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(54) **WNT SURROGATE MOLECULES AND USES THEREOF**

- (71) Applicant: **Surrozen Operating, Inc.**, South San Francisco, CA (US)
- (72) Inventors: **Yang Li**, Mountain View, CA (US); **Tom Zhiye Yuan**, Union City, CA (US); **Aaron Ken Sato**, Burlingame, CA (US); **Wen-Chen Yeh**, Belmont, CA (US); **Claudia Yvonne Janda**, Palo Alto, CA (US); **Tristan William Fowler**, San Francisco, CA (US); **Helene Baribault**, Redwood City, CA (US); **Kuo-Pao Lai**, Campbell, CA (US); **Liqin Xie**, South San Francisco, CA (US); **Randall J. Brezski**, South San Francisco, CA (US); **Chenggang Lu**, South San Francisco, CA (US)
- (73) Assignee: **Surrozen Operating, Inc.**, South San Francisco, CA (US)
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Primary Examiner — Meera Natarajan
Assistant Examiner — Cheom-Gil Cheong
(74) *Attorney, Agent, or Firm* — Cooley LLP

(57) **ABSTRACT**

The present invention provides Wnt pathway agonists and related compositions, which may be used in any of a variety of therapeutic methods for the treatment of diseases.

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Specification includes a Sequence Listing.

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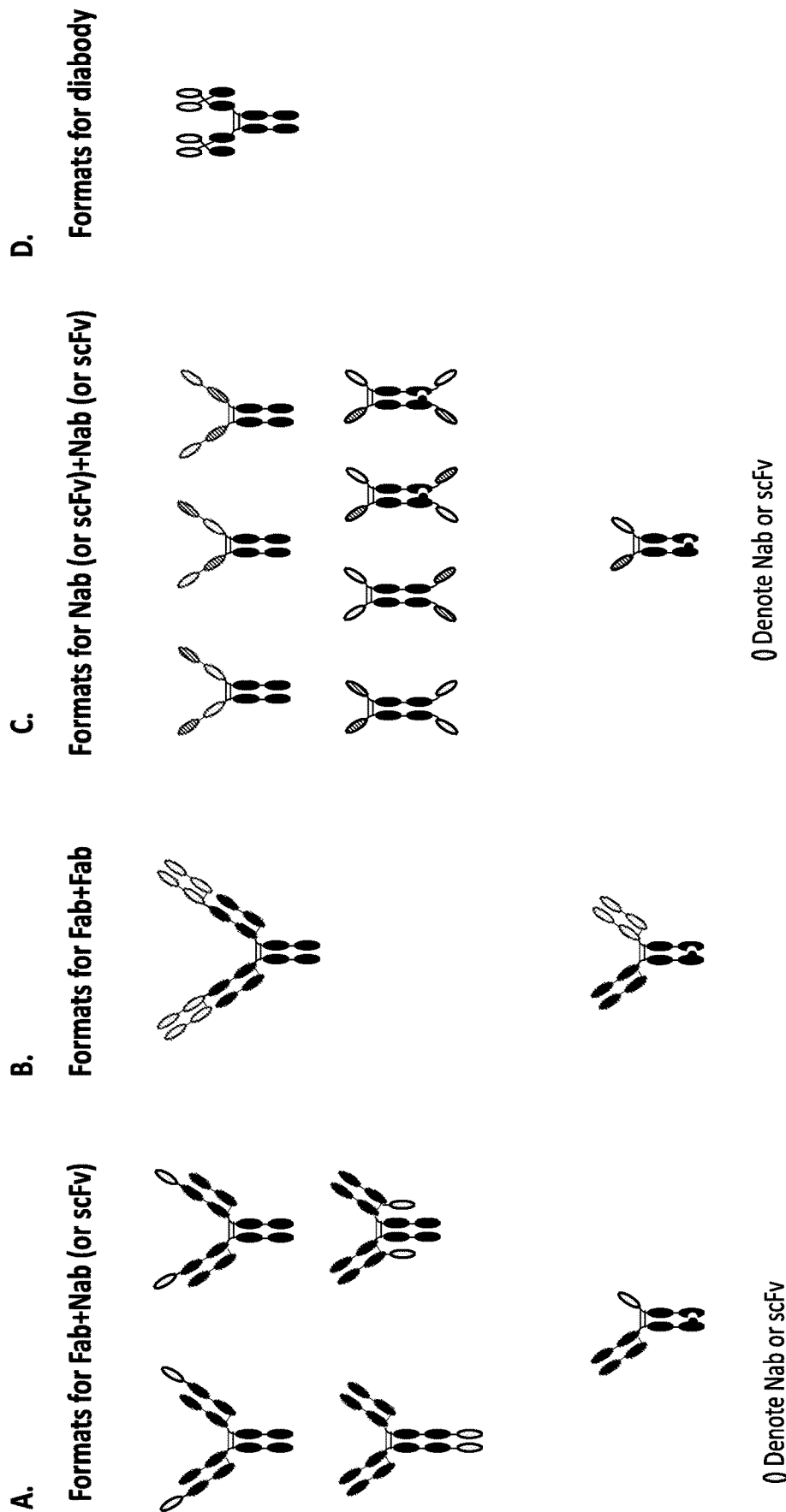


FIG. 1

B. SEC chromatography-protein and 293STF activity traces

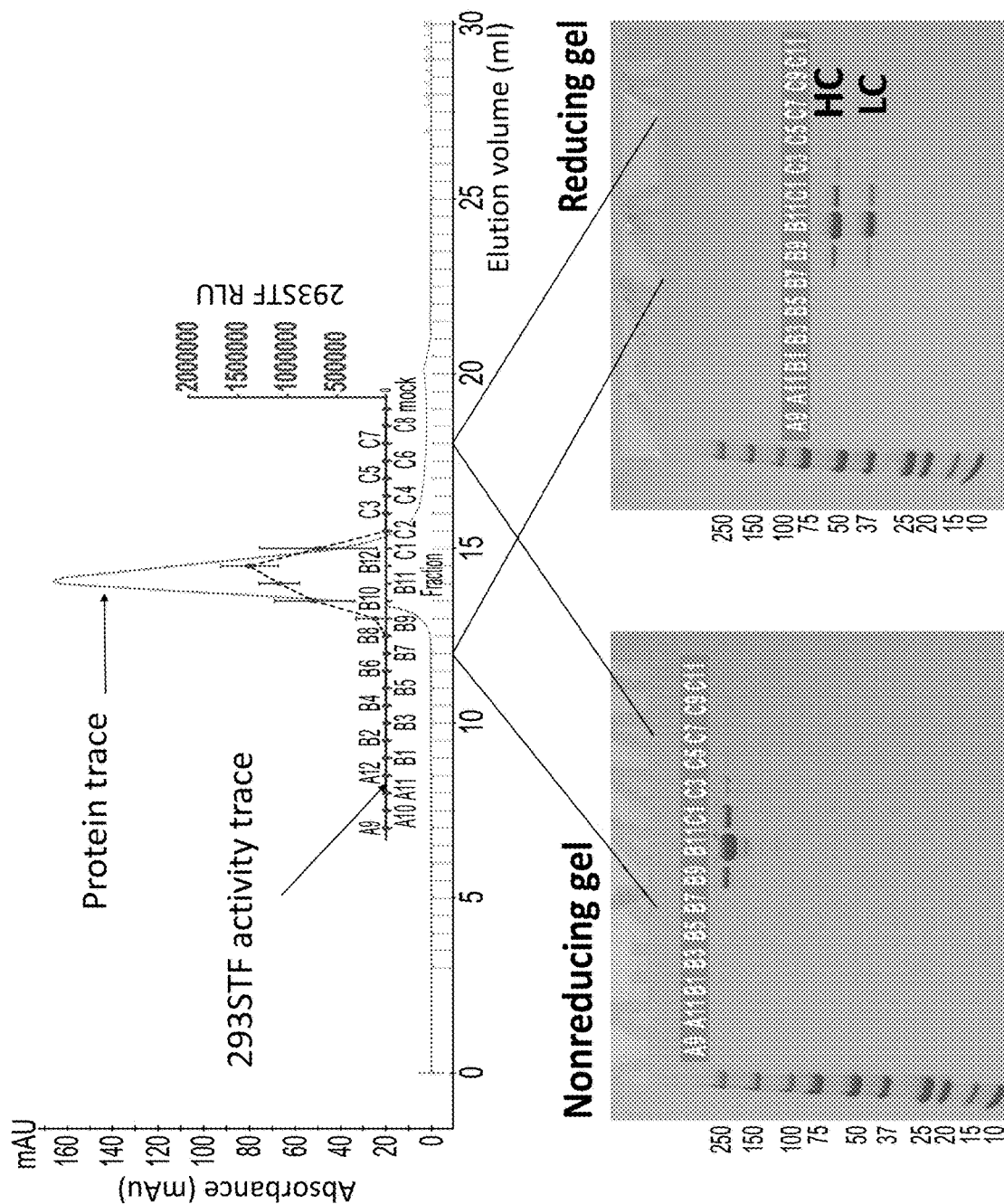
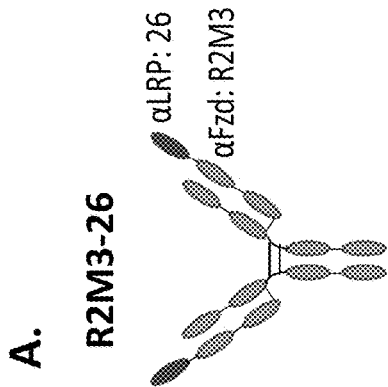


FIG. 2

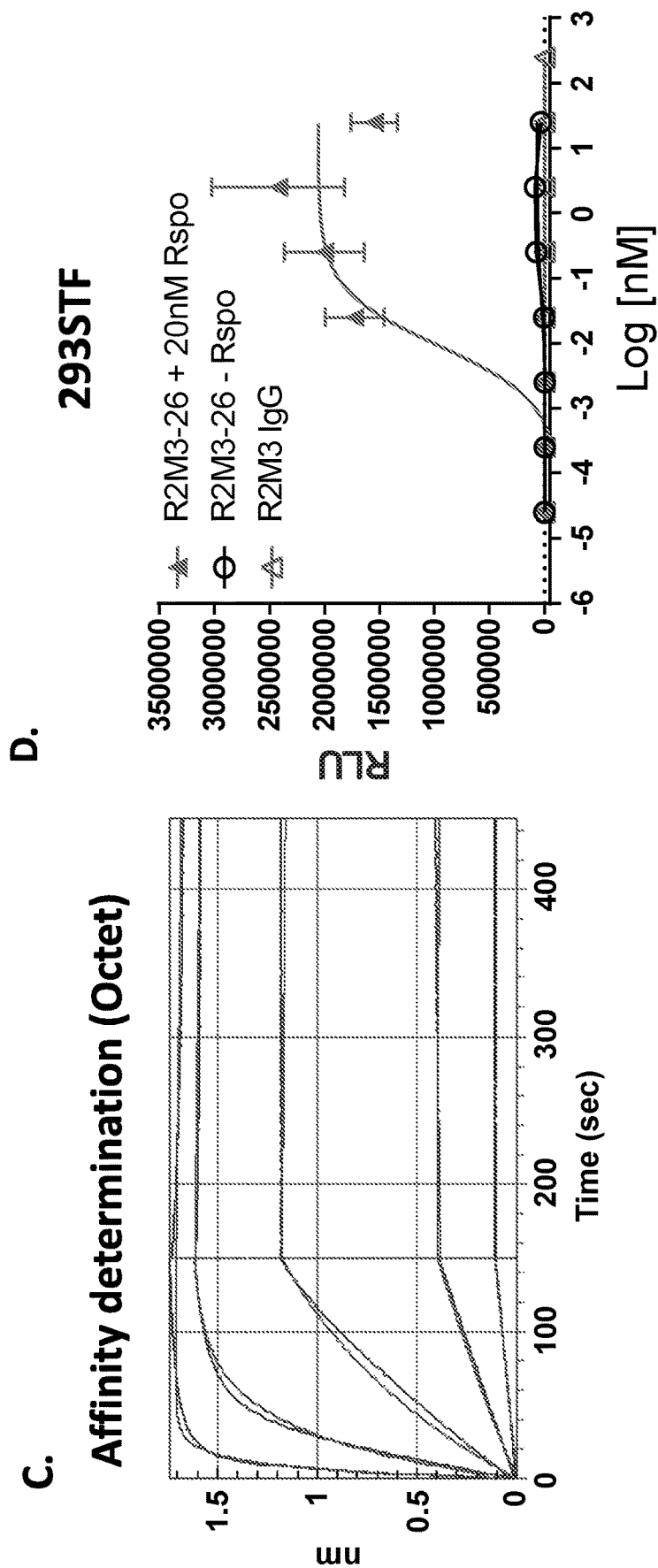


FIG. 2 (contd.)

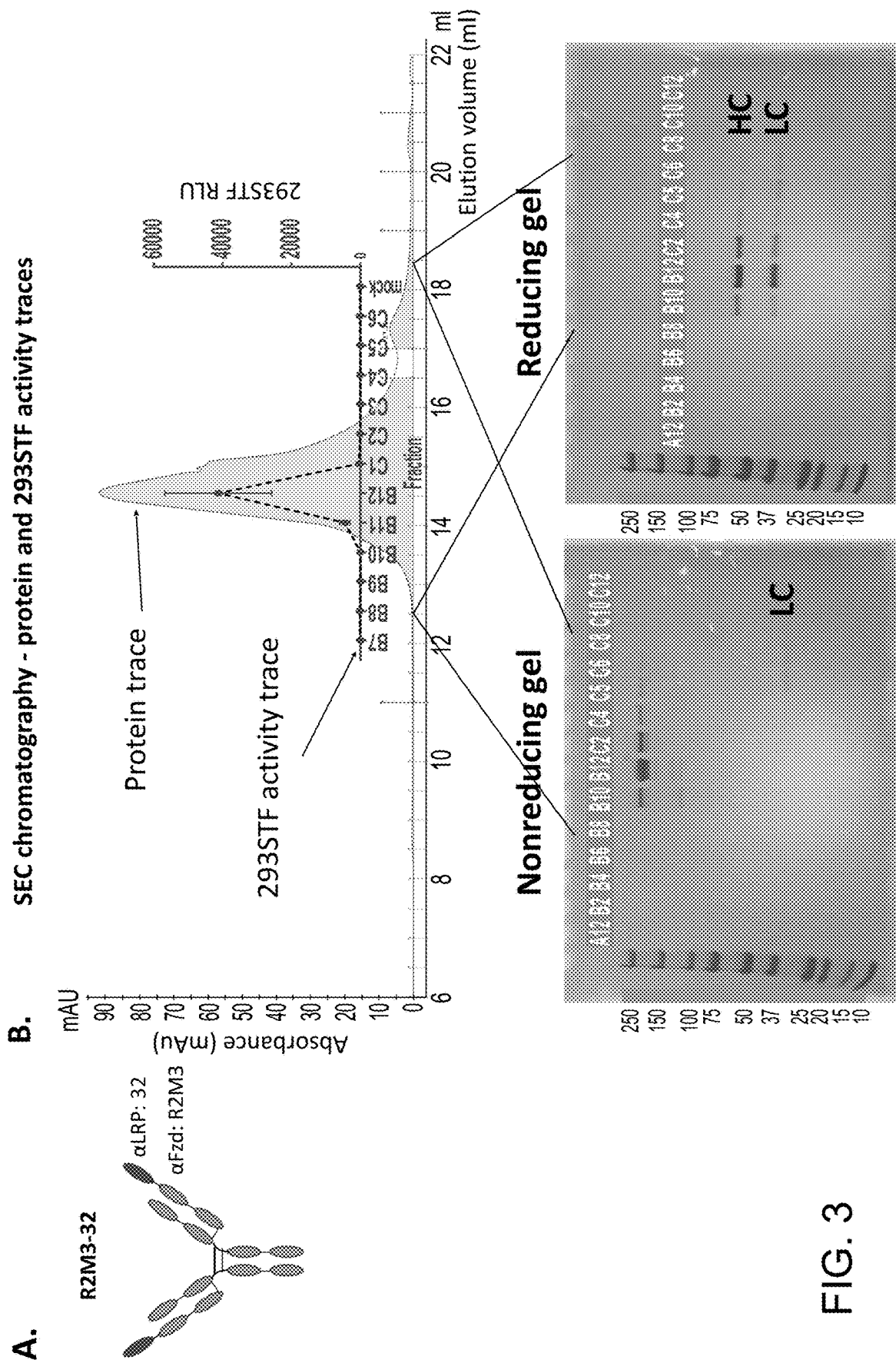
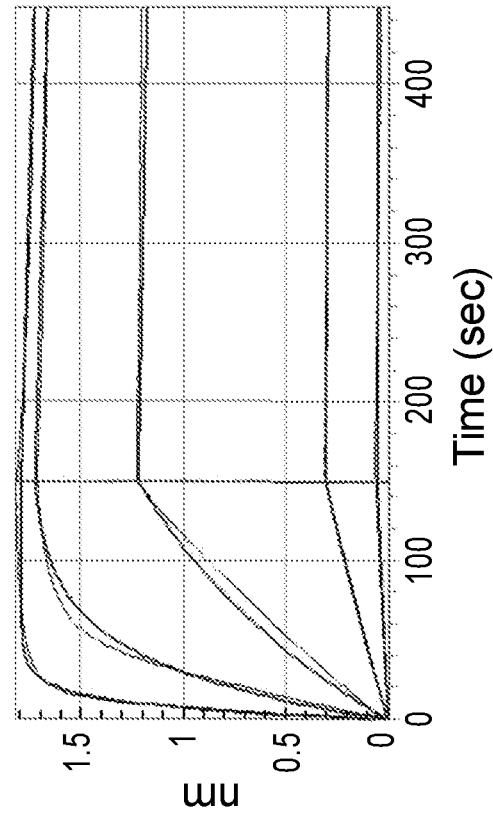


FIG. 3

C.

Affinity determination (Octet)



D.

293STF

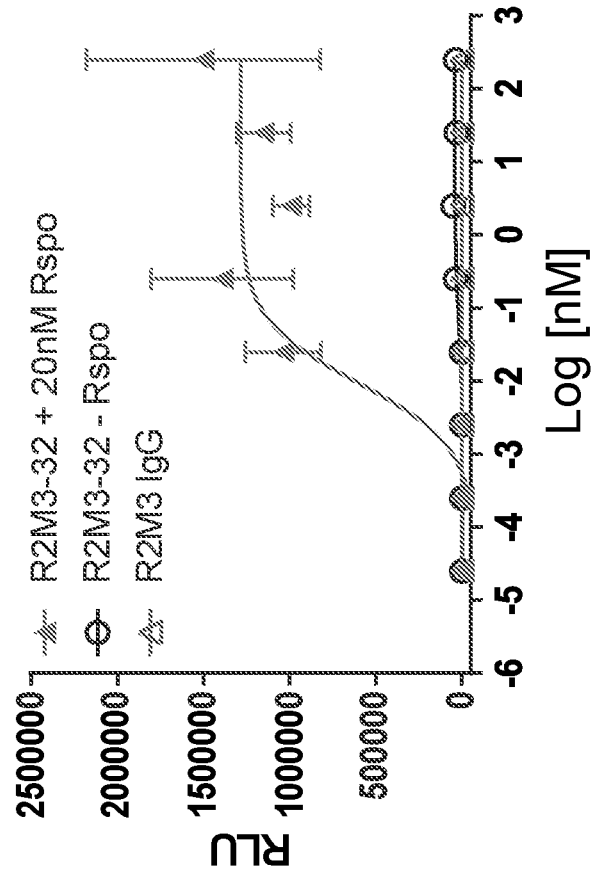


FIG. 3 (contd.)

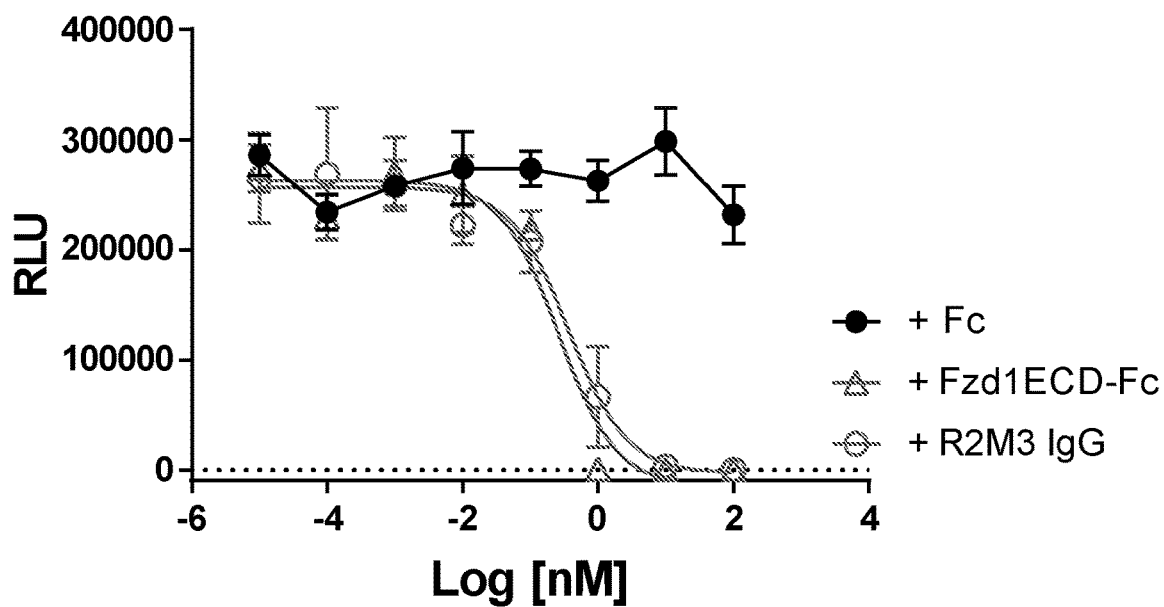
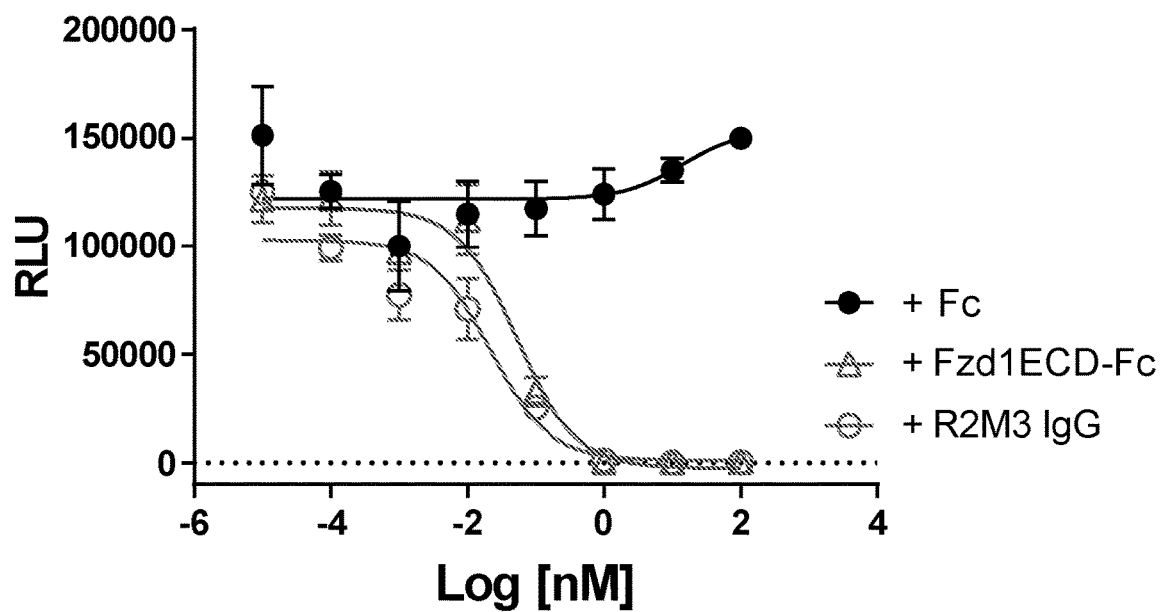
A.**B.**

FIG. 4

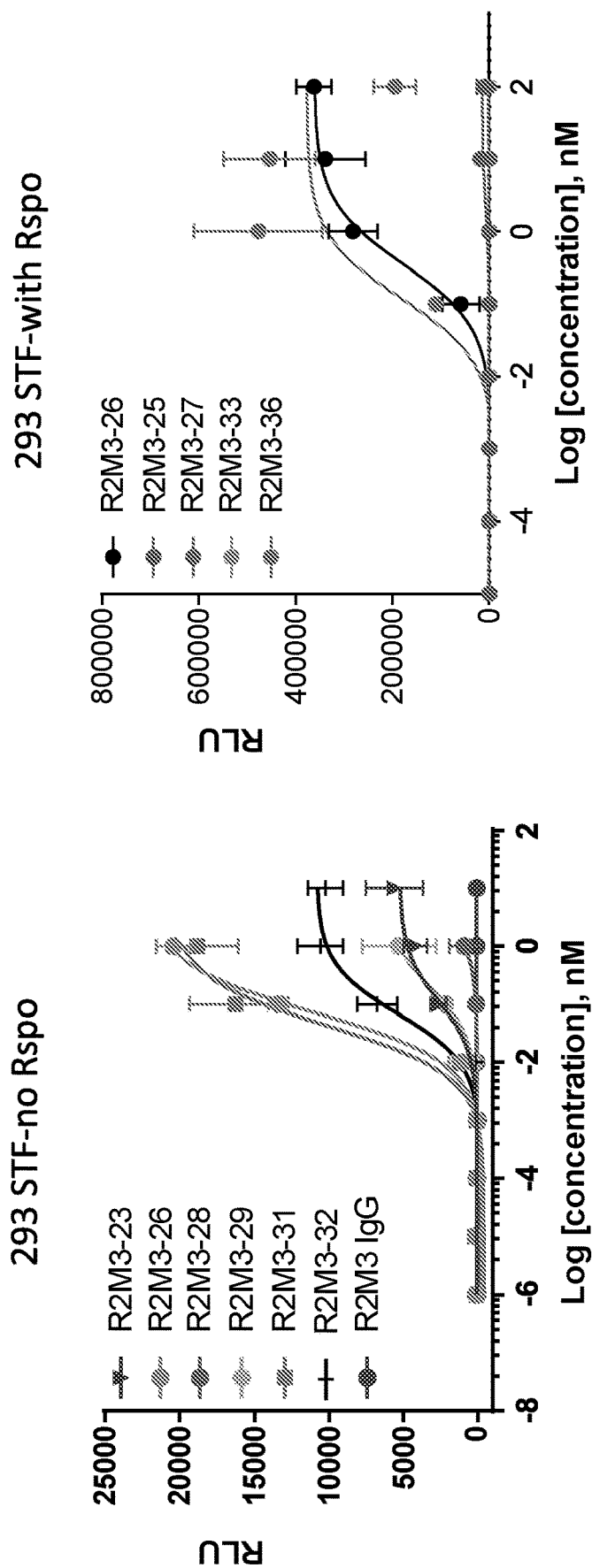


FIG. 5

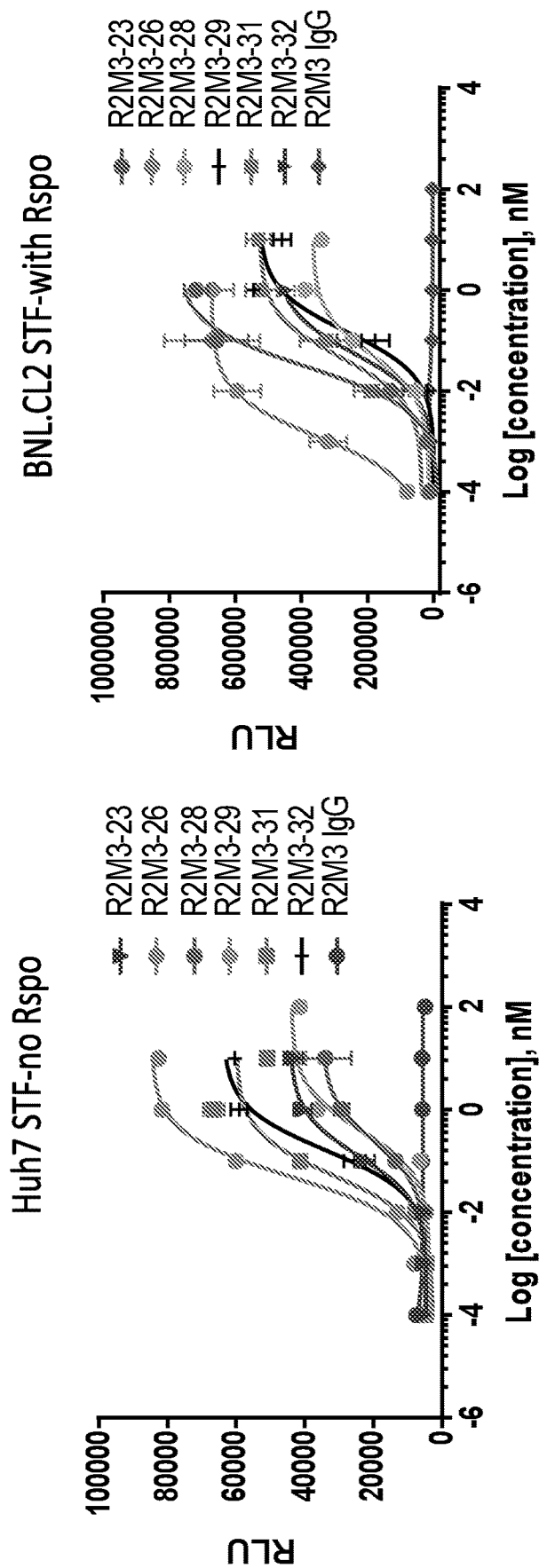


FIG. 5 (contd.)

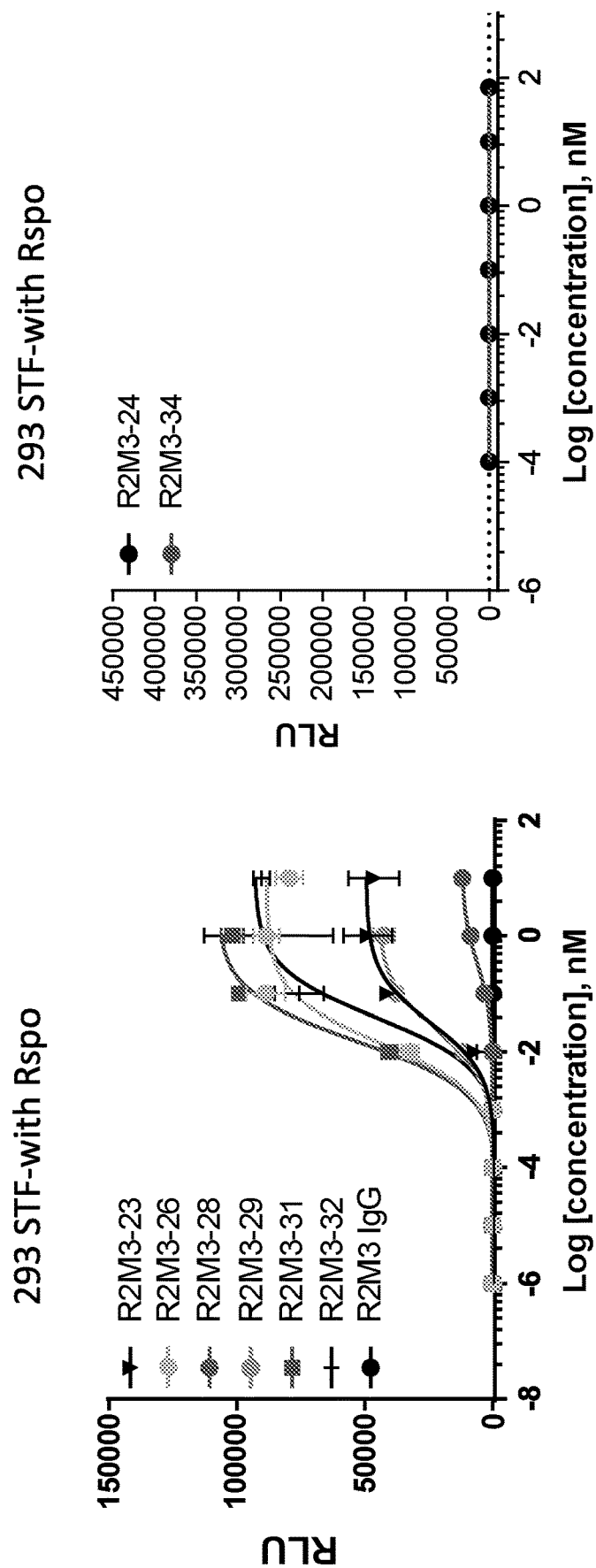


FIG. 5 (contd.)

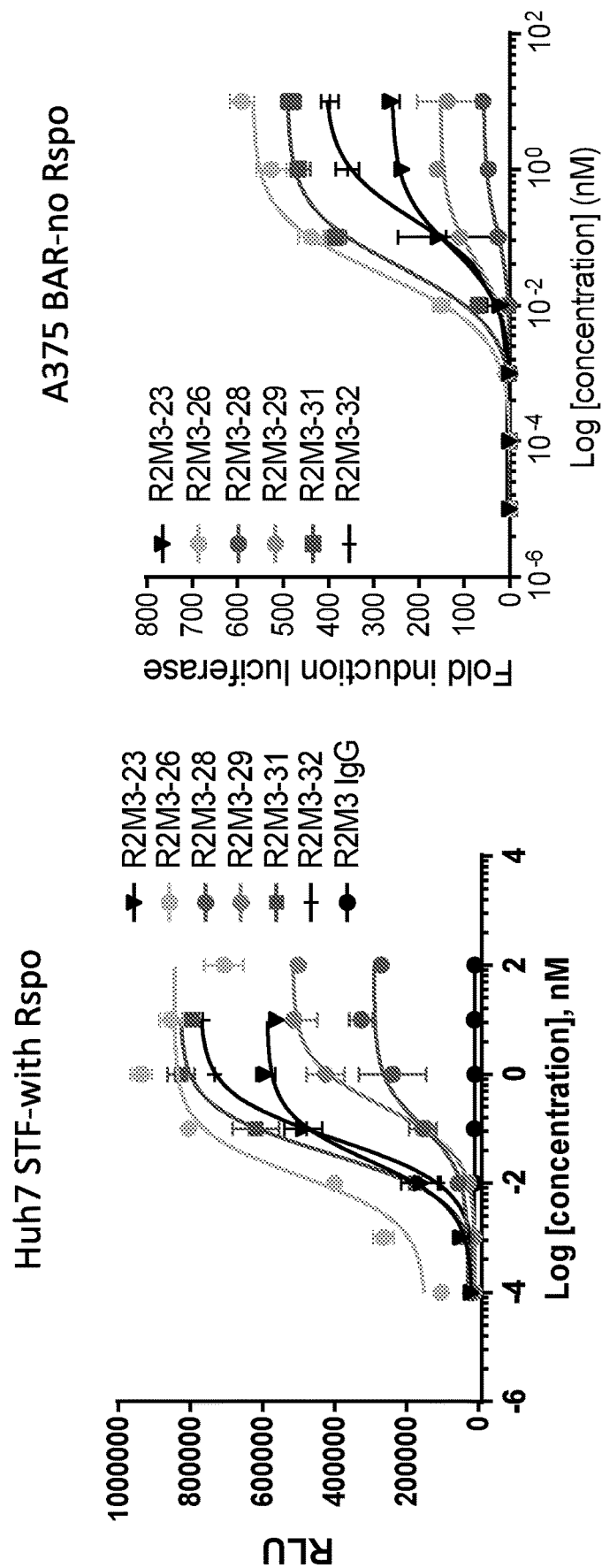


FIG. 5 (contd.)

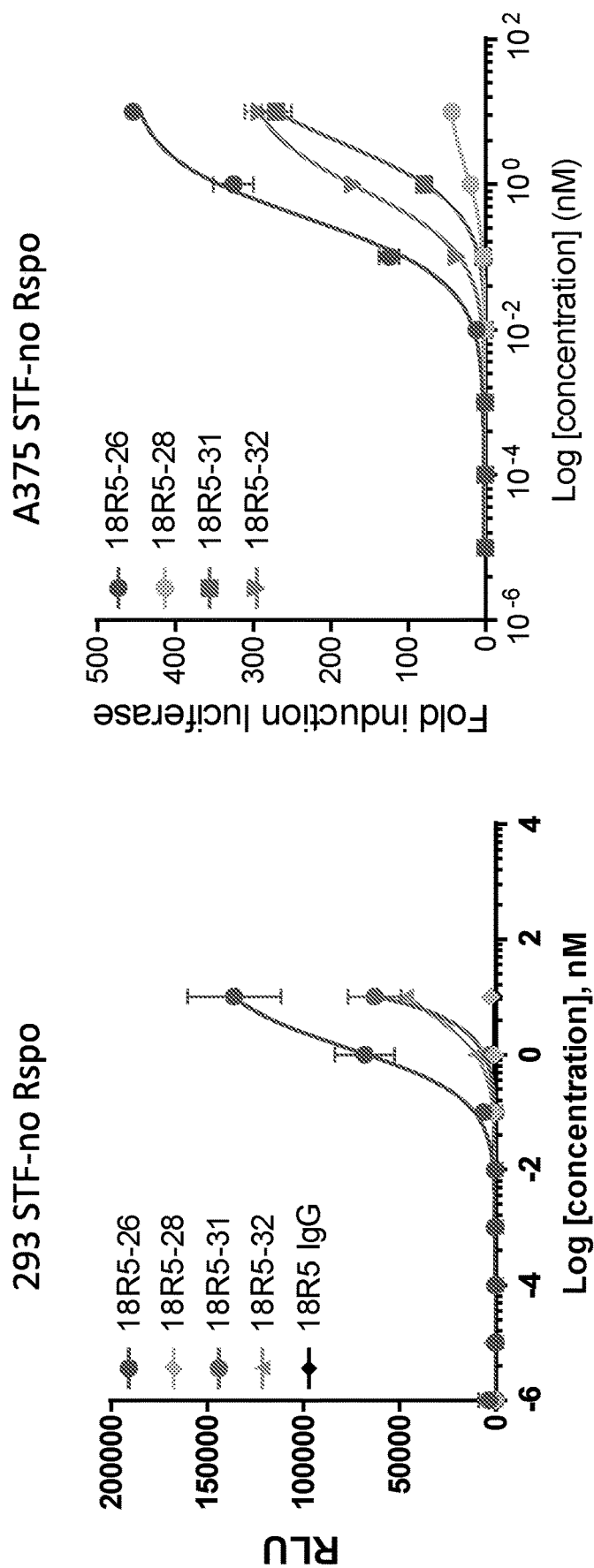


FIG. 6

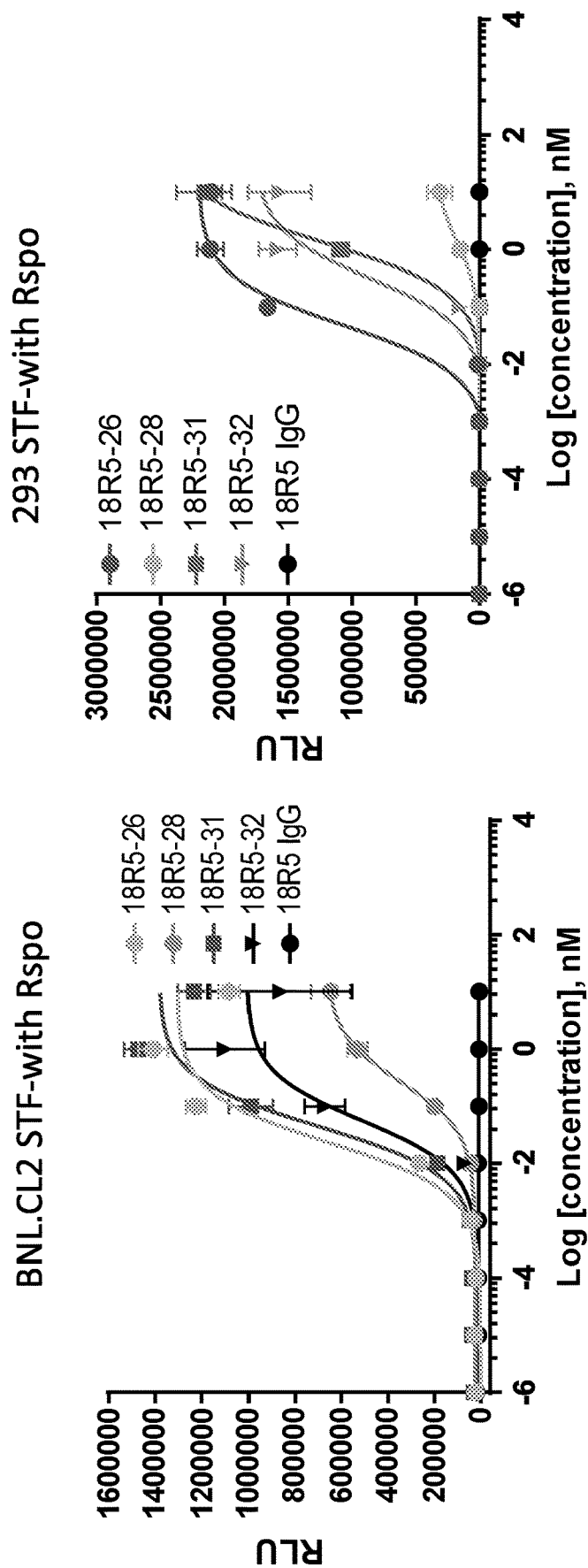


FIG. 6 (contd.)

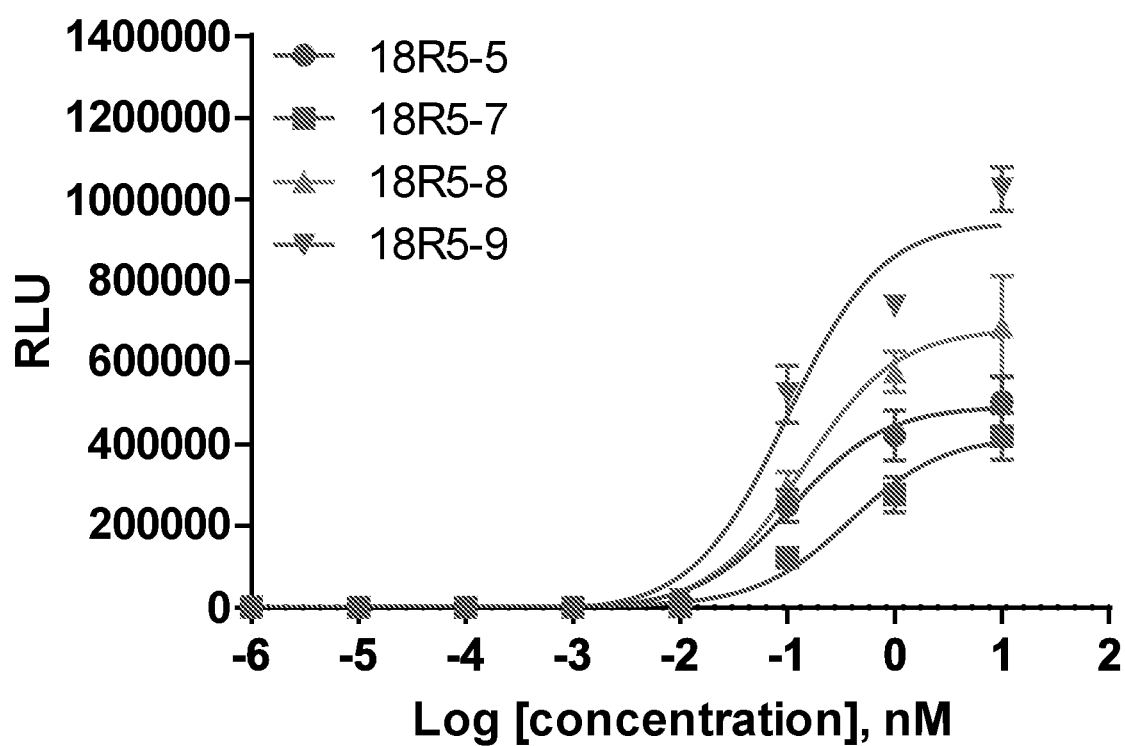
293 STF (with Rspo)

FIG. 7

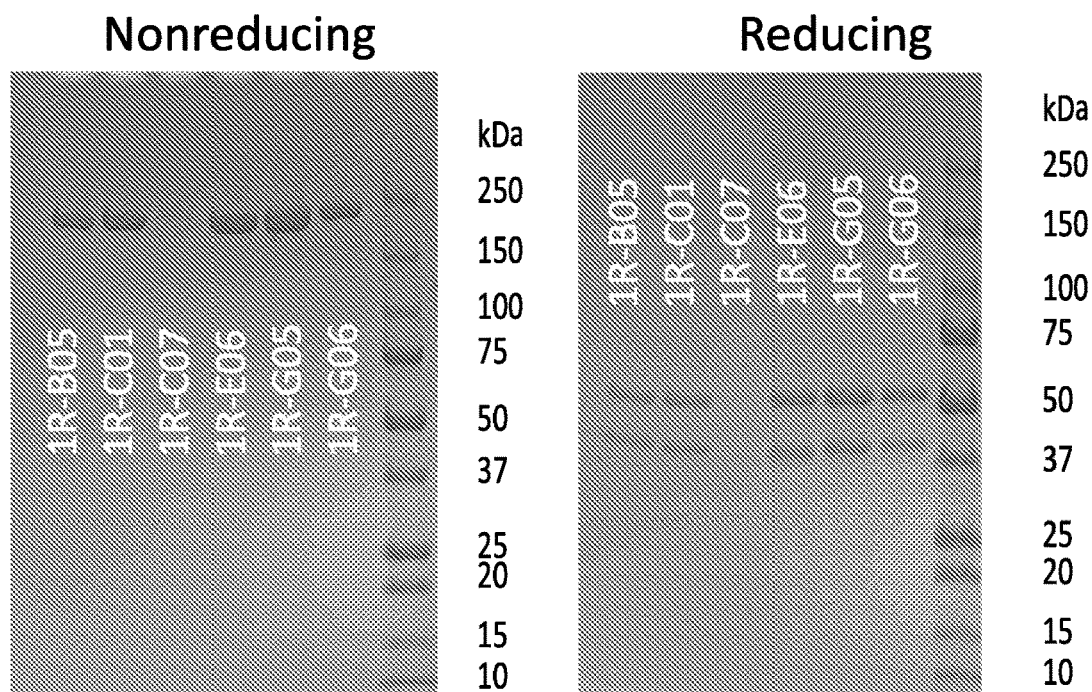
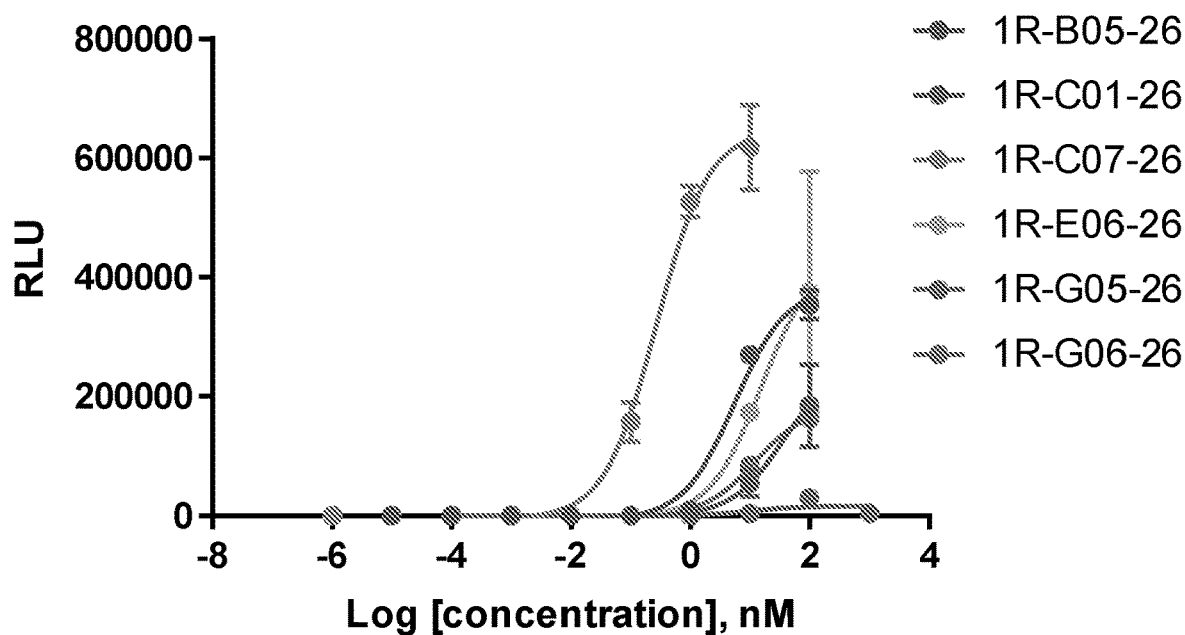
A.**B.**

FIG. 8

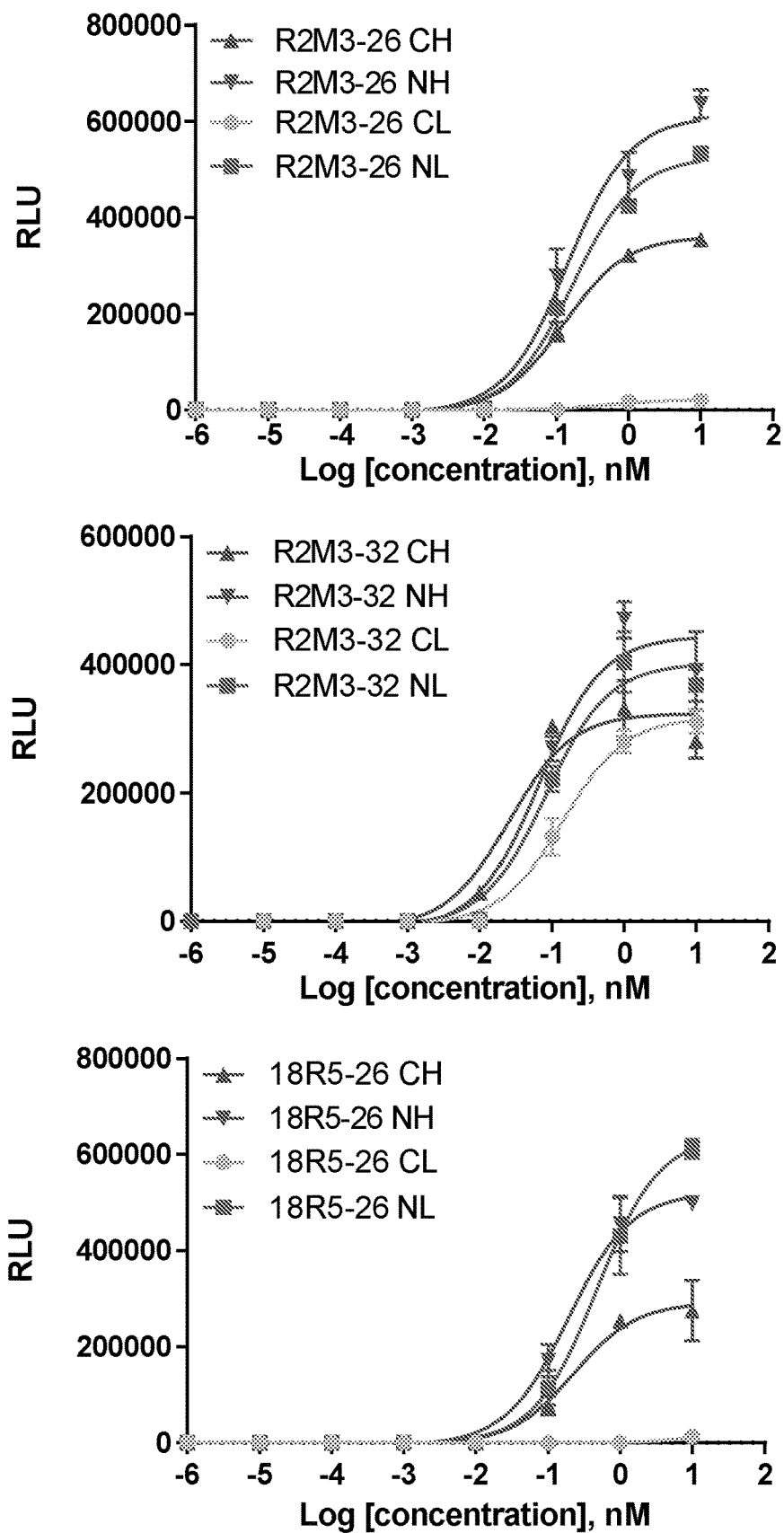


FIG. 9

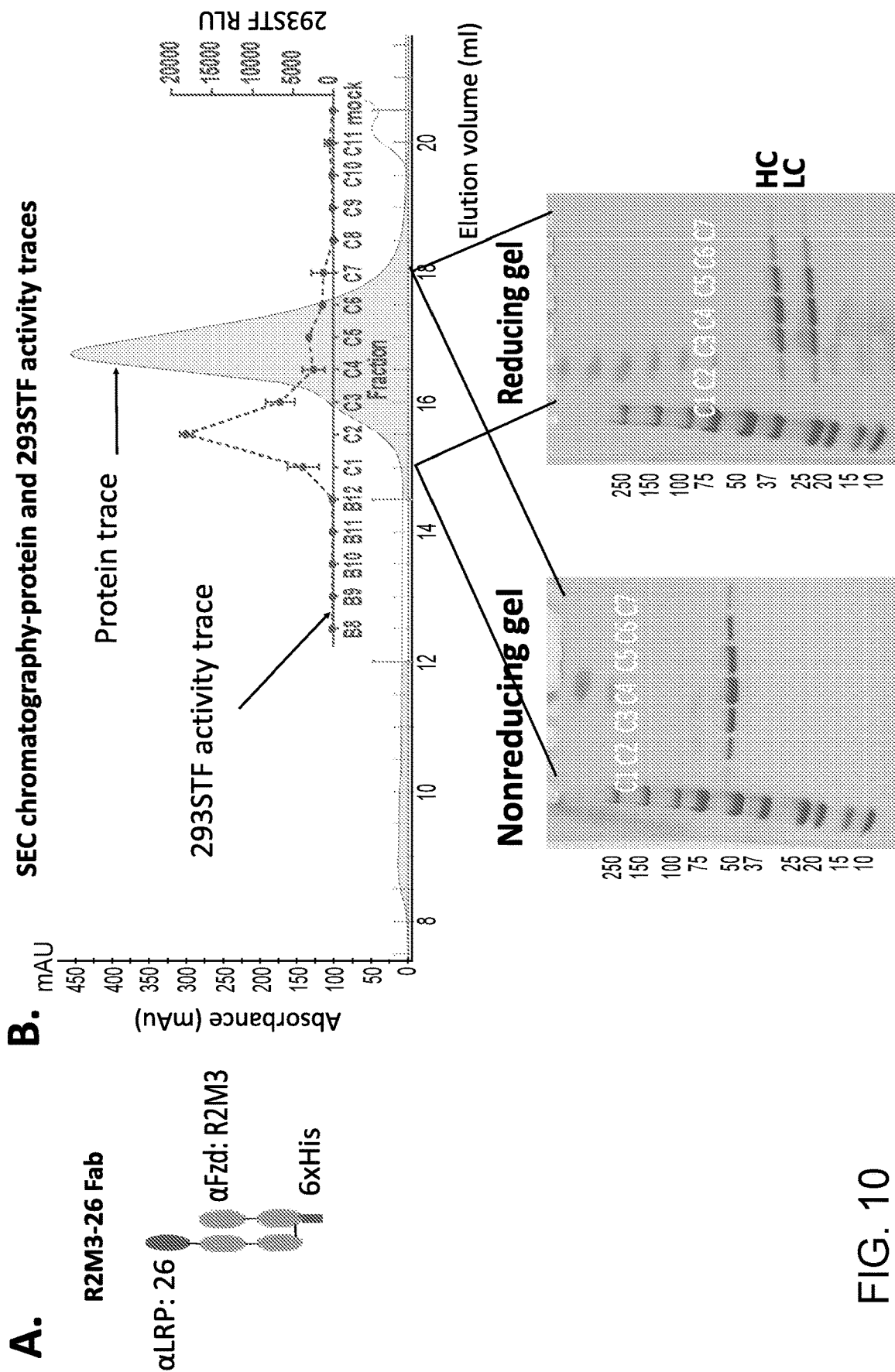


FIG. 10

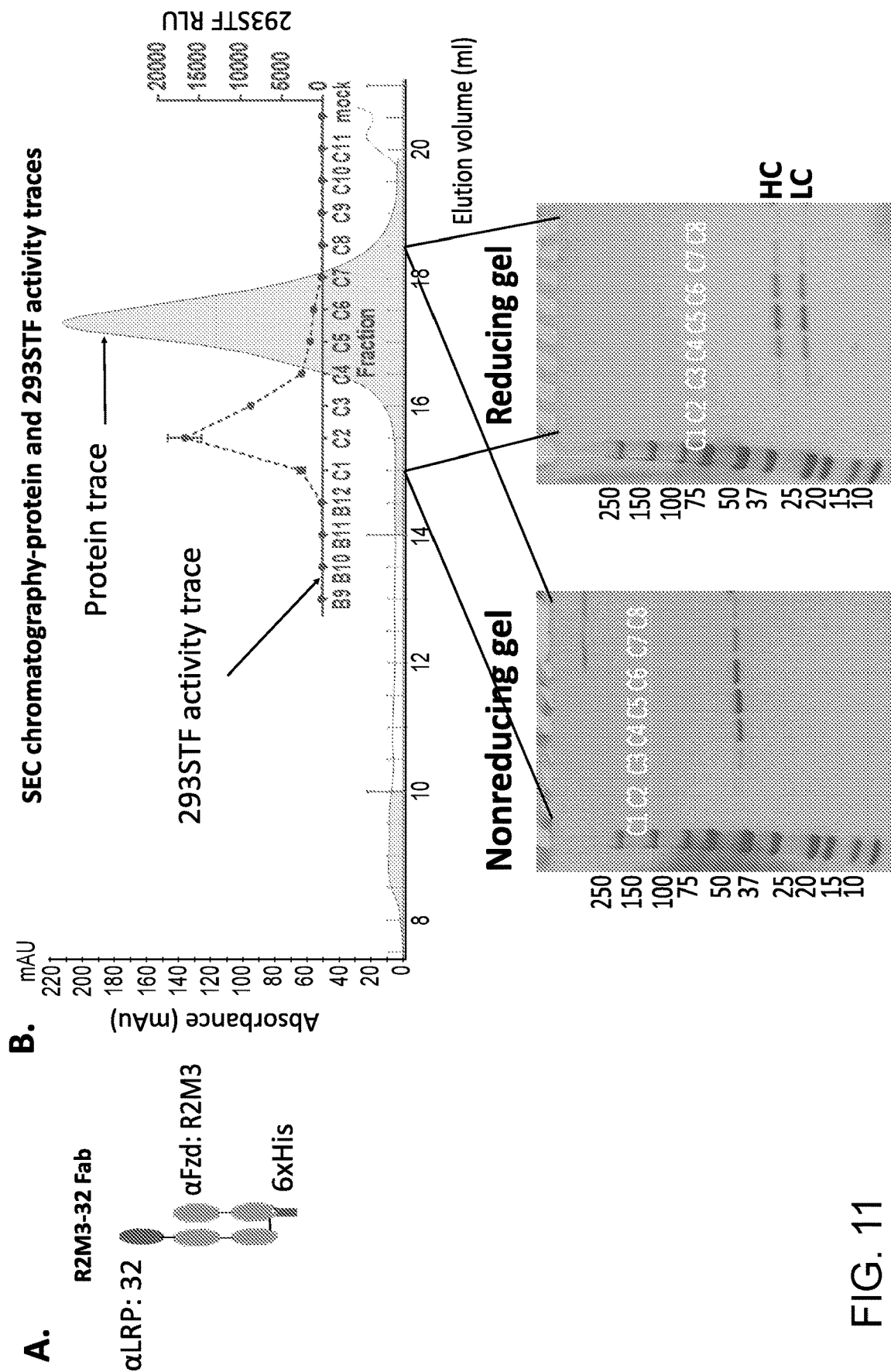
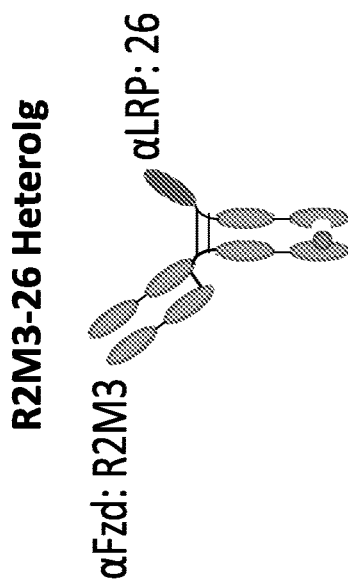


FIG. 11

A. **293 STF reporter assay with Rspo**



B.

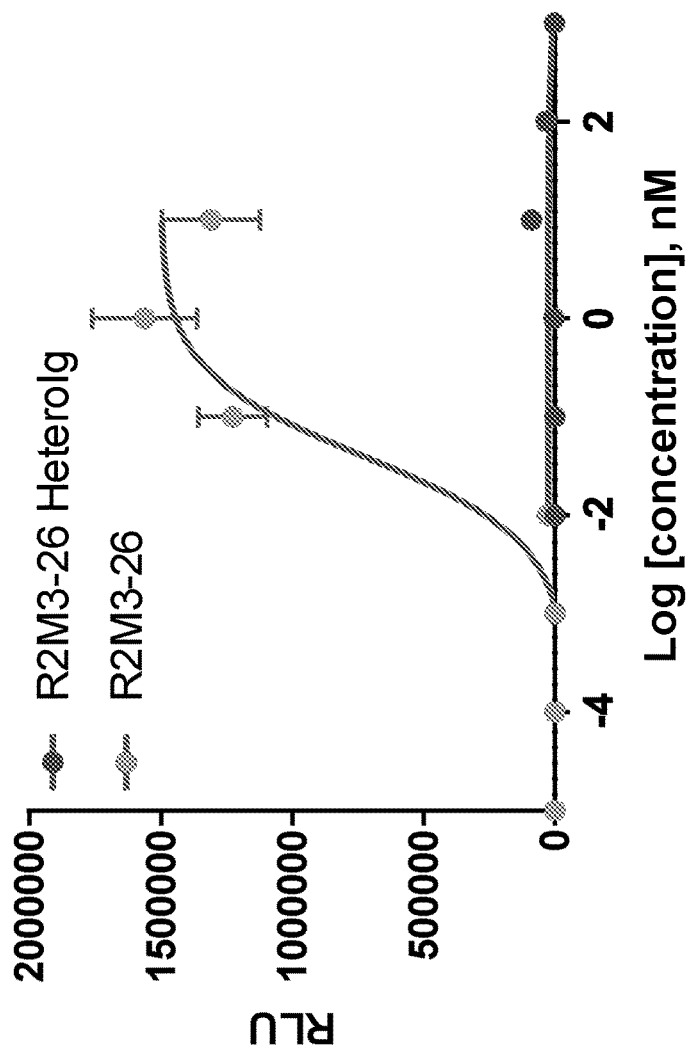


FIG. 12



FIG. 13

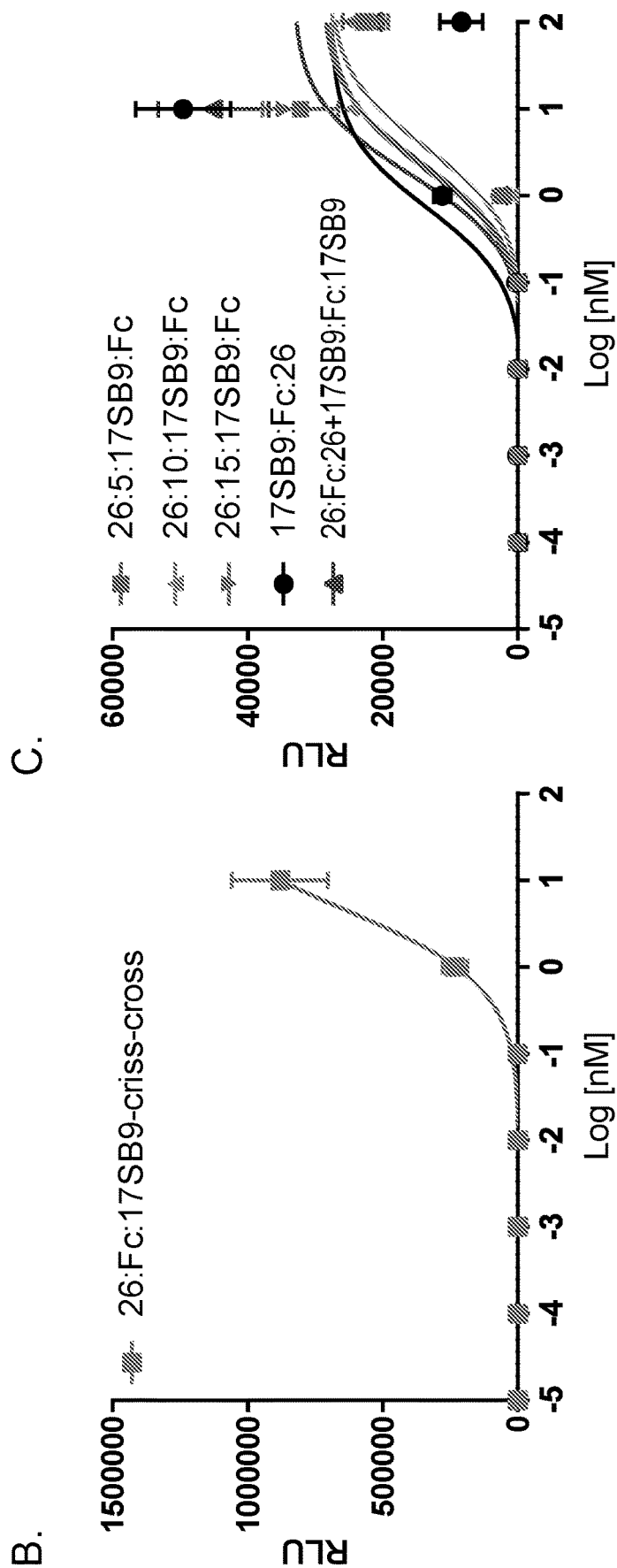


FIG. 13 (contd.)

D.

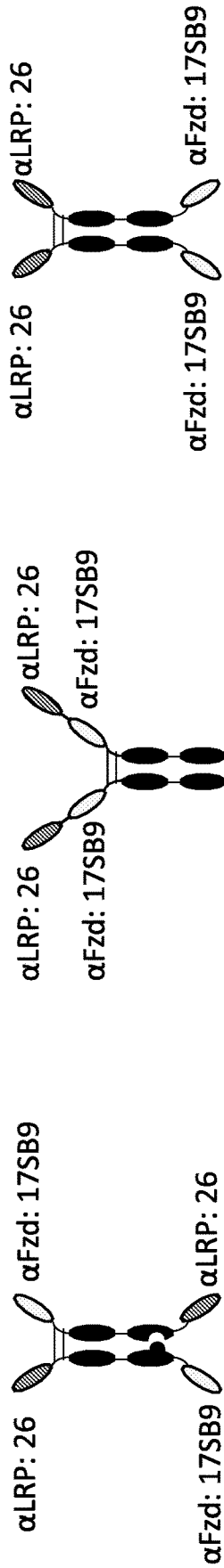
26:5:17SB9:Fc**26:10:17SB9:Fc****26:15:17SB9:Fc****26:Fc:17SB9-criss-cross****26:Fc:17SB9****17SB9:Fc:26****26:Fc:26+17SB9:Fc:17SB9**

FIG. 13 (contd.)

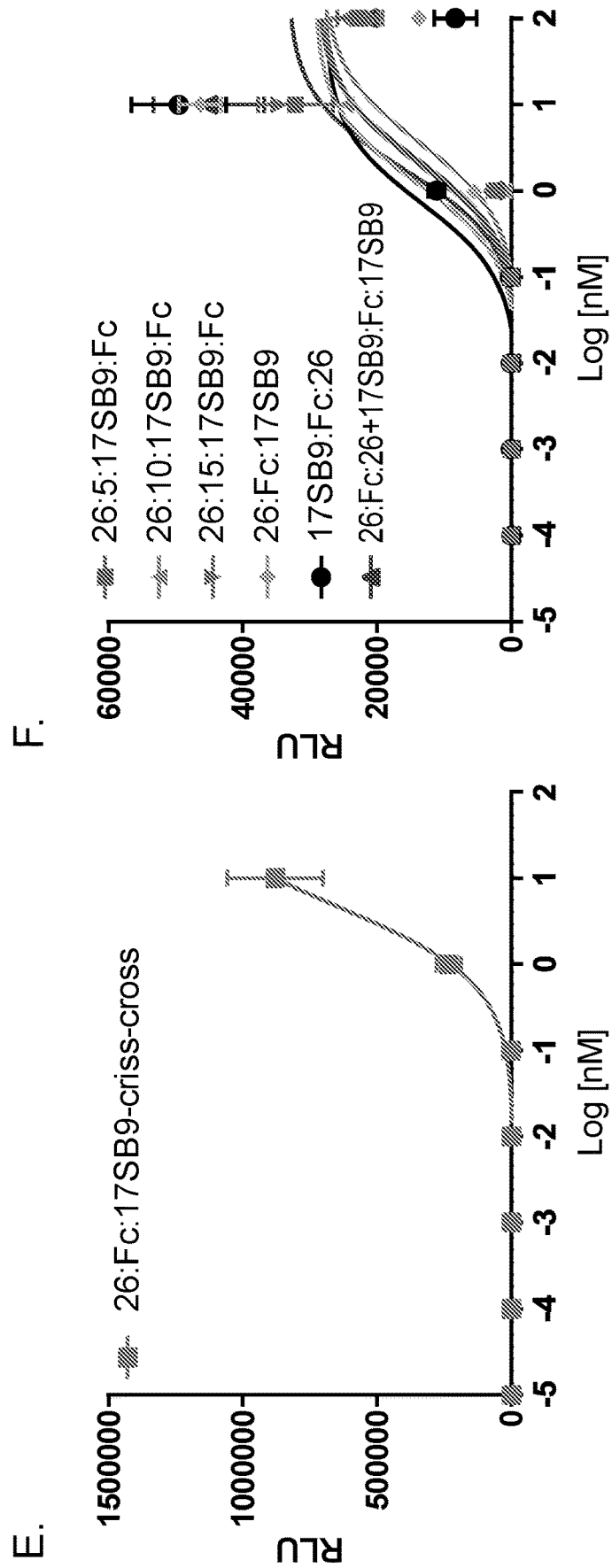


FIG. 13 (contd.)

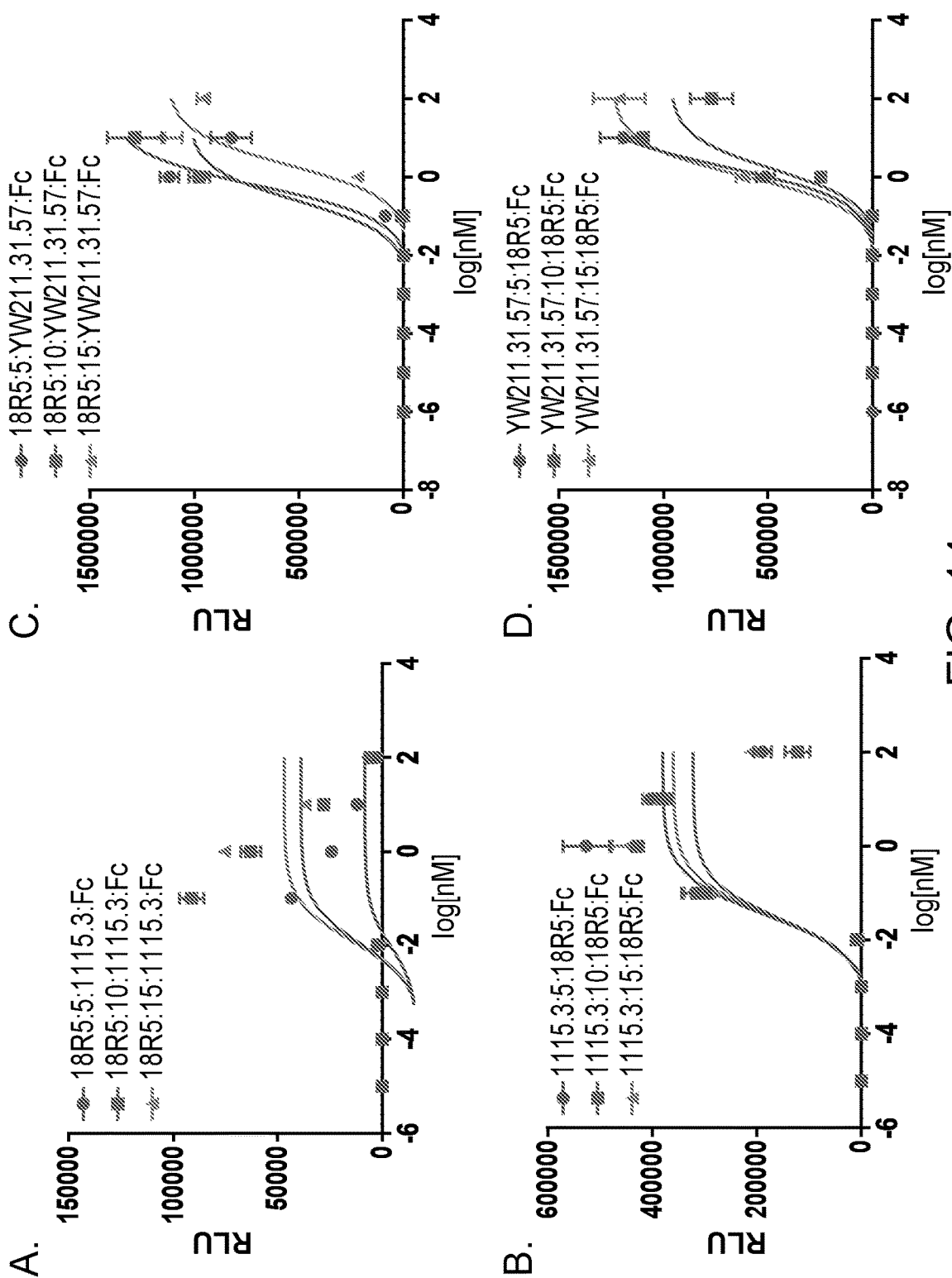
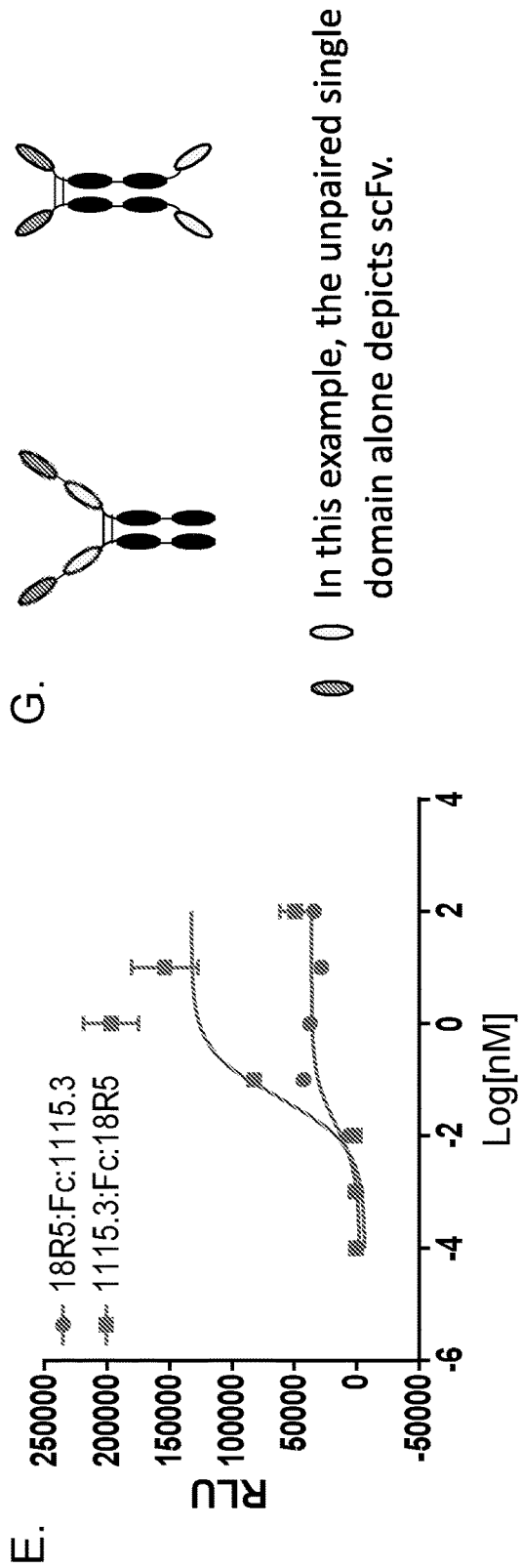
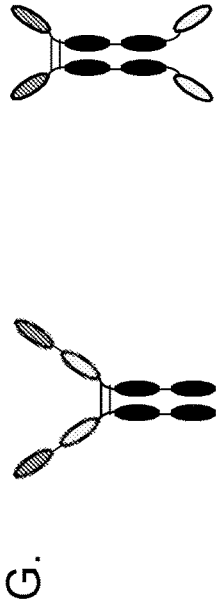


FIG. 14



○ In this example, the unpaired single domain alone depicts scFv.

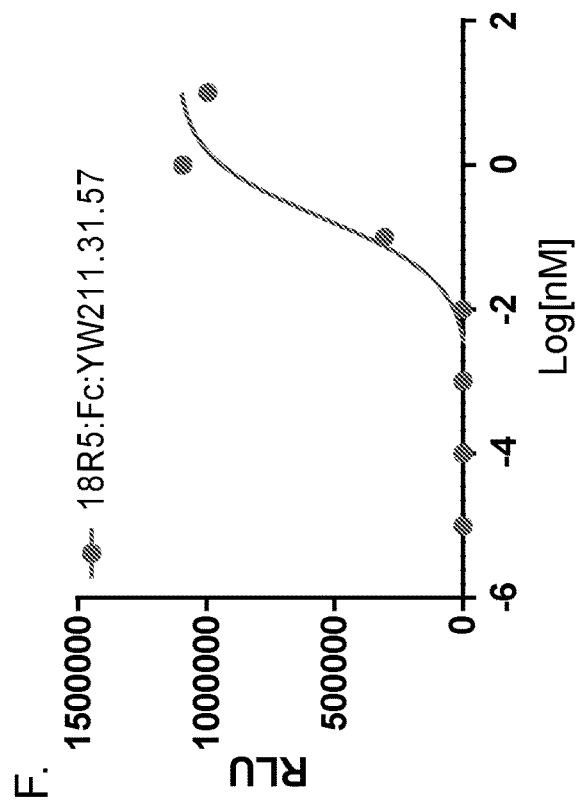


FIG. 14 (contd.)

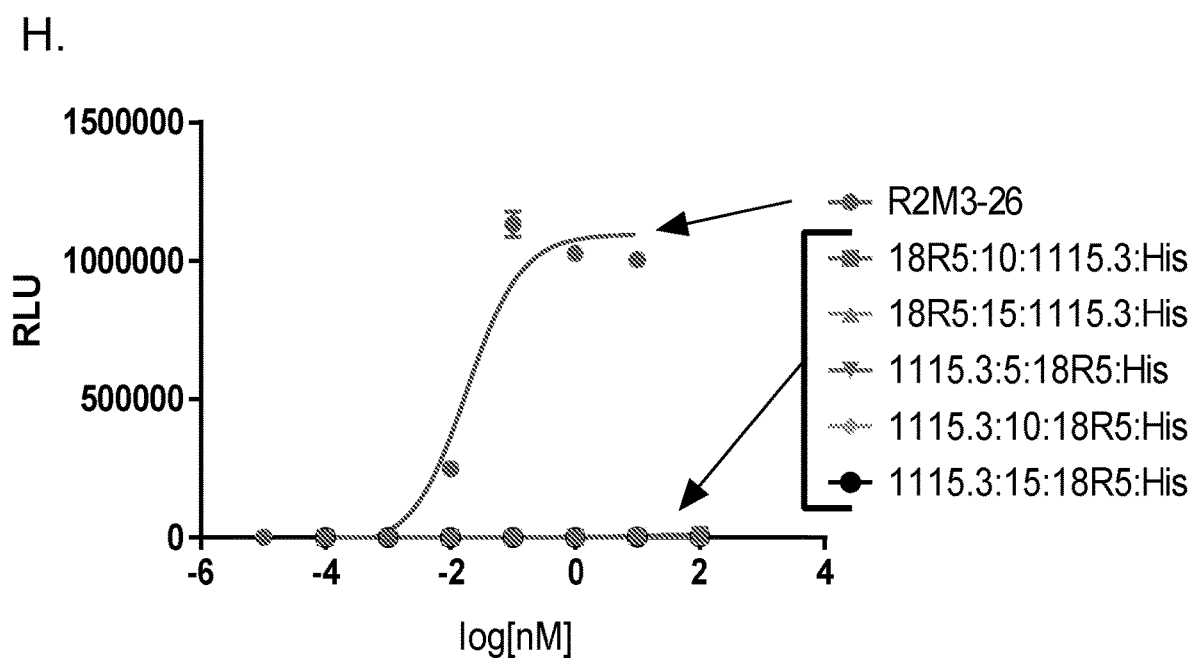


FIG. 14 (contd.)

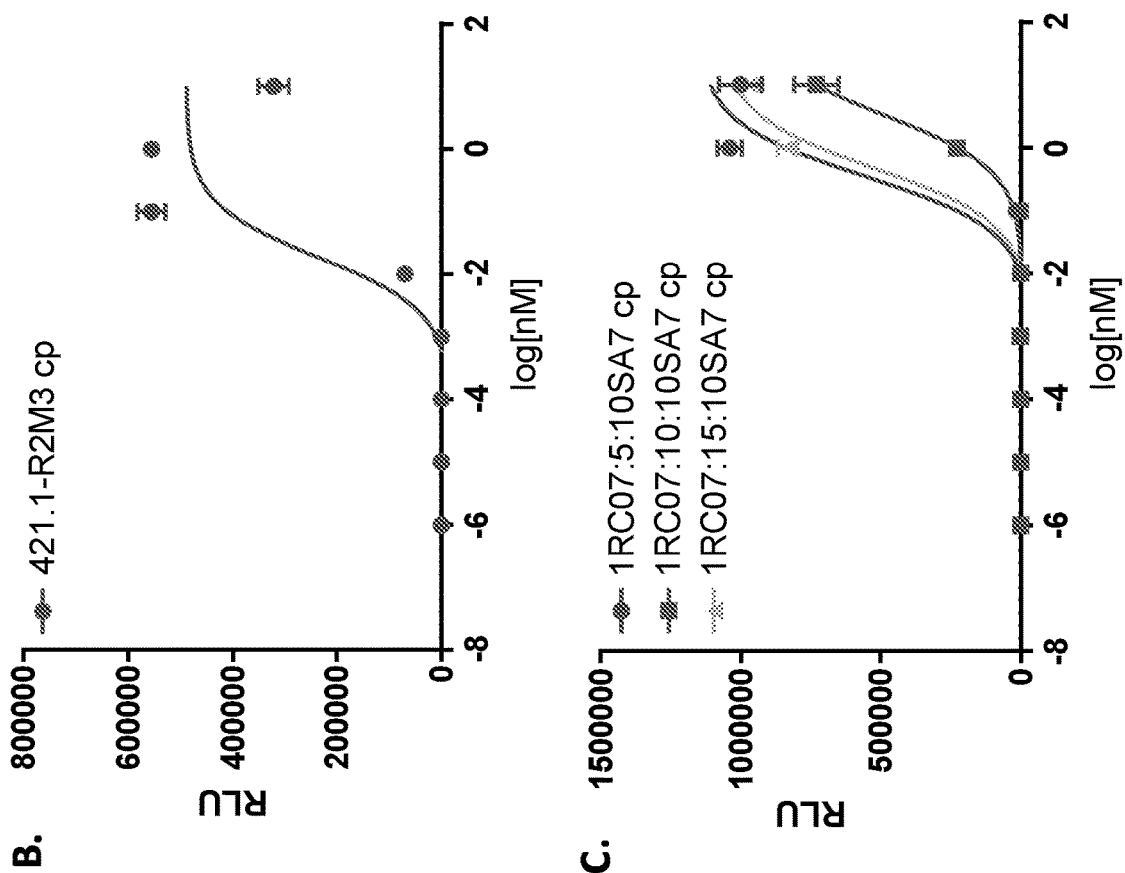


FIG. 15

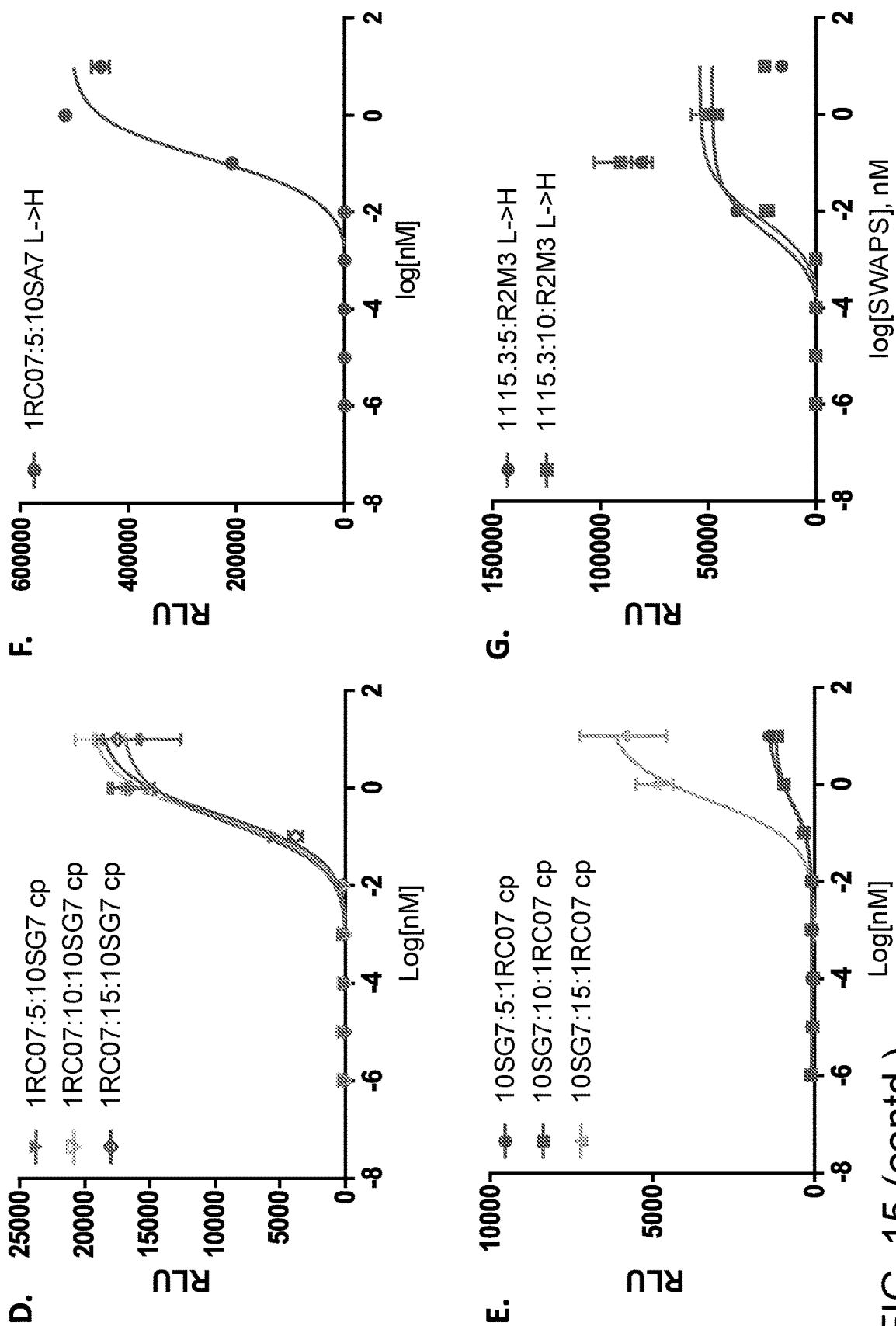


FIG. 15 (contd.)

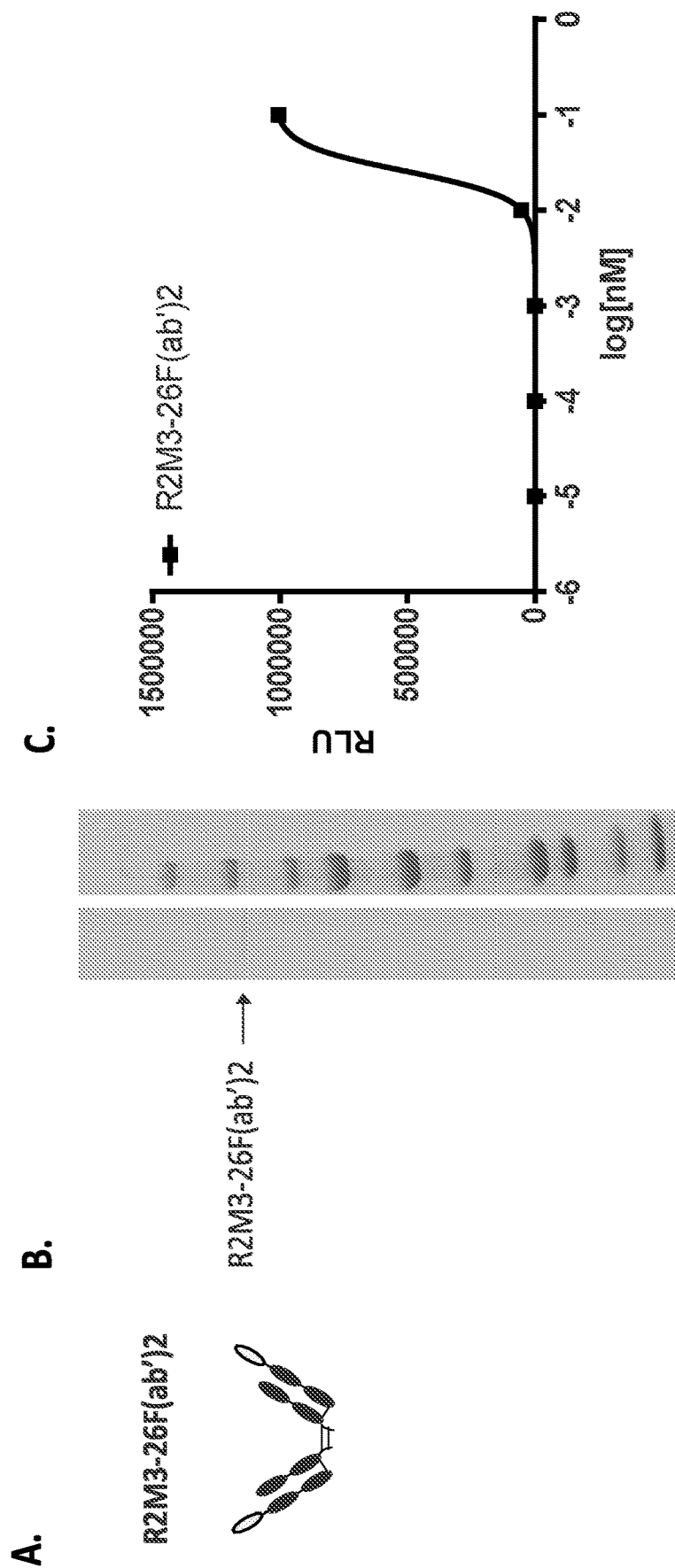
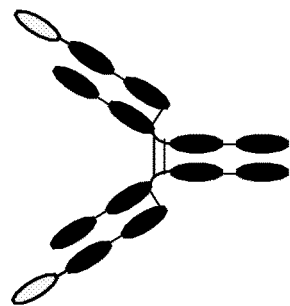


FIG. 16



B.

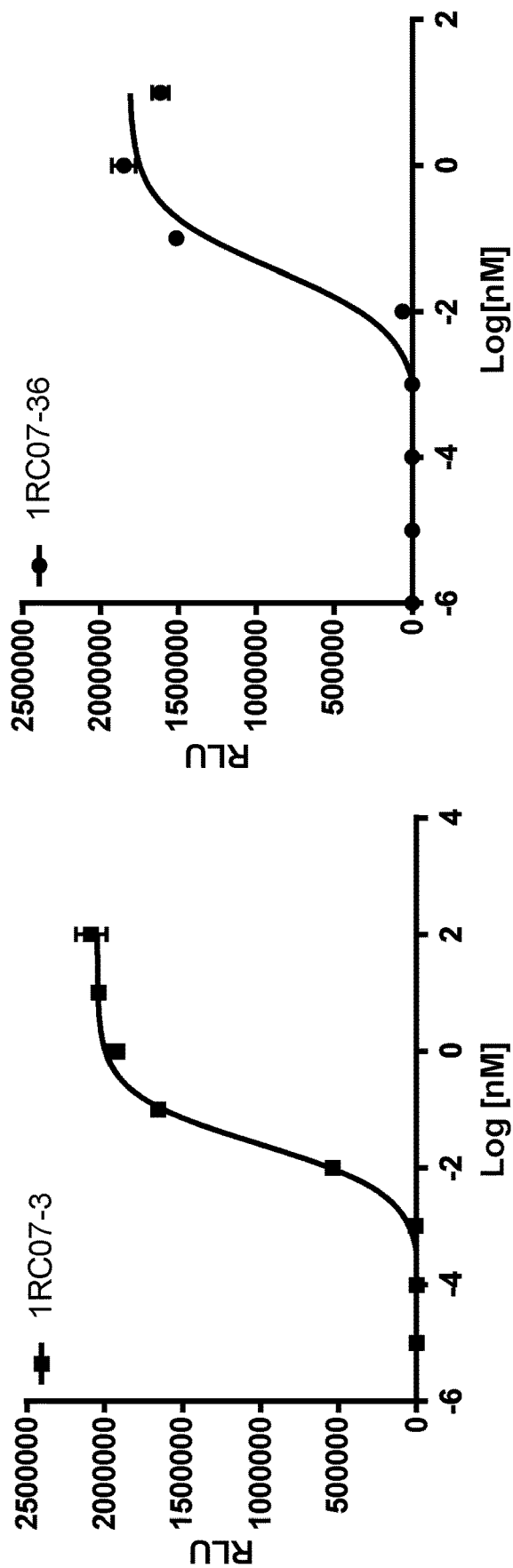


FIG. 17

C.

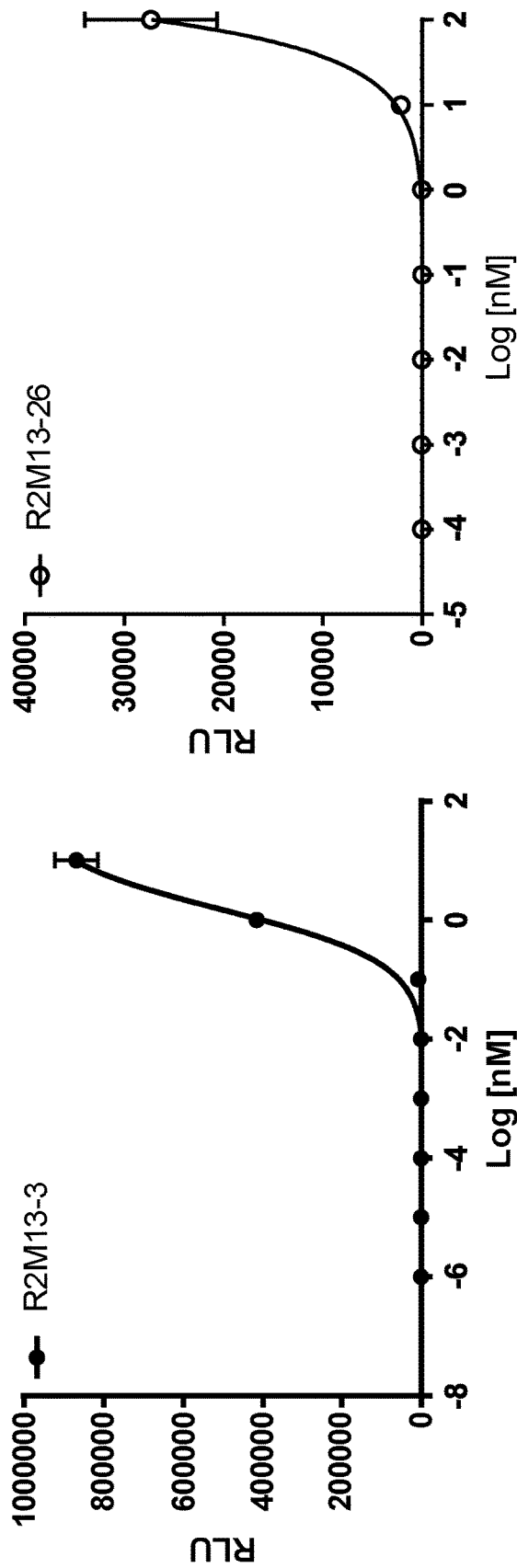
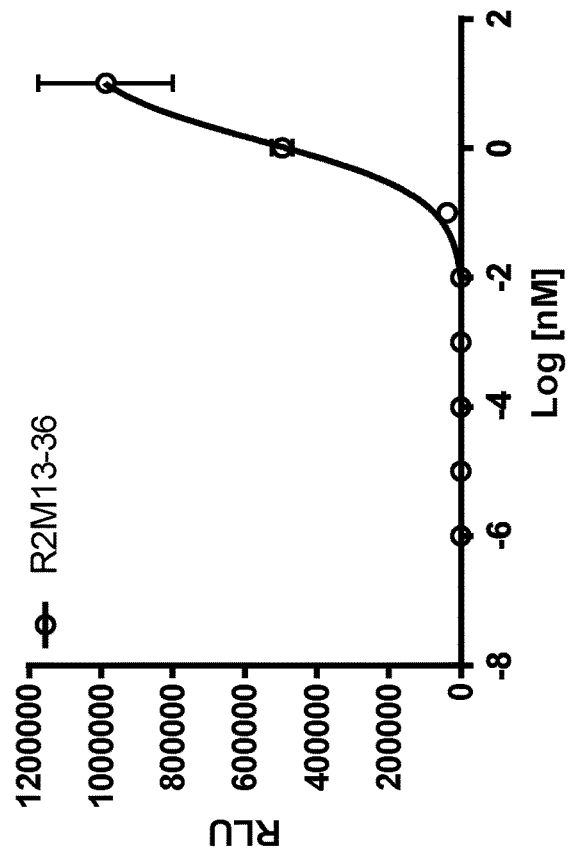


FIG. 17 (contd.)

D.

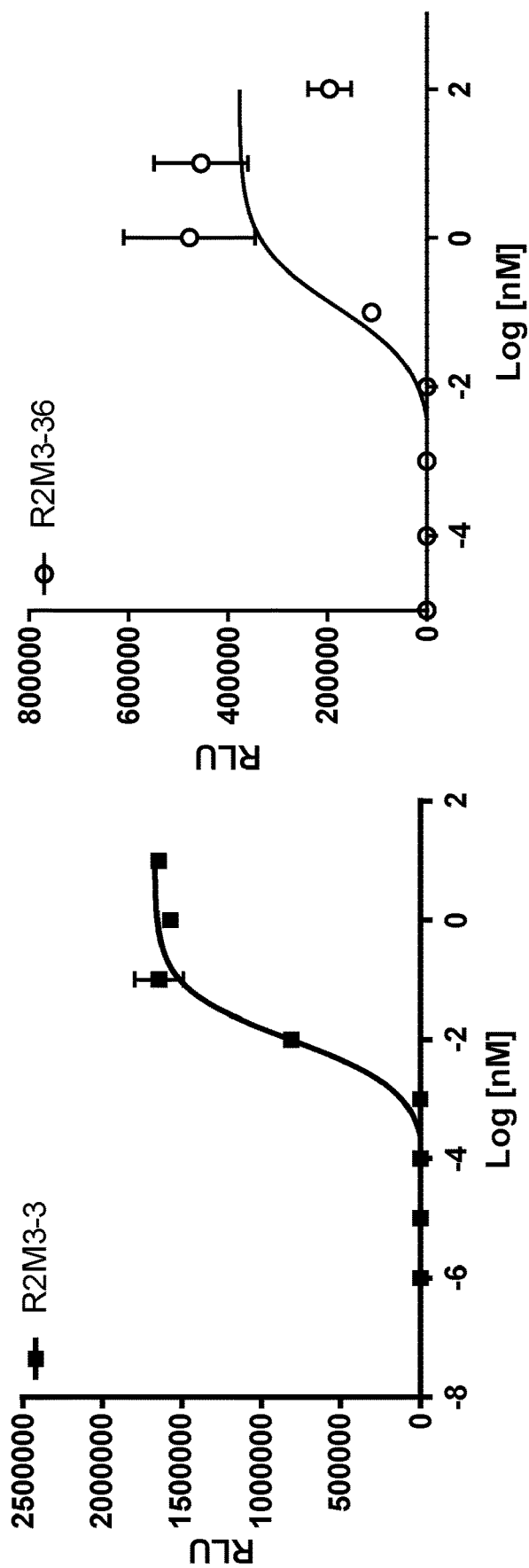


FIG. 17 (contd.)

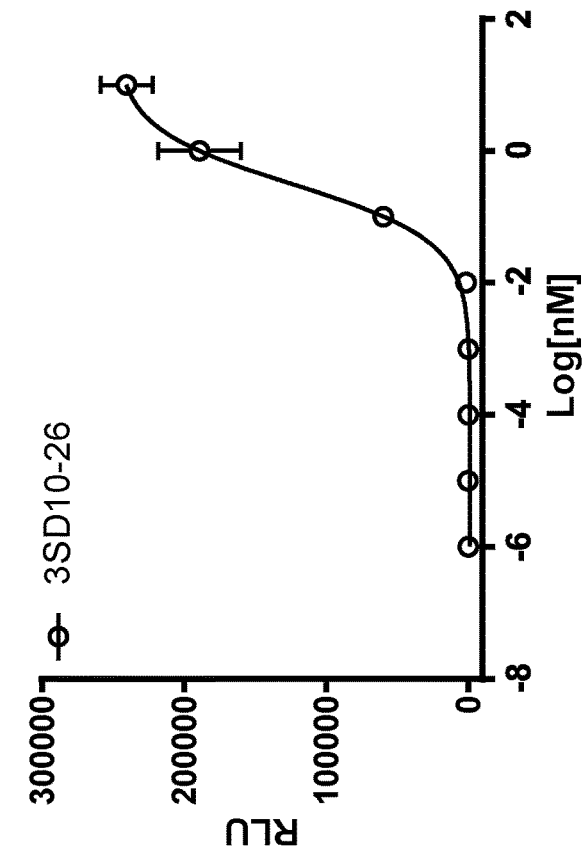
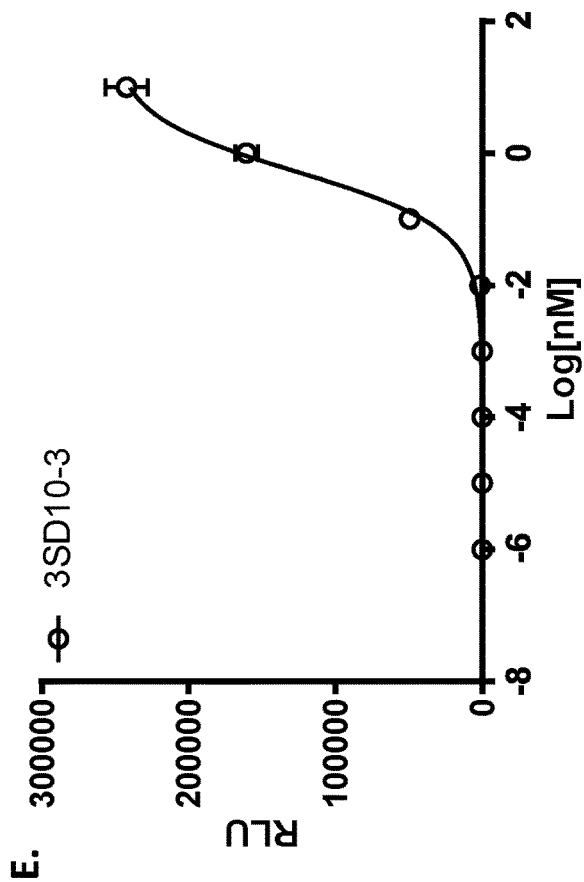
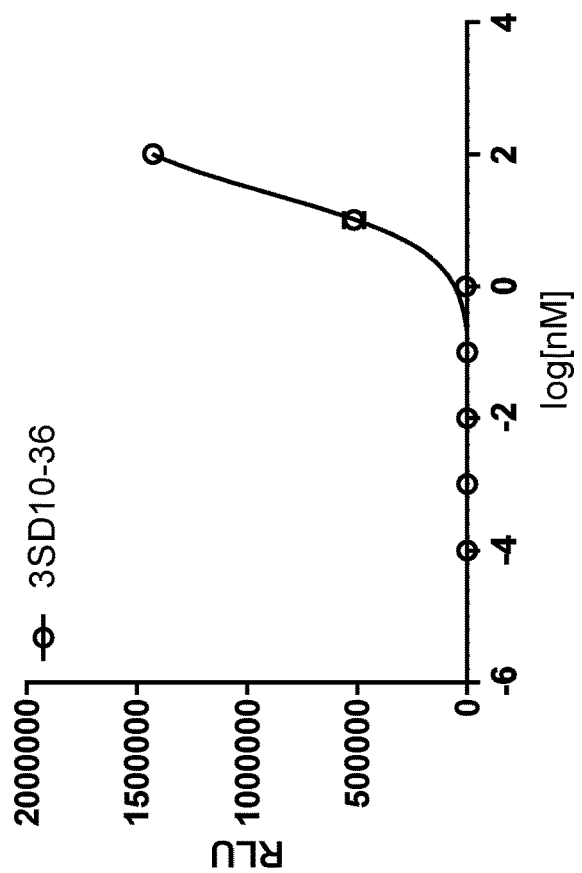
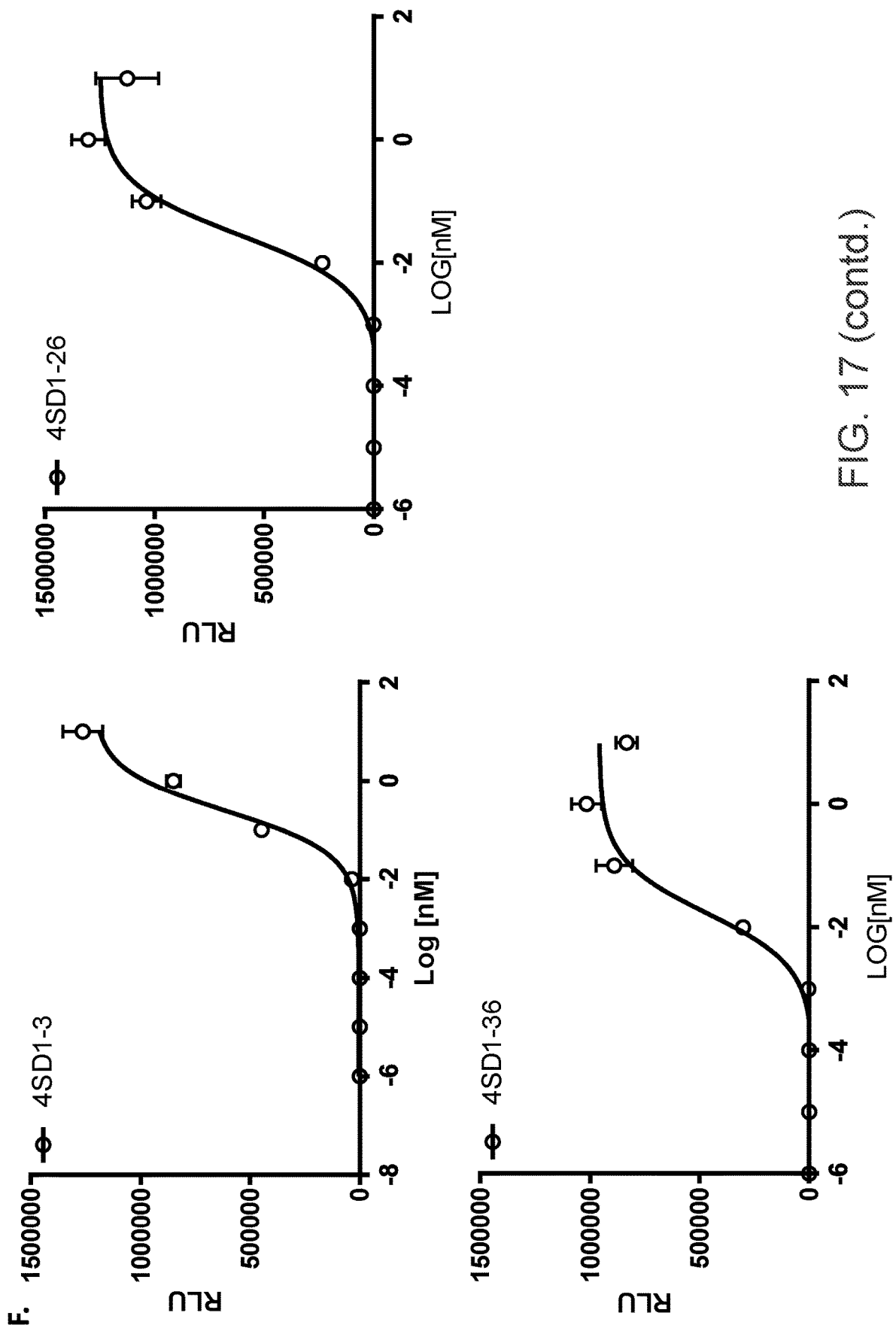


FIG. 17 (contd.)



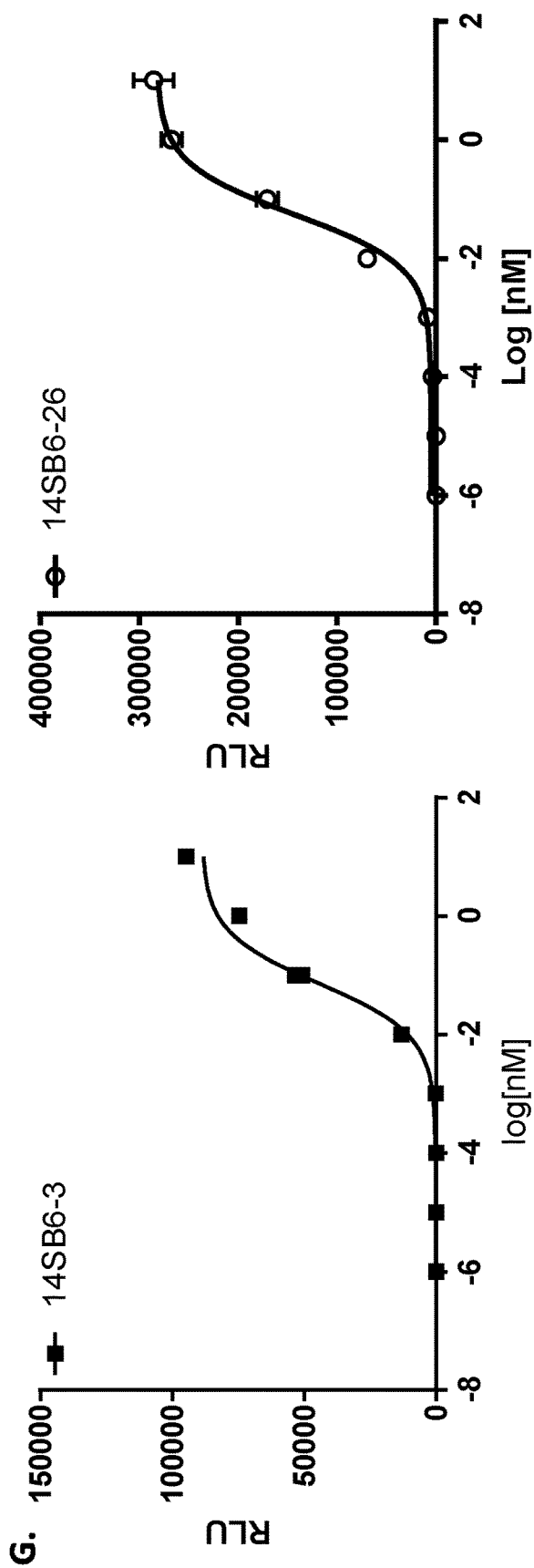
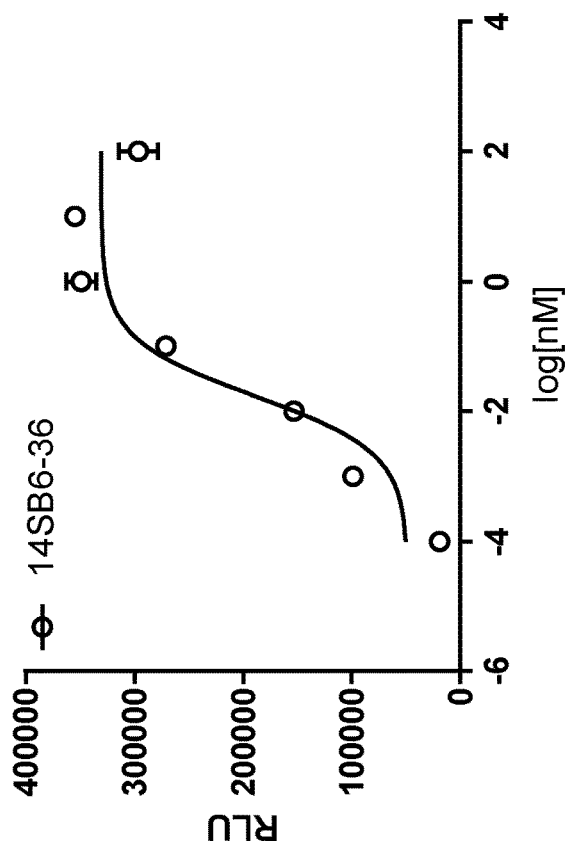


FIG. 17 (contd.)

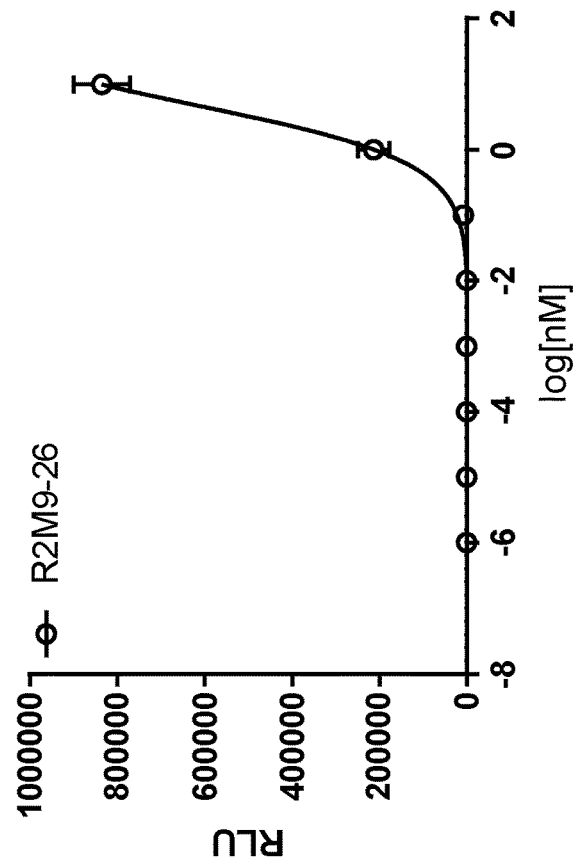
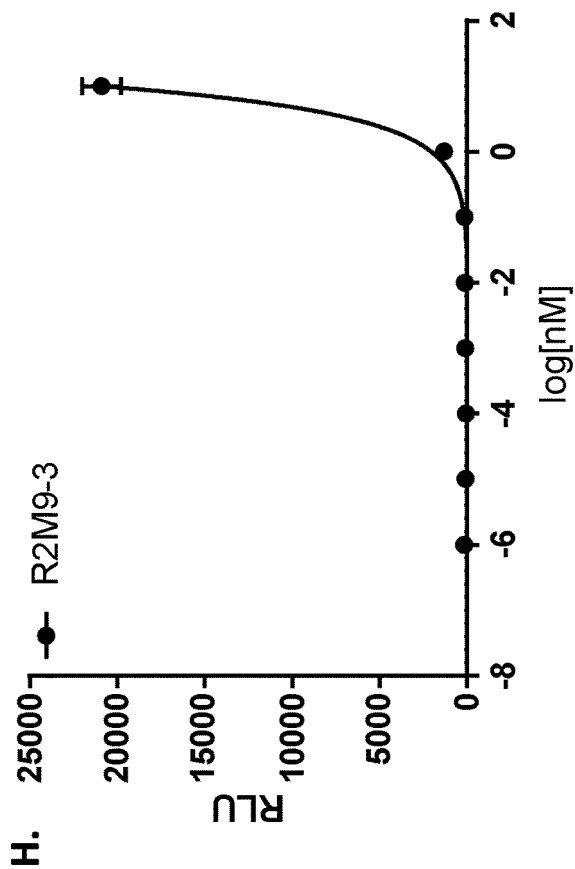
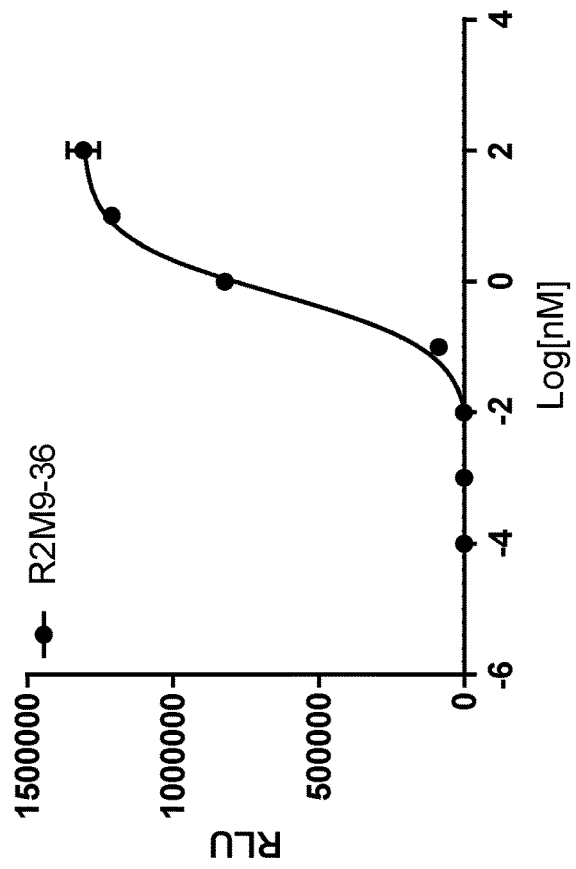


FIG. 17 (contd.)

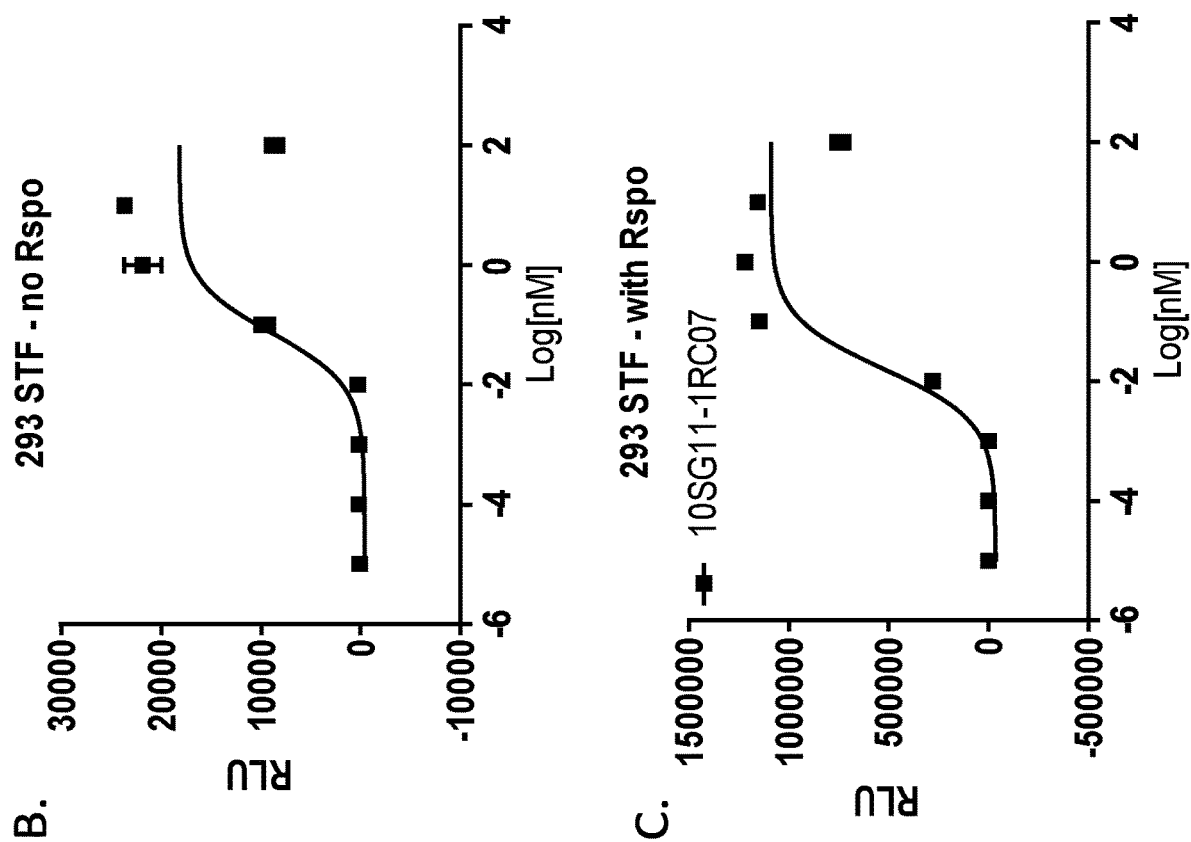


FIG. 18

FIG. 19

Leader = italics

Linker = underlined>

VHH/sdAb or VH or VL = bold

*MYRMQLLS***SCIALSLALVTNSDVQLVESGGGLVQPGGSLRLSCAASG***SIFMINTMAWYRQAPGNQRE*
LVATIRPVVSETTYADAVKGRFTISRDN*AKNTVYLQMNSLKSEDTAIYYCNAKRPWGTRDEYWGQG*
TLVTVSSGSGGS*QTVVLQEPSLSVSPGGTVTLTCGLSSGSVSTNYYPSWYQQTPGQAPRTLIYYTNT*
*RSSDVPERFSGSIVGNKAALTITGAQPDD**ESVYFCLLYLGRGIWVFGGGTKLTVLGQPKAAPSVTLFP*
*PSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTT**PSKQSNNKYAASSYLSLTPEQWK*
*SHRSYSCQVTHEGSTVEKTVAPTECS** (SEQ ID NO:89)

*MYRMQLLS***SCIALSLALVTNSDVQLVESGGGLVQAGGSLRLACAGSGRIFAIYDIAWYRHPPGNQREL**
VAMIRPVVTEIDYADSVKGRFTISRNNAMKT*VYLQMNNLKPEDTAVYYCNAKRPWGSRDEYWGQG*
TQVTVSSGSGGS*QTVVLQEPSLSVSPGGTVTLTCGLSSGSVSTNYYPSWYQQTPGQAPRTLIYYTNT*
*RSSDVPERFSGSIVGNKAALTITGAQPDD**ESVYFCLLYLGRGIWVFGGGTKLTVLGQPKAAPSVTLFP*
*PSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTT**PSKQSNNKYAASSYLSLTPEQWK*
*SHRSYSCQVTHEGSTVEKTVAPTECS** (SEQ ID NO:90)

*MYRMQLLS***SCIALSLALVTNSEVQLVESGGGLVQAGGSLRVSCAASGGTFSRYHMGWFRQAPGKER**
EFVSAITWSSGGR*TYADFKGRFTISRDDARN**TVYLMSSSLKPEDTAVYYCALTWAPTPTNRRSDY*
AYWGQGTQVTVSSGSGGS*QTVVLQEPSLSVSPGGTVTLTCGLSSGSVSTNYYPSWYQQTPGQAPR*
*TLIYYTNTRSSDVPERFSGSIVGNKAALTITGAQPDD**ESVYFCLLYLGRGIWVFGGGTKLTVLGQPKAA*
*PSVTLFPSSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTT**PSKQSNNKYAASSYLSL*
*TPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS** (SEQ ID NO:91)

*MYRMQLLS***SCIALSLALVTNSEVQLVESGGGLVQAGGSLRLACAGSGRIFAIYDMAWYRQAPGNQRE**
LVATIRPVVSETTYADAVKGRFTISR*SNAMKT**VYLQMNSLKSEDTAIYYCNAKRPWGTRDEYWGQG*
TLVTVSSGSGGS*QTVVLQEPSLSVSPGGTVTLTCGLSSGSVSTNYYPSWYQQTPGQAPRTLIYYTNT*
*RSSDVPERFSGSIVGNKAALTITGAQPDD**ESVYFCLLYLGRGIWVFGGGTKLTVLGQPKAAPSVTLFP*
*PSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTT**PSKQSNNKYAASSYLSLTPEQWK*
*SHRSYSCQVTHEGSTVEKTVAPTECS** (SEQ ID NO:92)

*MYRMQLLS***SCIALSLALVTNSAVQLVESGGGLVQAGGSLRLACAGSGRIFAIYDIAWYRQAPGNQREL**
VATIRPVVSETTYADAVKGRFTISR*SNAMKT**VYLQMNSLKSEDTAIYYCNAKRPWGTRDEYWGQG*
LVTVSSGSGGS*QTVVLQEPSLSVSPGGTVTLTCGLSSGSVSTNYYPSWYQQTPGQAPRTLIYYTNT*
*SSDVPERFSGSIVGNKAALTITGAQPDD**ESVYFCLLYLGRGIWVFGGGTKLTVLGQPKAAPSVTLFP*
*SSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTT**PSKQSNNKYAASSYLSLTPEQWKS*
*HRSYSCQVTHEGSTVEKTVAPTECS** (SEQ ID NO:93)

*MYRMQLLS***SCIALSLALVTNSAVQLVDSGGGLVQAGGSLRLSCAVSGRTFSMYDMGWFRQAPGKER**
EFVASIRWSSGNTWYADSMKGRFTISR*DN**AKNTVYLQMNSLKPEDTAVYYCYANIYYTRRAPEEYW*
GQGT**LVTVSSGSGGS***QTVVLQEPSLSVSPGGTVTLTCGLSSGSVSTNYYPSWYQQTPGQAPRTLIY*
*YTNTRSSDVPERFSGSIVGNKAALTITGAQPDD**ESVYFCLLYLGRGIWVFGGGTKLTVLGQPKAAPSV*
*TLFPSSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTT**PSKQSNNKYAASSYLSLTPE*
*QWKSHRSYSCQVTHEGSTVEKTVAPTECS** (SEQ ID NO:94)

*MYRMQLLS***SCIALSLALVTNSEVQLVQSGAEVKKPGASVKVSCKASGYTFTSYGISWVRQAPGQGLEW**
MGWISAYNGNTNYAQKLQGRVTMTTDTSTSTAYMELRSLRSDDTAVYYCASSKEKATYYYGMDVW
GQGT**TVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVT***SWNSGALTSGVHTFPAVL*
QSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFL
FPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVL
HQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDI
*AVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFC**SCVMHEALHNHYTQKSLSL*
*SPGK** (SEQ ID NO:95)

FIG. 19 (contd.)

**MYRMQLLSICIALSLALVTNSEVQLVESGGGLVQPGGSLRLSCTSSANINSIETLGWYRQAPGKQRELI
ANMRGGGYMKYAGSLKGRFTMSTESAKNTLYLQMNSLKPEDTAVYYCYARTQRMGVVNSYWGQ
GTLVTVSSGSGGSDIELTQPPSVSVAPGQTARISCSGDNIGSFYVHWYQQKPGQAPVLVIYDKSNRP
SGIPERFSGSNSGNTATLTISGTQAEDEADYYCQSYANTLSLVFGGGTKLTVLGQPKAAPSVTLFPPS
SEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTTPSKQSNNKYAASSYLSLTPEQWKSH
RSYSCQVTHEGSTVEKTVAPTECS*** (SEQ ID NO:96)

**MYRMQLLSICIALSLALVTNSQVQLVESGGGLVQPGGSLRLSCTSSANINSIETLGWYRQAPGKQREL
IANMRGGGYMKYAGSLKGRFTMSTESAKNTLYLQMNSLKPEDTAVYFCNAVTYDGYTIRGGGTLVT
VSSGSGGSDIELTQPPSVSVAPGQTARISCSGDNIGSFYVHWYQQKPGQAPVLVIYDKSNRPSGIPER
FSGSNSGNTATLTISGTQAEDEADYYCQSYANTLSLVFGGGTKLTVLGQPKAAPSVTLFPPSSEELQA
NKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTTPSKQSNNKYAASSYLSLTPEQWKSHRSYSCQ
VTHEGSTVEKTVAPTECS*** (SEQ ID NO:97)

**MYRMQLLSICIALSLALVTNSEVQLVESGGGLVQPGGSLRLSCTSSANINSIETLGWYRQAPGKQRELI
ANMRGGGYMKYADSVQGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCAAQFRNDYGLRYQSTNN
YWGQGTTLVTVSSGSGGSDIELTQPPSVSVAPGQTARISCSGDNIGSFYVHWYQQKPGQAPVLVIYDK
SNRPSGIPERFSGSNSGNTATLTISGTQAEDEADYYCQSYANTLSLVFGGGTKLTVLGQPKAAPSVTL
FPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTTPSKQSNNKYAASSYLSLTPEQ
WKSHRSYSCQVTHEGSTVEKTVAPTECS*** (SEQ ID NO:98)

**MYRMQLLSICIALSLALVTNSQVQLVESGGGLVQPGGSLRLSCTSSANINSIETLGWYRQAPGKQREL
IANMRGGGYMKYAGSLKGRFTMSTESAKNTVYLQMNSLKPEDSAVYYCNANYRGNRYWGQGTTLV
TVSSGSGGSDIELTQPPSVSVAPGQTARISCSGDNIGSFYVHWYQQKPGQAPVLVIYDKSNRPSGIPE
RFSGSNSGNTATLTISGTQAEDEADYYCQSYANTLSLVFGGGTKLTVLGQPKAAPSVTLFPPSSEELQ
ANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTTPSKQSNNKYAASSYLSLTPEQWKSHRSYSC
QVTHEGSTVEKTVAPTECS*** (SEQ ID NO:99)

**MYRMQLLSICIALSLALVTNSDVQLVESGGGLVQAGGSLRLACAGSGRIFAIYDIAWYRHPPGNQREL
VAMIRPVVTEIDYADSVKGRFTISRNNAMKTIVYLQMNNLKPEDTAVYYCNAKRPWGSRDEYWGQG
TQVTVSSGSGGSDIELTQPPSVSVAPGQTARISCSGDNIGSFYVHWYQQKPGQAPVLVIYDKSNRPS
GIPERFSGSNSGNTATLTISGTQAEDEADYYCQSYANTLSLVFGGGTKLTVLGQPKAAPSVTLFPPSS
EELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTTPSKQSNNKYAASSYLSLTPEQWKSHR
SYSCQVTHEGSTVEKTVAPTECS*** (SEQ ID NO:100)

**MYRMQLLSICIALSLALVTNSEVQLVESGGGLVQAGGSLRVSCAASGGTFSRYHMGWFRQAPGKER
EFVSAITWSSGRTYYADFKGRFTISRDDARNIVYLQMSSLKPEDTAVYYCALTWAPTPTNRRSDY
AYWGQGTQVTVSSGSGGSDIELTQPPSVSVAPGQTARISCSGDNIGSFYVHWYQQKPGQAPVLVIY
DKSNRPSGIPERFSGSNSGNTATLTISGTQAEDEADYYCQSYANTLSLVFGGGTKLTVLGQPKAAPSV
TLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTTPSKQSNNKYAASSYLSLTPE
QWKSHRSYSCQVTHEGSTVEKTVAPTECS*** (SEQ ID NO:101)

**MYRMQLLSICIALSLALVTNSAVQLVESGGGLVQAGGSLRLACAGSGRIFAIYDIAWYRQAPGNQREL
VATIRPVVSETTYADAVKGRFTISRNSAMKTIVYLQMNSLKSEDTAIYYCNAKRPWGTRDEYWGQGT
LVTVSSGSGGSDIELTQPPSVSVAPGQTARISCSGDNIGSFYVHWYQQKPGQAPVLVIYDKSNRPSGI
PERFSGSNSGNTATLTISGTQAEDEADYYCQSYANTLSLVFGGGTKLTVLGQPKAAPSVTLFPPSSEE
LQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTTPSKQSNNKYAASSYLSLTPEQWKSHRSY
SCQVTHEGSTVEKTVAPTECS*** (SEQ ID NO:102)

**MYRMQLLSICIALSLALVTNSAVQLVDSGGGLVQAGGSLRLSCAVSGRTFSMYDMGWFRQAPGKER
EFVASIRWSSGNTWYADSMKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCYANIYYTRRAPEEYW
GGGTLVTVSSGSGGSDIELTQPPSVSVAPGQTARISCSGDNIGSFYVHWYQQKPGQAPVLVIYDKSN
RPSGIPERFSGSNSGNTATLTISGTQAEDEADYYCQSYANTLSLVFGGGTKLTVLGQPKAAPSVTLFP
PSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTTPSKQSNNKYAASSYLSLTPEQWK
SHRSYSCQVTHEGSTVEKTVAPTECS*** (SEQ ID NO:103)

FIG. 19 (contd.)

MYRMQLLSICALSLALVTNSEVQLVESGGGLVQPGGSLRLSCAASGFTFSHYTL~~SWVRQAPGKGL~~
WVSVISGDGSYTYADSVKGRFTISRNNAMKT~~Y~~LQMNSLR~~AEDTAVYYCARNFIKYVFANWGQGT~~
LTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG
LYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKHTCPCPAPEAAAGGPSVFLFPPK
PKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD
WLNGKEYKCKVSNKALGAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVE
WESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNV~~FSCSV~~MHEALHNHYTQKSLSLSPG
K* (SEQ ID NO:104)

MDMRVPAQLLGLLLLWLRGARC~~DVQLVESGGGLVQAGGSLRLACAGSGRIFAIYDIAWYRHPPGNQ~~
REL~~VAMIRPVVTEIDYADSVKGRFTISRNNAMKT~~Y~~LQMNNLKPEDTAVYYCNAKRPWGSRDEYWG~~
QGTQVT~~VSSGSGSGDVMTQSP~~LS~~LPVTPGEPASISCRSSQSL~~LH~~SNGYNYLDWYLQKPGQSPQLLI~~
YLG~~SNRASGVPDRFSGSGSDFTLKISRVEAEDVG~~VYYCMQ~~GTHWPYTFGQGT~~KLEIKRTVAAPSV
FIFPPSDEQLKSGTASV~~VCLLNNFY~~P~~REAKVQWKVDNALQSGNSQESVTEQDSKDSTYLSSTLTLSK~~
ADYEKHKVYACEVTHQGLSSPVTKSFNRGEC* (SEQ ID NO:105)

MDMRVPAQLLGLLLLWLRGARC~~DVQLVESGGGLVQAGGSLRLACAGSGRIFAIYDIAWYRHPPGNQ~~
REL~~VAMIRPVVTEIDYADSVKGRFTISRNNAMKT~~Y~~LQMNNLKPEDTAVYYCNAKRPWGSRDEYWG~~
QGTQVT~~VSSGSGSGDIVMTHPLSLSVTPGEPASISCRSSRL~~LD~~DDGNTYLDWYLQKPGQSPQLLI~~
HTL~~SHRASGVPDRFSGSGSDFTLKISRVEAEDVG~~VYYCMQ~~SIQLPWTFGQGT~~KVEIKRTVAAPSV
FIFPPSDEQLKSGTASV~~VCLLNNFY~~P~~REAKVQWKVDNALQSGNSQESVTEQDSKDSTYLSSTLTLSK~~
ADYEKHKVYACEVTHQGLSSPVTKSFNRGEC* (SEQ ID NO:106)

MDMRVPAQLLGLLLLWLRGARC~~DVQLVESGGGLVQAGGSLRLACAGSGRIFAIYDIAWYRHPPGNQ~~
REL~~VAMIRPVVTEIDYADSVKGRFTISRNNAMKT~~Y~~LQMNNLKPEDTAVYYCNAKRPWGSRDEYWG~~
QGTQVT~~VSSGSGSGSYVLTQPPSVSVSPGQTASITCSGDKVGHKYASWYQQKPGQSPVLVIYEDSQ~~
RPSGIPVRFSGSNSGNTATLTISGTQAMDEADYYCQAWDSSTDV~~VFGG~~TKLTVLGQPKAAPSVTLF
PPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNNKYAASSYLSLTPEQW
KSHRSYSCQVTHEGSTVEKTVAPTECS* (SEQ ID NO:107)

MDMRVPAQLLGLLLLWLRGARC~~DVQLVESGGGLVQAGGSLRLACAGSGRIFAIYDIAWYRHPPGNQ~~
REL~~VAMIRPVVTEIDYADSVKGRFTISRNNAMKT~~Y~~LQMNNLKPEDTAVYYCNAKRPWGSRDEYWG~~
QGTQVT~~VSSGSGSGQSVVTQPPSVSGAPGQRVTISCTRSSNIGAGYDVHWYQQHPGTAPKLLIFG~~
NSIRPSGVPDRFSGSKSGTSATLGITGLQTGDEADYYCGTWDSSLSAWVFGG~~GT~~KLTVLGQPKAAPSV
VTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNNKYAASSYLSLT
EQWKSHRSYSCQVTHEGSTVEKTVAPTECS* (SEQ ID NO:108)

MDMRVPAQLLGLLLLWLRGARC~~DVQLVESGGGLVQAGGSLRLACAGSGRIFAIYDIAWYRHPPGNQ~~
REL~~VAMIRPVVTEIDYADSVKGRFTISRNNAMKT~~Y~~LQMNNLKPEDTAVYYCNAKRPWGSRDEYWG~~
QGTQVT~~VSSGSGSGEIVLTQSPATLSLSPGEGATLSCRASQSVGTYLTWYRQKPGQAPRLIYDASN~~
RATGIPARFSGSGAGTDFTLKISRVEAEDVG~~VYYCMQATQFPLTFGQGT~~KVEIKRTVAAPSVFIFPPSD
EQLKSGTASV~~VCLLNNFY~~P~~REAKVQWKVDNALQSGNSQESVTEQDSKDSTYLSSTLTLSKADYEK~~H
KVYACEVTHQGLSSPVTKSFNRGEC* (SEQ ID NO:109)

MDMRVPAQLLGLLLLWLRGARC~~DVQLVESGGGLVQAGGSLRLACAGSGRIFAIYDIAWYRHPPGNQ~~
REL~~VAMIRPVVTEIDYADSVKGRFTISRNNAMKT~~Y~~LQMNNLKPEDTAVYYCNAKRPWGSRDEYWG~~
QGTQVT~~VSSGSGSGDIQLTQSPSSLSASVGDRVTITCRASRSISSYFNWYQQKPGKAPKLLIYAASSL~~
QSGVPSRFSGSRSGTDFTLTISLQPEDFATYYCQQA~~DTFP~~TFGQGT~~RL~~EIKRTVAAPSVFIFPPSDE
QLKSGTASV~~VCLLNNFY~~P~~REAKVQWKVDNALQSGNSQESVTEQDSKDSTYLSSTLTLSKADYEK~~H
VYACEVTHQGLSSPVTKSFNRGEC* (SEQ ID NO:110)

MDMRVPAQLLGLLLLWLRGARC~~QVQLQESGPGLVKPSETLSLTCTVSGGSISGNNYYWG~~WIRQPPG
KGLEWIGSIYFTGGTYYNPSLKSRVTISVDTSKNQ~~FS~~LKLS~~SVTAADTAVYYCAR~~VMLITDAFDI~~WGQ~~
GTMVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQ
SGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKHTCPCPAPELLGGPSVFLFP
PKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ
DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAV

FIG. 19 (contd.)

EWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSP
GKGSGSGHHHHH** (SEQ ID NO:111)

MDMRVPAQLLGLLLLWLRGARCQLQLQESGSLVKPSQTLSTCAVSGGSISSGGYSWSWIRQPP
GKGLEWIGSIYHSGSTYYNPSLKSRLTISVDTSKNQFSLKLSSVTAADTAVYYCARFYDILTGYSYF
DYWGQGTLTVTSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTF
PAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGP
SVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSV
LTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFY
PSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQK
LSLSLSPGK153HHHHH** (SEQ ID NO:112)

MDMRVPAQLLGLLLLWLRGARCQVQLQQWGAGLLKPSETLSLTCVSGASFSGHYWTWIRQPPGK
GLEWIGEIDHTGSTNYEPSLRSRVTISVDTSKNQFSLNLKSVTAADTAVYYCARGGQGGYDWGHYH
GLDVWGQGTTLVTSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVH
TFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAG
GPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVV
SVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKG
FYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYT
QKSLSLSPGKGSGSGHHHHH* (SEQ ID NO:113)

MDMRVPAQLLGLLLLWLRGARCQVQLQESGPGLVKPSETLSLCTVSGGSISSSSYYWGWRQHPG
KGLEWIGIYYSGSTYYNPSLKSRLTISVDTSKNQFSLKLNSVTAADTAVYYCARATYGGDAFDIWG
QGTMTVTSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQ
SSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLF
PPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLH
QDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIA
VEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL
PGKGSGSGHHHHH** (SEQ ID NO:114)

MDMRVPAQLLGLLLLWLRGARCQVQLQESGPGLVKPSETLSLCTVSGGAISGTSYFWGWIRQPPG
KGLEWIGSIYYTGNTYYNPSLKSRLTVSVDTSKNQFSLKLNSVTAADTATYYCARIGIAVAAPVDHW
GQGTTLVTSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVL
QSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFL
FPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVL
HQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDI
AVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL
SPGKGSGSGHHHHH* (SEQ ID NO:115)

MDMRVPAQLLGLLLLWLRGARCQLQLQESGPGLVKPSQTLSTCTVSGGSISSGGYYWSWIRQHPG
KGLEWIGIYYSGSTYYNPSLKSRLTISVDTSKNQFSLKLSSVTAADTAVYYCARVRDYDSSGYYY
DYFDYWGQGTTLVTSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV
HTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL
GGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV
VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVK
GFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHY
TQKSLSLSPGKGSGSGHHHHH** (SEQ ID NO:116)

FIG. 19 (contd.)

MDMRVPAQLLGLLLLWLRGARCEVQLVQSGAEVKKPGASVKVSCASGYTFTSYGISWVRQAPGQG
LEWMGWISAYNGNTNYAQLQGRVTMTTDTSTSTAYMELRSLRSDDTAVYYCASSKEKATYYYYGMD
VWGQGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPA
VLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPS
VFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVL
TVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFY
PSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQK
SLSLSPGKSGSGSDVQLVESGGGLVQAGGSLRLACAGSGRIFAIYDIAWYRHPPGNQRELVAMIRP
VVTEIDYADSVKGRFTISRNNAMKTIVYLQMNNLKPEDTAVYYCNAKRPWGSRDEYWGQGTQVTVS
S* (SEQ ID NO:117)

MDMRVPAQLLGLLLLWLRGARCDVQLVESGGGLVQAGGSLRLACAGSGRIFAIYDIAWYRHPPGNQ
RELVAMIRPVVTEIDYADSVKGRFTISRNNAMKTIVYLQMNNLKPEDTAVYYCNAKRPWGSRDEYWG
QGTQVTVSSGSGSGEVQLVQSGAEVKKPGASVKVSCASGYTFTSYGISWVRQAPGQGLEWMGWI
SAYNGNTNYAQLQGRVTMTTDTSTSTAYMELRSLRSDDTAVYYCASSKEKATYYYYGMDVWGQGT
TVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGL
YSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPK
KDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWL
NGKEYKCKVSNKALGAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWE
SNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK*
(SEQ ID NO:118)

MDMRVPAQLLGLLLLWLRGARCAVVLQEPSLSVSPGGTVTLTCGLSSGSVSTNYYPSWYQQTPGQ
APRTLIYYTNTRSSDVPERFSGSIVGNKAALTITGAQPDDSVYFCLLYLGRGIWVFGGGTKLTVLGQP
KAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTTPSKQSNNKYAASSY
LSLTPEQWKSQRSYSCQVTHEGSTVEKTVAPTECSGSGSDVQLVESGGGLVQAGGSLRLACAGS
GRIFAIYDIAWYRHPPGNQRELVAMIRPVVTEIDYADSVKGRFTISRNNAMKTIVYLQMNNLKPEDTAV
YYCNAKRPWGSRDEYWGQGTQVTVSS* (SEQ ID NO:119)

MDMRVPAQLLGLLLLWLRGARCDVQLVESGGGLVQAGGSLRLACAGSGRIFAIYDIAWYRHPPGNQ
RELVAMIRPVVTEIDYADSVKGRFTISRNNAMKTIVYLQMNNLKPEDTAVYYCNAKRPWGSRDEYWG
QGTQVTVSSGSGSGQAVVLQEPSLSVSPGGTVTLTCGLSSGSVSTNYYPSWYQQTPGQAPRTLIY
TNTRSSDVPERFSGSIVGNKAALTITGAQPDDSVYFCLLYLGRGIWVFGGGTKLTVLGQPKAAPSV
TLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTTPSKQSNNKYAASSYLSLTPEQ
WKSQRSYSCQVTHEGSTVEKTVAPTECS* (SEQ ID NO:120)

MDMRVPAQLLGLLLLWLRGARCEVQLVQSGAEVKKPGASVKVSCASGYTFTSYGISWVRQAPGQ
GLEWMGWISAYNGNTNYAQLQGRVTMTTDTSTSTAYMELRSLRSDDTAVYYCASSKEKATYYYYG
MDVWGQGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHT
FPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCGSGSGHHHHHH* (SEQ
ID NO:122)

MYRMQLLSIALSLALVTNSAVQLVDSGGGLVQAGGSLRLSCAVSGRTFSMYDMGWFRQAPGKER
EFVASIRWSSGNTWYADSMKGRFTISRDNANTVYLQMNSLKPEDTAVYYCYANIYYTRAPEEYW
GQGTLTVTSSGSGGSQTIVLQEPSLSVSPGGTVTLTCGLSSGSVSTNYYPSWYQQTPGQAPRTLIY
YTNRSSDVPERFSGSIVGNKAALTITGAQPDDSVYFCLLYLGRGIWVFGGGTKLTVLGQPKAAPSV
TLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTTPSKQSNNKYAASSYLSLTPE
QWKSQRSYSCQVTHEGSTVEKTVAPTECS* (SEQ ID NO:123)

MDMRVPAQLLGLLLLWLRGARCEVQLVQSGAEVKKPGASVKVSCASGYTFTSYGISWVRQAPGQ
GLEWMGWISAYNGNTNYAQLQGRVTMTTDTSTSTAYMELRSLRSDDTAVYYCASSKEKATYYYYG
MDVWGQGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHT
FPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCGSGSGHHHHHH* (SEQ
ID NO:124)

FIG. 19 (contd.)

**MDMRVPAQLLGLLLLWLRGARCQAVVLQEPSLSVSPGGTVTLTCGLSSGSVSTNYYPSWYQQTPG
QAPRTLIIYTNTRSSDVPERFSGSIVGNKAALTITGAQPDDSVYFCLLYLGRGIWVFGGGTKLTVLG
QPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTTPSKQSNNKYAAS
SYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS*** (SEQ ID NO:125)

**MDMRVPAQLLGLLLLWLRGARCEVQLVQSGAEVKKPGASVKVSCKASGYTFTSYGISWVRQAPGQ
GLEWMGWISAYNGNTNYAQKLQGRVTMTTDTSTSTAYMELRSLRSDDTAVYYCASSKEKATYYYG
MDVWGQGTITVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHT
FPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAG
GPSVFLFPPKPKDTLMISRTPEVTCVWVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVV
SVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLWCLVK
GFYPSDIAVEWESNGQPENNYKTTPVLDSGGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHY
TQKSLSLSPGK*** (SEQ ID NO:126)

**MDMRVPAQLLGLLLLWLRGARCQVQLVESGGGLVQAGGSLRLACAGSGRIFAIYDIAWYRHPPGNQ
RELAMIRPVVTEIDYADSVKGRFTISRNNAMKTYYLQMNNLKPEDTAVYYCNAKRPWGSRDEYWG
QGTQVTVSSDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVWVDVSHEDPEVKFNWY
VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQPRE
PQVYTLPPSREEMTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPVLDSGGSFFLVSKLTV
DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKSGSGHHHHHH*** (SEQ ID NO:127)

**MDMRVPAQLLGLLLLWLRGARCQVQLVESGGGLVQAGGSLRLACAGSGRIFAIYDIAWYRHPPGNQ
RELAMIRPVVTEIDYADSVKGRFTISRNNAMKTYYLQMNNLKPEDTAVYYCNAKRPWGSRDEYWG
QGTQVTVSSDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVWVDVSHEDPEVKFNWY
VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQPRE
PQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPVLDSGGSFFLYSKLTV
DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKGGSGSAVQQVESGGGLVQPGGSLRLSCAA
SGFTLDYYAISWFRQAPGKKRELVADITSGGSTNYADSVKGRFTISRDNANTYIYLMNSLKPEDTA
VYFCNAVTYNGYTIWGQGTQVTVSS*** (SEQ ID NO:128)

**MDMRVPAQLLGLLLLWLRGARCQVQLVESGGGLVQPGGSLRLSCTSSANINSIETLGWYRQAPGKQ
RELIANMRGGGYMKYAGSLKGRFTMSTESAKNTMYLQMNSLKPEDTAVYYCYVKLRDDDYVYRGQ
GTQVTVSSGSGSGSYVLTQPPSVSVSPGQTASITCSGDKVGHKYASWYQQKPGQSPVLVIYEDSQR
PSGIPVRFSGSNSGNTATLTISGTQAMDEADYYCQAWDSSTDVVFGGGKLTVLGQPKAAPSVTLFPP
PSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTTPSKQSNNKYAASSYLSLTPEQWK
SHRSYSCQVTHEGSTVEKTVAPTECS*** (SEQ ID NO:134)

**MDMRVPAQLLGLLLLWLRGARCQVKLEESGGGLVQAGGSLRLSCAASGRIFSIYDMGWFRQAPGK
EREFVSGIRWGGTSYADSVKGRFTISKDNANTYIYLMNNLKAEDTAVYYCGSRGYWGGGTLTVV
SSGSGSGSYVLTQPPSVSVSPGQTASITCSGDKVGHKYASWYQQKPGQSPVLVIYEDSQRPSGIPVR
FSGSNSGNTATLTISGTQAMDEADYYCQAWDSSTDVVFGGGKLTVLGQPKAAPSVTLFPPSSEELQ
ANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTTPSKQSNNKYAASSYLSLTPEQWKSHRSYSC
QVTHEGSTVEKTVAPTECS*** (SEQ ID NO:135)

**MDMRVPAQLLGLLLLWLRGARCQVQLVESGGGLVQPGGSLRLSCTSSANINSIETLGWYRQAPGKQ
RELIANMRGGGYMKYAGSLKGRFTMSTESAKNTMYLQMNSLKPEDTAVYYCYVKLRDDDYVYRGQ
GTQVTVSSGSGSGSGDIQMTQSPSSLSASVGDRTITCRASQSISSYLNWYQQKPGKAPKLLIYAA
SSLQSGVPSRFSGSGSGTDFTLTISLQPEDFATYYCQQSYSTPLTFGGGKVEIKRTVAAPSVFIFPP
SDEQLKSGTASVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYE
KHKVYACEVTHQGLSSPVTKSFNRGEC*** (SEQ ID NO:136)

**MDMRVPAQLLGLLLLWLRGARCQVQLVESGGGLVQAGGSLRLACAGSGRIFAIYDIAWYRHPPGNQ
RELAMIRPVVTEIDYADSVKGRFTISRNNAMKTYYLQMNNLKPEDTAVYYCNAKRPWGSRDEYWG
QGTQVTVSSGSGSGDIQMTQSPSSLSASVGDRTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSL
QSGVPSRFSGSGSGTDFTLTISLQPEDFATYYCQQSYSTPLTFGGGKVEIKRTVAAPSVFIFPPSDE
QLKSGTASVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHK
VYACEVTHQGLSSPVTKSFNRGEC*** (SEQ ID NO:137)

FIG. 19 (contd.)

**MDMRVPAQLLGLLLLWLRGARCQVKLEESGGGLVQAGGSLRLSCAASGRIFSIYDMGWFRQAPGK
EREFVSGIRWSSGTSYADSVKGRFTISKDNAKNTIYLQMNNLKAEDTAVYYCGSRGYWGQGLTVT
SSGSGSGDIQMTQSPSSLSASVGDRVITICRASQSISSYLNWYQQKPKAPKLLIYAASSLQSGVPSR
FSGSGSGTDFLTISLQPEDFATYYCQQSYSTPLTFGGGKVEIKRTVAAPSVFIFPPSDEQLKSGTA
SVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSLSTLTLSKADYEKHKVYACEVT
HQLGLSSPVTKSFNRGEC*** (SEQ ID NO:138)

**MDMRVPAQLLGLLLLWLRGARCQVQLVESGGGLVQPGGSLRLSCTSSANINSIETLGWYRQAPGKQ
RELIANMRGGGYMKYAGSLKGRFTMSTESAKNTMYLQMNLSLKPEDTAVYYCYVKLRDDDYVYRGQ
GTQVTVSSGSGSGQAVVLQEPSLSVSPGGTVTLTCGLSSGSVSTNYYPSWYQQTPGQAPRTLIYYT
NTRSSDVPERFSGSIVGNKAALTITGAQPDDSVYFCLLYLGRGIWVFGGGTKLTVLGQPKAAPSVTL
FPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNNKYAASSYLSLTPEQ
WKSHRSYSQCQVTHEGSTVEKTVAPTECS*** (SEQ ID NO:139)

**MDMRVPAQLLGLLLLWLRGARCQVKLEESGGGLVQAGGSLRLSCAASGRIFSIYDMGWFRQAPGK
EREFVSGIRWSSGTSYADSVKGRFTISKDNAKNTIYLQMNNLKAEDTAVYYCGSRGYWGQGLTVT
SSGSGSGQAVVLQEPSLSVSPGGTVTLTCGLSSGSVSTNYYPSWYQQTPGQAPRTLIYYTNTRSSDV
PERFSGSIVGNKAALTITGAQPDDSVYFCLLYLGRGIWVFGGGTKLTVLGQPKAAPSVTLFPPSSEEL
QANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNNKYAASSYLSLTPEQWKSHRSYS
CQVTHEGSTVEKTVAPTECS*** (SEQ ID NO:140)

**MDMRVPAQLLGLLLLWLRGARCQVQLVESGGGLVQPGGSLRLSCTSSANINSIETLGWYRQAPGKQ
RELIANMRGGGYMKYAGSLKGRFTMSTESAKNTMYLQMNLSLKPEDTAVYYCYVKLRDDDYVYRGQ
GTQVTVSSGSGSGDIQMTQSPSSLSASVGDRVITICRASQGISSYLAWYQQKPKAPKLLIYAASNLL
GGVPSRFSGSGSGTDFLTISLQPEDFATYYCQQTYSTPWTFGQGTKEIKRTVAAPSVFIFPPSDE
QLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSLSTLTLSKADYEKHK
VYACEVTHQLGLSSPVTKSFNRGEC*** (SEQ ID NO:141)

**MDMRVPAQLLGLLLLWLRGARCQVQLVESGGGLVQAGGSLRLACAGSGRIFAIYDIAWYRHPPGNQ
RELVAMIRPVVTEIDYADSVKGRFTISRNNAMKTYYLQMNNLKPEDTAVYYCNAKRPWGSRDEYWG
QGTQVTVSSGSGSGDIQMTQSPSSLSASVGDRVITICRASQGISSYLAWYQQKPKAPKLLIYAASNLL
LGGVPSRFSGSGSGTDFLTISLQPEDFATYYCQQTYSTPWTFGQGTKEIKRTVAAPSVFIFPPSD
EQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSLSTLTLSKADYEKHK
VYACEVTHQLGLSSPVTKSFNRGEC*** (SEQ ID NO:142)

**MDMRVPAQLLGLLLLWLRGARCQVKLEESGGGLVQAGGSLRLSCAASGRIFSIYDMGWFRQAPGK
EREFVSGIRWSSGTSYADSVKGRFTISKDNAKNTIYLQMNNLKAEDTAVYYCGSRGYWGQGLTVT
SSGSGSGDIQMTQSPSSLSASVGDRVITICRASQGISSYLAWYQQKPKAPKLLIYAASNLLGGVPSR
FSGSGSGTDFLTISLQPEDFATYYCQQTYSTPWTFGQGTKEIKRTVAAPSVFIFPPSDEQLKSGT
ASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSLSTLTLSKADYEKHKVYACEV
THQLGLSSPVTKSFNRGEC*** (SEQ ID NO:143)

**MDMRVPAQLLGLLLLWLRGARCQVQLVESGGGLVQPGGSLRLSCTSSANINSIETLGWYRQAPGKQ
RELIANMRGGGYMKYAGSLKGRFTMSTESAKNTMYLQMNLSLKPEDTAVYYCYVKLRDDDYVYRGQ
GTQVTVSSGSGSGDIQMTQSPSSLSASVGDRVITICRASQSISSYLNWYQQKPKAPKLLIYAASSLQ
SGVPSRFSGSGSGTDFLTISLQPEDFATYYCQQSYSTPLTFGGGKVEIKRTVAAPSVFIFPPSDEQ
LKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSLSTLTLSKADYEKHKV
YACEVTHQLGLSSPVTKSFNRGEC*** (SEQ ID NO:144)

**MDMRVPAQLLGLLLLWLRGARCQVQLVESGGGLVQAGGSLRLACAGSGRIFAIYDIAWYRHPPGNQ
RELVAMIRPVVTEIDYADSVKGRFTISRNNAMKTYYLQMNNLKPEDTAVYYCNAKRPWGSRDEYWG
QGTQVTVSSGSGSGDIQMTQSPSSLSASVGDRVITICRASQSISSYLNWYQQKPKAPKLLIYAASSL
QSGVPSRFSGSGSGTDFLTISLQPEDFATYYCQQSYSTPLTFGGGKVEIKRTVAAPSVFIFPPSDE
QLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSLSTLTLSKADYEKHK
VYACEVTHQLGLSSPVTKSFNRGEC*** (SEQ ID NO:145)

FIG. 19 (contd.)

**MDMRVPAQLLGLLLLWLRGARCQVKLEESGGGLVQAGGSLRLSCAASGRIFSIYDMGWFRQAPGK
EREFVSGIRWSGGTSYADSVKGRFTISKDNAKNTIYLQMNNLKAEDTAVYYCGSRGYWGQGLTVT
SSGGSGSDIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSR
FSGSGSGTDFTLTISLQPEDFATYYCQQSYSTPLTFGGGTKEIKRTVAAPSVFIFPPSDEQLKSGTA
SVVCLLNFPYAPREKQVQWKVDNALQSGNSQESVTEQDSKDYSLSTLTLSKADYEKHKVYACEVT
HQLSSPVTKSFNRGEC*** (SEQ ID NO:146)

**MDMRVPAQLLGLLLLWLRGARCQVQVLESVGGGLVQPGGSLRLSCTSSANINSIETLGWYRQAPGKQ
RELIANMRGGGYMKYAGSLKGRFTMSTESAKNTMYLQMNLSLKPEDTAVYYCYVKLRDDDYVYRGQ
GTQVTVSSGGSGSDIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQ
SGVPSRFSGSGSGTDFTLTISLQPEDFATYYCQQSYSTPLTFGGGTKEIKRTVAAPSVFIFPPSDEQ
LKSGTASVVCLLNFPYAPREKQVQWKVDNALQSGNSQESVTEQDSKDYSLSTLTLSKADYEKHKV
YACEVTHQLSSPVTKSFNRGEC*** (SEQ ID NO:147)

**MDMRVPAQLLGLLLLWLRGARCQVQVLESVGGGLVQAGGSLRLACAGSGRIFAIYDIAWYRHPPGNQ
RELAMIRPVVTEIDYADSVKGRFTISRNNAMKTYYLQMNLSLKPEDTAVYYCNAKRPWGSRDEYWG
QGTQVTVSSGGSGSDIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSL
QSGVPSRFSGSGSGTDFTLTISLQPEDFATYYCQQSYSTPLTFGGGTKEIKRTVAAPSVFIFPPSDE
QLKSGTASVVCLLNFPYAPREKQVQWKVDNALQSGNSQESVTEQDSKDYSLSTLTLSKADYEKHK
YACEVTHQLSSPVTKSFNRGEC*** (SEQ ID NO:148)

**MDMRVPAQLLGLLLLWLRGARCQVKLEESGGGLVQAGGSLRLSCAASGRIFSIYDMGWFRQAPGK
EREFVSGIRWSGGTSYADSVKGRFTISKDNAKNTIYLQMNNLKAEDTAVYYCGSRGYWGQGLTVT
SSGGSGSDIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSR
FSGSGSGTDFTLTISLQPEDFATYYCQQSYSTPLTFGGGTKEIKRTVAAPSVFIFPPSDEQLKSGTA
SVVCLLNFPYAPREKQVQWKVDNALQSGNSQESVTEQDSKDYSLSTLTLSKADYEKHKVYACEVT
HQLSSPVTKSFNRGEC*** (SEQ ID NO:149)

**MDMRVPAQLLGLLLLWLRGARCQVQVLESVGGGLVQPGGSLRLSCTSSANINSIETLGWYRQAPGKQ
RELIANMRGGGYMKYAGSLKGRFTMSTESAKNTMYLQMNLSLKPEDTAVYYCYVKLRDDDYVYRGQ
GTQVTVSSGGSGSDIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQ
SGVPSRFSGSGSGTDFTLTISLQPEDFATYYCQQSYRTPFTFGPGTKVDIKRTVAAPSVFIFPPSDEQ
LKSGTASVVCLLNFPYAPREKQVQWKVDNALQSGNSQESVTEQDSKDYSLSTLTLSKADYEKHKV
YACEVTHQLSSPVTKSFNRGEC*** (SEQ ID NO:150)

**MDMRVPAQLLGLLLLWLRGARCQVQVLESVGGGLVQAGGSLRLACAGSGRIFAIYDIAWYRHPPGNQ
RELAMIRPVVTEIDYADSVKGRFTISRNNAMKTYYLQMNLSLKPEDTAVYYCNAKRPWGSRDEYWG
QGTQVTVSSGGSGSDIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSL
QSGVPSRFSGSGSGTDFTLTISLQPEDFATYYCQQSYRTPFTFGPGTKVDIKRTVAAPSVFIFPPSDE
QLKSGTASVVCLLNFPYAPREKQVQWKVDNALQSGNSQESVTEQDSKDYSLSTLTLSKADYEKHK
YACEVTHQLSSPVTKSFNRGEC*** (SEQ ID NO:151)

**MDMRVPAQLLGLLLLWLRGARCQVKLEESGGGLVQAGGSLRLSCAASGRIFSIYDMGWFRQAPGK
EREFVSGIRWSGGTSYADSVKGRFTISKDNAKNTIYLQMNNLKAEDTAVYYCGSRGYWGQGLTVT
SSGGSGSDIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSR
FSGSGSGTDFTLTISLQPEDFATYYCQQSYRTPFTFGPGTKVDIKRTVAAPSVFIFPPSDEQLKSGTA
SVVCLLNFPYAPREKQVQWKVDNALQSGNSQESVTEQDSKDYSLSTLTLSKADYEKHKVYACEVT
HQLSSPVTKSFNRGEC*** (SEQ ID NO:152)

**MDMRVPAQLLGLLLLWLRGARCEVQLLQSGAEVKKPGSSVKVSCASGGTFTYRYLHWVRQAPGQ
GLEWMGGIPIFGTGNYAQKFQGRVTITADESTSTAYMELSSLRSEDYAVYYCASSMVRVPYGGMD
VWGQGLTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPA
VLQSSGLYSLSSVTPVSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGFS
VFLFPPKPKDLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVL
TVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFY
PSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQK
SLSLSPGK*** (SEQ ID NO:153)

FIG. 19 (contd.)

**MDMRVPAQLLGLLLLWLRGARCEVQLVESGGGLVKPGGSLRLSCAASGFNFGIYSMTWVRQAPGK
GLEWISYISGDSGYTNYADSVKGRFTISRDDSKNTLYLQMNSLKTEDTAVYYCARVGGWFDPLQ
QGTLTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQ
SSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLF
PPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLH
QDWLNGKEYKCKVSNKALGAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIA
VEWESNGQPENNYKTTPVLDSGGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSL
PGK* (SEQ ID NO:154)**

**MDMRVPAQLLGLLLLWLRGARCEVQLVESGGGLVKPGGSLRLSCAASGFTFTNYAMSWVRQAPGK
GLEWVSAISGSGSTYYADSVKGRFTISRDDSKNTLYLQMNSLKTEDTAVYYCARATGFGTVFDY
WGQGLTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAV
LQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSV
FLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVL
VLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFY
SDIAVEWESNGQPENNYKTTPVLDSGGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKS
LSLSPGK* (SEQ ID NO:155)**

**MDMRVPAQLLGLLLLWLRGARCEVQLVQSGGGLVKPGGSLRLSCAASGFTFSSYSMNWVRQAPG
KGLEWVSYIENDGSITTYADSVKGRFTISRDDSKNTLYLQMNSLKTEDTAVYYCARAPYYYGSGSLF
RLDYWGQGLTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVH
TFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAG
GPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVV
SVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVK
FYPSDIAVEWESNGQPENNYKTTPVLDSGGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYT
QKSLSLSPGK* (SEQ ID NO:156)**

**MDMRVPAQLLGLLLLWLRGARCQVQLVQSGAEVKKPGSSVKVSCKASGYTFTNNFMHWVRQAPG
QGLEWMGWINPNSGGTKYAQKFQGRVTITADESTSTAYMELSSLRSED TAVYYCARSVGEVGATM
LGIGVWYWFDPWGQGLTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSG
ALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPC
PAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY
NSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQV
SLTCLVKGFYPSDIAVEWESNGQPENNYKTTPVLDSGGSFFLYSKLTVDKSRWQQGNVFCFSVMH
EALHNHYTQKSLSLSPGK* (SEQ ID NO:157)**

**MDMRVPAQLLGLLLLWLRGARCQVQLVESGGGLVQAGGSLRLACAGSGRIFAIYDIAWYRHPPGNQ
REL VAMIRPVVTEIDYADSVKGRFTISRNNAMKT VYLQMNNLKPEDTAVYYCNAKRPWGSRDEYWG
QGTQVTVSSDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWY
VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQPRE
PQVYTLPPSREEMTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPVLDSGGSFFLVSKLT
DKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGKGSGSAVQQVESGGGLVQPGGSLRLSCAA
SGFTLDYYAISWFRQAPGKKREL VADITSGGSTNYADSVKGRFTISRDNAKNTIYLQMNSLKPEDTA
VYFCNAVITYNGYTIWGGGTQVTVSSGSGSGHHHHH* (SEQ ID NO:2192)**

**MDMRVPAQLLGLLLLWLRGARAVQQVESGGGLVQPGGSLRLSCAASGFTLDYYAISWFRQAPGKK
REL VADITSGGSTNYADSVKGRFTISRDNAKNTIYLQMNSLKPEDTAVYFCNAVITYNGYTIWGGGTQ
VTVSSDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGV
EVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQPREPQVY
TLPPSREEMTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPVLDSGGSFFLYSKLTVDKSR
WQQGNVFCFSVMHEALHNHYTQKSLSLSPGKGSGSDVQLVESGGGLVQAGGSLRLACAGSGRIF
AIYDIAWYRHPPGNQREL VAMIRPVVTEIDYADSVKGRFTISRNNAMKT VYLQMNNLKPEDTAVYYC
NAKRPWGSRDEYWGQGTQVTVSS* (SEQ ID NO:2193)**

FIG. 19 (contd.)

**MDMRVPAQLLGLLLLWLRGARCDVQLVESGGGLVQAGGSLRLACAGSGRIFAIYDIAWYRHPPGNQ
RELVAMIRPVVTEIDYADSVKGRFTISRNNAMKTVYLQMNNLKPEDTAVYYCNAKRPWGSRDEYWG
QGTQVTVSSGSGSGSAVQQVESGGGLVQPGGSLRLSCAASGFTLDYYAISWFRQAPGKKRELVADI
TSGGSTNYADSVKGRFTISRDNANTYILQMNSLKPEDTAVYFCNAVITYNGYTIWGQGTQVTVSSDK
THTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKT
KPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQPREPQVYTLPPSRE
EMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNV
FSCSVMHEALHNHYTQKSLSLSPGK* (SEQ ID NO:2194)**

**MDMRVPAQLLGLLLLWLRGARCDVQLVESGGGLVQAGGSLRLACAGSGRIFAIYDIAWYRHPPGNQ
RELVAMIRPVVTEIDYADSVKGRFTISRNNAMKTVYLQMNNLKPEDTAVYYCNAKRPWGSRDEYWG
QGTQVTVSSGSGSGSGSGSAVQQVESGGGLVQPGGSLRLSCAASGFTLDYYAISWFRQAPGKKR
ELVADITSGGSTNYADSVKGRFTISRDNANTYILQMNSLKPEDTAVYFCNAVITYNGYTIWGQGTQV
TVSSDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVE
VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQPREPQVY
TLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRW
QQGNVFSCSVMHEALHNHYTQKSLSLSPGK* (SEQ ID NO:2195)**

**MDMRVPAQLLGLLLLWLRGARCDVQLVESGGGLVQAGGSLRLACAGSGRIFAIYDIAWYRHPPGNQ
RELVAMIRPVVTEIDYADSVKGRFTISRNNAMKTVYLQMNNLKPEDTAVYYCNAKRPWGSRDEYWG
QGTQVTVSSGSGSGSGSGSGSAVQQVESGGGLVQPGGSLRLSCAASGFTLDYYAISWFRQA
PGKKRELVADITSGGSTNYADSVKGRFTISRDNANTYILQMNSLKPEDTAVYFCNAVITYNGYTIWG
QGTQVTVSSDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWY
VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQPRE
PQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLT
VDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK* (SEQ ID NO:2196)**

**MDMRVPAQLLGLLLLWLRGARCAVQQVESGGGLVQPGGSLRLSCAASGFTLDYYAISWFRQAPGK
KRELVADITSGGSTNYADSVKGRFTISRDNANTYILQMNSLKPEDTAVYFCNAVITYNGYTIWGQGT
QVTVSSDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDG
VEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQPREPQV
YTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKS
RWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKGSGSDVQLVESGGGLVQAGGSLRLACAGSGR
IFAIYDIAWYRHPPGNQRELVAMIRPVVTEIDYADSVKGRFTISRNNAMKTVYLQMNNLKPEDTAVYY
CNAKRPWGSRDEYWGQGTQVTVSS* (SEQ ID NO:2197)**

**MDMRVPAQLLGLLLLWLRGARCDVQLVESGGGLVQAGGSLRLACAGSGRIFAIYDIAWYRHPPGNQ
RELVAMIRPVVTEIDYADSVKGRFTISRNNAMKTVYLQMNNLKPEDTAVYYCNAKRPWGSRDEYWG
QGTQVTVSSDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWY
VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQPRE
PQVYTLPPSREEMTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLT
VDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKGSGSDVQLVESGGGLVQAGGSLRLACAG
SGRIFAIYDIAWYRHPPGNQRELVAMIRPVVTEIDYADSVKGRFTISRNNAMKTVYLQMNNLKPEDT
AVYYCNAKRPWGSRDEYWGQGTQVTVSS* (SEQ ID NO:2198)**

**MDMRVPAQLLGLLLLWLRGARCAVQQVESGGGLVQPGGSLRLSCAASGFTLDYYAISWFRQAPGK
KRELVADITSGGSTNYADSVKGRFTISRDNANTYILQMNSLKPEDTAVYFCNAVITYNGYTIWGQGT
QVTVSSDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDG
VEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQPREPQV
YTLPPSREEMTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKS
RWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKGSGSAVQQVESGGGLVQPGGSLRLSCAASGFT
LDYYAISWFRQAPGKKRELVADITSGGSTNYADSVKGRFTISRDNANTYILQMNSLKPEDTAVYF
CNAVITYNGYTIWGQGTQVTVSSGSGSGHHHHHH* (SEQ ID NO:2199)**

FIG. 19 (contd.)

MDMRVPAQLLGLLLLWLRGARCEVQLVESGGGLVQPGGSLRLSCAASGFTFSHYTLSWVRQAPGK
GLEWVSISGDGSYTYADSVKGRFTISSDNSKNTLYLQMNSLRAEDTAVYYCARNFIKYVFANWG
QGTLTVTVSSGGGGSGGGGSGGGGSDIELTQPPSVSVAPGQTARISCSGDNIGSFYVHWYQQKPGQ
APVLVIYDKSNRPSGIPERFSGSNSGNTATLTISGTQAEDEADYYCQSYANTLSLVFGGGTKLTVLG
SGSGDVKLVESGGGLVKLGSLRLSCAASGFSFSTSYMSWVRQTPEKRELVAAINLNGGSTYYS
DTVKGRFTISRDNANTLYLQMSSLKSEDTAFYYCASELAGYGTPFAYWGQGTLTVTSAGSAASGS
SGGSSSGADIVLTQSPATLSVTPGDSVSLSCASQSSISYNLHWYQQKSHESPRLLIKYTSQSISGIPS
RFGSGSGTDFTLTINNVEDDFGMYFCQQSNSWPLTFGAGTKLEVKGSGSGDKTHTCPPCPAPEA
AGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYR
VSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLV
KGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNH
YTQKSLSLSPGK* (SEQ ID NO:2200)

MDMRVPAQLLGLLLLWLRGARCEVQLVESGGGLVQPGGSLRLSCAASGFTFSHYTLSWVRQAPGK
GLEWVSISGDGSYTYADSVKGRFTISSDNSKNTLYLQMNSLRAEDTAVYYCARNFIKYVFANWG
QGTLTVTVSSGGGGSGGGGSGGGGSDIELTQPPSVSVAPGQTARISCSGDNIGSFYVHWYQQKPGQ
APVLVIYDKSNRPSGIPERFSGSNSGNTATLTISGTQAEDEADYYCQSYANTLSLVFGGGTKLTVLG
SGSGSGSGSDVKLVESGGGLVKLGSLRLSCAASGFSFSTSYMSWVRQTPEKRELVAAINLNGG
STYYSDTVKGRFTISRDNANTLYLQMSSLKSEDTAFYYCASELAGYGTPFAYWGQGTLTVTSAGS
AASGSSSGSSSGADIVLTQSPATLSVTPGDSVSLSCASQSSISYNLHWYQQKSHESPRLLIKYTSQSI
SGIPSRFGSGSGTDFTLTINNVEDDFGMYFCQQSNSWPLTFGAGTKLEVKGSGSGDKTHTCPPC
PAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY
NSTYRVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQV
SLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMH
EALHNHYTQKSLSLSPGK* (SEQ ID NO:2201)

MDMRVPAQLLGLLLLWLRGARCEVQLVESGGGLVQPGGSLRLSCAASGFTFSHYTLSWVRQAPGK
GLEWVSISGDGSYTYADSVKGRFTISSDNSKNTLYLQMNSLRAEDTAVYYCARNFIKYVFANWG
QGTLTVTVSSGGGGSGGGGSGGGGSDIELTQPPSVSVAPGQTARISCSGDNIGSFYVHWYQQKPGQ
APVLVIYDKSNRPSGIPERFSGSNSGNTATLTISGTQAEDEADYYCQSYANTLSLVFGGGTKLTVLG
SGSGSGSGSGSDVKLVESGGGLVKLGSLRLSCAASGFSFSTSYMSWVRQTPEKRELVAAI
NLNGGSTYYSDTVKGRFTISRDNANTLYLQMSSLKSEDTAFYYCASELAGYGTPFAYWGQGTLVT
VSAGSAASGSSSGSSSGADIVLTQSPATLSVTPGDSVSLSCASQSSISYNLHWYQQKSHESPRLLIK
YTSQSISGIPSRFGSGSGTDFTLTINNVEDDFGMYFCQQSNSWPLTFGAGTKLEVKGSGSGDKTH
TCPPEAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
REEQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQPREPQVYTLPPSREEM
TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCF
SVMHEALHNHYTQKSLSLSPGK* (SEQ ID NO:2202)

MDMRVPAQLLGLLLLWLRGARCVDKLVESGGGLVKLGSLRLSCAASGFSFSTSYMSWVRQTPEK
RLELVAAINLNGGSTYYSDTVKGRFTISRDNANTLYLQMSSLKSEDTAFYYCASELAGYGTPFAYW
GGTLTVTSAGSAASGSSSGSSSGADIVLTQSPATLSVTPGDSVSLSCASQSSISYNLHWYQQKSH
ESPRLLIKYTSQSISGIPSRFGSGSGTDFTLTINNVEDDFGMYFCQQSNSWPLTFGAGTKLEVKG
SGSEVQLVESGGGLVQPGGSLRLSCAASGFTFSHYTLSWVRQAPGKGLEWVSISGDGSYTYAD
SVKGRFTISSDNSKNTLYLQMNSLRAEDTAVYYCARNFIKYVFANWGQGTLTVTVSSGGGGSGGGG
SGGGGSDIELTQPPSVSVAPGQTARISCSGDNIGSFYVHWYQQKPGQAPVLVIYDKSNRPSGIPERF
SGSNSGNTATLTISGTQAEDEADYYCQSYANTLSLVFGGGTKLTVLGSGSGDKTHTCPPCPAPEAA
GGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV
VSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVK
GFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHY
TQKSLSLSPGK* (SEQ ID NO:2203)

FIG. 19 (contd.)

MDMRVPAQLLGLLLLWLRGARCDVKLVESGGGLVKLGGSRLRLSCAASGFSFSTSYMSWVRQTPEK
RLELVAAINLNGGSTYYSDTVKGRFTISRDNANTLYLQMSSLKSEDTAFYYCASELAGYGTFFAYW
GQGTLVTVSAGSAASGSSGGSSSGADIVLTQSPATLSVTPGDSVLSCKASQSSISYNLHWYQQKSH
ESPRLLIKYTSQSISGIPSRFSGSGSGTDFTLTINNVEDDFGMYFCQQSNSWPLTFGAGTKLEVKG
SGSGSGSGGEVQLVESGGGLVQPGGSLRLSCAASGFTFSHYTLWVRQAPGKGLEWVSVISGDGS
YTTYADSVKGRFTISSDNSKNTLYLQMNSLRAEDTAVYYCARNFIKYVFANWGQGTLVTVSSGGGG
SGGGSGGGGSDIELTQPPSVSVAPGQTARISCSGDNIGSFYVHWYQQKPGQAPVLVIYDKSNRPS
GIPERFSGSNSGNTATLTISGTQAEDEADYYCQSYANTLSLVFGGGTKLTVLGSGSGDKTHTCPPCP
APEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYN
STYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSL
TCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSCVMHEA
LHNHYTQKSLSLSPGK* (SEQ ID NO:2204)

MDMRVPAQLLGLLLLWLRGARCDVKLVESGGGLVKLGGSRLRLSCAASGFSFSTSYMSWVRQTPEK
RLELVAAINLNGGSTYYSDTVKGRFTISRDNANTLYLQMSSLKSEDTAFYYCASELAGYGTFFAYW
GQGTLVTVSAGSAASGSSGGSSSGADIVLTQSPATLSVTPGDSVLSCKASQSSISYNLHWYQQKSH
ESPRLLIKYTSQSISGIPSRFSGSGSGTDFTLTINNVEDDFGMYFCQQSNSWPLTFGAGTKLEVKG
SGSGSGSGGSGGEVQLVESGGGLVQPGGSLRLSCAASGFTFSHYTLWVRQAPGKGLEWVSVI
SGDGSYTTYADSVKGRFTISSDNSKNTLYLQMNSLRAEDTAVYYCARNFIKYVFANWGQGTLVTVS
SGGGSGGGGSGGGGSDIELTQPPSVSVAPGQTARISCSGDNIGSFYVHWYQQKPGQAPVLVIYD
KSNRPSGIPERFSGSNSGNTATLTISGTQAEDEADYYCQSYANTLSLVFGGGTKLTVLGSGSGDKTH
TCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQPREPQVYTLPPSREEM
TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFC
SVMHEALHNHYTQKSLSLSPGK* (SEQ ID NO:2205)

MDMRVPAQLLGLLLLWLRGARCEVQLVESGGGLVQPGGSLRLSCAASGFTFSHYTLWVRQAPGK
GLEWVSVISGDGSYTTYADSVKGRFTISSDNSKNTLYLQMNSLRAEDTAVYYCARNFIKYVFANWG
QGTLVTVSSGGGGSGGGGSGGGGSDIELTQPPSVSVAPGQTARISCSGDNIGSFYVHWYQQKPGQ
APVLVIYDKSNRPSGIPERFSGSNSGNTATLTISGTQAEDEADYYCQSYANTLSLVFGGGTKLTVLG
SGSGEVQLVESGGGLVQPGGSLRLSCAASGFTFSYISWVRQAPGKGLEWVAEISPYSYSTYYAD
SVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCALRARPPIRLHPRGSVMDYWGQGTLVTVSSGS
AASGSSGGSSSGADIQMTQSPSSLSASVGDRTITCRASQDVSTAVAWYQQKPGKAPKLLIYSASF
LYSGVPSRFSGSGSGTDFTLTISLQPEDFATYYCQQSYTTPPTFGQGTKEIKGSGSGDKTHTCPP
CPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ
YNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQ
VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSCVM
HEALHNHYTQKSLSLSPGK* (SEQ ID NO:2206)

MDMRVPAQLLGLLLLWLRGARCEVQLVESGGGLVQPGGSLRLSCAASGFTFSHYTLWVRQAPGK
GLEWVSVISGDGSYTTYADSVKGRFTISSDNSKNTLYLQMNSLRAEDTAVYYCARNFIKYVFANWG
QGTLVTVSSGGGGSGGGGSGGGGSDIELTQPPSVSVAPGQTARISCSGDNIGSFYVHWYQQKPGQ
APVLVIYDKSNRPSGIPERFSGSNSGNTATLTISGTQAEDEADYYCQSYANTLSLVFGGGTKLTVLG
SGSGSGSGGEVQLVESGGGLVQPGGSLRLSCAASGFTFSYISWVRQAPGKGLEWVAEISPYS
STYYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCALRARPPIRLHPRGSVMDYWGQGTLV
TVSSGSAASGSSGGSSSGADIQMTQSPSSLSASVGDRTITCRASQDVSTAVAWYQQKPGKAPKLL
IYSASFLYSGVPSRFSGSGSGTDFTLTISLQPEDFATYYCQQSYTTPPTFGQGTKEIKGSGSGDKT
HTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTK
PREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQPREPQVYTLPPSREE
MTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNV
FCSCVMHEALHNHYTQKSLSLSPGK* (SEQ ID NO:2207)

FIG. 19 (contd.)

MDMRVPAQLLGLLLLWLRGARCEVQLVESGGGLVQPGGSLRLSCAASGFTFSHYTLSWVRQAPGK
GLEWVSVISGDGSYTYADSVKGRFTISSDNSKNTLYLQMNSLRAEDTAVYYCARNFIKYVFANWG
QGTLTVTVSSGGGGSGGGSGGGGSDIELTQPPSVSVAPGQTARISCSGDNIGSFYVHWYQQKPGQ
APVLVIYDKSNRPSGIPERFSGSNSGNTATLTISGTQAEDEADYYCQSYANTLSLVFGGGTKLTVLG
SGSGSGSGSGSGGGEVQLVESGGGLVQPGGSLRLSCAASGFTFSYIISWVRQAPGKGLEWVAEI
SPYSGSTYYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCALRARPPIRLHPRGSVMYDYG
QGTLTVTVSSGSAASGSSGGSSSGADIQMTQSPSSLSASVGDRVTITCRASQDVSTAVAWYQQKPG
KAPKLLIYSASFLYSGVPSRFSGSGSGTDFTLTISLQPEDFATYYCQQSYTTPPTFGGQTKVEIKGS
GSGDKHTCPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEV
HNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQPREPQVYTL
PPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGGSFFLYSKLTVDKSRWQ
QGNVFSCSVMHEALHNHYTQKSLSLSPGK* (SEQ ID NO:2208)

MDMRVPAQLLGLLLLWLRGARCEVQLVESGGGLVQPGGSLRLSCAASGFTFSYIISWVRQAPGK
GLEWVAEISPYSGSTYYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCALRARPPIRLHPRG
SVMYDYGQGTLTVTVSSGSAASGSSGGSSSGADIQMTQSPSSLSASVGDRVTITCRASQDVSTAVA
WYQQKPGKAPKLLIYSASFLYSGVPSRFSGSGSGTDFTLTISLQPEDFATYYCQQSYTTPPTFGGQ
TKVEIKGS GSGGEVQLVESGGGLVQPGGSLRLSCAASGFTFSHYTLSWVRQAPGKGLEWVSVISGD
GSYTYADSVKGRFTISSDNSKNTLYLQMNSLRAEDTAVYYCARNFIKYVFANWGQGTLTVTVSSGG
GGSGGGGGSGGGGSDIELTQPPSVSVAPGQTARISCSGDNIGSFYVHWYQQKPGQAPVLVIYDKSNR
PSGIPERFSGSNSGNTATLTISGTQAEDEADYYCQSYANTLSLVFGGGTKLTVLGSGSGDKHTCPC
CPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ
YNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQ
VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGGSFFLYSKLTVDKSRWQQGNVFSCSVM
HEALHNHYTQKSLSLSPGK* (SEQ ID NO:2209)

MDMRVPAQLLGLLLLWLRGARCEVQLVESGGGLVQPGGSLRLSCAASGFTFSYIISWVRQAPGK
GLEWVAEISPYSGSTYYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCALRARPPIRLHPRG
SVMYDYGQGTLTVTVSSGSAASGSSGGSSSGADIQMTQSPSSLSASVGDRVTITCRASQDVSTAVA
WYQQKPGKAPKLLIYSASFLYSGVPSRFSGSGSGTDFTLTISLQPEDFATYYCQQSYTTPPTFGGQ
TKVEIKGS GSGSGSGSGGEVQLVESGGGLVQPGGSLRLSCAASGFTFSHYTLSWVRQAPGKGLEWVS
VISGDGSYTYADSVKGRFTISSDNSKNTLYLQMNSLRAEDTAVYYCARNFIKYVFANWGQGTLTV
SSGGGGSGGGGGSGGGGSDIELTQPPSVSVAPGQTARISCSGDNIGSFYVHWYQQKPGQAPVLVIY
DKSNRPSGIPERFSGSNSGNTATLTISGTQAEDEADYYCQSYANTLSLVFGGGTKLTVLGSGSGDKT
HTCPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTK
PREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQPREPQVYTLPPSREE
MTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGGSFFLYSKLTVDKSRWQQGNVF
SCSVMHEALHNHYTQKSLSLSPGK* (SEQ ID NO:2210)

MDMRVPAQLLGLLLLWLRGARCEVQLVESGGGLVQPGGSLRLSCAASGFTFSYIISWVRQAPGK
GLEWVAEISPYSGSTYYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCALRARPPIRLHPRG
SVMYDYGQGTLTVTVSSGSAASGSSGGSSSGADIQMTQSPSSLSASVGDRVTITCRASQDVSTAVA
WYQQKPGKAPKLLIYSASFLYSGVPSRFSGSGSGTDFTLTISLQPEDFATYYCQQSYTTPPTFGGQ
TKVEIKGS GSGSGSGSGSGGGEVQLVESGGGLVQPGGSLRLSCAASGFTFSHYTLSWVRQAPGK
GLEWVSVISGDGSYTYADSVKGRFTISSDNSKNTLYLQMNSLRAEDTAVYYCARNFIKYVFANWG
QGTLTVTVSSGGGGSGGGGGSGGGGSDIELTQPPSVSVAPGQTARISCSGDNIGSFYVHWYQQKPGQ
APVLVIYDKSNRPSGIPERFSGSNSGNTATLTISGTQAEDEADYYCQSYANTLSLVFGGGTKLTVLG
SGSGDKHTCPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE
VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQPREPQVY
LPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGGSFFLYSKLTVDKSRW
QQGNVFSCSVMHEALHNHYTQKSLSLSPGK* (SEQ ID NO:2211)

FIG. 19 (contd.)

MDMRVPAQLLGLLLLWLRGARCEVQLVESGGGLVQPGGSLRLSCAASGFTFSHYTL~~SWVRQAPGK~~
GLEWVSVISGDG~~SY~~TY~~Y~~ADSVKGRFTISSD~~NS~~KNTLYLQMN~~SL~~RAEDTAVYYCARNFIKYVFANWG
QGTLTVTSSGGGGSGGGGGSGGGGSDIELTQPPSVSVAPGQTARISCSGDNIGSFYVHWYQQKPGQ
APVLVIYDKSNRPSGIPERFSGSNSGNTATLTISGTQAEDEADYYCQSYANTLSLVFGGGTKLTVLG
SGSGDKHTHTCPPCPAPEAAGGPSVFLFPPKPKD~~TL~~MISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE
VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQPREPQVYT
LPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK~~TT~~PPVLDSDGSFFLYSKLTVDKSRW
QQGNVFSCSVMHEALHNHYTQKSLSLSPGKSGSGSDV~~KL~~VESGGGLV~~KL~~GGS~~LR~~LS~~CA~~ASGFSFS
TSYMSWVRQTPEKRL~~EL~~VAAINLNGGSTY~~Y~~SDTVKGRFTISRDN~~AK~~N~~TL~~YLQMS~~SL~~KSEDTAFYYC
ASELAGYGT~~PF~~FAYWGQGT~~LV~~TVSAGSAASGSSGGSSSGADIVLTQSPATLSVTPGDSVSL~~SCKASQ~~
SISYNLHWYQQKSHESPRLLIKYTSQSIGIPSRFSGSGSGTDFTLTINN~~V~~ETEDFGMYFCQQSNSWP
LTFGAGTKLEVK* (SEQ ID NO:2212)

MDMRVPAQLLGLLLLWLRGARCDV~~KL~~VESGGGLV~~KL~~GGS~~LR~~LS~~CA~~ASGFSFS~~TS~~SYMSWVRQTPEK
R~~LE~~LVA~~AI~~NLNGGSTY~~Y~~SDTVKGRFTISRDN~~AK~~N~~TL~~YLQMS~~SL~~KSEDTAFYYC~~ASE~~LAGYGT~~PF~~FAYW
GQGT~~LV~~TVSAGSAASGSSGGSSSGADIVLTQSPATLSVTPGDSVSL~~SCKASQ~~SISYNLHWYQQKSH
ESPRLLIKYTSQSIGIPSRFSGSGSGTDFTLTINN~~V~~ETEDFGMYFCQQSNSWPLTFGAGTKLEV~~KGS~~
GSGDKHTHTCPPCPAPEAAGGPSVFLFPPKPKD~~TL~~MISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEV
HNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQPREPQVYTL
PPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK~~TT~~PPVLDSDGSFFLYSKLTVDKSRWQ
QQGNVFSCSVMHEALHNHYTQKSLSLSPGKSGSGEVQLVESGGGLVQPGGSLRLSCAASGFTFSH
YTL~~SWVRQAPGK~~GLEWVSVISGDG~~SY~~TY~~Y~~ADSVKGRFTISSD~~NS~~KNTLYLQMN~~SL~~RAEDTAVYYCA
RNF~~IKY~~VFANWGQGT~~LV~~TVSGGGGSGGGGGSGGGGSDIELTQPPSVSVAPGQTARISCSGDNIGSF
YVHWYQQKPGQAPVLVIYDKSNRPSGIPERFSGSNSGNTATLTISGTQAEDEADYYCQSYANTLSLV
FGGGTKLTVL* (SEQ ID NO:2213)

MDMRVPAQLLGLLLLWLRGARCAVVLQEP~~SL~~SVSPGGTVLTCLGSSGSVSTNYYPSWYQDTPG
QDPRTLIYYTNRSSDVPERFSGSIVGNKAALTITGAQPDD~~ES~~VYFCLLYLGRGIWVFGGGTKLTVLG
QPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNNKYAAK
SYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS* (SEQ ID NO:2214)

MDMRVPAQLLGLLLLWLRGARCAIVLTQSPAIMSASPG~~E~~KVTISCSANSSVRFMFWYQKKPGSKPK
PLIYRTSNLASGVPARFSGSGSGTSYSLTISSMEAE~~DA~~ATYYCQYHSYPWTFGGGKLEIKRTVAA
PSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLESTL
TL~~SK~~ADYEKHKVYACEVTHQGLSSPVTKSFNRGEC* (SEQ ID NO:2215)

MDMRVPAQLLGLLLLWLRGARCAVQLQQSGPELVKPGASVRISCKASGYTFTTYYIHWLKDRPGQG
LEWIGWIFPGNVNTKYNAKFKGKATLTADKSSSTAYMQLSSLTSEDSAVYFCAREELQYYFDYWG
GSTLTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLKSVVTPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCGSGSGEVQLVQSGAEVKKPGASVKVSCKASGYTFTSYGISWVRKAPGGGLEWMGWISAYNGNTNYAQKLQGRVTMTTDTSTSTAYME
LRLSRDDTAVYYCASSKEKATYYYGMDVWGKGTTTVTSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLKSVVTPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKHTHTCPPCPAPEAAGGPSVFLFPPKPKD~~TL~~MISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK~~TT~~PPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK* (SEQ ID NO:2216)

MDMRVPAQLLGLLLLWLRGARCSYVLTQPPSVSVSPGQTASITCSGDKVGHKYASWYQDKPGQDP
VLVIYEDSQRPSGIPVRFSGSNSGNTATLTISGTQAMDEADYYCQAWDSSTDVVFGGGKLTVLGQ
PKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNNKYAAKS
YLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS* (SEQ ID NO:2217)

MDMRVPAQLLGLLLLWLRGARCDIQMTQSPSSLSASVGD~~RV~~TITCRASQSISSYLNWYQKKPGKKP
KLLIYAASSLQSGVPSRFSGSGSGTDFTLTIS~~SL~~QPEDFATYYCQQSYSTPLTFGGGKVEIKRTVAA
PSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLESTL
TL~~SK~~ADYEKHKVYACEVTHQGLSSPVTKSFNRGEC* (SEQ ID NO:2218)

FIG. 19 (contd.)

**MDMRVPAQLLGLLLLWLRGARCQVQLQQWGAGLLKPSETLSLTCAVSGASFSGHYWTWIRKPPGK
GLEWIGEIDHTGSTNYEPSLRSRVTISVDTSKNQFSLNLKSVTAADTAVYYCARGGQGGYDWGHYH
GLDVGKGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVH
TFPAVLQSSGLYSLESVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCGSGSGSEVQLVESGGG
LVQPGGSLRLSCAASGFTFSSYAMHWVRDAPGKGLEWVASISSTSGSKYYADSVKGRFTISRDN
KNTLYLQMNSLRAEDTAVYSCAKTYDFWSGYYTFDYWGDGTLTVTSSASTKGPSVFPLAPSSKST
SGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSKVVTVPSSSLGTQTYICNV
HKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHED
PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTIS
KAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSF
FLYSKLTVDKSRWQQGNVFCSSVMHEALHNHYTQKSLSLSPGK* (SEQ ID NO:2219)**

**MDMRVPAQLLGLLLLWLRGARCSYVLTQPPSVSVSPGQTASITCSGDKVGHKYASWYQDKPGQDP
VLVIYEDSQRPSGIPVRFSGSNSGNTATLTISGTQAMDEADYYCQAWDSSTDVVFGGGKLTVLGQ
PKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNKYAAKS
YLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS* (SEQ ID NO:2220)**

**MDMRVPAQLLGLLLLWLRGARCDIQTQSPSSLSASVGDRVTITCRASQSISSYLNWYQKKPGKKP
KLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQSYSTPLTFGGGKVEIKRTVAA
PSVFIFPPSDEQLKSGTASVCLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLESTL
TLKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC* (SEQ ID NO:2221)**

**MDMRVPAQLLGLLLLWLRGARCQVQLQQWGAGLLKPSETLSLTCAVSGASFSGHYWTWIRKPPGK
GLEWIGEIDHTGSTNYEPSLRSRVTISVDTSKNQFSLNLKSVTAADTAVYYCARGGQGGYDWGHYH
GLDVGKGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVH
TFPAVLQSSGLYSLESVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCGSGSGSGSGSEVQLV
ESGGGLVQPGGSLRLSCAASGFTFSSYAMHWVRDAPGKGLEWVASISSTSGSKYYADSVKGRFTI
SRDNKNTLYLQMNSLRAEDTAVYSCAKTYDFWSGYYTFDYWGDGTLTVTSSASTKGPSVFPLAP
SSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSKVVTVPSSSLGTQ
TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVD
VSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAP
IEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLD
SDGSFFLYSKLTVDKSRWQQGNVFCSSVMHEALHNHYTQKSLSLSPGK* (SEQ ID NO:2222)**

**MDMRVPAQLLGLLLLWLRGARCSYVLTQPPSVSVSPGQTASITCSGDKVGHKYASWYQDKPGQDP
VLVIYEDSQRPSGIPVRFSGSNSGNTATLTISGTQAMDEADYYCQAWDSSTDVVFGGGKLTVLGQ
PKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNKYAAKS
YLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS* (SEQ ID NO:2223)**

**MDMRVPAQLLGLLLLWLRGARCDIQTQSPSSLSASVGDRVTITCRASQSISSYLNWYQKKPGKKP
KLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQSYSTPLTFGGGKVEIKRTVAA
PSVFIFPPSDEQLKSGTASVCLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLESTL
TLKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC* (SEQ ID NO:2224)**

**MDMRVPAQLLGLLLLWLRGARCQVQLQQWGAGLLKPSETLSLTCAVSGASFSGHYWTWIRKPPGK
GLEWIGEIDHTGSTNYEPSLRSRVTISVDTSKNQFSLNLKSVTAADTAVYYCARGGQGGYDWGHYH
GLDVGKGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVH
TFPAVLQSSGLYSLESVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCGSGSGSGSGSGSSGG
EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYAMHWVRDAPGKGLEWVASISSTSGSKYYADSVK
GRFTISRDNKNTLYLQMNSLRAEDTAVYSCAKTYDFWSGYYTFDYWGDGTLTVTSSASTKGPSV
FPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSKVVTVPSSSL
GTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTC
VVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKA
LGAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTP
PVLDSDGSFFLYSKLTVDKSRWQQGNVFCSSVMHEALHNHYTQKSLSLSPGK* (SEQ ID NO:2225)**

FIG. 19 (contd.)

**MDMRVPAQLLGLLLLWLRGARCSYVLTQPPSVSVSPGQTASITCSGDKVGHKYASWYQDKPGQDP
VLVIYEDSQRPSGIPVRFSGSNSGNTATLTISGTQAMDEADYYCQAWDSSTDVVFVGGGTCLTVLGQ
PKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTPSKQSNNKYAASK
YLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS*** (SEQ ID NO:2226)

**MDMRVPAQLLGLLLLWLRGARCDIQMTQSPSSLSAYVGDRVITITCRVSQGISSYLNWYQKKPGKKP
KLLIYAASSLQSGVPSRFSGSGSGTDFTLTISLQPEDFATYYCQQTYTIPFTFGPGTKVDIKRTVAAP
SVFIFPPSDEQLKSGTASVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSTYSLESTLT
LTKADYEKHKVYACEVTHQGLSPVTKSFNRGEC*** (SEQ ID NO:2227)

**MDMRVPAQLLGLLLLWLRGARCQVQLQQWGAGLLKPSETLSLTCAVSGASFSGHYWTWIRKPPGK
GLEWIGEIDHTGSTNYEPSLRSRVTISVDTSKNQFSLNLKSVTAADTAVYYCARGGQGGYDWGHYH
GLDVWGKGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVH
TFPAVLQSSGLYSLESVVTPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCGSGSGGEVQLVESVGG
LVQPGGSLRLSCAASGFTFSSYAMHWVRDAPGKGLEWISTISGSGGSTYYADSVKGRFTISRDNK
NTLYLQMNSLRADDTAVYYCARAGYGRYYYGMDVWGDGTTVTVSSASTKGPSVFPLAPSSKSTSG
GTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLKSVVTPSSSLGTQTYICNVNHK
PSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPE
VKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKA
KGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPVLDSDGSFFL
YSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK*** (SEQ ID NO:2228)

**MDMRVPAQLLGLLLLWLRGARCSYVLTQPPSVSVSPGQTASITCSGDKVGHKYASWYQDKPGQDP
VLVIYEDSQRPSGIPVRFSGSNSGNTATLTISGTQAMDEADYYCQAWDSSTDVVFVGGGTCLTVLGQ
PKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTPSKQSNNKYAASK
YLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS*** (SEQ ID NO:2229)

**MDMRVPAQLLGLLLLWLRGARCDIQMTQSPSSLSAYVGDRVITITCRVSQGISSYLNWYQKKPGKKP
KLLIYAASSLQSGVPSRFSGSGSGTDFTLTISLQPEDFATYYCQQTYTIPFTFGPGTKVDIKRTVAAP
SVFIFPPSDEQLKSGTASVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSTYSLESTLT
LTKADYEKHKVYACEVTHQGLSPVTKSFNRGEC*** (SEQ ID NO:2230)

**MDMRVPAQLLGLLLLWLRGARCQVQLQQWGAGLLKPSETLSLTCAVSGASFSGHYWTWIRKPPGK
GLEWIGEIDHTGSTNYEPSLRSRVTISVDTSKNQFSLNLKSVTAADTAVYYCARGGQGGYDWGHYH
GLDVWGKGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVH
TFPAVLQSSGLYSLESVVTPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCGSGSGGSGSGGEVQLV
ESVGGLVQPGGSLRLSCAASGFTFSSYAMHWVRDAPGKGLEWISTISGSGGSTYYADSVKGRFTIS
RDNSKNTLYLQMNSLRADDTAVYYCARAGYGRYYYGMDVWGDGTTVTVSSASTKGPSVFPLAPSS
KSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLKSVVTPSSSLGTQTYIC
NVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVDVS
HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIE
KTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPVLDSD
DGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK*** (SEQ ID NO:2231)

**MDMRVPAQLLGLLLLWLRGARCSYVLTQPPSVSVSPGQTASITCSGDKVGHKYASWYQDKPGQDP
VLVIYEDSQRPSGIPVRFSGSNSGNTATLTISGTQAMDEADYYCQAWDSSTDVVFVGGGTCLTVLGQ
PKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTPSKQSNNKYAASK
YLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS*** (SEQ ID NO:2232)

**MDMRVPAQLLGLLLLWLRGARCDIQMTQSPSSLSAYVGDRVITITCRVSQGISSYLNWYQKKPGKKP
KLLIYAASSLQSGVPSRFSGSGSGTDFTLTISLQPEDFATYYCQQTYTIPFTFGPGTKVDIKRTVAAP
SVFIFPPSDEQLKSGTASVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSTYSLESTLT
LTKADYEKHKVYACEVTHQGLSPVTKSFNRGEC*** (SEQ ID NO:2233)

FIG. 19 (contd.)

**MDMRVPAQLLGLLLLWLRGARCQVQLQQWGAGLLKPSETLSLTCAVSGASFSGHYWTWIRKPPGK
GLEWIGEIDHTGSTNYEPSLRSRVTISVDTSKNQFSLNLKSVTAADTAVYYCARGGQGGYDWGHYH
GLDWGKGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVH
TFPAVLQSSGLYSLESVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCGSGSGSGSGSGSSGG
EVQLVESVGGGLVQPGGSLRLSCAASGFTFSSYAMHWVRDAPGKGLEWISTISGSGGSTYYADSVK
GRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARAGYGRYYYGMDVWGDGTTVTVSSASTKGPSVF
PLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSKVVTVPSSSL
GTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTC
VVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKA
LGAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTP
PVLDSGDSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK* (SEQ ID NO:2234)**

**MDMRVPAQLLGLLLLWLRGARCDIQTQSPSSLSAYVGDRVITITCRVSQGISSYLNWYQKKPGKKP
KLLIYAASSLQSGVPSRFSGSGSGTDFTLTISLQPEDFATYYCQQTYPFTFGPGTKVDIKRTVAAP
SVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSTYSLESTLT
SKADYEKHKVYACEVTHQGLSPVTKSFNRGEC* (SEQ ID NO:2235)**

**MDMRVPAQLLGLLLLWLRGARCSYVLTQPPSVSVSPGQTASITCSGDKVGHKYASWYQDKPGQDP
VLVIYEDSQRPSGIPVRFSGSNSGNTATLTISGTQAMDEADYYCQAWDSSTDVVFGGGKLTVLGQ
PKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNNKYAAKS
YLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS* (SEQ ID NO:2236)**

**MDMRVPAQLLGLLLLWLRGARCEVQLVESVGGGLVQPGGSLRLSCAASGFTFSSYAMHWVRDAPGK
GLEWISTISGSGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARAGYGRYYYGMD
VWGDGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPA
VLQSSGLYSLSKVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCGSGSGSQVQLQQWGAGLLK
PSETLSLTCAVSGASFSGHYWTWIRKPPGKGLEWIGEIDHTGSTNYEPSLRSRVTISVDTSKNQFSL
NLKSVTAADTAVYYCARGGQGGYDWGHYHGLDWGKGTTVTVSSASTKGPSVFPLAPSSKSTSGG
TAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLESVVTVPSSSLGTQTYICNVNHK
SNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEV
KFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAK
GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPVLDSDGDSFFLY
SKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK* (SEQ ID NO:2237)**

**MDMRVPAQLLGLLLLWLRGARCDIQTQSPSSLSAYVGDRVITITCRVSQGISSYLNWYQKKPGKKP
KLLIYAASSLQSGVPSRFSGSGSGTDFTLTISLQPEDFATYYCQQTYPFTFGPGTKVDIKRTVAAP
SVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSTYSLESTLT
SKADYEKHKVYACEVTHQGLSPVTKSFNRGEC* (SEQ ID NO:2238)**

**MDMRVPAQLLGLLLLWLRGARCSYVLTQPPSVSVSPGQTASITCSGDKVGHKYASWYQDKPGQDP
VLVIYEDSQRPSGIPVRFSGSNSGNTATLTISGTQAMDEADYYCQAWDSSTDVVFGGGKLTVLGQ
PKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNNKYAAKS
YLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS* (SEQ ID NO:2239)**

**MDMRVPAQLLGLLLLWLRGARCEVQLVESVGGGLVQPGGSLRLSCAASGFTFSSYAMHWVRDAPGK
GLEWISTISGSGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARAGYGRYYYGMD
VWGDGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPA
VLQSSGLYSLSKVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCGSGSGSGSGSQVQLQQWG
AGLLKPSETLSLTCAVSGASFSGHYWTWIRKPPGKGLEWIGEIDHTGSTNYEPSLRSRVTISVDTSK
NQFSLNLKSVTAADTAVYYCARGGQGGYDWGHYHGLDWGKGTTVTVSSASTKGPSVFPLAPSSK
STSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLESVVTVPSSSLGTQTYICN
VNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSH
EDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEK
TISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPVLDSD
GSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK* (SEQ ID NO:2240)**

FIG. 19 (contd.)

**MDMRVPAQLLGLLLLWLRGARCDIQMTQSPSSLSAYVGDRVITICRVSQGISSYLNWYQKKPGKKP
KLLIYAASSLQSGVPSRFSGSGSGTDFTLTISLQPEDFATYYCQQTYPFTFGPGTKVDIKRTVAAP
SVFIFPPSDEQLKSGTASVCLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSTYSLESTLT
SKADYEKHKVYACEVTHQGLSPVTKSFNRGEC*** (SEQ ID NO:2241)

**MDMRVPAQLLGLLLLWLRGARCSYVLTQPPSVSVSPGQTASITCSGDKVGHKYASWYQDKPGQDP
VLVIYEDSQRPSGIPVRFSGSNSGNTATLTISGTQAMDEADYYCQAWDSSTDVVFGGGKLTVLGQ
PKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNNKYAAKS
YLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS*** (SEQ ID NO:2242)

**MDMRVPAQLLGLLLLWLRGARCEVQLVESVGGGLVQPGGSLRLSCAASGFTFSSYAMHWVRDAPGK
GLEWISTISGSGGSTYYADSVKGRFTISRDNKNTLYLQMNSLRAEDTAVYYCARAGYGRYYGMD
VWGDGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPA
VLQSSGLYSLKSVVTPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCGSGSGSGSGSGSGGQVQ
LQQWGAGLLKPSETLSLTCAVSGASFSGHYWTWIRKPPGKGLEWIGEIDHTGSTNYEPSLRSRVTIS
VDTSKNQFSLNLKSVTAADTAVYYCARGGQGGYDWGHYHGLDVWGKGTTVTVSSASTKGPSVFPL
APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLKSVVTPSSSLGT
QTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVV
VDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALG
APIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPV
LDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK*** (SEQ ID NO:2243)

**MDMRVPAQLLGLLLLWLRGARCCVQLQQWGAGLLKPSETLSLTCAVSGASFSGHYWTWIRQPPGK
GLEWIGEIDHTGSTNYEPSLRSRVTISVDTSKNQFSLNLKSVTAADTAVYYCARGGQGGYDWGHYH
GLDVWGQGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVH
TFPAVLQSSGLYSLSSVTPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCGSGSGHHHHHH***
(SEQ ID NO:2244)

**MDMRVPAQLLGLLLLWLRGARCDIQMTQSPSSLSASVGDRVITICRASQSISSYLNWYQQKPGKAP
KLLIYAASSLQSGVPSRFSGSGSGTDFTLTISLQPEDFATYYCQQSYSTPLTFGGGKVEIKRTVAA
PSVFIFPPSDEQLKSGTASVCLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSTYLSSTL
TLKADYEKHKVYACEVTHQGLSPVTKSFNRGEC*** (SEQ ID NO:2245)

**MDMRVPAQLLGLLLLWLRGARCSYVLTQPPSVSVSPGQTASITCSGDKVGHKYASWYQQKPGQSP
VLVIYEDSQRPSGIPVRFSGSNSGNTATLTISGTQAMDEADYYCQAWDSSTDVVFGGGKLTVLGQ
PKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNNKYAASS
YLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECSGSGSGEVQLVESGGGLVQPGGSLRLSCAA
SGFTFSSYAMHWVRQAPGKGLEWVASISSTSGSKYYADSVKGRFTISRDNKNTLYLQMNSLRAE
DTAVYSCAKTYDFWGSYYTFDYWGQGTLLTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDY
FPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTPSSSLGTQTYICNVNHKPSNTKVDKKVEP
KSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEV
HNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQPREPQVYTL
PPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPVLDSDGSFFLYSKLTVDKSRWQ
QGNVFCFSVMHEALHNHYTQKSLSLSPGK*** (SEQ ID NO:2246)

**MDMRVPAQLLGLLLLWLRGARCDVQLVESGGGLVKLGSLRLSCAASGFSFSTSYMSWVRQTPEK
RLELVAAILNNGGSTYYSDTVKGRFTISRDNKNTLYLQMSSLKSEDTAFYYCASELAGYGTPIYAYW
GQGTLVTVSAASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVL
QSSGLYSLSSVTPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCGSGSGHHHHHH*** (SEQ ID
NO:2247)

FIG. 19 (contd.)

MDMRVPAQLLGLLLLWLRGARCAAA

LTQSPATLSVTPGDSVSL

SCKASQSSISYNLHWYQQKSHE
SPRLLIKYTSSQISGIPSRFSGSGSGTDFTLTINNVEDDFGMYFCQQSNSWPLTFGAGTKLEVKRTV
AAPSVFIFPPSDEQLKSGTASVCLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSS
TLTSLKADYEKHKVYACEVTHQGLSSPVTKSFNRGECGGGGSEVQLVQSGAEVKKPGASVKVSCK
ASGYTFTSYGISWVRQAPGQGLEWMGWISAYNGNTNYAQKLQGRVTMTTDTSTSTAYMELRSLRS
DDTAVYYCASSKEKATYYYGMDVWGQGTITVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDY
FPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEP
KSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEV
HNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQPREPQVYTL
PPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPVLDSGDSFFLYSKLTVDKSRWQ
QGNVFSCSVMHEALHNHYTQKSLSLSPGKGSGSG HHHHHH* (SEQ ID NO:2248)

MDMRVPAQLLGLLLLWLRGARCAAA

LTQSPATLSVTPGDSVSL

SCKASQSSISYNLHWYQQKSHE
SPRLLIKYTSSQISGIPSRFSGSGSGTDFTLTINNVEDDFGMYFCQQSNSWPLTFGAGTKLEVKRTV
AAPSVFIFPPSDEQLKSGTASVCLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSS
TLTSLKADYEKHKVYACEVTHQGLSSPVTKSFNRGECGGGGSGGGGSEVQLVQSGAEVKKPGASV
KVSCKASGYTFTSYGISWVRQAPGQGLEWMGWISAYNGNTNYAQKLQGRVTMTTDTSTSTAYMEL
RSLRSDDTAVYYCASSKEKATYYYGMDVWGQGTITVSSASTKGPSVFPLAPSSKSTSGGTAALGC
LVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVD
KKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV
DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQPREP
QVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPVLDSGDSFFLYSKLTVD
KSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKGSGSGHHHHHH* (SEQ ID NO:2249)

MDMRVPAQLLGLLLLWLRGARCEVQLVESGGGLVQPGGSLRLSCAASGFTFSHYTLSSWVRQAPGK
GLEWVSVISGDSYTYADSVKGRFTISSDNSKNTLYLQMNSLRAEDTAVYYCARNFIKYVFANWG
QGTLTVTVSSGGGGSGGGGSGGGGSDIELTQPPSVSVAPGQTARISCSGDNIGSFYVHWYQQKPGQ
APVLVIYDKSNRPSGIPERFSGSNSGNTATLTISGTQAEDEADYYCQSYANTLSLVFGGGTKLTVLG
SGSGDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE
VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQPREPQVY
LPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPVLDSGDSFFLYSKLTVDKSRW
QQGNVFSCSVMHEALHNHYTQKSLSLSPGKGSGSGEVQLVESGGGLVQPGGSLRLSCAASGFTFT
SYISWVRQAPGKGLEWVAEISPYSGSTYYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYC
ALRARPPIRLHPRGSVMGYWGQGLTVTVSSGSAASGSSGGSSSGADIQMTQSPSSLSASVGDRVTI
TCRASQDVSTAVAWYQQKPGKAPKLLIYSASFLYSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYC
QQSYTTPPTFGQGTKEIK* (SEQ ID 2250)

MDMRVPAQLLGLLLLWLRGARCEVQLVQSGAEVKKPGASVKVSCKASGYTFTSYGISWVRQAPGQ
GLEWMGWISAYNGNTNYAQKLQGRVTMTTDTSTSTAYMELRSLRSDDTAVYYCASSKEKATYYYG
MDVWGQGTITVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHT
FPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAG*
(SEQ ID 2251)

MDMRVPAQLLGLLLLWLRGARCDIQMTQSPSSLSASVGDRVTITCRASQSISSYNLWYQQKPGKAPK
LLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPLTFGGGTKEIKGSGSGSY
VLTQPPSVSVSPGQTASITCSGDKVGHKYASWYQQKPGQSPVLVIYEDSQRPSGIPVRFSGSNSGNT
ATLTISGTQAMDEADYYCQAWDSSTDVVFGGGTKLTVLGQPKAAPSVTLFPPSSEELQANKATLVCLI
SDFYPGAVTVAWKADSSPVKAGVETTPSKQSNKYAASSYLSLTPEQWKSHRSYSCQVTHEGSTV
EKTVAPECS* (SEQ ID 2252)

FIG. 19 (contd.)

MDMRVPAQLLGLLLLWLRGARCCVQLVQSGAEVKKPGSSSVKVSCKASGYTFTYRYLHWVRQAPGQ
GLEWMGRIIPVLKITNYAQKFQGRVTITADESTSTAYMELSSLRSEDVAVYYCAVDDAFDIWGQGT
MTVTVSSGSGSGQVQLQQWAGLLKPSETLSLTCAVSGASFSGHYWTWIRQPPGKGLEWIGEIDHTGS
TNYEPSLRSRVTISVDTSKNQFSLNLKSVTAADTAVYYCARGGQGGYDWGHYHGLDVWGQGT
TVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSL
SSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKHTCPCPAPEAAAGGPSVFLFPPKPKDT
LMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVSVLT
TVLHQQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESN
GQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSSVMHEALHNHYTQKSLSLSPGK*
(SEQ ID 2253)

MDMRVPAQLLGLLLLWLRGARCEVQLVQSGAEVKKPGASVKVSCKASGYTFTSYGISWVRQAPGQ
GLEWMGWISAYNGNTNYAQKLQGRVTMTTDTSTSTAYMELRSLRSDDTAVYYCASSKEKATYYYYG
MDVWGQGT
TVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHT
FPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKHTCPCPAPEAAAG
GPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV
SVLT
TVLHQQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKG
FYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSSVMHEALHNHYT
QKSLSLSPGK* (SEQ ID NO:2254)

MDMRVPAQLLGLLLLWLRGARCEVQLVESGGGLVQPGGSLRLSCAASGFTFSHYTL
SWVRQAPGK
GLEWVSVISGDG
SYTYADSVKGRFTISSDNSKNTLYLQMNSLRAEDTAVYYCARNFIKYVFANWG
QGTLTVTVSSGGGGSGGGGSGGGGSDIELTQPPSVSVAPGQTARISCSGDNIGSFYVHWYQQKPGQ
APVLVIYDKSNRPSGIPERFSGSNSGNTATLTISGTQAEDEADYYCQSYANTLSLVFGGGTKLTVLG
SGSGDVKLVESGGGLVKLGSLRLSCAASGFSFSTSYMSWVRQTPEKRL
ELVAAILNNGGSTYYSDTVKGRFTISRDNKNTLYLQMSSLKSEDTAFYYCASELAGYGT
PFAYWGQGT
LTVSAGSAASGS
SGGSSSGADIVLTQSPATLSVTPGDSVSLSCKASQSSISYNLHWYQQKSHESPRLLIKYTSQ
SISGIPSRFSGSGSGTDFTLTINNVEDDFGMYFCQQSNSWPLTFGAGTKLEVKGSGSGHHHHHH*
(SEQ ID NO:225)

5

MDMRVPAQLLGLLLLWLRGARCEVQLVESGGGLVQPGGSLRLSCAASGFTFSHYTL
SWVRQAPGK
GLEWVSVISGDG
SYTYADSVKGRFTISSDNSKNTLYLQMNSLRAEDTAVYYCARNFIKYVFANWG
QGTLTVTVSSGGGGSGGGGSGGGGSDIELTQPPSVSVAPGQTARISCSGDNIGSFYVHWYQQKPGQ
APVLVIYDKSNRPSGIPERFSGSNSGNTATLTISGTQAEDEADYYCQSYANTLSLVFGGGTKLTVLG
SGSGGSGSGDVKLVESGGGLVKLGSLRLSCAASGFSFSTSYMSWVRQTPEKRL
ELVAAILNNGGSTYYSDTVKGRFTISRDNKNTLYLQMSSLKSEDTAFYYCASELAGYGT
PFAYWGQGT
LTVSAGS
AASGSSSGSSSGADIVLTQSPATLSVTPGDSVSLSCKASQSSISYNLHWYQQKSHESPRLLIKYTSQ
SISGIPSRFSGSGSGTDFTLTINNVEDDFGMYFCQQSNSWPLTFGAGTKLEVKGSGSGHHHHHH*
(SEQ ID NO:2256)

MDMRVPAQLLGLLLLWLRGARCEVQLVESGGGLVQPGGSLRLSCAASGFTFSHYTL
SWVRQAPGK
GLEWVSVISGDG
SYTYADSVKGRFTISSDNSKNTLYLQMNSLRAEDTAVYYCARNFIKYVFANWG
QGTLTVTVSSGGGGSGGGGSGGGGSDIELTQPPSVSVAPGQTARISCSGDNIGSFYVHWYQQKPGQ
APVLVIYDKSNRPSGIPERFSGSNSGNTATLTISGTQAEDEADYYCQSYANTLSLVFGGGTKLTVLG
SGSGGSGSGGSSGGDVKLVESGGGLVKLGSLRLSCAASGFSFSTSYMSWVRQTPEKRL
ELVAAILNNGGSTYYSDTVKGRFTISRDNKNTLYLQMSSLKSEDTAFYYCASELAGYGT
PFAYWGQGT
LTVSAGS
AASGSSSGSSSGADIVLTQSPATLSVTPGDSVSLSCKASQSSISYNLHWYQQKSHESPRLLIK
YTSQ
SISGIPSRFSGSGSGTDFTLTINNVEDDFGMYFCQQSNSWPLTFGAGTKLEVKGSGSGHHHH
HH* (SEQ ID NO:2257)

FIG. 19 (contd.)

MDMRVPAQLLGLLLLWLRGARC DVKLVESGGGLVKLGGSRLRLSCAASGFSFSTSYMSWVRQTPEK
RLELVAAINLNGGSTYYSDTVKGRFTISRDN AKNTLYLQMSSLKSEDTAFYYCASELAGYGTPFAYW
GQGT LVTVSAGSAASGSSGGSSSGADIVLTQSPATLSVTPGDSVSLSCKASQSSISYNLHWYQQKSH
ESPRLLIKYTSQSISGIPSRFSGSGSGTDFTLTINN VETEDFGMYFCQQSNSWPLTFGAGTKLEVKG
SGSEVQLVESGGGLVQPGGSLRLSCAASGFTFSHYT LSWVRQAPGKGLEWVSVISGDGSYTYAD
SVKGRFTISSDNSKNTLYLQMNSLRAEDTAVYYCARNFIKYVFANWGQGT LVTVSSGGGGSGGGG
SGGGGSDIELTQPPSVSVAPGQTARISCSGDNIGSFYVHWYQQKPGQAPVLVIYDKSNRPSGIPERF
SGSNSGNTATLTISGTQAEDEADYYCQSYANTLSLVFGGGTKLTVLGSGSGHHHHHHH* (SEQ ID
NO:2258)

MDMRVPAQLLGLLLLWLRGARC DVKLVESGGGLVKLGGSRLRLSCAASGFSFSTSYMSWVRQTPEK
RLELVAAINLNGGSTYYSDTVKGRFTISRDN AKNTLYLQMSSLKSEDTAFYYCASELAGYGTPFAYW
GQGT LVTVSAGSAASGSSGGSSSGADIVLTQSPATLSVTPGDSVSLSCKASQSSISYNLHWYQQKSH
ESPRLLIKYTSQSISGIPSRFSGSGSGTDFTLTINN VETEDFGMYFCQQSNSWPLTFGAGTKLEVKG
GSGGSGSGSEVQLVESGGGLVQPGGSLRLSCAASGFTFSHYT LSWVRQAPGKGLEWVSVISGDGS
YTYADSVKGRFTISSDNSKNTLYLQMNSLRAEDTAVYYCARNFIKYVFANWGQGT LVTVSSGGGG
SGGGGSGGGGSDIELTQPPSVSVAPGQTARISCSGDNIGSFYVHWYQQKPGQAPVLVIYDKSNRPS
GIPERFSGSNSGNTATLTISGTQAEDEADYYCQSYANTLSLVFGGGTKLTVLGSGSGHHHHHHH*
(SEQ ID NO:2259)

MDMRVPAQLLGLLLLWLRGARC DVKLVESGGGLVKLGGSRLRLSCAASGFSFSTSYMSWVRQTPEK
RLELVAAINLNGGSTYYSDTVKGRFTISRDN AKNTLYLQMSSLKSEDTAFYYCASELAGYGTPFAYW
GQGT LVTVSAGSAASGSSGGSSSGADIVLTQSPATLSVTPGDSVSLSCKASQSSISYNLHWYQQKSH
ESPRLLIKYTSQSISGIPSRFSGSGSGTDFTLTINN VETEDFGMYFCQQSNSWPLTFGAGTKLEVKG
GSGGSGSGGSSGGEVQLVESGGGLVQPGGSLRLSCAASGFTFSHYT LSWVRQAPGKGLEWVSVI
SGDGSYTYADSVKGRFTISSDNSKNTLYLQMNSLRAEDTAVYYCARNFIKYVFANWGQGT LVTVS
SGGGGSGGGGSGGGGSDIELTQPPSVSVAPGQTARISCSGDNIGSFYVHWYQQKPGQAPVLVIYD
KSNRPSGIPERFSGSNSGNTATLTISGTQAEDEADYYCQSYANTLSLVFGGGTKLTVLGSGSGHHH
HHH* (SEQ ID NO:2260)

MDMRVPAQLLGLLLLWLRGARCEVQLVESGGGLVQPGGSLRLSCAASGFTFSHYT LSWVRQAPGK
GLEWVSVISGDGSYTYADSVKGRFTISSDNSKNTLYLQMNSLRAEDTAVYYCARNFIKYVFANWG
QGT LVTVSSGGGGSGGGGSGGGGSDIELTQPPSVSVAPGQTARISCSGDNIGSFYVHWYQQKPGQ
APVLVIYDKSNRPSGIPERFSGSNSGNTATLTISGTQAEDEADYYCQSYANTLSLVFGGGTKLTVLG
SGSGEVQLVESGGGLVQPGGSLRLSCAASGFTFTSYISWVRQAPGKGLEWVAEISPYSGSTYYAD
SVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCALRARPPIRLHPRGSVM DYWGQGT LVTVSSGS
AASGSSGGSSSGADIQMTQSPSSLSASVGDRVITICRASQDVSTAVAWYQQKPGKAPKLLIYSASF
LYSGVPSRFSGSGSGTDFTLTISLQPEDFATYYCQQSYTTPPTFGQGTKVEIKSGSGHHHHHHH*
(SEQ ID NO:2261)

MDMRVPAQLLGLLLLWLRGARCEVQLVESGGGLVQPGGSLRLSCAASGFTFSHYT LSWVRQAPGK
GLEWVSVISGDGSYTYADSVKGRFTISSDNSKNTLYLQMNSLRAEDTAVYYCARNFIKYVFANWG
QGT LVTVSSGGGGSGGGGSGGGGSDIELTQPPSVSVAPGQTARISCSGDNIGSFYVHWYQQKPGQ
APVLVIYDKSNRPSGIPERFSGSNSGNTATLTISGTQAEDEADYYCQSYANTLSLVFGGGTKLTVLG
SGSGGSGSGSEVQLVESGGGLVQPGGSLRLSCAASGFTFTSYISWVRQAPGKGLEWVAEISPYSG
STYYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCALRARPPIRLHPRGSVM DYWGQGT L
TVSSGSAASGSSGGSSSGADIQMTQSPSSLSASVGDRVITICRASQDVSTAVAWYQQKPGKAPKLL
IYSASFLYSGVPSRFSGSGSGTDFTLTISLQPEDFATYYCQQSYTTPPTFGQGTKVEIKSGSGHHH
HHH* (SEQ ID NO:2262)

FIG. 19 (contd.)

MDMRVPAQLLGLLLLWLRGARCEVQLVESGGGLVQPGGSLRLSCAASGFTFESHYTLWVRQAPGK
GLEWVSVISGDGSGYTYADSVKGRFTISSDNSKNTLYLQMNSLRAEDTAVYYCARNFIKYVFANWG
QGTLVTVSSGGGGSGGGSGGGGSDIELTQPPSVSVAPGQTARISCSGDNIGSFYVHWYQQKPGQ
APVLVIYDKSNRPSGIPERFSGSNSGNTATLTISGTQAEDEADYYCQSYANTLSLVFGGGTKLTVLG
SGSGSGSGSGSGGGEVQLVESGGGLVQPGGSLRLSCAASGFTFESHYTLWVRQAPGKGLEWVAEI
SPYSGSTYYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCALRARPPIRLHPRGSVMMDYWG
QGTLVTVSSGSAASGSSGGSSSGADIQMTQSPSSLSASVGDRVITITCRASQDVSTAVAWYQQKPG
KAPKLLIYSASFLYSGVPSRFSGSGSGTDFTLTISLQPEDFATYYCQSYTTPPTFGGQTKVEIKGS
SGSGHHHHH* (SEQ ID NO:2263)

MDMRVPAQLLGLLLLWLRGARCEVQLVESGGGLVQPGGSLRLSCAASGFTFESHYTLWVRQAPGK
GLEWVAEISPYSGSTYYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCALRARPPIRLHPRG
SVMMDYWGQGTLVTVSSGSAASGSSGGSSSGADIQMTQSPSSLSASVGDRVITITCRASQDVSTAVA
WYQQKPGKAPKLLIYSASFLYSGVPSRFSGSGSGTDFTLTISLQPEDFATYYCQSYTTPPTFGGQ
TKVEIKGS GSGGEVQLVESGGGLVQPGGSLRLSCAASGFTFESHYTLWVRQAPGKGLEWVSVISGD
GSYTYADSVKGRFTISSDNSKNTLYLQMNSLRAEDTAVYYCARNFIKYVFANWGQGTLVTVSSGG
GGSGGGSGGGGSDIELTQPPSVSVAPGQTARISCSGDNIGSFYVHWYQQKPGQAPVLVIYDKSNR
PSGIPERFSGSNSGNTATLTISGTQAEDEADYYCQSYANTLSLVFGGGTKLTVLGSGSGHHHHHHH*
(SEQ ID NO:2264)

MDMRVPAQLLGLLLLWLRGARCEVQLVESGGGLVQPGGSLRLSCAASGFTFESHYTLWVRQAPGK
GLEWVAEISPYSGSTYYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCALRARPPIRLHPRG
SVMMDYWGQGTLVTVSSGSAASGSSGGSSSGADIQMTQSPSSLSASVGDRVITITCRASQDVSTAVA
WYQQKPGKAPKLLIYSASFLYSGVPSRFSGSGSGTDFTLTISLQPEDFATYYCQSYTTPPTFGGQ
TKVEIKGS GSGGEVQLVESGGGLVQPGGSLRLSCAASGFTFESHYTLWVRQAPGKGLEWVS
VISGDGSYTYADSVKGRFTISSDNSKNTLYLQMNSLRAEDTAVYYCARNFIKYVFANWGQGTLVT
SSGGGGSGGGGSGGGGSDIELTQPPSVSVAPGQTARISCSGDNIGSFYVHWYQQKPGQAPVLVIY
DKSNRPSGIPERFSGSNSGNTATLTISGTQAEDEADYYCQSYANTLSLVFGGGTKLTVLGSGSGHH
HHH* (SEQ ID NO:2265)

MDMRVPAQLLGLLLLWLRGARCEVQLVESGGGLVQPGGSLRLSCAASGFTFESHYTLWVRQAPGK
GLEWVAEISPYSGSTYYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCALRARPPIRLHPRG
SVMMDYWGQGTLVTVSSGSAASGSSGGSSSGADIQMTQSPSSLSASVGDRVITITCRASQDVSTAVA
WYQQKPGKAPKLLIYSASFLYSGVPSRFSGSGSGTDFTLTISLQPEDFATYYCQSYTTPPTFGGQ
TKVEIKGS GSGGSGSGSGSGGGEVQLVESGGGLVQPGGSLRLSCAASGFTFESHYTLWVRQAPGK
GLEWVSVISGDGSGYTYADSVKGRFTISSDNSKNTLYLQMNSLRAEDTAVYYCARNFIKYVFANWG
QGTLVTVSSGGGGSGGGGSGGGGSDIELTQPPSVSVAPGQTARISCSGDNIGSFYVHWYQQKPGQ
APVLVIYDKSNRPSGIPERFSGSNSGNTATLTISGTQAEDEADYYCQSYANTLSLVFGGGTKLTVLG
SGSGHHHHHHH* (SEQ ID NO:2266)

MDMRVPAQLLGLLLLWLRGARCEVQLVESGGGLVQPGGSLRLSCAASGFTFESHYTLWVRQAPGK
GLEWVAEISPYSGSTYYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCALRARPPIRLHPRG
SVMMDYWGQGTLVTVSSGSAASGSSGGSSSGADIQMTQSPSSLSASVGDRVITITCRASQDVSTAVA
WYQQKPGKAPKLLIYSASFLYSGVPSRFSGSGSGTDFTLTISLQPEDFATYYCQSYTTPPTFGGQ
TKVEIKGS GSGDKHTCPPEAAGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFN
WYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQP
REPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPVLDSDGSFFLYSKL
TVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGKGSGSGGEVQLVESGGGLVQPGGSLRLSCA
ASGFTFESHYTLWVRQAPGKGLEWVSVISGDGSGYTYADSVKGRFTISSDNSKNTLYLQMNSLRAE
DTAVYYCARNFIKYVFANWGQGTLVTVSSGGGGSGGGGSGGGGSDIELTQPPSVSVAPGQTARIS
CSGDNIGSFYVHWYQQKPGQAPVLVIYDKSNRPSGIPERFSGSNSGNTATLTISGTQAEDEADYYCQ
SYANTLSLVFGGGTKLTVL* (SEQ ID NO:2267)

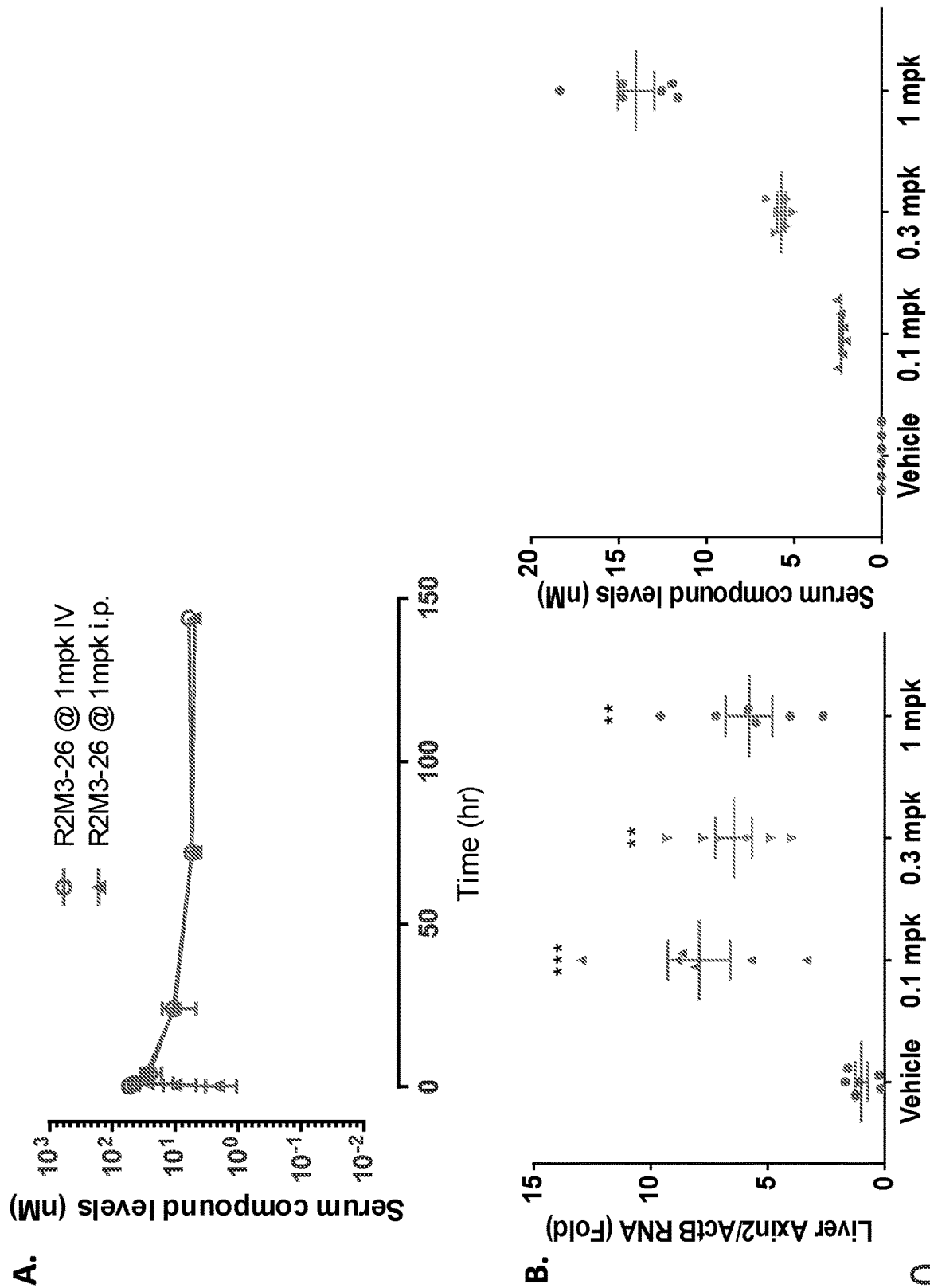


FIG. 20

A.

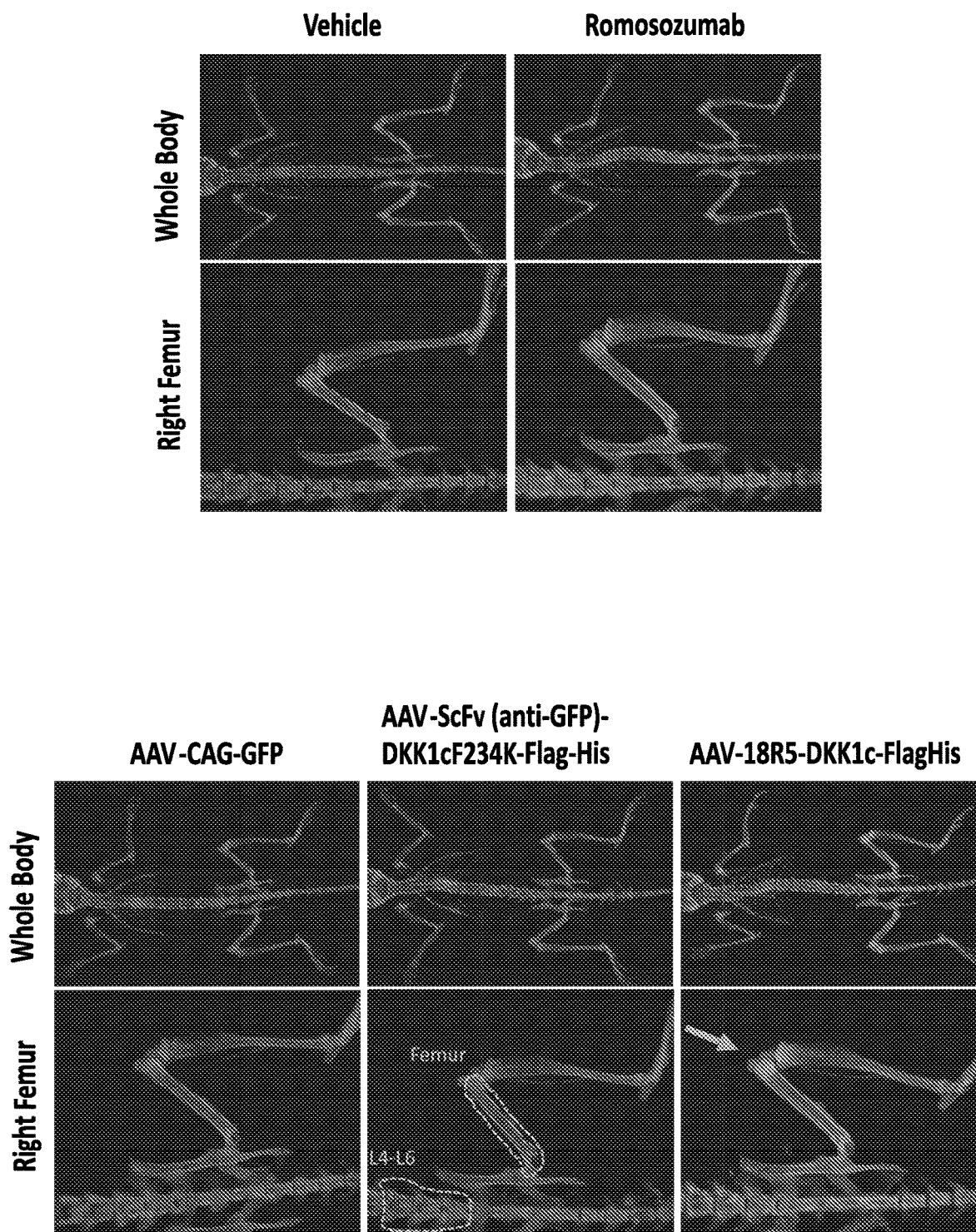


FIG. 21A

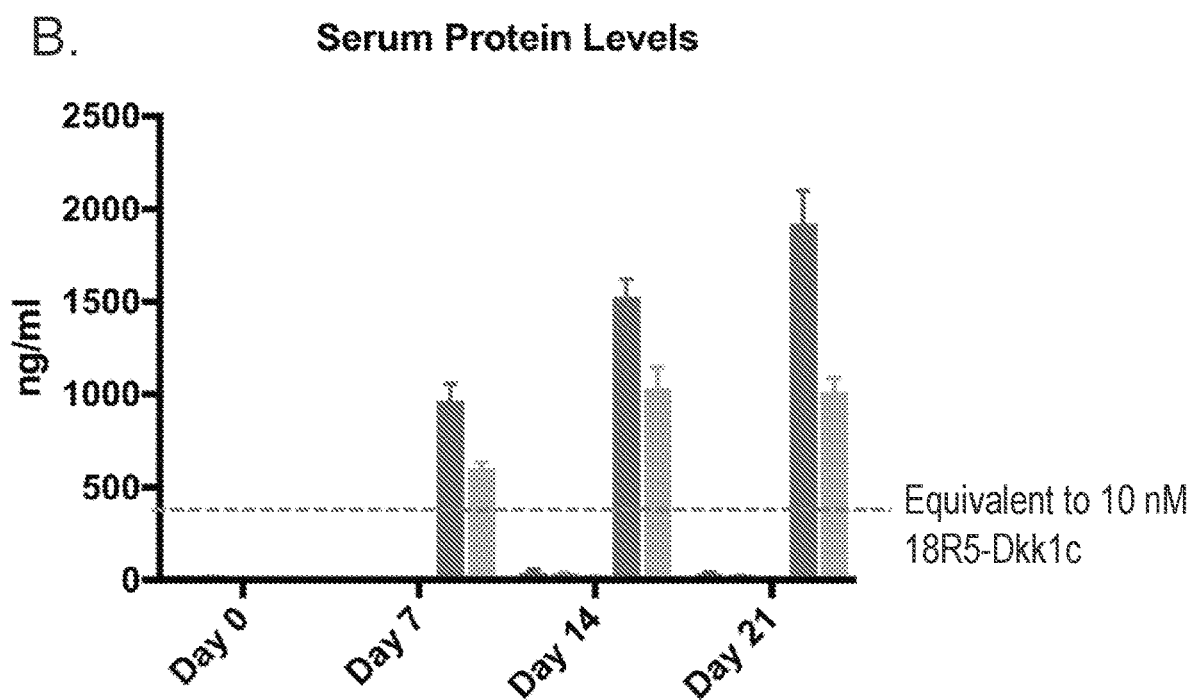


FIG. 21B

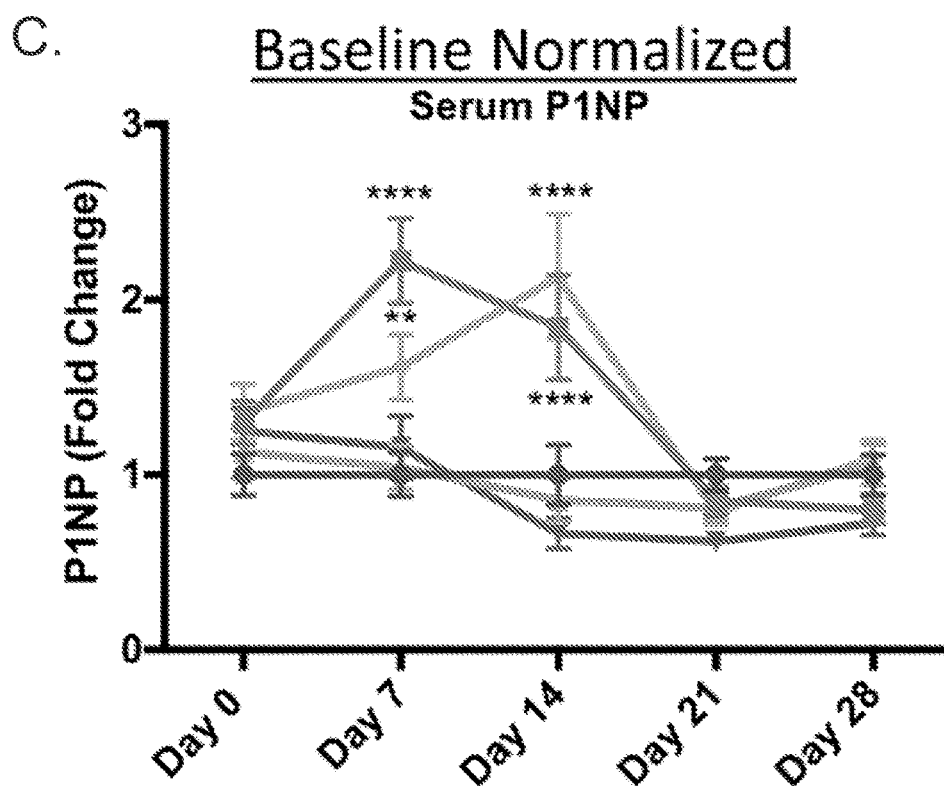


FIG. 21C

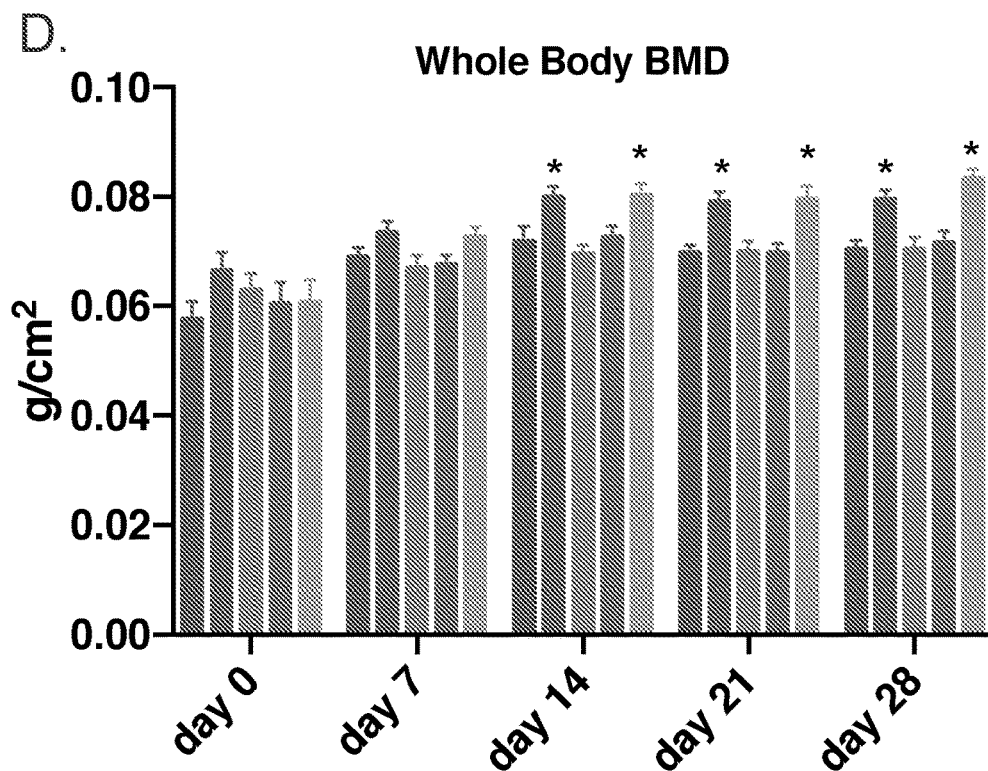
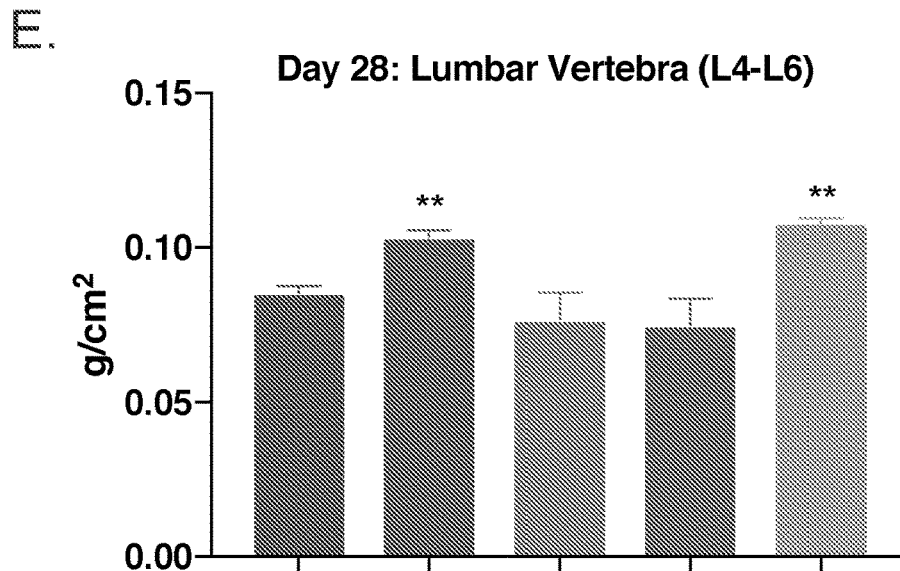


FIG. 21D



Vehicle

Romosozumab

AAV-CAG-GFP

AAV-ScFv (anti-GFP)-DKK1cF234K-Flag-His

AAV-18R5-DKK1c-FlagHis

** P value <0.05

**** P value <0.000.1

FIG. 21E

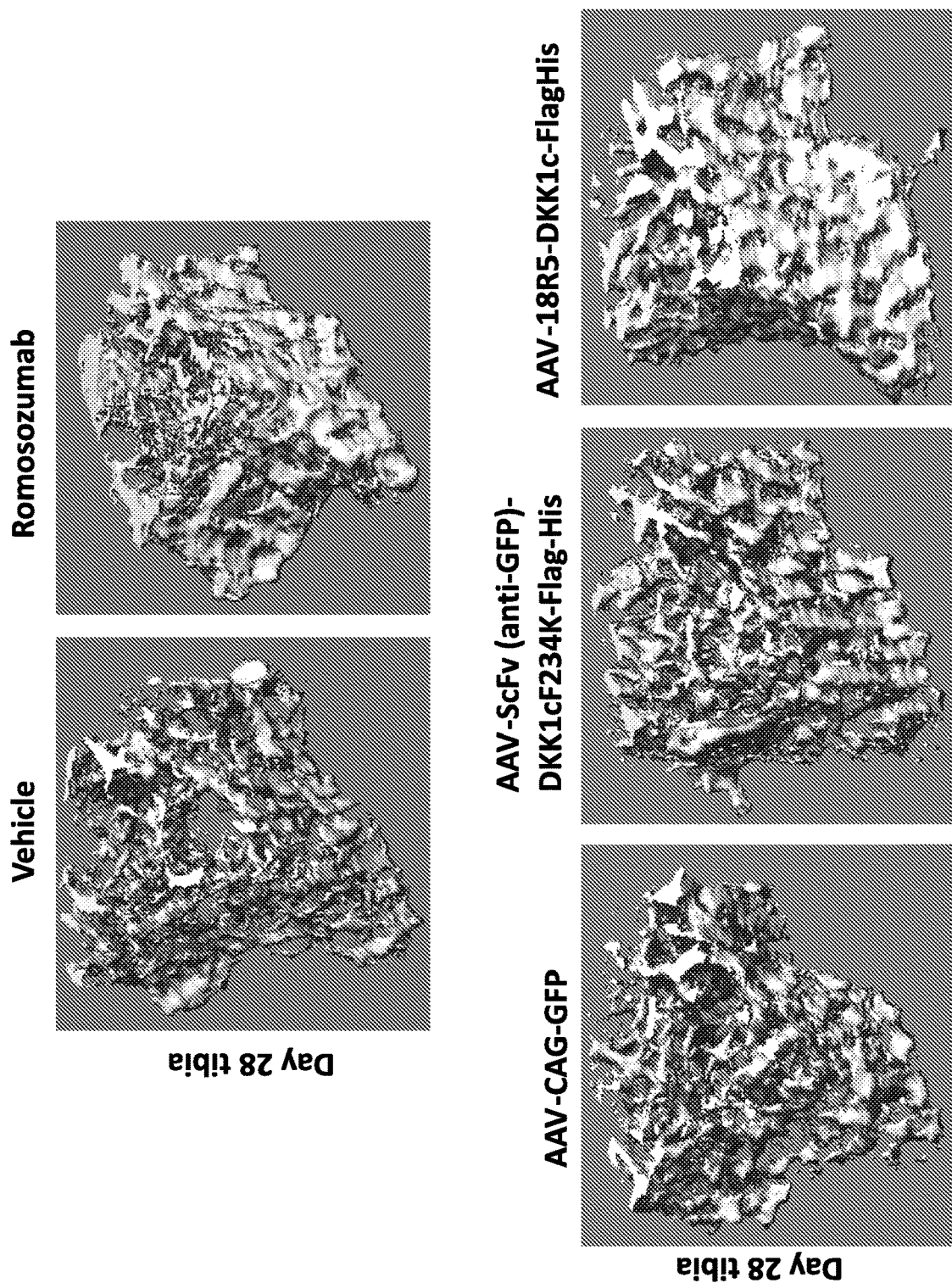


FIG. 22A

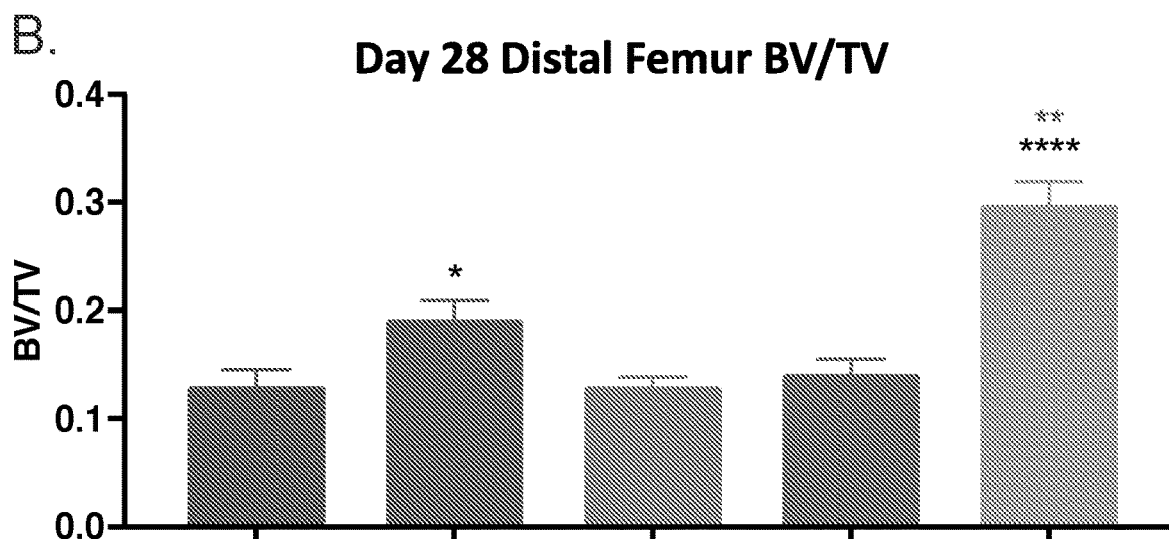
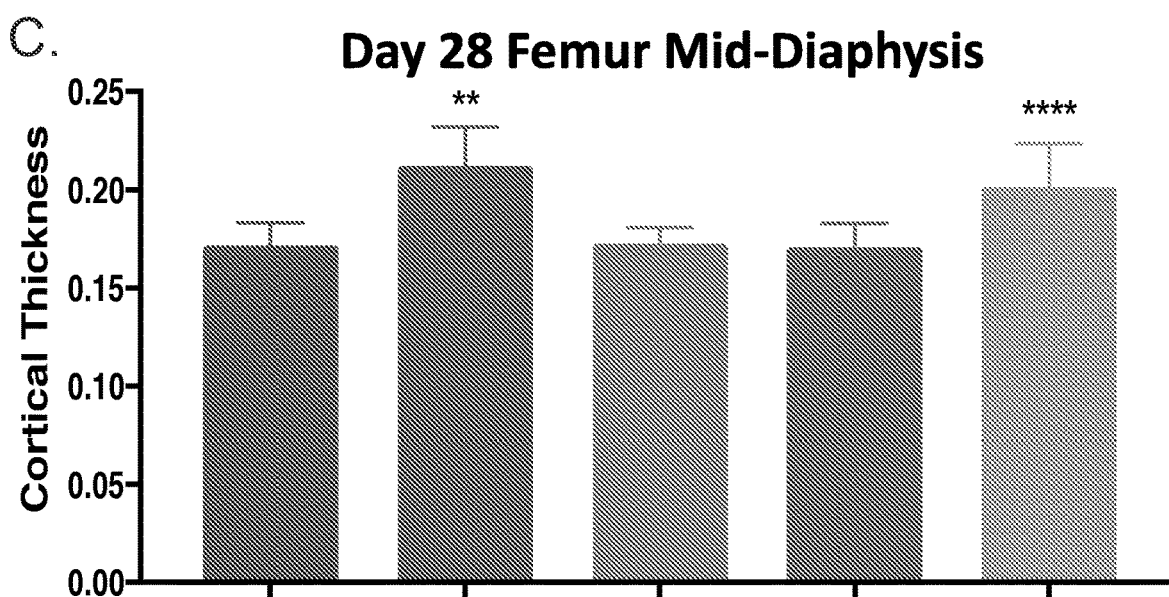
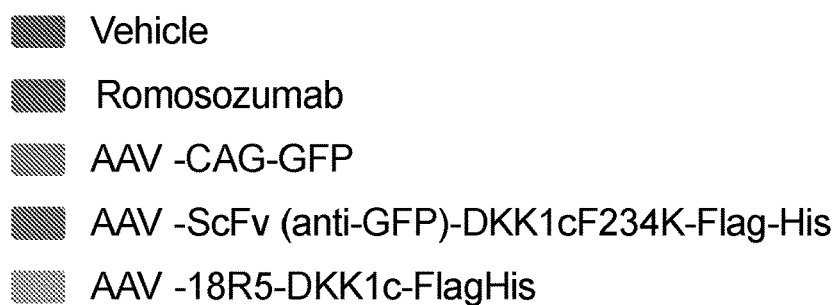


FIG. 22B



**** P value <0.0001

FIG. 22C

D.

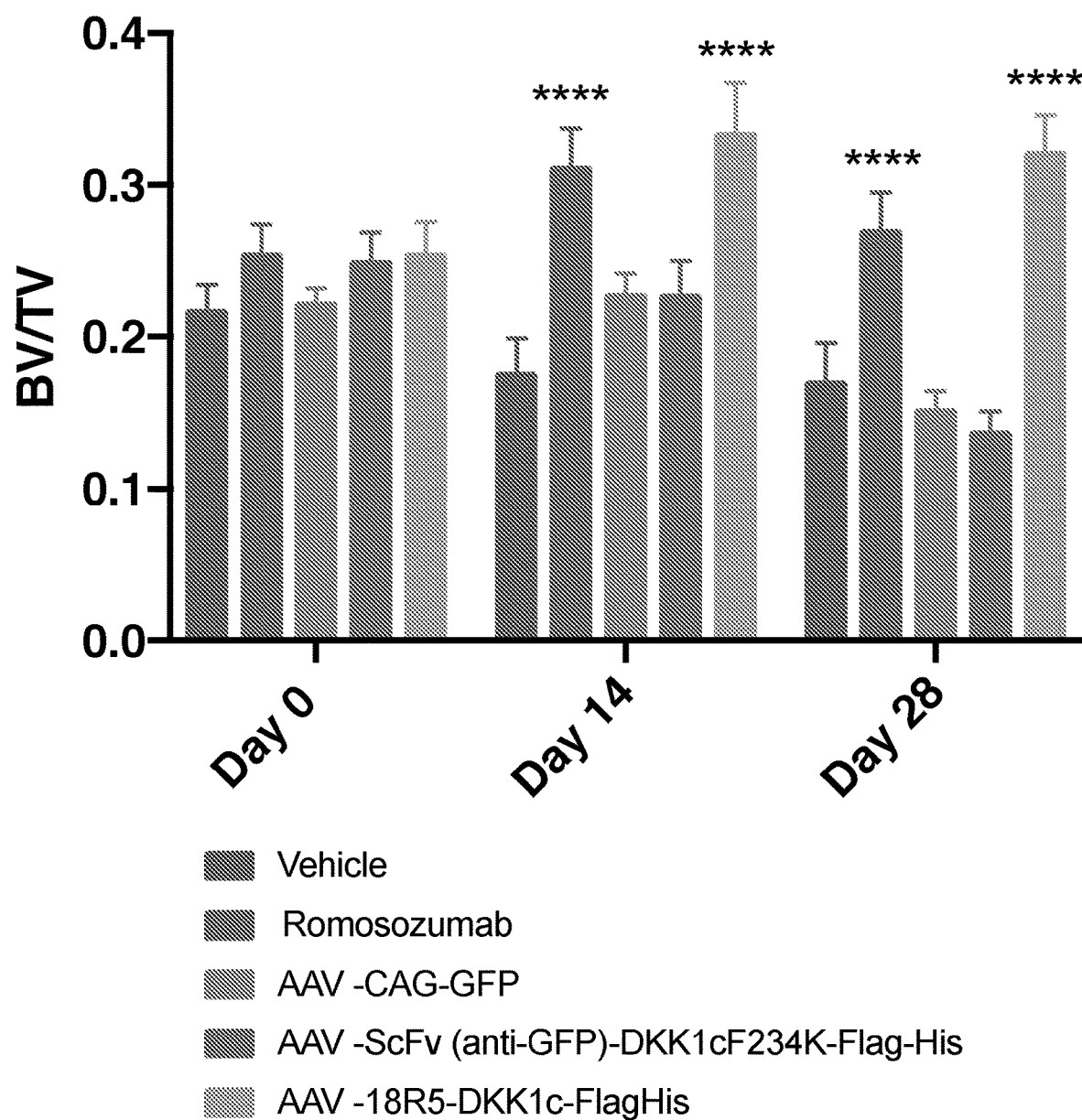


FIG. 22D

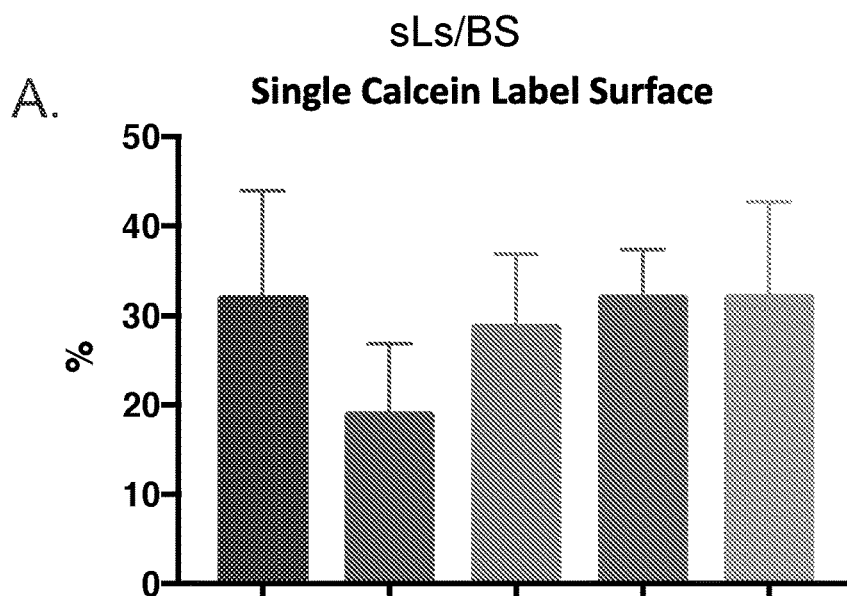


FIG. 23A

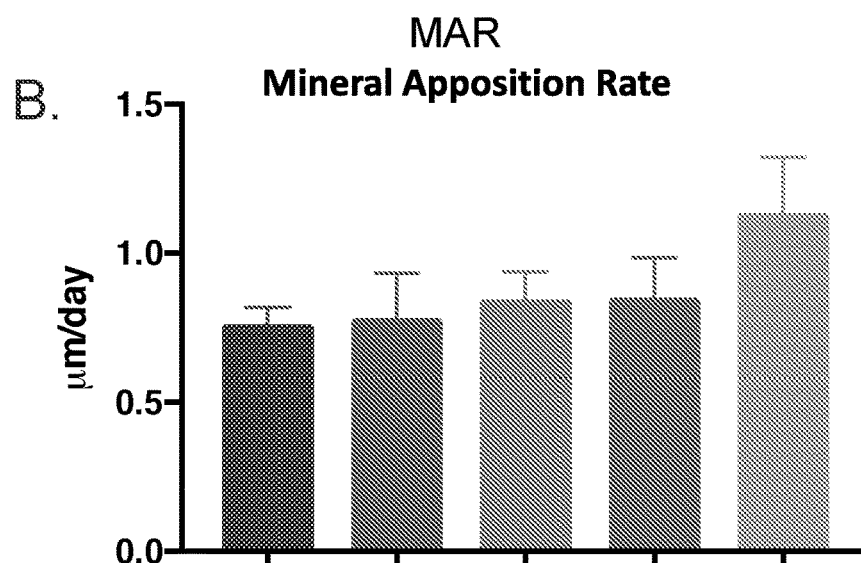
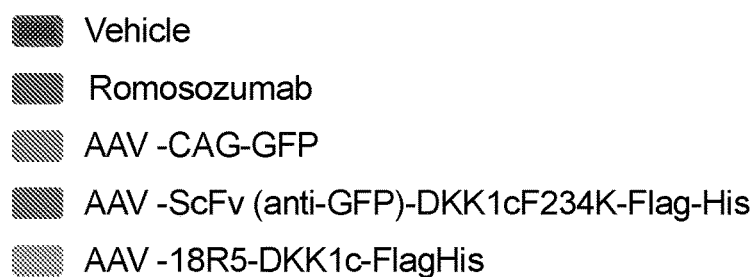


FIG. 23B

FIG. 24A-C

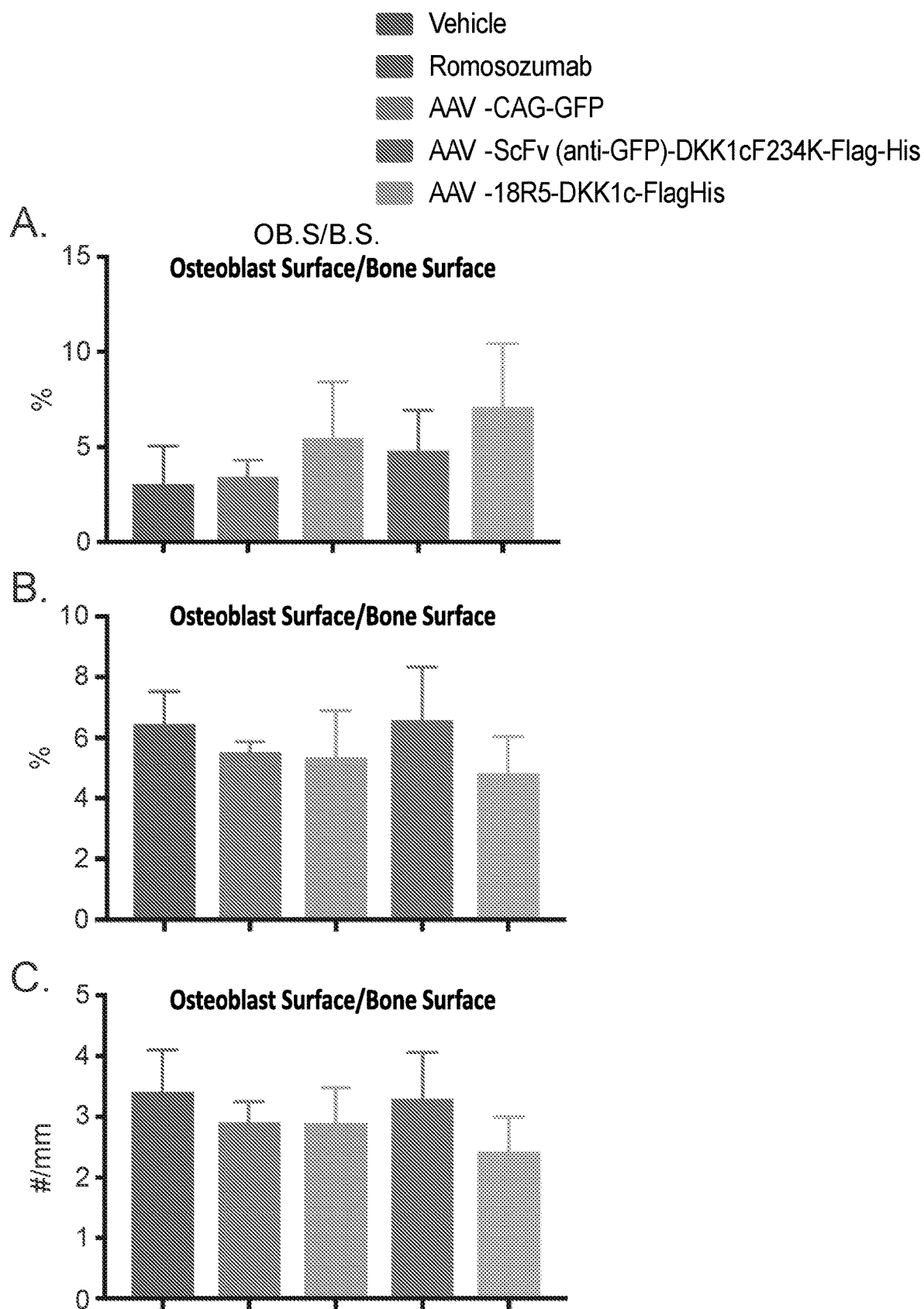


FIG. 24D

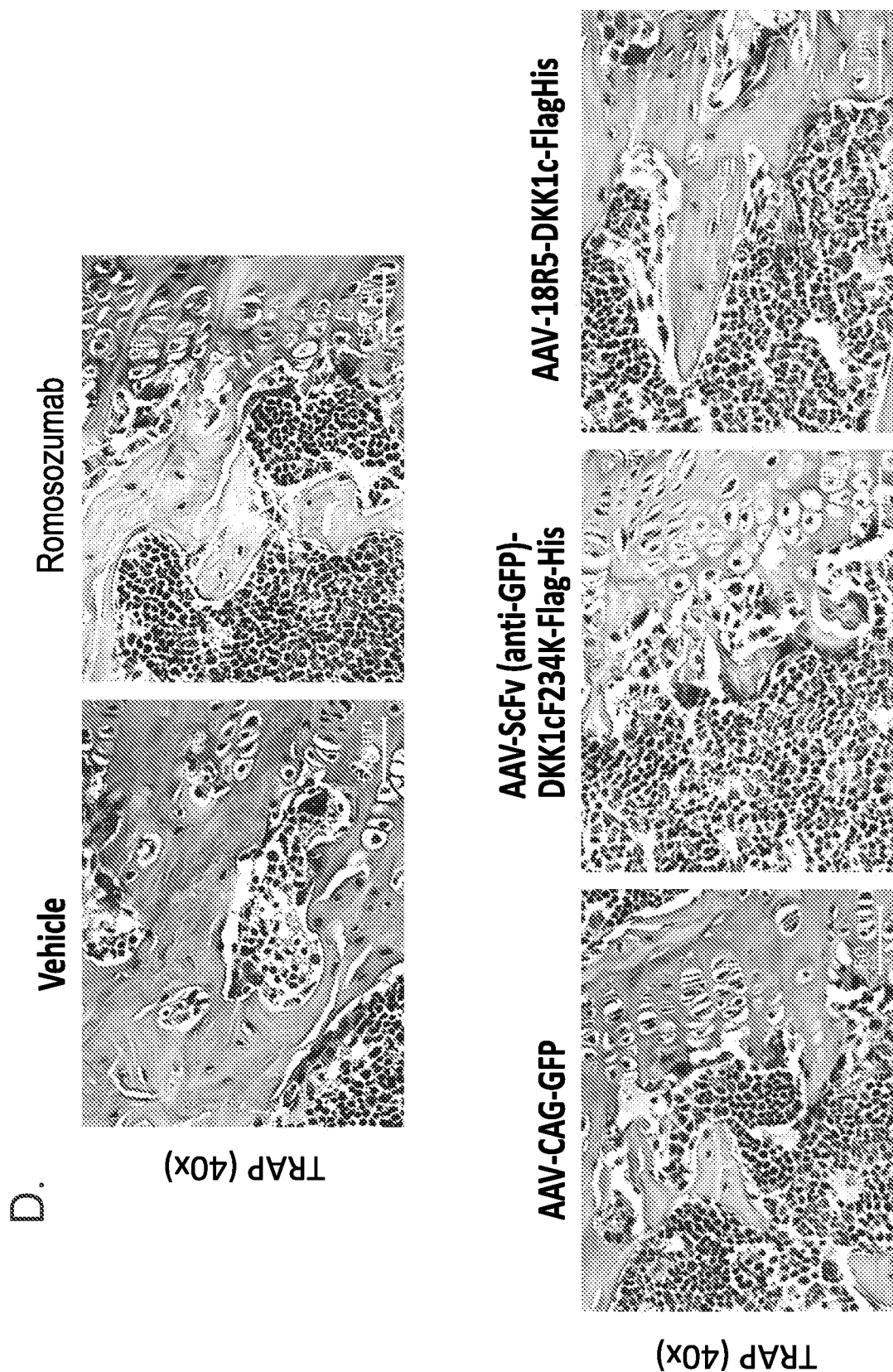


FIG. 25A

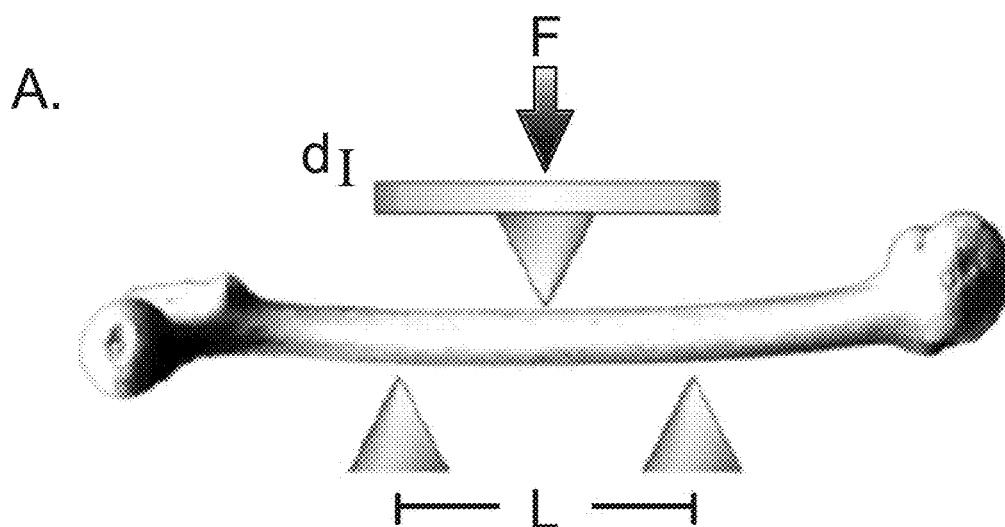


FIG. 25B

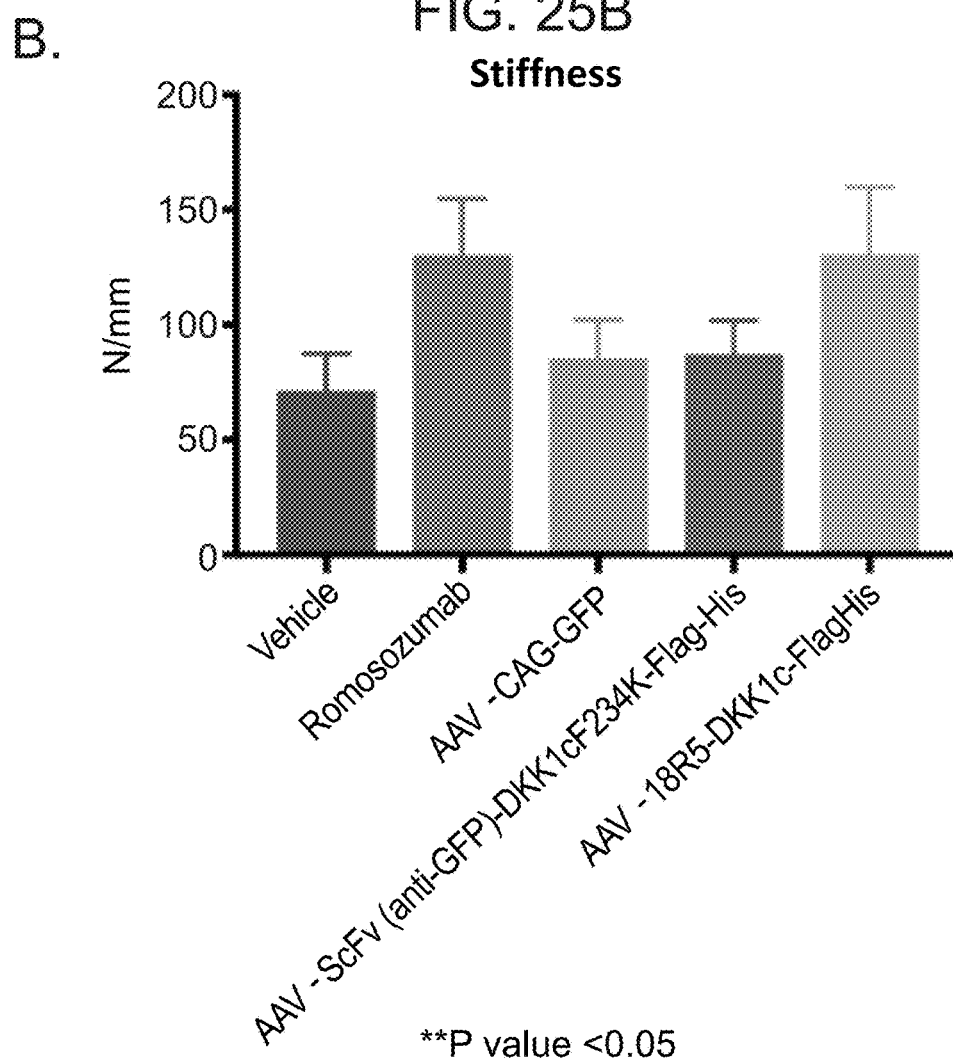
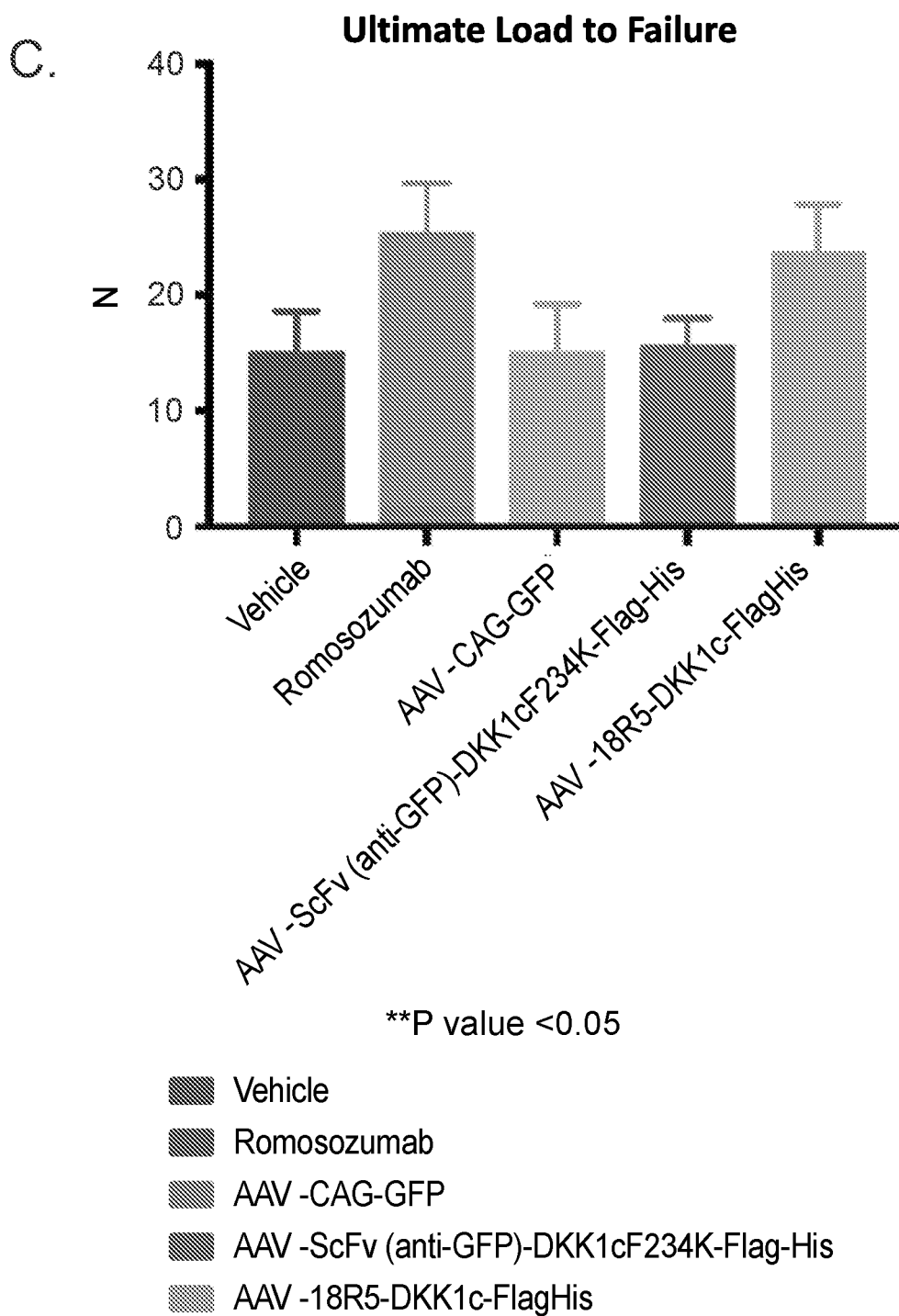
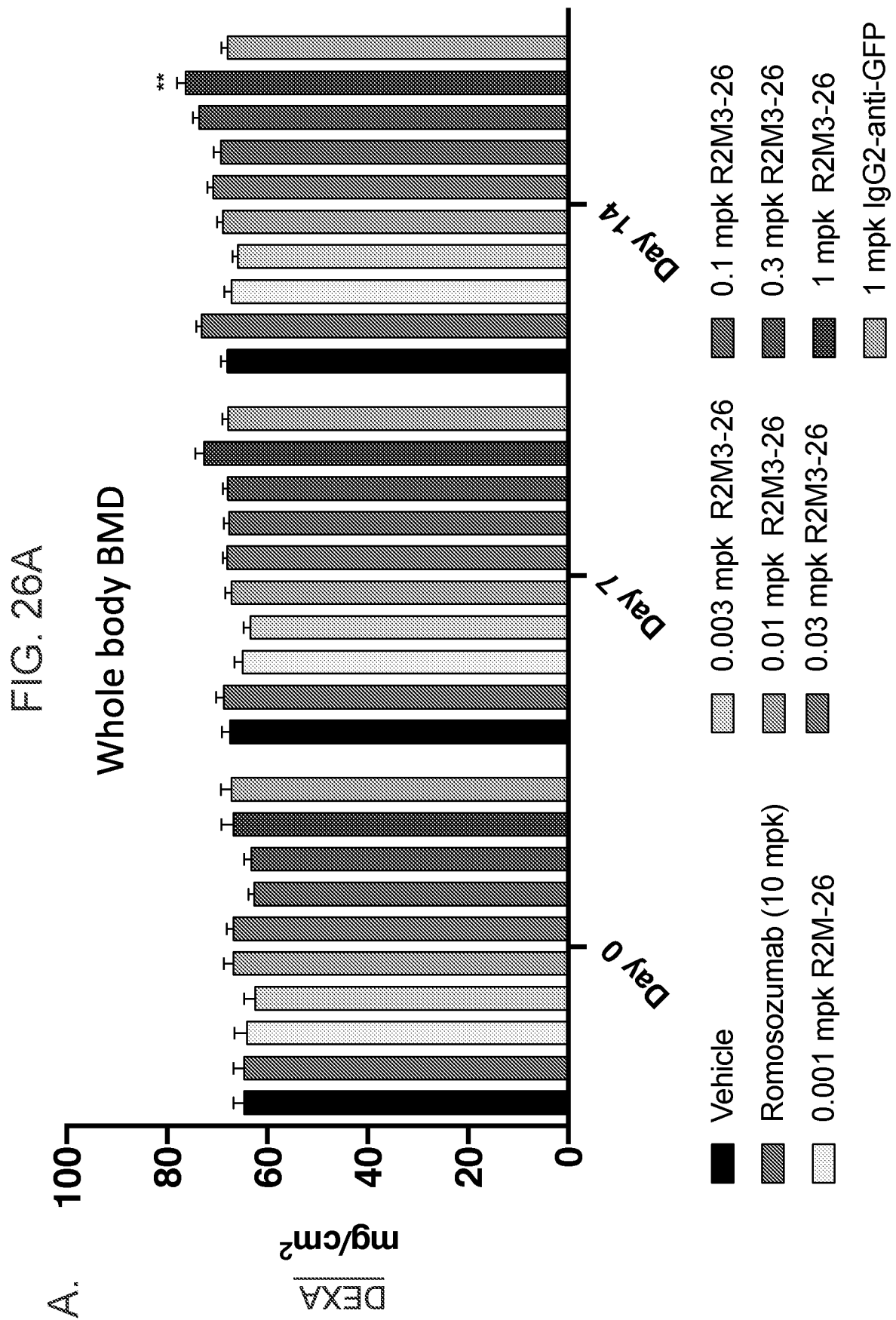


FIG. 25C





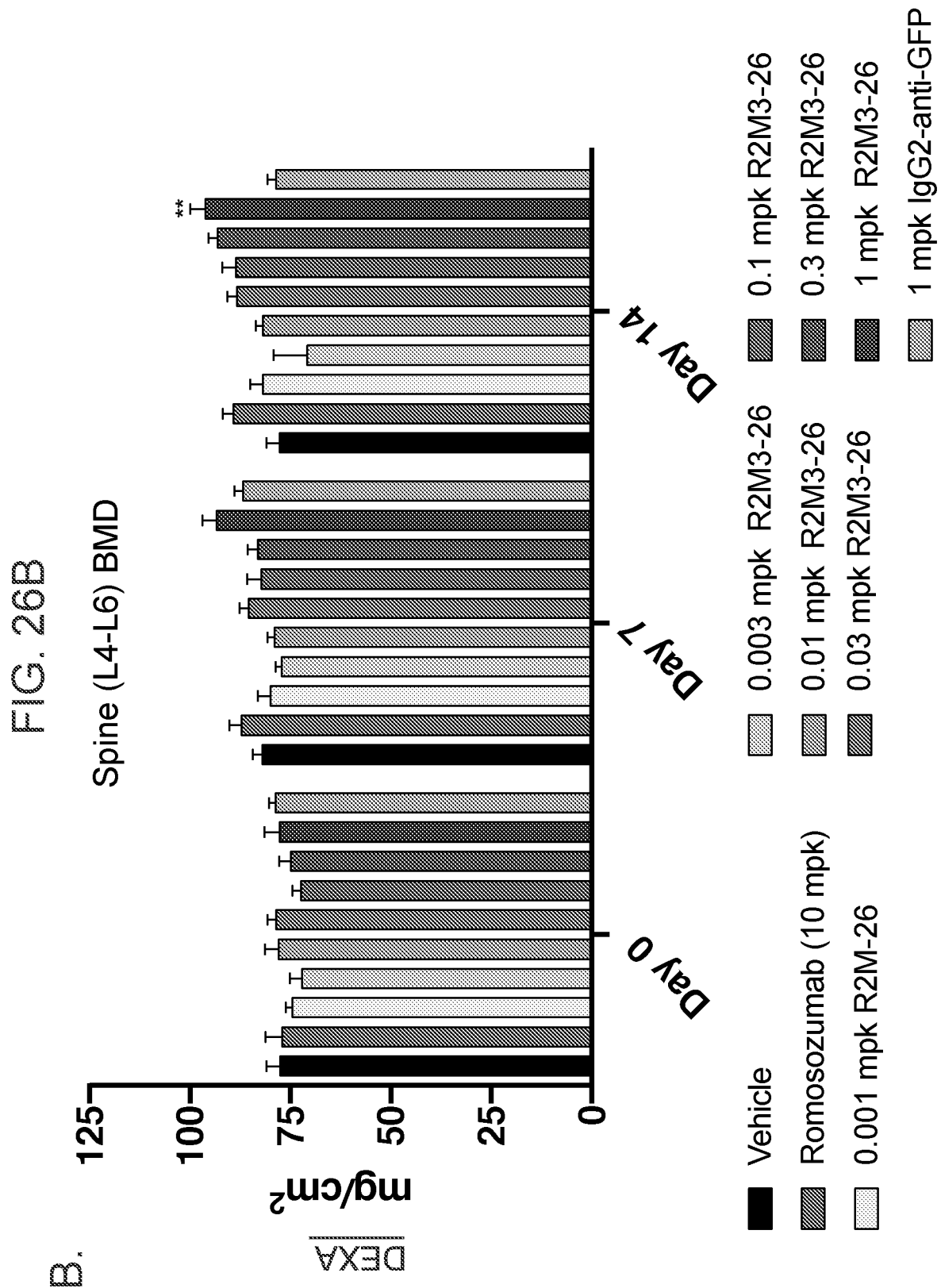


FIG. 26C

Proximal tibia μ CT

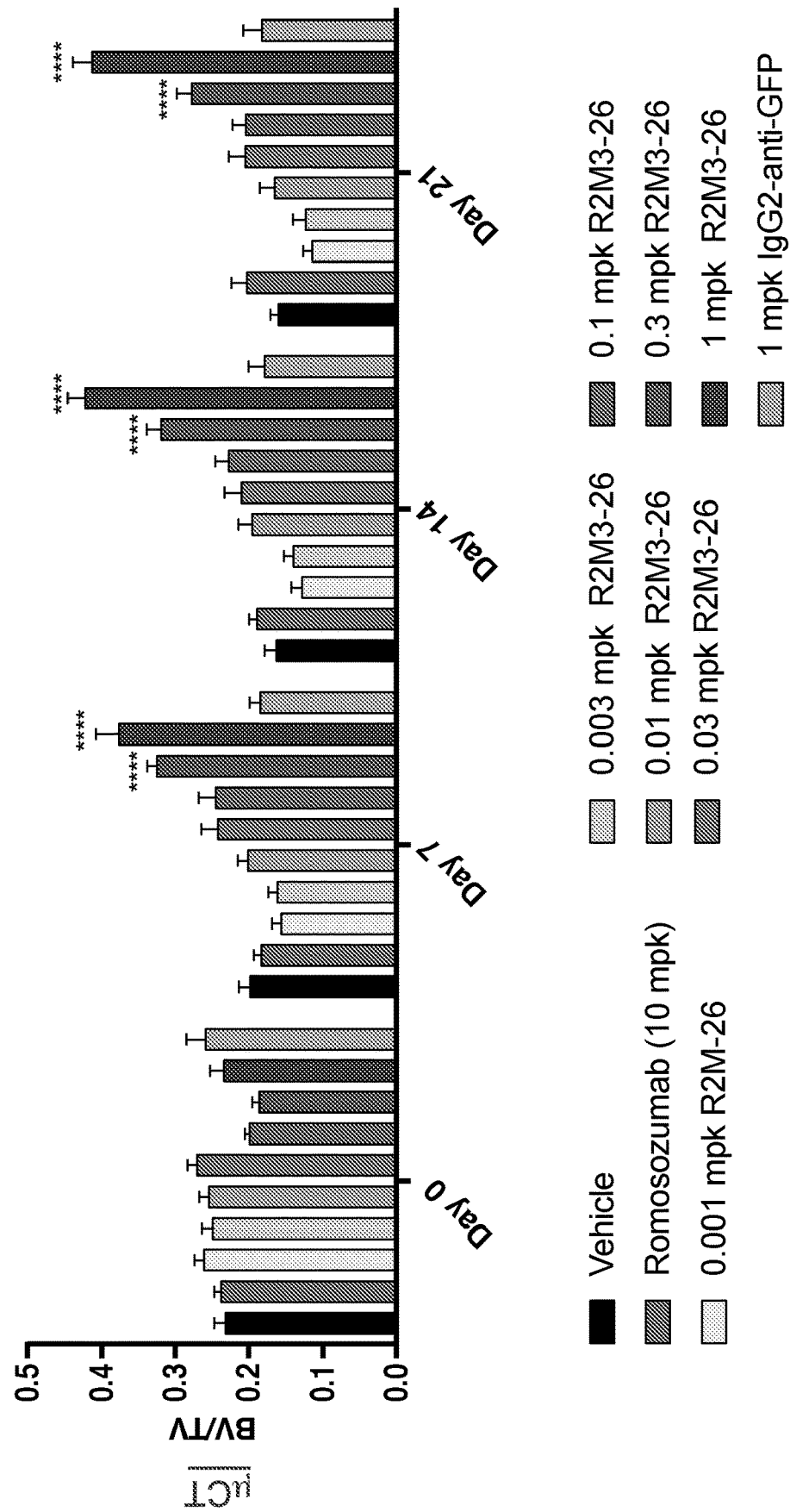
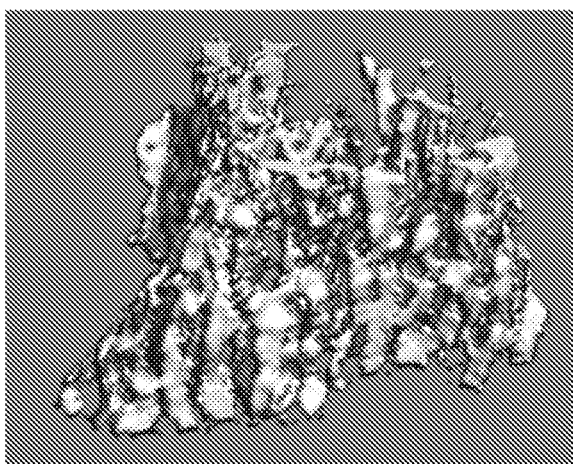


FIG. 26D

D.

Vehicle



1 mpk R2M3-26

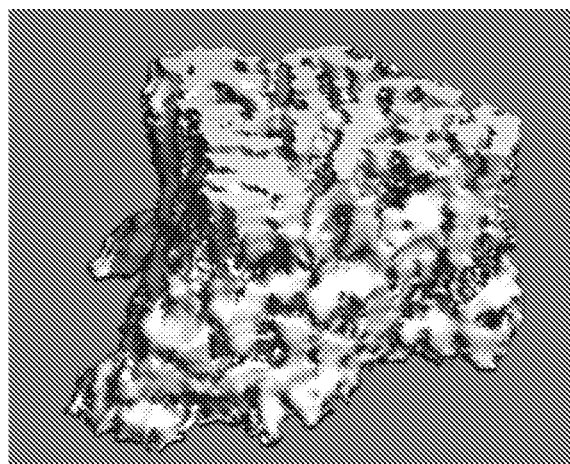
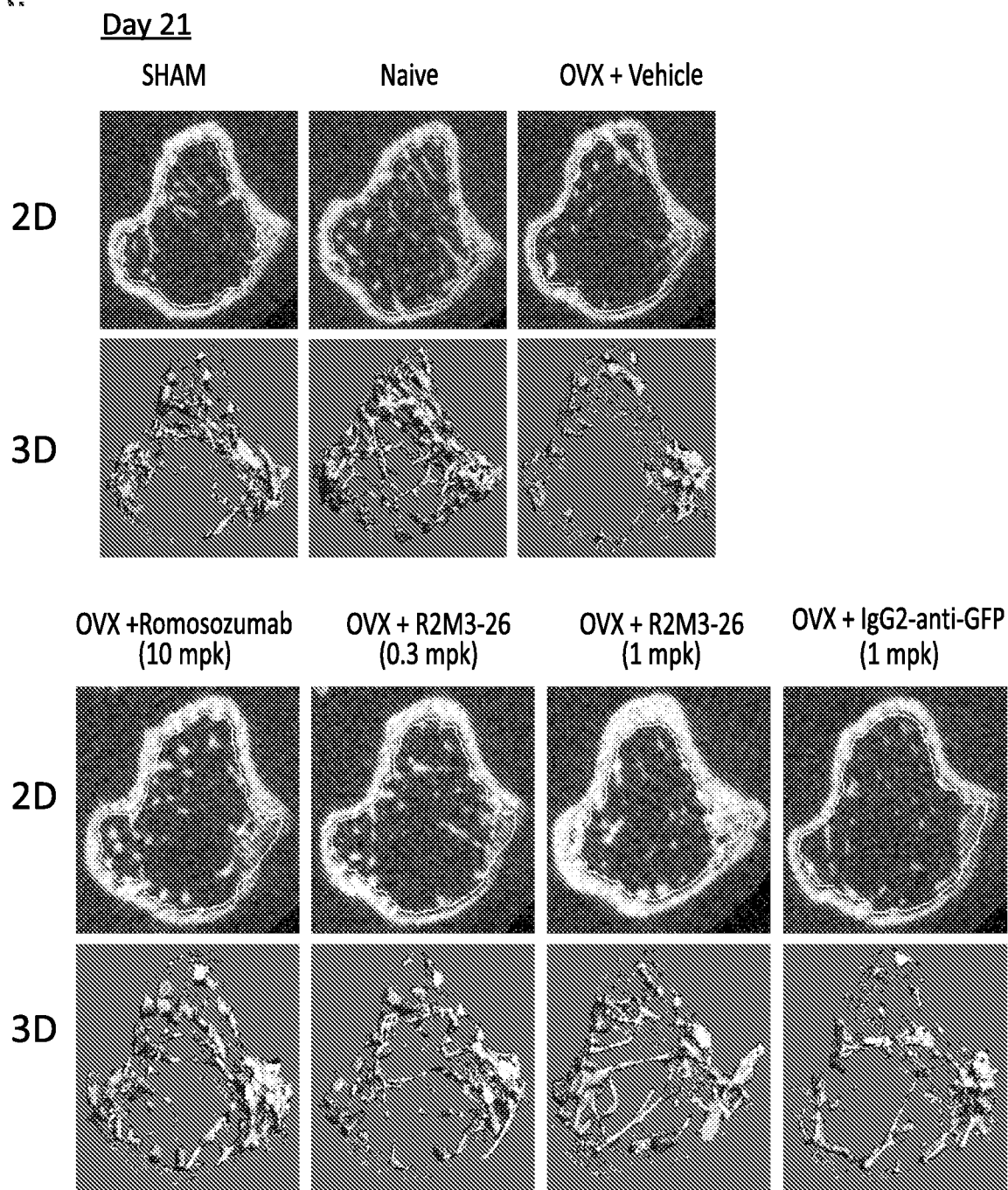


FIG. 27A

A.



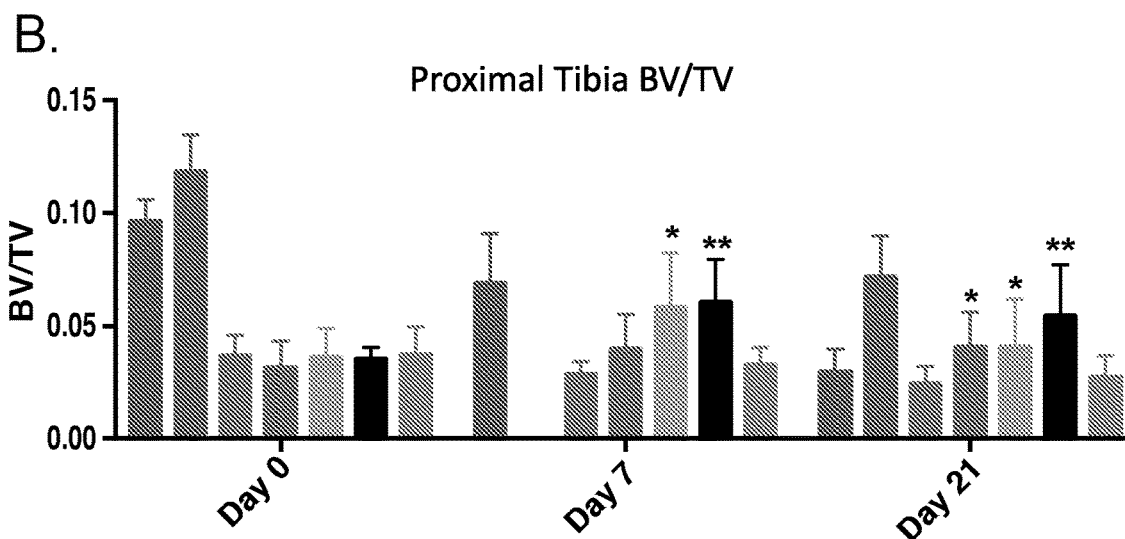
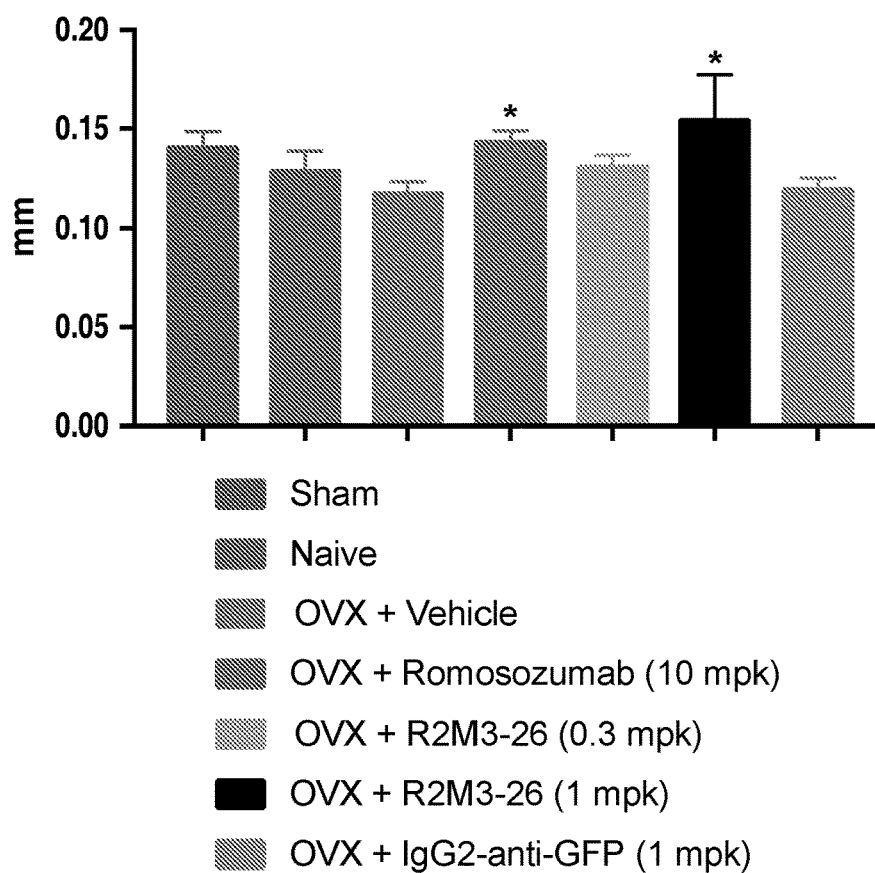


FIG. 27B

C. Day 21 Proximal Tibia Cortical Thickness



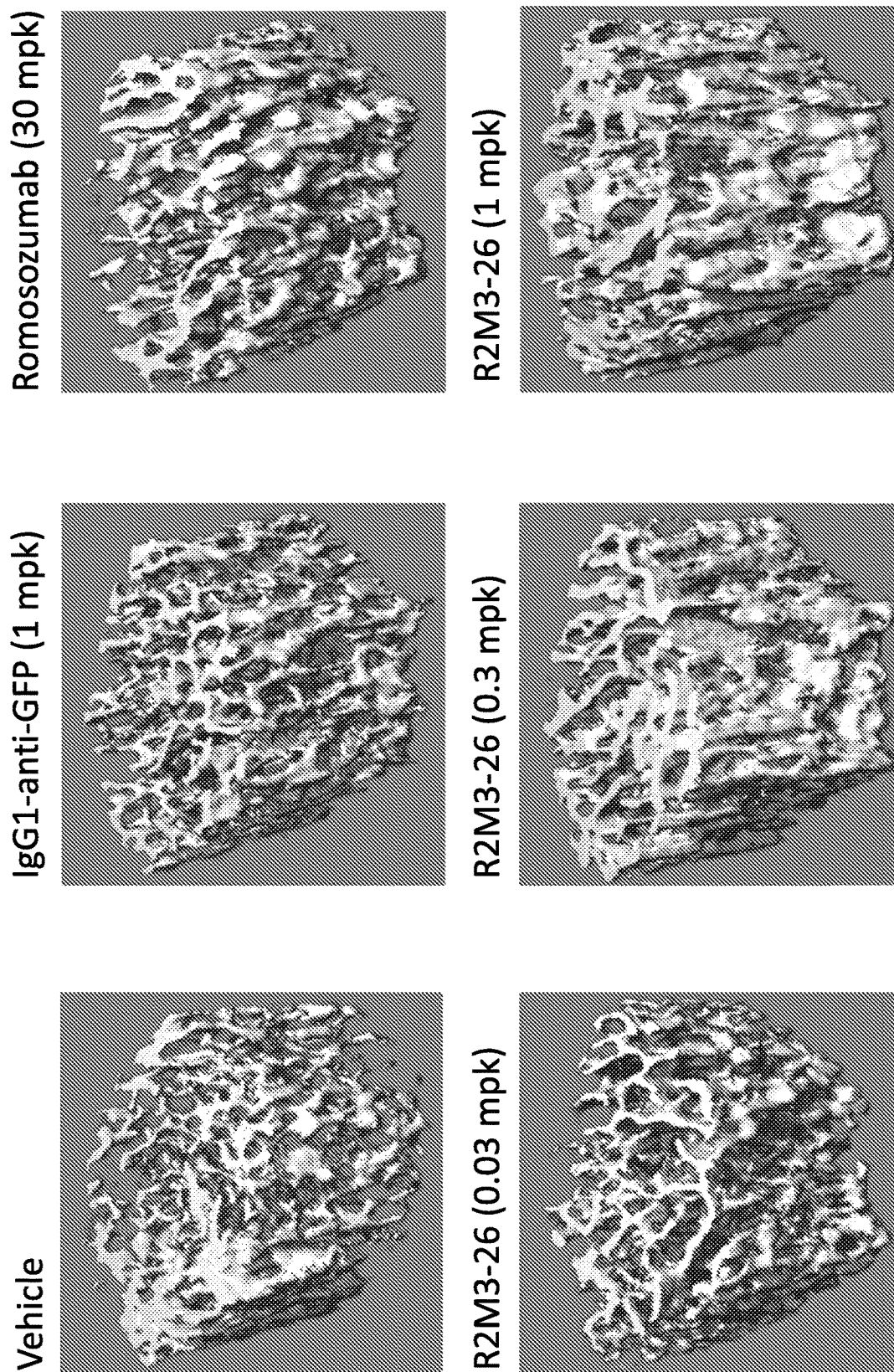
*P value <0.05

** P value <0.0001 Compared to OVX + Veh

FIG. 27C

A.

FIG. 28A



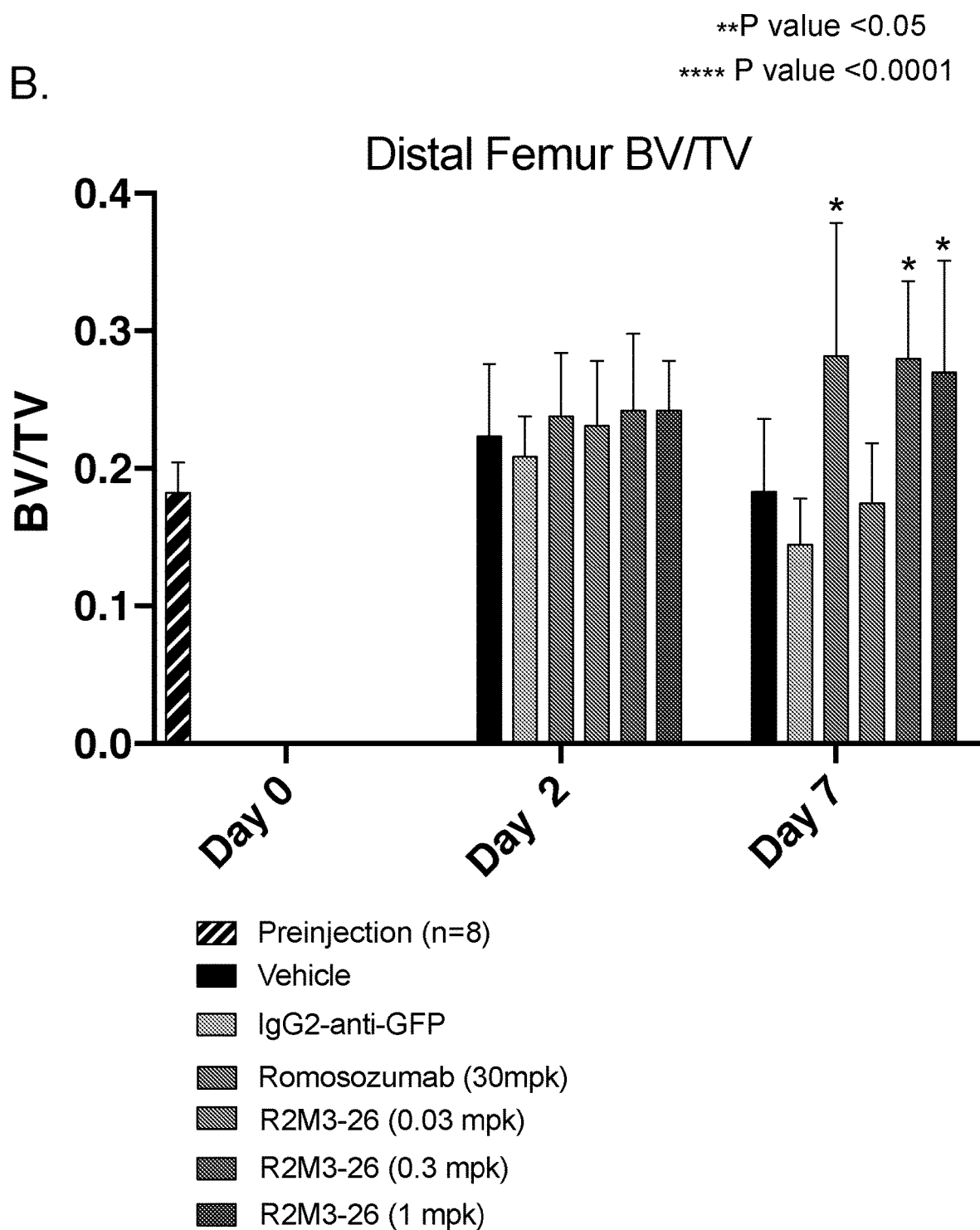


FIG. 28B

C.

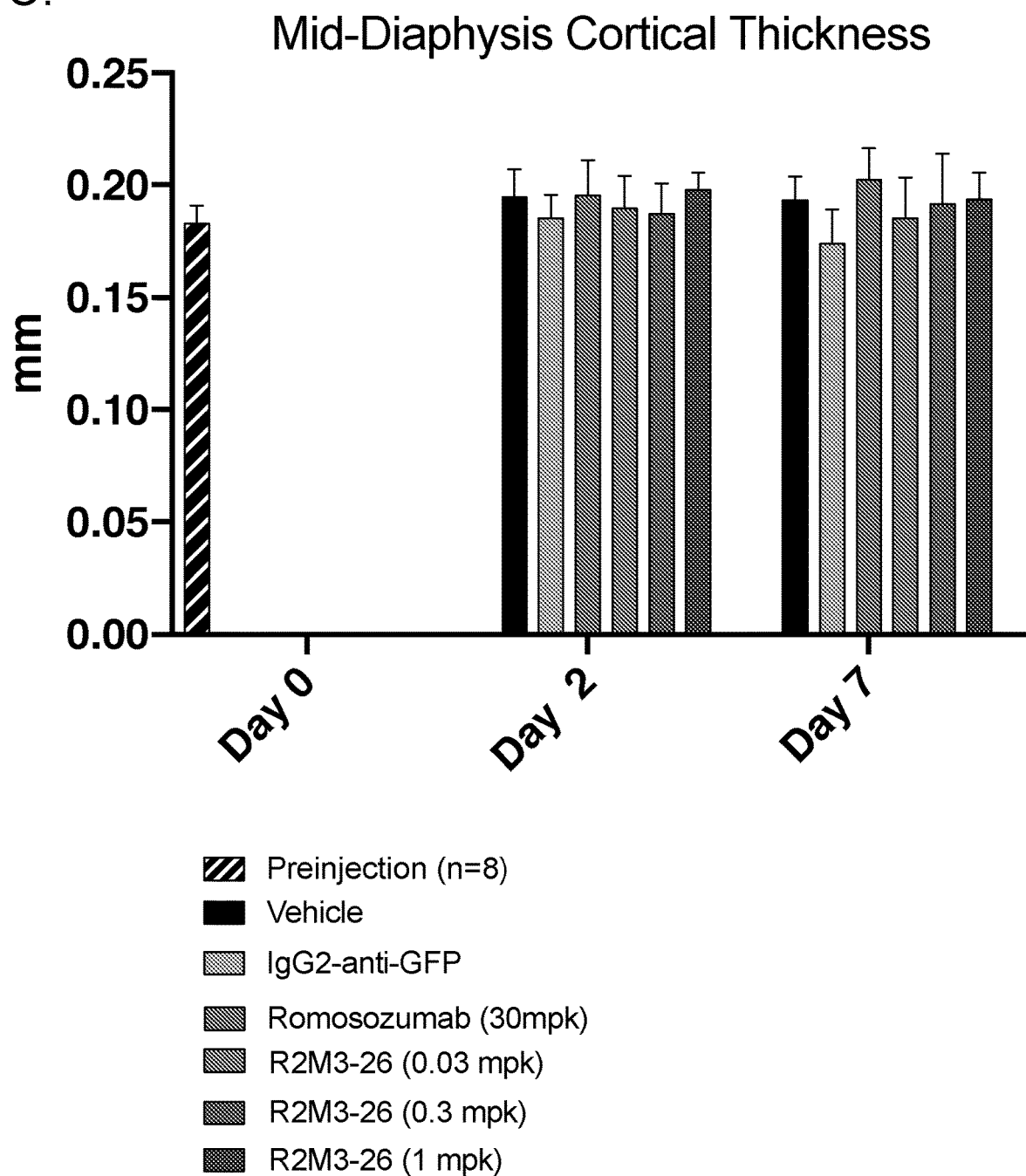


FIG. 28C

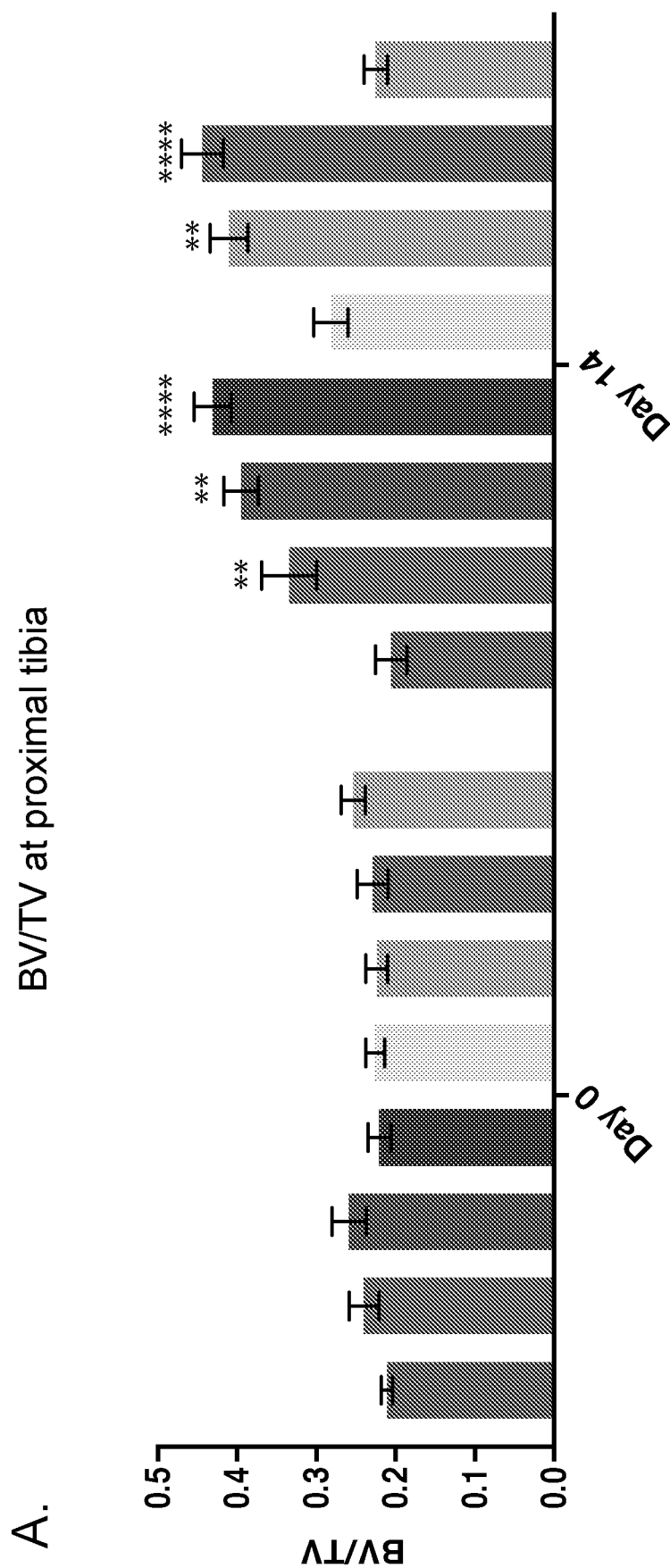


FIG. 29A

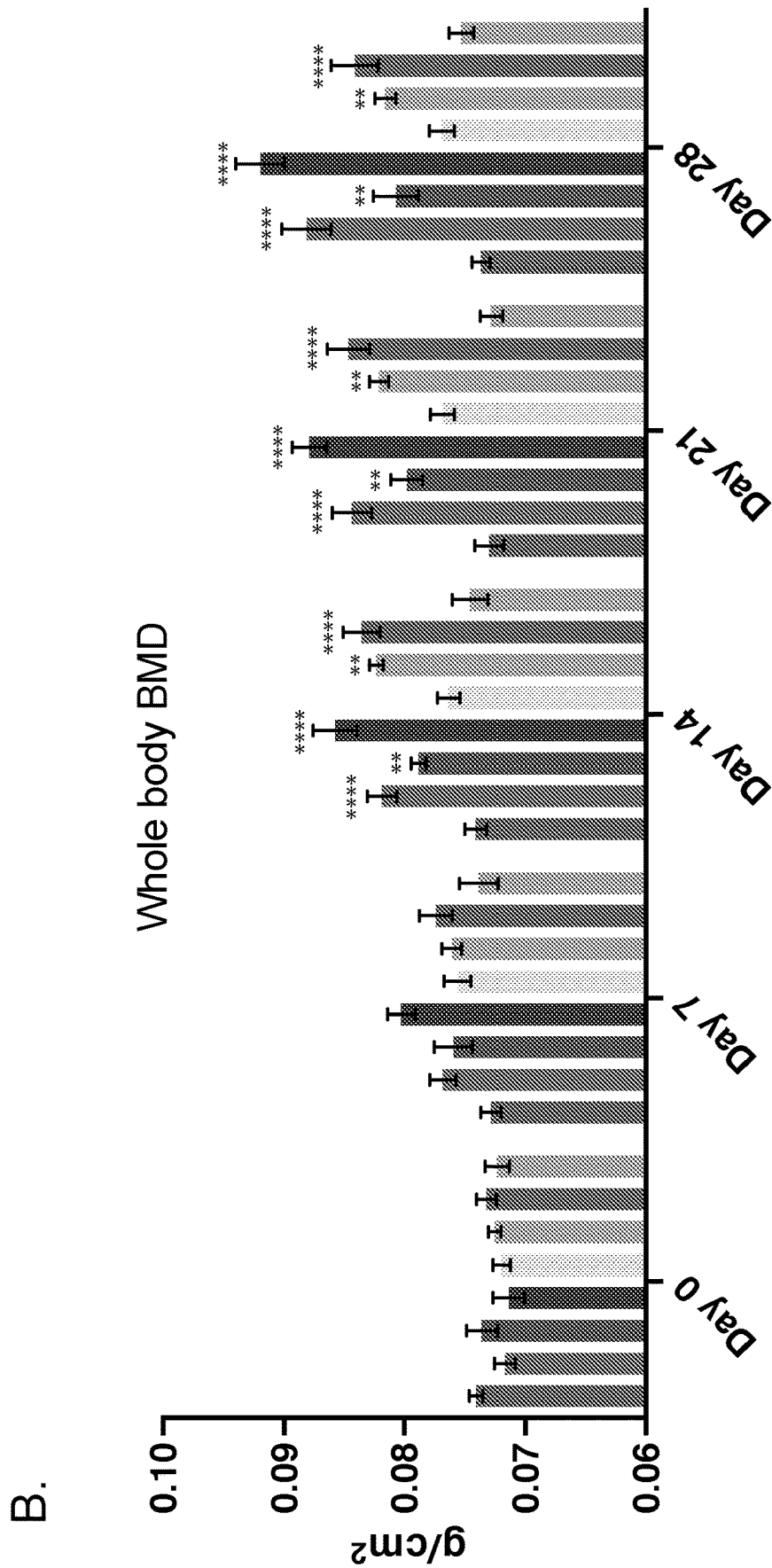


FIG. 29B

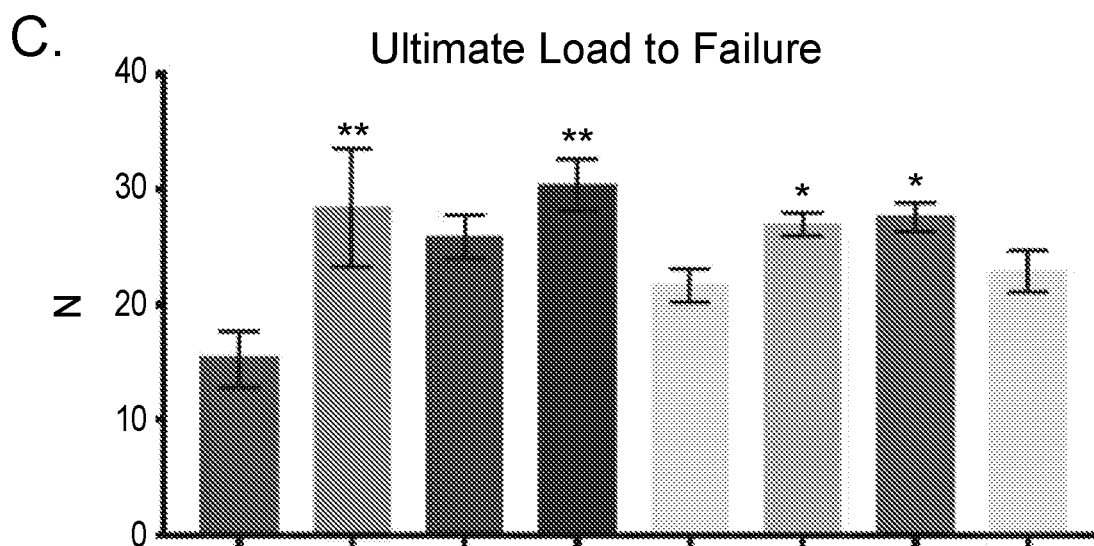


FIG. 29C

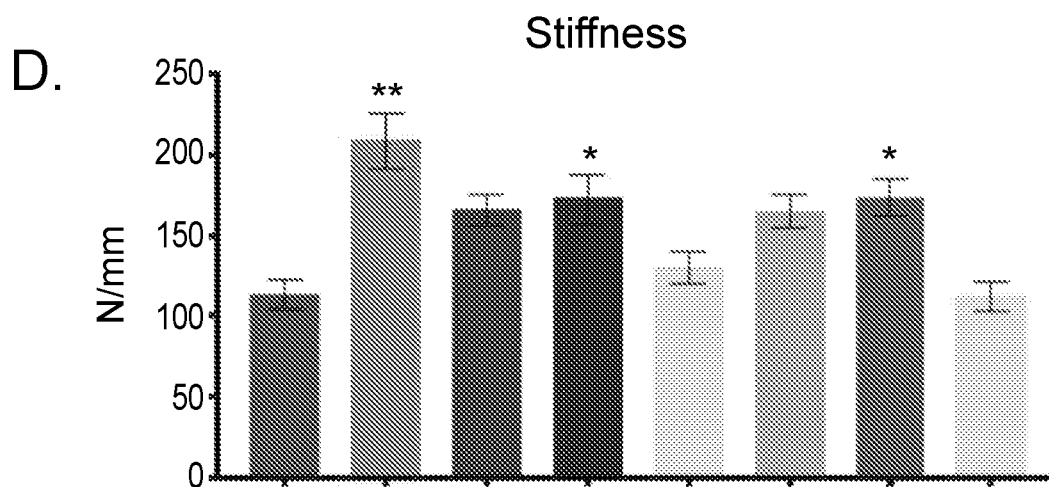
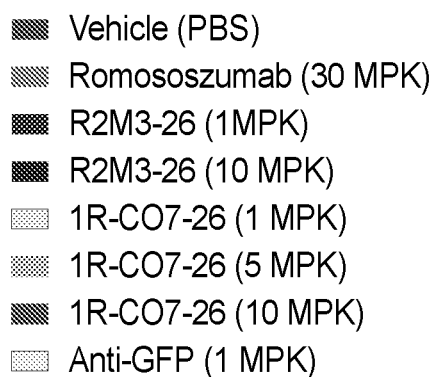


FIG. 29D

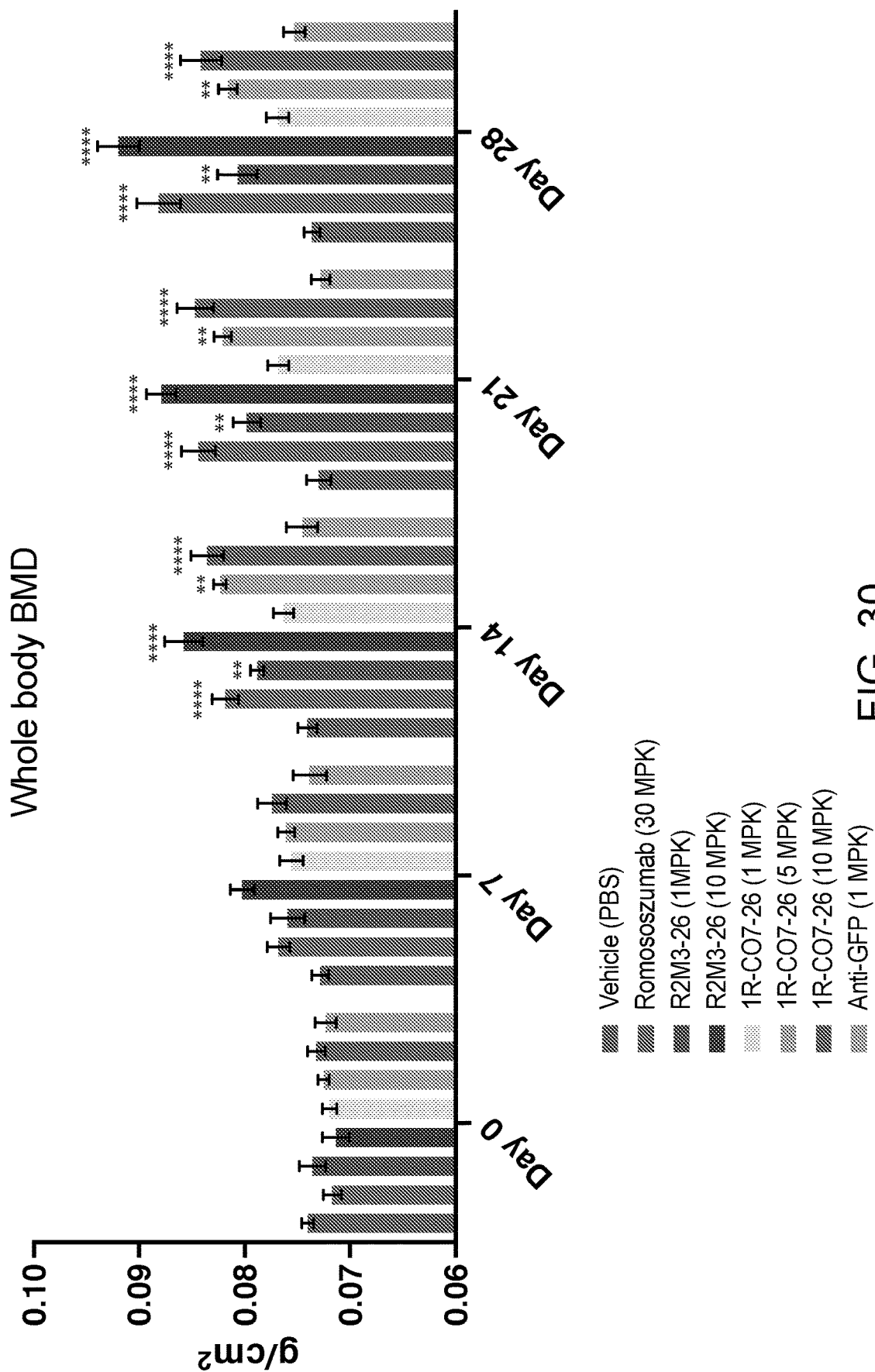


FIG. 30

FIG. 31

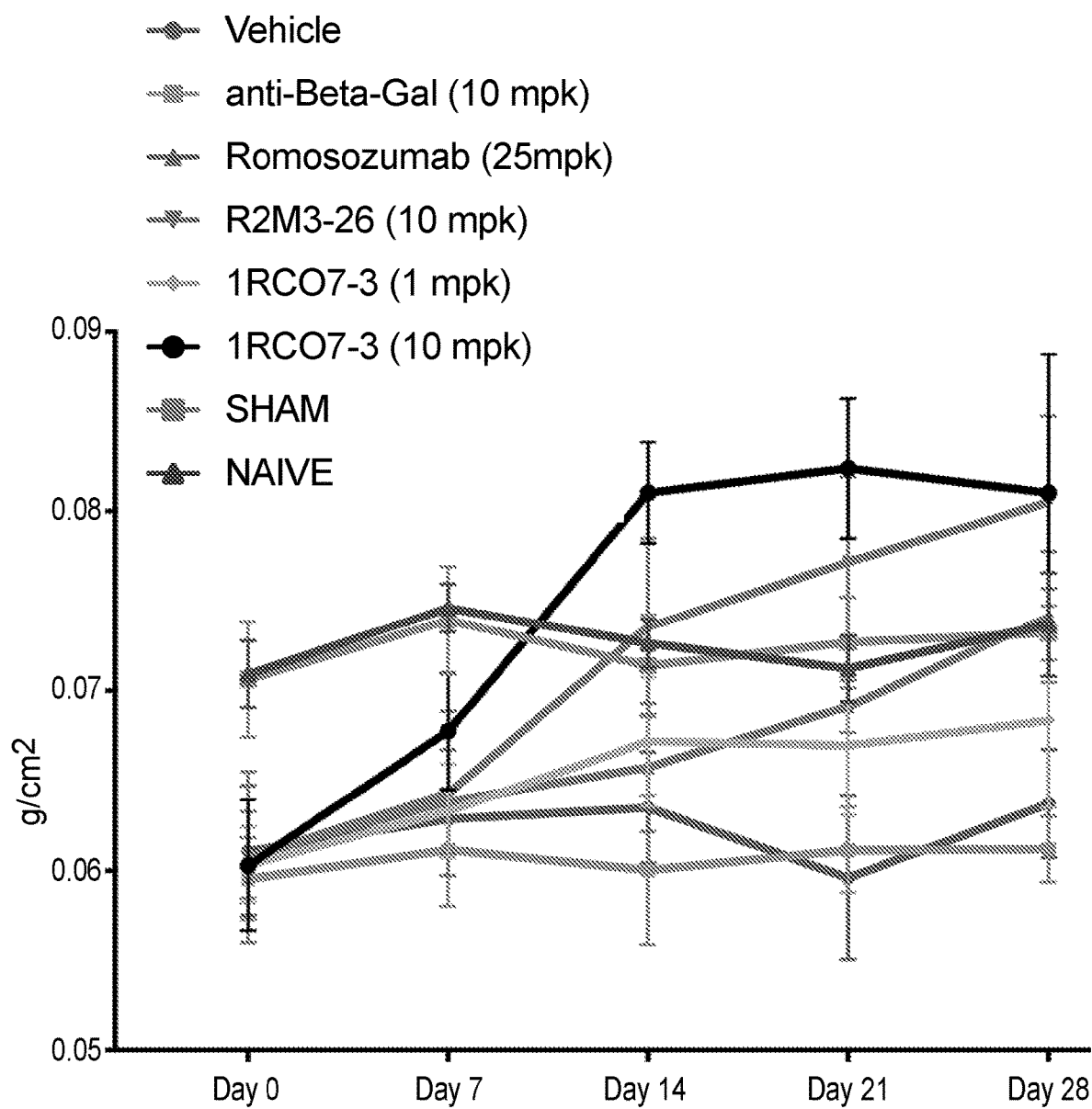


FIG. 32

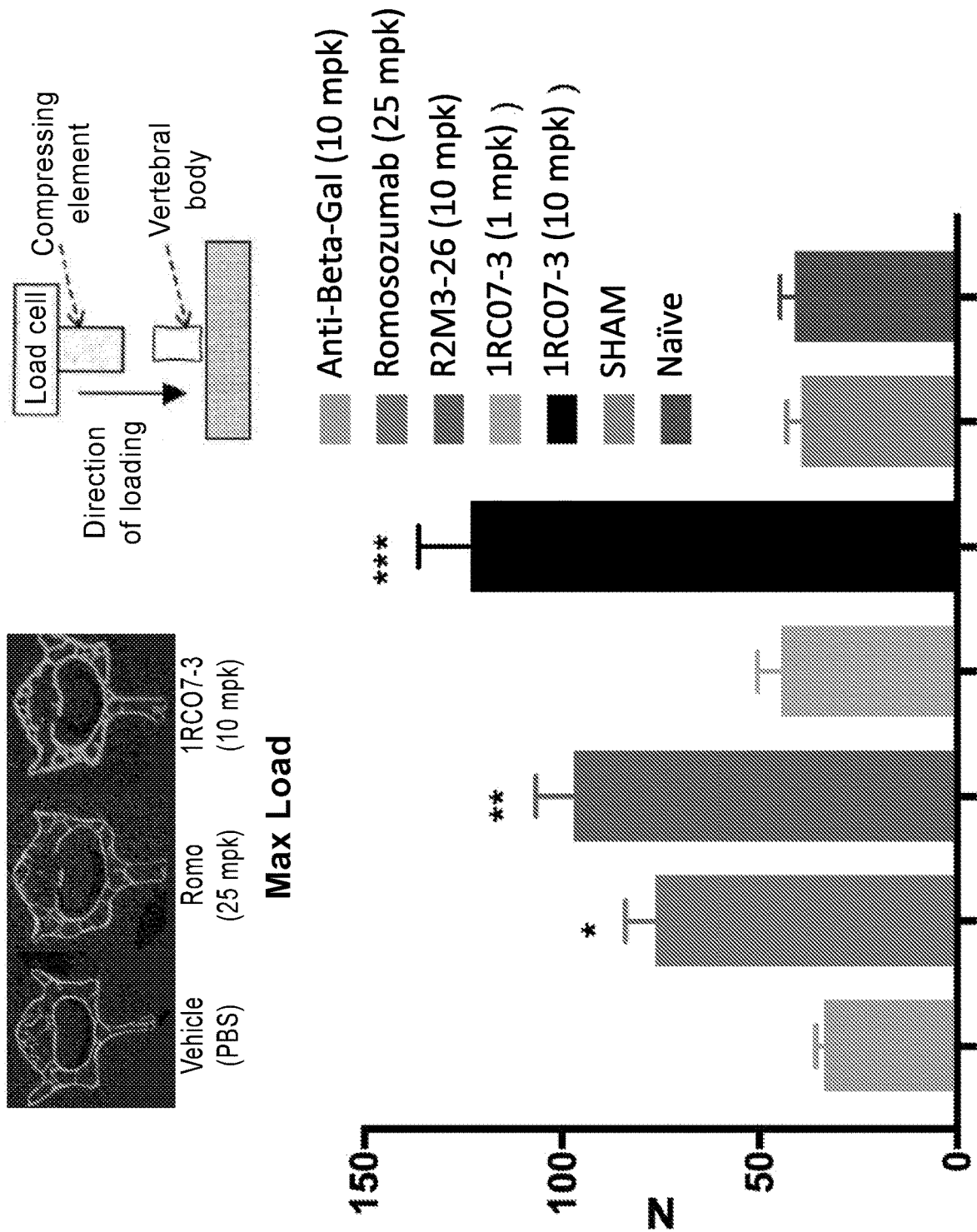


FIG. 33A 1 week of treatment

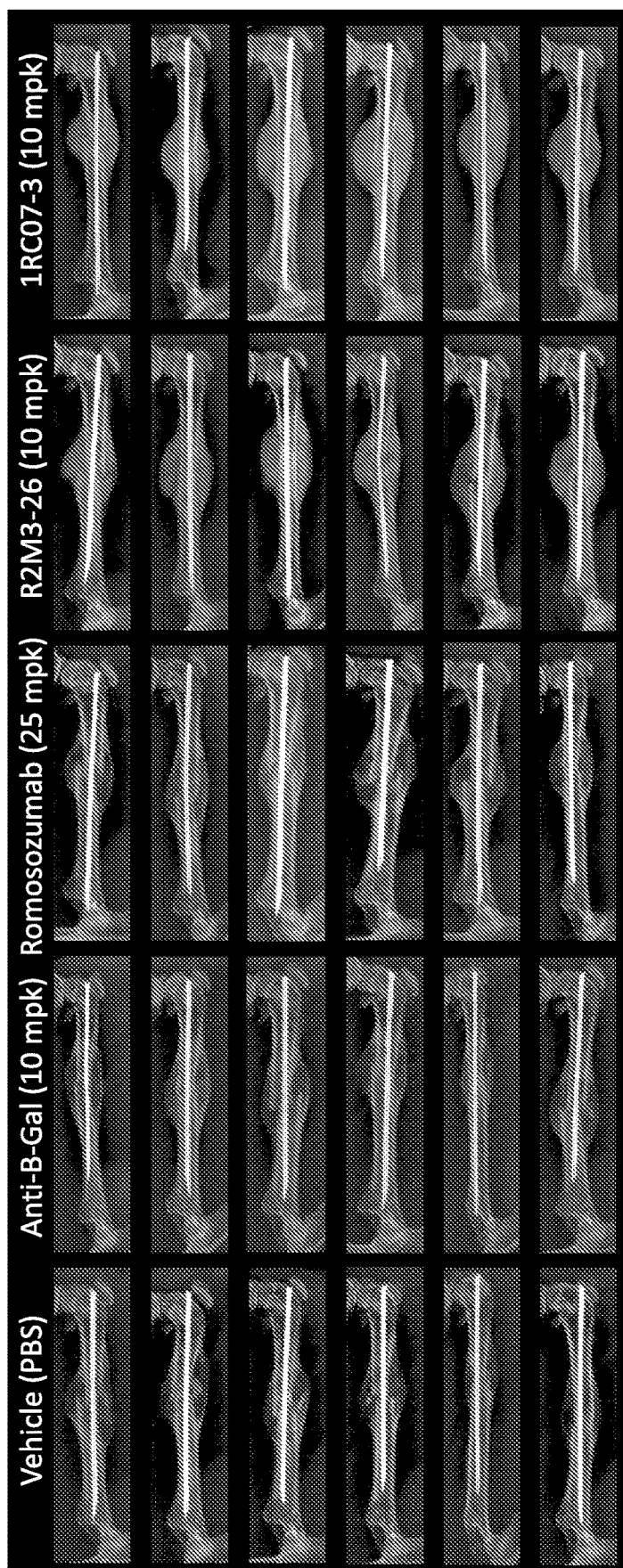


FIG. 33B 6 week of treatment

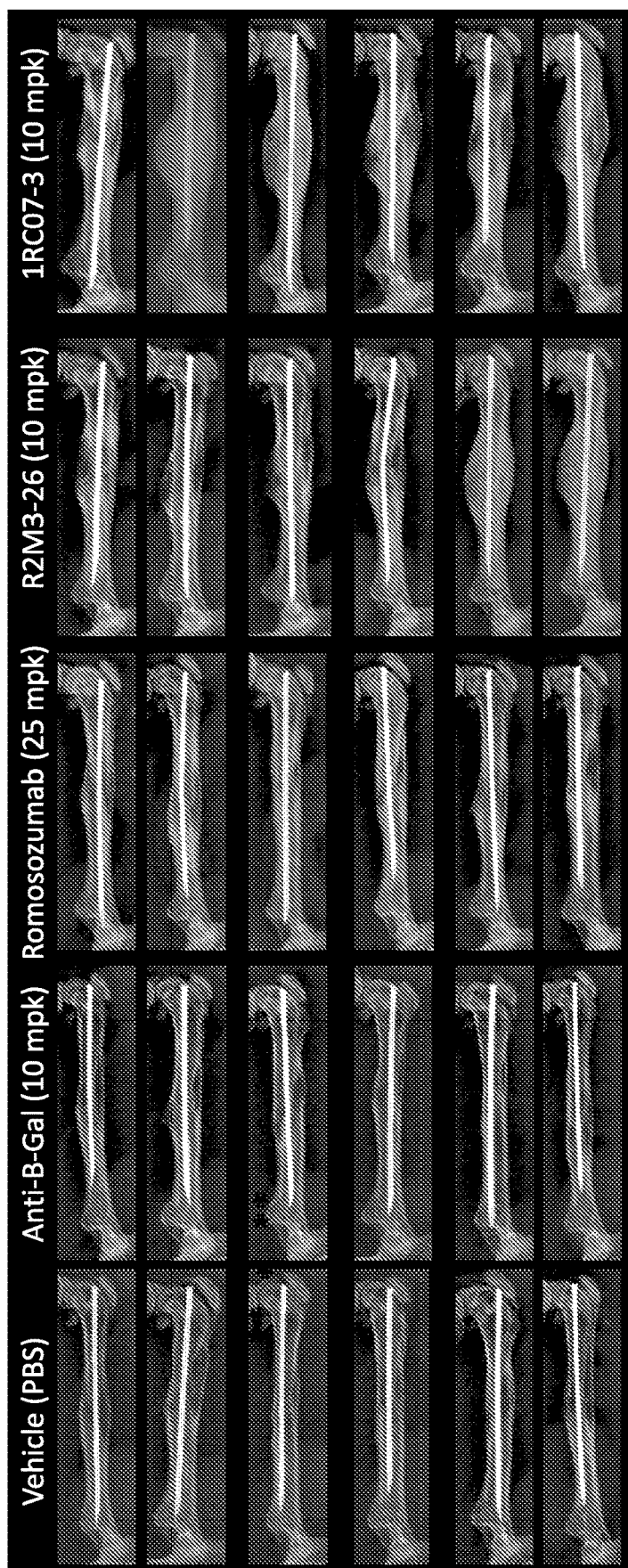


FIG. 33C

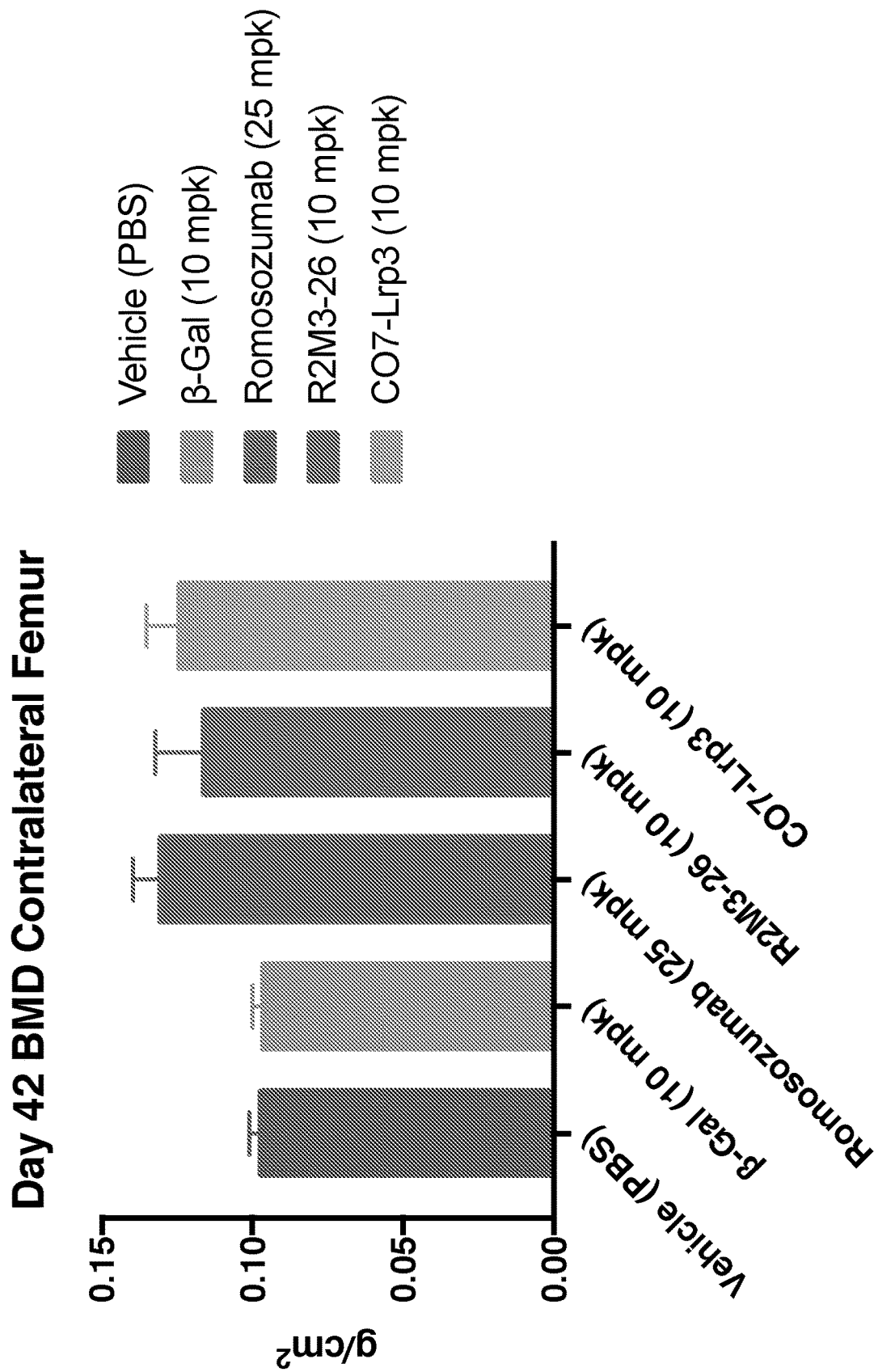
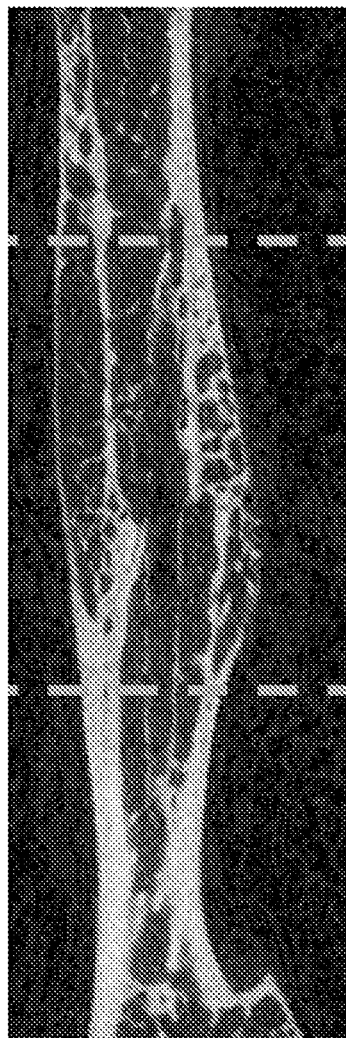
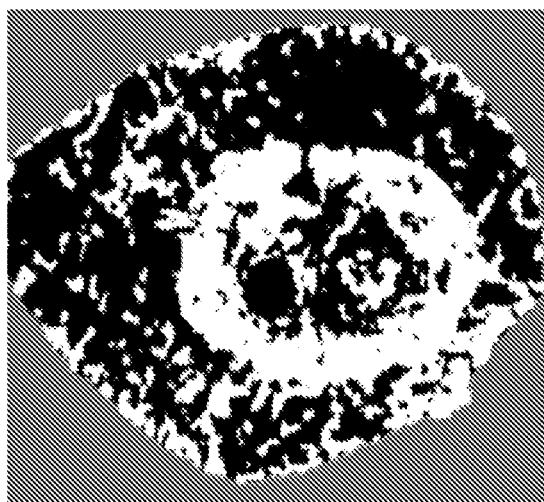
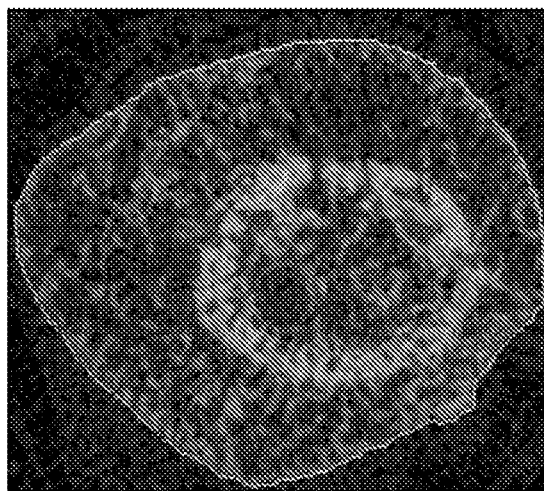


FIG. 33D

400 SLICES, 4.2 MM



Bone area



Tissue area

FIG. 33D (contd.)

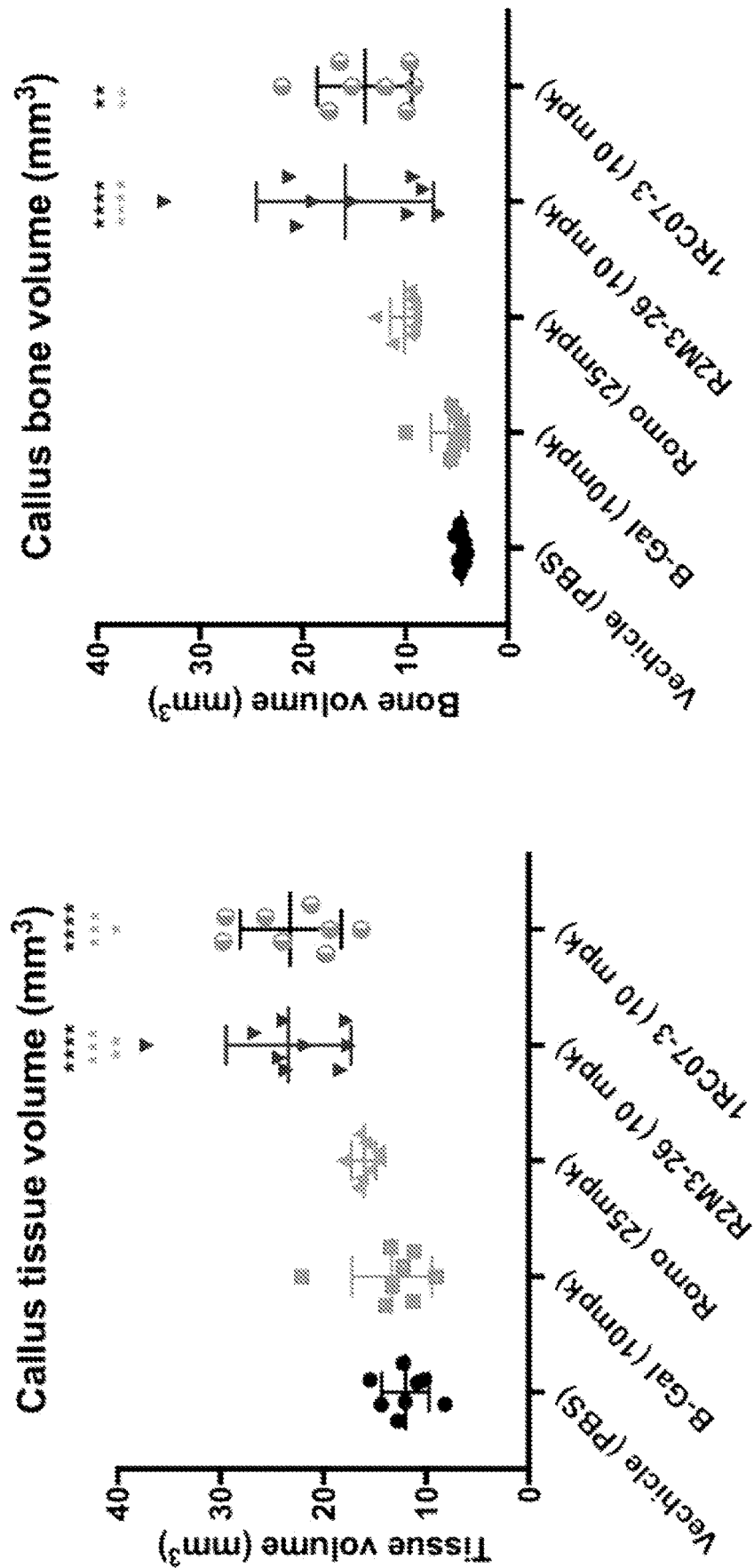


FIG. 33D (contd.)

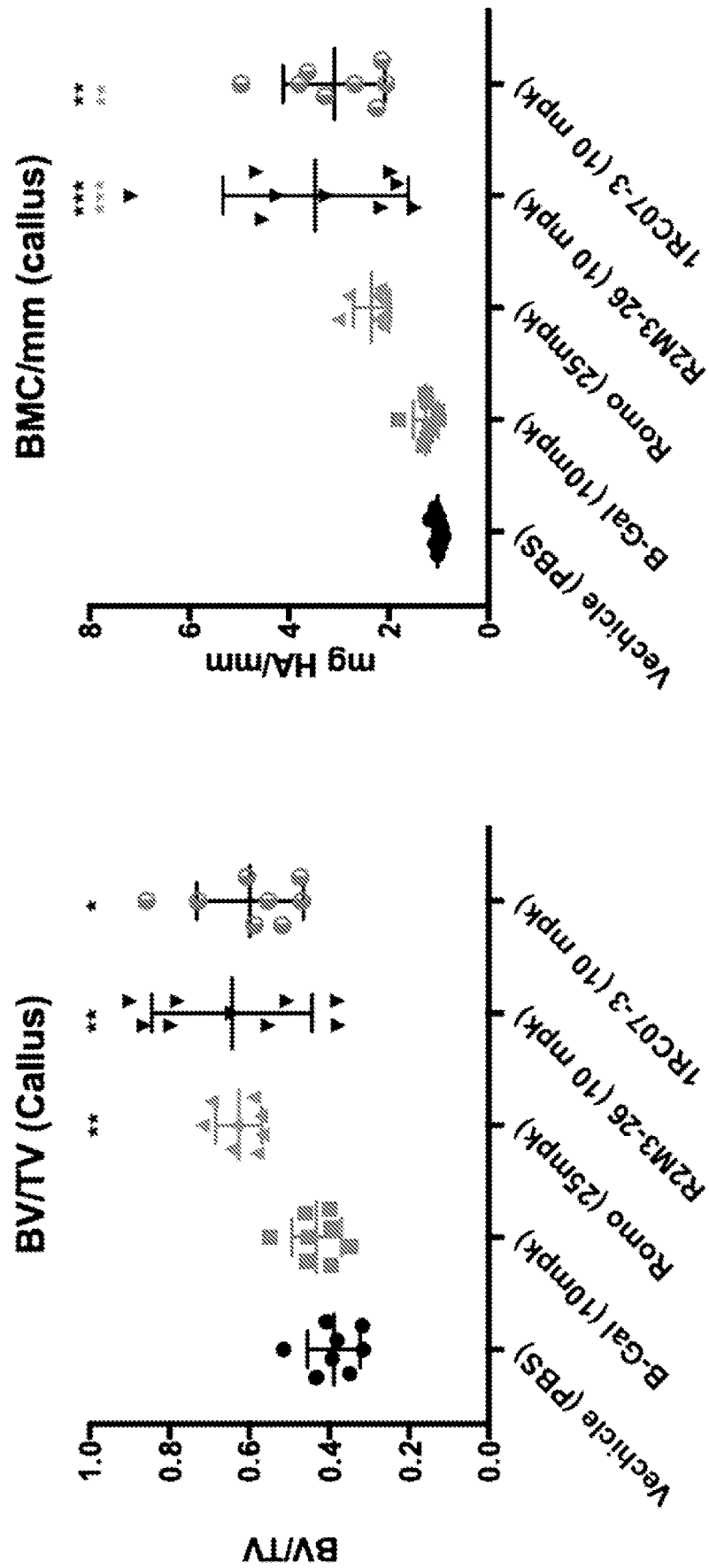


FIG. 33D (contd.)

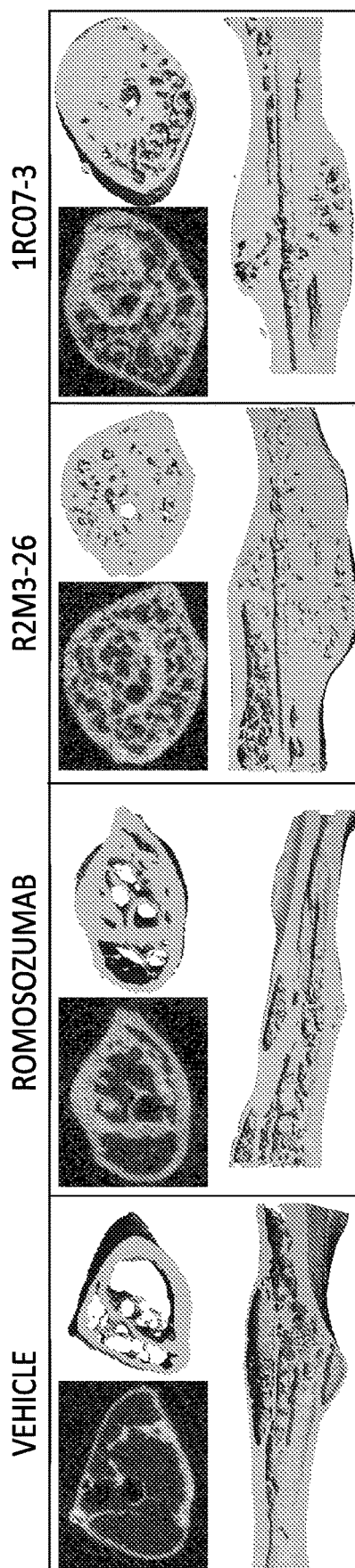


FIG. 34

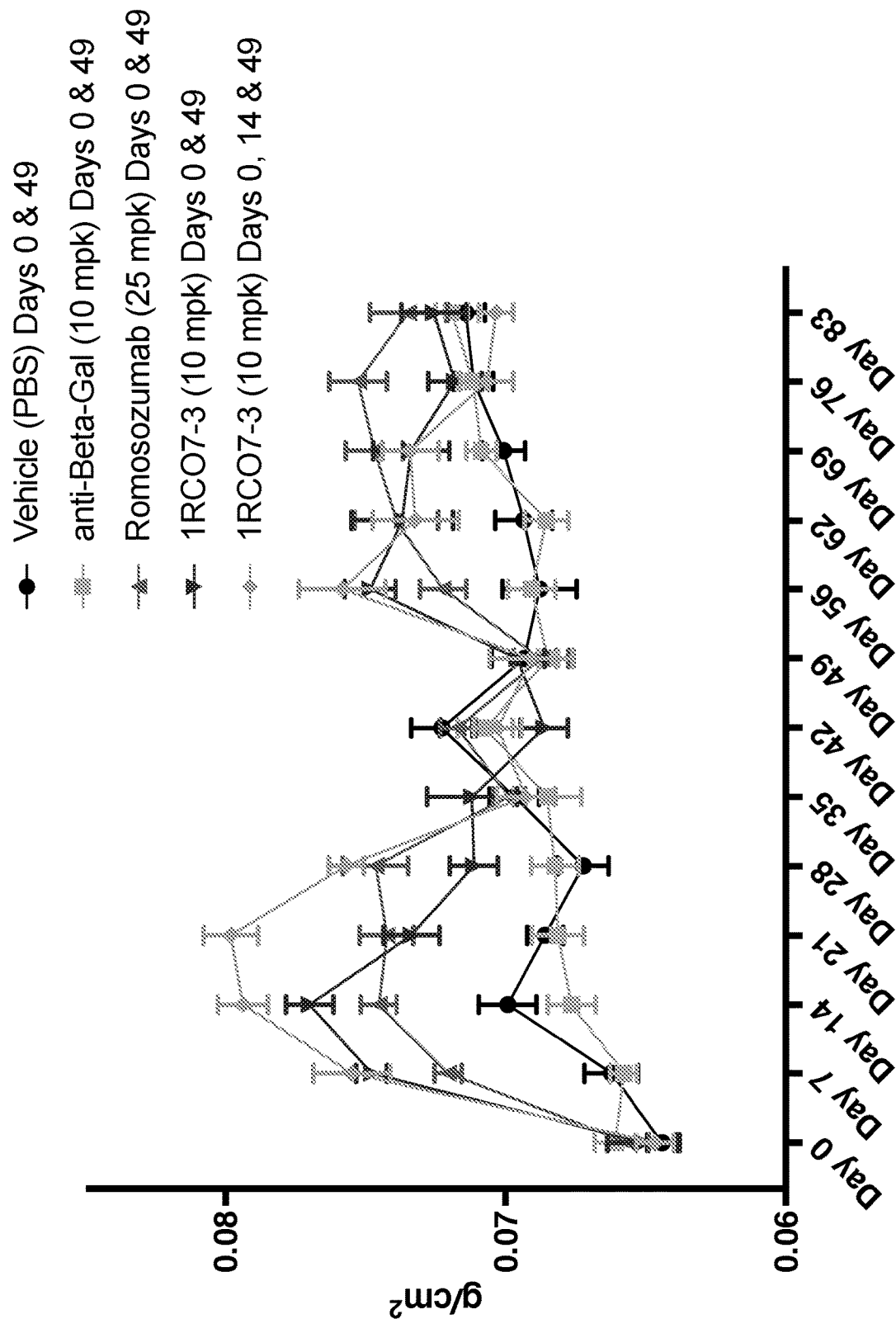


FIG. 35

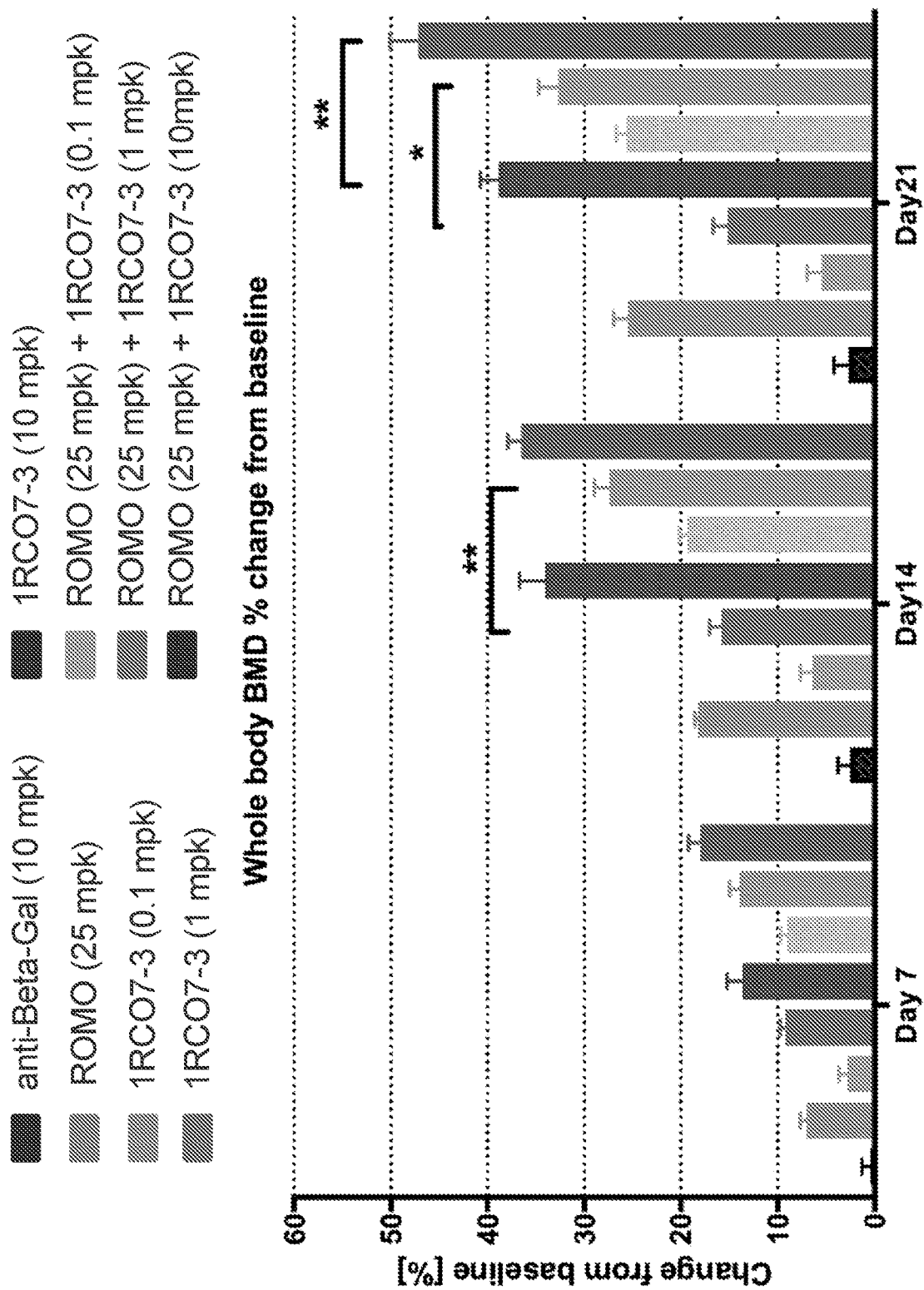
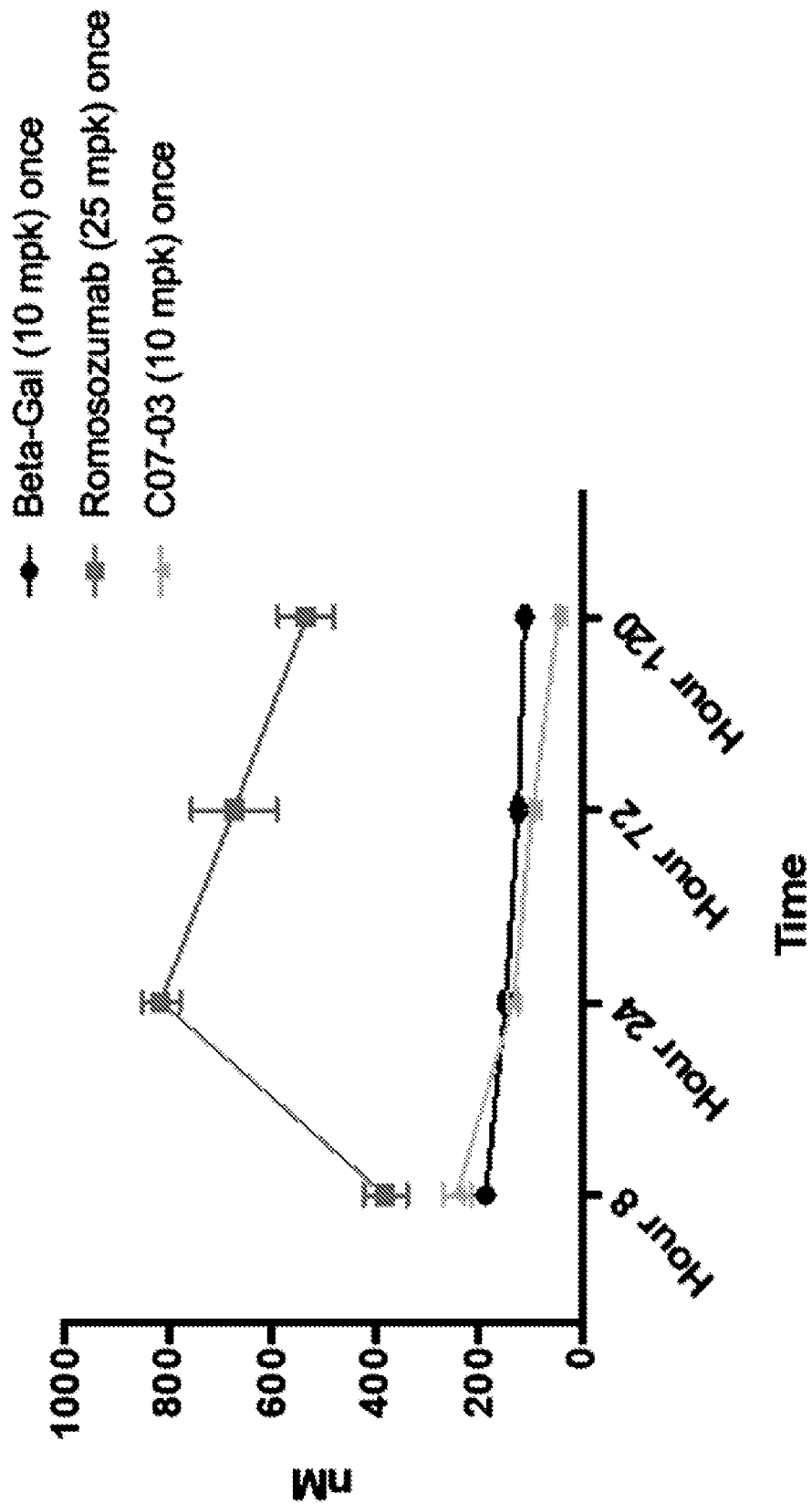


FIG. 36
Protein Serum Exposure



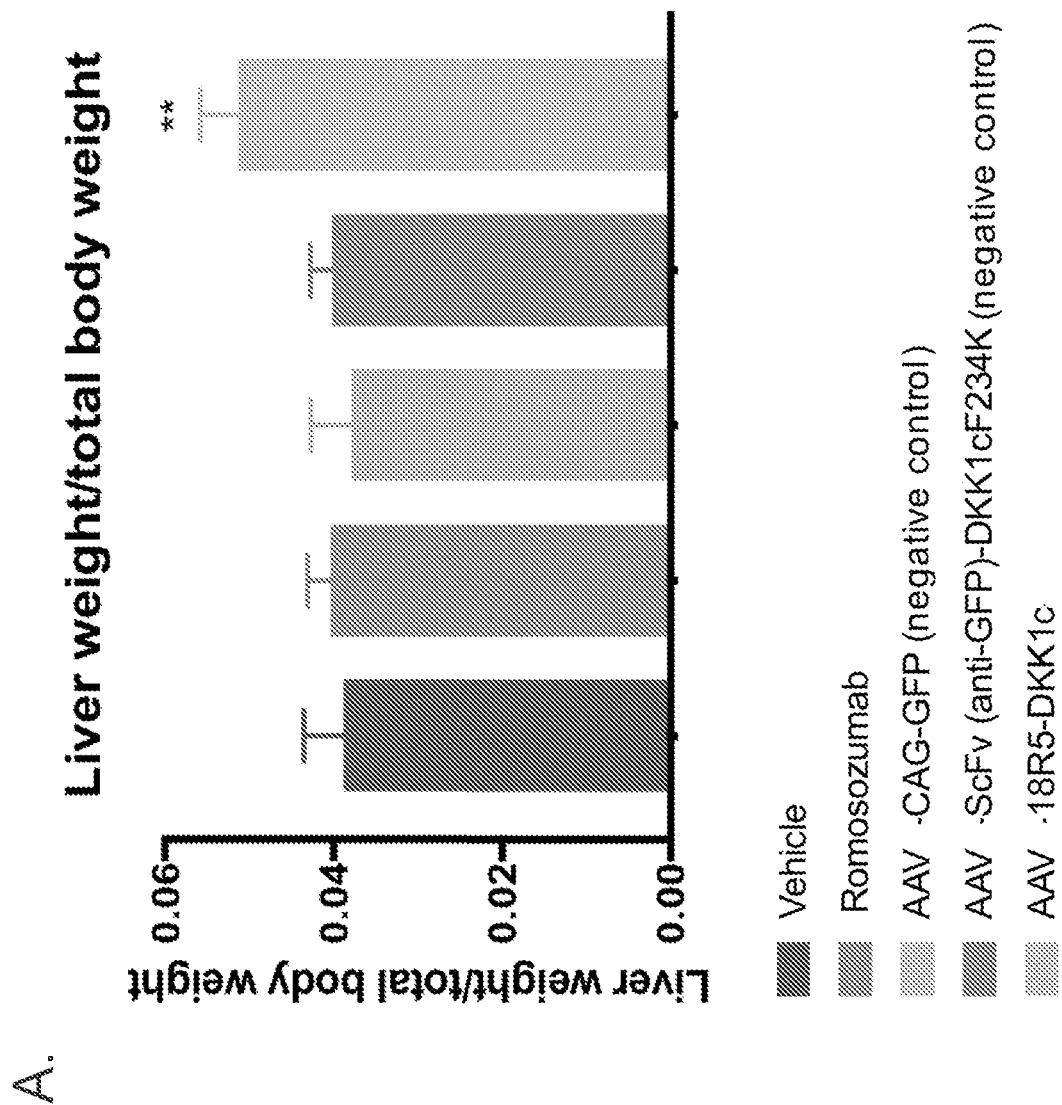


FIG. 37

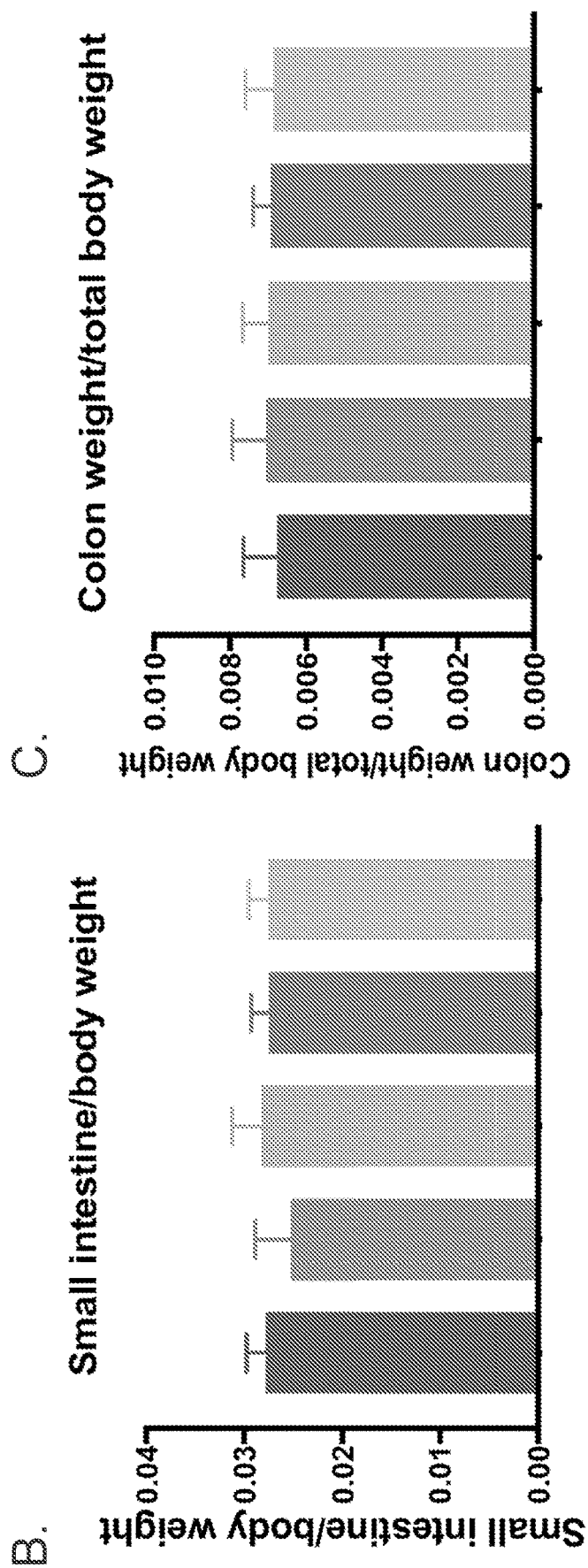
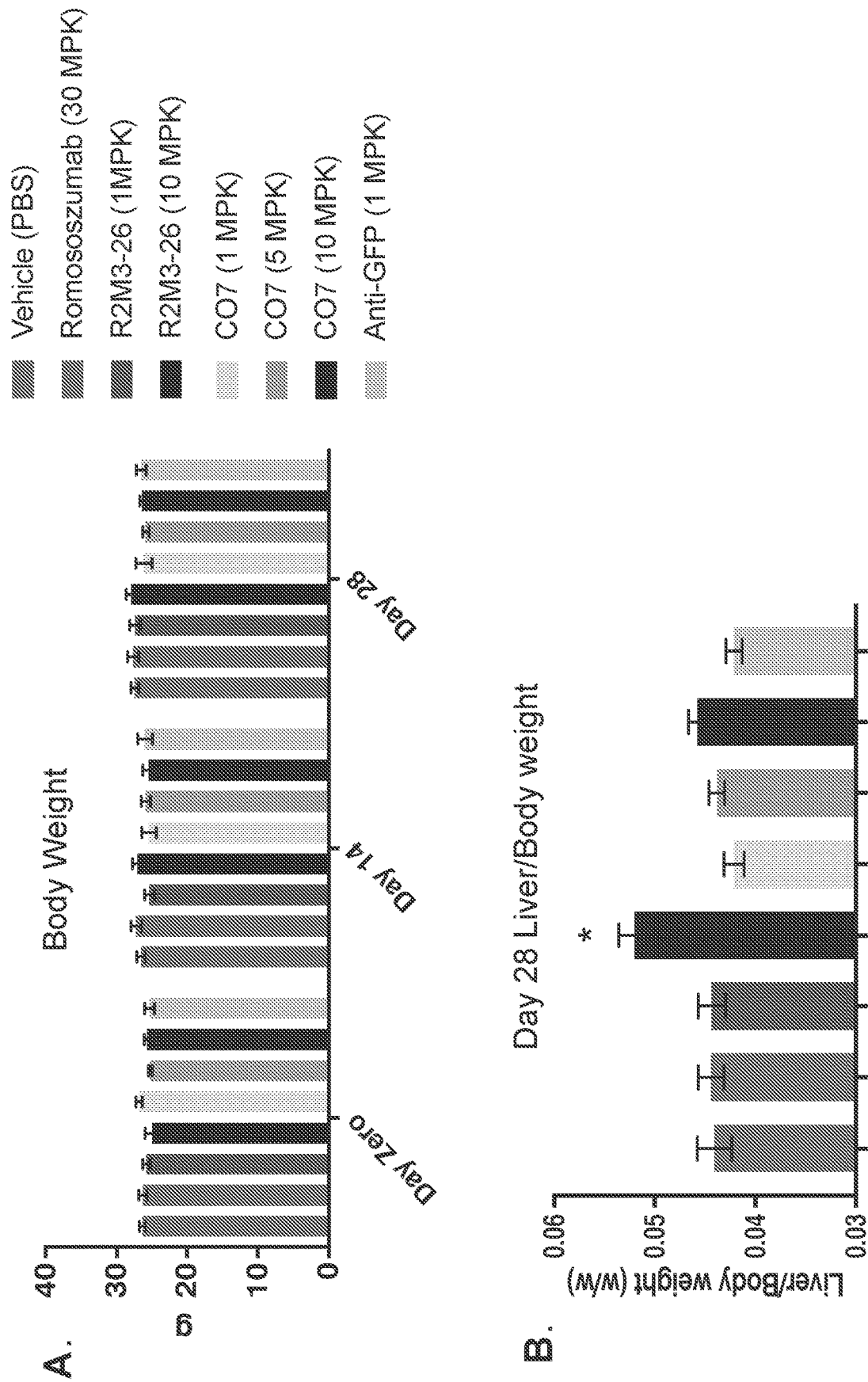
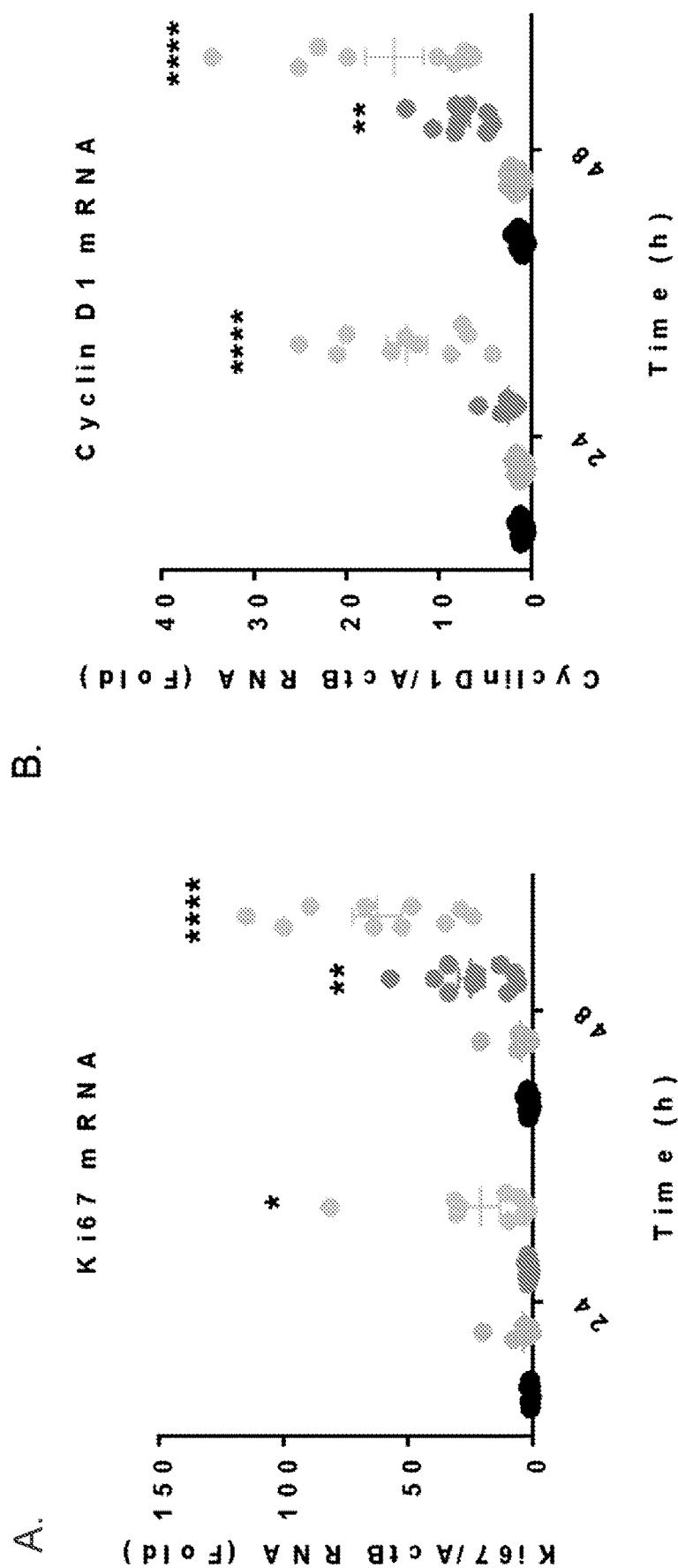


FIG. 37 (contd.)

FIG. 38





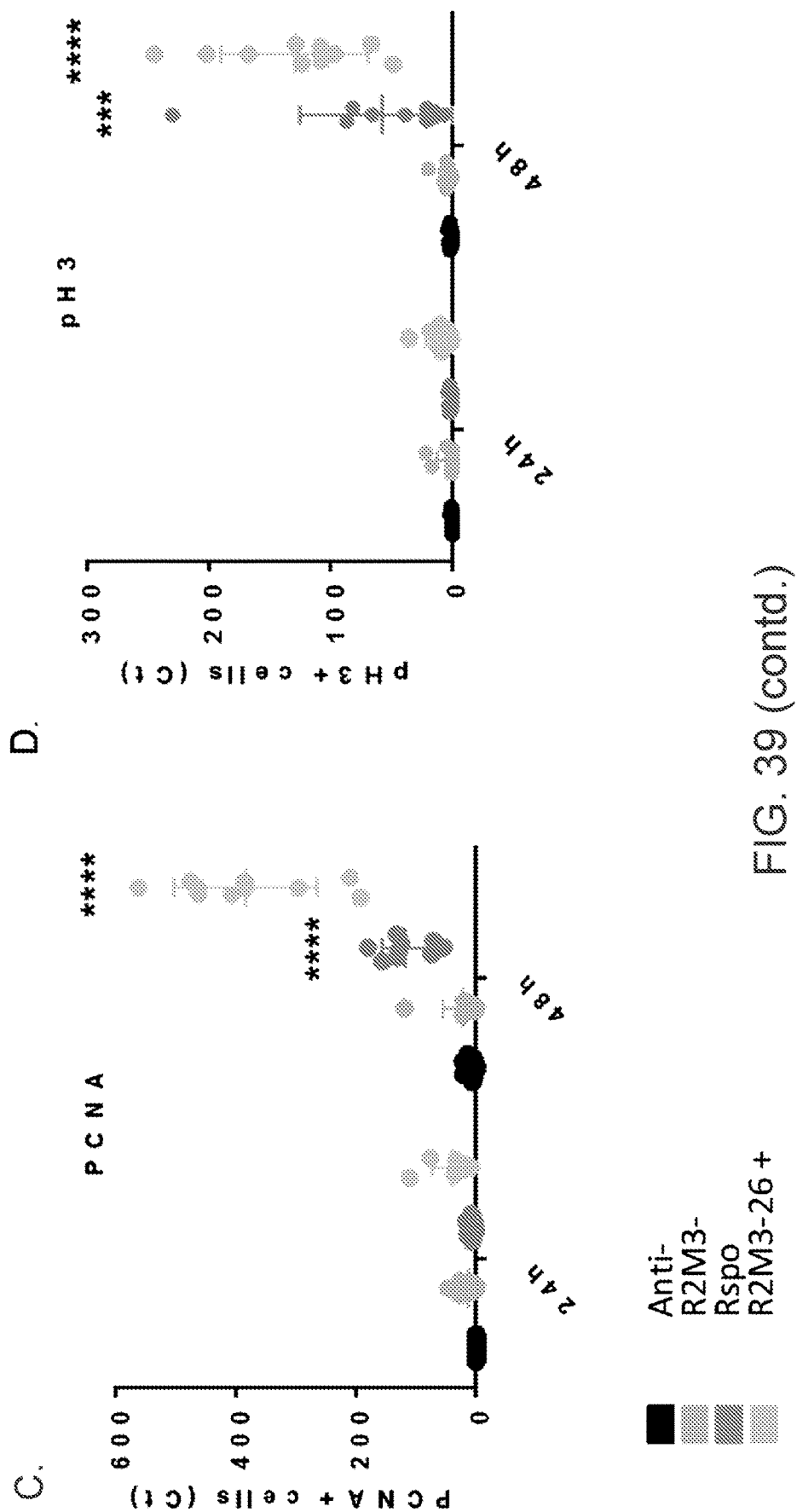
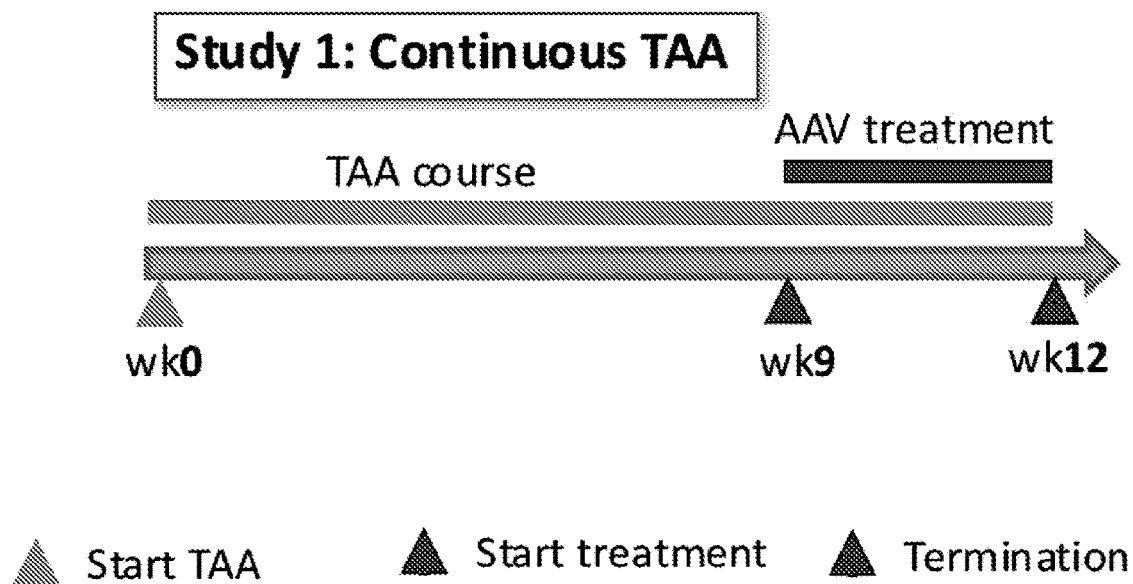


FIG. 39 (contd.)

FIG. 40A

A.



B.

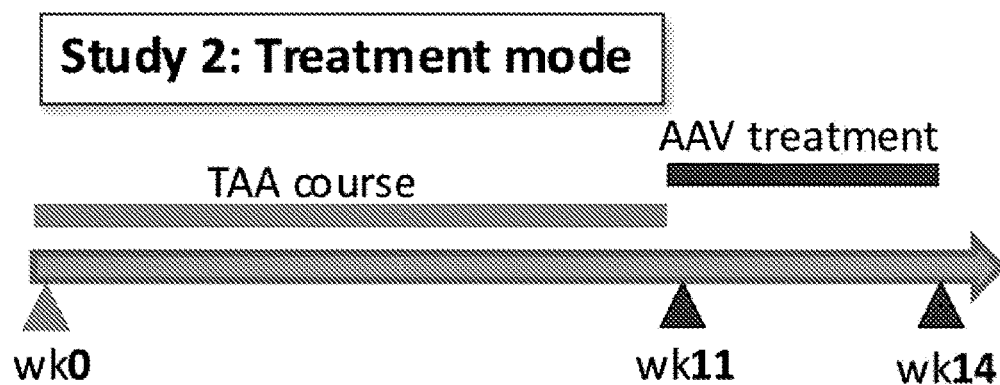


FIG. 40B

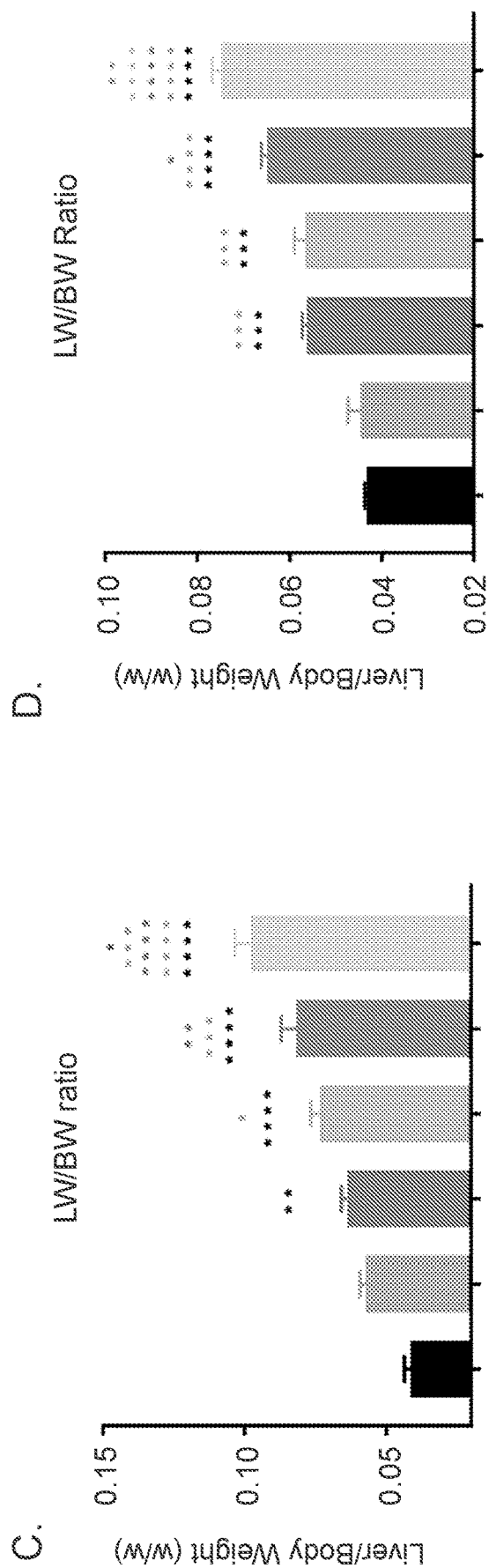


FIG. 40 (contd.)

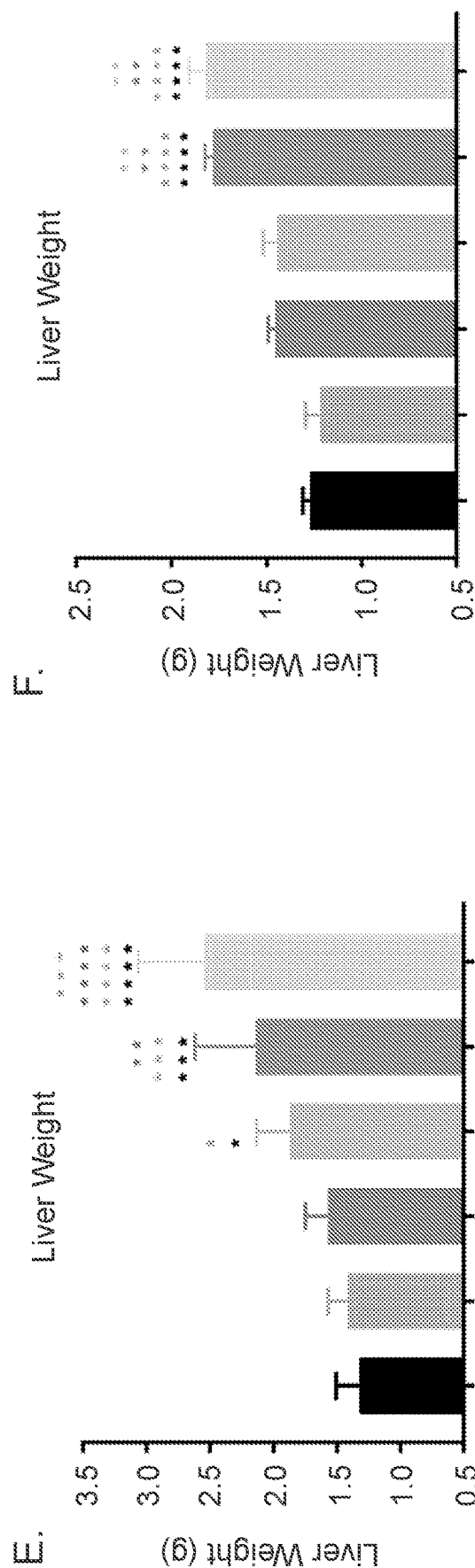
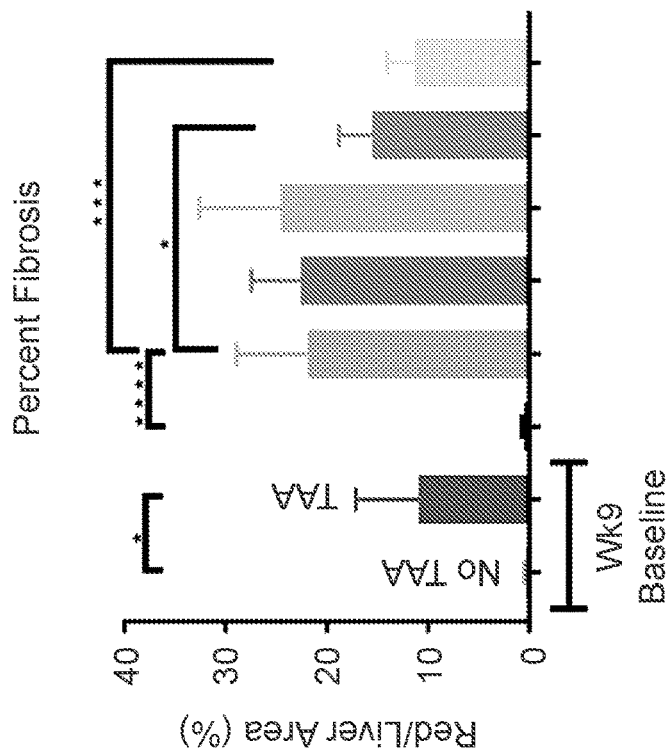
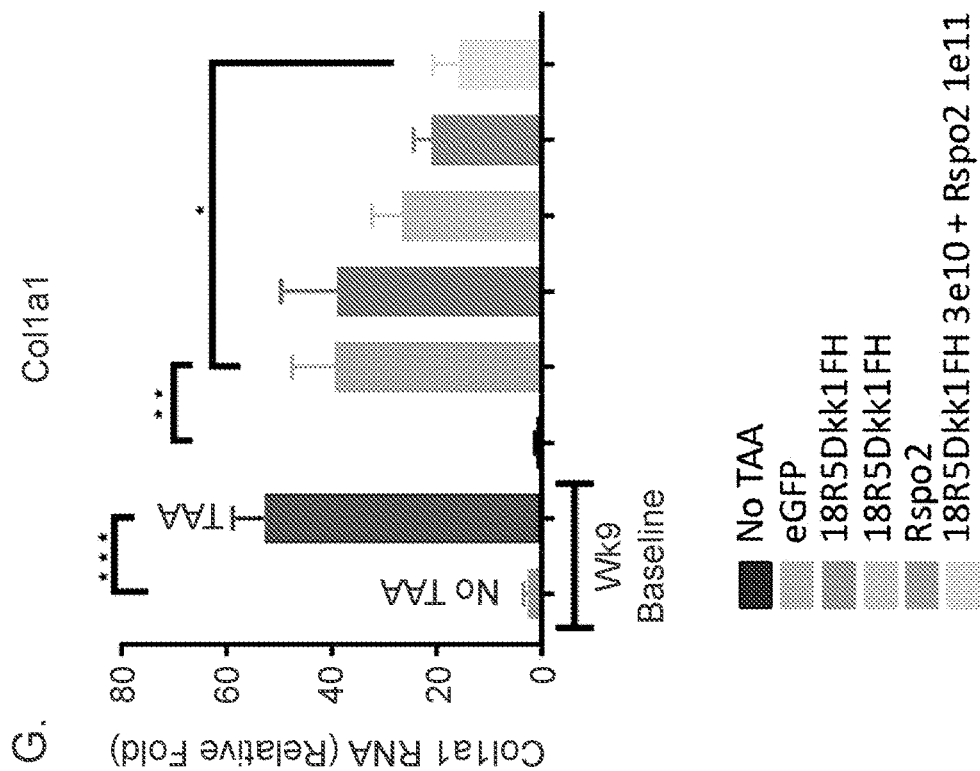


FIG. 40 (contd.)



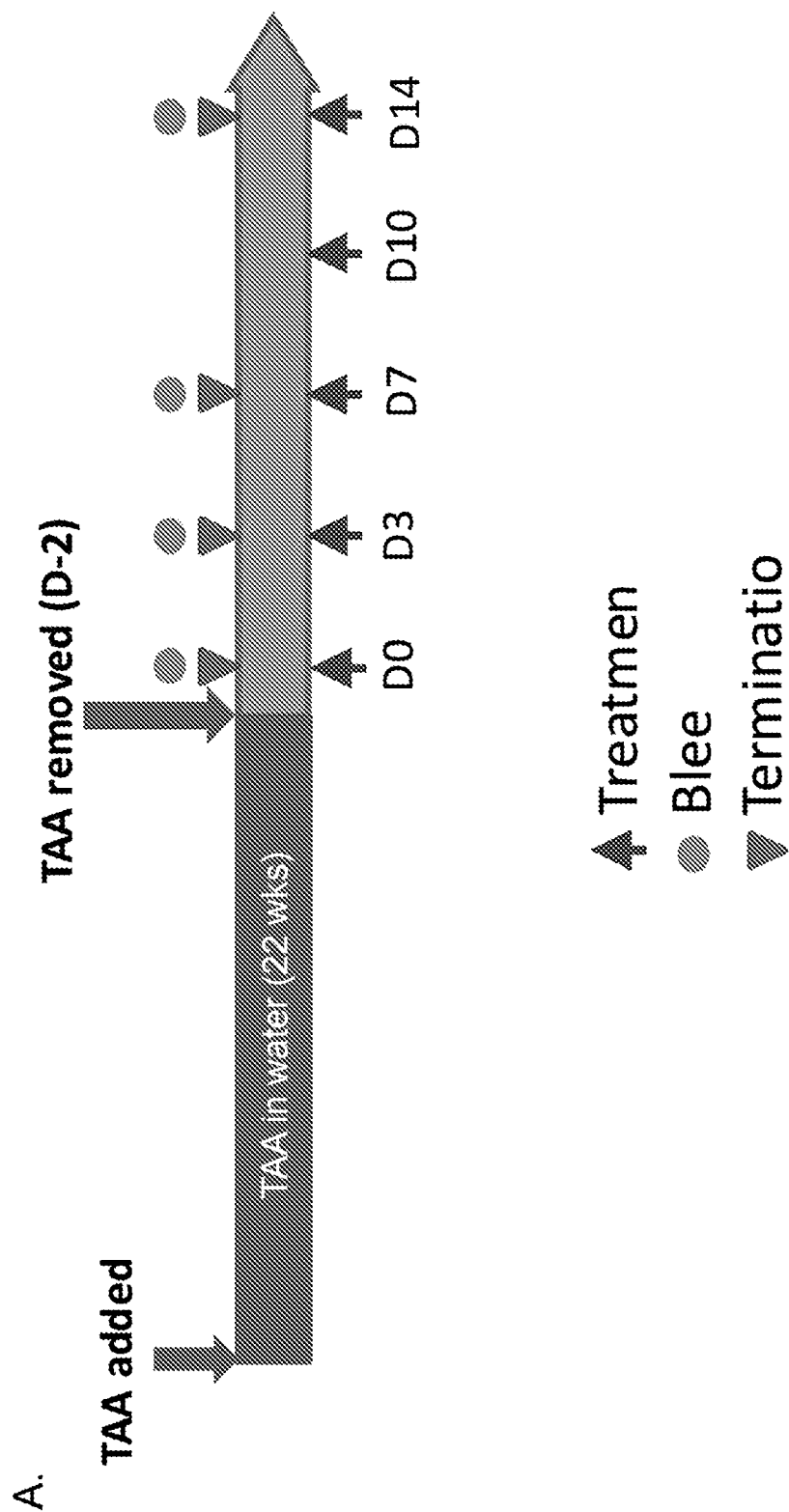
H.



G.

FIG. 40 (contd.)

FIG. 41A



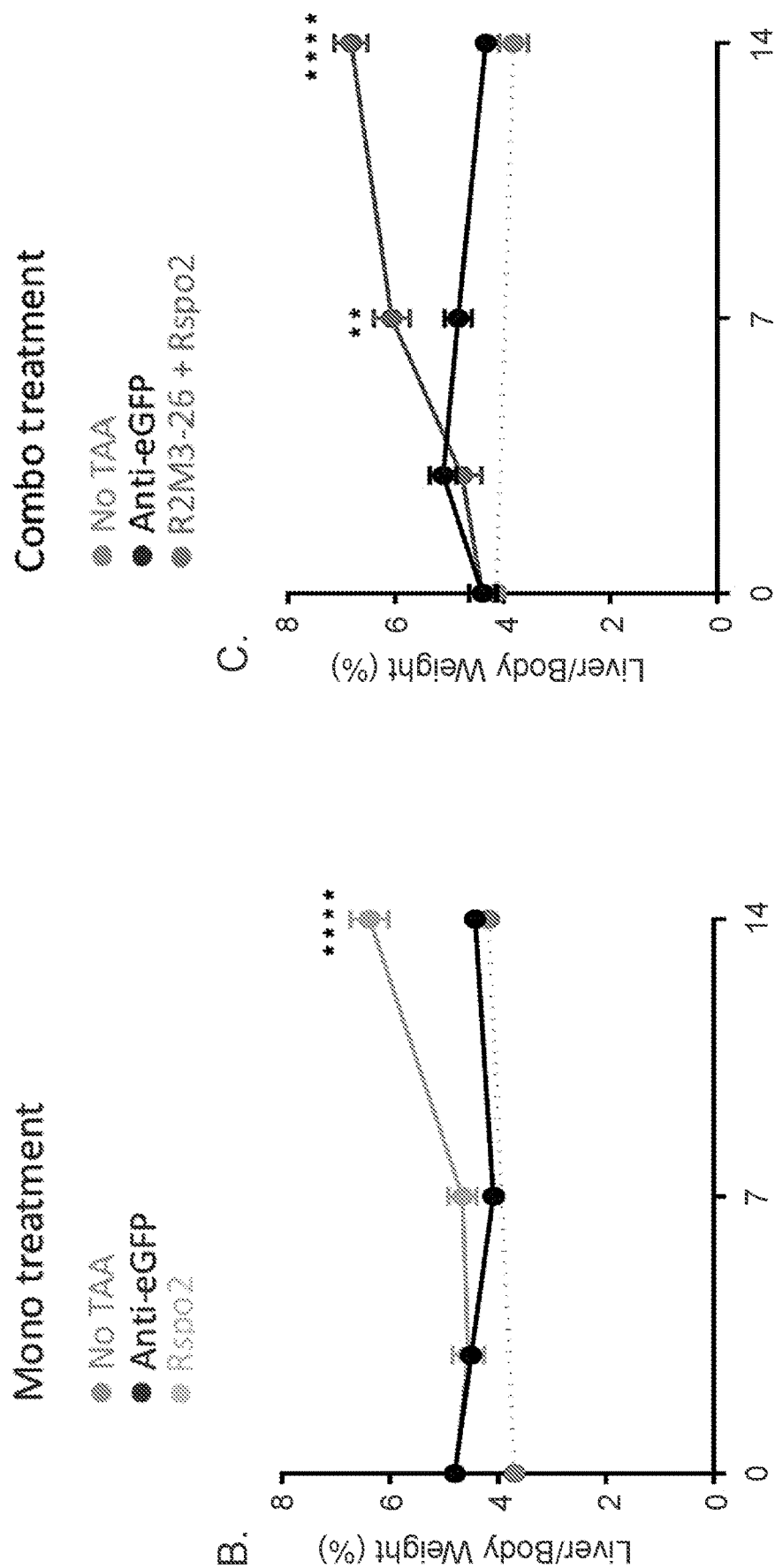


FIG. 41 (contd.)

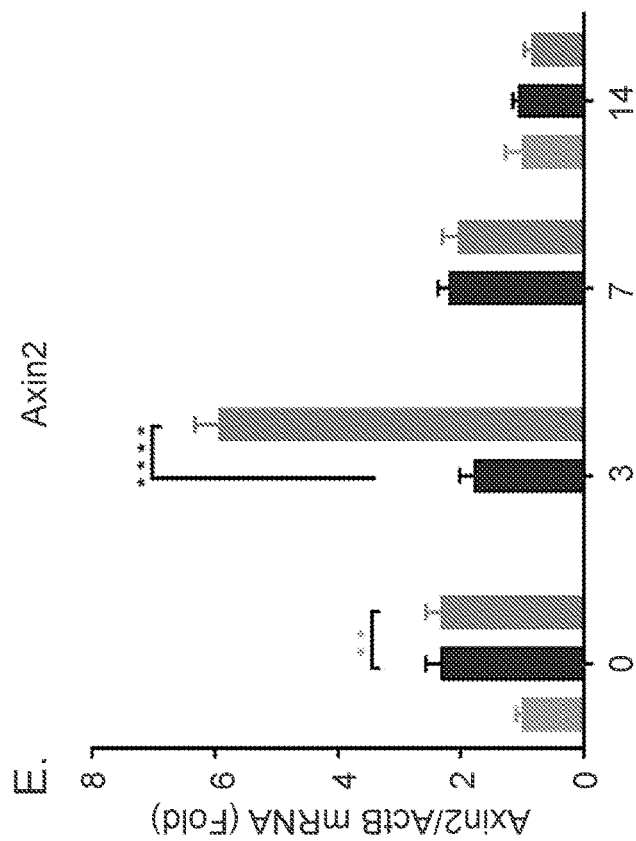
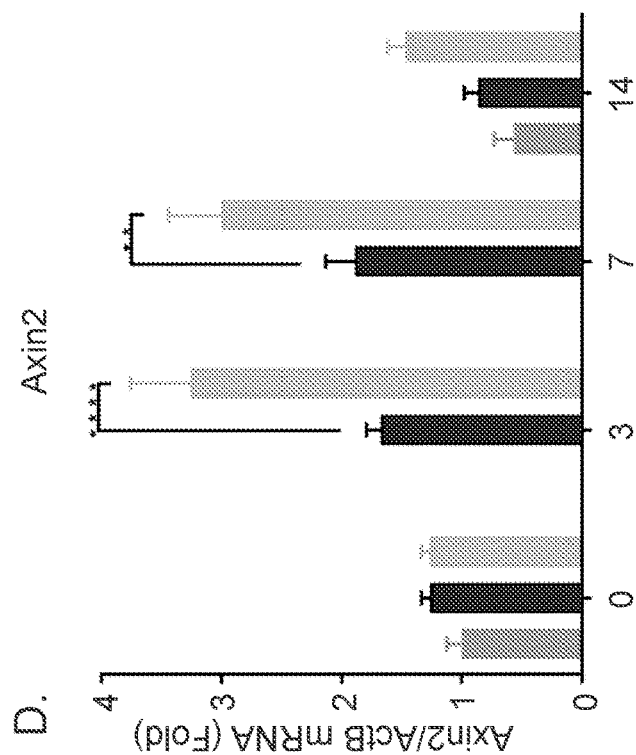


FIG. 41 (contd.)

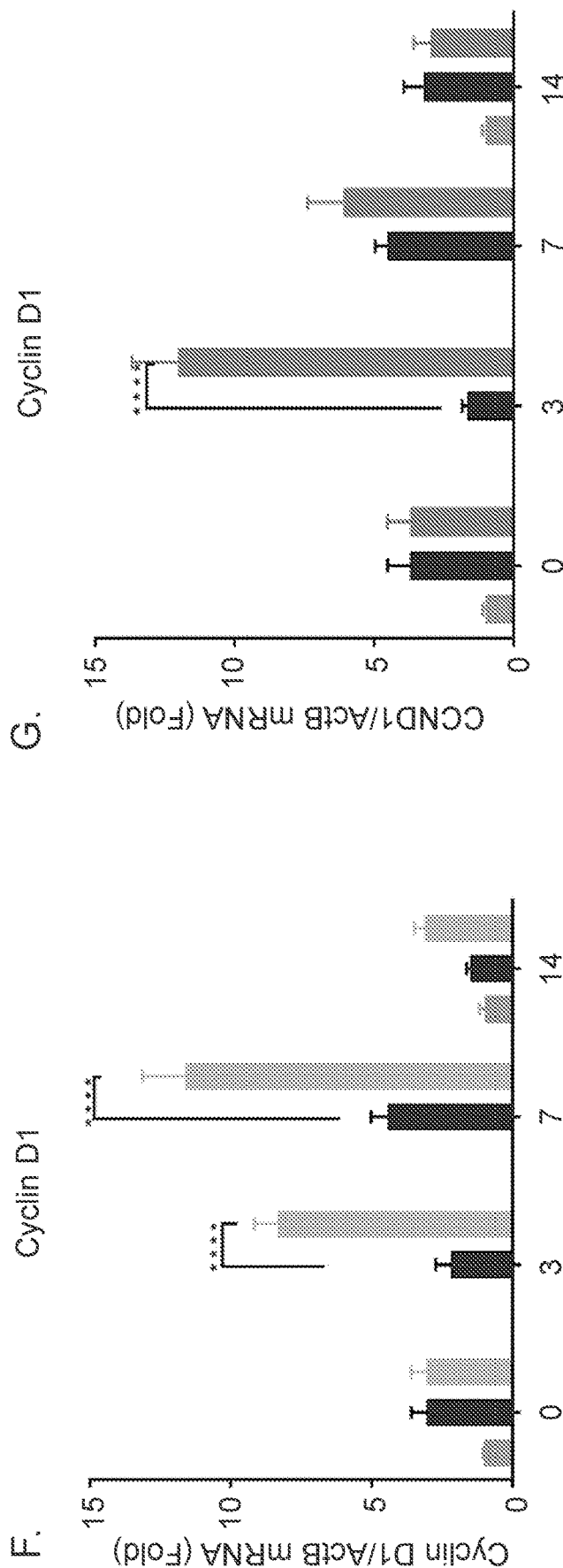


FIG. 41 (contd.)

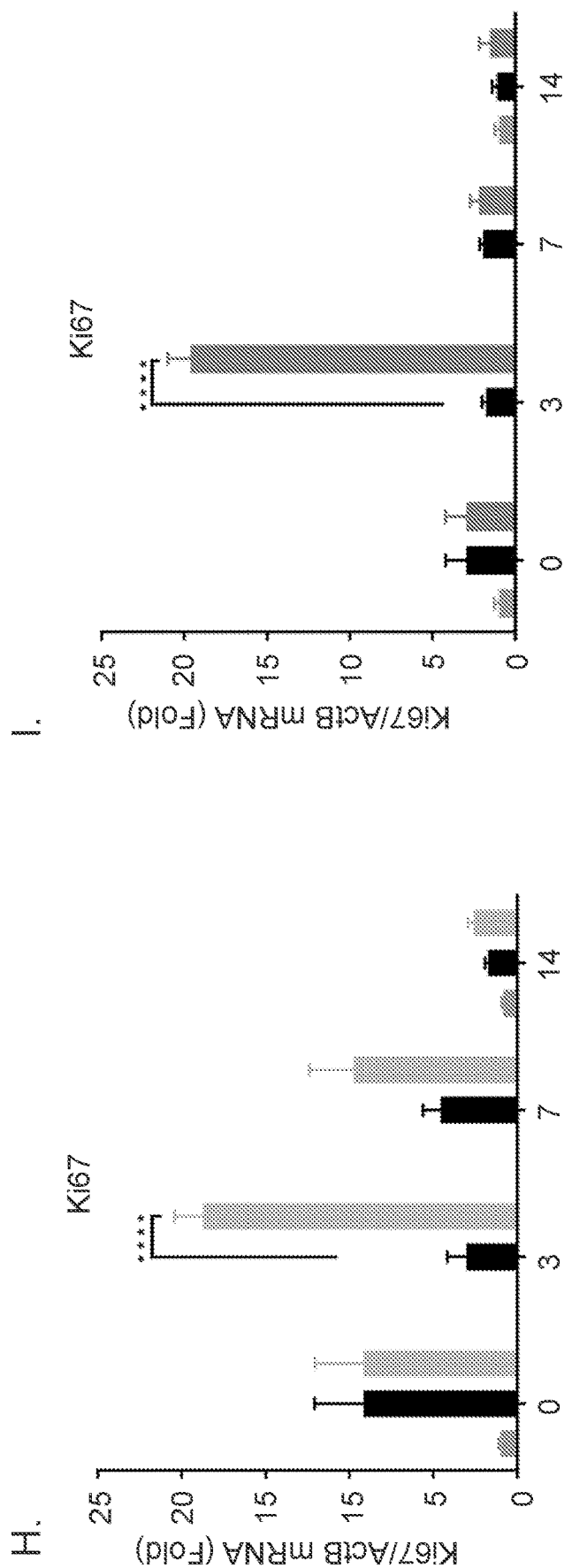


FIG. 41 (contd.)

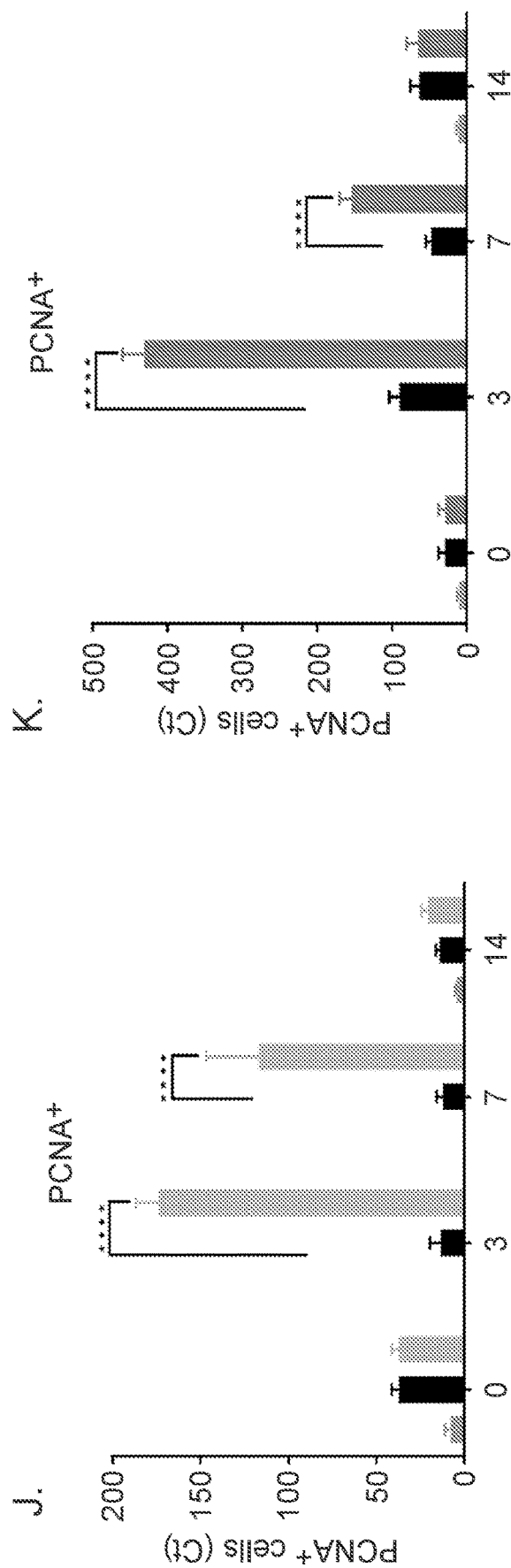


FIG. 41 (contd.)

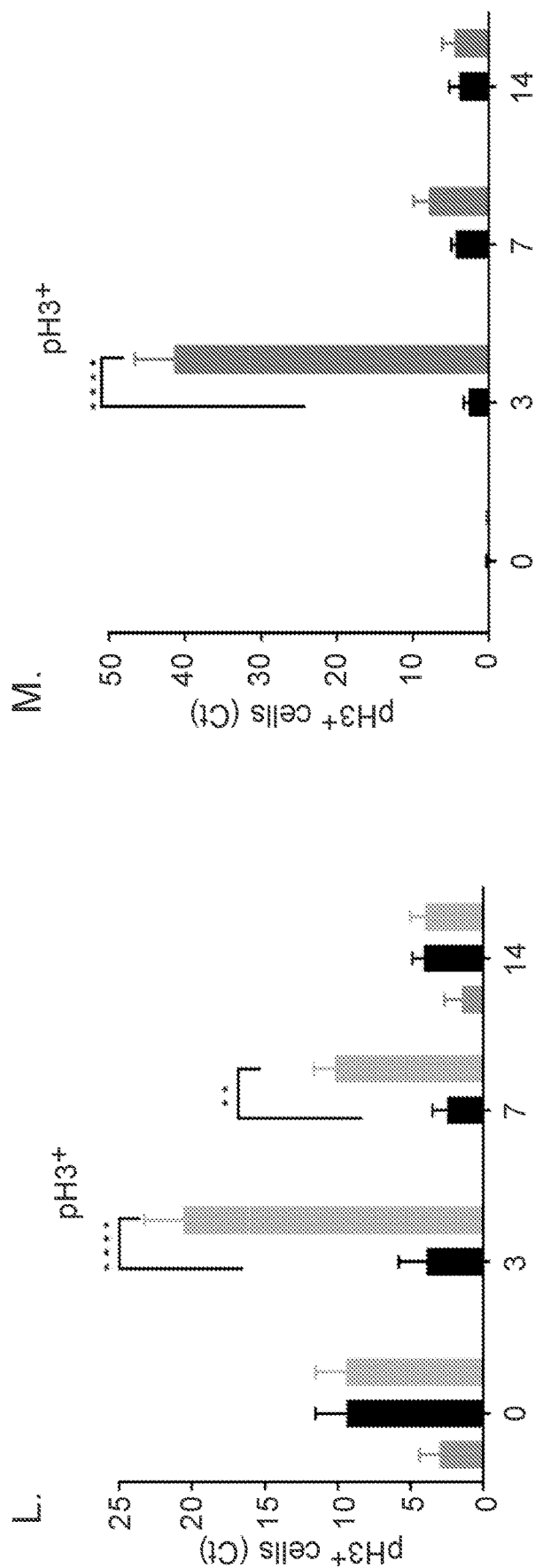


FIG. 41 (contd.)

FIG. 41N

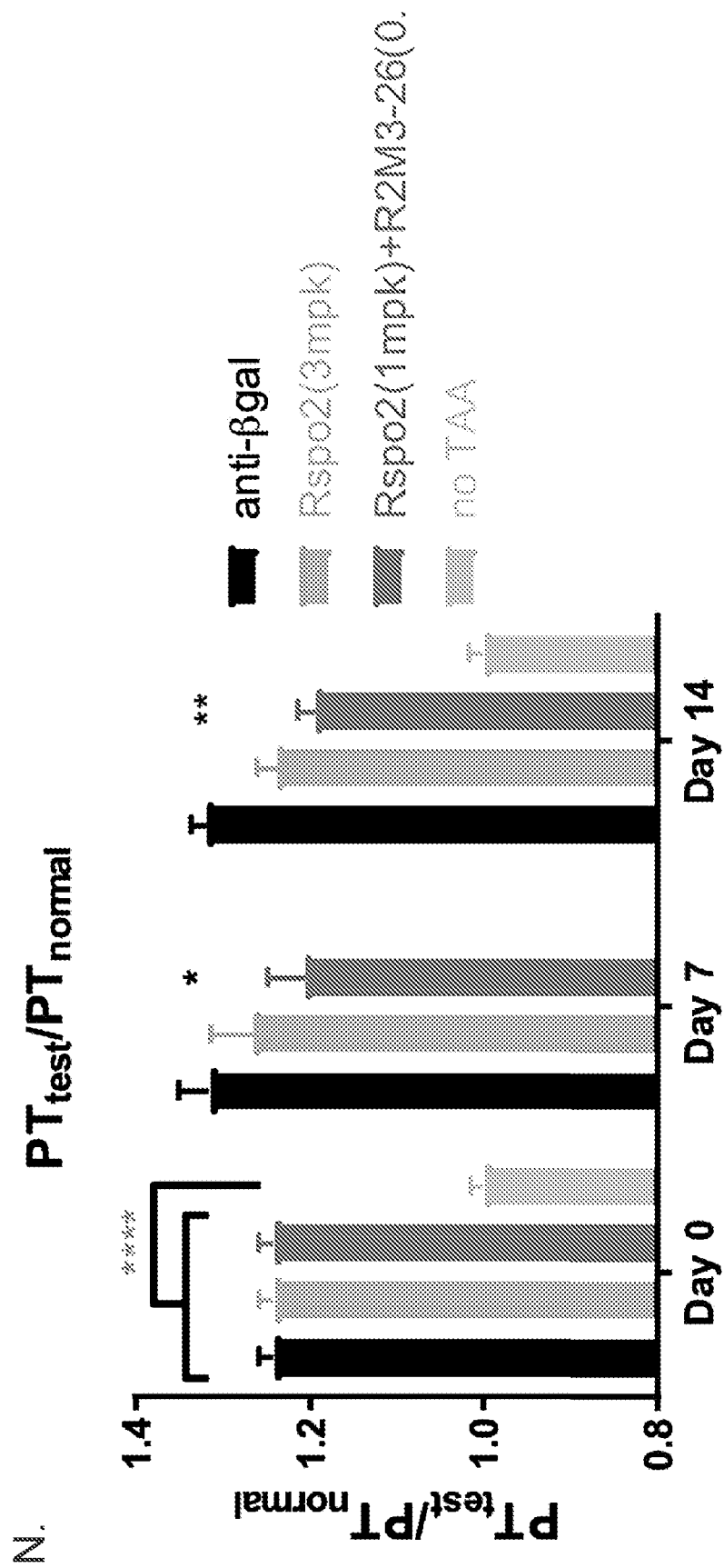


FIG. 42

A.

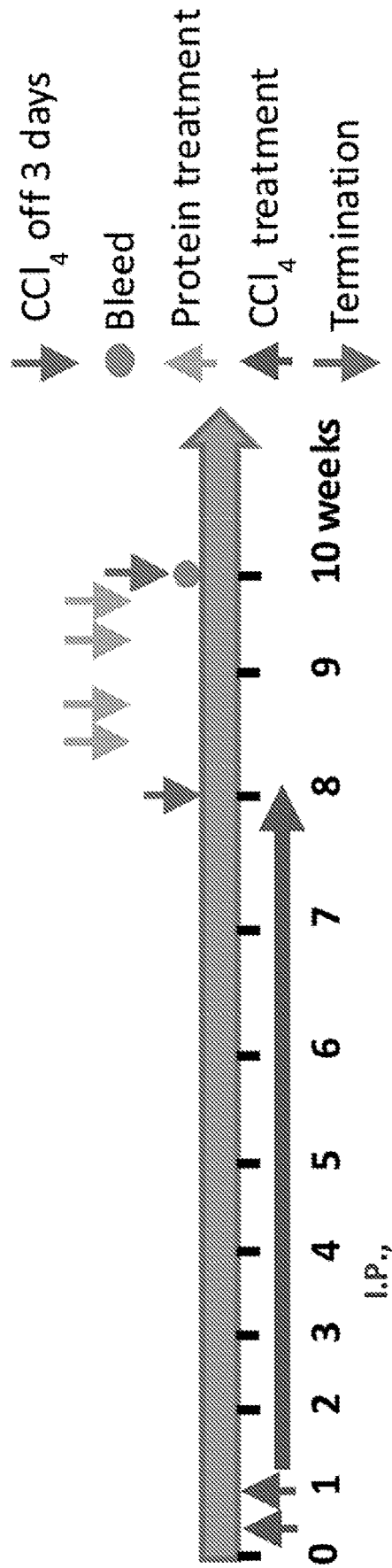


FIG. 42 (contd.)

B.

Liver weight/body weight

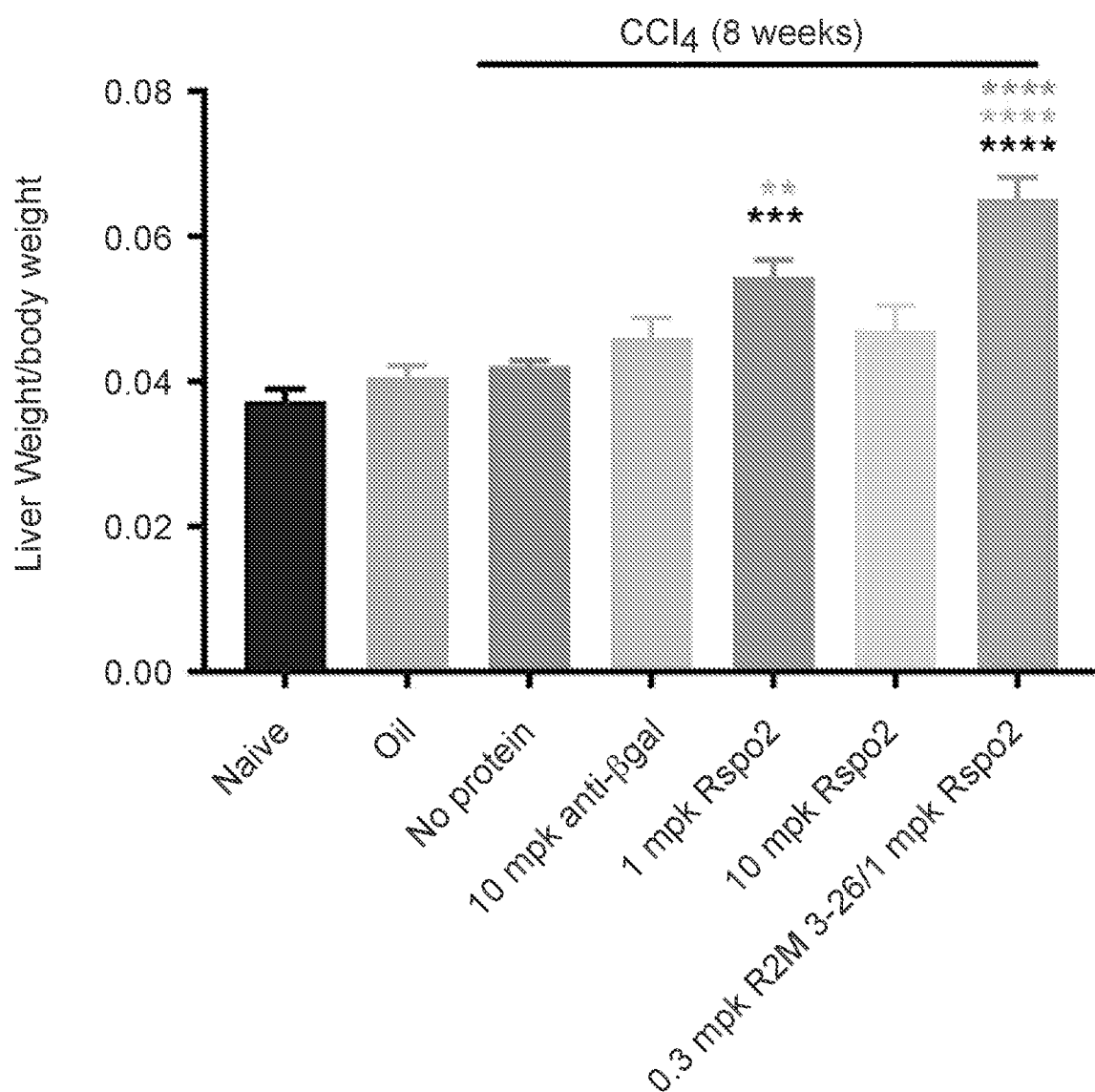


FIG. 42 (contd.)

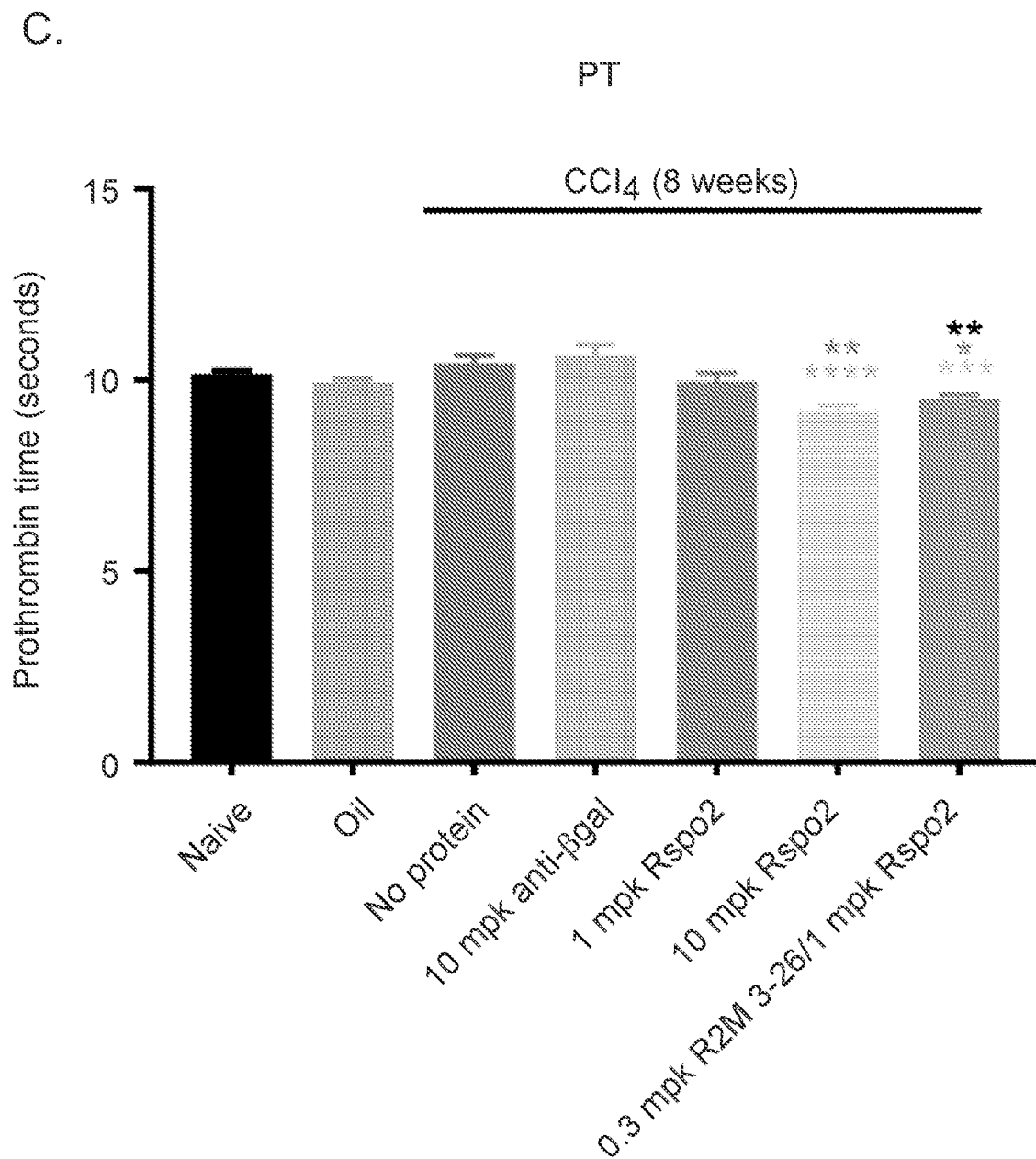


FIG. 42 (contd.)

D.

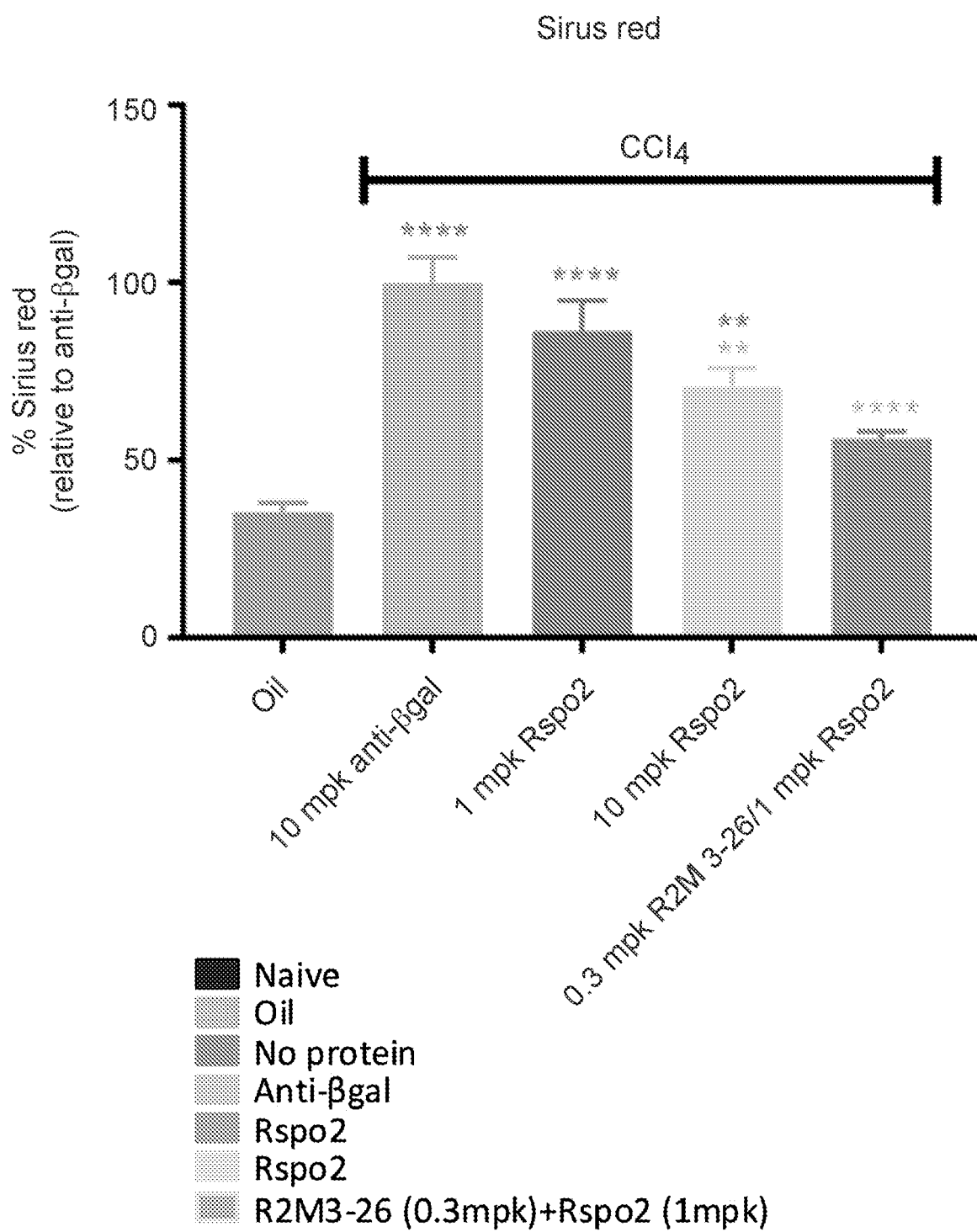


FIG. 43

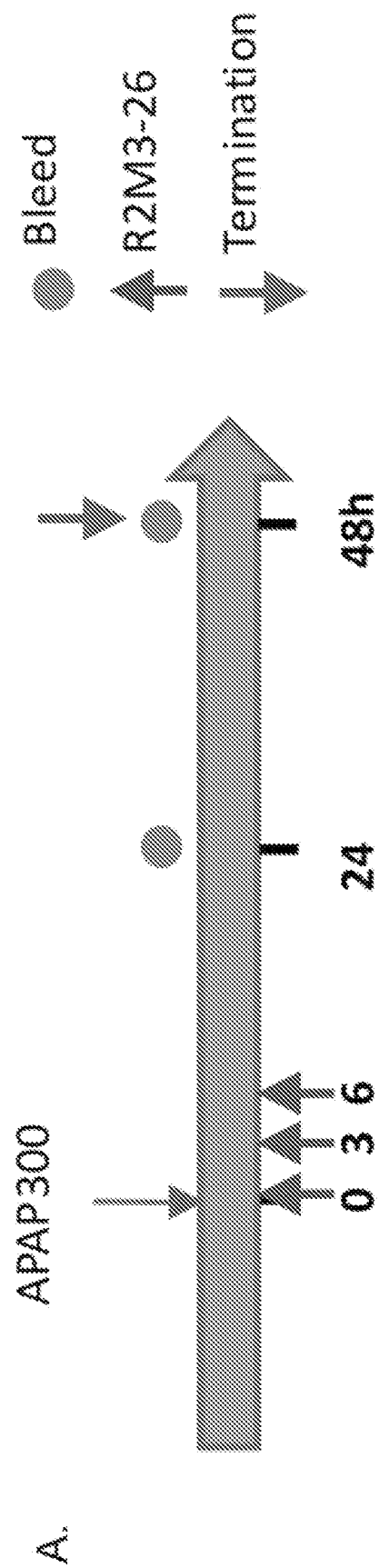
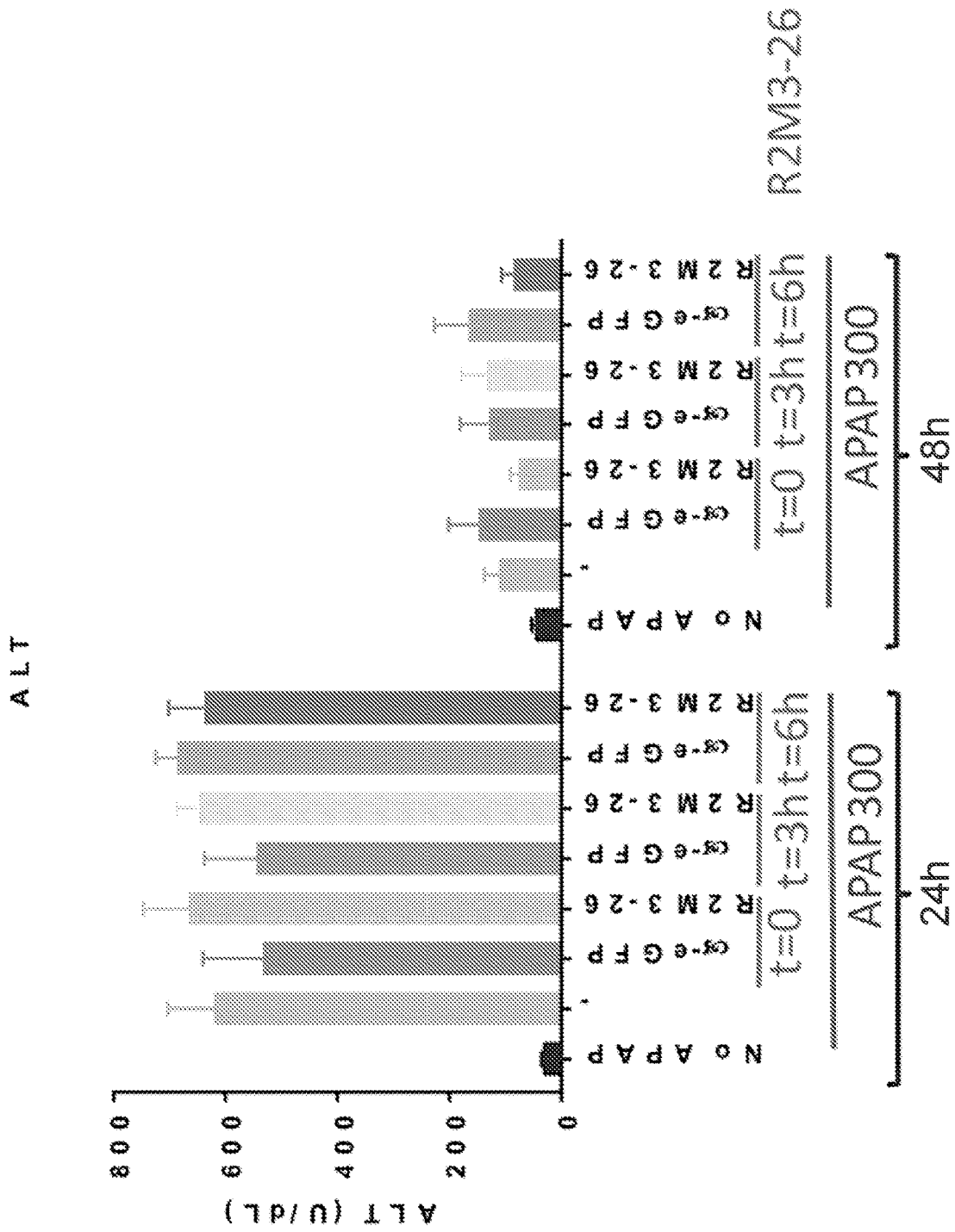
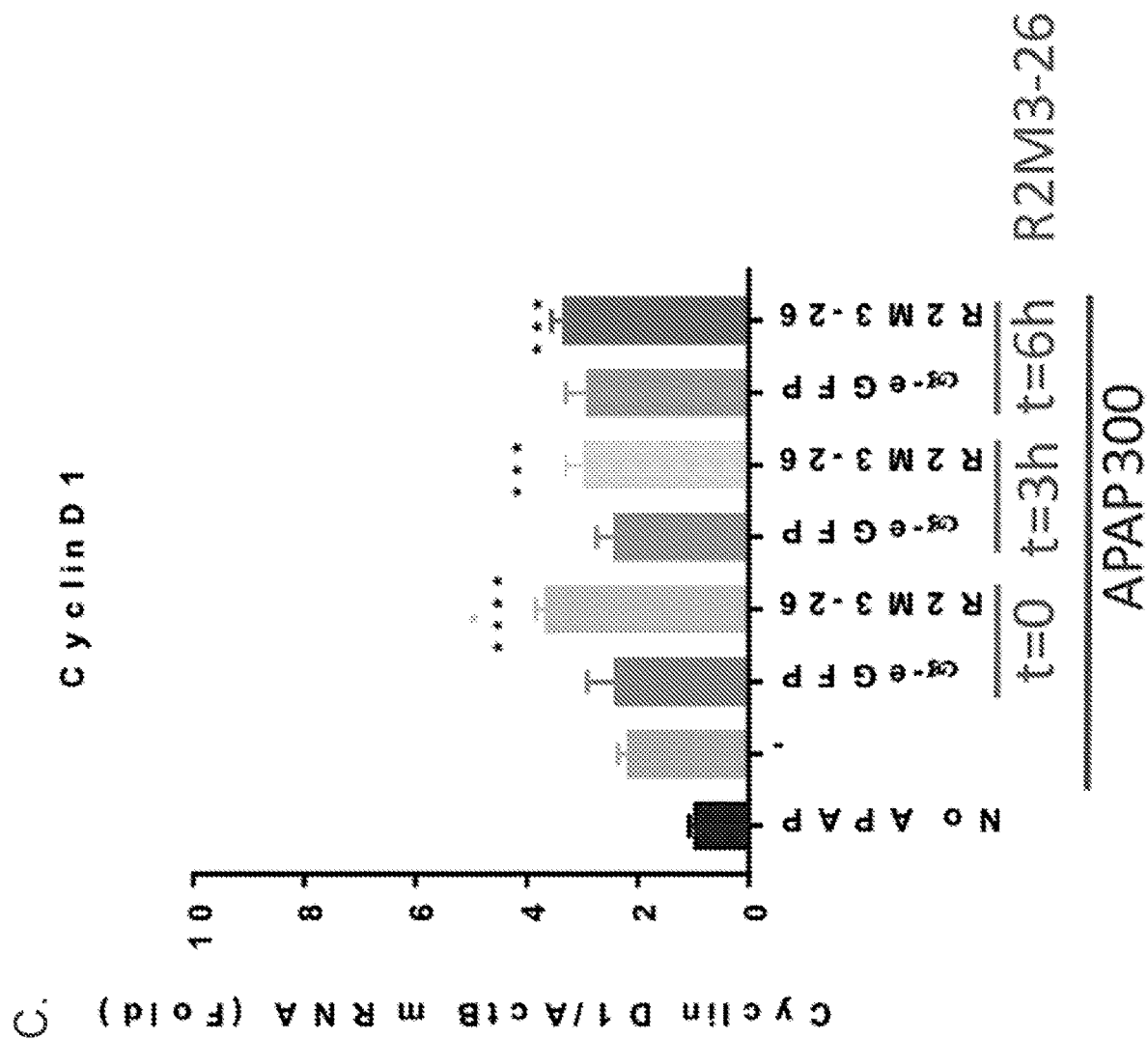


FIG. 43 (contd.)

B.





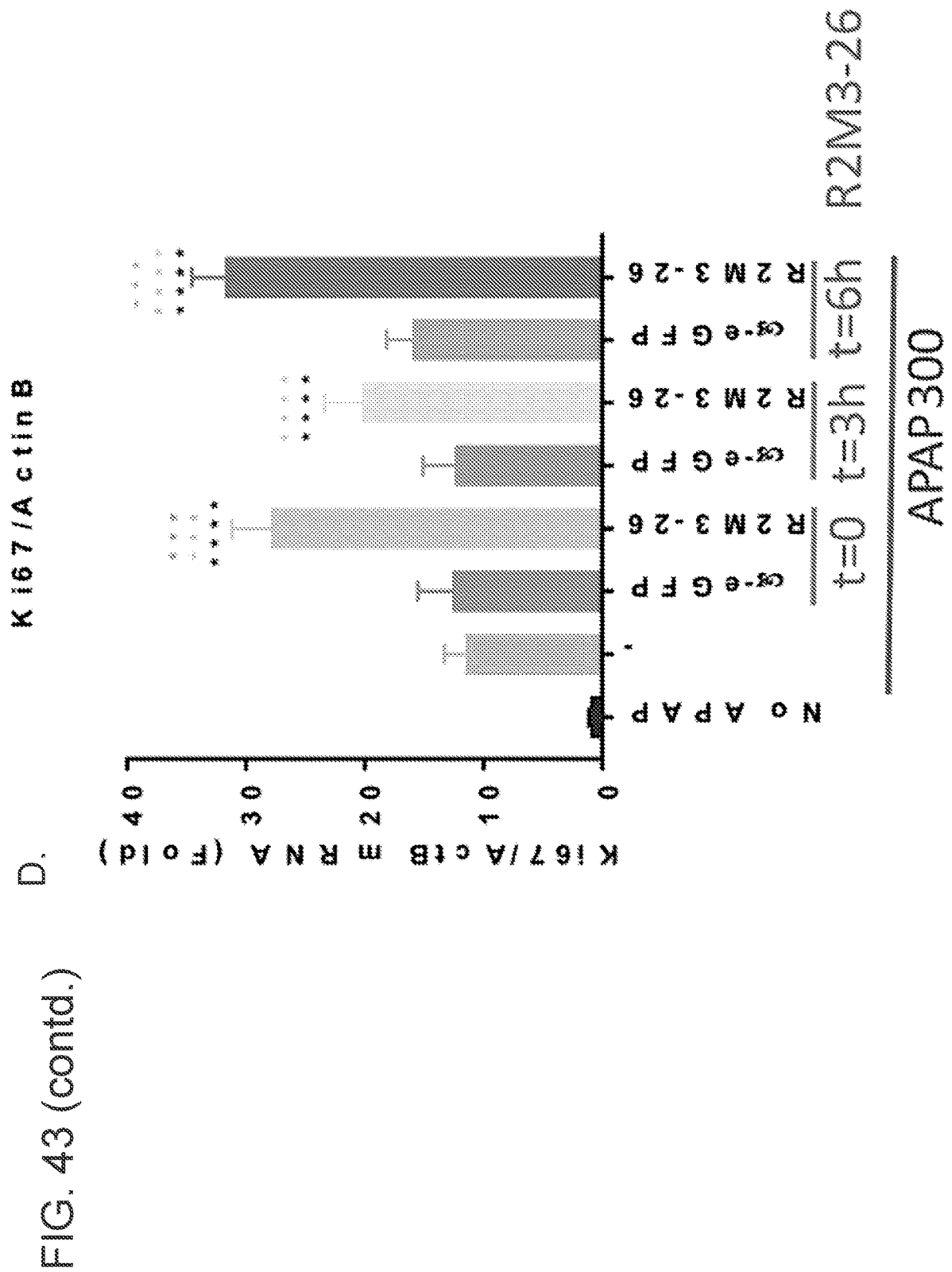
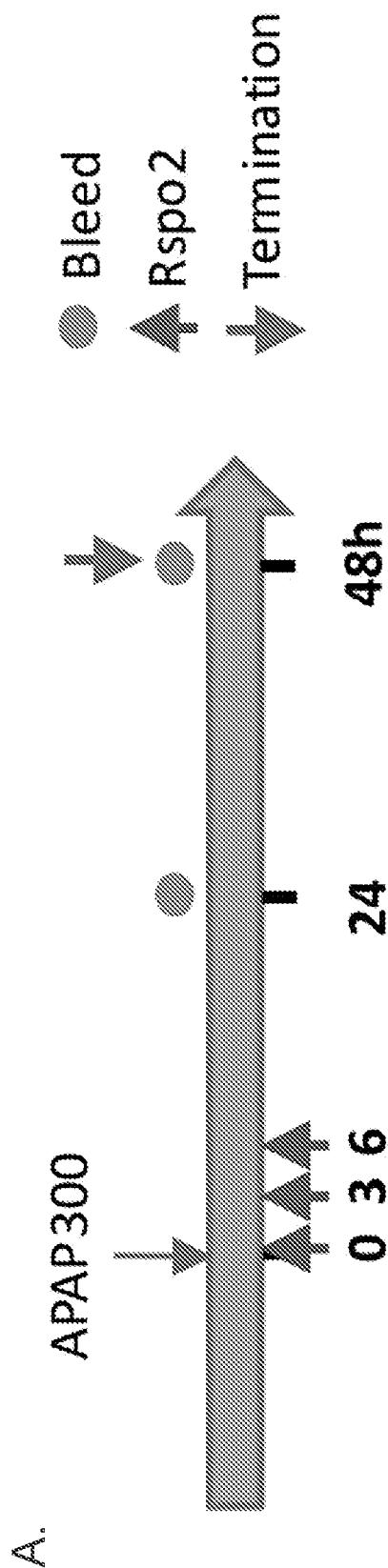
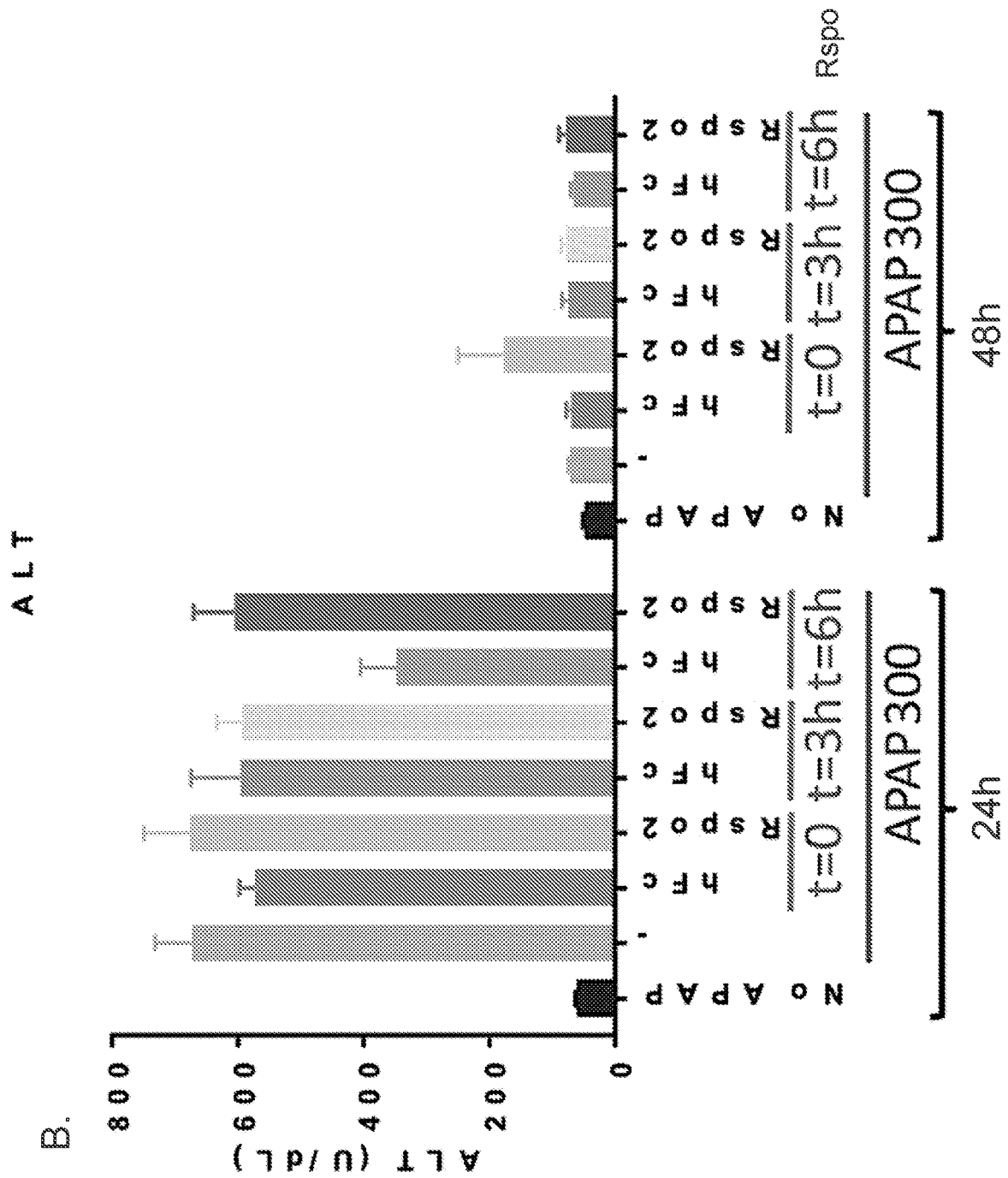


FIG. 44





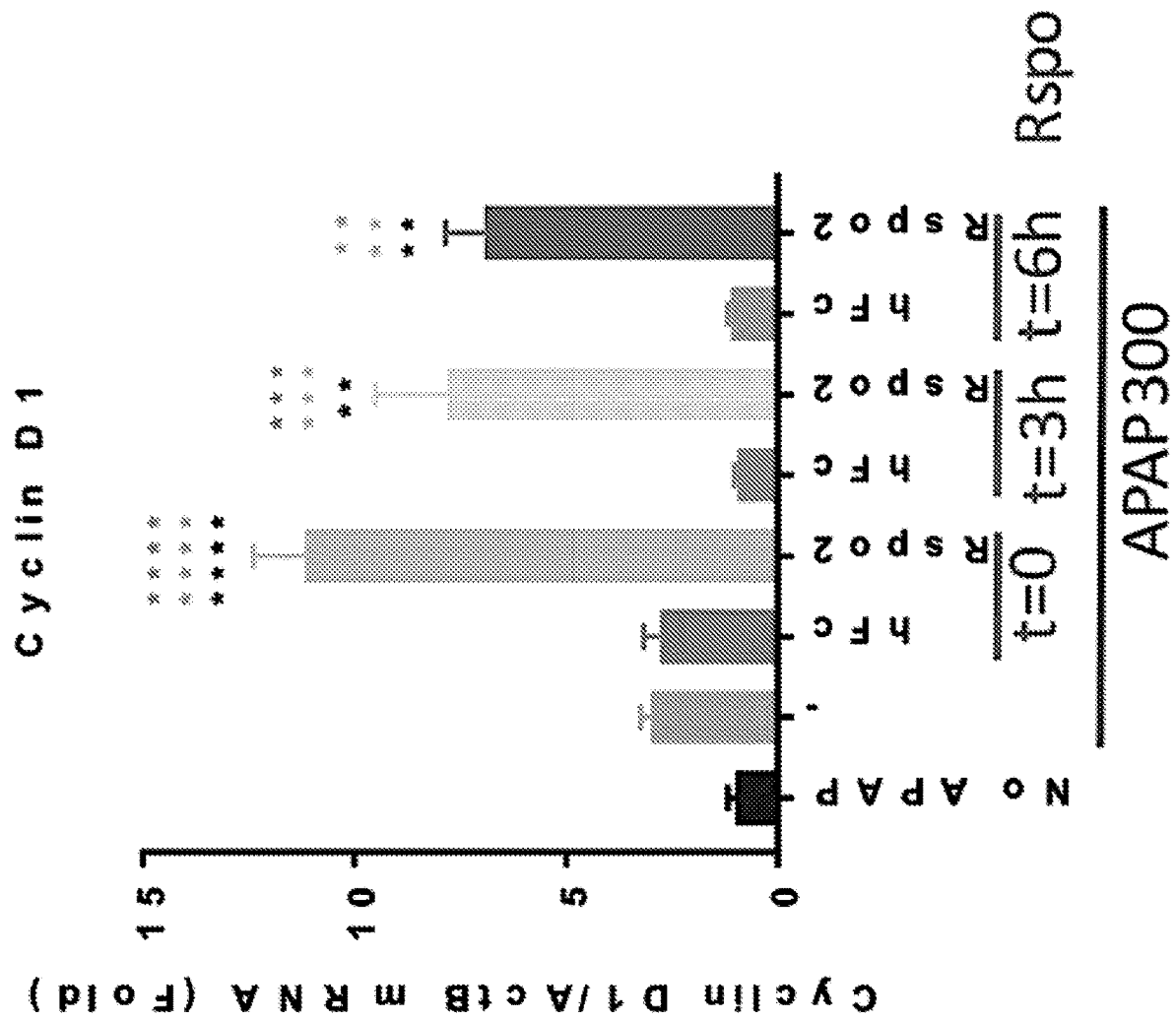


FIG. 44 (contd.) C.

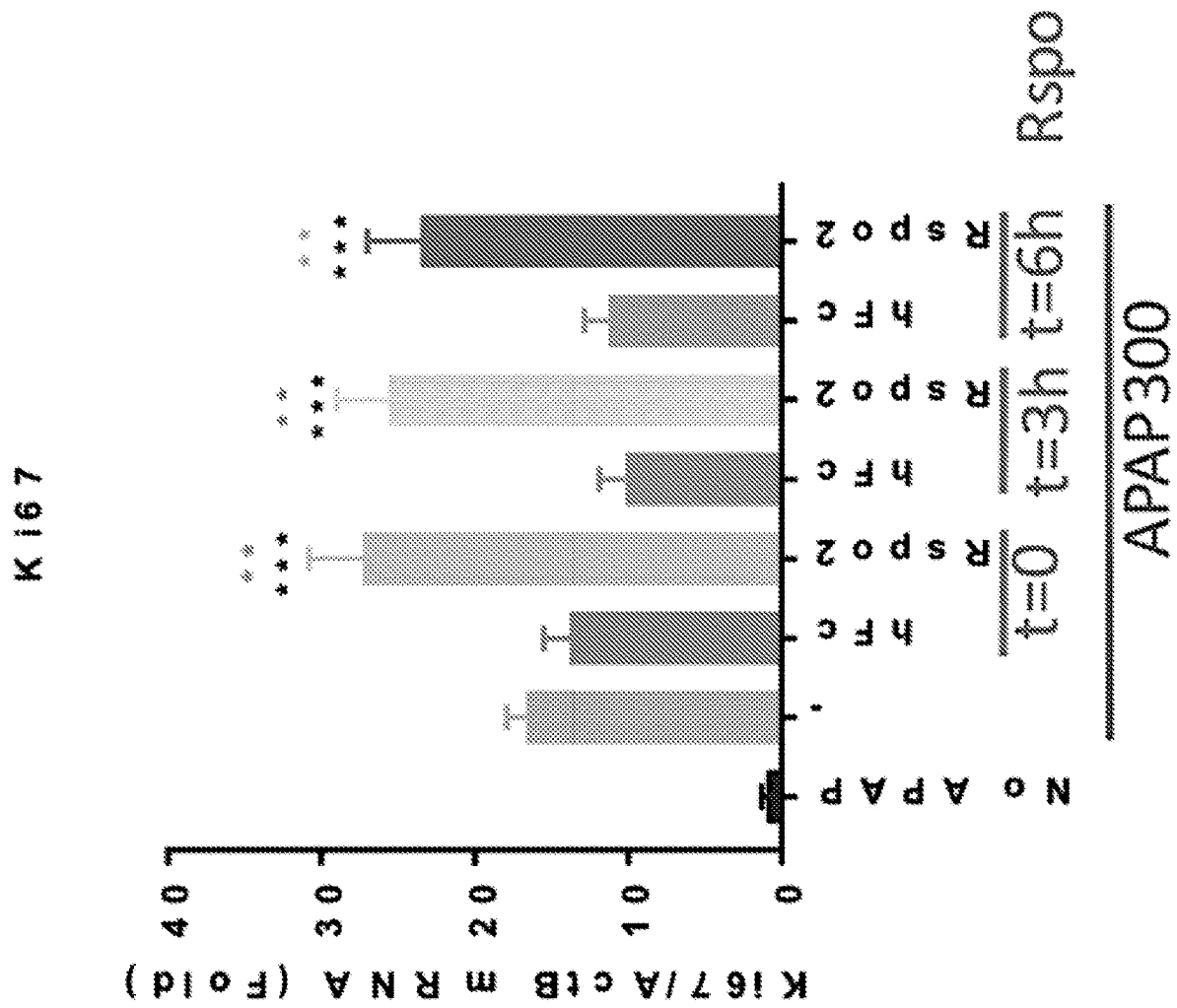


FIG. 44 (contd.) D.

FIG. 45

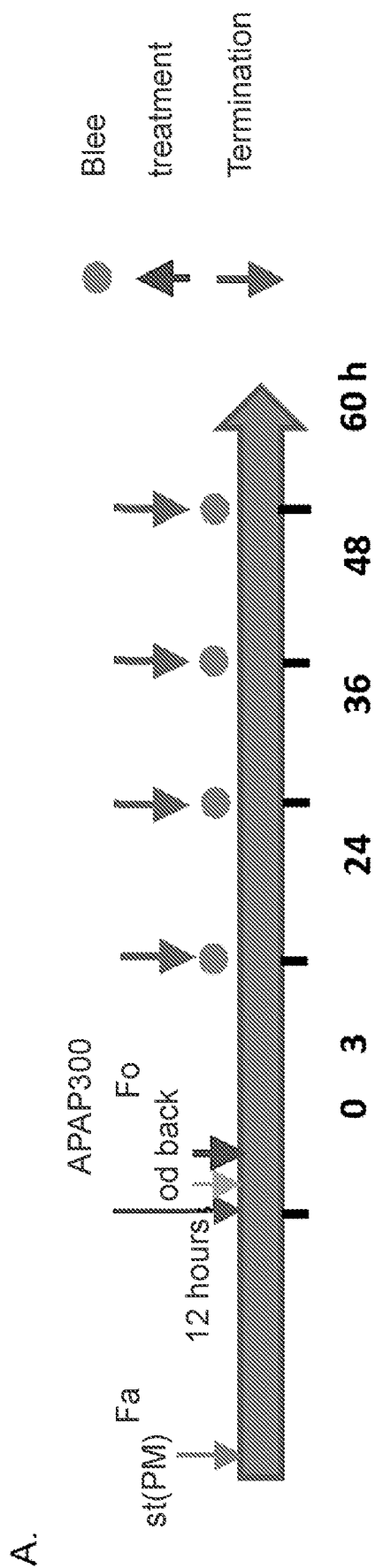
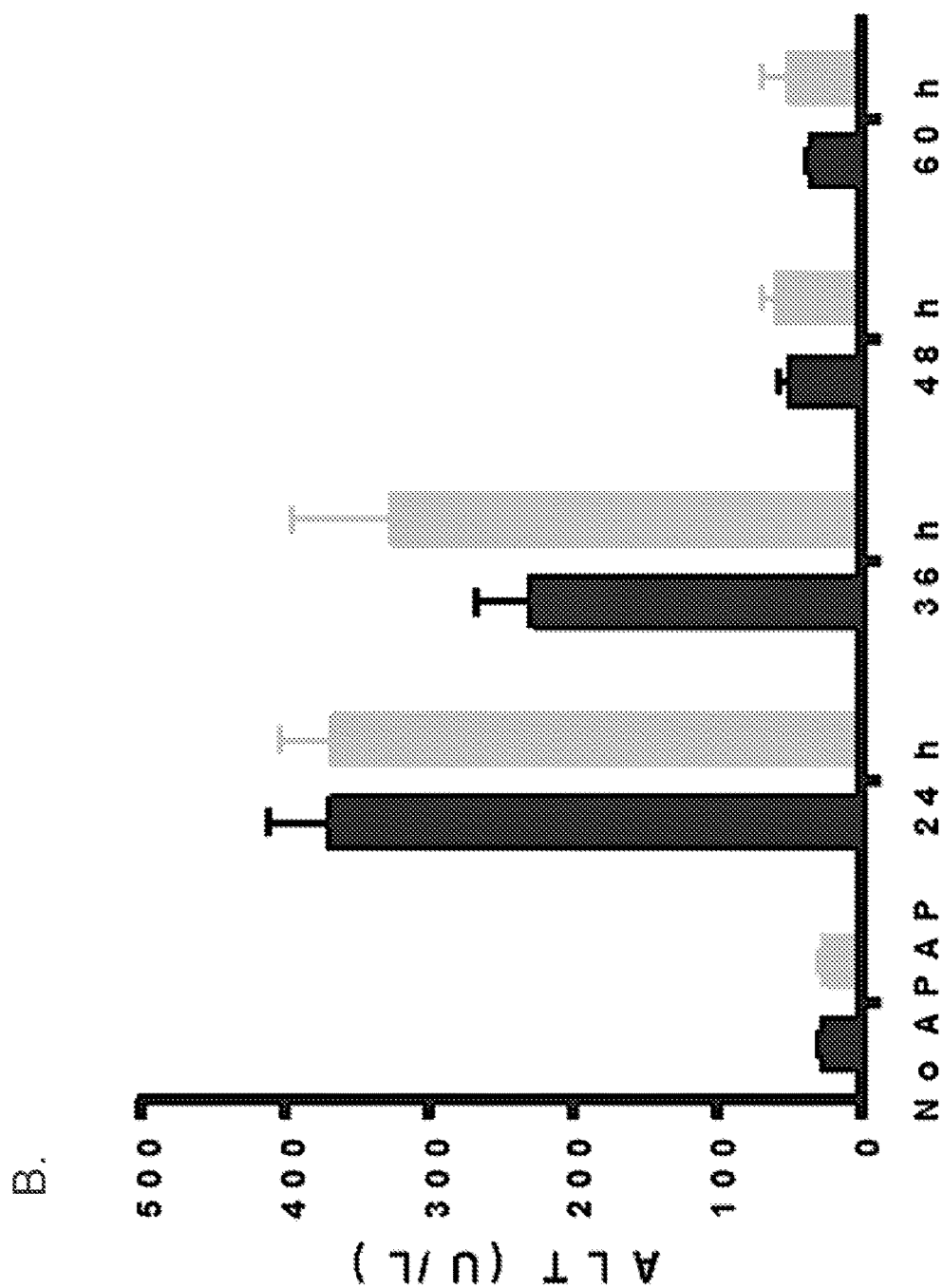
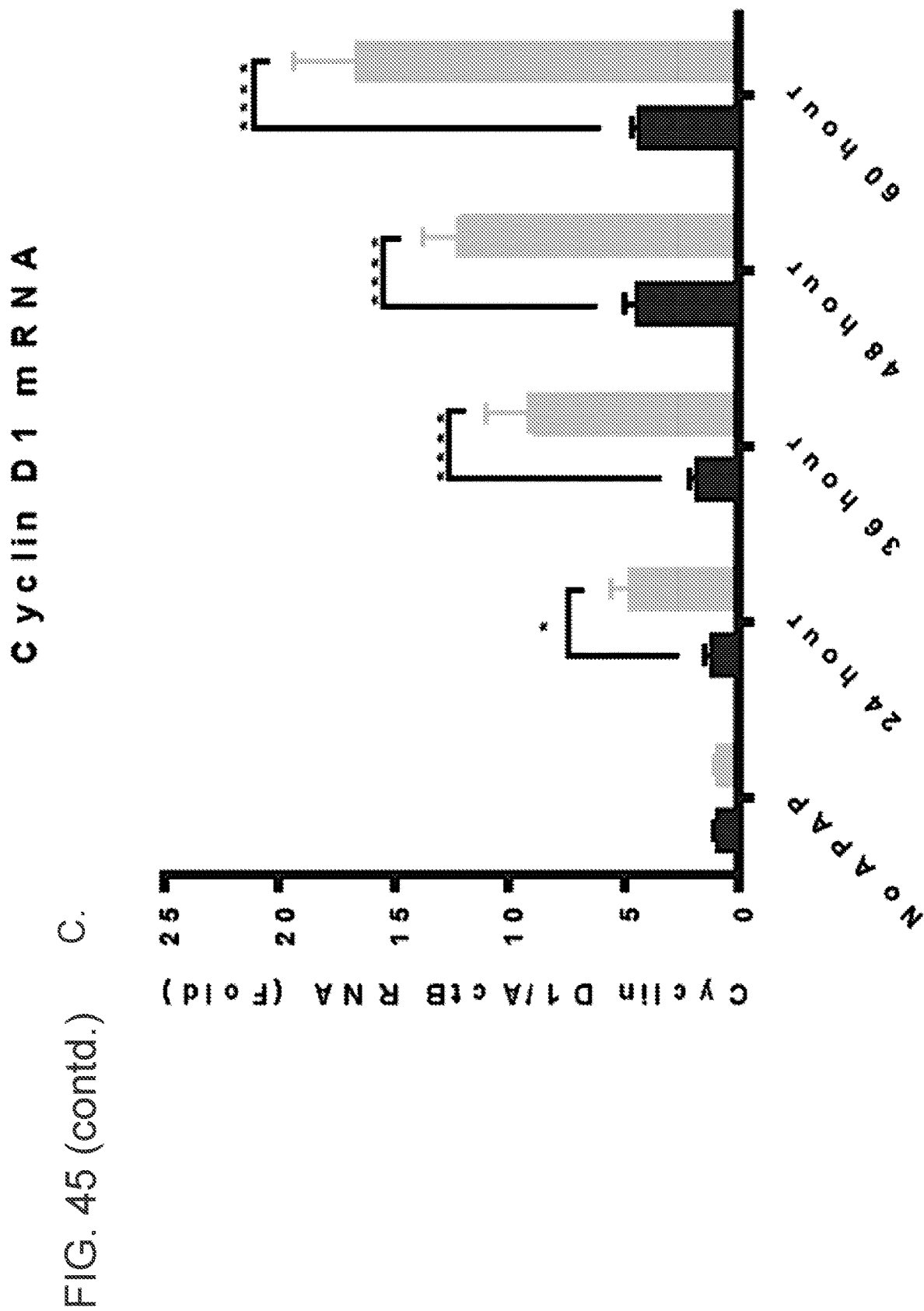


FIG. 45 (contd.)





K i 6 7 m R N A

FIG. 45 (contd.) D.

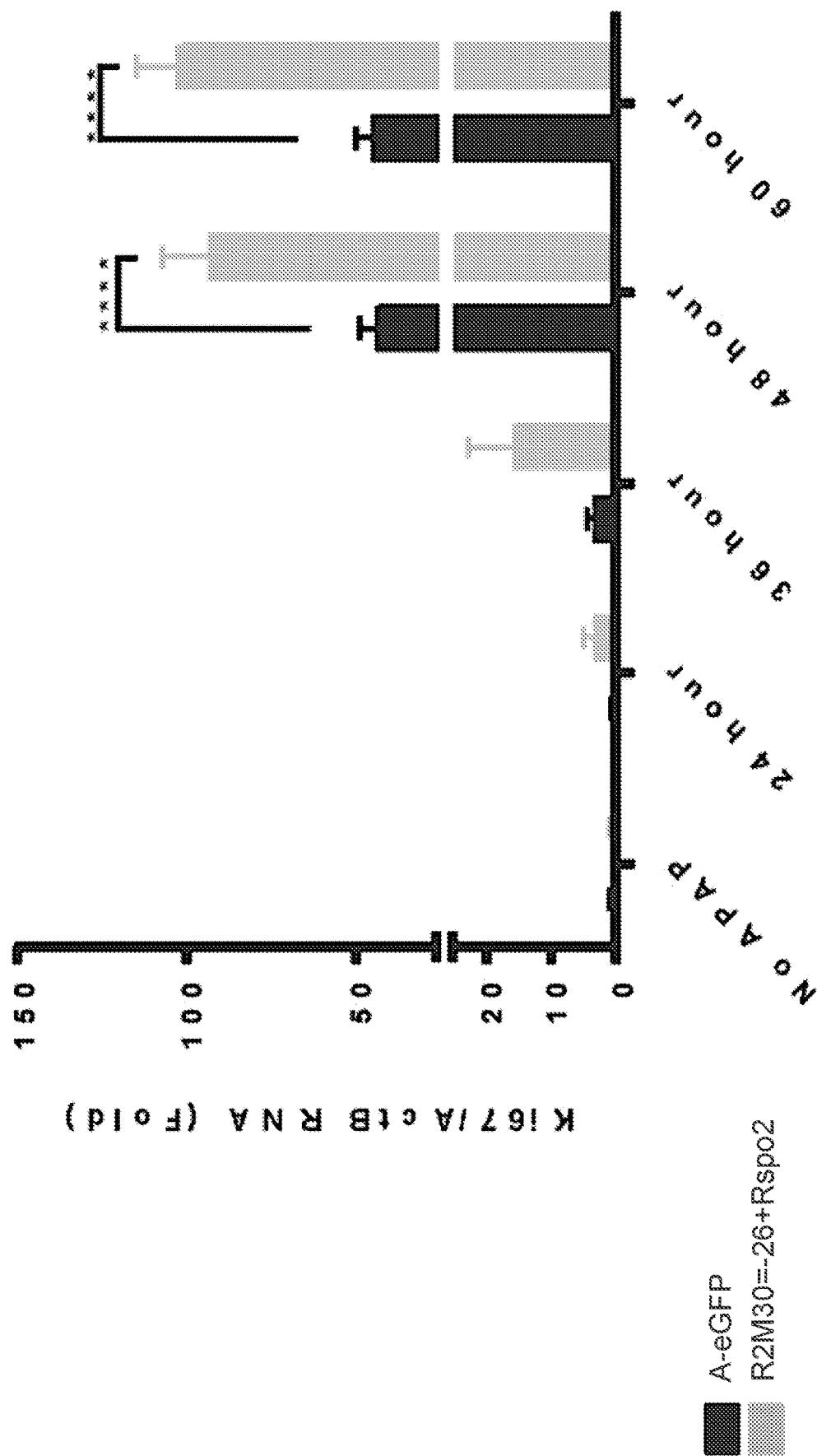


FIG. 46

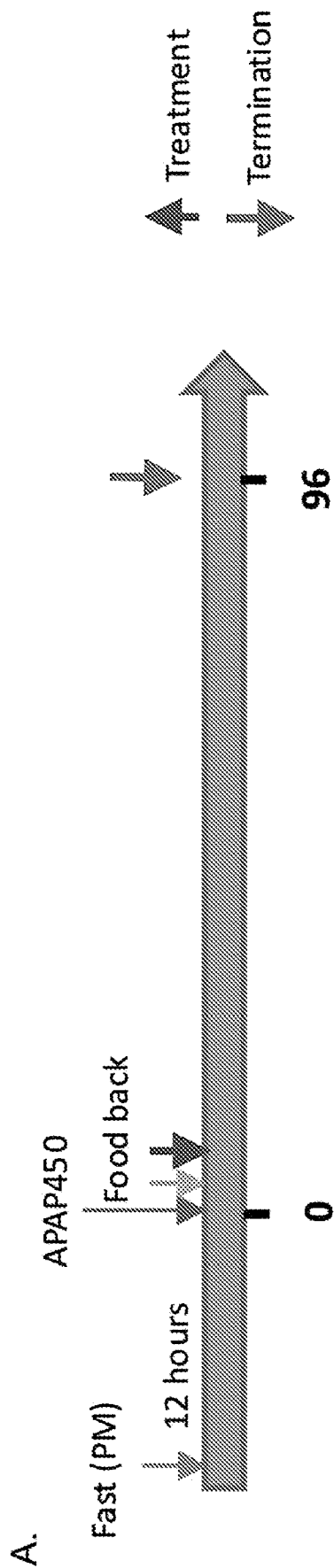


FIG. 46 (contd.)

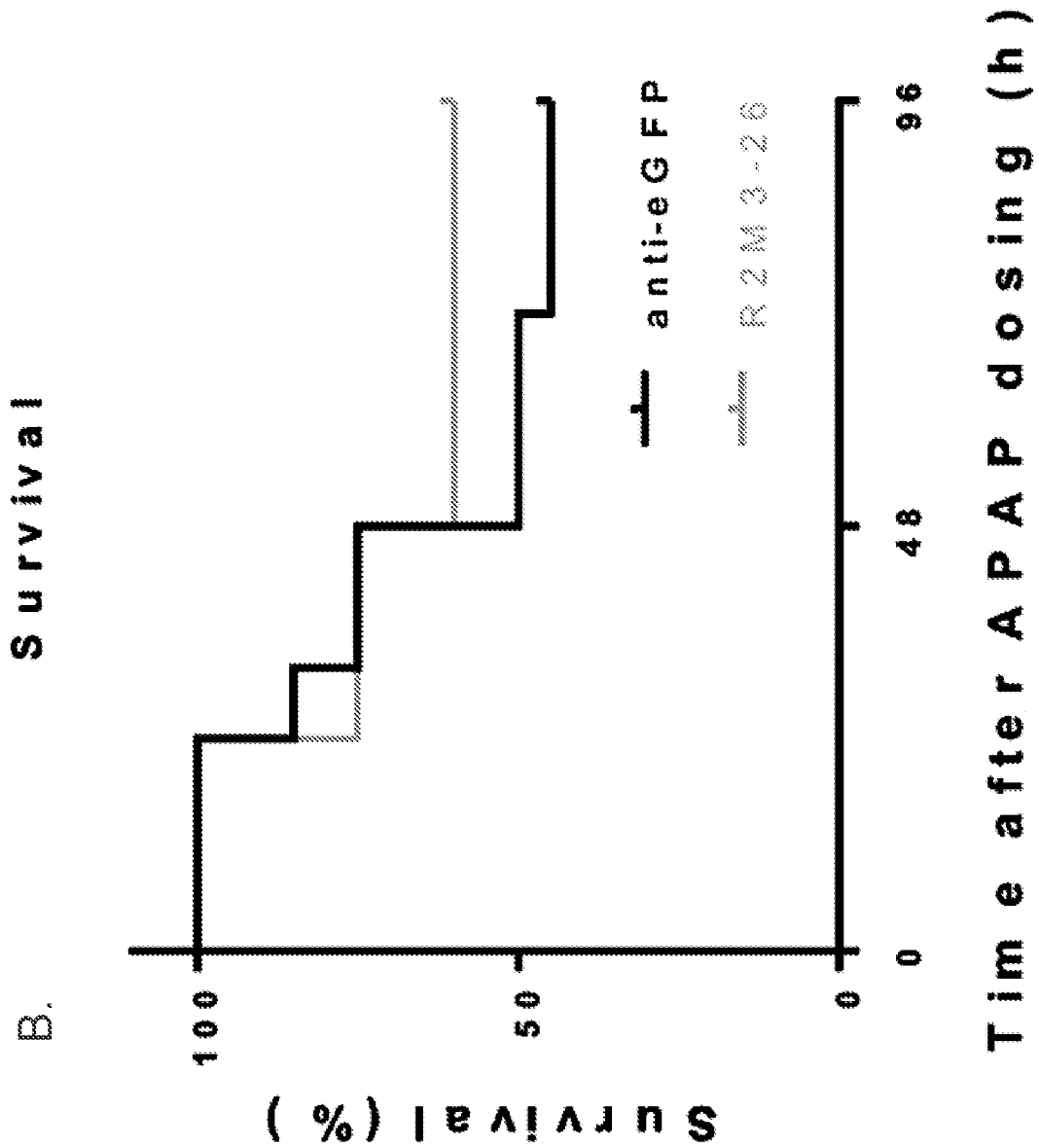


FIG. 46 (contd.)

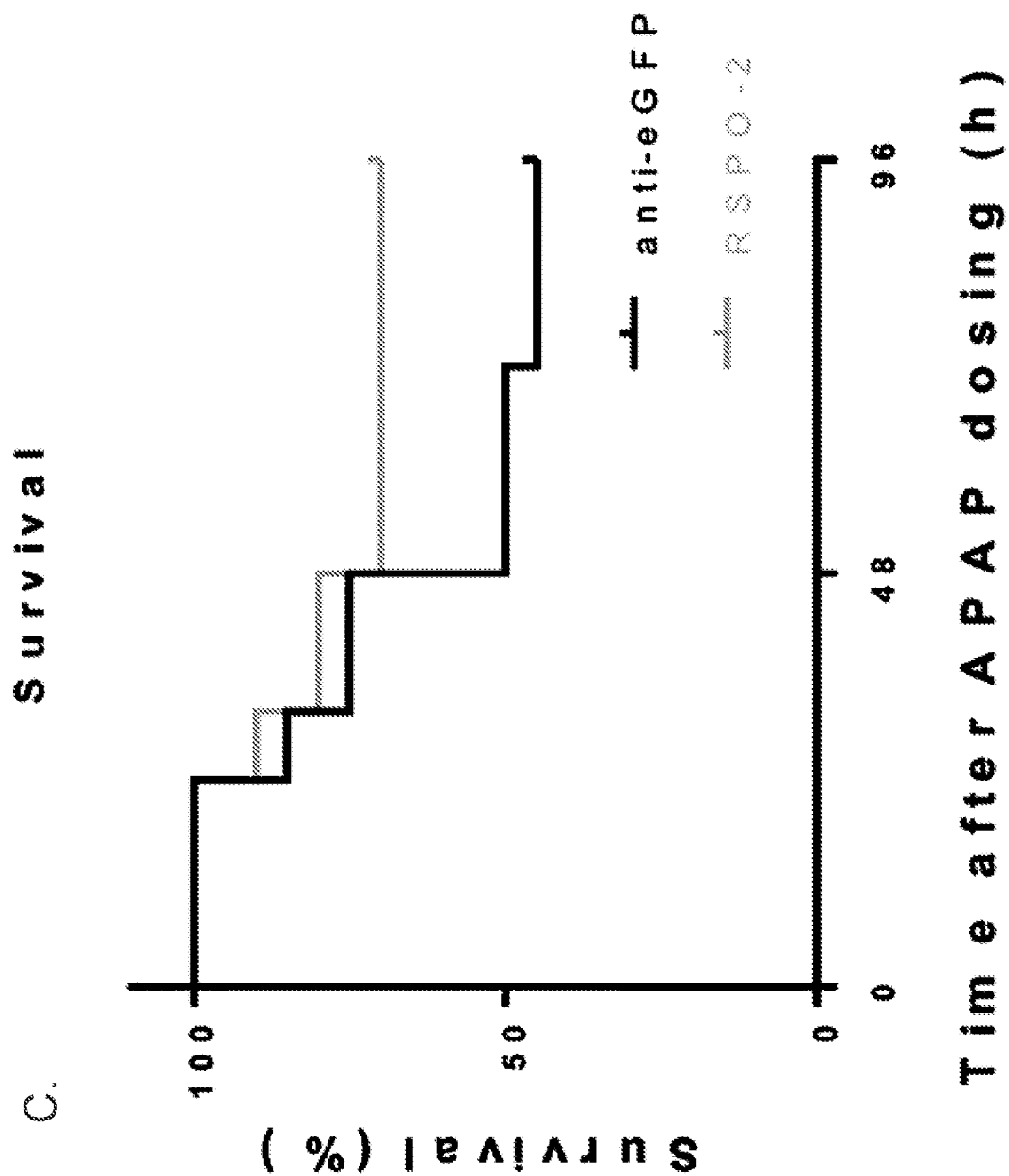
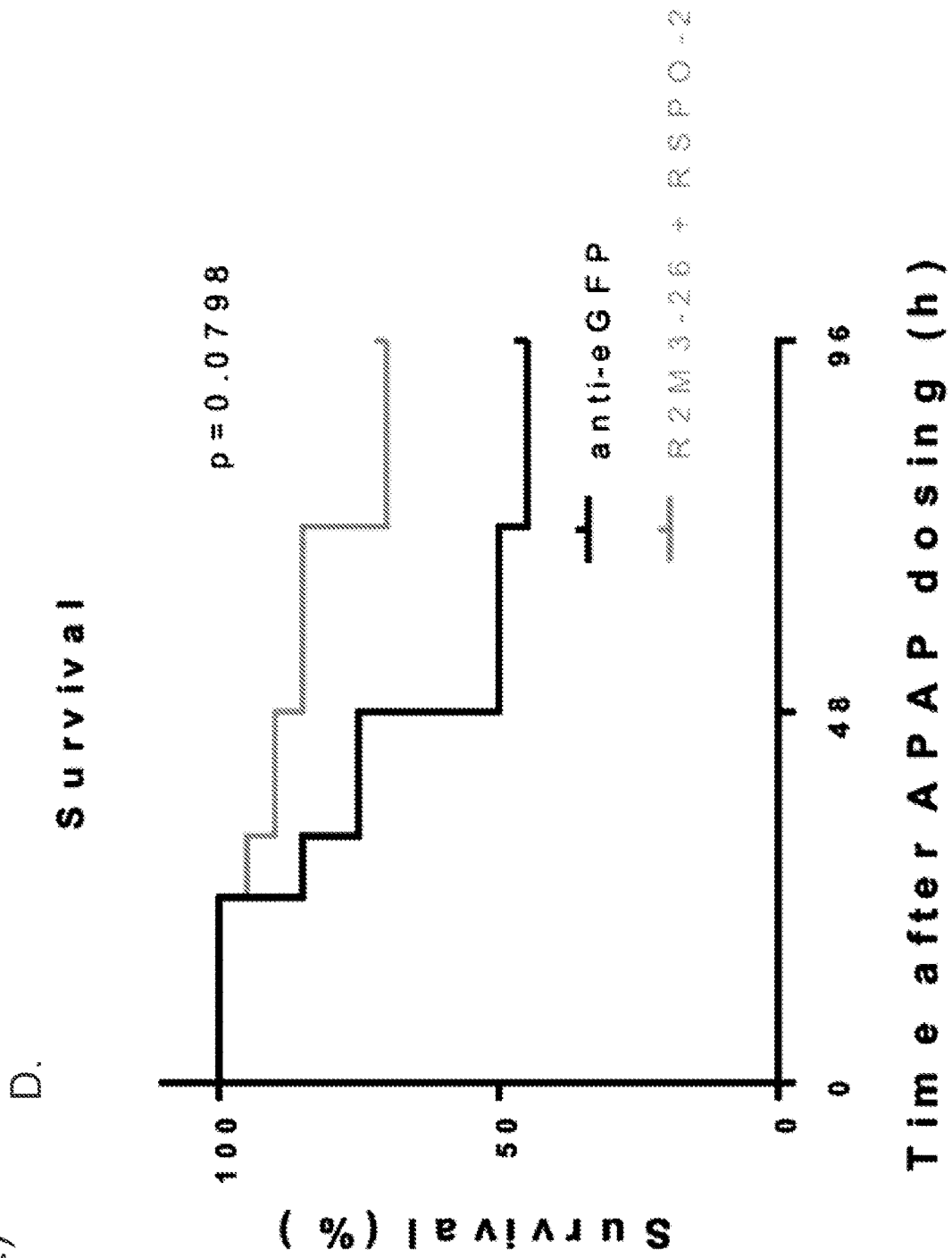


FIG. 46 (contd.)



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WNT SURROGATE MOLECULES AND USES THEREOF**CROSS REFERENCE TO RELATED APPLICATIONS**

This application is a National Stage Application under 35 U.S.C. § 371 of International Application No. PCT/US2018/066616, filed Dec. 19, 2018, which claims the benefit under 35 U.S.C. § 119(e) of U.S. Provisional Application No. 62/607,875, filed Dec. 19, 2017, U.S. Provisional Application No. 62/641,217, filed Mar. 9, 2018, and U.S. Provisional Application No. 62/680,522, filed Jun. 4, 2018, each of which is incorporated by reference herein in its entirety.

STATEMENT REGARDING SEQUENCE LISTING

The Sequence Listing associated with this application is provided in text format in lieu of a paper copy, and is hereby incorporated by reference into the specification. The name of the text file containing the Sequence Listing is SRZN_006_03WO_ST25.txt. The text file is 1.2 MB, was created on Dec. 19, 2018, and is being submitted electronically via EFS-Web.

BACKGROUND**Technical Field**

The present invention relates generally to Wnt signaling pathway agonist molecules, compositions, and methods of using the same. Such molecules are useful, for example, in modulating Wnt signaling pathways.

Description of the Related Art

Wnt (“Wingless-related integration site” or “Wingless and Int-1” or “Wingless-Int”) ligands and their signals play key roles in the control of development, homeostasis and regeneration of many essential organs and tissues, including bone, liver, skin, stomach, intestine, kidney, central nervous system, mammary gland, taste bud, ovary, cochlea and many other tissues (reviewed, e.g., by Clevers, Loh, and Nusse, 2014; 346:1248012). Modulation of Wnt signaling pathways has potential for treatment of degenerative diseases and tissue injuries.

One of the challenges for modulating Wnt signaling as a therapeutic is the existence of multiple Wnt ligands and Wnt receptors, Frizzled 1-10 (Fzd1-10), with many tissues expressing multiple and overlapping Fzds. Canonical Wnt signals also involve Low-density lipoprotein (LDL) receptor-related protein 5 (LRP5) or Low-density lipoprotein (LDL) receptor-related protein 6 (LRP6) as co-receptors, which are broadly expressed in various tissues, in addition to Fzds. Accordingly, there is clearly a need in the art for binding moieties that specifically bind to one or more Fzd, LRP5, or LRP6 to modulate Wnt signaling pathways. The present invention addresses this need.

BRIEF SUMMARY

In various embodiments, the present invention provides WNT surrogate molecules and related uses thereof.

In one embodiment, the disclosure provides a soluble, bivalent, bispecific Wnt surrogate molecule, wherein the Wnt surrogate molecule comprises: (i) one or more regions

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that specifically binds to one or more Frizzled (Fzd) receptor (a Fzd binding region); and (ii) one or more regions that specifically binds to a Low-density lipoprotein (LDL) receptor-related protein 5 (LRP5) and/or a Low-density lipoprotein (LDL) receptor-related protein 6 (LRP6) (a LRP5/6 binding region).

In particular embodiments, the Wnt surrogate molecule comprises two or more Fzd binding regions and two or more LRP5/6 binding regions. In particular embodiments, one or more Fzd binding regions comprise one or more antigen-binding fragments of an antibody. In particular embodiments, one or more antigen-binding fragments are selected from the group consisting of: IgG, scFv, Fab, and VHH or sdAb

In particular embodiments, any of the Fzd antigen-binding fragments comprise: (i) CDRH1, CDRH2 and CDRH3 sequences set forth for any of the antibodies of Tables 1A or 1B; and/or (ii) CDRL1, CDRL2 and CDRL3 sequences set forth for any of the antibodies of Tables 1A or 1B, or a variant of said Fzd binding region comprising one or more amino acid modifications, wherein said variant comprises less than 8 amino acid substitutions in said CDR sequences. In particular embodiments, any of the Fzd binding regions comprise an amino acid sequence having at least 90% identity to any of the sequences set forth in SEQ ID NOs:1-65 or 129-132, or an antigen-binding fragment thereof.

In particular embodiments, any of the Fzd binding regions bind to one or more of Frizzled 1 (Fzd1), Frizzled 2 (Fzd2), Frizzled 3 (Fzd3), Frizzled 4 (Fzd4), Frizzled 5 (Fzd5), Frizzled 6 (Fzd6), Frizzled 7 (Fzd7), Frizzled 8 (Fzd8), Frizzled 9 (Fzd9), and Frizzled 10 (Fzd10). In particular embodiments, any of the Fzd binding region binds to two or more of Frizzled 1 (Fzd1), Frizzled 2 (Fzd2), Frizzled 3 (Fzd3), Frizzled 4 (Fzd4), Frizzled 5 (Fzd5), Frizzled 6 (Fzd6), Frizzled 7 (Fzd7), Frizzled 8 (Fzd8), Frizzled 9 (Fzd9), and Frizzled 10 (Fzd10). In particular embodiments, any of the Fzd binding region binds to: (i) Fzd1, Fzd2, Fzd7 and Fzd9; (ii) Fzd1, Fzd2 and Fzd7; (iii) Fzd5 and Fzd8; (iv) Fzd5, Fzd7 and Fzd8; (v) Fzd1, Fzd4, Fzd5 and Fzd8; (vi) Fzd1, Fzd2, Fzd5, Fzd7 and Fzd8; (vii) Fzd4 and Fzd9; (viii) Fzd9 and Fzd10; (ix) Fzd5, Fzd8 and Fzd10; or (x) Fzd4, Fzd5 and Fzd8; Fzd1, Fzd5, Fzd7 and Fzd8.

In particular embodiments, any of the surrogate molecules comprise one or more LRP5/6 binding regions comprise one or more antigen-binding fragments of an antibody. In particular embodiments, the one or more antigen-binding fragments are selected from the group consisting of: IgG, scFv, Fab, and VHH or sdAb. In particular embodiments, any of the one or more LRP5/6 binding regions or antigen-binding fragments comprise: (i) CDRH1, CDRH2 and CDRH3 sequences set forth for any of the antibodies of Table 2; and/or (ii) CDRL1, CDRL2 and CDRL3 sequences set forth for any of the antibodies of Table 2, or a variant of said LRP5/6 binding region comprising one or more amino acid modifications, wherein said variant comprises less than 8 amino acid substitutions in said CDR sequences. In particular embodiments, any of the one or more LRP5/6 binding regions comprise an amino acid sequence having at least 90% identity to any of the sequences set forth in SEQ ID NOs:66-88 or 133, or an antigen-binding fragment thereof.

In particular embodiments of any of the Wnt surrogate molecules, the Fzd binding region comprising a Fab, and the LRP5/6 binding region comprising a VHH or sdAb. In particular embodiments, the Fab is present within a full immunoglobulin (Ig), optionally an IgG, comprising a light chain and a heavy chain. In particular embodiments, the

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LRP5/6 binding region is fused to the N-terminus or the C-terminus of the heavy chain. In particular embodiments, the LRP5/6 binding region is fused to the N-terminus or the C-terminus of the light chain. In certain embodiments, the LRP5/6 binding region is fused to the N-terminus of the heavy chain of the full Ig or the N-terminus of the light chain of the full Ig. In certain embodiments, the LRP5/6 binding region is fused to the C-terminus of the heavy chain of the full Ig or the C-terminus of the light chain of the full Ig. In certain embodiments, the variable light chain region of the LRP5/6 binding Fab is fused to the N-terminus of the variable heavy chain region of the full Ig. In certain embodiments, the variable light chain region of the LRP5/6 binding Fab is fused to the N-terminus of the variable heavy chain region of the full Ig, and the variable heavy chain region of the LRP5/6 binding Fab is fused to the N-terminus of the variable light chain region of the full IgG. In particular embodiments, any of the LRP5/6 binding region is fused to the heavy chain or the light chain via one or more linker moiety.

In certain embodiments of any of the Wnt surrogate molecules, the Fzd binding region comprises a VHH or sdAb and the LRP5/6 binding region comprises a Fab. In particular embodiments, the Fab is present within a full immunoglobulin (Ig), optionally an IgG, comprising a light chain and a heavy chain. In certain embodiments, the Fzd binding region is fused to the N-terminus or the C-terminus of the heavy chain. In certain embodiments, the Fzd binding region is fused to the N-terminus or the C-terminus of the light chain. In some embodiment, the Fzd binding region is fused to the N-terminus of the heavy chain of the full Ig or the N-terminus of the light chain of the full Ig. In some embodiments, the Fzd binding region is fused to the C-terminus of the heavy chain of the full Ig or the C-terminus of the light chain of the full Ig. In some embodiments, the variable light chain region of the Fzd binding Fab is fused to the N-terminus of the variable heavy chain region of the full Ig. In some embodiments, the variable light chain region of the Fzd binding Fab is fused to the N-terminus of the variable heavy chain region of the full Ig, and the variable heavy chain region of the Fzd binding Fab is fused to the N-terminus of the variable light chain region of the full IgG. In certain embodiments, any of the Fzd binding region is fused to the heavy chain or the light chain via one or more linker moiety.

In another embodiment, any of the Fzd binding region comprises a Fab or Fv, and the LRP5/6 binding region comprises a Fab or Fv. In particular embodiments, the Fab of the Fzd binding region or the Fab or Fv of the LRP5/6 binding region is present within a full immunoglobulin (Ig), optionally an IgG, comprising a light chain and a heavy chain. In particular embodiments, only one of the Fab of the Fzd binding region or the Fab of the LRP5/6 binding region is present within the full immunoglobulin (Ig). In particular embodiments, the Fab or Fv of the Fzd binding region is present within the full Ig. In particular embodiments, the Fab or Fv of the LRP5/6 binding region is fused to the N-terminus of the Ig. In particular embodiments, the Fab of the LRP5/6 binding region is fused to the C-terminus of the Ig. In a further embodiment, the Fab of the LRP5/6 binding region is present within the full Ig. In particular embodiments, the Fab of the Fzd binding region is fused to the N-terminus of the Ig. In particular embodiments, the Fab of the Fzd binding region is fused to the C-terminus of the Ig. In some embodiments, the variable light chain region of the Fzd binding Fab is fused to the N-terminus of the variable heavy chain region of the full Ig, and the variable heavy

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chain region of the Fzd binding Fab is fused to the N-terminus of the variable light chain region of the full IgG. In some embodiments, the variable light chain region of the LRP5/6 binding Fv is fused to the N-terminus of the variable heavy chain region of the full Ig, and the variable heavy chain region of the LRP5/6 binding Fv is fused to the N-terminus of the variable light chain region of the full IgG. In some embodiments, the variable heavy chain region of the LRP5/6 binding Fv is fused to the N-terminus of the variable heavy chain region of the full Ig, and the variable light chain region of the LRP5/6 binding Fv is fused to the N-terminus of the variable light chain region of the full IgG. In some embodiments, variable light chain region of the Fzd binding Fv is fused to the N-terminus of the variable heavy chain region of the full Ig, and the variable heavy chain region of the Fzd binding Fv is fused to the N-terminus of the variable light chain region of the full IgG. In some embodiments, the variable heavy chain region of the Fzd binding Fv is fused to the N-terminus of the variable heavy chain region of the full Ig, and the variable light chain region of the Fzd binding Fv is fused to the N-terminus of the variable light chain region of the full IgG.

In another embodiment, any of the Fzd binding regions comprises a VHH or sdAb, and the LRP5/6 binding region comprises a VHH or sdAb. In particular embodiments, the Fzd binding region is fused to the Lrp5/6 binding region, and wherein the Fzd binding region or the LRP5/6 binding region is fused to an Fc region. In particular embodiments, the Fzd binding region is fused to the N-terminus of an Fc region, and wherein the LRP5/6 binding region is fused to the C-terminus of an Fc region. In particular embodiments, the Fzd binding region is fused to the C-terminus of an Fc region, and wherein the LRP5/6 binding region is fused to the N-terminus of an Fc region.

In a further embodiment, any of the antibodies or one or more antigen-binding fragment thereof, is humanized. In a further embodiment, any of the Wnt surrogate molecules binds to one or more Fzd receptor with a K_D of 50 μ M or lower. In further embodiments, any of the Wnt surrogate molecules binds to LRP5 and/or LRP6 with a K_D of 50 μ M or lower.

In a further embodiment, any of the Wnt surrogate molecule modulates a Wnt signaling pathway in a cell, optionally a mammalian cell. In particular embodiments, the Wnt surrogate molecule increases signaling via the Wnt signaling pathway in the cell. In particular embodiments, the Wnt signaling pathway is a canonical Wnt signaling pathway or a non-canonical Wnt signaling pathway.

In a related embodiment, the present disclosure provides an isolated polynucleotide encoding a polypeptide sequence comprising one or more of the Fzd binding regions and/or one or more of the LRP5/6 binding regions of a Wnt surrogate molecule. In particular embodiments, the present disclosure provides an expression vector comprising the isolated polynucleotide. In further particular embodiments, the present disclosure provides an isolated host cell comprising the expression vector.

In a related embodiment, the present disclosure provides a pharmaceutical composition comprising a physiologically acceptable excipient, diluent, or carrier, and a therapeutically effective amount of any of the Wnt surrogate molecules disclosed herein.

In related embodiments, the present disclosure provides a method for agonizing a Wnt signaling pathway in a cell, comprising contacting the cell any of the Wnt surrogate molecules, wherein the Wnt surrogate molecule is an agonist of a Wnt signaling pathway.

In particular embodiments, the present disclosure provides a method for treating a subject having a disease or disorder associated with reduced Wnt signaling, comprising administering to the subject an effective amount of the pharmaceutical composition, wherein the Wnt surrogate molecule is an agonist of a Wnt signaling pathway. In particular embodiments, the disease or disorder is selected from the group consisting of: bone fractures, osteoporosis (e.g., post-menopausal osteoporosis), osteoporotic fractures, spinal fusion, vertebral compression fracture, pre-operative spinal surgery optimization, osseointegration of orthopedic devices, tendon-bone integration, tooth growth and regeneration, dental implantation, periodontal diseases, maxillo-facial reconstruction, osteonecrosis of the jaw, osteoarthritis (OA), muscular dystrophy, muscle atrophy resulting from sarcopenia or cachexia, alopecia, hearing loss, including regeneration of inner and outer auditory hair cells, vestibular hypofunction, macular degeneration, vitreoretinopathy, diseases of retinal degeneration, including diabetic retinopathy, diseases/disorders affecting the integrity of the blood brain barrier, Fuchs' dystrophy, stroke, traumatic brain injury, Alzheimer's disease, multiple sclerosis, spinal cord injuries, oral mucositis, short bowel syndrome, inflammatory bowel diseases (IBD), including Crohn's disease (CD) and ulcerative colitis (UC), in particular IBD with fistula formation, metabolic syndrome, diabetes, dyslipidemia, pancreatitis, exocrine pancreatic insufficiency, wound healing, diabetic foot ulcers, coronary artery disease, acute kidney injuries, chronic kidney diseases, chronic obstructive pulmonary diseases (COPD), pulmonary fibrosis, including idiopathic pulmonary fibrosis, acute liver failure, acute alcoholic liver injuries, chronic liver diseases with hepatitis C virus (HCV), HCV subjects post-antiviral drug therapies, chronic liver diseases with hepatitis B virus (HBV), fibrosis, HBV subjects post-antiviral drug therapies, chronic alcoholic liver diseases, alcoholic hepatitis, non-alcoholic fatty liver diseases and non-alcoholic steatohepatitis (NASH), cirrhosis, and chronic liver insufficiencies of all causes. In certain embodiments, the disease or disorder is a bone disease or disorder. In particular embodiments, the disease or disorder is a bone disease or disorder, and the Wnt surrogate molecule binds Fzd1, Fzd2, and FZD7, and binds LRP5 and/or LRP6. In certain embodiments, the disease or disorder is a bone disease or disorder, and the Wnt surrogate molecule binds Fzd1, Fzd2, FZD7, Fzd5 and Fzd8, and also binds LRP5 and/or LRP6.

In another related embodiment, the present disclosure provides a method for increasing bone mineral density, increasing bone volume, increasing bone cortical thickness, increasing bone mineral apposition rate, increasing bone stiffness, increasing bone biomechanical strength, increasing resistance to bone fracture, decreasing bone resorption, or decreasing bone loss associated with osteoporosis, in a subject in need thereof, comprising providing to the subject an effective amount of a pharmaceutical composition comprising a Wnt surrogate molecule, wherein the Wnt surrogate molecule is an agonist of a Wnt signaling pathway. In certain embodiments, the Wnt surrogate molecule binds Fzd1, Fzd2, and FZD7, and binds LRP5 and/or LRP6. In certain embodiments, the Wnt surrogate molecule binds Fzd1, Fzd2, FZD7, Fzd5 and Fzd8, and also binds LRP5 and/or LRP6.

In particular embodiments, methods of the invention, including those related to treating or preventing a bone disease or disorder, such as osteoporosis (e.g., post-menopausal osteoporosis), further comprise providing the subject an antiresorptive agent (in combination with a Wnt surrogate

molecule). Examples of anti-resorptive agents include, but are not limited to, bisphosphonates or selective estrogen receptor modulators. Antiresorptive agents are used to increase bone strength in individuals with osteoporosis and include five principal classes of agents: bisphosphonates, estrogens, selective estrogen receptor modulators (SERMs), calcitonin and monoclonal antibodies such as denosumab, any of which may be used. Illustrative examples of antiresorptive agents include, but are not limited to: bisphosphonates, e.g., alendronate-generic medication (Brand name: Fosamax™, Fosamax™ Plus D), risedronate (Brand name: Actonel™, Actonel™ with Calcium), ibandronate (Brand name: Boniva™), and zoledronic acid (Brand name: Reclast™); other antiresorptives, e.g., estrogen therapy or hormone therapy, raloxifene (Brand name: Evista™), and denosumab (Prolia™); and anabolic medication, e.g., teriparatide (Forteo™).

In a further related embodiment, the present disclosure provides a method for increasing liver to body weight ratio, promoting liver regeneration, increasing liver cell proliferation or mitosis, decreasing liver fibrosis, optionally following a chronic liver injury, increasing hepatocyte function, or decreasing coagulation time in liver, in a subject in need thereof, comprising providing to the subject an effective amount of a pharmaceutical composition comprising a Wnt surrogate molecule, wherein the Wnt surrogate molecule is an agonist of a Wnt signaling pathway.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGS. 1A-D. Schematic diagrams of illustrative formats of Wnt surrogate molecules.

FIGS. 2A-2D. Characterization of a Wnt surrogate molecule, R2M3-26.

FIGS. 3A-3D. Characterization of a Wnt surrogate molecule, R2M3-32.

FIGS. 4A-4B. Graphs showing that R2M3-26 and R2M3-32 activities can be inhibited by soluble Fzd ECD and by R2M3 IgG alone without the Lrp binding arm.

FIG. 5. Characterization of illustrative R2M3-Lrp6 binder fusions in 293, Huh7, A375, and BNL.CL2 Wnt dependent reporter assays.

FIG. 6. Characterization of illustrative 18R5-Lrp6 binder fusions in 293, A375, and BNL.CL2 Wnt dependent reporter assays.

FIG. 7. Characterization of illustrative 18R5-Lrp5 binder fusions in 293 Wnt dependent reporter assays.

FIG. 8A-8B. Characterization of illustrative Fzd binders-Lrp6 binder 26 fusions in 293 Wnt dependent reporter assays.

FIG. 9. SAR analysis of illustrative Wnt surrogate molecules in the IgG-Nab fusion format.

FIG. 10A-10B. Characterization of R2M3-26 in the Fab format in 293 Wnt dependent reporter assays.

FIG. 11A-11B. Characterization of R2M3-32 in the Fab format in 293 Wnt dependent reporter assays.

FIG. 12A-12B. Characterization of R2M3-26 in the Hetero-Ig format in 293 Wnt dependent reporter assays.

FIG. 13. Characterization of 26-17SB9 in the VHH/sdAb-VHH/sdAb format, in different tandem formats, and on different ends of the Fc fragment in 293 Wnt dependent reporter assays.

FIG. 14A-14H. Characterization of 18R5-LRP6 Binder Fusions in tandem scFv formats in 293 Wnt dependent reporter assays.

FIG. 15A-15G. Characterization of various Wnt Surrogate molecules in the Fab-IgG format in 293 Wnt dependent reporter assays.

FIG. 16A-16C. Characterization of R2M3-26 in the F(ab')₂ format in 293 Wnt dependent reporter assays.

FIG. 17A-17H. Characterization of additional Wnt surrogate molecules in 293 Wnt dependent reporter assays

FIG. 18A-18C. A. Schematic diagram of the 2Fv-Ig format. B-C. Characterization of the Wnt surrogate molecule, 10SG11-1RC07.

FIG. 19. Sequences of polypeptide chains of illustrative Wnt surrogates molecules.

FIG. 20A-20B. In vivo PK/PD characterization of R2M3-26

FIGS. 21A-21E. Images and graphs showing that systemic expression of 18R5-DKK1c for 14 days results in increased bone mineral density. *P value<0.05; ** P value<0.0001. For each time point, the bars from left to right are as follows: vehicle (diamond), romosozumab (square), AAV CAG-GFP (triangle), AAV ScFv (anti-GFP)-DKK1cF234K-Flag-His (inverted triangle), and AAV 18R5-DKK1c-FLagHis (circle).

FIGS. 22A-22D. Images and graphs showing that systemic expression of 18R5-DKK1c for 14 days or 28 days results in increased bone volume. For each time point, the bars from left to right are as follows: vehicle, romosozumab, AAV CAG-GFP, AAV ScFv (anti-GFP)-DKK1cF234K-Flag-His, and AAV 18R5-DKK1c-FLagHis. *P value<0.05; ** P value<0.0001, ****P value<0.0001.

FIGS. 23A-23B. Graphs showing the dynamic parameters of bone formation based on fluorochrome labelling. For each time point, the bars from left to right are as follows: vehicle, romosozumab, AAV CAG-GFP, AAV ScFv (anti-GFP)-DKK1cF234K-Flag-His, and AAV 18R5-DKK1c-FlagHis.

FIGS. 24A-24D. Graphs and images showing that systemic expression of 18R5-DKK1c results in increased osteoblast and reduced osteoclast on bone surface. For each time point, the bars from left to right are as follows: vehicle, romosozumab, AAV CAG-GFP, AAV ScFv (anti-GFP)-DKK1cF234K-Flag-His, and AAV 18R5-DKK1c-FlagHis. ** P value<0.05.

FIGS. 25A-25C. Diagram of assay for bone stiffness and fracture and graphs showing ultimate load to failure and stiffness in mice treated as indicated.

FIGS. 26A-26D. Graphs and images showing that systemic treatment with R2M3-26 results in rapid and sustained increase in bone after one week. For each timepoint, the bars from left to right correspond to the treatments indicated from top to bottom. **** indicates P value<0.0001.

FIGS. 27A-27C. Images and graphs showing that R2M3-26 treatment rapidly reverses the bone loss associated with ovariectomy-induced osteoporosis. For each time point, the bars from left to right correspond to the treatments indicated from top to bottom.

FIGS. 28A-28C. Images and graphs showing that a single injection of R2M3-26 rapidly increases bone volume.

FIGS. 29A-29D. Graphs showing that high doses of R2M3-26 and 1R-C07-26 significantly and rapidly increase bone formation in naïve mice. For each timepoint, the bars from left to right correspond to the treatments indicated from top to bottom.

FIG. 30. Graphs showing that R2M3-26 and 1R-C07-3 increase bone mineral density in naïve mice. For each timepoint, the bars from left to right correspond to the treatments indicated from top to bottom.

FIG. 31. Graph showing changes in whole body bone mineral density (BMD) measured weekly in ovariectomized mice as compared with naïve and sham surgery operated mice.

FIG. 32. Changes in vertebral mineral density (image shown) and changes the vertebral resistance to compression fracture in vertebra isolated from mice after various treatments (bar graph) as measured in newton units of force (N) after 4 weeks of treatment.

FIGS. 33A-D. Test of Wnt surrogate molecules in an Einhorn fracture model. Radiographs of the callus after 1 week (A) and 6 weeks (B) of treatment with Wnt surrogate molecules are shown. Graphs of changes in whole body bone mineral density (BMD) in contralateral femur are shown (C). Scatter plots showing changes in callus tissue volume, callus bone volume, bone volume/tissue volume ratio (BV/TV), and bone mineral content per millimeter (BMC/mm) as shown in (D) along with representative images of bone slices are shown.

FIG. 34. Graph with changes in whole body bone mineral density (BMD) measured weekly with different Wnt surrogate molecule dosing schedules is shown.

FIG. 35. Graph with changes in whole body bone mineral density (BMD) measured weekly from mice treated with different Wnt surrogate molecule alone and in combination with Romosozumab is shown.

FIG. 36. Levels of therapeutic molecules in serum as measured by ELISA. These data accompany gene expression data presented in Table 4.

FIGS. 37A-37C. Liver (A), small intestine (B) and colon (C) to body weight ratio after treatment with AAV-delivered Wnt surrogates. (**) p<0.01. For each graph, the treatments shown from left to right correspond to those in the legend from top to bottom.

FIGS. 38A-38B. Body weight (A) and liver to body weight (B) ratio after treatment with recombinantly produced Wnt surrogate proteins. (*) p<0.05. For each time point, the treatments shown from left to right correspond to those in the legend from top to bottom.

FIGS. 39A-39D. Induction of proliferation markers in response to R2M3-26 and Rspo2 recombinant proteins. Liver Ki67 (A) and cyclinD1 (B) mRNA expression. Average count of PCNA (C) or phospho-histone H3 (D) positive nuclei per 10× field after immunohistochemistry staining with PCNA and phospho-histone H3 antibodies respectively. (*) p<0.05, (**) p<0.01, (***) p<0.001, (****) p<0.0001. For each time point, the treatments shown from left to right correspond to those in the legend from top to bottom.

FIGS. 40A-40H. Efficacy of AAV-delivered Wnt surrogate and R-Spondin in a thioacetamide-induced chronic liver disease model. Design for study 1 (A) and study 2 (B). Liver to body weight ratio (C-D), liver weight (E-F), liver collagen A1 mRNA expression (G) and percentage red area in liver histological sections stained with Sirius red (H) in response to AAV-delivered wnt surrogate and R-spondin in study 1 (C, E, G and H) and study 2 (D, F, H). (*) p<0.05, (**) p<0.01, (***) p<0.001, (****) p<0.0001. For each graph, the treatments shown from left to right correspond to those in the legend from top to bottom (not including baseline).

FIGS. 41A-41N. Efficacy of recombinantly produced Wnt surrogate and R-Spondin in a thioacetamide-induced chronic liver disease model. Study design (A). D-2, D0, D3, D7, D10, D14 represents days relative to the start of treatment with recombinant proteins. Liver to body weight ratio (B, C), liver axin2 mRNA (D, E), cyclinD1 mRNA (F, G) and Ki67 mRNA (H, I) expression, average count of PCNA (J, K) or phospho-histone H3 (L, M) positive nuclei per 10×

field after immunohistochemistry staining with PCNA and phospho-histone H3 antibodies respectively, in a study using Rspo2 mono treatment (B, D, F, H, J, L) or R2M3-26/Rspo2 combination treatment (C, E, G, I, K, M). Pro-thrombin time ratio relative to the average pro-thrombin time in plasma collected from control naïve mice without TAA exposure (N). (*) $p < 0.05$, (**) $p < 0.01$, (****) $p < 0.0001$. No TAA treatment is indicated by the dashed line. For each bar graph timepoint, the treatments shown from left to right correspond to those in the legend shown above from top to bottom.

FIGS. 42A-42C. Efficacy of recombinantly produced Wnt surrogate and R-Spondin in a CC14-induced chronic liver disease model. Study design (A). Liver to body weight ratio (B) pro-thrombin time (C) and sirius red staining (D) in response to CC14 treatment, R2M3-26 and Rspo2. (*) $p < 0.05$, (**) $p < 0.01$, (****) $p < 0.0001$. For each graph, the treatments shown from left to right correspond to those in the legend from top to bottom (not including baseline).

FIGS. 43A-43D. Induction of proliferation markers in response to recombinantly produced Wnt surrogate in an acetaminophen-induced acute liver injury model. Study design (A). Serum level of alanine transferase at 24 and 48 hours after treatment with acetaminophen (B). Relative cyclinD1 (C) and Ki67 (D) mRNA expression. (*) $p < 0.05$, (****) $p < 0.0001$.

FIGS. 44A-44D. Induction of proliferation markers in response to R-Spondin in an acetaminophen-induced acute liver injury model. Study design (A). Serum level of alanine transferase at 24 and 48 hours after treatment with acetaminophen (B). Relative cyclinD1 (C) and Ki67 (D) mRNA expression. (**) $p < 0.01$, (****) $p < 0.0001$.

FIGS. 45A-45D. Induction of proliferation markers in response to Wnt surrogate and R-Spondin in an acetaminophen-induced acute liver injury model. Study design (A). Serum level of alanine transferase at 24, 36, 48 and 60 hours after treatment with acetaminophen (B). Relative cyclinD1 (C) and Ki67 (D) mRNA expression. (*) $p < 0.05$, (****) $p < 0.0001$. For each time point, the treatments shown from left to right correspond to those in the legend from top to bottom.

FIGS. 46A-46D. Efficacy of recombinantly produced Wnt surrogate and R-Spondin on the survival of mice after acetaminophen-induced liver injury. Study design (A). Survival curve of mice treated with the control anti-eGFP control protein or R2M3-26 (B), Rspo2 (C) or a combination of R2M3-26 and Rspo2 (D) recombinant proteins.

DETAILED DESCRIPTION

The present disclosure relates to Wnt surrogate molecules that bind to one or more Fzd receptor and one or more LRP5 or LRP6 receptor and modulate a downstream Wnt signaling pathway. In particular embodiments, the Wnt surrogate molecules activate a Wnt signaling pathway or increase signaling via a Wnt signaling pathway. In particular embodiments, the Wnt surrogate molecules disclosed herein comprise: (i) one or more antibodies or antigen-binding fragments thereof that specifically bind to one or more Fzd receptor, including antibodies or antigen-binding fragments thereof having particular Fzd receptor specificity and/or functional properties; and (ii) one or more antibodies or antigen-binding fragments thereof that specifically bind to LRP5 and/or LRP6. Certain embodiments encompass specific structural formats or arrangements of the Fzd binding region(s) and LRP5/6 binding region(s) of Wnt surrogate

molecules advantageous in increasing downstream Wnt pathway signaling and related biological effects.

Embodiments of the invention pertain to the use of Wnt surrogate molecules for the diagnosis, assessment and treatment of diseases and disorders associated with Wnt signaling pathways. In certain embodiments, the subject Wnt surrogate molecules are used to modulate a Wnt signaling pathway in a cell or tissue. In certain embodiments, the subject Wnt surrogate molecules are used in the treatment or prevention of diseases and disorders associated with aberrant or deregulated (e.g., reduced) Wnt signaling, or for which modulating, e.g., increasing, Wnt signaling would provide a therapeutic benefit.

The practice of the present invention will employ, unless indicated specifically to the contrary, conventional methods of virology, immunology, microbiology, molecular biology and recombinant DNA techniques within the skill of the art, many of which are described below for the purpose of illustration. Such techniques are explained fully in the literature. See, e.g., *Current Protocols in Molecular Biology or Current Protocols in Immunology*, John Wiley & Sons, New York, N.Y. (2009); Ausubel et al., *Short Protocols in Molecular Biology*, 3rd ed., Wiley & Sons, 1995; Sambrook and Russell, *Molecular Cloning: A Laboratory Manual* (3rd Edition, 2001); Maniatis et al. *Molecular Cloning: A Laboratory Manual* (1982); *DNA Cloning: A Practical Approach*, vol. I & II (D. Glover, ed.); *Oligonucleotide Synthesis* (N. Gait, ed., 1984); *Nucleic Acid Hybridization* (B. Hames & S. Higgins, eds., 1985); *Transcription and Translation* (B. Hames & S. Higgins, eds., 1984); *Animal Cell Culture* (R. Freshney, ed., 1986); Perbal, *A Practical Guide to Molecular Cloning* (1984) and other like references.

As used in this specification and the appended claims, the singular forms “a,” “an” and “the” include plural references unless the content clearly dictates otherwise.

Throughout this specification, unless the context requires otherwise, the word “comprise”, or variations such as “comprises” or “comprising”, will be understood to imply the inclusion of a stated element or integer or group of elements or integers but not the exclusion of any other element or integer or group of elements or integers.

Each embodiment in this specification is to be applied mutatis mutandis to every other embodiment unless expressly stated otherwise.

Standard techniques may be used for recombinant DNA, oligonucleotide synthesis, and tissue culture and transformation (e.g., electroporation, lipofection). Enzymatic reactions and purification techniques may be performed according to manufacturer's specifications or as commonly accomplished in the art or as described herein. These and related techniques and procedures may be generally performed according to conventional methods well known in the art and as described in various general and more specific references that are cited and discussed throughout the present specification. Unless specific definitions are provided, the nomenclature utilized in connection with, and the laboratory procedures and techniques of, molecular biology, analytical chemistry, synthetic organic chemistry, and medicinal and pharmaceutical chemistry described herein are those well-known and commonly used in the art. Standard techniques may be used for recombinant technology, molecular biological, microbiological, chemical syntheses, chemical analyses, pharmaceutical preparation, formulation, and delivery, and treatment of subjects.

Embodiments of the present invention relate to antibodies and antigen-binding fragments thereof that bind to one or more Fzd receptor. Sequences of illustrative antibodies, or

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antigen-binding fragments, or complementarity determining regions (CDRs) thereof, are set forth in SEQ ID NOs:1-65 or 129-132, Tables 1A and 1B, and Table 3. Anti-Fzd antibodies and antigen-binding fragments there that may be used or present in the Wnt surrogate molecules disclosed herein include, but are not limited to, those described in the U.S. provisional application No. 62/607,877, titled Anti-Frizzled Antibodies and Methods of Use, filed on Dec. 19, 2017.

Embodiments of the present invention relate to antibodies and antigen-binding fragments thereof that bind to LRP5 and/or LRP6. Sequences of illustrative antibodies, or antigen-binding fragments, or complementarity determining regions (CDRs) thereof, are set forth in SEQ ID NOs:66-88 or 133, Tables 2A and 2B, and Table 3. Anti-LRP5/6 antibodies and antigen-binding fragments there that may be used or present in the Wnt surrogate molecules disclosed herein include, but are not limited to, those described in the U.S. provisional application No. 62/607,879, titled Anti-LRP5/6 Antibodies and Methods of Use, filed on Dec. 19, 2017.

As is well known in the art, an antibody is an immunoglobulin molecule capable of specific binding to a target, such as a carbohydrate, polynucleotide, lipid, polypeptide, etc., through at least one epitope recognition site, located in the variable region of the immunoglobulin molecule. As used herein, the term encompasses not only intact polyclonal or monoclonal antibodies, but also fragments thereof (such as dAb, Fab, Fab', F(ab')₂, Fv), single chain (scFv), VHH or sdAb, synthetic variants thereof, naturally occurring variants, fusion proteins comprising an antibody or an antigen-binding fragment thereof, humanized antibodies, chimeric antibodies, and any other modified configuration of the immunoglobulin molecule that comprises an antigen-binding site or fragment (epitope recognition site) of the required specificity. "Diabodies" or 2scFV-Ig antibodies, are multivalent or multispecific fragments constructed by gene fusion (WO94/13804; P. Holliger et al., Proc. Natl. Acad. Sci. USA 90 6444-6448, 1993) are also a particular form of antibody contemplated herein. Minibodies comprising a scFv joined to a CH3 domain are also included herein (S. Hu et al., Cancer Res., 56, 3055-3061, 1996). See e.g., Ward, E. S. et al., Nature 341, 544-546 (1989); Bird et al., Science, 242, 423-426, 1988; Huston et al., PNAS USA, 85, 5879-5883, 1988); PCT/US92/09965; WO94/13804; P. Holliger et al., Proc. Natl. Acad. Sci. USA 90 6444-6448, 1993; Y. Reiter et al., Nature Biotech, 14, 1239-1245, 1996; S. Hu et al., Cancer Res., 56, 3055-3061, 1996.

The term "antigen-binding fragment" as used herein refers to a polypeptide fragment that contains at least one CDR of an immunoglobulin heavy and/or light chain, or of a VHH or sdAb, that binds to the antigen of interest, in particular to one or more Fzd receptor or LRP5 or LRP6 receptor. In this regard, an antigen-binding fragment of the herein described antibodies may comprise 1, 2, 3, 4, 5, or all 6 CDRs of a VH and VL sequence set forth herein from antibodies that bind one or more Fzd receptor or LRP5 and/or LRP6. In particular embodiments, an antigen-binding fragment may comprise all three VH CDRs or all three VL CDRs. Similarly, an antigen binding fragment thereof may comprise all three CDRs of a VHH or sdAb. An antigen-binding fragment of a Fzd-specific antibody is capable of binding to a Fzd receptor. An antigen-binding fragment of a LRP5/6-specific antibody is capable of binding to a LRP5 and/or LRP6 receptor. As used herein, the term encompasses not only isolated fragments but also polypeptides comprising an antigen-binding fragment of an antibody disclosed

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herein, such as, for example, fusion proteins comprising an antigen-binding fragment of an antibody disclosed herein, such as, e.g., a fusion protein comprising a VHH or sdAb that binds one or more Fzd receptors and a VHH or sdAb that binds LRP5 and/or LRP6.

The term "antigen" refers to a molecule or a portion of a molecule capable of being bound by a selective binding agent, such as an antibody, and additionally capable of being used in an animal to produce antibodies capable of binding to an epitope of that antigen. In certain embodiments, a binding agent (e.g., a Wnt surrogate molecule or binding region thereof) is said to specifically bind an antigen when it preferentially recognizes its target antigen in a complex mixture of proteins and/or macromolecules. In certain embodiments, a Wnt surrogate molecule or binding region thereof (e.g., an antibody or antigen-binding fragment thereof) is said to specifically bind an antigen when the equilibrium dissociation constant is $\leq 10^{-7}$ or $\leq 10^{-8}$ M. In some embodiments, the equilibrium dissociation constant may be $\leq 10^{-9}$ M or $\leq 10^{-10}$ M.

In certain embodiments, antibodies and antigen-binding fragments thereof as described herein include a heavy chain and a light chain CDR set, respectively interposed between a heavy chain and a light chain framework region (FR) set which provide support to the CDRs and define the spatial relationship of the CDRs relative to each other. As used herein, the term "CDR set" refers to the three hypervariable regions of a heavy or light chain V region. Proceeding from the N-terminus of a heavy or light chain, these regions are denoted as "CDR1," "CDR2," and "CDR3" respectively. An antigen-binding site, therefore, includes six CDRs, comprising the CDR set from each of a heavy and a light chain V region. A polypeptide comprising a single CDR, (e.g., a CDR1, CDR2 or CDR3) is referred to herein as a "molecular recognition unit." Crystallographic analysis of a number of antigen-antibody complexes has demonstrated that the amino acid residues of CDRs form extensive contact with bound antigen, wherein the most extensive antigen contact is with the heavy chain CDR3. Thus, the molecular recognition units are primarily responsible for the specificity of an antigen-binding site.

As used herein, the term "FR set" refers to the four flanking amino acid sequences which frame the CDRs of a CDR set of a heavy or light chain V region. Some FR residues may contact bound antigen; however, FRs are primarily responsible for folding the V region into the antigen-binding site, particularly the FR residues directly adjacent to the CDRs. Within FRs, certain amino residues and certain structural features are very highly conserved. In this regard, all V region sequences contain an internal disulfide loop of around 90 amino acid residues. When the V regions fold into a binding-site, the CDRs are displayed as projecting loop motifs which form an antigen-binding surface. It is generally recognized that there are conserved structural regions of FRs which influence the folded shape of the CDR loops into certain "canonical" structures—regardless of the precise CDR amino acid sequence. Further, certain FR residues are known to participate in non-covalent interdomain contacts which stabilize the interaction of the antibody heavy and light chains.

The structures and locations of immunoglobulin CDRs and variable domains may be determined by reference to Kabat, E. A. et al., Sequences of Proteins of Immunological Interest, 4th Edition. US Department of Health and Human Services, 1987, and updates thereof, now available on the Internet (immuno.bme.nwu.edu). Alternatively, CDRs may be determined by using "IMGT®, the international ImMu-

noGeneTics information System® available at www.imgt.org (see, e.g., Lefranc, M.-P. et al. (1999) *Nucleic Acids Res.*, 27:209-212; Ruiz, M. et al. (2000) *Nucleic Acids Res.*, 28:219-221; Lefranc, M.-P. (2001) *Nucleic Acids Res.*, 29:207-209; Lefranc, M.-P. (2003) *Nucleic Acids Res.*, 31:307-310; Lefranc, M.-P. et al. (2004) *In Silico Biol.*, 5, 0006 [Epub], 5:45-60 (2005)); Lefranc, M.-P. et al. (2005) *Nucleic Acids Res.*, 33:D593-597; Lefranc, M.-P. et al. (2009) *Nucleic Acids Res.*, 37:D1006-1012; Lefranc, M.-P. et al. (2015) *Nucleic Acids Res.*, 43:D413-422).

A “monoclonal antibody” refers to a homogeneous antibody population wherein the monoclonal antibody is comprised of amino acids (naturally occurring and non-naturally occurring) that are involved in the selective binding of an epitope. Monoclonal antibodies are highly specific, being directed against a single epitope. The term “monoclonal antibody” encompasses not only intact monoclonal antibodies and full-length monoclonal antibodies, but also fragments thereof (such as Fab, Fab', F(ab')₂, Fv), single chain (scFv), VHH or sdAb, variants thereof, fusion proteins comprising an antigen-binding fragment of a monoclonal antibody, humanized monoclonal antibodies, chimeric monoclonal antibodies, and any other modified configuration of the immunoglobulin molecule that comprises an antigen-binding fragment (epitope recognition site) of the required specificity and the ability to bind to an epitope, including Wnt surrogate molecules disclosed herein. It is not intended to be limited as regards the source of the antibody or the manner in which it is made (e.g., by hybridoma, phage selection, recombinant expression, transgenic animals, etc.). The term includes whole immunoglobulins as well as the fragments etc. described above under the definition of “antibody”.

The proteolytic enzyme papain preferentially cleaves IgG molecules to yield several fragments, two of which (the F(ab) fragments) each comprise a covalent heterodimer that includes an intact antigen-binding site. The enzyme pepsin is able to cleave IgG molecules to provide several fragments, including the F(ab')₂ fragment which comprises both antigen-binding sites. An Fv fragment for use according to certain embodiments of the present invention can be produced by preferential proteolytic cleavage of an IgM, and on rare occasions of an IgG or IgA immunoglobulin molecule. Fv fragments are, however, more commonly derived using recombinant techniques known in the art. The Fv fragment includes a non-covalent V_H:V_L heterodimer including an antigen-binding site which retains much of the antigen recognition and binding capabilities of the native antibody molecule. Inbar et al. (1972) *Proc. Nat. Acad. Sci. USA* 69:2659-2662; Hochman et al. (1976) *Biochem* 15:2706-2710; and Ehrlich et al. (1980) *Biochem* 19:4091-4096.

In certain embodiments, single chain Fv or scFV antibodies are contemplated. For example, Kappa bodies (III et al., *Prot. Eng.* 10: 949-57 (1997); minibodies (Martin et al., *EMBO J* 13: 5305-9 (1994); diabodies (Holliger et al., *PNAS* 90: 6444-8 (1993); or Janusins (Traunecker et al., *EMBO J* 10: 3655-59 (1991) and Traunecker et al., *Int. J. Cancer Suppl.* 7: 51-52 (1992)), may be prepared using standard molecular biology techniques following the teachings of the present application with regard to selecting antibodies having the desired specificity. In still other embodiments, bispecific or chimeric antibodies may be made that encompass the ligands of the present disclosure. For example, a chimeric antibody may comprise CDRs and framework regions from different antibodies, while bispecific antibodies may be generated that bind specifically to one or more Fzd receptor through one binding domain and to a second molecule

through a second binding domain. These antibodies may be produced through recombinant molecular biological techniques or may be physically conjugated together.

A single chain Fv (scFv) polypeptide is a covalently linked V_H:V_L heterodimer which is expressed from a gene fusion including V_H- and V_L-encoding genes linked by a peptide-encoding linker. Huston et al. (1988) *Proc. Nat. Acad. Sci. USA* 85(16):5879-5883. A number of methods have been described to discern chemical structures for converting the naturally aggregated—but chemically separated—light and heavy polypeptide chains from an antibody V region into an scFv molecule which will fold into a three dimensional structure substantially similar to the structure of an antigen-binding site. See, e.g., U.S. Pat. Nos. 5,091,513 and 5,132,405, to Huston et al.; and U.S. Pat. No. 4,946,778, to Ladner et al.

In certain embodiments, an antibody as described herein is in the form of a diabody. Diabodies are multimers of polypeptides, each polypeptide comprising a first domain comprising a binding region of an immunoglobulin light chain and a second domain comprising a binding region of an immunoglobulin heavy chain, the two domains being linked (e.g. by a peptide linker) but unable to associate with each other to form an antigen binding site: antigen binding sites are formed by the association of the first domain of one polypeptide within the multimer with the second domain of another polypeptide within the multimer (WO94/13804).

A dAb fragment of an antibody consists of a V_H domain (Ward, E. S. et al., *Nature* 341, 544-546 (1989)).

Where bispecific antibodies are to be used, these may be conventional bispecific antibodies, which can be manufactured in a variety of ways (Holliger, P. and Winter G. *Current Opinion Biotechnol.* 4, 446-449 (1993)), e.g. prepared chemically or from hybrid hybridomas, or may be any of the bispecific antibody fragments mentioned above. Diabodies and scFv can be constructed without an Fc region, using only variable domains, potentially reducing the effects of anti-idiotypic reaction.

Bispecific diabodies, as opposed to bispecific whole antibodies, may also be particularly useful because they can be readily constructed and expressed in *E. coli*. Diabodies (and many other polypeptides such as antibody fragments) of appropriate binding specificities can be readily selected using phage display (WO94/13804) from libraries. If one arm of the diabody is to be kept constant, for instance, with a specificity directed against antigen X, then a library can be made where the other arm is varied and an antibody of appropriate specificity selected. Bispecific whole antibodies may be made by knobs-into-holes engineering (J. B. B. Ridgeway et al., *Protein Eng.*, 9, 616-621, 1996).

In certain embodiments, the antibodies described herein may be provided in the form of a UniBody®. A UniBody® is an IgG4 antibody with the hinge region removed (see GenMab Utrecht, The Netherlands; see also, e.g., US20090226421). This proprietary antibody technology creates a stable, smaller antibody format with an anticipated longer therapeutic window than current small antibody formats. IgG4 antibodies are considered inert and thus do not interact with the immune system. Fully human IgG4 antibodies may be modified by eliminating the hinge region of the antibody to obtain half-molecule fragments having distinct stability properties relative to the corresponding intact IgG4 (GenMab, Utrecht). Halving the IgG4 molecule leaves only one area on the UniBody® that can bind to cognate antigens (e.g., disease targets) and the UniBody® therefore binds univalently to only one site on target cells.

In certain embodiments, the antibodies of the present disclosure may take the form of a VHH or sdAb. VHH or sdAb technology was originally developed following the discovery and identification that camelidae (e.g., camels and llamas) possess fully functional antibodies that consist of heavy chains only and therefore lack light chains. These heavy-chain only antibodies contain a single variable domain (V_{HH}) and two constant domains (C_{H2} , C_{H3}). The cloned and isolated single variable domains have full antigen binding capacity and are very stable. These single variable domains, with their unique structural and functional properties, form the basis of "VHH or sdAb". VHH or sdAb are encoded by single genes and are efficiently produced in almost all prokaryotic and eukaryotic hosts e.g. *E. coli* (see e.g. U.S. Pat. No. 6,765,087), molds (for example *Aspergillus* or *Trichoderma*) and yeast (for example *Saccharomyces*, *Kluyvermyces*, *Hansenula* or *Pichia* (see e.g. U.S. Pat. No. 6,838,254). The production process is scalable and multi-kilogram quantities of VHH or sdAb have been produced. VHH or sdAb may be formulated as a ready-to-use solution having a long shelf life. The VHH or sdAb method (see, e.g., WO 06/079372) is a proprietary method for generating VHH or sdAb against a desired target, based on automated high-throughput selection of B-cells. VHH or sdAb are single-domain antigen-binding fragments of camelid-specific heavy-chain only antibodies. VHH antibodies or sdAb, typically have a small size of around 15 kDa.

In certain embodiments, the antibodies or antigen-binding fragments thereof as disclosed herein are humanized. This refers to a chimeric molecule, generally prepared using recombinant techniques, having an antigen-binding site derived from an immunoglobulin from a non-human species and the remaining immunoglobulin structure of the molecule based upon the structure and/or sequence of a human immunoglobulin. The antigen-binding site may comprise either complete variable domains fused onto constant domains or only the CDRs grafted onto appropriate framework regions in the variable domains. Epitope binding sites may be wild type or modified by one or more amino acid substitutions. This eliminates the constant region as an immunogen in human individuals, but the possibility of an immune response to the foreign variable region remains (LoBuglio, A. F. et al., (1989) *Proc Natl Acad Sci USA* 86:4220-4224; Queen et al., *PNAS* (1988) 86:10029-10033; Riechmann et al., *Nature* (1988) 332:323-327). Illustrative methods for humanization of the anti-Fzd antibodies disclosed herein include the methods described in U.S. Pat. No. 7,462,697.

Another approach focuses not only on providing human-derived constant regions, but modifying the variable regions as well so as to reshape them as closely as possible to human form. It is known that the variable regions of both heavy and light chains contain three complementarity-determining regions (CDRs) which vary in response to the epitopes in question and determine binding capability, flanked by four framework regions (FRs) which are relatively conserved in a given species and which putatively provide a scaffolding for the CDRs. When nonhuman antibodies are prepared with respect to a particular epitope, the variable regions can be "reshaped" or "humanized" by grafting CDRs derived from nonhuman antibody on the FRs present in the human antibody to be modified. Application of this approach to various antibodies has been reported by Sato, K., et al., (1993) *Cancer Res* 53:851-856. Riechmann, L., et al., (1988) *Nature* 332:323-327; Verhoeven, M., et al., (1988) *Science* 239:1534-1536; Kettleborough, C. A., et al., (1991) *Protein Engineering* 4:773-778; Maeda, H., et al., (1991) *Human*

Antibodies Hybridoma 2:124-134; Gorman, S. D., et al., (1991) *Proc Natl Acad Sci USA* 88:4181-4185; Tempest, P. R., et al., (1991) *Bio/Technology* 9:266-271; Co, M. S., et al., (1991) *Proc Natl Acad Sci USA* 88:2869-2873; Carter, P., et al., (1992) *Proc Natl Acad Sci USA* 89:4285-4289; and Co, M. S. et al., (1992) *J Immunol* 148:1149-1154. In some embodiments, humanized antibodies preserve all CDR sequences (for example, a humanized mouse antibody which contains all six CDRs from the mouse antibodies). In other embodiments, humanized antibodies have one or more CDRs (one, two, three, four, five, six) which are altered with respect to the original antibody, which are also termed one or more CDRs "derived from" one or more CDRs from the original antibody.

In certain embodiments, the antibodies of the present disclosure may be chimeric antibodies. In this regard, a chimeric antibody is comprised of an antigen-binding fragment of an antibody operably linked or otherwise fused to a heterologous Fc portion of a different antibody. In certain embodiments, the heterologous Fc domain is of human origin. In other embodiments, the heterologous Fc domain may be from a different Ig class from the parent antibody, including IgA (including subclasses IgA1 and IgA2), IgD, IgE, IgG (including subclasses IgG1, IgG2, IgG3, and IgG4), and IgM. In further embodiments, the heterologous Fc domain may be comprised of CH2 and CH3 domains from one or more of the different Ig classes. As noted above with regard to humanized antibodies, the antigen-binding fragment of a chimeric antibody may comprise only one or more of the CDRs of the antibodies described herein (e.g., 1, 2, 3, 4, 5, or 6 CDRs of the antibodies described herein), or may comprise an entire variable domain (VL, VH or both).

Wnt Surrogates

The disclosure provides, in certain aspects, Wnt surrogate molecules that bind both one or more Fzd receptors and one or both of LRP5 and/or LRP6. Wnt surrogate molecules may also be referred to as "Wnt surrogates" or "Wnt mimetics." In particular embodiments, the Wnt surrogate molecules bind one or more human Fzd receptors and one or both of a human LRP5 and/or a human LRP6.

In certain embodiments, a Wnt surrogate molecule is capable of modulating or modulates Wnt signaling events in a cell contacted with the Wnt surrogate molecule. In certain embodiments, the Wnt surrogate molecule increases Wnt signaling, e.g., via the canonical Wnt/ β -catenin pathway. In certain embodiments, the Wnt surrogate molecule specifically modulates the biological activity of a human Wnt signaling pathway.

Wnt surrogate molecules of the present invention are biologically active in binding to one or more Fzd receptor and to one or more of LRP5 and LRP6, and in activation of Wnt signaling, i.e., the Wnt surrogate molecule is a Wnt agonist. The term "Wnt agonist activity" refers to the ability of an agonist to mimic the effect or activity of a Wnt protein binding to a frizzled protein and/or LRP5 or LRP6. The ability of the Wnt surrogate molecules and other Wnt agonists disclosed herein to mimic the activity of Wnt can be confirmed by a number of assays. Wnt agonists typically initiate a reaction or activity that is similar to or the same as that initiated by the receptor's natural ligand. In particular, the Wnt agonists disclosed herein activate, enhance or increase the canonical Wnt/ β -catenin signaling pathway. As used herein, the term "enhances" refers to a measurable increase in the level of Wnt/ β -catenin signaling compared with the level in the absence of a Wnt agonist, e.g., a Wnt surrogate molecule disclosed herein. In particular embodi-

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ments, the increase in the level of Wnt/ β -catenin signaling is at least 10%, at least 20%, at least 50%, at least two-fold, at least five-fold, at least 10-fold, at least 20-fold, at least 50-fold, or at least 100-fold as compared to the level of Wnt/ β -catenin signaling in the absence of the Wnt agonist, e.g., in the same cell type. Methods of measuring Wnt/ β -catenin signaling are known in the art and include those described herein.

In particular embodiments, Wnt surrogate molecules disclosed herein are bispecific, i.e., they specifically bind to two or more different epitopes, e.g., one or more Fzd receptor, and LRP5 and/or LRP6.

In particular embodiments, Wnt surrogate molecules disclosed herein are multivalent, e.g., they comprise two or more regions that each specifically bind to the same epitope, e.g., two or more regions that bind to an epitope within one or more Fzd receptor and/or two or more regions that bind to an epitope within LRP5 and/or LRP6. In particular embodiments, they comprise two or more regions that bind to an epitope within one or more Fzd receptor and two or more regions that bind to an epitope within LRP5 and/or LRP6. In certain embodiments, Wnt surrogate molecules comprise a ratio of the number of regions that bind one or more Fzd receptor to the number of regions that bind LRP5 and/or LRP6 of or about: 1:1, 2:1, 3:1, 4:1, 5:1, 6:1, 2:3, 2:5, 2:7, 7:2, 5:2, 3:2, 3:4, 3:5, 3:7, 3:8, 8:3, 7:3, 5:3, 4:3, 4:5, 4:7, 4:9, 9:4, 7:4, 5:4, 6:7, 7:6, 1:2, 1:3, 1:4, 1:5, or 1:6. In certain embodiments, Wnt surrogate molecules are bispecific and multivalent.

Wnt surrogate molecules disclosed herein may have any of a variety of different structural formats or configurations. Wnt surrogate molecules may comprise polypeptides and/or non-polypeptide binding moieties, e.g., small molecules. In particular embodiments, Wnt surrogate molecules comprise both a polypeptide region and a non-polypeptide binding moiety. In certain embodiments, Wnt surrogate molecules may comprise a single polypeptide, or they may comprise two or more, three or more, or four or more polypeptides. In certain embodiments, one or more polypeptides of a Wnt surrogate molecule are antibodies or antigen-binding fragments thereof. In certain embodiments, Wnt surrogates comprise two antibodies or antigen binding fragments thereof, one that binds one or more Fzd and one that binds LRP5 and/or LRP6. In certain embodiments, the Wnt surrogates comprises one, two, three, or four polypeptides, e.g., linked or bound to each other or fused to each other.

When the Wnt surrogate molecules comprise a single polypeptide, they may be a fusion protein comprising one or more Fzd binding domain and one or more LRP5/6 binding domain. The binding domains may be directly fused or they may be connected via a linker, e.g., a polypeptide linker, including but not limited to any of those disclosed herein.

When the Wnt surrogate molecules comprise two or more polypeptides, the polypeptides may be linked via covalent bonds, such as, e.g., disulfide bonds, and/or noncovalent interactions. For example, heavy chains of human immunoglobulin IgG interact at the level of their CH3 domains directly, whereas, at the level of their CH2 domains, they interact via the carbohydrate attached to the asparagine (Asn) N84.4 in the DE turn. In particular embodiments, the Wnt surrogate molecules comprise one or more regions derived from an antibody or antigen-binding fragment thereof, e.g., antibody heavy chains or antibody light chains or fragments thereof. In certain embodiments, a Wnt surrogate polypeptide comprises two antibody heavy chain regions (e.g., hinge regions) bound together via one or more disulfide bond. In certain embodiments, a Wnt surrogate

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polypeptide comprises an antibody light chain region (e.g., a CL region) and an antibody heavy chain region (e.g., a CH1 region) bound together via one or more disulfide bond.

Wnt surrogate polypeptides may be engineered to facilitate binding between two polypeptides. For example, Knob-into-holes amino acid modifications may be introduced into two different polypeptides to facilitate their binding. Knob-into-holes amino acid (AA) changes is a rational design strategy developed in antibody engineering, used for heterodimerization of the heavy chains, in the production of bispecific IgG antibodies. AA changes are engineered in order to create a knob on the C_H3 of the heavy chains from a first antibody and a hole on the C_H3 of the heavy chains of a second antibody. The knob may be represented by a tyrosine (Y) that belongs to the 'very large' IMGT volume class of AA, whereas the hole may be represented by a threonine (T) that belongs to the 'small' IMGT volume class. Other means of introducing modifications into polypeptides to facilitate their binding are known and available in the art. For example, specific amino acids may be introduced and used for cross-linking, such as Cysteine to form an intermolecular disulfide bond.

Wnt surrogate molecules may have a variety of different structural formats, including but not limited to those shown in FIG. 1.

In one embodiment, a Wnt surrogate molecule comprises an scFv or antigen-binding fragment thereof fused to a VHH or sdAb or antigen-binding fragment thereof. In certain embodiments, the scFv specifically binds one or more Fzd receptor, and the VHH or sdAb specifically binds LRP5 and/or LRP6. In certain embodiments, the scFv specifically binds LRP5 and/or LRP6, and the VHH or sdAb specifically binds one or more Fzd receptor. In particular embodiments, the scFv or antigen-binding fragment thereof is fused directly to the VHH or sdAb or antigen-binding fragment thereof, whereas in other embodiments, the two binding regions are fused via a linker moiety. In particular embodiments, the VHH or sdAb is fused to the N-terminus of the scFv, while in other embodiments, the VHH or sdAb is fused to the C-terminus of the scFv. In particular embodiments, the scFv is described herein or comprises any of the CDR sets described herein. In particular embodiments, the VHH or sdAb is described herein or comprises any of the CDR sets disclosed herein.

In various embodiments, including but not limited to those depicted in FIG. 1A, a Wnt surrogate molecule comprises one or more Fab or antigen-binding fragment thereof and one or more VHH or sdAb or antigen-binding fragment thereof (or alternatively, one or more scFv or antigen-binding fragment thereof). In certain embodiments, the Fab specifically binds one or more Fzd receptor, and the VHH or sdAb (or scFv) specifically binds LRP5 and/or LRP6. In certain embodiments, the Fab specifically binds LRP5 and/or LRP6, and the VHH or sdAb (or scFv) specifically binds one or more Fzd receptor. In certain embodiments, the VHH or sdAb (or scFv) is fused to the N-terminus of the Fab, while in some embodiments, the VHH or sdAb (or scFv) is fused to the C-terminus of the Fab. In particular embodiments, the Fab is present in a full IgG format, and the VHH or sdAb (or scFv) is fused to the N-terminus and/or C-terminus of the IgG light chain. In particular embodiments, the Fab is present in a full IgG format, and the VHH or sdAb (or scFv) is fused to the N-terminus and/or C-terminus of the IgG heavy chain. In particular embodiments, two or more VHH or sdAb (or scFvs) are fused to the IgG at any combination of these locations.

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Fabs may be converted into a full IgG format that includes both the Fab and Fc fragments, for example, using genetic engineering to generate a fusion polypeptide comprising the Fab fused to an Fc region, i.e., the Fab is present in a full IgG format. The Fc region for the full IgG format may be derived from any of a variety of different Fcs, including but not limited to, a wild-type or modified IgG1, IgG2, IgG3, IgG4 or other isotype, e.g., wild-type or modified human IgG1, human IgG2, human IgG3, human IgG4, human IgG4Pro (comprising a mutation in core hinge region that prevents the formation of IgG4 half molecules), human IgA, human IgE, human IgM, or the modified IgG1 referred to as IgG1 LALAPG. The L₂₃₅A, P₃₂₉G (LALA-PG) variant has been shown to eliminate complement binding and fixation as well as Fc-γ dependent antibody-dependent cell-mediated cytotoxicity (ADCC) in both murine IgG2a and human IgG1. These LALA-PG substitutions allow a more accurate translation of results generated with an “effectorless” antibody framework scaffold between mice and primates. In particular embodiments of any of the IgG disclosed herein, the IgG comprises one or more of the following amino acid substitutions: N297G, N297A, N297E, L234A, L235A, or P236G.

Non-limiting examples of bivalent and bispecific Wnt surrogate molecules that are bivalent towards both the one or more Fzd receptor and the LRP5 and/or LRP6 are provided as the top four structures depicted in FIG. 1A, where the VHH or sdAb or scFv is depicted in white, and the Fab or IgG is depicted in black. As shown, the VHH or sdAb (or scFvs) may be fused to the N-termini of both light chains, to the N-termini of both heavy chains, to the C-termini of both light chains, or to the C-termini of both heavy chains. It is further contemplated, e.g., that VHH or sdAb (or scFvs) could be fused to both the N-termini and C-termini of the heavy and/or light chains, to the N-termini of the light chains and the heavy chains, to the C-termini of the heavy and light chains, to the N-termini of the heavy chains and C-termini of the light chains, or to the C-termini of the heavy chains and the N-termini of the light chains. In other related embodiments, two or more VHH or sdAb (or scFvs) may be fused together, optionally via a linker moiety, and fused to the Fab or IgG at one or more of these locations. In a related embodiment, the Wnt surrogate molecule has a Hetero-IgG format, whereas the Fab is present as a half antibody, and one or more VHH or sdAb (or scFv) is fused to one or more of the N-terminus of the Fc, the N-terminus of the Fab, the C-terminus of the Fc, or the C-terminus of the Fab. A bispecific but monovalent to each receptor version of this format is depicted at the bottom of FIG. 1A. In certain embodiments, the Fab or antigen-binding fragment (or IgG) thereof is fused directly to the VHH or sdAb (or scFv) or antigen-binding fragment thereof, whereas in other embodiments, the binding regions are fused via a linker moiety. In particular embodiments, the Fab is described herein or comprises any of the CDR sets described herein. In particular embodiments, the VHH or sdAb or scFv is described herein or comprises any of the CDR sets disclosed herein.

In various embodiments, including but not limited to those depicted in FIG. 1B, a Wnt surrogate molecule comprises one or more Fab or antigen-binding fragment thereof that binds one or more Fzd receptor and one or more Fab or antigen-binding fragment thereof that binds LRP5 and/or LRP6. In certain embodiments, it comprises two Fab or antigen-binding fragments thereof that bind one or more Fzd receptor and/or two Fab or antigen-binding fragments thereof that bind LRP5 and/or LRP6. In particular embodiments, one or more of the Fab is present in a full IgG format, and in certain embodiments, both Fab are present in a full

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IgG format. In certain embodiments, the Fab in full IgG format specifically binds one or more Fzd receptor, and the other Fab specifically binds LRP5 and/or LRP6. In certain embodiments, the Fab specifically binds one or more Fzd receptor, and the Fab in full IgG format specifically binds LRP5 and/or LRP6. In certain embodiments, the Fab specifically binds LRP5 and/or LRP6, and the Fab in full IgG format specifically binds one or more Fzd receptor. In certain embodiments, the Fab is fused to the N-terminus of the IgG, e.g., to the heavy chain or light chain N-terminus, optionally via a linker. In certain embodiments, the Fab is fused to the N-terminus of the heavy chain of the IgG and not fused to the light chain. In particular embodiments, the two heavy chains can be fused together directly or via a linker. An example of such a bispecific and bivalent with respect to both receptors is shown at the top of FIG. 1B. In other related embodiments, two or more VHH or sdAb may be fused together, optionally via a linker moiety, and fused to the Fab or IgG at one or more of these locations. In a related embodiment, the Wnt surrogate molecule has a Hetero-IgG format, whereas one of the Fab is present as a half antibody, and the other Fab is fused to one or more of the N-terminus of the Fc, the N-terminus of the Fab, or the C-terminus of the Fc. A bispecific but monovalent to each receptor version of this format is depicted at the bottom of FIG. 1B. In certain embodiments, the Fab or antigen-binding fragment thereof is fused directly to the other Fab or IgG or antigen-binding fragment thereof, whereas in other embodiments, the binding regions are fused via a linker moiety. In particular embodiments, the one or both of the two Fabs are described herein or comprise any of the CDR sets described herein.

In certain embodiments, Wnt surrogate molecules have a format as described in PCT Application Publication No. WO2017/136820, e.g., a Fabs-in-tandem IgG (FIT-IG) format. Shiyong Gong, Fang Ren, Danqing Wu, Xuan Wu & Chengbin Wu (2017). FIT-IG also include the formats disclosed in “Fabs-in-tandem immunoglobulin is a novel and versatile bispecific design for engaging multiple therapeutic targets” mAbs, 9:7, 1118-1128, DOI: 10.1080/19420862.2017.1345401. In certain embodiments, FIT-IGs combine the functions of two antibodies into one molecule by re-arranging the DNA sequences of two parental monoclonal antibodies into two or three constructs and co-expressing them in mammalian cells. Examples of FIT-IG formats and constructs are provided in FIGS. 1A and 1B and FIGS. 2A and 2B of PCT Application Publication No. WO2017/136820. In certain embodiments, FIT-IGs require no Fc mutation; no scFv elements; and no linker or peptide connector. The Fab-domains in each arm work “in tandem” forming a tetravalent bi-specific antibody with four active and independent antigen binding sites that retain the biological function of their parental antibodies. In particular embodiments, Wnt surrogates comprises a Fab and an IgG. In certain embodiments, the Fab binder LC is fused to the HC of the IgG, e.g., by a linker of various length in between. In various embodiment, the Fab binder HC can be fused or unfused to the LC of the IgG. A variation of this format has been called Fabs-in-tandem IgG (or FIT-Ig).

In particular embodiments, Wnt surrogate molecules comprise two or more VHH or sdAb (or scFvs), including at least one that binds one or more Fzd receptor and at least one that binds LRP5 and/or LRP6. In certain embodiments, one of the binding regions is a VHH or sdAb and the other is an scFv. Wnt mimetic molecules comprising two or more VHH or sdAb (or scFvs) may be formatted in a variety of configurations, including but not limited to those depicted in

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FIG. 1C. In certain bispecific, bivalent formats, two or more VHH or sdAb (or scFvs) are fused in tandem or fused to two different ends of an Fc, optionally via one or more linkers. Where linkers are present, the linker and its length may be the same or different between the VHH or sdAb (or scFv) and the other VHH or sdAb (or scFv), or between the VHH or sdAb and Fc. For example, in certain embodiments, the VHH or sdAb is fused to the N-terminus and/or C-terminus of the IgG heavy chain. In particular embodiments, two or more VHH or sdAb are fused to the IgG at any combination of these locations. Non-limiting examples of bivalent and bispecific Wnt surrogate molecules of this format are depicted as the top seven structures depicted in FIG. 1C, where the first VHH or sdAb is depicted in white, the Fc or IgG is depicted in black, and the second VHH or sdAb is depicted as light gray. In various embodiments, both VHH or sdAb may be fused to the N-termini of the Fc, to the C-termini of the Fc, or one or more VHH or sdAb may be fused to either or both of an N-terminus or C-terminus of the Fc. In a related embodiment, the Wnt surrogate molecule has a Hetero-IgG format, whereas one VHH or sdAb is present as a half antibody, and the other is fused to the N-terminus of the Fc or the C-terminus of the Fc. A bispecific but monovalent to each receptor version of this format is depicted at the bottom of FIG. 1C. In certain embodiments, the VHH or sdAb is fused directly to the other VHH or sdAb whereas in other embodiments, the binding regions are fused via a linker moiety. In particular embodiments, the VHH or sdAb are described herein or comprises any of the CDR sets described herein. In various embodiments, any of these formats may comprise one or more scFvs in place of one or more VHH or sdAb.

In certain embodiments, a Wnt surrogate molecule is formatted as a diabody. As shown in FIG. 1D, the binders against Fzd and LRP can also be linked together in a diabody (or DART) configuration. The diabody can also be in a single chain configuration. If the diabody is fused to an Fc, this will create a bivalent bispecific format. Without fusion to Fc, this would be a monovalent bispecific format. In certain embodiments; a diabody is a noncovalent dimer scFv fragment that consists of the heavy-chain variable (VH) and light-chain variable (VL) regions connected by a small peptide linker. Another form of diabody is a single-chain (Fv)₂ in which two scFv fragments are covalently linked to each other.

As discussed, Wnt surrogate molecules, in various embodiments, comprise one or more antibodies or antigen-binding fragments thereof disclosed herein. Thus, in particular embodiments, a Wnt surrogate comprises two polypeptides, wherein each polypeptide comprises an Nab or scFv that binds LRP5/6 and an Nab or scFv that binds one or more Wnts, optionally wherein one of the binding domains is an scFv and the other is an Nab. In certain embodiments, a Wnt surrogate comprises three polypeptides, wherein the first polypeptide comprises an antibody heavy chain and the second polypeptide comprises an antibody light chain, wherein the antibody heavy chain and light chain bind LRP5/6 or one or more Fzds, and wherein the third polypeptide comprises a VHH or sdAb fused to a heavy chain Fc region, wherein the VHH or sdAb binds to either LRP5/6 or one or more Fzds. In other embodiments, Wnt polypeptides comprise four polypeptides, including two heavy chain polypeptides and two light chain polypeptides,

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wherein the two heavy chains and two light chains bind LRP5/6 or one or more Fzds, and further comprise one or more Nab or scFv fused to one or more of the heavy chains and/or light chains, wherein the Nab or scFv binds to LRP5/6 or one or more Fzds. In another illustrative embodiment, a Wnt surrogate comprises at least four polypeptides, including two heavy chain polypeptides and two light chain polypeptides that bind either LRP5/6 or one or more Fzds, wherein the Wnt surrogate further comprises a Fab that binds either LRP5/6 or one or more Fzds. For example, the Fab may comprise two polypeptides, each fused to one of the two heavy chain polypeptides, and two polypeptides, each fused to one of the two light chain polypeptides, or it may comprise two polypeptides each fused to one of the two heavy chain polypeptides and two additional polypeptides, each bound to one of the two polypeptides fused to the heavy chain polypeptides, thus making a second Fab. Other configurations may be used to produce the Wnt surrogates disclosed herein.

In particular embodiments, a Wnt surrogate molecule comprises a Fzd binding region, e.g., an anti-Fzd antibody, or antigen-binding fragment thereof, fused or bound to a polypeptide that specifically binds to one or more Fzd receptor. In particular embodiments, the polypeptide that specifically binds to one or more Fzd receptor is an antibody or antigen-binding fragment thereof. If certain embodiments, it is an antibody or antigen-binding fragment thereof disclosed herein or in the U.S. provisional patent application Ser. No. 62/607,877, titled, "Anti-Frizzled antibodies and Methods of Use," filed on Dec. 19, 2017, which is incorporated herein by reference in its entirety. In particular embodiments, the Fzd binding domain comprises the three heavy chain CDRs and/or the three light chain CDRs disclosed for any of the illustrative antibodies or fragments thereof that bind to one or more Fzd receptor provided in Table 1A. In particular embodiments, the Fzd binding domain comprises the three heavy chain CDRs and/or the three light chain CDRs disclosed for any of the illustrative antibodies or fragments thereof that bind to one or more Fzd receptor provided in Table 1A, wherein the CDRs collectively comprise one, two, three, four, five, six, seven, or eight amino acid modifications, e.g., substitutions, deletions, or additions. In certain embodiments, the Fzd binding domain is a VHH or sdAb or was derived from a VHH or sdAb, so Table 1A only includes the three heavy chain CDRs. In particular embodiments, the Fzd binding domain comprises the three CDR HC sequences provided in Table 1A or variants wherein the CDRs collectively comprise one, two, three, four, five, six, seven or eight amino acid modifications. In particular embodiments, the Fzd binding domain comprises the heavy chain fragment and/or light chain fragment of any of the illustrative antibodies or fragments thereof that bind to one or more Fzd receptor provided in Table 1B or SEQ ID NOs:1-65 or 129-132 (or an antigen-binding fragment or variant of either). In certain embodiments, the Fzd binding domain is an Fab or was derived from an Fab, so the heavy chain of Table 1B includes VH and CH1 sequence, but not CH2 or CH3 sequences. In certain embodiments, the Fzd binding domain is a VHH or sdAb or was derived from a VHH or sdAb, so Table 1B includes the VHH domain. In certain embodiments, the Fzd binding region is a polypeptide, e.g., an antibody or antigen-binding fragment thereof, that competes with any of these antibodies for binding to one or more Fzd receptor.

TABLE 1A

Anti-Fzd Antibody Clone IDs and CDR sequences													
Clone ID	Initial Binding	CDRH1	CDRH1 SEQ ID	CDRH2	CDRH2 SEQ ID	CDRH3	CDRH3 SEQ ID	CDRL1	CDRL1 SEQ ID	CDRL2	CDRL2 SEQ ID	CDRL3	CDRL3 SEQ ID
00 1S - A0 1	Fzd 1	YTFTS YGIS	391 GWIS AYNG NTNY A	570 CARASAW TPYGAFDI W	752 SGSSSNI GSHTVS	1156 SNY QRP S	1256 CAAW DGSLEF GHVV T	1265					
00 1S - B0 1	Fzd 1	GSISS GGYS WS	283 GSIYH SGSTY YN	547 CARFYDIL TGYSYFDY W	818 RSSRSLL DTDDGN TYLD	1142 TLS HRA S	1259 CMQSI QLPW TF	1295					
00 1S - E0 1	Fzd 1	GSISN YYWS	282 GEIDR SGDT NYN	488 CARVRARR FLVSDRSF DIW	945 SGNTLGS HYVS	1155 QDS KRP S	1246 CQVW DSSTV VF	1431					
00 1S - F0 1	Fzd 1	GSISG NNYY ZG	281 GSIYF TGGT YYN	546 CARVMLIT DAFDIW	942 RSSQSLL HSNGYN YLD	1138 LGS NRA S	1237 CMQG THWP YTF	1289					
00 1S - G 01	Fzd 1	GSISS SSYY WG	285 GYIYY SGSTY YN	589 CARATYGG	760 TRSSSNI GAGYDV DAFDIW H	1161 GNS IRPS	1220 CGTW DSSLS AWVF	1267					
00 1S - H0 1	Fzd 1	GSISS GGYY WS	284 GYIYY SGSTY YN	589 CARHAGFY GLADYFDY W	875 RSSQSLL HSNGYN YLD	1138 LGS KRA S	1236 CMQA LQIPP TF	1280					
00 1S - A0 2	Fzd 1	GSISS GGYY WS	284 GYIYY SGSTY YN	589 CARGKGYS YGYGKDW FDPW	845 QASQDI GKYLN	1041 DAS NLE T	1185 CQON DYLPL TF	1332					
00 1S - E0 2	Fzd 1	GSISG NNYY WG	280 GSIYF TGGT YYN	546 CARVMLIT DAFDIW	942 RSSQSLL HSNGYN YLD	1138 LGS NRA S	1237 CMQG THWP YTF	1289					
00 1S - G 02	Fzd 1	GAISG TSYF WG	266 GSIYY TGNT YYN	548 CARIGIAVA APVDHW	882 RASQSV GTYLT	1110 DAS NRA T	1188 CMQA TQFPL TF	1284					
00 1S - H0 2	Fzd 1	GSISS SSYY WG	285 GYIYY SGSTY YN	589 CARATYGG DAFDIW	760 TRSSSNI GAGYDV H	1161 GNS IRPS	1220 CGTW DSSLS AWVF	1267					
00 1S - A0 3	Fzd 1	GSISS GGYY WS	284 GYIYY SGSTY YN	589 CARVRDYY DSSGYYD YFDYW	946 RASRSISS YFN	1128 AAS SLQ S	1175 CQQA DTFPP TF	1314					
00 1S - B0 3	Fzd 1	ASFS GHY WT	158 GEID HTGS TNYE	487 CARGGQG GYDWGHY HGLDVW	835 SGDKVG HKYAS	1154 EDS QRP S	1199 CQAW DSSTD VVF	1301					

TABLE 1A-continued

Anti-Fzd Antibody Clone IDs and CDR sequences													
Clone ID	Initial Binding	CDRH1	CDRH1 SEQ ID	CDRH2	CDRH2 SEQ ID	CDRH3	CDRH3 SEQ ID	CDRL1	CDRL1 SEQ ID	CDRL2	CDRL2 SEQ ID	CDRL3	CDRL3 SEQ ID
00 1S - H0 8	Fzd 5	RAFT DNV MA	329 ATISG GGGS TFDD	466 CAAASSLTS TPYDLW	678								
00 1S - A0 9	Fzd 5	RSFRT NALG	333 AAIS WTG GSTY YA	422 CNTVTYTG GSYKNYW	1005								
00 1S - B0 9	Fzd 5	SIDSI NAM A	356 AALTS GGITY HA	428 CNVITIVRG MGPRAYW	1006								
00 1S - C0 9	Fzd 5	SIFSIN AMG	357 ATIQS GGRT NYA	465 CNVITIVRG MGPRAYW	1006								
00 1S - C0 7	Fzd 8	YTFTS YGIS	391 GWIS AYNG NTNY A	570 CARDGTPF YSGSYYGS W	772 QGDSLRT YYAS	1052 GKN NRP S	1219 CNSRD NSGK HKVF	1300					
00 1S - D0 7	Fzd 8	GTFSS YAIS	295 GRIIPI LGIAN YA	529 CARVPTSP YDILTGPF YW	944 RSSQSLL HSNGFN YVD	1136 FGS YRA S	1206 CMQN LQTP WTF	1291					
00 1S - E0 7	Fzd 8	ASVSS NSAA WN	159 YRSK WYN DYA	542 CARWKNY FDPW	953 RASQGIR SDLA	1070 AAS TLES	1177 CLQDY SYPRT F	1273					
00 1S - H0 7	Fzd 8	FTFSS YAMS	228 STISG GGGS TYYA	646 CAKDLVP WGSSAFNI W	704 RASQSVS SYLA	1121 GAS SRA T	1213 CQQY GSSPP TF	1410					
00 4S - E0 5	Fzd 5	FTFST YEMN	243 SGVS WNG SRTH YV	618 CARGQSEK WWSGLYG MDVW	856 RASQGIS SALA	1076 AAS ALQ S	1165 CQQT YSTPR TF	1394					
00 4S - E0 3	Fzd 5	GTFST YAIS	298 GWIN SGNG NTKY S	565 CWTGLLW FGESTDAF DIW	1031 RASQSIS SYLN	1098 AAS SLQ S	1175 CQQS YSTPL TF	1372					
00 4S - G 06	Fzd 5	GTFTY RYLH	307 GGIPI FGTG NYA	501 CASSMVR VPYYYGM DVW	964 RASQSIS SYLN	1098 AAS SLQ S	1175 CQQS YSTPL TF	1372					
00 1S - D0 9	Fzd 8	GPFN LFAM G	272 AGISR TGGN TGYA	445 CASKTTINS GWSREYH YW	958								

TABLE 1A-continued

Anti-Fzd Antibody Clone IDs and CDR sequences													
Clone ID	Initial Binding	CDRH1	CDRH1 SEQ ID	CDRH2	CDRH2 SEQ ID	CDRH3	CDRH3 SEQ ID	CDRL1	CDRL1 SEQ ID	CDRL2	CDRL2 SEQ ID	CDRL3	CDRL3 SEQ ID
00 1S - E0 9	Fzd 8	GPEN LFAM G	272 AGISR TGGN TGYA		445 CASKTTINS GWSREYH YW		958						
00 1S - F0 9	Fzd 8	GFFSS FTMG	268 AAISR NGVY TRFA		409 CNALAPGV RGSW		987						
00 1S - G 09	Fzd 8	SLFRL NGM G	360 ATIST RGTT HYA		467 CTDEESW		1011						
00 1S - H0 9	Fzd 8	GPEN LLAM G	273 AGISR TGGN TGYA		445 CASKTTINS GWSREYH YW		958						
00 1S - A1 0	Fzd 8	SVVN FVVM G	364 AAITS GGST NYA		425 CNRVGSRE YSYW		1001						
00 1S - B1 0	Fzd 8	AAIG RTSDL YTMG	352 YKVK WNG ERTYY L		404 CNAVITYN GYTIW		994						
00 1S - G 12	Fzd 1	SIFSS NTIY	359 ALITT SGNT NYA		455 CNAGAPA WTYRMGT YYPQFGS W		986						
00 2S - A0 1	Fzd 1	STFST YAM G	362 AAISG SGEN TYYA		408 CVKFGMN LGYSGYDY W		1028						
00 2S - B0 1	Fzd 1	STFSN YAM G	361 AAIS WGG GSTFY S		411 CAAGPIAR WYRGDM DYW		681						
00 2S - C0 1	Fzd 1	RMFS NYA MG	331 AAISS GGSG TYYS		410 CAAGPIAR WYRGDM DYW		681						
00 2S - D0	Fzd 1	RTDG GYVM G	337 ATVT WRTG TTY A		469 CAAGPIAR WYRGDM DYW		681						
1 00 2S - E0 1	Fzd 1	RTFSS AAM G	345 AAIS WSGS TAYY A		421 CATLTPYG TVASY		974						

TABLE 1A-continued

Anti-Fzd Antibody Clone IDs and CDR sequences													
Clone ID	Initial Binding	CDRH1	CDRH1 SEQ ID	CDRH2	CDRH2 SEQ ID	CDRH3	CDRH3 SEQ ID	CDRL1	CDRL1 SEQ ID	CDRL2	CDRL2 SEQ ID	CDRL3	CDRL3 SEQ ID
00 2S - F0 1	Fzd 1	RTFSS YAM G	347 AAVN WSG GSTY YA	430	CAAVFLSR NYEIQEYY RYQ	689							
00 2S - G 01	Fzd 1	RTFSS YAM G	347 AAIS WSG GSTY YA	418	CAAGPIAR WYRGDM DYW	681							
00 2S - H0 1	Fzd 1	RSFST YPMG	336 TVISG SGGS TYYS	676	CAAGPIAR WYRGDM DYW	681							
00 2S - A0 2	Fzd 1	RRFTT YGM G	332 AAVT WRSG STYYA	436	CYLEGPLD VYW	1032							
00 2S - B0 2	Fzd 1	RTFN RHV MG	341 AAIS WSG DSTY YA	415	CAKLGSS WLREYDY W	724							
00 2S - C0 2	Fzd 1	RTFR AYAM G	342 SAIS WSG GSTY YA	603	CAAGPIAR WYRGDM DYW	681							
00 2S - D0 2	Fzd 1	RTFSE YAM G	343 AAIS WSG GSTH YA	417	CNADSLRG IDYW	984							
00 2S - E0 2	Fzd 1	FTFRE YAMT	199 SGISR DGGR TSYS	613	CAPRVLVT APSGGMD YW	734							
00 2S - F0 2	Fzd 1	GDFT NYA MA	267 AAVN WRG DGTY YS	429	CAAVFLSR NYEIQEYY RYQ	689							
00 2S - G 02	Fzd 1	RTFG TWA MG	340 AAISY NGFS TYYS	424	CAAGPIAR WYRGDM DYW	681							
00 2S - H0 2	Fzd 1	RTFSS YAM G	347 AAIS WSG GSTY YA	418	CAAGPIAR WYRGDM DYW	681							
00 2S - D0 3	Fzd 1	RTFGS YAM G	339 AAIS WSG GSTY YA	418	CAAGPIAR WYRGDM DYW	681							

TABLE 1A-continued

Anti-Fzd Antibody Clone IDs and CDR sequences													
Clone ID	Initial Binding	CDRH1	CDRH1 SEQ ID	CDRH2	CDRH2 SEQ ID	CDRH3	CDRH3 SEQ ID	CDRL1	CDRL1 SEQ ID	CDRL2	CDRL2 SEQ ID	CDRL3	CDRL3 SEQ ID
00 2S - E0 3	Fzd 1	SIFSIY AMG	358 AVVA TGGA TNYA	481 CNMRGN WYREGRP AEFLSW	1000								
00 2S - F0 3	Fzd 1	RTSSS YAM G	353 AAIS WSG GSTY YA	418 CAAGPIAR WYRGDM DYW	681								
00 2S - G 03	Fzd 1	RTFGS YAM G	339 AAIS WSG GSTY YA	418 CAAGPIAR WYRGDM DYW	681								
00 2S - H0 3	Fzd 1	QTFT AYAM G	327 AAIS WSGS ATHY A	420 CNAWVLV AGSRGTSA DYW	996								
00 2S - A0 4	Fzd 1	RTFSS YAM G	347 AAIS WSGR STYYA	419 CAAGPIAR WYRGDM DYW	681								
00 2S - B0 4	Fzd 1	RTFSS YAM G	347 AAIS WSG GSTY YA	418 CAAGPNYS WFMPSSS RLIW	682								
00 2S - C0 4	Fzd 1	RRFTT YGM G	332 AAVT WRA GSTY YA	435 CSADKLDY LDDQPFKT WDYW	1010								
00 2S - D0 4	Fzd 1	GTSSST YAM G	309 AAIN RSGG STYYA	405 CAAVFLSR NYEIQEYY RYQ	689								
00 2S - E0 4	Fzd 1	GTFST YAM G	300 AAIS WSG DSTY YL	416 CAAGPIAR WYRGDM DYW	681								
00 4S - H0 4	Fzd 5	GTFSS YAIS	295 GWIS TYNG ATNY A	577 CARGGAG RFGEGMD VW	826 RASQSI SYLN	1098 AAS SLQ S	1175 CQQS YSTPL TF	1372					
00 1S - A0 4	Fzd 5	YTFTS YGIS	391 GWIS AYNG NTNY A	577 CASSKEKA TYYYGMD VW	963 GLSSGSV STNYIPS	1035 YTN TRS S	1263 CLLYL GRGI WVF	1271					
00 1S - D0 3	Fzd 5	GTFSS YAIS	295 GRIIPI LGIAN YA	529 CARLDPGY YYGMDVW	886 TGTSSDV GGYNSV S	1159 DVT KRP S	1196 CFSYA GSRF	1266					

TABLE 1A-continued

Anti-Fzd Antibody Clone IDs and CDR sequences													
Clone ID	Initial Binding	CDRH1	CDRH1 SEQ ID	CDRH2	CDRH2 SEQ ID	CDRH3	CDRH3 SEQ ID	CDRL1	CDRL1 SEQ ID	CDRL2	CDRL2 SEQ ID	CDRL3	CDRL3 SEQ ID
00 1S - F0 3	Fzd 5	GTFSS YAIS	295 GGIIP FGTA NYA		499 CARVIFSTV TTTNDIW		939 TRSSGSI ASNYVQ		1160 END KRP S		1202 CQSYD YDHR WVF		1430
00 4S - E0 4	Fzd 5	YTFSG YYLH	374 GTVT PILGT ANYA		549 CARVDGSG YYGIDYW		933 RASQSI SYLN		1098 AAS SLQ S		1175 CQQS YSTPL TF		1372
00 4S - A0 6	Fzd 5	GSFS NYAIS	278 GRIIP LGSA NYA		530 CARTYLKA FDIW		930 RASQSI SYLN		1098 AAS SLQ S		1175 CQQS YSTPL TF		1372
00 4S - F0 4	Fzd 5	YTFN NFMH	383 GRIN PNSG GTNY A		537 CARDREFDN WFDPW		788 RASQGI SALA		1076 AAS TLQ S		1179 CQQS YNTF WTF		1351
00 1S - C0 3	Fzd 5	GTFSS YAIS	295 GRIIP LGIAN YA		529 CAREGRSR VYGGNSFD YW		808 RSSQSL RRNGHN YVD		1139 MG SNR AP		1238 CMHG LHPPF TF		1279
00 3S - A0 1	Fzd 1	YIFTD YYMH	368 GGIIP FGTA NYA		499 CARMSSDY YDSSGYR RGMDVW		895 RASQGI NNLN		1072 GAS TLQ S		1215 CQQA DSFPP TF		1312
00 3S - E0 1	Fzd 1	YIFTD YYMH	368 GGIIP FGTA NYA		499 CARAWKG LWFGGTF DYW		761 RASQSI SYLN		1098 AAS SLQ S		1175 CQQS YSTPL TF		1372
00 3S - F0 1	Fzd 1	GTFSS YAIS	295 GWIN AGNG NTTY A		558 CARLAFDI W		885 RASQSI SYLN		1098 AAS SLQ S		1175 CQQS YSTPL TF		1372
00 3S - A0 2	Fzd 1	YTFTG YYMH	379 GWIN AGNG NTTY A		558 CAKDRGN YGDYLDY W		707 RASQGI NYLA		1074 EVS SVQ G		1204 CQQS YSTPL AF		1370
00 3S - C0 2	Fzd 1	FTFSN SDM N	214 ALISY DGSH TYYA		454 CTRGSRIG WFDPW		1015 RASQSIG RWLA		1084 AAS RLQ S		1171 CQQG FNFPL TF		1325
00 3S - E0 2	Fzd 1	GTFSS YTIS	296 GGIIP SGKT DYA		505 CARARGG DSPLSL		749 RASQGI NNLN		1072 TAS SLQ S		1258 CLQDY SYPYT F		1274
00 3S - F0 2	Fzd 1	GTFRS YAIN	292 GGIIP FGTA NYA		499 CARGGWR PDYYGSGS YYSPDYW		840 RASQSVS SDLA		1115 GAS TRA T		1217 CQQY ETWP VLTF		1405

TABLE 1A-continued

Anti-Fzd Antibody Clone IDs and CDR sequences													
Clone ID	Initial Binding	CDRH1	CDRH1 SEQ ID	CDRH2	CDRH2 SEQ ID	CDRH3	CDRH3 SEQ ID	CDRL1	CDRL1 SEQ ID	CDRL2	CDRL2 SEQ ID	CDRL3	CDRL3 SEQ ID
00 3S - G 02	Fzd 1	FTFGT YWVT	196 SGITG SGGR TFYA		616 CARMKDW FGAFDIW		894 RASESVS SSSFA		1056 GAS TRA T		1217 CQQY NNWP PNYTF		1420
00 3S - C0 3	Fzd 1	FTFSR YAMS	220 SYISG DSGY TNYA		658 CARGLVIA TNWFDPW		849 QANQDI SNYLN		1038 AAS SLQ S		1175 CQQT YNPPR TF		1389
00 3S - D0 3	Fzd 1	YTFTS YYMH	392 GWIN TYNG NTNY P		567 CAESLTSTA DW		691 RASQGIS NNLN		1072 AAS SLQ R		1174 CQQS YSTPF TF		1368
00 3S - E0 3	Fzd 1	YIFTD YYMH	368 NPTT GNTG YA		586 CARNVEGA TSFPEFDY W		898 RASQGIS NNLN		1072 SAS NLQ S		1252 CQQS YSPPP YTF		1364
00 3S - H0 3	Fzd 1	GTFSS YAIS	295 GGIPI FGTA NYA		499 CAKDIGSS WYYMDV W		701 RASQGIS SYLN		1098 AAS SLQ S		1175 CQQS YSTPL TF		1372
00 3S - A0 4	Fzd 1	FTFGT YWVT	196 SGITG SGGR TFYA		616 CARMKDW FGAFDIW		894 RTSERSSI SSFA		1148 GAS TRA T		1217 CQQY NNWP RNYTF		1420
00 3S - C0 4	Fzd 1	FAVSS SYMS	168 ASIW FDGS NQDY A		463 CAPNESGN VDYW		733 RASQGIS NNLN		1072 KAS SLE N		1225 CQQS YSTPH TF		1339
00 3S - D0 4	Fzd 1	FTFSS YAM H	227 SAISG SGGS TYYA		600 CARDHGSS WYQNTDA FDIW		774 QASQDI GNYLN		1042 DVS NLE R		1195 CQHL NSYPP GDTF		1304
00 3S - G 04	Fzd 1	FRFIS HPIH	177 GRVIP ILGVT NYA		545 CASSSDYG DYLKEPNY GMDVW		966 RASQGIS SYLN		1098 AAS SLQ S		1175 CQQS YSTPL TF		1372
00 3S - D0 5	Fzd 2	FTFSN YAMT	216 SAIGT GGGT YYA		595 CATAYRRP GGLDVW		969 RSSQSLL HSDGKTY LY		1134 LGS NRA S		1237 CMQN THWP LTR		1293
00 3S - E0 5	Fzd 2	FTFSS YTMS	236 GRIKS KANG GTTD YA		535 CARGSSSW YDW		863 KSSQSLL HSDGKTY LY		1036 LGS NRA S		1237 CMQN THWP LTR		1293
00 3S - A0 6	Fzd 2	FTFA DYG MH	188 SYISS GSYTI YYS		659 CARGTFD WLLSPSYD YW		865 RASQGIS NNLN		1072 AAS RLES		1170 CQQS YSTPL TF		1372

TABLE 1A-continued

Anti-Fzd Antibody Clone IDs and CDR sequences													
Clone ID	Initial Binding	CDRH1	CDRH1 SEQ ID	CDRH2	CDRH2 SEQ ID	CDRH3	CDRH3 SEQ ID	CDRL1	CDRL1 SEQ ID	CDRL2	CDRL2 SEQ ID	CDRL3	CDRL3 SEQ ID
00 3S - C0 6	Fzd 2	FTFSN YGM H	217 SAISN SGGS TYYA	601	CTSSFLTGS QPSGYW	1018	RASQDIS SYLA	1065	AAS SLQ S	1175	CQQS YRTPL TF	1353	
00 3S - G 06	Fzd 2	FTFSD YGM H	207 SSTSG SGGN SKYS	642	CARHNPGY MGYYYGM DVW	877	RASQSVS SNLA	1116	DAS NRA T	1188	CQHR TSWPL TF	1307	
00 3S - H0 6	Fzd 2	GTFSS YTIS	296 GLVD PEDG ETIYA	520	CTILPAAAA GTYYYYG MDVW	1012	RASQRV GNNLA	1083	DAS IRAT	1184	CQQY KDWP TF	1415	
00 3S - B0 7	Fzd 2	FTFSD HYMS	205 SSITR TPSG GTTE YA	639	CARDGGY W	768	RASQSV GSYLA	1109	GSS NRA A	1221	CQQY GTSLL TF	1414	
00 3S - D0 7	Fzd 2	YTFTN NFMH	383 GIINP SGGS TSYA	513	RRYCSSTSC YPRDAFDI W	759	QASQGIS NNLN	1049	LGS DRA S	1233	CQQS YSTPF TF	1368	
00 3S - E0 7	Fzd 2	YTFTN NFMH	383 GWIN PNSG GTKY A	563	CARSVGEV GATMLGIG VWYWFDP W	926	RASQSIG SYLN	1098	AAS SLQ S	1175	CQQS YSTPF TF	1368	
00 3S - A0 8	Fzd 2	FTFSN YAMT	216 SAIGT GGGT YYA	595	CATAYRRP GGLDVW	969	RSSQSLL HSDGKTY LY	1134	LGF NRA S	1232	CMQN THWP LTR	1293	
00 3S - C0 8	Fzd 2	LTVST NFMS	324 AGIG WDST NIGY A	440	CARDLVAA RPSNWDY W	782	RASQGIR NDLG	1069	GAS TLQ R	1214	CQQS YSTPR VTF	1374	
00 3S - E0 8	Fzd 2	FTFR NSAM H	201 STISG SGGS TYYS	647	CARGGGYS SSW	829	RSSRSLL HSDGKTY LY	1143	LGS NRA S	1237	CMQS SHWP KTF	1298	
00 3S - G 09	Fzd 4	FTFD HNP MN	194 SAIGA GGGT YYA	593	CASPTVTR R	960	RASQSIG SYLN	1098	AAS SLQ S	1175	CQQS YSTPL TF	1372	
00 3S - C1 0	Fzd 4	GTFSS YAIS	295 GWIN AGNG NTTY A	558	CARHYGYS GSYPDW	880	RASQSIG SYLN	1098	AAS SLQ S	1175	CQQS YSTPL TF	1372	
00 3S - D1 0	Fzd 4	FNFGI YSMT	172 SYISG DSGY TNYA	658	CARVGP GWFDPW	936	RASQGIS SYLA	1076	AAS NLL G	1167	CQQT YSTP WTF	1396	

TABLE 1A-continued

Anti-Fzd Antibody Clone IDs and CDR sequences													
Clone ID	Initial Binding	CDRH1	CDRH1 SEQ ID	CDRH2	CDRH2 SEQ ID	CDRH3	CDRH3 SEQ ID	CDRL1	CDRL1 SEQ ID	CDRL2	CDRL2 SEQ ID	CDRL3	CDRL3 SEQ ID
00 3S - E1 0	Fzd 4	FTFSS YAM H	227 AGISA SGGS TYYA	442	CARPSTTG TKAFDIW	901	RASQSIG SNLD	1085	AAS TLET	1178	CQQS YSVPD TF	1380	
00 3S - A1 1	Fzd 4	GTFSS YAIS	295 GWIN AGNG NTTY A	558	CARHYGGS GSDPDW	880	RASQSIS ZYZN	1103	ZAS SLQ S	1264	CQQS YSTPL TF	1372	
00 3S - G 11	Fzd 4	GTFSS YAIS	295 GRIIPI FGTV NYA	528	CARGARLD YW	820	RASQSIS SYLN	1098	AAS SLQ S	1175	CQQS YSTPL TF	1372	
00 3S - H1 1	Fzd 4	YTFTG YYMH	379 GGIPI FGTP HYA	502	CASTDPSS GLDYW	967	RASQSIG SNLD	1085	DAS SLES	1189	CQQSF IMPLT F	1341	
00 3S - C1 2	Fzd 4	GTFSS YAIS	295 GWIN PNSG GTNY A	564	CARGGSSD VR	838	RASQSIS SYLN	1098	AAS SLQ S	1175	CQQS YSTPL TF	1372	
00 3S - F1 2	Fzd 4	FTFSS YAM H	227 SVIST SGDT VLYT	652	CARGGSSD VR	838	RASQSIS SYLN	1098	AAS SLQ S	1175	CQQS YSTPL TF	1372	
00 4S - B0 1	Fzd 4	GTFSS YAIS	295 GIINP SGGS TSYA	513	CAKDGTV R	698	RAIQSISS YLN	1054	AAS SLQ S	1175	CQQS YSTPL TF	1372	
00 4S - C0 1	Fzd 4	FTFSN HYTS	213 STISS SGGR TFYA	650	CARASRID GGWPIIDH L	754	RASQDIR DELA	1062	AAS TLQ S	1179	CQQA DSFPL TF	1311	
00 4S - D0 1	Fzd 4	FTFTN YAMS	248 SAISG SGGS TYYA	600	CARATGFG TVVPDYW	757	RASQSIS SYLN	1098	AAS SLQ S	1175	CQQS YSTPL TF	1372	
00 4S - E0 1	Fzd 4	GTFSS YAIS	295 GWIN AGNG NTTY A	558	CARHYGGS GSDPDW	880	ZACLRIS YLN	1163	FAS SLQ S	1205	CQQS YSTPL TF	1372	
00 4F - F0 1	Fzd 4	GTFSS YAIS	295 GWIN AGNG NTTY A	558	CARDGVE	773	RASQGIS NWLA	1073	DAS SLQ S	1190	CQQS HITPY TF	1344	
00 4S - H0 1	Fzd 4	FTFSN YAM H	215 ALMS PDGTI IYYA	456	CAKGIWGD YGAFDIW	717	RASQSIS SYLN	1098	AAS SLQ S	1175	CQQS YSTPL TF	1372	

TABLE 1A-continued

Anti-Fzd Antibody Clone IDs and CDR sequences													
Clone ID	Initial Binding	CDRH1	CDRH1 SEQ ID	CDRH2	CDRH2 SEQ ID	CDRH3	CDRH3 SEQ ID	CDRL1	CDRL1 SEQ ID	CDRL2	CDRL2 SEQ ID	CDRL3	CDRL3 SEQ ID
00 4S - B0 2	Fzd 4	FTFSS YGM H	230 SSINN SSRTV FYA	630 CAKDH LAV ADAHGR	700 RASQS IS SYLN	1098 AAS SLQ S	1175 CQQS YSTPL TF	1372					
00 4S - E0 2	Fzd 4	FTFSS YAM H	227 AVISY DGSN EYYA	474 CAGGE VYE L	692 RASQS IS SYLN	1098 AAS SLQ S	1175 CQQS YSTPL TF	1372					
00 4S - F0 2	Fzd 4	FTFST YAM H	242 AVISS DGNN KYYT	473 CAAPD VVV TADGYW	685 RASQG IS SALA	1076 AAS TLQ S	1179 CQQA NTVPF TF	1322					
00 4S - G 02	Fzd 4	FTFA NYA MN	190 ALISY DGGT KYA	453 CAKTL VTS HALHIW	728 RASQS IS SYLN	1098 AAS SLQ S	1175 CQQS YSTPL TF	1372					
00 4S - H0 2	Fzd 4	FTFA NYA MH	189 ALISY DGGN KYA	452 CAKTL VTS HALHIW	728 RASQS IS SYLN	1098 AAS SLQ S	1175 CQQS YSTPL TF	1372					
00 1S - E0 3	Fzd 5	GSFS GYW H	276 GEIN HSGS TNYN	489 CARGR RLV RFTVTS AF DIW	858 TGSSS NI GAGFGV H	1158 SDR NRP S	1255 CQSYD SSLRA SVF	1429					
00 - B0 5	Fzd 5	GTFSS YAIS	295 GGIPI LGIAN YA	504 CARIP KPR GYSYGD N GSW	883 RSSQS LL HSNGNT YLD	1137 LGS DRT S	1234 CMQS LQTPY TF	1297					
00 4S - A0 7	Fzd 6	GNFK NYGIT	271 GRIIP ALGT ANYA	525 CARQY CSG GSCYPDA F DIR	908 RASQD IR SALA	1063 QAS SLIS	1245 CQQS YSMP QTF	1361					
00 4S - B0 7	Fzd 6	FTFSS YSMN	233 GVISK DGDN KYA	553 CASSR DGY NRLAFDI W	965 QASQD IR NYLN	1043 AAS SLQ S	1175 CQQS RFTW F	1347					
00 4S - A0 8	Fzd 6	GTFSS YAIS	295 GRIPI LGIAN YA	529 CARDG GD YGMVW	767 RASQS IS SYLN	1098 AAS SLQ S	1175 CQQS YSTPL TF	1372					
00 4S - B0 8	Fzd 6	YTFTN NFMH	383 PNSG GTNY A	537 CASQNY YG SGSYPGF D YW	961 RASQS IS SYLN	1098 AAS SLQ S	1175 CQQS YSTPL TF	1372					
00 4S - D0 8	Fzd 6	YTFTY RYLH	394 GGIPI FGTA NYA	499 CATHD SSG YYSFDY W	973 RASQS IS SYLN	1098 AAS SLQ S	1175 CQQS YSTPL TF	1372					

TABLE 1A-continued

Anti-Fzd Antibody Clone IDs and CDR sequences													
Clone ID	Initial Binding	CDRH1	CDRH1 SEQ ID	CDRH2	CDRH2 SEQ ID	CDRH3	CDRH3 SEQ ID	CDRL1	CDRL1 SEQ ID	CDRL2	CDRL2 SEQ ID	CDRL3	CDRL3 SEQ ID
004S-EO8	Fzd 6	FSVSS NYM N	187 SAIGT GGGT YYA	595 CTTRTYDSS GYETQNY YMDVW	1024 RSSRSLL HSNGNT YLQ	1144 LGS NRA S	1237 CVQTT QSPLT F	1434					
004S-G08	Fzd 6	FTFSD YYMS	208 AAISY DESN KFYA	423 CARSAVAG AFDIW	916 RASQSI SYLN	1098 AAS SLQ S	1175 CQQS YSTPL TF	1372					
004S-A09	Fzd 6	FTFRD YAM N	198 SGIS WNS GSIGY A	615 CARRSGYS GSVYYYG MDVW	913 RASQSI SYLN	1098 AAS SLQ S	1175 CQQS YSTPL TF	1372					
004S-B09	Fzd 6	FTFSS FGMH	221 AGIN WNG GSVV YA	441 CARGPSHQ HTFDIW	854 RASQGI SALA	1076 AAS SLQ S	1175 CQQS YSHTA FTF	1357					
004S-C09	Fzd 6	YTFTN NFMH	383 GGPD PEDG ETIYA	492 CARVGRGY SPDYW	937 RASQSI SYLN	1098 AAS SLQ S	1175 CQQS YSTPL TF	1372					
004S-EO9	Fzd 6	DTFS NYVIS	163 GRISA YNGY KSYA	538 CARSSGYV GWFDPW	924 RASQSI SYLN	1098 AAS SLQ S	1175 CQQS YSTPL TF	1372					
004S-F09	Fzd 6	FTFSN YYTS	218 SYISG AGGS TEYA	657 CARLPRRS GKGSAFDI W	888 RASQSI SYLN	1098 AAS SLQ S	1175 CQQS YSTPL TF	1372					
004S-H09	Fzd 6	GTFSS YTIS	296 NPNS GNTG YA	583 CARVGATS AGGMDV W	935 RASQSI SYLN	1098 AAS SLQ S	1175 CQQS YSTPL TF	1372					
004S-C10	Fzd 6	YIFTD YYMH	368 GLVD PEDG ETIYA	520 CAHSDFFS GLSPGDW	693 RASQSI SYLN	1098 AAS SLQ S	1175 CQQS YSTPL TF	1372					
004S-D10	Fzd 6	FTFSN SDM N	214 SSIST SGGS TYYA	637 CARGSYW	864 RASQNI NYLA	1081 RAS TLQ S	1249 CQQY SSYPY TI	1425					
004S-E10	Fzd 6	TTLNK YAIS	365 GRITP VVG TNYA	539 CALSSSWY GGFDYW	731 RASQSI SYLN	1098 AAS SLQ S	1175 CQQS YSTPL TF	1372					
004S-F10	Fzd 6	GFTFS DHY	269 ALVG YDGS QQFY G	458 CNTGIPML YW	1003 RASQSI SYLN	1098 AAS SLQ S	1175 CQQS YSTPL TF	1372					

TABLE 1A-continued

Anti-Fzd Antibody Clone IDs and CDR sequences													
Clone ID	Initial Binding	CDRH1	CDRH1 SEQ ID	CDRH2	CDRH2 SEQ ID	CDRH3	CDRH3 SEQ ID	CDRL1	CDRL1 SEQ ID	CDRL2	CDRL2 SEQ ID	CDRL3	CDRL3 SEQ ID
004S- G10	Fzd 6	FTFSD YYMS	208 SAISG SGFTY YA		599 CARVSRGF APDYW		948 RASQSIS SYLN		1098 AAS SLQ S		1175 CQQS YSTPL TF		1372
004S- A11	Fzd 6	GTFSS YAIS	295 GRIIPI LGIAN YA		529 CARESVNN YYMDVW		813 RASQSIS SYLN		1098 AAS SLQ S		1175 CQQS YSTPL TF		1372
004S- C11	Fzd 6	FTFSS YAM H	227 ALTSY DGSK KFYA		457 CAKTGRGY AFDIW		726 RASQSIS SYLN		1098 AAS SLQ S		1175 CQQS YSTPL TF		1372
004S- E11	Fzd 6	FTFSS YNM N	232 KANG GTTD YA		535 CAKAGQQ LDW		696 RASQSIS SYLN		1098 AAS SLQ S		1175 CQQS YSTPL TF		1372
004S- H11	Fzd 6	FTFSS SAM Q	249 GGIPI FGTA NYA		499 CATVQTNY YDSSGRFS YRAHYFDY W		977 RASQSIS RWLA		1094 AAS SLQ S		1175 CQQY VSYPL TF		1426
004S- A12	Fzd 6	YTFN NFMH	383 GRIN PNSG GTNY A		537 CARGQGY SGWYRGD AFDIW		855 RASQSIS SYLN		1098 AAS SLQ S		1175 CQQS YSTPL TF		1372
004S- D12	Fzd 6	FAFD DYA MH	165 KAYG GTTE YA		490 CAKDRGYS SGWYLDY W		708 RASQSIS SYLN		1098 AAS SLQ S		1175 CQQS YSTPL TF		1372
005S- H01	Fzd 7	FNFSS YTMR	173 SVIYG GGNT NYA		653 CARGGSG GNLSYW		836 RASQSIS SYLN		1098 AAS SLQ S		1175 CQQS YSTPL TF		1372
005S- A02	Fzd 7	GTFSS YAIS	295 GMIIP FLGIT NYA		521 CTRPYDAF DIW		1016 RASQSIS SYLN		1098 AAS SLQ S		1175 CQQS YSTPL TF		1372
005S- C02	Fzd 8	YTFAS YGM H	373 GWIN AGNG NTTY A		558 CARLSVWK WEQVTN WFDPW		890 RASQSIS SYLN		1098 AAS SLQ S		1175 CQQS YSTPL TF		1372
005S- E02	Fzd 8	GTFTS YAIS	305 GWIN AGNG NTKY S		557 CTTGLEFPYY RYNWNND AFDIW		1022 RASQSIS SYLN		1098 AAS SLQ S		1175 CQQS YSTPL TF		1372
005S- A03	Fzd 8	GTFSS YAIS	295 GWM NPNS GNTG YA		583 CAKWHIG ATGNWFD PW		729 RASQSIS SYLN		1098 AAS SLQ S		1175 CQQS YSTPL TF		1372

TABLE 1A-continued

Anti-Fzd Antibody Clone IDs and CDR sequences													
Clone ID	Initial Binding	CDRH1	CDRH1 SEQ ID	CDRH2	CDRH2 SEQ ID	CDRH3	CDRH3 SEQ ID	CDRL1	CDRL1 SEQ ID	CDRL2	CDRL2 SEQ ID	CDRL3	CDRL3 SEQ ID
005S-H03	Fzd 8	YTFTN NFMH	383 GGIFP IYGIS TYA	494 CARDRPTS SWYAFDY	792 RASQGIS>NNLN	1072 DAS TLQ T	1193 CQQSF SAPITF	1342					
005S-F04	Fzd 8	FSFSS TAMS	181 SYISS SGSIT HYA	670 CARYGDYD DYW	954 RASQGIS SYLN	1098 AAS SLQ S	1175 CQQS YSTPL TF	1372					
005S-H04	Fzd 8	YTFTN NFMH	383 GWIN AGNG NTTY A	558 CARVATGN AFDIW	932 RASQGIS SYLN	1098 AAS SLQ S	1175 CQQS YSTPL TF	1372					
005S-B05	Fzd 8	FTFSS YWM H	239 AGISG SGKT TFYA	444 CARGGLLF DYW	831 QASQDIS NYLN	1046 KAS SLES	1226 CQQS YSTPR TF	1373					
005S-F05	Fzd 8	FTFTS SAVQ	251 GWM NPNS GNTG YA	583 CARRTAVA GTIDYW	914 RASQGIS SYLN	1098 AAS SLQ S	1175 CQQS YSTPL TF	1372					
005S-G05	Fzd 8	GTFSS YAIS	295 GWIS PYNG NTNY A	573 CARGGWT NYGGNLDY W	841 RASQGIS SYLN	1098 AAS SLQ S	1175 CQQS YSTPL TF	1372					
005S-H05	Fzd 8	YTFTS YYMH	392 GRIN PNSG GTNY A	537 CARVPDF WSGYLDY W	943 RASQGIS RTLZ	1075 AAS SLQ S	1175 CQQT YSMPI TF	1392					
005S-D06	Fzd 8	YTFTY RYLH	394 GGIPI FGTA NYA	499 CARDSYPY GMDVW	800 RASQGIS SYLN	1098 AAS SLQ S	1175 CQQS YSTPL TF	1372					
005S-F06	Fzd 8	GTFSS YAIS	295 GRVIP ILGVT NYA	545 CAREYLG S FDIW	815 RASQSV GSNLA	1108 GAS SRA T	1213 CQQY GSSPP FTF	1409					
005S-A07	Fzd 9	FTFTG SAVQ	247 GGILP IYGT KYA	509 CARGARLY GFDYW	822 RASQSVS RNLA	1114 GAS TRA T	1217 CQQR SNWPI TF	1335					
005S-B07	Fzd 9	FTFTS SAVQ	251 GWM NPNS GNTG YA	583 CARGRGQ QWLTGY GMDVW	857 RASQGIS SALA	1076 GAS TLQ S	1215 CLQDY NYPFT F	1272					
005S-C07	Fzd 9	FTFSS YSMN	233 SYIEN DGSIT TYA	654 CARAPYYY GSGSLFRL DYW	748 RASQGIS SYLN	1098 AAS SLQ S	1175 CQQS YSTPL TF	1372					

TABLE 1A-continued

Anti-Fzd Antibody Clone IDs and CDR sequences													
Clone ID	Initial Binding	CDRH1	CDRH1 SEQ ID	CDRH2	CDRH2 SEQ ID	CDRH3	CDRH3 SEQ ID	CDRL1	CDRL1 SEQ ID	CDRL2	CDRL2 SEQ ID	CDRL3	CDRL3 SEQ ID
005S-D07	Fzd 9	GTFN SYAIA	291 GGIIPI FGTA NYA	499 CARAGSGY YNFDYW	740 RASQSIN RWLA	1090 AAS SLQ S	1175 CQQT YNIPIT F	1388					
005S-F07	Fzd 9	FSFSS YGM H	182 AYINS RGSL MYA	483 CAKTKLPI W	727 RASQSIN RNYLG	1089 AAS SRV T	1176 CQQY DSWP PTF	1402					
005S-G07	Fzd 9	GSFS GYAI N	274 GGIIPI FGTA NYA	499 CATGYYYD YYFDYW	972 RASQGIS>NNLN	1072 AAS SLQ S	1175 CQHY YNLPL TF	1309					
005S-H07	Fzd 9	GTFT NNF MH	303 GLVD PEDG ETIYA	520 CARTYRIV GATPRYYY YGMDVW	931 RASQTIN NQLA	1125 KAS NLE T	1224 CQQA NSFPV TF	1318					
005S-B08	Fzd 9	YIFTD YYMH	368 GWIN PNSG GTIYA	562 CARGPRDS GYPPGGAF DIW	853 RASQSIS SYLN	1098 AAS SLQ S	1175 CQQS YSTPL TF	1372					
005S-D08	Fzd 9	FAFSS HWM H	166 SAIDG SGGG TYA	592 CARDRLG WAHWYFD LW	794 RASQSIS SYLN	1098 AAS SLQ S	1175 CQQS YSTPL TF	1372					
005S-G08	Fzd 9	YTFTG YYMH	379 GWIN AGNG NTTY A	558 CARDRDY W	787 QTSQDIN>NNLN	1053 KAS SLES	1226 CQQS YSPP TF	1366					
005S-C09	Fzd 9	FTFSS YGM H	230 SAIGT GGGT YYA	595 CALLVGAA RGISYYYY GMDVW	730 QASQDIS NYLN	1046 AAS TLQ S	1179 CLQHK SPPTF	1276					
005S-D09	Fzd 9	YTFTS YAM H	389 GWIN AGNG NTTY A	558 CARDRPYS SGWYYPAF DIW	793 RASQSVS SNQLA	1117 GAS TRA T	1217 CQQR YNWP PSITF	1339					
005S-E09	Fzd 9	FNL RYNM N	175 SRISN SGSLV YYA	627 CARDADSS GYRYDAF DIW	762 RASQSVS SNLA	1116 DAS NRA T	1188 CQQR>NNWL YTF	1334					
005S-A10	Fzd 9	YTFTD YYMH	376 GIINP SGGG TSYA	513 CARHVGSGT YNNWF DPW	878 RASQSIS SYLN	1098 AAS SLQ S	1175 CQQS YSTPL TF	1372					
005S-D10	Fzd 9	YTFTS YYMH	392 GWM SPNS ANTG YA	583 CARGGPIH YYYYYYMD VW	834 RASQGIS>NNLN	1072 AAS TLQ S	1179 CQQT NLFPPY TF	1385					

TABLE 1A-continued

Anti-Fzd Antibody Clone IDs and CDR sequences													
Clone ID	Initial Binding	CDRH1	CDRH1 SEQ ID	CDRH2	CDRH2 SEQ ID	CDRH3	CDRH3 SEQ ID	CDRL1	CDRL1 SEQ ID	CDRL2	CDRL2 SEQ ID	CDRL3	CDRL3 SEQ ID
005S-H10	Fzd 9	GAFS TSSIS	265	GRIIP VLGT ANYA	534	CAKGGWR SSFDPW	715	RASQSVS SNLA	1116	GAS TRA T	1217	CQQY NSWP LTF	1421
005S-B11	Fzd 9	YTFTS YDIN	390	GGFD PEDG ETIYA	492	CAKAGDW GLYGMDV W	695	RASQSVS RWLA	1094	AAS SLQ S	1175	CQQT NTFPF TF	1386
005S-C11	Fzd 9	FTFTG SAVQ	247	GGILP IYGT KYA	509	CARGARLY GPDYW	822	RASQSVS RKLA	1113	GAS TRA T	1217	CQQR SNWPI TF	1335
005S-D11	Fzd 9	YTFTN NFMH	383	GWIN PNSG DTKF A	561	CAREANYD ILTGIRPD AFDIW	806	RASQSLR SKLA	1106	GAS TRA T	1217	CQQY ANSP WTF	1401
005S-E11	Fzd 9	GTFSS YAIS	295	GWIN AGNG NTKY S	557	CTTTEYSSS PDYYYGM DVW	1025	QASQDIS NYLN	1046	GAS TLQ S	1215	CQQLS RYPSL F	1331
005S-G11	Fzd 9	GTFT RNSIS	304	GGIPI FGTA NYA	499	CARSSDLRI FDYW	922	RASQSVS SNLA	1116	GAS NRP T	1209	CQQY GSSPY TF	1413
005S-H11	Fzd 10	YTFAS YDIH	372	GWIN AGNG NTTY A	558	CARDGIW DIFDYW	769	RASQSVS SYLN	1098	AAS SLQ S	1175	CQQS YSTPL TF	1372
005S-E12	Fzd 10	YIFTD YYMH	368	GVIFP VYPT PDYA	551	CARGGSTG YYGMDVW	839	RASQSV GRWMA	1107	AAS SLQ S	1175	CQQA NTFPF TF	1321
005S-F12	Fzd 10	GTFSS YAIS	295	GRIVP IVDV VKYA	541	CARDTCSS TSCSPDYW	801	RASQSVS SYLN	1098	AAS SLQ S	1175	CQQS YSTPL TF	1372
006S-A01	Fzd 10	FTFSS YSMN	233	SAIGT GGGT YYA	595	CAREGWF GESPPGM DVW	810	RASQSVS SYLN	1098	AAS SLQ S	1175	CQQS YSTPL TF	1372
006S-F01	Fzd 10	YTFTR YAVH	385	GWIS TFND NTNY A	576	CASPTGMT TNFDYW	959	RASQSVS SYLN	1098	AAS SLQ S	1175	CQQS YSTPL TF	1372
006S-H01	Fzd 10	YIFTD YYMH	368	GGIPI FGTA NYA	499	CAKGSYYY DSSGYW DAFDIW	723	RASQGIS NNLN	1072	AAS NLE T	1166	CQQT SSTPL TF	1387

TABLE 1A-continued

Anti-Fzd Antibody Clone IDs and CDR sequences													
Clone ID	Initial Binding	CDRH1	CDRH1 SEQ ID	CDRH2	CDRH2 SEQ ID	CDRH3	CDRH3 SEQ ID	CDRL1	CDRL1 SEQ ID	CDRL2	CDRL2 SEQ ID	CDRL3	CDRL3 SEQ ID
00 6S - A0 2	Fzd 10	YIFTD YYMH	368 GGIIP LFGTT DYA	507 CARDITGA DGMDVW	775 RASQSIG SYLN	1098 AAS SLQ S	1175 CQQS YSTPL TF	1372					
00 6S - D0 2	Fzd 10	GTFSS YAIS	295 GRIIP TVGT ANYA	533 CARDVCSG GSCSPDV W	802 RASQSIG NNLN	1072 DAS SLES	1189 CQQT YNTPR TF	1390					
00 6S - E0 2	Fzd 10	FTFSS SATQ	250 GGIIP FGTA NYA	499 CARDGSSG WYSPNAF DIW	770 RASQSIG NNLN	1072 AAS SLQ S	1175 CLQH NGYPI TF	1277					
00 6S - H0 2	Fzd 10	FTFR MYG MH	200 SRISP DGRT TTYA	628 CARSPRW YDAFDIW	920 RSSQSLL HSNGYN YLD	1138 RVS SRF S	1251 CMQG THWP PTF	1288					
00 6S - A0 3	Fzd 10	YIFTD YYMH	368 GWIN AGNG NTTY A	558 CARDPIMF GDQPGWF DPW	784 RASESVS SNLA	1055 GAS SRA T	1213 CQQY NKSPS F	1419					
00 6S - B0 3	Fzd 10	GTFSS YAIS	295 GWIN AGNG NTKY A	556 CAREGYDF WSGPYAF DIW	811 RASQTIS RYLN	1126 EVS SLQ G	1203 CQQS YSTP WTF	1378					
00 6S - C0 3	Fzd 10	GTFSS NVIS	293 GGIIP FGTA NYA	499 CARGGYYY GMDVW	843 RASQSIG SYLN	1098 AAS SLQ S	1175 CQQS YSTPL TF	1372					
01 4S - B0 1	Fzd 1	YIFTD YYMH	368 GGIIP FGTA NYA	499 CARMSSDY YDSSGYR RGMDVW	895 RASQSIG NNLN	1072 GAS TLQ S	1215 CQQA DSFPP TF	1312					
01 4S - D0 1	Fzd 4	GTFSS YAIS	295 GWIN AGNG NTTY A	558 CARHYGGS GSYPDW	880 RASQSIG SHZN	1096 AAS SLQ S	1175 CQQS YSTPL TF	1372					
01 4S - E0 1	Fzd 4	GTFSS YAIS	295 GWIN AGNG NTTY A	558 CARHYGGS GSYPDW	880 RASQSIZ ZYZN	1105 AAS SLQ S	1175 CQQS YSTPL TF	1372					
01 4S - G 01	Fzd 4	GTFSS YAIS	295 GWM NPNN GNTT YA	581 CARHYGGS GNRWDW	879 RASQSIG SYLN	1098 AAS SLQ S	1175 CQQS YSTPF TF	1368					
01 4S - A0 2	Fzd 4	FTFSS NAM H	223 SGISG SGGS TYA	608 CAKPGIAA AGTNNWF DPW	725 RASQSIG SALA	1076 GAS TVE S	1218 CQQS YSTPR TF	1373					

TABLE 1A-continued

Anti-Fzd Antibody Clone IDs and CDR sequences													
Clone ID	Initial Binding	CDRH1	CDRH1 SEQ ID	CDRH2	CDRH2 SEQ ID	CDRH3	CDRH3 SEQ ID	CDRL1	CDRL1 SEQ ID	CDRL2	CDRL2 SEQ ID	CDRL3	CDRL3 SEQ ID
01 4S - B0 2	Fzd 4	FTFSS YAM H	227 SGISG SGSST YYA		611 CARPSTTSF GMDVW		902 RASQSVS SNLA		1116 GAS TRA T		1217 CQQY DTPLR TF		1403
01 4S - C0 2	Fzd 5	YTFTS YYMH	392 PNSG GTNY A		537 CARVPDF WSGYLDY W		943 RASQGIS SALA		1076 AAS SLQ S		1175 CQQT YSMPI TF		1392
01 4S - D0 2	Fzd 5	GTFST YAIS	299 GIINP SGGS TSYA		513 CARAKGSG WVVGSAF DIW		744 RASQGIS SYLN		1098 AAS SLQ S		1175 CQQS YSTPL TF		1372
01 4S - E0 2	Fzd 5	FTFSD SYMS	206 GFIRS KAYG GTTE YA		490 CARATQEL LLPYGMDV W		758 RASQGIS SYLN		1098 AAS SLQ S		1175 CQQS YSTPL TF		1372
01 4S - F0 2	Fzd 5	YTFTS YYMH	392 GRIN PNSG GTNY A		537 CARVPDF WSGYLDY W		943 RASQGV STZLS		1079 AAS SLQ S		1175 CQQT YSMPI TF		1392
01 4S - G 02	Fzd 6	YTFTS YYMN	392 GIISP SGGS TSYA		516 CARWGDY GDLYYFDY W		951 RASQGIS SALA		1076 ATS TLQ S		1183 CQQV NSYPP TF		1399
01 4S - H0 2	Fzd 6	YIFTD YYMH	368 GRIN PNSG GTNY A		537 CARARSSG WTDAPDI W		751 RASQSVS SWLA		1120 AAS TLQ T		1180 CQQS YSTPT F		1376
01 4S - A0 3	Fzd 6	GTFSS YAIS	295 GWIN AGNG NTTY A		558 CARHYGYS GSYPDW		880 RASQGIS SYLN		1098 AAS SLQ S		1175 CQQS YSTPL TF		1372
01 4S - B0 3	Fzd 6	FTFSS YNM N	232 GRIKS KANG GTTD TA		535 CARAGDSP DYW		739 RASQGIS SYLN		1098 AAS SLQ S		1175 CQQS YSTPL TF		1372
01 4S - E0 3	Fzd 8	GTFSS YAIS	295 GWIS PYNG YTKY A		574 CARAMWS YGQQNAF DIW		745 RASQGIS SYLN		1098 AAS SLQ S		1175 CQQS YSTPL TF		1372
01 4S - G 03	Fzd 8	FTFTS SAVQ	251 GWM NPNS GNTG YA		583 CARRTAVA GTIDYW		914 RASQGIS SYLN		1098 AAS SLQ S		1175 CQQS YSTPL TF		1372
01 4S - H0 3	Fzd 8	YTFTS SAIH	387 GRIN PNSG GTNY A		537 CARVKWEL AIDYW		940 RASQGIS SYLN		1098 AAS SLQ S		1175 CQQS YSTPL TF		1372

TABLE 1A-continued

Anti-Fzd Antibody Clone IDs and CDR sequences													
Clone ID	Initial Binding	CDRH1	CDRH1 SEQ ID	CDRH2	CDRH2 SEQ ID	CDRH3	CDRH3 SEQ ID	CDRL1	CDRL1 SEQ ID	CDRL2	CDRL2 SEQ ID	CDRL3	CDRL3 SEQ ID
01 4S - B0 4	Fzd 8	YIFTD YYMH	368 GWM NPNS GNTG YA	583 CARGGSRY DFWSGHW YFDLW	837 RASQGIS NYLA	1074 AAS SLQ S	1175 CQQS YSTPF TF	1368					
01 4S - E0 4	Fzd 8	YTFTG YYMH	379 GRIN PNSG GTNY A	537 CARDVPKL VTRGVAYG MDVW	804 RASQGIS SYLN	1098 AAS SLQ S	1175 CQQS YSTPL TF	1372					
01 4S - F0 4	Fzd 8	YSFTT YGM N	371 GWIN AGNG NTTY A	558 CARAAAGS YGGGYW	736 RASQGIS NNLN	1072 EAS SVA S	1197 CQQS YTSTP LNSF	1381					
01 4S - G 04	Fzd 8	FTFSS YGMS	231 SAISG SGGS TYYA	600 CARDLTPF TQQQLVLG LL	780 RASQSVS GYLA	1112 GAS TRA A	1216 CQQY NYWP PAF	1423					
01 4S - H0 4	Fzd 8	FTFTS SAVQ	251 GRIVP AIGFT QYA	540 CARSGYNR RGYFDYW	919 RASQGIS SYLN	1098 AAS SLQ S	1175 CQQS YSTPL TF	1372					
01 4S - A0 5	Fzd 8	GTFS YAIS	295 GGIPI FGTA NYA	499 CARVTLGA SVDAPDIW	949 RASQGIS NNLN	1072 DAS SLES	1189 CLQH NSLPF TF	1278					
01 4S - B0 5	Fzd 8	GTFS YAIS	295 GWVS PNTG NTVY A	587 CTTDRRYS TYFDLW	1021 RASQSVS SNLA	1116 GVS NRA T	1223 CQQY NIWP RTF	1418					
01 4S - C0 5	Fzd 8	YTFAS YGM H	373 GWIN AGNG NTTY A	558 CARLSVWK WEQVTN WFDPW	890 RASQGIS SYLN	1098 AAS SLQ S	1175 CQQS YSTPL TF	1372					
01 4S - D0 5	Fzd 8	GTFTS YAIS	305 GWIN AGNG NTKY S	557 CTTGLFPYY RYNWNND AFDIW	1022 RASQGIS SYLN	1098 AAS SLQ S	1175 CQQS YSTPL TF	1372					
01 4S - F0 5	Fzd 9	FTFTG SAVQ	247 GGILP IYGT KYA	509 CARGARLY GCDYW	821 RASQZVS RZZA	1127 GAS TRA T	1217 CQQR SNWPI TF	1335					
01 4S - G 05	Fzd 9	FTFSS SWM H	226 SAIGT GGGT YYA	595 CARKVKGY CSGGSCYG YW	884 RVSQGIS SALA	1151 AAS SLQ S	1175 CQQT SVPW TF	1384					
01 4S - H0 5	Fzd 9	FTFSN YAMT	216 STISG SGVS TFYA	648 CARHGRIA ADIW	876 RASQGIS SYLN	1098 AAS SLQ S	1175 CQQS YSTPL TF	1372					

TABLE 1A-continued

Anti-Fzd Antibody Clone IDs and CDR sequences													
Clone ID	Initial Binding	CDRH1	CDRH1 SEQ ID	CDRH2	CDRH2 SEQ ID	CDRH3	CDRH3 SEQ ID	CDRL1	CDRL1 SEQ ID	CDRL2	CDRL2 SEQ ID	CDRL3	CDRL3 SEQ ID
01 4S - A0 6	Fzd 9	FTFZZ SZVQ	254 GGILP IYGT KYA	509 CARGARLY GPDYW	822 RASQSVS RNLA	1114 GAS TRA T	1217 CQQR SNWPI TF	1335					
01 4S - B0 6	Fzd 9	FTFSS YSMN	233 SYIEN DGSIT TYA	654 CARAPYYY GSGSLFRL DYW	748 RASQSIG SYLN	1098 AAS SLQ S	1175 CQQS YSTPL TF	1372					
01 4S - C0 6	Fzd 10	FTFTG SAVQ	247 GGILP IYGT KYA	509 CARGARLY GPDYW	822 RASQSVS RNLA	1114 GAS TRA T	1217 CQQR SNWPI TF	1335					
01 4S - D0 6	Fzd 10	FTFSR YAM H	219 SGIGV GGGT YYA	605 CARDAYN WFDPR	763 RASQSIG RYLN	1095 AAS SLQ S	1175 CQQR YSTPL TF	1340					
01 4S - F0 6	Fzd 10	YIFTD YYMH	368 GVIFP VYPT PDYA	551 CARGGSTG YYGMDVW	839 RASQSV GRWMA	1107 AAS SLQ S	1175 CQQA NTFPF TF	1321					
01 4S - G 06	Fzd 10	FTFSS YAM H	227 SAIGA GGGT YYA	593 CARDAYN WFDPW	764 RASQSIG SYLN	1098 AAS SLQ S	1175 CQQS YSTPL TF	1372					
01 4S - H0 6	Fzd 10	FTFSS YDM N	229 SAIGT GGGT YYA	595 CARDAYN WFDPW	764 RASQSIG SYLN	1098 AAS SLQ S	1175 CQQS YSTPL TF	1372					
01 4S - A0 7	Fzd 10	FTFSN AQM S	210 SAIGT GGGT YYA	595 CAREGSYY DWYFDLW	809 RASQSIG SRLA	1080 GAS NRA S	1208 CQY NHWP PLFTF	1417					
01 7S - E0 8	Fzd 8	IIFSP NDM G	313 ALISS GGST SYA	450 CHFGVASV GLNYW	980								
01 7S - H0 8	Fzd 8	RTFSS FVMG	346 AAVS ASGG YTWY A	432 CNLAQRGE TYW	998								
01 7S - A0 9	Fzd 8	LAFN GYTM G	317 AAIS WSD NTYY A	414 CAAGFPTV FVVDGEYD YW	680								
01 7S - B0 9	Fzd 8	FTLDY YAIS	255 ADITS GGST NYA	437 CNAVITYN GYTIW	994								

TABLE 1A-continued

Anti-Fzd Antibody Clone IDs and CDR sequences													
Clone ID	Initial Binding	CDRH1	CDRH1 SEQ ID	CDRH2	CDRH2 SEQ ID	CDRH3	CDRH3 SEQ ID	CDRL1	CDRL1 SEQ ID	CDRL2	CDRL2 SEQ ID	CDRL3	CDRL3 SEQ ID
017S- C09	Fzd 8	LTFS YTVG	319 ASST GGGV FENY A	464 CNAV TYN GYTIW	994								
018S- D06	Fzd 4	RIFSS YAQA	330 PRIPS DSTTF YA	590 CEVHN FGA TYW	979								
018S- E06	Fzd 4	RTFS NYV MG	344 AVISR SGGN TYYT	472 CNAV STD WTTDYW	992								
018S- F06	Fzd 4	RTFST YGM G	349 AAIS WSD NTYY A	414 CNSF PLRL HDW	1002								
018S- G06	Fzd 5	LAIDD YYMV	318 SYIST SDGS TYYA	673 CNAV TYN GYSIW	993								
018S- H06	Fzd 5	LAFN GYTM G	317 AQIS WTG GSTD YA	460 CNAD YGT WYGIGW	985								
018S- A07	Fzd 5	LAFN GYTM G	317 AAIS WMS NTYY A	412 CNMGL GY SEYRPLGY W	999								
018S- B07	Fzd 5	SAFS NYA MG	355 AAIT WSGA RTYY A	426 CNAV WKF GTTHW	995								
018S- C07	Fzd 7	LTIDD YYVV	323 SYISA GDGF TYYA	656 CNAV TYN GYTIW	994								
017S- F09	Fzd 4	GSFS GYYS	277 GEIN HSGS TNYN	489 CARDL RFY SSSWRRV GMDVW	778 RSSR SLL HTSGYNY LD	1147 LGS NRA S	1237 CMQ G TRWP TF	1290					
017S- G09	Fzd 4	YTITT YAIH	396 GWIN ADTG DTAY S	555 CARG WTTI SSLGVW	872 RSSR SLL HTNGYN YLD	1146 LGS NRA S	1237 CMQ A LQTPL TF	1281					
017S- H09	Fzd 5	NIFRI YAIA	326 AALT GQRT TNYA ASIT	427 CNTV TYNA GCYKKYW	1004								

TABLE 1A-continued

Anti-Fzd Antibody Clone IDs and CDR sequences													
Clone ID	Initial Binding	CDRH1	CDRH1 SEQ ID	CDRH2	CDRH2 SEQ ID	CDRH3	CDRH3 SEQ ID	CDRL1	CDRL1 SEQ ID	CDRL2	CDRL2 SEQ ID	CDRL3	CDRL3 SEQ ID
01 7S - A1 0	Fzd 5	LAFN GYTM G	317	WNG RYTTY A	462	CNARLDAV YGHSTRYDS W	988						
01 7S - B1 0	Fzd 5	NFFS NYPL G	325	GAISR TGSG TFYA	484	CAAGVTGS WRYW	684						
01 7S - C1 0	Fzd 8	RSFSN YRVA	335	AVSW SVGM TYYA	479	CNAVITYN GYTIW	994						
01 7S - D1 0	Fzd 8	GTFG SYAV G	288	GLISR NAGN TLYA	518	CNAVNGRL NYW	991						
01 7S - E1 0	Fzd 8	RTFSS YSLA	348	AAVS ASGA NTYY A	431	CAAPQSPN MYIRTDQL WWYKYW	687						
01 8S - D0 7	Fzd 1	RSFST YPMG	336	TVISG SGGS TYYA	675	CAAGPTLP FRYW	683						
01 8S - E0 7	Fzd 1	RAFS NYA MG	328	AAIN WSG DSAY YA	406	CNARLSFA GGMGYW	989						
01 8S - F0 7	Fzd 1	IKSMF DMNF MG	314	AFITR GGTT RYG	438	CNAVSTD WTRDYW	992						
01 8S - G 07	Fzd 1	LTIDD YYMV	322	SYIGT SDGT TYYA	655	CNAVITYN GYTIW	994						
01 8S - H0 7	Fzd 4	RVFSS YAQA	354	AGIAS DSTTF YA	439	CKVHNFGA TYW	983						
01 8S - A0 8	Fzd 4	RIFSS YAQA	330	ASIPS DGTT FYA	461	CKVHNFEA TYW	982						
01 8S - B0 8	Fzd 4	LTFST YGM G	321	AAIN WSGR STVY A	407	CNSFPLRL HDW	1002						

TABLE 1A-continued

Anti-Fzd Antibody Clone IDs and CDR sequences													
Clone ID	Initial Binding	CDRH1	CDRH1 SEQ ID	CDRH2	CDRH2 SEQ ID	CDRH3	CDRH3 SEQ ID	CDRL1	CDRL1 SEQ ID	CDRL2	CDRL2 SEQ ID	CDRL3	CDRL3 SEQ ID
01 8S - C0 8	Fzd 4	RTLSS YVVG	351 ALISL SGAS TYYA		449 CNAVSTD WTTDYW		992						
01 8S - D0 8	Fzd 5	IKSMF DMNF MG	314 AFITR GGTT RYG		438 CNAVSTD WTRDYW		992						
01 8S - E0 8	Fzd 5	RTDG MQA MG	338 GAIT WSLG SAFY A		486 CNVLAQN DGDYRTYG		1007						
01 8S - F0 8	Fzd 5	RTFSS FVMG	346 AAVS ASGG YTWY A		432 CNAVWKF GTTHW		995						
01 8S - G 08	Fzd 5	RTFSS FVMG	346 AAVS ASGG YTWY A		432 CNAVCKFG TTHW		990						
01 8S - H0 8	Fzd 5	RTFSS FVMG	346 AAVT ASGG YAWY A		434 CNAVWKF GTTHW		995						
01 8S - A0 9	Fzd 8	ITFSF NSVG	316 AVFIA GYGA YYA		470 CNGVTYN GYTIW		997						
01 8S - B0 9	Fzd 8	HDFS STYG VG	310 ATIS WGG TNIA		468 CAAQKPYY NGHFIYAD DKHYDHW		688						
01 8S - C0 9	Fzd 8	ITFGF DSVG	315 AVFN AGYR AYYA		471 CNAVTYN GYTIW		994						
01 8S - D0 9	Fzd 8	RTFS WYS MG	350 AAVS WSGV STYYP		433 CNAVTYN GYTIW		994						
01 8S - E0 9	Fzd 8	ITFSF NSVG	316 AVFIA GYGA YYA		470 CIGVTYNG YTIG		981						
01 8S - F0 9	Fzd 8	RTDG MQA MG	338 GAIT WSLG IAFYA		485 CNVLAQN DGDYRTY W		1008						

TABLE 1A-continued

Anti-Fzd Antibody Clone IDs and CDR sequences													
Clone ID	Initial Binding	CDRH1	CDRH1 SEQ ID	CDRH2	CDRH2 SEQ ID	CDRH3	CDRH3 SEQ ID	CDRL1	CDRL1 SEQ ID	CDRL2	CDRL2 SEQ ID	CDRL3	CDRL3 SEQ ID
01 8S - G 09	Fzd 8	HDFS STYG VG	311 AAIS WRGT NIA		413 CAAQKPYY NGHFYAD DKHYDHW	688							
02 1S - A0 1	Fzd 8	DSVS SNSA AWN	160 GRAY YKSR WYYD YA		524 CVRDLRPS GDLNFDY W	1029 RASQSIG SSLH		1087 YAS QSV S		1260 CHQS GRVP VTF	1268		
02 1S - C0 1	Fzd 1	GSISS GGYS WS	283 GSIYH SGSTY YN		547 CARFYDIL NGYSYFDY W	817 RSSRSL DTDDGN TYLD		1142 TLS HRA S		1259 CMQSI QLPW TF	1295		
02 1S - D0 1	Fzd 1	FTFSS YGM H	230 AVISY DGSN KYA		475 CAKGSVFG LKAGGYAD YW	721 RSSQSLV HSDGNT YLS		1140 KISN RFS		1230 CMQA TQFP TF	1283		
02 1S - E0 2	Fzd 8	YTFTS YGIS	391 GWIS AYNG NTNY A		570 CARDGTPF YSGSYG W	772 QGDRLT YYAS		1052 GKN NRP S		1219 CNSRD NSGK HKVF	1300		
02 1S - G 02	Fzd 8	DSVS SNSG AWN	162 GRTY YRSKY YNGY A		544 PRLDYW	1034 RSSQSL DSDDGN TYLD		1133 MLS SRA P		1240 CMQR LEFPY TF	1294		
02 1S - A0 3	Fzd 8	DSVS SNSA AWN	160 GRTY YRSK WYN DYA		542 CARSQATG ERFDYW	921 RSSQNIF QSLN		1131 SAS SLQ S		1254 CQQS YNSPI TF	1349		
02 2S - H0 6	Fzd 4	FTFSS YAMS	228 SVIST SGGT VLYT		652 CADGSGTS HR	690 RASQSIG SYLN		1098 AAS SLQ S		1175 CQQS YSTPL TF	1372		
02 2S - A1 1	Fzd 10	YIFTD YYMH	368 GGIFP IFGTA NYA		493 CAKGSYYY DNSGYW DAFDIW	722 RASQGIS NNIN		1071 AAS NLE T		1166 CQQT YSIPFT F	1391		
O M P- 18 R5		GFTFS HYTSL	270 VISGD GSYT YYAD SVKG		677 NFIKYVFA N	1033 SGDKLGK KYAS or SGDNIGS FYVH		1152 EKD or NRP 1153 SG or DKS NRP SG		1200 SSFAG or NSLE 1201 or QSYA NTLSL	1435 or 1436		
02 7S - H0 2	Fzd 5	FTFSS YAMS	228 SAISG SGGS TYA		600 CAKGLWG PLLNW	718 RASQSVS SNYLS		1118 GAS SRA P		1212 CQQR TNWP PRVTF	1337		
02 7S - B0 3	Fzd 8	DSVS SNSA TWN	161 GRTY YRSK WYSD YA		543 CTRGNNW VGLANW	1014 SGTSSNI GAGYDV H		1157 GNN NRP S		1219 CSAW DDNL NGVV F	1432		

TABLE 1A-continued

Anti-Fzd Antibody Clone IDs and CDR sequences													
Clone ID	Initial Binding	CDRH1	CDRH1 SEQ ID	CDRH2	CDRH2 SEQ ID	CDRH3	CDRH3 SEQ ID	CDRL1	CDRL1 SEQ ID	CDRL2	CDRL2 SEQ ID	CDRL3	CDRL3 SEQ ID
027S-E01	Fzd 5	RSFSI YNTA	334 AAIS WSG GSTY YA	418	CNVITIVRG MGPRAYW	1006							
004S-D05	Fzd 5	LTFSI YAM H	320 SAISG DGAL TYYA	597	CARGVYPY SSKHKPSY YYYGMDV W	870	QASQDIS NYLN	1046	AAS SLQ S	1175	CQQS YSTPL FTF	1371	
004S-D04	Fzd 5	YDFTT YGIH	367 GGVI PAFG ATDY S	511	CARGYYYG MDVW	874	RASQSIG SYLN	1098	AAS SLQ S	1175	CQQS YSTPL TF	1372	
004S-B05	Fzd 5	GTFSS YAIS	295 GWIN AGNG NTTY A	558	CASGLGYF DYW	957	RASQSIG SYLN	1098	AAS SLQ S	1175	CQQS YSTPL TF	1372	
004S-G03	Fzd 5	YTFTN NFMH	383 GGIPI FGTP HYA	502	CARTLTTPP YYYGMDV W	929	RASQSIG SYLN	1098	AAS SLQ S	1175	CQQS YSTPL TF	1372	
004S-F03	Fzd 5	FTFSN SDM N	214 SAIGT GGDT YYA	594	CTRDLYGG YRDYW	1013	KSSQSLL HSDGYTY LY	1036	LGS NRA S	1237	CMQG LQTP WTF	1286	
004S-C04	Fzd 5	YIFTG YYMH	369 GRIN PNSG GTNY A	537	CARGGEYS SGWYTYYY YGMDVW	827	RATQTIS TYLN	1129	AAS RLQ S	1171	CQQY YSYP WTS	1427	
004S-B06	Fzd 5	YTFTY RYLH	394 GMIN PIGGS INYA	522	CARDVMD VW	803	RASQSIG NNLN	1072	AAS ALQ S	1165	CQHL NNFPPL TF	1303	
004S-F06	Fzd 5	FSVG SNYM T	186 SSISS GNSYI YYA	634	CARGPKT MWEDRPD YW	851	RASQSIG SYLN	1098	AAS SLQ S	1175	CQQS YSTPL TF	1372	
004S-A04	Fzd 5	FTFST YSMI	245 GFIRS KDYG GTTE YA	491	CARTLGGA VAGTHRDI W	892	RASQSIG NNLN	1072	GAS SLQ S	1211	CQQS HSSPR TF	1346	
004S-A05	Fzd 5	FTFSS YVMS	237 SAIGT GGGT YYA	595	CARGSSGY YVAW	862	RASQSIG SYLN	1098	AAS SLQ S	1175	CQQS YSTPL TF	1372	
004S-F05	Fzd 5	FTFSN HYMS	212 AGVSI DANK KYA	447	CARDQND SWYRSDY W	785	RSSQSLL HSDGYTY LY	1135	LGS HRA S	1235	CMQG LQTPH TF	1285	

TABLE 1A-continued

Anti-Fzd Antibody Clone IDs and CDR sequences													
Clone ID	Initial Binding	CDRH1	CDRH1 SEQ ID	CDRH2	CDRH2 SEQ ID	CDRH3	CDRH3 SEQ ID	CDRL1	CDRL1 SEQ ID	CDRL2	CDRL2 SEQ ID	CDRL3	CDRL3 SEQ ID
003S-CO1	Fzd 1	GTFSS YAIS	295 GRIN PNSG GTNY A	537	CARGSGYD FFDYGMD VW	861	RASQSIG NNLN	1092	AAS SLQ S	1175	CQQS YNTPF TF	1350	
003S-HO1	Fzd 1	DTFS NYVL S	164 GLVD PEDG ETIYA	520	CAKASTPM VQGAPDY W	697	RASQSIG SNLD	1085	AAS TLQ S	1179	CQQN YATPR TF	1333	
003S-HO2	Fzd 1	GTFN RYAIT	289 GGIPI FGTA NYA	499	CATTQGVY SSSWYGG GRAFDIW	976	RASQSIG SYLN	1098	AAS SLQ S	1175	CQQS YSTPL TF	1372	
003S-HO4	Fzd 1	YTFTY RYLH	394 GRIN PNSG GTNY A	537	CWGSYY GDYW	1030	RASQGIS NNLN	1072	AAS SLQ S	1175	CQQA NSFPI TF	1316	
003S-AO5	Fzd 2	FTFSS YAM H	227 SSISW NSGR VDYA	638	CARGSGIA ASGSYW	860	TSSQSLL HSDGKTY LY	1162	LGS NRA S	1237	CMQG THWP YTF	1289	
003S-BO5	Fzd 2	FTFSN AWM S	211 STIAG SGGR TYYS	643	RGRGAPQ PYYYGYM DVW	709	KSSQSLL HSDGKTY LY	1036	LGS NRA S	1237	CMQS LQSPL TF	1296	
003S-FO5	Fzd 2	FSFST YTMS	184 SRING DGSS TRYA	624	CARAIVGA TGLNRFKA FDIW	743	KSSQSLL HSDGKTY LY	1036	LGS NRA S	1237	CMQN THWP LTR	1293	
003S-GO5	Fzd 2	STFTN AWM S	363 SAIGT GGGT YYA	595	CARDRVTL RGGYSYGT DAFDIW	796	RSSRSLL HSNGNT YLR	1145	LAS RRA S	1231	CIQNT HWPL TR	1270	
003S-HO5	Fzd 2	FTLST YNM N	257 SRINY DGSA TTYA	626	CARDRDIV VVPQQRG EGGFDPW	786	KSSQSLL HSDGKTY LY	1036	MG SYR AS	1239	CMQG THWP LTF	1287	
003S-AO7	Fzd 2	FTFSS YAMS	228 SAISG SGGS TYYA	600	CAKGGRD GYKGYFDY W	714	KSSQSLL HSDGKTY LY	1036	LGS NRA S	1237	CMQN THWP LTL	1292	
003S-CO7	Fzd 2	FSFRS YSMS	178 SAIGT GGGT YYA	595	CTTTTVTTS W	1026	RTSQSVS SNLA	1150	DAS NRA S	1187	CQQY GSSPY NF	1412	
003S-FO7	Fzd 2	FSFSS YGMS	183 SHISS GGAT IDYA	619	CARDGGY W	768	RASQSIG SYLN	1098	AAS SLQ S	1175	CQQS YSTPL TF	1372	

TABLE 1A-continued

Anti-Fzd Antibody Clone IDs and CDR sequences													
Clone ID	Initial Binding	CDRH1	CDRH1 SEQ ID	CDRH2	CDRH2 SEQ ID	CDRH3	CDRH3 SEQ ID	CDRL1	CDRL1 SEQ ID	CDRL2	CDRL2 SEQ ID	CDRL3	CDRL3 SEQ ID
00 3S - G 07	Fzd 2	FTFSS YWM H	239 SYISG DSGY TNYA	658 CARDNGYC SGGSCYAT YYGMDVVR	783 RASQAIS SYLA	1060 KAS TLD T	1228 CQQA DTFPF TF	1313					
00 3S - B0 8	Fzd 2	FTFSS ZZMH	240 AVISY DGSN RZYA	476 CARSYIDS SGYPRKDA FDIW	927 KSSQSLL HSDGKTY LY	1036 LGS NRA S	1237 CMQT LKAPL TF	1299					
00 3S - F0 8	Fzd 2	ZSVSS NYMS	401 SRINS DGSTI SYA	625 CARARLLG GYTPDR MDVW	750 RSSQYLS SAYLA	1141 GAS SRA T	1213 CQQY GSSPT F	1411					
00 3S - H0 8	Fzd 2	FTFN RHAL S	197 ALISS NGDH KYYT	451 CARDLMV GRNKLDY W	777 QASQGIS NNLN	1049 AAS SLQ S	1175 CQQS YSTPA FTF	1367					
00 3S - A0 9	Fzd 2	FTFSS SNM N	225 SGISG SGSST YYA	611 CARGRVW SSRDYVW	859 RSSQSLL HSDGYTY LY	1135 LGS NRA S	1237 CMQG THWP LTF	1287					
00 3S - B0 9	Fzd 2	FNIRR ZNMZ	174 SAIGT GGGT YYA	595 CARGDSGS YRDYVW	823 RSSESLL HSDGKTY LY	1130 LGS NRA S	1237 CTQTV QFPIT F	1433					
00 3S - C0 9	Fzd 2	FTFSS SAMH	224 SGISG SGTTT YYR	612 CARRLIAV AGAEFDP W	912 RASQGIS NNLN	1072 SAS NLQ S	1252 CQQS YSTP WTF	1378					
00 3S - F0 9	Fzd 4	FTFSN SDM N	214 KAYG GTTE YA	536 CARQYFDP YW	909 RASQSIG SYLN	1098 AAS SLQ S	1175 CQQS YSTPL TF	1372					
00 3S - H0 9	Fzd 4	FTFSS FGMH	221 SVISS GGSP YYA	651 CATASGDF DYW	968 RASQSIG SNLN	1086 RAS TLES	1248 CQQT YTTPR F	1398					
00 3S - A1 0	Fzd 4	FTFD DYA MH	191 AIVSY DGTY KYYS	448 CARQTRG GTTDGVW	907 RASQGIS NNLN	1072 YAS SLQ S	1262 CQQS HSPPG TF	1345					
00 3S - B1 0	Fzd 4	FTFSS HSTH	222 SAISA SGDS TFYA	596 CARPIVGA TAFDIW	900 RASQSIG SYLN S	1098 AAS SLQ TF	1175 CQQS YSTPL	1372					
00 3S - G 10	Fzd 4	FTZSS YSMN	264 SYSSG NSGY TNYA	674 CARGVGS GAFDIW	868 RASQSIV SYLN	1104 DAS NLQ S	1186 CQQG YSAP WTF	1329					

TABLE 1A-continued

Anti-Fzd Antibody Clone IDs and CDR sequences													
Clone ID	Initial Binding	CDRH1	CDRH1 SEQ ID	CDRH2	CDRH2 SEQ ID	CDRH3	CDRH3 SEQ ID	CDRL1	CDRL1 SEQ ID	CDRL2	CDRL2 SEQ ID	CDRL3	CDRL3 SEQ ID
00 3S - B1 1	Fzd 4	FTFSD YYMS	208 SAIDG AGRT YYT	591 CARAIPGD YDYW	742 RASQSIS SYLN	1098 AAS SLQ S	1175 CQQS YSTPL TF	1372					
00 3S - C1 1	Fzd 4	FTFST YAM H	252 GGIIP FGIAN YA	496 CARTGRGY YGMDVW	928 RASQSIS SNLD	1085 AAS TLQ S	1179 CQQS YSTPR TF	1373					
00 3S - D1 1	Fzd 4	FTFSS YSMS	234 SYISG DSGY TNYA	658 CARAGVAT IAPDYW	741 RASQSIS SYLN	1098 AAS SLQ S	1175 CQQS YSTPL TF	1372					
00 3S - F1 1	Fzd 4	FTFD DYG MH	192 SAISG SGGS TYYA	600 CTTPNYD SR	1023 RASQSIS SYLN	1098 AAS SLQ S	1175 CQQS YSTPL TF	1372					
00 3S - E1 2	Fzd 4	GTFSS YAIS	295 GWIN AGNG NTTY A	558 CARHYGGS GSDPDW	880 ZPZQTZZ SHLN	1164 PAS SLQ S	1242 CQQS YSTPL TF	1372					
00 4S - A0 1	Fzd 4	FTFST YGM H	244 SYISS SSSAI YYA	671 CARGGLDG PIDYR	830 RASQGIS NNLN	1072 AAS TLQ S	1179 CQQG NNFPF TF	1326					
00 4S - G 01	Fzd 4	FTVSS HSMG	260 SLVSF DGSK EHYA	621 CARLGSTP DYW	887 RASQGIS NNLN	1072 AAS SLQ S	1175 CQQY YTPY TF	1428					
00 4S - C0 2	Fzd 4	FTFSS YGM H	230 AVISY DGSN KYA	475 CASDPVTA ATR	956 RASQSIS SYLN	1098 AAS SLQ S	1175 CQQS YSTPL TF	1372					
00 4S - D0 2	Fzd 4	FSFSS YGMS	183 SGISG SGRS TYA	610 CAKDGW	699 RASQSIS SYLN	1098 AAS SLQ S	1175 CQQS YSTPL TF	1372					
00 4S - A0 3	Fzd 4	FTFSS YAM H	227 WNG GSTG YA	606 CARPAGSA QNWFPD W	899 RASQGIS NNLN	1072 DAS NLE T	1185 CHQSY SIPRTF	1269					
00 4S - B0 3	Fzd 4	FSFSR YGMS	180 SGVG GSGG STZYA	617 CARDGSW	771 RASQDV DTWLA	1066 DAS TLET	1191 CQQG YNIP WTF	1328					
00 4S - C0 3	Fzd 4	YTFTS YAIS	388 GIINP SGGS TSYA	513 CARQIGWE LMPDIW	906 QASQDIS SYLN	1047 AAS TLQ S	1179 CQQA SFPLTF	1315					

TABLE 1A-continued

Anti-Fzd Antibody Clone IDs and CDR sequences													
Clone ID	Initial Binding	CDRH1	CDRH1 SEQ ID	CDRH2	CDRH2 SEQ ID	CDRH3	CDRH3 SEQ ID	CDRL1	CDRL1 SEQ ID	CDRL2	CDRL2 SEQ ID	CDRL3	CDRL3 SEQ ID
00 4S - C0 5	Fzd 5	ZZZTD YYZQ	403 GGM NZNR GNTG YA	510 CANGSYA QHLW	732 RASQSIS SYLN	1098 AAS SLQ S	1175 CQQS YSTPL TF	1372					
00 4S - G 05	Fzd 5	FTFSS YWM H	239 STISP SGLYI YQA	649 CAKDKVPY SYGPNFDY W	703 RASQSIS SYLN	1098 AAS SLQ S	1175 CQQS YSTPL TF	1372					
00 4S - E0 6	Fzd 5	FFFSG YWM S	169 QDGS EKYY V	459 CARVFPPLH DYW	934 QASQDIS NYLN	1046 KAS SLES	1226 CQQA NSFPY TF	1319					
00 4S - C0 6	Fzd 5	FPFST FSMN	176 AGIS WNS GTIDY A	446 CARSGPAA MVYYYYG MDVW	918 RSSQNVS SYLA	1132 GAS TRA T	1217 CQHR ANWP QTF	1305					
00 4S - E0 7	Fzd 6	FTLSS HHM N	256 SAIGT GGGT YYA	595 CAAPDYW	686 RASQSIS SYLN	1098 AAS SLQ S	1175 CQQS YSTPL TF	1372					
00 4S - F0 7	Fzd 6	FSFSK KYMT	179 SSIDG NGDH VFYA	629 CARPYYYD SSGYDPM GDYW	904 QASQDIT NYLN	1048 KAS TLES	1229 CQQS YSAPY TF	1356					
00 4S - C0 8	Fzd 6	FTVSS NYM N	261 SAIGT GGGT YYA	595 CAQGTYW	735 RASQSIS SYLN	1098 AAS SLQ S	1175 CQQS YSTPL TF	1372					
00 4S - F0 8	Fzd 6	FTFD DYIM N	193 SAVS GNGG GTFY A	604 CARGGNY GSGDYW	833 RASQSIS ZWLA	1102 EAS TLQ S	1198 CQQT YTPPF TF	1397					
00 4S - G 09	Fzd 6	GTLN NHTL S	308 GRIIPI FGTA NYA	526 CARDRRGY GMDVW	795 RASQAIS NSLA	1058 DAS NLE T	1185 CQQA YSFP WTF	1324					
00 4S - B1 0	Fzd 6	FTFSD YYMS	208 SGIN WNS AKIGY V	607 CARIGAGG AFDIW	881 RASQSIS SYLN	1098 AAS SLQ S	1175 CQQS YSTPL TF	1372					
00 4S - H1 0	Fzd 6	FIFSD YYMS	170 AVITS GGTF KYA	477 CARNGIAA AEDYW	896 RASQSIS TYLS	1101 GAS SLES	1210 CQQS YSPPF TF	1362					
00 4S - B1 1	Fzd 6	FTFSS SWM H	226 SGIS WNS GSIGY A	615 CARYSSGG SLDYW	955 RASQSIS SYLN	1098 AAS SLQ S	1175 CQQS YSTPL TF	1372					

TABLE 1A-continued

Anti-Fzd Antibody Clone IDs and CDR sequences													
Clone ID	Initial Binding	CDRH1	CDRH1 SEQ ID	CDRH2	CDRH2 SEQ ID	CDRH3	CDRH3 SEQ ID	CDRL1	CDRL1 SEQ ID	CDRL2	CDRL2 SEQ ID	CDRL3	CDRL3 SEQ ID
00 4S - D1 1	Fzd 6	YZFZZ ZYM	400 GRIN PNSG GTNY A	537 CARARSSG WTDAPDI W	751 RASQSVS SWLA	1120 AAS TLQ T	1180 CQQS YSTPT F	1376					
00 4S - F1 1	Fzd 6	FTFSS YAMS	228 SSISG GGRH TYYA	632 CARPYSSS RQGDYW	903 RASQSIG SYLN	1098 AAS SLQ S	1175 CQQS YSTPL TF	1372					
00 4S - G 11	Fzd 6	YIFTD YMH	368 GWIN PNSG GTNY A	564 CARDRPGF DPW	790 RASQSVS SYLA	1121 GAS SRA T	1213 CQQY AISYTF	1400					
00 4S - B1 2	Fzd 6	FTFSS YWIH	238 SYISG DSGY TNYA GWM	658 CAKGIRWF DPW	716 RASQSIG SYLN	1098 AAS SLQ S	1175 CQQS YSTPL TF	1372					
00 4S - C1 2	Fzd 6	YIFTD YMH	368 NPNS GNTG YA	583 CASSHYAP GMDVW	962 RASQGIS SYLA	1076 RTS TLES	1250 CQQS YSTP WTF	1378					
00 4S - F1 2	Fzd 7	FTVG NNY MS	258 SSITT TSTLY A	640 CARGKEGR YSNYEAA W	844 RASQSIG SYLN	1098 AAS TLQ T	1180 CQQS YSIPFT F	1358					
00 5S - B0 1	Fzd 7	FTFRS YGM H	202 SLISG SGDN TNYA	620 CARREPLY SSRRGAFDI W	910 RASQSIG SYLN	1098 AAS SLQ S	1175 CQQS YSTPL TF	1372					
00 5S - C0 1	Fzd 7	FTFSS YSMS	234 SAISG SGGS TYYA	600 CTRTIVGAT PHYW	1017 RASQGIS NNLN	1072 KAS SLQ S	1227 CQQS YSLPY TF	1360					
00 5S - F0 1	Fzd 7	FTVSS NYMS	262 SAISG SGAT TTYA	598 CAKGAGY GSGSWQA AW	711 RASQSVS SSYLS	1119 GAS SRA T	1213 CQQR YKSYT F	1338					
00 5S - B0 2	Fzd 8	YSFTN YAM H	370 GRIIPI FGTA ZYA	527 CARGTFLE WLLTNYG MDVW	866 RASQSIG SYLN	1098 AAS SLQ S	1175 CQQS YSTPL TF	1372					
00 5S - D0 2	Fzd 8	GTFSS YVIS	297 GWIG PHNG NTNY A	554 CATGWPR YYYGMDV W	971 RASQSVS SNLA	1116 NTS NRA T	1241 CQHY NNWP FTF	1308					
00 5S - G 02	Fzd 8	YTFTS YYMH	392 GGIPI FGTA ZYA	500 CARLPYYD FWSGYG GRTGFDY W	889 RASQSVS TNLA	1122 DAS NRA T	1188 CQQR SNWP PQITF	1336					

TABLE 1A-continued

Anti-Fzd Antibody Clone IDs and CDR sequences													
Clone ID	Initial Binding	CDRH1	CDRH1 SEQ ID	CDRH2	CDRH2 SEQ ID	CDRH3	CDRH3 SEQ ID	CDRL1	CDRL1 SEQ ID	CDRL2	CDRL2 SEQ ID	CDRL3	CDRL3 SEQ ID
00 5S - H0 2	Fzd 8	YTFTY RYLH	394 GWIN AGNG NTTY A	558 CARASLYY DYVWGSY RHYYFDY W	753 QASQDIS HYLN	1044 AAS SLQ S	1175 CQQS YSTPL TF	1372					
00 5S - B0 3	Fzd 8	GTFSS YAIS	295 GIINP SGGR TTYA	512 CATSFGGG WIVVDTSL WYW	975 RASQSIN SNLA	1091 GAS SRA T	1213 CQQY GSSPY TF	1413					
00 5S - C0 3	Fzd 8	GSFS GYAIS	275 GGIPI FGTA NYA	499 CRVDAFDI W	1009 RASQSVS SSYLS	1119 DTS NRA T	1194 CQQY GSSPI TF	1408					
00 5S - E0 3	Fzd 8	FTFTS SAVQ NYA	251 GGIPI FGTA W	499 CARSSGW QNRPAFDI	923 RTSQSISS YLN S	1149 AAS TSQ	1181 CQQSF SSWTF	1343					
00 5S - F0 3	Fzd 8	YTFTY RYLH	394 GWIN AGNG NTKY S	557 CATDLPVR KGFTYYDIL TGSYGMD VW	970 RASQSISS SYLN	1098 AAS SLQ S	1175 CQQS YSTPL TF	1372					
00 5S - B0 4	Fzd 8	YTFTN NFMH	383 GGIPI FGTA NHA	498 CARGLRYF DWPQGIY YYYGMDV W	848 QASHDI NIALN	1039 AAS SLQ S	1175 CQQS YSSPL TF	1365					
00 5S - C0 4	Fzd 8	YTFST YYMH	392 GRIN PNSG GTNY A	537 CARGLLF DYW	831 RASQSISS SYLN	1098 AAS SLQ S	1175 CQQS YSTPL TF	1372					
00 5S - D0 4	Fzd 8	FTFTS YSMS	246 STIGT GGGT YYA	645 CARVGWL RFLDYW	938 RASQSISS SYLN	1098 AAS SLQ S	1175 CQQS YSTPL TF	1372					
00 5S - G 04	Fzd 8	GTFSS YAIS	295 GWM SPSSG NAGY A	585 CARNFLR AFDIW	897 RASQSISS SYLN	1098 AAS SLQ S	1175 CQQS YSTPL TF	1372					
00 5S - A0 5	Fzd 8	FAFSS YAMS	167 SRIDT DGST TYVA	622 CARAPSYS SGWYVRW	747 RASQSISS SYLN	1098 AAS SLQ S	1175 CQQS YSTPL TF	1372					
00 5S - C0 5	Fzd 8	YTFTY YAM H	395 GIINP SGGS TSYA	513 CARELLPM TTVTSPFI W	812 RASQGIS NNLN	1072 RAS SLQ S	1247 CQQA NSYPL TF	1320					
00 5S - E0 5	Fzd 8	GTFSS YAIS	295 GGIPI FGTA NYA	499 CAIRAFDI W	694 RASQSISS SYLN	1098 AAS SLQ S	1175 CQQS YSTPL TF	1372					

TABLE 1A-continued

Anti-Fzd Antibody Clone IDs and CDR sequences													
Clone ID	Initial Binding	CDRH1	CDRH1 SEQ ID	CDRH2	CDRH2 SEQ ID	CDRH3	CDRH3 SEQ ID	CDRL1	CDRL1 SEQ ID	CDRL2	CDRL2 SEQ ID	CDRL3	CDRL3 SEQ ID
00 5S - C0 6	Fzd 8	ZTFSZ YDM H	402 SSISS SSHY KYA		635 CARVRSKA VAGTLPKR LFDIW		947 RASQSVS SSYLS		1119 AAS RRA T		1172 CQQY SNWP FTF		1424
00 5S - E0 6	Fzd 8	YTFTS YYMH	392 GWM NPNS GNTG YA		583 CARGNPTS GHIVVPA ATFSDYW		850 QASQDIS NRLN		1045 SAS RLQI		1253 CQQS YRTPR TF		1354
00 5S - G 06	Fzd 8	GTFSS ZTIS	302 GWM NPDS GKTG YA		579 CARWAFPI PNAPDIW		950 RASQSIG SYLN		1098 AAS SLQ S		1175 CQQS YSTPL TF		1372
00 5S - H0 6	Fzd 8	YTFTN NFMH	383 GGIFP IYGIS TYA		494 CARDRPSS SWYAPDY W		791 RASQSIG NNLN		1072 DAS TLQ T		1193 CQQS SAPITF		1342
00 5S - A0 8	Fzd 9	GTFSS YAIS	301 GGIPI FGTA NYA		499 CARGGLLR FGDGW MGMDVW		832 RASQSIG SKSLA		1097 GAS TRA T		1217 CQQY GIAPT F		1407
00 5S - C0 8	Fzd 9	YTFTD YHM H	375 GWIN AGNG NTTY A		558 CARASSWY LHYYYGM DVW		755 RASQSIG SYLN		1098 AAS SLQ S		1175 CQQS YSTPL TF		1372
00 5S - E0 8	Fzd 9	FIFSS YAMS	171 SSISA AGAY KYA		631 CARRGYSS GWRDAFD IW		911 RASQSIG SYLN		1098 AAS SLQ S		1175 CQQS YSTPL TF		1372
00 5S - F0 8	Fzd 9	YTFTS YYMH	392 GWIN AGNG NTTY A		558 CAKDVNY W		710 QASQSIG NYLN		1050 AAS SLQ S		1175 CQQT YSTPT TF		1395
00 5S - H0 8	Fzd 9	GTFSS YAIS	295 GRIPI LGTP NYA		531 CARDRLAF DYW		789 RASQSIG SYLN		1098 AAS SLQ S		1175 CQQS YSTPL TF		1372
00 5S - A0 9	Fzd 9	FAFSS HWM H	166 SAISV SGGT TFYA		602 CARWGKR LRGSPYYF DYW		952 RASQSIG SNLD		1085 RAS TLQ S		1249 CQQS YSTPS F		1375
00 5S - F0 9	Fzd 9	FTFSI YGM H	209 SGIS WNS GNIG YA		614 CARGPLPT KIGGHYM DVW		852 RASQSIG SYLN		1098 AAS SLQ S		1175 CQQS YSTPL TF		1372
00 5S - B1 0	Fzd 9	FTFST XWM S	241 AVMY SGGT TYA		478 CARLSYYY DSSGPKGD AFDIW		891 RASQSIG NNLN		1072 AAS SLQ S		1175 CQQG NNFPL TF		1327

TABLE 1A-continued

Anti-Fzd Antibody Clone IDs and CDR sequences													
Clone ID	Initial Binding	CDRH1	CDRH1 SEQ ID	CDRH2	CDRH2 SEQ ID	CDRH3	CDRH3 SEQ ID	CDRL1	CDRL1 SEQ ID	CDRL2	CDRL2 SEQ ID	CDRL3	CDRL3 SEQ ID
005S-C10	Fzd 9	FSLSS YGM H	185 SSISS SSSYI YYA	636 CARSGMV KWLRSFDY W	917 RASQDIG SFLA	1061 AAS SLQ S	1175 CQKY NRAPF TF	1310					
005S-E10	Fzd 9	FTFSS SAM Q	249 GVIN PGSG GTSY N	552 CARGYGDY VWGENYF DYW	873 RASQSIG SYLN	1098 AAS SLQ S	1175 CQQS YSTPL TF	1372					
005S-F10	Fzd 9	YTLSN YGIS	397 GWIS AYNG DTKY A	568 CARFDYFG GMDVW	816 RASQSIG SYLN	1098 AAS SLQ S	1175 CQQS YSTPL TF	1372					
005S-G10	Fzd 9	YTFTR YAVH	385 GGIIP FFNT VNYA	495 CAADRSPY YYDSSGY PDADFIDW	679 RASQGIS>NNLN	1072 QAS SLD S	1244 CQQS YNVPY TF	1352					
005S-A11	Fzd 9	FTFSS YDM N	229 SGIS WNS GYIGY A	615 CAKGSLLL GYGMDV W	720 RASQSIG>NNLN	1092 DAS TLK R	1192 CQQS YNTPR TF	1350					
005S-B12	Fzd 10	FTZSS YDM H	263 SSISG LGGS TYZA	633 CAREAGTT GGWFDP W	805 RASQSIG SYLN	1098 AAS SLQ S	1175 CQQS YSTPL TF	1372					
005S-D12	Fzd 10	FTFSD HYM D	204 STIGP AGDT YYP	644 CARASTSG DYSLW	756 RASQSVS TSYLA	1123 GAS TRA T	1217 CQQY GASP WTF	1406					
006S-B01	Fzd 10	YTFTN YCTR	384 GLVC PSDG STSYA	519 CARRTSAS DIW	915 RASQSIG SYLN	1098 AAS SLQ S	1175 CQQS YSTPL TF	1372					
006S-C01	Fzd 10	FTFTZ SAVQ	253 GGPD PEDG ETIYA	492 CTTDPLELP WYW	1020 RASQGIS SALA	1076 SAS NLQ S	1252 CQQAI SFPLTF	1315					
006S-E01	Fzd 10	YTFTG YYMH	379 GIINP SSGR TDYA	514 CARDLTY YDSSGHSP LGAFDIW	781 RASQSVT SSLA	1124 GAS TRA T	1217 CQQY NDWP PTF	1416					
006S-G01	Fzd 10	FTFSD FGM N	203 AGISG GGGS TDYA	443 CARSDSF WYYYGMD VW	797 KSSQSVL YSSNNK NYLA	1037 STN TRS S	1257 CQHR NFF	1306					
006S-B02	Fzd 10	VSFS GYA MH	366 AYINS GSSE MNY A	482 CAREEWEL FGMDVW	807 RASQSIG SYLN	1098 AAS SLQ S	1175 CQQS YSTPL TF	1372					

TABLE 1A-continued

Anti-Fzd Antibody Clone IDs and CDR sequences													
Clone ID	Initial Binding	CDRH1	CDRH1 SEQ ID	CDRH2	CDRH2 SEQ ID	CDRH3	CDRH3 SEQ ID	CDRL1	CDRL1 SEQ ID	CDRL2	CDRL2 SEQ ID	CDRL3	CDRL3 SEQ ID
006S-G02	Fzd 10	YTVTS YAM H	399 GGIIPI FGTA KYA	497 CAKGGQW LYGMDVW	713 RASQSIS SYLN	1098 AAS SLQ S	1175 CQQS YSTPL TF	1372					
014S-A01	Fzd 1	YTFTS YYMH	392 GWVS PSSG NTAY A	588 CARDEGA GYYYYYM DVW	766 RASQSIS SYLN	1098 AAS SLQ S	1175 CQQS YSTPL TF	1372					
014S-C01	Fzd 2	FTFSN YAMT	216 SAIGT GGGT YYA	595 CATAYRRP GGLDVW	969 RSSQSLL HSDGKTY LY	1134 LGS NRA S	1237 CMQN THWP LTR	1293					
014S-F01	Fzd 4	FTFSS YAM H	227 SVIST SGDT VLYT	652 CARGSSD VR	838 RASQSIS SYLN	1098 AAS SLQ S	1175 CQQS YSTPL TF	1372					
014S-H01	Fzd 4	FTFSN YGM H	217 SYISS SSSTI YYA	672 CARAALGY CTGGVCPP VDYW	737 RASQGIS NNLN	1072 AAS RLQ S	1171 CQQS YSPPL TF	1363					
014S-C03	Fzd 7	YTFTN NFMH	383 GIIIZP GGGR TIYA	517 CAKGDYG ALDYW	712 RASQSIS SYLN	1098 AAS SLQ S	1175 CQQS YSTPL TF	1372					
014S-D03	Fzd 7	FNFS YTMR	173 SVIYG GGNT NYA	653 CARGGSG GNLSYW	836 RASQSIS SYLN	1098 AAS SLQ S	1175 CQQS YSTPL TF	1372					
014S-F03	Fzd 8	YTFTN NFMH	383 GGIIIP LFGTA NYA	506 CARLVVRG GYGMDV W	893 RASQSIS SYLN	1098 AAS SLQ S	1175 CQQS YSTPL TF	1372					
014S-A04	Fzd 8	GTFSS YAIS	295 GWIS SFNG NTKY A	575 CARADDYY DSSGYYYG FDYW	738 RASQSIS SYLN	1098 AAS SLQ S	1175 CQQS YSTPL TF	1372					
014S-C04	Fzd 8	FTFSS YTMN	235 SRING DGSN TNYA	623 CARGWAG FDYW	871 RASQSIS SYLN	1098 AAS SLQ S	1175 CQQS YSTPL TF	1372					
014S-D04	Fzd 8	HTFS GYHI H	312 GWIN AGNG NTTY A	558 CARDLSPM VRGVISGM DVW	779 RASQSIS SYLN	1098 AAS SLQ S	1175 CQQS YSTPL TF	1372					
014S-E05	Fzd 8	YTFTN NFMH	383 GIIISP GGGR TIYA	515 CAKGDYG ALDYW	712 RASQSIS SYLN	1098 AAS SLQ S	1175 CQQS YSTPL TF	1372					

TABLE 1A-continued

Anti-Fzd Antibody Clone IDs and CDR sequences													
Clone ID	Initial Binding	CDRH1	CDRH1 SEQ ID	CDRH2	CDRH2 SEQ ID	CDRH3	CDRH3 SEQ ID	CDRL1	CDRL1 SEQ ID	CDRL2	CDRL2 SEQ ID	CDRL3	CDRL3 SEQ ID
01 4S - EO 6	Fzd 10	FTFG NYD MN	195 SSLS WNS GTIVY A		641 CARDSSSG WYASYYG MDVW		798 RASQSIS RYLN		1095 AAS SLQ S		1175 CLQH HSYPF TF		1275
02 7S - CO 2	Fzd 5	YTLTT WYM X	398 GWM NPNS GNTA YA		582 CARGALG MDVW		819 QASQDIS NYLN		1046 AAS SLH T		1173 CQESY SSPYT F		1302
02 7S - EO 3	Fzd 8	YTFTG HYM H	378 GWM NPNS GNTG TA		583 CARGTGGF DYW		867 RASQSIS SYLN		1098 AAS SLQ S		1175 CQQS YSTPY TF		1379
02 7S - FO 3	Fzd 8	YTFTG HYIH	377 GWM NPISG NTGY A		580 CARSTPFD PW		925 RASHDIG TFLA		1057 AAS TLQ S		1179 CQQS YRTPY TF		1355
02 7S - G 03	Fzd 8	YTFTH SYIH	380 GWIN AKSG GTFY A		559 CARGDYDF WSGYHEY YYYGMDV W		824 QATQNI KKYLN		1051 KAS TLES		1229 CQQS YSTPL TF		1372
02 7S - HO 3	Fzd 8	YTFTS YYMH	392 GWIN PNSG GTNY A		564 CARAPLDG SGSYVVD W		746 RSSQSLL HSNGYN YLD		1138 LGS NRA S		1237 CMQA LQTPQ TF		1282
02 7S - AO 4	Fzd 8	YTFTN HFMH	382 GWIS PNRG GTNY A		571 CARDCSGG SCYSHFDY W		765 RASQSIS RSLA		1093 AAS NLQ S		1169 CQQA YSFPQ TF		1323
02 7S - BO 4	Fzd 8	FTVG SWY MS	259 SAIGT GGGT YYA		595 CAKDITPY GDYSILSH W		702 RASQAIS NYLN		1059 AAS SLQ S		1175 CQQT SPPLT F		1383
02 7S - CO 4	Fzd 8	YTFTS HWM H	386 GGIPI FGTT NYA		503 CARDSSSW YSYYYYYM DVW		799 RASQGIN NYLA		1067 QAS NLE S		1243 CQQT YSSPL TF		1393
02 7S - DO 4	Fzd 8	YTFTT YFMH	393 GWIY PNSG GTKY A		578 CTTDLRYD SSGPAAFDI W		1019 QASQDI DNYLN		1040 AAS SLQ S		1175 CQQS YSTPV TF		1377
02 7S - EO 4	Fzd 8	FTFSD HYMS	205 SGISG SGGT TYYA		609 CATYGDFG YFDLW		978 RASQGIR NDLG		1069 AAS TLQ S		1179 CQQA YSFP WTF		1324
02 7S - CO 5	Fzd 8	GSFST SVFG	279 GRIIP LFGTT NYA		532 CVKDRAW GFDYW		1027 RASQGIR NDLA		1068 AAS SLQ R		1174 CQQS YSKPT F		1359

TABLE 1A-continued

Anti-Fzd Antibody Clone IDs and CDR sequences													
Clone ID	Initial Binding	CDRH1	CDRH1 SEQ ID	CDRH2	CDRH2 SEQ ID	CDRH3	CDRH3 SEQ ID	CDRL1	CDRL1 SEQ ID	CDRL2	CDRL2 SEQ ID	CDRL3	CDRL3 SEQ ID
02 7S - D0 5	Fzd 8	YTFTS YYMH	392 GWIN PKSG GTNY A		560 CARGGFVF DYW		828 QASQDIS NYLN		1046 ASS TLQ T		1182 CQQS YSAPY TF		1356
02 7S - E0 5	Fzd 8	GTFSS YAIS	295 GMIN PSGG STTYA		523 CARQAGLH CSSTSCYLG NWFDPW		905 RASQGIT KSLA		1077 AAS NLQ L		1168 CQQY NTFPI TF		1422
02 7S - F0 5	Fzd 8	GTFN RYGIS	290 GGIIP RLGA TDYA		508 CAKGNWA FDIW		719 RASQSIG TYLA		1100 GAS TRA T		1217 CQQY GSSPT F		1411
02 7S - G 05	Fzd 8	GTFSS YAIS	295 GWIS PYNG NTKY A		572 CARGVWT TPMGGGG NWFDPW		869 RASQSIG SYLN		1098 YAS SLQ N		1261 CQQS YSTPF TF		1368
02 7S - H0 5	Fzd 8	GTFG NYGI N	286 GWIN PNSG GTNY A		564 CARETTY YGMVW		814 RASQSIG TYLN		1088 DAS NLE T		1185 CQQA NSFPL TF		1317
02 7S - A0 6	Fzd 8	GTFSS YAIN	294 GVID PSTG GTNY A		550 CARVLPD SSGWYRG YYYYYGM DVW		941 RASQGIS NNLN		1072 KAS SLES		1226 CQQA NSFPI TF		1316
02 7S - C0 6	Fzd 8	GTFTS YPIS	306 GWIN TYNG NTIYA		566 CARDLDSG FDLW		776 RASQGV GDYLA		1078 DAS NLQ S		1186 CQQH NAYPL TF		1330
02 7S - D0 6	Fzd 8	GTFSS YAIS	295 GWIS AYNG HTNY A		569 CARGGYSY GTVFDYW		842 RASQDIS SWLA		1064 KAS TLES		1229 CQQS YGAPL TF		1348
02 7S - E0 6	Fzd 8	YTFTK DYM H	381 GGIPI FGTA NYA		499 CARGLPPA AGGGGYF QHW		847 RASQNV NDWLA		1082 SAS NLQ S		1252 CQQS YSTPF TF		1368
02 7S - F0 6	Fzd 8	FTFSS YAM H	227 AVTW YDGS NKYY A		480 CAKDLVPY CSGGSCPP SGW		705 RASQSIG SYLN		1098 GAS NLQ S		1207 CQQS YSTPL TF		1372
02 7S - G 06	Fzd 5	YTFTD YYMH FA	376 GWM SPNS GNAG		584 CARGKSGS FDYW		846 RASQSV NNTYVA T		1111 GTS TRA TF		1222 CQQY DTSP		1404
02 7S - H0 6	Fzd 5	YTFTG HYIH	377 GIINP SGGS TSYA		513 CARGFCSG GSCLWYG MDVW		825 RASQSIG SYLN		1098 AAS SLQ S		1175 CQQS YTTPF TF		1382

TABLE 1A-continued

Anti-Fzd Antibody Clone IDs and CDR sequences													
Clone ID	Initial Binding	CDRH1	CDRH1 SEQ ID	CDRH2	CDRH2 SEQ ID	CDRH3	CDRH3 SEQ ID	CDRL1	CDRL1 SEQ ID	CDRL2	CDRL2 SEQ ID	CDRL3	CDRL3 SEQ ID
02 7S - A0 7	Fzd 5	GTFG SYAIT	287 GGIIPI FGTA NYA		499 CAKNGW YFDLW		706 RASQSI TNVN		1099 AAS SLQ S		1175 CQQS YSTPY TF		1379
02 9S - B0 1	Fzd 1	GTFSS YAIS	295 GRIN PHNG NTNY A		1461 CARATRV AAGTVHF QHW		1472 KSSQSVL HSSNNK NYLA		1498 STN TRS S		1257 CQQY YSTPF TF		1544
02 9S - D0 1	Fzd 2	YTFTR YYIH	1452 GWM NPNS GNTG YA		583 CARVRFLE EMDVW		1494 RASQSL SWLA		1512 DAS TLQ S		1520 CQQA SFPLTF		1315
02 9S - C0 2	Fzd 2	GTFSS YGIS	1457 GIINP SGGS TSYA		513 CARGDIVA TMGMKKV DYYYMD VW		1478 RASQDIS NNLN		1500 GAS HLQ T		1522 CQQA NSFPV TF		1318
02 9S - F0 2	Fzd 2	YTFTR YYLH	1453 GWM NPNS GNTG YA		583 CARGIGYW		1481 RASQGIS NYLA		1074 AAS RLQ T		1515 CLQYN TYPW TF		1528
02 9S - H0 2	Fzd 2	GTFST YAIS	298 GDIPI FGSA NYA		1458 CARELGLG WFPDPW		1476 RSSQSLL HSNGYN YLD		1138 LGS SRA S		1525 CMQA LQTPL TF		1281
03 0S - A0 2	Fzd 7	YTFD YYMH	1449 GWM NPNS GSTG YA		1467 CARGDINY GNFDYW		1477 RASQSI SYLN		1098 KAS TLH N		1523 CQQA SFPLTF		1315
03 0S - B0 2	Fzd 3	YTFD YYMH	1449 GWM NPNS GNTG YA		583 CARQGSY SMGLDPW		1488 RASQSIT TYLN		1511 KTS SLQ S		1524 CQGG DSFPY TF		1531
02 9S - E0 3	Fzd 3	YTFG YYMH	379 GWIN PNSG NTGY A		1463 CARSYYGV IDAFDIW		1492 RASQSI SYLN		1098 AAS SLQ T		1516 CQGSF RLPLT F		1532
02 9S - G 03	Fzd 3	YFTN YYMH	1451 GWM NPNS GNTG YA		583 CAREDDF WSGGGM DVW		1474 RASQSI YLN		1509 AAS SLQ S		1175 CQGS WRFP YTF		1535
03 0S - E0 3	Fzd 3	FTFSD YYMS	1438 SAISG SGHS TYYA		1468 CAREGLRG WSIFDIW		1475 KSSQSVL YSSNNK NYLA		1037 WA STR ES		1526 CQGY YSTPP TF		1545
02 9S - D0 5	Fzd 3	YTFD HYFH	1447 GWA NPSS GNTG TA		1462 CARSRLRW DWYFDLW		1491 RASQTIS SYLN		1513 DAS NLE T		1185 CQGS YSIPLT F		1536

TABLE 1A-continued

Anti-Fzd Antibody Clone IDs and CDR sequences													
Clone ID	Initial Binding	CDRH1	CDRH1 SEQ ID	CDRH2	CDRH2 SEQ ID	CDRH3	CDRH3 SEQ ID	CDRL1	CDRL1 SEQ ID	CDRL2	CDRL2 SEQ ID	CDRL3	CDRL3 SEQ ID
03 OS - H0 3	Fzd 3	FSFSS HAMS	1437 SAIGT GGGT YYA	595 CANPKHY W	1470 RASQGV STYLA	1505 AAS SLQ S	1175 CQQY YSSPQ TF					1543	
02 9S - B0 6	Fzd 3	YTFSR HYIH	1446 GWM NPNS GNTG YA	583 CARGGHT NHW	1479 RASQSVS GYSSGWY SWLA	1120 AAS SLQ S	1175 CQQA FRFP TF					1529	
02 9S - E0 6	Fzd 3	YWFT ASYM H	1455 GWM KPDS GNTG YA	1465 CARRSSSW GWYFDLW	1489 RASQNIN SWLA	1506 AAS SLQ S	1175 CQQY YSFPL TF					1542	
2 9S - H0 6	Fzd 3	YTFAK YYIH	1444 GWM NPNS GNTG YA	583 CARHKRHT PYAFDIW	1487 RASQSIG SYLN	1098 AAS SLQ S	1175 CQQS HSTPL TF					1533	
02 9S - G 07	Fzd 3	YTFTD SYIH	1448 GWIS AYNG NTNY A	570 CARGSGYF DLW	1486 RASQSIG KWLA	1510 GAS TLQ S	1215 CQQA YSFP WTF					1324	
02 9S - H0 8	Fzd 3	YTFTG HYM H	378 GWM NPNS GNTG YA	583 CARVGDY DRFNWYF DLW	1493 RASRTVY NFLA	1514 DAS NLR T	1519 CQQS YSTP TF					1537	
02 9S - F0 9	Fzd 3	GTFSS YAIT	1456 GWIS AYNG NTNY A	570 CARANRGL RKNYYYG MDVW	1471 RASQSIA RYLN	1507 GAS SLQ S	1211 CQQS YNTP WTF					1351	
03 OS - F0 4	Fzd 3	YTFTS SYIH	1454 GIINP SGGG AVYA	1459 CARWTTV VTGAAFDI W	1495 RASQGIR NDLN	1502 DAS NLG T	1518 CQQS RIPPTF					1534	
02 9S - A1 0	Fzd 3	YSFTG YYLH	1442 GWIN PNSG GTNY A	564 CARDHGT MIAVAGTF DYYYYMD VW	1473 RASQGIS KYLA	1503 AAS SLQ S	1175 CQQS YSTP WTF					1538	
02 9S - B1 1	Fzd 3	YTFN GYYM H	1445 GIVN PSGG GTNY A	1460 CARGGNY GRWLQPW YFDLW	1480 QASQDIS NYLN	1046 GAS ALR S	1521 CQQT KSFPL TF					1540	
02 9S - D1 1	Fzd 3	HTFTS HYM H	1440 GWM NPNS ANAG YA	1466 CARGLGYF DLW	1483 RASQDIS RGLG	1501 AAS TLY R	1517 CQQA YSFP WTF					1324	
03 OS - H0 5	Fzd 7	YSFTN YYMH	1443 GWM NPNS GNTG YA	583 CARSPDF WSGEGYF DLW	1490 RASQSIG NYLN	1508 AAS SLQ S	1175 CQQA NSFPL TF					1530	

TABLE 1A-continued

Anti-Fzd Antibody Clone IDs and CDR sequences												
Clone ID	Initial Binding	CDRH1 CDRH1	CDRH1 SEQ ID	CDRH2 CDRH2	CDRH2 SEQ ID	CDRH3 CDRH3	CDRH3 SEQ ID	CDRL1 CDR11	CDRL1 SEQ ID	CDRL2 CDR12	CDRL2 SEQ ID	CDRL3 CDR13
03 OS - A06	Fzd 7	YMFT GHD MH	1441 GRIIPI LGIAN YA	529 CARGINGD YGLDYYM DVW	1482 RASQAIG RRLA	1499 AAS SLQ S	1175 CQQY DTYW TF	1541				
02 9S - C12	Fzd 7	YTFTG YYMH	379 GWM NPNS GNTG YA	583 CARGMEY W	1484 RASQGIS SYLA	1076 AAS TLQ S	1179 CLQYN TYPW TF	1528				
03 OS - C06	Fzd 7	YTFTG YYIH	1450 GWM DPNS GYTG YA	1464 CARGPADF WSGYKND YFDFW	1485 RASQGIS SWLA	1504 DAS SLQ S	1190 CQQS YSTPY SF	1539				
4A12	Fzd 5	<u>GYTFT</u> <u>NYDI</u> <u>N</u>	1439 <u>WIYP</u> <u>RDGS</u> <u>TKYN</u> <u>EKFK</u> <u>G</u>	1469 <u>CVRSANG</u> <u>FAY</u>	1496 <u>KASQDV</u> <u>GTAVA</u>	1497 <u>WA</u> <u>STR</u> <u>HT</u>	1527 <u>QQYS</u> <u>TYPLT</u>	1546				

TABLE 1B

Anti-Fzd Antibody Clone IDs, Heavy Chain (HC) and Light Chain (LC) Seq ID Nos, and Binding Characteristics			
Clone ID	HC SID NO	LC SID NO	Confirmed Binding
001S-B01	1	38	Fzd1, 2, 7, 9
001S-E02	2	39	Fzd1, 2, 7
001S-G02	3	40	Fzd1, 2, 7
001S-H02	4	41	Fzd1, 2, 7
001S-A03	5	42	Fzd1, 2, 7, 9
001S-B03	6	43	Fzd1, 2, 7
004S-G06	7	44	Fzd5, 8
002S-B01	8		Fzd1
002S-C02	9		Fzd1
002S-E02	10		Fzd1
002S-G02	11		Fzd1
002S-F03	12		Fzd1
002S-A04	13		Fzd1
002S-B04	14		Fzd1
002S-D04	15		Fzd1
004S-H04	16	45	Fzd5
001S-A04	17	46	Fzd1, 2, 5, 7, 8
003S-E07	18	47	Fzd2
003S-D10	19	48	Fzd4
004S-B08	20	49	Fzd6
004S-D08	21	50	Fzd6
004S-C09	22	51	Fzd6
004S-F10	23	52	Fzd6
004S-A11	24	53	Fzd6
004S-A12	25	54	Fzd6
005S-B07	26	55	Fzd9
005S-D08	27	56	Fzd9
005S-E09	28	57	Fzd9
005S-H10	29	58	Fzd9
005S-B11	30	59	Fzd9
005S-D11	31	60	Fzd9
014S-G02	32	61	Fzd6
014S-B04	33	62	Fzd8
014S-B06	34	63	Fzd9
014S-G06	35	64	Fzd10
014S-A07	36	65	Fzd10
017S-B09	37		Fzd8
004S-D01	129	130	Fzd4
004S-E09	131	132	Fzd6
004S-F09			Fzd6

TABLE 1B-continued

Anti-Fzd Antibody Clone IDs, Heavy Chain (HC) and Light Chain (LC) Seq ID Nos, and Binding Characteristics			
Clone ID	HC SID NO	LC SID NO	Confirmed Binding
004S-H09			Fzd6
004S-B10			Fzd6
004S-C10			Fzd6
004S-F10			Fzd6
004S-G10			Fzd6
004S-A11			Fzd6
004S-B11			n.b.
004S-D11			Fzd6
004S-E11			n.b.
004S-F11			Fzd6
004S-G11			Fzd6
004S-A12			Fzd6
004S-B12			Fzd6
004S-C12			n.b.
004S-D12			n.b.
004S-F12			n.b.
004S-F12			n.b.
004S-G12			n.b.
005S-B02			n.b.
005S-C02			n.b.
005S-D02			Fzd5, 8
005S-E02			Fzd5, 8
005S-H02			Fzd5, 8
005S-A03			Fzd5, 8
005S-C03			n.s.
005S-E03			n.s.
005S-F03			Fzd8
005S-B04			Fzd5, 8
005S-F04			n.b.
005S-G04			Fzd5, 8
005S-H04			n.b.
005S-E05			n.b.
005S-G05			Fzd5, 8
005S-H05			Fzd5, 8
005S-D06			Fzd8
005S-F06			n.b.
005S-G06			n.b.
005S-A07			Fzd9, 10
005S-B07			Fzd9
005S-A08			Fzd9

TABLE 1B-continued

Anti-Fzd Antibody Clone IDs, Heavy Chain (HC) and Light Chain (LC) Seq ID Nos. and Binding Characteristics			
Clone ID	HC SID NO	LC SID NO	Confirmed Binding
005S-B08			Fzd9
005S-D08			Fzd9
005S-E08			Fzd9
005S-F08			n.b.
005S-C09			Fzd9
005S-D09			Fzd9
005S-E09			Fzd9
005S-F09			Fzd9
005S-A10			Fzd9
005S-B10			Fzd9
005S-E10			Fzd9
005S-H10			Fzd9
005S-B11			Fzd9
005S-D11			Fzd9
005S-G11			n.b.
005S-H11			n.b.
005S-E12			Fzd10
006S-A01			Fzd10
006S-H01			n.b.
006S-A02			Fzd10
006S-D02			n.b.
006S-H02			Fzd10
006S-A03			n.b.
006S-B03			n.b.
006S-C03			n.b.
014S-A01			Fzd1, 2, 7
014S-B02			n.b.
014S-G02			Fzd6
014S-B03			n.b.
014S-C03			Fzd1, 2, 7
014S-A04			n.b.
014S-B04			Fzd8
014S-B05			Fzd5, 8
014S-B06			Fzd9
014S-F06			n.s.
014S-G06			Fzd10
014S-A07			Fzd10
017S-E08			Fzd8
017S-H08			n.b.
017S-A09			n.b.
017S-B09			Fzd8
018S-F06			Fzd4
018S-H06			n.b.
018S-B07			n.b.
017S-A10			n.b.
017S-B10			n.b.
017S-D10			n.b.
018S-H08			n.b.
018S-B09			Fzd5, 8
021S-A01			n.b.
021S-E02			Fzd5, 8
021S-G02			n.s.
021S-A03			n.b.
029S-B01			n.b.
029S-D01			Fzd1, 2, 7
029S-C02			Fzd1, 2, 7
029S-H02			Fzd1
030S-A02			Fzd7
029S-E06			Fzd2, 6, 3
030S-F04			Fzd3
030S-H05			Fzd7
030S-A06			Fzd1, 2, 7, 5
029S-C12			Fzd7
030S-C06			Fzd1
001S-A01			Fzd1, 2, 7
001S-H01			Fzd1, 2, 7

In certain embodiment, the Fzd binding domain may be selected from any binding domain that binds a Fzd with an affinity of, e.g., a K_D of at least about 1×10^{-4} M, at least about 1×10^{-5} M, at least about 1×10^{-6} M, at least about 1×10^{-7} M, at least about 1×10^{-8} M, at least about 1×10^{-9} M, or at least about 1×10^{-10} M. In certain embodiment, the Fzd binding domain may be selected from any binding domain

that binds one or more Fzd receptor at high affinity, e.g., a K_D of less than about 1×10^{-7} M, less than about 1×10^{-8} M, less than about 1×10^{-9} M, or less than about 1×10^{-10} M. In certain embodiment, the Fzd binding domain may be selected from any binding domain that binds Fzd at high affinity, e.g. a K_D of less than or equal to about 1×10^{-4} M, less than or equal to about 1×10^{-5} M, less than or equal to about 1×10^{-6} M, less than or equal to about 1×10^{-7} M, less than or equal to about 1×10^{-8} M, less than or equal to about 1×10^{-9} M, or at least about 1×10^{-10} M in the context of a Wnt surrogate molecule.

Suitable Fzd binding domains include, without limitation, de novo designed Fzd binding proteins, antibody derived binding proteins, e.g. scFv, Fab, etc. and other portions of antibodies that specifically bind to one or more Fzd proteins; VHH or single domain antibody derived binding domains; knottin-based engineered scaffolds; norrin and engineered binding fragments derived therefrom, naturally occurring Fzd binding domains, and the like. A Fzd binding domain may be affinity selected to enhance binding to a desired Fzd protein or plurality of Fzd proteins, e.g. to provide tissue selectivity.

In some embodiments, the Fzd binding domain binds to one, two, three, four, five or more different frizzled proteins, e.g. one or more of human frizzled proteins Fzd1, Fzd2, Fzd3, Fzd4, Fzd5, Fzd6, Fzd7, Fzd8, Fzd9, Fzd10. In some embodiments, the Fzd binding domain binds to Fzd1, Fzd2, and Fzd 7. In some embodiments, the Fzd binding domain binds to Fzd1, Fzd2, Fzd5, Fzd7 and Fzd8. In other embodiments the Fzd binding domain is selective for one or more frizzled protein of interest, e.g. having a specificity for the one or more desired frizzled protein of at least 10-fold, 25-fold, 50-fold, 100-fold, 200-fold or more relative to other frizzled proteins.

In certain embodiments, the Fzd binding domain comprises the six CDR regions of the pan specific frizzled antibody OMP-18R5 (vantictumab). In certain embodiments, the Fzd binding domain is an scFv comprising the six CDR regions of the pan-specific frizzled antibody OMP-18R5 (vantictumab). See, for example, U.S. Pat. No. 8,507,442, herein specifically incorporated by reference. For example, the CDR sequences of OMP-18R5 include a heavy chain CDR1 comprising GFTFSHYTSL (SEQ ID NO:270), a heavy chain CDR2 comprising VISGDGSHYTY-ADSVK (SEQ ID NO:677), and a heavy chain CDR3 comprising NFIKYVFAN (SEQ ID NO:1033), and (ii) a light chain CDR1 comprising SGDKLGKKYAS (SEQ ID NO:1152) or SGDNIHSFYVH (SEQ ID NO:1153), a light chain CDR2 comprising EKDNRPSPG (SEQ ID NO:1200) or DKSNRPSPG (SEQ ID NO:1201), and a light chain CDR3 comprising SSFAGNSLE (SEQ ID NO:1435) or QSY-ANTLSL (SEQ ID NO:1436). In particular embodiments, the Fzd binding domain is an antibody or derivative thereof, including without limitation scFv, minibodies, VHH or single domain antibodies (sdAb) and various antibody mimetics comprising any of these CDR sequences. In certain embodiments, these CDR sequences comprise one or more amino acid modifications.

In other embodiments, the Fzd binding domain comprises a variable region sequence, or the CDRs thereof, from any of a number of frizzled specific antibodies, which are known in the art and are commercially available, or can be generated de novo. Any of the frizzled polypeptides can be used as an immunogen or in screening assays to develop an antibody. Non-limiting examples of frizzled binding domains include antibodies available from Biolegend, e.g. Clone CH3A4A7 specific for human frizzled 4 (CD344);

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Clone W3C4E11 specific for human Fzd9 (CD349); antibodies available from Abcam, e.g. ab64636 specific for Fzd7; ab83042 specific for human Fzd4; ab77379 specific for human Fzd7; ab75235 specific for human Fzd8; ab102956 specific for human Fzd9; and the like. Other examples of suitable antibodies are described in, inter alia, US Patent application 20140105917; US Patent application 20130230521; US Patent application 20080267955; US Patent application 20080038272; US Patent application 20030044409; etc., each herein specifically incorporated by reference.

The Fzd binding region of a Wnt surrogate molecule may be an engineered protein that is selected for structural homology to the frizzled binding region of a wnt protein. Such proteins can be identified by screening a structure database for homologies. The initial protein thus identified, for example the microbial Bh1478 protein. The native protein is then engineered to provide amino acid substitutions that increase affinity, and may further be selected by affinity maturation for increased affinity and selectivity in binding to the desired frizzled protein. Non-limiting examples of frizzled binding moieties include the Fz27 and Fz27-B12 proteins.

In particular embodiments, a Wnt surrogate molecule comprises an LRP5/6 binding domain, e.g., an anti-LRP5/6 antibody, or antigen-binding fragment thereof, fused to a polypeptide that specifically binds to one or more Fzd receptors. In particular embodiments, the polypeptide that specifically binds to LRP5/6 is an antibody or antigen-binding fragment thereof. If certain embodiments, it is an antibody or antigen-binding fragment thereof disclosed in the U.S. provisional patent application No. 62/607,879, titled, "Anti-LRP5/6 Antibodies and Methods of Use," filed on Dec. 19, 2017, which is incorporated herein by reference

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in its entirety. In particular embodiments, the LRP5/6 binding domain comprises the three heavy chain CDRs and/or the three light chain CDRs disclosed for any of the illustrative antibodies or fragments thereof that bind to LRP5 and/or LRP6 provided in Table 2A. In particular embodiments, the LRP5/6 binding domain comprises the three heavy chain CDRs and/or the three light chain CDRs disclosed for any of the illustrative antibodies or fragments thereof that bind to one or more Fzd receptor provided in Table 2A, wherein the CDRs collectively comprise one, two, three, four, five, six, seven, or eight amino acid modifications, e.g., substitutions, deletions, or additions. In certain embodiments, the LRP5/6 binding domain is a VHH or sdAb or was derived from a VHH or sdAb, so Table 2A only includes the three heavy chain CDRs. In certain embodiments, the LRP5/6 binding domain comprises the three heavy chain CDRs shown in Table 2A or variants wherein the CDRs collectively comprise one, two, three, four, five, six, seven or eight amino acid modifications. In particular embodiments, the LRP5/6 binding domain comprises the heavy chain fragment and/or light chain fragment of any of the illustrative antibodies or fragments thereof that bind to LRP5 and/or LRP6 provided in Table 2B or SEQ ID NOs:66-88 or 133 (or an antigen-binding fragment or variant of either). In certain embodiments, the LRP5/6 binding domain is an Fab or was derived from an Fab, so Table 2B includes VH and CH1 sequence, but not CH2 or CH3 sequences. In certain embodiments, the LRP5/6 binding domain is a VHH or sdAb or was derived from a VHH or sdAb, so Table 2B includes the VHH domain. In certain embodiments, the LRP5/6 binding region is a polypeptide, e.g., an antibody or antigen-binding fragment thereof, that competes with one of these antibodies for binding to LRP5 and/or LRP6.

TABLE 2A

Anti-LRP5/6 Antibody Clone IDs and CDR sequences.											
Clone ID	Confirmed Binding	CDRH1	CDRH1 SEQ ID	CDRH2	CDRH2 SEQ ID	CDRH3	CDRH3 SEQ ID	CDRL1	CDRL1 SEQ ID	CDRL2	CDRL2 SEQ ID
001S-C08	LRP6e1e2	YTIISNYIH	1682	GMINPSGGST YA	1762	CAIVRGKKWYFDLW	1842	RASQYISNYLN	2098	AASSLQS	2110
001S-C10	LRP6e1e2	RTFGTYPNG	1632	AAISWGRYA YA	1700	CYARTVIGGFGAPRA HW	2061				
001S-D10	LRP6e1e2	RTFSRYAMA	1642	AAIRWSGGGT YYA	1689	CAASMEAMNSLRVN KERYQSW	1836				
001S-E10	LRP6e1e2	LTFSNAAMA	1614	AAISRSGANTA YS	1696	CTLVNEIKTW	2039				
001S-F10	LRP6e1e2	RTFSSYAMA	1645	AAIKWSGTNT YYA	1684	CAASMEAMNSLRVN KERYQSW	1834				
001S-G10	LRP6e1e2	RTFSRYVMG	1644	AAITWRGGST YYA	1706	CATGPNISY	1987				
001S-A11	LRP6e1e2	RTFGNYDMG	1630	AGIRWSGSTL YA	1709	CYARTVIGGFGAPRA HW	2062				
001S-B11	LRP6e1e2	RRFTTYGMG	1623	AAVTWRSGST YYA	1708	CAAGSTVVAEFNYW	1828				
001S-C11	LRP6e1e2	SISFNTMG	1659	AVITGGDTSYS	1741	CNKVNAITKL	2025				
001S-E11	LRP6e1e2	RTLRSYVMG	1651	AAISRSGDRIY YS	1697	CTLVNEIKTW	2040				
001S-F11	LRP6e1e2	RTFSSYAMS	1646	AVIGRSGGIKY YA	1736	CATRRPFNSYNTEQS YDSW	1989				
001S-G11	LRP6e1e2	SIFRLGTMV	1655	ASIGKSGSTNYA	1719	CKQHPNGYR	2005				
001S-H11	LRP6e1e2	RTLSSFAMG	1652	ATISRSNGNTY YA	1732	CNLEWNSGAGY W	2026				
001S-A12	LRP6e1e2	IAPRYDMG	1608	AAITWNGRSS DYA	1704	CAAVTGRFPYGRPPR EKDYW	1838				
001S-B12	LRP6e1e2	RLISYVALA	1622	AAISRNGDKS HYS	1694	CTLVNEIKTW	2038				
001S-C12	LRP6e1e2	RTFSNYAVG	1641	AAISRFGGSTY YV	1693	CAADRIENYLGRIYD PSEYEY	1824				

TABLE 2A-continued

Anti-LRP5/6 Antibody Clone IDs and CDR sequences.														
Clone ID	Confirmed Binding	CDRH1	CDRH1 SEQ ID	CDRH2	CDRH2 SEQ ID	CDRH3	CDRH3 SEQ ID	CDRL1	CDRL1 SEQ ID	CDRL2	CDRL2 SEQ ID	CDRL3	CDRL3 SEQ ID	
001S-D12	LRP6e1e2	RFFSRVAMG	1643	GAISRSNNNT YYA	1744	CTLVNEIKTW	2041							
001S-F12	LRP6e1e2	RTFRSYTMG	1637	AAISGSGGTT YA	1690	CNADIKTTTYSPLRNYW	2009							
008S-B01	LRP5	TIFSINTMG	1664	ATMTSGGNT NYA	1734	CYRQWASSWGAR NYEYW	2064							
008S-C01	LRP5	NINSIETLG	1617	ANMRGGY MKYA	1716	CHGRDYGSNAPQYW	2001							
008S-D01	LRP5	NINSIETLG	1617	ANMRGGY MKYA	1716	CYVKLRDDDYVYR	2065							
008S-E01	LRP5	NINSIETLG	1617	ANMRGGY MKYA	1716	CNAVTYNGYTIR	2023							
008S-G01	LRP5	NINSIETLG	1617	ANMRGGY MKYA	1716	CYARTQRMGVVNSYW	2060							
008S-A02	LRP5	NINSIETLG	1617	ANMRGGY MKYA	1716	CNAVTFGGNTIR	2021							
008S-C02	LRP5	NINSIETLG	1617	ANMRGGY MKYA	1716	CNAVTYDGY	2022							
008S-D02	LRP5	NINSIETLG	1617	ANMRGGY MKYA	1716	CAAQFRNDYGLRYQ STNNYW	1832							
008S-E02	LRP5	NINSIETLG	1617	ANMRGGY MKYA	1716	CNAVYRGNRYW	2019							
009S-C01	LRP6e3e4	GSFSGYYWT	1595	GEINHSNATN YN	1745	CVRYAWPEFDHW	2053	RASQVSNY LN	2089	AASSLQG	2110	CQQSYSVPYTF	2178	
009S-B02	LRP6e3e4	GSLSGYWS	1596	GEINHSNSTYN	1746	CVRYAWPEFDHW	2055	RASQISNYLN	2090	AASSLQS	2110	CQQSYSILPLTF	2170	
009S-C02	LRP6e3e4	GSFSDYWS	1594	GEINHSNSTYN	1746	CVRYAWPEFDHW	2054	RASQISNYLN	2090	AASSLQS	2110	CQQSYSMLTF	2171	
009S-D02	LRP6e3e4	GTFSSYALS	1603	GGIIPFGTANYA	1749	CVYGRDPDYW	2056	SGSSSNVGN NYVS	2105	DNDKRPS	2122	CESWDSLSSE VF	2139	
010S-A02	LRP6e1e2	HTFSSYAMG	1607	AAISQSGYVRYA	1691	CKIYGLNGQPLGSW	2003							
010S-B02	LRP6e1e2	RTFNSGTMG	1634	AAITWRGGIT YYA	1705	CNADGYSWGRSG RRELEW	2008							

TABLE 2A-continued

Anti-LRP5/6 Antibody Clone IDs and CDR sequences.													
Clone ID	Confirmed Binding	CDRH1	CDRH1 SEQ ID	CDRH2	CDRH2 SEQ ID	CDRH3	CDRH3 SEQ ID	CDRL1	CDRL1 SEQ ID	CDRL2	CDRL2 SEQ ID	CDRL3	CDRL3 SEQ ID
010S-D02	LRP6e1e2	RTFSYAVG	1647	AAISYSGGSTK YA	1702	CAASVYISRRDSYG YW	1837						
010S-E02	LRP6e1e2	LSSGRPFSSY VMG	1612	AAISWSGGST KYA	1701	CKLQVRPIGYSSAYS NYW	2004						
010S-F02	LRP6e1e2	RSFNSYVIG	1625	AAIRWSGDNT YYA	1688	CAASMEAMNSLRVN KERYIQSW	1835						
009S-E02	LRP6e1e2	RRFTTYGMG	1623	AAVTWRSGST YYA	1708	CAAGSTVVAEFNYW	1829						
009S-F02	LRP6e1e2	RTFSYAMG	1649	AAISRSGGIYYA	1698	CNTVRPLMAW	2029						
009S-G02	LRP6e1e2	SIFSIIYAMG	1658	AVITSGGKTYYA	1740	CYADSRSSWYDEYLE HW	2058						
009S-H02	LRP6e1e2	SIVRSLPMA	1660	ATINDAQRYA	1727	CNTSPYMHVDVW	2027						
009S-A03	LRP6e1e2	RTFSVYGVG	1648	AAVSASGGYT WYA	1707	CKAAPRWGGATAYW	2002						
010S-G02	LRP6e1e2	SIVRSLPMA	1660	ATINDAQRYA	1727	CNTSPYMHVDVW	2028						
010S-A03	LRP6e1e2	RTFRRYAMG	1636	ATISASGGNTA YA	1731	CNAPAWLYDDDYW	2020						
009S-B03	LRP6e1e2	RTFSNYAVG	1641	AAISRFGGSTY YV	1693	CAADRIENYLGRYD PSEYEW	1825						
010S-B03	LRP6e1e2	RTFSNYAVG	1641	AAISRFGGSTY YA	1692	CHAKQLRNGQMYTYW	1999						
010S-D03	LRP6e1e2	ISSVYGMG	1609	AAIOWSADNT FYA	1686	CAARTSGGLPHYRRS DHWDTW	1833						
009S-C03	LRP6e1e2	LPFSRYAMA	1610	AGMSGEGRN TKYR	1713	CSSRGYW	2034						
009S-D03	LRP6e1e2	SIFSDGAMG	1656	AVISGGRGTGYA	1737	CNTYPPFIYKKGYPFW	2030						
009S-E03	LRP6e1e2	RRFTTYGMG	1623	AAVTWRSGST YYA	1708	CAAGSTVVAEFNYW	1830						
009S-F03	LRP6e1e2	RTFSSYAMS	1646	AVIGRSGGIKY YA	1736	CATRRPFNSYNTEQS YDSW	1990						

TABLE 2A-continued

Clone ID	Confirmed Binding	Anti-LRP5/6 Antibody Clone IDs and CDR sequences.									
		CDRH1	CDRH1 SEQ ID	CDRH2	CDRH2 SEQ ID	CDRH3	CDRH3 SEQ ID	CDRH3	CDRH3 SEQ ID	CDRL1	CDRL1 SEQ ID
010S-E03	LRP6e1e2	RSVSIYPMG	1628	AAINMSGDST KYA	1685	CNAVVGLSRRIDNIW	2024				
010S-F03	LRP6e1e2	RTFSRYVMG	1644	AAITWRGGST YYA	1706	CATGNSIY	1988				
009S-G03	LRP6e1e2	RSVSSYNMG	1629	AAISRRGGIIEYG	1695	CHAVENILGRFVDYW	2000				
009S-H03	LRP6e1e2	SIFSINTMG	1657	AVITSGKTVYA	1740	CYADSRSSWYDEYLE HW	2057				
009S-A04	LRP6e1e2	RTLSAYDMG	1650	GGIRMSGGTT LYP	1753	CYARTVIGGFGAFRA HW	2063				
009S-B04	LRP6e3e4	SIFMINTMA	1654	ATIRPVVSETT YA	1728	CNAKRPWGTRDEYW	2018				
010S-G03	LRP6e3e4	RSPNSYTTT	1624	AAIRSGSGSTF YA	1687	CNAASTVTAMPYYG PDYW	2006				
009S-C04	LRP6e3e4	FRFSISTMG	1553	AVITGGRTM DG	1743	CNAFVRSDEDRYDYW	2011				
009S-D04	LRP6e3e4	TIVSIYRIN	1665	AGITSGRTIYA	1712	CNAASTVTAMPYYG PDYW	2007				
010S-H03	LRP6e3e4	RIFSIYDMG	1621	SGIRMSGGTS YA	1789	CSSRGYW	2035				
009S-E04	LRP6e3e4	RIFAIDIA	1618	AMIRPVVTEID YA	1715	CNAKRPWGSRDEYW	2012				
010S-A04	LRP6e3e4	SLFSFNAV	1662	ASISSGRTNYA	1722	CSKGGVYGGTYVPD SW	2032				
009S-F04	LRP6e3e4	RLSSSFAMG	1627	ARISRGDGYT DEA	1718	CAAVQAVIGGTLTTA YDYW	1839				
010S-B04	LRP6e3e4	RVLSYYAMA	1653	AGITRGGATTY YS	1711	CAAGPNWSTNREY DYW	1827				
009S-G04	LRP6e3e4	GTFSRYHMG	1601	SAITWSGGRT YYA	1788	CALTWAPTPTNRRS DYAYW	1872				
009S-H04	LRP6e3e4	RIFAIDMA	1619	ATIRPVVSETT YA	1728	CNAKRPWGTRDEYW	2017				

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TABLE 2A-continued

Clone ID	Confirmed Binding	Anti-LRP5/6 Antibody Clone IDs and CDR sequences.										CDRL3 SEQ ID
		CDRH1 SEQ ID	CDRH1	CDRH2 SEQ ID	CDRH2	CDRH2 SEQ ID	CDRH3	CDRH3 SEQ ID	CDRL1 SEQ ID	CDRL2 SEQ ID	CDRL3 SEQ ID	
010S-C04	LRP6e3e4		SLFSFNAMG	1661	ASISSGSRNTYA	1723	CSKGGVYGTYVPD SW	2033				
010S-D04	LRP6e3e4		RIFAIYDIA	1618	ATIRPVVTQID YA	1730	CNAKRPWGSRDEYW	2015				
010S-E04	LRP6e3e4		RTFGSDVMG	1631	ALTGWGDGS TTYE	1714	CAAAARRSGTYDIGQY LRESAYVEW	1820				
010S-F04	LRP6e3e4		RTFSRYAMG	1643	AAITRSGSNTY YA	1703	CAADPRGVTLPRATA YEYW	1823				
009S-A05	LRP6e3e4		RTFSDYSMG	1639	AGISWIADNR YYA	1710	CTAGRSRYLYGSSLN GPYDYW	2036				
010S-G04	LRP6e3e4		VIFALYDIA	1666	ATIRPVVTETD YA	1729	CNAKRPWGSRDEYW	2014				
010S-H04	LRP6e3e4		RSFSDPFMG	1626	ATISWSGSSA NYE	1733	CAAAYSYSQYGSSYS YW	1821				
010S-A05	LRP6e3e4		LSFSSYAMG	1611	AAISRSGVSTY YA	1699	CAAKPGVLATTESRH DYW	1831				
010S-C05	LRP6e3e4		RTFNIDDMG	1633	ASIRWSGQSP YYA	1720	CNAETYSGNTIW	2010				
010S-D05	LRP6e3e4		RTFSDYSMA	1638	AGISWIADNR YYA	1710	CAGDRSRYLYGDSLRLR GPYGYW	1841				
010S-E05	LRP6e3e4		SVFTTFAKG	1663	ASITASSDRTF YA	1725	CAAYSTFNTDVASM KPDYW	1840				
010S-F05	LRP6e3e4		RIFSIFYDIA	1620	ATIRPVVTETD YA	1729	CNAKRPWGSRDEYW	2013				
013S-G04	LRP6e3e4		RIFAIYDIA	1618	ATIRPVVSETT YA	1728	CNAKRPWGTRDEYW	2016				
013S-H04	LRP6e3e4		RFSMYDMG	1640	ASIRWSGSGNT WYA	1721	CYANIYYTTRAPEEYW	2059				
013S-A05	LRP6e3e4		RTFNTYAMG	1635	ASVSWRYDRT YYT	1726	CAADTNWRAGPRV GIDEYAYW	1826				
013S-B05	LRP6e3e4		FAFSTTAMS	1549	STINPGGLSKS YA	1806	CTKGGIQ	2037				

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TABLE 2A-continued

Anti-LRP5/6 Antibody Clone IDs and CDR sequences.													
Clone ID	Confirmed Binding	CDRH1	CDRH1 SEQ ID	CDRH2	CDRH2 SEQ ID	CDRH3	CDRH3 SEQ ID	CDRL1	CDRL1 SEQ ID	CDRL2	CDRL2 SEQ ID	CDRL3	CDRL3 SEQ ID
013S-C05	LRP6e3e4	NIPPIDMS	1616	ATVTSGGRINYA	1735	CNVDRTLYGKYKEYW	2031						
013S-D05	LRP6e3e4	RIFSIYDMG	1621	SGIRMSGGTS YA	1789	CGSRGYW	1998						
013S-E05	LRP6e3e4	YTFTYRYLH	1681	GGIIPIFGTADYA	1748	CARDWELYGMDVW	1907	RASQSISSVYN	2094	AASSLQS	2110	CQQSYSTPLTF	2174
013S-F05	LRP6e3e4	GTFSYALS	1603	GIINPSGGSTSYA YA	1761	CARAGYDSSGGYAF DIW	1882	RASQSISSVYN	2094	AASSLQS	2110	CQQSYSTPLTF	2174
013S-G05	LRP6e3e4	YTFTYRYLH	1681	GGVPIPGTAD YA	1755	CASDIWDDAFDTW	1969	RASQDIISNY LN	2079	AASTLQS	2113	CQQGNSEFPYTF	2152
010S-G06	LRP6e3e4	FSPETYGMS	1555	SGISGSGRTH YA	1792	CARDLDYW	1897	QASQDIISNY LN	2077	AASSLQS	2110	CQQSYRIHWTFF	2163
009S-B05	LRP6e3e4	FTFDAYAMH	1560	STLSGDANNA YYA	1811	CARGSGWSNYYG MDVW	1931	RASQSISSVYN	2094	AASSLQS	2110	CQQSYSTPLTF	2174
009S-C05	LRP6e3e4	YTFTYRYLH	1681	GRILPVLKITNYA	1768	CAVDDDAFDIW	1996						
009S-D05	LRP6e3e4	FTLRNHWLS	1591	SAISGSGGSTY YA	1786	CATRTGYSGFNFW AFDIW	1991	RASQSISSVYN	2904	AASSLQS	2110	CQQSYSTPLTF	2174
009S-E05	LRP6e3e4	YTFTNPFMH	1676	GHVDPGDGET IYA	1756	CARDWGIAGAGDYI YYGMDVW	1908	RASQGINSY LA	2081	DAKGLHP	2114	CQQSYGAPLSF	2166
009S-F05	LRP6e3e4	FTFDDYGMS	1561	SAITGGGTYYA	1781	CARLGSYGSPYYIYG MDVW	1959	RASQSISSVYN	2094	AASSLQS	2110	CQQSYSTPLTF	2174
009S-G05	LRP6e3e4	FTFSDYYMS	1568	SGVSWNGSRT HYA	1799	CANKDSGLV	1852	QASQDIISNY LN	2077	AASTLQR	2112	CQQSYGAPLTF	2167
009S-C06	LRP6e3e4	YTFASYDIH	1671	GWMNPNSG NTGYA	1776	CARATGSGWYTDIG YW	1883	RASRNINRY LN	2099	AASSLLS	2109	CQQSYNVPFTF	2162
009S-D06	LRP6e3e4	FTFSSHSTH	1573	STISDTNSGTY YA	1807	CACAQATGWSGYIT FDYW	1843	RASQSISSVYN	2094	AASSLQS	2110	CQQSYSTPLTF	2174
009S-E06	LRP6e3e4	FTFTDYGLH	1587	AVISYGSNKY YA	1739	CASGYSGHYLYYGM DVM	1973	RASQSISSVYN	2094	AASSLQS	2110	CQQSYSTPLTF	2174
009S-F06	LRP6e3e4	YTFTYRYLH	1681	GGIIPIFGTAN YA	1749	CATEAALDAFDIW	1985	RASQSISSVYN	2094	AASSLQS	2110	CQQSYSTPLTF	2174
009S-G06	LRP6e3e4	YIFTDYTMH	1669	GWINPNSGCT NYA	1774	CARDFLGTGTGYW	1892	RASQNIIGLY LN	2088	DASSLQR	2121	CQQSYSTPYTF	2176

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TABLE 2A-continued

Anti-LRP5/6 Antibody Clone IDs and CDR sequences.													
Clone ID	Confirmed Binding	CDRH1	CDRH1 SEQ ID	CDRH2	CDRH2 SEQ ID	CDRH3	CDRH3 SEQ ID	CDRH3	CDRL1 SEQ ID	CDRL2 SEQ ID	CDRL3	CDRL3 SEQ ID	CDRL3
009S-H06	LRP6e3e4	FTFSSAMH	1574	SAITGGSTYYA	1783	CAKGGDYFYIYGM DVIW	1856	RASQSISSYLN	2094	AASLQS	2110	CQQSYSTPLTF	2174
009S-A07	LRP6e3e4	YTFTYRYLH	1681	GGIIPIFGTAN YA	1749	CATAYGSSSLNIDYW	1980	RASQSISSYLN	2094	AASLQS	2110	CQQSYSTPLTF	2174
009S-B07	LRP6e3e4	YTFTGYMH	1675	GWINPNSGGT NYA	1774	CVKDGGSPLAYAFD IW	2049	RASQSISSYLN	2904	AASLQS	2110	CQQSYSTPLTF	2174
009S-D07	LRP6e3e4	FPPRYYGMS	1551	ARIGNNGSI VYA	1717	CARDYSDRSGIDYW	1910	RSSQSLHLS NGNYLD	2102	LGSNRAS	2131	CMQATQPPLTF	2146
009S-F07	LRP6e3e4	GTFSSYALS	1603	GIINPSSGSTS YA	1761	CARAAGNFWSGYYT FDYW	1876	RASQSISSYLN	2094	AASLQS	2110	CQQSYSTPLTF	2174
009S-G07	LRP6e3e4	YTFTYRYLH	1681	GGIIPIFGTAN YA	1749	CARGSYGMDVW	1947	RASQGISNY LA	2083	DASNLET	2115	CLQDFSPFWTF	2140
009S-H07	LRP6e3e4	YTFTGYMH	1675	GWMNPNSG NTGYA	1776	CASSVVPAGPAGVY AFDIW	1975	RASQSISSYLN	2094	AASLQS	2110	CQQSYSTPLTF	2174
009S-A08	LRP6e3e4	GTFSSHAIN	1602	GWISANNGN TDYA	1775	CARDQDYGDIYGY YYGMDVW	1902	RASQGISNY LA	2083	GSSTLQS	2127	CQQTYSIPPTF	2181
011S-C01	LRP6e3e4	LTFTSHGMS	1615	SYVSDSGSSVY YA	1818	CARHPGSGGYSYA WYIYYGMDVW	1956	RASQSISSYLN	2094	AASLQS	2110	CQQSYSTPLTF	2174
009S-C08	LRP6e3e4	FSFNTFGIH	1556	AVISYDGSNKY YA	1738	CAKSIAAACTGYYG MDVW	1868	RASQSISSYLN	2094	AASLQS	2110	CQQSYSTPLTF	2174
009S-D08	LRP6e3e4	YTFTSYDIN	1679	GGIIPIFGTAN YA	1749	CARGPYIFDYW	1939	RASQGISNN LN	2082	DASSLES	2120	CLQHNSYPPTF	2143
011S-F01	LRP6e3e4	FSFSDYMS	1558	SGISESGRTY YA	1790	CASAADFDYW	1966	RASQDISNY LN	2079	AASLQS	2110	CLQDYSYPRTF	2141
009S-E08	LRP6e3e4	TGFTGYIYH	1668	GWMNPNSG NTGYA	1776	CARGYGDYDLW	1951	QASQDISNY LN	2077	DASSLES	2120	CQQSYRYPTF	2165
009S-F08	LRP6e3e4	DTFANYGFS	1547	GKYNAGNGN TTYA	1777	CAKGWLDYDLW	1866	QASQDISNY LN	2077	DASSLES	2120	CQQSYSTSITF	2177
009S-G08	LRP6e3e4	FTFSDFAMT	1566	SYISGDSGYTN YA	1813	CARLGSPGPYYIYM DVIW	1961	RASQSISSYLN	2094	AASLQS	2110	CQQSYSTPLTF	2174
009S-H08	LRP6e3e4	YTFTDYPMN	1673	GIINPSGDSTR FA	1758	CARDDGLGMDVW	1888	QASQDISNY LA	2076	AASLQS	2110	CQQSYSTPLTF	2174

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TABLE 2A-continued

Clone ID	Confirmed Binding	Anti-LRP5/6 Antibody Clone IDs and CDR sequences.										CDRL3 SEQ ID
		CDRH1 SEQ ID	CDRH2	CDRH2 SEQ ID	CDRH3	CDRH3 SEQ ID	CDRH3	CDRL1 SEQ ID	CDRL2 SEQ ID	CDRL2	CDRL3	
009S-A09	LRP6e3e4	YTFTRYLH 1681	GRIIPILGSTNYA	1767	CTTDLWDYW	2047	QASQGITNY LN	2078	AASSLQS 2110	CLQDYTDPTTF	2142	
011S-F02	LRP6e3e4	FTFSTYGMH 1584	SSISVSGTTH YA	1804	CARGGSGYYAFDIW	1929	PASQSISSYLN	2094	AASSLQS 2110	CQQSYSTPLTF	2174	
011S-G02	LRP6e3e4	YTFTSYAMN 1678	GGIPIFGTAN YA	1749	CARDASGGSTGWY FDSW	1886	PASQGISSYLA	2087	AASSLQS 2110	CQQAYSFPWTF	2150	
011S-A03	LRP6e3e4	FTFSSYWMH 1580	STISGSGRTY YA	1808	CATSPYGVFTLDYW	1993	PASQSISSYLN	2094	AASSLQS 2110	CQQSYSTPLTF	2174	
011S-C03	LRP6e3e4	YTFSYRYLH 1672	GGIPIFGTAN YA	1749	CASTVTITDAFDIW	1977	QASQDISNY LN	2077	DASSLES 2120	CQQSYSFPPPTF	2168	
011S-D03	LRP6e3e4	FSPDDYGMS 1554	SVISSGGTIYYA	1812	CARHLSGSLSYGYM DVW	1954	PASQSISSYLA	2093	AASTLQS 2113	CQQSYSTPLTF	2174	
011S-F03	LRP6e3e4	FTFSSYAMS 1577	SAISGSGSTY YA	1786	CAKGGRDGYKGYFD YW	1859	KSQSVLYTT TNENHIA	2073	WASSRKS 2135	CQQYVSTPYTF	2189	
011S-C04	LRP6e1e2	GTENSNAIS 1598	GWINPNSG NTGYA	1776	CARDYVSGSYNYG MDVW	1912	GASQSVPR NSLA	2066	GASQAT 2124	CQQYHNWPP EYTF	2184	
011S-D04	LRP6e1e2	YTFTSYDIN 1679	GIINPSGGSTS YA	1761	CAREAYYYYGMDVW	1915	PASQSISSYLN	2094	AASSLQS 2110	CQQSYSTPLTF	2174	
011S-H04	LRP6e1e2	YIFTDYMH 1669	GRIIPILGRANYA	1765	CARGGYSTLDYW	1932	QASQDISNY LN	2077	AASTLQS 2113	CQQSFSTPRTF	2156	
008S-F02	LRP5	YTFTNYCMH 1677	GIINPSDGSTS HA	1757	CAKDMVHLIIVALAID YW	1851	RSSQSLIHS DGYTLY	2101	TLSYRAS 2134	CMQALEALPTF	2144	
010S-C06	LRP6e1e2	FTFNSYSMD 1563	SSISPRGGSTY YA	1802	CAPYYDKSAKPLRSY PDHW	1875	PASQSISSYLN	2094	AASSLQS 2110	CQQSYSTPLTF	2174	
010S-E06	LRP6e3e4	LTVSSNYMS 1615	SGISWNSGIG YA	1796	CARGSDCSGSGCYYS PDYW	1944	PASQSISSYLN	2094	AASSLQS 2110	CQQSYSTPLTF	2174	
010S-F06	LRP6e3e4	FTFSSWMH 1575	SAITGGGTYYA	1781	CAREVAVKDYYYYY MDVW	1921	PASQSISSYLN	2094	AASSLQS 2110	CQQSYSTPLTF	2174	
010S-H06	LRP6e3e4	YTFTSYDIN 1679	GRIIPILGRANYA	1766	CARERGATGRAFDIW	1918	PASQSISSYLN	2094	AASSLQS 2110	CQQSYSTPLTF	2174	
010S-A07	LRP6e3e4	FTFSSYAMH 1576	ASISSTSGSKYYA	1724	CAKTYDFWSGYTTF DYW	1870	PASQSISSYLN	2094	AASSLQS 2110	CQQSYSTPLTF	2174	

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TABLE 2A-continued

Anti-LRP5/6 Antibody Clone IDs and CDR sequences.																	
Clone ID	Confirmed Binding	CDRH1	SEQ ID	CDRH1	CDRH2	CDRH2	SEQ ID	CDRH2	CDRH3	CDRH3	SEQ ID	CDRH3	SEQ ID	CDRL1	CDRL2	CDRL3	CDRL3 SEQ ID
010S-B07	LRP6e3e4	FTFSDYMS	1568	SMISYNGGRA FYA	1800	CARGNPYYFDYW	1937	RASQSISSYLN	2094	AASSLQS	2110	CQQSYSTPLTF	2174				
010S-C07	LRP6e3e4	FTPSKTDH	1569	STITDSEGT YA	1810	CAKGGDYIIYYGMDVW	1858	RASQSISSYLN	2094	AASSLQS	2110	CQQSYSTPLTF	2174				
010S-D07	LRP6e3e4	YFTYRYLH	1681	GGIPIPGTAN YA	1749	CANGLEDAYAFDIW	1873	RASQSISSYLN	2094	AASSLQS	2110	CQQSYSTPLTF	2174				
009S-D05	LRP6e3e4	FTLRHNL	1591	SAISGSGST YA	1786	CATRTGYSYGFNFW AFDIW	1992	RASQSISSYLN	2094	AASSLQS	2110	CQQSYSTPLTF	2174				
009S-E05	LRP6e3e4	YFTNNFMH	1676	GHVDPDGET IYA	1756	CARDWGIAAGDYY YIGMDVW	1909	RASQGINSY LA	2081	DAKGLHP	2114	CQQSYSAPLSF	2166				
010S-E07	LRP6e3e4	YFTYRYLH	1681	GGIPIPGTAN YA	1749	CAKDDPSLYGMDVW	1845	RASQSISSYLN	2094	AASSLQS	2110	CQQSYSTPLTF	2174				
009S-F05	LRP6e3e4	FTPDDYMS	1561	SAITGGGTYYA	1781	CARLGSYGSPYYYG MDVW	1960	RASQSISSYLN	2094	AASSLQS	2110	CQQSYSTPLTF	2174				
010S-F07	LRP6e3e4	YFTYRYLH	1681	GGIPIPGTAN YA	1749	CARLDYGETEGNDW	1958	RASQSVYSN LA	2097	DTSNRAT	2123	CQQYNNWPP ITF	2185				
010S-G07	LRP6e3e4	FTFSSYAMH	1576	STISGSGST YA	1809	CAPAGYGRYYGMD VW	1880	RVSQGISSYLN	2103	AASSLQS	2110	CQQTYTIPPTF	2182				
009S-G05	LRP6e3e4	FTFSDYMS	1568	SGVSNMGSRT HYA	1799	CAKDSGLV	1853	QASQDISNY LN	2077	AASLQR	2112	CQQSYSAPLTF	2167				
010S-H07	LRP6e3e4	YFTYRYLH	1681	GGIPIPGTAN YA	1749	CARDDSNGAFDIW	1890	QASQDISNY LN	2077	GTSNLQS	2128	CQQSYSTPYTF	2176				
010S-A08	LRP6e3e4	HTFLTIDIN	1606	GRITPRLGIAN YA	1770	CASYFGVMDVW	1979	RASQSISSYLN	2094	AASSLQS	2110	CQQSYSTPLTF	2174				
009S-A07	LRP6e3e4	YFTYRYLH	1681	GGIPIPGTAN YA	1749	CATAYGSSSLNIDW	1981	RASQSISSYLN	2094	AASSLQS	2110	CQQSYSTPLTF	2174				
009S-B07	LRP6e3e4	YFTGYM	1675	GWNNNSGCT NYA	1774	CVKDGSPFLAYAFD IW	2050	RASQSISSYLN	2094	AASSLQS	2110	CQQSYSTPLTF	2174				
009S-B06	LRP6e3e4	YFTYRYLH	1681	GGIPIPGTAN YA	1749	CAPALTDAGSPDYW	1874	RVSQSISSYLN	2103	AASSLQS	2110	CQQSYSTPLTF	2174				
010S-B08	LRP6e3e4	YFTYRYLH	1681	GGIIPVGTAD YA	1751	CARDREQIILDW	1904	RASQGISNN LN	2082	DASNLET	2115	CQQSYTSLTF	2179				

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TABLE 2A-continued

Anti-LRP5/6 Antibody Clone IDs and CDR sequences.															
Clone ID	Confirmed Binding	CDRH1	CDRH1 SEQ ID	CDRH2	CDRH2 SEQ ID	CDRH3	CDRH3 SEQ ID	CDRH3	CDRH3 SEQ ID	CDRL1	CDRL1 SEQ ID	CDRL2	CDRL2 SEQ ID	CDRL3	CDRL3 SEQ ID
010S-C08	LRP6e3e4	PTFTFCGMH	1582	STITSSGGSTYYA	1809	CARAGIAAAPGSRNY YGMVDVW	1878	PASQSISSVYN	2094	AASSLQS	2110	CQQSYSTPLTF	2174		
009S-C06	LRP6e3e4	YTPASYDIH	1671	GWNPNNSG NTGYA	1776	CARATSGWYTDLG YW	1884	RASRNINRY LN	2099	AASSLLS	2109	CQQSYNVPTTF	2162		
009S-D06	LRP6e3e4	PTFSSHSTH	1573	STISDTSNGTY YA	1807	CAKAQATGSGYYT FDYW	1844	PASQSISSVYN	2094	AASSLQS	2110	CQQSYSTPLTF	2174		
010S-D08	LRP6e3e4	PTFSSSNMH	1575	SAITGGGTYYA	1781	CAKEDYDSSGYYYY FQHW	1855	PASQSISSVYN	2094	AASSLQS	2110	CQQSYSTPLTF	2174		
009S-E06	LRP6e3e4	PTFTDYGLH	1587	AVISYGSNKY YA	1739	CASGYSYGLYYYGM DVW	1974	PASQSISSVYN	2094	AASSLQS	2110	CQQSYSTPLTF	2174		
010S-E08	LRP6e3e4	YSFTRTDMH	1670	GYISAYTGHTS YA	1778	CARDLGGTADYW	1898	PASQSISSVYN	2094	ZASSLQS	2137	CQQSYSTPLTF	2174		
010S-F08	LRP6e3e4	LTPDDHAMH	1613	SYISSSGRTIFYA	1815	CVRGDSGWGILYYV MDVW	2052	PASQSISSVYN	2094	AASSLQS	2110	CQQSYSTPLTF	2174		
009S-F06	LRP6e3e4	YTFTYRYLH	1681	GGIIPITGTAN YA	1749	CATEAALDAFDIW	1986	PASQSISSVYN	2094	AASSLQS	2110	CQQSYSTPLTF	2174		
010S-G08	LRP6e3e4	YIFTDYMH	1669	GGFDPEDGET IYA	1747	CARGGPNEDHYF DIW	1927	RASQSVRSS DLA	2096	GSSSRAT	2126	CQQYGRSPRY SF	2183		
010S-H08	LRP6e3e4	PTFZNAWMS	1590	SGISGSGSTY YA	1793	CARGRKKHYYYGM DVW	1942	PASQSISSVYN	2094	AASSLQS	2110	CQQSYSTPLTF	2174		
010S-A09	LRP6e3e4	PTFTTYMS	1586	SGISWNGGKT HYV	1794	CARGGDFDYW	1925	QASQDIANY LN	2075	AASSLQS	2110	CQQSYSTPYTF	2176		
010S-B09	LRP6e3e4	GTFSSYALS	1603	GWINPNSGDT NYA	1773	CARGEQLVWGF PW	1924	PASQSISSVYN	2092	KASSLES	2130	CQQSYDSPWTF	2159		
009S-G06	LRP6e3e4	YIFTDYMH	1669	GWINPNSGKT NYA	1774	CARDFLGSTGDYW	1893	PASQNIQY LN	2088	DASSLQR	2121	CQQSYSTPYTF	2176		
010S-C09	LRP6e3e4	YTFTYRYLH	1681	GGIIPITGTAN YA	1749	CARDEVEGGMDVW	1891	PASQSISSVYN	2094	AASSLQS	2110	CQQSYSTPLTF	2174		
009S-H06	LRP6e3e4	PTFSSSNMH	1574	SAITGGSTYYA	1783	CAKGGDYFYYYGM DVW	1857	PASQSISSVYN	2094	AASSLQS	2110	CQQSYSTPLTF	2174		
010S-D09	LRP6e3e4	GTFSSYTIS	1603	GGIVPAYRRA NYA	1754	CAKGGYELDYW	1865	QASQDIANY LN	2077	AASSLQS	2110	CQQIHSYPLTF	2155		

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TABLE 2A-continued

Anti-LRP5/6 Antibody Clone IDs and CDR sequences.												
Clone ID	Confirmed Binding	CDRH1	CDRH1 SEQ ID	CDRH2	CDRH2 SEQ ID	CDRH3	CDRH3 SEQ ID	CDRL1	CDRL1 SEQ ID	CDRL2	CDRL2 SEQ ID	CDRL3 SEQ ID
010S-E09	LRP6e3e4	GDLSIYTN	1593	GWINAGNGN TYYA	1772	CARGDSSGYYYAF DIW	1926	RASQSISSVLN	2094	AASSLQS	2110	CQQSYSTPLTF 2174
009S-A07	LRP6e3e4	YTFYRYLH	1681	GGIPIPGTAN YA	1749	CATAYGSSSLNIDYW	1982	RASQSISSVLN	2094	AASSLQS	2110	CQQSYSTPLTF 2174
009S-B07	LRP6e3e4	YTFGYTMH	1675	GWINPNSGGT NYA	1774	CVKDGSPPLAYAFD IW	2051	RASQSISSVLN	2094	AASSLQS	2110	CQQSYSTPLTF 2174
009S-D08	LRP6e3e4	YTFTSYDIN	1679	GGIPIPGTAN YA	1749	CARGPYDFDYW	1940	RASQISNNLN LN	2082	DASSLES	2120	CLQHNSYPPTF 2143
010S-F09	LRP6e3e4	FTFDEYAMH	1562	STISGSGSTY YA	1809	CASAKNDFHSGYFA FDYW	1968	RASQSISSVLN	2094	AASSLQS	2110	CQQSYSTPLTF 2174
010S-G09	LRP6e3e4	GTENTHTIT	1599	GMWPNMSG NTGYA	1776	CARGNLDFDYW	1936	QASQDINNY LN	2077	DASNLET	2115	CQQSYSTPLTF 2174
010S-H09	LRP6e3e4	FTFSDHYMS	1567	SAISGSDRTY YA	1787	CARYSGYDFDYW	1965	RASQGINY LN	2084	AASLQS	2113	CQQGYCTPP MF 2153
010S-A10	LRP6e3e4	FSPSSYSMN	1559	SYISSSSSTIYYA	1816	CARGSGYGPYYG MDVW	1946	RASQSISSVLN	2094	AASSLQS	2110	CQQSYSTPLTF 2174
009S-D07	LRP6e3e4	FPPRYYGMS	1551	ARIGNGGSI VYA	1717	CARDYSDRSIDYW	1911	RSSQSLHS NGNYLD	2102	LGSNRAS	2131	CMQATQFPLTF 2146
010S-B10	LRP6e3e4	FAPKDYTMT	1548	SAIGAGGTY YA	1779	CARBSALYSSSWYYY YIGMDVW	1920	RASQSISSVLN	2094	GTSSLHT	2129	CQQANSFPPTF 2148
010S-C10	LRP6e3e4	FTFSSYAMS	1577	SAISGSGSTY YA	1786	CAKGGEDGYKYFD YW	1860	KSSQSLSSS SNRDSL A	2072	WASSRKS	2135	CQQYNNIPYSF 2187
009S-E07	LRP6e3e4	YTFGYVIH	1674	ZHVPDEGETI YA	1819	CARGPAAGILGWFD PW	1938	RASQSISSVLN	2094	AASSLQS	2110	CQQSYSTPLTF 2174
010S-D10	LRP6e3e4	YIFDYYMH	1669	GMWPNMSG NTGYA	1776	CARTLSGYSSSWYVF DIW	1964	RASQSISSVLN	2094	AASSLQS	2110	CQQSYSTPLTF 2174
010S-E10	LRP6e3e4	FTFSSYSMN	1579	SGISWNSGTT GYS	1797	CARDHSSGWRHYFD YW	1895	RASQSISSVLN	2094	AASSLQS	2110	CQQSYSTPLTF 2174
010S-F10	LRP6e3e4	FTFNSDMN	1570	SYISGNSGYN YA	1814	CASGSYSDFDYW	1971	RASQISNYLN	2090	AASLQS	2111	CQQANSFPPTF 2148
010S-G10	LRP6e3e4	GTFSSYALS	1603	GRINPNSGGT IYA	1769	CAREGGYDFDYW	1919	RASQGINY LA	2083	AASSLQS	2110	CQQSYSTPWTTF 2175

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TABLE 2A-continued

Anti-LRP5/6 Antibody Clone IDs and CDR sequences.														
Clone ID	Confirmed Binding	CDRH1	CDRH1 SEQ ID	CDRH2	CDRH2 SEQ ID	CDRH3	CDRH3 SEQ ID	CDRL1	CDRL1 SEQ ID	CDRL2	CDRL2 SEQ ID	CDRL3	CDRL3 SEQ ID	
009S-F07	LRP6e3e4	GTFSYAIS	1603	GIINPSSGGSTS YA	1761	CARAAGNFWSGYYT FDYW	1877	RASQSISSYLN	2094	AASSLQS	2110	CQQSYSTPLTF	2174	
009S-G07	LRP6e3e4	YTFTYRYLH	1681	GGIIPFPGTAN YA	1749	CARGSYGMDVW	1948	RASQGISNY LA	2083	DASNLET	2115	CLQDFSPFWTF	2140	
010S-H10	LRP6e3e4	YTFTSYMH	1680	GWINPNSGGT NYA	1774	CAREAAEIPVGAFDIW	1914	KSSHLLYSS DNKNYLA	2071	WSSTRES	2136	CQQYYSTPQTF	2188	
010S-A11	LRP6e3e4	PTFNSDMN	1570	SYISNSGYTN YA	1814	CASGSYYSDFDYW	1972	RASQSIZNYLN	2095	ZASTLES	2138	CQQANSPPPTF	2148	
010S-B11	LRP6e3e4	PTFRNYAIH	1564	SAITGGDTYYA	1780	CARDGGIRDFDYW	1894	QASQDISNY LN	2077	AASTLQS	2113	CQQSYSTPLTF	2174	
010S-C11	LRP6e3e4	YTFTYRYLH	1681	GGIIPFPGTAN YA	1749	CAADDLGLLELHW	1822	RASQSISSYLN	2094	AASSLQS	2110	CQQSYSTPLTF	2174	
009S-H07	LRP6e3e4	YTFTGYMH	1675	GWNPNSG NTGYA	1776	CASSVVPAGPAGVY AFDIW	1976	RASQSISSYLN	2094	AASSLQS	2110	CQQSYSTPLTF	2174	
009S-A08	LRP6e3e4	GTFSHAIN	1602	GWISANNGN TDYA	1775	CARDQYGDYGYW YYGMDVW	1903	RASQGISNY LA	2083	GSSTLQS	2127	CQQTYSIPPTF	2181	
010S-D11	LRP6e3e4	YTFTYRYLH	1681	GGIIPVFGTAN YA	1752	CATDEYSSSYAFDIW	1983	RASQSVSSN LA	2097	GASTRAT	2125	CQQFDRSPLTF	2151	
010S-E11	LRP6e3e4	PTFSAHGMH	1565	SGISESGGSTY YA	1791	CARGGSYGYVAFD IW	1943	RASQSISSYLN	2094	AASSLQS	2110	CQQSYSTPLTF	2174	
010S-F11	LRP6e3e4	YTFTYRYLH	1681	GGIIPFPGTAN YA	1749	CARDSDWGVDPW	1905	RASQSISSYLN	2094	AASSLQS	2110	CQQSYSTPLTF	2174	
010S-G11	LRP6e3e4	YTFTYRYLH	1681	GRIPVLKITNYA	1768	CAVDDDAFDIW	1997	RASQSISSYLN	2094	AASSLQS	2110	CQQSYSTPLTF	2174	
010S-H11	LRP6e3e4	YTFTYRYLH	1681	GGIIPFPGTAN YA	1749	CAKDGTDGRDPDW	1846	RASQDISSYLA	2080	SASTLQS	2133	CQQSNSFPYTF	2157	
009S-B08	LRP6e3e4	PTFTSSAVQ	1589	GWINAGNGN TTYA	1772	CARRGGDVTVPAAV YAMDVW	1963	RASQSISSYLN	2094	ZASSLQS	2137	CQQSYSTPLTF	2174	
010S-A12	LRP6e3e4	VTFSTRYPIS	1667	GGIIPFPGTAN YA	1749	CAKDSGNYGYGMD VW	1854	RASQSISSYLN	2094	AASSLQS	2110	CQQSYSTPLTF	2174	
010S-B12	LRP6e3e4	PTFSSYDMH	1578	SGITSNGGATY YA	1798	CARGTTGKYTYYG MDVW	1949	RASQSISSYLN	2094	AASSLQS	2110	CQQSYSTPLTF	2174	

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TABLE 2A-continued

Anti-LRP5/6 Antibody Clone IDs and CDR sequences.													
Clone ID	Confirmed Binding	CDRH1	SEQ ID	CDRH1	CDRH2	CDRH2	SEQ ID	CDRH3	CDRH3	CDRH3	SEQ ID	CDRL1	SEQ ID
010S-C12	LRP6e3e4	FTFSNYWIH	1571	SAITGGGTYYA	1781	CTTAGYKAARSVYP RIPNFDYW	2044	RASQISSYLN	2094	AASLQS	2110	CQOSYSTPLTF	2174
010S-D12	LRP6e3e4	YTFTYRLH	1681	GRIIPIFGTANYA	1763	CAREBGVGMVDV	1917	PRQSIGSW LA	2100	DASNLQS	2116	CQOSSSTPYTF	2158
010S-E12	LRP6e3e4	FTFSSYAMH	1576	SAIGAGGTY YA	1779	CARGVSSGYYYYG MDVW	1950	RASQISSYLN	2094	AASLQS	2110	CQOSYSTPLTF	2174
010S-F12	LRP6e3e4	FTVSSNYMS	1592	SAITGGGTYYA	1781	CARAGTNWGGWYF DLW	1879	RASQISRD LA	2085	AASLQS	2113	CQOSYSPPTF	2172
010S-G12	LRP6e3e4	FALSGYYMS	1550	SSISSSTYIRYA	1803	CATVTGYSSAGAFDIW	1995	RASQISSYLN	2094	AASLQS	2110	CQOSYSTPLTF	2174
011S-A01	LRP6e3e4	FTFSTHAFH	1583	SAIRGSGERTY YA	1784	CARDLRNWGSPYW YFDLW	1901	RASQISSYLN	2094	AASLQS	2110	CQOSYSTPLTF	2174
011S-B01	LRP6e3e4	GTFSHYTIS	1600	GWINAGN TKYS	1771	CAKGSGLMDVW	1864	RASQISNY LA	2083	AASLQS	2108	CQOSYRTPPTF	2164
011S-C01	LRP6e3e4	LTFTSHGMS	1615	SYVSDSGSVY YA	1818	CARHPGSGGYSA WYYYGMDVW	1957	RASQISSYLN	2094	AASLQS	2110	CQOSYSTPLTF	2174
011S-D01	LRP6e3e4	GTISDYTVS	1605	GIINPSGGSTS YA	1761	CARGYYDFDYW	1953	RASQISNY LA	2083	AASLQS	2110	CQOSYSTPLTF	2174
009S-C08	LRP6e3e4	FSPNTFGIH	1556	AVISYDGSNKY YA	1738	CAKSTAAAGTGYG MDVW	1869	RASQISSYLN	2094	AASLQS	2110	CQOSYSTPLTF	2174
011S-E01	LRP6e3e4	FPEZYYSMN	1552	SAISGRDGRTY YA	1785	CAKDLGIQLDPDYED YW	1847	RASQISSA LA	2086	AASLQS	2113	CQOSYSSPPTF	2173
009S-D08	LRP6e3e4	YTFTSYDIN	1679	GGIPIFGTAN YA	1749	CARGPYDFDYW	1941	RASQISNN LN	2082	DASSLES	2120	CLOHNSYPPTF	2143
011S-F01	LRP6e3e4	FSPSDYYMS	1558	SGISESGGRTY YA	1790	CASAADFDYW	1967	RASQISNY LN	2079	AASLQS	2110	CLODYSYPTF	2141
009S-E08	LRP6e3e4	YGFTGYVIH	1668	GWMPNSG NTGYA	1776	CARGYGDYDLW	1952	QASQISNY LN	2077	DASSLES	2120	CQOSYRPTF	2165
009S-F08	LRP6e3e4	DTFANYGFS	1547	GKVNAGN TTYA	1777	CAKGWLDYDYW	1867	QASQISNY LN	2077	DASSLES	2120	CQOSYSTSTF	2177
011S-G01	LRP6e3e4	YTFTYRYLH	1681	GGIPIFGTAN YA	1750	CTTDDYGDYQGM VW	2046	RASQISSYLN	2094	AASLQS	2110	CQOSYSTPLTF	2174

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TABLE 2A-continued

Anti-LRP5/6 Antibody Clone IDs and CDR sequences.													
Clone ID	Confirmed Binding	CDRH1	CDRH1 SEQ ID	CDRH2	CDRH2 SEQ ID	CDRH3	CDRH3 SEQ ID	CDRL1	CDRL1 SEQ ID	CDRL2	CDRL2 SEQ ID	CDRL3	CDRL3 SEQ ID
011S-H01	LRP6e3e4	YTFYRYLH	1681	GGIPIPGTAN YA	1749	CTDDYGLTHLDYW	2045	RASQSISSVLN	2094	AASSLQS	2110	CQQSYSTPLTF	2174
011S-A02	LRP6e3e4	GTPSSYAIS	1603	GWWPNSG NTGYA	1776	CARDKGYAFDIW	1896	RSSQSLHLS NGNYILD	2102	AASSLQS	2110	CMQALQTPITF	2145
011S-B02	LRP6e3e4	YSPRTDMH	1670	GYISAYTGHTS YA	1778	CARDLGGTADYW	1899	RZSQSZSYLN	2104	AASSLQS	2110	CQQSYSTPLTF	2174
011S-C02	LRP6e3e4	FTFSTYSMN	1585	SGISWNSGRI GYA	1795	CARDVGAFDIW	1906	QASQDINNY LN	2077	AASILQS	2107	CQQSYSTPPTF	2169
009S-G08	LRP6e3e4	FTFDFPMT	1566	SYISGDSGYTN YA	1813	CARLGSYPGYYYM DVM	1962	RASQSISSVLN	2094	AASSLQS	2110	CQQSYSTPLTF	2174
011S-D02	LRP6e3e4	FTFSSYAMS	1577	SSISGSGVTY YA	1801	CARGNTYYYYGCM DVM	1928	RASQSISSVLN	2094	AASSLQS	2110	CQQSYSTPLTF	2174
009S-H08	LRP6e3e4	YTFTDYPMN	1673	GIINPSGDSTR FA	1758	CARDGGLGGMVDW	1889	QASQDINNY LA	2076	AASSLQS	2110	CQQSYSTPLTF	2174
011S-E02	LRP6e3e4	YTFYRYLH	1681	GGIPIPGTAN YA	1749	CATDYGDIYYGMDYW	1984	RASQSISSVLN	2094	AASSLQS	2110	CQQSYSTPLTF	2174
009S-A09	LRP6e3e4	YTFYRYLH	1681	GRILPLGSTNYA	1767	CTTDLWDYW	2048	QASQGITNY LN	2078	AASSLQS	2110	CLQDYTDPPTF	2142
011S-F02	LRP6e3e4	FTFSTYGMH	1584	SSISVSSGTH YA	1805	CARGSGSYYYAFDIW	1930	RASQSISSVLN	2094	AASSLQS	2110	CQQSYSTPLTF	2174
011S-G02	LRP6e3e4	YTFTSYAMN	1678	GGIPIPGTAN YA	1749	CARDASGGSTGWY FDSW	1887	RASQSISSVLA	2087	AASSLQS	2110	CQQAYSPPTF	2150
011S-H02	LRP6e3e4	YTFNNPMH	1676	GIINPSGGSTS YA	1761	CARGLYKRYSYGYG MDVM	1935	RASQSISSVLN	2094	AASSLQS	2110	CQQSYSTPLTF	2174
009S-B09	LRP6e3e4	FSFNTYAMN	1557	AVTSYDGGKK NYA	1742	CARDAGGDYDW	1885	QASQDINNY LN	2077	AASSLQS	2110	CQQSYNTPTF	2161
009S-C09	LRP6e3e4	GTFHTYGLS	1597	GGIPIPGTAN YA	1749	CARGSGWSGLDYW	1945	QASQDINNY LN	2077	DASNET	2115	CQQSYTPPTF	2180
011S-A03	LRP6e3e4	FTFSSYWMH	1580	STISGSGRTY YA	1808	CATSPYGVFTLDYW	1994	RASQSISSVLN	2094	AASSLQS	2110	CQQSYSTPLTF	2174
011S-B03	LRP6e3e4	GTPSZYAIS	1604	GIINPSGGSTN YA	1760	CARAGWSGVGYG MDVM	1881	RASQSISSVLN	2094	AASSLQS	2110	CQQSYSTPLTF	2174

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TABLE 2A-continued

Anti-LRP5/6 Antibody Clone IDs and CDR sequences.													
Clone ID	Confirmed Binding	CDRH1	CDRH1 SEQ ID	CDRH2	CDRH2 SEQ ID	CDRH3	CDRH3 SEQ ID	CDRL1	CDRL1 SEQ ID	CDRL2	CDRL2 SEQ ID	CDRL3	CDRL3 SEQ ID
011S-C03	LRP6e3e4	YTPSYRLH	1672	GGIPIPGTAN YA	1749	CASIVTTDAFDIW	1978	QASQDISNY LN	2077	DASSLES	2120	CQQSYSPFPPTF	2168
011S-D03	LRP6e3e4	FSPDDYGMS	1554	SVISSGGTIYYA	1812	CARHLSSGSLSYVGM DVM	1955	RASQISSVLA	2093	AASLTQS	2113	CQQSYSTPLTF	2174
009S-F09	LRP6e3e4	YSFRTDMH	1670	GYISAYTGHTS YA	1778	CARDLGGTADYW	1900	RASQISSVYN	2094	AASSLQS	2110	CQQSYSTPLTF	2174
011S-E03	LRP6e3e4	FTFSSYAMS	1577	SAISGSGGSTY YA	1786	CAKGGEDGYKGYFD YW	1861	KSSHSLSTIS TNRNQLA	2070	WASSRKS	2135	CQQYNNWPY TF	2186
009S-G09	LRP6e3e4	FTFRRHSMN	1572	SYSSGNSGYT NYA	1817	CARGDLEFDYW	1923	RASQIGINY LA	2083	SASSLQS	2132	CQQGYNTPRTF	2154
011S-F03	LRP6e3e4	FTFSSYAMS	1577	SAISGSGGSTY YA	1786	CAKGGEDGYKGYFD YW	1862	KSSQSVLYTT TNRNHIA	2074	WASSRKS	2135	CQQYYSTPYTF	2189
009S-H09	LRP6e3e4	FTFSSYAMS	1577	SAISGSGGSTY YA	1786	CAKGGEDGYKGYFD YW	1863	KSSHSLSTIS TNRNHIA	2069	WASSRKS	2135	CQQYNNIPYSF	2187
011S-G03	LRP6e3e4	YTFYRYLH	1681	GRILPIHGIANYA	1764	CAREYSYGFRYW	1922	RASQISSVLA	2087	DASNLET	2115	CQQANSLETF	2149
009S-A10	LRP6e3e4	FTFTSSAMQ	1588	GIINPSSGGSTIYA	1759	CASGPTYDLYSLDVW	1970	RASQISRW LA	2091	AASSLQS	2110	CQQAYSFPWTF	2150
009S-B10	LRP6e3e4	YIFTDYMH	1669	GWINAGN TTVA	1772	CAKVASGWSWPFDIW	1871	RASQISSVYN	2094	AASSLQS	2110	CQQSYSTPLTF	2174
011S-B04	LRP6e1e2	YTFTSYDIN	1679	GIINPSSGGSTS YA	1761	CTREHSYYYGMDVW	2042	RASQISSVYN	2094	AASSLQS	2110	CQQSYSTPLTF	2174
011S-C04	LRP6e1e2	GTFNSNAIS	1598	GWMNPNSG NTGYA	1776	CARDYYGSGSYNYG MDVW	1913	GASQSVPR NSLA	2066	GASQAT	2124	CQQYHNWPP EYTF	2184
011S-D04	LRP6e1e2	YTFTSYDIN	1679	GIINPSSGGSTS YA	1761	CAREAYYYYGMDVW	1916	RASQISSVYN	2094	AASSLQS	2110	CQQSYSTPLTF	2174

TABLE 2A-continued

Anti-LRP5/6 Antibody Clone IDs and CDR sequences.																
Clone ID	Confirmed Binding	CDRH1	CDRH1 SEQ ID	CDRH2	CDRH2 SEQ ID	CDRH3	CDRH3 SEQ ID	CDRH3	CDRH3 SEQ ID	CDRL1	CDRL1 SEQ ID	CDRL2	CDRL2 SEQ ID	CDRL3	CDRL3 SEQ ID	
011S-E04	LRP6e1e2	PTFSSZZMH	1581	SAITGGGTZYA	1782	CAKDLGRAAGSMD	1850	WASQSVRG	2106	DASNRAA	2117	COHRSNWPLTF	2147			
						VW		NYVA								
011S-F04	LRP6e1e2	YIFTDYNMH	1669	GRILPILGRANYA	1765	CARGGYSTLDYW	1933	HGSQDISNY	2067	DASNROS	2119	CQQSFSSTPRTF	2156			
								LN								
011S-H04	LRP6e1e2	YIFTDYNMH	1669	GRILPILGRANYA	1765	CARGGYSTLDYW	1934	QASQDISNY	2077	AASSTLOS	2113	CQQSFSSTPRTF	2156			
								LN								
011S-A05	LRP6e1e2	PTFSSYAMH	1576	SAITGGGTYYA	1781	CAKDLGRAAGSMD	1848	WASQSVRG	2106	DASNRAA	2118	COHRSNWPLTF	2147			
						VW		NYVA								
011S-B05	LRP6e1e2	YZFTDYNMH	1683	GWMNPNSG	1776	CTFVANGLDYW	2043	RASQSISSVLN	2094	AASSTLOS	2110	CQQSYSTPLTF	2174			
				NTGYA												
011S-C05	LRP6e1e2	PTFSSYAMH	1576	SAITGGGTYYA	1781	CAKDLGRAAGSMD	1849	WASQSVRG	2106	DASNRAA	2117	COHRSNWPLTF	2147			
						VW		NYVA								
1115.3	LRP6e1e2	GFSTST		NLNGGS		ELAGYGTPEAY		KASQSISSVLN		YTSQIS		QQSNSWPLT				
421.1	LRP6e1e2	GYTFTY		FPGNVNT		BELOYTFDY		SANSSVRMF		FTSNLAS		QOYHSYPWT				
YW211.31.	LRP6e3e4	GFTFTSY		SPYSGS		RARPPIRLHPRGSVM		RASQDVSTA		SASFLYS		QOSYTPPT				
57						DY		VA								

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TABLE 2B

Anti-LRP5/6 Antibody Clone IDs, Heavy Chain (HC) Seq ID Nos, and Binding Characteristics.		
Clone ID	HC Seq ID NO	Confirmed Binding
001S-F11	66	LRP6e1e2
009S-G02	67	LRP6e1e2
009S-A03	68	LRP6e1e2
009S-D03	69	LRP6e1e2
009S-F03	70	LRP6e1e2
009S-H03	71	LRP6e1e2
009S-A04	72	LRP6e1e2
009S-B04	73	LRP6e3e4
009S-D04	74	LRP6e3e4
009S-E04	75	LRP6e3e4
009S-F04	76	LRP6e3e4
009S-G04	77	LRP6e3e4
009S-H04	78	LRP6e3e4
009S-A05	79	LRP6e3e4
013S-G04	80	LRP6e3e4
013S-H04	81	LRP6e3e4
013S-C05	82	LRP6e3e4
013S-D05	83	LRP6e3e4
013S-G04	84	LRP6e3e4
013S-H04	85	LRP6e3e4
013S-A05	86	LRP6e3e4
013S-C05	87	LRP6e3e4
013S-D05	88	LRP6e3e4
008S-D01	133	LRP5

In certain embodiment, the LRP5/6 binding domain may be selected from any binding domain that binds LRP5 or LRP6 with a K_D of less than or equal to about 1×10^{-4} M, less than or equal to about 1×10^{-5} M, less than or equal to about 1×10^{-6} M, less than or equal to about 1×10^{-7} M, less than or equal to about 1×10^{-8} M, less than or equal to about 1×10^{-9} M, or less than or equal to about 1×10^{-10} M in the context of a Wnt surrogate molecule. In certain embodiment, the LRP5/6 binding domain may be selected from any binding domain that binds LRP5 or LRP6 with a K_D of greater than or equal to about 1×10^{-4} M, greater than or equal to about 1×10^{-5} M, greater than or equal to about 1×10^{-6} M, greater than or equal to about 1×10^{-7} M, greater than or equal to about 1×10^{-8} M, greater than or equal to about 1×10^{-9} M, or greater than about 1×10^{-10} M in the context of a Wnt surrogate molecule. In certain embodiment, the LRP5/6 binding domain may be selected from any binding domain that binds LRP5 or LRP6 at high affinity, e.g. a K_D of less than about 1×10^{-7} M, less than about 1×10^{-8} M, less than about 1×10^{-9} M, or less than about 1×10^{-10} M.

Other suitable LRP5/6 binding domains include, without limitation, de novo designed LRP5/6 binding proteins, antibody derived binding proteins, e.g. scFv, Fab, etc. and other portions of antibodies that specifically bind to one or more Fzd proteins; VHH or sdAb derived binding domains; knottin-based engineered scaffolds; naturally occurring LRP5/6, including without limitation, DKK1, DKK2, DKK3, DKK4, sclerostin; Wise; fusions proteins comprising any of the above; derivatives of any of the above; variants of any of the above; and biologically active fragments of any of the above, and the like. A LRP5/6 binding domain may be affinity selected to enhance binding.

Members of the Dickkopf (DKK) gene family (see Krupnik et al. (1999) Gene 238(2):301-13) include DKK-1, DKK-2, DKK-3, and DKK-4, and the DKK-3 related protein Soggy (Sgy). hDKKs 1-4 contain two distinct cysteine-rich domains in which the positions of 10 cysteine residues are highly conserved between family members. Exemplary sequences of human Dkk genes and proteins are publicly available, e.g., Genbank accession number NM_014419

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(soggy-1); NM_014420 (DKK4); AF177394 (DKK-1); AF177395 (DKK-2); NM_015881 (DKK3); and NM_014421 (DKK2). In some embodiments of the invention, the Lrp6 binding moiety is a DKK1 peptide, including without limitation the C-terminal domain of human DKK1. The C-terminal domain may comprise the sequence:

(SEQ ID NO: 2190)
 10 KMYHTKGQEGSVCLRSSDCASGLCCARHFWSKICKPVLKEGQVCTKH
 RRKGS HGLEIFQRCYCGEGLSCRIQKDDHHQASNSSRLHTCQRH

(see Genbank accession number NP_036374) or a biologically active fragment thereof.

15 Binding of DKK proteins to LRP5/6 are discussed, for example in Brott and Sokol Mol. Cell. Biol. 22 (17), 6100-6110 (2002); and Li et al. J. Biol. Chem. 277 (8), 5977-5981 (2002), each herein specifically incorporated by reference. The corresponding region of human DKK2 (Genbank reference NP_055236) may comprise the sequence: KMSHIKGHEGDPCLRSSDCIEGFCCARHFWTKICK-
 20 PVLHQGEVCTKQRKKGSH GLEIFQRCD-
 CAKGLSCKVWKDATYSSKARLHVCQK (SEQ ID NO:2191) or a biologically active fragment thereof.

25 Antibodies that specifically bind to LRP5 or LRP6 are known in the art and are commercially available, or can be generated de novo. LRP5, LRP6 or fragments thereof can be used as an immunogen or in screening assays to develop an antibody. Examples of known antibodies include, without limitation, those described in Gong et al. (2010) PLoS One. 5(9):e12682; Ettenberg et al. (2010) Proc Natl Acad Sci USA. 107(35):15473-8; and those commercially available from, for example Santa Cruz biotechnology antibody clone 1A12, which was raised against synthetic LRP5/6 of human origin and binds to both the full length and proteolytic fragment of LRP6 and LRP5 of mouse and human origin; the monoclonal antibody 2611; Cell Signaling Technology antibody specific for LRP5 (D80F2), catalog number 5731; etc.

40 In certain embodiments, Wnt surrogate molecules disclosed herein comprise one or more polypeptides comprising two or more binding regions. For example, the two or more binding regions may be two or more Fzd binding regions or two or more LRP5/6 binding regions, or they may comprise one or more Fzd binding region and one or more LRP5/6 binding region. The binding regions may be directly joined or contiguous, or may be separated by a linker, e.g. a polypeptide linker, or a non-peptidic linker, etc. The length of the linker, and therefore the spacing between the binding domains can be used to modulate the signal strength, and can be selected depending on the desired use of the Wnt surrogate molecule. The enforced distance between binding domains can vary, but in certain embodiments may be less than about 100 angstroms, less than about 90 angstroms, less than about 80 angstroms, less than about 70 angstroms, less than about 60 angstroms, or less than about 50 angstroms. In some embodiments the linker is a rigid linker, in other embodiments the linker is a flexible linker. In certain embodiments where the linker is a peptide linker, it may be from about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 21, 22, 23, 24, 25, 26, 27, 28, 29, 30 or more amino acids in length, and is of sufficient length and amino acid composition to enforce the distance between binding domains. In some embodiments, the linker comprises or consists of one or more glycine and/or serine residues.

In particular embodiments, a Wnt surrogate molecule comprises a polypeptide sequence having at least 90%, at

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least 95%, at least 98% or at least 99% identity to a polypeptide sequence disclosed in any of SEQ ID NOs:89-128 or 134-157, or having at least 90%, at least 95%, at least 98% or at least 99% identity to an antigen-binding fragment of a polypeptide sequence disclosed in any of SEQ ID NOs:89-128 or 134-157. In certain embodiments, the Wnt surrogate molecules comprises or consists of a polypeptide sequence set forth in any of SEQ ID NOs:89-128 or 134-157, or an antigen-binding fragment thereof. In particular embodiments, the antigen-binding fragment binds one or more Fzd receptors and also binds LRP5 and/or LRP6.

Wnt surrogate molecule can be multimerized, e.g. through an Fc domain, by concatenation, coiled coils, polypeptide zippers, biotin/avidin or streptavidin multimerization, and the like. The Wnt surrogate molecules can also be joined to a moiety such as PEG, Fc, etc., as known in the art to enhance stability *in vivo*.

In certain embodiments, a Wnt surrogate molecule directly activates canonical Wnt signaling through binding to one or more Fzd proteins and to LRP5/6, particularly by binding to these proteins on a cell surface, e.g., the surface of a human cell. The direct activation of Wnt signaling by a Wnt surrogate molecule is in contrast to potentiation of Wnt signaling, which enhances activity only when native Wnt proteins are present.

Wnt surrogate molecules may activate Wnt signaling, e.g., by mimicking the effect or activity of a Wnt protein binding to a frizzled protein. The ability of the Wnt surrogate molecules of the invention to mimic the activity of Wnt can be confirmed by a number of assays. The Wnt surrogate molecules typically initiate a reaction or activity that is similar to or the same as that initiated by the receptor's natural ligand. In particular, the Wnt surrogate molecules of the invention enhance the canonical Wnt/ β -catenin signaling pathway. As used herein, the term "enhances" refers to a measurable increase in the level of Wnt/ β -catenin signaling compared with the level in the absence of a Wnt surrogate molecule of the invention.

Various methods are known in the art for measuring the level of canonical Wnt/ β -catenin signaling. These include, but are not limited to assays that measure: Wnt/ β -catenin target gene expression; TCF reporter gene expression; β -catenin stabilization; LRP phosphorylation; Axin translocation from cytoplasm to cell membrane and binding to LRP. The canonical Wnt/ β -catenin signaling pathway ultimately leads to changes in gene expression through the transcription factors TCF7, TCF7L1, TCF7L2 (a.k.a., TCF4), and LEF. The transcriptional response to Wnt activation has been characterized in a number of cells and tissues. As such, global transcriptional profiling by methods well known in the art can be used to assess Wnt/ β -catenin signaling activation or inhibition.

Changes in Wnt-responsive gene expression are generally mediated by TCF and LEF transcription factors. A TCF reporter assay assesses changes in the transcription of TCF/LEF controlled genes to determine the level of Wnt/ β -catenin signaling. A TCF reporter assay was first described by Korinek, V. et al., 1997. Also known as TOP/FOP this method involves the use of three copies of the optimal TCF motif CCTTTGATC, or three copies of the mutant motif CCTTTGGCC, upstream of a minimal c-Fos promoter driving luciferase expression (pTOPFL-ASH and pFOPFL-ASH, respectively) to determine the transactivational activity of endogenous p-catenin/TCF4. A higher ratio of these two reporter activities (TOP/FOP) indicates higher β -catenin/TCF4 activity, whereas a lower ratio of these two reporter activities indicates lower β -catenin/TCF4 activity.

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Various other reporter transgenes that respond to Wnt signals exist intact in animals and therefore, effectively reflect endogenous Wnt signaling. These reporters are based on a multimerized TCF binding site, which drives expression of LacZ or GFP, which are readily detectable by methods known in the art. These reporter genes include: TOP-GAL, BAT-GAL, ins-TOPE FP, ins-TOPGAL, LEF-EGFP, Axin2-LacZ, Axin2-d2EGFP, Lgr5tm1 (cre/ERT2), TOPdGFP.

The recruitment of dephosphorylated β -catenin to the membrane, stabilization and phosphorylation status of β -catenin, and translocation of β -catenin to the nucleus (Klapholz-Brown Z et al., PLoS One. 2(9) e945, 2007), in some cases mediated by complex formation with TCF transcription factors and TNK1 are key steps in the Wnt signaling pathway. Stabilization is mediated by Dishevelled family proteins that inhibit the "destruction" complex so that degradation of intracellular β -catenin is reduced, and translocation of β -catenin to the nucleus follows thereafter. Therefore, measuring the level and location of β -catenin in a cell is a good reflection of the level of Wnt/ β -catenin signaling. A non-limiting example of such an assay is the "BioImage β -Catenin Redistribution Assay" (Thermo Scientific) which provides recombinant U2OS cells that stably express human β -catenin fused to the C-terminus of enhanced green fluorescent protein (EGFP). Imaging and analysis is performed with a fluorescence microscope or HCS platform allowing the levels and distribution of EGFP- β -catenin to be visualized.

Another way, in which the destruction complex is inhibited, is by removal of Axin by recruitment of Axin to the cytoplasmic tail of the Wnt co-receptor LRP. Axin has been shown to bind preferentially to a phosphorylated form of the LRP tail. Visualization of Axin translocation, for example with a GFP-Axin fusion protein, is therefore another method for assessing levels of Wnt/ β -catenin signaling.

In certain embodiments, a Wnt surrogate molecule enhances or increases canonical Wnt pathway signaling, e.g., β -catenin signaling, by at least 30%, 35%, 40%, 45%, 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, 110%, 150%, 200%, 250%, 300%, 400% or 500%, as compared to the β -catenin signaling induced by a neutral substance or negative control as measured in an assay described above, for example as measured in the TOPFlash assay. A negative control may be included in these assays. In particular embodiments, Wnt surrogate molecules may enhance β -catenin signaling by a factor of 2 $\times$, 5 $\times$, 10 $\times$, 100 $\times$, 1000 $\times$, 10000 $\times$ or more as compared to the activity in the absence of the Wnt surrogate molecule when measured in an assay described above, for example when measured in the TOPFlash assay, or any of the other assays mentioned herein.

"Wnt gene product" or "Wnt polypeptide" when used herein encompass native sequence Wnt polypeptides, Wnt polypeptide variants, Wnt polypeptide fragments and chimeric Wnt polypeptides. In particular embodiments, a Wnt polypeptide is a native human full length mature Wnt protein.

For example, human native sequence Wnt proteins of interest in the present application include the following: Wnt-1 (GenBank Accession No. NM_005430); Wnt-2 (GenBank Accession No. NM_003391); Wnt-2B (Wnt-13) (GenBank Accession No. NM_004185 (isoform 1), NM_024494.2 (isoform 2)), Wnt-3 (RefSeq: NM_030753), Wnt3a (GenBank Accession No. NM_033131), Wnt-4 (GenBank Accession No. NM_030761), Wnt-5A (GenBank Accession No. NM_003392), Wnt-5B (GenBank Accession No. NM_032642), Wnt-6 (GenBank Accession No.

NM_006522), Wnt-7A (GenBank Accession No. NM_004625), Wnt-7B (GenBank Accession No. NM_058238), Wnt-8A (GenBank Accession No. NM_058244), Wnt-8B (GenBank Accession No. NM_003393), Wnt-9A (Wnt-14) (GenBank Accession No. NM_003395), Wnt-9B (Wnt-15) (GenBank Accession No. NM_003396), Wnt-1 OA (GenBank Accession No. NM_025216), Wnt-10B (GenBank Accession No. NM_003394), Wnt-11 (GenBank Accession No. NM_004626), Wnt-16 (GenBank Accession No. NM_016087)). Although each member has varying degrees of sequence identity with the family, all encode small (i.e., 39-46 kD), acylated, palmitoylated, secreted glycoproteins that contain 23-24 conserved cysteine residues whose spacing is highly conserved (McMahon, A P et al., Trends Genet. 1992; 8: 236-242; Miller, J R. Genome Biol. 2002; 3(1): 3001.1-3001.15). Other native sequence Wnt polypeptides of interest include orthologs of the above from any mammal, including domestic and farm animals, and zoo, laboratory or pet animals, such as dogs, cats, cattle, horses, sheep, pigs, goats, rabbits, rats, mice, frogs, zebra fish, fruit fly, worm, etc.

"Wnt pathway signaling" or "Wnt signaling" is used herein to refer to the mechanism by which a biologically active Wnt exerts its effects upon a cell to modulate a cell's activity. Wnt proteins modulate cell activity by binding to Wnt receptors, including proteins from the Frizzled (Fzd) family of proteins, proteins from the ROR family of proteins, the proteins LRP5, LRP6 from the LRP family of proteins, the protein FRL1/crypto, and the protein Derailed/Ryk. Once activated by Wnt binding, the Wnt receptor(s) will activate one or more intracellular signaling cascades. These include the canonical Wnt signaling pathway; the Wnt/planar cell polarity (Wnt/PCP) pathway; the Wnt-calcium (Wnt/Ca²⁺) pathway (Giles, R H et al. (2003) Biochim Biophys Acta 1653, 1-24; Peifer, M. et al. (1994) Development 120: 369-380; Papkoff, J. et al (1996) Mol. Cell Biol. 16: 2128-2134; Veeman, M. T. et al. (2003) Dev. Cell 5: 367-377); and other Wnt signaling pathways as is well known in the art.

For example, activation of the canonical Wnt signaling pathway results in the inhibition of phosphorylation of the intracellular protein β -catenin, leading to an accumulation of β -catenin in the cytosol and its subsequent translocation to the nucleus where it interacts with transcription factors, e.g. TCF/LEF, to activate target genes. Activation of the Wnt/PCP pathway activates RhoA, c-Jun N-terminal kinase (JNK), and nemo-like kinase (NLK) signaling cascades to control such biological processes as tissue polarity and cell movement. Activation of the Wnt/Ca²⁺ by, for example, binding of Wnt-4, Wnt-5A or Wnt-11, elicits an intracellular release of calcium ions, which activates calcium sensitive enzymes like protein kinase C (PKC), calcium-calmodulin dependent kinase II (CamKII) or calcineurin (CaCN). By assaying for activity of the above signaling pathways, the biological activity of an antibody or antigen-binding fragment thereof, e.g., a Wnt surrogate molecule, can be readily determined.

In certain embodiments, functional properties of Wnt surrogate molecules may be assessed using a variety of methods known to the skilled person, including e.g., affinity/binding assays (for example, surface plasmon resonance, competitive inhibition assays), cytotoxicity assays, cell viability assays, cell proliferation or differentiation assays in response to a Wnt, cancer cell and/or tumor growth inhibition using in vitro or in vivo models, including but not limited to any described herein. The Wnt surrogate mol-

ecules described herein may also be tested for effects on Fzd receptor internalization, in vitro and in vivo efficacy, etc. Such assays may be performed using well-established protocols known to the skilled person (see e.g., Current Protocols in Molecular Biology (Greene Publ. Assoc. Inc. & John Wiley & Sons, Inc., NY, NY); Current Protocols in Immunology (Edited by: John E. Coligan, Ada M. Kruisbeek, David H. Margulies, Ethan M. Shevach, Warren Strober 2001 John Wiley & Sons, NY, NY); or commercially available kits.

In certain embodiments, a Fzd-binding region of a Wnt surrogate molecule (e.g., an antigen-binding fragment of an anti-Fzd antibody) comprises one or more of the CDRs of the anti-Fzd antibodies described herein. In certain embodiments, a LRP5/6-binding region of a Wnt surrogate molecule (e.g., an antigen-binding fragment of an anti-LRP5/6 antibody) comprises one or more of the CDRs of the anti-LRP5/6 antibodies described herein. In this regard, it has been shown in some cases that the transfer of only the VHCDR3 of an antibody can be performed while still retaining desired specific binding (Barbas et al., *PNAS* (1995) 92: 2529-2533). See also, McLane et al., *PNAS* (1995) 92:5214-5218, Barbas et al., *J. Am. Chem. Soc.* (1994) 116:2161-2162.

Also disclosed herein is a method for obtaining an antibody or antigen binding domain specific for a Fzd receptor, the method comprising providing by way of addition, deletion, substitution or insertion of one or more amino acids in the amino acid sequence of a VH domain set out herein or a VH domain which is an amino acid sequence variant of the VH domain, optionally combining the VH domain thus provided with one or more VL domains, and testing the VH domain or VH/VL combination or combinations to identify a specific binding member or an antibody antigen binding domain specific for one or more Fzd receptor and optionally with one or more desired properties. The VL domains may have an amino acid sequence which is substantially as set out herein. An analogous method may be employed in which one or more sequence variants of a VL domain disclosed herein are combined with one or more VH domains.

In particular embodiments, Wnt surrogate molecules are water soluble. By "water soluble" it is meant a composition that is soluble in aqueous buffers in the absence of detergent, usually soluble at a concentration that provides a biologically effective dose of the polypeptide. Compositions that are water soluble form a substantially homogeneous composition that has a specific activity that is at least about 5% that of the starting material from which it was purified, usually at least about 10%, 20%, or 30% that of the starting material, more usually about 40%, 50%, or 60% that of the starting material, and may be about 50%, about 90% or greater. Wnt surrogate molecules disclosed herein typically form a substantially homogeneous aqueous solution at concentrations of at least 25 μ M and higher, e.g. at least 25 μ M, 40 μ M, or 50 μ M, usually at least 60 μ M, 70 μ M, 80 μ M, or 90 μ M, sometimes as much as 100 μ M, 120 μ M, or 150 μ M. In other words, Wnt surrogate molecules disclosed herein typically form a substantially homogeneous aqueous solution at concentrations of about 0.1 mg/ml, about 0.5 mg/ml, of about 1 mg/ml or more.

An antigen or epitope that "specifically binds" or "preferentially binds" (used interchangeably herein) to an antibody or antigen-binding fragment thereof is a term well understood in the art, and methods to determine such specific or preferential binding are also well known in the art. A molecule, e.g., a Wnt surrogate molecule, is said to exhibit "specific binding" or "preferential binding" if it reacts or

associates more frequently, more rapidly, with greater duration and/or with greater affinity with a particular cell or substance than it does with alternative cells or substances. A molecule or binding region thereof, e.g., a Wnt surrogate molecule or binding region thereof, “specifically binds” or “preferentially binds” to a target antigen, e.g., a Fzd receptor, if it binds with greater affinity, avidity, more readily, and/or with greater duration than it binds to other substances. For example, a Wnt surrogate molecule or binding region thereof that specifically or preferentially binds to the Fzd1 receptor is an antibody that binds to the Fzd1 receptor with greater affinity, avidity, more readily, and/or with greater duration than it binds to other Fzd receptors or non-Fzd proteins. It is also understood by reading this definition that, for example, a Wnt surrogate molecule or binding region thereof that specifically or preferentially binds to a first target may or may not specifically or preferentially bind to a second target. As such, “specific binding” or “preferential binding” does not necessarily require (although it can include) exclusive binding. Generally, but not necessarily, reference to binding means preferential binding.

In some embodiments, any of the one or more Fzd binding region of a Wnt surrogate molecule binds to one, two, three, four, five or more different frizzled proteins, e.g., one or more of human frizzled proteins Fzd1, Fzd2, Fzd3, Fzd4, Fzd5, Fzd6, Fzd7, Fzd8, Fzd9, Fzd10. In some embodiments, any of the Fzd binding regions binds to Fzd1, Fzd2, Fzd5, Fzd7 and Fzd8. In various embodiments, any of the Fzd binding regions binds to: (i) Fzd1, Fzd2, Fzd7 and Fzd9; (ii) Fzd1, Fzd2 and Fzd7; (iii) Fzd5 and Fzd8; (iv) Fzd5, Fzd7 and Fzd8; (v) Fzd1, Fzd4, Fzd5 and Fzd8; (vi) Fzd1, Fzd2, Fzd5, Fzd7 and Fzd8; (vii) Fzd4 and Fzd9; (viii) Fzd9 and Fzd10; (ix) Fzd5, Fzd8 and Fzd10; or (x) Fzd4, Fzd5 and Fzd8; Fzd1, Fzd5, Fzd7 and Fzd8. In some embodiments, the Fzd binding region is selective for one or more Fzd protein of interest, e.g. having a specificity for the one or more desired Fzd protein of at least 10-fold, 25-fold, 50-fold, 100-fold, 200-fold or more relative to other Fzd proteins. In some embodiments, any of the one or more Fzd binding region of a Wnt surrogate molecule is monospecific and binds or specifically binds to only one of Fzd1, Fzd2, Fzd3, Fzd4, Fzd5, Fzd6, Fzd7, Fzd8, Fzd9, or Fzd10.

In some embodiments, any of the one or more LRP5/6 binding region of a Wnt surrogate molecule binds to one or both of LRP5/6. For convenience, the term “LRP5/6” is used to refer collectively to either or both of LRP5 and/or LRP6.

Immunological binding generally refers to the non-covalent interactions of the type which occur between an immunoglobulin molecule and an antigen for which the immunoglobulin is specific, for example by way of illustration and not limitation, as a result of electrostatic, ionic, hydrophilic and/or hydrophobic attractions or repulsion, steric forces, hydrogen bonding, van der Waals forces, and other interactions. The strength, or affinity of immunological binding interactions can be expressed in terms of the dissociation constant (K_d) of the interaction, wherein a smaller K_d represents a greater affinity. Immunological binding properties of selected polypeptides can be quantified using methods well known in the art. One such method entails measuring the rates of antigen-binding site/antigen complex formation and dissociation, wherein those rates depend on the concentrations of the complex partners, the affinity of the interaction, and on geometric parameters that equally influence the rate in both directions. Thus, both the “on rate constant” (K_{on}) and the “off rate constant” (K_{off}) can be determined by calculation of the concentrations and the

actual rates of association and dissociation. The ratio of K_{off}/K_{on} enables cancellation of all parameters not related to affinity, and is thus equal to the dissociation constant K_d . See, generally, Davies et al. (1990) *Annual Rev. Biochem.* 59:439-473.

In certain embodiments, the Wnt surrogate molecules or binding regions thereof described herein have an affinity of less than about 10,000, less than about 1000, less than about 100, less than about 10, less than about 1 or less than about 0.1 nM, and in some embodiments, the antibodies may have even higher affinity for one or more Fzd receptor or LRP5 or LRP6 receptor.

The constant regions of immunoglobulins show less sequence diversity than the variable regions, and are responsible for binding a number of natural proteins to elicit important biochemical events. In humans, there are five different classes of antibodies including IgA (which includes subclasses IgA1 and IgA2), IgD, IgE, IgG (which includes subclasses IgG1, IgG2, IgG3, and IgG4), and IgM. The distinguishing features between these antibody classes are their constant regions, although subtler differences may exist in the V region.

The Fc region of an antibody interacts with a number of Fc receptors and ligands, imparting an array of important functional capabilities referred to as effector functions. For IgG, the Fc region comprises Ig domains CH2 and CH3 and the N-terminal hinge leading into CH2. An important family of Fc receptors for the IgG class are the Fc gamma receptors (FcγRs). These receptors mediate communication between antibodies and the cellular arm of the immune system (Raghavan et al., 1996, *Annu Rev Cell Dev Biol* 12:181-220; Ravetch et al., 2001, *Annu Rev Immunol* 19:275-290). In humans this protein family includes FcγRI (CD64), including isoforms FcγRIa, FcγRIb, and FcγRIc; FcγRII (CD32), including isoforms FcγRIIa (including allotypes H131 and R131), FcγRIIb (including FcγRIIb-1 and FcγRIIb-2), and FcγRIIc; and FcγRIII (CD16), including isoforms FcγRIIIa (including allotypes V158 and F158) and FcγRIIIb (including allotypes FcγRIIIb-NA1 and FcγRIIIb-NA2) (Jefferis et al., 2002, *Immunol Lett* 82:57-65). These receptors typically have an extracellular domain that mediates binding to Fc, a membrane spanning region, and an intracellular domain that may mediate some signaling event within the cell. These receptors are expressed in a variety of immune cells including monocytes, macrophages, neutrophils, dendritic cells, eosinophils, mast cells, platelets, B cells, large granular lymphocytes, Langerhans’ cells, natural killer (NK) cells, and T cells. Formation of the Fc/FcγR complex recruits these effector cells to sites of bound antigen, typically resulting in signaling events within the cells and important subsequent immune responses such as release of inflammation mediators, B cell activation, endocytosis, phagocytosis, and cytotoxic attack.

The ability to mediate cytotoxic and phagocytic effector functions is a potential mechanism by which antibodies destroy targeted cells. The cell-mediated reaction wherein nonspecific cytotoxic cells that express FcγRs recognize bound antibody on a target cell and subsequently cause lysis of the target cell is referred to as antibody dependent cell-mediated cytotoxicity (ADCC) (Raghavan et al., 1996, *Annu Rev Cell Dev Biol* 12:181-220; Ghetie et al., 2000, *Annu Rev Immunol* 18:739-766; Ravetch et al., 2001, *Annu Rev Immunol* 19:275-290). The cell-mediated reaction wherein nonspecific cytotoxic cells that express FcγRs recognize bound antibody on a target cell and subsequently cause phagocytosis of the target cell is referred to as antibody dependent cell-mediated phagocytosis (ADCP).

All FcγRs bind the same region on Fc, at the N-terminal end of the Cg2 (CH2) domain and the preceding hinge. This interaction is well characterized structurally (Sondermann et al., 2001, J Mol Biol 309:737-749), and several structures of the human Fc bound to the extracellular domain of human FcγRIIIb have been solved (pdb accession code 1E4K) (Sondermann et al., 2000, Nature 406:267-273.) (pdb accession codes 1IIS and 1IX) (Radaev et al., 2001, J Biol Chem 276:16469-16477.)

The different IgG subclasses have different affinities for the FcγRs, with IgG1 and IgG3 typically binding substantially better to the receptors than IgG2 and IgG4 (Jefferis et al., 2002, Immunol Lett 82:57-65). All FcγRs bind the same region on IgG Fc, yet with different affinities: the high affinity binder FcγRI has a K_d for IgG1 of 10^{-8} M⁻¹, whereas the low affinity receptors FcγRII and FcγRIII generally bind at 10^{-6} and 10^{-5} respectively. The extracellular domains of FcγRIIIa and FcγRIIIb are 96% identical; however, FcγRIIIb does not have an intracellular signaling domain. Furthermore, whereas FcγRI, FcγRIIa/c, and FcγRIIIa are positive regulators of immune complex-triggered activation, characterized by having an intracellular domain that has an immunoreceptor tyrosine-based activation motif (ITAM), FcγRIIb has an immunoreceptor tyrosine-based inhibition motif (ITIM) and is therefore inhibitory. Thus the former are referred to as activation receptors, and FcγRIIb is referred to as an inhibitory receptor. The receptors also differ in expression pattern and levels on different immune cells. Yet another level of complexity is the existence of a number of FcγR polymorphisms in the human proteome. A particularly relevant polymorphism with clinical significance is V158/F158 FcγRIIIa. Human IgG1 binds with greater affinity to the V158 allotype than to the F158 allotype. This difference in affinity, and presumably its effect on ADCC and/or ADCP, has been shown to be a significant determinant of the efficacy of the anti-CD20 antibody rituximab (Rituxan®, a registered trademark of IDEC Pharmaceuticals Corporation). Subjects with the V158 allotype respond favorably to rituximab treatment; however, subjects with the lower affinity F158 allotype respond poorly (Cartron et al., 2002, Blood 99:754-758). Approximately 10-20% of humans are V158/V158 homozygous, 45% are V158/F158 heterozygous, and 35-45% of humans are F158/F158 homozygous (Lehrbecher et al., 1999, Blood 94:4220-4232; Cartron et al., 2002, Blood 99:754-758). Thus 80-90% of humans are poor responders, that is, they have at least one allele of the F158 FcγRIIIa.

The Fc region is also involved in activation of the complement cascade. In the classical complement pathway, C1 binds with its C1q subunits to Fc fragments of IgG or IgM, which has formed a complex with antigen(s). In certain embodiments of the invention, modifications to the Fc region comprise modifications that alter (either enhance or decrease) the ability of a Fcγ-specific antibody as described herein to activate the complement system (see e.g., U.S. Pat. No. 7,740,847). To assess complement activation, a complement-dependent cytotoxicity (CDC) assay may be performed (See, e.g., Gazzano-Santoro et al., J. Immunol. Methods, 202:163 (1996)).

Thus in certain embodiments, the present invention provides anti-Fcγ antibodies having a modified Fc region with altered functional properties, such as reduced or enhanced CDC, ADCC, or ADCP activity, or enhanced binding affinity for a specific FcγR or increased serum half-life. Other modified Fc regions contemplated herein are described, for example, in issued U.S. Pat. Nos. 7,317,091; 7,657,380; 7,662,925; 6,538,124; 6,528,624; 7,297,775; 7,364,731;

Published U.S. Applications US2009092599; US20080131435; US20080138344; and published International Applications WO2006/105338; WO2004/063351; WO2006/088494; WO2007/024249.

In certain embodiments, Wnt surrogate molecules comprise antibody variable domains with the desired binding specificities fused to immunoglobulin constant domain sequences. In certain embodiments, the fusion is with an Ig heavy chain constant domain, comprising at least part of the hinge, C_H2, and C_H3 regions. In particular embodiments, the first heavy-chain constant region (C_H1) containing the site necessary for light chain bonding, present in at least one of the fusions. DNAs encoding the immunoglobulin heavy chain fusions and, if desired, the immunoglobulin light chain, are inserted into separate expression vectors, and are co-transfected into a suitable host cell. This provides for greater flexibility in adjusting the mutual proportions of the three polypeptide fragments in embodiments when unequal ratios of the three polypeptide chains used in the construction provide the optimum yield of the desired bispecific antibody. It is, however, possible to insert the coding sequences for two or all three polypeptide chains into a single expression vector when the expression of at least two polypeptide chains in equal ratios results in high yields or when the ratios have no significant effect on the yield of the desired chain combination.

Wnt surrogate molecules disclosed herein may also be modified to include an epitope tag or label, e.g., for use in purification or diagnostic applications. There are many linking groups known in the art for making antibody conjugates, including, for example, those disclosed in U.S. Pat. No. 5,208,020 or EP Patent 0 425 235 B1, and Chari et al., Cancer Research 52: 127-131 (1992). The linking groups include disulfide groups, thioether groups, acid labile groups, photolabile groups, peptidase labile groups, or esterase labile groups, as disclosed in the above-identified patents, disulfide and thioether groups being preferred.

In certain embodiments, anti-LRP5/6 antibodies and antigen-binding fragments thereof and/or anti-Fcγ antibodies and antigen-binding fragments thereof present within a Wnt surrogate molecule are monoclonal. In certain embodiments, they are humanized.

The present invention further provides in certain embodiments an isolated nucleic acid encoding a polypeptide present in a Wnt surrogate molecule disclosed herein. Nucleic acids include DNA and RNA. These and related embodiments may include polynucleotides encoding antibody fragments that bind one or more Fcγ receptors and/or LRP5 or LRP6 as described herein. The term "isolated polynucleotide" as used herein shall mean a polynucleotide of genomic, cDNA, or synthetic origin, or some combination thereof, which by virtue of its origin, the isolated polynucleotide: (1) is not associated with all or a portion of a polynucleotide in which the isolated polynucleotide is found in nature; (2) is linked to a polynucleotide to which it is not linked in nature, or (3) does not occur in nature as part of a larger sequence. An isolated polynucleotide may include naturally occurring and/or artificial sequences.

The term "operably linked" means that the components to which the term is applied are in a relationship that allows them to carry out their inherent functions under suitable conditions. For example, a transcription control sequence "operably linked" to a protein coding sequence is ligated thereto so that expression of the protein coding sequence is achieved under conditions compatible with the transcriptional activity of the control sequences.

The term "control sequence" as used herein refers to polynucleotide sequences that can affect expression, processing or intracellular localization of coding sequences to which they are ligated or operably linked. The nature of such control sequences may depend upon the host organism. In particular embodiments, transcription control sequences for prokaryotes may include a promoter, ribosomal binding site, and transcription termination sequence. In other particular embodiments, transcription control sequences for eukaryotes may include promoters comprising one or a plurality of recognition sites for transcription factors, transcription enhancer sequences, transcription termination sequences and polyadenylation sequences. In certain embodiments, "control sequences" can include leader sequences and/or fusion partner sequences.

The term "polynucleotide" as referred to herein means single-stranded or double-stranded nucleic acid polymers. In certain embodiments, the nucleotides comprising the polynucleotide can be ribonucleotides or deoxyribonucleotides or a modified form of either type of nucleotide. Said modifications include base modifications such as bromouridine, ribose modifications such as arabinoside and 2',3'-dideoxyribose and internucleotide linkage modifications such as phosphorothioate, phosphorodithioate, phosphoroselenoate, phosphorodiselenoate, phosphoroanilothioate, phosphoranilate and phosphoramidate. The term "polynucleotide" specifically includes single and double stranded forms of DNA.

The term "naturally occurring nucleotides" includes deoxyribonucleotides and ribonucleotides. The term "modified nucleotides" includes nucleotides with modified or substituted sugar groups and the like. The term "oligonucleotide linkages" includes oligonucleotide linkages such as phosphorothioate, phosphorodithioate, phosphoroselenoate, phosphorodiselenoate, phosphoroanilothioate, phosphoranilate, phosphoramidate, and the like. See, e.g., LaPlanche et al., 1986, Nucl. Acids Res., 14:9081; Stec et al., 1984, J. Am. Chem. Soc., 106:6077; Stein et al., 1988, Nucl. Acids Res., 16:3209; Zon et al., 1991, Anti-Cancer Drug Design, 6:539; Zon et al., 1991, OLIGONUCLEOTIDES AND ANALOGUES: A PRACTICAL APPROACH, pp. 87-108 (F. Eckstein, Ed.), Oxford University Press, Oxford England; Stec et al., U.S. Pat. No. 5,151,510; Uhlmann and Peyman, 1990, Chemical Reviews, 90:543, the disclosures of which are hereby incorporated by reference for any purpose. An oligonucleotide can include a detectable label to enable detection of the oligonucleotide or hybridization thereof.

The term "vector" is used to refer to any molecule (e.g., nucleic acid, plasmid, or virus) used to transfer coding information to a host cell. The term "expression vector" refers to a vector that is suitable for transformation of a host cell and contains nucleic acid sequences that direct and/or control expression of inserted heterologous nucleic acid sequences. Expression includes, but is not limited to, processes such as transcription, translation, and RNA splicing, if introns are present.

As will be understood by those skilled in the art, polynucleotides may include genomic sequences, extra-genomic and plasmid-encoded sequences and smaller engineered gene segments that express, or may be adapted to express, proteins, polypeptides, peptides and the like. Such segments may be naturally isolated, or modified synthetically by the skilled person.

As will be also recognized by the skilled artisan, polynucleotides may be single-stranded (coding or antisense) or double-stranded, and may be DNA (genomic, cDNA or

synthetic) or RNA molecules. RNA molecules may include HnRNA molecules, which contain introns and correspond to a DNA molecule in a one-to-one manner, and mRNA molecules, which do not contain introns. Additional coding or non-coding sequences may, but need not, be present within a polynucleotide according to the present disclosure, and a polynucleotide may, but need not, be linked to other molecules and/or support materials. Polynucleotides may comprise a native sequence or may comprise a sequence that encodes a variant or derivative of such a sequence.

It will be appreciated by those of ordinary skill in the art that, as a result of the degeneracy of the genetic code, there are many nucleotide sequences that encodes an antibody as described herein. Some of these polynucleotides bear minimal sequence identity to the nucleotide sequence of the native or original polynucleotide sequence encoding a polypeptide within a Wnt surrogate molecule. Nonetheless, polynucleotides that vary due to differences in codon usage are expressly contemplated by the present disclosure. In certain embodiments, sequences that have been codon-optimized for mammalian expression are specifically contemplated.

Therefore, in another embodiment of the invention, a mutagenesis approach, such as site-specific mutagenesis, may be employed for the preparation of variants and/or derivatives of the polypeptides described herein. By this approach, specific modifications in a polypeptide sequence can be made through mutagenesis of the underlying polynucleotides that encode them. These techniques provide a straightforward approach to prepare and test sequence variants, for example, incorporating one or more of the foregoing considerations, by introducing one or more nucleotide sequence changes into the polynucleotide.

Site-specific mutagenesis allows the production of mutants through the use of specific oligonucleotide sequences which encode the DNA sequence of the desired mutation, as well as a sufficient number of adjacent nucleotides, to provide a primer sequence of sufficient size and sequence complexity to form a stable duplex on both sides of the deletion junction being traversed. Mutations may be employed in a selected polynucleotide sequence to improve, alter, decrease, modify, or otherwise change the properties of the polynucleotide itself, and/or alter the properties, activity, composition, stability, or primary sequence of the encoded polypeptide.

In certain embodiments, the inventors contemplate the mutagenesis of the polynucleotide sequences that encode a polypeptide present in a Wnt surrogate molecule, to alter one or more properties of the encoded polypeptide, such as the binding affinity, or the function of a particular Fc region, or the affinity of the Fc region for a particular FcγR. The techniques of site-specific mutagenesis are well-known in the art, and are widely used to create variants of both polypeptides and polynucleotides. For example, site-specific mutagenesis is often used to alter a specific portion of a DNA molecule. In such embodiments, a primer comprising typically about 14 to about 25 nucleotides or so in length is employed, with about 5 to about 10 residues on both sides of the junction of the sequence being altered.

As will be appreciated by those of skill in the art, site-specific mutagenesis techniques have often employed a phage vector that exists in both a single stranded and double stranded form. Typical vectors useful in site-directed mutagenesis include vectors such as the M13 phage. These phages are readily commercially available and their use is generally well-known to those skilled in the art. Double-stranded plasmids are also routinely employed in site

directed mutagenesis that eliminates the step of transferring the gene of interest from a plasmid to a phage.

The preparation of sequence variants of the selected peptide-encoding DNA segments using site-directed mutagenesis provides a means of producing potentially useful species and is not meant to be limiting as there are other ways in which sequence variants of peptides and the DNA sequences encoding them may be obtained. For example, recombinant vectors encoding the desired peptide sequence may be treated with mutagenic agents, such as hydroxylamine, to obtain sequence variants. Specific details regarding these methods and protocols are found in the teachings of Maloy et al., 1994; Segal, 1976; Prokop and Bajpai, 1991; Kubly, 1994; and Maniatis et al., 1982, each incorporated herein by reference, for that purpose.

In many embodiments, one or more nucleic acids encoding a polypeptide of a Wnt surrogate molecule are introduced directly into a host cell, and the cell incubated under conditions sufficient to induce expression of the encoded polypeptides. The Wnt surrogate polypeptides of this disclosure may be prepared using standard techniques well known to those of skill in the art in combination with the polypeptide and nucleic acid sequences provided herein. The polypeptide sequences may be used to determine appropriate nucleic acid sequences encoding the particular polypeptide disclosed thereby. The nucleic acid sequence may be optimized to reflect particular codon "preferences" for various expression systems according to standard methods well known to those of skill in the art.

According to certain related embodiments there is provided a recombinant host cell which comprises one or more constructs as described herein, e.g., a vector comprising a nucleic acid encoding a Wnt surrogate molecule or polypeptide thereof; and a method of production of the encoded product, which method comprises expression from encoding nucleic acid therefor. Expression may conveniently be achieved by culturing under appropriate conditions recombinant host cells containing the nucleic acid. Following production by expression, an antibody or antigen-binding fragment thereof, may be isolated and/or purified using any suitable technique, and then used as desired.

Polypeptides, and encoding nucleic acid molecules and vectors, may be isolated and/or purified, e.g. from their natural environment, in substantially pure or homogeneous form, or, in the case of nucleic acid, free or substantially free of nucleic acid or genes of origin other than the sequence encoding a polypeptide with the desired function. Nucleic acid may comprise DNA or RNA and may be wholly or partially synthetic. Reference to a nucleotide sequence as set out herein encompasses a DNA molecule with the specified sequence, and encompasses a RNA molecule with the specified sequence in which U is substituted for T, unless context requires otherwise.

Systems for cloning and expression of a polypeptide in a variety of different host cells are well known. Suitable host cells include bacteria, mammalian cells, yeast and baculovirus systems. Mammalian cell lines available in the art for expression of a heterologous polypeptide include Chinese hamster ovary cells, HeLa cells, baby hamster kidney cells, NSO mouse melanoma cells and many others. A common, preferred bacterial host is *E. coli*.

The expression of polypeptides, e.g., antibodies and antigen-binding fragments thereof, in prokaryotic cells such as *E. coli* is well established in the art. For a review, see for example Pluckthun, A. *Bio/Technology* 9: 545-551 (1991). Expression in eukaryotic cells in culture is also available to those skilled in the art as an option for production of

antibodies or antigen-binding fragments thereof, see recent reviews, for example Ref. M. E. (1993) *Curr. Opinion Biotech.* 4: 573-576; Trill J. J. et al. (1995) *Curr. Opinion Biotech* 6: 553-560.

Suitable vectors can be chosen or constructed, containing appropriate regulatory sequences, including promoter sequences, terminator sequences, polyadenylation sequences, enhancer sequences, marker genes and other sequences as appropriate. Vectors may be plasmids, viral e.g. phage, or phagemid, as appropriate. For further details see, for example, *Molecular Cloning: a Laboratory Manual*: 2nd edition, Sambrook et al., 1989, Cold Spring Harbor Laboratory Press. Many known techniques and protocols for manipulation of nucleic acid, for example in preparation of nucleic acid constructs, mutagenesis, sequencing, introduction of DNA into cells and gene expression, and analysis of proteins, are described in detail in *Current Protocols in Molecular Biology*, Second Edition, Ausubel et al. eds., John Wiley & Sons, 1992, or subsequent updates thereto.

The term "host cell" is used to refer to a cell into which has been introduced, or which is capable of having introduced into it, a nucleic acid sequence encoding one or more of the herein described polypeptides, and which further expresses or is capable of expressing a selected gene of interest, such as a gene encoding any herein described polypeptide. The term includes the progeny of the parent cell, whether or not the progeny are identical in morphology or in genetic make-up to the original parent, so long as the selected gene is present. Accordingly there is also contemplated a method comprising introducing such nucleic acid into a host cell. The introduction may employ any available technique. For eukaryotic cells, suitable techniques may include calcium phosphate transfection, DEAE-Dextran, electroporation, liposome-mediated transfection and transduction using retrovirus or other virus, e.g. vaccinia or, for insect cells, baculovirus. For bacterial cells, suitable techniques may include calcium chloride transformation, electroporation and transfection using bacteriophage. The introduction may be followed by causing or allowing expression from the nucleic acid, e.g. by culturing host cells under conditions for expression of the gene. In one embodiment, the nucleic acid is integrated into the genome (e.g. chromosome) of the host cell. Integration may be promoted by inclusion of sequences which promote recombination with the genome, in accordance with standard techniques.

The present invention also provides, in certain embodiments, a method which comprises using a construct as stated above in an expression system in order to express a particular polypeptide such as a Wnt mimetic molecule as described herein. The term "transduction" is used to refer to the transfer of genes from one bacterium to another, usually by a phage. "Transduction" also refers to the acquisition and transfer of eukaryotic cellular sequences by retroviruses. The term "transfection" is used to refer to the uptake of foreign or exogenous DNA by a cell, and a cell has been "transfected" when the exogenous DNA has been introduced inside the cell membrane. A number of transfection techniques are well known in the art and are disclosed herein. See, e.g., Graham et al., 1973, *Virology* 52:456; Sambrook et al., 2001, *MOLECULAR CLONING, A LABORATORY MANUAL*, Cold Spring Harbor Laboratories; Davis et al., 1986, *BASIC METHODS IN MOLECULAR BIOLOGY*, Elsevier; and Chu et al., 1981, *Gene* 13:197. Such techniques can be used to introduce one or more exogenous DNA moieties into suitable host cells.

The term "transformation" as used herein refers to a change in a cell's genetic characteristics, and a cell has been

transformed when it has been modified to contain a new DNA. For example, a cell is transformed where it is genetically modified from its native state. Following transfection or transduction, the transforming DNA may recombine with that of the cell by physically integrating into a chromosome of the cell, or may be maintained transiently as an episomal element without being replicated, or may replicate independently as a plasmid. A cell is considered to have been stably transformed when the DNA is replicated with the division of the cell. The term “naturally occurring” or “native” when used in connection with biological materials such as nucleic acid molecules, polypeptides, host cells, and the like, refers to materials which are found in nature and are not manipulated by a human. Similarly, “non-naturally occurring” or “non-native” as used herein refers to a material that is not found in nature or that has been structurally modified or synthesized by a human.

The terms “polypeptide” “protein” and “peptide” and “glycoprotein” are used interchangeably and mean a polymer of amino acids not limited to any particular length. The term does not exclude modifications such as myristylation, sulfation, glycosylation, phosphorylation and addition or deletion of signal sequences. The terms “polypeptide” or “protein” means one or more chains of amino acids, wherein each chain comprises amino acids covalently linked by peptide bonds, and wherein said polypeptide or protein can comprise a plurality of chains non-covalently and/or covalently linked together by peptide bonds, having the sequence of native proteins, that is, proteins produced by naturally-occurring and specifically non-recombinant cells, or genetically-engineered or recombinant cells, and comprise molecules having the amino acid sequence of the native protein, or molecules having deletions from, additions to, and/or substitutions of one or more amino acids of the native sequence. The terms “polypeptide” and “protein” specifically encompass Wnt surrogate molecules, Fzd binding regions thereof, LRP5/6 binding regions thereof, antibodies and antigen-binding fragments thereof that bind to a Fzd receptor or a LRP5 or LRP6 receptor disclosed herein, or sequences that have deletions from, additions to, and/or substitutions of one or more amino acid of any of these polypeptides. Thus, a “polypeptide” or a “protein” can comprise one (termed “a monomer”) or a plurality (termed “a multimer”) of amino acid chains.

The term “isolated protein,” “isolated Wnt surrogate molecule or “isolated antibody” referred to herein means that a subject protein, Wnt surrogate molecule, or antibody: (1) is free of at least some other proteins with which it would typically be found in nature; (2) is essentially free of other proteins from the same source, e.g., from the same species, (3) is expressed by a cell from a different species; (4) has been separated from at least about 50 percent of polynucleotides, lipids, carbohydrates, or other materials with which it is associated in nature; (5) is not associated (by covalent or noncovalent interaction) with portions of a protein with which the “isolated protein” is associated in nature; (6) is operably associated (by covalent or noncovalent interaction) with a polypeptide with which it is not associated in nature; or (7) does not occur in nature. Such an isolated protein can be encoded by genomic DNA, cDNA, mRNA or other RNA, or may be of synthetic origin, or any combination thereof. In certain embodiments, an isolated protein may comprise naturally-occurring and/or artificial polypeptide sequences. In certain embodiments, the isolated protein is substantially free from proteins or polypeptides or other contaminants that

are found in its natural environment that would interfere with its use (therapeutic, diagnostic, prophylactic, research or otherwise).

Amino acid sequence modification(s) of any of the polypeptides (e.g., Wnt surrogate molecules or Fzd binding regions or LRP5/6 binding regions thereof) described herein are contemplated. For example, it may be desirable to improve the binding affinity and/or other biological properties of the Wnt surrogate molecule. For example, amino acid sequence variants of a Wnt surrogate molecule may be prepared by introducing appropriate nucleotide changes into a polynucleotide that encodes the antibody, or a chain thereof, or by peptide synthesis. Such modifications include, for example, deletions from, and/or insertions into and/or substitutions of, residues within the amino acid sequences of the antibody. Any combination of deletion, insertion, and substitution may be made to arrive at the final Wnt surrogate molecule, provided that the final construct possesses the desired characteristics (e.g., high affinity binding to one or more Fzd and/or LRP5/6 receptor). The amino acid changes also may alter post-translational processes of the antibody, such as changing the number or position of glycosylation sites. Any of the variations and modifications described above for polypeptides of the present invention may be included in antibodies of the present invention.

The present disclosure provides variants of any of the polypeptides (e.g., Wnt surrogate molecules or Fzd binding regions or LRP5/6 binding regions thereof, or antibodies or antigen-binding fragments thereof) disclosed herein. In certain embodiments, a variant has at least 90%, at least 95%, at least 98%, or at least 99% identity to a polypeptide disclosed herein. In certain embodiments, such variant polypeptides bind to one or more Fzd receptor, and/or to one or more LRP5/6 receptor, at least about 50%, at least about 70%, and in certain embodiments, at least about 90% as well as a Wnt surrogate molecule specifically set forth herein. In further embodiments, such variant Wnt surrogate molecules bind to one or more Fzd receptor, and/or to one or more LRP5/6 receptor, with greater affinity than the Wnt surrogate molecules set forth herein, for example, that bind quantitatively at least about 105%, 106%, 107%, 108%, 109%, or 110% as well as an antibody sequence specifically set forth herein.

In particular embodiments, the Wnt surrogate molecule or a binding region thereof, e.g., a Fab, scFv, or VHH or sdAb may comprise: a) a heavy chain variable region comprising: i. a CDR1 region that is identical in amino acid sequence to the heavy chain CDR1 region of a selected antibody described herein; ii. a CDR2 region that is identical in amino acid sequence to the heavy chain CDR2 region of the selected antibody; and iii. a CDR3 region that is identical in amino acid sequence to the heavy chain CDR3 region of the selected antibody; and/or b) a light chain variable domain comprising: i. a CDR1 region that is identical in amino acid sequence to the light chain CDR1 region of the selected antibody; ii. a CDR2 region that is identical in amino acid sequence to the light chain CDR2 region of the selected antibody; and iii. a CDR3 region that is identical in amino acid sequence to the light chain CDR3 region of the selected antibody; wherein the antibody specifically binds a selected target (e.g., one or more Fzd receptors or LRP5 or LRP6 receptors). In a further embodiment, the antibody, or antigen-binding fragment thereof, is a variant antibody or antigen-binding fragment thereof wherein the variant comprises a heavy and light chain identical to the selected antibody except for up to 8, 9, 10, 11, 12, 13, 14, 15, or more amino acid substitutions in the CDR regions of the VH and VL

regions. In this regard, there may be 1, 2, 3, 4, 5, 6, 7, 8, or in certain embodiments, 9, 10, 11, 12, 13, 14, 15 more amino acid substitutions in the CDR regions of the selected antibody. Substitutions may be in CDRs either in the VH and/or the VL regions. (See e.g., Muller, 1998, Structure 6:1153-1167).

In particular embodiments, the Wnt surrogate molecule or a binding region thereof, e.g., a Fab, scFv, or VHH or sdAb may have: a) a heavy chain variable region having an amino acid sequence that is at least 80% identical, at least 95% identical, at least 90%, at least 95% or at least 98% or 99% identical, to the heavy chain variable region of an antibody or antigen-binding fragments thereof described herein; and/or b) a light chain variable region having an amino acid sequence that is at least 80% identical, at least 85%, at least 90%, at least 95% or at least 98% or 99% identical, to the light chain variable region of an antibody or antigen-binding fragments thereof described herein. The amino acid sequence of illustrative antigen-binding fragments thereof are set forth in SEQ ID NOs:1-128.

A polypeptide has a certain percent "sequence identity" to another polypeptide, meaning that, when aligned, that percentage of amino acids are the same when comparing the two sequences. Sequence similarity can be determined in a number of different manners. To determine sequence identity, sequences can be aligned using the methods and computer programs, including BLAST, available over the world wide web at ncbi.nlm.nih.gov/BLAST/. Another alignment algorithm is FASTA, available in the Genetics Computing Group (GCG) package, from Madison, Wis., USA, a wholly owned subsidiary of Oxford Molecular Group, Inc. Other techniques for alignment are described in *Methods in Enzymology*, vol. 266: Computer Methods for Macromolecular Sequence Analysis (1996), ed. Doolittle, Academic Press, Inc., a division of Harcourt Brace & Co., San Diego, Calif., USA. Of particular interest are alignment programs that permit gaps in the sequence. The Smith-Waterman is one type of algorithm that permits gaps in sequence alignments. See *Meth. Mol. Biol.* 70: 173-187 (1997). Also, the GAP program using the Needleman and Wunsch alignment method can be utilized to align sequences. See *J. Mol. Biol.* 48: 443-453 (1970).

Of interest is the BestFit program using the local homology algorithm of Smith and Waterman (*Advances in Applied Mathematics* 2: 482-489 (1981)) to determine sequence identity. The gap generation penalty will generally range from 1 to 5, usually 2 to 4 and in many embodiments will be 3. The gap extension penalty will generally range from about 0.01 to 0.20 and in many instances will be 0.10. The program has default parameters determined by the sequences inputted to be compared. Preferably, the sequence identity is determined using the default parameters determined by the program. This program is available also from Genetics Computing Group (GCG) package, from Madison, Wis., USA.

Another program of interest is the FastDB algorithm. FastDB is described in *Current Methods in Sequence Comparison and Analysis, Macromolecule Sequencing and Synthesis, Selected Methods and Applications*, pp. 127-149, 1988, Alan R. Liss, Inc. Percent sequence identity is calculated by FastDB based upon the following parameters: Mismatch Penalty: 1.00; Gap Penalty: 1.00; Gap Size Penalty: 0.33; and Joining Penalty: 30.0.

In particular embodiments, the Wnt surrogate molecule or a binding region thereof, e.g., a Fab, scFv, or VHH or sdAb may comprise: a) a heavy chain variable region comprising: i. a CDR1 region that is identical in amino acid sequence to the heavy chain CDR1 region of a selected antibody

described herein; ii. a CDR2 region that is identical in amino acid sequence to the heavy chain CDR2 region of the selected antibody; and iii. a CDR3 region that is identical in amino acid sequence to the heavy chain CDR3 region of the selected antibody; and b) a light chain variable domain comprising: i. a CDR1 region that is identical in amino acid sequence to the light chain CDR1 region of the selected antibody; ii. a CDR2 region that is identical in amino acid sequence to the light chain CDR2 region of the selected antibody; and iii. a CDR3 region that is identical in amino acid sequence to the light chain CDR3 region of the selected antibody; wherein the antibody specifically binds a selected target (e.g., a Fzd receptor, such as Fzd1). In a further embodiment, the antibody, or antigen-binding fragment thereof, is a variant antibody wherein the variant comprises a heavy and light chain identical to the selected antibody except for up to 8, 9, 10, 11, 12, 13, 14, 15, or more amino acid substitutions in the CDR regions of the VH and VL regions. In this regard, there may be 1, 2, 3, 4, 5, 6, 7, 8, or in certain embodiments, 9, 10, 11, 12, 13, 14, 15 more amino acid substitutions in the CDR regions of the selected antibody. Substitutions may be in CDRs either in the VH and/or the VL regions. (See e.g., Muller, 1998, Structure 6:1153-1167).

Determination of the three-dimensional structures of representative polypeptides (e.g., variant Fzd binding regions or LRP5/6 binding regions of Wnt surrogate molecules as provided herein) may be made through routine methodologies such that substitution, addition, deletion or insertion of one or more amino acids with selected natural or non-natural amino acids can be virtually modeled for purposes of determining whether a so derived structural variant retains the space-filling properties of presently disclosed species. See, for instance, Donate et al., 1994 *Prot. Sci.* 3:2378; Bradley et al., *Science* 309: 1868-1871 (2005); Schueler-Furman et al., *Science* 310:638 (2005); Dietz et al., *Proc. Nat. Acad. Sci. USA* 103:1244 (2006); Dodson et al., *Nature* 450:176 (2007); Qian et al., *Nature* 450:259 (2007); Raman et al. *Science* 327:1014-1018 (2010). Some additional non-limiting examples of computer algorithms that may be used for these and related embodiments, such as for rational design of binding regions include VMD which is a molecular visualization program for displaying, animating, and analyzing large biomolecular systems using 3-D graphics and built-in scripting (see the website for the Theoretical and Computational Biophysics Group, University of Illinois at Urbana-Champaign, at ks.uiuc.edu/Research/vmd/). Many other computer programs are known in the art and available to the skilled person and which allow for determining atomic dimensions from space-filling models (van der Waals radii) of energy-minimized conformations; GRID, which seeks to determine regions of high affinity for different chemical groups, thereby enhancing binding, Monte Carlo searches, which calculate mathematical alignment, and CHARMM (Brooks et al. (1983) *J. Comput. Chem.* 4:187-217) and AMBER (Weiner et al (1981) *J. Comput. Chem.* 106: 765), which assess force field calculations, and analysis (see also, Eisenfield et al. (1991) *Am. J. Physiol.* 261:C376-386; Lybrand (1991) *J. Pharm. Belg.* 46:49-54; Froimowitz (1990) *Biotechniques* 8:640-644; Burbam et al. (1990) *Proteins* 7:99-111; Pedersen (1985) *Environ. Health Perspect.* 61:185-190; and Kini et al. (1991) *J. Biomol. Struct. Dyn.* 9:475-488). A variety of appropriate computational computer programs are also commercially available, such as from Schrödinger (Munich, Germany).

Compositions

Pharmaceutical compositions comprising a Wnt surrogate molecule described herein and one or more pharmaceutically acceptable diluent, carrier, or excipient are also disclosed. In particular embodiments, the pharmaceutical composition further comprises one or more Wnt polypeptides or Norrin polypeptides.

In further embodiments, pharmaceutical compositions comprising a polynucleotide comprising a nucleic acid sequence encoding a Wnt surrogate molecule described herein and one or more pharmaceutically acceptable diluent, carrier, or excipient are also disclosed. In particular embodiments, the pharmaceutical composition further comprises one or more polynucleotides comprising a nucleic acid sequence encoding a Wnt polypeptide or Norrin polypeptide. In certain embodiments, the polynucleotides are DNA or mRNA, e.g., a modified mRNA. In particular embodiments, the polynucleotides are modified mRNAs further comprising a 5' cap sequence and/or a 3' tailing sequence, e.g., a polyA tail. In other embodiments, the polynucleotides are expression cassettes comprising a promoter operatively linked to the coding sequences. In certain embodiments, the nucleic acid sequence encoding the Wnt surrogate molecule and the nucleic acid sequence encoding the Wnt polypeptide or Norrin polypeptide are present in the same polynucleotide.

In further embodiments, pharmaceutical compositions comprising an expression vector, e.g., a viral vector, comprising a polynucleotide comprising a nucleic acid sequence encoding a Wnt surrogate molecule described herein and one or more pharmaceutically acceptable diluent, carrier, or excipient are also disclosed. In particular embodiments, the pharmaceutical composition further comprises an expression vector, e.g., a viral vector, comprising a polynucleotide comprising a nucleic acid sequence encoding a Wnt polypeptide or Norrin polypeptide. In certain embodiments, the nucleic acid sequence encoding the Wnt surrogate molecule and the nucleic acid sequence encoding the Wnt polypeptide or Norrin polypeptide are present in the same polynucleotide, e.g., expression cassette.

The present invention further contemplates a pharmaceutical composition comprising a cell comprising an expression vector comprising a polynucleotide comprising a promoter operatively linked to a nucleic acid encoding a Wnt surrogate molecule and one or more pharmaceutically acceptable diluent, carrier, or excipient. In particular embodiments, the pharmaceutical composition further comprises a cell comprising an expression vector comprising a polynucleotide comprising a promoter operatively linked to a nucleic acid sequence encoding a Wnt polypeptide or a Norrin polypeptide. In certain embodiments, the nucleic acid sequence encoding the Wnt surrogate molecule and the nucleic acid sequence encoding the Wnt polypeptide or Norrin polypeptide are present in the same polynucleotide, e.g., expression cassette and/or in the same cell. In particular embodiments, the cell is a heterologous cell or an autologous cell obtained from the subject to be treated. In particular embodiments, the cell is a stem cell, e.g., an adipose-derived stem cell or a hematopoietic stem cell.

The present disclosure contemplates pharmaceutical compositions comprising a first molecule for delivery of a Wnt surrogate molecule as a first active agent and a second molecule for delivery of a Wnt polypeptide or Norrin polypeptide. The first and second molecule may be the same type of molecule or different types of molecules. For example, in certain embodiments, the first and second molecule may each be independently selected from the follow-

ing types of molecules: polypeptides, small organic molecules, nucleic acids encoding the first or second active agent (optionally DNA or mRNA, optionally modified RNA), vectors comprising a nucleic acid sequence encoding the first or second active agent (optionally expression vectors or viral vectors), and cells comprising a nucleic acid sequence encoding the first or second active agent (optionally an expression cassette).

The subject molecules, alone or in combination, can be combined with pharmaceutically-acceptable carriers, diluents, excipients and reagents useful in preparing a formulation that is generally safe, non-toxic, and desirable, and includes excipients that are acceptable for mammalian, e.g., human or primate, use. Such excipients can be solid, liquid, semisolid, or, in the case of an aerosol composition, gaseous. Examples of such carriers, diluents and excipients include, but are not limited to, water, saline, Ringer's solutions, dextrose solution, and 5% human serum albumin. Supplementary active compounds can also be incorporated into the formulations. Solutions or suspensions used for the formulations can include a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial compounds such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating compounds such as ethylenediaminetetraacetic acid (EDTA); buffers such as acetates, citrates or phosphates; detergents such as Tween 20 to prevent aggregation; and compounds for the adjustment of tonicity such as sodium chloride or dextrose. The pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. In particular embodiments, the pharmaceutical compositions are sterile.

Pharmaceutical compositions may further include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, or phosphate buffered saline (PBS). In some cases, the composition is sterile and should be fluid such that it can be drawn into a syringe or delivered to a subject from a syringe. In certain embodiments, it is stable under the conditions of manufacture and storage and is preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be, e.g., a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, sodium chloride in the composition. Prolonged absorption of the internal compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate and gelatin.

Sterile solutions can be prepared by incorporating the anti-Fzd antibody or antigen-binding fragment thereof (or encoding polynucleotide or cell comprising the same) in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are

prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, methods of preparation are vacuum drying and freeze-drying that yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

In one embodiment, the pharmaceutical compositions are prepared with carriers that will protect the antibody or antigen-binding fragment thereof against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. The materials can also be obtained commercially. Liposomal suspensions can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art.

It may be advantageous to formulate the pharmaceutical compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subject to be treated; each unit containing a predetermined quantity of active antibody or antigen-binding fragment thereof calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms are dictated by and directly dependent on the unique characteristics of the antibody or antigen-binding fragment thereof and the particular therapeutic effect to be achieved, and the limitations inherent in the art of compounding such an active antibody or antigen-binding fragment thereof for the treatment of individuals.

The pharmaceutical compositions can be included in a container, pack, or dispenser, e.g. syringe, e.g. a prefilled syringe, together with instructions for administration.

The pharmaceutical compositions of the invention encompass any pharmaceutically acceptable salts, esters, or salts of such esters, or any other compound which, upon administration to an animal comprising a human, is capable of providing (directly or indirectly) the biologically active antibody or antigen-binding fragment thereof.

The present invention includes pharmaceutically acceptable salts of a Wnt surrogate molecule described herein. The term "pharmaceutically acceptable salt" refers to physiologically and pharmaceutically acceptable salts of the compounds of the invention: i.e., salts that retain the desired biological activity of the parent compound and do not impart undesired toxicological effects thereto. A variety of pharmaceutically acceptable salts are known in the art and described, e.g., in "Remington's Pharmaceutical Sciences", 17th edition, Alfonso R. Gennaro (Ed.), Mark Publishing Company, Easton, Pa., USA, 1985 (and more recent editions thereof), in the "Encyclopaedia of Pharmaceutical Technology", 3rd edition, James Swarbrick (Ed.), Informa Healthcare USA (Inc.), NY, USA, 2007, and in J. Pharm. Sci. 66: 2 (1977). Also, for a review on suitable salts, see "Handbook of Pharmaceutical Salts: Properties, Selection, and Use" by Stahl and Wermuth (Wiley-VCH, 2002).

Pharmaceutically acceptable base addition salts are formed with metals or amines, such as alkali and alkaline earth metals or organic amines. Metals used as cations comprise sodium, potassium, magnesium, calcium, and the like. Amines comprise N—N'-dibenzylethylenediamine,

chloroprocaine, choline, diethanolamine, dicyclohexylamine, ethylenediamine, N-methylglucamine, and procaine (see, for example, Berge et al., "Pharmaceutical Salts," J. Pharma Sci., 1977, 66, 119). The base addition salts of said acidic compounds are prepared by contacting the free acid form with a sufficient amount of the desired base to produce the salt in the conventional manner. The free acid form may be regenerated by contacting the salt form with an acid and isolating the free acid in the conventional manner. The free acid forms differ from their respective salt forms somewhat in certain physical properties such as solubility in polar solvents, but otherwise the salts are equivalent to their respective free acid for purposes of the present invention.

In some embodiments, the pharmaceutical composition provided herein comprise a therapeutically effective amount of a Wnt surrogate molecule or pharmaceutically acceptable salt thereof in admixture with a pharmaceutically acceptable carrier, diluent and/or excipient, for example saline, phosphate buffered saline, phosphate and amino acids, polymers, polyols, sugar, buffers, preservatives and other proteins. Exemplary amino acids, polymers and sugars and the like are octylphenoxy polyethoxy ethanol compounds, polyethylene glycol monostearate compounds, polyoxyethylene sorbitan fatty acid esters, sucrose, fructose, dextrose, maltose, glucose, mannitol, dextran, sorbitol, inositol, galactitol, xylitol, lactose, trehalose, bovine or human serum albumin, citrate, acetate, Ringer's and Hank's solutions, cysteine, arginine, camitine, alanine, glycine, lysine, valine, leucine, polyvinylpyrrolidone, polyethylene and glycol. Preferably, this formulation is stable for at least six months at 4° C.

In some embodiments, the pharmaceutical composition provided herein comprises a buffer, such as phosphate buffered saline (PBS) or sodium phosphate/sodium sulfate, tris buffer, glycine buffer, sterile water and other buffers known to the ordinarily skilled artisan such as those described by Good et al. (1966) Biochemistry 5:467. The pH of the buffer may be in the range of 6.5 to 7.75, preferably 7 to 7.5, and most preferably 7.2 to 7.4.

Methods of Use

The present disclosure also provides methods for using the Wnt surrogate molecules disclosed herein, e.g., to modulate a Wnt signaling pathway, e.g., to increase Wnt signaling, and the administration of a Wnt surrogate molecule disclosed herein in a variety of therapeutic settings. Provided herein are methods of treatment using a Wnt surrogate molecule. In one embodiment, a Wnt surrogate molecule is provided to a subject having a disease involving inappropriate or deregulated Wnt signaling, e.g., reduced Wnt signaling.

Increasing Wnt Pathway Signaling and Related Therapeutic Methods

In certain embodiments, a Wnt surrogate molecule may be used to increase Wnt signaling in a tissue or cell. Thus, in some aspects, the present invention provides a method for increasing Wnt signaling or enhancing Wnt signaling in a tissue or cell, comprising contacting the tissue or cell with an effective amount of a Wnt surrogate molecule or pharmaceutically acceptable salt thereof disclosed herein, wherein the a Wnt surrogate molecule is a Wnt signaling pathway agonist. In some embodiments, contacting occurs in vitro, ex vivo, or in vivo. In particular embodiments, the cell is a cultured cell, and the contacting occurs in vitro. In certain embodiments, the method comprises further contacting the tissue or cell with one or more Wnt polypeptides or Norrin polypeptides.

In related aspects, the present invention provides a method for increasing Wnt signaling in a tissue or cell,

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comprising contacting the tissue or cell with an effective amount of a polynucleotide comprising a Wnt surrogate molecule disclosed herein. In certain embodiments, the target tissue or cell is also contacted with a polynucleotide comprising a nucleic acid sequence that encodes a Wnt polypeptide or a Norrin polypeptide. In certain embodiments, the polynucleotides are DNA or mRNA, e.g., a modified mRNA. In particular embodiments, the polynucleotides are modified mRNAs further comprising a 5' cap sequence and/or a 3' tailing sequence, e.g., a polyA tail. In other embodiments, the polynucleotides are expression cassettes comprising a promoter operatively linked to the coding sequences. In certain embodiments, the nucleic acid sequence encoding the Wnt surrogate molecule and the nucleic acid sequence encoding the Wnt polypeptide or Norrin polypeptide are present in the same polynucleotide.

In related aspects, the present invention provides a method for increasing Wnt signaling in a tissue or cell, comprising contacting the tissue or cell with an effective amount of a vector comprising a nucleic acid sequence encoding a Wnt surrogate molecule. In certain embodiments, the tissue or cell is also contacted with a vector comprising a nucleic acid sequence that encodes a Wnt polypeptide or a Norrin polypeptide. In certain embodiments, the vector is an expression vector, and may comprise a promoter operatively linked to the nucleic acid sequence. In particular embodiments, the vector is a viral vector. In certain embodiments, the nucleic acid sequence encoding a Wnt surrogate molecule and the nucleic acid sequence encoding the Wnt polypeptide or Norrin polypeptide are present in the same vector, e.g., in the same expression cassette.

In related aspects, the present invention provides a method for increasing Wnt signaling in a tissue, comprising contacting the tissue with an effective amount of a cell comprising a nucleic acid sequence encoding a Wnt surrogate molecule of the present invention. In certain embodiments, the tissue is also contacted with a cell comprising a nucleic acid sequence that encodes a Wnt polypeptide or Norrin polypeptide. In certain embodiments, the nucleic acid sequence encoding the Wnt surrogate molecule and the nucleic acid sequence encoding the Wnt polypeptide or Norrin polypeptide are present in the same cell. In particular embodiments, the cell is a heterologous cell or an autologous cell obtained from the subject to be treated. In certain embodiments, the cell was transduced with a vector comprising an expression cassette encoding the Wnt surrogate molecule or the Wnt polypeptide or Norrin polypeptide. In particular embodiments, the cell is a stem cell, e.g., an adipose-derived stem cell or a hematopoietic stem cell.

Wnt surrogate molecules disclosed herein may be used in to treat a disease, disorder or condition, for example, by increasing Wnt signaling in a targeted cell, tissue or organ. Thus, in some aspects, the present invention provides a method for treating a disease or condition in a subject in need thereof, e.g., a disease or disorder associated with reduced Wnt signaling, or for which increased Wnt signaling would provide a therapeutic benefit, comprising contacting the subject with an effective amount of a composition of the present disclosure. In particular embodiments, the composition is a pharmaceutical composition comprising any of: a Wnt surrogate molecule; a polynucleotide comprising a nucleic acid sequence encoding a Wnt surrogate molecule, e.g., a DNA or mRNA, optionally a modified mRNA; a vector comprising a nucleic acid sequence encoding a Wnt surrogate molecule, e.g., an expression vector or viral vector; or a cell comprising a nucleic acid sequence encoding a

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Wnt surrogate molecule, e.g., a cell transduced with an expression vector or viral vector encoding a Wnt surrogate molecule. In particular embodiments, the disease or condition is a pathological disease or disorder, or an injury, e.g., an injury resulting from a wound. In certain embodiments, the wound may be the result of another therapeutic treatment. In certain embodiments, the disease or condition comprises impaired tissue repair, healing or regeneration, or would benefit from increased tissue repair, healing or regeneration. In some embodiments, contacting occurs *in vivo*, i.e., the subject composition is administered to a subject.

In certain embodiments, the method comprises further contacting the subject with a pharmaceutical composition comprising one or more Wnt polypeptides or Norrin polypeptides. The present disclosure contemplates contacting a subject with a first molecule for delivery of a Wnt surrogate molecule as a first active agent and a second molecule for delivery of a Wnt polypeptide or Norrin polypeptide. The first and second molecule may be the same type of molecule or different types of molecules. For example, in certain embodiments, the first and second molecule may each be independently selected from the following types of molecules: polypeptides, small organic molecules, nucleic acids encoding the first or second active agent (optionally DNA or mRNA, optionally modified RNA), vectors comprising a nucleic acid sequence encoding the first or second active agent (optionally expression vectors or viral vectors), and cells comprising a nucleic acid sequence encoding the first or second active agent (optionally an expression cassette).

In related aspects, the present invention provides a method for treating a disease or condition, e.g., a disease or disorder associated with reduced Wnt signaling, or for which increased Wnt signaling would provide a therapeutic benefit, comprising contacting a subject in need thereof with a pharmaceutical composition comprising an effective amount of a polynucleotide comprising a nucleic acid sequence encoding a Wnt surrogate molecule disclosed herein. In certain embodiments, the subject is also contacted with a pharmaceutical composition comprising an effective amount of a polynucleotide comprising a nucleic acid sequence that encodes a Wnt polypeptide or a Norrin polypeptide. In certain embodiments, the polynucleotides are DNA or mRNA, e.g., a modified mRNA. In particular embodiments, the polynucleotides are modified mRNAs further comprising a 5' cap sequence and/or a 3' tailing sequence, e.g., a polyA tail. In other embodiments, the polynucleotides are expression cassettes comprising a promoter operatively linked to the coding sequences. In certain embodiments, the nucleic acid sequence encoding the Wnt surrogate molecule and the nucleic acid sequence encoding the Wnt polypeptide or Norrin polypeptide are present in the same polynucleotide.

In related aspects, the present invention provides a method for treating a disease or condition, e.g., a disease or disorder associated with reduced Wnt signaling, or for which increased Wnt signaling would provide a therapeutic benefit, comprising contacting a subject in need thereof with a pharmaceutical composition comprising an effective amount of a vector comprising a nucleic acid sequence encoding a Wnt surrogate molecule. In certain embodiments, the subject is also contacted with a pharmaceutical composition comprising an effective amount of a vector comprising a nucleic acid sequence that encodes a Wnt polypeptide or a Norrin polypeptide. In certain embodiments, the vector is an expression vector, and may comprise a promoter operatively linked to the nucleic acid sequence. In particular embodiments, the vector is a viral vector. In certain embodiments,

the nucleic acid sequence encoding the Wnt surrogate molecule and the nucleic acid sequence encoding the Wnt polypeptide or Norrin polypeptide are present in the same vector, e.g., in the same expression cassette.

In related aspects, the present invention provides a method for treating a disease or condition, e.g., a disease or disorder associated with reduced Wnt signaling, or for which increased Wnt signaling would provide a therapeutic benefit, comprising contacting a subject in need thereof with a pharmaceutical composition comprising an effective amount of a cell comprising a nucleic acid sequence encoding a Wnt surrogate molecule. In certain embodiments, the subject is also contacted with a cell comprising a nucleic acid sequence that encodes a Wnt polypeptide or a Norrin polypeptide. In certain embodiments, the nucleic acid sequence encoding the Wnt surrogate molecule and the nucleic acid sequence encoding the Wnt polypeptide or Norrin polypeptide are present in the same cell. In particular embodiments, the cell is a heterologous cell or an autologous cell obtained from the subject to be treated. In certain embodiments, the cell was transduced with a vector comprising an expression cassette encoding the Wnt surrogate molecule or the Wnt polypeptide or Norrin polypeptide. In particular embodiments, the cell is a stem cell, e.g., an adipose-derived stem cell or a hematopoietic stem cell.

Wnt signaling plays key roles in the developmental process and maintenance of stem cells. Reactivation of Wnt signals is associated with regeneration and repair of most tissues after injuries and diseases. Wnt surrogate molecule molecules are expected to provide benefit of healing and tissue repair in response to injuries and diseases. Causes of tissue damage and loss include but are not limited to aging, degeneration, hereditary conditions, infection and inflammation, traumatic injuries, toxins/metabolic-induced toxicities, or other pathological conditions. Wnt signals and enhancers of Wnt signals have been shown to activate adult, tissue-resident stem cells. In some embodiments, the compounds of the invention are administered for use in treating diseased or damaged tissue, for use in tissue regeneration and for use in cell growth and proliferation, and/or for use in tissue engineering.

Human diseases associated with mutations of the Wnt pathway provide strong evidence for enhancement of Wnt signals in the treatment and prevention of diseases. Preclinical in vivo and in vitro studies provide additional evidence of involvement of Wnt signals in many disease conditions and further support utilization of a Wnt surrogate molecule in various human diseases. For example, compositions of the present invention may be used to promote or increase bone growth or regeneration, bone grafting, healing of bone fractures, treatment of osteoporosis and osteoporotic fractures, spinal fusion, spinal cord injuries, including vertebral compression fractures, pre-operative spinal surgery optimization, osseointegration of orthopedic devices, tendon-bone integration, tooth growth and regeneration, dental implantation, periodontal diseases, maxillofacial reconstruction, and osteonecrosis of the jaw. They may also be used in the treatment of alopecia; enhancing regeneration of sensory organs, e.g. treatment of hearing loss, including regeneration of inner and outer auditory hair cells treatment of vestibular hypofunction, treatment of macular degeneration, treatment of retinopathies, including vitreoretinopathy, diabetic retinopathy, other diseases of retinal degeneration, Fuchs' dystrophy, other cornea disease, etc.; treatment of stroke, traumatic brain injury, Alzheimer's disease, multiple sclerosis, multiple dystrophy, muscle atrophy as a result of sarcopenia or cachexia, and other conditions affecting the

degeneration or integrity of the blood brain barrier; The compositions of this invention may also be used in treatment of oral mucositis, treatment of short bowel syndrome, inflammatory bowel diseases (IBD), including Crohn's disease (CD) and ulcerative colitis (UC), in particular CD with fistula formation, other gastrointestinal disorders; treatment of metabolic syndrome, dyslipidemia, treatment of diabetes, treatment of pancreatitis, conditions where exocrine or endocrine pancreas tissues are damaged; conditions where enhanced epidermal regeneration is desired, e.g., epidermal wound healing, treatment of diabetic foot ulcers, syndromes involving tooth, nail, or dermal hypoplasia, etc., conditions where angiogenesis is beneficial; treatment of myocardial infarction, coronary artery disease, heart failure; enhanced growth of hematopoietic cells, e.g. enhancement of hematopoietic stem cell transplants from bone marrow, mobilized peripheral blood, treatment of immunodeficiencies, graft versus host diseases, etc.; treatment of acute kidney injuries, chronic kidney diseases; treatment of lung diseases, chronic obstructive pulmonary diseases (COPD), pulmonary fibrosis, including idiopathic pulmonary fibrosis, enhanced regeneration of lung tissues. The compositions of the present invention may also be used in enhanced regeneration of liver cells, e.g. liver regeneration, treatment of cirrhosis, enhancement of liver transplantations, treatment of acute liver failure, treatment of chronic liver diseases with hepatitis C or B virus infection or post-antiviral drug therapies, alcoholic liver diseases, including alcoholic hepatitis, non-alcoholic liver diseases with steatosis or steatohepatitis, and the like. The compositions of this invention may treat diseases and disorders including, without limitation, conditions in which regenerative cell growth is desired.

Human genetics involving loss-of-function or gain-of-function mutations in Wnt signaling components show strong evidence supporting enhancing Wnt signals for bone growth. Conditions in which enhanced bone growth is desired may include, without limitation, fractures, grafts, ingrowth around prosthetic devices, osteoporosis, osteoporotic fractures, spinal fusion, vertebral compression fractures, pre-operative optimization for spinal surgeries, osteonecrosis of the jaw, dental implantation, periodontal diseases, maxillofacial reconstruction, and the like. Wnt surrogate molecules enhance and promotes Wnt signals which are critical in promoting bone regeneration. Methods for regeneration of bone tissues benefit from administration of the compounds of the invention, which can be systemic or localized. In some embodiments, bone marrow cells are exposed to molecules of the invention, such that stem cells within that marrow become activated.

In some embodiments, bone regeneration is enhanced by contacting a responsive cell population, e.g. bone marrow, bone progenitor cells, bone stem cells, etc. with an effective dose of a Wnt surrogate molecule disclosed herein. Methods for regeneration of bone tissues benefit from administration of the Wnt surrogate molecule which can be systemic or localized. In some such embodiments, the contacting is performed in vivo. In other such embodiments, the contacting is performed ex vivo. The molecule may be localized to the site of action, e.g. by loading onto a matrix, which is optionally biodegradable, and optionally provides for a sustained release of the active agent. Matrix carriers include, without limitation, absorbable collagen sponges, ceramics, hydrogels, polymeric microspheres, nanoparticles, bone cements, and the like.

In particular embodiments, compositions comprising one or more Wnt surrogate molecule disclosed herein (or a polynucleotide encoding a Wnt surrogate molecule, or a

vector or cell comprising a polynucleotide encoding a Wnt surrogate molecule) are used to treat or prevent a bone disease or disorder, including but not limited to any of the following, or to treat or prevent an injury associated with, but not limited to, any of the following: osteoporosis, osteoporotic fractures, bone fractures including vertebral compression fractures, non-union fractures, delayed union fractures, spinal fusion, osteonecrosis, osteonecrosis of the jaw, hip, femoral head, etc., osseointegration of implants (e.g., to accelerate recovery following partial or total knee or hip replacement), osteogenesis imperfecta, bone grafts, tendon repair, maxillofacial surgery, dental implant, all other bone disorders or defects resulting from genetic diseases, degeneration, aging, drugs, or injuries. In one embodiment, Wnt surrogate molecules that bind Fzd1, Fzd 2, and Fzd 7, and also LRP5 and/or LRP6, are used to treat or prevent any bone disease or disorder. In one embodiment, Wnt surrogate molecules that bind Fzd1, Fzd 2, Fzd 5, Fzd 7 and Fzd 8, and also LRP5 and/or LRP6, are used to treat or prevent any bone disease or disorder. Other Fzd molecules that bind to additional Fzd receptors can also be used with LRP5 and/or LRP6 binders.

In particular embodiments, compositions and methods disclosed herein may be used to: increase bone mineral density, increase bone volume (e.g., tibia and/or femur bone volume), increase cortical thickness (e.g., in trabecular region or in femur mid-diaphysis), increase mineral apposition rate, increase the number of osteoblasts and/or decrease the number of osteoclasts (e.g., in bone), increase bone stiffness, increase the ultimate load to fracture point, improve bone resistance to fracture, decrease bone resorption, decrease bone loss associated with osteoporosis, or increase biochemical strength of bone, in a subject. In one embodiment, Wnt surrogate molecules that bind Fzd1, Fzd 2, and Fzd 7 are used for any of these indicated uses. In one embodiment, Wnt surrogate molecules that bind Fzd1, Fzd 2, Fzd 5, Fzd 7 and Fzd 8 are used for any of these indicated uses.

Methods disclosed herein, including methods for treating or preventing a bone disease or disorder include methods that comprise providing to a subject in need thereof both a Wnt surrogate molecule and an antiresorptive agent. In certain embodiments, the methods are used for the treatment of osteoporosis, optionally post-menopausal osteoporosis.

The disclosure also provides a method for inhibiting or reducing bone resorption in a subject in need thereof, comprising providing to the subject an effective amount of a Wnt surrogate molecule, wherein the Wnt surrogate molecule is an agonist of a Wnt signaling pathway. In certain embodiments, the method further comprises providing to the subject an antiresorptive agent. In certain embodiments, the subject has been diagnosed with or is at risk for osteoporosis, optionally postmenopausal osteoporosis. A variety of antiresorptive agents are known in the art and include, but are not limited to, those disclosed herein.

When a Wnt surrogate molecule is provide to the subject in combination with another therapeutic agent, such as an antiresorptive agent, the two agent may be provided in the same or different pharmaceutical compositions. They may be provided to the subject at the same time, at different times, e.g., simultaneously, consecutively, or during overlapping or non-overlapping time periods. In certain embodiments, the two agents are therapeutically active in the subject during an overlapping time period.

Compositions comprising one or more Wnt surrogate molecule disclosed herein (or a polynucleotide encoding a Wnt surrogate molecule, or a vector or cell comprising a

polynucleotide encoding a Wnt surrogate molecule) can be used for the in vivo treatment of skeletal tissue deficiencies. By "skeletal tissue deficiency", it is meant a deficiency in bone or other skeletal connective tissue at any site where it is desired to restore the bone or connective tissue, no matter how the deficiency originated, e.g. whether as a result of surgical intervention, removal of tumor, ulceration, implant, fracture, or other traumatic or degenerative conditions. The compositions of the present invention can be used as part of a regimen for restoring cartilage function to a connective tissue, for the repair of defects or lesions in cartilage tissue such as degenerative wear and arthritis, trauma to the tissue, displacement of torn meniscus, meniscectomy, a luxation of a joint by a torn ligament, malalignment of joints, bone fracture, or by hereditary disease.

A Wnt surrogate molecule may also be used for treatment of periodontal diseases. Periodontal diseases are a leading cause of tooth loss and are linked to multiple systemic conditions. In some embodiments, tooth or underlying bone regeneration is enhanced by contacting a responsive cell population. In some such embodiments, the contacting is performed in vivo. In other such embodiments, the contacting is performed ex vivo, with subsequent implantation of the activated stem or progenitor cells. The molecule may be localized to the site of action, e.g. by loading onto a matrix, which is optionally biodegradable, and optionally provides for a sustained release of the active agent. Matrix carriers include, without limitation, absorbable collagen sponges, ceramics, hydrogels, bone cements, polymeric microspheres, nanoparticles, and the like.

Studies have shown that biology of Wnt signaling and R-spondins are capable of promoting sensory hair cell regeneration in the inner ear following injuries, aging, or degeneration. Loss of sensory hair cells in the inner ear involved in hearing loss or vestibular hypofunction may also benefit from the compositions of the invention. In the inner ear, the auditory organ houses mechanosensitive hair cells required for translating sound vibration to electric impulses. The vestibular organs, comprised of the semicircular canals (SSCs), the utricle, and the saccule, also contain sensory hair cells in order to detect head position and motion. Compositions of the present invention can be used, for example, in an infusion; in a matrix or other depot system; or other topical application to the ear for enhancement of auditory regeneration.

A Wnt surrogate molecule may also be used in regeneration of retinal tissue. In the adult mammalian retina, Muller glia cells are capable of regenerating retinal cells, including photoreceptors, for example after neurotoxic injury in vivo. Wnt signaling and enhancers of Wnt signals can promote proliferation of Muller glia-derived retinal progenitors after damage or during degeneration. The compositions of the invention may also be used in the regeneration of tissues and other cell types in the eye. For examples age-related macular degeneration (AMD), other retina degenerative diseases, cornea diseases, Fuchs' dystrophy, vitreoretinopathy, hereditary diseases, etc. can benefit from the compositions of the present inventions. AMD is characterized by progressively decreased central vision and visual acuity. Fuchs' dystrophy is characterized by progressive loss of cornea endothelial cells. Wnt signal and enhancing of Wnt signal can promote regeneration of cornea endothelium, retina epithelium, etc. in the eye tissue. In other embodiments, compositions of the present invention can be used, for example, in an infusion; in a matrix or other depot system; or other topical application to the eye for retinal regeneration and treatment of macular degeneration.

Specific populations of proliferating cells for homeostatic renewal of hepatocytes have been identified through lineage tracing studies, for example Axin2-positive cells in pericentral region. Lineage tracing studies also identified additional potential liver progenitor cells, including but not limited to Lgr-positive cells. The self-renewing liver cells and other populations of potential progenitor cells, including Lgr5-positive and Axin2-positive cells, are identified to be capable of regeneration responding to Wnt signals and/or R-spondins following injuries. Numerous preclinical models of acute liver injury and failure and chronic liver diseases showed recovery and regeneration of hepatocytes benefit from enhancing Wnt signals.

In certain embodiments, compositions comprising a Wnt surrogate molecule disclosed herein (or a polynucleotide encoding a Wnt surrogate molecule, or a vector or cell comprising a polynucleotide encoding a Wnt surrogate molecule) are used to promote liver regeneration, reduce fibrosis, and/or improve liver function. In certain embodiments, compositions and methods disclosed herein are used to: increase liver weight, increase the liver to body weight ratio, increase the number of PCNA and pH3 positive nuclei in liver, increase expression of Ki67 and/or Cyclin D1 in liver, increase liver cell proliferation and/or mitosis, decrease fibrosis following chronic liver injury, or increase hepatocyte function.

In particular embodiments, the compositions of this invention may be used in treatment of acute liver failure, acute alcoholic liver injuries, treatment of chronic liver diseases with hepatitis C or B virus infection or post-antiviral drug therapies, chronic alcoholic liver diseases, alcoholic hepatitis, non-alcoholic fatty liver diseases and non-alcoholic steatohepatitis (NASH), treatment of cirrhosis and severe chronic liver diseases of all causes, and enhanced regeneration of liver cells. Methods for regeneration of liver tissue benefit from administration of the compounds of the invention, which can be systemic or localized. These include, but are not limited to, methods of systemic administration and methods of localized administration e.g. by injection into the liver tissue, by injection into veins or blood vessels leading into the liver, by implantation of a sustained release formulation, and the like.

In particular embodiments, compositions comprising a Wnt surrogate molecule disclosed herein (or a polynucleotide encoding a Wnt surrogate molecule, or a vector or cell comprising a polynucleotide encoding a Wnt surrogate molecule) are used to treat or prevent a liver disease or disorder, including but not limited to, or to treat or prevent a liver injury or disorder resulting from any of the following: acute liver failure (all causes), chronic liver failure (all causes), cirrhosis, liver fibrosis (all causes), portal hypertension, alcoholic liver diseases including alcoholic hepatitis, nonalcoholic steatohepatitis (NASH), nonalcoholic fatty liver disease (NAFLD) (fatty liver), alcoholic hepatitis, hepatitis C virus-induced liver diseases (HCV), hepatitis B virus-induced liver diseases (HBV), other viral hepatitis (e.g., hepatitis A virus-induced liver diseases (HAV) and hepatitis D virus-induced liver diseases (HDV)), primary biliary cirrhosis, autoimmune hepatitis, liver surgery, liver injury, liver transplantation, "small for size" syndrome in liver surgery and transplantation, congenital liver disease and disorders, any other liver disorder or defect resulting from genetic diseases, degeneration, aging, drugs, or injuries.

Wnt signals play an important role in regeneration of various epithelial tissues. Various epidermal conditions benefit from treatment with the compounds of the present

invention. Mucositis occurs when there is a breakdown of the rapidly divided epithelial cells lining the gastro-intestinal tract, leaving the mucosal tissue open to ulceration and infection. The part of the epithelial lining that covers the mouth, called the oral mucosa, is one of the most sensitive parts of the body and is particularly vulnerable to chemotherapy and radiation. Oral mucositis is probably the most common, debilitating complication of cancer treatments, particularly chemotherapy and radiation. In addition, the compositions of the invention may also benefit treatment of short bowel syndrome, inflammatory bowel diseases (IBD), or other gastrointestinal disorders. Other epidermal conditions include epidermal wound healing, diabetic foot ulcers, syndromes involving tooth, nail, or dermal hypoplasia, and the like. Molecules of the present invention may be used in all these conditions, where regenerative cells are contacted with compounds of the invention. Methods for regeneration of epithelial tissues benefit from administration of the compounds of the invention, which can be systemic or localized. Contacting can be, for example, topical, including intradermal, subdermal, in a gel, lotion, cream etc. applied at targeted site, etc.

In addition to skin and gastrointestinal tract, Wnt signals and enhancement and promotion of Wnt signals also play an important role in repair and regeneration of tissues including pancreas, kidney, and lung in preclinical models. A Wnt surrogate molecule may benefit various disease conditions involving exocrine and endocrine pancreas, kidney, or lung. The Wnt surrogate molecules may be used in treatment of metabolic syndrome; treatment of diabetes, treatment of acute or chronic pancreatitis, exocrine pancreatic insufficiency, treatment of acute kidney injuries, chronic kidney diseases, treatment of lung diseases, including but not limited to chronic obstructive pulmonary diseases (COPD), pulmonary fibrosis, in particular idiopathic pulmonary fibrosis (IPF), and other conditions that cause loss of lung epithelial tissues. Methods for regeneration of these tissues benefit from administration of the compounds of the invention, which can be systemic or localized.

Epidermal Wnt signaling, in coordination with signaling via other development factors, is critical for adult hair follicle regeneration. Hair loss is a common problem, and androgenetic alopecia, often called male pattern baldness, is the most common form of hair loss in men. In some embodiments, hair follicle regeneration is enhanced by contacting a responsive cell population with a molecule of the present invention. In some such embodiments, the contacting is performed *in vivo*. In other such embodiments, the contacting is performed *ex vivo*. The molecule may be localized to the site of action, e.g. topical lotions, gels, creams and the like.

Stroke, traumatic brain injury, Alzheimer's disease, multiple sclerosis and other conditions affecting the blood brain barrier (BBB) may be treated with a Wnt surrogate molecule. Angiogenesis is critical to ensure the supply of oxygen and nutrients to many tissues throughout the body, and is especially important for the CNS as the neural tissue is extremely sensitive to hypoxia and ischemia. CNS endothelial cells which form the BBB differ from endothelial cells in non-neural tissue, in that they are highly polarized cells held together by tight junctions and express specific transporters. Wnt signaling regulates CNS vessel formation and/or function. Conditions in which the BBB is compromised can benefit from administration of the compounds of the invention, which can be systemic or localized e.g. by direct injection, intrathecal administration, implantation of sustained release formulations, and the like. In

addition, Wnt signal is actively involved in neurogenesis and plays a role of neuroprotection following injury. The compositions of the present invention may also be used in treatment of spinal cord injuries, other spinal cord diseases, stroke, traumatic brain injuries, etc.

Wnt signals also play a role in angiogenesis. A Wnt surrogate molecule may benefit conditions where angiogenesis is beneficial, treatment of myocardial infarction, coronary artery disease, heart failure, diabetic retinopathy, etc., and conditions from hereditary diseases. Methods for regeneration of these tissues benefit from administration of the compounds of the invention, which can be systemic or localized.

In certain embodiments, methods of the present invention promote tissue regeneration, e.g., in a tissue subjected to damage or tissue or cell reduction or loss. The loss or damage can be anything which causes the cell number to diminish, including diseases or injuries. For example, an accident, an autoimmune disorder, a therapeutic side-effect or a disease state could constitute trauma. Tissue regeneration increases the cell number within the tissue and preferably enables connections between cells of the tissue to be re-established, and more preferably the functionality of the tissue to be regained.

The terms “administering” or “introducing” or “providing”, as used herein, refer to delivery of a composition to a cell, to cells, tissues and/or organs of a subject, or to a subject. Such administering or introducing may take place in vivo, in vitro or ex vivo.

In particular embodiments, a pharmaceutical composition is administered parenterally, e.g., intravenously, orally, rectally, or by injection. In some embodiments, it is administered locally, e.g., topically or intramuscularly. In some embodiments, a composition is administered to target tissues, e.g., to bone, joints, ear tissue, eye tissue, gastrointestinal tract, skin, a wound site or spinal cord. Methods of the invention may be practiced in vivo or ex vivo. In some embodiments, the contacting of a target cell or tissue with a Wnt surrogate molecule is performed ex vivo, with subsequent implantation of the cells or tissues, e.g., activated stem or progenitor cells, into the subject. The skilled artisan can determine an appropriate site of and route of administration based on the disease or disorder being treated.

The dose and dosage regimen may depend upon a variety of factors readily determined by a physician, such as the nature of the disease or disorder, the characteristics of the subject, and the subject's history. In particular embodiments, the amount of a Wnt surrogate molecule administered or provided to the subject is in the range of about 0.01 mg/kg to about 50 mg/kg, 0.1 mg/kg to about 500 mg/kg, or about 0.1 mg/kg to about 50 mg/kg of the subject's body weight.

The terms “treatment”, “treating” and the like are used herein to generally mean obtaining a desired pharmacologic and/or physiologic effect. The effect may be prophylactic in terms of completely or partially preventing a disease or symptom thereof, e.g. reducing the likelihood that the disease or symptom thereof occurs in the subject, and/or may be therapeutic in terms of a partial or complete cure for a disease and/or adverse effect attributable to the disease. “Treatment” as used herein covers any treatment of a disease in a mammal, and includes: (a) preventing the disease from occurring in a subject which may be predisposed to the disease but has not yet been diagnosed as having it; (b) inhibiting the disease, i.e., arresting its development; or (c) relieving the disease, i.e., causing regression of the disease. The therapeutic agent (e.g., a Wnt surrogate molecule) may be administered before, during or after the onset of disease

or injury. The treatment of ongoing disease, where the treatment stabilizes or reduces the undesirable clinical symptoms of the patient, is of particular interest. Such treatment is desirably performed prior to complete loss of function in the affected tissues. The subject therapy will desirably be administered during the symptomatic stage of the disease, and in some cases after the symptomatic stage of the disease. In some embodiments, the subject method results in a therapeutic benefit, e.g., preventing the development of a disorder, halting the progression of a disorder, reversing the progression of a disorder, etc. In some embodiments, the subject method comprises the step of detecting that a therapeutic benefit has been achieved. The ordinarily skilled artisan will appreciate that such measures of therapeutic efficacy will be applicable to the particular disease being modified, and will recognize the appropriate detection methods to use to measure therapeutic efficacy.

Other embodiments relate, in part, to the use of the Wnt surrogate molecules disclosed herein to promote or enhance the growth or proliferation of cells, tissues and organoids, for example, by contacting cells or tissue with one or more Wnt surrogate, optionally in combination with a Norrin or Rspndin polypeptide. In certain embodiments, the cells or tissue are contacted ex vivo, in vitro, or in vivo. Such methods may be used to generate cells, tissue or organoids for therapeutic use, e.g., to be transplanted or grafted into a subject. They may also be used to generate cells, tissue or organoids for research use. The Wnt surrogate molecules have widespread applications in non-therapeutic methods, for example in vitro research methods.

The invention provides a method for tissue regeneration of damaged tissue, such as the tissues discussed above, comprising administering a Wnt surrogate molecule to cells. The Wnt surrogate molecule may be administered directly to the cells in vivo, administered to a subject orally, intravenously, or by other methods known in the art, or administered to ex vivo cells. In some embodiments where the Wnt surrogate molecule is administered to ex vivo cells, these cells may be transplanted into a subject before, after or during administration of the Wnt surrogate molecule.

Wnt signaling is a key component of stem cell culture. For example, the stem cell culture media as described in WO2010/090513, WO2012/014076, Sato et al., 2011 (GASTROENTEROLOGY 2011; 141: 1762-1772) and Sato et al., 2009 (Nature 459, 262-5). The Wnt surrogate molecules disclosed herein are suitable alternatives to Rspndin for use in these stem cell culture media, or may be combined with Rspndin.

Accordingly, in one embodiment, the disclosure provides a method for enhancing the proliferation of stem cells comprising contacting stem cells with one or more Wnt surrogate molecules disclosed herein. In one embodiment, the disclosure provides a cell culture medium comprising one or more Wnt surrogate molecules disclosed herein. In some embodiments, the cell culture medium may be any cell culture medium already known in the art that normally comprises Wnt or Rspndin, but wherein the Wnt or Rspndin is replaced (wholly or partially) or supplemented by Wnt surrogate molecule(s) disclosed herein. For example, the culture medium may be as described in as described in WO2010/090513, WO2012/014076, Sato et al., 2011 (GASTROENTEROLOGY 2011; 141: 1762-1772) and Sato et al., 2009 (Nature 459, 262-5), which are hereby incorporated by reference in their entirety.

Stem cell culture media often comprise additional growth factors. This method may thus additionally comprise supplying the stem cells with a growth factor. Growth factors

commonly used in cell culture medium include epidermal growth factor (EGF, (Peprotech), Transforming Growth Factor-alpha (TGF-alpha, Peprotech), basic Fibroblast Growth Factor (bFGF, Peprotech), brain-derived neurotrophic factor (BDNF, R&D Systems), Hepatocyte Growth Factor (HGF) and Keratinocyte Growth Factor (KGF, Peprotech, also known as FGF7). EGF is a potent mitogenic factor for a variety of cultured ectodermal and mesodermal cells and has a profound effect on the differentiation of specific cells in vivo and in vitro and of some fibroblasts in cell culture. The EGF precursor exists as a membrane-bound molecule which is proteolytically cleaved to generate the 53-amino acid peptide hormone that stimulates cells. EGF or other mitogenic growth factors may thus be supplied to the stem cells. During culturing of stem cells, the mitogenic growth factor may be added to the culture medium every second day, while the culture medium is refreshed preferably every fourth day. In general, a mitogenic factor is selected from the groups consisting of: i) EGF, TGF-alpha, and KGF; ii) EGF, TGF-alpha, and FGF7; iii) EGF, TGF-alpha, and FGF; iv) EGF and KGF; v) EGF and FGF7; vi) EGF and a FGF; vii) TGF-alpha and KGF; viii) TGF-alpha, and FGF7; ix) or from TGF-alpha and a FGF. In certain embodiments, the disclosure includes a stem cell culture media comprising a Wnt surrogate molecule disclosed herein, e.g., optionally in combination with one or more of the growth factors or combinations thereof described herein.

These methods of enhancing proliferation of stem cells can be used to grow new organoids and tissues from stem cells, as for example described in WO2010/090513 WO02012/014076, Sato et al., 2011 (GASTROENTEROLOGY 2011; 141: 1762-1772) and Sato et al., 2009 (Nature 459, 262-5).

In some embodiments, the Wnt surrogate molecules are used to enhance stem cell regeneration. Illustrative stem cells of interest include but are not limited to: muscle satellite cells; hematopoietic stem cells and progenitor cells derived therefrom (U.S. Pat. No. 5,061,620); neural stem cells (see Morrison et al. (1999) Cell 96: 737-749); embryonic stem cells; mesenchymal stem cells; mesodermal stem cells; liver stem cells; adipose-tissue derived stem cells, etc.

Other embodiments of the present invention relate, in part, to diagnostic applications for detecting the presence of cells or tissues expressing one or more Fzd receptors or LRP5 or LRP6 receptors. Thus, the present disclosure provides methods of detecting one or more Fzd receptor or LRP5 or LRP6 receptor in a sample, such as detection of cells or tissues expressing Fzd1. Such methods can be applied in a variety of known detection formats, including, but not limited to immunohistochemistry (IHC), immunocytochemistry (ICC), in situ hybridization (ISH), whole-mount in situ hybridization (WISH), fluorescent DNA in situ hybridization (FISH), flow cytometry, enzyme immunoassay (EIA), and enzyme linked immuno-assay (ELISA), e.g., by detecting binding of a Wnt surrogate molecule.

ISH is a type of hybridization that uses a labeled complementary DNA or RNA strand (i.e., primary binding agent) to localize a specific DNA or RNA sequence in a portion or section of a cell or tissue (in situ), or if the tissue is small enough, the entire tissue (whole mount ISH). One having ordinary skill in the art would appreciate that this is distinct from immunohistochemistry, which localizes proteins in tissue sections using an antibody as a primary binding agent. DNA ISH can be used on genomic DNA to determine the structure of chromosomes. Fluorescent DNA ISH (FISH) can, for example, be used in medical diagnostics to assess chromosomal integrity. RNA ISH (hybridization histochem-

istry) is used to measure and localize mRNAs and other transcripts within tissue sections or whole mounts.

In various embodiments, the Wnt surrogate molecules described herein are conjugated to a detectable label that may be detected directly or indirectly. In this regard, an antibody "conjugate" refers to a Wnt surrogate molecule that is covalently linked to a detectable label. In the present invention, DNA probes, RNA probes, monoclonal antibodies, antigen-binding fragments thereof, and antibody derivatives thereof, such as a single-chain-variable-fragment antibody or an epitope tagged antibody, may all be covalently linked to a detectable label. In "direct detection", only one detectable antibody is used, i.e., a primary detectable antibody. Thus, direct detection means that the antibody that is conjugated to a detectable label may be detected, per se, without the need for the addition of a second antibody (secondary antibody).

A "detectable label" is a molecule or material that can produce a detectable (such as visually, electronically or otherwise) signal that indicates the presence and/or concentration of the label in a sample. When conjugated to an antibody, the detectable label can be used to locate and/or quantify the target to which the specific antibody is directed. Thereby, the presence and/or concentration of the target in a sample can be detected by detecting the signal produced by the detectable label. A detectable label can be detected directly or indirectly, and several different detectable labels conjugated to different specific-antibodies can be used in combination to detect one or more targets.

Examples of detectable labels, which may be detected directly, include fluorescent dyes and radioactive substances and metal particles. In contrast, indirect detection requires the application of one or more additional antibodies, i.e., secondary antibodies, after application of the primary antibody. Thus, the detection is performed by the detection of the binding of the secondary antibody or binding agent to the primary detectable antibody. Examples of primary detectable binding agents or antibodies requiring addition of a secondary binding agent or antibody include enzymatic detectable binding agents and hapten detectable binding agents or antibodies.

In some embodiments, the detectable label is conjugated to a nucleic acid polymer which comprises the first binding agent (e.g., in an ISH, WISH, or FISH process). In other embodiments, the detectable label is conjugated to an antibody which comprises the first binding agent (e.g., in an IHC process).

Examples of detectable labels which may be conjugated to Wnt surrogate molecules used in the methods of the present disclosure include fluorescent labels, enzyme labels, radioisotopes, chemiluminescent labels, electrochemiluminescent labels, bioluminescent labels, polymers, polymer particles, metal particles, haptens, and dyes.

Examples of fluorescent labels include 5-(and 6)-carboxyfluorescein, 5- or 6-carboxyfluorescein, 6-(fluorescein)-5-(and 6)-carboxamido hexanoic acid, fluorescein isothiocyanate, rhodamine, tetramethylrhodamine, and dyes such as Cy2, Cy3, and Cy5, optionally substituted coumarin including AMCA, PerCP, phycobiliproteins including R-phycoerythrin (RPE) and allophycoerythrin (APC), Texas Red, Princeton Red, green fluorescent protein (GFP) and analogues thereof, and conjugates of R-phycoerythrin or allophycoerythrin, inorganic fluorescent labels such as particles based on semiconductor material like coated CdSe nanocrystallites.

Examples of polymer particle labels include micro particles or latex particles of polystyrene, PMMA or silica,

which can be embedded with fluorescent dyes, or polymer micelles or capsules which contain dyes, enzymes or substrates.

Examples of metal particle labels include gold particles and coated gold particles, which can be converted by silver stains. Examples of haptens include DNP, fluorescein isothiocyanate (FITC), biotin, and digoxigenin. Examples of enzymatic labels include horseradish peroxidase (HRP), alkaline phosphatase (ALP or AP), β -galactosidase (GAL), glucose-6-phosphate dehydrogenase, β -N-acetylglucosaminidase, β -glucuronidase, invertase, Xanthine Oxidase, firefly luciferase and glucose oxidase (GO). Examples of commonly used substrates for horseradish peroxidase include 3,3'-diaminobenzidine (DAB), diaminobenzidine with nickel enhancement, 3-amino-9-ethylcarbazole (AEC), Benzidine dihydrochloride (BDHC), Hanker-Yates reagent (HYR), Indophane blue (IB), tetramethylbenzidine (TMB), 4-chloro-1-naphthol (CN), α -naphthol peryonin (α -NP), o-dianisidine (OD), 5-bromo-4-chloro-3-indolylphosphate (BCIP), Nitro blue tetrazolium (NBT), 2-(p-iodophenyl)-3-p-nitrophenyl-5-phenyl tetrazolium chloride (INT), tetranitro blue tetrazolium (TNBT), 5-bromo-4-chloro-3-indoxyl-beta-D-galactoside/ferro-ferricyanide (BCIG/FF).

Examples of commonly used substrates for Alkaline Phosphatase include Naphthol-AS-B 1-phosphate/fast red TR (NABP/FR), Naphthol-AS-MX-phosphate/fast red TR (NAMP/FR), Naphthol-AS-B1-phosphate/-fast red TR (NABP/FR), Naphthol-AS-MX-phosphate/fast red TR (NAMP/FR), Naphthol-AS-B1-phosphate/new fuschin (NABP/NF), bromochloroindolyl phosphate/nitroblue tetrazolium (BCIP/NBT), 5-Bromo-4-chloro-3-indolyl-b-d-galactopyranoside (BCIG).

Examples of luminescent labels include luminol, isoluminol, acridinium esters, 1,2-dioxetanes and pyridopyridazines. Examples of electrochemiluminescent labels include ruthenium derivatives. Examples of radioactive labels include radioactive isotopes of iodide, cobalt, selenium, tritium, carbon, sulfur and phosphorous.

Detectable labels may be linked to the antibodies described herein or to any other molecule that specifically binds to a biological marker of interest, e.g., an antibody, a nucleic acid probe, or a polymer. Furthermore, one of ordinary skill in the art would appreciate that detectable labels can also be conjugated to second, and/or third, and/or fourth, and/or fifth binding agents or antibodies, etc. Moreover, the skilled artisan would appreciate that each additional binding agent or antibody used to characterize a biological marker of interest may serve as a signal amplification step. The biological marker may be detected visually using, e.g., light microscopy, fluorescent microscopy, electron microscopy where the detectable substance is for example a dye, a colloidal gold particle, a luminescent reagent. Visually detectable substances bound to a biological marker may also be detected using a spectrophotometer. Where the detectable substance is a radioactive isotope detection can be visually by autoradiography, or non-visually using a scintillation counter. See, e.g., Larsson, 1988, Immunocytochemistry: Theory and Practice, (CRC Press, Boca Raton, Fla.); Methods in Molecular Biology, vol. 80 1998, John D. Pound (ed.) (Humana Press, Totowa, N.J.).

The invention further provides kits for detecting one or more Fzd or LRP5/6 receptor or cells or tissues expressing one or more Fzd or LRP5/6 receptors in a sample, wherein the kits contain at least one antibody, polypeptide, polynucleotide, vector or host cell as described herein. In certain embodiments, a kit may comprise buffers, enzymes, labels,

substrates, beads or other surfaces to which the antibodies of the invention are attached, and the like, and instructions for use.

All of the above U.S. patents, U.S. patent application publications, U.S. patent applications, foreign patents, foreign patent applications and non-patent publications referred to in this specification and/or listed in the Application Data Sheet, are incorporated herein by reference, in their entirety.

From the foregoing it will be appreciated that, although specific embodiments of the invention have been described herein for purposes of illustration, various modifications may be made without deviating from the spirit and scope of the invention. Accordingly, the invention is not limited except as by the appended claims.

EXAMPLES

Example 1

Illustrative Wnt Surrogate Molecule Formats

Wnt surrogates having a variety of different formats disclosed herein were made. These included the following illustrative formats, each comprising a binding domain ("binder") that binds to one or more Fzd receptor and a binding domain ("binder") that binds to an LRP5 and/or LRP6 receptor.

As shown in FIG. 1A, if a binder to one receptor is a Fab and to the other receptor is a Nab or scFv, they can be put together in several different configurations. In certain instances, the Fab binder first can be reformatted to a full IgG format, then the Nab binder can be fused to any of the 4 available termini of the IgG. For example, the Nab can be fused to the N-terminus of the IgG light chain (LC, the fusion will be referred to as NL, shown in top left), N-terminus of the IgG heavy chain (HC, the fusion will be referred to as NH, shown in top right), the C-terminus of LC (the fusion will be referred to as CL, shown in middle right), and the C-terminus of HC (the fusion will be referred to as CH, shown in middle left). The linkers and the length of the linkers between the IgG and the Nab can be varied. These four formats are bispecific and bivalent, they are bivalent binders toward each of the receptors. An alternative way to put the two binders together is the Hetero-Ig format where the Fab binder is presented as a half antibody, and the Nab is fused to the N-terminus of an Fc (shown in lower middle). The two halves may be brought together by mutations in the CH3 domain (such as the knobs-into-holes) that favor the formation of the heterodimer. The linker and its length between the Nab binder and the Fc can be varied. This format would be bispecific but monovalent toward each receptor. The Nab part of any of the formats described in this example can also be replaced by scFv fragments of binders as well.

As shown in FIG. 1B, if a binder to one receptor is a Fab and to the other receptor is also a Fab, they can be put together in several different configurations. In one approach, one Fab binder is first reformatted to a full IgG format (shown in top). The second Fab binder can be fused to the N-terminus of the IgG. The two HC can be fused together with a linker in between. The LCs can be fused or unfused. The linker and its length can be varied. This format is a bispecific and bivalent format. Alternatively, the second Fab binder LC can be fused to the HC of the IgG with a linker of various length in between. The second Fab binder HC can be fused or unfused to the LC of the IgG. A variation of this format has been called Fabs-in-tandem IgG (or FIT-Ig). In

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another approach, the two binders can be brought together as a Hetero-Ig by mutations in the CH3 domain that favors the heterodimer assembly, the two arms will each bind to one receptor (shown in bottom). This format is a bispecific and monovalent binder.

As shown in FIG. 1C, if a binder to one receptor is a Nab and to the other receptor is also a Nab, they can be put together in several different configurations. In the bispecific bivalent formats, in certain instances, the two Nab binders can be fused together in tandem (shown in top row) or fused to the two-different ends of the Fc (shown in middle row). The linker and its length between Nab and Nab or Nab and Fc can be varied. Alternatively, the two Nabs can be assembled together as Hetero-Ig to generate a bispecific and monovalent format (shown in bottom row). Similar to FIG. 1A, the Nab domains here can also be replaced by an scFv domain of a binder. In all the examples, the Nab and scFv can be mixed in certain combinations as well.

As shown in FIG. 1D, the binders against Fzd and LRP can also be linked together in a diabody (or DART) configuration. The diabody can also be in a single chain configuration. If the diabody is fused to an Fc, this will create a bivalent bispecific format. Without fusion to Fc, this would be a monovalent bispecific format.

A number of Wnt surrogates representing different configurations were produced. These included the Wnt surrogates described in Table 3. These illustrative Wnt surrogates include one, two or three polypeptides, the sequences of which are provided as Sequence 1, Sequence 2, and/or Sequence 3. The sequence may include a leader peptide sequence, a Nab sequence, a linker, and/or a heavy or light chain sequence. Annotated sequences are provided in FIG. 19, in which the leader peptide sequence is italicized, the linker sequence is underlined, the Nab sequence is shown in bold, and the remaining sequence is the heavy chain or light chain sequence. The Fzd binder IDs and LRP binder IDs correspond to the clone numbers provided in Tables 1A-B and 2A-B for various Fzd binding or LRP5/6 binding antibodies or antigen-binding fragments thereof.

The Wnt surrogates beginning with "R2M3" include different LRP6 binding domains fused to the N-terminus of a light chain region of the anti-Fzd antibody or antigen-

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binding fragment thereof named 001S-A04. The first six Wnt surrogates beginning with "18R5" in Table 3 include different LRP6 binding domains fused to the N-terminus of the anti-Fzd antibody or antigen-binding fragment thereof named 18R5. The Wnt surrogates beginning with "1R" include the anti-LRP6 antibody of antigen-binding fragment thereof named "009S-E04" fused to the N-terminus of different anti-Fzd antibodies or antigen-binding fragment thereof. For "R2M3-26CH," the LRP6 binding region is fused to the C-terminus of the Fzd binding region. For "R2M3-26NH," the LRP6 binding region is fused to the N-terminus of the Fzd binding region. For "R2M3-26CL," the LRP6 binding region is fused to the C-terminus of the Fzd binding region. For "R2M3-26NL," the LRP6 binding region is fused to the N-terminus of the Fzd binding region. For "R2M3-26Fab" and "R2M3-32Fab," the LRP6 binding region is fused to the N-terminus of the Fzd binding region. For "Hetero-Ig," the LRP6 binding region is fused to the N-terminus of human Fc_{hole}, and paired with Fzd binder light chain and heavy chain human IgG1_{knob}. The Wnt surrogates beginning with "17SB9" include different LRP6 binding domains fused to the N-terminus of a light chain region of the anti-Fzd antibody or antigen-binding fragment thereof named 017S-B09. The Wnt surrogates beginning with "1R-C07" include different LRP6 binding domains fused to the N-terminus of a light chain region of the anti-Fzd antibody or antigen-binding fragment thereof named 001S-B03. The Wnt surrogates beginning with "R2M13" include different LRP6 binding domains fused to the N-terminus of a light chain region of the anti-Fzd antibody or antigen-binding fragment thereof named 004S-G06. The Wnt surrogates beginning with "3SD10" include different LRP6 binding domains fused to the N-terminus of a light chain region of the anti-Fzd antibody or antigen-binding fragment thereof named 003S-D10. The Wnt surrogates beginning with "4SD1" include different LRP6 binding domains fused to the N-terminus of a light chain region of the anti-Fzd antibody or antigen-binding fragment thereof named 004S-D01. The Wnt surrogates beginning with "14SB6" include different LRP6 binding domains fused to the N-terminus of a light chain region of the anti-Fzd antibody or antigen-binding fragment thereof named 014S-B06.

TABLE 3

Wnt Surrogate Sequences					
Name	Fzd binder ID	LRP binder ID	Sequence 1 SEQ ID NO	Sequence 2 SEQ ID NO	Sequence 3 SEQ ID NO
R2M3-23	001S-A04	009S-B04	89	95	N/A
R2M3-26	001S-A04	009S-E04	90	95	N/A
R2M3-28	001S-A04	009S-G04	91	95	N/A
R2M3-29	001S-A04	009S-H04	92	95	N/A
R2M3-31	001S-A04	013S-G04	93	95	N/A
R2M3-32	001S-A04	013S-H04	94	95	N/A
18R5-5	18R5	008S-G01	96	104	N/A
18R5-7	18R5	008S-C02	97	104	N/A
18R5-8	18R5	008S-D02	98	104	N/A
18R5-9	18R5	008S-E02	99	104	N/A
18R5-26	18R5	009S-E04	100	104	N/A
18R5-28	18R5	009S-G04	101	104	N/A
18R5-31	18R5	013S-G04	102	104	N/A
18R5-32	18R5	013S-H04	103	104	N/A
1R-B05-26	001S-E02	009S-E04	105	111	N/A
1R-C01-26	001S-B01	009S-E04	106	112	N/A
1R-C07-26	001S-B03	009S-E04	107	113	N/A
1R-E06-26	001S-H02	009S-E04	108	114	N/A
1R-G05-26	001S-G02	009S-E04	109	115	N/A
1R-G06-26	001S-A03	009S-E04	110	116	N/A
R2M3-26CH	001S-A04	009S-E04	125	117	N/A

TABLE 3-continued

Wnt Surrogate Sequences					
Name	Fzd binder ID	LRP binder ID	Sequence 1 SEQ ID NO	Sequence 2 SEQ ID NO	Sequence 3 SEQ ID NO
R2M3-26NH	001S-A04	009S-E04	125	118	N/A
R2M3-26CL	001S-A04	009S-E04	119	2254	N/A
R2M3-26NL	001S-A04	009S-E04	120	2254	N/A
R2M3-26Fab	001S-A04	009S-E04	120	122	N/A
R2M3-26F(ab') ₂	001S-A04	009S-E04	120	2252	
R2M3-32Fab	001S-A04	013S-H04	123	122	N/A
R2M3-26Hetero-Ig	001S-A04	009S-E04	125	126	127
26-17SB9	017S-B09	009S-E04	128	N/A	N/A
26:Fc:17SB9-criss-cross	017S-B09	009S-E04	2192	2193	N/A
26:5:17SB9:Fc	017S-B09	009S-E04	2194	N/A	N/A
26:10:17SB9:Fc	017S-B09	009S-E04	2195	N/A	N/A
26:15:17SB9:Fc	017S-B09	009S-E04	2196	N/A	N/A
17SB9:Fc:26	017S-B09	009S-E04	2197	N/A	N/A
26:Fc:26+17SB9:Fc:17SB9	017S-B09	009S-E04	2198	2199	N/A
1R-C07-3	001S-B03	008S-D01	134	113	N/A
1R-C07-36	001S-B03	013S-D05	135	113	N/A
R2M13-3	004S-G06	008S-D01	136	153	N/A
R2M13-26	004S-G06	009S-E04	137	153	N/A
R2M13-36	004S-G06	013S-D05	138	153	N/A
R2M3-3	001S-A04	008S-D01	139	2254	N/A
R2M3-36	001S-A04	013S-D05	140	2254	N/A
3SD10-3	003S-D10	008S-D01	141	154	N/A
3SD10-26	003S-D10	009S-E04	142	154	N/A
3SD10-36	003S-D10	013S-D05	143	154	N/A
4SD1-3	004S-D01	008S-D01	144	155	N/A
4SD1-26	004S-D01	009S-E04	145	155	N/A
4SD1-36	004S-D01	013S-D05	146	155	N/A
14SB6-3	014S-B06	008S-D01	147	156	N/A
14SB6-26	014S-B06	009S-E04	148	156	N/A
14SB6-36	014S-B06	013S-D05	149	156	N/A
R2M9-3	003S-E07	008S-D01	150	157	N/A
R2M9-26	003S-E07	009S-E04	151	157	N/A
R2M9-36	003S-E07	013S-D05	152	157	N/A
18R5:5:1115.3:Fc	18R5	1115.3	2200	N/A	N/A
18R5:10:1115.3:Fc	18R5	1115.3	2201	N/A	N/A
18R5:15:1115.3:Fc	18R5	1115.3	2202	N/A	N/A
1115.3:5:18R5:Fc	18R5	1115.3	2203	N/A	N/A
1115.3:10:18R5:Fc	18R5	1115.3	2204	N/A	N/A
1115.3:15:18R5:Fc	18R5	1115.3	2205	N/A	N/A
18R5:5:YW211.31.57:Fc	18R5	YW211.31.57	2206	N/A	N/A
18R5:10:YW211.31.57:Fc	18R5	YW211.31.57	2207	N/A	N/A
18R5:15:YW211.31.57:Fc	18R5	YW211.31.57	2208	N/A	N/A
YW211.31.57:5:18R5:Fc	18R5	YW211.31.57	2209	N/A	N/A
YW211.31.57:10:18R5:Fc	18R5	YW211.31.57	2210	N/A	N/A
YW211.31.57:15:18R5:Fc	18R5	YW211.31.57	2211	N/A	N/A
18R5:Fc:1115.3	18R5	1115.3	2212	N/A	N/A
1115.3:Fc:18R5	18R5	1115.3	2213	N/A	N/A
18R5:Fc:YW211.31.57	18R5	YW211.31.57	2250	N/A	N/A
YW211.31.57:Fc:18R5	18R5	YW211.31.57	2267	N/A	N/A
421.1-R2M3 cp	001S-A04	421.1	2214	2215	2216
1RC07:5:10SA7 cp	1RC07	10SA7	2217	2218	2219
1RC07:10:10SA7 cp	1RC07	10SA7	2217	2218	2222
1RC07:15:10SA7 cp	1RC07	10SA7	2217	2218	2225
1RC07:5:10SG7 cp	1RC07	10SG7	2217	2227	2228
1RC07:10:10SG7 cp	1RC07	10SG7	2217	2227	2231
1RC07:15:10SG7 cp	1RC07	10SG7	2217	2227	2234
10SG7:5:1RC07 cp	1RC07	10SG7	2227	2217	2237
10SG7:10:1RC07 cp	1RC07	10SG7	2227	2217	2240
10SG7:15:1RC07 cp	1RC07	10SG7	2227	2217	2243
1RC07:5:10SA7 L->H	1RC07	10SA7	2244	2245	2246
1115.3:5:R2M3 L->H	001S-A04	1115.3	2247	125	2248
1115.3:10:R2M3 L->H	001S-A04	1115.3	2247	125	2249
10SG11-1RC07	1RC07	10SG11	2252	2253	N/A
18R5:5:1115.3:His	18R5	1115.3	2255	N/A	N/A
18R5:10:1115.3:His	18R5	1115.3	2256	N/A	N/A
18R5:15:1115.3:His	18R5	1115.3	2257	N/A	N/A
1115.3:5:18R5:His	18R5	1115.3	2258	N/A	N/A
1115.3:10:18R5:His	18R5	1115.3	2259	N/A	N/A
1115.3:15:18R5:His	18R5	1115.3	2260	N/A	N/A
18R5:5:YW211.31.57:His	18R5	YW211.31.57	2261	N/A	N/A
18R5:10:YW211.31.57:His	18R5	YW211.31.57	2262	N/A	N/A

TABLE 3-continued

Wnt Surrogate Sequences					
Name	Fzd binder ID	LRP binder ID	Sequence 1 SEQ ID NO	Sequence 2 SEQ ID NO	Sequence 3 SEQ ID NO
18R5:15:YW211.31.57:His	18R5	YW211.31.57	2263	N/A	N/A
YW211.31.57:5:18R5:His	18R5	YW211.31.57	2264	N/A	N/A
YW211.31.57:10:18R5:His	18R5	YW211.31.57	2265	N/A	N/A
YW211.31.57:15:18R5:His	18R5	YW211.31.57	2266	N/A	N/A

Example 2

Characterization of a Wnt Surrogate Molecule,
R2M3-26

The R2M3-26 molecule consists of a Fzd binder (R2M3) and a LRP6 binder (26). The LRP6 binder 26 was fused to the N-terminus of R2M3 LC with a 5-amino acid linker as depicted in FIG. 2A. R2M3 was in the form of an IgG. The protein was purified by Protein A affinity column followed by a size-exclusion-chromatography (SEC) step. The absorbance trace from the SEC and the SDS-PAGE gels of the SEC fractions were shown in FIG. 2B. The ability of R2M3-26 to activate canonical Wnt signaling was tested in a Wnt responding 293 reporter cell line (293STF). The 293STF reporter activity traces across the SEC fractions were shown in FIG. 2B, the peak of the reporter activity correlated with the peak of the proteins. The peak fraction was further characterized by a dose response in 293STF cells in the absence and presence of R-spondin (FIG. 2D). R2M3-26 induced reporter activity in a dose dependent manner and was enhanced by the presence of R-spondin similar to a natural Wnt ligand, while R2M3 IgG alone without the attachment of the LRP binding arm did not induce reporter activity. The ability of R2M3-26 to interact with its target, a Fzd1 ECD, was performed in Octet interaction assay (FIG. 2C), and the results showed that the fusion of the LRP6 binding arm, 26, did not affect R2M3 interaction with its target, Fzd.

Example 3

Characterization of a Wnt Surrogate Molecule,
R2M3-32

The R2M3-32 molecule consists of a Fzd binder (R2M3) and a LRP6 binder (32). The LRP6 binder 32 was fused to the N-terminus of R2M3 LC with a 5-amino acid linker as depicted in FIG. 3A. R2M3 was in the form of an IgG. The protein was purified by Protein A affinity column followed by a size-exclusion-chromatography (SEC) step. The absorbance trace from the SEC and the SDS-PAGE gels of the SEC fractions were shown in FIG. 3B. The ability of R2M3-32 to activate canonical Wnt signaling was tested in a Wnt responding 293 reporter cell line (293STF). The 293STF reporter activity traces across the SEC fractions were shown in FIG. 3B, the peak of the reporter activity correlated with the peak of the proteins. The peak fraction was further characterized by a dose response in 293STF cells in the absence and presence of R-spondin (FIG. 3D). R2M3-32 induced reporter activity in a dose dependent manner and was enhanced by the presence of R-spondin, while R2M3 IgG alone without the attachment of the LRP binding arm did not induce reporter activity. The ability of R2M3-32 to interact with its target, a Fzd1 extracellular

domain (ECD), was performed in Octet interaction assay (FIG. 3C). The results showed that the fusion of the LRP6 binding arm, 32, did not affect R2M3 interaction with its target, Fzd.

Example 4

R2M3-26 and R2M3-32 Activities can be Inhibited
by Soluble Fzd ECD and by R2M3 IgG Alone
without the LRP Binding Arm

The ability of soluble Fzd extracellular domain (ECD) or R2M3 IgG alone to inhibit Wnt surrogates was determined using the 293STF reporter assay. The Fzd1 ECD-Fc or R2M3 IgG was titrated into the 293STF reporter assay, at a fixed concentration of R2M3-26 or R2M3-32. In a dose dependent manner, both Fzd1 ECD-Fc and R2M3 IgG inhibited R2M3-26 (FIG. 4A) and R2M3-32 (FIG. 4B) induced reporter signaling, while the negative control molecule, Fc alone had no impact.

Example 5

Characterization of R2M3-LRP6 Binder Fusions in
293, Huh7, A375, BNL.CL2 Wnt Dependent
Reporter Assays

The Fzd binder, R2M3, was fused to additional LRP6 binders, 23, 25, 26, 27, 28, 29, 31, 32, 33, and 36. The LRP6 binders were Nab and were fused to the N-terminus of R2M3 LC with a 5-amino acid linker. These proteins were purified by Protein A affinity column followed by a SEC step. The fusion proteins were tested in Wnt dependent reporter assays in 293, Huh7, A375, and BNL.CL2 cell lines, and activated Wnt signaling to various levels. R2M3 was also fused to two non-LRP6 binder Nabs, 24 and 34, in the same format as the Lrp6 binders. These two non-binders displayed no activity in Wnt dependent 293 reporter assay (FIG. 5), suggesting that Wnt activities observed with R2M3 fusions to 23, 25, 26, 27, 28, 29, 31, 32, 33, and 36 are dependent on the presence of both Fzd and Lrp mimicking the nature ligand function.

Example 6

Characterization of 18R5-LRP6 Binder Fusions in
293, A375, and BNL.CL2 Wnt Dependent Reporter
Assays

The Fzd binder, 18R5, was fused to LRP6 binders, 26, 28, 31, 32. The LRP6 binders were Nab and were fused to the N-terminus of 18R5 LC with a 5-amino acid linker. These proteins were purified by Protein A affinity column followed by a SEC step. The fusion proteins were tested in Wnt

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dependent reporter assays in 293, A375, and BNL.CL2 cell lines and demonstrated ability to activate Wnt signaling (FIG. 6).

Example 7

Characterization of 18R5-LRP5 Binder Fusions in 293 Wnt Dependent Reporter Assays

The Fzd binder, 18R5, was fused to LRP5 binders, 5, 7, 8, 9. The LRP5 binders were Nab and were fused to the N-terminus of 18R5 LC with a 5-amino acid linker. These proteins were purified by Protein A affinity column followed by a SEC step. The fusion proteins were tested in a Wnt dependent reporter assays in 293 cells and were able to activate Wnt signaling (FIG. 7).

Example 8

Characterization of Various Fzd Binders-LRP6 Binder 26 Fusions in 293 Wnt Dependent Reporter Assays

The various Fzd binders, 1R-B05, 1R-C01, 1R-C07, 1R-E01, 1R-E06, 1R-G05, 1R-G06, 1R-H04, in IgG format were fused to LRP6 binders, 26. The LRP6 binder Nab was fused to the N-terminus of various Fzd binder LC with a 5-amino acid linker. These proteins were purified by Protein A affinity column followed by a SEC step. The SDS-PAGE gel analysis of the SEC peak fractions were shown in FIG. 8A. The fusion proteins were tested in a Wnt dependent reporter assays in 293 cells in the presence of Rspo and were able to activate Wnt signaling (FIG. 8B).

Example 9

SAR Analysis of the IgG-Nab Fusion Format

SAR analysis of the IgG-Nab fusions was performed by rotating the attachment location of the Nab to the different termini of the IgG HC or LC as depicted in FIG. 1A. CH indicates attaching the Nab to the C-terminus of heavy chain; NH indicates attaching the Nab to the N-terminus of heavy chain; CL indicates attaching the Nab to the C-terminus of light chain; NL indicates attaching the Nab to the N-terminus of light chain. Three pairs of IgG-Nab fusions SARs were shown, the pairs were between R2M3 and 26, between R2M3 and 32, and between 18R5 and 26. The assays were performed on Wnt responsive 293 reporter cells in the presence of Rspo and activated Wnt signaling to various levels (FIG. 9). These results demonstrate that the attachment location of the fusion and the geometry between the Fzd and LRP binding domains play roles in the ability of the Wnt surrogates to activate Wnt signaling.

Example 10

Characterization of R2M3-26 in the Fab Format

The molecule R2M3-26 Fab consists of a Fzd binder (R2M3) and a LRP6 binder (26). The LRP6 binder 26 was fused to the N-terminus of R2M3 LC with a 5-amino acid linker as depicted in FIG. 10A. R2M3 was in the form of a Fab. The protein was purified by Ni-NTA affinity column followed by a size-exclusion-chromatography (SEC) step. The absorbance trace from the SEC and the SDS-PAGE gels of the SEC fractions are shown in FIG. 10B. The ability of

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R2M3-26 as a Fab format to activate canonical Wnt signaling was tested in a Wnt responding 293 reporter cells (293STF). The 293STF reporter activity traces across the SEC fractions are shown in FIG. 10B. Unlike when R2M3 was in the IgG format shown in FIG. 2, the peak of the reporter activity from the R2M3 in the Fab format did not correlate with the peak of the proteins. These results suggest that R2M3-26 fusion in the Fab format is ineffective in inducing canonical Wnt signaling as detected by a reporter assay.

Example 11

Characterization of R2M3-32 in the Fab Format

The molecule R2M3-32 Fab consists of a Fzd binder (R2M3) and a LRP6 binder (32). The Lrp6 binder 32 was fused to the N-terminus of R2M3 LC with a 5-amino acid linker as depicted in FIG. 11A. R2M3 was in the form of a Fab. The protein was purified by Ni-NTA affinity column followed by a size-exclusion-chromatography (SEC) step. The absorbance trace from the SEC and the SDS-PAGE gels of the SEC fractions were shown in FIG. 11B. The ability of R2M3-32 as a Fab format to activate canonical Wnt signaling was tested in a Wnt responding 293 reporter cells (293STF). The 293STF reporter activity traces across the SEC fractions are shown in FIG. 11B. Unlike when R2M3 was in the IgG format shown in FIG. 3, the peak of the reporter activity from the R2M3 in the Fab format did not correlate with the peak of the proteins. These results suggest that R2M3-32 fusion in the Fab format is ineffective in inducing canonical Wnt signaling as detected by a reporter assay.

Example 12

Characterization of R2M3-26 in the Heterolg Format

The molecule R2M3-26 Heterolg consists of a Fzd binder (R2M3) and a LRP6 binder (26) as depicted in FIG. 12A and described in FIG. 1A. The protein was purified by Protein A affinity column followed by a size-exclusion-chromatography (SEC) step. The peak fraction from the SEC column was tested in a dose response in the Wnt responsive 293STF reporter cells in the absence or presence of R-spondin (FIG. 12B). Compared to R2M3-26 in the IgG format (as described in FIG. 2), R2M3-26 Heterolg was ineffective in inducing canonical Wnt signaling as detected in the 293 reporter assay.

Example 13

Characterization of 26-17SB9 in the Nab-Nab Format

The molecule 26-17SB9 Nab-Fc-Nab consists of a LRP6 binder (26) and a Fzd binder (17SB9) as depicted in FIG. 13A and described in FIG. 1C. The protein was purified by Protein A affinity column followed by a size-exclusion-chromatography (SEC) step. The peak fraction from the SEC column was tested in a dose response in the Wnt responsive 293STF reporter cells in the absence or presence of R-spondin (FIG. 13B). 26-17SB9 in the Nab-Fc-Nab format induced canonical Wnt signaling as detected in the 293 reporter assay.

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Additional combinations of 26 and 17SB9 were also constructed (FIG. 13C) and tested in 293 reporter assays. As shown in FIGS. 13D and 13E, these various combinations where 26 and 17SB9 were arranged in different tandem formats or on different ends of the Fc fragment all activated Wnt signaling to various levels in the presence of 20 nM R-spondin

Example 14

Characterization of 18R5-LRP6 Binder Fusions in Tandem scFv Formats

The Fzd binder 18R5, the LRP6E1E2 binder 1115.3 (as described in PCT Publication WO2009/064944), and the LRP6E3E4 binder YW211.31.57 (as described in PCT Publication WO2011/119661) were converted into scFv format. 1115.3_scFv or YW211.31.57_scFv is assembled to the N-terminus of 18R5_scFv with a 5, 10 or 15-amino acid linker and 18R5_scFv C-terminus is fused to a human Fc domain. In another set of examples, 1115.3_scFv or YW211.31.57_scFv is assembled to the C-terminus of 18R5_scFv with a 5, 10 or 15-amino acid linker and human Fc domain is fused to the C-terminus of LRP binders. These formats are depicted in FIG. 14G left panel. In another example, 18R5_scFv and LRP binder, 1115.3_scFv, or LRP binder, YW211.31.57_scFv were fused to the two ends of a human Fc domain (as depicted in FIG. 14G right panel). These proteins were purified by Protein A affinity column followed by a SEC step.

The fusion proteins were tested in a Wnt dependent reporter assays in 293 cells. 18R5_scFv-1115.3_scFv-Fc and 1115.3_scFv-18R5_scFv-Fc with 5, 10 or 15-mer linker were able to activate Wnt signaling (FIGS. 14A and 14B). 18R5_scFv-YW211.31.57_scFv-Fc and YW211.31.57_scFv-18R5_scFv-Fc with different linkers activated Wnt signaling (FIGS. 14C and 14D). In addition, 18R5_scFv and 1115.3_scFv or YW211.31.57 fused to the two ends of Fc also activated Wnt signaling (FIGS. 14E and F). While all of these scFv formats activated Wnt signaling, potency and overall maximal efficacy may differ depends on the binder combination, linker length, and relative orientation.

In another example, 1115.3_scFv or YW211.31.57_scFv is assembled to the N-terminus or C-terminus of 18R5_scFv with a 5, 10 or 15-amino acid linker without the further fusion of Fc, to create a bispecific but monovalent binding to each of Fzd or LRP. As shown in FIG. 14H, the 1115.3_scFv and 18R5_scFv fusions were in effective in activating Wnt signaling in the presence of 20 nM R-spondin in 293 reporter cells.

Example 15

Generation of Wnt Surrogate Molecules in the Fab-IgG Format

Wnt mimetic or surrogate molecules can be generated in various formats when both the FZD and LRP binders are Fabs. Various approaches, such as charge paring, "knobs-in-holes", crossover of the heavy and light chains of Fabs, can be employed to ensure proper heavy and light chain pairing. Two examples are given below.

1. Charge-pairing (cp) approach for Fab-on-IgG format: The heavy chain (VH-CH1) domain of an anti-LRP6 Fab, through a linker of 5, 10, or 15-mer amino acids, was fused in tandem with the N-terminus of the heavy

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chain (VH-CH1-CH2-CH3) of an anti-FZD binder. Both VH-CH1 domains of anti-LRP6 and anti-FZD contain three amino acid mutations (Q39D, Q105D, S183K in the anti-LRP6 Fab; Q39K, Q105K, S183E in anti-FZD Fab) each for proper paring with their own partner light chains, which also contain three complementary amino acid mutations (Q38K, A/S43K, S176E in anti-LRP6 light chain; Q38D, A/S43D, S176K in the anti-FZD light chain). The order of the anti-LRP6 and anti-FZD Fabs could be reversed, where the anti-FZD binder is a Fab and is fused to anti-LRP binder which is in IgG format (FIG. 15A).

2. HC-LC cross over approach for Fab-on-IgG format: The light chain (VL-CL) domains of anti-LRP6 binder was, through a linker of 5, 10, or 15-mer amino acids, fused in tandem with the N-terminus of the heavy chain (VH-CH1-CH2-CH3) of an anti-FZD binder. The second construct was VH-CH1 of the anti-LRP6 binder and the third construct was VL-CL of the anti-FZD binder. Similar to the example above, the order of the anti-LRP6 and the anti-FZD binders could be reversed, where anti-FZD binder Fab is fused to the N-terminus of the anti-LRP binder which is in IgG format (FIG. 15A).

Several different pairs of LRP and FZD binders were assembled in these formats and tested in a Wnt responding 293 reporter cell line (293STF). As an example, the anti LRP6E1E2 binder 421.1 (as described in PCT Publication WO2009/064944) was fused to the N-terminus of anti-FZD binder, R2M3, using the charge paring approach to generate, 421.1-R2M3 cp. 421.1-R2M3 cp dose-dependently activated Wnt signaling in the 293 reporter assay (FIG. 15B). An anti-FZD binder, 1RC07, was fused to the N-terminus of an anti-LRP binder, 10SA7, with 5, 10, or 15-mer linkers. All three fusion proteins activated Wnt signaling (FIG. 15C). The anti-FZD binder, 1RC07, was further fused with the anti-LRP binder, 10SG7, either with 1RC07 in the Fab format fused to the N-terminus of 10SG7 in IgG format, or in the reverse order where 10SG7 as Fab fused to the N-terminus of 1RC07 as IgG with either 5, 10, or 15-mer amino acid linkers. All fusion molecules activated Wnt signaling while some preference of orientation and linker length was observed (FIGS. 15D and 15E).

The HC-LC crossover Fab-IgG format was also tested. The anti-FZD binder 1RC07 LC was fused to the N-terminus of the anti-LRP6 binder 10SA7 HC to generate 1RC07-5: 10SA7 L→H. The LC of anti-LRP6E1E2 binder 1115.3 (as described in PCT Publication WO2009/064944) was fused to the N-terminus of the anti-FZD binder R2M3 HC with 5 or 10-mer linkers to generate 1115.3:5:R2M3 L→H or 1115.3:10:R2M3 L→H, respectively. These molecules also activated Wnt signaling (FIGS. 15F and 15G).

Example 16

Characterization of R2M3-26 in the F(ab')₂ Format

R2M3-26 IgG1 was digested by IDES (Promega, WI) at 37° C. for 2 hours. Vast majority of the digested product was R2M3-26F(ab')₂ (FIG. 16A), some partially digested product with one Fab still attached to Fc (designated as R2M3-26F(ab')₂-Fc here) was also detected, and no uncleaved R2M3-26 was detected. The cleaved product was purified by anti-Lambda resin to remove the Fc fragment, then a SEC polishing was followed to separate the R2M3-26F(ab')₂ from R2M3-26F(ab')₂-Fc. The SDS-PAGE gel of the final purified protein is shown in FIG. 16B. The R2M3-26F(ab')₂

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activity was measured in STF assay in HEK293 cells. R2M3-26F(ab')₂ was able to activate Wnt signaling (FIG. 16C).

Example 17

Characterization of Additional Wnt Surrogate Molecules

The FZD binders were fused to LRP binders. The LRP5 or 6 binders were Nabs (or VHs) in this example and were fused to the N-terminus of FZD binders LC with a 5-amino acid linker (as represented in FIG. 17A). These proteins were purified by Protein A affinity column followed by a SEC step. The purified proteins were tested in Wnt dependent reporter assay in 293 cells (FIG. 17B, C, D, H), or 293 cells co-transfected with FZD4 expression construct (FIG. 17E, F), or 293 cells co-transfected with FZD9 expression construct (FIG. 17G) in the presence of 20 nM R-spondin. These molecules activated Wnt signaling with varying levels of potency and efficacy.

Example 18

Characterization of 10SG11-1RC07 in the 2Fv-Ig Format

The molecule 10SG11-1RC07 consists of an N-terminal LRP binder (10SG11) and a Fzd binder (1RC07). The Fv of 10SG11 was fused to the N-terminus of 1RC07 with a 5-amino acid linker as depicted in FIG. 18A. 1RC07 was in the form of an IgG1 with the Fc mutations L234A/L235A/P329G. The protein was purified by a Protein A affinity column followed by an SEC step. The fusion protein was tested in Wnt dependent reporter assays in 293 cell lines and demonstrated the ability to activate Wnt signaling (FIG. 18B-C).

Example 19

In Vivo PK/PD Characterization of R2M3-26

Six-week old C57Bl/6J male mice were obtained from Jackson Laboratories (Bar Harbor, Me., USA) and were housed 3 per cage. All animal experimentation was in accordance with the criteria of the "Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Sciences. Protocols for animal experimentation were approved by the Surrozen Institutional Animal Care and Use Committee. Mice were acclimatized a minimum of two days prior to initiating experiments. Mice had unlimited access to purified, laboratory-grade acidified water and were fed ad libitum (2018 Teklad global 18% protein rodent diet) Mice were kept 12/12-hour light/dark cycle in a 30% to 70% humidity environment and room temperature ranging from 20° C. to 26° C.

For the pharmacokinetic (PK) study (FIG. 20A), n=3 per group was used. Mice were dosed with R2M3-26 (with effectorless Fc mutations) at 1 mg/kg (10 ml/kg in saline) either using intravenous (IV) or intraperitoneal (i.p.) injections. Mice were anesthetized with isoflurane and blood was removed from the retro-orbital plexus, tail vein or heart at 10 minutes, 30 minutes, 1, 4, 24, 72 or 144 hours after injection. Blood was allowed to coagulate at room temperature, followed by centrifugation for 7 minutes at 8,000 g. The serum was removed and stored at -20° C. until the measurement of

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serum R2M3-26 concentrations by ELISA with Anti Human IgG Fc Fragment (Jackson Immuno Research Labs NC9747692).

For the pharmacodynamic (PD) study (FIG. 20B), n=6 per group was used. Mice were injected i.p. with R2M3-26 at the indicated doses (10 ml/kg in saline). Control mice received saline only. Eight hours later, mice were anesthetized with isoflurane and the blood was collected by cardiac puncture. Blood was allowed to coagulate at room temperature, followed by centrifugation for 7 minutes at 10,000 g. The serum was removed and stored at -20° C. until the measurement of serum R2M3-26 concentrations by ELISA. A portion of the left liver lobe was snap-frozen in liquid nitrogen and stored at -80° C. for RNA analysis. RNA was extracted from liver samples using the MagMAX™ mir-Vana™ Total RNA Isolation Kit (ThermoFisher, A27828). cDNA was produced using the high-Capacity cDNA Reverse Transcription Kit (ThermoFisher, 43-688-14). Axin2 mRNA expression was measured by using TaqMan® Fast Advanced Master Mix (ThermoFisher, 4444963) and the Mm00443610_m1 Axin2 Probe (ThermoFisher, 4331182).

These studies showed that R2M3-26 was stable, highly bioavailable and active in vivo, as shown by the induction of Axin2 mRNA expression.

Example 20

In Vivo Bone Model and Characterization of AAV-Delivered Wnt Surrogates

In vivo experiments were conducted by infecting mice with an AAV vector that expressed Flag- and His-tagged 18R5-DKK1c protein (AAV-18R5-DKK1c-FlagHis). 18R5-DKK1c is a fusion protein containing the frizzled binding antibody, 18R5, in scFv format, fused to DKK1c, as described in PCT Publication WO2016/040895, e.g., FIG. 5. Control mice were treated with vehicle only, romosozumab, an AAV vector that expressed green fluorescent protein (GFP) (AAV-CAG-GFP), or an AAV vector that expressed a fusion protein comprising an anti-GFP scFv fused to a mutant DKK1c (AAV-ScFv (anti-GFP)-DKK1cF234K-FlagHis). 28 days after infection, animals were sacrificed and bone mineral density, bone volume and other characteristics were measured. As shown in FIGS. 21A-21E, systemic expression of 18R5-DKK1c resulted in significantly increased bone mineral density as early as 14 days of 18R5-DKK1c systemic expression, as determined by dual X-ray absorptiometry (DEXA) scan. Systemic expression of 18R5-DKK1c increased bone mineral density (BMD) as measured by DEXA scan (FIG. 21A) and increased levels of the serum P1NP bone formation marker in naïve mice (FIG. 21C). Serum levels of AAV-ScFv (anti-GFP)-DKK1cF234K and 18R5-DKK1c were detected in the serum and found to be well above the in vitro determined EC50 (FIG. 21B). AAV-CAG-GFP and AAV-ScFv (anti-GFP)-DKK1cF234K were negative controls. Romosozumab was a positive control, and vehicle only was a negative control. 18R5-DKK1c also increase bone density in lumbar vertebra and whole body, as shown in FIGS. 20D and 20E, wherein * indicates P value<0.05 and ** indicates P value<0.0001. 18R5-DKK1c expression through AAV also increased bone volume in tibia and femur and cortical thickness in femur mid-diaphysis at 28 days after treatment in naïve mice as measured by micro CT, as shown in FIGS. 22A-22D, wherein **** indicates P value<0.0001.

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Systemic expression of 18R5-DKK1c resulted in significantly increased mineral apposition rate from baseline to single label in last 8 days, as shown in FIGS. 23A and 23B.

Systemic expression of 18R5-DKK1c also resulted in increased osteoblast numbers and decreased osteoclast numbers, as shown in FIGS. 24A-24D.

18R5-DKK1c treatment increased bone stiffness and ultimate load to fracture in biomechanical testing, suggesting improved resistance to fracture, as shown in FIGS. 25A-25C.

These studies demonstrated that systemic expression of 18R5-DKK1c using AAV increased bone mineral density (BMD) as measured by DEXA, and also showed that 18R5-DKK1c increased bone volume as measured by micro CT as early as 14 days of treatment. 18R5-DKK1c also increased cortical thickness 28 days after treatment. Systemic expression of 18R5-DKK1c resulted in significantly increased mineral apposition rate, increased osteoblast numbers, and decreased osteoclast numbers. It also increased bone stiffness and ultimate load to fracture, suggesting improved resistance to fracture.

Example 21

In Vivo Bone Model and Characterization of Wnt Surrogates Produced as Recombinant Proteins

In vivo experiments were conducted by treating mice with recombinantly produced R2M3-26 protein at various dosages via i.p. injection. Control mice were treated with vehicle only (negative control), romosozumab (positive control), anti-Beta-Galactosidase (negative control), or IgG2-anti-GFP (negative control). Bone mineral density (BMD) measured by DEXA and bone volume measured by micro CT were monitored longitudinally at indicated time points. Four weeks after treatment, animals were sacrificed and bone characteristics were measured. For single injection of R2M3-26, the experimental data was monitored and is shown for two weeks after treatment.

Treatment with recombinant R2M3-26 induced rapid and sustained increase of bone mineral density (BMD) and bone volume in naïve mice, as shown in FIGS. 26A-26D. Both bone volume and BMD increased rapidly, suggesting resistance to fracture.

The ovariectomy induced osteoporosis model is a well-established high-hurdle model for determining the ability of an anabolic therapy to overcome the bone loss associated with hormone ablation (Zhou, S. et. al., Journal of Cellular Biochemistry, PMID: 11455579). Treatment with recombinant R2M3-26 treatment reversed bone loss in an ovariectomy-induced osteoporosis mouse model, as shown in FIGS. 27A-27C. Increased cortical thickness was observed in the trabecular regions, suggesting increased compressive strength. R2M3-26 treatment increased femur mid-diaphysis cortical bone thickness after 42 days as measured by micro CT, as shown by FIG. 27D. BMD was also increased by R2M3-26 as measured by DEXA, shown in FIG. 27E.

A single injection of R2M3-26 was sufficient to induce rapid bone formation and bone volume within one week, as shown in FIGS. 28A-28C, wherein * indicates P value<0.05.

High dose treatment with R2M3-26 and 1R-C07-26 rapidly and significantly increased bone volume and bone mineral density, and improved biomechanical strength of the bone (ultimate load to failure and stiffness), as shown in FIGS. 29A-29D. 1R-C07-26 showed a robust and significant effect on bone accrual that persisted through 28 days. Both

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R2M3-26 and 1R-C07-26 significantly increased the resistance to fracture after 28 days of treatment by biomechanical testing.

High dose treatment with R2M3-26 and 1R-C07-3 rapidly and significantly increased bone volume, bone mineral density, and cortical thickness after only 14 days of treatment, as shown in FIG. 30A-E. 1R-C07-3 at 10 mpk appeared more effective at increasing bone mass than any other treatment tested in this preclinical model.

These studies demonstrate that recombinant protein treatment can induce rapid and sustained increase of bone mineral density and bone volume in naïve mice and mouse osteoporosis model. Both bone volume and bone mineral density (BMD) increased rapidly, suggesting resistance to fracture. IgG2-anti-GFP is a negative control. Anti-Beta Galactosidase (anti-βgal) was a negative control.

An additional experiment was done to determine systemic skeletal effects of Wnt surrogate molecules in an ovariectomy-induced model of osteoporosis. 057BL/6 females, 4 weeks old at time of ovariectomy, (n=8/group) were compared to sham surgery operated as well as age-matched naïve mice. Animals were injected i.p. with recombinant Wnt surrogate molecules 7 months after surgery and when the onset of osteoporosis was confirmed. Experimental groups included R2M3-26, 1RC07-3, anti-Bgal (Ab control), and vehicle (PBS). Sub-cutaneous injection of romosozumab was done to another cohort of mice for comparison. Animal were treated twice weekly, and followed for 4 weeks.

As shown in FIG. 31, whole body bone mineral density (BMD) was measured weekly using dual-energy X-Ray absorptiometry (DEXA) and treatment with Wnt surrogate molecules can not only reverse but even surpass the total BMD seen in naïve or non-surgical animals. After 4 weeks of treatment, animals were assessed for the vertebral resistance to compression fracture.

Treatment with Wnt surrogate molecules significantly increased the vertebral resistance to compression fracture, as shown by fracture analysis (FIG. 32). 1RC07-3 most robustly increases the Max Force required to compressively fracture the vertebra.

The Einhorn fracture model (Bonnarens F, Einhorn T A. J Orthop Res. 1984; 2(1):97-101.PMID: 6491805) with delayed treatment with Wnt surrogate molecules was used to determine ability of this therapy to induce fracture healing. Delayed treatment with either 1RC07-3 or R2M3-26 was tested to determine if either molecule was capable of contributing to increased fracture healing after a mid-traverse femoral fracture. C57BL/6 females, 16 weeks old at time of fracture (n=8/group) were used. The presence of a cartilaginous callus 2 weeks after fracture was confirmed in all animals before the start of treatment. With delayed treatment to allow for callus formation, a pure osteogenic signal could be elicited, with rapid mineralization of the already existing callus.

Animals were injected i.p. with recombinant Wnt surrogate molecules with the following experimental groups: R2M3-26, 1RC07-3, anti-Bgal (Ab control), and vehicle (PBS). Sub-cutaneous injection of romosozumab was done to another cohort of mice for comparison. Animal were treated twice weekly, and followed for 6 weeks. Radiography was used to visualize changes in mineralization of the callus throughout the experiment (FIGS. 33A and B). An increase in the mineralization and size of the resulting callus with Wnt surrogate treatment is apparent at both 1 and 6 weeks of treatment. One week was sufficient to induce rapid mineralization that is predictive of rapid bone fracture healing and resistance to fracture. 1RC07-3 appeared to

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induce mineralization to a greater extent than R2M3-26. Radiographs taken after 6 weeks of treatment show the persistence of the highly mineralized callus in the 1RCO7-3 group, while some of the fracture callus in the R2M3-26 group has diminished (FIGS. 33B and D).

Whole body DEXA was measured throughout the experiment to examine bone mineral density in not only the fractured femur, but also the contralateral, non-fractured, femur where the expected increases in bone mineral density occurred after treatment (FIGS. 33C and D). This provides reduced risk of secondary fracture in the already fractured limb and the appendicular skeleton. BMD of contralateral femur at 42 days are shown in FIG. 33C.

Following 6-weeks of treatment after the confirmation of callus, we scanned the femurs with micro-computed tomography and determined multiple parameters which have been associated with an increased resistance to fracture after healing. Callus tissue volume, the bone volume within that callus, and especially bone mineral content are all significantly increased within the region of interest examined (FIG. 33D). Qualitatively, the reconstructions show the prevalence of thick osteoid and mineral within the treated fracture. These parameters suggest a robust resistance to fracture and indicate that delayed treatment with Wnt surrogate molecules, after the spontaneous cartilaginous callus formation, can initiate a rapid and significant increase in bone formation.

In another experiment, the dosing schedule was tested to determine whether Wnt surrogate molecule therapy can induce a significant bone anabolic effect, how long that effect persists, and after washout, to determine how the bone responds to additional treatment. Systemic skeletal effects were compared with variable dosing of 1RCO7-3 on anabolic effect, washout, and redosing after establishing baseline. C57BL/6 females, 12 weeks old (n=8/group) were injected i.p. with recombinant Wnt surrogate molecules with the following experimental groups: two groups with 1 RCO7-3, one with anti- β gal (Ab control), and one with vehicle (PBS) on day 0 of the experiment. Sub-cutaneous injection of romosozumab was done to another cohort of mice on day 0 for comparison (romosozumab is an anti-sclerostin antibody (Saag et al., N Engl J Med. 2017 Oct. 12; 377(15):1417-1427; PMID:28892457) that may reverse the bone loss associated with osteoporosis). Animals in 1 RCO7-3 treated groups had significant and rapid induction of bone formation by 14 days (FIG. 34). One group received a second injection at day 14 to determine if the bone anabolic effect could be further enhanced. Interestingly, regardless of treatment, all treatment effects were reversed and normalized after 35 days. A 2-week period was allowed to return to baseline levels prior to a second round of treatment. On day 49, a second round of treatment was done to all experimental groups. The Wnt surrogate treated animals responded rapidly, however not to the same magnitude as with the initial treatment (FIG. 34). For all groups, 5 weeks after the last injection, new bone formation ceased. This indicates that a single injection is capable of significantly increasing bone formation, however the anabolic effect is rapidly lost. This suggests that an anti-resorptive agent may be required in combination with Wnt surrogate therapy to maintain the anabolic effect.

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The mechanism of action of romosozumab relies upon the stimulation of bone formation by removing an inhibitor (sclerostin) of endogenous Wnt signaling. An experiment was done treating animals with Wnt surrogate molecules plus romosozumab to determine if Wnt surrogate molecule treatment was capable of synergizing with romosozumab in a combination study.

C57BL/6 males, 10 weeks old (n=8/group) were injected i.p. with recombinant Wnt surrogate molecules in combination with romosozumab in the following experimental groups: 1RCO7-3 (0.1 mpk), 1RCO7-3 (1 mpk), 1RCO7-3 (10 mpk), 1RCO7-3 (0.1 mpk)+romosozumab (25 mpk), 1RCO7-3 (1 mpk)+romosozumab (25 mpk), 1RCO7-3 (10 mpk)+romosozumab (25 mpk), romosozumab (25 mpk) alone, anti-Bgal (Ab control), and vehicle (PBS). Animals were treated twice weekly and followed for 3 weeks.

Whole body BMD was measured weekly and results are presented in FIG. 35. Conclusions from this study are that endogenous romosozumab can stimulate additional bone growth in the presence of high dose 1 RCO7-3. These data further suggest that peak anabolic action has not yet been reached with 10 mpk 1RCO7-3 treatments. These data also show that romosozumab can stimulate bone formation even in the presence of 1RCO7-3. Overall, This study shows that Wnt surrogate molecule treatment can synergize with romosozumab to enhance the bone anabolic effect after only 21 days of twice weekly treatment.

Changes in gene expression in whole bone were measured in a time course after Wnt surrogate molecule therapy in mice to assess how this therapy regulates expression of genetic markers related to proliferation and osteogenesis. C57BL/6 females, 13 weeks old (n=5/group) were injected i.p. with 1RCO7-3 or anti-Bgal (Ab control) once. Sub-cutaneous injection of romosozumab was done to another cohort of mice for comparison. Cohorts of animals were sacrificed at 8, 24, 48 and 120 hours after treatment, and tibia bones and serum were isolated and flash frozen for RNA extraction. ELISA was used to measure levels of therapeutic molecules in serum over the course of the experiment as described above (FIG. 36).

For purification of RNA from bone, the excised tibia from freshly sacrificed animals were processed as follows: the ends of the tibia were clipped to expose marrow cavity and marrow cavity was flushed with ice cold saline through 30 gauge needle. Steps were taken to ensure all muscle tissue and cartilage has been removed; bone appeared completely white with no red marrow component residual. Tibias were placed in 1.5 mL Eppendorf tubes and flash frozen in liquid nitrogen. To lyse, a single tissue lyser bead was placed in the tube with bone and Trizol was added directly to frozen bone and bead. Tissue lyser at high speed was used to completely homogenize. Homogenates were then subject to chloroform extraction to separate the nucleic acid phase. Further isolation and purification was carried out using an RNeasy mini kit (Qiagen).

RNA Isolated from tibias was tested for relative transcript levels of Runt Related Transcription Factor 2 (RunX2), Collagen Type I Alpha 1 Chain (Col1A1), Dentin Matrix Acidic Phosphoprotein 1 (Dmp1), Alkaline phosphatase (Alp), Receptor activator of nuclear factor kappa-B ligand (RankL), Dickkopf WNT Signaling Pathway Inhibitor 1 (Dkk1), sclerostin (Sost), Cyclin D1 (Cnd1), Axin2, and Ki67.

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TABLE 4

Changes in gene expression in bone with Wnt surrogate molecule (1RC07-3) and romosozumab (Rxmab) Therapy in mice				
Fold Induction +/- SEM relative to Bgal mean value				
gene	Time(hr)	Bgal	Rzmab	1RC073
RunX2	24	1.0 +/- 0.3	8.8 +/- 4.4	9.6 +/- 3.6**
	48	1.0 +/- 0.7	1.9 +/- 1.0	1.5 +/- 0.5
ColA1	24	1.0 +/- 0.2	3.9 +/- 2.0	3.0 +/- 1.0
	48	1.0 +/- 0.5	7.0 +/- 3.3	2.7 +/- 1.5
Dmp1	24	1.0 +/- 0.2	2.7 +/- 1.1	4.8 +/- 2.9
	48	1.0 +/- 0.6	1.6 +/- 0.7	3.8 +/- 1.8
Alp	24	1.0 +/- 0.4	18.3 +/- 11.7	16.5 +/- 6.4*
	48	1.0 +/- 0.6	4.0 +/- 1.8	5.0 +/- 1.9
RankL	24	1.0 +/- 0.3	8.6 +/- 4.5	8.7 +/- 3.3
	48	1.0 +/- 0.7	1.5 +/- 0.6	4.3 +/- 1.4
Dkk1	24	1.0 +/- 0.2	10.5 +/- 5.1	17.9 +/- 6.0**
	48	1.0 +/- 0.5	4.3 +/- 2.5	2.8 +/- 1.3
Sost	24	1.0 +/- 0.2	2.1 +/- 1.0	2.5 +/- 0.9
	48	1.0 +/- 0.6	5.1 +/- 2.3	9.0 +/- 4.2**
Cnd1	24	1.0 +/- 0.4	3.3 +/- 0.8	3.4 +/- 1.8
	48	1.0 +/- 0.6	2.5 +/- 1.2	2.4 +/- 1.2
Axin2	24	1.0 +/- 0.5	3.2 +/- 1.1	6.1 +/- 1.8**
	48	1.0 +/- 0.8	3.6 +/- 2.3	4.6 +/- 1.6*
Ki67	24	1.0 +/- 0.3	7.7 +/- 4.3	10.0 +/- 3.2
	48	1.0 +/- 0.5	1.0 +/- 0.5	25.5 +/- 12.2 ***

* p<0.05, ** p<0.005, 2-way ANOVA comparison with Bgal control

*** p<0.001, 2-way ANOVA comparison with both Bgal and romosozumab

In comparison to anti-sclerostin antibody (romosozumab) treatment, gene expression signatures over the time points were distinct with Wnt surrogate molecule therapy, with induction of more robust Axin2 and Ki67 expression than that caused by romosozumab treatment.

Example 22

In Vivo Liver Regeneration Model and Characterization of AAV-Delivered Wnt Surrogates

In vivo experiments were conducted by infecting approximately 8-weeks old C57BL/6J mice with an AAV vector that expressed Flag- and His-tagged 18R5-DKK1c protein (AAV-18R5-DKK1c-FlagHis). 18R5-DKK1c is a fusion protein containing the frizzled binding antibody, 18R5, in scFv format fused to DKK1c, as described in PCT Publication WO2016/040895, e.g., FIG. 5. Control mice were either injected sub-cutaneously with phosphate-buffered saline (PBS) only or romosozumab (10 mg/kg), or injected intravenously (IV) with an AAV vector that expressed green fluorescent protein (GFP) (AAV-CAG-GFP), or an AAV vector that expressed a fusion protein comprising an anti-GFP scFv fused to a mutant DKK1c (AAV-ScFv (anti-GFP)-DKK1cF234K-Flag-His). 28 days after infection, animals were weighed and sacrificed. The liver was weighed, and the liver to body weight ratio calculated. The content of the small intestine and colon was removed by flushing with phosphate-buffered saline and gentle pressure to expel the content. The small intestine and colon were then weighed.

Systemic expression of 18R5-DKK1c-FlagHis resulted in a significant liver weight increase (FIG. 37A). Systemic expression of the negative controls, eGFP or anti-eGFP-Dkk1cF234K, did not affect the liver to body weight ratio. Administration of the romosozumab recombinant protein or vehicle control, did not affect the liver to body weight ratio.

None of the treatments affected the small intestine (FIG. 37B) or colon (FIG. 37C) to body weight ratio.

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These studies showed that 18R5-DKK1c-FlagHis increases liver weight, but not that of the small or large intestines. This suggests that 18R5-DKK1c-FlagHis can promote liver regeneration.

Example 23

In Vivo Liver Regeneration Model and Characterization of Recombinantly Produced Wnt Surrogates

In vivo experiments were conducted by treating mice with recombinantly produced anti-eGFP, R2M3-26, 1R-C07-26, romosozumab or Rspo2 proteins at various dosages. The Rspo2 protein is a fusion protein between a short splice variant of the Rspo2 gene and a human Fc fragment.

In one study, mice were housed 4 per cage and n=8 per treatment group were used. Approximately 8-weeks old C57BL/6J mice were administered recombinant proteins, anti-eGFP (1 mg/kg), R2M3-26 (1 or 10 mg/kg) or 1R-C07-26 (1, 5 or 10 mg/kg), twice weekly intra-peritoneally (i.p.) for four weeks. In addition, groups of mice were administered romosozumab (30 mg/kg) or PBS vehicle control, subcutaneously.

Mice were weighed at the beginning and throughout the treatment. None of the treatment with recombinant proteins affected total body weight significantly (FIG. 38A). On day 28, the liver was weighed, and the liver to body weight ratio calculated (FIG. 38B). The highest dose of R2M3-26 (10 mg/kg) resulted in a significant increase in liver to body weight ratio. None of the other treatment affected the liver weight significantly.

The increase in liver weight in response to R2M3-26 suggest that this recombinant protein can promote liver regeneration.

In another study, mice were housed 5 per cage and n=10 per treatment group were used. Approximately 8-weeks old C57BL/6J mice received a single i.p. injection containing anti-eGFP (0.56 mg/kg), R2M3-26 (0.3 mg/kg) or Rspo2 (0.46 mg/kg) alone, or with a combination of R2M3-26 (0.1 mg/kg) and Rspo2 (0.46 mg/kg).

24 or 48 hours after injection, mice were euthanized. A portion of the left liver lobe was snap-frozen in liquid nitrogen and stored at -80° C. for RNA analysis. Cyclin D1 and Ki67 expression were measured by performing qPCR using the Mm00432359_m1 Cnd1 probe and the Mm01278617_m1 Ki67 probe (Thermofisher, 4331182). An additional portion of the left liver lobe was fixed in formalin and embedded in paraffin for immunohistochemistry analysis. Sections were stained with the anti-proliferating cell nuclear antigen (PCNA) (Abcam, ab18197) or anti-phospho-histone H3 (pH3) rabbit antibodies (Abcam, ab47297). The number of positive nuclei were counted using the image processing software, Image J.

Rspo2 alone increased Ki67 (FIG. 39A) and CyclinD1 (FIG. 39B) mRNA expression. In combination with R2M3-26, Rspo2 increased Ki67 and CyclinD1 expression further than with Rspo2 alone at 24 and 48 hours after treatment with recombinant proteins. Rspo2 alone increased the number of PCNA (FIG. 39C) and pH3 (FIG. 39D) positive nuclei in liver sections. In combination with R2M3-26, Rspo2 increased the number of PCNA and pH3 positive nuclei further than with Rspo2 alone at 48 hours after treatment with recombinant proteins.

These studies show that the proliferation markers, Ki67 mRNA, CyclinD1 mRNA and PCNA-positive nuclei, and the pH3 mitotic marker are induced by the R2M3-26 and

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Rspo2 recombinant proteins and suggest that these recombinant proteins can promote liver regeneration.

Example 24

In Vivo Chronic Liver Injury Model and Characterization of AAV-Delivered Wnt Surrogates

Two in vivo experiments were conducted in two, thioacetamide (TAA)- and CC14-induced, liver cirrhosis mouse models, thioacetamide (TAA)- and CC14-induced, to test the effect of AAV vectors expressing 18R5-DKK1c-FlagHis or Rspo2 protein on chronic liver injury. TAA was added to the drinking water of 6 weeks old C57BL/6J mice at a concentration of 300 mg/L throughout the TAA treatment duration. Mice were housed 5 per cage and groups of n=10 were used, except for the control groups without TAA treatment where n=5 per group was used.

In Study 1 (FIGS. 40A, 40C, 40E, 40G-H), mice with (n=10) or without (n=5) TAA treatment were weighed and sacrificed after 9 weeks of TAA addition into the drinking water, to measure baseline values. Livers were weighed and liver samples were collected for mRNA and histological analysis. TAA supplementation was maintained in the drinking water of the remaining mice and they were injected IV with AAV vectors that expressed an enhanced green fluorescent protein (eGFP) (3e10 genomic particles (GC)), 18R5-DKK1c-FlagHis (3e10 or 1e11 GC) or Rspo2 protein (1e11 GC) or a combination of 18R5-DKK1c-FlagHis (3e10 GC) and Rspo2 (1e11 GC). 5 age-matched naïve animals (no TAA) were kept as a negative control. Three weeks after AAV injection, all mice were weighed and euthanized. Livers were weighed and liver samples were collected for mRNA and histological analysis.

Treatment with 18R5Dkk1FH or Rspo2 resulted in a significant increase of liver weight (FIG. 40C) and liver to body weight ratio (FIG. 40E) in mice undergoing a continuous exposure to TAA. Treatment with a combination of 18R5Dkk1FH and Rspo2 resulted in a further increase in liver weight and liver to body weight ratio beyond that observed with either treatment alone. The combination 18R5Dkk1FH and Rspo2 treatment resulted in a decrease of the fibrosis marker Colla1 mRNA expression (FIG. 40G). Histological liver sections were stained with Sirius red to visualize the accumulation of collagen in fibrotic area (FIG. 40H). Quantification of the percentage of red area, using Image J analysis software, showed a significant increase of fibrotic area in TAA-treated mice. A combination of 18R5Dkk1FH and Rspo2 resulted in a reversal of fibrotic area increase when compared to mice treated with the eGFP negative control. Treatment with Rspo2 alone also resulted in a significant but smaller reversal than the combination treatment.

In Study 2, (FIGS. 40B, 40D, 40F), mice were exposed to TAA-supplemented water for 11 weeks, and were returned to standard drinking thereafter, two days prior to AAV treatment. At the start of AAV treatment, mice exposed to TAA (n=10) or not (n=5) were weighed and sacrificed to collect liver samples for baseline measurements. The remaining mice were injected with AAV vectors that expressed an enhanced green fluorescent protein (eGFP)

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(1.3e11 genomic particles (GC)), 18R5-DKK1c-FlagHis (3e10 or 1e11 GC) or Rspo2 protein (1e11 GC) or a combination of 18R5-DKK1c-FlagHis (3e10 GC) and Rspo2 (1e11 GC). 5 age-matched naïve animals (no TAA) were kept as a negative control. Three weeks after AAV injection, all mice were weighed and euthanized. Livers were weighed and liver samples were collected for mRNA and histological analysis.

A similar liver weight and liver to body weight increase was observed in mice treated with 18R5-Dkk1c-FlagHis and Rspo2 either alone or in combination than what was observed in Study 1 (FIGS. 40D, 40F).

These studies show that 18R5-Dkk1c-FlagHis and Rspo2 can increase liver weight in a TAA-induced liver cirrhosis model and reduce fibrosis markers. These results suggest that 18R5-Dkk1c-FlagHis and Rspo2 can promote liver tissue repair after chronic liver injury.

Example 25

In Vivo Chronic Liver Injury Models and Characterization of Recombinantly Produced Wnt Surrogates

In vivo experiments were conducted in thioacetamide (TAA)-induced and CC14-induced mouse models of liver cirrhosis by treating mice with recombinantly produced anti-eGFP, R2M3-26 and Rspo2 proteins.

In the TAA-induced cirrhosis model, six weeks old male mice were exposed to TAA-supplemented drinking water (300 mg/L) for approximately 22 weeks (FIG. 41A). TAA exposure was removed two days prior to beginning of treatment with recombinant proteins and mice were provided with fresh drinking water. Mice were housed 5 per cage and n=10 per treatment group were used. In a mono treatment study (FIGS. 41B, 41D, 41F, 41H, 41J, 41L), mice were injected i.p. with anti-eGFP (1 mg/kg) or Rspo2 (1 mg/kg) twice weekly. In a combination treatment study (FIGS. 41C, 41E, 41G, 41I, 41K, 41M), mice were injected i.p. with anti-eGFP (1.3 mg/kg) or a combination of R2M3-26 (0.3 mg/kg) and Rspo2 (1 mg/kg) twice weekly. Mice were then weighed and sacrificed at day 3, 7 or 14 days after beginning of treatment. Groups of control mice without exposure to TAA (n=5 per group) were euthanized at day 0 and day 14 in both studies.

Treatment with Rspo2 protein alone or in combination with R2M3-26 resulted in an increase in liver to body weight ratio (FIGS. 41B and 41C) and a transient stimulation of the Wnt signaling pathway as shown by an increase in Axin2 expression (FIGS. 41D and 41E). Treatment with Rspo2 protein alone or in combination with R2M3-26 induced the following proliferation markers: cyclinD1 (FIGS. 41F, 41G) and Ki67 (FIGS. 41H and 41I) mRNA expression, PCNA (FIGS. 41J, 41K) and pH3 (FIGS. 41L, 41M) positive nuclei.

In an additional study, plasma was collected for prothrombin time measurement. Coagulation time is impaired in TAA-induced cirrhosis model as exemplified by an increase in pro-thrombin (PT) test to normal value ratio in mice exposed to TAA when compared to normal mice without TAA exposure (FIG. 41N). Treatment with Rspo2 (1 mg/kg)

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and R2M3-26 (0.3 mg/kg) reversed the prolongation in PT time as shown by the decrease in PT_{test}/PT_{normal} ratio at day 7 and day 14 after biweekly Rspo2 and R2M3-26 treatment.

These studies show that Rspo2 and R2M3-26 can stimulate liver cell proliferation and improve hepatocytes functional activity such as pro-thrombin time in a TAA-induced liver cirrhosis model. These results suggest that Rspo2 and R2M3-26 can promote liver tissue repair in chronic liver disease.

In the CC14-induced cirrhosis model, six-weeks old C57BL/6J male mice were injected i.p. with 2 ml/kg CC14 in mineral oil, twice weekly for 8 weeks (FIG. 42A). 3 days after the last CC14 injection, mice were injected i.p. twice weekly with the following recombinant proteins: anti- β -galactosidase (10 mg/kg), Rspo2 (1 or 10 mg/kg), or a combination of R2M3-26 (0.3 mg/kg) and Rspo2 (1 mg/kg). Three additional control groups were included: one group injected with CC14 but no proteins, one group injected with mineral oil, and one untreated age-matched naïve group. n=8 were used for each group. After two weeks of treatment with recombinant proteins, mice were weighed and sacrificed. Plasma was collected for pro-thrombin time measurement. Livers were weighed and liver samples were collected for histological analysis.

Treatment with R2M3-26 and Rspo2 resulted in a significant increase in liver to body weight ratio when compared to treatment with anti- β -galactosidase negative control (FIG. 42B). Treatment with Rspo2 (10 mg/kg) or a combination of R2M3-26 and Rspo2 resulted in a significant decrease in prothrombin time (FIG. 42C). Treatment with Rspo2 (10 mg/kg) or a combination of R2M3-26 and Rspo2 resulted in a significant reversal in fibrotic area, induced by CC14 (FIG. 42D).

This study showed that Rspo2 and R2M3-26 can induce an increase in liver weight, improve hepatocytes functional activity such as pro-thrombin time and reduce fibrosis markers in a CC14-induced liver cirrhosis model. These results suggest that Rspo2 and R2M3-26 can promote liver tissue repair in chronic liver disease.

Example 26

In Vivo Acute Liver Injury Model and Characterization of Recombinantly Produced Wnt Surrogates

In vivo experiments were conducted using acetaminophen-induced mouse models of acute liver injury by treating mice with recombinantly produced anti-eGFP, R2M3-26 and Rspo2 proteins.

Eight-week old C57BL/6 male mice were housed 5 per cage. n=10 were used per group. Mice were fasted overnight for 12 hours. Acetaminophen (APAP) was administered i.p. at a sublethal dose (300 mg/kg).

In a first study, anti-eGFP (0.3 mpk) or R2M3-26 (0.3 mpk) were injected i.p. either immediately after or 3 or 6 hours after APAP injection (FIG. 43). Serum samples were collected at 24 and 48 hours after APAP injection for ALT measurements. Mice were sacrificed 48 hours after APAP injection and liver samples were collected for mRNA analysis.

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R2M3-26 treatment did not affect ALT level significantly (FIG. 43B). Treatment with R2M3-26 induced cyclinD1 (FIG. 43C) and Ki67 (FIG. 43D) mRNA significantly, beyond the level induced by APAP treatment alone.

In a second study, human Fc (0.46 mg/kg) or Rspo2 (0.46 mg/kg) were injected i.p. either immediately after or 3 or 6 hours after APAP injection (FIG. 44). Serum samples were collected at 24 and 48 hours after APAP injection. Liver samples were collected at 48 hours after APAP injection. Rspo2 treatment did not affect ALT serum level significantly (FIG. 44B). Treatment with Rspo2 induced cyclinD1 (FIG. 44C) and Ki67 (FIG. 43D) mRNA significantly, beyond the level induced by APAP treatment alone.

In a third study, anti-eGFP (0.56 mg/kg) or a combination of R2M3-26 (0.1 mg/kg) and Rspo2 (0.46 mg/kg) were injected i.p. 3 hours after APAP administration. Serum and liver samples were collected at 24, 36, 48 and 60 hours after APAP injection for ALT measurements and mRNA analysis.

R2M3-26 and Rspo2 combination treatment did not affect ALT level significantly (FIG. 45B). Treatment with R2M3-26 and Rspo2 induced cyclinD1 (FIG. 45C) and Ki67 (FIG. 45D) mRNA significantly, beyond the level induced by APAP treatment alone.

An additional study was performed to evaluate the effect of Rspo2 and R2M3-26 on the survival of mice treated with a dose of 600 mg/kg of acetaminophen (FIG. 46). Anti-eGFP (0.3 mg/kg), R2M3-26 (0.3 mg/kg), Rspo2 (0.46 mg/kg) or a combination of R2M3-26 (0.1 mg/kg) and Rspo2 (0.46 mg/kg) were injected i.p. 3 hours after APAP administration. Mice were monitored several times daily over the next 96 hours. A consistent trend in improving survival was observed in groups treated with R2M3-26 (FIG. 46B), Rspo2 (FIG. 46C) or a combination of R2M3-26 and Rspo2 (FIG. 46D).

These studies show that Rspo2 and R2M3-26 can induce proliferation markers beyond that induced spontaneously in APAP-induced acute injury model. These results suggest that Rspo2 and R2M3-26 can enhance liver tissue repair after an acute liver injury.

The various embodiments described above can be combined to provide further embodiments. All of the U.S. patents, U.S. patent application publications, U.S. patent applications, foreign patents, foreign patent applications and non-patent publications referred to in this specification and/or listed in the Application Data Sheet are incorporated herein by reference, in their entirety. Aspects of the embodiments can be modified, if necessary to employ concepts of the various patents, applications and publications to provide yet further embodiments. These and other changes can be made to the embodiments in light of the above-detailed description.

In general, in the following claims, the terms used should not be construed to limit the claims to the specific embodiments disclosed in the specification and the claims, but should be construed to include all possible embodiments along with the full scope of equivalents to which such claims are entitled. Accordingly, the claims are not limited by the disclosure.

SEQUENCE LISTING

The patent contains a lengthy sequence listing. A copy of the sequence listing is available in electronic form from the USPTO web site (<https://seqdata.uspto.gov/?pageRequest=docDetail&DocID=US11773171B2>). An electronic copy of the sequence listing will also be available from the USPTO upon request and payment of the fee set forth in 37 CFR 1.19(b)(3).

What is claimed is:

1. A soluble, multivalent, multispecific Wnt surrogate molecule, wherein the Wnt surrogate molecule comprises:

(i) one or more Fzd binding regions that specifically binds to one or more Frizzled (Fzd) receptors, wherein the one or more Fzd binding regions comprise one or more antibody or antigen-binding fragment of the antibody, wherein the one or more antibody or the antigen-binding fragment comprises: a CDRH1 of SEQ ID NO: 307, a CDRH2 of SEQ ID NO: 501, a CDRH3 of SEQ ID NO: 964, a CDRL1 of SEQ ID NO: 1098, a CDRL2 of SEQ ID NO: 1175, and a CDRL3 of SEQ ID NO: 1372; and

(ii) one or more LRP5/6 binding regions that specifically binds to a Low-density lipoprotein (LDL) receptor-related protein 5 (LRP5) and/or a Low-density lipoprotein (LDL) receptor-related protein 6 (LRP6), wherein the one or more LRP5/6 binding regions comprise one or more antibody or antigen-binding fragment of the antibody, wherein the one or more antibody or the antigen-binding fragment comprises: a CDRH1 of SEQ ID NO: 1618, a CDRH2 of SEQ ID NO: 1715, and a CDRH3 of SEQ ID NO: 2012.

2. The Wnt surrogate molecule of claim 1, wherein the Wnt surrogate molecule comprises one or more polypeptides having at least 90% identity to the sequence set forth in SEQ ID NO: 137 and one or more polypeptide having at least 90% identity to the sequence set forth in SEQ ID NO: 153.

3. The Wnt surrogate molecule of claim 1, wherein the Fzd binding region comprises a full immunoglobulin G (IgG), comprising two light chains and two heavy chains, and the LRP5/6 binding region comprises a VHH.

4. The Wnt surrogate molecule of claim 3, wherein each of the one or more LRP5/6 binding regions is fused to the N-terminus of one of the light chains.

5. A pharmaceutical composition comprising a physiologically acceptable excipient, diluent, or carrier, and the Wnt surrogate molecule according to claim 1.

6. The Wnt Surrogate molecule of claim 4, wherein each of the one or more LRP5/6 binding regions is fused to one of the light chains via a linker moiety.

7. The Wnt surrogate molecule of claim 6, wherein the Wnt surrogate molecule comprises:

(i) two Fzd binding regions, wherein each of the Fzd binding regions comprise one or more antibody or antigen-binding fragment thereof, wherein the one or more antibody or the antigen-binding fragment thereof comprises: a CDRH1 of SEQ ID NO: 307, a CDRH2 of SEQ ID NO: 501, a CDRH3 of SEQ ID NO: 964, a CDRL1 of SEQ ID NO: 1098, a CDRL2 of SEQ ID NO: 1175, and a CDRL3 of SEQ ID NO: 1372; and

(ii) two LRP5/6 binding regions, wherein each of the LRP5/6 binding regions comprise one or more antibody or antigen-binding fragment thereof, wherein the one or more antibody or the antigen-binding fragment thereof comprises: a CDRH1 of SEQ ID NO: 1618, a CDRH2 of SEQ ID NO: 1715, and a CDRH3 of SEQ ID NO: 2012.

8. The Wnt surrogate molecule of claim 6, wherein the Wnt surrogate molecule comprises two polypeptides having at least 90% identity to the sequence set forth in SEQ ID NO: 137 and two polypeptides having at least 90% identity to the sequence set forth in SEQ ID NO: 153.

9. A pharmaceutical composition comprising the physiologically acceptable excipient, diluent, or carrier, and the Wnt surrogate molecule of claim 7.

10. A pharmaceutical composition comprising the physiologically acceptable excipient, diluent, or carrier, and the Wnt surrogate molecule of claim 8.

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