40%, 50%, 60%, 70% or 80% of the level of glycosylation of a fully glycosylated hyaluronidase. In particular, N-partially glycosylated hylaruonidases retain at least an N-acetylglucosamine moiety at each of the N-linked glycosylation sites. In some examples, 1, 2, 3, 4, 5, or 6 of the N-glycosylation sites corresponding to amino acids N82, N166, N235, N254, N368, and N393 of SEQ ID NO: 107 minimally contain an N-acetylglucosamine moiety. In other examples, 3, 4, 5, or 6 of the N-glycosylation sites corresponding to amino acids N82, N166, N235, N254, N368, and N393 of SEQ ID NO: 107 are glycosylated at the level of glycosylation of a fully glycosylated hyaluronidase at each of the 3, 4, 5, or 6 N-glycosylation sites. In further examples, 1, 2, or 3 of the the N-glycosylation sites corresponding to amino acids N82, N166, N235, N254, N368, and N393 of SEQ ID NO: 107 are fully deglycosylated. In these examples, typically, amino acids N82, N166 or N254 are fully deglycosylated.

[0148] Exemplary N-partially glycosylated C-terminally truncated PH20 polypeptides are from any species, such as any set forth in any of SEQ ID NOS: 107-109, 111-120, 197 and 198, or allelic variants or other variants thereof. Allelic variants and other variants are known to one of skill in the art, and include polypeptides having 60%, 70%, 80%, 90%, 91%, 92%, 93%, 94%, 95% or more sequence identity to any of SEQ ID NOS: NOS:107-120, 197 and 198, or truncated

forms thereof. The N-partially glycosylated C-terminally truncated PH20 polypeptides provided herein also include hybrid, fusion and chimeric PH20 polypeptides, and PH20 conjugates. For example, the partially deglycosylated C-terminally truncated PH20 polypeptides provided herein can be conjugated to a polymer, such as dextran, a polyethylene glycol (pegylation (PEG)) or sialyl moiety, or other such polymers, such as natural or sugar polymers. In other examples, the N-partially glycosylated C-terminally truncated PH20 polypeptide is linked or fused to a domain such as an Fc domain from an IgG immunoglobulin.

[0149] Included amongst the glycosylated or partially glycosylated C-terminally truncated polypeptides provided herein are those that are truncated at the C-terminus by 2 amino acids up to 44 amino acids compared to the wild type PH20 set forth in SEQ ID NO:107 (precursor polypeptide) or 108 (mature polypeptide), or allelic or species variants thereof. Thus, C-terminally truncated PH20 polypeptides include any having C-terminal truncations to generate polypeptides containing amino acid 1 to amino acid 450, 451, 452, 453, 454, 455, 456, 457, 458, 459, 460, 461, 462, 463, 464, 465, 466, 467, 468, 469, 470, 471,472, 473, 474, 475, 476, 477, 478, 479, 480, 481, 482, 483, 484, 485, 486, 487, 488, 489, 490, 491, 492, 493, 494, 495, 496, 497, 498, 499, 500, 501, 502, 503, 504, 505, 506 or 507 of the sequence of amino acids set forth in SEQ ID NO: 107, or corresponding positions in an allelic or species variant thereof, with 2, 3, 4, 5, or 6 of the N-glycosylation sites, corresponding to amino acids N82, N166, N235, N254, N368, and N393 of SEQ ID NO: 107, glycosylated. When expressed in mammalian cells, the 35 amino acid N-terminal signal sequence is cleaved during processing, and the mature form of the protein is secreted. Thus, provided herein are mature C-terminally truncated PH20 polypeptides that contain amino acids 36 to 450, 451, 452, 453, 454, 455, 456, 457, 458, 459, 460, 461, 462, 463, 464, 465, 466, 467, 468, 469, 470, 471, 472, 473, 474, 475, 476, 477, 478, 479, 480, 481, 482, 483, 484, 485, 486, 487, 488, 489, 490, 491, 492, 493, 494, 495, 496, 497, 498, 499, 500, 501, 502, 503, 504, 505, 506 or 507 of the sequence of amino acids set forth in SEQ ID NO: 107 or corresponding positions in an allelic or species variant thereof, with 3, 4, 5, or 6 of the N-glycosylation sites, corresponding to amino acids N82, N166, N235, N254, N368, and N393 of SEQ ID NO: 107, glycosylated.

[0150] Table 2 provides non-limiting examples of exemplary C-terminally truncated PH20 polypeptides that can be glycosylated or partially deglycosylated. In Table 2 below, the length (in amino acids) of the precursor and mature polypeptides, and the sequence identifier (SEQ ID NO) in which exemplary amino acid sequences of the precursor and mature polypeptides of the C-terminally truncated PH20 proteins are set forth, are provided. The wild-type PH20 polypeptide also is included in Table 2 for comparison.

Table 2. Exemplary C-terminally truncated PH20 polypeptides

Polypeptide	Precursor (amino acids)	Precursor SEQ ID NO	Mature (amino acids)	Mature SEQ ID NO
SPAM1-VASL	509	1	474	108
SPAM1-SSVA	507	3	472	55
SPAM1-ISSV	506	45	471	97
SPAM1-IISS	505	4	470	56
SPAM1-LIIS	504	46	469	98
SPAM1-FLII	503	5	468	57
SPAM1-LFLI	502	47	467	99
SPAM1-ILFL	501	6	466	58
SPAM1-SILF	500	48	465	100
SPAM1-VSIL	499	7	464	59
SPAM1-IVSI	498	49	463	101
SPAM1-FIVS	497	8	462	60
SPAM1-MFIV	496	50	461	102
SPAM1-TMFI	495	9	460	61
SPAM1-ATMF	494	51	459	103
SPAM1-SATM	493	10	458	62
SPAM1-LSAT	492	52	457	104
SPAM1-TLSA	491	11	456	63

(continued)

Polypeptide	Precursor (amino acids)	Precursor SEQ ID NO	Mature (amino acids)	Mature SEQ ID NO
SPAM1-PSTL	489	12	454	64
SPAMI-STLS	490	13	455	65
SPAMI-SPST	488	53	453	105
SPAM1-ASPS	487	14	452	66
SPAM1-NASP	486	54	451	106
SPAM1-YNAS	485	15	450	67
SPAM1-FYNA	484	16	449	68
SPAM1-IFYN	483	17	448	69
SPAM1-QIFY	482	18	447	70
SPAM1-PQIF	481	19	446	71
SPAM1-EPQI	480	20	445	72
SPAMI-EEPQ	479	21	444	73
SPAM1-TEEP	478	22	443	74
SPAM1-ETEE	477	23	442	75
SPAM1-METE	476	24	441	76
SPAM1-PMET	475	25	440	77
SPAM1-PPME	474	26	439	78
SPAM1-KPPM	473	27	438	79
SPAM1-LKPP	472	28	437	80
SPAM1-FLKP	471	29	436	81
SPAM1-AFLK	470	30	435	82
SPAM1-DAFL	469	31	434	83
SPAM1-IDAF	468	32	433	84
SPAM1-CIDA	467	33	432	85
SPAM1-VCID	466	34	431	86
SPAM1-GVCI	465	35	430	87
SPAM1-GDVC	464	36	429	88
SPAM1-IADG	462	37	427	89
SPAM1-VCIA	460	38	425	90
SPAM1-VDVC	458	39	423	91
SPAM1-DAVD	456	40	421	92
SPAM1-DTDA	454	41	419	93
SPAM1-VKDT	452	42	417	94
SPAM1-ADVK	450	43	415	95

[0151] The N-glycosylated and partially deglycosylated C-terminal truncated PH20 polypeptides provided herein include those that are soluble, i.e. partition into the aqueous phase of a Triton® X-114 solution, and those that are insoluble, i.e. partition into the detergent phase of a Triton® X-114 solution. The partially deglycosylated C-terminally truncated PH20 polypeptides provided herein can have 10%, 20%, 30%, 40%, 50%, 60%, 70% or 80% of the level of glycosylation

of a fully glycosylated hyaluronidase. Alternatively, the partially deglycosylated C-terminal truncated PH20 polypeptides can have 1, 2 or 3 of the N-glycosylation sites, corresponding to amino acids N82, N166 and N254 of SEQ ID NO: 107, that are not glycosylated. Minimally, to be glycosylated, an N-glycosylation site contains at least an N-acetylglucosamine moiety.

[0152] In some examples, the N-partially glycosylated C-terminally truncated polypeptides provided herein are soluble, i.e., are not GPI-anchored. This can be assessed, for example, using a Triton® X-114 assay following incubation with PI-PLC or PI-PLD, as described below and in Example 4. For example, PH20 polypeptides that are C-terminally truncated at or 5' to the amino acid position corresponding to amino acid residue position 490 of the PH20 polypeptide set forth in SEQ ID NO:107 typically are soluble when expressed in a mammalian expression system (see, e.g. Example 3). These polypeptides are soluble by virtue of the fact that they completely lack the GPI-anchor attachment signal sequence. In other examples, the partially glycosylated C-terminally truncated polypeptides provided herein are insoluble and membrane-bound when expressed in a mammalian expression system. For example, PH20 polypeptides that are C-terminally truncated at or 3' of the amino acid position corresponding to amino acid position 500 of the PH20 polypeptide set forth in SEQ ID NO:107 typically are insoluble when expressed in a mammalian expression system (see, e.g. Example 3). The C-terminally truncated polypeptides provided herein can be partially glycosylated in that 3, 4, 5, or 6 of the N-glycosylation sites corresponding to amino acids N82, N166, N235, N254, N368, and N393 of SEQ ID NO: 107 are glycosylated.

[0153] Soluble partially glycosylated C-terminal truncated PH20 polypeptides provided herein include those that are truncated but retain at least one or more amino acid residues located in the GPI-anchor attachment signal, and those that completely lack the GPI-anchor attachment signal sequence and the ω-site. Thus, instead of having a GPI-anchor covalently attached to the C-terminus of the protein in the ER and being anchored to the extracellular leaflet of the plasma membrane, these polypeptides are secreted. These C-terminal truncated soluble PH20 polypeptides can be partially glycosylated such that 3, 4, 5, or 6 of the N-glycosylation sites are glycosylated. Exemplary soluble C-terminally truncated PH20 polypeptides that lack the GPI-anchor attachment signal sequence are from any species, such as any set forth in any of SEQ ID NOS: 107-109, 111-120, 197 and 198, or allelic variants or other variants thereof. These partially glycosylated soluble C-terminal truncated PH20 polypeptides have C-terminal truncations to generate polypeptides containing amino acids 1 to 450, 451, 452, 453, 454, 455, 456, 457, 458, 459, 460, 461, 462, 463, 464, 465, 466, 467, 468, 469, 470, 471, 472, 473, 474, 475, 476, 477, 478, 479, 480, 481, 482, 483, 484, 485, 486, 487, 488, 489, 490, 491, 492, 493, 494, 495, 496, 497, 498, 499 or 500 of the sequence of amino acids set forth in SEQ ID NO: 107. Upon cleavage of the N-terminal signal sequence following expression in mammalian cells, the mature partially glycosylated soluble C-terminal truncated PH20 polypeptides polypeptides contain amino acids 36 to 450, 451, 452, 453, 454, 455, 456, 457, 458, 459, 460, 461, 462, 463, 464, 465, 466, 467, 468, 469, 470, 471, 472, 473, 474, 475, 476, 477, 478, 479, 480, 481, 482, 483, 484, 485, 486, 487, 488, 489, 490, 491, 492, 493, 494, 495, 496, 497, 498, 499 or 500 of the sequence of amino acids set forth in SEQ ID NO: 107. In some examples, the C-terminally GPI-anchor signal sequence truncated soluble PH20 polypeptides are partially glycosylated, containing, for example, at least an N-acetylglucosamine at 3, 4, 5, or 6 of the N-glycosylation sites corresponding to amino acids N82, N166, N235, N254, N368, and N393 of SEQ ID NO: 107. In other examples, the C-terminally GPI-anchor signal sequence truncated soluble PH20 polypeptides have 10%, 20%, 30%, 40%, 50%, 60%, 70% or 80% of the level of glycosylation of a fully glycosylated hyaluronidase.

[0154] Partially deglycosylated C-terminal truncated PH20 polypeptides that retain at least one amino acid in the GPI-anchor attachment signal sequence provided herein are partially deglycosylated extended soluble PH20 polypeptides. In some examples, the partially deglycosylated C-terminal truncated PH20 polypeptides are not glycosylated at 1, 2 or 3 of the N-glycosylation sites corresponding to amino acids N82, N166 and N254 of SEQ ID NO: 107. These partially deglycosylated extended soluble PH20 polypeptides contain amino acids 1 to 491, 492, 493, 494, 495, 496, 497, 498, 499 or 500 of the sequence of amino acids set forth in SEQ ID NO: 107. When expressed in mammalian cells, the 35 amino acid N-terminal signal sequence is cleaved during processing, and the mature form of the protein is secreted. Thus, the mature form of the partially deglycosylated esPH20 polypeptides contain amino acids 36 to 491, 492, 493, 494, 495, 496, 497, 498, 499 or 500 of the sequence of amino acids set forth in SEQ ID NO: 107. Mature human forms of partially glycosylated esPH20 polypeptides provided herein include those set forth in SEQ ID NOS: 59-63 and 100-104 containing at least an N-acetylglucosamine at 3, 4, 5 or 6 of the N-glycosylation sites corresponding to amino acids N82, N166, N235, N254, N368, and N393 of SEQ ID NO: 107. In some examples, the extent of glycosylation is reduced by treatment with a endoglycosidase. Thus, the partially deglycosylated C-terminally truncated PH20 polypeptides that contain at least one amino acid in the GPI-anchor attachment signal sequence provided herein can have 10%, 20%, 30%, 40%, 50%, 60%, 70% or 80% of the level of glycosylation of a fully glycosylated hyaluronidase.

[0155] Also provided herein are partially deglycosylated C-terminally truncated PH20 polypeptides that are not soluble, that is, they are attached to the cell membrane and therefore not secreted into the media upon expression. The C-terminal truncated PH20 polypeptides that are not soluble can be partially deglycosylated as long as they retain hyaluronidase activity. Exemplary partially glycosylated mature C-terminally truncated PH20 polypeptides that are not soluble are those that contain amino acids corresponding to amino acid positions 36 to 501, 502, 503, 504, 505, 506 or 507 of

SEQ ID NO:107. Hence, partially glycosylated C-terminally truncated PH20 polypeptides provided herein that are not soluble include those that are 466, 467, 468, 469, 470, 471 or 472 amino acids in length, such as those set forth in SEQ ID NOS: 55-58 and 97-99, that retain at least 10%, 20%, 30%, 40%, 50%, 60%, 70% or 80% of the level of glycosylation of a fully glycosylated hyaluronidase. In some examples, 3, 4, 5, or 6 of the N-glycosylation sites corresponding to amino acids N82, N166, N235, N254, N368, and N393 of SEQ ID NO: 107 are glycosylated. In other examples, the 1, 2, 3, 4, 5 or 6 of the N-glycosylation sites, corresponding to amino acids N82, N166, N235, N254, N368, and N393 of SEQ ID NO: 107, contain at least an N-acetylglucosamine moiety.

[0156] The partially glycosylated C-terminally truncated polypeptides provided herein can exhibit hyaluronidase activity that is increased or decreased compared to the wild-type GPI-anchored form of the PH20. Additionally, the partially deglycosylated C-terminally truncated PH20 polypeptides are neutral active, that is, they retain hyaluronidase activity at neutral pH. For example, the C-terminal truncated PH20 polypeptides provided herein can exhibit hyaluronidase activity that is 1%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 110%, 120%, 130%, 140%, 150%, 200%, 300%, 400%, 500%, 1000% or more of the hyaluronidase activity exhibited by the wildtype GPI-anchored form. In some examples, partially glycosylated C-terminal truncated PH20 polypeptides exhibit increased hyaluronidase activity compared to the wildtype GPI-anchored form.

[0157] The C-terminal truncated PH20 polypeptides provided herein may also be N-glycosylated. The N-glycosylated and N-partially glycosylated hyaluronidases provided herein also include hybrid, fusion and chimeric N-glycosyated and partially deglycosylated hyaluronidases, and N-glycosylated and partially deglycosylated hyaluronidase conjugates.

3. Additional Modifications

[0158] The PH20 polypeptides included herein, including human esPH20 polypeptides, N-glycosylated and N-partially glycosylated C-terminally truncated PH20 polypeptides and partially glycosylated PH20 polypeptides, also include those that contain chemical or posttranslational modifications and those that do not contain chemical or posttranslational modifications. Such modifications include, but are not limited to, pegylation, sialation, albumination, glycosylation, famysylation, carboxylation, hydroxylation, phosphorylation, and other polypeptide modifications known in the art. Thus, C-terminally truncated PH20 polypeptides, including esPH20 polypeptides, provided herein can contain other modifications that are or are not in the primary sequence of the polypeptide, including, but not limited to, of a carbohydrate moiety, a polyethylene glycol (PEG) moiety, a silation moiety, an Fc domain from immunoglobulin G, or any other domain or moiety. For example, such additional modifications can be made to increase the stability or serum half-life of the protein. The C-terminally truncated PH20 polypeptides, including esPH20 polypeptides, provided herein can be conjugated or fused to any moiety using any method known in the art, including chemical and recombinant methods, providing the resulting polypeptide retains hyaluronidase activity.

Decreased immunogenicity

[0159] The PH20 polypeptides provided herein, including the human esPH20 polypeptides, can be made to have decreased immunogenicity. Decreased immunogenicity can be effected by sequence changes that elimiminate antigenic epitopes from the polypeptide or by altering post-translational modifications. For example, altering the glycosylation of the peptide is contemplated, so long as the polypeptides minimally contain at least N-acetylglucosamine at amino acid residues N235, N368 and N393 of SEQ ID NO:107.

[0160] For example, the PH20 polypeptides can be modified such that they lack fucose, particularly bifucosylation.. In particular, the PH20 polypeptides provided herein are not bifucosylated. This can be achieved by expressing and producing the PH20 polypeptide in a host cells, typically insect host cells, that do not effect bifucosylation. Fucose is a deoxyhexose that is present in a wide variety of organisms, including mammals, insects and plants. Fucosylated glycans are synthesized by fucosyl-tranferases. See, e.g., Ma et al., Glycobiology, 15(2):158R-184R, (2006); Nakayama et al., J. Biol. Chem., 276:16100-16106 (2001); and Sturla et al., Glycobiology, 15(10):924-935 (2005). In humans, fucose frequently exists as a terminal modification to glycan structures, and the presence of fucose α1,6-linked to N-acetylglucosamine has been shown to be important in glycoprotein processing and recognition. In insects, N-glycan core structures exhibit bifucosylation with α1,6- and α1,3-linkages. Insect cell core fucosylation with α1,3-linkages generates a carbohydrate epitope that is immunogenic in humans (see, e.g., US Patent Application No. 20070067855). For example, PH20 polypeptides provided herein, including esPH20 polypeptides, can be generated in host cells that are incapable of bifucosylating the polypeptide. Thus, while insect cells or other cells that bifucosylate can be used for expression of the polypeptides, typically mammalian cells, such as CHO cells, are used.

[0161] In some examples, defucosylated, or fucose-deficient PH20 polypeptides can be generated in insect cells with modified glycosylation pathways, through the use of baculovirus expression vectors containing eukaryotic oligosaccharide processing genes, thereby creating "mammalianized" insect cell expression systems (see, e.g., US Patent No. 6,461,863). Alternatively, antigenicity can be eliminated by expression of PH20 polypeptides in insect cells lacking α1,3-

fucosylatransferase (FT3) (see, e.g., US Patent Application No. 20070067855). In other examples, defucosylated or fucose-deficient PH20 polypeptides can be generated, for example, in cell lines that produce defucosylated proteins, including Lec13 CHO cells deficient in protein fucosylation (Ripka et al. Arch. Biochem. Biophys. 249:533-545 (1986); U.S. Pat. Appl. No. 2003/0157108; and WO 2004/056312), and knockout cell lines, such as alpha-1,6-fucosyltransferase gene, FUT8, knockout CHO cells (Yamane-Ohnuki et al. *Biotech. Bioeng.* 87: 614 (2004)).

Conjugation to polymers

[0162] In some examples, the esPH20 polypeptides and other C-terminally truncated PH20 polypeptides, including partially glycosylated PH20 polypeptides, provided herein are conjugated to polymers. Exemplary polymers that can be conjugated to the PH20 polypeptides, include natural and synthetic homopolymers, such as polyols (i.e. poly-OH), polyamines (i.e. poly-NH_2) and polycarboxylic acids (i.e. poly-COOH), and further heteropolymers i.e. polymers comprising one or more different coupling groups *e.g.* a hydroxyl group and amine groups. Examples of suitable polymeric molecules include polymeric molecules selected from among polyalkylene oxides (PAO), such as polyalkylene glycols (PAG), including polyethylene glycols (PEG), methoxypolyethylene glycols (mPEG) and polypropylene glycols, PEG-glycidyl ethers (Epox-PEG), PEG-oxycarbonylimidazole (CDI-PEG), branched polyethylene glycols (PEGs), polyvinyl alcohol (PVA), polycarboxylates, polyvinylpyrrolidone, poly-D,L-amino acids, polyethylene-co-maleic acid anhydride, polystyrene-co-maleic acid anhydride, dextrans including carboxymethyl-dextrans, heparin, homologous albumin, celluloses, including methylcellulose, carboxymethylcellulose, ethylcellulose, hydroxyethylcellulose, carboxyethylcellulose and hydroxypropylcellulose, hydrolysates of chitosan, starches such as hydroxyethyl-starches and hydroxypropyl-starches, glycogen, agaroses and derivatives thereof, guar gum, pullulan, inulin, xanthan gum, carrageenan, pectin, alginic acid hydrolysates and bio-polymers.

[0163] Typically, the polymers are polyalkylene oxides (PAO), such as polyethylene oxides, such as PEG, typically mPEG, which, in comparison to polysaccharides such as dextran, pullulan and the like, have few reactive groups capable of cross-linking. Typically, the polymers are non-toxic polymeric molecules such as (methoxy)polyethylene glycol (mPEG) which can be covalently conjugated to the esPH20 polypeptides and other C-terminally truncated PH20 polypeptides (*e.g.*, to attachment groups on the protein surface) using a relatively simple chemistry.

[0164] Suitable polymeric molecules for attachment to the esPH20 polypeptides and other C-terminally truncated PH20 polypeptides include, but are not limited to, polyethylene glycol (PEG) and PEG derivatives such as methoxy-polyethylene glycols (mPEG), PEG-glycidyl ethers (Epox-PEG), PEG-oxycarbonylimidazole (CDI-PEG), branched PEGs, and polyethylene oxide (PEO) (see *e.g.* Roberts et al., Advanced Drug Delivery Review 2002, 54: 459-476; Harris and Zalipsky (eds.) "Poly(ethylene glycol), Chemistry and Biological Applications" ACS Symposium Series 680, 1997; Mehvar et al., J. Pharm. Pharmaceut. Sci., 3(1):125-136, 2000; Harris and Chess (2003) Nat Rev Drug Discov. 2(3):214-21; and Tsubery, J Biol. Chem 279(37):38118-24, 2004). The polymeric molecule can be of a molecular weight typically ranging from about 3 kDa to about 60 kDa. In some embodiments the polymeric molecule that is conjugated to a PH20 polypeptide provided herein has a molecular weight of 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60 or more than 60 kDa.

[0165] Various methods of modifying polypeptides by covalently attaching (conjugating) a PEG or PEG derivative (i.e. "PEGylation") are known in the art (see *e.g.,* U.S. 2006/0104968; U.S. 5,672,662; U.S. 6,737,505; and U.S. 2004/0235734). Techniques for PEGylation include, but are not limited to, specialized linkers and coupling chemistries (see *e.g.,* Harris, Adv. Drug Deliv. Rev. 54:459-476, 2002), attachment of multiple PEG moieties to a single conjugation site (such as via use of branched PEGs; see *e.g.,* Veronese et al., Bioorg. Med. Chem. Lett. 12:177-180, 2002), site-specific PEGylation and/or mono-PEGylation (see *e.g.,* Chapman et al., Nature Biotech. 17:780-783, 1999), and site-directed enzymatic PEGylation (see *e.g.,* Sato, Adv. Drug Deliv. Rev., 54:487-504, 2002) (see, also, for example, Lu and Felix (1994) Int. J. Peptide Protein Res. 43:127-138; Lu and Felix (1993) Peptide Res. 6:142-6, 1993; Felix et al. (1995) Int. J. Peptide Res. 46:253-64; Benhar et al. (1994) J. Biol. Chem. 269:13398-404; Brumeanu et al. (1995) J Immunol. 154:3088-95; see also, Caliceti et al. (2003) Adv. Drug Deliv. Rev. 55(10):1261-77 and Molineux (2003) Pharmacotherapy 23 (8 Pt 2):3S-8S). Methods and techniques described in the art can produce proteins having 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more than 10 PEG or PEG derivatives attached to a single protein molecule (see *e.g.,* U.S. 2006/0104968).

[0166] Numerous reagents for PEGylation have been described in the art. Such reagents include, but are not limited to, N-hydroxysuccinimidyl (NHS) activated PEG, succinimidyl mPEG, mPEG2-N-hydroxysuccinimide, mPEG succinimidyl alpha-methylbutanoate, mPEG succinimidyl propionate, mPEG succinimidyl butanoate, mPEG carboxymethyl 3-hydroxybutanoic acid succinimidyl ester, homobifunctional PEG-succinimidyl propionate, homobifunctional PEG propionaldehyde, homobifunctional PEG butyraldehyde, PEG maleimide, PEG hydrazide, p-nitrophenyl-carbonate PEG, mPEG-benzotriazole carbonate, propionaldehyde PEG, mPEG butryaldehyde, branched mPEG2 butyraldehyde, mPEG acetyl, mPEG piperidone, mPEG methylketone, mPEG "linkerless" maleimide, mPEG vinyl sulfone, mPEG thiol, mPEG orthopyridylthioester, mPEG orthopyridyl disulfide, Fmoc-PEG-NHS, Boc-PEG-NHS, vinylsulfone PEG-NHS, acrylate

PEG-NHS, fluorescein PEG-NHS, and biotin PEG-NHS (see *e.g.,* Monfardini et al., Bioconjugate Chem. 6:62-69, 1995; Veronese et al., J. Bioactive Compatible Polymers 12:197-207, 1997; U.S. 5,672,662; U.S. 5,932,462; U.S. 6,495,659; U.S. 6,737,505; U.S. 4,002,531; U.S. 4,179,337; U.S. 5,122,614; U.S. 5,183,550; U.S. 5,324, 844; U.S. 5,446,090; U.S. 5,612,460; U.S. 5,643,575; U.S. 5,766,581; U.S. 5,795, 569; U.S. 5,808,096; U.S. 5,900,461; U.S. 5,919,455; U.S. 5,985,263; U.S. 5,990, 237; U.S. 6,113,906; U.S. 6,214,966; U.S. 6,258,351; U.S. 6,340,742; U.S. 6,413,507; U.S. 6,420,339; U.S. 6,437,025; U.S. 6,448,369; U.S. 6,461,802; U.S. 6,828,401; U.S. 6,858,736; U.S. 2001/0021763; U.S. 2001/0044526; U.S. 2001/0046481; U.S. 2002/0052430; U.S. 2002/0072573; U.S. 2002/0156047; U.S. 2003/0114647; U.S. 2003/0143596; U.S. 2003/0158333; U.S. 2003/0220447; U.S. 2004/0013637; US 2004/0235734; U.S. 2005/000360; U.S. 2005/0114037; U.S. 2005/0171328; U.S. 2005/0209416; EP 01064951; EP 0822199; WO 00176640; WO 0002017; WO 0249673; WO 9428024; and WO 0187925).

Other modifications

[0167] The esPH20 polypeptides and other C-terminally truncated PH20 polypeptides provided herein also include fusions and conjugates thereof.

E. Methods of Producing Nucleic Acids Encoding Extended Soluble PH20 and other Soluble PH20 Hyaluronidases, and Polypeptides Thereof

[0168] Polypeptides of extended soluble PH20, C-terminal truncated PH20 hyaluronidases, and partially glycosylated PH20 hyaluronidases set forth herein, and nucleic acid molecules encoding such polypeptides, can be obtained by methods well known in the art for recombinant protein expression and protein purification. For example, the DNA can be obtained from cloned DNA (e.g. from a DNA library), by chemical synthesis, by cDNA cloning, or by the cloning of genomic DNA or fragments thereof, purified from the desired cell. When the polypeptides are produced by recombinant means, any method known to those of skill in the art for identification of nucleic acids that encode desired genes can be used. Any method available in the art can be used to obtain a full length (i.e. encompassing the entire coding region) cDNA or genomic DNA encoding a desired PH20 enzyme, such as from a cell or tissue source. Modified or variant, including truncated forms such as provided herein, can be engineered from a wildtype polypeptide using standard recombinant DNA methods.

[0169] Polypeptides can be cloned or isolated using any available methods known in the art for cloning and isolating nucleic acid molecules. Such methods include PCR amplification of nucleic acids and screening of libraries, including nucleic acid hybridization screening, antibody-based screening and activity-based screening.

[0170] Methods for amplification of nucleic acids can be used to isolate nucleic acid molecules encoding a desired polypeptide, including for example, polymerase chain reaction (PCR) methods. PCR can be carried out using any known methods or procedures in the art. Exemplary of such methods include use of a Perkin-Elmer Cetus thermal cycler and Taq polymerase (Gene Amp). A nucleic acid containing material can be used as a starting material from which a desired polypeptide-encoding nucleic acid molecule can be isolated. For example, DNA and mRNA preparations, cell extracts, tissue extracts from an appropriate source (e.g. testis, prostate, breast), fluid samples (*e.g.* blood, serum, saliva), samples from healthy and/or diseased subjects can be used in amplification methods. The source can be from any eukaryotic species including, but not limited to, vertebrate, mammalian, human, porcine, bovine, feline, avian, equine, canine, and other primate sources. Nucleic acid libraries also can be used as a source of starting material. Primers can be designed to amplify a desired polypeptide. For example, primers can be designed based on expressed sequences from which a desired polypeptide is generated. Primers can be designed based on back-translation of a polypeptide amino acid sequence. If desired, degenerate primers can be used for amplification. Oligonucleotide primers that hybridize to sequences at the 3' and 5' termini of the desired sequence can be uses as primers to amplify by PCR sequences from a nucleic acid sample. Primers can be used to amplify the entire full-length PH20, or a truncated sequence thereof, such as a nucleic acid encoding any of the soluble PH20 polypeptides provided herein. Nucleic acid molecules generated by amplification can be sequenced and confirmed to encode a desired polypeptide.

[0171] Additional nucleotide sequences can be joined to a polypeptide-encoding nucleic acid molecule, including linker sequences containing restriction endonuclease sites for the purpose of cloning the synthetic gene into a vector, for example, a protein expression vector or a vector designed for the amplification of the core protein coding DNA sequences. Furthermore, additional nucleotide sequences specifying functional DNA elements can be operatively linked to a polypeptide-encoding nucleic acid molecule. Examples of such sequences include, but are not limited to, promoter sequences designed to facilitate intracellular protein expression, and secretion sequences, for example heterologous signal sequences, designed to facilitate protein secretion. Such sequences are known to those of skill in the art. For example, exemplary heterologous signal sequences include, but are not limited to, human and mouse kappa IgG heterologous signal sequences set forth in SEQ ID NO:144 and 145, respectively. Additional nucleotide residues sequences such as sequences of bases specifying protein binding regions also can be linked to enzyme-encoding nucleic acid molecules.

Such regions include, but are not limited to, sequences of residues that facilitate or encode proteins that facilitate uptake of an enzyme into specific target cells, or otherwise alter pharmacokinetics of a product of a synthetic gene.

[0172] In addition, tags or other moieties can be added, for example, to aid in detection or affinity purification of the polypeptide. For example, additional nucleotide residues sequences such as sequences of bases specifying an epitope tag or other detectable marker also can be linked to enzyme-encoding nucleic acid molecules. Exemplary of such sequences include nucleic acid sequences encoding a His tag (e.g., 6xHis, HHHHHH; SEQ ID NO:142) or Flag Tag (DYKDDDDK; SEQ ID NO:143).

[0173] The identified and isolated nucleic acids can then be inserted into an appropriate cloning vector. A large number of vector-host systems known in the art can be used. Possible vectors include, but are not limited to, plasmids or modified viruses, but the vector system must be compatible with the host cell used. Such vectors include, but are not limited to, bacteriophage such as lambda derivatives, or plasmids such as pCMV4, pBR322 or pUC plasmid derivatives or the Bluescript vector (Stratagene, La Jolla, CA). Other expression vectors include the HZ24 expression vector exemplified herein (set forth in SEQ ID NO:140). The insertion into a cloning vector can, for example, be accomplished by ligating the DNA fragment into a cloning vector which has complementary cohesive termini. If the complementary restriction sites used to fragment the DNA are not present in the cloning vector, the ends of the DNA molecules can be enzymatically modified. Alternatively, any site desired can be produced by ligating nucleotide sequences (linkers) onto the DNA termini; these ligated linkers can include specific chemically synthesized oligonucleotides encoding restriction endonuclease recognition sequences. In an alternative method, the cleaved vector and protein gene can be modified by homopolymeric tailing. Insertion can be effected using TOPO cloning vectors (INVITROGEN, Carlsbad, CA).

[0174] Recombinant molecules can be introduced into host cells via, for example, transformation, transfection, infection, electroporation and sonoporation, so that many copies of the gene sequence are generated. In specific embodiments, transformation of host cells with recombinant DNA molecules that incorporate the isolated protein gene, cDNA, or synthesized DNA sequence enables generation of multiple copies of the gene. Thus, the gene can be obtained in large quantities by growing transformants, isolating the recombinant DNA molecules from the transformants and, when necessary, retrieving the inserted gene from the isolated recombinant DNA.

[0175] In addition to recombinant production, soluble PH20, including any esPH20 provided herein, can be produced by direct peptide synthesis using solid-phase techniques (see e.g., Stewart et al. (1969) Solid-Phase Peptide Synthesis, WH Freeman Co., San Francisco; Merrifield J (1963) JAm Chem Soc., 85:2149-2154). *In vitro* protein synthesis can be performed using manual techniques or by automation. Automated synthesis can be achieved, for example, using Applied Biosystems 431A Peptide Synthesizer (Perkin Elmer, Foster City CA) in accordance with the instructions provided by the manufacturer. Various fragments of a polypeptide can be chemically synthesized separately and combined using chemical methods.

1. Vectors and Cells

[0176] For recombinant expression of one or more of the desired proteins, such as any described herein, the nucleic acid containing all or a portion of the nucleotide sequence encoding the protein can be inserted into an appropriate expression vector, *i.e.,* a vector that contains the necessary elements for the transcription and translation of the inserted protein coding sequence. The necessary transcriptional and translational signals also can be supplied by the native promoter for PH20 genes, and/or their flanking regions.

[0177] Also provided are vectors that contain a nucleic acid encoding the enzyme. Cells containing the vectors also are provided. The cells include eukaryotic and prokaryotic cells, and the vectors are any suitable for use therein.

[0178] Prokaryotic and eukaryotic cells, including endothelial cells, containing the vectors are provided. Such cells include bacterial cells, yeast cells, fungal cells, Archea, plant cells, insect cells and animal cells. The cells are used to produce a protein thereof by growing the above-described cells under conditions whereby the encoded protein is expressed by the cell, and recovering the expressed protein. For purposes herein, for example, soluble PH20 polypeptides, including extended soluble PH20 polypeptides, can be secreted into the medium.

[0179] A host cells strain can be chosen for its ability to modulate the expression of the inserted sequences or to process the expressed protein in the desired fashion. Such modifications of the polypeptide include, but are not limited to, acetylation, carboxylation, glycosylation, phosphorylation, lipidation and acylation. Post-translational processing can impact the folding and/or function of the polypeptide. Different host cells, such as, but not limited to, CHO (DG44, DXB11, CHO-K1), HeLa, MCDK, 293 and WI38 have specific cellular machinery and characteristic mechanisms for such post-translational activities and can be chosen to ensure the correct modification and processing of the introduced protein. Generally, the choice of cell is one that is capable of introducing N-linked glycosylation into the expressed polypeptide. Hence, eukaryotic cells containing the vectors are provided. Exemplary of eukaryotic cells are mammalian Chinese Hamster Ovary (CHO) cells. For example, CHO cells deficient in dihydrofolate reductase (e.g. DG44 cells) are used to produce polypeptides provided herein. Note that bacterial expression of an extended soluble PH20 or C-terminally truncated PH20 provided herein will not result in a catalytically active polypeptide, but when combined with proper

glycosylation machinery, the PH20 can be artificially glycosylated.

[0180] Provided are vectors that contain a sequence of nucleotides that encodes the hyaluronidase polypeptide, including extended soluble PH20 polypeptides and other C-terminal truncated PH20 polypeptides, coupled to the native or heterologous signal sequence, as well as multiple copies thereof. The vectors can be selected for expression of the enzyme protein in the cell or such that the enzyme protein is expressed as a secreted protein.

[0181] A variety of host-vector systems can be used to express the protein coding sequence. These include but are not limited to mammalian cell systems infected with virus (*e.g.* vaccinia virus, adenovirus and other viruses); insect cell systems infected with virus (*e.g.* baculovirus); microorganisms such as yeast containing yeast vectors; or bacteria transformed with bacteriophage, DNA, plasmid DNA, or cosmid DNA. The expression elements of vectors vary in their strengths and specificities. Depending on the host-vector system used, any one of a number of suitable transcription and translation elements can be used.

[0182] Any methods known to those of skill in the art for the insertion of DNA fragments into a vector can be used to construct expression vectors containing a chimeric gene containing appropriate transcriptional/translational control signals and protein coding sequences. These methods can include *in vitro* recombinant DNA and synthetic techniques and *in vivo* recombinants (genetic recombination). Expression of nucleic acid sequences encoding protein, or domains, derivatives, fragments or homologs thereof, can be regulated by a second nucleic acid sequence so that the genes or fragments thereof are expressed in a host transformed with the recombinant DNA molecule(s). For example, expression of the proteins can be controlled by any promoter/enhancer known in the art. In a specific embodiment, the promoter is not native to the genes for a desired protein. Promoters which can be used include but are not limited to the SV40 early promoter (Bemoist and Chambon, Nature 290:304-310 (1981)), the promoter contained in the 3' long terminal repeat of Rous sarcoma virus (Yamamoto et al. Cell 22:787-797 (1980)), the herpes thymidine kinase promoter (Wagner et al., Proc. Natl. Acad. Sci. USA 78:1441-1445 (1981)), the regulatory sequences of the metallothionein gene (Brinster et al., Nature 296:39-42 (1982)); prokaryotic expression vectors such as the b-lactamase promoter (Jay et al., (1981) Proc. Natl. Acad. Sci. USA 78:5543) or the *tac* promoter (DeBoer et al., Proc. Natl. Acad. Sci. USA 80:21-25 (1983)); see also "Useful Proteins from Recombinant Bacteria": in Scientific American 242:79-94 (1980)); plant expression vectors containing the nopaline synthetase promoter (Herrara-Estrella et al., Nature 303:209-213 (1984)) or the cauliflower mosaic virus 35S RNA promoter (Gardner et al., Nucleic Acids Res. 9:2871 (1981)), and the promoter of the photosynthetic enzyme ribulose bisphosphate carboxylase (Herrera-Estrella et al., Nature 310:115-120 (1984)); promoter elements from yeast and other fungi such as the Gal4 promoter, the alcohol dehydrogenase promoter, the phosphoglycerol kinase promoter, the alkaline phosphatase promoter, and the following animal transcriptional control regions that exhibit tissue specificity and have been used in transgenic animals: elastase I gene control region which is active in pancreatic acinar cells (Swift et al., Cell 38:639-646 (1984); Omitz et al., Cold Spring Harbor Symp. Quant. Biol. 50:399-409 (1986); MacDonald, Hepatology 7:425-515 (1987)); insulin gene control region which is active in pancreatic beta cells (Hanahan et al., Nature 315:115-122 (1985)), immunoglobulin gene control region which is active in lymphoid cells (Grosschedl et al., Cell 38:647-658 (1984); Adams et al., Nature 318:533-538 (1985); Alexander et al., Mol. Cell Biol. 7:1436-1444 (1987)), mouse mammary tumor virus control region which is active in testicular, breast, lymphoid and mast cells (Leder et al., Cell 45:485-495 (1986)), albumin gene control region which is active in liver (Pinckert et al., Genes and Devel. 1:268-276 (1987)), alpha-fetoprotein gene control region which is active in liver (Krumlauf et al., Mol. Cell. Biol. 5:1639-1648 (1985); Hammer et al., Science 235:53-58 1987)), alpha-1 antitrypsin gene control region which is active in liver (Kelsey et al, Genes and Devel. 1:161-171 (1987)), beta globin gene control region which is active in myeloid cells (Magram et al., Nature 315:338-340 (1985); Kollias et al., Cell 46:89-94 (1986)), myelin basic protein gene control region which is active in oligodendrocyte cells of the brain (Readhead et al., Cell 48:703-712 (1987)), myosin light chain-2 gene control region which is active in skeletal muscle (Shani, Nature 314:283-286 (1985)), and gonadotrophic releasing hormone gene control region which is active in gonadotrophs of the hypothalamus (Mason et al., Science 234:1372-1378 (1986)).

[0183] In a specific embodiment, a vector is used that contains a promoter operably linked to nucleic acids encoding a PH20 protein, or a domain, fragment, derivative or homolog, thereof, one or more origins of replication, and optionally, one or more selectable markers (*e.g.,* an antibiotic resistance gene). Depending on the expression system, specific initiation signals also are required for efficient translation of a PH20 sequence. These signals include the ATG initiation codon and adjacent sequences. In cases where the initiation codon and upstream sequences of PH20 or soluble forms thereof are inserted into the appropriate expression vector, no additional translational control signals are needed. In cases where only coding sequence, or a portion thereof, is inserted, exogenous transcriptional control signals including the ATG initiation codon must be provided. Furthermore, the initiation codon must be in the correct reading frame to ensure transcription of the entire insert. Exogenous transcriptional elements and initiation codons can be of various origins, both natural and synthetic. The efficiency of expression can be enhanced by the inclusion of enhancers appropriate to the cell system in use (Scharf et al. (1994) Results Probl Cell Differ 20:125-62; Bittner et al. (1987) Methods in Enzymol, 153:516-544).

[0184] Exemplary plasmid vectors for transformation of *E. coli* cells, include, for example, the pQE expression vectors

(available from Qiagen, Valencia, CA; see also literature published by Qiagen describing the system). pQE vectors have a phage T5 promoter (recognized by *E. coli* RNA polymerase) and a double lac operator repression module to provide tightly regulated, high-level expression of recombinant proteins in *E. coli,* a synthetic ribosomal binding site (RBS II) for efficient translation, a 6XHis tag coding sequence, t_0 and T1 transcriptional terminators, ColE1 origin of replication, and a beta-lactamase gene for conferring ampicillin resistance. The pQE vectors enable placement of a 6xHis tag at either the N- or C-terminus of the recombinant protein. Such plasmids include pQE 32, pQE 30, and pQE 31 which provide multiple cloning sites for all three reading frames and provide for the expression of N-terminally 6xHis-tagged proteins. Other exemplary plasmid vectors for transformation of *E. coli* cells, include, for example, the pET expression vectors (see, U.S patent 4,952,496; available from NOVAGEN, Madison, WI; see, also literature published by Novagen describing the system). Such plasmids include pET 11a, which contains the T71ac promoter, T7 terminator, the inducible *E. coli* lac operator, and the lac repressor gene; pET 12a-c, which contains the T7 promoter, T7 terminator, and the *E. coli* ompT secretion signal; and pET 15b and pET19b (NOVAGEN, Madison, WI), which contain a His-Tag™ leader sequence for use in purification with a His column and a thrombin cleavage site that permits cleavage following purification over the column, the T7-lac promoter region and the T7 terminator.

[0185] Exemplary of a vector for mammalian cell expression is the HZ24 expression vector. The HZ24 expression vector was derived from the pCI vector backbone (Promega). It contains DNA encoding the Beta-lactamase resistance gene (AmpR), an F1 origin of replication, a Cytomegalovirus immediate-early enhancer/promoter region (CMV), and an SV40 late polyadenylation signal (SV40). The expression vector also has an internal ribosome entry site (IRES) from the ECMV virus (Clontech) and the mouse dihydrofolate reductase (DHFR) gene. Cells transfected with such a vector can be cultured in chemically defined medium in the absence of hypoxanthine and thymidine, followed by further gene amplification with increasing concentrations of methotrexate. Such methods are described herein in Examples 13 and 15.

2. Expression

[0186] PH20 polypeptides, including esPH20 polypeptides and C-terminally truncated PH20 polypeptides provided herein, can be produced by any method known to those of skill in the art including in vivo and in vitro methods. Desired proteins can be expressed in any organism suitable to produce the required amounts and forms of the proteins, such as for example, needed for administration and treatment. Expression hosts include prokaryotic and eukaryotic organisms such as *E. coli,* yeast, plants, insect cells, mammalian cells, including human cell lines and transgenic animals. Expression hosts can differ in their protein production levels as well as the types of post-translational modification that are present on the expressed proteins. The choice of expression host can be made based on these and other factors, such as regulatory and safety considerations, production costs and the need and methods for purification.

[0187] Many expression vectors are available and known to those of skill in the art and can be used for expression of proteins. The choice of expression vector will be influenced by the choice of host expression system. In general, expression vectors can include transcriptional promoters and optionally enhancers, translational signals, and transcriptional and translational termination signals. Expression vectors that are used for stable transformation typically have a selectable marker which allows selection and maintenance of the transformed cells. In some cases, an origin of replication can be used to amplify the copy number of the vector.

[0188] Soluble hyaluronidase polypeptides, including esPH20, and other C-terminally truncated PH20 polypeptides, also can be utilized or expressed as protein fusions. For example, an enzyme fusion can be generated to add additional functionality to an enzyme. Examples of enzyme fusion proteins include, but are not limited to, fusions of a signal sequence, a tag such as for localization, *e.g.* a his_6 tag or a myc tag, or a tag for purification, for example, a GST fusion, a sequence for directing protein secretion and/or membrane association and other sequences used to increase half-life such as an Fc fusion.

[0189] For long-term, high-yield production of recombinant proteins, stable expression is desired. For example, cell lines that stably express a soluble PH20, such as an esPH20, or another C-terminally truncated PH20 polypeptide, can be transformed using expression vectors that contain viral origins of replication or endogenous expression elements and a selectable marker gene. Following the introduction of the vector, cells can be allowed to grow for 1-2 days in an enriched media before they are switched to selective media. The purpose of the selectable marker is to confer resistance to selection, and its presence allows growth and recovery of cells that successfully express the introduced sequences. Resistant cells of stably transformed cells can be proliferated using tissue culture techniques appropriate to the cell types.

[0190] Any number of selection systems can be used to recover transformed cell lines. These include, but are not limited to, the herpes simplex virus thymidine kinase (Wigler M et al. (1977) Cell, 11:223-32) and adenine phosphoribosyltransferase (Lowy I et al. (1980) Cell, 22:817-23) genes, which can be employed in TK- or APRT- cells, respectively. Also, antimetabolite, antibiotic or herbicide resistance can be used as the basis for selection. For example, DHFR, which confers resistance to methotrexate (Wigler M et al. (1980) Proc. Natl. Acad. Sci, 77:3567-70); npt, which confers resistance to the aminoglycosides neomycin and G-418 (Colbere-Garapin F et al. (1981) J. Mol. Biol., 150:1-14); and als or pat, which confer resistance to chlorsulfuron and phosphinotricin acetyltransferase, respectively, can be used. Additional

selectable genes have been described, for example, trpB, which allows cells to utilize indole in place of typtophan or hisD, which allows cells to utilize histinol in place of histidine (Hartman SC and RC Mulligan (1988) Proc. Natl. Acad. Sci, 85:8047-51). Visible markers, such as but not limited to, anthocyanins, beta glucuronidase and its substrate, GUS, and luciferase and its substrate luciferin, also can be used to identify transformants and also to quantify the amount of transient or stable protein expression attributable to a particular vector system (Rhodes CA et al. (1995) Methods Mol. Biol. 55:121-131).

[0191] The presence and expression of soluble PH20 polypeptides, including esPH20, and other C-terminal truncated PH20 polypetpides, can be monitored. For example, detection of a functional polypeptide can be determined by testing the conditioned media for hyaluronidase enzyme activity under appropriate conditions. Section G below provides exemplary assays to assess the solubility and activity of expressed proteins.

a. Prokaryotic Cells

[0192] Prokaryotes, especially *E. coli,* provide a system for producing large amounts of proteins. Transformation of *E. coli* is simple and rapid technique well known to those of skill in the art. Expression vectors for *E.coli* can contain inducible promoters, such promoters are useful for inducing high levels of protein expression and for expressing proteins that exhibit some toxicity to the host cells. Examples of inducible promoters include the lac promoter, the trp promoter, the hybrid tac promoter, the T7 and SP6 RNA promoters and the temperature regulated λPL promoter.

[0193] Proteins, such as any provided herein, can be expressed in the cytoplasmic environment of *E. coli.* The cytoplasm is a reducing environment and for some molecules, this can result in the formation of insoluble inclusion bodies. Reducing agents such as dithiothreotol and β-mercaptoethanol and denaturants, such as guanidine-HCl and urea can be used to resolubilize the proteins. An alternative approach is the expression of proteins in the periplasmic space of bacteria which provides an oxidizing environment and chaperonin-like and disulfide isomerases and can lead to the production of soluble protein. Typically, a leader sequence is fused to the protein to be expressed which directs the protein to the periplasm. The leader is then removed by signal peptidases inside the periplasm. Examples of periplasmic-targeting leader sequences include the pelB leader from the pectate lyase gene and the leader derived from the alkaline phosphatase gene. In some cases, periplasmic expression allows leakage of the expressed protein into the culture medium. The secretion of proteins allows quick and simple purification from the culture supernatant. Proteins that are not secreted can be obtained from the periplasm by osmotic lysis. Similar to cytoplasmic expression, in some cases proteins can become insoluble and denaturants and reducing agents can be used to facilitate solubilization and refolding. Temperature of induction and growth also can influence expression levels and solubility, typically temperatures between 25 °C and 37 °C are used. Typically, bacteria produce aglycosylated proteins. Thus, if proteins require glycosylation for function, glycosylation can be added *in vitro* after purification from host cells.

b. Yeast Cells

[0194] Yeasts such as *Saccharomyces cerevisae, Schizosaccharomyces pombe, Yarrowia lipolytica, Kluyveromyces lactis* and *Pichia pastoris* are well known yeast expression hosts that can be used for production of proteins, such as any described herein. Yeast can be transformed with episomal replicating vectors or by stable chromosomal integration by homologous recombination. Typically, inducible promoters are used to regulate gene expression. Examples of such promoters include GAL1, GAL7 and GAL5 and metallothionein promoters, such as CUP1, AOX1 or other *Pichia* or other yeast promoter. Expression vectors often include a selectable marker such as LEU2, TRP1, HIS3 and URA3 for selection and maintenance of the transformed DNA. Proteins expressed in yeast are often soluble. Co-expression with chaperonins such as Bip and protein disulfide isomerase can improve expression levels and solubility. Additionally, proteins expressed in yeast can be directed for secretion using secretion signal peptide fusions such as the yeast mating type alpha-factor secretion signal from *Saccharomyces cerevisae* and fusions with yeast cell surface proteins such as the Aga2p mating adhesion receptor or the *Arxula adeninivorans* glucoamylase. A protease cleavage site such as for the Kex-2 protease, can be engineered to remove the fused sequences from the expressed polypeptides as they exit the secretion pathway. Yeast also is capable of glycosylation at Asn-X-Ser/Thr motifs.

c. Insect Cells

[0195] Insect cells, particularly using baculovirus expression, are useful for expressing polypeptides such as hyaluronidase polypeptides. Insect cells express high levels of protein and are capable of most of the post-translational modifications used by higher eukaryotes. Baculovirus have a restrictive host range which improves the safety and reduces regulatory concerns of eukaryotic expression. Typical expression vectors use a promoter for high level expression such as the polyhedrin promoter of baculovirus. Commonly used baculovirus systems include the baculoviruses such as *Autographa californica* nuclear polyhedrosis virus (AcNPV), and the *bombyx mori* nuclear polyhedrosis virus (BmNPV)

and an insect cell line such as Sf9 derived from *Spodoptera frugiperda, Pseudaletia unipuncta* (A7S) and *Danaus plexippus* (DpN1). For high-level expression, the nucleotide sequence of the molecule to be expressed is fused immediately downstream of the polyhedrin initiation codon of the virus. Mammalian secretion signals are accurately processed in insect cells and can be used to secrete the expressed protein into the culture medium. In addition, the cell lines *Pseudaletia unipuncta* (A7S) and *Danaus plexippus* (DpN1) produce proteins with glycosylation patterns similar to mammalian cell systems. Exemplary insect cells are those that have been altered to reduce immunogenicity, including those with "mammalianized" baculoviruse expression vectors and those lacking the enzyme FT3.

[0196] An alternative expression system in insect cells is the use of stably transformed cells. Cell lines such as the Schnieder 2 (S2) and Kc cells (*Drosophila melanogaster*) and C7 cells (*Aedes albopictus*) can be used for expression. The *Drosophila* metallothionein promoter can be used to induce high levels of expression in the presence of heavy metal induction with cadmium or copper. Expression vectors are typically maintained by the use of selectable markers such as neomycin and hygromycin.

d. Mammalian Cells

[0197] Mammalian expression systems can be used to express proteins including soluble hyaluronidase polypeptides. Expression constructs can be transferred to mammalian cells by viral infection such as adenovirus or by direct DNA transfer such as liposomes, calcium phosphate, DEAE-dextran and by physical means such as electroporation and microinjection. Expression vectors for mammalian cells typically include an mRNA cap site, a TATA box, a translational initiation sequence (Kozak consensus sequence) and polyadenylation elements. IRES elements also can be added to permit bicistronic expression with another gene, such as a selectable marker. Such vectors often include transcriptional promoter-enhancers for high-level expression, for example the SV40 promoter-enhancer, the human cytomegalovirus (CMV) promoter and the long terminal repeat of Rous sarcoma virus (RSV). These promoter-enhancers are active in many cell types. Tissue and cell-type promoters and enhancer regions also can be used for expression. Exemplary promoter/enhancer regions include, but are not limited to, those from genes such as elastase I, insulin, immunoglobulin, mouse mammary tumor virus, albumin, alpha fetoprotein, alpha 1 antitrypsin, beta globin, myelin basic protein, myosin light chain 2, and gonadotropic releasing hormone gene control. Selectable markers can be used to select for and maintain cells with the expression construct. Examples of selectable marker genes include, but are not limited to, hygromycin B phosphotransferase, adenosine deaminase, xanthine-guanine phosphoribosyl transferase, aminoglycoside phosphotransferase, dihydrofolate reductase (DHFR) and thymidine kinase. For example, expression can be performed in the presence of methotrexate to select for only those cells expressing the DHFR gene. Fusion with cell surface signaling molecules such as TCR-ζ and $Fc_{\varepsilon}RI$-γ can direct expression of the proteins in an active state on the cell surface.

[0198] Many cell lines are available for mammalian expression including mouse, rat human, monkey, chicken and hamster cells. Exemplary cell lines include but are not limited to CHO, Balb/3T3, HeLa, MT2, mouse NS0 (nonsecreting) and other myeloma cell lines, hybridoma and heterohybridoma cell lines, lymphocytes, fibroblasts, Sp2/0, COS, NIH3T3, HEK293, 293S, 2B8, and HKB cells. Cell lines also are available adapted to serum-free media which facilitates purification of secreted proteins from the cell culture media. Examples include CHO-S cells (Invitrogen, Carlsbad, CA, cat # 11619-012) and the serum free EBNA-1 cell line (Pham et al., (2003) Biotechnol. Bioeng. 84:332-42.). Cell lines also are available that are adapted to grow in special mediums optimized for maximal expression. For example, DG44 CHO cells are adapted to grow in suspension culture in a chemically defined, animal product-free medium.

e. Plants

[0199] Transgenic plant cells and plants can be used to express proteins such as any described herein. Expression constructs are typically transferred to plants using direct DNA transfer such as microprojectile bombardment and PEG-mediated transfer into protoplasts, and with agrobacterium-mediated transformation. Expression vectors can include promoter and enhancer sequences, transcriptional termination elements and translational control elements. Expression vectors and transformation techniques are usually divided between dicot hosts, such as *Arabidopsis* and tobacco, and monocot hosts, such as corn and rice. Examples of plant promoters used for expression include the cauliflower mosaic virus promoter, the nopaline syntase promoter, the ribose bisphosphate carboxylase promoter and the ubiquitin and UBQ3 promoters. Selectable markers such as hygromycin, phosphomannose isomerase and neomycin phosphotransferase are often used to facilitate selection and maintenance of transformed cells. Transformed plant cells can be maintained in culture as cells, aggregates (callus tissue) or regenerated into whole plants. Transgenic plant cells also can include algae engineered to produce hyaluronidase polypeptides. Because plants have different glycosylation patterns than mammalian cells, this can influence the choice of protein produced in these hosts.

3. Purification Techniques

[0200] Host cells transformed with a nucleic acid sequence encoding a soluble PH20, including esPH20, and other C-terminal truncated PH20 polypeptides, can be cultured under conditions suitable for the expression and recovery of the encoded protein from cell culture. The protein produced by a recombinant cell is generally secreted, but may be contained intracellularly depending on the sequence and/or the vector used. As will be understood by those of skill in the art, expression vectors containing nucleic acid encoding PH20 can be designed with signal sequences that facilitate direct secretion of PH20 through prokaryotic or eukaryotic cell membrane.

[0201] Thus, method for purification of polypeptides from host cells will depend on the chosen host cells and expression systems. For secreted molecules, proteins are generally purified from the culture media after removing the cells. For intracellular expression, cells can be lysed and the proteins purified from the extract. When transgenic organisms such as transgenic plants and animals are used for expression, tissues or organs can be used as starting material to make a lysed cell extract. Additionally, transgenic animal production can include the production of polypeptides in milk or eggs, which can be collected, and if necessary, the proteins can be extracted and further purified using standard methods in the art.

[0202] Proteins, such as soluble PH20 polypeptides, including esPH20 polypeptides, or other C-terminal truncated PH20 polypeptides, can be purified using standard protein purification techniques known in the art including but not limited to, SDS-PAGE, size fractionation and size exclusion chromatography, ammonium sulfate precipitation and ionic exchange chromatography, such as anion exchange. Affinity purification techniques also can be utilized to improve the efficiency and purity of the preparations. For example, antibodies, receptors and other molecules that bind PH20 hyaluronidase enzymes can be used in affinity purification. For example, soluble PH20 can be purified from conditioned media.

[0203] Expression constructs also can be engineered to add an affinity tag to a protein such as a myc epitope, GST fusion or His_6 and affinity purified with myc antibody, glutathione resin and Ni-resin, respectively. Such tags can be joined to the nucleotide sequence encoding a soluble PH20 as described elsewhere herein, which can facilitate purification of soluble proteins. For example, soluble PH20 can be expressed as a recombinant protein with one or more additional polypeptide domains added to facilitate protein purification. Such purification facilitating domains include, but are not limited to, metal chelating peptides such as histidine-tryptophan modules that allow purification on immobilized metals, protein A domains that allow purification on immobilized immunoglobulin and the domain utilized in the FLAGS extension/affinity purification system (Immunex Corp., Seattle Wash.). The inclusion of a cleavable linker sequence such as Factor XA or enterokinase (Invitrogen, San Diego, CA) between the purification domain and the expressed PH20 polypeptide is useful to facilitate purification. One such expression vector provides for expression of a fusion protein containing a soluble PH20 and contains nucleic acid encoding 6 histidine residues followed by thioredoxin and an enterokinase cleavage site. The histidine residues facilitate purification on IMIAC (immobilized metal ion affinity chromatography), while the enterokinase cleavage site provides a means for purifying the polypeptide from the fusion protein.

[0204] Purity can be assessed by any method known in the art including gel electrophoresis, orthogonal HPLC methods, staining and spectrophotometric techniques. The expressed and purified protein can be analyzed using any assay or method known to one of skill in the art, for example, any described in Section G. These include assays based on the physical and/or functional properties of the protein, including, but not limited to, analysis by gel electrophoresis, immunoassay and assays of hyaluronidase activity.

[0205] Depending on the expression system and host cells used, the resulting polypeptide can be heterogeneous due to peptidases present in the culture medium upon production and purification. For example, culture of soluble PH20 in CHO cells can result in a mixture of heterogeneous polypeptides. An exemplary protocol for the generation, production and purification of a soluble PH20 (e.g. rHuPH20) is described in Examples 13-15 below. Similarly, for example, expression of a nucleic acid encoding a polypeptide having a sequence of amino acids 36-497 set forth in SEQ ID NO:60, can result in a heterogeneous mixture of polypeptides variably including polypeptides that end at 497, 496, 495, 494, 493, 492, 491, 490, 489 or shorter.

F. Preparation, Formulation and Administration of Extended Soluble PH20 Polypeptides, and Other Soluble PH20 Polypeptides

[0206] Pharmaceutical compositions of soluble PH20 polypeptides, including esPH20, are provided herein for administration. The soluble PH20 polypeptides can be formulated separately, or can be co-formulated or co-administered with pharmaceutical formulations of other therapeutic agents, for example, as described in Section G. The compounds can be formulated into suitable pharmaceutical preparations such as solutions, suspensions, tablets, dispersible tablets, pills, capsules, powders, sustained release formulations or elixirs, for oral administration, as well as transdermal patch preparation and dry powder inhalers. Typically, the compounds are formulated into pharmaceutical compositions using techniques and procedures well known in the art (see e.g., Ansel Introduction to Pharmaceutical Dosage Forms, Fourth

Edition, 1985, 126).

[0207] Typically, a therapeutically effective dosage is contemplated. The amount of a selected soluble PH20 to be administered for the treatment of a disease or condition can be determined by standard clinical techniques. In addition, *in vitro* assays and animal models can be employed to help identify optimal dosage ranges. The precise dosage, which can be determined empirically, can depend on the particular enzyme, the route of administration, the type of disease to be treated and the seriousness of the disease.

[0208] Hence, it is understood that the precise dosage and duration of treatment is a function of the disease being treated and can be determined empirically using known testing protocols or by extrapolation from *in vivo* or *in vitro* test data. It is to be noted that concentrations and dosage values also can vary with the severity of the condition to be alleviated. It is to be further understood that for any particular subject, specific dosage regimens should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the compositions, and that the concentration ranges set forth herein are exemplary only and are not intended to limit the scope or use of compositions and combinations containing them. The compositions can be administered hourly, daily, weekly, monthly, yearly or once. Generally, dosage regimens are chosen to limit toxicity. It should be noted that the attending physician would know how to and when to terminate, interrupt or adjust therapy to lower dosage due to toxicity, or bone marrow, liver or kidney or other tissue dysfunctions. Conversely, the attending physician would also know how to and when to adjust treatment to higher levels if the clinical response is not adequate (precluding toxic side effects).

[0209] Pharmaceutically acceptable compositions are prepared in view of approvals for a regulatory agency or other agency prepared in accordance with generally recognized pharmacopeia for use in animals and in humans. Compositions can take the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, and sustained release formulations. A composition can be formulated as a suppository, with traditional binders and carriers such as triglycerides. Oral formulation can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, and other such agents. The formulation should suit the mode of administration.

[0210] Pharmaceutical compositions can include carriers such as a diluent, adjuvant, excipient, or vehicle with which an soluble PH20 is administered. Examples of suitable pharmaceutical carriers are described in "Remington's Pharmaceutical Sciences" by E. W. Martin. Such compositions will contain a therapeutically effective amount of the compound, generally in purified form, together with a suitable amount of carrier so as to provide the form for proper administration to the patient. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, and sesame oil. Water is a typical carrier when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions also can be employed as liquid carriers, particularly for injectable solutions. Compositions can contain along with an active ingredient: a diluent such as lactose, sucrose, dicalcium phosphate, or carboxymethylcellulose; a lubricant, such as magnesium stearate, calcium stearate and talc; and a binder such as starch, natural gums, such as gum acacia, gelatin, glucose, molasses, polyvinylpyrrolidine, celluloses and derivatives thereof, povidone, crospovidones and other such binders known to those of skill in the art. Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, and ethanol. A composition, if desired, also can contain minor amounts of wetting or emulsifying agents, or pH buffering agents, for example, acetate, sodium citrate, cyclodextrine derivatives, sorbitan monolaurate, triethanolamine sodium acetate, triethanolamine oleate, and other such agents.

[0211] Formulations of pharmaceutically therapeutically active compounds and derivatives thereof are provided for administration to humans and animals in unit dosage forms or multiple dosage forms. For example compounds can be formulated as tablets, capsules, pills, powders, granules, sterile parenteral solutions or suspensions, and oral solutions or suspensions, and oil water emulsions containing suitable quantities of the compounds or pharmaceutically acceptable derivatives thereof. Each unit dose contains a predetermined quantity of therapeutically active compound sufficient to produce the desired therapeutic effect, in association with the required pharmaceutical carrier, vehicle or diluent. Examples of unit dose forms include ampoules and syringes and individually packaged tablets or capsules. Unit dose forms can be administered in fractions or multiples thereof. A multiple dose form is a plurality of identical unit dosage forms packaged in a single container to be administered in segregated unit dose form. Examples of multiple dose forms include vials, bottles of tablets or capsules or bottles of pints or gallons. Hence, multiple dose form is a multiple of unit doses that are not segregated in packaging. Generally, dosage forms or compositions containing active ingredient in the range of 0.005% to 100% with the balance made up from non-toxic carrier can be prepared.

[0212] Compositions provided herein typically are formulated for administration by subcutaneous route, although other routes of administration are contemplated, such as any route known to those of skill in the art including intramuscular, intraperitineal, intravenous, intradermal, intralesional, intraperitoneal injection, epidural, vaginal, rectal, local, otic, transdermal administration or any route. Formulations suited for such routes are known to one of skill in the art. Administration can be local, topical or systemic depending upon the locus of treatment. Local administration to an area in need

of treatment can be achieved by, for example, but not limited to, local infusion during surgery, topical application, *e.g.,* in conjunction with a wound dressing after surgery, by injection, by means of a catheter, by means of a suppository, or by means of an implant. Compositions also can be administered with other biologically active agents, either sequentially, intermittently or in the same composition.

[0213] The most suitable route in any given case depends on a variety of factors, such as the nature of the disease, the tolerance of the subject to a particular administration route, the severity of the disease, and the particular composition that is used. Typically, the compositions provided herein are administered parenterally. In some examples, soluble PH20 compositions are administered so that they reach the interstitium of skin or tissues, thereby degrading the interstitial space for subsequent delivery of a therapeutic agent. Thus, in some examples, direct administration under the skin, such as by subcutaneous administration methods, is contemplated. Thus, in one example, local administration can be achieved by injection, such as from a syringe or other article of manufacture containing a injection device such as a needle. In another example, local administration can be achieved by infusion, which can be facilitated by the use of a pump or other similar device. Other modes of administration also are contemplated. Pharmaceutical compositions can be formulated in dosage forms appropriate for each route of administration.

[0214] Administration methods can be employed to decrease the exposure of selected soluble PH20 polypeptides to degradative processes, such as proteolytic degradation and immunological intervention via antigenic and immunogenic responses. Examples of such methods include local administration at the site of treatment. Pegylation of therapeutics has been reported to increase resistance to proteolysis, increase plasma half-life, and decrease antigenicity and immunogenicity. Examples of pegylation methodologies are known in the art (see for example, Lu and Felix, Int. J. Peptide Protein Res., 43: 127-138, 1994; Lu and Felix, Peptide Res., 6: 142-6, 1993; Felix et al., Int. J. Peptide Res., 46 : 253-64, 1995; Benhar et al., J. Biol. Chem., 269: 13398-404, 1994; Brumeanu et al., J Immunol., 154: 3088-95, 1995; see also, Caliceti et al. (2003) Adv. Drug Deliv. Rev. 55(10):1261-77 and Molineux (2003) Pharmacotherapy 23 (8 Pt 2):3S-8S). Pegylation also can be used in the delivery of nucleic acid molecules *in vivo.* For example, pegylation of adenovirus can increase stability and gene transfer (see, e.g., Cheng et al. (2003) Pharm. Res. 20(9): 1444-51).

1. Injectables, solutions and emulsions

[0215] Parenteral administration, generally characterized by injection or infusion, either subcutaneously, intramuscularly, intravenous or intradermally is contemplated herein. Injectables can be prepared in conventional forms, either as liquid solutions or suspensions, solid forms suitable for solution or suspension in liquid prior to injection, or as emulsions. Suitable excipients are, for example, water, saline, dextrose, glycerol or ethanol. The pharmaceutical compositions may contain other minor amounts of non-toxic auxiliary substances such as wetting or emulsifying agents, pH buffering agents, stabilizers, solubility enhancers, and other such agents, such as for example, sodium acetate, sodium phosphate, sorbitan monolaurate, triethanolamine oleate and cyclodextrins. Implantation of a slow-release or sustained-release system, such that a constant level of dosage is maintained (see, e. g., U. S. Patent No. 3,710,795) is also contemplated herein. The percentage of active compound contained in such parenteral compositions is highly dependent on the specific nature thereof, as well as the activity of the compound and the needs of the subject.

[0216] Injectables are designed for local and systemic administration. For purposes herein, local administration is desired for direct administration to the affected interstitium. Preparations for parenteral administration include sterile solutions ready for injection, sterile dry soluble products, such as lyophilized powders, ready to be combined with a solvent just prior to use, including hypodermic tablets, sterile suspensions ready for injection, sterile dry insoluble products ready to be combined with a vehicle just prior to use and sterile emulsions. The solutions may be either aqueous or nonaqueous. If administered intravenously, suitable carriers include physiological saline or phosphate buffered saline (PBS), and solutions containing thickening and solubilizing agents, such as glucose, polyethylene glycol, and polypropylene glycol and mixtures thereof.

[0217] Pharmaceutically acceptable carriers used in parenteral preparations include aqueous vehicles, nonaqueous vehicles, antimicrobial agents, isotonic agents, buffers, antioxidants, local anesthetics, suspending and dispersing agents, emulsifying agents, sequestering or chelating agents and other pharmaceutically acceptable substances. Examples of aqueous vehicles include Sodium Chloride Injection, Ringers Injection, Isotonic Dextrose Injection, Sterile Water Injection, Dextrose and Lactated Ringers Injection. Nonaqueous parenteral vehicles include fixed oils of vegetable origin, cottonseed oil, corn oil, sesame oil and peanut oil. Antimicrobial agents in bacteriostatic or fungistatic concentrations can be added to parenteral preparations packaged in multiple-dose containers, which include phenols or cresols, mercurials, benzyl alcohol, chlorobutanol, methyl and propyl p-hydroxybenzoic acid esters, thimerosal, benzalkonium chloride and benzethonium chloride. Isotonic agents include sodium chloride and dextrose. Buffers include phosphate and citrate. Antioxidants include sodium bisulfate. Local anesthetics include procaine hydrochloride. Suspending and dispersing agents include sodium carboxymethylcelluose, hydroxypropyl methylcellulose and polyvinylpyrrolidone. Emulsifying agents include Polysorbate 80 (TWEEN 80). A sequestering or chelating agent of metal ions include EDTA. Pharmaceutical carriers also include ethyl alcohol, polyethylene glycol and propylene glycol for water miscible vehicles

and sodium hydroxide, hydrochloric acid, citric acid or lactic acid for pH adjustment.

[0218] The concentration of the pharmaceutically active compound is adjusted so that an injection or infusion provides an effective amount to produce the desired pharmacological effect, such as glycemic control. The exact dose depends on the age, weight and condition of the patient or animal as is known in the art. The unit-dose parenteral preparations can be packaged in, for example, an ampoule, a cartridge, a vial or a syringe with a needle. The volume of liquid solution or reconstituted powder preparation, containing the pharmaceutically active compound, is a function of the disease to be treated and the particular article of manufacture chosen for package. All preparations for parenteral administration must be sterile, as is known and practiced in the art.

[0219] In one example, pharmaceutical preparation can be in liquid form, for example, solutions, syrups or suspensions. If provided in liquid form, the pharmaceutical preparations can be provided as a concentrated preparation to be diluted to a therapeutically effective concentration before use. Such liquid preparations can be prepared by conventional means with pharmaceutically acceptable additives such as suspending agents (*e.g.,* sorbitol syrup, cellulose derivatives or hydrogenated edible fats); emulsifying agents (*e.g.,* lecithin or acacia); non-aqueous vehicles (*e.g.,* almond oil, oily esters, or fractionated vegetable oils); and preservatives (*e.g.,* methyl or propyl-p-hydroxybenzoates or sorbic acid). In another example, pharmaceutical preparations can be presented in lyophilized form for reconstitution with water or other suitable vehicle before use.

Lyophilized Powders

[0220] Of interest herein are lyophilized powders, which can be reconstituted for administration as solutions, emulsions and other mixtures. They may also be reconstituted and formulated as solids or gels.

[0221] The sterile, lyophilized powder is prepared by dissolving a compound of inactive enzyme in a buffer solution. The buffer solution may contain an excipient which improves the stability or other pharmacological component of the powder or reconstituted solution, prepared from the powder. Subsequent sterile filtration of the solution followed by lyophilization under standard conditions known to those of skill in the art provides the desired formulation. Briefly, the lyophilized powder is prepared by dissolving an excipient, such as dextrose, sorbital, fructose, corn syrup, xylitol, glycerin, glucose, sucrose or other suitable agent, in a suitable buffer, such as citrate, sodium or potassium phosphate or other such buffer known to those of skill in the art. Then, a selected enzyme is added to the resulting mixture, and stirred until it dissolves. The resulting mixture is sterile filtered or treated to remove particulates and to insure sterility, and apportioned into vials for lyophilization. Each vial will contain a single dosage or multiple dosages of the compound. The lyophilized powder can be stored under appropriate conditions, such as at about 4 °C to room temperature. Reconstitution of this lyophilized powder with an appropriate buffer solution provides a formulation for use in parenteral administration.

2. Topical Administration

[0222] Topical mixtures are prepared as described for the local and systemic administration. The resulting mixture can be a solution, suspension, emulsions or the like and are formulated as creams, gels, ointments, emulsions, solutions, elixirs, lotions, suspensions, tinctures, pastes, foams, aerosols, irrigations, sprays, suppositories, bandages, dermal patches or any other formulations suitable for topical administration.

[0223] The compounds or pharmaceutically acceptable derivatives thereof may be formulated as aerosols for topical application, such as by inhalation (see, e.g., U. S. Patent Nos. 4,044,126,4,414,209, and 4,364,923, which describe aerosols for delivery of a steroid useful for treatment inflammatory diseases, particularly asthma). These formulations for administration to the respiratory tract can be in the form of an aerosol or solution for a nebulizer, or as a microfine powder for insufflation, alone or in combination with an inert carrier such as lactose. In such a case, the particles of the formulation will typically have diameters of less than 50 microns, or less than 10 microns.

[0224] The compounds can be formulated for local or topical application, such as for topical application to the skin and mucous membranes, such as in the eye, in the form of gels, creams, and lotions and for application to the eye or for intracisternal or intraspinal application. Topical administration is contemplated for transdermal delivery and also for administration to the eyes or mucosa, or for inhalation therapies. Nasal solutions of the active compound alone or in combination with other pharmaceutically acceptable excipients also can be administered.

[0225] Formulations suitable for transdermal administration are provided. They can be provided in any suitable format, such as discrete patches adapted to remain in intimate contact with the epidermis of the recipient for a prolonged period of time. Such patches contain the active compound in optionally buffered aqueous solution of, for example, 0.1 to 0.2 M concentration with respect to the active compound. Formulations suitable for transdermal administration also can be delivered by iontophoresis (see, *e.g., Pharmaceutical Research* 3(6):318 (1986)) and typically take the form of an optionally buffered aqueous solution of the active compound.

3. Compositions for other routes of administration

[0226] Depending upon the condition treated other routes of administration, such as topical application, transdermal patches, oral and rectal administration are also contemplated herein. For example, pharmaceutical dosage forms for rectal administration are rectal suppositories, capsules and tablets for systemic effect. Rectal suppositories include solid bodies for insertion into the rectum which melt or soften at body temperature releasing one or more pharmacologically or therapeutically active ingredients. Pharmaceutically acceptable substances utilized in rectal suppositories are bases or vehicles and agents to raise the melting point. Examples of bases include cocoa butter (theobroma oil), glycerin-gelatin, carbowax (polyoxyethylene glycol) and appropriate mixtures of mono-, di- and triglycerides of fatty acids. Combinations of the various bases may be used. Agents to raise the melting point of suppositories include spermaceti and wax. Rectal suppositories may be prepared either by the compressed method or by molding. The typical weight of a rectal suppository is about 2 to 3 gm. Tablets and capsules for rectal administration are manufactured using the same pharmaceutically acceptable substance and by the same methods as for formulations for oral administration.

[0227] Formulations suitable for rectal administration can be provided as unit dose suppositories. These can be prepared by admixing the active compound with one or more conventional solid carriers, for example, cocoa butter, and then shaping the resulting mixture.

[0228] For oral administration, pharmaceutical compositions can take the form of, for example, tablets or capsules prepared by conventional means with pharmaceutically acceptable excipients such as binding agents (*e.g.,* pregelatinized maize starch, polyvinyl pyrrolidone or hydroxypropyl methylcellulose); fillers (*e.g.,* lactose, microcrystalline cellulose or calcium hydrogen phosphate); lubricants (*e.g.,* magnesium stearate, talc or silica); disintegrants (*e.g.,* potato starch or sodium starch glycolate); or wetting agents (*e.g.,* sodium lauryl sulphate). The tablets can be coated by methods well-known in the art.

[0229] Formulations suitable for buccal (sublingual) administration include, for example, lozenges containing the active compound in a flavored base, usually sucrose and acacia or tragacanth; and pastilles containing the compound in an inert base such as gelatin and glycerin or sucrose and acacia.

[0230] Pharmaceutical compositions also can be administered by controlled release formulations and/or delivery devices (see, *e.g.,* in U.S. Patent Nos. 3,536,809; 3,598,123; 3,630,200; 3,845,770; 3,847,770; 3,916,899; 4,008,719; 4,687,610; 4,769,027; 5,059,595; 5,073,543; 5,120,548; 5,354,566; 5,591,767; 5,639,476; 5,674,533 and 5,733,566).

[0231] Various delivery systems are known and can be used to administer selected soluble PH20 polypeptides, such as but not limited to, encapsulation in liposomes, microparticles, microcapsules, recombinant cells capable of expressing the compound, receptor mediated endocytosis, and delivery of nucleic acid molecules encoding selected soluble PH20 polypeptides such as retrovirus delivery systems.

[0232] Hence, in certain embodiments, liposomes and/or nanoparticles also can be employed with administration of soluble PH20 polypeptides. Liposomes are formed from phospholipids that are dispersed in an aqueous medium and spontaneously form multilamellar concentric bilayer vesicles (also termed multilamellar vesicles (MLVs)). MLVs generally have diameters of from 25 nm to 4 μm. Sonication of MLVs results in the formation of small unilamellar vesicles (SUVs) with diameters in the range of 200 to 500 angstroms containing an aqueous solution in the core.

[0233] Phospholipids can form a variety of structures other than liposomes when dispersed in water, depending on the molar ratio of lipid to water. At low ratios, the liposomes form. Physical characteristics of liposomes depend on pH, ionic strength and the presence of divalent cations. Liposomes can show low permeability to ionic and polar substances, but at elevated temperatures undergo a phase transition which markedly alters their permeability. The phase transition involves a change from a closely packed, ordered structure, known as the gel state, to a loosely packed, less-ordered structure, known as the fluid state. This occurs at a characteristic phase-transition temperature and results in an increase in permeability to ions, sugars and drugs.

[0234] Liposomes interact with cells via different mechanisms: endocytosis by phagocytic cells of the reticuloendothelial system such as macrophages and neutrophils; adsorption to the cell surface, either by nonspecific weak hydrophobic or electrostatic forces, or by specific interactions with cell-surface components; fusion with the plasma cell membrane by insertion of the lipid bilayer of the liposome into the plasma membrane, with simultaneous release of liposomal contents into the cytoplasm; and by transfer of liposomal lipids to cellular or subcellular membranes, or vice versa, without any association of the liposome contents. Varying the liposome formulation can alter which mechanism is operative, although more than one can operate at the same time. Nanocapsules can generally entrap compounds in a stable and reproducible way. To avoid side effects due to intracellular polymeric overloading, such ultrafine particles (sized around 0.1 μm) should be designed using polymers able to be degraded *in vivo.* Biodegradable polyalkyl-cyanoacrylate nanoparticles that meet these requirements are contemplated for use herein, and such particles can be easily made.

4. Dosage and Administration

[0235] The soluble PH20 polypeptides, including esPH20, provided herein can be formulated as pharmaceutical compositions for single dosage or multiple dosage administration. The selected hyaluronan degrading enzyme is included in an amount sufficient to exert a therapeutically useful effect in the absence of undesirable side effects on the patient treated. The therapeutically effective concentration can be determined empirically by testing the polypeptides in known *in vitro* and *in vivo* systems such as by using the assays provided herein or known in the art (see e.g., Taliani et al. (1996) Anal. Biochem., 240: 60-67; Filocamo et al. (1997) J Virology, 71: 1417-1427; Sudo et al. (1996) Antiviral Res. 32: 9-18; Buffard et al. (1995) Virology, 209:52-59; Bianchi et al. (1996) Anal. Biochem., 237: 239-244; Hamatake et al. (1996) Intervirology 39:249-258; Steinkuhler et al. (1998) Biochem., 37:8899-8905; D'Souza et al. (1995) J Gen. Virol., 76:1729-1736; Takeshita et al. (1997) Anal. Biochem., 247:242-246; see also e.g., Shimizu et al. (1994) J. Virol. 68:8406-8408; Mizutani et al. (1996) J. Virol. 70:7219-7223; Mizutani et al. (1996) Biochem. Biophys. Res. Commun., 227:822-826; Lu et al. (1996) Proc. Natl. Acad. Sci (USA), 93:1412-1417; Hahm et al., (1996) Virology, 226:318-326; Ito et al. (1996) J. Gen. Virol., 77:1043-1054; Mizutani et al. (1995) Biochem. Biophys. Res. Commun., 212:906-911; Cho et al. (1997) J. Virol. Meth. 65:201-207 and then extrapolated therefrom for dosages for humans.

[0236] Typically, a therapeutically effective dose of a soluble PH20 enzyme is at or about 10 Unit (U) to 500,000 Units, 100 Units to 100,000 Units, 500 Units to 50,000 Units, 1000 Units to 10,000 Units, 5000 Units to 7500 Units, 5000 Units to 50,000 Units, or 1,000 Units to 10,000 Units, generally 1,000 to 50,000 Units, in a stabilized solution or suspension or a lyophilized from. The formulations can be provided in unit-dose forms such as, but not limited to, ampoules, syringes and individually packaged tablets or capsules. The dispersing agent can be administered alone, or with other pharmacologically effective agent or therapeutic agent in a total volume of 0.1 -100 ml, 1 -50 ml, 10- 50 ml, 10-30 ml, 1-20 ml, or 1-10 ml, typically 10-50 ml.

[0237] For example, a soluble PH20, including esPH20, can be administered subcutaneously at or about 10 U, 20 U, 30 U, 40 U, 50 U, 100 U, 150 U, 200 U, 250 U, 300 U, 350 U, 400 U, 450 U, 500 U, 600 U, 700 U, 800 U, 900 U, 1000 U, 2,000 U, 3,000 U, 4,000 Units, 5,000 U or more. In some examples, dosages can be provided as a ratio of amount of a soluble PH20 to therapeutic agent administered. For example, a soluble PH20 polypeptide can be administered at 1 hyaluronidase U/therapeutic agent U (1:1) to 50:1 or more, for example, at or about 1:1, 2:1, 3:1, 4:1, 5:1, 6:1, 7:1, 8:1, 9:1, 10:1, 11:1, 12:1, 13:1, 14:1, 15:1, 20:1, 25:1, 30:1, 35:1, 40:1, 45:1, 50:1 or more. Typically, volumes of injections or infusions of a soluble PH20 contemplated herein are from at or about 0.01 mL, 0.05 mL, 0.1 mL, 0.2 mL, 0.3 mL, 0.4 mL, 0.5 mL, 1 mL, 2 mL, 3 mL, 4 mL, 5 mL, 6 mL, 7 mL, 8 mL, 9 ml, 10 ml, 20 ml, 30 ml, 40 ml, 50 ml or more. The soluble PH20 can be provided as a stock solution at or about 100 U/ml, 150 U/ml, 200 U/ml, 300 U/ml, 400 U/ml, 500 U/mL, 600 U/mL, 800 U/mL or 1000 U/mL, or can be provided in a more concentrated form, for example at or about 2000 U/ml, 3000 Units/ml, 4000 U/ml, 5000 U/ml, 8000 U/ml, 10,000 U/mL or 20,000 U/mL for use directly or for dilution to the effective concentration prior to use. The soluble PH20 can be provided as a liquid or lyophilized formulation.

5. Packaging, Articles of Manufacture and Kits

[0238] Pharmaceutical compounds of soluble PH20 polypeptides, including esPH20, or nucleic acids encoding such polypeptides, or a derivative or variant thereof can be packaged as articles of manufacture containing packaging material, a pharmaceutical composition which is effective for treating a disease or disorder, and a label that indicates that the soluble PH20 or nucleic acid molecule is to be used for treating the disease or disorder. Combinations of a selected soluble PH20 hyaluronidase, or derivative or variant thereof and an therapeutic agent also can be packaged in an article of manufacture.

[0239] The articles of manufacture provided herein contain packaging materials. Packaging materials for use in packaging pharmaceutical products are well known to those of skill in the art. See, for example, U.S. Patent Nos. 5,323,907, 5,052,558 and 5,033,252. Examples of pharmaceutical packaging materials include, but are not limited to, blister packs, bottles, tubes, inhalers, pumps, bags, vials, containers, syringes, bottles, and any packaging material suitable for a selected formulation and intended mode of administration and treatment. The articles of manufacture can include a needle or other injection device so as to facilitate administration (e.g. sub-epidermal administration) for local injection purposes. A wide array of formulations of the compounds and compositions provided herein are contemplated including a soluble PH20, such as an esPH20, and a therapeutic agent known to treat a particular disease or disorder. The choice of package depends on the soluble PH20 and/or therapeutic agent, and whether such compositions will be packaged together or separately. In one example, the soluble PH20 can be packaged as a mixture with the therapeutic agent. In another example, the components can be packaged as separate compositions

[0240] Selected soluble PH20 polypeptides, such as esPH20 polypeptides, therapeutic agents and/or articles of manufacture thereof also can be provided as kits. Kits can include a pharmaceutical composition described herein and an item for administration provided as an article of manufacture. For example a soluble PH20 polypeptide can be supplied with a device for administration, such as a syringe, an inhaler, a dosage cup, a dropper, or an applicator. The compositions

can be contained in the item for administration or can be provided separately to be added later. The kit can, optionally, include instructions for application including dosages, dosing regimens and instructions for modes of administration. Kits also can include a pharmaceutical composition described herein and an item for diagnosis. For example, such kits can include an item for measuring the concentration, amount or activity of the selected protease in a subject.

G. Assays

[0241] Soluble PH20 polypeptides provided herein, including esPH20 polypeptides, are soluble and retain a hyaluronidase enzymatic activity. N-glycosylated or N-partially glycosylated PH20 polypeptides provided herein retain a hyaluronidase enzymatic activity. The activity of a PH20 provided herein is or is about 30%, 40%, 50%, 60%, 70%, 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more compared to the activity of a corresponding PH20 that is not C-terminally truncated or N-partially glycosylated. The activity of a soluble PH20 hyaluronidase polypeptide, such as an esPH20, can be assessed using methods well known in the art. These methods include, for example, a microturbidity assay and a microtiter assay using biotinylated hyaluronic acid. Activity and assessments can be performed on conditioned medium or supernatants or on purified protein. The solublity of a protein also can be determined, for example, by a Triton® X-114 partition assay. In all assays, the activity or solubility of a soluble PH20 can be compared to a control, for example, a full length PH20 lacking C-terminal truncations.

1. Hyaluronidase Activity

[0242] The activity of a soluble PH20 polypeptide can be assessed using methods well known in the art. For example, the USP XXII assay for hyaluronidase determines activity indirectly by measuring the amount of undegraded hyaluronic acid, or hyaluronan, (HA) substrate remaining after the enzyme is allowed to react with the HA for 30 min at 37 °C (USP XXII-NF XVII (1990) 644-645 United States Pharmacopeia Convention, Inc, Rockville, MD). A Hyaluronidase Reference Standard (USP) or National Formulary (NF) Standard Hyaluronidase solution can be used in an assay to ascertain the activity, in units, of any hyaluronidase.

[0243] In one example, activity is measured using a microturbidity assay, as described in Example 12. This is based on the formation of an insoluble precipitate when hyaluronic acid binds with serum albumin. The activity is measured by incubating hyaluronidase with sodium hyaluronate (hyaluronic acid) for a set period of time (e.g. 10 minutes) and then precipitating the undigested sodium hyaluronate with the addition of acidified serum albumin. The turbidity of the resulting sample is measured at 640 nm after an additional development period. The decrease in turbidity resulting from hyaluronidase activity on the sodium hyaluronate substrate is a measure of hyaluronidase enzymatic activity.

[0244] In another example, hyaluronidase activity is measured using a microtiter assay in which residual biotinylated hyaluronic acid is measured following incubation with hyaluronidase (see e.g. Frost and Stern (1997) Anal. Biochem. 251:263-269, U.S. Patent Publication No. 20050260186). In Example 4, the hyaluronidase activity of truncated human PH20 hyaluronidase is determined using biotinylated hyaluronic acid. The free carboxyl groups on the glucuronic acid residues of hyaluronic acid are biotinylated, and the biotinylated hyaluronic acid substrate is covalently coupled to a microtiter plate. Following incubation with hyaluronidase, the residual biotinylated hyaluronic acid substrate is detected using an avidin-peroxidase reaction, and compared to that obtained following reaction with hyaluronidase standards of known activity. As the-substrate is covalently bound to the microtiter plate, artifacts such as pH-dependent displacement of the biotinylated substrate does not occur. The sensitivity permits rapid measurement of hyaluronidase activity from cultured cells and biological samples with an inter-assay variation of less than 10%.

[0245] Other assays to measure hyaluronidase activity also are known in the art and can be used in the methods herein (see e.g. Delpech et al., (1995) Anal. Biochem. 229:35-41; Takahashi et al., (2003) Anal. Biochem. 322:257-263).

[0246] Many hyaluronidase assays have been based upon the measurement of the generation of new reducing N-acetylamino groups (Bonner and Cantey, Clin. Chim. Acta 13:746-752, 1966), or loss of viscosity (De Salegui et al., Arch. Biochem. Biophys. 121:548-554, 1967) or turbidity (Dorfman and Ott, J. Biol. Chem. 172:367, 1948). With purified substrates all of these methods suffice for determination of the presence or absence of endoglucosamidic activity.

[0247] Substantially purified glycosaminoglycan substrates can also be used in a Gel Shift Assay. Glycosaminoglycans are mixed with recombinant PH20, such as a soluble PH20, to test for endoglucosidase activity that results in a shift in substrate mobility within the gel. Exemplary of such substrates include, but are not limited to, chondroitin-4 and 6 sulfate, dermatan sulfate, heparan-sulfate, which can be obtained from Sigma Chemical. Human umbilical cord Hyaluronan can be obtained from ICN. For example, each test substrate can be diluted to at or about 0.1 mg/ml in a buffer range from pH 3.5-7.5. In such an exemplary assay, at or about 10 μl samples of purified soluble PH20 or conditioned media from PH20 expressing cells can be mixed with at or about 90 μl of test substrate in desired buffer and incubated for 3 hours at 37 °C. Following incubation, samples are neutralized with sample buffer (Tris EDTA pH 8.0, Bromophenol Blue and glycerol) followed by electrophoresis. Glycosaminoglycans can be detected using any method known in the art, for example, glycosaminoglycans can be detected by staining the gels using 0.5% Alcian Blue in 3% Glacial Acetic Acid

overnight followed by destaining in 7% Glacial Acetic Acid. Degradation is determined by comparison of substrate mobility in the presence and absence of enzyme.

[0248] Hyaluronidase activity can also be detected by substrate gel zymography (Guentenhoner et al., 1992, Matrix 388-396). In this assay a sample is applied to a SDS-PAGE gel containing hyaluronic acid and the proteins in the sample separated by electrophoresis. The gel is then incubated in an enzyme assay buffer and subsequently stained to detect the hyaluronic acid in the gel. Hyaluronidase activity is visualized as a cleared zone in the substrate gel.

[0249] The ability of a soluble PH20 polypeptide to act as a spreading or diffusing agent also can be assessed. For example, trypan blue dye can be injected subcutaneously with or without a soluble PH20 into the lateral skin on each side of nude mice. The dye area is then measured, such as with a microcaliper, to determine the ability of the hyaluronan degrading enzyme to act as a spreading agent (U.S. Patent No. 20060104968). The effect of co-administration of hyaluronidase with another agent, for example a therapeutic agent, on the pharmacokinetic and pharmacodynamic properties of that agent also can be assessed *in vivo* using animal model and/or human subjects, such as in the setting of a clinical trial.

[0250] The functional activity of a soluble PH20, such as esPH20 can be compared and/or normalized to a reference standared using any of these assays. This can be done to determine what a functionally equivalent amount of a soluble PH20 is. For example, the ability of a soluble PH20 to act as a spreading or diffusing agent can be assessed by injecting it into the lateral skin of mice with trypan blue, and the amount required to achieve the same amount of diffusion as, for example, 100 units of a Hyaluronidase Reference Standard, can be determined. The amount of soluble PH20 required is, therefore, functionally equivalent to 100 hyauronidase units.

2. Solubility

[0251] Solubility of a hyaluronidase can be determined by any method known to one of the skill in the art. One method for determining solubility is by detergent partitioning. For example, a soluble PH20 polypeptide can be distinguished, for example, by its partitioning into the aqueous phase of a Triton® X-114 solution at 37 °C (Bordier et al., (1981) J. Biol. Chem., 256:1604-1607). For example, the solubility of the PH20 polypeptides described herein is assessed as described in Example 4. Membrane-anchored hyaluronidases, such as lipid-anchored hyaluronidases, including GPI-anchored hyaluronidases, will partition into the detergent-rich phase, but will partition into the detergent-poor or aqueous phase following treatment with Phospholipase C. Phospholipase C is an enzyme that cleaves the phospho-glycerol bond found in GPI-anchored proteins. Treatment with PLC will cause release of GPI-linked proteins from the outer cell membrane.

[0252] Another method for assessing solubility is to determine whether a PH20 polypeptide is GPI-anchored. A GPI-anchored PH20 polypeptide is bound to the cell membrane and therefore insoluble. To determine whether a PH20 polypeptide is GPI-anchored, one can assess solubility before and after PLC/PLD hydrolysis, and also use predictive algorithms to identify a GPI-anchor attachment signal sequence. GPI-anchored proteins can be identified by their solubilization after specific enzymatic or chemical cleavage, in conjunction with detergent partitioning (e.g., in Triton® X-114), antibody recognition, and metabolic radioactive labeling.

[0253] A common method used to demonstrate that a protein has a GPI anchor is its release from the cell surface or its solubilization by treating with bacterial PI-PLC or trypanosome-derived GPI-specific phospholipase C (GPI-PLC). These enzymes cleave a diacylglycerol in the membrane and produce the immunoreactive glycan epitope (CRD) on the protein, which can be detected by Western blotting with antibodies produced against the GPI of trypanosomes. One common problem with this approach especially encountered in mammalian cells is that the lipases cannot cleave a GPI anchor in which the inositol is acylated. These require prior treatment with mild alkali to remove the fatty acid on the inositol ring. Alternatively, serum-derived GPI-specific phospholipase D can be used to cleave GPI anchors. This enzyme cleaves between the inositol ring and the phosphatidic acid moiety and is not inhibited by inositol acylation. Hydrofluoric acid cleaves GPI anchors between the inositol ring and phosphatidic acid and also cleaves the phosphodiester linkages between any phosphoethanolamines and mannosyl residues. Dilute nitrous acid is particularly useful in the study of GPI anchors because it cleaves specifically between the nonacetylated glucosamine and the inositol ring, releasing the protein-bound glycan (now containing a diagnostic anhydromannose moiety) and phosphatidylinositol. In combination with CRD antibodies, composition analyses, radioactive labeling with myo-inositol, ethanolamine, glucosamine, mannose, or fatty acids and chromatographic or detergent partitioning methods, these degradation methods represent a powerful set of tools to study GPI anchors on proteins.

[0254] Various *in silico* methods and algorithms have been developed that can be used to identify GPI-anchor attachment signal consensus sequences in polypeptides (see, e.g. Udenfriend et al. (1995) Methods Enzymol. 250:571-582; Eisenhaber et al., (1999) J. Biol. Chem. 292: 741-758, Kronegg and Buloz, (1999); "Detection/prediction of GPI cleavage site (GPI-anchor) in a protein (DGPI)", 129.194.185.165/dgpi/; Fankhauser et al., (2005) Bioinformatics 21:1846-1852; Omaetxebarria et al., (2007) Proteomics 7:1951-1960; Pierleoni et al., (2008) BMC Bioinformatics 9:392); including those that are readily available on bioinformatic websites, such as the ExPASy Proteomics tools site (expasy.ch/tools/). Thus, one of skill in the art can determine whether a PH20 polypeptide contains a GPI-anchor attachment signal sequence,

and, therefore, whether the PH20 polypeptide is a GPI-anchored protein.

H. Methods of Treatment and Uses of Extended Soluble PH20 and other Soluble PH20 and Combination Therapy

[0255] Various forms of PH20 hyaluronidases have been prepared and approved for therapeutic use in humans. For example, animal-derived hyaluronidase preparations include Vitrase® (ISTA Pharmaceuticals), a purified ovine testicular hyaluronidase, and Amphadase® (Amphastar Pharmaceuticals), a bovine testicular hyaluronidase. Hylenex® (Halozyme Therapeutics) is a human recombinant hyaluronidase produced by genetically engineered Chinese Hamster Ovary (CHO) cells containing nucleic acid encoding for soluble rHuPH20. Approved therapeutic uses for hyaluronidase include use as an adjuvant to increase the absorption and dispersion of other therapeutic agents, for hypodermoclysis (subcutaneous fluid administration), and as an adjunct in subcutaneous urography for improving resorption of radiopaque agents. In addition to these indications hyaluronidases can be used as a therapeutic or cosmetic agent for the treatment of additional diseases and conditions.

[0256] Hyaluronidases have also been used to enhance the activity of chemotherapeutics and/or the accessibility of tumors to chemotherapeutics (Schuller et al., 1991, Proc. Amer. Assoc. Cancer Res. 32:173, abstract no. 1034; Czejka et al., 1990, Pharmazie 45:H.9). Combination chemotherapy with hyaluronidase is effective in the treatment of a variety of cancers including urinary bladder cancer (Horn et al., 1985, J. Surg. Oncol. 28:304-307), squamous cell carcinoma (Kohno et al., 94, J. Cancer Res. Oncol. 120:293-297), breast cancer (Beckenlehner et al., 1992, J. Cancer Res. Oncol. 118:591-596), and gastrointestinal cancer (Scheithauer et al., 1988, Anticancer Res. 8:391-396). Hyaluronidase is effective as the sole therapeutic agent in the treatment of brain cancer (gliomas) (PCT published application no. WO88/02261, published Apr. 7, 1988). Administration of hyaluronidase also induces responsiveness of previously chemotherapy-resistant tumors of the pancreas, stomach, colon, ovaries, and breast (Baumgartner et al., 1988, Reg. Cancer Treat. 1:55-58; Zanker et al., 1986, Proc. Amer. Assoc. Cancer Res. 27:390). Unfortunately, the contaminants and non human nature of such hyaluronidases result in anaphylactic reactions.

[0257] In addition to its indirect anticancer effects, hyaluronidases also have direct anticarcinogenic effects. Hyaluronidase prevents growth of tumors transplanted into mice (De Maeyer et al., 1992, Int. J. Cancer 51:657-660) and inhibits tumor formation upon exposure to carcinogens (Pawlowski et al., 1979, Int. J. Cancer 23:105-109; Haberman et al., 1981, Proceedings of the 17th Annual Meeting of the American Society of Clinical Oncology, Washington, D.C., 22:105, abstract no. 415).

[0258] In particular, PH20 hyaluronidase can be used to treat hyaluronan-associated diseases or conditions associated with high interstitial fluid pressure, such as disc pressure, proliferative disorders, such as cancer and benign prostatic hyperplasia, and edema. Edema can result from or be manifested in, for example, from organ transplant, stroke or brain trauma. Proliferative disorders include, but are not limited to, cancer, smooth muscle cell proliferation, systemic sclerosis, cirrhosis of the liver, adult respiratory distress syndrome, idiopathic cardiomyopathy, lupus erythematosus, retinopathy, e.g., diabetic retinopathy or other retinopathies, cardiac hyperplasia, reproductive system associated disorders, such as benign prostatic hyperplasia (BPH) and ovarian cysts, pulmonary fibrosis, endometriosis, fibromatosis, harmatomas, lymphangiomatosis, sarcoidosis, desmoid tumors. Cancers include solid and lymphatic/blood tumors and metastatic disease, and undifferentiated tumors. The tumors amenable to treatment typically exhibit cellular and/or stromal expression of a hyaluronan, compared to a non-cancerous tissue of the same tissue type or compared to a non-metastatic tumor of the same tumor-type. Cancers include any one or more of ovarian cancer, in situ carcinoma (ISC), squamous cell carcinoma (SCC), prostate cancer, pancreatic cancer, other gastric cancers, non-small cell lung cancer, breast cancer, brain cancer and colon cancer.

[0259] Hence, PH20 hyaluronidases have multiple uses, including and in addition to their use as a spreading agent. Hyaluronidase is commonly used, for example, for peribulbar block in local anesthesia prior ophthalmic surgery. The presence of the enzyme prevents the need for additional blocks and speeds the time to the onset of akinesia (loss of eye movement). Peribulbar and sub-Tenon's block are the most common applications of hyaluronidase for ophthalmic procedures. Hyaluronidase also can promote akinesia in cosmetic surgery, such as blepharoplasties and face lifts. It is understood that soluble PH20 hyaluronidases provided herein, including esPH20 hyaluronidases, can be used in any method of treatment or combination therapy for which a PH20 hyaluronidase is used (see e.g., U.S. Publication Nos. US20040268425; US20050260186; US20060104968; and U.S. Appl. Serial Nos. 12/381,844, 12/386,249, 12/387,225 and 12/386,222).

Exemplary therapeutic and cosmetic uses for hyaluronidase are described below.

1. Use as a Spreading Agent and Combination Therapy

[0260] As noted above, hyaluronidase is a spreading or diffusing substance which modifies the permeability of connective tissue through the hydrolysis of hyaluronic acid, a polysaccharide found in the intercellular ground substance of connective tissue, and of certain specialized tissues, such as the umbilical cord and vitreous humor. When no spreading

factor is present, materials injected subcutaneously, such as drugs, proteins, peptides and nucleic acid, spread very slowly. Co-injection with hyaluronidase, however, can cause rapid spreading. The rate of diffusion is proportional to the amount of enzyme, and the extent of diffusion is proportional to the volume of solution.

[0261] PH20, including soluble PH20 such as esPH20 provided herein, can be used to promote or enhance the delivery agents and molecules to any of a variety of mammalian tissues *in vivo.* It can be used to facilitate the diffusion and, therefore, promote the delivery, of small molecule pharmacologic agents as well as larger molecule pharmacologic agents, such as proteins, nucleic acids and ribonucleic acids, and macromolecular compositions than can contain a combination of components including, but not limited to, nucleic acids, proteins, carbohydrates, lipids, lipid-based molecules and drugs (see e.g. U.S. Publication Nos. US20040268425; US20050260186; and US20060104968). PH20, including soluble PH20 such as esPH20 can be co-administered and/or co-formulated with a therapeutic agent to improve the bioavailability as well as pharmacokinetic (PK) and/or pharmacodynamic (PD) characteristics of co-formulated or co-administered agents. PK/PD parameters that can be improved by using soluble PH20, such as esPH20, include such measures as C_{max} (the maximal concentration of agent achieved following absorption in, *e.g.,* the bloodstream), T_{max} (the time required to achieve maximal concentration), $T_{1/2}$ (the time required for the concentration to fall by half), C_{min} (the minimal concentration of agent following metabolism and excretion), AUC (area under the curve of concentration versus time, a measure of the overall amount of bioavailability), concentrations in various tissues of interest (including, *e.g.,* the rate of achieving desired concentrations, the overall levels, and the duration of maintaining desired levels), and E_{max} (the maximal effect achieved).

[0262] The methods of treatment provided herein include combination therapies with a therapeutic agent for the treatment of a disease or disorder for which the therapeutic agent threats. Any therapeutic agent that ameliorates and or otherwise lessens the severity of a disease or condition can be combined with a soluble PH20 provided herein in order to increase the bioavailability of such therapeutic agent. In particular, soluble PH20 polypeptides provided herein, such as esPH20s, can be used in each and all of the combinations described in applications see e.g., U.S. Publication Nos. US20040268425; US20050260186; US20060104968 and U.S. Appl. Serial Nos. 12/381,844, 12/386,249, 12/387,225 and 12/386,222 in place of the disclosed hyaluronidase or hyaluronidase degrading enzyme.

[0263] Soluble PH20 polypeptides provided herein, in particular esPH20 polypeptides, can be administered prior, subsequently, intermittently or simultaneously to the therapeutic agent preparation. Generally, the soluble PH20 is administered prior to or simultaneously with administration of the therapeutic agent preparation to permit the soluble PH20 to degrade the hyaluronic acid in the interstitial space. The soluble PH20 can be administered at a site different from the site of administration of the therapeutic molecule or the soluble PH20 can be administered at a site the same as the site of administration of the therapeutic molecule.

[0264] Examples of pharmaceutical, therapeutic and cosmetic agents and molecules that can be administered with hyaluronidase include, but are not limited to, a chemotherapeutic or anticancer agent, an analgesic agent, an antibiotic agent, an anti-inflammatory agent, an antimicrobial agent, an amoebicidal agent, a trichomonocidal agent, an anti-parkinson agent, an anti-malarial agent, an anticonvulsant agent, an anti-depressant agent, an anti-arthritics agent, an anti-fungal agent, an antihypertensive agent, an antipyretic agent, an anti-parasitic agent, an antihistamine agent, an alpha-adrenergic agonist agent, an alpha blocker agent, an anesthetic agent, a bronchial dilator agent, a biocide agent, a bactericide agent, a bacteriostatic agent, a beta adrenergic blocker agent, a calcium channel blocker agent, a cardiovascular drug agent, a contraceptive agent, a cosmetic or esthetic agent, a decongestant agent, a diuretic agent, a depressant agent, a diagnostic agent, an electrolyte agent, a hypnotic agent, a hormone agent, a hyperglycemic agent, a muscle relaxant agent, a muscle contractant agent, an ophthalmic agent, a parasympathomimetic agent, a psychic energizer agent, a sedative agent, a sleep inducer, a sympathomimetic agent, a tranquilizer agent, a urinary agent, a vaginal agent, a viricide agent, a vitamin agent, a non-steroidal anti-inflammatory agent, or an angiotensin converting enzyme inhibitor agent, and any combination thereof. In particular, therapeutic agents include antibodies, including monoclonal antibodies, bisphosphonates, insulins and immunoglobulins.

[0265] For example, exemplary antibiotic agents include, but are not limited to, Aminoglycosides; Amphenicols; Ansamycins; Carbacephems; Carbapenems; Cephalosporins or Cephems; Cephamycins; Clavams; Cyclic lipopeptides; Diaminopyrimidines; Ketolides; Lincosamides; Macrolides; Monobactams; Nitrofurans; Oxacephems; Oxazolidinones; Penems, thienamycins and miscellaneous beta-lactams; Penicillins; Polypeptides antibiotics; Quinolones; Sulfonamides; Sulfones; Tetracyclines; and other antibiotics (such as Clofoctols, Fusidic acids, Hexedines, Methenamines, Nitrofurantoins Nitroxolines, Ritipenems, Taurolidines, Xibomols).

[0266] Also included among exemplary therapeutic agents are blood modifiers such as antihemophilic factors, anti-inhibitor coagulent complexes, antithrombin IIIs, coagulations Factor Vhs, coagulation Factor VIIIs, coagulation Factor IXs, plasma protein fractions, von Willebrand factors; antiplatelet agents (including, for example, abciximabs, anagrelides, cilostazols, clopidogrel bisulfates, dipyridamoles, epoprostenols, eptifibatides, tirofibans; colony stimulating factors (CSFs) (including, for example, Granulocyte CSFs and Granulocyte Macrophage CSFs); erythropoiesis stimulators (including, for example, erythropoietins such as darbepoetin alfas) and epoetin alfas; hemostatics and albumins (including, for example, aprotinins, combinations of antihemophilic factors and plasma, Desmopressin Acetates, and albumins); immune

globulins, as well as hepatitis B immune globulins; thrombin inhibitors (including for example direct thrombin inhibitors and lepirudin), and drotecogin alfas; anticoagulants (including, for example, dalteparins, enoxaperins and other heparins, and warfarins).

[0267] Other exemplary therapeutic agents that can be combined by co-administration and/or co-formulation with a soluble PH20, such as an esPH20, include, but are not limited to, Adalimumabs, Agalsidase Betas, Alefacepts, Ampicillins, Anakinras, Antipoliomyelitic Vaccines, Anti-Thymocytes, Azithromycins, Becaplermins, Caspofungins, Cefazolins, Cefepimes, Cefotetans, Ceftazidimes, Ceftriaxones, Cetuximabs, Cilastatins, Clavulanic Acids, Clindamycins, Darbepoetin Alfas, Deaclizumabs, Diphtheria, Diphtheria antitoxins, Diphtheria Toxoids, Efalizumabs, Epinephrines, Erythropoietin Alphas, Etanercepts, Filgrastims, Fluconazoles, Follicle-Stimulating Hormones, Follitropin Alphas, Follitropin Betas, Fosphenyloins, Gadodiamides, Gadopentetates, Gatifloxacins, Glatiramers, GM-CSF's, Goserelins, Goserelin acetates, Granisetrons, *Haemophilus Influenza* B's, Haloperidols, Hepatitis vaccines, Hepatitis A Vaccines, Hepatitis B Vaccines, Ibritumomab Tiuxetans, Ibritumomabs, Tiuxetans, Immunoglobulins, Hemophilus influenza vaccines, Influenza Virus Vaccines, Infliximabs, Insulins, Insulin Glargines, Interferons, Interferon alphas, Interferon Betas, Interferon Gammas, Interferon alpha-2a's, Interferon alpha-2b's, Interferon alpha-1's, Interferon alpha-n3's, Interferon Betas, Interferon Beta-1a's, Interferon Gammas, Interferon alpha-consensus, Iodixanols, Iohexols, Iopamidols, Ioversols, Ketorolacs, Laronidases, Levofloxacins, Lidocaines, Linezolids, Lorazepams, Measles Vaccines, Measles virus, Mumps viruses, Measles-Mumps-Rubella Virus Vaccines, Rubella vaccines, Medroxyprogesterones, Meropenems, Methylprednisolones, Midazolams, Morphines, Octreotides, Omalizumabs, Ondansetrons, Palivizumabs, Pantoprazoles, Pegaspargases, Pegfilgrastims, Peg-Interferon Alfa-2a's, Peg-Interferon Alfa-2b's, Pegvisomants, Pertussis vaccines, Piperacillins, Pneumococcal Vaccines and Pneumococcal Conjugate Vaccines, Promethazines, Reteplases, Somatropins, Sulbactams, Sumatriptans, Tazobactams, Tenecteplases, Tetanus Purified Toxoids, Ticarcillins, Tositumomabs, Triamcinotones, Triamcinolone Acetonides, Triamcinolone hexacetonides, Vancomycins, Varicella Zoster immunoglobulins, Varicella vaccines, other vaccines, Alemtuzumabs, Alitretinoins, Allopurinols, Altretamines, Amifostines, Anastrozoles, Arsenics, Arsenic Trioxides, Asparaginases, Bacillus Calmette-Guerin (BCG) vaccines, BCG Live, Bexarotenes, Bleomycins, Busulfans, Busulfan intravenous, Busulfan orals, Calusterones, Capecitabines, Carboplatins, Carmustines, Carmustines with Polifeprosans, Celecoxibs, Chlorambucils, Cisplatins, Cladribines, Cyclophosphamides, Cytarabines, Cytarabine liposomals, Dacarbazines, Dactinomycins, Daunorubicin liposomals, Daunorubicins, Daunomycins, Denileukin Diftitoxes, Dexrazoxanes, Docetaxels, Doxorubicins, Doxorubicin liposomals, Dromostanolone propionates, Elliott's B Solutions, Epirubicins, Epoetin alfas, Estramustines, Etoposides, Etoposide phosphates, Etoposide VP-16s, Exemestanes, Floxuridines, Fludarabines, Fluorouracils, 5-Fluorouracils, Fulvestrants, Gemcitabines, Gemtuzumabs, Ozogamicins, Gemtuzumab ozogamicins, Hydroxyureas, Idarubicins, Ifosfamides, Imatinib mesylates, Irinotecans, Letrozoles, Leucovorins, Levamisoles, Lomustines, CCNUs, Mechlorethamines, Nitrogen mustards, Megestrols, Megestrol acetates, Melphalans, L-PAMs, Mercaptopurines, 6-Mercaptopurines, Mesnas, Methotrexates, Methoxsalens, Mitomycins, Mitomycin C's, Mitotanes, Mitoxantrones, Nandrolones, Nandrolone Phenpropionates, Nofetumomabs, Oprelvekins, Oxaliplatins, Paclitaxels, Pamidronates, Pegademases, Pentostatins, Pipobromans, Plicamycins, Mithramycins, Porfimers, Porfimer sodiums, Procarbazines, Quinacrines, Rasburicases, Rituximabs, Sargramostims, Streptozocins, Talcs, Tamoxifens, Temozolomides, Teniposides, Testolactones, Thioguanines, 6-Thioguanines, Triethylenethiophosphoramides (Thiotepas), Topotecans, Toremifenes, Trastuzumabs, Tretinoins, Uracil Mustards, Valrubicins, Vinblastines, Vincristines, Vinorelbines, Zoledronates, Acivicins, Aclarubicins, Acodazoles, Acronines, Adozelesins, Aldesleukins, Retinoic Acids, Alitretinoins, 9-Cis-Retinoic Acids, Alvocidibs, Ambazones, Ambomycins, Ametantrones, Aminoglutethimides, Amsacrines, Anaxirones, Ancitabines, Anthramycins, Apaziquones, Argimesnas, Asperlins, Atrimustines, Azacitidines, Azetepas, Azotomycins, Banoxantrones, Batabulins, Batimastats, Benaxibines, Bendamustines, Benzodepas, Bicalutamides, Bietaserpines, Biricodars, Bisantrenes, Bisnafide Dimesylates, Bizelesins, Bortezomibs, Brequinars, Bropirimines, Budotitanes, Cactinomycins, Canertinibs, Caracemides, Carbetimers, Carboquones, Carmofurs, Carubicins, Carzelesins, Cedefingols, Cemadotins, Chiorambucils, Cioteronels, Cirolemycins, Clanfenurs, Clofarabines, Crisnatols, Decitabines, Dexniguldipines, Dexormaplatins, Dezaguanines, Diaziquones, Dibrospidiums, Dienogests, Dinalins, Disermolides, Dofequidars, Doxifluridines, Droloxifenes, Duazomycins, Ecomustines, Edatrexates, Edotecarins, Eflomithines, Elacridars, Elinafides, Elsamitrucins, Emitefurs, Enloplatins, Enpromates, Enzastaurins, Epipropidines, Eptaloprosts, Erbulozoles, Esorubicins, Etanidazoles, Etoglucids, Etoprines, Exisulinds, Fadrozoles, Fazarabines, Fenretinides, Fluoxymesterones, Flurocitabines, Fosquidones, Fostriecins, Fotretamines, Galarubicins, Galocitabines, Geroquinols, Gimatecans, Gimeracils, Gloxazones, Glufosfamides, Ilmofosines, Ilomastats, Imexons, Improsulfans, Indisulams, Inproquones, Interleukins, Interleukin-2s, recombinant Interleukins, Intoplicines, Iobenguanes, Iproplatins, Irsogladines, Ixabepilones, Ketotrexates, L-Alanosines, Lanreotides, Lapatinibs, Ledoxantrones, Leuprolides, Leuprorelins, Lexacalcitols, Liarozoles, Lobaplatins, Lometrexols, Lonafarnibs, Losoxantrones, Lurtotecans, Mafosfamides, Mannosulfans, Marimastats, Masoprocols, Maytansines, Mechiorethamines, Melengestrols, Meiphalans, Menogarils, Mepitiostanes, Metesinds, Metomidates, Metoprines, Meturedepas, Miboplatins, Miproxifenes, Misonidazoles, Mitindomides, Mitocarcins, Mitocromins, Mitoflaxones, Mitogillins, Mitoguazones, Mitomalcins, Mitonafides, Mitoquidones, Mitospers, Mitozolomides, Mivobulins, Mizoribines, Mofarotenes, Mopidamols, Mubritinibs, Mycophenolic Acids,

Nedaplatins, Neizarabines, Nemorubicins, Nitracrines, Nocodazoles, Nogalamycins, Nolatrexeds, Nortopixantrones, Ormaplatins, Ortataxels, Oteracils, Oxisurans, Oxophenarsines, Patubilones, Peldesines, Peliomycins, Pelitrexols, Pemetrexeds, Pentamustines, Peplomycins, Perfosfamides, Perifosines, Picoplatins, Pinafides, Piposulfans, Pirfenidones, Piroxantrones, Pixantrones, Plevitrexeds, Plomestanes, Porfiromycins, Prednimustines, Propamidines, Prospidiums, Pumitepas, Puromycins, Pyrazofurins, Ranimustines, Riboprines, Ritrosulfans, Rogletimides, Roquinimexs, Rufocromomycins, Sabarubicins, Safingols, Satraplatins, Sebriplatins, Semustines, Simtrazenes, Sizofirans, Sobuzoxanes, Sorafenibs, Sparfosates, Sparfosic Acids, Sparsomycins, Spirogermaniums, Spiromustines, Spiroplatins, Squalamines, Streptonigrins, Streptovarycins, Sufosfamides, Sulofenurs, Tacedinalines, Talisomycins, Tallimustines, Tariquidars, Tauromustines, Tecogalans, Tegafurs, Teloxantrones, Temoporfins, Teroxirones, Thiamiprines, Tiamiprines, Tiazofurins, Tilomisoles, Tilorones, Timcodars, Timonacics, Tirapazamines, Topixantrones, Trabectedins, Ecteinascidin 743, Trestolones, Triciribines, Trilostanes, Trimetrexates, Triplatin Tetranitrates, Triptorelins, Trofosfarnides, Tubulozoles, Ubenimexs, Uredepas, Vaispodars, Vapreotides, Verteporfins, Vinbiastines, Vindesines, Vinepidines, Vinflunines, Vinformides, Vinglycinates, Vinleucinols, Vinleurosines, Vinrosidines, Vintriptols, Vinzolidines, Vorozoles, Xanthomycin A's, Guamecyclines, Zeniplatins, Zilascorbs [2-H], Zinostatins, Zorubicins, Zosuquidars, Acetazolamides, Acyclovirs, Adipiodones, Alatrofloxacins, Alfentanils, Allergenic extracts, Alpha 1-proteinase inhibitors, Aiprostadils, Amikacins, Amino acids, Aminocaproic acids, Aminophyllines, Amitriptylines, Amobarbitals, Amrinones, Analgesics, Anti-poliomyelitic vaccines, Anti-rabic serums, Anti-tetanus immunoglobulins, tetanus vaccines, Antithrombin III's, Antivenom serums, Argatrobans, Arginines, Ascorbic acids, Atenolols, Atracuriums, Atropines, Aurothioglucoses, Azathioprines, Aztreonams, Bacitracins, Baclofens, Basiliximabs, Benzoic acids, Benztropines, Betamethasones, Biotins, Bivalirudins, Botulism antitoxins, Bretyliums, Bumetanides, Bupivacaines, Buprenorphines, Butorphanols, Calcitonins, Calcitriols, Calciums, Capreomycins, Carboprosts, Carnitines, Cefaniandoles, Cefoperazones, Cefotaximes, Cefoxitins, Ceftizoximes, Cefuroximes, Chioramphenicols, Chioroprocaines, Chioroquines, Chlorothiazides, Chiorpromazines, Chondroitinsulfuric acids, Choriogonadotropin alfas, Chromiums, Cidofovirs, Cimetidines, Ciprofloxacins, Cisatracuriums, Clonidines, Codeines, Coichicines, Colistins, Collagens, Corticorelin ovine triflutates, Corticotrophins, Cosyntropins, Cyanocobalamins, Cyclosporines, Cysteines, Dacliximabs, Dalfopristins, Dalteparins, Danaparoids, Dantrolenes, Deferoxamines, Desmopressins, Dexamethasones, Dexmedetomidines, Dexpanthenols, Dextrans, Iron dextrans, Diatrizoic acids, Diazepams, Diazoxides, Dicyclomines, Digibinds, Digoxins, Dihydroergotamines, Diltiazems, Diphenhydramines, Dipyridamoles, Dobutamines, Dopamines, Doxacuriums, Doxaprams, Doxercalciferols, Doxycyclines, Droperidols,Dyphyllines, Edetic acids, Edrophoniums, Enalaprilats, Ephedrines, Epoprostenols, Ergocalciferols, Ergonovines, Ertapenems, Erythromycins, Esmolols, Estradiols, Estrogenics, Ethacrynic acids, Ethanolamines, Ethanols, Ethiodized oils, Etidronic acids, Etomidates, Factor VIII's, Famotidines, Fenoldopams, Fentanyls, Flumazenils, Fluoresceins, Fluphenazines, Folic acids, Fomepizoles, Fomivirsens, Fondaparinuxs, Foscarnets, Fosphenytoins, Furosemides, Gadoteridols, Gadoversetamides, Ganciclovirs, Gentamicins, Glucagons, Glucoses, Glycines, Glycopyrrolates, Gonadorelins, Gonadotropin chorionics, Haemophilus B polysaccarides, Hemins, Herbals, Histamines, Hydralazines, Hydrocortisones, Hydromorphones, Hydroxocobalamins, Hydroxyzines, Hyoscyamines, Ibutilides, Imiglucerases, Indigo carmines, Indomethacins, Iodides, Iopromides, Iothalamic acids, Ioxaglic acids, Ioxilans, Isoniazids, Isoproterenols, Japanese encephalitis vaccines, Kanamycins, Ketamines, Labetalols, Lepirudins, Levobupivacaines, Levothyroxines, Lincomycins, Liothyronines, Luteinising hormones, Lyme disease vaccines, Mangafodipirs, Manthtols, Meningococcal polysaccharide vaccines, Meperidines, Mepivacaines, Mesoridazines, Metaraminols, Methadones, Methocarbamols, Methohexitals, Methyldopates, Methylergonovines, Metoclopramides, Metoprolols, Metronidazoles, Minocyclines, Mivacuriums, Morrhuic acids, Moxifloxacins, Muromonab-CD3s, Mycophenolate mofetils, Nafcillins, Nalbuphines, Nalmefenes, Naloxones, Neostigmines, Niacinamides, Nicardipines, Nitroglycerins, Nitroprussides, Norepinephrines, Orphenadrines, Oxacillins, Oxymorphones, Oxytetracyclines, Oxytocins, Pancuroniums, Panthenols, Pantothenic acids, Papaverines, Peginterferon-alpha (e.g. interferon alpha 2a or 2b), Penicillin Gs, Pentamidines, Pentazocines, Pentobarbitals, Perfiutrens, Perphenazines, Phenobarbitals, Phentolamines, Phenylephrines, Phenytoins, Physostigmines, Phytonadiones, Polymyxin bs, Pralidoximes, Prilocaines, Procainamides, Procaines, Prochiorperazines, Progesterones, Propranolols, Pyridostigmine hydroxides, Pyridoxines, Quinidines, Quinupristins, Rabies immunoglobulins, Rabies vaccines, Ranitidines, Remifentanils, Riboflavins, Rifampins, Ropivacaines, Samariums, Scopolamines, Seleniums, Sermorelins, Sincalides, Somatrems, Spectinomycins, Streptokinases, Streptomycins, Succinylcholines, Sufentanils, Sulfamethoxazoles, Tacrolirnuss, Terbutalines, Teriparatides, Testosterones, Tetanus antitoxins, Tetracaines, Tetradecyl sulfates, Theophyllines, Thiamines, Thiethylperazines, Thiopentals, Thyroid stimulating hormones, Tinzaparins, Tirofibans, Tobramycins, Tolazolines, Tolbutamides, Torsemides, Tranexamic acids, Treprostinils, Trifluoperazines, Trimethobenzamides, Trimethoprims, Tromethamines, Tuberculins, Typhoid vaccines, Urofollitropins, Urokinases, Vaiproic acids, Vasopressins, Vecuroniums, Verapamils, Voriconazoles, Warfarins, Yellow fever vaccines, Zidovudines, Zincs, Ziprasidone hydrochiorides, Aclacinomycins, Actinomycins, Adriamycins, Azaserines, 6-Azauridines, Carzinophilins, Chromomycins, Denopterins, 6-Diazo-5-Oxo-L-Norleucines, Enocitabines, Loxuridines, Olivomycines, Pirarubicins, Piritreximes, Pteropterins, Tagafurs, Tubercidins, Alteplases, Arcitumomabs, bevacizumabs, Botulinum Toxin Type A's, Botulinum Toxin Type B's, Capromab Pendetides, Daclizumabs, Dornase alfas, Drotrecogin alfas, Imciromab Pentetates, and Iodine-131's.

[0268] In particular, therapeutic agents include, but are not limited to, immunoglobulins, Interferon beta, Interferon alpha-2as, Interferon alpha-Is, Interferon alpha-n3s, Interferon beta-1, Interferon beta-las, Interferon gamma-lbs, Peg-interferon alpha-2 and Peginterferon alpha-2bs, insulin, a bisphosphate (e.g. Pamidronates or Zoledronates), Docetaxels, Doxorubincins, Doxorubicin liposomals and bevacizumabs.

2. Use to Remove Excess Glycosaminoglycanases

[0269] Provided herein are methods for treating hyaluronan-associated diseases and conditions by administration of a composition containing a soluble PH20, typically a soluble hyaluronidase either alone or in combination with or in addition to another treatment and/or agent. Hyaluronan-associated conditions and diseases are diseases and conditions in which hyaluronan levels are elevated as cause, consequence or otherwise observed in the disease or condition, and can be treated by administration of a composition hyaluronidases, such as a soluble PH20, either alone or in combination with or in addition to another treatment and/or agent.

[0270] Typically, hyaluronan-associated diseases and conditions are associated with elevated hyaluronan expression in a tissue, cell, or body fluid (e.g. tumor tissue or tumor-associated tissue, blood, or interstitial space) compared to a control, e.g. another tissue, cell or body fluid. The elevated hyaluronan expression can be elevated compared to a normal tissue, cell or body fluid, for example, a tissue, cell or body fluid that is analogous to the sample being tested, but isolated from a different subject, such as a subject that is normal (i.e. does not have a disease or condition, or does not have the type of disease or condition that the subject being tested has), for example, a subject that does not have a hyaluronan-associated disease or condition. The elevated hyaluronan expression can be elevated compared to an analogous tissue from another subject that has a similar disease or condition, but whose disease is not as severe and/or is not hyaluronan-associated or expresses relatively less hyaluronan and thus is hyaluronan-associated to a lesser degree. For example, the subject being tested can be a subject with a hyaluronan-associated cancer, where the HA amounts in the tissue, cell or fluid are relatively elevated compared to a subject having a less severe cancer, such as an early stage, differentiated or other type of cancer. In another example, the cell, tissue or fluid contains elevated levels of hyaluronan compared to a control sample, such as a fluid, tissue, extract (e.g. cellular or nuclear extract), nucleic acid or peptide preparation, cell line, biopsy, standard or other sample, with a known amount or relative amount of HA, such as a sample, for example a tumor cell line, known to express relatively low levels of HA, such as exemplary tumor cell lines described herein that express low levels of HA, for example, the HCT 116 cell line, the HT29 cell line, the NCI H460 cell line, the DU145 cell line, the Capan-1 cell line, and tumors from tumor models generated using such cell lines.

[0271] In some cases, hyaluronan-associated diseases and conditions are associated with increased interstitial fluid pressure, decreased vascular volume, and/or increased water content in a tissue, such as a tumor. In one example, treatment with the compositions and compounds provided herein ameliorates one or more of these symptoms or other symptoms associated with the disease or condition, for example, improves survival or quality of life of the subject over time, or inhibits tumor growth.

[0272] Exemplary hyaluronan-associated diseases and conditions that can be treated using the provided enzymes, compositions and methods, include, but are not limited to, hyaluronan-rich cancers, for example, tumors, including solid tumors such as late-stage cancers, a metastatic cancers, undifferentiated cancers, ovarian cancer, in situ carcinoma (ISC), squamous cell carcinoma (SCC), prostate cancer, pancreatic cancer, non-small cell lung cancer, breast cancer, colon cancer and other cancers.

[0273] Also exemplary of hyaluronan-associated diseases and conditions are diseases that are associated with elevated interstitial fluid pressure, such as diseases associated with disc pressure, and edema, for example, edema caused by organ transplant, stroke, brain trauma or other injury. Exemplary hyaluronan-associated diseases and conditions include diseases and conditions associated with elevated interstitial fluid pressure, decreased vascular volume, and/or increased water content in a tissue, including cancers, disc pressure and edema. In one example, treatment of the hyaluronan-associated condition, disease or disorder includes amelioration, reduction, or other beneficial effect on one or more of increased interstitial fluid pressure (IFP), decreased vascular volume, and increased water content in a tissue.

[0274] Typically, the hyaluronan-associated disease or condition is associated with increased HA expression, for example, in a diseased tissue, for example, a tumor. In one example, HALOs (pericellular matrix regions that are rich in proteoglycans, including hyaluronan) form in a tissue of the subject, for example, in a diseased tissue. In another example, the presence of HALOs is detected in an in vitro culture of cells from a tissue of the subject, for example, a diseased tissue.

a. Use in cancer treatment

[0275] Hyaluronidase has direct anticarcinogenic effects by degradation of hyaluronic acid in tumors. Thus, soluble PH20 hyaluronidases, such as esPH20, can be used to treat tumors, in particular, tumors that are hyaluronan rich. The hyaluronan-rich cancer can be a cancer in which the cancer cells produce HALOs, cancers that have elevated expression

of hyaluronan (as determined by immunostaining, e.g. histological staining of sections from the tumor), cancers that have elevated HAS2 (Hyaluronan synthase 2), cancers that do not produce hyaluronidase (HYAL1) *in vitro*. Hyaluronan-rich cancers can be identified by any method for assessing hyaluronan expression, and other known methods for assaying protein/mRNA expression.

[0276] Several hyaluronan-rich cancers have been identified. In some cases, hyaluronan expression correlates with poor prognosis, for example, decreased survival rate and/or recurrence-free survival rate, metastases, angiogenesis, cancer cell invasion into other tissues/areas, and other indicators of poor prognosis. Such correlation has been observed, for example, in hyaluronan-rich tumors including ovarian cancer, SCC, ISC, prostate cancer, lung cancer, including non-small-cell lung cancer (NSCLC), breast cancer, colon cancer and pancreatic cancer (see, for example, Maarit et al., Cancer Research, 60:150-155 (2000); Karvinen et al., British Journal of Dermatology, 148:86-94 (2003); Lipponen et al., Eur. Journal of Cancer, 849-856 (2001); Pirinen et al., Int. J. Cancer: 95: 12-17 (2001); Auvinen et al., American Journal of Pathology, 156(2):529-536 (2000); Ropponen et al., Cancer Research, 58: 342-347 (1998)). Thus, hyaluronan-rich cancers can be treated by administration of a hyaluronidase, such as a soluble PH20, to treat one or more symptoms of the cancer. Hyaluronan-rich tumors include, but are not limited to, prostate, breast, colon, ovarian, stomach, head and neck and other tumors and cancers.

[0277] Hyaluronidases can also be used to increase the sensitivity of tumors that are resistant to conventional chemotherapy. For example, a hyaluronidase, such as soluble PH20, can be administered to a patient having a tumor associated with a HYAL1 defect in an amount effective to increase diffusion around the tumor site (e.g., to facilitate circulation and/or concentrations of chemotherapeutic agents in and around the tumor site), inhibit tumor cell motility, such as by hyaluronic acid degradation, and/or to lower the tumor cell apoptosis threshold. This can bring the tumor cell(s) to a state of anoikis, which renders the tumor cell more susceptible to the action of chemotherapeutic agents. Administration of a hyaluronidase can induce responsiveness of previously chemotherapy-resistant tumors of the pancreas, stomach, colon, ovaries, and breast (Baumgartner et al. (1988) Reg. Cancer Treat. 1:55-58; Zanker et al. (1986) Proc. Amer. Assoc. Cancer Res. 27:390). Thus, in addition to treatment of a cancer with a soluble PH20 alone, the compositions and methods provided herein also can be used to treat hyaluronan-associated cancers by administration of a soluble PH20 in combination with, for example, simultaneously or prior to, a chemotherapeutic or other anti-cancer agent or treatment. In this example, the hyaluronidase, such as a soluble PH20, typically enhances penetration of chemotherapeutic or other anti-cancer agents into solid tumors, thereby treating the disease.

[0278] Compositions containing soluble PH20 can be injected intratumorally with anti-cancer agents or intravenously for disseminated cancers or hard to reach tumors. The anticancer agent can be a chemotherapeutic, an antibody, a peptide, or a gene therapy vector, virus or DNA. Additionally, hyaluronidase can be used to recruit tumor cells into the cycling pool for sensitization in previously chemorefractory tumors that have acquired multiple drug resistance (St Croix et al., (1998) Cancer Lett September 131(1): 35-44).

[0279] Exemplary anti-cancer agents that can be administered after, coincident with or before administration of a soluble PH20, such as an esPH20, include, but are not limited to Acivicins; Aclarubicins; Acodazoles; Acronines; Adozelesins; Aldesleukins; Alemtuzumabs; Alitretinoins (9-Cis-Retinoic Acids); Allopurinols; Altretamines; Alvocidibs; Ambazones; Ambomycins; Ametantrones; Amifostines; Aminoglutethimides; Amsacrines; Anastrozoles; Anaxirones; Ancitabines; Anthramycins; Apaziquones; Argimesnas; Arsenic Trioxides; Asparaginases; Asperlins; Atrimustines; Azacitidines; Azetepas; Azotomycins; Banoxantrones; Batabulins; Batimastats; BCG Live; Benaxibines; Bendamustines; Benzodepas; Bexarotenes; Bevacizumab; Bicalutamides; Bietaserpines; Biricodars; Bisantrenes; Bisantrenes; Bisnafide Dimesylates; Bizelesins; Bleomycins; Bortezomibs; Brequinars; Bropirimines; Budotitanes; Busulfans; Cactinomycins; Calusterones; Canertinibs; Capecitabines; Caracemides; Carbetimers; Carboplatins; Carboquones; Carmofurs; Carmustines with Polifeprosans; Carmustines; Carubicins; Carzelesins; Cedefingols; Celecoxibs; Cemadotins; Chlorambucils; Cioteronels; Cirolemycins; Cisplatins; Cladribines; Clanfenurs; Clofarabines; Crisnatols; Cyclophosphamides; Cytarabine liposomals; Cytarabines; Dacarbazines; Dactinomycins; Darbepoetin Alfas; Daunorubicin liposomals; Daunorubicins/Daunomycins; Daunorubicins; Decitabines; Denileukin Diftitoxes; Dexniguldipines; Dexonnaplatins; Dexrazoxanes; Dezaguanines; Diaziquones; Dibrospidiums; Dienogests; Dinalins; Disermolides; Docetaxels; Dofequidars; Doxifluridines; Doxorubicin liposomals; Doxorubicin HCL; Docorubicin HCL liposome injection; Doxorubicins; Droloxifenes; Dromostanolone Propionates; Duazomycins; Ecomustines; Edatrexates; Edotecarins; Eflornithines; Elacridars; Elinafides; Elliott's B Solutions; Elsamitrucins; Emitefurs; Enloplatins; Enpromates; Enzastaurins; Epipropidines; Epirubicins; Epoetin alfas; Eptaloprosts; Erbulozoles; Esorubicins; Estramustines; Etanidazoles; Etoglucids; Etoposide phosphates; Etoposide VP-16s; Etoposides; Etoprines; Exemestanes; Exisulinds; Fadrozoles; Fazarabines; Fenretinides; Filgrastims; Floxuridines; Fludarabines; Fluorouracils; 5-fluorouracils; Fluoxymesterones; Flurocitabines; Fosquidones; Fostriecins; Fostriecins; Fotretamines; Fulvestrants; Galarubicins; Galocitabines; Gemcitabines; Gemtuzumabs/Ozogamicins; Geroquinols; Gimatecans; Gimeracils; Gloxazones; Glufosfamides; Goserelin acetates; Hydroxyureas; Ibritumomabs/Tiuxetans; Idarubicins; Ifosfamides; Ilmofosines; Ilomastats; Imatinib mesylates; Imexons; Improsulfans; Indisulams; Inproquones; Interferon alfa-2as; Interferon alfa-2bs; Interferon Alfas; Interferon Betas; Interferon Gammas; Interferons; Interleukin-2s and other Interleukins (including recombinant Interleukins); Intoplicines; Ioben-

guanes [131-1]; Iproplatins; Irinotecans; Irsogladines; Ixabepilones; Ketotrexates; L-Alanosines; Lanreotides; Lapatinibs; Ledoxantrones; Letrozoles; Leucovorins; Leuprolides; Leuprorelins (Leuprorelides); Levamisoles; Lexacalcitols; Liarozoles; Lobaplatins; Lometrexols; Lomustines/CCNUs; Lomustines; Lonafarnibs; Losoxantrones; Lurtotecans; Mafosfamides; Mannosulfans; Marimastats; Masoprocols; Maytansines; Mechlorethamines; Mechlorethamines/Nitrogen mustards; Megestrol acetates; Megestrols; Melengestrols; Melphalans; MelphalansIL-PAMs; Menogarils; Mepitiostanes; Mercaptopurines; 6-Mecaptopurine; Mesnas; Metesinds; Methotrexates; Methoxsalens; Metomidates; Metoprines; Meturedepas; Miboplatins; Miproxifenes; Misonidazoles; Mitindomides; Mitocarcins; Mitocromins; Mitoflaxones; Mitogillins; Mitoguazones; Mitomalcins; Mitomycin Cs; Mitomycins; Mitonafides; Mitoquidones; Mitospers; Mitotanes; Mitoxantrones; Mitozolomides; Mivobulins; Mizoribines; Mofarotenes; Mopidamols; Mubritinibs; Mycophenolic Acids; Nandrolone Phenpropionates; Nedaplatins; Nelzarabines; Nemorubicins; Nitracrines; Nocodazoles; Nofetumomabs; Nogalamycins; Nolatrexeds; Nortopixantrones; Octreotides; Oprelvekins; Ormaplatins; Ortataxels; Oteracils; Oxaliplatins; Oxisurans; Oxophenarsines; Paclitaxels; Pamidronates; Patubilones; Pegademases; Pegaspargases; Pegfilgrastims; Peldesines; Peliomycins; Pelitrexols; Pemetrexeds; Pentamustines; Pentostatins; Peplomycins; Perfosfamides; Perifosines; Picoplatins; Pinafides; Pipobromans; Piposulfans; Pirfenidones; Piroxantrones; Pixantrones; Plevitrexeds; Plicamycid Mithramycins; Plicamycins; Plomestanes; Plomestanes; Porfimer sodiums; Porfimers; Porfiromycins; Prednimustines; Procarbazines; Propamidines; Prospidiums; Pumitepas; Puromycins; Pyrazofurins; Quinacrines; Ranimustines; Rasburicases; Riboprines; Ritrosulfans; Rituximabs; Rogletimides; Roquinimexs; Rufocromomycins; Sabarubicins; Safingols; Sargramostims; Satraplatins; Sebriplatins; Semustines; Simtrazenes; Sizofirans; Sobuzoxanes; Sorafenibs; Sparfosates; Sparfosic Acids; Sparsomycins; Spirogermaniums; Spiromustines; Spiroplatins; Spiroplatins; Squalamines; Streptonigrins; Streptovarycins; Streptozocins; Sufosfamides; Sulofenurs; Sunitinib Malate; 6-TG; Tacedinalines; Talcs; Talisomycins; Tallimustines; Tamoxifens; Tariquidars; Tauromustines; Tecogalans; Tegafurs; Teloxantrones; Temoporfins; Temozolomides; Teniposides/VM-26s; Teniposides; Teroxirones; Testolactones; Thiamiprines; Thioguanines; Thiotepas; Tiamiprines; Tiazofurins; Tilomisoles; Tilorones; Timcodars; Timonacics; Tirapazamines; Topixantrones; Topotecans; Toremifenes; Tositumomabs; Trabectedins (Ecteinascidin 743); Trastuzumabs; Trestolones; Tretinoins/ATRA; Triciribines; Trilostanes; Trimetrexates; Triplatin Tetranitrates; Triptorelins; Trofosfamides; Tubulozoles; Ubenimexs; Uracil Mustards; Uredepas; Valrubicins; Valspodars; Vapreotides; Verteporfins; Vinblastines; Vincristines; Vindesines; Vinepidines; Vinflunines; Vinformides; Vinglycinates; Vinleucinols; Vinleurosines; Vinorelbines; Vinrosidines; Vintriptols; Vinzolidines; Vorozoles; Xanthomycin A's (Guamecyclines); Zeniplatins; Zilascorbs [2-H]; Zinostatins; Zoledronate; Zorubicins; and Zosuquidars, for example:

Aldesleukins (e.g. PROLEUKIN®); Alemtuzumabs (e.g. CAMPATH®); Alitretinoins (e.g. PANRETIN®); Allopurinols (e.g. ZYLOPRIM®); Altretamines (e.g. HEXALEN®); Amifostines (e.g. ETHYOL®); Anastrozoles (e.g. ARIMIDEX®); Arsenic Trioxides (e.g. TRISENOX®); Asparaginases (e.g. ELSPAR®); BCG Live (e.g. TICE® BCG); Bexarotenes (e.g. TARGRETIN®); Bevacizumab (AVASTIN®); Bleomycins (e.g. BLENOXANE®); Busulfan intravenous (e.g. BUSULFEX®); Busulfan orals (e.g. MYLERAN™); Calusterones (e.g. METHOSARB®); Capecitabines (e.g. XELODA®); Carboplatins (e.g. PARAPLATIN®); Carmustines (e.g. BCNU®, BiCNU®); Carmustines with Polifeprosans (e.g. GLIADEL® Wafer); Celecoxibs (e.g. CELEBREX®); Chlorambucils (e.g. LEUKERAN®); Cisplatins (e.g. PLATINOL®); Cladribines (e.g. LEUSTATIN®, 2-CdA®); Cyclophosphamides (e.g. CYTOXAN®, NEOSAR®); Cytarabines (e.g. CYTOSAR-U®); Cytarabine liposomals (e.g. DepoCyt®); Dacarbazines (e.g. DTIC-Domeυ): Dactinomycins (e.g. COSMEGEN®); Darbepoetin Alfas (e.g. ARANESP®); Daunorubicin liposomals (e. g. DANUOXOME®); Daunorubicins/Daunomycins (e.g. CERUBIDINE®); Denileukin Diftitoxes (e.g. ONTAK®); Dexrazoxanes (e.g. ZINECARD®); Docetaxels (e.g. TAXOTERE®); Doxorubicins (e.g. ADRIAMYCIN®, RUBEX®); Doxorubicin liposomals, including Docorubicin HCL liposome injections (e.g. DOXIL®); Dromostanolone propionates (e.g. DROMOSTANOLONE® and MASTERONE® Injection); Elliott's B Solutions (e.g. Elliott's B Solution®); Epirubicins (e.g. ELLENCE®); Epoetin alfas (e.g. EPOGEN®); Estramustines (e.g. EMCYT®); Etoposide phosphates (e.g. ETOPOPHOS®); Etoposide VP-16s (e.g. VEPESID®); Exemestanes (e.g. AROMASIN®); Filgrastims (e.g. NEUPOGEN®); Floxuridines (e.g. FUDR®); Fludarabines (e.g. FLUDARA®); Fluorouracils incl. 5-FUs (e.g. ADRUCIL®); Fulvestrants (e.g. FASLODEX®); Gemcitabines (e.g. GEMZAR®); Gemtuzumabs/Ozogamicins (e.g. MYLOTARG®); Goserelin acetates (e.g. ZOLADEX®); Hydroxyureas (e.g. HYDREA®); Ibritumomabs/Tiuxetans (e.g. ZEVALIN®); Idarubicins (e.g. IDAMYCIN®); Ifosfamides (e.g. IFEX®); Imatinib mesylates (e.g. GLEEVEC®); Interferon alfa-2as (e.g. ROFERON-A®); Interferon alfa-2bs (e.g. INTRON A®); Irinotecans (e.g. CAMPTOSAR®); Letrozoles (e.g. FEMARA®); Leucovorins (e.g. WELLCOVORIN®, LEUCOVORIN®); Levamisoles (e.g. ERGAMISOL®); Lomustines/CCNUs (e.g. CeeBU®); Mechlorethamines/Nitrogen mustards (e.g. MUSTARGEN®); Megestrol acetates (e.g. MEGACE®); Melphalans/L-PAMs (e.g. ALKERAN®); Mercaptopurine incl. 6-MPs (e.g. PURINETHOL®); Mesnas (e.g. MESNEX®); Methotrexates; Methoxsalens (e.g. UVADEX®); Mitomycin Cs (e.g. MUTAMYCIN®, MITOZYTREX®); Mitotanes (e.g. LYSODREN®); Mitoxantrones (e.g. NOVANTRONE®); Nandrolone Phenpropionates (e.g. DURABOLIN-50®); Nofetumomabs (e.g. VERLUMA®); Oprelvekins (e.g. NEUMEGA®); Oxaliplatins (e.g. ELOXATIN®); Paclitaxels (e.g. PAXENE®, TAXOL®); Pamidronates (e.g. AREDIA®); Pegademases (e.g. ADAGEN®); Pegaspargases (e.g. ONCASPAR®); Pegfilgrastims (e.g. NEULASTA®); Pentostatins (e.g. NIPENT®); Pipobromans (e.g. VERCYTE®); Plicamycin/Mithramycins (e.g. MITHRACIN®); Porfimer sodiums (e.g. PHOTOFRIN®); Procar-

bazines (e.g. MATULANE®); Quinacrines (e.g. ATABRINE®); Rasburicases (e.g. ELITEK®); Rituximabs (e.g. RITUXAN®); Sargramostims (e.g. PROKINE®); Streptozocins (e.g. ZANOSAR®); Sunitinib Malates (e.g. SUTENT®); Talcs (e.g. SCLEROSOL®); Tamoxifens (e.g. NOLVADEX®); Temozolomides (e.g. TEMODAR®); Teniposides/VM-26s (e.g. VUMON®); Testolactones (e.g. TESLAC®); Thioguanines incl. 6-TG; Thiotepas (e.g. THIOPLEX®); Topotecans (e.g. HYCAMTIN®); Toremifenes (e.g. FARESTON®); Tositumomabs (e.g. BEXXAR®); Trastuzumabs (e.g. HERCEPTIN®); Tretinoins/ATRA (e.g. VESANOID®); Uracil Mustards; Valrubicins (e.g. VALSTAR®); Vinblastines (e.g. VELBAN®); Vincristines (e.g. ONCOVIN®); Vinorelbines (e.g. NAVELBINE®); and Zoledronates (e.g. ZOMETA®).

[0280] In one example, a soluble PH20, such as an esPH20, for example, PEGylated rHuPH20, is administered to a subject after, coincident with or before administration of one or more of docetaxel (e.g. TAXOTERE®), Doxorubicin liposomal (e.g. DOXIL®), Sunitinib Malate (e.g. SUTENT®) or Bevacizumab (AVASTIN®).

[0281] Hence, soluble PH20 polypeptides provided herein can be used in the treatment of metastatic and non-metastatic cancers, including those that have decreased endogenous hyaluronidase activity relative to non-cancerous cells. Hyaluronidases can be used as a chemotherapeutic agent alone or in combination with other chemotherapeutics. Exemplary cancers include, but are not limited to, small lung cell carcinoma, squamous lung cell carcinoma, and cancers of the breast, ovaries, head and neck, or any other cancer associated with depressed levels of hyaluronidase activity or decreased hyaluronic acid catabolism.

b. Use in treatment of glycosaminoglycan accumulation in the brain

[0282] Hyaluronic acid levels are elevated in a number of cerebrospinal pathologic conditions. Levels of cerebrospinal hyaluronic acid are normally less than 200 μg/L in adults (Laurent et al. (1996) Acta Neurol Scand September 94(3):194-206), but can elevate to levels of over 8000 μg/L in diseases such as meningitis, spinal stenosis, head injury and cerebral infarction. Hyaluronidases, such as, for example, soluble rHuPH20, can be utilized to degrade critically élevated levels of substrate.

[0283] The lack of effective lymphatics in the brain also can lead to life threatening edema following head trauma. Hyaluronic acid accumulation is a result of increased synthesis by hyaluronic acid synthases and decreased degradation. Accumulation of hyaluronic acid can initially serve the beneficial purpose of increasing water content in the damaged tissue to facilitate leukocyte extravasation, but continued accumulation can be lethal. Administration of hyaluronidase, such as intrathecally or intravenously, to a patient suffering from head trauma can serve to remove tissue hyaluronic acid accumulation and the water associated with it.

[0284] Soluble PH20 also can be used in the treatment of edema associated with brain tumors, particularly that associated with glioblastoma multiform. The edema associated with brain tumors results from the accumulation of hyaluronic acid in the non-cancerous portions of the brain adjacent the tumor. Administration of a soluble PH20 hyaluronidase to the sites of hyaluronic acid accumulation (e.g., by intravenous injection or via a shunt) can relieve the edema associated with such malignancies by degrading the excess hyaluronic acid at these sites.

c. Use in treatment of glycosaminoglycan accumulation in cardiovascular disease

[0285] Soluble PH20 hyaluronidases can be used in the treatment of some cardiovascular disease. Administration of hyaluronidase in animal models following experimental myocardial infarct can reduce infarct size (Maclean, et al (1976) Science 194(4261):199-200). One proposed mechanism by which this can occur is by reducing hyaluronic acid accumulation that occurs following ischemia reperfusion. Reduction of infarct size is believed to occur from increased lymph drainage and increased tissue oxygenation and reduction of myocardial water content.

[0286] Soluble PH20 hyaluronidases also can be used to limit coronary plaques from arteriosclerosis. Such plaques accumulate glycosaminoglycans and mediate macrophage and foam cell adhesion (Kolodgie et al. (2002) Arterioscler Thromb Vasc Biol. 22(10):1642-8).

d. Use in vitrectomy and ophthalmic disorders and conditions

[0287] Hyaluronidase, such as a soluble PH20, can be used to minimize the detachment or tearing of the retina during vitrectomy. This could cause, for example, the vitreous body to become uncoupled or "disinserted" from the retina, prior to removal of the vitreous body. Such disinsertion or uncoupling of the vitreous body can minimize the likelihood that further tearing or detachment of the retina will occur as the vitreous body is removed.

[0288] Hyaluronidase, such as a soluble PH20, can be used for various ophthalmic applications, including the vitrectomy adjunct application described in U.S. Pat. No. 5,292,509. The use of a highly purified hyaluronidase, such as, for example, soluble PH20 provided herein, is preferable for intraocular procedures to minimize immunogenicity and toxicity.

[0289] Soluble PH20 hyaluronidases can be used to treat and/or prevent ophthalmic disorders by, for example, preventing neovascularization and increasing the rate of clearance from the vitreous of materials toxic to the retina. A soluble

PH20 hyaluronidase can be administered in an amount effective to liquefy the vitreous humor of the eye without causing toxic damage to the eye. Liquefaction of the vitreous humor increases the rate of liquid exchange from the vitreal chamber. This increase in exchange removes the contaminating materials whose presence can cause ophthalmologic and retinal damage.

[0290] Soluble PH20 hyaluronidases also can be used to reduce postoperative pressure. Hyaluronic acid has been used in eye primarily as a spacer during cataract and intraocular lens surgical procedures. It also is used in other ocular surgical procedures such as glaucoma, vitreous and retina surgery and in corneal transplantation. A common side effect occurring in postoperative cataract patients is a significant early, and occasionally prolonged, rise in intraocular pressure. Such a condition is sometimes serious, especially in patients with glaucomatous optic disc changes. Hyaluronidase, such as soluble PH20, can be co-administered with hyaluronic acid to the eye prior to surgery to reduce postoperative pressure in the eye. The hyaluronidase is administered in an amount effective to reduce the intraocular pressure to pre-operative levels by breaking down the hyaluronic acid without decreasing its effectiveness during surgery nor causing side effects in the patient (U.S. Patent No. 6,745,776).

[0291] Soluble PH20 hyaluronidases also can be administered to patients with glaucoma to remove glycosaminoglycans from the trabecular meshwork and reduce intraocular pressure, and can be applied to the vitreous to promote the resolution of vitreous hemorrhages (i.e. extravasation of blood into the vitreous), which can occur in connection with conditions such as diabetic retinopathy, retinal neovascularization, retinal vein occlusion, posterior vitreous detachment, retinal tears, ocular traumas and the like. The presence of vitreous hemorrhages, which are typically slow to resolve, can delay, complicate or prevent procedures that require the retina to be visualized through the vitreous for diagnosis and/or for treatment procedures such as laser photocoagulation and the like which are often primary treatments for conditions such as proliferative diabetic retinopathy.

e. Use in hypodermocyclsis

[0292] Hypodermoclysis, the infusion of fluids and electrolytes into the hypodermis of the skin, is a useful and simple hydration technique suitable for mildly to moderately dehydrated adult patients, especially the elderly. Although considered safe and effective, the most frequent adverse effect is mild subcutaneous edema that can be treated by local massage or systemic diuretics. Approximately 3 L can be given in a 24-hour period at two separate sites. Common infusion sites include the chest, abdomen, thighs and upper arms. Solutions used in hypodermoclysis include, for example, normal saline, half-normal saline, glucose with saline and 5% glucose. Potassium chloride also can be added to the solution. The addition of a hyaluronidase, such as a soluble PH20, to the solution can enhance fluid absorption and increase the overall rate of administration.

f. Use in gene therapy

[0293] The efficacy of most gene delivery vehicles *in vivo* does not correspond to the efficacy found observed *in vitro.* Glycosaminoglycans can hinder the transfer and diffusion of DNA and viral vectors into many cell types. The levels such extracellular matrix material can hinder the process considerably. Administration of hyaluronidase, such as a soluble PH20, can open channels in the extracellular matrix, thus enhancing delivery of gene therapy. For example, soluble PH20 can be administered with collagenase to facilitate transduction of DNA *in vivo* (Dubensky et al. (1984) Proc Natl Acad Sci USA 81(23):7529-33). Hyaluronidase also can enhance gene therapy using adeno-associated virus (Favre et al, (2000) Gene Therapy 7(16):1417-20). The channels opened following administration of hyaluronidase are of a size that typically enhance diffusion of smaller molecules such as retroviruses, adenoviruses, adeno-associated viruses and DNA complexes (as well as other therapeutic and pharmacological agents of interest). The pores are not so large, however, as to promote the dislocation and movement of cells.

[0294] In some examples, viruses can be engineered to express hyaluronidase, such as a soluble PH20, to facilitate their replication and spread within a target tissue. The target tissue can be, for example, a cancerous tissue whereby the virus is capable of selective replication within the tumor. The virus also can be a non-lytic virus wherein the virus selectively replicates under a tissue specific promoter. As the viruses replicate, the co-expression of hyaluronidase with viral genes can facilitate the spread of the virus *in vivo.*

g. Cosmetic uses

[0295] Hyaluronidases, such as a soluble PH20, can be by administered to remove glycosaminoglycans involved in the accumulation of cellulite and to promote lymphatic flow. For example, soluble PH20 can be used for the treatment of cellulite. The hyaluronidase can be administered through repeated subcutaneous injections, through transdermal delivery in the form of ointments or creams or through the use of injectable slow release formulations to promote the continual degradation of glycosaminoglycans and prevent their return.

[0296] Hyaluronidase, such as a soluble PH20, also can be used to treat conditions such as "pigskin" edema or "orange peel" edema. Hyaluronidases can effect depolymerization of the long mucopolysaccharide chains that can accumulate in the dermis and which are responsible for the retention of bound water and of the slowing, by capillary compression, of the diffusion of organic liquids, which eliminate metabolic wastes. Such retention of water and wastes associated with fat overloading of the lipocytes, constitutes classical "pigskin" edema or "orange peel" edema. Depolymerization can cut the long chains of mucopolysaccharides into shorter chains, resulting in the elimination of the bound water and wastes and restoration of the venous and lymphatic circulation, culminating in the disappearance of local edema.

h. Use in organ transplantation

[0297] The content of hyaluronic acid in an organ can increase with inflammation. An increased concentration of hyaluronic acid has been observed in tissue from different organs characterized by inflammatory-immunological injury such as alveolitis (Nettelbladt et al. (1991) Am. Rev. Resp. Dis. 139: 759-762) and myocardial infarction (Waldenstrom et al. (1991) J. Clin. Invest. 88(5): 1622-1628). Other examples include allograft rejection after a renal (Ha'llgren et al. (1990) J. Exp. Med. 171: 2063-2076; Wells et al. (1990) Transplantation 50: 240-243), small bowel (Wallander et al. (1993) Transplant. Int. 6: 133-137) or cardiac (Haellgren et al. (1990) J Clin Invest 185:668-673) transplantation; or a myocardial inflammation of viral origin (Waldenstrdm et al. (1993) Eur. J. Clin. Invest. 23: 277-282). The occurrence of interstitial edemas in connection with the grafting of an organ constitutes a severe problem in the field of transplantation surgery. Grafts with interstitial edemas can swell to such a degree that the function is temporarily be lost. In some instances, the swelling can cause disruption of the kidney, resulting in a massive hemorrhage. Hyaluronidases, such as a soluble PH20, can be used to degrade accumulated glycosaminoglycans in an organ transplant. Removal of such glycosaminoglycans promotes removal of water from the graft and thus enhances organ function.

i. Use in pulmonary disease

[0298] Levels of hyaluronic acid in broncheoalveolar lavages (BAL) from normal individuals are generally below 15 ng/ml. Hyaluronic acid levels in BAL rise dramatically in conditions of respiratory distress (Bjermer et al.(1987) Br Med J (Clin Res Ed) 295(6602):803-6). The increased hyaluronic acid in the lung can prevent oxygen diffusion and gas exchange as well as activating neutrophil and macrophage responses. Purified preparations of soluble PH20, such as any provided herein, can be delivered by either pulmonary or intravenous delivery to patients presenting with such conditions to reduce hyaluronan levels. Hyaluronidases, such as a soluble PH20, also can be administered to patients suffering from other pulmonary complications that are associated with elevated glycosaminoglycans or to enhance the delivery of other co delivered molecules to the lung.

3. Other uses

[0299] In further examples of its therapeutic use, hyaluronidase, such as a soluble PH20 including esPH20 provided herein, can be used for such purposes as an antidote to local necrosis from paravenous injection of necrotic substances such as vinka alkaloids (Few et al. (1987) Amer. J. Matern. Child Nurs. 12, 23-26), treatment of ganglion cysts (Paul et al. (1997) J Hand Surg. 22 (2): 219-21) and treatment of tissue necrosis due to venous insufficiency (Elder et al. (1980) Lancet 648-649). Soluble PH20 also can be used to treat ganglion cysts (also known as a wrist cyst, Bible cyst, or dorsal tendon cyst), which are the most common soft tissue mass of the hand and are fluid filled sacs that can be felt below the skin.

[0300] Hyaluronidases, such as soluble PH20, can be used in the treatment of spinal cord injury by degrading chondroitin sulfate proteoglycans (CSPGs). Following spinal cord injury, glial scars containing CSPGs are produced by astrocytes. CSPGs play a crucial role in the inhibition of axon growth. In addition, the expression of CSPG has been shown to increase following injury of the central nervous system (CNS). Soluble PH20 also can be utilized for the treatment of herniated disks in a process known as chemonucleolysis. Chondroitinase ABC, an enzyme cleaving similar substrates as hyaluronidase, can induce the reduction of intradiscal pressure in the lumbar spine. There are three types of disk injuries. A protruded disk is one that is intact but bulging. In an extruded disk, the fibrous wrapper has torn and the NP has oozed out, but is still connected to the disk. In a sequestered disk, a fragment of the NP has broken loose from the disk and is free in the spinal canal. Chemonucleolysis is typically effective on protruded and extruded disks, but not on sequestered disk injuries.

I. Examples

[0301] The following examples are included for illustrative purposes only.

[0302] The invention is defined in the claims.

Example 1

Generation of Human PH20 Hyaluronidase Carboxy-terminus Deletion Mutants

[0303] In this example, a series of human PH20 hyaluronidase carboxy-terminus deletion mutants were generated. Mature human PH20 hyaluronidase, or sperm adhesion molecule 1 (SPAM1), contains 474 amino acids while the mature carboxy-terminus deletion mutants generated in this example ranged in length from 472 amino acids to 415 amino acids.

[0304] DNA oligonucleotides encoding truncated human PH20 hyaluronidase carboxy-terminus deletion mutants from amino acid A507 to amino acid K450 were synthesized according to standard DNA synthesis protocols. The parent DNA sequence was a codon-optimized human PH20 hyaluronidase, the nucleotide sequence of which is set forth in SEQ ID NO:2. This codon-optimized human PH20 hyaluronidase contained an heterologous immunoglobulin kappa (IgK) signal sequence, set forth in SEQ ID NO:144. Additionally, the sequences contained a 5' NheI and a 3' BamHI restriction site to allow cloning into the HZ24 plasmid (SEQ ID NO:140). The human PH20 hyaluronidase carboxy-terminus deletion mutant nucleotide sequences are set forth in SEQ ID NOS:146-185 and 199-201. The synthetic DNA sequences were digested with NheI and BamHI restriction enzymes and cloned into a similarly digested HZ24 plasmid to generate a mutant SPAM1-HZ24 plasmid for each individual clone.

[0305] The human PH20 hyaluronidase carboxy-terminus deletion mutants are set forth in Table 3. The SPAM1 mutants are identified by the 4 amino acids at the C-terminal end of the proteins. Also set forth are the length, in amino acids, of the precursor and mature carboxy-terminus deletion mutants.

Table 3. Human PH20 hyaluronidase carboxy-terminus deletion mutants.

Mutant	Precursor (amino acids)	Precursor SEQ ID NO	Mature (amino acids)	Mature SEQ ID NO
SPAM1-VASL	509	1	474	108
SPAM1-SSVA	507	3	472	55
SPAM1-IISS	505	4	470	56
SPAM1-FLII	503	5	468	57
SPAM1-LFLI	502	47	467	99
SPAM1-ILFL	501	6	466	58
SPAM1-SILF	500	48	465	100
SPAM1-VSIL	499	7	464	59
SPAM1-IVSI	498	49	463	101
SPAM1-FIVS	497	8	462	60
SPAM1-TMFI	495	9	460	61
SPAM1-SATM	493	10	458	62
SPAM1-TLSA	491	11	456	63
SPAM1-PSTL	489	12	454	64
SPAM1-STLS	490	13	455	65
SPAM1-ASPS	487	14	452	66
SPAM1-YNAS	485	15	450	67
SPAM1-FYNA	484	16	449	68
SPAM1-IFYN	483	17	448	69
SPAM1-QIFY	482	18	447	70
SPAM1-PQIF	481	19	446	71
SPAM1-EPQI	480	20	445	72
SPAM1-EEPQ	479	21	444	73
SPAM1-TEEP	478	22	443	74

(continued)

Table 3. Human PH20 hyaluronidase carboxy-terminus deletion mutants.				
Mutant	Precursor (amino acids)	Precursor SEQ ID NO	Mature (amino acids)	Mature SEQ ID NO
SPAM1-ETEE	477	23	442	75
SPAM1-METE	476	24	441	76
SPAM1-PMET	475	25	440	77
SPAM1-PPME	474	26	439	78
SPAM1-KPPM	473	27	438	79
SPAM1-LKPP	472	28	437	80
SPAM1-FLKP	471	29	436	81
SPAM1-AFLK	470	30	435	82
SPAM1-DAFL	469	31	434	83
SPAM1-IDAF	468	32	433	84
SPAM1-CIDA	467	33	432	85
SPAM1-VCID	466	34	431	86
SPAM1-GVCI	465	35	430	87
SPAM1-DGVC	464	36	429	88
SPAM1-IADG	462	37	427	89
SPAM1-VCIA	460	38	425	90
SPAM1-VDVC	458	39	423	91
SPAM1-DAVD	456	40	421	92
SPAM1-DTDA	454	41	419	93
SPAM1-VKDT	452	42	417	94
SPAM1-ADVK	450	43	415	95

Example 2

Expression of Human PH20 Hyaluronidase Carboxy-terminus Deletion Mutants

[0306] In this example, the human PH20 hyaluronidase carboxy-terminus deletion mutants generated in Example 1 were expressed in CHO-S cells. Additionally, rHuPH20 and His-tagged PH20 were expressed in each of four strains of lectin resistant CHO mutants, including Lec1 (Cat No. CRL-1735, ATCC), Lec2 (Cat No. CRL-1736, ATCC), Lec8 (Cat No. CRL-1737, ATCC) and Pro-5 (Cat No. CRL-1781). The expression of PH20 in Lec mutant cells is further discussed in Example 9 below.

A. Transient Expression in CHO-S Cells in 6-well plates

[0307] The mutant PH20-HZ24 plasmids generated in Example 1 were transiently infected into CHO-S cells (derived from Chinese Hamster Ovary CHO K1 cells) using GeneJuice® (Novagen) according to the manufacturer's instructions. In short, the CHO-S cells were grown in CD CHO medium supplemented with L-glutamine. Prior to transfection, the CHO-S cells were plated in 6-well plates, with approximately $5x10^5$ cells per well, and grown overnight at 37 °C with 5 % CO_2. The medium was then removed and the CHO-S cells were washed 2 times with 1 mL serum-free medium. GeneJuice® was mixed with serum-free media followed by the addition of 2 μg mutant-HZ24 DNA. After incubating at room temperature for 5-15 minutes, the GeneJuice®/DNA mixture was added dropwise to an individual well containing the washed CHO-S cells. After 4 hours, the medium was replaced with 1 mL CD-CHO medium supplemented with L-glutamine and the cells were incubated for 72 hours at 37 °C with 5 % CO_2. Following expression, the media and cells were harvested separately.

B. Transient Expression in CHO Cells in 10 cm cell culture dishes

[0308] The mutant PH20-HZ24 plasmids generated in Example 1 were transiently infected into CHO-S cells using GeneJuice® (Novagen) according to the manufacturer's instructions. Alternatively, HZ24-PH20, (SEQ ID NO:108, encoding rHuPH20), PH20sHis, (SEQ ID NO: 187, encoding his-tagged PH20) and HZ24-mut(B/S) (SEQ ID NO:122, encoding PH20 truncated at amino acid 482), were transiently infected into four strains of lectin resistant CHO mutants, including Lec1 (Cat No. CRL-1735, ATCC), Lec2 (Cat No. CRL-1736, ATCC), Lec8 (Cat No. CRL-1737, ATCC) and Pro-5 (Cat No. CRL-1781), using GeneJuice® (Novagen) according to the manufacturer's instructions.

[0309] In short, CHO-S cells were maintained in CD-CHO medium supplemented with 8 mM GlutaMax. Lectin resistant CHO mutant cells were grown in DMEM medium supplemented with 10% FBS. Prior to transfection, the CHO cells were plated in 10 cm cell culture dishes, with approximately $3x10^6$ cells per well and grown overnight in DMEM medium supplemented with 10% FBS at 37 °C with 5 % CO_2. The medium was then removed and the monolayer of cells was washed 2 times with 10 mL serum-free medium. 36 μL GeneJuice® was mixed with 1.2 mL DMEM and incubated at room temperature for 5 minutes. Following incubation, 12 μg DNA was added and mixed gently. After incubating at room temperature for 15 minutes, the GeneJuice®/DNA mixture was added dropwise the monolayer of CHO cells and the cell culture dish was shaken gently to allow for mixing. The plate was incubated for 4 hours at 37 °C with 5 % CO_2. After 4 hours, the medium was replaced with 12 mL detergent free CD DG44 medium supplemented with Glutamax-1 and the cells were incubated for 48 hours at 37 °C with 5 % CO_2. Following expression, the media and cells were harvested separately.

Example 3

Solubility of Human PH20 Hyaluronidase Carboxy-terminus Deletion Mutants

[0310] In this example, following transient expression, as described in Example 2 above, the media and cells were harvested separately and analyzed for PH20 expression and solublility by Western blot analysis. Solubility of the C-terminus truncation mutants was determined by examining whether the expressed protein was present in the growth media or in the cells. C-terminus deletion mutants from 455 to 472 amino acids in length, corresponding to SEQ ID NOS:55-65 and 99-101, contain amino acid residues from the GPI-anchor which serves to attach the protein to the cell membrane. Cells expressing these mutants were treated with phosphoinositol-phospholipase C (PI-PLC), which cleaves the GPI-anchor allowing the release of soluble protein into the media, and the presence of PH20 in the resulting media and cells was determined by Western blot analysis.

A. Western blot analysis

[0311] Non-reduced samples were run on a 4-20% Tris-Glycine gel and transferred to PVDF membrane using iBlot (Invitrogen). For the Western blot, rabbit anti-PH20 IgG (0.5 μg/mL) was used as the primary antibody and HRP-conjugated goat anti-rabbit IgG (0.1 ng/mL, Cat# DC03L, EMD) was used as the secondary antibody. Evidence of expression is determined by a band at approximately 66 kDa, corresponding to recombinant human PH20 hyaluronidase.

B. PI-PLC Treatment

1. Transient Expression in 6-well plates

[0312] Following expression of rHuPH20 in CHO-S cells for 72 hours, as described in Example 2A above, the media and cells were harvested separately. The cells were washed with serum-free media followed by the addition of 2 mL serum-free media per well. PI-PLC (0.5 units/well) was added to each well and the cells were incubated in the PI-PLC for 2 hours. The resultant media and cells were analyzed by Western blot analysis as described above.

2. Transient Expression in 10 cm tissue culture dishes

[0313] Two plates each of rHuPH20 expressing CHO-S cells, one for treatment with PI-PLC and one without treatment with PI-PLC, were prepared for each C-terminus mutant as described in Example 2B above. Following expression for 48 hours, for cells untreated with PI-PLC, the media and cells were harvested separately. The harvested media was spun down, concentrated to a volume of 10 mL, and buffer exchanged into PBS using an Amicon 30kD MWCO concentrator. The cells were rinsed with cold PBS and scraped and resuspended in 1.2 mL PBS with protease inhibitor Set III (Cat No. 539134, Calbiochem). The resuspended cells were briefly sonicated to prepare whole-cell extract. For PI-PLC treatment of cells, following expression for 48 hours, the untreated media was harvested, as described above. The

cells were rinsed once with fresh CD DG44 medium with Glutamax-1, and the media was replaced with 12 mL fresh detergent free CD DG44 medium supplemented with Glutamax-1 with 3.0 units PI-PLC per dish, and the cells were incubated for 2 hours at 37 °C with 5 % CO_2. After 2 hours, the PI-PLC media and cells were harvested separately, as described above. The resultant untreated media and cells, and PI-PLC treated media and cells, were analyzed by Western blot analysis as described above.

C. Results

[0314] The results are described in Table 4 below. Four mutants, ILFL (SEQ ID NO58), SILF (SEQ ID NO:100), VSIL (SEQ ID NO:59) and IVSI (SEQ ID NO:101), exhibited low expression of PH20. Western blot analysis shows that human PH20 hyaluronidase carboxy-terminus deletion mutants shorter than F500 (SEQ ID NOS:59-95 and 100-101) are expressed in the media, as evidenced by a protein band at approximately 66 kDa. Human PH20 hyaluronidase carboxy-terminus deletion mutants with lengths between L501 and A507 (SEQ ID NOS:55-58 and 99) are expressed in the cells. Upon treatment of these cells with PI-PLC, human PH20 hyaluronidase is released into the media, as evidenced by a protein band at approximately 66 kDa. Treatment of cells from human PH20 hyaluronidase carboxy-terminus deletion mutants, corresponding to SEQ ID NOS:59-65 and 100-101, with PI-PLC had no effect since these proteins were initially expressed into the media.

Table 4. Human PH20 hyaluronidase carboxy-terminus deletion mutant expression.

Mutant	Mature (AA)	Protein Expression	Expressed in Media	Expressed in Media Following Addition of PI-PLC
SPAM1-VASL (SEQ ID NO:108)	474	YES	NO	YES
SPAM1-SSVA (SEQ ID NO:55)	472	YES	NO	YES
SPAM1-IISS (SEQ ID NO:56)	470	YES	NO	YES
SPAM1-FLII (SEQ ID NO:57)	468	YES	NO	YES
SPAM1-LFLI (SEQ ID NO:99)	467	YES	NO	YES
SPAM1-ILFL (SEQ ID NO:58)	466	WEAK	NO	YES
SPAM1-SILF (SEQ ID NO:100)	465	WEAK	WEAK/YES	Initially in media
SPAM1-VSIL (SEQ ID NO:59)	464	WEAK	YES	Initially in media
SPAM1-IVSI (SEQ ID NO:101)	463	WEAK	YES	Initially in media
SPAM1-FIVS (SEQ ID NO:60)	462	YES	YES	Initially in media
SPAM1-TMFI (SEQ ID NO:61)	460	YES	YES	Initially in media
SPAM1-SATM (SEQ ID NO:62)	458	YES	YES	Initially in media
SPAM1-TLSA (SEQ ID NO:63)	456	YES	YES	Initially in media
SPAM1-STLS (SEQ ID NO:65)	455	YES	YES	Initially in media

(continued)

Table 4. Human PH20 hyaluronidase carboxy-terminus deletion mutant expression.

Mutant	Mature (AA)	Protein Expression	Expressed in Media	Expressed in Media Following Addition of PI-PLC
SPAM1-PSTL (SEQ ID NO:64)	454	YES	YES	Initially in media
SPAM1-ASPS (SEQ ID NO:66)	452	YES	YES	n/a
SPAM1-YNAS (SEQ ID NO:67)	450	YES	YES	n/a
SPAM1-FYNA (SEQ ID NO:68)	449	YES	YES	n/a
SPAM1-IFYN (SEQ ID NO:69)	448	YES	YES	n/a
SPAM1-QIFY (SEQ ID NO:70)	447	YES	YES	n/a
SPAM1-PQIF (SEQ ID NO:71)	446	YES	YES	n/a
SPAM1-EPQI (SEQ ID NO:72)	445	YES	YES	n/a
SPAM1-EEPQ (SEQ ID NO:73)	444	YES	YES	n/a
SPAM1-TEEP (SEQ ID NO:74)	443	YES	YES	n/a
SPAM1-ETEE (SEQ ID NO:75)	442	YES	YES	n/a
SPAM1-METE (SEQ ID NO:76)	441	YES	YES	n/a
SPAM1-PMET (SEQ ID NO:77)	440	YES	YES	n/a
SPAM1-PPME (SEQ ID NO:78)	439	YES	YES	n/a
SPAM1-KPPM (SEQ ID NO:79)	438	YES	YES	n/a
SPAM1-LKPP (SEQ ID NO:80)	437	YES	YES	n/a
SPAM1-FLKP (SEQ ID NO:81)	436	YES	YES	n/a
SPAM1-AFLK (SEQ ID NO:82)	435	YES	YES	n/a
SPAM1-DAFL (SEQ ID NO:83)	434	YES	YES	n/a
SPAM1-IDAF (SEQ ID NO:84)	433	YES	YES	n/a
SPAM1-CIDA (SEQ ID NO:85)	432	YES	YES	n/a

(continued)

Table 4. Human PH20 hyaluronidase carboxy-terminus deletion mutant expression.				
Mutant	Mature (AA)	Protein Expression	Expressed in Media	Expressed in Media Following Addition of PI-PLC
SPAM1-VCID (SEQ ID NO:86)	431	YES	YES	n/a
SPAM1-GVCI (SEQ ID NO:87)	430	YES	YES	n/a
SPAM1-DGVC (SEQ ID NO:88)	429	YES	YES	n/a
SPAM1-IADG (SEQ ID NO:89)	427	YES	YES	n/a
SPAM1-VCIA (SEQ ID NO:90)	425	YES	YES	n/a
SPAM1-VDVC (SEQ ID NO:91)	423	YES	YES	n/a
SPAM1-DAVD (SEQ ID NO:92)	421	YES	YES	n/a
SPAM1-DTDA (SEQ ID NO:93)	419	YES	YES	n/a
SPAM1-VKDT (SEQ ID NO:94)	417	YES	YES	n/a
SPAM1-ADVK (SEQ ID NO:95)	415	YES	YES	n/a

Example 4

Solubility of Human PH20 Hyaluronidase Carboxy-terminus Deletion Mutants using Triton® X-114 Assay

[0315] In this example, the solubility of the human PH20 hyaluronidase carboxy-terminus deletion mutants was tested using a Triton® X-114 assay. In this assay, soluble PH20 hyaluronidases will partition into the aqueous phase of a Triton® X-114 solution warmed to 37 °C (modification as described by Bordier et al., (1981) J. Biol. Chem., 256:1604-7) while membrane-anchored PH20 hyaluronidases will partition into the detergent rich phase.

[0316] For this purpose, 2% (v/v) Triton® X-114 in PBS at 0 °C was added to 200 μL of tissue culture media or cell extract, as prepared in Example 3B above, and the samples were incubated on ice. For separation, the sample was overlaid on a 30 μL sucrose cushion (6% w/v) containing 0.06% Triton® X-114 at 4 °C in a microfuge tube. The samples were heated to 37 °C for 3 minutes to induce phase separation and centrifuged for 3 min at 4000g at room temperature. Aqueous and detergent phases were removed for SDS-PAGE analysis and Western blotting. Rabbit anti-PH20 IgG (0.5 μg/mL) was used as the primary antibody and HRP-conjugated goat anti-Rabbit IgG (0.1 ng/mL, Cat# DC03L, EMD) was used as the secondary antibody. Full length human PH20, which partitions strongly into the detergent phase, was used as a control.

[0317] The results of the solubility of the carboxy-terminus deletion mutants are shown in Table 5. Human PH20 hyaluronidase carboxy-terminus deletion mutants up to F500 (precursor SEQ ID NOS:7-13 and 48-49 or mature SEQ ID NOS:59-65 and 100-101) partition into the aqueous phase and are therefore soluble. Human PH20 hyaluronidase carboxy-terminus deletion mutants longer than F500 (SEQ ID NOS:55-58 and 99) partition into the detergent phase and are insoluble. Full length PH20 is also insoluble.

Table 5. Solubility of human PH20 hyaluronidase carboxy-terminus deletion mutants			
Mutant	SEQ ID NO	Mature (AA)	Soluble
SPAM1-VASL	108	474	NO

(continued)

Table 5. Solubility of human PH20 hyaluronidase carboxy-terminus deletion mutants			
Mutant	SEQ ID NO	Mature (AA)	Soluble
SPAM1-SSVA	55	472	NO
SPAM1-IISS	56	470	NO
SPAM1-FLII	57	468	NO
SPAM1-LFLI	99	467	NO
SPAM1-ILFL	58	466	NO
SPAM1-SILF	100	465	YES
SPAM1-VSIL	59	464	YES
SPAM1-IVSI	101	463	YES
SPAM1-FIVS	60	462	YES
SPAM1-TMFI	61	460	YES
SPAM1-SATM	62	458	YES
SPAM1-TLSA	63	456	YES
SPAM1-PSTL	64	454	YES
SPAM1-STLS	65	455	YES

Example 5

Hyaluronidase Activity of Human PH20 Hyaluronidase Carboxy-terminus Deletion Mutants

[0318] In this example, the human PH20 hyaluronidase carboxy-terminus deletion mutants were tested for their PH20 hyaluronidase activity using a microtiter assay with biotinylated-hyaluronic acid (biotinylated-HA or bHA). The human PH20 hyaluronidase carboxy-terminus deletion mutants were tested for hyaluronidase activity at both pH 7.4 and pH 5.5.

[0319] In short, a 4xBHX 96-well plate was coated with biotinylated-HA (1.1 MDa). The 72 hour post transfection supernatant from cells transfected with human PH20 hyaluronidase carboxy-terminus deletion mutants was diluted in buffer at either pH 7.4 or pH 5.5 and added to individual wells of the plate and allowed to incubate at 37 °C for 90 minutes. The reaction was terminated by addition of 4M guanidine HCl. The wells were washed 4x with Phosphate Buffered Saline with Tween20 (PBST) to remove any digested biotinylated-HA followed by addition of streptavidin-HRP for 1 hour at room temperature. The wells were washed 4x with PBST and the plate was developed with TMB. The plate was read at 450 nm using an ELISA plate reader. Hyaluronidase activity (in Units/mL) was determined by interpolating the measured absorbance at 450 nm with a hyaluronidase reference standard curve. Full length mature human PH20 hyaluronidase and untransfected CHO cells were used as positive and negative controls.

[0320] The results are shown in Tables 6 and 6A, below. Human PH20 hyaluronidase carboxy-terminus deletion mutants shorter than 1430, corresponding to SPAM1-GDVC to SPAM1-ADVK (SEQ ID NOS:88-95), are inactive. Human PH20 hyaluronidase carboxy-terminus deletion mutants ending at 1498 (SEQ ID NO:101), L499 (SEQ ID NO:59), F500 (SEQ ID NO:100), L501 (SEQ ID NO:58) and 1502 (SEQ ID NO:99) have little detectable activity due to low expression level. All other human PH20 hyaluronidase carboxy-terminus deletion mutants (SEQ ID NOS:55-57 and 60-87) are active hyaluronidases at both pH 7.4 and pH 5.5.

Table 6. Hyaluronidase Activity				
Deletion Mutant	**Precursor (AA)**	**Mature (AA)**	**pH 7.4 Activity (Units/ml)**	**pH 5.5 Activity (Units/ml)**
SPAM1-SSVA (SEQ ID NO: 55)	507	472	1.4715	1.125
SPAM1-IISS (SEQ ID NO: 56)	505	470	1.458	0.837

(continued)

Table 6. Hyaluronidase Activity				
Deletion Mutant	**Precursor (AA)**	**Mature (AA)**	**pH 7.4 Activity (Units/ml)**	**pH 5.5 Activity (Units/ml)**
SPAM1-FLII (SEQ ID NO: 57)	503	468	0.9405	0.6345
SPAM1-ILFL (SEQ ID NO: 58)	501	466	0.0405	0.0405
SPAM1-VSIL (SEQ ID NO: 59)	499	464	0.02025	0.045
SPAM1-FIVS (SEQ ID NO: 60)	497	462	0.1755	0.216
SPAM1-TMFI (SEQ ID NO: 61)	495	460	0.45	0.612
SPAM1-SATM (SEQ ID NO: 62)	493	458	0.5715	0.7335
SPAM1-TLSA (SEQ ID NO: 63)	491	456	0.3645	0.5625
SPAM1-STLS (SEQ ID NO: 65)	490	455	0.819	1.2375
SPAM1-PSTL (SEQ ID NO: 64)	489	454	1.557	1.089
SPAM1-ASPS (SEQ ID NO: 66)	487	452	1.017	0.9225
SPAM1-YNAS (SEQ ID NO: 67)	485	450	1.8765	1.74825
SPAM1-FYNA (SEQ ID NO: 68)	484	449	1.4985	1.26225
SPAM1-IFYN (SEQ ID NO: 69)	483	448	2.45025	2.3085
SPAM1-QIFY (SEQ ID NO: 70)	482	447	2.03175	1.647
SPAM1-PQIF (SEQ ID NO: 71)	481	446	1.818	1.701
SPAM1-EPQI (SEQ ID NO: 72)	480	445	2.1825	1.6425
SPAM1-EEPQ (SEQ ID NO: 73)	479	444	1.917	2.0745
SPAM1-TEEP (SEQ ID NO: 74)	478	443	1.764	1.584
SPAM1-ETEE (SEQ ID NO: 75)	477	442	2.088	2.0475
SPAM1-METE (SEQ ID NO: 76)	476	441	1.332	1.278
SPAM1-PMET (SEQ ID NO: 77)	475	440	2.223	2.0925

(continued)

Table 6. Hyaluronidase Activity				
Deletion Mutant	**Precursor (AA)**	**Mature (AA)**	**pH 7.4 Activity (Units/ml)**	**pH 5.5 Activity (Units/ml)**
SPAM1-PPME (SEQ ID NO: 78)	474	439	1.2105	1.341
SPAM1-KPPM (SEQ ID NO: 79)	473	438	0.8595	0.91575
SPAM1-LKPP (SEQ ID NO: 80)	472	437	0.5445	0.9
SPAM1-FLKP (SEQ ID NO: 81)	471	436	3.321	2.79
SPAM1-AFLK (SEQ ID NO: 82)	470	435	3.204	2.925
SPAM1-DAFL (SEQ ID NO: 83)	469	434	2.3895	2.2365
SPAM1-IDAF (SEQ ID NO: 84)	468	433	0.5625	0.62775
SPAM1-CIDA (SEQ ID NO: 85)	467	432	0.5535	0.4725
SPAM1-VCID (SEQ ID NO: 86)	466	431	0	0.2115
SPAM1-GVCI (SEQ ID NO: 87)	465	430	0.441	0.468
SPAM1-DGVC (SEQ ID NO: 88)	464	429	0	0.045
SPAM1-IADG (SEQ ID NO: 89)	462	427	0	0.00225
SPAM1-VCIA (SEQ ID NO: 90)	460	425	0	0.0135
SPAM1-VDVC (SEQ ID NO: 91)	458	423	0.0495	0.0585
SPAM1-DAVD (SEQ ID NO: 92)	456	421	0	0.0675
SPAM1-DTDA (SEQ ID NO: 93)	454	419	0	0.054
SPAM1-VKDT (SEQ ID NO: 94)	452	417	0.054	0.0225
SPAM1-ADVK (SEQ ID NO: 95)	450	415	0.063	0.0405
VASL (SEQ ID NO:108)	509	474	1.8045	0.891
VASL + PLC (SEQ ID NO: 108)	509	474	3.96	2.313
HZ24-PH20 (SEQ ID NO: 109)	482	447	0.499	0.726188
CHO-S	n/a	n/a	0	0.012375

Table 6A. Hyaluronidase Activity				
Deletion Mutant	**Precursor (AA)**	**Mature (AA)**	**pH 7.4 Activity (Units/ml)**	**pH 5.5 Activity (Units/ml)**
SPAM1-SSVA (SEQ ID NO:55)	507	472	1.782	1.256
SPAM1-IISS (SEQ ID NO: 56)	505	470	1.863	0.932
SPAMI-FLII (SEQ ID NO: 57)	503	468	1.094	0.648
SPAMI-LFLI (SEQ ID NO: 99)	502	467	0.608	0.324
SPAM1-ILFL (SEQ ID NO: 58)	501	466	0.446	0.122
SPAM1-SILF (SEQ ID NO: 100)	500	465	0.365	0.162
SPAM1-VSIL (SEQ ID NO: 59)	499	464	0.486	0.122
SPAM1-IVSI (SEQ ID NO: 101)	498	463	0.527	0.203
SPAMI-FIVS (SEQ IDNO: 60)	497	462	0.365	0.162
SPAM1-TMFI (SEQ ID NO: 61)	495	460	0.689	0.770
SPAMI-SATM (SEQ ID NO:62)	493	458	0.689	0.851
SPAM1-TLSA (SEQ ID NO:63)	491	456	0.851	0.729
SPAM1-PSTL (SEQ ID NO:64)	489	454	1.985	3.321
SPAM1-ASPS (SEQ ID NO:66)	487	452	1.134	1.580

Example 6

Glycan Analysis of rHuPH20 by LC-MS

[0321] In this example, a glycan analysis study of rHuPH20 (SEQ ID NO:122) was performed by mass spectral analysis of trypsin digested PH20.

[0322] Briefly, rHuPH20 (as produced in Example 15C), was lyophilized and resuspended in buffer containing 6M guanidine HCL, 0.002 M EDTA and 0.02 M Tris, pH 8.28 to a final concentration of 0.5 mg/mL. DTT (10 mM final concentration) was added and the protein/DTT mixture was incubated for 1 hour at 37 °C. Following reduction, iodoacetamide was added to a final concentration of 20 mM. Finally, trypsin (1:25 w/w) was added and the mixture was incubated for 20 hours at 37 °C.

[0323] The tryptic digests were analyzed by LC-MS. Briefly, the tryptic digests were injected onto a C18 reverse phase column using the conditions set forth in Table 7 below. MS data was collected on a Q-TOF Ultima mass spectrometer using electrospray ionization (ESI) in positive ion mode. Data was acquired from m/z 200-1950 in MS mode. The glycopeptides were analyzed using GlycoMod software (www.expasy.ch/tools/glycomod/) to determine the glycan type.

Table 7. LC-MS parameters and settings			
Parameter	**Setting**		
Column	Phenomenex Synergi Hydro-RP		
Column Temperature	30°C		
Mobile Phase A	Deionized water containing 0.2% formic acid		
Mobile Phase B	Acetonitrile containing 0.2% formic acid		
Gradient	Time (min)	%A	%B
	0.0	97.0	3.0
	5.0	97.0	3.0
	144.0	60.0	40.0
	150.0	10.0	90.0
	160.0	10.0	90.0
	161.0	97.0	3.0
	180.0	97.0	3.0
Flow Rate	0.2 mL/min		
Injection Volume	5 μL		
Run Time (total)	180 minutes		

[0324] Human PH20 hyaluronidase has one O-glycosylation site at T475. The site is occupied by a core type 1 glycan that has one or two sialic acids. rHuPH20 is glycosylated at six different asparagine residues, including N82, N166, N235, N254, N368, and N393. The results show that N254 is approximately 75% occupied, N393 is approximately 85% occupied, and the four remaining sites, N82, N166, N235 and N368, are greater than 99% occupied. All of the three types of N-glycans, high mannose, hybrid and complex types, are present in rHuPH20. In general, rHuPH20 contains about 45% high mannose glycans, 45% complex glycans and 10% hybrid glycans. About 35% of the total glycans are anionic, of which 25% contain a sialic acid and the remaining 10% contain an unknown anionic group, possibly a phosphate group. Most of the complex glycans are fucosylated and the anionic complex glycans contain mostly one sialic acid while a few of them contain two sialic acids. Each asparagine residue has about 90% of one type of glycan and a small proportion of the other two types of glycans, with the exception of N235. The major glycan type for each residue is set forth in Table 8 below. Residues N82, N166 and N254 are occupied by complex glycans. Residues N368 and N393 are occupied by high mannose glycans. Residue N235 is occupied by approximately 80% high mannose glycans, with approximately 20% complex glycans.

Table 8. Types of N-glycans at asparagine residues in rHuPH20		
Glycan Site	**High Mannose glycans**	**Complex Glycans**
N82		X
N166		X
N235	~ 80%	~ 20%
N254		X
N368	X	
N393	X	

Example 7

Deglycosylation of Human PH20 Hyaluronidase by treatment with Endoglycosidases

[0325] In this example, human PH20 hyaluronidase was deglycosylated by treatment of purified rHuPH20 (SEQ ID NO:122) with various glycosidases and hyaluronidase activity was assessed. Human PH20 hyaluronidase is glycosylated at six different asparagine residues, including N82, N166, N235, N254, N368, and N393. Five glycosidases were used to generate deglycosylated human PH20 hyaluronidase, including: PNGaseF (New England Biolabs, Cat. No. P0704S, Lot #34), which cleaves all N-glycans; EndoF1, which cleaves high mannose and hybrid type glycans; EndoF2, which cleaves biantennary complex type glycans; EndoF3, which cleaves biantennary and more branched complex glycans; and EndoH (New England Biolabs, Cat. No. P0702S), which cleaves high mannose and hybrid type glycans. Therefore, treatment with PNGaseF results in complete deglycosylation whereas treatment with endoglycosidases results in only partial deglycosylation.

[0326] For complete deglycosylation, purified rHuPH20 (0.1 mg/mL final concentration) was incubated with PNGaseF (50,000 units/mL) in 50 mM phosphate buffer pH 7.2 overnight at 37 °C. For partial deglycosylation, purified rHuPH20 (0.5 mg/mL final concentration) was incubated with 0.3 units/mL of endoglycosidase (either EndoF 1, EndoF2, EndoF3 or EndoH) or a mixture all four endoglycosidases in 50 mM sodium acetate buffer pH 5.0 overnight at 35 °C. Deglycosylation of rHuPH20 was analyzed by the shift in the mobility of PH20 by SDS-PAGE. Hyaluronidase enzymatic activity was determined as described in Example 5.

[0327] Human PH20 hyaluronidase has a molecular weight of approximately 66 kDa. Treatment with EndoF1, EndoH or a mixture of EndoF1, EndoF2, EndoF3 and EndoH resulted in partially deglycosylated human PH20 hyaluronidase as determined by SDS-PAGE mobility shift to a molecular weight of approximately 56 kDa. Treatment with PNGaseF resulted in complete deglycosylation of human PH20 hyaluronidase. Partial deglycosylation of rHuPH20 did not result in inactivation of hyaluronidase enzymatic activity whereas exhaustive digestion with PNGaseF to completely remove N-glycans resulted in the total loss of hyaluronidase enzymatic activity (see Table 9 below).

Table 9. Effect of glycosidase treatment on rHuPH20 activity

rHuPH20 (U/ml)	Control PH20	EndoF1	EndoF2	EndoF3	EndoH	EndoF1, F2,F3,H	PNGaseF
1.0000	0.3195	0.2983	0.2573		0.2965	0.2144	1.9315
0.2000	0.7910	0.7656	0.6048	0.5880	0.7435	0.5366	1.9173
0.0400	1.4299	1.3450	1.3117	1.2255	1.3584	1.3877	1.9926
0.0080	1.8397	1.7338	1.6900	1.6698	1.6998	1.8418	1.9172

Example 8

Treatment of Human PH20 Hyaluronidase with Glycosylation inhibitors

[0328] In this example, rHuPH20 (SEQ ID NO:122) was transiently expressed in the presence of each of two glycosylation inhibitors and hyaluronidase secretion and activity were assessed. Kifunensine is a potent inhibitor mannosidase I, an enzyme involved in glycan processing (see e.g., Elbein et al., J Biol Chem, 265:15599-15605 (1990)). Tunicamycin is a mixture of homologous nucleoside antibiotics that inhibit the enzyme GlcNAc phospho-transferase (GPT), thereby blocking the synthesis of all N-glycans (see e.g., Böhme et al., Eur. J. Biochem. 269:977-988 (2002)).

[0329] Briefly, $1x10^6$ HZ24-2B2 cells expressing rHuPH20 (see Example 14 below) were seeded in 24 mL complete CD-CHO medium in two 125 mL flasks. Tunicamycin (dissolved in DMSO) or Kifunensine (freshly dissolved in water) was added to a final concentration of 5 μg/mL (containing 12 μL DMSO). As a control, one flask was seeded with $1x10^6$ HZ24-2B2 cells expressing rHuPH20 and 12 μL DMSO was added as a vehicle control. Following addition of either tunicamycin or kifunensine, the cells were incubated for 4-6 hours at 37 °C with 5% CO_2. Following expression, a 2 mL culture was removed and centrifuged for 5 minutes at 500g. The supernatant was stored at 4 °C and the cell pellets were stored at -20 °C. The remaining 22 mL cultures were centrifuged for 5 minutes at 500g. The supernatant was stored at 4 °C. The cells were resuspended in 22 mL complete CD-CHO medium in the original two 125 mL flasks. Tunicamycin or Kifunensine was added to the culture at a final concentration of 5 μg/mL and the cells were incubated at 37 °C with 5% CO_2. Two mL (2 mL) cultures were removed from each flask at approximately every 24 hours post changing medium. For each time point, the supernatant was stored at 4 °C and the cell pellets were stored at -20 °C. The expression of rHuPH20 was analyzed by Western blot analysis and the hyaluronidase activity was measured using the biotinylated HA enzymatic assay (as described in Examples 3 and 5 above).

[0330] The results are shown in Tables 10-13 below, which set forth the number of viable cells and the PH20 activity. As shown in Tables 10-11, tunicamycin inhibits PH20 activity in both tissue culture media and inside the cell and also results in a complete loss of cell viability. Additionally, one hour of treatment with tunicamycin resulted in the accumulation of deglycosylated human PH20 hyaluronidase inside the cell, as determined by SDS-PAGE mobility shift to a molecular weight of approximately 56 kDa in the cell pellet fractions. As shown in Tables 12-13, kifunensine did not affect the activity of PH20 while western blot analysis revealed kifunensine inhibited the expression and secretion of rHuPH20 in treated cells.

Table 10. Effect of Tunicamycin on Cell Viability and PH20 activity in Tissue Culture Media

Time (hours)	With Tunicamycin		Without Tunicamycin	
	Viable Cells ($x10^6$)	PH20 activity (U/mL)	Viable Cells ($x10^6$)	PH20 activity (U/mL)
0	1.04	0.50	1.04	0.00
1	1.04	2.80	1.04	1.50
2	1.04	5.00	1.04	3.00
4	0.910	8.80	1.30	7.00
25	1.08	5.80	1.32	82.50
49	0.200	6.80	2.72	171.30
73	0.080	7.80	3.80	331.00
91	0	7.50	6.25	313.30

Table 11. Effect of Tunicamycin on Cell Viability and PH20 activity in Cell Pellets

Time (hours)	With Tunicamycin		Without Tunicamycin	
	Viable Cells ($x10^6$)	PH20 activity (U/mL)	Viable Cells ($x10^6$)	PH20 activity (U/mL)
0	1.04	34.50	1.04	35.00
1	1.04	38.00	1.04	38.10
2	1.04	34.00	1.04	36.60
4	0.910	18.00	1.30	31.90
25	1.08	1.00	1.32	14.40
49	0.200	0.80	2.72	33.10
73	0.080	0.30	3.80	67.50
91	0	0.30	6.25	79.40

Table 12. Effect of Kifunensine on Cell Viability and PH20 activity in Tissue Culture Media

Time (hours)	With Kifunensine		Without Kifunensine	
	Viable Cells ($x10^6$)	PH20 activity (U/mL)	Viable Cells ($x10^6$)	PH20 activity (U/mL)
0	1	0.4	1	0.45
6	1	23.85	1	15.75
24	1.2	129.6	1.4	75.6
50	2.1	299.7	2.4	206.55
72	3	535.95	4.4	444.15
96	3.7	945	6.3	726.3

(continued)

Table 12. Effect of Kifunensine on Cell Viability and PH20 activity in Tissue Culture Media				
Time (hours)	With Kifunensine		Without Kifunensine	
	Viable Cells (x10^6)	PH20 activity (U/mL)	Viable Cells (x10^6)	PH20 activity (U/mL)
144	5.8	2968.65	8.5	2241

Table 13. Effect of Kifunensine on Cell Viability and PH20 activity in Cell Pellets				
Time (hours)	With Kifunensine		Without Kifunensine	
	Viable Cells (x10^6)	PH20 activity (U/mL)	Viable Cells (x10^6)	PH20 activity (U/mL)
0	1	22.25	1	23
6	1	21.25	1	27
24	1.2	27.75	1.4	14.45
50	2.1	43	2.4	26
72	3	98.75	4.4	52.75
96	3.7	208.75	6.3	167.5
144	5.8	497.25	8.5	107

Example 9

Transient Expression of rHuPH20 in Lectin resistant CHO mutants

[0331] In this example, rHuPH20 was transiently expressed in four Lectin resistant CHO mutants and hyaluronidase secretion and activity were assessed. The Lectin resistant CHO mutants are summarized in Table 14 below. Pro$^-$5 cells lack the galactosyltransferase β4galT-6 causing a reduction in galactosylated *N*-glycans (see, e.g., Lee et al. J. Biol. Chem. 276:13924-13934 (2001)). Lec1 cells lack *N*-acetylglucosaminyltranferase I activity and therefore do not synthesize complex or hybrid glycans (see, e.g., Chen and Stanley, Glycobiology, 13:43-50 (2003)). Lec2 and Lec8 are deficient in nucleotide-sugar transporters, which transport nucleotide-sugars across the ER or golgi membrane. Lec2 cells are unable to translocate CMP-sialic acid (namely CMP-NeuAc) therefore causing the expression of asialo cell surfaces (see, e.g., Eckhardt et al., J. Biol. Chem. 273:20189-20195 (1998)). Lec8 cells are unable to translocate UDP-galactose therefore causing glycans devoid of galactose (see, e.g., Bakker et al., Glycobiology, 15:193-201 (2005)).

Table 14. Lectin resistant CHO mutants		
CHO line	Biochemical Change	Genetic Change
Pro$^-$5 (parent)	↓ Gal on N-glycans	No expression of *β4galt6*
Lec1	↓ GlcNAc-TI	Insertion/deletion in *Mgat1* ORF
Lec2	↓ CMP-sialic acid Golgi transporter	Mutation in *Slc35a1* ORF
Lec8	↓ UDP-Gal Golgi transporter	Mutation in *Slc35a2* ORF

[0332] In brief, PH20sHis (encoding his-tagged PH20, SEQ ID NO:187) wa transiently expressed in each of four strains of lectin resistant CHO mutants, including Lec1 (Cat No. CRL-1735, ATCC), Lec2 (Cat No. CRL-1736, ATCC), Lec8 (Cat No. CRL-1737, ATCC) and Pro$^-$5 (Cat No. CRL-1781) as described in Example 2A above. Additionally, HZ24-mut(B/S) (encoding PH20 truncated at amino acid 482, SEQ ID NO:122) was transiently expressed in Pro$^-$5 cells and as negative control, Pro$^-$ 5 cells were subjected to a mock transfection. The resulting cell culture media was analyzed by Western blot analysis and hyaluronidase activity was measured using the biotinylated HA enzymatic assay (as described in Examples 3 and 5 above).

[0333] The results show that rHuPH20 expressed in the Lec mutants is secreted into the medium, as evidenced by a

protein band at approximately 66 kDa. The results of the bHA enzymatic assay are set forth in Table 15 below, which sets for the lectin resistant CHO mutant, the PH20 encoding plasmid used to transfect the cells, and the PH20 activity at pH5.5 for both a 1:27 and 1:81 dilution. rHuPH20 expressed by Lec mutant cells is enzymatically active.

Table 15. PH20 activity (U/mL) of rHuPH20 transiently expressed in Lec mutant cells.

Lectin Mutant	Pro-5	Pro-5	Lec1	Lec2	Lec8	Pro-5
Plasmid	**HZ24-mut(B/S)**	**HZ24-PH20sHis**	**HZ24-PH20sHis**	**HZ24-PH20sHis**	**HZ24-PH20sHis**	**Mock transfection**
PH20 Activity (1:27)	0.6615	0.297	0.54	0.675	0.2565	0.081
PH20 Activity (1:81)	1.1745	0.6075	0.7695	1.053	0.567	0.1215

Example 10

Site-Directed Mutagenesis of Human PH20 Hyaluronidase N-glycosylation Sites

[0334] In this example, N-glycan site specific human PH20 hyaluronidase deglycosylation mutants were generated and their secretion patterns and hyaluronidase enzymatic activity were assessed. The N-glycan site specific deglycosylation mutants and glycan types are set forth in Table 16 below.

[0335] PH20sHis (SEQ ID NO:210) was used as a template for mutagenesis of each asparagine residue to alanine using QuikChange® Site-Directed Mutagenesis Kit (Cat No. 200518, Stratagene). The protein encoded by the template DNA corresponds to PH20sHis (SEQ ID NO:187), a human PH20 clone that contains a HexaHis tag (SEQ ID NO:142) after amino acid S490. Wild type PH20sHis and deglycosylated mutants are set forth in Table 16. Six single mutants were generated, one for each of the N-glycosylation sites. Additionally, three double mutants and a triple mutant were generated for asparagines N82, N166 and N254, all of which are occupied by complex type glycans. Finally, a double mutant N368A/N393A was generated, lacking high mannose glycans. The mutants were transfected into CHO-S cells and expression was performed as described in Example 2A. Secretion into the media and hyaluronidase activity were determined as described in Examples 3 and 5, above.

[0336] The results are shown in Table 16 below, which sets forth the mutation, the glycan types, whether the protein was secreted into the media and the hyaluronidase activity at both pH 5.5 and pH 7.4. Western blot analysis showed that mutation of residues N82, N166, N235 and N254 had no effect on secretion of the rHuPH20 protein into the media. Alternatively, mutation of residues N368A and N368A/N393A prevented PH20 expression and secretion, as evidenced by a lack of protein at approximately 66 kDa in the media. Mutation of residue N393A resulted in reduced protein expression, but rHuPH20 was observed in the media, as evidenced by a protein band at approximately 66 kDa. Mutation of a residues N82, N166 and/or N254 had no effect on rHuPH20 activity. These residues are occupied by complex glycans. In contrast, mutation of residues N235, N368 and/or N393, which contain high mannose glycans, resulted in a complete loss of detectable activity in the media due to a lack of secretion.

Table 16. Human PH20 Hyaluronidase Deglycosylation Mutants

Mutant	SEQ ID NO	Glycan Type	Secretion	Activity pH 5.5	Activity pH 7.4
PH20sHis (parent)	187	Both	YES	YES	YES
N82A	202	Complex	YES	YES	YES
N166A	203	Complex	YES	YES	YES
N235A	204	High Mannose (80%) Complex (20%)	YES	NO	NO
N254A	205	Complex	YES	YES	YES

(continued)

Table 16. Human PH20 Hyaluronidase Deglycosylation Mutants					
Mutant	SEQ ID NO	Glycan Type	Secretion	Activity pH 5.5	Activity pH 7.4
N368A	188	High Mannose	NO	NO	NO
N393A	189	High Mannose	YES (WEAK)	NO	NO
N82A/N166A	206	Complex	YES	YES	YES
N82A/N254A	207	Complex	YES	YES	YES
N166A/N254A	208	Complex	YES	YES	YES
N82A/N166A/N254A	209	Complex	YES	YES	YES
N368A/N393A	190	High Mannose	NO	NO	NO

[0337] Immunofluorescent analysis of CHO cells with an anti-PH20 antibody was used to visualize the expression of the N-glycan site specific deglycosylation mutants N368A, N393A and N368A/N393A. CHO cells were seeded for monolayer culture onto 8-well chamber slides with 200 μL of cells at 2.5 x 10^4 cells per ml of Dulbecco's Modified Eagle Medium (DMEM) containing 10 % fetal bovine serum (FBS) and grown at 37 °C in a humidified atmosphere of 5 % CO_2. Cells were transfected 36 hours later at 80 % confluency using Lipofectamine™ 2000 (Invitrogen) as follows. DNA (0.4 μg in 50 μL of DMEM without serum) and Lipofectamine™ 2000 (1 μL in DMEM without serum) were mixed gently for 20 minutes at room temperature and then added to each well containing cells and 100 μL serum free medium. Mixing was effected by gently rocking the plate back and forth. The cells were then incubated at 37 °C in a CO_2 incubator for 4-6 hours after which the medium was replaced with medium containing 10 % FBS. At 48 hours post-transfection, the cells on the chamber slides were fixed with 4 % paraformaldehyde for 15 minutes. The cells were washed 3x with PBS and 200 μL of a 1 % NP-40/PBS solution was added and incubated for 30 minutes at room temperature. The cells were washed 3x with PBS and stored at 4 °C prior to immunolabeling.

[0338] To immunolabel the cells, the samples were blocked with 15 % normal goat serum for 30 minutes at room temperature. The cells were incubated with a 1:20 solution of anti-PH20 rabbit IgG diluted in 5 % normal goat serum in PBS for 2 hours. Finally, the cells were washed 3x with PBS followed by incubation with a FITC-conjugated goat anti-rabbit IgG for 1 hour followed by visualization. In addition, the mounting solution contained DAPI allowing for nuclei staining. Immunofluorescent analysis using the anti-PH20 antibody showed that N368A and N393A mutations caused PH20 to accumulate inside the cells.

Summary of N-glycosylation studies

[0339] As exhibited in Examples 7-10 above, N-linked glycosylation is essential for proper folding and enzymatic activity of rHuPH20. Complete deglycosylation of rHuPH20, effected by exhaustive digestion with PNGaseF or by inhibition of glycosylation during biosynthesis by treatment with tunicamyicn, abolished all detectible enzymatic activity. In addition, unglycosylated rHuPH20 was shown to accumulate in the cell. In contrast, partially deglycosylated rHuPH20, effected by treatment with kifunensine or by expression in Lec mutants, retained enzymatic activity. Finally, detailed mutational analysis using site-directed mutagenesis revealed that the presence of high mannose type glycans is necessary for production of soluble, enzymatically active rHuPH20.

Example 11

Generation of a soluble rHuPH20 -expressing cell line

[0340] The HZ24 plasmid (set forth in SEQ ID NO:140) was used to transfect Chinese Hamster Ovary (CHO cells) (see *e.g.* U.S. Patent Application Nos. 10,795,095, 11/065,716 and 11/238,171). The HZ24 plasmid vector for expression of soluble rHuPH20 contains a pCI vector backbone (Promega), DNA encoding amino acids 1-482 of human PH20 hyaluronidase (SEQ ID NO:110), an internal ribosomal entry site (IRES) from the ECMV virus (Clontech), and the mouse dihydrofolate reductase (DHFR) gene. The pCI vector backbone also includes DNA encoding the Beta-lactamase resistance gene (AmpR), an f1 origin of replication, a Cytomegalovirus immediate-early enhancer/promoter region (CMV), a chimeric intron, and an SV40 late polyadenylation signal (SV40). The DNA encoding the soluble rHuPH20 construct contains an NheI site and a Kozak consensus sequence prior to the DNA encoding the methionine at amino acid position

1 of the native 35 amino acid signal sequence of human PH20, and a stop codon following the DNA encoding the tyrosine corresponding to amino acid position 482 of the human PH20 hyaluronidase set forth in SEQ ID NO:107, followed by a BamHI restriction site. The construct pCI-PH20-IRES-DHFR-SV40pa (HZ24), therefore, results in a single mRNA species driven by the CMV promoter that encodes amino acids 1-482 of human PH20 (set forth in SEQ ID NO:109 and amino acids 1-186 of mouse dihydrofolate reductase (set forth in SEQ ID NO:141), separated by the internal ribosomal entry site (IRES).

[0341] Non-transfected DG44 CHO cells growing in GIBCO Modified CD-CHO media for DHFR(-) cells, supplemented with 4 mM Glutamine and 18 ml/L Plurionic F68/L (Gibco), were seeded at 0.5 x 10^6 cells/ml in a shaker flask in preparation for transfection. Cells were grown at 37 °C in 5% CO_2 in a humidified incubator, shaking at 120 rpm. Exponentially growing non-transfected DG44 CHO cells were tested for viability prior to transfection.

[0342] Sixty million viable cells of the non-transfected DG44 CHO cell culture were pelleted and resuspended to a density of 2 $\times$ 10^7 cells in 0.7 mL of 2x transfection buffer (2x HeBS: 40 mM Hepes, pH 7.0,274 mM NaCl, 10 mM KCl, 1.4 mM Na_2HPO_4, 12 mM dextrose). To each aliquot of resuspended cells, 0.09 mL (250 μg) of the linear HZ24 plasmid (linearized by overnight digestion with Cla I (New England Biolabs) was added, and the cell/DNA solutions were transferred into 0.4 cm gap BTX (Gentronics) electroporation cuvettes at room temperature. A negative control electroporation was performed with no plasmid DNA mixed with the cells. The cell/plasmid mixes were electroporated with a capacitor discharge of 330 V and 960 μF or at 350 V and 960 μF.

[0343] The cells were removed from the cuvettes after electroporation and transferred into 5 mL of Modified CD-CHO media for DHFR(-) cells, supplemented with 4 mM Glutamine and 18 ml/L Plurionic F68/L (Gibco), and allowed to grow in a well of a 6-well tissue culture plate without selection for 2 days at 37° C in 5% CO_2 in a humidified incubator.

[0344] Two days post-electroporation, 0.5 mL of tissue culture media was removed from each well and tested for the presence of hyaluronidase activity, using the microturbidity assay described in Example 12. Results are shown in Table 17.

Table 17. Initial Hyaluronidase Activity of HZ24 Transfected DG44 CHO cells at 40 hours post-transfection

	Dilution	Activity Units/ml
Transfection 1 330V	1 to 10	0.25
Transfection 2 350V	1 to 10	0.52
Negative Control	1 to 10	0.015

[0345] Cells from Transfection 2 (350V) were collected from the tissue culture well, counted and diluted to 1 $\times$ 10^4 to 2 $\times$ 10^4 viable cells per mL. A 0.1 mL aliquot of the cell suspension was transferred to each well of five, 96 well round bottom tissue culture plates. One hundred microliters of CD-CHO media (GIBCO) containing 4 mM GlutaMAX™-1 supplement (GIBCO™, Invitrogen Corporation) and without hypoxanthine and thymidine supplements were added to the wells containing cells (final volume 0.2 mL).

[0346] Ten clones were identified from the 5 plates grown without methotrexate (Table 18).

Table 18. Hyaluronidase activity of identified clones

Plate/Well ID	Relative Hyaluronidase
1C3	261
2C2	261
3D3	261
3E5	243
3C6	174
2G8	103
1B9	304
2D9	273
4D10	302

[0347] Six HZ24 clones were expanded in culture and transferred into shaker flasks as single cell suspensions. Clones 3D3, 3E5, 2G8, 2D9, 1E11, and 4D10 were plated into 96-well round bottom tissue culture plates using a two-dimensional

infinite dilution strategy in which cells were diluted 1:2 down the plate, and 1:3 across the plate, starting at 5000 cells in the top left hand well. Diluted clones were grown in a background of 500 non-transfected DG44 CHO cells per well, to provide necessary growth factors for the initial days in culture. Ten plates were made per subclone, with 5 plates containing 50 nM methotrexate and 5 plates without methotrexate.

[0348] Clone 3D3 produced 24 visual subclones (13 from the no methotrexate treatment, and 11 from the 50 nM methotrexate treatment. Significant hyaluronidase activity was measured in the supernatants from 8 of the 24 subclones (>50 Units/mL), and these 8 subclones were expanded into T-25 tissue culture flasks. Clones isolated from the methotrexate treatment protocol were expanded in the presence of 50 nM methotrexate. Clone 3D35M was further expanded in 500 nM methotrexate giving rise to clones producing in excess of 1,000 Units/ml in shaker flasks (clone 3D35M; or Gen1 3D35M). A master cell bank (MCB) of the 3D35M cells was then prepared.

Example 12

Determination of hyaluronidase activity of soluble rHuPH20

[0349] Hyaluronidase activity of soluble rHuPH20 in samples such as cell cultures, purification fractions and purified solutions was determined using a turbidometric assay, which is based on the formation of an insoluble precipitate when hyaluronic acid binds with serum albumin. The activity is measured by incubating soluble rHuPH20 with sodium hyaluronate (hyaluronic acid) for a set period of time (10 minutes) and then precipitating the undigested sodium hyaluronate with the addition of acidified serum albumin. The turbidity of the resulting sample is measured at 640 nm after a 30 minute development period. The decrease in turbidity resulting from enzyme activity on the sodium hyaluronate substrate is a measure of the soluble rHuPH20 hyaluronidase activity. The method is performed using a calibration curve generated with dilutions of a soluble rHuPH20 assay working reference standard, and sample activity measurements are made relative to this calibration curve.

[0350] Dilutions of the sample were prepared in Enzyme Diluent Solution. The Enzyme Diluent Solution was prepared by dissolving 33.0 $\pm$ 0.05 mg of hydrolyzed gelatin in 25.0 mL of the 50 mM PIPES Reaction Buffer (140 mM NaCl, 50 mM PIPES, pH 5.5) and 25.0 mL of SWFI, and diluting 0.2 mL of 25% Buminate solution into the mixture and vortexing for 30 seconds. This was performed within 2 hours of use and stored on ice until needed. The samples were diluted to an estimated 1-2 U/mL. Generally, the maximum dilution per step did not exceed 1:100 and the initial sample size for the first dilution was not be less than 20 μL. The minimum sample volumes needed to perform the assay were: In-process Samples, FPLC Fractions: 80 μL; Tissue Culture Supematants:1 mL; Concentrated Material 80 μL; Purified or Final Step Material: 80 μL. The dilutions were made in triplicate in a Low Protein Binding 96-well plate, and 30 μL of each dilution was transferred to Optilux black/clear bottom plates (BD BioSciences).

[0351] Dilutions of known soluble rHuPH20 with a concentration of 2.5 U/mL were prepared in Enzyme Diluent Solution to generate a standard curve and added to the Optilux plate in triplicate. The dilutions included 0 U/mL, 0.25 U/mL, 0.5 U/mL, 1.0 U/mL, 1.5 U/mL, 2.0 U/mL, and 2.5 U/mL. "Reagent blank" wells that contained 60 μL of Enzyme Diluent Solution were included in the plate as a negative control. The plate was then covered and warmed on a heat block for 5 minutes at 37°C. The cover was removed and the plate was shaken for 10 seconds. After shaking, the plate was returned to the heat block and the MULTIDROP 384 Liquid Handling Device was primed with the warm 0.25 mg/mL sodium hyaluronate solution (prepared by dissolving 100 mg of sodium hyaluronate (LifeCore Biomedical) in 20.0 mL of SWFI. This was mixed by gently rotating and/or rocking at 2-8 °C for 2-4 hours, or until completely dissolved). The reaction plate was transferred to the MULTIDROP 384 and the reaction was initiated by pressing the start key to dispense 30 μL sodium hyaluronate into each well. The plate was then removed from the MULTIDROP 384 and shaken for 10 seconds before being transferred to a heat block with the plate cover replaced. The plate was incubated at 37 °C for 10 minutes

[0352] The MULTIDROP 384 was prepared to stop the reaction by priming the machine with Serum Working Solution and changing the volume setting to 240 μL. (25 mL of Serum Stock Solution [1 volume of Horse Serum (Sigma) was diluted with 9 volumes of 500 mM Acetate Buffer Solution and the pH was adjusted to 3.1 with hydrochloric acid] in 75 mL of 500 mM Acetate Buffer Solution). The plate was removed from the heat block and placed onto the MULTIDROP 384 and 240 μL of serum Working Solutions was dispensed into the wells. The plate was removed and shaken on a plate reader for 10 seconds. After a further 15 minutes, the turbidity of the samples was measured at 640 nm and the hyaluronidase activity (in U/mL) of each sample was determined by fitting to the standard curve.

[0353] Specific activity (Units/mg) was calculated by dividing the hyaluronidase activity (U/ml) by the protein concentration (mg/mL).

Example 13

Production and Purification of Gen1 Human sPH20

A. 5 L Bioreactor Process

[0354] A vial of 3D35M was thawed and expanded from shaker flasks through 1 L spinner flasks in CD-CHO media (Invitrogen, Carlsbad Calif.) supplemented with 100 nM Methotrexate and GlutaMAX™-1 (Invitrogen). Cells were transferred from spinner flasks to a 5 L bioreactor (Braun) at an inoculation density of 4×10^5 viable cells per ml. Parameters were temperature Setpoint 37°C, pH 7.2 (starting Setpoint), with Dissolved Oxygen Setpoint 25% and an air overlay of 0-100 cc/min. At 168 hrs, 250 ml of Feed #1 Medium (CD CHO with 50 g/L Glucose) was added. At 216 hours, 250 ml of Feed #2 Medium (CD CHO with 50 g/L Glucose and 10 mM Sodium Butyrate) was added, and at 264 hours 250 ml of Feed #2 Medium was added. This process resulted in a final productivity of 1600 Units per ml with a maximal cell density of 6×10^6 cells/ml. The addition of sodium butyrate was to dramatically enhance the production of soluble rHuPH20 in the final stages of production.

[0355] Conditioned media from the 3D35M clone was clarified by depth filtration and tangential flow diafiltration into 10 mM Hepes pH 7.0. Soluble rHuPH20 was then purified by sequential chromatography on Q Sepharose (Pharmacia) ion exchange, Phenyl Sepharose (Pharmacia) hydrophobic interaction chromatography, phenyl boronate (Prometics) and Hydroxapatite Chromatography (Biorad, Richmond, CA).

[0356] Soluble rHuPH20 bound to Q Sepharose and eluted at 400 mM NaCl in the same buffer. The eluate was diluted with 2M ammonium sulfate to a final concentration of 500 mM ammonium sulfate and passed through a Phenyl Sepharose (low sub) column, followed by binding under the same conditions to a phenyl boronate resin. The soluble rHuPH20 was eluted from the phenyl sepharose resin in Hepes pH 6.9 after washing at pH 9.0 in 50 mM bicine without ammonium sulfate. The eluate was loaded onto a ceramic hydroxyapatite resin at pH 6.9 in 5 mM potassium phosphate and 1 mM $CaCl_2$ and eluted with 80 mM potassium phosphate, pH 7.4 with 0.1 mM $CaCl_2$.

[0357] The resultant purified soluble rHuPH20 possessed a specific activity in excess of 65,000 USP Units/mg protein by way of the microturbidity assay (Example 12) using the USP reference standard. Purified sPH20 eluted as a single peak from 24 to 26 minutes from a Pharmacia 5RPC styrene divinylbenzene column with a gradient between 0.1% TFA/H_2O and 0.1% TFA/90% acetonitrile/10% H_20 and resolved as a single broad 61 kDa band by SDS electrophoresis that reduced to a sharp 51 kDa band upon treatment with PNGASE-F. N-terminal amino acid sequencing revealed that the leader peptide had been efficiently removed.

B. Upstream Cell Culture Expansion Process into 100 L Bioreactor Cell Culture

[0358] A scaled-up process was used to separately purify soluble rHuPH20 from four different vials of 3D35M cell to produce 4 separate batches of sHuPH20; HUA0406C, HUA0410C, HUA0415C and HUA0420C. Each vial was separately expanded and cultured through a 125 L bioreactor, then purified using column chromatography. Samples were taken throughout the process to assess such parameters as enzyme yield. The description of the process provided below sets forth representative specifications for such things as bioreactor starting and feed media volumes, transfer cell densities, and wash and elution volumes. The exact numbers vary slightly with each batch, and are detailed in Tables 24 to 30.

[0359] Four vials of 3D35M cells were thawed in a 37°C water bath, CD CHO containing 100 nM methotrexate and 40 mL/L GlutaMAX was added and the cells were centrifuged. The cells were re-suspended in a 125 mL shake flask with 20 mL of fresh media and placed in a 37°C, 7% CO_2 incubator. The cells were expanded up to 40 mL in the 125 mL shake flask. When the cell density reached 1.5 - 2.5 x 10^6 cells/mL, the culture was expanded into a 125 mL spinner flask in a 100 mL culture volume. The flask was incubated at 37°C, 7% CO_2. When the cell density reached 1.5 - 2.5 x 10^6 cells/mL, the culture was expanded into a 250 mL spinner flask in 200 mL culture volume, and the flask was incubated at 37°C, 7% CO_2. When the cell density reached 1.5 - 2.5 x 10^6 cells/mL, the culture was expanded into a 1 L spinner flask in 800 mL culture volume and incubated at 37°C, 7% CO_2. When the cell density reached 1.5 - 2.5 x 10^6 cells/mL, the culture was expanded into a 6 L spinner flask in 5 L culture volume and incubated at 37°C, 7% CO_2. When the cell density reached 1.5 - 2.5 x 10^6 cells/mL, the culture was expanded into a 36 L spinner flask in 20 L culture volume and incubated at 37°C, 7% CO_2.

[0360] A 125 L reactor was sterilized with steam at 121°C, 20 PSI and 65 L of CD CHO media was added. Before use, the reactor was checked for contamination. When the cell density in the 36 L spinner flasks reached 1.8 -2.5 x 10^6 cells/mL, 20 L cell culture were transferred from the 36L spinner flasks to the 125 L bioreactor (Braun), resulting a final volume of 85 L and a seeding density of approximately 4×10^5 cells/mL. Parameters were temperature setpoint, 37°C; pH: 7.2; Dissolved oxygen: 25% ± 10%; Impeller Speed 50 rpm; Vessel Pressure 3 psi; Air Sparge 1 L/ min.; Air Overlay: 1 L/min. The reactor was sampled daily for cell counts, pH verification, media analysis, protein production and retention. Nutrient feeds were added during the run. At Day 6,3.4 L of Feed #1 Medium (CD CHO + 50 g/L Glucose + 40 mL/L

GlutaMAX™-1) was added, and culture temperature was changed to 36.5°C. At day 9,3.5 L of Feed #2 (CD CHO + 50 g/L Glucose + 40 mL/L GlutaMAX™-1 + 1.1 g/L Sodium Butyrate) was added, and culture temperature was changed to 36°C. At day 11,3.7 L of Feed #3 (CD CHO + 50 g/L Glucose + 40 mL/L GlutaMAX™-1 + 1.1 g/L Sodium Butyrate) was added, and the culture temperature was changed to 35.5°C. The reactor was harvested at 14 days or when the viability of the cells dropped below 50%. The process resulted in production of soluble rHuPH20 with an enzymatic activity of 1600 Units/ml with a maximal cell density of 8 million cells/mL. At harvest, the culture was sampled for mycoplasma, bioburden, endotoxin, and virus *in vitro* and *in vivo,* transmission electron microscopy (TEM) for viral particles, and enzyme activity.

[0361] The one hundred liter bioreactor cell culture harvest was filtered through a series of disposable capsule filters having a polyethersulfone medium (Sartorius): first through a 8.0 μm depth capsule, a 0.65 μm depth capsule, a 0.22 μm capsule, and finally through a 0.22 μm Sartopore 2000 cm^2 filter and into a 100 L sterile storage bag. The culture was concentrated 10× using two TFF with Spiral Polyethersulfone 30 kDa MWCO filters (Millipore), followed by a 6× buffer exchange with 10 mM HEPES, 25 mM Na_2SO_4, pH 7.0 into a 0.22 μm final filter into a 20 L sterile storage bag. Table 19 provides monitoring data related to the cell culture, harvest, concentration and buffer exchange steps.

Table 19. Monitoring data for cell culture, harvest, concentration and buffer exchange steps.

Parameter	HUA0406C	HUA04010C	HUA0415C	HUA0420C
Time from thaw to inoculate 100 L bioreactor (days)	21	19	17	18
100 L inoculation density (× 10^6 cells/mL)	0.45	0.33	0.44	0.46
Doubling time in logarithmic growth (hr)	29.8	27.3	29.2	23.5
Max. cell density (× 10^6 cells/mL)	5.65	8.70	6.07	9.70
Harvest viability (%)	41	48	41	41
Harvest titer (U/ml)	1964	1670	991	1319
Time in 100-L bioreactor (days)	13	13	12	13
Clarified harvest volume (mL)	81800	93300	91800	89100
Clarified harvest enzyme assay (U/mL)	2385	1768	1039	1425
Concentrate enzyme assay (U/mL)	22954	17091	8561	17785
Buffer exchanged concentrate enzyme assay (U/mL)	15829	11649	9915	8679
Filtered buffer exchanged concentrate enzyme assay (U/mL)	21550	10882	9471	8527
Buffer exchanged concentrate volume (mL)	10699	13578	12727	20500
Ratio enzyme units concentration/ harvest	0.87	0.96	1.32	1.4

[0362] A Q Sepharose (Pharmacia) ion exchange column (3 L resin, Height = 20 cm, Diameter = 14 cm) was prepared. Wash samples were collected for a determination of pH, conductivity and endotoxin (LAL) assay. The column was equilibrated with 5 column volumes of 10 mM Tris, 20 mM Na_2SO_4, pH 7.5. The concentrated, diafiltered harvest was loaded onto the Q column at a flow rate of 100 cm/hr. The column was washed with 5 column volumes of 10 mM Tris, 20 mM Na_2SO_4, pH 7.5 and 10 mM Hepes, 50 mM NaCl, pH 7.0. The protein was eluted with 10 mM Hepes, 400 mM NaCl, pH 7.0 and filtered through a 0.22 μm final filter into a sterile bag.

[0363] Phenyl-Sepharose (Pharmacia) hydrophobic interaction chromatography was next performed. A Phenyl-Sepharose (PS) column (9.1 L resin, Height = 29 cm, Diameter = 20cm) was prepared. The column was equilibrated with 5 column volumes of 5 mM potassium phosphate, 0.5 M ammonium sulfate, 0.1 mM $CaCl_2$, pH 7.0. The protein eluate from above was supplemented with 2M ammonium sulfate, 1 M potassium phosphate and 1 M $CaCl_2$ stock solutions to

final concentrations of 5 mM, 0.5 M and 0.1 mM, respectively. The protein was loaded onto the PS column at a flow rate of 100 cm/hr. 5 mM potassium phosphate, 0.5 M ammonium sulfate and 0.1 mM $CaCl_2$ pH 7.0 was added at 100 cm/hr. The flow through was passed through a 0.22 μm final filter into a sterile bag.

[0364] The PS-purified protein was the loaded onto an aminophenyl boronate column (ProMedics) (6.3 L resin, Height = 20 cm, Diameter = 20cm) that had been equilibrated with 5 column volumes of 5 mM potassium phosphate, 0.5 M ammonium sulfate. The protein was passed through the column at a flow rate of 100 cm/hr, and the column was washed with 5 mM potassium phosphate, 0.5 M ammonium sulfate, pH 7.0. The column was then washed with 20 mM bicine, 100 mM NaCl, pH 9.0 and the protein eluted with 50 mM Hepes, 100 mM NaCl pH 6.9 through a sterile filter and into a 20 L sterile bag. The eluate was tested for bioburden, protein concentration and enzyme activity.

[0365] A hydroxyapatite (HAP) column (BioRad) (1.6 L resin, Height = 10 cm, Diameter = 14 cm) was equilibrated with 5 mM potassium phosphate, 100 mM NaCl, 0.1 mM $CaCl_2$ pH 7.0. Wash samples were collected and tested for pH, conductivity and endotoxin (LAL assay. The aminophenyl boronate purified protein was supplemented with potassium phosphate and $CaCl_2$ to yield final concentrations of 5 mM potassium phosphate and 0.1 mM $CaCl_2$ and loaded onto the HAP column at a flow rate of 100 cm/hr. The column was washed with 5 mM potassium phosphate pH 7.0, 100 mM NaCl, 0.1 mM $CaCl_2$, then 10 mM potassium phosphate pH 7.0, 100 mM NaCl, 0.1 mM $CaCl_2$ pH. The protein was eluted with 70 mM potassium phosphate pH 7.0 and filtered through a 0.22 μm filter into a 5 L sterile storage bag. The eluate was tested for bioburden, protein concentration and enzyme activity.

[0366] The HAP-purified protein was then pumped through a 20 nM viral removal filter via a pressure tank. The protein was added to the DV20 pressure tank and filter (Pall Corporation), passing through an Ultipor DV20 Filter with 20 nm pores (Pall Corporation) into a sterile 20 L storage bag. The filtrate was tested for protein concentration, enzyme activity, oligosaccharide, monosaccharide and sialic acid profiling, and process-related impurities. The protein in the filtrate was then concentrated to 1 mg/mL using a 10 kD molecular weight cut off (MWCO) Sartocon Slice tangential flow filtration (TFF) system (Sartorius). The filter was first prepared by washing with a Hepes/saline solution (10 mM Hepes, 130 mM NaCl, pH 7.0) and the permeate was sampled for pH and conductivity. Following concentration, the concentrated protein was sampled and tested for protein concentration and enzyme activity. A 6× buffer exchange was performed on the concentrated protein into the final buffer: 10 mM Hepes, 130 mM NaCl, pH 7.0. The concentrated protein was passed though a 0.22 μm filter into a 20 L sterile storage bag. The protein was sampled and tested for protein concentration, enzyme activity, free sulfydryl groups, oligosaccharide profiling and osmolarity.

[0367] Tables 20 to 26 provide monitoring data related to each of the purification steps described above, for each 3D35M cell lot.

Table 20. Q sepharose column data

Parameter	HUA0406C	HUA0410C	HUA0415C	HUA0420C
Load volume (mL)	10647	13524	12852	20418
Load Volume/Resin Volume ratio	3.1	4.9	4.5	7.3
Column Volume (mL)	2770	3840	2850	2880
Eluate volume (mL)	6108	5923	5759	6284
Protein Conc. of Eluate (mg/mL)	2.8	3.05	2.80	2.86
Eluate Enzyme Assay (U/mL)	24493	26683	18321	21052
Enzyme Yield (%)	65	107	87	76

Table 21. Phenyl Sepharose column data

Parameter	HUA0406C	HUA0410C	HUA0415C	HUA0420C
Volume Before Stock Solution Addition (mL)	5670	5015	5694	6251
Load Volume (mL)	7599	6693	7631	8360
Column Volume (mL)	9106	9420	9340	9420
Load Volume/Resin Volume ratio	0.8	0.71	0.82	0.89
Eluate volume (mL)	16144	18010	16960	17328
Protein Cone of Eluate (mg/mL)	0.4	0.33	0.33	0.38

(continued)

Table 21. Phenyl Sepharose column data				
Parameter	**HUA0406C**	**HUA0410C**	**HUA0415C**	**HUA0420C**
Eluate Enzyme Assay (U/mL)	8806	6585	4472	7509
Protein Yield (%)	41	40	36	37
Enzyme Yield (%)	102	88	82	96

Table 22. Amino Phenyl Boronate column data				
Parameter	**HUA0406C**	**HUA0410C**	**HUA0415C**	**HUA0420C**
Load Volume (mL)	16136	17958	16931	17884
Load Volume/Resin Volume ratio	2.99	3.15	3.08	2.98
Column Volume (mL)	5400	5700	5500	5300
Eluate volume (mL)	17595	22084	20686	19145
Protein Conc. of Eluate (mg/mL)	0.0	0.03	0.03	0.04
Protein Conc. of Filtered Eluate (mg/mL)	not tested	0.03	0.00	0.04
Eluate Enzyme Assay (U/mL)	4050	2410	1523	4721
Protein Yield (%)	0	11	11	12
Enzyme Yield (%)	not determined	41	40	69

Table 23. Hydroxyapatite column data				
Parameter	**HUA0406C**	**HUA0410C**	**HUA0415C**	**HUA0420C**
Volume Before Stock Solution Addition (mL)	16345	20799	20640	19103
Load Volume/Resin Volume ratio	10.95	13.58	14.19	12.81
Column Volume (mL)	1500	1540	1462	1500
Load volume (mL)	16429	20917	20746	19213
Eluate volume (mL)	4100	2415	1936	2419
Protein Conc. of Eluate (mg/mL)	not tested	0.24	0.17	0.23
Protein Conc. of Filtered Eluate (mg/mL)	NA	NA	0.17	NA
Eluate Enzyme Assay (U/mL)	14051	29089	20424	29826
Protein Yield (%)	Not tested	93	53	73
Enzyme Yield (%)	87	118	140	104

Table 24. DV20 filtration data				
Parameter	**HUA0406C**	**HUA0410C**	**HUA0415C**	**HUA0420C**
Start volume (mL)	4077	2233	1917	2419
Filtrate Volume (mL)	4602	3334	2963	3504
Protein Conc. of Filtrate (mg/mL)	0.1	NA	0.09	NA
Protein Conc. of Filtered Eluate (mg/mL)	NA	0.15	0.09	0.16

(continued)

Table 24. DV20 filtration data				
Parameter	**HUA0406C**	**HUA0410C**	**HUA0415C**	**HUA0420C**
Protein Yield (%)	not tested	93	82	101

Table 25. Final concentration data				
Parameter	**HUA0406C**	**HUA0410C**	**HUA0415C**	**HUA0420C**
Start volume (mL)	4575	3298	2963	3492
Concentrate Volume (mL)	562	407	237	316
Protein Conc. of Concentrate (mg/mL)	0.9	1.24	1.16	1.73
Protein Yield (%)	111	102	103	98

Table 26. Buffer Exchange into Final Formulation data				
Parameter	**HUA0406C**	**HUA0410C**	**HUA0415C**	**HUA0420C**
Start Volume (mL)	562	407	237	316
Final Volume Buffer Exchanged Concentrate (mL)	594	516	310	554
Protein Conc. of Concentrate (mg/mL)	1.00	0.97	0.98	1.00
Protein Conc. of Filtered Concentrate (mg/mL)	0.95	0.92	0.95	1.02
Protein Yield (%)	118	99	110	101

[0368] The purified and concentrated soluble rHuPH20 protein was aseptically filled into sterile vials with 5 mL and 1 mL fill volumes. The protein was passed though a 0.22 μm filter to an operator controlled pump that was used to fill the vials using a gravimetric readout. The vials were closed with stoppers and secured with crimped caps. The closed vials were visually inspected for foreign particles and then labeled. Following labeling, the vials were flash-frozen by submersion in liquid nitrogen for no longer than 1 minute and stored at ≤-15 °C (-20 ± 5 °C).

Example 14

Production Gen2 Cells Containing Soluble human PH20 (rHuPH20)

[0369] The Gen1 3D35M cell line described in Example 13 was adapted to higher methotrexate levels to produce generation 2 (Gen2) clones. 3D35M cells were seeded from established methotrexate-containing cultures into CD CHO medium containing 4mM GlutaMAX-1™ and 1.0 μM methotrexate. The cells were adapted to a higher methotrexate level by growing and passaging them 9 times over a period of 46 days in a 37°C, 7% CO_2 humidified incubator. The amplified population of cells was cloned out by limiting dilution in 96-well tissue culture plates containing medium with 2.0 μM methotrexate. After approximately 4 weeks, clones were identified and clone 3E10B was selected for expansion. 3E10B cells were grown in CD CHO medium containing 4 mM GlutaMAX-1™ and 2.0 μM methotrexate for 20 passages. A master cell bank (MCB) of the 3E10B cell line was created and frozen and used for subsequent studies.

[0370] Amplification of the cell line continued by culturing 3E10B cells in CD CHO medium containing 4 mM GlutaMAX-1™ and 4.0 μM methotrexate. After the 12th passage, cells were frozen in vials as a research cell bank (RCB). One vial of the RCB was thawed and cultured in medium containing 8.0 μM methotrexate. After 5 days, the methotrexate concentration in the medium was increased to 16.0 μM, then 20.0 μM 18 days later. Cells from the 8th passage in medium containing 20.0 μM methotrexate were cloned out by limiting dilution in 96-well tissue culture plates containing CD CHO medium containing 4 mM GlutaMAX-1™ and 20.0 μM methotrexate. Clones were identified 5-6 weeks later and clone 2B2 was selected for expansion in medium containing 20.0 μM methotrexate. After the 11th passage, 2B2 cells were frozen in vials as a research cell bank (RCB).

[0371] The resultant 2B2 cells are dihydrofolate reductase deficient (dhfr-) DG44 CHO cells that express soluble

recombinant human PH20 (rHuPH20). The soluble PH20 is present in 2B2 cells at a copy number of approximately 206 copies/cell. Southern blot analysis of Spe I-, Xba I- and BamH I/Hind III-digested genomic 2B2 cell DNA using a rHuPH20-specific probe revealed the following restriction digest profile: one major hybridizing band of ~7.7 kb and four minor hybridizing bands (~13.9, ~6.6, ~5.7 and ~4.6 kb) with DNA digested with Spe I; one major hybridizing band of ~5.0 kb and two minor hybridizing bands (~13.9 and ~6.5 kb) with DNA digested with Xba I; and one single hybridizing band of ~1.4 kb observed using 2B2 DNA digested with BamH I/Hind III. Sequence analysis of the mRNA transcript indicated that the derived cDNA (SEQ ID NO:139) was identical to the reference sequence (SEQ ID NO:110) except for one base pair difference at position 1131, which was observed to be a thymidine (T) instead of the expected cytosine (C). This is a silent mutation, with no effect on the amino acid sequence.

Example 15

A. Production of Gen2 soluble rHuPH20 in 300 L Bioreactor Cell Culture

[0372] A vial of HZ24-2B2 was thawed and expanded from shaker flasks through 36L spinner flasks in CD-CHO media (Invitrogen, Carlsbad, CA) supplemented with 20 μM methotrexate and GlutaMAX-1™ (Invitrogen). Briefly, the vial of cells was thawed in a 37 °C water bath, media was added and the cells were centrifuged. The cells were re-suspended in a 125 mL shake flask with 20 mL of fresh media and placed in a 37°C, 7% CO_2 incubaor. The cells were expanded up to 40 mL in the 125 mL shake flask. When the cell density reached greater than 1.5 x 10^6 cells/mL, the culture was expanded into a 125 mL spinner flask in a 100 mL culture volume. The flask was incubated at 37°C, 7% CO_2. When the cell density reached greater than 1.5 x 10^6 cells/mL, the culture was expanded into a 250 mL spinner flask in 200 mL culture volume, and the flask was incubated at 37°C, 7% CO_2. When the cell density reached greater than 1.5 x 10^6 cells/mL, the culture was expanded into a 1 L spinner flask in 800 mL culture volume and incubated at 37°C, 7% CO_2. When the cell density reached greater than 1.5 x 10^6 cells/mL the culture was expanded into a 6 L spinner flask in 5000 mL culture volume and incubated at 37°C, 7% CO_2. When the cell density reached greater than 1.5 x 10^6 cells/mL the culture was expanded into a 36 L spinner flask in 32 L culture volume and incubated at 37°C, 7% CO_2.

[0373] A 400 L reactor was sterilized and 230 mL of CD-CHO media was added. Before use, the reactor was checked for contamination. Approximately 30 L cells were transferred from the 36L spinner flasks to the 400 L bioreactor (Braun) at an inoculation density of 4.0 × 10^5 viable cells per ml and a total volume of 260L. Parameters were temperature setpoint, 37°C; Impeller Speed 40-55 RPM; Vessel Pressure: 3 psi; Air Sparge 0.5- 1.5 L/Min.; Air Overlay: 3 L/ min. The reactor was sampled daily for cell counts, pH verification, media analysis, protein production and retention. Also, during the run nutrient feeds were added. At 120 hrs (day 5), 10.4L of Feed #1 Medium (4x CD-CHO + 33 g/L Glucose + 160 mL/L Glutamax-1™ + 83 mL/L Yeastolate + 33 mg/L rHuInsulin) was added. At 168 hours (day 7), 10.8 L of Feed #2 (2× CD-CHO + 33 g/L Glucose + 80 mL/L Glutamax-1™ + 167 mL/L Yeastolate + 0.92 g/L Sodium Butyrate) was added, and culture temperature was changed to 36.5°C. At 216 hours (day 9), 10.8 L of Feed #3 (1× CD-CHO + 50 g/L Glucose + 50 mL/L Glutamax-1™ + 250 mL/L Yeastolate + 1.80 g/L Sodium Butyrate) was added, and culture temperature was changed to 36° C. At 264 hours (day 11), 10.8 L of Feed #4 (1× CD-CHO + 33 g/L Glucose + 33 mL/L Glutamax-1™ + 250 mL/L Yeastolate + 0.92 g/L Sodium Butyrate) was added, and culture temperature was changed to 35.5 °C. The addition of the feed media was observed to dramatically enhance the production of soluble rHuPH20 in the final stages of production. The reactor was harvested at 14 or 15 days or when the viability of the cells dropped below 40%. The process resulted in a final productivity of 17,000 Units per ml with a maximal cell density of 12 million cells/mL. At harvest, the culture was sampled for mycoplasma, bioburden, endotoxin and viral *in vitro* and *in vivo,* Transmission Electron Microscopy (TEM) and enzyme activity.

[0374] The culture was pumped by a peristaltic pump through four Millistak filtration system modules (Millipore) in parallel, each containing a layer of diatomaceous earth graded to 4-8 μm and a layer of diatomaceous earth graded to 1.4-1.1 μm, followed by a cellulose membrane, then through a second single Millistak filtration system (Millipore) containing a layer of diatomaceous earth graded to 0.4-0.11 μm and a layer of diatomaceous earth graded to <0.1 μm, followed by a cellulose membrane, and then through a 0.22 μm final filter into a sterile single use flexible bag with a 350 L capacity. The harvested cell culture fluid was supplemented with 10 mM EDTA and 10 mM Tris to a pH of 7.5. The culture was concentrated 10× with a tangential flow filtration (TFF) apparatus using four Sartoslice TFF 30 kDa molecular weight cut-off (MWCO) polyether sulfone (PES) filter (Sartorious), followed by a 10× buffer exchange with 10 mM Tris, 20mM Na_2SO_4, pH 7.5 into a 0.22 μm final filter into a 50 L sterile storage bag.

[0375] The concentrated, diafiltered harvest was inactivated for virus. Prior to viral inactivation, a solution of 10% Triton® X-100, 3% tri (n-butyl) phosphate (TNBP) was prepared. The concentrated, diafiltered harvest was exposed to 1% Triton® X-100, 0.3% TNBP for 1 hour in a 36 L glass reaction vessel immediately prior to purification on the Q column.

B. Purification of Gen2 soluble rHuPH20

[0376] A Q Sepharose (Pharmacia) ion exchange column (9 L resin, H= 29 cm, D= 20 cm) was prepared. Wash samples were collected for a determination of pH, conductivity and endotoxin (LAL) assay. The column was equilibrated with 5 column volumes of 10 mM Tris, 20 mM Na2SO4, pH 7.5. Following viral inactivation, the concentrated, diafiltered harvest was loaded onto the Q column at a flow rate of 100 cm/hr. The column was washed with 5 column volumes of 10 mM Tris, 20 mM Na2SO4, pH 7.5 and 10 mM Hepes, 50 mM NaCl, pH7.0. The protein was eluted with 10 mM Hepes, 400 mM NaCl, pH 7.0 into a 0.22 μm final filter into sterile bag. The eluate sample was tested for bioburden, protein concentration and hyaluronidase activity. A_{280} absorbance reading were taken at the beginning and end of the exchange.

[0377] Phenyl-Sepharose (Pharmacia) hydrophobic interaction chromatography was next performed. A Phenyl-Speharose (PS) column (19-21 L resin, H=29 cm, D= 30 cm) was prepared. The wash was collected and sampled for pH, conductivity and endotoxin (LAL assay). The column was equilibrated with 5 column volumes of 5 mM potassium phosphate, 0.5 M ammonium sulfate, 0.1 mM CaCl2, pH 7.0. The protein eluate from the Q sepharose column was supplemented with 2M ammonium sulfate, 1 M potassium phosphate and 1 M $CaCl_2$ stock solutions to yield final concentrations of 5 mM, 0.5 M and 0.1 mM, respectively. The protein was loaded onto the PS column at a flow rate of 100 cm/hr and the column flow thru collected. The column was washed with 5 mM potassium phosphate, 0.5 M ammonium sulfate and 0.1 mM CaC12 pH 7.0 at 100 cm/hr and the wash was added to the collected flow thru. Combined with the column wash, the flow through was passed through a 0.22 μm final filter into a sterile bag. The flow through was sampled for bioburden, protein concentration and enzyme activity.

[0378] An aminophenyl boronate column (Prometics) was prepared. The wash was collected and sampled for pH, conductivity and endotoxin (LAL assay). The column was equilibrated with 5 column volumes of 5 mM potassium phosphate, 0.5 M ammonium sulfate. The PS flow through containing purified protein was loaded onto the aminophenyl boronate column at a flow rate of 100 cm/hr. The column was washed with 5 mM potassium phosphate, 0.5 M ammonium sulfate, pH 7.0. The column was washed with 20 mM bicine, 0.5 M ammonium sulfate, pH 9.0. The column was washed with 20 mM bicine, 100 mM sodium chloride, pH 9.0. The protein was eluted with 50 mM Hepes, 100 mM NaCl, pH 6.9 and passed through a sterile filter into a sterile bag. The eluted sample was tested for bioburden, protein concentration and enzyme activity.

[0379] The hydroxyapatite (HAP) column (Biorad) was prepared. The wash was collected and tested for pH, conductivity and endotoxin (LAL assay). The column was equilibrated with 5 mM potassium phosphate, 100 mM NaCl, 0.1 mM $CaCl_2$, pH 7.0. The aminophenyl boronate purified protein was supplemented to final concentrations of 5 mM potassium phosphate and 0.1 mM $CaCl_2$ and loaded onto the HAP column at a flow rate of 100 cm/hr. The column was washed with 5 mM potassium phosphate, pH 7, 100 mM NaCl, 0.1 mM $CaCl_2$. The column was next washed with 10 mM potassium phosphate, pH 7, 100 mM NaCl, 0.1 mM $CaCl_2$. The protein was eluted with 70 mM potassium phosphate, pH 7.0 and passed through a 0.22μm sterile filter into a sterile bag. The eluted sample was tested for bioburden, protein concentration and enzyme activity.

[0380] The HAP purified protein was then passed through a viral removal filter. The sterilized Viosart filter (Sartorius) was first prepared by washing with 2 L of 70 mM potassium phosphate, pH 7.0. Before use, the filtered buffer was sampled for pH and conductivity. The HAP purified protein was pumped via a peristaltic pump through the 20 nM viral removal filter. The filtered protein in 70 mM potassium phosphate, pH 7.0 was passed through a 0.22 μm final filter into a sterile bag. The viral filtered sample was tested for protein concentration, enzyme activity, oligosaccharide, monosaccharide and sialic acid profiling. The sample also was tested for process related impurities.

[0381] The protein in the filtrate was then concentrated to 10 mg/mL using a 10 kD molecular weight cut off (MWCO) Sartocon Slice tangential flow filtration (TFF) system (Sartorius). The filter was first prepared by washing with 10 mM histidine, 130 mM NaCl, pH 6.0 and the permeate was sampled for pH and conductivity. Following concentration, the concentrated protein was sampled and tested for protein concentration and enzyme activity. A 6× buffer exchange was performed on the concentrated protein into the final buffer: 10 mM histidine, 130 mM NaCl, pH 6.0. Following buffer exchange, the concentrated protein was passed though a 0.22 μm filter into a 20 L sterile storage bag. The protein was sampled and tested for protein concentration, enzyme activity, free sulfydryl groups, oligosaccharide profiling and osmolarity.

[0382] The sterile filtered bulk protein was then asceptically dispensed at 20 mL into 30 mL sterile Teflon vials (Nalgene). The vials were then flash frozen and stored at-20 ± 5°C.

C. Comparison of production and purification of Gen1 soluble rHuPH20 and Gen2 soluble rHuPH20

[0383] The production and purification of Gen2 soluble rHuPH20 in a 300L bioreactor cell culture contained some changes in the protocols compared to the production and purification Gen1 soluble rHuPH20 in a 100L bioreactor cell culture (described in Example 13B). Table 27 sets forth exemplary differences, in addition to simple scale up changes, between the methods.

Table 27.

Process Difference	Gen1 soluble rHuPH20	Gen2 soluble rHuPH20
Cell line	3D35M	2B2
Media used to expand cell inoculum	Contains 0.10 μM methotrexate (0.045 mg/L)	Contains 20 μM methotrexate (9 mg/L)
Media in 6L cultures onwards	Contains 0.10 μM methotrexate	Contains no methotrexate
36 L spinner flask	No instrumentation 20 L operating volume.	Equipped with instrumentation that monitors and controls pH, dissolved oxygen, sparge and overlay gas flow rate. 32 L operating volume
Final operating volume in bioreactor	Approx. 100 L in a 125 L bioreactor (initial culture volume + 65 L)	Approx. 300L in a 400L bioreactor (initial culture volume + 260L)
Culture media in final bioreactor	No rHuInsulin	5.0 mg/L rHuInsulin
Media feed volume	Scaled at 4% of the bioreactor cell culture volume i.e. 3.4, 3.5 and 3.7 L, resulting in a target bioreactor volume of ~92 L.	Scaled at 4% of the bioreactor cell culture volume i.e. 10.4, 10.8, 11.2 and 11.7 L, resulting in a target bioreactor volume of ~303L.
Media feed	Feed #1 Medium: CD CHO + 50 g/L Glucose + 8mM GlutaMAX™-1 Feed #2 (CD CHO + 50 g/L Glucose + 8 mM GlutaMAX + 1.1 g/L Sodium Butyrate Feed #3: CD CHO + 50 g/L Glucose + 8 mM GlutaMAX + 1.1 g/L Sodium Butyrate	Feed #1 Medium: 4× CD CHO + 33 g/L Glucose + 32 mM Glutamax + 16.6 g/L Yeastolate + 33 mg/L rHuInsulin Feed #2: 2× CD CHO + 33 g/L Glucose + 16 mM Glutamax + 33.4 g/L Yeastolate + 0.92 g/L Sodium Butyrate Feed #3: 1 × CD CHO + 50 g/L Glucose + 10 mM Glutamax + 50 g/L Yeastolate + 1.80 g/L Sodium Butyrate Feed #4:1× CD CHO+ 33 g/L Glucose + 6.6 mM Glutamax + 50 g/L Yeastolate + 0.92 g/L Sodium Butyrate
Filtration of bioreactor cell culture	Four polyethersulfone filters (8.0 μm, 0.65 μm, 0.22 μm and 0.22 μm) in series 100 L storage bag	1st stage - Four modules in parallel, each with a layer of diatomaceous earth graded to 4-8 μm and a layer of diatomaceous earth graded to 1.4-1.1 μm, followed by a cellulose membrane. 2nd stage -single module containing a layer of diatomaceous earth graded to 0.4-0.11 μm and a layer of diatomaceous earth graded to <0.1 μm, followed by a cellulose membrane. 3rd stage - 0.22 μm polyethersulfone filter 300L storage bag Harvested cell culture is supplemented with 10 mM EDTA, 10 mM Tris to a pH of 7.5.
Concentration and buffer exchange prior to chromatography	Concentrate with 2 TFF with Millipore Spiral Polyethersulfone 30K MWCO Filter Buffer Exchange the Concentrate 6× with 10 mM Hepes, 25 mM NaCl, pH 7.0 20L sterile storage bag	Concentrate using four Sartorius Sartoslice TFF 30K MWCO Filter Buffer Exchange the Concentrate 10× with 10 mM Tris, 20 mM Na2SO4, pH 7.5 50L sterile storage bag

(continued)

Table 27.		
Process Difference	**Gen1 soluble rHuPH20**	**Gen2 soluble rHuPH20**
Viral inactivation prior to chromatography	None	Viral inactivation performed with the addition of a 1% Triton® X-100, 0.3% Tributyl Phosphate, pH 7.5,
1st purification step (Q sepharose)	No absorbance reading	A280 measurements at the beginning and end
Viral filtration after chromatography	Pall DV-20 filter (20 nm)	Sartorius Virosart filter (20 nm)
Concentration and buffer exchange after chromatography	Hepes/saline pH 7.0 buffer Protein concentrated to 1 mg/ml	Histidine/saline, pH 6.0 buffer Protein concentrated to 10 mg/ml

Example 16

Determination of sialic acid and monosaccharide content

[0384] The sialic acid and monosaccharide content of soluble rHuPH20 can be assessed by reverse phase liquid chromatography (RPLC) following hydrolysis with trifluoroacetic acid. In one example, the sialic acid and monosaccharide content of purified hyaluronidase lot # HUB0701E (1.2 mg/mL; produced and purified essentially as described in Example 15) was determined. Briefly, 100 μg sample was hydrolyzed with 40 % (v/v) trifluoroacetic acid at 100 °C for 4 hours in duplicate. Following hydrolysis, the samples were dried down and resuspended in 300 μL water. A 45 μL aliquot from each re-suspended sample was transferred to a new tube and dried down, and 10 μL of a 10 mg/mL sodium acetate solution was added to each. The released monosaccharides were fluorescently labeled by the addition of 50 μL of a solution containing 30 mg/mL 2-aminobenzoic acid, 20 mg/mL sodium cyanoborohydride, approximately 40 mg/mL sodium acetate and 20 mg/mL boric acid in methanol. The mixture was incubated for 30 minutes at 80 °C in the dark. The derivitization reaction was quenched by the addition of 440 μL of mobile phase A (0.2% (v/v) n-butylamine, 0.5% (v/v) phosphoric acid, 1% (v/v) tetrahydrofuran). A matrix blank of water also was hydrolyzed and derivitized as described for the hyaluronidase sample as a negative control. The released monosaccharides were separated by RPLC using an Octadecyl (C_{18}) reverse phase column (4.6 x 250 mm, 5 μm particle size; J.T. Baker) and monitored by fluorescence detection (360 nm excitation, 425 nm emission). Quantitation of the monosaccharide content was made by comparison of the chromatograms from the hyaluronidase sample with chromatograms of monosaccharide standards including N-D-glucosamine (GlcN), *N*-D-galactosamine (GalN), galactose, fucose and mannose. Table 28 presents the molar ratio of each monosaccharide per hyaluronidase molecule.

Table 28. Monosaccharide content of soluble rHuPH20						
Lot	**Replicate**	**GlcN**	**GalN**	**Galactose**	**Mannose**	**Fucose**
HUB0701E	**1**	14.28	0.07*	6.19	25.28	2.69
	2	13.66	0.08*	6.00	24.34	2.61
	Average	13.97	0.08*	6.10	24.81	2.65
* GalN results were below the limit of detection						

Example 17

C-terminal heterogeneity of soluble rHuPH20 from 3D35M and 2B2 cells

[0385] C-terminal sequencing was performed on two lots of sHuPH20 produced and purified from 3D35M cells in a 100 L bioreactor volume (Lot HUA0505MA) and 2B2 cells in a 300L bioreactor volume (Lot HUB0701EB). The lots were separately digested with endoproteinase Asp-N, which specifically cleaves peptide bonds N-terminally at aspartic and cysteic acid. This releases the C-terminal portion of the soluble rHuPH20 at the aspartic acid at position 431 of SEQ ID

NO:122. The C-terminal fragments were separated and characterized to determine the sequence and abundance of each population in Lot HUA0505MA and Lot HUB0701EB.

[0386] It was observed that the soluble rHuPH20 preparations from 3D35M cells and 2B2 cells displayed heterogeneity, and contained polyepeptides that differed from one another in their C-terminal sequence (Tables 30 and 31). This heterogeneity is likely the result of C-terminal cleavage of the expressed 447 amino acid polypeptide (SEQ ID NO: 122) by peptidases present in the cell culture medium or other solutions during the production and purification process. The polypeptides in the soluble rHuPH20 preparations have amino acid sequences corresponding to amino acids 1-447, 1-446, 1-445, 1-444 and 1-443 of the soluble rHuPH20 sequence set forth SEQ ID NO:122. The full amino acid sequence of each of these polypeptides is forth in SEQ ID NOS: 122 to 126, respectively. As noted in tables 29 and 30, the abundance of each polypeptide in the soluble rHuPH20 preparations from 3D35M cells and 2B2 cells differs.

Table 29. Analysis of C-terminal fragments from Lot HUA0505MA							
Fragment	**Amino acid position (relative to SEQ ID NO: 122)**	**Sequence**	**Theor. mass**	**Exp. Mass**	**Error**	**Elution time**	**Abundance**
D28a	431-447	DAFKLPPMETEEPQIF Y (SEQ ID NO:191)	2053.97	2054.42	0.45	99.87	0.2%
D28b	431-446	DAFKLPPMETEEPQIF (SEQ ID NO:192)	1890.91	1891.28	0.37	97.02	18.4%
D28c	431-445	DAFKLPPMETEEPQI (SEQ ID NO:193)	1743.84	1744.17	0.33	86.4	11.8%
D28d	431-444	DAFKLPPME T EEPQ (SEQ ID NO:194)	1630.70	1631.07	0.32	74.15	56.1%
D28e	431-443	DAFKLPPMETEEP (SEQ ID NO:195)	1502.70	1502.98	0.28	77.36	13.6%
D28f	431-442	DAFKLPPMETEE (SEQ ID NO:196)	1405.64	ND	N/A	N/A	0.0%

Table 30. Analysis of C-terminal fragments from Lot HUB0701EB							
Fragment	**Amino acid position (relative to SEQ ID NO: 122)**	**Sequence**	**Theor. mass**	**Exp. Mass**	**Error**	**Elution time**	**Abund -ance**
D28a	431-447	DAFKLPPMETEEPQIF Y (SEQ ID NO:191)	2053.97	2054.42	0.45	99.89	1.9%
D28b	431-446	DAFKLPPMETEEPQIF (SEQ ID NO:192)	1890.91	1891.36	0.45	96.92	46.7%
D28c	431-445	DAFKLPPMETEEPQI (SEQ ID NO:193)	1743.84	1744.24	0.40	85.98	16.7%
D28d	431-444	DAFKLPPMETEEPQ (SEQ ID NO:194)	1630.70	1631.14	0.39	73.9	27.8%
D28e	431-443	DAFKLPPMETEEP (SEQ ID NO:195)	1502.70	1503.03	0.33	77.02	6.9%
D28f	431-442	DAFKLPPMETEE (SEQ ID NO:196)	1405.64	ND	N/A	N/A	0.0%

The present disclosure also includes the following items.

ITEMS:

[0387]

1. An extended soluble PH20 hyaluronidase (esPH20).
2. An esPH20 of item 1 that is N-glycosylated, N-partially glycosylated or is deglycosylated.
3. An esPH20 of item 1 or item 2, wherein the N-partially glycosylated polypeptide comprises at least an N-acetylglucosamine moiety linked to each of at least three asparagine (N) residues.
4. An esPH20 of item 3, wherein the three asparagine residues are amino acid residues 235, 368 and 393 of SEQ ID NO: 107 or are residues corresponding to amino acid residues 235, 368 and 393 of SEQ ID NO: 107.
5. An esPH20 of any of items 1-4 that is selected from among:

   a polypeptide that consists of the sequence of amino acids set forth in any of SEQ ID NOS: 60-63 and 102-104; or a polypeptide that contains amino acid substitutions in the sequence of amino acids set forth in any of SEQ ID NOS: 60-63 and 102-104, whereby the amino acid-substituted polypeptide consists of a sequence of amino acids that has at least 85%, 87%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity with the corresponding sequence of amino acids set forth in any of SEQ ID NOS: 60-63 and 102-104.

6. An esPH20 of any of items 1-5 that is neutral active.
7. An esPH20 of any of items 1-5 that is a human esPH20.
8. A esPH20 of any of items 1-4 that is a chimpanzee esPH20.
9. An esPH20 of item 5 that consists of the sequence of amino acids set forth in any of SEQ ID NOS: 60-63 and 102-104.
10. An esPH20 of any of items 1-4 that is selected from among:

    a polypeptide that consists of the sequence of amino acids set forth as amino acids 36-491, 36-492, 36-493, 36-494, 36-495, 36-496, 36-497, 36-498, 36-499 or 36-500 of SEQ ID NO: 107; or
    a polypeptide that contains amino acid substitutions in the sequence of amino acids set forth as amino acids 36-491, 36-492, 36-493, 36-494, 36-495, 36-496, 36-497, 36-498, 36-499 or 36-500 of SEQ ID NO: 107, whereby the amino acid-substituted polypeptide consists of a sequence of amino acids that has at least 85%, 87%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity with the corresponding sequence

of amino acids set forth as amino acids 36-491, 36-492, 36-493, 36-494, 36-495, 36-496, 36-497, 36-498, 36-499 or 36-500 of SEQ ID NO: 107.

11. An esPH20 of item 10 that consists of the sequence of amino acids set forth as amino acids 36-491, 36-492, 36-493, 36-494, 36-495, 36-496, 36-497, 36-498, 36-499 or 36-500 of SEQ ID NO: 107.

12. A truncated PH20 hyaluronidase that consists of the sequence of amino acids set forth as amino acids 1-465, 1-491, 1-492, 1-493, 1-494, 1-495, 1-496, 1-497, 1-498, 1-499 or 1-500 of SEQ ID NO: 107; or
contains amino acid substitutions in the sequence of amino acids set forth as amino acids 1-465, 1-491, 1-492, 1-493, 1-494, 1-495, 1-496, 1-497, 1-498, 1-499 or 1-500 of SEQ ID NO: 107, whereby the amino acid-substituted PH20 hyaluronidase consists of a sequence of amino acids that has at least 85%, 87%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity with the sequence of amino acids set forth as amino acids 1-465, 1-491, 1-492, 1-493, 1-494, 1-495, 1-496, 1-497, 1-498, 1-499 or 1-500 of SEQ ID NO: 107.

13. A truncated PH20 hyaluronidase that consists of the sequence of amino acids set forth as amino acids 36-465, 36-484, 36-485, 36-486, 36-487, 36-488 or 36-489 of SEQ ID NO: 107, or contains amino acid substitutions in the sequence of amino acids set forth as amino acids 36-465, 36-484, 36-485, 36-486, 36-487, 36-488 or 36-489 of SEQ ID NO: 107, whereby the amino acid-substituted PH20 hyaluronidase consists of a sequence of amino acids that has at least 85%, 87%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity with the sequence of amino acids set forth as amino acids 36-465, 36-484, 36-485, 36-486, 36-487, 36-488 or 36-489 of SEQ ID NO: 107.

14. A PH20 of item 12 or item 13 that is N-glycosylated, N-partially glycosylated or is deglycosylated.

15. A PH20 of any of items 12-14 that is neutral active.

16. A truncated PH20 hyaluronidase glycoprotein that consists of the sequence of amino acids set forth in any of SEQ ID NOS: 55-106, or contains amino acid substitutions in the sequence of amino acids set forth in any of SEQ ID NOS: 55-106, whereby the amino acid-substituted PH20 hyaluronidase consists of a sequence of amino acids that has at least 85%, 87%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity with the sequence of amino acids set forth in any of SEQ ID NOS: 55-106, wherein the PH20 hyaluronidase glycoprotein is N-glycosylated but is not glycosylated at one or more amino acid residues corresponding to amino acid residues 82, 166 or 254 of SEQ ID NO: 107.

17. A PH20 of item 6 that comprises at least an N-acetylglucosamine moiety linked to each of at least three asparagine (N) residues.

18. A PH20 of item 17, wherein the three asparagine residues are amino acid residues 235, 368 and 393 of SEQ ID NO:107 or residues corresponding to amino acid residues 235, 368 and 393 of SEQ ID NO:107.

19. A PH20 of any of items 16-18 that is neutral active.

20. A PH20 of any of items 16-19 that is soluble.

21. A PH20 of any of items 16-20, wherein the PH20 is selected from among human, chimpanzee, rhesus monkey and cynomolgus monkey.

22. An esPH20 or PH20 of any of items 1-21 that is modified by modification selected from among sialation, albumination, farnysylation, carboxylation, hydroxylation and phosphorylation.

23. An esPH20 or PH20 of any of items 1-21 that is modified by a polymer.

24. An esPH20 or PH20 of item 23, wherein the polymer is dextran or PEG.

25. A PH20 hyaluronidase glycoprotein, comprising the sequence of amino acids set forth in SEQ ID NO: 107, or a truncated neutral active form thereof, or a sequence of amino acids that has at least 85%, 87%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity with the sequence of amino acids set forth in SEQ ID NO: 107 or truncated forms thereof, wherein the PH20 hyaluronidase glycoprotein is N-glycosylated but is not glycosylated at one or more amino acid residues corresponding to amino acid residues 82, 166 or 254 of SEQ ID NO: 107.

26. An esPH20 or PH20 of any of items 1-25, wherein the polypeptide is modified to have decreased immunogenicity in a human compared to the unmodified polypeptide.

27. The esPH20 or PH20 of item 26, wherein the primary sequence is altered to eliminate epitopes that contribute to antigenicity.

28. An esPH20 or PH20 of any of items 1-25, wherein the polypeptide is produced by expression in a host cell that does not bifucosylate the polypeptide.

29. The esPH20 or PH20 of item 28, wherein the host cell is a mammalian host cell or a mammalianized insect cell expression system.

30. An esPH20 or PH20 of any of items 1-25 that is not bifucosylated.

31. The esPH20 or PH20 of any of items 1-30 that is substantially purified or isolated.

32. A conjugate, comprising the esPH20 or PH20 of any of items 1-31.

33. A conjugate of item 32, wherein the esPH20 or PH20 is conjugated to a moiety selected from among a mul-

timerization domain, toxin, detectable label or drug.

34. A conjugate of item 33, wherein the esPH20 or PH20 is conjugated to an Fc domain.

35. A conjugate of item 32, wherein the esPH20 or PH20 is conjugated to a polymer.

36. A conjugate of item 35, wherein the polymer is dextran or PEG.

37. A conjugate of any of items 32-36, wherein the conjugated moiety is linked directly or via a linker to the C-terminus or N-terminus of the esPH20 or PH20.

38. A nucleic acid molecule encoding the esPH20 or PH20 of any of items 1-21, wherein the nucleic acid molecule does not encode a full-length PH20 but a encodes a truncated PH20 or encodes an esPH20.

39. An expression vector, comprising a polynucleotide that consists of the sequence of nucleotides encoding a PH20 or esPH20 of any of items 1-21 each including or immediately followed by a stop codon operatively inserted into the vector for expression of the PH20 or esPH20 of any of items 1-21.

40. The nucleic acid molecule of item 38 or item 39 that encodes an esPH20 polypeptide that consists of amino acids of 36-491, 36-492, 36-493, 36-494, 36-495, 36-496, 36-497, 36-498, 36-499 or 36-500 of SEQ ID NO: 107; or a polypeptide that contains amino acid substitutions in the sequence of amino acids set forth as amino acids 36-491, 36-492, 36-493, 36-494, 36-495, 36-496, 36-497, 36-498, 36-499 or 36-500 of SEQ ID NO: 107, whereby the amino acid-substituted esPH20 polypeptide consists of a sequence of amino acids that has at least 85%, 87%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity with the sequence of amino acids set forth as amino acids of 36-491, 36-492, 36-493, 36-494, 36-495, 36-496, 36-497, 36-498, 36-499 or 36-500 of SEQ ID NO: 107.

41. A nucleic acid molecule that is an expression vector, comprising a polynucleotide that consists of the sequence of nucleotides set forth in any of SEQ ID NOS: 150-154, 200-201 and 213-215, or degenerates thereof and includes a stop codon to produce a truncated PH20 or esPH20 encoded by any of SEQ ID NOS: 150-154, 200-201 and 213-215, wherein the polynucleotide is operatively linked for expression to produce the encoded truncated PH20 or esPH20.

42. The nucleic acid of item 38 that encodes an esPH20 that consists of amino acids 36-491, 36-492, 36-493, 36-494, 36-495, 36-496, 36-497 or 36-498 of SEQ ID NO:107.

43. A vector, comprising the nucleic acid of any of items 38, 40 and 42.

44. A cell, comprising a vector of item 39 or 43 or a nucleic acid molecule of any of items 38 and 40-42.

45. The cell of item 44 that is a CHO cell.

46. A composition, comprising an esPH20 or PH20 polypeptide of any of items 1-31.

47. A composition, comprising a plurality of esPH20 or PH20 polypeptides of any of items 1-21.

48. A composition, comprising a plurality of esPH20 or PH20 polypeptides of any of items 22-31.

49. The composition of item 47, wherein the plurality of esPH20 polypeptides is encoded by a nucleic acid molecule of any of items 38 and 40-42 or a vector of item 39 or item 43.

50. The composition of any of items 46-49, wherein the esPH20 or PH20 polypeptides are secreted from CHO cells.

51. A composition of any of items 46-50, that is a pharmaceutical composition.

52. A composition of item 51 comprising an additional therapeutic agent.

53. The composition of item 52, wherein the therapeutic agent is formulated with the composition or in a separate composition.

54. The composition of item 52 or item 53, wherein the therapeutic agent is selected from among a chemotherapeutic agent, an analgesic agent, an anti-inflammatory agent, an antimicrobial agent, an amoebicidal agent, a trichomonocidal agent, an anti-parkinson agent, an anti-malarial agent, an anticonvulsant agent, an anti-depressant agent, and antiarthritics agent, an anti-fungal agent, an antihypertensive agent, an antipyretic agent, an anti-parasite agent, an antihistamine agent, an alpha-adrenargic agonist agent, an alpha blocker agent, an anesthetic agent, a bronchial dilator agent, a biocide agent, a bactericide agent, a bacteriostat agent, a beta adrenergic blocker agent, a calcium channel blocker agent, a cardiovascular drug agent, a contraceptive agent, a decongestant agent, a diuretic agent, a depressant agent, a diagnostic agent, a electrolyte agent, a hypnotic agent, a hormone agent, a hyperglycemic agent, a muscle relaxant agent, a muscle contractant agent, an ophthalmic agent, a parasympathomimetic agent, a psychic energizer agent, a sedative agent, a sympathomimetic agent, a tranquilizer agent, an urinary agent, a vaginal agent, a viricide agent, a vitamin agent, a non-steroidal anti-inflammatory agent, an angiotensin converting enzyme inhibitor agent, a polypeptide, a protein, a nucleic acid, a drug, an organic molecule or a sleep inducer.

55. The composition of any of items 52-54, wherein the therapeutic agent is selected from among an antibody, an immunoglobulin, a bisphosphonate, a cytokine, a chemotherapeutic agent and an insulin.

56. The composition of item 55, wherein the insulin is a fast-acting insulin and the bisphosphonate is zoledronic acid.

57. A method for treating a hyaluronan-associated disease or condition, comprising administering to a subject an esPH20 or PH20 of any of items 1-31 or a composition of any of items 46-56.

58. A method for treating an excess of glycosaminoglycans; for treating a tumor; for treating glycosaminoglycan accumulation in the brain; for treating a cardiovascular disorder; for treating an ophthalmic disorder; for treating

pulmonary disease; for increasing penetration of chemotherapeutic agents into solid tumors; for treating cellulite; for treating a proliferative disorder; or for increasing bioavailability of drugs and other therapeutic agents, comprising administering to a subject an esPH20 or PH20 of any of items 1-31 or a composition of any of items 46-56.

59. The method of item 58, wherein the proliferative disorder is benign prostatic hyperplasia.

60. The method of item 58, or the composition of any of items 52-56, wherein the additional therapeutic agent is selected from among Acivicins; Aclarubicins; Acodazoles; Acronines; Adozelesins; Aldesleukins; Alemtuzumabs; Alitretinoins (9-Cis-Retinoic Acids); Allopurinols; Altretamines; Alvocidibs; Ambazones; Ambomycins; Ametantrones; Amifostines; Aminoglutethimides; Amsacrines; Anastrozoles; Anaxirones; Ancitabines; Anthramycins; Apaziquones; Argimesnas; Arsenic Trioxides; Asparaginases; Asperlins; Atrimustines; Azacitidines; Azetepas; Azotomycins; Banoxantrones; Batabulins; Batimastats; BCG Live; Benaxibines; Bendamustines; Benzodepas; Bexarotenes; Bevacizumab; Bicalutamides; Bietaserpines; Biricodars; Bisantrenes; Bisantrenes; Bisnafide Dimesylates; Bizelesins; Bleomycins; Bortezomibs; Brequinars; Bropirimines; Budotitanes; Busulfans; Cactinomycins; Calusterones; Canertinibs; Capecitabines; Caracemides; Carbetimers; Carboplatins; Carboquones; Carmofurs; Carmustines with Polifeprosans; Carmustines; Carubicins; Carzelesins; Cedefingols; Celecoxibs; Cemadotins; Chlorambucils; Cioteronels; Cirolemycins; Cisplatins; Cladribines; Clanfenurs; Clofarabines; Crisnatols; Cyclophosphamides; Cytarabine liposomals; Cytarabines; Dacarbazines; Dactinomycins; Darbepoetin Alfas; Daunorubicin liposomals; Daunorubicins/Daunomycins; Daunorubicins; Decitabines; Denileukin Diftitoxes; Dexniguldipines; Dexonnaplatins; Dexrazoxanes; Dezaguanines; Diaziquones; Dibrospidiums; Dienogests; Dinalins; Disermolides; Docetaxels; Dofequidars; Doxifluridines; Doxorubicin liposomals; Doxorubicin HCL; Docorubicin HCL liposome injection; Doxorubicins; Droloxifenes; Dromostanolone Propionates; Duazomycins; Ecomustines; Edatrexates; Edotecarins; Eflornithines; Elacridars; Elinafides; Elliott's B Solutions; Elsamitrucins; Emitefurs; Enloplatins; Enpromates; Enzastaurins; Epipropidines; Epirubicins; Epoetin alfas; Eptaloprosts; Erbulozoles; Esorubicins; Estramustines; Etanidazoles; Etoglucids; Etoposide phosphates; Etoposide VP-16s; Etoposides; Etoprines; Exemestanes; Exisulinds; Fadrozoles; Fazarabines; Fenretinides; Filgrastims; Floxuridines; Fludarabines; Fluorouracils; 5-fluorouracils; Fluoxymesterones; Flurocitabines; Fosquidones; Fostriecins; Fostriecins; Fotretamines; Fulvestrants; Galarubicins; Galocitabines; Gemcitabines; Gemtuzumabs/Ozogamicins; Geroquinols; Gimatecans; Gimeracils; Gloxazones; Glufosfamides; Goserelin acetates; Hydroxyureas; Ibritumomabs/Tiuxetans; Idarubicins; Ifosfamides; Ilmofosines; Ilomastats; Imatinib mesylates; Imexons; Improsulfans; Indisulams; Inproquones; Interferon alfa-2as; Interferon alfa-2bs; Interferon Alfas; Interferon Betas; Interferon Gammas; Interferons; Interleukin-2s and other Interleukins (including recombinant Interleukins); Intoplicines; Iobenguanes [131-I]; Iproplatins; Irinotecans; Irsogladines; Ixabepilones; Ketotrexates; L-Alanosines; Lanreotides; Lapatinibs; Ledoxantrones; Letrozoles; Leucovorins; Leuprolides; Leuprorelins (Leuprorelides); Levamisoles; Lexacalcitols; Liarozoles; Lobaplatins; Lometrexols; Lomustines/CCNUs; Lomustines; Lonafamibs; Losoxantrones; Lurtotecans; Mafosfamides; Mannosulfans; Marimastats; Masoprocols; Maytansines; Mechlorethamines; Mechlorethamines/Nitrogen mustards; Megestrol acetates; Megestrols; Melengestrols; Melphalans; MelphalansIL-PAMs; Menogarils; Mepitiostanes; Mercaptopurines; 6-Mecaptopurine; Mesnas; Metesinds; Methotrexates; Methoxsalens; Metomidates; Metoprines; Meturedepas; Miboplatins; Miproxifenes; Misonidazoles; Mitindomides; Mitocarcins; Mitocromins; Mitoflaxones; Mitogillins; Mitoguazones; Mitomalcins; Mitomycin Cs; Mitomycins; Mitonafides; Mitoquidones; Mitospers; Mitotanes; Mitoxantrones; Mitozolomides; Mivobulins; Mizoribines; Mofarotenes; Mopidamols; Mubritinibs; Mycophenolic Acids; Nandrolone Phenpropionates; Nedaplatins; Nelzarabines; Nemorubicins; Nitracrines; Nocodazoles; Nofetumomabs; Nogalamycins; Nolatrexeds; Nortopixantrones; Octreotides; Oprelvekins; Ormaplatins; Ortataxels; Oteracils; Oxaliplatins; Oxisurans; Oxophenarsines; Paclitaxels; Pamidronates; Patubilones; Pegademases; Pegaspargases; Pegfilgrastims; Peldesines; Peliomycins; Pelitrexols; Pemetrexeds; Pentamustines; Pentostatins; Peplomycins; Perfosfamides; Perifosines; Picoplatins; Pinafides; Pipobromans; Piposulfans; Pirfenidones; Piroxantrones; Pixantrones; Plevitrexeds; Plicamycid Mithramycins; Plicamycins; Plomestanes; Plomestanes; Porfimer sodiums; Porfimers; Porfiromycins; Prednimustines; Procarbazines; Propamidines; Prospidiums; Pumitepas; Puromycins; Pyrazofurins; Quinacrines; Ranimustines; Rasburicases; Riboprines; Ritrosulfans; Rituximabs; Rogletimides; Roquinimexs; Rufocromomycins; Sabarubicins; Safingols; Sargramostims; Satraplatins; Sebriplatins; Semustines; Simtrazenes; Sizofirans; Sobuzoxanes; Sorafenibs; Sparfosates; Sparfosic Acids; Sparsomycins; Spirogermaniums; Spiromustines; Spiroplatins; Spiroplatins; Squalamines; Streptonigrins; Streptovarycins; Streptozocins; Sufosfamides; Sulofenurs; Sunitinib Malate; 6-TG; Tacedinalines; Talcs; Talisomycins; Tallimustines; Tamoxifens; Tariquidars; Tauromustines; Tecogalans; Tegafurs; Teloxantrones; Temoporfins; Temozolomides; Teniposides/VM-26s; Teniposides; Teroxirones; Testolactones; Thiamiprines; Thioguanines; Thiotepas; Tiamiprines; Tiazofurins; Tilomisoles; Tilorones; Timcodars; Timonacics; Tirapazamines; Topixantrones; Topotecans; Toremifenes; Tositumomabs; Trabectedins (Ecteinascidin 743); Trastuzumabs; Trestolones; Tretinoins/ATRA; Triciribines; Trilostanes; Trimetrexates; Triplatin Tetranitrates; Triptorelins; Trofosfamides; Tubulozoles; Ubenimexs; Uracil Mustards; Uredepas; Vairubicins; Valspodars; Vapreotides; Verteporfins; Vinblastines; Vincristines; Vindesines; Vinepidines; Vinflunines; Vinformides; Vinglycinates; Vinleucinols; Vinleurosines; Vinorelbines; Vinrosidines; Vintriptols; Vinzolidines; Vorozoles; Xanthomycin A's (Gua-

mecyclines); Zeniplatins; Zilascorbs [2-H]; Zinostatins; Zoledronate; Zorubicins; and Zosuquidars.

61. The method of item 59, wherein PH20 or esPH20 is pegylated.

62. Use of a esPH20 or PH20 of any of items 1-31 or a composition of any of items 46-56 in the formulation of a medicament for treating a hyaluronan-associated disease or condition.

63. A pharmaceutical composition, comprising a polypeptide of any of items 1-30 for treating a hyaluronan-associated disease or condition.

64. A pharmaceutical composition of any of items 46-56 for treating a hyaluronan-associated disease or condition.

65. Use of a esPH20 or PH20 of any of items 1-31 or a composition of any of items 46-56 for the formulation of a medicatment for treating an excess of glycosaminoglycans; for treating a tumor; for treating glycosaminoglycan accumulation in the brain; for treating a cardiovascular disorder; for treating an ophthalmic disorder; for treating pulmonary disease; for increasing penetration of chemotherapeutic agents into solid tumors; for treating cellulite; for treating a proliferative disorder; or for increasing bioavailability of drugs and other therapeutic agents.

66. The use of item 65, wherein the proliferative disorder is benign prostatic hyperplasia.

67. A pharmaceutical composition, comprising a polypeptide of any of items 1-31, for use in treating an excess of glycosaminoglycans; for treating a tumor; for treating glycosaminoglycan accumulation in the brain; for treating a cardiovascular disorder; for treating an ophthalmic disorder; for treating pulmonary disease; for increasing penetration of chemotherapeutic agents into solid tumors; for treating cellulite; for treating a proliferative disorder; or for increasing bioavailability of drugs and other therapeutic agents.

68. A pharmaceutical composition of any of items 46-56 for use in treating an excess of glycosaminoglycans; for treating a tumor; for treating glycosaminoglycan accumulation in the brain; for treating a cardiovascular disorder; for treating an ophthalmic disorder; for treating pulmonary disease; for increasing penetration of chemotherapeutic agents into solid tumors; for treating cellulite; for treating a proliferative disorder; or for increasing bioavailability of drugs and other therapeutic agents.

69. The pharmaceutical composition of item 67 or item 68, wherein the proliferative disorder is benign prostatic hyperplasia.

[0388] Since modifications will be apparent to those of skill in this art, it is intended that this invention be limited only by the scope of the appended claims.

SEQUENCE LISTING

[0389]

<110> Halozyme, Inc.

<120> Extended Soluble PH20 Polypeptides and
Uses Thereof

<130> 186030

<150> US 61/201,384
<151> 2008-12-09

<150> US 61/281,240
<151> 2009-11-13

<160> 215

<170> FastSEQ for Windows Version 4.0

<210> 1
<211> 1530
<212> DNA
<213> Homo sapiens

<220>
<223> Human PH20 cDNA encoding amino acids 1-509

<400> 1

```
atgggagtgc taaaattcaa gcacatcttt ttcagaagct ttgttaaatc aagtggagta 60
tcccagatag ttttcacctt ccttctgatt ccatgttgct tgactctgaa tttcagagca 120
cctcctgtta ttccaaatgt gcctttcctc tgggcctgga atgccccaag tgaattttgt 180
cttggaaaat ttgatgagcc actagatatg agcctcttct ctttcatagg aagcccccga 240
ataaacgcca ccgggcaagg tgttacaata ttttatgttg atagacttgg ctactatcct 300
tacatagatt caatcacagg agtaactgtg aatggaggaa tcccccagaa gatttcctta 360
caagaccatc tggacaaagc taagaaagac attacatttt atatgccagt agacaatttg 420
ggaatggctg ttattgactg ggaagaatgg agacccactt gggcaagaaa ctggaaacct 480
aaagatgttt acaagaatag gtctattgaa ttggttcagc aacaaaatgt acaacttagt 540
ctcacagagg ccactgagaa agcaaaacaa gaatttgaaa aggcagggaa ggatttcctg 600
gtagagacta taaaattggg aaaattactt cggccaaatc acttgtgggg ttattatctt 660
tttccggatt gttacaacca tcactataag aaacccggtt acaatggaag ttgcttcaat 720
gtagaaataa aaagaaatga tgatctcagc tggttgtgga atgaaagcac tgctctttac 780
ccatccattt atttgaacac tcagcagtct cctgtagctg ctacactcta tgtgcgcaat 840
cgagttcggg aagccatcag agtttccaaa atacctgatg caaaaagtcc acttccggtt 900
tttgcatata cccgcatagt ttttactgat caagttttga aattcctttc tcaagatgaa 960
cttgtgtata catttggcga aactgttgct ctgggtgctt ctggaattgt aatatgggga 1020
accctcagta taatgcgaag tatgaaatct tgcttgctcc tagacaatta catggagact 1080
atactgaatc cttacataat caacgtcaca ctagcagcca aaatgtgtag ccaagtgctt 1140
tgccaggagc aaggagtgtg tataaggaaa aactggaatt caagtgacta tcttcacctc 1200
aacccagata attttgctat tcaacttgag aaaggtggaa agttcacagt acgtggaaaa 1260
ccgacacttg aagacctgga gcaattttct gaaaaatttt attgcagctg ttatagcacc 1320
ttgagttgta aggagaaagc tgatgtaaaa gacactgatg ctgttgatgt gtgtattgct 1380
gatggtgtct gtatagatgc ttttctaaaa cctcccatgg agacagaaga acctcaaatt 1440
ttctacaatg cttcaccctc cacactatct gccacaatgt tcattgttag tattttgttt 1500
cttatcattt cttctgtagc gagtttgtaa                                  1530
```

<210> 2
<211> 1506
<212> DNA
<213> Artificial Sequence

<220>
<223> Codon Optimized PH20 with restriction sites

<400> 2

```
gctagcatgg acatgagggt accagcccag ctgttgggcc ttcttctgct ttggctccct 60
ggggcaaggg gacttaactt cagagctccc cctgtaatcc caaacgtccc cttcctgtgg 120
gcatggaacg cccctagcga attctgtttg gggaaatttg acgaacctct ggacatgtct 180
ctgttttctt ttatcggtag ccctcgaatc aacgctaccg gacaggggtg gacaatcttt 240
tacgtggatc gtctgggcta ttacccttac atcgacagca ttactggggt gaccgtgaac 300
ggaggcattc ctcagaaaat atcactgcag gaccacctgg acaaagctaa gaaggatatt 360
acattctaca tgccagtgga caatctcgga atggctgtga ttgactggga ggagtggaga 420
cctacttggg cccgaaattg gaagcccaag gatgtgtata agaaccgatc cattgagctg 480
gtgcagcagc agaacgtgca gctgtccctt acagaagcca ccgaaaaagc taagcaggaa 540
ttcgagaaag ctgggaagga tttcctggtt gagacaataa aacttgggaa actgcttaga 600
cctaaccacc tctggggcta ttatttgttc cccgattgct acaatcacca ctataagaag 660
cccggctaca acggatcttg tttcaacgtc gaaattaaaa gaaacgatga cctctcttgg 720
ttgtggaatg agtctactgc actctacccc tctatatatc tcaacacaca gcagtcccca 780
gtagccgcta ccctctacgt tcggaataga gtgagggaag ccatcagggt gtccaagatc 840
cccgatgcca aatctccccc cccagtgttt gcctacacca ggatcgtgtt taccgaccag 900
gtgttgaagt tcctgagcca ggacgagctc gtgtatactt ttggcgagac agttgctctg 960
ggagcctctg ggatcgtgat ctggggcact ctgagtatta tgcgatcaat gaagtcctgc 1020
ctgctgttgg ataactacat ggagacaatc ctgaacccat atatcattaa cgtgactttg 1080
gccgctaaga tgtgttcaca ggtgctgtgt caagagcagg gagtatgcat taggaagaac 1140
tggaactcaa gcgattatct ccatctgaac ccagataatt ttgcaatcca gctggaaaag 1200
ggtggtaaat tcactgtccg agggaagccc acactcgagg atctcgaaca gtttagtgaa 1260
aagttctact gtagctgcta tagcactctg agttgtaagg aaaaagccga tgtgaaagac 1320
actgatgccg ttgacgtttg catcgccgat ggggtgtgta ttgacgcctt tctcaagcca 1380
ccaatggaga ctgaggagcc ccagatcttt tacaacgcct ccccctccac cttgagtgct 1440
accatgttca tcgtatctat acttttcctg attatatcaa gcgtggcaag cctctgataa 1500
ggatcc                                                          1506
```

<210> 3
<211> 507
<212> PRT
<213> Homo sapiens

<220>
<223> HuPH20 precursor 1-507

<400> 3

```
Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1               5                  10                  15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
            20                  25                  30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
        35                  40                  45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
    50                  55                  60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
65                  70                  75                  80
Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
                85                  90                  95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
            100                 105                 110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
        115                 120                 125
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
    130                 135                 140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145                 150                 155                 160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
                165                 170                 175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
            180                 185                 190
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
```

```
          195                 200                 205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
    210                 215                 220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
225                 230                 235                 240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
                245                 250                 255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
            260                 265                 270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
        275                 280                 285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
    290                 295                 300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
305                 310                 315                 320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
                325                 330                 335
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
            340                 345                 350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
        355                 360                 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
    370                 375                 380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
385                 390                 395                 400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
                405                 410                 415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
            420                 425                 430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
        435                 440                 445
Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
    450                 455                 460
Ile Asp Ala Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile
465                 470                 475                 480
Phe Tyr Asn Ala Ser Pro Ser Thr Leu Ser Ala Thr Met Phe Ile Val
                485                 490                 495
Ser Ile Leu Phe Leu Ile Ile Ser Ser Val Ala
            500                 505
```

<210> 4
<211> 505
<212> PRT
<213> Homo sapiens

<220>
<223> HuPH20 precursor 1-505

<400> 4

```
Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1               5                  10                  15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
            20                  25                  30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
        35                  40                  45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
    50                  55                  60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
65                  70                  75                  80
Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
                85                  90                  95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
```

```
                100                 105                 110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
            115                 120                 125
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
    130                 135                 140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145                 150                 155                 160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
                165                 170                 175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
                180                 185                 190
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
            195                 200                 205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
    210                 215                 220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
225                 230                 235                 240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
                245                 250                 255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
                260                 265                 270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
            275                 280                 285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
    290                 295                 300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
305                 310                 315                 320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
                325                 330                 335
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
                340                 345                 350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
            355                 360                 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
    370                 375                 380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
385                 390                 395                 400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
                405                 410                 415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
                420                 425                 430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
            435                 440                 445
Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
    450                 455                 460
Ile Asp Ala Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile
465                 470                 475                 480
Phe Tyr Asn Ala Ser Pro Ser Thr Leu Ser Ala Thr Met Phe Ile Val
                485                 490                 495
Ser Ile Leu Phe Leu Ile Ile Ser Ser
                500                 505
```

<210> 5
<211> 503
<212> PRT
<213> Homo sapiens

<220>
<223> HuPH20 precursor 1-503

<400> 5

Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys

```
 1               5                  10                  15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
            20                  25                  30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
        35                  40                  45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
    50                  55                  60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
65                  70                  75                  80
Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
                85                  90                  95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
            100                 105                 110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
        115                 120                 125
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
    130                 135                 140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145                 150                 155                 160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
                165                 170                 175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
            180                 185                 190
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
        195                 200                 205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
    210                 215                 220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
225                 230                 235                 240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
                245                 250                 255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
            260                 265                 270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
        275                 280                 285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
    290                 295                 300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
305                 310                 315                 320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
                325                 330                 335
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
            340                 345                 350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
        355                 360                 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
    370                 375                 380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
385                 390                 395                 400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
                405                 410                 415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
            420                 425                 430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
        435                 440                 445
Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
    450                 455                 460
Ile Asp Ala Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile
465                 470                 475                 480
Phe Tyr Asn Ala Ser Pro Ser Thr Leu Ser Ala Thr Met Phe Ile Val
                485                 490                 495
Ser Ile Leu Phe Leu Ile Ile
            500
```

<210> 6
<211> 501
<212> PRT
<213> Homo sapiens

<220>
<223> HuPH20 precursor 1-501

<400> 6

```
Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1               5                  10                  15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
            20                  25                  30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
        35                  40                  45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
    50                  55                  60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
65                  70                  75                  80
Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
                85                  90                  95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
            100                 105                 110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
        115                 120                 125
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
    130                 135                 140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145                 150                 155                 160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
                165                 170                 175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
            180                 185                 190
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
        195                 200                 205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
    210                 215                 220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
225                 230                 235                 240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
                245                 250                 255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
            260                 265                 270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
        275                 280                 285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
    290                 295                 300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
305                 310                 315                 320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
                325                 330                 335
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
            340                 345                 350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
        355                 360                 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
    370                 375                 380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
385                 390                 395                 400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
                405                 410                 415
```

```
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
            420                 425                 430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
        435                 440                 445
Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
    450                 455                 460
Ile Asp Ala Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile
465                 470                 475                 480
Phe Tyr Asn Ala Ser Pro Ser Thr Leu Ser Ala Thr Met Phe Ile Val
                485                 490                 495
Ser Ile Leu Phe Leu
            500
```

<210> 7
<211> 499
<212> PRT
<213> Homo sapiens

<220>
<223> HuPH20 precursor 1-499

<400> 7

```
Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1               5                  10                  15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
            20                  25                  30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
        35                  40                  45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
    50                  55                  60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
65                  70                  75                  80
Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
                85                  90                  95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
            100                 105                 110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
        115                 120                 125
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
    130                 135                 140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145                 150                 155                 160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
                165                 170                 175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
            180                 185                 190
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
        195                 200                 205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
    210                 215                 220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
225                 230                 235                 240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
                245                 250                 255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
            260                 265                 270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
        275                 280                 285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
    290                 295                 300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
305                 310                 315                 320
```

```
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
                325                 330                 335
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
            340                 345                 350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
        355                 360                 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
    370                 375                 380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
385                 390                 395                 400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
                405                 410                 415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
            420                 425                 430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
        435                 440                 445
Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
    450                 455                 460
Ile Asp Ala Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile
465                 470                 475                 480
Phe Tyr Asn Ala Ser Pro Ser Thr Leu Ser Ala Thr Met Phe Ile Val
                485                 490                 495
Ser Ile Leu
```

<210> 8
<211> 497
<212> PRT
<213> Homo sapiens

<220>
<223> HuPH20 precursor 1-497

<400> 8

```
Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1               5                  10                  15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
            20                  25                  30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
        35                  40                  45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
    50                  55                  60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
65                  70                  75                  80
Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
                85                  90                  95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
            100                 105                 110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
        115                 120                 125
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
    130                 135                 140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145                 150                 155                 160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
                165                 170                 175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
            180                 185                 190
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
        195                 200                 205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
    210                 215                 220
```

```
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
225                 230                 235                 240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
                245                 250                 255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
            260                 265                 270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
        275                 280                 285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
    290                 295                 300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
305                 310                 315                 320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
                325                 330                 335
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
            340                 345                 350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
        355                 360                 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
    370                 375                 380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
385                 390                 395                 400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
                405                 410                 415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
            420                 425                 430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
        435                 440                 445
Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
    450                 455                 460
Ile Asp Ala Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile
465                 470                 475                 480
Phe Tyr Asn Ala Ser Pro Ser Thr Leu Ser Ala Thr Met Phe Ile Val
                485                 490                 495
Ser
```

<210> 9
<211> 495
<212> PRT
<213> Homo sapiens

<220>
<223> HuPH20 precursor 1-495

<400> 9

```
Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1               5                  10                  15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
            20                  25                  30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
        35                  40                  45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
    50                  55                  60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
65                  70                  75                  80
Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
                85                  90                  95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
            100                 105                 110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
        115                 120                 125
```

```
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
    130                 135                 140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145                 150                 155                 160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
                165                 170                 175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
            180                 185                 190
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
        195                 200                 205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
    210                 215                 220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
225                 230                 235                 240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
                245                 250                 255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
            260                 265                 270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
        275                 280                 285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
    290                 295                 300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
305                 310                 315                 320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
                325                 330                 335
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
            340                 345                 350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
        355                 360                 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
    370                 375                 380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
385                 390                 395                 400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
                405                 410                 415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
            420                 425                 430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
        435                 440                 445
Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
    450                 455                 460
Ile Asp Ala Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile
465                 470                 475                 480
Phe Tyr Asn Ala Ser Pro Ser Thr Leu Ser Ala Thr Met Phe Ile
                485                 490                 495
```

<210> 10
<211> 493
<212> PRT
<213> Homo sapiens

<220>
<223> HuPH20 precursor 1-493

<400> 10

```
Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1               5                  10                  15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
            20                  25                  30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
        35                  40                  45
```

```
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
    50                  55                  60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
65                  70                  75                  80
Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
                85                  90                  95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
            100                 105                 110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
        115                 120                 125
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
    130                 135                 140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145                 150                 155                 160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
                165                 170                 175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
            180                 185                 190
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
        195                 200                 205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
    210                 215                 220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
225                 230                 235                 240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
                245                 250                 255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
            260                 265                 270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
        275                 280                 285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
    290                 295                 300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
305                 310                 315                 320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
                325                 330                 335
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
            340                 345                 350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
        355                 360                 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
    370                 375                 380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
385                 390                 395                 400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
                405                 410                 415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
            420                 425                 430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
        435                 440                 445
Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
    450                 455                 460
Ile Asp Ala Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile
465                 470                 475                 480
Phe Tyr Asn Ala Ser Pro Ser Thr Leu Ser Ala Thr Met
                485                 490
```

<210> 11
<211> 491
<212> PRT
<213> Homo sapiens

<220>
<223> HuPH20 precursor 1-491

<400> 11

```
Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1               5                  10                  15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
            20                  25                  30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
        35                  40                  45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
    50                  55                  60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
65                  70                  75                  80
Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
                85                  90                  95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
            100                 105                 110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
        115                 120                 125
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
    130                 135                 140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145                 150                 155                 160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
                165                 170                 175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
            180                 185                 190
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
        195                 200                 205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
    210                 215                 220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
225                 230                 235                 240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
                245                 250                 255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
            260                 265                 270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
        275                 280                 285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
    290                 295                 300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
305                 310                 315                 320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
                325                 330                 335
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
            340                 345                 350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
        355                 360                 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
    370                 375                 380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
385                 390                 395                 400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
                405                 410                 415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
            420                 425                 430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
        435                 440                 445
Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
    450                 455                 460
Ile Asp Ala Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile
```

```
465                 470                 475                 480
Phe Tyr Asn Ala Ser Pro Ser Thr Leu Ser Ala
                485                 490
```

<210> 12
<211> 489
<212> PRT
<213> Homo sapiens

<220>
<223> HuPH20 precursor 1-489

<400> 12

```
Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1               5                  10                  15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
            20                  25                  30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
        35                  40                  45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
    50                  55                  60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
65                  70                  75                  80
Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
                85                  90                  95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
            100                 105                 110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
        115                 120                 125
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
    130                 135                 140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145                 150                 155                 160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
                165                 170                 175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
            180                 185                 190
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
        195                 200                 205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
    210                 215                 220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
225                 230                 235                 240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
                245                 250                 255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
            260                 265                 270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
        275                 280                 285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
    290                 295                 300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
305                 310                 315                 320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
                325                 330                 335
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
            340                 345                 350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
        355                 360                 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
    370                 375                 380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
```

```
385                 390                 395                 400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
                405                 410                 415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
            420                 425                 430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
        435                 440                 445
Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
    450                 455                 460
Ile Asp Ala Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile
465                 470                 475                 480
Phe Tyr Asn Ala Ser Pro Ser Thr Leu
                485
```

<210> 13
<211> 490
<212> PRT
<213> Homo sapiens

<220>
<223> HuPH20 precursor 1-490

<400> 13

```
Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1               5                  10                  15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
            20                  25                  30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
        35                  40                  45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
    50                  55                  60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
65                  70                  75                  80
Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
                85                  90                  95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
            100                 105                 110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
        115                 120                 125
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
    130                 135                 140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145                 150                 155                 160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
                165                 170                 175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
            180                 185                 190
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
        195                 200                 205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
    210                 215                 220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
225                 230                 235                 240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
                245                 250                 255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
            260                 265                 270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
        275                 280                 285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
    290                 295                 300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
```

```
305                 310                 315                 320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
                325                 330                 335
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
            340                 345                 350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
        355                 360                 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
    370                 375                 380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
385                 390                 395                 400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
                405                 410                 415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
            420                 425                 430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
        435                 440                 445
Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
    450                 455                 460
Ile Asp Ala Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile
465                 470                 475                 480
Phe Tyr Asn Ala Ser Pro Ser Thr Leu Ser
                485                 490
```

<210> 14
<211> 487
<212> PRT
<213> Homo sapiens

<220>
<223> HuPH20 precursor 1-487

<400> 14

```
Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1               5                  10                  15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
            20                  25                  30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
        35                  40                  45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
    50                  55                  60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
65                  70                  75                  80
Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
                85                  90                  95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
            100                 105                 110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
        115                 120                 125
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
    130                 135                 140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145                 150                 155                 160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
                165                 170                 175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
            180                 185                 190
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
        195                 200                 205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
    210                 215                 220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
```

```
225                 230                 235                 240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
                245                 250                 255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
            260                 265                 270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
        275                 280                 285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
    290                 295                 300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
305                 310                 315                 320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
                325                 330                 335
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
            340                 345                 350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
        355                 360                 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
    370                 375                 380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
385                 390                 395                 400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
                405                 410                 415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
            420                 425                 430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
        435                 440                 445
Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
    450                 455                 460
Ile Asp Ala Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile
465                 470                 475                 480
Phe Tyr Asn Ala Ser Pro Ser
                485
```

<210> 15
<211> 485
<212> PRT
<213> Homo sapiens

<220>
<223> HuPH20 precursor 1-485

<400> 15

```
Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1               5                  10                  15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
            20                  25                  30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
        35                  40                  45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
    50                  55                  60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
65                  70                  75                  80
Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
                85                  90                  95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
            100                 105                 110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
        115                 120                 125
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
    130                 135                 140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
```

```
145                 150                 155                 160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
                165                 170                 175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
            180                 185                 190
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
        195                 200                 205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
    210                 215                 220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
225                 230                 235                 240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
                245                 250                 255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
            260                 265                 270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
        275                 280                 285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
    290                 295                 300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
305                 310                 315                 320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
                325                 330                 335
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
            340                 345                 350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
        355                 360                 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
    370                 375                 380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
385                 390                 395                 400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
                405                 410                 415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
            420                 425                 430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
        435                 440                 445
Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
    450                 455                 460
Ile Asp Ala Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile
465                 470                 475                 480
Phe Tyr Asn Ala Ser
                485
```

<210> 16
<211> 484
<212> PRT
<213> Homo sapiens

<220>
<223> HuPH20 precursor 1-484

<400> 16

```
Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1               5                  10                  15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
            20                  25                  30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
        35                  40                  45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
    50                  55                  60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
```

```
65                  70                  75                  80
Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
                85                  90                  95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
            100                 105                 110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
        115                 120                 125
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
    130                 135                 140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145                 150                 155                 160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
                165                 170                 175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
            180                 185                 190
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
        195                 200                 205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
    210                 215                 220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
225                 230                 235                 240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
                245                 250                 255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
            260                 265                 270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
        275                 280                 285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
    290                 295                 300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
305                 310                 315                 320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
                325                 330                 335
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
            340                 345                 350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
        355                 360                 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
    370                 375                 380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
385                 390                 395                 400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
                405                 410                 415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
            420                 425                 430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
        435                 440                 445
Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
    450                 455                 460
Ile Asp Ala Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile
465                 470                 475                 480
Phe Tyr Asn Ala
```

<210> 17
<211> 483
<212> PRT
<213> Homo sapiens

<220>
<223> HuPH20 precursor 1-483

<400> 17

```
Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1               5                  10                  15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
            20                  25                  30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
        35                  40                  45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
    50                  55                  60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
65                  70                  75                  80
Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
                85                  90                  95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
            100                 105                 110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
        115                 120                 125
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
    130                 135                 140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145                 150                 155                 160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
                165                 170                 175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
            180                 185                 190
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
        195                 200                 205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
    210                 215                 220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
225                 230                 235                 240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
                245                 250                 255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
            260                 265                 270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
        275                 280                 285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
    290                 295                 300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
305                 310                 315                 320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
                325                 330                 335
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
            340                 345                 350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
        355                 360                 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
    370                 375                 380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
385                 390                 395                 400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
                405                 410                 415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
            420                 425                 430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
        435                 440                 445
Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
    450                 455                 460
Ile Asp Ala Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile
465                 470                 475                 480
Phe Tyr Asn
```

<210> 18
<211> 482
<212> PRT
<213> Homo sapiens

<220>
<223> HuPH20 precursor 1-482

<400> 18

```
Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1               5                  10                  15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
            20                  25                  30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
        35                  40                  45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
    50                  55                  60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
65                  70                  75                  80
Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
                85                  90                  95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
            100                 105                 110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
        115                 120                 125
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
    130                 135                 140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145                 150                 155                 160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
                165                 170                 175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
            180                 185                 190
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
        195                 200                 205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
    210                 215                 220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
225                 230                 235                 240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
                245                 250                 255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
            260                 265                 270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
        275                 280                 285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
    290                 295                 300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
305                 310                 315                 320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
                325                 330                 335
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
            340                 345                 350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
        355                 360                 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
    370                 375                 380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
385                 390                 395                 400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
                405                 410                 415
```

```
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
            420                 425                 430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
        435                 440                 445
Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
    450                 455                 460
Ile Asp Ala Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile
465                 470                 475                 480
Phe Tyr
```

<210> 19
<211> 481
<212> PRT
<213> Homo sapiens

<220>
<223> HuPH20 precursor 1-481

<400> 19

```
Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1               5                  10                  15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
            20                  25                  30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
        35                  40                  45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
    50                  55                  60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
65                  70                  75                  80
Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
                85                  90                  95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
            100                 105                 110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
        115                 120                 125
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
    130                 135                 140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145                 150                 155                 160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
                165                 170                 175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
            180                 185                 190
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
        195                 200                 205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
    210                 215                 220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
225                 230                 235                 240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
                245                 250                 255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
            260                 265                 270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
        275                 280                 285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
    290                 295                 300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
305                 310                 315                 320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
                325                 330                 335
```

```
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
            340                 345                 350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
        355                 360                 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
    370                 375                 380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
385                 390                 395                 400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
                405                 410                 415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
            420                 425                 430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
        435                 440                 445
Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
    450                 455                 460
Ile Asp Ala Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile
465                 470                 475                 480
Phe
```

<210> 20
<211> 480
<212> PRT
<213> Homo sapiens

<220>
<223> HuPH20 precursor 1-480

<400> 20

```
Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1               5                  10                  15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
            20                  25                  30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
        35                  40                  45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
    50                  55                  60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
65                  70                  75                  80
Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
                85                  90                  95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
            100                 105                 110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
        115                 120                 125
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
    130                 135                 140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145                 150                 155                 160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
                165                 170                 175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
            180                 185                 190
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
        195                 200                 205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
    210                 215                 220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
225                 230                 235                 240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
                245                 250                 255

Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
            260                 265                 270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
        275                 280                 285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
    290                 295                 300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
305                 310                 315                 320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
                325                 330                 335
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
            340                 345                 350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
        355                 360                 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
    370                 375                 380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
385                 390                 395                 400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
                405                 410                 415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
            420                 425                 430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
        435                 440                 445
Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
    450                 455                 460
Ile Asp Ala Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile
465                 470                 475                 480
```

<210> 21
<211> 479
<212> PRT
<213> Homo sapiens

<220>
<223> HuPH20 precursor 1-479

<400> 21

```
Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1               5                  10                  15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
            20                  25                  30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
        35                  40                  45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
    50                  55                  60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
65                  70                  75                  80
Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
                85                  90                  95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
            100                 105                 110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
        115                 120                 125
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
    130                 135                 140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145                 150                 155                 160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
                165                 170                 175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
            180                 185                 190
```

```
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
        195                 200                 205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
    210                 215                 220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
225                 230                 235                 240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
                245                 250                 255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
            260                 265                 270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
        275                 280                 285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
    290                 295                 300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
305                 310                 315                 320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
                325                 330                 335
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
            340                 345                 350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
        355                 360                 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
    370                 375                 380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
385                 390                 395                 400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
                405                 410                 415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
            420                 425                 430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
        435                 440                 445
Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
    450                 455                 460
Ile Asp Ala Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln
465                 470                 475
```

<210> 22
<211> 478
<212> PRT
<213> Homo sapiens

<220>
<223> HuPH20 precursor 1-478

<400> 22

```
Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1               5                  10                  15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
            20                  25                  30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
        35                  40                  45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
    50                  55                  60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
65                  70                  75                  80
Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
                85                  90                  95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
            100                 105                 110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
        115                 120                 125

Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
    130                 135                 140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145                 150                 155                 160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
                165                 170                 175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
            180                 185                 190
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
        195                 200                 205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
    210                 215                 220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
225                 230                 235                 240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
                245                 250                 255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
            260                 265                 270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
        275                 280                 285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
    290                 295                 300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
305                 310                 315                 320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
                325                 330                 335
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
            340                 345                 350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
        355                 360                 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
    370                 375                 380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
385                 390                 395                 400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
                405                 410                 415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
            420                 425                 430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
        435                 440                 445
Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
    450                 455                 460
Ile Asp Ala Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro
465                 470                 475
```

<210> 23
<211> 477
<212> PRT
<213> Homo sapiens

<220>
<223> HuPH20 precursor 1-477

<400> 23

```
Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1               5                  10                  15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
            20                  25                  30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
        35                  40                  45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
    50                  55                  60
```

```
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
65                  70                  75                  80
Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
                85                  90                  95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
            100                 105                 110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
        115                 120                 125
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
    130                 135                 140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145                 150                 155                 160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
                165                 170                 175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
            180                 185                 190
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
        195                 200                 205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
    210                 215                 220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
225                 230                 235                 240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
                245                 250                 255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
            260                 265                 270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
        275                 280                 285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
    290                 295                 300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
305                 310                 315                 320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
                325                 330                 335
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
            340                 345                 350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
        355                 360                 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
    370                 375                 380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
385                 390                 395                 400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
                405                 410                 415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
            420                 425                 430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
        435                 440                 445
Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
    450                 455                 460
Ile Asp Ala Phe Leu Lys Pro Pro Met Glu Thr Glu Glu
465                 470                 475
```

<210> 24
<211> 476
<212> PRT
<213> Homo sapiens

<220>
<223> HuPH20 precursor 1-476

<400> 24

```
Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1               5                  10                  15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
            20                  25                  30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
        35                  40                  45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
    50                  55                  60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
65                  70                  75                  80
Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
                85                  90                  95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
            100                 105                 110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
        115                 120                 125
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
    130                 135                 140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145                 150                 155                 160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
                165                 170                 175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
            180                 185                 190
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
        195                 200                 205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
    210                 215                 220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
225                 230                 235                 240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
                245                 250                 255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
            260                 265                 270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
        275                 280                 285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
    290                 295                 300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
305                 310                 315                 320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
                325                 330                 335
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
            340                 345                 350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
        355                 360                 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
    370                 375                 380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
385                 390                 395                 400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
                405                 410                 415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
            420                 425                 430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
        435                 440                 445
Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
    450                 455                 460
Ile Asp Ala Phe Leu Lys Pro Pro Met Glu Thr Glu
465                 470                 475
```

<210> 25
<211> 475
<212> PRT
<213> Homo sapiens

<220>
<223> HuPH20 precursor 1-475

<400> 25

```
Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1               5                  10                  15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
            20                  25                  30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
        35                  40                  45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
    50                  55                  60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
65                  70                  75                  80
Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
                85                  90                  95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
            100                 105                 110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
        115                 120                 125
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
    130                 135                 140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145                 150                 155                 160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
                165                 170                 175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
            180                 185                 190
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
        195                 200                 205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
    210                 215                 220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
225                 230                 235                 240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
                245                 250                 255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
            260                 265                 270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
        275                 280                 285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
    290                 295                 300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
305                 310                 315                 320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
                325                 330                 335
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
            340                 345                 350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
        355                 360                 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
    370                 375                 380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
385                 390                 395                 400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
                405                 410                 415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
            420                 425                 430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp

        435                 440                 445
Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
    450                 455                 460
Ile Asp Ala Phe Leu Lys Pro Pro Met Glu Thr
465                 470                 475
```

<210> 26
<211> 474
<212> PRT
<213> Homo sapiens

<220>
<223> HuPH20 precursor 1-474

<400> 26

```
Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1               5                  10                  15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
            20                  25                  30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
        35                  40                  45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
    50                  55                  60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
65                  70                  75                  80
Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
                85                  90                  95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
            100                 105                 110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
        115                 120                 125
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
    130                 135                 140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145                 150                 155                 160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
                165                 170                 175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
            180                 185                 190
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
        195                 200                 205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
    210                 215                 220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
225                 230                 235                 240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
                245                 250                 255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
            260                 265                 270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
        275                 280                 285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
    290                 295                 300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
305                 310                 315                 320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
                325                 330                 335
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
            340                 345                 350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
        355                 360                 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
```

```
    370                 375                 380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
385                 390                 395                 400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
                405                 410                 415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
            420                 425                 430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
        435                 440                 445
Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
    450                 455                 460
Ile Asp Ala Phe Leu Lys Pro Pro Met Glu
465                 470
```

<210> 27
<211> 473
<212> PRT
<213> Homo sapiens

<220>
<223> HuPH20 precursor 1-473

<400> 27

```
Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1               5                  10                  15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
            20                  25                  30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
        35                  40                  45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
    50                  55                  60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
65                  70                  75                  80
Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
                85                  90                  95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
            100                 105                 110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
        115                 120                 125
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
    130                 135                 140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145                 150                 155                 160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
                165                 170                 175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
            180                 185                 190
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
        195                 200                 205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
    210                 215                 220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
225                 230                 235                 240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
                245                 250                 255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
            260                 265                 270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
        275                 280                 285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
    290                 295                 300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu

305                 310                 315                 320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
                325                 330                 335
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
            340                 345                 350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
        355                 360                 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
    370                 375                 380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
385                 390                 395                 400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
                405                 410                 415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
            420                 425                 430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
        435                 440                 445
Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
    450                 455                 460
Ile Asp Ala Phe Leu Lys Pro Pro Met
465                 470
```

<210> 28
<211> 472
<212> PRT
<213> Homo sapiens

<220>
<223> HuPH20 precursor 1-472

<400> 28

```
Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1               5                  10                  15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
            20                  25                  30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
        35                  40                  45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
    50                  55                  60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
65                  70                  75                  80
Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
                85                  90                  95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
            100                 105                 110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
        115                 120                 125
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
    130                 135                 140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145                 150                 155                 160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
                165                 170                 175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
            180                 185                 190
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
        195                 200                 205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
    210                 215                 220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
225                 230                 235                 240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
```

```
                    245                 250                 255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
                260                 265                 270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
            275                 280                 285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
        290                 295                 300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
305                 310                 315                 320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
                325                 330                 335
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
            340                 345                 350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
        355                 360                 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
    370                 375                 380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
385                 390                 395                 400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
                405                 410                 415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
            420                 425                 430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
        435                 440                 445
Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
    450                 455                 460
Ile Asp Ala Phe Leu Lys Pro Pro
465                 470
```

<210> 29
<211> 471
<212> PRT
<213> Homo sapiens

<220>
<223> HuPH20 precursor 1-471

<400> 29

```
Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1               5                  10                  15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
            20                  25                  30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
        35                  40                  45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
    50                  55                  60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
65                  70                  75                  80
Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
                85                  90                  95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
            100                 105                 110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
        115                 120                 125
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
    130                 135                 140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145                 150                 155                 160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
                165                 170                 175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe

            180                 185                 190
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
        195                 200                 205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
    210                 215                 220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
225                 230                 235                 240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
                245                 250                 255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
            260                 265                 270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
        275                 280                 285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
    290                 295                 300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
305                 310                 315                 320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
                325                 330                 335
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
            340                 345                 350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
        355                 360                 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
    370                 375                 380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
385                 390                 395                 400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
                405                 410                 415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
            420                 425                 430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
        435                 440                 445
Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
    450                 455                 460
Ile Asp Ala Phe Leu Lys Pro
465                 470
```

<210> 30
<211> 470
<212> PRT
<213> Homo sapiens

<220>
<223> HuPH20 precursor 1-470

<400> 30

```
Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1               5                  10                  15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
            20                  25                  30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
        35                  40                  45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
    50                  55                  60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
65                  70                  75                  80
Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
                85                  90                  95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
            100                 105                 110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
```

```
          115                 120                 125
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
    130                 135                 140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145                 150                 155                 160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
                165                 170                 175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
            180                 185                 190
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
        195                 200                 205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
    210                 215                 220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
225                 230                 235                 240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
                245                 250                 255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
            260                 265                 270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
        275                 280                 285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
    290                 295                 300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
305                 310                 315                 320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
                325                 330                 335
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
            340                 345                 350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
        355                 360                 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
    370                 375                 380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
385                 390                 395                 400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
                405                 410                 415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
            420                 425                 430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
        435                 440                 445
Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
    450                 455                 460
Ile Asp Ala Phe Leu Lys
465                 470
```

<210> 31
<211> 469
<212> PRT
<213> Homo sapiens

<220>
<223> HuPH20 precursor 1-469

<400> 31

```
Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1               5                  10                  15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
            20                  25                  30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
        35                  40                  45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe

    50                  55                  60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
65                  70                  75                  80
Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
                85                  90                  95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
            100                 105                 110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
        115                 120                 125
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
    130                 135                 140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145                 150                 155                 160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
                165                 170                 175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
            180                 185                 190
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
        195                 200                 205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
    210                 215                 220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
225                 230                 235                 240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
                245                 250                 255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
            260                 265                 270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
        275                 280                 285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
    290                 295                 300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
305                 310                 315                 320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
                325                 330                 335
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
            340                 345                 350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
        355                 360                 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
    370                 375                 380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
385                 390                 395                 400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
                405                 410                 415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
            420                 425                 430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
        435                 440                 445
Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
    450                 455                 460
Ile Asp Ala Phe Leu
465
```

<210> 32
<211> 468
<212> PRT
<213> Homo sapiens

<220>
<223> HuPH20 precursor 1-468

<400> 32

```
Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1               5                  10                  15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
            20                  25                  30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
        35                  40                  45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
    50                  55                  60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
65                  70                  75                  80
Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
                85                  90                  95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
            100                 105                 110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
        115                 120                 125
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
    130                 135                 140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145                 150                 155                 160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
                165                 170                 175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
            180                 185                 190
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
        195                 200                 205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
    210                 215                 220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
225                 230                 235                 240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
                245                 250                 255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
            260                 265                 270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
        275                 280                 285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
    290                 295                 300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
305                 310                 315                 320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
                325                 330                 335
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
            340                 345                 350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
        355                 360                 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
    370                 375                 380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
385                 390                 395                 400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
                405                 410                 415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
            420                 425                 430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
        435                 440                 445
Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
    450                 455                 460
Ile Asp Ala Phe
465
```

<210> 33

<211> 467
<212> PRT
<213> Homo sapiens

<220>
<223> HuPH20 precursor 1-467

<400> 33

```
Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1               5                  10                  15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
            20                  25                  30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
        35                  40                  45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
    50                  55                  60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
65                  70                  75                  80
Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
                85                  90                  95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
            100                 105                 110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
        115                 120                 125
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
    130                 135                 140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145                 150                 155                 160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
                165                 170                 175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
            180                 185                 190
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
        195                 200                 205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
    210                 215                 220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
225                 230                 235                 240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
                245                 250                 255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
            260                 265                 270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
        275                 280                 285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
    290                 295                 300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
305                 310                 315                 320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
                325                 330                 335
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
            340                 345                 350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
        355                 360                 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
    370                 375                 380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
385                 390                 395                 400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
                405                 410                 415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
            420                 425                 430

Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
        435                 440                 445
Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
    450                 455                 460
Ile Asp Ala
465
```

<210> 34
<211> 466
<212> PRT
<213> Homo sapiens

<220>
<223> HuPH20 precursor 1-466

<400> 34

```
Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1               5                  10                  15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
            20                  25                  30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
        35                  40                  45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
    50                  55                  60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
65                  70                  75                  80
Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
                85                  90                  95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
            100                 105                 110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
        115                 120                 125
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
    130                 135                 140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145                 150                 155                 160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
                165                 170                 175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
            180                 185                 190
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
        195                 200                 205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
    210                 215                 220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
225                 230                 235                 240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
                245                 250                 255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
            260                 265                 270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
        275                 280                 285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
    290                 295                 300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
305                 310                 315                 320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
                325                 330                 335
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
            340                 345                 350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
        355                 360                 365
```

```
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
    370                 375                 380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
385                 390                 395                 400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
                405                 410                 415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
            420                 425                 430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
        435                 440                 445
Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
    450                 455                 460
Ile Asp
465
```

<210> 35
<211> 465
<212> PRT
<213> Homo sapiens

<220>
<223> HuPH20 precursor 1-465

<400> 35

```
Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1               5                  10                  15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
            20                  25                  30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
        35                  40                  45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
    50                  55                  60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
65                  70                  75                  80
Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
                85                  90                  95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
            100                 105                 110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
        115                 120                 125
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
    130                 135                 140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145                 150                 155                 160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
                165                 170                 175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
            180                 185                 190
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
        195                 200                 205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
    210                 215                 220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
225                 230                 235                 240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
                245                 250                 255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
            260                 265                 270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
        275                 280                 285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
    290                 295                 300

Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
305                 310                 315                 320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
                325                 330                 335
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
            340                 345                 350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
        355                 360                 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
    370                 375                 380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
385                 390                 395                 400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
                405                 410                 415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
            420                 425                 430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
        435                 440                 445
Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
    450                 455                 460
Ile
465
```

<210> 36
<211> 464
<212> PRT
<213> Homo sapiens

<220>
<223> HuPH20 precursor 1-464

<400> 36

```
Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1               5                  10                  15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
            20                  25                  30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
        35                  40                  45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
    50                  55                  60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
65                  70                  75                  80
Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
                85                  90                  95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
            100                 105                 110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
        115                 120                 125
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
    130                 135                 140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145                 150                 155                 160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
                165                 170                 175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
            180                 185                 190
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
        195                 200                 205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
    210                 215                 220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
225                 230                 235                 240
```

```
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
                245                 250                 255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
            260                 265                 270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
        275                 280                 285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
    290                 295                 300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
305                 310                 315                 320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
                325                 330                 335
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
            340                 345                 350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
        355                 360                 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
    370                 375                 380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
385                 390                 395                 400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
                405                 410                 415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
            420                 425                 430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
        435                 440                 445
Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
    450                 455                 460
```

<210> 37
<211> 462
<212> PRT
<213> Homo sapiens

<220>
<223> HuPH20 precursor 1-462

<400> 37

```
Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1               5                  10                  15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
            20                  25                  30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
        35                  40                  45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
    50                  55                  60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
65                  70                  75                  80
Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
                85                  90                  95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
            100                 105                 110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
        115                 120                 125
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
    130                 135                 140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145                 150                 155                 160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
                165                 170                 175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
            180                 185                 190

Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
        195                 200                 205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
    210                 215                 220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
225                 230                 235                 240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
                245                 250                 255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
            260                 265                 270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
        275                 280                 285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
    290                 295                 300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
305                 310                 315                 320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
                325                 330                 335
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
            340                 345                 350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
        355                 360                 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
    370                 375                 380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
385                 390                 395                 400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
                405                 410                 415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
            420                 425                 430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
        435                 440                 445
Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly
    450                 455                 460
```

<210> 38

<211> 460
<212> PRT
<213> Homo sapiens

<220>
<223> HuPH20 precursor 1-460

<400> 38

```
Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1               5                  10                  15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
            20                  25                  30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
        35                  40                  45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
    50                  55                  60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
65                  70                  75                  80
Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
                85                  90                  95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
            100                 105                 110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
        115                 120                 125
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
    130                 135                 140
```

```
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145                 150                 155                 160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
                165                 170                 175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
            180                 185                 190
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
        195                 200                 205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
    210                 215                 220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
225                 230                 235                 240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
                245                 250                 255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
            260                 265                 270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
        275                 280                 285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
    290                 295                 300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
305                 310                 315                 320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
                325                 330                 335
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
            340                 345                 350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
        355                 360                 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
    370                 375                 380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
385                 390                 395                 400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
                405                 410                 415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
            420                 425                 430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
        435                 440                 445
Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala
    450                 455                 460
```

<210> 39
<211> 458
<212> PRT
<213> Homo sapiens

<220>
<223> HuPH20 precursor 1-458

<400> 39

```
Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1               5                  10                  15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
            20                  25                  30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
        35                  40                  45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
    50                  55                  60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
65                  70                  75                  80
Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
                85                  90                  95

Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
            100                 105                 110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
        115                 120                 125
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
    130                 135                 140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145                 150                 155                 160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
                165                 170                 175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
            180                 185                 190
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
        195                 200                 205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
    210                 215                 220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
225                 230                 235                 240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
                245                 250                 255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
            260                 265                 270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
        275                 280                 285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
    290                 295                 300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
305                 310                 315                 320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
                325                 330                 335
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
            340                 345                 350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
        355                 360                 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
    370                 375                 380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
385                 390                 395                 400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
                405                 410                 415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
            420                 425                 430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
        435                 440                 445
Val Lys Asp Thr Asp Ala Val Asp Val Cys
    450                 455
```

<210> 40

<211> 456
<212> PRT
<213> Homo sapiens

<220>
<223> HuPH20 precursor 1-456

<400> 40

```
Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1               5                  10                  15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
            20                  25                  30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
        35                  40                  45
```

```
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
    50                  55                  60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
65                  70                  75                  80
Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
                85                  90                  95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
            100                 105                 110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
        115                 120                 125
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
    130                 135                 140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145                 150                 155                 160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
                165                 170                 175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
            180                 185                 190
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
        195                 200                 205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
    210                 215                 220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
225                 230                 235                 240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
                245                 250                 255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
            260                 265                 270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
        275                 280                 285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
    290                 295                 300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
305                 310                 315                 320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
                325                 330                 335
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
            340                 345                 350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
        355                 360                 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
    370                 375                 380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
385                 390                 395                 400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
                405                 410                 415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
            420                 425                 430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
        435                 440                 445
Val Lys Asp Thr Asp Ala Val Asp
    450                 455
```

<210> 41
<211> 454
<212> PRT
<213> Homo sapiens

<220>
<223> HuPH20 precursor 1-454

<400> 41

```
Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1               5                  10                  15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
            20                  25                  30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
        35                  40                  45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
    50                  55                  60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
65                  70                  75                  80
Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
                85                  90                  95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
            100                 105                 110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
        115                 120                 125
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
    130                 135                 140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145                 150                 155                 160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
                165                 170                 175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
            180                 185                 190
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
        195                 200                 205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
    210                 215                 220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
225                 230                 235                 240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
                245                 250                 255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
            260                 265                 270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
        275                 280                 285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
    290                 295                 300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
305                 310                 315                 320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
                325                 330                 335
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
            340                 345                 350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
        355                 360                 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
    370                 375                 380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
385                 390                 395                 400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
                405                 410                 415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
            420                 425                 430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
        435                 440                 445
Val Lys Asp Thr Asp Ala
    450
```

<210> 42
<211> 452
<212> PRT
<213> Homo sapiens

<220>
<223> HuPH20 precursor 1-452

<400> 42

```
Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1               5                  10                  15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
            20                  25                  30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
        35                  40                  45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
    50                  55                  60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
65                  70                  75                  80
Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
                85                  90                  95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
            100                 105                 110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
        115                 120                 125
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
    130                 135                 140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145                 150                 155                 160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
                165                 170                 175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
            180                 185                 190
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
        195                 200                 205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
    210                 215                 220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
225                 230                 235                 240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
                245                 250                 255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
            260                 265                 270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
        275                 280                 285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
    290                 295                 300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
305                 310                 315                 320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
                325                 330                 335
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
            340                 345                 350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
        355                 360                 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
    370                 375                 380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
385                 390                 395                 400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
                405                 410                 415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
            420                 425                 430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
        435                 440                 445
Val Lys Asp Thr
```

450

<210> 43
<211> 450
<212> PRT
<213> Homo sapiens

<220>
<223> HuPH20 precursor 1-450

<400> 43

```
Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1               5                  10                  15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
            20                  25                  30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
        35                  40                  45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
    50                  55                  60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
65                  70                  75                  80
Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
                85                  90                  95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
            100                 105                 110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
        115                 120                 125
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
    130                 135                 140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145                 150                 155                 160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
                165                 170                 175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
            180                 185                 190
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
        195                 200                 205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
    210                 215                 220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
225                 230                 235                 240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
                245                 250                 255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
            260                 265                 270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
        275                 280                 285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
    290                 295                 300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
305                 310                 315                 320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
                325                 330                 335
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
            340                 345                 350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
        355                 360                 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
    370                 375                 380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
385                 390                 395                 400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
```

```
                    405                 410                 415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
            420                 425                 430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
        435                 440                 445
Val Lys
    450
```

<210> 44
<211> 508
<212> PRT
<213> Homo sapiens

<220>
<223> HuPH20 precursor 1-508

<400> 44

```
Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1               5                  10                  15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
            20                  25                  30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
        35                  40                  45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
    50                  55                  60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
65                  70                  75                  80
Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
                85                  90                  95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
            100                 105                 110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
        115                 120                 125
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
    130                 135                 140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145                 150                 155                 160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
                165                 170                 175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
            180                 185                 190
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
        195                 200                 205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
    210                 215                 220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
225                 230                 235                 240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
                245                 250                 255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
            260                 265                 270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
        275                 280                 285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
    290                 295                 300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
305                 310                 315                 320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
                325                 330                 335
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
            340                 345                 350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
```

```
          355                 360                 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
    370                 375                 380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
385                 390                 395                 400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
                405                 410                 415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
            420                 425                 430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
        435                 440                 445
Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
    450                 455                 460
Ile Asp Ala Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile
465                 470                 475                 480
Phe Tyr Asn Ala Ser Pro Ser Thr Leu Ser Ala Thr Met Phe Ile Val
                485                 490                 495
Ser Ile Leu Phe Leu Ile Ile Ser Ser Val Ala Ser
            500                 505
```

<210> 45
<211> 506
<212> PRT
<213> Homo sapiens

<220>
<223> HuPH20 precursor 1-506

<400> 45

```
Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1               5                  10                  15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
            20                  25                  30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
        35                  40                  45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
    50                  55                  60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
65                  70                  75                  80
Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
                85                  90                  95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
            100                 105                 110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
        115                 120                 125
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
    130                 135                 140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145                 150                 155                 160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
                165                 170                 175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
            180                 185                 190
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
        195                 200                 205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
    210                 215                 220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
225                 230                 235                 240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
                245                 250                 255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
```

```
                260                 265                 270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
        275                 280                 285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
    290                 295                 300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
305                 310                 315                 320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
                325                 330                 335
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
                340                 345                 350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
        355                 360                 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
    370                 375                 380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
385                 390                 395                 400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
                405                 410                 415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
                420                 425                 430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
        435                 440                 445
Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
    450                 455                 460
Ile Asp Ala Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile
465                 470                 475                 480
Phe Tyr Asn Ala Ser Pro Ser Thr Leu Ser Ala Thr Met Phe Ile Val
                485                 490                 495
Ser Ile Leu Phe Leu Ile Ile Ser Ser Val
                500                 505
```

<210> 46
<211> 504
<212> PRT
<213> Homo sapiens

<220>
<223> HuPH20 precursor 1-504

<400> 46

```
Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1               5                  10                  15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
            20                  25                  30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
        35                  40                  45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
    50                  55                  60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
65                  70                  75                  80
Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
                85                  90                  95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
            100                 105                 110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
        115                 120                 125
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
    130                 135                 140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145                 150                 155                 160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
```

```
                    165                 170                 175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
                180                 185                 190
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
        195                 200                 205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
    210                 215                 220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
225                 230                 235                 240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
                245                 250                 255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
            260                 265                 270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
        275                 280                 285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
    290                 295                 300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
305                 310                 315                 320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
                325                 330                 335
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
            340                 345                 350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
        355                 360                 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
    370                 375                 380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
385                 390                 395                 400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
                405                 410                 415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
            420                 425                 430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
        435                 440                 445
Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
    450                 455                 460
Ile Asp Ala Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile
465                 470                 475                 480
Phe Tyr Asn Ala Ser Pro Ser Thr Leu Ser Ala Thr Met Phe Ile Val
                485                 490                 495
Ser Ile Leu Phe Leu Ile Ile Ser
            500
```

<210> 47
<211> 502
<212> PRT
<213> Homo sapiens

<220>
<223> HuPH20 precursor 1-502

<400> 47

```
Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1               5                  10                  15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
            20                  25                  30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
        35                  40                  45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
    50                  55                  60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
```

```
65                  70                  75                  80
Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
                85                  90                  95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
            100                 105                 110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
        115                 120                 125
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
    130                 135                 140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145                 150                 155                 160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
                165                 170                 175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
            180                 185                 190
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
        195                 200                 205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
    210                 215                 220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
225                 230                 235                 240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
                245                 250                 255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
            260                 265                 270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
        275                 280                 285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
    290                 295                 300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
305                 310                 315                 320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
                325                 330                 335
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
            340                 345                 350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
        355                 360                 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
    370                 375                 380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
385                 390                 395                 400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
                405                 410                 415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
            420                 425                 430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
        435                 440                 445
Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
    450                 455                 460
Ile Asp Ala Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile
465                 470                 475                 480
Phe Tyr Asn Ala Ser Pro Ser Thr Leu Ser Ala Thr Met Phe Ile Val
                485                 490                 495
Ser Ile Leu Phe Leu Ile
            500
```

<210> 48
<211> 500
<212> PRT
<213> Homo sapiens

<220>
<223> HuPH20 precursor 1-500

<400> 48

```
Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1               5                  10                  15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
            20                  25                  30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
        35                  40                  45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
    50                  55                  60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
65                  70                  75                  80
Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
                85                  90                  95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
            100                 105                 110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
        115                 120                 125
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
    130                 135                 140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145                 150                 155                 160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
                165                 170                 175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
            180                 185                 190
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
        195                 200                 205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
    210                 215                 220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
225                 230                 235                 240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
                245                 250                 255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
            260                 265                 270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
        275                 280                 285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
    290                 295                 300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
305                 310                 315                 320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
                325                 330                 335
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
            340                 345                 350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
        355                 360                 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
    370                 375                 380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
385                 390                 395                 400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
                405                 410                 415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
            420                 425                 430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
        435                 440                 445
Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
    450                 455                 460
Ile Asp Ala Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile
465                 470                 475                 480
```

```
Phe Tyr Asn Ala Ser Pro Ser Thr Leu Ser Ala Thr Met Phe Ile Val
                485                 490                 495
Ser Ile Leu Phe
            500
```

<210> 49
<211> 498
<212> PRT
<213> Homo sapiens

<220>
<223> HuPH20 precursor 1-498

<400> 49

```
Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1               5                  10                  15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
            20                  25                  30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
        35                  40                  45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
    50                  55                  60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
65                  70                  75                  80
Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
                85                  90                  95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
            100                 105                 110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
        115                 120                 125
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
    130                 135                 140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145                 150                 155                 160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
                165                 170                 175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
            180                 185                 190
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
        195                 200                 205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
    210                 215                 220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
225                 230                 235                 240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
                245                 250                 255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
            260                 265                 270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
        275                 280                 285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
    290                 295                 300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
305                 310                 315                 320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
                325                 330                 335
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
            340                 345                 350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
        355                 360                 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
    370                 375                 380
```

```
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
385                 390                 395                 400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
                405                 410                 415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
            420                 425                 430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
        435                 440                 445
Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
    450                 455                 460
Ile Asp Ala Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile
465                 470                 475                 480
Phe Tyr Asn Ala Ser Pro Ser Thr Leu Ser Ala Thr Met Phe Ile Val
                485                 490                 495
Ser Ile
```

<210> 50
<211> 496
<212> PRT
<213> Homo sapiens

<220>
<223> HuPH20 precursor 1-496

<400> 50

```
Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1               5                  10                  15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
            20                  25                  30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
        35                  40                  45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
    50                  55                  60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
65                  70                  75                  80
Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
                85                  90                  95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
            100                 105                 110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
        115                 120                 125
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
    130                 135                 140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145                 150                 155                 160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
                165                 170                 175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
            180                 185                 190
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
        195                 200                 205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
    210                 215                 220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
225                 230                 235                 240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
                245                 250                 255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
            260                 265                 270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
        275                 280                 285
```

```
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
    290                 295                 300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
305                 310                 315                 320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
                325                 330                 335
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
            340                 345                 350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
        355                 360                 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
    370                 375                 380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
385                 390                 395                 400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
                405                 410                 415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
            420                 425                 430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
        435                 440                 445
Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
    450                 455                 460
Ile Asp Ala Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile
465                 470                 475                 480
Phe Tyr Asn Ala Ser Pro Ser Thr Leu Ser Ala Thr Met Phe Ile Val
                485                 490                 495
```

<210> 51
<211> 494
<212> PRT
<213> Homo sapiens

<220>
<223> HuPH20 precursor 1-494

<400> 51

```
Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1               5                  10                  15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
            20                  25                  30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
        35                  40                  45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
    50                  55                  60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
65                  70                  75                  80
Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
                85                  90                  95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
            100                 105                 110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
        115                 120                 125
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
    130                 135                 140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145                 150                 155                 160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
                165                 170                 175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
            180                 185                 190
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
        195                 200                 205
```

```
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
    210                 215                 220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
225                 230                 235                 240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
                245                 250                 255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
            260                 265                 270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
        275                 280                 285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
    290                 295                 300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
305                 310                 315                 320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
                325                 330                 335
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
            340                 345                 350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
        355                 360                 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
    370                 375                 380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
385                 390                 395                 400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
                405                 410                 415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
            420                 425                 430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
        435                 440                 445
Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
    450                 455                 460
Ile Asp Ala Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile
465                 470                 475                 480
Phe Tyr Asn Ala Ser Pro Ser Thr Leu Ser Ala Thr Met Phe
                485                 490
```

<210> 52
<211> 492
<212> PRT
<213> Homo sapiens

<220>
<223> HuPH20 precursor 1-492

<400> 52

```
Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1               5                  10                  15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
            20                  25                  30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
        35                  40                  45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
    50                  55                  60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
65                  70                  75                  80
Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
                85                  90                  95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
            100                 105                 110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
        115                 120                 125
```

```
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
    130                 135                 140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145                 150                 155                 160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
                165                 170                 175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
            180                 185                 190
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
        195                 200                 205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
    210                 215                 220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
225                 230                 235                 240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
                245                 250                 255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
            260                 265                 270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
        275                 280                 285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
    290                 295                 300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
305                 310                 315                 320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
                325                 330                 335
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
            340                 345                 350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
        355                 360                 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
    370                 375                 380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
385                 390                 395                 400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
                405                 410                 415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
            420                 425                 430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
        435                 440                 445
Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
    450                 455                 460
Ile Asp Ala Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile
465                 470                 475                 480
Phe Tyr Asn Ala Ser Pro Ser Thr Leu Ser Ala Thr
                485                 490
```

<210> 53
<211> 488
<212> PRT
<213> Homo sapiens

<220>
<223> HuPH20 precursor 1-488

<400> 53

```
Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1               5                  10                  15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
            20                  25                  30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
        35                  40                  45
```

```
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
    50                  55                  60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
65                  70                  75                  80
Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
                85                  90                  95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
            100                 105                 110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
        115                 120                 125
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
    130                 135                 140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145                 150                 155                 160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
                165                 170                 175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
            180                 185                 190
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
        195                 200                 205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
    210                 215                 220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
225                 230                 235                 240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
                245                 250                 255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
            260                 265                 270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
        275                 280                 285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
    290                 295                 300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
305                 310                 315                 320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
                325                 330                 335
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
            340                 345                 350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
        355                 360                 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
    370                 375                 380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
385                 390                 395                 400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
                405                 410                 415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
            420                 425                 430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
        435                 440                 445
Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
    450                 455                 460
Ile Asp Ala Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile
465                 470                 475                 480
Phe Tyr Asn Ala Ser Pro Ser Thr
                485
```

<210> 54
<211> 486
<212> PRT
<213> Homo sapiens

<220>
<223> HuPH20 precursor 1-486

<400> 54

```
Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1               5                  10                  15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
            20                  25                  30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
        35                  40                  45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
    50                  55                  60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
65                  70                  75                  80
Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
                85                  90                  95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
            100                 105                 110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
        115                 120                 125
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
    130                 135                 140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145                 150                 155                 160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
                165                 170                 175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
            180                 185                 190
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
        195                 200                 205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
    210                 215                 220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
225                 230                 235                 240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
                245                 250                 255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
            260                 265                 270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
        275                 280                 285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
    290                 295                 300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
305                 310                 315                 320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
                325                 330                 335
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
            340                 345                 350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
        355                 360                 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
    370                 375                 380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
385                 390                 395                 400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
                405                 410                 415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
            420                 425                 430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
        435                 440                 445
Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
    450                 455                 460
Ile Asp Ala Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile
```

```
465                 470                 475                 480
Phe Tyr Asn Ala Ser Pro
                485
```

<210> 55
<211> 472
<212> PRT
<213> Homo sapiens

<220>
<223> mature HuPH20 36-507

<400> 55

```
Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro Phe Leu Trp
 1               5                  10                  15
Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe Asp Glu Pro
            20                  25                  30
Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala
        35                  40                  45
Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
    50                  55                  60
Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
65                  70                  75                  80
Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile
                85                  90                  95
Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
            100                 105                 110
Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
        115                 120                 125
Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn Val Gln Leu
    130                 135                 140
Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
145                 150                 155                 160
Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
                165                 170                 175
Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
            180                 185                 190
His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
        195                 200                 205
Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
    210                 215                 220
Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
225                 230                 235                 240
Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
                245                 250                 255
Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
            260                 265                 270
Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
        275                 280                 285
Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
    290                 295                 300
Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
305                 310                 315                 320
Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
                325                 330                 335
Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
            340                 345                 350
Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
        355                 360                 365
Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
    370                 375                 380
Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys

385                 390                 395                 400
Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
                405                 410                 415
Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys Ile Asp Ala
            420                 425                 430
Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile Phe Tyr Asn
        435                 440                 445
Ala Ser Pro Ser Thr Leu Ser Ala Thr Met Phe Ile Val Ser Ile Leu
    450                 455                 460
Phe Leu Ile Ile Ser Ser Val Ala
465                 470
```

<210> 56
<211> 470
<212> PRT
<213> Homo sapiens

<220>
<223> mature HuPH20 36-505

<400> 56

```
Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro Phe Leu Trp
 1               5                  10                  15
Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe Asp Glu Pro
            20                  25                  30
Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala
        35                  40                  45
Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
    50                  55                  60
Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
65                  70                  75                  80
Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile
                85                  90                  95
Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
            100                 105                 110
Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
        115                 120                 125
Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn Val Gln Leu
    130                 135                 140
Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
145                 150                 155                 160
Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
                165                 170                 175
Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
            180                 185                 190
His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
        195                 200                 205
Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
    210                 215                 220
Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
225                 230                 235                 240
Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
                245                 250                 255
Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
            260                 265                 270
Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
        275                 280                 285
Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
    290                 295                 300
Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
305                 310                 315                 320
Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
```

```
                    325                 330                 335
Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
            340                 345                 350
Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
        355                 360                 365
Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
    370                 375                 380
Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
385                 390                 395                 400
Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
                405                 410                 415
Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys Ile Asp Ala
            420                 425                 430
Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile Phe Tyr Asn
        435                 440                 445
Ala Ser Pro Ser Thr Leu Ser Ala Thr Met Phe Ile Val Ser Ile Leu
    450                 455                 460
Phe Leu Ile Ile Ser Ser
465                 470
```

<210> 57
<211> 468
<212> PRT
<213> Homo sapiens

<220>
<223> mature HuPH20 36-503

<400> 57

```
Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro Phe Leu Trp
 1               5                  10                  15
Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe Asp Glu Pro
            20                  25                  30
Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala
        35                  40                  45
Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
    50                  55                  60
Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
65                  70                  75                  80
Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile
                85                  90                  95
Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
            100                 105                 110
Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
        115                 120                 125
Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn Val Gln Leu
    130                 135                 140
Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
145                 150                 155                 160
Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
                165                 170                 175
Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
            180                 185                 190
His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
        195                 200                 205
Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
    210                 215                 220
Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
225                 230                 235                 240
Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
                245                 250                 255
Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
```

```
                260                 265                 270
Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
        275                 280                 285
Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
    290                 295                 300
Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
305                 310                 315                 320
Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
                325                 330                 335
Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
                340                 345                 350
Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
        355                 360                 365
Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
    370                 375                 380
Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
385                 390                 395                 400
Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
                405                 410                 415
Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys Ile Asp Ala
                420                 425                 430
Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile Phe Tyr Asn
        435                 440                 445
Ala Ser Pro Ser Thr Leu Ser Ala Thr Met Phe Ile Val Ser Ile Leu
    450                 455                 460
Phe Leu Ile Ile
465
```

<210> 58
<211> 466
<212> PRT
<213> Homo sapiens

<220>
<223> mature HuPH20 36-501

<400> 58

```
Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro Phe Leu Trp
 1               5                  10                  15
Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe Asp Glu Pro
            20                  25                  30
Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala
        35                  40                  45
Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
    50                  55                  60
Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
65                  70                  75                  80
Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile
                85                  90                  95
Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
            100                 105                 110
Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
        115                 120                 125
Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn Val Gln Leu
    130                 135                 140
Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
145                 150                 155                 160
Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
                165                 170                 175
Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
            180                 185                 190
His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile

        195                 200                 205
Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
    210                 215                 220
Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
225                 230                 235                 240
Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
                245                 250                 255
Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
            260                 265                 270
Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
        275                 280                 285
Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
    290                 295                 300
Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
305                 310                 315                 320
Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
                325                 330                 335
Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
            340                 345                 350
Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
        355                 360                 365
Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
    370                 375                 380
Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
385                 390                 395                 400
Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
                405                 410                 415
Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys Ile Asp Ala
            420                 425                 430
Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile Phe Tyr Asn
        435                 440                 445
Ala Ser Pro Ser Thr Leu Ser Ala Thr Met Phe Ile Val Ser Ile Leu
    450                 455                 460
Phe Leu
465
```

<210> 59
<211> 464
<212> PRT
<213> Homo sapiens

<220>
<223> mature HuPH20 36-499

<400> 59

```
Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro Phe Leu Trp
 1               5                  10                  15
Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe Asp Glu Pro
            20                  25                  30
Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala
        35                  40                  45
Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
    50                  55                  60
Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
65                  70                  75                  80
Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile
                85                  90                  95
Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
            100                 105                 110
Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
        115                 120                 125
Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn Val Gln Leu
```

```
    130                 135                 140
Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
145                 150                 155                 160
Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
                165                 170                 175
Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
            180                 185                 190
His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
        195                 200                 205
Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
    210                 215                 220
Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
225                 230                 235                 240
Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
                245                 250                 255
Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
            260                 265                 270
Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
        275                 280                 285
Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
    290                 295                 300
Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
305                 310                 315                 320
Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
                325                 330                 335
Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
            340                 345                 350
Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
        355                 360                 365
Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
    370                 375                 380
Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
385                 390                 395                 400
Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
                405                 410                 415
Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys Ile Asp Ala
            420                 425                 430
Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile Phe Tyr Asn
        435                 440                 445
Ala Ser Pro Ser Thr Leu Ser Ala Thr Met Phe Ile Val Ser Ile Leu
    450                 455                 460
```

<210> 60
<211> 462
<212> PRT
<213> Homo sapiens

<220>
<223> esHuPH20 36-497

<400> 60

```
Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro Phe Leu Trp
 1               5                  10                  15
Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe Asp Glu Pro
            20                  25                  30
Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala
        35                  40                  45
Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
    50                  55                  60
Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
65                  70                  75                  80
Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile

                85                  90                  95
Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
            100                 105                 110
Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
        115                 120                 125
Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn Val Gln Leu
    130                 135                 140
Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
145                 150                 155                 160
Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
                165                 170                 175
Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
            180                 185                 190
His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
        195                 200                 205
Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
    210                 215                 220
Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
225                 230                 235                 240
Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
                245                 250                 255
Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
            260                 265                 270
Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
        275                 280                 285
Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
    290                 295                 300
Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
305                 310                 315                 320
Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
                325                 330                 335
Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
            340                 345                 350
Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
        355                 360                 365
Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
    370                 375                 380
Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
385                 390                 395                 400
Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
                405                 410                 415
Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys Ile Asp Ala
            420                 425                 430
Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile Phe Tyr Asn
        435                 440                 445
Ala Ser Pro Ser Thr Leu Ser Ala Thr Met Phe Ile Val Ser
    450                 455                 460
```

<210> 61

<211> 460
<212> PRT
<213> Homo sapiens

<220>
<223> esHuPH20 36-495

<400> 61

```
Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro Phe Leu Trp
 1               5                  10                  15
Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe Asp Glu Pro
            20                  25                  30
Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala
```

```
          35                  40                  45
Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
      50                  55                  60
Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
65                  70                  75                  80
Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile
                  85                  90                  95
Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
             100                 105                 110
Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
         115                 120                 125
Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn Val Gln Leu
     130                 135                 140
Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
145                 150                 155                 160
Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
                 165                 170                 175
Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
             180                 185                 190
His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
         195                 200                 205
Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
     210                 215                 220
Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
225                 230                 235                 240
Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
                 245                 250                 255
Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
             260                 265                 270
Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
         275                 280                 285
Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
     290                 295                 300
Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
305                 310                 315                 320
Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
                 325                 330                 335
Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
             340                 345                 350
Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
         355                 360                 365
Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
     370                 375                 380
Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
385                 390                 395                 400
Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
                 405                 410                 415
Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys Ile Asp Ala
             420                 425                 430
Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile Phe Tyr Asn
         435                 440                 445
Ala Ser Pro Ser Thr Leu Ser Ala Thr Met Phe Ile
     450                 455                 460
```

<210> 62
<211> 458
<212> PRT
<213> Homo sapiens

<220>
<223> esHuPH20 36-493

<400> 62

```
Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro Phe Leu Trp
 1               5                  10                  15
Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe Asp Glu Pro
            20                  25                  30
Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala
        35                  40                  45
Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
    50                  55                  60
Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
65                  70                  75                  80
Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile
                85                  90                  95
Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
            100                 105                 110
Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
        115                 120                 125
Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn Val Gln Leu
    130                 135                 140
Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
145                 150                 155                 160
Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
                165                 170                 175
Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
            180                 185                 190
His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
        195                 200                 205
Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
    210                 215                 220
Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
225                 230                 235                 240
Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
                245                 250                 255
Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
            260                 265                 270
Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
        275                 280                 285
Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
    290                 295                 300
Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
305                 310                 315                 320
Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
                325                 330                 335
Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
            340                 345                 350
Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
        355                 360                 365
Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
    370                 375                 380
Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
385                 390                 395                 400
Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
                405                 410                 415
Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys Ile Asp Ala
            420                 425                 430
Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile Phe Tyr Asn
        435                 440                 445
Ala Ser Pro Ser Thr Leu Ser Ala Thr Met
    450                 455
```

<210> 63
<211> 456
<212> PRT
<213> Homo sapiens

<220>
<223> esHuPH20 36-491

<400> 63

```
Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro Phe Leu Trp
 1               5                  10                  15
Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe Asp Glu Pro
            20                  25                  30
Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala
        35                  40                  45
Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
    50                  55                  60
Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
65                  70                  75                  80
Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile
                85                  90                  95
Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
            100                 105                 110
Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
        115                 120                 125
Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn Val Gln Leu
    130                 135                 140
Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
145                 150                 155                 160
Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
                165                 170                 175
Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
            180                 185                 190
His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
        195                 200                 205
Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
    210                 215                 220
Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
225                 230                 235                 240
Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
                245                 250                 255
Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
            260                 265                 270
Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
        275                 280                 285
Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
    290                 295                 300
Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
305                 310                 315                 320
Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
                325                 330                 335
Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
            340                 345                 350
Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
        355                 360                 365
Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
    370                 375                 380
Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
385                 390                 395                 400
Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
                405                 410                 415
Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys Ile Asp Ala
            420                 425                 430
Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile Phe Tyr Asn
        435                 440                 445

                    Ala Ser Pro Ser Thr Leu Ser Ala
                450                 455
```

<210> 64

<211> 454
<212> PRT
<213> Homo sapiens

<220>
<223> mature HuPH20 36-489

<400> 64

```
Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro Phe Leu Trp
 1               5                  10                  15
Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe Asp Glu Pro
            20                  25                  30
Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala
        35                  40                  45
Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
    50                  55                  60
Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
65                  70                  75                  80
Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile
                85                  90                  95
Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
            100                 105                 110
Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
        115                 120                 125
Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn Val Gln Leu
    130                 135                 140
Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
145                 150                 155                 160
Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
                165                 170                 175
Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
            180                 185                 190
His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
        195                 200                 205
Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
    210                 215                 220
Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
225                 230                 235                 240
Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
                245                 250                 255
Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
            260                 265                 270
Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
        275                 280                 285
Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
    290                 295                 300
Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
305                 310                 315                 320
Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
                325                 330                 335
Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
            340                 345                 350
Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
        355                 360                 365
Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
    370                 375                 380
Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
385                 390                 395                 400
```

```
Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
                405                 410                 415
Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys Ile Asp Ala
            420                 425                 430
Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile Phe Tyr Asn
        435                 440                 445
Ala Ser Pro Ser Thr Leu
    450
```

<210> 65
<211> 455
<212> PRT
<213> Homo sapiens

<220>
<223> mature HuPH20 36-490

<400> 65

```
Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro Phe Leu Trp
 1               5                  10                  15
Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe Asp Glu Pro
            20                  25                  30
Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala
        35                  40                  45
Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
    50                  55                  60
Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
65                  70                  75                  80
Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile
                85                  90                  95
Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
            100                 105                 110
Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
        115                 120                 125
Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn Val Gln Leu
    130                 135                 140
Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
145                 150                 155                 160
Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
                165                 170                 175
Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
            180                 185                 190
His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
        195                 200                 205
Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
    210                 215                 220
Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
225                 230                 235                 240
Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
                245                 250                 255
Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
            260                 265                 270
Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
        275                 280                 285
Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
    290                 295                 300
Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
305                 310                 315                 320
Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
                325                 330                 335
Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
            340                 345                 350

Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
        355                 360                 365
Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
    370                 375                 380
Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
385                 390                 395                 400
Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
                405                 410                 415
Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys Ile Asp Ala
            420                 425                 430
Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile Phe Tyr Asn
        435                 440                 445
Ala Ser Pro Ser Thr Leu Ser
    450                 455
```

<210> 66
<211> 452
<212> PRT
<213> Homo sapiens

<220>
<223> mature HuPH20 36-487

<400> 66

```
Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro Phe Leu Trp
 1               5                  10                  15
Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe Asp Glu Pro
            20                  25                  30
Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala
        35                  40                  45
Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
    50                  55                  60
Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
65                  70                  75                  80
Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile
                85                  90                  95
Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
            100                 105                 110
Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
        115                 120                 125
Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn Val Gln Leu
    130                 135                 140
Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
145                 150                 155                 160
Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
                165                 170                 175
Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
            180                 185                 190
His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
        195                 200                 205
Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
    210                 215                 220
Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
225                 230                 235                 240
Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
                245                 250                 255
Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
            260                 265                 270
Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
        275                 280                 285
Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
    290                 295                 300
```

```
Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
305                 310                 315                 320
Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
                325                 330                 335
Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
            340                 345                 350
Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
        355                 360                 365
Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
    370                 375                 380
Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
385                 390                 395                 400
Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
                405                 410                 415
Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys Ile Asp Ala
            420                 425                 430
Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile Phe Tyr Asn
        435                 440                 445
Ala Ser Pro Ser
    450
```

<210> 67
<211> 450
<212> PRT
<213> Homo sapiens

<220>
<223> mature HuPH20 36-485

<400> 67

```
Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro Phe Leu Trp
 1               5                  10                  15
Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe Asp Glu Pro
            20                  25                  30
Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala
        35                  40                  45
Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
    50                  55                  60
Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
65                  70                  75                  80
Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile
                85                  90                  95
Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
            100                 105                 110
Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
        115                 120                 125
Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn Val Gln Leu
    130                 135                 140
Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
145                 150                 155                 160
Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
                165                 170                 175
Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
            180                 185                 190
His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
        195                 200                 205
Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
    210                 215                 220
Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
225                 230                 235                 240
Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
                245                 250                 255

Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
            260                 265                 270
Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
        275                 280                 285
Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
    290                 295                 300
Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
305                 310                 315                 320
Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
                325                 330                 335
Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
            340                 345                 350
Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
        355                 360                 365
Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
    370                 375                 380
Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
385                 390                 395                 400
Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
                405                 410                 415
Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys Ile Asp Ala
            420                 425                 430
Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile Phe Tyr Asn
        435                 440                 445
Ala Ser
    450
```

<210> 68

<211> 449
<212> PRT
<213> Homo sapiens

<220>
<223> Mature HuPH20 36-484

<400> 68

```
Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro Phe Leu Trp
 1               5                  10                  15
Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe Asp Glu Pro
            20                  25                  30
Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala
        35                  40                  45
Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
    50                  55                  60
Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
65                  70                  75                  80
Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile
                85                  90                  95
Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
            100                 105                 110
Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
        115                 120                 125
Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn Val Gln Leu
    130                 135                 140
Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
145                 150                 155                 160
Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
                165                 170                 175
Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
            180                 185                 190
His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
        195                 200                 205
```

```
Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
    210                 215                 220
Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
225                 230                 235                 240
Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
                245                 250                 255
Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
            260                 265                 270
Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
        275                 280                 285
Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
    290                 295                 300
Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
305                 310                 315                 320
Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
                325                 330                 335
Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
            340                 345                 350
Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
        355                 360                 365
Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
    370                 375                 380
Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
385                 390                 395                 400
Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
                405                 410                 415
Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys Ile Asp Ala
            420                 425                 430
Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile Phe Tyr Asn
        435                 440                 445
Ala
```

<210> 69
<211> 448
<212> PRT
<213> Homo sapiens

<220>
<223> Mature HuPH20 36-483

<400> 69

```
Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro Phe Leu Trp
 1               5                  10                  15
Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe Asp Glu Pro
            20                  25                  30
Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala
        35                  40                  45
Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
    50                  55                  60
Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
65                  70                  75                  80
Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile
                85                  90                  95
Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
            100                 105                 110
Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
        115                 120                 125
Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn Val Gln Leu
    130                 135                 140
Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
145                 150                 155                 160

Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
                165                 170                 175
Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
            180                 185                 190
His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
        195                 200                 205
Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
    210                 215                 220
Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
225                 230                 235                 240
Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
                245                 250                 255
Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
            260                 265                 270
Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
        275                 280                 285
Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
    290                 295                 300
Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
305                 310                 315                 320
Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
                325                 330                 335
Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
            340                 345                 350
Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
        355                 360                 365
Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
    370                 375                 380
Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
385                 390                 395                 400
Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
                405                 410                 415
Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys Ile Asp Ala
            420                 425                 430
Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile Phe Tyr Asn
        435                 440                 445
```

<210> 70
<211> 447
<212> PRT

<213> Homo sapiens

<220>
<223> Mature HuPH20 36-482

<400> 70

```
Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro Phe Leu Trp
 1               5                  10                  15
Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe Asp Glu Pro
            20                  25                  30
Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala
        35                  40                  45
Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
    50                  55                  60
Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
65                  70                  75                  80
Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile
                85                  90                  95
Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
            100                 105                 110
Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
        115                 120                 125
```

```
Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn Val Gln Leu
    130                 135                 140
Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
145                 150                 155                 160
Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
                165                 170                 175
Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
            180                 185                 190
His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
        195                 200                 205
Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
    210                 215                 220
Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
225                 230                 235                 240
Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
                245                 250                 255
Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
            260                 265                 270
Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
        275                 280                 285
Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
    290                 295                 300
Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
305                 310                 315                 320
Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
                325                 330                 335
Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
            340                 345                 350
Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
        355                 360                 365
Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
    370                 375                 380
Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
385                 390                 395                 400
Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
                405                 410                 415
Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys Ile Asp Ala
            420                 425                 430
Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile Phe Tyr
        435                 440                 445
```

<210> 71
<211> 446
<212> PRT
<213> Homo sapiens

<220>
<223> Mature HuPH20 36-481

<400> 71

```
Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro Phe Leu Trp
 1               5                  10                  15
Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe Asp Glu Pro
            20                  25                  30
Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala
        35                  40                  45
Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
    50                  55                  60
Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
65                  70                  75                  80
Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile
                85                  90                  95

Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
            100                 105                 110
Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
        115                 120                 125
Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn Val Gln Leu
    130                 135                 140
Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
145                 150                 155                 160
Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
                165                 170                 175
Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
            180                 185                 190
His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
        195                 200                 205
Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
    210                 215                 220
Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
225                 230                 235                 240
Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
                245                 250                 255
Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
            260                 265                 270
Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
        275                 280                 285
Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
    290                 295                 300
Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
305                 310                 315                 320
Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
                325                 330                 335
Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
            340                 345                 350
Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
        355                 360                 365
Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
    370                 375                 380
Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
385                 390                 395                 400
Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
                405                 410                 415
Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys Ile Asp Ala
            420                 425                 430
Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile Phe
        435                 440                 445
```

<210> 72
<211> 445

<212> PRT
<213> Homo sapiens

<220>
<223> Mature HuPH20 36-480

<400> 72

```
Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro Phe Leu Trp
 1               5                  10                  15
Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe Asp Glu Pro
            20                  25                  30
Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala
        35                  40                  45
Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
    50                  55                  60
```

```
Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
65                  70                  75                  80
Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile
                85                  90                  95
Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
            100                 105                 110
Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
        115                 120                 125
Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn Val Gln Leu
    130                 135                 140
Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
145                 150                 155                 160
Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
                165                 170                 175
Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
            180                 185                 190
His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
        195                 200                 205
Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
    210                 215                 220
Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
225                 230                 235                 240
Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
                245                 250                 255
Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
            260                 265                 270
Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
        275                 280                 285
Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
    290                 295                 300
Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
305                 310                 315                 320
Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
                325                 330                 335
Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
            340                 345                 350
Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
        355                 360                 365
Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
    370                 375                 380
Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
385                 390                 395                 400
Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
                405                 410                 415
Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys Ile Asp Ala
            420                 425                 430
Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile
        435                 440                 445
```

<210> 73
<211> 479
<212> PRT
<213> Homo sapiens

<220>
<223> Mature HuPH20 36-479

<400> 73

```
Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1               5                  10                  15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
            20                  25                  30

Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
        35                  40                  45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
    50                  55                  60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
65                  70                  75                  80
Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
                85                  90                  95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
            100                 105                 110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
        115                 120                 125
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
    130                 135                 140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145                 150                 155                 160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
                165                 170                 175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
            180                 185                 190
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
        195                 200                 205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
    210                 215                 220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
225                 230                 235                 240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
                245                 250                 255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
            260                 265                 270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
        275                 280                 285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
    290                 295                 300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
305                 310                 315                 320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
                325                 330                 335
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
            340                 345                 350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
        355                 360                 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
    370                 375                 380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
385                 390                 395                 400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
                405                 410                 415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
            420                 425                 430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
        435                 440                 445
Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
    450                 455                 460
Ile Asp Ala Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln
465                 470                 475
```

<210> 74
<211> 443
<212> PRT
<213> Homo sapiens

<220>
<223> Mature HuPH20 36-478

<400> 74

```
Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro Phe Leu Trp
 1               5                  10                  15
Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe Asp Glu Pro
            20                  25                  30
Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala
        35                  40                  45
Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
    50                  55                  60
Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
65                  70                  75                  80
Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile
                85                  90                  95
Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
            100                 105                 110
Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
        115                 120                 125
Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn Val Gln Leu
    130                 135                 140
Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
145                 150                 155                 160
Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
                165                 170                 175
Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
            180                 185                 190
His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
        195                 200                 205
Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
    210                 215                 220
Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
225                 230                 235                 240
Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
                245                 250                 255
Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
            260                 265                 270
Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
        275                 280                 285
Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
    290                 295                 300
Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
305                 310                 315                 320
Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
                325                 330                 335
Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
            340                 345                 350
Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
        355                 360                 365
Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
    370                 375                 380
Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
385                 390                 395                 400
Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
                405                 410                 415
Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys Ile Asp Ala
            420                 425                 430
Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro
        435                 440
```

<210> 75
<211> 442
<212> PRT
<213> Homo sapiens

<220>
<223> Mature HuPH20 36-477

<400> 75

```
Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro Phe Leu Trp
 1               5                  10                  15
Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe Asp Glu Pro
            20                  25                  30
Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala
        35                  40                  45
Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
    50                  55                  60
Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
65                  70                  75                  80
Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile
                85                  90                  95
Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
            100                 105                 110
Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
        115                 120                 125
Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn Val Gln Leu
    130                 135                 140
Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
145                 150                 155                 160
Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
                165                 170                 175
Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
            180                 185                 190
His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
        195                 200                 205
Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
    210                 215                 220
Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
225                 230                 235                 240
Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
                245                 250                 255
Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
            260                 265                 270
Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
        275                 280                 285
Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
    290                 295                 300
Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
305                 310                 315                 320
Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
                325                 330                 335
Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
            340                 345                 350
Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
        355                 360                 365
Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
    370                 375                 380
Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
385                 390                 395                 400
Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
                405                 410                 415
Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys Ile Asp Ala
            420                 425                 430
Phe Leu Lys Pro Pro Met Glu Thr Glu Glu
```

435 440

<210> 76
<211> 441
<212> PRT
<213> Homo sapiens

<220>
<223> Mature HuPH20 36-476

<400> 76

```
Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro Phe Leu Trp
 1               5                  10                  15
Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe Asp Glu Pro
            20                  25                  30
Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala
        35                  40                  45
Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
    50                  55                  60
Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
65                  70                  75                  80
Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile
                85                  90                  95
Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
            100                 105                 110
Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
        115                 120                 125
Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn Val Gln Leu
    130                 135                 140
Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
145                 150                 155                 160
Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
                165                 170                 175
Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
            180                 185                 190
His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
        195                 200                 205
Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
    210                 215                 220
Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
225                 230                 235                 240
Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
                245                 250                 255
Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
            260                 265                 270
Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
        275                 280                 285
Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
    290                 295                 300
Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
305                 310                 315                 320
Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
                325                 330                 335
Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
            340                 345                 350
Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
        355                 360                 365
Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
    370                 375                 380
Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
385                 390                 395                 400
Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp

                405                 410                 415
Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys Ile Asp Ala
            420                 425                 430
Phe Leu Lys Pro Pro Met Glu Thr Glu
        435                 440
```

<210> 77
<211> 440
<212> PRT

<213> Homo sapiens

<220>
<223> Mature HuPH20 36-475

<400> 77

```
Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro Phe Leu Trp
 1               5                  10                  15
Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe Asp Glu Pro
            20                  25                  30
Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala
        35                  40                  45
Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
    50                  55                  60
Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
65                  70                  75                  80
Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile
                85                  90                  95
Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
            100                 105                 110
Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
        115                 120                 125
Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn Val Gln Leu
    130                 135                 140
Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
145                 150                 155                 160
Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
                165                 170                 175
Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
            180                 185                 190
His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
        195                 200                 205
Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
    210                 215                 220
Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
225                 230                 235                 240
Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
                245                 250                 255
Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
            260                 265                 270
Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
        275                 280                 285
Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
    290                 295                 300
Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
305                 310                 315                 320
Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
                325                 330                 335
Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
            340                 345                 350
Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
        355                 360                 365
Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
```

```
    370                 375                 380
Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
385                 390                 395                 400
Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
                405                 410                 415
Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys Ile Asp Ala
            420                 425                 430
Phe Leu Lys Pro Pro Met Glu Thr
        435                 440
```

<210> 78
<211> 439
<212> PRT
<213> Homo sapiens

<220>
<223> Mature HuPH20 36-474

<400> 78

```
Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro Phe Leu Trp
 1               5                  10                  15
Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe Asp Glu Pro
            20                  25                  30
Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala
        35                  40                  45
Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
    50                  55                  60
Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
65                  70                  75                  80
Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile
                85                  90                  95
Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
            100                 105                 110
Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
        115                 120                 125
Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn Val Gln Leu
    130                 135                 140
Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
145                 150                 155                 160
Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
                165                 170                 175
Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
            180                 185                 190
His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
        195                 200                 205
Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
    210                 215                 220
Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
225                 230                 235                 240
Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
                245                 250                 255
Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
            260                 265                 270
Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
        275                 280                 285
Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
    290                 295                 300
Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
305                 310                 315                 320
Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
                325                 330                 335
Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys

            340                 345                 350
Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
        355                 360                 365
Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
    370                 375                 380
Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
385                 390                 395                 400
Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
                405                 410                 415
Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys Ile Asp Ala
            420                 425                 430
Phe Leu Lys Pro Pro Met Glu
        435
```

<210> 79
<211> 438

<212> PRT
<213> Homo sapiens

<220>
<223> Mature HuPH20 36-473

<400> 79

```
Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro Phe Leu Trp
 1               5                  10                  15
Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe Asp Glu Pro
            20                  25                  30
Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala
        35                  40                  45
Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
    50                  55                  60
Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
65                  70                  75                  80
Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile
                85                  90                  95
Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
            100                 105                 110
Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
        115                 120                 125
Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn Val Gln Leu
    130                 135                 140
Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
145                 150                 155                 160
Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
                165                 170                 175
Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
            180                 185                 190
His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
        195                 200                 205
Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
    210                 215                 220
Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
225                 230                 235                 240
Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
                245                 250                 255
Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
            260                 265                 270
Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
        275                 280                 285
Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
    290                 295                 300
Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
```

```
305                 310                 315                 320
Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
                325                 330                 335
Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
            340                 345                 350
Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
        355                 360                 365
Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
    370                 375                 380
Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
385                 390                 395                 400
Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
                405                 410                 415
Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys Ile Asp Ala
            420                 425                 430
Phe Leu Lys Pro Pro Met
        435
```

<210> 80
<211> 437
<212> PRT
<213> Homo sapiens

<220>
<223> Mature HuPH20 36-472

<400> 80

```
Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro Phe Leu Trp
 1               5                  10                  15
Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe Asp Glu Pro
            20                  25                  30
Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala
        35                  40                  45
Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
    50                  55                  60
Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
65                  70                  75                  80
Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile
                85                  90                  95
Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
            100                 105                 110
Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
        115                 120                 125
Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn Val Gln Leu
    130                 135                 140
Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
145                 150                 155                 160
Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
                165                 170                 175
Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
            180                 185                 190
His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
        195                 200                 205
Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
    210                 215                 220
Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
225                 230                 235                 240
Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
                245                 250                 255
Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
            260                 265                 270
Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
        275                 280                 285
Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
    290                 295                 300
Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
305                 310                 315                 320
Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
                325                 330                 335
Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
            340                 345                 350
Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
        355                 360                 365
Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
    370                 375                 380
Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
385                 390                 395                 400
Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
                405                 410                 415
Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys Ile Asp Ala
            420                 425                 430
Phe Leu Lys Pro Pro
        435
```

<210> 81
<211> 436
<212> PRT

<213> Homo sapiens

<220>
<223> Mature HuPH20 36-471

<400> 81

```
Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro Phe Leu Trp
 1               5                  10                  15
Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe Asp Glu Pro
            20                  25                  30
Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala
        35                  40                  45
Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
    50                  55                  60
Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
65                  70                  75                  80
Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile
                85                  90                  95
Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
            100                 105                 110
Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
        115                 120                 125
Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn Val Gln Leu
    130                 135                 140
Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
145                 150                 155                 160
Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
                165                 170                 175
Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
            180                 185                 190
His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
        195                 200                 205
Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
    210                 215                 220
Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
225                 230                 235                 240
Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
```

```
                    245                 250                 255
Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
                260                 265                 270
Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
            275                 280                 285
Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
        290                 295                 300
Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
305                 310                 315                 320
Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
                325                 330                 335
Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
            340                 345                 350
Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
        355                 360                 365
Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
    370                 375                 380
Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
385                 390                 395                 400
Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
                405                 410                 415
Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys Ile Asp Ala
            420                 425                 430
Phe Leu Lys Pro
        435
```

<210> 82
<211> 435
<212> PRT
<213> Homo sapiens

<220>
<223> Mature HuPH20 36-470

<400> 82

```
Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro Phe Leu Trp
 1               5                  10                  15
Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe Asp Glu Pro
            20                  25                  30
Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala
        35                  40                  45
Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
    50                  55                  60
Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
65                  70                  75                  80
Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile
                85                  90                  95
Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
            100                 105                 110
Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
        115                 120                 125
Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn Val Gln Leu
    130                 135                 140
Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
145                 150                 155                 160
Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
                165                 170                 175
Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
            180                 185                 190
His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
        195                 200                 205
Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu

    210                 215                 220
Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
225                 230                 235                 240
Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
                245                 250                 255
Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
            260                 265                 270
Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
        275                 280                 285
Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
    290                 295                 300
Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
305                 310                 315                 320
Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
                325                 330                 335
Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
            340                 345                 350
Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
        355                 360                 365
Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
    370                 375                 380
Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
385                 390                 395                 400
Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
                405                 410                 415
Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys Ile Asp Ala
            420                 425                 430
Phe Leu Lys
        435
```

<210> 83
<211> 434

<212> PRT
<213> Homo sapiens

<220>
<223> Mature HuPH20 36-469

<400> 83

```
Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro Phe Leu Trp
 1               5                  10                  15
Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe Asp Glu Pro
            20                  25                  30
Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala
        35                  40                  45
Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
    50                  55                  60
Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
65                  70                  75                  80
Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile
                85                  90                  95
Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
            100                 105                 110
Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
        115                 120                 125
Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn Val Gln Leu
    130                 135                 140
Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
145                 150                 155                 160
Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
                165                 170                 175
Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
```

```
                180                 185                 190
His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
        195                 200                 205
Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
    210                 215                 220
Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
225                 230                 235                 240
Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
                245                 250                 255
Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
            260                 265                 270
Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
        275                 280                 285
Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
    290                 295                 300
Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
305                 310                 315                 320
Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
                325                 330                 335
Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
            340                 345                 350
Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
        355                 360                 365
Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
    370                 375                 380
Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
385                 390                 395                 400
Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
                405                 410                 415
Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys Ile Asp Ala
            420                 425                 430
Phe Leu
```

<210> 84
<211> 433
<212> PRT
<213> Homo sapiens

<220>
<223> Mature HuPH20 36-468

<400> 84

```
Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro Phe Leu Trp
 1               5                  10                  15
Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe Asp Glu Pro
            20                  25                  30
Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala
        35                  40                  45
Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
    50                  55                  60
Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
65                  70                  75                  80
Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile
                85                  90                  95
Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
            100                 105                 110
Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
        115                 120                 125
Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn Val Gln Leu
    130                 135                 140
Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala

145                 150                 155                 160
Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
                165                 170                 175
Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
            180                 185                 190
His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
        195                 200                 205
Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
    210                 215                 220
Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
225                 230                 235                 240
Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
                245                 250                 255
Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
            260                 265                 270
Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
        275                 280                 285
Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
    290                 295                 300
Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
305                 310                 315                 320
Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
                325                 330                 335
Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
            340                 345                 350
Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
        355                 360                 365
Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
    370                 375                 380
Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
385                 390                 395                 400
Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
                405                 410                 415
Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys Ile Asp Ala
            420                 425                 430
Phe
```

<210> 85
<211> 432
<212> PRT
<213> Homo sapiens

<220>
<223> Mature HuPH20 36-467

<400> 85

```
Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro Phe Leu Trp
 1               5                  10                  15
Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe Asp Glu Pro
            20                  25                  30
Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala
        35                  40                  45
Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
    50                  55                  60
Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
65                  70                  75                  80
Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile
                85                  90                  95
Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
            100                 105                 110
Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val

        115                 120                 125
Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn Val Gln Leu
    130                 135                 140
Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
145                 150                 155                 160
Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
                165                 170                 175
Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
            180                 185                 190
His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
        195                 200                 205
Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
    210                 215                 220
Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
225                 230                 235                 240
Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
                245                 250                 255
Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
            260                 265                 270
Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
        275                 280                 285
Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
    290                 295                 300
Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
305                 310                 315                 320
Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
                325                 330                 335
Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
            340                 345                 350
Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
        355                 360                 365
Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
    370                 375                 380
Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
385                 390                 395                 400
Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
                405                 410                 415
Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys Ile Asp Ala
            420                 425                 430
```

<210> 86
<211> 431
<212> PRT
<213> Homo sapiens

<220>
<223> Mature HuPH20 36-466

<400> 86

```
Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro Phe Leu Trp
 1               5                  10                  15
Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe Asp Glu Pro
            20                  25                  30
Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala
        35                  40                  45
Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
    50                  55                  60
Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
65                  70                  75                  80
Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile
                85                  90                  95
Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
```

```
                100                 105                 110
Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
        115                 120                 125
Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn Val Gln Leu
    130                 135                 140
Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
145                 150                 155                 160
Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
                165                 170                 175
Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
            180                 185                 190
His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
        195                 200                 205
Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
    210                 215                 220
Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
225                 230                 235                 240
Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
                245                 250                 255
Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
            260                 265                 270
Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
        275                 280                 285
Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
    290                 295                 300
Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
305                 310                 315                 320
Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
                325                 330                 335
Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
            340                 345                 350
Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
        355                 360                 365
Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
    370                 375                 380
Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
385                 390                 395                 400
Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
                405                 410                 415
Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys Ile Asp
            420                 425                 430
```

<210> 87
<211> 430
<212> PRT
<213> Homo sapiens

<220>
<223> Mature HuPH20 36-465

<400> 87

```
Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro Phe Leu Trp
 1               5                  10                  15
Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe Asp Glu Pro
            20                  25                  30
Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala
        35                  40                  45
Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
    50                  55                  60
Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
65                  70                  75                  80
Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile

                85                  90                  95
Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
            100                 105                 110
Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
        115                 120                 125
Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn Val Gln Leu
    130                 135                 140
Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
145                 150                 155                 160
Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
                165                 170                 175
Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
            180                 185                 190
His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
        195                 200                 205
Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
    210                 215                 220
Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
225                 230                 235                 240
Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
                245                 250                 255
Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
            260                 265                 270
Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
        275                 280                 285
Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
    290                 295                 300
Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
305                 310                 315                 320
Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
                325                 330                 335
Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
            340                 345                 350
Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
        355                 360                 365
Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
    370                 375                 380
Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
385                 390                 395                 400
Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
                405                 410                 415
Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys Ile
            420                 425                 430
```

<210> 88
<211> 429
<212> PRT
<213> Homo sapiens

<220>
<223> Mature HuPH20 36-464

<400> 88

```
Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro Phe Leu Trp
 1               5                  10                  15
Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe Asp Glu Pro
            20                  25                  30
Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala
        35                  40                  45
Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
    50                  55                  60
Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro

65                  70                  75                  80
Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile
                85                  90                  95
Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
            100                 105                 110
Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
        115                 120                 125
Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Asn Val Gln Leu
    130                 135                 140
Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
145                 150                 155                 160
Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
                165                 170                 175
Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
            180                 185                 190
His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
        195                 200                 205
Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
    210                 215                 220
Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
225                 230                 235                 240
Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
                245                 250                 255
Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
            260                 265                 270
Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
        275                 280                 285
Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
    290                 295                 300
Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
305                 310                 315                 320
Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
                325                 330                 335
Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
            340                 345                 350
Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
        355                 360                 365
Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
    370                 375                 380
Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
385                 390                 395                 400
Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
                405                 410                 415
Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
            420                 425
```

<210> 89
<211> 427
<212> PRT
<213> Homo sapiens

<220>
<223> Mature HuPH20 36-462

<400> 89

```
Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro Phe Leu Trp
 1               5                  10                  15
Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe Asp Glu Pro
            20                  25                  30
Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala
        35                  40                  45
Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
```

```
    50                  55                  60
Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
65                  70                  75                  80
Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile
                85                  90                  95
Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
            100                 105                 110
Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
        115                 120                 125
Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn Val Gln Leu
    130                 135                 140
Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
145                 150                 155                 160
Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
                165                 170                 175
Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
            180                 185                 190
His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
        195                 200                 205
Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
    210                 215                 220
Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
225                 230                 235                 240
Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
                245                 250                 255
Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
            260                 265                 270
Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
        275                 280                 285
Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
    290                 295                 300
Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
305                 310                 315                 320
Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
                325                 330                 335
Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
            340                 345                 350
Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
        355                 360                 365
Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
    370                 375                 380
Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
385                 390                 395                 400
Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
                405                 410                 415
Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly
            420                 425
```

<210> 90
<211> 425
<212> PRT
<213> Homo sapiens

<220>
<223> Mature HuPH20 36-460

<400> 90

```
Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro Phe Leu Trp
 1               5                  10                  15
Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe Asp Glu Pro
            20                  25                  30
Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala

        35                  40                  45
Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
    50                  55                  60
Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
65                  70                  75                  80
Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile
                85                  90                  95
Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
            100                 105                 110
Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
        115                 120                 125
Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn Val Gln Leu
    130                 135                 140
Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
145                 150                 155                 160
Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
                165                 170                 175
Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
            180                 185                 190
His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
        195                 200                 205
Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
    210                 215                 220
Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
225                 230                 235                 240
Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
                245                 250                 255
Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
            260                 265                 270
Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
        275                 280                 285
Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
    290                 295                 300
Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
305                 310                 315                 320
Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
                325                 330                 335
Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
            340                 345                 350
Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
        355                 360                 365
Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
    370                 375                 380
Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
385                 390                 395                 400
Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
                405                 410                 415
Thr Asp Ala Val Asp Val Cys Ile Ala
            420                 425
```

<210> 91
<211> 423
<212> PRT
<213> Homo sapiens

<220>
<223> Mature HuPH20 36-458

<400> 91

```
Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro Phe Leu Trp
 1               5                  10                  15
Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe Asp Glu Pro
            20                  25                  30
Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala
        35                  40                  45
Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
    50                  55                  60
Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
65                  70                  75                  80
Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile
                85                  90                  95
Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
            100                 105                 110
Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
        115                 120                 125
Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn Val Gln Leu
    130                 135                 140
Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
145                 150                 155                 160
Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
                165                 170                 175
Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
            180                 185                 190
His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
        195                 200                 205
Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
    210                 215                 220
Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
225                 230                 235                 240
Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
                245                 250                 255
Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
            260                 265                 270
Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
        275                 280                 285
Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
    290                 295                 300
Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
305                 310                 315                 320
Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
                325                 330                 335
Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
            340                 345                 350
Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
        355                 360                 365
Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
    370                 375                 380
Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
385                 390                 395                 400
Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
                405                 410                 415
Thr Asp Ala Val Asp Val Cys
            420
```

<210> 92
<211> 421
<212> PRT
<213> Homo sapiens

<220>
<223> Mature HuPH20 36-456

<400> 92

Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro Phe Leu Trp

```
 1               5                  10                  15
Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe Asp Glu Pro
            20                  25                  30
Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala
        35                  40                  45
Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
    50                  55                  60
Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
65                  70                  75                  80
Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile
                85                  90                  95
Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
            100                 105                 110
Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
        115                 120                 125
Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn Val Gln Leu
    130                 135                 140
Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
145                 150                 155                 160
Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
                165                 170                 175
Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
            180                 185                 190
His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
        195                 200                 205
Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
    210                 215                 220
Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
225                 230                 235                 240
Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
                245                 250                 255
Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
            260                 265                 270
Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
        275                 280                 285
Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
    290                 295                 300
Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
305                 310                 315                 320
Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
                325                 330                 335
Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
            340                 345                 350
Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
        355                 360                 365
Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
    370                 375                 380
Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
385                 390                 395                 400
Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
                405                 410                 415
Thr Asp Ala Val Asp
            420
```

<210> 93
<211> 419
<212> PRT
<213> Homo sapiens

<220>
<223> Mature HuPH20 36-454

<400> 93

```
Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro Phe Leu Trp
 1               5                  10                  15
Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe Asp Glu Pro
            20                  25                  30
Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala
        35                  40                  45
Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
    50                  55                  60
Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
65                  70                  75                  80
Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile
                85                  90                  95
Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
            100                 105                 110
Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
        115                 120                 125
Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn Val Gln Leu
    130                 135                 140
Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
145                 150                 155                 160
Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
                165                 170                 175
Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
            180                 185                 190
His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
        195                 200                 205
Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
    210                 215                 220
Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
225                 230                 235                 240
Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
                245                 250                 255
Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
            260                 265                 270
Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
        275                 280                 285
Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
    290                 295                 300
Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
305                 310                 315                 320
Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
                325                 330                 335
Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
            340                 345                 350
Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
        355                 360                 365
Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
    370                 375                 380
Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
385                 390                 395                 400
Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
                405                 410                 415
Thr Asp Ala
```

<210> 94
<211> 417
<212> PRT
<213> Homo sapiens

<220>
<223> Mature HuPH20 36-452

<400> 94

```
Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro Phe Leu Trp
 1               5                  10                  15
Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe Asp Glu Pro
            20                  25                  30
Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala
        35                  40                  45
Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
    50                  55                  60
Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
65                  70                  75                  80
Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile
                85                  90                  95
Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
            100                 105                 110
Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
        115                 120                 125
Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn Val Gln Leu
    130                 135                 140
Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
145                 150                 155                 160
Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
                165                 170                 175
Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
            180                 185                 190
His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
        195                 200                 205
Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
    210                 215                 220
Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
225                 230                 235                 240
Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
                245                 250                 255
Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
            260                 265                 270
Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
        275                 280                 285
Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
    290                 295                 300
Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
305                 310                 315                 320
Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
                325                 330                 335
Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
            340                 345                 350
Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
        355                 360                 365
Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
    370                 375                 380
Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
385                 390                 395                 400
Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
                405                 410                 415
Thr
```

<210> 95
<211> 415

<212> PRT
<213> Homo sapiens

<220>
<223> Mature HuPH20 36-450

<400> 95

```
Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro Phe Leu Trp
 1               5                  10                  15
Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe Asp Glu Pro
            20                  25                  30
Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala
        35                  40                  45
Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
    50                  55                  60
Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
65                  70                  75                  80
Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile
                85                  90                  95
Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
            100                 105                 110
Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
        115                 120                 125
Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn Val Gln Leu
    130                 135                 140
Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
145                 150                 155                 160
Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
                165                 170                 175
Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
            180                 185                 190
His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
        195                 200                 205
Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
    210                 215                 220
Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
225                 230                 235                 240
Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
                245                 250                 255
Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
            260                 265                 270
Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
        275                 280                 285
Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
    290                 295                 300
Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
305                 310                 315                 320
Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
                325                 330                 335
Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
            340                 345                 350
Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
        355                 360                 365
Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
    370                 375                 380
Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
385                 390                 395                 400
Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys
                405                 410                 415
```

<210> 96
<211> 473
<212> PRT
<213> Homo sapiens

<220>
<223> Mature HuPH20 36-508

<400> 96

```
Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro Phe Leu Trp
 1               5                  10                  15
Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe Asp Glu Pro
            20                  25                  30
Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala
        35                  40                  45
Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
    50                  55                  60
Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
65                  70                  75                  80
Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile
                85                  90                  95
Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
            100                 105                 110
Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
        115                 120                 125
Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn Val Gln Leu
    130                 135                 140
Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
145                 150                 155                 160
Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
                165                 170                 175
Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
            180                 185                 190
His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
        195                 200                 205
Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
    210                 215                 220
Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
225                 230                 235                 240
Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
                245                 250                 255
Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
            260                 265                 270
Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
        275                 280                 285
Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
    290                 295                 300
Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
305                 310                 315                 320
Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
                325                 330                 335
Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
            340                 345                 350
Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
        355                 360                 365
Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
    370                 375                 380
Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
385                 390                 395                 400
Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
                405                 410                 415
Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys Ile Asp Ala
            420                 425                 430
Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile Phe Tyr Asn
        435                 440                 445
Ala Ser Pro Ser Thr Leu Ser Ala Thr Met Phe Ile Val Ser Ile Leu
    450                 455                 460

Phe Leu Ile Ile Ser Ser Val Ala Ser
465                 470
```

<210> 97
<211> 471
<212> PRT
<213> Homo sapiens

<220>
<223> Mature HuPH20 36-506

<400> 97

```
Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro Phe Leu Trp
 1               5                  10                  15
Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe Asp Glu Pro
            20                  25                  30
Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala
        35                  40                  45
Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
    50                  55                  60
Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
65                  70                  75                  80
Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile
                85                  90                  95
Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
            100                 105                 110
Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
        115                 120                 125
Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn Val Gln Leu
    130                 135                 140
Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
145                 150                 155                 160
Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
                165                 170                 175
Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
            180                 185                 190
His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
        195                 200                 205
Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
    210                 215                 220
Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
225                 230                 235                 240
Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
                245                 250                 255
Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
            260                 265                 270
Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
        275                 280                 285
Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
    290                 295                 300
Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
305                 310                 315                 320
Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
                325                 330                 335
Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
            340                 345                 350
Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
        355                 360                 365
Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
    370                 375                 380
Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
385                 390                 395                 400
```

```
Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
                405                 410                 415
Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys Ile Asp Ala
            420                 425                 430
Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile Phe Tyr Asn
        435                 440                 445
Ala Ser Pro Ser Thr Leu Ser Ala Thr Met Phe Ile Val Ser Ile Leu
    450                 455                 460
Phe Leu Ile Ile Ser Ser Val
465                 470
```

<210> 98
<211> 469
<212> PRT
<213> Homo sapiens

<220>
<223> Mature HuPH20 36-504

<400> 98

```
Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro Phe Leu Trp
 1               5                  10                  15
Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe Asp Glu Pro
            20                  25                  30
Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala
        35                  40                  45
Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
    50                  55                  60
Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
65                  70                  75                  80
Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile
                85                  90                  95
Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
            100                 105                 110
Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
        115                 120                 125
Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn Val Gln Leu
    130                 135                 140
Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
145                 150                 155                 160
Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
                165                 170                 175
Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
            180                 185                 190
His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
        195                 200                 205
Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
    210                 215                 220
Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
225                 230                 235                 240
Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
                245                 250                 255
Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
            260                 265                 270
Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
        275                 280                 285
Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
    290                 295                 300
Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
305                 310                 315                 320
Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
                325                 330                 335

Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
            340                 345                 350
Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
        355                 360                 365
Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
    370                 375                 380
Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
385                 390                 395                 400
Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
                405                 410                 415
Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys Ile Asp Ala
            420                 425                 430
Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile Phe Tyr Asn
        435                 440                 445
Ala Ser Pro Ser Thr Leu Ser Ala Thr Met Phe Ile Val Ser Ile Leu
    450                 455                 460
Phe Leu Ile Ile Ser
465
```

<210> 99
<211> 467
<212> PRT
<213> Homo sapiens

<220>
<223> Mature HuPH20 36-502

<400> 99

```
Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro Phe Leu Trp
 1               5                  10                  15
Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe Asp Glu Pro
            20                  25                  30
Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala
        35                  40                  45
Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
    50                  55                  60
Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
65                  70                  75                  80
Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile
                85                  90                  95
Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
            100                 105                 110
Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
        115                 120                 125
Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn Val Gln Leu
    130                 135                 140
Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
145                 150                 155                 160
Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
                165                 170                 175
Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
            180                 185                 190
His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
        195                 200                 205
Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
    210                 215                 220
Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
225                 230                 235                 240
Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
                245                 250                 255
Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
            260                 265                 270
```

```
Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
        275                 280                 285
Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
    290                 295                 300
Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
305                 310                 315                 320
Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
                325                 330                 335
Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
            340                 345                 350
Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
        355                 360                 365
Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
    370                 375                 380
Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
385                 390                 395                 400
Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
                405                 410                 415
Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys Ile Asp Ala
            420                 425                 430
Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile Phe Tyr Asn
        435                 440                 445
Ala Ser Pro Ser Thr Leu Ser Ala Thr Met Phe Ile Val Ser Ile Leu
    450                 455                 460
Phe Leu Ile
465
```

<210> 100
<211> 465
<212> PRT
<213> Homo sapiens

<220>
<223> Mature HuPH20 36-500

<400> 100

```
Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro Phe Leu Trp
 1               5                  10                  15
Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe Asp Glu Pro
            20                  25                  30
Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala
        35                  40                  45
Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
    50                  55                  60
Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
65                  70                  75                  80
Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile
                85                  90                  95
Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
            100                 105                 110
Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
        115                 120                 125
Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn Val Gln Leu
    130                 135                 140
Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
145                 150                 155                 160
Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
                165                 170                 175
Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
            180                 185                 190
His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
        195                 200                 205

Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
    210                 215                 220
Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
225                 230                 235                 240
Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
                245                 250                 255
Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
            260                 265                 270
Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
        275                 280                 285
Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
    290                 295                 300
Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
305                 310                 315                 320
Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
                325                 330                 335
Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
            340                 345                 350
Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
        355                 360                 365
Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
    370                 375                 380
Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
385                 390                 395                 400
Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
                405                 410                 415
Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys Ile Asp Ala
            420                 425                 430
Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile Phe Tyr Asn
        435                 440                 445
Ala Ser Pro Ser Thr Leu Ser Ala Thr Met Phe Ile Val Ser Ile Leu
    450                 455                 460
Phe
465
```

<210> 101
<211> 463
<212> PRT
<213> Homo sapiens

<220>
<223> Mature HuPH20 36-498

<400> 101

```
Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro Phe Leu Trp
 1               5                  10                  15
Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe Asp Glu Pro
            20                  25                  30
Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala
        35                  40                  45
Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
    50                  55                  60
Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
65                  70                  75                  80
Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile
                85                  90                  95
Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
            100                 105                 110
Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
        115                 120                 125
Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn Val Gln Leu
    130                 135                 140
```

```
Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
145                 150                 155                 160
Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
                165                 170                 175
Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
            180                 185                 190
His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
        195                 200                 205
Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
    210                 215                 220
Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
225                 230                 235                 240
Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
                245                 250                 255
Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
            260                 265                 270
Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
        275                 280                 285
Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
    290                 295                 300
Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
305                 310                 315                 320
Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
                325                 330                 335
Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
            340                 345                 350
Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
        355                 360                 365
Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
    370                 375                 380
Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
385                 390                 395                 400
Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
                405                 410                 415
Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys Ile Asp Ala
            420                 425                 430
Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile Phe Tyr Asn
        435                 440                 445
Ala Ser Pro Ser Thr Leu Ser Ala Thr Met Phe Ile Val Ser Ile
    450                 455                 460
```

<210> 102
<211> 461
<212> PRT
<213> Homo sapiens

<220>
<223> esHuPH20 36-496

<400> 102

```
Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro Phe Leu Trp
 1               5                  10                  15
Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe Asp Glu Pro
            20                  25                  30
Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala
        35                  40                  45
Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
    50                  55                  60
Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
65                  70                  75                  80
Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile
                85                  90                  95

Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
            100                 105                 110
Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
        115                 120                 125
Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn Val Gln Leu
    130                 135                 140
Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
145                 150                 155                 160
Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
                165                 170                 175
Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
            180                 185                 190
His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
        195                 200                 205
Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
    210                 215                 220
Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
225                 230                 235                 240
Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
                245                 250                 255
Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
            260                 265                 270
Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
        275                 280                 285
Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
    290                 295                 300
Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
305                 310                 315                 320
Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
                325                 330                 335
Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
            340                 345                 350
Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
        355                 360                 365
Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
    370                 375                 380
Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
385                 390                 395                 400
Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
                405                 410                 415
Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys Ile Asp Ala
            420                 425                 430
Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile Phe Tyr Asn
        435                 440                 445
Ala Ser Pro Ser Thr Leu Ser Ala Thr Met Phe Ile Val
    450                 455                 460
```

<210> 103

<211> 459
<212> PRT
<213> Homo sapiens

<220>
<223> esHuPH20 36-494

<400> 103

```
Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro Phe Leu Trp
 1               5                  10                  15
Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe Asp Glu Pro
            20                  25                  30
Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala
        35                  40                  45
```

```
Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
    50                  55                  60
Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
65                  70                  75                  80
Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile
                85                  90                  95
Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
            100                 105                 110
Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
        115                 120                 125
Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Asn Val Gln Leu
    130                 135                 140
Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
145                 150                 155                 160
Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
                165                 170                 175
Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
            180                 185                 190
His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
        195                 200                 205
Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
    210                 215                 220
Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
225                 230                 235                 240
Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
                245                 250                 255
Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
            260                 265                 270
Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
        275                 280                 285
Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
    290                 295                 300
Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
305                 310                 315                 320
Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
                325                 330                 335
Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
            340                 345                 350
Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
        355                 360                 365
Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
    370                 375                 380
Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
385                 390                 395                 400
Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
                405                 410                 415
Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys Ile Asp Ala
            420                 425                 430
Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile Phe Tyr Asn
        435                 440                 445
Ala Ser Pro Ser Thr Leu Ser Ala Thr Met Phe
    450                 455
```

<210> 104
<211> 457
<212> PRT
<213> Homo sapiens

<220>
<223> esHuPH20 36-492

<400> 104

```
Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro Phe Leu Trp
 1               5                  10                  15
Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe Asp Glu Pro
            20                  25                  30
Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala
        35                  40                  45
Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
    50                  55                  60
Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
65                  70                  75                  80
Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile
                85                  90                  95
Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
            100                 105                 110
Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
        115                 120                 125
Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn Val Gln Leu
    130                 135                 140
Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
145                 150                 155                 160
Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
                165                 170                 175
Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
            180                 185                 190
His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
        195                 200                 205
Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
    210                 215                 220
Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
225                 230                 235                 240
Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
                245                 250                 255
Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
            260                 265                 270
Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
        275                 280                 285
Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
    290                 295                 300
Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
305                 310                 315                 320
Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
                325                 330                 335
Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
            340                 345                 350
Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
        355                 360                 365
Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
    370                 375                 380
Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
385                 390                 395                 400
Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
                405                 410                 415
Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys Ile Asp Ala
            420                 425                 430
Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile Phe Tyr Asn
        435                 440                 445
Ala Ser Pro Ser Thr Leu Ser Ala Thr
    450                 455
```

<210> 105
<211> 453
<212> PRT
<213> Homo sapiens

<220>
<223> Mature HuPH20 36-488

<400> 105

```
Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro Phe Leu Trp
 1               5                  10                  15
Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe Asp Glu Pro
            20                  25                  30
Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala
        35                  40                  45
Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
    50                  55                  60
Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
65                  70                  75                  80
Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile
                85                  90                  95
Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
            100                 105                 110
Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
        115                 120                 125
Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn Val Gln Leu
    130                 135                 140
Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
145                 150                 155                 160
Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
                165                 170                 175
Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
            180                 185                 190
His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
        195                 200                 205
Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
    210                 215                 220
Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
225                 230                 235                 240
Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
                245                 250                 255
Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
            260                 265                 270
Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
        275                 280                 285
Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
    290                 295                 300
Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
305                 310                 315                 320
Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
                325                 330                 335
Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
            340                 345                 350
Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
        355                 360                 365
Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
    370                 375                 380
Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
385                 390                 395                 400
Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
                405                 410                 415
Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys Ile Asp Ala
            420                 425                 430
Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile Phe Tyr Asn
        435                 440                 445
Ala Ser Pro Ser Thr
```

450

<210> 106
<211> 451
<212> PRT
<213> Homo sapiens

<220>
<223> Mature HuPH20 36-486

<400> 106

```
Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro Phe Leu Trp
 1               5                  10                  15
Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe Asp Glu Pro
            20                  25                  30
Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala
        35                  40                  45
Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
    50                  55                  60
Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
65                  70                  75                  80
Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile
                85                  90                  95
Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
            100                 105                 110
Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
        115                 120                 125
Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn Val Gln Leu
    130                 135                 140
Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
145                 150                 155                 160
Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
                165                 170                 175
Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
            180                 185                 190
His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
        195                 200                 205
Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
    210                 215                 220
Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
225                 230                 235                 240
Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
                245                 250                 255
Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
            260                 265                 270
Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
        275                 280                 285
Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
    290                 295                 300
Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
305                 310                 315                 320
Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
                325                 330                 335
Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
            340                 345                 350
Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
        355                 360                 365
Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
    370                 375                 380
Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
385                 390                 395                 400
Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
```

```
                    405                 410                 415
Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys Ile Asp Ala
            420                 425                 430
Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile Phe Tyr Asn
        435                 440                 445
Ala Ser Pro
    450
```

<210> 107
<211> 509
<212> PRT
<213> Homo sapiens

<220>
<223> Human PH20 1-509

<400> 107

```
Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1               5                  10                  15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
            20                  25                  30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
        35                  40                  45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
    50                  55                  60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
65                  70                  75                  80
Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
                85                  90                  95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
            100                 105                 110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
        115                 120                 125
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
    130                 135                 140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145                 150                 155                 160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
                165                 170                 175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
            180                 185                 190
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
        195                 200                 205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
    210                 215                 220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
225                 230                 235                 240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
                245                 250                 255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
            260                 265                 270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
        275                 280                 285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
    290                 295                 300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
305                 310                 315                 320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
                325                 330                 335
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
            340                 345                 350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
```

```
          355                 360                 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
    370                 375                 380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
385                 390                 395                 400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
                405                 410                 415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
            420                 425                 430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
        435                 440                 445
Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
    450                 455                 460
Ile Asp Ala Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile
465                 470                 475                 480
Phe Tyr Asn Ala Ser Pro Ser Thr Leu Ser Ala Thr Met Phe Ile Val
                485                 490                 495
Ser Ile Leu Phe Leu Ile Ile Ser Ser Val Ala Ser Leu
            500                 505
```

<210> 108
<211> 474
<212> PRT
<213> Homo sapiens

<220>
<223> Mature Human PH20 36-509

<400> 108

```
Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro Phe Leu Trp
 1               5                  10                  15
Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe Asp Glu Pro
            20                  25                  30
Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala
        35                  40                  45
Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
    50                  55                  60
Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
65                  70                  75                  80
Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile
                85                  90                  95
Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
            100                 105                 110
Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
        115                 120                 125
Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn Val Gln Leu
    130                 135                 140
Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
145                 150                 155                 160
Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
                165                 170                 175
Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
            180                 185                 190
His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
        195                 200                 205
Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
    210                 215                 220
Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
225                 230                 235                 240
Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
                245                 250                 255
Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
```

```
                260                 265                 270
Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
        275                 280                 285
Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
    290                 295                 300
Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
305                 310                 315                 320
Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
                325                 330                 335
Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
                340                 345                 350
Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
        355                 360                 365
Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
    370                 375                 380
Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
385                 390                 395                 400
Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
                405                 410                 415
Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys Ile Asp Ala
                420                 425                 430
Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile Phe Tyr Asn
        435                 440                 445
Ala Ser Pro Ser Thr Leu Ser Ala Thr Met Phe Ile Val Ser Ile Leu
    450                 455                 460
Phe Leu Ile Ile Ser Ser Val Ala Ser Leu
465                 470
```

<210> 109
<211> 482
<212> PRT
<213> Homo sapiens

<220>
<223> Precursor rHuPH20 1-482

<400> 109

```
Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1               5                  10                  15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
            20                  25                  30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
        35                  40                  45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
    50                  55                  60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
65                  70                  75                  80
Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
                85                  90                  95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
            100                 105                 110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
        115                 120                 125
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
    130                 135                 140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145                 150                 155                 160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
                165                 170                 175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
            180                 185                 190
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
```

```
          195                 200                 205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
    210                 215                 220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
225                 230                 235                 240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
                245                 250                 255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
            260                 265                 270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
        275                 280                 285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
    290                 295                 300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
305                 310                 315                 320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
                325                 330                 335
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
            340                 345                 350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
        355                 360                 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
    370                 375                 380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
385                 390                 395                 400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
                405                 410                 415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
            420                 425                 430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
        435                 440                 445
Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
    450                 455                 460
Ile Asp Ala Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile
465                 470                 475                 480
Phe Tyr
```

<210> 110
<211> 1446
<212> DNA
<213> Homo sapiens

<220>
<223> DNA encoding souble rHuPH20 precursor

<400> 110

```
atgggagtgc taaaattcaa gcacatcttt ttcagaagct ttgttaaatc aagtggagta 60
tcccagatag ttttcacctt ccttctgatt ccatgttgct tgactctgaa tttcagagca 120
cctcctgtta ttccaaatgt gcctttcctc tgggcctgga atgccccaag tgaattttgt 180
cttggaaaat ttgatgagcc actagatatg agcctcttct ctttcatagg aagcccccga 240
ataaacgcca ccgggcaagg tgttacaata ttttatgttg atagacttgg ctactatcct 300
tacatagatt caatcacagg agtaactgtg aatggaggaa tcccccagaa gatttcctta 360
caagaccatc tggacaaagc taagaaagac attacatttt atatgccagt agacaatttg 420
ggaatggctg ttattgactg ggaagaatgg agacccactt gggcaagaaa ctggaaacct 480
aaagatgttt acaagaatag gtctattgaa ttggttcagc aacaaaatgt acaacttagt 540
ctcacagagg ccactgagaa agcaaaacaa gaatttgaaa aggcagggaa ggatttcctg 600
gtagagacta taaaattggg aaaattactt cggccaaatc acttgtgggg ttattatctt 660
tttccggatt gttacaacca tcactataag aaacccggtt acaatggaag ttgcttcaat 720
gtagaaataa aaagaaatga tgatctcagc tggttgtgga atgaaagcac tgctctttac 780
ccatccattt atttgaacac tcagcagtct cctgtagctg ctacactcta tgtgcgcaat 840
cgagttcggg aagccatcag agtttccaaa atacctgatg caaaaagtcc acttccggtt 900

tttgcatata cccgcatagt ttttactgat caagttttga aattcctttc tcaagatgaa 960
cttgtgtata catttggcga aactgttgct ctgggtgctt ctggaattgt aatatgggga 1020
accctcagta taatgcgaag tatgaaatct tgcttgctcc tagacaatta catggagact 1080
atactgaatc cttacataat caacgtcaca ctagcagcca aaatgtgtag ccaagtgctt 1140
tgccaggagc aaggagtgtg tataaggaaa aactggaatt caagtgacta tcttcacctc 1200
aacccagata attttgctat tcaacttgag aaaggtggaa agttcacagt acgtggaaaa 1260
ccgacacttg aagacctgga gcaattttct gaaaaatttt attgcagctg ttatagcacc 1320
ttgagttgta aggagaaagc tgatgtaaaa gacactgatg ctgttgatgt gtgtattgct 1380
gatggtgtct gtatagatgc ttttctaaaa cctcccatgg agacagaaga acctcaaatt 1440
ttctac                                                          1446
```

<210> 111
<211> 553
<212> PRT
<213> Bos taurus

<220>
<223> Bovine PH20

<400> 111

```
Met Arg Met Leu Arg Arg His His Ile Ser Phe Arg Ser Phe Ala Gly
 1               5                  10                  15
Ser Ser Gly Thr Pro Gln Ala Val Phe Thr Phe Leu Leu Leu Pro Cys
            20                  25                  30
Cys Leu Ala Leu Asp Phe Arg Ala Pro Pro Leu Ile Ser Asn Thr Ser
        35                  40                  45
Phe Leu Trp Ala Trp Asn Ala Pro Val Glu Arg Cys Val Asn Arg Arg
    50                  55                  60
Phe Gln Leu Pro Pro Asp Leu Arg Leu Phe Ser Val Lys Gly Ser Pro
65                  70                  75                  80
Gln Lys Ser Ala Thr Gly Gln Phe Ile Thr Leu Phe Tyr Ala Asp Arg
                85                  90                  95
Leu Gly Tyr Tyr Pro His Ile Asp Glu Lys Thr Gly Lys Thr Val Phe
            100                 105                 110
Gly Gly Ile Pro Gln Leu Gly Asn Leu Lys Ser His Met Glu Lys Ala
        115                 120                 125
Lys Asn Asp Ile Ala Tyr Tyr Ile Pro Asn Asp Ser Val Gly Leu Ala
    130                 135                 140
Val Ile Asp Trp Glu Asn Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys
145                 150                 155                 160
Pro Lys Asp Val Tyr Arg Asp Glu Ser Val Glu Leu Val Leu Gln Lys
                165                 170                 175
Asn Pro Gln Leu Ser Phe Pro Glu Ala Ser Lys Ile Ala Lys Val Asp
            180                 185                 190
Phe Glu Thr Ala Gly Lys Ser Phe Met Gln Glu Thr Leu Lys Leu Gly
        195                 200                 205
Lys Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp
    210                 215                 220
Cys Tyr Asn His Asn His Asn Gln Pro Thr Tyr Asn Gly Asn Cys Pro
225                 230                 235                 240
Asp Val Glu Lys Arg Arg Asn Asp Asp Leu Glu Trp Leu Trp Lys Glu
                245                 250                 255
Ser Thr Ala Leu Phe Pro Ser Val Tyr Leu Asn Ile Arg Leu Lys Ser
            260                 265                 270
Thr Gln Asn Ala Ala Leu Tyr Val Arg Asn Arg Val Gln Glu Ala Ile
        275                 280                 285
Arg Leu Ser Lys Ile Ala Ser Val Glu Ser Pro Leu Pro Val Phe Val
    290                 295                 300
Tyr Ala Arg Pro Val Phe Thr Asp Gly Ser Ser Thr Tyr Leu Ser Gln
305                 310                 315                 320
Gly Asp Leu Val Asn Ser Val Gly Glu Ile Val Ser Leu Gly Ala Ser
                325                 330                 335
Gly Ile Ile Met Trp Gly Ser Leu Asn Leu Ser Leu Ser Met Gln Ser
```

```
                340                 345                 350
Cys Met Asn Leu Gly Thr Tyr Leu Asn Thr Thr Leu Asn Pro Tyr Ile
        355                 360                 365
Ile Asn Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys His
    370                 375                 380
Asn Glu Gly Val Cys Thr Arg Lys His Trp Asn Ser Ser Asp Tyr Leu
385                 390                 395                 400
His Leu Asn Pro Met Asn Phe Ala Ile Gln Thr Gly Glu Gly Gly Lys
                405                 410                 415
Tyr Thr Val Pro Gly Thr Val Thr Leu Glu Asp Leu Gln Lys Phe Ser
            420                 425                 430
Asp Thr Phe Tyr Cys Ser Cys Tyr Ala Asn Ile His Cys Lys Lys Arg
        435                 440                 445
Val Asp Ile Lys Asn Val His Ser Val Asn Val Cys Met Ala Glu Asp
    450                 455                 460
Ile Cys Ile Asp Ser Pro Val Lys Leu Gln Pro Ser Asp His Ser Ser
465                 470                 475                 480
Ser Gln Glu Ala Ser Thr Thr Thr Phe Ser Ser Ile Ser Pro Ser Thr
                485                 490                 495
Thr Thr Ala Thr Val Ser Pro Cys Thr Pro Glu Lys His Ser Pro Glu
            500                 505                 510
Cys Leu Lys Val Arg Cys Ser Glu Val Ile Pro Asn Val Thr Gln Lys
        515                 520                 525
Ala Cys Gln Ser Val Lys Leu Lys Asn Ile Ser Tyr Gln Ser Pro Ile
    530                 535                 540
Gln Asn Ile Lys Asn Gln Thr Thr Tyr
545                 550
```

<210> 112
<211> 545
<212> PRT
<213> Oryctolagus cuniculus

<220>
<223> Rabbit PH20

<400> 112

```
Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Gly Ser Ala Val Glu
 1               5                  10                  15
Leu Ser Gly Val Phe Gln Ile Val Phe Ile Phe Leu Leu Ile Pro Cys
            20                  25                  30
Cys Leu Thr Ala Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
        35                  40                  45
Phe Leu Trp Ala Trp Asn Ala Pro Thr Glu Phe Cys Leu Gly Lys Ser
    50                  55                  60
Gly Glu Pro Leu Asp Met Ser Leu Phe Ser Leu Phe Gly Ser Pro Arg
65                  70                  75                  80
Lys Asn Lys Thr Gly Gln Gly Ile Thr Ile Phe Tyr Val Asp Arg Leu
                85                  90                  95
Gly Tyr Tyr Pro Tyr Ile Asp Pro His Thr Gly Ala Ile Val His Gly
            100                 105                 110
Arg Ile Pro Gln Leu Gly Pro Leu Gln Gln His Leu Thr Lys Leu Arg
        115                 120                 125
Gln Glu Ile Leu Tyr Tyr Met Pro Lys Asp Asn Val Gly Leu Ala Val
    130                 135                 140
Ile Asp Trp Glu Glu Trp Leu Pro Thr Trp Leu Arg Asn Trp Lys Pro
145                 150                 155                 160
Lys Asp Ile Tyr Arg Ile Lys Ser Ile Glu Leu Val Lys Ser Gln His
                165                 170                 175
Pro Gln Tyr Asn His Ser Tyr Ala Thr Glu Lys Ala Lys Arg Asp Phe
            180                 185                 190
Glu Lys Ala Gly Lys Asp Phe Met Glu Glu Thr Leu Lys Leu Gly Arg
```

```
                195                 200                 205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
    210                 215                 220
Tyr Asn His His Tyr Asp Lys Pro Asn Leu Tyr Lys Gly Ser Cys Phe
225                 230                 235                 240
Asp Ile Glu Lys Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Lys Glu
                245                 250                 255
Ser Thr Ala Leu Phe Pro Ser Val Tyr Leu Thr Ser Arg Ala Arg Ser
            260                 265                 270
Ala Thr Ala Leu Ser Lys Leu Tyr Val Val Arg Asn Arg Val His Glu
        275                 280                 285
Ala Ile Arg Val Ser Lys Ile Pro Asp Asp Lys Ser Pro Leu Pro Asn
    290                 295                 300
Phe Val Tyr Thr Arg Leu Val Phe Thr Asp Gln Ile Phe Gln Phe Leu
305                 310                 315                 320
Ser His His Asp Leu Val Tyr Thr Ile Gly Glu Ile Val Ala Leu Gly
                325                 330                 335
Ala Ser Gly Ile Val Val Trp Gly Ser Gln Ser Leu Ala Arg Ser Met
            340                 345                 350
Lys Ser Cys Leu His Leu Asp Asn Tyr Met Lys Thr Ile Leu Asn Pro
        355                 360                 365
Tyr Leu Ile Asn Val Thr Leu Ala Ala Lys Met Cys Asn Gln Val Leu
    370                 375                 380
Cys Gln Glu Gln Gly Val Cys Thr Arg Lys Asn Trp Asn Pro Asn Asp
385                 390                 395                 400
Tyr Leu His Leu Asn Pro Gly Asn Phe Ala Ile Gln Leu Gly Ser Asn
                405                 410                 415
Gly Thr Tyr Lys Val Asp Gly Lys Pro Thr Leu Thr Asp Leu Glu Gln
            420                 425                 430
Phe Ser Lys Asn Phe Gln Cys Ser Cys Tyr Thr Asn Leu Asn Cys Lys
        435                 440                 445
Glu Arg Thr Asp Met Asn Asn Val Arg Thr Val Asn Val Cys Ala Val
    450                 455                 460
Glu Asn Val Cys Ile Asp Thr Asn Val Gly Pro Gln Ala Val Thr Tyr
465                 470                 475                 480
Ala Pro Lys Glu Lys Lys Asp Val Ala His Ile Leu Ser Asn Thr Thr
                485                 490                 495
Ser Ile Asn Ser Ser Thr Thr Met Ser Leu Pro Phe Pro Arg Lys His
            500                 505                 510
Val Ser Gly Cys Leu Leu Val Leu Cys Met Tyr Ser Gln Tyr Leu Asn
        515                 520                 525
Ile Cys Tyr Arg Leu Val Ala Ile Gly Ile Gln His Gly Tyr Tyr Leu
    530                 535                 540
Lys
545
```

<210> 113
<211> 114
<212> PRT
<213> Ovis aries

<220>
<223> Sheep PH20 (partial)

<400> 113

```
Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Leu Ser Lys Ile
 1               5                  10                  15
Ala Ser Val Glu Ser Pro Leu Pro Val Phe Val Tyr His Arg Pro Val
            20                  25                  30
Phe Thr Asp Gly Ser Ser Thr Tyr Leu Ser Gln Gly Asp Leu Val Asn
        35                  40                  45
Ser Val Gly Glu Ile Val Ala Leu Gly Ala Ser Gly Ile Ile Met Trp

    50                  55                  60
Gly Ser Leu Asn Leu Ser Leu Thr Met Gln Ser Cys Met Asn Leu Gly
65                  70                  75                  80
Asn Tyr Leu Asn Thr Thr Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
                85                  90                  95
Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
            100                 105                 110
Ile Arg
```

<210> 114
<211> 510
<212> PRT
<213> Macaca fascicularis

<220>
<223> Cynomolgus monkey PH20

<400> 114

```
Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1               5                  10                  15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
            20                  25                  30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Ile Ile Pro Asn Val Pro
        35                  40                  45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
    50                  55                  60
Asn Glu Pro Leu Asp Met Ser Leu Phe Thr Leu Met Gly Ser Pro Arg
65                  70                  75                  80
Ile Asn Val Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
                85                  90                  95
Gly Tyr Tyr Pro Tyr Ile Asp Leu Thr Thr Gly Val Thr Val His Gly
            100                 105                 110
Gly Ile Pro Gln Lys Val Ser Leu Gln Asp His Leu Asp Lys Ser Lys
        115                 120                 125
Gln Asp Ile Leu Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
    130                 135                 140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145                 150                 155                 160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
                165                 170                 175
Val Gln Leu Ser Leu Pro Gln Ala Thr Asp Lys Ala Lys Gln Glu Phe
            180                 185                 190
Glu Lys Ala Gly Lys Asp Phe Met Leu Glu Thr Ile Lys Leu Gly Arg
        195                 200                 205
Ser Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
    210                 215                 220
Tyr Asn His His Tyr Arg Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asp
225                 230                 235                 240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
                245                 250                 255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Val Val
            260                 265                 270
Val Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
        275                 280                 285
Ser Lys Ile Pro Asp Ala Lys Asn Pro Leu Pro Val Phe Val Tyr Ala
    290                 295                 300
Arg Leu Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Arg Glu Glu
305                 310                 315                 320
Leu Val Ser Thr Leu Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
                325                 330                 335
Val Ile Trp Gly Ser Leu Ser Ile Thr Arg Ser Met Lys Ser Cys Leu
```

```
                340                 345                 350
Leu Leu Asp Thr Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
            355                 360                 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
        370                 375                 380
Gly Val Cys Ile Arg Lys Asp Trp Asn Ser Ser Asp Tyr Leu His Leu
385                 390                 395                 400
Asn Pro Asp Asn Phe Asp Ile Arg Leu Glu Lys Gly Gly Lys Phe Thr
                    405                 410                 415
Val His Gly Lys Pro Thr Val Glu Asp Leu Glu Glu Phe Ser Glu Lys
                420                 425                 430
Phe Tyr Cys Ser Cys Tyr Thr Asn Leu Ser Cys Lys Glu Lys Ala Asp
            435                 440                 445
Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
        450                 455                 460
Ile Asp Ala Ser Leu Lys Pro Pro Val Glu Thr Glu Gly Ser Pro Pro
465                 470                 475                 480
Ile Phe Tyr Asn Thr Ser Ser Ser Thr Val Ser Thr Thr Met Phe Ile
                    485                 490                 495
Val Asn Ile Leu Phe Leu Ile Ile Ser Ser Val Ala Ser Leu
                500                 505                 510
```

<210> 115
<211> 529
<212> PRT
<213> Cavia porcellus

<220>
<223> Guinea Pig PH20

<400> 115

```
Met Gly Ala Phe Thr Phe Lys His Ser Phe Phe Gly Ser Phe Val Glu
 1               5                  10                  15
Cys Ser Gly Val Leu Gln Thr Val Phe Ile Phe Leu Leu Ile Pro Cys
            20                  25                  30
Cys Leu Ala Asp Lys Arg Ala Pro Pro Leu Ile Pro Asn Val Pro Leu
        35                  40                  45
Leu Trp Val Trp Asn Ala Pro Thr Glu Phe Cys Ile Gly Gly Thr Asn
    50                  55                  60
Gln Pro Leu Asp Met Ser Phe Phe Ser Ile Val Gly Thr Pro Arg Lys
65                  70                  75                  80
Asn Ile Thr Gly Gln Ser Ile Thr Leu Tyr Tyr Val Asp Arg Leu Gly
                85                  90                  95
Tyr Tyr Pro Tyr Ile Asp Pro His Thr Gly Ala Ile Val His Gly Gly
            100                 105                 110
Leu Pro Gln Leu Met Asn Leu Gln Gln His Leu Arg Lys Ser Arg Gln
        115                 120                 125
Asp Ile Leu Phe Tyr Met Pro Thr Asp Ser Val Gly Leu Ala Val Ile
    130                 135                 140
Asp Trp Glu Glu Trp Arg Pro Thr Trp Thr Arg Asn Trp Arg Pro Lys
145                 150                 155                 160
Asp Ile Tyr Arg Asn Lys Ser Ile Glu Leu Val Lys Ser Gln His Pro
                165                 170                 175
Gln Tyr Asn His Ser Tyr Ala Val Ala Val Ala Lys Arg Asp Phe Glu
            180                 185                 190
Arg Thr Gly Lys Ala Phe Met Leu Glu Thr Leu Lys Leu Gly Lys Ser
        195                 200                 205
Leu Arg Pro Ser Ser Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr
    210                 215                 220
Asn Thr His Phe Thr Lys Pro Asn Tyr Asp Gly His Cys Pro Pro Ile
225                 230                 235                 240
Glu Leu Gln Arg Asn Asn Asp Leu Gln Trp Leu Trp Asn Asp Ser Thr
```

```
                    245                 250                 255
Ala Leu Tyr Pro Ser Val Tyr Leu Thr Ser Arg Val Arg Ser Ser Gln
                260                 265                 270
Asn Gly Ala Leu Tyr Val Arg Asn Arg Val His Glu Ser Ile Arg Val
            275                 280                 285
Ser Lys Leu Met Asp Asp Lys Asn Pro Leu Pro Ile Tyr Val Tyr Ile
        290                 295                 300
Arg Leu Val Phe Thr Asp Gln Thr Thr Thr Phe Leu Glu Leu Asp Asp
305                 310                 315                 320
Leu Val His Ser Val Gly Glu Ile Val Pro Leu Gly Val Ser Gly Ile
                325                 330                 335
Ile Ile Trp Gly Ser Leu Ser Leu Thr Arg Ser Leu Val Ser Cys Ile
            340                 345                 350
Gly Leu Glu Asn Tyr Met Lys Gly Thr Leu Leu Pro Tyr Leu Ile Asn
        355                 360                 365
Val Thr Leu Ala Ala Lys Met Cys Gly Gln Val Leu Cys Lys Asn Gln
    370                 375                 380
Gly Ile Cys Thr Arg Lys Asp Trp Asn Thr Asn Thr Tyr Leu His Leu
385                 390                 395                 400
Asn Ala Thr Asn Phe Asp Ile Glu Leu Gln Gln Asn Gly Lys Phe Val
                405                 410                 415
Val His Gly Lys Pro Ser Leu Glu Asp Leu Gln Glu Phe Ser Lys Asn
            420                 425                 430
Phe His Cys Ser Cys Tyr Thr Asn Val Ala Cys Lys Asp Arg Leu Asp
        435                 440                 445
Val His Asn Val Arg Ser Val Asn Val Cys Thr Ala Asn Asn Ile Cys
    450                 455                 460
Ile Asp Ala Val Leu Asn Phe Pro Ser Leu Asp Asp Asp Asp Glu Pro
465                 470                 475                 480
Pro Ile Thr Asp Asp Thr Ser Gln Asn Gln Asp Ser Ile Ser Asp Ile
                485                 490                 495
Thr Ser Ser Ala Pro Pro Ser Ser His Ile Leu Pro Lys Asp Leu Ser
            500                 505                 510
Trp Cys Leu Phe Leu Leu Ser Ile Phe Ser Gln His Trp Lys Tyr Leu
        515                 520                 525
Leu
```

<210> 116
<211> 512
<212> PRT
<213> Rattus norvegicus

<220>
<223> Rat PH20

<400> 116

```
Met Gly Glu Leu Gln Phe Lys Trp Leu Phe Trp Arg Ser Phe Ala Glu
 1               5                  10                  15
Ser Gly Gly Thr Phe Gln Thr Val Leu Ile Phe Leu Phe Ile Pro Tyr
            20                  25                  30
Ser Leu Thr Val Asp Tyr Arg Ala Thr Pro Val Leu Ser Asp Thr Thr
        35                  40                  45
Phe Val Trp Val Trp Asn Val Pro Thr Glu Ala Cys Val Glu Asn Val
    50                  55                  60
Thr Glu Pro Ile Asp Leu Ser Phe Phe Ser Leu Ile Gly Ser Pro Arg
65                  70                  75                  80
Lys Thr Ala Ile Gly Gln Pro Val Thr Leu Phe Tyr Val Asp Arg Leu
                85                  90                  95
Gly Asn Tyr Pro His Ile Asp Ala Gln Gln Thr Glu His His Gly Gly
            100                 105                 110
Ile Pro Gln Lys Gly Asp Leu Thr Thr His Leu Val Lys Ala Lys Glu
```

```
          115                 120                 125
Asp Val Glu Arg Tyr Ile Pro Thr Asp Lys Leu Gly Leu Ala Ile Ile
      130                 135                 140
Asp Trp Glu Glu Trp Arg Pro Thr Trp Met Arg Asn Trp Thr Pro Lys
145                 150                 155                 160
Asp Ile Tyr Arg Asn Lys Ser Ile Glu Leu Val Gln Ala Ala Asp Pro
                  165                 170                 175
Ala Ile Asn Ile Thr Glu Ala Thr Val Arg Ala Lys Ala Gln Phe Glu
              180                 185                 190
Gly Ala Ala Lys Glu Phe Met Glu Gly Thr Leu Lys Leu Gly Lys His
          195                 200                 205
Ile Arg Pro Lys His Leu Trp Gly Phe Tyr Leu Phe Pro Asp Cys Tyr
      210                 215                 220
Asn Asn Lys Phe Gln Val Asp Asn Tyr Asp Gly Gln Cys Pro Asp Val
225                 230                 235                 240
Glu Lys Lys Arg Asn Asp Asp Leu Asp Trp Leu Trp Lys Glu Ser Thr
                  245                 250                 255
Gly Leu Tyr Pro Ser Val Tyr Leu Lys Lys Asp Leu Lys Ser Ser Arg
              260                 265                 270
Lys Ala Thr Leu Tyr Val Arg Tyr Arg Val Leu Glu Ser Ile Arg Val
          275                 280                 285
Ser Lys Val Ser Asp Glu Ser Asn Pro Val Pro Ile Phe Val Tyr Ile
      290                 295                 300
Arg Leu Val Phe Thr Asp His Val Ser Glu Tyr Leu Leu Glu Asp Asp
305                 310                 315                 320
Leu Val Asn Thr Ile Gly Glu Ile Val Ala Gln Gly Thr Ser Gly Ile
                  325                 330                 335
Ile Ile Trp Asp Ala Met Ser Leu Ala Gln Arg Ser Ala Gly Cys Pro
              340                 345                 350
Ile Leu Arg Gln Tyr Met Lys Thr Thr Leu Asn Pro Tyr Ile Val Asn
          355                 360                 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Thr Leu Cys Lys Glu Lys
      370                 375                 380
Gly Met Cys Ser Arg Lys Thr Glu Ser Ser Asp Ala Tyr Leu His Leu
385                 390                 395                 400
Asp Pro Ser Ser Phe Ser Ile Asn Val Thr Glu Ala Gly Lys Tyr Glu
                  405                 410                 415
Val Leu Gly Lys Pro Glu Val Lys Asp Leu Glu Tyr Phe Ser Glu His
              420                 425                 430
Phe Lys Cys Ser Cys Phe Ser Lys Met Thr Cys Glu Glu Thr Ser Asp
          435                 440                 445
Met Arg Ser Ile Gln Asp Val Asn Val Cys Met Gly Asp Asn Val Cys
      450                 455                 460
Ile Lys Ala Thr Leu Gly Pro Asn Ser Ala Phe His Leu Leu Pro Gly
465                 470                 475                 480
Lys Gly Leu Leu Leu Met Thr Thr Leu Ala His Ile Leu His His Leu
                  485                 490                 495
Pro His Asp Ile Phe Val Phe Pro Trp Lys Met Leu Val Ser Thr Pro
              500                 505                 510
```

<210> 117
<211> 512
<212> PRT
<213> Mus musculus

<220>
<223> Mouse PH20

<400> 117

```
Met Gly Glu Leu Arg Phe Lys His Leu Phe Trp Gly Ser Phe Val Glu
 1               5                  10                  15
Ser Gly Gly Thr Phe Gln Thr Val Leu Ile Phe Leu Leu Ile Pro Cys
```

```
                20                  25                  30
Ser Leu Thr Val Asp Tyr Arg Ala Ala Pro Ile Leu Ser Asn Thr Thr
        35                  40                  45
Phe Leu Trp Ile Trp Asn Val Pro Thr Glu Arg Cys Val Gly Asn Val
    50                  55                  60
Asn Asp Pro Ile Asp Leu Ser Phe Phe Ser Leu Ile Gly Ser Pro Arg
65                  70                  75                  80
Lys Thr Ala Thr Gly Gln Pro Val Thr Leu Phe Tyr Val Asp Arg Leu
                85                  90                  95
Gly Leu Tyr Pro His Ile Asp Ala Asn Gln Ala Glu His Tyr Gly Gly
            100                 105                 110
Ile Pro Gln Arg Gly Asp Tyr Gln Ala His Leu Arg Lys Ala Lys Thr
        115                 120                 125
Asp Ile Glu His Tyr Ile Pro Asp Asp Lys Leu Gly Leu Ala Ile Ile
    130                 135                 140
Asp Trp Glu Glu Trp Arg Pro Thr Trp Leu Arg Asn Trp Lys Pro Lys
145                 150                 155                 160
Asp Asn Tyr Arg Asn Lys Ser Ile Glu Leu Val Gln Ser Thr Asn Pro
                165                 170                 175
Gly Leu Ser Ile Thr Glu Ala Thr Gln Lys Ala Ile Gln Gln Phe Glu
            180                 185                 190
Glu Ala Gly Arg Lys Phe Met Glu Gly Thr Leu His Leu Gly Lys Phe
        195                 200                 205
Leu Arg Pro Asn Gln Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr
    210                 215                 220
Asn Asn Lys Phe Gln Asp Pro Lys Tyr Asp Gly Gln Cys Pro Ala Val
225                 230                 235                 240
Glu Lys Lys Arg Asn Asp Asn Leu Lys Trp Leu Trp Lys Ala Ser Thr
                245                 250                 255
Gly Leu Tyr Pro Ser Val Tyr Leu Lys Lys Asp Leu Lys Ser Asn Arg
            260                 265                 270
Gln Ala Thr Leu Tyr Val Arg Tyr Arg Val Val Glu Ala Ile Arg Val
        275                 280                 285
Ser Lys Val Gly Asn Ala Ser Asp Pro Val Pro Ile Phe Val Tyr Ile
    290                 295                 300
Arg Leu Val Phe Thr Asp Arg Thr Ser Glu Tyr Leu Leu Glu Asp Asp
305                 310                 315                 320
Leu Val Asn Thr Ile Gly Glu Ile Val Ala Leu Gly Thr Ser Gly Ile
                325                 330                 335
Ile Ile Trp Asp Ala Met Ser Leu Ala Gln Arg Ala Ala Gly Cys Pro
            340                 345                 350
Ile Leu His Lys Tyr Met Gln Thr Thr Leu Asn Pro Tyr Ile Val Asn
        355                 360                 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Thr Leu Cys Asn Glu Lys
    370                 375                 380
Gly Met Cys Ser Arg Arg Lys Glu Ser Ser Asp Val Tyr Leu His Leu
385                 390                 395                 400
Asn Pro Ser His Phe Asp Ile Met Leu Thr Glu Thr Gly Lys Tyr Glu
                405                 410                 415
Val Leu Gly Asn Pro Arg Val Gly Asp Leu Glu Tyr Phe Ser Glu His
            420                 425                 430
Phe Lys Cys Ser Cys Phe Ser Arg Met Thr Cys Lys Glu Thr Ser Asp
        435                 440                 445
Val Lys Asn Val Gln Asp Val Asn Val Cys Val Gly Asp Asn Val Cys
    450                 455                 460
Ile Lys Ala Lys Val Glu Pro Asn Pro Ala Phe Tyr Leu Leu Pro Gly
465                 470                 475                 480
Lys Ser Leu Leu Phe Met Thr Thr Leu Gly His Val Leu Tyr His Leu
                485                 490                 495
Pro Gln Asp Ile Phe Val Phe Pro Arg Lys Thr Leu Val Ser Thr Pro
            500                 505                 510
```

<210> 118
<211> 520
<212> PRT
<213> Ovis aries

<220>
<223> Sheep PH20

<400> 118

```
Leu Asp Phe Pro Ala Pro Pro Leu Ile Ser Asn Thr Ser Phe Leu Trp
 1               5                  10                  15
Ala Trp Asn Ala Pro Ala Glu Arg Cys Val Lys Ile Phe Lys Leu Pro
            20                  25                  30
Pro Asp Leu Arg Leu Phe Ser Val Lys Gly Ser Pro Gln Lys Ser Ala
        35                  40                  45
Thr Gly Gln Phe Ile Thr Leu Phe Tyr Ala Asp Arg Leu Gly Tyr Tyr
    50                  55                  60
Pro His Ile Asp Glu Lys Thr Gly Asn Thr Val Tyr Gly Gly Ile Pro
65                  70                  75                  80
Gln Leu Gly Asn Leu Lys Asn His Leu Glu Lys Ala Lys Lys Asp Ile
                85                  90                  95
Ala Tyr Tyr Ile Pro Asn Asp Ser Val Gly Leu Ala Val Ile Asp Trp
            100                 105                 110
Glu Asn Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
        115                 120                 125
Tyr Arg Asp Glu Ser Val Glu Leu Val Leu Gln Lys Asn Pro Gln Leu
    130                 135                 140
Ser Phe Pro Glu Ala Ser Lys Ile Ala Lys Val Asp Phe Glu Thr Ala
145                 150                 155                 160
Gly Lys Ser Phe Met Gln Glu Thr Leu Lys Leu Gly Lys Leu Leu Arg
                165                 170                 175
Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
            180                 185                 190
Asn Tyr Asn Gln Pro Thr Tyr Asn Gly Asn Cys Ser Asp Leu Glu Lys
        195                 200                 205
Arg Arg Asn Asp Asp Leu Asp Trp Leu Trp Lys Glu Ser Thr Ala Leu
    210                 215                 220
Phe Pro Ser Val Tyr Leu Asn Ile Lys Leu Lys Ser Thr Pro Lys Ala
225                 230                 235                 240
Ala Phe Tyr Val Arg Asn Arg Val Gln Glu Ala Ile Arg Leu Ser Lys
                245                 250                 255
Ile Ala Ser Val Glu Ser Pro Leu Pro Val Phe Val Tyr His Arg Pro
            260                 265                 270
Val Phe Thr Asp Gly Ser Ser Thr Tyr Leu Ser Gln Gly Asp Leu Val
        275                 280                 285
Asn Ser Val Gly Glu Ile Val Ala Leu Gly Ala Ser Gly Ile Ile Met
    290                 295                 300
Trp Gly Ser Leu Asn Leu Ser Leu Thr Met Gln Ser Cys Met Asn Leu
305                 310                 315                 320
Gly Asn Tyr Asn Thr Thr Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
                325                 330                 335
Ala Ala Lys Met Cys Ser Gln Val Leu Cys His Asp Glu Gly Val Cys
            340                 345                 350
Thr Arg Lys Gln Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Ile
        355                 360                 365
Met Asn Phe Ala Ile Gln Thr Gly Lys Gly Gly Lys Tyr Thr Val Pro
    370                 375                 380
Gly Lys Val Thr Leu Glu Asp Leu Gln Thr Phe Ser Asp Lys Phe Tyr
385                 390                 395                 400
Cys Ser Cys Tyr Ala Asn Ile Asn Cys Lys Lys Arg Val Asp Ile Lys
                405                 410                 415
Asn Val His Ser Val Asn Val Cys Met Ala Glu Asp Ile Cys Ile Glu
            420                 425                 430
```

```
Gly Pro Val Lys Leu Gln Pro Ser Asp His Ser Ser Ser Gln Asn Glu
        435                 440                 445
Ala Ser Thr Thr Thr Val Ser Ser Ile Ser Pro Ser Thr Thr Ala Thr
    450                 455                 460
Thr Val Val Ser Pro Cys Thr Pro Glu Lys Gln Ser Pro Glu Cys Leu
465                 470                 475                 480
Lys Val Arg Cys Leu Glu Ala Ile Ala Asn Val Thr Gln Thr Gly Cys
                485                 490                 495
Gln Gly Val Lys Trp Lys Asn Thr Ser Ser Gln Ser Gln Ser Ser Ile
            500                 505                 510
Gln Asn Ile Lys Asn Gln Thr Thr
        515                 520
```

<210> 119
<211> 474
<212> PRT
<213> Bos taurus

<220>
<223> Cow PH20

<400> 119

```
Met Gly Met Phe Arg Arg His His Ile Ser Phe Arg Ser Phe Ala Gly
 1               5                  10                  15
Ser Ser Gly Thr Pro Gln Ala Val Phe Thr Phe Leu Leu Leu Pro Cys
            20                  25                  30
Cys Leu Ala Leu Asp Phe Arg Ala Pro Pro Leu Ile Ser Asn Thr Ser
        35                  40                  45
Phe Leu Trp Ala Trp Asn Ala Pro Val Glu Arg Cys Val Asn Arg Arg
    50                  55                  60
Phe Gln Leu Pro Pro Asp Leu Arg Leu Phe Ser Val Lys Gly Ser Pro
65                  70                  75                  80
Gln Lys Ser Ala Thr Gly Gln Phe Ile Thr Leu Phe Tyr Ala Asp Arg
                85                  90                  95
Leu Gly Tyr Tyr Pro His Ile Asp Glu Lys Thr Gly Lys Thr Val Phe
            100                 105                 110
Gly Gly Ile Pro Gln Leu Gly Asn Leu Lys Ser His Leu Glu Lys Ala
        115                 120                 125
Lys Asn Asp Ile Ala Tyr Tyr Ile Pro Asn Asp Ser Val Gly Leu Ala
    130                 135                 140
Val Ile Asp Trp Glu Asn Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys
145                 150                 155                 160
Pro Lys Asp Val Tyr Arg Asp Glu Ser Val Glu Leu Val Leu Gln Lys
                165                 170                 175
Asn Pro Gln Leu Ser Phe Pro Glu Ala Ser Lys Ile Ala Lys Val Asp
            180                 185                 190
Phe Glu Thr Ala Gly Lys Ser Phe Met Gln Glu Thr Leu Lys Leu Gly
        195                 200                 205
Lys Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp
    210                 215                 220
Cys Tyr Asn His Asn His Asn Gln Pro Thr Tyr Asn Gly Asn Cys Pro
225                 230                 235                 240
Asp Val Glu Lys Arg Arg Asn Asp Asp Leu Glu Trp Leu Trp Lys Glu
                245                 250                 255
Ser Thr Ala Leu Phe Pro Ser Val Tyr Leu Asn Ile Arg Leu Lys Ser
            260                 265                 270
Thr Gln Asn Ala Ala Leu Tyr Val Arg Asn Arg Val Gln Glu Ala Ile
        275                 280                 285
Arg Leu Ser Lys Ile Ala Ser Val Glu Ser Pro Leu Pro Val Phe Val
    290                 295                 300
Tyr Ala Arg Pro Val Phe Thr Asp Gly Ser Ser Thr Tyr Leu Ser Gln
305                 310                 315                 320

Gly Asp Leu Val Asn Ser Val Gly Glu Ile Val Ser Leu Gly Ala Ser
                325                 330                 335
Gly Ile Ile Met Trp Gly Ser Leu Asn Leu Ser Leu Ser Val Gln Ser
            340                 345                 350
Cys Met Asn Leu Gly Thr Tyr Leu Asn Thr Thr Leu Asn Pro Tyr Ile
        355                 360                 365
Ile Asn Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys His
    370                 375                 380
Asp Gly Gly Val Cys Thr Arg Lys His Trp Asn Ser Ser Asp Tyr Leu
385                 390                 395                 400
His Leu Asn Pro Met Asn Phe Ala Ile Gln Thr Gly Glu Gly Gly Lys
                405                 410                 415
Tyr Thr Val Pro Gly Thr Leu Thr Leu Glu Asp Leu Gln Lys Phe Ser
            420                 425                 430
Asp Thr Phe Tyr Cys Ser Cys Tyr Ser Asn Leu Ser Cys Lys Lys Arg
        435                 440                 445
Val Asp Ile Lys Asn Val His Ser Val Asp Val Cys Met Ala Glu Asp
    450                 455                 460
Val Cys Ile Asp Ala Phe Leu Lys Pro Pro
465                 470
```

<210> 120
<211> 517
<212> PRT
<213> Ovis aries

<220>
<223> Sheep PH20 (Halozyme)

<400> 120

```
Asp Phe Arg Ala Pro Pro Leu Ile Ser Asn Thr Ser Phe Leu Trp Ala
 1               5                  10                  15
Trp Asn Ala Pro Ala Glu Arg Cys Ile Lys Ile Phe Lys Leu Pro Pro
            20                  25                  30
Asp Leu Arg Leu Phe Ser Val Lys Gly Ser Pro Gln Lys Ser Ala Thr
        35                  40                  45
Gly Gln Phe Ile Thr Leu Phe Tyr Ala Asp Arg Leu Gly Tyr Tyr Pro
    50                  55                  60
His Ile Asp Glu Lys Thr Gly Asn Thr Val Tyr Gly Gly Ile Pro Gln
65                  70                  75                  80
Leu Gly Asn Leu Lys Asn His Leu Glu Lys Ala Lys Lys Asp Ile Ala
                85                  90                  95
Tyr Tyr Ile Pro Asn Asp Ser Val Gly Leu Ala Val Ile Asp Trp Glu
            100                 105                 110
Asn Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val Tyr
        115                 120                 125
Arg Asp Glu Ser Val Glu Leu Val Leu Gln Lys Asn Pro Gln Leu Ser
    130                 135                 140
Phe Pro Glu Ala Ser Lys Ile Ala Lys Val Asp Phe Glu Thr Ala Gly
145                 150                 155                 160
Lys Ser Phe Met Gln Glu Thr Leu Lys Leu Gly Lys Leu Leu Arg Pro
                165                 170                 175
Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His Asn
            180                 185                 190
Tyr Asn Gln Pro Thr Tyr Asn Gly Asn Cys Ser Asp Leu Glu Lys Arg
        195                 200                 205
Arg Asn Asp Asp Leu Asp Trp Leu Trp Lys Glu Ser Thr Ala Leu Phe
    210                 215                 220
Pro Ser Val Tyr Leu Asn Ile Lys Leu Lys Ser Thr Pro Lys Ala Ala
225                 230                 235                 240
Phe Tyr Val Arg Asn Arg Val Gln Glu Ala Ile Arg Leu Ser Lys Ile
                245                 250                 255
```

```
Ala Ser Val Glu Ser Pro Leu Pro Val Phe Val Tyr His Arg Pro Val
            260                 265                 270
Phe Thr Asp Gly Ser Ser Thr Tyr Leu Ser Gln Gly Asp Leu Val Asn
        275                 280                 285
Ser Val Gly Glu Ile Val Ala Leu Gly Ala Ser Gly Ile Ile Met Trp
    290                 295                 300
Gly Ser Leu Asn Leu Ser Leu Thr Met Gln Ser Cys Met Asn Leu Gly
305                 310                 315                 320
Asn Tyr Leu Asn Thr Thr Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
                325                 330                 335
Ala Ala Lys Met Cys Ser Gln Val Leu Cys His Asp Glu Gly Val Cys
            340                 345                 350
Thr Arg Lys Gln Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Met
        355                 360                 365
Asn Phe Ala Ile Gln Thr Gly Lys Gly Gly Lys Tyr Thr Val Pro Gly
    370                 375                 380
Lys Val Thr Leu Glu Asp Leu Gln Thr Phe Ser Asp Lys Phe Tyr Cys
385                 390                 395                 400
Ser Cys Tyr Ala Asn Ile Asn Cys Lys Lys Arg Val Asp Ile Lys Asn
                405                 410                 415
Val His Ser Val Asn Val Cys Met Ala Glu Asp Ile Cys Ile Glu Gly
            420                 425                 430
Pro Val Lys Leu Gln Pro Ser Asp His Ser Ser Ser Gln Asn Glu Ala
        435                 440                 445
Ser Thr Thr Thr Val Ser Ser Ile Ser Pro Ser Thr Thr Ala Thr Thr
    450                 455                 460
Val Ser Pro Cys Thr Pro Glu Lys Gln Ser Pro Glu Cys Leu Lys Val
465                 470                 475                 480
Arg Cys Leu Glu Ala Ile Ala Asn Val Thr Gln Thr Gly Cys Gln Gly
                485                 490                 495
Val Lys Trp Lys Asn Thr Ser Ser Gln Ser Ser Ile Gln Asn Ile Lys
            500                 505                 510
Asn Gln Thr Thr Tyr
        515
```

<210> 121
<211> 1620
<212> DNA
<213> Ovis aries

<220>
<223> DNA encoding sheep PH20 in SEQ ID NO:118

<400> 121

```
ttttccgtgt tggttggctc tggacttcag agcacccccc ctcatttcaa acacttcttt 60
cctctgggcc tggaatgccc cagctgaacg ttgtattaaa atctttaaac tacctccaga 120
tctgagactc ttctctgtaa aaggaagccc ccaaaaaagt gctacgggac aatttattac 180
attattttat gctgatagac ttggctacta tcctcatata gatgaaaaaa caggcaacac 240
tgtatatgga ggaattcccc agttgggaaa cttaaaaaat catttggaaa aagccaaaaa 300
agacattgcc tattacatac caaatgacag cgtgggcttg gcggtcattg actgggaaaa 360
ctggaggcct acctgggcaa gaaactggaa acctaaagat gtttacaggg atgagtctgt 420
tgagttggtt ctgcaaaaaa atccacaact cagtttccca gaggcttcca agattgcaaa 480
agtggatttt gagacagcag gaaagagttt catgcaagag actttaaaac tgggaaaatt 540
acttcggcca aatcacttat ggggttatta tctttttcct gattgttaca atcataatta 600
taaccagcct acttacaatg gaaattgctc tgatttagaa aaaaggagaa atgatgatct 660
cgactggttg tggaaggaaa gcactgccct tttcccttct gtttatttga atatcaagtt 720
aaaatctact ccaaaagctg ccttctatgt tcgtaatcgt gtccaggaag ccattcggtt 780
gtctaaaata gcgagtgttg aaagtccact tcccgttttt gtatatcacc gtccagtttt 840
tactgatggg tcttcaacat acctttctca gggtgacctt gtgaattcgg ttggtgagat 900
cgttgctcta ggtgcctctg ggattataat gtggggcagt ctcaatctaa gcctaactat 960
gcaatcttgc atgaacctag gcaattactt gaacactaca ctgaatcctt acataatcaa 1020
cgtcacccta gcagccaaaa tgtgcagcca agtgctttgc cacgatgaag gagtgtgtac 1080

aaggaaacaa tggaattcaa gcgactatct tcacctgaac ccaatgaatt ttgctattca 1140
aactgggaaa ggtggaaaat acacagtacc tgggaaagtc acacttgaag acctgcaaac 1200
gttttctgat aaattttatt gcagttgtta tgccaacatc aactgtaaga agagagttga 1260
tataaaaaat gttcatagtg ttaatgtatg tatggcagaa gacatttgta tagagggccc 1320
tgtgaagtta caacccagtg atcattcctc tagccagaat gaggcatcta ctaccaccgt 1380
cagcagtatc tcaccctcta ctacagccac cacagtatct ccatgtactc ctgagaaaca 1440
gtcccctgag tgcctcaaag tcaggtgttt ggaagccatc gccaacgtca cccaaacggg 1500
gtgtcaaggt gttaaatgga agaacacttc cagtcagtca agtattcaaa atattaaaaa 1560
tcaaacaacc tattaaaata taaattcagt gcttataaaa aaaaaaaaaa aaaaaaaaaa 1620
```

<210> 122
<211> 447
<212> PRT
<213> Homo sapiens

<220>
<223> soluble rHuPH20 36-482

<400> 122

```
Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro Phe Leu Trp
 1               5                  10                  15
Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe Asp Glu Pro
            20                  25                  30
Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala
        35                  40                  45
Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
    50                  55                  60
Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
65                  70                  75                  80
Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile
                85                  90                  95
Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
            100                 105                 110
Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
        115                 120                 125
Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn Val Gln Leu
    130                 135                 140
Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
145                 150                 155                 160
Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
                165                 170                 175
Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
            180                 185                 190
His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
        195                 200                 205
Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
    210                 215                 220
Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
225                 230                 235                 240
Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
                245                 250                 255
Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
            260                 265                 270
Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
        275                 280                 285
Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
    290                 295                 300
Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
305                 310                 315                 320
Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
                325                 330                 335
Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys

            340                 345                 350
Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
        355                 360                 365
Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
    370                 375                 380
Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
385                 390                 395                 400
Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
                405                 410                 415
Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys Ile Asp Ala
            420                 425                 430
Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile Phe Tyr
        435                 440                 445
```

<210> 123
<211> 446

<212> PRT
<213> Homo sapiens

<220>
<223> soluble rHuPH20 36-481

<400> 123

```
Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro Phe Leu Trp
 1               5                  10                  15
Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe Asp Glu Pro
            20                  25                  30
Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala
        35                  40                  45
Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
    50                  55                  60
Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
65                  70                  75                  80
Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile
                85                  90                  95
Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
            100                 105                 110
Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
        115                 120                 125
Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn Val Gln Leu
    130                 135                 140
Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
145                 150                 155                 160
Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
                165                 170                 175
Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
            180                 185                 190
His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
        195                 200                 205
Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
    210                 215                 220
Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
225                 230                 235                 240
Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
                245                 250                 255
Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
            260                 265                 270
Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
        275                 280                 285
Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
    290                 295                 300
Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
```

```
305                 310                 315                 320
Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
                325                 330                 335
Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
            340                 345                 350
Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
        355                 360                 365
Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
    370                 375                 380
Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
385                 390                 395                 400
Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
                405                 410                 415
Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys Ile Asp Ala
            420                 425                 430
Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile Phe
        435                 440                 445
```

<210> 124
<211> 445
<212> PRT
<213> Homo sapiens

<220>
<223> soluble rHuPH20 36-480

<400> 124

```
Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro Phe Leu Trp
 1               5                  10                  15
Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe Asp Glu Pro
            20                  25                  30
Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala
        35                  40                  45
Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
    50                  55                  60
Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
65                  70                  75                  80
Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile
                85                  90                  95
Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
            100                 105                 110
Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
        115                 120                 125
Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn Val Gln Leu
    130                 135                 140
Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
145                 150                 155                 160
Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
                165                 170                 175
Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
            180                 185                 190
His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
        195                 200                 205
Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
    210                 215                 220
Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
225                 230                 235                 240
Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
                245                 250                 255
Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
            260                 265                 270
Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
        275                 280                 285
Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
    290                 295                 300
Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
305                 310                 315                 320
Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
                325                 330                 335
Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
            340                 345                 350
Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
        355                 360                 365
Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
    370                 375                 380
Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
385                 390                 395                 400
Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
                405                 410                 415
Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys Ile Asp Ala
            420                 425                 430
Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile
        435                 440                 445
```

<210> 125
<211> 444
<212> PRT

<213> Homo sapiens

<220>
<223> soluble rHuPh20 36-479

<400> 125

```
Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro Phe Leu Trp
 1               5                  10                  15
Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe Asp Glu Pro
            20                  25                  30
Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala
        35                  40                  45
Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
    50                  55                  60
Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
65                  70                  75                  80
Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile
                85                  90                  95
Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
            100                 105                 110
Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
        115                 120                 125
Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn Val Gln Leu
    130                 135                 140
Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
145                 150                 155                 160
Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
                165                 170                 175
Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
            180                 185                 190
His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
        195                 200                 205
Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
    210                 215                 220
Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
225                 230                 235                 240
Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
```

```
                    245                 250                 255
Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
                260                 265                 270
Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
            275                 280                 285
Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
        290                 295                 300
Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
305                 310                 315                 320
Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
                325                 330                 335
Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
                340                 345                 350
Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
            355                 360                 365
Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
        370                 375                 380
Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
385                 390                 395                 400
Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
                405                 410                 415
Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys Ile Asp Ala
                420                 425                 430
Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln
            435                 440
```

<210> 126
<211> 443
<212> PRT
<213> Homo sapiens

<220>
<223> soluble rHuPH20 36-478

<400> 126

```
Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro Phe Leu Trp
 1               5                  10                  15
Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe Asp Glu Pro
            20                  25                  30
Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala
        35                  40                  45
Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
    50                  55                  60
Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
65                  70                  75                  80
Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile
                85                  90                  95
Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
            100                 105                 110
Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
        115                 120                 125
Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn Val Gln Leu
    130                 135                 140
Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
145                 150                 155                 160
Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
                165                 170                 175
Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
            180                 185                 190
His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
        195                 200                 205
Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu

    210                 215                 220
Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
225                 230                 235                 240
Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
                245                 250                 255
Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
            260                 265                 270
Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
        275                 280                 285
Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
    290                 295                 300
Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
305                 310                 315                 320
Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
                325                 330                 335
Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
            340                 345                 350
Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
        355                 360                 365
Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
    370                 375                 380
Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
385                 390                 395                 400
Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
                405                 410                 415
Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys Ile Asp Ala
            420                 425                 430
Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro
        435                 440
```

<210> 127
<211> 442

<212> PRT
<213> Homo sapiens

<220>
<223> soluble rHuPh20 36-477

<400> 127

```
Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro Phe Leu Trp
 1               5                  10                  15
Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe Asp Glu Pro
            20                  25                  30
Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala
        35                  40                  45
Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
    50                  55                  60
Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
65                  70                  75                  80
Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile
                85                  90                  95
Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
            100                 105                 110
Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
        115                 120                 125
Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn Val Gln Leu
    130                 135                 140
Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
145                 150                 155                 160
Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
                165                 170                 175
Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
```

```
                180                 185                 190
His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
        195                 200                 205
Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
    210                 215                 220
Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
225                 230                 235                 240
Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
                245                 250                 255
Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
            260                 265                 270
Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
        275                 280                 285
Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
    290                 295                 300
Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
305                 310                 315                 320
Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
                325                 330                 335
Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
            340                 345                 350
Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
        355                 360                 365
Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
    370                 375                 380
Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
385                 390                 395                 400
Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
                405                 410                 415
Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys Ile Asp Ala
            420                 425                 430
Phe Leu Lys Pro Pro Met Glu Thr Glu Glu
        435                 440
```

<210> 128
<211> 467
<212> PRT
<213> Homo sapiens

<220>
<223> sHuPH20 precursor 1-467

<400> 128

```
Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1               5                  10                  15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
            20                  25                  30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
        35                  40                  45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
    50                  55                  60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
65                  70                  75                  80
Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
                85                  90                  95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
            100                 105                 110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
        115                 120                 125
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
    130                 135                 140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145                 150                 155                 160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
                165                 170                 175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
            180                 185                 190
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
        195                 200                 205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
    210                 215                 220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
225                 230                 235                 240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
                245                 250                 255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
            260                 265                 270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
        275                 280                 285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
    290                 295                 300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
305                 310                 315                 320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
                325                 330                 335
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
            340                 345                 350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
        355                 360                 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
    370                 375                 380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
385                 390                 395                 400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
                405                 410                 415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
            420                 425                 430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
        435                 440                 445
Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
    450                 455                 460
Ile Asp Ala
465
```

<210> 129
<211> 477
<212> PRT
<213> Homo sapiens

<220>
<223> sHuPH20 precursor 1-477

<400> 129

```
Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1               5                  10                  15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
            20                  25                  30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
        35                  40                  45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
    50                  55                  60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
65                  70                  75                  80
Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
```

```
                    85                  90                  95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
            100                 105                 110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
        115                 120                 125
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
    130                 135                 140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145                 150                 155                 160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
                165                 170                 175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
            180                 185                 190
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
        195                 200                 205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
    210                 215                 220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
225                 230                 235                 240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
                245                 250                 255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
            260                 265                 270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
        275                 280                 285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
    290                 295                 300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
305                 310                 315                 320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
                325                 330                 335
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
            340                 345                 350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
        355                 360                 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
    370                 375                 380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
385                 390                 395                 400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
                405                 410                 415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
            420                 425                 430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
        435                 440                 445
Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
    450                 455                 460
Ile Asp Ala Phe Leu Lys Pro Pro Met Glu Thr Glu Glu
465                 470                 475
```

<210> 130
<211> 478
<212> PRT
<213> Homo sapiens

<220>
<223> sHuPH20 precursor 1-478

<400> 130

```
Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1               5                  10                  15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
```

```
                 20                  25                  30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
        35                  40                  45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
    50                  55                  60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
65                  70                  75                  80
Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
                85                  90                  95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
            100                 105                 110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
        115                 120                 125
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
    130                 135                 140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145                 150                 155                 160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
                165                 170                 175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
            180                 185                 190
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
        195                 200                 205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
    210                 215                 220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
225                 230                 235                 240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
                245                 250                 255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
            260                 265                 270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
        275                 280                 285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
    290                 295                 300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
305                 310                 315                 320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
                325                 330                 335
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
            340                 345                 350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
        355                 360                 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
    370                 375                 380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
385                 390                 395                 400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
                405                 410                 415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
            420                 425                 430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
        435                 440                 445
Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
    450                 455                 460
Ile Asp Ala Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro
465                 470                 475
```

<210> 131
<211> 479
<212> PRT

<213> Homo sapiens

<220>
<223> sHuPH20 precursor 1-479

<400> 131

```
Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1               5                  10                  15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
            20                  25                  30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
        35                  40                  45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
    50                  55                  60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
65                  70                  75                  80
Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
                85                  90                  95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
            100                 105                 110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
        115                 120                 125
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
    130                 135                 140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145                 150                 155                 160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
                165                 170                 175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
            180                 185                 190
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
        195                 200                 205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
    210                 215                 220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
225                 230                 235                 240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
                245                 250                 255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
            260                 265                 270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
        275                 280                 285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
    290                 295                 300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
305                 310                 315                 320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
                325                 330                 335
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
            340                 345                 350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
        355                 360                 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
    370                 375                 380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
385                 390                 395                 400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
                405                 410                 415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
            420                 425                 430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
        435                 440                 445
Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
    450                 455                 460

Ile Asp Ala Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln
465                 470                 475
```

<210> 132
<211> 480
<212> PRT
<213> Homo sapiens

<220>
<223> sHuPH20 precursor 1-480

<400> 132

```
Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1               5                  10                  15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
            20                  25                  30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
        35                  40                  45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
    50                  55                  60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
65                  70                  75                  80
Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
                85                  90                  95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
            100                 105                 110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
        115                 120                 125
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
    130                 135                 140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145                 150                 155                 160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
                165                 170                 175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
            180                 185                 190
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
        195                 200                 205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
    210                 215                 220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
225                 230                 235                 240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
                245                 250                 255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
            260                 265                 270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
        275                 280                 285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
    290                 295                 300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
305                 310                 315                 320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
                325                 330                 335
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
            340                 345                 350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
        355                 360                 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
    370                 375                 380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
385                 390                 395                 400
```

```
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
                405                 410                 415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
            420                 425                 430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
        435                 440                 445
Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
    450                 455                 460
Ile Asp Ala Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile
465                 470                 475                 480
```

<210> 133
<211> 481
<212> PRT
<213> Homo sapiens

<220>
<223> sHuPH20 precursor 1-481

<400> 133

```
Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1               5                  10                  15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
            20                  25                  30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
        35                  40                  45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
    50                  55                  60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
65                  70                  75                  80
Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
                85                  90                  95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
            100                 105                 110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
        115                 120                 125
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
    130                 135                 140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145                 150                 155                 160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
                165                 170                 175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
            180                 185                 190
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
        195                 200                 205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
    210                 215                 220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
225                 230                 235                 240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
                245                 250                 255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
            260                 265                 270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
        275                 280                 285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
    290                 295                 300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
305                 310                 315                 320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
                325                 330                 335
```

```
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
            340                 345                 350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
        355                 360                 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
    370                 375                 380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
385                 390                 395                 400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
                405                 410                 415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
            420                 425                 430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
        435                 440                 445
Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
    450                 455                 460
Ile Asp Ala Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile
465                 470                 475                 480
Phe
```

<210> 134
<211> 483
<212> PRT
<213> Homo sapiens

<220>
<223> sHuPH20 precursor 1-483

<400> 134

```
Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1               5                  10                  15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
            20                  25                  30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
        35                  40                  45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
    50                  55                  60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
65                  70                  75                  80
Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
                85                  90                  95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
            100                 105                 110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
        115                 120                 125
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
    130                 135                 140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145                 150                 155                 160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
                165                 170                 175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
            180                 185                 190
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
        195                 200                 205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
    210                 215                 220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
225                 230                 235                 240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
                245                 250                 255
```

```
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
            260                 265                 270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
        275                 280                 285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
    290                 295                 300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
305                 310                 315                 320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
                325                 330                 335
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
            340                 345                 350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
        355                 360                 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
    370                 375                 380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
385                 390                 395                 400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
                405                 410                 415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
            420                 425                 430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
        435                 440                 445
Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
    450                 455                 460
Ile Asp Ala Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile
465                 470                 475                 480
Phe Tyr Asn
```

<210> 135
<211> 432
<212> PRT
<213> Homo sapiens

<220>
<223> sHuPH20 mature 36-467

<400> 135

```
Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro Phe Leu Trp
 1               5                  10                  15
Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe Asp Glu Pro
            20                  25                  30
Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala
        35                  40                  45
Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
    50                  55                  60
Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
65                  70                  75                  80
Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile
                85                  90                  95
Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
            100                 105                 110
Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
        115                 120                 125
Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn Val Gln Leu
    130                 135                 140
Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
145                 150                 155                 160
Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
                165                 170                 175

Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
            180                 185                 190
His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
        195                 200                 205
Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
    210                 215                 220
Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
225                 230                 235                 240
Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
                245                 250                 255
Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
            260                 265                 270
Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
        275                 280                 285
Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
    290                 295                 300
Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
305                 310                 315                 320
Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
                325                 330                 335
Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
            340                 345                 350
Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
        355                 360                 365
Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
    370                 375                 380
Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
385                 390                 395                 400
Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
                405                 410                 415
Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys Ile Asp Ala
            420                 425                 430
```

<210> 136
<211> 448
<212> PRT
<213> Homo sapiens

<220>
<223> sHuPH20 mature 36-483

<400> 136

```
Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro Phe Leu Trp
 1               5                  10                  15
Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe Asp Glu Pro
            20                  25                  30
Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg Ile Asn Ala
        35                  40                  45
Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu Gly Tyr Tyr
    50                  55                  60
Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly Gly Ile Pro
65                  70                  75                  80
Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys Lys Asp Ile
                85                  90                  95
Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val Ile Asp Trp
            100                 105                 110
Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro Lys Asp Val
        115                 120                 125
Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn Val Gln Leu
    130                 135                 140
Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe Glu Lys Ala
145                 150                 155                 160
```

```
Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys Leu Leu Arg
                165                 170                 175
Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys Tyr Asn His
            180                 185                 190
His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn Val Glu Ile
        195                 200                 205
Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser Thr Ala Leu
    210                 215                 220
Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val Ala Ala Thr
225                 230                 235                 240
Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val Ser Lys Ile
                245                 250                 255
Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr Arg Ile Val
            260                 265                 270
Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu Leu Val Tyr
        275                 280                 285
Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile Val Ile Trp
    290                 295                 300
Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu Leu Leu Asp
305                 310                 315                 320
Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn Val Thr Leu
                325                 330                 335
Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln Gly Val Cys
            340                 345                 350
Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu Asn Pro Asp
        355                 360                 365
Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr Val Arg Gly
    370                 375                 380
Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys Phe Tyr Cys
385                 390                 395                 400
Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp Val Lys Asp
                405                 410                 415
Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys Ile Asp Ala
            420                 425                 430
Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile Phe Tyr Asn
        435                 440                 445
```

<210> 137
<211> 509
<212> PRT
<213> Homo sapiens

<220>
<223> precursor PH20 variant P48A

<400> 137

```
Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1               5                  10                  15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
            20                  25                  30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Ala
        35                  40                  45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
    50                  55                  60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
65                  70                  75                  80
Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
                85                  90                  95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
            100                 105                 110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
        115                 120                 125
```

```
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
    130                 135                 140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145                 150                 155                 160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
                165                 170                 175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
            180                 185                 190
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
        195                 200                 205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
    210                 215                 220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
225                 230                 235                 240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
                245                 250                 255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
            260                 265                 270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
        275                 280                 285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
    290                 295                 300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
305                 310                 315                 320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
                325                 330                 335
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
            340                 345                 350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
        355                 360                 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
    370                 375                 380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
385                 390                 395                 400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
                405                 410                 415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
            420                 425                 430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
        435                 440                 445
Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
    450                 455                 460
Ile Asp Ala Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile
465                 470                 475                 480
Phe Tyr Asn Ala Ser Pro Ser Thr Leu Ser Ala Thr Met Phe Ile Val
                485                 490                 495
Ser Ile Leu Phe Leu Ile Ile Ser Ser Val Ala Ser Leu
            500                 505
```

<210> 138
<211> 509
<212> PRT
<213> Homo sapiens

<220>
<223> precursor PH20 variant L499W

<400> 138

```
Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1               5                  10                  15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
            20                  25                  30
```

```
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
        35                  40                  45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
    50                  55                  60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
65                  70                  75                  80
Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
                85                  90                  95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
            100                 105                 110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
        115                 120                 125
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
    130                 135                 140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145                 150                 155                 160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
                165                 170                 175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
            180                 185                 190
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
        195                 200                 205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
    210                 215                 220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
225                 230                 235                 240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
                245                 250                 255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
            260                 265                 270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
        275                 280                 285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
    290                 295                 300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
305                 310                 315                 320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
                325                 330                 335
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
            340                 345                 350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
        355                 360                 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
    370                 375                 380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
385                 390                 395                 400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
                405                 410                 415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
            420                 425                 430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
        435                 440                 445
Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
    450                 455                 460
Ile Asp Ala Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile
465                 470                 475                 480
Phe Tyr Asn Ala Ser Pro Ser Thr Leu Ser Ala Thr Met Phe Ile Val
                485                 490                 495
Ser Ile Trp Phe Leu Ile Ile Ser Ser Val Ala Ser Leu
            500                 505
```

<210> 139
<211> 1449
<212> DNA
<213> Homo sapiens

<220>
<223> Gen2 mRNA sequence

<400> 139

atgggagtgc taaaattcaa gcacatcttt ttcagaagct ttgttaaatc aagtggagta 60
tcccagatag ttttcacctt ccttctgatt ccatgttgct tgactctgaa tttcagagca 120
cctcctgtta ttccaaatgt gcctttcctc tgggcctgga atgccccaag tgaattttgt 180
cttggaaaat ttgatgagcc actagatatg agcctcttct ctttcatagg aagcccccga 240
ataaacgcca ccgggcaagg tgttacaata ttttatgttg atagacttgg ctactatcct 300
tacatagatt caatcacagg agtaactgtg aatggaggaa tcccccagaa gatttcctta 360
caagaccatc tggacaaagc taagaaagac attacatttt atatgccagt agacaatttg 420
ggaatggctg ttattgactg ggaagaatgg agacccactt gggcaagaaa ctggaaacct 480
aaagatgttt acaagaatag gtctattgaa ttggttcagc aacaaaatgt acaacttagt 540
ctcacagagg ccactgagaa agcaaaacaa gaatttgaaa aggcagggaa ggatttcctg 600
gtagagacta taaaattggg aaaattactt cggccaaatc acttgtgggg ttattatctt 660
tttccggatt gttacaacca tcactataag aaacccggtt acaatggaag ttgcttcaat 720
gtagaaataa aaagaaatga tgatctcagc tggttgtgga atgaaagcac tgctctttac 780
ccatccattt atttgaacac tcagcagtct cctgtagctg ctacactcta tgtgcgcaat 840
cgagttcggg aagccatcag agtttccaaa atacctgatg caaaaagtcc acttccggtt 900
tttgcatata cccgcatagt ttttactgat caagttttga aattcctttc tcaagatgaa 960
cttgtgtata catttggcga aactgttgct ctgggtgctt ctggaattgt aatatgggga 1020
accctcagta taatgcgaag tatgaaatct tgcttgctcc tagacaatta catggagact 1080
atactgaatc cttacataat caacgtcaca ctagcagcca aaatgtgtag tcaagtgctt 1140
tgccaggagc aaggagtgtg tataaggaaa aactggaatt caagtgacta tcttcacctc 1200
aacccagata attttgctat tcaacttgag aaaggtggaa agttcacagt acgtggaaaa 1260
ccgacacttg aagacctgga gcaattttct gaaaaatttt attgcagctg ttatagcacc 1320
ttgagttgta aggagaaagc tgatgtaaaa gacactgatg ctgttgatgt gtgtattgct 1380
gatggtgtct gtatagatgc ttttctaaaa cctcccatgg agacagaaga acctcaaatt 1440
ttctactga 1449

<210> 140
<211> 6630
<212> DNA
<213> Artificial Sequence

<220>
<223> HZ24 vector

<400> 140

tcaatattgg ccattagcca tattattcat tggttatata gcataaatca atattggcta 60
ttggccattg catacgttgt atctatatca taatatgtac atttatattg gctcatgtcc 120
aatatgaccg ccatgttggc attgattatt gactagttat taatagtaat caattacggg 180
gtcattagtt catagcccat atatggagtt ccgcgttaca taacttacgg taaatggccc 240
gcctggctga ccgcccaacg acccccgccc attgacgtca ataatgacgt atgttcccat 300
agtaacgcca atagggactt tccattgacg tcaatgggtg gagtatttac ggtaaactgc 360
ccacttggca gtacatcaag tgtatcatat gccaagtccg ccccctattg acgtcaatga 420
cggtaaatgg cccgcctggc attatgccca gtacatgacc ttacgggact ttcctacttg 480
gcagtacatc tacgtattag tcatcgctat taccatggtg atgcggtttt ggcagtacac 540
caatgggcgt ggatagcggt ttgactcacg gggatttcca agtctccacc ccattgacgt 600
caatgggagt ttgttttggc accaaaatca acgggacttt ccaaaatgtc gtaataaccc 660
cgccccgttg acgcaaatgg gcggtaggcg tgtacggtgg gaggtctata taagcagagc 720
tcgtttagtg aaccgtcaga tcactagaag ctttattgcg gtagtttatc acagttaaat 780
tgctaacgca gtcagtgctt ctgacacaac agtctcgaac ttaagctgca gaagttggtc 840
gtgaggcact gggcaggtaa gtatcaaggt tacaagacag gtttaaggag accaatagaa 900
actgggcttg tcgagacaga gaagactctt gcgtttctga taggcaccta ttggtcttac 960
tgacatccac tttgcctttc tctccacagg tgtccactcc cagttcaatt acagctctta 1020
aggctagagt acttaatacg actcactata ggctagcatg ggagtgctaa aattcaagca 1080
catcttttc agaagctttg ttaaatcaag tggagtatcc cagatagttt tcaccttcct 1140
tctgattcca tgttgcttga ctctgaattt cagagcacct cctgttattc caaatgtgcc 1200

```
tttcctctgg gcctggaatg ccccaagtga attttgtctt ggaaaatttg atgagccact 1260
agatatgagc ctcttctctt tcataggaag cccccgaata aacgccaccg ggcaaggtgt 1320
tacaatattt tatgttgata gacttggcta ctatccttac atagattcaa tcacaggagt 1380
aactgtgaat ggaggaatcc cccagaagat ttccttacaa gaccatctgg acaaagctaa 1440
gaaagacatt acattttata tgccagtaga caatttggga atggctgtta ttgactggga 1500
agaatggaga cccacttggg caagaaactg gaaacctaaa gatgtttaca agaataggtc 1560
tattgaattg gttcagcaac aaaatgtaca acttagtctc acagaggcca ctgagaaagc 1620
aaaacaagaa tttgaaaagg cagggaagga tttcctggta gagactataa aattgggaaa 1680
attacttcgg ccaaatcact tgtggggtta ttatcttttt ccggattgtt acaaccatca 1740
ctataagaaa cccggttaca atggaagttg cttcaatgta gaaataaaaa gaaatgatga 1800
tctcagctgg ttgtggaatg aaagcactgc tctttaccca tccatttatt tgaacactca 1860
gcagtctcct gtagctgcta cactctatgt gcgcaatcga gttcgggaag ccatcagagt 1920
ttccaaaata cctgatgcaa aaagtccact tccggttttt gcatataccc gcatagtttt 1980
tactgatcaa gttttgaaat tcctttctca agatgaactt gtgtatacat ttggcgaaac 2040
tgttgctctg ggtgcttctg gaattgtaat atggggaacc ctcagtataa tgcgaagtat 2100
gaaatcttgc ttgctcctag acaattacat ggagactata ctgaatcctt acataatcaa 2160
cgtcacacta gcagccaaaa tgtgtagcca agtgctttgc caggagcaag gagtgtgtat 2220
aaggaaaaac tggaattcaa gtgactatct tcacctcaac ccagataatt ttgctattca 2280
acttgagaaa ggtggaaagt tcacagtacg tggaaaaccg acacttgaag acctggagca 2340
attttctgaa aaattttatt gcagctgtta tagcaccttg agttgtaagg agaaagctga 2400
tgtaaaagac actgatgctg ttgatgtgtg tattgctgat ggtgtctgta tagatgcttt 2460
tctaaaacct cccatggaga cagaagaacc tcaaattttc tactgaggat ccatagctaa 2520
cgcccctctc cctccccccc ccctaacgtt actggccgaa gccgcttgga ataaggccgg 2580
tgtgcgtttg tctatatgtt attttccacc atattgccgt cttttggcaa tgtgagggcc 2640
cggaaacctg gccctgtctt cttgacgagc attcctaggg gtctttcccc tctcgccaaa 2700
ggaatgcaag gtctgttgaa tgtcgtgaag gaagcagttc ctctggaagc ttcttgaaga 2760
caaacaacgt ctgtagcgac cctttgcagg cagcggaacc ccccacctgg cgacaggtgc 2820
ctctgcggcc aaaagccacg tgtataagat acacctgcaa aggcggcaca accccagtgc 2880
cacgttgtga gttggatagt tgtggaaaga gtcaaatggc tctcctcaag cgtattcaac 2940
aaggggctga aggatgccca gaaggtaccc cattgtatgg gatctgatct ggggcctcgg 3000
tgcacatgct ttacatgtgt ttagtcgagg ttaaaaaaac gtctaggccc cccgaaccac 3060
ggggacgtgg ttttcctttg aaaaacacga tgataagctt gccacaaccc acagcggccg 3120
ctgccatcat ggttcgacca ttgaactgca tcgtcgccgt gtcccaaaat atggggattg 3180
gcaagaacgg agacctaccc tggcctccgc tcaggaacga gttcaagtac ttccaaagaa 3240
tgaccacaac ctcttcagtg gaaggtaaac agaatctggt gattatgggt aggaaaacct 3300
ggttctccat tcctgagaag aatcgacctt taaaggacag aattaatata gttctcagta 3360
gagaactcaa agaaccacca cgaggagctc attttcttgc caaaagtttg gatgatgcct 3420
taagacttat tgaacaaccg gaattggcaa gtaaagtaga catggtttgg atagtcggag 3480
gcagttctgt ttaccaggaa gccatgaatc aaccaggcca cctcagactc tttgtgacaa 3540
ggatcatgca ggaatttgaa agtgacacgt ttttcccaga aattgatttg gggaaatata 3600
aacttctccc agaataccca ggcgtcctct ctgaggtcca ggaggaaaaa ggcatcaagt 3660
ataagtttga agtctacgag aagaaagact aaacgcgtgg tacctctaga gtcgacccgg 3720
gcggccgctt cgagcagaca tgataagata cattgatgag tttggacaaa ccacaactag 3780
aatgcagtga aaaaaatgct ttatttgtga aatttgtgat gctattgctt tatttgtaac 3840
cattataagc tgcaataaac aagttaacaa caacaattgc attcatttta tgtttcaggt 3900
tcaggggggag atgtgggagg ttttttaaag caagtaaaac ctctacaaat gtggtaaaat 3960
cgataaggat ccgggctggc gtaatagcga agaggcccgc accgatcgcc cttcccaaca 4020
gttgcgcagc ctgaatggcg aatggacgcg ccctgtagcg gcgcattaag cgcggcgggt 4080
gtggtggtta cgcgcagcgt gaccgctaca cttgccagcg ccctagcgcc cgctcctttc 4140
gctttcttcc cttcctttct cgccacgttc gccggctttc cccgtcaagc tctaaatcgg 4200
gggctccctt tagggttccg atttagtgct ttacggcacc tcgaccccaa aaaacttgat 4260
tagggtgatg gttcacgtag tgggccatcg ccctgataga cggtttttcg ccctttgacg 4320
ttggagtcca cgttctttaa tagtggactc ttgttccaaa ctggaacaac actcaaccct 4380
atctcggtct attcttttga tttataaggg attttgccga tttcggccta ttggttaaaa 4440
aatgagctga tttaacaaaa atttaacgcg aattttaaca aaatattaac gcttacaatt 4500
tcctgatgcg gtatttttctc cttacgcatc tgtgcggtat ttcacaccgc atatggtgca 4560
ctctcagtac aatctgctct gatgccgcat agttaagcca gccccgacac ccgccaacac 4620
ccgctgacgc gccctgacgg gcttgtctgc tcccggcatc cgcttacaga caagctgtga 4680
ccgtctccgg gagctgcatg tgtcagaggt tttcaccgtc atcaccgaaa cgcgcgagac 4740
gaaagggcct cgtgatacgc ctatttttat aggttaatgt catgataata atggtttctt 4800
agacgtcagg tggcactttt cggggaaatg tgcgcggaac ccctatttgt ttatttttct 4860
aaatacattc aaatatgtat ccgctcatga gacaataacc ctgataaatg cttcaataat 4920
attgaaaaag gaagagtatg agtattcaac atttccgtgt cgcccttatt ccctttttg 4980
```

```
cggcattttg ccttcctgtt tttgctcacc cagaaacgct ggtgaaagta aaagatgctg 5040
aagatcagtt gggtgcacga gtgggttaca tcgaactgga tctcaacagc ggtaagatcc 5100
ttgagagttt tcgccccgaa gaacgttttc caatgatgag cacttttaaa gttctgctat 5160
gtggcgcggt attatcccgt attgacgccg ggcaagagca actcggtcgc cgcatacact 5220
attctcagaa tgacttggtt gagtactcac cagtcacaga aaagcatctt acggatggca 5280
tgacagtaag agaattatgc agtgctgcca taaccatgag tgataacact gcggccaact 5340
tacttctgac aacgatcgga ggaccgaagg agctaaccgc ttttttgcac aacatggggg 5400
atcatgtaac tcgccttgat cgttgggaac cggagctgaa tgaagccata ccaaacgacg 5460
agcgtgacac cacgatgcct gtagcaatgg caacaacgtt gcgcaaacta ttaactggcg 5520
aactacttac tctagcttcc cggcaacaat taatagactg gatggaggcg gataaagttg 5580
caggaccact tctgcgctcg gcccttccgg ctggctggtt tattgctgat aaatctggag 5640
ccggtgagcg tgggtctcgc ggtatcattg cagcactggg gccagatggt aagccctccc 5700
gtatcgtagt tatctacacg acggggagtc aggcaactat ggatgaacga aatagacaga 5760
tcgctgagat aggtgcctca ctgattaagc attggtaact gtcagaccaa gtttactcat 5820
atatacttta gattgattta aaacttcatt tttaatttaa aaggatctag gtgaagatcc 5880
tttttgataa tctcatgacc aaaatccctt aacgtgagtt ttcgttccac tgagcgtcag 5940
accccgtaga aaagatcaaa ggatcttctt gagatccttt ttttctgcgc gtaatctgct 6000
gcttgcaaac aaaaaaacca ccgctaccag cggtggtttg tttgccggat caagagctac 6060
caactctttt tccgaaggta actggcttca gcagagcgca gataccaaat actgttcttc 6120
tagtgtagcc gtagttaggc caccacttca agaactctgt agcaccgcct acatacctcg 6180
ctctgctaat cctgttacca gtggctgctg ccagtggcga taagtcgtgt cttaccgggt 6240
tggactcaag acgatagtta ccggataagg cgcagcggtc gggctgaacg gggggttcgt 6300
gcacacagcc cagcttggag cgaacgacct acaccgaact gagataccta cagcgtgagc 6360
tatgagaaag cgccacgctt cccgaaggga gaaaggcgga caggtatccg gtaagcggca 6420
gggtcggaac aggagagcgc acgagggagc ttccaggggg aaacgcctgg tatctttata 6480
gtcctgtcgg gtttcgccac ctctgacttg agcgtcgatt tttgtgatgc tcgtcagggg 6540
ggcggagcct atggaaaaac gccagcaacg cggccttttt acggttcctg gcctttgct 6600
ggcctttgc tcacatggct cgacagatct                                  6630
```

<210> 141
<211> 186
<212> PRT
<213> Mus musculus

<220>
<223> dihydrofolate reductase

<400> 141

```
Val Arg Pro Leu Asn Cys Ile Val Ala Val Ser Gln Asn Met Gly Ile
 1               5                  10                  15
Gly Lys Asn Gly Asp Leu Pro Trp Pro Pro Leu Arg Asn Glu Phe Lys
            20                  25                  30
Tyr Phe Gln Arg Met Thr Thr Thr Ser Ser Val Glu Gly Lys Gln Asn
        35                  40                  45
Leu Val Ile Met Gly Arg Lys Thr Trp Phe Ser Ile Pro Glu Lys Asn
    50                  55                  60
Arg Pro Leu Lys Asp Arg Ile Asn Ile Val Leu Ser Arg Glu Leu Lys
65                  70                  75                  80
Glu Pro Pro Arg Gly Ala His Phe Leu Ala Lys Ser Leu Asp Asp Ala
                85                  90                  95
Leu Arg Leu Ile Glu Gln Pro Glu Leu Ala Ser Lys Val Asp Met Val
            100                 105                 110
Trp Ile Val Gly Gly Ser Ser Val Tyr Gln Glu Ala Met Asn Gln Pro
        115                 120                 125
Gly His Leu Arg Leu Phe Val Thr Arg Ile Met Gln Glu Phe Glu Ser
    130                 135                 140
Asp Thr Phe Phe Pro Glu Ile Asp Leu Gly Lys Tyr Lys Leu Leu Pro
145                 150                 155                 160
Glu Tyr Pro Gly Val Leu Ser Glu Val Gln Glu Glu Lys Gly Ile Lys
                165                 170                 175
Tyr Lys Phe Glu Val Tyr Glu Lys Lys Asp
            180                 185
```

<210> 142
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> Hexa His Tag

<400> 142

```
His His His His His His
 1               5
```

<210> 143
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Flag Tag

<400> 143

```
Asp Tyr Lys Asp Asp Asp Asp Lys
 1               5
```

<210> 144
<211> 22
<212> PRT
<213> Homo sapiens

<220>
<223> Human IgK signal peptide

<400> 144

Met Asp Met Arg Val Pro Ala Gln Leu Leu Gly Leu Leu Leu Leu Trp
1 5 10 15
Leu Pro Gly Ala Arg Gly
20

<210> 145
<211> 21
<212> PRT
<213> Mus musculus

<220>
<223> Mouse IgK signal peptide

<400> 145

Met Glu Thr Asp Thr Leu Leu Leu Trp Val Leu Leu Leu Trp Val Pro
1 5 10 15
Gly Ser Thr Gly Asp
20

<210> 146
<211> 1500
<212> DNA
<213> Artificial Sequence

<220>
<223> SPAM1-SSVA codon optimized DNA with NheI and BamHI restriction sites

<400> 146

gctagcatgg acatgagggt accagcccag ctgttgggcc ttcttctgct ttggctccct 60
ggggcaaggg gacttaactt cagagctccc cctgtaatcc caaacgtccc cttcctgtgg 120
gcatggaacg cccctagcga attctgtttg gggaaatttg acgaacctct ggacatgtct 180
ctgttttctt ttatcggtag ccctcgaatc aacgctaccg gacaggggtt gacaatcttt 240
tacgtggatc gtctgggcta ttacccttac atcgacagca ttactggggt gaccgtgaac 300
ggaggcattc ctcagaaaat atcactgcag gaccacctgg acaaagctaa gaaggatatt 360
acattctaca tgccagtgga caatctcgga atggctgtga ttgactggga ggagtggaga 420
cctacttggg cccgaaattg gaagcccaag gatgtgtata agaaccgatc cattgagctg 480
gtgcagcagc agaacgtgca gctgtccctt acagaagcca ccgaaaaagc taagcaggaa 540
ttcgagaaag ctgggaagga tttcctggtt gagacaataa aacttgggaa actgcttaga 600
cctaaccacc tctggggcta ttatttgttc cccgattgct acaatcacca ctataagaag 660
cccggctaca acggatcttg tttcaacgtc gaaattaaaa gaaacgatga cctctcttgg 720
ttgtggaatg agtctactgc actctacccc tctatatatc tcaacacaca gcagtcccca 780
gtagccgcta ccctctacgt tcggaataga gtgagggaag ccatcagggt gtccaagatc 840
cccgatgcca aatctccccc cccagtgttt gcctacacca ggatcgtgtt taccgaccag 900
gtgttgaagt tcctgagcca ggacgagctc gtgtatactt ttggcgagac agttgctctg 960
ggagcctctg ggatcgtgat ctggggcact ctgagtatta tgcgatcaat gaagtcctgc 1020
ctgctgttgg ataactacat ggagacaatc ctgaacccat atatcattaa cgtgactttg 1080
gccgctaaga tgtgttcaca ggtgctgtgt caagagcagg gagtatgcat taggaagaac 1140
tggaactcaa gcgattatct ccatctgaac ccagataatt ttgcaatcca gctggaaaag 1200
ggtggtaaat tcactgtccg agggaagccc acactcgagg atctcgaaca gtttagtgaa 1260
aagttctact gtagctgcta tagcactctg agttgtaagg aaaaagccga tgtgaaagac 1320
actgatgccg ttgacgtttg catcgccgat ggggtgtgta ttgacgcctt tctcaagcca 1380
ccaatggaga ctgaggagcc ccagatcttt tacaacgcct ccccctccac cttgagtgct 1440
accatgttca tcgtatctat actttcctg attatatcaa gcgtggcatg ataaggatcc 1500

<210> 147

<211> 1494
<212> DNA
<213> Artificial Sequence

<220>
<223> SPAM1-IISS codon optimized DNA with NheI and BamHI restriction sites

<400> 147

```
gctagcatgg acatgagggt accagcccag ctgttgggcc ttcttctgct ttggctccct 60
ggggcaaggg gacttaactt cagagctccc cctgtaatcc caaacgtccc cttcctgtgg 120
gcatggaacg cccctagcga attctgtttg gggaaatttg acgaacctct ggacatgtct 180
ctgttttctt ttatcggtag ccctcgaatc aacgctaccg gacagggggt gacaatcttt 240
tacgtggatc gtctgggcta ttacccttac atcgacagca ttactggggt gaccgtgaac 300
ggaggcattc ctcagaaaat atcactgcag gaccacctgg acaaagctaa gaaggatatt 360
acattctaca tgccagtgga caatctcgga atggctgtga ttgactggga ggagtggaga 420
cctacttggg cccgaaattg gaagcccaag gatgtgtata agaaccgatc cattgagctg 480
gtgcagcagc agaacgtgca gctgtccctt acagaagcca ccgaaaaagc taagcaggaa 540
ttcgagaaag ctgggaagga tttcctggtt gagacaataa aacttgggaa actgcttaga 600
cctaaccacc tctggggcta ttatttgttc cccgattgct acaatcacca ctataagaag 660
cccggctaca acggatcttg tttcaacgtc gaaattaaaa gaaacgatga cctctcttgg 720
ttgtggaatg agtctactgc actctacccc tctatatatc tcaacacaca gcagtcccca 780
gtagccgcta ccctctacgt tcggaataga gtgagggaag ccatcagggt gtccaagatc 840
cccgatgcca aatctccccc cccagtgttt gcctacacca ggatcgtgtt taccgaccag 900
gtgttgaagt tcctgagcca ggacgagctc gtgtatactt ttggcgagac agttgctctg 960
ggagcctctg ggatcgtgat ctggggcact ctgagtatta tgcgatcaat gaagtcctgc 1020
ctgctgttgg ataactacat ggagacaatc ctgaacccat atatcattaa cgtgactttg 1080
gccgctaaga tgtgttcaca ggtgctgtgt caagagcagg gagtatgcat taggaagaac 1140
tggaactcaa gcgattatct ccatctgaac ccagataatt ttgcaatcca gctggaaaag 1200
ggtggtaaat tcactgtccg agggaagccc acactcgagg atctcgaaca gtttagtgaa 1260
aagttctact gtagctgcta tagcactctg agttgtaagg aaaaagccga tgtgaaagac 1320
actgatgccg ttgacgtttg catcgccgat ggggtgtgta ttgacgcctt tctcaagcca 1380

ccaatggaga ctgaggagcc ccagatcttt tacaacgcct ccccctccac cttgagtgct 1440
accatgttca tcgtatctat actttcctg attatatcaa gctgataagg atcc       1494
```

<210> 148
<211> 1488
<212> DNA
<213> Artificial Sequence

<220>
<223> SPAM1-FLII codon optimized DNA with NheI and BamHI restriction sites

<400> 148

```
gctagcatgg acatgagggt accagcccag ctgttgggcc ttcttctgct ttggctccct 60
ggggcaaggg gacttaactt cagagctccc cctgtaatcc caaacgtccc cttcctgtgg 120
gcatggaacg cccctagcga attctgtttg gggaaatttg acgaacctct ggacatgtct 180
ctgttttctt ttatcggtag ccctcgaatc aacgctaccg gacagggggt gacaatcttt 240
tacgtggatc gtctgggcta ttacccttac atcgacagca ttactggggt gaccgtgaac 300
ggaggcattc ctcagaaaat atcactgcag gaccacctgg acaaagctaa gaaggatatt 360
acattctaca tgccagtgga caatctcgga atggctgtga ttgactggga ggagtggaga 420
cctacttggg cccgaaattg gaagcccaag gatgtgtata agaaccgatc cattgagctg 480
gtgcagcagc agaacgtgca gctgtccctt acagaagcca ccgaaaaagc taagcaggaa 540
ttcgagaaag ctgggaagga tttcctggtt gagacaataa aacttgggaa actgcttaga 600
cctaaccacc tctggggcta ttatttgttc cccgattgct acaatcacca ctataagaag 660
cccggctaca acggatcttg tttcaacgtc gaaattaaaa gaaacgatga cctctcttgg 720
ttgtggaatg agtctactgc actctacccc tctatatatc tcaacacaca gcagtcccca 780
gtagccgcta ccctctacgt tcggaataga gtgagggaag ccatcagggt gtccaagatc 840
cccgatgcca aatctcccct cccagtgttt gcctacacca ggatcgtgtt taccgaccag 900
gtgttgaagt tcctgagcca ggacgagctc gtgtatactt ttggcgagac agttgctctg 960
ggagcctctg ggatcgtgat ctggggcact ctgagtatta tgcgatcaat gaagtcctgc 1020
ctgctgttgg ataactacat ggagacaatc ctgaacccat atatcattaa cgtgactttg 1080
gccgctaaga tgtgttcaca ggtgctgtgt caagagcagg gagtatgcat taggaagaac 1140
tggaactcaa gcgattatct ccatctgaac ccagataatt ttgcaatcca gctggaaaag 1200
ggtggtaaat tcactgtccg agggaagccc acactcgagg atctcgaaca gtttagtgaa 1260
aagttctact gtagctgcta tagcactctg agttgtaagg aaaaagccga tgtgaaagac 1320
actgatgccg ttgacgtttg catcgccgat ggggtgtgta ttgacgcctt tctcaagcca 1380
ccaatggaga ctgaggagcc ccagatcttt tacaacgcct ccccctccac cttgagtgct 1440
accatgttca tcgtatctat acttttcctg attatatgat aaggatcc              1488
```

<210> 149
<211> 1482
<212> DNA
<213> Artificial Sequence

<220>
<223> SPAM1-ILFL codon optimized DNA with NheI and BamHI restriction sites

<400> 149

```
gctagcatgg acatgagggt accagcccag ctgttgggcc ttcttctgct ttggctccct 60
ggggcaaggg gacttaactt cagagctccc cctgtaatcc caaacgtccc cttcctgtgg 120
gcatggaacg cccctagcga attctgtttg gggaaatttg acgaacctct ggacatgtct 180
ctgttttctt ttatcggtag ccctcgaatc aacgctaccg gacagggggt gacaatcttt 240
tacgtggatc gtctgggcta ttacccttac atcgacagca ttactggggt gaccgtgaac 300
ggaggcattc ctcagaaaat atcactgcag gaccacctgg acaaagctaa gaaggatatt 360
acattctaca tgccagtgga caatctcgga atggctgtga ttgactggga ggagtggaga 420
cctacttggg cccgaaattg gaagcccaag gatgtgtata agaaccgatc cattgagctg 480
gtgcagcagc agaacgtgca gctgtccctt acagaagcca ccgaaaaagc taagcaggaa 540
ttcgagaaag ctgggaagga tttcctggtt gagacaataa aacttgggaa actgcttaga 600
cctaaccacc tctggggcta ttatttgttc cccgattgct acaatcacca ctataagaag 660
cccggctaca acggatcttg tttcaacgtc gaaattaaaa gaaacgatga cctctcttgg 720
ttgtggaatg agtctactgc actctacccc tctatatatc tcaacacaca gcagtcccca 780
gtagccgcta ccctctacgt tcggaataga gtgagggaag ccatcagggt gtccaagatc 840
```

cccgatgcca aatctcccct cccagtgttt gcctacacca ggatcgtgtt taccgaccag 900
gtgttgaagt tcctgagcca ggacgagctc gtgtatactt ttggcgagac agttgctctg 960
ggagcctctg ggatcgtgat ctggggcact ctgagtatta tgcgatcaat gaagtcctgc 1020
ctgctgttgg ataactacat ggagacaatc ctgaacccat atatcattaa cgtgactttg 1080
gccgctaaga tgtgttcaca ggtgctgtgt caagagcagg gagtatgcat taggaagaac 1140
tggaactcaa gcgattatct ccatctgaac ccagataatt ttgcaatcca gctggaaaag 1200
ggtggtaaat tcactgtccg agggaagccc acactcgagg atctcgaaca gtttagtgaa 1260
aagttctact gtagctgcta tagcactctg agttgtaagg aaaaagccga tgtgaaagac 1320
actgatgccg ttgacgtttg catcgccgat ggggtgtgta ttgacgcctt tctcaagcca 1380
ccaatggaga ctgaggagcc ccagatcttt tacaacgcct ccccctccac cttgagtgct 1440
accatgttca tcgtatctat acttttcctg tgataaggat cc 1482

<210> 150
<211> 1476
<212> DNA
<213> Artificial Sequence

<220>
<223> SPAM1-VSIL codon optimized DNA with NheI and BamHI restriction sites

<400> 150

gctagcatgg acatgagggt accagcccag ctgttgggcc ttcttctgct ttggctccct 60
ggggcaaggg gacttaactt cagagctccc cctgtaatcc caaacgtccc cttcctgtgg 120
gcatggaacg cccctagcga attctgtttg gggaaatttg acgaacctct ggacatgtct 180
ctgttttctt ttatcggtag ccctcgaatc aacgctaccg gacaggggtt gacaatcttt 240
tacgtggatc gtctgggcta ttacccttac atcgacagca ttactggggt gaccgtgaac 300
ggaggcattc ctcagaaaat atcactgcag gaccacctgg acaaagctaa gaaggatatt 360
acattctaca tgccagtgga caatctcgga atggctgtga ttgactggga ggagtggaga 420
cctacttggg cccgaaattg gaagcccaag gatgtgtata agaaccgatc cattgagctg 480
gtgcagcagc agaacgtgca gctgtccctt acagaagcca ccgaaaaagc taagcaggaa 540
ttcgagaaag ctgggaagga tttcctggtt gagacaataa aacttgggaa actgcttaga 600
cctaaccacc tctggggcta ttatttgttc cccgattgct acaatcacca ctataagaag 660
cccggctaca acggatcttg tttcaacgtc gaaattaaaa gaaacgatga cctctcttgg 720
ttgtggaatg agtctactgc actctacccc tctatatatc tcaacacaca gcagtcccca 780
gtagccgcta ccctctacgt tcggaataga gtgagggaag ccatcagggt gtccaagatc 840
cccgatgcca aatctcccct cccagtgttt gcctacacca ggatcgtgtt taccgaccag 900
gtgttgaagt tcctgagcca ggacgagctc gtgtatactt ttggcgagac agttgctctg 960
ggagcctctg ggatcgtgat ctggggcact ctgagtatta tgcgatcaat gaagtcctgc 1020
ctgctgttgg ataactacat ggagacaatc ctgaacccat atatcattaa cgtgactttg 1080
gccgctaaga tgtgttcaca ggtgctgtgt caagagcagg gagtatgcat taggaagaac 1140
tggaactcaa gcgattatct ccatctgaac ccagataatt ttgcaatcca gctggaaaag 1200
ggtggtaaat tcactgtccg agggaagccc acactcgagg atctcgaaca gtttagtgaa 1260
aagttctact gtagctgcta tagcactctg agttgtaagg aaaaagccga tgtgaaagac 1320
actgatgccg ttgacgtttg catcgccgat ggggtgtgta ttgacgcctt tctcaagcca 1380
ccaatggaga ctgaggagcc ccagatcttt tacaacgcct ccccctccac cttgagtgct 1440
accatgttca tcgtatctat actttgataa ggatcc 1476

<210> 151
<211> 1470
<212> DNA
<213> Artificial Sequence

<220>
<223> SPAM1-FIVS codon optimized DNA with NheI and BamHI restriction sites

<400> 151

```
gctagcatgg acatgagggt accagcccag ctgttgggcc ttcttctgct ttggctccct 60
ggggcaaggg gacttaactt cagagctccc cctgtaatcc caaacgtccc cttcctgtgg 120
gcatggaacg cccctagcga attctgtttg gggaaatttg acgaacctct ggacatgtct 180
ctgttttctt ttatcggtag ccctcgaatc aacgctaccg gacagggggt gacaatcttt 240
tacgtggatc gtctgggcta ttacccttac atcgacagca ttactggggt gaccgtgaac 300

ggaggcattc ctcagaaaat atcactgcag gaccacctgg acaaagctaa gaaggatatt 360
acattctaca tgccagtgga caatctcgga atggctgtga ttgactggga ggagtggaga 420
cctacttggg cccgaaattg gaagcccaag gatgtgtata agaaccgatc cattgagctg 480
gtgcagcagc agaacgtgca gctgtccctt acagaagcca ccgaaaaagc taagcaggaa 540
ttcgagaaag ctgggaagga tttcctggtt gagacaataa aacttgggaa actgcttaga 600
cctaaccacc tctggggcta ttatttgttc cccgattgct acaatcacca ctataagaag 660
cccggctaca acggatcttg tttcaacgtc gaaattaaaa gaaacgatga cctctcttgg 720
ttgtggaatg agtctactgc actctacccc tctatatatc tcaacacaca gcagtcccca 780
gtagccgcta ccctctacgt tcggaataga gtgagggaag ccatcagggt gtccaagatc 840
cccgatgcca aatctcccct cccagtgttt gcctacacca ggatcgtgtt taccgaccag 900
gtgttgaagt tcctgagcca ggacgagctc gtgtatactt ttggcgagac agttgctctg 960
ggagcctctg ggatcgtgat ctggggcact ctgagtatta tgcgatcaat gaagtcctgc 1020
ctgctgttgg ataactacat ggagacaatc ctgaacccat atatcattaa cgtgactttg 1080
gccgctaaga tgtgttcaca ggtgctgtgt caagagcagg gagtatgcat taggaagaac 1140
tggaactcaa gcgattatct ccatctgaac ccagataatt ttgcaatcca gctggaaaag 1200
ggtggtaaat tcactgtccg agggaagccc acactcgagg atctcgaaca gtttagtgaa 1260
aagttctact gtagctgcta tagcactctg agttgtaagg aaaaagccga tgtgaaagac 1320
actgatgccg ttgacgtttg catcgccgat ggggtgtgta ttgacgcctt tctcaagcca 1380
ccaatggaga ctgaggagcc ccagatcttt tacaacgcct ccccctccac cttgagtgct 1440
accatgttca tcgtatcttg ataaggatcc                                  1470
```

<210> 152
<211> 1464
<212> DNA
<213> Artificial Sequence

<220>
<223> SPAM1-TMFI codon optimized DNA with NheI and BamHI restriction sites

<400> 152

```
gctagcatgg acatgagggt accagcccag ctgttgggcc ttcttctgct ttggctccct 60
ggggcaaggg gacttaactt cagagctccc cctgtaatcc caaacgtccc cttcctgtgg 120
gcatggaacg cccctagcga attctgtttg gggaaatttg acgaacctct ggacatgtct 180
ctgttttctt ttatcggtag ccctcgaatc aacgctaccg gacagggggt gacaatcttt 240
tacgtggatc gtctgggcta ttacccttac atcgacagca ttactggggt gaccgtgaac 300
ggaggcattc ctcagaaaat atcactgcag gaccacctgg acaaagctaa gaaggatatt 360
acattctaca tgccagtgga caatctcgga atggctgtga ttgactggga ggagtggaga 420
cctacttggg cccgaaattg gaagcccaag gatgtgtata agaaccgatc cattgagctg 480
gtgcagcagc agaacgtgca gctgtccctt acagaagcca ccgaaaaagc taagcaggaa 540
ttcgagaaag ctgggaagga tttcctggtt gagacaataa aacttgggaa actgcttaga 600
cctaaccacc tctggggcta ttatttgttc cccgattgct acaatcacca ctataagaag 660
cccggctaca acggatcttg tttcaacgtc gaaattaaaa gaaacgatga cctctcttgg 720
ttgtggaatg agtctactgc actctacccc tctatatatc tcaacacaca gcagtcccca 780
gtagccgcta ccctctacgt tcggaataga gtgagggaag ccatcagggt gtccaagatc 840
cccgatgcca aatctccccc cccagtgttt gcctacacca ggatcgtgtt taccgaccag 900
gtgttgaagt tcctgagcca ggacgagctc gtgtatactt ttggcgagac agttgctctg 960
ggagcctctg ggatcgtgat ctggggcact ctgagtatta tgcgatcaat gaagtcctgc 1020
ctgctgttgg ataactacat ggagacaatc ctgaacccat atatcattaa cgtgactttg 1080
gccgctaaga tgtgttcaca ggtgctgtgt caagagcagg gagtatgcat taggaagaac 1140
tggaactcaa gcgattatct ccatctgaac ccagataatt ttgcaatcca gctggaaaag 1200
ggtggtaaat tcactgtccg agggaagccc acactcgagg atctcgaaca gtttagtgaa 1260
aagttctact gtagctgcta tagcactctg agttgtaagg aaaaagccga tgtgaaagac 1320
actgatgccg ttgacgtttg catcgccgat ggggtgtgta ttgacgcctt tctcaagcca 1380
ccaatggaga ctgaggagcc ccagatcttt tacaacgcct ccccctccac cttgagtgct 1440
accatgttca tctgataagg atcc                                       1464
```

<210> 153
<211> 1458
<212> DNA
<213> Artificial Sequence

<220>
<223> SPAM1-SATM codon optimized DNA with NheI and BamHI restriction sites

<400> 153

```
gctagcatgg acatgagggt accagcccag ctgttgggcc ttcttctgct ttggctccct 60
ggggcaaggg gacttaactt cagagctccc cctgtaatcc caaacgtccc cttcctgtgg 120
gcatggaacg cccctagcga attctgtttg gggaaatttg acgaacctct ggacatgtct 180
ctgttttctt ttatcggtag ccctcgaatc aacgctaccg gacaggggggt gacaatcttt 240
tacgtggatc gtctgggcta ttacccttac atcgacagca ttactggggt gaccgtgaac 300
ggaggcattc ctcagaaaat atcactgcag gaccacctgg acaaagctaa gaaggatatt 360
acattctaca tgccagtgga caatctcgga atggctgtga ttgactggga ggagtggaga 420
cctacttggg cccgaaattg gaagcccaag gatgtgtata agaaccgatc cattgagctg 480
gtgcagcagc agaacgtgca gctgtccctt acagaagcca ccgaaaaagc taagcaggaa 540
ttcgagaaag ctgggaagga tttcctggtt gagacaataa aacttgggaa actgcttaga 600
cctaaccacc tctggggcta ttatttgttc cccgattgct acaatcacca ctataagaag 660
cccggctaca acggatcttg tttcaacgtc gaaattaaaa gaaacgatga cctctcttgg 720
ttgtggaatg agtctactgc actctacccc tctatatatc tcaacacaca gcagtcccca 780
gtagccgcta ccctctacgt tcggaataga gtgagggaag ccatcagggt gtccaagatc 840
cccgatgcca aatctcccct cccagtgttt gcctacacca ggatcgtgtt taccgaccag 900
gtgttgaagt tcctgagcca ggacgagctc gtgtatactt ttggcgagac agttgctctg 960
ggagcctctg ggatcgtgat ctggggcact ctgagtatta tgcgatcaat gaagtcctgc 1020
ctgctgttgg ataactacat ggagacaatc ctgaacccat atatcattaa cgtgactttg 1080
gccgctaaga tgtgttcaca ggtgctgtgt caagagcagg gagtatgcat taggaagaac 1140
tggaactcaa gcgattatct ccatctgaac ccagataatt ttgcaatcca gctggaaaag 1200
ggtggtaaat tcactgtccg agggaagccc acactcgagg atctcgaaca gtttagtgaa 1260
aagttctact gtagctgcta tagcactctg agttgtaagg aaaaagccga tgtgaaagac 1320
actgatgccg ttgacgtttg catcgccgat ggggtgtgta ttgacgcctt tctcaagcca 1380
ccaatggaga ctgaggagcc ccagatcttt tacaacgcct ccccctccac cttgagtgct 1440
accatgtgat aaggatcc                                          1458
```

<210> 154
<211> 1452
<212> DNA
<213> Artificial Sequence

<220>
<223> SPAM1-TLSA codon optimized DNA with NheI and BamHI restriction sites

<400> 154

```
gctagcatgg acatgagggt accagcccag ctgttgggcc ttcttctgct ttggctccct 60
ggggcaaggg gacttaactt cagagctccc cctgtaatcc caaacgtccc cttcctgtgg 120
gcatggaacg cccctagcga attctgtttg gggaaatttg acgaacctct ggacatgtct 180
ctgttttctt ttatcggtag ccctcgaatc aacgctaccg gacaggggggt gacaatcttt 240
tacgtggatc gtctgggcta ttacccttac atcgacagca ttactggggt gaccgtgaac 300
ggaggcattc ctcagaaaat atcactgcag gaccacctgg acaaagctaa gaaggatatt 360
acattctaca tgccagtgga caatctcgga atggctgtga ttgactggga ggagtggaga 420
cctacttggg cccgaaattg gaagcccaag gatgtgtata agaaccgatc cattgagctg 480
gtgcagcagc agaacgtgca gctgtccctt acagaagcca ccgaaaaagc taagcaggaa 540
ttcgagaaag ctgggaagga tttcctggtt gagacaataa aacttgggaa actgcttaga 600
cctaaccacc tctggggcta ttatttgttc cccgattgct acaatcacca ctataagaag 660
cccggctaca acggatcttg tttcaacgtc gaaattaaaa gaaacgatga cctctcttgg 720
ttgtggaatg agtctactgc actctacccc tctatatatc tcaacacaca gcagtcccca 780
gtagccgcta ccctctacgt tcggaataga gtgagggaag ccatcagggt gtccaagatc 840
cccgatgcca aatctcccct cccagtgttt gcctacacca ggatcgtgtt taccgaccag 900
gtgttgaagt tcctgagcca ggacgagctc gtgtatactt ttggcgagac agttgctctg 960
ggagcctctg ggatcgtgat ctggggcact ctgagtatta tgcgatcaat gaagtcctgc 1020
ctgctgttgg ataactacat ggagacaatc ctgaacccat atatcattaa cgtgactttg 1080
gccgctaaga tgtgttcaca ggtgctgtgt caagagcagg gagtatgcat taggaagaac 1140
tggaactcaa gcgattatct ccatctgaac ccagataatt ttgcaatcca gctggaaaag 1200
ggtggtaaat tcactgtccg agggaagccc acactcgagg atctcgaaca gtttagtgaa 1260
aagttctact gtagctgcta tagcactctg agttgtaagg aaaaagccga tgtgaaagac 1320
actgatgccg ttgacgtttg catcgccgat ggggtgtgta ttgacgcctt tctcaagcca 1380
```

ccaatggaga ctgaggagcc ccagatcttt tacaacgcct ccccctccac cttgagtgct 1440
tgataaggat cc 1452

<210> 155
<211> 1446
<212> DNA
<213> Artificial Sequence

<220>
<223> SPAM1-PSTL codon optimized DNA with NheI and BamHI restriction sites

<400> 155

gctagcatgg acatgagggt accagcccag ctgttgggcc ttcttctgct ttggctccct 60
ggggcaaggg gacttaactt cagagctccc cctgtaatcc caaacgtccc cttcctgtgg 120
gcatggaacg cccctagcga attctgtttg gggaaatttg acgaacctct ggacatgtct 180
ctgttttctt ttatcggtag ccctcgaatc aacgctaccg gacaggggtg gacaatcttt 240
tacgtggatc gtctgggcta ttacccttac atcgacagca ttactggggt gaccgtgaac 300
ggaggcattc ctcagaaaat atcactgcag gaccacctgg acaaagctaa gaaggatatt 360
acattctaca tgccagtgga caatctcgga atggctgtga ttgactggga ggagtggaga 420
cctacttggg cccgaaattg gaagcccaag gatgtgtata agaaccgatc cattgagctg 480
gtgcagcagc agaacgtgca gctgtccctt acagaagcca ccgaaaaagc taagcaggaa 540
ttcgagaaag ctgggaagga tttcctggtt gagacaataa aacttgggaa actgcttaga 600
cctaaccacc tctggggcta ttatttgttc cccgattgct acaatcacca ctataagaag 660
cccggctaca acggatcttg tttcaacgtc gaaattaaaa gaaacgatga cctctcttgg 720
ttgtggaatg agtctactgc actctacccc tctatatatc tcaacacaca gcagtcccca 780
gtagccgcta ccctctacgt tcggaataga gtgagggaag ccatcagggt gtccaagatc 840
cccgatgcca aatctcccct cccagtgttt gcctacacca ggatcgtgtt taccgaccag 900
gtgttgaagt tcctgagcca ggacgagctc gtgtatactt ttggcgagac agttgctctg 960
ggagcctctg ggatcgtgat ctggggcact ctgagtatta tgcgatcaat gaagtcctgc 1020
ctgctgttgg ataactacat ggagacaatc ctgaacccat atatcattaa cgtgactttg 1080
gccgctaaga tgtgttcaca ggtgctgtgt caagagcagg gagtatgcat taggaagaac 1140
tggaactcaa gcgattatct ccatctgaac ccagataatt ttgcaatcca gctggaaaag 1200
ggtggtaaat tcactgtccg agggaagccc acactcgagg atctcgaaca gtttagtgaa 1260
aagttctact gtagctgcta tagcactctg agttgtaagg aaaaagccga tgtgaaagac 1320
actgatgccg ttgacgtttg catcgccgat ggggtgtgta ttgacgcctt tctcaagcca 1380
ccaatggaga ctgaggagcc ccagatcttt tacaacgcct ccccctccac cttgtgataa 1440
ggatcc 1446

<210> 156
<211> 1440
<212> DNA
<213> Artificial Sequence

<220>
<223> SPAM1-ASPS codon optimized DNA with NheI and BamHI restriction sites

<400> 156

```
gctagcatgg acatgagggt accagcccag ctgttgggcc ttcttctgct ttggctccct 60
ggggcaaggg gacttaactt cagagctccc cctgtaatcc caaacgtccc cttcctgtgg 120
gcatggaacg cccctagcga attctgtttg gggaaatttg acgaacctct ggacatgtct 180
ctgttttctt ttatcggtag ccctcgaatc aacgctaccg gacagggggt gacaatcttt 240
tacgtggatc gtctgggcta ttacccttac atcgacagca ttactggggt gaccgtgaac 300
ggaggcattc ctcagaaaat atcactgcag gaccacctgg acaaagctaa gaaggatatt 360
acattctaca tgccagtgga caatctcgga atggctgtga ttgactggga ggagtggaga 420
cctacttggg cccgaaattg gaagcccaag gatgtgtata agaaccgatc cattgagctg 480
gtgcagcagc agaacgtgca gctgtccctt acagaagcca ccgaaaaagc taagcaggaa 540
ttcgagaaag ctgggaagga tttcctggtt gagacaataa aacttgggaa actgcttaga 600
cctaaccacc tctggggcta ttatttgttc cccgattgct acaatcacca ctataagaag 660
cccggctaca acggatcttg tttcaacgtc gaaattaaaa gaaacgatga cctctcttgg 720
ttgtggaatg agtctactgc actctacccc tctatatatc tcaacacaca gcagtcccca 780
gtagccgcta ccctctacgt tcggaataga gtgagggaag ccatcagggt gtccaagatc 840

cccgatgcca aatctcccct cccagtgttt gcctacacca ggatcgtgtt taccgaccag 900
gtgttgaagt tcctgagcca ggacgagctc gtgtatactt ttggcgagac agttgctctg 960
ggagcctctg ggatcgtgat ctggggcact ctgagtatta tgcgatcaat gaagtcctgc 1020
ctgctgttgg ataactacat ggagacaatc ctgaacccat atatcattaa cgtgactttg 1080
gccgctaaga tgtgttcaca ggtgctgtgt caagagcagg gagtatgcat taggaagaac 1140
tggaactcaa gcgattatct ccatctgaac ccagataatt ttgcaatcca gctggaaaag 1200
ggtggtaaat tcactgtccg agggaagccc acactcgagg atctcgaaca gtttagtgaa 1260
aagttctact gtagctgcta tagcactctg agttgtaagg aaaaagccga tgtgaaagac 1320
actgatgccg ttgacgtttg catcgccgat ggggtgtgta ttgacgcctt tctcaagcca 1380
ccaatggaga ctgaggagcc ccagatcttt tacaacgcct ccccctcctg ataaggatcc 1440
```

<210> 157
<211> 1434
<212> DNA
<213> Artificial Sequence

<220>
<223> SPAM1-YNAS codon optimized DNA with NheI and BamHI restriction sites

<400> 157

```
gctagcatgg acatgagggt accagcccag ctgttgggcc ttcttctgct ttggctccct 60
ggggcaaggg gacttaactt cagagctccc cctgtaatcc caaacgtccc cttcctgtgg 120
gcatggaacg cccctagcga attctgtttg gggaaatttg acgaacctct ggacatgtct 180
ctgttttctt ttatcggtag ccctcgaatc aacgctaccg gacagggggt gacaatcttt 240
tacgtggatc gtctgggcta ttacccttac atcgacagca ttactggggt gaccgtgaac 300
ggaggcattc ctcagaaaat atcactgcag gaccacctgg acaaagctaa gaaggatatt 360
acattctaca tgccagtgga caatctcgga atggctgtga ttgactggga ggagtggaga 420
cctacttggg cccgaaattg gaagcccaag gatgtgtata agaaccgatc cattgagctg 480
gtgcagcagc agaacgtgca gctgtccctt acagaagcca ccgaaaaagc taagcaggaa 540
ttcgagaaag ctgggaagga tttcctggtt gagacaataa aacttgggaa actgcttaga 600
cctaaccacc tctggggcta ttatttgttc cccgattgct acaatcacca ctataagaag 660
cccggctaca acggatcttg tttcaacgtc gaaattaaaa gaaacgatga cctctcttgg 720
ttgtggaatg agtctactgc actctacccc tctatatatc tcaacacaca gcagtcccca 780
gtagccgcta ccctctacgt tcggaataga gtgagggaag ccatcagggt gtccaagatc 840
cccgatgcca aatctccccc cccagtgttt gcctacacca ggatcgtgtt taccgaccag 900
gtgttgaagt tcctgagcca ggacgagctc gtgtatactt ttggcgagac agttgctctg 960
ggagcctctg ggatcgtgat ctggggcact ctgagtatta tgcgatcaat gaagtcctgc 1020
ctgctgttgg ataactacat ggagacaatc ctgaacccat atatcattaa cgtgactttg 1080
gccgctaaga tgtgttcaca ggtgctgtgt caagagcagg gagtatgcat taggaagaac 1140
tggaactcaa gcgattatct ccatctgaac ccagataatt ttgcaatcca gctggaaaag 1200
ggtggtaaat tcactgtccg agggaagccc acactcgagg atctcgaaca gtttagtgaa 1260
aagttctact gtagctgcta tagcactctg agttgtaagg aaaaagccga tgtgaaagac 1320
actgatgccg ttgacgtttg catcgccgat ggggtgtgta ttgacgcctt tctcaagcca 1380
ccaatggaga ctgaggagcc ccagatcttt tacaacgcct cctgataagg atcc       1434
```

<210> 158
<211> 1431
<212> DNA
<213> Artificial Sequence

<220>
<223> SPAM1-FYNA codon optimized DNA with NheI and BamHI restriction sites

<400> 158

```
gctagcatgg acatgagggt accagcccag ctgttgggcc ttcttctgct ttggctccct 60
ggggcaaggg gacttaactt cagagctccc cctgtaatcc caaacgtccc cttcctgtgg 120
gcatggaacg cccctagcga attctgtttg gggaaatttg acgaacctct ggacatgtct 180
ctgttttctt ttatcggtag ccctcgaatc aacgctaccg gacagggggt gacaatcttt 240
tacgtggatc gtctgggcta ttacccttac atcgacagca ttactggggt gaccgtgaac 300
ggaggcattc ctcagaaaat atcactgcag gaccacctgg acaaagctaa gaaggatatt 360
```

```
acattctaca tgccagtgga caatctcgga atggctgtga ttgactggga ggagtggaga 420
cctacttggg cccgaaattg gaagcccaag gatgtgtata agaaccgatc cattgagctg 480
gtgcagcagc agaacgtgca gctgtccctt acagaagcca ccgaaaaagc taagcaggaa 540
ttcgagaaag ctgggaagga tttcctggtt gagacaataa aacttgggaa actgcttaga 600
cctaaccacc tctggggcta ttatttgttc cccgattgct acaatcacca ctataagaag 660
cccggctaca acggatcttg tttcaacgtc gaaattaaaa gaaacgatga cctctcttgg 720
ttgtggaatg agtctactgc actctacccc tctatatatc tcaacacaca gcagtcccca 780
gtagccgcta ccctctacgt tcggaataga gtgagggaag ccatcagggt gtccaagatc 840
cccgatgcca aatctcccct cccagtgttt gcctacacca ggatcgtgtt taccgaccag 900
gtgttgaagt tcctgagcca ggacgagctc gtgtatactt ttggcgagac agttgctctg 960
ggagcctctg ggatcgtgat ctggggcact ctgagtatta tgcgatcaat gaagtcctgc 1020
ctgctgttgg ataactacat ggagacaatc ctgaacccat atatcattaa cgtgactttg 1080
gccgctaaga tgtgttcaca ggtgctgtgt caagagcagg gagtatgcat taggaagaac 1140
tggaactcaa gcgattatct ccatctgaac ccagataatt ttgcaatcca gctggaaaag 1200
ggtggtaaat tcactgtccg agggaagccc acactcgagg atctcgaaca gtttagtgaa 1260
aagttctact gtagctgcta tagcactctg agttgtaagg aaaaagccga tgtgaaagac 1320
actgatgccg ttgacgtttg catcgccgat ggggtgtgta ttgacgcctt tctcaagcca 1380
ccaatggaga ctgaggagcc ccagatcttt tacaacgcct gataaggatc c          1431
```

<210> 159
<211> 1428
<212> DNA
<213> Artificial Sequence

<220>
<223> SPAM1-IFYN codon optimized DNA with NheI and BamHI restriction sites

<400> 159

```
gctagcatgg acatgagggt accagcccag ctgttgggcc ttcttctgct ttggctccct 60
ggggcaaggg gacttaactt cagagctccc cctgtaatcc caaacgtccc cttcctgtgg 120
gcatggaacg cccctagcga attctgtttg gggaaatttg acgaacctct ggacatgtct 180
ctgttttctt ttatcggtag ccctcgaatc aacgctaccg gacaggggtg gacaatcttt 240
tacgtggatc gtctgggcta ttacccttac atcgacagca ttactggggt gaccgtgaac 300
ggaggcattc ctcagaaaat atcactgcag gaccacctgg acaaagctaa gaaggatatt 360
acattctaca tgccagtgga caatctcgga atggctgtga ttgactggga ggagtggaga 420
cctacttggg cccgaaattg gaagcccaag gatgtgtata agaaccgatc cattgagctg 480
gtgcagcagc agaacgtgca gctgtccctt acagaagcca ccgaaaaagc taagcaggaa 540
ttcgagaaag ctgggaagga tttcctggtt gagacaataa aacttgggaa actgcttaga 600
cctaaccacc tctggggcta ttatttgttc cccgattgct acaatcacca ctataagaag 660
cccggctaca acggatcttg tttcaacgtc gaaattaaaa gaaacgatga cctctcttgg 720
ttgtggaatg agtctactgc actctacccc tctatatatc tcaacacaca gcagtcccca 780
gtagccgcta ccctctacgt tcggaataga gtgagggaag ccatcagggt gtccaagatc 840
cccgatgcca aatctcccct cccagtgttt gcctacacca ggatcgtgtt taccgaccag 900
gtgttgaagt tcctgagcca ggacgagctc gtgtatactt ttggcgagac agttgctctg 960
ggagcctctg ggatcgtgat ctggggcact ctgagtatta tgcgatcaat gaagtcctgc 1020
ctgctgttgg ataactacat ggagacaatc ctgaacccat atatcattaa cgtgactttg 1080
gccgctaaga tgtgttcaca ggtgctgtgt caagagcagg gagtatgcat taggaagaac 1140
tggaactcaa gcgattatct ccatctgaac ccagataatt ttgcaatcca gctggaaaag 1200
ggtggtaaat tcactgtccg agggaagccc acactcgagg atctcgaaca gtttagtgaa 1260
aagttctact gtagctgcta tagcactctg agttgtaagg aaaaagccga tgtgaaagac 1320
actgatgccg ttgacgtttg catcgccgat ggggtgtgta ttgacgcctt tctcaagcca 1380
ccaatggaga ctgaggagcc ccagatcttt tacaactgat aaggatcc              1428
```

<210> 160
<211> 1425
<212> DNA
<213> Artificial Sequence

<220>
<223> SPAM1-QIFY codon optimized DNA with NheI and BamHI restriction sites

<400> 160

```
gctagcatgg acatgagggt accagcccag ctgttgggcc ttcttctgct ttggctccct 60
ggggcaaggg gacttaactt cagagctccc cctgtaatcc caaacgtccc cttcctgtgg 120
gcatggaacg cccctagcga attctgtttg gggaaatttg acgaacctct ggacatgtct 180
ctgttttctt ttatcggtag ccctcgaatc aacgctaccg gacaggggtg gacaatcttt 240
tacgtggatc gtctgggcta ttacccttac atcgacagca ttactggggt gaccgtgaac 300
ggaggcattc ctcagaaaat atcactgcag gaccacctgg acaaagctaa gaaggatatt 360
acattctaca tgccagtgga caatctcgga atggctgtga ttgactggga ggagtggaga 420
cctacttggg cccgaaattg gaagcccaag gatgtgtata agaaccgatc cattgagctg 480
gtgcagcagc agaacgtgca gctgtccctt acagaagcca ccgaaaaagc taagcaggaa 540
ttcgagaaag ctgggaagga tttcctggtt gagacaataa aacttgggaa actgcttaga 600
cctaaccacc tctggggcta ttatttgttc cccgattgct acaatcacca ctataagaag 660
cccggctaca acggatcttg tttcaacgtc gaaattaaaa gaaacgatga cctctcttgg 720
ttgtggaatg agtctactgc actctacccc tctatatatc tcaacacaca gcagtcccca 780
gtagccgcta ccctctacgt tcggaataga gtgagggaag ccatcagggt gtccaagatc 840
cccgatgcca aatctccccc cccagtgttt gcctacacca ggatcgtgtt taccgaccag 900
gtgttgaagt tcctgagcca ggacgagctc gtgtatactt ttggcgagac agttgctctg 960
ggagcctctg ggatcgtgat ctggggcact ctgagtatta tgcgatcaat gaagtcctgc 1020
ctgctgttgg ataactacat ggagacaatc ctgaacccat atatcattaa cgtgactttg 1080
gccgctaaga tgtgttcaca ggtgctgtgt caagagcagg gagtatgcat taggaagaac 1140
tggaactcaa gcgattatct ccatctgaac ccagataatt ttgcaatcca gctggaaaag 1200
ggtggtaaat tcactgtccg agggaagccc acactcgagg atctcgaaca gtttagtgaa 1260
aagttctact gtagctgcta tagcactctg agttgtaagg aaaaagccga tgtgaaagac 1320
actgatgccg ttgacgtttg catcgccgat ggggtgtgta ttgacgcctt tctcaagcca 1380
ccaatggaga ctgaggagcc ccagatcttt tactgataag gatcc                 1425
```

<210> 161
<211> 1422
<212> DNA
<213> Artificial Sequence

<220>
<223> SPAM1-PQIF codon optimized DNA with NheI and BamHI restriction sites

<400> 161

```
gctagcatgg acatgagggt accagcccag ctgttgggcc ttcttctgct ttggctccct 60
ggggcaaggg gacttaactt cagagctccc cctgtaatcc caaacgtccc cttcctgtgg 120
gcatggaacg cccctagcga attctgtttg gggaaatttg acgaacctct ggacatgtct 180
ctgttttctt ttatcggtag ccctcgaatc aacgctaccg gacaggggtg gacaatcttt 240
tacgtggatc gtctgggcta ttacccttac atcgacagca ttactggggt gaccgtgaac 300
ggaggcattc ctcagaaaat atcactgcag gaccacctgg acaaagctaa gaaggatatt 360
acattctaca tgccagtgga caatctcgga atggctgtga ttgactggga ggagtggaga 420
cctacttggg cccgaaattg gaagcccaag gatgtgtata agaaccgatc cattgagctg 480
gtgcagcagc agaacgtgca gctgtccctt acagaagcca ccgaaaaagc taagcaggaa 540
ttcgagaaag ctgggaagga tttcctggtt gagacaataa aacttgggaa actgcttaga 600
cctaaccacc tctggggcta ttatttgttc cccgattgct acaatcacca ctataagaag 660
cccggctaca acggatcttg tttcaacgtc gaaattaaaa gaaacgatga cctctcttgg 720
ttgtggaatg agtctactgc actctacccc tctatatatc tcaacacaca gcagtcccca 780
gtagccgcta ccctctacgt tcggaataga gtgagggaag ccatcagggt gtccaagatc 840
cccgatgcca aatctcccct cccagtgttt gcctacacca ggatcgtgtt taccgaccag 900
gtgttgaagt tcctgagcca ggacgagctc gtgtatactt ttggcgagac agttgctctg 960
ggagcctctg ggatcgtgat ctggggcact ctgagtatta tgcgatcaat gaagtcctgc 1020
ctgctgttgg ataactacat ggagacaatc ctgaacccat atatcattaa cgtgactttg 1080
gccgctaaga tgtgttcaca ggtgctgtgt caagagcagg gagtatgcat taggaagaac 1140
tggaactcaa gcgattatct ccatctgaac ccagataatt ttgcaatcca gctggaaaag 1200
ggtggtaaat tcactgtccg agggaagccc acactcgagg atctcgaaca gtttagtgaa 1260
aagttctact gtagctgcta tagcactctg agttgtaagg aaaaagccga tgtgaaagac 1320
actgatgccg ttgacgtttg catcgccgat ggggtgtgta ttgacgcctt tctcaagcca 1380
ccaatggaga ctgaggagcc ccagatcttt tgataaggat cc                    1422
```

<210> 162
<211> 1419
<212> DNA
<213> Artificial Sequence

<220>
<223> SPAM1-EPQI codon optimized DNA with NheI and BamHI restriction sites

<400> 162

```
gctagcatgg acatgagggt accagcccag ctgttgggcc ttcttctgct ttggctccct 60
ggggcaaggg gacttaactt cagagctccc cctgtaatcc caaacgtccc cttcctgtgg 120
gcatggaacg cccctagcga attctgtttg gggaaatttg acgaacctct ggacatgtct 180
ctgttttctt ttatcggtag ccctcgaatc aacgctaccg gacaggggtg gacaatcttt 240
tacgtggatc gtctgggcta ttacccttac atcgacagca ttactggggt gaccgtgaac 300
ggaggcattc ctcagaaaat atcactgcag gaccacctgg acaaagctaa gaaggatatt 360
acattctaca tgccagtgga caatctcgga atggctgtga ttgactggga ggagtggaga 420
cctacttggg cccgaaattg gaagcccaag gatgtgtata agaaccgatc cattgagctg 480
gtgcagcagc agaacgtgca gctgtccctt acagaagcca ccgaaaaagc taagcaggaa 540
ttcgagaaag ctgggaagga tttcctggtt gagacaataa aacttgggaa actgcttaga 600
cctaaccacc tctggggcta ttatttgttc cccgattgct acaatcacca ctataagaag 660
cccggctaca acggatcttg tttcaacgtc gaaattaaaa gaaacgatga cctctcttgg 720
ttgtggaatg agtctactgc actctacccc tctatatatc tcaacacaca gcagtcccca 780
gtagccgcta ccctctacgt tcggaataga gtgagggaag ccatcagggt gtccaagatc 840
cccgatgcca aatctcccct cccagtgttt gcctacacca ggatcgtgtt taccgaccag 900
gtgttgaagt tcctgagcca ggacgagctc gtgtatactt ttggcgagac agttgctctg 960
ggagcctctg ggatcgtgat ctggggcact ctgagtatta tgcgatcaat gaagtcctgc 1020
ctgctgttgg ataactacat ggagacaatc ctgaacccat atatcattaa cgtgactttg 1080
gccgctaaga tgtgttcaca ggtgctgtgt caagagcagg gagtatgcat taggaagaac 1140
tggaactcaa gcgattatct ccatctgaac ccagataatt ttgcaatcca gctggaaaag 1200
ggtggtaaat tcactgtccg agggaagccc acactcgagg atctcgaaca gtttagtgaa 1260
aagttctact gtagctgcta tagcactctg agttgtaagg aaaaagccga tgtgaaagac 1320
actgatgccg ttgacgtttg catcgccgat ggggtgtgta ttgacgcctt tctcaagcca 1380
ccaatggaga ctgaggagcc ccagatctga taaggatcc                    1419
```

<210> 163
<211> 1416
<212> DNA
<213> Artificial Sequence

<220>
<223> SPAM1-EEPQ codon optimized DNA with NheI and BamHI restriction sites

<400> 163

```
gctagcatgg acatgagggt accagcccag ctgttgggcc ttcttctgct ttggctccct 60
ggggcaaggg gacttaactt cagagctccc cctgtaatcc caaacgtccc cttcctgtgg 120
gcatggaacg cccctagcga attctgtttg gggaaatttg acgaacctct ggacatgtct 180
ctgttttctt ttatcggtag ccctcgaatc aacgctaccg gacagggggt gacaatcttt 240
tacgtggatc gtctgggcta ttacccttac atcgacagca ttactggggt gaccgtgaac 300
ggaggcattc ctcagaaaat atcactgcag gaccacctgg acaaagctaa gaaggatatt 360
acattctaca tgccagtgga caatctcgga atggctgtga ttgactggga ggagtggaga 420
cctacttggg cccgaaattg gaagcccaag gatgtgtata agaaccgatc cattgagctg 480
gtgcagcagc agaacgtgca gctgtccctt acagaagcca ccgaaaaagc taagcaggaa 540
ttcgagaaag ctgggaagga tttcctggtt gagacaataa aacttgggaa actgcttaga 600
cctaaccacc tctggggcta ttatttgttc cccgattgct acaatcacca ctataagaag 660
cccggctaca acggatcttg tttcaacgtc gaaattaaaa gaaacgatga cctctcttgg 720
ttgtggaatg agtctactgc actctacccc tctatatatc tcaacacaca gcagtcccca 780
gtagccgcta ccctctacgt tcggaataga gtgagggaag ccatcagggt gtccaagatc 840
cccgatgcca aatctccccc cccagtgttt gcctacacca ggatcgtgtt taccgaccag 900
gtgttgaagt tcctgagcca ggacgagctc gtgtatactt ttggcgagac agttgctctg 960
ggagcctctg ggatcgtgat ctggggcact ctgagtatta tgcgatcaat gaagtcctgc 1020
ctgctgttgg ataactacat ggagacaatc ctgaacccat atatcattaa cgtgactttg 1080
gccgctaaga tgtgttcaca ggtgctgtgt caagagcagg gagtatgcat taggaagaac 1140
tggaactcaa gcgattatct ccatctgaac ccagataatt ttgcaatcca gctggaaaag 1200

ggtggtaaat tcactgtccg agggaagccc acactcgagg atctcgaaca gtttagtgaa 1260
aagttctact gtagctgcta tagcactctg agttgtaagg aaaaagccga tgtgaaagac 1320
actgatgccg ttgacgtttg catcgccgat ggggtgtgta ttgacgcctt tctcaagcca 1380
ccaatggaga ctgaggagcc ccagtgataa ggatcc                           1416
```

<210> 164
<211> 1413
<212> DNA
<213> Artificial Sequence

<220>
<223> SPAM1-TEEP codon optimized DNA with NheI and BamHI restriction sites

<400> 164

```
gctagcatgg acatgagggt accagcccag ctgttgggcc ttcttctgct ttggctccct 60
ggggcaaggg gacttaactt cagagctccc cctgtaatcc caaacgtccc cttcctgtgg 120
gcatggaacg cccctagcga attctgtttg gggaaatttg acgaacctct ggacatgtct 180
ctgttttctt ttatcggtag ccctcgaatc aacgctaccg gacagggggt gacaatcttt 240
tacgtggatc gtctgggcta ttacccttac atcgacagca ttactggggt gaccgtgaac 300
ggaggcattc ctcagaaaat atcactgcag gaccacctgg acaaagctaa gaaggatatt 360
acattctaca tgccagtgga caatctcgga atggctgtga ttgactggga ggagtggaga 420
cctacttggg cccgaaattg gaagcccaag gatgtgtata agaaccgatc cattgagctg 480
gtgcagcagc agaacgtgca gctgtccctt acagaagcca ccgaaaaagc taagcaggaa 540
ttcgagaaag ctgggaagga tttcctggtt gagacaataa aacttgggaa actgcttaga 600
cctaaccacc tctggggcta ttatttgttc cccgattgct acaatcacca ctataagaag 660
cccggctaca acggatcttg tttcaacgtc gaaattaaaa gaaacgatga cctctcttgg 720
ttgtggaatg agtctactgc actctacccc tctatatatc tcaacacaca gcagtcccca 780
gtagccgcta ccctctacgt tcggaataga gtgagggaag ccatcagggt gtccaagatc 840
cccgatgcca aatctccccc cccagtgttt gcctacacca ggatcgtgtt taccgaccag 900
gtgttgaagt tcctgagcca ggacgagctc gtgtatactt ttggcgagac agttgctctg 960
ggagcctctg ggatcgtgat ctggggcact ctgagtatta tgcgatcaat gaagtcctgc 1020
ctgctgttgg ataactacat ggagacaatc ctgaacccat atatcattaa cgtgactttg 1080
gccgctaaga tgtgttcaca ggtgctgtgt caagagcagg gagtatgcat taggaagaac 1140
tggaactcaa gcgattatct ccatctgaac ccagataatt ttgcaatcca gctggaaaag 1200
ggtggtaaat tcactgtccg agggaagccc acactcgagg atctcgaaca gtttagtgaa 1260
aagttctact gtagctgcta tagcactctg agttgtaagg aaaaagccga tgtgaaagac 1320
actgatgccg ttgacgtttg catcgccgat ggggtgtgta ttgacgcctt tctcaagcca 1380
ccaatggaga ctgaggagcc ctgataagga tcc                              1413
```

<210> 165
<211> 1410
<212> DNA
<213> Artificial Sequence

<220>
<223> SPAM1-ETEE codon optimized DNA with NheI and BamHI restriction sites

<400> 165

```
gctagcatgg acatgagggt accagcccag ctgttgggcc ttcttctgct ttggctccct 60
ggggcaaggg gacttaactt cagagctccc cctgtaatcc caaacgtccc cttcctgtgg 120
gcatggaacg cccctagcga attctgtttg gggaaatttg acgaacctct ggacatgtct 180
ctgttttctt ttatcggtag ccctcgaatc aacgctaccg gacagggggt gacaatcttt 240
tacgtggatc gtctgggcta ttacccttac atcgacagca ttactggggt gaccgtgaac 300
ggaggcattc ctcagaaaat atcactgcag gaccacctgg acaaagctaa gaaggatatt 360
acattctaca tgccagtgga caatctcgga atggctgtga ttgactggga ggagtggaga 420
cctacttggg cccgaaattg gaagcccaag gatgtgtata agaaccgatc cattgagctg 480
gtgcagcagc agaacgtgca gctgtccctt acagaagcca ccgaaaaagc taagcaggaa 540
ttcgagaaag ctgggaagga tttcctggtt gagacaataa aacttgggaa actgcttaga 600
cctaaccacc tctggggcta ttatttgttc cccgattgct acaatcacca ctataagaag 660
cccggctaca acggatcttg tttcaacgtc gaaattaaaa gaaacgatga cctctcttgg 720
ttgtggaatg agtctactgc actctacccc tctatatatc tcaacacaca gcagtcccca 780
```

gtagccgcta ccctctacgt tcggaataga gtgagggaag ccatcagggt gtccaagatc 840
cccgatgcca aatctcccct cccagtgttt gcctacacca ggatcgtgtt taccgaccag 900
gtgttgaagt tcctgagcca ggacgagctc gtgtatactt ttggcgagac agttgctctg 960
ggagcctctg ggatcgtgat ctggggcact ctgagtatta tgcgatcaat gaagtcctgc 1020
ctgctgttgg ataactacat ggagacaatc ctgaacccat atatcattaa cgtgactttg 1080
gccgctaaga tgtgttcaca ggtgctgtgt caagagcagg gagtatgcat taggaagaac 1140
tggaactcaa gcgattatct ccatctgaac ccagataatt ttgcaatcca gctggaaaag 1200
ggtggtaaat tcactgtccg agggaagccc acactcgagg atctcgaaca gtttagtgaa 1260
aagttctact gtagctgcta tagcactctg agttgtaagg aaaaagccga tgtgaaagac 1320
actgatgccg ttgacgtttg catcgccgat ggggtgtgta ttgacgcctt tctcaagcca 1380
ccaatggaga ctgaggagtg ataaggatcc 1410

<210> 166
<211> 1407
<212> DNA
<213> Artificial Sequence

<220>
<223> SPAM1-METE codon optimized DNA with NheI and BamHI restriction sites

<400> 166

gctagcatgg acatgagggt accagcccag ctgttgggcc ttcttctgct ttggctccct 60
ggggcaaggg gacttaactt cagagctccc cctgtaatcc caaacgtccc cttcctgtgg 120
gcatggaacg cccctagcga attctgtttg gggaaatttg acgaacctct ggacatgtct 180
ctgttttctt ttatcggtag ccctcgaatc aacgctaccg gacaggggtg gacaatcttt 240
tacgtggatc gtctgggcta ttacccttac atcgacagca ttactggggt gaccgtgaac 300
ggaggcattc ctcagaaaat atcactgcag gaccacctgg acaaagctaa gaaggatatt 360
acattctaca tgccagtgga caatctcgga atggctgtga ttgactggga ggagtggaga 420
cctacttggg cccgaaattg gaagcccaag gatgtgtata agaaccgatc cattgagctg 480
gtgcagcagc agaacgtgca gctgtccctt acagaagcca ccgaaaaagc taagcaggaa 540
ttcgagaaag ctgggaagga tttcctggtt gagacaataa aacttgggaa actgcttaga 600
cctaaccacc tctggggcta ttatttgttc cccgattgct acaatcacca ctataagaag 660
cccggctaca acggatcttg tttcaacgtc gaaattaaaa gaaacgatga cctctcttgg 720
ttgtggaatg agtctactgc actctacccc tctatatatc tcaacacaca gcagtcccca 780
gtagccgcta ccctctacgt tcggaataga gtgagggaag ccatcagggt gtccaagatc 840
cccgatgcca aatctcccct cccagtgttt gcctacacca ggatcgtgtt taccgaccag 900
gtgttgaagt tcctgagcca ggacgagctc gtgtatactt ttggcgagac agttgctctg 960
ggagcctctg ggatcgtgat ctggggcact ctgagtatta tgcgatcaat gaagtcctgc 1020
ctgctgttgg ataactacat ggagacaatc ctgaacccat atatcattaa cgtgactttg 1080
gccgctaaga tgtgttcaca ggtgctgtgt caagagcagg gagtatgcat taggaagaac 1140
tggaactcaa gcgattatct ccatctgaac ccagataatt ttgcaatcca gctggaaaag 1200
ggtggtaaat tcactgtccg agggaagccc acactcgagg atctcgaaca gtttagtgaa 1260
aagttctact gtagctgcta tagcactctg agttgtaagg aaaaagccga tgtgaaagac 1320
actgatgccg ttgacgtttg catcgccgat ggggtgtgta ttgacgcctt tctcaagcca 1380
ccaatggaga ctgagtgata aggatcc 1407

<210> 167
<211> 1404
<212> DNA
<213> Artificial Sequence

<220>
<223> SPAM1-PMET codon optimized DNA with NheI and BamHI restriction sites

<400> 167

```
gctagcatgg acatgagggt accagcccag ctgttgggcc ttcttctgct ttggctccct 60
ggggcaaggg gacttaactt cagagctccc cctgtaatcc caaacgtccc cttcctgtgg 120
gcatggaacg cccctagcga attctgtttg gggaaatttg acgaacctct ggacatgtct 180
ctgttttctt ttatcggtag ccctcgaatc aacgctaccg gacagggggt gacaatcttt 240
tacgtggatc gtctgggcta ttacccttac atcgacagca ttactggggt gaccgtgaac 300
ggaggcattc ctcagaaaat atcactgcag gaccacctgg acaaagctaa gaaggatatt 360

acattctaca tgccagtgga caatctcgga atggctgtga ttgactggga ggagtggaga 420
cctacttggg cccgaaattg gaagcccaag gatgtgtata agaaccgatc cattgagctg 480
gtgcagcagc agaacgtgca gctgtccctt acagaagcca ccgaaaaagc taagcaggaa 540
ttcgagaaag ctgggaagga tttcctggtt gagacaataa aacttgggaa actgcttaga 600
cctaaccacc tctggggcta ttatttgttc cccgattgct acaatcacca ctataagaag 660
cccggctaca acggatcttg tttcaacgtc gaaattaaaa gaaacgatga cctctcttgg 720
ttgtggaatg agtctactgc actctacccc tctatatatc tcaacacaca gcagtcccca 780
gtagccgcta ccctctacgt tcggaataga gtgagggaag ccatcagggt gtccaagatc 840
cccgatgcca aatctccccc cccagtgttt gcctacacca ggatcgtgtt taccgaccag 900
gtgttgaagt tcctgagcca ggacgagctc gtgtatactt ttggcgagac agttgctctg 960
ggagcctctg ggatcgtgat ctggggcact ctgagtatta tgcgatcaat gaagtcctgc 1020
ctgctgttgg ataactacat ggagacaatc ctgaacccat atatcattaa cgtgactttg 1080
gccgctaaga tgtgttcaca ggtgctgtgt caagagcagg gagtatgcat taggaagaac 1140
tggaactcaa gcgattatct ccatctgaac ccagataatt ttgcaatcca gctggaaaag 1200
ggtggtaaat tcactgtccg agggaagccc acactcgagg atctcgaaca gtttagtgaa 1260
aagttctact gtagctgcta tagcactctg agttgtaagg aaaaagccga tgtgaaagac 1320
actgatgccg ttgacgtttg catcgccgat ggggtgtgta ttgacgcctt tctcaagcca 1380
ccaatggaga cttgataagg atcc                                       1404
```

<210> 168
<211> 1401
<212> DNA
<213> Artificial Sequence

<220>
<223> SPAM1-PPME codon optimized DNA with NheI and BamHI restriction sites

<400> 168

```
gctagcatgg acatgagggt accagcccag ctgttgggcc ttcttctgct ttggctccct 60
ggggcaaggg gacttaactt cagagctccc cctgtaatcc caaacgtccc cttcctgtgg 120
gcatggaacg cccctagcga attctgtttg gggaaatttg acgaacctct ggacatgtct 180
ctgttttctt ttatcggtag ccctcgaatc aacgctaccg gacaggggtg gacaatcttt 240
tacgtggatc gtctgggcta ttacccttac atcgacagca ttactggggt gaccgtgaac 300
ggaggcattc ctcagaaaat atcactgcag gaccacctgg acaaagctaa gaaggatatt 360
acattctaca tgccagtgga caatctcgga atggctgtga ttgactggga ggagtggaga 420
cctacttggg cccgaaattg gaagcccaag gatgtgtata agaaccgatc cattgagctg 480
gtgcagcagc agaacgtgca gctgtccctt acagaagcca ccgaaaaagc taagcaggaa 540
ttcgagaaag ctgggaagga tttcctggtt gagacaataa aacttgggaa actgcttaga 600
cctaaccacc tctggggcta ttatttgttc cccgattgct acaatcacca ctataagaag 660
cccggctaca acggatcttg tttcaacgtc gaaattaaaa gaaacgatga cctctcttgg 720
ttgtggaatg agtctactgc actctacccc tctatatatc tcaacacaca gcagtcccca 780
gtagccgcta ccctctacgt tcggaataga gtgagggaag ccatcagggt gtccaagatc 840
cccgatgcca aatctcccct cccagtgttt gcctacacca ggatcgtgtt taccgaccag 900
gtgttgaagt tcctgagcca ggacgagctc gtgtatactt ttggcgagac agttgctctg 960
ggagcctctg ggatcgtgat ctggggcact ctgagtatta tgcgatcaat gaagtcctgc 1020
ctgctgttgg ataactacat ggagacaatc ctgaacccat atatcattaa cgtgactttg 1080
gccgctaaga tgtgttcaca ggtgctgtgt caagagcagg gagtatgcat taggaagaac 1140
tggaactcaa gcgattatct ccatctgaac ccagataatt ttgcaatcca gctggaaaag 1200
ggtggtaaat tcactgtccg agggaagccc acactcgagg atctcgaaca gtttagtgaa 1260
aagttctact gtagctgcta tagcactctg agttgtaagg aaaaagccga tgtgaaagac 1320
actgatgccg ttgacgtttg catcgccgat ggggtgtgta ttgacgcctt tctcaagcca 1380
ccaatggagt gataaggatc c                                          1401
```

<210> 169
<211> 1398
<212> DNA
<213> Artificial Sequence

<220>
<223> SPAM1-KPPM codon optimized DNA with NheI and BamHI restriction sites

<400> 169

```
gctagcatgg acatgagggt accagcccag ctgttgggcc ttcttctgct ttggctccct 60
ggggcaaggg gacttaactt cagagctccc cctgtaatcc caaacgtccc cttcctgtgg 120
gcatggaacg cccctagcga attctgtttg gggaaatttg acgaacctct ggacatgtct 180
ctgttttctt ttatcggtag ccctcgaatc aacgctaccg gacaggggtg gacaatcttt 240
tacgtggatc gtctgggcta ttacccttac atcgacagca ttactggggt gaccgtgaac 300
ggaggcattc ctcagaaaat atcactgcag gaccacctgg acaaagctaa gaaggatatt 360
acattctaca tgccagtgga caatctcgga atggctgtga ttgactggga ggagtggaga 420
cctacttggg cccgaaattg gaagcccaag gatgtgtata agaaccgatc cattgagctg 480
gtgcagcagc agaacgtgca gctgtccctt acagaagcca ccgaaaaagc taagcaggaa 540
ttcgagaaag ctgggaagga tttcctggtt gagacaataa aacttgggaa actgcttaga 600
cctaaccacc tctggggcta ttatttgttc cccgattgct acaatcacca ctataagaag 660
cccggctaca acggatcttg tttcaacgtc gaaattaaaa gaaacgatga cctctcttgg 720
ttgtggaatg agtctactgc actctacccc tctatatatc tcaacacaca gcagtcccca 780
gtagccgcta ccctctacgt tcggaataga gtgagggaag ccatcagggt gtccaagatc 840
cccgatgcca aatctcccct cccagtgttt gcctacacca ggatcgtgtt taccgaccag 900
gtgttgaagt tcctgagcca ggacgagctc gtgtatactt ttggcgagac agttgctctg 960
ggagcctctg ggatcgtgat ctggggcact ctgagtatta tgcgatcaat gaagtcctgc 1020
ctgctgttgg ataactacat ggagacaatc ctgaacccat atatcattaa cgtgactttg 1080
gccgctaaga tgtgttcaca ggtgctgtgt caagagcagg gagtatgcat taggaagaac 1140
tggaactcaa gcgattatct ccatctgaac ccagataatt ttgcaatcca gctggaaaag 1200
ggtggtaaat tcactgtccg agggaagccc acactcgagg atctcgaaca gtttagtgaa 1260
aagttctact gtagctgcta tagcactctg agttgtaagg aaaaagccga tgtgaaagac 1320
actgatgccg ttgacgtttg catcgccgat ggggtgtgta ttgacgcctt tctcaagcca 1380
ccaatgtgat aaggatcc                                          1398
```

<210> 170
<211> 1395
<212> DNA
<213> Artificial Sequence

<220>
<223> SPAM1-LKPP codon optimized DNA with NheI and BamHI restriction sites

<400> 170

```
gctagcatgg acatgagggt accagcccag ctgttgggcc ttcttctgct ttggctccct 60
ggggcaaggg gacttaactt cagagctccc cctgtaatcc caaacgtccc cttcctgtgg 120
gcatggaacg cccctagcga attctgtttg gggaaatttg acgaacctct ggacatgtct 180
ctgttttctt ttatcggtag ccctcgaatc aacgctaccg gacagggggt gacaatcttt 240
tacgtggatc gtctgggcta ttacccttac atcgacagca ttactggggt gaccgtgaac 300
ggaggcattc ctcagaaaat atcactgcag gaccacctgg acaaagctaa gaaggatatt 360
acattctaca tgccagtgga caatctcgga atggctgtga ttgactggga ggagtggaga 420
cctacttggg cccgaaattg gaagcccaag gatgtgtata agaaccgatc cattgagctg 480
gtgcagcagc agaacgtgca gctgtccctt acagaagcca ccgaaaaagc taagcaggaa 540
ttcgagaaag ctgggaagga tttcctggtt gagacaataa aacttgggaa actgcttaga 600
cctaaccacc tctggggcta ttatttgttc cccgattgct acaatcacca ctataagaag 660
cccggctaca acggatcttg tttcaacgtc gaaattaaaa gaaacgatga cctctcttgg 720
ttgtggaatg agtctactgc actctacccc tctatatatc tcaacacaca gcagtcccca 780
gtagccgcta ccctctacgt tcggaataga gtgagggaag ccatcagggt gtccaagatc 840
cccgatgcca aatctccccc cccagtgttt gcctacacca ggatcgtgtt taccgaccag 900
gtgttgaagt tcctgagcca ggacgagctc gtgtatactt ttggcgagac agttgctctg 960
ggagcctctg ggatcgtgat ctggggcact ctgagtatta tgcgatcaat gaagtcctgc 1020
ctgctgttgg ataactacat ggagacaatc ctgaacccat atatcattaa cgtgactttg 1080
gccgctaaga tgtgttcaca ggtgctgtgt caagagcagg gagtatgcat taggaagaac 1140
tggaactcaa gcgattatct ccatctgaac ccagataatt ttgcaatcca gctggaaaag 1200
ggtggtaaat tcactgtccg agggaagccc acactcgagg atctcgaaca gtttagtgaa 1260
aagttctact gtagctgcta tagcactctg agttgtaagg aaaaagccga tgtgaaagac 1320
actgatgccg ttgacgtttg catcgccgat ggggtgtgta ttgacgcctt tctcaagcca 1380
ccatgataag gatcc                                                  1395
```

<210> 171
<211> 1392
<212> DNA
<213> Artificial Sequence

<220>
<223> SPAM1-FLKP codon optimized DNA with NheI and BamHI restriction sites

<400> 171

```
gctagcatgg acatgagggt accagcccag ctgttgggcc ttcttctgct ttggctccct 60
ggggcaaggg gacttaactt cagagctccc cctgtaatcc caaacgtccc cttcctgtgg 120
gcatggaacg cccctagcga attctgtttg gggaaatttg acgaacctct ggacatgtct 180
ctgttttctt ttatcggtag ccctcgaatc aacgctaccg gacaggggtg gacaatcttt 240
tacgtggatc gtctgggcta ttacccttac atcgacagca ttactggggt gaccgtgaac 300
ggaggcattc ctcagaaaat atcactgcag gaccacctgg acaaagctaa gaaggatatt 360
acattctaca tgccagtgga caatctcgga atggctgtga ttgactggga ggagtggaga 420
cctacttggg cccgaaattg gaagcccaag gatgtgtata agaaccgatc cattgagctg 480
gtgcagcagc agaacgtgca gctgtccctt acagaagcca ccgaaaaagc taagcaggaa 540
ttcgagaaag ctgggaagga tttcctggtt gagacaataa aacttgggaa actgcttaga 600
cctaaccacc tctggggcta ttatttgttc cccgattgct acaatcacca ctataagaag 660
cccggctaca acggatcttg tttcaacgtc gaaattaaaa gaaacgatga cctctcttgg 720
ttgtggaatg agtctactgc actctacccc tctatatatc tcaacacaca gcagtcccca 780
gtagccgcta ccctctacgt tcggaataga gtgagggaag ccatcagggt gtccaagatc 840
cccgatgcca aatctccccc cccagtgttt gcctacacca ggatcgtgtt taccgaccag 900
gtgttgaagt tcctgagcca ggacgagctc gtgtatactt ttggcgagac agttgctctg 960
ggagcctctg ggatcgtgat ctggggcact ctgagtatta tgcgatcaat gaagtcctgc 1020
ctgctgttgg ataactacat ggagacaatc ctgaacccat atatcattaa cgtgactttg 1080
gccgctaaga tgtgttcaca ggtgctgtgt caagagcagg gagtatgcat taggaagaac 1140
tggaactcaa gcgattatct ccatctgaac ccagataatt ttgcaatcca gctggaaaag 1200
ggtggtaaat tcactgtccg agggaagccc acactcgagg atctcgaaca gtttagtgaa 1260
aagttctact gtagctgcta tagcactctg agttgtaagg aaaaagccga tgtgaaagac 1320
actgatgccg ttgacgtttg catcgccgat ggggtgtgta ttgacgcctt tctcaagcca 1380
tgataaggat cc                                                    1392
```

<210> 172
<211> 1389
<212> DNA
<213> Artificial Sequence

<220>
<223> SPAM1-AFLK codon optimized DNA with NheI and BamHI restriction sites

<400> 172

```
gctagcatgg acatgagggt accagcccag ctgttgggcc ttcttctgct ttggctccct 60
ggggcaaggg gacttaactt cagagctccc cctgtaatcc caaacgtccc cttcctgtgg 120
gcatggaacg cccctagcga attctgtttg gggaaatttg acgaacctct ggacatgtct 180
ctgttttctt ttatcggtag ccctcgaatc aacgctaccg gacaggggtg gacaatcttt 240
tacgtggatc gtctgggcta ttacccttac atcgacagca ttactggggt gaccgtgaac 300
ggaggcattc ctcagaaaat atcactgcag gaccacctgg acaaagctaa gaaggatatt 360
acattctaca tgccagtgga caatctcgga atggctgtga ttgactggga ggagtggaga 420
cctacttggg cccgaaattg gaagcccaag gatgtgtata agaaccgatc cattgagctg 480
gtgcagcagc agaacgtgca gctgtccctt acagaagcca ccgaaaaagc taagcaggaa 540
ttcgagaaag ctgggaagga tttcctggtt gagacaataa aacttgggaa actgcttaga 600
cctaaccacc tctggggcta ttatttgttc cccgattgct acaatcacca ctataagaag 660
cccggctaca acggatcttg tttcaacgtc gaaattaaaa gaaacgatga cctctcttgg 720
ttgtggaatg agtctactgc actctacccc tctatatatc tcaacacaca gcagtcccca 780
gtagccgcta ccctctacgt tcggaataga gtgagggaag ccatcagggt gtccaagatc 840
cccgatgcca aatctccccc cccagtgttt gcctacacca ggatcgtgtt taccgaccag 900
gtgttgaagt tcctgagcca ggacgagctc gtgtatactt ttggcgagac agttgctctg 960
ggagcctctg ggatcgtgat ctggggcact ctgagtatta tgcgatcaat gaagtcctgc 1020
ctgctgttgg ataactacat ggagacaatc ctgaacccat atatcattaa cgtgactttg 1080
gccgctaaga tgtgttcaca ggtgctgtgt caagagcagg gagtatgcat taggaagaac 1140
tggaactcaa gcgattatct ccatctgaac ccagataatt ttgcaatcca gctggaaaag 1200

ggtggtaaat tcactgtccg agggaagccc acactcgagg atctcgaaca gtttagtgaa 1260
aagttctact gtagctgcta tagcactctg agttgtaagg aaaaagccga tgtgaaagac 1320
actgatgccg ttgacgtttg catcgccgat ggggtgtgta ttgacgcctt tctcaagtga 1380
taaggatcc                                                        1389
```

<210> 173
<211> 1386
<212> DNA
<213> Artificial Sequence

<220>
<223> SPAM1-DAFL codon optimized DNA with NheI and BamHI restriction sites

<400> 173

```
gctagcatgg acatgagggt accagcccag ctgttgggcc ttcttctgct ttggctccct 60
ggggcaaggg gacttaactt cagagctccc cctgtaatcc caaacgtccc cttcctgtgg 120
gcatggaacg cccctagcga attctgtttg gggaaatttg acgaacctct ggacatgtct 180
ctgttttctt ttatcggtag ccctcgaatc aacgctaccg gacagggggt gacaatcttt 240
tacgtggatc gtctgggcta ttacccttac atcgacagca ttactggggt gaccgtgaac 300
ggaggcattc ctcagaaaat atcactgcag gaccacctgg acaaagctaa gaaggatatt 360
acattctaca tgccagtgga caatctcgga atggctgtga ttgactggga ggagtggaga 420
cctacttggg cccgaaattg gaagcccaag gatgtgtata agaaccgatc cattgagctg 480
gtgcagcagc agaacgtgca gctgtccctt acagaagcca ccgaaaaagc taagcaggaa 540
ttcgagaaag ctgggaagga tttcctggtt gagacaataa aacttgggaa actgcttaga 600
cctaaccacc tctggggcta ttatttgttc cccgattgct acaatcacca ctataagaag 660
cccggctaca acggatcttg tttcaacgtc gaaattaaaa gaaacgatga cctctcttgg 720
ttgtggaatg agtctactgc actctacccc tctatatatc tcaacacaca gcagtcccca 780
gtagccgcta ccctctacgt tcggaataga gtgagggaag ccatcagggt gtccaagatc 840
cccgatgcca aatctccccc cccagtgttt gcctacacca ggatcgtgtt taccgaccag 900
gtgttgaagt tcctgagcca ggacgagctc gtgtatactt ttggcgagac agttgctctg 960
ggagcctctg ggatcgtgat ctggggcact ctgagtatta tgcgatcaat gaagtcctgc 1020
ctgctgttgg ataactacat ggagacaatc ctgaacccat atatcattaa cgtgactttg 1080
gccgctaaga tgtgttcaca ggtgctgtgt caagagcagg gagtatgcat taggaagaac 1140
tggaactcaa gcgattatct ccatctgaac ccagataatt ttgcaatcca gctggaaaag 1200
ggtggtaaat tcactgtccg agggaagccc acactcgagg atctcgaaca gtttagtgaa 1260
aagttctact gtagctgcta tagcactctg agttgtaagg aaaaagccga tgtgaaagac 1320
actgatgccg ttgacgtttg catcgccgat ggggtgtgta ttgacgcctt tctctgataa 1380
ggatcc                                                           1386
```

<210> 174
<211> 1383
<212> DNA
<213> Artificial Sequence

<220>
<223> SPAM1-IDAF codon optimized DNA with NheI and BamHI restriction sites

<400> 174

```
gctagcatgg acatgagggt accagcccag ctgttgggcc ttcttctgct ttggctccct 60
ggggcaaggg gacttaactt cagagctccc cctgtaatcc caaacgtccc cttcctgtgg 120
gcatggaacg cccctagcga attctgtttg gggaaatttg acgaacctct ggacatgtct 180
ctgttttctt ttatcggtag ccctcgaatc aacgctaccg gacagggggt gacaatcttt 240
tacgtggatc gtctgggcta ttacccttac atcgacagca ttactggggt gaccgtgaac 300
ggaggcattc ctcagaaaat atcactgcag gaccacctgg acaaagctaa gaaggatatt 360
acattctaca tgccagtgga caatctcgga atggctgtga ttgactggga ggagtggaga 420
cctacttggg cccgaaattg gaagcccaag gatgtgtata agaaccgatc cattgagctg 480
gtgcagcagc agaacgtgca gctgtccctt acagaagcca ccgaaaaagc taagcaggaa 540
ttcgagaaag ctgggaagga tttcctggtt gagacaataa aacttgggaa actgcttaga 600
cctaaccacc tctggggcta ttatttgttc cccgattgct acaatcacca ctataagaag 660
cccggctaca acggatcttg tttcaacgtc gaaattaaaa gaaacgatga cctctcttgg 720
ttgtggaatg agtctactgc actctacccc tctatatatc tcaacacaca gcagtcccca 780
```

gtagccgcta ccctctacgt tcggaataga gtgagggaag ccatcagggt gtccaagatc 840
cccgatgcca aatctcccct cccagtgttt gcctacacca ggatcgtgtt taccgaccag 900
gtgttgaagt tcctgagcca ggacgagctc gtgtatactt ttggcgagac agttgctctg 960
ggagcctctg ggatcgtgat ctggggcact ctgagtatta tgcgatcaat gaagtcctgc 1020
ctgctgttgg ataactacat ggagacaatc ctgaacccat atatcattaa cgtgactttg 1080
gccgctaaga tgtgttcaca ggtgctgtgt caagagcagg gagtatgcat taggaagaac 1140
tggaactcaa gcgattatct ccatctgaac ccagataatt ttgcaatcca gctggaaaag 1200
ggtggtaaat tcactgtccg agggaagccc acactcgagg atctcgaaca gtttagtgaa 1260
aagttctact gtagctgcta tagcactctg agttgtaagg aaaaagccga tgtgaaagac 1320
actgatgccg ttgacgtttg catcgccgat ggggtgtgta ttgacgcctt ttgataagga 1380
tcc 1383

<210> 175
<211> 1380
<212> DNA
<213> Artificial Sequence

<220>
<223> SPAM1-CIDA codon optimized DNA with NheI and BamHI restriction sites

<400> 175

gctagcatgg acatgagggt accagcccag ctgttgggcc ttcttctgct ttggctccct 60
ggggcaaggg gacttaactt cagagctccc cctgtaatcc caaacgtccc cttcctgtgg 120
gcatggaacg cccctagcga attctgtttg gggaaatttg acgaacctct ggacatgtct 180
ctgttttctt ttatcggtag ccctcgaatc aacgctaccg gacaggggtt gacaatcttt 240
tacgtggatc gtctgggcta ttacccttac atcgacagca ttactggggt gaccgtgaac 300
ggaggcattc ctcagaaaat atcactgcag gaccacctgg acaaagctaa gaaggatatt 360
acattctaca tgccagtgga caatctcgga atggctgtga ttgactggga ggagtggaga 420
cctacttggg cccgaaattg gaagcccaag gatgtgtata agaaccgatc cattgagctg 480
gtgcagcagc agaacgtgca gctgtccctt acagaagcca ccgaaaaagc taagcaggaa 540
ttcgagaaag ctgggaagga tttcctggtt gagacaataa aacttgggaa actgcttaga 600
cctaaccacc tctggggcta ttatttgttc cccgattgct acaatcacca ctataagaag 660
cccggctaca acggatcttg tttcaacgtc gaaattaaaa gaaacgatga cctctcttgg 720
ttgtggaatg agtctactgc actctacccc tctatatatc tcaacacaca gcagtcccca 780
gtagccgcta ccctctacgt tcggaataga gtgagggaag ccatcagggt gtccaagatc 840
cccgatgcca aatctcccct cccagtgttt gcctacacca ggatcgtgtt taccgaccag 900
gtgttgaagt tcctgagcca ggacgagctc gtgtatactt ttggcgagac agttgctctg 960
ggagcctctg ggatcgtgat ctggggcact ctgagtatta tgcgatcaat gaagtcctgc 1020
ctgctgttgg ataactacat ggagacaatc ctgaacccat atatcattaa cgtgactttg 1080
gccgctaaga tgtgttcaca ggtgctgtgt caagagcagg gagtatgcat taggaagaac 1140
tggaactcaa gcgattatct ccatctgaac ccagataatt ttgcaatcca gctggaaaag 1200
ggtggtaaat tcactgtccg agggaagccc acactcgagg atctcgaaca gtttagtgaa 1260
aagttctact gtagctgcta tagcactctg agttgtaagg aaaaagccga tgtgaaagac 1320
actgatgccg ttgacgtttg catcgccgat ggggtgtgta ttgacgcctg ataaggatcc 1380

<210> 176
<211> 1377
<212> DNA
<213> Artificial Sequence

<220>
<223> SPAM1-VCID codon optimized DNA with NheI and BamHI restriction sites

<400> 176

```
gctagcatgg acatgagggt accagcccag ctgttgggcc ttcttctgct ttggctccct 60
ggggcaaggg gacttaactt cagagctccc cctgtaatcc caaacgtccc cttcctgtgg 120
gcatggaacg cccctagcga attctgtttg gggaaatttg acgaacctct ggacatgtct 180
ctgttttctt ttatcggtag ccctcgaatc aacgctaccg gacagggggt gacaatcttt 240
tacgtggatc gtctgggcta ttacccttac atcgacagca ttactggggt gaccgtgaac 300
ggaggcattc ctcagaaaat atcactgcag gaccacctgg acaaagctaa gaaggatatt 360

acattctaca tgccagtgga caatctcgga atggctgtga ttgactggga ggagtggaga 420
cctacttggg cccgaaattg gaagcccaag gatgtgtata agaaccgatc cattgagctg 480
gtgcagcagc agaacgtgca gctgtccctt acagaagcca ccgaaaaagc taagcaggaa 540
ttcgagaaag ctgggaagga tttcctggtt gagacaataa aacttgggaa actgcttaga 600
cctaaccacc tctggggcta ttatttgttc cccgattgct acaatcacca ctataagaag 660
cccggctaca acggatcttg tttcaacgtc gaaattaaaa gaaacgatga cctctcttgg 720
ttgtggaatg agtctactgc actctacccc tctatatatc tcaacacaca gcagtcccca 780
gtagccgcta ccctctacgt tcggaataga gtgagggaag ccatcagggt gtccaagatc 840
cccgatgcca aatctcccct cccagtgttt gcctacacca ggatcgtgtt taccgaccag 900
gtgttgaagt tcctgagcca ggacgagctc gtgtatactt ttggcgagac agttgctctg 960
ggagcctctg ggatcgtgat ctggggcact ctgagtatta tgcgatcaat gaagtcctgc 1020
ctgctgttgg ataactacat ggagacaatc ctgaacccat atatcattaa cgtgactttg 1080
gccgctaaga tgtgttcaca ggtgctgtgt caagagcagg gagtatgcat taggaagaac 1140
tggaactcaa gcgattatct ccatctgaac ccagataatt ttgcaatcca gctggaaaag 1200
ggtggtaaat tcactgtccg agggaagccc acactcgagg atctcgaaca gtttagtgaa 1260
aagttctact gtagctgcta tagcactctg agttgtaagg aaaaagccga tgtgaaagac 1320
actgatgccg ttgacgtttg catcgccgat ggggtgtgta ttgactgata aggatcc    1377
```

<210> 177
<211> 1374
<212> DNA
<213> Artificial Sequence

<220>
<223> SPAM1-GVCI codon optimized DNA with NheI and BamHI restriction sites

<400> 177

```
gctagcatgg acatgagggt accagcccag ctgttgggcc ttcttctgct ttggctccct 60
ggggcaaggg gacttaactt cagagctccc cctgtaatcc caaacgtccc cttcctgtgg 120
gcatggaacg cccctagcga attctgtttg gggaaatttg acgaacctct ggacatgtct 180
ctgttttctt ttatcggtag ccctcgaatc aacgctaccg gacagggggt gacaatcttt 240
tacgtggatc gtctgggcta ttacccttac atcgacagca ttactggggt gaccgtgaac 300
ggaggcattc ctcagaaaat atcactgcag gaccacctgg acaaagctaa gaaggatatt 360
acattctaca tgccagtgga caatctcgga atggctgtga ttgactggga ggagtggaga 420
cctacttggg cccgaaattg gaagcccaag gatgtgtata agaaccgatc cattgagctg 480
gtgcagcagc agaacgtgca gctgtccctt acagaagcca ccgaaaaagc taagcaggaa 540
ttcgagaaag ctgggaagga tttcctggtt gagacaataa aacttgggaa actgcttaga 600
cctaaccacc tctggggcta ttatttgttc cccgattgct acaatcacca ctataagaag 660
cccggctaca acggatcttg tttcaacgtc gaaattaaaa gaaacgatga cctctcttgg 720
ttgtggaatg agtctactgc actctacccc tctatatatc tcaacacaca gcagtcccca 780
gtagccgcta ccctctacgt tcggaataga gtgagggaag ccatcagggt gtccaagatc 840
cccgatgcca aatctcccct cccagtgttt gcctacacca ggatcgtgtt taccgaccag 900
gtgttgaagt tcctgagcca ggacgagctc gtgtatactt ttggcgagac agttgctctg 960
ggagcctctg ggatcgtgat ctggggcact ctgagtatta tgcgatcaat gaagtcctgc 1020
ctgctgttgg ataactacat ggagacaatc ctgaacccat atatcattaa cgtgactttg 1080
gccgctaaga tgtgttcaca ggtgctgtgt caagagcagg gagtatgcat taggaagaac 1140
tggaactcaa gcgattatct ccatctgaac ccagataatt ttgcaatcca gctggaaaag 1200
ggtggtaaat tcactgtccg agggaagccc acactcgagg atctcgaaca gtttagtgaa 1260
aagttctact gtagctgcta tagcactctg agttgtaagg aaaaagccga tgtgaaagac 1320
actgatgccg ttgacgtttg catcgccgat ggggtgtgta tttgataagg atcc       1374
```

<210> 178
<211> 1371
<212> DNA
<213> Artificial Sequence

<220>
<223> SPAM1-DGVC codon optimized DNA with NheI and BamHI restriction sites

<400> 178

```
gctagcatgg acatgagggt accagcccag ctgttgggcc ttcttctgct ttggctccct 60

ggggcaaggg gacttaactt cagagctccc cctgtaatcc caaacgtccc cttcctgtgg 120
gcatggaacg cccctagcga attctgtttg gggaaatttg acgaacctct ggacatgtct 180
ctgttttctt ttatcggtag ccctcgaatc aacgctaccg gacagggggt gacaatcttt 240
tacgtggatc gtctgggcta ttacccttac atcgacagca ttactggggt gaccgtgaac 300
ggaggcattc ctcagaaaat atcactgcag gaccacctgg acaaagctaa gaaggatatt 360
acattctaca tgccagtgga caatctcgga atggctgtga ttgactggga ggagtggaga 420
cctacttggg cccgaaattg gaagcccaag gatgtgtata agaaccgatc cattgagctg 480
gtgcagcagc agaacgtgca gctgtccctt acagaagcca ccgaaaaagc taagcaggaa 540
ttcgagaaag ctgggaagga tttcctggtt gagacaataa aacttgggaa actgcttaga 600
cctaaccacc tctggggcta ttatttgttc cccgattgct acaatcacca ctataagaag 660
cccggctaca acggatcttg tttcaacgtc gaaattaaaa gaaacgatga cctctcttgg 720
ttgtggaatg agtctactgc actctacccc tctatatatc tcaacacaca gcagtcccca 780
gtagccgcta ccctctacgt tcggaataga gtgagggaag ccatcagggt gtccaagatc 840
cccgatgcca aatctccccc cccagtgttt gcctacacca ggatcgtgtt taccgaccag 900
gtgttgaagt tcctgagcca ggacgagctc gtgtatactt ttggcgagac agttgctctg 960
ggagcctctg ggatcgtgat ctggggcact ctgagtatta tgcgatcaat gaagtcctgc 1020
ctgctgttgg ataactacat ggagacaatc ctgaacccat atatcattaa cgtgactttg 1080
gccgctaaga tgtgttcaca ggtgctgtgt caagagcagg gagtatgcat taggaagaac 1140
tggaactcaa gcgattatct ccatctgaac ccagataatt ttgcaatcca gctggaaaag 1200
ggtggtaaat tcactgtccg agggaagccc acactcgagg atctcgaaca gtttagtgaa 1260
aagttctact gtagctgcta tagcactctg agttgtaagg aaaaagccga tgtgaaagac 1320
actgatgccg ttgacgtttg catcgccgat ggggtgtgtt gataaggatc c          1371
```

<210> 179
<211> 1365
<212> DNA
<213> Artificial Sequence

<220>
<223> SPAM1-IADG codon optimized DNA with NheI and BamHI restriction sites

<400> 179

gctagcatgg acatgagggt accagcccag ctgttgggcc ttcttctgct ttggctccct 60
ggggcaaggg gacttaactt cagagctccc cctgtaatcc caaacgtccc cttcctgtgg 120
gcatggaacg cccctagcga attctgtttg gggaaatttg acgaacctct ggacatgtct 180
ctgttttctt ttatcggtag ccctcgaatc aacgctaccg gacaggggtg gacaatcttt 240
tacgtggatc gtctgggcta ttacccttac atcgacagca ttactggggt gaccgtgaac 300
ggaggcattc ctcagaaaat atcactgcag gaccacctgg acaaagctaa gaaggatatt 360
acattctaca tgccagtgga caatctcgga atggctgtga ttgactggga ggagtggaga 420
cctacttggg cccgaaattg gaagcccaag gatgtgtata agaaccgatc cattgagctg 480
gtgcagcagc agaacgtgca gctgtccctt acagaagcca ccgaaaaagc taagcaggaa 540
ttcgagaaag ctgggaagga tttcctggtt gagacaataa aacttgggaa actgcttaga 600
cctaaccacc tctggggcta ttatttgttc cccgattgct acaatcacca ctataagaag 660
cccggctaca acggatcttg tttcaacgtc gaaattaaaa gaaacgatga cctctcttgg 720
ttgtggaatg agtctactgc actctacccc tctatatatc tcaacacaca gcagtcccca 780
gtagccgcta ccctctacgt tcggaataga gtgagggaag ccatcagggt gtccaagatc 840
cccgatgcca aatctcccct cccagtgttt gcctacacca ggatcgtgtt taccgaccag 900
gtgttgaagt tcctgagcca ggacgagctc gtgtatactt ttggcgagac agttgctctg 960
ggagcctctg ggatcgtgat ctggggcact ctgagtatta tgcgatcaat gaagtcctgc 1020
ctgctgttgg ataactacat ggagacaatc ctgaacccat atatcattaa cgtgactttg 1080
gccgctaaga tgtgttcaca ggtgctgtgt caagagcagg gagtatgcat taggaagaac 1140
tggaactcaa gcgattatct ccatctgaac ccagataatt ttgcaatcca gctggaaaag 1200
ggtggtaaat tcactgtccg agggaagccc acactcgagg atctcgaaca gtttagtgaa 1260
aagttctact gtagctgcta tagcactctg agttgtaagg aaaaagccga tgtgaaagac 1320
actgatgccg ttgacgtttg catcgccgat gggtgataag gatcc 1365

<210> 180
<211> 1359
<212> DNA
<213> Artificial Sequence

<220>
<223> SPAM1-VCIA codon optimized DNA with NheI and BamHI restriction sites

<400> 180

gctagcatgg acatgagggt accagcccag ctgttgggcc ttcttctgct ttggctccct 60
ggggcaaggg gacttaactt cagagctccc cctgtaatcc caaacgtccc cttcctgtgg 120
gcatggaacg cccctagcga attctgtttg gggaaatttg acgaacctct ggacatgtct 180
ctgttttctt ttatcggtag ccctcgaatc aacgctaccg gacaggggtg gacaatcttt 240
tacgtggatc gtctgggcta ttacccttac atcgacagca ttactggggt gaccgtgaac 300
ggaggcattc ctcagaaaat atcactgcag gaccacctgg acaaagctaa gaaggatatt 360
acattctaca tgccagtgga caatctcgga atggctgtga ttgactggga ggagtggaga 420
cctacttggg cccgaaattg gaagcccaag gatgtgtata agaaccgatc cattgagctg 480
gtgcagcagc agaacgtgca gctgtccctt acagaagcca ccgaaaaagc taagcaggaa 540
ttcgagaaag ctgggaagga tttcctggtt gagacaataa aacttgggaa actgcttaga 600
cctaaccacc tctggggcta ttatttgttc cccgattgct acaatcacca ctataagaag 660
cccggctaca acggatcttg tttcaacgtc gaaattaaaa gaaacgatga cctctcttgg 720
ttgtggaatg agtctactgc actctacccc tctatatatc tcaacacaca gcagtcccca 780
gtagccgcta ccctctacgt tcggaataga gtgagggaag ccatcagggt gtccaagatc 840
cccgatgcca aatctcccct cccagtgttt gcctacacca ggatcgtgtt taccgaccag 900
gtgttgaagt tcctgagcca ggacgagctc gtgtatactt ttggcgagac agttgctctg 960
ggagcctctg ggatcgtgat ctggggcact ctgagtatta tgcgatcaat gaagtcctgc 1020
ctgctgttgg ataactacat ggagacaatc ctgaacccat atatcattaa cgtgactttg 1080
gccgctaaga tgtgttcaca ggtgctgtgt caagagcagg gagtatgcat taggaagaac 1140
tggaactcaa gcgattatct ccatctgaac ccagataatt ttgcaatcca gctggaaaag 1200
ggtggtaaat tcactgtccg agggaagccc acactcgagg atctcgaaca gtttagtgaa 1260
aagttctact gtagctgcta tagcactctg agttgtaagg aaaaagccga tgtgaaagac 1320
actgatgccg ttgacgtttg catcgcctga taaggatcc 1359

<210> 181
<211> 1353

<212> DNA
<213> Artificial Sequence

<220>
<223> SPAM1-VDVC codon optimized DNA with NheI and BamHI restriction sites

<400> 181

```
gctagcatgg acatgagggt accagcccag ctgttgggcc ttcttctgct ttggctccct 60
ggggcaaggg gacttaactt cagagctccc cctgtaatcc caaacgtccc cttcctgtgg 120
gcatggaacg cccctagcga attctgtttg gggaaatttg acgaacctct ggacatgtct 180
ctgttttctt ttatcggtag ccctcgaatc aacgctaccg gacagggggt gacaatcttt 240
tacgtggatc gtctgggcta ttacccttac atcgacagca ttactggggt gaccgtgaac 300
ggaggcattc ctcagaaaat atcactgcag gaccacctgg acaaagctaa gaaggatatt 360
acattctaca tgccagtgga caatctcgga atggctgtga ttgactggga ggagtggaga 420
cctacttggg cccgaaattg gaagcccaag gatgtgtata agaaccgatc cattgagctg 480
gtgcagcagc agaacgtgca gctgtccctt acagaagcca ccgaaaaagc taagcaggaa 540
ttcgagaaag ctgggaagga tttcctggtt gagacaataa aacttgggaa actgcttaga 600
cctaaccacc tctggggcta ttatttgttc cccgattgct acaatcacca ctataagaag 660
cccggctaca acggatcttg tttcaacgtc gaaattaaaa gaaacgatga cctctcttgg 720
ttgtggaatg agtctactgc actctacccc tctatatatc tcaacacaca gcagtcccca 780
gtagccgcta ccctctacgt tcggaataga gtgagggaag ccatcagggt gtccaagatc 840
cccgatgcca aatctccccc cccagtgttt gcctacacca ggatcgtgtt taccgaccag 900
gtgttgaagt tcctgagcca ggacgagctc gtgtatactt ttggcgagac agttgctctg 960
ggagcctctg ggatcgtgat ctggggcact ctgagtatta tgcgatcaat gaagtcctgc 1020
ctgctgttgg ataactacat ggagacaatc ctgaacccat atatcattaa cgtgactttg 1080
gccgctaaga tgtgttcaca ggtgctgtgt caagagcagg gagtatgcat taggaagaac 1140
tggaactcaa gcgattatct ccatctgaac ccagataatt ttgcaatcca gctggaaaag 1200
ggtggtaaat tcactgtccg agggaagccc acactcgagg atctcgaaca gtttagtgaa 1260
aagttctact gtagctgcta tagcactctg agttgtaagg aaaaagccga tgtgaaagac 1320
actgatgccg ttgacgtttg ctgataagga tcc                              1353
```

<210> 182
<211> 1347
<212> DNA
<213> Artificial Sequence

<220>
<223> SPAM1-DAVD codon optimized DNA with NheI and BamHI restriction sites

<400> 182

gctagcatgg acatgagggt accagcccag ctgttgggcc ttcttctgct ttggctccct 60
ggggcaaggg gacttaactt cagagctccc cctgtaatcc caaacgtccc cttcctgtgg 120
gcatggaacg cccctagcga attctgtttg gggaaatttg acgaacctct ggacatgtct 180
ctgttttctt ttatcggtag ccctcgaatc aacgctaccg gacaggggtg gacaatcttt 240
tacgtggatc gtctgggcta ttacccttac atcgacagca ttactggggt gaccgtgaac 300
ggaggcattc ctcagaaaat atcactgcag gaccacctgg acaaagctaa gaaggatatt 360
acattctaca tgccagtgga caatctcgga atggctgtga ttgactggga ggagtggaga 420
cctacttggg cccgaaattg gaagcccaag gatgtgtata agaaccgatc cattgagctg 480
gtgcagcagc agaacgtgca gctgtccctt acagaagcca ccgaaaaagc taagcaggaa 540
ttcgagaaag ctgggaagga tttcctggtt gagacaataa aacttgggaa actgcttaga 600
cctaaccacc tctggggcta ttatttgttc cccgattgct acaatcacca ctataagaag 660
cccggctaca acggatcttg tttcaacgtc gaaattaaaa gaaacgatga cctctcttgg 720
ttgtggaatg agtctactgc actctacccc tctatatatc tcaacacaca gcagtcccca 780
gtagccgcta ccctctacgt tcggaataga gtgagggaag ccatcagggt gtccaagatc 840
cccgatgcca aatctcccct cccagtgttt gcctacacca ggatcgtgtt taccgaccag 900
gtgttgaagt tcctgagcca ggacgagctc gtgtatactt ttggcgagac agttgctctg 960
ggagcctctg ggatcgtgat ctggggcact ctgagtatta tgcgatcaat gaagtcctgc 1020
ctgctgttgg ataactacat ggagacaatc ctgaacccat atatcattaa cgtgactttg 1080
gccgctaaga tgtgttcaca ggtgctgtgt caagagcagg gagtatgcat taggaagaac 1140
tggaactcaa gcgattatct ccatctgaac ccagataatt ttgcaatcca gctggaaaag 1200
ggtggtaaat tcactgtccg agggaagccc acactcgagg atctcgaaca gtttagtgaa 1260
aagttctact gtagctgcta tagcactctg agttgtaagg aaaaagccga tgtgaaagac 1320
actgatgccg ttgactgata aggatcc 1347

<210> 183
<211> 1341
<212> DNA
<213> Artificial Sequence

<220>
<223> SPAM1-DTDA codon optimized DNA with NheI and BamHI restriction sites

<400> 183

gctagcatgg acatgagggt accagcccag ctgttgggcc ttcttctgct ttggctccct 60
ggggcaaggg gacttaactt cagagctccc cctgtaatcc caaacgtccc cttcctgtgg 120
gcatggaacg cccctagcga attctgtttg gggaaatttg acgaacctct ggacatgtct 180
ctgttttctt ttatcggtag ccctcgaatc aacgctaccg gacaggggtg gacaatcttt 240
tacgtggatc gtctgggcta ttacccttac atcgacagca ttactggggt gaccgtgaac 300
ggaggcattc ctcagaaaat atcactgcag gaccacctgg acaaagctaa gaaggatatt 360
acattctaca tgccagtgga caatctcgga atggctgtga ttgactggga ggagtggaga 420
cctacttggg cccgaaattg gaagcccaag gatgtgtata agaaccgatc cattgagctg 480
gtgcagcagc agaacgtgca gctgtccctt acagaagcca ccgaaaaagc taagcaggaa 540
ttcgagaaag ctgggaagga tttcctggtt gagacaataa aacttgggaa actgcttaga 600
cctaaccacc tctggggcta ttatttgttc cccgattgct acaatcacca ctataagaag 660
cccggctaca acggatcttg tttcaacgtc gaaattaaaa gaaacgatga cctctcttgg 720
ttgtggaatg agtctactgc actctacccc tctatatatc tcaacacaca gcagtcccca 780
gtagccgcta ccctctacgt tcggaataga gtgagggaag ccatcagggt gtccaagatc 840
cccgatgcca aatctcccct cccagtgttt gcctacacca ggatcgtgtt taccgaccag 900
gtgttgaagt tcctgagcca ggacgagctc gtgtatactt ttggcgagac agttgctctg 960
ggagcctctg ggatcgtgat ctggggcact ctgagtatta tgcgatcaat gaagtcctgc 1020
ctgctgttgg ataactacat ggagacaatc ctgaacccat atatcattaa cgtgactttg 1080
gccgctaaga tgtgttcaca ggtgctgtgt caagagcagg gagtatgcat taggaagaac 1140
tggaactcaa gcgattatct ccatctgaac ccagataatt ttgcaatcca gctggaaaag 1200

ggtggtaaat tcactgtccg agggaagccc acactcgagg atctcgaaca gtttagtgaa 1260
aagttctact gtagctgcta tagcactctg agttgtaagg aaaaagccga tgtgaaagac 1320
actgatgcct gataaggatc c 1341

<210> 184
<211> 1335
<212> DNA
<213> Artificial Sequence

<220>
<223> SPAM1-VKDT codon optimized DNA with NheI and BamHI restriction sites

<400> 184

```
gctagcatgg acatgagggt accagcccag ctgttgggcc ttcttctgct ttggctccct 60
ggggcaaggg gacttaactt cagagctccc cctgtaatcc caaacgtccc cttcctgtgg 120
gcatggaacg cccctagcga attctgtttg gggaaatttg acgaacctct ggacatgtct 180
ctgttttctt ttatcggtag ccctcgaatc aacgctaccg gacagggggt gacaatcttt 240
tacgtggatc gtctgggcta ttacccttac atcgacagca ttactggggt gaccgtgaac 300
ggaggcattc ctcagaaaat atcactgcag gaccacctgg acaaagctaa gaaggatatt 360
acattctaca tgccagtgga caatctcgga atggctgtga ttgactggga ggagtggaga 420
cctacttggg cccgaaattg gaagcccaag gatgtgtata agaaccgatc cattgagctg 480
gtgcagcagc agaacgtgca gctgtccctt acagaagcca ccgaaaaagc taagcaggaa 540
ttcgagaaag ctgggaagga tttcctggtt gagacaataa aacttgggaa actgcttaga 600
cctaaccacc tctggggcta ttatttgttc cccgattgct acaatcacca ctataagaag 660
cccggctaca acggatcttg tttcaacgtc gaaattaaaa gaaacgatga cctctcttgg 720
ttgtggaatg agtctactgc actctacccc tctatatatc tcaacacaca gcagtcccca 780
gtagccgcta ccctctacgt tcggaataga gtgagggaag ccatcagggt gtccaagatc 840
cccgatgcca aatctcccct cccagtgttt gcctacacca ggatcgtgtt taccgaccag 900
gtgttgaagt tcctgagcca ggacgagctc gtgtatactt ttggcgagac agttgctctg 960
ggagcctctg ggatcgtgat ctggggcact ctgagtatta tgcgatcaat gaagtcctgc 1020
ctgctgttgg ataactacat ggagacaatc ctgaacccat atatcattaa cgtgactttg 1080
gccgctaaga tgtgttcaca ggtgctgtgt caagagcagg gagtatgcat taggaagaac 1140
tggaactcaa gcgattatct ccatctgaac ccagataatt ttgcaatcca gctggaaaag 1200
ggtggtaaat tcactgtccg agggaagccc acactcgagg atctcgaaca gtttagtgaa 1260
aagttctact gtagctgcta tagcactctg agttgtaagg aaaaagccga tgtgaaagac 1320
acttgataag gatcc                                                  1335
```

<210> 185
<211> 1329
<212> DNA
<213> Artificial Sequence

<220>
<223> SPAM1-ADVK codon optimized DNA with NheI and BamHI restriction sites

<400> 185

```
gctagcatgg acatgagggt accagcccag ctgttgggcc ttcttctgct ttggctccct 60
ggggcaaggg gacttaactt cagagctccc cctgtaatcc caaacgtccc cttcctgtgg 120
gcatggaacg cccctagcga attctgtttg gggaaatttg acgaacctct ggacatgtct 180
ctgttttctt ttatcggtag ccctcgaatc aacgctaccg gacagggggt gacaatcttt 240
tacgtggatc gtctgggcta ttacccttac atcgacagca ttactggggt gaccgtgaac 300
ggaggcattc ctcagaaaat atcactgcag gaccacctgg acaaagctaa gaaggatatt 360
acattctaca tgccagtgga caatctcgga atggctgtga ttgactggga ggagtggaga 420
cctacttggg cccgaaattg gaagcccaag gatgtgtata agaaccgatc cattgagctg 480
gtgcagcagc agaacgtgca gctgtccctt acagaagcca ccgaaaaagc taagcaggaa 540
ttcgagaaag ctgggaagga tttcctggtt gagacaataa aacttgggaa actgcttaga 600
cctaaccacc tctggggcta ttatttgttc cccgattgct acaatcacca ctataagaag 660
cccggctaca acggatcttg tttcaacgtc gaaattaaaa gaaacgatga cctctcttgg 720
ttgtggaatg agtctactgc actctacccc tctatatatc tcaacacaca gcagtcccca 780
gtagccgcta ccctctacgt tcggaataga gtgagggaag ccatcagggt gtccaagatc 840
cccgatgcca aatctcccct cccagtgttt gcctacacca ggatcgtgtt taccgaccag 900
```

gtgttgaagt tcctgagcca ggacgagctc gtgtatactt ttggcgagac agttgctctg 960
ggagcctctg ggatcgtgat ctggggcact ctgagtatta tgcgatcaat gaagtcctgc 1020
ctgctgttgg ataactacat ggagacaatc ctgaacccat atatcattaa cgtgactttg 1080
gccgctaaga tgtgttcaca ggtgctgtgt caagagcagg gagtatgcat taggaagaac 1140
tggaactcaa gcgattatct ccatctgaac ccagataatt ttgcaatcca gctggaaaag 1200
ggtggtaaat tcactgtccg agggaagccc acactcgagg atctcgaaca gtttagtgaa 1260
aagttctact gtagctgcta tagcactctg agttgtaagg aaaaagccga tgtgaaatga 1320
taaggatcc 1329

<210> 186
<211> 35
<212> PRT
<213> Homo sapiens

<220>
<223> PH20 signal peptide

<400> 186

Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
1 5 10 15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
20 25 30
Cys Leu Thr
35

<210> 187
<211> 496
<212> PRT
<213> Artificial Sequence

<220>
<223> PH20sHis

<400> 187

```
Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1               5                  10                  15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
            20                  25                  30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
        35                  40                  45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
    50                  55                  60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
65                  70                  75                  80
Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
                85                  90                  95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
            100                 105                 110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
        115                 120                 125
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
    130                 135                 140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145                 150                 155                 160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
                165                 170                 175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
            180                 185                 190
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
        195                 200                 205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
    210                 215                 220
```

```
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
225                 230                 235                 240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
                245                 250                 255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
            260                 265                 270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
        275                 280                 285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
    290                 295                 300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
305                 310                 315                 320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
                325                 330                 335
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
            340                 345                 350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
        355                 360                 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
    370                 375                 380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
385                 390                 395                 400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
                405                 410                 415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
            420                 425                 430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
        435                 440                 445
Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
    450                 455                 460
Ile Asp Ala Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile
465                 470                 475                 480
Phe Tyr Asn Ala Ser Pro Ser Thr Leu Ser His His His His His His
                485                 490                 495
```

<210> 188
<211> 496
<212> PRT
<213> Artificial Sequence

<220>
<223> PH20sHis-N368A (mut1)

<400> 188

```
Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1               5                  10                  15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
            20                  25                  30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
        35                  40                  45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
    50                  55                  60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
65                  70                  75                  80
Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
                85                  90                  95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
            100                 105                 110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
        115                 120                 125
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
    130                 135                 140
```

```
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145                 150                 155                 160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
                165                 170                 175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
            180                 185                 190
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
        195                 200                 205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
    210                 215                 220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
225                 230                 235                 240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
                245                 250                 255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
            260                 265                 270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
        275                 280                 285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
    290                 295                 300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
305                 310                 315                 320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
                325                 330                 335
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
            340                 345                 350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Ala
        355                 360                 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
    370                 375                 380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
385                 390                 395                 400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
                405                 410                 415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
            420                 425                 430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
        435                 440                 445
Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
    450                 455                 460
Ile Asp Ala Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile
465                 470                 475                 480
Phe Tyr Asn Ala Ser Pro Ser Thr Leu Ser His His His His His His
                485                 490                 495
```

<210> 189
<211> 496
<212> PRT
<213> Artificial Sequence

<220>
<223> PH20sHis-N393A (mut2)

<400> 189

```
Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1               5                  10                  15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
            20                  25                  30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
        35                  40                  45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
    50                  55                  60
```

```
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
65                  70                  75                  80
Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
                85                  90                  95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
            100                 105                 110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
        115                 120                 125
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
    130                 135                 140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145                 150                 155                 160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
                165                 170                 175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
            180                 185                 190
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
        195                 200                 205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
    210                 215                 220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
225                 230                 235                 240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
                245                 250                 255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
            260                 265                 270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
        275                 280                 285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
    290                 295                 300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
305                 310                 315                 320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
                325                 330                 335
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
            340                 345                 350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
        355                 360                 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
    370                 375                 380
Gly Val Cys Ile Arg Lys Asn Trp Ala Ser Ser Asp Tyr Leu His Leu
385                 390                 395                 400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
                405                 410                 415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
            420                 425                 430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
        435                 440                 445
Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
    450                 455                 460
Ile Asp Ala Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile
465                 470                 475                 480
Phe Tyr Asn Ala Ser Pro Ser Thr Leu Ser His His His His His His
                485                 490                 495
```

<210> 190
<211> 496
<212> PRT
<213> Artificial Sequence

<220>

<223> PH20sHis-N368A/N393A (mut3)

<400> 190

```
Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1               5                  10                  15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
            20                  25                  30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
        35                  40                  45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
    50                  55                  60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
65                  70                  75                  80
Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
                85                  90                  95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
            100                 105                 110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
        115                 120                 125
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
    130                 135                 140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145                 150                 155                 160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
                165                 170                 175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
            180                 185                 190
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
        195                 200                 205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
    210                 215                 220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
225                 230                 235                 240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
                245                 250                 255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
            260                 265                 270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
        275                 280                 285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
    290                 295                 300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
305                 310                 315                 320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
                325                 330                 335
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
            340                 345                 350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Ala
        355                 360                 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
    370                 375                 380
Gly Val Cys Ile Arg Lys Asn Trp Ala Ser Ser Asp Tyr Leu His Leu
385                 390                 395                 400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
                405                 410                 415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
            420                 425                 430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
        435                 440                 445
Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
    450                 455                 460
Ile Asp Ala Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile
465                 470                 475                 480
Phe Tyr Asn Ala Ser Pro Ser Thr Leu Ser His His His His His His
```

485 490 495

<210> 191
<211> 17
<212> PRT
<213> Homo sapiens

<220>
<223> C-terminal peptide cleaved from rHuPH20 aa 431-447

<400> 191

Asp Ala Phe Lys Leu Pro Pro Met Glu Thr Glu Glu Pro Gln Ile Phe
1 5 10 15
Tyr

<210> 192
<211> 16
<212> PRT
<213> Homo sapiens

<220>
<223> C-terminal peptide cleaved from rHuPH20 aa 431-446

<400> 192

Asp Ala Phe Lys Leu Pro Pro Met Glu Thr Glu Glu Pro Gln Ile Phe
1 5 10 15

<210> 193
<211> 15
<212> PRT
<213> Homo sapiens

<220>
<223> C-terminal peptide cleaved from rHuPH20 aa 431-445

<400> 193

Asp Ala Phe Lys Leu Pro Pro Met Glu Thr Glu Glu Pro Gln Ile
1 5 10 15

<210> 194
<211> 14
<212> PRT
<213> Homo sapiens

<220>
<223> C-terminal peptide cleaved from rHuPH20 aa 431-444

<400> 194

Asp Ala Phe Lys Leu Pro Pro Met Glu Thr Glu Glu Pro Gln
1 5 10

<210> 195

<211> 13
<212> PRT
<213> Homo sapiens

<220>
<223> C-terminal peptide cleaved from rHuPH20 aa 431-443

<400> 195

Asp Ala Phe Lys Leu Pro Pro Met Glu Thr Glu Glu Pro
1 5 10

<210> 196
<211> 12
<212> PRT
<213> Artificial Sequence

<220>
<223> C-terminal peptide cleaved from rHuPH20 aa 431-442

<400> 196

Asp Ala Phe Lys Leu Pro Pro Met Glu Thr Glu Glu
1 5 10

<210> 197
<211> 510
<212> PRT
<213> Pan troglodytes

<220>
<223> Chimpanzee PH20

<400> 197

```
Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1               5                  10                  15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
            20                  25                  30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
        35                  40                  45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
    50                  55                  60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
65                  70                  75                  80
Ile Asn Val Thr Gly Gln Asp Val Thr Ile Phe Tyr Val Asp Arg Leu
                85                  90                  95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
            100                 105                 110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
        115                 120                 125
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
    130                 135                 140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145                 150                 155                 160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
                165                 170                 175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
            180                 185                 190
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
        195                 200                 205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
    210                 215                 220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
225                 230                 235                 240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
                245                 250                 255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
            260                 265                 270
```

```
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Gln Glu Ala Ile Arg Val
        275                 280                 285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Val Tyr Thr
    290                 295                 300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
305                 310                 315                 320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
                325                 330                 335
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
            340                 345                 350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
        355                 360                 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
    370                 375                 380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
385                 390                 395                 400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
                405                 410                 415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
            420                 425                 430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
        435                 440                 445
Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
    450                 455                 460
Ile Asp Ala Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Ser Gln Ile
465                 470                 475                 480
Phe Tyr Asn Ala Ser Pro Ser Thr Leu Ser Ala Thr Met Phe Ile Asp
                485                 490                 495
Leu Cys Asp Leu Tyr Leu Val Pro Thr Ser Tyr Leu Ile Leu
            500                 505                 510
```

<210> 198
<211> 512
<212> PRT
<213> Macaca mulatta

<220>
<223> Rhesus Monkey PH20

<400> 198

```
Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1               5                  10                  15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
            20                  25                  30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Ile Ile Pro Asn Val Pro
        35                  40                  45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
    50                  55                  60
Asn Glu Pro Leu Asp Met Ser Leu Phe Thr Leu Met Gly Ser Pro Arg
65                  70                  75                  80
Ile Asn Ile Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
                85                  90                  95
Gly Tyr Tyr Pro Tyr Ile Asp Leu Thr Thr Gly Val Thr Val His Gly
            100                 105                 110
Gly Ile Pro Gln Lys Val Ser Leu Gln Asp His Leu Asp Lys Ser Lys
        115                 120                 125
Gln Asp Ile Leu Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
    130                 135                 140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145                 150                 155                 160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
                165                 170                 175
```

```
Val Gln Leu Ser Leu Pro Gln Ala Thr Asp Lys Ala Lys Gln Glu Phe
            180                 185                 190
Glu Lys Ala Gly Lys Asp Phe Met Leu Glu Thr Ile Lys Leu Gly Arg
        195                 200                 205
Ser Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
    210                 215                 220
Tyr Asn His His Tyr Arg Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asp
225                 230                 235                 240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
                245                 250                 255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Val Val
            260                 265                 270
Val Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
        275                 280                 285
Ser Lys Ile Pro Asp Ala Lys Asn Pro Leu Pro Val Phe Val Tyr Ala
    290                 295                 300
Arg Leu Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Arg Glu Glu
305                 310                 315                 320
Leu Val Ser Thr Leu Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
                325                 330                 335
Val Ile Trp Gly Ser Leu Ser Ile Thr Arg Ser Met Lys Ser Cys Leu
            340                 345                 350
Leu Leu Asp Thr Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
        355                 360                 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
    370                 375                 380
Gly Val Cys Ile Arg Lys Asp Trp Asn Ser Ser Asp Tyr Leu His Leu
385                 390                 395                 400
Asn Pro Asp Asn Phe Asp Ile Arg Leu Glu Lys Gly Gly Lys Phe Thr
                405                 410                 415
Val His Gly Lys Pro Thr Val Glu Asp Leu Glu Glu Phe Ser Glu Lys
            420                 425                 430
Phe Tyr Cys Ser Cys Tyr Thr Asn Leu Ser Cys Lys Glu Lys Ala Asp
        435                 440                 445
Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
    450                 455                 460
Ile Asp Ala Ser Leu Lys Pro Pro Val Glu Thr Glu Gly Ser Pro Pro
465                 470                 475                 480
Ile Phe Tyr Asn Thr Ser Ser Ser Thr Val Ser Thr Thr Met Phe Ile
                485                 490                 495
Trp Arg Leu Glu Val Trp Asp Gln Gly Ile Ser Arg Ile Gly Phe Phe
            500                 505                 510
```

<210> 199
<211> 1485
<212> DNA
<213> Artificial Sequence

<220>
<223> SPAM1-LFLI codon optimized DNA with NheI and BamHI

<400> 199

```
gctagcatgg acatgagggt accagcccag ctgttgggcc ttcttctgct ttggctccct 60
ggggcaaggg gacttaactt cagagctccc cctgtaatcc caaacgtccc cttcctgtgg 120
gcatggaacg cccctagcga attctgtttg gggaaatttg acgaacctct ggacatgtct 180
ctgttttctt ttatcggtag ccctcgaatc aacgctaccg gacagggggt gacaatcttt 240
tacgtggatc gtctgggcta ttacccttac atcgacagca ttactggggt gaccgtgaac 300
ggaggcattc ctcagaaaat atcactgcag gaccacctgg acaaagctaa gaaggatatt 360
acattctaca tgccagtgga caatctcgga atggctgtga ttgactggga ggagtggaga 420
cctacttggg cccgaaattg gaagcccaag gatgtgtata agaaccgatc cattgagctg 480
gtgcagcagc agaacgtgca gctgtccctt acagaagcca ccgaaaaagc taagcaggaa 540
ttcgagaaag ctgggaagga tttcctggtt gagacaataa aacttgggaa actgcttaga 600

cctaaccacc tctggggcta ttatttgttc cccgattgct acaatcacca ctataagaag 660
cccggctaca acggatcttg tttcaacgtc gaaattaaaa gaaacgatga cctctcttgg 720
ttgtggaatg agtctactgc actctacccc tctatatatc tcaacacaca gcagtcccca 780
gtagccgcta ccctctacgt tcggaataga gtgagggaag ccatcagggt gtccaagatc 840
cccgatgcca aatctccccc cccagtgttt gcctacacca ggatcgtgtt taccgaccag 900
gtgttgaagt tcctgagcca ggacgagctc gtgtatactt ttggcgagac agttgctctg 960
ggagcctctg ggatcgtgat ctggggcact ctgagtatta tgcgatcaat gaagtcctgc 1020
ctgctgttgg ataactacat ggagacaatc ctgaacccat atatcattaa cgtgactttg 1080
gccgctaaga tgtgttcaca ggtgctgtgt caagagcagg gagtatgcat taggaagaac 1140
tggaactcaa gcgattatct ccatctgaac ccagataatt ttgcaatcca gctggaaaag 1200
ggtggtaaat tcactgtccg agggaagccc acactcgagg atctcgaaca gtttagtgaa 1260
aagttctact gtagctgcta tagcactctg agttgtaagg aaaaagccga tgtgaaagac 1320
actgatgccg ttgacgtttg catcgccgat ggggtgtgta ttgacgcctt tctcaagcca 1380
ccaatggaga ctgaggagcc ccagatcttt tacaacgcct ccccctccac cttgagtgct 1440
accatgttca tcgtatctat actttcctg atttgataag gatcc                 1485
```

<210> 200
<211> 1479
<212> DNA
<213> Artificial Sequence

<220>
<223> SPAM1-SILF codon optimized DNA with NheI and BamHI

<400> 200

```
gctagcatgg acatgagggt accagcccag ctgttgggcc ttcttctgct ttggctccct 60
ggggcaaggg gacttaactt cagagctccc cctgtaatcc caaacgtccc cttcctgtgg 120
gcatggaacg cccctagcga attctgtttg gggaaatttg acgaacctct ggacatgtct 180
ctgttttctt ttatcggtag ccctcgaatc aacgctaccg gacaggggtg gacaatcttt 240
tacgtggatc gtctgggcta ttacccttac atcgacagca ttactggggt gaccgtgaac 300
ggaggcattc ctcagaaaat atcactgcag gaccacctgg acaaagctaa gaaggatatt 360
acattctaca tgccagtgga caatctcgga atggctgtga ttgactggga ggagtggaga 420
cctacttggg cccgaaattg gaagcccaag gatgtgtata agaaccgatc cattgagctg 480
gtgcagcagc agaacgtgca gctgtccctt acagaagcca ccgaaaaagc taagcaggaa 540
ttcgagaaag ctgggaagga tttcctggtt gagacaataa aacttgggaa actgcttaga 600
cctaaccacc tctggggcta ttatttgttc cccgattgct acaatcacca ctataagaag 660
cccggctaca acggatcttg tttcaacgtc gaaattaaaa gaaacgatga cctctcttgg 720
ttgtggaatg agtctactgc actctacccc tctatatatc tcaacacaca gcagtcccca 780
gtagccgcta ccctctacgt tcggaataga gtgagggaag ccatcagggt gtccaagatc 840
cccgatgcca aatctccccc cccagtgttt gcctacacca ggatcgtgtt taccgaccag 900
gtgttgaagt tcctgagcca ggacgagctc gtgtatactt ttggcgagac agttgctctg 960
ggagcctctg ggatcgtgat ctggggcact ctgagtatta tgcgatcaat gaagtcctgc 1020
ctgctgttgg ataactacat ggagacaatc ctgaacccat atatcattaa cgtgactttg 1080
gccgctaaga tgtgttcaca ggtgctgtgt caagagcagg gagtatgcat taggaagaac 1140
tggaactcaa gcgattatct ccatctgaac ccagataatt ttgcaatcca gctggaaaag 1200
ggtggtaaat tcactgtccg agggaagccc acactcgagg atctcgaaca gtttagtgaa 1260
aagttctact gtagctgcta tagcactctg agttgtaagg aaaaagccga tgtgaaagac 1320
actgatgccg ttgacgtttg catcgccgat ggggtgtgta ttgacgcctt tctcaagcca 1380
ccaatggaga ctgaggagcc ccagatcttt tacaacgcct ccccctccac cttgagtgct 1440
accatgttca tcgtatctat acttttctga taaggatcc                       1479
```

<210> 201
<211> 1473
<212> DNA
<213> Artificial Sequence

<220>
<223> SPAM1-IVSI codon optimized DNA with NheI and BamHI

<400> 201

```
gctagcatgg acatgagggt accagcccag ctgttgggcc ttcttctgct ttggctccct 60
ggggcaaggg gacttaactt cagagctccc cctgtaatcc caaacgtccc cttcctgtgg 120
gcatggaacg cccctagcga attctgtttg gggaaatttg acgaacctct ggacatgtct 180
```

```
ctgttttctt ttatcggtag ccctcgaatc aacgctaccg gacagggggt gacaatcttt 240
tacgtggatc gtctgggcta ttacccttac atcgacagca ttactggggt gaccgtgaac 300
ggaggcattc ctcagaaaat atcactgcag gaccacctgg acaaagctaa gaaggatatt 360
acattctaca tgccagtgga caatctcgga atggctgtga ttgactggga ggagtggaga 420
cctacttggg cccgaaattg gaagcccaag gatgtgtata agaaccgatc cattgagctg 480
gtgcagcagc agaacgtgca gctgtccctt acagaagcca ccgaaaaagc taagcaggaa 540
ttcgagaaag ctgggaagga tttcctggtt gagacaataa aacttgggaa actgcttaga 600
cctaaccacc tctggggcta ttatttgttc cccgattgct acaatcacca ctataagaag 660
cccggctaca acggatcttg tttcaacgtc gaaattaaaa gaaacgatga cctctcttgg 720
ttgtggaatg agtctactgc actctacccc tctatatatc tcaacacaca gcagtcccca 780
gtagccgcta ccctctacgt tcggaataga gtgagggaag ccatcagggt gtccaagatc 840
cccgatgcca aatctcccct cccagtgttt gcctacacca ggatcgtgtt taccgaccag 900
gtgttgaagt tcctgagcca ggacgagctc gtgtatactt ttggcgagac agttgctctg 960
ggagcctctg ggatcgtgat ctggggcact ctgagtatta tgcgatcaat gaagtcctgc 1020
ctgctgttgg ataactacat ggagacaatc ctgaacccat atatcattaa cgtgactttg 1080
gccgctaaga tgtgttcaca ggtgctgtgt caagagcagg gagtatgcat taggaagaac 1140
tggaactcaa gcgattatct ccatctgaac ccagataatt ttgcaatcca gctggaaaag 1200
ggtggtaaat tcactgtccg agggaagccc acactcgagg atctcgaaca gtttagtgaa 1260
aagttctact gtagctgcta tagcactctg agttgtaagg aaaaagccga tgtgaaagac 1320
actgatgccg ttgacgtttg catcgccgat ggggtgtgta ttgacgcctt tctcaagcca 1380
ccaatggaga ctgaggagcc ccagatcttt tacaacgcct ccccctccac cttgagtgct 1440
accatgttca tcgtatctat atgataagga tcc                           1473
```

<210> 202
<211> 496
<212> PRT
<213> Artificial Sequence

<220>
<223> PH20sHis N82A

<400> 202

```
Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1               5                  10                  15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
            20                  25                  30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
        35                  40                  45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
    50                  55                  60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
65                  70                  75                  80
Ile Ala Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
                85                  90                  95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
            100                 105                 110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
        115                 120                 125
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
    130                 135                 140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145                 150                 155                 160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
                165                 170                 175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
            180                 185                 190
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
        195                 200                 205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
    210                 215                 220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
225                 230                 235                 240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
```

```
                245                 250                 255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
            260                 265                 270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
        275                 280                 285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
    290                 295                 300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
305                 310                 315                 320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
                325                 330                 335
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
            340                 345                 350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
        355                 360                 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
    370                 375                 380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
385                 390                 395                 400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
                405                 410                 415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
            420                 425                 430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
        435                 440                 445
Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
    450                 455                 460
Ile Asp Ala Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile
465                 470                 475                 480
Phe Tyr Asn Ala Ser Pro Ser Thr Leu Ser His His His His His His
                485                 490                 495
```

<210> 203
<211> 496
<212> PRT
<213> Artificial Sequence

<220>
<223> PH20sHis N166A

<400> 203

```
Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1               5                  10                  15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
            20                  25                  30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
        35                  40                  45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
    50                  55                  60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
65                  70                  75                  80
Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
                85                  90                  95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
            100                 105                 110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
        115                 120                 125
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
    130                 135                 140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145                 150                 155                 160
Lys Asp Val Tyr Lys Ala Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
```

```
                    165                 170                 175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
                180                 185                 190
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
        195                 200                 205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
    210                 215                 220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
225                 230                 235                 240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
                245                 250                 255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
            260                 265                 270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
        275                 280                 285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
    290                 295                 300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
305                 310                 315                 320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
                325                 330                 335
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
            340                 345                 350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
        355                 360                 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
    370                 375                 380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
385                 390                 395                 400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
                405                 410                 415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
            420                 425                 430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
        435                 440                 445
Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
    450                 455                 460
Ile Asp Ala Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile
465                 470                 475                 480
Phe Tyr Asn Ala Ser Pro Ser Thr Leu Ser His His His His His His
                485                 490                 495
```

<210> 204
<211> 496
<212> PRT
<213> Artificial Sequence

<220>
<223> PH20sHis N235A

<400> 204

```
Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1               5                  10                  15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
            20                  25                  30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
        35                  40                  45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
    50                  55                  60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
65                  70                  75                  80
Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
```

```
                    85                  90                  95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
                100                 105                 110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
            115                 120                 125
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
        130                 135                 140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145                 150                 155                 160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
                165                 170                 175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
            180                 185                 190
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
        195                 200                 205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
    210                 215                 220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Ala Gly Ser Cys Phe Asn
225                 230                 235                 240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
                245                 250                 255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
            260                 265                 270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
        275                 280                 285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
    290                 295                 300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
305                 310                 315                 320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
                325                 330                 335
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
            340                 345                 350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
        355                 360                 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
    370                 375                 380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
385                 390                 395                 400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
                405                 410                 415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
            420                 425                 430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
        435                 440                 445
Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
    450                 455                 460
Ile Asp Ala Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile
465                 470                 475                 480
Phe Tyr Asn Ala Ser Pro Ser Thr Leu Ser His His His His His His
                485                 490                 495
```

<210> 205
<211> 496
<212> PRT
<213> Artificial Sequence

<220>
<223> PH20sHis N254A

<400> 205

Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys

```
 1               5                  10                  15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
            20                  25                  30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
        35                  40                  45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
    50                  55                  60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
65                  70                  75                  80
Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
                85                  90                  95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
            100                 105                 110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
        115                 120                 125
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
    130                 135                 140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145                 150                 155                 160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
                165                 170                 175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
            180                 185                 190
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
        195                 200                 205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
    210                 215                 220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
225                 230                 235                 240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Ala Glu Ser
                245                 250                 255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
            260                 265                 270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
        275                 280                 285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
    290                 295                 300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
305                 310                 315                 320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
                325                 330                 335
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
            340                 345                 350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
        355                 360                 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
    370                 375                 380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
385                 390                 395                 400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
                405                 410                 415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
            420                 425                 430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
        435                 440                 445
Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
    450                 455                 460
Ile Asp Ala Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile
465                 470                 475                 480
Phe Tyr Asn Ala Ser Pro Ser Thr Leu Ser His His His His His His
                485                 490                 495
```

<210> 206
<211> 496
<212> PRT
<213> Artificial Sequence

<220>
<223> PH20sHis N82A/N166A

<400> 206

```
Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1               5                  10                  15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
            20                  25                  30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
        35                  40                  45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
    50                  55                  60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
65                  70                  75                  80
Ile Ala Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
                85                  90                  95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
            100                 105                 110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
        115                 120                 125
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
    130                 135                 140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145                 150                 155                 160
Lys Asp Val Tyr Lys Ala Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
                165                 170                 175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
            180                 185                 190
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
        195                 200                 205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
    210                 215                 220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
225                 230                 235                 240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Asn Glu Ser
                245                 250                 255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
            260                 265                 270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
        275                 280                 285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
    290                 295                 300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
305                 310                 315                 320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
                325                 330                 335
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
            340                 345                 350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
        355                 360                 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
    370                 375                 380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
385                 390                 395                 400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
                405                 410                 415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
            420                 425                 430
```

```
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
        435                 440                 445
Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
    450                 455                 460
Ile Asp Ala Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile
465                 470                 475                 480
Phe Tyr Asn Ala Ser Pro Ser Thr Leu Ser His His His His His His
                485                 490                 495
```

<210> 207
<211> 496
<212> PRT
<213> Artificial Sequence

<220>
<223> PH20sHis N82A/N254A

<400> 207

```
Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1               5                  10                  15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
            20                  25                  30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
        35                  40                  45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
    50                  55                  60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
65                  70                  75                  80
Ile Ala Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
                85                  90                  95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
            100                 105                 110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
        115                 120                 125
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
    130                 135                 140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145                 150                 155                 160
Lys Asp Val Tyr Lys Asn Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
                165                 170                 175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
            180                 185                 190
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
        195                 200                 205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
    210                 215                 220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
225                 230                 235                 240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Ala Glu Ser
                245                 250                 255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
            260                 265                 270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
        275                 280                 285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
    290                 295                 300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
305                 310                 315                 320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
                325                 330                 335
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
            340                 345                 350
```

```
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
        355                 360                 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
    370                 375                 380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
385                 390                 395                 400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
                405                 410                 415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
            420                 425                 430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
        435                 440                 445
Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
    450                 455                 460
Ile Asp Ala Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile
465                 470                 475                 480
Phe Tyr Asn Ala Ser Pro Ser Thr Leu Ser His His His His His His
                485                 490                 495
```

<210> 208
<211> 496
<212> PRT
<213> Artificial Sequence

<220>
<223> PH20sHis N166A/N254A

<400> 208

```
Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1               5                  10                  15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
            20                  25                  30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
        35                  40                  45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
    50                  55                  60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
65                  70                  75                  80
Ile Asn Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
                85                  90                  95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
            100                 105                 110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
        115                 120                 125
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
    130                 135                 140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145                 150                 155                 160
Lys Asp Val Tyr Lys Ala Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
                165                 170                 175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
            180                 185                 190
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
        195                 200                 205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
    210                 215                 220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
225                 230                 235                 240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Ala Glu Ser
                245                 250                 255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
            260                 265                 270
```

```
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
        275                 280                 285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
    290                 295                 300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
305                 310                 315                 320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
                325                 330                 335
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
            340                 345                 350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
        355                 360                 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
    370                 375                 380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
385                 390                 395                 400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
                405                 410                 415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
            420                 425                 430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
        435                 440                 445
Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
    450                 455                 460
Ile Asp Ala Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile
465                 470                 475                 480
Phe Tyr Asn Ala Ser Pro Ser Thr Leu Ser His His His His His His
                485                 490                 495
```

<210> 209
<211> 496
<212> PRT
<213> Artificial Sequence

<220>
<223> PH20sHis N82A/N166A/N254A

<400> 209

```
Met Gly Val Leu Lys Phe Lys His Ile Phe Phe Arg Ser Phe Val Lys
 1               5                  10                  15
Ser Ser Gly Val Ser Gln Ile Val Phe Thr Phe Leu Leu Ile Pro Cys
            20                  25                  30
Cys Leu Thr Leu Asn Phe Arg Ala Pro Pro Val Ile Pro Asn Val Pro
        35                  40                  45
Phe Leu Trp Ala Trp Asn Ala Pro Ser Glu Phe Cys Leu Gly Lys Phe
    50                  55                  60
Asp Glu Pro Leu Asp Met Ser Leu Phe Ser Phe Ile Gly Ser Pro Arg
65                  70                  75                  80
Ile Ala Ala Thr Gly Gln Gly Val Thr Ile Phe Tyr Val Asp Arg Leu
                85                  90                  95
Gly Tyr Tyr Pro Tyr Ile Asp Ser Ile Thr Gly Val Thr Val Asn Gly
            100                 105                 110
Gly Ile Pro Gln Lys Ile Ser Leu Gln Asp His Leu Asp Lys Ala Lys
        115                 120                 125
Lys Asp Ile Thr Phe Tyr Met Pro Val Asp Asn Leu Gly Met Ala Val
    130                 135                 140
Ile Asp Trp Glu Glu Trp Arg Pro Thr Trp Ala Arg Asn Trp Lys Pro
145                 150                 155                 160
Lys Asp Val Tyr Lys Ala Arg Ser Ile Glu Leu Val Gln Gln Gln Asn
                165                 170                 175
Val Gln Leu Ser Leu Thr Glu Ala Thr Glu Lys Ala Lys Gln Glu Phe
            180                 185                 190
```

```
Glu Lys Ala Gly Lys Asp Phe Leu Val Glu Thr Ile Lys Leu Gly Lys
        195                 200                 205
Leu Leu Arg Pro Asn His Leu Trp Gly Tyr Tyr Leu Phe Pro Asp Cys
    210                 215                 220
Tyr Asn His His Tyr Lys Lys Pro Gly Tyr Asn Gly Ser Cys Phe Asn
225                 230                 235                 240
Val Glu Ile Lys Arg Asn Asp Asp Leu Ser Trp Leu Trp Ala Glu Ser
                245                 250                 255
Thr Ala Leu Tyr Pro Ser Ile Tyr Leu Asn Thr Gln Gln Ser Pro Val
            260                 265                 270
Ala Ala Thr Leu Tyr Val Arg Asn Arg Val Arg Glu Ala Ile Arg Val
        275                 280                 285
Ser Lys Ile Pro Asp Ala Lys Ser Pro Leu Pro Val Phe Ala Tyr Thr
    290                 295                 300
Arg Ile Val Phe Thr Asp Gln Val Leu Lys Phe Leu Ser Gln Asp Glu
305                 310                 315                 320
Leu Val Tyr Thr Phe Gly Glu Thr Val Ala Leu Gly Ala Ser Gly Ile
                325                 330                 335
Val Ile Trp Gly Thr Leu Ser Ile Met Arg Ser Met Lys Ser Cys Leu
            340                 345                 350
Leu Leu Asp Asn Tyr Met Glu Thr Ile Leu Asn Pro Tyr Ile Ile Asn
        355                 360                 365
Val Thr Leu Ala Ala Lys Met Cys Ser Gln Val Leu Cys Gln Glu Gln
    370                 375                 380
Gly Val Cys Ile Arg Lys Asn Trp Asn Ser Ser Asp Tyr Leu His Leu
385                 390                 395                 400
Asn Pro Asp Asn Phe Ala Ile Gln Leu Glu Lys Gly Gly Lys Phe Thr
                405                 410                 415
Val Arg Gly Lys Pro Thr Leu Glu Asp Leu Glu Gln Phe Ser Glu Lys
            420                 425                 430
Phe Tyr Cys Ser Cys Tyr Ser Thr Leu Ser Cys Lys Glu Lys Ala Asp
        435                 440                 445
Val Lys Asp Thr Asp Ala Val Asp Val Cys Ile Ala Asp Gly Val Cys
    450                 455                 460
Ile Asp Ala Phe Leu Lys Pro Pro Met Glu Thr Glu Glu Pro Gln Ile
465                 470                 475                 480
Phe Tyr Asn Ala Ser Pro Ser Thr Leu Ser His His His His His His
                485                 490                 495
```

<210> 210
<211> 1494
<212> DNA
<213> Artificial Sequence

<220>
<223> PH20sHis DNA

<400> 210

```
atgggagtgc taaaattcaa gcacatcttt ttcagaagct ttgttaaatc aagtggagta 60
tcccagatag ttttcacctt ccttctgatt ccatgttgct tgactcttaa cttcagagct 120
ccccctgtaa tcccaaacgt ccccttcctg tgggcatgga acgcccctag cgaattctgt 180
ttggggaaat ttgacgaacc tctggacatg tctctgtttt cttttatcgg tagccctcga 240
atcaacgcta ccggacaggg ggtgacaatc ttttacgtgg atcgtctggg ctattaccct 300
tacatcgaca gcattactgg ggtgaccgtg aacggaggca ttcctcagaa aatatcactg 360
caggaccacc tggacaaagc taagaaggat attacattct acatgccagt ggacaatctc 420
ggaatggctg tgattgactg ggaggagtgg agacctactt gggcccgaaa ttggaagccc 480
aaggatgtgt ataagaaccg atccattgag ctggtgcagc agcagaacgt gcagctgtcc 540
cttacagaag ccaccgaaaa agctaagcag gaattcgaga aagctgggaa ggatttcctg 600
gttgagacaa taaaacttgg gaaactgctt agacctaacc acctctgggg ctattatttg 660
ttccccgatt gctacaatca ccactataag aagcccggct acaacggatc ttgtttcaac 720
gtcgaaatta aaagaaacga tgacctctct tggttgtgga atgagtctac tgcactctac 780
ccctctatat atctcaacac acagcagtcc ccagtagccg ctaccctcta cgttcggaat 840

agagtgaggg aagccatcag ggtgtccaag atccccgatg ccaaatctcc cctcccagtg 900
tttgcctaca ccaggatcgt gtttaccgac caggtgttga agttcctgag ccaggacgag 960
ctcgtgtata cttttggcga gacagttgct ctgggagcct ctgggatcgt gatctggggc 1020
actctgagta ttatgcgatc aatgaagtcc tgcctgctgt tggataacta catggagaca 1080
atcctgaacc catatatcat taacgtgact ttggccgcta agatgtgttc acaggtgctg 1140
tgtcaagagc agggagtatg cattaggaag aactggaact caagcgatta tctccatctg 1200
aacccagata attttgcaat ccagctggaa aagggtggta aattcactgt ccgagggaag 1260
cccacactcg aggatctcga acagtttagt gaaaagttct actgtagctg ctatagcact 1320
ctgagttgta aggaaaaagc cgatgtgaaa gacactgatg ccgttgacgt ttgcatcgcc 1380
gatggggtgt gtattgacgc ctttctcaag ccaccaatgg agactgagga gccccagatc 1440
ttttacaacg cctccccctc caccttgagt catcatcatc atcatcatta gtaa       1494
```

<210> 211
<211> 1497
<212> DNA
<213> Artificial Sequence

<220>
<223> SPAM1-ISSV

<400> 211

gctagcatgg acatgagggt accagcccag ctgttgggcc ttcttctgct ttggctccct 60
ggggcaaggg gacttaactt cagagctccc cctgtaatcc caaacgtccc cttcctgtgg 120
gcatggaacg cccctagcga attctgtttg gggaaatttg acgaacctct ggacatgtct 180
ctgttttctt ttatcggtag ccctcgaatc aacgctaccg gacagggggt gacaatcttt 240
tacgtggatc gtctgggcta ttacccttac atcgacagca ttactggggt gaccgtgaac 300
ggaggcattc ctcagaaaat atcactgcag gaccacctgg acaaagctaa gaaggatatt 360
acattctaca tgccagtgga caatctcgga atggctgtga ttgactggga ggagtggaga 420
cctacttggg cccgaaattg gaagcccaag gatgtgtata agaaccgatc cattgagctg 480
gtgcagcagc agaacgtgca gctgtccctt acagaagcca ccgaaaaagc taagcaggaa 540
ttcgagaaag ctgggaagga tttcctggtt gagacaataa aacttgggaa actgcttaga 600
cctaaccacc tctggggcta ttatttgttc cccgattgct acaatcacca ctataagaag 660
cccggctaca acggatcttg tttcaacgtc gaaattaaaa gaaacgatga cctctcttgg 720
ttgtggaatg agtctactgc actctacccc tctatatatc tcaacacaca gcagtcccca 780
gtagccgcta ccctctacgt tcggaataga gtgagggaag ccatcagggt gtccaagatc 840
cccgatgcca aatctccccc cccagtgttt gcctacacca ggatcgtgtt taccgaccag 900
gtgttgaagt tcctgagcca ggacgagctc gtgtatactt ttggcgagac agttgctctg 960
ggagcctctg ggatcgtgat ctggggcact ctgagtatta tgcgatcaat gaagtcctgc 1020
ctgctgttgg ataactacat ggagacaatc ctgaacccat atatcattaa cgtgactttg 1080
gccgctaaga tgtgttcaca ggtgctgtgt caagagcagg gagtatgcat taggaagaac 1140
tggaactcaa gcgattatct ccatctgaac ccagataatt ttgcaatcca gctggaaaag 1200
ggtggtaaat tcactgtccg agggaagccc acactcgagg atctcgaaca gtttagtgaa 1260
aagttctact gtagctgcta tagcactctg agttgtaagg aaaaagccga tgtgaaagac 1320
actgatgccg ttgacgtttg catcgccgat ggggtgtgta ttgacgcctt tctcaagcca 1380
ccaatggaga ctgaggagcc ccagatcttt tacaacgcct ccccctccac cttgagtgct 1440
accatgttca tcgtatctat acttttcctg attatatcaa gcgtgtgata aggatcc 1497

<210> 212
<211> 1491
<212> DNA
<213> Artificial Sequence

<220>
<223> SPAM1-LIIS

<400> 212

gctagcatgg acatgagggt accagcccag ctgttgggcc ttcttctgct ttggctccct 60
ggggcaaggg gacttaactt cagagctccc cctgtaatcc caaacgtccc cttcctgtgg 120
gcatggaacg cccctagcga attctgtttg gggaaatttg acgaacctct ggacatgtct 180
ctgttttctt ttatcggtag ccctcgaatc aacgctaccg gacagggggt gacaatcttt 240
tacgtggatc gtctgggcta ttacccttac atcgacagca ttactggggt gaccgtgaac 300
ggaggcattc ctcagaaaat atcactgcag gaccacctgg acaaagctaa gaaggatatt 360
acattctaca tgccagtgga caatctcgga atggctgtga ttgactggga ggagtggaga 420

cctacttggg cccgaaattg gaagcccaag gatgtgtata agaaccgatc cattgagctg 480
gtgcagcagc agaacgtgca gctgtccctt acagaagcca ccgaaaaagc taagcaggaa 540
ttcgagaaag ctgggaagga tttcctggtt gagacaataa aacttgggaa actgcttaga 600
cctaaccacc tctggggcta ttatttgttc cccgattgct acaatcacca ctataagaag 660
cccggctaca acggatcttg tttcaacgtc gaaattaaaa gaaacgatga cctctcttgg 720
ttgtggaatg agtctactgc actctacccc tctatatatc tcaacacaca gcagtcccca 780
gtagccgcta ccctctacgt tcggaataga gtgagggaag ccatcagggt gtccaagatc 840
cccgatgcca aatctccccc cccagtgttt gcctacacca ggatcgtgtt taccgaccag 900
gtgttgaagt tcctgagcca ggacgagctc gtgtatactt ttggcgagac agttgctctg 960
ggagcctctg ggatcgtgat ctggggcact ctgagtatta tgcgatcaat gaagtcctgc 1020
ctgctgttgg ataactacat ggagacaatc ctgaacccat atatcattaa cgtgactttg 1080
gccgctaaga tgtgttcaca ggtgctgtgt caagagcagg gagtatgcat taggaagaac 1140
tggaactcaa gcgattatct ccatctgaac ccagataatt ttgcaatcca gctggaaaag 1200
ggtggtaaat tcactgtccg agggaagccc acactcgagg atctcgaaca gtttagtgaa 1260
aagttctact gtagctgcta tagcactctg agttgtaagg aaaaagccga tgtgaaagac 1320
actgatgccg ttgacgtttg catcgccgat ggggtgtgta ttgacgcctt tctcaagcca 1380
ccaatggaga ctgaggagcc ccagatcttt tacaacgcct ccccctccac cttgagtgct 1440
accatgttca tcgtatctat acttttcctg attatatcat gataaggatc c 1491

<210> 213
<211> 1467
<212> DNA
<213> Artificial Sequence

<220>
<223> SPAM1-MFIV

<400> 213

gctagcatgg acatgagggt accagcccag ctgttgggcc ttcttctgct ttggctccct 60
ggggcaaggg gacttaactt cagagctccc cctgtaatcc caaacgtccc cttcctgtgg 120
gcatggaacg cccctagcga attctgtttg gggaaatttg acgaacctct ggacatgtct 180
ctgttttctt ttatcggtag ccctcgaatc aacgctaccg gacaggggtg gacaatcttt 240
tacgtggatc gtctgggcta ttacccttac atcgacagca ttactggggt gaccgtgaac 300
ggaggcattc ctcagaaaat atcactgcag gaccacctgg acaaagctaa gaaggatatt 360
acattctaca tgccagtgga caatctcgga atggctgtga ttgactggga ggagtggaga 420
cctacttggg cccgaaattg gaagcccaag gatgtgtata agaaccgatc cattgagctg 480
gtgcagcagc agaacgtgca gctgtccctt acagaagcca ccgaaaaagc taagcaggaa 540
ttcgagaaag ctgggaagga tttcctggtt gagacaataa aacttgggaa actgcttaga 600
cctaaccacc tctggggcta ttatttgttc cccgattgct acaatcacca ctataagaag 660
cccggctaca acggatcttg tttcaacgtc gaaattaaaa gaaacgatga cctctcttgg 720
ttgtggaatg agtctactgc actctacccc tctatatatc tcaacacaca gcagtcccca 780
gtagccgcta ccctctacgt tcggaataga gtgagggaag ccatcagggt gtccaagatc 840
cccgatgcca aatctccccc cccagtgttt gcctacacca ggatcgtgtt taccgaccag 900
gtgttgaagt tcctgagcca ggacgagctc gtgtatactt ttggcgagac agttgctctg 960
ggagcctctg ggatcgtgat ctggggcact ctgagtatta tgcgatcaat gaagtcctgc 1020
ctgctgttgg ataactacat ggagacaatc ctgaacccat atatcattaa cgtgactttg 1080
gccgctaaga tgtgttcaca ggtgctgtgt caagagcagg gagtatgcat taggaagaac 1140
tggaactcaa gcgattatct ccatctgaac ccagataatt ttgcaatcca gctggaaaag 1200
ggtggtaaat tcactgtccg agggaagccc acactcgagg atctcgaaca gtttagtgaa 1260
aagttctact gtagctgcta tagcactctg agttgtaagg aaaaagccga tgtgaaagac 1320
actgatgccg ttgacgtttg catcgccgat ggggtgtgta ttgacgcctt tctcaagcca 1380
ccaatggaga ctgaggagcc ccagatcttt tacaacgcct ccccctccac cttgagtgct 1440
accatgttca tcgtatgata aggatcc 1467

<210> 214
<211> 1461
<212> DNA
<213> Artificial Sequence

<220>
<223> SPAM1-ATMF

<400> 214

gctagcatgg acatgagggt accagcccag ctgttgggcc ttcttctgct ttggctccct 60
ggggcaaggg gacttaactt cagagctccc cctgtaatcc caaacgtccc cttcctgtgg 120
gcatggaacg cccctagcga attctgtttg gggaaatttg acgaacctct ggacatgtct 180
ctgttttctt ttatcggtag ccctcgaatc aacgctaccg gacaggggtg gacaatcttt 240
tacgtggatc gtctgggcta ttacccttac atcgacagca ttactggggt gaccgtgaac 300
ggaggcattc ctcagaaaat atcactgcag gaccacctgg acaaagctaa gaaggatatt 360
acattctaca tgccagtgga caatctcgga atggctgtga ttgactggga ggagtggaga 420
cctacttggg cccgaaattg gaagcccaag gatgtgtata agaaccgatc cattgagctg 480
gtgcagcagc agaacgtgca gctgtccctt acagaagcca ccgaaaaagc taagcaggaa 540
ttcgagaaag ctgggaagga tttcctggtt gagacaataa aacttgggaa actgcttaga 600
cctaaccacc tctggggcta ttatttgttc cccgattgct acaatcacca ctataagaag 660
cccggctaca acggatcttg tttcaacgtc gaaattaaaa gaaacgatga cctctcttgg 720
ttgtggaatg agtctactgc actctacccc tctatatatc tcaacacaca gcagtcccca 780
gtagccgcta ccctctacgt tcggaataga gtgagggaag ccatcagggt gtccaagatc 840
cccgatgcca aatctccccc cccagtgttt gcctacacca ggatcgtgtt taccgaccag 900
gtgttgaagt tcctgagcca ggacgagctc gtgtatactt ttggcgagac agttgctctg 960
ggagcctctg ggatcgtgat ctggggcact ctgagtatta tgcgatcaat gaagtcctgc 1020
ctgctgttgg ataactacat ggagacaatc ctgaacccat atatcattaa cgtgactttg 1080
gccgctaaga tgtgttcaca ggtgctgtgt caagagcagg gagtatgcat taggaagaac 1140
tggaactcaa gcgattatct ccatctgaac ccagataatt ttgcaatcca gctggaaaag 1200
ggtggtaaat tcactgtccg agggaagccc acactcgagg atctcgaaca gtttagtgaa 1260
aagttctact gtagctgcta tagcactctg agttgtaagg aaaaagccga tgtgaaagac 1320
actgatgccg ttgacgtttg catcgccgat ggggtgtgta ttgacgcctt tctcaagcca 1380
ccaatggaga ctgaggagcc ccagatcttt tacaacgcct ccccctccac cttgagtgct 1440
accatgttct gataaggatc c 1461

<210> 215
<211> 1455
<212> DNA
<213> Artificial Sequence

<220>
<223> SPAM1-LSAT

<400> 215

```
gctagcatgg acatgagggt accagcccag ctgttgggcc ttcttctgct ttggctccct 60
ggggcaaggg gacttaactt cagagctccc cctgtaatcc caaacgtccc cttcctgtgg 120
gcatggaacg cccctagcga attctgtttg gggaaatttg acgaacctct ggacatgtct 180
ctgttttctt ttatcggtag ccctcgaatc aacgctaccg gacaggggtg gacaatcttt 240
tacgtggatc gtctgggcta ttacccttac atcgacagca ttactggggt gaccgtgaac 300
ggaggcattc ctcagaaaat atcactgcag gaccacctgg acaaagctaa gaaggatatt 360
acattctaca tgccagtgga caatctcgga atggctgtga ttgactggga ggagtggaga 420
cctacttggg cccgaaattg gaagcccaag gatgtgtata agaaccgatc cattgagctg 480
gtgcagcagc agaacgtgca gctgtccctt acagaagcca ccgaaaaagc taagcaggaa 540
ttcgagaaag ctgggaagga tttcctggtt gagacaataa aacttgggaa actgcttaga 600
cctaaccacc tctggggcta ttatttgttc cccgattgct acaatcacca ctataagaag 660
cccggctaca acggatcttg tttcaacgtc gaaattaaaa gaaacgatga cctctcttgg 720
ttgtggaatg agtctactgc actctacccc tctatatatc tcaacacaca gcagtcccca 780
gtagccgcta ccctctacgt tcggaataga gtgagggaag ccatcagggt gtccaagatc 840
cccgatgcca aatctccccc cccagtgttt gcctacacca ggatcgtgtt taccgaccag 900
gtgttgaagt tcctgagcca ggacgagctc gtgtatactt ttggcgagac agttgctctg 960
ggagcctctg ggatcgtgat ctggggcact ctgagtatta tgcgatcaat gaagtcctgc 1020
ctgctgttgg ataactacat ggagacaatc ctgaacccat atatcattaa cgtgactttg 1080
gccgctaaga tgtgttcaca ggtgctgtgt caagagcagg gagtatgcat taggaagaac 1140
tggaactcaa gcgattatct ccatctgaac ccagataatt ttgcaatcca gctggaaaag 1200
ggtggtaaat tcactgtccg agggaagccc acactcgagg atctcgaaca gtttagtgaa 1260
aagttctact gtagctgcta tagcactctg agttgtaagg aaaaagccga tgtgaaagac 1320
actgatgccg ttgacgtttg catcgccgat ggggtgtgta ttgacgcctt tctcaagcca 1380
ccaatggaga ctgaggagcc ccagatcttt tacaacgcct ccccctccac cttgagtgct 1440
acctgataag gatcc                                                  1455
```

Claims

1. An extended soluble PH20 hyaluronidase (esPH20), wherein:

   the esPH20 polypeptide is active at neutral pH;
   the esPH20 polypeptide is soluble; and
   the esPH20 polypeptide is selected from among:

   a polypeptide that consists of the sequence of amino acids set forth as amino acid residues 36-495, 36-496, 36-497, 36-498, 36-499 or 36-500 of SEQ ID NO: 107; or
   a polypeptide that contains amino acid substitutions in the sequence of amino acids set forth as amino acids 36-495, 36-496, 36-497, 36-498, 36-499 or 36-500 of SEQ ID NO: 107, whereby the amino acid-substituted polypeptide consists of a sequence of amino acids that has at least 95% sequence identity with the corresponding sequence of amino acids set forth as amino acids 36-495, 36-496, 36-497, 36-498, 36-499 or 36-500 of SEQ ID NO: 107.

2. The esPH20 of claim 1 that consists of the sequence of amino acids set forth as amino acids 36-495, 36-496, 36-497, 36-498, 36-499 or 36-500 of SEQ ID NO: 107.

3. The esPH20 of claim 1 or claim 2 that is N-glycosylated.

4. The esPH20 of any of claims 1-3, wherein the esPH20 is N-glycosylated and comprises at least an N-acetylglucosamine moiety linked to each of at least three asparagine (N) residues.

5. The esPH20 of any of claims 1-4 that is purified or isolated.

6. A conjugate, comprising the esPH20 of any of claims 1-5, wherein the esPH20 is soluble.

7. The conjugate of claim 6, wherein the esPH20 is conjugated to a polymer.

8. The conjugate of claim 7, wherein the polymer is dextran or PEG.

9. The conjugate of any of claims 6-8, wherein the esPH20 is conjugated to a moiety selected from among a multimerization domain, toxin, detectable label and drug.

10. The conjugate of any of claims 6-9, wherein the esPH20 is conjugated to a moiety that is an Fc domain.

11. A nucleic acid molecule encoding the esPH20 of any of claims 1-5, wherein the nucleic acid molecule does not encode a full-length PH20 hyaluronidase but encodes the truncated PH20 hyaluronidase.

12. A vector, comprising a polynucleotide that consists of a sequence of nucleotides encoding the esPH20 of any of claims 1-5, wherein the sequence of nucleotides includes or is immediately followed by a stop codon operatively inserted into the vector for expression of the esPH20 of any of claims 1-5.

13. The vector of claim 12 that is a virus vector.

14. An isolated cell or cell culture, comprising the vector of claim 12 or claim 13 or a nucleic acid molecule of claim 11.

15. The cell or cell culture of claim 14, wherein the cell is a CHO cell or the cell culture comprises a CHO cell.

16. A composition, comprising an esPH20 of any of claims 1-5 or a conjugate of any of claims 6-10.

17. A pharmaceutical composition, comprising an esPH20 of any of claims 1-5 or a conjugate of any of claims 6-10.

18. The pharmaceutical composition of claim 17, comprising an additional therapeutic agent.

19. The pharmaceutical composition of claim 18, wherein the therapeutic agent is selected from among:
a chemotherapeutic agent, an analgesic agent, an anti-inflammatory agent, an antimicrobial agent, an amoebicidal agent, a trichomonacidal agent, an anti-parkinson agent, an anti-malarial agent, an anticonvulsant agent, an antidepressant agent, an antiarthritics agent, an anti-fungal agent, an antihypertensive agent, an antipyretic agent, an anti-parasite agent, an antihistamine agent, an alpha-adrenergic agonist agent, an alpha blocker agent, an anesthetic agent, a bronchial dilator agent, a biocide agent, a bactericide agent, a bacteriostat agent, a beta adrenergic blocker agent, a calcium channel blocker agent, a cardiovascular drug agent, a contraceptive agent, a decongestant agent, a diuretic agent, a depressant agent, a diagnostic agent, a electrolyte agent, a hypnotic agent, a hormone agent, a hyperglycemic agent, a muscle relaxant agent, a muscle contractant agent, an ophthalmic agent, a parasympathomimetic agent, a psychic energizer agent, a sedative agent, a sympathomimetic agent, a tranquilizer agent, an urinary agent, a vaginal agent, a viricide agent, a vitamin agent, a non-steroidal anti-inflammatory agent, an angiotensin converting enzyme inhibitor agent, a polypeptide, a protein, a nucleic acid, a drug, an organic molecule a sleep inducer; an antibody, an immunoglobulin, a cytokine and an insulin.

20. The pharmaceutical composition of claim 18 or claim 19, wherein the therapeutic agent comprises an antibody.

21. The pharmaceutical composition of claim 18, wherein the additional therapeutic agent is selected from among Acivicins; Aclarubicins; Acodazoles; Acronines; Adozelesins; Aldesleukins; Alemtuzumabs; Alitretinoins (9-Cis-Retinoic Acids); Allopurinols; Altretamines; Alvocidibs; Ambazones; Ambomycins; Ametantrones; Amifostines; Aminoglutethimides; Amsacrines; Anastrozoles; Anaxirones; Ancitabines; Anthramycins; Apaziquones; Argimesnas; Arsenic Trioxides; Asparaginases; Asperlins; Atrimustines; Azacitidines; Azetepas; Azotomycins; Banoxantrones; Batabulins; Batimastats; BCG Live; Benaxibines; Bendamustines; Benzodepas; Bexarotenes; Bevacizumab; Bicalutamides; Bietaserpines; Biricodars; Bisantrenes; Bisantrenes; Bisnafide Dimesylates; Bizelesins; Bleomycins; Bortezomibs; Brequinars; Bropirimines; Budotitanes; Busulfans; Cactinomycins; Calusterones; Canertinibs; Capecitabines; Caracemides; Carbetimers; Carboplatins; Carboquones; Carmofurs; Carmustines with Polifeprosans; Carmustines; Carubicins; Carzelesins; Cedefingols; Celecoxibs; Cemadotins; Chlorambucils; Cioteronels; Cirolemycins; Cisplatins; Cladribines; Clanfenurs; Clofarabines; Crisnatols; Cyclophosphamides; Cytarabine liposomals; Cytarabines; Dacarbazines; Dactinomycins; Darbepoetin Alfas; Daunorubicin liposomals; Daunorubicins/Daunomycins; Daunorubicins; Decitabines; Denileukin Diftitoxes; Dexniguldipines; Dexonas; Dexrazoxanes; Dezaguanines; Diaziquones; Dibrospidiums; Dienogests; Dinalins; Disermolides; Docetaxels; Dofequidars; Doxifluridines; Doxorubicin liposomals; Doxorubicin HCL; Doxorubicin HCl liposome injection; Doxorubicins; Droloxifenes; Dromostanolone Propionates; Duazomycins; Ecomustines; Edatrexates; Edotecarins; Eflornithines; Elacridars; Elinafides; Elliott's B Solutions; Elsamitrucins; Emitefurs; Enloplatins; Enpromates; Enzastaurins; Epipropidines; Epirubicins; Epoetin alfas; Eptaloprosts; Erbulozoles; Esorubicins; Estramustines; Etanidazoles; Etoglucids; Etoposide

phosphates; Etoposide VP-16s; Etoposides; Etoprines; Exemestanes; Exisulinds; Fadrozoles; Fazarabines; Fenretinides; Filgrastims; Floxuridines; Fludarabines; Fluorouracils; 5-fluorouracils; Fluoxymesterones; Flurocitabines; Fosquidones; Fostriecins; Fotretamines; Fulvestrants; Galarubicins; Galocitabines; Gemcitabines; Gemtuzumabs/Ozogamicins; Geroquinols; Gimatecans; Gimeracils; Gloxazones; Glufosfamides; Goserelin acetates; Hydroxyureas; Ibritumomabs/Tiuxetans; Idarubicins; Ifosfamides; Ilmofosines; Ilomastats; Imatinib mesylates; Imexons; Improsulfans; Indisulams; Inproquones; Interferon α-2as; Interferon α-2bs; Interferon Alphas; Interferon Betas; Interferon Gammas; Interferons; Interleukin-2s and other Interleukins (including recombinant Interleukins); Intoplicines; Iobenguanes [131-1]; Iproplatins; Irinotecans; Irsogladines; Ixabepilones; Ketotrexates; L-Alanosines; Lanreotides; Lapatinibs; Ledoxantrones; Letrozoles; Leucovorins; Leuprolides; Leuprorelins (Leuprolides); Levamisoles; Lexacalcitols; Liarozoles; Lobaplatins; Lometrexols; Lomustines/CCNUs; Lomustines; Lonafanibs; Losoxantrones; Lurtotecans; Mafosfamides; Mannosulfans; Marimastats; Masoprocols; Maytansines; Mechlorethamines; Mechlorethamines/Nitrogen mustards; Megestrol acetates; Megestrols; Melengestrols; Melphalans; L-PAMs; Menogarils; Mepitiostanes; Mercaptopurines; 6-Mercaptopurine; Mesnas; Metesinds; Methotrexates; Methoxsalens; Metomidates; Metoprines; Meturedepas; Miboplatins; Miproxifenes; Misonidazoles; Mitindomides; Mitocarcins; Mitocromins; Mitoflaxones; Mitogillins; Mitoguazones; Mitomalcins; Mitomycin Cs; Mitomycins; Mitonafides; Mitoquidones; Mitospers; Mitotanes; Mitoxantrones; Mitozolomides; Mivobulins; Mizoribines; Mofarotenes; Mopidamols; Mubritinibs; Mycophenolic Acids; Nandrolone Phenpropionates; Nedaplatins; Nelarabines; Nemorubicins; Nitracrines; Nocodazoles; Nofetumomabs; Nogalamycins; Nolatrexeds; Nortopixantrones; Octreotides; Oprelvekins; Ormaplatins; Ortataxels; Oteracils; Oxaliplatins; Oxisurans; Oxophenarsines; Paclitaxels; Pamidronates; Patupilones; Pegademases; Pegaspargases; Pegfilgrastims; Peldesines; Peliomycins; Pelitrexols; Pemetrexeds; Pentamustines; Pentostatins; Peplomycins; Perfosfamides; Perifosines; Picoplatins; Pinafides; Pipobromans; Piposulfans; Pirfenidones; Piroxantrones; Pixantrones; Plevitrexeds; Plicamycin Mithramycins; Plicamycins; Plomestanes; Plomestanes; Porfimer sodiums; Porfimers; Porfiromycins; Prednimustines; Procarbazines; Propamidines; Prospidiums; Pumitepas; Puromycins; Pyrazofurins; Quinacrines; Ranimustines; Rasburicases; Riboprines; Ritrosulfans; Rituximabs; Rogletimides; Roquinimexs; Rufocromomycins; Sabarubicins; Safingols; Sargramostims; Satraplatins; Sebriplatins; Semustines; Simtrazenes; Sizofirans; Sobuzoxanes; Sorafenibs; Sparfosates; Sparfosic Acids; Sparsomycins; Spirogermaniums; Spiromustines; Spiroplatins; Spiroplatins; Squalamines; Streptonigrins; Streptovarycins; Streptozocins; Sufosfamides; Sulofenurs; Sunitinib Malate; 6-TG; Tacedinalines; Talcs; Talisomycins; Tallimustines; Tamoxifens; Tariquidars; Tauromustines; Tecogalans; Tegafurs; Teloxantrones; Temoporfins; Temozolomides; Teniposides/VM-26s; Teniposides; Teroxirones; Testolactones; Thiamiprines; Thioguanines; Thiotepas; Tiamiprines; Tiazofurins; Tilomisoles; Tilorones; Timcodars; Timonacics; Tirapazamines; Topixantrones; Topotecans; Toremifenes; Tositumomabs; Trabectedins (Ecteinascidin 743); Trastuzumabs; Trestolones; Tretinoins/ATRA; Triciribines; Trilostanes; Trimetrexates; Triplatin Tetranitrates; Triptorelins; Trofosfamides; Tubulozoles; Ubenimexs; Uracil Mustards; Uredepas; Valrubicins; Valspodars; Vapreotides; Verteporfins; Vinblastines; Vincristines; Vindesines; Vinepidines; Vinflunines; Vinformides; Vinglycinates; Vinleucinols; Vinleurosines; Vinorelbines; Vinrosidines; Vintriptols; Vinzolidines; Vorozoles; Xanthomycin A's (Guamecyclines); Zeniplatins; Zilascorbs [2-H]; Zinostatins; Zoledronate; Zorubicins; and Zosuquidars.

22. The pharmaceutical composition of any of claims 17-21 for use in treating a hyaluronan-associated disease or condition; for use in treating an excess of glycosaminoglycans; for use in treating a tumor; for use in treating glycosaminoglycan accumulation in the brain; for use in treating a cardiovascular disorder; for use in treating an ophthalmic disorder; for use in treating pulmonary disease; for use in treating cellulite; or for use in treating a proliferative disorder.

23. The pharmaceutical composition of any of claims 17-21 for increasing penetration of chemotherapeutic agents into solid tumors or for increasing bioavailability of drugs and other therapeutic agents.

24. A combination, comprising:

a first composition containing an esPH20 polypeptide of any of claims 1-5 or a conjugate of any of claims 6-10; and a second composition comprising a therapeutic agent.

25. The vector of claim 13 for use for treating a tumor.

26. The pharmaceutical composition of any of claims 17-21 for use for treating a tumor.

27. An esPH20 polypeptide of any of claims 1-5 or a conjugate of any of claims 6-10, for use in combination therapy with a therapeutic agent, wherein the esPH20 is for administration prior, subsequently, intermittently or simultane-

ously to the therapeutic agent.

28. The pharmaceutical composition for use of claim 22 or claim 26 or the esPH20 polypeptide for use according to claim 27, wherein the esPH20 polypeptide is administered so that it reaches the interstitium of skin or tissues.

29. The pharmaceutical composition or the esPH20 polypeptide for use of claim 28, wherein the esPH20 polypeptide is administered by subcutaneous administration methods.

Patentansprüche

1. Erweitert lösliche PH20-Hyaluronidase (extended soluble PH20 hyaluronidase, esPH20), wobei:

das esPH20-Polypeptid bei neutralem pH-Wert aktiv ist; das esPH20-Polypeptid löslich ist; und
das esPH20-Polypeptid ausgewählt ist aus:

einem Polypeptid, welches aus der Sequenz von Aminosäuren von Aminosäurenresten 36-495, 36-496, 36-497, 36-498, 36-499 oder 36-500 von SEQ ID NO: 107 besteht; oder
einem Polypeptid, welches Aminosäuresubstitutionen in der Sequenz von Aminosäuren von Aminosäurenresten 36-495, 36-496, 36-497, 36-498, 36-499 oder 36-500 von SEQ ID NO: 107 enthält,
wobei das Aminosäure-substituierte Polypeptid aus einer Sequenz von Aminosäuren besteht, welche mindestens 95% Sequenz-Identität mit der entsprechenden Aminosäuresequenz von Aminosäuren 36-495, 36-496, 36-497, 36-498, 36-499 oder 36-500 von SEQ ID NO: 107 aufweist.

2. esPH20 gemäß Anspruch 1, welche aus der Sequenz von Aminosäuren 36-495, 36-496, 36-497, 36-498, 36-499 oder 36-500 von SEQ ID NO: 107 besteht.

3. esPH20 gemäß Anspruch 1 oder Anspruch 2, welche N-glycosiliert ist.

4. esPH20 gemäß irgendeinem der Ansprüche 1-3, wobei die esPH20 N-glycosiliert ist und mindestens eine N-Acetylglucosamin-Gruppe verbunden mit jeweils mindestens drei Asparagin (N)-Resten umfasst.

5. esPH20 gemäß irgendeinem der Ansprüche 1-4 welche aufgereinigt oder isoliert ist.

6. Konjugat, umfassend die esPH20 gemäß irgendeinem der Ansprüche 1-5, wobei die esPH20 löslich ist.

7. Konjugat gemäß Anspruch 6, wobei die esPH20 an ein Polymer konjugiert ist.

8. Konjugat gemäß Anspruch 7, wobei das Polymer Dextran oder PEG ist.

9. Konjugat gemäß irgendeinem der Ansprüche 6-8, wobei die esPH20 an eine Gruppe konjugiert ist, welche ausgewählt ist aus einer/einem Multimerisierungs-Domäne, Toxin, detektierbaren Marker und Arzneimittel.

10. Konjugat gemäß irgendeinem der Ansprüche 6-9, wobei die esPH20 an eine Gruppe konjugiert ist, welche eine Fc-Domäne ist.

11. Nukleinsäuremolekül, welches die esPH20 gemäß irgendeinem der Ansprüche 1-5 codiert, wobei das Nukleinsäuremolekül keine PH20-Hyalonuridase von voller Länge (full length) codiert, aber die trunkierte PH20-Hyalonuridase codiert.

12. Vektor, umfassend ein Polynukleotid, welches aus einer Sequenz von Nukleotiden besteht, welche die esPH20 gemäß irgendeinem der Ansprüche 1-5 codiert, wobei die Sequenz von Nukleotiden ein Stop-Codon einschließt oder direkt davon gefolgt wird, welches operativ in den Vektor zur Expression der esPH20 gemäß irgendeinem der Ansprüche 1-5 eingefügt ist.

13. Vektor gemäß Anspruch 12, welcher ein Virus-Vektor ist.

14. Isolierte Zelle oder Zellkultur, umfassend den Vektor gemäß Anspruch 12 oder 13 oder ein Nukleinsäuremolekül

gemäß Anspruch 11.

15. Zelle oder Zellkultur gemäß Anspruch 14, wobei die Zelle eine CHO-zelle ist oder die Zellkultur eine CHO-zelle umfasst.

16. Zusammensetzung, umfassend eine esPH20 gemäß irgendeinem der Ansprüche 1-5 oder ein Konjugat gemäß irgendeinem der Ansprüche 6-10.

17. Pharmazeutische Zusammensetzung, umfassend eine esPH20 gemäß irgendeinem der Ansprüche 1-5 oder ein Konjugat gemäß irgendeinem der Ansprüche 6-10.

18. Pharmazeutische Zusammensetzung gemäß Anspruch 17, umfassend ein zusätzliches therapeutisches Agens.

19. Pharmazeutische Zusammensetzung gemäß Anspruch 18, wobei das therapeutische Agens ausgewählt wird aus: einem chemotherapeutischen Agens, einem schmerzlindernden Agens, einem entzündungshemmenden Agens, einem antimikrobischen Agens, einem amoebiciden Agens, einem trichomonaciden Agens, einem Anti-Parkinson-Agens, einem Anti-Malaria-Agens, einem krampflösenden Agens, einem Anti-Depressiva-Agens, einem Anti-Arthrose-Agens, einem anti-fungalen Agens, einem Anti-Hypertensivum-Agens, einem fiebersenkenden Agens, einem anti-parasitären Agens, einem Antihistamin-Agens, einem alpha-adrenergenen Agonist-Agens, einem Alpha-Blocker-Agens, einem anaesthetischen Agens, einem Bronchial-Dilatator-Agens, einem biociden Agens, einem bacteriociden Agens, einem bacteriostatischen Agens, einem beta-adrenergenen Blocker-Agens, einem Calcium-Kanal-Blocker-Agens, einem cardiovasculären Arzneistoff-Agens, einem kontrazeptiven Agens, einem abschwellenden Agens, einem diuretischen Agens, einem Depressiva-Agens, einem diagnostischen Agens, einem Elektrolyt-Agens, einem hypnotischen Agens, einem Hormon-Agens, einem hyperglycaemischen Agens, einem Muskelentspannungs-Agens, einem Muskel-Kontraktions-Agens, einem ophthalmischen Agens, einem parasympathomimetischen Agens, einem psychisch-stimulierendem Agens, einem Sedativum-Agens, einem sympathomimetischen Agens, einem Beruhigungsmittel-Agens, einem urinalen Agens, einem vaginalen Agens, einem viriciden Agens, einem Vitamin-Agens, einem nicht-steroidalen anti-inflammatorischen Agens, einem Angiotensin-umwandelndes-Enzym-Inhibitor-Agens, einem Polypeptid, einem Protein, einer Nukleinsäure, einem Arzneistoff, einem organischen Molekül, einem Schlaf-Induzierer, einem Antikörper, einem Immunoglobulin, einem Cytokin und einem Insulin.

20. Pharmazeutische Zusammensetzung gemäß Anspruch 18 oder Anspruch 19, wobei das therapeutische Agens einen Antikörper umfasst.

21. Pharmazeutische Zusammensetzung gemäß Anspruch 18, wobei das zusätzliche therapeutische Agens ausgewählt wird aus Acivicinen; Aclarubicinen; Acodazolen; Acroninen; Adozelesinen; Aldesleukinen; Alemtuzumabs; Alitretinoinen (9-Cis-Retinsäuren); Allopurinolen; Altretaminen; Alvocidiben; Ambazonen; Ambomycinen; Ametantronen; Amifostinen; Aminoglutethimiden; Amsacrinen; Anastrozolen; Anaxironen; Ancitabinen; Anthramycinen; Apaziquonen; Argimesnanen; Arsentrioxiden; Asparaginasen; Asperlinen; Atrimustinen; Azacitidinen; Azetepanen; Azotomycinen; Banoxantronen; Batabulinen; Batimastaten; lebendes BCG (BCG Live); Benaxibinen; Bendamustinen; Benzodepanen; Bexarotenen; Bevacizumab; Bicalutamiden; Bietaserpinen; Biricodaren; Bisantrenen; Bisantrenen; Bisnafide-Dimesylaten; Bizelesinen; Bleomycinen; Bortezomiben; Brequinaren; Bropiriminen; Budotitanen; Busulfanen; Cactinomycinen; Calusteronen; Canertiniben; Capecitabinen; Caracemiden; Carbetimeren; Carboplatinen; Carboquonen; Carmofuren; Carmustinen mit Polifeprosanen; Carmustinen; Carubicinen; Carzelesinen; Cedefingolen; Celecoxiben; Cemadotinen; Chlorambucilen; Cioteronelen; Cirolemycinen; Cisplatinen; Cladribinen; Clanfenuren; Clofarabinen; Crisnatolen; Cyclophosphamiden; liposomalen Cytarabinen (Cytarabin liposomals); Cytarabinen; Dacarbazinen; Dactinomycinen; Darbepoetin-Alfas; liposomalen Daunorubicinen (Daunorubicin liposomals); Daunorubicinen/Daunomycinen; Daunorubicinen; Decitabinen; Denileukin-Diftitoxen; Dexniguldipinen; Dexonanen; Dexrazoxanen; Dezaguaninen; Diaziquonen; Dibrospidiumen; Dienogestinen; Dinalinen; Disermoliden; Docetaxelen; Dofequidaren; Doxifluridinen; liposomalen Doxorubicinen (Doxorubicin liposomals); Doxorubicin-HCl; lipsomale Doxorubicin-HCl-Injektion; Doxorubicinen; Droloxifenen; Dromostanolon-Propionaten; Duazomycinen; Ecomustinen; Edatrexaten; Edotecarinen; Eflornithinen; Elacridaren; Elinafiden; Elliott's-B-Lösungen; Elsamitrucinen; Emitefuren; Enloplatinen; Enpromaten; Enzastaurinen; Epipropidinen; Epirubicinen; Epoetin-Alfas; Eptaloprosten; Erbulozolen; Esorubicinen; Estramustinen; Etanidazolen; Etogluciden; Etoposid-Phosphaten; Etoposid-VP-16s; Etoposiden; Etoprinen; Exemestanen; Exisulinden; Fadrozolen; Fazarabinen; Fenretiniden; Filgrastimen; Floxuridinen; Fludarabinen; Fluorouracilen; 5-Fluorouracilen; Fluoxymesteronen; Flurocitabinen; Fosquidonen; Fostriecinen; Fotretaminen; Fulvestranten; Galarubicinen; Galocitabinen; Gemcitabinen; Gemtuzumabs/Ozogamicinen; Geroquinolen; Gimatecanen; Gimeracilen; Gloxazonen; Glufosfamiden; Goserelin-Acetaten; Hydroxyureas; Ibritu-

momabs/Tiuxetanen; Idarubicinen; Ifosfamiden; Ilmofosinen; Ilomastaten; Imatinib-Mesylaten; Imexonen; Improsulfanen; Indisulamen; Inproquonen; Interferon-α-2as; Interferon-α-2bs; Interferon-Alphas; Interferon-Betas; Interferon-Gammas; Interferonen; Interleukin-2s und anderen Interleukinen (rekombinante Interleukine einschließend); Intoplicinen; Iobenguanen [131-I]; Iproplatinen; Irinotecanen; Irsogladinen; Ixabepilonen; Ketotrexaten; L-Alanosinen; Lanreotiden; Lapatiniben; Ledoxantronen; Letrozolen; Leucovorinen; Leuproliden; Leuprorelinen (Leuproliden); Levamisolen; Lexacalcitolen; Liarozolen; Lobaplatinen; Lometrexolen; Lomustinen/CCNUs; Lomustinen; Lonafarniben; Losoxantronen; Lurtotecanen; Mafosfamiden; Mannosulfanen; Marimastaten; Masoprocolen; Maytansinen; Mechlorethaminen; Mechlorethaminen/Stickstoff-Senfen (Nitrogen mustards); Megestrol-Acetaten; Megestrolen; Melengestrolen; Melphalanen; L-PAMs; Menogarilen; Mepitiostanen; Mercaptopurinen; 6-Mercaptopurinen; Mesnanen; Metesinden; Methotrexaten; Methoxsalenen; Metomidaten; Metoprinen; Meturedepanen; Miboplatinen; Miproxifenen; Misonidazolen; Mitindomiden; Mitocarcinen; Mitocrominen; Mitoflaxones; Mitogillins; Mitoguazones; Mitomalcins; Mitomycin-Cs; Mitomycinen; Mitonafiden; Mitoquidonen; Mitosperen; Mitotanen; Mitoxantronen; Mitozolomiden; Mivobulinen; Mizoribinen; Mofarotenen; Mopidamolen; Mubritiniben; Mycophenolische Säuren; Nandrolon-Phenpropionaten; Nedaplatinen; Nelarabinen; Nemorubicinen; Nitracrinen; Nocodazolen; Nofetumomabs; Nogalamycinen; Nolatrexeden; Nortopixantronen; Octreotiden; Oprelvekinen; Ormaplatinen; Ortataxels; Oteracilen; Oxaliplatinen; Oxisuranen; Oxophenarsinen; Paclitaxels; Pamidronaten; Patupilonen; Pegademasen; Pegaspargasen; Pegfilgrastimen; Peldesinen; Peliomycinen; Pelitrexolen; Pemetrexeds; Pentamustinen; Pentostatinen; Peplomycinen; Perfosfamiden; Perifosinen; Picoplatinen; Pinafiden; Pipobromanen; Piposulfanen; Pirfenidonen; Piroxantronen; Pixantronen; Plevitrexeden; Plicamycin-Mithramycinen; Plicamycinen; Plomestanen; Plomestanen; Porfimer-Natriums; Porfimeren; Porfiromycinen; Prednimustinen; Procarbazinen; Propamidinen; Prospidiumen; Pumitepanen; Puromycinen; Pyrazofurinen; Quinacrinen; Ranimustinen; Rasburicasen; Riboprinen; Ritrosulfanen; Rituximabs; Rogletimiden; Roquinimexen; Rufocromomycinen; Sabarubicinen; Safingolen; Sargramostimen; Satraplatinen; Sebriplatinen; Semustinen; Simtrazenen; Sizofiranen; Sobuzoxanen; Sorafeniben; Sparfosaten; Sparfosischen Säuren (Sparfosic Acids); Sparsomycinen; Spirogermaniumen; Spiromustinen; Spiroplatinen; Spiroplatinen; Squalaminen; Streptonigrinen; Streptovarycinen; Streptozocinen; Sufosfamiden; Sulofenuren; Sunitinib-Malat; 6-TG; Tacedinalinen; Talkumen; Talisomycinen; Tallimustinen; Tamoxifenen; Tariquidaren; Tauromustinen; Tecogalanen; Tegafuren; Teloxantronen; Temoporfinen; Temozolomiden; Teniposiden/VM-26s; Teniposiden; Teroxironen; Testolactonen; Thiamiprinen; Thioguaninen; Thiotepanen; Tiamiprinen; Tiazofurinen; Tilomisolen; Tiloronen; Timcodaren; Timonacicen; Tirapazaminen; Topixantronen; Topotecanen; Toremifenen; Tositumomabs; Trabectedinen (Ecteinascidin 743); Trastuzumabs; Trestolonen; Tretinoinen/ATRA; Triciribinen; Trilostanen; Trimetrexaten; Triplatin-Tetranitraten; Triptorelinen; Trofosfamiden; Tubulozolen; Ubenimexen; Uracil-Senfen (Uracil Mustards); Uredepanen; Valrubicinen; Valspodaren; Vapreotiden; Verteporfinen; Vinblastinen; Vincristinen; Vindesinen; Vinepidinen; Vinfluninen; Vinformiden; Vinglycinaten; Vinleucinolen; Vinleurosinen; Vinorelbinen; Vinrosidinen; Vintriptolen; Vinzolidinen; Vorozolen; Xanthomycin-A's (Guamecyclinen); Zeniplatinen; Zilascorben [2-H]; Zinostatinen; Zoledronaten; Zorubicinen; und Zosuquidaren.

22. Pharmazeutische Zusammensetzung gemäß irgendeinem der Ansprüche 17-21 zur Verwendung für die Behandlung einer/eines Hyaluronan-assoziierten Krankheit oder Zustands; zur Verwendung für die Behandlung eines Glycosaminglycan-Überschusses; zur Verwendung für die Behandlung eines Tumors; zur Verwendung für die Behandlung einer Glycosaminglycan-Anreicherung im Gehirn; zur Verwendung für die Behandlung einer kardiovaskulären Störung; zur Verwendung für die Behandlung einer ophthalmischen Störung; zur Verwendung für die Behandlung einer Lungenkrankeit; zur Verwendung für die Behandlung von Cellulite; oder zur Verwendung für die Behandlung einer proliferativen Störung.

23. Pharmazeutische Zusammensetzung gemäß irgendeinem der Ansprüche 17-21 zur Erhöhung des Eindringens von chemotherapeutischen Agenzien in einen soliden Tumor oder zur Erhöhung der Bioverfügbarkeit von Arzneistoffen und anderen therapeutischen Agenzien.

24. Kombination, umfassend:

eine erste Zusammensetzung, welche ein esPH20-Polypeptid gemäß irgendeinem der Ansprüche 1-5 oder ein Konjugat gemäß irgendeinem der Ansprüche 6-10 enthält; und
eine zweite Zusammensetzung, umfassend einen therapeutischen Agens.

25. Vektor gemäß Anspruch 13 zur Verwendung für die Behandlung eines Tumors.

26. Pharmazeutische Zusammensetzung gemäß irgendeinem der Ansprüche 17-21 zur Verwendung für die Behandlung eines Tumors.

27. esPH20-Polypeptid gemäß irgendeinem der Ansprüche 1-5 oder Konjugat gemäß irgendeinem der Ansprüche 6-10, zur Verwendung in Kombinationstherapie mit einem therapeutischen Agens, wobei die esPH20 zur Verabreichung vor, während, in Abständen zu oder gleichzeitig mit dem therapeutischen Agens ist.

28. Pharmazeutische Zusammensetzung zur Verwendung gemäß Anspruch 22 oder Anspruch 26 oder esPH20-Polypeptid zur Verwendung gemäß Anspruch 27, wobei das esPH20-Polypeptid so verabreicht wird, dass es das Interstitium der Haut oder von Geweben erreicht.

29. Pharmazeutische Zusammensetzung oder esPH20-Polypeptid zur Verwendung gemäß Anspruch 28, wobei das esPH20-Polypeptid durch subkutane Verabreichungsverfahren verabreicht wird.

Revendications

1. Hyaluronidase PH20 étendue soluble (esPH20), dans laquelle :

   le polypeptide esPH20 est actif à pH neutre ;
   le polypeptide esPH20 est soluble ; et
   le polypeptide esPH20 est choisi parmi :

   un polypeptide qui est constitué de la séquence d'acides aminés présentée comme des résidus d'acides aminés 36 à 495, 36 à 496, 36 à 497, 36 à 498, 36 à 499 ou 36 à 500 de SEQ ID NO : 107 ; ou
   un polypeptide qui contient des substitutions d'acides aminés dans la séquence d'acides aminés présentée comme des acides aminés 36 à 495, 36 à 496, 36 à 497, 36 à 498, 36 à 499 ou 36 à 500 de SEQ ID NO : 107, selon lequel le polypeptide à substitutions d'acides aminés est constitué d'une séquence d'acides aminés qui présente une identité de séquence d'au moins 95 % avec la séquence d'acides aminés correspondante présentée comme des acides aminés 36 à 495, 36 à 496, 36 à 497, 36 à 498, 36 à 499 ou 36 à 500 de SEQ ID NO : 107.

2. esPH20 selon la revendication 1 qui est constituée de la séquence d'acides aminés présentée comme des acides aminés 36 à 495, 36 à 496, 36 à 497, 36 à 498, 36 à 499 ou 36 à 500 de SEQ ID NO : 107.

3. esPH20 selon la revendication 1 ou la revendication 2 qui est N-glycosylée.

4. esPH20 selon l'une quelconque des revendications 1 à 3, dans laquelle l'esPH20 est N-glycosylée et comprend au moins une fraction N-acétylglucosamine liée à chacun d'au moins trois résidus d'asparagine (N).

5. esPH20 selon l'une quelconque des revendications 1 à 4 qui est purifiée ou isolée.

6. Conjugué, comprenant l'esPH20 selon l'une quelconque des revendications 1 à 5, dans lequel l'esPH20 est soluble.

7. Conjugué selon la revendication 6, dans lequel l'esPH20 est conjuguée à un polymère.

8. Conjugué selon la revendication 7, dans lequel le polymère est le dextrane ou le PEG.

9. Conjugué selon l'une quelconque des revendications 6 à 8, dans lequel l'esPH20 est conjuguée à une fraction choisie parmi un domaine de multimérisation, une toxine, une étiquette détectable et un médicament.

10. Conjugué selon l'une quelconque des revendications 6 à 9, dans lequel l'esPH20 est conjuguée à une fraction qui est un domaine Fc.

11. Molécule d'acide nucléique codant pour l'esPH20 selon l'une quelconque des revendications 1 à 5, dans laquelle la molécule d'acide nucléique ne code pas pour une hyaluronidase PH20 de pleine longueur mais code pour une hyaluronidase PH20 tronquée.

12. Vecteur, comprenant un polynucléotide qui est constitué d'une séquence de nucléotides codant pour l'esPH20 selon l'une quelconque des revendications 1 à 5, dans lequel la séquence de nucléotides comprend ou est immédiatement suivie d'un codon d'arrêt opérationnellement inséré dans le vecteur pour l'expression de l'esPH20 selon l'une

quelconque des revendications 1 à 5.

13. Vecteur selon la revendication 12 qui est un vecteur viral.

14. Cellule isolée ou culture cellulaire, comprenant le vecteur selon la revendication 12 ou la revendication 13 ou une molécule d'acide nucléique selon la revendication 11.

15. Cellule ou culture cellulaire selon la revendication 14, dans laquelle la cellule est une cellule CHO ou la culture cellulaire comprend une cellule CHO.

16. Composition, comprenant une esPH20 selon l'une quelconque des revendications 1 à 5 ou un conjugué selon l'une quelconque des revendications 6 à 10.

17. Composition pharmaceutique, comprenant une esPH20 selon l'une quelconque des revendications 1 à 5 ou un conjugué selon l'une quelconque des revendications 6 à 10.

18. Composition pharmaceutique selon la revendication 17, comprenant un agent thérapeutique supplémentaire.

19. Composition pharmaceutique selon la revendication 18, dans laquelle l'agent thérapeutique est choisi parmi : un agent de chimiothérapie, un agent analgésique, un agent anti-inflammatoire, un agent antimicrobien, un agent amoebicide, un agent trichomonacide, un agent antiparkinsonien, un agent antipaludéen, un agent anticonvulsivant, un agent antidépresseur, et un agent anti-arthritique, un agent antifongique, un agent antihypertenseur, un agent antipyrétique, un agent antiparasitaire, un agent antihistaminique, un agent agoniste alpha-adrénergique, un agent alpha-bloquant, un agent anesthésique, un agent bronchodilatateur, un agent biocide, un agent bactéricide, un agent bactériostatique, un agent bloquant bêta-adrénergique, un agent inhibiteur des canaux calciques, un agent médicamenteux cardio-vasculaire, un agent contraceptif, un agent décongestionnant, un agent diurétique, un agent dépresseur, un agent de diagnostic, un agent d'électrolyte, un agent hypnotique, un agent hormonal, un agent hyperglycémiant, un agent relaxant musculaire, un agent contractant musculaire, un agent ophtalmique, un agent parasympathomimétique, un agent psychostimulant, un agent sédatif, un agent sympathomimétique, un agent tranquillisant, un agent urinaire, un agent vaginal, un agent virucide, un agent vitaminique, un agent anti-inflammatoire non stéroïdien, un agent inhibiteur de l'enzyme de conversion de l'angiotensine, un polypeptide, une protéine, un acide nucléique, un médicament, une molécule organique, un agent d'endormissement; un anticorps, une immunoglobuline, un bisphosphonate, une cytokine et une insuline.

20. Composition pharmaceutique selon la revendication 18 ou la revendication 19, dans laquelle l'agent thérapeutique comprend un anticorps.

21. Composition pharmaceutique selon la revendication 18, dans laquelle l'agent thérapeutique supplémentaire est choisi parmi les acivicines; aclarubicines; acodazoles; acronines; adozélésines; aldesleukines; alemtuzumabs; alitrétinoïnes (acides 9-cis-rétinoïques) ; allopurinols; altrétamines; alvocidibs; ambazones; ambomycines; amétantrones; amifostines; aminoglutéthimides; amsacrines; anastrozoles; anaxirones; ancitabines; anthramycines ; apaziquones; argimesnas; trioxydes d'arsénic; asparaginases; asperlines; atrimustines; azacitidines; azétépas; azotomycines; banoxantrones ; batabulines; batimastats; BCG vivant; bénaxibines ; bendamustines ; benzodepas ; bexarotènes ; bévacizumab ; bicalutamides; biétaserpines ; biricodars ; bisantrènes ; bisantrènes ; dimésylates de bisnafide ; bizélésines; bléomycines; bortézomibs ; bréquinars ; bropirimines ; budotitanes ; busulfanes; cactinomycines ; calustérones; canertinibs ; capécitabines ; caracémides ; carbétimers ; carboplatines ; carboquones ; carmofurs ; carmustines avec des poliféprosans ; carmustines ; carubicines ; carzélésines; cédéfingols; célécoxibs; cémadotines; chlorambucils; ciotéronels; cirolémycines; cisplatines; cladribines; clanfénur ; clofarabines; crisnatols; cyclophosphamides; cytarabines liposomales; cytarabines; dacarbazines ; dactinomycines ; darbépoétines α; daunorubicines liposomales; daunorubicines/daunomycines ; daunorubicines ; décitabines ; denileukine diftitox ; dexniguldipines; dexonas ; dexrazoxanes; dézaguanines ; diaziquones ; dibrospidiums ; diénogests ; dinalines; disermolides; docétaxels; doféquidars ; doxifluridines; doxorubicines liposomales; doxorubicine HCl; injection liposomale de doxorubicine HCl; doxorubicines ; droloxifènes; propionates de dromostanolone; duazomycines ; écomustines ; édatrexates ; édotécarines; éflornithines; élacridars; élinafides; solutions B d'Elliott; elsamitrucines ; émitéfurs ; enloplatines ; enpromates ; enzastaurines; épipropidines ; épirubicines ; époétines α; éptaloprosts; erbulozoles; ésorubicines ; estramustines ; étanidazoles; étoglucides; phosphates d'étoposide ; étoposide VP-16; étoposides ; étoprines ; exemestanes ; exisulinds; fadrozoles ; fazarabines; fenrétinides ; filgrastimes; floxuridines; fludarabines; fluorouracils; 5-fluorouracils; fluoxymestérones; flurocitabines; fosquidones ;

fostriécines ; fotrétamines; fulvestrants; galarubicines; galocitabines; gemcitabines; gemtuzumabs/ozogamicines; géroquinols; gimatécans; giméracils; gloxazones; glufosfamides; acétate de goséréline; hydroxyurées; ibritumomabs/tiuxétans; idarubicines; ifosfamides; ilmofosines; ilomastats; mésylates d'imatinib ; iméxons; improsulfanes; indisulams; inproquones; interféron α-2as ; interféron α-2bs; interférons α; interférons β; interférons γ; interférons; interleukin-2s et autres interleukines (y compris les interleukines recombinantes) ; intoplicines; iobenguanes [131-I]; iproplatines; irinotécanes ; irsogladines; ixabépilones; kétotrexates; L-alanosines; lanréotides; lapatinibs; lédoxantrones; létrozoles; leucovorines; leuprolides; leuprorelines (leuprolides); lévamisoles; lexacalcitols; liarozoles; lobaplatines; lométrexols ; lomustines/ccnus; lomustines ; lonafarnibs ; losoxantrones ; lurtotécanes; mafosfamides; mannosulfanes; marimastats ; masoprocols; maytansines ; méchloréthamines; méchloréthamines/moutardes à l'azote; acétates de mégestrol ; mégestrols; mélengestrols; melphalans; L-PAMs ; ménogarils; mépitiostanes ; mercaptopurines ; 6-mercaptopurine ; mesnas ; métésinds ; méthotrexates ; méthoxsalènes ; métomidates ; métoprines ; méturédépas ; miboplatines; miproxifènes ; misonidazoles; mitindomides; mitocarcines ; mitocrominines; mitoflaxones; mitogillines; mitoguazones ; mitomalcines; mitomycine Cs ; mitomycines ; mitonafides ; mitoquidones ; mitospers ; mitotanes ; mitoxantrones ; mitozolomides; mivobulines; mizoribines; mofarotènes; mopidamols; mubritinibs; acides mycophénoliques; phenpropionates de nandrolone ; nédaplatines; nélarabines ; némorubicines ; nitracrines; nocodazoles; nofétumomabs ; nogalamycines; nolatrexeds; nortopixantrones ; octréotides ; oprélvékines; ormaplatines; ortataxels; otéracils; oxaliplatines; oxisurans ; oxophénarsines ; paclitaxels; pamidronates ; patupilones ; pégadémases ; pégaspargases ; pegfilgrastimes ; peldésines ; péliomycines; pelitrexols; pémétrexeds ; pentamustines ; pentostatines ; péplomycines; perfosfamides ; périfosines ; picoplatines; pinafides ; pipobromanes; piposulfanes; pirfénidones; piroxantrones ; pixantrones ; plevitrexeds; plicamycines mithramycines ; plicamycines; plomestanes; plomestanes; porfimères sodiques ; porfimères ; porfiromycines ; prednimustines ; procarbazines ; propamidines ; prospidiums ; pumitépas ; puromycines ; pyrazofurines ; quinacrines; ranimustines ; rasburicases; riboprines ; ritrosulfans; rituximabs; roglétimides; roquinimexs; rufocromomycines ; sabarubicines ; safingols; sargramostimes ; satraplatines; sébriplatines; sémustines ; simtrazènes; sizofiranes; sobuzoxanes ; sorafénibs ; sparfosates ; acides sparfosiques ; sparsomycines ; spirogermaniums ; spiromustines ; spiroplatines; spiroplatines; squalamines; streptonigrines ; streptovarycines ; streptozocines ; sufosfamides; sulofénurs; malate de sunitinib ; 6-TG; tacédinalines; talcs; talisomycines; tallimustines; tamoxifènes; tariquidars ; tauromustines ; técogalans; tégafurs ; téloxantrones ; témoporfines ; témozolomides ; téniposides/VM-26s; teniposides; teroxirones; testolactones; thiamiprines ; thioguanines ; thiotépas ; tiamiprines ; tiazofurines ; tilomisoles; tilorones; timcodars ; timonacics; tirapazamines ; topixantrones ; topotécanes ; torémifènes ; tositumomabs ; trabectédines (éctéinascidine 743); trastuzumabs ; trestolones; trétinoïnes/ATRA; triciribines; trilostanes; trimétrexates; téetranitrates de triplatine; triptorélines; trofosfamides ; tubulozoles; ubénimexs ; moutardes à l'uracil ; urédépas ; valrubicines; valspodars; vapréotides ; vertéporfines ; vinblastines; vincristines ; vindésines ; vinépidines; vinflunines; vinformides ; vinglycinates; vinleucinols; vinleurosines; vinorelbines; vinrosidines ; vintriptols; vinzolidines; vorozoles; xanthomycine A's (guamécyclines) ; zéniplatines; zilascorbs [2-H] ; zinostatines ; zolédronate; zorubicines; et zosuquidars.

22. Composition pharmaceutique selon l'une quelconque des revendications 17 à 21 à utiliser dans le traitement d'une maladie ou d'une affection associée au hyaluronane ; à utiliser dans le traitement d'un excès de glycosaminoglycanes; à utiliser dans le traitement d'une tumeur; à utiliser dans le traitement de l'accumulation de glycosaminoglycanes dans le cerveau ; à utiliser dans le traitement d'un trouble cardiovasculaire ; à utiliser dans le traitement d'un trouble ophtalmique ; à utiliser dans le traitement d'une maladie pulmonaire ; à utiliser dans le traitement de la cellulite ; ou à utiliser dans le traitement d'un trouble prolifératif.

23. Composition pharmaceutique à utiliser selon l'une quelconque des revendications 17 à 21, pour augmenter la pénétration d'agents de chimiothérapie dans des tumeurs solides ou pour augmenter la biodisponibilité des médicaments et d'autres agents thérapeutiques.

24. Combinaison, comprenant :

une première composition contenant un polypeptide esPH20 selon l'une quelconque des revendications 1 à 5 ou un conjugué selon l'une quelconque des revendications 6 à 10 ; et
une seconde composition comprenant un agent thérapeutique.

25. Vecteur selon la revendication 13 à utiliser pour le traitement d'une tumeur.

26. Composition pharmaceutique selon l'une quelconque des revendications 17 à 21 à utiliser pour le traitement d'une tumeur.

27. Polypeptide esPH20 selon l'une quelconque des revendications 1 à 5 ou conjugué selon l'une quelconque des revendications 6 à 10, à utiliser dans une thérapie combinatoire avec un agent thérapeutique, dans lequel l'esPH20 est destinée à être administrée avant, après, par intermittence ou simultanément avec l'agent thérapeutique.

28. Composition pharmaceutique à utiliser selon la revendication 22 ou la revendication 26 ou polypeptide esPH20 à utiliser selon la revendication 27, où le polypeptide esPH20 est administré de manière à atteindre l'interstitium de la peau ou des tissus.

29. Composition pharmaceutique ou polypeptide esPH20 à utiliser selon la revendication 28, où le polypeptide esPH20 est administré par un procédé d'administration sous-cutanée.

```
HumanPH20          MGVLKFKHIFFRSFVKSSGVSQIVFTFLLIPCCLTLNFRAPPVIPNVPFLWAWNAPSEFC 60
ChimpanzeePH20     MGVLKFKHIFFRSFVKSSGVSQIVFTFLLIPCCLTLNFRAPPVIPNVPFLWAWNAPSEFC 60
                   ************************************************************

HumanPH20          LGKFDEPLDMSLFSFIGSPRINATGQGVTIFYVDRLGYYPYIDSITGVTVNGGIPQKISL 120
ChimpanzeePH20     LGKFDEPLDMSLFSFIGSPRINVTGQDVTIFYVDRLGYYPYIDSITGVTVNGGIPQKISL 120
                   **********************.***.********************************

HumanPH20          QDHLDKAKKDITFYMPVDNLGMAVIDWEEWRPTWARNWKPKDVYKNRSIELVQQQNVQLS 180
ChimpanzeePH20     QDHLDKAKKDITFYMPVDNLGMAVIDWEEWRPTWARNWKPKDVYKNRSIELVQQQNVQLS 180
                   ************************************************************

HumanPH20          LTEATEKAKQEFEKAGKDFLVETIKLGKLLRPNHLWGYYLFPDCYNHHYKKPGYNGSCFN 240
ChimpanzeePH20     LTEATEKAKQEFEKAGKDFLVETIKLGKLLRPNHLWGYYLFPDCYNHHYKKPGYNGSCFN 240
                   ************************************************************

HumanPH20          VEIKRNDDLSWLWNESTALYPSIYLNTQQSPVAATLYVRNRVREAIRVSKIPDAKSPLPV 300
ChimpanzeePH20     VEIKRNDDLSWLWNESTALYPSIYLNTQQSPVAATLYVRNRVQEAIRVSKIPDAKSPLPV 300
                   ************************************:***********************

HumanPH20          FAYTRIVFTDQVLKFLSQDELVYTFGETVALGASGIVIWGTLSIMRSMKSCLLLDNYMET 360
ChimpanzeePH20     FVYTRIVFTDQVLKFLSQDELVYTFGETVALGASGIVIWGTLSIMRSMKSCLLLDNYMET 360
                   *.***********************************************************

HumanPH20          ILNPYIINVTLAAKMCSQVLCQEQGVCIRKNWNSSDYLHLNPDNFAIQLEKGGKFTVRGK 420
ChimpanzeePH20     ILNPYIINVTLAAKMCSQVLCQEQGVCIRKNWNSSDYLHLNPDNFAIQLEKGGKFTVRGK 420
                   ************************************************************

HumanPH20          PTLEDLEQFSEKFYCSCYSTLSCKEKADVKDTDAVDVCIADGVCIDAFLKPPMETEEPQI 480
ChimpanzeePH20     PTLEDLEQFSEKFYCSCYSTLSCKEKADVKDTDAVDVCIADGVCIDAFLKPPMETEESQI 480
                   ************************************************************.**

HumanPH20          FYNASPSTLSATMFI-VSILFLIISSVASL 509
ChimpanzeePH20     FYNASPSTLSATMFIDLCDLYLVPTSYLIL 510
                   *************** :. *:*: :*   *
```

FIG. 1

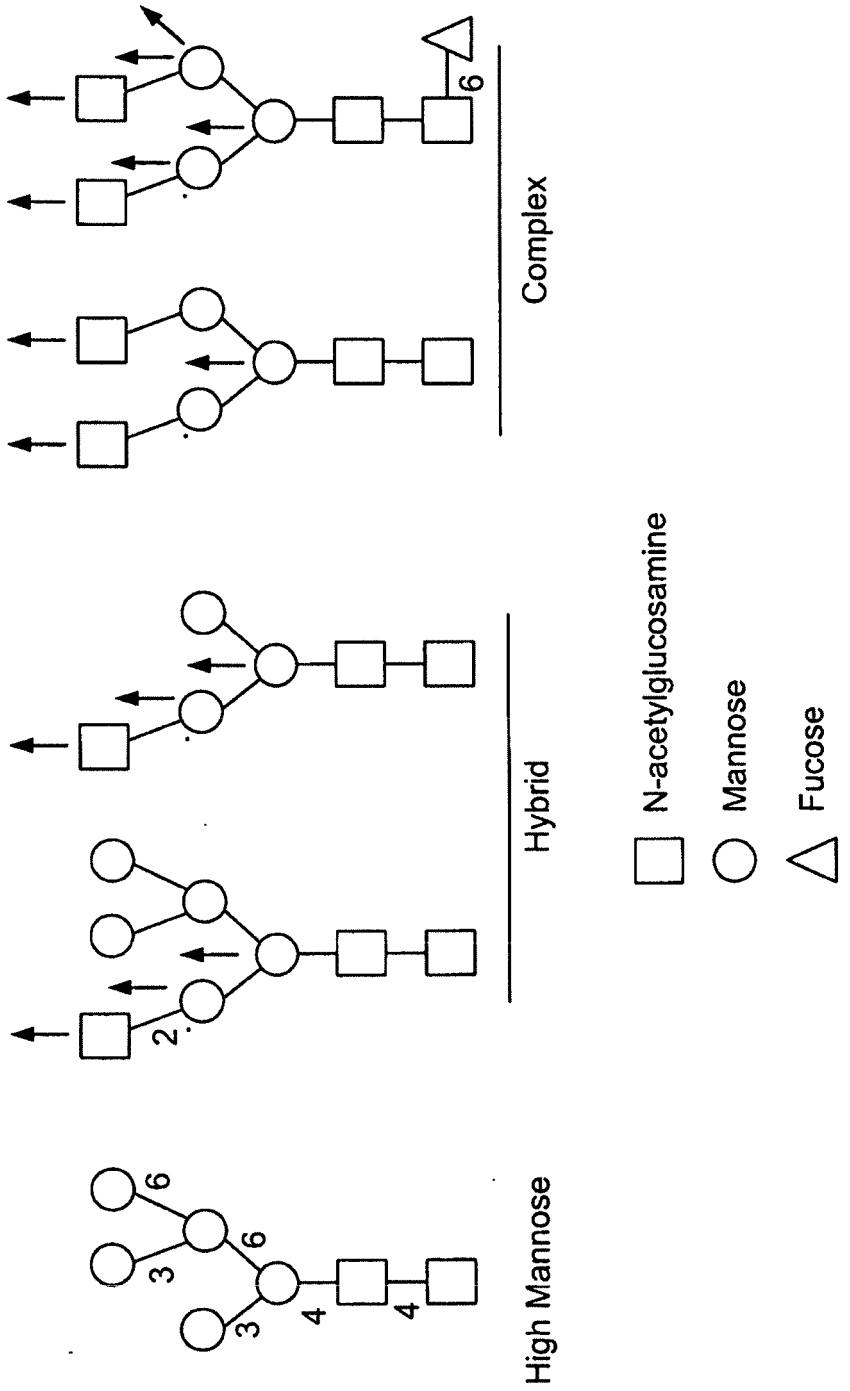
High Mannose
Hybrid
Complex
N-acetylglucosamine
Mannose
Fucose
2
3
3
4
4
6
6
6

FIG. 2

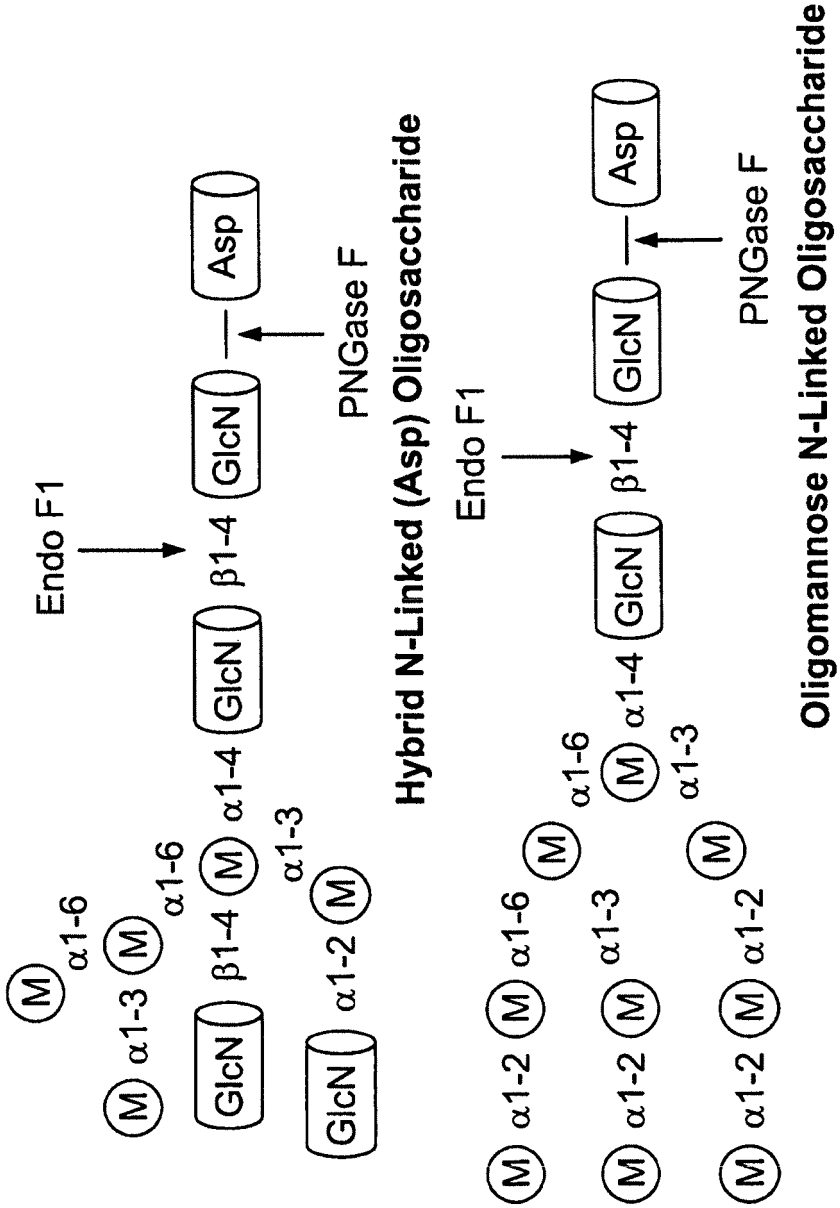
Endo F1
PNGase F
Hybrid N-Linked (Asp) Oligosaccharide
Endo F1
PNGase F
Oligomannose N-Linked Oligosaccharide
M
α1-6
α1-3
β1-4
α1-4
α1-2
GlcN
Asp

FIG. 3A

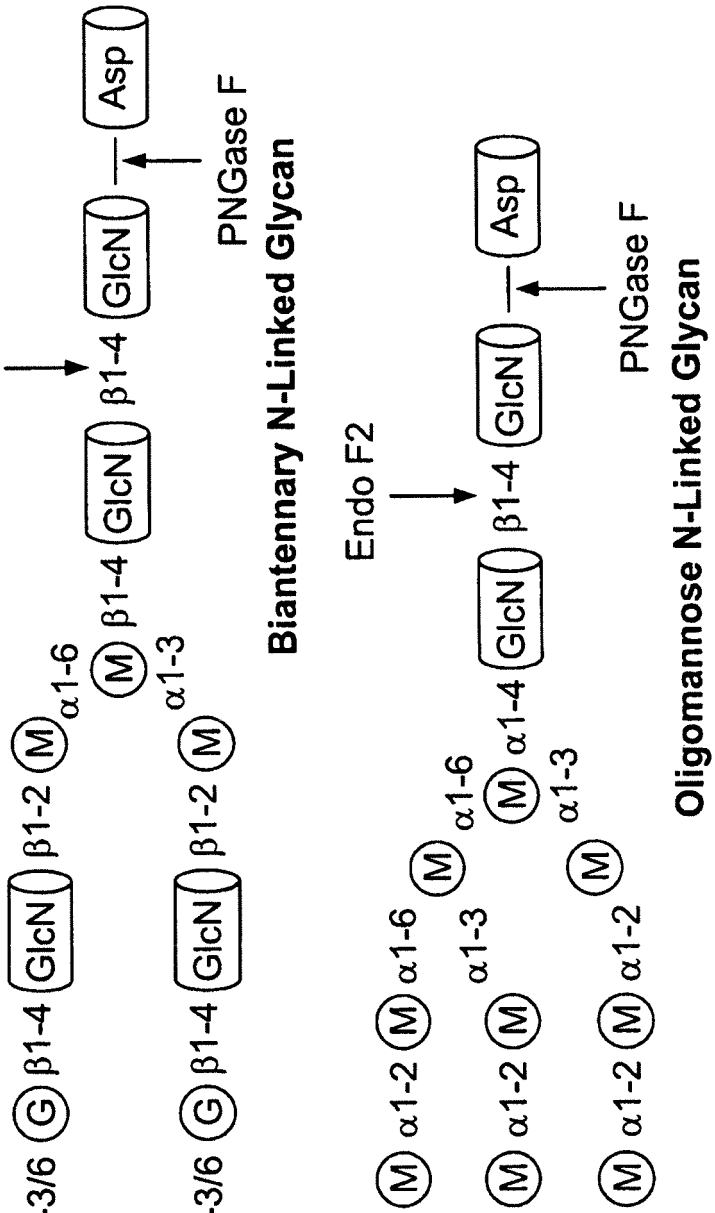
Endo F2
PNGase F
Biantennary N-Linked Glycan
S
α2-3/6
G
β1-4
GlcN
β1-2
M
α1-6
α1-3
β1-4
GlcN
GlcN
Asp
Endo F2
PNGase F
Oligomannose N-Linked Glycan
α1-2
α1-6
α1-3
α1-4
α1-2
α1-3
α1-2

FIG. 3B

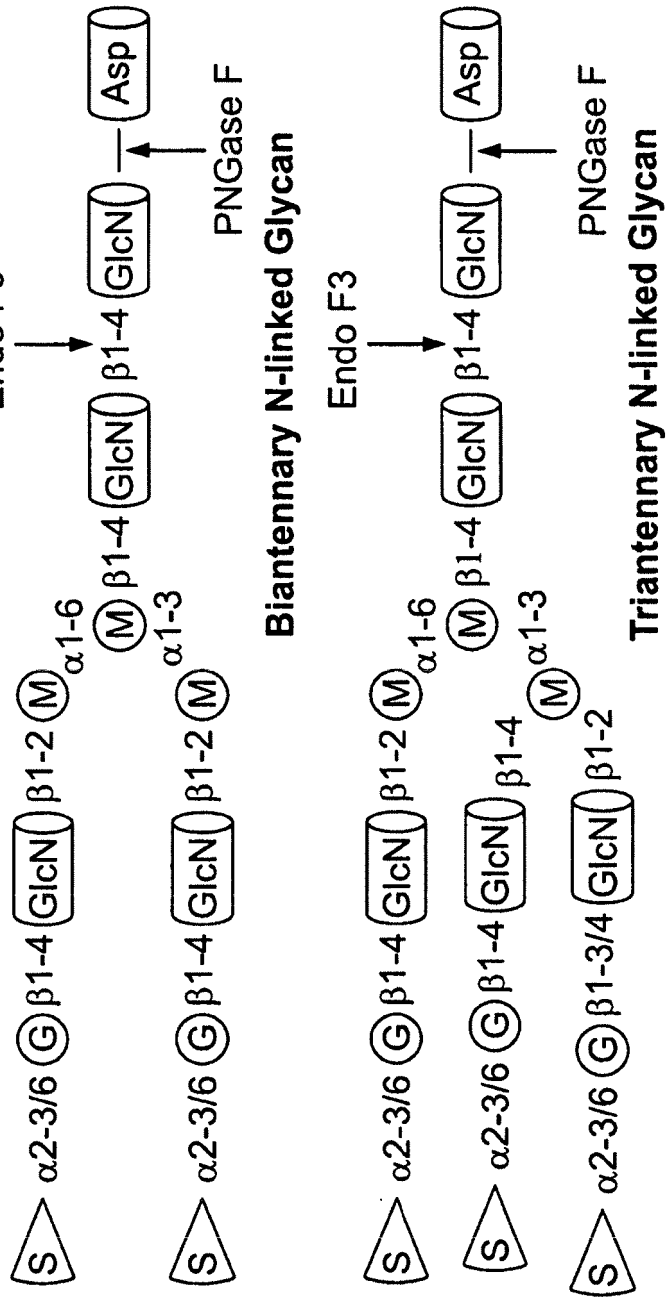
Endo F3
PNGase F
Biantennary N-linked Glycan
Endo F3
PNGase F
Triantennary N-linked Glycan
S
α2-3/6
G
β1-4
GlcN
β1-2
M
α1-6
α1-3
β1-4
β1-3/4
Asp

FIG. 3C

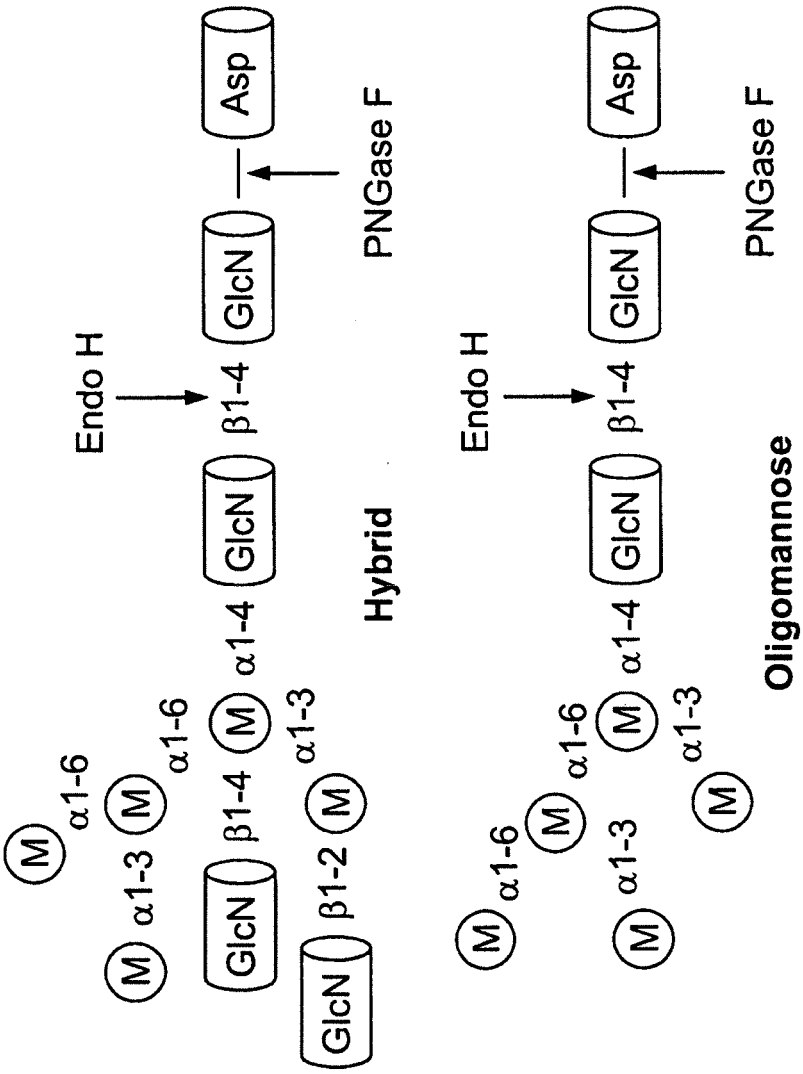
Endo H
PNGase F
Asp
GlcN
β1-4
α1-4
α1-6
α1-3
β1-2
M
Hybrid
Oligomannose

FIG. 3D

REFERENCES CITED IN THE DESCRIPTION

This list of references cited by the applicant is for the reader's convenience only. It does not form part of the European patent document. Even though great care has been taken in compiling the references, errors or omissions cannot be excluded and the EPO disclaims all liability in this regard.

Patent documents cited in the description

- US 20040268425 A **[0017] [0259] [0261] [0262]**
- US 20050260186 A **[0017] [0244] [0259] [0261] [0262]**
- US 20060104968 A **[0017] [0165] [0249] [0259] [0261] [0262]**
- US 381844 A **[0017] [0262]**
- US 12386249 B **[0017] [0262]**
- US 12387225 B **[0017] [0259] [0262]**
- US 12386222 B **[0017] [0259] [0262]**
- US 20070067855 A **[0160] [0161]**
- US 6461863 B **[0161]**
- US 20030157108 A **[0161]**
- WO 2004056312 A **[0161]**
- US 5672662 A **[0165] [0166]**
- US 6737505 B **[0165] [0166]**
- US 20040235734 A **[0165] [0166]**
- US 5932462 A **[0166]**
- US 6495659 B **[0166]**
- US 4002531 A **[0166]**
- US 4179337 A **[0166]**
- US 5122614 A **[0166]**
- US 5183550 A **[0166]**
- US 5324 A **[0166]**
- US 844 A **[0166]**
- US 5446090 A **[0166]**
- US 5612460 A **[0166]**
- US 5643575 A **[0166]**
- US 5766581 A **[0166]**
- US 5795 A **[0166]**
- US 569 A **[0166]**
- US 5808096 A **[0166]**
- US 5900461 A **[0166]**
- US 5919455 A **[0166]**
- US 5985263 A **[0166]**
- US 5990 A **[0166]**
- US 237 A **[0166]**
- US 6113906 A **[0166]**
- US 6214966 B **[0166]**
- US 6258351 B **[0166]**
- US 6340742 B **[0166]**
- US 6413507 B **[0166]**
- US 6420339 B **[0166]**
- US 6437025 B **[0166]**
- US 6448369 B **[0166]**
- US 6461802 B **[0166]**
- US 6828401 B **[0166]**
- US 6858736 B **[0166]**
- US 20010021763 A **[0166]**
- US 20010044526 A **[0166]**
- US 20010046481 A **[0166]**
- US 20020052430 A **[0166]**
- US 20020072573 A **[0166]**
- US 20020156047 A **[0166]**
- US 20030114647 A **[0166]**
- US 20030143596 A **[0166]**
- US 20030158333 A **[0166]**
- US 20030220447 A **[0166]**
- US 20040013637 A **[0166]**
- US 2005000360 A **[0166]**
- US 20050114037 A **[0166]**
- US 20050171328 A **[0166]**
- US 20050209416 A **[0166]**
- EP 01064951 A **[0166]**
- EP 0822199 A **[0166]**
- WO 00176640 A **[0166]**
- WO 0002017 A **[0166]**
- WO 0249673 A **[0166]**
- WO 9428024 A **[0166]**
- WO 0187925 A **[0166]**
- US 4952496 A **[0184]**
- US 3710795 A **[0215]**
- US 4044126 A **[0223]**
- US 4414209 A **[0223]**
- US 4364923 A **[0223]**
- US 3536809 A **[0230]**
- US 3598123 A **[0230]**
- US 3630200 A **[0230]**
- US 3845770 A **[0230]**
- US 3847770 A **[0230]**
- US 3916899 A **[0230]**
- US 4008719 A **[0230]**
- US 4687610 A **[0230]**
- US 4769027 A **[0230]**
- US 5059595 A **[0230]**
- US 5073543 A **[0230]**
- US 5120548 A **[0230]**
- US 5354566 A **[0230]**
- US 5591767 A **[0230]**
- US 5639476 A **[0230]**
- US 5674533 A **[0230]**
- US 5733566 A **[0230]**
- US 5323907 A **[0239]**
- US 5052558 A **[0239]**
- US 5033252 A **[0239]**
- WO 8802261 A **[0256]**
- US 1238184412386249 A **[0259]**
- US 5292509 A **[0288]**
- US 6745776 B **[0290]**

- US 10795095 A **[0340]**
- US 11065716 B **[0340]**
- US 11238171 B **[0340]**
- US 61201384 B **[0389]**
- US 61281240 B **[0389]**

Non-patent literature cited in the description

- **LAURENT TC et al.** *FASEB J,* 1992, vol. 6, 2397-2404 **[0002]**
- **BORDIER et al.** *J. Biol. Chem.,* 1981, vol. 256, 1604-7 **[0024] [0118] [0315]**
- **UDENFRIEND et al.** *Methods Enzymol.,* 1995, vol. 250, 571-582 **[0042] [0117] [0254]**
- **EISENHABER et al.** *J. Biol. Chem.,* 1999, vol. 292, 741-758 **[0042] [0117] [0254]**
- **KRONEGG ; BULOZ.** *Detection/prediction of GPI cleavage site (GPI-anchor) in a protein (DGPI),* 1999 **[0042]**
- **FANKHAUSER et al.** *Bioinformatics,* 2005, vol. 21, 1846-1852, 129.194.185.165/dgpi **[0042]**
- **OMAETXEBARRIA et al.** *Proteomics,* 2007, vol. 7, 1951-1960 **[0042] [0117] [0254]**
- **PIERLEONI et al.** *BMC Bioinformatics,* 2008, vol. 9, 392, expasy.ch/tools **[0042]**
- *J. Biol. Chem.,* 1968, vol. 243, 3557-3559 **[0047]**
- Computational Molecular Biology. Oxford University Press, 1988 **[0055] [0056]**
- Biocomputing: Informatics and Genome Projects. Academic Press, 1993 **[0055] [0056]**
- Computer Analysis of Sequence Data. Humana Press, 1994 **[0055] [0056]**
- **HEINJE, G.** Sequence Analysis in Molecular Biology. Academic Press, 1987 **[0055]**
- Sequence Analysis Primer. M Stockton Press, 1991 **[0055] [0056]**
- **CARILLO, H. ; LIPTON, D.** *SIAM J Applied Math,* 1988, vol. 48, 1073 **[0055]**
- Sequence Analysis in Molecular Biology. Academic Press, 1987 **[0056]**
- **CARILLO et al.** *SIAM J Applied Math,* 1988, vol. 48, 1073 **[0056] [0057]**
- **PEARSON et al.** *Proc. Natl. Acad. Sci. USA,* 1988, vol. 85, 2444 **[0057]**
- **DEVEREUX, J. et al.** *Nucleic Acids Research,* 1984, vol. 12 (I), 387 **[0057]**
- **ATSCHUL, S.F. et al.** *J Mol Biol,* 1990, vol. 215, 403 **[0057]**
- Guide to Huge Computers. Academic Press, 1994 **[0057]**
- **NEEDLEMAN et al.** *J. Mol. Biol.,* 1970, vol. 48, 443 **[0057]**
- **SMITH ; WATERMAN.** *Adv. Appl. Math.,* 1981, vol. 2, 482 **[0057]**
- **GRIBSKOV et al.** *Nucl. Acids Res.,* 1986, vol. 14, 6745 **[0057]**
- National Biomedical Research Foundation. ATLAS OF PROTEIN SEQUENCE AND STRUCTURE. 1979, 353-358 **[0057]**
- *Biochem.,* 1972, vol. 11, 1726 **[0107]**
- **CHERR et al.** *Matrix Biology,* 2001, vol. 20, 515-525 **[0113]**
- **WHITE et al.** *J. Cell Sci.,* 2000, vol. 113 (4), 721-727 **[0116]**
- **PIERLEONI et al.** *BMC Bioinformatics,* 2008, vol. 9, 392 **[0116] [0117] [0254]**
- **KRONEGG ; BULOZ.** *Detection/prediction of GPI cleavage site (GPI-anchor) in a protein (DGPI),* 1999, 129.194.185.165/dgpi **[0117] [0254]**
- **FANKHAUSER et al.** *Bioinformatics,* 2005, vol. 21, 1846-1852 **[0117] [0254]**
- **LIN et al.** *J. Biol. Chem.,* 1994, vol. 125, 1157-1163 **[0118]**
- **MA et al.** *Glycobiology,* 2006, vol. 15 (2), 158R-184R **[0160]**
- **NAKAYAMA et al.** *J. Biol. Chem.,* 2001, vol. 276, 16100-16106 **[0160]**
- **STURLA et al.** *Glycobiology,* 2005, vol. 15 (10), 924-935 **[0160]**
- **RIPKA et al.** *Arch. Biochem. Biophys.,* 1986, vol. 249, 533-545 **[0161]**
- **ROBERTS et al.** *Advanced Drug Delivery Review,* 2002, vol. 54, 459-476 **[0164]**
- Poly(ethylene glycol), Chemistry and Biological Applications. ACS Symposium Series, 1997, 680 **[0164]**
- **MEHVAR et al.** *J. Pharm. Pharmaceut. Sci.,* 2000, vol. 3 (1), 125-136 **[0164]**
- **HARRIS ; CHESS.** *Nat Rev Drug Discov.,* 2003, vol. 2 (3), 214-21 **[0164]**
- **TSUBERY.** *J Biol. Chem,* 2004, vol. 279 (37), 38118-24 **[0164]**
- **HARRIS.** *Adv. Drug Deliv. Rev.,* 2002, vol. 54, 459-476 **[0165]**
- **VERONESE et al.** *Bioorg. Med. Chem. Lett.,* 2002, vol. 12, 177-180 **[0165]**
- **CHAPMAN et al.** *Nature Biotech.,* 1999, vol. 17, 780-783 **[0165]**
- **SATO.** *Adv. Drug Deliv. Rev.,* 2002, vol. 54, 487-504 **[0165]**
- **LU ; FELIX.** *Int. J. Peptide Protein Res.,* 1994, vol. 43, 127-138 **[0165] [0214]**
- **LU ; FELIX.** *Peptide Res.,* 1993, vol. 6, 142-6 **[0165] [0214]**
- **FELIX et al.** *Int. J. Peptide Res.,* 1995, vol. 46, 253-64 **[0165] [0214]**
- **BENHAR et al.** *J. Biol. Chem.,* 1994, vol. 269, 13398-404 **[0165] [0214]**
- **BRUMEANU et al.** *J Immunol.,* 1995, vol. 154, 3088-95 **[0165]**

- **CALICETI et al.** *Adv. Drug Deliv. Rev.,* 2003, vol. 55 (10), 1261-77 **[0165] [0214]**
- **MOLINEUX.** *Pharmacotherapy,* 2003, vol. 23 (2), 3S-8S **[0165] [0214]**
- **MONFARDINI et al.** *Bioconjugate Chem.,* 1995, vol. 6, 62-69 **[0166]**
- **VERONESE et al.** *J. Bioactive Compatible Polymers,* 1997, vol. 12, 197-207 **[0166]**
- **STEWART et al.** Solid-Phase Peptide Synthesis. WH Freeman Co, 1969 **[0175]**
- **MERRIFIELD J.** *JAm Chem Soc.,* 1963, vol. 85, 2149-2154 **[0175]**
- **BEMOIST ; CHAMBON.** *Nature,* 1981, vol. 290, 304-310 **[0182]**
- **YAMAMOTO et al.** *Cell,* 1980, vol. 22, 787-797 **[0182]**
- **WAGNER et al.** *Proc. Natl. Acad. Sci. USA,* 1981, vol. 78, 1441-1445 **[0182]**
- **BRINSTER et al.** *Nature,* 1982, vol. 296, 39-42 **[0182]**
- **JAY et al.** *Proc. Natl. Acad. Sci. USA,* 1981, vol. 78, 5543 **[0182]**
- **DEBOER et al.** *Proc. Natl. Acad. Sci. USA,* 1983, vol. 80, 21-25 **[0182]**
- Useful Proteins from Recombinant Bacteria. *Scientific American,* 1980, vol. 242, 79-94 **[0182]**
- **HERRARA-ESTRELLA et al.** *Nature,* 1984, vol. 303, 209-213 **[0182]**
- **GARDNER et al.** *Nucleic Acids Res.,* 1981, vol. 9, 2871 **[0182]**
- **HERRERA-ESTRELLA et al.** *Nature,* 1984, vol. 310, 115-120 **[0182]**
- **SWIFT et al.** *Cell,* 1984, vol. 38, 639-646 **[0182]**
- **OMITZ et al.** *Cold Spring Harbor Symp. Quant. Biol.,* 1986, vol. 50, 399-409 **[0182]**
- **MACDONALD.** *Hepatology,* 1987, vol. 7, 425-515 **[0182]**
- **HANAHAN et al.** *Nature,* 1985, vol. 315, 115-122 **[0182]**
- **GROSSCHEDL et al.** *Cell,* 1984, vol. 38, 647-658 **[0182]**
- **ADAMS et al.** *Nature,* 1985, vol. 318, 533-538 **[0182]**
- **ALEXANDER et al.** *Mol. Cell Biol.,* 1987, vol. 7, 1436-1444 **[0182]**
- **LEDER et al.** *Cell,* 1986, vol. 45, 485-495 **[0182]**
- **PINCKERT et al.** *Genes and Devel.,* 1987, vol. 1, 268-276 **[0182]**
- **KRUMLAUF et al.** *Mol. Cell. Biol.,* 1985, vol. 5, 1639-1648 **[0182]**
- **HAMMER et al.** *Science,* 1987, vol. 235, 53-58 **[0182]**
- **KELSEY et al.** *Genes and Devel.,* 1987, vol. 1, 161-171 **[0182]**
- **MAGRAM et al.** *Nature,* 1985, vol. 315, 338-340 **[0182]**
- **KOLLIAS et al.** *Cell,* 1986, vol. 46, 89-94 **[0182]**
- **READHEAD et al.** *Cell,* 1987, vol. 48, 703-712 **[0182]**
- **SHANI.** *Nature,* 1985, vol. 314, 283-286 **[0182]**
- **MASON et al.** *Science,* 1986, vol. 234, 1372-1378 **[0182]**
- **SCHARF et al.** *Results Probl Cell Differ,* 1994, vol. 20, 125-62 **[0183]**
- **BITTNER et al.** *Methods in Enzymol,* 1987, vol. 153, 516-544 **[0183]**
- **WIGLER M et al.** *Cell,* 1977, vol. 11, 223-32 **[0190]**
- **LOWY I et al.** *Cell,* 1980, vol. 22, 817-23 **[0190]**
- **WIGLER M et al.** *Proc. Natl. Acad. Sci,* 1980, vol. 77, 3567-70 **[0190]**
- **COLBERE-GARAPIN F et al.** *J. Mol. Biol,* 1981, vol. 150, 1-14 **[0190]**
- **HARTMAN SC ; RC MULLIGAN.** *Proc. Natl. Acad. Sci,* 1988, vol. 85, 8047-51 **[0190]**
- **RHODES CA et al.** *Methods Mol. Biol.,* 1995, vol. 55, 121-131 **[0190]**
- **PHAM et al.** *Biotechnol. Bioeng.,* 2003, vol. 84, 332-42 **[0198]**
- **ANSEL.** Introduction to Pharmaceutical Dosage Forms. 1985, 126 **[0206]**
- **BRUMEANU et al.** *J Immunol,* 1995, vol. 154, 3088-95 **[0214]**
- **CHENG et al.** *Pharm. Res.,* 2003, vol. 20 (9), 1444-51 **[0214]**
- **TALIANI et al.** *Anal. Biochem.,* 1996, vol. 240, 60-67 **[0235]**
- **FILOCAMO et al.** *J Virology,* 1997, vol. 71, 1417-1427 **[0235]**
- **SUDO et al.** *Antiviral Res.,* 1996, vol. 32, 9-18 **[0235]**
- **BUFFARD et al.** *Virology,* 1995, vol. 209, 52-59 **[0235]**
- **BIANCHI et al.** *Anal. Biochem.,* 1996, vol. 237, 239-244 **[0235]**
- **HAMATAKE et al.** *Intervirology,* 1996, vol. 39, 249-258 **[0235]**
- **STEINKUHLER et al.** *Biochem.,* 1998, vol. 37, 8899-8905 **[0235]**
- **D'SOUZA et al.** *J Gen. Virol.,* 1995, vol. 76, 1729-1736 **[0235]**
- **TAKESHITA et al.** *Anal. Biochem.,* 1997, vol. 247, 242-246 **[0235]**
- **SHIMIZU et al.** *J. Virol.,* 1994, vol. 68, 8406-8408 **[0235]**
- **MIZUTANI et al.** *J. Virol.,* 1996, vol. 70, 7219-7223 **[0235]**
- **MIZUTANI et al.** *Biochem. Biophys. Res. Commun.,* 1996, vol. 227, 822-826 **[0235]**
- **LU et al.** *Proc. Natl. Acad. Sci (USA),* 1996, vol. 93, 1412-1417 **[0235]**
- **HAHM et al.** *Virology,* 1996, vol. 226, 318-326 **[0235]**
- **ITO et al.** *J. Gen. Virol.,* 1996, vol. 77, 1043-1054 **[0235]**
- **MIZUTANI et al.** *Biochem. Biophys. Res. Commun.,* 1995, vol. 212, 906-911 **[0235]**
- **CHO et al.** *J. Virol. Meth.,* 1997, vol. 65, 201-207 **[0235]**
- **FROST ; STERN.** *Anal. Biochem.,* 1997, vol. 251, 263-269 **[0244]**

- **DELPECH et al.** *Anal. Biochem.,* 1995, vol. 229, 35-41 **[0245]**
- **TAKAHASHI et al.** *Anal. Biochem.,* 2003, vol. 322, 257-263 **[0245]**
- **BONNER ; CANTEY.** *Clin. Chim. Acta,* 1966, vol. 13, 746-752 **[0246]**
- **DE SALEGUI et al.** *Arch. Biochem. Biophys.,* 1967, vol. 121, 548-554 **[0246]**
- **DORFMAN ; OTT.** *J. Biol. Chem.,* 1948, vol. 172, 367 **[0246]**
- **GUENTENHONER et al.** *Matrix,* 1992, 388-396 **[0248]**
- **BORDIER et al.** *J. Biol. Chem.,* 1981, vol. 256, 1604-1607 **[0251]**
- **SCHULLER et al.** *Proc. Amer. Assoc. Cancer Res.,* 1991, vol. 32, 173 **[0256]**
- **CZEJKA et al.** *Pharmazie,* 1990, vol. 45, H.9 **[0256]**
- **HORN et al.** *J. Surg. Oncol.,* 1985, vol. 28, 304-307 **[0256]**
- **KOHNO et al.** *J. Cancer Res. Oncol.,* vol. 120, 293-297 **[0256]**
- **BECKENLEHNER et al.** *Cancer Res. Oncol.,* 1992, vol. 118, 591-596 **[0256]**
- **SCHEITHAUER et al.** *Anticancer Res.,* 1988, vol. 8, 391-396 **[0256]**
- **BAUMGARTNER et al.** *Reg. Cancer Treat.,* 1988, vol. 1, 55-58 **[0256] [0277]**
- **ZANKER et al.** *Proc. Amer. Assoc. Cancer Res.,* 1986, vol. 27, 390 **[0256] [0277]**
- **DE MAEYER et al.** *Int. J. Cancer,* 1992, vol. 51, 657-660 **[0257]**
- **PAWLOWSKI et al.** *Int. J. Cancer,* 1979, vol. 23, 105-109 **[0257]**
- **HABERMAN et al.** *Proceedings of the 17th Annual Meeting of the American Society of Clinical Oncology,* 1981, vol. 22, 105 **[0257]**
- **MAARIT et al.** *Cancer Research,* 2000, vol. 60, 150-155 **[0276]**
- **KARVINEN et al.** *British Journal of Dermatology,* 2003, vol. 148, 86-94 **[0276]**
- **LIPPONEN et al.** *Eur. Journal of Cancer,* 2001, 849-856 **[0276]**
- **PIRINEN et al.** *Int. J. Cancer,* 2001, vol. 95, 12-17 **[0276]**
- **AUVINEN et al.** *American Journal of Pathology,* 2000, vol. 156 (2), 529-536 **[0276]**
- **ROPPONEN et al.** *Cancer Research,* 1998, vol. 58, 342-347 **[0276]**
- **ST CROIX et al.** *Cancer Lett,* 1998, vol. 131 (1), 35-44 **[0278]**
- **LAURENT et al.** *Acta Neurol Scand September,* 1996, vol. 94 (3), 194-206 **[0282]**
- **MACLEAN et al.** *Science,* 1976, vol. 194 (4261), 199-200 **[0285]**
- **KOLODGIE et al.** *Arterioscler Thromb Vasc Biol.,* 2002, vol. 22 (10), 1642-8 **[0286]**
- **DUBENSKY et al.** *Proc Natl Acad Sci USA,* 1984, vol. 81 (23), 7529-33 **[0293]**
- **FAVRE et al.** *Gene Therapy,* 2000, vol. 7 (16), 1417-20 **[0293]**
- **NETTELBLADT et al.** *Am. Rev. Resp. Dis.,* 1991, vol. 139, 759-762 **[0297]**
- **WALDENSTROM et al.** *J. Clin. Invest.,* 1991, vol. 88 (5), 1622-1628 **[0297]**
- **HA'LLGREN et al.** *J. Exp. Med.,* 1990, vol. 171, 2063-2076 **[0297]**
- **WELLS et al.** *Transplantation,* 1990, vol. 50, 240-243 **[0297]**
- **WALLANDER et al.** *Transplant. Int.,* 1993, vol. 6, 133-137 **[0297]**
- **HAELLGREN et al.** *J Clin Invest,* 1990, vol. 185, 668-673 **[0297]**
- **WALDENSTRDM et al.** *Eur. J. Clin. Invest.,* 1993, vol. 23, 277-282 **[0297]**
- **BJERMER et al.** *Br Med J (Clin Res Ed),* 1987, vol. 295 (6602), 803-6 **[0298]**
- **FEW et al.** *Amer. J. Matern. Child Nurs,* 1987, vol. 12, 23-26 **[0299]**
- **PAUL et al.** *J Hand Surg.,* 1997, vol. 22 (2), 219-21 **[0299]**
- **ELDER et al.** *Lancet,* 1980, 648-649 **[0299]**
- **ELBEIN et al.** *J Biol Chem,* 1990, vol. 265, 15599-15605 **[0328]**
- **BÖHME et al.** *Eur. J. Biochem.,* 2002, vol. 269, 977-988 **[0328]**
- **LEE et al.** *J. Biol. Chem.,* 2001, vol. 276, 13924-13934 **[0331]**
- **CHEN ; STANLEY.** *Glycobiology,* 2003, vol. 13, 43-50 **[0331]**
- **ECKHARDT et al.** *J. Biol. Chem.,* 1998, vol. 273, 20189-20195 **[0331]**
- **BAKKER et al.** *Glycobiology,* 2005, vol. 15, 193-201 **[0331]**