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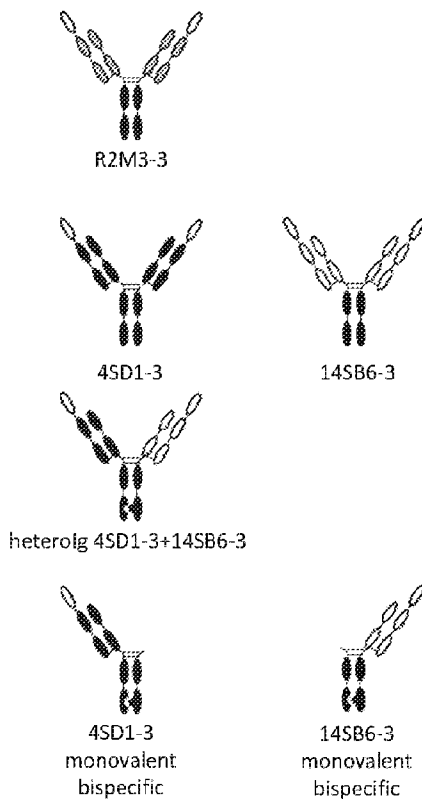
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(54) Titre : MOLECULES DE SUBSTITUTION DE WNT MULTISPECIFIQUES ET LEURS UTILISATIONS
(54) Title: MULTI-SPECIFIC WNT SURROGATE MOLECULES AND USES THEREOF

FIG. 3A



(57) **Abrégé/Abstract:**

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

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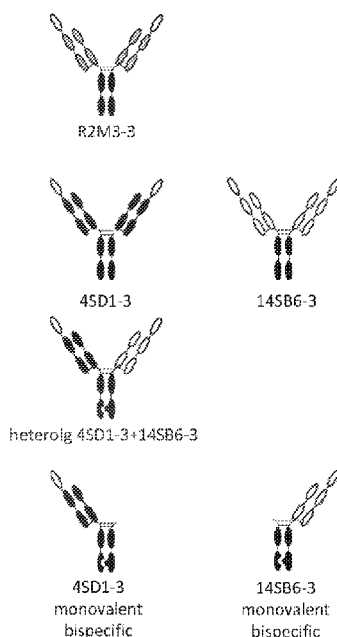
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- as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))

(54) Title: MULTI-SPECIFIC WNT SURROGATE MOLECULES AND USES THEREOF

FIG. 3A



(57) Abstract: The present disclosure provides Wnt pathway agonists and related compositions, which may be used in any of a variety of therapeutic methods for the treatment of diseases.

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MULTI-SPECIFIC WNT SURROGATE MOLECULES AND USES THEREOF

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims priority to U.S. Provisional Application No.

- 5 62/694,339, filed on July 5, 2018, U.S. Provisional Application No. 62/782,122, filed on December 19, 2018, and U.S. Provisional Application No. 62/797,772, filed on January 28, 2019, each of which is incorporated by reference herein in its entirety.

10 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_008_03WO_ST25.txt. The text file is 878 KB, was created on July 5, 2019, and is being submitted electronically via EFS-Web.
- 15

BACKGROUND

Technical Field

- The present disclosure relates generally to Wnt signaling pathway agonist molecules, compositions, and methods of using the same. Such molecules are useful, for example, in modulating Wnt signaling pathways.
- 20

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.
- 25
- 30 One of the challenges for modulating Wnt signaling as a therapeutic is the existence of multiple Wnt ligands and Wnt receptors, Frizzled 1-10 (Fzd1-

10), with many tissues expressing multiple and overlapping Fzds. Canonical Wnt signals also involve Low-density lipoprotein (LDL) receptor-related protein 5 (LRP5) or Low-density lipoprotein (LDL) receptor-related protein 6 (LRP6) as co-receptors, which are broadly expressed in various tissues, in addition to Fzds.

- 5 Ratios of Fzd to LRP binding moieties have not been previously explored to modulate signaling levels, and to confer tissue and/or functional specificity.

The Wnt signaling pathway is subdivided into canonical (β -catenin dependent) and non-canonical (β -catenin independent) pathways. The non-canonical pathway can be further divided into two distinct branches – the Planar
10 Cell Polarity (PCP) pathway and the Wnt/ Ca^{2+} pathway. Binding of certain Wnt ligands with certain Fzd receptors, or combinations of Fzd receptors can trigger the different pathways, and/or confer tissue and functional specificity.

Accordingly, there is clearly a need in the art for binding moieties that specifically bind to one or more Fzd, LRP5, or LRP6 to modulate the different Wnt
15 signaling pathways. Also a need exists to create binding moieties with certain ratios of co-receptors (e.g., Fzd and LRP receptors) to modulate signaling levels, and to confer tissue and/or functional specificity. The present disclosure addresses these needs.

20 BRIEF SUMMARY

In various embodiments, the present disclosure provides Wnt surrogate molecules and related uses thereof.

In one aspect, the present disclosure provides a multispecific Wnt surrogate molecule, wherein the Wnt surrogate molecule comprises: (i) a plurality
25 of regions that each specifically bind to a set of one or more Fzd receptor epitopes (Fzd binding regions), wherein at least two Fzd binding regions bind to the same or different sets of one or more Fzd receptor epitopes; and (ii) one or more regions that specifically bind to a Low-density lipoprotein (LDL) receptor-related protein 5 (LRP5) and/or a LDL receptor-related protein 6 (LRP6) (LRP5/6 binding regions).

In some embodiments, at least two Fzd binding regions bind to different sets of one or more Fzd receptors, different sets of one or more epitopes within the same set of one or more Fzd receptors, or a combination thereof.

In some embodiments, each Fzd binding region binds to one or more
 5 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 some embodiments, at least one Fzd binding region binds to: (i) Fzd1, Fzd2, Fzd7, and Fzd9; (ii) Fzd1, Fzd2, and Fzd7; (iii) Fzd5 and Fzd8; (iv)
 10 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; (x) Fzd4, Fzd5, and Fzd8; or (xi) Fzd1, Fzd5, Fzd7, and Fzd8.

In some embodiments, the plurality of Fzd binding regions comprises:
 (i) a first Fzd binding region that binds to a first set of one or more Fzd receptors,
 15 and (ii) a second Fzd binding region that binds to a second, different set of one or more Fzd receptors. In some embodiments, the first Fzd binding region binds to one or more of Fzd1, Fzd2, Fzd3, Fzd4, Fzd5, Fzd6, Fzd7, Fzd8, Fzd9, and Fzd10, and the second Fzd binding region binds to one or more of Fzd1, Fzd2, Fzd3, Fzd4, Fzd5, Fzd6, Fzd7, Fzd8, Fzd9, and Fzd10. In some embodiments, the
 20 first Fzd binding region binds to Fzd4 and the second Fzd binding region binds to Fzd9.

In some embodiments, the plurality of Fzd binding regions comprises:
 (i) a first Fzd binding region that binds to a first set of one or more epitopes within a set of one or more Fzd receptors, and (ii) a second Fzd binding region that binds to
 25 a second, different set of one or more epitopes within the same set of one or more Fzd receptors.

In some embodiments, the Wnt surrogate binds to at least one Fzd receptor that induces non-canonical Wnt signaling; and the second Fzd binding region binds to at least one Fzd receptor that induces canonical Wnt signaling. In a further
 30 embodiment, the Wnt surrogate binding to the first Fzd receptor and second Fzd receptor results in canonical Wnt signaling; or non-canonical Wnt signaling.

In some embodiments, at least one Fzd binding region binds monospecifically to a single Fzd receptor. In some embodiments, the at least one Fzd binding region binds monospecifically to Fzd1, Fzd2, Fzd3, Fzd4, Fzd5, Fzd6, Fzd7, Fzd8, Fzd9, or Fzd10.

5 In some embodiments, at least one Fzd binding region binds to a region of a Fzd receptor that (i) does not include the cysteine rich domain (CRD) of the Fzd receptor or (ii) includes less than the entire CRD of the FZD receptor or (iii) partially overlap with the CRD of the FZD receptor.

In some embodiments, the at least one Fzd binding region binds to a
10 hinge region of the Fzd receptor. In some embodiments, the hinge region comprises an amino acid sequence having at least 90% identity, at least 95% identity, or at least 98% identity to any of the sequences set forth in SEQ ID NO:98-107.

In some embodiments, the at least one Fzd binding region binds to
15 an N-terminal region upstream of the CRD of the Fzd receptor. In some embodiments, the N-terminal region comprises an amino acid sequence having at least 90% identity, at least 95% identity, or at least 98% identity to SEQ ID NO:108.

In some embodiments, at least one of the Fzd binding regions
20 comprises one or more antigen-binding fragments of an antibody. In some embodiments, the one or more antigen-binding fragments are selected from the group consisting of: IgG, scFv, Fab, and VHH or sdAbs. In some embodiments, the one or more antigen-binding fragments are humanized.

In some embodiments, at least one Fzd binding region comprises an
25 amino acid sequence having at least 90% identity to any of the sequences set forth in Table 1A, Table 1B, SEQ ID NOs: 1-73, or an antigen-binding fragment thereof.

In some embodiments, the one or more LRP5/6 binding regions
comprises one or more antigen-binding fragments of an antibody. In some
embodiments, the one or more antigen-binding fragments are selected from the
30 group consisting of: IgG, scFv, Fab, and VHH or sdAbs. In some embodiments, the one or more antigen-binding fragments are humanized.

In some embodiments, the one or more LRP5/6 binding regions comprise an amino acid sequence having at least 90% identity to any of the sequences set forth in Table 2A, Table 2B, or SEQ ID NOs: 74-97, or an antigen-binding fragment thereof.

5 In some embodiments, the Wnt surrogate molecule comprises two or more LRP5/6 binding regions.

In some embodiments, the Fzd binding regions and the LRP5/6 binding regions are in a ratio of Fzd:LRP5/6.

In some embodiments, the Fzd binding regions and the LRP5/6 binding regions are in a ratio of Fzd_n:LRP5/6_n (F_n:L_n), wherein F and L are integers between 1 and 9, inclusive, and n is an integer between 1 and 4 inclusive.

In some embodiments, the Fzd binding regions and the LRP5/6 binding regions are in a ratio of Fzd:LRP5/6 selected from the group consisting of: 1:1, 2:1, 3:1, 4:1, 5:1, 6:1, 2:1 2:3, 2:5, 2:7, 7:2, 5:2, 3:2, 3:4, 3:5, 3:7, 3:8, 8:3, 7:3, 15 5:3, 4:3, 4:5, 4:7, 4:9, 9:4, 7:4, 5:4, 6:7, 7:6, 1:2, 1:3, 1:4, 1:5, 1:6, 2:1 (with two Fzd binders and one LRP binder), 1:2 (with one Fzd binder and two LRP binders), 2:1:1 (with two different LRP binders), 1:1:2 (with two different Fzd binders), 1:1:1 (two different Fzd binders and one LRP binder or one Fzd binder and two different LRP binders) and 1:1:1:1 (all different Fzd and LRP binders).

20 In some embodiments, the ratio of Fzd binding regions to LRP5/6 binding regions (Fzd:LRP5/6) comprises 2 Fzd binding regions and 2 LRP5/6 binding regions; 2 Fzd binding regions and 1 LRP5/6 binding region; or 1 Fzd binding region and 2 LRP5/6 binding regions.

In some embodiments, the ratio of Fzd binding regions to LRP5/6 binding regions (Fzd:LRP5/6) comprises a first Fzd binding region, a second Fzd binding region, and 1 LRP5/6 binding region; or a first Fzd binding regions, a second Fzd binding regions, a first LRP5/6 binding region, and a second LRP5/6 binding region. In further embodiments, the first Fzd and second Fzd binding regions bind to different Fzd receptors, or bind to the same Fzd receptor on 25 different regions/epitope, and the first LRP5/6 and second LRP5/6 binding regions bind to different epitopes or to different LRP proteins.

In some embodiments, the LRP binding regions comprise a first LRP binding region that binds to a first set of one or more LRP receptors, and a second LRP binding region that binds to a second, different set of one or more LRP receptors.

5 In some embodiments, the Wnt surrogate molecule comprises a structural format selected from the group consisting of: hetero-Ig, diabody (DART), tandem diabody (DART), diabody-Fc, Fabs-in-tandem, Fabs-in-tandem IgG (FIT-Ig), Fv-IgG, and tandem scFv.

10 In some embodiments, the Wnt surrogate molecule comprises: (i) a first light chain and a first heavy chain forming a first Fzd binding region, and (ii) a second light chain and a second heavy chain forming a second Fzd binding region, wherein the first and second Fzd binding regions bind to different sets of one or more Fzd receptor epitopes.

15 In some embodiments, the Wnt surrogate molecule comprises a first LRP5/6 binding region fused to an N-terminus of the first light chain, a C-terminus of the first light chain, an N-terminus of the first heavy chain, or a C-terminus of the first heavy chain. In some embodiments, the Wnt surrogate molecule comprises second LRP5/6 binding region fused to an N-terminus of the second light chain, a C-terminus of the second light chain, an N-terminus of the second heavy chain, or
20 a C-terminus of the second heavy chain.

In some embodiments, the first and second heavy chains are connected to each other. In some embodiments, the first heavy chain comprises a first CH3 domain, the second heavy chain comprises a second CH3 domain, and the first and second CH3 domains are connected to each other. In some
25 embodiments, the first and second CH3 domains are connected to each other via knobs-into-holes mutations. In some embodiments, the first heavy chain and/or the second heavy chain comprise an amino acid sequence having at least 90% identity, at least 95% identity, or at least 98% identity to any of the sequences set forth in SEQ ID NOs:110, 112, 114, 116, 118, 120, or 122 (or shown in Table 5 or
30 Table 6A), and (ii) the first light chain and/or the second light chain comprise an amino acid sequence having at least 90% identity to any of the sequences set forth

in SEQ ID NOs:109, 111, 113, 115, 117, 119, or 121 (or shown in Table 5 or Table 6A). In some embodiments, the Wnt surrogate molecule comprises one or more sequences (e.g., two or three sequences) having at least 90%, at least 95%, at least 98% or at least 99% sequence identity to a sequence disclosed in Table 5 or Table 6A. In particular embodiments, the Wnt surrogate molecule comprises the sequences set forth for any Wnt surrogate molecule disclosed in Table 5 or Table 6A, or sequences having at least 90%, at least 95%, at least 98%, or at least 99% identity to such sequences.

In another aspect, the Wnt surrogate molecule has a structure as set forth in Table 6B.

In some embodiments, the Wnt surrogate molecule modulates a Wnt signaling pathway in a cell, optionally a mammalian cell. In some embodiments, the Wnt surrogate molecule increases signaling via the Wnt signaling pathway in the cell. In some embodiments, the Wnt signaling pathway is a canonical Wnt signaling pathway. In some embodiments, the Wnt signaling pathway is a non-canonical Wnt signaling pathway.

In another aspect, the present disclosure provides a pharmaceutical composition comprising a pharmaceutically acceptable excipient, diluent, or carrier, and a Wnt surrogate molecule according to any of the embodiments herein.

In another aspect, the present disclosure provides a method for agonizing a Wnt signaling pathway in a cell, comprising contacting the cell with a Wnt surrogate molecule according to any of the embodiments herein, wherein the Wnt surrogate molecule is an agonist of a Wnt signaling pathway.

In another aspect, the present disclosure provides a method for treating a subject having a disease or disorder, comprising administering to the subject an effective amount of a pharmaceutical composition of any of the embodiments herein, wherein the Wnt surrogate molecule is an agonist of a Wnt signaling pathway.

In some embodiments, the disease or disorder is associated with reduced or impaired Wnt signaling, and/or wherein the subject would benefit from increased Wnt signaling. In some embodiments, the disease or disorder is selected

from the group consisting of: bone fractures, stress fractures, vertebral compression fractures, osteoporosis, osteoporotic fractures, non-union fractures, delayed union fractures, spinal fusion, pre-operative optimization for spine surgeries, osteonecrosis, osseointegration of implants or orthopedic devices,

5 osteogenesis imperfecta, bone grafts, tendon repair, tendon-bone integration, tooth growth and regeneration, maxillofacial surgery, dental implantation, periodontal diseases, maxillofacial reconstruction, osteonecrosis of the jaw, hip or femoral head, avascular necrosis, alopecia, hearing loss, vestibular hypofunction, macular degeneration, age-related macular degeneration (AMD), vitreoretinopathy,

10 retinopathy, diabetic retinopathy, diseases of retinal degeneration, Fuchs' dystrophy, cornea diseases, stroke, traumatic brain injury, Alzheimer's disease, multiple sclerosis, muscular dystrophy, muscle atrophy caused by sarcopenia or cachexia, diseases affecting blood brain barrier (BBB), spinal cord injuries, spinal cord diseases, oral mucositis, short bowel syndrome, inflammatory bowel diseases

15 (IBD), metabolic syndrome, diabetes, dyslipidemia, pancreatitis, exocrine pancreatic insufficiency, wound healing, diabetic foot ulcers, pressure sores, venous leg ulcers, epidermolysis bullosa, dermal hypoplasia, myocardial infarction, coronary artery disease, heart failure, hematopoietic cell disorders, immunodeficiencies, graft versus host diseases, acute kidney injuries, chronic

20 kidney diseases, chronic obstructive pulmonary diseases (COPD), idiopathic pulmonary fibrosis, acute liver failure of all causes, acute liver failure drug-induced, alcoholic liver diseases, chronic liver failure of all causes, cirrhosis, liver fibrosis of all causes, portal hypertension, chronic liver insufficiency of all causes, nonalcoholic steatohepatitis (NASH), nonalcoholic fatty liver disease (NAFLD)

25 (fatty liver), alcoholic hepatitis, hepatitis C virus-induced liver diseases (HCV), hepatitis B virus-induced liver diseases (HBV), other viral hepatitis (e.g., hepatitis A virus-induced liver diseases (HAV) and hepatitis D virus-induced liver diseases (HDV)), primary biliary cirrhosis, autoimmune hepatitis, liver surgery, liver injury, liver transplantation, "small for size" syndrome in liver surgery and transplantation,

30 congenital liver disease and disorders, any other liver disorder or defect resulting from genetic diseases, degeneration, aging, drugs, and injuries.

In some embodiments, the disease or disorder is a bone disease or disorder. In some embodiments, the Wnt surrogate molecule binds: (i) Fzd1, Fzd2, and Fzd7; or (ii) Fzd1, Fzd2, Fzd5, Fzd7, and Fzd8.

5 BRIEF DESCRIPTION OF THE DRAWINGS

The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

FIG. 1. Schematic diagrams of illustrative formats of Wnt surrogate molecules. Different VHH, Fv, or scFv, Diabody, or Fabs containing various VL and VH regions directed against different Fzd receptors and Lrp receptors are combined in various ratios. The different colors represent different binders (which can bind to the same target or different targets).

FIG. 2A. Schematic diagram of a Fzd receptor including a cysteine rich domain (CRD), hinge region, and N-terminal region.

FIG. 2B. Schematic diagram of a Wnt surrogate molecule with binding specificity for the Fzd receptor hinge region.

FIG. 2C. Binding kinetics of 1791-3 and 1291-3 Wnt surrogate molecules.

FIG. 2D. In vitro activity of 1791-3 and 1291-3 Wnt surrogate molecules.

FIG. 3A. Schematic diagrams of monospecific and multispecific Wnt surrogate molecules.

FIG. 3B. In vitro activity of Wnt surrogate molecules in 293STF cells.

FIG. 3C. In vitro activity of Wnt surrogate molecules in 293STF cells overexpressing Fzd4 (293STF Fzd4OE).

FIG. 3D. In vitro activity of Wnt surrogate molecules in 293STF cells overexpressing Fzd9 (293STF Fzd9OE).

FIG. 3C. In vitro activity of Wnt surrogate molecules in 293STF cells overexpressing Fzd4 and Fzd9 (293STF Fzd4OE + Fzd9OE).

FIGS. 4A-4E show sequence alignments of the hinge region of various Fzds (SEQ ID NOs: 2251-2260).

FIGS. 5A-5C show the structure of heterologous molecules containing soluble ligands for different Fzd receptors together with Lrp5, and their
5 in vitro activity in 293STF cells.

FIGS. 6A-6E show the structures of Wnt surrogate molecules with different ratios of Fzd to Lrp binders and their impact on Wnt3a activation of beta-catenin-dependent signaling.

FIGS. 7A-7D show structures containing heterodimerization of two
10 different Lrp binders together with Fzd binders and their in vitro activity in 293STF cells.

FIGS. 8A-8J show 1:1 bivalent bispecific L1/F1 tandem scFv molecules are not efficient in activating β -catenin dependent WNT signaling. (A) Diagram of 1:1 bivalent bispecific L1/F1 tandem scFv constructs. Each circle
15 represents a scFv domain, the thin black line at the end of each molecule represent the 6xHis tag. (B) Ni resin purified tandem scFv molecules were separated on 4-15% SDS-PAGE gel. Left panel, from left to right: L1-F1 tandem scFv with 5-mer, 10-mer and 15-mer linkers under reducing (R, lanes 1-3) or nonreducing (NR, lanes 4-6) conditions. Right panel, from left to right, F1-L1
20 tandem scFv with 5-mer, 10-mer and 15-mer linkers under reducing (R, lanes 1-3) or nonreducing (NR, lanes 4-6) conditions. C) and D) The dose dependent STF activity of the Ni resin purified tandem scFv with L1 fused to the N-terminus of F1 (C), or F1 fused to the N-terminus of L1 (D). E) The dose dependent STF activity of the monomer peak fractions from the size exclusive column (SEC) polished
25 tandem scFv with L1 fused to the N-terminus of F1. F) The dose dependent STF activity of the monomer peak fractions from the SEC polished tandem scFv with F1 fused to the N-terminus of L1. G) and H) The STF activity across the SEC fractions of various tandem scFv. The arrows on each panel indicate the position of the monomeric tandem scFv protein. I) The elution profile of the protein standard on
30 the SEC column, thick arrow indicates the expected position of the monomeric

form of tandem scFv molecules. J) The addition of anti-His antibody to L1:5:F1 induced significant activation of β -catenin dependent WNT signaling.

FIG. 9. 1:1 bivalent bispecific L1/F1 tandem scFv molecules are not efficient in activating β -catenin dependent WNT signaling. The dose dependent STF activity of the tandem scFvs, purified either from Ni column alone or additionally purified from SEC column, with L1 fused to the N-terminus of F1 and F1 fused to the N-terminus of L1 comparing to recombinant WNT3A and the surrogate WNT, 18R5-DKK1c. These data are identical to Fig. 1C-1F, except with the positive control molecule data included.

FIGS. 10A-10F. Increasing the valency of L1 and F1 tandem scFv by fusing to a Fc domain significantly increased the activity in Wnt signal. A) Diagram of F1 and L1 tandem scFv fused to Fc domain to generate the 2:2 tetravalent bispecific formats. B-C) The STF activity across the SEC fractions of the tandem scFv-Fc molecules. D) The elution profile of the protein standard on the SEC column, thick arrow indicates the expected position of the monomeric form of tandem scFv-Fc molecules. E-F) The dose dependent STF activity of the tandem scFv-Fc molecules from the protein peak fractions corresponding to the monomeric forms of the molecules from SEC column.

FIGS. 11A-11B. The STF activity and Octet binding profiles of the 2:2 tetravalent bispecific F1/L1 molecules. A) The dose dependent STF activity of the F1/L1 bivalent tandem scFv molecules from the protein peak fractions corresponding to the monomeric forms comparing to the recombinant WNT3A and 18R5-DKK1c. These data are identical to Fig. 2E and 2F, except with the positive control molecule data included. B) The binding affinity of the various 2:2 tetravalent bispecific tandem scFv molecules to FZD1 and LRP6E1E2 measured on the Octet.

FIGS. 12A-12E. The 2:2 tetravalent bispecific molecules, consisting of the two F1 and two L2 binding arms, are highly potent in inducing Wnt signaling. A) Diagrams representing the 2:2 tetravalent bispecific molecule formats consisting of the FZD binder F1 and the LRP6E3E4 binder L2. The STF activities across the SEC fractions of these various 2:2 molecules are shown below the format diagrams. B-C) The dose dependent STF activities of the 2:2 tetravalent bispecific

molecules consisting of F1 and L2 binding arms in both orientations, from the protein peak fractions corresponding to the monomeric forms of each molecules from SEC column. D) The activity of the 2:2 tetravalent bispecific molecules from the combination of F1 and L2 needs the presence of both FZD and LRP6 binding
 5 arms, as the substitution of either binding arm by the neutral anti-GFP scFv fragment resulted in no activity. E) Interaction of the molecules from D) to their respective receptors were determined by Octet. The binders L1, L2, and F1 in the IgG1 format were also included as comparators.

FIGS. 13A-13B. 1:1 bivalent bispecific L2/F1 tandem scFv molecules
 10 are not efficient in activating β -catenin dependent WNT signaling. A) The STF activity across the SEC fractions of the 1:1 bivalent bispecific tandem scFv molecules between F1/L2 in both orientations. The molecular format diagrams are also shown on top. The arrows in each panel indicate the position of the monomeric forms of the proteins. B) The dose dependent STF activities of the
 15 molecules shown in A) from the protein peak fractions corresponding to the monomeric forms of each molecule from SEC column.

FIGS. 14A-14I. The 2:2 tetravalent bispecific molecules, consisting of the two F2 and two L1 or L2 binding arms, activate Wnt signaling. A) The molecular format diagrams of the 2:2 tetravalent bispecific molecules consisting of
 20 F2 and L1 binding arms and the STF activities across the SEC column of these various surrogate WNT molecules. The arrows in each panel indicate the position of the monomeric forms of the proteins. B) The dose dependent STF activities of the 2:2 tetravalent bispecific molecules consisting of F2 and L1 binding arms in both orientations, from the protein peak fractions corresponding to the monomeric
 25 forms of each molecules from SEC column. These surrogate WNT agonists have higher potency but lower efficacy compared to WNT3A in activating Wnt signal. C) and D) The 1:1 bivalent bispecific molecular formats and STF activities across SEC column fractions for molecules consisting of F2 and L2 combinations in both orientations. The F2-L2 orientation does not appear to be active while the reverse
 30 L2-F2 orientation seems to be active in the 1:1 format. The arrows in each panel indicate the position of the monomeric forms of the proteins. E) The dose

dependent STF activities of the 1:1 bivalent bispecific L2-F2 molecules from the protein peak fractions corresponding to the monomeric forms of each molecules from SEC column. F) and H) The 2:2 tetravalent bispecific molecular formats and STF activities across SEC column fractions for molecules consisting of F2 and L2 combinations in both orientations. The arrows in each panel indicate the position of the monomeric forms of the proteins. G) and I) The dose dependent STF activities of the 2:2 tetravalent bispecific L2/F2 molecules of F) and H) from the protein peak fractions corresponding to the monomeric forms of each molecules from SEC column.

FIGS. 15A-15B. FZD specific profile of F1, F2, F3, and the STF activities of the 1:1 bivalent bispecific F2/L1 molecules. A) The binding affinity and specificity of F1, F2, and F3 to all 10 FZDs measured on Octet. B) The STF activity across the SEC fractions of the 1:1 bivalent bispecific tandem scFv molecules between F2/L1 in both orientations. The molecular format diagrams are also shown on top. The arrows in each panel indicate the position of the monomeric forms of the proteins. The 1:1 bivalent bispecific format is ineffective in inducing Wnt/ β -catenin signaling.

FIGS 16A-16D. The 2:2 tetravalent bispecific molecules, consisting of the two F3 and two L1 or L2 binding arms, activate Wnt signaling. A) Diagrams representing the 2:2 tetravalent bispecific molecule formats consisting of the FZD binder F3 and the LRP6 binders L1 or L2 where the FZD binder is attached to the N-terminus of the LRP binders. The STF activities across the SEC fractions of these two 2:2 molecules are shown below the format diagrams. B) Diagrams representing the 2:2 tetravalent bispecific molecule formats consisting of the FZD binder F3 and the LRP6 binders L1 or L2 where the LRP binder is attached to the N-terminus of the FZD binder, the reverse orientation of molecules in A). The STF activities across the SEC fractions of these two 2:2 molecules are shown below the format diagrams. C,D) The dose dependent STF activities of the 2:2 tetravalent bispecific molecules from the protein peak fractions corresponding to the monomeric forms of each molecules from SEC column. C) and D) correspond to molecules from A) and B), respectively.

FIG. 17. The 1:1 bivalent bispecific L1/F3 or L2/F3 tandem scFv molecules are not efficient in activating β -catenin dependent WNT signaling. The STF activity across the SEC fractions of the 1:1 bivalent bispecific tandem scFv molecules between F3/L1 or F3/L2 in both orientations. The molecular format diagrams are also shown on top. The arrows in each panel indicate the position of the monomeric forms of the proteins. The 1:1 bivalent bispecific format is ineffective in inducing Wnt/ β -catenin signaling.

FIGS. 18A-18B. The 2:2 tetravalent bispecific dumbbell format has similar activity to the 2:2 tetravalent bispecific tandem scFv-Fc format. A) The dose dependent STF activity of the protein peak fractions corresponding to the monomeric forms of each molecules from SEC column. The surrogate WNT agonists tested here are the combination of F1 and L1 in the 2:2 tetravalent bispecific dumbbell format. This format is active, however, show a much lower efficacy compare to WNT3A. There is a preference for L1 to be on the N-terminus of Fc. B) The dose dependent STF activity of the protein peak fractions corresponding to the monomeric forms of each molecules from SEC column. The surrogate WNT agonists tested here are the combination of F1 and L2 in the 2:2 tetravalent bispecific dumbbell format. There is also a preference for L2 to be on the N-terminus of Fc.

FIGS. 19A-19C. Various 1:1 bivalent bispecific tandem scFv molecules show little to no activity. A) Sequential binding of FZD8, followed by 1:1 bivalent bispecific tandem scFv molecules, F3:5:L2 and L2:5:F3, then followed by additional of LRP6E3E4 on Octet show that the 1:1 tandem scFv molecules can simultaneously engage both FZD and LRP. B) The diagram of various molecules with the 1:1 stoichiometry between FZD and LRP binders. C) The dose response of the various molecules in B) showed no induction of STF signals.

FIG 20A-20B. Various 1:1 bispecific scFv molecules show little to no activity. The diagrams of various combinations of 1:1 bivalent bispecific scFv molecules between F3/L2 and F2/L2 binder pairs. B) The dose response of the various molecules depicted in A,B) showed no induction of STF signals.

FIGS. 21A-21K. Exploring different stoichiometries of FZD and LRP binders and combining binders of different receptor specificities or epitopes in the 2:2 tetravalent multispecific formats. A,D) The diagrams of molecules with different stoichiometries between FZD and LRP binders, such as 2 FZD binders and 1 LRP binders (2:1) or 1 FZD and 2 LRP binders (1:2). B,C) Dose response of molecules in A) in STF reporter assays. E,F) Dose response of molecules in D) in STF reporter assays. G) Molecular formats of 2:2 tetravalent trispecific molecule where the two FZD binders are of different FZD specificity (1:1:2). H) Dose response of molecules in G) in STF reporter assays. I) Molecular formats of 2:2 tetravalent trispecific molecule where the two FZD binders are of different FZD specificity together with only one LRP binder (1:1:1:0). J) Dose response of the molecules in I) in STF reporter assays. K) Molecular formats of 2:2 tetravalent trispecific molecule where the two FZD binders and the two LRP binders are all of different FZD or LRP specificities (1:1:1:1). H) Dose response of the molecules in K) in STF reporter assays.

DETAILED DESCRIPTION

The present disclosure relates to multispecific Wnt surrogate molecules that specifically bind to a plurality of different Fzd receptors and epitopes and to LRP5 and/or LRP6 in order to modulate a Wnt signaling pathway. In particular embodiments, the Wnt surrogate molecules activate a Wnt signaling pathway or increase signaling via a Wnt signaling pathway. In certain aspects, the Wnt surrogate molecules of the present disclosure have: (i) a plurality of regions that each specifically bind to a set of one or more Frizzled (Fzd) receptors and/or epitopes, referred to herein as “Fzd binding regions;” and (ii) one or more regions that specifically bind to a LRP5 and/or a LRP6, referred to herein as “LRP5/6 binding regions.” Certain embodiments encompass specific structural formats or arrangements of the Fzd binding regions and the LRP5/6 binding regions that are advantageous in modulating Wnt signaling pathways and related biological effects, e.g., for the treatment of diseases and disorders associated with Wnt signaling.

In particular embodiments, the Wnt surrogate molecules disclosed herein include multiple Fzd binding regions with binding specificities for different Fzd receptors and/or epitopes. For example, a Wnt surrogate molecule may include at least two Fzd binding regions that each bind to different sets of one or more Fzd receptors, different sets of one or more epitopes within the same set of one or more Fzd receptors, or a combination thereof. Each Fzd binding region may be monospecific, bispecific, trispecific, etc. for a different Fzd receptor epitope or plurality of Fzd receptor epitopes. Such multispecific Wnt surrogate molecules are capable of selectively activating specific combinations of Fzd receptors, while reducing or eliminating activation of non-targeted Fzd receptors. Embodiments of the present disclosure are advantageous for selectively modulating Wnt signaling in a target cell type and/or for the treatment of a specific disease or disorder, e.g., by reducing off-target effects.

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

The practice of the present disclosure 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.

5 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.

 As used herein, "A and/or B" encompasses one or more of A or B, and combinations thereof such as A and B.

10 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.

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

 Standard techniques may be used for recombinant DNA, oligonucleotide synthesis, and tissue culture and transformation (e.g., electroporation, lipofection). Enzymatic reactions and purification techniques may
20 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
25 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,
30 microbiological, chemical syntheses, chemical analyses, pharmaceutical preparation, formulation, and delivery, and treatment of subjects.

Embodiments of the present disclosure relate to antibodies and antigen-binding fragments thereof that bind to one or more Fzd receptors. Sequences of illustrative antibodies, or antigen-binding fragments, or complementarity determining regions (CDRs) thereof, are set forth in SEQ ID
5 NOs:1-73, Tables 1A and 1B, and Table 5.

Embodiments of the present disclosure relate to antibodies and antigen-binding fragments thereof that bind to LRP5 and/or LRP6. Sequences of illustrative antibodies, or antigen-binding fragments, or complementarity determining regions (CDRs) thereof, are set forth in SEQ ID NOs:74-97, Tables 2A
10 and 2B, and Table 5.

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,
15 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® (Nabs; also referred to as VHH or single-domain antibodies (sdAbs)), synthetic variants thereof, naturally occurring variants, fusion proteins comprising an antibody or an antigen-binding fragment thereof, humanized
20 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 gene fusion (WO94/13804; P. Holliger et al., Proc. Natl. Acad. Sci. USA 90 6444-6448, 1993) are also a particular form of
25 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
30 6444-6448, 1993; Y. Reiter et al., Nature Biotech, 14, 1239-1245, 1996; S. Hu et al., Cancer Res., 56, 3055-3061, 1996.

The term "antigen-binding fragment" as used herein refers to a polypeptide fragment that contains at least one CDR of an immunoglobulin heavy and/or light chain, or of a VHH or sdAb, that binds to the antigen of interest, in particular to one or more Fzd receptors, or to LRP5 and/or LRP6. 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 receptors or LRP5 and/or LRP6. In particular embodiments, an antigen-binding fragment may comprise all three VH CDRs or all three VL CDRs. Similarly, an antigen-binding fragment thereof may comprise all three CDRs of a VHH or sdAb. An antigen-binding fragment of a Fzd-specific antibody is capable of binding to a Fzd receptor. An antigen-binding fragment of a LRP5/6-specific antibody is capable of binding to a LRP5 and/or LRP6 receptor. As used herein, the term encompasses not only isolated fragments but also polypeptides comprising an antigen-binding fragment of an antibody disclosed herein, such as, for example, fusion proteins comprising an antigen-binding fragment of an antibody disclosed herein, such as, e.g., a fusion protein comprising a VHH or sdAb that binds one or more Fzd receptors and a VHH or sdAb that binds LRP5 and/or LRP6.

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

In certain embodiments, antibodies and antigen-binding fragments thereof as described herein include a heavy chain and a light chain CDR set,

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

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

The structures and locations of immunoglobulin CDRs and variable domains may be determined by reference to Kabat, E. A. et al., Sequences of Proteins of Immunological Interest. 4th Edition. US Department of Health and Human Services. 1987, and updates thereof, now available on

the Internet (immuno.bme.nwu.edu). The Abgenisis software from Distributed Bio (South San Francisco, CA) was used to map the specificity determining regions of the antibodies disclosed herein, which include the Kabat definition of CDRs. (Padlan et al. FASEB J. 9, 133-139 (1995).

5 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"

10 encompasses not only intact monoclonal antibodies and full-length monoclonal antibodies, but also fragments thereof (such as Fab, Fab', F(ab')₂, Fv), single chain (scFv), VHH or sdAbs, variants thereof, fusion proteins comprising an antigen-binding fragment of a monoclonal antibody, humanized monoclonal antibodies, chimeric monoclonal antibodies, and any other modified

15 configuration of the immunoglobulin molecule that comprises an antigen-binding fragment (epitope recognition site) of the required specificity and the ability to bind to an epitope, including Wnt surrogate molecules disclosed herein. It is not intended to be limited as regards the source of the antibody or the manner in which it is made (e.g., by hybridoma, phage selection,

20 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

25 covalent heterodimer that includes an intact antigen-binding site. The enzyme pepsin is able to cleave IgG molecules to provide several fragments, including the F(ab')₂ fragment which comprises both antigen-binding sites. An Fv fragment for use according to certain embodiments of the present disclosure can be produced by preferential proteolytic cleavage of an IgM, and on rare occasions of an IgG or

30 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.

5 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
10 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
15 antibodies, while bispecific antibodies may be generated that bind specifically to one or more Fzd receptors 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.

20 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
25 separated—light and heavy polypeptide chains from an antibody V region into an scFv molecule which will fold into a three dimensional structure substantially similar to the structure of an antigen-binding site. See, e.g., U.S. Patent Nos. 5,091,513 and 5,132,405, to Huston et al.; and U.S. Patent No. 4,946,778, to Ladner et al.

30 In certain embodiments, an antibody as described herein is in the form of a diabody. Diabodies are multimers of polypeptides, each polypeptide

comprising a first domain comprising a binding region of an immunoglobulin light chain and a second domain comprising a binding region of an immunoglobulin heavy chain, the two domains being linked (e.g., by a peptide linker) but unable to associate with each other to form an antigen binding site: antigen binding sites are
5 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
10 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
15 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
20 (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)).

25 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
30 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
5 UniBody® therefore binds univalently to only one site on target cells.

In certain embodiments, the antibodies of the present disclosure may take the form of a VHH or sdAb. VHH or sdAb technology was originally developed following the discovery and identification that *camelidae* (e.g., camels and llamas) possess fully functional antibodies that consist of heavy chains only and therefore
10 lack light chains. These heavy-chain only antibodies contain a single variable domain(V_HH) and two constant domains (C_H2, C_H3). The cloned and isolated single variable domains have full antigen binding capacity and are very stable. These single variable domains, with their unique structural and functional properties, form the basis of "VHH or sdAbs". VHH or sdAbs are encoded by single genes and are
15 efficiently produced in almost all prokaryotic and eukaryotic hosts, e.g., *E. coli* (see, e.g., U.S. Pat. No. 6,765,087), molds (for example *Aspergillus* or *Trichoderma*) and yeast (for example *Saccharomyces*, *Kluyvermyces*, *Hansenula* or *Pichia* (see, e.g., U.S. Pat. No. 6,838,254). The production process is scalable and multi-kilogram quantities of VHH or sdAbs have been produced. VHH or sdAbs
20 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 VHH or sdAbs against a desired target, based on automated high-throughput selection of B-cells. VHH or sdAbs are single-domain antigen-binding fragments of camelid-specific heavy-chain only antibodies. VHH or sdAbs typically
25 have a small size of around 15 kDa.

In certain embodiments, the antibodies or antigen-binding fragments thereof as disclosed herein are humanized. This refers to a chimeric molecule, generally prepared using recombinant techniques, having an antigen-binding site derived from an immunoglobulin from a non-human species and the remaining
30 immunoglobulin structure of the molecule based upon the structure and/or sequence of a human immunoglobulin. The antigen-binding site may comprise

either complete variable domains fused onto constant domains or only the CDRs grafted onto appropriate framework regions in the variable domains. Epitope binding sites may be wild type or modified by one or more amino acid substitutions. This eliminates the constant region as an immunogen in human individuals, but the possibility of an immune response to the foreign variable region remains (LoBuglio, A. F. et al., (1989) *Proc Natl Acad Sci USA* 86:4220-4224; Queen et al., *PNAS* (1988) 86:10029-10033; Riechmann et al., *Nature* (1988) 332:323-327). Illustrative methods for humanization of the anti-Fzd antibodies disclosed herein include the methods described in U.S. Pat. No. 7,462,697.

Another approach focuses not only on providing human-derived constant regions, but also 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., 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 antibody operably linked or otherwise fused to a heterologous Fc portion of a different antibody. In certain embodiments, the heterologous Fc domain is of human origin. In other embodiments, the heterologous Fc domain may be from a different Ig class from the parent antibody, including IgA (including subclasses IgA1 and IgA2), IgD, IgE, IgG (including subclasses IgG1, IgG2, IgG3, and IgG4), and IgM. In further embodiments, the heterologous Fc domain may be comprised of CH2 and CH3 domains from one or more of the different Ig classes. As noted above with regard to humanized antibodies, the antigen-binding fragment of a chimeric antibody may comprise only one or more of the CDRs of the antibodies described herein (e.g., 1, 2, 3, 4, 5, or 6 CDRs of the antibodies described herein), or may comprise an entire variable domain (VL, VH or both).

Wnt Surrogates

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

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

Wnt surrogate molecules of the present disclosure are biologically active in binding to one or more Fzd receptors and to one or more of LRP5 and

LRP6, and in activation of Wnt signaling, i.e., the Wnt surrogate molecule is a Wnt agonist. The term "Wnt agonist activity" refers to the ability of an agonist to mimic the effect or activity of a Wnt protein binding to an Fzd receptor and/or LRP5 or LRP6. The ability of the Wnt surrogate molecules and other Wnt agonists disclosed

5 herein to mimic the activity of Wnt can be confirmed by a number of assays. Wnt agonists typically initiate a reaction or activity that is similar to or the same as that initiated by the receptor's natural ligand. In particular, the Wnt agonists disclosed herein activate, enhance or increase the canonical Wnt/ β -catenin signaling pathway. As used herein, the term "enhances" refers to a measurable increase in

10 the level of Wnt/ β -catenin signaling compared with the level in the absence of a Wnt agonist, e.g., a Wnt surrogate molecule disclosed herein. In particular embodiments, the increase in the level of Wnt/ β -catenin signaling is at least 10%, at least 20%, at least 50%, at least two-fold, at least five-fold, at least 10-fold, at least 20-fold, at least 50-fold, or at least 100-fold as compared to the level of

15 Wnt/ β -catenin signaling in the absence of the Wnt agonist, e.g., in the same cell type. Methods of measuring Wnt/ β -catenin signaling are known in the art and include those described herein. Wnt surrogate molecules disclosed herein are multispecific, i.e., they specifically bind to two or more different epitopes. At least one epitope is within one or more Fzd receptors and at least one epitope binds to

20 LRP5 and/or LRP6. In particular embodiments, multispecific Wnt surrogate molecules are multispecific with respect to Fzd receptor binding, i.e., they specifically bind to two or more different types of Fzd receptors, two or more different epitopes within a single type of Fzd receptor, or a combination thereof. For example, a multispecific Wnt surrogate molecule may bind to two or more of

25 Fzd1, Fzd2, Fzd3, Fzd4, Fzd5, Fzd6, Fzd7, Fzd8, Fzd9, and Fzd10. In certain embodiments, a multispecific Wnt surrogate molecule 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; (x) Fzd4, Fzd5, and Fzd8; or (xi) Fzd1, Fzd5, Fzd7 and Fzd8.

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In certain embodiments, a Wnt surrogate molecule that is multispecific with respect to Fzd binding includes at least one Fzd binding region that binds to a plurality of different Fzd receptor epitopes, e.g., epitopes within different Fzd receptors, different epitopes within the same Fzd receptor, or combinations thereof. For example, the Wnt surrogate molecule may include at least one Fzd binding region that binds to two or more Fzd receptors, e.g., two or more of Fzd1, Fzd2, Fzd3, Fzd4, Fzd5, Fzd6, Fzd7, Fzd8, Fzd9, and Fzd10. As another example, the Wnt surrogate molecule may include at least one Fzd binding region that 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; (x) Fzd4, Fzd5 and Fzd8; or (xi) Fzd1, Fzd5, Fzd7 and Fzd8.

Alternatively, or in combination, in certain embodiments, a Wnt surrogate that is multispecific with respect to Fzd binding includes at least two Fzd binding regions that each bind to different sets of one or more Fzd receptor epitopes, e.g., epitopes within different Fzd receptors, different epitopes within the same Fzd receptor, or combinations thereof. A set of one or more Fzd receptor epitopes may include one, two, three, four, five, six, seven, eight, nine, ten, or more Fzd receptor epitopes, such that each Fzd binding region may be monospecific, bispecific, trispecific, tetraspecific, etc.

In certain embodiments, a multispecific Wnt surrogate includes two or more Fzd binding regions, wherein one or more of these Fzd binding regions specifically binds only one Fzd receptor or receptor epitope. In certain embodiments, two or more, three or more, or four or more Fzd binding regions within the multispecific Wnt surrogate each specifically bind only one Fzd receptor or receptor epitope, wherein at least two or more, at least three or more, or at least four or more of the Fzd binding regions specifically bind different Fzd receptors and/or receptor epitopes.

In certain embodiments, the Wnt surrogate molecule includes a first Fzd binding region that binds to a first set of one or more Fzd receptor epitopes,

and a second Fzd binding region that binds to a second, different set of one or more Fzd receptor epitopes. For example, the first Fzd binding region may bind to a first set of one or more Fzd receptors, and the second Fzd binding region may bind to a second, different set of one or more Fzd receptors. Alternatively or in combination, the first Fzd binding region may bind to a first set of one or more epitopes within a set of one or more Fzd receptors, and the second Fzd binding region may bind to a second, different set of one or more epitopes within the same set of one or more Fzd receptors. In certain embodiments, the first Fzd binding region binds to one or more of Fzd1, Fzd2, Fzd3, Fzd4, Fzd5, Fzd6, Fzd7, Fzd8, Fzd9, and Fzd10. In certain embodiments, the second Fzd binding region binds to one or more of Fzd1, Fzd2, Fzd3, Fzd4, Fzd5, Fzd6, Fzd7, Fzd8, Fzd9, and Fzd10.

In certain embodiments, the Wnt surrogate molecule includes a first Fzd binding region and a second Fzd binding region, wherein: the first Fzd binding region binds to Fzd1 and the second Fzd binding region binds to Fzd2; the first Fzd binding region binds to Fzd1 and the second Fzd binding region binds to Fzd3; the first Fzd binding region binds to Fzd1 and the second Fzd binding region binds to Fzd4; the first Fzd binding region binds to Fzd1 and the second Fzd binding region binds to Fzd5; the first Fzd binding region binds to Fzd1 and the second Fzd binding region binds to Fzd6; the first Fzd binding region binds to Fzd1 and the second Fzd binding region binds to Fzd7; the first Fzd binding region binds to Fzd1 and the second Fzd binding region binds to Fzd8; the first Fzd binding region binds to Fzd1 and the second Fzd binding region binds to Fzd9; or the first Fzd binding region binds to Fzd1 and the second Fzd binding region binds to Fzd10.

In certain embodiments, the Wnt surrogate molecule includes a first Fzd binding region and a second Fzd binding region, wherein: the first Fzd binding region binds to Fzd2 and the second Fzd binding region binds to Fzd3; the first Fzd binding region binds to Fzd2 and the second Fzd binding region binds to Fzd4; the first Fzd binding region binds to Fzd2 and the second Fzd binding region binds to Fzd5; the first Fzd binding region binds to Fzd2 and the second Fzd binding region binds to Fzd6; the first Fzd binding region binds to Fzd2 and the second Fzd binding region binds to Fzd7; the first Fzd binding region binds to Fzd2 and the second Fzd binding region binds to Fzd8; the first Fzd binding region binds to Fzd2 and the second Fzd binding region binds to Fzd9; or the first Fzd binding region binds to Fzd2 and the second Fzd binding region binds to Fzd10.

binding region binds to Fzd7; the first Fzd binding region binds to Fzd2 and the second Fzd binding region binds to Fzd8; the first Fzd binding region binds to Fzd2 and the second Fzd binding region binds to Fzd9; or the first Fzd binding region binds to Fzd2 and the second Fzd binding region binds to Fzd10.

5 In certain embodiments, the Wnt surrogate molecule includes a first Fzd binding region and a second Fzd binding region, wherein: the first Fzd binding region binds to Fzd3 and the second Fzd binding region binds to Fzd4; the first Fzd binding region binds to Fzd3 and the second Fzd binding region binds to Fzd5; the first Fzd binding region binds to Fzd3 and the second Fzd binding region binds to
10 Fzd6; the first Fzd binding region binds to Fzd3 and the second Fzd binding region binds to Fzd7; the first Fzd binding region binds to Fzd3 and the second Fzd binding region binds to Fzd8; the first Fzd binding region binds to Fzd3 and the second Fzd binding region binds to Fzd9; or the first Fzd binding region binds to Fzd3 and the second Fzd binding region binds to Fzd10.

15 In certain embodiments, the Wnt surrogate molecule includes a first Fzd binding region and a second Fzd binding region, wherein: the first Fzd binding region binds to Fzd4 and the second Fzd binding region binds to Fzd5; the first Fzd binding region binds to Fzd4 and the second Fzd binding region binds to Fzd6; the first Fzd binding region binds to Fzd4 and the second Fzd binding region binds to
20 Fzd7; the first Fzd binding region binds to Fzd4 and the second Fzd binding region binds to Fzd8; the first Fzd binding region binds to Fzd4 and the second Fzd binding region binds to Fzd9; or the first Fzd binding region binds to Fzd4 and the second Fzd binding region binds to Fzd10.

 In certain embodiments, the Wnt surrogate molecule includes a first
25 Fzd binding region and a second Fzd binding region, wherein: the first Fzd binding region binds to Fzd5 and the second Fzd binding region binds to Fzd6; the first Fzd binding region binds to Fzd5 and the second Fzd binding region binds to Fzd7; the first Fzd binding region binds to Fzd5 and the second Fzd binding region binds to Fzd8; the first Fzd binding region binds to Fzd5 and the second Fzd binding region
30 binds to Fzd9; or the first Fzd binding region binds to Fzd5 and the second Fzd binding region binds to Fzd10.

In certain embodiments, the Wnt surrogate molecule includes a first Fzd binding region and a second Fzd binding region, wherein: the first Fzd binding region binds to Fzd6 and the second Fzd binding region binds to Fzd7; the first Fzd binding region binds to Fzd6 and the second Fzd binding region binds to Fzd8; the first Fzd binding region binds to Fzd6 and the second Fzd binding region binds to Fzd9; or the first Fzd binding region binds to Fzd6 and the second Fzd binding region binds to Fzd10.

In certain embodiments, the Wnt surrogate molecule includes a first Fzd binding region and a second Fzd binding region, wherein: the first Fzd binding region binds to Fzd7 and the second Fzd binding region binds to Fzd8; the first Fzd binding region binds to Fzd7 and the second Fzd binding region binds to Fzd9; or the first Fzd binding region binds to Fzd7 and the second Fzd binding region binds to Fzd10.

In certain embodiments, the Wnt surrogate molecule includes a first Fzd binding region and a second Fzd binding region, wherein: the first Fzd binding region binds to Fzd8 and the second Fzd binding region binds to Fzd9; or the first Fzd binding region binds to Fzd8 and the second Fzd binding region binds to Fzd10. In certain embodiments, the Wnt surrogate molecule includes a first Fzd binding region and a second Fzd binding region, wherein the first Fzd binding region binds to Fzd9 and the second Fzd binding region binds to Fzd10.

For each of the combinations of Fzd receptors disclosed above, it is understood that, in certain embodiments, the first or second binding region may specifically bind only the indicated Fzd, or it may also bind additional Fzds. For example, wherein the first Fzd binding region binds to Fzd1 and the second Fzd binding region binds to Fzd2, the first Fzd binding region may specifically bind only Fzd1, or it may also bind to one or more other Fzds in addition to Fzd1. Similarly, the second Fzd binding region may specifically bind only Fzd2, or it may also bind to one or more other Fzds in addition to Fzd2. However, the first and second Fzd binding regions bind to different sets of Fzd receptors.

In certain embodiments, the first and second binding regions may specifically bind to different epitopes within the same Fzd receptor. For example,

the first binding region may bind to a first epitope in Fzd1, and the second binding region may bind to a second, different epitope in Fzd1. The first binding region may bind to a first epitope in Fzd2, and the second binding region may bind to a second, different epitope in Fzd2. The first binding region may bind to a first epitope in Fzd3, and the second binding region may bind to a second, different epitope in Fzd3. The first binding region may bind to a first epitope in Fzd4, and the second binding region may bind to a second, different epitope in Fzd4. The first binding region may bind to a first epitope in Fzd5, and the second binding region may bind to a second, different epitope in Fzd5. The first binding region may bind to a first epitope in Fzd6, and the second binding region may bind to a second, different epitope in Fzd6. The first binding region may bind to a first epitope in Fzd7, and the second binding region may bind to a second, different epitope in Fzd7. The first binding region may bind to a first epitope in Fzd8, and the second binding region may bind to a second, different epitope in Fzd8. The first binding region may bind to a first epitope in Fzd9, and the second binding region may bind to a second, different epitope in Fzd9. The first binding region may bind to a first epitope in Fzd10, and the second binding region may bind to a second, different epitope in Fzd10.

For each of the combinations of Fzd receptor epitopes disclosed above, it is understood that, in certain embodiments, the first or second binding regions may specifically bind only the indicated epitope, or may also bind additional epitopes. For example, wherein the first binding region binds to a first epitope in Fzd1, and the second binding region binds to a second, different epitope in Fzd1, the first binding region may specifically bind only the first epitope in Fzd1, or it may also bind to one or more other epitopes in Fzd1 or other Fzd receptors. Similarly, the second Fzd binding region may specifically bind only the second epitope in Fzd1, or it may also bind to one or more other epitopes in Fzd1 or other Fzd receptors. In embodiments where the first and second binding regions specifically bind to the same Fzd receptor or receptors, the first and second binding regions bind to different epitopes within the same receptor(s).

In particular embodiments, multispecific Wnt surrogate molecules are multispecific with respect to LRP5/6 binding, i.e., they specifically bind to two or more different epitopes within LRP5 and/or LRP6. In certain embodiments, a multispecific Wnt surrogate molecule includes a first LRP5/6 binding region that binds to a first
5 epitope within LRP5 and/or LRP6, and a second LRP5/6 binding region that binds to a second, different epitope within LRP5 and/or LRP6.

For each of the combinations of the LRP receptor epitopes, it is understood that, in certain embodiments, the first or second binding regions may specifically bind only the indicated epitope, or may also bind additional epitopes.
10 For example, wherein the first binding region binds to a first epitope in LRP5E1E2, and the second binding region binds to a second, different epitope in LRP5E1E2, the first binding region may specifically bind only the first epitope in LRP5E1E2, or it may also bind to one or more other epitopes in LRP5E1E2 or other LRP receptors. Similarly, the second LRP binding region may specifically bind only the
15 second epitope in LRP5E1E2, or it may also bind to one or more other epitopes in LRP5E1E2 or other LRP receptors. In embodiments where the first and second binding regions specifically bind to the same LRP receptor or receptors, the first and second binding regions bind to different epitopes within the same receptor(s). Similar epitope binding can occur for LRP5E3E4, LRP6E1E2, and LRP6E3E4. In
20 particular embodiments, multispecific Wnt surrogate molecules are multispecific with respect to both Fzd binding and LRP5/6 binding. For example, a Wnt surrogate molecule may include a plurality of Fzd binding regions that each specifically bind to a different set of one or more Fzd receptors, and a plurality of LRP5/6 binding regions that each specifically bind to a different epitope within
25 LRP5 and/or LRP6. It shall be appreciated that the various embodiments of Fzd binding regions and LRP5/6 binding regions disclosed herein may be combined in many ways to generate multispecific Wnt surrogate molecules with any desired combination of Fzd and LRP5/6 binding specificity.

In particular embodiments, Wnt surrogate molecules disclosed herein
30 are multivalent, e.g., they comprise two or more regions that each specifically bind to the same epitope, e.g., two or more regions that bind to an epitope within one or

more Fzd receptors and/or two or more regions that bind to an epitope within LRP5 and/or LRP6. In particular embodiments, they comprise two or more regions that bind to an epitope within one or more Fzd receptors and two or more regions that bind to an epitope within LRP5 and/or LRP6.

5 In particular embodiments, Wnt surrogate molecule comprise Fzd binding regions and LRP5/6 binding regions in a ratio of $F_{zn}:LRP5/6_n$ ($F_n:L_n$), wherein F and L are integers between 1 and 9, inclusive, and n is an integer between 1 and 4 inclusive.

10 In certain embodiments, Wnt surrogate molecules comprise a ratio of the number of regions that bind one or more Fzd receptors to the number of regions that bind LRP5 and/or LRP6 of or about: 1:1, 2:1, 3:1, 4:1, 5:1, 6:1, 2:1 2:3, 2:5, 2:7, 7:2, 5:2, 3:2, 3:4, 3:5, 3:7, 3:8, 8:3, 7:3, 5:3, 4:3, 4:5, 4:7, 4:9, 9:4, 7:4, 5:4, 6:7, 7:6, 1:2, 1:3, 1:4, 1:5, 1:6, 2:1 (with two Fzd binders and one LRP binder), 1:2 (with one Fzd binder and two LRP binders), 2:1:1 (with two different LRP binders), 15 1:1:2 (with two different Fzd binders), 1:1:1 (two different Fzd binders and one LRP binder or one Fzd binder and two different LRP binders) and 1:1:1:1 (all different Fzd and LRP binders). Varying the ratios of Fzd binding moieties to LRP5/6 binding moieties can confer tissue and/or functional specificity and modulate signaling levels. In certain embodiments, Wnt surrogate molecules are 20 multispecific and multivalent.

Wnt surrogate molecules disclosed herein may have any of a variety of different structural formats or configurations. Wnt surrogate molecules may comprise polypeptides and/or non-polypeptide binding moieties, e.g., small molecules. In particular embodiments, Wnt surrogate molecules comprise both a 25 polypeptide region and a non-polypeptide binding moiety. In certain embodiments, Wnt surrogate molecules may comprise a single polypeptide, or they may comprise two or more, three or more, or four or more polypeptides. In certain embodiments, the Wnt surrogates comprises one, two, three, or four polypeptides, e.g., linked or bound to each other or fused to each other.

30 When the Wnt surrogate molecules comprise a single polypeptide, they may be a fusion protein comprising one or more Fzd binding regions (also

referred to herein as “Fzd binding domains”) and one or more LRP5/6 binding regions (also referred to herein as “LRP5/6 binding domains”). The binding regions may be directly fused or they may be connected via a linker, e.g., a polypeptide or chemical linker, including but not limited to any of those disclosed herein.

5 When the Wnt surrogate molecules comprise two or more polypeptides, the polypeptides may be linked via covalent bonds, such as, e.g., disulfide bonds, and/or noncovalent interactions. For example, heavy chains of human immunoglobulin IgG interact at the level of their CH3 domains directly, whereas, at the level of their CH2 domains, they interact via the carbohydrate
10 attached to the asparagine (Asn) N84.4 in the DE turn.

 Wnt surrogate polypeptides may be engineered to facilitate binding between two polypeptides. For example, knobs-into-holes amino acid modifications may be introduced into two different polypeptides to facilitate their binding. Knobs-into-holes amino acid (AA) changes is a rational design strategy developed in
15 antibody engineering, used for heterodimerization of the heavy chains, in the production of bispecific IgG antibodies. AA changes are engineered in order to create a knob on the CH3 of the heavy chains from a first antibody and a hole on the CH3 of the heavy chains of a second antibody. The knob may be represented by a tyrosine (Y) that belongs to the 'very large' IMGT volume class of AA, whereas
20 the hole may be represented by a threonine (T) that belongs to the 'small' IMGT volume class. Other means of introducing modifications into polypeptides to facilitate their binding are known and available in the art. For example, specific amino acids may be introduced and used for cross-linking, such as Cysteine to form an intermolecular disulfide bond.

25 In particular embodiments, the Wnt surrogate molecules comprise one or more binding regions derived from an antibody or antigen-binding fragment thereof, e.g., antibody heavy chains or antibody light chains or fragments thereof. In certain embodiments, one or more polypeptides of a Wnt surrogate molecule are antibodies or antigen-binding fragments thereof. In certain embodiments, Wnt
30 surrogates comprise two antibodies or antigen-binding fragments thereof, e.g., one that binds one or more Fzd receptors and one that binds LRP5 and/or LRP6. In

certain embodiments, Wnt surrogates comprise three antibodies or antigen-binding fragments thereof, e.g., one that binds a first set of one or more Fzd receptor epitopes, one that binds a second, different set of one or more Fzd receptor epitopes, and one that binds LRP5 and/or LRP6. In certain embodiments, Wnt

5 surrogates comprise four antibodies or antigen-binding fragments thereof, e.g., one that binds a first set of one or more Fzd receptor epitopes, one that binds a second, different set of one or more Fzd receptor epitopes, one that binds a first epitope within LRP5 and/or LRP6, and one that binds a second, different epitope within LRP5 and/or LRP6.

10 In certain embodiments, a Wnt surrogate molecule includes a polypeptide comprising two antibody heavy chain regions (e.g., hinge regions) bound together via one or more disulfide bond. In certain embodiments, a Wnt surrogate molecule includes a polypeptide comprising an antibody light chain region (e.g., a C_L region) and an antibody heavy chain region (e.g., a C_H1 region)

15 bound together via one or more disulfide bonds.

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

In one embodiment, a Wnt surrogate molecule comprises an scFv or antigen-binding fragment thereof fused to a VHH or sdAb or antigen-binding

20 fragment thereof. In certain embodiments, the scFv specifically binds one or more Fzd receptor epitopes, and the VHH or sdAb specifically binds LRP5 and/or LRP6. In certain embodiments, the scFv specifically binds LRP5 and/or LRP6, and the VHH or sdAb specifically binds one or more Fzd receptor epitopes. In particular embodiments, the scFv or antigen-binding fragment thereof is fused directly to the

25 VHH or sdAb or antigen-binding fragment thereof, whereas in other embodiments, the two binding regions are fused via a linker moiety. In particular embodiments, the VHH or sdAb is fused to the N-terminus of the scFv, while in other embodiments, the VHH or sdAb is fused to the C-terminus of the scFv. In particular embodiments, the scFv is described herein or comprises any of the CDR sets

30 described herein. In particular embodiments, the VHH or sdAb is described herein or comprises any of the CDR sets disclosed herein.

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

Fabs may be converted into a full IgG format that includes both the Fab and Fc fragments, for example, using genetic engineering to generate a fusion polypeptide comprising the Fab fused to an Fc region, i.e., the Fab is present in a full IgG format. The Fc region for the full IgG format may be derived from any of a variety of different Fcs, including but not limited to, a wild-type or modified IgG1, IgG2, IgG3, IgG4 or other isotype, e.g., wild-type or modified human IgG1, human IgG2, human IgG3, human IgG4, human IgG4Pro (comprising a mutation in core hinge region that prevents the formation of IgG4 half molecules), human IgA, human IgE, human IgM, or the modified IgG1 referred to as IgG1 LALAPG. The L234A, L235A, P329G (LALA-PG) variant has been shown to eliminate complement binding and fixation as well as Fc- γ dependent antibody-

dependent cell-mediated cytotoxicity (ADCC) in both murine IgG2a and human IgG1. These LALA-PG substitutions allow a more accurate translation of results generated with an “effectorless” antibody framework scaffold between mice and primates. In particular embodiments of any of the IgG disclosed herein, the IgG comprises one or more of the following amino acid substitutions: N297G, N297A, N297E, L234A, L235A, or P236G.

Non-limiting examples of bivalent and bispecific Wnt surrogate molecules that are bivalent towards both the one or more Fzd receptor epitopes and the LRP5 and/or LRP6 are provided as the top four structures depicted in FIG. 1, where the VHH or sdAbs or scFvs are depicted as single solid ovals in red, blue or yellow, and the Fab or IgG is depicted in blue. As shown, the VHH or sdAbs (or scFvs) may be fused to the N-termini of both light chains, to the N-termini of both heavy chains, to the C-termini of both light chains, or to the C-termini of both heavy chains. It is further contemplated, e.g., that VHH or sdAbs (or scFvs) could be fused to both the N-termini and C-termini of the heavy and/or light chains, to the N-termini of the light chains and the heavy chains, to the C-termini of the heavy and light chains, to the N-termini of the heavy chains and C-termini of the light chains, or to the C-termini of the heavy chains and the N-termini of the light chains. In other related embodiments, two or more VHH or sdAbs (or scFvs) may be fused together, optionally via a linker moiety, and fused to the Fab or IgG at one or more of these locations. In particular embodiments, the two or more VHH or sdAbs (or scFvs) may each bind to a different set of one or more Fzd receptor epitopes.

In a related embodiment, the Wnt surrogate molecule has a hetero-Ig format, whereas the Fab is present as a half antibody, and one or more VHH or sdAb (or scFv) is fused to one or more of the N-terminus of the Fc, the N-terminus of the Fab, the C-terminus of the Fc, or the C-terminus of the Fab. In particular embodiments, two or more VHH or sdAbs (or scFvs) are fused to the N-terminus of the Fc, the N-terminus of the Fab, the C-terminus of the Fc, or the C-terminus of the Fab, wherein each of the VHH or sdAbs (or scFvs) binds a different set of one or more Fzd receptor epitopes. A bispecific but monovalent to each receptor version of this format is depicted in FIG. 1C, 1D, 1E, 1F, which may be modified to

include two or more Fzd binding regions, wherein at least two of the Fzd binding regions bind to different sets of one or more Fzd receptor epitopes. In certain embodiments, the Fab or antigen-binding fragment (or IgG) thereof is fused directly to the VHH or sdAb (or scFv) or antigen-binding fragment thereof, whereas in other
5 embodiments, the binding regions are fused via a linker moiety. In particular embodiments, the Fab is described herein or comprises any of the CDR sets described herein. In particular embodiments, the VHH or sdAbs or scFvs are described herein or comprises any of the CDR sets disclosed herein.

 In various embodiments, including but not limited to those depicted in
10 FIG. 1V, 1W, 1X, 1AA, a Wnt surrogate molecule comprises one or more Fabs or antigen-binding fragment thereof that binds one or more Fzd receptor epitopes and one or more Fabs or antigen-binding fragment thereof that binds LRP5 and/or LRP6. In certain embodiments, it comprises two Fab or antigen-binding fragments thereof that bind different sets of one or more Fzd receptor epitopes and/or two
15 Fab or antigen-binding fragments thereof that bind LRP5 and/or LRP6. In particular embodiments, one or more of the Fabs are present in a full IgG format, and in certain embodiments, both Fabs are present in a full IgG format. In certain embodiments, the Fabs in full IgG format specifically binds one or more Fzd receptor epitopes, and the other Fabs specifically binds LRP5 and/or LRP6. In
20 certain embodiments, the Fabs specifically bind different sets of one or more Fzd receptor epitopes, and the Fabs in full IgG format specifically binds LRP5 and/or LRP6. In certain embodiments, the Fabs specifically binds LRP5 and/or LRP6, and the Fabs in full IgG format specifically bind different sets of one or more Fzd receptor epitopes. In certain embodiments, the Fab is fused to the N-terminus of
25 the IgG, e.g., to the heavy chain or light chain N-terminus, optionally via a linker. In certain embodiments, the Fab is fused to the N-terminus of the heavy chain of the IgG and not fused to the light chain. In particular embodiments, the two heavy chains can be fused together directly or via a linker. An example of such a bispecific and bivalent with respect to both receptors is shown in FIG. 1V, 1W, 1X
30 and 1AA. In other related embodiments, two or more VHH or sdAbs may be fused together, optionally via a linker moiety, and fused to the Fab or IgG at one or more

of these locations. In a related embodiment, the Wnt surrogate molecule has a hetero-IgG format, whereas one of the Fab is present as a half antibody, and the other Fab is fused to one or more of the N-terminus of the Fc, the N-terminus of the Fab, or the C-terminus of the Fc. A bispecific but monovalent to each receptor
5 version of this format is depicted in FIG. 1D, which may be modified to includes one or more additional Fab, wherein two or more Fab bind to different sets of Fzd receptor epitopes. In certain embodiments, the Fab or antigen-binding fragment thereof is fused directly to the other Fab or IgG or antigen-binding fragment thereof, whereas in other embodiments, the binding regions are fused via a linker
10 moiety. In particular embodiments, the one or both of the two Fabs are described herein or comprise any of the CDR sets described herein.

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

the FIT-Ig comprises two or more Fzd binding domains, wherein at least two or the Fzd binding regions bind to different sets of one or more Fzd receptor epitopes, e.g., different sets of one or more Fzd receptors or different sets of one or more epitopes within the same Fzd receptor(s).

5 In particular embodiments, Wnt surrogate molecules comprise two or more VHH or sdAbs (or scFvs), including at least one that binds one or more Fzd receptor epitopes and at least one that binds LRP5 and/or LRP6. In certain embodiments, one of the binding regions is a VHH or sdAb and the other is an scFv. In particular embodiments, the Wnt surrogate molecules comprises three or
10 more VHH or sdAbs (or scFvs), including at least two that binds different sets of one or more Fzd receptor epitopes and at least one that binds LRP5 and/or LRP6. Wnt surrogate molecules comprising two or more VHH or sdAbs (or scFvs) may be formatted in a variety of configurations, including but not limited to those depicted in FIG. 1K, 1L, 1M, 1N, 1O, 1P, 1Q, 1S, 1T. In certain bispecific, bivalent formats,
15 two or more VHH or sdAbs (or scFvs) are fused in tandem or fused to two different ends of an Fc, optionally via one or more linkers. Where linkers are present, the linker and its length may be the same or different between the VHH or sdAb (or scFv) and the other VHH or sdAb (or scFv), or between the VHH or sdAb and Fc. For example, in certain embodiments, the VHH or sdAb is fused to the N-terminus
20 and/or C-terminus of the IgG heavy chain. In particular embodiments, two or more VHH or sdAbs are fused to the IgG at any combination of these locations. Non-limiting examples of bivalent and bispecific Wnt surrogate molecules of this format are depicted as the structures depicted in FIG. 1K, 1L, 1M, 1N, 1O, 1P, 1Q., where the first VHH or sdAb is depicted in blue, the Fc or IgG is depicted in blue, and the
25 second VHH or sdAb is depicted as red. In various embodiments, both VHH or sdAbs may be fused to the N-termini of the Fc, to the C-termini of the Fc, or one or more VHH or sdAb may be fused to either or both of an N-terminus or C-terminus of the Fc. In a related embodiment, the Wnt surrogate molecule has a hetero-IgG format, whereas one VHH or sdAb is present as a half antibody, and the other is
30 fused to the N-terminus of the Fc or the C-terminus of the Fc. A bispecific but monovalent to each receptor version of this format is depicted in FIG. 1E. In certain

embodiments, the VHH or sdAb is fused directly to the other VHH or sdAb, whereas in other embodiments, the binding regions are fused via a linker moiety. In particular embodiments, the VHH or sdAbs are described herein or comprises any of the CDR sets described herein. In various embodiments, any of these
5 formats may comprise one or more scFvs in place of one or more VHH or sdAbs.

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

In certain embodiments, two or more diabodies, scFvs, and/or VHH or sdAbs can be fused in tandem in a multivalent format, with or without being
20 fused to an Fc (FIG. 1A, 1B, 1F, 1U). In particular embodiments, at least one of the diabodies, scFvs, and/or VHH or sdAbs binds one or more Fzd receptor epitopes, and at least one of the diabodies, scFvs, and/or VHH or sdAbs binds LRP5 and/or LRP6.

In various embodiments, including but not limited those depicted in
25 FIG. 1G or 1H, a Wnt surrogate molecule comprises two or more Fabs or antigen-binding fragments thereof that each bind a different set of one or more Fzd receptor epitopes, and one or more VHH or sdAbs or antigen-binding fragments thereof (or, alternatively or in combination, one or more scFvs or antigen-binding fragments thereof), e.g., that bind LRP5/6. In certain embodiments, a first Fab
30 specifically binds a first set of one or more Fzd receptor epitopes, a second Fab specifically binds a second, different set of one or more Fzd receptor epitopes, and

the VHH or sdAb (or scFv) specifically binds LRP5 and/or LRP6. In certain embodiments, the VHH or sdAb (or scFv) is fused to the N-terminus of the Fabs, while in some embodiments, the VHH or sdAb (or scFv) is fused to the C-terminus of the Fabs. In particular embodiments, the Wnt surrogate molecule has a hetero-Ig format, as depicted in FIG. 1G, 1H, 1AG in which the first and second Fabs are each present as a half antibody, and one or more VHH or sdAbs (or scFvs) are fused to one or more of the N-terminus of the Fc, the N-terminus of the Fab, the C-terminus of the Fc (e.g., FIG. 1Y), or the C-terminus of the Fab. The first and second Fabs may be connected to each other via knobs-into-holes mutations in their respective Fcs, e.g., within the CH3 domain.

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

polypeptides and two light chain polypeptides that bind either LRP5/6 or one or more Fzd receptor epitopes, wherein the Wnt surrogate further comprises a Fab that binds either LRP5/6 or one or more Fzd receptor epitopes. For example, the Fab may comprise two polypeptides, each fused to one of the two heavy chain
5 polypeptides, and two polypeptides, each fused to one of the two light chain polypeptides, or it may comprise two polypeptides each fused to one of the two heavy chain polypeptides and two additional polypeptides, each bound to one of the two polypeptides fused to the heavy chain polypeptides, thus making a second Fab. Other configurations may be used to produce the Wnt surrogates disclosed
10 herein. In particular embodiments of any of these formats, they comprise at least two or more Fzd binding regions, which each bind to a different set of Fzd receptor epitopes.

In some embodiments, the differing ratios of Fzd binding regions to LRP binding regions (Fzd:LRP) is represented in FIG. 1AB, 1AC, 1AD, 1AE, 1AF,
15 1AG, 1AH, 1AI, 1AJ, 1AK, 1AL, 1AM. In particular embodiments, one or more Fabs bind to one or more Fzd receptors or to different epitopes in the same Fzd receptor, and one or more VHH or sdAbs (or scFvs) bind to one or more LRP receptors or different epitopes in the same LRP receptor.

In particular embodiments, a Wnt surrogate molecule includes a first
20 light chain and first heavy chain forming a first Fzd binding region, and a second light chain and second heavy chain forming a second Fzd binding region, with the first and second Fzd binding regions binding to different sets of one or more Fzd receptor epitopes. In certain embodiments, the first and second heavy chains are connected to each other. For example, the first heavy chain may include a first
25 CH3 domain, the second heavy chain may include a second CH3 domain, and the first and second CH3 domains may be connected to each other, e.g., via knobs-into-holes mutations. In certain embodiments, the first heavy chain and/or the second heavy chain comprise an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99% identity to any of the sequences set forth
30 in SEQ ID NOs:110, 112, 114, 116, 118, 120, and 122. In certain embodiments, the first light chain and/or the second light chain comprise an amino acid sequence

having at least 90%, at least 95%, at least 98%, or at least 99% identity to any of the sequences set forth in SEQ ID NOs:109, 111, 113, 115, 117, 119, and 121. In some embodiments, one or more heavy chain comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99% identity to any of the sequences disclosed in Table 5. In some embodiments, one or more light chain comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99% identity to any of the sequences disclosed in Table 5.

In certain embodiments, the Wnt surrogate molecule includes a first LRP5/6 binding region and/or a second LRP5/6 binding region, each of which may be or include a Fab or scFv. The first and second LRP5/6 binding regions may bind to the same epitope within LRP5/6, or may bind to different epitopes within LRP5/6. The first LRP5/6 binding region may be fused to an N-terminus of the first light chain, a C-terminus of the first light chain, an N-terminus of the first heavy chain, or a C-terminus of the first heavy chain. The second LRP5/6 binding region may be fused to an N-terminus of the second light chain, a C-terminus of the second light chain, an N-terminus of the second heavy chain, or a C-terminus of the second heavy chain.

In particular embodiments, a Wnt surrogate molecule comprises an Fzd binding region, e.g., an anti-Fzd antibody, or antigen-binding fragment thereof, fused or bound to a polypeptide that specifically binds to one or more Fzd receptors. In particular embodiments, the polypeptide that specifically binds to one or more Fzd receptors is an antibody or antigen-binding fragment thereof. In certain embodiments, it is an antibody or antigen-binding fragment thereof disclosed herein or in U.S. Provisional Patent Application No. 62/607,877, titled, "Anti-Frizzled antibodies and Methods of Use," Attorney Docket No. SRZN-004/00US, filed on December 19, 2017, which is incorporated herein by reference in its entirety.

In particular embodiments, at least one Fzd binding region of a Wnt surrogate molecule includes one or more antigen-binding fragments of an antibody. For example, the one or more antigen-binding fragments may be or be

derived from an IgG, scFv, Fab, or VHH or sdAb. In certain embodiments, the one or more antigen-binding fragments are humanized.

In particular embodiments, the Fzd binding region comprises the three heavy chain CDRs and/or the three light chain CDRs disclosed for any of the illustrative antibodies or fragments thereof that bind to one or more Fzd receptors provided in Table 1A. In particular embodiments, the Fzd binding region comprises the three heavy chain CDRs and/or the three light chain CDRs disclosed for any of the illustrative antibodies or fragments thereof that bind to one or more Fzd receptors provided in Table 1A, wherein the CDRs collectively comprise one, two, three, four, five, six, seven, or eight amino acid modifications, e.g., substitutions, deletions, or additions. In certain embodiments, the Fzd binding region is a VHH or sdAb or was derived from a VHH or sdAb, so Table 1A only includes the three heavy chain CDRs. In particular embodiments, the Fzd binding region comprises the three CDR HC sequences provided in Table 1A or variants wherein the CDRs collectively comprise one, two, three, four, five, six, seven or eight amino acid modifications.

In particular embodiments, the Fzd binding region comprises the heavy chain fragment and/or light chain fragment of any of the illustrative antibodies or fragments thereof that bind to one or more Fzd receptors provided in Table 1B or SEQ ID NOs:1-73 (or an antigen-binding fragment or variant of either). In certain embodiments, the Fzd binding region is a Fab or was derived from a Fab, so the heavy chain of Table 1B includes VH and CH1 sequences, but not CH2 or CH3 sequences. In certain embodiments, the Fzd binding region is a VHH or sdAb or was derived from a VHH or sdAb, so Table 1B includes the VHH domain. In certain embodiments, the Fzd binding region is a polypeptide, e.g., an antibody or antigen-binding fragment thereof, that competes with any of these antibodies for binding to one or more Fzd receptors.

In particular embodiments, the Fzd binding region includes an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99% identity to any of the sequences set forth in Table 1A, Table 1B, or SEQ ID NOs: 1-73, or an antigen-binding fragment thereof. Binding characteristics of clones listed

in Table 1B were determined and are shown in Table 1B. Heavy chain CDRs are designated CDRH1, CDRH2 and CDRH3, and light chain CDRs are designated CDRL1, CDRL2, and CDRL3.

Table 1A: Anti-Fzd Antibody Clone IDs and CDR sequences

Clone ID	Initial Binding	CDRH1	SID NO.	CDRH2	SID NO.	CDRH3	SID NO.
001S-A01	Fzd1	YTFTSYGIS	391	GWISAYNGNTNYA	570	CARASAWTPYGAFDIW	752
001S-B01	Fzd1	GSISSGGYSWS	283	GSIIHSGSTYYN	547	CARFYDILTGYSYFDYW	818
001S-E01	Fzd1	GSISNYYWS	282	GEIDRSGDTNYN	488	CARVRARRFLVSDRSAFDIW	945
001S-F01	Fzd1	GSISGNYYZG	281	GSIIYFTGGTYYN	546	CARVMLITDAFDIW	942
001S-G01	Fzd1	GSISSSYYWG	285	GYIYSGSTYYN	589	CARATYGGDAFDIW	760
001S-H01	Fzd1	GSISSGGYYWS	284	GYIYSGSTYYN	589	CARHAGFYGLADYFDYW	875
001S-A02	Fzd1	GSISSGGYYWS	284	GYIYSGSTYYN	589	CARGKGYSYGYGKDWFDPW	845
001S-E02	Fzd1	GSISGNYYWG	280	GSIIYFTGGTYYN	546	CARVMLITDAFDIW	942
001S-G02	Fzd1	GAISGTSYFWG	266	GSIIYTGNTYYN	548	CARIGIAVAAPVDHW	882
001S-H02	Fzd1	GSISSSYYWG	285	GYIYSGSTYYN	589	CARATYGGDAFDIW	760
001S-A03	Fzd1	GSISSGGYYWS	284	GYIYSGSTYYN	589	CARVRDYYDSSGYYYDYFDYW	946
001S-B03	Fzd1	ASFSGHYWT	158	GEIDHTGSTNYE	487	CARGGQGGYDWGHYHGLDVW	835
001S-H08	Fzd5	RAFTDNVMA	329	ATISGGGGSTFDD	466	CAAASSLTSTPYDLW	678
001S-A09	Fzd5	RSFRTNALG	333	AAISWTGGSTYYA	422	CNTVTYTGGSYKNYW	1005
001S-B09	Fzd5	SIDSINAMA	356	AALTSGGITYHA	428	CNVITIVRGMGPRAW	1006
001S-C09	Fzd5	SIFSINAMG	357	ATIQSGGRTNYA	465	CNVITIVRGMGPRAW	1006
001S-C07	Fzd8	YTFTSYGIS	391	GWISAYNGNTNYA	570	CARDGTPFYSGSYYGSW	772
001S-D07	Fzd8	GTFSSYAIS	295	GRIIPILGIANYA	529	CARVPTSPYDILTGPFDYW	944
001S-E07	Fzd8	ASVSSNSAAWN	159	GRTYYRSKYNDYA	542	CARWKNYFDPW	953
001S-H07	Fzd8	FTFSSYAMS	228	STISGGGGSTYYA	646	CAKDLVPWGSSAFNIW	704
004S-E05	Fzd5	FTFSTYEMN	243	SGVSWNGSRTHYV	618	CARGQSEKWWSGLYGMDVW	856
004S-E03	Fzd5	GTFSTYAIS	298	GWINSNGNGNTKYS	565	CWTGLLWFGESTDAFDIW	1031
004S-G06	Fzd5	GTFTYRYLH	307	GGIIPIFGTGNYA	501	CASSMVRVPYYPYGMVDW	964
001S-D09	Fzd8	GPFNLFAMG	272	AGISRTGGNTGYA	445	CASKTTINSWGSREYHYW	958
001S-E09	Fzd8	GPFNLFAMG	272	AGISRTGGNTGYA	445	CASKTTINSWGSREYHYW	958

Clone ID	Initial Binding	CDRH1	SID NO.	CDRH2	SID NO.	CDRH3	SID NO.
001S-F09	Fzd8	GFFSSFTMG	268	AAISRNGVYTRFA	409	CNALAPGVVRSW	987
001S-G09	Fzd8	SLFRLNGMG	360	ATISTRGTTHYA	467	CTDEESW	1011
001S-H09	Fzd8	GPFNLLAMG	273	AGISRTGGTGYA	445	CASKTTINSOWSREYHY W	958
001S-A10	Fzd8	SVVNFVVMG	364	AAITSGGSTNYA	425	CNRVGSREYSYW	1001
001S-B10	Fzd8	RTSDLYTMG	352	AAIGYKVKWNGERT YYL	404	CNAVTYNGYTIW	994
001S-G12	Fzd1	SIFSSNTIY	359	ALITSGNTNYA	455	CNAGAPAWTYRMGTYY PQFGSW	986
002S-A01	Fzd1	STFSTYAMG	362	AAISGGGENTYYA	408	CVKFGMNLGYSYDYW	1028
002S-B01	Fzd1	STFSNYAMG	361	AAISWGGGSTFYs	411	CAAGPIARWYRGDM DY W	681
002S-C01	Fzd1	RMFSNYAMG	331	AAISSGGSGTYYS	410	CAAGPIARWYRGDM DY W	681
002S-D01	Fzd1	RTDGGYVMG	337	ATVTWRTGTYYA	469	CAAGPIARWYRGDM DY W	681
002S-E01	Fzd1	RTFSSAAMG	345	AAISWSGSTAYYA	421	CATLTPYGTVAS Y	974
002S-F01	Fzd1	RTFSSYAMG	347	AAVNWSGGSTYYA	430	CAAVFLSRNYEIQEYYRY Q	689
002S-G01	Fzd1	RTFSSYAMG	347	AAISWSGGSTYYA	418	CAAGPIARWYRGDM DY W	681
002S-H01	Fzd1	RSFSTYPMG	336	TVISGGSGTYYS	676	CAAGPIARWYRGDM DY W	681
002S-A02	Fzd1	RRFTTYGMG	332	AAVTWRSGSTYYA	436	CYLEGPLDVY W	1032
002S-B02	Fzd1	RTFNRHVMG	341	AAISWSGDSTYYA	415	CAKLGSSWLREYDY W	724
002S-C02	Fzd1	RTFRAYAMG	342	SAISWSGGSTYYA	603	CAAGPIARWYRGDM DY W	681
002S-D02	Fzd1	RTFSEYAMG	343	AAISWSGGSTHYA	417	CNADSLRGIDY W	984
002S-E02	Fzd1	FTFREYAMT	199	SGISRDGGRTSYS	613	CAPRVLVTAPSGGMDY W	734
002S-F02	Fzd1	GDFTNYAMA	267	AAVNWRGDGTYYs	429	CAAVFLSRNYEIQEYYRY Q	689
002S-G02	Fzd1	RTFGTWAMG	340	AAISYNGFSTYYs	424	CAAGPIARWYRGDM DY W	681
002S-H02	Fzd1	RTFSSYAMG	347	AAISWSGGSTYYA	418	CAAGPIARWYRGDM DY W	681
002S-D03	Fzd1	RTFGSYAMG	339	AAISWSGGSTYYA	418	CAAGPIARWYRGDM DY W	681
002S-E03	Fzd1	SIFSIYAMG	358	AVVATGGATNYA	481	CNMRGNWYREGRPAEF LSW	1000
002S-F03	Fzd1	RTSSSYAMG	353	AAISWSGGSTYYA	418	CAAGPIARWYRGDM DY W	681

Clone ID	Initial Binding	CDRH1	SID NO.	CDRH2	SID NO.	CDRH3	SID NO.
002S-G03	Fzd1	RTFGSYAMG	339	AAISWSGGSTYYA	418	CAAGPIARWYRGDM DY W	681
002S-H03	Fzd1	QTFTAYAMG	327	AAISWSGSATHYA	420	CNAWVLVAGSRGTSAD YW	996
002S-A04	Fzd1	RTFSSYAMG	347	AAISWSGRSTYYA	419	CAAGPIARWYRGDM DY W	681
002S-B04	Fzd1	RTFSSYAMG	347	AAISWSGGSTYYA	418	CAAGPNYSWFMPSSRL IW	682
002S-C04	Fzd1	RRFTTYGMG	332	AAVTWRAGSTYYA	435	CSADKLDYDDQPFKTW DYW	1010
002S-D04	Fzd1	GTSSTYAMG	309	AAINRSGGSTYYA	405	CAAVFLSRNYEIQEYRY Q	689
002S-E04	Fzd1	GTFSTYAMG	300	AAISWSGDSTYYL	416	CAAGPIARWYRGDM DY W	681
004S-H04	Fzd5	GTFSSY AIS	295	GWISTYNGATNYA	577	CARGGAGRFEGGMDV W	826
001S-A04	Fzd5	YTFTSYGIS	391	GWISAYNGNTNYA	570	CASSKEKATYYYGMDV W	963
001S-D03	Fzd5	GTFSSY AIS	295	GRIIPILGIANYA	529	CARLDPGYYYGMDVW	886
001S-F03	Fzd5	GTFSSY AIS	295	GGIPIFGTANYA	499	CARVIFSTVTTTNDIW	939
004S-E04	Fzd5	YTFSGYYLH	374	GTVPILGTANYA	549	CARVDGSGYYGIDYW	933
004S-A06	Fzd5	GSFSNY AIS	278	GRIIPILGSANYA	530	CARTYLKAFDIW	930
004S-F04	Fzd5	YTFTNNFMH	383	GRINPNSGGTNYA	537	CARDRFDNWFDPW	788
001S-C03	Fzd5	GTFSSY AIS	295	GRIIPILGIANYA	529	CAREGRSRVYGGNSFDY W	808
003S-A01	Fzd1	YIFTDYMH	368	GGIPIFGTANYA	499	CARMSSDYDSSGGYRR GMDVW	895
003S-E01	Fzd1	YIFTDYMH	368	GGIPIFGTANYA	499	CARAWKGLWFEGGTFD YW	761
003S-F01	Fzd1	GTFSSY AIS	295	GWINAGNGNTTYA	558	CARLAFDIW	885
003S-A02	Fzd1	YTFTGYMH	379	GWINAGNGNTTYA	558	CAKDRGNYG DYLDYW	707
003S-C02	Fzd1	FTFSNSDMN	214	ALISYDGSHTYYA	454	CTRGSRIGWFDPW	1015
003S-E02	Fzd1	GTFSSYTIS	296	GGIPISGKTDYA	505	CARARGGDSPLSL	749
003S-F02	Fzd1	GTFRSYAIN	292	GGIPIFGTANYA	499	CARGGWRPDY YGSGSY YSFDYW	840
003S-G02	Fzd1	FTFGTYWVT	196	SGITGSGGRTFYA	616	CARMKDWFGAFDIW	894
003S-C03	Fzd1	FTFSRYAMS	220	SYISGDSGYTNYA	658	CARGLVIATNWFDPW	849
003S-D03	Fzd1	YTFTSYMH	392	GWINTYNGNTNYP	567	CAESLTSTADW	691
003S-E03	Fzd1	YIFTDYMH	368	GWVNPTTGNTGYA	586	CARNVEGATSFPEFDYW	898
003S-H03	Fzd1	GTFSSY AIS	295	GGIPIFGTANYA	499	CAKDIGSSWYYYMDVW	701
003S-A04	Fzd1	FTFGTYWVT	196	SGITGSGGRTFYA	616	CARMKDWFGAFDIW	894
003S-C04	Fzd1	FAVSSSYMS	168	ASIWFDGSNQDYA	463	CAPNESGNVDYW	733

Clone ID	Initial Binding	CDRH1	SID NO.	CDRH2	SID NO.	CDRH3	SID NO.
003S-D04	Fzd1	FTFSSYAMH	227	SAISGSGGSTYYA	600	CARDHGSSWYQNTDAF DIW	774
003S-G04	Fzd1	FRFISHPIH	177	GRVIPILGVTNYA	545	CASSSDYGDYKPEPNYG MDVW	966
003S-D05	Fzd2	FTFSNYAMT	216	SAIGTGGGTYYA	595	CATAYRRPGGLDVW	969
003S-E05	Fzd2	FTFSSYTMS	236	GRIKSKANGGTTDYA	535	CARGSSSWYDW	863
003S-A06	Fzd2	FTFADYGMH	188	SYISSGSYTIYYS	659	CARGTFDWLLSPSYDYW	865
003S-C06	Fzd2	FTFSNYGMH	217	SAISNSGGSTYYA	601	CTSSFLTGSQPSGYW	1018
003S-G06	Fzd2	FTFSDYGMH	207	SSTSGSGGNSKYS	642	CARHNPGYMGYYYGM DVW	877
003S-H06	Fzd2	GTFSSYTIS	296	GLVDPEDGETIYA	520	CTILPAAAAGTYYYYGM DVW	1012
003S-B07	Fzd2	FTFSDHYMS	205	SSITRTPSGGTTEYA	639	CARDGGYW	768
003S-D07	Fzd2	YTFTNNFMH	383	GIINPSGGSTSYA	513	CARATSLGRRYCSSTSCY PRDAFDIW	759
003S-E07	Fzd2	YTFTNNFMH	383	GWINPNSGGTKYA	563	CARSVGEVGATMLGIGV WYWFDPW	926
003S-A08	Fzd2	FTFSNYAMT	216	SAIGTGGGTYYA	595	CATAYRRPGGLDVW	969
003S-C08	Fzd2	LTVSTNFMS	324	AGIGWDSTNIGYA	440	CARDLVAARPSNWDYW	782
003S-E08	Fzd2	FTFRNSAMH	201	STISGSGGSTYYYS	647	CARGGGYSSSW	829
003S-G09	Fzd4	FTFDHNP MN	194	SAIGAGGGTYYA	593	CASPTVTRR	960
003S-C10	Fzd4	GTFSSY AIS	295	GWINAGNGNTTYA	558	CARHYYGSGSYPDW	880
003S-D10	Fzd4	FNFGIYSMT	172	SYISGDSGYTNYA	658	CARVGP GGWFDPW	936
003S-E10	Fzd4	FTFSSYAMH	227	AGISASGGSTYYA	442	CARPSTTGTKAFDIW	901
003S-A11	Fzd4	GTFSSY AIS	295	GWINAGNGNTTYA	558	CARHYYGSGSYPDW	880
003S-G11	Fzd4	GTFSSY AIS	295	GRIPIFGTVNYA	528	CARGARLDYW	820
003S-H11	Fzd4	YTFTGYM H	379	GGIPIFGTPHYA	502	CASTDPSSGLDYW	967
003S-C12	Fzd4	GTFSSY AIS	295	GWINPNSGGTNYA	564	CARGGSSDVR	838
003S-F12	Fzd4	FTFSSYAMH	227	SVISTSGDTVLYT	652	CARGGSSDVR	838
004S-B01	Fzd4	GTFSSY AIS	295	GIINPSGGSTSYA	513	CAKDG VVR	698
004S-C01	Fzd4	FTFSNHYTS	213	STISSSGGRTFYA	650	CARASRIDGGWP IIDLHL	754
004S-D01	Fzd4	FTFTNYAMS	248	SAISGSGGSTYYA	600	CARATGFGTVVFDYW	757
004S-E01	Fzd4	GTFSSY AIS	295	GWINAGNGNTTYA	558	CARHYYGSGSYPDW	880
004S-F01	Fzd4	GTFSSY AIS	295	GWINAGNGNTTYA	558	CARDGVE	773
004S-H01	Fzd4	FTFSNYAMH	215	ALMSPDGTIIYYA	456	CAKGIVGDYGAFDIW	717
004S-B02	Fzd4	FTFSSYGMH	230	SSINNSSRTVFYA	630	CAKDH LAVADAHGR	700
004S-E02	Fzd4	FTFSSYAMH	227	AVISYDGSNEYA	474	CAGGEVYEL	692
004S-F02	Fzd4	FTFSTYAMH	242	AVISSDGNNKYYT	473	CAAPDVVVTDAGYYW	685
004S-G02	Fzd4	FTFANYAMN	190	ALISYDGGTKYYA	453	CAKTLVTSHALHIW	728

Clone ID	Initial Binding	CDRH1	SID NO.	CDRH2	SID NO.	CDRH3	SID NO.
004S-H02	Fzd4	FTFANYAMH	189	ALISYDGGNKYYA	452	CAKTLVTSHALHIW	728
001S-E03	Fzd5	GSFSGYYWH	276	GEINHSGSTNYN	489	CARGRRLVRFTVTSAFDIW	858
001S-B05	Fzd5	GTFSSYAIS	295	GGIIPILGIANYA	504	CARIPKPRGYSYGDNGSW	883
004S-A07	Fzd6	GNFKNYGIT	271	GRIIPALGTANYA	525	CARQYCSGGSCYPDAFDIR	908
004S-B07	Fzd6	FTFSSYSMN	233	GVISKDGDNKYYA	553	CASSRDGYNRLAFDIW	965
004S-A08	Fzd6	GTFSSYAIS	295	GRIIPILGIANYA	529	CARDGGDYGMVDVW	767
004S-B08	Fzd6	YTFTNNFMH	383	GRINPNSGGTNYA	537	CASQNYGSGSYPGFDYW	961
004S-D08	Fzd6	YTFTYRYLH	394	GGIIPIFTANYA	499	CATHDSSGYYSFDYW	973
004S-E08	Fzd6	FSVSSNYMN	187	SAIGTGGGTYYA	595	CTTRTYDSSGYETQNYMDVW	1024
004S-G08	Fzd6	FTFSDYYMS	208	AAISYDESNKFYA	423	CARSAVAGAFDIW	916
004S-A09	Fzd6	FTFRDYAMN	198	SGISWNSGSIGYA	615	CARRSGYSGSVYYYGMVDVW	913
004S-B09	Fzd6	FTFSSFGMH	221	AGINWNGGSSVYYA	441	CARGPSHQHTFDIW	854
004S-C09	Fzd6	YTFTNNFMH	383	GGFDPEDGETIYA	492	CARVGRGYSFDYW	937
004S-E09	Fzd6	DTFSNYVIS	163	GRISAYNGYKSYA	538	CARSSGYVGWFDPW	924
004S-F09	Fzd6	FTFSNYTIS	218	SYISGAGGSTEYA	657	CARLPRRSGKGSAFDIW	888
004S-H09	Fzd6	GTFSSYTIS	296	GWMNPNSGNTGYA	583	CARVGATSAGGMVDVW	935
004S-C10	Fzd6	YIFTDYYMH	368	GLVDPEDGETIYA	520	CAHSDFSGLSFGDW	693
004S-D10	Fzd6	FTFSNSDMN	214	SSISTSGGSTYYA	637	CARGSYW	864
004S-E10	Fzd6	TTLNKYAIS	365	GRITPVVGVNTYA	539	CALSSSWYGGFDYW	731
004S-F10	Fzd6	GFTFSDHY	269	ALVGYDGSQQFYG	458	CNTGIPMLYW	1003
004S-G10	Fzd6	FTFSDYYMS	208	SAISGSGFTYYA	599	CARVSRGFAFDYW	948
004S-A11	Fzd6	GTFSSYAIS	295	GRIIPILGIANYA	529	CARESVNNYYYMDVW	813
004S-C11	Fzd6	FTFSSYAMH	227	ALTSYDGSKKFYA	457	CAKTGRGYAFDIW	726
004S-E11	Fzd6	FTFSSYNMN	232	GRIKSKANGGTTDYA	535	CAKAGQQLDW	696
004S-H11	Fzd6	FTFTSSAMQ	249	GGIIPIFTANYA	499	CATVQTNYYDSSGRFSYRAHYFDYW	977
004S-A12	Fzd6	YTFTNNFMH	383	GRINPNSGGTNYA	537	CARGQGYSSGWYRGDAFDIW	855
004S-D12	Fzd6	FAFDDYAMH	165	GFIRSKAYGGTTEYA	490	CAKDRGYSSGWYLDYW	708
005S-H01	Fzd7	FNFSYTMIR	173	SVIYGGGNTNYA	653	CARGGSGGNLSYW	836
005S-A02	Fzd7	GTFSSYAIS	295	GMIIPFLGITNYA	521	CTRPYDAFDIW	1016
005S-C02	Fzd8	YTFASYGMH	373	GWINAGNGNTTYA	558	CARLSVWKWEQVTNWFDPW	890

Clone ID	Initial Binding	CDRH1	SID NO.	CDRH2	SID NO.	CDRH3	SID NO.
005S-E02	Fzd8	GTFTSYAIS	305	GWINAGNGNTKYS	557	CTTGLFPYYRYNWNNDADFIDW	1022
005S-A03	Fzd8	GTFSSYAIS	295	GWMNPNSGNTGYA	583	CAKWHIGATGNWFDPW	729
005S-H03	Fzd8	YTFTNNFMH	383	GGIFPIYGISTYA	494	CARDRPTSSWYAFDYW	792
005S-F04	Fzd8	FSFSSTAMS	181	SYISSSGSITHYA	670	CARYGDYGDYW	954
005S-H04	Fzd8	YTFTNNFMH	383	GWINAGNGNTTYA	558	CARVATGNAFDIW	932
005S-B05	Fzd8	FTFSSYWMH	239	AGISGSGKTTYA	444	CARGGLLDYW	831
005S-F05	Fzd8	FTFTSSAVQ	251	GWMNPNSGNTGYA	583	CARRTAVAGTIDYW	914
005S-G05	Fzd8	GTFSSYAIS	295	GWISPYNGNTNYA	573	CARGGWTNYGGNLDYW	841
005S-H05	Fzd8	YTFTSYMH	392	GRINPNSGGTNYA	537	CARVPDFWSGYLDYW	943
005S-D06	Fzd8	YTFTYRYLH	394	GGIPIFGTANYA	499	CARDSYPYGMVDW	800
005S-F06	Fzd8	GTFSSYAIS	295	GRVIPILGVTNYA	545	CAREYLGSDIW	815
005S-A07	Fzd9	FTFTGSAVQ	247	GGILPIYGTTKYA	509	CARGARLYGFDYW	822
005S-B07	Fzd9	FTFTSSAVQ	251	GWMNPNSGNTGYA	583	CARGRQQLWTGYYGMDVW	857
005S-C07	Fzd9	FTFSSYSMN	233	SYIENDGSITTYA	654	CARAPYYYGSGSLFRLDYW	748
005S-D07	Fzd9	GTFNSYAIA	291	GGIPIFGTANYA	499	CARAGSGYYNFDYW	740
005S-F07	Fzd9	FSFSSYGMH	182	AYINSRGSMLMYA	483	CAKTKLPIW	727
005S-G07	Fzd9	GSFSGYAIN	274	GGIPIFGTANYA	499	CATGYYDYFDYW	972
005S-H07	Fzd9	GTFTNNFMH	303	GLVDPEDGETIYA	520	CARTYRIVGATPRYYYYGMDVW	931
005S-B08	Fzd9	YIFTDYMH	368	GWINPNSGGTIYA	562	CARGPRDSGYYPGGAFDIW	853
005S-D08	Fzd9	FAFSSHWMH	166	SAIDGSGGSTYYA	592	CARDRQLGWAHWYFDLW	794
005S-G08	Fzd9	YTFTGYMH	379	GWINAGNGNTTYA	558	CARDRDYW	787
005S-C09	Fzd9	FTFSSYGMH	230	SAIGTGGSTYYA	595	CALLVGAARGISYYYYGMDVW	730
005S-D09	Fzd9	YTFTSYAMH	389	GWINAGNGNTTYA	558	CARDRPYSSGWYYPAFDIW	793
005S-E09	Fzd9	FNLRRYNMN	175	SRISNSGSLVYYA	627	CARDADSSGYRYDAFDIW	762
005S-A10	Fzd9	YTFTDYMH	376	GIINPSGGSTSYA	513	CARHVGSGTYNNWFDPW	878
005S-D10	Fzd9	YTFTSYMH	392	GWMSPNSANTGYA	583	CARGGPIHYYYYYMDVW	834
005S-H10	Fzd9	GAFSTSSIS	265	GRIIPVLGTANYA	534	CAKGGWRSSFDPW	715
005S-B11	Fzd9	YTFTSYDIN	390	GGFDPEDGETIYA	492	CAKAGDWGLYGMVDW	695
005S-C11	Fzd9	FTFTGSAVQ	247	GGILPIYGTTKYA	509	CARGARLYGFDYW	822

Clone ID	Initial Binding	CDRH1	SID NO.	CDRH2	SID NO.	CDRH3	SID NO.
005S-D11	Fzd9	YTFTNNFMH	383	GWINPNNSGDTKFA	561	CAREANYDILTGYIRPDA FDIW	806
005S-E11	Fzd9	GTFSYYAIS	295	GWINAGNGNTKYS	557	CTTEYSSSPDYGYGMD VW	1025
005S-G11	Fzd9	GTFTNRNSIS	304	GGIPIFGTANYA	499	CARSSDLRIFDYW	922
005S-H11	Fzd10	YTFASYDIH	372	GWINAGNGNTTYA	558	CARDGIWDIFDYW	769
005S-E12	Fzd10	YIFTDYMH	368	GVIFPVYTPDYA	551	CARGGSTGYGMDVW	839
005S-F12	Fzd10	GTFSYYAIS	295	GRIVPIVDVVKYA	541	CARDTCSSTSCSPDYW	801
006S-A01	Fzd10	FTFSYSMN	233	SAIGTGGGTYYA	595	CAREGWFGESPFGMDV W	810
006S-F01	Fzd10	YTFTRYAVH	385	GWISTFNDNTNYA	576	CASPTGMTTNFDYW	959
006S-H01	Fzd10	YIFTDYMH	368	GGIPIFGTANYA	499	CAKGSYYYDSSGYWDA FDIW	723
006S-A02	Fzd10	YIFTDYMH	368	GGIPLFGTTDYA	507	CARDITGADGMDVW	775
006S-D02	Fzd10	GTFSYYAIS	295	GRIIPTVGTANYA	533	CARDVCSGGSCSPDVW	802
006S-E02	Fzd10	FTFTSSATQ	250	GGIPIFGTANYA	499	CARDGSSGWYSPNAFDI W	770
006S-H02	Fzd10	FTFRMYGMH	200	SRISPDGRTTYA	628	CARSPRWYDAFDIW	920
006S-A03	Fzd10	YIFTDYMH	368	GWINAGNGNTTYA	558	CARDPIMFGDQPGWFD PW	784
006S-B03	Fzd10	GTFSYYAIS	295	GWINAGNGNTKYA	556	CAREGYDFWSGPYA FDI W	811
006S-C03	Fzd10	GTFSNNVIS	293	GGIPIFGTANYA	499	CARGGYYYGMDVW	843
014S-B01	Fzd1	YIFTDYMH	368	GGIPIFGTANYA	499	CARMSSDYDSSGYRR GMDVW	895
014S-D01	Fzd4	GTFSYYAIS	295	GWINAGNGNTTYA	558	CARHYGSGSYPDW	880
014S-E01	Fzd4	GTFSYYAIS	295	GWINAGNGNTTYA	558	CARHYGSGSYPDW	880
014S-G01	Fzd4	GTFSYYAIS	295	GWMPNPNNGNTTYA	581	CARHYGSGNYRDW	879
014S-A02	Fzd4	FTFSSNAMH	223	SGISGSGGSTYYA	608	CAKPGIAAAGTNNWFD PW	725
014S-B02	Fzd4	FTFSSYAMH	227	SGISGSGGSTYYA	611	CARPSTTSFGMDVW	902
014S-C02	Fzd5	YTFTSYMH	392	GRINPNSSGGTNYA	537	CARVPDFWSGYLDYW	943
014S-D02	Fzd5	GTFTSYAIS	299	GIINPSGGSTSYA	513	CARAKGSGWYVGS AFDI W	744
014S-E02	Fzd5	FTFSDSYMS	206	GFIRSKAYGGTTEYA	490	CARATQELLPPYGMDV W	758
014S-F02	Fzd5	YTFTSYMH	392	GRINPNSSGGTNYA	537	CARVPDFWSGYLDYW	943
014S-G02	Fzd6	YTFTSYMN	392	GIISPSGGSTSYA	516	CARWGDYGDLYFDYW	951
014S-H02	Fzd6	YIFTDYMH	368	GRINPNSSGGTNYA	537	CARARSSGWTDAFDIW	751
014S-A03	Fzd6	GTFSYYAIS	295	GWINAGNGNTTYA	558	CARHYGSGSYPDW	880
014S-B03	Fzd6	FTFSSYNMN	232	GRIKSKANGGTTDYA	535	CARAGDSPDYW	739

Clone ID	Initial Binding	CDRH1	SID NO.	CDRH2	SID NO.	CDRH3	SID NO.
014S-E03	Fzd8	GTFSSY AIS	295	GWISPYNGYTKYA	574	CARAMWSYGQNAFDI W	745
014S-G03	Fzd8	FTFTSSAVQ	251	GWMNPNSGNTGYA	583	CARRTAVAGTIDYW	914
014S-H03	Fzd8	YTFTSSAIH	387	GRINPNSSGGTNYA	537	CARVKWELAIIDYW	940
014S-B04	Fzd8	YIFTDYYMH	368	GWMNPNSGNTGYA	583	CARGGSRYDFWSGHWW FDLW	837
014S-E04	Fzd8	YTFTGYMH	379	GRINPNSSGGTNYA	537	CARDVVKLVTRGVAYG MDVW	804
014S-F04	Fzd8	YSFTTYGMN	371	GWINAGNGNTTYA	558	CARAAAGSYGGGYW	736
014S-G04	Fzd8	FTFSSYGMS	231	SAISGSGGTYA	600	CARDLTPFTQQQLVLGLL	780
014S-H04	Fzd8	FTFTSSAVQ	251	GRIVPAIGFTQYA	540	CARSGYNRRGYFDYW	919
014S-A05	Fzd8	GTFSSY AIS	295	GGIPIFGTANYA	499	CARVTLGASVDAFDIW	949
014S-B05	Fzd8	GTFSSY AIS	295	GWVSPNTGNTVYA	587	CTTDRRYSTYFDLW	1021
014S-C05	Fzd8	YTFASYGMH	373	GWINAGNGNTTYA	558	CARLSVWKWEQVTNWF DPW	890
014S-D05	Fzd8	GTFTSY AIS	305	GWINAGNGNTKYS	557	CTTGLFPYYRYNWNND A FDIW	1022
014S-F05	Fzd9	FTFTGSAVQ	247	GGILPIYGTTKYA	509	CARGARLYGCDYW	821
014S-G05	Fzd9	FTFSSSWMH	226	SAIGTGGGTYA	595	CARKVKGYCSGGSCYGY W	884
014S-H05	Fzd9	FTFSNYAMT	216	STISGSGVSTFYA	648	CARHGRIAAIDW	876
014S-A06	Fzd9	FTFZZSZVQ	254	GGILPIYGTTKYA	509	CARGARLYGFDYW	822
014S-B06	Fzd9	FTFSSYSMN	233	SYIENDGSITTYA	654	CARAPYYYGSGSLFRLDY W	748
014S-C06	Fzd10	FTFTGSAVQ	247	GGILPIYGTTKYA	509	CARGARLYGFDYW	822
014S-D06	Fzd10	FTFSRYAMH	219	SGIGVGGGTYA	605	CARDAYNWFDPR	763
014S-F06	Fzd10	YIFTDYYMH	368	GVIFPVYPTPDYA	551	CARGGSTGYGMDVW	839
014S-G06	Fzd10	FTFSSYAMH	227	SAIGAGGGTYA	593	CARDAYNWFDPW	764
014S-H06	Fzd10	FTFSSYDMN	229	SAIGTGGGTYA	595	CARDAYNWFDPW	764
014S-A07	Fzd10	FTFSNAQMS	210	SAIGTGGGTYA	595	CAREGSYYDWYFDLW	809
017S-E08	Fzd8	IIFSPNDMG	313	ALISSGGSTSYA	450	CHFGVASVGLNYW	980
017S-H08	Fzd8	RTFSSFVMG	346	AAVSASGGYTWYA	432	CNLAQRGETYW	998
017S-A09	Fzd8	LAFNGYTMG	317	AAISWSDNTTYA	414	CAAGFPTVFVVDGEYDY W	680
017S-B09	Fzd8	FTLDYYAIS	255	ADITSGGSTNYA	437	CNAVTYNGYTIW	994
017S-C09	Fzd8	LTFSDYTVG	319	ASSTGGGVFENYA	464	CNAVTYNGYTIW	994
018S-D06	Fzd4	RIFSSYAQA	330	PRIPSDSTTFYA	590	CEVHNFGATYW	979
018S-E06	Fzd4	RTFSNYVMG	344	AVISRSGGNTYYT	472	CNAVSTDWTTDYW	992
018S-F06	Fzd4	RTFSTYGMG	349	AAISWSDNTTYA	414	CNSFPLRLHDW	1002
018S-G06	Fzd5	LAIDDYYMV	318	SYISTSDGSTTYA	673	CNAVTYNGYSIW	993

Clone ID	Initial Binding	CDRH1	SID NO.	CDRH2	SID NO.	CDRH3	SID NO.
018S-H06	Fzd5	LAFNGYTMG	317	AQISWTGGSTDYA	460	CNADYGTWYGIGW	985
018S-A07	Fzd5	LAFNGYTMG	317	AAISWMSNTYYA	412	CNMGLGYSEYRPLGYW	999
018S-B07	Fzd5	SAFSNYAMG	355	AAITWSGARTYYA	426	CNAVWKFGTTHW	995
018S-C07	Fzd7	LTIDDDYYVV	323	SYISAGDGFYYA	656	CNAVTYNGYTIW	994
017S-F09	Fzd4	GSFSGYYWS	277	GEINHSGSTNYN	489	CARDLRFYSSSWRRVGM DVW	778
017S-G09	Fzd4	YTITTYAIH	396	GWINADTGDAYS	555	CARGWTTISLGVW	872
017S-H09	Fzd5	NIFRIYAIA	326	AALTGQRTTNYA	427	CNTVTYNAGCYKKYW	1004
017S-A10	Fzd5	LAFNGYTMG	317	ASITWNGRYTYA	462	CNARLDAVYGHRSRYDS W	988
017S-B10	Fzd5	NFFSNYPLG	325	GAISRTGSGTFYA	484	CAAGVTGSWRYW	684
017S-C10	Fzd8	RSFSNYRVA	335	AVSWSVGMTYYA	479	CNAVTYNGYTIW	994
017S-D10	Fzd8	GTFGSYAVG	288	GLISRNAGNTLYA	518	CNAVNGRLNYW	991
017S-E10	Fzd8	RTFSSYSLA	348	AAVSASGANTYYA	431	CAAPQSPNMYIIRDQL WWYKYW	687
018S-D07	Fzd1	RSFSTYPMG	336	TVISGSGGSTYYA	675	CAAGPTLPFRYW	683
018S-E07	Fzd1	RAFSNYAMG	328	AAINWSGDSAYYA	406	CNARLSFAGGMGYW	989
018S-F07	Fzd1	IKSMFDMNFMG	314	AFITRGGTTRYG	438	CNAVSTDWTRDYW	992
018S-G07	Fzd1	LTIDDDYVMV	322	SYIGTSDGTTYA	655	CNAVTYNGYTIW	994
018S-H07	Fzd4	RVFSSYAQA	354	AGIASDSTTFYA	439	CKVHNFGATYW	983
018S-A08	Fzd4	RIFSSYAQA	330	ASIPSDGTTFYA	461	CKVHNFEATYW	982
018S-B08	Fzd4	LTFSTYGMG	321	AAINWSGRSTVYA	407	CNSFPLRLHDW	1002
018S-C08	Fzd4	RTLSSYVVG	351	ALISLSGASTYYA	449	CNAVSTDWTTDYW	992
018S-D08	Fzd5	IKSMFDMNFMG	314	AFITRGGTTRYG	438	CNAVSTDWTRDYW	992
018S-E08	Fzd5	RTDGMQAMG	338	GAITWSLGSIFYA	486	CNVLAQNDGDYRTYG	1007
018S-F08	Fzd5	RTFSSFVMG	346	AAVSASGGYTWYA	432	CNAVWKFGTTHW	995
018S-G08	Fzd5	RTFSSFVMG	346	AAVSASGGYTWYA	432	CNAVCKFGTTHW	990
018S-H08	Fzd5	RTFSSFVMG	346	AAVTASGGYAWYA	434	CNAVWKFGTTHW	995
018S-A09	Fzd8	ITFSFNSVG	316	AVFIAGYGAYYA	470	CNGVTYNGYTIW	997
018S-B09	Fzd8	HDFSSTYGVG	310	ATISWGGTNIA	468	CAAQKPYYNGHFYADDK HYDHW	688
018S-C09	Fzd8	ITFGFDSVG	315	AVFNAGYRAYYA	471	CNAVTYNGYTIW	994
018S-D09	Fzd8	RTFSWYSMG	350	AAVSWSGVSTYYP	433	CNAVTYNGYTIW	994
018S-E09	Fzd8	ITFSFNSVG	316	AVFIAGYGAYYA	470	CIGVTYNGYTIG	981
018S-F09	Fzd8	RTDGMQAMG	338	GAITWSLGIIFYA	485	CNVLAQNDGDYRTYW	1008
018S-G09	Fzd8	HDFSSTYGVG	311	AAISWRGTNIA	413	CAAQKPYYNGHFYADDK HYDHW	688
021S-A01	Fzd8	DSVSSNSAAWN	160	GRAYKSRWYYDYA	524	CVRDLRPSGDLNFDYW	1029
021S-C01	Fzd1	GSISSGGYSWS	283	GSIHSGSTYYN	547	CARFYDILNGYSYFDYW	817

Clone ID	Initial Binding	CDRH1	SID NO.	CDRH2	SID NO.	CDRH3	SID NO.
021S-D01	Fzd1	FTFSSYGMH	230	AVISYDGSNKYYA	475	CAKGSVFGLKAGGYADY W	721
021S-E02	Fzd8	YTFTSYGIS	391	GWISAYNGNTNYA	570	CARDGTPFYSGSYGWSW	772
021S-G02	Fzd8	DSVSSNSGAWN	162	GRTYYRSKYNGYA	544	PRLDYW	1034
021S-A03	Fzd8	DSVSSNSAAWN	160	GRTYYRSKWYNDYA	542	CARSQATGERFDYW	921
022S-H06	Fzd4	FTFSSYAMS	228	SVISTSGGTVLYT	652	CADGSGTSHR	690
022S-A11	Fzd10	YIFTDYMH	368	GGIFPIFGTANYA	493	CAKGSYYYDMSGYYWD AFDIW	722
OMP-18R5		GFTFSHYTSL	270	VISGDGSYTYADSV KG	677	NFIKYVFAN	1033
027S-H02	Fzd5	FTFSSYAMS	228	SAISGSGGSTYYA	600	CAKGLWGPLLNW	718
027S-B03	Fzd8	DSVSSNSATWN	161	GRTYYRSKWYSDYA	543	CTRGNNVWGLANW	1014
027S-E01	Fzd5	RSFSIYNTA	334	AAISWSGGSTYYA	418	CNVITIVRGMGPRAW	1006
004S-D05	Fzd5	LTFSIYAMH	320	SAISGDGALTYA	597	CARGVYPYSSKHKPSYYY YGMDVW	870
004S-D04	Fzd5	YDFTTYGIH	367	GGVIPAFGATDYS	511	CARGYYGMDVW	874
004S-B05	Fzd5	GTFSSYALS	295	GWINAGNGNTTYA	558	CASGLGYFDYW	957
004S-G03	Fzd5	YTFTNNFMH	383	GGIPIFGTPHYA	502	CARTLTTPPYGMDVW	929
004S-F03	Fzd5	FTFSNSDMN	214	SAIGTGGDTYYA	594	CTRDLYGGYRDYW	1013
004S-C04	Fzd5	YIFTGYMH	369	GRINPNSGGTNYA	537	CARGGEYSSGWYTYYYY GMDVW	827
004S-B06	Fzd5	YTFTYRYLH	394	GMINPIGGSINYA	522	CARDVMDVW	803
004S-F06	Fzd5	FSVGSNYMT	186	SSISSGNSIYYA	634	CARGPKTMWEDRPDY W	851
004S-A04	Fzd5	FTFSTYSMI	245	GFIRSKDYGGTTEYA	491	CARLTGGAVAGTHR DY W	892
004S-A05	Fzd5	FTFSSYVMS	237	SAIGTGGGTYYA	595	CARGSSGYVAW	862
004S-F05	Fzd5	FTFSNHYMS	212	AGVSIDANKKYA	447	CARDQNSWYRSDYW	785
003S-C01	Fzd1	GTFSSYALS	295	GRINPNSGGTNYA	537	CARGSGYDFFDYGMDV W	861
003S-H01	Fzd1	DTFSNYVLS	164	GLVDPEDGETIYA	520	CAKASTPMVQGPADYW	697
003S-H02	Fzd1	GTFNRYAIT	289	GGIPIFGTANYA	499	CATTQGVYSSSWYGGG RAFDIW	976
003S-H04	Fzd1	YTFTYRYLH	394	GRINPNSGGTNYA	537	CWGGSYGYDYW	1030
003S-A05	Fzd2	FTFSSYAMH	227	SSISWNSGRVDYA	638	CARGSGIAASGSYW	860
003S-B05	Fzd2	FTFSNAWMS	211	STIAGSGGRTYYS	643	CAKDSIGRRGRGAPQPY YYYGMDVW	709
003S-F05	Fzd2	FSFSTYTMS	184	SRINGDGSSTRYA	624	CARAIVGATGLNRFKAF DIW	743
003S-G05	Fzd2	STFTNAWMS	363	SAIGTGGGTYYA	595	CARDRVTLRGGYSYGT D AFDIW	796

Clone ID	Initial Binding	CDRH1	SID NO.	CDRH2	SID NO.	CDRH3	SID NO.
003S-H05	Fzd2	FTLSTYNMN	257	SRINYDGSATTYA	626	CARDRDIVVPAQRGEG GFDPW	786
003S-A07	Fzd2	FTFSSYAMS	228	SAISGSGGSTYYA	600	CAKGGRDGYKGYFDYW	714
003S-C07	Fzd2	FSFRSYSMS	178	SAIGTGGGTYYA	595	CTTTVTTSW	1026
003S-F07	Fzd2	FSFSSYGMS	183	SHISSGGATIDYA	619	CARDGGYW	768
003S-G07	Fzd2	FTFSSYWMH	239	SYISGDSGYTNYA	658	CARDNGYCSGGSCYATY YGMDVR	783
003S-B08	Fzd2	FTFSSZMH	240	AVISYDGSNRZYA	476	CARSYYDSSGYPRKDAF DIW	927
003S-F08	Fzd2	ZSVSSNYMS	401	SRINSDGSTISYA	625	CARARLLGGYYTPDRMD VW	750
003S-H08	Fzd2	FTFNRHALS	197	ALISSNGDHKYYT	451	CARDLMVGRNKLDYW	777
003S-A09	Fzd2	FTFSSSNMN	225	SGISGSGSSTYYA	611	CARGRVWSSRDYW	859
003S-B09	Fzd2	FNIRRZNMZ	174	SAIGTGGGTYYA	595	CARGDSGSYRDYW	823
003S-C09	Fzd2	FTFSSSAMH	224	SGISGSGTTTYR	612	CARRLIAVAGAEFDPW	912
003S-F09	Fzd4	FTFSNSDMN	214	GRIKSKAYGGTTEYA	536	CARQYYFDYW	909
003S-H09	Fzd4	FTFSSFGMH	221	SVISSGGSPYYA	651	CATASGDFDYW	968
003S-A10	Fzd4	FTFDDYAMH	191	AIVSYDGTYYKYS	448	CARQTRGGTTDGW	907
003S-B10	Fzd4	FTFSSHSTH	222	SAISASGDSTFYA	596	CARPIVGATAFDIW	900
003S-G10	Fzd4	FTZSSYSMN	264	SYSSGNSGYTNYA	674	CARGVVGSGAFDIW	868
003S-B11	Fzd4	FTFSDYYMS	208	SAIDGAGRTYYT	591	CARAIPGDYDYW	742
003S-C11	Fzd4	FTFTSYAMH	252	GGIIPFIANYA	496	CARTGRGYGMVDVW	928
003S-D11	Fzd4	FTFSSYSMS	234	SYISGDSGYTNYA	658	CARAGVATIAFDYW	741
003S-F11	Fzd4	FTFDDYGMH	192	SAISGSGGSTYYA	600	CTTPNYYDSR	1023
003S-E12	Fzd4	GTFSSYAIS	295	GWINAGNGNTTYA	558	CARHYGSGSYPDW	880
004S-A01	Fzd4	FTFSTYGMH	244	SYISSSSAIYYA	671	CARGGLDGPIDYR	830
004S-G01	Fzd4	FTVSSHSMG	260	SLVSFDGSKEHYA	621	CARLGSTPDYW	887
004S-C02	Fzd4	FTFSSYGMH	230	AVISYDGSNKYYA	475	CASDPVTAATR	956
004S-D02	Fzd4	FSFSSYGMS	183	SGISGSGRSTYYA	610	CAKDGW	699
004S-A03	Fzd4	FTFSSYAMH	227	SGINWNGGSTGYA	606	CARPAGSAQNWFDPW	899
004S-B03	Fzd4	FSFSRYGMS	180	SGVGSGSGSTZYA	617	CARDGSW	771
004S-C03	Fzd4	YTFTSYAIS	388	GIINPSGGSTSYA	513	CARQIGWELMPDIW	906
004S-C05	Fzd5	ZZZTDYYZQ	403	GGMNZNRGNTGYA	510	CANGSYAQHLW	732
004S-G05	Fzd5	FTFSSYWMH	239	STISPSGLIYQA	649	CAKDKVPYSYGNFDYW	703
004S-E06	Fzd5	FFFSGYWMS	169	ANIKQDGSSEKYYV	459	CARVFPLHDYW	934
004S-C06	Fzd5	FPFSTFSMN	176	AGISWNSGTIDYA	446	CARSGPAAMVYYYYGM DVW	918
004S-E07	Fzd6	FTLSSHMMN	256	SAIGTGGGTYYA	595	CAAPDYW	686

Clone ID	Initial Binding	CDRH1	SID NO.	CDRH2	SID NO.	CDRH3	SID NO.
004S-F07	Fzd6	FSFSKKYMT	179	SSIDGNGDHVFYA	629	CARPYYYDSSGYDPMGD YW	904
004S-C08	Fzd6	FTVSSNYMN	261	SAIGTGGGTYYA	595	CAQGTYW	735
004S-F08	Fzd6	FTFDDYYMN	193	SAVSGNGGGTFYA	604	CARGGNYGSGDYW	833
004S-G09	Fzd6	GTLNNHTLS	308	GRIIPIFGTANYA	526	CARDRRGYGMDVW	795
004S-B10	Fzd6	FTFSDYYMS	208	SGINWNSAKIGYV	607	CARIGAGGAFDIW	881
004S-H10	Fzd6	FIFSDYYMS	170	AVITSGGTFKYYA	477	CARNGIAAAEDYW	896
004S-B11	Fzd6	FTFSSSWMH	226	SGISWNSGSIGYA	615	CARYSSGGS LDYW	955
004S-D11	Fzd6	YZFZZZYM	400	GRINPNSGGTNYA	537	CARARSSGWTD AFDIW	751
004S-F11	Fzd6	FTFSSYAMS	228	SSISGGGRHTYYA	632	CARPYSSSRQGDYW	903
004S-G11	Fzd6	YIFTDYYMH	368	GWINPNSGGTNYA	564	CARDRPGFDPW	790
004S-B12	Fzd6	FTFSSYWIH	238	SYISGDSGYTNYA	658	CAKGIRWFDPW	716
004S-C12	Fzd6	YIFTDYYMH	368	GWMPNSGNTGYA	583	CASSHYAPGMDVW	962
004S-F12	Fzd7	FTVGNNYMS	258	SSITTSTLYA	640	CARGKEGRYSNEYAAW	844
005S-B01	Fzd7	FTFRSYGMH	202	SLISGSGDNTNYA	620	CARREPLYSSRRGA FDIW	910
005S-C01	Fzd7	FTFSSYSMS	234	SAISGSGGSTYYA	600	CTRTIVGATPHYW	1017
005S-F01	Fzd7	FTVSSNYMS	262	SAISGSGATTTYA	598	CAKGAGYGS GS WQAA W	711
005S-B02	Fzd8	YSFTNYAMH	370	GRIIPIFGTAZYA	527	CARGTFLEWLLTNYGMD VW	866
005S-D02	Fzd8	GTFSSYVIS	297	GWIGPHNGNTNYA	554	CATGWPRYYYGMDVW	971
005S-G02	Fzd8	YTFTSYMH	392	GGIPIFGTAZYA	500	CARLPYYDFWSGYGGRTGFDYW	889
005S-H02	Fzd8	YTFTYRYLH	394	GWINAGNGNTTYA	558	CARASLYDYVWGSYRHYFDYW	753
005S-B03	Fzd8	GTFSSYAIS	295	GIINPSGGRTTYA	512	CATSFGGGWIVVDTSLW YW	975
005S-C03	Fzd8	GSFSGYAIS	275	GGIPIFGTANYA	499	CRVDAFDIW	1009
005S-E03	Fzd8	FTFTSSAVQ	251	GGIPIFGTANYA	499	CARSSGWQNRFAFDIW	923
005S-F03	Fzd8	YTFTYRYLH	394	GWINAGNGNTKYS	557	CATDLPVRKGFTYYDILT GSYGMDVW	970
005S-B04	Fzd8	YTFTNNFMH	383	GGIPIFGTANHA	498	CARGLRYFDWPQGIYY YGMDVW	848
005S-C04	Fzd8	YTFTSYMH	392	GRINPNSGGTNYA	537	CARGGLLDYW	831
005S-D04	Fzd8	FTFSTYSMS	246	STIGTGGGTYYA	645	CARVGWLRFLDYW	938
005S-G04	Fzd8	GTFSSYAIS	295	GWMSPPSSGNAGYA	585	CARNNFLRAFDIW	897
005S-A05	Fzd8	FAFSSYAMS	167	SRIDTDGSTTVYA	622	CARAPSYSSGWYVRW	747
005S-C05	Fzd8	YTFTYAMH	395	GIINPSGGSTSYA	513	CARELLPMTT VTSPFIW	812
005S-E05	Fzd8	GTFSSYAIS	295	GGIPIFGTANYA	499	CAIRA FDIW	694

Clone ID	Initial Binding	CDRH1	SID NO.	CDRH2	SID NO.	CDRH3	SID NO.
005S-C06	Fzd8	ZTFSZYDMH	402	SSISSSSHYKYA	635	CARVRSKAVAGTLPKRLF DIW	947
005S-E06	Fzd8	YTFTSYMH	392	GWMNPNSGNTGYA	583	CARGNPTSGHIVVPAATFSDYW	850
005S-G06	Fzd8	GTFSZZTIS	302	GWMNPDSGKTGYA	579	CARWAFPIPNAFDIW	950
005S-H06	Fzd8	YTFTNNFMH	383	GGIFPIYGISTYA	494	CARDRPSSSWYAFDYW	791
005S-A08	Fzd9	GTFSZYAIS	301	GGIPIFGTANYA	499	CARGGLLRFGDGWGMGMDVW	832
005S-C08	Fzd9	YTFTDYHMH	375	GWINAGNGNTTYA	558	CARASSWYLHYGYMDVW	755
005S-E08	Fzd9	FIFSZYAMS	171	SSISAAGAYKYA	631	CARRGYSSGWRDAFDIW	911
005S-F08	Fzd9	YTFTSYMH	392	GWINAGNGNTTYA	558	CAKDVNYW	710
005S-H08	Fzd9	GTFSZYAIS	295	GRIIPILGTPNYA	531	CARDRLAFDYW	789
005S-A09	Fzd9	FAFSSHWMH	166	SAISVSGGTTFYA	602	CARWKGRLRGSPYFDYW	952
005S-F09	Fzd9	FTFSIYGMH	209	SGISWNSGNIGYA	614	CARGPLPTKIGGHYMDVW	852
005S-B10	Fzd9	FTFSTXWMS	241	AVMYSGGTTYA	478	CARLSYYYDSSGPKGDAFDIW	891
005S-C10	Fzd9	FSLSSYGMH	185	SSISSSSYIYYA	636	CARSGMVKWLRSFDYW	917
005S-E10	Fzd9	FTFTSSAMQ	249	GVINPGSGGTSYN	552	CARGYGDYVWGENYFDYW	873
005S-F10	Fzd9	YTLSNYGIS	397	GWISAYNGDTKYA	568	CARFDYFGGMDVW	816
005S-G10	Fzd9	YTFTRYAVH	385	GGIIPFFNTVNYA	495	CAADRSPPYYDSSGYYPDAFDIW	679
005S-A11	Fzd9	FTFSSYDMN	229	SGISWNSGYIGYA	615	CAKGSLLLGYGMDVW	720
005S-B12	Fzd10	FTZSSYDMH	263	SSISGLGGSTYZA	633	CAREAGTTGGWFDPW	805
005S-D12	Fzd10	FTFSDHYMD	204	STIGPAGDTYYP	644	CARASTSGDYSW	756
006S-B01	Fzd10	YTFTNYCTR	384	GLVCPSDGSTSYA	519	CARRTSASDIW	915
006S-C01	Fzd10	FTFTZSAVQ	253	GGFDPEDGETIYA	492	CTTDPLELPWYW	1020
006S-E01	Fzd10	YTFTGYMH	379	GIINPSSGRTDYA	514	CARDLTYYYDSSGHSPLGAFDIW	781
006S-G01	Fzd10	FTFSDFGMN	203	AGISGGGGSTDYA	443	CARDSDFWYYYGMDVW	797
006S-B02	Fzd10	VFSFGYAMH	366	AYINSGSSEMNYA	482	CAREEWELFGMDVW	807
006S-G02	Fzd10	YTVTSYAMH	399	GGIPIFGTAKYA	497	CAKGGQWLYGMDVW	713
014S-A01	Fzd1	YTFTSYMH	392	GWVSPSSGNTAYA	588	CARDEGAGYYYYYMDVW	766
014S-C01	Fzd2	FTFSNYAMT	216	SAIGTGGGTYA	595	CATAYRRPGGLDVW	969
014S-F01	Fzd4	FTFSSYAMH	227	SVISTSGDVTLYT	652	CARGGSSDVR	838

Clone ID	Initial Binding	CDRH1	SID NO.	CDRH2	SID NO.	CDRH3	SID NO.
014S-H01	Fzd4	FTFSNYGMH	217	SYISSSSSTIYYA	672	CARAALGYCTGGVCPV DYW	737
014S-C03	Fzd7	YTFTNNFMH	383	GIIZPGGGRTIYA	517	CAKGDYGALDYW	712
014S-D03	Fzd7	FNFSYTMR	173	SVIYGGGNTNYA	653	CARGGSGGNLSYW	836
014S-F03	Fzd8	YTFTNNFMH	383	GGIIPLFGTANYA	506	CARLVVRGGYGMDVW	893
014S-A04	Fzd8	GTFSSY AIS	295	GWISSFNGNTKYA	575	CARADDYYDSSGYYYGF DYW	738
014S-C04	Fzd8	FTFSSYTMN	235	SRINGDGSNTNYA	623	CARGWAGFDYW	871
014S-D04	Fzd8	HTFSGYHIH	312	GWINAGNGNTTYA	558	CARDLSPMVRGVISGM DVW	779
014S-E05	Fzd8	YTFTNNFMH	383	GIISPGGGRTIYA	515	CAKGDYGALDYW	712
014S-E06	Fzd10	FTFGNYDMN	195	SSLSWNSGTIVYA	641	CARDSSSGWYASYGMDVW	798
027S-C02	Fzd5	YTLTTWYMX	398	GWMNPNSGNTAYA	582	CARGALGMDVW	819
027S-E03	Fzd8	YTFTGHYMH	378	GWMNPNSGNTGYA	583	CARGTGGFDYW	867
027S-F03	Fzd8	YTFTGHYIH	377	GWMNPISGNTGYA	580	CARSTPFDPW	925
027S-G03	Fzd8	YTFTHSYIH	380	GWINAKSGGTFYA	559	CARGDYDFWSGYHEYYY YGMDVW	824
027S-H03	Fzd8	YTFTSYMH	392	GWINPNSGGTNYA	564	CARAPLDGSGSYVDW	746
027S-A04	Fzd8	YTFTNHFMH	382	GWISPNRGGTNYA	571	CARDCSGGSCSYHFDYW	765
027S-B04	Fzd8	FTVGSWYMS	259	SAIGTGGGTYYA	595	CAKDITPYGDYSILSHW	702
027S-C04	Fzd8	YTFTSHWMH	386	GGIIPIFGTTNYA	503	CARDSSSWYSYYYYYMD VW	799
027S-D04	Fzd8	YTFTTYFMH	393	GWYIPNSGGTKYA	578	CTTDLRYDSSGPAAFDIW	1019
027S-E04	Fzd8	FTFSDHYMS	205	SGISGSGGTTYA	609	CATYGDFGYFDLW	978
027S-C05	Fzd8	GSFSTSVFG	279	GRIIPLFGTTNYA	532	CVKDRAWGFDYW	1027
027S-D05	Fzd8	YTFTSYMH	392	GWINPKSGGTNYA	560	CARGGFVFDYW	828
027S-E05	Fzd8	GTFSSY AIS	295	GMINPSGGSTTYA	523	CARQAGLHCSSTSCYLG NWFDPW	905
027S-F05	Fzd8	GTFNRYGIS	290	GGIIPRLGATDYA	508	CAKGNWAFDIW	719
027S-G05	Fzd8	GTFSSY AIS	295	GWISPYNGNTKYA	572	CARGVWTTPMGGGGN WFDPW	869
027S-H05	Fzd8	GTFGNYGIN	286	GWINPNSGGTNYA	564	CARETTDYYYGMDVW	814
027S-A06	Fzd8	GTFSSYAIN	294	GVIDPSTGGTNYA	550	CARVLPDSSGWYRGYY YYGMDVW	941
027S-C06	Fzd8	GTFTSYPIIS	306	GWINTYNGNTIYA	566	CARDLDSGFDLW	776
027S-D06	Fzd8	GTFSSY AIS	295	GWISAYNGHTNYA	569	CARGGYSYGTVFDYW	842
027S-E06	Fzd8	YTFTKDYM H	381	GGIIPIFGTANYA	499	CARGLPPAAGGGGYFQ HW	847
027S-F06	Fzd8	FTFSSYAMH	227	AVTWYDGSNKYYA	480	CAKDLVPYCSGGSCPPSG W	705

Clone ID	Initial Binding	CDRH1	SID NO.	CDRH2	SID NO.	CDRH3	SID NO.
027S-G06	Fzd5	YTFTDYMH	376	GWMSPNNGNAGFA	584	CARGKSGSFDYW	846
027S-H06	Fzd5	YTFTGHYIH	377	GIINPSGGSTSYA	513	CARGFCSGGSLWYGM DVW	825
027S-A07	Fzd5	GTFGSYAIT	287	GGIPIFGTANYA	499	CAKDNGWYFDLW	706
029S-B01	Fzd1	GTFSSYAIS	295	GRINPHNGNTNYA	1461	CARATRVSAAGTVHFQHW	1472
029S-D01	Fzd2	YTFTRYYIH	1452	GWMNPNSGNTGYA	583	CARVRFLEEMDVW	1494
029S-C02	Fzd2	GTFSSYGIS	1457	GIINPSGGSTSYA	513	CARGDIVATMGMMKKVD YYYMDVW	1478
029S-F02	Fzd2	YTFTRYLH	1453	GWMNPNSGNTGYA	583	CARGIGYW	1481
029S-H02	Fzd2	GTFSTY AIS	298	GDIPIFGSANYA	1458	CAREGLGWFDPW	1476
030S-A02	Fzd7	YTFTDYMH	1449	GWMNPNSGNTGYA	1467	CARGDINYGNFDYW	1477
030S-B02	Fzd3	YTFTDYMH	1449	GWMNPNSGNTGYA	583	CARQGGSYSMGLDPW	1488
029S-E03	Fzd3	YTFTGYMH	379	GWINPNNGNTGYA	1463	CARSYYGVIDAFDIW	1492
029S-G03	Fzd3	YTFTNYMH	1451	GWMNPNSGNTGYA	583	CAREDDFWSGGMDV W	1474
030S-E03	Fzd3	FTFSDYMS	1438	SAISGSGHSTYYA	1468	CAREGLRGWSIFDIW	1475
029S-D05	Fzd3	YTFTDHYFH	1447	GWANPSSGNTGTA	1462	CARSRLRWDWYFDLW	1491
030S-H03	Fzd3	FSFSSHAMS	1437	SAIGTGGGTYYA	595	CANPKHYW	1470
029S-B06	Fzd3	YTFSRHYIH	1446	GWMNPNSGNTGYA	583	CARGGHTGYSSGWYNH W	1479
029S-E06	Fzd3	YWFTASYMH	1455	GWMPKPSGNTGYA	1465	CARRSSSWGWFYDLW	1489
029S-H06	Fzd3	YTFAYYIH	1444	GWMNPNSGNTGYA	583	CARHKRHTPYAFDIW	1487
029S-G07	Fzd3	YTFTDSYIH	1448	GWISAYNGNTNYA	570	CARGSGYFDLW	1486
029S-H08	Fzd3	YTFTGHYMH	378	GWMNPNSGNTGYA	583	CARVGDYDRFNWYFDL W	1493
029S-F09	Fzd3	GTFSSYAIT	1456	GWISAYNGNTNYA	570	CARANRGLRKNYYYGM DVW	1471
030S-F04	Fzd3	YTFTSSYIH	1454	GIINPSGGGAVYA	1459	CARWTTVVTGAAFDIW	1495
029S-A10	Fzd3	YSFTGYLH	1442	GWINPNSSGNTNYA	564	CARDHGTMIAGVAGTFDY YYYMDVW	1473
029S-B11	Fzd3	YTFNGYMH	1445	GIVNPSGGGNTNYA	1460	CARGGNYGRWLQPWF DLW	1480
029S-D11	Fzd3	HTFTSHYMH	1440	GWMNPNSANAGY A	1466	CARGLGYFDLW	1483
030S-H05	Fzd7	YSFTNYMH	1443	GWMNPNSGNTGYA	583	CARSPDFWSGEGYFDLW	1490
030S-A06	Fzd7	YMFTGHDMH	1441	GRIIPILGIANYA	529	CARGIHGDYGLDYMD VW	1482
029S-C12	Fzd7	YTFTGYMH	379	GWMNPNSGNTGYA	583	CARGMEYW	1484
030S-C06	Fzd7	YTFTGYIH	1450	GWMDPNSSGTGYA	1464	CARGPADFWSGYKNDY FDFW	1485

Clone ID	Initial Binding	CDRH1	SID NO.	CDRH2	SID NO.	CDRH3	SID NO.
4A12	Fzd5	GYTFTNYDIN	1439	WIYPRDGSTKYNEFKG	1469	CVRSAWGFAY	1496
1791	Fzd7	TYAMH	2190	RIRSKSNYYAKNYDDSVKD	2193	ENYGGREFDY	2196
1291	Fzd7	SYAMS	2191	TISDGGSYTRYDPDKLKG	2194	VGGRRDYFDY	2197
18R5	Fzd7	GFTFSHYTLS	2192	VISGDGSYTYYADSVKG	2195	NFIKYVFAN	2198

Table 1A, cont.

Clone ID	Initial Binding	CDRL1	SID NO.	CDRL2	SID NO.	CDRL3	SID NO.
001S-A01	Fzd1	SGSSSNIGSHTVS	1156	SNYQRPS	1256	CAAWDGSFLGHWWVF	1265
001S-B01	Fzd1	RSSRSLDITDDGNTYLD	1142	TLSHRAS	1259	CMQSIQLPWTF	1295
001S-E01	Fzd1	SGNTLGSHYVS	1155	QDSKRPS	1246	CQVWDSSTVVF	1431
001S-F01	Fzd1	RSSQSLLHSNGYNYLD	1138	LGSNRAS	1237	CMQGTHWPYTF	1289
001S-G01	Fzd1	TRSSSNIGAGYDVH	1161	GNSIRPS	1220	CGTWDSSLSAWVF	1267
001S-H01	Fzd1	RSSQSLLHSNGYNYLD	1138	LGSKRAS	1236	CMQALQIPPTF	1280
001S-A02	Fzd1	QASQDIGKYLN	1041	DASNLET	1185	CQQNDYLPPLTF	1332
001S-E02	Fzd1	RSSQSLLHSNGYNYLD	1138	LGSNRAS	1237	CMQGTHWPYTF	1289
001S-G02	Fzd1	RASQSVGTLYT	1110	DASNRAT	1188	CMQATQFPLTF	1284
001S-H02	Fzd1	TRSSSNIGAGYDVH	1161	GNSIRPS	1220	CGTWDSSLSAWVF	1267
001S-A03	Fzd1	RASRSISSYFN	1128	AASSLQS	1175	CQQADTFPPTF	1314
001S-B03	Fzd1	SGDKVGHKYAS	1154	EDSQRPS	1199	CQAWDSSTDVVF	1301
001S-H08	Fzd5						
001S-A09	Fzd5						
001S-B09	Fzd5						
001S-C09	Fzd5						
001S-C07	Fzd8	QGDSLRTYYAS	1052	GKNNRPS	1219	CNSRDNSGKHKVF	1300
001S-D07	Fzd8	RSSQSLLHSNGFN YVD	1136	FGSYRAS	1206	CMQNLQTPWTF	1291
001S-E07	Fzd8	RASQGIRSDLA	1070	AASTLES	1177	CLQDYSYPRTF	1273
001S-H07	Fzd8	RASQSVSSYLA	1121	GASSRAT	1213	CQQYGSSPPTF	1410
004S-E05	Fzd5	RASQGISSALA	1076	AASALQS	1165	CQQTYSTPRTF	1394
004S-E03	Fzd5	RASQSISSYLN	1098	AASSLQS	1175	CQQSYSTPLTF	1372
004S-G06	Fzd5	RASQSISSYLN	1098	AASSLQS	1175	CQQSYSTPLTF	1372
001S-D09	Fzd8						

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Clone ID	Initial Binding	CDRL1	SID NO.	CDRL2	SID NO.	CDRL3	SID NO.
001S-E09	Fzd8						
001S-F09	Fzd8						
001S-G09	Fzd8						
001S-H09	Fzd8						
001S-A10	Fzd8						
001S-B10	Fzd8						
001S-G12	Fzd1						
002S-A01	Fzd1						
002S-B01	Fzd1						
002S-C01	Fzd1						
002S-D01	Fzd1						
002S-E01	Fzd1						
002S-F01	Fzd1						
002S-G01	Fzd1						
002S-H01	Fzd1						
002S-A02	Fzd1						
002S-B02	Fzd1						
002S-C02	Fzd1						
002S-D02	Fzd1						
002S-E02	Fzd1						
002S-F02	Fzd1						
002S-G02	Fzd1						
002S-H02	Fzd1						
002S-D03	Fzd1						
002S-E03	Fzd1						
002S-F03	Fzd1						
002S-G03	Fzd1						
002S-H03	Fzd1						
002S-A04	Fzd1						
002S-B04	Fzd1						
002S-C04	Fzd1						
002S-D04	Fzd1						
002S-E04	Fzd1						
004S-H04	Fzd5	RASQSISSYLN	1098	AASSLQS	1175	CQQSYSTPLTF	1372
001S-A04	Fzd5	GLSSGSVSTNYYP S	1035	YTNRSS	1263	CLLYLGRGIWVF	1271

Clone ID	Initial Binding	CDRL1	SID NO.	CDRL2	SID NO.	CDRL3	SID NO.
001S-D03	Fzd5	TGTSSDVGGYNSV S	1159	DVTKRPS	1196	CFSYAGSRF	1266
001S-F03	Fzd5	TRSSGSIASNYVQ	1160	ENDKRPS	1202	CQSYDYDHRWVF	1430
004S-E04	Fzd5	RASQSISSYLN	1098	AASSLQS	1175	CQQSYSTPLTF	1372
004S-A06	Fzd5	RASQSISSYLN	1098	AASSLQS	1175	CQQSYSTPLTF	1372
004S-F04	Fzd5	RASQGISSALA	1076	AASTLQS	1179	CQQSYNTPWTF	1351
001S-C03	Fzd5	RSSQSLLRRNGHN YVD	1139	MGSNRAP	1238	CMHGLHPPFTF	1279
003S-A01	Fzd1	RASQGISNNLN	1072	GASTLQS	1215	CQQADSFPTF	1312
003S-E01	Fzd1	RASQSISSYLN	1098	AASSLQS	1175	CQQSYSTPLTF	1372
003S-F01	Fzd1	RASQSISSYLN	1098	AASSLQS	1175	CQQSYSTPLTF	1372
003S-A02	Fzd1	RASQGISNYLA	1074	EVSSVQG	1204	CQQSYSTPLAF	1370
003S-C02	Fzd1	RASQSIGRWLA	1084	AASRLQS	1171	CQQGFNFPLTF	1325
003S-E02	Fzd1	RASQGISNNLN	1072	TASSLQS	1258	CLQDYSYPYTF	1274
003S-F02	Fzd1	RASQSVSSDLA	1115	GASTRAT	1217	CQQYETWPVLTF	1405
003S-G02	Fzd1	RASESVSSSFA	1056	GASTRAT	1217	CQQYNNWPPNYTF	1420
003S-C03	Fzd1	QANQDISNYLN	1038	AASSLQS	1175	CQQTYNPPRTF	1389
003S-D03	Fzd1	RASQGISNNLN	1072	AASSLQR	1174	CQQSYSTPFTF	1368
003S-E03	Fzd1	RASQGISNNLN	1072	SASNLQS	1252	CQQSYSPPPYTF	1364
003S-H03	Fzd1	RASQSISSYLN	1098	AASSLQS	1175	CQQSYSTPLTF	1372
003S-A04	Fzd1	RTSERSSISSFA	1148	GASTRAT	1217	CQQYNNWPRNYTF	1420
003S-C04	Fzd1	RASQGISNNLN	1072	KASSLEN	1225	CQQSYSTPHTF	1369
003S-D04	Fzd1	QASQDIGNYLN	1042	DVSNLER	1195	CQHLSYPPGDTF	1304
003S-G04	Fzd1	RASQSISSYLN	1098	AASSLQS	1175	CQQSYSTPLTF	1372
003S-D05	Fzd2	RSSQSLLHSDGKT YLY	1134	LGSNRAS	1237	CMQNTHWPLTR	1293
003S-E05	Fzd2	KSSQSLLHSDGKT YLY	1036	LGSNRAS	1237	CMQNTHWPLTR	1293
003S-A06	Fzd2	RASQGISNNLN	1072	AASRLQS	1170	CQQSYSTPLTF	1372
003S-C06	Fzd2	RASQDISSYLA	1065	AASSLQS	1175	CQQSYRTPLTF	1353
003S-G06	Fzd2	RASQSVSSNLA	1116	DASNRAT	1188	CQHRTSWPLTF	1307
003S-H06	Fzd2	RASQRVGNLA	1083	DASIRAT	1184	CQQYKDWPTF	1415
003S-B07	Fzd2	RASQSVGSYLA	1109	GSSNRAS	1221	CQQYGTSLTF	1414
003S-D07	Fzd2	QASQGISNNLN	1049	LGSDRAS	1233	CQQSYSTPFTF	1368
003S-E07	Fzd2	RASQSISSYLN	1098	AASSLQS	1175	CQQSYSTPFTF	1368
003S-A08	Fzd2	RSSQSLLHSDGKT YLY	1134	LGFNRAS	1232	CMQNTHWPLTR	1293
003S-C08	Fzd2	RASQGIRNDLG	1069	GASTLQR	1214	CQQSYSTPRVTF	1374

Clone ID	Initial Binding	CDRL1	SID NO.	CDRL2	SID NO.	CDRL3	SID NO.
003S-E08	Fzd2	RSSRSLHSDGKTY LY	1143	LGSNRAS	1237	CMQSSHWPKTF	1298
003S-G09	Fzd4	RASQSISSYLN	1098	AASSLQS	1175	CQQSYSTPLTF	1372
003S-C10	Fzd4	RASQSISSYLN	1098	AASSLQS	1175	CQQSYSTPLTF	1372
003S-D10	Fzd4	RASQGISSYLA	1076	AASNLLG	1167	CQQTYSTPWTF	1396
003S-E10	Fzd4	RASQSIGSNLD	1085	AASTLET	1178	CQQSYSVPDF	1380
003S-A11	Fzd4	RASQSISSYZN	1103	ZASSLQS	1264	CQQSYSTPLTF	1372
003S-G11	Fzd4	RASQSISSYLN	1098	AASSLQS	1175	CQQSYSTPLTF	1372
003S-H11	Fzd4	RASQSIGSNLD	1085	DASSLES	1189	CQQSFIMPLTF	1341
003S-C12	Fzd4	RASQSISSYLN	1098	AASSLQS	1175	CQQSYSTPLTF	1372
003S-F12	Fzd4	RASQSISSYLN	1098	AASSLQS	1175	CQQSYSTPLTF	1372
004S-B01	Fzd4	RAIQSISSYLN	1054	AASSLQS	1175	CQQSYSTPLTF	1372
004S-C01	Fzd4	RASQDIRDELA	1062	AASTLQS	1179	CQQADSFPLTF	1311
004S-D01	Fzd4	RASQSISSYLN	1098	AASSLQS	1175	CQQSYSTPLTF	1372
004S-E01	Fzd4	ZACLRISSYLN	1163	FASSLQS	1205	CQQSYSTPLTF	1372
004S-F01	Fzd4	RASQGISNWLA	1073	DASSLQS	1190	CQQSHITPYTF	1344
004S-H01	Fzd4	RASQSISSYLN	1098	AASSLQS	1175	CQQSYSTPLTF	1372
004S-B02	Fzd4	RASQSISSYLN	1098	AASSLQS	1175	CQQSYSTPLTF	1372
004S-E02	Fzd4	RASQSISSYLN	1098	AASSLQS	1175	CQQSYSTPLTF	1372
004S-F02	Fzd4	RASQGISSALA	1076	AASTLQS	1179	CQQANTVPFTF	1322
004S-G02	Fzd4	RASQSISSYLN	1098	AASSLQS	1175	CQQSYSTPLTF	1372
004S-H02	Fzd4	RASQSISSYLN	1098	AASSLQS	1175	CQQSYSTPLTF	1372
001S-E03	Fzd5	TGSSSNIGAGFGV H	1158	SDRNRPS	1255	CQSYDSSLRASVF	1429
001S-B05	Fzd5	RSSQSLHNSGNT YLD	1137	LGSDRTS	1234	CMQSLQTPYTF	1297
004S-A07	Fzd6	RASQDIRSALA	1063	QASSLIS	1245	CQQSYSMPTQTF	1361
004S-B07	Fzd6	QASQDIRNYLN	1043	AASSLQS	1175	CQQSSRFWTF	1347
004S-A08	Fzd6	RASQSISSYLN	1098	AASSLQS	1175	CQQSYSTPLTF	1372
004S-B08	Fzd6	RASQSISSYLN	1098	AASSLQS	1175	CQQSYSTPLTF	1372
004S-D08	Fzd6	RASQSISSYLN	1098	AASSLQS	1175	CQQSYSTPLTF	1372
004S-E08	Fzd6	RSSRSLHNSGNT YLQ	1144	LGSNRAS	1237	CVQTTQSPLTF	1434
004S-G08	Fzd6	RASQSISSYLN	1098	AASSLQS	1175	CQQSYSTPLTF	1372
004S-A09	Fzd6	RASQSISSYLN	1098	AASSLQS	1175	CQQSYSTPLTF	1372
004S-B09	Fzd6	RASQGISSALA	1076	AASSLQS	1175	CQQSYSHATFTF	1357
004S-C09	Fzd6	RASQSISSYLN	1098	AASSLQS	1175	CQQSYSTPLTF	1372
004S-E09	Fzd6	RASQSISSYLN	1098	AASSLQS	1175	CQQSYSTPLTF	1372

Clone ID	Initial Binding	CDRL1	SID NO.	CDRL2	SID NO.	CDRL3	SID NO.
004S-F09	Fzd6	RASQSISSYLN	1098	AASSLQS	1175	CQQSYSTPLTF	1372
004S-H09	Fzd6	RASQSISSYLN	1098	AASSLQS	1175	CQQSYSTPLTF	1372
004S-C10	Fzd6	RASQSISSYLN	1098	AASSLQS	1175	CQQSYSTPLTF	1372
004S-D10	Fzd6	RASQNINNYLA	1081	RASTLQS	1249	CQQYSSYPYTI	1425
004S-E10	Fzd6	RASQSISSYLN	1098	AASSLQS	1175	CQQSYSTPLTF	1372
004S-F10	Fzd6	RASQSISSYLN	1098	AASSLQS	1175	CQQSYSTPLTF	1372
004S-G10	Fzd6	RASQSISSYLN	1098	AASSLQS	1175	CQQSYSTPLTF	1372
004S-A11	Fzd6	RASQSISSYLN	1098	AASSLQS	1175	CQQSYSTPLTF	1372
004S-C11	Fzd6	RASQSISSYLN	1098	AASSLQS	1175	CQQSYSTPLTF	1372
004S-E11	Fzd6	RASQSISSYLN	1098	AASSLQS	1175	CQQSYSTPLTF	1372
004S-H11	Fzd6	RASQSISRWLA	1094	AASSLQS	1175	CQQYVSYPLTF	1426
004S-A12	Fzd6	RASQSISSYLN	1098	AASSLQS	1175	CQQSYSTPLTF	1372
004S-D12	Fzd6	RASQSISSYLN	1098	AASSLQS	1175	CQQSYSTPLTF	1372
005S-H01	Fzd7	RASQSISSYLN	1098	AASSLQS	1175	CQQSYSTPLTF	1372
005S-A02	Fzd7	RASQSISSYLN	1098	AASSLQS	1175	CQQSYSTPLTF	1372
005S-C02	Fzd8	RASQSISSYLN	1098	AASSLQS	1175	CQQSYSTPLTF	1372
005S-E02	Fzd8	RASQSISSYLN	1098	AASSLQS	1175	CQQSYSTPLTF	1372
005S-A03	Fzd8	RASQSISSYLN	1098	AASSLQS	1175	CQQSYSTPLTF	1372
005S-H03	Fzd8	RASQGISNNLN	1072	DASTLQT	1193	CQQSFSAPIF	1342
005S-F04	Fzd8	RASQSISSYLN	1098	AASSLQS	1175	CQQSYSTPLTF	1372
005S-H04	Fzd8	RASQSISSYLN	1098	AASSLQS	1175	CQQSYSTPLTF	1372
005S-B05	Fzd8	QASQDISNYLN	1046	KASSLES	1226	CQQSYSTPRTF	1373
005S-F05	Fzd8	RASQSISSYLN	1098	AASSLQS	1175	CQQSYSTPLTF	1372
005S-G05	Fzd8	RASQSISSYLN	1098	AASSLQS	1175	CQQSYSTPLTF	1372
005S-H05	Fzd8	RASQGISRTLZ	1075	AASSLQS	1175	CQQTYSMPITF	1392
005S-D06	Fzd8	RASQSISSYLN	1098	AASSLQS	1175	CQQSYSTPLTF	1372
005S-F06	Fzd8	RASQSVGSNLA	1108	GASSRAT	1213	CQQYGSSPPFTF	1409
005S-A07	Fzd9	RASQSVSRNLA	1114	GASTRAT	1217	CQQRSNWPITF	1335
005S-B07	Fzd9	RASQGISSALA	1076	GASTLQS	1215	CLQDYNYPFTF	1272
005S-C07	Fzd9	RASQSISSYLN	1098	AASSLQS	1175	CQQSYSTPLTF	1372
005S-D07	Fzd9	RASQSINRWLA	1090	AASSLQS	1175	CQQTYNIPITF	1388
005S-F07	Fzd9	RASQSINRNYLG	1089	AASSRVT	1176	CQQYDSWPPTF	1402
005S-G07	Fzd9	RASQGISNNLN	1072	AASSLQS	1175	CQHYYNLPITF	1309
005S-H07	Fzd9	RASQTINNQLA	1125	KASNLET	1224	CQQANSFPVTF	1318
005S-B08	Fzd9	RASQSISSYLN	1098	AASSLQS	1175	CQQSYSTPLTF	1372
005S-D08	Fzd9	RASQSISSYLN	1098	AASSLQS	1175	CQQSYSTPLTF	1372
005S-G08	Fzd9	QTSQDINNNLN	1053	KASSLES	1226	CQQSYSSPPTF	1366

Clone ID	Initial Binding	CDRL1	SID NO.	CDRL2	SID NO.	CDRL3	SID NO.
005S-C09	Fzd9	QASQDISNYLN	1046	AASTLQS	1179	CLQHKSFPTF	1276
005S-D09	Fzd9	RASQSVSSNQLA	1117	GASTRAT	1217	CQQRYNWPPSITF	1339
005S-E09	Fzd9	RASQSVSSNLA	1116	DASNRAT	1188	CQQRNNWLYTF	1334
005S-A10	Fzd9	RASQSISSYLN	1098	AASSLQS	1175	CQQSYSTPLTF	1372
005S-D10	Fzd9	RASQGISNNLN	1072	AASTLQS	1179	CQQTNLFPYTF	1385
005S-H10	Fzd9	RASQSVSSNLA	1116	GASTRAT	1217	CQQYNWPLTF	1421
005S-B11	Fzd9	RASQSISSRWLA	1094	AASSLQS	1175	CQQTNTFPPTF	1386
005S-C11	Fzd9	RASQSVSRKLA	1113	GASTRAT	1217	CQQRSNWPITF	1335
005S-D11	Fzd9	RASQSLRSKLA	1106	GASTRAT	1217	CQQYANSPWTF	1401
005S-E11	Fzd9	QASQDISNYLN	1046	GASTLQS	1215	CQQLSRYPRLF	1331
005S-G11	Fzd9	RASQSVSSNLA	1116	GASNRPT	1209	CQQYGSSPYTF	1413
005S-H11	Fzd10	RASQSISSYLN	1098	AASSLQS	1175	CQQSYSTPLTF	1372
005S-E12	Fzd10	RASQSVGRWMA	1107	AASSLQS	1175	CQQANTFPPTF	1321
005S-F12	Fzd10	RASQSISSYLN	1098	AASSLQS	1175	CQQSYSTPLTF	1372
006S-A01	Fzd10	RASQSISSYLN	1098	AASSLQS	1175	CQQSYSTPLTF	1372
006S-F01	Fzd10	RASQSISSYLN	1098	AASSLQS	1175	CQQSYSTPLTF	1372
006S-H01	Fzd10	RASQGISNNLN	1072	AASNLET	1166	CQQTSTPLTF	1387
006S-A02	Fzd10	RASQSISSYLN	1098	AASSLQS	1175	CQQSYSTPLTF	1372
006S-D02	Fzd10	RASQGISNNLN	1072	DASSLES	1189	CQQTYNTPRTF	1390
006S-E02	Fzd10	RASQGISNNLN	1072	AASSLQS	1175	CLQHNGYPITF	1277
006S-H02	Fzd10	RSSQSLHSHNGYN YLD	1138	RVSSRFS	1251	CMQGTHWPPTF	1288
006S-A03	Fzd10	RASESVSSNLA	1055	GASSRAT	1213	CQQYNKSPSF	1419
006S-B03	Fzd10	RASQTISRVLN	1126	EVSSLQG	1203	CQQSYSTPWTF	1378
006S-C03	Fzd10	RASQSISSYLN	1098	AASSLQS	1175	CQQSYSTPLTF	1372
014S-B01	Fzd1	RASQGISNNLN	1072	GASTLQS	1215	CQQADSFPTF	1312
014S-D01	Fzd4	RASQSISSHZN	1096	AASSLQS	1175	CQQSYSTPLTF	1372
014S-E01	Fzd4	RASQSISSYLN	1105	AASSLQS	1175	CQQSYSTPLTF	1372
014S-G01	Fzd4	RASQSISSYLN	1098	AASSLQS	1175	CQQSYSTPPTF	1368
014S-A02	Fzd4	RASQGISSALA	1076	GASTVES	1218	CQQSYSTPRTF	1373
014S-B02	Fzd4	RASQSVSSNLA	1116	GASTRAT	1217	CQQYDTPRTF	1403
014S-C02	Fzd5	RASQGISSALA	1076	AASSLQS	1175	CQQTYSMPITF	1392
014S-D02	Fzd5	RASQSISSYLN	1098	AASSLQS	1175	CQQSYSTPLTF	1372
014S-E02	Fzd5	RASQSISSYLN	1098	AASSLQS	1175	CQQSYSTPLTF	1372
014S-F02	Fzd5	RASQGVSTZLS	1079	AASSLQS	1175	CQQTYSMPITF	1392
014S-G02	Fzd6	RASQGISSALA	1076	ATSTLQS	1183	CQQVNSYPPTF	1399
014S-H02	Fzd6	RASQSVSSWLA	1120	AASTLQT	1180	CQQSYSTPTF	1376

Clone ID	Initial Binding	CDRL1	SID NO.	CDRL2	SID NO.	CDRL3	SID NO.
014S-A03	Fzd6	RASQSISSYLN	1098	AASSLQS	1175	CQQSYSTPLTF	1372
014S-B03	Fzd6	RASQSISSYLN	1098	AASSLQS	1175	CQQSYSTPLTF	1372
014S-E03	Fzd8	RASQSISSYLN	1098	AASSLQS	1175	CQQSYSTPLTF	1372
014S-G03	Fzd8	RASQSISSYLN	1098	AASSLQS	1175	CQQSYSTPLTF	1372
014S-H03	Fzd8	RASQSISSYLN	1098	AASSLQS	1175	CQQSYSTPLTF	1372
014S-B04	Fzd8	RASQGISNYLA	1074	AASSLQS	1175	CQQSYSTPFTF	1368
014S-E04	Fzd8	RASQSISSYLN	1098	AASSLQS	1175	CQQSYSTPLTF	1372
014S-F04	Fzd8	RASQGISNNLN	1072	EASSVAS	1197	CQQSYTSTPLNSF	1381
014S-G04	Fzd8	RASQSVSGYLA	1112	GASTRAA	1216	CQQYNYWPPAF	1423
014S-H04	Fzd8	RASQSISSYLN	1098	AASSLQS	1175	CQQSYSTPLTF	1372
014S-A05	Fzd8	RASQGISNNLN	1072	DASSLES	1189	CLQHNSLPFTF	1278
014S-B05	Fzd8	RASQSVSSNLA	1116	GVSNRAT	1223	CQQYNIWPRTF	1418
014S-C05	Fzd8	RASQSISSYLN	1098	AASSLQS	1175	CQQSYSTPLTF	1372
014S-D05	Fzd8	RASQSISSYLN	1098	AASSLQS	1175	CQQSYSTPLTF	1372
014S-F05	Fzd9	RASQZVSRZZA	1127	GASTRAT	1217	CQQRSNWPITF	1335
014S-G05	Fzd9	RVSQGISSALA	1151	AASSLQS	1175	CQQTFSVPWTF	1384
014S-H05	Fzd9	RASQSISSYLN	1098	AASSLQS	1175	CQQSYSTPLTF	1372
014S-A06	Fzd9	RASQSVSRNLA	1114	GASTRAT	1217	CQQRSNWPITF	1335
014S-B06	Fzd9	RASQSISSYLN	1098	AASSLQS	1175	CQQSYSTPLTF	1372
014S-C06	Fzd10	RASQSVSRNLA	1114	GASTRAT	1217	CQQRSNWPITF	1335
014S-D06	Fzd10	RASQSISSYLN	1095	AASSLQS	1175	CQQRYSTPLTF	1340
014S-F06	Fzd10	RASQSVGRWMA	1107	AASSLQS	1175	CQQANTFPFTF	1321
014S-G06	Fzd10	RASQSISSYLN	1098	AASSLQS	1175	CQQSYSTPLTF	1372
014S-H06	Fzd10	RASQSISSYLN	1098	AASSLQS	1175	CQQSYSTPLTF	1372
014S-A07	Fzd10	RASQNIGSRLA	1080	GASNRAS	1208	CQQYNHWPLFTF	1417
017S-E08	Fzd8						
017S-H08	Fzd8						
017S-A09	Fzd8						
017S-B09	Fzd8						
017S-C09	Fzd8						
018S-D06	Fzd4						
018S-E06	Fzd4						
018S-F06	Fzd4						
018S-G06	Fzd5						
018S-H06	Fzd5						
018S-A07	Fzd5						
018S-B07	Fzd5						

Clone ID	Initial Binding	CDRL1	SID NO.	CDRL2	SID NO.	CDRL3	SID NO.
018S-C07	Fzd7						
017S-F09	Fzd4	RSSRSLHTSGYNY LD	1147	LGSNRAS	1237	CMQGTRWPTF	1290
017S-G09	Fzd4	RSSRSLHTNGYNY LD	1146	LGSNRAS	1237	CMQALQTPLTF	1281
017S-H09	Fzd5						
017S-A10	Fzd5						
017S-B10	Fzd5						
017S-C10	Fzd8						
017S-D10	Fzd8						
017S-E10	Fzd8						
018S-D07	Fzd1						
018S-E07	Fzd1						
018S-F07	Fzd1						
018S-G07	Fzd1						
018S-H07	Fzd4						
018S-A08	Fzd4						
018S-B08	Fzd4						
018S-C08	Fzd4						
018S-D08	Fzd5						
018S-E08	Fzd5						
018S-F08	Fzd5						
018S-G08	Fzd5						
018S-H08	Fzd5						
018S-A09	Fzd8						
018S-B09	Fzd8						
018S-C09	Fzd8						
018S-D09	Fzd8						
018S-E09	Fzd8						
018S-F09	Fzd8						
018S-G09	Fzd8						
021S-A01	Fzd8	RASQSIGSSLH	1087	YASQSVS	1260	CHQSGRVPVTF	1268
021S-C01	Fzd1	RSSRSLDTHDGN TYLD	1142	TLSHRAS	1259	CMQSIQLPWTF	1295
021S-D01	Fzd1	RSSQSLVHSDGNT YLS	1140	KISNRFS	1230	CMQATQFPHTF	1283
021S-E02	Fzd8	QGDSLRTYYAS	1052	GKNNRPS	1219	CNSRDNSGKHKVF	1300
021S-G02	Fzd8	RSSQSLDSDHGN TYLD	1133	MLSSRAP	1240	CMQRLEFPYTF	1294

Clone ID	Initial Binding	CDRL1	SID NO.	CDRL2	SID NO.	CDRL3	SID NO.
021S-A03	Fzd8	RSSQNIFQSLN	1131	SASSLQS	1254	CQQSYNSPITF	1349
022S-H06	Fzd4	RASQSISSYLN	1098	AASSLQS	1175	CQQSYSTPLTF	1372
022S-A11	Fzd10	RASQGISNNIN	1071	AASNLET	1166	CQQTYSIPFTF	1391
OMP-18R5		SGDKLGKKYAS or SGDNIGSFYVH	1152 or 1153	EKDNRPSPG or DKSNRPSPG	1200 or 1201	SSFAGNSLE or QSYANTLSL	1435 or 1436
027S-H02	Fzd5	RASQSVSSNYLS	1118	GASSRAP	1212	CQQRNTWPPRVTF	1337
027S-B03	Fzd8	SGTSSNIGAGYDV H	1157	GNNNRPS	1219	CSAWDDNLNGVVF	1432
027S-E01	Fzd5						
004S-D05	Fzd5	QASQDISNYLN	1046	AASSLQS	1175	CQQSYSTPLTF	1371
004S-D04	Fzd5	RASQSISSYLN	1098	AASSLQS	1175	CQQSYSTPLTF	1372
004S-B05	Fzd5	RASQSISSYLN	1098	AASSLQS	1175	CQQSYSTPLTF	1372
004S-G03	Fzd5	RASQSISSYLN	1098	AASSLQS	1175	CQQSYSTPLTF	1372
004S-F03	Fzd5	KSSQSLLHSDGYT YLY	1036	LGSNRAS	1237	CMQGLQTPWTF	1286
004S-C04	Fzd5	RATQTISTYLN	1129	AASRLQS	1171	CQQYYSYPWTS	1427
004S-B06	Fzd5	RASQGISNNLN	1072	AASALQS	1165	CQHLNNEPLTF	1303
004S-F06	Fzd5	RASQSISSYLN	1098	AASSLQS	1175	CQQSYSTPLTF	1372
004S-A04	Fzd5	RASQGISNNLN	1072	GASSLQS	1211	CQQSHSSPRTF	1346
004S-A05	Fzd5	RASQSISSYLN	1098	AASSLQS	1175	CQQSYSTPLTF	1372
004S-F05	Fzd5	RSSQSLLHSDGYT YLY	1135	LGSHRAS	1235	CMQGLQTPHTF	1285
003S-C01	Fzd1	RASQSISSYLN	1092	AASSLQS	1175	CQQSYNTPFTF	1350
003S-H01	Fzd1	RASQSIGSNLD	1085	AATLQS	1179	CQQNYATPRTF	1333
003S-H02	Fzd1	RASQSISSYLN	1098	AASSLQS	1175	CQQSYSTPLTF	1372
003S-H04	Fzd1	RASQGISNNLN	1072	AASSLQS	1175	CQQANSEPTF	1316
003S-A05	Fzd2	TSSQSLLHSDGKT YLY	1162	LGSNRAS	1237	CMQGTHWPYTF	1289
003S-B05	Fzd2	KSSQSLLHSDGKT YLY	1036	LGSNRAS	1237	CMQSLQSPLTF	1296
003S-F05	Fzd2	KSSQSLLHSDGKT YLY	1036	LGSNRAS	1237	CMQNTHWPLTR	1293
003S-G05	Fzd2	RSSRSLLHSNGNT YLR	1145	LASRRAS	1231	CIQNTHWPLTR	1270
003S-H05	Fzd2	KSSQSLLHSDGKT YLY	1036	MGSYRAS	1239	CMQGTHWPLTF	1287
003S-A07	Fzd2	KSSQSLLHSDGKT YLY	1036	LGSNRAS	1237	CMQNTHWPLTL	1292
003S-C07	Fzd2	RTSQSVSSNLA	1150	DASNRAS	1187	CQQYGSSPYNF	1412
003S-F07	Fzd2	RASQSISSYLN	1098	AASSLQS	1175	CQQSYSTPLTF	1372

Clone ID	Initial Binding	CDRL1	SID NO.	CDRL2	SID NO.	CDRL3	SID NO.
003S-G07	Fzd2	RASQAISYLA	1060	KASTLDT	1228	CQQADTFPFTF	1313
003S-B08	Fzd2	KSSQSLLHSDGKTYLY	1036	LGSNRAS	1237	CMQTLKAPLTF	1299
003S-F08	Fzd2	RSSQYLSSAYLA	1141	GASSRAT	1213	CQQYGSSPTF	1411
003S-H08	Fzd2	QASQGISNNLN	1049	AASSLQS	1175	CQQSYSTPAFTF	1367
003S-A09	Fzd2	RSSQSLLHSDGYTYLY	1135	LGSNRAS	1237	CMQGTHWPLTF	1287
003S-B09	Fzd2	RSSESLLHSDGKTYLY	1130	LGSNRAS	1237	CTQTVQFPITF	1433
003S-C09	Fzd2	RASQGISNNLN	1072	SASNLQS	1252	CQQSYSTPWTF	1378
003S-F09	Fzd4	RASQSISSYLN	1098	AASSLQS	1175	CQQSYSTPLTF	1372
003S-H09	Fzd4	RASQSIGSNLN	1086	RASTLES	1248	CQQTYTTPRF	1398
003S-A10	Fzd4	RASQGISNNLN	1072	YASSLQS	1262	CQQSHSPPGTF	1345
003S-B10	Fzd4	RASQSISSYLN	1098	AASSLQS	1175	CQQSYSTPLTF	1372
003S-G10	Fzd4	RASQSIVSYLN	1104	DASNLQS	1186	CQQGYSAPWTF	1329
003S-B11	Fzd4	RASQSISSYLN	1098	AASSLQS	1175	CQQSYSTPLTF	1372
003S-C11	Fzd4	RASQSIGSNLD	1085	AASTLQS	1179	CQQSYSTPRTF	1373
003S-D11	Fzd4	RASQSISSYLN	1098	AASSLQS	1175	CQQSYSTPLTF	1372
003S-F11	Fzd4	RASQSISSYLN	1098	AASSLQS	1175	CQQSYSTPLTF	1372
003S-E12	Fzd4	ZPZQTZZSHLN	1164	PASSLQS	1242	CQQSYSTPLTF	1372
004S-A01	Fzd4	RASQGISNNLN	1072	AASTLQS	1179	CQQGNNFPFTF	1326
004S-G01	Fzd4	RASQGISNNLN	1072	AASSLQS	1175	CQQYYTYPYTF	1428
004S-C02	Fzd4	RASQSISSYLN	1098	AASSLQS	1175	CQQSYSTPLTF	1372
004S-D02	Fzd4	RASQSISSYLN	1098	AASSLQS	1175	CQQSYSTPLTF	1372
004S-A03	Fzd4	RASQGISNNLN	1072	DASNLET	1185	CHQSYSIPRTF	1269
004S-B03	Fzd4	RASQDVDTWLA	1066	DASTLET	1191	CQQGYNIPWTF	1328
004S-C03	Fzd4	QASQDISSYLN	1047	AASTLQS	1179	CQQAISFPLTF	1315
004S-C05	Fzd5	RASQSISSYLN	1098	AASSLQS	1175	CQQSYSTPLTF	1372
004S-G05	Fzd5	RASQSISSYLN	1098	AASSLQS	1175	CQQSYSTPLTF	1372
004S-E06	Fzd5	QASQDISNYLN	1046	KASSLES	1226	CQQANSFPYTF	1319
004S-C06	Fzd5	RSSQNVSSYLA	1132	GASTRAT	1217	CQHRANWPQTF	1305
004S-E07	Fzd6	RASQSISSYLN	1098	AASSLQS	1175	CQQSYSTPLTF	1372
004S-F07	Fzd6	QASQDITNYLN	1048	KASTLES	1229	CQQSYSAPYTF	1356
004S-C08	Fzd6	RASQSISSYLN	1098	AASSLQS	1175	CQQSYSTPLTF	1372
004S-F08	Fzd6	RASQSISSWLA	1102	EASTLQS	1198	CQQTYTPPFTF	1397
004S-G09	Fzd6	RASQAISNSLA	1058	DASNLET	1185	CQQAYSFPWTF	1324
004S-B10	Fzd6	RASQSISSYLN	1098	AASSLQS	1175	CQQSYSTPLTF	1372
004S-H10	Fzd6	RASQSISSYLS	1101	GASSLES	1210	CQQSYSPFTF	1362

Clone ID	Initial Binding	CDRL1	SID NO.	CDRL2	SID NO.	CDRL3	SID NO.
004S-B11	Fzd6	RASQSISSYLN	1098	AASSLQS	1175	CQQSYSTPLTF	1372
004S-D11	Fzd6	RASQSVSSWLA	1120	AASTLQT	1180	CQQSYSTPTF	1376
004S-F11	Fzd6	RASQSISSYLN	1098	AASSLQS	1175	CQQSYSTPLTF	1372
004S-G11	Fzd6	RASQSVSSYLA	1121	GASSRAT	1213	CQQYAI SYTF	1400
004S-B12	Fzd6	RASQSISSYLN	1098	AASSLQS	1175	CQQSYSTPLTF	1372
004S-C12	Fzd6	RASQGISSYLA	1076	RTSTLES	1250	CQQSYSTPWTF	1378
004S-F12	Fzd7	RASQSISSYLN	1098	AASTLQT	1180	CQQSY SIPTF	1358
005S-B01	Fzd7	RASQSISSYLN	1098	AASSLQS	1175	CQQSYSTPLTF	1372
005S-C01	Fzd7	RASQGISNNLN	1072	KASSLQS	1227	CQQSYSLPYTF	1360
005S-F01	Fzd7	RASQSVSSSYLS	1119	GASSRAT	1213	CQQRYKSYTF	1338
005S-B02	Fzd8	RASQSISSYLN	1098	AASSLQS	1175	CQQSYSTPLTF	1372
005S-D02	Fzd8	RASQSVSSNLA	1116	NTSNRAT	1241	CQHYNWNPFTF	1308
005S-G02	Fzd8	RASQSVSTNLA	1122	DASNRAT	1188	CQQRSNWPPQITF	1336
005S-H02	Fzd8	QASQDISHYLN	1044	AASSLQS	1175	CQQSYSTPLTF	1372
005S-B03	Fzd8	RASQSINSNLA	1091	GASSRAT	1213	CQQYGSSPYTF	1413
005S-C03	Fzd8	RASQSVSSSYLS	1119	DTSNRAT	1194	CQQYGSSPITF	1408
005S-E03	Fzd8	RTSQSISSYLN	1149	AASTSQS	1181	CQQSFSSWTF	1343
005S-F03	Fzd8	RASQSISSYLN	1098	AASSLQS	1175	CQQSYSTPLTF	1372
005S-B04	Fzd8	QASHDINIALN	1039	AASSLQS	1175	CQQSYSSPLTF	1365
005S-C04	Fzd8	RASQSISSYLN	1098	AASSLQS	1175	CQQSYSTPLTF	1372
005S-D04	Fzd8	RASQSISSYLN	1098	AASSLQS	1175	CQQSYSTPLTF	1372
005S-G04	Fzd8	RASQSISSYLN	1098	AASSLQS	1175	CQQSYSTPLTF	1372
005S-A05	Fzd8	RASQSISSYLN	1098	AASSLQS	1175	CQQSYSTPLTF	1372
005S-C05	Fzd8	RASQGISNNLN	1072	RASSLQS	1247	CQQANSYPLTF	1320
005S-E05	Fzd8	RASQSISSYLN	1098	AASSLQS	1175	CQQSYSTPLTF	1372
005S-C06	Fzd8	RASQSVSSSYLS	1119	AASRRAT	1172	CQQYSNWPFTF	1424
005S-E06	Fzd8	QASQDISNRLN	1045	SASRLQI	1253	CQQSYRTPTF	1354
005S-G06	Fzd8	RASQSISSYLN	1098	AASSLQS	1175	CQQSYSTPLTF	1372
005S-H06	Fzd8	RASQGISNNLN	1072	DASTLQT	1193	CQQSFSAPIF	1342
005S-A08	Fzd9	RASQSISSKSLA	1097	GASTRAT	1217	CQQYGIAPTF	1407
005S-C08	Fzd9	RASQSISSYLN	1098	AASSLQS	1175	CQQSYSTPLTF	1372
005S-E08	Fzd9	RASQSISSYLN	1098	AASSLQS	1175	CQQSYSTPLTF	1372
005S-F08	Fzd9	QASQGISNYLN	1050	AASSLQS	1175	CQQTYSTPTTF	1395
005S-H08	Fzd9	RASQSISSYLN	1098	AASSLQS	1175	CQQSYSTPLTF	1372
005S-A09	Fzd9	RASQSIGSNLD	1085	RASTLQS	1249	CQQSYSTPSF	1375
005S-F09	Fzd9	RASQSISSYLN	1098	AASSLQS	1175	CQQSYSTPLTF	1372
005S-B10	Fzd9	RASQGISNNLN	1072	AASSLQS	1175	CQQGNNFPLTF	1327

Clone ID	Initial Binding	CDRL1	SID NO.	CDRL2	SID NO.	CDRL3	SID NO.
005S-C10	Fzd9	RASQDIGSFLA	1061	AASSLQS	1175	CQKYNRAPFTF	1310
005S-E10	Fzd9	RASQSISSYLN	1098	AASSLQS	1175	CQQSYSTPLTF	1372
005S-F10	Fzd9	RASQSISSYLN	1098	AASSLQS	1175	CQQSYSTPLTF	1372
005S-G10	Fzd9	RASQGISNNLN	1072	QASSLDS	1244	CQQSYNVPYTF	1352
005S-A11	Fzd9	RASQSISSNNLN	1092	DASTLKR	1192	CQQSYNTPRTF	1350
005S-B12	Fzd10	RASQSISSYLN	1098	AASSLQS	1175	CQQSYSTPLTF	1372
005S-D12	Fzd10	RASQSVSTSYLA	1123	GASTRAT	1217	CQQYGASPWTF	1406
006S-B01	Fzd10	RASQSISSYLN	1098	AASSLQS	1175	CQQSYSTPLTF	1372
006S-C01	Fzd10	RASQGISSALA	1076	SASNLQS	1252	CQQAISFPLTF	1315
006S-E01	Fzd10	RASQSVTSSLA	1124	GASTRAT	1217	CQQYNDWPPTF	1416
006S-G01	Fzd10	KSSQSVLYSSNNK NYLA	1037	STNTRSS	1257	CQHRNFF	1306
006S-B02	Fzd10	RASQSISSYLN	1098	AASSLQS	1175	CQQSYSTPLTF	1372
006S-G02	Fzd10	RASQSISSYLN	1098	AASSLQS	1175	CQQSYSTPLTF	1372
014S-A01	Fzd1	RASQSISSYLN	1098	AASSLQS	1175	CQQSYSTPLTF	1372
014S-C01	Fzd2	RSSQSLHSDGKT YLY	1134	LGSNRAS	1237	CMQNTHWPLTR	1293
014S-F01	Fzd4	RASQSISSYLN	1098	AASSLQS	1175	CQQSYSTPLTF	1372
014S-H01	Fzd4	RASQGISNNLN	1072	AASRLQS	1171	CQQSYSPPLTF	1363
014S-C03	Fzd7	RASQSISSYLN	1098	AASSLQS	1175	CQQSYSTPLTF	1372
014S-D03	Fzd7	RASQSISSYLN	1098	AASSLQS	1175	CQQSYSTPLTF	1372
014S-F03	Fzd8	RASQSISSYLN	1098	AASSLQS	1175	CQQSYSTPLTF	1372
014S-A04	Fzd8	RASQSISSYLN	1098	AASSLQS	1175	CQQSYSTPLTF	1372
014S-C04	Fzd8	RASQSISSYLN	1098	AASSLQS	1175	CQQSYSTPLTF	1372
014S-D04	Fzd8	RASQSISSYLN	1098	AASSLQS	1175	CQQSYSTPLTF	1372
014S-E05	Fzd8	RASQSISSYLN	1098	AASSLQS	1175	CQQSYSTPLTF	1372
014S-E06	Fzd10	RASQSISSYLN	1095	AASSLQS	1175	CLQHHSYPFTF	1275
027S-C02	Fzd5	QASQDISNYLN	1046	AASSLHT	1173	CQESYSSPYTF	1302
027S-E03	Fzd8	RASQSISSYLN	1098	AASSLQS	1175	CQQSYSTPYTF	1379
027S-F03	Fzd8	RASHDIGTFLA	1057	AASLQSL	1179	CQQSYRTPYTF	1355
027S-G03	Fzd8	QATQNIKKYLN	1051	KASTLES	1229	CQQSYSTPLTF	1372
027S-H03	Fzd8	RSSQSLHSHNGYN YLD	1138	LGSNRAS	1237	CMQALQTPQTF	1282
027S-A04	Fzd8	RASQSISSYLA	1093	AASNLQS	1169	CQQAYSFPQTF	1323
027S-B04	Fzd8	RASQAISNYLN	1059	AASSLQS	1175	CQQTFSPPLTF	1383
027S-C04	Fzd8	RASQGINNYLA	1067	QASNLQS	1243	CQQTYSSPLTF	1393
027S-D04	Fzd8	QASQDIDNYLN	1040	AASSLQS	1175	CQQSYSTPVTF	1377
027S-E04	Fzd8	RASQGIRNDLG	1069	AASLQSL	1179	CQQAYSFPWTF	1324

Clone ID	Initial Binding	CDRL1	SID NO.	CDRL2	SID NO.	CDRL3	SID NO.
027S-C05	Fzd8	RASQGIRNDLA	1068	AASSLQR	1174	CQQSYSKPTF	1359
027S-D05	Fzd8	QASQDISNYLN	1046	ASSTLQT	1182	CQQSYSAPYTF	1356
027S-E05	Fzd8	RASQGITKSLA	1077	AASNQLQ	1168	CQQYNTFPITF	1422
027S-F05	Fzd8	RASQSISTYLA	1100	GASTRAT	1217	CQQYGSSTPF	1411
027S-G05	Fzd8	RASQSISSYLN	1098	YASSLQN	1261	CQQSYSTPPTF	1368
027S-H05	Fzd8	RASQSIGTYLN	1088	DASNLET	1185	CQQANSFPLTF	1317
027S-A06	Fzd8	RASQGISNNLN	1072	KASSLES	1226	CQQANSFPITF	1316
027S-C06	Fzd8	RASQGVGDYLA	1078	DASNLQS	1186	CQQHNAYPLTF	1330
027S-D06	Fzd8	RASQDISSWLA	1064	KASTLES	1229	CQQSYGAPLTF	1348
027S-E06	Fzd8	RASQNVNDWLA	1082	SASNLQS	1252	CQQSYSTPPTF	1368
027S-F06	Fzd8	RASQSISSYLN	1098	GASNLQS	1207	CQQSYSTPLTF	1372
027S-G06	Fzd5	RASQSVNNTYVA	1111	GTSTRAT	1222	CQQYDTSPTF	1404
027S-H06	Fzd5	RASQSISSYLN	1098	AASSLQS	1175	CQQSYTTPPTF	1382
027S-A07	Fzd5	RASQSISTNVN	1099	AASSLQS	1175	CQQSYSTPYTF	1379
029S-B01	Fzd1	KSSQSVLHSSNNK NYLA	1498	STNTRSS	1257	CQQYYSTPPTF	1544
029S-D01	Fzd2	RASQSLSSWLA	1512	DASTLQS	1520	CQQAISFPLTF	1315
029S-C02	Fzd2	RASQDISNNLN	1500	GASHLQT	1522	CQQANSFPVTF	1318
029S-F02	Fzd2	RASQGISNYLA	1074	AASRLQT	1515	CLQYNTYPWTF	1528
029S-H02	Fzd2	RSSQSVLLHSNGYN YLD	1138	LGSSRAS	1525	CMQALQTPLTF	1281
030S-A02	Fzd7	RASQSISSYLN	1098	KASTLHN	1523	CQQAISFPLTF	1315
030S-B02	Fzd3	RASQSITTYLN	1511	KTSSLQS	1524	CQQGDSFPYTF	1531
029S-E03	Fzd3	RASQSISSYLN	1098	AASSLQT	1516	CQQSFRPLTF	1532
029S-G03	Fzd3	RASQSISSYLN	1509	AASSLQS	1175	CQQSWRFPYTF	1535
030S-E03	Fzd3	KSSQSVLYSSNNK NYLA	1037	WASTRES	1526	CQQYYSTPPTF	1545
029S-D05	Fzd3	RASQTISSYLN	1513	DASNLET	1185	CQQSYSIPLTF	1536
030S-H03	Fzd3	RASQGVSTYLA	1505	AASSLQS	1175	CQQYYSSPQTF	1543
029S-B06	Fzd3	RASQSVSSWLA	1120	AASSLQS	1175	CQQAIFRPPTF	1529
029S-E06	Fzd3	RASQNINSWLA	1506	AASSLQS	1175	CQQYYSFPLTF	1542
029S-H06	Fzd3	RASQSISSYLN	1098	AASSLQS	1175	CQQSHSTPLTF	1533
029S-G07	Fzd3	RASQSISKWLA	1510	GASTLQS	1215	CQQAYSFPWTF	1324
029S-H08	Fzd3	RASRTVYNFLA	1514	DASNLRT	1519	CQQSYSTPPTF	1537
029S-F09	Fzd3	RASQSIARYLN	1507	GASSLQS	1211	CQQSYNTPWTF	1351
030S-F04	Fzd3	RASQGIRNDLN	1502	DASNLGT	1518	CQQSSRIPPTF	1534
029S-A10	Fzd3	RASQGISKYLA	1503	AASSLQS	1175	CQQSYSTPWTF	1538
029S-B11	Fzd3	QASQDISNYLN	1046	GASALRS	1521	CQQTKSFPLTF	1540

Clone ID	Initial Binding	CDRL1	SID NO.	CDRL2	SID NO.	CDRL3	SID NO.
029S-D11	Fzd3	RASQDISRGLG	1501	AASTLYR	1517	CQQAYSPWTF	1324
030S-H05	Fzd7	RASQSIGNYLN	1508	AASSLQS	1175	CQQANSFPLTF	1530
030S-A06	Fzd7	RASQAIGRRLA	1499	AASSLQS	1175	CQQYDTYWTF	1541
029S-C12	Fzd7	RASQGISSYLA	1076	AASTLQS	1179	CLQYNTYPWTF	1528
030S-C06	Fzd7	RASQGISSWLA	1504	DASSLQS	1190	CQQSYSTPYSF	1539
4A12	Fzd5	KASQDVGTAVA	1497	WASTRHT	1527	QQYSTYPLT	1546
1791	Fzd7	KASENVLNYSVS	2199	GASNRYT	2202	GQSYRYP	2205
1291	Fzd7	KSSQSLLYSSNQ KNYLAW	2200	WASTRES	2203	QQYYSY	2206
18R5	Fzd7	SGDNIGS FYVH	2201	DKSNRPSG	2204	QSYA NTLSL	2207

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

Clone ID	HC SID NO	LC SID NO	Confirmed Binding
001S-B01	1	38	Fzd1,2,7,9
001S-E02	2	39	Fzd1,2,7
001S-G02	3	40	Fzd1,2,7
001S-H02	4	41	Fzd1,2,7
001S-A03	5	42	Fzd1,2,7,9
001S-B03	6	43	Fzd1,2,7
004S-G06	7	44	Fzd5,8
002S-B01	8		Fzd1
002S-C02	9		Fzd1
002S-E02	10		Fzd1
002S-G02	11		Fzd1
002S-F03	12		Fzd1
002S-A04	13		Fzd1
002S-B04	14		Fzd1
002S-D04	15		Fzd1
004S-H04	16	45	Fzd5
001S-A04	17	46	Fzd1,2,5,7,8

Clone ID	HC SID NO	LC SID NO	Confirmed Binding
003S-E07	18	47	Fzd2
003S-D10	19	48	Fzd4
004S-B08	20	49	Fzd6
004S-D08	21	50	Fzd6
004S-C09	22	51	Fzd6
004S-F10	23	52	Fzd6
004S-A11	24	53	Fzd6
004S-A12	25	54	Fzd6
005S-B07	26	55	Fzd9
005S-D08	27	56	Fzd9
005S-E09	28	57	Fzd9
005S-H10	29	58	Fzd9
005S-B11	30	59	Fzd9
005S-D11	31	60	Fzd9
014S-G02	32	61	Fzd6
014S-B04	33	62	Fzd8
014S-B06	34	63	Fzd9
014S-G06	35	64	Fzd10
014S-A07	36	65	Fzd10
017S-B09	37		Fzd8
004S-D01	66	67	Fzd4
004S-E09	68	69	Fzd6
anti-FZD7-1791	70	71	Fzd7
anti-FZD7-1291	72	73	Fzd7

Clone ID	HC SID NO	LC SID NO	Confirmed Binding
004S-B10			Fzd6
004S-C10			Fzd6
004S-F10			Fzd6
004S-G10			Fzd6
004S-A11			Fzd6
004S-B11			n.b.
004S-D11			Fzd6
004S-E11			n.b.
004S-F11			Fzd6
004S-G11			Fzd6
004S-A12			Fzd6
004S-B12			Fzd6
004S-C12			n.b.
004S-D12			n.b.
004S-F12			n.b.
004S-F12			n.b.
004S-G12			n.b.
005S-B02			n.b.
005S-C02			n.b.
005S-D02			Fzd5,8
005S-E02			Fzd5,8
005S-H02			Fzd5,8
005S-A03			Fzd5,8
005S-C03			n.s.
005S-E03			n.s.
005S-F03			Fzd8
005S-B04			Fzd5,8
005S-F04			n.b.

Clone ID	HC SID NO	LC SID NO	Confirmed Binding
005S-G04			Fzd5,8
005S-H04			n.b.
005S-E05			n.b.
005S-G05			Fzd5,8
005S-H05			Fzd5,8
005S-D06			Fzd8
005S-F06			n.b.
005S-G06			n.b.
005S-A07			Fzd9,10
005S-B07			Fzd9
005S-A08			Fzd9
005S-B08			Fzd9
005S-D08			Fzd9
005S-E08			Fzd9
005S-F08			n.b.
005S-C09			Fzd9
005S-D09			Fzd9
005S-E09			Fzd9
005S-F09			Fzd9
005S-A10			Fzd9
005S-B10			Fzd9
005S-E10			Fzd9
005S-H10			Fzd9
005S-B11			Fzd9
005S-D11			Fzd9
005S-G11			n.b.
005S-H11			n.b.
005S-E12			Fzd10

Clone ID	HC SID NO	LC SID NO	Confirmed Binding
006S-A01			Fzd10
006S-H01			n.b.
006S-A02			Fzd10
006S-D02			n.b.
006S-H02			Fzd10
006S-A03			n.b.
006S-B03			n.b.
006S-C03			n.b.
014S-A01			Fzd1,2,7
014S-B02			n.b.
014S-G02			Fzd6
014S-B03			n.b.
014S-C03			Fzd1,2,7
014S-A04			n.b.
014S-B04			Fzd8
014S-B05			Fzd5,8
014S-B06			Fzd9
014S-F06			n.s.
014S-G06			Fzd10
014S-A07			Fzd10
017S-E08			Fzd8
017S-H08			n.b.
017S-A09			n.b.
017S-B09			Fzd8
018S-F06			Fzd4
018S-H06			n.b.
018S-B07			n.b.
017S-A10			n.b.

Clone ID	HC SID NO	LC SID NO	Confirmed Binding
017S-B10			n.b.
017S-D10			n.b.
018S-H08			n.b.
018S-B09			Fzd5,8
021S-A01			n.b.
021S-E02			Fzd5,8
021S-G02			n.s.
021S-A03			n.b.
029S-B01			n.b.
029S-D01			Fzd1,2,7
029S-C02			Fzd1,2,7
029S-H02			Fzd1
030S-A02			Fzd7
029S-E06			Fzd2, 6, 3
030S-F04			Fzd3
030S-H05			Fzd7
030S-A06			Fzd1,2,7,5
029S-C12			Fzd7
030S-C06			Fzd1
001S-A01			Fzd1,2,7
001S-H01			Fzd1,2,7

In certain embodiments, the Fzd binding region may be selected from any binding domain that binds an Fzd receptor epitope with an affinity of, e.g., a K_D of at least about 1×10^{-4} M, at least about 1×10^{-5} M, at least about 1×10^{-6} M, at least about 1×10^{-7} M, at least about 1×10^{-8} M, at least about 1×10^{-9} M, at least about 1×10^{-10} M, at least about 1×10^{-11} M, at least about 1×10^{-12} M, at least about 1×10^{-13} M, at least about 1×10^{-14} M, or at least about 1×10^{-15} M. In certain embodiment, the Fzd binding region may be selected from any binding domain that

binds one or more Fzd receptor epitopes at high affinity, e.g., a K_D of less than about 1×10^{-7} M, less than about 1×10^{-8} M, less than about 1×10^{-9} M, less than about 1×10^{-10} M, less than about 1×10^{-11} M, less than about 1×10^{-12} M, less than about 1×10^{-13} M, less than about 1×10^{-14} M, or less than about 1×10^{-15} M.

- 5 In certain embodiment, the Fzd binding region may be selected from any binding domain that binds an Fzd receptor epitope at high affinity, e.g. a K_D of less than or equal to about 1×10^{-4} M, less than or equal to about 1×10^{-5} M, less than or equal to about 1×10^{-6} M, less than or equal to about 1×10^{-7} M, less than or equal to about 1×10^{-8} M, less than or equal to about 1×10^{-9} M, less than or equal to about 1×10^{-10} M, less than or equal to about 1×10^{-11} M, less than or equal to about 1×10^{-12} M, less than or equal to about 1×10^{-13} M, less than or equal to about 1×10^{-14} M, or less than or equal to about 1×10^{-15} M in the context of a Wnt surrogate molecule.

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

- In some embodiments, the Fzd binding region 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, and
 25 Fzd10. In some embodiments, the Fzd binding region binds to Fzd1, Fzd2, and Fzd 7. In some embodiments, the Fzd binding region binds to Fzd1, Fzd2, Fzd5, Fzd7, and Fzd8. In other embodiments the Fzd binding region 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
 30 more relative to other frizzled proteins.

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

In certain embodiments, the Fzd binding region comprises the six CDR regions of anti-FZD7-1791 or anti-FZD7-1291. Anti-FZD7-1791 and anti-FZD7-1291 are antibodies that bind to different epitopes within the hinge region of Fzd7, as described in PCT Patent Publication Nos. WO2016/205551 and WO2016/205566, herein specifically incorporated by reference. In certain embodiments, the Fzd binding region is an scFv comprising the six CDR regions of anti-FZD7-1791 or anti-FZD7-1291. For example, the CDR sequences of anti-FZD7-1791 include (i) a heavy chain CDR1 comprising TYAMH (SEQ ID NO:2190), a heavy chain CDR2 comprising RIRSKSNNYAKNYDDSVKD (SEQ ID NO:2193), and a heavy chain CDR3 comprising ENYGGRFDY (SEQ ID NO:2196), and (ii) a light chain CDR1 comprising KASENVLNYS (SEQ ID NO:2199), a light chain CDR2 comprising GASNRYT (SEQ ID NO:2202), and a light chain CDR3 comprising GQSYRYP (SEQ ID NO:2205). The heavy chain sequence of anti-FZD7-1791 includes SEQ ID NO:70 and the light chain sequence of anti-FZD7-

1791 includes SEQ ID NO:71. As another example, the CDR sequences of anti-FZD7-1291 include (i) a heavy chain CDR1 comprising SYAMS (SEQ ID NO:2191), a heavy chain CDR2 comprising TISDGGSYTRYDPDKLKG (SEQ ID NO:2194), and a heavy chain CDR3 comprising VGGRRDYFDY (SEQ ID NO:2197), and (ii) a light chain CDR1 comprising KSSQSLLYSSNQKNYLAW (SEQ ID NO:2200), a light chain CDR2 comprising WASTRES (SEQ ID NO:2203), and a light chain CDR3 comprising QQYYSTP (SEQ ID NO:2206). The heavy chain sequence of anti-FZD7-1291 includes SEQ ID NO:72 and the light chain sequence of anti-FZD7-1291 includes SEQ ID NO:73. In some embodiments, In particular embodiments, the Fzd binding region is an antibody or derivative thereof, including without limitation scFv, minibodies, VHH or sdAbs and various antibody mimetics comprising any of these CDR sequences. In certain embodiments, these CDR sequences comprise one or more amino acid modifications.

In other embodiments, the Fzd binding region comprises a variable region sequence, or the CDRs thereof, from any of a number of frizzled specific antibodies, which are known in the art and are commercially available, or can be generated de novo. Any of the frizzled polypeptides can be used as an immunogen or in screening assays to develop an antibody. Non-limiting examples of frizzled binding domains include antibodies available from Biolegend, e.g., Clone CH3A4A7 specific for human frizzled 4 (CD344); Clone W3C4E11 specific for human Fzd9 (CD349); antibodies available from Abcam, e.g., ab64636 specific for Fzd7; ab83042 specific for human Fzd4; ab77379 specific for human Fzd7; ab75235 specific for human Fzd8; ab102956 specific for human Fzd9; and the like. Other examples of suitable antibodies are described in, inter alia, U.S. Patent Application No. 20140105917; U.S. Patent Application No. 20130230521; U.S. Patent Application No. 20080267955; U.S. Patent Application No. 20080038272; U.S. Patent Application No. 20030044409; etc., each herein specifically incorporated by reference.

The Fzd binding region of a Wnt surrogate molecule may be an engineered protein that is selected for structural homology to the frizzled binding region of a Wnt protein. Such proteins can be identified by screening a structure

database for homologies. The initial protein thus identified, for example the microbial Bh1478 protein. The native protein is then engineered to provide amino acid substitutions that increase affinity, and may further be selected by affinity maturation for increased affinity and selectivity in binding to the desired frizzled protein. Non-limiting examples of frizzled binding moieties include the Fz27 and Fz27-B12 proteins.

In particular embodiments, a Wnt surrogate molecule comprises an LRP5/6 binding region, e.g., an anti-LRP5/6 antibody, or antigen-binding fragment thereof, fused to a polypeptide that specifically binds to one or more Fzd receptor epitopes. In particular embodiments, the polypeptide that specifically binds to LRP5/6 is an antibody or antigen-binding fragment thereof. If certain embodiments, it is an antibody or antigen-binding fragment thereof disclosed in the U.S. Provisional Patent Application No. 62/607,879, titled, "Anti-LRP5/6 Antibodies and Methods of Use," Attorney Docket No. SRZN-005/00US, filed on December 19, 2017, which is incorporated herein by reference in its entirety.

In particular embodiments, at least one LRP5/6 binding region of a Wnt surrogate molecule includes one or more antigen-binding fragments of an antibody. For example, the one or more antigen-binding fragments may be or be derived from an IgG, scFv, Fab, or VHH or sdAb. In certain embodiments, the one or more antigen-binding fragments are humanized.

In particular embodiments, the LRP5/6 binding region comprises the three heavy chain CDRs and/or the three light chain CDRs disclosed for any of the illustrative antibodies or fragments thereof that bind to LRP5 and/or LRP6 provided in Table 2A. In particular embodiments, the LRP5/6 binding region comprises the three heavy chain CDRs and/or the three light chain CDRs disclosed for any of the illustrative antibodies or fragments thereof that bind to LRP5 and/or LRP6 provided in Table 2A, wherein the CDRs collectively comprise one, two, three, four, five, six, seven, or eight amino acid modifications, e.g., substitutions, deletions, or additions. In certain embodiments, the LRP5/6 binding region is a VHH or sdAb or was derived from a VHH or sdAb, so Table 2A only includes the three heavy chain CDRs. In certain embodiments, the LRP5/6 binding region comprises the three

heavy chain CDRs shown in Table 2A or variants wherein the CDRs collectively comprise one, two, three, four, five, six, seven or eight amino acid modifications. In particular embodiments, the LRP5/6 binding region comprises the heavy chain fragment and/or light chain fragment of any of the illustrative antibodies or fragments thereof that bind to LRP5 and/or LRP6 provided in Table 2B or SEQ ID NOs:74-97 (or an antigen-binding fragment or variant of either). In certain embodiments, the LRP5/6 binding region is a Fab or was derived from a Fab, so Table 2B includes VH and CH1 sequence, but not CH2 or CH3 sequences. In certain embodiments, the LRP5/6 binding region is a VHH or sdAb or was derived from a VHH or sdAb, so Table 2B includes the VHH domain. In certain embodiments, the LRP5/6 binding region is a polypeptide, e.g., an antibody or antigen-binding fragment thereof, that competes with one of these antibodies for binding to LRP5 and/or LRP6.

In particular embodiments, the LRP5/6 binding region includes an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99% identity to any of the sequences set forth in Table 2A, Table 2B, or SEQ ID NOs:74-97, or an antigen-binding fragment thereof. Binding characteristics for clones listed in Table 2B were determined and are shown in Table 2B.

Table 2A: Anti-LRP5/6 Antibody Clone IDs and CDR sequences.

Clone ID	Confirmed Binding	CDRH1	SID NO.	CDRH2	SID NO.	CDRH3	SID NO.
001S-C08	LRP6e1e2	YTISNYYIH	1682	GMINPSGGSTTYA	1762	CAIVRGKKWYFDLW	1842
001S-C10	LRP6e1e2	RTFGTYPNG	1632	AAISWGGRTAYA	1700	CYARTVIGGFGAFRAHW	2061
001S-D10	LRP6e1e2	RTFSRYAMA	1642	AAIRWSGGGTYA	1689	CAASMEAMNSLRVNKERYQ SW	1836
001S-E10	LRP6e1e2	LTFSNAAMA	1614	AAISRSGANTAYS	1696	CTLVNEIKTWW	2039
001S-F10	LRP6e1e2	RTFSSYAMA	1645	AAIKWSGTNTTYA	1684	CAASMEAMNSLRVNKERYQ SW	1834
001S-G10	LRP6e1e2	RTFSRYVMG	1644	AAITWRGGSTTYA	1706	CATGPNSIY	1987
001S-A11	LRP6e1e2	RTFGNYDMG	1630	AGIRWSGSTLYA	1709	CYARTVIGGFGAFRAHW	2062
001S-B11	LRP6e1e2	RRFTTYGMG	1623	AAVTWRSGSTTYA	1708	CAAGSTVVAEFNYW	1828
001S-C11	LRP6e1e2	SISSFNTMG	1659	AVITTTGGDTSYS	1741	CNKVNATKL	2025
001S-E11	LRP6e1e2	RTLRYSMG	1651	AAISRSGDRIYYS	1697	CTLVNEIKTWW	2040

Clone ID	Confirmed Binding	CDRH1	SID NO.	CDRH2	SID NO.	CDRH3	SID NO.
001S-F11	LRP6e1e2	RTFSSYAMS	1646	AVIGRSGGIKYA	1736	CATRRPFNSYNTEQSYDSW	1989
001S-G11	LRP6e1e2	SIFRLGTMY	1655	ASIGKSGSTNYA	1719	CKQHPNGYR	2005
001S-H11	LRP6e1e2	RTLSSFAMG	1652	ATISRSGGNTYYA	1732	CNLREWNNSGAGYW	2026
001S-A12	LRP6e1e2	IAFRYYDMG	1608	AAITWNGRSSDYA	1704	CAAVFTGRFYGRPPREKYDYW	1838
001S-B12	LRP6e1e2	RLLSYALA	1622	AAISRNGDKSHYS	1694	CTLVNEIKTWW	2038
001S-C12	LRP6e1e2	RTFSNYAVG	1641	AAISRFGGSTYYV	1693	CAADRIENYLGRYYDPSEYEW	1824
001S-D12	LRP6e1e2	RTFSRYAMG	1643	GAISRSGNNTYYA	1744	CTLVNEIKTWW	2041
001S-F12	LRP6e1e2	RTFRSYTMG	1637	AAISGSGGSTTYA	1690	CNADIKTTTYSPLRNYW	2009
008S-B01	LRP5	TIFSINTMG	1664	ATMTSGGNTNYA	1734	CYRRQWASSWGARNYEYW	2064
008S-C01	LRP5	NINSIETLG	1617	ANMRGGGYMKYA	1716	CHGRDYGSNAPQYW	2001
008S-D01	LRP5	NINSIETLG	1617	ANMRGGGYMKYA	1716	CYVKLRDDDDVYR	2065
008S-E01	LRP5	NINSIETLG	1617	ANMRGGGYMKYA	1716	CNAVTYNGYTIR	2023
008S-G01	LRP5	NINSIETLG	1617	ANMRGGGYMKYA	1716	CYARTQRMGVVNSYW	2060
008S-A02	LRP5	NINSIETLG	1617	ANMRGGGYMKYA	1716	CNAVTFGGNTIR	2021
008S-C02	LRP5	NINSIETLG	1617	ANMRGGGYMKYA	1716	CNAVTYDGY	2022
008S-D02	LRP5	NINSIETLG	1617	ANMRGGGYMKYA	1716	CAAQFRNDYGLRYQSTNNYW	1832
008S-E02	LRP5	NINSIETLG	1617	ANMRGGGYMKYA	1716	CNANYRGNRYW	2019
009S-C01	LRP6e3e4	GSFSGYYWT	1595	GEINHSGATNYN	1745	CVRYAWPEFDHW	2053
009S-B02	LRP6e3e4	GSLSGYYWS	1596	GEINHSGSTNYN	1746	CVRYAWPEFDHW	2055
009S-C02	LRP6e3e4	GSFSDYYWS	1594	GEINHSGSTNYN	1746	CVRYAWPEFDHW	2054
009S-D02	LRP6e3e4	GTFSSYALS	1603	GGIPIFGTANYA	1749	CVYGRDFDYW	2056
010S-A02	LRP6e1e2	HTFSSYAMG	1607	AAISQSGYVRYA	1691	CKIYGLNGQPLGSW	2003
010S-B02	LRP6e1e2	RTFNSGTMG	1634	AAITWRGGITYA	1705	CNADGYSWDGRSGRRLELW	2008
010S-D02	LRP6e1e2	RTFSSYAVG	1647	AAISYSGGSTKYA	1702	CAASVYISRRDSYGYW	1837
010S-E02	LRP6e1e2	LSSGRPFSSYVM G	1612	AAISWSGGSTKYA	1701	CKLQVRPIGYSSAYSRYW	2004
010S-F02	LRP6e1e2	RSFNSYVIG	1625	AAIRWSGDNTYYA	1688	CAASMEAMNSLRVNKERYQ SW	1835
009S-E02	LRP6e1e2	RRFTTYGMG	1623	AAVTWRSGSTYYA	1708	CAAGSTVVAEFNYW	1829
009S-F02	LRP6e1e2	RTFSYYAMG	1649	AAISRSGGIYYA	1698	CNTRVPLLAW	2029
009S-G02	LRP6e1e2	SIFSIYAMG	1658	AVITSGGKTVYA	1740	CYADSRSSWYDEYLEHW	2058
009S-H02	LRP6e1e2	SIVRSLPMA	1660	ATINDAQRYA	1727	CNTSPYMHDVW	2027
009S-A03	LRP6e1e2	RTFSVYGVG	1648	AAVSASGGYTWYA	1707	CKAAPRWGGATAYW	2002
010S-G02	LRP6e1e2	SIVRSLPMA	1660	ATINDAQRYA	1727	CNTSPYMHDVW	2028
010S-A03	LRP6e1e2	RTFRRYAMG	1636	ATISASGGNTAYA	1731	CNAPAWLYDDDYW	2020
009S-B03	LRP6e1e2	RTFSNYAVG	1641	AAISRFGGSTYYV	1693	CAADRIENYLGRYYDPSEYEW	1825
010S-B03	LRP6e1e2	RTFSNYAVG	1641	AAISRFGGSTYYA	1692	CHAKQLRNGQMYTYW	1999
010S-D03	LRP6e1e2	ISSVYGMG	1609	AAIQWSADNTFYA	1686	CAARTSGGLFHYRRSDHWD TW	1833

Clone ID	Confirmed Binding	CDRH1	SID NO.	CDRH2	SID NO.	CDRH3	SID NO.
009S-C03	LRP6e1e2	LPFSRYAMA	1610	AGMSGEGRNTKYR	1713	CSSRGYW	2034
009S-D03	LRP6e1e2	SIFSDGAMG	1656	AVISGGRTGYA	1737	CNTYFPPIYKKGYPFW	2030
009S-E03	LRP6e1e2	RRFTTYGMG	1623	AAVTWRSGSTYYA	1708	CAAGSTVVAEFNYW	1830
009S-F03	LRP6e1e2	RTFSSYAMS	1646	AVIGRSGGIKYA	1736	CATRRPFSYNTEQSYDSW	1990
010S-E03	LRP6e1e2	RSVSIYPMG	1628	AAINWSGDSTKYA	1685	CNAVVVGLSRRIDNIW	2024
010S-F03	LRP6e1e2	RTFSRYVMG	1644	AAITWRGGSTYYA	1706	CATGPNSIY	1988
009S-G03	LRP6e1e2	RSVSSYNMG	1629	AAISRRGGIIEYG	1695	CHAVENILGRFVDYW	2000
009S-H03	LRP6e1e2	SIFSINTMG	1657	AVITSGGKTYA	1740	CYADSRSSWYDEYLEHW	2057
009S-A04	LRP6e1e2	RTLSAYDMG	1650	GGIRWSGGTTLYP	1753	CYARTVIGGFGAFRAHW	2063
009S-B04	LRP6e3e4	SIFMINTMA	1654	ATIRPVVSETTYA	1728	CNAKRPWGTRDEYW	2018
010S-G03	LRP6e3e4	RSFNSYTTT	1624	AAIRGSSGSTFYA	1687	CNAASTVTAWPYGPDYW	2006
009S-C04	LRP6e3e4	FRFSISTMG	1553	AYITGGGRTMDG	1743	CNAFVRSDFDYDYW	2011
009S-D04	LRP6e3e4	TIVSIYRIN	1665	AGITSSGRTIYA	1712	CNAASTVTAWPYGPDYW	2007
010S-H03	LRP6e3e4	RIFSIYDMG	1621	SGIRWSGGTSYA	1789	CSSRGYW	2035
009S-E04	LRP6e3e4	RIFAIYDIA	1618	AMIRPVVTEIDYA	1715	CNAKRPWGSRDEYW	2012
010S-A04	LRP6e3e4	SLFSFNAV G	1662	ASISSGGRTNYA	1722	CSKGGVYGGTYVPDSW	2032
009S-F04	LRP6e3e4	RSLSFAMG	1627	ARISRGDGYTDEA	1718	CAAVQAVIGGTLTAYDYW	1839
010S-B04	LRP6e3e4	RVLSYYAMA	1653	AGITRGGATTYY S	1711	CAAGPNWSTRNREYDYW	1827
009S-G04	LRP6e3e4	GTFSRYHMG	1601	SAITWSGGRTYYA	1788	CALTWAPTPTNRRSDYAYW	1872
009S-H04	LRP6e3e4	RIFAIYDMA	1619	ATIRPVVSETTYA	1728	CNAKRPWGTRDEYW	2017
010S-C04	LRP6e3e4	SLFSFNAMG	1661	ASISSGSRTNYA	1723	CSKGGVYGGTYVPDSW	2033
010S-D04	LRP6e3e4	RIFAIYDIA	1618	ATIRPVVTQIDYA	1730	CNAKRPWGSRDEYW	2015
010S-E04	LRP6e3e4	RTFGSDVMG	1631	ALTGWGDGSTTYE	1714	CAAARRSGTYDIGQYLRESAYV FW	1820
010S-F04	LRP6e3e4	RTFSRYAMG	1643	AAITRSGSNTYYA	1703	CAADPRGVTLPRTAYEYW	1823
009S-A05	LRP6e3e4	RTFSDYSMG	1639	AGISWIADNRYA	1710	CTAGRSRYLYGSSLNGPYDYW	2036
010S-G04	LRP6e3e4	VIFALYDIA	1666	ATIRPVVTETDYA	1729	CNAKRPWGSRDEYW	2014
010S-H04	LRP6e3e4	RSFSDFFMG	1626	ATISWSGSSANYE	1733	CAAAYSYSQYSSSYW	1821
010S-A05	LRP6e3e4	LSFSSYAMG	1611	AAISRSGVSTYYA	1699	CAAKFGVLATTESRHDYW	1831
010S-C05	LRP6e3e4	RTFNIDDMG	1633	ASIRWSGQSPYYA	1720	CNAETYSGNTIW	2010
010S-D05	LRP6e3e4	RTFSDYSMA	1638	AGISWIADNRYA	1710	CAGDRSRYLYGDSLPGPYGYW	1841
010S-E05	LRP6e3e4	SVFTTFAKG	1663	ASITASSDRTFYA	1725	CAAYSTFNTDVASMKPDYW	1840
010S-F05	LRP6e3e4	RIFSIYDIA	1620	ATIRPVVTETDYA	1729	CNAKRPWGSRDEYW	2013
013S-G04	LRP6e3e4	RIFAIYDIA	1618	ATIRPVVSETTYA	1728	CNAKRPWGTRDEYW	2016
013S-H04	LRP6e3e4	RTFSMYDMG	1640	ASIRWSSGNTWYA	1721	CYANIYYTRRAPEEYW	2059
013S-A05	LRP6e3e4	RTFNTYAMG	1635	ASVSWRYDRTYT	1726	CAADTNWRAGPRVGIDEYAY W	1826
013S-B05	LRP6e3e4	FAFSTTAMS	1549	STINPGGLSKSYA	1806	CTKGGIQ	2037
013S-C05	LRP6e3e4	NIFPIDDMS	1616	ATVTSGGRINYA	1735	CNVDRTLYGKYKEYW	2031

Clone ID	Confirmed Binding	CDRH1	SID NO.	CDRH2	SID NO.	CDRH3	SID NO.
013S-D05	LRP6e3e4	RIFSIYDMG	1621	SGIRWSGGTSYA	1789	CGSRGYW	1998
013S-E05	LRP6e3e4	YTFTYRYLH	1681	GGIPIFGTADYA	1748	CARDWELYGMDVW	1907
013S-F05	LRP6e3e4	GTFSSYAIS	1603	GIINPSGGSTSYA	1761	CARAGYYDSSGYAFDIW	1882
013S-G05	LRP6e3e4	YTFTYRYLH	1681	GGVIPIFGTADYA	1755	CASDIVVDDAFDTW	1969
010S-G06	LRP6e3e4	FSFETYGMS	1555	SGISGSGGRTHYA	1792	CARDLDYW	1897
009S-B05	LRP6e3e4	FTFDAYAMH	1560	STLSGDANNAYYA	1811	CARGGSGWSNYYGMDVW	1931
009S-C05	LRP6e3e4	YTFTYRYLH	1681	GRIIPVLKITNYA	1768	CAVVDDAFDIW	1996
009S-D05	LRP6e3e4	FTLRNHWLS	1591	SAISGSGGSTYYA	1786	CATRTGYSYGFNFWAFDIW	1991
009S-E05	LRP6e3e4	YTFTNNFMH	1676	GHVDPGDGETIYA	1756	CARDWGIAAAGDYYYYGMDVW	1908
009S-F05	LRP6e3e4	FTFDDYGMS	1561	SAIGTGGGTYA	1781	CARLGSGSPYYYYGMDVW	1959
009S-G05	LRP6e3e4	FTFSDYYMS	1568	SGVSWNGSRTHYA	1799	CAKDSGLV	1852
009S-C06	LRP6e3e4	YTFASYDIH	1671	GWMNPNSGNTGYA	1776	CARATGSGWYTDLGW	1883
009S-D06	LRP6e3e4	FTFSSHSTH	1573	STISDTNSGTYA	1807	CAKAQATGWSGYTDFDYW	1843
009S-E06	LRP6e3e4	FTFTDYGLH	1587	AVISYGGSNKYYA	1739	CASGYSYGLYYYGMDVW	1973
009S-F06	LRP6e3e4	YTFTYRYLH	1681	GGIPIFGTANYA	1749	CATEAALDAFDIW	1985
009S-G06	LRP6e3e4	YIFTDYMH	1669	GWINPNSSGGTNYA	1774	CARDFLGSTGDYW	1892
009S-H06	LRP6e3e4	FTFSSSAMH	1574	SAIGTGGGTYA	1783	CAKGGDYFYYYYGMDVW	1856
009S-A07	LRP6e3e4	YTFTYRYLH	1681	GGIPIFGTANYA	1749	CATAYGSSSLNIDYW	1980
009S-B07	LRP6e3e4	YTFTGYMH	1675	GWINPNSSGGTNYA	1774	CVKDGGSFPLAYAFDIW	2049
009S-D07	LRP6e3e4	FPFRYMGMS	1551	ARIGWNGGSIVYA	1717	CARDYSDRSGIDYW	1910
009S-F07	LRP6e3e4	GTFSSYAIS	1603	GIINPSGGSTSYA	1761	CARAAGNFWSGYTFDYW	1876
009S-G07	LRP6e3e4	YTFTYRYLH	1681	GGIPIFGTANYA	1749	CARGSYGMDVW	1947
009S-H07	LRP6e3e4	YTFTGYMH	1675	GWMNPNSGNTGYA	1776	CASSVVPAGPAGVYAFDIW	1975
009S-A08	LRP6e3e4	GTFSSHAIN	1602	GWISANNNGNTDYA	1775	CARDQDYGDYGWYYYGMDVW	1902
011S-C01	LRP6e3e4	LTFTSHGMS	1615	SYVSDSGSSVYYA	1818	CARHPGSFGGYSAWYYYGMDVW	1956
009S-C08	LRP6e3e4	FSFNTFGIH	1556	AVISYDGSNKYYA	1738	CAKSIAAAGTGYGMDVW	1868
009S-D08	LRP6e3e4	YTFTSYDIN	1679	GGIPIFGTANYA	1749	CARGPYDFDYW	1939
011S-F01	LRP6e3e4	FSFSDYYMS	1558	SGISESGGRTYYA	1790	CASAADF DYW	1966
009S-E08	LRP6e3e4	YGFTGYYIH	1668	GWMNPNSGNTGYA	1776	CARGYGDYDLW	1951
009S-F08	LRP6e3e4	DTFANYGFS	1547	GXVNAGNGNTTYA	1777	CAKGWLD FDYW	1866
009S-G08	LRP6e3e4	FTFSDFAMT	1566	SYISGDSGYTNYA	1813	CARLGSPGPYYYMDVW	1961
009S-H08	LRP6e3e4	YTFTDYFMN	1673	GIINPSGDSTRFA	1758	CARDDGLGGMDVW	1888
009S-A09	LRP6e3e4	YTFTYRYLH	1681	GRIIPILGSTNYA	1767	CTTDLWDYW	2047
011S-F02	LRP6e3e4	FTFSTYGMH	1584	SSISVSSGTTTHYA	1804	CARGGSGSYAFDIW	1929

Clone ID	Confirmed Binding	CDRH1	SID NO.	CDRH2	SID NO.	CDRH3	SID NO.
011S-G02	LRP6e3e4	YTFTSYAMN	1678	GGIPIFGTANYA	1749	CARDASGGSTGWYFDSW	1886
011S-A03	LRP6e3e4	FTFSSYWMH	1580	STISGSGGRTYYA	1808	CATSPYGVFTLDYW	1993
011S-C03	LRP6e3e4	YTFSYRYLH	1672	GGIPIFGTANYA	1749	CASTVTDAFDIW	1977
011S-D03	LRP6e3e4	FSFDDYGMS	1554	SVISSGGTIYYA	1812	CARHLSSGYLSYYGMDVW	1954
011S-F03	LRP6e3e4	FTFSSYAMS	1577	SAISGSGGSTYYA	1786	CAKGGRDGYKGYFDYW	1859
011S-C04	LRP6e1e2	GTFNSNAIS	1598	GWMNPNSGNTGY A	1776	CARDYYGSGSYNYGMDVW	1912
011S-D04	LRP6e1e2	YTFTSYDIN	1679	GIINPSGGSTSYA	1761	CAREAYYYYYGMDVW	1915
011S-H04	LRP6e1e2	YIFTDYYMH	1669	GRIIPILGRANYA	1765	CARGGYSTLDYW	1932
008S-F02	LRP5	YTFTNYCMH	1677	GIINPSDGGSTSHA	1757	CAKDMVHLIVALAIYW	1851
010S-C06	LRP6e1e2	FTFNSYSMD	1563	SSISPRGGSTYYA	1802	CAPYYDKSAKPLRSYFDHW	1875
010S-E06	LRP6e3e4	LTVSSNYMS	1615	SGISWNSGSIGYA	1796	CARGSDCSGGSCYYSFDYW	1944
010S-F06	LRP6e3e4	FTFSSSWMH	1575	SAIGTGGGTYYA	1781	CAREVAVKDYYYYYMDVW	1921
010S-H06	LRP6e3e4	YTFTSYDIN	1679	GRIIPILGRNTYA	1766	CAREERGATGRAFDIW	1918
010S-A07	LRP6e3e4	FTFSSYAMH	1576	ASISSTSGSKYYA	1724	CAKTYDFWSGYTFDYW	1870
010S-B07	LRP6e3e4	FTFSDYYMS	1568	SMISYNGGRAFYA	1800	CARGNPYYFDYW	1937
010S-C07	LRP6e3e4	FTFSKTDH	1569	STITDSTRGTYYA	1810	CAKGGDYYYYYYMDVW	1858
010S-D07	LRP6e3e4	YTFTYRYLH	1681	GGIPIFGTANYA	1749	CANGLEDAYAFDIW	1873
009S-D05	LRP6e3e4	FTLRNHWLS	1591	SAISGSGGSTYYA	1786	CATRTGYSYGFNFWAFAFDIW	1992
009S-E05	LRP6e3e4	YTFTNNFMH	1676	GHVDPGDGETIYA	1756	CARDWGIAAAGDYYYYYGMDV W	1909
010S-E07	LRP6e3e4	YTFTYRYLH	1681	GGIPIFGTANYA	1749	CAKDDFSLYGMDVW	1845
009S-F05	LRP6e3e4	FTFDDYGMS	1561	SAIGTGGGTYYA	1781	CARLGSYGSPPYYGMDVW	1960
010S-F07	LRP6e3e4	YTFTYRYLH	1681	GGIPIFGTANYA	1749	CARLDYGETEGNGDW	1958
010S-G07	LRP6e3e4	FTFSSYAMH	1576	STISGSGGSTYYA	1809	CARAGYGRYYGMDVW	1880
009S-G05	LRP6e3e4	FTFSDYYMS	1568	SGVSWNGSRTHYA	1799	CAKDSGLV	1853
010S-H07	LRP6e3e4	YTFTYRYLH	1681	GGIPIFGTANYA	1749	CARDDSMGAFAFDIW	1890
010S-A08	LRP6e3e4	HTFLTYDIN	1606	GRITPRLGIANYA	1770	CASYFGVMDVW	1979
009S-A07	LRP6e3e4	YTFTYRYLH	1681	GGIPIFGTANYA	1749	CATAYGSSSLNIDYW	1981
009S-B07	LRP6e3e4	YTFTGYMH	1675	GWINPNSGGTNYA	1774	CVKDGGSFPLAYAFDIW	2050
009S-B06	LRP6e3e4	YTFTYRYLH	1681	GGIPIFGTANYA	1749	CAPALTDAGSFDYW	1874
010S-B08	LRP6e3e4	YTFTYRYLH	1681	GGIIPVFGTADYA	1751	CARDREQQILDYW	1904
010S-C08	LRP6e3e4	FTFSTFGMH	1582	STITSSGGSTYYA	1809	CARAGIAAAGSRNYYGMDV W	1878
009S-C06	LRP6e3e4	YTFASYDIH	1671	GWMNPNSGNTGY A	1776	CARATGSGWYTDLGYW	1884
009S-D06	LRP6e3e4	FTFSSHSTH	1573	STISDTNSGTYYA	1807	CAKAQATGWSGYTFDYW	1844
010S-D08	LRP6e3e4	FTFSSSWMH	1575	SAIGTGGGTYYA	1781	CAKEDYDSSGYYYYYFQHW	1855
009S-E06	LRP6e3e4	FTFTDYGLH	1587	AVISYGGSNKYA	1739	CASGYSYGLYYGMDVW	1974

Clone ID	Confirmed Binding	CDRH1	SID NO.	CDRH2	SID NO.	CDRH3	SID NO.
010S-E08	LRP6e3e4	YSFTRTDMH	1670	GYISAYTGHTSYA	1778	CARDLGGTADYW	1898
010S-F08	LRP6e3e4	LTFDDHAMH	1613	SYISSSGRTIFYA	1815	CVRGDSGWGILYYVMDVW	2052
009S-F06	LRP6e3e4	YTFTYRYLH	1681	GGIIPFGTANYA	1749	CATEAALDAFDIW	1986
010S-G08	LRP6e3e4	YIFTDYYMH	1669	GGFDPEDGETIYA	1747	CARGGGPNEHDYFDYW	1927
010S-H08	LRP6e3e4	FTFZNAWMS	1590	SGISGSGGSTYYA	1793	CARGRGKKNYYYGMDVW	1942
010S-A09	LRP6e3e4	FTFSTYYMS	1586	SGISWNGGKTHYV	1794	CARGGDFDYW	1925
010S-B09	LRP6e3e4	GTFSSYAIS	1603	GWINPNSGDTNYA	1773	CARGEQWLWVGFDPW	1924
009S-G06	LRP6e3e4	YIFTDYYMH	1669	GWINPNSGGTNYA	1774	CARDFLGSTGDYW	1893
010S-C09	LRP6e3e4	YTFTYRYLH	1681	GGIIPFGTANYA	1749	CARDEVEGGMDVW	1891
009S-H06	LRP6e3e4	FTFSSSAMH	1574	SAIGTGGSTYYA	1783	CAKGGDYFYYYGMDVW	1857
010S-D09	LRP6e3e4	GTFSSYTIS	1603	GGIVPAYRRANYA	1754	CAKGGYELDYW	1865
010S-E09	LRP6e3e4	GDLSIYTIN	1593	GWINAGNGNTTYA	1772	CARGGDSSGYYYAFDIW	1926
009S-A07	LRP6e3e4	YTFTYRYLH	1681	GGIIPFGTANYA	1749	CATAYGSSSLNIDYW	1982
009S-B07	LRP6e3e4	YTFTGYMH	1675	GWINPNSGGTNYA	1774	CVKDGGSFPLAYAFDIW	2051
009S-D08	LRP6e3e4	YTFTSYDIN	1679	GGIIPFGTANYA	1749	CARGPYFDYW	1940
010S-F09	LRP6e3e4	FTFDEYAMH	1562	STISGSGGSTYYA	1809	CASAKNDFWSGYFAFDYW	1968
010S-G09	LRP6e3e4	GTFNTHIT	1599	GWMNPNSGNTGY A	1776	CARGNLDFDYW	1936
010S-H09	LRP6e3e4	FTFSDHYMS	1567	SAISSGSDRTYYA	1787	CARYSGYDFDYW	1965
010S-A10	LRP6e3e4	FSFSSYSMN	1559	SYISSSSSTIYYA	1816	CARGSGYGPYGGMDVW	1946
009S-D07	LRP6e3e4	FPFRYYGMS	1551	ARIGWNGGSIVYA	1717	CARDYSDRSIDYW	1911
010S-B10	LRP6e3e4	FAFKDYMT	1548	SAIGAGGGTYA	1779	CARESALYSSSWYYYGMDV W	1920
010S-C10	LRP6e3e4	FTFSSYAMS	1577	SAISGSGGSTYYA	1786	CAKGGRDGYKGYFDYW	1860
009S-E07	LRP6e3e4	YTFTGYIH	1674	ZHVPEDGETIYA	1819	CARGPAAIGILGWFDPW	1938
010S-D10	LRP6e3e4	YIFTDYYMH	1669	GWMNPNSGNTGY A	1776	CARTLSGYSSSWYVFDYW	1964
010S-E10	LRP6e3e4	FTFSSYSMN	1579	SGISWNSGTTGYS	1797	CARDHSSGWRHYFDYW	1895
010S-F10	LRP6e3e4	FTFSNSDMN	1570	SYISGNSGYTNYA	1814	CASGSYYSDFDYW	1971
010S-G10	LRP6e3e4	GTFSSYAIS	1603	GRINPNGGGTIYA	1769	CAREGGYFDYW	1919
009S-F07	LRP6e3e4	GTFSSYAIS	1603	GIINPSGGSTSYA	1761	CARAAGNFWSGYTFDYW	1877
009S-G07	LRP6e3e4	YTFTYRYLH	1681	GGIIPFGTANYA	1749	CARGSYGMDVW	1948
010S-H10	LRP6e3e4	YTFTSYMH	1680	GWINPNSGGTNYA	1774	CAREAAEIPVGAFDIW	1914
010S-A11	LRP6e3e4	FTFSNSDMN	1570	SYISGNSGYTNYA	1814	CASGSYYSDFDYW	1972
010S-B11	LRP6e3e4	FTFRNYAIH	1564	SAIGTGGDTYYA	1780	CARDGGIRDFDYW	1894
010S-C11	LRP6e3e4	YTFTYRYLH	1681	GGIIPFGTANYA	1749	CAADDLGLLEHYW	1822
009S-H07	LRP6e3e4	YTFTGYMH	1675	GWMNPNSGNTGY A	1776	CASSVVPAGPAGVYAFDIW	1976
009S-A08	LRP6e3e4	GTFSSHAIN	1602	GWISANNGNTDYA	1775	CARDQDYGDYGWYYYGMDV W	1903

Clone ID	Confirmed Binding	CDRH1	SID NO.	CDRH2	SID NO.	CDRH3	SID NO.
010S-D11	LRP6e3e4	YTFTYRYLH	1681	GGIIPVFGTANYA	1752	CATDEYSSSYAFDIW	1983
010S-E11	LRP6e3e4	FTFSAHGMH	1565	SGISESGGSTYYA	1791	CARGRGYSYGYAFDIW	1943
010S-F11	LRP6e3e4	YTFTYRYLH	1681	GGIIPIFGTANYA	1749	CARDSDWGVVDPW	1905
010S-G11	LRP6e3e4	YTFTYRYLH	1681	GRIIPVLKITNYA	1768	CAVVDDAFDIW	1997
010S-H11	LRP6e3e4	YTFTYRYLH	1681	GGIIPIFGTANYA	1749	CAKDGTDGRFDPW	1846
009S-B08	LRP6e3e4	FTFTSSAVQ	1589	GWINAGNGNTTYA	1772	CARRGGDVTVAAYYAMDVW	1963
010S-A12	LRP6e3e4	VTFSRYPIS	1667	GGIIPIFGTANYA	1749	CAKDSGNYGYGMDVW	1854
010S-B12	LRP6e3e4	FTFSSYDMH	1578	SGITSNGGATYYA	1798	CARGTTGKGYYYGMDVW	1949
010S-C12	LRP6e3e4	FTFSNYWIH	1571	SAIGTGGGTYA	1781	CTTAGYKAARRSVYPRIFNFDY W	2044
010S-D12	LRP6e3e4	YTFTYRYLH	1681	GRIIPIFGTANYA	1763	CAREEGVGGMDVW	1917
010S-E12	LRP6e3e4	FTFSSYAMH	1576	SAIGAGGGTYA	1779	CARGVSSGYYYYGMDVW	1950
010S-F12	LRP6e3e4	FTVSSNYMS	1592	SAIGTGGGTYA	1781	CARAGTNWGGWYFDLW	1879
010S-G12	LRP6e3e4	FALSGYYMS	1550	SSISSSTYIRYA	1803	CATVTGYSSAGAFDIW	1995
011S-A01	LRP6e3e4	FTFSTHAFH	1583	SAIRGSGERTYYA	1784	CARDLRNWGSPYWYFDLW	1901
011S-B01	LRP6e3e4	GTFSHYTIS	1600	GWINAGNGNTKYS	1771	CAKGGSLDMDVW	1864
011S-C01	LRP6e3e4	LTFTSHGMS	1615	SYVSDSGSSVYYA	1818	CARHPGSFGGYSYAWYYYYG MDVW	1957
011S-D01	LRP6e3e4	GTISDYTVS	1605	GIINPSGGSTSYA	1761	CARGYYDFDYW	1953
009S-C08	LRP6e3e4	FSFNTFGIH	1556	AVISYDGSNKYYA	1738	CAKSIAAAGTGYGMDVW	1869
011S-E01	LRP6e3e4	FPFZYYSMN	1552	SAISGRDGRTYA	1785	CAKDLGIQLPDYFDYW	1847
009S-D08	LRP6e3e4	YTFTSYDIN	1679	GGIIPIFGTANYA	1749	CARGPYFDYW	1941
011S-F01	LRP6e3e4	FSFSDYYMS	1558	SGISESGGRTYYA	1790	CASAADF DYW	1967
009S-E08	LRP6e3e4	YGFTGYYIH	1668	GWMNPNSGNTGY A	1776	CARGYGDYDLW	1952
009S-F08	LRP6e3e4	DTFANYGFS	1547	GXVNAGNGNTTYA	1777	CAKGWLDFDYW	1867
011S-G01	LRP6e3e4	YTFTYRYLH	1681	GGIIPLFGTANYA	1750	CTTDDYGDQYGMVDW	2046
011S-H01	LRP6e3e4	YTFTYRYLH	1681	GGIIPIFGTANYA	1749	CTTDDYGD LTHLDYW	2045
011S-A02	LRP6e3e4	GTFSYSAIS	1603	GWMNPNSGNTGY A	1776	CARDKGYAFDIW	1896
011S-B02	LRP6e3e4	YSFTRTDMH	1670	GYISAYTGHTSYA	1778	CARDLGGTADYW	1899
011S-C02	LRP6e3e4	FTFSTYSMN	1585	SGISWNSGRIGYA	1795	CARDVGAFDIW	1906
009S-G08	LRP6e3e4	FTFSDFAMT	1566	SYISGDSGYTNYA	1813	CARLGSPGPYYYMDVW	1962
011S-D02	LRP6e3e4	FTFSSYAMS	1577	SSISGSGGVTYA	1801	CARGGNTYYYYGMDVW	1928
009S-H08	LRP6e3e4	YTFTDYFMN	1673	GIINPSGDSTRFA	1758	CARDDGLGGMDVW	1889
011S-E02	LRP6e3e4	YTFTYRYLH	1681	GGIIPIFGTANYA	1749	CATDYGDYGYGMDVW	1984
009S-A09	LRP6e3e4	YTFTYRYLH	1681	GRIIPILGSTNYA	1767	CTTDLWDYW	2048
011S-F02	LRP6e3e4	FTFSTYGMH	1584	SSISVSSGTHHYA	1805	CARGGSGSYAFDIW	1930
011S-G02	LRP6e3e4	YTFTSYAMN	1678	GGIIPIFGTANYA	1749	CARDASGGSTGWYFDSW	1887

Clone ID	Confirmed Binding	CDRH1	SID NO.	CDRH2	SID NO.	CDRH3	SID NO.
011S-H02	LRP6e3e4	YFTNNFMH	1676	GIINPSGGSTSYA	1761	CARGLYKRYSGYGMDVW	1935
009S-B09	LRP6e3e4	FSFNTYAMN	1557	AVTSYDGGKKNYA	1742	CARDAGGDYDYW	1885
009S-C09	LRP6e3e4	GTFHTYGLS	1597	GGIIPFGTANYA	1749	CARGSGWSGLDYW	1945
011S-A03	LRP6e3e4	FTFSSYWMH	1580	STISGSGGRTYYA	1808	CATSPYGVFTLDYW	1994
011S-B03	LRP6e3e4	GTFSZYAIS	1604	GIINPSGGSTNYA	1760	CARAGYWSGYGYGMDVW	1881
011S-C03	LRP6e3e4	YTFSYRYLH	1672	GGIIPFGTANYA	1749	CASTVTDDAFDIW	1978
011S-D03	LRP6e3e4	FSFDDYGMS	1554	SVISSGGTIYYA	1812	CARHLSSGYLSYGYGMDVW	1955
009S-F09	LRP6e3e4	YSFTRTDMH	1670	GYISAYTGHTSYA	1778	CARDLGGTADYW	1900
011S-E03	LRP6e3e4	FTFSSYAMS	1577	SAISGSGGSTYYA	1786	CAKGGRDGYKGYFDYW	1861
009S-G09	LRP6e3e4	FTFSRHSMN	1572	SYSSGNSGYTNYA	1817	CARGDLEFDYW	1923
011S-F03	LRP6e3e4	FTFSSYAMS	1577	SAISGSGGSTYYA	1786	CAKGGRDGYKGYFDYW	1862
009S-H09	LRP6e3e4	FTFSSYAMS	1577	SAISGSGGSTYYA	1786	CAKGGRDGYKGYFDYW	1863
011S-G03	LRP6e3e4	YFTYRYLH	1681	GRIPIHGIANYA	1764	CAREYSYGYFRYW	1922
009S-A10	LRP6e3e4	FTFTSSAMQ	1588	GIINPSGGSTIYA	1759	CASGDTYDLYSLDVW	1970
009S-B10	LRP6e3e4	YIFTDYMH	1669	GWINAGNGNTTYA	1772	CAKVASGWSWPFDIW	1871
011S-B04	LRP6e1e2	YTFTSYDIN	1679	GIINPSGGSTSYA	1761	CTREHSYYYYGMDVW	2042
011S-C04	LRP6e1e2	GTFNSNAIS	1598	GWMNPNSGNTGY A	1776	CARDYYGSGSYNYGMDVW	1913
011S-D04	LRP6e1e2	YTFTSYDIN	1679	GIINPSGGSTSYA	1761	CAREAYYYYYGMDVW	1916
011S-E04	LRP6e1e2	FTFSSZZMH	1581	SAIGTGGGTZYA	1782	CAKDLGRAAAGSMDVW	1850
011S-F04	LRP6e1e2	YIFTDYMH	1669	GRIIPILGRANYA	1765	CARGGYSTLDYW	1933
011S-H04	LRP6e1e2	YIFTDYMH	1669	GRIIPILGRANYA	1765	CARGGYSTLDYW	1934
011S-A05	LRP6e1e2	FTFSSYAMH	1576	SAIGTGGGTYYA	1781	CAKDLGRAAAGSMDVW	1848
011S-B05	LRP6e1e2	YZFTDYMH	1683	GWMNPNSGNTGY A	1776	CTRVAWGGLDYW	2043
011S-C05	LRP6e1e2	FTFSSYAMH	1576	SAIGTGGGTYYA	1781	CAKDLGRAAAGSMDVW	1849
1115.3	LRP6e1e2	GFSFSTS	2208	NLNGGS	2211	ELAGYGTPEFAY	2214
421.1	LRP6e1e2	GYTFTTY	2209	FPGNVNT	2212	EELQYYFDY	2215
YW211.31.57	LRP6e3e4	GFTFTSY	2210	SPYSGS	2213	RARPPIRLHPRGSVMDY	2216

Table 2A, cont.

Clone ID	Confirmed Binding	CDRL1	SID NO.	CDRL2	SID NO.	CDRL3	SID NO.
001S-C08	LRP6e1e2	RASQYISNYLN	2098	AASSLQS	2110	CQQSYITPLTF	2160
001S-C10	LRP6e1e2						
001S-D10	LRP6e1e2						
001S-E10	LRP6e1e2						
001S-F10	LRP6e1e2						
001S-G10	LRP6e1e2						
001S-A11	LRP6e1e2						

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Clone ID	Confirmed Binding	CDRL1	SID NO.	CDRL2	SID NO.	CDRL3	SID NO.
001S-B11	LRP6e1e2						
001S-C11	LRP6e1e2						
001S-E11	LRP6e1e2						
001S-F11	LRP6e1e2						
001S-G11	LRP6e1e2						
001S-H11	LRP6e1e2						
001S-A12	LRP6e1e2						
001S-B12	LRP6e1e2						
001S-C12	LRP6e1e2						
001S-D12	LRP6e1e2						
001S-F12	LRP6e1e2						
008S-B01	LRP5						
008S-C01	LRP5						
008S-D01	LRP5						
008S-E01	LRP5						
008S-G01	LRP5						
008S-A02	LRP5						
008S-C02	LRP5						
008S-D02	LRP5						
008S-E02	LRP5						
009S-C01	LRP6e3e4	RASQRVSNYLN	2089	AASSLQG	2110	CQQSYSPYTF	2178
009S-B02	LRP6e3e4	RASQSIYNLN	2090	AASSLQS	2110	CQQSYSLPLTF	2170
009S-C02	LRP6e3e4	RASQSIYNLN	2090	AASSLQS	2110	CQQSYSMPPLTF	2171
009S-D02	LRP6e3e4	SGSSSNVGNVVS	2105	DNDKRPS	2122	CESWDSSLSSEVF	2139
010S-A02	LRP6e1e2						
010S-B02	LRP6e1e2						
010S-D02	LRP6e1e2						
010S-E02	LRP6e1e2						
010S-F02	LRP6e1e2						
009S-E02	LRP6e1e2						
009S-F02	LRP6e1e2						
009S-G02	LRP6e1e2						
009S-H02	LRP6e1e2						
009S-A03	LRP6e1e2						
010S-G02	LRP6e1e2						
010S-A03	LRP6e1e2						
009S-B03	LRP6e1e2						

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Clone ID	Confirmed Binding	CDRL1	SID NO.	CDRL2	SID NO.	CDRL3	SID NO.
010S-B03	LRP6e1e2						
010S-D03	LRP6e1e2						
009S-C03	LRP6e1e2						
009S-D03	LRP6e1e2						
009S-E03	LRP6e1e2						
009S-F03	LRP6e1e2						
010S-E03	LRP6e1e2						
010S-F03	LRP6e1e2						
009S-G03	LRP6e1e2						
009S-H03	LRP6e1e2						
009S-A04	LRP6e1e2						
009S-B04	LRP6e3e4						
010S-G03	LRP6e3e4						
009S-C04	LRP6e3e4						
009S-D04	LRP6e3e4						
010S-H03	LRP6e3e4						
009S-E04	LRP6e3e4						
010S-A04	LRP6e3e4						
009S-F04	LRP6e3e4						
010S-B04	LRP6e3e4						
009S-G04	LRP6e3e4						
009S-H04	LRP6e3e4						
010S-C04	LRP6e3e4						
010S-D04	LRP6e3e4						
010S-E04	LRP6e3e4						
010S-F04	LRP6e3e4						
009S-A05	LRP6e3e4						
010S-G04	LRP6e3e4						
010S-H04	LRP6e3e4						
010S-A05	LRP6e3e4						
010S-C05	LRP6e3e4						
010S-D05	LRP6e3e4						
010S-E05	LRP6e3e4						
010S-F05	LRP6e3e4						
013S-G04	LRP6e3e4						
013S-H04	LRP6e3e4						
013S-A05	LRP6e3e4						
013S-B05	LRP6e3e4						

Clone ID	Confirmed Binding	CDRL1	SID NO.	CDRL2	SID NO.	CDRL3	SID NO.
013S-C05	LRP6e3e4						
013S-D05	LRP6e3e4						
013S-E05	LRP6e3e4	RASQSISSYLN	2094	AASSLQS	2110	CQQSYSTPLTF	2174
013S-F05	LRP6e3e4	RASQSISSYLN	2094	AASSLQS	2110	CQQSYSTPLTF	2174
013S-G05	LRP6e3e4	RASQDISNYLN	2079	AASTLQS	2113	CQQGNSFPYTF	2152
010S-G06	LRP6e3e4	QASQDISNYLN	2077	AASSLQS	2110	CQQSYRIHWTF	2163
009S-B05	LRP6e3e4	RASQSISSYLN	2094	AASSLQS	2110	CQQSYSTPLTF	2174
009S-C05	LRP6e3e4						
009S-D05	LRP6e3e4	RASQSISSYLN	2094	AASSLQS	2110	CQQSYSTPLTF	2174
009S-E05	LRP6e3e4	RASQGINSYLA	2081	DAKGLHP	2114	CQQSYSAPLSF	2166
009S-F05	LRP6e3e4	RASQSISSYLN	2094	AASSLQS	2110	CQQSYSTPLTF	2174
009S-G05	LRP6e3e4	QASQDISNYLN	2077	AASTLQR	2112	CQQSYSAPLTF	2167
009S-C06	LRP6e3e4	RASRNINRYLN	2099	AASSLLS	2109	CQQSYNVPFTF	2162
009S-D06	LRP6e3e4	RASQSISSYLN	2094	AASSLQS	2110	CQQSYSTPLTF	2174
009S-E06	LRP6e3e4	RASQSISSYLN	2094	AASSLQS	2110	CQQSYSTPLTF	2174
009S-F06	LRP6e3e4	RASQSISSYLN	2094	AASSLQS	2110	CQQSYSTPLTF	2174
009S-G06	LRP6e3e4	RASQNIGLYLN	2088	DASSLQR	2121	CQQSYSTPYTF	2176
009S-H06	LRP6e3e4	RASQSISSYLN	2094	AASSLQS	2110	CQQSYSTPLTF	2174
009S-A07	LRP6e3e4	RASQSISSYLN	2094	AASSLQS	2110	CQQSYSTPLTF	2174
009S-B07	LRP6e3e4	RASQSISSYLN	2094	AASSLQS	2110	CQQSYSTPLTF	2174
009S-D07	LRP6e3e4	RSSQSLHSNGY NYLD	2102	LGSNRAS	2131	CMQATQFPLTF	2146
009S-F07	LRP6e3e4	RASQSISSYLN	2094	AASSLQS	2110	CQQSYSTPLTF	2174
009S-G07	LRP6e3e4	RASQGISNYLA	2083	DASNLET	2115	CLQDFSFPWTF	2140
009S-H07	LRP6e3e4	RASQSISSYLN	2094	AASSLQS	2110	CQQSYSTPLTF	2174
009S-A08	LRP6e3e4	RASQGISNYLA	2083	GSSTLQS	2127	CQQTYSIPPTF	2181
011S-C01	LRP6e3e4	RASQSISSYLN	2094	AASSLQS	2110	CQQSYSTPLTF	2174
009S-C08	LRP6e3e4	RASQSISSYLN	2094	AASSLQS	2110	CQQSYSTPLTF	2174
009S-D08	LRP6e3e4	RASQGISNNLN	2082	DASSLES	2120	CLQHNSYPFTF	2143
011S-F01	LRP6e3e4	RASQDISNYLN	2079	AASSLQS	2110	CLQDYSYPRTF	2141
009S-E08	LRP6e3e4	QASQDISNYLN	2077	DASSLES	2120	CQQSYRYPTF	2165
009S-F08	LRP6e3e4	QASQDISNYLN	2077	DASSLES	2120	CQQSYSTSITF	2177
009S-G08	LRP6e3e4	RASQSISSYLN	2094	AASSLQS	2110	CQQSYSTPLTF	2174
009S-H08	LRP6e3e4	QASQDISNYLA	2076	AASSLQS	2110	CQQSYSTPLTF	2174
009S-A09	LRP6e3e4	QASQGITNYLN	2078	AASSLQS	2110	CLQDYTDPTF	2142
011S-F02	LRP6e3e4	RASQSISSYLN	2094	AASSLQS	2110	CQQSYSTPLTF	2174
011S-G02	LRP6e3e4	RASQGISSYLA	2087	AASSLQS	2110	CQQAYSFPWTF	2150
011S-A03	LRP6e3e4	RASQSISSYLN	2094	AASSLQS	2110	CQQSYSTPLTF	2174

Clone ID	Confirmed Binding	CDRL1	SID NO.	CDRL2	SID NO.	CDRL3	SID NO.
011S-C03	LRP6e3e4	QASQDISNYLN	2077	DASSLES	2120	CQQSYSPPTTF	2168
011S-D03	LRP6e3e4	RASQSISSYLA	2093	AASTLQS	2113	CQQSYSTPLTF	2174
011S-F03	LRP6e3e4	KSSQSVLYTTTNR NHIA	2073	WASSRKS	2135	CQQYSTPYTF	2189
011S-C04	LRP6e1e2	GASQSVPRNSLA	2066	GASQRAT	2124	CQQYHNWPPEYTF	2184
011S-D04	LRP6e1e2	RASQSISSYLN	2094	AASSLQS	2110	CQQSYSTPLTF	2174
011S-H04	LRP6e1e2	QASQDISNYLN	2077	AASTLQS	2113	CQQSFSTPRTF	2156
008S-F02	LRP5	RSSQSLHSDGYT YLY	2101	TLSYRAS	2134	CMQALEALFTF	2144
010S-C06	LRP6e1e2	RASQSISSYLN	2094	AASSLQS	2110	CQQSYSTPLTF	2174
010S-E06	LRP6e3e4	RASQSISSYLN	2094	AASSLQS	2110	CQQSYSTPLTF	2174
010S-F06	LRP6e3e4	RASQSISSYLN	2094	AASSLQS	2110	CQQSYSTPLTF	2174
010S-H06	LRP6e3e4	RASQSISSYLN	2094	AASSLQS	2110	CQQSYSTPLTF	2174
010S-A07	LRP6e3e4	RASQSISSYLN	2094	AASSLQS	2110	CQQSYSTPLTF	2174
010S-B07	LRP6e3e4	RASQSISSYLN	2094	AASSLQS	2110	CQQSYSTPLTF	2174
010S-C07	LRP6e3e4	RASQSISSYLN	2094	AASSLQS	2110	CQQSYSTPLTF	2174
010S-D07	LRP6e3e4	RASQSISSYLN	2094	AASSLQS	2110	CQQSYSTPLTF	2174
009S-D05	LRP6e3e4	RASQSISSYLN	2094	AASSLQS	2110	CQQSYSTPLTF	2174
009S-E05	LRP6e3e4	RASQGINSYLA	2081	DAKGLHP	2114	CQQSYSAPLSF	2166
010S-E07	LRP6e3e4	RASQSISSYLN	2094	AASSLQS	2110	CQQSYSTPLTF	2174
009S-F05	LRP6e3e4	RASQSISSYLN	2094	AASSLQS	2110	CQQSYSTPLTF	2174
010S-F07	LRP6e3e4	RASQSVYSNLA	2097	DTSNRAT	2123	CQQYNNWPPITF	2185
010S-G07	LRP6e3e4	RVSQGISSYLN	2103	AASSLQS	2110	CQQTYPITFTF	2182
009S-G05	LRP6e3e4	QASQDISNYLN	2077	AASTLQR	2112	CQQSYSAPLTF	2167
010S-H07	LRP6e3e4	QASQDISNYLN	2077	GTSNLQS	2128	CQQSYSTPYTF	2176
010S-A08	LRP6e3e4	RASQSISSYLN	2094	AASSLQS	2110	CQQSYSTPLTF	2174
009S-A07	LRP6e3e4	RASQSISSYLN	2094	AASSLQS	2110	CQQSYSTPLTF	2174
009S-B07	LRP6e3e4	RASQSISSYLN	2094	AASSLQS	2110	CQQSYSTPLTF	2174
009S-B06	LRP6e3e4	RVSQSISSYLN	2103	AASSLQS	2110	CQQSYSTPLTF	2174
010S-B08	LRP6e3e4	RASQGISNNLN	2082	DASNLET	2115	CQQSYTSRLTF	2179
010S-C08	LRP6e3e4	RASQSISSYLN	2094	AASSLQS	2110	CQQSYSTPLTF	2174
009S-C06	LRP6e3e4	RASRNINRYLN	2099	AASSLLS	2109	CQQSYNVPPTF	2162
009S-D06	LRP6e3e4	RASQSISSYLN	2094	AASSLQS	2110	CQQSYSTPLTF	2174
010S-D08	LRP6e3e4	RASQSISSYLN	2094	AASSLQS	2110	CQQSYSTPLTF	2174
009S-E06	LRP6e3e4	RASQSISSYLN	2094	AASSLQS	2110	CQQSYSTPLTF	2174
010S-E08	LRP6e3e4	RASQSISSYLN	2094	ZASSLQS	2137	CQQSYSTPLTF	2174
010S-F08	LRP6e3e4	RASQSISSYLN	2094	AASSLQS	2110	CQQSYSTPLTF	2174
009S-F06	LRP6e3e4	RASQSISSYLN	2094	AASSLQS	2110	CQQSYSTPLTF	2174
010S-G08	LRP6e3e4	RASQSVRSSDLA	2096	GSSSRAT	2126	CQQYGRSPRYSF	2183

Clone ID	Confirmed Binding	CDRL1	SID NO.	CDRL2	SID NO.	CDRL3	SID NO.
010S-H08	LRP6e3e4	RASQSISSYLN	2094	AASSLQS	2110	CQQSYSTPLTF	2174
010S-A09	LRP6e3e4	QASQDIANYLN	2075	AASSLQS	2110	CQQSYSTPYTF	2176
010S-B09	LRP6e3e4	RASQSISSRYLN	2092	KASSLES	2130	CQQSYDSPWTF	2159
009S-G06	LRP6e3e4	RASQNIIGLYLN	2088	DASSLQR	2121	CQQSYSTPYTF	2176
010S-C09	LRP6e3e4	RASQSISSYLN	2094	AASSLQS	2110	CQQSYSTPLTF	2174
009S-H06	LRP6e3e4	RASQSISSYLN	2094	AASSLQS	2110	CQQSYSTPLTF	2174
010S-D09	LRP6e3e4	QASQDISNYLN	2077	AASSLQS	2110	CQQIHSYPLTF	2155
010S-E09	LRP6e3e4	RASQSISSYLN	2094	AASSLQS	2110	CQQSYSTPLTF	2174
009S-A07	LRP6e3e4	RASQSISSYLN	2094	AASSLQS	2110	CQQSYSTPLTF	2174
009S-B07	LRP6e3e4	RASQSISSYLN	2094	AASSLQS	2110	CQQSYSTPLTF	2174
009S-D08	LRP6e3e4	RASQGISNNLN	2082	DASSLES	2120	CLQHNSYPFTF	2143
010S-F09	LRP6e3e4	RASQSISSYLN	2094	AASSLQS	2110	CQQSYSTPLTF	2174
010S-G09	LRP6e3e4	QASQDISNYLN	2077	DASNLET	2115	CQQSYSTPLTF	2174
010S-H09	LRP6e3e4	RASQGISNYLN	2084	AASTLQS	2113	CQQGYGTPPMF	2153
010S-A10	LRP6e3e4	RASQSISSYLN	2094	AASSLQS	2110	CQQSYSTPLTF	2174
009S-D07	LRP6e3e4	RSSQSLLHSNGY NYLD	2102	LGSNRAS	2131	CMQATQFPLTF	2146
010S-B10	LRP6e3e4	RASQSISSYLN	2094	GTSSLHT	2129	CQQANSFPFTF	2148
010S-C10	LRP6e3e4	KSSQSILSSSSNR DSL A	2072	WASSRKS	2135	CQQYINIPYSF	2187
009S-E07	LRP6e3e4	RASQSISSYLN	2094	AASSLQS	2110	CQQSYSTPLTF	2174
010S-D10	LRP6e3e4	RASQSISSYLN	2094	AASSLQS	2110	CQQSYSTPLTF	2174
010S-E10	LRP6e3e4	RASQSISSYLN	2094	AASSLQS	2110	CQQSYSTPLTF	2174
010S-F10	LRP6e3e4	RASQSISSYLN	2090	AASTLES	2111	CQQANSFPPTF	2148
010S-G10	LRP6e3e4	RASQGISNYLA	2083	AASSLQS	2110	CQQSYSTPWTF	2175
009S-F07	LRP6e3e4	RASQSISSYLN	2094	AASSLQS	2110	CQQSYSTPLTF	2174
009S-G07	LRP6e3e4	RASQGISNYLA	2083	DASNLET	2115	CLQDFSFPWTF	2140
010S-H10	LRP6e3e4	KSSHLLYSSDNK NYLA	2071	WSSTRES	2136	CQQYYSTPQTF	2188
010S-A11	LRP6e3e4	RASQSIZNYLN	2095	ZASTLES	2138	CQQANSFPPTF	2148
010S-B11	LRP6e3e4	QASQDISNYLN	2077	AASTLQS	2113	CQQSYSTPLTF	2174
010S-C11	LRP6e3e4	RASQSISSYLN	2094	AASSLQS	2110	CQQSYSTPLTF	2174
009S-H07	LRP6e3e4	RASQSISSYLN	2094	AASSLQS	2110	CQQSYSTPLTF	2174
009S-A08	LRP6e3e4	RASQGISNYLA	2083	GSSTLQS	2127	CQQTYSIPPTF	2181
010S-D11	LRP6e3e4	RASQSVSSNLA	2097	GASTRAT	2125	CQQFDRSPLTF	2151
010S-E11	LRP6e3e4	RASQSISSYLN	2094	AASSLQS	2110	CQQSYSTPLTF	2174
010S-F11	LRP6e3e4	RASQSISSYLN	2094	AASSLQS	2110	CQQSYSTPLTF	2174
010S-G11	LRP6e3e4	RASQSISSYLN	2094	AASSLQS	2110	CQQSYSTPLTF	2174
010S-H11	LRP6e3e4	RASQDISSYLA	2080	SASTLQS	2133	CQQSNSFPYTF	2157

Clone ID	Confirmed Binding	CDRL1	SID NO.	CDRL2	SID NO.	CDRL3	SID NO.
009S-B08	LRP6e3e4	RASQSISSYLN	2094	ZASSLQS	2137	CQQSYSTPLTF	2174
010S-A12	LRP6e3e4	RASQSISSYLN	2094	AASSLQS	2110	CQQSYSTPLTF	2174
010S-B12	LRP6e3e4	RASQSISSYLN	2094	AASSLQS	2110	CQQSYSTPLTF	2174
010S-C12	LRP6e3e4	RASQSISSYLN	2094	AASSLQS	2110	CQQSYSTPLTF	2174
010S-D12	LRP6e3e4	RPSQSIGSWLA	2100	DASNLQS	2116	CQQSSSTPYTF	2158
010S-E12	LRP6e3e4	RASQSISSYLN	2094	AASSLQS	2110	CQQSYSTPLTF	2174
010S-F12	LRP6e3e4	RASQGISRDLA	2085	AASTLQS	2113	CQQSYSPPTF	2172
010S-G12	LRP6e3e4	RASQSISSYLN	2094	AASSLQS	2110	CQQSYSTPLTF	2174
011S-A01	LRP6e3e4	RASQSISSYLN	2094	AASSLQS	2110	CQQSYSTPLTF	2174
011S-B01	LRP6e3e4	RASQGISNYLA	2083	AASSLHS	2108	CQQSYRTPLTF	2164
011S-C01	LRP6e3e4	RASQSISSYLN	2094	AASSLQS	2110	CQQSYSTPLTF	2174
011S-D01	LRP6e3e4	RASQGISNYLA	2083	AASSLQS	2110	CQQSYSTPLTF	2174
009S-C08	LRP6e3e4	RASQSISSYLN	2094	AASSLQS	2110	CQQSYSTPLTF	2174
011S-E01	LRP6e3e4	RASQGISSALA	2086	AASTLQS	2113	CQQSYSSPPTF	2173
009S-D08	LRP6e3e4	RASQGISNNLN	2082	DASSLES	2120	CLQHNSYPPTF	2143
011S-F01	LRP6e3e4	RASQDISNYLN	2079	AASSLQS	2110	CLQDYSYPTF	2141
009S-E08	LRP6e3e4	QASQDISNYLN	2077	DASSLES	2120	CQQSYRYPTF	2165
009S-F08	LRP6e3e4	QASQDISNYLN	2077	DASSLES	2120	CQQSYSTSITF	2177
011S-G01	LRP6e3e4	RASQSISSYLN	2094	AASSLQS	2110	CQQSYSTPLTF	2174
011S-H01	LRP6e3e4	RASQSISSYLN	2094	AASSLQS	2110	CQQSYSTPLTF	2174
011S-A02	LRP6e3e4	RSSQSLHSNGY NYLD	2102	AASSLQS	2110	CMQALQTPITF	2145
011S-B02	LRP6e3e4	RZSQSZSZYLN	2104	AASSLQS	2110	CQQSYSTPLTF	2174
011S-C02	LRP6e3e4	QASQDISNYLN	2077	AASILQS	2107	CQQSYSIPPTF	2169
009S-G08	LRP6e3e4	RASQSISSYLN	2094	AASSLQS	2110	CQQSYSTPLTF	2174
011S-D02	LRP6e3e4	RASQSISSYLN	2094	AASSLQS	2110	CQQSYSTPLTF	2174
009S-H08	LRP6e3e4	QASQDISNYLA	2076	AASSLQS	2110	CQQSYSTPLTF	2174
011S-E02	LRP6e3e4	RASQSISSYLN	2094	AASSLQS	2110	CQQSYSTPLTF	2174
009S-A09	LRP6e3e4	QASQGITNYLN	2078	AASSLQS	2110	CLQDYTDPTF	2142
011S-F02	LRP6e3e4	RASQSISSYLN	2094	AASSLQS	2110	CQQSYSTPLTF	2174
011S-G02	LRP6e3e4	RASQGISSYLA	2087	AASSLQS	2110	CQQAYSFPWTF	2150
011S-H02	LRP6e3e4	RASQSISSYLN	2094	AASSLQS	2110	CQQSYSTPLTF	2174
009S-B09	LRP6e3e4	QASQDISNYLN	2077	AASSLQS	2110	CQQSYNTPTF	2161
009S-C09	LRP6e3e4	QASQDISNYLN	2077	DASNLET	2115	CQQSYTTPPTF	2180
011S-A03	LRP6e3e4	RASQSISSYLN	2094	AASSLQS	2110	CQQSYSTPLTF	2174
011S-B03	LRP6e3e4	RASQSISSYLN	2094	AASSLQS	2110	CQQSYSTPLTF	2174
011S-C03	LRP6e3e4	QASQDISNYLN	2077	DASSLES	2120	CQQSYSPPTF	2168
011S-D03	LRP6e3e4	RASQSISSYLA	2093	AASTLQS	2113	CQQSYSTPLTF	2174

Clone ID	Confirmed Binding	CDRL1	SID NO.	CDRL2	SID NO.	CDRL3	SID NO.
009S-F09	LRP6e3e4	RASQSISSYLN	2094	AASSLQS	2110	CQQSYSTPLTF	2174
011S-E03	LRP6e3e4	KSSHLLSTSTNR NQLA	2070	WASSRKS	2135	CQQYNNWPYTF	2186
009S-G09	LRP6e3e4	RASQGISNYLA	2083	SASSLQS	2132	CQQGYNTPRTF	2154
011S-F03	LRP6e3e4	KSSQSVLYTTTNR NHIA	2074	WASSRKS	2135	CQQYYSTPYTF	2189
009S-H09	LRP6e3e4	KSSHLLSTSTNR NHIA	2069	WASSRKS	2135	CQQYINIPYSF	2187
011S-G03	LRP6e3e4	RASQGISSYLA	2087	DASNLET	2115	CQQANSLFTF	2149
009S-A10	LRP6e3e4	RASQISRWLA	2091	AASSLQS	2110	CQQAYSPWTF	2150
009S-B10	LRP6e3e4	RASQSISSYLN	2094	AASSLQS	2110	CQQSYSTPLTF	2174
011S-B04	LRP6e1e2	RASQSISSYLN	2094	AASSLQS	2110	CQQSYSTPLTF	2174
011S-C04	LRP6e1e2	GASQSVPRNSLA	2066	GASQRAT	2124	CQQYHNWPPEYTF	2184
011S-D04	LRP6e1e2	RASQSISSYLN	2094	AASSLQS	2110	CQQSYSTPLTF	2174
011S-E04	LRP6e1e2	WASQSVRGNYV A	2106	DASNRAA	2117	CQHRSNWPLTF	2147
011S-F04	LRP6e1e2	HGSQDISNYLN	2067	DASNRQS	2119	CQQSFSTPRTF	2156
011S-H04	LRP6e1e2	QASQDISNYLN	2077	AASTLQS	2113	CQQSFSTPRTF	2156
011S-A05	LRP6e1e2	WASQSVRGNYV A	2106	DASNRAA	2117	CQHRSNWPLTF	2147
011S-B05	LRP6e1e2	RASQSISSYLN	2094	AASSLQS	2110	CQQSYSTPLTF	2174
011S-C05	LRP6e1e2	WASQSVRGNYV A	2106	DASNRAA	2117	CQHRSNWPLTF	2147
1115.3	LRP6e1e2	KASQISYNLH	2217	YTSQIS	2220	QQSNSWPLT	2223
421.1	LRP6e1e2	SANSSVRFMF	2218	RTSNLAS	2221	QQYHSPWT	2224
YW211.31 .57	LRP6e3e4	RASQDVSTAVA	2219	SASFLYS	2222	QQSYTTPPT	2225

Table 2B. Anti-LRP5/6 Antibody Clone IDs, Heavy Chain (HC) Seq ID Nos, and Binding Characteristics.

Clone ID	HC Seq ID NO	Confirmed Binding
001S-F11	74	LRP6e1e2
009S-G02	75	LRP6e1e2
009S-A03	76	LRP6e1e2
009S-D03	77	LRP6e1e2
009S-F03	78	LRP6e1e2
009S-H03	79	LRP6e1e2
009S-A04	80	LRP6e1e2

009S-B04	81	LRP6e3e4
009S-D04	82	LRP6e3e4
009S-E04	83	LRP6e3e4
009S-F04	84	LRP6e3e4
009S-G04	85	LRP6e3e4
009S-H04	86	LRP6e3e4
009S-A05	87	LRP6e3e4
013S-G04	88	LRP6e3e4
013S-H04	89	LRP6e3e4
013S-C05	90	LRP6e3e4
013S-D05	91	LRP6e3e4
013S-G04	92	LRP6e3e4
013S-H04	93	LRP6e3e4
013S-A05	94	LRP6e3e4
013S-C05	95	LRP6e3e4
013S-D05	96	LRP6e3e4
008S-D01	97	LRP5

In certain embodiments, the LRP5/6 binding region may be selected from any binding domain that binds LRP5 or LRP6 with a K_D of less than or equal to about 1×10^{-4} M, less than or equal to about 1×10^{-5} M, less than or equal to about 1×10^{-6} M, less than or equal to about 1×10^{-7} M, less than or equal to about 1×10^{-8} M, less than or equal to about 1×10^{-9} M, less than or equal to about 1×10^{-10} M, less than or equal to about 1×10^{-11} M, less than or equal to about 1×10^{-12} M, less than or equal to about 1×10^{-13} M, less than or equal to about 1×10^{-14} M, or less than or equal to 1×10^{-15} M in the context of a Wnt surrogate molecule.

In certain embodiment, the LRP5/6 binding region may be selected from any binding domain that binds LRP5 or LRP6 with a K_D of greater than or equal to about 1×10^{-4} M, greater than or equal to about 1×10^{-5} M, greater than or equal to about 1×10^{-6} M, greater than or equal to about 1×10^{-7} M, greater than or equal to about 1×10^{-8} M, greater than or equal to about 1×10^{-9} M, greater than about 1×10^{-10} M, greater than or equal to about 1×10^{-11} M, greater than or equal to about 1×10^{-12} M, greater than or equal to about 1×10^{-13} M, greater than or equal to about 1×10^{-14} M, or greater than or equal to 1×10^{-15} M in the context of a Wnt surrogate molecule. In certain embodiment, the LRP5/6 binding region may be selected from any binding domain that binds LRP5 or LRP6 at high affinity, e.g. a K_D of less than about 1×10^{-7} M, less than about 1×10^{-8} M, less than about 1×10^{-9} M, or less than about 1×10^{-10} M.

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

Members of the Dickkopf (DKK) gene family (see Krupnik et al. (1999) Gene 238(2):301-13) include DKK-1, DKK-2, DKK-3, and DKK-4, and the

DKK-3 related protein Soggy (Sgy). hDKKs 1-4 contain two distinct cysteine-rich domains in which the positions of 10 cysteine residues are highly conserved between family members. Exemplary sequences of human Dkk genes and proteins are publicly available, e.g., Genbank accession number NM_014419 (soggy-1);

5 NM_014420 (DKK4); AF177394 (DKK-1); AF177395 (DKK-2); NM_015881 (DKK3); and NM_014421 (DKK2). In some embodiments of the disclosure, 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:

10 KMYHTKGQEGSVCLRSSDCASGLCCARHFWKICKPVLKEGQVCTKHRRKGSH
GLEIFQRCYCGEGLSRIQKDDHHQASNSSRLHTCQRH (SEQ ID NO:2249) (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

15 reference. The corresponding region of human DKK2 (Genbank reference NP_055236) may comprise the sequence:

KMSHIKGHEGDPCLRSSDCIEGFCCARHFWTKICKPVLHQGEVCTKQRKKGSHG
LEIFQRCDCAKGLSCKVWKDATYSSKARLHVCQK (SEQ ID NO:2250), or a biologically active fragment thereof.

20 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.

25 (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 LRP6 and LRP5 of mouse and human origin; the monoclonal antibody 2B11; Cell Signaling Technology antibody specific for LRP5

30 (D80F2), catalog number 5731; etc.

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

In particular embodiments, a Wnt surrogate molecule comprises a polypeptide sequence having at least 90%, at least 95%, at least 98% or at least 99% identity to a polypeptide sequence disclosed in any of SEQ ID NOs: 109-124 or 125-157, or having at least 90%, at least 95%, at least 98% or at least 99%
25 identity to an antigen-binding fragment of a polypeptide sequence disclosed in any of SEQ ID NOs: 109-124 or 125-157. In certain embodiments, the Wnt surrogate molecules comprises or consists of a polypeptide sequence set forth in any of SEQ ID NOs: 109-124 or 125-147, or an antigen-binding fragment thereof. In particular
embodiments, the antigen-binding fragment binds one or more Fzd receptors and
30 also binds LRP5 and/or LRP6.

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

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

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

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 -
25 catenin stabilization; LRP phosphorylation; Axin translocation from cytoplasm to cell membrane and binding to LRP. The canonical Wnt/ β -catenin signaling pathway ultimately leads to changes in gene expression through the transcription factors TCF7, TCF7L1, TCF7L2 and LEF. The transcriptional response to Wnt activation has been characterized in a number of cells and tissues. As such, global
30 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.,
5 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 (pTOPFI_ASH and pFOPFI_ASH, respectively) to determine the transactivational activity of endogenous p-catenin/TCF4. A higher ratio of these two reporter
10 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
15 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
20 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
25 intracellular β -catenin is reduced, and translocation of β -catenin to the nucleus follows thereafter. Therefore, measuring the level and location of β -catenin in a cell is a good reflection of the level of Wnt/ β -catenin signaling. A non-limiting example of such an assay is the "Bioluminescence β -Catenin Redistribution Assay" (Thermo Scientific) which provides recombinant U2OS cells that stably express human β -
30 catenin fused to the C-terminus of enhanced green fluorescent protein (EGFP).

Imaging and analysis is performed with a fluorescence microscope or HCS platform allowing the levels and distribution of EGFP- β -catenin to be visualized.

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

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

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

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

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

"Wnt pathway signaling" or "Wnt signaling" is used herein to refer to the mechanism by which a biologically active Wnt exerts its effects upon a cell to modulate a cell's activity. Wnt proteins modulate cell activity by binding to Wnt receptors, including proteins from the Frizzled (Fzd) family of proteins, proteins from the ROR family of proteins, the proteins LRP5, LRP6 from the LRP family of proteins, the protein FRL1/crypto, and the protein Derailed/Ryk. Once activated by Wnt binding, the Wnt receptor(s) will activate one or more intracellular signaling cascades. These include the canonical Wnt signaling pathway; the Wnt/planar cell polarity (Wnt/PCP) pathway; the Wnt-calcium (Wnt/Ca²⁺) pathway (Giles, 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/Ca²⁺ by, for example, binding of Wnt-4, Wnt-5A or Wnt-11, elicits an intracellular release of calcium ions, which activates calcium sensitive enzymes like protein kinase C (PKC), calcium-calmodulin dependent kinase II (CamKII) or calcineurin (CaCN). By assaying for activity of the above signaling pathways, the biological activity of an antibody or antigen-binding fragment thereof, e.g., a Wnt surrogate molecule, can be readily determined.

In certain embodiments, functional properties of Wnt surrogate molecules may be assessed using a variety of methods known to the skilled person, including e.g., affinity/binding assays (for example, surface plasmon resonance, competitive inhibition assays), cytotoxicity assays, cell viability assays, cell proliferation or differentiation assays in response to a Wnt, cancer cell and/or tumor growth inhibition using in vitro or in vivo models, including but not limited to any described herein. The Wnt surrogate molecules 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, an Fzd binding region of a Wnt surrogate molecule (e.g., an antigen-binding fragment of an anti-Fzd antibody) comprises one or more of the CDRs of the anti-Fzd antibodies described herein. In certain embodiments, a LRP5/6 binding region of a Wnt surrogate molecule (e.g., an antigen-binding fragment of an anti-LRP5/6 antibody) comprises one or more of the CDRs of the anti-LRP5/6 antibodies described herein. In this regard, it has been shown in some cases that the transfer of only the VHCDR3 of an antibody

can be performed while still retaining desired specific binding (Barbas et al., *PNAS* (1995) 92: 2529-2533). See also, McLane et al., *PNAS* (1995) 92:5214-5218, Barbas et al., *J. Am. Chem. Soc.* (1994) 116:2161-2162.

Also disclosed herein is a method for obtaining an antibody or
5 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
10 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
15 VL domain disclosed herein are combined with one or more VH domains.

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

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

In some embodiments, any of the one or more Fzd binding regions of a Wnt surrogate molecule binds to one, two, three, four, five or more different frizzled receptors, e.g., one or more of human frizzled receptors Fzd1, Fzd2, Fzd3, Fzd4, Fzd5, Fzd6, Fzd7, Fzd8, Fzd9, Fzd10. In some embodiments, any of the Fzd binding regions binds to Fzd1, Fzd2, Fzd5, Fzd7 and Fzd8. In various embodiments, any of the Fzd binding regions binds to: (i) Fzd1, Fzd2, Fzd7 and Fzd9; (ii) Fzd1, Fzd2 and Fzd7; (iii) Fzd5 and Fzd8; (iv) Fzd5, Fzd7 and Fzd8; (v) Fzd1, Fzd4, Fzd5 and Fzd8; (vi) Fzd1, Fzd2, Fzd5, Fzd7 and Fzd8; (vii) Fzd4 and Fzd9; (viii) Fzd9 and Fzd10; (ix) Fzd5, Fzd8 and Fzd10; (x) Fzd4, Fzd5 and Fzd8; or (xi) Fzd1, Fzd5, Fzd7 and Fzd8.

In some embodiments, the Fzd binding region is selective for one or more Fzd receptors of interest, e.g. having a specificity for the one or more desired Fzd receptors of at least 10-fold, 25-fold, 50-fold, 100-fold, 200-fold or more relative to other Fzd receptors. In some embodiments, any of the one or more Fzd binding regions of a Wnt surrogate molecule is multispecific and binds or specifically binds to a plurality of Fzd receptors, e.g., two or more of Fzd1, Fzd2, Fzd3, Fzd4, Fzd5, Fzd6, Fzd7, Fzd8, Fzd9, or Fzd10. For example, any of the one or more Fzd binding regions may be bispecific, trispecific, tetraspecific, and so on. In some embodiments, any of the one or more Fzd binding regions of a Wnt surrogate molecule is monospecific and binds or specifically binds to a single Fzd receptor, e.g., only one of Fzd1, Fzd2, Fzd3, Fzd4, Fzd5, Fzd6, Fzd7, Fzd8, Fzd9, or Fzd10.

In some embodiments, a monospecific Fzd binding region binds to a region of an Fzd receptor that does not include the cysteine rich domain (CRD) of the Fzd receptor, or includes less than the entire CRD of the FZD receptor. As illustrated in FIG. 2A, sequences within the CRD show strong homology between the 10 Fzd receptors, with homologies being even higher between subfamily members. Accordingly, certain embodiments of the monospecific Fzd binding regions disclosed herein do not bind to the CRD, or bind only to a subset of the CRD.

In some embodiments, a Fzd binding region, e.g., a monospecific Fzd binding region, binds to an epitope comprising at least a portion of the extracellular domain after the CRD, referred to herein as the “hinge region” of a Fzd receptor (see FIG. 2A). In particular embodiments, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or 100% of the epitope is present within the hinge region of a Fzd receptor. As illustrated in FIGS. 4A-4E, the hinge regions of the extracellular domain of Fzd receptors show highly divergent sequences. Sequences of illustrative Fzd receptor hinge regions are set forth in SEQ ID NOs:98-107 and in Table 3 below. In certain embodiments, the hinge region includes an amino acid sequence having at least

90%, at least 95%, at least 98%, or at least 99% identity to any of the sequences set forth in SEQ ID NOs:98-107.

Table 3: Fzd hinge region sequences

SID NO.	Fzd	Hinge Region Sequence
98	Fzd1	CVGQNTSDKGTPTPSLLPEFWTSNPQHGGGGHRRGGFPGGA GASERGKFSC
99	Fzd2	CVGQNHSEDGAPALLTTAPPPGLQPGAGGTPGGPGGGGAP PRYATLEHPFHC
100	Fzd3	CDEPYPRLLVDLNLAGEPTEGAPVAVQRDYGFWC
101	Fzd4	CMEGPGDEEVPLPHKTPIQPGEEC
102	Fzd5	CMDYNRSEATTAPPRPFAKPTLPGPPGAPASGGEC
103	Fzd6	CDETVPVTFDPHTEFLGPQKKTEQVQRDIGFWC
104	Fzd7	CVGQNTSDGSGGAGGSPTAYPTAPYLPDPPFTAMSPSDGRG RLSFPPFSC
105	Fzd8	CMDYNRTDLTTAAPSPRRRLPPPPPGEQPPSGSGHGRPPGA RPPHRGGGRGGGGGDAAAPPARGGGGGGKARPPGGGAAP C
106	Fzd9	CMEAPENATAGPAEPHKGLGMLPVAPRPAPPPGDLGPGAGG SGTC
107	Fzd10	CMEAPNNGSDEPTRGSLFPPLFRPQRPHSAQEHLKDGGP GRGGC

5

In some embodiments, a monospecific Fzd binding region binds to an epitope comprising at least a portion of an N-terminal region upstream of the CRD of the Fzd receptor (FIG. 2A). In particular embodiments, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or 100% of the epitope is present within the N-terminal region of a Fzd receptor. The sequence of an illustrative N-terminal region is set forth in SEQ ID NO:108 and in Table 4 below. In certain embodiments, the N-terminal region includes an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99% identity to SEQ ID NO:108.

15

Table 4: Fzd N-terminal region sequences

SEQ ID NO	Fzd	Hinge Region Sequence
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108	Fzd1	QAAGQGPGQGPGGQQPPPPQQQSGQQYN
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In some embodiments, any of the one or more LRP5/6 binding regions of a Wnt surrogate molecule binds to one or both of LRP5/6. For convenience, the term "LRP5/6" is used to refer collectively to either or both of LRP5 and/or LRP6.

5 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,
10 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
15 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
20 dissociation. The ratio of K_{off}/K_{on} enables cancellation of all parameters not related to affinity, and is thus equal to the dissociation constant K_d . See, generally, Davies et al. (1990) *Annual Rev. Biochem.* 59:439-473.

In certain embodiments, the Wnt surrogate molecules or binding regions thereof described herein have an affinity of less than about 10,000, less
25 than about 1000, less than about 100, less than about 10, less than about 1, less than about 0.1, less than about 0.01, less than about 0.001, less than about 0.0001, less than about 0.00001, or less than about 0.000001 nM, and in some embodiments, the antibodies may have even higher affinity for one or more Fzd receptor epitopes or LRP5 or LRP6 receptor.

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

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

The ability to mediate cytotoxic and phagocytic effector functions is a potential mechanism by which antibodies destroy targeted cells. The cell-mediated reaction wherein nonspecific cytotoxic cells that express Fc γ Rs 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 Fc γ Rs 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 Fc γ Rs 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 Fc γ RIIIb 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 Fc γ Rs, 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 Fc γ Rs bind the same region on IgG Fc, yet with different affinities: the high affinity binder Fc γ RI has a K_d for IgG1 of 10^{-8} M $^{-1}$, whereas the low affinity receptors Fc γ RII and Fc γ RIII generally bind at 10^{-6} and 10^{-5} respectively. The extracellular domains of Fc γ RIIIa and Fc γ RIIIb are 96% identical; however, Fc γ RIIIb does not have an intracellular signaling domain. Furthermore, whereas Fc γ RI, Fc γ RIIa/c, and Fc γ RIIIa are positive regulators of immune complex-triggered activation, characterized by having an intracellular domain that has an immunoreceptor tyrosine-based activation motif (ITAM), Fc γ RIIIb has an immunoreceptor tyrosine-based inhibition motif (ITIM) and is therefore inhibitory. Thus the former are referred to as activation receptors, and Fc γ RIIIb 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 Fc γ R polymorphisms in the human proteome. A particularly relevant polymorphism with clinical significance is V158/F158 Fc γ RIIIa. Human IgG1 binds with greater affinity to the V158 allotype than to the F158 allotype. This difference in affinity, and presumably its effect on

5 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 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 Fc γ RIIIa.

15 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 present disclosure, modifications to the Fc region comprise modifications that alter (either enhance or decrease) the ability of a Fzd-specific

20 antibody as described herein to activate the complement system (see e.g., U.S. Patent No. 7,740,847). To assess complement activation, a complement-dependent cytotoxicity (CDC) assay may be performed (See, e.g., Gazzano-Santoro et al., J. Immunol. Methods, 202:163 (1996)).

Thus in certain embodiments, the present disclosure provides anti-

25 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 Fc γ R or increased serum half-life. Other modified Fc regions contemplated herein are described, for example, in issued U.S. Patent Nos. 7,317,091; 7,657,380; 7,662,925; 6,538,124; 6,528,624; 7,297,775; 7,364,731;

30 published U.S. Application Nos. US2009092599; US20080131435;

US20080138344; and published International Application Nos. WO2006/105338; WO2004/063351; WO2006/088494; WO2007/024249.

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

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

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

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

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

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

The term "polynucleotide" as referred to herein means single-stranded or double-stranded nucleic acid polymers. In certain embodiments, the

nucleotides comprising the polynucleotide can be ribonucleotides or deoxyribonucleotides or a modified form of either type of nucleotide. Said modifications include base modifications such as bromouridine, ribose modifications such as arabinoside and 2',3'-dideoxyribose and internucleotide
5 linkage modifications such as phosphorothioate, phosphorodithioate, phosphoroselenoate, phosphorodiselenoate, phosphoroanilothioate, phosphoraniladate and phosphoroamidate. The term "polynucleotide" specifically includes single and double stranded forms of DNA.

The term "naturally occurring nucleotides" includes
10 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, phosphoraniladate,
15 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
20 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,
25 plasmid, or virus) used to transfer coding information to a host cell. The term "expression vector" refers to a vector that is suitable for transformation of a host cell and contains nucleic acid sequences that direct and/or control expression of inserted heterologous nucleic acid sequences. Expression includes, but is not limited to, processes such as transcription, translation, and RNA splicing, if introns
30 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
5 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
10 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
15 variant or derivative of such a sequence.

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

25 Therefore, in another embodiment of the present disclosure, a mutagenesis approach, such as site-specific mutagenesis, may be employed for the preparation of variants and/or derivatives of the polypeptides described herein. By this approach, specific modifications in a polypeptide sequence can be made through mutagenesis of the underlying polynucleotides that encode them. These
30 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
5 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
10 the properties, activity, composition, stability, or primary sequence of the encoded polypeptide.

In certain embodiments, the inventors contemplate the mutagenesis of the polynucleotide sequences that encode a polypeptide present in a Wnt surrogate molecule, to alter one or more properties of the encoded polypeptide,
15 such as the binding affinity, or the function of a particular Fc region, or the affinity of the Fc region for a particular FcγR. The techniques of site-specific mutagenesis are well-known in the art, and are widely used to create variants of both polypeptides and polynucleotides. For example, site-specific mutagenesis is often used to alter a specific portion of a DNA molecule. In such embodiments, a primer
20 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
25 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
30 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
5 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
10 incorporated herein by reference, for that purpose.

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

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

Polypeptides, and encoding nucleic acid molecules and vectors, may be isolated and/or purified, e.g., from their natural environment, in substantially pure or homogeneous form, or, in the case of nucleic acid, free or substantially free of nucleic acid or genes of origin other than the sequence encoding a polypeptide
5 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.

10 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
15 others. A common, preferred bacterial host is *E. coli*.

The expression of polypeptides, e.g., antibodies and antigen-binding fragments thereof, in prokaryotic cells such as *E. coli* is well established in the art. For a review, see for example Pluckthun, A. Bio/Technology 9: 545-551 (1991). Expression in eukaryotic cells in culture is also available to those skilled in the art
20 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
25 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
30 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
5 introduced, or which is capable of having introduced into it, a nucleic acid
sequence encoding one or more of the herein described polypeptides, and which
further expresses or is capable of expressing a selected gene of interest, such as a
gene encoding any herein described polypeptide. The term includes the progeny of
the parent cell, whether or not the progeny are identical in morphology or in genetic
10 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
15 transduction using retrovirus or other virus, e.g. vaccinia or, for insect cells,
baculovirus. For bacterial cells, suitable techniques may include calcium chloride
transformation, electroporation and transfection using bacteriophage. The
introduction may be followed by causing or allowing expression from the nucleic
acid, e.g. by culturing host cells under conditions for expression of the gene. In one
20 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 disclosure also provides, in certain embodiments, a
method which comprises using a construct as stated above in an expression
25 system in order to express a particular polypeptide such as a Wnt mimetic
molecule as described herein. The term "transduction" is used to refer to the
transfer of genes from one bacterium to another, usually by a phage.
"Transduction" also refers to the acquisition and transfer of eukaryotic cellular
sequences by retroviruses. The term "transfection" is used to refer to the uptake of
30 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.,
5 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
10 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
15 the division of the cell. The term "naturally occurring" or "native" when used in connection with biological materials such as nucleic acid molecules, polypeptides, host cells, and the like, refers to materials which are found in nature and are not manipulated by a human. Similarly, "non-naturally occurring" or "non-native" as used herein refers to a material that is not found in nature or that has been
20 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
25 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
30 and specifically non-recombinant cells, or genetically-engineered or recombinant cells, and comprise molecules having the amino acid sequence of the native

protein, or molecules having deletions from, additions to, and/or substitutions of one or more amino acids of the native sequence. The terms "polypeptide" and "protein" specifically encompass Wnt surrogate molecules, Fzd binding regions thereof, LRP5/6 binding regions thereof, antibodies and antigen-binding fragments thereof that bind to a Fzd receptor or a LRP5 or LRP6 receptor disclosed herein, or sequences that have deletions from, additions to, and/or substitutions of one or more amino acid of any of these polypeptides. Thus, a "polypeptide" or a "protein" can comprise one (termed "a monomer") or a plurality (termed "a multimer") of amino acid chains.

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

 Amino acid sequence modification(s) of any of the polypeptides (e.g., Wnt surrogate molecules or Fzd binding regions or LRP5/6 binding regions thereof) described herein are contemplated. For example, it may be desirable to improve the binding affinity and/or other biological properties of the Wnt surrogate

molecule. For example, amino acid sequence variants of a Wnt surrogate molecule may be prepared by introducing appropriate nucleotide changes into a polynucleotide that encodes the antibody, or a chain thereof, or by peptide synthesis. Such modifications include, for example, deletions from, and/or
5 insertions into and/or substitutions of, residues within the amino acid sequences of the antibody. Any combination of deletion, insertion, and substitution may be made to arrive at the final Wnt surrogate molecule, provided that the final construct possesses the desired characteristics (e.g., high affinity binding to one or more Fzd and/or LRP5/6 receptor). The amino acid changes also may alter post-translational
10 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 disclosure may be included in antibodies of the present disclosure.

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

In particular embodiments, the Wnt surrogate molecule or a binding region thereof, e.g., a Fab, scFv, or VHH or sdAb may comprise: a) a heavy chain variable region comprising: i. a CDR1 region that is identical in amino acid
30 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
5 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 receptor epitopes or LRP5 or LRP6 receptors). In a further embodiment, the antibody, or
10 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
15 more amino acid substitutions in the CDR regions of the selected antibody. Substitutions may be in CDRs either in the VH and/or the VL regions. (See, e.g., Muller, 1998, Structure 6:1153-1167).

In particular embodiments, the Wnt surrogate molecule or a binding region thereof, e.g., a Fab, scFv, or VHH or sdAb, may have: a) a heavy chain
20 variable region having an amino acid sequence that is at least 80% identical, at least 95% identical, at least 90%, at least 95% or at least 98% or 99% identical, to the heavy chain variable region of an antibody or antigen-binding fragments thereof described herein; and/or b) a light chain variable region having an amino acid sequence that is at least 80% identical, at least 85%, at least 90%, at least
25 95% or at least 98% or 99% identical, to the light chain variable region of an antibody or antigen-binding fragments thereof described herein. The amino acid sequence of illustrative antigen-binding fragments thereof are set forth in SEQ ID NOs:1-97 and 109-157.

A polypeptide has a certain percent "sequence identity" to another
30 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
5 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.
10 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
15 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
20 to be compared. Preferably, the sequence identity is determined using the default parameters determined by the program. This program is available also from Genetics Computing Group (GCG) package, from Madison, Wis., USA.

Another program of interest is the FastDB algorithm. FastDB is described in *Current Methods in Sequence Comparison and Analysis*,
25 *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.

30 In particular embodiments, the Wnt surrogate molecule or a binding region thereof, e.g., a Fab, scFv, or VHH or sdAb may comprise: a) a heavy chain

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

Determination of the three-dimensional structures of representative polypeptides (e.g., variant Fzd binding regions or LRP5/6 binding regions of Wnt surrogate molecules as provided herein) may be made through routine methodologies such that substitution, addition, deletion or insertion of one or more amino acids with selected natural or non-natural amino acids can be virtually modeled for purposes of determining whether a so derived structural variant retains the space-filling properties of presently disclosed species. See, for instance, Donate et al., 1994 *Prot. Sci.* 3:2378; Bradley et al., *Science* 309: 1868-1871 (2005); Schueler-Furman et al., *Science* 310:638 (2005); Dietz et al., *Proc. Nat. Acad. Sci. USA* 103:1244 (2006); Dodson et al., *Nature* 450:176 (2007); Qian et al., *Nature* 450:259 (2007); Raman et al. *Science* 327:1014-1018 (2010). Some additional non-limiting examples of computer algorithms that may be used for

these and related embodiments, such as for rational design of binding regions include VMD which is a molecular visualization program for displaying, animating, and analyzing large biomolecular systems using 3-D graphics and built-in scripting (see the website for the Theoretical and Computational Biophysics Group, University of Illinois at Urbana-Champaign, at ks.uiuc.edu/Research/vmd/). Many other computer programs are known in the art and available to the skilled person and which allow for determining atomic dimensions from space-filling models (van der Waals radii) of energy-minimized conformations; GRID, which seeks to determine regions of high affinity for different chemical groups, thereby enhancing binding, Monte Carlo searches, which calculate mathematical alignment, and CHARMM (Brooks et al. (1983) *J. Comput. Chem.* 4:187-217) and AMBER (Weiner et al (1981) *J. Comput. Chem.* 106: 765), which assess force field calculations, and analysis (see also, Eisenfield et al. (1991) *Am. J. Physiol.* 261:C376-386; Lybrand (1991) *J. Pharm. Belg.* 46:49-54; Froimowitz (1990) *Biotechniques* 8:640-644; Burbam et al. (1990) *Proteins* 7:99-111; Pedersen (1985) *Environ. Health Perspect.* 61:185-190; and Kini et al. (1991) *J. Biomol. Struct. Dyn.* 9:475-488). A variety of appropriate computational computer programs are also commercially available, such as from Schrödinger (Munich, Germany).

Compositions

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

In further embodiments, pharmaceutical compositions comprising a polynucleotide comprising a nucleic acid sequence encoding a Wnt surrogate molecule described herein and one or more pharmaceutically acceptable diluent, carrier, or excipient are also disclosed. In particular embodiments, the pharmaceutical composition further comprises one or more polynucleotides comprising a nucleic acid sequence encoding a Wnt polypeptide or Norrin polypeptide. In certain embodiments, the polynucleotides are DNA or mRNA, e.g.,

a modified mRNA. In particular embodiments, the polynucleotides are modified mRNAs further comprising a 5' cap sequence and/or a 3' tailing sequence, e.g., a polyA tail. In other embodiments, the polynucleotides are expression cassettes comprising a promoter operatively linked to the coding sequences. In certain
5 embodiments, the nucleic acid sequence encoding the Wnt surrogate molecule and the nucleic acid sequence encoding the Wnt polypeptide or Norrin polypeptide are present in the same polynucleotide.

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

 The present disclosure further contemplates a pharmaceutical
20 composition comprising a cell comprising an expression vector comprising a polynucleotide comprising a promoter operatively linked to a nucleic acid encoding a Wnt surrogate molecule and one or more pharmaceutically acceptable diluent, carrier, or excipient. In particular embodiments, the pharmaceutical composition further comprises a cell comprising an expression vector comprising a
25 polynucleotide comprising a promoter operatively linked to a nucleic acid sequence encoding a Wnt polypeptide or a Norrin polypeptide. In certain embodiments, the nucleic acid sequence encoding the Wnt surrogate molecule and the nucleic acid sequence encoding the Wnt polypeptide or Norrin polypeptide are present in the same polynucleotide, e.g., expression cassette and/or in the same cell. In
30 particular embodiments, the cell is a heterologous cell or an autologous cell

obtained from the subject to be treated. In particular embodiments, the cell is a stem cell, e.g., an adipose-derived stem cell or a hematopoietic stem cell.

The present disclosure contemplates pharmaceutical compositions comprising a first molecule for delivery of a Wnt surrogate molecule as a first active agent and a second molecule for delivery of a Wnt polypeptide or Norrin polypeptide. The first and second molecule may be the same type of molecule or different types of molecules. For example, in certain embodiments, the first and second molecule may each be independently selected from the 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, propylene glycol or other synthetic solvents; antibacterial compounds such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating compounds such as ethylenediaminetetraacetic acid (EDTA); buffers such as acetates, citrates or phosphates; detergents such as Tween 20 to prevent aggregation; and compounds for the adjustment of tonicity such as sodium chloride or dextrose. The

pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. In particular embodiments, the pharmaceutical compositions are sterile.

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

Sterile solutions can be prepared by incorporating the anti-Fzd antibody or antigen-binding fragment thereof (or encoding polynucleotide or cell comprising the same) in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, methods of preparation are vacuum drying and freeze-drying that yields a powder of the active ingredient

plus any additional desired ingredient from a previously sterile-filtered solution thereof.

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

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

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

The pharmaceutical compositions of the present disclosure 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 disclosure includes pharmaceutically acceptable salts of a Wnt surrogate molecule described herein. The term “pharmaceutically acceptable salt” refers to physiologically and pharmaceutically acceptable salts of the compounds of the present disclosure: 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, chlorprocaine, choline, diethanolamine, dicyclohexylamine, ethylenediamine, N-methylglucamine, and procaine (see, for example, Berge et al., “Pharmaceutical Salts,” J. Pharma Sci., 1977, 66, 119). The base addition salts of said acidic compounds are prepared by contacting the free acid form with a sufficient amount of the desired base to produce the salt in the conventional manner. The free acid form may be regenerated by contacting the salt form with an acid and isolating the free acid in the conventional manner. The free acid forms differ from their respective salt forms somewhat in certain physical properties such as solubility in polar solvents, but otherwise the salts are equivalent to their respective free acid for purposes of the present disclosure.

In some embodiments, the pharmaceutical composition provided herein comprise a therapeutically effective amount of a Wnt surrogate molecule or pharmaceutically acceptable salt thereof in admixture with a pharmaceutically acceptable carrier, diluent and/or excipient, for example saline, phosphate buffered

saline, phosphate and amino acids, polymers, polyols, sugar, buffers, preservatives and other proteins. Exemplary amino acids, polymers and sugars and the like are octylphenoxy polyethoxy ethanol compounds, polyethylene glycol monostearate compounds, polyoxyethylene sorbitan fatty acid esters, sucrose, fructose, dextrose, maltose, glucose, mannitol, dextran, sorbitol, inositol, galactitol, xylitol, lactose, trehalose, bovine or human serum albumin, citrate, acetate, Ringer's and Hank's solutions, cysteine, arginine, carnitine, alanine, glycine, lysine, valine, leucine, polyvinylpyrrolidone, polyethylene and glycol. Preferably, this formulation is stable for at least six months at 4° C.

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

Methods of Use

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

Agonizing Wnt Pathway Signaling and Related Therapeutic Methods

25 In certain embodiments, a Wnt surrogate molecule may be used to agonize a Wnt signaling pathway in a tissue or a cell. Agonizing the Wnt signaling pathway may include, for example, increasing Wnt signaling or enhancing Wnt signaling in a tissue or cell. Thus, in some aspects, the present disclosure provides a method for agonizing a Wnt signaling pathway in a cell, comprising contacting the tissue or cell with an effective amount of a Wnt surrogate molecule or pharmaceutically acceptable salt thereof disclosed herein, wherein the a Wnt

surrogate molecule is a Wnt signaling pathway agonist. In some embodiments, contacting occurs in vitro, ex vivo, or in vivo. In particular embodiments, the cell is a cultured cell, and the contacting occurs in vitro. In certain embodiments, the method comprises further contacting the tissue or cell with one or more Wnt

5 polypeptides or Norrin polypeptides.

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

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

30 In related aspects, the present disclosure provides a method for agonizing Wnt signaling in a tissue, comprising contacting the tissue with an effective

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

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

tissue repair, healing or regeneration. In some embodiments, contacting occurs in vivo, i.e., the subject composition is administered to a subject.

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

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

and the nucleic acid sequence encoding the Wnt polypeptide or Norrin polypeptide are present in the same polynucleotide.

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

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

embodiments, the cell is a stem cell, e.g., an adipose-derived stem cell or a hematopoietic stem cell.

Wnt signaling plays key roles in the developmental process and maintenance of stem cells. Reactivation of Wnt signals is associated with
5 regeneration and repair of most tissues after injuries and diseases. Wnt surrogate molecule molecules are expected to provide benefit of healing and tissue repair in response to injuries and diseases. Causes of tissue damage and loss include but are not limited to aging, degeneration, hereditary conditions, infection and inflammation, traumatic injuries, toxins/metabolic-induced toxicities, or other
10 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 present disclosure 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.

15 Human diseases associated with mutations of the Wnt pathway provide strong evidence for enhancement of Wnt signals in the treatment and prevention of diseases. Preclinical in vivo and in vitro studies provide additional evidence of involvement of Wnt signals in many disease conditions and further support utilization of a Wnt surrogate molecule in various human diseases. For
20 example, compositions of the present disclosure may be used to promote or increase bone growth or regeneration, bone grafting, healing of bone fractures, stress fractures, vertebral compression fractures, treatment of osteoporosis and osteoporotic fractures, spinal fusion, osseointegration of orthopedic devices, tendon-bone integration, tooth growth and regeneration, dental implantation,
25 periodontal diseases, maxillofacial reconstruction, and osteonecrosis of the jaw. They may also be used in the treatment of alopecia; enhancing regeneration of sensory organs, e.g. treatment of hearing loss, treatment of vestibular hypofunction, treatment of macular degeneration, treatment of vitreoretinopathy, other diseases of retinal degeneration, Fuchs' dystrophy, other cornea disease,
30 etc.; treatment of stroke, traumatic brain injury, Alzheimer's disease, multiple sclerosis, muscular dystrophy, muscle atrophy caused by sarcopenia or cachexia,

and other conditions affecting the blood brain barrier; treatment of spinal cord injuries, other spinal cord diseases. The compositions of this present disclosure 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, dyslipidemia, 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), idiopathic pulmonary fibrosis (IPF), enhanced regeneration of lung tissues. The compositions of the present disclosure 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, alcoholic hepatitis, non-alcoholic liver diseases with steatosis or steatohepatitis (NASH), and the like. The compositions of this present disclosure may treat diseases and disorders including, without limitation, conditions in which regenerative cell growth is desired.

Human genetics involving loss-of-function or gain-of-function mutations in Wnt signaling components show strong evidence supporting enhancing Wnt signals for bone growth. Conditions in which enhanced bone growth is desired may include, without limitation, fractures, grafts, ingrowth around prosthetic devices, osteoporosis, osteoporotic fractures, spinal fusion, osteonecrosis of the jaw, dental implantation, periodontal diseases, maxillofacial reconstruction, and the like. Wnt surrogate molecules enhance and promotes Wnt signals which are critical in promoting bone regeneration. Methods for regeneration

of bone tissues benefit from administration of the compounds of the present disclosure, which can be systemic or localized. In some embodiments, bone marrow cells are exposed to molecules of the present disclosure, such that stem cells within that marrow become activated.

5 In some embodiments, bone regeneration is enhanced by contacting a responsive cell population, e.g., bone marrow, bone progenitor cells, bone stem cells, etc. with an effective dose of a Wnt surrogate molecule disclosed herein. Methods for regeneration of bone tissues benefit from administration of the Wnt surrogate molecule which can be systemic or localized. In some such
10 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,
15 polymeric microspheres, nanoparticles, bone cements, and the like.

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

Fzd 8, and also LRP5 and/or LRP6, are used to treat or prevent any bone disease or disorder.

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

Compositions comprising one or more Wnt surrogate molecule
15 disclosed herein (or a polynucleotide encoding a Wnt surrogate molecule, or a vector or cell comprising a polynucleotide encoding a Wnt surrogate molecule) can be used for the in vivo treatment of skeletal tissue deficiencies. By "skeletal tissue deficiency", it is meant a deficiency in bone or other skeletal connective tissue at any site where it is desired to restore the bone or connective tissue, no matter how
20 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 disclosure can be used as part of a regimen for restoring cartilage function to a connective tissue, for the repair of defects or lesions in cartilage tissue such as degenerative wear and arthritis,
25 trauma to the tissue, displacement of torn meniscus, meniscectomy, a luxation of a joint by a torn ligament, malalignment of joints, bone fracture, or by hereditary disease.

A Wnt surrogate molecule may also be used for treatment of periodontal diseases. Periodontal diseases are a leading cause of tooth loss and
30 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
5 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
10 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 present disclosure. 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
15 utricle, and the saccule, also contain sensory hair cells in order to detect head position and motion. Compositions of the present disclosure can be used, for example, in an infusion; in a matrix or other depot system; or other topical application to the ear for enhancement of auditory regeneration.

A Wnt surrogate molecule may also be used in regeneration of retinal
20 tissue. In the adult mammalian retina, Muller glia cells are capable of regenerating retinal cells, including photoreceptors, for example after neurotoxic injury in vivo. Wnt signaling and enhancers of Wnt signals can promote proliferation of Muller glia-derived retinal progenitors after damage or during degeneration. The compositions of the present disclosure may also be used in the regeneration of
25 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 disclosure. AMD is characterized by progressively decreased central vision and visual acuity. Fuchs' dystrophy is characterized by
30 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 disclosure 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.

5 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
10 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.

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

 In particular embodiments, the compositions of this disclosure may
25 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
30 regeneration of liver tissue benefit from administration of the compounds of the present disclosure, 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.

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

20 Wnt signals play an important role in regeneration of various epithelial tissues. Various epidermal conditions benefit from treatment with the compounds of the present disclosure. 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
25 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 present disclosure may also benefit treatment of short bowel syndrome,
30 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 disclosure may be used in all these conditions, where regenerative cells are contacted with compounds of the present disclosure. Methods for regeneration of epithelial tissues benefit from administration of the compounds of the present disclosure, which can be systemic or localized. Contacting can be, for example, topical, including intradermal, subdermal, in a gel, lotion, cream etc. applied at targeted site, etc.

In addition to skin and gastrointestinal tract, Wnt signals and enhancement and promotion of Wnt signals also play an important role in repair and regeneration of tissues including pancreas, kidney, and lung in preclinical models. A Wnt surrogate molecule may benefit various disease conditions involving exocrine and endocrine pancreas, kidney, or lung. The Wnt surrogate molecules may be used in treatment of metabolic syndrome; treatment of diabetes, treatment of acute or chronic pancreatitis, exocrine pancreatic insufficiency, treatment of acute kidney injuries, chronic kidney diseases, treatment of lung diseases, including but not limited to chronic obstructive pulmonary diseases (COPD), other conditions that cause loss of lung epithelial tissues. Methods for regeneration of these tissues benefit from administration of the compounds of the present disclosure, 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 disclosure. In some such embodiments, the contacting is performed in vivo. In other such embodiments, the contacting is performed ex vivo. The molecule may be localized to the site of action, e.g. topical lotions, gels, creams and the like.

Stroke, traumatic brain injury, Alzheimer's disease, multiple sclerosis and other conditions affecting the blood brain barrier (BBB) may be treated with a Wnt surrogate molecule. Angiogenesis is critical to ensure the supply of oxygen

and nutrients to many tissues throughout the body, and is especially important for the CNS as the neural tissue is extremely sensitive to hypoxia and ischemia. CNS endothelial cells which form the BBB differ from endothelial cells in non-neural tissue, in that they are highly polarized cells held together by tight junctions and
5 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 present disclosure, 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
10 involved in neurogenesis and plays a role of neuroprotection following injury. The compositions of the present disclosure may also be used in treatment of spinal cord injuries, other spinal cord diseases, stroke, traumatic brain injuries, etc.

Wnt signals also play a role in angiogenesis. A Wnt surrogate molecule may benefit conditions where angiogenesis is beneficial, treatment of
15 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 present disclosure, which can be systemic or localized.

In certain embodiments, methods of the present disclosure promote
20 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
25 enables connections between cells of the tissue to be re-established, and more preferably the functionality of the tissue to be regained.

The terms "administering" or "introducing" or "providing", as used herein, refer to delivery of a composition to a cell, to cells, tissues and/or organs of a subject, or to a subject. Such administering or introducing may take place in vivo,
30 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 present disclosure may be practiced in vivo or ex vivo. In some embodiments, the contacting of a target cell or tissue with a Wnt surrogate molecule is performed ex vivo, with subsequent implantation of the cells or tissues, e.g., activated stem or progenitor cells, into the subject. The skilled artisan can determine an appropriate site of and route of administration based on the disease or disorder being treated.

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

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

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

Other embodiments relate, in part, to the use of the Wnt surrogate molecules disclosed herein to promote or enhance the growth or proliferation of cells, tissues and organoids, for example, by contacting cells or tissue with one or more Wnt surrogate, optionally in combination with a Norrin or Rspodin 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 present disclosure 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
5 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
10 disclosure provides a cell culture medium comprising one or more Wnt surrogate molecules disclosed herein. In some embodiments, the cell culture medium may be any cell culture medium already known in the art that normally comprises Wnt or Rspondin, but wherein the Wnt or Rspondin is replaced (wholly or partially) or supplemented by Wnt surrogate molecule(s) disclosed herein. For example, the
15 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.
20 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
25 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
30 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) 5 EGF and FGF7; vi) EGF and a FGF; vii) TGF-alpha and KGF; viii) TGF-alpha, and FGF7; ix) or from TGF-alpha and a FGF. In certain embodiments, the disclosure includes a stem cell culture media comprising a Wnt surrogate molecule disclosed herein, e.g., optionally in combination with one or more of the growth factors or combinations thereof described herein.

10 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., 2011 (GASTROENTEROLOGY 2011; 141: 1762-1772) and Sato et al., 2009 (Nature 459, 262-5).

 In some embodiments, the Wnt surrogate molecules are used to 15 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. Patent 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.

20 Other embodiments of the present disclosure relate, in part, to diagnostic applications for detecting the presence of cells or tissues expressing one or more Fzd receptors or LRP5 or LRP6 receptors. Thus, the present disclosure provides methods of detecting one or more Fzd receptors or LRP5 or LRP6 receptors in a sample, such as detection of cells or tissues expressing Fzd1. 25 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), e.g., by detecting binding of a Wnt 30 surrogate molecule.

ISH is a type of hybridization that uses a labeled complementary DNA or RNA strand (i.e., primary binding agent) to localize a specific DNA or RNA sequence in a portion or section of a cell or tissue (*in situ*), or if the tissue is small enough, the entire tissue (whole mount ISH). One having ordinary skill in the art
5 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
10 measure and localize mRNAs and other transcripts within tissue sections or whole mounts.

In various embodiments, the Wnt surrogate molecules described herein are conjugated to a detectable label that may be detected directly or indirectly. In this regard, an antibody "conjugate" refers to a Wnt surrogate
15 molecule that is covalently linked to a detectable label. In the present disclosure, 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
20 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
25 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
30 several different detectable labels conjugated to different specific-antibodies can be used in combination to detect one or more targets.

Examples of detectable labels, which may be detected directly, include fluorescent dyes and radioactive substances and metal particles. In contrast, indirect detection requires the application of one or more additional antibodies, i.e., secondary antibodies, after application of the primary antibody.

5 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.

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

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

Examples of fluorescent labels include 5-(and 6)-carboxyfluorescein,
20 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
25 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
30 dyes, or polymer micelles or capsules which contain dyes, enzymes or substrates.

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

Examples of commonly used substrates for Alkaline Phosphatase include Naphthol-AS-B 1-phosphate/fast red TR (NABP/FR), Naphthol-AS-MX-phosphate/fast red TR (NAMP/FR), Naphthol-AS-B1-phosphate/- fast red TR (NABP/FR), Naphthol-AS-MX-phosphate/fast red TR (NAMP/FR), Naphthol-AS-B1-phosphate/new fuchsin (NABP/NF), bromochloroindolyl phosphate/nitroblue tetrazolium (BCIP/NBT), 5-Bromo-4-chloro-3-indolyl-beta-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 iodine, cobalt, selenium, tritium, carbon, sulfur and phosphorous.

Detectable labels may be linked to the antibodies described herein or to any other molecule that specifically binds to a biological marker of interest, e.g., an antibody, a nucleic acid probe, or a polymer. Furthermore, one of ordinary skill in the art would appreciate that detectable labels can also be conjugated to

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

The present disclosure further provides kits for detecting one or more Fzd or LRP5/6 receptor or cells or tissues expressing one or more Fzd or LRP5/6 receptors in a sample, wherein the kits contain at least one antibody, polypeptide, polynucleotide, vector or host cell as described herein. In certain embodiments, a kit may comprise buffers, enzymes, labels, substrates, beads or other surfaces to which the antibodies of the present disclosure 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 present disclosure have been described herein for purposes of illustration, various modifications may be made without deviating from the spirit and scope of the present disclosure. Accordingly, the present disclosure is not limited except as by the appended claims.

EXAMPLES

Example 1

Multispecific Wnt Surrogate Molecules

A number of multispecific Wnt surrogate molecules representing different configurations were produced, as further described in the following Examples. These included the Wnt surrogate molecules disclosed in Table 5 below, which comprise the sequences set forth in SEQ ID NOs:109-157. The specific Fzd and LRP binding elements used for Wnt surrogate molecules presented in these examples are listed in Tables 1A and 1B and 2A and 2B.

Table 5. Wnt surrogate molecule sequences

Lrp VHH or sdAb = italics

anti-Fzd light chain = underline

anti-Fzd heavy chain = bold

Name	SEQ ID NO	Sequence
R2M3-3 LC	109	MDMRVPAQLLGLLLLWLRGARCDVQLVESGGGLVQPGGSLRLSCTSSANINSIE TLGWYRQAPGKQRELIANMRGGGYMKYAGSLKGRFTMSTESAKNTMYLQMNS LKPEDTAVYYCYVKLRDDDYVYRGQGTQVTVSSGSGSGQAVVLQEPSSLVSPG GTVTLTCLGSSGSVSTNYYPSWYQQTPGQAPRTLIIYTNTRSSDVPERFSGSIV GNKAALTITGAQPDDSVYFCLLYLGRGIWVFGGGTKLTVLGQPKAAPSVTLFP PSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTPSKQSNKYA ASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS
R2M3-3 HC	110	MDMRVPAQLLGLLLLWLRGARCEVQLVQSGAEVKKPGASVKVSCKASGYTFT SYGISWVRQAPGGGLEWMGWISAYNGNTNYAQKLQGRVTMTTDTSTSTAYM ELRSLRSDDTAVYYCASSKEKATYYGMDVWGQGTITVTVSSASTKGPSVFPL APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLY SLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPE AAGGPSVFLFPPKPKDITLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHN AKTKPREEQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAK KGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNY KTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSL SPGK
1291-3 LC	111	MDMRVPAQLLGLLLLWLRGARCDVQLVESGGGLVQPGGSLRLSCTSSANINSIE TLGWYRQAPGKQRELIANMRGGGYMKYAGSLKGRFTMSTESAKNTMYLQMNS LKPEDTAVYYCYVKLRDDDYVYRGQGTQVTVSSGSGSGDVVMSQSPSSLAVS VGEKVTMSCKSSQSLLYSSNQKNYLAWYQQKPGQSPKLLIYWASTRESGVPR FTGSGSGTDFTLTISSVKAKDLAVYYCQQYYSYPTFGGGTKLEIKRTVAAPSVFIF PPSDEQLKSGTASVCLLNFFYPREAKVQWKVDNALQSGNSQESVTEQDSKD STYLSLSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
1291-3 HC	112	MDMRVPAQLLGLLLLWLRGARCEVQLVESGGGLVQPGGSLKLSCAASGFTFS SYAMSWVRQTPEKRLWVATISDGGSYTRYPDCLKGRFTISRDNANKNNLYLQ MSHLKSEDTAMYYCARVGGRRDYFDYWGQGTTLTVSSASTKGPSVFPLAPSS KSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSV VTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGP SVFLFPPKPKDITLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKP REEQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQPR EPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPV LDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK

Name	SEQ ID NO	Sequence
1791-3 LC	113	MDMRVPAQLLGLLLLWLRGARCDVQLVESGGGLVQPGGSLRLSCTSSANINSIE TLGWYRQAPGKQRELIANMRGGGYMKYAGSLKGRFTMSTESAKNTMYLQMNS LKPEDTAVYYCYVKLRDDDDYVYRGQGTQVTVSSGSGSGDIQMTQSPKSSMSMS VGERVTLRCKASENVLYVSWYQQKPEQSPKLLIYGASNRYTGVPDRFTGSGS ATDFTLTISVQAEDLADYHCGQSYRYPTFGAGTKLELKRTVAAPSVFIFPPSDE QLKSGTASVVCLLNNFYPRKAVQWKVDNALQSGNSQESVTEQDSKIDSTYLSL STLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
1791-3 HC	114	MDMRVPAQLLGLLLLWLRGARCEVQPVESGGGLVQPKGSLKLSAASGFTFN TYAMHWVRQAPGKGLEWVARIRSKSNYAKNYDDSVKDRFTISRDDSQSMYLY LQMNNLKTEDTAMYYCVRENYGGRFDYWGQGTTLTVSSASTKGPSVFPLAPS SKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSS VVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGG PSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTK PREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQP REPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTP VLDSGDSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK
4SD1-3 LC	115	MDMRVPAQLLGLLLLWLRGARCDVQLVESGGGLVQPGGSLRLSCTSSANINSIE TLGWYRQAPGKQRELIANMRGGGYMKYAGSLKGRFTMSTESAKNTMYLQMNS LKPEDTAVYYCYVKLRDDDDYVYRGQGTQVTVSSGSGSGDIQMTQSPSSLSASV GDRVITICRASQSISSYLNWYQQKPKGAPKLLIYAASSLQSGVPSRFSGSGSGT DFTLTISLQPEDFATYYCQQSYSTPLTFGGGTKEIKRTVAAPSVFIFPPSDEQL KSGTASVVCLLNNFYPRKAVQWKVDNALQSGNSQESVTEQDSKIDSTYLSLST LTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
4SD1-3HC	116	MDMRVPAQLLGLLLLWLRGARCEVQLVESGGGLVQPKGSLRLSCAASGFTFT NYAMSWVRQAPGKGLEWVSAISGSGGSTYYADSVKGRFTISRDDSNTLYLQ MNSLKTEDTAVYYCARATGFGTVVFDYWGQGTTLTVSSASTKGPSVFPLAPS SKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSS VVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGG PSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTK PREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQP REPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTP VLDSGDSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK
14SB6-3 LC	117	MDMRVPAQLLGLLLLWLRGARCDVQLVESGGGLVQPGGSLRLSCTSSANINSIE TLGWYRQAPGKQRELIANMRGGGYMKYAGSLKGRFTMSTESAKNTMYLQMNS LKPEDTAVYYCYVKLRDDDDYVYRGQGTQVTVSSGSGSGDIQMTQSPSSLSASV GDRVITICRASQSISSYLNWYQQKPKGAPKLLIYAASSLQSGVPSRFSGSGSGT DFTLTISLQPEDFATYYCQQSYSTPLTFGGGTKEIKRTVAAPSVFIFPPSDEQL KSGTASVVCLLNNFYPRKAVQWKVDNALQSGNSQESVTEQDSKIDSTYLSLST LTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
14SB6-3 HC	118	MDMRVPAQLLGLLLLWLRGARCEVQLVQSGGGGLVQPKGSLRLSCAASGFTFS SYSMNWVRQAPGKGLEWVSYIENDGSITTYADSVKGRFTISRDDSKNTLYLQM NSLKTEDTAVYYCARAPYYYGSGSLFRLDYWGQGTTLTVSSASTKGPSVFPL APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLY SLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPE AAGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHN AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKA KGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNY KTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSL SPGK
4SD1-3 LC	119	MDMRVPAQLLGLLLLWLRGARCDVQLVESGGGLVQPGGSLRLSCTSSANINSIE TLGWYRQAPGKQRELIANMRGGGYMKYAGSLKGRFTMSTESAKNTMYLQMNS LKPEDTAVYYCYVKLRDDDDYVYRGQGTQVTVSSGSGSGDIQMTQSPSSLSASV GDRVITICRASQSISSYLNWYQQKPKGAPKLLIYAASSLQSGVPSRFSGSGSGT DFTLTISLQPEDFATYYCQQSYSTPLTFGGGTKEIKRTVAAPSVFIFPPSDEQL KSGTASVVCLLNNFYPRKAVQWKVDNALQSGNSQESVTEQDSKIDSTYLSLST LTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

Name	SEQ ID NO	Sequence
4SD1-3 HC-holes	120	MDMRVPAQLLGLLLLWLRGARCEVQLVESGGGLVKPGGSLRLSCAASGFTFT NYAMSWVRQAPGKGLEWVSAISGGSTYYADSVKGRFTISRDDSKNTLYLQ MNSLKTEDTAVYYCARATGFGTVVFDYWGGGTLTVSSASTKGPSVFPLAPS SKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSS VVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGG PSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTK PREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQP REPQVYTLPPSREEMTKNQVSLSCAVKGFYPSPDIAVEWESNGQPENNYKTPP VLDSGDSFFLVSKLTVDKSRWQQGNVFSCSVMEALHNHYTQKSLSLSPGKG SGSGHHHHHH
14SB6-3 LC	121	MDMRVPAQLLGLLLLWLRGARCDVQLVESGGGLVQPGGSLRLSCTSSANINSI TLGWYRQAPGKQRELIANMRGGGYMKYAGSLKGRFTMSTESAKNTMYLQMNS LKPEDTAVYYCYVKLRDDDYVYRGQGTQVTVSSGSGSGDIQMTQSPSSLSASV GDRVTITCRASQSISSYLNWYQQKPKGAPKLLIYAASSLQSGVPSRFSGSGSGT DFTLTISLQPEDFATYYCQQSYSTPLTFGGGTKEIKRTVAAPSVFIFPPSDEQL KSGTASVCLLNFFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSLSS LTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
14SB6-3 HC-knobs	122	MDMRVPAQLLGLLLLWLRGARCEVQLVQSGGGGLVKPGGSLRLSCAASGFTFS SYSMNWVRQAPGKGLEWVSYIENDGSITTYADSVKGRFTISRDDSKNTLYLQM NSLKTEDTAVYYCARAPYYYGSGSLFRLDYWGQGTTLTVSSASTKGPSVFPL APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLY SLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPE AAGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHN AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKA KGQPREPQVYTLPPSREEMTKNQVSLWCLVKGFYPSPDIAVEWESNGQPENNY KTPPVLDSDGDSFFLYSKLTVDKSRWQQGNVFSCSVMEALHNHYTQKSLSL SPGKGSGSGDYKDDDDK
Fc-knobs	123	MDMRVPAQLLGLLLLWLRGARCEFDKTHTCPPCPAPEAAGGPSVFLFPPKPKD TLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV VSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQPREPQVYTLPPSRE EMTKNQVSLWCLVKGFYPSPDIAVEWESNGQPENNYKTPPVLDSDGDSFFLYSK LTVDKSRWQQGNVFSCSVMEALHNHYTQKSLSLSPGKGSGSGDYKDDDDK
Fc-holes	124	MDMRVPAQLLGLLLLWLRGARCEFDKTHTCPPCPAPEAAGGPSVFLFPPKPKD TLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV VSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQPREPQVYTLPPSRE EMTKNQVSLSCAVKGFYPSPDIAVEWESNGQPENNYKTPPVLDSDGDSFFLVSKL TVDKSRWQQGNVFSCSVMEALHNHYTQKSLSLSPGKGSGSGHHHHHHH
4SC10-3 LC	125	MDMRVPAQLLGLLLLWLRGARCDVQLVESGGGLVQPGGSLRLSCTSSANINSI <u>ETL</u> <u>GWYRQAPGKQRELIANMRGGGYMKYAGSLKGRFTMSTESAKNTMYLQM</u> <u>NSLKPEDTAVYYCYVKLRDDDYVYRGQGTQVTVSSGSGSGDIQMTQSPSSLS</u> <u>ASVGDRVTITCRASQSISSYLNWYQQKPKGAPKLLIYAASSLQSGVPSRFSGSG</u> <u>SGTDFTLTISLQPEDFATYYCQQSYSTPLTFGGGTKEIKRTVAAPSVFIFPPSD</u> <u>EQLKSGTASVCLLNFFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSL</u> <u>SSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC</u>
4SC10-3 HC	126	MDMRVPAQLLGLLLLWLRGARCQVQLVQSGAEVKKPGASVKVSCKASGYIFT DYMHVVRQAPGGGLEWMGLVDPEDGETIYAEKFQGRVTMTRDTSTSTVYM ELSSLRSEDVAVYYCAHSDFSGLSFGDWGQGTTLTVSSASTKGPSVFPLAPS SKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSS VVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGG PSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTK PREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQP REPQVYTLPPSREEMTKNQVSLTCLVKGFYPSPDIAVEWESNGQPENNYKTPP VLDSGDSFFLYSKLTVDKSRWQQGNVFSCSVMEALHNHYTQKSLSLSPGK

Name	SEQ ID NO	Sequence
heterolg 4SC10- 3+1RC07-3 LC1	127	MDMRVPAQLLGLLLLWLRGARCQVQLVESGGGLVQPGGSLRLSCTSSANINSIE TLGWYRQAPGKQRELIANMRGGGYMKYAGSLKGRFTMSTESAKNTMYLQMNS LKPEDTAVYYCYVKLRDDDDYVYRGQGTQVTVSSGSGSGSDIQMTQSPSSLSASV GDRVITICRASQSISSYLNWYQKKPGKKPKLLIYAASSLQSGVPSRFSGSGSGT DFTLTISLQPEDFATYYCQYSYSTPLTFGGGKVEIKRTVAAPSVFIFPPSDEQL KSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSTYSLEST LTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
heterolg 4SC10- 3+1RC07-3 HC1	128	MDMRVPAQLLGLLLLWLRGARCQVQLVQSGAEVKKPGASVKVSCKASGYIFT DYMHWRDAPGQGLEWMGLVDPEDGETIYAEKFQGRVTMTRDTSTSTVYM ELSSLRSEDVAVYYCAHSDFFSGLSFGDWGDGLTVTVSSASTKGPSVFPLAPS SKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLKS VVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGG PSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTK PREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQP REPQVYTLPPSREEMTKNQVSLSCAVKGFYPDSIAVEWESNGQPENNYKTPP VLDSGDSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKG SGSGHHHHHH
heterolg 4SC10- 3+1RC07-3 LC2	129	MDMRVPAQLLGLLLLWLRGARCQVQLVESGGGLVQPGGSLRLSCTSSANINSIE TLGWYRQAPGKQRELIANMRGGGYMKYAGSLKGRFTMSTESAKNTMYLQMNS LKPEDTAVYYCYVKLRDDDDYVYRGQGTQVTVSSGSGSGSYVLTQPPSVSVSPG QTASITCSGDKVGHKYASWYQDKPGQDPVLVIYEDSQRPSGIPVRFSGNSGN TATLTISGTQAMDEADYYCAWDSSTDVVFGGGKLTVLGQPKAAPSVTLFPPS SEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTPSKQSNNKYAAK SYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS
heterolg 4SC10- 3+1RC07-3 HC2	130	MDMRVPAQLLGLLLLWLRGARCQVQLQWAGALLKPSETLSLTCAVSGASFS GHYWTWIRKPPGKGLEWIGEIDHTGSTNYEPSLRSRVTISVDTSKNQFSLNLKS VTAADTAVYYCARGGQGGYDWGHYHGLDVWGKGTITVTVSSASTKGPSVFPL APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLY SLESVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPE AAGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHN AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKA KGQPREPQVYTLPPSREEMTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNY KTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL SPGKGGSGGDYKDDDDK
1RC07-3 monovalent bispecific LC1	129	MDMRVPAQLLGLLLLWLRGARCQVQLVESGGGLVQPGGSLRLSCTSSANINSIE TLGWYRQAPGKQRELIANMRGGGYMKYAGSLKGRFTMSTESAKNTMYLQMNS LKPEDTAVYYCYVKLRDDDDYVYRGQGTQVTVSSGSGSGSYVLTQPPSVSVSPG QTASITCSGDKVGHKYASWYQDKPGQDPVLVIYEDSQRPSGIPVRFSGNSGN TATLTISGTQAMDEADYYCAWDSSTDVVFGGGKLTVLGQPKAAPSVTLFPPS SEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTPSKQSNNKYAAK SYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS
1RC07-3 monovalent bispecific HC	130	MDMRVPAQLLGLLLLWLRGARCQVQLQWAGALLKPSETLSLTCAVSGASFS GHYWTWIRKPPGKGLEWIGEIDHTGSTNYEPSLRSRVTISVDTSKNQFSLNLKS VTAADTAVYYCARGGQGGYDWGHYHGLDVWGKGTITVTVSSASTKGPSVFPL APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLY SLESVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPE AAGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHN AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKA KGQPREPQVYTLPPSREEMTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNY KTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL SPGKGGSGGDYKDDDDK
1RC07-3 monovalent bispecific Fc	131	MDMRVPAQLLGLLLLWLRGARCEFDKTHTCPPCPAPEAAGGPSVFLFPPKPKD TLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV VSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQPREPQVYTLPPSR EEMTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLV SKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKGSGSGHHHHHH

Name	SEQ ID NO	Sequence
R2M3-26 LC	132	MDMRVPAQLLGLLLLWLRGARCDVQLVESGGGLVQAGGSLRLACAGSGRIFAI YDIAWYRHPPGNQREL VAMIRPVVTEIDYADSVKGRFTISRNNAMKTVYLQMN LKPEDTAVYYCNAKRPWGS RDEYWGQGTQVTVSSGSGSGQAVVLQEPSLSVS PGGTVTLTCGLSSGSVSTNYYP SWYQQTPGQAPRTLIIYTNTRSSDVPERFSG SIVGNKAALTITGAQPDDSVYFCLLYLGRGIWWFGGGTKLTVLGQPKAAPSVTL FPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTTPSKQSNK YAASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS
R2M3-26 HC	110	MDMRVPAQLLGLLLLWLRGARCEVQLVQSGAEVKKPGASVKVSCKASGYTFT SYGISWVRQAPGQGLEWMGWISAYNGNTNYAQKLQGRVTMTTDTSTSTAYM ELRSLRSDDTAVYYCASSKEKATYYGMDVWGQGTQVTVSSASTKGPSVFPL APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLY SLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPE AAGGPSVFLFPPKPKD TLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHN AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYCKVSNKALGAPIEKTISKA KGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNY KTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL SPGK
R2M3mut-26 LC	132	MDMRVPAQLLGLLLLWLRGARCDVQLVESGGGLVQAGGSLRLACAGSGRIFAI YDIAWYRHPPGNQREL VAMIRPVVTEIDYADSVKGRFTISRNNAMKTVYLQMN LKPEDTAVYYCNAKRPWGS RDEYWGQGTQVTVSSGSGSGQAVVLQEPSLSVS PGGTVTLTCGLSSGSVSTNYYP SWYQQTPGQAPRTLIIYTNTRSSDVPERFSG SIVGNKAALTITGAQPDDSVYFCLLYLGRGIWWFGGGTKLTVLGQPKAAPSVTL FPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTTPSKQSNK YAASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS
R2M3mut-26 HC	133	MDMRVPAQLLGLLLLWLRGARCEVQLVQSGAEVKKPGASVKVSCKASGYTFT SYGISWVRQAPGQGLEWMGWISAYNGNTNYAQKLQGRVTMTTDTSTSTAYM ELRSLRSDDTAVYYCASSKEKATYYAGMDVWGQGTQVTVSSASTKGPSVFPL APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLY SLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPE AAGGPSVFLFPPKPKD TLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHN AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYCKVSNKALGAPIEKTISKA KGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNY KTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL SPGKGSGSGHHHHHH
R2M3-3 LC	109	MDMRVPAQLLGLLLLWLRGARCDVQLVESGGGLVQPGGSLRLSCTSSANINSIE TLGWYRQAPGKQRELIANMRGGGYMKYAGSLKGRFTMTESAKNTMYLQMNS LKPEDTAVYYCYVKLRDDDDYVYRGQGTQVTVSSGSGSGQAVVLQEPSLSVSPG GTVTLTCGLSSGSVSTNYYP SWYQQTPGQAPRTLIIYTNTRSSDVPERFSGSIV GNKAALTITGAQPDDSVYFCLLYLGRGIWWFGGGTKLTVLGQPKAAPSVTLFP PSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTTPSKQSNKYA ASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS
R2M3-3 HC	110	MDMRVPAQLLGLLLLWLRGARCEVQLVQSGAEVKKPGASVKVSCKASGYTFT SYGISWVRQAPGQGLEWMGWISAYNGNTNYAQKLQGRVTMTTDTSTSTAYM ELRSLRSDDTAVYYCASSKEKATYYGMDVWGQGTQVTVSSASTKGPSVFPL APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLY SLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPE AAGGPSVFLFPPKPKD TLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHN AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYCKVSNKALGAPIEKTISKA KGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNY KTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL SPGK

Name	SEQ ID NO	Sequence
R2M3mut-3 LC	109	MDMRVPAQLLGLLLLWLRGARC DVQLVESGGGLVQPGGSLRLSCTSSANINSIE TLGWYRQAPGKQRELIANMRGGGYMKYAGSLKGRFTMSTESAKNTMYLQMNS LKPEDTAVYYCYVKLRDDDDYVYRGQGTQVTVSSGSGSGQAVVLQEPSSLVSPG GTVTLTCGLSSGSVSTNYYPSWYQQTPGQAPRTLIIYTNTRSSDVPERFSGSIV GNKAALTITGAQPDDSVYFCLLYLGRGIWWFGGGTKLTVLGQPKAAPSVTLFP PSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTTPSKQSNNKYA ASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS
R2M3mut-3 HC	133	MDMRVPAQLLGLLLLWLRGARCEVQLVQSGAEVKKPGASVKVSCKASGYTFT SYGISWVRQAPGQGLEWMGWISAYNGNTNYAQKLQGRVTMTTDTSTSTAYM ELRSLRSDDTAVYYCASSKEKATYYAGMDVWGQGT TVTVSSASTKGPSVFPL APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLY SLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPE AAGGPSVFLFPPKPKD TLMISRTP E VTCVVDVSHEDPEVKFNWYVDGVEVHN AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKA KGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNY KTTTPVLDS DGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL SPGKGSGSGSHHHHHH
1RC07-3 (2:2) LC	134	MDMRVPAQLLGLLLLWLRGARC DVQLVESGGGLVQPGGSLRLSCTSSANINSIE TLGWYRQAPGKQRELIANMRGGGYMKYAGSLKGRFTMSTESAKNTMYLQMNS LKPEDTAVYYCYVKLRDDDDYVYRGQGTQVTVSSGSGSGSYVLTQPPSVSVSPG QTASITCSGDKVGHKYASWYQQKPGQSPVLVIYEDSQRPSGIPVRFSGSNSGN TATLTISGTQAMDEADYYCQAWDSSTDVVFGGGTKLTVLGQPKAAPSVTLFPPS SEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTTPSKQSNNKYAAS SYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS
1RC07-3 (2:2) HC	135	MDMRVPAQLLGLLLLWLRGARCQVQLQQWGAGLLKPSETLSLTCAVSGASFS GHYWTWIRQPPGKGLEWIGEIDHTGSTNYEPSLRSRVTISVDTSKNQFSLNLKS VTAADTAVYYCARGGQGGYDWGHYHGLDVWGQGT TVTVSSASTKGPSVFPL APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLY SLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPE AAGGPSVFLFPPKPKD TLMISRTP E VTCVVDVSHEDPEVKFNWYVDGVEVHN AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKA KGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNY KTTTPVLDS DGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL SPGK
1RC07-3 (2:1) LC1	129	MDMRVPAQLLGLLLLWLRGARC DVQLVESGGGLVQPGGSLRLSCTSSANINSIE TLGWYRQAPGKQRELIANMRGGGYMKYAGSLKGRFTMSTESAKNTMYLQMNS LKPEDTAVYYCYVKLRDDDDYVYRGQGTQVTVSSGSGSGSYVLTQPPSVSVSPG QTASITCSGDKVGHKYASWYQDKPGQDPVLVIYEDSQRPSGIPVRFSGSNSGN TATLTISGTQAMDEADYYCQAWDSSTDVVFGGGTKLTVLGQPKAAPSVTLFPPS SEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTTPSKQSNNKYAAK SYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS
1RC07-3 (2:1) HC1	130	MDMRVPAQLLGLLLLWLRGARCQVQLQQWGAGLLKPSETLSLTCAVSGASFS GHYWTWIRKPPGKGLEWIGEIDHTGSTNYEPSLRSRVTISVDTSKNQFSLNLKS VTAADTAVYYCARGGQGGYDWGHYHGLDVWGKGTT TVTVSSASTKGPSVFPL APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLY SLESVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPE AAGGPSVFLFPPKPKD TLMISRTP E VTCVVDVSHEDPEVKFNWYVDGVEVHN AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKA KGQPREPQVYTLPPSREEMTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNY KTTTPVLDS DGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL SPGKGSGGDYKDDDDK

Name	SEQ ID NO	Sequence
1RC07-3 (2:1) LC2	136	MDMRVPAQLLGLLLLWLRGARCSYVLTQPPSVSVSPGQTASITCSGDKVGHKY ASWYQKKPGQKPVLYIEDSQRPSGIPVRFSGSNSGNTATLTISGTQAMDEADY YCQAWDSSTDVVFVGGGKLTVLGQPKAAPSVTLFPPSSEELQANKATLVCLISD FYPGAVTVAWKADSSPVKAGVETTTPSKQSNKYAAESYLSLTPEQWKSHRSY SCQVTHEGSTVEKTVAPTECS
1RC07-3 (2:1) HC2	137	MDMRVPAQLLGLLLLWLRGARCQVQLQQWGAGLLKPSETLSLTCAVSGASF GHYWTWIRDPGKGLEWIGEIDHTGSTNYEPSLSRVRTISVDTSKNQFSLNLKS VTAADTAVYYCARGGGGYDWGHYHGLDVWGDGTTTVSSASTKGPSVFPL APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLY SLKSVVTPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPE AAGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHN AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKA KGQPREPQVYTLPPSREEMTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNY KTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL SPGKGGSGSGHHHHHH
R2M3- 26NL-26NH (1:2) LC	132	MDMRVPAQLLGLLLLWLRGARCDVQLVESGGGLVQAGGSLRLACAGSGRIFAI YDIAWYRHPPGNQRELVAMIRPVVTEIDYADSVKGRFTISRNNAMKTVYLMNN LKPEDTAVYYCNAKRPWGSRDEYWGQGTQVTVSSGSGSGQAVVLQEPSLSVS PGGTVTLTCGLSSGSVSTNYPSWYQQTPGQAPRTLIYYTNTRSSDVPERFSG SIVGNKAALTITGAQPDDSVYFCLLYLGRGIWWFGGGTKLTVLGQPKAAPSVTL FPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNK YAASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS
R2M3- 26NL-26NH (1:2) HC	138	MDMRVPAQLLGLLLLWLRGARCDVQLVESGGGLVQAGGSLRLACAGSGRIFAI YDIAWYRHPPGNQRELVAMIRPVVTEIDYADSVKGRFTISRNNAMKTVYLMNN LKPEDTAVYYCNAKRPWGSRDEYWGQGTQVTVSSGSGSGEVQLVQSGAEVK KPGASVKVSCKASGYTFTSYGISWVRQAPGQGLEWMGWISAYNGNTNYAQK LQGRVTMTTDTSTSTAYMELRSLRSDDTAVYYCASSKEKATYYYGMDVWGQGT TTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGAL TSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKKVEP KSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCK VSNKALGAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLWCLNGKFYPS DIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSV MHEALHNHYTQKSLSLSPGKGGSGGDYKDDDDK
R2M3- 26NL-26NH (1:2) Fc	131	MDMRVPAQLLGLLLLWLRGARCEFDKHTHTCPPCPAPEAAGGPSVFLFPPKPKD TLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV VSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQPREPQVYTLPPSR EEMTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLV SKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKGGSGSGHHHHHH
R2M3- 26NL-26CL (1:2) LC	139	MDMRVPAQLLGLLLLWLRGARCDVQLVESGGGLVQAGGSLRLACAGSGRIFAI YDIAWYRHPPGNQRELVAMIRPVVTEIDYADSVKGRFTISRNNAMKTVYLMNN LKPEDTAVYYCNAKRPWGSRDEYWGQGTQVTVSSGSGSGQAVVLQEPSLSVS PGGTVTLTCGLSSGSVSTNYPSWYQQTPGQAPRTLIYYTNTRSSDVPERFSG SIVGNKAALTITGAQPDDSVYFCLLYLGRGIWWFGGGTKLTVLGQPKAAPSVTL FPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNK YAASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECSGSGSGDVQLVES GGGLVQAGGSLRLACAGSGRIFAIYDIAWYRHPPGNQRELVAMIRPVVTEIDYA DSVKGRFTISRNNAMKTVYLMNNLKPEDTAVYYCNAKRPWGSRDEYWGQGT QVTVSS
R2M3- 26NL-26CL (1:2) HC_Fab	140	MDMRVPAQLLGLLLLWLRGARCEVQLVQSGAEVKKPGASVKVSCKASGYTFT SYGISWVRQAPGQGLEWMGWISAYNGNTNYAQKLQGRVTMTTDTSTSTAYM ELRSLRSDDTAVYYCASSKEKATYYYGMDVWGQGTQVTVSSASTKGPSVFPL APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLY SLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCGSGSGHHHHHH

Name	SEQ ID NO	Sequence
R2M3-26NL-26CH (1:2) LC	132	MDMRVPAQLLGLLLLWLRGARCDVQLVESGGGLVQAGGSLRLACAGSGRIFAIYDIAWYRHPPGNQREL VAMIRPVVTEIDYADSVKGRFTISRNNAMKTVY LQMNN LKPEDTAVYYCNAKRPWGS RDEYWGQGTQVTVSSGSGSGQAVVLQEPSLSVS PGGTVTLTCGLSSGSVSTNY YPSWYQQTPGQAPRTLIYYTNRSSDVPERFSG SIVGNKAALTITGAQPDDES VYFCLLYLGRGIWWFGGGTKLTVLGQPKAAPSVTL FPPSSEELQANKATLVCLIS DFIYPGAVTVAWKADSSPVKAGVETTTTPSKQSNNK YAASSYLSLTPEQWKSHRSY SCQVTHEGSTVEKTVAPTECS
R2M3-26NL-26CH (1:2) HC	141	MDMRVPAQLLGLLLLWLRGARCEVQLVQSGAEVKKPGASVKVSCKASGYTFTSYGISWVRQAPGQGLEWMGWISAYNGNTNYAQKLQGRVTMTTDTSTSTAYMELRSLRSDDTAVYYCASSKEKATYYYGMDVWGQGT TVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVT VSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNV FSCSV MHEALHNHYTQKSLSLSPGKSGSGSDVQLVESGGGLVQAGGSLRLACAGSGRIFAIYDIAWYRHPPGNQREL VAMIRPVVTEIDYADSVKGRFTISRNNAMKTVY LQMNN LKPEDTAVYYCNAKRPWGS RDEYWGQGTQVTVSS
R2M3-26NL-26CH (1:2) Fc	131	MDMRVPAQLLGLLLLWLRGARCEFDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLVSKLTVDKSRWQQGNV FSCSV MHEALHNHYTQKSLSLSPGKSGSGHHHHHH
1RC07-3+R2M3 (1:1:1) LC1	129	MDMRVPAQLLGLLLLWLRGARCDVQLVESGGGLVQPGGSLRLSCTSSANINSITLGWYRQAPGKQRELIANMRGGGYMKYAGSLKGRFTMTSESANTMYLQMNS LKPEDTAVYYCYVKLRDDDDYVYRGQGTQVTVSSGSGSGSYVLTQPPSVSVSPGQTASITCSGDKVGHKYASWYQDKPGQDPVLVIYEDSQRPSGIPVRFSGSNSGN TATLTISGTQAMDEADYYCQAWDSSTDVVFSGGTKLTVLGQPKAAPSVTLFPPSSEELQANKATLVCLIS DFIYPGAVTVAWKADSSPVKAGVETTTTPSKQSNNKYAAKSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS
1RC07-3+R2M3 (1:1:1) HC1	130	MDMRVPAQLLGLLLLWLRGARCQVQLQQWGAGLLKPSETLSLTCAVSGASFS GHYWTWIRKPPGKGLEWIGEIDHTGSTNYEPSLRSRVTISVDTSKNQFSLNLKS VTAADTAVYYCARGGQGGYDWGHYHGLDVWGKGTTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVT VSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNV FSCSV MHEALHNHYTQKSLSLSPGKGSGG DYKDDDDK
1RC07-3+R2M3 (1:1:1) LC2	142	MDMRVPAQLLGLLLLWLRGARCQAVVLQEPSLSVSPGGTVTLTCGLSSGSVSTNY YPSWYQKTPGQKPRTLIYYTNRSSDVPERFSGSIVGNKAALTITGAQPDDES VYFCLLYLGRGIWWFGGGTKLTVLGQPKAAPSVTLFPPSSEELQANKATLVCLIS DFIYPGAVTVAWKADSSPVKAGVETTTTPSKQSNNKYAAESYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS

Name	SEQ ID NO	Sequence
1RC07-3+R2M3 (1:1:1) HC2	143	MDMRVPAQLLGLLLLWLRGARCEVQLVQSGAEVKKPGASVKVSCKASGYTFTSYGISWVRDAPGQGLEWMGWISAYNGNTNYAQKLQGRVTMTTDTSTSTAYMELRSLRSDDTAVYYCASSKEKATYYYGMDVWGDGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLKSVVTPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDITLMISRTPPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKGSGSGHHHHHH
1RC07-3+R2M13 (1:1:1) LC1	129	MDMRVPAQLLGLLLLWLRGARCDVQLVESGGGLVQPGGSLRLSCTSSANINSIE TLGWYRQAPGKQRELIANMRGGGYMKYAGSLKGRFTMTESAKNTMYLQMNS LKPEDTAVYYCYVKLRDDDDYVYRGQGTQVTVSSGSGSGSYVLTQPPSVSVSPGQTASITCSGDKVGHKYASWYQDKPGQDPVLVIYEDSQRPSGIPVRFSGSNSGN TATLTISGTQAMDEADYYCQAWDSSTDVVFGGGTGLTVLGQPKAAPSVTLFPPS SEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTTPSKQSNNKYAAK SYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS
1RC07-3+R2M13 (1:1:1) HC1	130	MDMRVPAQLLGLLLLWLRGARCDVQLVQSGAEVKKPGSETLSLTCAVSGASFS GHYWTWIRKPPGKGLEWIGEIDHTGSTNYEPSLRSRVTISVDTSKNQFSLNLKS VTAADTAVYYCARGGQGGYDWGHYHGLDVWGKGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLY SLESVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDITLMISRTPPEVTCVVDVSHEDPEVKFNWYVDGVEVHN AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNY KTTTPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKGSGSGDYKDDDDK
1RC07-3+R2M13 (1:1:1) LC2	144	MDMRVPAQLLGLLLLWLRGARCDIQMTQSPSSLSASVGDRTITCRASQSISSY LNWWYQKKPGKPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTITSLQPEDFATYY CQQSYSTPLTFGGGTKEIKRTVAAPSVFIFPPSDEQLKSGTASVCLLNNFYPR EAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLESTLTLSKADYEKHKVYACE VTHQGLSSPVTKSFNRGEC
1RC07-3+R2M13 (1:1:1) HC2	145	MDMRVPAQLLGLLLLWLRGARCEVQLVQSGAEVKKPGSSVKVSCKASGGTFT YRYLHWVRDAPGQGLEWMGGIPIFGTGNIAQKFQGRVTITADESTSTAYMEL SSLRSED TAVYYCASSMVRVPYYGMDVWGDGTLTVSSASTKGPSVFPLAP SSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLK SVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAG GPSVFLFPPKPKDITLMISRTPPEVTCVVDVSHEDPEVKFNWYVDGVEVHN AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQ PREPQVYTLPPSREEMTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTTP VLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKGSGSGHHHHHH
1RC07-3+5SH5 (1:1:1) LC1	129	MDMRVPAQLLGLLLLWLRGARCDVQLVESGGGLVQPGGSLRLSCTSSANINSIE TLGWYRQAPGKQRELIANMRGGGYMKYAGSLKGRFTMTESAKNTMYLQMNS LKPEDTAVYYCYVKLRDDDDYVYRGQGTQVTVSSGSGSGSYVLTQPPSVSVSPGQTASITCSGDKVGHKYASWYQDKPGQDPVLVIYEDSQRPSGIPVRFSGSNSGN TATLTISGTQAMDEADYYCQAWDSSTDVVFGGGTGLTVLGQPKAAPSVTLFPPS SEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTTPSKQSNNKYAAK SYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS

Name	SEQ ID NO	Sequence
1RC07-3+5SH5 (1:1:1) HC1	130	MDMRVPAQLLGLLLLWLRGARCQVQLQQWGAGLLKPSETLSLTCAVSGASFSGHYWTWIRKPPGKGLEWIGEIDHTGSTNYEPSLRSRVTISVDTSKNQFSLNLKSVTAADTAVYYCARGGGGGYDWGHYHGLDVWGKGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLESVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKGSGGGDYKDDDDK
1RC07-3+5SH5 (1:1:1) LC2	146	MDMRVPAQLLGLLLLWLRGARCDIQTQSPSSLSASVGDRVTITCRASQGISSALAWYQKKPGKPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISLQPEDFATYYCQQTYSMPITFGQGTREIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLESTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
1RC07-3+5SH5 (1:1:1) HC2	147	MDMRVPAQLLGLLLLWLRGARCQVQLVQSGAEVKKPGASVKVSCASGYTFTSYMHVWRDAPGQGLEWMGRINPNSGGTNYAQKFQGRVTMTRDTSTSTVYMESSLSRSED TAVYYCARVPDFWSGYLDYWGDTLTVTSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLKSVVTVPPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKGSGSGHHHHHH
1RC07-3-26 NL (2:1:1) LC1	129	MDMRVPAQLLGLLLLWLRGARCDVQLVESGGGLVQPGGSLRLSCTSSANINSIELGWYRQAPGKQRELIANMRGGGYMKYAGSLKGRFTMTESAKNTMYLQMNSLKPEDTAVYYCYVKLRDDDDYVYRGQGTQVTVSSGSGSGSYVLTQPPSVSVSPGQTASITCSGDKVGHKYASWYQDKPGQDPVLVIYEDSQRPSGIPVRFSGSNSGTATLTISGTQAMDEADYYCQAWDSSTDVVFGGGTKLTVLGQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNKYAAKSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS
1RC07-3-26 NL (2:1:1) HC1	130	MDMRVPAQLLGLLLLWLRGARCQVQLQQWGAGLLKPSETLSLTCAVSGASFSGHYWTWIRKPPGKGLEWIGEIDHTGSTNYEPSLRSRVTISVDTSKNQFSLNLKSVTAADTAVYYCARGGGGGYDWGHYHGLDVWGKGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLESVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKGSGGGDYKDDDDK
1RC07-3-26 NL (2:1:1) LC2	148	MDMRVPAQLLGLLLLWLRGARCDVQLVESGGGLVQAGGSLRLACAGSGRIFAIYDIAWYRHPPGNQRELVAMIRPVVTEIDYADSVKGRFTISRNNAMKTVYLMNNLKPEDTAVYYCNAKRPWGSRDEYWGQGTQVTVSSGSGSGSYVLTQPPSVSVSPGQTASITCSGDKVGHKYASWYQKKPGQKPVLYIYEDSQRPSGIPVRFSGSNSGNTATLTISGTQAMDEADYYCQAWDSSTDVVFGGGTKLTVLGQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNKYAAESYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS

Name	SEQ ID NO	Sequence
1RC07-3-26 NL (2:1:1) HC2	137	MDMRVPAQLLGLLLLWLRGARCQVQLQQWGAGLLKPSETLSLTCAVSGASFS GHYWTWIRDPGKGLEWIGEIDHTGSTNYEPSLRSRVTISVDTSKNQFSLNLKS VTAADTAVYYCARGGGGGYDWGHYHGLDVWGDGTTVTVSSASTKGPSVFPL APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLY SLKSVVTPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPE AAGGSPVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKA KGQPREPQVYTLPPSREEMTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNY KTTTPVLDSGGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL SPGKGGSGSGHHHHHH
1RC07-3-26 NH (2:1:1) LC	149	MDMRVPAQLLGLLLLWLRGARCQVQLQQWGAGLLKPSETLSLTCAVSGASFS ASWYQQKPGQSPVLVIYEDSQRPSGIPVRFSGSNSGNTATLTISGTQAMDEADY YCQAWDSSTDVVFSGGGLTKLTVLGQPKAAPSVTLFPPSSEELQANKATLVCLISD FYPGAVTVAWKADSSPVKAGVETTPSKQSNKYAASSYLSTPEQWKS SHRSY SCQVTHEGSTVEKTVAPTECS
1RC07-3-26 NH (2:1:1) HC1	150	MDMRVPAQLLGLLLLWLRGARCQVQLVESGGGLVQAGGSLRLACAGSGRIFAI YDIAWYRHPPGNQRELVAMIRPVTEIDYADSVKGRFTISRNNAMKTVYLMNN LKPEDTAVYYCNAKRPWGSRDYWGQGTQVTVSSGGSGSQVQLQQWGAGL LKPSETLSLTCAVSGASFSGHYWTWIRQPPGKGLEWIGEIDHTGSTNYEPSLR SRVTISVDTSKNQFSLNLKS VTAADTAVYYCARGGGGGYDWGHYHGLDVWG QGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSG ALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKKV EPKSCDKTHTCPPCPAPEAAGGSPVFLFPPKPKDTLMISRTPEVTCVVVDVSH EDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYK CKVSNKALGAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLWCLVKGFY PSDIAVEWESNGQPENNYKTTTPVLDSGGSFFLVSKLTVDKSRWQQGNVFSC SVMHEALHNHYTQKSLSLSPGKGGSGGDKDDDDK
1RC07-3-26 NH (2:1:1) HC2	151	MDMRVPAQLLGLLLLWLRGARCQVQLVESGGGLVQAGGSLRLSCTSSANINSIE TLGWYRQAPGKQRELIANMRGGGYMKYAGSLKGRFTMTESAKNTMYLMNNS LKPEDTAVYYCYVKLRDDDYVYRGQGTQVTVSSGGSGSQVQLQQWGAGLLK PSETLSLTCAVSGASFSGHYWTWIRQPPGKGLEWIGEIDHTGSTNYEPSLRSR VTISVDTSKNQFSLNLKS VTAADTAVYYCARGGGGGYDWGHYHGLDVWGQ TTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGAL TSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKKVEP KSCDKTHTCPPCPAPEAAGGSPVFLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCK VSNKALGAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLSCAVKGFYPS DIAVEWESNGQPENNYKTTTPVLDSGGSFFLVSKLTVDKSRWQQGNVFSCSV MHEALHNHYTQKSLSLSPGKGGSGSGHHHHHH
1RC07- 26+4SD1- 3_(1:1:1:1) LC1	152	MDMRVPAQLLGLLLLWLRGARCQVQLVESGGGLVQAGGSLRLACAGSGRIFAI YDIAWYRHPPGNQRELVAMIRPVTEIDYADSVKGRFTISRNNAMKTVYLMNN LKPEDTAVYYCNAKRPWGSRDYWGQGTQVTVSSGGSGSSVLTQPPSVSVS PGQTASITCSGDKVGHKYASWYQDKPGQDPVLVIYEDSQRPSGIPVRFSGSNS GNTATLTISGTQAMDEADYYCQAWDSSTDVVFSGGGLTKLTVLGQPKAAPSVTLF PPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTPSKQSNKY AAKSYLSLTPEQWKS SHRSYSCQVTHEGSTVEKTVAPTECS
1RC07- 26+4SD1- 3_(1:1:1:1) HC1	130	MDMRVPAQLLGLLLLWLRGARCQVQLQQWGAGLLKPSETLSLTCAVSGASFS GHYWTWIRKPPGKGLEWIGEIDHTGSTNYEPSLRSRVTISVDTSKNQFSLNLKS VTAADTAVYYCARGGGGGYDWGHYHGLDVWGKGTTVTVSSASTKGPSVFPL APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLY SLESVTVTPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPE AAGGSPVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKA KGQPREPQVYTLPPSREEMTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNY KTTTPVLDSGGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL SPGKGGSGGDKDDDDK

Name	SEQ ID NO	Sequence
1RC07-26+4SD1-3_(1:1:1:1) LC2	153	MDMRVPAQLLGLLLLWLRGARC DVQLVESGGGLVQPGGSLRLSCTSSANINSIE TLGWYRQAPGKQRELIANMRGGGYMKYAGSLKGRFTMSTESAKNTMYLQMNS LKPEDTAVYYCYVKLRDDDDYVYRGQGTQVTVSSGGSGSDIQMTQSPSSLSASV GDRVITICRASQSISSYLNWYQKKPGKAPKLLIYAASSLQSGVPSRFSGSGSGT DFTLTISLQPEDFATYYCQQSYSTPLTFGGG TKVEIKRTVAAPSVFIFPPSDEQL KSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSTYSLEST LTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
1RC07-26+4SD1-3_(1:1:1:1) HC2	154	MDMRVPAQLLGLLLLWLRGARCEVQLVESGGGLVQPGGSLRLSCAASGFTFT NYAMSWVRDAPGKGLEWVSAISGGGSTYYADSVKGRFTISRDDSKNTLYLQ MNSLKTEDTAVYYCARATGFGTVVFDYWGDTLVTVSSASTKGPSVFPLAPS SKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLKS VVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGG PSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTK PREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQP REPQVYTLPPSREEMTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTPP VLDSGDSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK SGSGHHHHHH
R2M9-26+1RC07-3(1:1:1:1) LC1	155	MDMRVPAQLLGLLLLWLRGARC DVQLVESGGGLVQAGGSLRLACAGSGRIFAI YDIAWYRHPPGNQRELVAMIRPVTEIDYADSVKGRFTISRNNAMKTVYLQMNN LKPEDTAVYYCNAKRPWGSRDYWGQGTQVTVSSGGSGSDIQMTQSPSSLSA SVGDRVITICRASQSISSYLNWYQDKPGKDPKLLIYAASSLQSGVPSRFSGSGS GTDFLTISLQPEDFATYYCQQSYRTPFTFGPGTKVDIKRTVAAPSVFIFPPSDE QLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSTYSLK STLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
R2M9-26+1RC07-3(1:1:1:1) HC1	156	MDMRVPAQLLGLLLLWLRGARCQVQLVQSGAEVVKPGSSVKVSCKASGYTFT NNFMHWVRKAPGQGLEWMGWINPNSGGTKYAQKFQGRVTITADESTSTAYM ELSSLRSED TAVYYCARSVGEVGATMLGIGVWYWFDPWGKGLTVTVSSASTK GPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVL QSSGLYSLESVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCP PCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVD GVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPI EKTISKAKGQPREPQVYTLPPSREEMTKNQVSLWCLVKGFYPSDIAVEWESNG QPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYT QKSLSLSPGKGGSGGDYKDDDDK
R2M9-26+1RC07-3(1:1:1:1) LC2	157	MDMRVPAQLLGLLLLWLRGARC DVQLVESGGGLVQPGGSLRLSCTSSANINSIE TLGWYRQAPGKQRELIANMRGGGYMKYAGSLKGRFTMSTESAKNTMYLQMNS LKPEDTAVYYCYVKLRDDDDYVYRGQGTQVTVSSGGSGSSYVLTQPPSVSVSPG QTASITCSGDKVGHKYASWYQKKPGQKPVLYIEDSQRPSGIPVRFSGSNSGN TATLTISGTQAMDEADYYCAWDSSTDVFGGGTKLTVLGQPKAAPSVTLFPPS SEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTPSKQSNNKYAAE SYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS
R2M9-26+1RC07-3(1:1:1:1) HC2	130	MDMRVPAQLLGLLLLWLRGARCQVQLQQWGAGLLKPSETLSLTCAVSGASFS GHYWTWIRKPPGKGLEWIGEIDHTGSTNYEPSLRSRVTISVDTSKNQFSLNLKS VTAADTAVYYCARGGGGGYDWGHYHGLDVWGKGTTVTVSSASTKGPSVFPL APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLY SLESVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPE AAGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHN AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKA KGQPREPQVYTLPPSREEMTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNY KTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL SPGKGGSGGDYKDDDDK

Example 2

Generation of Wnt Surrogate Molecules with Binding Specificity for the Fzd Receptor Hinge Region

Active Wnt surrogate molecules were generated comprising various combinations of Fzd binders that bind the Fzd receptor hinge region (see FIG. 2A) and LRP binders. Two antibodies that bind to the hinge region of Fzd7, anti-FZD7-1791 (SEQ ID NOS:70-71) and anti-FZD7-1291 (SEQ ID NOS:72-73), have been described in WO2016/205551 and WO2016/205566. These two antibodies were used to demonstrate that binders to the Fzd hinge region yield active Wnt surrogate molecules.

Both anti-FZD7-1791 and anti-FZD7-1291 were cloned into human IgG1 framework with LALA-PG mutations in Fc to reduce effector functions. LRP5 binder #3 (008S-D01; SEQ ID NO:97) was cloned in frame to the N-termini of the light chains of both antibodies as depicted in FIG. 2B. Both recombinant appended IgG proteins, named 1791-3 (SEQ ID NOS:113-114) and 1291-3 (SEQ ID NOS:111-112), respectively, 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 spinning down the cell pellet. The media was incubated with Protein A resin (REPLIGEN, Waltham, MA) for collecting proteins containing human IgG-Fc portion. Proteins were eluted with 10 mM glycine, pH 3.5 from Protein A resin. 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). The peak fractions were analyzed by SDS-Polyacrylamide Gel Electrophoresis (SDS-PAGE) to confirm the content.

Antibodies that bind to the hinge region of Fzd1 and Fzd2 were utilized to demonstrate that, in addition to the Fzd7 hinge, other Fzd hinge regions also yield active Wnt surrogate molecules. Recombinant Fab fragments of these antibodies were produced from Expi293F cells (Thermo Fisher Scientific, Waltham, MA) via transient transfection. The Fabs were purified from the culture media with Nickel resin and further polished with size exclusion chromatography (SEC).

The anti-FZD1 hinge and anti-FZD2 hinge antibodies were cloned into human IgG1 framework with LALA-PG mutations in Fc to reduce effector functions. LRP5 binder #3 (008S-D01; SEQ ID NO:97) or LRP5/6 binder #36 (013S-D05; SEQ ID NOS: 91 and 96) were cloned in frame to the N-termini of the light chains of respective

antibodies as depicted in FIG. 2B. The recombinant appended IgG 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 spinning down the cell pellet. The media was incubated with Protein A resin (REPLIGEN, Waltham, MA) for collecting proteins containing human IgG-Fc portion. Proteins were eluted with 10 mM glycine, pH 3.5 from Protein A resin. 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). The peak fractions were analyzed by SDS-Polyacrylamide Gel Electrophoresis (SDS-PAGE) to confirm the content. The results indicated that these antibodies also yielded active Wnt surrogate molecules.

Example 3

Binding Kinetics of Hinge Region-Specific Wnt Surrogate Molecules

Binding kinetics of 1791-3 and 1291-3 to either Fzd7 CRD or Fzd7 CRD with the extracellular hinge region sequences (Fzd7 CRD+hinge) 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. N-terminal biotinylated Fzd7 CRD and Fzd7 CRD+hinge proteins were captured on the SA biosensor. Following capture of biotinylated-Fzd7, the SA biosensor with captured biotinylated-Fzd7 was dipped into wells containing the relevant antibodies at 7 different concentrations in running buffer plus a well with only running buffer as a reference channel. KD was determined by global fitting. As shown in FIG. 2C, both of these antibody fusion proteins only bound to the Fzd7 protein with the hinge region, and not to the Fzd7 CRD domain alone.

Example 4

In Vitro Activity of Hinge Region-Specific Wnt Surrogate Molecules

The ability of 1791-3 and 1291-3 to activate Wnt signaling was assessed in the 293 STF cell line, where the β -Catenin luciferase reporter plasmid Super TOP Flash (STF) was stably integrated. As shown in FIG. 2D, both 1791-3 and 1291-3 activated Wnt signaling as judged by the induction of luciferase reporter in these cells in

the present of 20 nM R-spondin 2 (RPSO). 1791-3 showed higher activity than 1291-3, even though 1291-3 had higher affinity to Fzd7 CRD+hinge, suggesting that potential differences in geometry between the two antibodies may contribute to differences in activity. Another Wnt surrogate molecule, R2M3-3 (Fzd binder 001S-A04; Lrp binder 008S-D01; SEQ ID NOs:109-110), which can engage Fzd1, Fzd2, Fzd7, Fzd5, and Fzd8, was also tested. As shown in FIG. 2D, the maximal effect from 1791-3 is approaching that of the multifamily specific R2M3-3.

The ability of Wnt surrogates comprising antibodies that bind the Fzd1 or Fzd2 hinge regions to activate Wnt signaling was assessed in the 293 STF cell line overexpressing either Fzd1 or Fzd2, where the β -Catenin luciferase reporter plasmid Super TOP Flash (STF) was stably integrated. For the Luciferase reporter assays, in each 96 well plate, 1 million cells were seeded, IWP2 (a wnt signaling inhibitor) was added at 3 μ M final concentration. 26 hours after seeding, compounds were added to the 96 well plates with triplicates and 10-fold series dilution from 100nM, and the highest concentration is 500nM. 18 hours later, cells were lysed with 100 μ l lysis buffer. From the above lysed cells, 20 μ l samples were transferred to opaque 96- well plates. Toward each well, 10 μ l of luciferase substrate was added. The plate was immediately placed in Molecular Device Lum96 plate reader and luciferase luminescence signals were collected. Data were processed with Prism7. These antibodies activated Wnt signaling as judged by the induction of luciferase reporter in these cells with either Fzd1 or Fzd 2 overexpression in the present of 20 nM R-spondin 2 (RPSO). These results demonstrate that Fzd hinge binding antibodies when assembled with LRP binders can induce Wnt signaling activation.

Example 5

Generation of Multispecific Wnt Surrogate Molecules

Sequences from monospecific Wnt surrogate molecules were combined to generate a multispecific Wnt surrogate molecule with a desired combination of Fzd binding specificity. As depicted in FIG. 3A, two monospecific Wnt surrogate molecules, 4SD1-3 (Fzd binder 004S-D01; Lrp binder 008S-D01; SEQ ID NOs:115-116), which binds Fzd4, and 14SB6-3 (Fzd binder 014S-B06; Lrp binder 008S-D01; SEQ ID NOs:117-118), which binds Fzd9, were combined using knobs-into-holes technology into a hetero-Ig Wnt surrogate molecule, hetero-Ig 4SD1-3+14SB6-3 (SEQ ID NOs:120

and122). Half of the hetero-Ig molecule came from 4SD1-3 with the introduction of the “holes” mutations in the CH3 domain (SEQ ID NO:120). This half of the molecule contained one binding arm to Fzd4 and one binding arm to LRP5. The other half of the hetero-Ig molecule came from 14SB6-3 with the introduction of the “knobs” mutations in the CH3 domain (SEQ ID NO:122). This half of the molecule contained one binding arm to Fzd9 and one binding arm to LRP5. The final Wnt surrogate, hetero-Ig 4SD1-3+14SB6-3, was a tetravalent tri-specific molecule containing one binding arm to Fzd4, one binding arm to Fzd9, and two binding arms to LRP5.

Two control molecules were also generated: 4SD1-3 monovalent bispecific (SEQ ID NO:120 and 123) and 14SB6-3 monovalent bispecific (FIG. 3A) SEQ ID NO:122 and 124). The 4SD1-3 monovalent bispecific molecule contained the 4SD1-3 half of the hetero-Ig with the “holes” mutations (SEQ ID NO:120) paired with an Fc with “knobs” mutations (SEQ ID NO:123), but without an Fab arm; therefore, this molecule was bispecific but monovalent against Fzd4 and LRP5. The 14SB6-3 monovalent bispecific contained the 14SB6-3 half of the hetero-Ig with the “knobs” mutations (SEQ ID NO:122) paired with an Fc with “holes” mutations (SEQ ID NO:124), but without an Fab arm; therefore, this molecule was bispecific but monovalent against Fzd9 and LRP5.

Hetero-Ig 4SD1-3+14SB6-3, 4SD1-3 monovalent bispecific, and 14SB6-3 monovalent bispecific were purified through a 4-step purification process. Since the Fc containing the “knobs” mutation also contains a FLAG tag, and the Fc containing the “holes” mutation also contains a His tag, these proteins were purified first by protein A and Ni-NTA affinity purification steps, followed by an SEC step; Anti-FLAG M2 beads were used as a final purification step.

Example 6

Characterization of Multispecific Wnt Surrogate Molecules

The ability of hetero-Ig 4SD1-3+14SB6-3, 4SD1-3 monovalent bispecific, and 14SB6-3 monovalent bispecific to activate Wnt signaling was assessed in Wnt-responsive 293STF reporter cells in the presence or absence of 20 nM RPSO (only data in the presence of RPSO is shown in FIGS. 3B-3E). R2M3-3, which can bind to Fzd1, Fzd2, Fzd7, Fzd5, and Fzd8, was also tested.

The 293STF cell line expresses low level of Fzd4 and no detectable Fzd9 expression by QPCR. All molecules other than R2M3-3 showed very little or no activity in the parental 293STF cells (FIG. 3B).

A retrovirus-based Fzd4 overexpression 293STF stable cell line was generated (293STF Fzd4OE). In this cell line, both R2M3-3 and 4SD1-3 (Fzd4 monospecific) potently activated Wnt signaling, while the hetero-Ig 4SD1-3+14SB6-3 showed very weak activity, and the Fzd9 monospecific molecule, 14SB6-3 was inactive (FIG. 3C).

Fzd9 was introduced into the parental 293STF cells through transient transfection to create the 293STF Fzd9OE cell line. In this cell line, both R2M3-3 and 14SB6-3 (Fzd9 monospecific) were able to activate Wnt signaling, while the hetero-Ig 4SD1-3+14SB6-3 showed very weak activity, and the Fzd4 monospecific molecule, 4SD1-3 was inactive (FIG. 3D).

Fzd4 and Fzd9 were co-introduced into 293STF cells through a combination of retroviral Fzd4 delivery and Fzd9 transient transfection to create the 293STF Fzd4OE + Fzd9OE cell line. In this cell line, R2M3-3, 4SD1-3, 14SB6-3, and hetero-Ig 4SD1-3+14SB6-3 were all fully active (FIG. 3E)

The two monovalent bispecific molecules, 4SD1-3 monovalent bispecific and 14SB6-3 monovalent bispecific, were inactive in all cells (FIGS. 3B-3E). These results demonstrated that for the two monospecific binders, the monovalent bispecific combination with a LRP5 binder is not able to induce or not able to efficiently induce Wnt signaling. Therefore, the activity observed with the hetero-Ig 4SD1-3+14SB6-3 was indeed the result of the engagement of both Fzd4 and Fzd9 proteins, together with LRP. These results further demonstrated that this approach can be applied to any combination of two or more Fzd binders of different specificities, thus enabling the generation of any desired combination of Fzd specificities through this process.

This example showed that two Fzd binders of different specificities can be combined in the hetero-Ig format to generate Wnt surrogate molecules of different specificities. This concept can also be extended to the use of two Fzd binders to the same Fzd on two different epitopes. The resulting hetero-Ig Wnt surrogate molecule would be a biparatopic molecule, which may be beneficial for further receptor clustering and activation of Wnt signaling.

EXAMPLE 7

Heterodimerization of Canonical and Noncanonical Signaling Fzd Receptors Together with LRP Receptor Binding Leads to Activation of Beta-catenin-dependent Signaling

Wnt signaling can be mediated through beta-catenin-dependent (canonical) and beta-catenin-independent (noncanonical) pathways. There are Fzd receptors that have been reported to activate beta-catenin-independent signaling such as Fzd6, while other Fzd receptors activate beta-catenin-dependent signaling. It has not been considered previously whether forced dimerization of a noncanonical Fzd receptor with Lrp or heterodimerizing a canonical and a noncanonical Fzd receptors with Lrp can lead to beta-catenin-dependent or beta-catenin-independent signaling. To test these ideas, the following examples were generated.

First, a multivalent molecule consisting of bivalent binding arms (004S-C10) toward Fzd6, a noncanonical receptor, was combined with bivalent binding arms (3; 004S-D01) toward Lrp5. The molecule is named 4SC10-3 (Fig. 5A top panel). Such a format has been shown to activate beta-catenin-dependent signaling when the Fzd binder is directed against a canonical Fzd receptor. However, as shown in Fig. 5A bottom panel, 4SC10-3 was not able to effectively activate beta-catenin-dependent signaling in 293STF reporter cells (in the presence of 20nM R-spondin), in contrast to the positive control molecule R2M3-26 (Fzd binder 001S-A04; Lrp binder 009S-E04) that engages Fzd1,2,7,5,8.

Next, a multivalent molecule consisting of 4SC10 and another Fzd binding arm, 1RC07 (001S-B03), which binds to Fzd1,2,7 (canonical Fzd receptors), was combined with bivalent binding arms (3) toward Lrp5. The molecule is named heterolg 4SC10-3+1RC07-3 (Fig. 5B top panel), and it activated beta-catenin-dependent signaling in 293STF reporter cells. To rule out the possibility that the 1RC07-3 arm alone is able to activate signaling, the 1RC07-3 monovalent bispecific molecule shown in Fig. 5C top panel was also tested. As shown in Fig. 5C, 1RC07-3 monovalent bispecific molecule is not able to effectively activate signaling. These results demonstrated for the first time that combining a canonical and a noncanonical Fzd receptors with Lrp can lead to beta-catenin-dependent signaling.

EXAMPLE 8

Exploring Impact of Different Ratios of Fzd to Lrp Binders in Wnt Surrogate Molecules on Beta-catenin-dependent Signaling

Multivalent formats produced potent and highly efficacious Wnt signaling activators. Experiments were designed to test the impact of the formats on endogenous Wnt signaling. To understand the bivalent/multivalent Lrp binding arms impact on endogenous Wnt ligands, an inactive surrogate molecule was generated with a null mutant of R2M3, R2M3mut (Y9A), and fused to either Lrp binders 3 or 26, and named R2M3mut-3 or R2M3mut-26, respectively. As shown in Fig. 6A, while the wild-type R2M3 surrogates, R2M3-3 and R2M3-26, are fully active in 293STF Wnt reporter cell line in the presence of 20nM R-spondin, R2M3mut-3 and R2M3mut-26 are inactive. To test the impact of the bivalent Lrp arms in the surrogate molecules on endogenous Wnt signaling, we tested these two inactive surrogate molecules in 293STF Wnt reporter cell line in the presence of 20% conditioned media containing Wnt3A. As shown in Fig. 6B, while R2M3mut-3 and R2M3mut-26 are inactive on their own, R2M3mut-3 synergized with Wnt3a in a dose responsive manner, and R2M3mut-26 antagonized Wnt3a in a dose responsive manner. This demonstrated that multivalent formats might have potential to interact with endogenous Wnt ligands.

To understand formats that reduce the potential interaction with endogenous Wnts and to further understand the required number of receptor binders for active signaling, we explored different number and ratios of Fzd and Lrp binders in a multivalent surrogate molecule.

Since bivalency of the Lrp binding arms may lead to synergy with endogenous Wnts, removing one Lrp binding arm was tested, so in the final molecule there are two Fzd binders and one Lrp binder; this molecule is referred to as 2:1. The original molecule format would be 2:2 as there are two Fzd binders and two Lrp binding arms. One example of the 2:1 molecule, 1RC07-3 (2:1) is shown in Fig. 6C left panel, in comparison to the 2:2 format. As shown in Fig. 6C right panel, 1RC07-3 (2:1) is active and able to induce Wnt signaling in 293STF reporter cells. This format, in principle, should lack the ability to synergize with endogenous Wnt ligands via cross-linking of Lrp co-receptors. The reverse ratio, one Fzd binder and two Lrp binders, as exemplified by a few formats in Fig. 6D, has also been constructed. In general, these molecules at best, showed modest induction of signaling (Fig. 6D).

Since heterodimerizing Fzd in the 2:2 format can induce Wnt signaling (shown in Fig. 3 and Fig. 5), a molecule was constructed heterodimerizing two different Fzd binders with one Lrp binder (1:1:1) format and tested for the ability to confer Wnt signaling. As shown in Fig. 6E, several examples of 1:1:1 molecules all activated Wnt signaling in 293STF reporter cells. Fzd binders tested in this structure include R2M3 (001S-A04), 1RC07 (001S-B03), R2M13 (004S-G06), and 5S-H5 (005S-H05)

EXAMPLE 9

Heterodimerization of Lrp Binders Together with Fzd Binding Leads to Activation of Wnt Signaling

In addition to heterodimerizing/multimerizing different Fzd receptor binders, the effects of heterodimerizing/multimerizing different Lrp co-receptor binders on Wnt signaling was also tested. As shown above, the two Fzd:one Lrp format alleviated synergy with endogenous Wnt ligands. To determine if heterodimerized/multimerized Lrp binders directed against two different regions on Lrp may also relieve synergistic effects of bivalent Lrp binder formats on endogenous Wnt ligands was tested. As shown in Fig. 7A, several examples were constructed in a 2:1:1 ratio, where two different Lrp binders (one binds to Lrp5E1E2, the other binds to Lrp6E3E4) were each fused to either the N-terminus of light chain of Fzd binder, 1RC07, as 1RC07-3-26 NL (2:1:1); or to the N-terminus of 1RC07 heavy chain as 1RC07-3-26 NH (2:1:1). Both molecules are highly active in inducing Wnt signaling as detected in a 293STF Wnt responsive reporter cells (Fig. 7B).

Finally, a multivalent molecule with two distinct Fzd binding arms and two distinct Lrp binding arms, termed 1:1:1:1 format as shown in Fig. 7C was generated. Both the 1RC07-26+4SD1-3 (1:1:1:1) and R2M9-26+1RC07-3 (1:1:1:1) combinations resulted in strong induction of Wnt signaling (Fig. 7D). R2M9 structure contains the 003S-E07 Fzd binding element.

The examples shown in Figs. 5-7 illustrate the various structures for induction of canonical or non-canonical Wnt signaling (or both), as well as the ability to synergize with, antagonize, or leave unaffected signaling via endogenous Wnt ligands within target tissues. This versatile collection of soluble Wnt surrogate ligands could be used for therapies with tailored Wnt signaling that may allow for maximum therapeutic effect and minimal side effects.

EXAMPLE 10**Structure-Function Analysis of Wnt Surrogate Molecules Containing Different Stoichiometric Ratios of Fzd and LRP Binders**

A surrogate WNT agonist generated by linking a FZD binder (18R5 antibody in scFv format) and the C-terminal portion of Dickkopf (DKK1c) into a single polypeptide chain (18R5-DKK1c) exhibited the ability to activate WNT/ β -catenin signaling (Janda et al., 2017, *Nature*, 545(7653):234–237). Combination of various FZD and LRP binding antibody fragments were generated based on this concept.

The sequences for Fzd binding scFvs, referred to as F1, F2, and F3, and LRP scFvs, referred to as L1 and L2, as well as linker sequences used to combine them are shown in Table 6A. Since these molecules have a monovalent binding arm to each target, we refer to this format as bivalent bispecific format (denoted as 1:1 to represent the stoichiometry of binding to one FZD and one LRP molecule).

Additional stoichiometries for the tandem scFv constructs were tested. A summary of Combinatorial Wnt surrogate constructs used for this analysis are shown in Table 6B.

Table 6A: Wnt surrogate components used to stoichiometric analysis

Component	SEQ ID NO	Designation	Specificity (Clone name)	Sequence
signal peptide	2234			MDMRVPAQLLGLLLLWLRGARC
antiFZD_sc Fv	2235	F1:	antiFZD1, 2,7,5,8 (18R5)	EVQLVESGGGLVQPGGSLRLSCAASGFTFSHYTLNW VRQAPGKGLEWWSVISGD GSYTYADSVKGRFTISSDNSKNTLYLQMNSLRAEDT AVYYCARNFIKYVFANWG QGTLVTVSSGGGGSGGGGSGGGGSDIELTQPPSVSV APGQTARISCSGDNIGSFY VHWYQQKPGQAPVLVIYDKSNRPSGIPERFSGSNSG NTATLTISGTQAEDEADYY CQSYANTLSLVFGGGTKLTVL
	2236	F2:	antiFZD1, 2,7 (R2H1)	EVQLVESGGGLVQPGGSLRLSCAASGFNISSSYIHWV RQAPGKGLEWWAYIYSSY GSTYYADSVKGRFTISADTSKNTAYLQMNSLRAEDTA VYYCARASWYALDYWG

				QGTLVTVSSGSAASGSSGGSSSGADIQMTQSPSSLS ASVGDRVTITCRASQSVSSAV AWYQQKPGKAPKLLIYSASSLYSGVPSRFSGSRSGTD FTLTISSLQPEDFATYYCQQ YWYGVAPITFGQGTKVEIK
	2237	F3:	antiFZD5, 8 (2919)	EVQLVESGGGLVQPGGSLRLSCAASGFNISYSYIHWV RQAPGKGLEWVASIYSSSGS TSYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVY YCARGAIDYWGGQTLTV SSGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDR VTITCRASQSVSSAVAWYQQK PGKAPKLLIYSASSLYSGVPSRFSGSRSGTDFTLTISS LQPEDFATYYCQQWYSSGHV LITFGQGTKVEIK
antiLRP_sc Fv	2238	L1:	antiLRP6E 1E2 (1115.3)	DVKLVESGGGLVVKLGGSLRLSCAASGFSFSTSYMSW VRQTPEKRLELVAAINLNGGS TYYSDTVKGRTISRDNKNTLYLQMSSLKSEDTAFY YCASELAGYGTFFAYWGQGT LVTVSAGSAASGSSGGSSSGADIVLTQSPATLSVTPG DSVSLSCASQSSISYNLHWYQQ KSHPRLLIKYSQSSIGIPSRFSGSGSGTDFTLTINN VETEDFGMYFCQQSNSWPLTFG AGTKLEVK
	2239	L2:	antiLRP6E 3E4 (G211)	EVQLVESGGGLVQPGGSLRLSCAASGFTFTSYIISWV RQAPGKGLEWVAEISPYSGST YYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVY CALRARPPIRLHPRGSVMDY WGQGTTLVTVSSGSAASGSSGGSSSGADIQMTQSPSS LSASVGDRVTITCRASQDVSTA VAWYQQKPGKAPKLLIYSASFLYSGVPSRFSGSGSGT DFTLTISSLQPEDFATYYCQQS YTPPTFGQGTKVEIK

Component	SEQ ID NO	Designation	Specificity (Clone name)	Sequence
antiGFP_sc Fv	2240			QVQLVESGGGLVQPGGSLRLSCAASGFT FSRYGMHWVRQAPGKGLEWWSGIGSIGS NTTYADSVKGRFTISRDNKNTLYLQMN SLRAEDTAVYYCARWYKTYIDVWGQGT LVT

				VSSGGGGSGGGGSGGGGSDIELTQPPSV SVAPGQTARISCSGDNLGKKYVYWYQQK PGQAPVLVIYGDDERPSGIPERFSGSNSG NTATLTISGTQAEDEADYYCASYDSSHILIV FGGGTKLTVL
linker between tandem scFv	2241	5-mer (5)		GSGSG
	2242	10-mer (10)		GSGSGGSGSG
	2243	15-mer (15)		GSGSGGSGSGGSSGG
hlgG1_Fc_ LALAPG	2244	Fc		DKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISR TPEVTCVVVDVSHEDPEVKFNWYVDG VEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNLN GKEYCKVSNKALGAPIEKTISKAKG QPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSD IAVEWESNGQPENNYKTTPVLDSGDS FFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYT QKSLSLSPGK
	2245	Fc knob- FLAG		DKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISR TPEVTCVVVDVSHEDPEVKFNWYVDGV EVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNLN KEYCKVSNKALGAPIEKTISKAKGQPP REPQVYTLPPSREEMTKNQVSLWCLVKGFYPSDIA VEWESNGQPENNYKTTPVLDSGDSFFL YSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKS LSLSPGKGGSGGDYKDDDDK
	2246	Fc_hole-His		DKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISR TPEVTCVVVDVSHEDPEVKFNWYVDGVE VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNLN EYKCKVSNKALGAPIEKTISKAKGQPRE PQVYTLPPSREEMTKNQVSLSCAVKGFYPSDIAVE WESNGQPENNYKTTPVLDSGDSFFLVSK LTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL SPGKGGSGGHHHHHH
linker before tags or Fc	2241			GSGSG
tags	2247	Flag		DYKDDDDK

	2248	His		HHHHHH
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Table 6B: Combinatorial Wnt surrogate Constructs

FZD:LRP ratio	Construct
1FZD:1LRP	L1:5:F1_His
	L1:10:F1_His
	L1:15:F1_His
	F1:5:L1_His
	F1:10:L1_His
	F1:15:L1_His
	L2:5:F1_His
	L2:10:F1_His
	L2:15:F1_His
	F1:5:L2_His
	F1:10:L2_His
	F1:15:L2_His
	L2:5:F2_His
	L2:15:F2_His
	F2:5:L2_His
	F2:15:L2_His
	L1:5:F2_His
	L1:15:F2_His
	F2:5:L1_His
	F2:15:L1_His
	F3:5:L1_His
	L1:5:F3_His
	F3:5:L2_His
	L2:5:F3_His
	F1:5:Fc_knob+L2:5:Fc_hole
	F1:5:αGFP:Fc_knob+L2:5:αGFP:Fc_hole
	F1:5:αGFP:Fc_knob+αGFP:5:L2:Fc_hole
	αGFP:5:F1:Fc_knob+L2:5:αGFP:Fc_hole
	F3:5:Fc_knob+L2:5:Fc_hole
	F3:5:αGFP:Fc_knob+αGFP:5:L2:Fc_hole
	αGFP:5:F3:Fc_knob+L2:5:αGFP:Fc_hole
	L2:5mer:αGFP:Fc_knob+αGFP:5mer:F2:Fc_hole
	F2:5:Fc_knob+L2:5:Fc_hole
	F2:5:αGFP:Fc_knob+αGFP:5:L2:Fc_hole
2FZD:2LRP	L1:5:F1:Fc
	L1:10:F1:Fc
	L1:15:F1:Fc
	F1:5:L1:Fc
	F1:10:L1:Fc
	F1:15:L1:Fc
	L2:5:F1:Fc
	L2:10:F1:Fc

FZD:LRP ratio	Construct
	L2:15:F1:Fc
	F1:5:L2:Fc
	F1:10:L2:Fc
	F1:15:L2:Fc
	L1:5:F2:Fc
	L1:15:F2:Fc
	F2:5:L1:Fc
	F2:15:L1:Fc
	L2:5:F2:Fc
	L2:15:F2:Fc
	F2:5:L2:Fc
	F2:15:L2:Fc
	F3:5:L1:Fc
	F3:5:L2:Fc
	L1:5:F3:Fc
	L2:5:F3:Fc
	F1:5:Fc:5:L1
	L1:5:Fc:5:F1
	F1:5:Fc:5:L2
	L2:5:Fc:5:F1
	F3:5:L2:Fc_knob+F2:5:L2:Fc_hole
	L2:5:F3:Fc_knob+L2:5:F2:Fc_hole
	L1:5:F3:Fc_knob+L2:5:F2:Fc_hole
	F3:5:L1:Fc_knob+F2:5:L2:Fc_hole
0FZD:2LRP	α GFP:5:L1:Fc
	L1:5: α GFP:Fc
	α GFP:5:L2:Fc
	L2:5: α GFP:Fc
2FZD:0LRP	α GFP:5:F1:Fc
	F1:5: α GFP:Fc
2FZD:1LRP	F2:5:L2:Fc_knob+F2:5: α GFP:Fc_hole
	L2:5:F2:Fc_knob+ α GFP:5:F2:Fc_hole
	α GFP:5:F2:Fc_knob+L2:5:F2:Fc_hole
	L2:5:F3:Fc_knob+ α GFP:5:F2:Fc_hole
1FZD:2LRP	F2:5:L2:Fc_knob+ α GFP:5:L2:Fc_hole
	L2:5:F2:Fc_knob+L2:5: α GFP:Fc_hole
	F3:5:L2:Fc_knob+ α GFP:5:L2:Fc_hole
	L2:5:F3:Fc_knob+L2:5: α GFP:Fc_hole

A set of molecules generated replaced the DKK1c component in the 18R5-DKK1c molecule (Janda et al., 2017, *Nature*, 545(7653):234–237) with LRP binding antibody fragments in scFv format to generate a tandem scFv molecule, depicted in Fig. 8A. The LRP binder is a LRP6 E1E2 domains binder, 1115.3 (US 8,715,941 B2), which is referred herein as L1. To test the impact of

geometry to activity, L1 was fused to either the N- or C-terminus of 18R5 scFv (referred herein as F1) with 5, 10, or 15 amino acid linkers in between. This set of six proteins were purified to near homogeneity via Ni-affinity column (Fig. 8B) and tested in WNT responsive 293 STF reporter cells. While linker length did not seem to significantly affect activity, having the LRP6 binder fused to the N-terminus of the FZD binder, resulted in much higher activity than the other orientation (compare Fig. 8C to Fig. 8D, note the differences in the y-axis). All of these new surrogate molecules showed lower E_{max} (maximal response) than did both WNT3A and 18R5-DKK1c tested in parallel (Fig. 9).

Since scFv fragments have tendencies to form higher molecular weight aggregates, to confirm whether the surrogate WNT activities came from the monomeric form of the tandem scFv molecules, these six proteins were purified on size-exclusion-chromatography (SEC) and the peak fractions corresponding to the monomeric forms were tested in the 293 STF reporter cells. In contrast to the Ni purified material before SEC, the SEC purified proteins significantly lost activity (compare Fig. 8E and 8F to 8C and 8D). The fractions across the SEC runs were assayed in the 293 STF reporter cells. Peak activities were identified across the column fractions; however, the STF activity peaks did not coincide with the protein peak of the monomeric molecules. Instead, in all cases, the STF activity peaked in the higher molecular weight fractions (Fig. 8G and 8H, SEC molecular weight standards shown in Fig. 8I), suggesting that the 1:1 tandem scFv molecules were ineffective in inducing β -catenin dependent signaling. These results suggest that efficient activation of WNT signaling requires multimerization of receptor complexes. The tandem scFv molecules, which contain a 6 x His tag, were artificially crosslinking with anti-His antibodies and tested in the 293 STF reporter assay. As shown in Fig. 8J, while L1:5:F1 from the monomeric fraction alone was not effective in activating β -catenin dependent signaling in the presence of an isotype control (anti-GFP) antibody, the addition of anti-His antibody significantly induced signaling, supporting the idea that multimerization of receptor complexes may be required for signaling.

Multivalent molecules were produced in a more defined and easily produced format by attaching L1 and F1 tandem scFv fusions to the N-terminus of an Fc fragment as depicted in Fig. 10A to generate a tetravalent bispecific format. In this format, there are two binding sites against each receptor target (also noted as 2:2 in later sections representing a stoichiometric ratio of two FZDs and two LRPs). These proteins were purified by Protein A affinity step followed by SEC. As shown in Fig. 10B and 10C, the activity peaks from the 293 STF assay across the SEC fractions coincided with the protein peaks that corresponded to the monomer species of the molecule shown in Fig. 10A. SEC molecular weight standards are shown in Fig. 10D. These new configurations demonstrated a correlation between STF and major protein peaks. Dose response curves of the peak fractions were also performed, while linker length did not seem to significantly affect activity, the relative orientation of the two binders did affect activity. Fusing L1 to the N-terminus of F1 is the preferred orientation for the combination of these two binders (compare Fig. 10E and 10F y-axis). The relative E_{max} of the activities of these multivalent bispecific FZD/LRP binding molecules as compared to WNT3A and 18R5-DKK1c are similar to that of the pre-SEC material shown in Fig. 8 (Fig. 11). The affinity of the binding arms to their respective receptors in this tetravalent bispecific format was determined using bio-layer interferometry on an Octet instrument. As shown in Fig. 11B the relative orientation and linker lengths had no impact on the affinity of the respective arms to their target receptors, suggesting that the effects of orientation on the reporter activity is not due to impact of format on binding but rather the geometry of the receptors assembled by the surrogate molecules.

DKK1c predominantly binds LRP E3E4 domain (Ahn et al., 2011, Cheng et al., 2011). To understand the impact of LRP epitope on activity, the fusion of F1 with an LRP6E3E4 binder, YW211.31.57 (US 8,846,041 B2) referred herein as L2, was tested in the 1:1 tandem scFv format where L2 was fused to either the N- or C-terminus of F1 scFv with 5, 10, or 15 amino acid linkers in between. While these fusion proteins behaved less well and expressed at lower levels compared to the L1/F1 fusion proteins (Fig. 13A), similar to L1/F1 fusion

proteins shown in Fig. 8, the 1:1 tandem scFv of L2/F1 fusions were also ineffective in inducing β -catenin dependent signaling in 293 STF reporter cells as seen both in activity tests across the SEC column (Fig. 13A) as well as titration of monomeric peak fractions from the SEC column (Fig. 13B). Fusion of the tandem
5 scFv L2/F1 molecules to an Fc fragment to generate a 2:2 tetravalent bispecific molecule efficiently activated canonical WNT signaling. As shown in Fig. 12A, the peak from the 293 STF assay across the SEC fractions coincided with the protein peak that corresponded to the monomeric species. These L2/F1 fusion
10 molecules are much more active than the L1/F1 fusion molecules with Emax in range similar to WNT3A and significantly improved potency beyond both WNT3A and 18R5-DKK1c (Fig. 12B). The L2 and F1 combinations are less sensitive to orientation; both orientations resulted in similar Emax, while having L2 on the N-terminus of F1 with a 15mer linker yielded higher potency (Fig. 12B and 12C).

As a control to verify that the 2:2 tetravalent bispecific molecules
15 activate WNT signaling requires binding to both FZD and LRP receptors, either the FZD binder (F1) or the LRP binders (L1 or L2) was replaced with an scFv fragment that binds GFP in the 2:2 tetravalent bispecific format. These molecules are referred to as tetravalent monospecific or 2:0 or 0:2 formats. As shown in Fig. 12D and 12E, while all of these molecules bind to their respective receptors,
20 none of these tetravalent monospecific molecules are active, demonstrating that the ability to activate WNT signaling requires the engagement of both FZD and LRP.

To generate WNT surrogate molecules with different FZD specificity and to understand whether the multivalency requirement for WNT
25 signaling is a general requirement beyond the FZD binder F1 (which binds FZD1,2,7,5,8, Gurney et al., 2012), fusion proteins were generated between additional FZD binders, R2H1 (US 2016/0194394 A1) which binds to FZD1,2,7 referred herein as F2, and 2919 (WO 2017/127933 A1) which binds to FZD5,8 referred herein as F3, with the two LRP6 binders L1 and L2. The specificity of F2
30 and F3 were verified in Octet binding assays (Fig. 15A)

F2/L1 combination in the 1:1 tandem scFv format were constructed with 5 and 15mer linkers. Similar to the F1 fusion data in Fig. 8, the peak STF activities across the SEC column broadly distributed in the higher molecular weight fractions and did not coincide with the peak of the monomeric form of the molecules (Fig. 15B). In contrast, a STF peak activity across the SEC column coincided with the monomeric forms when the tandem scFvs were fused to Fc fragments in the 2:2 tetravalent bispecific format (Fig. 14A). A second STF activity peak on the SEC column was also observed in the higher molecular weight fractions that were not observed with the F1 fusion molecules (Fig. 14A). The dose response curves from the monomeric SEC peak fractions on 293 STF reporter cells showed slight preference for L1 fusion to the N-terminus of F2 for higher activity (Fig. 14B) similar to the fusions with F1 (Fig. 10).

Fusions between F2 and L2 were also constructed in the various formats (Table 6B). While on SEC column, the peak STF activities did not coincide with the monomeric forms of F2 fused to the N-terminus of L2 in the 1:1 format (Fig. 14C), it appears that the monomeric 1:1 forms of the reverse orientation where L2 was fused to the N-terminus of F2 were active (Fig. 14D). Dose response of the peak fractions in this orientation showed weaker activity compared to WNT3A with a preference for the 5mer linker (Fig. 14E). The 2:2 tetravalent bispecific format from the fusion of these tandem scFvs to the N-terminus of Fc yielded highly potent molecules. On SEC column, the peak STF activities coincided with protein peak that's consistent with the monomeric forms (Fig. 14F and 14H), and dose responses in 293 STF reporter cells showed potent and efficacious molecules, exceeded the activities of the F1/L2 fusions shown in Fig. 3 (Fig. 14G and 14I).

The 1:1 bivalent bispecific tandem scFv format and the 2:2 tetravalent bispecific fusions to Fc were also constructed between F3 and the two LRP binders. Similar to the observation with F1 fusions shown in Fig. 8, no robust activities were observed for the 1:1 tandem scFv fusion molecules between F3 and L1 or L2 (Fig. 17). In contrast, 293 STF reporter activities of the fusion molecules between F3, LRP binders, and Fc in the 2:2 tetravalent

bispecific format from the SEC column fractions coincided to the protein peak of the monomeric forms of the molecules (Fig. 16A and 16B). It is interesting to note that similar to fusions with F1 and F2, the dose response of the peak SEC fractions show that the L2-F3 fusions are highly active in both orientations, while L1-F3 fusions are less active and show preference of L1 on the N-terminus of F3 (Fig. 16C and 16D). All of these results together suggest that multivalency of FZD and LRP binding with the stoichiometry of two FZDs and two LRPs are required for consistent and efficient activation of β -catenin dependent signaling, at least with the binders tested.

10 Having established the multivalency requirement for efficient induction of canonical WNT signaling, alternative 2:2 tetravalent bispecific format where the two scFv binders are attached to the two ends of a Fc fragment as shown in Fig. 18A and 18B were explored (termed dumbbell format). The fusion of F1 with L1 in this dumbbell format minimally activated WNT signaling in reporter assay (Fig. 18A). Interestingly, there is also a preference for the orientation of the L1 molecule where its attachment C-terminal to Fc affected activity. The fusions of F1 and L2 in this format also activated WNT signaling with similar preference in orientation for the LRP binding arm (Fig. 18B). The activity across the SEC columns were also tested for these molecules, and as shown in Fig. 18C, peak STF reporter activities coincided with the protein peak corresponding to the monovalent forms of the proteins.

25 To understand whether the activity from the 2:2 tetravalent bispecific format is due to the simultaneous binding of the same surrogate molecule to multiple FZDs and LRPs or whether it is due to a unique geometry provided by the format that does not require the multivalent binding to receptors, Octet binding studies to assess whether the 1:1 tandem scFv format allows simultaneous binding to both FZD and LRP. As shown in Fig. 19A, by sequential addition of FZD8 CRD to sensor surface, followed by 1:1 tandem scFv L2:5:F3 or F3:5:L2, and then LRP6 E3E4, a stepwise increase in binding signal was observed. This suggests that the 1:1 tandem scFv molecules are capable of binding to both receptors simultaneously, and therefore, the lack of activity from

these molecules in inducing WNT signaling is not due to inability to crosslink FZD and LRP receptors.

To determine if the 2:2 tetravalent bispecific format still engages the receptors in the 1:1 fashion, however, through a unique geometry that allows signaling not provided by the 1:1 tandem scFv format. We constructed 4 different variations of the possible 1:1 geometry on the framework of the 2:2 tetravalent bispecific molecule as depicted in Fig. 19B and Fig. 20. None of these 4 molecules induced WNT signaling (Fig. 19C and Fig. 20A) suggesting that cross-linking multiple copies of the receptors are required for efficient signaling.

To further understand the required ratio of FZD to LRP receptors, either 2 FZD binders with 1 LRP binder (2:1) or 1 FZD binder combined with 2 LRP binders (1:2) were constructed as depicted in Fig. 21A and 21D. As shown in Fig. 21, removing either 1 FZD binder or 1 LRP binder from the 2:2 format still activated β -catenin dependent signaling. Since the 1:1 formats shown in Fig. 19 did not activate signaling, these results further supports the notion that simultaneous engagement of the multiple FZDs and LRPs are necessary for efficient signaling. As removing 1 LRP binder having much less impact on the activity than removing 1 FZD binder, these results also suggest that dimerization of FZD receptors is more important for signaling complex formation.

The multivalency requirement for the efficient induction of canonical WNT signaling is established, however, since each of the FZD binding arms in the surrogate molecules have the specificity to several different FZD receptors, it is not clear whether signaling can come from heterodimerization of different FZD receptors. To address this question, two FZD binders that have non-overlapping specificity, F2 and F3, were combined into one molecule as depicted in Fig. 21G. While either F2 or F3 in the 1:2 format (1 FZD binder combined with 2 copies of LRP binders, Fig. 21B and 21C) showed low to very low activities in the 293STF reporter cells, respectively, the combination of F2 and F3 together with 2 copies of LRP binders L2 into one molecule (we termed this 1:1:2 format with the stoichiometry of one FZD plus one different FZD plus two LRPs) yielded highly potent and efficacious surrogate molecules (Fig. 21H). To further confirm this, a

molecule with F2 and F3 and one copy of LRP binder L2 was constructed (1:1:1:0 format with the stoichiometry of one FZD plus one different FZD plus one LRP binder, Table 6B and Fig. 21I). This molecule is also highly active (Fig. 21J), even though F2 or F3 alone with one copy of L2 showed no activity (Fig. 20).

- 5 Since F2 and F3 bind to different FZDs, these results suggest that heterodimerization of FZD leads to β -catenin dependent signaling.

As a final configuration, we also constructed surrogate molecules with two different FZD binding arms and two different LRP binding arms (1:1:1:1 format) as depicted in Fig. 21K. It is interesting to note that although surrogate
10 molecules containing the LRP binder L1 consistently yielded much lower E_{max} compared to WNT3A, when combined with L2 in the context of 1:1:1:1 format, yielded highly potent and efficacious molecules (Fig. 21L).

All the observation together demonstrate a new surrogate WNT platform that is flexible, can produce any FZD/LRP specificity combination, and
15 can generate highly potent and active molecules.

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

25 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.

CLAIMS

What is claimed is:

1. A multispecific Wnt surrogate molecule, wherein the Wnt surrogate molecule comprises:
 - (i) a plurality of regions that each specifically bind to a set of one or more Frizzled (Fzd) receptor epitopes (Fzd binding regions), wherein at least two Fzd binding regions bind to different sets of one or more Fzd receptor epitopes; and
 - (ii) one or more regions that specifically bind to a Low-density lipoprotein (LDL) receptor-related protein 5 (LRP5) and/or a LDL receptor-related protein 6 (LRP6) (LRP5/6 binding regions).
2. The Wnt surrogate molecule of claim 1, wherein the at least two Fzd binding regions bind to different sets of one or more Fzd receptors, different sets of one or more epitopes within the same set of one or more Fzd receptors, or a combination thereof.
3. The Wnt surrogate molecule of any one of claims 1-2, wherein each Fzd binding region 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).
4. The Wnt surrogate molecule of any one of claims 1-3, wherein at least one Fzd binding region binds to: (i) Fzd1, Fzd2, Fzd7, and Fzd9; (ii) Fzd1, Fzd2, and Fzd7; (iii) Fzd5 and Fzd8; (iv) Fzd5, Fzd7, and Fzd8; (v) Fzd1, Fzd4, Fzd5, and Fzd8; (vi) Fzd1, Fzd2, Fzd5, Fzd7, and Fzd8; (vii) Fzd4 and Fzd9; (viii) Fzd9 and Fzd10; (ix) Fzd5, Fzd8, and Fzd10; (x) Fzd4, Fzd5, and Fzd8; or (xi) Fzd1, Fzd5, Fzd7 and Fzd8.
5. The Wnt surrogate molecule of any one of claims 1-4, wherein the plurality of Fzd binding regions comprises:

(i) a first Fzd binding region that binds to a first set of one or more Fzd receptors, and

(ii) a second Fzd binding region that binds to a second, different set of one or more Fzd receptors.

6. The Wnt surrogate molecule of claim 5, wherein:

(i) the first Fzd binding region binds to one or more of Fzd1, Fzd2, Fzd3, Fzd4, Fzd5, Fzd6, Fzd7, Fzd8, Fzd9, and Fzd10, and

(ii) the second Fzd binding region binds to one or more of Fzd1, Fzd2, Fzd3, Fzd4, Fzd5, Fzd6, Fzd7, Fzd8, Fzd9, and Fzd10.

7. The Wnt surrogate molecule of claim 5, wherein the first Fzd binding region binds to Fzd4 and the second Fzd binding region binds to Fzd9.

8. The Wnt surrogate molecule of any one of claims 1-7, wherein the plurality of Fzd binding regions comprises:

(i) a first Fzd binding region that binds to a first set of one or more epitopes within a set of one or more Fzd receptors, and

(ii) a second Fzd binding region that binds to a second, different set of one or more epitopes within the same set of one or more Fzd receptors.

9. The Wnt surrogate of claim 8, wherein:

a) the first Fzd binding region binds to at least one Fzd receptor that induces non-canonical Wnt signaling; and

b) the second Fzd binding region binds to at least one Fzd receptor that induces canonical Wnt signaling.

10. The Wnt surrogate of claim 9, wherein binding of the first Fzd receptor and second Fzd receptor results in:

a. canonical Wnt signaling; or

b. non-canonical Wnt signaling.

11. The Wnt surrogate molecule of any one of claims 1-10, wherein at least one Fzd binding region binds monospecifically to a single Fzd receptor.
12. The Wnt surrogate molecule of claim 11, wherein the at least one Fzd binding region binds monospecifically to Fzd1, Fzd2, Fzd3, Fzd4, Fzd5, Fzd6, Fzd7, Fzd8, Fzd9, or Fzd10.
13. The Wnt surrogate molecule of any one of claims 1-12, wherein at least one Fzd binding region binds to a region of a Fzd receptor that (i) does not include the cysteine rich domain (CRD) of the Fzd receptor or (ii) includes less than the entire CRD of the FZD receptor or (iii) overlaps with the CRD of the Fzd receptor.
14. The Wnt surrogate molecule of claim 13, wherein the at least one Fzd binding region binds to a hinge region of the Fzd receptor.
15. The Wnt surrogate molecule of claim 14, wherein the hinge region comprises an amino acid sequence having at least 90% identity to any of the sequences set forth in SEQ ID NOs:98-107.
16. The Wnt surrogate molecule of claim 13 wherein the at least one Fzd binding region binds to an N-terminal region upstream of the CRD of the Fzd receptor.
17. The Wnt surrogate molecule of claim 16, wherein the N-terminal region comprises an amino acid sequence having at least 90% identity to SEQ ID NO:108.
18. The Wnt surrogate molecule of any one of claims 1-17, wherein at least one of the Fzd binding regions comprises one or more antigen-binding fragments of an antibody.

19. The Wnt surrogate molecule of claim 18, wherein the one or more antigen-binding fragments are selected from the group consisting of: IgG, Fv, scFv, Fab, and VHH or sdAbs.
20. The Wnt surrogate molecule of any one of claims 18-19, wherein the one or more antigen-binding fragments are humanized.
21. The Wnt surrogate molecule of any one of claims 1-20, wherein at least one Fzd binding region comprises an amino acid sequence having at least 90% identity to any of the sequences set forth in Table 1A, Table 1B, or SEQ ID NOs:1-73, or an antigen-binding fragment thereof.
22. The Wnt surrogate molecule of any one of claims 1-21, wherein the one or more LRP5/6 binding regions comprises one or more antigen-binding fragments of an antibody.
23. The Wnt surrogate molecule of claim 22, wherein the one or more antigen-binding fragments are selected from the group consisting of: IgG, Fv, scFv, Fab, and VHH or sdAb.
24. The Wnt surrogate molecule of any one of claims 22-23, wherein the one or more antigen-binding fragments are humanized.
25. The Wnt surrogate molecule of any one of claims 1-24, wherein the one or more LRP5/6 binding regions comprise an amino acid sequence having at least 90% identity to any of the sequences set forth in Table 2A, Table 2B, or SEQ ID NOs:74-97, or an antigen-binding fragment thereof.
26. The Wnt surrogate molecule of any one of claims 1-25, comprising one or more LRP5/6 binding regions.

27. The Wnt surrogate molecule of any one of claims 1-26, wherein the Fzd binding regions and the LRP5/6 binding regions are in a ratio of Fzd_n:LRP5/6_n (F_n:L_n), wherein F and L are integers between 1 and 9, inclusive, and n is an integer between 1 and 4 inclusive.

28. The Wnt surrogate molecule of any one of claims 1-27, wherein the Fzd binding regions and the LRP5/6 binding regions are in a ratio of Fzd:LRP5/6 selected from the group consisting of: 1:1, 2:1, 3:1, 4:1, 5:1, 6:1, 2:1 2:3, 2:5, 2:7, 7:2, 5:2, 3:2, 3:4, 3:5, 3:7, 3:8, 8:3, 7:3, 5:3, 4:3, 4:5, 4:7, 4:9, 9:4, 7:4, 5:4, 6:7, 7:6, 1:2, 1:3, 1:4, 1:5, 1:6, 2:1 (with two Fzd binders and one LRP binder), 1:2 (with one Fzd binder and two LRP binders), 2:1:1 (with two different LRP binders), 1:1:2 (with two different Fzd binders), 1:1:1 (two different Fzd binders and one LRP binder or one Fzd binder and two different LRP binders) and 1:1:1:1 (all different Fzd and LRP binders)..

29. The Wnt surrogate molecule of claim 28, wherein the ratio of Fzd binding regions to LRP5/6 binding regions (Fzd:LRP5/6) comprises:

- a) 2 Fzd binding regions and 2 LRP5/6 binding regions;
- b) 2 Fzd binding regions and 1 LRP5/6 binding region; and
- c) 1 Fzd binding region and 2 LRP5/6 binding regions.

30. The Wnt surrogate molecule of claim 28, wherein the ratio of Fzd binding regions to LRP5/6 binding regions (Fzd:LRP5/6) comprises:

- a) a first Fzd binding region, a second Fzd binding region, and 1 LRP5/6 binding region; or
 - b) a first Fzd binding regions, a second Fzd binding regions, a first LRP5/6 binding region, and a second LRP5/6 binding region,
- wherein the first Fzd and second Fzd binding regions bind to same or different Fzd receptors, or binding to the same Fzd receptor on different regions/epitope, and the first LRP5/6 and second LRP5/6 binding regions bind to different epitopes or to same or different LRP proteins.

31. The Wnt surrogate molecule of any one of claims 1-30, wherein two or more LRP binding regions comprise:

- (i) a first LRP binding region that binds to a first set of one or more LRP receptors, and
- (ii) a second LRP binding region that binds to a second, different set of one or more LRP receptors.

32. The Wnt surrogate molecule of any one of claims 1-31, comprising a structural format selected from the group consisting of: hetero-Ig, diabody (DART), tandem diabody (DART), diabody-Fc, Fabs-in-tandem, Fabs-in-tandem IgG (FIT-Ig), Fv-IgG, and tandem scFv.

33. The Wnt surrogate molecule of any one of claims 1-32, comprising (i) a first light chain and a first heavy chain forming a first Fzd binding region, and (ii) a second light chain and a second heavy chain forming a second Fzd binding region, wherein the first and second Fzd binding regions bind to same or different sets of one or more Fzd receptor epitopes.

34. The Wnt surrogate molecule of claim 33, comprising a first LRP5/6 binding region fused to an N-terminus of the first light chain, a C-terminus of the first light chain, an N-terminus of the first heavy chain, or a C-terminus of the first heavy chain.

35. The Wnt surrogate molecule of any one of claims 33-34, comprising a second LRP5/6 binding region fused to an N-terminus of the second light chain, a C-terminus of the second light chain, an N-terminus of the second heavy chain, or a C-terminus of the second heavy chain.

36. The Wnt surrogate molecule of any one of claims 33-35, wherein the first and second heavy chains are connected to each other.

37. The Wnt surrogate molecule of claim 36, wherein the first heavy chain comprises a first CH3 domain, the second heavy chain comprises a second CH3 domain, and the first and second CH3 domains are connected to/interact with each other.
38. The Wnt surrogate molecule of claim 37, wherein the first and second CH3 domains are connected to each other via knobs-into-holes mutations.
39. The Wnt surrogate molecule of any one of claims 33-38, wherein: (i) the first heavy chain and/or the second heavy chain comprise an amino acid sequence having at least 90% identity to any of the sequences set forth in SEQ ID NOs:110, 112, 114, 116, 118, 120, and 122, and (ii) the first light chain and/or the second light chain comprise an amino acid sequence having at least 90% identity to any of the sequences set forth in SEQ ID NOs:109, 111, 113, 115, 117, 119, and 121.
40. The Wnt surrogate molecule of claim 1, comprising one or more sequences set forth in Table 5 or Table 6A.
41. The Wnt surrogate molecule of claim 41, having a structure as set forth in Table 6B.
42. The Wnt surrogate molecule of any of claims 1-41, which modulates a Wnt signaling pathway in a cell, optionally a mammalian cell.
43. The Wnt surrogate molecule of claim 42, which increases signaling via the Wnt signaling pathway in the cell.
44. The Wnt surrogate molecule of any one of claims 42-43, wherein the Wnt signaling pathway is a canonical Wnt signaling pathway.

45. The Wnt surrogate molecule of any one of claims 42-43, wherein the Wnt signaling pathway is a non-canonical Wnt signaling pathway.

46. A pharmaceutical composition comprising a pharmaceutically acceptable excipient, diluent, or carrier, and the Wnt surrogate molecule according to any of claims 1-45.

47. A method for agonizing a Wnt signaling pathway in a cell, comprising contacting the cell with the Wnt surrogate molecule according to any of claims 1-45 or the pharmaceutical composition of claim 45, wherein the Wnt surrogate molecule is an agonist of a Wnt signaling pathway.

48. A method for treating a subject having a disease or disorder, comprising administering to the subject an effective amount of the Wnt surrogate molecule according to any of claims 1-45 or the pharmaceutical composition of claim 44, wherein the Wnt surrogate molecule is an agonist of a Wnt signaling pathway.

49. The method of claim 48, wherein the disease or disorder is associated with reduced or impaired Wnt signaling, and/or wherein the subject would benefit from increased Wnt signaling.

50. The method of any one of claims 48-49, wherein the disease or disorder is selected from the group consisting of: bone fractures, stress fractures, vertebral compression fractures, osteoporosis, osteoporotic fractures, non-union fractures, delayed union fractures, spinal fusion, pre-operative optimization for spine surgeries, osteonecrosis, osseointegration of implants or orthopedic devices, osteogenesis imperfecta, bone grafts, tendon repair, tendon-bone integration, tooth growth and regeneration, maxillofacial surgery, dental implantation, periodontal diseases, maxillofacial reconstruction, osteonecrosis of the jaw, hip or femoral head, avascular necrosis, alopecia, hearing loss, vestibular hypofunction, macular degeneration, age-related macular degeneration (AMD), vitreoretinopathy, retinopathy, diabetic

retinopathy, diseases of retinal degeneration, Fuchs' dystrophy, cornea diseases, stroke, traumatic brain injury, Alzheimer's disease, multiple sclerosis, muscular dystrophy, muscle atrophy caused by sarcopenia or cachexia, diseases affecting blood brain barrier (BBB), spinal cord injuries, spinal cord diseases, oral mucositis, short bowel syndrome, inflammatory bowel diseases (IBD), metabolic syndrome, diabetes, dyslipidemia, pancreatitis, exocrine pancreatic insufficiency, wound healing, diabetic foot ulcers, pressure sores, venous leg ulcers, epidermolysis bullosa, dermal hypoplasia, myocardial infarction, coronary artery disease, heart failure, hematopoietic cell disorders, immunodeficiencies, graft versus host diseases, acute kidney injuries, chronic kidney diseases, chronic obstructive pulmonary diseases (COPD), idiopathic pulmonary fibrosis, acute liver failure of all causes, acute liver failure drug-induced, alcoholic liver diseases, chronic liver failure of all causes, cirrhosis, liver fibrosis of all causes, portal hypertension, chronic liver insufficiency of all causes, nonalcoholic steatohepatitis (NASH), nonalcoholic fatty liver disease (NAFLD) (fatty liver), alcoholic hepatitis, hepatitis C virus-induced liver diseases (HCV), hepatitis B virus-induced liver diseases (HBV), other viral hepatitis (e.g., hepatitis A virus-induced liver diseases (HAV) and hepatitis D virus-induced liver diseases (HDV)), primary biliary cirrhosis, autoimmune hepatitis, liver surgery, liver injury, liver transplantation, "small for size" syndrome in liver surgery and transplantation, congenital liver disease and disorders, any other liver disorder or defect resulting from genetic diseases, degeneration, aging, drugs, and injuries.

51. The method of any one of claims 48-49, wherein the disease or disorder is a bone disease or disorder.

52. The method of claim 51, wherein the Wnt surrogate molecule binds: (i) Fzd1, Fzd2, and Fzd7; or (ii) Fzd1, Fzd2, Fzd5, Fzd7, and Fzd8.

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FIG. 1

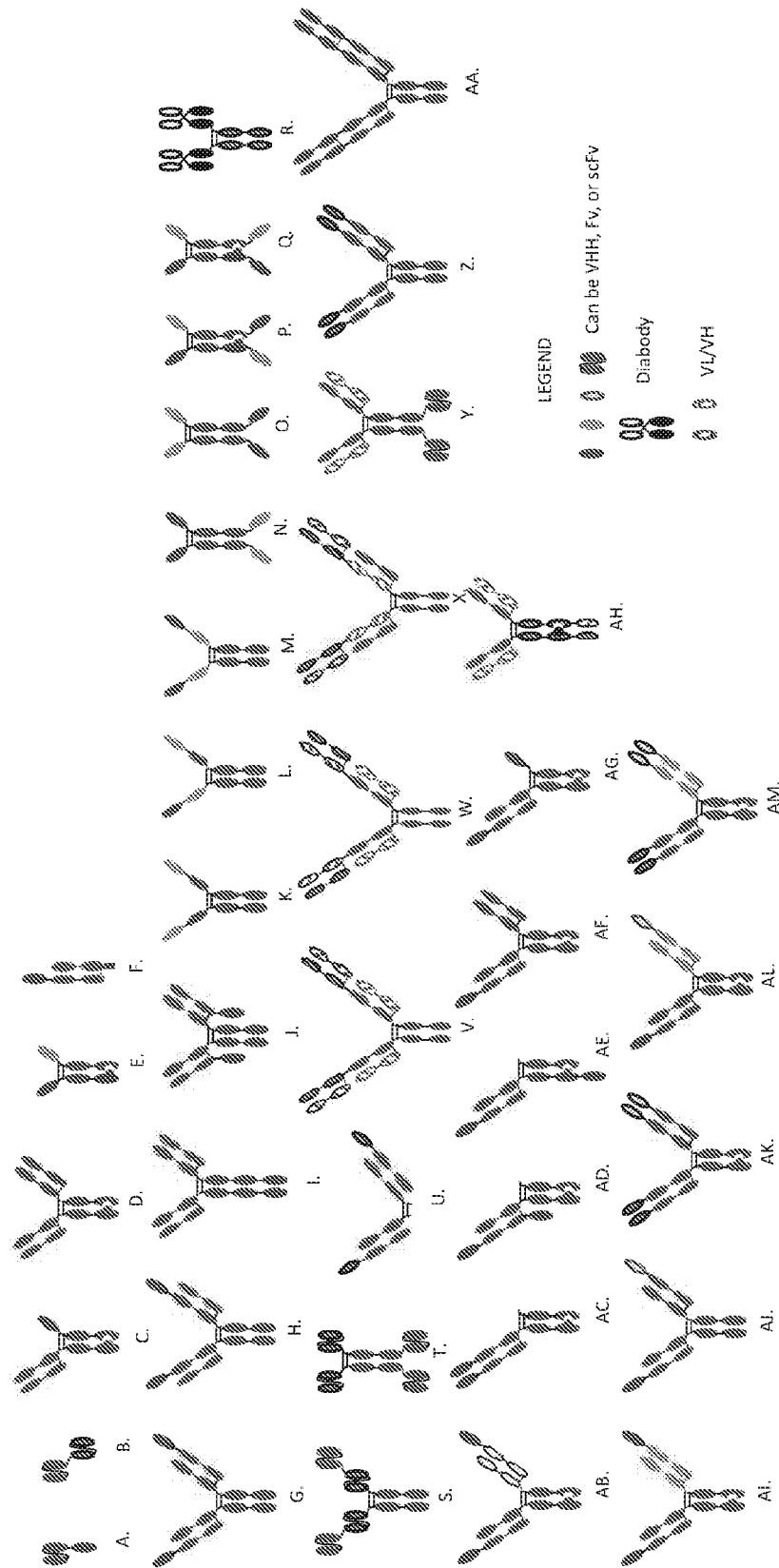


FIG. 2A

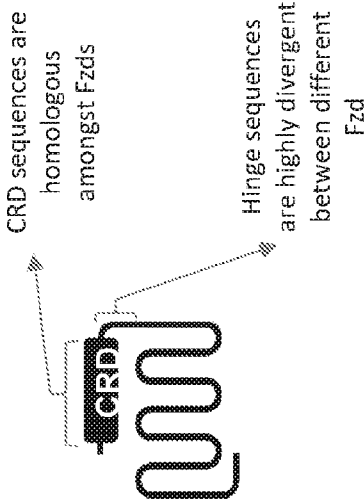


FIG. 2B

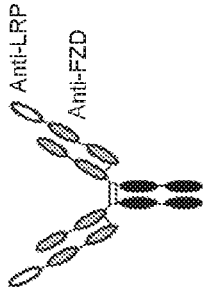


FIG. 2C

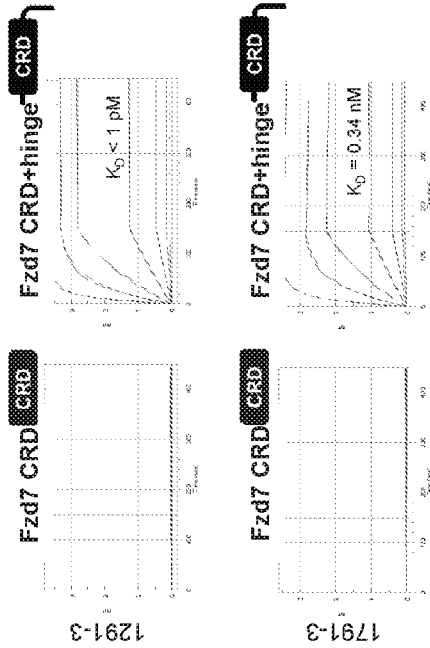


FIG. 2D

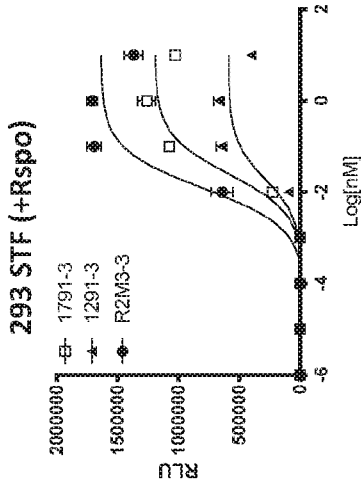


FIG. 3A

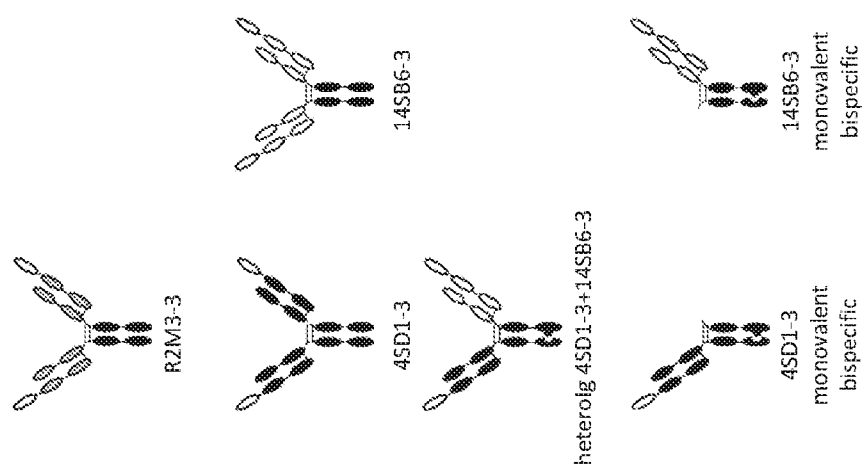


FIG. 3B

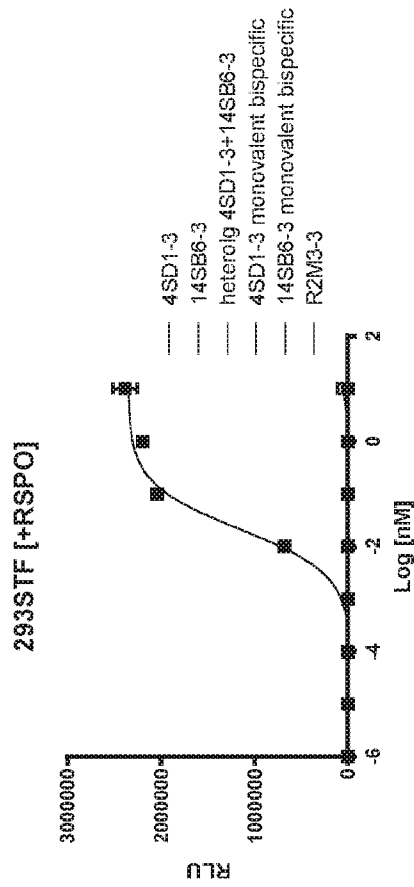


FIG. 3C

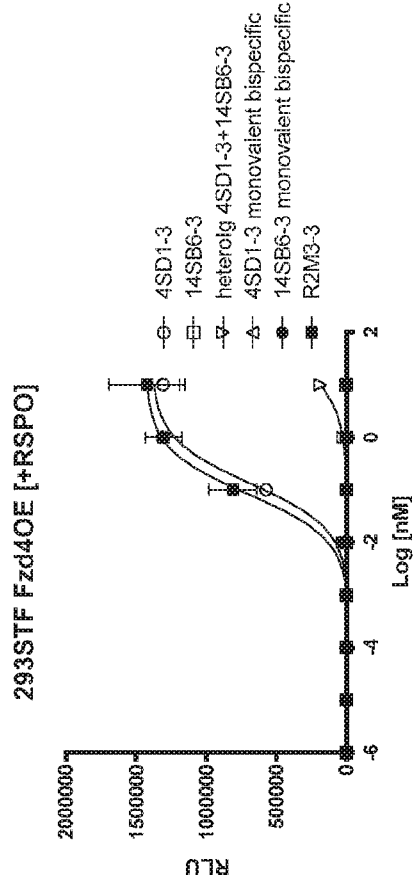


FIG. 3D

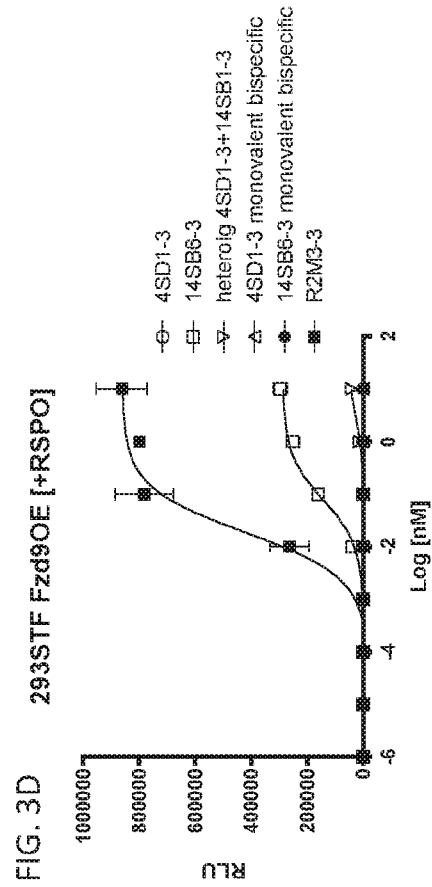
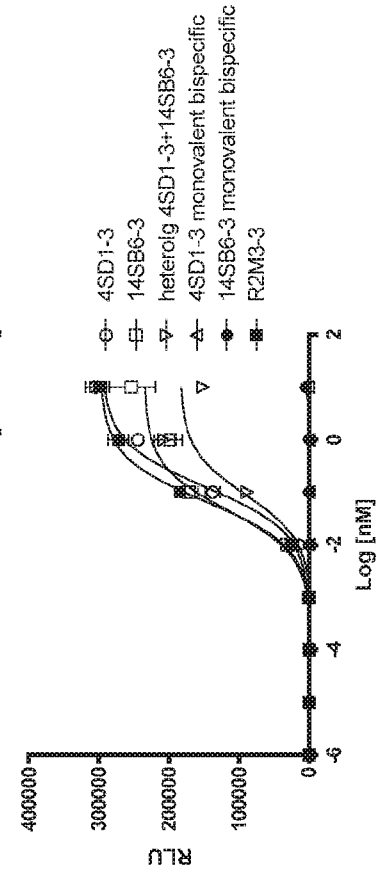


FIG. 3E



hzd3-hinge	-----GAPVAVQPDIGTWC	33
hzd6-hinge	-----KTEIQVQPDIGTWC	33
hzd5-hinge	-----	35
hzd8-hinge	APPARGGGGKARFEGGKAF	61
hzd9-hinge	-----C	45
hzd10-hinge	-----C	45
hzd4-hinge	LPKRTPI-QPE-----EC	24
hzd7-hinge	CPPTAM-SPDGGKGLSPPSC	49
hzd1-hinge	-----ASD-----HGKESC	50
hzd2-hinge	-----APPTAULSHGPHSC	52

FIG. 4A

Percent Identity Matrix - created by Clustal2.1

1: hFzd7-hinge 100.00 44.12 39.47
 2: hFzd1-hinge 44.12 100.00 44.68
 3: hFzd2-hinge 39.47 44.68 100.00

CLUSTAL O(1.2.4) multiple sequence alignment

```

hFzd7-hinge  CVGQNTSGSGCA-----GGSPAYAYAPYLDPFFETAMSSSDSGRLS  44
hFzd1-hinge  CVGQNTSGTPIPSILFEWISNPOHGGGHHGGPTGGAG-----AST-----R  45
hFzd2-hinge  CVGQNHSGDGPALL--TTAPPGLQPGASSTTSG--PSSGGS-----RPPHYATLE  47
*****+;. : ** +.

```

FIG. 4B

```

hFzd7-hinge  RPFSC 49
hFzd1-hinge  GKFSC 50
hFzd2-hinge  RPFSC 52
* +

```

Percent Identity Matrix - created by Clustal2.1

1: hFzd5-hinge 100.00 47.06
 2: hFzd8-hinge 47.06 100.00

CLUSTAL O(1.2.4) multiple sequence alignment

```

hFzd5-hinge  CNDYNRSEATTAPEPEPAKXETLPGPGAPASGSEC----- 36
hFzd8-hinge  CNDYNRDLATAPSPRPRIIP--PPFGDQPPSGSGHGPFGAPPIHGGGSGSGDA 58
*****+; *** * * * *** +.

```

```

hFzd5-hinge  ----- 36
hFzd8-hinge  APPARGSGGGAHPFGGGAAPC  81

```

FIG. 4C

```
# Percent Identity Matrix - created by Clustal2.1
1: hFzd3-linker 100.00 39.39
2: hFzd6-linker 39.39 100.00

CLUSTAL O(1.2.4) multiple sequence alignment
hFzd3-hinge      CDEEYFELVDLNLAGEPIEGAFVAVQRDYGEWC 33
hFzd6-hinge      CDEEYFVFDFHFIPLGEGQNKIEVDVQRDIGEWG 33
                *** * .* :          **** *****
```

FIG. 4D

```
# Percent Identity Matrix - created by Clustal2.1
1: hFzd9-linker 100.00 47.62
2: hFzd10-linker 47.62 100.00

CLUSTAL O(1.2.4) multiple sequence alignment
hFzd9-hinge      CMEAPENATNGFAEPHFGGLGMLFVAFRPARPYGO-----LGRGAGGSHIC 45
hFzd10-hinge      CMEAPNKG----SDEPTGSGSLPPLFRPQRPMSAQEHLEKDGEGKGGC 46
                >>>>>.a.  **:**:** >>>>> . > * > * >
```

FIG. 4E

FIG. 5A

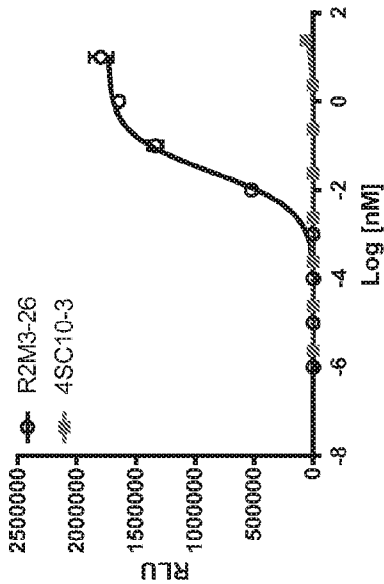
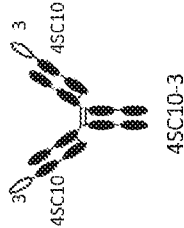


FIG. 5B

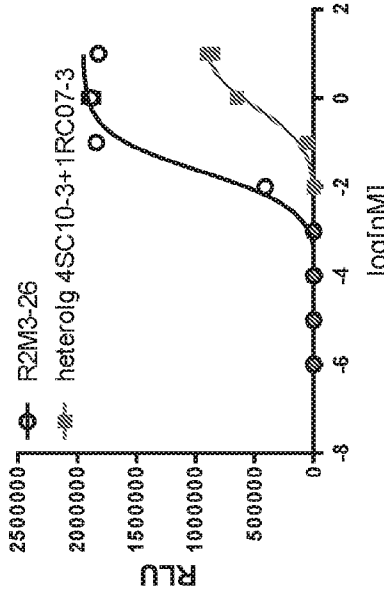
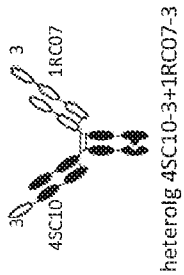


FIG. 5C

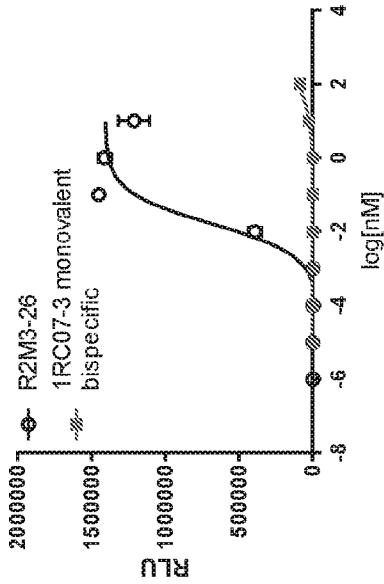
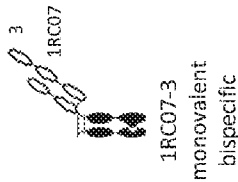


FIG. 6A

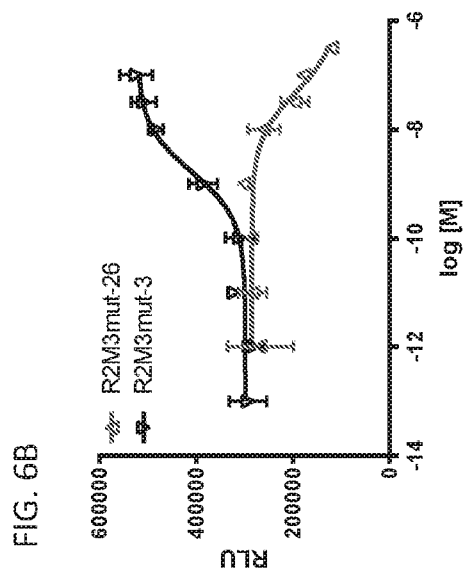
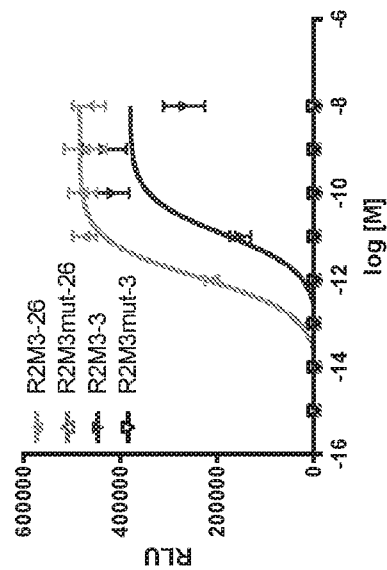


FIG. 6C

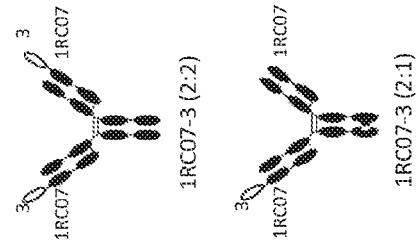
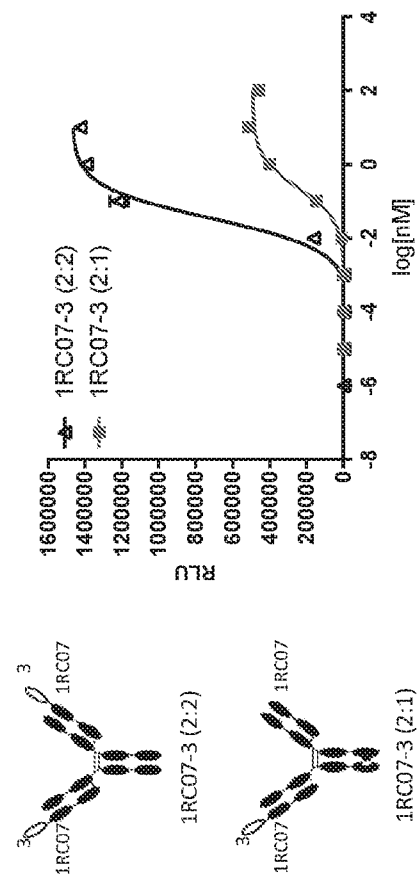
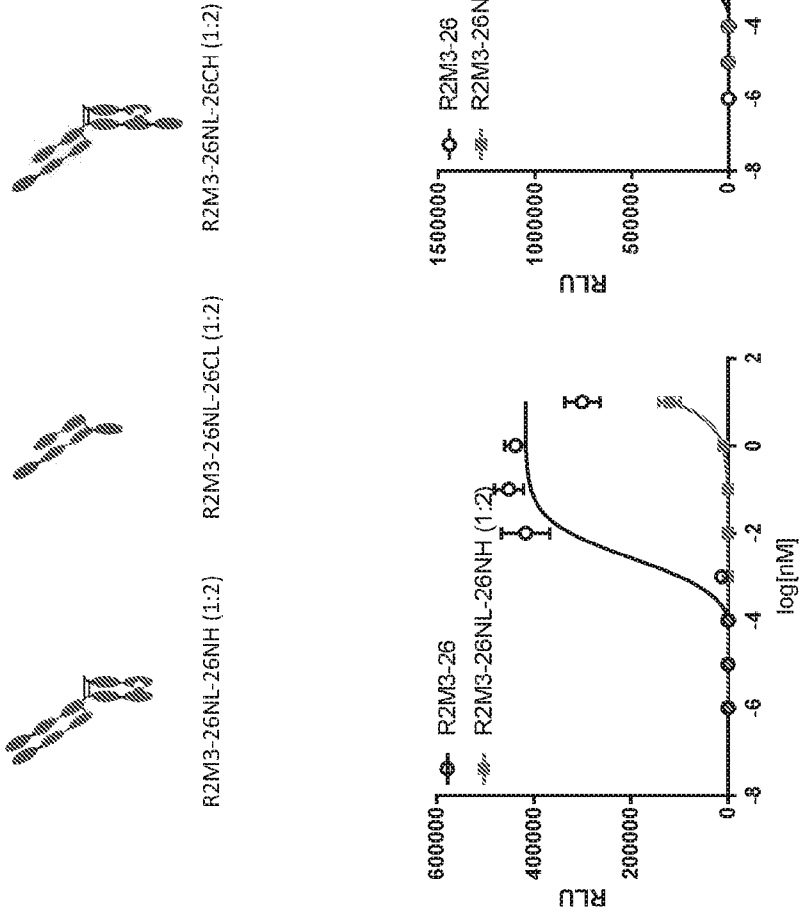


FIG. 6D



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FIG. 6E

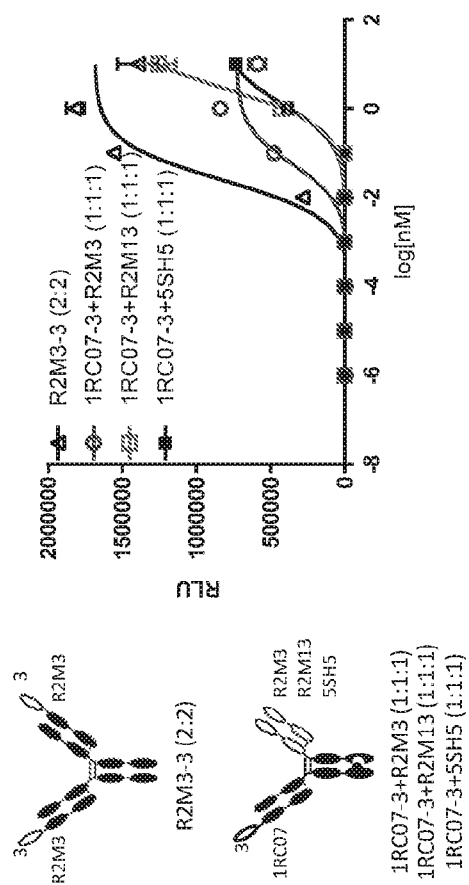


FIG. 7A

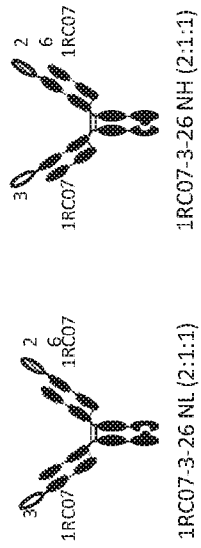


FIG. 7B

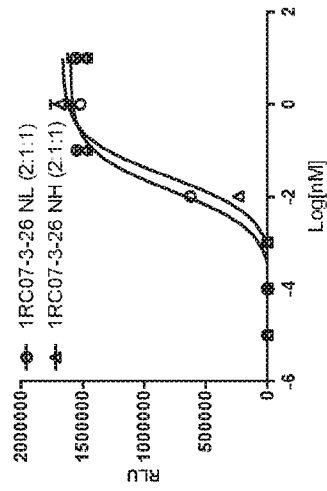


FIG. 7C

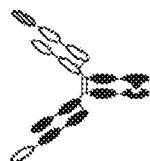


FIG. 7D

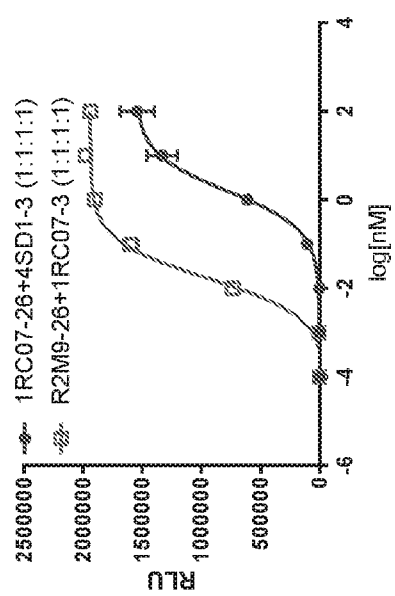
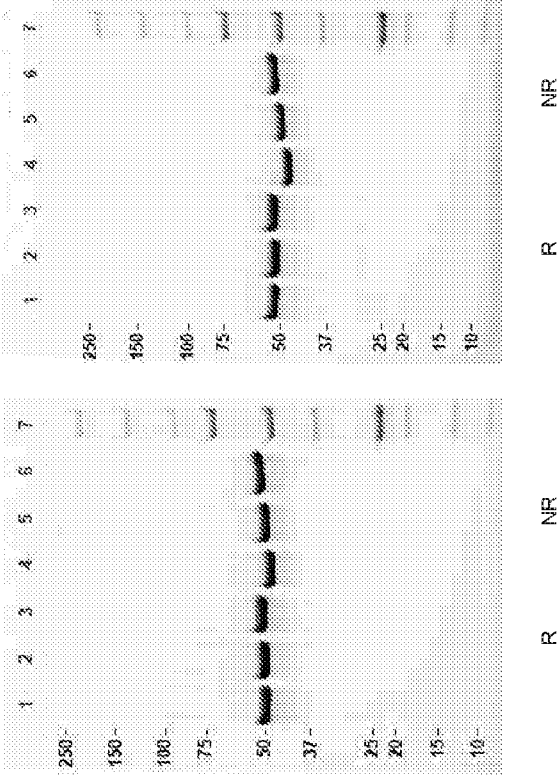


FIG. 8A



FIG. 8B



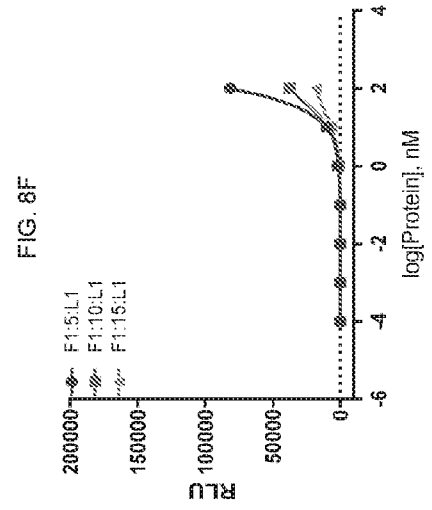
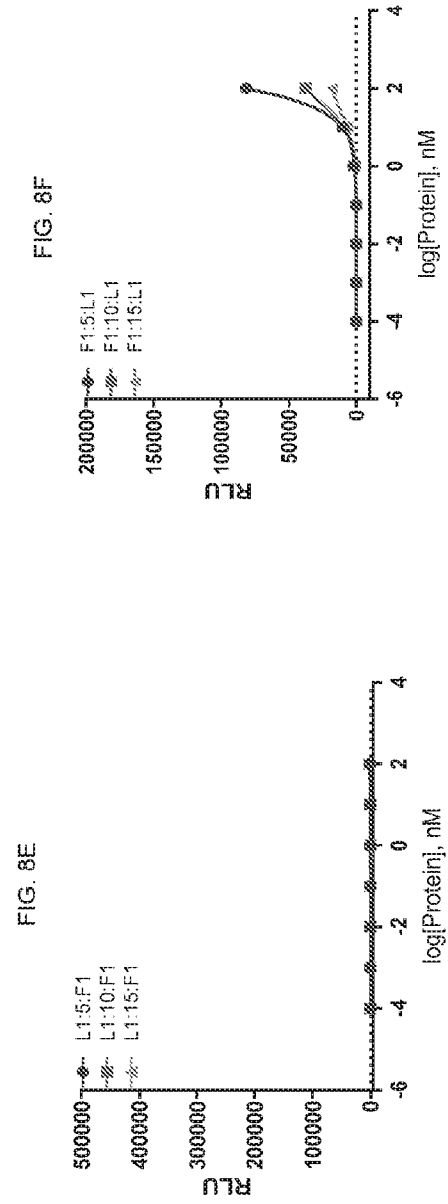
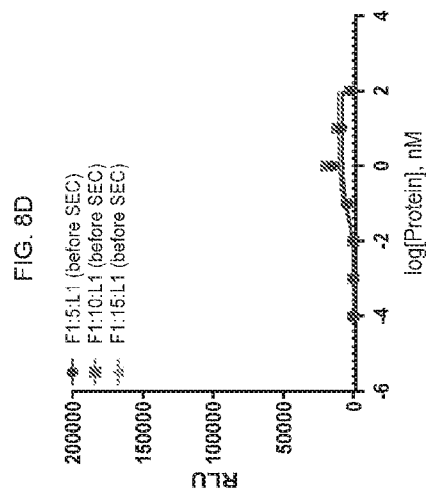
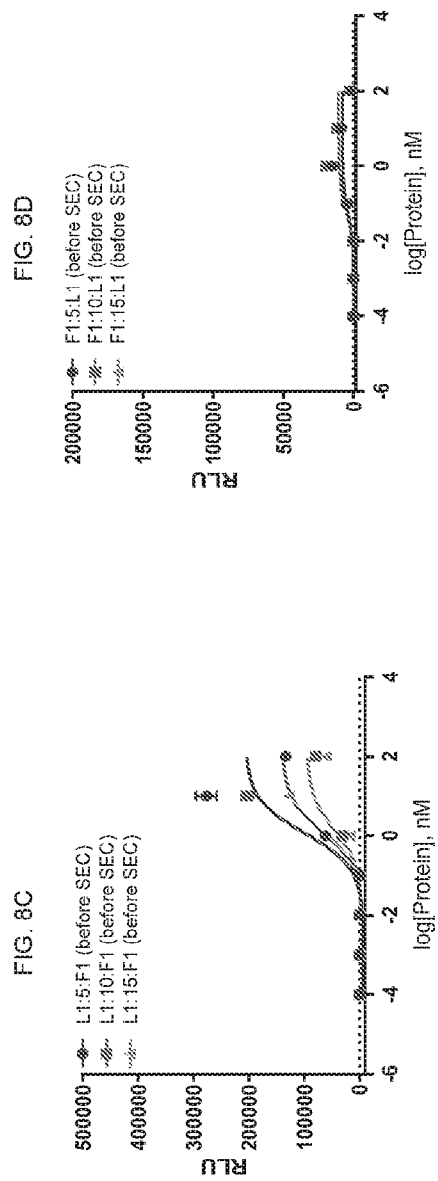


FIG. 8H

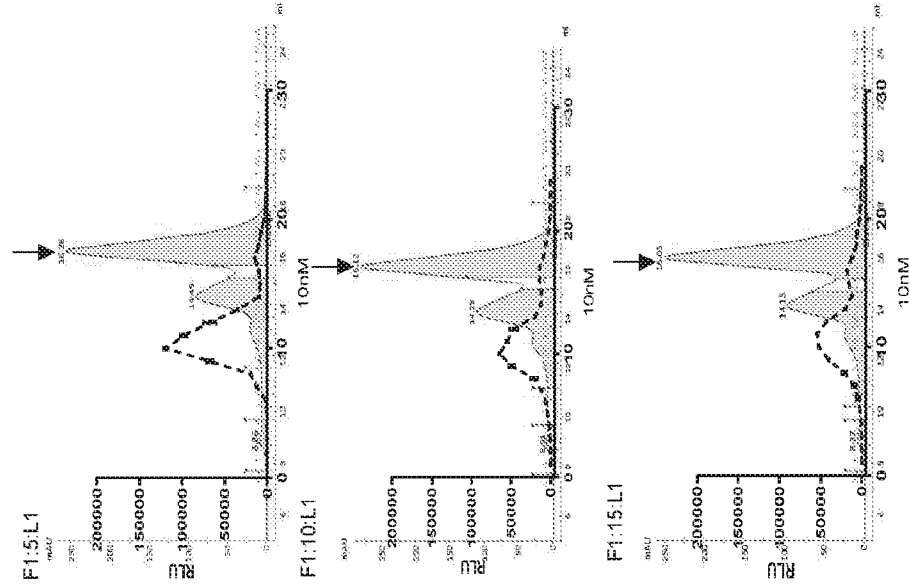


FIG. 8G

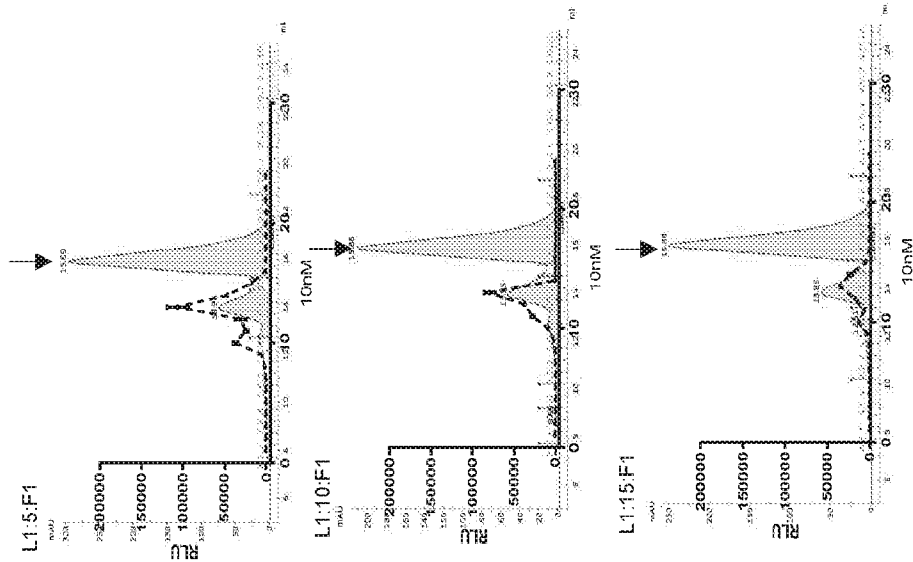


FIG. 8J

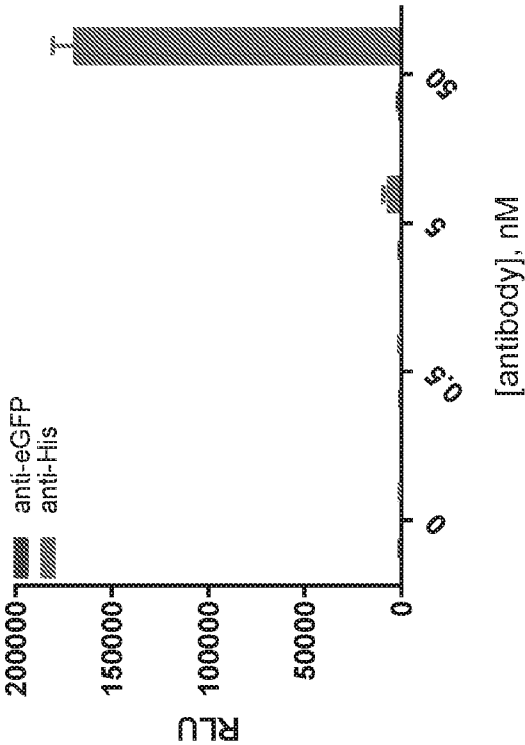


FIG. 8I

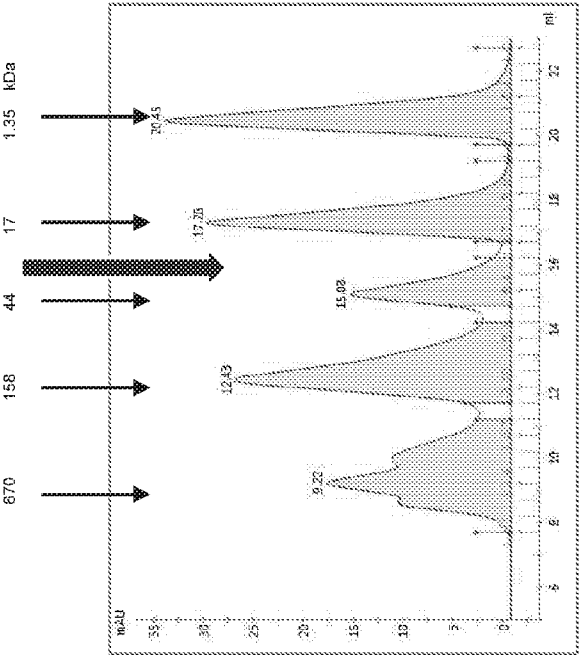
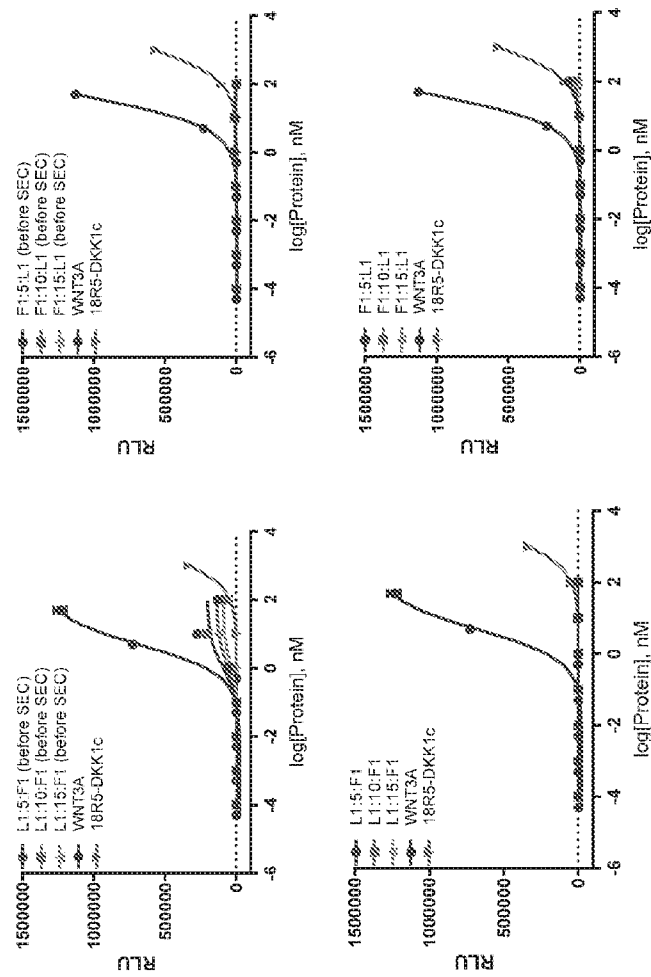


FIG. 9



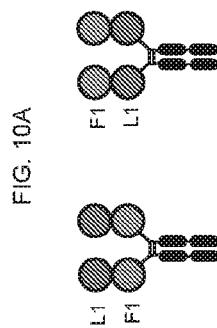


FIG. 10B

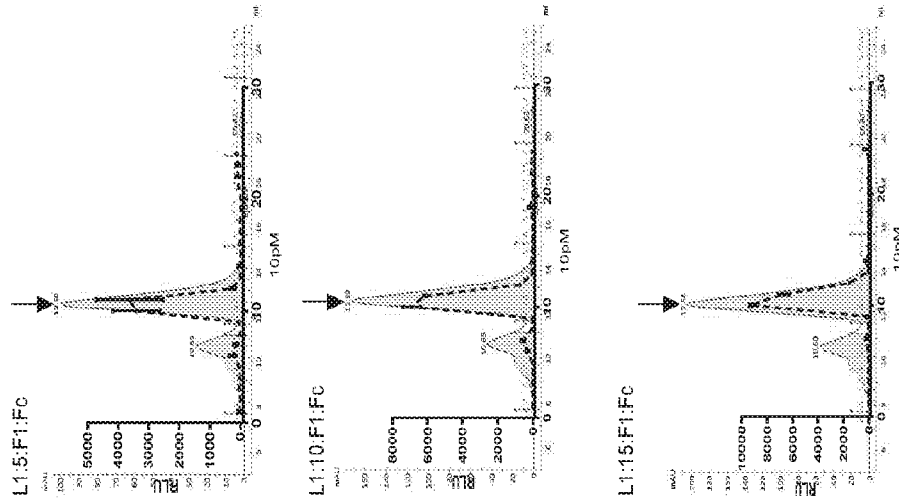
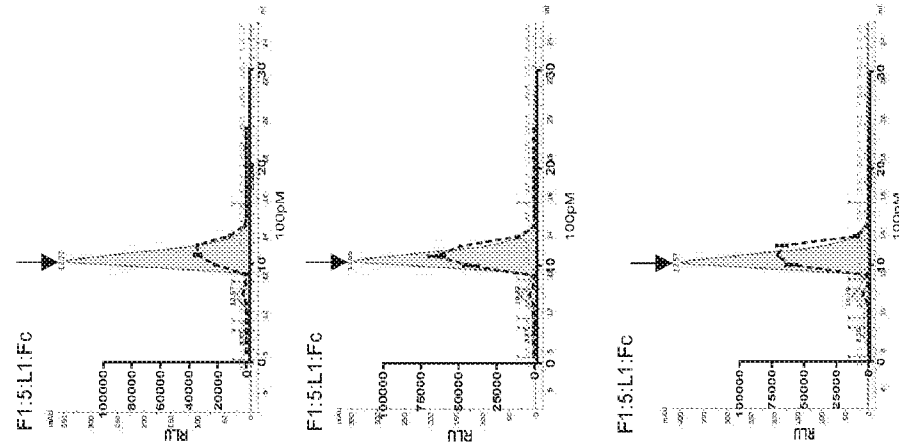
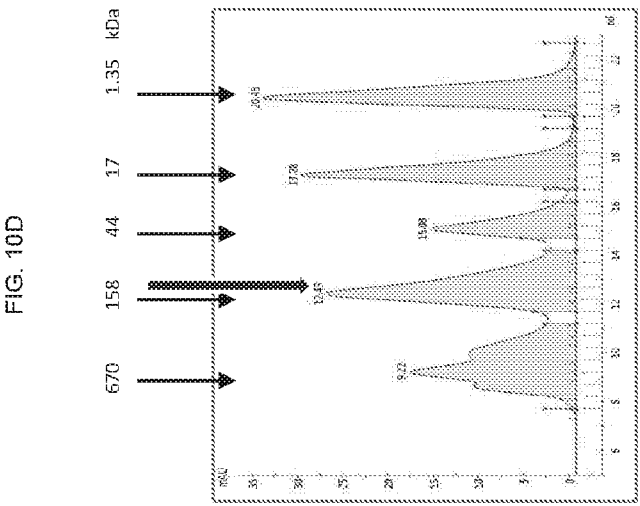
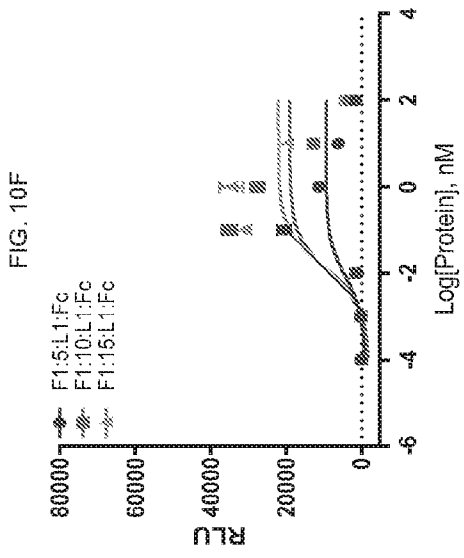
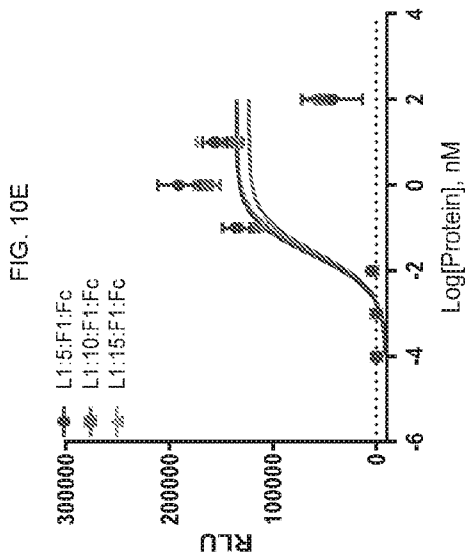


FIG. 10C





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FIG. 11A

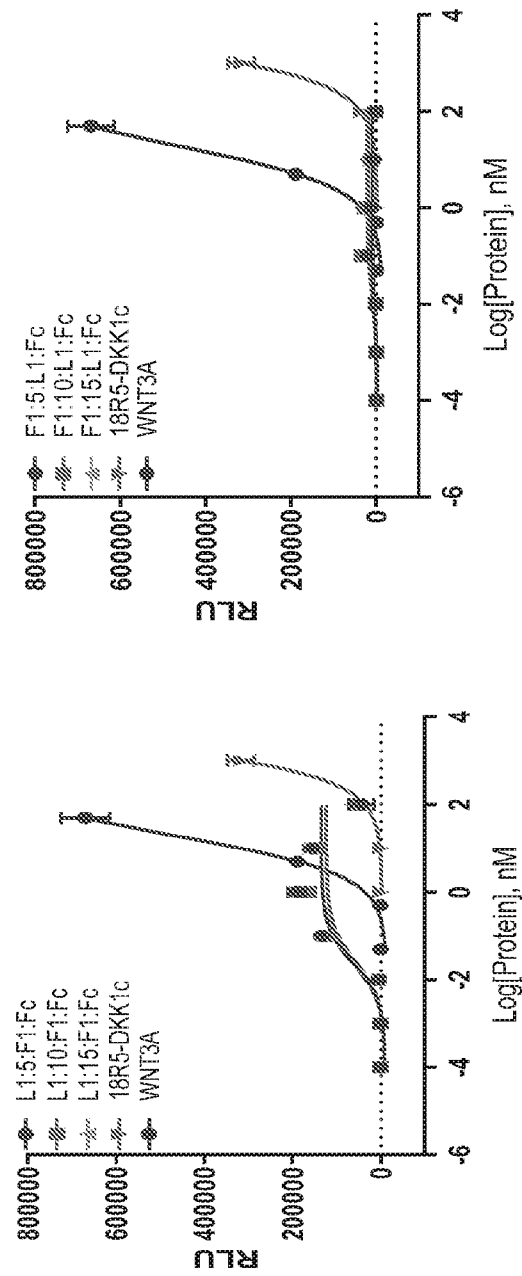
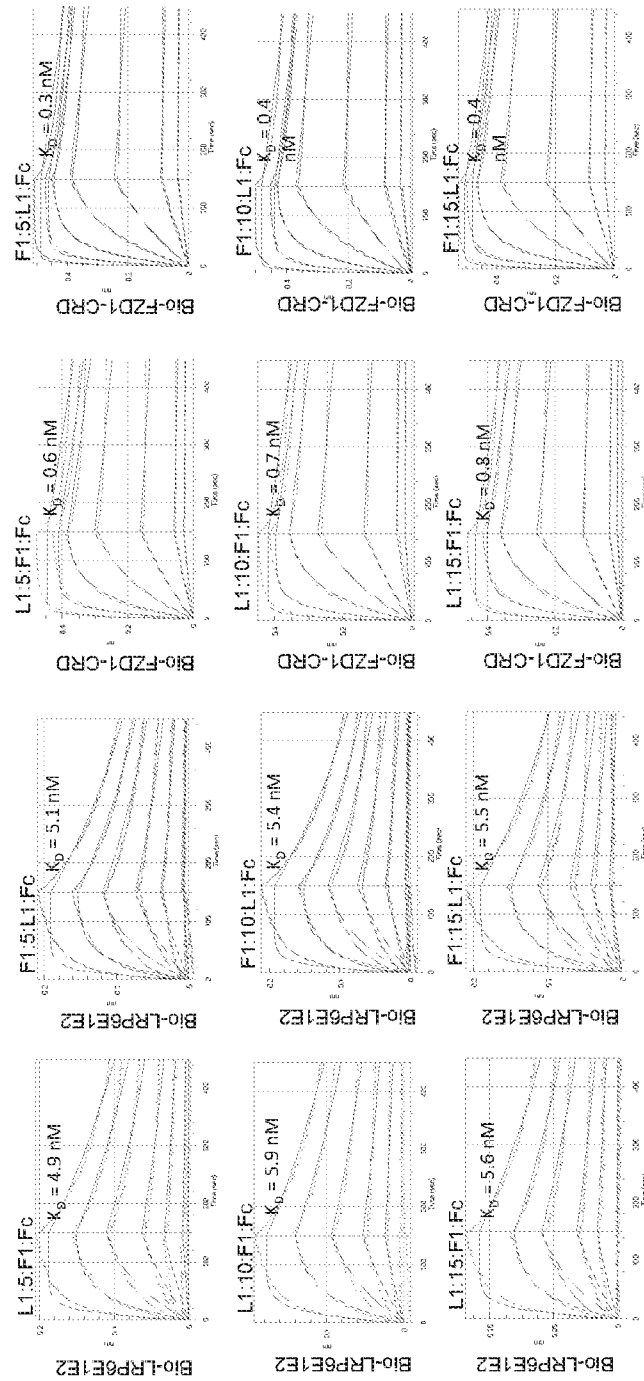
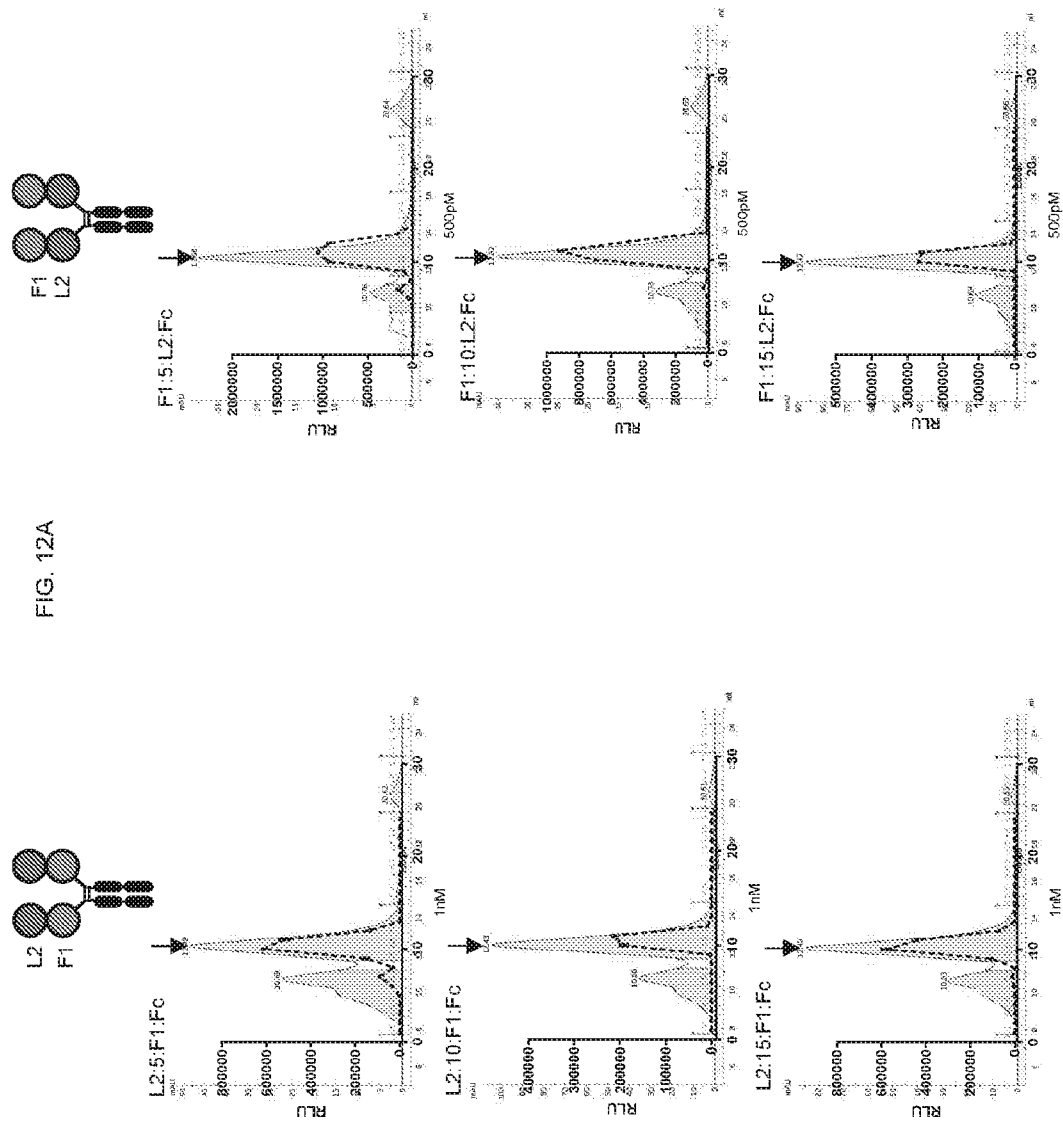


FIG. 11B





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FIG. 12C

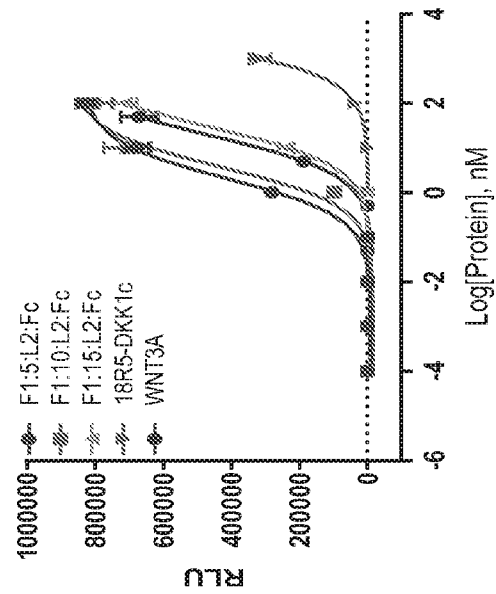
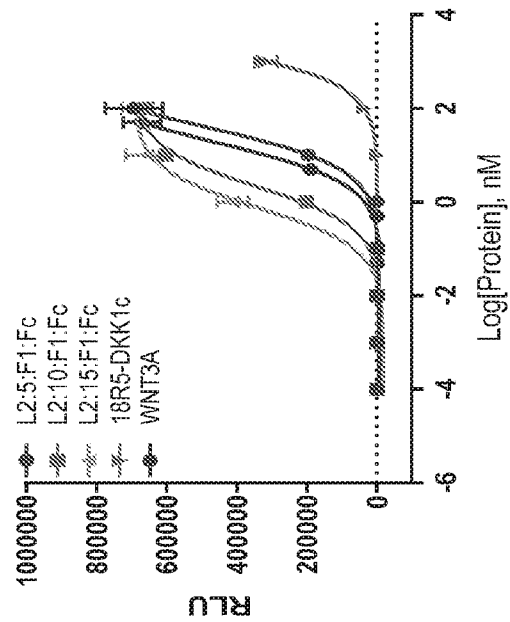


FIG. 12B



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FIG. 12D

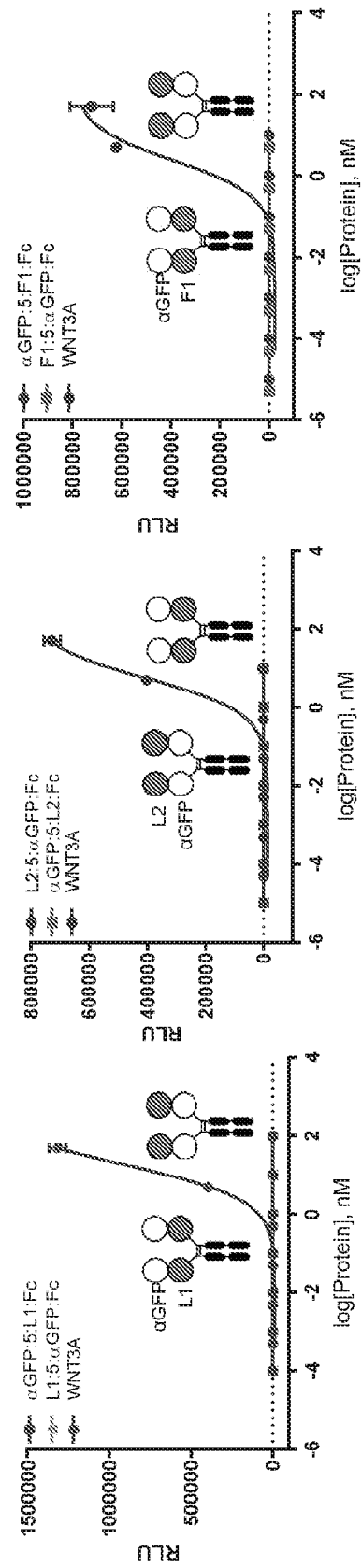


FIG. 12E

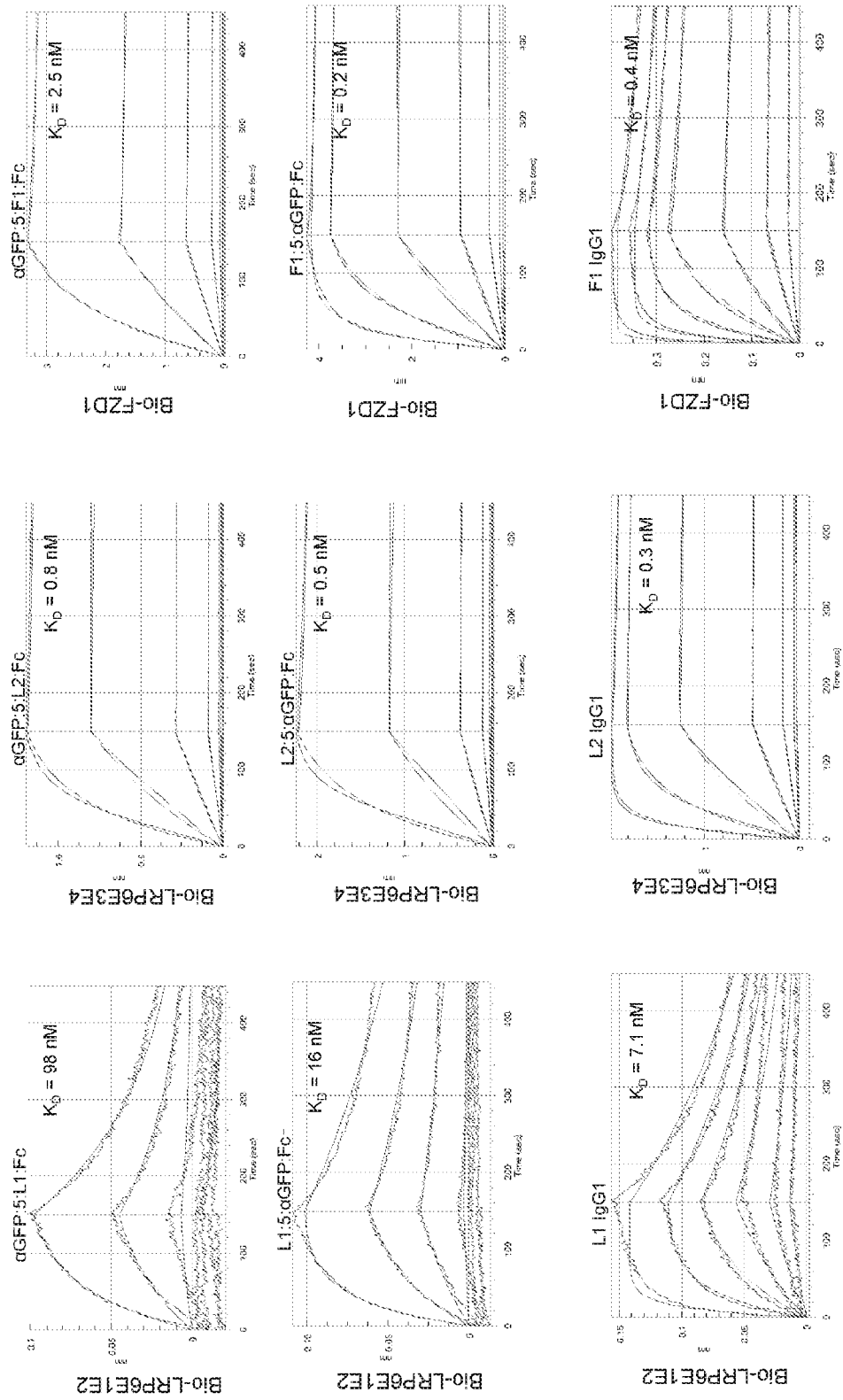
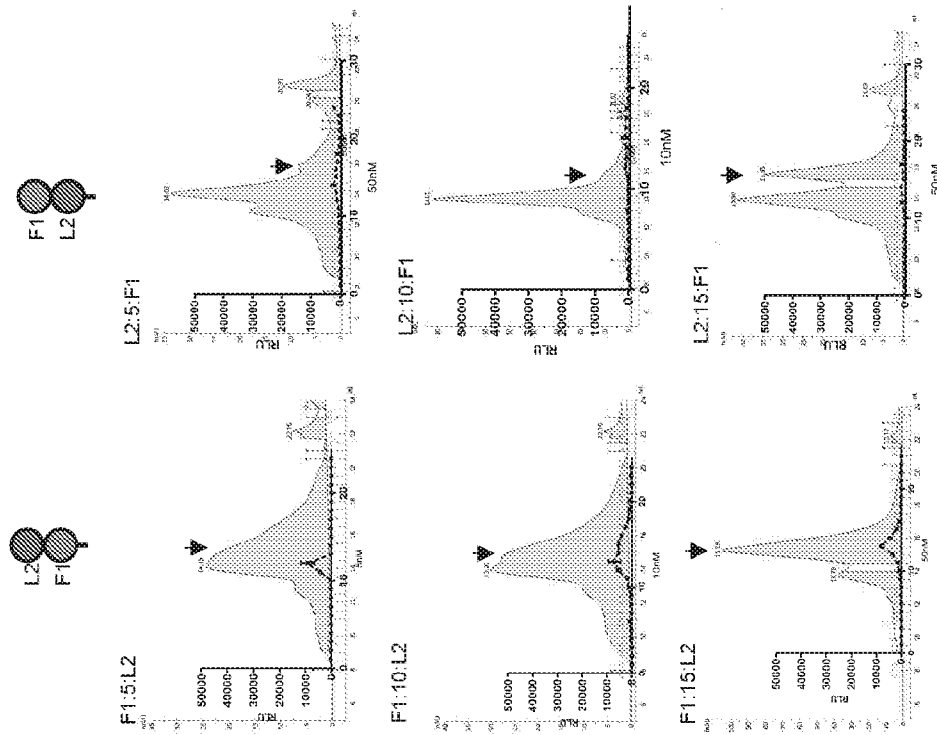


FIG. 13A



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FIG. 13B

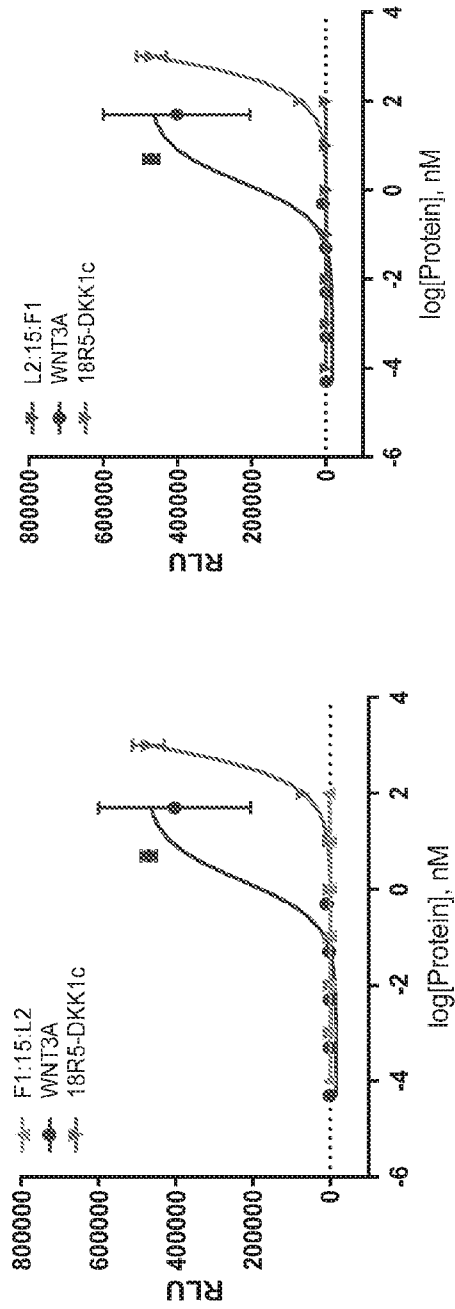
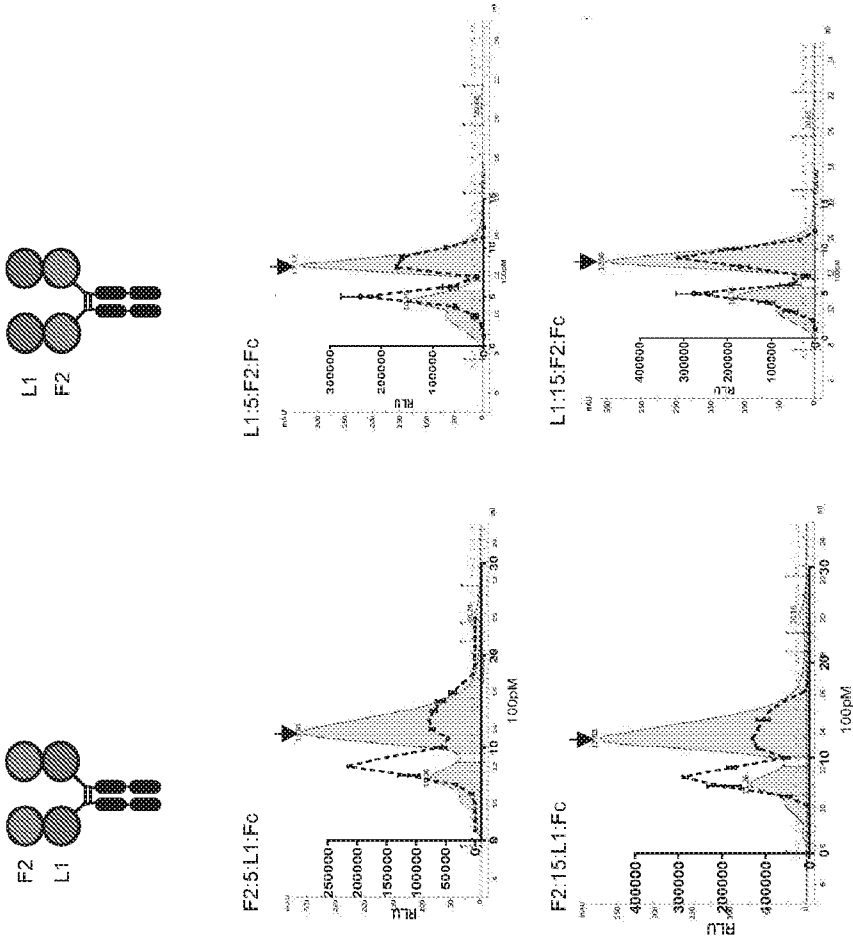


FIG. 14A



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FIG. 14B

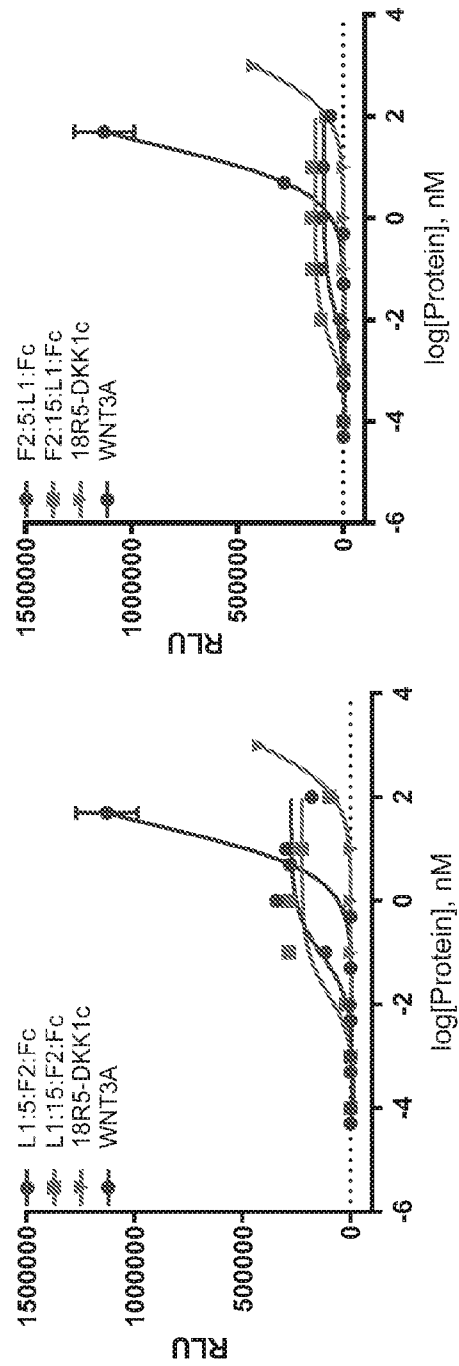


FIG. 14D

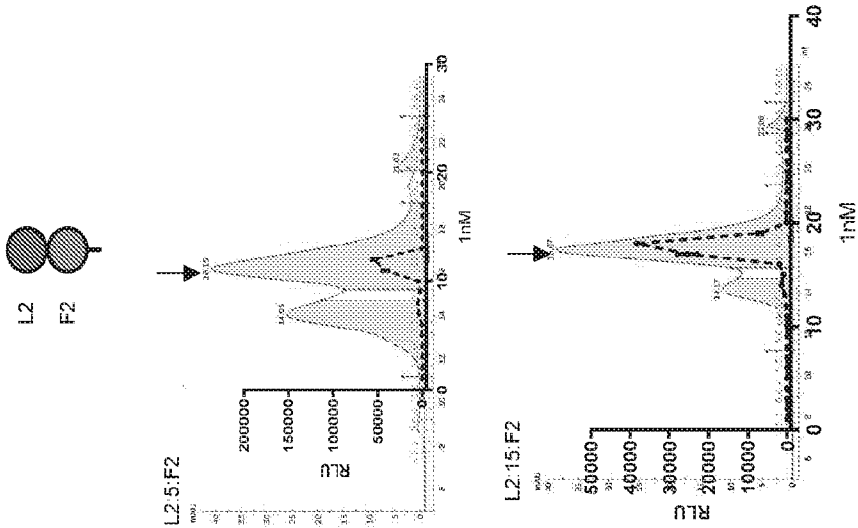
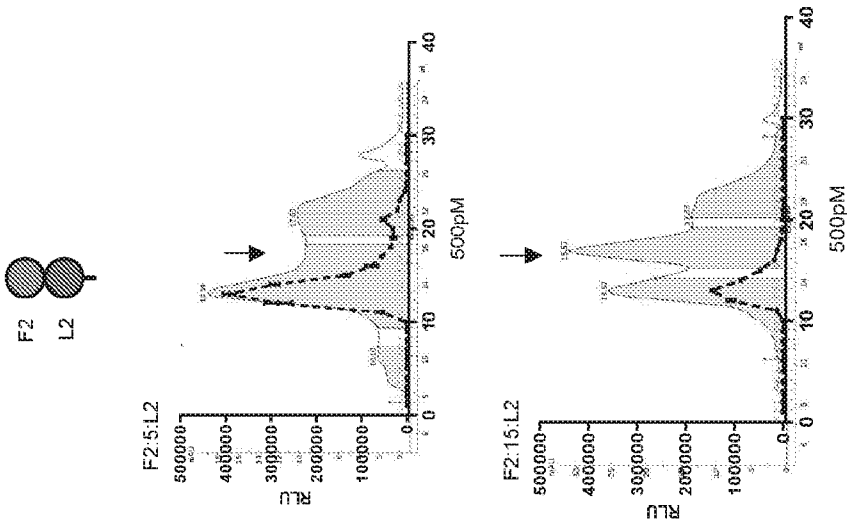


FIG. 14C



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FIG. 14E

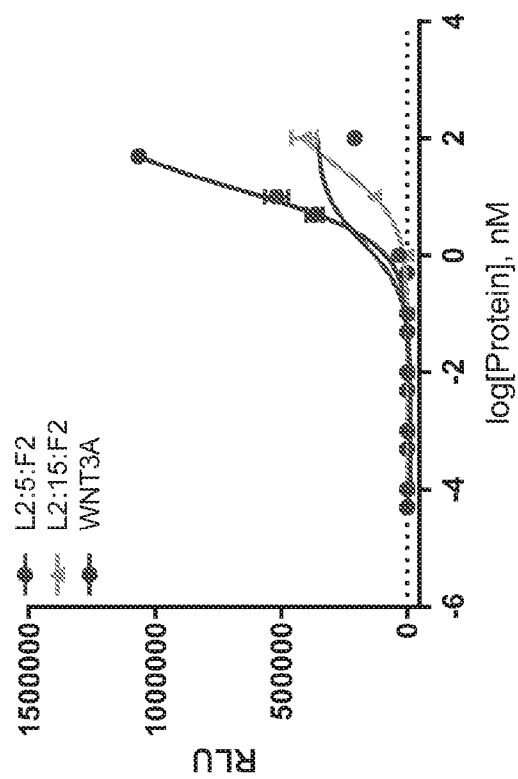


FIG. 14F

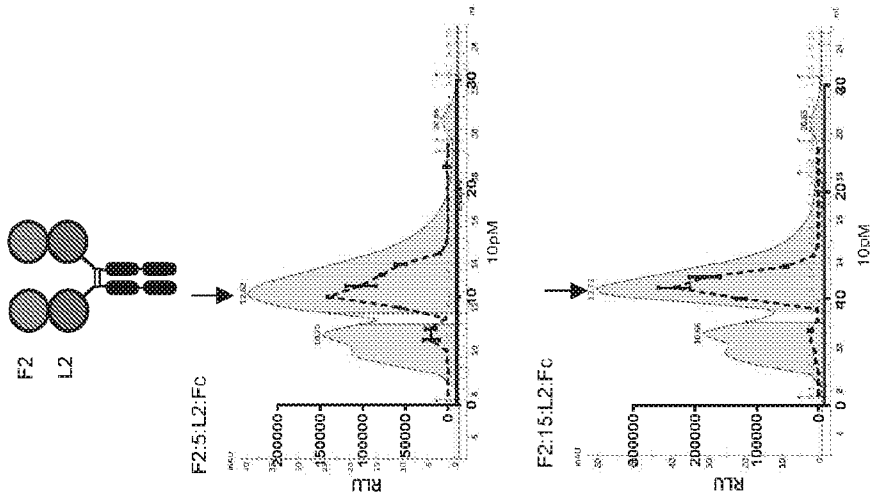


FIG. 14G

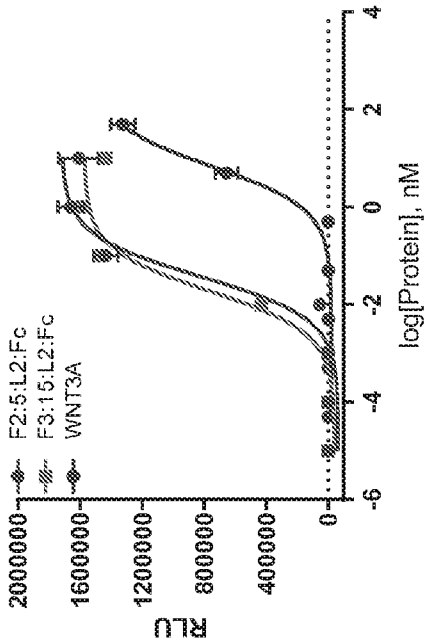


FIG. 14H

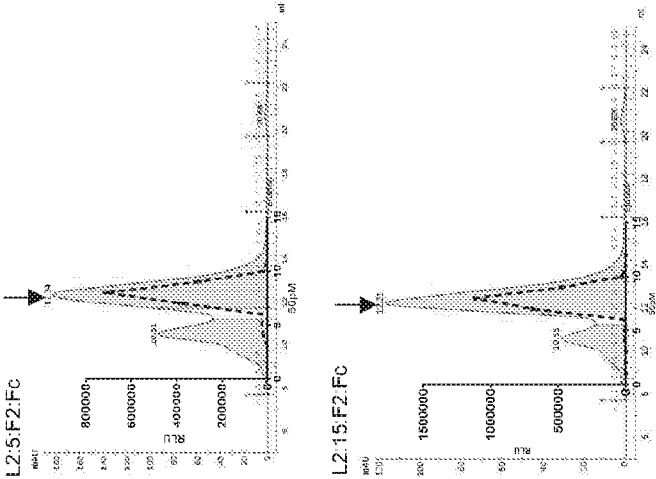
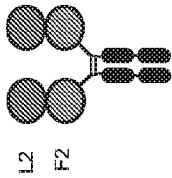


FIG. 14I

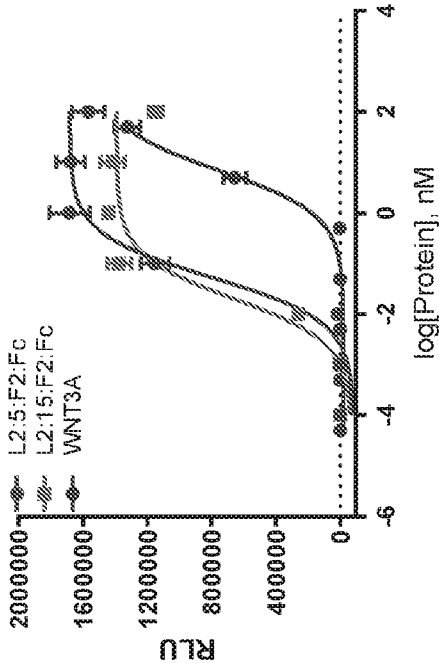


FIG. 15A

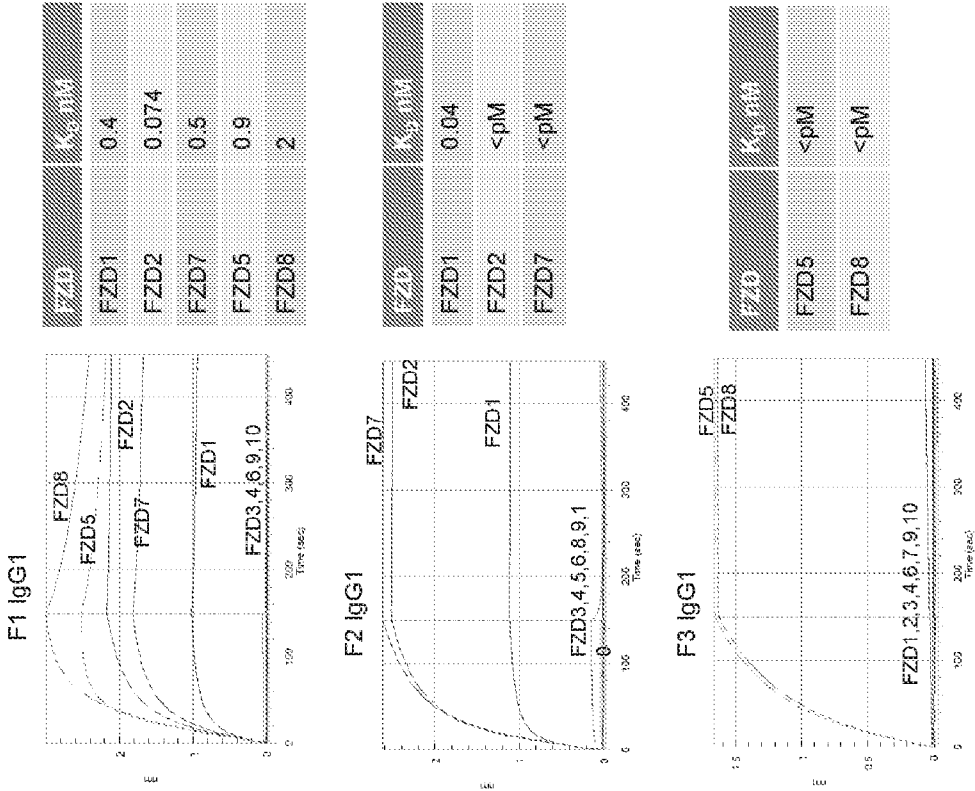
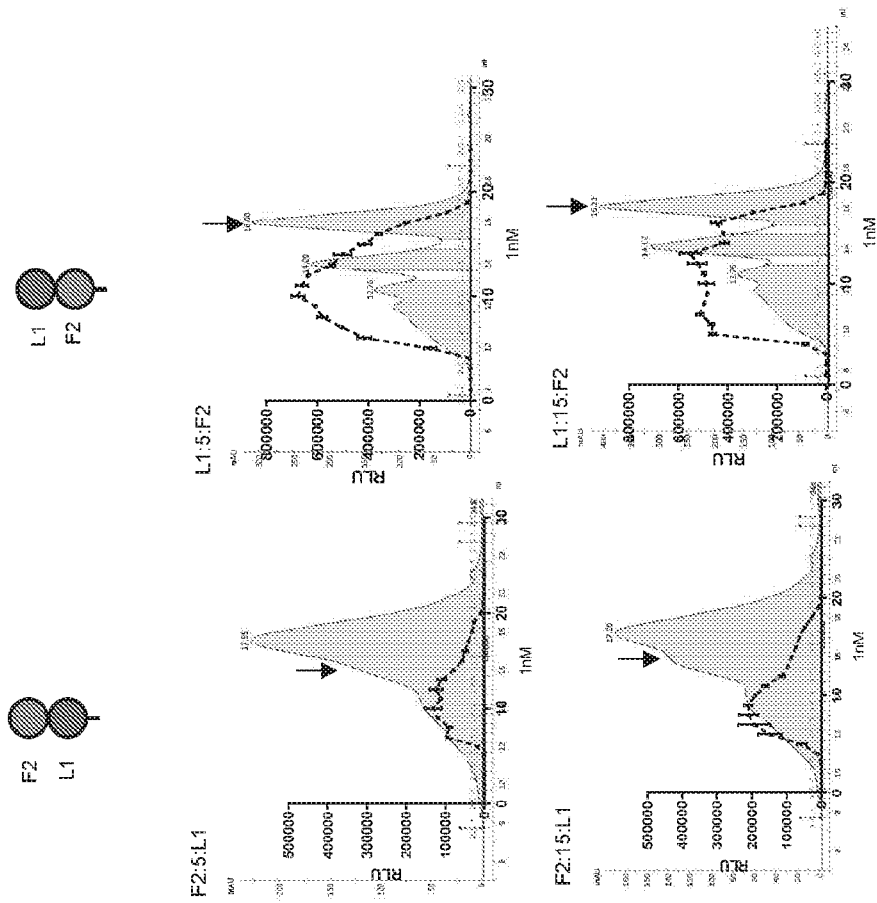
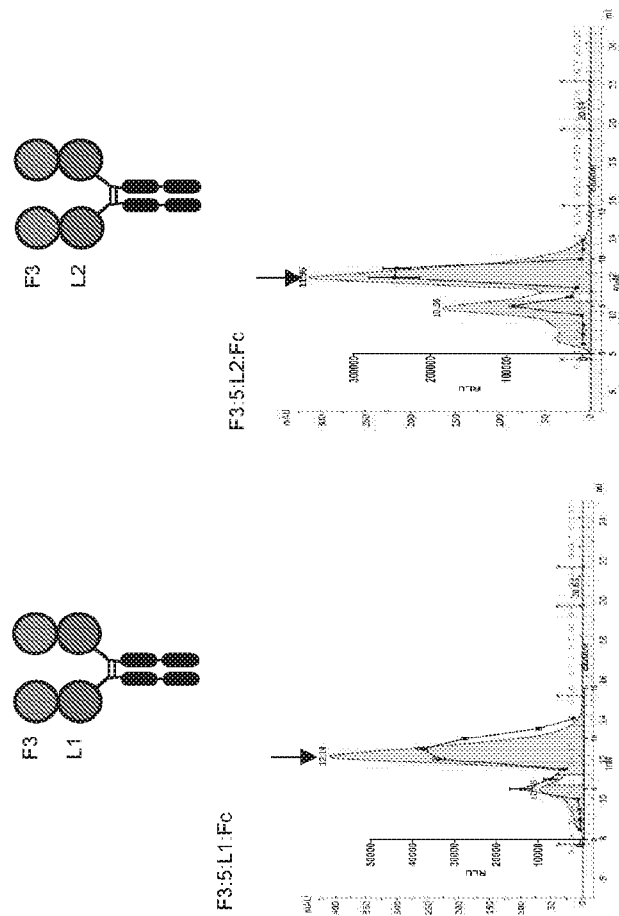


FIG. 15B



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FIG. 16A



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FIG. 16B

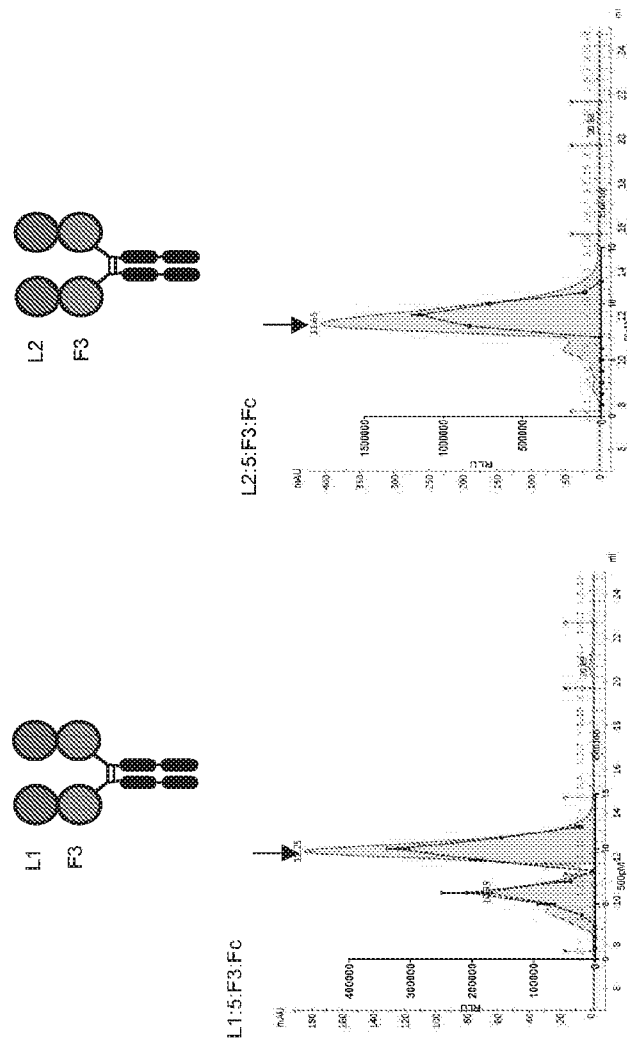


FIG. 16D

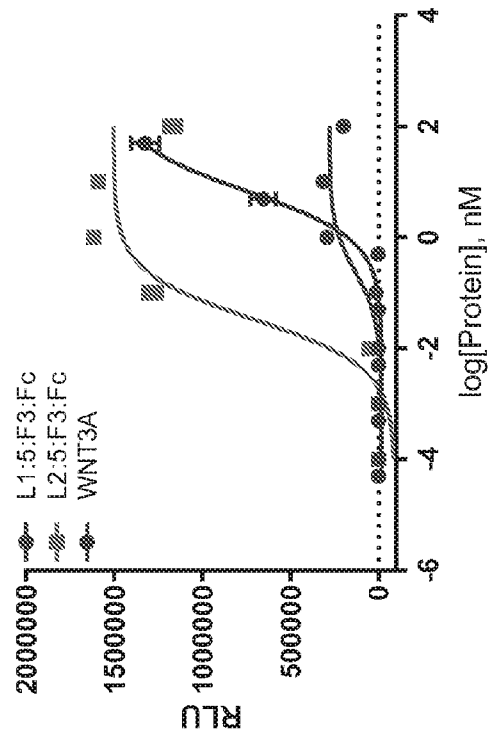
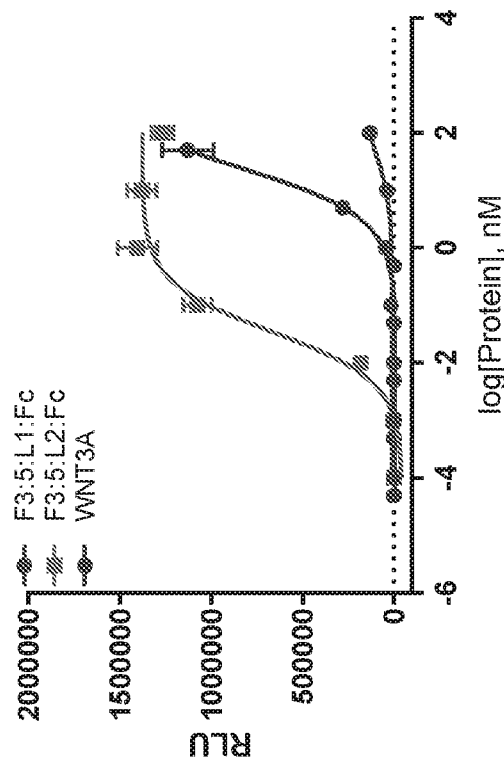


FIG. 16C



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FIG. 17

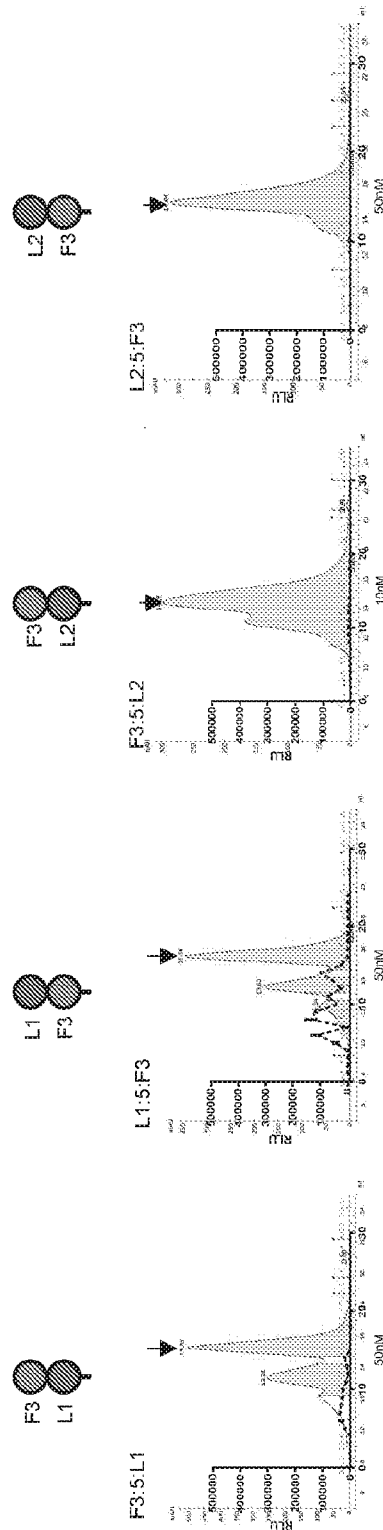


FIG. 18B

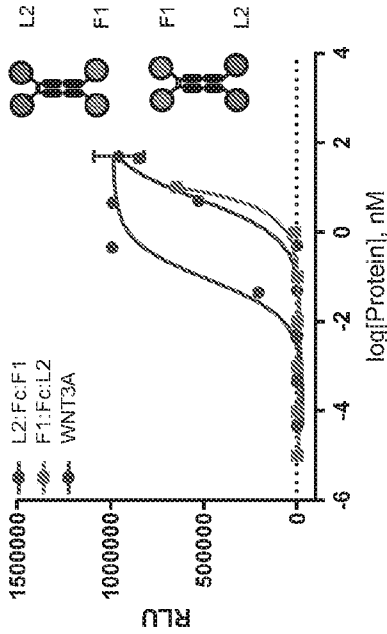


FIG. 18A

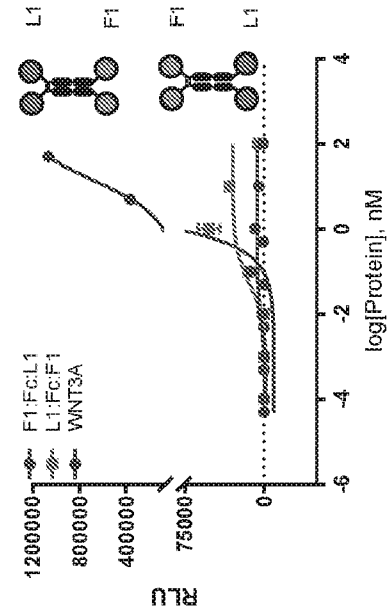


FIG. 18C

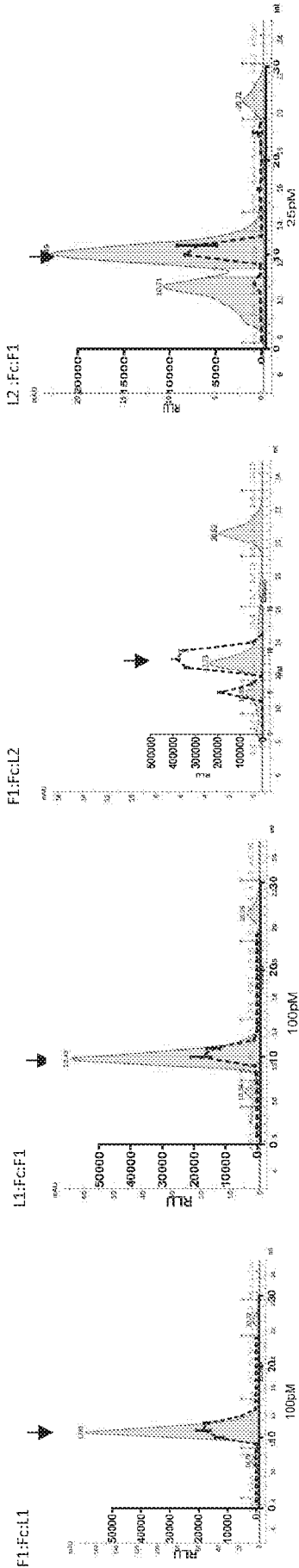


FIG. 19A

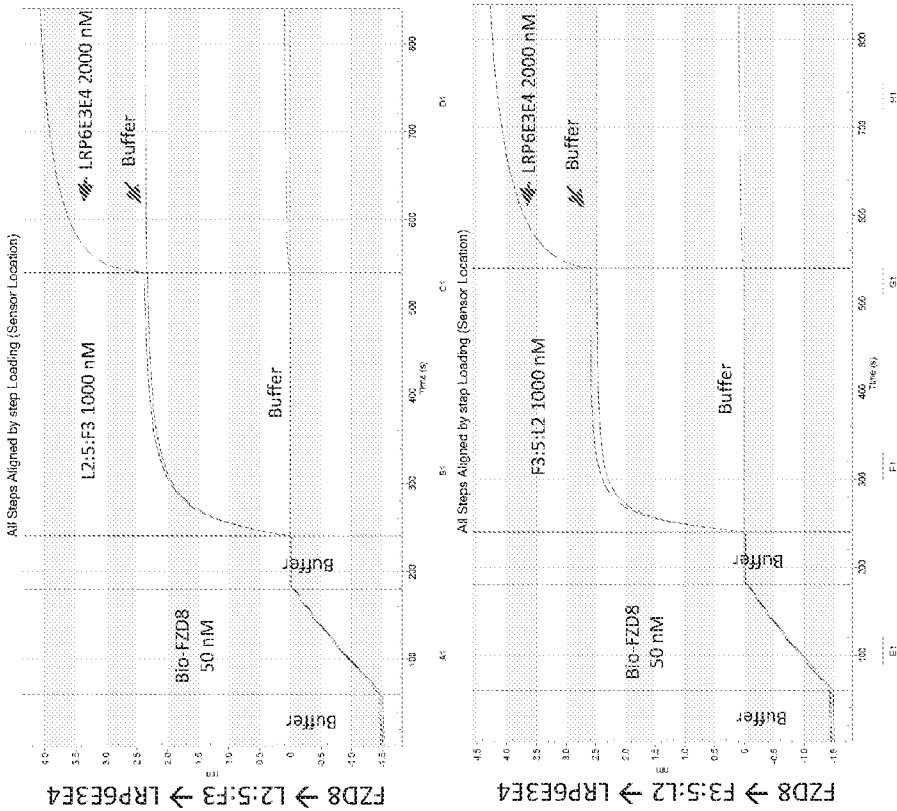


FIG. 19B

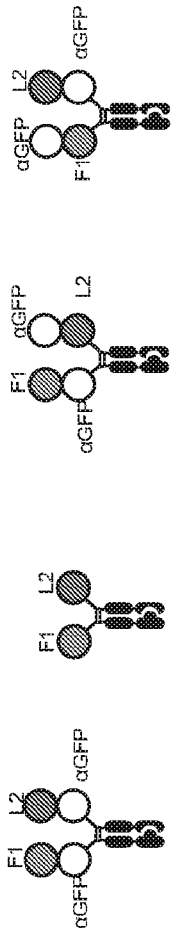


FIG. 19C

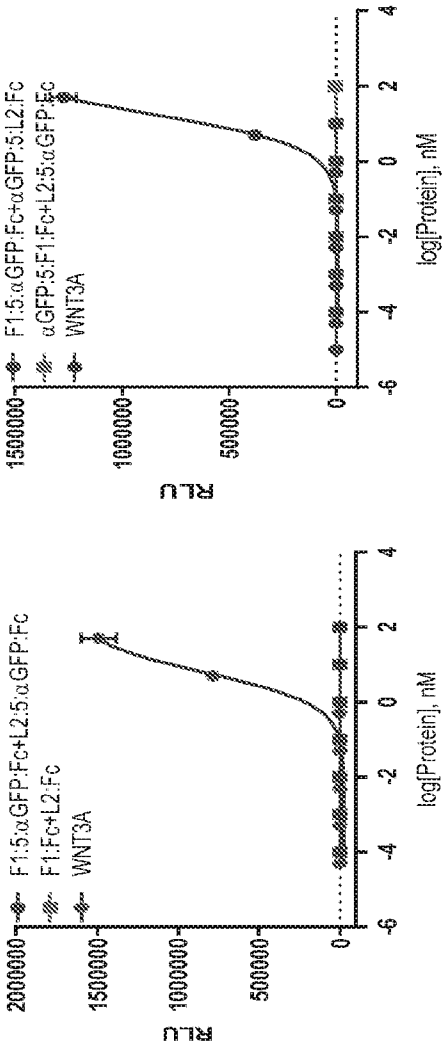


FIG. 20A

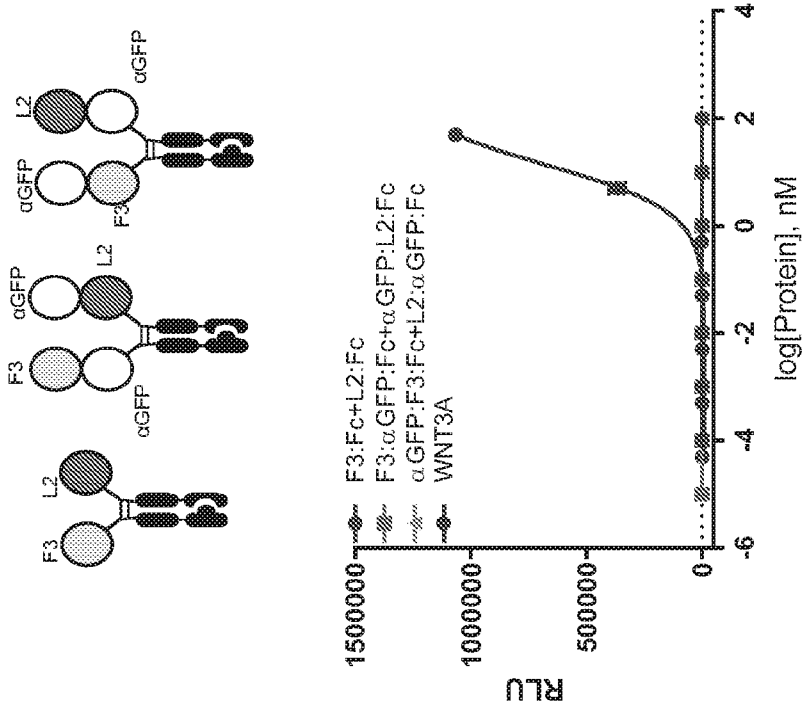


FIG. 20B

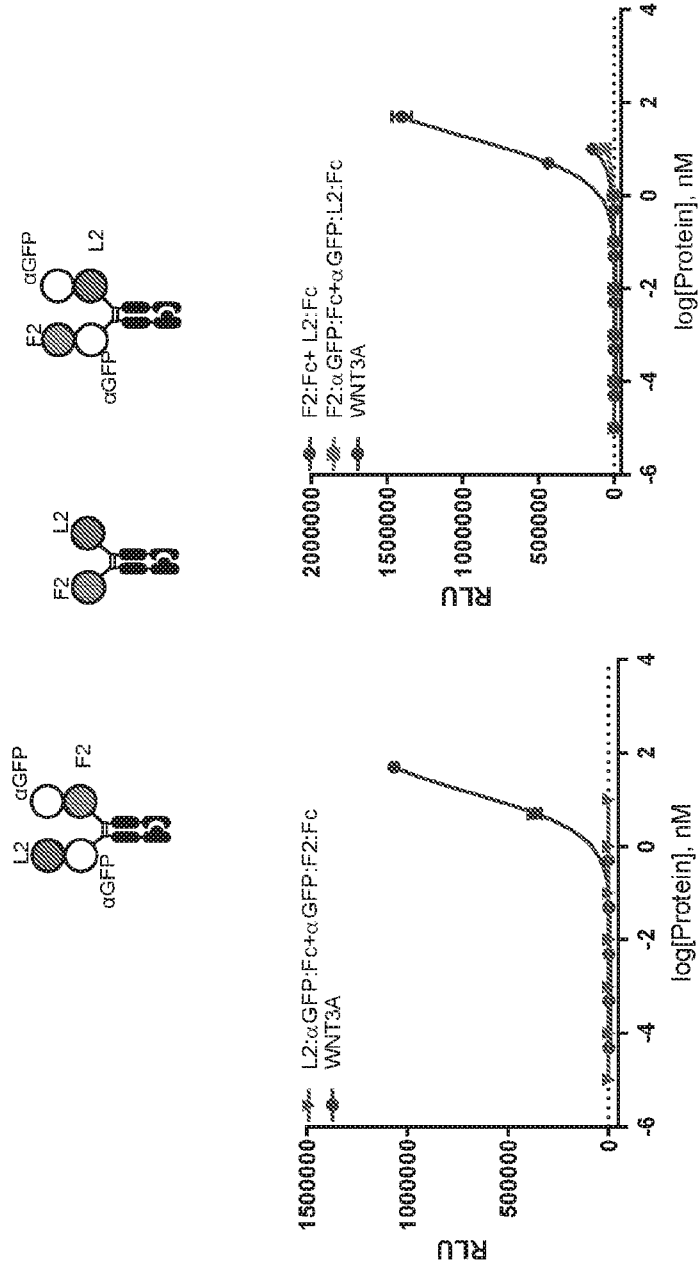


FIG. 21A

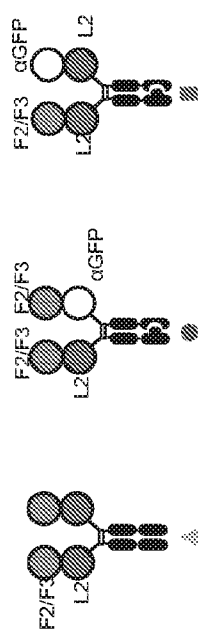
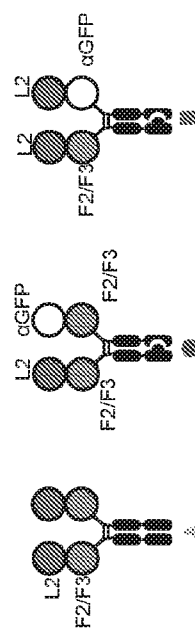


FIG. 21D



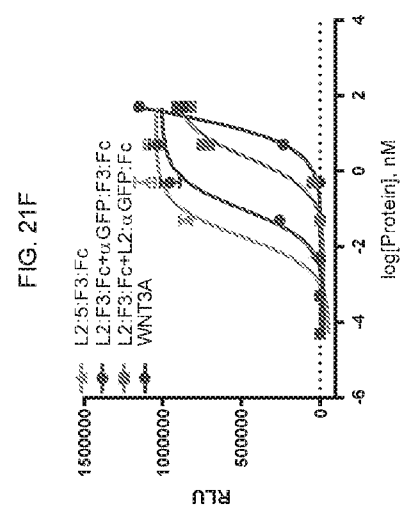
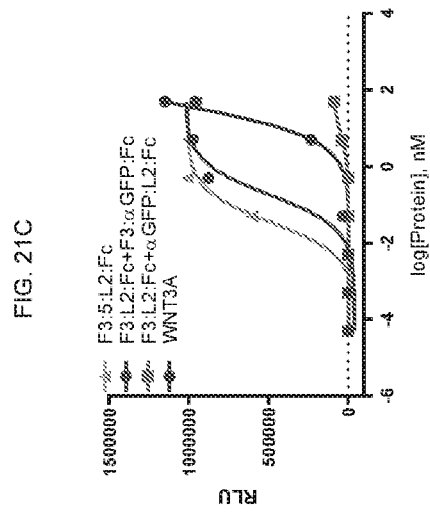
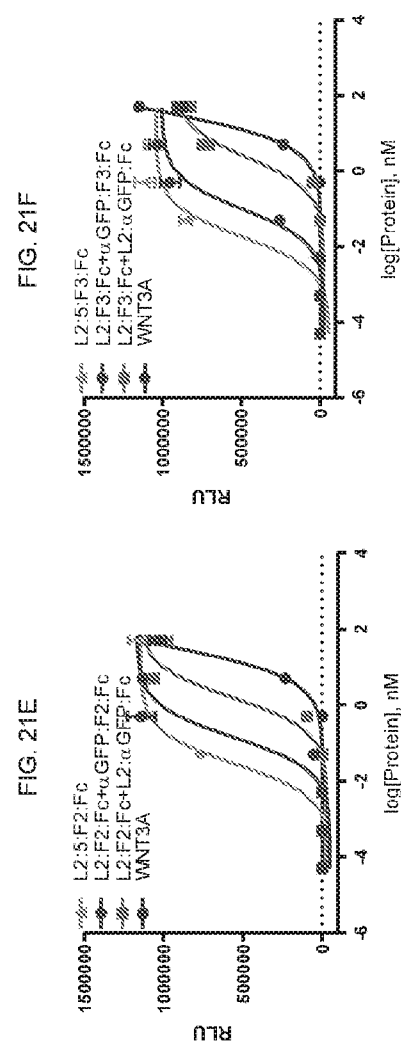
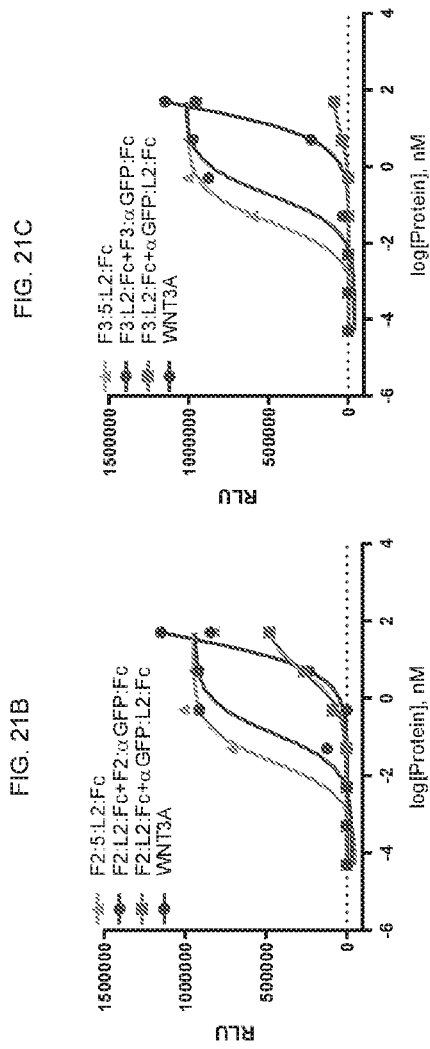


FIG. 211

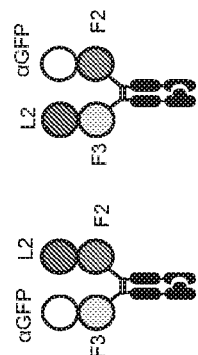


FIG. 21G

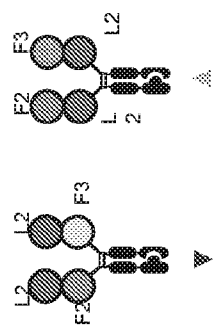


FIG. 21J

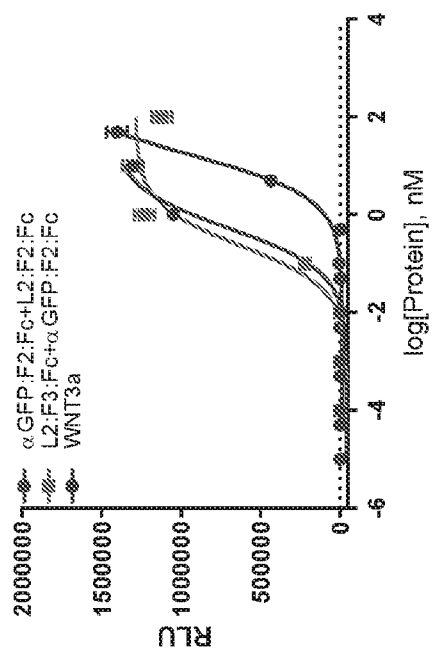
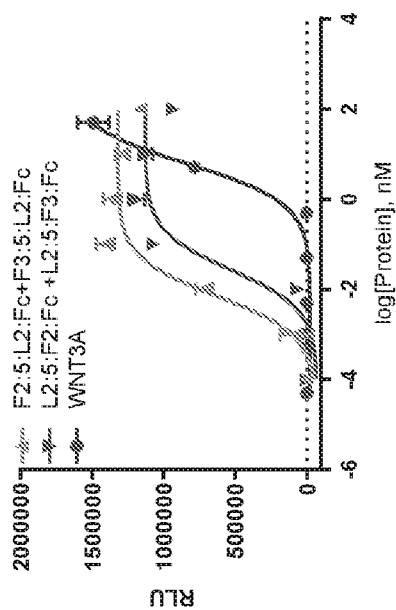


FIG. 21H



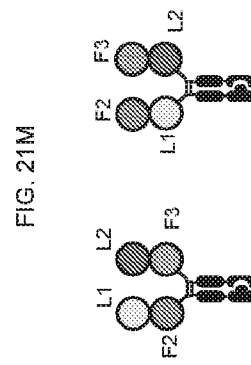
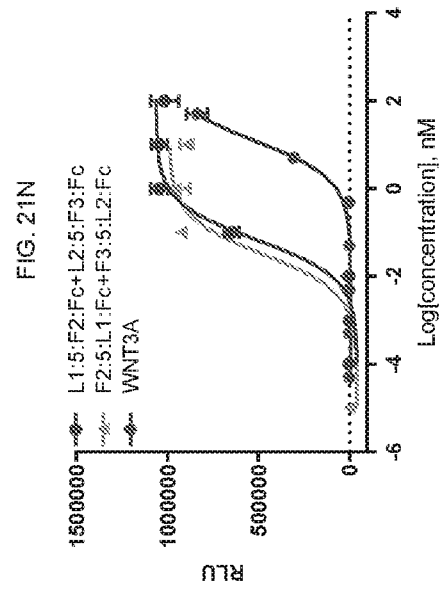
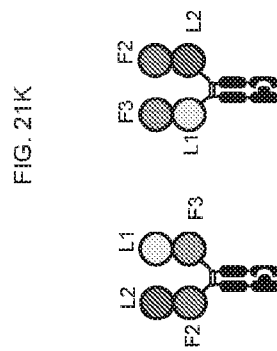
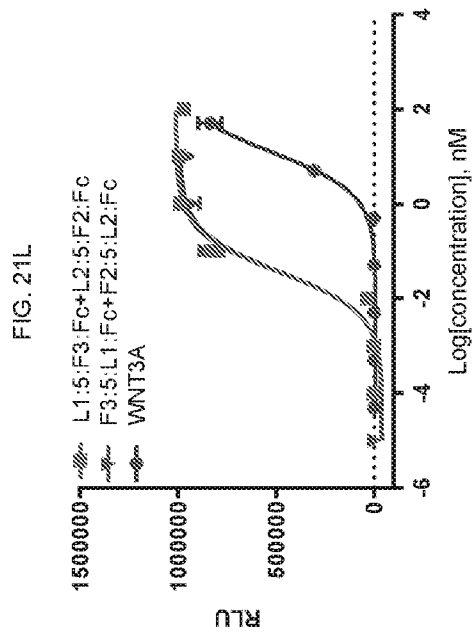
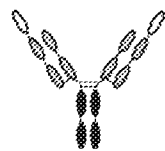


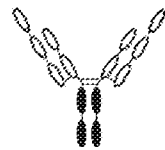
FIG. 3A



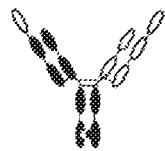
R2M3-3



4SD1-3



14SB6-3



heterolog 4SD1-3+14SB6-3



4SD1-3
monovalent
bispecific



14SB6-3
monovalent
bispecific