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Application Number: 62607877 Document Date: 12/19/2017

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Form Revision Date: August 26, 2013

Doc Code: **TR.PROV**
Document Description: Provisional Cover Sheet (SB16)

PTO/SB/16 (11-08)
Approved for use through 05/31/2015. OMB 0651-0032
U.S. Patent and Trademark Office: U.S. DEPARTMENT OF COMMERCE
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Provisional Application for Patent Cover Sheet

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c)

Inventor(s)

Inventor 1 — Remove

Given Name	Middle Name	Family Name	City	State	Country
Yang		Li	Mountain View	CA	US

Inventor 2 — Remove

Given Name	Middle Name	Family Name	City	State	Country
Tom	Zhiye	Yuan	Union City	CA	US

Inventor 3 — Remove

Given Name	Middle Name	Family Name	City	State	Country
Aaron	Ken	Sato	Burlingame	CA	US

Inventor 4 — Remove

Given Name	Middle Name	Family Name	City	State	Country
Wen-Chen		Yeh	Belmont	CA	US

All Inventors Must Be Listed – Additional Inventor Information blocks may be generated within this form by selecting the **Add** button. — Add

Title of Invention	ANTI-FRIZZLED ANTIBODIES AND METHODS OF USE
Attorney Docket Number (if applicable)	SRZN-004/00US 328202-2016

Correspondence Address

Direct all correspondence to (select one):

(•) The address corresponding to Customer Number	( ) Firm or Individual Name
Customer Number	58249

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

(•) No.

( ) Yes, the invention was made by an agency of the United States Government. The U.S. Government agency name is:

( ) Yes, the invention was under a contract with an agency of the United States Government. The name of the U.S. Government agency and Government contract number are:

EFS - Web 1.0.1

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Entity Status
Applicant asserts small entity status under 37 CFR 1.27 or applicant certifies micro entity status under 37 CFR 1.29

- (●) Applicant asserts small entity status under 37 CFR 1.27
- ( ) Applicant certifies micro entity status under 37 CFR 1.29. Applicant must attach form PTO/SB/15A or B or equivalent.
- ( ) No

Warning

Petitioner/applicant is cautioned to avoid submitting personal information in documents filed in a patent application that may contribute to identity theft. Personal information such as social security numbers, bank account numbers, or credit card numbers (other than a check or credit card authorization form PTO-2038 submitted for payment purposes) is never required by the USPTO to support a petition or an application. If this type of personal information is included in documents submitted to the USPTO, petitioners/applicants should consider redacting such personal information from the documents before submitting them to USPTO. Petitioner/applicant is advised that the record of a patent application is available to the public after publication of the application (unless a non-publication request in compliance with 37 CFR 1.213(a) is made in the application) or issuance of a patent. Furthermore, the record from an abandoned application may also be available to the public if the application is referenced in a published application or an issued patent (see 37 CFR1.14). Checks and credit card authorization forms PTO-2038 submitted for payment purposes are not retained in the application file and therefore are not publicly available.

Signature

Please see 37 CFR 1.4(d) for the form of the signature.

Signature	/Carol D. Laherty/			Date (YYYY-MM-DD)	2017-12-19
First Name	Carol D.	Last Name	Laherty	Registration Number (If appropriate)	51909

This collection of information is required by 37 CFR 1.51. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 8 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **This form can only be used when in conjunction with EFS-Web. If this form is mailed to the USPTO, it may cause delays in handling the provisional application.**

Privacy Act Statement

The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or paten. Accordingly, pursuant to the requirements of the Act, please be advised that : (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether disclosure of these records is required by the Freedom of Information Act.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, t o a n other federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	SRZN-004/00US 328202-2016
	Application Number	
Title of Invention	ANTI-FRIZZLED ANTIBODIES AND METHODS OF USE	

The application data sheet is part of the provisional or nonprovisional application for which it is being submitted. The following form contains the bibliographic data arranged in a format specified by the United States Patent and Trademark Office as outlined in 37 CFR 1.76.
This document may be completed electronically and submitted to the Office in electronic format using the Electronic Filing System (EFS) or the document may be printed and included in a paper filed application.

Secrecy Order 37 CFR 5.2:

☐ Portions or all of the application associated with this Application Data Sheet may fall under a Secrecy Order pursuant to 37 CFR 5.2 (Paper filers only. Applications that fall under Secrecy Order may not be filed electronically.)

Inventor Information:

Inventor 1 Remove

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
	Yang		Li	

Residence Information (Select One) ◉ US Residency ○ Non US Residency ○ Active US Military Service

City	Mountain View	State/Province	CA	Country of Residence	US

Mailing Address of Inventor:

Address 1	1142 Cuesta Drive		
Address 2			
City	Mountain View	State/Province	CA
Postal Code	94040	Country	US

Inventor 2 Remove

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
	Tom	Zhiye	Yuan	

Residence Information (Select One) ◉ US Residency ○ Non US Residency ○ Active US Military Service

City	Union City	State/Province	CA	Country of Residence	US

Mailing Address of Inventor:

Address 1	33755 15th Street		
Address 2			
City	Union City	State/Province	CA
Postal Code	94587	Country	US

Inventor 3 Remove

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
	Aaron	Ken	Sato	

Residence Information (Select One) ◉ US Residency ○ Non US Residency ○ Active US Military Service

PTO/AIA/14 (11-15)
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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	SRZN-004/00US 328202-2016
	Application Number	
Title of Invention	ANTI-FRIZZLED ANTIBODIES AND METHODS OF USE	

City	Burlingame	State/Province	CA	Country of Residence	US

Mailing Address of Inventor:

Address 1	1640 Coronado Way		
Address 2			
City	Burlingame	State/Province	CA
Postal Code	94010	Country	US

Inventor 4 Remove

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
	Wen-Chen		Yeh	

Residence Information (Select One) (•) US Residency ( ) Non US Residency ( ) Active US Military Service

City	Belmont	State/Province	CA	Country of Residence	US

Mailing Address of Inventor:

Address 1	2920 Belmont Woods Way		
Address 2			
City	Belmont	State/Province	CA
Postal Code	94010	Country	US

All Inventors Must Be Listed - Additional Inventor Information blocks may be generated within this form by selecting the **Add** button. Add

Correspondence Information:

Enter either Customer Number or complete the Correspondence Information section below.
For further information see 37 CFR 1.33(a).

☐ **An Address is being provided for the correspondence Information of this application.**

Customer Number	58249		
Email Address		Add Email	Remove Email

Application Information:

Title of the Invention	ANTI-FRIZZLED ANTIBODIES AND METHODS OF USE		
Attorney Docket Number	SRZN-004/00US 328202-2016	Small Entity Status Claimed	☒
Application Type	Provisional		
Subject Matter	Utility		
Total Number of Drawing Sheets (if any)	0	Suggested Figure for Publication (if any)	

PTO/AIA/14 (11-15)
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	Application Number	
Title of Invention	ANTI-FRIZZLED ANTIBODIES AND METHODS OF USE	

Filing By Reference:

Only complete this section when filing an application by reference under 35 U.S.C. 111(c) and 37 CFR 1.57(a). Do not complete this section if application papers including a specification and any drawings are being filed. Any domestic benefit or foreign priority information must be provided in the appropriate section(s) below (i.e., "Domestic Benefit/National Stage Information" and "Foreign Priority Information").

For the purposes of a filing date under 37 CFR 1.53(b), the description and any drawings of the present application are replaced by this reference to the previously filed application, subject to conditions and requirements of 37 CFR 1.57(a).

Application number of the previously filed application	Filing date (YYYY-MM-DD)	Intellectual Property Authority or Country

Publication Information:

☐ Request Early Publication (Fee required at time of Request 37 CFR 1.219)

☐ **Request Not to Publish.** I hereby request that the attached application not be published under 35 U.S.C. 122(b) and certify that the invention disclosed in the attached application **has not and will not** be the subject of an application filed in another country, or under a multilateral international agreement, that requires publication at eighteen months after filing.

Representative Information:

Representative information should be provided for all practitioners having a power of attorney in the application. Providing this information in the Application Data Sheet does not constitute a power of attorney in the application (see 37 CFR 1.32).
Either enter Customer Number or complete the Representative Name section below. If both sections are completed the customer Number will be used for the Representative Information during processing.

Please Select One:	◉ Customer Number	○ US Patent Practitioner	○ Limited Recognition (37 CFR 11.9)
Customer Number	58249		

Domestic Benefit/National Stage Information:

This section allows for the applicant to either claim benefit under 35 U.S.C. 119(e), 120, 121, 365(c), or 386(c) or indicate National Stage entry from a PCT application. Providing benefit claim information in the Application Data Sheet constitutes the specific reference required by 35 U.S.C. 119(e) or 120, and 37 CFR 1.78.
When referring to the current application, please leave the "Application Number" field blank.

Prior Application Status			Remove
Application Number	Continuity Type	Prior Application Number	Filing or 371(c) Date (YYYY-MM-DD)

Additional Domestic Benefit/National Stage Data may be generated within this form by selecting the **Add** button.

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	SRZN-004/00US 328202-2016
	Application Number	
Title of Invention	ANTI-FRIZZLED ANTIBODIES AND METHODS OF USE	

Foreign Priority Information:

This section allows for the applicant to claim priority to a foreign application. Providing this information in the application data sheet constitutes the claim for priority as required by 35 U.S.C. 119(b) and 37 CFR 1.55. When priority is claimed to a foreign application that is eligible for retrieval under the priority document exchange program (PDX)[i] the information will be used by the Office to automatically attempt retrieval pursuant to 37 CFR 1.55(i)(1) and (2). Under the PDX program, applicant bears the ultimate responsibility for ensuring that a copy of the foreign application is received by the Office from the participating foreign intellectual property office, or a certified copy of the foreign priority application is filed, within the time period specified in 37 CFR 1.55(g)(1).

Remove

Application Number	Country[i]	Filing Date (YYYY-MM-DD)	Access Code[i] (if applicable)

Additional Foreign Priority Data may be generated within this form by selecting the **Add** button.

Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications

☐ This application (1) claims priority to or the benefit of an application filed before March 16, 2013 and (2) also contains, or contained at any time, a claim to a claimed invention that has an effective filing date on or after March 16, 2013.
NOTE: By providing this statement under 37 CFR 1.55 or 1.78, this application, with a filing date on or after March 16, 2013, will be examined under the first inventor to file provisions of the AIA.

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	SRZN-004/00US 328202-2016
	Application Number	
Title of Invention	ANTI-FRIZZLED ANTIBODIES AND METHODS OF USE	

Authorization or Opt-Out of Authorization to Permit Access:

When this Application Data Sheet is properly signed and filed with the application, applicant has provided written authority to permit a participating foreign intellectual property (IP) office access to the instant application-as-filed (see paragraph A in subsection 1 below) and the European Patent Office (EPO) access to any search results from the instant application (see paragraph B in subsection 1 below).

Should applicant choose not to provide an authorization identified in subsection 1 below, applicant **must opt-out** of the authorization by checking the corresponding box A or B or both in subsection 2 below.

NOTE: This section of the Application Data Sheet is **ONLY** reviewed and processed with the **INITIAL** filing of an application. After the initial filing of an application, an Application Data Sheet cannot be used to provide or rescind authorization for access by a foreign IP office(s). Instead, Form PTO/SB/39 or PTO/SB/69 must be used as appropriate.

1. Authorization to Permit Access by a Foreign Intellectual Property Office(s)

A. Priority Document Exchange (PDX) - Unless box A in subsection 2 (opt-out of authorization) is checked, the undersigned hereby **grants the USPTO authority** to provide the European Patent Office (EPO), the Japan Patent Office (JPO), the Korean Intellectual Property Office (KIPO), the State Intellectual Property Office of the People's Republic of China (SIPO), the World Intellectual Property Organization (WIPO), and any other foreign intellectual property office participating with the USPTO in a bilateral or multilateral priority document exchange agreement in which a foreign application claiming priority to the instant patent application is filed, access to: (1) the instant patent application-as-filed and its related bibliographic data, (2) any foreign or domestic application to which priority or benefit is claimed by the instant application and its related bibliographic data, and (3) the date of filing of this Authorization. See 37 CFR 1.14(h)(1).

B. Search Results from U.S. Application to EPO - Unless box B in subsection 2 (opt-out of authorization) is checked, the undersigned hereby **grants the USPTO authority** to provide the EPO access to the bibliographic data and search results from the instant patent application when a European patent application claiming priority to the instant patent application is filed. See 37 CFR 1.14(h)(2).

The applicant is reminded that the EPO's Rule 141(1) EPC (European Patent Convention) requires applicants to submit a copy of search results from the instant application without delay in a European patent application that claims priority to the instant application.

2. Opt-Out of Authorizations to Permit Access by a Foreign Intellectual Property Office(s)

☒ A. Applicant **DOES NOT** authorize the USPTO to permit a participating foreign IP office access to the instant application-as-filed. If this box is checked, the USPTO will not be providing a participating foreign IP office with any documents and information identified in subsection 1A above.

☒ B. Applicant **DOES NOT** authorize the USPTO to transmit to the EPO any search results from the instant patent application. If this box is checked, the USPTO will not be providing the EPO with search results from the instant application.

NOTE: Once the application has published or is otherwise publicly available, the USPTO may provide access to the application in accordance with 37 CFR 1.14.

PTO/AIA/14 (11-15)
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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	SRZN-004/00US 328202-2016
	Application Number	
Title of Invention	ANTI-FRIZZLED ANTIBODIES AND METHODS OF USE	

Applicant Information:

Providing assignment information in this section does not substitute for compliance with any requirement of part 3 of Title 37 of CFR to have an assignment recorded by the Office.

Applicant 1

If the applicant is the inventor (or the remaining joint inventor or inventors under 37 CFR 1.45), this section should not be completed. The information to be provided in this section is the name and address of the legal representative who is the applicant under 37 CFR 1.43; or the name and address of the assignee, person to whom the inventor is under an obligation to assign the invention, or person who otherwise shows sufficient proprietary interest in the matter who is the applicant under 37 CFR 1.46. If the applicant is an applicant under 37 CFR 1.46 (assignee, person to whom the inventor is obligated to assign, or person who otherwise shows sufficient proprietary interest) together with one or more joint inventors, then the joint inventor or inventors who are also the applicant should be identified in this section.

Clear

◉ Assignee	○ Legal Representative under 35 U.S.C. 117	○ Joint Inventor
○ Person to whom the inventor is obligated to assign.	○ Person who shows sufficient proprietary interest	

If applicant is the legal representative, indicate the authority to file the patent application, the inventor is:

Name of the Deceased or Legally Incapacitated Inventor:	

If the Applicant is an Organization check here. ☒

Organization Name	Surrozen, Inc.

Mailing Address Information For Applicant:

Address 1	171 Oyster Point Blvd.		
Address 2	Suite 400		
City	South San Francisco	**State/Province**	CA
Country	US	Postal Code	94080
Phone Number		Fax Number	
Email Address			

Additional Applicant Data may be generated within this form by selecting the Add button.

Assignee Information including Non-Applicant Assignee Information:

Providing assignment information in this section does not substitute for compliance with any requirement of part 3 of Title 37 of CFR to have an assignment recorded by the Office.

PTO/AIA/14 (11-15)
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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	SRZN-004/00US 328202-2016
	Application Number	
Title of Invention	ANTI-FRIZZLED ANTIBODIES AND METHODS OF USE	

Assignee 1

Complete this section if assignee information, including non-applicant assignee information, is desired to be included on the patent application publication. An assignee-applicant identified in the "Applicant Information" section will appear on the patent application publication as an applicant. For an assignee-applicant, complete this section only if identification as an assignee is also desired on the patent application publication.

If the Assignee or Non-Applicant Assignee is an Organization check here. ☐

Prefix	**Given Name**	Middle Name	**Family Name**	Suffix

Mailing Address Information For Assignee including Non-Applicant Assignee:

Address 1			
Address 2			
City		**State/Province**	
Country		Postal Code	
Phone Number		Fax Number	
Email Address			

Additional Assignee or Non-Applicant Assignee Data may be generated within this form by selecting the Add button.

Signature:

NOTE: This Application Data Sheet must be signed in accordance with 37 CFR 1.33(b). **However, if this Application Data Sheet is submitted with the INITIAL filing of the application and either box A or B is not checked in subsection 2 of the "Authorization or Opt-Out of Authorization to Permit Access" section, then this form must also be signed in accordance with 37 CFR 1.14(c).**

This Application Data Sheet **must** be signed by a patent practitioner if one or more of the applicants is a **juristic entity** (e.g., corporation or association). If the applicant is two or more joint inventors, this form must be signed by a patent practitioner, **all** joint inventors who are the applicant, or one or more joint inventor-applicants who have been given power of attorney (e.g., see USPTO Form PTO/AIA/81) on behalf of **all** joint inventor-applicants.

See 37 CFR 1.4(d) for the manner of making signatures and certifications.

Signature	/Carol D. Laherty/			Date (YYYY-MM-DD)	2017-12-19
First Name	Carol D.	Last Name	Laherty	Registration Number	51,909

Additional Signature may be generated within this form by selecting the Add button.

PTO/AIA/14 (11-15)
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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	SRZN-004/00US 328202-2016
	Application Number	
Title of Invention	ANTI-FRIZZLED ANTIBODIES AND METHODS OF USE	

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Privacy Act Statement

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The information provided by you in this form will be subject to the following routine uses:

1 The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether the Freedom of Information Act requires disclosure of these records.

2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.

3 A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.

4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).

5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent CooperationTreaty.

6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).

7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.

8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspections or an issued patent.

9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

ANTI-FRIZZLED ANTIBODIES AND METHODS OF USE

BACKGROUND

Technical Field

The present invention relates generally to anti-Frizzled antibodies and antigen-binding fragments thereof, compositions, and methods of using the same. Such antibodies are useful, for example, in modulating Wnt signaling pathways.

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_004_00US_ST25.txt. The text file is 122 KB, was created on December 19, 2017, and is being submitted electronically via EFS-Web.

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

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tissues, in addition to Fzds. Accordingly, there is clearly a need in the art for binding moieties, such as antibodies, that specifically bind to one or more Fzd, LRP5, or LRP6. The present invention addresses this need.

BRIEF SUMMARY

In various embodiments, the present invention provides anti-Fzd antibodies and antigen-binding fragments thereof and related methods of use.

In one embodiments, the disclosure provides an isolated antibody, or an antigen-binding fragment thereof, that binds to one or more Frizzled receptor, comprising a sequence comprising: (i) CDRH1, CDRH2 and CDRH3 sequences set forth for any of the antibodies of Table 1A; and/or (ii) CDRL1, CDRL2 and CDRL3 sequences set forth for any of the antibodies of Table 1A, or a variant of said antibody, or antigen-binding fragment thereof, comprising one or more amino acid modifications, wherein said variant comprises less than 8 amino acid substitutions in said CDR sequences. In particular embodiments, the antibody or antigen-binding fragment thereof comprises a heavy chain variable region comprising an amino acid sequence having at least 90% identity to the amino acid sequence set forth in any of SEQ ID NOs:1-37 or a heavy chain variable region comprising the amino acid sequence set forth in any of SEQ ID NOs:1-37. In particular embodiments, the antibody, or antigen-binding fragment thereof, comprises a light chain variable region comprising an amino acid sequence having at least 90% identity to the amino acid sequence set forth in any of SEQ ID NOs:38-65 or a light chain variable region comprising the amino acid sequence set forth in any of SEQ ID NOs:38-65.

In particular embodiments, any of the antibodies, or antigen-binding fragments thereof, are humanized. In certain embodiments, any of the antibodies, or antigen-binding fragments thereof, are a single chain antibody, a scFv, a univalent antibody lacking a hinge region, a nanobody, or a minibody. In particular embodiments, any of the antibodies, or antigen-binding fragments thereof, are a VHH nanobody. In particular embodiments, any of the antibodies, or antigen-binding fragments thereof, are a Fab or a Fab' fragment.

In certain embodiments, any of the antibodies, or antigen-binding fragments thereof, are a fusion protein. In certain embodiments, the antibody,

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or antigen-binding fragment thereof, is fused to a polypeptide sequence that binds LRP5 or LRP6. In certain embodiments, the polypeptide sequence that binds LRP5 or LRP6 is an antibody, or an antigen-binding fragment thereof, that binds to LRP5 or LRP6.

In particular embodiments of any of the antibodies, or antigen-binding fragments thereof, the antibody, or antigen-binding fragment thereof, binds 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 certain embodiments, any of the antibodies, or antigen-binding fragments thereof, bind 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 certain embodiments, any of the antibodies, or antigen-binding fragments thereof, bind 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 a related embodiment, the disclosure provides an isolated antibody, or an antigen-binding fragment thereof, that competes with any of the antibodies disclosed herein for binding to a human Fzd receptor.

In particular embodiments, any of the antibodies, or antigen-binding fragments thereof, bind to the Fzd with a KD of 50 μM or lower.

In particular embodiments, any of the antibodies, or antigen-binding fragments thereof, modulate a Wnt signaling pathway in a cell, optionally a mammalian cell. In particular embodiments, any of the antibodies, or antigen-binding fragments thereof increase signaling via a Wnt signaling pathway in the cell. In particular embodiments, any of the antibodies, or antigen-binding fragments thereof decrease signaling via a Wnt signaling pathway in the cell. In certain embodiments, the Wnt signaling pathway is a canonical Wnt signaling pathway or a non-canonical Wnt signaling pathway.

In a further related embodiment, the present disclosure provides an isolated polynucleotide encoding an antibody, or antigen-binding fragment

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thereof, disclosed herein. In certain embodiments, the present disclosure provides an expression vector comprising the isolated polynucleotide and an isolated host cell comprising the expression vector.

In another embodiment, the present disclosure provides a pharmaceutical composition comprising a physiologically acceptable excipient, diluent, or carrier, and a therapeutically effective amount of the isolated antibody, or antigen-binding fragment thereof, disclosed herein.

In a further embodiment, the present disclosure provides a method for agonizing a Wnt signaling pathway in a cell, comprising contacting the cell with an isolated antibody, or antigen-binding fragment thereof, disclosed herein that increases Wnt signaling. In particular embodiments, the antibody, or antigen-binding fragment thereof, is a fusion protein comprising a polypeptide sequence that binds LRP5 or LRP6.

In another embodiment, the present disclosure provides a method for inhibiting a Wnt signaling pathway in a cell, comprising contacting the cell with the isolated antibody, or antigen-binding fragment thereof, disclosed herein the inhibits Wnt signaling.

In another embodiment, the present disclosure includes 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 a pharmaceutical composition comprising , an isolated antibody, or antigen-binding fragment thereof, disclosed herein that 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, osteoporotic fractures, spinal fusion, osseointegration of orthopedic devices, tendon-bone integration, tooth growth and regeneration, dental implantation, periodontal diseases, maxillofacial reconstruction, osteonecrosis of the jaw, alopecia, hearing loss, vestibular hypofunction, macular degeneration, vitreoretinopathy, diseases of retinal degeneration, Fuchs' dystrophy, stroke, traumatic brain injury, Alzheimer's disease, multiple sclerosis, spinal cord injuries, oral mucositis, short bowel syndrome, inflammatory bowel diseases (IBD), metabolic syndrome, diabetes, pancreatitis, exocrine pancreatic insufficiency, wound healing, diabetic foot ulcers, coronary artery disease, acute kidney injuries, chronic

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kidney diseases, chronic obstructive pulmonary diseases (COPD), 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, non-alcoholic fatty liver diseases and non-alcoholic steatohepatitis (NASH), cirrhosis, and chronic liver insufficiencies of all causes.

In a related embodiment, the present disclosure provides a method for treating a subject having a disease or disorder associated with increased or enhanced Wnt signaling, comprising administering to the subject an effective amount of the pharmaceutical composition comprising an isolated antibody, or antigen-binding fragment thereof, disclosed herein that is an inhibitor of a Wnt signaling pathway. In certain embodiments, the disease or disorder is selected from the group consisting of: tumors and cancers, degenerative disorders, fibrosis, heart failure, coronary artery disease, heterotopic ossification, osteopetrosis, and congenital high bone mass disorders.

DETAILED DESCRIPTION

The present disclosure relates to antibodies and antigen-binding fragments thereof that specifically bind to one or more Fzd receptor, including antibodies having particular Fzd receptor specificity and/or functional properties. One embodiment of the invention encompasses specific humanized antibodies and fragments thereof capable of binding to one or more Fzd receptor and modulate downstream Wnt pathway signaling and related biological effects.

Embodiments of the invention pertain to the use of anti-Fzd antibodies or antigen-binding fragments thereof for the diagnosis, assessment and treatment of diseases and disorders associated with Wnt signaling pathways. In certain embodiments, the subject antibodies and antigen-binding fragments thereof are used to modulate a Wnt signaling pathway in a cell or tissue. In certain embodiments, the subject antibodies and antigen-binding

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fragments thereof are used in the treatment or prevention of diseases and disorders associated with aberrant or deregulated (e.g., either increased or reduced) Wnt signaling, or for which either decreasing or 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.*,

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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 antigen-binding fragments, or complementarity determining regions (CDRs) thereof, are set forth in Table 1A and SEQ ID NOs:1-65.

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), Nanobodies®, 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", multivalent or multispecific fragments constructed by

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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 that binds to the antigen of interest, in particular to one or more Fzd 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. An antigen-binding fragment of the Fzd-specific antibodies described herein is capable of binding to a Fzd receptor. As used herein, the term encompasses not only isolated fragments but also polypeptides comprising an antigen-binding fragment of an antibody disclosed herein, such as, for example, fusion proteins comprising an antigen-binding fragment of an antibody disclosed herein.

In certain embodiments, an antibody or antigen-binding fragment thereof, modulates Wnt signaling events in a cell contacted with the antibody or antigen-binding fragment thereof. In certain embodiments, the antibody or antigen-binding fragment thereof increases Wnt signaling, while in other embodiments, it decreases Wnt signaling. In certain embodiments, the antibody or antigen-binding fragment thereof binds specifically to and/or modulates the biological activity of the human Wnt signaling pathway.

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, an antibody is

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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, an antibody is said to specifically bind an antigen when the equilibrium dissociation constant is $\leq 10^{-7}$ or 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).

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), Nanobodies®, 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. 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

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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 (Ill *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

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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, a Fzd binding 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 VH 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).

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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 Nanobody®. Nanobody 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 “Nanobodies”. Nanobodies® 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 Nanobodies® have been produced. Nanobodies® may be formulated as a ready-to-use solution having a long shelf life. The Nanoclone® method (see, e.g., WO 06/079372) is a proprietary method for generating Nanobodies® against a desired target, based on automated high-throughput selection of B-cells. Nanobodies are single-domain antigen-binding fragments

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of camelid-specific heavy-chain only antibodies. Nanobodies, also referred to as VHH antibodies, typically have a small size of around 15 kDa.

In certain embodiments, the anti-Fzd 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. patent 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; Verhoeyen, M.,

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et al., (1988) *Science* 239:1534-1536; Kettleborough, C. A., *et al.*, (1991) *Protein Engineering* 4:773-3783; 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 anti-Fzd 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 anti-Fzd 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).

In certain embodiments, antibodies or antigen-binding fragments thereof disclosed herein include fusion proteins, e.g., Wnt signaling pathway agonist fusion proteins, also referred to herein as “Wnt surrogates.” Wnt surrogates of the present invention are usually biologically active in binding to a cognate Frizzled receptor, and in activation of Wnt signaling, i.e., the surrogate

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. The ability of the agonists of the invention to mimic the activity of Wnt can be confirmed by a number of assays. The agonists of the invention 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 agonists 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 an agonist of the invention.

In particular embodiments, a Wnt signaling pathway agonist fusion protein (or Wnt surrogate) comprises an anti-Fzd antibody, or antigen-binding fragment thereof, disclosed herein fused to a polypeptide that specifically binds to LRP5 and/or LRP6. In particular embodiments, the polypeptide that specifically binds to LRP5 and/or LRP6 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 titled, "Anti-LRP5/6 antibodies and Methods of Use," Attorney docket number SRZN-005/00US, filed on December 19, 2017, which is incorporated herein by reference in its entirety.

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; nanobody derived binding domains; knottin-based engineered scaffolds; naturally occurring LRP5/6, including without limitation, DKK1, DKK2, DKK3, DKK4, sclerostin; Wise; fusion 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

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

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: KMSHIKGHEGDPCLRSSDCIEGFCCARHFWTKICKPVLHQGEVCTKQRKKGS HGLEIFQRCDCAKGLSCKVWKDATYSSKARLHVCQK or a biologically active fragment thereof.

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 U S A. 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 LRP 6 and LRP 5 of mouse and human origin; the monoclonal antibody 2B11; Cell Signaling Technology antibody specific for LRP5 (D80F2), catalog number 5731; etc.

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In some embodiments, the LRP5/6 binding domain or element may be selected from any domain that binds LRP5/6 at high affinity, e.g. a K_D of at least about 1 x 10^{-7} M, at least about 1 x 10^{-8} M, at least about 1 x 10^{-9} M, at least about 1 x 10^{-10} M. 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; nanobody derived binding domains; knottin-based engineered scaffolds; naturally occurring LRP5/6 binding proteins or polypeptides, including without limitation, Norrin, DKK1, DKK2, DKK3, DKK4, sclerostin; and the like. In certain embodiments the LRP5/6 binding domain is a c-terminal portion of DKK1. A LRP5/6 binding domain may be affinity selected to enhance binding.

The anti-Fzd antibody, or antigen binding fragment thereof, and the LRP5/6 binding domain may be directly joined, or may be separated by a linker, e.g. a polypeptide linker, or a non-peptidic linker, etc. The region of the Wnt surrogate that binds one or more Fzd receptor and the polypeptide that binds LRP5 and/or LRP6 may be contiguous or 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. 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. 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.

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A Wnt surrogate 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 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 direct 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 is in contrast to potentiation of Wnt signaling, which enhances activity only when native Wnt proteins are present.

Wnt surrogates of the present 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 surrogates of the invention to mimic the activity of Wnt can be confirmed by a number of assays. The Wnt surrogates 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 surrogates 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 of the invention.

In certain embodiments, an antibody or antigen-binding fragment thereof disclosed herein inhibits Wnt pathway signaling. In particular embodiments, binding of an anti-Fzd antibody or antigen-binding fragment thereof blocks or inhibits the binding of endogenous Wnt to one or more Fzd receptor on a cell surface, thus reducing or inhibiting Wnt signaling.

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

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through the transcription factors TCF7, TCF7L1, TCF7L2 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.

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-TOPEGFP, 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 TNIK are key steps in the Wnt signaling pathway. Stabilization is mediated by Disheveled family proteins that inhibit the "destruction" complex so that degradation of intracellular β-catenin is reduced, and translocation of β-catenin

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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 U20S 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 signaling pathway agonist 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 agonists may enhance β-catenin signaling by a factor of 2x, 5x, 10x, 100x, 1000x, 10000x or more as compared to the activity in the absence of the agonist when measured in an assay described above, for example when measured in the TOPFlash assay, or any of the other assays mentioned herein.

In certain embodiments, a Wnt signaling pathway antagonist or inhibitor inhibits or decreases canonical Wnt pathway signaling, e.g., β-catenin signaling, by at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 75%, 80%, 90%, 95%, or 100%, as compared to the β-catenin signaling observed in the presence of a neutral substance or negative control as measured in an assay

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described above, for example as measured in the TOPFlash assay. A positive control may be included in these assays.

"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.

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"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/Ca^{2+}) pathway (Giles, RH 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/Ca^{2+} 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, can be readily determined.

In certain embodiments, functional properties of anti-Fzd antibodies and antigen-binding fragments thereof 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

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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. Other assays may test the ability of antibodies described herein to block normal Wnt/Fzd-mediated responses. The antibodies and antigen-binding fragments thereof 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 antibody or antigen-binding fragment thereof, e.g., a Wnt surrogate, comprises one or more of the CDRs of the 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.

Marks et al (*Bio/Technology*, 1992, 10:779-783) describe methods of producing repertoires of antibody variable domains in which consensus primers directed at or adjacent to the 5' end of the variable domain area are used in conjunction with consensus primers to the third framework region of human VH genes to provide a repertoire of VH variable domains lacking a CDR3. Marks et al further describe how this repertoire may be combined with a CDR3 of a particular antibody. Using analogous techniques, the CDR3-derived sequences of the presently described antibodies may be shuffled with repertoires of VH or VL domains lacking a CDR3, and the shuffled complete VH or VL domains combined with a cognate VL or VH domain to provide an antibody or antigen-binding fragment thereof that binds one or more Fzd receptors. The repertoire may then be displayed in a suitable host system such

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as the phage display system of WO92/01047 so that suitable antibodies or antigen-binding fragments thereof may be selected. A repertoire may consist of at least from about 10^4 individual members and upwards by several orders of magnitude, for example, to about from 10^6 to 10^8 or 10^{10} or more members. Analogous shuffling or combinatorial techniques are also disclosed by Stemmer (Nature, 1994, 370:389-391), who describes the technique in relation to a β-lactamase gene but observes that the approach may be used for the generation of antibodies.

A further alternative is to generate novel VH or VL regions carrying one or more CDR-derived sequences of the herein described invention embodiments using random mutagenesis of one or more selected VH and/or VL genes to generate mutations within the entire variable domain. Such a technique is described by Gram et al (1992, Proc. Natl. Acad. Sci., USA, 89:3576-3580), who used error-prone PCR. Another method which may be used is to direct mutagenesis to CDR regions of VH or VL genes. Such techniques are disclosed by Barbas et al., (1994, Proc. Natl. Acad. Sci., USA, 91:3809-3813) and Schier et al (1996, J. Mol. Biol. 263:551-567).

In certain embodiments, a specific VH and/or VL of the antibodies described herein may be used to screen a library of the complementary variable domain to identify antibodies with desirable properties, such as increased affinity for one or more Fzd receptor. Such methods are described, for example, in Portolano *et al.*, J. Immunol. (1993) 150:880-887; Clarkson *et al.*, Nature (1991) 352:624-628.

Other methods may also be used to mix and match CDRs to identify antibodies having desired binding activity, such as binding to one or more Fzd receptor. For example: Klimka *et al.*, *British Journal of Cancer* (2000) 83: 252-260, describe a screening process using a mouse VL and a human VH library with CDR3 and FR4 retained from the mouse VH. After obtaining antibodies, the VH was screened against a human VL library to obtain antibodies that bound antigen. Beiboer *et al.*, J. Mol. Biol. (2000) 296:833-849 describe a screening process using an entire mouse heavy chain and a human

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light chain library. After obtaining antibodies, one VL was combined with a human VH library with the CDR3 of the mouse retained. Antibodies capable of binding antigen were obtained. Rader *et al.*, PNAS (1998) 95:8910-8915 describe a process similar to Beiboer et al above.

These just-described techniques are, in and of themselves, known as such in the art. The skilled person will, however, be able to use such techniques to obtain antibodies or antigen-binding fragments thereof according to several embodiments of the invention described herein, using routine methodology in the art.

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, anti-Fzd antibodies, and antigen-binding fragments thereof, 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 homogenous 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

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greater. Anti-Fzd antibodies and antigen-binding fragments thereof, including Wnt surrogates, of the present invention typically form a substantially homogeneous aqueous solution at concentrations of at least 25 μM and higher, e.g. at least 25 μM, 40 μM, or 50 μM, usually at least 60 μM, 70 μM, 80 μM, or 90 μM, sometimes as much as 100 μM, 120 μM, or 150 μM. In other words, compositions of the present invention 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 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. An antibody "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, an antibody 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, an antibody (or moiety or epitope) 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 the anti-Fzd antibody, or antigen-binding fragment thereof, 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 antibody

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based signaling agonist binds to Fzd1 , Fzd2, Fzd5, Fzd7 and Fzd8. In various embodiments, the anti-Fzd antibody, or antigen-binding fragment thereof, 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 frizzled binding moiety 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.

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 embodiment, the anti-Fzd antibodies bind one or more Fzd receptors 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

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than or equal to about 1 x 10^{-7} M, less than or equal to about 1 x 10^{-8} M, less than or equal to about 1 x 10^{-9} M, or at least about 1 x 10^{-10} M. In certain embodiments, the anti-Fzd antibodies described herein bind one or more Fzd receptor with a K_D of less than about 10,000 nM, less than about 1000 nM, less than about 100 nM, less than about 10 nM, less than about 1 nM or less than about 0.1 nM, and in some embodiments, the antibodies may have even higher affinity for one or more Fzd receptor. In certain embodiments, the anti-Fzd antibodies described herein have an affinity K_D of about 100, 150, 155, 160, 170, 175, 180, 185, 190, 191, 192, 193, 194, 195, 196, 197, 198 or 199 picomolar, and in some embodiments, the antibodies may have even higher affinity for one or more Fzd receptor.

An antibody or antigen-binding fragment thereof according to certain embodiments includes antibodies and antigen binding fragments thereof that compete for binding to one or more Fzd receptor with any antibody described herein which both (i) specifically binds to the one or more Fzd receptor and/or (ii) comprises a VH and/or VL domain (or a VH and/or VL CDR set) disclosed herein, or (iii) comprises a VH CDR3 disclosed herein, or a variant of any of these. Competition between antibodies may be assayed easily *in vitro*, for example using ELISA and/or by tagging a specific reporter molecule to one antibody which can be detected in the presence of other untagged antibodies, to enable identification of specific antibodies which bind the same epitope or an overlapping epitope. Thus, there is provided herein a specific antibody or antigen-binding fragment thereof, comprising a human antibody antigen-binding site which competes with an antibody described herein that binds to one or more Fzd receptor.

In this regard, as used herein, the terms “competes with”, "inhibits binding" and "blocks binding" (*e.g.*, referring to inhibition/blocking of binding of a Wnt to one or more Fzd receptor or referring to inhibition/blocking of binding of an anti-Fzd antibody to a Fzd receptor) are used interchangeably and encompass both partial and complete inhibition/blocking. The inhibition/blocking of a Wnt to one or more Fzd receptor preferably reduces or alters the normal

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level or type of cell signaling that occurs when the Wnt binds to the Fzd receptor without inhibition or blocking. Inhibition and blocking are also intended to include any measurable decrease in the binding of a Wnt to a Fzd receptor when in contact with an anti-Fzd antibody as disclosed herein as compared to the ligand not in contact with an anti-Fzd antibody, *e.g.*, the blocking of binding of the Wnt to the Fzd receptor by at least about 10%, 20%, 30%, 40%, 50%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%.

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

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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 1IIX) (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

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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 (Lehrnbecher *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 Fzd-specific antibody as described herein to activate the complement system (see *e.g.*, U.S.

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Patent 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-Fzd 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. Patents 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.

Thus, in certain embodiments, antibody variable domains with the desired binding specificities are 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. It is preferred to have 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.

Antibodies of the present invention (and antigen-binding fragments and variants thereof) may also be modified to include an epitope tag or label, *e.g.*, for use in purification or diagnostic applications. There are many

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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 another contemplated embodiment, a Fzd-specific antibody or antigen-binding fragment thereof as described herein may be conjugated or operably linked to another therapeutic compound, referred to herein as a conjugate. The conjugate may be a cytotoxic agent, a chemotherapeutic agent, a cytokine, an anti-angiogenic agent, a tyrosine kinase inhibitor, a toxin, a radioisotope, or other therapeutically active agent. Chemotherapeutic agents, cytokines, anti-angiogenic agents, tyrosine kinase inhibitors, and other therapeutic agents have been described above, and all of these aforementioned therapeutic agents may find use as antibody conjugates.

Immunoconjugates may be made using a variety of bifunctional protein coupling agents such as N-succinimidyl-3-(2-pyridyldithio)propionate (SPDP), succinimidyl-4-(N-maleimidomethyl)cyclohexane-1-carboxylate, iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCL), active esters (such as disuccinimidyl suberate), aldehydes (such as glutareldehyde), bis-azido compounds (such as bis (p-azidobenzoyl)hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as toluene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene). Particular coupling agents include N-succinimidyl-3-(2-pyridyldithio)propionate (SPDP) (Carlsson *et al.*, Biochem. J. 173:723-737 [1978]) and N-succinimidyl-4-(2-pyridylthio)pentanoate (SPP) to provide for a disulfide linkage. The linker may be a “cleavable linker” facilitating release of one or more cleavable components. For example, an acid-labile linker may be used (Cancer Research 52: 127-131 (1992); U.S. Pat. No. 5,208,020).

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In certain embodiments, anti-LRP5/6 antibodies and antigen-binding fragments thereof are monoclonal antibodies. In certain embodiments, they are humanized.

The present invention further provides in certain embodiments an isolated nucleic acid encoding an antibody or antigen-binding fragment thereof as described herein, for instance, a nucleic acid that codes for one or more CDR or VH or VL domain as described herein. Nucleic acids include DNA and RNA. These and related embodiments may include polynucleotides encoding antibodies that bind one or more Fzd receptors 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.

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

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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, phoshoraniladate and phosphoroamidate. 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, phoshoraniladate, phosphoroamidate, 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

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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.

Therefore, according to these and related embodiments, the present disclosure also provides polynucleotides encoding the anti-Fzd antibodies and antigen-binding fragments thereof described herein. In certain embodiments, polynucleotides are provided that comprise some or all of a polynucleotide sequence encoding an antibody or antigen-binding fragment thereof as described herein and complements of such polynucleotides.

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 that encode antibodies that bind

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to a Fzd receptor. 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 antibodies 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 an antibody disclosed herein, or an antigen-binding fragment thereof, to alter one or more properties of the encoded polypeptide, such as the binding affinity of the antibody or the antigen-binding fragment thereof, 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.

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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; Kuby, 1994; and Maniatis *et al.*, 1982, each incorporated herein by reference, for that purpose.

In many embodiments, the nucleic acids encoding a subject monoclonal antibody are introduced directly into a host cell, and the cell incubated under conditions sufficient to induce expression of the encoded antibody. The antibodies of this disclosure are 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 antibody disclosed thereby. The nucleic acid sequence may be optimized to reflect particular codon "preferences" for various

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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; a nucleic acid encoding any antibody, CDR, VH or VL domain, or antigen-binding fragment 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.

Antibodies or antigen-binding fragments thereof as provided herein, 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 antibodies and antigen-binding fragments thereof in prokaryotic cells such as *E. coli* is well established in the art. For a

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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 antibodies, and which further expresses or is capable of expressing a selected gene of interest, such as a gene encoding any herein described antibody. 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

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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 Fzd-specific antibody 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

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"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 the antibodies that bind to a Fzd receptor of the present disclosure, or sequences that have deletions from, additions to, and/or substitutions of one or more amino acid of an anti-Fzd antibody. 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" or “isolated antibody” referred to herein means that a subject protein or antibody is (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

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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, of may be of synthetic origin, or any combination thereof. 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 the antibodies described herein are contemplated. For example, it may be desirable to improve the binding affinity and/or other biological properties of the antibody. For example, amino acid sequence variants of an antibody 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 antibody, provided that the final construct possesses the desired characteristics (*e.g.*, high affinity binding to one or more Fzd 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 the antibodies and antigen-binding fragments thereof disclosed herein. In certain embodiments, such variant antibodies or antigen-binding fragments, or CDRs thereof, bind to one or more Fzd receptor at least about 50%, at least about 70%, and in certain embodiments, at least about 90% as well as an antibody sequence specifically set forth herein. In further embodiments, such variant antibodies or antigen-

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binding fragments, or CDRs thereof, bind to one or more Fzd receptor with greater affinity than the antibodies 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 antibody or antigen-binding fragment thereof, e.g., a Fab, scFv, Nanobody®, or Wnt surrogate, 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). 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, a subject antibody or antigen-binding fragments thereof, e.g., a Fab, scFv, Nanobody®, or Wnt surrogate, 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%

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or 99% identical, to the heavy chain variable region of an anti-Fzd 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 anti-Fzd 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:1-65.

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

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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 antibody 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., 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).

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Determination of the three-dimensional structures of representative polypeptides (*e.g.*, variant Fzd-specific antibodies as provided herein, for instance, an antibody protein having an antigen-binding fragment 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 Fzd-specific antibodies antigen-binding domains thereof as provided herein, 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-Champagne, 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

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computer programs are also commercially available, such as from Schrödinger (Munich, Germany).

In another embodiment of invention, the anti-Fzd antibodies and humanized versions thereof are derived from rabbit monoclonal antibodies, and in particular are generated using RabMAb® technology. These antibodies are advantageous as they require minimal sequence modifications, thereby facilitating retention of functional properties after humanization using mutational lineage guided (MLG) humanization technology (see e.g., U.S. Patent No. 7,462,697). Thus, illustrative methods for making the anti-Fzd antibodies of the present disclosure include the RabMab® rabbit monoclonal antibody technology described, for example, in U.S. Patents 5,675,063 and 7,429,487. In this regard, in certain embodiments, the anti-Fzd antibodies of the disclosure are produced in rabbits. In particular embodiments, a rabbit-derived immortal B-lymphocyte capable of fusion with a rabbit splenocyte is used to produce a hybrid cell that produces an antibody. The immortal B-lymphocyte does not detectably express endogenous immunoglobulin heavy chain and may contain, in certain embodiments, an altered immunoglobulin heavy chain-encoding gene.

Compositions

Pharmaceutical compositions comprising an anti-Fzd antibody or antigen-binding fragment thereof, e.g., a Wnt surrogate, 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 an anti-Fzd antibody or antigen-binding fragment thereof, e.g., a Wnt surrogate, 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

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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 anti-Fzd antibody or antigen-binding fragment thereof, e.g., a Wnt surrogate, 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 an anti-Fzd antibody or antigen-binding fragment thereof, e.g., a Wnt surrogate, 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 anti-Fzd antibody or antigen-binding fragment thereof, e.g., a Wnt surrogate, 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 an anti-Fzd antibody or antigen-binding fragment thereof described herein 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

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acid sequence encoding the anti-Fzd antibody or antigen-binding fragment thereof, e.g., a Wnt surrogate, 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 anti-Fzd antibody or antigen-binding fragment thereof, e.g., a Wnt surrogate, 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).

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,

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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 to allow it to be drawn into a syringe and provided to a subject using 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.

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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

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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 the anti-Fzd antibodies or antigen-binding fragments thereof, e.g., Wnt surrogates, 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

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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 an anti-Fzd antibody or antigen-binding fragment thereof, e.g., a Wnt surrogate, described herein 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, carnitine, alanine, glycine, lysine, valine, leucine, polyvinylpyrrolidone, polyethylene and glycol. Preferably, this formulation is stable for at least six months at 4º 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 Fzd-specific antibodies, antigen-binding fragments thereof, e.g., Wnt surrogates, disclosed herein, e.g., to modulate a Wnt signaling pathway, e.g., to increase or

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decrease Wnt signaling, and the administration of Fzd-specific antibodies, antigen-binding fragments thereof, and Wnt surrogates disclosed herein in a variety of therapeutic settings. Provided herein are methods of treatment using the antibodies that bind one or more Fzd receptors or antigen-binding fragments thereof. In one embodiment, an antibody, or antigen-binding fragment thereof, of the present invention is provided to a subject having a disease involving inappropriate or deregulated Wnt signaling, e.g., increased or reduced Wnt signaling.

Increasing Wnt Pathway Signaling and Related Therapeutic Methods

In certain embodiments, an anti-Fzd antibody or antigen-binding fragment thereof, e.g., a Wnt surrogate, 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 an anti-Fzd antibody or antigen-binding fragment thereof, e.g., a Wnt surrogate, disclosed herein, wherein the anti-Fzd antibody or antigen-binding fragment thereof 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, comprising contacting the tissue or cell with an effective amount of a polynucleotide comprising an anti-Fzd antibody or antigen-binding fragment thereof, e.g., a Wnt surrogate, of the present invention. 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

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embodiments, the polynucleotides are expression cassettes comprising a promoter operatively linked to the coding sequences. In certain embodiments, the nucleic acid sequence encoding the anti-Fzd antibody or antigen-binding fragment thereof, e.g., a Wnt surrogate, 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 an anti-Fzd antibody or antigen-binding fragment thereof, e.g., a Wnt surrogate. 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 the anti-Fzd antibody or antigen-binding fragment thereof, e.g., a Wnt surrogate, 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 an anti-Fzd antibody or antigen-binding fragment thereof, e.g., a Wnt surrogate, 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 anti-Fzd antibody or antigen-binding fragment thereof, e.g., a Wnt surrogate, 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

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comprising an expression cassette encoding the anti-Fzd antibody or antigen-binding fragment thereof, e.g., a Wnt surrogate, 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.

Anti-Fzd antibodies and antigen-binding fragments thereof, e.g., Wnt surrogates, 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: an anti-Fzd antibody or antigen-binding fragment thereof, e.g., a Wnt surrogate; a polynucleotide comprising a nucleic acid sequence encoding an anti-Fzd antibody or antigen-binding fragment thereof, e.g., a Wnt surrogate,, e.g., a DNA or mRNA, optionally a modified mRNA; a vector comprising a nucleic acid sequence encoding an anti-Fzd antibody or antigen-binding fragment thereof, e.g., a Wnt surrogate,, e.g., an expression vector or viral vector; or a cell comprising a nucleic acid sequence encoding an anti-Fzd antibody or antigen-binding fragment thereof, e.g., a Wnt surrogate, e.g., a cell transduced with an expression vector or viral vector encoding an anti-Fzd antibody or antigen-binding fragment thereof, e.g., a Wnt surrogate. 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.

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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 an anti-Fzd antibody or antigen-binding fragment thereof, e.g., a Wnt surrogate, 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 an anti-Fzd antibody or antigen-binding fragment thereof, e.g., a Wnt surrogate, 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 anti-Fzd antibody or antigen-binding

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fragment thereof, e.g., a Wnt surrogate, 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 an anti-Fzd antibody or antigen-binding fragment thereof, e.g., a Wnt surrogate. 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 anti-Fzd antibody or antigen-binding fragment thereof, e.g., a Wnt surrogate, 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 an anti-Fzd antibody or antigen-binding fragment thereof, e.g., a Wnt surrogate. 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 anti-Fzd antibody or antigen-binding fragment thereof, e.g., a Wnt surrogate, and the nucleic acid sequence encoding the Wnt polypeptide or Norrin polypeptide are present in the same

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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 anti-Fzd antibody or antigen-binding fragment thereof, e.g., a Wnt surrogate, 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. Anti-Fzd antibody or antigen-binding fragment thereof, e.g., a Wnt surrogate, 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 an anti-Fzd antibody or antigen-binding fragment thereof, e.g., a Wnt surrogate, 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, 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;

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enhancing regeneration of sensory organs, e.g. treatment of hearing loss, treatment of vestibular hypofunction, treatment of macular degeneration, treatment of vitreoretinopathy, other diseases of retinal degeneration, Fuchs' dystrophy, other cornea disease, etc.; treatment of stroke, traumatic brain injury, Alzheimer's disease, multiple sclerosis and other conditions affecting the blood brain barrier; treatment of spinal cord injuries, other spinal cord diseases. The compositions of this invention may also be used in treatment of oral mucositis, treatment of short bowel syndrome, inflammatory bowel diseases (IBD), other gastrointestinal disorders; treatment of metabolic syndrome; 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), 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, 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

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around prosthetic devices, osteoporosis, osteoporotic fractures, spinal fusion, osteonecrosis of the jaw, dental implantation, periodontal diseases, maxillofacial reconstruction, and the like. An anti-Fzd antibody or antigen-binding fragment thereof, e.g., a Wnt surrogate, enhances 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 an anti-Fzd antibody or antigen-binding fragment thereof, e.g., a Wnt surrogate, disclosed herein. Methods for regeneration of bone tissues benefit from administration of the anti-Fzd antibody or antigen-binding fragment thereof, e.g., a Wnt surrogate disclosed herein, 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.

Compositions comprising one or more anti-Fzd antibody or antigen-binding fragment thereof, e.g., a Wnt surrogate, disclosed herein 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,

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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.

An anti-Fzd antibody or antigen-binding fragment thereof, e.g., a Wnt surrogate, 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.

An anti-Fzd antibody or antigen-binding fragment thereof, e.g., a Wnt surrogate, may also be used in regeneration of retinal tissue. In the adult mammalian retina, Muller glia cells are capable of regenerating retinal cells,

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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 peri-central 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. 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, 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

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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.

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. An anti-Fzd antibody or antigen-binding fragment thereof, e.g., a Wnt surrogate, may benefit various disease conditions involving exocrine and endocrine pancreas, kidney, or lung. The anti-Fzd antibody or antigen-binding fragment thereof, e.g., a Wnt surrogate, may be used in treatment of metabolic

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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), 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 an anti-Fzd antibody or antigen-binding fragment thereof, e.g., a Wnt surrogate. 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

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treatment of spinal cord injuries, other spinal cord diseases, stroke, traumatic brain injuries, etc.

Wnt signals also play a role in angiogenesis. An anti-Fzd antibody or antigen-binding fragment thereof, e.g., a Wnt surrogate, may benefit conditions where angiogenesis is beneficial, treatment of myocardial infarction, coronary artery disease, heart failure, 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.

Reducing Wnt Pathway Signaling and Related Therapeutic Methods

In certain embodiments, an anti-Fzd antibody or antigen-binding fragment thereof, may be used to decrease or inhibit Wnt signaling in a tissue or cell. Thus, in some aspects, the present invention provides a method for decreasing Wnt signaling or inhibiting Wnt signaling in a tissue or cell, comprising contacting the tissue or cell with an effective amount of an anti-Fzd antibody, or antigen-binding fragment thereof, disclosed herein, wherein the anti-Fzd antibody or antigen-binding fragment thereof is a Wnt signaling pathway antagonist or inhibitor. 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 related aspects, the present invention provides a method for decreasing or inhibiting Wnt signaling in a tissue or cell, comprising contacting the tissue or cell with an effective amount of a polynucleotide comprising an

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anti-Fzd antibody or antigen-binding fragment thereof, of the present invention, wherein the anti-Fzd antibody or antigen-binding fragment thereof is a Wnt signaling pathway antagonist or inhibitor. 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 related aspects, the present invention provides a method for decreasing or inhibiting 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 an anti-Fzd antibody or antigen-binding fragment thereof, wherein the anti-Fzd antibody or antigen-binding fragment thereof is a Wnt signaling pathway antagonist or inhibitor. 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 related aspects, the present invention provides a method for decreasing or inhibiting Wnt signaling in a tissue, comprising contacting the tissue with an effective amount of a cell comprising a nucleic acid sequence encoding an anti-Fzd antibody or antigen-binding fragment thereof, wherein the anti-Fzd antibody or antigen-binding fragment thereof is a Wnt signaling pathway antagonist or inhibitor. 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 anti-Fzd antibody or antigen-binding fragment thereof, wherein the anti-Fzd antibody or antigen-binding fragment thereof is a Wnt signaling pathway antagonist or inhibitor. In particular embodiments, the cell is a stem cell, e.g., an adipose-derived stem cell or a hematopoietic stem cell.

Anti-Fzd antibodies and antigen-binding fragments thereof, wherein the anti-Fzd antibody or antigen-binding fragment thereof is a Wnt

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signaling pathway antagonist or inhibitor, may be used in to treat a disease, disorder or condition, for example, by decreasing or inhibiting Wnt signaling in a 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 increased or deregulated Wnt signaling, or for which decreased Wnt signaling would provide a therapeutic benefit, comprising contacting the subject with an effective amount of a composition comprising an anti-Fzd antibody or antigen-binding fragment thereof, wherein the anti-Fzd antibody or antigen-binding fragment thereof is a Wnt signaling pathway antagonist or inhibitor. In particular embodiments, the composition is a pharmaceutical composition comprising any of: an anti-Fzd antibody or antigen-binding fragment thereof; a polynucleotide comprising a nucleic acid sequence encoding an anti-Fzd antibody or antigen-binding fragment thereof, e.g., a DNA or mRNA, optionally a modified mRNA; a vector comprising a nucleic acid sequence encoding an anti-Fzd antibody or antigen-binding fragment thereof, e.g., an expression vector or viral vector; or a cell comprising a nucleic acid sequence encoding an anti-Fzd antibody or antigen-binding fragment thereof, e.g., a cell transduced with an expression vector or viral vector encoding an anti-Fzd antibody or antigen-binding fragment thereof. In particular embodiments, the disease or condition is a pathological disease or disorder, or an injury. In some embodiments, contacting occurs in vivo, i.e., the subject composition is administered to a subject.

In related aspects, the present invention provides a method for treating a disease or condition, e.g., a disease or disorder associated with increased Wnt signaling, or for which reduced 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 an anti-Fzd antibody or antigen-binding fragment thereof, wherein the antibody or antigen-binding fragment thereof is a Wnt signaling pathway antagonist or inhibitor, disclosed herein. In certain embodiments, the polynucleotides are DNA or mRNA, e.g., a modified

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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 related aspects, the present invention provides a method for treating a disease or condition, e.g., a disease or disorder associated with increased Wnt signaling, or for which decreased 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 an anti-Fzd antibody or antigen-binding fragment thereof, wherein the antibody or antigen-binding fragment thereof is a Wnt signaling pathway antagonist or inhibitor. 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 related aspects, the present invention provides a method for treating a disease or condition, e.g., a disease or disorder associated with increased Wnt signaling, or for which decreased 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 an anti-Fzd antibody or antigen-binding fragment thereof, wherein the antibody or antigen-binding fragment thereof is a Wnt signaling pathway antagonist or inhibitor. 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 anti-Fzd antibody or antigen-binding fragment thereof. In particular embodiments, the cell is a stem cell, e.g., an adipose-derived stem cell or a hematopoietic stem cell.

In certain embodiments, methods of treating or preventing diseases or disorders in a subject in need thereof, by providing to the subject an effective amount of an anti-Fzd antibody, or an antigen-binding fragment

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thereof, wherein the antibody or the antigen-binding fragment thereof is an inhibitor of a Wnt signaling pathway, may be used to treat a cancer or tumor, e.g., a solid or liquid tumor. Examples of cancers and tumors that may be treated include, but are not limited to: colon tumors (e.g. colon cancer or adenoma), stomach tumors (e.g., stomach cancer), small intestine tumors (e.g., small intestinal cancer), liver tumors (e.g., liver cancer), pancreas tumors (e.g., pancreatic cancer), lung tumors (e.g., lung cancer), ovary tumors (e.g., ovarian cancer), kidney (e.g., kidney cancer), brain tumors (e.g., brain cancer), spinal cord tumors (e.g., spinal cord cancer), skin tumors (e.g., skin cancer or melanoma), head and neck tumors (e.g., head and neck cancer), gastointestinal tract tumors (e.g., gastrointestinal cancer, esophageal cancer, oral mucosa cancer, tongue cancer, stomach cancer, intestinal cancer, colon cancer), breast tumors (e.g., breast cancer), prostate tumors (e.g., prostate cancer), bone tumors (e.g., bone cancer), vascular tumors, Wilms tumor, leukemina/lymphoma, soft tissue tumors (e.g., soft tissue sarcoma or synovial sarcoma) and metastatic cancers, etc.

In certain embodiments, methods of treating or preventing diseases or disorders in a subject in need thereof, by providing to the subject an effective amount of an anti-Fzd antibody, or an antigen-binding fragment thereof, wherein the antibody or the antigen-binding fragment thereof is an inhibitor of a Wnt signaling pathway, may be used to treat degenerative diseases. Examples of degenerative diseases that may be treated include, but are not limited to osteoarthritis, cartilage degeneration, sports injuries (e.g., cartilage injury), retinopathy, atherosclerosis, neurodegenerative disorders, and vascular disorders e.g. vasculitis, conditions with abnormal angiogenesis.

In certain embodiments, methods of treating or preventing diseases or disorders in a subject in need thereof, by providing to the subject an effective amount of an anti-Fzd antibody, or an antigen-binding fragment thereof, wherein the antibody or the antigen-binding fragment thereof is an inhibitor of a Wnt signaling pathway, may be used to treat fibrosis. Examples of fibrosis that may be treated include, but are not limited to, lung fibrosis

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(including but not limited to COPD), kidney fibrosis (e.g. end stage renal failure), liver fibrosis, congenital liver storage diseases, and cardiac fibrosis.

In certain embodiments, methods of treating or preventing diseases or disorders in a subject in need thereof, by providing to the subject an effective amount of an anti-Fzd antibody, or an antigen-binding fragment thereof, wherein the antibody or the antigen-binding fragment thereof is an inhibitor of a Wnt signaling pathway, may be used to treat heart failure, e.g., congestive heart failure, systolic heart failure, heart failure with preserved ejection fraction, or coronary artery disease.

In certain embodiments, methods of treating or preventing diseases or disorders in a subject in need thereof, by providing to the subject an effective amount of an anti-Fzd antibody, or an antigen-binding fragment thereof, wherein the antibody or the antigen-binding fragment thereof is an inhibitor of a Wnt signaling pathway, may be used to treat heterotopic ossification, osteopetrosis, or congenital high bone mass disorders.

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 tissue-specific Wnt signal enhancing 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.

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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 anti-Fzd antibody or antigen-binding fragment thereof, e.g., a Wnt surrogate, 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., anti-Fzd antibody or antigen-binding fragment thereof) 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

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particular disease being modified, and will recognize the appropriate detection methods to use to measure therapeutic efficacy.

Promoting Cell, Tissue and Organoid Growth and Related Methods

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 Rspondin 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 201 1; 141: 1762-1772) and Sato et al., 2009 (Nature 459, 262-5). The Wnt surrogate molecules disclosed herein are suitable alternatives to Rspondin for use in these stem cell culture media, or may be combined with Rspondin.

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

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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 Rspondin, but wherein the Wnt or Rspondin 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 201 1; 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 embdoiments, 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.

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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 WO2012/014076, Sato et al., 201 1 (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.

Diagnostic and Related Methods

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. Thus, the present disclosure provides methods of detecting one or more Fzd 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 immuno-assay (EIA), and enzyme linked immuno-assay (ELISA). In particular embodiments, a method comprises contacting a tissue or cell, e.g., obtained from a subject, with an antibody or antigen-binding fragment thereof disclosed herein, and then determining an amount of binding of the antibody or antigen-binding fragment thereof to the tissue or cell, thus determining the presence of or an amount of the Fzd receptor(s) in the tissue or cell.

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

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

In various embodiments, the antibodies and antigen-binding fragments thereof described herein are conjugated to a detectable label that may be detected directly or indirectly. In this regard, an antibody "conjugate" refers to an anti-Fzd antibody or antigen-binding fragment thereof 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

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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 antibodies 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.

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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-acetylglucosamimidase, β-glucuronidase, invertase, Xanthine Oxidase, firefly luciferase and glucose oxidase (GO). Examples of commonly used substrates for horseradishperoxidase 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-naphtol (CN), .alpha.-naphtol pyronin (.alpha.-NP), o-dianisidine (OD), 5-bromo-4-chloro-3-indolylphosp- hate (BCIP), Nitro blue tetrazolium (NBT), 2-(p-iodophenyl)-3-p-nitropheny- l-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,

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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 receptor or cells or tissues expressing one or more Fzd 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.

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EXAMPLES

EXAMPLE 1

CHARACTERIZATION OF ANTI-FZD ANTIBODIES

Antibody Fab, scFv and VHH nanobody fragments disclosed herein were sequenced and sub-cloned into mammalian expression vectors for expression, purification, and characterization of binding affinities to various Fzd receptors.

Soluble recombinant proteins were prepared by transfection of respective expression vectors into Expi293F cells (Thermo Fisher Scientific, Waltham, MA) according to the manufacturer's instructions. Briefly, four days after the transfection, cell culture medium was collected after spin down the cell pellet. The media were incubated with either Protein A resin (REPLIGEN, Waltham, MA) for collecting proteins containing human IgG-Fc portion, or Nickel affinity resin (Roche, Basel, Switzerland) for collecting proteins conjugated with His-tag. Proteins were eluted with 10 mM glycine, pH 3.5 from Protein A resin, or with 150 mM imidazole, pH 7.4 from Nickel affinity resin, respectively.

Subsequently, the protein elutes were fractionated and further purified by size-exclusion chromatography (SEC). SEC was performed by a fast protein liquid chromatography using a Superdex 200 Increase 10/300 GL (GE Healthcare, Pittsburgh, PA) in HBS buffer (10 mM HEPES, 150 mM NaCl, pH7.4). Each protein was injected onto the column at a volume of 475 µl or 500 µl. The absorbance at 280 nm was monitored, and the 500 µl fractions of all elutes were collected. Each collected faction near main peak was further analyzed by SDS-Polyacrylamide Gel Electrophoresis (SDS-PAGE) to confirm the content. SDS-PAGE was performed using Tris-HCl 4-15% gel (Bio-Rad, Hercules, CA) under both non-reducing and reducing conditions. The samples were prepared in Laemmli sample buffer and heated at 100ºC for 5 min.

Protein concentrations were determined using a NanoDrop Spectrophotometer (Thermo Scientific) by the direct UV A280 method. The

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relationship of absorbance to protein concentration is linear based on Beer-Lamber equation, $A = \varepsilon l c$; A is the absorbance value, ε is the wavelength-dependent extinction coefficient, l is the path length in centimeters, and c is the protein concentration. The experimental extinction coefficients of all produced proteins were estimated by their amino acid sequences.

Binding kinetics of antibody fragments to a variety of Fzd cysteine rich domain (CRD) protein targets (Fzd1, Fzd2, Fzd3, Fzd4, Fzd5, Fzd6, Fzd7, Fzd8, Fzd9 and/or Fzd10 CRDs) was determined by bio-layer interferometry (BLI) using Octet Red 96 (PALL ForteBio, Fremont, CA) instruments at 30° C, 1000 rpm with streptavidin (SA) biosensors. C-terminal biotinylated Fzd CRD recombinant protein was diluted to 20 nM in the running buffer (PBS, 0.05% Tween-20, 0.5% BSA, pH 7.2) and captured to the SA biosensor until coupling length reached 0.2 nm. Following capture of the Fzd CRD, the SA biosensor with captured biotinylated-Fzd CRD was dipped into wells containing the relevant antibody fragment at 7 different concentrations (0, 1.37, 4.12, 12.4, 37, 111.1, 333.3, 1000 nM) in running buffer, plus a well with only running buffer as a reference channel. K_D was determined by global fitting, 1:1 binding model according to manufacturer recommended settings.

Table 1A provides the heavy chain CDRs (CDRH1, CDRH2, and CDRH3) and light chain CDRs (CDRL1, CDRL2, and CDRL3) for the indicated antibody clones. Where light chain CDRs are not provided, the antibody fragment did not comprise a light chain. Table 1A also indicates the initial Fzd receptor the antibody fragment was shown to bind. The Fzd receptor to which each clone was initially identified as binding was determined by detection of phage-displayed antibody fragments bound to target antigen immobilized on Nunc Maxisorb microtiter plates (Thermo Fisher Scientific, Waltham, MA) by single-dose or dose-dependent ELISA. Detection of bound phage was determined calorimetrically by turnover of TMB substrate (Thermo Fisher Scientific, Waltham, MA) at 415 nm by anti-M13-HRP antibody (GE Healthcare, Pittsburgh, PA). Clones were identified as binding to a Fzd receptor when the fold OD 450 nm over background was greater than a threshold level.

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Table 1A: Clone IDs and CDR sequences.

Clone ID	Initial Binding	CDRH1	CDRH2	CDRH3	CDRL1	CDRL2	CDRL3
001S-A01	Fzd1	YTFTSYGIS	GWISAYNGNTNYA	CARASAWTPYGAFDIW	SGSSSNIGSHTVS	SNYQRPS	CAAWDGSLFGHWVF
001S-B01	Fzd1	GSISSGGYSWS	GSIYHSGSTYYN	CARFYYDILTGYSYFDYW	RSSRSLLDTDDGNTYLD	TLSHRAS	CMQSIQLPWTF
001S-E01	Fzd1	GSISNYYWS	GEIDRSGDTNYN	CARVRARRFLVSDRSAFDIW	SGNTLGSHYVS	QDSKRPS	CQVWDSSTVVF
001S-F01	Fzd1	GSISGNNYYZG	GSIYFTGGTYYN	CARVMLITDAFDIW	RSSQSLLHSNGYNYLD	LGSNRAS	CMQGTHWPYTF
001S-G01	Fzd1	GSISSSSYYWG	GYIYYSGSTYYN	CARATYGGDAFDIW	TRSSSNIGAGYDVH	GNSIRPS	CGTWDSSLSAWVF
001S-H01	Fzd1	GSISSGGYYWS	GYIYYSGSTYYN	CARHAGFYGLADYFDYW	RSSQSLLHSNGYNYLD	LGSKRAS	CMQALQIPPTF
001S-A02	Fzd1	GSISSGGYYWS	GYIYYSGSTYYN	CARGKGYSYGYGKDWFDPW	QASQDIGKYLN	DASNLET	CQQNDYLPLTF
001S-E02	Fzd1	GSISGNNYYWG	GSIYFTGGTYYN	CARVMLITDAFDIW	RSSQSLLHSNGYNYLD	LGSNRAS	CMQGTHWPYTF
001S-G02	Fzd1	GAISGTSYFWG	GSIYYTGNTYYN	CARIGIAVAAPVDHW	RASQSVGTYLT	DASNRAT	CMQATQFPLTF
001S-H02	Fzd1	GSISSSSYYWG	GYIYYSGSTYYN	CARATYGGDAFDIW	TRSSSNIGAGYDVH	GNSIRPS	CGTWDSSLSAWVF
001S-A03	Fzd1	GSISSGGYYWS	GYIYYSGSTYYN	CARVRDYYDSSGYYYDYFDYW	RASRSISSYFN	AASSLQS	CQQADTFPPTF
001S-B03	Fzd1	ASFSGHYWT	GEIDHTGSTNYE	CARGGQGGYDWGHYHGLDVW	SGDKVGHKYAS	EDSQRPS	CQAWDSSTDVVF
001S-H08	Fzd5	RAFTDNVMA	ATISGGGGSTFDD	CAAASSLTSTPYDLW			
001S-A09	Fzd5	RSFRTNALG	AAISWTGGSTYYA	CNTVTYTGGSYKNYW			
001S-B09	Fzd5	SIDSINAMA	AALTSGGITYHA	CNVITIVRGMGPRAYW			
001S-C09	Fzd5	SIFSINAMG	ATIQSGGRTNYA	CNVITIVRGMGPRAYW			
001S-C07	Fzd8	YTFTSYGIS	GWISAYNGNTNYA	CARDGTPFYSGSYYGSW	QGDSLRTYYAS	GKNNRPS	CNSRDNSGKHKVF
001S-D07	Fzd8	GTFSSYAIS	GRIIPILGIANYA	CARVPTSPYDILTGPFDYW	RSSQSLLHSNGFNYVD	FGSYRAS	CMQNLQTPWTF
001S-E07	Fzd8	ASVSSNSAAWN	GRTYYRSKWYNDYA	CARWKNYFDPW	RASQGIRSDLA	AASTLES	CLQDYSYPRTF
001S-H07	Fzd8	FTFSSYAMS	STISGGGGSTYYA	CAKDLVPWGSSAFNIW	RASQSVSSYLA	GASSRAT	CQQYGSSPPTF
004S-E05	Fzd5	FTFSTYEMN	SGVSWNGSRTHYV	CARGQSEKWWSGLYGMDVW	RASQGISSALA	AASALQS	CQQTYSTPRTF
004S-E03	Fzd5	GTFSTYAIS	GWINSGNGNTKYS	CWTGLLWFGESTDAFDIW	RASQSISSYLN	AASSLQS	CQQSYSTPLTF
004S-G06	Fzd5	GTFTYRYLH	GGIIPIFGTGNYA	CASSMVRVPYYYGMDVW	RASQSISSYLN	AASSLQS	CQQSYSTPLTF
001S-D09	Fzd8	GPFNLFAMG	AGISRTGGNTGYA	CASKTTINSGWSREYHYW			
001S-E09	Fzd8	GPFNLFAMG	AGISRTGGNTGYA	CASKTTINSGWSREYHYW			
001S-F09	Fzd8	GFFSSFTMG	AAISRNGVYTRFA	CNALAPGVRGSW			
001S-G09	Fzd8	SLFRLNGMG	ATISTRGTTHYA	CTDEESW			
001S-H09	Fzd8	GPFNLLAMG	AGISRTGGNTGYA	CASKTTINSGWSREYHYW			
001S-A10	Fzd8	SVVNFVVMG	AAITSGGSTNYA	CNRVGSREYSYW			
001S-B10	Fzd8	RTSDLYTMG	AAIGYKVKWNGERTYYL	CNAVTYNGYTIW			
001S-G12	Fzd1	SIFSSNTIY	ALITTSGNTNYA	CNAGAPAWTYRMGTYYPQFGSW			
002S-A01	Fzd1	STFSTYAMG	AAISGSGENTYYA	CVKFGMNLGYSGYDYW			
002S-B01	Fzd1	STFSNYAMG	AAISWGGGSTFYS	CAAGPIARWYRGDMDYW			
002S-C01	Fzd1	RMFSNYAMG	AAISSGGSGTYYS	CAAGPIARWYRGDMDYW			
002S-D01	Fzd1	RTDGGYVMG	ATVTWRTGTTYYA	CAAGPIARWYRGDMDYW			
002S-E01	Fzd1	RTFSSAAMG	AAISWSGSTAYYA	CATLTPYGTVASY			
002S-F01	Fzd1	RTFSSYAMG	AAVNWSGGSTYYA	CAAVFLSRNYEIQEYYRYQ			
002S-G01	Fzd1	RTFSSYAMG	AAISWSGGSTYYA	CAAGPIARWYRGDMDYW			
002S-H01	Fzd1	RSFSTYPMG	TVISGSGGSTYYS	CAAGPIARWYRGDMDYW			
002S-A02	Fzd1	RRFTTYGMG	AAVTWRSGSTYYA	CYLEGPLDVYW			

Clone ID	Initial Binding	CDRH1	CDRH2	CDRH3	CDRL1	CDRL2	CDRL3
002S-B02	Fzd1	RTFNRHVMG	AAISWSGDSTYYA	CAKLGGSSWLREYDYW			
002S-C02	Fzd1	RTFRAYAMG	SAISWSGGSTYYA	CAAGPIARWYRGDMDYW			
002S-D02	Fzd1	RTFSEYAMG	AAISWSGGSTHYA	CNADSLRGIDYW			
002S-E02	Fzd1	FTFREYAMT	SGISRDGGRTSYS	CAPRVLVTAPSGGMDYW			
002S-F02	Fzd1	GDFTNYAMA	AAVNWRGDGTYYS	CAAVFLSRNYEIQEYYRYQ			
002S-G02	Fzd1	RTFGTWAMG	AAISYNGFSTYYS	CAAGPIARWYRGDMDYW			
002S-H02	Fzd1	RTFSSYAMG	AAISWSGGSTYYA	CAAGPIARWYRGDMDYW			
002S-D03	Fzd1	RTFGSYAMG	AAISWSGGSTYYA	CAAGPIARWYRGDMDYW			
002S-E03	Fzd1	SIFSIYAMG	AVVATGGATNYA	CNMRGNWYREGRPAEFLSW			
002S-F03	Fzd1	RTSSSYAMG	AAISWSGGSTYYA	CAAGPIARWYRGDMDYW			
002S-G03	Fzd1	RTFGSYAMG	AAISWSGGSTYYA	CAAGPIARWYRGDMDYW			
002S-H03	Fzd1	QTFTAYAMG	AAISWSGSATHYA	CNAWVLVAGSRGTSADYW			
002S-A04	Fzd1	RTFSSYAMG	AAISWSGRSTYYA	CAAGPIARWYRGDMDYW			
002S-B04	Fzd1	RTFSSYAMG	AAISWSGGSTYYA	CAAGPNYSWFMPSSSRLIW			
002S-C04	Fzd1	RRFTTYGMG	AAVTWRAGSTYYA	CSADKLDYLDDQPFKTWDYW			
002S-D04	Fzd1	GTSSTYAMG	AAINRSGGSTYYA	CAAVFLSRNYEIQEYYRYQ			
002S-E04	Fzd1	GTFSTYAMG	AAISWSGDSTYYL	CAAGPIARWYRGDMDYW			
004S-H04	Fzd5	GTFSSYAIS	GWISTYNGATNYA	CARGGAGRFGEGMDVW	RASQSISSYLN	AASSLQS	CQQSYSTPLTF
001S-A04	Fzd5	YTFTSYGIS	GWISAYNGNTNYA	CASSKEKATYYYGMDVW	GLSSGSVSTNYYPS	YTNTRSS	CLLYLGRGIWVF
001S-D03	Fzd5	GTFSSYAIS	GRIIPILGIANYA	CARLDPGYYYGMDVW	TGTSSDVGGYNSVS	DVTKRPS	CFSYAGSRF
001S-F03	Fzd5	GTFSSYAIS	GGIIPIFGTANYA	CARVIFSTVTTTNDIW	TRSSGSIASNYVQ	ENDKRPS	CQSYDYDHRWVF
004S-E04	Fzd5	YTFSGYYLH	GTVTPILGTANYA	CARVDGSGYYGIDYW	RASQSISSYLN	AASSLQS	CQQSYSTPLTF
004S-A06	Fzd5	GSFSNYAIS	GRIIPILGSANYA	CARTYLKAFDIW	RASQSISSYLN	AASSLQS	CQQSYSTPLTF
004S-F04	Fzd5	YTFTNNFMH	GRINPNSGGTNYA	CARDRFDNWFDPW	RASQGISSALA	AASTLQS	CQQSYNTPWTF
001S-C03	Fzd5	GTFSSYAIS	GRIIPILGIANYA	CAREGRSRVYGGNSFDYW	RSSQSLLRRNGHNYVD	MGSNRAP	CMHGLHPPFTF
003S-A01	Fzd1	YIFTDYYMH	GGIIPIFGTANYA	CARMSSDYYDSSGYYRRGMDVW	RASQGISNNLN	GASTLQS	CQQADSFPPTF
003S-E01	Fzd1	YIFTDYYMH	GGIIPIFGTANYA	CARAWKGLWFGEGTFDYW	RASQSISSYLN	AASSLQS	CQQSYSTPLTF
003S-F01	Fzd1	GTFSSYAIS	GWINAGNGNTTYA	CARLAFDIW	RASQSISSYLN	AASSLQS	CQQSYSTPLTF
003S-A02	Fzd1	YTFTGYYMH	GWINAGNGNTTYA	CAKDRGNYGDYLDYW	RASQGISNYLA	EVSSVQG	CQQSYSTPLAF
003S-C02	Fzd1	FTFSNSDMN	ALISYDGSHTYYA	CTRGSRIGWFDPW	RASQSIGRWLA	AASRLQS	CQQGFNFPLTF
003S-E02	Fzd1	GTFSSYTIS	GGIIPISGKTDYA	CARARGGDSPLSL	RASQGISNNLN	TASSLQS	CLQDYSYPYTF
003S-F02	Fzd1	GTFRSYAIN	GGIIPIFGTANYA	CARGGWRPDYYGSGSYYSFDYW	RASQSVSSDLA	GASTRAT	CQQYETWPVLTF
003S-G02	Fzd1	FTFGTYWVT	SGITGSGGRTFYA	CARMKDWFGAFDIW	RASESVSSSSFA	GASTRAT	CQQYNNWPPNYTF
003S-C03	Fzd1	FTFSRYAMS	SYISGDSGYTNYA	CARGLVIATNWFDPW	QANQDISNYLN	AASSLQS	CQQTYNPPRTF
003S-D03	Fzd1	YTFTSYYMH	GWINTYNGNTNYP	CAESLTSTADW	RASQGISNNLN	AASSLQR	CQQSYSTPFTF
003S-E03	Fzd1	YIFTDYYMH	GWVNPTTGNTGYA	CARNVEGATSFPEFDYW	RASQGISNNLN	SASNLQS	CQQSYSPPPYTF
003S-H03	Fzd1	GTFSSYAIS	GGIIPIFGTANYA	CAKDIGSSWYYYMDVW	RASQSISSYLN	AASSLQS	CQQSYSTPLTF
003S-A04	Fzd1	FTFGTYWVT	SGITGSGGRTFYA	CARMKDWFGAFDIW	RTSERSSISSFA	GASTRAT	CQQYNNWPRNYTF
003S-C04	Fzd1	FAVSSSYMS	ASIWFDGSNQDYA	CAPNESGNVDYW	RASQGISNNLN	KASSLEN	CQQSYSTPHTF
003S-D04	Fzd1	FTFSSYAMH	SAISGSGGSTYYA	CARDHGSSWYQNTDAFDIW	QASQDIGNYLN	DVSNLER	CQHLNSYPPGDTF
003S-G04	Fzd1	FRFISHPIH	GRVIPILGVTNYA	CASSSDYGDYLKEPNYGMDVW	RASQSISSYLN	AASSLQS	CQQSYSTPLTF

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Clone ID	Initial Binding	CDRH1	CDRH2	CDRH3	CDRL1	CDRL2	CDRL3
003S-D05	Fzd2	FTFSNYAMT	SAIGTGGGTYYA	CATAYRRPGGLDVW	RSSQSLLHSDGKTYLY	LGSNRAS	CMQNTHWPLTR
003S-E05	Fzd2	FTFSSYTMS	GRIKSKANGGTTDYA	CARGSSSWYDW	KSSQSLLHSDGKTYLY	LGSNRAS	CMQNTHWPLTR
003S-A06	Fzd2	FTFADYGMH	SYISSGSYTIYYS	CARGTFDWLLSPSYDYW	RASQGISNNLN	AASRLES	CQQSYSTPLTF
003S-C06	Fzd2	FTFSNYGMH	SAISNSGGSTYYA	CTSSFLTGSQPSGYW	RASQDISSYLA	AASSLQS	CQQSYRTPLTF
003S-G06	Fzd2	FTFSDYGMH	SSTSGSGGNSKYS	CARHNPGYMGYYYGMDVW	RASQSVSSNLA	DASNRAT	CQHRTSWPLTF
003S-H06	Fzd2	GTFSSYTIS	GLVDPEDGETIYA	CTILPAAAAGTYYYYGMDVW	RASQRVGNNLA	DASIRAT	CQQYKDWPTF
003S-B07	Fzd2	FTFSDHYMS	SSITRTPSGGTTEYA	CARDGGYW	RASQSVGSYLA	GSSNRAA	CQQYGTSLLTF
003S-D07	Fzd2	YTFTNNFMH	GIINPSGGSTSYA	CARATSLGRRYCSSTSCYPRDAFDIW	QASQGISNNLN	LGSDRAS	CQQSYSTPFTF
003S-E07	Fzd2	YTFTNNFMH	GWINPNSGGTKYA	CARSVGEVGATMLGIGVWYWFDPW	RASQSISSYLN	AASSLQS	CQQSYSTPFTF
003S-A08	Fzd2	FTFSNYAMT	SAIGTGGGTYYA	CATAYRRPGGLDVW	RSSQSLLHSDGKTYLY	LGFNRAS	CMQNTHWPLTR
003S-C08	Fzd2	LTVSTNFMS	AGIGWDSTNIGYA	CARDLVAARPSNWDYW	RASQGIRNDLG	GASTLQR	CQQSYSTPRVTF
003S-E08	Fzd2	FTFRNSAMH	STISGSGGSTYYS	CARGGGYSSSW	RSSRSLLHSDGKTYLY	LGSNRAS	CMQSSHWPKTF
003S-G09	Fzd4	FTFDHNPMN	SAIGAGGGTYYA	CASPTVTRR	RASQSISSYLN	AASSLQS	CQQSYSTPLTF
003S-C10	Fzd4	GTFSSYAIS	GWINAGNGNTTYA	CARHYYGSGSYPDW	RASQSISSYLN	AASSLQS	CQQSYSTPLTF
003S-D10	Fzd4	FNFGIYSMT	SYISGDSGYTNYA	CARVGPGGWFDPW	RASQGISSYLA	AASNLLG	CQQTYSTPWTF
003S-E10	Fzd4	FTFSSYAMH	AGISASGGSTYYA	CARPSTTGTKAFDIW	RASQSIGSNLD	AASTLET	CQQSYSVPDTF
003S-A11	Fzd4	GTFSSYAIS	GWINAGNGNTTYA	CARHYYGSGSYPDW	RASQSISZYZN	ZASSLQS	CQQSYSTPLTF
003S-G11	Fzd4	GTFSSYAIS	GRIIPIFGTVNYA	CARGARLDYW	RASQSISSYLN	AASSLQS	CQQSYSTPLTF
003S-H11	Fzd4	YTFTGYYMH	GGIIPIFGTPHYA	CASTDPSSGLDYW	RASQSIGSNLD	DASSLES	CQQSFIMPLTF
003S-C12	Fzd4	GTFSSYAIS	GWINPNSGGTNYA	CARGGSSDVR	RASQSISSYLN	AASSLQS	CQQSYSTPLTF
003S-F12	Fzd4	FTFSSYAMH	SVISTSGDTVLYT	CARGGSSDVR	RASQSISSYLN	AASSLQS	CQQSYSTPLTF
004S-B01	Fzd4	GTFSSYAIS	GIINPSGGSTSYA	CAKDGVVR	RAIQSISSYLN	AASSLQS	CQQSYSTPLTF
004S-C01	Fzd4	FTFSNHYTS	STISSSGGRTFYA	CARASRIDGGWPIIDHL	RASQDIRDELA	AASTLQS	CQQADSFPLTF
004S-D01	Fzd4	FTFTNYAMS	SAISGSGGSTYYA	CARATGFGTVVFDYW	RASQSISSYLN	AASSLQS	CQQSYSTPLTF
004S-E01	Fzd4	GTFSSYAIS	GWINAGNGNTTYA	CARHYYGSGSYPDW	ZACLRIISYLN	FASSLQS	CQQSYSTPLTF
004S-F01	Fzd4	GTFSSYAIS	GWINAGNGNTTYA	CARDGVE	RASQGISNWLA	DASSLQS	CQQSHITPYTF
004S-H01	Fzd4	FTFSNYAMH	ALMSPDGTIIYYA	CAKGIVGDYGAFDIW	RASQSISSYLN	AASSLQS	CQQSYSTPLTF
004S-B02	Fzd4	FTFSSYGMH	SSINNSSRTVFYA	CAKDHLAVADAHGR	RASQSISSYLN	AASSLQS	CQQSYSTPLTF
004S-E02	Fzd4	FTFSSYAMH	AVISYDGSNEYYA	CAGGEVYEL	RASQSISSYLN	AASSLQS	CQQSYSTPLTF
004S-F02	Fzd4	FTFSTYAMH	AVISSDGNNKYYT	CAAPDVVVTADGYYW	RASQGISSALA	AASTLQS	CQQANTVPFTF
004S-G02	Fzd4	FTFANYAMN	ALISYDGGTKYYA	CAKTLVTSHALHIW	RASQSISSYLN	AASSLQS	CQQSYSTPLTF
004S-H02	Fzd4	FTFANYAMH	ALISYDGGNKYYA	CAKTLVTSHALHIW	RASQSISSYLN	AASSLQS	CQQSYSTPLTF
001S-E03	Fzd5	GSFSGYYWH	GEINHSGSTNYN	CARGRRLVRFTVTSAFDIW	TGSSSNIGAGFGVH	SDRNRPS	CQSYDSSLRASVF
001S-B05	Fzd5	GTFSSYAIS	GGIIPILGIANYA	CARIPKPRGYSYGDNGSW	RSSQSLLHSNGNTYLD	LGSDRTS	CMQSLQTPYTF
004S-A07	Fzd6	GNFKNYGIT	GRIIPALGTANYA	CARQYCSGGSCYPDAFDIR	RASQDIRSALA	QASSLIS	CQQSYSMPQTF
004S-B07	Fzd6	FTFSSYSMN	GVISKDGDNKYYA	CASSRDGYNRLAFDIW	QASQDIRNYLN	AASSLQS	CQQSSRFWTF
004S-A08	Fzd6	GTFSSYAIS	GRIIPILGIANYA	CARDGGDYGMDVW	RASQSISSYLN	AASSLQS	CQQSYSTPLTF
004S-B08	Fzd6	YTFTNNFMH	GRINPNSGGTNYA	CASQNYYGSGSYPGFDYW	RASQSISSYLN	AASSLQS	CQQSYSTPLTF
004S-D08	Fzd6	YTFTYRYLH	GGIIPIFGTANYA	CATHDSSGYYSFDYW	RASQSISSYLN	AASSLQS	CQQSYSTPLTF
004S-E08	Fzd6	FSVSSNYMN	SAIGTGGGTYYA	CTTRTYDSSGYYETQNYYMDVW	RSSRSLLHSNGNTYLQ	LGSNRAS	CVQTTQSPLTF
004S-G08	Fzd6	FTFSDYYMS	AAISYDESNKFYA	CARSAVAGAFDIW	RASQSISSYLN	AASSLQS	CQQSYSTPLTF

Clone ID	Initial Binding	CDRH1	CDRH2	CDRH3	CDRL1	CDRL2	CDRL3
004S-A09	Fzd6	FTFRDYAMN	SGISWNSGSIGYA	CARRSGYSGSVYYYYGMDVW	RASQSISSYLN	AASSLQS	CQQSYSTPLTF
004S-B09	Fzd6	FTFSSFGMH	AGINWNGGSVVYA	CARGPSHQHTFDIW	RASQGISSALA	AASSLQS	CQQSYSHTAFTF
004S-C09	Fzd6	YTFTNNFMH	GGFDPEDGETIYA	CARVGRGYSFDYW	RASQSISSYLN	AASSLQS	CQQSYSTPLTF
004S-E09	Fzd6	DTFSNYVIS	GRISAYNGYKSYA	CARSSGYVGWFDPW	RASQSISSYLN	AASSLQS	CQQSYSTPLTF
004S-F09	Fzd6	FTFSNYYTS	SYISGAGGSTEYA	CARLPRRSGKGSAFDIW	RASQSISSYLN	AASSLQS	CQQSYSTPLTF
004S-H09	Fzd6	GTFSSYTIS	GWMNPNSGNTGYA	CARVGATSAGGMDVW	RASQSISSYLN	AASSLQS	CQQSYSTPLTF
004S-C10	Fzd6	YIFTDYYMH	GLVDPEDGETIYA	CAHSDFFSGLSFGDW	RASQSISSYLN	AASSLQS	CQQSYSTPLTF
004S-D10	Fzd6	FTFSNSDMN	SSISTSGGSTYYA	CARGSYW	RASQNINNYLA	RASTLQS	CQQYSSYPYTI
004S-E10	Fzd6	TTLNKYAIS	GRITPVVGVTNYA	CALSSSWYGGFDYW	RASQSISSYLN	AASSLQS	CQQSYSTPLTF
004S-F10	Fzd6	GFTFSDHY	ALVGYDGSQQFYG	CNTGIPMLYW	RASQSISSYLN	AASSLQS	CQQSYSTPLTF
004S-G10	Fzd6	FTFSDYYMS	SAISGSGFTYYA	CARVSRGFAFDYW	RASQSISSYLN	AASSLQS	CQQSYSTPLTF
004S-A11	Fzd6	GTFSSYAIS	GRIIPILGIANYA	CARESVNNYYYMDVW	RASQSISSYLN	AASSLQS	CQQSYSTPLTF
004S-C11	Fzd6	FTFSSYAMH	ALTSYDGSKKFYA	CAKTGRGYAFDIW	RASQSISSYLN	AASSLQS	CQQSYSTPLTF
004S-E11	Fzd6	FTFSSYNMN	GRIKSKANGGTTDYA	CAKAGQQLDW	RASQSISSYLN	AASSLQS	CQQSYSTPLTF
004S-H11	Fzd6	FTFTSSAMQ	GGIIPIFGTANYA	CATVQTNYYDSSGRFSYRAHYFDYW	RASQSISRWLA	AASSLQS	CQQYVSYPLTF
004S-A12	Fzd6	YTFTNNFMH	GRINPNSGGTNYA	CARGQGYSSGWYRGDAFDIW	RASQSISSYLN	AASSLQS	CQQSYSTPLTF
004S-D12	Fzd6	FAFDDYAMH	GFIRSKAYGGTTEYA	CAKDRGYSSGWYLDYW	RASQSISSYLN	AASSLQS	CQQSYSTPLTF
005S-H01	Fzd7	FNFSSYTMR	SVIYGGGNTNYA	CARGGSGGNLSYW	RASQSISSYLN	AASSLQS	CQQSYSTPLTF
005S-A02	Fzd7	GTFSSYAIS	GMIIPFLGITNYA	CTRPYDAFDIW	RASQSISSYLN	AASSLQS	CQQSYSTPLTF
005S-C02	Fzd8	YTFASYGMH	GWINAGNGNTTYA	CARLSVWKWEQVTNWFDPW	RASQSISSYLN	AASSLQS	CQQSYSTPLTF
005S-E02	Fzd8	GTFTSYAIS	GWINAGNGNTKYS	CTTGLFPYYRYNWNNDAFDIW	RASQSISSYLN	AASSLQS	CQQSYSTPLTF
005S-A03	Fzd8	GTFSSYAIS	GWMNPNSGNTGYA	CAKWHIGATGNWFDPW	RASQSISSYLN	AASSLQS	CQQSYSTPLTF
005S-H03	Fzd8	YTFTNNFMH	GGIFPIYGISTYA	CARDRPTSSWYAFDYW	RASQGISNNLN	DASTLQT	CQQSFSAPITF
005S-F04	Fzd8	FSFSSTAMS	SYISSSGSITHYA	CARYGDYGDYW	RASQSISSYLN	AASSLQS	CQQSYSTPLTF
005S-H04	Fzd8	YTFTNNFMH	GWINAGNGNTTYA	CARVATGNAFDIW	RASQSISSYLN	AASSLQS	CQQSYSTPLTF
005S-B05	Fzd8	FTFSSYWMH	AGISGSGKTTFYA	CARGGLLFDYW	QASQDISNYLN	KASSLES	CQQSYSTPRTF
005S-F05	Fzd8	FTFTSSAVQ	GWMNPNSGNTGYA	CARRTAVAGTIDYW	RASQSISSYLN	AASSLQS	CQQSYSTPLTF
005S-G05	Fzd8	GTFSSYAIS	GWISPYNGNTNYA	CARGGWTNYGGNLDYW	RASQSISSYLN	AASSLQS	CQQSYSTPLTF
005S-H05	Fzd8	YTFTSYYMH	GRINPNSGGTNYA	CARVPDFWSGYLDYW	RASQGISRTLZ	AASSLQS	CQQTYSMPITF
005S-D06	Fzd8	YTFTYRYLH	GGIIPIFGTANYA	CARDSYPYGMDVW	RASQSISSYLN	AASSLQS	CQQSYSTPLTF
005S-F06	Fzd8	GTFSSYAIS	GRVIPILGVTNYA	CAREYLGSFDIW	RASQSVGSNLA	GASSRAT	CQQYGSSPPFTF
005S-A07	Fzd9	FTFTGSAVQ	GGILPIYGTTKYA	CARGARLYGFDYW	RASQSVSRNLA	GASTRAT	CQQRSNWPITF
005S-B07	Fzd9	FTFTSSAVQ	GWMNPNSGNTGYA	CARGRGQQWLTGYYGMDVW	RASQGISSALA	GASTLQS	CLQDYNYPFTF
005S-C07	Fzd9	FTFSSYSMN	SYIENDGSITTYA	CARAPYYYGSGSLFRLDYW	RASQSISSYLN	AASSLQS	CQQSYSTPLTF
005S-D07	Fzd9	GTFNSYAIA	GGIIPIFGTANYA	CARAGSGYYNFDYW	RASQSINRWLA	AASSLQS	CQQTYNIPITF
005S-F07	Fzd9	FSFSSYGMH	AYINSRGSLMYYA	CAKTKLPIW	RASQSINRNYLG	AASSRVT	CQQYDSWPPTF
005S-G07	Fzd9	GSFSGYAIN	GGIIPIFGTANYA	CATGYYYDYYFDYW	RASQGISNNLN	AASSLQS	CQHYYNLPLTF
005S-H07	Fzd9	GTFTNNFMH	GLVDPEDGETIYA	CARTYRIVGATPRYYYYGMDVW	RASQTINNQLA	KASNLET	CQQANSFPVTF
005S-B08	Fzd9	YIFTDYYMH	GWINPNSGGTIYA	CARGPRDSGYYPGGAFDIW	RASQSISSYLN	AASSLQS	CQQSYSTPLTF
005S-D08	Fzd9	FAFSSHWMH	SAIDGSGGSTYYA	CARDRQLGWAHWYFDLW	RASQSISSYLN	AASSLQS	CQQSYSTPLTF
005S-G08	Fzd9	YTFTGYYMH	GWINAGNGNTTYA	CARDRDYW	QTSQDINNNLN	KASSLES	CQQSYSSPPTF

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Clone ID	Initial Binding	CDRH1	CDRH2	CDRH3	CDRL1	CDRL2	CDRL3
005S-C09	Fzd9	FTFSSYGMH	SAIGTGGGTYYA	CALLVGAARGISYYYYYGMDVW	QASQDISNYLN	AASTLQS	CLQHKSFPTF
005S-D09	Fzd9	YTFTSYAMH	GWINAGNGNTTYA	CARDRPYSSGWYYPAFDIW	RASQSVSSNQLA	GASTRAT	CQQRYNWPPSITF
005S-E09	Fzd9	FNLRRYNMN	SRISNSGSLVYYA	CARDADSSGYYRYDAFDIW	RASQSVSSNLA	DASNRAT	CQQRNNWLYTF
005S-A10	Fzd9	YTFTDYYMH	GIINPSGGSTSYA	CARHVYGSGTYNNWFDPW	RASQSISSYLN	AASSLQS	CQQSYSTPLTF
005S-D10	Fzd9	YTFTSYYMH	GWMSPNSANTGYA	CARGGPIHYYYYYYMDVW	RASQGISNNLN	AASTLQS	CQQTNLFPYTF
005S-H10	Fzd9	GAFSTSSIS	GRIIPVLGTANYA	CAKGGWRSSFDPW	RASQSVSSNLA	GASTRAT	CQQYNSWPLTF
005S-B11	Fzd9	YTFTSYDIN	GGFDPEDGETIYA	CAKAGDWGLYGMDVW	RASQSISRWLA	AASSLQS	CQQTNTFPFTF
005S-C11	Fzd9	FTFTGSAVQ	GGILPIYGTTKYA	CARGARLYGFDYW	RASQSVSRKLA	GASTRAT	CQQRSNWPITF
005S-D11	Fzd9	YTFTNNFMH	GWINPNSGDTKFA	CAREANYDILTGYIRPDAFDIW	RASQSLRSKLA	GASTRAT	CQQYANSPWTF
005S-E11	Fzd9	GTFSSYAIS	GWINAGNGNTKYS	CTTTEYSSSPDYYYGMDVW	QASQDISNYLN	GASTLQS	CQQLSRYPSLF
005S-G11	Fzd9	GTFTRNSIS	GGIIPIFGTANYA	CARSSDLRIFDYW	RASQSVSSNLA	GASNRPT	CQQYGSSPYTF
005S-H11	Fzd10	YTFASYDIH	GWINAGNGNTTYA	CARDGIWDIFDYW	RASQSISSYLN	AASSLQS	CQQSYSTPLTF
005S-E12	Fzd10	YIFTDYYMH	GVIFPVYPTPDYA	CARGGSTGYYGMDVW	RASQSVGRWMA	AASSLQS	CQQANTFPFTF
005S-F12	Fzd10	GTFSSYAIS	GRIVPIVDVVKYA	CARDTCSSTSCSPDYW	RASQSISSYLN	AASSLQS	CQQSYSTPLTF
006S-A01	Fzd10	FTFSSYSMN	SAIGTGGGTYYA	CAREGWFGESPFGMDVW	RASQSISSYLN	AASSLQS	CQQSYSTPLTF
006S-F01	Fzd10	YTFTRYAVH	GWISTFNDNTNYA	CASPTGMTTNFDYW	RASQSISSYLN	AASSLQS	CQQSYSTPLTF
006S-H01	Fzd10	YIFTDYYMH	GGIIPIFGTANYA	CAKGSYYYDSSGYYWDAFDIW	RASQGISNNLN	AASNLET	CQQTSSTPLTF
006S-A02	Fzd10	YIFTDYYMH	GGIIPLFGTTDYA	CARDITGADGMDVW	RASQSISSYLN	AASSLQS	CQQSYSTPLTF
006S-D02	Fzd10	GTFSSYAIS	GRIIPTVGTANYA	CARDVCSGGSCSPDVW	RASQGISNNLN	DASSLES	CQQTYNTPRTF
006S-E02	Fzd10	FTFTSSATQ	GGIIPIFGTANYA	CARDGSSGWYSPNAFDIW	RASQGISNNLN	AASSLQS	CLQHNGYPITF
006S-H02	Fzd10	FTFRMYGMH	SRISPDGRTTTYA	CARSPRWYDAFDIW	RSSQSLLHSNGYNYLD	RVSSRFS	CMQGTHWPPTF
006S-A03	Fzd10	YIFTDYYMH	GWINAGNGNTTYA	CARDPIMFGDQPGWFDPW	RASESVSSNLA	GASSRAT	CQQYNKSPSF
006S-B03	Fzd10	GTFSSYAIS	GWINAGNGNTKYA	CAREGYDFWSGPYAFDIW	RASQTISRYLN	EVSSLQG	CQQSYSTPWTF
006S-C03	Fzd10	GTFSSNVIS	GGIIPIFGTANYA	CARGGYYYGMDVW	RASQSISSYLN	AASSLQS	CQQSYSTPLTF
014S-B01	Fzd1	YIFTDYYMH	GGIIPIFGTANYA	CARMSSDYYDSSGYYRRGMDVW	RASQGISNNLN	GASTLQS	CQQADSFPPTF
014S-D01	Fzd4	GTFSSYAIS	GWINAGNGNTTYA	CARHYYGSGSYPDW	RASQSISSHZN	AASSLQS	CQQSYSTPLTF
014S-E01	Fzd4	GTFSSYAIS	GWINAGNGNTTYA	CARHYYGSGSYPDW	RASQSIZZYZN	AASSLQS	CQQSYSTPLTF
014S-G01	Fzd4	GTFSSYAIS	GWMNPNNGNTTYA	CARHYYGSGNYRDW	RASQSISSYLN	AASSLQS	CQQSYSTPFTF
014S-A02	Fzd4	FTFSSNAMH	SGISGSGGSTYYA	CAKPGIAAAGTNNWFDPW	RASQGISSALA	GASTVES	CQQSYSTPRTF
014S-B02	Fzd4	FTFSSYAMH	SGISGSGSSTYYA	CARPSTTSFGMDVW	RASQSVSSNLA	GASTRAT	CQQYDTPLRTF
014S-C02	Fzd5	YTFTSYYMH	GRINPNSGGTNYA	CARVPDFWSGYLDYW	RASQGISSALA	AASSLQS	CQQTYSMPITF
014S-D02	Fzd5	GTFSTYAIS	GIINPSGGSTSYA	CARAKGSGWYVGSAFDIW	RASQSISSYLN	AASSLQS	CQQSYSTPLTF
014S-E02	Fzd5	FTFSDSYMS	GFIRSKAYGGTTEYA	CARATQELLLPYGMDVW	RASQSISSYLN	AASSLQS	CQQSYSTPLTF
014S-F02	Fzd5	YTFTSYYMH	GRINPNSGGTNYA	CARVPDFWSGYLDYW	RASQGVSTZLS	AASSLQS	CQQTYSMPITF
014S-G02	Fzd6	YTFTSYYMN	GIISPSGGSTSYA	CARWGDYGDLYYFDYW	RASQGISSALA	ATSTLQS	CQQVNSYPPTF
014S-H02	Fzd6	YIFTDYYMH	GRINPNSGGTNYA	CARARSSGWTDAFDIW	RASQSVSSWLA	AASTLQT	CQQSYSTPTF
014S-A03	Fzd6	GTFSSYAIS	GWINAGNGNTTYA	CARHYYGSGSYPDW	RASQSISSYLN	AASSLQS	CQQSYSTPLTF
014S-B03	Fzd6	FTFSSYNMN	GRIKSKANGGTTDYA	CARAGDSPDYW	RASQSISSYLN	AASSLQS	CQQSYSTPLTF
014S-E03	Fzd8	GTFSSYAIS	GWISPYNGYTKYA	CARAMWSYGQQNAFDIW	RASQSISSYLN	AASSLQS	CQQSYSTPLTF
014S-G03	Fzd8	FTFTSSAVQ	GWMNPNSGNTGYA	CARRTAVAGTIDYW	RASQSISSYLN	AASSLQS	CQQSYSTPLTF
014S-H03	Fzd8	YTFTSSAIH	GRINPNSGGTNYA	CARVKWELAIDYW	RASQSISSYLN	AASSLQS	CQQSYSTPLTF

Clone ID	Initial Binding	CDRH1	CDRH2	CDRH3	CDRL1	CDRL2	CDRL3
014S-B04	Fzd8	YIFTDYYMH	GWMNPNSGNTGYA	CARGGSRYDFWSGHWYFDLW	RASQGISNYLA	AASSLQS	CQQSYSTPFTF
014S-E04	Fzd8	YTFTGYYMH	GRINPNSGGTNYA	CARDVPKLVTRGVAYGMDVW	RASQSISSYLN	AASSLQS	CQQSYSTPLTF
014S-F04	Fzd8	YSFTTYGMN	GWINAGNGNTTYA	CARAAAGSYGGGYW	RASQGISNNLN	EASSVAS	CQQSYTSTPLNSF
014S-G04	Fzd8	FTFSSYGMS	SAISGSGGSTYYA	CARDLTPFTQQQLVLGLL	RASQSVSGYLA	GASTRAA	CQQYNYWPPAF
014S-H04	Fzd8	FTFTSSAVQ	GRIVPAIGFTQYA	CARSGYNRRGYFDYW	RASQSISSYLN	AASSLQS	CQQSYSTPLTF
014S-A05	Fzd8	GTFSSYAIS	GGIIPIFGTANYA	CARVTLGASVDAFDIW	RASQGISNNLN	DASSLES	CLQHNSLPFTF
014S-B05	Fzd8	GTFSSYAIS	GWVSPNTGNTVYA	CTTDRRYSTYFDLW	RASQSVSSNLA	GVSNRAT	CQQYNIWPRTF
014S-C05	Fzd8	YTFASYGMH	GWINAGNGNTTYA	CARLSVWKWEQVTNWFDPW	RASQSISSYLN	AASSLQS	CQQSYSTPLTF
014S-D05	Fzd8	GTFTSYAIS	GWINAGNGNTKYS	CTTGLFPYYRYNWNNDAFDIW	RASQSISSYLN	AASSLQS	CQQSYSTPLTF
014S-F05	Fzd9	FTFTGSAVQ	GGILPIYGTTKYA	CARGARLYGCDYW	RASQZVSRZZA	GASTRAT	CQQRSNWPITF
014S-G05	Fzd9	FTFSSSWMH	SAIGTGGGTYYA	CARKVKGYCSGGSCYGYW	RVSQGISSALA	AASSLQS	CQQTFSVPWTF
014S-H05	Fzd9	FTFSNYAMT	STISGSGVSTFYA	CARHGRIAADIW	RASQSISSYLN	AASSLQS	CQQSYSTPLTF
014S-A06	Fzd9	FTFZZSZVQ	GGILPIYGTTKYA	CARGARLYGFDYW	RASQSVSRNLA	GASTRAT	CQQRSNWPITF
014S-B06	Fzd9	FTFSSYSMN	SYIENDGSITTYA	CARAPYYYGSGSLFRLDYW	RASQSISSYLN	AASSLQS	CQQSYSTPLTF
014S-C06	Fzd10	FTFTGSAVQ	GGILPIYGTTKYA	CARGARLYGFDYW	RASQSVSRNLA	GASTRAT	CQQRSNWPITF
014S-D06	Fzd10	FTFSRYAMH	SGIGVGGGTYYA	CARDAYNWFDPR	RASQSISRYLN	AASSLQS	CQQRYSTPLTF
014S-F06	Fzd10	YIFTDYYMH	GVIFPVYPTPDYA	CARGGSTGYYGMDVW	RASQSVGRWMA	AASSLQS	CQQANTFPFTF
014S-G06	Fzd10	FTFSSYAMH	SAIGAGGGTYYA	CARDAYNWFDPW	RASQSISSYLN	AASSLQS	CQQSYSTPLTF
014S-H06	Fzd10	FTFSSYDMN	SAIGTGGGTYYA	CARDAYNWFDPW	RASQSISSYLN	AASSLQS	CQQSYSTPLTF
014S-A07	Fzd10	FTFSNAQMS	SAIGTGGGTYYA	CAREGSYYDWYFDLW	RASQNIGSRLA	GASNRAS	CQQYNHWPPLFTF
017S-E08	Fzd8	IIFSPNDMG	ALISSGGSTSYA	CHFGVASVGLNYW			
017S-H08	Fzd8	RTFSSFVMG	AAVSASGGYTWYA	CNLAQRGETYW			
017S-A09	Fzd8	LAFNGYTMG	AAISWSDNTYYA	CAAGFPTVFVVDGEYDYW			
017S-B09	Fzd8	FTLDYYAIS	ADITSGGSTNYA	CNAVTYNGYTIW			
017S-C09	Fzd8	LTFSDYTVG	ASSTGGGVFENYA	CNAVTYNGYTIW			
018S-D06	Fzd4	RIFSSYAQA	PRIPSDSTTFYA	CEVHNFGATYW			
018S-E06	Fzd4	RTFSNYVMG	AVISRSGGNTYYT	CNAVSTDWTTDYW			
018S-F06	Fzd4	RTFSTYGMG	AAISWSDNTYYA	CNSFPLRLHDW			
018S-G06	Fzd5	LAIDDYYMV	SYISTSDGSTYYA	CNAVTYNGYSIW			
018S-H06	Fzd5	LAFNGYTMG	AQISWTGGSTDYA	CNADYGTWYGIGW			
018S-A07	Fzd5	LAFNGYTMG	AAISWMSNTYYA	CNMGLGYSEYRPLGYW			
018S-B07	Fzd5	SAFSNYAMG	AAITWSGARTYYA	CNAVWKFGTTHW			
018S-C07	Fzd7	LTIDDYYVV	SYISAGDGFTYYA	CNAVTYNGYTIW			
017S-F09	Fzd4	GSFSGYYWS	GEINHSGSTNYN	CARDLRFYSSSWRRVGMDVW	RSSRSLLHTSGYNYLD	LGSNRAS	CMQGTRWPTF
017S-G09	Fzd4	YTITTYAIH	GWINADTGDTAYS	CARGWTTISSLGVW	RSSRSLLHTNGYNYLD	LGSNRAS	CMQALQTPLTF
017S-H09	Fzd5	NIFRIYAIA	AALTGQRTTNYA	CNTVTYNAGCYKKYW			
017S-A10	Fzd5	LAFNGYTMG	ASITWNGRYTYYA	CNARLDAVYGHSRYDSW			
017S-B10	Fzd5	NFFSNYPLG	GAISRTGSGTFYA	CAAGVTGSWRYW			
017S-C10	Fzd8	RSFSNYRVA	AVSWSVGMTYYA	CNAVTYNGYTIW			
017S-D10	Fzd8	GTFGSYAVG	GLISRNAGNTLYA	CNAVNGRLNYW			
017S-E10	Fzd8	RTFSSYSLA	AAVSASGANTYYA	CAAPQSPNMYIRTDQLWWYKYW			

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Clone ID	Initial Binding	CDRH1	CDRH2	CDRH3	CDRL1	CDRL2	CDRL3
018S-D07	Fzd1	RSFSTYPMG	TVISGSGGSTYYA	CAAGPTLPFRYW			
018S-E07	Fzd1	RAFSNYAMG	AAINWSGDSAYYA	CNARLSFAGGMGYW			
018S-F07	Fzd1	IKSMFDMNFMG	AFITRGGTTRYG	CNAVSTDWTRDYW			
018S-G07	Fzd1	LTIDDYYMV	SYIGTSDGTTYYA	CNAVTYNGYTIW			
018S-H07	Fzd4	RVFSSYAQA	AGIASDSTTFYA	CKVHNFGATYW			
018S-A08	Fzd4	RIFSSYAQA	ASIPSDGTTFYA	CKVHNFEATYW			
018S-B08	Fzd4	LTFSTYGMG	AAINWSGRSTVYA	CNSFPLRLHDW			
018S-C08	Fzd4	RTLSSYVVG	ALISLSGASTYYA	CNAVSTDWTTDYW			
018S-D08	Fzd5	IKSMFDMNFMG	AFITRGGTTRYG	CNAVSTDWTRDYW			
018S-E08	Fzd5	RTDGMQAMG	GAITWSLGSAFYA	CNVLAQNDGDYRTYG			
018S-F08	Fzd5	RTFSSFVMG	AAVSASGGYTWYA	CNAVWKFGTTHW			
018S-G08	Fzd5	RTFSSFVMG	AAVSASGGYTWYA	CNAVCKFGTTHW			
018S-H08	Fzd5	RTFSSFVMG	AAVTASGGYAWYA	CNAVWKFGTTHW			
018S-A09	Fzd8	ITFSFNSVG	AVFIAGYGAYYA	CNGVTYNGYTIW			
018S-B09	Fzd8	HDFSSTYGVG	ATISWGGTNIA	CAAQKPYYNGHFYADDKHYDHW			
018S-C09	Fzd8	ITFGFDSVG	AVFNAGYRAYYA	CNAVTYNGYTIW			
018S-D09	Fzd8	RTFSWYSMG	AAVSWSGVSTYYP	CNAVTYNGYTIW			
018S-E09	Fzd8	ITFSFNSVG	AVFIAGYGAYYA	CIGVTYNGYTIG			
018S-F09	Fzd8	RTDGMQAMG	GAITWSLGIAFYA	CNVLAQNDGDYRTYW			
018S-G09	Fzd8	HDFSSTYGVG	AAISWRGTNIA	CAAQKPYYNGHFYADDKHYDHW			
021S-A01	Fzd8	DSVSSNSAAWN	GRAYYKSRWYYDYA	CVRDLRPSGDLNFDYW	RASQSIGSSLH	YASQSVS	CHQSGRVPVTF
021S-C01	Fzd1	GSISSGGYSWS	GSIYHSGSTYYN	CARFYYDILNGYSYFDYW	RSSRSLLDTDDGNTYLD	TLSHRAS	CMQSIQLPWTF
021S-D01	Fzd1	FTFSSYGMH	AVISYDGSNKYYA	CAKGSVFGLKAGGYADYW	RSSQSLVHSDGNTYLS	KISNRFS	CMQATQFPHTF
021S-E02	Fzd8	YTFTSYGIS	GWISAYNGNTNYA	CARDGTPFYSGSYYGSW	QGDSLRTYYAS	GKNNRPS	CNSRDNSGKHKVF
021S-G02	Fzd8	DSVSSNSGAWN	GRTYYRSKYYNGYA	PRLDYW	RSSQSLLDSDDGNTYLD	MLSSRAP	CMQRLEFPYTF
021S-A03	Fzd8	DSVSSNSAAWN	GRTYYRSKWYNDYA	CARSQATGERFDYW	RSSQNIFQSLN	SASSLQS	CQQSYNSPITF
022S-H06	Fzd4	FTFSSYAMS	SVISTSGGTVLYT	CADGSGTSHR	RASQSISSYLN	AASSLQS	CQQSYSTPLTF
022S-A11	Fzd10	YIFTDYYMH	GGIFPIFGTANYA	CAKGSYYYDNSGYYWDAFDIW	RASQGISNNIN	AASNLET	CQQTYSIPFTF

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Table 1B provides clone IDs, the sequence identifier number of the antibody heavy chain fragment and/or light chain fragment, if present, for illustrative clones. Table 1B also provides data demonstrating binding of these clones to various Fzd receptors. The Kd values were determined by BLI as described above. Blank entries denote that the binding to the specific Fzd receptor has not yet been determined. As shown in Table 1B, a subset of antibody fragments exhibited mono-specificity for a single Fzd, or specificity for a sub-family of Fzd as determined by binding affinity in Octet BLI.

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Table 1B: Clone IDs, Heavy Chain (HC) and Light Chain (LC) Seq ID Nos, and Binding Characteristics.

			Confirmed	Kd (nM)									
Clone ID	HC SID NO	LC SID NO	Binding	Fz1	Fz2	Fz3	Fz4	Fz5	Fz6	Fz7	Fz8	Fz9	Fz10
001S-B01	1	38	Fzd1,2,7,9	**	**	n.b.	n.b.	n.b.	n.b.	**	n.b.	*	n.b.
001S-E02	2	39	Fzd1,2,7	*	**	n.b.	n.b.	n.b.	n.b.	*	n.b.	n.b.	n.b.
001S-G02	3	40	Fzd1,2,7	**	**	n.b.	n.b.	n.b.	n.b.	**	n.b.	n.b.	n.b.
001S-H02	4	41	Fzd1,2,7	**	**	n.b.	n.b.	n.b.	n.b.	**	n.b.	n.b.	n.b.
001S-A03	5	42	Fzd1,2,7,9	*	**	n.b.	n.b.	n.b.	n.b.	**	n.b.	**	n.b.
001S-B03	6	43	Fzd1,2,7	*	*	n.b.	n.b.	n.b.	n.b.	*	n.b.	n.b.	n.b.
004S-G06	7	44	Fzd5					*					
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	*	*	n.b.	n.b.	*	n.b.	*	*	n.b.	n.b.
003S-E07	18	47	Fzd2		**								
003S-D10	19	48	Fzd4	n.b.	n.b.		*	n.b.	n.b.		n.b.	n.b.	n.b.
004S-B08	20	49	Fzd6	n.b.	n.b.		n.b.	n.b.	**		n.b.	n.b.	n.b.
004S-D08	21	50	Fzd6	n.b.	n.b.		n.b.	n.b.	**		n.b.	n.b.	n.b.
004S-C09	22	51	Fzd6	n.b.	n.b.		n.b.	n.b.	**		n.b.	n.b.	n.b.
004S-F10	23	52	Fzd6	n.b.	n.b.		n.b.	n.b.	**		n.b.	n.b.	n.b.
004S-A11	24	53	Fzd6	n.b.	n.b.		n.b.	n.b.	**		n.b.	n.b.	n.b.
004S-A12	25	54	Fzd6	n.b.	n.b.		n.b.	n.b.	**		n.b.	n.b.	n.b.
005S-B07	26	55	Fzd9	n.b.	n.b.		n.b.	n.b.	n.b.		n.b.	**	n.b.
005S-D08	27	56	Fzd9	n.b.	n.b.		n.b.	n.b.	n.b.		n.b.	**	n.b.
005S-E09	28	57	Fzd9	n.b.	n.b.		n.b.	n.b.	n.b.		n.b.	**	n.b.
005S-H10	29	58	Fzd9	n.b.	n.b.		n.b.	n.b.	n.b.		n.b.	**	n.b.
005S-B11	30	59	Fzd9	n.b.	n.b.		n.b.	n.b.	n.b.		n.b.	**	n.b.
005S-D11	31	60	Fzd9	n.b.	n.b.		n.b.	n.b.	n.b.		n.b.	**	n.b.
014S-G02	32	61	Fzd6	n.b.	n.b.		n.b.	n.b.	414		n.b.	n.b.	n.b.
014S-B04	33	62	Fzd8	n.b.	n.b.		n.b.	n.b.	n.b.		**	n.b.	n.b.
014S-B06	34	63	Fzd9	n.b.	n.b.		n.b.	n.b.	n.b.		n.b.	**	n.b.
014S-G06	35	64	Fzd10	n.b.	n.b.		n.b.	n.b.	n.b.		n.b.	n.b.	**
014S-A07	36	65	Fzd10	n.b.	n.b.		n.b.	n.b.	n.b.		n.b.	n.b.	**
017S-B09	37		Fzd8	n.b.	n.b.		n.b.	n.b.	n.b.		*	n.b.	n.b.

* Indicates < 100 nM; ** indicates 100-2000 nM

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EXAMPLE 2

ALANINE-SCANNING MUTATION OF AN ANTI-FZD ANTIBODY FRAGMENT

One antibody fragment, clone 001S-A04, was selected for alanine-scanning mutagenesis of CDRs, and the respective Fzd1 binding affinities of the various mutants were determined by Octet BLI as described in Example 1. As shown in Table 2, a large number of mutants bound Fzd1 with a similar affinity as the wild type antibody fragment, demonstrating that the Fzd1 antibodies and antigen-binding fragments thereof can tolerate amino acid modifications within the CDRs.

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Table 2. 001S-A04 alanine-scanning mutant CDR sequences and K_D (nM) to biotinylated-Fzdd1 CRD as determined by Octet BLI (n.b. indicates no binding observed).

Clone ID	Kd (nM)	CDRH1	CDRH2	CDRH3	CDRL1	CDRL2	CDRL3
WT	*	YTFTSYGIS	GWISAYNGNTNYA	CASSKEKATYYYGMDVW	GLSSGSVSTNYYPS	YTNTRSS	CLLYLGRGIWVF
VLL1A	*	YTFTSYGIS	GWISAYNGNTNYA	CASSKEKATYYYGMDVW	GLSSGSVSTNYYPS	YTNTRSS	CALYLGRGIWVF
VLL2A	*	YTFTSYGIS	GWISAYNGNTNYA	CASSKEKATYYYGMDVW	GLSSGSVSTNYYPS	YTNTRSS	CLAYLGRGIWVF
VLY3A	*	YTFTSYGIS	GWISAYNGNTNYA	CASSKEKATYYYGMDVW	GLSSGSVSTNYYPS	YTNTRSS	CLLALGRGIWVF
VLL4A	*	YTFTSYGIS	GWISAYNGNTNYA	CASSKEKATYYYGMDVW	GLSSGSVSTNYYPS	YTNTRSS	CLLYAGRGIWVF
VLG5A	*	YTFTSYGIS	GWISAYNGNTNYA	CASSKEKATYYYGMDVW	GLSSGSVSTNYYPS	YTNTRSS	CLLYLARGIWVF
VLR6A	*	YTFTSYGIS	GWISAYNGNTNYA	CASSKEKATYYYGMDVW	GLSSGSVSTNYYPS	YTNTRSS	CLLYLGAGIWVF
VLG7A	*	YTFTSYGIS	GWISAYNGNTNYA	CASSKEKATYYYGMDVW	GLSSGSVSTNYYPS	YTNTRSS	CLLYLGRAIWVF
VLI8A	*	YTFTSYGIS	GWISAYNGNTNYA	CASSKEKATYYYGMDVW	GLSSGSVSTNYYPS	YTNTRSS	CLLYLGRGAWVF
VLW9A	**	YTFTSYGIS	GWISAYNGNTNYA	CASSKEKATYYYGMDVW	GLSSGSVSTNYYPS	YTNTRSS	CLLYLGRGIAVF
VLV10A	*	YTFTSYGIS	GWISAYNGNTNYA	CASSKEKATYYYGMDVW	GLSSGSVSTNYYPS	YTNTRSS	CLLYLGRGIWAF
VHK2A	*	YTFTSYGIS	GWISAYNGNTNYA	CASSAEKATYYYGMDVW	GLSSGSVSTNYYPS	YTNTRSS	CLLYLGRGIWVF
VHK4A	*	YTFTSYGIS	GWISAYNGNTNYA	CASSKEAATYYYGMDVW	GLSSGSVSTNYYPS	YTNTRSS	CLLYLGRGIWVF
VHT6A	**	YTFTSYGIS	GWISAYNGNTNYA	CASSKEKAAYYYGMDVW	GLSSGSVSTNYYPS	YTNTRSS	CLLYLGRGIWVF
VHY7A	**	YTFTSYGIS	GWISAYNGNTNYA	CASSKEKATAYYGMDVW	GLSSGSVSTNYYPS	YTNTRSS	CLLYLGRGIWVF
VHY8A	n.b.	YTFTSYGIS	GWISAYNGNTNYA	CASSKEKATYAYGMDVW	GLSSGSVSTNYYPS	YTNTRSS	CLLYLGRGIWVF
VHY9A	**	YTFTSYGIS	GWISAYNGNTNYA	CASSKEKATYYAGMDVW	GLSSGSVSTNYYPS	YTNTRSS	CLLYLGRGIWVF
VHG10A	*	YTFTSYGIS	GWISAYNGNTNYA	CASSKEKATYYYAMDVW	GLSSGSVSTNYYPS	YTNTRSS	CLLYLGRGIWVF
VHV13A	*	YTFTSYGIS	GWISAYNGNTNYA	CASSKEKATYYYGMDAW	GLSSGSVSTNYYPS	YTNTRSS	CLLYLGRGIWVF

* Indicates < 100; ** indicates > 100

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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 application, foreign patents, foreign patent application 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, application 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.

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CLAIMS

What is claimed is:

1. An isolated antibody, or an antigen-binding fragment thereof, that binds to one or more Frizzled receptor, comprising a sequence comprising:

(i) CDRH1, CDRH2 and CDRH3 sequences set forth for any of the antibodies of Table 1A; and/or

(ii) CDRL1, CDRL2 and CDRL3 sequences set forth for any of the antibodies of Table 1A,

or a variant of said antibody, or antigen-binding fragment thereof, comprising one or more amino acid modifications, wherein said variant comprises less than 8 amino acid substitutions in said CDR sequences.

2. The isolated antibody, or antigen-binding fragment thereof, of claim 1, comprising a heavy chain variable region comprising an amino acid sequence having at least 90% identity to the amino acid sequence set forth in any of SEQ ID NOs:1-37.

3. The isolated antibody, or antigen-binding fragment thereof, of claim 2, comprising a heavy chain variable region comprising the amino acid sequence set forth in any of SEQ ID NOs:1-37.

4. The isolated antibody, or antigen-binding fragment thereof, of any of claims 1-3, comprising a light chain variable region comprising an amino acid sequence having at least 90% identity to the amino acid sequence set forth in any of SEQ ID NOs:38-65.

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5. The isolated antibody, or antigen-binding fragment thereof, of claim 4, comprising a light chain variable region comprising the amino acid sequence set forth in any of SEQ ID NOs:38-65.

6. The isolated antibody, or antigen-binding fragment thereof, of any of claims 1-5, wherein the antibody, or antigen-binding fragment thereof, is humanized.

7. The isolated antibody, or antigen-binding fragment thereof, of any of claims 1-6, wherein the antibody, or antigen-binding fragment thereof, is a single chain antibody, a scFv, a univalent antibody lacking a hinge region, a nanobody, or a minibody.

8. The isolated antibody, or antigen-binding fragment thereof, of claim 7, wherein the antibody, or antigen-binding fragment thereof, is a VHH nanobody.

9. The isolated antibody, or antigen-binding fragment thereof, of claim 1, wherein the antibody, or antigen-binding fragment thereof, is a Fab or a Fab' fragment.

10. The isolated antibody, or antigen-binding fragment thereof, of any of claims 1-9, wherein the antibody, or antigen-binding fragment thereof, is a fusion protein.

11. The isolated antibody, or antigen-binding fragment thereof, of claim 10, wherein the antibody, or antigen-binding fragment thereof, is fused to a polypeptide sequence that binds LRP5 or LRP6.

12. The isolated antibody, or antigen-binding fragment thereof, of claim 11, wherein the polypeptide sequence that binds LRP5 or LRP6 is an antibody, or an antigen-binding fragment thereof, that binds to LRP5 or LRP6.

13. The isolated antibody, or antigen-binding fragment thereof, of any of claims 1-12, wherein the antibody, or antigen-binding fragment thereof, binds to one or more of Frizzled 1 (Fzd1), Frizzled 2 (Fzd2), Frizzled 3 (Fzd3), Frizzled 4 (Fzd4), Frizzled 5

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(Fzd5), Frizzled 6 (Fzd6), Frizzled 7 (Fzd7), Frizzled 8 (Fzd8), Frizzled 9 (Fzd9), and Frizzled 10 (Fzd10).

14. The isolated antibody, or antigen-binding fragment thereof, of claim 13, wherein the antibody, or antigen-binding fragment thereof, 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).

15. The isolated antibody, or antigen-binding fragment thereof, of claim 14, wherein the antibody, or antigen-binding fragment thereof, 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.

16. An isolated antibody, or an antigen-binding fragment thereof, that competes with the antibody of any of claims 1-15 for binding to a human Frizzled.

17. An isolated antibody, or antigen-binding fragment thereof, of any of claims 1-16, that binds to the Fzd with a KD of 50 μM or lower.

18. The isolated antibody, or antigen-binding fragment thereof, of any of claims 1-17, which modulates a Wnt signaling pathway in a cell, optionally a mammalian cell.

19. The isolated antibody, or antigen-binding fragment thereof, of claim 18, which increases signaling via the Wnt signaling pathway in the cell.

20. The isolated antibody, or antigen-binding fragment thereof, of claim 18, which decreases signaling via the Wnt signaling pathway in the cell.

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21. The isolated antibody, or antigen-binding fragment thereof, of any of claims 18-20, wherein the Wnt signaling pathway is a canonical Wnt signaling pathway.

22. The isolated antibody, or antigen-binding fragment thereof, of any of claims 18-20, wherein the Wnt signaling pathway is a non-canonical Wnt signaling pathway

23. An isolated polynucleotide encoding the isolated antibody, or antigen-binding fragment thereof, according to any of claims 1-22.

24. An expression vector comprising the isolated polynucleotide of claim 24.

25. An isolated host cell comprising the expression vector of claim 24.

26. A pharmaceutical composition comprising a physiologically acceptable excipient, diluent, or carrier, and a therapeutically effective amount of the isolated antibody, or antigen-binding fragment thereof, according to any of claims 1-22.

27. A method for agonizing a Wnt signaling pathway in a cell, comprising contacting the cell with the isolated antibody, or antigen-binding fragment thereof, according to claim 19.

28. The method of claim 26, wherein the antibody, or antigen-binding fragment thereof, is a fusion protein comprising a polypeptide sequence that binds LRP5 or LRP6.

29. A method for inhibiting a Wnt signaling pathway in a cell, comprising contacting the cell with the isolated antibody, or antigen-binding fragment thereof, according to claim 20.

30. 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

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the pharmaceutical composition of claim 26, wherein the isolated antibody, or antigen-binding fragment thereof is an agonist of a Wnt signaling pathway.

31. The method of claim 30, wherein the disease or disorder is wherein the disease or disorder is selected from the group consisting of: bone fractures, osteoporosis, osteoporotic fractures, spinal fusion, osseointegration of orthopedic devices, tendon-bone integration, tooth growth and regeneration, dental implantation, periodontal diseases, maxillofacial reconstruction, osteonecrosis of the jaw, alopecia, hearing loss, vestibular hypofunction, macular degeneration, vitreoretinopathy, diseases of retinal degeneration, Fuchs' dystrophy, stroke, traumatic brain injury, Alzheimer's disease, multiple sclerosis, spinal cord injuries, oral mucositis, short bowel syndrome, inflammatory bowel diseases (IBD), metabolic syndrome, diabetes, pancreatitis, exocrine pancreatic insufficiency, wound healing, diabetic foot ulcers, coronary artery disease, acute kidney injuries, chronic kidney diseases, chronic obstructive pulmonary diseases (COPD), 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, non-alcoholic fatty liver diseases and non-alcoholic steatohepatitis (NASH), cirrhosis, and chronic liver insufficiencies of all causes.

32. A method for treating a subject having a disease or disorder associated with increased or enhanced Wnt signaling, comprising administering to the subject an effective amount of the pharmaceutical composition of claim 26, wherein the isolated antibody, or antigen-binding fragment thereof is an inhibitor of a Wnt signaling pathway.

33. The method of claim 31, wherein the disease or disorder is selected from the group consisting of: tumors and cancers, degenerative disorders, fibrosis, heart failure, coronary artery disease, heterotopic ossification, osteopetrosis, and congenital high bone mass disorders.

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ABSTRACT OF THE DISCLOSURE

The present invention provides anti-Fzd monoclonal antibodies and related compositions, which may be used in any of a variety of therapeutic methods for the treatment of diseases.

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Electronic Patent Application Fee Transmittal

Application Number:	
Filing Date:	
Title of Invention:	ANTI-FRIZZLED ANTIBODIES AND METHODS OF USE
First Named Inventor/Applicant Name:	Yang Li
Filer:	Carol Denise Laherty/Lynnette Harvey Jones
Attorney Docket Number:	SRZN-004/00US 328202-2016

Filed as Small Entity

Filing Fees for Provisional

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
PROVISIONAL APPLICATION FILING FEE	2005	1	130	130
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Extension-of-Time:				
Miscellaneous:				
			Total in USD ($)	**130**

Electronic Acknowledgement Receipt

EFS ID:	31280066
Application Number:	62607877
International Application Number:	
Confirmation Number:	1506
Title of Invention:	ANTI-FRIZZLED ANTIBODIES AND METHODS OF USE
First Named Inventor/Applicant Name:	Yang Li
Customer Number:	58249
Filer:	Carol Denise Laherty/Lynnette Harvey Jones
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Attorney Docket Number:	SRZN-004/00US 328202-2016
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Deposit Account	501283
Authorized User	Lynnette Harvey Jones
The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows: 37 CFR 1.17 (Patent application and reexamination processing fees) 37 CFR 1.19 (Document supply fees)	

37 CFR 1.21 (Miscellaneous fees and charges)

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Provisional Cover Sheet (SB16)	SRZN-004-00US_Transmittal.pdf	608385 2213a4e90149ed87a2777d641458f4ee1c74da65	no	4
Warnings:					
This is not a USPTO supplied Provisional Cover Sheet SB16 form.					
Information:					
2	Application Data Sheet	SRZN-004-00US_ADS.pdf	1481201 918a3440600e5f06d9baf2ed3e71a06daeb10e36	no	9
Warnings:					
Information:					
This is not an USPTO supplied ADS fillable form					
3	Sequence Listing (Text File)	SRZN_004_00US_ST25.txt	124138	no	-
Warnings:					
Information:					
4		SRZN-004-00US_Application.pdf	676581 5473052879dc69c872c02e9b82f009f22454bff7	yes	101

Multipart Description/PDF files in .zip description

Document Description	Start	End
Specification	1	95
Claims	96	100
Abstract	101	101

Warnings:

Information:

5	Fee Worksheet (SB06)	fee-info.pdf	29989 06897e877992c3954e8fba69e8796853ccff7370	no	2
Warnings:					
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			Total Files Size (in bytes):	2920294	

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New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

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Sequence Listing was accepted.
If you need help call the Patent Electronic Business Center at (866)
217-9197 (toll free).
Reviewer: Anjum, Durreshwar
Timestamp: [year=2017; month=12; day=21; hr=12; min=22; sec=42; ms=852;
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Validated By CRFValidator v 1.0.5

Application No: 62607877 Version No: 1.0

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Output Set:

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Finished: 2017-12-19 21:13:21.695
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Total Errors: 0
No. of SeqIDs Defined: 65
Actual SeqID Count: 65

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Input Set:

Output Set:

Started: 2017-12-19 21:13:20.488
Finished: 2017-12-19 21:13:21.695
Elapsed: 0 hr(s) 0 min(s) 1 sec(s) 207 ms
Total Warnings: 65
Total Errors: 0
No. of SeqIDs Defined: 65
Actual SeqID Count: 65

Error code	Error Description
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SEQUENCE LISTING

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<140> US 62/607,877
<141> 2017-12-19

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35 40 45

Trp Ile Gly Ser Ile Tyr His Ser Gly Ser Thr Tyr Tyr Asn Pro Ser
50 55 60

Leu Lys Ser Arg Leu Thr Ile Ser Val Asp Thr Ser Lys Asn Gln Phe
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Ser Leu Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr
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Cys Ala Arg Phe Tyr Tyr Asp Ile Leu Thr Gly Tyr Ser Tyr Phe Asp
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Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys
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Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly

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Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val
            180                 185                 190

Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn
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Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro
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Lys Ser Cys
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Asn Tyr Tyr Trp Gly Trp Ile Arg Gln Pro Pro Gly Lys Gly Leu Glu
        35                  40                  45

Trp Ile Gly Ser Ile Tyr Phe Thr Gly Gly Thr Tyr Tyr Asn Pro Ser
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Leu Lys Ser Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn Gln Phe
65                  70                  75                  80
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Ser Leu Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr
                85                  90                  95

Cys Ala Arg Val Met Leu Ile Thr Asp Ala Phe Asp Ile Trp Gly Gln
            100                 105                 110

Gly Thr Met Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val
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Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala
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Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser
145                 150                 155                 160

Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val
                165                 170                 175

Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro
            180                 185                 190

Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys
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Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys
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Ser Tyr Phe Trp Gly Trp Ile Arg Gln Pro Pro Gly Lys Gly Leu Glu
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Trp Ile Gly Ser Ile Tyr Tyr Thr Gly Asn Thr Tyr Tyr Asn Pro Ser
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Leu Lys Ser Arg Leu Thr Val Ser Val Asp Thr Ser Lys Asn Gln Phe
65                  70                  75                  80

Ser Leu Asn Leu Asn Ser Val Thr Ala Ala Asp Thr Ala Thr Tyr Tyr
                85                  90                  95

Cys Ala Arg Ile Gly Ile Ala Val Ala Ala Pro Val Asp His Trp Gly
            100                 105                 110

Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser
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Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala
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Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val
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Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala
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Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val
            180                 185                 190

Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His
        195                 200                 205

Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys
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Ser Tyr Tyr Trp Gly Trp Ile Arg Gln His Pro Gly Lys Gly Leu Glu
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Trp Ile Gly Tyr Ile Tyr Tyr Ser Gly Ser Thr Tyr Tyr Asn Pro Ser
    50                  55                  60

Leu Lys Ser Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn Gln Phe
65                  70                  75                  80

Ser Leu Lys Leu Asn Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr
                85                  90                  95

Cys Ala Arg Ala Thr Tyr Gly Gly Asp Ala Phe Asp Ile Trp Gly Gln
            100                 105                 110

Gly Thr Met Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val
        115                 120                 125

Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala
    130                 135                 140

Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser
145                 150                 155                 160

Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val
                165                 170                 175

Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro
            180                 185                 190

Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys
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Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys
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Gly Tyr Tyr Trp Ser Trp Ile Arg Gln His Pro Gly Lys Gly Leu Glu
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Trp Ile Gly Tyr Ile Tyr Tyr Ser Gly Ser Thr Tyr Tyr Asn Pro Ser
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Leu Lys Ser Arg Val Thr Ile Ser Val Asp Lys Ser Lys Asn Gln Phe
65                  70                  75                  80


Ser Leu Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr
                85                  90                  95


Cys Ala Arg Val Arg Asp Tyr Tyr Asp Ser Ser Gly Tyr Tyr Tyr Asp
            100                 105                 110


Tyr Phe Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala
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Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser
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Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe
145                 150                 155                 160


Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly
                165                 170                 175


Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu
            180                 185                 190


Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr
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Val Glu Pro Lys Ser Cys
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Tyr Trp Thr Trp Ile Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Ile
        35                  40                  45


Gly Glu Ile Asp His Thr Gly Ser Thr Asn Tyr Glu Pro Ser Leu Arg
    50                  55                  60


Ser Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn Gln Phe Ser Leu
65                  70                  75                  80


Asn Leu Lys Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys Ala
                85                  90                  95


Arg Gly Gly Gln Gly Gly Tyr Asp Trp Gly His Tyr His Gly Leu Asp
            100                 105                 110


Val Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Ala Ser Thr Lys
        115                 120                 125


Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly
    130                 135                 140


Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro
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145                 150                 155                 160

Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr
                165                 170                 175

Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val
            180                 185                 190

Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn
        195                 200                 205

Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro
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Lys Ser Cys
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Tyr Leu His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
        35                  40                  45

Gly Gly Ile Ile Pro Ile Phe Gly Thr Gly Asn Tyr Ala Gln Lys Phe
    50                  55                  60

Gln Gly Arg Val Thr Ile Thr Ala Asp Glu Ser Thr Ser Thr Ala Tyr
65                  70                  75                  80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95
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```
Ala Ser Ser Met Val Arg Val Pro Tyr Tyr Tyr Gly Met Asp Val Trp
            100                 105                 110

Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro
        115                 120                 125

Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr
    130                 135                 140

Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr
145                 150                 155                 160

Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro
                165                 170                 175

Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr
            180                 185                 190

Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn
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His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser
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Cys
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Ala Met Gly Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Glu Phe Val
        35                  40                  45
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Ala Ala Ile Ser Trp Gly Gly Gly Ser Thr Phe Tyr Ser Gly Ser Val
    50                  55                  60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Ser Ala Lys Asn Thr Val His
65                  70                  75                  80

Leu Gln Met Asp Ser Leu Ile Pro Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Ala Gly Pro Ile Ala Arg Trp Tyr Arg Gly Asp Met Asp Tyr Trp
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Ala Met Gly Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Glu Phe Val
        35                  40                  45

Ser Ala Ile Ser Trp Ser Gly Gly Ser Thr Tyr Tyr Ala Gly Ser Val
    50                  55                  60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Ser Ala Lys Asn Thr Val His
65                  70                  75                  80

Leu Gln Met Asp Ser Leu Ile Pro Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Ala Gly Pro Ile Ala Arg Trp Tyr Arg Gly Asp Met Asp Tyr Trp
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Gly Lys Gly Thr Leu Val Thr Val Ser Ser
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20 25 30

Ala Met Thr Trp Val Arg Gln Ser Pro Gly Lys Arg Leu Glu Trp Val
35 40 45

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United States Patent and Trademark Office
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Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NUMBER	FILING or 371(c) DATE	GRP ART UNIT	FIL FEE REC'D	ATTY.DOCKET.NO	TOT CLAIMS	IND CLAIMS
62/607,877	12/19/2017		130	SRZN-004/00US 328202-2016		

CONFIRMATION NO. 1506

FILING RECEIPT

58249
COOLEY LLP
ATTN: Patent Group
1299 Pennsylvania Avenue, NW
Suite 700
Washington, DC 20004

Date Mailed: 01/30/2018

Receipt is acknowledged of this provisional patent application. It will not be examined for patentability and will become abandoned not later than twelve months after its filing date. Any correspondence concerning the application must include the following identification information: the U.S. APPLICATION NUMBER, FILING DATE, NAME OF APPLICANT, and TITLE OF INVENTION. Fees transmitted by check or draft are subject to collection. Please verify the accuracy of the data presented on this receipt. **If an error is noted on this Filing Receipt, please submit a written request for a Filing Receipt Correction. Please provide a copy of this Filing Receipt with the changes noted thereon. If you received a "Notice to File Missing Parts" for this application, please submit any corrections to this Filing Receipt with your reply to the Notice. When the USPTO processes the reply to the Notice, the USPTO will generate another Filing Receipt incorporating the requested corrections**

Inventor(s)

Yang Li, Mountain View, CA;
Tom Zhiye Yuan, Union City, CA;
Aaron Ken Sato, Burlingame, CA;
Wen-Chen Yeh, Belmont, CA;

Applicant(s)

Surrozen, Inc., South San Francisco, CA;

Power of Attorney:
Carol Laherty--51909

Permission to Access Application via Priority Document Exchange: No

Permission to Access Search Results: No

Applicant may provide or rescind an authorization for access using Form PTO/SB/39 or Form PTO/SB/69 as appropriate.

If Required, Foreign Filing License Granted: 01/29/2018

The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is **US 62/607,877**

Projected Publication Date: None, application is not eligible for pre-grant publication

Non-Publication Request: No

Early Publication Request: No

**** SMALL ENTITY ****

Title

ANTI-FRIZZLED ANTIBODIES AND METHODS OF USE

Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications: No

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Since the rights granted by a U.S. patent extend only throughout the territory of the United States and have no effect in a foreign country, an inventor who wishes patent protection in another country must apply for a patent in a specific country or in regional patent offices. Applicants may wish to consider the filing of an international application under the Patent Cooperation Treaty (PCT). An international (PCT) application generally has the same effect as a regular national patent application in each PCT-member country. The PCT process **simplifies** the filing of patent applications on the same invention in member countries, but **does not result** in a grant of "an international patent" and does not eliminate the need of applicants to file additional documents and fees in countries where patent protection is desired.

Almost every country has its own patent law, and a person desiring a patent in a particular country must make an application for patent in that country in accordance with its particular laws. Since the laws of many countries differ in various respects from the patent law of the United States, applicants are advised to seek guidance from specific foreign countries to ensure that patent rights are not lost prematurely.

Applicants also are advised that in the case of inventions made in the United States, the Director of the USPTO must issue a license before applicants can apply for a patent in a foreign country. The filing of a U.S. patent application serves as a request for a foreign filing license. The application's filing receipt contains further information and guidance as to the status of applicant's license for foreign filing.

Applicants may wish to consult the USPTO booklet, "General Information Concerning Patents" (specifically, the section entitled "Treaties and Foreign Patents") for more information on timeframes and deadlines for filing foreign patent applications. The guide is available either by contacting the USPTO Contact Center at 800-786-9199, or it can be viewed on the USPTO website at http://www.uspto.gov/web/offices/pac/doc/general/index.html.

For information on preventing theft of your intellectual property (patents, trademarks and copyrights), you may wish to consult the U.S. Government website, http://www.stopfakes.gov. Part of a Department of Commerce initiative, this website includes self-help "toolkits" giving innovators guidance on how to protect intellectual property in specific countries such as China, Korea and Mexico. For questions regarding patent enforcement issues, applicants may call the U.S. Government hotline at 1-866-999-HALT (1-866-999-4258).

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Title 35, United States Code, Section 184

Title 37, Code of Federal Regulations, 5.11 & 5.15

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Surrozen 2021
Merck v. Surrozen PGR2026-00027
Page 136

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Doc Code: PA..
Document Description: Power of Attorney

PTO/AIA/82B (07-13)
Approved for use through 01/31/2018. OMB 0651-0035
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TRANSMITTAL FOR POWER OF ATTORNEY TO ONE OR MORE REGISTERED PRACTITIONERS

NOTE: This form is to be submitted with the Power of Attorney by Applicant form (PTO/AIA/82B) to identify the application to which the Power of Attorney is directed, in accordance with 37 CFR 1.5, unless the application number and filing date are identified in the Power of Attorney by Applicant form. If neither form PTO/AIA/82A nor form PTO/AIA82B identifies the application to which the Power of Attorney is directed, the Power of Attorney will not be recognized in the application.

Application Number	62/607,877
Filing Date	December 19, 2017
First Named Inventor	Yang LI
Title	ANTI-FRIZZLED ANTIBODIES AND METHODS OF USE
Art Unit	N/A
Examiner Name	N/A
Attorney Docket Number	SRZN-004/00US 328202-2016

SIGNATURE of Applicant or Patent Practitioner

Signature	/Carol D. Laherty/	Date (Optional)	August 24, 2018
Name	Carol D. Laherty	Registration Number	51,909
Title (if Applicant is a juristic entity)			
Applicant Name (if Applicant is a juristic entity)			

NOTE: This form must be signed in accordance with 37 CFR 1.33. See 37 CFR 1.4(d) for signature requirements and certifications. If more than one applicant, use multiple forms.

☐ *Total of 1 forms are submitted.

163174077 v1

Doc Code: PA..
Document Description: Power of Attorney

PTO/AIA/82B (07-13)
Approved for use through 01/31/2015. OMB 0651-0035
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
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POWER OF ATTORNEY BY APPLICANT

I hereby revoke all previous powers of attorney given in the application identified in either the attached transmittal letter or the boxes below.

Application Number	Filing Date
62/607,877	December 19, 2017

(Note: The boxes above may be left blank if information is provided on form PTO/AIA/82A.)

[X] I hereby appoint the Patent Practitioner(s) associated with the following Customer Number as my/our attorney(s) or agent(s), and to transact all business in the United States Patent and Trademark Office connected therewith for the application referenced in the attached transmittal letter (form PTO/AIA/82A) or identified above: 58249

OR

[ ] I hereby appoint Practitioner(s) named in the attached list (form PTO/AIA/82C) as my/our attorney(s) or agent(s), and to transact all business in the United States Patent and Trademark Office connected therewith for the patent application referenced in the attached transmittal letter (form PTO/AIA/82A) or identified above. (Note: Complete form PTO/AIA/82C.)

Please recognize or change the correspondence address for the application identified in the attached transmittal letter or the boxes above to:

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I am the Applicant (if the Applicant is a juristic entity, list the Applicant name in the box):

Surrozen, Inc.

[ ] Inventor or Joint Inventor (title not required below)

[ ] Legal Representative of a Deceased or Legally Incapacitated Inventor (title not required below)

[X] Assignee or Person to Whom the Inventor is Under an Obligation to Assign (provide signer's title if applicant is a juristic entity)

[ ] Person Who Otherwise Shows Sufficient Proprietary Interest (e.g., a petition under 37 CFR 1.46(b)(2) was granted in the application or is concurrently being filed with this document) (provide signer's title if applicant is a juristic entity)

SIGNATURE of Applicant for Patent

The undersigned (whose title is supplied below) is authorized to act on behalf of the applicant (e.g., where the applicant is a juristic entity).

Signature		Date (Optional)	Aug. 17, 2018
Name	Wen-Chen Yeh		
Title	Chief Scientific Officer		

NOTE: Signature - This form must be signed by the applicant in accordance with 37 CFR 1.33. See 37 CFR 1.4 for signature requirements and certifications. If more than one applicant, use multiple forms.

[X] Total of 1 forms are submitted.

163174212 v1

Electronic Acknowledgement Receipt

EFS ID:	33530829
Application Number:	62607877
International Application Number:	
Confirmation Number:	1506
Title of Invention:	ANTI-FRIZZLED ANTIBODIES AND METHODS OF USE
First Named Inventor/Applicant Name:	Yang Li
Customer Number:	58249
Filer:	Carol Denise Laherty/Lynnette Harvey Jones
Filer Authorized By:	Carol Denise Laherty
Attorney Docket Number:	SRZN-004/00US 328202-2016
Receipt Date:	24-AUG-2018
Filing Date:	19-DEC-2017
Time Stamp:	14:19:12
Application Type:	Provisional

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Power of Attorney	SRZN-004-00US_Transmittal-POA.pdf	640714 7168abee0fc46d8bb3cdad3a94fa117fa3c46dad	no	2

Warnings:

Information:	
Total Files Size (in bytes):	640714

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

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APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
62/607,877	12/19/2017	Yang Li	SRZN-004/00US 328202-2016

CONFIRMATION NO. 1506

POA ACCEPTANCE LETTER

58249
COOLEY LLP
ATTN: IP Docketing Department
1299 Pennsylvania Avenue, NW
Suite 700
Washington, DC 20004

OC000000101902195

Date Mailed: 08/29/2018

NOTICE OF ACCEPTANCE OF POWER OF ATTORNEY

This is in response to the Power of Attorney filed 08/24/2018.

The Power of Attorney in this application is accepted. Correspondence in this application will be mailed to the above address as provided by 37 CFR 1.33.

Questions about the contents of this notice and the requirements it sets forth should be directed to the Office of Data Management, Application Assistance Unit, at **(571) 272-4000** or **(571) 272-4200** or **1-888-786-0101**.

/zabraha/