

TETRAVALENT FZD AND WNT CO-RECEPTOR BINDING ANTIBODY MOLECULES AND USES THEREOF

SEQUENCE LISTING

The instant application contains a Sequence Listing which has been submitted electronically
5 in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy,
created on December 16, 2021, is named 117946_PD606WO_FINAL.txt and is 989,432
bytes in size.

BACKGROUND

Wnt signaling pathways are critical for embryonic development and tissue homeostasis in
10 adults. Wnt signaling is initiated when a Frizzled (FZD) receptor on the cell surface
membrane binds with a Wnt ligand. Wnt ligands are secreted growth factors that regulate
various cellular processes such as proliferation, differentiation, survival and migration.

Nineteen Wnt ligands exist in humans that interact with a network of ten Frizzled cell surface
receptors (FZD) and one of several co-receptors that guide the selective engagement of
15 different intracellular signaling branches (Wodarz, A. and Nusse, R. Annu. Rev. Cell Dev.
Biol. 14, 59–88 (1998); Angers, S and Moon, R.T., transduction. Nat. Rev. Mol. Cell Biol.
10, 468–477 (2009)). FZDs have conserved structural features including seven hydrophobic
transmembrane domains and a cysteine-rich ligand-binding domain. FZDs are known to
function in three distinct signaling pathways, known as the Wnt planar cell polarity (PCP)
20 pathway, the canonical Wnt/ β -catenin pathway, and the Wnt/calcium pathway. The presence
of Wnt co-receptors is also required to direct the differential engagement of the intracellular
signaling cascades listed above. For example, Wnt ligands bind to a Frizzled receptor and a
member of the low-density lipoprotein receptor-related proteins 5 and 6 (LRP5/6) co-
receptor family to activate the Wnt/ β -catenin pathway, or with a receptor tyrosine kinase-like
25 orphan receptors 1 and 2 (ROR1/2), related to receptor tyrosine kinase (RYK) or protein
tyrosine kinase 7 (PTK7) co-receptor to activate alternate β -catenin-independent signaling
pathways.

Wnt ligands are universally important for the control of tissue stem cells self-renewal and
regulation of many progenitor cell populations, but the hydrophobicity and sensitive tertiary
30 structure of Wnt proteins makes their biochemical purification challenging and their use *in*

vitro and *in vivo* inefficient. Described herein are tetravalent binding antibody molecules that activate a Wnt signaling pathway and methods for their use.

SUMMARY OF THE INVENTION

Described herein are tetravalent binding antibody molecules that activate a Wnt signaling pathway and methods for their use. The tetravalent binding antibody molecules bind to both an FZD receptor, e.g., Frizzled Class Receptor 1 (FZD1), Frizzled Class Receptor 2 (FZD2), Frizzled Class Receptor 3 (FZD3), Frizzled Class Receptor 4 (FZD4), Frizzled Class Receptor 5 (FZD5), Frizzled Class Receptor 6 (FZD6), Frizzled Class Receptor 7 (FZD7), Frizzled Class Receptor 8 (FZD8), Frizzled Class Receptor 9 (FZD9), or Frizzled Class Receptor 10 (FZD10) and a Wnt co-receptor, e.g., LRP5 or LRP6 (LRP5/6), thereby activating a Wnt signaling pathway. In an embodiment, the tetravalent binding antibody molecules bind to both a FZD4 receptor and LRP5 and/or LRP6 and activate the Wnt/ β -catenin signaling pathway. The tetravalent binding antibody molecules of this invention are also referred herein as “FZD Agonists”, Frizzled and LRP5/6 Agonist (FLAG), and in some embodiments as “ANTs”.

The tetravalent binding antibody molecules include an Fc domain comprised of CH2 and CH3 domains or fragment thereof comprising the CH3 domain, and a first bivalent binding domain that interacts with one or more FZD receptor, e.g., one or more of FZD1, FZD2, FZD3, FZD4, FZD5, FZD6, FZD7, FZD8, FZD9, and FZD10, and a second bivalent binding domain that binds a WNT co-receptor, e.g., LRP5 or LRP6, wherein the FZD binding domain is linked to one terminus of the Fc domain and the co-receptor binding domain is linked to the other terminus of the Fc domain. Thus, the binding domain for the FZD receptor and the binding domain for the WNT co-receptor are not directly linked rather they are separated by the Fc domain, or fragment thereof comprising the CH3 domain.

The Fc domain of the FZD Agonists may be an Fc domain of an immunoglobulin with or without effector function. The immunoglobulin may be an IgG, e.g., an IgG1. In an embodiment of this invention the tetravalent binding antibody molecule comprises two polypeptides containing an Fc region that dimerize via the intrinsic ability of the Fc region in each polypeptide to dimerize or via a knob-in-holes configuration within the Fc. Thus, the Fc dimer may be a heterodimer or a homodimer. Methods for dimerizing peptides via a knob-in-hole configuration are described in WO2018/026942, inventors Van Dyk et al., Carter P.

(2001) J. Immunol. Methods 248, 7–15; Ridgway et al. (1996) Protein Eng. 9, 617–621; Merchant, et al. (1998) Nat. Biotechnol. 16, 677–681, and; Atwell et al., (1997) J. Mol. Biol. 270, 26–35, all incorporated herein by reference.

In an embodiment, each of the binding domains of the FZD Agonists described herein are
5 bivalent and each may be monospecific, having two binding sites for the same epitope of an FZD receptor, e.g., FZD4, or Wnt co-receptor, e.g. LRP5/6, or bispecific having two binding sites with each site binding a different epitope on an FZD or Wnt co-receptor, e.g., a Wnt1 binding (domain E1-E2 within the extracellular domain of LRP5/6) site and a Wnt3 binding site (domain E3-E4 within the extracellular domain of LRP5/6) within the LRP5/6 co-
10 receptor. In an embodiment, the LRP5/6 binding domain binds to a Wnt3A site (domain E3-E4) on LRP5 and binds to a Wnt3A site (domain E3-E4) on LRP6.

In embodiments of this invention the FZD binding domain linked to the Fc domain of the FZD Agonist comprises one or more immunoglobulin heavy-chain variable domain (VH) fragments and/or one or more immunoglobulin light-chain variable domain (VL) fragments
15 that bind the FZD, e.g., FZD4. In an embodiment of this invention the FZD binding domain may comprise Fabs, a diabody or single chain variable fragments (scFv) single-domain antibody fragments, e.g., V_{HH}, or combinations thereof that bind to the same or different epitopes on the FZD.

In an embodiment of this invention the VHs and/or VLs of the FZD binding domain binds
20 FZD4 or FZD5 and comprise the light chain CDRs and the heavy chain CDRs of a FZD4 or FZD5 binding antibody of Table 1, Table 2, or Table 6, and/or comprise light chain CDRs and heavy chain CDRs that are 50%, 55%, 60%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to the CDRs of an FZD4 antibody of Table 1, Table 2 or Table 6, and still retain binding to the FZD4 or FZD5 receptor. For example, in an embodiment of the
25 invention, the FZD binding domain may comprise a first heavy chain (CDR-H1), a second heavy chain (CDR-H2), and/or a third heavy chain (CDR-H3), wherein the VH that binds FZD may comprise CDR-H1 of SEQ ID NO: 24, SEQ ID NO: 365, or SEQ ID NO: 893, a CDR-H2 of SEQ ID NO: 51, SEQ ID NO: 61, SEQ ID NO: 462, or SEQ ID NO: 894 and/or CDR-H3 of SEQ ID NO: 79, SEQ ID NO: 90, SEQ ID NO: 484, or SEQ ID NO: 895 and a
30 first light chain (CDR-L1), a second light chain (CDR-L2), and/or a third light chain (CDR-L3), wherein the VL that binds FZD may comprise CDR-L1 of SEQ ID NO: 1, SEQ ID NO:

3 or SEQ ID NO: 12, a CDR-L2 of SEQ ID NO: 2, SEQ ID NO: 3 or SEQ ID NO: 12 and/or a CDR-L3 of SEQ ID NO: 3, SEQ ID NO: 12, SEQ ID NO: 285, or SEQ ID NO: 896.

In an embodiment of this invention the co-receptor (LRP5/6) binding domain linked to the Fc domain of the FZD Agonist comprises one or more immunoglobulin heavy-chain variable domain (VH) fragments and/or one or more immunoglobulin light-chain variable domain (VL) fragments that bind to the Wnt co-receptor, e.g., LRP5 and/or LRP6. For example, in an embodiment of the invention, the LRP binding domain may comprise a first heavy chain (CDR-H1), a second heavy chain (CDR-H2), and/or a third heavy chain (CDR-H3), wherein the VH that binds LRP may comprise a CDR-H1 of SEQ ID NO: 527, SEQ ID NO: 528, SEQ ID NO: 536, SEQ ID NO: 716, or SEQ ID NO: 720, a CDR-H2 of SEQ ID NO: 552, SEQ ID NO: 553, or SEQ ID NO: 566, SEQ ID NO: 785, or SEQ ID NO: 791 and/or a CDR-H3 of SEQ ID NO: 584, SEQ ID NO: 585, SEQ ID NO: 586 or SEQ ID NO: 603, SEQ ID NO: 856 or SEQ ID NO: 862 CDR-H3 and and a first light chain (CDR-L1), a second light chain (CDR-L2), and/or a third light chain (CDR-L3), wherein the VL that binds LRP may comprise CDR-L1 of SEQ ID NO: 1, a CDR-L2 of SEQ ID NO: 2, or SEQ ID NO: 491 and/or a CDR-L3 of SEQ ID NO: 130, SEQ ID NO: 492, SEQ ID NO: 493, SEQ ID NO: 510, SEQ ID NO: 623 or SEQ ID NO: 665.

In an embodiment of this invention, the Wnt co-receptor binding domain is bivalent and may comprise a diabody, or may comprise a Fab, a single chain variable fragment (scFv) or a single domain antibody fragments (V_{HH}) or combinations thereof for binding to the same or different epitopes on the co-receptor. In embodiments of this invention the VHs and VLs of the Wnt coreceptor binding domain comprise the light chain CDRs and/or the heavy chain CDRs of a LRP5 and/or LRP6 binding antibody of Table 3, Table 4 or Table 6, or comprise light chain CDRs and/or heavy chain CDRs that are 50%, 55%, 60%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to the CDRs of an LRP5 and/or LRP6 antibody of Table 3, Table 4 or Table 6, and still retain binding to the LRP5 and/or LRP6 co-receptor.

In an embodiment of this invention the Wnt co-receptor binding domain linked to the Fc domain of the FZD Agonists described herein comprises a diabody, formed by two peptides each peptide comprising a heavy-chain variable domain (VH or VH domain) linked to a light-chain variable domain (VL or VL domain) wherein the VH and the VL from one peptide pair with the VL and VH of the other peptide forming the diabody. In this configuration, the binding domain has two binding sites that bind to the Wnt co-receptor, e.g., LRP5 or LRP6.

The diabody may be monospecific binding the same site on the co-receptor or may be bispecific (bs) binding two different sites on the co-receptor. By using a knobs-in-holes Fc configuration, the peptides comprising the VH and VL linked to Fc regions, can be non-identical but will still pair to form a bispecific binding domain capable of binding to two
5 different sites on the Wnt co-receptor (e.g. LRP5 or LRP6).

The peptides forming the diabodies, the V_HH, the scFv, and the Fabs that form the binding domains may be derived from an antibody selected for its binding to a desired target, a “source antibody”. For the FZD binding domain, the “FZD source antibody” may be an antibody that binds to one or more of the FZD receptor(s), e.g., one or more of FZD1, FZD2,
10 FZD3, FZD4, FZD5, FZD6, FZD7, FZD8, FZD9, and FZD10, and antagonizes Wnt signaling or inhibits Wnt binding to the given FZD receptor(s). Alternatively, the FZD source antibody may be an antibody that binds to the FZD receptor(s) without antagonizing Wnt signaling or without inhibiting Wnt binding to the FZD receptor. Likewise, for the co-receptor binding domain, the “co-receptor source antibody” may be an antibody that binds to the Wnt co-
15 receptor, e.g., LRP5/6, and antagonizes Wnt signaling or inhibits Wnt binding to the Wnt co-receptor. Alternatively, the co-receptor source antibody may be an antibody that binds to a co-receptor, e.g., LRP5/6, without antagonizing Wnt signaling or without inhibiting Wnt binding to the co-receptor.

In an embodiment of this invention the FZD binding domain of the FZD Agonist may bind
20 specifically to a specific FZD, e.g., FZD4, with a higher affinity than to other FZDs, i.e., FZD1, FZD2, FZD3, FZD5, FZD6, FZD7, FZD8, FZD9, and FZD10, or may be pan-specific, binding to one or more other members of the FZD receptor family. In an embodiment the FZD binding domain binds specifically to one FZD with an affinity greater than 10-fold over the binding to any other Frizzled family member.

25 In an embodiment of this invention the FZD Agonist binds to FZD4, a “FZD4 Agonist”. The FZD4 binding domain of the FZD4 Agonist may bind specifically to FZD4, binding with a higher affinity to FZD4 over other FZDs, or may be pan-specific, binding to FZD4 and one or more other members of the FZD receptor family, e.g., Frizzled Class Receptor 1 (FZD1), Frizzled Class Receptor 2(FZD2), Frizzled Class Receptor 3 (FZD3), Frizzled Class Receptor
30 5 (FZD5), Frizzled Class Receptor 6 (FZD6), Frizzled Class Receptor 7 (FZD7), Frizzled Class Receptor 8 (FZD8), Frizzled Class Receptor 9 (FZD9), or Frizzled Class Receptor 10

(FZD10). In an embodiment the FZD binding domain binds specifically to FZD4 with an affinity greater than 10-fold over any other Frizzled family member listed above.

In an embodiment of this invention the FZD Agonist binds to FZD5, a “FZD5 Agonist.” The FZD5 binding domain of the FZD5 Agonist may bind specifically to FZD5, binding with a
5 higher affinity to FZD5 over other FZDs, or may be panspecific, binding to FZD5 and one or more other members of the FZD receptor family, e.g., FZD1, FZD2, FZD3, FZD4, FZD6, FZD7, FZD8, FZD9, or FZD10. In an embodiment the FZD binding domain binds specifically to FZD5 with an affinity greater than 10-fold over any other Frizzled family member listed above.

10 In an embodiment of the FZD Agonists of this invention the Wnt co-receptor binding domain is a monospecific bivalent LRP5/6 co-receptor binding domain and binds to a single epitope on the LRP5 and/or LRP6 co-receptor, e.g., an epitope of the LRP5 and/or LRP6 coreceptor that binds to Wnt1(E1-E2 domain of LRP5 or LRP6) or binds Wnt3a (E3-E4 domain of LRP5 or LRP6). In an embodiment of this invention the co-receptor binding domain is a
15 bispecific bivalent LRP5/6 binding domain that binds to two epitopes within the LRP5 and/or LRP6 co-receptor extracellular domain, e.g., the co-receptor binding domain interacts with the Wnt1 (E1-E2) and Wnt3 (E3-E4) epitopes of the LRP5 and/or LRP6 co-receptor. In an embodiment of this invention the co-receptor binding domain is a bispecific bivalent binding domain that binds to an extracellular domain of LRP5 and LPR6, e.g., the domain interacts
20 with the Wnt1 (E1-E2) epitope of the LRP5 co-receptor and the Wnt1 (E1-E2) epitope of the LRP6 co-receptor LRP5, or the domain interacts with the Wnt3a (E3-E4) epitope of the LRP5 co-receptor and the Wnt3a (E3-E4) epitope of the LRP6 co-receptor or alternatively the domain interacts with a Wnt1 (E1-E2) epitope or LRP5 co-receptor and a Wnt3a (E3-E4) epitope of LPR6 co-receptor or vis versa.

25 Various formats of tetravalent binding antibody molecules described herein are depicted in Figure 6. In a particular format, Diabody-Fc-Fab, an LRP5/6 binding diabody is linked to the N-terminus of an Fc domain and two Fabs are linked to the C-terminus of the Fc domain wherein the Fab is linked to the CH3 of the Fc domain via the Fab heavy chain (VH) variable

domain. Alternatively, the Fab is linked to the CH3 of the Fc domain via the variable region (VL) of the light chain.

We previously reported multivalent binding molecules comprising an Fc domain, a FZD binding domain and a Wnt co-receptor (LRP5/6) binding domain on opposite termini of the Fc domain, e.g., a molecule having a FZD4 diabody linked one terminus of an Fc domain and a LRP5/6-binding diabody linked to the other terminus of the Fc domain, see PCT/IB2019/051174 inventors Angers et al. and PCT/IB2020/055463 inventors Angers et al., both incorporated in their entirety by reference.

It has been reported that Wnt- β -catenin signaling, specifically through activation of FZD4, is important for vasculature development and for adult vasculature homeostasis. More specifically, it is critical for barrier function at the blood-retina and blood-brain barriers (BRB and BBB). Defects in FZD4 signaling can lead to endothelial cell permeability defects and genetic mutations within this pathway are known to lead to vascular defects (e.g. Norrie disease, FEVR). At the blood-retina barrier, the extracellular ligand Norrin predominantly activates a FZD4-TSPAN12-LRP5 complex to regulate endothelial cell-cell interactions, barrier functions and permeability (Wang et al. (2012) Norrin/Frizzled4 signaling in retinal vascular development and blood brain barrier plasticity. Cell. 151:1332–1344). At the blood-brain barrier the secreted Wnt7a/b growth factor chiefly activates the FZD4-GPR124-LRP6 receptor complex (Chang et al., (2017). GPR124 is essential for blood-brain barrier integrity in central nervous system disease. (Nat. Med. 23: 450–460). The FZD4 Agonists described herein, e.g., the configurations having a diabody binding domain for a LRP5/6 and an FZD4 binding domain comprised of two Fab fragments that bind FZD4, wherein the binding domains are on opposite termini of an Fc domain, produce a particularly stable and homogenous molecule with an unexpectedly high level of Wnt- β -catenin signaling pathway activation in endothelial cells that translates into increased barrier function and decreased vascular permeability (Figure 11). In essence, the FZD4 Agonists described herein function as Norrin and Wnt7a/b mimetic molecules.

This invention also includes methods for using the FZD Agonists described herein. Described herein are methods to activate a Wnt signaling pathway, e.g., the Wnt/ β -catenin signaling pathway, using the tetravalent binding antibody molecules of this invention, which are contemplated to promote the proximity of FZD receptors and Wnt co-receptors, e.g., one or more of FZD1, FZD2, FZD3, FZD4, FZD5, FZD6, FZD7, FZD8, FZD9, and FZD10

receptors and LRP5 and/or LRP6 co-receptors, on a cell wherein binding by the FZD Agonists to both FZD receptor(s) and the LRP5 and/or LRP6 co-receptor(s) activates the Wnt signaling pathway.

- 5 Blood-retina barrier (BRB) formation and retinal angiogenesis require β catenin signaling induced by the ligand norrin (NDP [Norrie disease protein]), the receptor FZD4, co-receptor LRP5, and the TSPAN12 (tetraspanin 12). As such, an aspect of this invention is a method for promoting and/or maintaining retinal vasculature barrier function and angiogenesis by treating eye tissue, e.g., retinal tissue, with an effective amount of a tetravalent FZD4 Agonists of this invention.
- 10 Also, an aspect of this invention is a method for promoting, restoring and/or maintaining the BRB and BBB functions by treating the BRB or BBB vasculature with an effective amount of a tetravalent FZD4 Agonist described herein. A further aspect of this invention is a method for treating a subject having a disorder or condition characterized by defective retinal or brain angiogenesis characterized by reduced endothelial cell barrier function leading to
- 15 vascular leakage by administering to such subject an effective amount of a FZD4 Agonists of this invention. A further aspect of this invention is a FZD4/LRP5 tetravalent binding antibody molecule or pharmaceutical composition for use in the treatment or prevention of a disorder or condition characterized by defective retinal or brain angiogenesis and/or characterized by reduced endothelial cell barrier function and/or vascular leakage. A further aspect of this
- 20 invention is a method of treating or preventing a disorder or condition characterized by defective retinal or brain angiogenesis and/or reduced endothelial cell barrier function and/or vascular leakage comprising administering to a person in need thereof a therapeutically effective amount of a FZD4/LRP5 tetravalent binding antibody molecule described herein. A further aspect of the invention is the use of a FZD4/LRP5 tetravalent binding antibody
- 25 molecule for the manufacture of a medicament for the treatment or prevention of a disorder or condition characterized by defective retinal or brain angiogenesis and/or reduced endothelial cell barrier function and/or vascular leakage. Such disorders or conditions include ocular disorders, including but are not limited to disorders of the retina or macula. Such disorders of the retina or macula include, but are not limited to diabetic retinopathy,
- 30 retinopathy of prematurity, Coats' disease, FEVR, Norrie disease, macular degeneration, diabetic macular edema, and pediatric vitreoretinopathies. Additional disorders or conditions

included in embodiments of this invention include but are not limited to Alzheimer's disease, epilepsy, multiple sclerosis, ischemia, and stroke.

An embodiment of this invention includes methods for producing vascularized cerebral organoids by promoting the barrier function of the vasculature network throughout the organoids, and thereby mimicking blood-brain-barrier function using an effective amount of a
5 tetravalent FZD4 Agonist described herein.

Also, an embodiment of this invention is a method of treating a subject suffering from a gastrointestinal disorder, including a subject having inflammation of all or part of the intestines, also known as inflammatory bowel disease, by administering to such subject an
10 effective amount of a pharmaceutical composition of this invention, e.g., a composition comprising a FZD5 Agonist. Examples of inflammatory bowel disease include, but are not limited to, Crohn's disease, and ulcerative colitis.

Also, an embodiment of this invention are methods for directing differentiation of iPS or other pluripotent stem cells (PSCs) towards various lineages by culturing these cells in the
15 presence of an effective amount of a tetravalent binding antibody molecule of this invention.

Also described herein are methods for making the tetravalent binding antibody molecules of this invention.

The modular aspects of this invention allow for mixing and matching binding domains derived from FZD-binding antibodies and LRP5/6-binding antibodies on opposite termini of
20 the Fc domain to generate a tetravalent binding antibody molecule that can engage a FZD-LRP5/6 co-receptor complexes to selectively activate Wnt signaling. The modularity and effectiveness of the tetravalent binding antibody molecules for activating Wnt signaling pathways described herein contrasts with the Wnt surrogates described in the prior art that consists of monovalent FZD and Wnt co-receptor binding ligands, or FZD and Wnt co-
25 receptor binding ligands wherein the binding ligands are not attached to opposite ends of an Fc domain.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1A and Figure 1B. Single point ELISAs. FZD4-binding antibodies isolated from affinity matured libraries of the known FZD4-binding antibodies 5044 (Figure 1A) and 5027
30 (Figure 1B) bind to FZD4 sites that compete with their parental antibody. The reaction was

stopped by adding 1M H₃PO₄ and the absorbance was measured spectrophotometrically at 450 nm in a microtiter plate reader; white=BSA; black and white stripe=Fc; gray=FZD4 + blocking antibody; and black=FZD4.

Figure 2. Epitope mapping of FZD4 antibodies. FZD4 and 5027 and 5044 have overlapping epitopes. The pan-FZD binder 5016 is a positive control showing that the antigens are functional, with the exception of “FZD4_Swap10”. Both FZD4-specific antibodies 5027 and 5044 are unable to bind to “FZD4 Swap 7” suggesting that these molecules bind to this region of the FZD ECD.

Figure 3A. Size-exclusion chromatography (SEC). Analysis of FZD4 antibodies as compared to Trastuzumab. Protein elution was monitored using absorbance at 280 nM.

Figure 3B. ELISA specificity. Measurements of the FZD4 antibodies determined against FZD4 and against FZD1 and FZD10, two members of the FZD family most-closely related to FZD4. The reaction was stopped by adding 1M H₃PO₄ and the absorbance was measured spectrophotometrically at 450 nm in a microtiter plate reader.

Figure 4. Phage clonal ELISA of synthetic antibodies targeting LRP5. The results demonstrate that the synthetic antibodies bound to LRP5. The absorbance was measured spectrophotometrically at 450 nm in a microtiter plate reader; gray=BSA; light gray=his-Fc; dark gray=LRP5

Figures 5A and 5B. Phage clonal ELISA of synthetic antibodies targeting LRP6. The results demonstrate the synthetic antibodies bound to LRP6. The absorbance was measured spectrophotometrically at 450 nm in a microtiter plate reader; black=BSA; gray=Fc; light gray=LRP6-Fc.

Figure 6. Modalities of tetravalent binding antibody molecules. Illustrated are: a diabody-Fc-diabody format having an FZD-binding monospecific diabody on the N-terminal of the Fc domain and a LPR5/6-binding bispecific diabody on the C-terminal of the Fc domain; a Diabody-Fc-scFv format having an N-terminal LPR5/6-binding bispecific diabody and two C-terminal FZD binding scFvs; an IgG-diabody format having two FZD-binding Fabs forming an N-terminal binding domain and a bispecific LRP5/6 binding diabody forming the C-terminal binding domain; an IgG-scFv format having two FZD-binding Fabs forming an N-terminal binding domain and two LRP5/6 binding scFvs forming the C-terminal binding

domain, and; a diabody-Fc-Fab format having a bispecific LRP5/6 binding diabody forming the N-terminal binding domain and two FZD-binding Fabs forming the C-terminal binding domain, wherein the Fabs are linked to the CH3 of the Fc domain via the Fab variable heavy region. It is specifically contemplated that in an alternate diabody-Fc-Fab format the Fabs
5 are linked to the CH3 of the Fc domain via the Fab variable light region. The various domains of the tetravalent molecules, VL, VH, CH1, CH2, CH3, CL1 and Fc, are joined via linkers, e.g., peptide linkers. The Fc domain, is formed by the dimerization of the CH2 and CH3 domains of the Hole construct Fc region and Knob construct Fc region. The various domains of the tetravalent molecules, VL, VH, CH1, CH2, CH3, CL1 and Fc, are joined via
10 linkers, e.g., peptide linkers.

Figure 7. FZD4 Agonist having a Diabody-Fc-Fab format. The Diabody-Fc-Fab format having an LRP5-binding bispecific diabody forming a bivalent bispecific N-terminal LRP5-binding domain and two FZD4-binding Fabs forming a bivalent monospecific C-terminal FZD4-binding domain and an Fc region with attenuated effector functions due to amino acid
15 mutations, e.g., N297G (NG) and D265A, (DANG) variants. The various domains of the tetravalent molecules, VL, VH, CH1, CH2, CH3, CL1 and Fc, are joined via linkers, e.g., peptide linkers.

Figure 8. FZD4 Agonists having a Diabody-Fc-Fab format (ANT) bind FZD4 with high selectivity. Figure 8A depicts the apparent selectivity of the FZD4 Agonists for the
20 recombinant extracellular domain (ECD) of 9 of the 10 FZD as determined by biolayer interferometry (BLI). Figure 8B demonstrates FZD agonists do not recognize common non-specific antigens. The FZD Agonists were tested at 100 nM for binding to a panel of antigens as described in Mouquet et al. Polyreactivity increases the apparent affinity of anti-HIV antibodies by heterologation. *Nature*. 2010 Sep;467(7315):591-595. DOI:
25 10.1038/nature09385, PMC3699875, and Jain T. et al. Biophysical properties of the clinical-stage antibody landscape. *Proceedings of the National Academy of Sciences of the United States of America*. 2017 Jan;114(5):944-949. DOI: 10.1073/pnas.1616408114, PMC5293111.

Figure 9. FZD4 Agonists (ANT) having a Diabody-Fc-Fab format (having a LRP-binding bispecific diabody and two FZD4-binding Fabs) are stable and monomeric in solution. Figure
30 9A presents the results of an analytical SEC analysis of FZD agonists as compared to trastuzumab IgG. Figure 9B presents the results of differential scanning fluorimetry demonstrating that the FZD4 Agonists in the Diabody-Fc-Fab format have thermal

denaturation profiles similar to that of trastuzumab, whereas a first generation diabody-Fc-diabody FZD4 modality (CM0199) is less optimal.

Figure 10. FZD4-LRP5 specific FZD4 Agonists having the Diabody-Fc-Fab format (ANT). FZD4-LRP5 specific FZD4 Agonists in this format stimulate FZD4 in mouse endothelial cell line (bEND3.1) and lead to an increase in Axin2 (beta catenin target gene) gene transcription in a concentration-dependent manner.

Figure 11A and Figure 11B depicts a FZD4-LRP5 specific agonist having the diabody-fc-diabody format promotes endothelial cell barrier functions in a mechanism opposing VEGF-induced permeability. Figure 11A depicts Immunofluorescence of ZO-1/CLDN3 and ZO-1/CLDN5 localization on bEnd.3 cell junctions. bEnd.3 cells were treated or not with 30nM of F4L5.13 (aka CM0199) and Norrin (NDP) in the presence or absence of VEGF (100ng/ml) for 1h. Starting from the top row and working downward: NT (non-treated) show no change in permeability; VEGF treatment of bEND3.1 cells leads to junction disassembly as seen by loss of plasma membrane staining of CLDN3, CLDN5 and ZO-1; Co-treatment of cells with VEGF and the FZD4 agonist CM0199 (F4L5.13) leads to a near-complete rescue of the effect of VEGF alone; the last row of Figure 11A shows co-treatment of cells with VEGF and NDP and similarly leads to a near-complete rescue of the effect of VEGF alone, suggesting that the FZD4 Agonists described herein function as Norrin and Wnt7a/b mimetic molecules. Figure 11B shows a transendothelial permeability assay quantifying the passage of FITC-dextran through a monolayer of bEnd.3 cells. Passage of FITC-dextran was measured after exposure of bEnd.3 cells to 100 ng/ml VEGF, 30 nM F4L5.13 or both or pretreated with VEGF for 1 h before treating with F4L5.13 for 1 h. Data are presented as mean $\pm$ SD, n = 5 independent experiments. Significance was calculated by one-way ANOVA with Bonferroni's multiple comparisons test (*P < 0.05 as compared to VEGF treatment).

Figure 12. Single point ELISA. FZD5 antibodies that bind the extracellular domain of FZD5 at a site overlapping with 2919 identified from affinity maturation libraries. New FZD5 antibodies bind FZD5 at a site overlapping with 2919 identified from affinity maturation libraries. Single point ELISAs were performed on 96-well Maxisorp plates coated with the ECDs of human FZD5 protein in the presence or absence of a saturating concentration of 2919 IgG. The absorbance was measured spectrophotometrically at 450 nm in a microtiter

plate reader; white with black stripes=BSA; black with white stripes=Fc; gray=FZD5 + blocking antibody; black=FZD5.

Figure 13. Single point ELISA. demonstrates new FZD5 antibodies from 2928 affinity maturation library selectively bind FZD5. New FZD5 antibodies from 2928 affinity maturation library selectively bind FZD5. Single point ELISAs were performed on 96-well Maxisorp plates coated with the ECDs of human FZD2, FZD5, or FZD8 protein. The absorbance was measured spectrophotometrically at 450 nm in a microtiter plate reader; black with white stripes=Fc; white with black stripes=FZD2; gray=FZD8; black=FZD5.

Figure 14. Luciferase assay. Pan-FZD/LRP6 ANT9 and FZD5-specific/LRP6 ANT59 activate Wnt signaling in cells. TOPFLASH cells were treated overnight with varying concentrations of FZD agonist or a non-targeting control molecule (CM0156) and TCF/LEF-driven luciferase expression was measured using a standard luciferase assay. Both molecules are able to activate FZD-mediated luciferase expression in a concentration-responsive manner. ANT9, which is able to bind to 7 of the 10 FZD receptor subtypes produces a higher maximal activation signal than the FZD5-specific ANT59.

Figure 15. Original format ANT39 and inverted format ANT39i. The FZD4 Agonist ANT39 having a Diabody-Fc-Fab format and FZD4 Agonist ANT39i having an IgG-Diabody format (having two FZD-binding Fabs forming an N-terminal binding domain and a bispecific LRP5/6 binding diabody forming the C-terminal binding domain) and an Fc domain. The FZD binding domain of ANT39i comprises two Fab fragments attached to the N- terminus of the Fc domain and each Fab binds an FZD. The LRP5/6 co-receptor binding domain is attached to the C- terminus of the Fc domain and is composed of a diabody that binds two different sites on the co-receptor, e.g., a Wnt1 site (E1-E2) and a Wnt3 site (E3-E4) on LRP5/6. The Fabs may be specific for a particular FZD, e.g. FZD4, or may be pan-specific, binding to more than one FZD, e.g., to FZD4 and one or more other FZD. The Fc region may have attenuated effector functions due to amino acid mutations, e.g., N297G (NG) and D265A, (DANG) variants. The various domains of the tetravalent molecules, VL, VH, CH1, CH2, CH3, CL1 and Fc, are joined via linkers, e.g., peptide linkers.

Figure 16A depicts FZD4 Agonist ANT39 having a Diabody-Fc-Fab format (having an LRP5-binding bispecific diabody forming a bivalent bispecific N-terminal LRP5-binding domain and two FZD4-binding Fabs forming a bivalent monospecific C-terminal FZD4-

- binding domain) with the Fc region having attenuated effector functions due amino acid mutations to N297G and D265A (DANG) variants or L234A, L235A, P331S (LALAPS) variants, and with the Fc region further comprising knob-in-hole heterodimerization variants Merrimack, Merchant or Merchant S:S (Merrimack CH3 mutations as described in
- 5 WO2018/026942A1, Merchant CH3 mutations as described in Merchant A.M. et al Nature Biotechnology 1998 vol 16 p677-681). Figure 16A discloses SEQ ID NOS 886, 892, 891, 886, 892, 891, 886, 892, 891, 886, 892, and 891, respectively, in order of appearance. Figure 16B depicts FZD4 Agonist ANT39i having an IgG-Fc-Diobody format (having two Fab fragments attached to the N- terminus of the Fc domain, each Fab binding to an FZD, and a
- 10 LRP5/6 co-receptor binding domain attached to the C- terminus of the Fc domain that is composed of a diabody that binds two different sites on the co-receptor) and an Fc region with attenuated effector functions due to DANG or LALAPS variants, and Merrimack, Merchant or Merchant S:S heterodimerization variants. Figure 16B discloses SEQ ID NOS 891, 886, 891, 886, 891, 886, 891, and 886, respectively, in order of appearance.
- 15 Figure 17. Thermostability of ANT39 variants. Figure 17 presents the results of differential scanning fluorimetry experiments demonstrating that the LALA variant of FZD4 agonist ANT39 (ANT39 LALA) has improved thermal stability relative to the parental ANT39 (containing DANG mutations in the Fc). Specifically, the LALA variant showed an improved thermal stability that is closer to the profile of a variant of Trastuzumab that
- 20 contains the same Knob/Hole Fc mutations as the ANT.
- Figure 18. FZD4 Agonist ANT42 having a Diabody-Fc-Fab format. FZD4 Agonist ANT42 having an LRP5-binding bispecific diabody forming a bivalent bispecific N-terminal LRP5-binding domain and two FZD4-binding Fabs forming a bivalent monospecific C-terminal FZD4-binding domain with the Fc region having attenuated effector functions due amino acid
- 25 mutations to N297G and D265A (DANG) variants or L234A, L235A, P331S (LALAPS) variants, and with the Fc region further comprising knob-in-hole heterodimerization variants Merrimack, Merchant or Merchant S:S (Merrimack CH3 mutations as described in WO2018/026942A1, Merchant CH3 mutations as described in Merchant A.M. et al Nature Biotechnology 1998 vol 16 p677-681). And FZD4 Agonist ANT42i having an IgG-Fc-
- 30 Diabody format (having two Fab fragments attached to the N- terminus of the Fc domain, each Fab binding to an FZD, and a LRP5/6 co-receptor binding domain attached to the C-terminus of the Fc domain that is composed of a diabody that binds two different sites on the

co-receptor) and an Fc region with attenuated effector functions due to DANG or LALAPS variants, and Merrimack, Merchant or Merchant S:S heterodimerization variants. Figure 18 discloses SEQ ID NOS 886, 892, 891, 891, 886, 886, 892, 891, 891, 886, 886, 892, 891, 891, 886, 886, 892, 891, 891, and 886, respectively, in order of appearance.

- 5 Figure 19. Antibody modalities tested for FZD agonism. A) Diabody-Fc-Diabody, VH and VL were tested both FZD or LRP binding variable domains; B) Diabody-Fc-scFv; C) scFv-Fc-Diabody; D) scFv-Fc-scFv; E) IgG-Diabody; F) IgG-scFv; G) Diabody-Fc-Fab; H) Diabody-CH3-Diabody; I) Fab-Diabody. In figure 19, molecules B-F, H-I, comprise N-terminal variable domains that bind LRP and the C-terminal variable domains bind FZD. In
10 figure 19, molecule G comprises a variable domain at the N-terminal that binds FZD and a variable domain at the C-terminal that binds LRP. These antibody formats marked with an * were tested using a Knobs-in-Holes Fc.

Figure 20. Multiple antibody architectures are able to elicit potent FZD agonism. Paratopes targeting pan-FZD and LRP6 were configured in various arrangements as described in table
15 14. Canonical Wnt pathway stimulation by each antibody was determined on wild-type HEK cells expressing the TOPFLASH reporter in a blinded manner by two different scientists. Data are presented as mean $\pm$ SD and are representative of 4 different experiments.

Figure 21. Expression Titers of various FZD agonist modalities. Various FZD agonist modalities were expressed in HEK cells, purified via protein A chromatography, and
20 expression titer was determined based on the absorbance at 280 nm. EC50 for FZD activation was determined on wild-type HEK cells expressing the TOPFLASH reporter in a blinded manner by two different scientists.

Figure 22. Organoid viability Assay. Mouse small intestine organoids were grown in the presence of 1 μ M LGK-974 to block endogenous Wnt secretion and treated with PBS, Wnt3a
25 conditioned media or FLAg molecules as indicated. Left, representative images from n=3 independent experiments. Right, quantification of organoid viability via CellTiter-Glo luminescence assay. Bars represent mean $\pm$ standard error from 3 independent experiments.

Figure 23. Mouse colon histology. Histological appearance of the mouse colon following DSS treatment cycle (7 days 2% DSS, 3 days 0.5% DSS) with intraperitoneal injection of
30 either control IgG or ANT59 (10 mg/kg) on days 4 and 7. (A) Images captured at 20X

magnification showing overall architecture. (B) Images captured at 100X showing rescue of mucosal integrity with ANT59 treatment.

Figure 24. (A) Body weight changes in mice throughout DSS treatment cycle (7 days 2% DSS, 3 days 0.5% DSS) with intraperitoneal injection of either control CM0156, PanFZD agonist or ANT59 (10 mg/kg) on days 4 and 7. (B) Left: Representative images of dissected colons from 6-8 mice per treatment group with centimeter scale for comparison. Right: colon length from each treatment group with bar representing mean colon length $\pm$ S.D. and individual data points displayed. *** indicates $p < 0.0001$ in one-way ANOVA, H2O indicates normal water (no DSS).

Figure 25. Characterization of FZD5/LRP6 ANTs. ANTs were expressed in HEK cells, purified via protein A chromatography, and expression titer was determined based on the absorbance at 280 nm. Using biolayer interferometry, the apparent affinity (avidity) of each molecule for recombinant Fc-fused human FZD5 was determined and selectivity against other human FZDs was measured. Dose-response curves for the activation of a LEF/TCF reporter gene in FZD-knockout (1,2,4,5,7) HEK293 cells overexpressing FZD5. Cells were seeded in 96-well dishes for 24 hours, then treated as indicated for 17 hours. Reporter activation was assessed using the Dual-Luciferase Reporter Assay System (Promega). Data are presented as mean $\pm$ SD for technical duplicates and representative of $n=3$ independent experiments.

DETAILED DESCRIPTION OF THE INVENTION

Described herein are tetravalent binding antibody molecules comprising an Fc domain, with or without effector function, a bivalent FZD binding domain and a bivalent LRP-binding domain, wherein the binding domains are attached to opposite ends of the Fc domain. In an embodiment, the FZD binding domain is attached to the carboxy terminus of the Fc region and the LRP co-receptor binding domain is attached to the amino terminus of the Fc domain. Alternatively, the FZD binding domain is attached to the amino terminus of the Fc region and the co-receptor binding domain is attached to the carboxy terminus of the Fc domain. The binding domains may be attached directly to the Fc domain or attached to the Fc domain via a linker. The FZD binding domain may bind to one or to more than one FZD receptor, i.e., one or more of FZD1, FZD2, FZD3, FZD4, FZD5, FZD6, FZD7, FZD8, FZD9, and FZD10.

In an embodiment of the invention the FZD binding domain is bivalent and comprises a diabody or comprises a scfv, a V_{HH} fragment, or an Fab fragment or combinations thereof

that bind FZD, and the co-receptor binding domain is bivalent and comprises a diabody or a V_HH fragment, an Fab, or a scFv or combinations thereof that bind the LRP5/6 co-receptor. In an embodiment of the invention the FZD binding domain is attached to the carboxy-terminus of the Fc domain and comprises two scfv, two V_HH fragments, two Fab fragments
5 or a diabody that bind FZD, and the co-receptor binding domain attached to the amino terminus of the Fc domain comprises a diabody, two V_HH fragments or two scFvs that binds to the LRP5/6 co-receptor. When attached to the carboxy terminus of the Fc domain the FZD-binding Fabs are linked to the CH3 of the Fc domain via the Fab variable heavy region or variable light region. In other embodiments the FZD binding domain is attached to the amino
10 terminus of the Fc domain and is comprised of two Fabs and the LRP5/6 co-receptor binding domain is attached to the carboxy terminus of the Fc domain and is comprised of a diabody or two scFvs that bind the co-receptor.

Figure 6 illustrates a tetravalent binding antibody molecule of this invention in the Diabody-Fc-scFv format having a LRP5/6 co-receptor binding domain, an Fc domain, and a FZD
15 binding domain. The Diabody-Fc-sFv comprises (i) an Fc domain, (ii) a bispecific diabody attached to the N-terminal of the Fc domain that binds two different sites on the co-receptor, e.g., a Wnt1 (E1-E2) site on LRP5/6, and a Wnt3 site (E3-E4) on LRP5/6, and (iii) a FZD binding domain comprising two FZD-binding scFv fragments attached to the carboxy terminus of the Fc domain. The scFv may be specific for a particular FZD, e.g. FZD4, or
20 may be pan-specific, binding to more than one FZD, e.g. to FZD4 and one or more other FZD.

An embodiment of this invention is a tetravalent binding antibody molecule in a Diabody-Fc-scFv format having (i) an Fc domain, (ii) a LRP5/6 co-receptor binding domain that comprises a bispecific diabody that binds two different sites on the co-receptor, e.g., a Wnt1
25 (E1-E2) site on LRP5/6, and a Wnt3 site (E3-E4) on LRP5/6, wherein the diabody is attached to the amino terminus of the Fc domain and (iii) a FZD binding domain, attached to the carboxy terminus of the Fc domain comprising two scFv fragments each binding FZD. The scFv may be specific for the FZD, or may be pan-specific, binding to the FZD and one or more other FZD.

30 Figure 6 also illustrates a tetravalent binding antibody molecule of this invention in the IgG-diabody format having (i) an Fc domain, (ii) a FZD binding domain that comprises of two Fab fragments attached to the N- terminus of the Fc domain, each Fab binding to an FZD,

and (iii) a LRP5/6 co-receptor binding domain attached to the C- terminus of the Fc domain that is composed of a diabody that binds two different sites on the co-receptor, e.g., a Wnt1 site (E1-E2) and a Wnt3 site (E3-E4) on LRP5/6. The Fabs may be specific for a particular FZD, e.g. FZD4, or may be pan-specific, binding to more than one FZD, e.g., to FZD4 and one or more other FZD.

An embodiment of this invention is a tetravalent binding antibody molecule in an IgG-Diabody format comprising (i) an Fc domain, (ii) an N-terminal binding domain for a FZD, comprising two FZD-binding Fabs and (ii) a C-terminal binding domain for a LRP5 and/or LRP6 co-receptor, comprising a LRP5/6 coreceptor-binding diabody. This FZD Agonist in the IgG-Diabody format comprises,

(1) a first and second heavy chain monomer, wherein each heavy chain monomer comprises a single-chain polypeptide comprising from N-terminus to C-terminus:

(a) a heavy chain variable domain (VH) that binds a FZD, linked to

(b) a heavy chain constant region domain 1 (CH1 domain), linked to

(c) an Fc region (or fragment thereof comprising a constant heavy chain domain 3 (CH3 domain)), linked to

(d) a peptide comprising a VH that binds a LRP5/6 co-receptor, linked to a light chain variable domain (VL) that binds a LRP5/6 co-receptor, and

(2) a first and second light chain monomer, each light chain monomer comprising from N terminus to C terminus a VL that binds the FZD, linked to a constant light chain domain 1 (CL1 domain).

The first and second heavy chain monomers dimerize via their Fc regions, or fragments thereof. The linker between the VH and VL that bind the LRP5/6 is of a length that promotes the pairing of the VH and VL of the first heavy chain monomer with the VL and VH of the second heavy chain monomer thereby forming a LRP5/6 co-receptor binding diabody. The FZD-binding Fabs are formed by the pairing of each heavy chain monomer with a light chain monomer such that the VH that binds FZD4 and CH1 of each of the heavy chain monomer, pairs with the VL that binds FZD4 and CL1 of the light chain monomers. In this IgG-Diabody format, the Fabs

form the FZD4-binding domain on the N-terminus of the Fc domain and the diabody forms the co-receptor-binding domain on the C-terminus of the Fc domain. The Fabs may be specific for one FZD, e.g., FZD4 or FZD5, or may be pan-specific, binding to more than one FZD, e.g., to FZD4 and/or FZD5, and in some cases more FZD. The Fc regions may dimerize via a knob-in-hole configuration. Methods for dimerizing peptides via a knob-in-hole configuration are described in WO2018/026942, inventors Van Dyk et al., Carter P. (2001) J. Immunol. Methods 248, 7–15; Ridgway et al. (1996) Protein Eng. 9, 617–621; Merchant, et al. (1998) Nat. Biotechnol. 16, 677–681, and; Atwell et al., (1997) J. Mol. Biol. 270, 26–35. The Fc regions may be Merrimack (knob chain: Q347M, Y349F, T350D, T366W and L368M; hole chain: S354I, E357L, T366S, L368A and Y407V), Merchant (knob chain: T366W; hole chain: T336S, L368A and Y407V) or Merchant S:S (Merchant mutations with additional S354C variant in the knob chain and Y349C in the hole chain). The Fc regions may also contain mutations that alter their effector function, e.g., the Fc region may have attenuated effector functions due to amino acid mutations, e.g., DANG variants and LALAPS variants.

Although in Figure 6 the peptides forming the diabody in the IgG-Diabody format are linked to the C-terminal of the Fc domain via their VH domain in a VH-VL orientation (N terminal to C terminal), in some embodiments, the peptides forming the diabody are linked to the C-terminal of the Fc domain via their VL domains in a VL-VH orientation (N-terminal to C-terminal). And, although the heavy chains are depicted as comprising a VH domain and a CH1 domain linked to the N-terminal of the Fc domain and the light chains are depicted as comprising a VL domain and CL1 domain to form the Fabs, in some embodiments (Diabody-Fc-Fab in Fig.6 and Fig. 7A) the diabodies are fused to the N-terminus of the Fc and the Fabs are fused to the C-terminus of the Fc. In order to do this, the CH3 domain of the Fc is fused directly to the heavy chain of the Fab via its VH domain (VH-CH1) or directly to the light chain via its VL domain (VL-CL) and where the light and heavy chains still associate to form the Fabs.

Figure 6 illustrates a tetravalent binding antibody molecule in a Diabody-Fc-Fab configuration having an LRP5/6-binding bispecific bivalent diabody forming the N-terminal binding domain, and two FZD-binding Fabs forming the C-terminal binding domain. The Fabs may be specific for a particular FZD, e.g. FZD4, or may be pan-specific, binding to

more than one FZD, e.g. FZD4 and one or more other FZD. See also Figure 7A, which illustrates a tetravalent binding antibody molecule in the Diabody-Fc-Fab format having an Fc in a knob-in-hole (KiH) configuration and an LRP5-binding bispecific bivalent diabody forming the N-terminal binding domain, and two FZD4-binding Fabs forming the C-terminal binding domain. Although Figures 6 and 7A illustrates the Fabs linked to the CH3 of the Fc domain (at the C-terminus) via the Fab variable heavy domain (VH), it is specifically contemplated that in an alternate diabody-Fc-Fab format the Fabs are linked to the CH3 of the Fc domain via the Fab variable light domain (VL). The various domains of the tetravalent molecules, VL, VH, CH1, CH2, CH3, CL1 and Fc, are joined via linkers, e.g., peptide linkers.

Also an embodiment of this invention is a tetravalent binding antibody molecule in the Diabody-Fc-Fab format comprising (i) an Fc domain, (ii) an N-terminal binding domain comprising a diabody that binds to the co-receptor, e.g., LRP5 and/or LRP6 co-receptor and (ii) a C-terminal binding domain comprising two Fab that bind to one or more FZD, e.g., FZD4 or FZD5. This FZD Agonist in the Diabody-Fc-Fab format comprises,

(1) a first and second heavy chain monomer, wherein each heavy chain monomer comprises a single-chain polypeptide comprising, from N-terminus to C-terminus:

(a) a peptide comprising a heavy chain variable (VH) domain that binds a LRP5/6 co-receptor and a light chain variable (VL) domain that binds a LRP5/6 co-receptor, linked to

(b) an Fc region (or fragment thereof comprising a constant heavy chain domain 3 (CH3 domain)), linked to

(c) a VH domain that binds a FZD, linked to

(d) a CH1 domain, and

(2) a first and second light chain monomer each light chain monomer comprising from N-terminus to C-terminus a VL domain that binds FZD, and a constant light chain domain 1 (CL1).

The first and second heavy chain monomers dimerize via the Fc regions or fragments thereof and a bivalent LRP5/6-binding diabody is formed by the pairing of the VH domain and VL

- domain that bind LRP5/6 of the first heavy chain monomer with the VL domain and VH domain that bind LRP5/6 of the second heavy chain monomer. The two FZD-binding Fabs are formed by the pairing of each heavy chain monomer with a light chain monomer such that the VL that binds the FZD and the CL1 of a light chain monomer pairs with the VH that binds the FZD and the CH1 of each of the heavy chain monomers. In this Diabody-Fc-Fab format, the diabody forms the LRP5/6 co-receptor binding domain on the amino terminus of the tetravalent molecule and the two Fabs form the FZD binding domain on the C-terminus of the tetravalent binding antibody molecule. The Fc regions may dimerize via a knob-in-hole configuration.
- 10 Methods for dimerizing peptides via a knob-in-hole configuration are described in WO2018/026942, inventors Van Dyk et al., Carter P. (2001) *J. Immunol. Methods* 248, 7–15; Ridgway et al. (1996) *Protein Eng.* 9, 617–621; Merchant, et al. (1998) *Nat. Biotechnol.* 16, 677–681, and; Atwell et al., (1997) *J. Mol. Biol.* 270, 26–35. The Fc regions may be Merrimack (knob chain: Q347M, Y349F, T350D, T366W and L368M; hole chain: S354I, E357L, T366S, L368A and Y407V), Merchant (knob chain: T366W; hole chain: T336S, L368A and Y407V) or Merchant S:S (Merchant mutations with additional S354C variant in the knob chain and Y349C in the hole chain). The Fc regions may also contain mutations that alter their effector function, e.g., the Fc region may have attenuated effector functions due to amino acid mutations, e.g., DANG variants and LALAPS variants.
- 20 Although in Figures 6 and 7A the peptides forming the diabody in the Diabody-Fc-Fab format are linked to the Fc domain via their VL domains, thus in a VH -VL orientation (from N-terminal to C-terminal), in some embodiments the orientation can be switched such that the peptides forming the diabody are linked to the N-terminal of the Fc domain via their VH domains, thus in a VL-VH orientation (from N-terminal to C-terminal). Also, although the heavy chains in the Diabody-Fc-Fab format are depicted as comprising a VH domain and a CH1 domain, which pair with the light chain comprising a VL and CL1 domain to form the Fabs, it is also contemplated that in some embodiments the variable and constant domains are switched such that the heavy chains comprise a VL domain and a CL1 domain and the light chains comprises the VH domain and CH1 domain and the heavy and light chains still pair to form the Fabs.
- 30

In an embodiment of this invention the binding moiety of the FZD binding domain is derived from an antibody, or an antibody fragment, that binds specifically to one FZD, e.g. FZD4 or

FZD5, or is pan-specific interacting with a specific FZD, e.g. FZD4 or FZD5, and one or more additional FZD receptors (an FZD source antibody), and the co-receptor binding domain comprises a binding moiety that is derived from an antibody or antibody fragment that binds to a LPR5 and/or LRP6 (a LRP5/6 coreceptor source antibody). In an embodiment of the invention the FZD-binding antibodies bind to an extracellular cysteine rich domain (CRD) of the FZD receptor. The antibody that binds FZD may be an antibody that binds the FZD receptor and antagonizes Wnt signaling or inhibits binding of a Wnt ligand to the FZD receptor. The antibody that binds FZD may be an antibody that binds the FZD receptor without antagonizing or inhibiting binding of a Wnt ligand to the FZD receptor. The antibody that binds FZD may be an antibody that binds FZD and enhances Wnt signaling. The antibody that binds the LRP5/6 co-receptor may be an antibody that binds the LRP5/6 co-receptor and antagonizes Wnt signaling or inhibits binding of a Wnt ligand to the co-receptor, or the antibody that binds the LRP5/6 co-receptor may be an antibody that binds the co-receptor without antagonizing Wnt or Norrin signaling or inhibiting binding of a Wnt or Norrin ligand to the co-receptor.

In an embodiment of this invention the LRP5/6 co-receptor binding domain binds to a single epitope on a co-receptor, e.g., an epitope that binds to the Wnt1 (E1-E2) or Wnt3 (E3-E4) interacting domain of LRP5/6. In an embodiment of this invention the LRP5/6 co-receptor binding domain binds to two epitopes within the co-receptor, e.g., a paratope that binds to the Wnt1 (E1-E2) interacting epitope and a paratope that binds to Wnt3 (E3-E4) epitope of LRP5/6. In an embodiment of this invention the multivalent binding molecule comprises a Fc domain, wherein the Fc domain is the Fc domain of an immunoglobulin or a fragment thereof comprising the CH3 domain. In an embodiment of the invention the immunoglobulin is an IgG. In an embodiment of this invention the IgG is an IgG₁.

In an embodiment of this invention the LRP5/6 binding domain comprises a diabody comprising two peptides each comprising a heavy chain variable domain (VH) that binds to LRP5/6 linked to a light-chain variable domain (VL) that binds LRP5/6 wherein the binding domain is formed by pairing of the VH and the VL from one peptide to the VL and VH of the other peptide thereby forming the LRP5/6 binding domain.

In the tetravalent binding antibody molecules of this invention both of the binding domains are bivalent and one or both of the bivalent binding domains may be bispecific for the respective FZD receptor, e.g., FZD4 or FZD5, or LRP5/6 co-receptor. For example, the

binding molecule may comprise an FZD binding domain that is bivalent and monospecific (each binding site binding to the same epitope) and the LRP 5/6 binding domain is bivalent and bispecific, binding to two different epitopes (the Wnt1 (E1-E2) and Wnt3 (E3-E4) sites on the LRP5/6 ectodomain). In an embodiment of this invention both binding domains are
5 bivalent and bispecific, each binding domain binding to two different epitopes on their respective target FZD receptor or LRP 5/6 co-receptor.

The VH and VL domains of the FZD binding domain of the tetravalent molecules of this invention may comprise the three light chain CDRs and three heavy chain CDRs of a FZD source antibody, e.g. the FZD4 or FZD5, binding antibodies of Table 1, Table 2 or Table 6,
10 or three light chain CDRs and three heavy chain CDRs that are at least 50%, at least 55%, at least 60%, at least 75, at least 80%, at least 85%, at least 90%, at least at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identical to the CDRs of the FZD source antibody, e.g., the FZD4 antibodies of Table 1, Table 2 or Table 6, and still retain binding to the FZD or FZD5 receptor bound by the source antibody.

15 The VH and VL domains of the LRP5/6 co-receptor binding domain of the tetravalent molecules of this invention may comprise the three light chain CDRs and three heavy chain CDRs of an LRP5/6 co-receptor source antibody, e.g., the LRP5/6 binding antibodies of Table 3, Table 4 or Table 6, or three light chain CDRs and three heavy chain CDRs that are at least 50%, at least 55%, at least 60%, at least 75%, at least 80%, at least 85%, at least 90%, at
20 least 95%, at least 96%, at least 97%, at least 98% or at least 99% identical to the VH and VL of the Wnt co-receptor source antibody, e.g., the LRP5/6 binding antibodies of Table 3, Table 4 or Table 6, and still bind to the LRP5/6 co-receptor.

In an embodiment of this invention the FZD binding domain of the tetravalent binding molecule of this invention binds FZD4 (an FZD4 Agonist) or FZD5 (FZD5 Agonist) or FZD4
25 and/or FZD5 and one or more other FZDs (a pan-FZD Agonist) and comprises

the CDR-H1, CDR-H2 and CDR-H3 and the CDR-L1, CDR-L2 and CDR-L3 of the antibodies of Table 1, Table 2 or Table 6, or CDRs that are at least 50%, at least 55%, at least 60%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identical to the CDR-H1, CDR-H2 and CDR-H3 and CDR-
30 L1, CDR-L2 and CDR-L3 of the antibodies of Table 1, Table 2 or Table 6, and still bind to FZD4 or FZD5,

and the LRP5/6 binding domain of the FZD4 Agonist or FZD5 Agonist or pan-FZD Agonist comprises

the CDR-H1, CDR-H2 and CDR-H3 and CDR-L1, CDR-L2 and CDR-L3 of the antibodies of Table 3, Table 4 or Table 6 or the CDRs are at least 50%, at least 55%, at least 60%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identical to the CDR-H1, CDR-H2 and CDR-H3 and CDR-L1, CDR-L2 and CDR-L3 of the antibodies in Table 3, Table 4 or Table 6, and still bind to LRP5 or LRP6.

In an embodiment, the tetravalent binding antibody molecule's FZD binding domain does not comprise a diabody, scFv, or Fab comprising the three heavy chain CDRs or three light chain CDRs of the FZD4-binding antibody 5044 in combination with a Wnt co-receptor binding domain comprising a diabody, scFv, or Fab comprising the three heavy chain CDRs and three light chain CDRs of LRP6-binding antibody 2542 and/or antibody 2539. In an embodiment, the tetravalent binding molecule does not comprise a diabody, scFv, or Fab, comprising the three heavy chain CDRs and three light chain CDRs of the FZD4-binding antibody 5027 in combination with a Wnt co-receptor binding domain comprising a diabody, scFv, or Fab comprising the three heavy chain CDRs and three light chain CDRs of LRP6-binding antibody 2542 and/or antibody 2539.

Also, an embodiment of this invention are the nucleic acid molecules encoding the tetravalent binding molecules described herein. An embodiment of this invention are the nucleic acid molecules encoding the polypeptides of the tetravalent binding molecules described herein comprising the heavy chain and light chain CDRs set forth in Tables 1, 2, 3, 4, 6. Also an embodiment of this invention are the nucleic acid molecules that encode the polypeptides of the tetravalent binding molecules, e.g., FZD5 Agonists or FZD4 Agonists, of Figure 7A and 7B that comprise the CDRs of Table 6. Also, an embodiment of this invention are the nucleic acid molecules that encode VH and VL domains comprising respectively the heavy chain and light chain CDRs set forth in Tables 1, 2, 3, 4, and 6. The nucleic acid molecules can be inserted into a vector and expressed in an appropriate host cell and then the tetravalent binding antibody molecules may be isolated from the cells using methods well known in the art. As such, also an aspect of this invention are expression cassettes and vectors comprising the nucleic acid molecules that encode the polypeptides of the tetravalent binding molecules, e.g., FZD4 or FZD5 Agonists, described herein, the VL and VH domains, the Fabs and the

diabodies comprising the CDRs of set forth in Tables 1, 2, 3, 4, and 6, and the Fc domains described herein. An aspect of this invention are the host cells expressing these expression cassettes and vectors.

As used in this invention, the term "vector" refers to a nucleic acid delivery vehicle or
5 plasmid that can be engineered to contain a nucleic acid molecule, e.g., a nucleic acid sequence encoding the tetravalent binding antibody molecules described herein. The vector that can express protein when inserted with a polynucleotide is called an expression vector. Vectors can be inserted into the host cell by transformation, transduction, or transfection, so that the carried genetic substances can be expressed in the host cell. Vectors are well known
10 to the technical personnel in the field, including but not limited to: plasmid; phagemid; cosmid; artificial chromosome such as yeast artificial chromosome (YAC), bacterial artificial chromosome (BAC), or P1 derived artificial chromosome (PAC); phage such as λ phage or M13 phage and animal viruses etc. Animal viruses may include but not limited to, reverse transcriptase virus (including lentivirus), adenovirus, adeno-associated virus, herpes virus (e.
15 g. herpes simplex virus), chicken pox virus, baculovirus, papilloma virus, and papova virus (such as SV40). A vector can contain multiple components that control expression of the tetravalent binding antibody molecules described herein, including but not limited to, promoters, e.g., viral or eukaryotic promoters, e.g., a CMV promoter, signal peptides, e.g., TRYP2 signal peptide, transcription initiation factor, enhancer, selection element, and
20 reporter gene. In addition, the vector may also contain replication initiation site(s).

As used in this invention, the term "host cell" refers to cells that can import expression cassettes and vectors, including but not limited to, prokaryotic cells such as Escherichia coli and Bacillus subtilis, fungal cells such as yeast and Aspergillus, insect cells such as S2
drosophila cells and Sf9, or animal cells, including human cells, e.g., fibroblast cells, CHO
25 cells, COS cells, NSO cells, HeLa cells, BHK cells, or HEK293 cells.

An embodiment of this invention is a pharmaceutical composition comprising a FZD Agonist or a nucleic acid molecule, expression cassette and vector encoding a FZD Agonist described herein and a pharmaceutically acceptable carrier, diluent or excipient. The pharmaceutical composition may further comprise an additional agent, e.g., a second therapeutic antibody
30 e.g. an anti-VEGF antibody (aflibercept, ranibizumab and bevacizumab), a growth factor, e.g., VEGF, or an agent that activates a Wnt pathway, e.g., the small molecule CHIR99021, a Norrin or R-Spondin, or a nucleic acid molecule, expression cassettes and vectors that encode

the agent. The pharmaceutical composition may consist of or consist essentially of a FZD Agonist, or a nucleic acid molecule, an expression cassette or vector encoding an FZD Agonist described herein, and a pharmaceutically acceptable diluent, carrier or excipient. Suitable carriers, diluents and excipients, and their formulations are described in Remington:

- 5 The Science and Practice of Pharmacy (19th ed.) ed. A. R. Gennaro, Mack Publishing Company, Easton, Pa. 1995. Typically, an appropriate amount of a pharmaceutically-acceptable salt is used in the formulation to render the formulation isotonic. Examples of the pharmaceutically-acceptable carrier include, but are not limited to, saline, Ringer's solution and dextrose solution. The pH of the solution may be e.g., from about 5 to about 8, from
10 about 5 to 7.5 or from about 6 to 7. Further carriers include sustained release preparations such as semipermeable matrices of solid hydrophobic polymers containing the agonist, which matrices are in the form of shaped articles, e.g., films, liposomes or microparticles. It will be apparent to those persons skilled in the art that certain carriers may be more preferable depending upon, for instance, the route of administration and concentration of the FZD
15 Agonists being administered.

- This invention also includes methods for using the FZD Agonists, described herein. An embodiment of this invention is a method for activating a Wnt signaling pathway in a cell, comprising contacting a cell having an FZD receptor and a LRP5/6 co-receptor, with a tetravalent binding antibody molecule of this invention that binds the FZD, e.g., FZD4, and
20 the LRP5/6 in an amount effective to activate Wnt signaling. It has been reported that the Norrin-FZD4 pathway plays a role in retinal angiogenesis (see Wang et al. Cell. 2012;151(6):1332–1344.; Braunger BM, Tamm ER. Adv Exp Med Biol. 2012; 723:679–683; Ohlmann A, Tamm ER. Prog Retin Eye Res. 2012;31(3):243–257 and; Ye et al. Trends Mol Med. 2010;16(9):417–425). Signaling through Norrin-FZD4 pathway is necessary for
25 development and maintenance of retinal vasculature. Mutations affecting genes of this pathway may result in several vitreoretinopathies, such as Norrie Disease, Familial Exudative Vitreoretinopathy (FEVR), and Pseudoglioma and Osteoporosis Syndrome. Additionally, Retinopathy of Prematurity (ROP) has been associated with mutations in this Norrin-FZD4 pathway, and Wnt-pathway mutations have been reported in Coats Disease and Persistent
30 Fetal Vasculature (PFV). FZD4 signaling activated by Norrin and/or WNT7A/B pathway is also associated with CNS blood brain barrier development and homeostasis. Genetic ablation of the Norrin, *FZD4*, *LRP5*, *LRP6* and the co-receptor Tetraspanin-12 (Tspan-12) result in defective angiogenesis and barrier disruption in the retinal and/or cerebellar vessels (Cho et

al. (2017) Neuron 95, 1056-1073; Zhou et al., (2014) J Clin Invest 124:3825–3846). Thus, a functional Wnt signaling system plays a key fundamental role in the development of a sufficient vascular and neural network in the eye and retina to support vision and in the CNS to support BBB development and homeostasis.

- 5 An aspect of this invention is a method for promoting and/or maintaining retinal vasculature by treating eye tissue, e.g., retinal tissue, with an effective amount of a pharmaceutical compositions comprising the tetravalent antibody molecules of this invention, e.g., tetravalent antibody molecules that binds FZD4 and LRP5/6, a FZD4 Agonists, having the structures illustrated in Figure 6 through local or systemic administration. Also, an aspect of this
- 10 invention is a method for promoting and/or maintaining BBB vasculature by treating a subject in need thereof with an effective amount of a pharmaceutical compositions of this invention, e.g., a composition comprising a FZD4 Agonists having the structures depicted in Figure 6. The BBB is initiated during development and its integrity remains vital for homeostasis and neural protection throughout life. A subject in need thereof includes a
- 15 subject having a neurological condition associated with BBB dysfunction, e.g., neurodegenerative diseases such as Alzheimer's disease, as well epilepsy, multiple sclerosis, and stroke.

- A further aspect of this invention is a method for treating a subject having a disorder characterized by vascular leakage, particularly retinal vascular leakage, and/or endothelial
- 20 cell leakage, and disorders characterized by reduced retinal or brain endothelial cell barrier functions or a compromised BBB or BRB, e.g., diabetic retinopathy, retinopathy of prematurity, Coat's disease, FEVR, Norrie disease, macular degeneration, diabetic macular edema, and pediatric vitreoretinopathies, by administering to such subject an effective amount of a pharmaceutical compositions of this invention, e.g., a composition comprising a
- 25 FZD4 Agonist having the structures depicted in Figure 6. An effective amount of such composition is an amount sufficient, e.g., to increase or restore endothelial cell barrier functions and thereby reducing vascular leakage in such subject. The subject may be a fetus. The FZD4 Agonists of this invention particularly the FZD4 Agonist in the diabody-Fc-Fab format comprising two Fab fragments forming the FZD4 binding domain on the carboxy
- 30 terminal of the Fc receptor and a binding domain for LRP5 and/or LRP6 composed of a diabody on the amino terminal of the Fc domain, e.g., as illustrated in Figure 6, activates FZD4 and β -catenin signaling in endothelial cells, promotes barrier functions and thereby

reduces endothelial cell permeability and significantly enhance angiogenesis. In particular, treatment of endothelial cells, in vivo, ex vivo or in vitro, with these FZD4 Agonists, preferably those with the diabody-Fc-Fab format, enhance the development and maintenance of retinal vasculature and/or the BRB and the BBB far more effectively than other molecules
5 that do not have this structure.

A further aspect of the invention is a method for treating a subject having inflammation of all or part of the intestines, also known as inflammatory bowel disease, by administering to such subject an effective amount of a pharmaceutical composition of this invention, e.g., a composition comprising a FZD5 Agonist. Examples of inflammatory bowel disease include,
10 but are not limited to, Crohn's disease, and ulcerative colitis. An effective amount of such composition is an amount sufficient to reduce, ameliorate, eliminate, or treat the inflammation. A subject in need thereof includes a subject having inflammation of the mucosal of the gastrointestinal tract. The methods disclosed herein may be practiced to reduce inflammation (e.g., inflammation associated with IBD or in a tissue affected by IBD,
15 such as gastrointestinal tract tissue, e.g., small intestine, large intestine, or colon), activate WNT signaling, or reduce any of the histological symptoms of IBD (e.g., those disclosed herein).

The FZD Agonists of the present invention may be administered systemically or locally, e.g., by injection (e.g. subcutaneous, intravenous, intraperitoneal, intrathecal, intraocular,
20 intravitreal, etc.), implantation, topically, or orally. Depending on the route of administration, the FZD Agonists may be coated in a material to protect the agonists from conditions that may inactivate the agonists. The tetravalent binding antibody molecules described herein may be dissolved or suspended in a pharmaceutically acceptable, preferably aqueous carrier. In addition, the composition comprising the FZD Agonists can contain
25 excipients, such as buffers, binding agents, blasting agents, diluents, flavors, lubricants, etc. An extensive listing of excipients that can be used in such a composition, can be, for example, taken from A. Kibbe, Handbook of Pharmaceutical Excipients (Kibbe, 2000). The tetravalent binding antibody molecules can also be administered together with immune stimulating substances, such as cytokines.

30 An embodiment of this invention includes a method for deriving cerebral organoids with a vascular network exhibiting barrier functions by using the tetravalent antibody molecules described herein. The tetravalent binding antibody molecules described herein that activate

FZD4 signaling are envisioned to promote barrier function within endothelial cells cultured with cerebral organoids and thereby promoting angiogenesis.

An embodiment of this invention includes a method for directed differentiation of multipotent or pluripotent stem cells (PSC) or induced pluripotent stem (iPS) cells comprising culturing the cells under conditions suitable for directed differentiation wherein said culturing conditions further comprise an effective amount of a tetravalent binding antibody molecule described herein. Studies in mouse and human PSCs have identified specific approaches to the addition of growth factors, including Wnt, which can induce PSC differentiation into different lineages. Methods for directed differentiation of PSCs comprising the activation of Wnt signaling are known in the art see e.g. Lam et al. (2014) Semin Nephrol 34(4); 445-461; Yucer et al. (September 6, 2017) Scientific Reports 7, Article number 10741. It is contemplated that the FZD Agonists, e.g. FZD4 Agonists, described herein can be used in an amount sufficient to effect activation of Wnt signaling pathways to direct differentiation of the PSCs to certain mesodermal lineages such as cardiomyocytes (cite Yoon et al. FZD4 Marks Lateral Plate Mesoderm and Signals with NORRIN to Increase Cardiomyocyte Induction from Pluripotent Stem Cell-Derived Cardiac Progenitors. Stem Cell Reports. 2018 Jan;10(1):87-100. DOI: 10.1016/j.stemcr.2017.11.008.PMID: 29249665).

An embodiment of this invention is a method for enhancing tissue regeneration in a subject in need thereof by activating Wnt signaling in such subject by administering to the subject in need thereof an effective amount of a FZD Agonists described herein.

An embodiment of this invention includes a method for promoting endothelial cell barrier functions in eye tissue, e.g., retinal tissue, in a subject in need thereof, by administering an effective amount of a tetravalent binding molecule of this invention that binds FZD4 and LPR5/6, an FZD4 Agonist. In a particular embodiment the FZD4 Agonist of this invention that binds to FZD4 and a binding domain that binds to LRP5 or/and LRP6 has a diabody-Fc-Fab structure depicted in Figure 6 and 7. In an embodiment of this invention the FZD4 Agonists for enhancing retinal angiogenesis comprise the light chain CDRs, i.e., CDR-L1, CDR-L2, and CDR-L3 and heavy chain CDRs, i.e., CDR-H1, CDR-H2 and CDR-H3 of the

FZD4-binding antibodies set forth in Tables 1, 2, and 6 and the LRP5/6-binding antibodies set forth in Tables 3, 4, and 6.

A subject as used herein may be any animal (e.g., a mammal), including, but not limited to, humans, non-human primates, horses, cows, dogs, cats, rodents, and the like. The subject
5 may be a fetus. Typically, the subject is human.

Effective dosages and schedules for administering the FZD Agonists and nucleic acids that encode them described herein may be determined empirically, and making such determinations is within the skill in the art. Those skilled in the art will understand that the dosage of such FZD Agonists that must be administered will vary depending on, for example,
10 the subject who will receive the antibody, the route of administration, the particular type of FZD Agonists used and other drugs being administered. Guidance in selecting appropriate doses for FZD Agonists is found in the literature on therapeutic uses of antibodies, e.g., Handbook of Monoclonal Antibodies, Ferrone, eds., Noyes Publications, Park Ridge, N.J., (1985) ch. 22 and pp. 303-357; Smith, Antibodies in Human Diagnosis and Therapy, Haber,
15 eds., Raven Press, New York (1977) pp. 365-389. The dosage ranges for the administration of the compositions are those large enough to produce the desired effect, e.g., promote endothelial cell barrier functions, vascular homeostasis, or enhance Wnt signaling. The dosage should not be so large as to cause adverse side effects, such as unwanted cross-reactions, anaphylactic reactions, and the like. Generally, the dosage will vary with the age,
20 condition, gender and the extent of the disease or disorder, in the patient and can be determined by one of skill in the art. The dosage can be adjusted by the individual physician in the event of any contraindications. Dosage can vary, and can be administered in one or more dose administrations daily, for one or several days. While individual needs vary, determination of optimal ranges of effective amounts of the vector is within the skill of the
25 art.

Also, an aspect of this invention is a method for making the tetravalent binding antibody molecules described herein. The amino acid sequences of FZD receptors, e.g. FZD4, and the Wnt co-receptors LRP5/6, and nucleotide sequences encoding FZD receptors and the Wnt co-receptors LRP5/6, as well as antibodies and libraries of antibodies that bind FZD, e.g., FZD4,
30 or the Wnt co-receptors LRP5/6, are readily available or can be generated using methods well known in the art (see e.g., U.S. publication no. 2015/0232554, inventors Gurney et al. and US publication no. 2016/0194394, inventors Sidhu et al. and US 20190040144, inventors Pan et

al.; U.S. publication no. 2017/0166636, inventors Wu et al.; U.S. publication no. 2016/0208018, inventors Chen et al.; U.S. publication no. 2016/0053022, inventors Macheda et al.; U.S. publication no. 2015/031293, inventors Damelin et al.). And a variety of methods are known in the art for generating and screening such phage display libraries for antibodies, and antibody fragments, scFv, Fab, VL, and VH possessing the desired binding characteristics. Such methods are reviewed, e.g., in Hoogenboom et al. in *Methods in Molecular Biology* 178:1-37 (O'Brien et al., ed., Human Press, Totowa, N.J., 2001) and further described, e.g., in the McCafferty et al., *Nature* 348:552-554; Clackson et al., *Nature* 352: 624-628 (1991); Marks et al., *J. Mol. Biol.* 222: 581-597 (1992); Marks and Bradbury, in *Methods in Molecular Biology* 248:161-175 (Lo, ed., Human Press, Totowa, N.J., 2003); Sidhu et al., *J. Mol. Biol.* 338(2): 299-310 (2004); Lee et al., *J. Mol. Biol.* 340(5): 1073-1093 (2004); Fellouse, *Proc. Natl. Acad. Sci. USA* 101(34): 12467-12472 (2004); and Lee et al., *J. Immunol. Methods* 284(1-2): 119-132(2004), all incorporated herein by reference. In certain phage display methods, repertoires of VH and VL genes are separately cloned by polymerase chain reaction (PCR) and recombined randomly in phage libraries, which can then be screened for antigen-binding phage as described in Winter et al., *Ann. Rev. Immunol.*, 12: 433-455 (1994). Phage typically display antibody fragments, either as single-chain Fv (scFv) fragments or as Fab fragments. Libraries from immunized sources provide high-affinity antibodies to the immunogen without the requirement of constructing hybridomas.

Alternatively, the naive repertoire can be cloned (e.g., from human) to provide a single source of antibodies to a wide range of non-self and also self antigens without any immunization as described by Griffiths et al., *EMBO J*, 12: 725-734 (1993). Finally, naive libraries can also be made synthetically by cloning unrearranged V-gene segments from stem cells, and using PCR primers containing random sequence to encode the highly variable CDR3 regions and to accomplish rearrangement in vitro, as described by Hoogenboom and Winter, *J. Mol. Biol.*, 227: 381-388 (1992). Patent publications describing human antibody phage libraries include, for example: U.S. Pat. No. 5,750,373, and US Patent Publication Nos. 2005/0079574, 2005/0119455, 2005/0266000, 2007/0117126, 2007/0160598, 2007/0237764, 2007/0292936, and 2009/0002360, all incorporated herein by reference. Antibodies or antibody fragments

isolated from human antibody libraries are considered human antibodies or human antibody fragments herein.

In an embodiment of this invention a tetravalent binding antibody molecule in a diabody-Fc-scFv format comprising a LRP5/6 coreceptor binding domain comprising LRP5/6 -binding
5 diabody and an FZD-binding domain comprising two FZD-binding scFvs is generated by,

- (a) selecting an Fc domain having a C-terminus and an N-terminus
- (b) identifying an antibody that binds to an FZD receptor (the “FZD source antibody”), and
- (c) identifying an antibody that binds LRP 5/6 co-receptor (“coreceptor source
10 antibody” or “LRP 5/6 source antibody”),
- (d) generating a nucleic acid molecule comprising a nucleotide sequence that encodes a polypeptide monomer comprising
 - (i) a peptide comprising a VL domain linked to a VH domain, the domains comprising the heavy chain and/or light chain CDRs of the antibody of step
15 b that bind the FZD receptor, or comprising heavy chain and/or light chain CDRs derived from the antibody of step b that still bind the FZD, linked to
 - (ii) an Fc domain of step a, linked to
 - (iii) a peptide comprising VL domain linked to a VH domain comprising the light chain and/or heavy chain CDRs of the antibody of step c, or
20 comprising CDRs derived from the antibody of step c and that still bind LRP 5/6 co-receptor,
- (e) expressing the nucleic acid molecule of step d to produce the polypeptide monomer and then dimerizing the polypeptide,

wherein the VH and VL that bind the FZD of each monomer form a scFv that binds FZD, and
25 the VH and VL domains that bind the LRP 5/6 coreceptor of one monomer bind the VL and VH that binds the Wnt coreceptor of another monomer forming a LRP5/6 co-receptor-binding diabody, and

wherein the polypeptide monomer dimerizes via the Fc regions to form a tetravalent binding antibody molecule comprising an Fc domain, a FZD-binding domain comprised of two FZD-binding scFvs, and a LRP5/6 coreceptor binding domain comprised of the diabody,

wherein the FZD binding domain and the LRP5/6 co-receptor binding domain are on opposite termini of Fc domain. It is contemplated that the peptides comprising the VL and VH domains that bind the FZD or the LRP may be linked to either the N or C terminus of the Fc domain via the VL domain or the VH domain provided the FZD binding domain and LRP binding domain are on opposite termini of the Fc domain. The FZD may be one or more of FZD1, FZD2, FZD3, FZD4, FZD5, FZD6, FZD7, FZD8, FZD9, and FZD10.

In an embodiment of this invention, the tetravalent binding antibody molecule has two FZD-binding Fabs, e.g., FZD4-binding Fabs, linked to one terminus of the Fc domain and two LRP5/6-binding scFvs or a LRP5/6-binding diabody linked to the other terminus of the Fc domain and is generated by,

(a) identifying the light chain complementary determining regions (CDR-L1, CDR-L2, and CDR-L3) and/or heavy chains complementary determining regions (CDR-H1, CDR-H2, and CDR-H3) of an antibody that binds to the FZD, e.g., FZD4 or FZD5, (the “FZD source antibody”) and

(b) identifying the CDR-L1, CDR-L2, and CDR-L3 and/or the CDR-H1, CDR-H2, and CDR-H3 of one or more antibodies that binds to LRP5 or LRP6, (the “LRP5/6 source antibody”),

(c) generating a nucleic acid molecule encoding a “heavy chain” polypeptide comprising

(i) a peptide comprising an immunoglobulin constant heavy chain region 1 (CH1 domain) linked to a VH domain comprising the CDR-H1, H2 and H3 of the antibody of step a), or a CDR-H1, CDR-H2 and CDR-H3 derived from the antibody of step a) that still binds the FZD4, linked to

(ii) an Fc region, linked to

(iii) a peptide comprising a VL domain comprising the CDR-L1, CDR-L2 and CDR-L3 of an antibody of step b) linked to a VH domain comprising

the CDR-H1, CDR-H2 and CDR-H3 of an antibody of step b), or CDR-H1, CDR-H2 and CDR-H3 derived from the antibody of step b) that binds to LRP5 or LRP6,

5 (d) generating a nucleic acid molecule comprising a nucleic acid sequence that encodes a "light chain" polypeptide comprising an immunoglobulin constant light region 1 (CL1) linked to a VL domain wherein the VL domain comprises the FZD light chain CDR-L1, CDR-L2 and CDR-L3 of the antibody in step a),

(e) expressing the nucleic acid molecules of (c) and (d) to produce the heavy chain polypeptide and the light chain polypeptide,

10 wherein two heavy chain polypeptides dimerize via their Fc regions and the VH that binds the FZD and CH1 domains of the heavy chain polypeptide pair with the VL that binds the FZD and CL1 domains of the light chain polypeptide forming two FZD Fabs and

wherein the VH and VL that binds LRP5/6 in each heavy chain polypeptide pair to form an scFv that binds LRP5/6, or the VH and VL that bind LRP5/6 of one heavy chain polypeptide
15 in the dimer pair with the VL and VH that bind the LRP5/6 of the other heavy chain polypeptide in the dimer to form a diabody, thereby forming the tetravalent binding antibody molecule comprising an Fc domain, two FZD Fabs linked to either the N or C terminus of the Fc domain and two LRP5/6-binding scFvs or a LRP5/6-binding diabody linked to the other terminus of the Fc domain.

20 The FZD source antibody may be an antibody that binds specifically to one FZD, e.g., FZD4, or is a pan-specific antibody binding FZD, e.g., FZD4 or FZD5, and one or more other FZD receptors and antagonizes Wnt signaling or inhibits Wnt binding to the receptor.

Alternatively, the FZD source antibody may be an antibody that binds specifically to one FZD, e.g., FZD4 or FZD5, or is a pan-specific antibody binding one FZD, e.g., FZD4 or
25 FZD5, and one or more other FZD receptors without antagonizing Wnt signaling or inhibiting Wnt binding to the receptor. The LRP source antibody may be an antibody that binds specifically to LRP5/6, or is panspecific binding to LRP5/6 and to one or more of the Wnt co-receptors, and antagonizes Wnt signaling or inhibits Wnt binding to the co-receptor.

Alternatively, the LRP5/6 source antibody may be an antibody that binds to the LRP 5/6 co-

receptor, or is panspecific binding to LRP5/6 and to one or more of the Wnt co-receptors, without antagonizing Wnt signaling or inhibiting Wnt binding to the LRP5/6 co-receptor.

The FZD source antibody may be an antibody fragment that binds the FZD receptor, e.g., an Fab, a VL or VH. The light chain and heavy chain CDRs, the VH and/or VL in the FZD binding domain of the FZD Agonists may be identical to the CDRs, the VH and/or VL of the FZD source antibody or may be at least 50%, at least 55%, at least 60%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identical to the CDRs, VH or VL of the source antibody and still retain binding to the FZD receptor. The CDRs, the VH and/or VL in the FZD binding domain of the FZD Agonists may be identical to the CDRs, the VH and/or VL of a FZD4-binding or FZD5-binding antibody of Table 1, Table 2 or Table 6, or may be at least 50%, at least 55%, at least 60%, 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identical to the CDRs, VH or VL of a FZD4-binding or FZD5-binding antibody of Table 1 or Table 2 or Table 6 and still retain binding to the FZD receptor.

Likewise, the Wnt co-receptor source antibody may be an antibody fragment, e.g. an Fab, a VL or a VH, that binds the LRP co-receptor, e.g., LRP5/6. The light chain CDRs and heavy chain CDRs, the VH and/or VL in the Wnt co-receptor binding domain of the FZD4 Agonists may be identical to the CDRs, the VH and/or VL of the Wnt co-receptor source antibody or may be at least at least 50%, at least 55%, at least 60%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identical to the CDRs, VHs or VLs of the source antibody and still retain binding to the LRP co-receptor. The light chain CDRs and heavy chain CDRs, the VH and/or VL in the LRP5/6 binding domain of the FZD Agonists may be identical to the light chain CDRs and heavy chain CDRs, the VH and/or VL of a LRP-binding antibody of Table 3, Table 4 or Table 6 or may be at least at least 50%, at least 55%, at least 60%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identical to the light chain CDRs and heavy chain CDRs, VH or VL of a LRP-binding antibody of Table 3, Table 4 or Table 6 and still retain binding to the LRP co- receptor.

In an embodiment of this invention, two polypeptides of the tetravalent binding antibody molecule dimerize via knob-in-hole configuration of their Fc sequences. The tetravalent binding antibody molecules of this invention may be generated by dimerizing two

polypeptides in a “knob-in-hole” configuration. The knob-in-hole configuration increases the modularity of this invention by facilitating the association of peptides that comprise binding moieties that bind different epitopes on a FZD receptor or LRP5/6 co-receptor or to epitopes on different members of the FZD receptor or co-receptor family, see e.g., Figure 6. Methods
5 for engineering Fc molecules via the knobs into holes design are well known in the art, see e.g., WO2018/026942, inventors Van Dyk et al., Carter P. (2001) *J. Immunol. Methods* 248, 7–15; Ridgway et al. (1996) *Protein Eng.* 9, 617–621; Merchant, et al. (1998) *Nat. Biotechnol.* 16, 677–681, and; Atwell et al., (1997) *J. Mol. Biol.* 270, 26–35.

Without wishing to be bound by theory, it is contemplated that the tetravalent binding
10 antibody molecules of this invention facilitate the interaction of a FZD receptor and an LRP5/6 co-receptor on a cell by promoting their proximity and stabilizing conformations of the receptor proteins that are favorable for activating Wnt signaling pathways. Another embodiment of this invention is a method for facilitating the interaction of a FZD receptor and an LRP5/6 co-receptor on a cell thereby activating a Wnt signaling pathway in the cell
15 comprising, a) selecting an Fc domain, or fragment thereof comprising a CH3 domain, having a C-terminus and an N-terminus b) linking a first bivalent binding domain, which binds the FZD receptor, on one terminus of the Fc domain and linking a second bivalent binding domain, which binds to the Wnt co-receptor, on the other terminus of the Fc domain thereby forming a tetravalent binding antibody molecule; c) contacting said tetravalent
20 binding antibody molecule with the cell expressing said FZD receptor and Wnt co-receptor under conditions wherein the FZD receptor and co-receptor both bind to the tetravalent binding antibody molecule thereby activating the Wnt signaling pathway. The Wnt co-receptor binding domain and FZD binding domain are bivalent and each comprise a VL and/or a VH, or V_HH domain and one or both of the binding domains may be monospecific.
25 In an embodiment of the invention one or both the Wnt co-receptor binding domain and FZD binding domain are bispecific. In an embodiment of the invention the Wnt co-receptor binding domain is bivalent and bispecific. The FZD binding domain may comprise a scFV that binds FZD, a V_HH that binds FZD, or an Fab that binds FZD, or combinations thereof, or a diabody that binds FZD. The Wnt co-receptor binding domain may comprise a scFV that
30 binds the LRP5/6 co-receptor, a V_HH that binds LRP5/6, an Fab that binds the LRP5/6 co-receptor, or combinations thereof, or a diabody that binds the LRP5/6 co-receptor. In an embodiment of the invention the FZD binding domain comprises two FZD-binding Fabs and

the Wnt co-receptor binding domain comprises a bispecific bivalent diabody that binds LRP5/6 on two different epitopes.

The tetravalent binding antibody molecules of this invention initiate the Wnt signaling pathway(s) that are stimulated by the FZD-co-receptor complexes, e.g., the β -catenin pathway stimulated by FZD-LRP5/6 complexes. Wnt ligands function by promoting the clustering of FZD receptors with co-receptors. Without wishing to be bound by theory, it is contemplated that the FZD Agonists described herein bind both the FZD receptor and its LRP5/6 co-receptor thereby forming a complex that mimics the binding of a Wnt molecule to the FZD receptor and LRP 5/6 co-receptor(s), which in turn activates Wnt signaling pathways, the Wnt β -catenin pathway.

An embodiment of this invention is a method for activating a Wnt signaling pathway comprising contacting a cell expressing a FZD receptor and its LRP5/6 co-receptor with an effective amount of the FZD Agonists of this invention comprising a FZD binding domain and a LRP5/6 co-receptor binding domain.

The FZD Agonists of this invention may be made recombinantly, e.g., by Gibson assembly (see Gibson et al. (2009) Nature Methods 6 (5): 343–345 and Gibson DG. (2011) Methods in Enzymology 498: 349–361), or the molecules may be made synthetically e.g., using commercial synthetic apparatuses, for example, automated synthesizers by Applied Biosystems, Inc., Beckman, etc. By using synthesizers, naturally occurring amino acids may be substituted with unnatural amino acids. The particular sequence and the manner of preparation will be determined by convenience, economics, purity required, and the like. If desired, various groups may be introduced into the peptide during synthesis or during expression, which allow for linking to other molecules or to a surface.

The binding domains of the FZD Agonists may be linked to the Fc domain via a linker. In some embodiments, adjacent VH and VL domains may be attached to each other via a peptide linker. In some embodiments adjacent constant domains and variable domains are attached via a peptide linker. The linker may be, e.g. a polypeptide linker, or a non-peptidic linker. In some embodiments the constant domains and variable domains of the FZD Agonists are attached to the Fc domain via a peptide linker. Suitable linkers are well known in the art, e.g., an XTEN linker (see WO2013120683, inventors Schellenberger et al.) In some embodiments, the peptide linker comprises at least 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, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or at least 100 amino acids. In some embodiments, the peptide linker is between 1 to 100, 5 to 75, 1 to 50, 5 to 50, 1 to 30, 1 to 25, 5 to 25, 5 to 20, 5 to 15, 5 to 10, 1-10 or 1-5 amino acids in length. The modular aspects of this invention allow for mixing and matching of binding domains derived from antibodies that bind to FZD receptor or antibodies that bind LRP5/6 co-receptor on the opposite termini of the Fc domain to generate a tetravalent binding antibody molecule that can engage FZD receptor – LRP5/6 co-receptor complexes to activate Wnt signaling.

The Fc domain of the FZD Agonists, with or without the linker, is of a length and flexibility that allows for the tetravalent binding antibody molecule of this invention to bind both the FZD receptor and its LRP5/6 co-receptor thereby stabilizing receptor conformations that are compatible with activation of downstream Wnt signaling pathways. In an embodiment of this invention the Fc domain, or fragment thereof comprising the CH3 domain, with or without the linker is greater than 100 amino acids spanning up to 300Å, greater than 125 amino acids spanning up to 375Å, greater than 150 amino acids spanning up to 450Å, greater than 175 amino acids spanning up to 525Å, or greater than 300 amino acids spanning up to 900Å. Preferably the Fc domain is about 200 amino acids to about 300 amino acids in length.

As used herein and in the appended claims, the singular forms "a", "an", and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a cell" includes a plurality of such cells and reference to "the peptide" includes reference to one or more peptides and equivalents thereof, e.g. polypeptides, known to those skilled in the art, and so forth.

An "affinity matured" antibody or "maturation of an antibody" refers to an antibody with one or more alterations in one or more hypervariable regions (HVRs), compared to a parent or source antibody which does not possess such alterations, such alterations resulting in an improvement in the affinity of the antibody for antigen or to other desired properties of the molecule.

By "comprising" it is meant that the recited elements are required in the composition/method/kit, but other elements may be included to form the

composition/method/kit etc. within the scope of the claim. For example, a composition comprising tetravalent binding antibody molecules is a composition that may comprise other elements in addition to the tetravalent binding antibody molecules, e.g. functional moieties such as polypeptides, small molecules, or nucleic acids bound, e.g. covalently bound, to the tetravalent binding antibody molecules; agents that promote the stability of the tetravalent binding antibody molecule composition, agents that promote the solubility of the tetravalent binding antibody molecule composition, adjuvants, etc. as will be readily understood in the art, with the exception of elements that are encompassed by any negative provisos.

By "consisting essentially of", it is meant a limitation of the scope of composition or method described to the specified materials or steps that do not materially affect the basic and novel characteristic(s) of the subject invention. For example, a tetravalent binding antibody molecule "consisting essentially of" a disclosed sequence has the amino acid sequence of the disclosed sequence plus or minus about 5 amino acid residues at the boundaries of the sequence based upon the sequence from which it was derived, e.g. about 5 residues, 4 residues, 3 residues, 2 residues or about 1 residue less than the recited bounding amino acid residue, or about 1 residue, 2 residues, 3 residues, 4 residues, or 5 residues more than the recited bounding amino acid residue.

By "consisting of", it is meant the exclusion from the composition, method, or kit of any element, step, or ingredient not specified in the claim. For example, a tetravalent binding antibody molecule "consisting of" a disclosed sequence consists only of the disclosed amino acid sequence.

Where a range of values is provided, it is understood that each intervening value, to the tenth of the unit of the lower limit unless the context clearly dictates otherwise, between the upper and lower limits of that range is also specifically disclosed. Each smaller range between any stated value or intervening value in a stated range and any other stated or intervening value in that stated range is encompassed within the invention. The upper and lower limits of these smaller ranges may independently be included or excluded in the range, and each range where either, neither or both limits are included in the smaller ranges is also encompassed within the invention, subject to any specifically excluded limit in the stated range. Where the

stated range includes one or both of the limits, ranges excluding either or both of those included limits are also included in the invention.

The basic antibody structural unit is known to comprise a tetramer. Each tetramer is composed of two identical pairs of polypeptide chains, each pair having one "light" (about 25 kDa) and one "heavy" chain (about 50-70 kDa). The amino-terminal portion of each chain includes a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. The carboxy-terminal portion of each chain defines a constant region primarily responsible for effector functions, e.g., binding Fc receptors and activation of antibody-dependent cellular cytotoxicity (ADCC) and complement dependent cytotoxicity (CDC). Methods for dimerizing peptides via a knob-in-hole configuration are described in WO2018/026942, inventors Van Dyk et al., Carter P. (2001) *J. Immunol. Methods* 248, 7–15; Ridgway et al. (1996) *Protein Eng.* 9, 617–621; Merchant, et al. (1998) *Nat. Biotechnol.* 16, 677–681, and; Atwell et al., (1997) *J. Mol. Biol.* 270, 26–35. The Fc regions may be Merrimack (knob chain: Q347M, Y349F, T350D, T366W and L368M; hole chain: S354I, E357L, T366S, L368A and Y407V), Merchant (knob chain: T366W; hole chain: T366S, L368A and Y407V) or Merchant S:S (Merchant mutations with additional S354C variant in the knob chain and Y349C in the hole chain). The Fc regions may also contain mutations that alter their effector function, e.g., the Fc region may have attenuated effector functions due to amino acid mutations, e.g., DANG variants and LALAPS variants. Methods are well known in the art for mitigating antibody effector function, including for example amino acid substitutions in the Fc regions, e.g., the N297G and D265A, N297G (DANG) variants, L234A, L235A, P331S (LALAPS), LALAPS Merchant, LALAPS Merchant S-S (Merchant A.M. et al *Nature Biotechnol* 1998 vol 16 p677-681) variants, or L234A, L235A, P329G (LALA-PG) substitutions, see e.g., Lo et al. "Effector Attenuating Substitutions that Maintain Antibody Stability and Reduce Toxicity in Mice. *The Journal of Biological Chemistry* Vol. 292, No. 9, pp. 3900–3908, March 3, 2017, incorporated herein by reference. In general, antibody molecules obtained from humans relate to any of the classes IgG, IgM, IgA, IgE and IgD, which differ from one another by the nature of the heavy chain present in the molecule. Certain classes have subclasses as well, such as IgG₁, IgG₂, and others. Furthermore, in humans, the light chain may be a kappa chain or a lambda chain.

Three highly divergent stretches within each of the heavy chain variable domain, VH or VH domain, and light chain variable domain, VL or VL domain, referred to as complementarity

determining regions (CDRs), are interposed between more conserved flanking stretches known as "framework regions", or "FRs". Thus, the term "FR" refers to amino acid sequences which are naturally found between, and adjacent to, CDRs in immunoglobulins. A VH domain typically has four FRs, referred to herein as VH framework region 1 (FR1), VH framework region 2 (FR2), VH framework region 3 (FR3), and VH framework region 4 (FR4). Similarly, a VL domain typically has four FRs, referred to herein as VL framework region 1 (FR1), VL framework region 2 (FR2), VL framework region 3 (FR3), and VL framework region 4 (FR4). In an antibody molecule, the three CDRs of a VL domain (CDR-L1, CDR-L2 and CDR-L3) and the three CDRs of a VH domain (CDR-H1, CDR-H2 and CDR-H3) are disposed relative to each other in three-dimensional space to form an antigen-binding site within the antibody variable region. The surface of the antigen-binding site is complementary to a three-dimensional surface of a bound antigen. The amino acid sequences of VL and VH domains may be numbered, and CDRs and FRs therein identified/defined, according to the Kabat numbering system (Kabat et al., 1991, Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md.) or the INTERNATIONAL IMMUNOGENETICS INFORMATION SYSTEM (IMGT numbering system; Lefranc et al., 2003, Development and Comparative Immunology 27:55-77), both incorporated herein by reference. One of ordinary skill in the art would possess the knowledge for numbering amino acid residues of a VL domain and of a VH domain, and identifying CDRs and FRs therein, according to a routinely employed numbering system such as the IMGT numbering system, the Kabat numbering system, and the like.

The term "antibody" as referred to herein includes whole antibodies and any antigen binding fragment (i.e., "antigen-binding portion") or single chain thereof. A "whole antibody" or full-length refers to a glycoprotein comprising at least two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds, or an antigen binding portion thereof. Each heavy chain is comprised of a heavy chain variable region or domain (abbreviated herein as VH) and a heavy chain constant region. The heavy chain constant region is comprised of three domains, CH1, CH2 and CH3. Each light chain is comprised of a light chain variable region or domain (abbreviated herein as VL) and a light chain constant region. The light chain constant region is comprised of one domain, CL or CL1. The VH and VL regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDR), interspersed with regions that are more conserved, termed framework regions

(FR). Each VH and VL is composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. The variable regions of the heavy and light chains contain a binding domain that interacts with an antigen. The constant regions of the antibodies may mediate the binding of the immunoglobulin to host tissues or factors, including various cells of the immune system (e.g., effector cells) and the first component (C1q) of the classical complement system.

The term "antigen-binding portion" or "antigen-binding fragment" of an antibody (or simply "antibody portion" or "antibody fragment"), as used herein, refers to one or more fragments, portions or domains of an antibody that retain the ability to specifically bind to an antigen. It has been shown that fragments of a full-length antibody can perform the antigen-binding function of an antibody. Examples of binding fragments encompassed within the term "antigen-binding portion" of an antibody include (i) an Fab fragment, a monovalent fragment consisting of the VL, VH, CL1 and CH1 domains; (ii) an F(ab')₂ fragment, a bivalent fragment comprising two F(ab)' fragments linked by a disulfide bridge at the hinge region; (iii) an Fd fragment consisting of the VH and CH1 domains; (iv) an Fv fragment consisting of the VL and VH domains of a single arm of an antibody; (v) a dAb fragment (Ward et al. (1989) Nature 241:544-546), which consists of a VH domain; and (vi) an isolated complementary determining region (CDR). Furthermore, although the two domains of the Fv fragment, VL and VH, are coded for by separate genes, they can be joined, using recombinant methods, by a synthetic linker that enables them to be made as a single contiguous chain in which the VL and VH regions pair to form monovalent molecules (known as single chain Fv (scFv); see e.g., Bird et al. (1988) Science 242:423-426; and Huston et al. (1988) Proc. Natl. Acad. Sci. USA 85:5879-5883). Such single chain antibodies are also intended to be encompassed within the term "antigen-binding portion" of an antibody. Other forms of single chain antibodies, such as diabodies, are also encompassed (see e.g., Holliger et al. (1993) PNAS. USA 90:6444-6448).

"Diabodies," or sometimes referred to herein as "Dia," as used herein are dimeric antibody fragments. In each polypeptide of the diabody, a heavy-chain variable domain (VH) is linked to a light-chain variable domain (VL) but unlike single-chain Fv fragments, the linker between the VL and VH is too short for intramolecular pairing and as such each antigen-binding site is formed by pairing of the VH and VL of one polypeptide with the VH and VL of the other polypeptide. Diabodies thus have two antigen-binding sites, and can be

monospecific or bispecific. (see, e.g., Holliger, P., et al. (1993) Proc. Natl. Acad. Sci. USA 90:6444-6448; Poljak, R. J., et al. (1994) Structure 2:1121-1123; Kontermann and Dubel eds., Antibody Engineering (2001) Springer-Verlag. New York. 790 pp. (ISBN 3-540-41354-5) incorporated herein by reference.

- 5 As used herein an "effective amount" of an agent, e.g., the tetravalent binding antibody molecules or a pharmaceutical composition comprising the molecules, refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired result. In some embodiments, a therapeutically effective amount is one that reduces the incidence and/or severity of, stabilizes one or more characteristics of, and/or delays onset of, one or more
- 10 symptoms of the disease, disorder, and/or condition. In some embodiments, the amount of a FZD Agonists administered to the subject is in the range of about 0.001mg/kg to 10mg/kg, 0.5 mg/kg to about 10 mg/kg, or about 0.5 mg/kg to about 1mg/kg of the subject's body weight. For example, in some embodiments the FZD4 Agonist may be applied to the eye in an amount of, e.g., about 0.02 -1.5 mg, about 0.05-1.0mg, or about 0.1-0.5 mg per eye.
- 15 As used herein, the term "epitope" includes any protein determinant capable of specific binding to an immunoglobulin or fragment thereof, or a T-cell receptor. The term "epitope" includes any protein determinant capable of specific binding to an immunoglobulin or T-cell receptor. Epitopic determinants usually consist of chemically active surface groupings of molecules such as amino acids or sugar side chains and usually have specific three-
- 20 dimensional structural characteristics, as well as specific charge characteristics. An antibody is said to specifically bind an antigen when the dissociation constant is $\leq 10\mu\text{M}$; e.g., ≤ 100 nM, preferably ≤ 10 nM and more preferably ≤ 1 nM.

The constant region of immunoglobulin molecules is also called the fragment crystallizable region, the "Fc region" or "Fc domain." The Fc domain is composed of two identical protein

25 fragments, derived from the second and third constant domains of the antibody's two heavy chains and the Fc domains of IgGs bear a highly conserved N-glycosylation site. Glycosylation of the Fc fragment is essential for Fc receptor-mediated activity. In an embodiment of the invention the Fc domain of the tetravalent binding antibody molecule is engineered such that it does not target the cell that binds the tetravalent binding antibody

30 molecule for ADCC or CDC-dependent death. In an embodiment of the invention the Fc

domain of the tetravalent binding antibody molecule is a peptide dimer in a knob-in-hole configuration. The peptide dimer may be a heterodimer.

The terms "individual," "subject," "host," and "patient," are used interchangeably herein and refer to any mammalian subject for whom diagnosis, treatment, or therapy is desired,
5 particularly humans.

"LRP", "LRP proteins" and "LRP receptors" is used herein to refer to members of the low density lipoprotein receptor-related protein family. These receptors are single-pass transmembrane proteins that bind and internalize ligands in the process of receptor-mediated endocytosis. LRP proteins LRP5 (e.g., LRP5: NP_002326.2) and LRP6 (e.g., LRP6:
10 NP_002327.2) are included in a Wnt receptor complex required for activation on the Wnt- β catenin signaling pathway. See also, for human/mouse LRP5 and LRP6: <https://www.uniprot.org/uniprot/O75197>, <https://www.uniprot.org/uniprot/Q91VN0>, <https://www.uniprot.org/uniprot/O75581>, <https://www.uniprot.org/uniprot/O88572>.

The term "polypeptide fragment" as used herein refers to a polypeptide that has an amino
15 terminal and/or carboxy-terminal deletion, but where the remaining amino acid sequence is identical to the corresponding positions in the naturally-occurring sequence deduced, for example, from a full-length cDNA sequence.

As used herein the term "paratope" includes the antigen binding site in the variable region of an antibody that binds to an epitope.

20 "Single-chain Fv" or "scFv" antibody fragments comprise the VH and VL domains of an antibody, wherein these domains are present in a single polypeptide chain. Generally, the Fv polypeptide further comprises a polypeptide linker between the VH and VL domains which enables the scFv to form the desired structure for antigen binding. For a review of scFv and other antibody fragments, see James D. Marks, Antibody Engineering, Chapter 2, Oxford
25 University Press (1995) (Carl K. Borrebaeck, Ed.).

"Single-domain antibody" (sdAb), or "nanobody", is an antibody fragment consisting of a single monomeric variable antibody domain. "VHH" or "VHH fragment" as used herein refers to a human VH that has been engineered to be independent of the light chain

(Nilvebrant et al. Curr Pharm Des. (2016) 22(43):6527-6537; Barthelemy et al., Journal of Biological Chemistry (2007) 283:3639-3654).

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
5 completely or partially preventing a disease or symptom thereof 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., slowing
10 or arresting its development; or (c) relieving the disease, i.e., causing regression of the disease. The therapeutic agent 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
15 therapy may be administered during the symptomatic stage of the disease, and in some cases after the symptomatic stage of the disease.

The ability of the tetravalent binding antibody molecules of this invention to activate Wnt signaling can be confirmed by a number of assays. The tetravalent binding antibody molecules of this invention typically initiate a reaction or activity that is similar to or the
20 same as that initiated by the FZD receptor's natural ligand. The tetravalent binding antibody molecules of this invention activates the Wnt signaling pathways, e.g., the canonical Wnt- β catenin signaling pathway. As used herein, the term "activates" refers to a measurable increase in the intracellular level of a Wnt signaling pathway, e.g., the Wnt- β catenin signaling pathway, compared with the level in the absence of a FZD Agonist of the
25 invention.

Various methods are known in the art for measuring the level of Wnt- β catenin activation. These include but are not limited to assays that measure: Wnt- β catenin target gene expression; LEF/TCF reporter gene expression (such as TopFLASH, superTopFLASH, pBAR); β catenin stabilization; LRP5/6 phosphorylation; Dishevelled phosphorylation; Axin
30 translocation from cytoplasm to cell membrane and binding to LRP5/6. The canonical Wnt- β catenin signaling pathway ultimately leads to changes in gene expression through the transcription factors TCF1, TCF7L1, TCF7L2 and LEF1. The transcriptional response to Wnt

activation has been characterized in a number of cells and tissues. As such, global transcriptional profiling by methods well known in the art can be used to assess Wnt- β catenin signaling activation.

- Changes in Wnt-responsive gene expression are generally mediated by TCF and LEF transcription factors. A TCF reporter assay assesses changes in the transcription of TCF/LEF controlled genes to determine the level of Wnt- β catenin signaling. A TCF reporter assay was first described by Korinek, V. et al., 1997. Also known as TOP/FOP this method involves the use of three copies of the optimal TCF motif CCTTTGATC, or three copies of the mutant motif CCTTTGGCC, upstream of a minimal c-Fos promoter driving luciferase expression (pTOPFLASH and pFOPFLASH, respectively) to determine the transactivational activity of endogenous β catenin/TCF. A higher ratio of these two reporter activities (TOP/FOP) indicates higher β catenin/TCF activity. A newer and more sensitive version of this reporter is called pBAR and contains 12 repeats of the TCF motifs (Biechele and Moon, *Methods Mol Biol.* 2008;468:99-110, PMID: 19099249).
- General methods in molecular and cellular biochemistry can be found in such standard textbooks as *Molecular Cloning: A Laboratory Manual*, 3rd Ed. (Sambrook et al., CSH Laboratory Press 2001); *Short Protocols in Molecular Biology*, 4th Ed. (Ausubel et al. eds., John Wiley & Sons 1999); *Protein Methods* (Bollag et al., John Wiley & Sons 1996); *Nonviral Vectors for Gene Therapy* (Wagner et al. eds., Academic Press 1999); *Viral Vectors* (Kaplift & Loewy eds., Academic Press 1995); *Immunology Methods Manual* (I. Lefkovits ed., Academic Press 1997); and *Cell and Tissue Culture: Laboratory Procedures in Biotechnology* (Doyle & Griffiths, John Wiley & Sons 1998).

Unless otherwise defined, scientific and technical terms used in connection with the present invention shall have the meanings that are commonly understood by those of ordinary skill in the art. Further, unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular. Generally, nomenclatures utilized in connection with, and techniques of, cell and tissue culture, molecular biology, and protein and oligo- or polynucleotide chemistry and hybridization described herein are those well-known and commonly used in the art. Standard techniques are used for recombinant DNA, oligonucleotide synthesis, and tissue culture and transformation (e.g., electroporation, lipofection). Enzymatic reactions and purification techniques are performed according to manufacturer's specifications or as commonly accomplished in the art or as described herein. The foregoing techniques and procedures are 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. See e.g., Sambrook et al. *Molecular Cloning: A Laboratory Manual* (2d ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989)). The nomenclatures utilized in connection with, and the laboratory procedures and techniques of, analytical chemistry, synthetic organic chemistry, and medicinal and pharmaceutical chemistry described herein are those well-known and commonly used in the art. Standard techniques are used for chemical syntheses, chemical analyses, pharmaceutical preparation, formulation, and delivery, and treatment of patients.

EXAMPLES

EXAMPLE I Identification and characterization of FZD4 binding or FZD5 binding Fab-phage.

A. FZD4 antibodies from affinity matured libraries of FZD4-binding antibody 5027 and 5044; FZD5 antibodies from affinity matured libraries of FZD5-binding antibody 2919 and 2928.

Affinity matured libraries of known FZD4-binding antibodies 5027 and 5044 and known FZD5-binding antibodies 2919 and 2928 were prepared using routine methods, essentially as described in US publication no. 2016/0194394, inventors Sidhu et al., see also Persson et al.

J. Mol. Biol., 2013 Feb 22; 425(4):803-11 <https://pubmed.ncbi.nlm.nih.gov/23219464/>, both incorporated herein in their entirety by reference.

The 6 CDRs of the heavy chain (CDR-H1, CDR-H2 and CDR-H3) and light chains (CDR-L1, CDR-L2 and CDR-L3) of antibodies 5044, 5027, 2919, and 2928 antibodies isolated from the affinity matured libraries are set forth in Table 1 and Table 2.

Single point ELISAs were performed on 96-well Maxisorp plates coated with the extracellular domains (ECDs) of human FZD4 protein in the presence or absence of a saturating concentration of 5027 diabody-Fc (a diabody comprising the VL and VH of 5027 linked to an Fc domain). The plates were incubated with monoclonal Fab-phage followed by incubation with horseradish peroxidase (HRP)-conjugated anti-M13 antibody. Wells were subsequently washed 8 times followed by incubations with 3,3',5,5'-tetramethylbenidine/H₂O₂ peroxidase (TMB) substrate for 5-10 min. The reaction was stopped by adding 1M H₃PO₄ and the absorbance was measured spectrophotometrically at 450 nm in a microtiter plate reader. The results of the assay are depicted in Figure 1 and Figure 2 and demonstrate that the newly identified FZD4 antibodies bind FZD4 at a site overlapping with the site recognized by antibody 5027. FZD4 binding antibodies 5027 and 5044 are described in US provisional application no. 62/885,781, incorporated herein by reference.

B. Epitope mapping of Lead FZD4 antibodies.

ELISA assays were performed in 384-well Maxisorp plates coated with FZD4 ECD wild-type (FZD4) or mutant FZD4 proteins (FZD_swap1-18) that replace segments of the FZD4 ECD with corresponding regions from FZD5. The plates were incubated with 10 nM IgG known to bind specifically to FZD4, i.e., 5044 and 5027, or to be panspecific, i.e., 5016 (binds FZD4, FZD5, and other FZD receptors), followed by incubation with horseradish peroxidase (HRP)-conjugated anti-Kappa light chain antibody. Phosphate buffered saline (PBS) and IgG 4275 which does not bind FZD4 or FZD5 were used as controls. The wells were washed 6 times followed by incubations with 3,3',5,5'-tetramethylbenidine/H₂O₂ peroxidase (TMB) substrate for 3-5 min. The reaction was stopped by adding 1M H₃PO₄ and the absorbance was measured spectrophotometrically at 450 nm in a microtiter plate reader, see Figure 2. The pan-FZD binder 5016 is a positive control showing that the antigens are functional, with the exception of "FZD4_Swap10". Both FZD4-specific antibodies 5027 and 5044 are unable

to bind to “FZD4 Swap 7”, suggesting that these molecules bind to this region of the FZD ECD.

C. Characterization of FZD4 IgG.

FZD4-binding full length IgGs were expressed via transient transfection in an Expi293 cell culture system, essentially as described in Tao et al., Tailored tetravalent antibodies potently and specifically activate Wnt/Frizzled pathways in cells, organoids and mice. *Elife*. 2019 Aug 27;8:e46134. doi: 10.7554/eLife.46134; PMID: 31452509. and purified via Protein A affinity chromatography. Briefly, cells were grown to a density of approximately 2.5×10^6 cells/ml in Expi293 Expression Media (Gibco) in baffled cell culture flasks and transfected with the appropriate vectors using FectoPRO transfection reagent (Polyplus-transfection) using standard manufacture protocols (ThermoFisher). Expression was allowed to proceed for 5 days at 37 °C and 8% CO₂ with shaking at 125 rpm. After expression, cells were removed by centrifugation and protein was purified from the conditioned media using rProtein A Sepharose (GE Healthcare). Purified protein was buffer exchanged into either PBS or a formulated stabilization buffer (36.8 mM citric acid, 63.2 mM Na₂HPO₄, 10% trehalose, 0.2 M L-arginine, 0.01% Tween-80, pH 6.0) for storage. Proteins concentrations were determined by absorbance at 280 nm and purity was confirmed by SDS-PAGE analysis.

Expression titers were determined as mg of purified protein per liter of mammalian cell culture. Size exclusion chromatography (SEC) results in Table A below are defined as “-”: evidence of multiple peaks on SEC trace, <50% monomeric species; “+”: >50% monomeric species, delayed retention time (>14 min.); “++”: >90% of major peak at/near expected retention time for a monomeric IgG. Standard retention time was determined by comparison to Trastuzumab.

Table A

TRAC ID	Expression Titer (mg/l)	SEC Result
13980	58	-
13981	40	-
13982	43	-
13983	65	++
13984	52	-
13985	61	++
13956	47	+
13957	52	+
13958	58	-
13959	46	+
13962	38	-
13963	56	-
13964	47	-
13965	74	++
13966	86	-
13967	87	-
13968	89	+
13969	72	-
13970	36	-
13971	46	++
13972	27	+
13973	41	+
13974	41	+
13975	41	++
13979	49	-

Trac ID corresponds to the antibody number in Table 1 and Table 2.

- D. Size-exclusion chromatography analysis and ELISA specificity measurements of the FZD4 IgGs.

Twenty micrograms of the FZD4 binding IgGs were separated over an AdvanceBio SEC, 300Å, 2.7 µm, 4.6 x 300 mm column in a mobile phase of PBS using an Agilent Bio-Inert HPLC. Protein elution was monitored using absorbance at 280 nM. The results are presented in Figure 3A.

- 10 ELISA specificity measurements of the FZD4 antibodies were determined against FZD1 and FZD10, the two FZD family member most closely related to FZD4. ELISA assays were performed in 384-well Maxisorp plates coated with FZD ECD wild-type or mutant proteins at a concentration of 1 µg/ml and excess binding sites were blocked with 0.5% BSA. The plates

were incubated with 10 nM of the FZD4 binding IgGs followed by incubation with horseradish peroxidase (HRP)-conjugated anti-Kappa light chain antibody. The wells were washed 6 times followed by incubations with 3,3',5,5'-tetramethylbenidine/H₂O₂ peroxidase (TMB) substrate for 3-5 min. The reaction was stopped by adding 1M H₃PO₄ and the
5 absorbance was measured spectrophotometrically at 450 nm in a microtiter plate reader. The results are presented in Figure 3B.

E. Identification of the CDRs of the FZD4 or FZD5 binding antibodies.

The amino acid sequences of the CDRs of the FZD4-binding and FZD5-binding immunoglobulins are set forth in Tables 1 and 2. The CDRs were identified according to the
10 INTERNATIONAL IMMUNOGENETICS INFORMATION SYSTEM (IMGT numbering system; Lefranc et al., 2003, Development and Comparative Immunology 27:55-77), and annotated as described in Persson et al. J Mol Biol. 2013 Feb 22;425(4):803-11, both incorporated herein by reference.

Table 1		FZD4 binding antibodies CDRs											
Antibody ID	Library	SEQ ID NO: L1	SEQ ID NO: L2	SEQ ID NO: L3	SEQ ID NO: H1	SEQ ID NO: H2	SEQ ID NO: H3	SEQ ID NO:	SEQ ID NO:				
5044	F	SVSSA	1	SASSLYS	2	WYYAPI	3	LSSYSM	24	YISSYYGYTY	51	PAPGHWGF	79
13953	5044AM	SVSSA	1	SASSLYS	2	GNYGPI	4	IAFYSI	25	YISPFSGITH	52	PAVGHLAGM	80
13954	5044AM	SVSSA	1	SASSLYS	2	GYAPI	5	IYSYSI	26	YISSYYGYTY	51	STVGHGGM	81
13955	5044AM	SVSSA	1	SASSLYS	2	WYYAPI	3	ISSYSI	27	YISPYYSYTY	53	PAPAHWGF	82
13956	5044AM	SVSSA	1	SASSLYS	2	GYVALI	6	LSSYSM	24	YISSYAGYTS	54	PALGHAGM	83
13957	5044AM	SVSSA	1	SASSLYS	2	WYFAPI	7	ISAYSI	28	YISPYFGLTG	55	PAPGHWGM	84
13958	5044AM	SVSSA	1	SASSLYS	2	WYYAPI	3	LSSYSI	29	YISSYYGYTY	51	PVAGHGGM	85
13959	5044AM	SVSSA	1	SASSLYS	2	GYNAPI	8	LYSYSM	30	FISSFYGYTD	56	PAVGHLAGL	86
13960	5044AM	SVSSA	1	SASSLYS	2	WYYAPI	3	LTSYSM	31	YISSYYGSTY	57	PAPGHWGM	84
13961	5044AM	SVSSA	1	SASSLYS	2	WYYAPI	3	ISSYSM	32	YISSYYSYTY	58	PAPYGAL	87
13962	5044AM	SVSSA	1	SASSLYS	2	GYFAPI	9	LFSYPM	33	YISPYYGYTN	59	QTAGHAGM	88
13963	5044AM	SVSSA	1	SASSLYS	2	WFNAPI	10	LSAYSM	34	YISSYYGYTY	51	PAPGHWGF	79
13964	5044AM	SVSSA	1	SASSLYS	2	SNYAPI	11	ISSHSM	35	YISPFSSFTH	60	QAPGVSGI	89
13965	5044AM	SVSSA	1	SASSLYS	2	WYNAPI	12	LSSYSM	24	YISSYDSITD	61	PAVGHMAF	90
13966	5044AM	SVSSA	1	SASSLYS	2	GFYAPI	13	LSSYSM	24	YISSYYSYTA	62	PTPGHGGL	91
13967	5044AM	SVSSA	1	SASSLYS	2	WYYAPI	3	ISAYAM	36	YISPYYGYTF	63	PAPGHGGM	92
13968	5044AM	SVSSA	1	SASSLYS	2	GYSAPI	14	ISTYSM	37	YISPHYGFTS	64	PAVGHLM	93
13969	5044AM	SVSSA	1	SASSLYS	2	WYFAPI	7	LHAFSM	38	YISPYYGYTY	65	PAPGHWGL	94
13970	5044AM	SVSSA	1	SASSLYS	2	GFYAPI	13	ISGYSI	39	YISSYYGYTF	66	TAPGHGAF	95
13971	5044AM	SVSSA	1	SASSLYS	2	GFYAPI	13	ISNYSI	40	IISNFGYTS	67	PALGHLAGM	96
13972	5044AM	SVSSA	1	SASSLYS	2	GYAGLI	15	ISAYSM	41	SISSYYGFTS	68	LAPGHPAL	97
13973	5044AM	SVSSA	1	SASSLYS	2	GFSSPI	16	LTSYAM	42	YISPYYGYTY	65	PAAGHLAL	98
13974	5044AM	SVSSA	1	SASSLYS	2	GYAPI	5	LYYSI	43	YISPSYGSTY	69	PIPGHLAF	99
13975	5044AM	SVSSA	1	SASSLYS	2	GHYAPI	17	LSSFSM	44	YISSFNSTF	70	PTWAHGAF	100
13976	5044AM	SVSSA	1	SASSLYS	2	SFYAPI	18	IASYSI	45	YISSYYGSTY	57	PVLAHSF	101
13977	5044AM	SVSSA	1	SASSLYS	2	GYRAPI	19	IHSNSM	46	YISPYYSFTS	71	QTPGHSGM	102
13978	5044AM	SVSSA	1	SASSLYS	2	WHRAPI	20	LSTNSM	47	YISPYYSFTY	72	QAPGPWGM	103
13979	5044AM	SVSSA	1	SASSLYS	2	SFYAPI	18	LHSFSM	48	FISSYYGYTY	73	PAPGHGAF	104

Table 1 cont'	FZD4 binding antibodies CDRs												
Antibody ID	Library	L1	SEQ ID NO:	L2	SEQ ID NO:	L3	SEQ ID NO:	H1	SEQ ID NO:	H2	SEQ ID NO:	H3	SEQ ID NO:
13980	5044AM	SVSSA	1	SASSLYS	2	GYAPI	5	LTSYSM	31	SISPYYSYTN	74	PTTAHMAL	105
13981	5044AM	SVSSA	1	SASSLYS	2	WYYAPI	3	ISSFSI	49	FINPYYSYTY	75	PAPGHWGM	84
13982	5044AM	SVSSA	1	SASSLYS	2	GYAPI	5	ISSYSM	32	YISSYYDYTY	76	PTPGHSGF	106
13983	5044AM	SVSSA	1	SASSLYS	2	GDFAPF	21	LPYYSM	50	IISYFGFTY	77	PAVGHGAL	107
13984	5044AM	SVSSA	1	SASSLYS	2	GYSSPI	22	ISSHSM	35	YISPYYSYTY	53	TAPGHPAM	108
13985	5044AM	SVSSA	1	SASSLYS	2	WFYAPI	23	ISSYSI	27	YISSNFGSTY	78	PVPAHGAF	109

Table 2A	FZD4 binding antibodies CDRs												
Antibody ID	Library	L1	SEQ ID NO:	L2	SEQ ID NO:	L3	SEQ ID NO:	H1	SEQ ID NO:	H2	SEQ ID NO:	H3	SEQ ID NO:
5027	F	SVSSA	1	SASSLYS2	2	SSVSLI	130	SSFYFM	148	TVVPLYDITY	985	AFPGSYHPM	199
13986	5027AM	SVSSA	1	SASSLYS2	2	ASYALI	110	STYFFI	139	TIYPYLNSTY	166	AYPGSYHPL	198
13987	5027AM	SVSSA	1	SASSLYS2	2	SNYALI	111	TSFYFM	140	SVYPYLDNTY	167	AFPGSYHPM	199
13988	5027AM	SVSSA	1	SASSLYS2	2	SSSFLI	112	SSFYFI	141	TVYSYIDITY	168	AFPGSYHPM	200
13989	5027AM	SVSSA	1	SASSLYS2	2	SSDSLJ	113	SAYYFI	142	TVYPYRGYTY	169	GYPLAYTPL	201
13990	5027AM	SVSSA	1	SASSLYS2	2	STHFLI	114	SYFYFM	143	SVYPYLSYTY	170	AFPGSYHPM	199
13991	5027AM	SVSSA	1	SASSLYS2	2	ASYSLI	115	SSFYFI	141	SVYPYLDFTY	171	ALQGHYHPM	202
13992	5027AM	SVSSA	1	SASSLYS2	2	SAYTLI	116	TSFYFM	144	AIYPYLDITY	172	AFPGSYLPM	203
13993	5027AM	SVSSA	1	SASSLYS2	2	SSVSLI	117	FTFYFM	145	SIYPYLNITY	173	AFPGSYHPM	199
13994	5027AM	SVSSA	1	SASSLYS2	2	SYYSLI	118	SSYYFI	146	TIYPYSDNTY	174	GFPGRYHPL	204
13995	5027AM	SVSSA	1	SASSLYS2	2	SSYYLI	119	SSFYVM	147	SIYSYGNITY	175	AFPLSYHPM	205
13996	5027AM	SVSSA	1	SASSLYS2	2	ASYALI	110	SSFYFM	148	AIYPYLSYTY	176	AFPGRYHGM	206
13997	5027AM	SVSSA	1	SASSLYS2	2	AYYFLI	120	SSFYYI	149	TVFPYLGRTY	177	AFPGSYTPL	207

Table 2A – cont'		FZD4 binding antibodies CDRs											
Antibody ID	Library	L1	SEQ ID NO:	L2	SEQ ID NO:	L3	SEQ ID NO:	H1	SEQ ID NO:	H2	SEQ ID NO:	H3	SEQ ID NO:
13998	5027AM	SVSSA	1	SASSLYS2	2	SSFSLI	121	STFYFM	150	SVYPYLNITY	178	AFPGAYSPM	208
13999	5027AM	SVSSA	1	SASSLYS2	2	SAYSIL	122	SAFYFM	151	TVYPYLSYTY	179	AFPGAYHPM	209
14000	5027AM	SVSSA	1	SASSLYS2	2	SSYALI	123	ASFYFM	152	TVYPYLNHTY	180	AFPGAYHPF	210
14001	5027AM	SVSSA	1	SASSLYS2	2	SYFSLI	124	SAFYFI	153	SIYPYLSYTY	181	AFPGAYHPM	209
14002	5027AM	SVSSA	1	SASSLYS2	2	SRFTLI	125	FPFYFM	154	SVYPYLNHTY	182	AYPGFYHPI	211
14003	5027AM	SVSSA	1	SASSLYS2	2	SSNTLI	126	SAFYFM	155	SVYSSLSHTY	183	AYPLSYHPM	212
14004	5027AM	SVSSA	1	SASSLYS2	2	SSFSLI	121	ASYYFM	156	SVYPYLDFTY	171	ALPGFYHPF	213
14005	5027AM	SVSSA	1	SASSLYS2	2	SSFALI	127	SVFYFM	157	SVYPYNDITY	184	GFPPTYHPL	214
14006	5027AM	SVSSA	1	SASSLYS2	2	ANYALI	128	SSLYYM	158	SVYPYLDNTH	185	AIPGFYHPI	215
14008	5027AM	SVSSA	1	SASSLYS2	2	ASYSIL	115	SNFYLM	159	SIYSYLNITY	186	AFPGSYHPM	199
14009	5027AM	SVSSA	1	SASSLYS2	2	SSASLI	129	FSFYFI	160	SIYPYLDFTH	187	AFPGSYHPL	216
14010	5027AM	SVSSA	1	SASSLYS2	2	SSYSIL	130	SAFYFM	155	AIYPYIGYTY	188	PFPAHYHPL	217
14011	5027AM	SVSSA	1	SASSLYS2	2	SSYSIL	130	SSLYFM	161	TIYPFRGTTY	189	AYPGRYHPL	218
14012	5027AM	SVSSA	1	SASSLYS2	2	SAFYLI	131	YFYFYM	162	AIYPYLGITY	190	AFPGSYHPL	216
14013	5027AM	SVSSA	1	SASSLYS2	2	SAYFLI	132	SSFYFI	141	SVYPYLGDTY	191	AFPGFYHPF	219
14015	5027AM	SVSSA	1	SASSLYS2	2	ASSSLI	133	TSYYFI	163	SVYSYLGITY	192	AFPGSYHPL	216
14016	5027AM	SVSSA	1	SASSLYS2	2	SIYSIL	134	SYFYFM	143	AIYPYLSYTY	176	AFPGSYHPM	199
14018	5027AM	SVSSA	1	SASSLYS2	2	ASYILI	110	FSFYFI	160	TVYPYLSHTY	193	AFPGSYHPM	199
14019	5027AM	SVSSA	1	SASSLYS2	2	STGSLI	135	SDFYFI	164	TIYPFIGNTY	194	AFPGSYHPF	220
14020	5027AM	SVSSA	1	SASSLYS2	2	SSHSLI	136	SSFYFM	148	SVYPYVDITY	195	AFPGFYHPM	221
14021	5027AM	SVSSA	1	SASSLYS2	2	SSYTLI	137	SSFYFM	165	TVYPYLSFTY	196	ALPGSYHPF	222
14022	5027AM	SVSSA	1	SASSLYS2	2	SYHYLI	138	STFYFM	150	SVYPYLDITY	197	AYPGSYHPL	198

Table 2B	FZD5 binding antibodies CDRs													SEQ ID NO.	H3	SEQ ID NO.
Antibody ID	Antigen Name	Selection ID	L1	SEQ ID NO.	L2	SEQ ID NO.	L3	SEQ ID NO.	H1	SEQ ID NO.	H2	SEQ ID NO.	SEQ ID NO.			
14023	FZD5	2919AM	SVSSA	1	SASSLYS	2	WYSGVHGLI	223	LIYTI	308	TIYPASSSTS	381	GAM			
14024	FZD5	2919AM	SVSSA	1	SASSLYS	2	WFSSAHVPF	224	ITPGM	309	TIFSSHGSTS	382	FGM			
14025	FZD5	2919AM	SVSSA	1	SASSLYS	2	WYSGVHGLI	225	ISYSYM	310	SIYSSSSTS	383	GAL			
14026	FZD5	2919AM	SVSSA	1	SASSLYS	2	WYTSVHGLI	226	ISFFYM	311	TIDSSTGTT	384	GAL			
14027	FZD5	2919AM	SVSSA	1	SASSLYS	2	WYASDHGLI	227	IPYFYM	312	SIYSSSSTS	385	GAM			
14028	FZD5	2919AM	SVSSA	1	SASSLYS	2	WFSPGNVLI	228	ISYYI	313	SIYSSSSTS	386	AAF			
14029	FZD5	2919AM	SVSSA	1	SASSLYS	2	WYSTRNLI	229	ISYAYM	314	SIYSSSSTS	387	YAF			
14030	FZD5	2919AM	SVSSA	1	SASSLYS	2	WYFSDHDLF	230	ITYFYM	315	SIYPSFGSTS	388	GGM			
14031	FZD5	2919AM	SVSSA	1	SASSLYS	2	WYSSGDVLI	231	IYYSYI	316	SIYSSNGSTS	389	GAM			
14032	FZD5	2919AM	SVSSA	1	SASSLYS	2	WYSSADVL	232	ILNTYM	317	SIYSSSSTA	387	GGL			
14033	FZD5	2919AM	SVSSA	1	SASSLYS	2	WYSSGHGLI	233	ISFYI	318	SIYPSSTS	390	GGL			
14034	FZD5	2919AM	SVSSA	1	SASSLYS	2	WYSSGHGLI	234	LAYSYM	319	SIYPSGDT	391	GAM			
14035	FZD5	2919AM	SVSSA	1	SASSLYS	2	WYSSNHIPI	235	IRYSYI	320	AIYSSSSTS	392	GAM			
14036	FZD5	2919AM	SVSSA	1	SASSLYS	2	WYSSSNVLI	236	ITYSYM	321	TIYPSGSTA	393	FAM			
14037	FZD5	2919AM	SVSSA	1	SASSLYS	2	WYFSDRVLI	237	IINTYM	322	SIYAPSSTA	394	GAI			
14038	FZD5	2919AM	SVSSA	1	SASSLYS	2	WYPSHVL	238	ISYSYM	310	TIYPSGSTA	393	GGM			
14039	FZD5	2919AM	SVSSA	1	SASSLYS	2	WYSSRHHLI	239	LANSYM	323	TIYSSGSTS	395	GAM			
14040	FZD5	2919AM	SVSSA	1	SASSLYS	2	WYSTGRVLF	240	LSYTYM	324	SIDPSSGSTS	396	FGM			
14041	FZD5	2919AM	SVSSA	1	SASSLYS	2	WFPTGHVLF	241	ISYSYI	325	TIYPSGSGTG	397	FGM			
14042	FZD5	2919AM	SVSSA	1	SASSLYS	2	WYAPRHVLI	242	ISYSYI	325	SIYSSSSTS	383	GAM			
14043	FZD5	2919AM	SVSSA	1	SASSLYS	2	WFTSGLVLF	243	LSYLYI	326	SIYPSSSSTS	398	GGM			
14044	FZD5	2919AM	SVSSA	1	SASSLYS	2	WYSSGHVLF	244	ISYAYI	327	SIYSPSSTS	399	GAM			
14045	FZD5	2919AM	SVSSA	1	SASSLYS	2	WYPGAPVLI	245	IAYSYM	328	SIYPSSSSTS	398	GAM			
14046	FZD5	2919AM	SVSSA	1	SASSLYS	2	WYSSSNVLI	236	FIWESLLVS	329	TIYPSGSTA	393	FAM			
14047	FZD5	2919AM	SVSSA	1	SASSLYS	2	WYSSANALF	246	ISHSYM	330	SIDSSGSTS	400	GAL			
14048	FZD5	2919AM	SVSSA	1	SASSLYS	2	WYSSGDVLI	231	LRYSYI	331	TIYASGSGTT	401	GAL			

Table 2B	FZD5 binding antibodies CDRs													SEQ ID NO:	H3	SEQ ID NO:
Antibody ID	Antigen Name	Selection ID	L1	SEQ ID NO:	L2	SEQ ID NO:	L3	SEQ ID NO:	H1	SEQ ID NO:	H2	SEQ ID NO:	SEQ ID NO:			
14049	FZD5	2919AM	SVSSA	1	SASSLYS	2	WYSSNHVLI	247	ISYAYM	314	SISPSDSSTS	402	GAL			
14050	FZD5	2919AM	SVSSA	1	SASSLYS	2	WYSAAHILF	248	ISYSYM	310	SIDPSSGLTS	403	GAL			
14051	FZD5	2919AM	SVSSA	1	SASSLYS	2	WFASGHVLI	249	IRYAYM	332	TIDSFSGSTS	404	YAM			
14052	FZD5	2919AM	SVSSA	1	SASSLYS	2	WFSSGDSLI	250	ITHLYM	333	SIYPSGGSTS	405	AAL			
14053	FZD5	2919AM	SVSSA	1	SASSLYS	2	WYASGNVLI	251	LSIFYM	334	TIYPSSTS	406	GAF			
14054	FZD5	2919AM	SVSSA	1	SASSLYS	2	WFSPGHLLI	252	LSYSYI	335	AIYPSSTS	407	GAM			
14055	FZD5	2919AM	SVSSA	1	SASSLYS	2	WYPSGHVLF	253	ISYSFM	336	SIYSTSGSTS	408	GGL			
14056	FZD5	2919AM	SVSSA	1	SASSLYS	2	WFAAGHVLI	254	IAYVGI	337	AITPSSNTS	409	FGF			
14057	FZD5	2919AM	SVSSA	1	SASSLYS	2	WFRSAHVLI	255	LSYSYM	338	SIDPSSSTA	410	FGF			
14058	FZD5	2919AM	SVSSA	1	SASSLYS	2	WFASGHILL	256	ISYSFM	336	SIYRSRGSTA	411	GAM			
14059	FZD5	2919AM	SVSSA	1	SASSLYS	2	WFSGGHALI	257	IAYFYI	339	TIDPSSGSTS	412	GGL			
14060	FZD5	2919AM	SVSSA	1	SASSLYS	2	WFASAHVLF	258	LSSSYM	340	SIGPSSGSTS	413	FGF			
14061	FZD5	2919AM	SVSSA	1	SASSLYS	2	WYSPGHVLI	259	LSYSYM	338	SIYPSSTS	398	GGL			
14062	FZD5	2919AM	SVSSA	1	SASSLYS	2	WYASGLVLI	260	IYYSYM	341	SIDPSSSTS	414	GGM			
14063	FZD5	2919AM	SVSSA	1	SASSLYS	2	WYTSGHVLF	261	ITYSYM	321	SIYPSSTS	415	GGM			
14064	FZD5	2919AM	SVSSA	1	SASSLYS	2	WFSPPHALI	262	LHYGGI	342	SISSFSSTS	416	FGF			
14066	FZD5	2919AM	SVSSA	1	SASSLYS	2	WYASGTDLI	263	ISYSYM	310	TIYSSSTA	417	GGM			
14067	FZD5	2919AM	SVSSA	1	SASSLYS	2	WYSSGRILLI	264	IGYAYM	343	SIYSPGSTA	418	GAL			
14068	FZD5	2919AM	SVSSA	1	SASSLYS	2	WFSSPHVLF	265	LSYSSI	344	SICPFCSTS	419	FGF			
14069	FZD5	2919AM	SVSSA	1	SASSLYS	2	WFASASSLF	266	LSYDGI	345	TIYSSGSTS	395	FGL			
14070	FZD5	2919AM	SVSSA	1	SASSLYS	2	WYPSHHVLF	267	LSYAYM	346	SIHPFDGSTS	420	GAL			
14071	FZD5	2919AM	SVSSA	1	SASSLYS	2	WYPAHHVLF	268	ISYSGI	347	SISSSSGSTA	421	FGM			
14072	FZD5	2919AM	SVSSA	1	SASSLYS	2	WFPSGNVLF	269	ISSSYM	348	TIDPYAGSTS	422	FAM			
14073	FZD5	2919AM	SVSSA	1	SASSLYS	2	WYSSNNVLI	270	LSYSYM	338	SIYPSGSTA	423	GGM			
14074	FZD5	2919AM	SVSSA	1	SASSLYS	2	WFSSRHVLF	271	LSYSGM	349	TIYPSSTS	406	FGM			
14075	FZD5	2919AM	SVSSA	1	SASSLYS	2	WYFSDHDLI	272	GFTISYFFM	350	SIYPSDSSTS	424	GAM			

Table 2B	FZD5 binding antibodies CDRs													
Antibody ID	Antigen Name	Selection ID	L1	SEQ ID NO:	L2	SEQ ID NO:	L3	SEQ ID NO:	H1	SEQ ID NO:	H2	SEQ ID NO:	H3	SEQ ID NO:
14076	FZD5	2919AM	SVSSA	1	SASSLYS	2	WYSSGGVLI	273	ISYFI	351	SIYSSGHTS	425	GAL	
14077	FZD5	2919AM	SVSSA	1	SASSLYS	2	WFPSGHVLF	274	ISYSYM	310	SIDPSSGSTS	396	FGM	
14078	FZD5	2919AM	SVSSA	1	SASSLYS	2	WYSSTHVLV	275	LSYTM	324	SIYSPGSTS	426	GGM	
14079	FZD5	2919AM	SVSSA	1	SASSLYS	2	WYSSGSALI	276	ISHAYM	352	SIYSPGSTA	418	FAL	
14080	FZD5	2919AM	SVSSA	1	SASSLYS	2	WFSPGSLI	277	LLFFYM	353	SIDPSSGFTS	427	GAM	
14081	FZD5	2919AM	SVSSA	1	SASSLYS	2	WYPSAHILF	278	IAYSYM	328	SIYPASSSTS	390	GGM	
14082	FZD5	2919AM	SVSSA	1	SASSLYS	2	WYGSGGILI	279	LSYFM	354	SIFSSSSTS	428	GAM	
14083	FZD5	2919AM	SVSSA	1	SASSLYS	2	WFTSGRDLF	280	GFTLISSI	355	SIYPAPSSTP	429	GGM	
14084	FZD5	2919AM	SVSSA	1	SASSLYS	2	WYLSRNILI	281	LSYSVI	335	SIYSSSSTS	383	GAM	
14085	FZD5	2919AM	SVSSA	1	SASSLYS	2	WFSGRDALF	282	ITNSYM	356	SIYSPSSTS	399	GGL	
14369	FZD5	2928AM	SVSSA	1	SASSLYS	2	AFYPI	283	ISFSSI	357	SIYPSYGSSF	430	YYAF	483
14370	FZD5	2928AM	SVSSA	1	SASSLYS	2	AFYPI	284	ISYSSM	358	TIYPYSSTL	431	YYAF	483
14371	FZD5	2928AM	SVSSA	1	SASSLYS	2	AHYFPI	285	ISYSSM	358	SIYSSYSTY	432	YYAM	484
14372	FZD5	2928AM	SVSSA	1	SASSLYS	2	AFYFPI	284	ISYSSM	358	SIYPSYSVTY	433	YYGM	485
14373	FZD5	2928AM	SVSSA	1	SASSLYS	2	SHYPI	286	LSFSSM	359	SIYPYGSTF	434	YYAM	484
14374	FZD5	2928AM	SVSSA	1	SASSLYS	2	AFYFPI	284	ISYSSM	358	SIYSSYSTY	432	YYGF	486
14375	FZD5	2928AM	SVSSA	1	SASSLYS	2	GFYPI	287	ISFGSI	360	SIYPSYSTF	435	YYAM	484
14376	FZD5	2928AM	SVSSA	1	SASSLYS	2	AFYPI	288	ITYSSI	361	SIYPAYSSTY	436	YYAF	483
14377	FZD5	2928AM	SVSSA	1	SASSLYS	2	SFYFPI	289	ISYSAI	362	SIYSSYSTF	437	YYAM	484
14378	FZD5	2928AM	SVSSA	1	SASSLYS	2	AFYFPI	284	ISYSSM	358	SIYSSYGSTY	438	YYAM	484
14379	FZD5	2928AM	SVSSA	1	SASSLYS	2	ADYFPI	290	ISYSSM	358	SIYPSYSTY	439	YYGM	485
14380	FZD5	2928AM	SVSSA	1	SASSLYS	2	AFYPI	288	ISYSSI	363	SIYSYGSTY	440	YYAM	484
14381	FZD5	2928AM	SVSSA	1	SASSLYS	2	AFYFPI	284	ISFSSI	357	TIYSLFGATF	441	YYGF	486
14382	FZD5	2928AM	SVSSA	1	SASSLYS	2	SFYFPI	289	ISYSSM	358	SIYPSYSTY	439	YYAF	483
14383	FZD5	2928AM	SVSSA	1	SASSLYS	2	AFHYPI	291	ISFSSI	357	SIYPSYSTY	439	YYAM	484
14384	FZD5	2928AM	SVSSA	1	SASSLYS	2	AFYPI	288	ISFSSI	357	SIYPAYGATF	442	YYGM	485

Table 2B	FZD5 binding antibodies CDRs														
Antibody ID	Antigen Name	Selection ID	L1	SEQ ID NO:	L2	SEQ ID NO:	L3	SEQ ID NO:	SEQ ID NO:	H1	SEQ ID NO:	H2	SEQ ID NO:	H3	SEQ ID NO:
14385	FZD5	2928AM	SVSSA	1	SASSLYS	2	AYYPI	292	ISYSSI	363	SIYSSYSTF	437	YYAF	483	
14386	FZD5	2928AM	SVSSA	1	SASSLYS	2	VFYPI	293	ISYSSM	358	SIYSSYSTY	432	YYAF	483	
14387	FZD5	2928AM	SVSSA	1	SASSLYS	2	SFYPI	289	ISYSSM	358	SIYPYSSTY	443	YYAM	484	
14388	FZD5	2928AM	SVSSA	1	SASSLYS	2	AFYFPI	284	LSYSSM	364	SIYPAYSSTY	436	YYGM	485	
14389	FZD5	2928AM	SVSSA	1	SASSLYS	2	ANYFPI	294	ISFGSI	360	SIYSSYSTY	432	YYAM	484	
14390	FZD5	2928AM	SVSSA	1	SASSLYS	2	GFYFPI	295	ISYSSI	363	SIYPSFGSTY	444	YYAM	484	
14391	FZD5	2928AM	SVSSA	1	SASSLYS	2	AFYFPI	284	ISYSSM	358	SIYPSFSPTY	445	YYGM	485	
14392	FZD5	2928AM	SVSSA	1	SASSLYS	2	AFYFPI	284	IAYSSM	365	SIYSSYGSTF	446	YYAF	483	
14393	FZD5	2928AM	SVSSA	1	SASSLYS	2	AYYFPI	296	ISYSSI	363	SIYPAYSPTY	447	YYAF	483	
14394	FZD5	2928AM	SVSSA	1	SASSLYS	2	AFIFPI	297	ISYSAM	366	SIYPSYSSTY	439	YYAL	487	
14395	FZD5	2928AM	SVSSA	1	SASSLYS	2	AYYPI	292	ISYGVI	367	SIYSAYSTF	448	YYAF	483	
14396	FZD5	2928AM	SVSSA	1	SASSLYS	2	SFYPI	289	ISYSSI	363	TIYPYDPTY	449	YYAF	483	
14397	FZD5	2928AM	SVSSA	1	SASSLYS	2	AFYFPI	284	LSYSSM	364	SIYSSYGSTY	438	YYAM	484	
14398	FZD5	2928AM	SVSSA	1	SASSLYS	2	AFYPI	288	ISYSSI	363	SIYPSYGSTY	450	YYGM	485	
14399	FZD5	2928AM	SVSSA	1	SASSLYS	2	AFYFPI	284	ISYSSM	358	SIPHYGATF	451	YYGM	485	
14400	FZD5	2928AM	SVSSA	1	SASSLYS	2	AFYPI	288	ISYSSI	363	SIYPSYGSTF	452	YYAM	484	
14401	FZD5	2928AM	SVSSA	1	SASSLYS	2	AFFPI	298	ISYSSI	363	SIYSSYSATY	453	YYAM	484	
14402	FZD5	2928AM	SVSSA	1	SASSLYS	2	SFYPI	299	ISYSSM	358	SIYPSYSSTY	439	YYAF	483	
14403	FZD5	2928AM	SVSSA	1	SASSLYS	2	AFYFPI	284	IPYGSM	368	SIYPSYGSTF	452	YYAM	484	
14404	FZD5	2928AM	SVSSA	1	SASSLYS	2	ALYPI	300	ISYSSM	358	SIYPSYSTF	435	YYAM	484	
14405	FZD5	2928AM	SVSSA	1	SASSLYS	2	AFYFPI	284	ISYSAM	366	SIYPYGPY	454	YYAF	483	
14406	FZD5	2928AM	SVSSA	1	SASSLYS	2	VFYPI	293	ISYSSI	363	SIYPFYGSTF	455	FYAF	488	
14407	FZD5	2928AM	SVSSA	1	SASSLYS	2	RFYFPI	301	ISYSSI	363	AIYPSYSTN	456	YYAM	484	
14408	FZD5	2928AM	SVSSA	1	SASSLYS	2	AFYPI	288	ISYSSI	363	SIYPYSSTH	457	YYAM	484	
14409	FZD5	2928AM	SVSSA	1	SASSLYS	2	LYFPI	302	IAYSAM	369	SIYPSYSTY	458	YYAM	484	
14410	FZD5	2928AM	SVSSA	1	SASSLYS	2	AFYFPI	284	LSFSSI	370	SIYPSYSTF	435	YYAF	483	

Table 2B	FZD5 binding antibodies CDRs													
Antibody ID	Antigen Name	Selection ID	L1	SEQ ID NO:	L2	SEQ ID NO:	L3	SEQ ID NO:	SEQ ID NO:	H1	SEQ ID NO:	H2	SEQ ID NO:	SEQ ID NO:
14411	FZD5	2928AM	SVSSA	1	SASSLYS	2	AYYFPI	296	ISYSSM	358	SIYSSVGSTY	438	YYGM	485
14412	FZD5	2928AM	SVSSA	1	SASSLYS	2	SSYFPI	303	ISYSSM	358	SIYSNVSSSY	459	YYAM	484
14413	FZD5	2928AM	SVSSA	1	SASSLYS	2	AFYFPI	284	ITYSSI	371	TIYPSVGSTY	460	YYAM	484
14414	FZD5	2928AM	SVSSA	1	SASSLYS	2	AFYFPI	284	ISYSSM	358	SIYPSFGSTF	461	YYAI	489
14415	FZD5	2928AM	SVSSA	1	SASSLYS	2	AHYFPI	285	LSYGSI	372	SIYPSVGSTY	450	YYGM	485
14416	FZD5	2928AM	SVSSA	1	SASSLYS	2	ASYFPI	304	ISYSSM	358	TIYPSVSSTY	462	YYAM	484
14417	FZD5	2928AM	SVSSA	1	SASSLYS	2	AFYFPI	284	IAYSSM	365	TIYSSVGATS	463	YYAF	483
14418	FZD5	2928AM	SVSSA	1	SASSLYS	2	AHYFPI	305	ISYSSI	363	SIYPSVSSTI	464	YYAM	484
14419	FZD5	2928AM	SVSSA	1	SASSLYS	2	AGFFPI	306	ISYSSI	363	TIYPPYGATY	465	YYGF	486
14420	FZD5	2928AM	SVSSA	1	SASSLYS	2	SSYFPI	307	ISYSSI	363	SIYSGYSATY	466	YYAM	484
14421	FZD5	2928AM	SVSSA	1	SASSLYS	2	AFYFPI	284	ITYSSM	373	SIYPSVGSTY	450	YYGF	486
14422	FZD5	2928AM	SVSSA	1	SASSLYS	2	AFYFPI	284	ISYSSI	363	TIYPSVSSTY	462	YYAM	484
14423	FZD5	2928AM	SVSSA	1	SASSLYS	2	AFYFPI	284	LGYSM	374	SIYPSVGSTY	450	YYAM	484
14424	FZD5	2928AM	SVSSA	1	SASSLYS	2	AFYFPI	284	ISYSSM	358	SIYPSVSATF	467	YFAL	490
14425	FZD5	2928AM	SVSSA	1	SASSLYS	2	AFYFPI	288	IGYSSI	375	TIYPSVSSTF	468	YYAF	483
14426	FZD5	2928AM	SVSSA	1	SASSLYS	2	AFYFPI	288	ITYGSI	376	AIYSSVGSTY	469	YYAF	483
14427	FZD5	2928AM	SVSSA	1	SASSLYS	2	AFYFPI	288	ISYSSM	358	SIFPYVGSTY	470	YYGF	486
14428	FZD5	2928AM	SVSSA	1	SASSLYS	2	AFYFPI	284	ISYSSM	358	SIYPFYSTTF	471	YYAM	484
14429	FZD5	2928AM	SVSSA	1	SASSLYS	2	SSYFPI	307	ISYGSM	377	SIYPSVSATF	467	YYGM	485
14430	FZD5	2928AM	SVSSA	1	SASSLYS	2	AFYFPI	288	ISYSSM	358	SIYPSVGSTS	472	YYGM	485
14431	FZD5	2928AM	SVSSA	1	SASSLYS	2	AFYFPI	284	IYVSSM	378	SIYTYGSTV	473	YYGM	485
14432	FZD5	2928AM	SVSSA	1	SASSLYS	2	AFYFPI	284	ISYSSI	363	TIYPNVSSY	474	YYAM	484
14433	FZD5	2928AM	SVSSA	1	SASSLYS	2	AFYFPI	284	IFYSSM	379	SIYPSVSATY	475	YYAM	484
14434	FZD5	2928AM	SVSSA	1	SASSLYS	2	AFYFPI	288	ISYSSM	358	SIYPGVSSTY	476	YYAM	484
14435	FZD5	2928AM	SVSSA	1	SASSLYS	2	AHYFPI	285	IAYSSM	365	TIYPSVSSTY	462	YYAM	484
14436	FZD5	2928AM	SVSSA	1	SASSLYS	2	AFYFPI	288	ISYSSI	363	SIYPSVSSTS	477	YYGM	485

Table 2B	FZD5 binding antibodies CDRs													
	Antibody ID	Antigen Name	Selection ID	L1	SEQ ID NO:	L2	SEQ ID NO:	L3	SEQ ID NO:	H1	SEQ ID NO:	H2	SEQ ID NO:	SEQ ID NO:
	14437	FZD5	2928AM	SVSSA	1	SASSLYS	2	AFYFPI	284	ISYSSM	358	SIYSGYGSTY	478	YYGM 485
	14438	FZD5	2928AM	SVSSA	1	SASSLYS	2	AFYFPI	284	ISYSSM	358	SIYPSYGSTY	450	YYGM 485
	14439	FZD5	2928AM	SVSSA	1	SASSLYS	2	AFYFPI	284	LSFSSM	359	SIYSGYSATY	466	YYAL 487
	14440	FZD5	2928AM	SVSSA	1	SASSLYS	2	AYYFPI	296	ISYSSM	358	SIYPSYGSTN	479	YYAF 483
	14441	FZD5	2928AM	SVSSA	1	SASSLYS	2	AFYFPI	284	LSYSSI	344	SIYPTYGSAY	480	YYGM 485
	14442	FZD5	2928AM	SVSSA	1	SASSLYS	2	AFYFPI	284	ITYSSM	373	TIYSSYGSTY	481	YYAM 484
	14443	FZD5	2928AM	SVSSA	1	SASSLYS	2	AFYFPI	284	ISFSSM	380	AIYPPYGSTY	482	YYGF 486
	14444	FZD5	2928AM	SVSSA	1	SASSLYS	2	AHYFPI	285	ISYSSM	358	SIYSSYGSTY	438	YYGM 485
	14445	FZD5	2928AM	SVSSA	1	SASSLYS	2	ASYFPI	304	LSYSSM	364	SIYPSYSSTF	435	YYAM 484
	14446	FZD5	2928AM	SVSSA	1	SASSLYS	2	AFYPI	288	ISYGSM	377	SIYPSYGSTY	450	YYGM 485

EXAMPLE 2: Identification and characterization of LRP binding synthetic antibodies

A. Phage Clonal ELISA of Synthetic Antibodies targeting LRP5 and LRP6.

Single point ELISAs were performed on 96-well Maxisorp plates coated with the ECDs of mouse LRP5-his protein or human Fc and blocked with BSA (0.5%). The plates were
5 incubated with monoclonal Fab-phage, or VH-phage and titers $>10^9$ phage/ml followed by incubation with horseradish peroxidase (HRP)-conjugated anti-M13 antibody. The wells were washed 8 times followed by incubations with 3,3',5,5'-tetramethylbenidine/ H_2O_2 peroxidase (TMB) substrate for 5-10 min. the reaction was stopped by adding 1M H_3PO_4 and the absorbance was measured spectrophotometrically at 450 nm in a microtiter plate reader.
10 The results are presented in Figure 4. The results demonstrate that the synthetic antibodies bound to LRP5. LRP5 binding antibodies, 2459, 2460 and 8716, are described in US provisional application no. 62/886,913, incorporated herein by reference.

Single point ELISAs were performed on 96-well Maxisorp plates coated with the ECDs of human LRP6-Fc protein chimeras. The plates were incubated with the monoclonal Fab-
15 phage, or VH-phage and titers $>10^9$ phage/ml followed by incubation with horseradish peroxidase (HRP)-conjugated anti-M13 antibody. The wells were washed 8 times followed by incubations with 3,3',5,5'-tetramethylbenidine/ H_2O_2 peroxidase (TMB) substrate for 5-10 min. the reaction was stopped by adding 1M H_3PO_4 and the absorbance was measured spectrophotometrically at 450 nm in a microtiter plate reader. The results are presented in
20 Figures 5A and 5B. The results demonstrate the synthetic antibodies bound to LRP6. LRP6 binding antibodies, 2539, 2540, and 2542 are described in US provisional application no. 62/886,918, incorporated herein by reference.

B. Identification of the CDRs of the synthetic antibodies targeting LRP5 and LRP6.

The CDRs of the LRP5-binding and LRP6-binding immunoglobulins set forth in Tables 3
25 and 4 were identified according to the INTERNATIONAL IMMUNOGENETICS INFORMATION SYSTEM (IMGT numbering system; Lefranc et al., 2003, Development and Comparative Immunology 27:55-77) and annotated as described in Persson et al. J Mol Biol. 2013 Feb 22;425(4):803-11, both incorporated herein by reference.

Table 3		LRP5-binding antibodies CDRs											
Antibody ID	Library	Antigen	SEQ ID NO:	L2	SEQ ID NO:	L3	SEQ ID NO:	H1	SEQ ID NO:	H2	SEQ ID NO:	H3	SEQ ID NO:
2459	F	mLRP5-his	SVSSA1	SASSLYS 2	492	ASYAPI	492	LSYYYM	527	SISSYGYTY	552	WSHVS GHYSGM	584
2460	F	mLRP5-his	SVSSA1	SASSLYS 2	130	SSYSLI	130	FSSSSI	528	SISSYGYTY	553	GGSGVSHYGSVYYSWWAL	585
8716	F	mLRP5-his	SVSSA1	SASSLYS 2	493	YWYYSP	493	FSSSSI	528	SISSYGYTY	553	SWAM	586
9931	F	mLRP5-his	SVSSA1	SASSLYS 2	494	SSSGHLI	494	LYYYSM	529	YISSYGYTS	554	WSHVVG AHYGWAI	587
9932	F	mLRP5-his	SVSSA1	SASSLYS 2	495	PYGYPI	495	IYYYSM	530	SISSYGYTS	555	SWWYWSYHGYAAM	588
9933	F	mLRP5-his	SVSSA1	SASSLYS 2	496	FHGLI	496	ISSYSM	531	SISSYGYTS	556	GYSYVWYGVYYHGYGAM	589
9934	F	mLRP5-his	SVSSA1	SASSLYS 2	497	YWFLI	497	LYYYI	531	SISSYGYTS	557	SGYGWYAM	590
9935	F	mLRP5-his	SVSSA1	SASSLYS 2	498	HSYGYPI	498	ISYSYM	530	SISSYGYTY	553	SYWGYWAAL	591
9936	F	mLRP5-his	SVSSA1	SASSLYS 2	499	YYAWYLI	499	ISYYSM	532	SISSYGYTY	552	GGVYYPSYAGWPYGM	592
9937	F	mLRP5-his	SVSSA1	SASSLYS 2	500	AAYSPI	500	ISSYSM	534	SISSYGYTY	558	HYAWWVGAF	593
9938	F	mLRP5-his	SVSSA1	SASSLYS 2	501	YYSWYPP	501	ISYSYM	310	SISSYGYTS	559	GGGAHGWGYSL	594
9939	F	mLRP5-his	SVSSA1	SASSLYS 2	502	SFYPI	502	LSSYSI	29	SISSYGYTS	560	SGPVYASYSWAWYYYGAL	595
9940	F	mLRP5-his	SVSSA1	SASSLYS 2	503	YYWYYP	503	LSYYI	533	SISSYSSY	561	YSWGAYGYGAM	596
9941	F	mLRP5-his	SVSSA1	SASSLYS 2	504	WGSP	504	FSSSSI	528	SISSSGSTY	562	YYHYSVPVYAAL	597
9942	F	mLRP5-his	SVSSA1	SASSLYS 2	505	YSSWYLI	505	LYYYSM	529	SISSSGSTY	562	SPYYGFYYS GFYHWVFGF	598
9943	F	mLRP5-his	SVSSA1	SASSLYS 2	506	GAYLI	506	LYSSM	534	YISSYGYTY	563	AAWGWHPAF	599
9944	F	mLRP5-his	SVSSA1	SASSLYS 2	507	HFYYPI	507	IYSSYI	535	YISSYGYTY	57	GGGFYAGGWYPYASYAWAF	600
9945	F	mLRP5-his	SVSSA1	SASSLYS 2	508	AFHSPI	508	LYSSM	534	YISSYGYTS	564	AWYSYVGL	601
9946	F	mLRP5-his	SVSSA1	SASSLYS 2	509	AWYPI	509	ISYSYM	538	SISSYGYTY	565	GVYGGGYWAGGYPAAL	602
12600	H	mLRP5-his	SVSSA1	SASDLYS 491	510	YAGAGLI	510	FTAYAM	536	SISSGGYTA	566	RSYFAL	603
12601	H	mLRP5-his	SVSSA1	SASDLYS 491	511	YSYPI	511	LGYYGI	537	GISSGGYTA	567	SISSSYHYFAL	604
12602	H	mLRP5-his	SVSSA1	SASDLYS 491	512	YYSRLI	512	FASAWM	538	RISPSGGYTA	568	STRWHSAYAYYSHAM	605
12603	H	mLRP5-his	SVSSA1	SASDLYS 491	513	SYFYPI	513	IGGAAM	539	GISASGGATA	569	SRPSWYWSYHYFAL	606
12604	H	mLRP5-his	SVSSA1	SASDLYS 491	514	GFFPI	514	FSSSSI	528	GISGSGATA	570	SYSGYSRGF	607
12605	H	mLRP5-his	SVSSA1	SASDLYS 491	515	SHSYPI	515	FASYGM	540	SISSPGGYTA	566	SYSFHYAWPRYHYGAL	608

Table 3		LRP5-binding antibodies CDRs													
Antibody ID	LRP5 – antibody		Antigen	L1	SEQ ID	L2	SEQ ID	L3	SEQ ID	H1	SEQ ID	H2	SEQ ID	H3	SEQ ID
	Library	NO:			NO:		NO:		NO:		NO:		NO:		NO:
12606	H	mLRP5-his	SVSSA I		SASDLYS	491	SHYYPI	286	IAGSSI	541	GISASGGYTA	571	YDSGYAWYYHDRAM	609	
12607	H	mLRP5-his	SVSSA I		SASDLYS	491	RGSGPI	516	LTGDW	542	GIYPSGGATA	572	FGSPHYGM	610	
12608	H	mLRP5-his	SVSSA I		SASDLYS	491	RYSGGLI	517	FTSYSM	543	GIYPSGGSTA	573	PRYYAAYSSGGF	611	
12609	H	mLRP5-his	SVSSA I		SASDLYS	491	SARYYYLI	518	LNAAM	544	GISASRGATA	574	SHSSGSRSRGL	612	
12610	H	mLRP5-his	SVSSA I		SASDLYS	491	SSYSLI	130	LAGAGM	545	RISTSGGYTA	575	YSFRYPYSYAM	613	
12611	H	mLRP5-his	SVSSA I		SASDLYS	491	SYRGYYLI	519	IGGAAM	539	GIYASGGATA	576	GSRYSGF	614	
13218	2539AM	mLRP5-his	SVSSA I		SASSLYS	2	FSWGLI	520	ISYYPM	546	YISPYSGYTF	577	HYFYFRWSRGM	615	
13219	2539AM	mLRP5-his	SVSSA I		SASSLYS	2	YSWLPI	521	IVFGSI	547	HIAPYYGFTY	578	VNFIRFRGM	616	
13378	2542AM	mLRP5-his	SVSSA I		SASSLYS	2	FFRPI	522	ISSNYI	548	SINPSYSYTF	579	AVRRSQGI	617	
13379	2539AM	mLRP5-his	SVSSA I		SASSLYS	2	YSRRLF	523	LYTSM	549	SISPFHGHTF	580	FPYFAWVGGM	618	
13380	2539AM	mLRP5-his	SVSSA I		SASSLYS	2	FIRVPI	524	ITYYSM	550	SITSYYGNNTD	581	SHYFPWTVL	619	
13558	2540AM	mLRP5-his	SVSSA I		SASSLYS	2	FYVPF	525	N/D		SIYPYYGFTD	582	ASYHASFDGL	620	
13559	2540AM	mLRP5-his	SVSSA I		SASSLYS	2	GYSGGQ	526	IADSSI	551	SISPYFSYTR	583	TSIFKRFAGM	621	

Table 4		LRP6 binding antibodies CDRs													
LRP6 – antibody															
Antibody ID	Library	Antigen	L1	SEQ ID NO:	L2	SEQ ID NO:	L3	SEQ ID NO:	H1	SEQ ID NO:	H2	SEQ ID NO:	H3	SEQ ID NO:	
2539	F	LRP6-Fc	SVSSA I		SASSLYS 2		YSWGPF 622		ISYSSI 363		YISSYYGYTY 51		AHYFPWAGAM 813		
2542	F	LRP6-Fc	SVSSA I		SASSLYS 2		YYWPI 623		ISSYYI 683		SIYSSYGYTS 741		TVRGSKKPYFSGWAM 814		
2540	F	LRP6-Fc	SVSSA I		SASSLYS 2		YYFLI 624		ISYSSI 363		SISPYYGYTY 742		SSYFPWFSA 815		
12612	H	LRP6-Fc	SVSSA I		SASDLYS 491		YRYYWRPI 625		FSGDAM 684		RISSSGGYTA 743		ASYYSNYYYGPAM 816		
12613	H	LRP6-Fc	SVSSA I		SASDLYS 491		GPWGLI 626		ISGAWM 685		RIYPSGGTTD 744		GWFSFRSYRSYYYSA 817		

Table 4		LRP6 binding antibodies CDRs													
LRP6 – antibody															
Antibody ID	Library	Antigen	L1	SEQ ID NO:	L2	SEQ ID NO:	L3	SEQ ID NO:	SEQ ID NO:	H1	SEQ ID NO:	H2	SEQ ID NO:	H3	SEQ ID NO:
I2614	H	LRP6-Fc	SVSSA	1	SASDLYS	491	RYSSPI	627	FTTYSI	686	GISTSGGYTD	745	SGHPRYYSRHSYGM	818	
I2615	H	LRP6-Fc	SVSSA	1	SASDLYS	491	YRYWYGPI	628	FAASGI	687	YISPSGGYTD	746	SYYSNYYYDDAM	819	
I2979	2539AM	LRP6-Fc	SVSSA	1	SASSLYS	2	YSWALF	629	IFSSM	688	GISSYYGFTY	747	AHYFPWAGAL	820	
I2980	2539AM	LRP6-Fc	SVSSA	1	SASSLYS	2	ISWGLI	630	IRYSSI	689	GIFSNYGPTT	748	AHYFRRARGL	821	
I2981	2539AM	LRP6-Fc	SVSSA	1	SASSLYS	2	YFSYPI	631	LSYSSI	344	FISSYYSFTH	749	SHYFPWAGAM	822	
I2982	2539AM	LRP6-Fc	SVSSA	1	SASSLYS	2	YRWALF	632	LAYSSI	690	SISSYYGFTY	750	AHYFPWAGGM	823	
I2983	2539AM	LRP6-Fc	SVSSA	1	SASSLYS	2	YSRILF	633	LRYASM	691	YIASFYGDTY	751	SYYYPRSRGM	824	
I2984	2539AM	LRP6-Fc	SVSSA	1	SASSLYS	2	YSLRPI	634	LSFSSI	370	HISPPYGYTH	752	SHYFPWAVAM	825	
I2985	2539AM	LRP6-Fc	SVSSA	1	SASSLYS	2	YSRGLI	635	IFYSSI	692	YISSYYSYTY	58	SHYFPWAVAM	825	
I2986	2539AM	LRP6-Fc	SVSSA	1	SASSLYS	2	YFWGLI	636	LSYSSM	364	NITPYYGYTS	753	SHYFPWFAAM	826	
I2987	2539AM	LRP6-Fc	SVSSA	1	SASSLYS	2	YSWGLI	637	INYSSI	693	GISSYYSYTY	754	ANYFPWAYAM	827	
I2988	2539AM	LRP6-Fc	SVSSA	1	SASSLYS	2	DSWGLF	638	ISYSSM	358	LITSYYGYTT	755	AHYFPWVVGGM	828	
I2989	2539AM	LRP6-Fc	SVSSA	1	SASSLYS	2	YYWGLI	639	LSYSSI	344	FISSYYGYTY	73	SHYFPWAGAL	829	
I3002	2540AM	LRP6-Fc	SVSSA	1	SASSLYS	2	YYYLJ	640	IAFSSI	694	SISSYYGYTS	555	SSYFPWFSAI	830	
I3003	2540AM	LRP6-Fc	SVSSA	1	SASSLYS	2	YYILJ	641	LFYSSM	695	FISPPYSFTN	756	SSYFPWLSDM	831	
I3004	2540AM	LRP6-Fc	SVSSA	1	SASSLYS	2	HSFPI	642	ISYSSM	358	NITSYYGYTT	757	SSYFPWVSGM	832	
I3005	2540AM	LRP6-Fc	SVSSA	1	SASSLYS	2	YHHLJ	643	ISFASM	696	SISPPYYSYTS	758	SSYFPWFSAI	815	
I3006	2540AM	LRP6-Fc	SVSSA	1	SASSLYS	2	YYSPI	644	IFYSSI	692	SISPPYGYTD	759	SSYLPWFSAI	833	
I3007	2540AM	LRP6-Fc	SVSSA	1	SASSLYS	2	YYYPJ	645	ISFYSI	697	SISSYYSYTD	760	SSYFPWFTAL	834	
I3008	2540AM	LRP6-Fc	SVSSA	1	SASSLYS	2	YHFLJ	646	ISYSSI	363	AIYSYYSYTI	761	SSYFPWVGGF	835	
I3009	2540AM	LRP6-Fc	SVSSA	1	SASSLYS	2	YYFPI	647	LSYSSI	344	SISPPYYSYTS	758	SSRFPWFYGL	836	
I3010	2540AM	LRP6-Fc	SVSSA	1	SASSLYS	2	YDFLJ	648	ISYSSM	358	SISSYYGYTS	555	SSYFPWFSAI	830	
I3394	2540AM	LRP6-Fc	SVSSA	1	SASSLYS	2	YYILJ	641	LFYASM	698	SISSYYGYTS	555	SSYFPWFSAI	815	
I3395	2540AM	LRP6-Fc	SVSSA	1	SASSLYS	2	YYYLJ	640	ISYSSI	363	SISPPYYGFTL	762	SSYFPWFSAI	815	
I3396	2540AM	LRP6-Fc	SVSSA	1	SASSLYS	2	YYFPI	647	IAYASI	699	SISSYYSYTY	763	SSYFPWFSAI	815	
I3397	2540AM	LRP6-Fc	SVSSA	1	SASSLYS	2	YYFPI	647	ITYSSI	361	SISSYYGYTA	764	SSYFPWSSAI	837	
I3398	2540AM	LRP6-Fc	SVSSA	1	SASSLYS	2	NSFLJ	649	ISRSSI	700	GISSYYGYTR	765	SHYFPWLSAI	838	

Table 4			LRP6 binding antibodies CDRs											
LRP6 – antibody														
Antibody ID	Library	Antigen	L1	SEQ ID NO:	L2	SEQ ID NO:	L3	SEQ ID NO:	H1	SEQ ID NO:	H2	SEQ ID NO:	SEQ ID NO:	
13399	2540AM	LRP6-Fc	SVSSA 1	SASSLYS 2	YYNPI	650	ISYASI	701	SISPPYGYTR	766	ASYYPWFSAM	839		
13400	2540AM	LRP6-Fc	SVSSA 1	SASSLYS 2	YGFLI	651	LDYSSI	702	SISPPYSYTT	767	SSYTPWRYAI	840		
13401	2540AM	LRP6-Fc	SVSSA 1	SASSLYS 2	YYFLI	624	ISYSSI	363	SISPPYSYTN	74	SSYFPWISAM	841		
13402	2540AM	LRP6-Fc	SVSSA 1	SASSLYS 2	YYFPI	647	IYYYSM	530	GISSYYSYTS	768	SSYFPWISAL	842		
13403	2540AM	LRP6-Fc	SVSSA 1	SASSLYS 2	YYFPI	647	ISYSSM	358	SISPPYYSFTS	769	SSRFPWISGM	843		
13404	2540AM	LRP6-Fc	SVSSA 1	SASSLYS 2	YNFLI	652	ISYSSI	363	SISPPYGYFTS	68	SSYFPWFSAL	830		
13405	2540AM	LRP6-Fc	SVSSA 1	SASSLYS 2	YYFPI	647	ISYSSI	363	SISPPYGYFTS	68	SPYFPWSSAL	844		
13406	2540AM	LRP6-Fc	SVSSA 1	SASSLYS 2	YYFPI	647	LSYSAI	703	YISPPYGYTY	65	ASYLPWFSAM	845		
13407	2540AM	LRP6-Fc	SVSSA 1	SASSLYS 2	SSFLI	653	LSGFGI	704	FISSPPYGYTA	770	SSYLPWITAM	846		
13408	2540AM	LRP6-Fc	SVSSA 1	SASSLYS 2	YVFLI	654	LSFASM	705	SISPPYGYTP	771	SAYFPWFAAL	847		
13409	2540AM	LRP6-Fc	SVSSA 1	SASSLYS 2	YYFPI	647	LAYNSI	706	SIFSPYSYTN	772	SSYFPWFSAM	815		
13410	2540AM	LRP6-Fc	SVSSA 1	SASSLYS 2	FYGP	655	ISFSSI	357	SISPPYGYTL	773	SSYFPWFFAM	848		
13411	2540AM	LRP6-Fc	SVSSA 1	SASSLYS 2	YFFLI	656	LYYSNI	707	YISPPYGYTS	774	SSYFPWFSAL	830		
13412	2540AM	LRP6-Fc	SVSSA 1	SASSLYS 2	YYGP	657	IHYYSI	708	SISPPYSFTD	775	SSYFPWFSAM	815		
13413	2540AM	LRP6-Fc	SVSSA 1	SASSLYS 2	YDFLI	648	ISFASI	709	SIYSPPYFTN	776	SSHFPWVSAL	849		
13414	2540AM	LRP6-Fc	SVSSA 1	SASSLYS 2	YYSPI	644	IYYSSM	378	SISPPYGYTS	557	SSYFPWVSAM	850		
13415	2540AM	LRP6-Fc	SVSSA 1	SASSLYS 2	YYFLI	624	LSYFSM	710	SIYSPPYGFTN	777	SSHFPWFSAM	851		
13422	2540AM	LRP6-Fc	SVSSA 1	SASSLYS 2	FYGP	655	LFYSSM	695	TISPPYGYTS	778	SSYFPWFSAL	830		
13428	2540AM	LRP6-Fc	SVSSA 1	SASSLYS 2	YTLPI	658	ISFSSM	380	HISPPYGYFTS	779	SSYFPWFSAL	830		
13430	2540AM	LRP6-Fc	SVSSA 1	SASSLYS 2	YRFPI	659	ISYFSI	711	SISPPYSYTN	74	SSYFPWFSAI	852		
13431	2540AM	LRP6-Fc	SVSSA 1	SASSLYS 2	YHLLI	660	LYYNSI	712	AIHPYPPGYTS	780	SSRFPWFPPAM	853		
13432	2540AM	LRP6-Fc	SVSSA 1	SASSLYS 2	YSFPI	661	LSYASI	713	SISPPYGYTG	781	SSYFPWFSAM	815		
13435	2540AM	LRP6-Fc	SVSSA 1	SASSLYS 2	YFLPI	662	LSYSSM	364	SISPPYGYFTY	782	SSYFPWFSAL	830		
13436	2540AM	LRP6-Fc	SVSSA 1	SASSLYS 2	YYFPI	647	ISYASM	714	SISPPYGYTD	783	SSYVPWFYAL	854		
13437	2540AM	LRP6-Fc	SVSSA 1	SASSLYS 2	YDNL	663	ITYSSI	361	SISPPYGYFTS	68	SSYFPWFSAM	815		

Table 4		LRP6 binding antibodies CDRs												
LRP6 – antibody														
Antibody ID	Library	Antigen	L1	SEQ ID NO:	L2	SEQ ID NO:	L3	SEQ ID NO:	H1	SEQ ID NO:	H2	SEQ ID NO:	SEQ ID NO:	
13438	2540AM	LRP6-Fc	SVSSA	1	SASSLYS	2	YLFPI	664	ISYYSI	715	SIYSYGYTT	784	ASYFPWFAM	855
13439	2540AM	LRP6-Fc	SVSSA	1	SASSLYS	2	YFFPI	665	LSHSSI	716	SISSHYGYTH	785	SSYFPWFFAL	856
13440	2540AM	LRP6-Fc	SVSSA	1	SASSLYS	2	YYIPI	666	IAYNSM	717	SISSYYSYTD	760	SSYFPWFSA	830
13441	2540AM	LRP6-Fc	SVSSA	1	SASSLYS	2	FNFLI	667	LSYSSI	344	YISSYGYTA	786	SSYYPWFSA	857
13442	2540AM	LRP6-Fc	SVSSA	1	SASSLYS	2	YNYLI	668	LAYSSI	690	SISSYGYTY	787	SSYLPWVSAL	858
12990	2542AM	LRP6-Fc	SVSSA	1	SASSLYS	2	YYWPI	623	ISLFEM	718	SIYSSYSYTS	788	SFNGAKKPYFSGWAM	859
12991	2542AM	LRP6-Fc	SVSSA	1	SASSLYS	2	YYWPI	623	IPSYFM	719	SIYSSYSYTA	789	NVVVSRKKPYFFRSAM	860
12992	2542AM	LRP6-Fc	SVSSA	1	SASSLYS	2	YYWPI	623	ISSSYM	348	SIYSSYSYTG	790	TVPGSKRPYFASLAI	861
12993	2542AM	LRP6-Fc	SVSSA	1	SASSLYS	2	YYWPI	623	ISSYFI	720	SIYPSYSYTS	791	NVRGFRKKPYFSRWAM	862
12994	2542AM	LRP6-Fc	SVSSA	1	SASSLYS	2	YYWPI	623	LSSYYM	721	SIYSRFSYTA	792	NVPGIKRPYFTAWAM	863
12995	2542AM	LRP6-Fc	SVSSA	1	SASSLYS	2	SYWPI	669	LHSYYI	722	NIYSSYGYTS	793	TIRGSKRPYFAASGL	864
12996	2542AM	LRP6-Fc	SVSSA	1	SASSLYS	2	YYWPI	623	LSSYYM	721	SIYSSYSYTS	788	TARENKKPYFSGWAM	865
12997	2542AM	LRP6-Fc	SVSSA	1	SASSLYS	2	YYRPI	670	IGSYYM	723	AIYSPYGYTS	794	TLPLSKKPYFSLWAF	866
12998	2542AM	LRP6-Fc	SVSSA	1	SASSLYS	2	YYWPI	623	LGSYYI	724	TIYSYYSYTS	795	TFHGSKKPYFSGRAM	867
12999	2542AM	LRP6-Fc	SVSSA	1	SASSLYS	2	YYWPI	623	LTSYYI	725	SIYPSYSYTS	791	SIRGSKKPYFSRLAL	868
13000	2542AM	LRP6-Fc	SVSSA	1	SASSLYS	2	YYWPI	623	ISTYYM	726	SIYPSYSYTS	791	SVRGSKKPYFSHWAM	869
13001	2542AM	LRP6-Fc	SVSSA	1	SASSLYS	2	YYWPI	623	ISSNYI	548	SIYSSFGYTS	796	NVRTSKKPYFPLWAM	870
13011	2460AM	LRP6-Fc	SVSSA	1	SASSLYS	2	ASNLI	671	IPLYTI	727	SISPSYTY	797	GVSGGSNYISIFRNSGM	871
													GGLSVSHYNYVHSFKWG	872
13012	2460AM	LRP6-Fc	SVSSA	1	SASSLYS	2	SSYSLI	130	IFSTSI	728	SITSAYSYTY	798	SCSSFCNYVSNYNARRA	873
13013	2460AM	LRP6-Fc	SVSSA	1	SASSLYS	2	SAITPI	672	ISYSTM	729	SIVSSYSRTY	799	GNARVSNYISSYYAWRA	874
13014	2460AM	LRP6-Fc	SVSSA	1	SASSLYS	2	FSYSPF	673	LSSSGM	730	AIYPFDGYTY	800	GGYGFSYYNSVTFYRWA	875
13015	2460AM	LRP6-Fc	SVSSA	1	SASSLYS	2	SSYNLI	674	FSSSSI	528	GIFSFSYTY	801		

Table 4		LRP6 binding antibodies CDRs											
Antibody ID	Library	Antigen	L1	SEQ ID NO:	L2	SEQ ID NO:	L3	SEQ ID NO:	H1	SEQ ID NO:	H2	SEQ ID NO:	SEQ ID NO:
13016	2460AM	LRP6-Fc	SVSSA	1	SASSLYS	2	SYNPF	675	IRGPSI	731	SISPFCEGFTY	802	VSGGVCNSLHNYFYKRG
13017	2460AM	LRP6-Fc	SVSSA	1	SASSLYS	2	SSTVPF	676	FSSTY/M	732	SITPSYGYTN	803	SSSDISIDNSIRYTWGAM
13018	2460AM	LRP6-Fc	SVSSA	1	SASSLYS	2	SSYSLI	130	INSSFM	733	SISPSYGYTY	804	GHSQVTNFISFYRRRA
13019	2460AM	LRP6-Fc	SVSSA	1	SASSLYS	2	SNYSLF	677	IFGYSM	734	SIFPCCSNTY	805	GVSGLDNNRTASYSRMG
13020	2460AM	LRP6-Fc	SVSSA	1	SASSLYS	2	STYSLI	678	FSSTI	735	SIAPSFGHTY	806	GAASVNHIGSLYVSWRG
13021	2460AM	LRP6-Fc	SVSSA	1	SASSLYS	2	SAFSLI	679	LSSTM	736	SISPYYSYTR	807	HGSGLSHNATTSSYYRSAI
13022	2460AM	LRP6-Fc	SVSSA	1	SASSLYS	2	SSYSLI	130	ISYTSI	737	SIFSTYSYTY	808	SSSAVSRNSIVYYSR TAM
13023	2460AM	LRP6-Fc	SVSSA	1	SASSLYS	2	SSYTP	680	FSSSV/M	738	SIAPSYSTY	809	GVCRVSNYSYFYSRGA
13024	2460AM	LRP6-Fc	SVSSA	1	SASSLYS	2	YSNSLI	681	ISYSTM	729	SITPYYSTTS	810	AISHVAHYDNDVIYSKWGI
13025	2460AM	LRP6-Fc	SVSSA	1	SASSLYS	2	SSYSLI	130	IASYSM	739	FISPYHSYTD	811	GHSVLSNYVSIHYWAG
13026	8716AM	LRP6-Fc	SVSSA	1	SASSLYS	2	YWAYFSP	682	FSYSSM	740	SISSRYGYTY	812	SWAM

EXAMPLE 3: Tetravalent binding antibody molecule formats

A. We generated various formats of tetravalent binding antibody molecules comprising pan-FZD and LRP5/6 antibody fragments, e.g., scFv, diabodies and Fab, on either end of an Fc domain, see Table 5 and Figure 6, and assayed their Wnt agonist activity. DNA fragments encoding antibody variable domains were either amplified by PCR from phagemid DNA template or were constructed by chemical synthesis (Twist Biosciences). The DNA fragments were cloned into mammalian expression vectors (pSCSTa). Bispecific diabodies and IgGs contained an optimized version of a “knobs-in-holes” heterodimeric Fc (Ridgway et al. Protein Eng. 9, 617–621 (1996)). Diabody domains were arranged in a VH-VL orientation with the variable domains separated by a short GGGGS linker (SEQ ID NO: 886), which favors intermolecular association between VH and VL domains and thus favors diabody formation. To produce diabody fusion constructs, the diabody chains were fused to human IgG1 Fc. Diabody-fc-diabody proteins were constructed as VH-x-VL-y-[human IgG1 Fc]-z-VH-x-VL where linkers are x= GGGGS (SEQ ID NO: 886), y= LEDKTHTKVEPKSS (SEQ ID NO: 887), and z= SGSETPGTSESATPESGGG (SEQ ID NO: 888). In this format, the human IgG1 Fc or knob-in-hole IgG1 Fc fragments spanned from position 234-478 (Kabat numbering). For scFv-Fc fusions, the variable domains were arranged in a VL-VH orientation and were connected by a long GTTAASGSSGGSSSGA linker (SEQ ID NO: 889), which favors intramolecular association between VH and VL domains and thus favors scFv formation. Variants with a Fab domain fused to the C-terminus of the Fc were generated via chemical synthesis (Twist Biosciences). For all constructs, the entire coding region was cloned into a mammalian expression vector in frame with the secretion signal peptide.

These various of tetravalent binding antibody molecules comprising pan-specific FZD and LRP5/6 antibody fragments were tested in a TOPFLASH assay to monitor beta catenin-mediated gene reporter activity. Proteins were compared against the native ligand Wnt3a. Assays were performed by plating TOPFLASH cells to ~70% confluency in a 96-well tissue culture treated plate. Agonists were diluted in DMEM to provide a final assay concentration of 0.046 nM – 100 nM and cells were treated overnight at 37°C under 5% CO₂. Luciferase expression was quantified using the Dual-Luciferase Reporter Assay System (Promega) in 96-well black plates in accordance with the manufacturer’s instructions. Briefly, HEK293T cells were transduced with lentivirus coding for the pBARl reporter (Biechele and Moon in *Wnt Signaling: Pathway Methods and Mammalian Models*, E. Vincan, Ed. (Humana Press, Totowa,

NJ, 2008), pp. 99–110) and with Renilla Luciferase as a control to generate a Wnt- β catenin signaling reporter cell line. $1-2 \times 10^3$ cells in 120 μ l were seeded in each well of 96-well plates for 24 hours prior to transfection or stimulation. The following day, FZD Agonists or Ab protein was added, and following 15-20 hours of stimulation, cells were lysed and luminescence was

5 measured in accordance with the dual luciferase protocol (Promega) using an Envision plate reader (PerkinElmer). For the FZD4 Agonist assay, FZD4 cDNA was transfected for 6 hours prior to adding the FZD Agonist. For the Wnt inhibition assays, Wnt1 was introduced by cDNA transfection or WNT3A protein was applied for 6 hours prior to the addition of Ab protein. All assays were repeated at least three times. The results are presented in Table 5. As shown in

10 Table 5, each of the tetravalent formats activate FZD signaling to differing degrees when clustering FZD4 and LRP5. These formats were also evaluated for stability, homogeneity and yield production from Expi293 (Figures 3 and 9). From these analyses, the Diabody-Fc-Fab format provides the best balance of activity, expression, stability. Finally, we applied the same modality arrangement for FZD5 and LRP6 and we observed potent agonist activity. The results

15 in Table 5 show that the various tetravalent modalities elicit WNT agonism and that engagement of 2 LRP5/6 epitopes produces WNT signaling activity (maxima) higher than with 1 LRP5/6 epitope.

Table 5

Molecule	Modality	Paratopes	Binding specificity (Paratope Stoichiometry)	Topflash EC50 (nM) Avg Stdev	% Wnt Maxima Avg Stdev	
Wnt3a	Recombinantly produced ligand	NA	FZD/LRP	6	3	100% ND
CM0011	FZD Diabody-Fc-LRP Diabody	5019 (FZD Diabody); 2539, 2542 (LRP diabody)	FZD1/2/4/5/7/8, LRP6 ¹ , LRP6 ³ (2:1:1)	0.3	0.1	63% 10%
ANT-4	FZD Diabody-Fc-LRP Fab	5016(FZD Diabody); 2540,2542 (LRP Fab)	FZD1/2/4/5/7/8, LRP6 ¹ , LRP6 ³ (2:1:1)	0.5	0.1	63% 6%
ANT-5	FZD Diabody-Fc-LRP Fab	5016(FZD Diabody); 2540,2542 (LRP Fab)	FZD1/2/4/5/7/8, LRP6 ¹ , LRP6 ³ (2:1:1)	0.5	0.1	59% 8%
CT0001	FZD IgG-LRP Diabody	5016(FZD IgG); 2539,2542 (LRP Diabody)	FZD1/2/4/5/7/8, LRP6 ¹ , LRP6 ³ (2:1:1)	0.3	0.1	57% 17%
CT0002	LRP Diabody-Fc-FZD scFv (VL-VH)	2539, 2542 (LRP Diabody); 5016 (FZD scFv)	FZD1/2/4/5/7/8, LRP6 ¹ , LRP6 ³ (2:1:1)	0.5	0.1	55% 22%
CM0126	FZD Diabody-Fc-LRP Diabody	5016(FZD Diabody); 2539, 2542 (LRP Diabody)	FZD1/2/4/5/7/8, LRP6 ¹ , LRP6 ³ (2:1:1)	0.3	0.2	52% 1%
ANT-1	LRP Diabody-Fc-FZD Fab	2540, 2542 (LRP Diabody); 5016 (FZD Fab)	FZD1/2/4/5/7/8, LRP6 ¹ , LRP6 ³ (2:1:1)	0.7	0.5	49% 22%
CT0003	FZD Diabody-Fc-LRP scFv	5016 (FZD Diabody); 2539, 2542 (LRP scFv)	FZD1/2/4/5/7/8, LRP6 ¹ , LRP6 ³ (2:1:1)	0.4	0.1	42% 16%
ANT-2	LRP Diabody-Fc-FZD Fab	2540, 2542(LRP Diabody); 5016(FZD Fab)	FZD1/2/4/5/7/8, LRP6 ¹ , LRP6 ³ (2:1:1)	0.3	0.1	38% 25%
CM0042	FZD Diabody-Fc-LRP Diabody	5019(FZD Diabody); 2539 (LRP Diabody)	FZD1/2/4/5/7/8, LRP6 ³ (2:2)	0.9	0.8	33% 22%
CM0302	FZD IgG-LRP Diabody	5056(FZD IgG)-2539(LRP Diabody)	FZD1/2/4/5/7/8/10, LRP6 ³ (2:2)	0.3	0.2	18% 8%
CM0303	FZD IgG-LRP scFv	5056(FZD IgG); 2539(LRP scFv)	FZD1/2/4/5/7/8/10, LRP6 ³ (2:2)	3.7	2.1	17% 13%
CM0299	FZD IgG-LRP Diabody	5016(FZD IgG)-, 2539(LRP Diabody)	FZD1/2/4/5/7/8, LRP6 ³ (2:2)	0.1	0.1	14% 5%

Table 5 Cont'

Molecule	Modality	Paratopes	Binding specificity (Paratope Stoichiometry)	Topflash EC ₅₀ (nM)	% Wnt Maxima	
	Recombinantly produced ligand			Avg Stdev	Avg	Stdev
Wnt3a		NA	FZD/LRP	6	3	100% ND
CM0300	FZD IgG-LRP scFv	5016(FZD IgG)-, 2539(LRP scFv)	FZD1/2/4/5/7/8, LRP6 ³ (2:2)	0.3	0.2	5% 2%
CM0301	FZD IgG-LRP scFv	5016(FZD IgG)-, 2542(LRP scFv)	FZD1/2/4/5/7/8, LRP6 ¹ (2:2)	0.2	0.2	1% 1%
CM0156	Diabody-Fc-Diabody	4275	no FZD/LRP binding	NA	NA	NA NA
4275	IgG	4275	no FZD/LRP binding	NA	NA	NA NA

B. Diabody-Fc-Fab format FZD4 Agonists.

FZD Agonists having a bispecific LRP5-binding diabody and a FZD4 binding domain comprising FZD4-binding Fabs (FZD4 Agonists), a FZD5 binding domain comprising FZD5-binding Fabs (FZD5 Agonists), or a FZD binding domain that binds multiple FZD (pan-FZD Agonist) were generated using a knob-in-holes system. Briefly, the constructs were generated by chemical synthesis (Twist Biosciences) or by standard molecular biology techniques in a mammalian expression vector (pSCSTa). Diabody constructs were arranged in a VH-VL manner with a short (GGGGS (SEQ ID NO: 886)) linker linking the VH and VL to favor intermolecular pairing. For bispecific diabody arrangements, the variable domains for paratopes A and B, respectively, were arranged as VH(A) – VL(B) on the Hole Fc chain and VH(B)-VL(A) on the Knob Fc chain to facilitate proper paratope formation. Diabodies were fused to the N-terminus of an optimized knob-in-holes heterodimeric Fc (Ridgway et al. Protein Eng. 9, 617–621 (1996) via a GGGSGGGGSEPKSS linker (SEQ ID NO: 890). The Fc region also contains the effector-null mutations D278A and N314G (Kabat numbering), corresponding to D655A/N297G (EU numbering). Fab domains were fused to the C-terminus of the heterodimeric Fc via a GGGSGGGSGGGSGGGSGSTG linker (SEQ ID NO: 891). Directly to this linker was fused the N-terminus of the Fab VH domain followed by CH1, terminating at T238 (Kabat numbering). This Fab pairs with a standard kappa light chain which was cloned as described above. For all constructs, the entire coding region was cloned into a mammalian expression vector in frame with the secretion signal peptide.

In addition, Diabody-fc-Fab formats were constructed as VH-x-VL-y-[human IgG1 Fc]-z-VH where linkers are x= GGGGS (SEQ ID NO: 886), y= GGGSGGGGSEPKSSDKTHT (SEQ ID NO: 892), and z= GGGSGGGSGGGSGGGSGSTG (SEQ ID NO: 891). Diabody domains were arranged in a VH-VL orientation with the variable domains separated by a short GGGGS linker (SEQ ID NO: 886), which favors intermolecular association between VH and VL domains and thus favors diabody formation. Further, the Fc region may exhibit attenuated effector functions due amino acid mutations to N297G and D265A (DANG) variants or L234A, L235A, P331S (LALAPS) variants, and with the Fc region further comprising knob-in-hole heterodimerization variants Merrimack, Merchant or Merchant S:S.

Figure 7 is an illustration of the Diabody-Fc-Fab format FZD4 Agonists having a LRP5 binding domain comprised of a diabody that is bivalent and bispecific for LRP5 and a FZD4

binding domain comprised of two FZD4 binding Fab fragments formed by a VL and CL1 of the light chain construct pairing with the VH and CH1 of each of the heavy chain hole and heavy chain knob constructs. Table 12 presents the amino acid sequences of heavy chains and light chains of FZD4 Agonists ANT's (Diabody-Fc-Fab format): the heavy chain knob construct (ANT16 knob), the heavy chain hole construct (ANT hole) and the light chain construct. The light chain and heavy chain variable CDRs are in bold underlined italics.

Figure 16A depicts Diabody-Fc-Fab format FZD4 Agonists having Fc regions with attenuated effector functions due to amino acid mutations, e.g., N297G (NG) and D265A, (DANG) variants, and/or LALAPS variants, and with the Fc region further comprising knob-in-hole heterodimerization variants Merrimack, Merchant or Merchant S:S

B. IgG-Diabody format FZD4 Agonists.

FZD Agonists having two FZD-binding Fabs forming an N-terminal binding domain and a bispecific LRP5/6 binding diabody forming the C-terminal binding domain and an Fc domain were generated using a knob-in-holes system. IgG-Diabody proteins were constructed as VH-[human IgG1 Fc]-y- VH-x-VL where linkers are x= GGGGS (SEQ ID NO: 886) and y= GGGSGGGSGGGSGGGSGSTG (SEQ ID NO: 891).

Figure 15 presents an illustration of the IgG-Diabody format FZD4 Agonists having an FZD binding domain comprising two Fab fragments attached to the N- terminus of the Fc domain with each Fab binding an FZD. The LRP5/6 co-receptor binding domain is attached to the C-terminus of the Fc domain and is composed of a diabody that binds two different sites on the co-receptor, e.g., a Wnt1 site (E1-E2) and a Wnt3 site (E3-E4) on LRP5/6. The Fabs may be specific for a particular FZD, e.g. FZD4, or may be pan-specific, binding to more than one FZD, e.g., to FZD4 and one or more other FZD.

Figure 16B depicts IgG-Diabody FZD4 Agonists having Fc regions with attenuated effector functions due to amino acid mutations, e.g., N297G (NG) and D265A, (DANG) variants, and/or LALAPS variants, and with the Fc region further comprising knob-in-hole heterodimerization variants Merrimack, Merchant or Merchant S:S. Table 13 presents the amino acid sequences of heavy chains and light chains of FZD4 Agonist, ANT39 (Diabody-Fc-Fab format) and ANT39wi (IgG-Diabody format): the heavy chain knob construct (ANT39 and ANT39i knob), the heavy chain hole construct (ANT39 and ANT39i hole) and the light chain construct. Also included in Table 13 are amino acid sequences of heavy chains

and light chains of FZD4 Agonist, ANT39 and ANT39i variants DANG, LALAPS, LALAPS Merchant and LALAPS Merchant S-S. The light chain and heavy chain variable CDRs are in bold underlined italics.

Table 6A		LRP5 Diabody Site 1					LRP5 Diabody Site 2				
Molecule	[1]	[2]	SEQ ID NO	[3]	SEQ ID NO	[4]	SEQ ID NO	[5]	SEQ ID NO	[6]	SEQ ID NO
		CDR-H1		CDR-H2		CDR-H3		CDR-L2		CDR-L3	
ANT16-Hole	D	FSSSI	528	SISSSYGYTY	553	SWAM	586	SASDLYS	491	YAGAGLI	510
ANT16-Knob	D	FTAYAM	536	SIYPSGGYTA	566	RSYYFAL	603	SASSLYS	2	YWAYYSPI	493
ANT18-Hole	N	LSYYM	527	SIYSSYGYTY	552	WSHVS GHYSGM	584	SASSLYS	2	SSYSLI	130
ANT18-Knob	N	FSSSI	528	SISSSYGYTY	553	GGSGVSHYGSVYYSWWAL	585	SASSLYS	2	ASYAPI	492
ANT20-Hole	N	LSYYM	527	SIYSSYGYTY	552	WSHVS GHYSGM	584	SASDLYS	491	YAGAGLI	510
ANT20-Knob	D	FTAYAM	536	SIYPSGGYTA	566	RSYYFAL	603	SASSLYS	2	ASYAPI	492
ANT21-Hole	N	FSSSI	528	SISSSYGYTY	553	SWAM	586	SASSLYS	2	SSYSLI	130
ANT21-Knob	N	FSSSI	528	SISSSYGYTY	553	GGSGVSHYGSVYYSWWAL	585	SASSLYS	2	YWAYYSPI	493
ANT36-Hole	N	LSYYM	527	SIYSSYGYTY	552	WSHVS GHYSGM	584	SASSLYS	2	SSYSLI	130
ANT36-Knob	N	FSSSI	528	SISSSYGYTY	553	GGSGVSHYGSVYYSWWAL	585	SASSLYS	2	ASYAPI	492
ANT39-Hole	D	FSSSI	528	SISSSYGYTY	553	SWAM	586	SASDLYS	491	YAGAGLI	510
ANT39-Knob	D	FTAYAM	536	SIYPSGGYTA	566	RSYYFAL	603	SASSLYS	2	YWAYYSPI	493
ANT42-Hole	D	FSSSI	528	SISSSYGYTY	553	SWAM	586	SASDLYS	491	YAGAGLI	510
ANT42-Knob	D	FTAYAM	536	SIYPSGGYTA	566	RSYYFAL	603	SASSLYS	2	YWAYYSPI	493

In all the molecules the LRP5 Diabody site 2 CDR-L1 is SVSSA (SEQ ID NO: 1)

Table 6B		FZD4 FAb							
Molecule	[7]	[8]	SEQ ID NO:	[9]	SEQ ID NO:	[10]	SEQ ID NO:	[11]	SEQ ID NO:
		CDR-H1		CDR-H2		CDR-H3		CDR-L3	
ANT16-Hole	T	LSSYSM	24	YISSYYGYTY	51	PAPGHWGF	79	WYYAPI	3
ANT16-Knob	T	LSSYSM	24	YISSYYGYTY	51	PAPGHWGF	79	WYYAPI	3
ANT18-Hole	T	LSSYSM	24	YISSYYGYTY	51	PAPGHWGF	79	WYYAPI	3
ANT18-Knob	T	LSSYSM	24	YISSYYGYTY	51	PAPGHWGF	79	WYYAPI	3
ANT20-Hole	T	LSSYSM	24	YISSYYGYTY	51	PAPGHWGF	79	WYYAPI	3
ANT20-Knob	T	LSSYSM	24	YISSYYGYTY	51	PAPGHWGF	79	WYYAPI	3
ANT21-Hole	T	LSSYSM	24	YISSYYGYTY	51	PAPGHWGF	79	WYYAPI	3
ANT21-Knob	T	LSSYSM	24	YISSYYGYTY	51	PAPGHWGF	79	WYYAPI	3
ANT36-Hole	T	LSSYSM	24	YISSYDSITD	61	PAVGHMAF	90	WYNAPI	12
ANT36-Knob	T	LSSYSM	24	YISSYDSITD	61	PAVGHMAF	90	WYNAPI	12
ANT39-Hole	T	LSSYSM	24	YISSYDSITD	61	PAVGHMAF	90	WYNAPI	12
ANT39-Knob	T	LSSYSM	24	YISSYDSITD	61	PAVGHMAF	90	WYNAPI	12
ANT42-Hole	T	SDFYFI	164	TIYPIFIGNTY	194	AFPGSYHPF	220	STGSLI	135
ANT42-Knob	T	SDFYFI	164	TIYPIFIGNTY	194	AFPGSYHPF	220	STGSLI	135

In all the molecules the FZD FAB CDR-L1 and CDR-L2 are respectively SVSSA (SEQ ID NO: 1) and SASSLYS (SEQ ID NO: 2)

Table 6C		LRP6 Diabody Site 1				LRP6 Diabody Site 2			
Molecule		[1]	[2]	SEQ ID NO	[3]	SEQ ID NO	[4]	SEQ ID NO	SEQ ID NO
			CDR-H1			CDR-H2	CDR-H3	CDR-L2	CDR-L3
ANT59-Hole	D	ISSYFI	720	SIYPSYSYTS	791	NVRGFRKPYFSRWAM	862	SASSLYS	2
ANT59-Knob	D	LSHSSI	716	SISSHYGYTH	785	SSYFPWFFAL	856	SASSLYS	2
ANT9-Hole	D	ISSYFI	720	SIYPSYSYTS	791	NVRGFRKPYFSRWAM	862	SASSLYS	2
ANT9-Knob	D	LSHSSI	716	SISSHYGYTH	785	SSYFPWFFAL	856	SASSLYS	2

In all the molecules the CDR-L1 of all LRP abs are SVSSA (SEQ ID NO: 1)

Table 6D		FZD Paratope								
Molecule	FZD Specificity	7	H1 (8)	SEQ ID NO	H2 (9)	SEQ ID NO	H3 (10)	SEQ ID NO	L3 (11)	SEQ ID NO
			CDR-H1		CDR-H2		CDR-H3		CDR-L3	
ANT59-Hole	5	N	IAYSSM	365	TIYPSYSSTY	462	YYAM	484	AHYFPI	285
ANT59-Knob	5	N	IAYSSM	365	TIYPSYSSTY	462	YYAM	484	AHYFPI	285
ANT9-Hole	1,2,4,5,7,8	N	IHSSSI	893	ATYSSFGSIT	894	YHHFPGYAL	895	GVYLF	896
ANT9-Knob	1,2,4,5,7,8	N	IHSSSI	893	ATYSSFGSIT	894	YHHFPGYAL	895	GVYLF	896

In all molecules the CDR-L1 for FZD FAB are SVSSA (SEQ ID NO: 1) and CDR-L2 are SASSLYS (SEQ ID NO: 2)

C. FZD Agonists are highly specific for FZD4, bind with high specificity and are stable in solution.

Using biolayer interferometry (BLI) we have found that FZD4 Agonists described herein are highly specific for FZD4 over other FZD receptors. Recombinant FZD ECD proteins were immobilized on BLI sensors. The FZD4 Agonists in the Diabody-Fc-Fab format, having a LRP5 binding domain comprised of a diabody that is bivalent and bispecific for LRP5 and a FZD4 binding domain comprised of two FZD4 binding Fab fragments, were tested at a concentration of 100 nM in a buffer of PBS+0.05% Tween-20 and 1% BSA for binding to the ECD proteins. The results are presented in Figure 8A. Controls in the assay included CM0199, a diabody-Fc-diabody format FZD agonist that recognizes FZD4 and LRP5 and Immunoglobulin 4275, which is an IgG that does not bind FZD or LRP.

The FZD4 Agonists also did not recognize common non-specific antigens. The FZD4 Agonists were tested at 100 nM for binding to a panel of antigens essentially as described in Monquet et al. "Polyreactivity increases the apparent affinity of anti-HIV antibodies by heterologation" Nature 2010 Sep 30;467(7315):591-5(PMC3699875), and Jain et al., "Biophysical properties of the clinical-stage antibody landscape" Proc Natl Acad Sci 2017 Jan 31;114(5):944-949. (PMC5293111). Controls in the assay included CM0199, a diabody-Fc-diabody format FZD agonist that recognizes FZD4 and LRP5 and immunoglobulin 6606, which is an IgG that is particularly prone to non-specific binding in this assay. The results are presented in Figures 8B.

The FZD4 Agonists comprising binding domains for FZD4 and LRP5 bind both FZD4 and LRP5 with high affinity. The apparent affinity of the FZD4 Agonists for recombinant ECD of FZD4 were determined by biolayer interferometry essentially as described in Elife. 2019 Aug 27; 8: e46134. Briefly, BLI assays were performed using an Octet HTX instrument (ForteBio). For measuring binding to antigen, FZD-Fc proteins were captured on AHQ BLI sensors (18-5001, ForteBio) to achieve a BLI response of 0.6–1 nm and remaining Fc-binding sites were saturated with human Fc (009-000-008, Jackson ImmunoResearch). FZD-coated or control (Fc-coated) sensors were transferred into 100-0.1 nM tetravalent FZD agonist in assay buffer (PBS, 1% BSA, 0.05% Tween20) and association was monitored for 300 s. Sensors were then transferred into assay buffer and dissociation was monitored for an

additional 300 s. Shake speed was 1000 rpm and temperature was 25°C. The results are presented in Table 7.

Table 7		
Molecule	FZD4 KD (nM)	LRP5 EC50 (nM)
CM0199	0.7	7.5
ANT16	0.6	1.4
ANT18	2.6	ND
ANT20	0.7	ND
ANT21	2.2	ND
ANT36	<0.1*	ND
ANT39	0.3	ND

The FZD4 Agonists were also analyzed by SEC as compared to trastuzumab IgG. The results are presented in Figure 9A and demonstrate that the diabody-Fc-Fab format Agonists are stable and homogenous in solution.

The FZD4 Agonists are also stable in solution. Purified FZD4 Agonists, ANT16, ANT18, ANT20, ANT21 and ANT 36 were resuspended to 1 mg/ml (except for ANT18, which was resuspended at 0.34 mg/ml) in 10 mM Histidine, 140 mM NaCl, 0.9% sucrose, pH 6 and stored either at 4°C or 40°C for a period of 6 days. Samples were removed at various time points, centrifuged to remove precipitated protein and residual protein concentration was measured. The results are presented in Tables 8 and 9.

Table 8				
Soluble Protein Remaining (%)				
Time at 40 degrees (days)	0	1	2	6
CM0199	100%	60%	64%	40%
ANT-16	100%	102%	104%	104%
ANT-18	100%	92%	83%	105%
ANT-20	100%	97%	67%	104%
ANT-21	100%	102%	75%	102%
ANT-36	100%	100%	149%	106%

Table 9		
Active Protein Remaining (%)		
Time at 40 degrees (days)	0	6
CM0199	100%	42%
ANT-16	100%	34%
ANT-18	100%	42%
ANT-20	100%	30%
ANT-21	100%	25%

- On Day 6, the amount of FZD4-specific binding sites remaining in the samples were quantified using BLI. Analysis by differential scanning fluorimetry showed that the FZD4
- 5 Agonists having a Diabody-Fc-Fab formats with an LRP-binding diabody on the N-terminal of the Fc domain and two FZD4-binding Fabs on the C-terminal of the Fc domain, have thermal denaturation profiles similar to that of Trastuzumab. IgGs generally display two peaks in a thermal stability assay, the first corresponding to CH2, the later to the Fab domain and CH3, see Figure 9B.
 - 10 The FZD4 Agonists were also assayed for induction of the beta-catenin target gene *AXIN2* in a mouse endothelial cell line (bEND3.1) and were shown to induce transcription in a concentration dependent manner. These results are presented in Figure 10.

TABLE 10			
Antibody No.		FZD recognized	Co-Receptor recognized
5019		FZD1, FZD2, FZD4, FZD5, FZD7, FZD8	
5027		FZD4	
5044		FZD4	
5016		FZD1, FZD2, FZD4, FZD5, FZD7, FZD8	
5056		FZD1, FZD 2, FZD 4, FZD5, FZD7, FZD8, FZD10	
2459			LRP5-W3
2460			LRP5-W1
8716			LRP5-W3

TABLE 10			
Antibody No.		FZD recognized	Co-Receptor recognized
12600			LRP5-W1
12608			LPR5-W1
2539			LRP6-W3
2540			LRP6-W3
2542			LRP6-W1
CM0199		FZD4	LRP5
6066		N/A Positive control for non-specificity assay	
4275		no FZD/LRP binding	

EXAMPLE 4. The FZD4 agonist was assayed for its ability to oppose the effect on cell junction disassembly and increased permeability mediated by VEGF, a cytokine released during tissue hypoxia. VEGF treatment of bEND3.1 cells leads to junction disassembly as seen by loss of plasma membrane staining of CLDN3, CLDN5 and ZO-1. Co-treatment of cells with VEGF and the FZD4 agonist leads to a near-complete rescue of this effect (Fig. 11). This decrease cell-cell junction stability mediated by VEGF treatment translates into increase endothelial cell permeability as monitor in a transendothelial permeability assay measuring the passage of 40-kDa FITC-dextran across a confluent endothelial monolayer of bEnd.3 grown on transwell filters. Co-treatment of cells with VEGF and the FZD4 agonist completely rescues that VEGF-mediated increase in cell permeability. These results indicate that the FZD4 agonist promotes endothelial cell barrier functions in a mechanism independent of VEGF.

A) Immunofluorescence of ZO-1 (green)/CLDN3(red) and ZO-1 (green)/CLDN5(red) localization on bEnd.3 cell junctions. bEnd.3 cells were treated or not with 30nM of F4L5.13 (aka CM0199) and Norrin in the presence or absence of VEGF (100ng/ml) for 1h. DAPI (blue) stain the nucleus. B) Transendothelial permeability was determined by measuring the passage of FITC-dextran through the bEnd.3 monolayer. Passage of FITC-dextran was measured after bEnd.3 treatment with VEGF (100ng/ml) and F4L5.13 (30nM) alone or

simultaneously or upon pre-treatment with VEGF for 1h followed by F4L5.13 treatment for 1h. Error bar indicate SEM, n=5. The results are presented in Figure 11.

EXAMPLE 5. New FZD5 antibodies bind FZD5 at a site overlapping with 2919 identified from affinity maturation libraries.

- 5 Single point ELISAs were performed on 96-well Maxisorp plates coated with the ECDs of human FZD5 protein in the presence or absence of a saturating concentration of 2919 IgG. The plates were incubated with the monoclonal Fab-phage followed by incubation with horseradish peroxidase (HRP)-conjugated anti-M13 antibody. Wells were subsequently washed 8 times followed by incubations with 3,3',5,5'-tetramethylbenidine/H₂O₂ peroxidase
10 (TMB) substrate for 5-10 min. the reaction was stopped by adding 1M H₃PO₄ and the absorbance was measured spectrophotometrically at 450 nm in a microtiter plate reader. The results are presented in Figure 12.

EXAMPLE 6. New FZD5 antibodies from 2928 affinity maturation library selectively bind FZD5.

- 15 Single point ELISAs were performed on 96-well Maxisorp plates coated with the ECDs of human FZD2, FZD5, or FZD8 protein. The plates were incubated with the monoclonal Fab-phage followed by incubation with horseradish peroxidase (HRP)-conjugated anti-M13 antibody. Wells were subsequently washed 8 times followed by incubations with 3,3',5,5'-tetramethylbenidine/H₂O₂ peroxidase (TMB) substrate for 5-10 min. the reaction was
20 stopped by adding 1M H₃PO₄ and the absorbance was measured spectrophotometrically at 450 nm in a microtiter plate reader. The results are presented in Figure 13.

EXAMPLE 7. Pan-FZD/LRP6 ANT9 and FZD5-specific/LRP6 ANT9 activate Wnt signaling in cells.

- TOPFLASH HEK293 cells were treated overnight with varying concentrations of FZD
25 agonist or a non-targeting control molecule (CM0156) and TCF/LEF-driven luciferase expression was measured using a standard luciferase assay. Both molecules are able to activate FZD-mediated luciferase expression in a concentration-responsive manner. ANT9,

which is able to bind to 7 of the 10 FZD receptor subtypes produces a higher maximal activation signal than the FZD5-specific ANT59.

In vivo Experiments

DSS induced colitis model

- 5 In figure 24, C57/BL6 mice were given 2% DSS in the drinking water for 7 days and 0.5% DSS for an additional 3 days to induce colitis. Control-FLAG, Pan-FLAG and ANT59 were administered via intraperitoneal injection on days 4 and 7 at a dosage of 10 mg/kg. Mice were weighed daily. On day 10 mice were euthanized and tissues were harvested for measurement of colon length and histology.

10 Histology

For histological analysis, harvested tissues were fixed in 4% paraformaldehyde and embedded in paraffin. Sections of 5 µm were stained with haematoxylin and eosin (H&E). Images were captured using a Nikon Eclipse microscope (figure 23).

Organoid Culture and Viability Measurement

- 15 Small intestine crypts were harvested from 8-week-old, female, C57BL/6 mice and cultured as previously described (O'Rourke et al., 2016). Organoid cultures were passaged and embedded in 25 µl Growth Factor Reduced Matrigel (Corning, 356231) and plated in triplicates in a 48-well plate. Organoid cultures were treated with DMSO, 1 µM LGK974, 1 µM LGK974 +50% WNT3A conditioned media, 1 µM LGK974 +30 nM Pan-FLAG, 1 µM
20 LGK974 +30 nM FZD2-FLAG, 1 µM LGK974 +30 nM FZD4-FLAG, 1 µM LGK974 +30 nM FZD5-FLAG, 1 µM LGK974 +30 nM FZD7-FLAG. Treatments were prepared in 250 µl of complete media, added to each well on day of passaging and changed every 2–3 days. At the endpoint (7 days), 150 µl Cell Titer-Glo3D (Promega) was added to 150 µl media in each well. Organoids were lysed on a rocking platform for 30 min at room temperature. The
25 luminescence reading was measured in duplicates for 20 µl lysate from each well on the Envision multilabel plate reader. The average luminescence reading for each condition was normalized to the control condition to calculate relative viability (figure 22).

EXAMPLE 8. Transient expression of 8 ANT39 variants. A series of eight ANT39 variants (Figures 16A and 16B) were transiently expressed in CHO cells using standard manufacture

lipid based protocols (ThermoFisher). Briefly cells were grown to a density of approximately 2.0×10^6 cells/ml in growth media and relevant DNAs were transfected with appropriate transfection reagent. For each variant two alternate input plasmid ratios were tested, either 1:1:2 or 2:1:3 (knob heavy chain : hole heavy chain : light chain). Conditioned media was

5 harvested 7 days later, purified by Protein A Sepharose and the titre measured.

Table 11

Molecule	DNA Ratio (Knob:Hole:LC)	Titer (mg/L)
ANT39	1:1:2	390
	2:1:3	290
ANT39i	1:1:2	260
	2:1:3	250
ANT39 LALAPS	1:1:2	330
	2:1:3	250
ANT39i LALAPS	1:1:2	300
	2:1:3	270
ANT39 LALAPS Merchant	1:1:2	340
	2:1:3	280
ANT39i LALAPS Merchant	1:1:2	240
	2:1:3	280
ANT39 LALAPS Merchant S:S	1:1:2	320
	2:1:3	190
ANT39i LALAPS Merchant S:S	1:1:2	300
	2:1:3	250
ANT42*	1:1:2	107
ANT42 LALA*	1:1:2	287
ANT42 LALAPS*	1:1:2	95
ANT42i LALAPS*	1:1:2	49

*Variants were transiently expressed in HEK293 cells

Those skilled in the art will recognize, or be able to ascertain, using no more than routine experimentation, numerous equivalents to the specific procedures described herein. Such

equivalents are considered to be within the scope of the inventions. Various substitutions, alterations and modifications may be made to the invention without departing from the spirit and scope of the invention. Other aspects, advantages, and modifications are within the scope of the invention.

- 5 The contents of all references, issued patents, and published patent applications cited through this application are hereby incorporated by reference. The appropriate component, process and methods of those patents, applications and other documents may be selected for the invention and embodiments thereof.

- 10 Throughout the specification and the claims which follow, unless the context requires otherwise, the word 'comprise', and variations such as 'comprises' and 'comprising', will be understood to imply the inclusion of a stated integer, step, group of integers or group of steps but not to the exclusion of any other integer, step, group of integers or group of steps.

- To clarify the use of and to hereby provide notice to the public, the phrases "at least one of <A>, , ... and <N>" or "at least one of <A>, , ... or <N>" or "at least one of <A>, , ... <N>, or combinations thereof" or "<A>, , ... and/or <N>" are defined by the Applicant in the broadest sense, superseding any other implied definitions hereinbefore or hereinafter unless expressly asserted by the Applicant to the contrary, to mean one or more elements selected from the group comprising A, B, ... and N. In other words, the phrases mean any combination of one or more of the elements A, B, ... or N including any one
15 element alone or the one element in combination with one or more of the other elements
20 which may also include, in combination, additional elements not listed. Unless otherwise indicated or the context suggests otherwise, as used herein, "a" or "an" means "at least one" or "one or more."

Table 12
Diabody-Fc-Fab amino acid sequences of the “heavy chain” hole construct, “heavy chain” knob construct and the “light chain” construct of the FZD4 Agonists, ANT16, ANT18, ANT20, ANT21, ANT39, and ANT42.

The CDRs of the chains are underlined, italicized. The CDRs may be replaced with the CDRs of another antibody to alter the binding specificity, e.g., the specificity could be altered to bind another site on the FZD or LPR5/6, or to another FZD or LPR.

FZD4 Agonist ANT16	EVQLVESGGGLVQPGGSLRLSCAASGFDFTA YAM HWVRQAPGKGLEWVAS IYPSGGGY TAYADSVKGRFTISADTSKNTAYLQ MNSLRAEDTAVYYCAR RSYVE ALDYWGQGLTVTVSSGGGSDIQMTQSPSSLASVGDRTITCRAS QSYSSA VAWYQQKPGK APKLLIY SASSL YSGVPSRFSGRSGTDFLTITISLQPEDFATYYCQQ YWAYYSPIT FGQGTKVEIKGGGGGGGSEPKTSDKTHT CPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVAVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYGSTYRVVSVLTVL HQDWLNGKEYCKVSNKALPAPIEKTISKAKGQPREPMVFDLPPSREEMTKNQVSLWCMVKGFYPSDIAVEWESNGQPENNY KTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCVMHEALHNHYTQKSLSLSPKGGGGGGGGSGTGEVQLVES GGGLVQPGGSLRLSCAASGF TLSSYSM HWVRQAPGKGLEWVA YISYYGYTYY ADSVKGRFTISADTSKNTAYLQMNSLRAED TAVYYCAR PAPGHW GFDYWGQGLTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFP AVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHNKPSNTKVDKKVEPKSCDKTHT (SEQ ID NO: 897)	EVQLVESGGGLVQPGGSLRLSCAASGF DESSSSH HWVRQAPGKGLEWVAS ISSSYGYTYY ADSVKGRFTISADTSKNTAYLQMN SLRAEDTAVYYCAR SW4MD YWGQGLTVTVSSGGGSDIQMTQSPSSLASVGDRTITCRAS QSYSSA VAWYQQKPGKAPKL LIY S4SDL YSGVPSRFSGRSGTDFLTITISLQPEDFATYYCQQ YAGAGLIT FGQGTKVEIKGGGGGGGSEPKSSDKTHTCPPCP APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVAVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYGSTYRVVSVLTVLHQDW LNGKEYCKVSNKALPAPIEKTISKAKGQPREPQVYTLPIRELMTSNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPPVL DSDGSFFLYSKLTVDKSRWQQGNVFCVMHEALHNHYTQKSLSLSPKGGGGGGGGSGTGEVQLVESGGGLVQ PGGSLRLSCAASGF TLSSYSM HWVRQAPGKGLEWVA YISYYGYTYY ADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYC ARP PAGHW GFDYWGQGLTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS GLYSLSSVVTVPSSSLGTQTYICNVNHNKPSNTKVDKKVEPKSCDKTHT (SEQ ID NO: 898)
Knob Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole Hc construct		
Hole		

FZD4 Agonist ANT18	Knob Hc construct	MNLLILTFVAAA/AEVQLVESGGGMVQPGGSLRLSCAASGFNFSSSSHHWVRQAPGKGLEWVA/SSSSYGYTYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARGGSGVSHYGSVYYSWW/ALDYWGQGTLVTVSSGGGGSDIQMTQSPSSLSASVGDRTVITTCRASQSLSSA/VAWYQQKPGKAPKLLIYSASSLYSGVPSRFSGSRSGTDFTLTISLQPEDFATYYCQQASYPITFGQGTKVEIKGGGGGGGSEPKTSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVAVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYGSTYRVVSVLTVLHQDWLNGKEYCKVSNKALPAPIEKTISKAKGQPREPMVFDLPPSREEMTKNQVSLWCMVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSCVMHEALHNHYTQKSLSLSPGKGGGGGGGGSGTGEVQLVESGGGLVQPGGSLRLSCAASGF/TLSSYSMHHWVRQAPGKGLEWVA/YISSYYGYTYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARPAPGHWGFDYWGGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHNKPSNTKVDKKVEPKSCDKTHT (SEQ ID NO: 900)
	Hole Hc construct	MNLLILTFVAAA/AEVQLVESGGGLVQPGGSLRLSCAASGFNLSSYYMHHWVRQAPGKGLEWVA/SSSYGYTYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARWSH/SGHYSGMDYWGQGTLVTVSSGGGGSDIQMTQSPSSLSASVGDRTVITCRASQSLSSA/VAWYQQKPGKAPKLLIYSASSLYSGVPSRFSGSRSGTDFTLTISLQPEDFATYYCQQSSYSLITFGQGTKVEIKGGGGGGGSEPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVAVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYGSTYRVVSVLTVLHQDWLNGKEYCKVSNKALPAPIEKTISKAKGQPREPVVYTLPIRELMTSNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFCSCVMHEALHNHYTQKSLSPGKGGGGGGGGSGTGEVQLVESGGGLVQPGGSLRLSCAASGF/TLSSYSMHHWVRQAPGKGLEWVA/YISSYYGYTYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARPAPGHWGFDYWGGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHNKPSNTKVDKKVEPKSCDKTHT (SEQ ID NO: 901)
Light Chain		MNLLILTFVAAA/ADIQMTQSPSSLSASVGDRTVITTCRASQSLSSA/VAWYQQKPGKAPKLLIYSASSLYSGVPSRFSGSRSGTDFTLTISLQPEDFATYYCQQWYYAPITFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSYSTLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 902)

FZD4 Agonist ANT20	Knob Hc construct	MNLLLILTFVAAA ¹ VAEVQLVESGGGLVQPGGSLRLSCAASGFD ² <u>FTAYAM</u> HWVRQAPGKGLEWVA ³ <u>SIYPSGGYTAY</u> ADSVKGRFTISADTSKNTAYLQMNSLR AEDTAVYYCAR ⁴ <u>RSYYFAL</u> DYWGQGTLLTVTSSGGGGSDIQMTQSPSSLSASVGDRTITCRASQ ⁵ <u>LSSAV</u> AWYQQKPGKAPKLLIY ⁶ <u>S4SSL</u> YSGVPSRFSRSGTDFTLTISSLQPEDFATYYCQQ ⁷ <u>ASY4PT</u> FGQGTKEIKGGGGSGGGSEPKTSDKTHTCPPCPAPELLGGPSVFLFPPKPKD ⁸ TLMSRTP ⁹ EVTCVVAVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYGSTYRVVSVLTVLHQDWLNGKEYCKVSNKALPAPIEK ¹⁰ TISKAKGQPREPMV ¹¹ FDLP ¹² PSREEMTKNQVSLWCMVKGFYPSDIAVEWESNGQPENNYK ¹³ TPPVLDSDGSFFLYSKLTVDKSRWQQGNV ¹⁴ SCSMHEALHNHYTQKSLSPGKGGSGGGSGGGSGGGSGGSGSTGEVQLVESGGGLVQPGGSLRLSCAASGFD ¹⁵ <u>TLSSYSM</u> HWVRQAPGKGLEWVA ¹⁶ <u>YISSYYGYTY</u> YADSVKGRFTISADTSKNTAYLQMNSLR AEDTAVYYCAR ¹⁷ <u>PAPGHWGF</u> DYWGQGTLLTVTSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHT (SEQ ID NO: 903)
	Hole Hc construct	MNLLLILTFVAAA ¹ VAEVQLVESGGGLVQPGGSLRLSCAASGFN ² <u>LSYYTM</u> HWVRQAPGKGLEWVA ³ <u>SIYSSYGYTY</u> YADSVKGRFTISADTSKNTAYLQMNSLR AEDTAVYYCAR ⁴ <u>WSHVSGHYS</u> GMDYWGQGTLLTVTSSGGGGSDIQMTQSPSSLSASVGDRTITCRASQ ⁵ <u>SLSSAV</u> AWYQQKPGKAPKLLIY ⁶ <u>S4SDL</u> YSGVPSRFSRSGTDFTLTISSLQPEDFATYYCQQ ⁷ <u>Y4GAGLIT</u> FGQGTKEIKGGGGSEPKTSDKTHTCPPCPAPELLGGPSVFLFPPKPKD ⁸ TLMSRTP ⁹ EVTCVVAVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYGSTYRVVSVLTVLHQDWLNGKEYCKVSNKALPAPIEK ¹⁰ TISKAKGQPREPQVY ¹¹ TLPPIRELMTSNQVSLSPGKGGSGGGSGGGSGGGSGGSGSTGEVQLVESGGGLVQPGGSLRLSCAASGFD ¹² <u>TLSSYSM</u> HWVRQAPGKGLEWVA ¹³ <u>YISSYYGYTY</u> YADSVKGRFTISADTSKNTAYLQMNSLR AEDTAVYYCAR ¹⁴ <u>PAPGHWGF</u> DYWGQGTLLTVTSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHT (SEQ ID NO: 904)
Light Chain		MNLLLILTFVAAA ¹ VADIQMTQSPSSLSASVGDRTITCRASQ ² <u>SLSSAV</u> AWYQQKPGKAPKLLIY ³ <u>S4SSL</u> YSGVPSRFSRSGRSGTDFLTISSLQPEDFATYYCQQ ⁴ <u>WYY4PT</u> FGQGTKEIKRTVAAPSVFIFPPSDEQLKSGTASV ⁵ VCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 902)

FZD4 Agonist ANT21	
Knob Hc construct	MNLLILTFVAAA VAEV QLVESGGGLVQPGGSLRLSCAASG FNFSSSS HHWVRQAPGKGLEWVA SSSSYGYTY YADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCAR GGSGVYYSWW ALDYWGQGTLVTVSSGGGGSDIQMTQSPSSLSASVGDRTITTCRASQ SSA VAWYQQKPGKAPKLLIY SASSL YSGVPSRFSGRSGTDFTLTISLQPEDFATYYC QQYW AYYSPT FGQGTKVEIKGGGGGGSEPKTSDKTHTCPPCP APPELLGGPSVFLFPPKPKDITLMSRPEETLCVVAISHEDPEVKFNWYVDGVEVHN AKTKPREEQYSGTYRVS LVTHQDNLNGKEYCKVSNKALPAPIEKTTISKAKGQPREPQVYTLPIRELMTSNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSCVMHEALHNHYTQKSLSPGKGGSGGGGGGGSGTGEVQLVESGGGLVQPGGSLRLSCAASG FTLSYSM HHWVRQAPGKGLEWVA YISSYGYTY YADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCAR PAPGHWGFD YWGGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHT (SEQ ID NO: 905)
Hole Hc construct	MNLLILTFVAAA VAEV QLVESGGGLVQPGGSLRLSCAASG FNFSSSS HHWVRQAPGKGLEWVA SSSSYGYTY YADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCAR SWAMD YWGQGTLVTVSSGGGGSDIQMTQSPSSLSASVGDRVTITCRASQ SSA VAWYQQKPGKAPKLLIY SASSL YSGVPSRFSGRSGTDFTLTISLQPEDFATYYC QQSSYSL ITFGQGTKVEIKGGGGSGGGSEPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDITLMISRTPETCVVAVSHEDPEVKFNWYVDGVEVHN AKTKPREEQYSGTYRVS LVTHQDNLNGKEYCKVSNKALPAPIEKTTISKAKGQPREPQVYTLPIRELMTSNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSCVMHEALHNHYTQKSLSPGKGGSGGGGGSGTGEVQLVESGGGLVQPGGSLRLSCAASG FTLSYSM HHWVRQAPGKGLEWVA YISSYGYTY YADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCAR PAPGHWGFD YWGGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHT (SEQ ID NO: 906)
Light Chain	MNLLILTFVAAA VA DIQMTQSPSSLSASVGDRVTITCRAS QSLSSA VAWYQQKPGKAPKLLIY SASSL YSGVPSRFSGRSGTDFLTISLQPEDFATYYC QQWYYA PTITFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCCLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 902)

FZD4 Agonist ANT39	Knob Hc construct	MNLLLLTFVAAVAEVQLVESGGGLVQPGGSLRLSCAASGFDFTAYAMHWVRQAPGKGLEWVA SIYPSGGYTAY ADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCAR RSYF ALDYWGQGTILVTVSSGGGSDIQMTQSPSSLSASVGDRTITCRASQSVSSAVAWYQQKPGKAPKLLIY S4SL YSGVPSRFSGRSGTDFTLTISLQPEDFATYYCQQYAWAY YSP TTFGQGTKEIKGGGGGGGSEPKTSDKTHTCPPCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVAVSHEDPEVKFNWYVDGVEVHNATKPREEQYGSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTIISKAKGQPREPMVFDLPPSREEMTKNQVSLWCMVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCVMHEALHNHYTQKSLSLSPGKGGGGGGGGSGGSTGEVQLVESGGGLVQPGGSLRLSCAASGFTLS SYSM HWVRQAPGKGLEWVA YISSYDSITD YADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCAR PAVGHMAFD YWGQGTILVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHNKPSNTKVDKKVEPKSCDKTHT (SEQ ID NO: 907)
	Hole Hc construct	MNLLLLTFVAAVAEVQLVESGGGLVQPGGSLRLSCAASGFD FS SSSHHWVRQAPGKGLEWVA SISSSYGYTY YADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCAR SW AMDYWGQGTILVTVSSGGGSDIQMTQSPSSLSASVGDRTITCRASQSVSSAVAWYQQKPGKAPKLLIY S4SDL YSGVPSRFSGRSGTDFTLTISLQPEDFATYYCQQYAG AGL ITFGQGTKEIKGGGGGGGSEPKSSDKTHTCPPCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVAVSHEDPEVKFNWYVDGVEVHNATKPREEQYGSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTIISKAKGQPREPVYTLPIRELMTSNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCVMHEALHNHYTQKSLSLSPGKGGGGGGSGGSTGEVQLVESGGGLVQPGGSLRLSCAASGFTLS SYSM HWVRQAPGKGLEWVA YISSYDSITD YADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCAR PAVGHMAFD YWGQGTILVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHNKPSNTKVDKKVEPKSCDKTHT (SEQ ID NO: 908)
Light Chain		MNLLLLTFVAAVADIQMTQSPSSLSASVGDRVTITCRASQSVSSAVAWYQQKPGKAPKLLIY S4SSL YSGVPSRFSGRSGTDFTLTISLQPEDFATYYCQQY YN NA PT TFGQGTKEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 909)

FZD4 Agonist ANT42	Knob Hc construct MNLLILTFVAAA¹VAEVQLVESGGGLVQPGGSLRLSCAASGFD²ETAYAM³HWVRQAPGKGLEWVA⁴SIYPSGGY⁵AYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCAR⁶RSY⁷YFALDYWGQGTLVTVSSGGGGSDIQMTQSPSSLSASVGDRVTITCRASQ⁸SI⁹SSAVAWYQQKPGKAPKLLIY¹⁰S¹¹SSL¹²YSGVPSRFSGRSGTDFTLTISSLQPEDFATYYCQQ¹³Y¹⁴W¹⁵AY¹⁶YSP¹⁷TFGGQGTKVEIKGGGGGGGSEPKTSDKTHTCPPCPAPPELLGGPSVFLFPPKPKD¹⁸TLMSIRTPETCVVA¹⁹VSHEDPEVKFNWYVDGVEVHNAKTKPREEQYGSTYRVVSVLTVLHQDWLNGKEYCKVSNKALPAPIEK²⁰TISKAKGQPREPMVFDLPPSREEMTKNQVSLWCMVKGFYPSDIAVEWESNGQPENNYK²¹TPPVLDSDGSFFLYSKLTVDKSRWQQGNV²²FSCVMHEALHNHYTQKSLSPGKGGGSGGGGGGGSGSTGEVQLVESGGGLVQPGGSLRLSCAASGF²³TSDFY²⁴F²⁵F²⁶HWVRQAPGKGLEWVA²⁷TIY²⁸PI²⁹GN³⁰TY³¹YADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCAR³²AFPGSY³³HP³⁴FDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVN³⁵NHKPSNTKVDK³⁶VEPKSCDK³⁷TH³⁸T (SEQ ID NO: 910)
Hole Hc construct	MNLLILTFVAAA¹VAEVQLVESGGGLVQPGGSLRLSCAASGFD²FS³SS⁴SH⁵HWVRQAPGKGLEWVA⁶SISSSYGY⁷TYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCAR⁸SW⁹AMDYWGQGTLVTVSSGGGGSDIQMTQSPSSLSASVGDRVTITCRASQ¹⁰SI¹¹SSAVAWYQQKPGKAPKLLIY¹²S¹³SSL¹⁴YSGVPSRFSGRSGTDFTLTISSLQPEDFATYYCQQ¹⁵Y¹⁶AG¹⁷AG¹⁸LITFGGQGTKVEIKGGGGSGGGGGSEPKSSDKTHTCPPCPAPPELLGGPSVFLFPPKPKD¹⁹TLMSIRTPETCVVA²⁰VSHEDPEVKFNWYVDGVEVHNAKTKPREEQYGSTYRVVSVLTVLHQDWLNGKEYCKVSNKALPAPIEK²¹TISKAKGQPREPQVYTLPIRELM²²TSNQVSLSCAVKGFYPSDIAVEWESNGQPENNYK²³TPPVLDSDGSFFLYSKLTVDKSRWQQGNV²⁴FSCVMHEALHNHYTQKSLSPGKGGSGGGSGGGGGSGSTGEVQLVESGGGLVQPGGSLRLSCAASGF²⁵TSDFY²⁶F²⁷F²⁸HWVRQAPGKGLEWVA²⁹TIY³⁰PI³¹GN³²TY³³YADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCAR³⁴AFPGSY³⁵HP³⁶FDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVN³⁷NHKPSNTKVDK³⁸VEPKSCDK³⁹TH⁴⁰T (SEQ ID NO: 911)
Light Chain	MNLLILTFVAAA¹VADIQMTQSPSSLSASVGDRVTITCRASQ²SI³SSAV⁴AWYQQKPGKAPKLLIY⁵S⁶SSL⁷YSGVPSRFSGRSGTDFTLTISSLQPEDFATYYCQQ⁸ST⁹GS¹⁰LITFGGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASV¹¹VCLLN¹²NFYPREAKVQW¹³KVDNALQSGNSQESVTEQDSKDS¹⁴TYSLSS¹⁵TLTL¹⁶SKADY¹⁷EKHKVYACEVTHQGLSSPV¹⁸TKSFNRGEC (SEQ ID NO: 912)

FZD5-LRP6 ANT 59	EVQLVESGGGLVQPGGSLRLSCAASGFDLSHSSHHWVRQAPGKGLEWVA SISSHYGYTH YADSVKGRFTISADTSKNTAYLQMN NSLRAEDTAVYYCAR SSYFPWF FALDYWGQGTLVTVSSGGGGSDIQMTQSPSSLSASVGDRTITCRASQ SLSSA VAWYQQK PKAPKLLIY SASSLY SGVPSRFSRSGTDFTLTISSLQPEDFATYYCQQ YYWPI TFGQGTKVEIKGGGGGGGSEPKTSDKTHT CPPCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVA VSHED PEVKFNWYVDGVEVHNAKTKPREEQYGSTYRVSVLT TVLHQDWLNGKEYKCKVSNKALPAPIEKTIISKAKGQPREPMVFDLPPSREEMTKNQVSLWCMVKGFYPSDIAVEWESNGQPENNY KTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVSCVMHEALHNHYTQKSLSPGKGGGGGGGGGGSGTGEVQLVES GGGLVQPGGSLRLSCAASGF NIAYSSM HWVRQAPGKGLEWVA TIYPSYSSTYY ADSVKGRFTISADTSKNTAYLQMNSLRAED TAVYYCAR YYAM DYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQ SSGLYSLSVTVTPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHT (SEQ ID NO: 913)
Knob Hc construct	EVQLVESGGGLVQPGGSLRLSCAASGFD ISSYF HHWVRQAPGKGLEWVA SIYPSYSTYY ADSVKGRFTISADTSKNTAYLQMN SLRAEDTAVYYCAR NI/RGFRKPYFSRW AMDYWGQGTLVTVSSGGGGSDIQMTQSPSSLSASVGDRTITCRASQ SLSSA VAW YQKPGKAPKLLIY SASSLY SGVPSRFSRSGTDFTLTISSLQPEDFATYYCQQ YFFPI TFGQGTKVEIKGGGGGGGSEPKSS DKTHTCPPCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVA VSHED PEVKFNWYVDGVEVHNAKTKPREEQYGSTYRVV SVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTIISKAKGQPREPQVYTLPPIRELMTSNQVSLSCAVKGFYPSDIAVEWESNGQP ENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVSCVMHEALHNHYTQKSLSPGKGGGGGGGGGGSGTGEV QLVESGGGLVQPGGSLRLSCAASGF NIAYSSM HWVRQAPGKGLEWVA TIYPSYSTYY ADSVKGRFTISADTSKNTAYLQMN LRAEDTAVYYCAR YYAM DYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTF PAVLQSSGLYSLSVTVTPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHT (SEQ ID NO: 914)
Hole Hc construct	DIQMTQSPSSLSASVGDRTITCRASQ SLSSA VAWYQQKPGKAPKLLIY SASSLY SGVPSRFSRSGTDFTLTISSLQPEDFATYY CQQ AHYFPI TFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDS TYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 915)
Light Chain	

pFZD-LRP6 ANT9	EVQLVESGGGLVQPGGSLRLSCAASGFDLSHSSHHWVRQAPGKGLEWVA SISSHYGYTHY ADSVKGRFTISADTSKNTAYLQMN NSLRAEDTAVYYCAR SSYFPWF FALDYWGQGTLVTVSSGGGGSDIQMTQSPSSLSASVGDRTITCRASQ SSA VAWYQQK PKAPKLLIY SA SLYSGVPSRFSRSGTDFTLTISSLQPEDFATYYCQQ YVWPI TFGGQTKVEIKGGGGGGGSEPKTSDKTH THTCPPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVAVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYGSTYRVSVLT TVLHQDWLNGKEYCKVSNKALPAPIEKTIISKAKGQPREPMVFDLPPSREEMTKNQVSLWCMVKGFYPSDIAVEWESNGQPENNY KTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCVMHEALHNHYTQKSLSPGKGGGGGGGGGGGGSTGEVQLVES GGGLVQPGGSLRLSCAASGFNIHSSHHWVRQAPGKGLEWVA ATYSFSGSITY ADSVKGRFTISADTSKNTAYLQMNSLRAEDT AVYYCAR YHH PF GYAL DYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFP AVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHT (SEQ ID NO: 916)
Knob Hc construct	EVQLVESGGGLVQPGGSLRLSCAASGFDLSYFHHWVRQAPGKGLEWVA SIYPSYSYTSY ADSVKGRFTISADTSKNTAYLQMN SLRAEDTAVYYCARNV RGFRKPYFSRW AMDYWGQGTLVTVSSGGGGSDIQMTQSPSSLSASVGDRTITCRASQ SSA VAW YQQKPGKAPKLLIY SA SLYSGVPSRFSRSGTDFTLTISSLQPEDFATYYCQQ YFFPI TFGGQTKVEIKGGGGGGGSEPKSS DKTHTCPPCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVAVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYGSTYRVV SVLTVLHQDWLNGKEYCKVSNKALPAPIEKTIISKAKGQPREPQVYTLPPIRELMTSNQVSLSCAVKGFYPSDIAVEWESNGQP ENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCVMHEALHNHYTQKSLSPGKGGGGGGGGGGGGSTGEV QLVESGGGLVQPGGSLRLSCAASGFNIHSSHHWVRQAPGKGLEWVA ATYSFSGSITY ADSVKGRFTISADTSKNTAYLQMNSL RAEDTAVYYCAR YHH PF GYAL DYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSG VHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHT (SEQ ID NO: 917)
Hole Hc construct	DIQMTQSPSSLSASVGDRTITCRASQ SLSSA VAWYQQKPGKAPKLLIY SA SLYSGVPSRFSRSGTDFTLTISSLQPEDFATYY CQQ GLVIL TFGGQTKVEIKRTVAAPSVFIFFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQ SGNSQESVTEQDSKDSYSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 918)
Light Chain	

diabody-Fc-diabody FZD4 CM0199	Knob Hc construct	EFEVQLVESGGGLVQPGGSLRLSCAASGFNSSFYFHHWVRQAPGKGLEWVA <u>TVPYLDYTY</u> ADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCAR <u>AFPGSYHPMD</u> YWGQGTLTVTVSSGGGGSDIQMTQSPSSLASVGDRTITCRASQ <u>SVSSA</u> VAWYQQKPGKAPKLLIY <u>S4SSL</u> YSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQ <u>SSYS</u> LTFGGQGTKVEIKLEDKTHTKVEPKTSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTTISKAKGQPREPMVFDLPPSREEMTKNQVSLWCMVKGFYPSDIAVEWESNGQPENNYKTTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSCVMHEALHNHYTQKSLSPGKSGSETPGTSESATPESGGGEVQLVESGGGLVQPGGSLRLSCAASGFN <u>FS</u> SSHHWVRQAPGKGLEWVA <u>SISSSYGYTY</u> ADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCAR <u>GGSG</u> L <u>SHYGS</u> VYYS <u>W</u> ALDYWGQGTLTVTVSSGGGGSDIQMTQSPSSLASVGDRTITCRASQ <u>SVSSA</u> VAWYQQKPGKAPKLLIY <u>S4SSL</u> YSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQ <u>ASYA</u> PTFGGQGTKVEIK (SEQ ID NO: 919)
	Hole Hc construct	EFEVQLVESGGGLVQPGGSLRLSCAASGFNSSFYFHHWVRQAPGKGLEWVA <u>TVPYLDYTY</u> ADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCAR <u>AFPGSYHPMD</u> YWGQGTLTVTVSSGGGGSDIQMTQSPSSLASVGDRTITCRASQ <u>SVSSA</u> VAWYQQKPGKAPKLLIY <u>S4SSL</u> YSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQ <u>SSYS</u> LTFGGQGTKVEIKLEDKTHTKVEPKSSDKTHNCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTTISKAKGQPREPQVYTLPIRELMTSNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSCVMHEALHNHYTQKSLSPGKSGSETPGTSESATPESGGGEVQLVESGGGLVQPGGSLRLSCAASGFN <u>N</u> SYYYHHWVRQAPGKGLEWVA <u>SISSSYGYTY</u> ADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCAR <u>W</u> <u>SHV</u> <u>SGHYSGM</u> DYWGQGTLTVTVSSGGGGSDIQMTQSPSSLASVGDRTITCRASQ <u>SVSSA</u> VAWYQQKPGKAPKLLIY <u>S4SSL</u> YSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQ <u>SSYS</u> LTFGGQGTKVEIK (SEQ ID NO: 920)

Table 13

Diabody-Fc-Fab and IgG-Diabody amino acid sequences of the “heavy chain” hole construct, “heavy chain” knob construct and the “light chain” construct of the FZD4 Agonists, ANT39 and ANT42, ANT39 and ANT42, and ANT39i and ANT42i having Fc domain amino acid mutations DANG, LALAPS, LALAPS and Merchant or LALAPS and Merchant S-S. Mature sequences do not include the 5' leader peptide.

5 The V-region CDRs of the chains are underlined, italicized and bolded, Fc null mutations are italicized, CH3 heterodimerisation mutations are underlined and italicized, CH3 cys disulphide bridges are bolded, and linkers are underlined.

ANT39i HC hole DANG

MNLLLLITFVAAA¹⁰VAEVQLVESGGGLVQPGGSLRLSCAASGFTLS¹⁵SYSMHWVRQAPGKGLEWVA²⁰YSSYDSITDYADSVKGRFTISAD
TSKNTAYLQMNSLRAEDTAVYYCAR²⁵PAVGHMAFDYWGQGTLT³⁰TVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS
WNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCTPCPAPELLGGPSVFLFPPKP
KDTLMISRTPETCVVAVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYGSTYRVVSVLTVLHQDWLNGKEYCKVSNKALPAPIE
KTISKAKGQPREPQVYTLPP/RE/MTLNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFL/LSKLTVDKSRWQQGNV
FSCVMHEALHNHYTQKSLSPGKGGSGGGSGGSGSTGEVQLVESGGGLVQPGGSLRLSCAASGFD³⁵FSSSSJHWWVRQAPG
KGLEWVA⁴⁰S⁴⁵SSSYGYTYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCAR⁴⁵SW/AMDYWGQGTLT⁵⁰TVSSGGGGSDIQMTQSPS
SLSASVGDRVTITCRASQSVSSAVAWYQQKPGKAPKLLIY⁵⁵SADLLYSGVPSRFSGRSGTDFTLTISLQPEDFATYYCQQ⁶⁰YAGAGLITFG
QGTVKEIK* (SEQ ID NO: 921)

ANT39i HC hole LALAPS

MNLLLLITFVAAA²⁰VAEVQLVESGGGLVQPGGSLRLSCAASGFTLS²⁵SYSMHWVRQAPGKGLEWVA³⁰YSSYDSITDYADSVKGRFTISAD
TSKNTAYLQMNSLRAEDTAVYYCAR³⁵PAVGHMAFDYWGQGTLT⁴⁰TVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS
WNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCTPCPAPEAAGGPSVFLFPPKP
KDTLMISRTPETCVVAVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYCKVSNKALPAPIE
KTISKAKGQPREPQVYTLPP/RE/MTLNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFL/LSKLTVDKSRWQQGNV
FSCVMHEALHNHYTQKSLSPGKGGSGGGSGGSGSTGEVQLVESGGGLVQPGGSLRLSCAASGFD⁴⁵FSSSSJHWWVRQAPG
KGLEWVA⁵⁰S⁵⁵SSSYGYTYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCAR⁶⁰SW/AMDYWGQGTLT⁶⁵TVSSGGGGSDIQMTQSPS
SLSASVGDRVTITCRASQSVSSAVAWYQQKPGKAPKLLIY⁷⁰SADLLYSGVPSRFSGRSGTDFTLTISLQPEDFATYYCQQ⁷⁵YAGAGLITFG
QGTVKEIK* (SEQ ID NO: 922)

ANT39i HC hole LALAPS Merchant

MNLLLLITFVAAA VAEVQLVESGGGLVQPGGSLRLSCAASGFTLSSYSMHWVRQAPGKGLEWVA YISSYDSITDYADSVKGRFTISAD
TSKNTAYLQMNSLRAEDTAVYYCAR PAVGHMAFDYWGQGTLLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS
WNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKP
KDTLMISRTPETCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPASI
ETISKAKGQPREPQVYTLPPSREEMTKNQVSLCĀVKGFYPSDIAVEWESNGQPENNYKTTPVLDSDGSFFLĴSKLTVDKSRWQQGN
VFSCVMHEALHNHYTQKSLSLSPGKGGGGGGGGSGTGEVQLVESGGGLVQPGGSLRLSCAASGFT DFSSSSJHWWVRQAP
GKGLEWVA ASJSSSYGYTYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCAR SWAMDYWGQGTLLVTVSSGGGGSDIQMTQS
PSSLSASVGDRVTITCRAS QSYSSAVAWYQQKPGKAPKLLIY SASDLYSGVPSRFSGRSGTDFTLTISSLQPEDFATYYCQQ YAGAGLIT
FGQGTKVEIK* (SEQ ID NO: 923)

ANT39i HC hole LALAPS Merchant S-S

MNLLLLITFVAAA VAEVQLVESGGGLVQPGGSLRLSCAASGFTLSSYSMHWVRQAPGKGLEWVA YISSYDSITDYADSVKGRFTISAD
TSKNTAYLQMNSLRAEDTAVYYCAR PAVGHMAFDYWGQGTLLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS
WNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKP
KDTLMISRTPETCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPASI
ETISKAKGQPREPQVCTLPPSREEMTKNQVSLCĀVKGFYPSDIAVEWESNGQPENNYKTTPVLDSDGSFFLĴSKLTVDKSRWQQGN
VFSCVMHEALHNHYTQKSLSLSPGKGGGGGGGGSGTGEVQLVESGGGLVQPGGSLRLSCAASGFT DFSSSSJHWWVRQAP
GKGLEWVA ASJSSSYGYTYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCAR SWAMDYWGQGTLLVTVSSGGGGSDIQMTQS
PSSLSASVGDRVTITCRAS QSYSSAVAWYQQKPGKAPKLLIY SASDLYSGVPSRFSGRSGTDFTLTISSLQPEDFATYYCQQ YAGAGLIT
FGQGTKVEIK* (SEQ ID NO: 924)

Mature ANT39i HC hole DANG

EVQLVESGGGLVQPGGSLRLSCAASGFTLSSYSMHWVRQAPGKGLEWVA YISSYDSITDYADSVKGRFTISADTSKNTAYLQMNSLRA
EDTAVYYCAR PAVGHMAFDYWGQGTLLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAV
LQSSGLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPETCVV
VAVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYGSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVY
TLPPREĴMTĴNQVSLCĀVKGFYPSDIAVEWESNGQPENNYKTTPVLDSDGSFFLĴSKLTVDKSRWQQGNVFCSCVMHEALHNHYT
QKSLSLSPGKGGGGGGGGSGTGEVQLVESGGGLVQPGGSLRLSCAASGFT DFSSSSJHWWVRQAPGKGLEWVA ASJSSSYGYTY

YYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARSW/AMDYWGQGTILVTVSSGGGSDIQMTQSPSSLSASVGDRVTITCRA
SQSVSSAVAWYQQKPGKAPKLLIYSASDLYSGVPSRFSGSRSGTDFTLTISLQPEDFATYYCQQYAGAGLITFGQGTKVEIK* (SEQ ID
 NO: 925)

Mature ANT39i HC hole LALAPS

5 EVQLVESGGGLVQPGGSLRLSCAASGFTLSSYSMHWVRQAPGKGLEWVAYISSYDSITDYADSVKGRFTISADTSKNTAYLQMNSLRA
 EDTAVYYCARPAVGHMAFDYWGQGTILVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAV
 LQSSGLYSLSSVTVTPSSSLGTQTYICNVNHNKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDITLMISRTPEVTCVV
 VDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPASPKEKTSKAKGQPREPQVY
 TLPPIR/MTNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFL/SKLTVDKSRWQQGNVVFSCVMHEALHNHYT
 QKSLSLSPGKGGSGGGSGGGSGGSTGEVQLVESGGGLVQPGGSLRLSCAASGFDFSSSSIHWVRQAPGKGLEWVAASISSYGYT
 10 YYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARSW/AMDYWGQGTILVTVSSGGGSDIQMTQSPSSLSASVGDRVTITCRA
SQSVSSAVAWYQQKPGKAPKLLIYSASDLYSGVPSRFSGSRSGTDFTLTISLQPEDFATYYCQQYAGAGLITFGQGTKVEIK* (SEQ ID
 NO: 926)

Mature ANT39i HC hole LALAPS Merchant

15 EVQLVESGGGLVQPGGSLRLSCAASGFTLSSYSMHWVRQAPGKGLEWVAYISSYDSITDYADSVKGRFTISADTSKNTAYLQMNSLRA
 EDTAVYYCARPAVGHMAFDYWGQGTILVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAV
 LQSSGLYSLSSVTVTPSSSLGTQTYICNVNHNKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDITLMISRTPEVTCVV
 VDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPASPKEKTSKAKGQPREPQVY
 TLPSREEMTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFL/SKLTVDKSRWQQGNVVFSCVMHEALHNHYT
 20 QKSLSLSPGKGGSGGGSGGGSGGSTGEVQLVESGGGLVQPGGSLRLSCAASGFDFSSSSIHWVRQAPGKGLEWVAASISSYGYT
YYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARSW/AMDYWGQGTILVTVSSGGGSDIQMTQSPSSLSASVGDRVTITCRA
SQSVSSAVAWYQQKPGKAPKLLIYSASDLYSGVPSRFSGSRSGTDFTLTISLQPEDFATYYCQQYAGAGLITFGQGTKVEIK* (SEQ ID
 NO: 927)

Mature ANT39i HC hole LALAPS Merchant S-S

25 EVQLVESGGGLVQPGGSLRLSCAASGFTLSSYSMHWVRQAPGKGLEWVAYISSYDSITDYADSVKGRFTISADTSKNTAYLQMNSLRA
 EDTAVYYCARPAVGHMAFDYWGQGTILVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAV
 LQSSGLYSLSSVTVTPSSSLGTQTYICNVNHNKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDITLMISRTPEVTCVV

VDVSHEDPEVKFNWYVDGVEVHNATKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPASEIETISKAKGQPREPQVC
 TLPPSREEMTKNQVSLVCAVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLJSKLTVDKSRWQQGNVFCVSMHEALHNHYT
 QKSLSLSPGKGGSGGGSGGSTGEVQLVESGGGLVQPGGSLRLSCAASGFD~~ESSSS~~HHWVRQAPGKGLEWVA~~SSSSYGYT~~
 IYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCAR~~SWAMD~~YWGQGTLLVTVSSGGGGSDIQMTQSPSSLSASVGDRTTITCRA
QSIVSSAVAWYQQKPGKAPKLLIYS4SDLYSGVPSRFSRSGTDFTLTLSLQPEDFATYYCQYAGAGLITFGQGTKVEIK* (SEQ ID
 NO: 928)

5

ANT39i HC knob DANG

MNLLLLITFVAAA~~VA~~EVQLVESGGGLVQPGGSLRLSCAASGFTLSSYS~~SM~~HHWVRQAPGKGLEWVA~~YISSYDSITDY~~ADSVKGRFTISAD
 TSKNTAYLQMNSLRAEDTAVYYCAR~~PAVGHMAFD~~YWGQGTLLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS
 WNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHNKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKP
 KDTLMISRTPETCVVAVSHEDPEVKFNWYVDGVEVHNATKPREEQYSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE
 KTISKAKGQPREP~~M~~~~V~~EDLPPSREEMTKNQVSL~~W~~CMVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQG
 NVFSCSVMEALHNHYTQKSLSLSPGKGGSGGGSGGSTGEVQLVESGGGLVQPGGSLRLSCAASGFD~~FTAYAM~~HHWVRQ
 APGKGLEWVA~~SIYPSGGYT~~AYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCAR~~RSYVFA~~LDYWGQGTLLVTVSSGGGGSDIQ
 MTQSPSSLSASVGDRTTITCRAS~~QSVSSA~~VAWYQQKPGKAPKLLIYS4SSLYSGVPSRFSRSGTDFTLTLSLQPEDFATYYCQQYWA
YYSPIITFGQGTKVEIK* (SEQ ID NO: 929)

15

ANT39i HC Knob LALAPS

MNLLLLITFVAAA~~VA~~EVQLVESGGGLVQPGGSLRLSCAASGFTLSSYS~~SM~~HHWVRQAPGKGLEWVA~~YISSYDSITDY~~ADSVKGRFTISAD
 TSKNTAYLQMNSLRAEDTAVYYCAR~~PAVGHMAFD~~YWGQGTLLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS
 WNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHNKPSNTKVDKKVEPKSCDKTHTCPPCPAPEA4GGPSVFLFPPKP
 KDTLMISRTPETCVVVDVSHEDPEVKFNWYVDGVEVHNATKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPASIE
 KTISKAKGQPREP~~M~~~~V~~EDLPPSREEMTKNQVSL~~W~~CMVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQG
 NVFSCSVMEALHNHYTQKSLSLSPGKGGSGGGSGGSTGEVQLVESGGGLVQPGGSLRLSCAASGFD~~FTAYAM~~HHWVRQ
 APGKGLEWVA~~SIYPSGGYT~~AYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCAR~~RSYVFA~~LDYWGQGTLLVTVSSGGGGSDIQ
 MTQSPSSLSASVGDRTTITCRAS~~QSVSSA~~VAWYQQKPGKAPKLLIYS4SSLYSGVPSRFSRSGTDFTLTLSLQPEDFATYYCQQYWA
YYSPIITFGQGTKVEIK* (SEQ ID NO: 930)

20

25

ANT39i HC Knob LALAPS Merchant

MNLLLLTFVAAA⁵VAEVQLVESGGGLVQPGGSLRLSCAASGFTLSSYS⁵MHWVRQAPGKGLEWVA⁵YSSYDSITDYADSVKGRFTISAD
TSKNTAYLQMNSLRAEDTAVYYCAR⁵PAVGHMAFDYWGQGT⁵LVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS
WNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKP
KDTLMISRTPETCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVSVLT⁵VLHQDWLNGKEYKCKVSNKALPASP
KTISKAKGQPREPQVYTLPPSREEMTKNQVSLVCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGN
VFSCVMHEALHNHYTQKSLSLSPGKGGGGGGGGSGTGEVQLVESGGGLVQPGGSLRLSCAASGFDFTAYAMHWVRQA
PGKGLEWVA⁵SIYPSGGYTAYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCAR⁵RSYFFALDYWGQGT⁵LVTVSSGGGGSDIQM
TQSPSSLASVGD⁵RVTTITCRASQSVSSAVAWYQQKPGKAPKLLIY⁵SASSLYSGVPSRFSGRSGTDFTLT⁵TISSLQPEDFATYYCQQY⁵W⁵AYY
SPITFGQGTKVEIK* (SEQ ID NO: 931)

10

ANT39i HC Knob LALAPS Merchant S-S

MNLLLLTFVAAA¹⁵VAEVQLVESGGGLVQPGGSLRLSCAASGFTLSSYS⁵MHWVRQAPGKGLEWVA⁵YSSYDSITDYADSVKGRFTISAD
TSKNTAYLQMNSLRAEDTAVYYCAR⁵PAVGHMAFDYWGQGT⁵LVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS
WNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKP
KDTLMISRTPETCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVSVLT⁵VLHQDWLNGKEYKCKVSNKALPASP
KTISKAKGQPREPQVYTLPPSREEMTKNQVSLVCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGN
VFSCVMHEALHNHYTQKSLSLSPGKGGGGGGGGSGTGEVQLVESGGGLVQPGGSLRLSCAASGFDFTAYAMHWVRQA
PGKGLEWVA⁵SIYPSGGYTAYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCAR⁵RSYFFALDYWGQGT⁵LVTVSSGGGGSDIQM
TQSPSSLASVGD⁵RVTTITCRASQSVSSAVAWYQQKPGKAPKLLIY⁵SASSLYSGVPSRFSGRSGTDFTLT⁵TISSLQPEDFATYYCQQY⁵W⁵AYY
SPITFGQGTKVEIK* (SEQ ID NO: 932)

20

Mature ANT39i HC knob DANG

EVQLVESGGGLVQPGGSLRLSCAASGFTLSSYS⁵MHWVRQAPGKGLEWVA⁵YSSYDSITDYADSVKGRFTISADTSKNTAYLQMNSLRA
EDTAVYYCAR⁵PAVGHMAFDYWGQGT⁵LVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAV
LQSSGLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELGGPSVFLFPPKPKDITLMISRTPETCVV
VAVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYGSTYRVSVLT⁵VLHQDWLNGKEYKCKVSNKALPAPIEKTTISKAKGQPREP⁵W⁵F
DLPPSREEMTKNQVSLVCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSCVMHEALHNHY
TQKSLSLSPGKGGGGGGGGSGTGEVQLVESGGGLVQPGGSLRLSCAASGFDFTAYAMHWVRQAPGKGLEWVA⁵SIYPSGG
YTAYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCAR⁵RSYFFALDYWGQGT⁵LVTVSSGGGGSDIQMTQSPSSLASVGD⁵RVTTI

25

TCRASQSLSS4VAWYQQKPGKAPKLLIYSASSLYSGVPSRFSRSGTDFTLTISSLQPEDFATYYCQQYW4YYSPTTFGGQGTKVEIK*
(SEQ ID NO: 933)

Mature ANT39i HC Knob LALAPS

5 EVQLVESGGGLVQPGGSLRLSCAASGFTLSSYSMHWVRQAPGKGLEWVAYISSYDSITDYADSVKGRFTISADTSKNTAYLQMNSLRA
EDTAVYYCARPAVGHMAFDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAV
LQSSGLYSLSVTVTPSSSLGTQTYICNVNHNKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDITLMISRTPEVTCVV
VDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPASEKTIISKAKGQPREPMVF
DLPPSREEMTKNQVSLICMVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSCVMHEALHNHY
TQKLSLSPGKGGSGGGSGGGSGTGEVQLVESGGGLVQPGGSLRLSCAASGFDFTAYAMHWVRQAPGKGLEWVASIYPSGG
10 YTAYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARRSYFFALDYWGQGTLVTVSSGGGGSDIQMTQSPSSLSASVGDRVTI
TCRASQSLSS4VAWYQQKPGKAPKLLIYSASSLYSGVPSRFSRSGTDFTLTISSLQPEDFATYYCQQYW4YYSPTTFGGQGTKVEIK*
(SEQ ID NO: 934)

Mature ANT39i HC Knob LALAPS Merchant

15 EVQLVESGGGLVQPGGSLRLSCAASGFTLSSYSMHWVRQAPGKGLEWVAYISSYDSITDYADSVKGRFTISADTSKNTAYLQMNSLRA
EDTAVYYCARPAVGHMAFDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAV
LQSSGLYSLSVTVTPSSSLGTQTYICNVNHNKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDITLMISRTPEVTCVV
VDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPASEKTIISKAKGQPREPQVY
TLPPSREEMTKNQVSLICLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSCVMHEALHNHY
TQKLSLSPGKGGSGGGSGGGSGTGEVQLVESGGGLVQPGGSLRLSCAASGFDFTAYAMHWVRQAPGKGLEWVASIYPSGG
20 YTAYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARRSYFFALDYWGQGTLVTVSSGGGGSDIQMTQSPSSLSASVGDRVTI
TCRASQSLSS4VAWYQQKPGKAPKLLIYSASSLYSGVPSRFSRSGTDFTLTISSLQPEDFATYYCQQYW4YYSPTTFGGQGTKVEIK*
(SEQ ID NO: 935)

Mature ANT39i HC Knob LALAPS Merchant S-S

25 EVQLVESGGGLVQPGGSLRLSCAASGFTLSSYSMHWVRQAPGKGLEWVAYISSYDSITDYADSVKGRFTISADTSKNTAYLQMNSLRA
EDTAVYYCARPAVGHMAFDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAV
LQSSGLYSLSVTVTPSSSLGTQTYICNVNHNKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDITLMISRTPEVTCVV

VDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPASEKTIKAKGQPREPQVY
 TLPCCREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVSCSVMHEALHNYH
 TQKLSLSPGKGGGGGGGGSGTGEVQLVESGGGLVQPGGSLRLSCAASGFDFTAYAMHWVRQAPGKGLEWVASIYPSGG
 YTAYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARRSYYFALDYWGQGTLLTVSSGGGGSDIQMTQSPSSLSASVGDRTI
 TCRASQSVSSAVAWYQQKPGKAPKLLIYASSLYSGVPSRFSRSGTDFLTITSLQPEDFATYYCQYWA~~YYSPI~~TFGQGTKVEIK*
 (SEQ ID NO: 936)

ANT39 Hc Hole DANG

MNLLLLTFVAAA~~VAE~~VQLVESGGGLVQPGGSLRLSCAASGFD~~ESSS~~HWWVRQAPGKGLEWVAS~~SSSYGYTY~~YADSVKGRFTISADT
 SKNTAYLQMNSLRAEDTAVYYCAR~~SW~~AMDYWGQGTLLTVSSGGGGSDIQMTQSPSSLSASVGDRTITTCRASQSVSSAVAWYQQK
 GKAPKLLIY~~SASDL~~YSGVPSRFSRSGTDFLTITSLQPEDFATYYCQY~~AGAGL~~TFGQGTKVEIKGGGGGGGGSEPKSSDKTHTCPP
 CPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVA~~V~~SHEDPEVKFNWYVDGVEVHNAKTKPREEQYGSTYRVVSVLTVLHQDWLN
 GKEYCKVSNKALPAPIEKTIKAKGQPREPQVYTLPPRE~~MT~~SNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFF
 L~~SK~~LTVDKSRWQQGNVSCSVMHEALHNYTKQSLSLSPGKGGGGGGGGSGTGEVQLVESGGGLVQPGGSLRLSCAAS
 GF~~TLSYS~~SMHWVRQAPGKGLEWVA~~YISSYDSITD~~YADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCAR~~PAVGHMAFD~~YWGQ
 GTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYI
 CNVNHKPSNTKVDKKVEPKSCDKTHT* (SEQ ID NO: 908)

ANT39 Hc Hole LALAPS

MNLLLLTFVAAA~~VAE~~VQLVESGGGLVQPGGSLRLSCAASGFD~~ESSS~~HWWVRQAPGKGLEWVAS~~SSSYGYTY~~YADSVKGRFTISADT
 SKNTAYLQMNSLRAEDTAVYYCAR~~SW~~AMDYWGQGTLLTVSSGGGGSDIQMTQSPSSLSASVGDRTITTCRASQSVSSAVAWYQQK
 GKAPKLLIY~~SASDL~~YSGVPSRFSRSGTDFLTITSLQPEDFATYYCQY~~AGAGL~~TFGQGTKVEIKGGGGGGGGSEPKSSDKTHTCPP
 CPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLN
 GKEYCKVSNKALPASEKTIKAKGQPREPQVYTLPPRE~~MT~~SNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFF
 L~~SK~~LTVDKSRWQQGNVSCSVMHEALHNYTKQSLSLSPGKGGGGGGGGSGTGEVQLVESGGGLVQPGGSLRLSCAAS
 GF~~TLSYS~~SMHWVRQAPGKGLEWVA~~YISSYDSITD~~YADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCAR~~PAVGHMAFD~~YWGQ
 GTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYI
 CNVNHKPSNTKVDKKVEPKSCDKTHT* (SEQ ID NO: 937)

ANT39 Hc Hole LALAPS Merchant

MNLLILTFVAAA~~V~~AEVQLVESGGGLVQPGGSLRLSCAASGFF~~DESSSS~~HWVRQAPGKGLEWVA~~SISSSYGYTY~~YADSVKGRFTISADT
SKNTAYLQMNSLRAEDTAVYYCAR~~SW/AMD~~YWGGTTLVTVSSGGGSDIQMTQSPSSLASVGDRTITCRAS~~QSI/SSA~~VAWYQQKP
GKAPKLLIY~~S4SDLY~~SGVPSRFSGRSGTDFTLTISLQPEDFATYYCQQ~~YAGAGL~~ITFGQGTKVEIKGGGGGGGGSEPKSSDKTHTCPP
CPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNLN
GKEYCKVSNKALPASEKTIKAKGQPREPQVYTLPPSREEMTKNQVSL~~SCA~~VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSF
FL~~/SKL~~TVDKSRWQQGNV~~FSCVM~~HEALHNHYTQKSLSLSPGKGGSGGGGGSGTGEVQLVESGGGLVQPGGSLRLSCAA
SGF~~TLSSYSM~~HWVRQAPGKGLEWVA~~YISSYDSITD~~YADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCAR~~PAVGHMAFDY~~WVG
QGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTY
ICNVNHHKPSNTKVDKKVEPKSCDKTHT* (SEQ ID NO: 938)

5

10

ANT39 Hc Hole LALAPS Merchant S-S

MNLLILTFVAAA~~V~~AEVQLVESGGGLVQPGGSLRLSCAASGFF~~DESSSS~~HWVRQAPGKGLEWVA~~SISSSYGYTY~~YADSVKGRFTISADT
SKNTAYLQMNSLRAEDTAVYYCAR~~SW/AMD~~YWGGTTLVTVSSGGGSDIQMTQSPSSLASVGDRTITCRAS~~QSI/SSA~~VAWYQQKP
GKAPKLLIY~~S4SDLY~~SGVPSRFSGRSGTDFTLTISLQPEDFATYYCQQ~~YAGAGL~~ITFGQGTKVEIKGGGGGGGGSEPKSSDKTHTCPP
CPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNLN
GKEYCKVSNKALPASEKTIKAKGQPREPQVCTLPPSREEMTKNQVSL~~SCA~~VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSF
FL~~/SKL~~TVDKSRWQQGNV~~FSCVM~~HEALHNHYTQKSLSLSPGKGGSGGGGGSGTGEVQLVESGGGLVQPGGSLRLSCAA
SGF~~TLSSYSM~~HWVRQAPGKGLEWVA~~YISSYDSITD~~YADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCAR~~PAVGHMAFDY~~WVG
QGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTY
ICNVNHHKPSNTKVDKKVEPKSCDKTHT* (SEQ ID NO: 939)

15

20

Mature ANT39 Hc Hole DANG

EVQLVESGGGLVQPGGSLRLSCAASGFF~~DESSSS~~HWVRQAPGKGLEWVA~~SISSSYGYTY~~YADSVKGRFTISADTSKNTAYLQMNSLRA
EDTAVYYCAR~~SW/AMD~~YWGGTTLVTVSSGGGSDIQMTQSPSSLASVGDRTITCRAS~~QSI/SSA~~VAWYQQKPGKAPKLLIY~~S4SDLY~~
SGVPSRFSGRSGTDFTLTISLQPEDFATYYCQQ~~YAGAGL~~ITFGQGTKVEIKGGGGGGGGSEPKSSDKTHTCPPAPELLGGPSVFLF
PPKPKDTLMISRTPEVTCVVVA~~V~~SHEDPEVKFNWYVDGVEVHNAKTKPREEQY~~G~~STYRVVSVLTVLHQDWLNLNKEYCKVSNKALP
APIEKTIKAKGQPREPQVYTLPP~~/RE/MT~~NQVSL~~SCA~~VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFL~~/SKL~~TVDKSRWQQ
GNVFSCVMHEALHNHYTQKSLSLSPGKGGSGGGGGSGTGEVQLVESGGGLVQPGGSLRLSCAASGFF~~TLSSYSM~~HWVRQ

25

APGKGLEWVA~~YISSYDSITDY~~ADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCAR~~PAVGHMAFDY~~WGQGTLVTVSSASTKGPS
VFPLAPSSKSTSGGTAAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDK
KVEPKSCDKTHT* (SEQ ID NO: 940)

Mature ANT39 Hc Hole LALAPS

5 EVQLVESGGGLVQPGGSLRLSCAASGEF~~DFSSSS~~~~HHWVRQ~~APGKGLEWVA~~SISSSYGYTY~~YADSVKGRFTISADTSKNTAYLQMNSLRA
EDTAVYYCAR~~SW/AMDY~~WGQGTLVTVSSGGGSDIQMTQSPSSLSASVGDRVITITCRAS~~QSI/SSA~~VAWYQQKPGKAPKLLIY~~S4SDLY~~
SGVPSRFSGRSGTDFTLTISLQPEDFAITYYCCQQ~~YAGAGL~~ITFGQGTKEIKGGGGGGGSEPKSSDKTHITCPPCPAPEAAGGPSVFLF
PPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
ASIEKTIKAKGQPREPQVYTLPP/RE/MTNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFL/SKLTVDKSRWQQ
10 GNVFSCVMHEALHNHYTQKSLSLSPGKGGGGGGGGGGSGTGEVQLVESGGGLVQPGGSLRLSCAASGF~~TLSSYS~~~~MHWVRQ~~
APGKGLEWVA~~YISSYDSITDY~~ADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCAR~~PAVGHMAFDY~~WGQGTLVTVSSASTKGPS
VFPLAPSSKSTSGGTAAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDK
KVEPKSCDKTHT* (SEQ ID NO: 941)

Mature ANT39 Hc Hole LALAPS Merchant

15 EVQLVESGGGLVQPGGSLRLSCAASGEF~~DFSSSS~~~~HHWVRQ~~APGKGLEWVA~~SISSSYGYTY~~YADSVKGRFTISADTSKNTAYLQMNSLRA
EDTAVYYCAR~~SW/AMDY~~WGQGTLVTVSSGGGSDIQMTQSPSSLSASVGDRVITITCRAS~~QSI/SSA~~VAWYQQKPGKAPKLLIY~~S4SDLY~~
SGVPSRFSGRSGTDFTLTISLQPEDFAITYYCCQQ~~YAGAGL~~ITFGQGTKEIKGGGGGGGSEPKSSDKTHITCPPCPAPEAAGGPSVFLF
PPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
ASIEKTIKAKGQPREPQVYTLPPSREEMTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFL/SKLTVDKSRWQ
20 QGNVFSCVMHEALHNHYTQKSLSLSPGKGGGGGGGGGGSGTGEVQLVESGGGLVQPGGSLRLSCAASGF~~TLSSYS~~~~MHWVR~~
QAPGKGLEWVA~~YISSYDSITDY~~ADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCAR~~PAVGHMAFDY~~WGQGTLVTVSSASTKG
SVFPLAPSSKSTSGGTAAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVD
KVEPKSCDKTHT* (SEQ ID NO: 942)

Mature ANT39 Hc Hole LALAPS Merchant S-S

25 EVQLVESGGGLVQPGGSLRLSCAASGEF~~DFSSSS~~~~HHWVRQ~~APGKGLEWVA~~SISSSYGYTY~~YADSVKGRFTISADTSKNTAYLQMNSLRA
EDTAVYYCAR~~SW/AMDY~~WGQGTLVTVSSGGGSDIQMTQSPSSLSASVGDRVITITCRAS~~QSI/SSA~~VAWYQQKPGKAPKLLIY~~S4SDLY~~
SGVPSRFSGRSGTDFTLTISLQPEDFAITYYCCQQ~~YAGAGL~~ITFGQGTKEIKGGGGGGGSEPKSSDKTHITCPPCPAPEAAGGPSVFLF

PPKPKD¹⁰TL¹⁰MISR¹⁰TPE¹⁰VC¹⁰VV¹⁰VD¹⁰VSHED¹⁰PE¹⁰VK¹⁰FN¹⁰W¹⁰Y¹⁰VD¹⁰GV¹⁰EV¹⁰HN¹⁰AK¹⁰TK¹⁰PREE¹⁰Q¹⁰YN¹⁰ST¹⁰Y¹⁰RV¹⁰SV¹⁰LT¹⁰VL¹⁰HQ¹⁰DW¹⁰LN¹⁰GE¹⁰Y¹⁰K¹⁰CK¹⁰V¹⁰SN¹⁰K¹⁰AL¹⁰P¹⁰
 AS¹⁰IE¹⁰K¹⁰TIS¹⁰K¹⁰AK¹⁰GQ¹⁰PRE¹⁰PQ¹⁰VC¹⁰TL¹⁰PP¹⁰SRE¹⁰EM¹⁰TK¹⁰NQ¹⁰VS¹⁰L¹⁰SC¹⁰AV¹⁰K¹⁰GF¹⁰YPS¹⁰DI¹⁰AV¹⁰EW¹⁰ES¹⁰NG¹⁰Q¹⁰PEN¹⁰NY¹⁰KT¹⁰TP¹⁰PV¹⁰LD¹⁰SD¹⁰GS¹⁰FF¹⁰L¹⁰/SK¹⁰LT¹⁰VD¹⁰K¹⁰SR¹⁰W¹⁰Q¹⁰
 QGN¹⁰VF¹⁰SC¹⁰SV¹⁰M¹⁰HE¹⁰AL¹⁰HN¹⁰HY¹⁰TQ¹⁰KS¹⁰LS¹⁰SP¹⁰GK¹⁰GG¹⁰SG¹⁰GG¹⁰SG¹⁰SG¹⁰ST¹⁰GE¹⁰V¹⁰QL¹⁰VE¹⁰SG¹⁰GL¹⁰V¹⁰Q¹⁰PG¹⁰SL¹⁰RL¹⁰SC¹⁰AA¹⁰SG¹⁰F¹⁰TLSSYS¹⁰M¹⁰HW¹⁰VR¹⁰
 QAP¹⁰GK¹⁰GLEW¹⁰VA¹⁰YISSYDSITDY¹⁰AD¹⁰SV¹⁰K¹⁰GR¹⁰FT¹⁰IS¹⁰AD¹⁰TS¹⁰KN¹⁰TA¹⁰YL¹⁰QM¹⁰NS¹⁰LR¹⁰AE¹⁰DT¹⁰AV¹⁰Y¹⁰CA¹⁰R¹⁰PAVGHMA¹⁰FD¹⁰YW¹⁰GQ¹⁰GL¹⁰VT¹⁰V¹⁰SS¹⁰AS¹⁰TK¹⁰GP¹⁰
 SV¹⁰FL¹⁰AP¹⁰SS¹⁰KS¹⁰TS¹⁰GG¹⁰TA¹⁰AL¹⁰GL¹⁰V¹⁰KD¹⁰YF¹⁰PE¹⁰PV¹⁰TV¹⁰SW¹⁰NS¹⁰GA¹⁰LT¹⁰SG¹⁰VH¹⁰TF¹⁰PA¹⁰VL¹⁰Q¹⁰SS¹⁰GL¹⁰YS¹⁰LS¹⁰SV¹⁰TV¹⁰VP¹⁰SS¹⁰LT¹⁰GT¹⁰Q¹⁰TY¹⁰IC¹⁰N¹⁰V¹⁰NH¹⁰K¹⁰PS¹⁰NT¹⁰K¹⁰VD¹⁰
 KK¹⁰VE¹⁰PK¹⁰SCD¹⁰K¹⁰TH¹⁰T* (SEQ ID NO: 943)

ANT39 Hc knob DANG

MN¹⁰LL¹⁰L¹⁰IT¹⁰FV¹⁰AA¹⁰VA¹⁰AE¹⁰V¹⁰QL¹⁰VE¹⁰SG¹⁰GL¹⁰V¹⁰Q¹⁰PG¹⁰SL¹⁰RL¹⁰SC¹⁰AA¹⁰SG¹⁰F¹⁰DFTAYAM¹⁰HW¹⁰VR¹⁰AP¹⁰GK¹⁰GLEW¹⁰VA¹⁰SIYPSGGYTAY¹⁰AD¹⁰SV¹⁰K¹⁰GR¹⁰FT¹⁰ISA¹⁰
 DT¹⁰SK¹⁰NT¹⁰AY¹⁰LQ¹⁰M¹⁰NS¹⁰LR¹⁰AE¹⁰DT¹⁰AV¹⁰Y¹⁰CA¹⁰R¹⁰RSYYFAL¹⁰DY¹⁰WG¹⁰Q¹⁰GL¹⁰VT¹⁰V¹⁰SS¹⁰GG¹⁰SG¹⁰DI¹⁰Q¹⁰MT¹⁰Q¹⁰SP¹⁰SS¹⁰LS¹⁰AS¹⁰V¹⁰G¹⁰DR¹⁰VT¹⁰IT¹⁰CR¹⁰AS¹⁰QSVSS4¹⁰VA¹⁰W¹⁰Y¹⁰
 QQ¹⁰K¹⁰PG¹⁰K¹⁰AP¹⁰K¹⁰LL¹⁰IY¹⁰S4SSL¹⁰YS¹⁰GV¹⁰PS¹⁰RF¹⁰SG¹⁰SR¹⁰SG¹⁰TD¹⁰FT¹⁰LT¹⁰TI¹⁰SS¹⁰LQ¹⁰PE¹⁰DE¹⁰AT¹⁰Y¹⁰YC¹⁰QQ¹⁰YW4YISP¹⁰TF¹⁰G¹⁰Q¹⁰TK¹⁰VE¹⁰IK¹⁰GG¹⁰SG¹⁰GG¹⁰SE¹⁰PK¹⁰SS¹⁰DK¹⁰T¹⁰
 HT¹⁰CP¹⁰CP¹⁰AP¹⁰ELL¹⁰GG¹⁰PS¹⁰VL¹⁰FP¹⁰PK¹⁰DT¹⁰LT¹⁰MIS¹⁰R¹⁰TP¹⁰EV¹⁰TC¹⁰V¹⁰V¹⁰AV¹⁰SHED¹⁰PE¹⁰V¹⁰K¹⁰FN¹⁰W¹⁰Y¹⁰VD¹⁰GV¹⁰EV¹⁰HN¹⁰AK¹⁰TK¹⁰PREE¹⁰Q¹⁰Y¹⁰G¹⁰ST¹⁰Y¹⁰RV¹⁰SV¹⁰LT¹⁰VL¹⁰HQ¹⁰
 DW¹⁰LN¹⁰GE¹⁰Y¹⁰K¹⁰CK¹⁰V¹⁰SN¹⁰K¹⁰AL¹⁰P¹⁰API¹⁰E¹⁰K¹⁰TIS¹⁰K¹⁰AK¹⁰GQ¹⁰PRE¹⁰PQ¹⁰VC¹⁰TL¹⁰PP¹⁰SRE¹⁰EM¹⁰TK¹⁰NQ¹⁰VS¹⁰L¹⁰SC¹⁰AV¹⁰K¹⁰GF¹⁰YPS¹⁰DI¹⁰AV¹⁰EW¹⁰ES¹⁰NG¹⁰Q¹⁰PEN¹⁰NY¹⁰KT¹⁰TP¹⁰PV¹⁰L¹⁰
 DSD¹⁰GS¹⁰FF¹⁰L¹⁰YS¹⁰KL¹⁰TV¹⁰DK¹⁰SR¹⁰W¹⁰Q¹⁰GN¹⁰VF¹⁰SC¹⁰SV¹⁰M¹⁰HE¹⁰AL¹⁰HN¹⁰HY¹⁰TQ¹⁰KS¹⁰LS¹⁰SP¹⁰GK¹⁰GG¹⁰SG¹⁰GG¹⁰SG¹⁰ST¹⁰GE¹⁰V¹⁰QL¹⁰VE¹⁰SG¹⁰GL¹⁰V¹⁰Q¹⁰PG¹⁰SL¹⁰
 RL¹⁰SC¹⁰AA¹⁰SG¹⁰F¹⁰TLSSYS¹⁰M¹⁰HW¹⁰VR¹⁰AP¹⁰GK¹⁰GLEW¹⁰VA¹⁰YISSYDSITDY¹⁰AD¹⁰SV¹⁰K¹⁰GR¹⁰FT¹⁰IS¹⁰AD¹⁰TS¹⁰KN¹⁰TA¹⁰YL¹⁰QM¹⁰NS¹⁰LR¹⁰AE¹⁰DT¹⁰AV¹⁰Y¹⁰CA¹⁰R¹⁰PAVGHMA¹⁰
¹⁵ FDY¹⁰WG¹⁰Q¹⁰GL¹⁰VT¹⁰V¹⁰SS¹⁰AS¹⁰TK¹⁰GPS¹⁰VF¹⁰PL¹⁰AP¹⁰SS¹⁰KS¹⁰TS¹⁰GG¹⁰TA¹⁰AL¹⁰GCL¹⁰V¹⁰KD¹⁰YF¹⁰PE¹⁰PV¹⁰TV¹⁰SW¹⁰NS¹⁰GA¹⁰LT¹⁰SG¹⁰VH¹⁰TF¹⁰PA¹⁰VL¹⁰Q¹⁰SS¹⁰GL¹⁰YS¹⁰LS¹⁰SV¹⁰TV¹⁰VP¹⁰SS¹⁰
 LG¹⁰TQ¹⁰TY¹⁰IC¹⁰N¹⁰V¹⁰NH¹⁰K¹⁰PS¹⁰NT¹⁰K¹⁰VD¹⁰KK¹⁰VE¹⁰PK¹⁰SCD¹⁰K¹⁰TH¹⁰T* (SEQ ID NO: 944)

ANT39 Hc knob LALAPS

MN¹⁰LL¹⁰L¹⁰IT¹⁰FV¹⁰AA¹⁰VA¹⁰AE¹⁰V¹⁰QL¹⁰VE¹⁰SG¹⁰GL¹⁰V¹⁰Q¹⁰PG¹⁰SL¹⁰RL¹⁰SC¹⁰AA¹⁰SG¹⁰F¹⁰DFTAYAM¹⁰HW¹⁰VR¹⁰AP¹⁰GK¹⁰GLEW¹⁰VA¹⁰SIYPSGGYTAY¹⁰AD¹⁰SV¹⁰K¹⁰GR¹⁰FT¹⁰ISA¹⁰
 DT¹⁰SK¹⁰NT¹⁰AY¹⁰LQ¹⁰M¹⁰NS¹⁰LR¹⁰AE¹⁰DT¹⁰AV¹⁰Y¹⁰CA¹⁰R¹⁰RSYYFAL¹⁰DY¹⁰WG¹⁰Q¹⁰GL¹⁰VT¹⁰V¹⁰SS¹⁰GG¹⁰SG¹⁰DI¹⁰Q¹⁰MT¹⁰Q¹⁰SP¹⁰SS¹⁰LS¹⁰AS¹⁰V¹⁰G¹⁰DR¹⁰VT¹⁰IT¹⁰CR¹⁰AS¹⁰QSVSS4¹⁰VA¹⁰W¹⁰Y¹⁰
 QQ¹⁰K¹⁰PG¹⁰K¹⁰AP¹⁰K¹⁰LL¹⁰IY¹⁰S4SSL¹⁰YS¹⁰GV¹⁰PS¹⁰RF¹⁰SG¹⁰SR¹⁰SG¹⁰TD¹⁰FT¹⁰LT¹⁰TI¹⁰SS¹⁰LQ¹⁰PE¹⁰DE¹⁰AT¹⁰Y¹⁰YC¹⁰QQ¹⁰YW4YISP¹⁰TF¹⁰G¹⁰Q¹⁰TK¹⁰VE¹⁰IK¹⁰GG¹⁰SG¹⁰GG¹⁰SE¹⁰PK¹⁰SS¹⁰DK¹⁰T¹⁰
 HT¹⁰CP¹⁰CP¹⁰AP¹⁰EA¹⁰GG¹⁰PS¹⁰VL¹⁰FP¹⁰PK¹⁰DT¹⁰LT¹⁰MIS¹⁰R¹⁰TP¹⁰EV¹⁰TC¹⁰V¹⁰VD¹⁰V¹⁰SHED¹⁰PE¹⁰V¹⁰K¹⁰FN¹⁰W¹⁰Y¹⁰VD¹⁰GV¹⁰EV¹⁰HN¹⁰AK¹⁰TK¹⁰PREE¹⁰Q¹⁰Y¹⁰G¹⁰ST¹⁰Y¹⁰RV¹⁰SV¹⁰LT¹⁰VL¹⁰HQ¹⁰
 DW¹⁰LN¹⁰GE¹⁰Y¹⁰K¹⁰CK¹⁰V¹⁰SN¹⁰K¹⁰AL¹⁰P¹⁰API¹⁰E¹⁰K¹⁰TIS¹⁰K¹⁰AK¹⁰GQ¹⁰PRE¹⁰PQ¹⁰VC¹⁰TL¹⁰PP¹⁰SRE¹⁰EM¹⁰TK¹⁰NQ¹⁰VS¹⁰L¹⁰SC¹⁰AV¹⁰K¹⁰GF¹⁰YPS¹⁰DI¹⁰AV¹⁰EW¹⁰ES¹⁰NG¹⁰Q¹⁰PEN¹⁰NY¹⁰KT¹⁰TP¹⁰PV¹⁰L¹⁰
 DSD¹⁰GS¹⁰FF¹⁰L¹⁰YS¹⁰KL¹⁰TV¹⁰DK¹⁰SR¹⁰W¹⁰Q¹⁰GN¹⁰VF¹⁰SC¹⁰SV¹⁰M¹⁰HE¹⁰AL¹⁰HN¹⁰HY¹⁰TQ¹⁰KS¹⁰LS¹⁰SP¹⁰GK¹⁰GG¹⁰SG¹⁰GG¹⁰SG¹⁰ST¹⁰GE¹⁰V¹⁰QL¹⁰VE¹⁰SG¹⁰GL¹⁰V¹⁰Q¹⁰PG¹⁰SL¹⁰
 RL¹⁰SC¹⁰AA¹⁰SG¹⁰F¹⁰TLSSYS¹⁰M¹⁰HW¹⁰VR¹⁰AP¹⁰GK¹⁰GLEW¹⁰VA¹⁰YISSYDSITDY¹⁰AD¹⁰SV¹⁰K¹⁰GR¹⁰FT¹⁰IS¹⁰AD¹⁰TS¹⁰KN¹⁰TA¹⁰YL¹⁰QM¹⁰NS¹⁰LR¹⁰AE¹⁰DT¹⁰AV¹⁰Y¹⁰CA¹⁰R¹⁰PAVGHMA¹⁰
²⁵ FDY¹⁰WG¹⁰Q¹⁰GL¹⁰VT¹⁰V¹⁰SS¹⁰AS¹⁰TK¹⁰GPS¹⁰VF¹⁰PL¹⁰AP¹⁰SS¹⁰KS¹⁰TS¹⁰GG¹⁰TA¹⁰AL¹⁰GCL¹⁰V¹⁰KD¹⁰YF¹⁰PE¹⁰PV¹⁰TV¹⁰SW¹⁰NS¹⁰GA¹⁰LT¹⁰SG¹⁰VH¹⁰TF¹⁰PA¹⁰VL¹⁰Q¹⁰SS¹⁰GL¹⁰YS¹⁰LS¹⁰SV¹⁰TV¹⁰VP¹⁰SS¹⁰
 LG¹⁰TQ¹⁰TY¹⁰IC¹⁰N¹⁰V¹⁰NH¹⁰K¹⁰PS¹⁰NT¹⁰K¹⁰VD¹⁰KK¹⁰VE¹⁰PK¹⁰SCD¹⁰K¹⁰TH¹⁰T* (SEQ ID NO: 945)

ANT39 Hc knob LALAPS Merchant

MNLLILTFVAAA⁵VAEVQLVESGGGLVQPGGSLRLSCAASGF**DF****TAYAM**HWVVRQAPGKGLEWVA**SIYPSGGYT**AYADSVKGRFTISA
 DTSKNTAYLQMNSLRAEDTAVYYCAR**RSYYF**ALDYWGQGTLLTVSSGGGGSDIQTQSPSSLSASVGDRVTITCRAS**QSI****SSA**VAWY
 QQKPGKAPKLLIY**S4SSL**YSGVPSRFSGRSGTDFTLTISSLQPEDFATYYCQQ**YW4IYSP**ITFGQGTKEIKGGGGGGGSEPKSSDKT
 HTCPCPAPEA4AGGPSVFLFPPKPKD¹⁰TLMSRTPETCVVDVSHEDPEVKFNWYVDGVEVHNATKPREEQYNSTYRVSVLTVLHQ
 DWLNGKEYCKVSNKALPASEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLVCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLD
 SDGSFFLYSKLTVDKSRWQQGNV¹⁵VFSCVMHEALHNHYTQKSLSPGKGGGGGGGGSGTGEVQLVESGGGLVQPGGSLR
 LSCAASGF**TLSSYS**MHWVVRQAPGKGLEWVA**YISSYDSITD**YADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCAR**PAVGHMAF**
 DYWGQGTLLTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSL
 GTQTYICNVNHKPSNTKVDKKVEPKSCDKTHT* (SEQ ID NO: 946)

ANT39 Hc knob LALAPS Merchant S-S

MNLLILTFVAAA¹⁰VAEVQLVESGGGLVQPGGSLRLSCAASGF**DF****TAYAM**HWVVRQAPGKGLEWVA**SIYPSGGYT**AYADSVKGRFTISA
 DTSKNTAYLQMNSLRAEDTAVYYCAR**RSYYF**ALDYWGQGTLLTVSSGGGGSDIQTQSPSSLSASVGDRVTITCRAS**QSI****SSA**VAWY
 QQKPGKAPKLLIY**S4SSL**YSGVPSRFSGRSGTDFTLTISSLQPEDFATYYCQQ**YW4IYSP**ITFGQGTKEIKGGGGGGGSEPKSSDKT
 HTCPCPAPEA4AGGPSVFLFPPKPKD¹⁵TLMSRTPETCVVDVSHEDPEVKFNWYVDGVEVHNATKPREEQYNSTYRVSVLTVLHQ
 DWLNGKEYCKVSNKALPASEKTISKAKGQPREPQVYTLPPCREEMTKNQVSLVCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLD
 SDGSFFLYSKLTVDKSRWQQGNV²⁰VFSCVMHEALHNHYTQKSLSPGKGGGGGGGGSGTGEVQLVESGGGLVQPGGSLR
 LSCAASGF**TLSSYS**MHWVVRQAPGKGLEWVA**YISSYDSITD**YADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCAR**PAVGHMAF**
 DYWGQGTLLTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSL
 GTQTYICNVNHKPSNTKVDKKVEPKSCDKTHT* (SEQ ID NO: 947)

Mature ANT39 Hc knob DANG

EVQLVESGGGLVQPGGSLRLSCAASGF**DF****TAYAM**HWVVRQAPGKGLEWVA**SIYPSGGYT**AYADSVKGRFTISADTSKNTAYLQMNSLR
 AEDTAVYYCAR**RSYYF**ALDYWGQGTLLTVSSGGGGSDIQTQSPSSLSASVGDRVTITCRAS**QSI****SSA**VAWYQQKPGKAPKLLIY**S4S**
SLYSGVPSRFSGRSGTDFTLTISSLQPEDFATYYCQQ**YW4IYSP**ITFGQGTKEIKGGGGGGGSEPKSSDKTHTCPCPAPELLGGPS
 VFLFPPKPKD²⁵TLMSRTPETCVVDVSHEDPEVKFNWYVDGVEVHNATKPREEQYGSTYRVSVLTVLHQDWLNGKEYCKVSN
 KALPAPIEKTISKAKGQPREP**MF**DLPPSREEMTKNQVSLVCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDK
 SRWQQGNVVFSCVMHEALHNHYTQKSLSPGKGGGGGGGGSGTGEVQLVESGGGLVQPGGSLRLSCAASGF**TLSSYS**M

HWVRQAPGKGLEWVA~~YISSYDSITD~~YADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCAR~~PAVGHMAFD~~YWGGGTLVTVSSA
STKGPSVFPLAPSSKSTGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSN
TKVDKKVEPKSCDKTHT* (SEQ ID NO: 948)

Mature ANT39 Hc knob LALAPS

5 EVQLVESGGGLVQPGGSLRLSCAASGEF~~DFTAYAMH~~WVRQAPGKGLEWVA~~SIYPSGGYTA~~YADSVKGRFTISADTSKNTAYLQMNSLR
AEDTAVYYCAR~~RSYYFAL~~DYWGQGTLVTVSSGGGSDIQMTQSPSSLSASVGDRTITCRAS~~QSISSA~~VAWYQQKPGKAPKLLIY~~SAS~~
~~SL~~YSGVPSRFSGRSGTDFTLTISLQPEDFATYYCQQY~~WAIYSPIT~~TFGQGTKVEIKGGGGGGGSEPKSSDKTHTCPPCPAPEAAGGPS
VFLFPPKPKDITLMISRTPETCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSN
KALPASEIETISKAKGQPREP~~MVE~~DLPPSREEMTKNQVSL~~LC~~MVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDK
SRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKGGGGGGGGSGSTGEVQLVESGGGLVQPGGSLRLS~~CAASGF~~~~TLSSYSM~~
10 HWVRQAPGKGLEWVA~~YISSYDSITD~~YADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCAR~~PAVGHMAFD~~YWGGGTLVTVSSA
STKGPSVFPLAPSSKSTGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSN
TKVDKKVEPKSCDKTHT* (SEQ ID NO: 949)

Mature ANT39 Hc knob LALAPS Merchant

15 EVQLVESGGGLVQPGGSLRLSCAASGEF~~DFTAYAMH~~WVRQAPGKGLEWVA~~SIYPSGGYTA~~YADSVKGRFTISADTSKNTAYLQMNSLR
AEDTAVYYCAR~~RSYYFAL~~DYWGQGTLVTVSSGGGSDIQMTQSPSSLSASVGDRTITCRAS~~QSISSA~~VAWYQQKPGKAPKLLIY~~SAS~~
~~SL~~YSGVPSRFSGRSGTDFTLTISLQPEDFATYYCQQY~~WAIYSPIT~~TFGQGTKVEIKGGGGGGGSEPKSSDKTHTCPPCPAPEAAGGPS
VFLFPPKPKDITLMISRTPETCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSN
KALPASEIETISKAKGQPREPQVYTLPPSREEMTKNQVSL~~LC~~MVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDK
SRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKGGGGGGGGSGSTGEVQLVESGGGLVQPGGSLRLS~~CAASGF~~~~TLSSYSM~~
20 HWVRQAPGKGLEWVA~~YISSYDSITD~~YADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCAR~~PAVGHMAFD~~YWGGGTLVTVSSA
STKGPSVFPLAPSSKSTGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSN
TKVDKKVEPKSCDKTHT* (SEQ ID NO: 950)

Mature ANT39 Hc knob LALAPS Merchant

25 EVQLVESGGGLVQPGGSLRLSCAASGEF~~DFTAYAMH~~WVRQAPGKGLEWVA~~SIYPSGGYTA~~YADSVKGRFTISADTSKNTAYLQMNSLR
AEDTAVYYCAR~~RSYYFAL~~DYWGQGTLVTVSSGGGSDIQMTQSPSSLSASVGDRTITCRAS~~QSISSA~~VAWYQQKPGKAPKLLIY~~SAS~~
~~SL~~YSGVPSRFSGRSGTDFTLTISLQPEDFATYYCQQY~~WAIYSPIT~~TFGQGTKVEIKGGGGGGGSEPKSSDKTHTCPPCPAPEAAGGPS

VLFPPKPKD TLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSN
KALPASEIETISKAKGQPREPQVYTLPPCREEMTKNQVSLVCLVKFGFYPSDIAVEWESNGQPENNYKTTPPVLDSGGSFFLYSKLTVDK
SRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKGGGGGGSGSTGEVQLVESGGGLVQPGGSLRLSCAASGFTLSYSYM
HWVRQAPGKGLEWVA~~YISSYDSITD~~YADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCAR~~PAVGHMAFD~~YWGGGTLVTVSSA
STKGPSVFPFLAPSSKSTSGGTAAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSN
TKVDKCKVEPKSCDKTHT* (SEQ ID NO: 951)

ANT39 LC

MNLLLLITFVAAAADIQMTQSPSSLSASVGDRVTITCRAS~~QSLSSA~~VAWYQQKPGKAPKLLIY~~SASSLY~~SGVPSRFSGRSGTDFTLTIS
SLQPEDFATYYCQQ~~WYNAPIT~~FGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVCLLNNFYPREAKVQWKVDNALQSGNSQESVT
EQDSKDYSLSSLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC* (SEQ ID NO: 909)

Mature ANT39 LC

DIQMTQSPSSLSASVGDRVTITCRAS~~QSLSSA~~VAWYQQKPGKAPKLLIY~~SASSLY~~SGVPSRFSGRSGTDFTLTISSLQPEDFATYYCQQ~~W
YNAPIT~~FGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSLSSLTLS
SKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 952)

ANT42i HC hole DANG

MNLLLLITFVAAA~~VAE~~VQLVESGGGLVQPGGSLRLSCAASGFTSD~~FYFJH~~WVRQAPGKGLEWVA~~TIYFFIG~~NTYYADSVKGRFTISAD
TSKNTAYLQMNSLRAEDTAVYYCAR~~AFPGSYH~~PF~~FD~~YWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAAALGCLVKDYFPEPVTVS
WNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKP
KDTLMISRTPEVTCVVVA~~V~~SHEDPEVKFNWYVDGVEVHNAKTKPREEQYGSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE
KTISKAKGQPREPQVYTLPP~~RE~~LM~~TS~~NQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFL~~L~~SKLTVDKSRWQQGNV
FSCSVMHEALHNHYTQKSLSLSPGKGGGGGGSGSTGEVQLVESGGGLVQPGGSLRLSCAASGFT~~DFSSSJH~~WVRQAPG
KGLEWVA~~ASISSYGYTY~~YADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCAR~~SWAMD~~YWGQGTLVTVSSGGGGSDIQMTQSPS
LSASVGDRVTITCRAS~~QSLSSA~~VAWYQQKPGKAPKLLIY~~SASDL~~YSGVPSRFSGRSGTDFTLTISSLQPEDFATYYCQQ~~YAGAGLIT~~FG
QGTKVEIK* (SEQ ID NO: 953)

ANT42i HC hole LALAPS

5 MNLLLILTFVAAA VAEVQLVESGGGLVQPGGSLRLSCAASGFTSD~~DFYF~~HWVRQAPGKGLEWVA~~TIYFFIGNTYY~~ADSVKGRFTISAD
 TSKNTAYLQMNSLRAEDTAVYYCAR~~AFPGSYHPFDY~~WGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS
 WNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKP
 KDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVSVLTVLHQDWLNGKEYCKVSNKALPASI
 KTISKAKGQPREPQVYTLPPRE/M~~TNQVSL~~CAVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFL/SKLTVDKSRWQQGNV
 FSCVMHEALHNHYTQKSLSPGKGGSGGGSGSGTGEVQLVESGGGLVQPGGSLRLSCAASGF~~DFSSSS~~HWVRQAPG
 KGLEWVA~~ASSSSYGYTY~~ADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCAR~~SWAMDY~~WGQGTLVTVSSGGGGSDIQMTQSPS
 SLSASVGDRVTITCRAS~~QSLSSA~~VAWYQQKPGKAPKLLIY~~SASDL~~YSGVPSRFSGSRSGTDFTLTISLQPEDFATYYCQQ~~YAGAGLIT~~FG
 10 QGTKVEIK* (SEQ ID NO: 954)

ANT42i HC hole LALAPS Merchant

15 MNLLLILTFVAAA VAEVQLVESGGGLVQPGGSLRLSCAASGFTSD~~DFYF~~HWVRQAPGKGLEWVA~~TIYFFIGNTYY~~ADSVKGRFTISAD
 TSKNTAYLQMNSLRAEDTAVYYCAR~~AFPGSYHPFDY~~WGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS
 WNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKP
 KDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVSVLTVLHQDWLNGKEYCKVSNKALPASI
 KTISKAKGQPREPQVYTLPPSREEMTKNQVSL~~CA~~VKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFL/SKLTVDKSRWQQGN
 VFSCVMHEALHNHYTQKSLSPGKGGSGGGSGSGTGEVQLVESGGGLVQPGGSLRLSCAASGF~~DFSSSS~~HWVRQAP
 GKGLEWVA~~SISSSYGYTY~~ADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCAR~~SWAMDY~~WGQGTLVTVSSGGGGSDIQMTQS
 PSSLSASVGDRVTITCRAS~~QSLSSA~~VAWYQQKPGKAPKLLIY~~SASDL~~YSGVPSRFSGSRSGTDFTLTISLQPEDFATYYCQQ~~YAGAGLIT~~
 20 FGQGTKVEIK* (SEQ ID NO: 955)

ANT42i HC hole LALAPS Merchant S-S

25 MNLLLILTFVAAA VAEVQLVESGGGLVQPGGSLRLSCAASGFTSD~~DFYF~~HWVRQAPGKGLEWVA~~TIYFFIGNTYY~~ADSVKGRFTISAD
 TSKNTAYLQMNSLRAEDTAVYYCAR~~AFPGSYHPFDY~~WGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS
 WNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKP
 KDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVSVLTVLHQDWLNGKEYCKVSNKALPASI
 KTISKAKGQPREPQVCTLPPSREEMTKNQVSL~~CA~~VKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFL/SKLTVDKSRWQQGN
 VFSCVMHEALHNHYTQKSLSPGKGGSGGGSGSGTGEVQLVESGGGLVQPGGSLRLSCAASGF~~DFSSSS~~HWVRQAP

GKGLEWVA~~SSSSYGYT~~YADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCAR~~SW~~AMDYWGQGT~~LVTVSSGGGSDIQMTQS~~
PSSLSASVGDRVTITCRA~~SQSSAV~~AWYQQKPGKAPKLLIY~~SAD~~LYSGVPSRFSGRSGTDFTLTISSLQPEDFATYYCQQ~~YAGAGLIT~~
FGQGTKVEIK* (SEQ ID NO: 956)

Mature ANT42i HC hole DANG

5 EVQLVESGGGLVQPGGSLRLSCAASGF~~TSDFYF~~HWVRQAPGKGLEWVA~~TIYFIGNTY~~YADSVKGRFTISADTSKNTAYLQMNSLRA
EDTAVYYCAR~~AFPGSYHP~~FDYWGQGT~~LVTVSSASTKGPSVF~~PLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAV
LQSSGLYSLSVVTVPSSSLGTQTYICNVNHNKPSNTKVDKKVEPKSCDKTHTCPPCPAPELGGPSVFLFPPKPKDTLMISRTPEVTCVV
VAVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYGSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVY
TLPP~~RE~~MT~~SNQVSL~~CAVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFL~~LSKLTVDKSRWQQGNV~~FSCSVMH~~EA~~LNHNYT
10 QKSLSPGKGGSGGGSGGGSGGSTGEVQLVESGGGLVQPGGSLRLSCAASGF~~DFSSSY~~HWVRQAPGKGLEWVA~~SSSSYGYT~~
YYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCAR~~SW~~AMDYWGQGT~~LVTVSSGGGSDIQMTQSPSSL~~SASVGDRVTITCRA
~~SQSSAV~~AWYQQKPGKAPKLLIY~~SAD~~LYSGVPSRFSGRSGTDFTLTISSLQPEDFATYYCQQ~~YAGAGLIT~~FGQGTKVEIK* (SEQ ID
NO: 957)

Mature ANT42i HC hole LALAPS

15 EVQLVESGGGLVQPGGSLRLSCAASGF~~TSDFYF~~HWVRQAPGKGLEWVA~~TIYFIGNTY~~YADSVKGRFTISADTSKNTAYLQMNSLRA
EDTAVYYCAR~~AFPGSYHP~~FDYWGQGT~~LVTVSSASTKGPSVF~~PLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAV
LQSSGLYSLSVVTVPSSSLGTQTYICNVNHNKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVV
VDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVY
TLPP~~RE~~MT~~SNQVSL~~CAVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFL~~LSKLTVDKSRWQQGNV~~FSCSVMH~~EA~~LNHNYT
20 QKSLSPGKGGSGGGSGGGSGGSTGEVQLVESGGGLVQPGGSLRLSCAASGF~~DFSSSY~~HWVRQAPGKGLEWVA~~SSSSYGYT~~
YYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCAR~~SW~~AMDYWGQGT~~LVTVSSGGGSDIQMTQSPSSL~~SASVGDRVTITCRA
~~SQSSAV~~AWYQQKPGKAPKLLIY~~SAD~~LYSGVPSRFSGRSGTDFTLTISSLQPEDFATYYCQQ~~YAGAGLIT~~FGQGTKVEIK* (SEQ ID
NO: 958)

Mature ANT42i HC hole LALAPS Merchant

25 EVQLVESGGGLVQPGGSLRLSCAASGF~~TSDFYF~~HWVRQAPGKGLEWVA~~TIYFIGNTY~~YADSVKGRFTISADTSKNTAYLQMNSLRA
EDTAVYYCAR~~AFPGSYHP~~FDYWGQGT~~LVTVSSASTKGPSVF~~PLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAV
LQSSGLYSLSVVTVPSSSLGTQTYICNVNHNKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVV

VDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPASEKTIKAKGQPREPQVY
 TLPSSREEMTKNQVSL_{SC}AVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFL_LSKLTVDKSRWQQGNVFCFSVMHEALHNHYT
 QKSLSLSPGKGGSGGGSGGSTGEVQLVESGGGLVQPGGSLRLSCAASGF~~DESSSS~~HWVRQAPGKGLEWVA~~SSSSYGYT~~
~~YY~~ADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCAR~~SW~~~~AM~~DYWGQGTILVTVSSGGGGSDIQMTQSPSSLSASVGDRVTITCRA
~~SQSLSS~~~~AV~~AWYQQKPGKAPKLLIY~~S~~~~ASDL~~YSGVPSRFSGSRSGTDFTLTISSLPQEDFATYYCQ~~YAGAGL~~ITFGQGTKEIK* (SEQ ID
 NO: 959)

Mature ANT42i HC hole LALAPS Merchant S-S

EVQLVESGGGLVQPGGSLRLSCAASGF~~TSDFYF~~HWVRQAPGKGLEWVA~~TIYFIGN~~TYADSVKGRFTISADTSKNTAYLQMNSLRA
 EDTAVYYCAR~~AFPGSYHP~~FDYWGQGTILVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAV
 LQSSGLYSLSSVTVTPSSSLGTQTYICNVNHNKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVV
 VDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPASEKTIKAKGQPREPQV
 TLPSSREEMTKNQVSL_{SC}AVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFL_LSKLTVDKSRWQQGNVFCFSVMHEALHNHYT
 QKSLSLSPGKGGSGGGSGGSTGEVQLVESGGGLVQPGGSLRLSCAASGF~~DESSSS~~HWVRQAPGKGLEWVA~~SSSSYGYT~~
~~YY~~ADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCAR~~SW~~~~AM~~DYWGQGTILVTVSSGGGGSDIQMTQSPSSLSASVGDRVTITCRA
~~SQSLSS~~~~AV~~AWYQQKPGKAPKLLIY~~S~~~~ASDL~~YSGVPSRFSGSRSGTDFTLTISSLPQEDFATYYCQ~~YAGAGL~~ITFGQGTKEIK* (SEQ ID
 NO: 960)

ANT42i HC knob DANG

MNLLLLITFVAAA_{AV}AEVQLVESGGGLVQPGGSLRLSCAASGF~~TSDFYF~~HWVRQAPGKGLEWVA~~TIYFIGN~~TYADSVKGRFTISAD
 TSKNTAYLQMNSLRAEDTAVYYCAR~~AFPGSYHP~~FDYWGQGTILVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS
 WNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHNKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKP
 KDTLMISRTPEVTCVVAVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYGSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE
 KTISKAKGQPREP~~M~~~~VD~~LPPSREEMTKNQVSL~~WC~~~~M~~KGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQG
 NVFSCVMHEALHNHYTQKSLSPGKGGSGGGSGGSTGEVQLVESGGGLVQPGGSLRLSCAASGF~~DTAYAM~~HWVRQ
 APGKGLEWVA~~SIYPSGGY~~TA_YADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCAR~~RSYF~~~~AL~~DYWGQGTILVTVSSGGGGSDIQ
 MTQSPSSLSASVGDRVTITCRAS~~OS~~~~SS~~~~AV~~AWYQQKPGKAPKLLIY~~S~~~~ASDL~~YSGVPSRFSGSRSGTDFTLTISSLPQEDFATYYCQ~~Y~~~~W~~~~A~~
~~YS~~~~PI~~ITFGQGTKEIK* (SEQ ID NO: 961)

ANT42i HC Knob LALAPS

5 MNLLLILTFVAAA/AEVQLVESGGGLVQPGGSLRLSCAASGFTSDFYFJHWWVRQAPGKGLEWVA TIYPFIGNNTYYADSVKGRFTISAD
TSKNTAYLQMNSLRAEDTAVYYCAR AFPGSYHPFDDYWGQGTLLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS
WNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKP
KDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPASI
KTISKAKGQPREP MVEDLPPSREEMTKNQVSL WCLVKGYFPSDIAVEWESNGQPENNYKTTPVLDSDGSFFLYSKLTVDKSRWQQG
NVFSCSVMEALHNHYTQKSLSLSPGKGGSGGGSGGSTGEVQLVESGGGLVQPGGSLRLSCAASGFT DFTAYAMHHWVRQ
APGKGLEWVA SIYPSGGYTAYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCAR RSYYFALDYWGQGTLLVTVSSGGGGSDIQ
MTQSPSSLSASVGDRVTITCRAS QSVSSAVAWYQQKPGKAPKLLIY SASSLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQY W W
10 YKSPITFGQGTKVEIK* (SEQ ID NO: 962)

ANT42i HC Knob LALAPS Merchant

15 MNLLLILTFVAAA/AEVQLVESGGGLVQPGGSLRLSCAASGFTSDFYFJHWWVRQAPGKGLEWVA TIYPFIGNNTYYADSVKGRFTISAD
TSKNTAYLQMNSLRAEDTAVYYCAR AFPGSYHPFDDYWGQGTLLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS
WNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKP
KDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPASI
KTISKAKGQPREPQVYTLPPSREEMTKNQVSL WCLVKGYFPSDIAVEWESNGQPENNYKTTPVLDSDGSFFLYSKLTVDKSRWQQGN
VFSCSVMEALHNHYTQKSLSLSPGKGGSGGGSGGSTGEVQLVESGGGLVQPGGSLRLSCAASGFT DFTAYAMHHWVRQA
PGKGLEWVA SIYPSGGYTAYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCAR RSYYFALDYWGQGTLLVTVSSGGGGSDIQM
TQSPSSLSASVGDRVTITCRAS QSVSSAVAWYQQKPGKAPKLLIY SASSLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQY W W
20 SPITFGQGTKVEIK* (SEQ ID NO: 963)

ANT42i HC Knob LALAPS Merchant S-S

25 MNLLLILTFVAAA/AEVQLVESGGGLVQPGGSLRLSCAASGFTSDFYFJHWWVRQAPGKGLEWVA TIYPFIGNNTYYADSVKGRFTISAD
TSKNTAYLQMNSLRAEDTAVYYCAR AFPGSYHPFDDYWGQGTLLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS
WNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKP
KDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPASI
KTISKAKGQPREPQVYTLPPSREEMTKNQVSL WCLVKGYFPSDIAVEWESNGQPENNYKTTPVLDSDGSFFLYSKLTVDKSRWQQGN
VFSCSVMEALHNHYTQKSLSLSPGKGGSGGGSGGSTGEVQLVESGGGLVQPGGSLRLSCAASGFT DFTAYAMHHWVRQA

PGKGLEWVA~~SIYPSGGYTAY~~ADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCAR~~RSYYFAL~~DYWGQGTLVTVSSGGGGSDIQMTQSPSSLSASVGD~~RV~~TITCRAS~~QSVSSAV~~AWYQQKPGKAPKLLIY~~SASSLYSGVPSRFSGRSGTDFTL~~TISSLQPEDFATYYCQ~~YW~~~~AYY~~~~SPT~~TFGGQGTKVEIK* (SEQ ID NO: 964)

Mature ANT42i HC knob DANG

5 EVQLVESGGGLVQPGGSLRLSCAASGF~~TSDFYFJH~~HWVRQAPGKGLEWVA~~TIYFIGNTY~~YADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCAR~~AFPGSYHPFDY~~WGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHNKPSNTKVDKKVEPKSCDKTHTCPPCPAPELGGPSVFLFPPKPKDTLMISRTPEVTCVVVAVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYGSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP~~MVF~~~~DL~~PPSREEMTKNQVSL~~WCMVK~~GFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSCVMHEALHNHYTQKLSLSPGKGGSGGGSGGSGTGEVQLVESGGGLVQPGGSLRLSCAASGF~~DFTAYAM~~HWVRQAPGKGLEWVA~~SIYPSGGYTAY~~ADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCAR~~RSYYFAL~~DYWGQGTLVTVSSGGGGSDIQMTQSPSSLSASVGD~~RV~~TITCRAS~~QSVSSAV~~AWYQQKPGKAPKLLIY~~SASSLYSGVPSRFSGRSGTDFTL~~TISSLQPEDFATYYCQ~~YW~~~~AYY~~~~SPT~~TFGGQGTKVEIK* (SEQ ID NO: 965)

Mature ANT42i HC Knob LALAPS

15 EVQLVESGGGLVQPGGSLRLSCAASGF~~TSDFYFJH~~HWVRQAPGKGLEWVA~~TIYFIGNTY~~YADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCAR~~AFPGSYHPFDY~~WGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHNKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP~~MVF~~~~DL~~PPSREEMTKNQVSL~~WCMVK~~GFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSCVMHEALHNHYTQKLSLSPGKGGSGGGSGGSGTGEVQLVESGGGLVQPGGSLRLSCAASGF~~DFTAYAM~~HWVRQAPGKGLEWVA~~SIYPSGGYTAY~~ADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCAR~~RSYYFAL~~DYWGQGTLVTVSSGGGGSDIQMTQSPSSLSASVGD~~RV~~TITCRAS~~QSVSSAV~~AWYQQKPGKAPKLLIY~~SASSLYSGVPSRFSGRSGTDFTL~~TISSLQPEDFATYYCQ~~YW~~~~AYY~~~~SPT~~TFGGQGTKVEIK* (SEQ ID NO: 966)

Mature ANT42i HC Knob LALAPS Merchant

25 EVQLVESGGGLVQPGGSLRLSCAASGF~~TSDFYFJH~~HWVRQAPGKGLEWVA~~TIYFIGNTY~~YADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCAR~~AFPGSYHPFDY~~WGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHNKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVV

VDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPASEIETISKAKGQPREPQVY
 TLPPSREEMTKNQVSLVCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFELYSKLTVDKSRWQQGNVFCSCVMHEALHNYH
 TQKLSLSPGKGGGGGGGGSGTGEVQLVESGGGLVQPGGSLRLSCAASGFDFTAYAMHWVRQAPGKGLEWVASIYPSGG
YTAYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYCARRSYYFALDYWGQGTLLTVSSGGGGSDIQMTQSPSSLSASVGDRTVI
 TCRASQSVSSAVAWYQQKPGKAPKLLIYSASSLYSGVPSRFSRSGTDFTLTISSLQPEDFATYYCQQYWAYYSPITFGGQGTKVEIK*
 (SEQ ID NO: 967)

Mature ANT42i HC Knob LALAPS Merchant S-S

EVQLVESGGGLVQPGGSLRLSCAASGFTSDYF~~YF~~HWVRQAPGKGLEWVATIYFIGNTYADSVKGRFTISADTSKNTAYLQMNSLRA
 EDTAVYCARAFPGSYHPFDYWGQGTLLTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAV
 LQSSGLYSLSVTVTPSSSLGTQTYICNVNHNKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVV
 VDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPASEIETISKAKGQPREPQVY
 TLPPCREEMTKNQVSLVCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFELYSKLTVDKSRWQQGNVFCSCVMHEALHNYH
 TQKLSLSPGKGGGGGGGGSGTGEVQLVESGGGLVQPGGSLRLSCAASGFDFTAYAMHWVRQAPGKGLEWVASIYPSGG
YTAYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYCARRSYYFALDYWGQGTLLTVSSGGGGSDIQMTQSPSSLSASVGDRTVI
 TCRASQSVSSAVAWYQQKPGKAPKLLIYSASSLYSGVPSRFSRSGTDFTLTISSLQPEDFATYYCQQYWAYYSPITFGGQGTKVEIK*
 (SEQ ID NO: 968)

ANT42 Hc Hole DANG

MNLLILTFVAAVAEVQLVESGGGLVQPGGSLRLSCAASGFD~~FSSS~~HWVRQAPGKGLEWVASISSSYGYTYADSVKGRFTISADT
 SKNTAYLQMNSLRAEDTAVYCARSWAMDYWGQGTLLTVSSGGGGSDIQMTQSPSSLSASVGDRTVITTCRASQSVSSAVAWYQQK
 GKAPKLLIYSASDLYSGVPSRFSRSGTDFTLTISSLQPEDFATYYCQQYAGAGLTFGGQGTKVEIKGGGGGGGGSEPKSSDKTHTCPP
 CPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVAVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYGYSTYRVVSVLTVLHQDWL
 N GKEYKCKVSNKALPAPIEKTIISKAKGQPREPQVYTLPPREMTSNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFF
 LJ/SKLTVDKSRWQQGNVFCSCVMHEALHNYTQKLSLSPGKGGGGGGGGSGTGEVQLVESGGGLVQPGGSLRLSCAAS
 GFTSDYF~~YF~~HWVRQAPGKGLEWVATIYFIGNTYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYCARAFPGSYHPFDYWGQ
 GTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYI
 CNVNHKPSNTKVDKKVEPKSCDKTHT* (SEQ ID NO: 911)

ANT42 Hc Hole LALAPS

5 MNLLLILTFVAAA~~VA~~AEVQLVESGGGLVQPGGSLRLSCAASGFF~~DESSSS~~HWVRQAPGKGLEWVA~~SSSSSYGYTY~~YADSVKGRFTISADT
 SKNTAYLQMNSLRAEDTAVYYCAR~~SW/AMD~~YWGQGTILVTVSSGGGSDIQMTQSPSSLSASVGDRVTITCRAS~~QSV/SSA~~VAWYQQKP
 GKAPKLLIY~~SASDL~~YSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQ~~YAGAGL~~ITFGQGTKVEIKGGGGGGGGSEPKSSDKTHTCPP
 CPAEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNATKPREEQYNSTYRVVSVLTVLHQDWLNLN
 GKEYCKVSNKALPASEKTISKAKGQPREPQVYTLPP/RE/MT~~SNQ~~SL~~SC~~AVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFF
 LJ~~SKL~~TVDKSRWQQGNVFSCVMEALHNHYTQKSLSLSPKGGGGGGGGGGSGTGEVQLVESGGGLVQPGGSLRLSCAAS
 GFT~~SDFFYF~~HWVRQAPGKGLEWVA~~TIYPIFIGN~~TYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCAR~~AFPGSYHPFD~~YWGQ
 GTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYI
 10 CNVNHKPSNTKVDKKVEPKSCDKTHT* (SEQ ID NO: 969)

ANT42 Hc Hole LALAPS Merchant

15 MNLLLILTFVAAA~~VA~~AEVQLVESGGGLVQPGGSLRLSCAASGFF~~DESSSS~~HWVRQAPGKGLEWVA~~SSSSSYGYTY~~YADSVKGRFTISADT
 SKNTAYLQMNSLRAEDTAVYYCAR~~SW/AMD~~YWGQGTILVTVSSGGGSDIQMTQSPSSLSASVGDRVTITCRAS~~QSV/SSA~~VAWYQQKP
 GKAPKLLIY~~SASDL~~YSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQ~~YAGAGL~~ITFGQGTKVEIKGGGGGGGGSEPKSSDKTHTCPP
 CPAEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNATKPREEQYNSTYRVVSVLTVLHQDWLNLN
 GKEYCKVSNKALPASEKTISKAKGQPREPQVYTLPPSREEMTKNQVSL~~SC~~AVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSF
 FL~~J~~SKLTVDKSRWQQGNVFSCVMEALHNHYTQKSLSLSPKGGGGGGGGGGSGTGEVQLVESGGGLVQPGGSLRLSCAA
 SGF~~TSDFYF~~HWVRQAPGKGLEWVA~~TIYPIFIGN~~TYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCAR~~AFPGSYHPFD~~YWG
 QGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTY
 20 ICNVNHKPSNTKVDKKVEPKSCDKTHT* (SEQ ID NO: 970)

ANT42 Hc Hole LALAPS Merchant S-S

25 MNLLLILTFVAAA~~VA~~AEVQLVESGGGLVQPGGSLRLSCAASGFF~~DESSSS~~HWVRQAPGKGLEWVA~~SSSSSYGYTY~~YADSVKGRFTISADT
 SKNTAYLQMNSLRAEDTAVYYCAR~~SW/AMD~~YWGQGTILVTVSSGGGSDIQMTQSPSSLSASVGDRVTITCRAS~~QSV/SSA~~VAWYQQKP
 GKAPKLLIY~~SASDL~~YSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQ~~YAGAGL~~ITFGQGTKVEIKGGGGGGGGSEPKSSDKTHTCPP
 CPAEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNATKPREEQYNSTYRVVSVLTVLHQDWLNLN
 GKEYCKVSNKALPASEKTISKAKGQPREPQVCTLPPSREEMTKNQVSL~~SC~~AVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSF
 FL~~J~~SKLTVDKSRWQQGNVFSCVMEALHNHYTQKSLSLSPKGGGGGGGGGGSGTGEVQLVESGGGLVQPGGSLRLSCAA

SGF~~TSDFYF~~HWVRQAPGKGLEWVA~~TIYPFIGN~~TYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCAR~~AFPGSYHHP~~FDYWG
QGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTY
ICNVNHKPSNTKVDKKVEPKSCDKTHT* (SEQ ID NO: 971)

Mature ANT42 Hc Hole DANG

5 EVQLVESGGGLVQPGGSLRLSCAASGEF~~DFSSSS~~~~HWVRQAPGKGLEWVA~~~~SISSSYGYTY~~ADSVKGRFTISADTSKNTAYLQMNSLRA
EDTAVYYCAR~~SW/AMD~~YWGGGTLVTVSSGGGSDIQMTQSPSSLASVGDRTITCRAS~~QSI/SSA~~VAWYQQKPGKAPKLLIY~~S4SDLY~~
SGVPSRFSGRSGTDFTLTISSLPQEDFAITYYCCQQ~~YAGAGL~~ITFGQGTKEIKGGGGGGGSEPKSSDKTHTCPPCPAPELGGPSVFLF
PPKPKDTLMISRTPEVTCVVAVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYGSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
APIEKTIISKAKGQPREPQVYTLPP/RE/MTLNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFL/SKLTVDKSRWQQ
10 GNVFSCVMHEALHNHYTQKSLSLSPGKGGGGGGGGSGTGEVQLVESGGGLVQPGGSLRLSCAASGEF~~TSDFYF~~HWVRQ
APGKGLEWVA~~TIYPFIGN~~TYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCAR~~AFPGSYHHP~~EDYWGQGTLVTVSSASTKGP
SVFPLAPSSKSTSGGTAAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVD
KKVEPKSCDKTHT* (SEQ ID NO: 972)

Mature ANT42 Hc Hole LALAPS

15 EVQLVESGGGLVQPGGSLRLSCAASGEF~~DFSSSS~~~~HWVRQAPGKGLEWVA~~~~SISSSYGYTY~~ADSVKGRFTISADTSKNTAYLQMNSLRA
EDTAVYYCAR~~SW/AMD~~YWGGGTLVTVSSGGGSDIQMTQSPSSLASVGDRTITCRAS~~QSI/SSA~~VAWYQQKPGKAPKLLIY~~S4SDLY~~
SGVPSRFSGRSGTDFTLTISSLPQEDFAITYYCCQQ~~YAGAGL~~ITFGQGTKEIKGGGGGGGSEPKSSDKTHTCPPCPAPEAAGGPSVFLF
PPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
ASIEKTIISKAKGQPREPQVYTLPP/RE/MTLNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFL/SKLTVDKSRWQQ
20 GNVFSCVMHEALHNHYTQKSLSLSPGKGGGGGGGGSGTGEVQLVESGGGLVQPGGSLRLSCAASGEF~~TSDFYF~~HWVRQ
APGKGLEWVA~~TIYPFIGN~~TYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCAR~~AFPGSYHHP~~EDYWGQGTLVTVSSASTKGP
SVFPLAPSSKSTSGGTAAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVD
KKVEPKSCDKTHT* (SEQ ID NO: 973)

Mature ANT42 Hc Hole LALAPS Merchant

25 EVQLVESGGGLVQPGGSLRLSCAASGEF~~DFSSSS~~~~HWVRQAPGKGLEWVA~~~~SISSSYGYTY~~ADSVKGRFTISADTSKNTAYLQMNSLRA
EDTAVYYCAR~~SW/AMD~~YWGGGTLVTVSSGGGSDIQMTQSPSSLASVGDRTITCRAS~~QSI/SSA~~VAWYQQKPGKAPKLLIY~~S4SDLY~~
SGVPSRFSGRSGTDFTLTISSLPQEDFAITYYCCQQ~~YAGAGL~~ITFGQGTKEIKGGGGGGGSEPKSSDKTHTCPPCPAPEAAGGPSVFLF

PPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
 ASIEKTSKAKGQPREPQVYTLPPSREEMTKNQVSL²CAVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLJ¹SKLTVDKSRWQ
 QGNVFCSCVMHEALHNHYTQKSLSLSPGKGGSGGGSGGGSGTGEVQLVESGGGLVQPGGSLRLSCAASGF~~TSDFYF~~HVVR
 QAPGKGLEWVA~~TIYFIGNTY~~YADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCAR~~AFPGSYHP~~FDYWGGGTLVTVSSASTKG
 PSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHNKPSNTKVD
 KKVEPKSCDKTHT* (SEQ ID NO: 974)

Mature ANT42 Hc Hole LALAPS Merchant S-S

EVQLVESGGGLVQPGGSLRLSCAASGF~~DESSSS~~HVVRQAPGKGLEWVA~~SISSSYGYTY~~YADSVKGRFTISADTSKNTAYLQMNSLRA
 EDTAVYYCAR~~SWAMDY~~WGGGTLVTVSSGGGGDIQMTQSPSSLSASVGDRVTITCRAS~~QSVSSA~~VAWYQQKPGKAPKLLIY~~S4SDLY~~
 SGVPSRFSGSRSGTDFTLTISSLPEDFATYYCQQY~~AGAGLI~~TFGQGTKVEIKGGGGGGGSEPKSSDKTHITCPPCPAPEAAGGPSVFLF
 PPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
 ASIEKTSKAKGQPREPQV¹CTLPPSREEMTKNQVSL²CAVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLJ¹SKLTVDKSRWQ
 QGNVFCSCVMHEALHNHYTQKSLSLSPGKGGSGGGSGGGSGTGEVQLVESGGGLVQPGGSLRLSCAASGF~~TSDFYF~~HVVR
 QAPGKGLEWVA~~TIYFIGNTY~~YADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCAR~~AFPGSYHP~~FDYWGGGTLVTVSSASTKG
 PSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHNKPSNTKVD
 KKVEPKSCDKTHT* (SEQ ID NO: 975)

ANT42 Hc knob DANG

MNLLILTFVAAA¹VAEVQLVESGGGLVQPGGSLRLSCAASGF~~DFTAYAM~~HVVRQAPGKGLEWVA~~SIYPSGGYT~~AYADSVKGRFTISA
 DTSKNTAYLQMNSLRAEDTAVYYCAR~~RSYF~~ALDYWGGGTLVTVSSGGGGDIQMTQSPSSLSASVGDRVTITCRAS~~QSVSSA~~VAWY
 QQKPGKAPKLLIY~~S4SSL~~YSGVPSRFSGSRSGTDFTLTISSLPEDFATYYCQQY~~W4YYSPT~~TFGQGTKVEIKGGGGGGGSEPKSSDKT
²⁰HTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVA¹VSHEDPEVKFNWYVDGVEVHNAKTKPREEQY²GSTYRVVSVLTVLHQ
 DWLNGKEYKCKVSNKALPAPIEKTSKAKGQPREP~~MV~~¹FDLPPSREEMTKNQVSL²CAVKGFYPSDIAVEWESNGQPENNYKTTPPVL
 DSDGSFFLYSKLTVDKSRWQQGNVFCSCVMHEALHNHYTQKSLSLSPGKGGSGGGSGGGSGTGEVQLVESGGGLVQPGGSL
 RLSCAASGF~~TSDFYF~~HVVRQAPGKGLEWVA~~TIYFIGNTY~~YADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCAR~~AFPGSYHP~~
²⁵~~FDY~~WGGGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSS
 LGTQTYICNVNHNKPSNTKVDKKVEPKSCDKTHT* (SEQ ID NO: 976)

ANT42 Hc knob LALAPS

5 MNLLILTFVAAA VAEVQLVESGGGLVQPGGSLRLSCAASGFDFTAYAMHWVVRQAPGKGLEWVA SIYPSGGYTAYADSVKGRFTISA
 DTSKNTAYLQMNSLRAEDTAVYYCAR RSYYFALDYWGGTGLVTVS SGGGGSDIQMTQSPSSLASVGDRTITCRAS QSVSSAVAWY
 QQKPGKAPKLLIY S4SSLYSGVPSRFSGRSGTDFTLTISLQPEDFATYYCQQY W4YISPITFGQGTKEIKGGGGGGGSEPKSSDKT
 HTCPPCAPEA 44GGPSVFLFPKPKDTLMSRTPETCVVDVSHEDPEVKFNWYVDGVEVHNATKPREEQYNSTYRVSVLTVLHQ
 DWLNGKEYCKVSNKALPASEKTISKAKGQPREP MVEDLPPSREEMTKNQVSL WCMVKGFYPSDIAVEWESNGQPENNYKTTPPVLD
 DSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSPGKGGGGGGGGSGTGEVQLVESGGGLVQPGGSL
 RLSCAASGFT SDFYFHWVVRQAPGKGLEWVA TIYPIFIGNTYYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCAR AFPGSYHP
 FDIYWGQGTGLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSS
 10 LGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHT* (SEQ ID NO: 977)

ANT42 Hc knob LALAPS Merchant

15 MNLLILTFVAAA VAEVQLVESGGGLVQPGGSLRLSCAASGFDFTAYAMHWVVRQAPGKGLEWVA SIYPSGGYTAYADSVKGRFTISA
 DTSKNTAYLQMNSLRAEDTAVYYCAR RSYYFALDYWGGTGLVTVS SGGGGSDIQMTQSPSSLASVGDRTITCRAS QSVSSAVAWY
 QQKPGKAPKLLIY S4SSLYSGVPSRFSGRSGTDFTLTISLQPEDFATYYCQQY W4YISPITFGQGTKEIKGGGGGGGSEPKSSDKT
 HTCPPCAPEA 44GGPSVFLFPKPKDTLMSRTPETCVVDVSHEDPEVKFNWYVDGVEVHNATKPREEQYNSTYRVSVLTVLHQ
 DWLNGKEYCKVSNKALPASEKTISKAKGQPREPQVYTLPPSREEMTKNQVSL WCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLD
 SDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSPGKGGGGGGGGSGTGEVQLVESGGGLVQPGGSLR
 LSCAASGFT TSDFYFHWVVRQAPGKGLEWVA TIYPIFIGNTYYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCAR AFPGSYHPF
 20 DYWGQGTGLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSS
 GTQTYICNVNHKPSNTKVDKKVEPKSCDKTHT* (SEQ ID NO: 978)

ANT42 Hc knob LALAPS Merchant S-S

25 MNLLILTFVAAA VAEVQLVESGGGLVQPGGSLRLSCAASGFDFTAYAMHWVVRQAPGKGLEWVA SIYPSGGYTAYADSVKGRFTISA
 DTSKNTAYLQMNSLRAEDTAVYYCAR RSYYFALDYWGGTGLVTVS SGGGGSDIQMTQSPSSLASVGDRTITCRAS QSVSSAVAWY
 QQKPGKAPKLLIY S4SSLYSGVPSRFSGRSGTDFTLTISLQPEDFATYYCQQY W4YISPITFGQGTKEIKGGGGGGGSEPKSSDKT
 HTCPPCAPEA 44GGPSVFLFPKPKDTLMSRTPETCVVDVSHEDPEVKFNWYVDGVEVHNATKPREEQYNSTYRVSVLTVLHQ
 DWLNGKEYCKVSNKALPASEKTISKAKGQPREPQVYTLPPSREEMTKNQVSL WCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLD
 SDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSPGKGGGGGGGGSGTGEVQLVESGGGLVQPGGSLR

LSCAASGF~~TSDFYFI~~HWVRQAPGKGLEWVA~~TIYPIFIGNTYY~~ADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCAR~~AFPGSYHHPF~~
DYWGQGTLLVTVSSATKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSL
GTQTYICNVNHNKPSNTKVDKKVEPKSCDKTHT* (SEQ ID NO: 979)

Mature ANT42 Hc knob DANG

5 EVQLVESGGGLVQPGGSLRLSCAASGF~~DFTAYAM~~HWVRQAPGKGLEWVA~~SIYPSGGYTA~~YADSVKGRFTISADTSKNTAYLQMNSLR
AEDTAVYYCAR~~RSYYFAL~~DYWGQGTLLVTVSSGGGSDIQMTQSPSSLSASVGDRTITCRAS~~QSISSA~~VAWYQQKPGKAPKLLIY~~SAS~~
~~SL~~YSGVPSRFSGRSGTDFTLTISSLQPEDFATYYCQQY~~WAIYSPIT~~TFGQGTKVEIKGGGGGGGSEPKSSDKTHTCPPCPAPELLGGPS
VFLFPPKPKDITLMISRTPETCVVVA~~VS~~HEDPEVKFNWYVDGVEVHNAKTKPREEQYGSTYRVVSVLTVLHQDWLNGKEYKCKVSN
KALPAPIEKTIISKAKGQPREP~~MV~~FDLPPSREEMTKNQVSL~~ICM~~VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDK
SRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKGGGGGGGGSGSTGEVQLVESGGGLVQPGGSLRLSCAASGF~~TSDFYFI~~
HWVRQAPGKGLEWVA~~TIYPIFIGNTYY~~ADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCAR~~AFPGSYHHPF~~DYWGQGTLLVTVSS
ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHNKPS
NTKVDKKVEPKSCDKTHT* (SEQ ID NO: 980)

Mature ANT42 Hc knob LALAPS

15 EVQLVESGGGLVQPGGSLRLSCAASGF~~DFTAYAM~~HWVRQAPGKGLEWVA~~SIYPSGGYTA~~YADSVKGRFTISADTSKNTAYLQMNSLR
AEDTAVYYCAR~~RSYYFAL~~DYWGQGTLLVTVSSGGGSDIQMTQSPSSLSASVGDRTITCRAS~~QSISSA~~VAWYQQKPGKAPKLLIY~~SAS~~
~~SL~~YSGVPSRFSGRSGTDFTLTISSLQPEDFATYYCQQY~~WAIYSPIT~~TFGQGTKVEIKGGGGGGGSEPKSSDKTHTCPPCPAPEAAGGPS
VFLFPPKPKDITLMISRTPETCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSN
KALPASTEKTIISKAKGQPREP~~MV~~FDLPPSREEMTKNQVSL~~ICM~~VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDK
SRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKGGGGGGGGSGSTGEVQLVESGGGLVQPGGSLRLSCAASGF~~TSDFYFI~~
HWVRQAPGKGLEWVA~~TIYPIFIGNTYY~~ADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCAR~~AFPGSYHHPF~~DYWGQGTLLVTVSS
ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHNKPS
NTKVDKKVEPKSCDKTHT* (SEQ ID NO: 981)

Mature ANT42 Hc knob LALAPS Merchant

25 EVQLVESGGGLVQPGGSLRLSCAASGF~~DFTAYAM~~HWVRQAPGKGLEWVA~~SIYPSGGYTA~~YADSVKGRFTISADTSKNTAYLQMNSLR
AEDTAVYYCAR~~RSYYFAL~~DYWGQGTLLVTVSSGGGSDIQMTQSPSSLSASVGDRTITCRAS~~QSISSA~~VAWYQQKPGKAPKLLIY~~SAS~~
~~SL~~YSGVPSRFSGRSGTDFTLTISSLQPEDFATYYCQQY~~WAIYSPIT~~TFGQGTKVEIKGGGGGGGSEPKSSDKTHTCPPCPAPEAAGGPS

VLFPPKPKD TLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSN
KALPASEIETISKAKGQPREPQVYTLPPSREEMTKNQVSLVCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDK
SRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKGGGGGGGGSGTGEVQLVESGGGLVQPGGSLRLSCAASGF~~TSDFYFI~~
HWVRQAPGKGLEWVA~~TIYFIGNTYY~~ADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCAR~~AFPGSVHHPFDYWGQGTLVTVSS~~
ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHHKPS
NTKVDKKVEPKSCDKTHT* (SEQ ID NO: 982)

Mature ANT42 Hc knob LALAPS Merchant S-S

EVQLVESGGGLVQPGGSLRLSCAASGF~~DFATAYAM~~HWVRQAPGKGLEWVA~~SIYPSGGVTA~~YADSVKGRFTISADTSKNTAYLQMNSLR
AEDTAVYYCAR~~RSYYFALD~~YWGQGTLLVTVSSGGGGSDIQTQSPSSLASVGDRTVTITCRAS~~QSVSSA~~VAWYQQKPGKAPKLLIY~~SAS~~
~~SL~~YSGVPSRFSGSRSGTDFLTISLQPEDFATYYCQQY~~W~~~~AYISPT~~TFGQGTKVEIKGGGGGGGGSEPKSSDKTHTCPPCPAPEAAGGPS
VLFPPKPKD TLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSN
KALPASEIETISKAKGQPREPQVYTLPPCREEMTKNQVSLVCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDK
SRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKGGGGGGGGSGTGEVQLVESGGGLVQPGGSLRLSCAASGF~~TSDFYFI~~
HWVRQAPGKGLEWVA~~TIYFIGNTYY~~ADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCAR~~AFPGSVHHPFDYWGQGTLVTVSS~~
ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHHKPS
NTKVDKKVEPKSCDKTHT* (SEQ ID NO: 983)

ANT42 LC

MNLLILTFVAAAADIQMTQSPSSLASVGDRTVTITCRAS~~QSVSSA~~VAWYQQKPGKAPKLLIY~~SASSL~~YSGVPSRFSGSRSGTDFLTIS
SLQPEDFATYYCQQ~~STGSL~~TFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVCLLNNFYPREAKVQWKVDNALQSGNSQESVTE
QDSKDSYSLSSITLTSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC* (SEQ ID NO: 912)

Mature ANT42 LC

DIQMTQSPSSLASVGDRTVTITCRAS~~QSVSSA~~VAWYQQKPGKAPKLLIY~~SASSL~~YSGVPSRFSGSRSGTDFLTISLQPEDFATYYCQQ~~S~~
~~TGSL~~TFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSYSLSSITL
SKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 984)

ANT80-1 Hole

MNLLLLTFVAAA VAEVQLVESGGGLVQPGGSLRLSAAASGF**TL****SYSY**HWVRQAPGKGLEWVA**SIYSYTGATD**YADSVKGRFTISADT
SKNTAYLQMNSLRAEDTAVYYCARY**YWSTNRILSYGGM**DYWGQGTLVTVSSGGGGSDIQMTQSPSSLSASVGDRVTITCRASQ**SVSSA**
VAWYQQKPGKAPKLLIYSASSLYSGVPSRFSGRSGTDFTLTISSLQPEDFATYYCQ**YFDPI**TFGQGTKVEIKGGGGGGGSEPKSSD
5 KTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVAVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYGSTYRVVSVLTVL
HQDWLNGKEYCKVSNKALPAPIEKTIISKAKGQPREPQVYTLPPIRELMTSNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTTPVL
DSDGSFFLVSKLTVDKSRWQQGNVFSCVMHEALHNHYTQKLSLSPGKGGGGGGGGSGSTGEVQLVESGGGLVQPGGSL
RLSAAASGF**NIAYSSM**HWVRQAPGKGLEWVA**TIYPSYSSTY**YADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARY**YYAMDY**
GQGTLLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQT
YICNVNHPKPSNTKVDKKVEPKSCDKTHT (SEQ ID NO: 986)

10

ANT80-2 Knob

MNLLLLTFVAAA VAEVQLVESGGGLVQPGGSLRLSAAASGF**DL****SHSS**IHWVRQAPGKGLEWVA**SISSHYGYTHY**ADSVKGRFTISAD
TSKNTAYLQMNSLRAEDTAVYYCARY**SYFPPWF**EALDYWGQGTLVTVSSGGGGSDIQMTQSPSSLSASVGDRVTITCRASQ**DLSTAVA**
WYQQKPGKAPKLLIY**SASF**LYSGVPSRFSGRSGTDFTLTISSLQPEDFATYYCQ**HYTTPPT**FGQGTKVEIKGGGGGGGSEPKTSDK
15 THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVAVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYGSTYRVVSVLTVLH
QDWLNGKEYCKVSNKALPAPIEKTIISKAKGQPREPMVFDLPPSREEMTKNQVSLWCMVKGFYPSDIAVEWESNGQPENNYKTTTPPV
LDSDGSFFLYSKLTVDKSRWQQGNVFSCVMHEALHNHYTQKLSLSPGKGGGGGGGGSGSTGEVQLVESGGGLVQPGGS
LRLSAAASGF**NIAYSSM**HWVRQAPGKGLEWVA**TIYPSYSSTY**YADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARY**YYAMDY**
WGQGTLLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGT
QTYICNVNHPKPSNTKVDKKVEPKSCDKTHT (SEQ ID NO: 987)

20

ANT98-1 Hole

MNLLLLTFVAAA VAEVQLVESGGGLVQPGGSLRLSAAASGF**TL****SYSY**HWVRQAPGKGLEWVA**SIYSYTGATD**YADSVKGRFTISADT
SKNTAYLQMNSLRAEDTAVYYCARY**YWSTNRILSYGGM**DYWGQGTLVTVSSGGGGSDIQMTQSPSSLSASVGDRVTITCRASQ**SVSSA**
VAWYQQKPGKAPKLLIY**SASS**LYSGVPSRFSGRSGTDFTLTISSLQPEDFATYYCQ**YFDPI**TFGQGTKVEIKGGGGGGGSEPKSSD
25 KTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVAVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYGSTYRVVSVLTVL
HQDWLNGKEYCKVSNKALPAPIEKTIISKAKGQPREPQVYTLPPIRELMTSNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTTPVL
DSDGSFFLVSKLTVDKSRWQQGNVFSCVMHEALHNHYTQKLSLSPGKGGGGGGGGSGSTGEVQLVESGGGLVQPGGSL

25

RLSCAASGFNIA~~YSSM~~HWVRQAPGKGLEWVA~~TIYPSYSTY~~YADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCAR~~DIYAMD~~YWGQGTAVTVSSASTKGPSVFPLAPSSKSTSGGTAAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQT
YICNVNHNKPSNTKVDKKVEPKSCDKTHT (SEQ ID NO: 988)

ANT98-2 Knob

5 MNLLILTFVAAA~~VAE~~VLVESGGGLVQPGGSLRLSCAASGF~~DLSSHSH~~HWVRQAPGKGLEWVA~~SISSHYGYTH~~YADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCAR~~SYFPPWF~~ALDYWGQGTAVTVSSGGGSDIQTQSPSSLASVGDRTITCRASQ~~DI~~STAVAWYQKPGKAPKLLIY~~SASF~~LYSGVPSRFSGRSGTDFTLTISLQPEDFATYYCQ~~HYTTP~~TFGQGTKVEIKGGGGGGGSEPKTSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVA~~VS~~HEDPEVKFNWYVDGVEVHNAKTKPREEQYGSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTIISKAKGQPREPMVFDLPPSREEMTKNQVSLWCMVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCVMHEALHNHYTQKSLSPGKGGGGGGGGSGSTGEVQLVESGGGLVQPGGSLRLSCAASGFNIA~~YSSM~~HWVRQAPGKGLEWVA~~TIYPSYSTY~~YADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCAR~~DIYAMD~~YWGQGTAVTVSSASTKGPSVFPLAPSSKSTSGGTAAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHNKPSNTKVDKKVEPKSCDKTHT (SEQ ID NO: 989)

15 **ANT99-1 Hole**

MNLLILTFVAAA~~VAE~~VLVESGGGLVQPGGSLRLSCAASGF~~TLSSSYH~~HWVRQAPGKGLEWVA~~SIYSYTGATD~~YADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCAR~~YWSTNRILSYGMD~~YWGQGTAVTVSSGGGSDIQTQSPSSLASVGDRTITCRASQ~~SI~~SSAVAWYQKPGKAPKLLIY~~SASS~~LYSGVPSRFSGRSGTDFTLTISLQPEDFATYYCQ~~YFDP~~TFGQGTKVEIKGGGGGGGSEPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVA~~VS~~HEDPEVKFNWYVDGVEVHNAKTKPREEQYGSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTIISKAKGQPREPQVYTLPIRELMTSNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCVMHEALHNHYTQKSLSPGKGGGGGGGGSGSTGEVQLVESGGGLVQPGGSLRLSCAASGFNIA~~YSSM~~HWVRQAPGKGLEWVA~~TIYPSYSTY~~YADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCAR~~YDMD~~YWGQGTAVTVSSASTKGPSVFPLAPSSKSTSGGTAAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHNKPSNTKVDKKVEPKSCDKTHT (SEQ ID NO: 990)

20

GQGTLVTVSSASTKGPSVFLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQT
YICNVNHHKPSNTKVDKKVEPKSCDKTHT (SEQ ID NO: 990)

ANT99-2 Knob

5 MNLLILTFVAAA VAEFVQLVESGGGLVQPGGSLRLSCAASGFDLSSSHJHWVRQAPGKGLEWVA~~SISSHYGYTHY~~ADSVKGRFTISAD
TSKNTAYLQMNSLRAEDTAVYYCAR~~SSYFPWF~~FALDYWGQGTLVTVSSGGGGSDIQMTQSPSSLSASVGDRTVTITCRASQ~~DI~~STAVA
WYQQKPGKAPKLLIY~~S4SFL~~YSGVPSRFSGRSGTDFTLTISSLPEDFATYYCQ~~HYTTP~~TFGQGTKVEIKGGGGGGGSEPKTSDK
THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVAVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYGSTYRVVSVLTVLH
QDWLNGKEYKCKVSNKALPAPIEKTIISKAKGQPREPMVFDLPPSREEMTKNQVSLWCMVKGFYPSDIAVEWESNGQPENNYKTTPPV
LDSDGSFFLYSKLTVDKSRWQQGNVFCVSMHEALHNHYTQKLSLSPGKGGGGGGGGSGTGEVQLVESGGGLVQPGGS
10 LRLSCAASGF~~NI4YSSM~~HVVRQAPGKGLEWVA~~TIYPSYSS~~TYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCAR~~YYDM~~DY
WGQGTLVTVSSASTKGPSVFLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGT
QTYICNVNHHKPSNTKVDKKVEPKSCDKTHT (SEQ ID NO: 991)

ANT80 ANT98 ANT99 LC

15 MNLLILTFVAAA VADIQMTQSPSSLSASVGDRTVTITCRASQ~~SVSSA~~VAWYQQKPGKAPKLLIY~~S4SSL~~YSGVPSRFSGRSGTDFTLTIS
SLQPEDFATYYCQ~~QAHYFP~~ITFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTE
QDSKDSYSLSTLTLSKADYEEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 992)

CM0042 (Dia-Fc-Dia)

20 MNLLILTFVAAA VAEFVQLVESGGGLVQPGGSLRLSCAASGF~~NI~~GSSJHWVRQAPGKGLEWVA~~SIYSAF~~ASTYADSVKGRFTISA
DTSKNTAYLQMNSLRAEDTAVYYCAR~~YHFPFGF~~ALDYWGQGTLVTVSSGGGGSDIQMTQSPSSLSASVGDRTVTITCRASQ~~SVSSA~~V
WYQQKPGKAPKLLIY~~S4SSL~~YSGVPSRFSGRSGTDFTLTISSLPEDFATYYCQ~~QGLYLF~~TFGQGTKVEIKLEDKTHTKVEPKSSDKTH
TCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD
WLNKEYKCKVSNKALPAPIEKTIISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLD
DGSFFLYSKLTVDKSRWQQGNVFCVSMHEALHNHYTQKLSLSPGKSGSETPGTSEATPESGGGEVQLVESGGGLVQPGGSLRLSC
25 AASGF~~NI~~YSSJHWVRQAPGKGLEWVA~~YISYYGY~~TYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCAR~~AHYFPW~~AGAMD
YWQGTGLVTVSSGGGGSDIQMTQSPSSLSASVGDRTVTITCRASQ~~SVSSA~~VAWYQQKPGKAPKLLIY~~S4SSL~~YSGVPSRFSGRSGTDFTL
TISSLPEDFATYYCQ~~YSWGF~~TFGQGTKVEIK (SEQ ID NO: 993)

CM0011 Hole (Dia-Fc-Dia)

MNLLILTFVAAA/AEFVQLVESGGGLVQPGGSLRLSCAASGFNIGSSJHWRQAPGKGLEWVA~~SIYS~~~~AF~~~~ASTSY~~ADSVKGRFTISA
 DTSKNTAYLQMNSLRAEDTAVYYCAR~~YHFPF~~~~FGAL~~DYWGQGTLLTVSSGGGGDIQMTQSPSSLSASVGDRTITCRASQ~~SI~~~~SS~~~~AA~~V
 WYQKPGKAPKLLIY~~S~~~~AS~~~~SL~~~~YS~~GVPSRFSGRSGTDFLTISLQPEDFATYYCQQ~~GV~~~~YLF~~TFGQGTKEIKLEDKTHTKVEPKSSDKTH
 5 NCPCPAPELLGGPSVFLPPKPKDITLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVSVLTVLHQD
 WLNKEYCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPRELMTSNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTPPVLDSD
 GSFFLVSKLTVDKSRWQQGNVFSCVMHEALHNHYTQKLSLSPGKSGSETPGTSEATPESGGGEVQLVESGGGLVQPGGSLRLSCA
 ASGFN~~ISSYY~~~~IHW~~~~VR~~~~Q~~~~A~~~~P~~~~G~~~~K~~~~G~~~~L~~~~E~~~~W~~~~V~~~~A~~~~S~~~~I~~~~Y~~~~S~~~~S~~~~Y~~~~G~~~~T~~~~S~~~~Y~~~~A~~~~D~~~~S~~~~V~~~~K~~~~G~~~~R~~~~F~~~~T~~~~I~~~~S~~~~A~~~~E~~~~D~~~~T~~~~A~~~~V~~~~Y~~~~Y~~~~C~~~~A~~~~R~~~~T~~~~V~~~~R~~~~G~~~~S~~~~K~~~~K~~~~P~~~~Y~~~~F~~~~S~~~~G~~~~W~~
~~AMD~~~~YW~~~~G~~~~Q~~~~G~~~~T~~~~L~~~~V~~~~T~~~~V~~~~S~~~~S~~~~G~~~~G~~~~G~~~~S~~~~D~~~~I~~~~Q~~~~M~~~~T~~~~Q~~~~S~~~~P~~~~S~~~~S~~~~L~~~~S~~~~A~~~~S~~~~V~~~~G~~~~D~~~~R~~~~V~~~~T~~~~I~~~~T~~~~C~~~~R~~~~A~~~~S~~~~Q~~~~S~~~~I~~~~S~~~~S~~~~A~~~~V~~~~A~~~~W~~~~Y~~~~Q~~~~K~~~~P~~~~G~~~~K~~~~A~~~~P~~~~K~~~~L~~~~L~~~~I~~~~Y~~~~S~~~~A~~~~S~~~~S~~~~L~~~~Y~~~~S~~~~G~~~~V~~~~P~~~~S~~~~R~~~~F~~~~S~~~~G~~~~S~~~~R~~~~S~~~~G~~~~T~~
 DFTLTISLQPEDFATYYCQQ~~YSW~~~~GPF~~TFGQGTKEIK (SEQ ID NO: 994)

10

CM0011 Knob (Dia-Fc-Dia)

MNLLILTFVAAA/AEFVQLVESGGGLVQPGGSLRLSCAASGFNIGSSJHWRQAPGKGLEWVA~~SIYS~~~~AF~~~~ASTSY~~ADSVKGRFTISA
 DTSKNTAYLQMNSLRAEDTAVYYCAR~~YHFPF~~~~FGAL~~DYWGQGTLLTVSSGGGGDIQMTQSPSSLSASVGDRTITCRASQ~~SI~~~~SS~~~~AA~~V
 WYQKPGKAPKLLIY~~S~~~~AS~~~~SL~~~~YS~~GVPSRFSGRSGTDFLTISLQPEDFATYYCQQ~~GV~~~~YLF~~TFGQGTKEIKLEDKTHTKVEPKTSDKTH
 15 TCPCPAPELLGGPSVFLPPKPKDITLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVSVLTVLHQD
 WLNKEYCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLWCMVKGFYPSDIAVEWESNGQPENNYKTPPVLD
 SDGSFFLYSKLTVDKSRWQQGNVFSCVMHEALHNHYTQKLSLSPGKSGSETPGTSEATPESGGGEVQLVESGGGLVQPGGSLRLS
 CAASGFN~~ISSYY~~~~IHW~~~~VR~~~~Q~~~~A~~~~P~~~~G~~~~K~~~~G~~~~L~~~~E~~~~W~~~~V~~~~A~~~~S~~~~I~~~~Y~~~~S~~~~S~~~~Y~~~~G~~~~T~~~~S~~~~Y~~~~A~~~~D~~~~S~~~~V~~~~K~~~~G~~~~R~~~~F~~~~T~~~~I~~~~S~~~~A~~~~E~~~~D~~~~T~~~~A~~~~V~~~~Y~~~~Y~~~~C~~~~A~~~~R~~~~A~~~~H~~~~Y~~~~F~~~~P~~~~W~~~~A~~~~G~~~~A~~~~M~~
 DYWGQGTLLTVSSGGGGDIQMTQSPSSLSASVGDRTITCRASQ~~SI~~~~SS~~~~AA~~VAWYQKPGKAPKLLIY~~S~~~~AS~~~~S~~~~L~~~~Y~~~~S~~~~G~~~~V~~~~P~~~~S~~~~R~~~~F~~~~S~~~~G~~~~S~~~~R~~~~S~~~~G~~~~T~~~~D~~~~F~~
 TLTISLQPEDFATYYCQQ~~YYW~~~~P~~TFGQGTKEIK (SEQ ID NO: 995)

20

CM0126 Hole (Dia-Fc-Dia)

MNLLILTFVAAA/AEFVQLVESGGGLVQPGGSLRLSCAASGFNIHSSJHWRQAPGKGLEWVA~~ATYS~~~~FGS~~~~ITY~~ADSVKGRFTISA
 DTSKNTAYLQMNSLRAEDTAVYYCAR~~YHHPF~~~~FGYAL~~DYWGQGTLLTVSSGGGGDIQMTQSPSSLSASVGDRTITCRASQ~~SI~~~~SS~~~~AA~~V
 WYQKPGKAPKLLIY~~S~~~~AS~~~~SL~~~~YS~~GVPSRFSGRSGTDFLTISLQPEDFATYYCQQ~~GV~~~~YLF~~TFGQGTKEIKLEDKTHTKVEPKSSDKTH
 25 NCPCPAPELLGGPSVFLPPKPKDITLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVSVLTVLHQD
 WLNKEYCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPRELMTSNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTPPVLDSD
 GSFFLVSKLTVDKSRWQQGNVFSCVMHEALHNHYTQKLSLSPGKSGSETPGTSEATPESGGGEVQLVESGGGLVQPGGSLRLSCA
 ASGFN~~ISSYY~~~~IHW~~~~VR~~~~Q~~~~A~~~~P~~~~G~~~~K~~~~G~~~~L~~~~E~~~~W~~~~V~~~~A~~~~S~~~~I~~~~Y~~~~S~~~~S~~~~Y~~~~G~~~~T~~~~S~~~~Y~~~~A~~~~D~~~~S~~~~V~~~~K~~~~G~~~~R~~~~F~~~~T~~~~I~~~~S~~~~A~~~~E~~~~D~~~~T~~~~A~~~~V~~~~Y~~~~Y~~~~C~~~~A~~~~R~~~~T~~~~V~~~~R~~~~G~~~~S~~~~K~~~~K~~~~P~~~~Y~~~~F~~~~S~~~~G~~~~W~~

25

AMDYWGQGTLVTVSSGGGSDIQMTQSPSSLASVGDRTITCRASQSISS4VAWYQQPGKAPKLLIYS4SSLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQYSWGPFTFGQGTKVEIK (SEQ ID NO: 996)

CM010126 Knob (Dia-Fc-Dia)

5 MNLLLITFVAAA VAEFEVQLVESGGGLVQPGGSLRLSCAASGFNNIHSSSHHWVRQAPGKGLEWVAATYSSFGSITYADSVKGRFTISA
 DTSKNTAYLQMNSLR AEDTAVYYCARYHHPFGFALDYWGQGTLVTVSSGGGGSDIQMTQSPSSLASVGDRTITCRASQSISS4V
 WYQQKPGKAPKLLIYS4SSLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQGGVYLFTFGQGTKVEIKLEDKTHTKVEPKTSDKTH
 TCPPCPAPELLGGPSVFLFPKPKDITLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD
 WLNKGKEYCKVSNKALPAPIEKTISKAKGQPREPMVFDLPSPREEMTKNQVSLWCMVKGFYPSDIAVEWESNGQPENNYKTTPPVLD
 SDGSFFLYSKLTVDKSRWQQGNVFCSCVMHEALHNHYTQKSLSLSPGKSGSETPGTSEATPESGGGEVQLVESGGGLVQPGGSLRLS
 10 CAASGFNSISS4HWVRQAPGKGLEWVAYISSYIGYTYADSVKGRFTISADTSKNTAYLQMNSLR AEDTAVYYCAR4HYFPWAGAM
 DYWGQGTLVTVSSGGGSDIQMTQSPSSLASVGDRTITCRASQSISS4VAWYQQPGKAPKLLIYS4SSLYSGVPSRFSGSRSGTDF
 TLTISSLQPEDFATYYCQQYHWPITFGQGTKVEIK (SEQ ID NO: 997)

CM010107 Hole (Dia-Fc-scFv)

15 MNLLLITFVAAA VAEFEVQLVESGGGLVQPGGSLRLSCAASGFNIGSSSHHWVRQAPGKGLEWVASIYS4FASTSYADSVKGRFTISA
 DTSKNTAYLQMNSLR AEDTAVYYCARYHHPFGFALDYWGQGTLVTVSSGGGGSDIQMTQSPSSLASVGDRTITCRASQSISS4V
 WYQQKPGKAPKLLIYS4SSLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQGGVYLFTFGQGTKVEIKLEDKTHTKVEPKSSDKTH
 NCPPCPAPELLGGPSVFLFPKPKDITLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD
 WLNKGKEYCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPRELMTSNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSD
 GSFFLVSKLTVDKSRWQQGNVFCSCVMHEALHNHYTQKSLSLSPGKSGSETPGTSEATPESGGGDIQMTQSPSSLASVGDRTITCR
 20 ASQSISS4VAWYQQKPGKAPKLLIYS4SSLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQYSWGPFTFGQGTKVEIKGTTAASG
 SSGSSSGAEVQLVESGGGLVQPGGSLRLSCAASGFNSISS4HWVRQAPGKGLEWVAYISSYIGYTYADSVKGRFTISADTSKNTAY
 LQMNSLR AEDTAVYYCAR4HYFPWAGAMDYWGQGTLVTVSS (SEQ ID NO: 998)

CM010107 Knob (Dia-Fc-scFv)

25 MNLLLITFVAAA VAEFEVQLVESGGGLVQPGGSLRLSCAASGFNIGSSSHHWVRQAPGKGLEWVASIYS4FASTSYADSVKGRFTISA
 DTSKNTAYLQMNSLR AEDTAVYYCARYHHPFGFALDYWGQGTLVTVSSGGGGSDIQMTQSPSSLASVGDRTITCRASQSISS4V
 WYQQKPGKAPKLLIYS4SSLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQGGVYLFTFGQGTKVEIKLEDKTHTKVEPKTSDKTH
 TCPPCPAPELLGGPSVFLFPKPKDITLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD

5 WLNKEYKCKVSNKALPAPIEKTISKAKGQPREPMVFDLPPSREEMTKNQVSLWCMV/KGFYPSDIAVEWESNGQPENNYKTTPPVLD
SDGSFFLYSKLTVDKSRWQQGNVFCSCVMHEALHNHYTQKSLSLSPGKSGSETPGTSEATPESGGGDIQMTQSPSSLSASVGDRTIT
CRASQYSS4VAVWYQKPKGAPKLLIY\$4SSL\$YSGVPSRFSGRSGTDFTLTISSLQPEDFATYYCQQY\$W/GPFTFGQGTKEIKGTTAA
SGSSGSSGAEVQLVESGGGLVQPGGSLRLSAAAGFNIS\$Y\$HWRQAPGKGLEWVA\$YSSYGYTY\$ADSVKGRFTISADTSKNT
AYLQMNSLRAEDTAVYYCAR\$HYFPWAGAMD\$YWGQGLVTVSS (SEQ ID NO: 999)

CM0108 Hole (Dia-Fc-scFv)

10 MNLLILTFVAAA/AEFVQLVESGGGLVQPGGSLRLSAAAGFNIG\$SS\$HWRQAPGKGLEWVA\$YYS4FAST\$YADSVKGRFTISA
DTSKNTAYLQMNSLRAEDTAVYYCAR\$YHFPFGFAL\$DYWGQGLVTVSSGGGGDIQMTQSPSSLSASVGDRTITCRASQYSS4VAV
WYQKPKGAPKLLIY\$4SSL\$YSGVPSRFSGRSGTDFTLTISSLQPEDFATYYCQQG\$YLF\$TFGQGTKEIKLEDKTHTKVEPKSSDKTH
NCPCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDV\$SHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD
WLNKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPRELMTSNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSD
GSFFLVSKLTVDKSRWQQGNVFCSCVMHEALHNHYTQKSLSLSPGKSGSETPGTSEATPESGGGDIQMTQSPSSLSASVGDRTITCR
ASQYSS4VAVWYQKPKGAPKLLIY\$4SSL\$YSGVPSRFSGRSGTDFTLTISSLQPEDFATYYCQQY\$W/P\$TFGQGTKEIKGTTAASGSS
GGSSGAEVQLVESGGGLVQPGGSLRLSAAAGFNIS\$Y\$YHWRQAPGKGLEWVA\$YSSYGYTY\$ADSVKGRFTISADTSKNTAYLQ
15 MNSLRAEDTAVYYCAR\$TVRGSKKPYFSGWAMD\$YWGQGLVTVSS (SEQ ID NO: 1000)

CM0108 Knob (Dia-Fc-scFv)

20 MNLLILTFVAAA/AEFVQLVESGGGLVQPGGSLRLSAAAGFNIG\$SS\$HWRQAPGKGLEWVA\$YYS4FAST\$YADSVKGRFTISA
DTSKNTAYLQMNSLRAEDTAVYYCAR\$YHFPFGFAL\$DYWGQGLVTVSSGGGGDIQMTQSPSSLSASVGDRTITCRASQYSS4VAV
WYQKPKGAPKLLIY\$4SSL\$YSGVPSRFSGRSGTDFTLTISSLQPEDFATYYCQQG\$YLF\$TFGQGTKEIKLEDKTHTKVEPKTSDKTH
TCPPCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDV\$SHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD
WLNKEYKCKVSNKALPAPIEKTISKAKGQPREPMVFDLPPSREEMTKNQVSLWCMV/KGFYPSDIAVEWESNGQPENNYKTTPPVLD
SDGSFFLYSKLTVDKSRWQQGNVFCSCVMHEALHNHYTQKSLSLSPGKSGSETPGTSEATPESGGGDIQMTQSPSSLSASVGDRTIT
CRASQYSS4VAVWYQKPKGAPKLLIY\$4SSL\$YSGVPSRFSGRSGTDFTLTISSLQPEDFATYYCQQY\$W/P\$TFGQGTKEIKGTTAAS
GSSGSSGAEVQLVESGGGLVQPGGSLRLSAAAGFNIS\$Y\$YHWRQAPGKGLEWVA\$YSSYGYTY\$ADSVKGRFTISADTSKNTA
25 YLQMNSLRAEDTAVYYCAR\$TVRGSKKPYFSGWAMD\$YWGQGLVTVSS (SEQ ID NO: 1001)

CM0108 Hole (Dia-Fc-scFv)

MNLLILTFVAAA/AEFVQLVESGGGLVQPGGSLRLSAAASGFNIGSSSHHWVRQAPKGLEWVA~~SIYS~~AF~~ASTSY~~ADSVKGRFTISA
 DTSKNTAYLQMNSLRAEDTAVYYCAR~~YHFPF~~FGFALDYWGQGTLLVTVSSGGGGSDIQMTQSPSSLSASVGDRTITCRASQ~~SISS~~SAVA
 WYQKPGKAPKLLIY~~S~~AS~~SL~~YSGVPSRFSGRSGTDFTLTISLQPEDFATYYCQQ~~G~~IV~~YLF~~TFGGQGTKVEIKLEDKTHTKVEPKSSDKTH
 NCPCPAPPELLGGPSVFLFPPKPKDITLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD
 WLNQKEYCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPRELMTSNQVSLCAVKGFYPSDIAVEWESNGQPENNYKTPPVLDSD
 GSFFLVSKLTVDKSRWQQGNVFSCVMHEALHNHYTQKLSLSPGKSGSETPGTSEATPESGGDIQMTQSPSSLSASVGDRTITCR
 ASQ~~SISS~~SAVAWYQQKPGKAPKLLIY~~S~~AS~~SL~~YSGVPSRFSGRSGTDFTLTISLQPEDFATYYCQQ~~Y~~W~~PI~~TFGGQGTKVEIKGTTAASGSS
 GSSSGAEVQLVESGGGLVQPGGSLRLSAAASGFN~~ISSY~~YHWVRQAPKGLEWVA~~SIYS~~GY~~TSY~~ADSVKGRFTISADTSKNTAYLQ
 MNSLRAEDTAVYYCAR~~TV~~RG~~SK~~KPY~~FSGWAMDYWGGGTLLVTVSS (SEQ ID NO: 1000)~~

CM0108 Knob (Dia-Fc-scFv)

MNLLILTFVAAA/AEFVQLVESGGGLVQPGGSLRLSAAASGFNIGSSSHHWVRQAPKGLEWVA~~SIYS~~AF~~ASTSY~~ADSVKGRFTISA
 DTSKNTAYLQMNSLRAEDTAVYYCAR~~YHFPF~~FGFALDYWGQGTLLVTVSSGGGGSDIQMTQSPSSLSASVGDRTITCRASQ~~SISS~~SAVA
 WYQKPGKAPKLLIY~~S~~AS~~SL~~YSGVPSRFSGRSGTDFTLTISLQPEDFATYYCQQ~~G~~IV~~YLF~~TFGGQGTKVEIKLEDKTHTKVEPKTSDKTH
 TCPCPAPPELLGGPSVFLFPPKPKDITLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD
 WLNQKEYCKVSNKALPAPIEKTISKAKGQPREPMVFDLPPSREEMTKNQVSLWCMVKGFYPSDIAVEWESNGQPENNYKTPPVLD
 SDGSFFLYSKLTVDKSRWQQGNVFSCVMHEALHNHYTQKLSLSPGKSGSETPGTSEATPESGGDIQMTQSPSSLSASVGDRTIT
 CRASQ~~SISS~~SAVAWYQQKPGKAPKLLIY~~S~~AS~~SL~~YSGVPSRFSGRSGTDFTLTISLQPEDFATYYCQQ~~Y~~W~~PI~~TFGGQGTKVEIKGTTAAS
 GSSSGSSGAEVQLVESGGGLVQPGGSLRLSAAASGFN~~ISSY~~YHWVRQAPKGLEWVA~~SIYS~~GY~~TSY~~ADSVKGRFTISADTSKNTA
 YLQMNSLRAEDTAVYYCAR~~TV~~RG~~SK~~KPY~~FSGWAMDYWGGGTLLVTVSS (SEQ ID NO: 1001)~~

CM0109 Hole (Dia-Fc-scFv)

MNLLILTFVAAA/AEFVQLVESGGGLVQPGGSLRLSAAASGFNIGSSSHHWVRQAPKGLEWVA~~SIYS~~AF~~ASTSY~~ADSVKGRFTISA
 DTSKNTAYLQMNSLRAEDTAVYYCAR~~YHFPF~~FGFALDYWGQGTLLVTVSSGGGGSDIQMTQSPSSLSASVGDRTITCRASQ~~SISS~~SAVA
 WYQKPGKAPKLLIY~~S~~AS~~SL~~YSGVPSRFSGRSGTDFTLTISLQPEDFATYYCQQ~~G~~IV~~YLF~~TFGGQGTKVEIKLEDKTHTKVEPKSSDKTH
 NCPCPAPPELLGGPSVFLFPPKPKDITLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD
 WLNQKEYCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPRELMTSNQVSLCAVKGFYPSDIAVEWESNGQPENNYKTPPVLDSD
 GSFFLVSKLTVDKSRWQQGNVFSCVMHEALHNHYTQKLSLSPGKSGSETPGTSEATPESGGDIQMTQSPSSLSASVGDRTITCR
 ASQ~~SISS~~SAVAWYQQKPGKAPKLLIY~~S~~AS~~SL~~YSGVPSRFSGRSGTDFTLTISLQPEDFATYYCQQ~~Y~~W~~PI~~TFGGQGTKVEIKGTTAASGSS

GGSSSGAEVQLVESGGGLVQPGGSLRLSCAASGFN~~ISSYYH~~WVRQAPGKGLEWVA~~SIYSSGYTSY~~ADSVKGRFTISADTSKNTAYLQ
MNSLRAEDTAVYYCAR~~TVRGSKKPYFSGWAMD~~YWGQGLVTVSS (SEQ ID NO: 1000)

CM0109 Knob (Dia-Fc-scFv)

5 MNLLILTFVAAAVAEFEVQLVESGGGLVQPGGSLRLSCAASGFN~~IGSSSH~~WVRQAPGKGLEWVA~~SIYSAFASTSY~~ADSVKGRFTISA
DTSKNTAYLQMNSLRAEDTAVYYCAR~~YHFPFEGFAL~~DYWGQGLVTVSSGGGSDIQMTQSPSSLASVGDRTITCRASQ~~SIYSSAV~~A
WYQQKPGKAPKLLIY~~SASSLY~~SGVPSRFSGRSGTDFLT~~TISSLPQEDF~~ATYYCQQ~~GGVYLF~~TFGQGTKEIKLEDKTHTKVEPKTSDKTH
TCPPCPAPPELLGGPSVFLFPPKPKDITLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD
WLNQKEYCKVSNKALPAPIEKTISKAKGQPREPMVFDLPSSREEMTKNQVSLWCMVKGFYPSDIAVEWESNGQPENNYKTTPPVLD
SDGSFFLYSKLTVDKSRWQQGNVSCSMHEALHNHYTQKSLSPGKSGSETPGTSEATPESGGGDIQMTQSPSSLASVGDRTIT
CRASQ~~SIYSSAV~~AWYQQKPGKAPKLLIY~~SASSLY~~SGVPSRFSGRSGTDFLT~~TISSLPQEDF~~ATYYCQQ~~YSWGPF~~TFGQGTKEIKGTAA
SGSSGSSSGAEVQLVESGGGLVQPGGSLRLSCAASGFN~~SIYSH~~WVRQAPGKGLEWVA~~YISSYGYTY~~ADSVKGRFTISADTSKNT
AYLQMNSLRAEDTAVYYCAR~~AHYFPWAGAMD~~YWGQGLVTVSS (SEQ ID NO: 999)

10

CT003 Hole (Dia-Fc-scFv)

15 MNLLILTFVAAAVAEFEVQLVESGGGLVQPGGSLRLSCAASGFN~~ISSYYH~~WVRQAPGKGLEWVA~~SIYSSGYTSY~~ADSVKGRFTISA
DTSKNTAYLQMNSLRAEDTAVYYCAR~~TVRGSKKPYFSGWAMD~~YWGQGLVTVSSGGGSDIQMTQSPSSLASVGDRTITCRASQ~~S~~
~~IYSSAV~~AWYQQKPGKAPKLLIY~~SASSLY~~SGVPSRFSGRSGTDFLT~~TISSLPQEDF~~ATYYCQQ~~YSWGPF~~TFGQGTKEIKLEDKTHTKVEP
KSSDKTHNCPPCPAPELLGGPSVFLFPPKPKDITLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSV
LTVLHQDWLNGKEYCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPIRELMTSNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKT
TPPVLDSDGSFFLVSKLTVDKSRWQQGNVSCSMHEALHNHYTQKSLSPGKSGSETPGTSEATPESGGGEVQLVESGGGLVQPG
GSLRLSCAASGFN~~HSYSH~~WVRQAPGKGLEWVA~~ATYSSFGSITY~~ADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCAR~~YHHPF~~
~~GYALD~~YWGQGLVTVSSGTTAASGSSGSSGADIQMTQSPSSLASVGDRTITCRASQ~~SIYSSAV~~AWYQQKPGKAPKLLIY~~SASSLYS~~
GVPSRFSGRSGTDFLT~~TISSLPQEDF~~ATYYCQQ~~GGVYLF~~TFGQGTKEIK (SEQ ID NO: 1002)

20

CT003 Knob (Dia-Fc-scFv)

25 MNLLILTFVAAAVAEFEVQLVESGGGLVQPGGSLRLSCAASGFN~~SIYSH~~WVRQAPGKGLEWVA~~YISSYGYTY~~ADSVKGRFTISA
DTSKNTAYLQMNSLRAEDTAVYYCAR~~AHYFPWAGAMD~~YWGQGLVTVSSGGGSDIQMTQSPSSLASVGDRTITCRASQ~~SIYSSAV~~
AWYQQKPGKAPKLLIY~~SASSLY~~SGVPSRFSGRSGTDFLT~~TISSLPQEDF~~ATYYCQQ~~YYPPI~~TFGQGTKEIKLEDKTHTKVEPKTSDKT
HTCPPCPAPELLGGPSVFLFPPKPKDITLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ

25

5 DWLNGKEYCKVSNKALPAPIEKTISKAKGQPREPMVFDLPPSREEMTKNQVSLWCMVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCVMHEALHNHYTQKSLSLSPGKSGSETPGTSEATPESGGGEVQLVESGGGLVQPGGSLRLSCAASGFTNHHSSHHVVRQAPGKGLEWVA4TYSSEFGSITYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCAR~~YHHPFGYALD~~YWGQGTLVTVSSGTTAASGSSGGSSGADIQMTQSPSSLSASVGDRVTITCRASQ~~SVSS4~~VAWYQQKPKGAPKLLIY~~\$4SSLYSGVPSRF~~SGRSRSGTDFTLTITSSLPEDFATYYCQQGVYLFITFGGQGTKVEIK (SEQ ID NO: 1003)

CM0112 Hole (scFv-Fc-Dia)

10 MNLLILTFVAAA/AEFDIQMTQSPSSLSASVGDRVTITCRASQ~~SVSS4~~VAWYQQKPKGAPKLLIY~~\$4SSLYSGVPSRF~~SGRSRSGTDFTLTITSSLPEDFATYYCQQGVYLFITFGGQGTKVEIKGTAAAGSSGSSGAEVQLVESGGGLVQPGGSLRLSCAASGFTNHHSSHHVVRQAPGKGLEWVA~~SVSS4F4STSY~~ADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCAR~~YHHPFGFAL~~DYWGQGTLVTVSSLEDKTHKVEPKSSDKTHNCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPIRELMTSNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCVMHEALHNHYTQKSLSLSPGKSGSETPGTSEATPESGGGEVQLVESGGGLVQPGGSLRLSCAASGFTNHHSSHHVVRQAPGKGLEWVA~~SVSSYGYTSY~~ADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCAR~~TV~~~~RGSKKPYFSGWAMD~~YWGQGTLVTVSSGGGGSDIQMTQSPSSLSASVGDRVTITCRASQ~~SVSS4~~VAWYQQKPKGAPKLLIY~~\$4SSLYSG~~VPSRFSGRSGTDFTLTITSSLPEDFATYYCQQ~~YSWGPFT~~ITFGGQGTKVEIK (SEQ ID NO: 1004)

CM0112 Knob (scFv-Fc-Dia)

20 MNLLILTFVAAA/AEFDIQMTQSPSSLSASVGDRVTITCRASQ~~SVSS4~~VAWYQQKPKGAPKLLIY~~\$4SSLYSGVPSRF~~SGRSRSGTDFTLTITSSLPEDFATYYCQQGVYLFITFGGQGTKVEIKGTAAAGSSGSSGAEVQLVESGGGLVQPGGSLRLSCAASGFTNHHSSHHVVRQAPGKGLEWVA~~SVSS4F4STSY~~ADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCAR~~YHHPFGFAL~~DYWGQGTLVTVSSLEDKTHKVEPKTSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPMVFDLPPSREEMTKNQVSLWCMVKGFYPSDIAVEWESNGQPENNYKTTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCVMHEALHNHYTQKSLSLSPGKSGSETPGTSEATPESGGGEVQLVESGGGLVQPGGSLRLSCAASGFTNHHSSHHVVRQAPGKGLEWVA~~YISSYGYTY~~ADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCAR~~4~~~~HYFPWAGAMD~~YWGQGTLVTVSSGGGGSDIQMTQSPSSLSASVGDRVTITCRASQ~~SVSS4~~VAWYQQKPKGAPKLLIY~~\$4SSLYSGVPSR~~FSGRSRSGTDFTLTITSSLPEDFATYYCQQ~~YVWPIT~~ITFGGQGTKVEIK (SEQ ID NO: 1005)

CM0110 Hole (scFv-Fc-scFv)

MNLLILTFVAAA/AEFDIQMTQSPSSLSASVGDRTTITCRASQ~~SVSS~~AVAWYQQKPKAPKLLIY~~SASSLY~~SGVPSRFSGRSGTDFTLT
ISSLPEDFATYYCQQ~~GLVLF~~TFGQGTKVEIKGTTAASGSSGSSGAEVQLVESGGGLVQPGGSLRLSCAASG~~FNIGSSSH~~HWWVRQAP
GKGLEWV~~A~~~~SIYS4F4ST~~SYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCAR~~YHFPFGFAL~~DYWGQGTLVTVSSLEDKTHTK
5 VEPKSSDKTHNCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV
VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTIKAKGQPREPQVYTLPIRELMTSNQVSLCAVKGFYPSDIAVEWESNGQPENN
YKTTTPVLDSDGSFFLVSKLTVDKSRWQQGNVFCVMHEALHNHYTQKLSLSPGKSGSETPGTSEATPESGGGDIQMTQSPSSLSA
SVGDRVTTITCRASQ~~SVSS~~AVAWYQQKPKAPKLLIY~~SASSLY~~SGVPSRFSGRSGTDFTLTISLQPEDFATYYCQQ~~YVWP~~TFGQGTKV
EIKGTTAASGSSGSSGAEVQLVESGGGLVQPGGSLRLSCAASG~~FNISSVYH~~HWVRQAPGKGLEWV~~A~~~~SIYSYGYS~~YADSVKGRFTIS
10 ADTSKNTAYLQMNSLRAEDTAVYYCAR~~TVRGSKKPYFSGWAMD~~YWGQGTLVTVSS (SEQ ID NO: 1006)

CM0110 Knob (scFv-Fc-scFv)

MNLLILTFVAAA/AEFDIQMTQSPSSLSASVGDRTTITCRASQ~~SVSS~~AVAWYQQKPKAPKLLIY~~SASSLY~~SGVPSRFSGRSGTDFTLT
ISSLPEDFATYYCQQ~~GLVLF~~TFGQGTKVEIKGTTAASGSSGSSGAEVQLVESGGGLVQPGGSLRLSCAASG~~FNIGSSSH~~HWWVRQAP
GKGLEWV~~A~~~~SIYS4F4ST~~SYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCAR~~YHFPFGFAL~~DYWGQGTLVTVSSLEDKTHTK
15 VEPKTSCLKTHNCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV
VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTIKAKGQPREPMVFDLPPSREEMTKNQVSLWCMVKGFYPSDIAVEWESNGQPEN
NYKTTTPVLDSDGSFFLVSKLTVDKSRWQQGNVFCVMHEALHNHYTQKLSLSPGKSGSETPGTSEATPESGGGDIQMTQSPSSLS
ASVGDRTTITCRASQ~~SVSS~~AVAWYQQKPKAPKLLIY~~SASSLY~~SGVPSRFSGRSGTDFTLTISLQPEDFATYYCQQ~~YSWGP~~TFGQGT
KVEIKGTTAASGSSGSSGAEVQLVESGGGLVQPGGSLRLSCAASG~~FNISSVH~~HWVRQAPGKGLEWV~~A~~~~YISSYGYTY~~YADSVKGRF
20 TISADTSKNTAYLQMNSLRAEDTAVYYCAR~~HYFPWAGAMD~~YWGQGTLVTVSS (SEQ ID NO: 1007)

CT001 CM0299 CM0300 CM0301 ANT1 LC

MNLLILTFVAAA/ADIQMTQSPSSLSASVGDRTTITCRASQ~~SVSS~~AVAWYQQKPKAPKLLIY~~SASSLY~~SGVPSRFSGRSGTDFTLTIS
SLQPEDFATYYCQQ~~GLVLF~~TFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTE
QDSKDSYSLSTLTLSKADYEEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 1008)

CT001 Hole (bsIgG-dia)

MNLLLILTFVAAA/AEVQLVESGGGLVQPGGSLRLSCAASGFNIHSSSHHWVRQAPGKGLEWVAATYSSFGSITYADSVKGRFTISADT
 SKNTAYLQMNSLRAEDTAVYYCARYHHPFGYALDYWGQGLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSW
 NSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPK
 5 DTLMSRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEK
 TISKAKGQPREPQVYTLPPIRELMTSNQVSLCAVKGFYPSDIAVEWESNGQPENNYKTTPVLDSDGSFFLVSKLTVDKSRWQQGNVF
 SCSVMHEALHNHYTQKSLSLSPGKSGSETPGTSEATPESGGGEVQLVESGGGLVQPGGSLRLSCAASGFNISSYHHWVRQAPGKGLE
 WVASYSSYGYTSYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARTVRGSKKPYFSGW4MDYWGQGLVTVSSGGGGSDI
 QMTQSPSSLSASVGDRVTITCRASQSSAVAWYQQKPGKAPKLLIYS4SSLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQYS
 10 WGPFTFGQGTKVEIK (SEQ ID NO: 1009)

CT001 Knob (bsIgG-dia)

MNLLLILTFVAAA/AEVQLVESGGGLVQPGGSLRLSCAASGFNIHSSSHHWVRQAPGKGLEWVAATYSSFGSITYADSVKGRFTISADT
 SKNTAYLQMNSLRAEDTAVYYCARYHHPFGYALDYWGQGLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSW
 NSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPK
 15 DTLMSRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEK
 TISKAKGQPREPQVYTLPPSREEMTKNQVSLWCMVKGFYPSDIAVEWESNGQPENNYKTTPVLDSDGSFFLVSKLTVDKSRWQQGN
 VFSCVMHEALHNHYTQKSLSLSPGKSGSETPGTSEATPESGGGEVQLVESGGGLVQPGGSLRLSCAASGFNISSYHHWVRQAPGKGLEWVA
YISSYGYTYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARHYFPWAG4MDYWGQGLVTVSSGGGGSDIQM
 TQSPSSLSASVGDRVTITCRASQSSAVAWYQQKPGKAPKLLIYS4SSLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQYYWPI
 20 TFGQGTKVEIK (SEQ ID NO: 1010)

CM0299 Hc (IgG-Dia)

MNLLLILTFVAAA/AEVQLVESGGGLVQPGGSLRLSCAASGFNIHSSSHHWVRQAPGKGLEWVAATYSSFGSITYADSVKGRFTISADT
 SKNTAYLQMNSLRAEDTAVYYCARYHHPFGYALDYWGQGLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSW
 NSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPK
 25 DTLMSRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEK
 TISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPVLDSDGSFFLVSKLTVDKSRWQQGNV
 FSCVMHEALHNHYTQKSLSLSPGKSGSETPGTSEATPESGGGEVQLVESGGGLVQPGGSLRLSCAASGFNISSYHHWVRQAPGKGLEWVA
YISSYGYTYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARHYFPWAG4MDYWGQGLVTVSSGGGGSDIQMT

QSPSSLASVGDRTVTITCRASQ~~SLSS~~AVAWYQQKPGKAPKLLIY~~SASSLY~~SGVPSRFSGRSGTDFTLTISSLQPEDFATYYCQ~~YSWGPF~~
TFGGQGTKVEIK (SEQ ID NO: 1011)

CM0300 He (IgG-scFv)

5 MNLLLITFVAAAVAEVQLVESGGGLVQPGGSLRLSCAASGFN~~IHSSSH~~HWVRQAPGKGLEWVA~~ATYSFSGSITY~~ADSVKGRFTISADT
SKNTAYLQMNSLRAEDTAVYYCAR~~YHHPFGYAL~~DYWGQGLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSW
NSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPPELLGGPSVFLFPPKPK
DTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEK
TISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVTDKSRWQQGNV
FSCVMHEALHNHYTQKSLSLSPGKSGSETPGTSEATPESGGGDIQMTQSPSSLSASVGDRVTITCRASQ~~SLSS~~AVAWYQQKPGKAPK
10 LLIY~~SASSLY~~SGVPSRFSGRSGTDFTLTISSLQPEDFATYYCQ~~YSWGPF~~TFGGQGTKVEIKGTTAASGSSGGSSSGAEVQLVESGGGLVQ
PGGSLRLSCAASGFN~~ISYSH~~HWVRQAPGKGLEWVA~~YSSSYGYTY~~ADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCAR~~4HY~~
~~FPWAGAMD~~YWGQGLVTVSS (SEQ ID NO: 1012)

CM0301 He (IgG-scFv)

15 MNLLLITFVAAAVAEVQLVESGGGLVQPGGSLRLSCAASGFN~~IHSSSH~~HWVRQAPGKGLEWVA~~ATYSFSGSITY~~ADSVKGRFTISADT
SKNTAYLQMNSLRAEDTAVYYCAR~~YHHPFGYAL~~DYWGQGLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSW
NSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPPELLGGPSVFLFPPKPK
DTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEK
TISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVTDKSRWQQGNV
FSCVMHEALHNHYTQKSLSLSPGKSGSETPGTSEATPESGGGDIQMTQSPSSLSASVGDRVTITCRASQ~~SLSS~~AVAWYQQKPGKAPK
20 LLIY~~SASSLY~~SGVPSRFSGRSGTDFTLTISSLQPEDFATYYCQ~~YHWP~~TFGGQGTKVEIKGTTAASGSSGGSSSGAEVQLVESGGGLVQ
GGSLRLSCAASGFN~~ISSYI~~HWVRQAPGKGLEWVA~~SYSSYGYTY~~ADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCAR~~TVRG~~
~~SKKPYFSGWAMD~~YWGQGLVTVSS (SEQ ID NO: 1013)

ANT1 Hole (Dia-Fc-Fab)

25 MNLLLITFVAAAVAEFEVQLVESGGGLVQPGGSLRLSCAASGFN~~ISSYI~~HWVRQAPGKGLEWVA~~SYSSYGYTY~~ADSVKGRFTISA
DTSKNTAYLQMNSLRAEDTAVYYCAR~~TVRGSKKPYFSGWAMD~~YWGQGLVTVSSGGGGSDIQMTQSPSSLSASVGDRVTITCRASQ~~S~~
~~ISS~~AVAWYQQKPGKAPKLLIY~~SASSLY~~SGVPSRFSGRSGTDFTLTISSLQPEDFATYYCQ~~YHWP~~TFGGQGTKVEIKLEDKTHTKVEPKS
SDKTHNCPPCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLT

VLHQDWLNGKEYCKVSNKALPAPIEKTISKAKGQPREPQVYTLPIRELMTSNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPP
VLDSGGSFELVSKLTVDKSRWQQGNVFCSCVMHEALHNHYTQKSLSLSPGKSGSETPGTSEATPESGGGEVQLVESGGGLVQPGGSL
RLSCAASGFNHHSSJHWWVRQAPGKGLEWVAATYSSFGSITYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCAR~~YHHHPFGYA~~
~~L~~DYWGQGTLVTVSSASTKGPSVFPLAPSSKSTGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSST
LGTQYICNVNHNKPSNTKVDKKVEPKSCDKTHT (SEQ ID NO: 1014)

ANT1 Knob (Dia-Fc-Fab)

MNLLLLITFVAAA/AEFVQLVESGGGLVQPGGSLRLSCAASGFNHHSSJHWWVRQAPGKGLEWVA~~SISPIYGYTY~~ADSVKGRFTISA
DTSKNTAYLQMNSLRAEDTAVYYCAR~~SSYFPWF~~ES4MDYWGQGTLVTVSSGGGGSDIQMTQSPSSLSASVGDRTITCRASQ~~SVSSAV~~
AWYQQKPGKAPKLLIY~~SASSLYSGVPSRFS~~SGSRSGTDFLTISLQPEDFATYYCQ~~YVWPI~~TFGQGTKEIKLEDKTHTKVEPKTSDKT
HTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ
DWLNGKEYCKVSNKALPAPIEKTISKAKGQPREPMVFDLPPSREEMTKNQVSLWCMVKGFYPSDIAVEWESNGQPENNYKTTPPVL
DSDGSFELVSKLTVDKSRWQQGNVFCSCVMHEALHNHYTQKSLSLSPGKSGSETPGTSEATPESGGGEVQLVESGGGLVQPGGSLRL
SCAASGFNHHSSJHWWVRQAPGKGLEWVAATYSSFGSITYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCAR~~RYHHHPFGYALD~~
YWGQGTLVTVSSASTKGPSVFPLAPSSKSTGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSTLG
TQYICNVNHNKPSNTKVDKKVEPKSCDKTHT (SEQ ID NO: 1015)

Fab-Dia Hc (Fab-Dia)

MNLLLLITFVAAA/AEVQLVESGGGLVQPGGSLRLSCAASGFNHHSSJHWWVRQAPGKGLEWVAATYSSFGSITYADSVKGRFTISADT
SKNTAYLQMNSLRAEDTAVYYCAR~~YHHHPFGYALD~~YWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSW
NSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSTLTQTYICNVNHNKPSNTKVDKKVEPKSCDKTHTGGSGGEVQLVESGGGLVQPGG
SLRLSCAASGF~~TISYSSJHWWVRQAPGKGLEWVA~~~~SISPIYGYTY~~ADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCAR~~SSYFPWF~~
~~S4MDYWGQGTLVTVSSGGGGSDIQMTQSPSSLSASVGDRTITCRASQ~~~~SVSSAV~~AWYQQKPGKAPKLLIY~~SASSLYSGVPSRFS~~SGSRSG
TDFLTISLQPEDFATYYCQ~~YVWPI~~TFGQGTKEIK (SEQ ID NO: 1016)

Fab-Dia LC (Fab-Dia)

MNLLLLITFVAAA/ADIQMTQSPSSLSASVGDRTITCRASQ~~SVSSAV~~AWYQQKPGKAPKLLIY~~SASSLYSGVPSRFS~~SGSRSGTDFLTIS
SLQPEDFATYYCQQGV~~YLF~~TFGQGTKEIKRTVAAPSVFIFPPSDEQLKSGTASVVCCLNNFYPREAKVQWKVDNALQSGNSQESVTE
QDSKDSYSLSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECGGGGEVQLVESGGGLVQPGGSLRLSCAASGF~~TISYYIH~~
WVRQAPGKGLEWVA~~SIYSSYGYTSY~~ADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCAR~~TVRGSKKPYESGW~~AMDYWGQGT

VTVSSGGGSDIQMTQSPSSLASVGDRTVITCRASQ~~SVSS4~~VAWYQQKPGKAPKLLIY~~S4SSL~~YSGVPSRFSGRSGTDFLTITSSLOPE
DFATYYCQ~~YYFL~~ITFGQGTKVEIK (SEQ ID NO: 1017)

CH3 (Dia-CH3-Dia)

5 MNLLILTFVAAAVAEFEVQLVESGGGLVQPGGSLRLSCAASGFNIGSSSHHWVRQAPGKGLEWVA~~SVSS4FASTSY~~ADSVKGRFTISA
DTSKNTAYLQMNSLRAEDTAVYYCAR~~YHFFPFGFAL~~DYWGQGTLLVTVSSGGGGSDIQMTQSPSSLASVGDRTVITCRASQ~~SVSS4~~VA
WYQQKPGKAPKLLIY~~S4SSL~~YSGVPSRFSGRSGTDFLTITSSLOPEDFATYYCQ~~QGGVYLF~~TFGQGTKEIKDKTHTKVEPKTSDKTHTC
PPCPGQPREPQVYITLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFS
CSVMHEALHNHYTQKSLSLSPGKGTAAAGSSGGSSGAGRTEVQLVESGGGLVQPGGSLRLSCAASGFN~~ISYS~~SHHWVRQAPGKGLE
WVA~~YSSYYGYTY~~ADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCAR~~HYFPWAGAMD~~YWGQGTLLVTVSSGGGGSDIQMTQ
10 SPSSLASVGDRTVITCRASQ~~SVSS4~~VAWYQQKPGKAPKLLIY~~S4SSL~~YSGVPSRFSGRSGTDFLTITSSLOPEDFATYYCQ~~YSWGPFT~~
FGQGTKVEIK (SEQ ID NO: 1018)

CM0156

133 EVQLVESGGGLVQPGGSLRLSCAASGFN~~ISYS~~MHWWVRQAPGKGLEWVA~~SISPIYSYTSY~~ADSVKGRFTISADTSKNTAYLQMNSLRA
EDTAVYYCAR~~PS4W/SHYYPSSSS4FD~~YWGQGTLLVTVSSGGGGSDIQMTQSPSSLASVGDRTVITCRASQ~~SVSS4~~VAWYQQKPGKAP
15 KLLIY~~S4SSL~~YSGVPSRFSGRSGTDFLTITSSLOPEDFATYYCQ~~QSSYSL~~TFGQGTKEIKLEDKTHTKVEPKSSDKTHTCPPCPAPPELLG
GPSVFLFPKPKDITLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCK
VSNKALPAPIEKTTISKAKGQPREPQVYITLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLT
DKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGKSGSETPGTSEATPESGGGEVQLVESGGGLVQPGGSLRLSCAASGFN~~ISYS~~YM
HWVRQAPGKGLEWVA~~SISPIYSYTSY~~ADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCAR~~PS4W/SHYYPSSSS4FD~~YWGQGT
20 LVTVSSGGGGSDIQMTQSPSSLASVGDRTVITCRASQ~~SVSS4~~VAWYQQKPGKAPKLLIY~~S4SSL~~YSGVPSRFSGRSGTDFLTITSSLOP
EDFATYYCQ~~QSSYSL~~ITFGQGTKVEIK (SEQ ID NO: 1019)

Table 14
Comparison of expression titers and monodispersity of FZD agonists following Protein A purification. See corresponding figure 21.

Molecule	Format	N-term Paratope	C-term Paratope	Expression titer mg/l	% Monomer	Monomer Yield (mg/l)
CM0042	Dia – Fc – Dia	5019	2539	8±4	43	3
CM0011	Dia – Fc – Dia	5019	2539/2542	12±3	81±5	10
CM0126	Dia – Fc – Dia	5016	2539/2542	8±0.7	ND	ND
CM0107	Dia – Fc – scFv	5019	2539	90	87	80
CM0108	Dia – Fc – scFv	5019	2542	47	89	40
CM0109	Dia – Fc – scFv	5019	2539/2542	79	86	70
CT003	Dia – Fc – scFv	2539/2542	5016	47	67	30
CM0112	scFv – Fc – Dia	5019	2539/2542	15	79	10
CM0110	scFv – Fc – scFv	5019	2539/2542	82	81	70
CT001	bsIgG-dia	5016	2539/2542	47±21	44±11	30
CM0299	IgG-dia	5016	2539	20±8	ND	ND
CM0300	IgG – scFv	5016	2539	134±38	66±7	100
CM0301	IgG – scFv	5016	2542	134±27	71±8	90
ANT1	Dia-Fc-Fab	5016	2539/2542	294±75	53±9	150
CH3	Dia-CH3-Dia	5019	2539	<1	ND	ND
Fab-Dia	Fab-Dia	5016	2540/2542	1	ND	ND

Table 15

Functional comparison of FZD agonists. Concentration-response curves of each FZD agonist were generated using the TOPFLASH assay. Figure 20 shows representative traces of the A) Diabody-Fc-Diabody and B) Diabody-Fc-Fab format overlaid with Wnt3a for comparison. Calculated EC50 and maximum efficacy relative to recombinant Wnt3a control were derived and are presented as the average ± SD.

Name	Format	N-term Paratope	C-term Paratope	EC ₅₀ (nM)	Maximal Signal (% Wnt3a maxima)
Wnt3a	Recombinant Protein	NA	NA	6±3	100
CM0042	Dia – Fc – Dia	5019	2539	0.9±0.8	33±22
CM0011	Dia – Fc – Dia	5019	2539/2542	0.3±0.1	63±10
CM0126	Dia – Fc – Dia	5016	2539/2542	0.3±0.2	52±1
CM0107	Dia-Fc-scFv	5019	2539	ND	ND
CM0108	Dia-Fc-scFv	5019	2542	ND	ND
CM0109	Dia-Fc-scFv	5019	2539/2542	ND	ND
CT003	Dia-Fc-scFv	2539/2542	5016	0.4±0.1	42±16
CM0112	scFv-Fc-Dia	5019	2539/2542	ND	ND
CM0110	scFv-Fc-scFv	5019	2539/2542	ND	ND
CT001	bsIgG-dia	5016	2539/2542	0.3±0.1	57±17
CM0299	IgG-dia	5016	2539	0.1±0.1	14±5
CM0300	IgG-scFv	5016	2539	0.3±0.2	5±2
CM0301	IgG-scFv	5016	2542	0.2±0.2	1±1
ANT1	Dia-Fc-Fab	5016	2539/2542	0.7±0.5	50±18
CH3	Dia-CH3-Dia	5019	2539	ND	22±10
Fab-Dia	Fab-Dia	5016	2540/2542	0.4±0.2	42±16

Table 16

FZD2-LRP6 CM0072	MNLLILTFVAAVAEFEVQLVESGGGLVQPGGSLRLSCAASGFNIIYSSJHVVVRQAPGKGLEWVA SIYPYGYTY YADSVKGR FTISADTSKNTAYLQMNSLRAEDTAVYYCAR YHYGLDY WGQGTLVTVSSGGGGSDIQMTQSPSSLSASVGDRTVITCRASQ S I SSAVAWYQQKPGKAPKLLIY S4SSL YSGVPSRFSGRSGTDFTLTISLQPEDFATYYCQ SYWHSYL ITFGQGTKEIKLEDKT HTKVEPKTSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ YNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTIISKAKGQPREPMVFDLPPSREEMTKNQVSLWCMVKGFYPSDI AVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCVMEALHNHYTQKSLSLSPGKSGSETPTGTSAT PESGGGEVQLVESGGGLVQPGGSLRLSCAASGFNIYSSJHVVVRQAPGKGLEWVA YISSYGYTY YADSVKGRFTISADTSKNT AYLQMNSLRAEDTAVYYCAR 4HYFPW AGAMDYWGQGTLVTVSSGGGGSDIQMTQSPSSLSASVGDRTVITCRASQ SI SS4VA WYQQKPGKAPKLLIY S4SSL YSGVPSRFSGRSGTDFTLTISLQPEDFATYYCQ QYYPIT ITFGQGTKEIK (SEQ ID NO: 1020)
Knob construct	MNLLILTFVAAVAEFEVQLVESGGGLVQPGGSLRLSCAASGFNIIYSSJHVVVRQAPGKGLEWVA SIYPYGYTY YADSVKGR FTISADTSKNTAYLQMNSLRAEDTAVYYCAR YHYGLDY WGQGTLVTVSSGGGGSDIQMTQSPSSLSASVGDRTVITCRASQ S I SSAVAWYQQKPGKAPKLLIY S4SSL YSGVPSRFSGRSGTDFTLTISLQPEDFATYYCQ SYWHSYL ITFGQGTKEIKLEDKT HTKVEPKSSDKTHNCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ YNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTIISKAKGQPREPQVYTLPIRELMTSNQVSLSCAVKGFYPSDIAV EWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCVMEALHNHYTQKSLSLSPGKSGSETPTGTSATPES GGGEVQLVESGGGLVQPGGSLRLSCAASGFNISSYYHVVVRQAPGKGLEWVA SIYSYGYTY YADSVKGRFTISADTSKNTAYL QMNSLRAEDTAVYYCAR TVRGSKKPYFSGW AMDYWGQGTLVTVSSGGGGSDIQMTQSPSSLSASVGDRTVITCRASQ SI SS4 VAWYQQKPGKAPKLLIY S4SSL YSGVPSRFSGRSGTDFTLTISLQPEDFATYYCQ QYSGP ITFGQGTKEIK (SEQ ID NO: 1021)
Hole construct	

FZD5-LRP6 CM0024	MNLLJLTFVAAVAEFEVQLVESGGGLVQPGGSLRLSCAASGFN<u>ISYSSJ</u>HHWVRQAPGKGLEWVA<u>SIYPSYSS</u>TYADSVKGR FTISADTSKNTAYLQMNSLRAEDTAVYYCAR<u>YY4MD</u>YWGGTLVTVSSGGGGSDIQMTQSPSSLSASVGDRVTITCRASQ<u>SVS</u> <u>S4</u>VAWYQQKPGKAPKLLIY<u>S4SSL</u>YSGVPSRFSGRSGTDFTLTISLQPEDFATYYCQQ4<u>FIYYP</u>ITFGQGTKVEIKLEDKTHTKV EPKTSKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPETCVVDVSHEDPEVKFNWYVDGVEVHNATKPREEQYNST YRVSVLTVLHQDWLNGKEYCKVSNKALPAPIEKTIISKAKGQPREPMVFDLPPSREEMTKNQVSLWCMVKGFYPSDIAVEW ESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCVSMHEALHNHYTQKSLSPGKSGSETPTGTSATPESGG GEVQLVESGGGLVQPGGSLRLSCAASGFN<u>ISYSSJ</u>HHWVRQAPGKGLEWVA<u>YISSYGYTY</u>YADSVKGRFTISADTSKNTAYLQM NSLRAEDTAVYYCAR4<u>HYFPW4G4MD</u>YWGGTLVTVSSGGGGSDIQMTQSPSSLSASVGDRVTITCRASQ<u>SVSS4</u>VAWYQQK GKAPKLLIY<u>S4SSL</u>YSGVPSRFSGRSGTDFTLTISLQPEDFATYYCQQ<u>YIYWP</u>ITFGQGTKVEIK (SEQ ID NO: 1022)
Knob construct	MNLLJLTFVAAVAEFEVQLVESGGGLVQPGGSLRLSCAASGFN<u>ISYSSJ</u>HHWVRQAPGKGLEWVA<u>SIYPSYSS</u>TYADSVKGR FTISADTSKNTAYLQMNSLRAEDTAVYYCAR<u>YY4MD</u>YWGGTLVTVSSGGGGSDIQMTQSPSSLSASVGDRVTITCRASQ<u>SVS</u> <u>S4</u>VAWYQQKPGKAPKLLIY<u>S4SSL</u>YSGVPSRFSGRSGTDFTLTISLQPEDFATYYCQQ4<u>FIYYP</u>ITFGQGTKVEIKLEDKTHTKV EPKSSDKTHNCPCPAPELLGGPSVFLFPPKPKDTLMISRTPETCVVDVSHEDPEVKFNWYVDGVEVHNATKPREEQYNST YRVSVLTVLHQDWLNGKEYCKVSNKALPAPIEKTIISKAKGQPREPQVYVTLPIRELMTSNQVSLSCAVKGFYPSDIAVEWES NGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCVSMHEALHNHYTQKSLSPGKSGSETPTGTSATPESGGG EVQLVESGGGLVQPGGSLRLSCAASGFN<u>ISYSSJ</u>HHWVRQAPGKGLEWVA<u>SIYPSYGYTY</u>YADSVKGRFTISADTSKNTAYLQMN SLRAEDTAVYYCAR<u>TVRGSKKPYFSGW4MD</u>YWGGTLVTVSSGGGGSDIQMTQSPSSLSASVGDRVTITCRASQ<u>SVSS4</u>VAWYQ QKPGKAPKLLIY<u>S4SSL</u>YSGVPSRFSGRSGTDFTLTISLQPEDFATYYCQQ<u>YSWGP</u>ITFGQGTKVEIK (SEQ ID NO: 1023)
Hole construct	

FZD7-LRP6 CM0172	MNLLJLTFVAAVAEFEVQLVESGGGLVQPGGSLRLSCAASGFN<u>ISSSSM</u>HWVVRQAPGKGLEWVA<u>SIYSYIGSTYY</u>ADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCAR<u>WYGM</u>DYWGQGTLVTVSSGGGGSDIQMTQSPSSLSASVGDVTVTITCRASQ<u>SISS4</u>VAWYQQKPGKAPKLLIY<u>S4SSL</u>YSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQ<u>PGSWYFPPI</u>TFGGQGTKVEIKLEDKTHTKVEPKTSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYCKVSNKALPAPIEKTISKAKGQPREPMVFDLPPSREEMTKNQVSLWCMVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCVMHEALHNHYTQKSLSPGKSGSETPTGTSESA TPESGGGEVQLVESGGGLVQPGGSLRLSCAASGFN<u>SIYSST</u>HWVVRQAPGKGLEWVA<u>YISSYIGYTY</u>YADSVKGRFTISADTSKN TAYLQMNSLRAEDTAVYYCAR<u>4HYFPW4G4MD</u>YWGQGTLVTVSSGGGGSDIQMTQSPSSLSASVGDVTVTITCRASQ<u>SVSS4</u>VAWYQQKPGKAPKLLIY<u>S4SSL</u>YSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQ<u>YIYWPI</u>TFGGQGTKVEIK (SEQ ID NO: 1024)
Knob construct	MNLLJLTFVAAVAEFEVQLVESGGGLVQPGGSLRLSCAASGFN<u>ISSSSM</u>HWVVRQAPGKGLEWVA<u>SIYSYIGSTYY</u>ADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCAR<u>WYGM</u>DYWGQGTLVTVSSGGGGSDIQMTQSPSSLSASVGDVTVTITCRASQ<u>SISS4</u>VAWYQQKPGKAPKLLIY<u>S4SSL</u>YSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQ<u>PGSWYFPPI</u>TFGGQGTKVEIKLEDKTHTKVEPKSSDKTHNCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYCKVSNKALPAPIEKTISKAKGQPREPQVYTLPIRELMTSNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCVMHEALHNHYTQKSLSPGKSGSETPTGTSESAT PESGGGEVQLVESGGGLVQPGGSLRLSCAASGFN<u>SISSYTH</u>HWVVRQAPGKGLEWVA<u>SIYSYIGYTS</u>YADSVKGRFTISADTSKNTA YLQMNSLRAEDTAVYYCAR<u>TVRGSKKPYFSGW4MD</u>YWGQGTLVTVSSGGGGSDIQMTQSPSSLSASVGDVTVTITCRASQ<u>SISS4</u>VAWYQQKPGKAPKLLIY<u>S4SSL</u>YSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQ<u>YSWGPFI</u>TFGGQGTKVEIK (SEQ ID NO: 1025)
Hole construct	

PanFZD-LRP6 CM0011	Knob construct MNLLLLITFVAAVAEFEVQLVESGGGLVQPGGSLRLSCAASGFNIGSSSHHWVRQAPGKGLEWVASIYS4F4STSYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARYHFPFGFALDYWGQGTLVTVSSGGGGSDIQMTQSPSSLSASVGDRVTITCRAQSVS4VAWYQQKPGKAPKLLIYS4SSLYSGVPSRFSGSRSGTDFTLTISLQPEDFATYYCQQGVYLFTFGGQGTKVEIKLEDKHTKVEPKTSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPETCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTSISKAGQPREPMVFDLPPSREEMTKNQVSLWCMVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSVMHEALHNHYTQKSLSPGKSGSETPGTSESATPESGGGEVQLVESGGGLVQPGGSLRLSCAASGFNISYSSHHWVRQAPGKGLEWVAYISSYGYTYYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCAR4HYFPWAGAMDYWGQGTLVTVSSGGGGSDIQMTQSPSSLSASVGDRVTITCRASQVS4VAWYQQKPGKAPKLLIYS4SSLYSGVPSRFSGSRSGTDFTLTISLQPEDFATYYCQQYVWPITFGGQGTKVEIK (SEQ ID NO: 1026)
	Hole construct MNLLLLITFVAAVAEFEVQLVESGGGLVQPGGSLRLSCAASGFNIGSSSHHWVRQAPGKGLEWVASIYS4F4STSYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARYHFPFGFALDYWGQGTLVTVSSGGGGSDIQMTQSPSSLSASVGDRVTITCRAQSVS4VAWYQQKPGKAPKLLIYS4SSLYSGVPSRFSGSRSGTDFTLTISLQPEDFATYYCQQGVYLFTFGGQGTKVEIKLEDKHTKVEPKSSDKTHNCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPETCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTSISKAGQPREPQVYTLPIRELMTSNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSVMHEALHNHYTQKSLSPGKSGSETPGTSESATPESGGGEVQLVESGGGLVQPGGSLRLSCAASGFNISYSSHHWVRQAPGKGLEWVASIYSYGYTYYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARTVRGSKKPYFSGWAMDYWGQGTLVTVSSGGGGSDIQMTQSPSSLSASVGDRVTITCRASQVS4VAWYQQKPGKAPKLLIYS4SSLYSGVPSRFSGSRSGTDFTLTISLQPEDFATYYCQQYVWPITFGGQGTKVEIK (SEQ ID NO: 1027)

FZD4-LRP6 CM0016	MNLLJLTFVAAAEFEVQLVESGGGLVQPGGSLRLSCAASGFN<u>ISYYI</u>HWVRQAPGKGLEWVA<u>SIYPSSGYTY</u>YADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCAR<u>SSFYI</u>MDYWGQGTLVTVSSGGGGSDIQMTQSPSSLSASVGDRVTTITCRASQ<u>SI</u><u>SS4</u>VAWYQQKPGKAPKLLIY<u>S4SSL</u>YSGVPSRFSGRSGTDFTLTISLQPEDFATYYCQ<u>SY44YL</u>FTFGQGTKEIKLEDKTHTKVEPKSSDKTHNCPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNATKPREEQYNSTYRVVSVLTVLHQDWLNGKEYCKVSNKALPAPIEKTSISKAGQPREPQVYTLPPIRELMTSNQVLSCAVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVSCSMHEALHNHYTQKSLSPGKSGSETPGTSESATPESGGGEVQLVESGGGLVQPGGSLRLSCAASGFN<u>ISYYI</u>HWVRQAPGKGLEWVA<u>SIYSYGYTSY</u>ADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCAR<u>TVRGSKKPYESGW</u>MDYWGQGTLVTVSSGGGGSDIQMTQSPSSLSASVGDRVTTITCRASQ<u>SI</u><u>SS4</u>VAWYQQKPGKAPKLLIY<u>S4SSL</u>YSGVPSRFSGRSGTDFTLTISLQPEDFATYYCQ<u>YSWGPF</u>TFGGQGTKEIK (SEQ ID NO: 1028)
Knob construct	MNLLJLTFVAAAEFEVQLVESGGGLVQPGGSLRLSCAASGFN<u>ISYYI</u>HWVRQAPGKGLEWVA<u>SIYPSSGYTY</u>YADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCAR<u>SSFYI</u>MDYWGQGTLVTVSSGGGGSDIQMTQSPSSLSASVGDRVTTITCRASQ<u>SI</u><u>SS4</u>VAWYQQKPGKAPKLLIY<u>S4SSL</u>YSGVPSRFSGRSGTDFTLTISLQPEDFATYYCQ<u>SY44YL</u>FTFGQGTKEIKLEDKTHTKVEPKTSDKTHNCPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNATKPREEQYNSTYRVVSVLTVLHQDWLNGKEYCKVSNKALPAPIEKTSISKAGQPREPQVVDLPPSREEMTKNQVSLWCMVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVSCSMHEALHNHYTQKSLSPGKSGSETPGTSESATPESGGGEVQLVESGGGLVQPGGSLRLSCAASGFN<u>ISYYI</u>HWVRQAPGKGLEWVA<u>YISYYGYTY</u>YADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCAR<u>HYFPW</u>AGMDYWGQGTLVTVSSGGGGSDIQMTQSPSSLSASVGDRVTTITCRASQ<u>SI</u><u>SS4</u>VAWYQQKPGKAPKLLIY<u>S4SSL</u>YSGVPSRFSGRSGTDFTLTISLQPEDFATYYCQ<u>QYI</u>WPTFGQGTKEIK (SEQ ID NO: 1029)
Hole construct	

WE CLAIM

1. A tetravalent binding antibody molecule comprising:
 - (a) an Fc domain or fragment thereof comprising a constant heavy chain domain 3 (CH3),
 - (b) a bivalent low-density lipoprotein receptor-related protein 5 (LRP5) binding domain
 - 5 and
 - (c) a bivalent Frizzled (FZD) binding domain,wherein the LRP5 binding domain is attached to one terminus of the Fc domain and the FZD binding domain is attached to the other end of the Fc domain,
wherein the LRP5 binding domain comprises a diabody that binds LRP5 and the FZD
10 binding domain comprises two scFv or two Fab that bind FZD4.
2. The tetravalent binding antibody molecule of claim 1, wherein
 - (a) the LRP5 binding domain diabody is attached to the N-terminal of the Fc domain, and
 - (b) the FZD4 binding domain is attached to the C-terminal of the Fc domain.
3. The tetravalent binding antibody molecule of claim 2, wherein
 - 15 (a) the LRP5 binding domain diabody is attached to the N-terminal of the Fc domain via a VL or VH of the diabody, and
 - (b) the FZD binding domain comprises two FZD binding Fabs fused to the C-terminal of the Fc, wherein each Fab is attached to the Fc domain via a heavy or light chain variable domain (VH or VL) of the Fab linked to the CH3 domain of the Fc domain.
- 20 4. A tetravalent binding antibody molecule comprising an N-terminal LRP5-binding diabody and a C-terminal domain comprising two FZD4-binding scFv, the tetravalent binding antibody molecule comprising
a dimer of a first and second monomer, wherein each monomer comprises a single-chain polypeptide comprising, from N-terminus to C-terminus:

- (a) a first peptide, said first peptide comprising a first heavy chain variable domain (VH) and a first light chain variable (VL) domain that bind LRP5,
- (b) an Fc region, or fragment thereof comprising a constant heavy chain domain 3 (CH3), and
- 5 (c) a second peptide, said second peptide comprising a second VL and a second VH that bind a FZD4, and
- a first and second light chain monomer, each light chain monomer comprising from N terminus to C terminus a VL that binds the FZD4, linked to a constant light chain domain 1 (CL1 domain),
- 10 wherein the first and second monomers dimerize via the Fc regions or fragments thereof, the first VH and VL of each monomer pairs with the first VH and VL of the other monomer forming a diabody that binds LRP5 and the second VL and VH of each monomer pair to form a scFvs that bind FZD4, and
- wherein the LRP5-binding diabody forms the N-terminal LRP5-binding domain of the
- 15 tetravalent binding antibody molecule and the two FZD4-binding scFvs form the C-terminal FZD-binding domain of the tetravalent binding antibody molecule.
5. The tetravalent binding antibody molecule of claim 1, wherein the FZD binding domain is attached to the N-terminal of the Fc domain and the LRP5 binding domain is attached to the C-terminal of the Fc domain.
- 20 6. The tetravalent binding antibody molecule of claim 5, wherein
- (a) the FZD binding domain comprises two Fabs that bind the FZD4, wherein each Fab is attached to N-terminal of the Fc domain via a heavy or light chain variable domain (VH or VL) of the Fab linked to the CH2 domain of the Fc domain, and
- (b) the LRP5 binding domain comprises a diabody or two scFv that bind LRP5, wherein
- 25 the diabody or two scFv are attached to the C-terminal of the Fc domain via a VL or VH of the diabody or scFv linked to the CH3 of the Fc domain.

7. A tetravalent binding antibody molecule comprising an Fc domain or fragment thereof comprising a constant heavy chain domain 3 (CH3), an N-terminal FZD4 binding domain comprising two FZD4-binding Fabs and a C-terminal LRP5 binding domain comprising a LRP5 binding diabody, the tetravalent binding antibody molecule comprising

5 (a) a first and second heavy chain monomer, wherein each heavy chain monomer comprises a single-chain polypeptide comprising from N-terminus to C-terminus:

(i) a heavy chain variable domain (VH) that binds FZD4, linked to

(ii) a heavy chain constant region domain 1 (CH1 domain), linked to

(iii) the CH2 domain of the Fc region linked to

10 (iv) a peptide comprising a VH that binds a LRP5 co-receptor, linked to a light chain variable domain (VL) that binds a LRP5 co-receptor, and

(b) a first and second light chain monomer, each light chain monomer comprising from N terminus to C terminus a VL that binds the FZD4, linked to a constant light chain domain 1 (CL1 domain),

15 wherein the first and second heavy chain monomers dimerize via their Fc regions, or fragments thereof,

wherein a linker between the VH and VL that binds the LRP5 is of a length that promotes the pairing of the VH and VL of the first heavy chain monomer with the VL and VH of the second heavy chain monomer thereby forming a LRP5 co-receptor binding diabody and the FZD-binding Fabs are formed by the pairing of each heavy chain monomer with a light chain monomer such that the VH that binds FZD4 and CH1 of each of the heavy chain monomer, pairs with the VL that binds FZD4 and CL1 of the light chain monomers, and

20

wherein the Fabs form the FZD4-binding domain on the N-terminus of the Fc domain and the diabody forms the LRP5 co-receptor-binding domain on the C-terminus of the Fc domain.

25

8. The tetravalent binding antibody molecule of any one of claims 1 to 7, wherein the LRP5 binding diabody is a bispecific bivalent LRP5 binding domain that binds to two epitopes within the LRP5 co-receptor extracellular domain.
9. The tetravalent binding antibody molecule of claim 8 wherein the LRP5 binding domain
5 interacts with the Wnt1 and Wnt3 epitopes of the LRP5 co-receptor.
10. The tetravalent binding antibody molecule of any one of claims 1 to 9, wherein the FZD binding domain is monospecific.
11. The tetravalent binding antibody molecule of any one of claims 1 to 10, wherein
10 the diabody of the LRP5 binding domain binds LRP5 and comprises heavy chain complementary determining regions CDR-H1, CDR-H2 and CDR-H3 and light chain complementary determining regions CDR-L1, CDR-L2 and CDR-L3, of an antibody set forth in the sequences in Table 3 or Table 6A.
12. The tetravalent binding antibody molecule of any one of claims 1 to 11 wherein the Fc domain or fragment thereof dimerize via a knob-in-hole configuration of the Fc regions or
15 fragments thereof.
13. The tetravalent binding antibody molecule of claim 12, wherein the Fc region of the first heavy chain monomer comprises the mutations T366S, L368A and Y407V, and the Fc region of the second heavy chain monomer comprises the mutation T366W, according to EU numbering.
14. The tetravalent binding antibody molecule of claim 13, wherein the Fc region of the first
20 heavy chain monomer further comprises the mutations S354I and E357L, and the Fc region of the second heavy chain monomer further comprises the mutations Q347M, Y349F, T350D and L368M, according to EU numbering.
15. The tetravalent binding antibody molecule of claim 13, wherein an additional disulfide bond is introduced between the Fc region of the first heavy chain monomer and the second heavy
25 chain monomer, preferably wherein the first heavy chain monomer comprises the mutation Y349C and the second heavy chain monomer comprises the mutation S354C, according to EU numbering.

16. The tetravalent binding antibody molecule of any one of claims 1 to 15, wherein the Fc domain lacks one or more effector functions.
17. The tetravalent binding antibody molecule of claim 16, wherein the Fc regions contain mutations that alter their effector function due to amino acid mutations, N297G (NG) and/or
5 D265A (DA) variants, according to EU numbering.
18. The tetravalent binding antibody molecule of claim 16, wherein the Fc regions contain mutations that alter their effector function due to amino acid mutations L234A, L235A and/or P331S, according to EU numbering.
19. The tetravalent binding antibody molecule of claim 18, wherein the Fc regions contain
10 mutations that alter their effector function due to amino acid mutations L234A and L235A (LALA).
20. The tetravalent binding antibody molecule of claim 19, wherein the Fc regions contain mutations that alter their effector function due to amino acid mutations L234A, L235A and P331S (LALAPS).
- 15 21. The tetravalent binding antibody molecule of any one of claims 1 to 20, wherein the LRP5 binding domain and FZD binding domain are each attached to the Fc domain by a linker.
22. The tetravalent binding antibody molecule of claim 21, wherein the linker comprises 1 to 100, 1 to 50, 1-30, 1-25, 1- 10, 1-6 amino acids, 1-5 amino acids, or 2-4 amino acids.
23. The tetravalent binding antibody molecule of claim 21 or 22, wherein the diabodies forming
20 the LRP5 co-receptor-binding domain were fused to the Fc domain via a GGGGSGGGGSEPKSSDKTHT (SEQ ID NO: 892) linker.
24. The tetravalent binding antibody molecule of any one of claims 21 to 23, wherein the FZD4-binding Fabs are fused to the Fc region via a GGGSGGGSGGGSGGGSGSTG (SEQ ID NO: 891) linker.

25. The tetravalent binding antibody molecule of any one of claims 1 to 24, wherein the VH that binds the LRP5 co-receptor is linked to the VL that binds a LRP5 co-receptor via a short GGGGS (SEQ ID NO: 886) linker.
26. The tetravalent binding antibody molecule of any one of claims 1 to 25, wherein the FZD
5 binding domain comprises two Fabs that bind FZD4.
27. The tetravalent binding antibody molecule of claim 26, wherein the FZD-binding Fab comprise light chain complementary determining regions CDR-L1, CDR-L2 and CDR-L3 and heavy chain CDRs, CDR-H1, CDR-H2 and CDR-H3 of an antibody set forth in the sequences in Table 1, Table 2 or Table 6.
- 10 28. The tetravalent binding antibody molecule of any one of claims 1 to 4 and 8 to 27 comprising,
- (a) a dimer of a first and second heavy chain monomer, each monomer comprising a single-chain polypeptide comprising, from N-terminus to C-terminus:
- 15 (1) a peptide comprising a heavy chain variable domain (VH) that binds LRP5 and a light chain variable domain (VL) that binds LRP5,
- (2) an Fc region, or fragment thereof comprising the CH3,
- (3) a VH that binds FZD4, and
- (4) a constant heavy chain domain 1 (CH1),
- wherein
- 20 (5) the VH that binds LRP5 comprises heavy chain CDRs (CDR-H1, CDR-H2 and CDR-H3), of an antibody set forth in the sequences in Table 3 or Table 6, and
- (b) the VL that binds LRP5 comprises light chain CDRs (CDR-L1, CDR-L2 and CDR-L3), of an antibody set forth in the sequences in Table 3 or Table 6,

(c) the VH that binds FZD4, comprises the heavy chain CDRs (CDR-H1, CDR-H2 and CDR-H3), of an antibody set forth in the sequences in Table 1, Table 2 or Table 6, and

5 (d) a third and fourth light chain monomer each comprising a VL that binds FZD4, and a constant light chain domain 1 (CL1), the VL that binds FZD4, comprising the light chain CDRs (CDR-L1, CDR-L2 and CDR-L3), of an antibody set forth in the sequences in Table 1, Table 2 or Table 6,

wherein the first and second heavy chain monomer dimerize via their Fc regions and the VL and VH that bind LRP5 of the first monomer pair with the VH and VL that bind LRP5 or of the
10 second monomer forming a bivalent diabody that binds LRP5, and

the CL1 and VLs that bind FZD4, of the third and fourth light chain monomers pair with the CH1 and VHs that bind FZD4, of the first and second heavy chain monomers forming two Fabs that bind FZD4, wherein the diabody forms the N-terminal bivalent LRP5 binding domain and the two Fabs form the C-terminal bivalent FZD4, binding domain.

15 29. The tetravalent binding antibody molecule of any one of claims 5 to 7 comprising,

(a) a dimer of a first and second heavy chain monomer, each monomer comprising a single-chain polypeptide comprising, from N-terminus to C-terminus:

(1) a VH that binds FZD4

(2) an Fc region, or fragment thereof comprising the CH3,

20 (3), a peptide comprising a VH that binds LRP5 and a VL that binds LRP5 and

(4) a constant heavy chain domain 1 (CH1),

wherein

(5) the VH that binds LRP5 comprises heavy chain CDRs (CDR-H1, CDR-H2 and CDR-H3), of an antibody set forth in the sequences in Table 3 or Table 6,

25 and

- (b) the VL that binds LRP5 comprises light chain CDRs (CDR-L1, CDR-L2 and CDR-L3), of an antibody set forth in the sequences in Table 3 or Table 6,
- (c) the VH that binds FZD4, comprises the heavy chain CDRs (CDR-H1, CDR-H2 and CDR-H3), of an antibody set forth in the sequences in Table 1, Table 2 or Table 6,
- 5 and
- (d) a third and fourth light chain monomer each comprising from N terminus to C terminus a VL that binds FZD4, and a constant light chain domain 1 (CL1), the VL that binds FZD4, comprising the light chain CDRs (CDR-L1, CDR-L2 and CDR-L3), of an antibody set forth in the sequences in Table 1, Table 2 or Table 6,
- 10 wherein the first and second heavy chain monomer dimerize via their Fc regions and the VL and VH that bind LRP5 of the first monomer pair with the VH and VL that bind LRP5 or of the second monomer forming a bivalent diabody that binds LRP5, and
- the CL1 and VLs that bind FZD4, of the third and fourth light chain monomers pair with the CH1 and VHs that bind FZD4, of the first and second heavy chain monomers forming two Fabs
- 15 that bind FZD4, wherein the diabody forms the C-terminal bivalent LRP5 binding domain and the two Fabs form the N-terminal bivalent FZD4, binding domain.
30. The tetravalent binding antibody molecule of claim 28 or 29, wherein the LRP5 binding diabody is bispecific, wherein
- 20 the CDRs of the VHs that bind LRP5 of the first heavy chain monomer and the CDRs of the VHs that bind LRP5 of the second heavy chain monomer are non-identical, and
- the CDRs of the VLs that bind LRP5 of the first heavy chain monomer and the CDRs of the VLs that bind LRP5 second heavy chain monomer are non-identical.
31. The tetravalent binding antibody molecule of claim 30, wherein
- in the first heavy chain monomer

- (a) the CDR-H1 and CDR-H2 of the VH that binds LRP5 comprise respectively FSSSSI (SEQ ID NO: 528) and SISSSYGYTY (SEQ ID NO: 553), or the CDR-H1 and CDR-H2 of the VH that binds LRP5 comprise respectively LSYYYM (SEQ ID NO: 527) and SIYSSYGYTY (SEQ ID NO: 552) and
- 5 (b) the CDR-L2 and CDR-L3 of the VL that binds LRP5 comprise respectively SASDLYS (SEQ ID NO: 491) and YAGAGLI (SEQ ID NO: 510), or the CDR-L2 and CDR-L3 of the VL that binds LRP5 comprise respectively SASSLYS (SEQ ID NO: 2) and SSYSLI (SEQ ID NO: 130), and
- in the second heavy chain monomer
- 10 (c) the CDR-H1 and CDR-H2 of the VH that binds LRP5 comprises respectively FTAYAM (SEQ ID NO: 536) and SIYPSGGYTA (SEQ ID NO: 566), or the CDR-H1 and CDR-H2 of the VH that binds LRP5 comprises respectively FSSSSI (SEQ ID NO: 528) and SISSSYGYTY (SEQ ID NO: 553) and
- (d) the CDR-L2 and the CDR-L3 of the VL that binds LRP5 comprises respectively
- 15 SASSLYS (SEQ ID NO: 2) and YWAYYSPI, or the CDR-L2 and the CDR-L3 of the VL that binds LRP5 comprises respectively SASSLYS (SEQ ID NO: 2) and ASYAPI.
32. The tetravalent binding antibody molecule of any one of claims 28 to 31, wherein in the first and second heavy chain monomer the CDR-H1 and CDR-H2 of the VH that binds FZD4 comprises respectively LSSYSM (SEQ ID NO: 24) and YISSYYGYTY (SEQ ID NO: 51), or
- 20 the CDR-H1 and CDR-H2 of the VH that binds FZD4 comprises respectively LSSYSM (SEQ ID NO: 24) and YISSYDSITD (SEQ ID NO: 61).
33. The tetravalent binding antibody molecule of any one of claims 28 to 32, wherein in the third and fourth light chain monomers the CDR-L1 and CDR-L2 of the VL that bind FZD4 comprise respectively SVSSA (SEQ ID NO: 1) and SASSLYS (SEQ ID NO: 2), and the CDR-
- 25 L3 of the VL that binds FZD4 comprises WYYAPI (SEQ ID NO: 3) or WYNAPI (SEQ ID NO: 12).

34. The tetravalent binding antibody molecule of any one of claims 28, 29, 30, 32 and 33, comprising a bivalent, bispecific LRP5 binding domain, wherein

(a) in the first heavy chain monomer,

5 the VH that binds LRP-5 comprises CDR-H1 of SEQ ID NO: 528, CDR-H2 of SEQ ID NO: 553 and CDR-H3 of SEQ ID NO: 586, and the VL that binds LRP-5 comprises CDR-L1 of SEQ ID NO: 1, CDR-L2 of SEQ ID NO: 491 and CDR-L3 of SEQ ID NO: 510 of ANT16-Hole of Table 6A, or

10 the VH that binds LRP-5 comprises CDR-H1 of SEQ ID NO: 527, CDR-H2 of SEQ ID NO: 552 and CDR-H3 of SEQ ID NO: 584 and the VL that binds LRP-5 comprises CDR-L1 of SEQ ID NO: 1, CDR-L2 of SEQ ID NO: 2 and CDR-L3 of SEQ ID NO: 130 of ANT18-Hole of Table 6A, or

15 the VH that binds LRP-5 comprises CDR-H1 of SEQ ID NO: 527, CDR-H2 of SEQ ID NO: 552 and CDR-H3 of SEQ ID NO: 584 and the VL that binds LRP-5 comprises CDR-L1 of SEQ ID NO: 1, CDR-L2 of SEQ ID NO: 491 and CDR-L3 of SEQ ID NO: 510 of ANT20-Hole of Table 6A, or

the VH that binds LRP-5 comprises CDR-H1 of SEQ ID NO: 528, CDR-H2 of SEQ ID NO: 553 and CDR-H3 of SEQ ID NO: 586 and the VL that binds LRP-5 comprises CDR-L1 of SEQ ID NO: 1, CDR-L2 of SEQ ID NO: 2 and CDR-L3 of SEQ ID NO: 130 of ANT21-Hole of Table 6A, or

20 the VH that binds LRP-5 comprises CDR-H1 of SEQ ID NO: 527, CDR-H2 of SEQ ID NO: 552 and CDR-H3 of SEQ ID NO: 584 and the VL that binds LRP-5 comprises CDR-L1 of SEQ ID NO: 1, CDR-L2 of SEQ ID NO: 2 and CDR-L3 of SEQ ID NO: 130 of ANT36-Hole of Table 6A, or

25 the VH that binds LRP-5 comprises CDR-H1 of SEQ ID NO: 528, CDR-H2 of SEQ ID NO: 553 and CDR-H3 of SEQ ID NO: 586 and the VL that binds LRP-5 comprises CDR-L1 of SEQ ID NO: 1, CDR-L2 of SEQ ID NO: 491 and CDR-L3 of SEQ ID NO: 510 of ANT39-Hole of Table 6A,

and

the VH that binds FZD4 comprises the FZD4 VH CDRs CDR-H1 of SEQ ID NO: 24, a CDR-H2 of SEQ ID NO: 51 and a CDR-H3 of SEQ ID NO: 79 of ANT16-Hole of Table 6B, or

5 the VH that binds FZD4 comprises the FZD4 VH CDRs CDR-H1 of SEQ ID NO: 24, a CDR-H2 of SEQ ID NO: 51 and a CDR-H3 of SEQ ID NO: 79 of ANT18-Hole of Table 6B, or

10 the VH that binds FZD4 comprises the FZD4 VH CDRs CDR-H1 of SEQ ID NO: 24, a CDR-H2 of SEQ ID NO: 51 and a CDR-H3 of SEQ ID NO: 79 of ANT20-Hole of Table 6B, or

the VH that binds FZD4 comprises the FZD4 VH CDRs CDR-H1 of SEQ ID NO: 24, a CDR-H2 of SEQ ID NO: 51 and a CDR-H3 of SEQ ID NO: 79 of ANT21-Hole of Table 6B, or

15 the VH that binds FZD4 comprises the FZD4 VH CDRs CDR-H1 of SEQ ID NO: 24, a CDR-H2 of SEQ ID NO: 61 and a CDR-H3 of SEQ ID NO: 90 of ANT36-Hole of Table 6B, or

the VH that binds FZD4 comprises the FZD4 VH CDRs CDR-H1 of SEQ ID NO: 24, a CDR-H2 of SEQ ID NO: 61 and a CDR-H3 of SEQ ID NO: 90 of ANT39-Hole of Table 6B,

20 wherein the FZD CDR-L1 and CDR-L2 are respectively SVSSA (SEQ ID NO: 1) and SASSLYS (SEQ ID NO: 2), and

(b) in the second monomer

25 the VH that binds LRP5 comprises CDR-H1 of SEQ ID NO: 536, CDR-H2 of SEQ ID NO: 566 and CDR-H3 of SEQ ID NO: 603 and the VL that binds LRP5 comprises CDR-L1 of SEQ ID NO: 1, CDR-L2 of SEQ ID NO: 2 and CDR-L3 of SEQ ID NO: 493 of ANT16-Knob of Table 6A, or

the VH that binds LRP5 comprises CDR-H1 of SEQ ID NO: 528, CDR-H2 of SEQ ID NO: 553 and CDR-H3 of SEQ ID NO: 585 and the VL that binds LRP5 comprises CDR-L1 of SEQ ID NO: 1, CDR-L2 of SEQ ID NO: 2 and CDR-L3 of SEQ ID NO: 492 of ANT18-Knob of Table 6A, or

5 the VH that binds LRP5 comprises CDR-H1 of SEQ ID NO: 536, CDR-H2 of SEQ ID NO: 566 and CDR-H3 of SEQ ID NO: 603 and the VL that binds LRP5 comprises CDR-L1 of SEQ ID NO: 1, CDR-L2 of SEQ ID NO: 2 and CDR-L3 of SEQ ID NO: 492 of ANT20-Knob of Table 6A, or

10 the VH that binds LRP5 comprises CDR-H1 of SEQ ID NO: 528, CDR-H2 of SEQ ID NO: 553 and CDR-H3 of SEQ ID NO: 585 and the VL that binds LRP5 comprises CDR-L1 of SEQ ID NO: 1, CDR-L2 of SEQ ID NO: 2 and CDR-L3 of SEQ ID NO: 493 of ANT21-Knob of Table 6A, or

15 the VH that binds LRP5 comprises CDR-H1 of SEQ ID NO: 528, CDR-H2 of SEQ ID NO: 553 and CDR-H3 of SEQ ID NO: 585 and the VL that binds LRP5 comprises CDR-L1 of SEQ ID NO: 1, CDR-L2 of SEQ ID NO: 2 and CDR-L3 of SEQ ID NO: 492 of ANT36-Knob of Table 6A, or the VH that binds LRP5 comprises CDR-H1 of SEQ ID NO: 536, CDR-H2 of SEQ ID NO: 566 and CDR-H3 of SEQ ID NO: 603 and the VL that binds LRP5 comprises CDR-L1 of SEQ ID NO: 1, CDR-L2 of SEQ ID NO: 2 and CDR-L3 of SEQ ID NO: 493 of
20 ANT39-Knob of Table 6A,

and the VH that binds FZD4 comprises,

FZD4 Fab CDR-H1 of SEQ ID NO: 24, a CDR-H2 of SEQ ID NO: 51 and a CDR-H3 of SEQ ID NO: 79 of ANT16 Knob of Table 6B, or

25 FZD4 Fab CDR-H1 of SEQ ID NO: 24, a CDR-H2 of SEQ ID NO: 51 and a CDR-H3 of SEQ ID NO: 79 of ANT18 Knob of Table 6B, or

FZD4 Fab CDR-H1 of SEQ ID NO: 24, a CDR-H2 of SEQ ID NO: 51 and a CDR-H3 of SEQ ID NO: 79 of ANT20 Knob of Table 6B, or

FZD4 Fab CDR-H1 of SEQ ID NO: 24, a CDR-H2 of SEQ ID NO: 51 and
a CDR-H3 of SEQ ID NO: 79 of ANT21 Knob of Table 6B, or

FZD4 Fab CDR-H1 of SEQ ID NO: 24, a CDR-H2 of SEQ ID NO: 61 and
a CDR-H3 of SEQ ID NO: 90 of ANT36 Knob of Table 6B, or

5 FZD4 Fab CDR-H1 of SEQ ID NO: 24, a CDR-H2 of SEQ ID NO: 61 and
a CDR-H3 of SEQ ID NO: 90 of ANT39 Knob of Table 6B,

and

(c) in each of the third and fourth monomers

10 the VL comprises the FZD4 Fab CDR-L1, CDR-L2 and CDR-L3 of SEQ ID NO:
3 of ANT16- Knob of Table 6B, or

the VL comprises the FZD4 Fab CDR-L1, CDR-L2 and CDR-L3 of SEQ ID NO:
3 of ANT18- Knob of Table 6B, or

the VL comprises the FZD4 Fab CDR-L1, CDR-L2 and CDR-L3 of SEQ ID NO:
3 of ANT20- Knob of Table 6B, or

15 the VL comprises the FZD4 Fab CDR-L1, CDR-L2 and CDR-L3 of SEQ ID NO:
3 of ANT21- Knob of Table 6B, or

the VL comprises the FZD4 Fab CDR-L1, CDR-L2 and CDR-L3 of SEQ ID NO:
12 of ANT36- Knob of Table 6B, or

20 the VL comprises the FZD4 Fab CDR-L1, CDR-L2 and CDR-L3 of SEQ ID NO:
12 of ANT39- Knob of Table 6B,

wherein the FZD4 Fab CDR-L1 comprises SVSSA (SEQ ID NO: 1) and CDR-L2
comprises SASSLYS (SEQ ID NO: 2).

35. The tetravalent binding antibody molecule of any one of claims 1 to 34 wherein the
tetravalent binding antibody molecule does not comprise a FZD binding domain comprising the

CDRs of the FZD-binding antibody 5044 in combination with the Wnt co-receptor binding domain comprising the CDRs of LRP6-binding antibody 2542 and/or antibody 2539.

36. The tetravalent binding antibody molecule of any one of claims 1 to 35, wherein the tetravalent binding antibody molecule comprises

- 5 (a) a first heavy chain comprising the amino acid sequence of the hole heavy chain construct of SEQ ID NO: 898, a second heavy chain comprising the amino acid a knob heavy chain construct of SEQ ID NO: 897 and a light chain comprising the amino acid sequence of the light chain construct of SEQ ID NO: 899 wherein the amino acid sequence of the CDRs are the amino acid sequence of the CDRs of ANT16; or
- 10 (b) a first heavy chain comprising the amino acid sequence of the hole heavy chain construct of SEQ ID NO: 901, a second heavy chain comprising the amino acid a knob heavy chain construct of SEQ ID NO: 900 and a light chain comprising the amino acid sequence of the light chain construct of SEQ ID NO: 902 wherein the amino acid sequence of the CDRs are the amino acid sequence of the CDRs of ANT 18; or
- 15 (c) a first heavy chain comprising the amino acid sequence of the hole heavy chain construct of SEQ ID NO: 904, a second heavy chain comprising the amino acid a knob heavy chain construct of SEQ ID NO: 903 and a light chain comprising the amino acid sequence of the light chain construct of SEQ ID NO: 902 wherein the amino acid sequence of the CDRs are the amino acid sequence of the CDRs of ANT20; or
- 20 (d) a first heavy chain comprising the amino acid sequence of the hole heavy chain construct of SEQ ID NO: 906, a second heavy chain comprising the amino acid a knob heavy chain construct of SEQ ID NO: 905 and a light chain comprising the amino acid sequence of the light chain construct of SEQ ID NO: 902 wherein the amino acid sequence of the CDRs are the amino acid sequence of the CDRs of ANT21; or
- 25 (e) a first heavy chain comprising the amino acid sequence of the hole heavy chain construct of SEQ ID NO: 908, a second heavy chain comprising the amino acid a knob heavy chain construct of SEQ ID NO: 907 and a light chain comprising the amino acid

sequence of the light chain construct of SEQ ID NO: 909 wherein the amino acid sequence of the CDRs are the amino acid sequence of the CDRs of ANT39; or

- (f) a first heavy chain comprising the amino acid sequence of the hole heavy chain construct selected from the group consisting of SEQ ID NO: 921; SEQ ID NO: 922; SEQ ID NO: 923; SEQ ID NO: 924; SEQ ID NO: 925; SEQ ID NO: 926; SEQ ID NO: 927; and SEQ ID NO: 928; a second heavy chain comprising the amino acid of a knob heavy chain construct selected from the group consisting of SEQ ID NO: 929; SEQ ID NO: 930; SEQ ID NO: 931; SEQ ID NO: 932; SEQ ID NO: 933; SEQ ID NO: 934; SEQ ID NO: 935; and SEQ ID NO: 936; and a light chain comprising the amino acid sequence of the light chain construct selected from the group consisting of SEQ ID NO: 909 and SEQ ID NO: 952.

37. The tetravalent binding antibody molecule of any one of claims 1 to 35, wherein the tetravalent binding antibody molecule comprises

- (a) a first heavy chain comprising the amino acid sequence of the hole heavy chain construct of SEQ ID NO: 921 or SEQ ID NO: 925, a second heavy chain comprising the amino acid a knob heavy chain construct of SEQ ID NO: 929 or SEQ ID NO: 933, and a light chain comprising the amino acid sequence of the light chain construct of SEQ ID NO: 909 or SEQ ID NO: 952; or

- b) a first heavy chain comprising the amino acid sequence of the hole heavy chain construct of SEQ ID NO: 922 or SEQ ID NO: 926, a second heavy chain comprising the amino acid a knob heavy chain construct of SEQ ID NO: 930 or SEQ ID NO: 934, and a light chain comprising the amino acid sequence of the light chain construct of SEQ ID NO: 909 or SEQ ID NO: 952; or

- c) a first heavy chain comprising the amino acid sequence of the hole heavy chain construct of SEQ ID NO: 923 or SEQ ID NO: 927, a second heavy chain comprising the amino acid a knob heavy chain construct of SEQ ID NO: 931 or SEQ ID NO: 935, and a light chain comprising the amino acid sequence of the light chain construct of SEQ ID NO: 909 or SEQ ID NO: 952; or

5 d) a first heavy chain comprising the amino acid sequence of the hole heavy chain construct of SEQ ID NO: 924 or SEQ ID NO: 928, a second heavy chain comprising the amino acid a knob heavy chain construct of SEQ ID NO: 932 or SEQ ID NO: 936, and a light chain comprising the amino acid sequence of the light chain construct of SEQ ID NO: 909 or SEQ ID NO: 952; or

10 e) a first heavy chain comprising the amino acid sequence of the hole heavy chain construct of SEQ ID NO: 908 or SEQ ID NO: 940, a second heavy chain comprising the amino acid a knob heavy chain construct of SEQ ID NO: 944 or SEQ ID NO: 948, and a light chain comprising the amino acid sequence of the light chain construct of SEQ ID NO: 909 or SEQ ID NO: 952; or

15 f) a first heavy chain comprising the amino acid sequence of the hole heavy chain construct of SEQ ID NO: 937 or SEQ ID NO: 941, a second heavy chain comprising the amino acid a knob heavy chain construct of SEQ ID NO: 945 or SEQ ID NO: 949, and a light chain comprising the amino acid sequence of the light chain construct of SEQ ID NO: 909 or SEQ ID NO: 952; or

20 g) a first heavy chain comprising the amino acid sequence of the hole heavy chain construct of SEQ ID NO: 938 or SEQ ID NO: 942, a second heavy chain comprising the amino acid a knob heavy chain construct of SEQ ID NO: 946 or SEQ ID NO: 950, and a light chain comprising the amino acid sequence of the light chain construct of SEQ ID NO: 909 or SEQ ID NO: 952; or

25 h) a first heavy chain comprising the amino acid sequence of the hole heavy chain construct of SEQ ID NO: 939 or SEQ ID NO: 943, a second heavy chain comprising the amino acid a knob heavy chain construct of SEQ ID NO: 947 or SEQ ID NO: 951, and a light chain comprising the amino acid sequence of the light chain construct of SEQ ID NO: 909 or SEQ ID NO: 952.

38. A pharmaceutical composition comprising a tetravalent binding antibody molecule of any one claims 1 to 37 and a pharmaceutically acceptable carrier.

39. A method for promoting endothelial cell barrier functions in a tissue comprising administering an effective amount of a tetravalent binding antibody molecule of any one of claims 1 to 37 to a tissue.
40. The method of claim 39, wherein the tissue is brain, kidney or eye tissue.
- 5 41. The method of claim 40, wherein the tetravalent binding antibody molecule is administered to eye tissue by intravitreal injection.
42. A method for increasing retinal or brain endothelial cell barrier functions, decreasing endothelial cell permeability, enhancing or restoring blood retina and blood brain barrier maintenance in a subject in need thereof comprising contacting an endothelial cell comprising an
- 10 FZD4 receptor and an LRP5 in a subject in need thereof with an effective amount of a tetravalent binding antibody molecule of any one of claim 1 to 37.
43. The method of claim 42, wherein the tetravalent binding antibody molecule is administered to the subject in need thereof by injection, topically, or orally.
44. The method of claim 42, wherein the tetravalent binding antibody molecule is administered
- 15 subcutaneously, intravenously, intraperitoneally, intrathecally, intravitreously, or intraocularly.
45. The tetravalent binding antibody molecule of any one of claims 1 to 37 or the pharmaceutical composition of claim 38 for use as a medicament.
46. The tetravalent binding antibody molecule or pharmaceutical composition of claim 45 for use in the treatment or prevention of a disorder or condition characterized by defective retinal or
- 20 brain angiogenesis and/or characterized by reduced endothelial cell barrier function and/or vascular leakage.
47. A method of treating or preventing a disorder or condition characterized by defective retinal or brain angiogenesis and/or reduced endothelial cell barrier function and/or vascular leakage comprising administering to a person in need thereof a therapeutically effective amount
- 25 of the tetravalent binding antibody molecule of any one of claims 1 to 37 or the pharmaceutical composition of claim 38.

48. Use of the tetravalent binding antibody molecule of claims 1 to 37 or the pharmaceutical composition of claim 38 for the manufacture of a medicament for the treatment or prevention of a disorder or condition characterized by defective retinal or brain angiogenesis and/or reduced endothelial cell barrier function and/or vascular leakage.
- 5 49. The tetravalent binding antibody molecule or pharmaceutical composition for use, method or use of any one of claims 46 to 48 wherein the disorder is selected from diabetic retinopathy, retinopathy of prematurity, Coats' disease, FEVR, Norrie disease, macular degeneration, diabetic macular edema, pediatric vitreoretinopathies, Alzheimer's disease, epilepsy, multiple sclerosis, stroke and ischemia.
- 10 50. The tetravalent binding antibody molecule of any one of claims 1 to 37 or the pharmaceutical composition of claim 37, for use in the treatment or prevention of an ocular disorder e.g. a disorder of the retina or macula e.g. selected from diabetic retinopathy, retinopathy of prematurity, Coats' disease, FEVR, Norrie disease, macular degeneration, diabetic macular edema and pediatric vitreoretinopathies or in the treatment or prevention of a disorder
15 selected from Alzheimer's disease, epilepsy, multiple sclerosis, stroke and ischemia.
51. A method of treating or preventing an ocular disorder e.g. a disorder of the retina or macula e.g. selected from diabetic retinopathy, retinopathy of prematurity, Coats' disease, FEVR, Norrie disease, macular degeneration, diabetic macular edema and pediatric vitreoretinopathies or in the treatment or prevention of a disorder selected from Alzheimer's
20 disease, epilepsy, multiple sclerosis, stroke and ischemia comprising administering to a person in need thereof a therapeutically effective amount of the tetravalent binding antibody molecule of any one of claims 1 to 37 or the pharmaceutical composition of claim 38.
52. Use of the tetravalent binding antibody molecule of claims 1 to 36 or the pharmaceutical composition of claim 38 for the manufacture of a medicament for the treatment or prevention of
25 an ocular disorder e.g. a disorder of the retina or macula e.g. selected from diabetic retinopathy, retinopathy of prematurity, Coats' disease, FEVR, Norrie disease, macular degeneration, diabetic macular edema and pediatric vitreoretinopathies or in the treatment or prevention of a disorder selected from Alzheimer's disease, epilepsy, multiple sclerosis, stroke and ischemia.