

MULTIVALENT BINDING MOLECULES ACTIVATING WNT SIGNALING AND USES THEREOF

This Application claims the benefit under 35 U.S.C. 119(e) of U.S. Provisional application no. 62/630,772 filed February 14, 2018, the entirety of which is incorporated herein by reference.

The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on February 12, 2019, is named 115773_PA895WO_SL.txt and is 220,360 bytes in size.

BACKGROUND

[0001] Wnt signaling pathways are critical for embryonic development and tissue homeostasis in adults. Wnt ligands are secreted growth factors that regulate various cellular processes such as proliferation, differentiation, survival and migration. Wnt ligands are universally important for the control of tissue stem cells self-renewal and regulation of many progenitor cell populations. The hydrophobicity and sensitive tertiary structure of Wnt proteins have rendered their biochemical purification challenging and their use *in vitro* and *in vivo* impracticable.

[0002] 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 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) pathway, the canonical Wnt/ β -catenin pathway, and the Wnt/calcium pathway. Activation of Wnt signaling pathways also require the presence of Wnt co-receptors to dictate the differential engagement of intracellular signaling cascades regulating the expression of genes effecting cellular machineries underlying the cellular processes 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 orphan receptors 1 and 2 (ROR1/2), related to receptor tyrosine kinase (RYK) or protein tyrosine kinase 7 (PTK7) co-

receptor to initiate the Wnt/PCP pathway or alternate β catenin-independent signaling pathways. The Wnt/ β -catenin pathway, sometimes referred to as the canonical pathway, culminates in the post-translational accumulation of the transcriptional effector β -catenin that interacts with T-cell factor/lymphoid enhancer factor (LEF/TCF) family of transcription factors to regulate the expression of context-specific genes.

SUMMARY OF THE INVENTION

[0003] Wnts require lipidation for function (Janda et al., *Science*. 337 , 59–64 (2012); Kadowaki et al., *Genes Dev.* 10 , 3116–3128 (1996)) and their hydrophobic nature complicates biochemical manipulation; consequently, only a few Wnts have been purified (Willert et al., *Nature* 423, 448–452 (2003). Furthermore, Wnts are inherently cross-reactive for multiple receptors, especially when overexpressed or applied at high dose (He et al. *Science*. 275 , 1652–1654 (1997); Andres et al. Systematic mapping of Wnt-Frizzled interactions reveals functional selectivity by distinct Wnt-Frizzled pairs. *Journal of Biological* (2015) (available at <http://www.jbc.org/content/early/2015/01/20/jbc.M114.12648.short>); Holmen et al., *J. Biol. Chem.* 277 , 34727–34735 (2002).). As a result, it has been impossible to activate Frizzled receptor complexes selectively to determine the specific functions of each in different contexts or to evaluate their therapeutic potential for degenerative conditions. The multivalent binding molecules and methods described herein activate preselected Frizzled receptor-coreceptor complexes selectively. Administration of the multivalent binding molecules described herein are contemplated to treat degenerative conditions by activating the appropriate Frizzled co-receptor complexes.

[0004] Described herein are methods to affect binding by a peptide to a FZD receptor and a Wnt co-receptor on a cell wherein binding by the peptide to both FZD receptor and the co-receptor activates a Wnt signaling pathway.

[0005] Also described herein are multivalent binding molecules that activate a Wnt signaling pathway and methods for their use. The multivalent binding molecules bind to both an FZD receptor and a Wnt co-receptor thereby activating a Wnt signaling pathway. The multivalent binding molecules of this invention are also referred herein as “FZD agonists” or “FZDag”. In a particular embodiment wherein the molecules of this invention bind a FZD and a LRP5/6 the molecules maybe referred to as “Frizzled and LRP5/6 Agonists” or “FLAgs”. The multivalent binding molecules comprise an Fc domain, or fragment thereof comprising the CH3 domain, and a first binding domain that binds a FZD receptor and a second binding domain that binds a Wnt co-receptor 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 co-receptor are not directly linked rather they are separated by the Fc domain, or fragment thereof comprising the CH3 domain. This configuration of binding domains produces an unexpectedly high level of Wnt signaling pathway activation. The FZD binding domain may be monovalent, having a single binding site (paratope) for a FZD receptor, or may be multivalent having more than one binding site for a FZD receptor, e.g., the binding domain may be bivalent, trivalent or tetravalent. The Wnt co-receptor binding domain may be monovalent, having a single binding site (paratope) for a Wnt co-receptor, or may be multivalent having more than one binding site for a Wnt co-receptor, e.g., the binding domain may be bivalent, trivalent or tetravalent.

[0006] The methods described herein for producing the multivalent binding molecules enable selective and robust activation of any FZD receptor complex *in vitro* and *in vivo*. Leveraging a panel of hundreds of synthetic antibodies targeting FZDs and their co-receptors, we generated multivalent binding molecules for selective and rational activation of one, two or multiple FZD receptors. The multivalent binding molecules of this invention are highly stable, amenable to large-scale production and facile purification, have predictable pharmacokinetics, and are contemplated to exhibit low immunogenicity.

[0007] In an embodiment of this invention the binding domains of the multivalent binding molecules described herein bind to one or more FZD receptors and an LRP, e.g., LRP 5 and/or LRP6 and are alternatively referred to herein as FLAgs. FLAgs that target particular FZDs and their LRP co-receptors will improve directed differentiation and cell therapy, sustain tissue organoid growth, and mobilize endogenous stem cells *in vivo* and promote tissue repair after injury and restore function following tissue degeneration.

[0008] The Fc domain of the FZD agonists may be an Fc domain of an immunoglobulin. The immunoglobulin may be an IgG, e.g., an IgG₁. In an embodiment of this invention the multivalent binding molecule is a peptide dimer wherein the peptides are dimerized via the intrinsic ability of Fc domain to dimerize or via a knob-in-holes configuration within the Fc which allows for specific assembly of two different peptides to produce multivalent binding domains. Methods for dimerizing peptides via a knob-in-hole configuration are described in WO2018/026942, inventors Van Dyk et al. incorporated herein by reference,

[0009] One or both of the multivalent binding domains of the FZD agonists described herein may be bivalent and monospecific, having two binding sites for the same epitope of

their respective receptor or co-receptor targets. One or both of binding domains may be bivalent and bispecific having two binding sites with each site binding a different epitope on their respective targets.

[0010] In an embodiment of this invention the FZD binding domain may comprise two single chain variable fragments (scFv) for binding to the same or different epitopes on the FZD receptor. In other embodiments of this invention the FZD binding domain comprises one or more heavy-chain variable domain (VH) fragments and/or one or more light-chain variable domain (VL) fragments that bind the FZD. In other embodiments of this invention the FZD binding domain consists of one or more single-domain antibody fragments that bind to FZD. In other embodiments of this invention the FZD binding domain comprises a FZD ligand or fragment thereof that binds the FZD receptor. In an embodiment of this invention the FZD binding domain comprises a synthetic peptide that binds the FZD, e.g., an affibody, an ankyrin repeat protein, a fibronectin repeat protein, a fynomer, or an anticalin. In an embodiment of this invention the FZD multivalent binding domain does not comprise scFv. The FZD ligand may be, e.g., a fragment of Wnt protein or of Norrin that binds the FZD receptor, or another natural or synthetic peptide that is affinity matured to interact with one or more FZD receptors. Norrin is a FZD4-specific ligand that, in complex with LRP5 and/or LRP6, is associated with activation of canonical Wnt signaling.

[0011] In an embodiment of this invention, the co-receptor binding domain may comprise two single chain variable fragments (scFv) for binding to the same or different epitopes on the co-receptor. In other embodiments of this invention the Wnt co-receptor binding domain comprises one or more heavy-chain variable domain (VH) fragments and/or one or more light-chain variable domain (VL) fragments that bind the Wnt co-receptor. In other embodiments of this invention the co-receptor binding domain consists of one or more single-domain antibody fragments that bind to the co-receptor. In an embodiment of this invention the Wnt co-receptor binding domain comprises a peptide that binds the Wnt co-receptor wherein the peptide is a fragment of a naturally occurring ligand that binds the Wnt co-receptor or is a synthetic peptide that binds the Wnt co-receptor, e.g., an affibody, an ankyrin repeat protein, a fibronectin repeat protein, a fynomer, or an anticalin. In another embodiment of this invention the co-receptor binding domain comprises a co-receptor ligand or fragment thereof that binds the co-receptor (for example the ligand Dkk1 for the co-receptor LRP5/6) or another natural or synthetic peptide affinity matured to interact with one or more co-receptors.

[0012] In an embodiment of this invention the co-receptor multivalent binding domains do not comprise scFv.

[0013] In an embodiment of this invention each binding domain of the molecules described herein may be formed by two peptides each peptide comprising a heavy-chain variable domain (VH) linked to a light-chain variable domain (VL) wherein the VH and the VL from one peptide pair with the VL and VH of the other peptide forming a diabody. In this configuration, the binding domain has two binding sites that bind to its target, i.e., the FZD binding domain has two binding sites for the FZD receptor and the co-receptor binding domain has two binding sites for the co-receptor. Using a knobs-in-holes Fc configuration, the peptides comprising the VH and VL can be engineered such that they are non-identical but still pair to form a bispecific binding domain capable of binding to two different sites on the FZD receptor or co-receptor (see Figure 3A).

[0014] In an embodiment of this invention one or both of the multivalent binding domains comprise two peptides forming a diabody on each terminus of the Fc domain. Each diabody has two binding sites for an epitope on their respective FZD receptor or co-receptor targets. The diabody may be monospecific wherein the binding sites bind the same epitope on the FZD receptor or co-receptor, or the diabody may be bispecific binding to two different epitopes on the FZD receptor or co-receptor.

[0015] The peptides forming the scFv or diabolies may be derived from an antibody that binds to a FZD receptor or from an antibody that binds to a Wnt co-receptor. For the FZD binding domain, the antibody may be an antibody that binds to one or more FZD receptors and antagonizes Wnt signaling or inhibits Wnt binding to given FZD receptor(s), or the antibody may be an antibody that binds to one or more FZD receptors without inhibiting Wnt binding to the FZD receptor. For the co-receptor binding domain, the antibody may be an antibody that binds to the co-receptor and antagonizes Wnt signaling or inhibits Wnt binding to the co-receptor or the antibody may be an antibody that binds to a co-receptor without inhibiting Wnt binding to the co-receptor.

[0016] The FZD binding domain may bind to one or more 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 4 (FZD4), Frizzled Class Receptor 5 (FZD5), Frizzled Class Receptor 6 (FZD6), Frizzled Class Receptor & (FZD7), Frizzled Class Receptor 1 Frizzled Class Receptor 8 (FZD8), Frizzled Class Receptor 9 (FZD9), or Frizzled Class Receptor 10 (FZD10). The co-receptor binding domain may bind to any Wnt

co-receptor, e.g., LRP5/6, PTK7, ROR1/2, RYK, GPR124, TSPAN12, or CD133. In an embodiment of this invention the co-receptor binding domain binds to LRP5 and/or LRP6. In an embodiment of this invention the co-receptor binding domain binds to a single epitope on a co-receptor, e.g., an epitope of the LRP protein that binds Wnt1 or Wnt3a. In an embodiment of this invention the co-receptor binding domain binds to two epitopes on a co-receptor, e.g., an epitope on an LRP that binds to Wnt1 and an epitope that binds to Wnt3a.

[0017] An embodiment of this invention includes methods for producing induced pluripotent stem (iPS) cells comprising culturing a somatic cell under conditions suitable for reprogramming the somatic cells in the presence of an effective amount of a multivalent binding molecule described herein. The multivalent binding molecule may be included in an amount to accelerate the generation of iPS cells as compared to the generation of iPS cells in the same culture conditions without the multivalent binding molecule.

[0018] 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 presence of an effective amount of a multivalent binding molecule described herein.

[0019] An embodiment of this invention includes methods for generating tissue organoids comprising culturing a tissue sample under conditions suitable for the generation of organoids in the presence of an effective amount of a multivalent binding molecule described herein as part of the culture cocktail. In an embodiment of this invention, the frequency of generating tissue organoids cultured in a medium comprising the multivalent binding molecule is enhanced as compared to organoids cultured in the same medium without the multivalent binding molecule. In an embodiment of this invention, the tissue organoids are generated more rapidly when cultured in a medium comprising the multivalent binding molecules as compared to tissue samples cultured in the same medium without the multivalent binding molecules.

An embodiment of this invention includes methods for enhancing the maintenance of tissue organoids comprising culturing an organoid in the presence of an effective amount of a multivalent binding molecule described herein as part of the culture cocktail. As described herein, the survival of tissue organoids cultured in a medium comprising the multivalent binding molecules is prolonged as compared to organoids cultured in the same medium without the multivalent binding molecule.

[0020] An aspect of this invention is a method for making the multivalent binding molecules described herein. In an embodiment of this invention the multivalent binding

molecule is generated by,

- a) selecting an Fc domain having a C-terminus and an N-terminus,
- b) identifying an antibody that binds to one or more FZD receptors and
- c) identifying an antibody that binds to one or more Wnt co-receptors,
- d) generating a nucleic acid molecule comprising a nucleotide sequence that encodes (i) the Fc domain of step a, (ii) a nucleotide sequence that encodes a VL and/or a VH of the antibody of step b, or a VL and/or a VH derived from the antibody of step b that binds the one or more FZD , and (iii) a nucleotide sequence that encodes a VL and a VH of the antibody of step c, or a VL and a VH derived from the antibody of step c that binds to the one or more Wnt receptors of step c,
- e) expressing the nucleic acid molecule of (d) to produce a polypeptide wherein the polypeptide dimerizes to form a multivalent binding molecule comprising an Fc domain, a FZD binding domain and a Wnt co-receptor binding domain wherein, the FZD binding domain comprises of the VL and VH of the antibody of step b or derived from the antibody of step b and is linked to one terminus of the Fc domain, and the Wnt co-receptor binding domain comprises the VL and VH of the antibody of step c or derived from the antibody of step c and is linked to the other terminus of the Fc domain thereby forming the multi-specific binding molecule.

The antibody in step (b) may be an antibody or antibody fragment that binds to one or more FZD receptors and antagonizes Wnt signaling or inhibits Wnt binding to the receptor. The antibody in step (b) may be an antibody or antibody fragment that binds to one or more FZD receptors without antagonizing Wnt signaling or inhibiting Wnt binding to the receptor. The antibody in step (c) may be an antibody or antibody fragment that binds to one or more of the Wnt co-receptors and antagonizes Wnt signaling or inhibits Wnt binding to the co-receptor, or binds to the co-receptor without antagonizing Wnt signaling or inhibiting Wnt binding to the co-receptor. The binding domains may be linked to the Fc domain via a linker. The modular aspects of this invention allows for mixing and matching binding domains of antibodies for any given FZD receptor and co-receptor on the termini of the Fc domain to generate a multivalent binding molecule that can engage multiple Frizzled receptor – co-

receptor complexes or to selectively engage a single Frizzled receptor-co-receptor complex to activate Wnt signaling.

[0021] The multivalent binding molecule comprises a peptide dimer configured to have an Fc domain and a binding domain that binds one or more FZD receptors and a second binding domain that binds one or more Wnt co-receptors wherein the FZD binding domain is linked to one terminus of the Fc and the co-receptor binding domain is linked to the other terminus of the Fc. Each binding domain may be monovalent or multivalent, e.g. bivalent, trivalent or tetravalent.

[0022] Also an embodiment of this invention are methods using the multivalent binding molecules, e.g., for producing induced pluripotent stem (iPS) cells, for directed differentiation of pluripotent stem cells, and for generating and/or maintaining tissue organoids, or to enhance tissue regeneration in a subject in need thereof.

[0023] Additional embodiments of this invention are methods for activating Wnt signaling pathways for the mobilization of endogenous stem/progenitor cell pools for regenerative medicine and for disorders or diseases associated with insufficient Wnt signaling.

BRIEF DESCRIPTION OF THE FIGURES

[0024] Figure 1A depicts the binding specificity of five antibodies selected for their binding to the extracellular domain (ECD) of human LRP6. LRP6-binding antibodies were selected from a synthetic antibody library by selecting for antibodies that bound the recombinant extracellular domain (ECD) of human LRP6. The antibodies were assayed by ELISA for binding to human LRP6, mouse LRP6, and mouse LRP5. Binding to an Fc peptide and bovine serum albumin (BSA) were included as negative controls.

[0025] Figure 1B depicts the results of a luciferase reporter assay monitoring Wnt signaling activation demonstrating that IgG 2539 and IgG 2542 (100 μ M) bind different sites on LRP6 ECD by their opposite effects on Wnt1 (Transient transfection) and Wnt3a (0.5 μ g/ml purified protein) stimulation. Anti-MBP antibody acts as control.

[0026] Figure 2A depicts a representative bispecific IgGs (Bi-IgG) and bispecific diabody (bi-diabody) comprising of a FZD binding domain (5019) and an LRP6-W1 (2942, L6¹) or -W3 (2539, L6³) binding domains on the same end of the Fc domain.

[0027] Figure 2B demonstrates that the bispecific IgGs (5019-2539 Bi-IgG and 5019-2542 Bi-IgG) do not activate Wnt signaling but rather act as antagonists of Wnt signaling, as

determined in a TOPFlash luciferase reporter assay in HEK293 cells.

[0028] Figure 2C -2G depict the binding of bispecific diabodies wherein the Fc domains are in a knob/hole configuration (K/H). Two resultant diabodies 5019-2539-K/H (FZD/LRP6-W3) and 5019-2542-K/H (FZD/LRP6-W1) retain the FZD binding profile of the original IgG as well as the LRP6 binding activity though very weak. FIG. 2C depicts the purified FZD-LRP6 diabodies: 5019-2539-K/H and 5019-2542-K/H. FIG. 2D depicts the FZD receptor binding profile of the 5019-diabody to FZD4, FZD5, and FZD7. The 5019 FZD IgG was previously characterized to bind to FZD1, 2, 4, 5, 7, 8. FIG. 2E depicts the FZD receptor binding profile of the bi-specific FZD/LRP6 diabody 5019-2539- K/H. FIG. 2F depicts the FZD receptor binding profile of the bi-specific FZD/LRP6 diabody 5019-2542-K/H. FIG. 2G demonstrates that the homo (2539-Fc and 2542-Fc) and hetero-diabodies (5019-2539-Fc and 5019-2542-Fc) having the binding domains on one terminus of the Fc domain interact with the LRP6 extra-cellular domain. Figure 2H demonstrates co-binding of the diabodies 5019-2539-K/H and 5019-2542-K/H to FZD CRD and LRP6 ECD in solution as determined in Bio-Layer Interferometry (BLI) assays.

[0029] Figure 2I demonstrates neither 5019-2539-K/H or 5019-2542-K/H, wherein the FZD and LRP6 receptors diabodies forming the binding domains are present on the same side of the Fc, are FZD agonists that activate a Wnt mediated pathways. The results demonstrate the 5019-2539-K/H diabody (selective for the Wnt3 site on LRP6) completely blocks the Wnt3-mediated pathway activation at 10nM and 50nM whereas the 5019-2542-K/H is less effective as revealed using the TOPFlash luciferase reporter assay in HEK293 cells.

[0030] Figure 2J depicts a comparison of the luciferase activity of a tetravalent binding molecule having binding domains comprising diabodies or scFvs. The molecules having binding domains comprising anti-FZD scFvs and anti-LRP diabodies ($F^{P*+P*}-L6^{1+3}$) exhibited similar activity to the molecules having binding domains comprising anti-FZD diabody and anti-LRP diabodies ($F^{P+P}-L6^{1+3}$). In contrast, as compared to the $F^{P+P}-L6^{1+3}$ molecules activity was significantly reduced for the molecules that contained anti-FZD diabodies but anti-LRP6 scFvs ($F^{P+P}-L6^{1*+3*}$) or scFvs at both ends ($F^{P*+P*}-L6^{1*+3*}$).

[0031] Figure 2K and Figure 2L demonstrate the differences in activity between a tetravalent binding molecule having binding domains comprising diabodies or scFvs were not due to differences in affinity, as BLI measurements showed comparable, high-affinity binding to LRP6 and FZD isoforms regardless of whether paratopes were presented in the diabody or scFv format.

[0032] Figure 3A a schematic representation of a tetravalent binding molecule wherein two FZD binding domains comprised of homo (recognizing the same epitope) or hetero (recognizing separate epitopes) diabodies are linked to one end of an Fc domain and two LRP6 binding domains comprised of homo or hetero diabodies are linked to the other end of the Fc domain.

[0033] Figure 3B depicts binding by the multivalent binding molecule 5019-Fc-2539 (F^{P+P} -L6³⁺³), and 5019-Fc-2542 (F^{P+P} -L6¹⁺¹), to FZD4, FZD5 and FZD7 ECDs. Binding to FZD receptors is detected using BLI assays.

[0034] Figure 3C depicts activation of the Wnt- β catenin signaling pathway by tetravalent binding molecules 5019-Fc-2539 (F^{P+P} -L6³⁺³), 5019-Fc-2542 (F^{P+P} -L6¹⁺¹), 5019-K/H-2539-2542 (F^{P+P} -L6¹⁺³), and purified Wnt3A (0.5 μ g/ml). The concentration of the molecules is indicated. The tetravalent binding molecules are agonists that robustly activate the Wnt- β catenin pathway in HEK293T cells as measured using the pBAR luciferase reporter assay. The 5019-Fc-2539 homodiabody binds to multiple FZD receptors (5019: FZD1, 2, 4, 5, 7, 8) and to the Wnt3a site on LRP6 (2539) and activates the reporter to levels comparable to purified Wnt ligands. The 5019-K/H-2539:2542 heterodiabody, which binds to both Wnt binding sites on LRP6 is more effective.

[0035] Figure 3D depicts Wnt- β catenin pathway activation by multivalent binding molecules having a FZD homodiabody (5019) linked through the Fc to either monospecific LRP6 homodiabody (5019-Fc-2539, 5019-Fc-2542) or bispecific LRP6 heterodiabody (5019-K/H-2539-2542, also known as 5019Ag or F^{P+P} -L6¹⁺³).

[0036] Figure 3E depicts the activation of Wnt- β catenin signaling by molecules comprising a monovalent binding domain for either the FZD receptor or the LRP6 co-receptor. Wnt- β catenin pathway activation was detected using pBAR luciferase reporter assays performed in HEK293T cells. 5019-MBP-K/H-2539-2542 contains one monovalent binding domain for FZD and still activates the Wnt pathway, but showing an 8-fold decrease in efficacy with respect to 5019Ag (which contains two FZD binding domains to the same epitope). 5019-K/H-2539-MBP, which retains only one LRP6-W3 binding domain in the C-terminus, exhibits much less efficacy. Importantly, minimal agonistic activity was detected for the two mono-FZD:mono-LRP6 diabodies 5019-MBP-K/H-2539-MBP and 5019-MBP-K/H-MBP-2542 as well as the one LRP6-W1 site diabody 5019-K/H-MBP-2542.

[0037] Figure 3F depicts Wnt- β catenin pathway activation by a tetravalent binding molecule in which an anti-LRP5 paratope targeting the WNT3A binding site was substituted

for the anti-LRP6 paratope targeting the WNT1 binding site to generate a molecule (F^{P+P} -L5/6³) that could recruit both co-receptors and observed activity similar to that of F^{P+P} -L6¹⁺³ ($EC_{50} = 4$ nM).

[0038] Figure 4A depicts Wnt- β catenin pathway activation in reporter cells without an endogenous FZD4 receptor (-FZD4) or modified to express the FZD4 receptor (+FZD4) by a multivalent binding molecules having FZD binding domains (homodiabodies in this case) specific for FZD4 on one side of the Fc domain and a co-receptor binding domain for LRP6 (2539 and 2542) on the other side of the Fc domain (FZD4Ag: 5038Ag/5038-K/H-2539-2542, 5044Ag/5044-K/H-2539-2542, 5048Ag/5048-K/H-2539-2542, 5063Ag/5063-K/H-2539-2542, 5080Ag/5080-K/H-2539-2542, 5081Ag/5081-K/H-2539-2542). Controls are the multivalent binding molecule 5019Ag (5019-K/H-2539-2542), and Norrin, an endogenous agonist of FZD4. The results demonstrate that replacing the 5019 FZD binding domain (recognizing FZD1, 2, 4, 5, 7, 8) within the 5019Ag/5019-K/H-2539:2542 (a pan-FZD agonist) with selective binding domains for FZD4, enabled the development of selective FZD4 agonists. HEK293T cells were transfected with pBARL (Wnt- β catenin luciferase reporter) and Rluc (normalization control), plasmids coding for the listed FZD agonists and with or without FZD4 and LRP6 cDNA. Norrin was used as a positive control for activation of FZD4. HEK293T cells express low to not detectable levels of FZD4 therefore FZD4 agonists were only able to activate the reporter gene in the presence of transfected FZD4 cDNA. In contrast, the pan-FZDag 5019-K/H-2539:2542 robustly activates Wnt- β catenin signaling in the absence or presence of FZD4 through activation of other endogenously expressed Frizzled in these cells.

[0039] Figure 4B depicts Wnt- β catenin pathway activation by multivalent binding molecules having binding domains (homodiabodies) specific for FZD2 (2876, 2890), FZD2/7 (2886) FZD6 (2747), or FZD9/10 (2969, 2974) on one side of the Fc and the LRP6 heterodiabody formed by 2539 and 2542 antibody fragments on the other side of the Fc. Wnt- β catenin pathway activation was evaluated using the pBARL assay in HEK293T cells.

[0040] Figure 4C depicts Wnt pathway activation by multivalent binding molecules having FZD binding domains that are pan-specific for FZD and derived from IgG that block Wnt binding to FZD and Wnt- β catenin signaling. The LRP6 binding domains in these molecules are on the c-terminus of the Fc and consist of a diabody formed by antibody 2539 and 2542, which have paratopes recognizing the Wnt3 and Wnt1 binding sites on LRP6 respectively.

[0041] Figure 4D depicts Wnt pathway activation by multivalent binding molecules having FZD binding domains that are pan-specific for FZD and derived from IgG that do not block Wnt binding to FZD and do not antagonize Wnt3-induced pathway activation. The LRP6 binding domains in these molecules are on the c-terminus of the Fc and consists of a diabody formed by antibody 2539 and 2542, which have paratopes recognizing the Wnt3 and Wnt1 binding sites on LRP6 respectively.

[0042] Figure 5 depicts a comparison of the FZD/LRP6 binding behavior of three tetravalent binding molecules of this invention. 5019-Fc-2539, 5019-Fc-2542, 5019-Fc-2539-2542 bind tightly to FZD but exhibit weaker LRP6 interaction (left graph) or FZD/LRP6 co-binding (middle graph). The FZD binding profile of 5019-K/H-2539-2542 (right graph) shows it recognizes FZD4, FZD5 and FZD7.

[0043] Figure 6A is an illustration of the top two propellers (E1-E2) of LRP5/6 known to mediate binding with Wnt1, and binding of the bottom 2 propellers (E3-E4) of LRP5/6 that are proximal to the plasma membrane and known to mediate interaction with Wnt3. Figure 6A also illustrates Wnt1 interacting with LRP5/6 and the FZD receptor and Wnt3 interacting with LRP5/6 and the FZD receptor.

[0044] Figure 6B is an illustration of a possible interaction of the FZD receptor and LRP5/6 receptor by the multivalent binding molecules 5019-Fc-2539, 5019-Fc-2542 and 5019-K/H-2539-2542.

[0045] Figure 6C demonstrates the multivalent binding molecules are agonists that robustly activate the Wnt- β catenin pathway in HEK293T cells as measured using the pBAR luciferase reporter assay. 5019-Fc-2539 homodiabody binds to multiple FZD receptors (5019 binds FZD1, 2, 4, 5, 7, 8) and to the Wnt3a site on LRP6 (2539) and activates the reporter to levels comparable to purified Wnt ligands. The 5019-K/H-2539:2542 heterodiabody, which binds to both Wnt3a and Wnt1 binding sites on LRP6, is more effective.

[0046] Figure 6D demonstrates 5019-K/H-2459:2460, a tetravalent binding molecule having an Fc domain in a knob-in-hole configuration and having a FZD binding domain (homodiabody) that is pan FZD-specific (5019) and a co-receptor binding domain that is bispecific (heterodiabody) for two sites on LRP5 (2459 binds Wnt1 binding site and 2460 binds Wnt3 binding site), also activates the Wnt- β catenin pathway in HEK293T cells.

[0047] Figure 7A demonstrates that by replacing the FZD binding domain within the 5019-K/H-2539:2542 (a pan-FZD agonist recognizing FZD1, 2, 4, 5, 7, 8) with a FZD binding domain specific for FZD5 (#2928), a selective FZD5 agonist was generated. HPAF-II

cells have been shown to depend on FZD5 signaling for their proliferation. Blocking Wnt-FZD5 signaling using the Wnt secretion inhibitor LGK974 (targeting the acyl-transferase Porcupine) leads to cell cycle arrest and inhibition of proliferation. Proliferation can be rescued with addition of exogenous Wnt3a conditioned media or with the addition of the FZD5 selective agonists (2928-K/H- 2539:2542) or pan-FZD agonist (5019-K/H-2539:2542) described herein. The FZD4 selective agonist 5038-K/H- 2539:2542 only has modest rescue ability.

[0048] Figure 7B demonstrates stimulation of C3H10T1/2 cells with a FZD2-specific FLaG led to robust induction of the osteogenic marker alkaline phosphatase (*ALPL*) to levels similar to those achieved with a Pan-FZD FLaG, whereas a FZD5-specific FLaG exhibited minimal activity.

[0049] Figure 8A and 8B demonstrates that the pan-FZDag (F^{P+P} -L6¹⁺³) of this invention fully substitute for exogenous Wnt3A conditioned media to rescue the growth inhibition of intestinal organoids when Wnt secretion is blocked with LGK974, a small molecule inhibitor of Porcupine (lower left photograph). Intestinal organoids isolated from mice grow in the presence of recombinant R-Spondin and require the presence of Wnt ligands secreted by the paneth cells. Figure 8A depicts inhibition of Wnt production using LGK974 leads to organoid death (upper right photograph). Exogenous application of Wnt3A conditioned media (lower right photograph) or FZDag (lower left photograph) rescues organoid growth in the presence of LGK974. The upper left photograph depicts organoids treated with DMSO without LGK974 as control. Figure 8B demonstrates that inhibition of Wnt production by LGK974, leading to organoid death, can be rescued by application of Wnt3A conditioned media or FZDag (F^{P+P} -L6¹⁺³), as quantified using CellTiter Glow® Assay, Promega.

[0050] Figure 9A and 9B depict an example of the plasmids encoding the peptides that dimerize in a knob-into-hole conformation to form the pan-FZDag 5019-KH-2539-2542(F^{P+P} -L6¹⁺³). Figure 9A depicts a plasmid encoding the peptide comprising an Fc region comprising a “knob” mutation, the VH and VL of panFZD antibody #5019, and the VL of LRP antibody #2542 and the VH of LRP antibody #2539. Figure 9B depicts a plasmid encoding the peptide comprising a nucleic acid encoding Fc region comprising a “hole” mutation, the VH and VL of pan-FZD antibody #5019, and the VH of LRP antibody #2542 and the VL of LRP antibody #2539. The peptides encoded by these plasmids form a heterodimer having tetravalent binding domains comprising a homo-diabody produced by pairing of the VH and VL of the pan specific FZD antibody #5019 and a bispecific

heterodiabody produced by the pairing of VL of LRP6 antibody #2539 and VH of LRP antibody #2542 from one peptide with the VH of LRP antibody #2539 and the VL of LRP antibody #2542 of the other peptide.

[0051] Figure 9C is a schematic representation of the heterodimer knob-into-hole configuration 5019-K/H-2539:2542 (F^{P+P} -L6¹⁺³). Using a knob-in-hole configuration within the Fc it is possible to increase the modularity of the molecule up to 4 different binding sites. For this molecule (5019-K/H-2539:2542) a pan-FZD homodiabody is engineered on one side of the Fc domain and an heterodiabody containing Wnt3 (2539) and Wnt1 (2542) LRP6 binding sites on the other side of the Fc domain

[0052] Figure 10A and 10B is an annotation of the domains of the nucleic acid sequence of the 5019-knob-2539:2542 multivalent binding molecule (SEQ ID NO: 21 plus an additional 3' TGA, and its complementary sequence).

[0053] Figure 11 A-F depict the design and validation of tetravalent binding molecules that bind FZD and LRP6 Wnt1 and Wnt3 binding sites (FLAgS) as activators of the Wnt- β catenin pathway. FIG. 11A depicts anti-FZD Fab inhibitory (*top*) and specific activity (*bottom*). FIG. 11B depicts inhibition of Wnt1 or Wnt3A signaling by the indicated LRP6 Abs in the diabody-Fc format. FIG. 11C depicts molecular architecture of tetravalent FLAgS. FIG. 11D shows dose response curves for the activation of a LEF/TCF reporter gene (*y-axis*) in HEK293T cells by serial dilutions of pan-specific FLAg proteins (F^{P+P} -L6¹⁺¹, F^{P+P} -L6³⁺³ and F^{P+P} -L6¹⁺³) (*x-axis*). FIG. 11E depicts the levels of β catenin protein in RKO cells after 30 min treatment with indicated concentrations of pan-FLAg (F^{P+P} -L6¹⁺³). FIG. 11F depicts the time course of β catenin and phosphorylated Dishevelled-2 (p-Dvl2) protein levels in RKO cells treated with 10 nM pan-FLAg (F^{P+P} -L6¹⁺³).

[0054] Figure 12A-Figure 12D depict the characterization and dissection of the FLAg F^{P+P} -L6¹⁺³ binding and activity. FIG. 12A and 12B depicts binding kinetics of F^{P+P} -L6¹⁺³ to nine of 10 human FZD CRDs to human LRP6 ECD. FIG. 12C demonstrates F^{P+P} -L6¹⁺³ behaved similarly to a conventional IgG and interacted with FcRn in a dose and pH dependent manner. FIG. 12D demonstrates F^{P+P} -L6¹⁺³ also behaved similarly to the IgG for interaction with other Fc effectors including complement (C1q), the natural killer cell marker CD16a, the B cell marker CD32a, and the monocyte and macrophage marker CD64.

[0055] Figures 13A and 13B demonstrate that treatment of with 30 nM F^{P+P} -L6¹⁺³ for three days caused robust induction of the mesoderm marker BRACHYURY and decreased expression of the pluripotency marker OCT4 to levels comparable to treatment with the

GSK3 inhibitor CHIR99021 at 6 μ M.

[0056] Figure 14 displays representative fluorescence images of small intestinal sections from *LGR5*-GFP mice treated with vehicle, C59 or pan-FLAG(F^{P+P} -L6¹⁺³) + C59. *LGR5*-GFP is expressed in the stem cells at the bottom of crypts. Cell nuclei were counterstained with DAPI.

DETAILED DESCRIPTION OF THE INVENTION

[0057] Described herein are multivalent binding molecules comprising an Fc domain, a FZD binding domain and a binding domain for a Wnt co-receptor wherein the binding domains are attached to opposite ends of the Fc domain. The multivalent binding molecules of this invention are agonists of a Wnt signaling pathway and are alternately referred to herein as FZD agonists or FZDag. 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 multispecific molecules described herein simultaneously bind to a FZD receptor and a Wnt co-receptor and thereby activate Wnt signaling pathways.

[0058] The modularity and effectiveness of the multivalent binding molecules for activating Wnt signaling pathways described herein contrasts with the Wnt surrogates described in the prior art which consists of monovalent FZD and LRP5/6 binding ligands, wherein the binding ligands are not attached to opposite ends of an Fc domain. In an embodiment of this invention the FZD binding domain comprise a binding moiety that is derived from antibodies or polypeptides that bind specifically to one or more FZD receptors and the co-receptor binding domain comprises a binding moiety that binds to a co-receptor, e.g., an LRP5/6, ROR1/2, RYK or PTK7. In an embodiment of the invention the antibodies or polypeptides that specifically bind to one or more FZD receptors bind to a cysteine rich domain (CRD) of one or more of the FZD receptor.

[0059] The amino acid sequences of FZD receptors and nucleotide sequences encoding FZD receptors, and antibodies and libraries of antibodies that bind FZD or the Wnt co-receptors LRP5/6, ROR1/2, RYK or PTK7 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.).

[0060] Methods for generating peptides or polypeptides that bind to a selected target are well known in the art, see for example Sidhu et al. *Methods in Enzymology* (2000) 328: 333-336. For example, a library of affibodies that bind a FZD or Wnt co-receptor may be obtained according to protocols known in the art (see, e.g., U.S. Pat. No. 5,831,012 and Lofblom et al., *FEBS Letters* 584 (2010) 2670-2680); a library of ankyrin repeat proteins used for the selection of a peptide that binds a FZD or Wnt co-receptor may be obtained according to protocols known in the art (see e.g., WO 02/020565, inventors Stumpp et al.) and a library of fibronectin repeat proteins used for the selection of a peptide that binds a FZD or a Wnt co-receptor may also be obtained according to protocols known in the art (see e.g., U.S. Patent No. 9,200,273, inventors Diem and Jacobs. The peptides that bind to a FZD or a Wnt co-receptors may also be fynomers, small binding proteins derived from the human Fyn SH3 domain or artificial receptor proteins, "anticalins", based on human apolipoprotein D, and may be generated using methods known in the art, see e.g., Silacci et al., *J. Biol. Chem* (2014) 289(20):14392-8 and Vogt and Skerra, *ChemBioChem* (2004) 5, 191-199).

[0061] Antibodies suitable as the source for antigen binding peptides as described herein may be isolated by screening combinatorial libraries for polypeptides with the desired activity or activities. For example, a variety of methods are known in the art for generating phage display libraries and screening such libraries for antibodies 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). 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. Antibodies or antibody fragments isolated from human antibody libraries are considered human antibodies or human antibody fragments herein.

[0062] Thus one of skill in the art would readily prepare an Fc domain and mix and match multivalent FZD binding domains and Wnt co-receptor binding domains having a desired specificity on the N and C terminals of the Fc domain to prepare the multivalent binding molecules to bind the desired FZD receptors and co-receptors and thereby activate specific Wnt pathways. These specific agonists would serve as powerful tools in enhancing cell proliferation, differentiation, organoid survival and maintenance, and tissue regeneration in vivo. These specific agonists also serve as powerful tools for profiling the FZD specificity involved in these processes. For example, as shown herein, the FZD5Ag but not FZD4Ag rescues the growth defect of LGK974-treated RNF43 mutant PDAC cell lines, highlighting the importance of FZD5 over FZD4 receptor in this process.

[0063] An embodiment of this invention is a method to effect binding by a peptide to a FZD receptor and a Wnt co-receptor on a cell wherein binding by said peptide to both FZD receptor and co-receptor activates a Wnt signaling pathway in cell. The method comprises selecting an Fc domain, or fragment thereof comprising a CH3 domain, having a C-terminus and an N-terminus, linking a first multivalent binding domain that binds the FZD receptor on one terminus of the Fc domain, and linking a second multivalent binding domain that binds to the Wnt co-receptor on the other terminus of the Fc domain thereby forming a multivalent binding molecule and then contacting the multivalent binding molecule with a cell expressing said FZD receptor and co-receptor under conditions to activate the Wnt signaling pathway.

[0064] In an embodiment of the invention the multivalent binding domains may comprise single chain variable fragments (ScFv) that bind to one or more FZD receptor, a ligand of the FZD receptor or co-receptor, or a fragment thereof that binds to the FZD receptor or the co-receptor. In another embodiment the binding domains do not comprise single chain variable

fragments (ScFv) that bind to one or more FZD receptor, a ligand of the FZD receptor or co-receptor, or a fragment thereof that binds to the FZD receptor or the co-receptor.

[0065] In an embodiment of the invention at least one of the FZD or co-receptor multivalent binding domain comprises a diabody having two peptides each peptide having a heavy-chain variable domain (VH) linked to a light-chain variable domain (VL), wherein the VH and the VL from one peptide pairs with the VL and VH of the other peptide such that the binding domain has two epitope-binding sites. The VH and VL domains may be the VH and VL of an antibody that binds to a Wnt binding site on the FZD receptor or co-receptor. A VH or VL derived from an antibody, the source antibody, may be 50%, 55%, 60%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to the VH and VL of the source antibody and still retain binding to the FZD receptor or co-receptor site bound by the antibody.

[0066] In an embodiment of this invention the multivalent binding molecules of this invention comprise the multivalent binding molecules of Table 1 (Table 1 comprises Tables 1A and 1B: Table 1A indicates nucleotide sequences and amino acid sequences of exemplified multivalent binding molecules of this invention; Table 1B indicates the nucleotide sequences of the various domains of the exemplified multivalent binding molecules). In an embodiment of this invention the multivalent binding molecules of this invention consist essentially of the multivalent binding molecules of Table 1. In an embodiment of this invention the multivalent binding molecules of this invention consist of the multivalent binding molecules of Table 1.

[0067] In an embodiment of this invention the multivalent binding domains comprise one or more of the VL and VH domains of the molecules of Table 1. In an embodiment of this invention the multivalent binding domains of the multivalent molecules consist essentially of one or more the VL and VH domains of the molecules of Table 1. In an embodiment of this invention the multivalent binding domains of the multivalent molecules consist of one or more of the VL and VH domains of the molecules of Table 1. In an embodiment of this invention the binding domains of the multivalent molecules described herein comprise VH and VL domains that are at least 75%, 80%, 85%, 90%, 95%, 98% or 99% identical to VH and VL of the molecules set forth in Table 1 and retain binding to the antigen bound by the molecules set forth in Table 1.

[0068] In an embodiment of this invention the binding domains of the multivalent molecules described herein comprise one or more complementarity determining regions

(CDRs) of the molecules set forth in Table 1. In an embodiment of this invention the binding domains of the multivalent molecules described herein comprise CDRs that are at least 75%, 80%, 85%, 90%, 95%, 98% or 99% identical to CDRs of the molecules set forth in Table 1 and retain binding to the antigen bound by the molecules set forth in Table 1.

[0069] The FZD receptor bound by the multivalent binding molecules of this invention may be FZD1, FZD2, FZD3, FZD4, FZD5, FZD6, FZD7, FZD8, FZD9, or FZD10. The FZD receptor may be FZD1, FZD2, FZD4, FZD5, FZD7 or FZD8. The multivalent binding molecules may bind to only one FZD receptor or may be pan-specific binding to more than one FZD receptor. The FZD multivalent binding domain may bind to e.g., FZD1, FZD2, FZD4, FZD5, FZD7 and FZD8. The FZD multivalent binding domain may specifically bind to one FZD receptor, e.g., FZD2, FZD4, FZD5, or FZD6.

[0070] In an embodiment of this invention the FZD binding domain is monospecific and binds to a single epitope on a FZD receptor. In an embodiment of this invention the FZD binding domain is bispecific and binds to two epitopes on an FZD receptor.

[0071] The co-receptor binding domain may bind to any Wnt co-receptor, e.g., LRP5/6, or ROR1/2. The multivalent co-receptor binding domain may bind to, e.g., LRP5/6, PTK7, ROR1/2, RYK, GPR12, TSPAN12 or CD133. In an embodiment of this invention the co-receptor multivalent binding domain binds to LRP5 or LRP6.

[0072] In an embodiment of this invention the co-receptor multivalent binding domain binds to a single epitope on a co-receptor, e.g., an epitope of LRP5/6 that binds Wnt1 or Wnt3. In an embodiment of this invention the co-receptor multivalent binding domain binds to two epitopes within a co-receptor, e.g., an epitope on LRP5/6 that binds to Wnt1 and an epitope that binds to Wnt3. The Wnt co-receptor bound by the multivalent binding molecules of this invention may be LRP5 or LRP6, PTK7, ROR1, ROR2, RYK, GPR124, TSPAN12 or CD133.

[0073] 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.

[0074] An embodiment of this invention is a method for activating a Wnt signaling pathway in a cell, comprising contacting a cell having a FZD receptor and a Wnt co-receptor with a multivalent binding molecule of this invention in an amount effective to activate Wnt signaling

[0075] In an embodiment of this invention at least one of the multivalent binding domains comprises an scFv that binds the FZD receptor or co-receptor, or comprises a ligand of the FZD receptor or co-receptor or a fragment of said ligand. In an embodiment of this invention at least one of the multivalent binding domains does not comprise an scFv that binds the FZD receptor or co-receptor and does not comprise a ligand of the FZD receptor or co-receptor or a fragment of said ligand.

[0076] In an embodiment of this invention the FZD multivalent binding domains comprise a FZD diabody and the co-receptor multivalent binding domain comprises a co-receptor diabody wherein the diabodies comprises two peptides each comprising a heavy-chain variable domain (VH) linked to a light-chain variable domain (VL) 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 binding domains.

[0077] The VH and VL of the FZD binding domain may be derived from an antibody that binds the FZD receptor and antagonizes Wnt signaling or inhibits binding of a Wnt ligand to the FZD receptor. The VH and VL of the FZD binding domain may be derived from an antibody that binds the FZD receptor without antagonizing or inhibiting binding of a Wnt ligand to the FZD receptor.

[0078] The VH and VL of the co-receptor binding domain may be derived from an antibody that binds the co-receptor and antagonizes Wnt signaling or inhibits binding of a Wnt ligand to the co-receptor. The VH and VL of the co-receptor binding domain may be derived from an antibody that binds the co-receptor without antagonizing Wnt signaling or inhibiting binding of a Wnt ligand to the co-receptor.

[0079] In the multivalent binding molecules of this invention one or both of the binding domains may be bivalent and one or both of the bivalent binding domains may be bispecific for the FZD receptor or for the co-receptor. In an embodiment of this invention both binding domains are bivalent and bispecific, each binding domain binding to two different epitopes on their respective target FZD receptor or co-receptor. For example, the binding molecule may comprise a FZD binding domain that is bivalent and bispecific (binding to two different epitopes) for FZD receptors, or the binding molecule may comprise a co-receptor binding domain that is bivalent and bispecific for a co-receptor.

[0080] In an embodiment of this invention the FZD binding domain is attached to the N-terminus of the Fc domain of the multivalent binding molecule and the co-receptor binding domain is attached to the C-terminus of the Fc domain. In an embodiment of this invention

the FZD binding domain is attached to the C-terminus of the Fc domain of the multivalent binding molecule and the co-receptor binding domain is attached to the N-terminus of the Fc domain.

[0081] Also an embodiment of this invention are the nucleic acid molecules encoding the multivalent binding molecules described herein, including expression cassettes and vectors comprising the nucleic acid molecules that encode the multivalent binding molecules. The nucleic acid molecules can be inserted into a vector and expressed in an appropriate host cell and then the multivalent binding molecules isolated from the cells using methods well known in the art. As used in this invention, the term "vector" refers to a nucleic acid delivery vehicle or plasmid that can be engineered to contain a nucleic acid molecule, e.g., a nucleic acid sequence encoding the multivalent binding 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 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.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 multivalent binding 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 reporter gene. In addition, the vector may also contain replication initiation site(s).

[0082] As used in this invention, the term "host cell" refers to cells that can import 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 cells, COS cells, NSO cells, HeLa cells, BHK cells, or HEK293 cells.

[0083] An embodiment of this invention is a pharmaceutical composition comprising a FZD agonist described herein and a pharmaceutically acceptable excipient. The pharmaceutical composition may further comprise an additional agent that activates a Wnt

pathway, e.g., a Norrin or R-Spondin. The pharmaceutical composition may consist or consist essentially of the multivalent binding molecules described herein and a pharmaceutically acceptable carrier or excipient. Suitable carriers and their formulations are described in Remington: 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 is preferably from about 5 to about 8, and more preferably from about 7 to about 7.5. Further carriers include sustained release preparations such as semipermeable matrices of solid hydrophobic polymers containing the antibody, 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 agonists being administered.

[0084] Wnt-signaling is a ubiquitous pathway that modulates cellular and tissue differentiation. For example, in regards to eye development a particular Wnt-pathway, the Norrin-FZD4 pathway, has been identified as playing a role in retinal angiogenesis. Signaling through Norrin-FZD4 pathway is necessary for development and maintenance of retinal vasculature. Mutations affecting genes of this pathway may result in several pediatric 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 pathway, and Wnt-pathway mutations have been reported in Coats Disease and Persistent Fetal Vasculature (PFV). The Norrin-FZD4 pathway is also associated with CNS blood vessel development. Genetic ablation of the Norrin, FZD4, Lrp5 and the co-receptor Tetraspanin-12 (Tspan-12) result in defective angiogenesis and barrier disruption in both retinal and cerebellar vessels (Cho et al. (2017) Neuron 95, 1056-1073; Zhou et al., (2014) J Clin Invest 124:3825-3846). It is specifically contemplated herein that the FZD4 agonists of this invention, particularly the FZD4 FLAgs comprising a FZD4 binding domain on one end of the Fc receptor and a binding domain for LRP5 and/or LRP6 on the other side of the Fc domain will strengthen barrier function and facilitate angiogenesis, e.g., treatment with the FZD4 FLAgs will facilitate the development and maintenance of retinal vasculature and/or the blood retinal barrier (BRB) and the blood brain barrier (BBB). Thus 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 FZD4 FLAgs through local or systemic administration. Also an aspect of this invention is a method for promoting and/or maintaining BBB vasculature by treating the BBB with an effective amount of a FZD4 FLAgs following systemic administration. A further aspect of this invention is a method for treating a subject having a disorder characterized by reduced retinal or brain angiogenesis by administering to such subject an effective amount of a FZD4 FLAgs, wherein the effective amount is an amount sufficient to increase retinal or brain angiogenesis in such subject. The subject may be a fetus.

[0085] Pathologically low levels of Wnt signaling have been associated with osteoporosis, polycystic kidney disease and neurodegenerative diseases. Controlled activation of Wnt pathway has been shown to promote regenerative processes such as tissue repair and wound-healing. Zhao J, Kim KA, and Abo A, Trends Biotechnol. 27(3):131-6 (Mar. 2009). See also, Logan CY and Nusse R, Annu. Rev. Cell. Dev. Biol. 20:781-810 (2004); Nusse R., Cell Res. 15(1):28-32 (Jan. 2005); Clevers H, Cell 127(3):469-80 (3 Nov. 2006). Proof- of- concept experiments have been done to show the role of Wnt signaling in osteoporosis or mucositis. Furthermore, it has been suggested that increasing of Wnt signaling might be beneficial for the treatment of diabetes and other metabolic diseases. Decreased Wnt signaling has been associated with metabolic disease. Loss-of-function LRP6^{R611C} mutation results in early coronary artery disease, metabolic syndrome and osteoporosis in human. Main A et al, Science 315:1278 (2007). "LRP5 loss-of-function mutation is associated with osteoporosis, impaired glucose metabolism and hypercholesterolaemia in human." Saarinen et al., Clin Endocrinol 72:481 (2010). Severe hypercholesterolemia, impaired fat tolerance, and advanced atherosclerosis in mice lacking both LRP5 and apoE. Magoori K. et al., JBC 1 1331 (2003). LRP5 is essential for normal cholesterol metabolism and glucose-induced insulin secretion in mice. Fujino et al., PNAS 100:229 (2003). TCF7L2 variant confers risk of type 2 diabetes. Grant et al., Nat Genet 38:320 (2006); Florez et al., N Engl J Med 355:241 (2006). An increase of Wnt signaling can be beneficial for treating metabolic diseases. Accordingly, the administration of the multivalent binding molecules of this invention to a subject with metabolic disease is useful for treating the subject's metabolic disease.

[0086] Inflammatory bowel disease (IBD) is a group of inflammatory conditions of the colon and small intestine. The major types of IBD are Crohn's disease and ulcerative colitis. RSP01 protein has been shown to ameliorate inflammatory bowel disease in an animal model. Zhao J et al., Gastroenterology 132:1331 (2007). Accordingly, the administration of

the multivalent binding molecule of this invention to a subject with IBD is useful for treating the subject's IBD.

[0087] Thus, an embodiment of this invention is a method for treating a subject having a condition associated with reduced Wnt signaling comprising administering to a subject in need thereof an effective amount of the FZD agonists of this invention. The condition may be e.g., osteoporosis, polycystic kidney disease, neurodegenerative diseases, mucositis, short bowel syndrome, bacterial translocation in the gastrointestinal mucosa, enterotoxigenic or enteropathic infectious diarrhea, celiac disease, non-tropical sprue, lactose intolerance and other conditions where dietary exposures cause blunting of the mucosal villi and malabsorption, atrophic gastritis and diabetes, bone fracture, tissue regeneration, e.g. tissue repair and wound healing, as well as metabolic diseases such as diabetes, and melanoma, Examples of damaged tissue that can be treated using methods of the invention include, but are not limited to, intestinal tissue, cardiac tissue, liver tissue, kidney tissue, skeletal muscle, brain tissue, bone tissue, connective tissue, and skin tissue. The multivalent binding molecules of this invention can be administered to a subject with a disease or condition characterized by a low Wnt signaling. The multivalent binding molecules of the invention are administered to the subject in an amount effective to increase Wnt signaling and to ameliorate the disease or condition in the subject.

[0088] Mucositis is a clinical complication of cancer therapy. Mucositis is caused by the cytotoxic effects of irradiation or chemotherapy on fast proliferating cells. Mucositis consists of epithelial damage mainly affecting the intestinal and oral mucosa. Clinical signs are severe pain of the oral cavity, nausea, diarrhea, malnutrition, and, in severe cases, sepsis and death. The symptoms can often lead to dose limitation of cancer therapy. There are no currently available treatments for oral or gastrointestinal- mucositis associated with chemotherapy or radiation therapy for solid tumors.

[0089] Oral mucositis is a common and often debilitating complication of cancer treatment. 50% of patients undergoing radiotherapy for head and neck cancer and 10- 15% of patients treated with 5-FU get grade 3-4 oral mucositis. RSP01 has been shown to ameliorate oral mucositis in an animal model. Zhao J et al., PNAS 106:2331 (2010).

[0090] Short bowel syndrome (SBS) results from functional or anatomic loss of extensive segments of small intestine, so that digestive and absorptive capacities are severely compromised. Each year, many people undergo resection of long segments of small intestine for various disorders, including trauma, inflammatory bowel disease, malignancy, mesenteric

ischemia and others. Various nonoperative procedures such as radiation can cause functional short-bowel syndrome. Current therapies for short-bowel syndrome include dietary approaches, total parenteral nutrition (TPN), intestinal transplantation, and nontransplantation abdominal operations. Although these treatments have contributed to the improved outcome of SBS patients, they only partially correct the underlying problem of reduced bowel function. No current therapy can accelerate the recovery of remaining small intestine in SBS patients. See, Seetharam and Rodrigues, *The Saudi Journal of Gastroenterology* 17, 229-235 (2011).

[0091] The adult mammalian gut constitutes one of the most rapidly self-renewing tissues, in which the intestinal mucosa comprises a continuous structure folded into the proliferative crypts and the differentiated villi. In response to mucosal disruption, the host initiates a healing response resulting in restoration of mucosal integrity and regeneration of the mucosal architecture. This process is heavily dependent on the proliferation of intestinal stem cells. Neal et al., *Journal of Surgical Research* 167, 1-8 (2010); van der Flier and Clevers, *Annual Review of Physiology* 71, 241-261 (2009).

[0092] Therefore, the factors that regulate the activity of intestinal stem cells play a dominant role in the ability of the host to respond to injury within the intestinal tract. Because Wnt proteins are the most important growth factors that support the proliferation of intestinal stem cells, enhancing Wnt signaling will increase the proliferation of intestinal epithelium. This will lead to increased number of small bowel villi and increased mucosal absorptive surface area.

[0093] Thus, in one embodiment, the multivalent binding molecules of this invention are administered to a person with short bowel syndrome. The multivalent binding molecules is administered in an amount sufficient to increase gastrointestinal mucosal absorptive surface area. The administration of the multivalent binding molecules of this invention has a successful outcome when the person with incident short bowel syndrome adapts to enteral feeding, or when the person with prevalent SBS absorbs nutrients from enteral feeds, or when the person decreases the amount of total parenteral nutrition required daily for the person to maintain weight.

[0094] Prevention of bacterial translocation. In one embodiment, the antibody of the invention is administered to a person at risk of septicemia caused by enteric bacteria. The multivalent binding molecules is administered in an amount sufficient to increase gastrointestinal mucosal integrity, thus preventing enteric bacteria from passing into the

bloodstream of the person. Decreased gastrointestinal mucosal integrity (as compared with the gastrointestinal mucosal integrity that is normal for the human population) is a major source of bloodstream infections and sepsis in critically ill patients. The administration of the multivalent binding molecules has a successful outcome when fewer cases of bacteremia and sepsis are observed in intensive care unit (ICU) patients than in patients to whom the multivalent binding molecules of this invention is not administered.

[0095] Accelerated recovery during or after enterotoxigenic or enteropathic infectious diarrhea. Infectious diarrhea is a major pediatric problem. In one embodiment, the multivalent binding molecules of the invention is administered in an amount sufficient to shorten the time to the end of diarrhea or the time to normal bowel movements. The multivalent binding molecules of this invention can be administered in addition to the standard of care, which includes oral or parenteral rehydration and sometimes, antibiotics. The administration of the multivalent binding molecules has a successful outcome when decrease hospitalizations, shorten hospitalizations, or a decrease the incidence of complications of dehydration and electrolyte abnormalities are observed in pediatric patients as compared with pediatric patients to whom the multivalent binding molecules of the invention is not administered.

[0096] Celiac disease, non-tropical sprue, lactose intolerance and other conditions where dietary exposures cause blunting of the mucosal villi and malabsorption. In one embodiment, the multivalent binding molecules of this invention is administered in an amount sufficient to increase mucosal absorptive surface area. The multivalent binding molecules of this invention can be administered in addition to the standard of care, which is primarily avoiding the offending foods and sometimes, dietary supplements. The administration of the multivalent binding molecules of the invention has a successful outcome when the person with celiac disease, non-tropical sprue, lactose intolerance or other condition adapts to enteral feeding, or when the person with any of the conditions absorbs nutrients from enteral feeds, or when the person decreases the amount of total parenteral nutrition required daily for the person to maintain weight.

[0097] Atrophic gastritis, specifically the form termed environmental metaplastic atrophic gastritis. Atrophic gastritis is a common condition in the elderly, currently treated with vitamin B12 injections. The patients have an increased risk of carcinoid tumors and adenocarcinoma. The administration of the multivalent binding molecules has a successful outcome when decreased the tumor incidence, in the case of carcinoid by decreasing gastrin

production from the metaplastic G cells, is observed by a medical expert. The multivalent binding molecules should not be administered to the subject if a medical expert determined that if the tumors are activated by increases in the Wnt pathway.

[0098] The FZD agonists of the present invention may be administered, e.g., by injection (e.g. subcutaneous, intravenous, intraperitoneal, etc.), topically, or orally. Depending on the route of administration, the active compound may be coated in a material to protect the compound from the action of acids and other natural conditions which may inactivate the compound. The multivalent binding molecules described herein may be dissolved or suspended in a pharmaceutically acceptable, preferably aqueous carrier. In addition, the composition can contain 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 multivalent binding molecules can also be administered together with immune stimulating substances, such as cytokines.

[0099] An embodiment of this invention includes a method for producing induced pluripotent stem (iPS) cells comprising culturing a somatic cell under conditions suitable for reprogramming the somatic cell wherein said culturing conditions further comprise a multivalent binding molecule described herein. Method for generating pluripotent stem cells are well known in the art, see e.g., Takahashi and Yamanaka, (2006), Induction of Pluripotent Stem Cells from Mouse Embryonic and Adult Fibroblast Cultures by Defined Factors, Cell 126, 663–676; Takahashi et al. (2007) Induction of Pluripotent Stem Cells from Adult Human Fibroblasts by Defined Factors Cell 131, 861–872; Yu et al. (2007). Induced pluripotent stem cell lines derived from human somatic cells. Science 318, 1917-1920; US patent no. 8,546,140, and; US patent No. 8,268,620. In an embodiment of this invention the multivalent binding molecules of this invention are included in the culture media in an amount sufficient to accelerate the generation of iPS cells.

[0100] 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 multivalent binding 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 multivalent binding molecules described herein can be used to effect activation of Wnt signaling pathways to direct differentiation of the PSCs.

[0101] 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 multivalent binding peptide described herein.

[0102] An embodiment of this invention includes a method for enhancing bone healing and/or regeneration in a subject in need thereof, e.g., a subject with osteoporosis or fracture, by administering an effective amount of a multivalent binding molecule described herein. In a particular embodiment the multivalent binding molecule of this invention comprises a binding domain that binds to FZD2 and a binding domain that binds to LRP5 or/and LRP6. The binding domains may be monovalent or multivalent, e.g., bivalent, trivalent or tetravalent, and monospecific or multispecific, e.g., bispecific.

[0103] A subject 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. Typically, the subject is human.

[0104] Effective dosages and schedules for administering the multivalent binding molecules 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, the subject that 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, 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. 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, condition, sex and extent of the inflammation 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 art.

[0105] In recent years, methods have been developed for culturing mini-organs called “organoids” that recapitulate the gross anatomy and cell type composition of different tissues. Remarkably, full organoids can be generated from a single tissue stem cell as first demonstrated with intestinal LGR5+ stem cells isolated from a mouse. It is known that components within the media that activate the Wnt- β catenin pathway are required for organoid derivatization, growth, survival and maintenance. Therefore, R-spondin and Wnt ligands, purified or provided as conditioned media are universally required to grow organoids from different tissues. However, purified Wnt proteins have generally low specific activity and are not able to sustain growth of organoids. As such those of skill in the art rely on the addition of Wnt3A conditioned media, or to the addition of small molecules, e.g., GSK3 inhibitors, to generate organoids. But the production of Wnt3A conditioned media is labor intensive, the characteristics of the conditioned media are inconsistent, and small molecule GSK3 inhibitors may robustly activate the pathway to levels that are toxic. The multivalent binding molecules described herein solve these problems as they are easy to produce and purify, have consistent reproducible characteristics, and activate Wnt specifically by selectively engaging the desired FZD receptor(s) and co-receptor(s) combination.

[0106] An embodiment of this invention includes a method for generating tissue organoids comprising culturing tissue in an effective amount of a multivalent binding molecule described herein. An organoid is a 3D multicellular in vitro tissue construct that mimics its corresponding in vivo organ, such that it can be used to study aspects of that organ in the tissue culture dish. Methods for generating organoids are well known in the art and epithelial organoids derived from adult stem cells in the various organs of the gastrointestinal tract, for example, almost all need agonists of Wnt signaling (among other signaling factors, including embedding in Matrigel) to both maintain the cells and to generate an in vivo-like complement of cell types. Wnt signaling also enhances inner ear organoid development in 3D culture, and has been used in the generation of kidney organoids, see e.g., Natalie de Souza (2018) Nature Methods 15(1): 23; DeJonge et al. (2016) PLoSOne 11(9), e0162508; Akkerman and Defize, (2017) Bioessays 39, 4, 1600244. The multivalent binding molecules of this invention can be included in the culture media of organoids in an amount sufficient to enhance their growth, survival and maintenance in culture. As such, an embodiment of this

invention includes a method for enhancing the culture of tissue organoids comprising a culture medium comprising an effective amount of a multivalent binding molecule described herein.

[0107] Also an aspect of this invention is a method for making the multivalent binding molecules described herein. In an embodiment of this invention the multivalent binding molecule is generated by,

- a) selecting an Fc domain having a C-terminus and an N-terminus
- b) identifying a peptide that binds to one or more FZD receptors, or identifying an antibody that binds to one or more FZD receptors, and
- c) identifying a peptide that binds to one or more Wnt co-receptors or identifying an antibody that binds to one or more Wnt co-receptors,
- d) generating a nucleic acid molecule comprising a nucleotide sequence that encodes (i) the Fc domain of step a, (ii) a nucleotide sequence that encodes the peptide of step b, or a nucleotide sequence that encodes a VL and/or a VH of the antibody of step b, or a nucleotide sequence that encodes a VL and/or a VH derived from the antibody of step b, that binds the one or more FZD receptors, and (iii) a nucleotide sequence that encodes the peptide of step c, or a nucleotide sequence that encodes the VL and/or the VH of the antibody of step c, or a nucleotide sequence that encodes the VL and/or the VH derived from the antibody of step c, that binds to the one or more Wnt co-receptors,
- e) expressing the nucleic acid molecule of (d) to produce a polypeptide wherein the polypeptide dimerizes to form a tetravalent binding molecule comprising (i) an Fc domain, (ii) a FZD binding domain and (iii) a Wnt co-receptor binding domain wherein, such that the FZD binding domain comprises of the peptide of step b, or the VL and/or VH of step b, and is linked to one terminus of the Fc domain, and the Wnt co-receptor binding domain comprises the peptide of step c or the VL and/or VH of step c and is linked to the other terminus of the Fc domain thereby forming the multi-specific binding molecule.

[0108] The peptide that binds to one or more of the FZD receptor may be a synthetic polypeptide, e.g., a synthetic peptide, an affibody, an ankyrin repeat protein, a fibronectin repeat protein, a fynomer, or an anticalin or a peptide of a naturally occurring protein that

binds the FZD receptor. The naturally occurring protein may be, e.g., a Wnt, e.g., Wnt-1, Wnt-2, Wnt-2b, Wnt-3a, Wnt-4, Wnt-5a, Wnt-5b, Wnt-6, Wnt-7a, Wnt-7a/b, Wnt-7b, Wnt-8a, Wnt-8b, Wnt-9a, Wnt-9b, Wnt-10a, Wnt-10b, Wnt-11, Wnt-16b. The peptide of step b may be multivalent, binding to more than one site on the FZD, e.g., bivalent, trivalent or tetravalent, and may be monospecific, binding to a single epitope, or multispecific, binding to more than one epitope on the FZD.

[0109] The peptide that binds to one or more of the Wnt co receptor may be a synthetic peptide, e.g., an affibody, an ankyrin repeat protein, a fibronectin repeat protein, a fynomer, or an anticalin, or a peptide of a naturally occurring protein that binds the Wnt co-receptor. The naturally occurring protein may be for example, a Wnt, e.g., Wnt-1, Wnt-2, Wnt-2b, Wnt-3a, Wnt-4, Wnt-5a, Wnt-5b, Wnt-6, Wnt-7a, Wnt-7a/b, Wnt-7b, Wnt-8a, Wnt-8b, Wnt-9a, Wnt-9b, Wnt-10a, Wnt-10b, Wnt-11 or Wnt-16b, or Dickkopf-1.

[0110] The peptide of step c may be multivalent binding to more than one epitope on the Wnt co-receptor, e.g., bivalent, trivalent or tetravalent, and may be monospecific binding to a single epitope or multispecific binding to more than one epitope on the Wnt co-receptor.

[0111] The naturally occurring protein that binds the FZD receptor and the naturally occurring protein that binds the Wnt co-receptor may be the same protein.

[0112] In an embodiment the peptide or antibody of step b may bind FZD2 and the peptide of step c may be a peptide of Wnt5a and the antibody of step c may be an antibody that binds to a site on the co-receptor that binds to Wnt5a.

[0113] In an embodiment the peptide or antibody of step b may bind FZD4 and the peptide of step c may be a peptide of one or more of Norrin, Wnt1, Wnt8, or Wnt5a and the antibody of step c may be antibody that binds to a site on the co-receptor that binds to Norrin, Wnt1, Wnt8, or Wnt5a.

[0114] In an embodiment the peptide or antibody of step b may bind FZD5 and the peptide of step c may be a peptide of one or more of Wnt7a, Wnt5a, Wnt10b, or Wnt2 and the antibody of step c may be an antibody that binds to a site on the co-receptor which site binds to one or more of Wnt7a, Wnt5a, Wnt10b, or Wnt2.

[0115] In an embodiment the peptide or antibody of step c binds LRP6 and/or LRP5, e.g., the peptide may be a peptide of Norrin, Wnt1 and/or Wnt3a, and the antibody of step c may be an antibody that binds to a site on LRP6/LRP5 which site binds to Norrin, Wnt1 and/or Wnt3a.

[0116] In an embodiment the peptide or antibody of step c may bind LRP6, e.g., the peptide may be a peptide of Wnt1 or Wnt3a, or both, and the antibody may be an antibody that binds a site on LRP6 that binds Wnt1 or Wnt3a.

[0117] In an embodiment the peptide or antibody of step c binds ROR1 and/or ROR2.

[0118] In an embodiment the peptide or antibody of step c may bind RYK.

[0119] In an embodiment the peptide or antibody of step c may bind PTK7.

[0120] In an embodiment, the peptide or antibody in step (b) may be a peptide or antibody that binds to one or more FZD receptors and antagonizes Wnt signaling or inhibits Wnt binding to the receptor. In an embodiment, the peptide or antibody in step (b) may be a peptide or antibody that binds to one or more FZD receptors without antagonizing Wnt signaling or inhibiting Wnt binding to the receptor. In an embodiment, the peptide or antibody in step (c) may be a peptide or antibody that binds to one or more of the Wnt co-receptors and antagonizes Wnt signaling or inhibits Wnt binding to the co-receptor. In an embodiment, the peptide or antibody of step (c) may be a peptide or antibody that binds to the Wnt co-receptor without antagonizing Wnt signaling or inhibiting Wnt binding to the co-receptor. The binding domains may be linked to the Fc domain via a linker. The modular aspects of this invention allows for mixing and matching of peptide or antibody VH and VL that bind to any given FZD receptor and Wnt co-receptor on the opposite termini of the Fc domain to generate a multivalent binding molecule that can engage multiple Frizzled receptor – co-receptor complexes or to selectively engage a single Frizzled receptor-co-receptor complex to activate Wnt signaling.

[0121] An embodiment of this invention is a method of making a multivalent binding molecule that activates a Wnt signaling pathway comprising

- a) selecting an Fc domain having a C-terminus and an N-terminus, e.g. an Fc domain of an immunoglobulin comprising a CH3 domain, e.g., an IgG, e.g., an IgG1,
- b) identifying an antibody having a binding specificity for one or more FZD receptor and
- c) identifying an antibody having a binding specificity for a Wnt co-receptor;
- d) generating a nucleic acid molecule comprising
 - (i) a nucleotide sequence that encodes the selected Fc domain,
 - (ii) a nucleotide sequence that encodes a VL and/or a VH derived from the antibody of step b, and
 - (iii) a nucleotide sequence that encodes a VL and/or a VH derived from the

antibody of step c,

d) expressing the nucleic acid molecule of (d) to produce a polypeptide which dimerizes via the Fc domain to form a multivalent binding molecule comprising (i) the Fc domain, (ii) a FZD binding domain and (iii) a Wnt co-receptor binding domain, such that the FZD binding domain is linked to one terminus of the Fc domain and the Wnt co-receptor binding domain is linked to the other terminus of the Fc domain thereby forming a multivalent binding molecule. In a preferred embodiment the multivalent binding molecule is a dimer of two polypeptides encoded by the nucleic acid molecule wherein the Fc domain is in a knob in hole configuration.. One or both of the binding domains may be multivalent binding domains. The antibody of step b may be an antibody fragment that binds the FZD receptor. The VH and/or VL in step d(ii) may be identical to the VH and/or VL of the antibody of step b). The antibody of step c may be an antibody fragment that binds the Wnt co-receptor. The VH and/or VL in step d(iii) may be identical to the VH and/or VL of the antibody of step c).

[0122] The multivalent 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 co-receptor or to different members of the same FZD receptor or co-receptor family, see e.g., Figure 3A. Methods 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 A. M., et al.. (1998) Nat. Biotechnol. 16, 677–681 and; et al., (1997) J. Mol. Biol. 270, 26–35.

[0123] Another embodiment of this invention is a method for facilitating the interaction of a FZD receptor and a co-receptor on a cell thereby activating a Wnt signaling pathway in the cell 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 multivalent binding domain, which binds the FZD receptor, on one terminus of the Fc domain and linking a second binding domain, which binds to the Wnt co-receptor, on the other terminus of the Fc domain thereby forming a binding molecule; c) contacting said multivalent binding 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 multivalent binding molecule thereby activating the Wnt signaling pathway. One or both of the binding domains may be monovalent or multivalent, e.g., bivalent, trivalent, or tetravalent. The FZD binding domain

may comprise a peptide of a naturally occurring protein that binds FZD, a synthetic peptide, e.g., a affibody, an ankyrin repeat protein, a fibronectin repeat protein, a fynomer, or an anticalin, that binds FZD, VH and/or VL fragments that bind FZD, a scFV that binds FZD, or a diabody that binds FZD. The Wnt co-receptor binding domain may comprise a peptide of a naturally occurring protein that binds the Wnt co-receptor, a synthetic peptide, e.g., an affibody, an ankyrin repeat protein, a fibronectin repeat protein, a fynomer or an anticalin, that binds to the Wnt co-receptor, VH and/or VL fragments that bind the Wnt co-receptor, a scFV that binds the Wnt co-receptor, or a diabody that binds the Wnt co-receptor.

[0124] An embodiment of this invention is a molecule comprising an Fc domain and two binding domains, the first domain binds to a FZD receptor and the second domain binds to a Wnt co-receptor, and these two moieties are linked together by a Fc domain, or fragment thereof comprising the CH3 domain, wherein one domain is linked to the N-terminus of the Fc receptor, and the other domain is linked to the C-terminus of the Fc receptor. The binding domains may be linked to the Fc receptor either directly or via a peptide linker, e.g. a polypeptide linker, or a non-peptidic linker. Suitable linkers are well known in the art, e.g., an XTEN linker (see WO2013120683, inventors Schellenberger et al.)

[0125] An embodiment of this invention is a method for activating a Wnt signaling pathway comprising contacting a cell expressing a FZD receptor and its co-receptor with an effective amount of the multivalent molecules of this invention. Without wishing to be bound by theory, it is contemplated that the multivalent molecules described herein bind both the FZD receptor and its co-receptor thereby forming a complex that mimics the binding of a Wnt molecule to the FZD receptor and co-receptor(s), which in turn activates Wnt signaling pathways.

[0126] The multivalent binding molecules 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 a 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.

[0127] In some embodiments, the binding domains are attached to the Fc domain via a peptide linker, e.g., an XTEN linker. In some embodiments, the peptide linker comprises at least 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 5 to 75, 5 to 50, 5 to 25, 5 to 20, 5 to 15, or 5 to 10 amino acids in length. The Fc domain with or without the linker are of a length and flexibility that allows for the multivalent binding molecule to bind both the FZD receptor and its co-receptor thereby activating a Wnt signaling pathway. 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, greater than 125 amino acids greater than 150 amino acids, greater than 175 amino acids or greater than 200 amino acids.

[0128] It must be noted that 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.

[0129] 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.

[0130] 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 multivalent binding molecules is a composition that may comprise other elements in addition to multivalent binding molecules, e.g. functional moieties such as polypeptides, small molecules, or nucleic acids bound, e.g. covalently bound, to the multivalent binding molecules; agents that promote the stability of the multivalent binding molecule composition, agents that promote the solubility of the multivalent binding molecule composition, adjuvants, etc. as will be readily understood in the art, with the exception of elements that are

encompassed by any negative provisos.

[0131] 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 multivalent binding 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.

[0132] 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 multivalent binding molecule "consisting of" a disclosed sequence consists only of the disclosed amino acid sequence.

[0133] 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.

[0134] 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 function. 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.

[0135] Three highly divergent stretches within each of the heavy chain variable domain, VH, and light chain variable domain, VL, 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). 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.

[0136] 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).

[0137] "Affibodies" are small, single domain proteins engineered to bind to a large number of target proteins or peptides with high affinity, imitating monoclonal antibodies. They are composed of a three-helix bundle based on the scaffold of one of the IgG-binding domains of staphylococcal protein A. This scaffold domain consists of 58 amino acids, 13 of which are randomized to generate affibody libraries with a large number of ligand variants. See, e.g., U.S. Pat. No. 5,831,012 and Lofblom et al. *FEBS Letters* 584 (2010) 2670-2680. Affibody molecules mimic antibodies have a molecular weight of about 6 kDa.

[0138] "Diabodies" 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, see e.g. Figure 3A. 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).

[0139] As used herein an "effective amount" of an agent, e.g., the multivalent binding 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 symptoms of the disease, disorder, and/or condition.

[0140] 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 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., $\leq 100 \text{ nM}$, preferably $\leq 10 \text{ nM}$ and more preferably $\leq 1 \text{ nM}$.

[0141] 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 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 multivalent molecule is engineered such that it does not target the cell binding the multivalent molecule for ADCC or CDC-dependent death. In an embodiment of the invention the Fc domain of the multivalent binding molecule is a peptide dimer in a knob-in-hole configuration. The peptide dimer may be a heterodimer.

[0142] 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, particularly humans.

[0143] "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 (GenBank Accession No. NM 002335.2) and LRP6 (GenBank Accession No. NM 002336.2) are included in a Wnt receptor complex required for activation on the Wnt- β catenin signaling pathway.

[0144] The term "polypeptide fragment" as used herein refers to a polypeptide that has an amino 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.

[0145] As used herein the term "paratope" includes the antigen binding site in the variable region of an antibody that binds to an epitope.

[0146] The terms "treatment", "treating" and the like are used herein to generally mean obtaining a desired pharmacologic and/or physiologic effect. The effect may be prophylactic in terms of completely or partially preventing a disease or symptom thereof and/or may be therapeutic in terms of a partial or complete cure for a disease and/or adverse effect attributable to the disease. "Treatment" as used herein covers any treatment of a disease in a mammal, and includes: (a) preventing the disease from occurring in a subject which may be predisposed to the disease but has not yet been diagnosed as having it; (b) inhibiting the disease, i.e., arresting its development; or (c) relieving the disease, i.e., causing regression of the disease. The therapeutic agent may be administered before, during or after the onset of disease or injury. The treatment of ongoing disease, where the treatment stabilizes or reduces the undesirable clinical symptoms of the patient, is of particular interest. Such treatment is desirably performed prior to complete loss of function in the affected tissues. The subject therapy may be administered during the symptomatic stage of the disease, and in some cases after the symptomatic stage of the disease.

[0147] The ability of the multivalent binding molecules of this invention to activate Wnt signaling can be confirmed by a number of assays. The multivalent binding molecules of this invention typically initiate a reaction or activity that is similar to or the same as that initiated by the FZD receptor's natural ligand. The multivalent binding 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 invention.

[0148] 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; Axin 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 LEF. The transcriptional response to Wnt activation has been characterized in a number of cells and tissues. As such, global transcriptional profiling by methods well known in the art can be used to assess Wnt- β catenin signaling activation.

[0149] 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).

[0150] 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)..

[0151] "Single-chain Fv" or "scFv" antibody fragments comprise the VH and VL domains of 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 University Press (1995) (Carl K. Borrebaeck, Ed.).

[0152] 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.

EXAMPLE I

1. Development of multivalent FZD agonists.

[0153] To make a multivalent binding molecule having a first binding domain comprising a FZD diabody and a second binding domain comprising a co-receptor diabody, we identified FZD specific antibodies from a synthetic Fab phage library (Library F; see US publication no. 2016/0194394, inventors Sidhu et al.) by selecting for those that bound to the cysteine rich domain (CRD) of FZD receptors using conventional phage-display technologies. Affinity or specificity maturation was carried out as needed. For example, a pan-FZD binding antibody, #5019 (which recognizes FZD1, 2, 4, 5, 7 and 8), was matured from a FZD7-derived antibody using the FZD4 CRD as antigen. Our previous work also identified several antibodies that are completely specific for FZD4 (5038, 5044, 5048, 5062, 5063, 5080, 5081) or for FZD5 (2928) (see, e.g., US20160194394, inventors Sidhu et al. and WO2017127933A1, inventors Pan et al.).

[0154] These FZD antibodies were used to prepare a FZD specific diabody. A diabody is an antibody form similar to single chain variable fragment (scFv), but it is a dimer of two peptides each encoding a VL and VH, however, unlike a scFv the linker between the VH and VL within the polypeptides is too short to allow for intramolecular complementation between the VH and VL domains. Therefore, the VH-VL fragment of one polypeptide dimerizes with the VH-VL fragment of another polypeptide in such a way to functionally reconstitute two antigen binding paratopes. Diabodies were generated having paratopes that were identical or non-identical, by forming dimers of the polypeptides having the same VL and VH thereby forming homodiabodies, or forming dimers from two polypeptides having different VL and

VH domain thereby forming heterodiabodies .

[0155] LRP6 antibodies were also selected from a synthetic antibody library by selecting those that bound the recombinant extracellular domain (ECD) of human LRP6. Five Fab with unique CDR regions were identified. After converting to IgG forms, they all display human LRP6 binding as well as mouse LRP6 binding. No LRP5 binding was detected via ELISA, demonstrating these antibodies are LRP6 specific (Figure 1A). LRP6 ECD contains four β -propeller motifs that alternate with four epidermal growth factor (EGF) like repeats. The first two β -propeller motifs are thought to be involved in Wnt1 binding and the last two are thought to be involved in Wnt3 binding, thus creating two potential epitopes for antibody binding. See Figure 6A. Epitope binding results suggest that these five antibodies bind two separate sites on LRP6 and could be divided into two groups with antibodies 2538, 2542, and 2543 binding the Wnt1 binding site and 2539 and 2540 binding the Wnt3 binding site on LRP6. In general, an antibody binding to the LRP6-Wnt1 site would be expected to block Wnt1-induced Wnt pathway activation.

[0156] To prepare the Fc N-terminal binding domain containing a homodiabody specific for a FZD, the VH and VL fragments, VH-1, VH-2, VL-1 and VL-2, of the selected FZD antibodies were amplified by PCR from the corresponding phagemid templates and isolated. Gibson assembly was then utilized to introduce the isolated fragments (VH-1 and VL-2) into an EcoRI/XhoI precut vector containing an Fc-knob region (pSCST backbone) (see Gibson et al. (2009). *Nature Methods*. 6 (5): 343–345 and Gibson DG. (2011). *Methods in Enzymology*. 498: 349–361). Gibson assembly was also utilized to introduce the fragments (VH-2 and VL-1) into the EcoRI/XhoI precut vector containing an Fc-hole region. Correct assembly was validated using DNA sequencing. The two plasmids (one pair, Fc-knob and Fc-hole) were then used to introduce the second binding domain at the C-terminus of the Fc domain.

[0157] The Fc-knob and Fc-hole configuration was needed to generate multivalent binding domains wherein one of the binding domains was a heterodiabody. However, the Fc-knob and Fc-hole configuration was not needed to prepare binding molecules that comprise homodiabodies on both the N and C termini of the Fc domain and thus for such binding molecules the VHs and VLs were linked to a wild-type Fc region and only one plasmid was used to generate the VH-VL containing polypeptides to form the homodimer. Optionally, a linker, e.g. a peptide linker, or a non-peptidic linker, can be present between the binding domains and the Fc domain .

[0158] To generate the C-terminal binding domain, an LRP5/6 antibody was identified and an LRP5/6 diabody was generated following the same protocol as described above for generating the FZD diabody. The C-terminal binding domain was generated by PCR amplifying the VH-3, VH-4, VL-3 and VL-4 fragments from the corresponding phagemid template for the LRP antibody and then isolating the amplified fragments. As described above, Gibson assembly was then utilized to introduce the VH-3 and VL-4 fragments into the PpuMI/BamHI site of the Fc-knob plasmid described above. Gibson assembly was used to insert the other VH-4 and VL-3 fragments into the PpuMI/BamHI cut of the Fc-hole plasmid

[0159] Two plasmids (one pair, Fc-knob and Fc-hole) with differing VL and VH sequences were used to generate a FZD or co-receptor binding domain that was bispecific, i.e., capable of binding to two different sites. Because a knob-into-hole configuration was not needed to generate a dimer having monospecific binding domains, only a single plasmid containing the wild-type Fc sequence was used if each of the binding domains were to be monospecific.

[0160] Figure 9A depicts a plasmid encoding the peptide comprising an Fc region comprising a “knob” mutation, the VH and VL of panFZD antibody #5019, and the VL of LRP antibody #2542 and the VH of LRP antibody #2539. Figure 9B depicts a plasmid encoding the peptide comprising a nucleic acid encoding Fc region comprising a “hole” mutation, the VH and VL of panFZD antibody #5019, and the VH of LRP antibody #2542 and the VL of LRP antibody #2539. The peptides encoded by these plasmids form a heterodimer having a multivalent binding site comprising a homodiabody derived from the pan specific FZD antibody #5019 and a multivalent binding site comprising a bispecific heterodiabody produced by the pairing of VL of LRP antibody #2539 and VH of LRP antibody #2542 from one peptide with the VH of LRP antibody #2539 and the VL of LRP antibody #2542 of the other peptide.

[0161] The resulting plasmids were then sequenced and the sequenced-verified plasmids were prepared using a PureLink HiPure Plasmid Filter Maxiprep Kit (Invitrogen) according to manufacturer’s instructions. The plasmids were then transfected into Expi293F cells (Thermo Fisher Scientific) and FectoPRO Reagent (Polyplus) was used for antibody expression according to the manufactory’s instructions. Typically, a scale of 200ml cell was used for a small batch antibody production.

[0162] Typically, 80h after transfection, the Expi 293F cell culture medium was harvested by centrifugation to pellet the cells and cellular debris. The supernatant was

transferred to a clean bottle and buffered with 10xPBS buffer. After 1h incubation with appropriate amount of Protein A beads (GE Healthcare), the beads were washed and the binding molecules were eluted according to the manufacturer instruction. Finally, the buffer was exchanged into PBS.

2. Heterodimeric multivalent binding molecules

[0163] Using the methods described above, we also generated tetravalent heterodimeric molecules which contain intact bispecific diabodies fused to each of the N-terminus and C-terminus of Fc domain (Knob/Hole), (Figure 2A and 3A). In particular, we generated a tetravalent binding molecule having a FZD binding homodiabody derived from antibody 5019 on the N-terminus of the Fc domain and a homodiabody derived from LRP6-W1 antibody 2542 (5019-Fc-2542) or LRP6-W3 antibody 2539 (5019-Fc-2539) on the C-terminus of the Fc domain. Surprisingly, both tetravalent molecules activated the Wnt pathway, but 5019-Fc-2542 had much less efficacy (FIG. 3C). Without wishing to be bound by theory this difference may reflect differences in capacity of LRP6-W1 and LRP6-W3 binding to activate Wnt signaling. Wnt binding of the LRP6-W3 site has been observed to be more effective in activating Wnt signaling than Wnt binding to the LRP6-W1 site.

[0164] We also generated a tetravalent trispecific binding molecule having a FZD binding homodiabody derived from antibody 5019 on the N-terminus of the Fc domain and a LRP heterodiabody derived from LRP6-W1 antibody 2542 and LRP6-W3 antibody 2539 on the C-terminus of the Fc domain (5019-K/H-2539-2542, named as 5019Ag) (Figure 5). 5019Ag is unexpectedly effective in activating Wnt signaling as compared to the molecules having a monospecific LRP6 homodiabody (Figure 3C). Nanomolar amounts of all three forms activate Wnt signaling determined by pBAR luciferase reporter assays (Figure 3D), indicating they are effective Wnt mimics. Without wishing to be bound by theory it is contemplated that engagement of a strong Wnt3A site and a weak Wnt1 site together is more effective than engagement of two strong Wnt3A sites. The two best multivalent binding molecules having an FZD binding domain and a LRP binding domain, “FLAgS”, had single-digit nanomolar potency ($EC_{50} \sim 5$ nM), which was virtually identical to the potency of purified Wnt3A, and displayed a bell-shaped dose response profile (Figure 11D). We interpret this as indicating that maximal stimulation requires multivalent binding of the FLAg and that decreased efficacy at higher concentrations is likely attributable to monovalent binding to either FZD or LRP6. We treated RKO cells, which express low levels of β catenin (Major et al. Science. 316 , 1043–1046 (2007)), with F^{P+P} -L6¹⁺³ which caused dose- and time-

dependent increases in β catenin protein levels and phosphorylation of DVL2, a hallmark of Wnt-FZD pathway activation (FIG. 11E and FIG. 11F). Thus, tetravalent FLAgs are modular, engineerable, human Ab modalities that function as synthetic agonists of FZD and LRP6.

[0165] To confirm the engineered affinity and specificity of the optimal FLAg F^{P+P} -L6¹⁺³, we used Bio-Layer Interferometry (BLI) to measure its binding kinetics to nine of the 10 human FZD CRDs and to human LRP6 ECD (FIG. 12A and FIG. 12B). The FLAg bound with affinities in the picomolar range ($K_D = 10$ -800 pM) to the six FZDs recognized by the FZD diabodies derived from the parent pan-FZD paratope (Pavlovic et al. 2018) but did not bind detectably to the other three FZDs. Moreover, affinity for LRP6 was in the nanomolar range ($K_D = 12$ nM) (FIG. 12B). We then used BLI to assess FLAg binding to various Fc receptors.

[0166] The FLAg behaved similarly to a conventional IgG and interacted with FcRn in a dose and pH dependent manner (FIG. 12C). Natural IgGs bind to FcRn at pH 6 but not at pH 7.4, and this enables recycling during pinocytosis and consequent long half-life in vivo. The FLAg also behaved similarly to the IgG for interaction with other Fc effectors including complement (C1q), the natural killer cell marker CD16a, the B cell marker CD32a, and the monocyte and macrophage marker CD64 (FIG. 12D). We conclude that the FLAg contains a functional Fc moiety that should confer effector functions and long half-life in vivo.

[0167] The modular design of the tetravalent F^{P+P} -L6¹⁺³ FLAg allowed us to dissect the contributions of each of the four paratopes to the intrinsic agonist activity by replacing each with a null paratope binding to the irrelevant antigen maltose-binding protein (MBP). We generated “mono-binding” molecules comprising an Fc domain and an FZD binding domain attached to one Fc domain terminus and LRP binding domain attached the other Fc domain terminus, but rather than having two binding sites for FZD or LRP within the diabodies, the binding domains have only a single or mono binding site, and one control maltose-binding-protein binding site, “MBP”. One MBP binding site was introduced into at least one binding domain of the molecules to generate five mono binding molecules. The 5019-MBP-K/H-2539-2542, which contains one FZD and one MBP binding site in the N-terminus, still activates the Wnt pathway, but has an 8-fold decrease in efficacy as compared to 5019Ag (FIG. 3E). Similarly, the 5019-K/H-2539-MBP, which retains only one LRP6-W3 site in the C-terminus, exhibits much less Wnt activation as compared to 5019Ag (Figure 3E). Minimal agonistic activity was detected for the two MBP-FZD/MBP-LRP6 molecules 5019-MBP-K/H-2539-MBP and 5019-MBP-K/H-MBP-2542 and the molecules having one LRP6-W1

diabody, 5019-K/H-MBP-2542 (Figure 3E). The results of these β catenin signaling assays showed that maximal stimulation was reduced significantly by disabling one anti-FZD paratope or the anti-LRP6 paratope for the WNT1 binding site and was completely ablated by disabling the anti-LRP6 paratope for the WNT3A binding site or by simultaneously disabling one anti-FZD paratope and either of the anti-LRP6 paratopes. We also substituted an anti-LRP5 paratope targeting the WNT3A binding site for the anti-LRP6 paratope targeting the WNT1 binding site to generate a molecule (F^{P+P} -L5/6³) that could recruit both co-receptors and observed activity similar to that of F^{P+P} -L6¹⁺³ (FIG. 3F, $EC_{50} = 4$ nM). Taken together, these data showed that optimal agonist activity is achieved with a molecule capable of recruiting two FZDs through a common epitope and LRP6 through two distinct epitopes, but activity can be modulated to intermediate levels by disabling one of the anti-FZD or anti-LRP6 paratopes. Moreover, molecules that could recruit FZD and two different co-receptors were generated by combining two anti-FZD paratopes with one paratope each for LRP5 and LRP6.

[0168] We also explored the requirements for geometric and spatial constraints imposed by the intermolecular diabody format by substituting diabody pairs with pairs of less constrained intramolecular single-chain variable fragments (scFvs) (FIG. 2J). Compared with F^{P+P} -L6¹⁺³, a FLA_g that contained anti-FZD scFvs (F^{P*+P*} -L6¹⁺³) exhibited similar activity, whereas activity was significantly reduced for FLA_gs that contained anti-LRP6 scFvs (F^{P+P} -L6^{1*+3*}) or scFvs at both ends (F^{P*+P*} -L6^{1*+3*}). These differences in activity were not due to differences in affinity, as BLI measurements showed comparable, high-affinity binding to LRP6 and FZD isoforms regardless of whether paratopes were presented in the diabody or scFv format (FIG. 2K and FIG. 2L). Taken together, these results showed that particular stoichiometries and geometries are required for the assembly of optimal FZD/LRP6 signaling complexes, and constraints are especially precise for LRP6, which requires engagement of two distinct epitopes in a specific geometry dictated by the diabody format. Notably, the looser constraints for FZD engagement enabled significant activation with a single anti-FZD paratope (FIG. 2D), which opens the door for further enhancing specificity or altering signaling by recruiting a different cell surface protein through an additional paratope in conjunction with an anti-FZD paratope at the N-termini of the heterodimeric Fc.

3. Other bispecific antibody forms

[0169] Bispecific molecules comprising a FZD binding domain of antibody #5019 and LRP6-W1 binding domain of antibody #2942 (5019/2942) or LRP6-W3 binding domain of

antibody #2539 (5019/2539) on the same terminus of an Fc domain were constructed and the corresponding proteins were purified (Figure 2A) and assayed for activation of Wnt signaling using pBAR luciferase reporter assays. These molecules failed to activate Wnt signaling. Notably, both bispecific molecules antagonized the activity of the Wnt ligand (Figure 2B). Without wishing to be bound by theory, the distance and flexibility between the two paratopes of these bispecific molecules might not recruit the FZD and LRP6 receptor in a suitable geometry for activation.

[0170] Bispecific molecules comprising a FZD diabody and an LRP diabody attached to the same terminus of an Fc domain were also generated using a knob in hole configuration. These diabodies designated 5019-2539-K/H (FZD/LRP-W3) and 5019-2542-K/H (FZD/LRP-W1) were assayed for FZD and LRP binding and Wnt pathway activation. Both diabodies retained the FZD binding profile of the original antibody as well as the LRP6 binding activity (FIGS. 2D -2G). Both molecules bound individually to the FZD receptor and the LRP co-receptor. 5019-2542-K/H displayed co-binding to both FZD and LRP in solution as determined with BLI assays (FIG. 2H) but no significant co-binding was observed with 5019-2539-K/H. Neither 5019-2539-K/H nor 5019-2542-K/H activated Wnt signaling as determined in pBAR luciferase reporter assays, similar to the results obtained with the homo-diabodies that bound to only a FZD receptor (5019-Fc) or co-receptor (2539-Fc) (FIG. 2I). Moreover, both 5019-2539-K/H (FZD/LRP-W3) and 5019-2542-K/H (FZD/LRP-W1) effectively inhibited Wnt3a mediated pathway activation (Figure 2I).

4. Wnt Pathway Signaling Assay

[0171] Wnt pathway activation was assayed in HEK293 cells using the pBAR luciferase reporter system that faithfully monitor the transcriptional activation of β catenin (Biechele and Moon, *Methods Mol Biol.* 2008;468:99-110, PMID: 19099249). Briefly, HEK293T cells stably expressing pBARLS and pSL9 Efl α -Renilla Luciferase constructs were seeded in 96-well plates at 1.5E4 cells/well. 24 hours following seeding, cells were treated with the indicated FZD agonists in triplicate at indicated concentrations or PBS vehicle control. 16.5 hours after treatment cells were lysed and luminescence was measured using Dual-Luciferase Reporter Assay System (Promega #E1960), according to manufacturer's protocol. Firefly luminescence was normalized to Renilla luminescence for each well, to control for cell number.

[0172] We assayed the agonist activity of multivalent molecules containing an N-terminal FZD diabody derived from an antibody fragment (Antibody #5019) that recognized several of

the FZD receptors (FZD 1, 2, 4, 5, 7, and 8) joined via the Fc domain to an LRP binding domain on the C-terminus of the Fc domain. The C-terminal LRP binding domains comprised a diabody derived from one of two LRP6 antibodies, #2539 and #2542, which bind to the Wnt3 site and Wnt1 site respectively (FIG. 6B). Nanomolar amounts of these multivalent binding molecules, denoted 5019-Fc-2539 and 5019-Fc-2542 activated the Wnt- β catenin pathway (FIG. 6C), however, treatment of cells with the molecule harboring the LRP6 antibody targeting the Wnt3 site, 5019-Fc-2539, led to an approximately 10 folds higher activation when compared to 5019-Fc-2542 (200 folds vs 20 folds over background respectively) (FIG. 6C).

[0173] Importantly, using a knob-hole system engineered within the Fc moiety we generated multivalent binding molecules (FIG. 1C) that contained a homodiabody for the pan-FZD binding domain on one end (#5019) and an heterodiabody forming LRP6 binding domain with binding sites for Wnt1 (#2542) and Wnt3 (#2539) 5019-K/H-2539:2542 on the other end (FIG. 6B). This configuration enabled the incorporation of 4 different binding sites within the molecule with different selectivity and affinity profiles, i.e., tetravalent and trispecific. When tested in the β -catenin luciferase reporter assay in HEK293 cells, this molecule has a 2 folds higher activation than 5019-Fc-2539 or approximately 400 folds over background (FIG. 6C).

[0174] We also substituted the binding sites for LRP6 for equivalent LRP5 binding sites (diabodies derived from 2459 and 2460 antibodies which both bind LRP5) within the knob-in-hole system along with the same pan-FZD diabody that binds FZD1, 2, 4, 5, 7, 8 (5019). This molecule 5019-K/H-2459:2460 was also able to activate the Wnt- β catenin pathway in HEK293T cells (FIG. 6D), albeit with lower efficacy than the agonist harboring the LRP6 diabodies.

5. Characterization of selective FZD agonists (Agonist modularity with binding domains derived from selective FZD and co- receptor antibody fragments)

[0175] To assess activities of our monospecific FZD agonists, we used cell-based assays that depend on particular FZD isoforms. We prepared multivalent binding molecules that only bound to one of the ten FZD receptors. Our previous work identified several antibodies that are completely specific for FZD4 (5038, 5044, 5048, 5062, 5063, 5080, 5081) (see, e.g., US20160194394, inventors Sidhu et al. and WO2017127933A1, inventors Pan et al.). Multivalent binding molecules comprised a FZD binding domain that was FZD4 specific and an LRP6 binding domain comprising a bispecific heterodiabody derived from antibodies

2539 and 2542 were generated using the Fc knob-in-hole system. These molecules could activate FZD4 signaling through the β -catenin pathway but only when co-transfected into HEK293 cells along with FZD4 cDNA. These FZD4 binding molecules could not activate FZD4 signaling or the β -catenin pathway in non-modified HEK293T cells, which express low levels of FZD4. Thus, this experiment demonstrates the specificity of the molecules for FZD4. 5019-K/H-2539-2542 (the pan-FZD agonist described above) can activate signaling in HEK293T cells even in the absence of FZD4 (FIG. 4A). This result is not surprising as Wnt-mediated activation of β -catenin signaling HEK293T cells occurs through FZD1, 2 and 7 (Voloshanenko et al. FASEB 2017 FASEB J. 2017 Nov;31(11):4832-4844; PMID: 28733458) and the 5919 FZD antibody binds to all three receptors.

[0176] In addition, we generated a FZD5 specific multivalent binding molecule using the binding domain of the FZD5 specific antibody 2928, which we previously characterized to bind only to FZD5 (Steinhart et al. Nat Med. 2017 Jan;23(1):60-68, PMID: 27869803; WO2017127933A1, inventors Pan et al.). We previously demonstrated that several *RNF43* mutant pancreatic ductal adenocarcinoma (PDAC) cell lines are dependent only on FZD5 signaling for their proliferation (Steinhart et al. 2017, PMID: 27869803). Indeed, genome-wide CRISPR essentiality/fitness screens in three *RNF43* mutant PDAC lines showed that FZD5 was one of the most essential genes for their growth whereas PDAC cell lines with WT *RNF43* did not exhibit this requirement for FZD5. When RNF43 mutant cells are treated with a Porcupine inhibitor (PORCNI; such as LGK-974) that inhibits the palmitoylation and activity of Wnt ligands, *RNF43* mutant cells stop proliferating.

[0177] Co-treatment of *RNF43* mutant cells with the pan-FZDag 5019-K/H-2539-2542 or with the selective FZD5 agonist 2928-K/H-2539-2542 led to robust rescue of cell proliferation blocked by LGK974. These results demonstrate that these two molecules were capable of activating FZD5 and induced Wnt signaling in these cells, thereby mimicking the action of endogenous Wnt ligands (FIG. 7B). In contrast, addition of the FZD4 specific agonist 5038-K/H-2539-2542 or a FZD2 specific agonist were unable to rescue the inhibition of proliferation mediated by LGK974.

[0178] RNAseq analysis has shown that *FZD2* is the predominant isoform in the mesenchymal stem cell line CH3H10T1/2 (Mouse ENCODE), suggesting that FZD2 may be responsible for the established role of Wnt proteins during osteogenic differentiation of mesenchymal cells (Day et al. Dev. Cell. 8, 739–750 (2005)). Stimulation of C3H10T1/2 cells with a FZD2-specific FFlag led to robust induction of the osteogenic marker alkaline

phosphatase (*ALPL*) to levels similar to those achieved with a Pan-FZD FLAg, whereas a FZD5-specific FLAg exhibited minimal activity (**FIG. 7B**).

6. Co-targeting with the tetravalent binding molecules

[0179] In addition to mixing and matching FZD multivalent binding domains and co-receptor binding domains with an Fc domain to achieve desired combinations, the existence of tetravalent paratopes in the current system provides an opportunity for targeting two FZD receptors and two co-receptors simultaneously with one molecule, ensuring the co-localization when applying in vivo. Considering the agonistic activity of 5019-MBP-K/H-2539:2542 shown above, the generation of a multivalent binding molecule having binding domains for selective FZD receptors by combining the binding regions within an heterodiabody at the N-terminus of the Fc domain. For example, the binding domains derived from antibody 5038 (binds FZD4) and 2928 (binds FZD5) would yield a FZD4 and FZD5 co-targeting molecule. The binding molecules can also be generated to have a co-receptor binding domain for specific or multiple co-receptors. For example, an LRP6/LRP5 co-targeting binding domain could be produced by combining the binding domains derived from the 2459 (binds Wnt1 binding site of LRP6) and 2539 (binds Wnt3a binding site of LRP6) antibodies on the C-terminal of the Fc domain. Likewise, the co-receptor binding domain may comprise a binding site for an LRP6 in combination with another co-receptor, e.g., ROR1/2, to initiate activation of both canonical and non-canonical Wnt signaling pathways in a single cell.

[0180] Also contemplated herein is a multivalent binding molecule having a tissue specific binding domain derived from a tissue specific antibody which would recruit the multivalent binding molecule to a desired tissue where it would then activate Wnt signaling by binding a FZD receptor and co-receptor. This is contemplated to be particularly useful when using the multivalent binding molecules in regenerative therapeutics when desired effects may need to be restricted to a specific tissue. To summarize, the tetravalent mode allows more designing flexibility to meet versatile functional requirements.

7. Multivalent binding molecules having a FZD binding domain and co-receptor binding domain can replace Wnt ligands to sustain intestinal organoid cultures.

[0181] The effect of the FZD agonists described herein on organoid survival and maintenance was assayed as follows. An 8-week old female C57BL/6 mouse was sacrificed, and small intestine crypts were harvested for organoid isolation (O'Rourke et al. 2016. Isolation, Culture, and Maintenance of Mouse Intestinal Stem Cells. *Bio Protoc.* 20:4).

Organoid cultures were passed by mechanical dissociation (O'Rourke 2016) and embedded in 25µl of Growth Factor Reduced Matrigel (Corning, 356231) in a 48 well plate. Organoids were plated in triplicates for each experimental condition. Complete organoid media (O'Rourke 2016) with experimental conditions (1µM LGK-974 +/- 40% Wnt3a conditioned media or +/- 50nM panFzd-5056 (a FZDag targeting FZD1, 2, 4, 6, 7, 8 but binding to an epitope that does not compete with Wnt ligands)) was added to each well on day of passaging and changed every 2-3 days. After one week, 150µl of Cell Titer Glo 3D (Promega) was added to 150µl of media in each well. Organoids were lysed on a rocking platform for 30 min at RT. The luminescence reading was measured in duplicates for 20µl of lysate from each well. The average luminescence reading for each condition was normalized to the DMSO condition to calculate viability.

[0182] Being pervasive stem cell niche factors, Wnts and R-spondins are required for the derivatization and maintenance of three-dimensional culture organoids from many tissues. In vitro, Wnt proteins secreted by paneth cells are sufficient to support the growth of mouse small intestine organoids in the presence of R-spondins. However, if Wnt release and activity is blocked with the PORCNI LGK974, the organoids can't proliferate and eventually die. Herein we demonstrate that a pan-FZD multivalent binding molecule of this invention, FZDag (F^{P+P}-L6¹⁺³) can rescue and sustain the growth of organoids in the presence of LGK974, suggesting that the molecule functionally mimics Wnt ligands (FIG. 8) and can substitute for Wnt proteins to support growth of tissue organoids,. Because Wnt ligands are integral components of the media required to grow many human tissue organoids, the antibody-derived FZD agonists of this invention are expected to promote the derivatization, survival and maintenance of organoids of different tissues when included in the culture media and thereby alleviate limitations associated with the use of conditioned media or purified Wnt proteins.

8. Multivalent binding molecules promote bone regeneration

[0183] A rat closed femoral fracture model is used to evaluate the regenerative properties of multivalent binding molecules of this invention having a first multivalent binding domain that binds FZD2, and a co-receptor binding domain that binds to LRP5 or LRP 6. The first multivalent binding domain may specifically bind FZD2 or may bind FZD2 and other FZD receptors.

[0184] Rats are administered vehicle or the multivalent binding molecule following unilateral closed femoral mid-diaphyseal fractures (see Bonnarens, and Einhorn, J. Orthop.

Res. 2, 97–101 (1984)). Briefly, an 18-gauge syringe needle is inserted into the medullary canal through the condyles. A transverse fracture of the femur is then created via blunt impact loading at the anterior (lateral) aspect of the thigh. One day after the fracture, rats are injected subcutaneously with either saline vehicle or multivalent binding molecules twice per week for 7 weeks. At termination, the intramedullary pins are removed and the fractured femurs will be analyzed by microCT.

[0185] The multivalent binding molecules having a multivalent domain that binds FZD2, and a second multivalent binding domain that binds to LRP5 or LRP6 significantly increases regeneration of bone in this model in comparison to bone regeneration by the vehicle alone.

EXAMPLE II -Synthetic antibodies targeting FZD and LRP6

[0186] We previously applied phage display to derive hundreds of synthetic Abs using nine recombinant FZD CRDs as antigens (FZD3 CRD could not be purified) (Steinhart et al. Nat. Med. 23 , 60 (2016); Pavlovic et al. MAbs (2018), doi: 10.1080/19420862.2018.1515565). Systematic characterization revealed a continuum of specificity profiles with some Abs displaying broad specificities, exemplified by a pan-FZD Ab (FP) that recognized FZD1/2/4/5/7/8 (FIG.11A), others displaying more restricted specificities, and some being monospecific (FIG. 11B). Functional characterization revealed that some antibodies competed with Wnt and inhibited β catenin signaling, whereas others were non-competitive and did not interfere with Wnt signaling (FIG. 11B). In total, we fully characterized 161 anti-FZD antibodies, including 47 inhibitors of Wnt signaling. Unexpectedly as discussed herein, regardless of whether or not they competed with Wnt and inhibited Wnt signaling, all the multivalent binding molecules that we generated by using these anti-FZD antibodies as the source of the FZD binding domains in conjunction with an LRP binding domain, e.g., a binding domain that bound to Wnt1 and/or Wnt3a binding sites on LRP 5/6, were agonists of the Wnt pathways.

EXAMPLE III - Phenotypic effects of FLAgs in cells, organoids and animals

[0187] Having established that FLAgs selectively engage FZD and LRP to activate Wnt-associated signaling pathways, we explored the phenotypic effects of these signals in progenitor stem cells (PSCs), organoids and animals. Modulation of Wnt- β catenin signaling activity is integral to most PSCs differentiation protocols (Huggins et al. Methods Mol. Biol. 1481 , 161–181(2016)). Treatment of human PSCs with WNT3A conditioned media, or small molecule inhibitors of GSK3, activates β catenin signaling, leads to primitive streak induction, and promotes mesodermal fate specification (Davidson et al. PNAS U.S.A. 109 , 4485–4490

(2012)). We evaluated FLAg activity in this context and found that treatment of human PSCs with 30 nM F^{P+P} -L6¹⁺³ for three days caused robust induction of the mesoderm marker BRACHYURY and decreased expression of the pluripotency marker OCT4 to levels comparable to treatment with the GSK3 inhibitor CHIR99021 at 6 μ M (FIG. 13A and FIG. 13B).

[0188] F^{P+P} -L6¹⁺³ recognizes mouse FZDs and LRP6, and it contains an Fc that interacts with the FcRn. It is contemplated that the Fc endows the molecule with a long, Ab-like, half-life in vivo. Thus, we tested whether F^{P+P} -L6¹⁺³ could interact with endogenous receptors in mice and accumulate to levels that would be sufficient to activate β catenin signaling and mobilize endogenous stem cell activity. Within the intestinal stem cell niche, Wnt proteins secreted by mesenchymal cells induce expression of β catenin target genes in stem cells at the bottom of the crypt to direct their self-renewal, and the target gene LGR5 is frequently used as a marker of stem cells in various tissues. Treatment of LGR5-GFP mice with LGK974 ablated Wnt production and caused rapid extinction of LGR5 expression and the linked GFP signal in crypt stem cells. Strikingly, GFP expression was rescued upon co-treatment with F^{P+P} -L6¹⁺³ by intraperitoneal injection (Figure 14 right panel). We conclude that F^{P+P} -L6¹⁺³ has a sufficient half-life and bioavailability to enable β catenin activation at levels that promote self-renewal of intestinal stem cells in the absence of endogenous Wnt.

EXAMPLE IV - Materials and Methods :

1. Ab selections and screens

[0189] The phage-displayed synthetic library F was used to select for Fabs that bound to Wnt receptors, as described (Persson et al. J. Mol. Biol. 425 , 803–811 (2013)). Briefly, Fc-tagged ECD protein (R&D Systems) was immobilized on Maxisorp immunoplates (ThermoFisher, catalog number 12-565-135) and used for positive binding selections with library phage pools that were first exposed to similarly immobilized Fc protein to deplete non-specific binders. After 4 rounds of binding selections, clonal phage were prepared and evaluated by phage ELISA (Birtalan et al. J. Mol. Biol. 377 , 1518–1528 (2008)). Clones that displayed at least 10-fold greater signal for binding to antigen compared with Fc were considered to be specific binders that were subjected to further characterization.

2. Recombinant proteins and reagents

[0190] Fc-tagged fusions of FZD1 (5988-FZ-050), FZD2 (1307-FZ-050), FZD4 (5847-FZ-050), FZD5 (1617-FZ-050), FZD7 (6178-FZ-050), FZD8 (6129-FZ-050), FZD9 (9175-FZ-050), FZD10 (3459-FZ-050) were purchased from R&D Systems. The Fc-tagged ECD of

FZD6 (residues 19-132, UniprotO60353-1) was expressed and purified from Expi293 cells using the pFUSE-hIgG1-Fc2 vector (Invivogen) and the single protomer species was separated from aggregated protein by size exclusion chromatography on a Superdex 200 (10/300) column (GE Healthcare). Fc-tagged ECD fusion proteins of human (1505-LR-025) and mouse (2960-LR-025) LRP6 and mouse LRP5 (7344-LR-025/CF) were purchased from R&D Systems. WNT1 (SRP4754-10ug), WNT2b (3900-WN-010/CF), WNT5a (645-WN-010/CF) and WNT3A (5036-WN-010/CF) were purchased from R&D Systems, and WNT3A conditioned media was prepared as described (PMID:12717451). Other proteins and chemicals were purchased from the following suppliers: FcRN (R&D, 8693-FC), C1q (Sigma, C1740), CD16a (R&D, 4325-FC), CD32a (R&D, 1330-CD/CF), CD64 (R&D, 1257-FC), LGK974 (Cayman Chemicals), the Porcupine Inhibitor C59 (Dalriada Therapeutics), and CHIR99021 (Sigma Aldrich).

3. Tetravalent binding molecules for FZD and LRP, “FLAgS”, and antibody cloning

[0191] DNA fragments encoding antibody (Ab) variable domains were either amplified by the PCR from phagemid DNA template or were constructed by chemical synthesis (Twist Biosciences). The DNA fragments were cloned into mammalian expression vectors (pSCSTa) designed for production of kappa light chains and human IgG1 heavy chains. Bispecific diabodies and IgGs contained an optimized version of a “knobs-in-holes” heterodimeric Fc (Ridgway et al. Protein Eng. 9, 617–621 (1996)). FLAgS and diabody-Fc fusions were arranged a VH-VL orientation with the variable domains separated by a short GGGGS (e.g. amino acids 121-125 of SEQ ID NO: 2) linker, which favors intermolecular association between VH and VL domains and thus favors diabody formation. To produce diabody-Fc fusion constructs, diabody chains were fused to human IgG1 Fc. FLAg proteins were constructed as VH-x-VL-y-[human IgG1 Fc]-z-VH-x-VL where linkers are x= GGGGS (e.g. amino acids 121-125 of SEQ ID NO: 2), y= LEDKTHTKVEPKSS (amino acids 232 to 245 of SEQ ID NO: 4), and z= SGSETPGTSESATPESGGG (amino acids 473 to 501 of SEQ ID NO: 4). 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 GTTAASGSSGSSSGA (SEQ ID NO: 75) linker, which favors intramolecular association between VH and VL domains and thus favors scFv formation. For all constructs, the entire coding region was cloned into a mammalian expression vector in frame with the secretion signal peptide.

4. Protein expression and purification

[0192] Antigen, Ab, and FLA_g proteins were produced in Expi293F (ThermoFisher) cells by transient transfection. 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.

5. In vitro binding assays

[0193] BLI assays were performed using an Octet HTX instrument (ForteBio). For measuring binding to antigen, Fc-tagged fusions of FZD receptors (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 nM Ab or FLA_g in assay buffer (PBS, 1% BSA, 0.05% Tween20) and association was monitored for 300 seconds. Sensors were then transferred into assay buffer and dissociation was monitored for an additional 300 seconds. Shake speed was 1000 rpm and temperature was 25 °C. End-point response values were taken after 295 seconds of association time. End-point data were analyzed by subtracting the Fc signal from the FZD-Fc signal and then normalizing the data to the highest binding signal.

[0194] For measuring binding to Fc receptors, Abs or FLA_gs were immobilized on AR2G sensors (18-5092, ForteBio) by amine coupling to achieve a BLI response of 0.6-3 nm and remaining sites were quenched with ethanolamine. Coated sensors were equilibrated in assay buffer (PBS, 1% BSA, 0.05% Tween20) and transferred into Fc receptor solutions. Association was monitored for 600 seconds, the sensors were transferred to assay buffer, and dissociation was monitored for 600 seconds. CD64 and all other Fc receptors were assayed at 50 nM or 300 nM, respectively, at pH 7.4, unless as indicated. Shake speed was 1000 rpm and temperature was 25 °C. End-point response values were taken at the end of the association phase and were normalized to isotype controls. Steady-state FcRN binding assays

were performed in a similar manner, except that FcRN was immobilized and serial dilutions (0.1 – 225 nM) of Ab or FLAg were assessed in solution. The association and disassociation times were 600 or 1200 seconds, respectively.

[0195] Surface plasmon resonance (SPR) assays were performed using a ProteOn XPR36 system (Bio-Rad). FZD-Fc or LRP-Fc proteins were immobilized to GLC sensor surface (176-5011) using standard amine coupling chemistry. Abs or FLAgs in assay buffer (PBS, 0.05% Tween20, 0.5% BSA) were injected at 40 μ l/min and association was monitored for 150 seconds. Assay buffer was then injected at 100 μ l/min and dissociation was monitored for 900 seconds. Assays were performed at 25 °C. Analysis was performed using a 1:1 Langmuir model and globally fit to determine k_{on} and k_{off} values using ProteOn Manager software. KD was calculated as the ratio of k_{off}/k_{on} .

6. Epitope binning

[0196] BLI epitope binning experiments were performed using an Octet HTX instrument (ForteBio). Fc fusions with FZD (FZD-Fc) or with LRP6 (LRP6-Fc) protein were immobilized on AHQ (18-5001, ForteBio) or AR2G (18-5092, ForteBio) BLI sensors, respectively. Coated sensors were transferred into 100 nM Ab in assay buffer (PBS, 1% BSA, 0.05% Tween20) for 240 seconds to achieve saturation of binding sites. Sensors were then transferred into 100 nM competing Ab in assay buffer for 180 seconds. Response at 20 seconds after exposure to competing Ab was measured and normalized to binding signal on unblocked antigen-coated sensors. Shake speed was 1000 rpm and temperature was 25 °C.

7. Cell lines

[0197] HPAF-II and HEK293T cell lines were maintained in DMEM containing 4.5 g/L D-glucose, Sodium pyruvate, L-glutamine (ThermoFisher #12430-054) and supplemented with 10% FBS (ThermoFisher) and Penicillin/Streptomycin (ThermoFisher #15140-163). CHO cells were maintained in DMEM/F12 (ThermoFisher #11320-033) supplemented with 10% FBS and penicillin/streptomycin. Cells were maintained at 37 °C and 5% CO₂.

8. Flow cytometry

[0198] Indirect immunofluorescence staining of cells was performed with 10 nM anti-FZD Fab for the CHO cell lines as previously described (Steinhart et al. 2017 Nat Med. Jan;23(1):60-68, PMID: 27869803). Alexa Fluor 488 AffiniPure F(ab')₂ was used as the secondary antibody (Jackson ImmunoResearch, 109-545-097). Anti-c-Myc IgG1 9E10 (primary antibody, ThermoFisher, MA1-980) and Alexa Fluor 488 IgG (secondary antibody, Life technologies, A11001) were used as controls for expression. All reagents were used as

per manufacturer's instructions.

9. Luciferase reporter assay

[0199] HEK293T cells were transduced with lentivirus coding for the pBARl's 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, FLAg or Ab protein was added, and following 15-20 hours of stimulation, cells were lysed and luminescence was measured in accordance with the dual luciferase protocol (Promega) using an Envision plate reader (PerkinElmer). For the FZD4-specific agonist assay, FZD4 cDNA was transfected for 6 hours prior to adding FLAg protein. 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.

10. Western blot assay

[0200] H1 ESCs were solubilized with lysis buffer (1% Nonidet P-40, 0.1% sodium dodecyl sulfate (SDS), 0.1% deoxycholic acid, 50 mM Tris (pH 7.4), 0.1 mM EGTA, 0.1 mM EDTA, 20 mM sodium fluoride (NaF), 1:500 protease inhibitors (Sigma) and 1 mM sodium orthovanadate (Na_3VO_4)). Lysate was incubated for 30 min at 4 °C, centrifuged at $14,000 \times g$ for 10 min, boiled in SDS sample buffer, separated by SDS-polyacrylamide gel electrophoresis, transferred onto a nitrocellulose membrane and Western blotted using indicated Abs. Ab detection was performed by a chemiluminescence-based detection system (ECL; ThermoFisher).

11. Crystal violet proliferation assay

[0201] HPAF-II cells were seeded at 500 cells per well, and after 24 hours, 100 nM LGK974 was added with or without 100 nM FLAg. Medium was changed and drug treatment was renewed every other day. Cells were fixed with ice-cold methanol after 7 days treatment. Cells were stained with 0.5% crystal violet solution in 25% methanol, destained in 10% acetic acid and quantified by measuring absorbance at 590 nm.

12. Immunofluorescence

[0202] H1 hES treated with FLAg and CHIR99021 for 3 days were washed with cold PBS, and fixed for 20 min with 4% PFA. Fixed cells were rinsed with PBS, permeabilized

with 0.3% triton for 10 min, and blocked with 1% BSA for 1 hour. Cells were incubated for 2 hours with primary Abs for BRACHYURY (R&D systems AF2085; goat; dilution 1:100) or OCT3/4 (Santa Cruz sc5279; mouse; dilution 1:100) in 1% BSA and 1 hour with Alexa Fluor 488-labeled donkey anti-goat or Alexa Fluor 568-labeled donkey anti-mouse Ab (FIG. 13A). Coverslips were mounted using Fluoromount (Sigma-Aldrich) and analyzed on a Zeiss LSM700 confocal microscope using a 60× oil objective (FIG. 13B). Images were assembled using ImageJ and Photoshop CS6 (Adobe Systems, Mountain View, CA).

13. Intestinal crypt self-renewal assay

[0203] 8–10 week-old *Lgr5-EGFP-IRES-creERT2* (B6.129P2-*Lgr5*^{tm1}(*cre/ERT2*)*Cle/J*) mice were purchased from The Jackson Laboratory (Bar Harbor, ME). All experiments were performed according to protocols approved by the Animal Care and Use Committee at the University of Toronto, and complied with the regulations of the Canadian Council on Animal Care and with the ARRIVE guidelines (Animal Research: Reporting in Vivo Experiments). F^{P+P} -*L6*¹⁺³ or a negative control Ab was reconstituted in 37 mM Citric Acid, 63 mM Na₂HPO₄, 10% trehalose, 0.2M L-Arginine, 0.01% polysorbate 80, pH 6.0. The Porcupine Inhibitor C59 was reconstituted with 0.5% methylcellulose mixed with 0.1% Tween 80 in ddH₂O. The mice (male and female) were divided into three groups (5-7 per group): vehicle, control (C59 and control Ab) or FLAg (C59 and F^{P+P} -*L6*¹⁺³). On day 1, mice were treated by intraperitoneal injection with vehicle, or 10 mg/kg control Ab or F^{P+P} -*L6*¹⁺³. The treatments were blinded to the investigators until the end of the experiment and were repeated every two days for a total three treatments. Starting on day 2, vehicle or 50 mg/kg C59 was administered by gavage to the vehicle group or the two experimental groups, respectively, twice a day with 8 hours interval for 4 days. On day 6, the mice were sacrificed. The whole intestinal tissue was harvested, cleaned with cold PBS, dehydrated with PBS, 30% sucrose, fixed with 4% paraformaldehyde and embedded in optimal cutting temperature compound (OCT). 8 μm OCT frozen sections were used for immunohistology. The intestinal EGFP crypts were analyzed using confocal microscopy (Zeiss LSM700). Representative fluorescence images of small intestinal sections from *LGR5*-GFP mice treated with vehicle, C59 or pan-FLAg(F^{P+P} -*L6*¹⁺³) + C59 are depicted in FIG. 14. *LGR5*-GFP is expressed in the stem cells at the bottom of crypts. Cell nuclei were counterstained with DAPI.

[0204] 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. 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.

Table 1A

ID	DNA	SEQ ID	Protein	SEQ ID
5019-knob-2539-2542	GAGGTTGAGCTGGTGGAGTCTGGCGTGGCTGGTGGAGCCAGGGGCTCACTCCGTTTGTCTGTGAGCTTCTGGCTTCAACA TCGGTTCTTCTATCACTGGTGGTGGTGGAGCCCGGTAAGGGCTGGAATGGTTGATCTATTTATTTCTGTTTGGCTCTA CTTCTTATGCCGATAGCGTCAAGGGCGTTTCACTATAAGCGCAGACATCAAAAACACAGCTACTACAAATGAACAGCTTA AGAGCTGAGGACACTGCCGCTATTATTGCTCGCTACCATTTCCGTTTGGTTTGGTCTGACTACTGGGTCAAGGAACCT GGTCACCGTCTCTCGGTGGAGTGGAGTGAGTGATATCCAGATGACCCAGTCCCGCTGCTCGCTCTGCGGCGATAGG GTCAACATCACTGCCGTGCCAGTCAAGTCCGTGTCCAGCGCTGTAGCTGTATCAACAGAACACAGAAAGCTCCGAAGCTTCT GATTACTCGGCATCAGCCTACTCTGGAGTCCCTTCTCGTGTAGCCGTTCCGGGACGGAATTCCTCTGACCATCAG CAGTCTGACGCGGAAGACTTCGCAACTTATTACTGTACGAAGGTGTTTACCTGTTACGTTCCGACAGGATACCAAGGTGAG ATCAAACTCGAGataaaactcacacAAAGTGGAGCCCAAACTTCTgataagaccactactgtcccaCCGTGCCAGCCTGAACCTCTG GGGGACCGTCACTCTTCTTCCCCCAAAACCAAGGACACCTCATGATCTCCGGACCCCTGAGTCAATGCGTGGTGGT GGAGTGAAGCCAGAACCTGAGGTCAAGTTCACTGGTACGCGGTGGAGGTGCAATAATGCCAAGACAAAGCCGCG CGAGGAGCAGTACAAACAGCAGTACCGTGTGGTCAAGCTCTCACCGTCTGCCAGGAGTGGCTGAATGGCAAGGAGTACAA GTGCAAGTCTTCAACAAAGCCCTCCAGCCCTCGAGAAACCATCTCAAAGCCAAAGGGAGCCCGGAGAACCAATGGTG TTTGACCTGCCCATCCCGGAGGAGATGACCAAGAACAGGTCAAGCTGTGGTGGTCAAGGCTTCTATCCAGCGACA TCGCGTGGAGTGGAGAGCAATGGCGAGCGGAGAACAACTACAAGACACGCTCCGCTGCTGGACTCCGACGGCTCTTCTT CTGTACAGCAAGCTCACCGTGGACAGAGCCGCTGGCAGCAGGGGAAAGTCTTCTCATGCTCGTGTGATGATGAGGCTCTGCAC AACCACTACAGCAGAAAGCTCTCCCTGTCTCCGGTAAAGCGCAGGAGACTCCCGGAGCTCAGAGTCGCGCACACCCG AAAGTGGTGGCGGA+B10GAGGTTCAGCTGGTGGAGTCTGGCGGTGGCTGTGGAGCCAGGGGCTCACTCCGTTTGTCTGT GCAGCTTCTGGCTTCAACATCTTATTCTTCTATCCAGTGGGTGAGGCTGAGGCTGAGGCTGGAATGGTGGTGCATATAT TCTCTTATTATGGCTATACTTATGCGATAGCGTCAAGGGCGGTTTCACTATAAGCGCAGACACATCCAAAAACACAGCTA CTTACAAATGAACAGCTTAAGAGCTGAGGACACTGCCGCTATTATTGTCTCGGCTCATTACTTCCGTGGGCTGGTGTATGG ACTACTGGGTCAAGGAACCTGTACCGTCTCTCGGTGGAGTGGCAGTGATATCCAGATGACCCAGTCCCGAGCTCCCT GTCCGCTCTGTGGCGATAGGGTCAACATCACCTGCCGTGCCAGTCCGTGTCCAGCGCTGTAGCCTGTATCAACAGAAAC CAGGAAAAGCTCCGAAGCTTCTGATTACTCGGCATCCAGCTCTACTCTGGAGTCCCTTCTCGCTTCTGTTAGCCGTTCCGGGA CGGATTTACTCTGACCATCAGCAGTCTGCAGCGGAAGACTTCGCACTTATTACTGTACGAATACTACTGCGCCGATCAGTTCTG GACAGGGTACCAAGGTGGAGATCAA	1	EVQLVESGGGLVQPGGSLRLCAASGFNIG SSSIHWVROAPGKGLWVASVAFSTSY ADSVKGRFTISADTSKNTAYLQMNSLRAED TAVYYCARYHPFGFALDYWGQGLTVTVS SGGGSDIQMTQSPSSLSASVGRVTITCR ASQSVSSAVAWYQQKPKAPKLLIYSASSL YSGVPSRFSGRSGTDFLTISLQPEDFAT YYCQQGVYLFITGGGTKEIKLEDKHTKV EPKTSKTHITCPPCPAPPELLGGPSVFLFPPK PKDTLMISRTPEVTCVVVDVSHEDPEVKFN WYVDGVEVHNAKTKPREEQYNSTYRVVS VLTVLHQDWLNGKEYKCKVSNKALPAPIE KTSKAKGQPREPMVFDLPPSREEMTKNQ VSLWCMVKGFYPSDIAVEWESNGQPENN YKTTTPVLDSGSEFLYSLTVDKSRWQQG NVFCSVMHEALHNHYTQKSLSLPGKSG SETPTGSEATPESGGGEVQLVESGGGLVQ PGGSLRLCAASGFNISYSIHWVROAPGK GLEWVAISSYYGTYADSVKGRFTISADT SKNTAYLQMNSLRAEDTAVYYCARAHYFP WAGAMDYWGQGLTVTVSSGGGSDIQ MTQSPSSLSASVGRVTITCRASQSVSSAV AWYQQKPKAPKLLIYSASSLYSGVPSRFS GSRSGTDFLTISLQPEDFATYYCQQYYW PITFGQGTKEIK	2

Table 1A

[illegible]

Table 1A

ID	DNA	SEQ ID	Protein	SEQ ID
5019- Fc- 2539	GAGGTTGAGCTGGTGGAGTCTGGCGGTGGCTGGTGGAGCCAGGGGCTCACTCCGTTTGTCTGTGGAGCTTCTGGCTTCAACA TCGGTTCTTCTATCACTGGGTGGTGGAGCCCGGGTAAAGGCTGGAATGGTTGGATCTATTATTTCTGCTTTTGCCTCTA CTTCTTATGCCGATAGCGTCAAGGGCGTTTCACTATAAGCGCAGACATCCAAAAACAGACCTACTACAAATGAACAGCTTA AGAGCTGAGGACACTGCCGTCTATTATTGTCTCGCTACCATTTCCGTTTGGTTTGTCTTGGACTACTGGGTCAAGGAACCT GGTCACCGTCTCTCGGTGGAGGTGGCAGTGATATCCAGATGACCCAGTCCCGCTCTCGCTCTGTCGGCGGATAGG GTCAACCATCACTGCCGTGCCAGTCCGTGTCCAGCGCTGTAGCTGTGATCAACAGAACAGGAAAGCTCCGAAGCTTCT GATTTACTCGGCATCAGCCTTACTCTGGAGTCCCTTCTCGCTTCTCTGGTAGCCGTTCCGGGACGGAATTTCACTCTGACCATCAG CAGTCTGCAGCGGAAGACTTCGCAACTTATTACTGTGACCAAGGTGTTACCTGTTCCAGTCCGACAGGTTACCAAGGTGGAG ATCAAACTCGAGgataaaactcacaaAAAGTTGAGCCCAATCTTCTgataagaccatacttgcacacgtgcccagcacctgaactcttgggggacc gtcagttcttcttcccccaaaacccaaggacacccctatgatctccgggacccctgaggtcacatgctggtgtgagcgtgagccacgaagacccctgaggtcaagtt caactggtacgtggacggcgtggagggtgacataatgccaagacaagccgaggaggagcagtaacacagcagtaaccgtgtggtcagcgtctcacgtctcgcacacag gactggtgaatggcaaggagtacaaagtgcaaggttctccaagaagccttccagcccccatcgagaacacatctccaagccaaagggcagccccgagagacacacag gtgtacacccctgccccatccgggaggagatgaccaagaaccaggctcagctgacctgctgggtcaaaaggtcttctccacagcagctcacagtggtgagagagcag aagcttctctatgctcgtgtagtcagaggtctctgcaacactacacagcaggaagccttctctctcgggtaaaAGCGCAGCGAGACTCCCGGAC CTCAGAGTCCGCCACACCCGAAAGTGGTGGCGGAGAGGTTACGTGGTGGAGTCTGGCGGTGGCTGTGTCAGCCAGCGGGGGCTC ACTCCGTTTGTCTGTGAGCTTCTGGCTTCAACATCTCTATTCTTATCCACTGGGTGCGTCAGGCCCGGGTAAGGGCGCTGGA ATGGGTTGCAATATTTCTTATTATGGCTATATTTATGCGGATAGCGTCAAGGGCGGTTTCACTATAAGCGCAGACACATC CAAAACACAGCCTACCTACAAATGAACAGCTTAAGAGCTGAGGACACTGCCGTCTATTATTGTCTCGCGCTCATTTACCTCCCGT GGGCTGGTGTATGGACTACTGGGTCAAGGAACCCCTGGTCACCGTCTCTCGGTGGAGGTGGCAGTGATATCCAGATGACCC AGTCCCGAGCTCCCTGTCCGCTCTGTGGCGATAGGTCACATCACTGCGGTGAGTCCGCTGTCAGCTGTCAGCGCTGTAGCC TGGTATCAACAGAAACAGGAAAGCTCGAAAGCTTCTGATTTACTCGGCATCCAGCCTTACTCTGGAGTCCCTTCTCGCTTCTCT GGTAGCCGTTCCGGGACGGAATTTCACTCTGACCATCAGCAGTCTGCAGCCGGAAGACTTCGCAACTTATTACTGTGACGAATACTC TTGGGGTCCGTTACGTTTCGACAGGGTACCAAGGTGGAGTCAAA	5	EVQLVESGGGLVQPGGSLRLCAASGFGNIG SSSIHWVROAPGKGLWVASVAFSTSY ADSVKGRFTISADTSKNTAYLQMNSRAED TAVYYCARYHPFGFALDYWGQGTLVTVS SGGGSDIQMTQSPSSLSASVGRVTITCR ASQSVSSAVAWYQQKPKAPKLLIYSASSL YSGVPSRFSGRSGTDFLTISLQPEDFAT YYCQQGVYLTFTGGGTKEIKLEDKHTKV EPKSSDKTHITCPPCPAPPELLGGPSVFLFPPK PKDTLMISRTPEVTCVVVDVSHEDPEVKFN WYVDGVEVHNAKTKPREEQYNSTYRVVS VLTVLHQDWLNGKEYCKVSNKALPAPIE KTSKAKGQPREPQVYTLPPSREEMTKNQ VSLTCLVKGFPYSDIAVEWESNGQPENNY KITTPVLDSDGSFELYSLTVDKSRWQQG NVFCSVMHEALHNHYTQKSLSLSPGKSG SETPTGSEATPESGGGEVQLVESGGGLVQ PGGSLRLCAASGFGNISYSIHWVROAPGK GLEWVAISSYGYTYADSVKGRFTISADT SKNTAYLQMNSRAEDTAVYYCARAHYFP WAGAMDYWGQGTITVTVSSGGGSDIQ MTQSPSSLSASVGRVTITCRASQSVSSAV AWYQQKPKAPKLLIYSASSLYSGVPSRFS GSRSGTDFLTISLQPEDFATYYCQQVSW GPFTFGQGTKEIK	6

Table 1A

[illegible]

Table 1A

ID	DNA	SEQ ID	Protein	SEQ ID
5038-knob-2539-2542	GAGGTTGAGCTGGAGTCTGGCGTGGCTGGTGGAGCCAGGGGCTCACTCCGTTTGTCTGTGAGCTTCTGGCTTCAACA TCTCTTATTATATGCACTGGGTGCGTCAGGCCCGGTGAGGCGCTGGAATGGGTGGTGTGATCTATTATTTATTTATGGCTATA CTTATTATGCCGATAGCGTCAAGGGCGTTTCACTATAAGCGAGACATCAAAAACACAGCTACTACAAATGAACAGCTTA AGAGCTGAGGACACTGCCGCTATTATTGTCTGCTCTTTCTTCTGGCTATGGACTACTGGGTCAAGGAACCTGGTCAAC CGTCTCTCGGGTGGAGTGGCAGTGATATCAGATGACCGCTCCAGCTCCGCTCTGTGGCGATAGGGTCAAC ATCACCTGCCGTGCCAGTCACTCGTGTCCAGCGCTGTAGCTGTATCAACAGAAACAGGAAAGCTCCGAAGCTTCTGATTTA CTCGGATCCAGCTCTACTCTGGAGTCCCTTCTCGTCTCTGTAGCGTTCGCGGACGGATTTCACCTGACCATCAGCAGCTCT GCAGCGGAAGACTTCGCAACTTATTACTGTGCAACATCCGTGCTGTGGTTACCTGATCACTGACGACAGGGTACCAAG GTGGAGATCAAACTCGAGcaaaactcacaaaAGTGGAGCCCAAACTTCTgataagacctactTGCACCGTGCACGACCTTG AACTCTGGGGGACCGTCAAGTCTCTCTCTCTCCCAAAACCAAGGACACCTCATGATCTCCCGACCCCTGAGGTACATGC GTGGTGTGGAGTGGAGCCAGAACCTGAGGTCAAGTTCAACTGCTGACGCGCTGGAGGTGCATAATGCCAAGACA AAGCCGCGAGGAGCAGTACAAAGCAGCAGTACCGTGTGGTCAAGCTCCTACCGTCTGCCAGGAGTGGCTGAATGGCAAG GAGTACAAGTCAAGGTCTCCAAAGCCCTCCAGCCCCATCGAGAAACCATCTCCAAAGCCAAAGGCGACCCCGAGAAC CAATGGTGTGACCTGCCCCATCCGGAGGAGATGACCAAGAACCAAGTCAAGCTGTGGTGCATGGTCAAGGCTTCTATCC CAGCGACATCGCGTGGAGTGGAGAGCAATGGCGAGCCGAGAACCACTACAAGACACGCTCCCGTGTGCTGACTCCGACGG CTCCTTCTCTGTACAGGAAGTCAAGTGGACAAGAGCGGTGGCAGAGGGGAACGTCTTCTCATGCTCGTGTGATGCATGAGG CTCTGCACAACTACAGCAGAAAGCTCTCCGTCTCCGGTAAAGCGCAGCGAGACTCCCGGACCTCAGAGTCCGC CACACCCGAAAGTGGTGGCGAGAGTTCAGCTGGTGGAGTCTGGCGTGGCTGTGCAAGCCAGGGGCTCACTCCGTTTGTCTC CTGTGAGCTTCTGGCTTCAACATCTTATTCTTCTATCCAGTGGGTGCTCAGGCCCCGGTAAAGGCTGGAATGGGTGCTGAT ATATTCTCTTATTATGGCTATACCTATTATGCCGATAGCTCAAGGCGGTTCACCTATAAGCGCAGACATCCAAAAACACAG CTTACCTACAAATGAACAGCTAAGAGCTGAGGACACTGCCGTCTATTGTGCTCGCTCATTTACTTCCGTTGGCTGGTGTCT ATGAGTACTGGGTCAAGAACCTGCTACCGTCTCTCGGGTGGAGTGGAGTGATATCCAGATGACCCAGTCCCGGAGCT CCCTGTCCGCTCTGTGGCGATAGGATCAACATCACCTGCGGTGCCAGTCACTCGCTGCCAGCGCTGAGCTGATCAACAG AAACAGGAAAGCTCCGAAGCTCTGATTTACTCGGATCCAGCTCTACTGAGGCTCTCTCGCTTCTCTGCTGAGCGCTTC GGAGGAGATTCTACTGTGACCATCAGAGTCTGAGCGGAAAGCTTCGCAACTTATTACTGTGAGCAATACTACTGCGCGATCAC GTTCCGACAGGGTACCAAGGTGGAGTCAAA	9	EVLVESGGGLVQPGGSLRLSCAASGFMNIS YYMHVVRQAPGKGLWVASVSYGYT YYADSVKGRFTISADTSKNITAYLQMNSLRA EDTAVYCARSSFSWAMDYWGQGLTVTV SSGGGSDIQMTQSPSSLSASVGDRTITC RASQSVSSAVAWYQQKPKAPKLIYSASS LYSGVPSRFSRSGTDFTLTISLQPEDFAT YYCQHPWWSGGYLITFGQGTKEIKLEDKT HTKVEPKTSDKTHITCPPCAPPELLGGPSVF LEPPKPKDTLMISRTPEVTCVVVDVSHEDP EVKFNWYVDGVEVHNAKTKPREEQNST YRVVSLTVLHQDWLNGKEYKCKVSNKAL PAPIETISKAKGQPREPMVFDLPPSREEM TKNQVSLWCMVKGFYPYSDIAVEWESNGQ PENNYKTTTPVLDSDGSFELYSLTVDKSR WQQGNVFCVSMHEALHNHYTQKSLSLS PGKSGSETPGTSESATPESGGGEVQLVESG GGLVQPGGSLRLSCAASGFMNISYSHWVR QAPGKGLWVAVYISSYGYTYADSVKGRF TISADTSKNITAYLQMNSLRAEDTAVYCAR AHYFPWAGAMDYWGQGLTVTVSSGGGG SDIQMTQSPSSLSASVGDRTITTCRASQSV SSAVAWYQQKPKAPKLIYSASSLYSGVP SRFSRSGTDFTLTISLQPEDFATYYCQ YYWPTFGGQTKVEIK	10

Table 1A

ID	DNA	SEQ ID	Protein	SEQ ID
5044-knob-2539-2542	GAGGTTGAGCTGGAGTCTGGCGGTGGCTGGTGCAGCCAGGGGCTCACTCCGTTTGCTGTGCTGTCAGCTTCTGGCTTCAACC TCTCTCTTATTTCTATGCACTGGGTGCGTCAGGCCCGGGTAAGGGCTGGAATGGGTGCATATATTTCTTATTTATGGCTATA CTTATTATGCCGATAGCGTCAAGGGCGTTTCACTATAAGCGCAGACATCAAAAACAGACCTACTACAAATGAACAGCTTA AGAGCTGAGGACACTGCCGTCTATTATTGTCTGCCGGCTCCGGGTCTTGGGTTTGTACTACTGGGTCAAGGAACCTGG TCACCGTCTCTCGGGTGGAGTGGCAGTGATATCAGATGACCCAGTCCCGAGCTCCTGTCCGCTCTGTGGCGGATAGGGT CACCATCACTGCCGTGCCAGTCAGTCCGTGTCCAGCGCTGTAGCTGGTATCAACAGAAACAGGAAAGCTCCGAAGCTTCTGA TTTACTCGGCATCCAGCTCTACTCTGGAGTCCCTTCTCGCTTCTGTAGCCGTTCGGGACGGATTTCACCTGTGACCATCAGCA GTCTGCAGCCGGAAGACTTCGCACTTATTACTGTAGCAATGTTACTACGCTCCGATCAGCTTCGGACAGGGTACCAAGTGGG GATCAAACTCGAGgacaaaactcacataAAAGTGGAGCCCCAAAATTTCTgataagaccataactTGCCACCCGTGCCAGCACCTGAACCTCC TGGGGGACCGTCAAGTCTTCTCTTCCCCCAAAACCAAGGACACCTCATGATCTCCGACCCCTGAGGTACATGCGTGGTG GTGGACGTGAGCCAGAACCTGAGGTCAAGTTCAACTGGTACGTGGACGGCTGGAGTGCATTAATGCCAAGACAAAGCCG CGCGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAAGCTCTCACCCGTCTGACCCAGGACTGGCTGAAATGGCAAGGAGTAC AAGTGAAGGTCTCAACAAAGCCCTCCAGCCCCATCGAGAAACCATCTCAAGCCAAAGGGCAGCCCGAGAACCAATGG TGTTGACCTGCCCTCCCGGAGGAGATGACCAAGAACCAAGGTCAAGTGGTGCATGGTCAAGGGCTTCTATCCACGCA CATCGCCGTGGAGTGGAGAGCAATGGGACGCCGAGAACAACTACAAGACCAAGCTCCCGTGTGGACTCCGACGGCTCCTTC TTCCTGTACAGCAAGCTACCGTGGACAAGACCGCTGGCAGGAGGAACTCTTCTCATGCTCCGTGATGCATGAGGCTCTGC ACAACCACTACCGCAGAAAGACCTCTCCCTGTCTCCGGTAAAGCGCAGCGAGACTCCCGGACCTCAGAGTCCGCGCACACC CGAAAGTGGTGGCGGAGGTTTCAGCTGGTGGAGTCTGGCGGTGGCTGGTGCAGCCAGGGGGCTCACTCCGTTTGTCTGTGC AGCTCTGGCTCAACATCTCTATTCTTATCCACTGGGTGCGTCAAGCCCGGGTAAGGGCTGGAATGGGTGGCATATATTT TTCCTATTATGGCTATATTATGCCGATAGCGTCAAGGGCCGTTTCACTATAAGCGCAGACACATCCAAAAACACAGCTACCT ACAAATGAACAGCTTAAGAGCTGAGGACACTGCCGTCTATTATGTCTCGGCTCACTTCCGTTGGGCTGGTGTGCTATGGACT ACTGGGGTCAAGGAACCTGTACCGCTCTCTCGGGTGGAGTGGCAGTGATATCCAGATGACCCAGTCCCGAGCTCCCTGTCT CGCTCTGTGGCGATAGGGTCAACCATCACCTGCCGTGCCAGTCCGTGTCCAGCGCTGTAGCTGGTATCAACAGAAACCA GGAAAGCTCCGAAGCTTCTGATTTACTCGGCATCCAGCTCTCACTGAGTCCCTTCTCGTCTCTGTTAGCCGTTCCGGGACG GATTCACCTCTGACCATCAGAGTCTGAGCCGGAAGACTTCGCAACTTATTACTGTAGCAATACTACTGCGCGATCACGTTCCG ACAGGTTACCAAGTGGAGATCAAA	13	EVQLVESGGGLVQPGGSLRLSCAASGFLNS SYSMHWRQAPAGKGLWVAYISSYGYT YYADSVKGRFTISADTSKNITAYLQMNSLRA EDTAVYYCARPAPGHWGFDYWGQGLTV TVSSGGGSDIQMTQSPSSLSASVGDRTI TCRASQSVSSA/AWYQQKPKAPKLLIYSA SSLYSYGVPSRFSRSGTDFTLTISLQPEDF ATYYCQQWYYAPITFGQGQTKVEIKEDKTH TKVEPKTSDKTHTCPPCAPPELLGGPSVFLF PPPKDITLMISRTPEVTCVVDVSHEDPEV KFNWYVDGVEVHNAKTKPREEQYNSTYR VVSITLVLHQDWLNGKEYCKVSNKALPA PIEKTISKAKGQPREPMVFDLPPSREMTK NQVSLWCMVKGFPSDIAVEWESNGQPE NNYKTTTPVLDSDGSFFLYSKLTVDKSRWQ QGNVFCVSMHEALHNHYTQKSLSLSPGK SGSETPTGTSATPESGGGEVQLVESGGGL VQPGGSLRLSCAASGFLNSYSSIHVWRQAP GKGLWVAYISSYGYTYADSVKGRFTISA DTSKNITAYLQMNSLRAEDTAVYYCARAHY FPWAGAMDYWGQGLTVTVSSGGGSDI QMTQSPSSLSASVGDRTITTCRASQSVSSA VAWYQQKPKAPKLLIYASLSYSGVPSRF SGSRSGTDFTLTISLQPEDFATYYCQQY WPIITFGQGQTKVEIK	14

Table 1A

ID	DNA	SEQ ID	Protein	SEQ ID
5044- hole- 2539- 2542	GAGGTTACGCTGGTGGAGTCTGGCGGTGGCTGGTGTGACGCGAGGGGGCTCACTCCGTTTGCTGTGCAAGTCTTGCGCTTCAACC TCTCTTTATTTATGCACTGGGTGCTCAGGCGCCCGGTGAAGGGCTGGAAATGGGTGCATATATTTCTTATTAATGAGCTATA CTTATTACCGGATAGCTCAAGGCGCTTCACTATAAGCGACAGCTCAAAACACAGCTACCTCAATGAACACGCTTA AGAGCTGAGGACAGCTGCCGTCTATTATTGTCTGCTCCCGGCTCCGGGTCAATGGGTTTGTACTCTGGGTCAGGAACACCTGG TCACCGTCTCTCGGTTGGAGTGGCAGTGATATCCAGATGACCAAGTCCCGAGCTCCCTGTCCGCTCTGTGGGCGATAGGT CACCATCACTCCGTCGCGAGTCACTCCGTGTCCAGCGCTAGCTGGTATCAACAGAAACAGGAAAGCTCGAAGCTTCTGA TTTACTCGGCATCCAGCTCTACTCTGGAGTCCCTTCTCGCTTCTGAGCTTCCGGGACGGATTTCACTCTGACCATCAGCA GTCTGACCGGAAGACTTCGCAACTTATTACTGTGCACAATGGTACTACAGCTCCGATCCAGCTTCGGACAGGGTACCAAGGTGGA GATCAAA-B16CTCGAGagcaaaacacaaAAAGTTGAGCCAAATCTTCTgataagaccataaTtGCCACCCTGCCAGCACCTGAA CTCTGGGGGACGCTGAGTCTTCTCTCCCCCAAAACCCAGGACACCTCATGATCTCCGAGCCCTCGAGGTACATGCTGCGT GGTGTGAGCTGAGCCAGCAAGACCTGAGGTCAAGTTCAACTGGTACTGTGACGCGGTGGAGGTGCATATGCCAAGACAAA GCCGGGAGGAGCAGTACAACAGCAGTACCGTGTGTGTCAGCGTCTCACCGTCTGCACAGGACTGGCTGAATGGCAAGGA GTACAAGTCAAGGTCTCCAAACAAAGCCCTCCAGCCCCATCGAGAAACCATCTCCAAAGCCAAAGGCGCCCGGAGAACCA CAGGTGTACACCTCTCCCCAGTGTGATGACCAACACGAGTCAAGCTGAGCTGCGCGCTCAAGCGCTTCTATCCCA CGCATCTCCGCTGGAGTGGGAGACATGGCAGCCGGAGACAACCTACAAGCTACGCTCCCGTCTGCTGAGCTCCGAGCT CCTTCTCTGTGAGCAAGTCAACGTGGACAGAGCAGGTGGCAGCGGGGAAAGCTTCTCATGCTGCTGTGATGATGAGGC TCTGCACAACTACAGCAAGAGCTCTCCTGTCTCCGGTAAAGCGGACAGAGACTCCCGGAGCTCAGAGTCCGCC ACACCCGAAAGTGGTGGCGAGAGTTACGCTGTGGAGTCTGGCGGTGGCTGGTGCAGCCAGGGGCTCACTCCGTTTGTC TGTGAGCTCTGGCTTCAACATCTCTTATTATTCACCTGGGTGGCTCAGGCCCGGTTAAGGGCTTGGAAATGGGTGTCATC TATTTATCTTCTGCTATCTTATGCGATAGCTCAAGGGCGTTTCACTATAAGCGACAGACATGCCAAAACACGAGC CTACCTACAATGAACAGTTAAGAGCTGAGGACACTCGCTCTATTATTGTCTCGCATCTTCTGGATCCAAAACACCGTACT TCTCTGTTGGGCTATGAGTACTGGGTTCAAGAACCCCTGGTCAACGCTCTCTCGGTGGAGGTGGCAGTGATATCCAGATGAC CCAGTCCCGAGCTCCTGTCCGCTCTGTGGCGATAGGGTACCATCACTCCGTGCCAGTCACTCCGTGCCAGCGCTGAG CCTGGTATCAACAGAAACCCGAAAGCTTCGATTATTCTCGGATCCAGCTCTACTCTGGAGTCCCTTCTCGCTTCT CTGTAGCCGTTCCGGGACGGATTCTACTGTCAACATCAGCAGTCTGACGCCGGAAGACTTCGCACTTATTACTGTGACCAATAC TCTTGGGCTCGTTCAGTTCGGACAGGGTACCAAGGTGGAGATCAAA	EVQLVESGGGLVQPGGSLRLSCAASGFLNLS SYSAHWVROAPKGLKEWVAIVSYGYT YFADSVKGRFTISADTSKNTAYLQMSLRA EDTAVYKCARPAPHWGFDYWGQGTLV TVSSGGGGSDIQMTQSPSSLSASGDRVTI TCRASQSVSAVAYWYQKPGKAPKLLVSA SSLYGVPSPRFSGRSGTDFLTISLQPEDF ATYYCQQWYYAPITFGQGTKEIKLEDKTH TKYVEPKSSDKTHNCPPEAPELGGPSVLF PKPKPDLTLMISRTPEVTCVVDVSHEDPEV KFNWYDGVGEVHNAKTPRREEQYNSTYR VWSVLTVLHQDLWNGKEYCKVSNKALPA PIEKTSKAGQPREQPVYTLPIREILMTSN QVLSCAVKGFYPSPDIAVEWESNGQPENN YKTTTPPVLDSDGSFFELYSKLTVDKSRWQQ GNVFCSMVHEALHNHYTKSLSPGKS GSETPTSESATPESGGEVQLVESGGGLV QPGGSLRLSCAASGFLNISYYIHVWROAPG KGLEWVAIVSYGYTSDVSKGRFTISAD TSKNTAYLQMSLRAEDTAVYKCARTVRG SKKPYFSGWAMDYWGQGTFLTVSSGGG GSDIQMTQSPSSLSASGDRVTITCRASQS VSSAVAYWYQKPGKAPKLLVSA PSRFSGRSGTDFLTISLQPEDFATYYCQ QYSWGPFTFGGQTKVEIK	15	16

Table 1A

D	DNA	SEQ ID	Protein	SEQ ID
5048- knob- 2539- 2542	GAGGTTGAGTCTGGCGGTGGCTGGTGCAGCCAGGGGGCTCACTCCGTTTGCTCTGTGAGCTTCTGGCTTCAACA TCTCTATTATATGACCTGGGTGGCTCAGGCCCGGCTGAAGGCTGGATGGGTGCATCTATTCTCTTATTATGGCTCTA CTTATTGCGCATAGCTCAAGGCGGTTTGACTATAGCGCAGACATCCAAACAGACCTCACTCAAAATGAACAGCTTA ATAGCTGAGGACAGCTGCCGTCTATTGCTCTGCTCTGGTGGGCTTGGCTTTGACTACTGGGGTCAAGAACCTCGTCA CGTCTCTGGGTGGAGTGGCAGTGATATCCAGATGACCCAGTCCCGAGCTCCCTGTCCGCTCTGTGGGCGATAGGGTCACC ATACATCGCGTGCAGTCAGTCCGTGTCCAGCGCTGTAGCTGGTATCAACAGAACAGGAAAGCTCGGAAGCTTCTGATTA CTCGGCATCAGCCCTCTACTCTGGAGTCCCTCTCGCTCTCTGTTAGCGGTTCCGGGCGGATTTCACTCTGACCATCAGCAGTCT GCAGCCGAAGACTTCGCAACTTATTACTGTGAGCAACATTACTCTGTTACGCTTCTCTGATCAGCTTCGCGGAGGGTACCAAG TGGAGATCAAACTCGAGataaaactcacaaaAGTGGAGGCCCAAACTTCTgataagaccctactTGCACACCTGCCACAGCTGAA CTCTGGGGGAGCTGAGTCTCTCTCTCCCCCAAAACCCAGGACACCTCATGATCTCCCGAGCCCTCGAGTCCACATGCGT GGTGTGAGCTGAGCCAGCAAGACCTGAGGTCAAGTTCAACTGGTAGCTGGACGGCTGGAGGTGCATATGCCAAGACAAA GCCGCGAGGAGCAGTACAAAGCAGTACCGTGTGGTTCAGCGTCTCACCCTCTGCACAGGACTGGCTGATGGCAAGGA GTACAAGTGAAGTCTTCCAAACAAAGCCCTCCAGCCCATCGAGAAACCATCTCCAAAGCCAAAGGCGCCCGCAGAACCA GTGATTTGCTGCCCCCTCCGGGAGAGATGACCAAGCAACAGTCAAGCTGTGTGTGTCAGTGGTCAAGGGCTTCTATCCCA CGCATCTGCCGTGGAGTGGGAGCAATGGCGAGCGGAGAAACACTACAAGACACCGCTCCCGTCTGGCTCCGACGCT CCTTCTCTGTACAGCAAGCTCAGCTGGACAGAGAGCGCTGGCAGACGGGAAGCTTCTCATGCTCCGTGTGTCATGAGGCT CTGCACAACTACACGAGAAGACCTTCCCTGTCTCGGGTTAAAGCGCAGGAGACTCCGGGACTCAGAGTCCGCCA CACCCGAAAGTGGTGGCGGAGAGTTCAGCTGGTGGAGTCTGGCGGTGGCTGGTGCAGCCAGGGGCTCACTCCGTTTGTCT GTGCACTTCTGGCTTCAACATCTTATTCTTATCAGCTGGGTGGGTGAGGGCTGAAGGGCTCGAATGGGTTGCAAT TACTCTATTATGCTATATTATGCGCATAGGTCAGGGCCGTTTCACTATAGCGCAGACATCCAAACAGACAGCC TACCTACAAATGAACACTGAAGCTGAGGACACTGCCGTCTATTATGTGCTCGGCTCACTACTTCCGCTGGTGGTGTCTAT GGACTACTGGGTCAAGGAACCTGTGACCGTCTCTCGGGTGGAGTGGCAGTGATCCAGATGACCCAGTCCCGAGCTCC CTGTCCGCTCTGTGGGATAGGTCACCATCACTCCGTCGCGCAGTCCGTGTCCAGCGCTGTAGCTGGTATCAACAGAA ACCAGGAAAGCTCCGAGCTTCTGATTTACTCGGCATCCAGCTCTACTCTGAGTCCCTTCTCGCTTCTGTGGTGGTCCG GACGATTTCACTCTGACCATCAGAGTCTGCAGCCGGAAGACTTCGCAACTTATTCTGTGAGCAATACTACTGCGCGCATCAGT TCGACAGGGTACCAAGTGGAGTCAAA	17	EVQLVESGGGLVQPGGSLRLSCAASGFNIS YYMHWRVQAPGKGLWEVASISYVST YADSVYKRTISDTSKNTAYLQMSLRV EDTAVYKCRSWAWAFDYWGQTLRT VSSGGGSDIQMTQSPSSLSASVGRVTIT CRASQSVSAVAWYQKPGKAPKLLIYAS SLYSGVPSRFSRSGTDFTLTISLQPEDFA TYYCQHYSVYASLTGQGQTKVEIKDKT HTKVEPKTSDKTHLTPCPAPPELLGGPSVF LPFPKPKDTHMISRTPEVTCVYVDVSHEDP EVKFNWYVDGEVHNAKTKPREEQYNST YRVVSVLT/LHQDWLNGKEYKCKVSNKAL PAPIETKSKAKGQPRPMVFDP/LPPSREEM TKNQSLWCMYKGFPSDIAVEWESNGQ PENNYKTTPPVLDSDGSFFLYSKLTVDKSR WQGNVNFCSVMHEALHNHYTQKLSLS PGKSGSETPTGTSATPESGGEGVQLVESG GGLVQPGGSLRLSCAASGFNISYSHWVR QAPGKGLWEWAVYISSYGYTYVADSVKGRF TISADTSKNTAYLQMSLRADETAVYCAR AHYFVPWAGAMDYWGQTLTVTVSSGGGG SDIQMTQSPSSLSASVGRVTITCRASQSV SSAVAWYQKPGKAPKLLIYASSLYSGVP SRFSRSGTDFTLTISLQPEDFAITYCQQ YYWPIITGGQTKVEIK	18

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			EVQLVESGGGLVQPGGSLRLCAASGFNIS YYMHVVRQAPGKGLWVASISSYSGT YYADSVKGRFTISADTSKNITAYLQMNSLRA EDTAVYYCARSWAWAFDYWGQGLTVT VSSGGGSDIQMTQSPSSLSASVGDRTIT CRASQSVSAVAWYQKPGKAPKLLIYSAS SLYSGVPSRFSGRSGTDFTLTISLQPEDFA TYVCOQHYSVYASLTFTGQGTKEIKLEDKT HTKVEPKSSDKTHNCPCPAPPELLGGPSVF LEPPKPKDTLMISRTPEVTCVVVDVSHEDP EVKFNWYVDGVEVHNAKTKPREEQVNST YRVSVLTVLHQDWLNLGKEYKCKVSNKAL PAPIETISKAKGQPREPQVYTLPPRELMT SNQVLSLCAVKGFYPSDIAVEWESNGQPE NNYKTTTPVLDSDGSFSLVSKLTVDKSRW QQGNVFSCSVMHAEALHNHTQKSLSPG KSGSETPTGSETAPESGGGEVQLVESGGG LVQPGGSLRLCAASGFNMISSYIHVVRQA PGKGLWVASISSYSGTYSADSVKGRFTIS ADTSKNITAYLQMNSLRAEDTAVYVCARTV RGSKKPYFSGWAMDYWGQGLTVTVSSG GGGSDIQMTQSPSSLSASVGDRTITCRAS QS/VSSAVAWYQKPGKAPKLLIYSASSLYS GVPSRFSGRSGTDFTLTISLQPEDFATYV CQQYSWGPFTFGQGTKEIK	

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ID	DNA	SEQ ID	Protein	SEQ ID
5062-knob-2539-2542	GAGGTTGAGTCTGGAGTCTGGCGTGGCTGGTGGAGCCAGGGGCTCACTCCGTTTGCTGTGGAGTCTGGCTTCAACatctctattattatctCACTGGGTGGTTCAGGCCCGGGTAAGGGCTGGAATGGGTGGATCTATTATCTTCTAGCTATACCTTATATGCCGATAGCTCAAGGCCGTTTCACTATAAGCGCAGACACATCAAAACACAGCTACTACAAATGAACGCTTAAAGAGCTGAGGACACTGCCGTCTATTATTGTCTGCTCTTCTAGCTTGGGCTATTGACTACTGGGTCAAGGAACCTGGTCAACGCTCTCTCGGGTGGAGGTGGCAGTGATCCAGATGACCCAGTCCCGAGCTCCCTGTCGGCTCTGTGGCGATAGGGTCAACCATCACCTGCGGTGCCAGTCAGTCCGTGTCCAGCGCTGTAGCTGTATCAACAGAAACAGGAAAGCTCCGAAGCTTCTGATTATCTCGGCATCCAGCTCTACTCTGGAGTCCCTCTCTGCTCTGGTACGCCGTTCCGGGACGGATTCACTCTGACCATGCGAGCTCTGCAGCCGGAAGACTTCGCAACTTATTACTGTACGCAATCTGTTGGTGGGTGTTTCTGTATCACGTTCCGACAGGTACCAAGGTGGAGATCAAACTCGAGataaaactcacacaAAAGTGGAGCCCAAACTTCTgataagaccatactTgccCACCGTGCCCAAGCACTGAACTCCTGGGGACCGTCAGTCTTCTCTTCCCCCAAAACCAAGGACACCTCATGATCTCCGGACCCCTGAGGTACATGCGTGGTGGTGAACGTGAGCCAGAACCTGAGGTCAAGTTCAACTGGTACGTGGCGGTGGAGGTGCATAATGCCAAGACAAAGCCGCGAGGAGCAGTACAACAGCAGTACCGTGTGTGTCAGCTCCTCACCGCTCTGCACCGAGACTGGCTGAATGGCAAGGAGTACAGTGCAAGGTCTCAACAAAGCCCTCCAGCCCCATCGAAGAACCATCTCAAGCAAGGGCAGCCCCGAGAACCAATGGTGTTGACCTGCCCATCCCGGAGGAGATGACCAAGAACCAAGTTCAGCTGGTGCATGGTCAAGGCTTCTATCCAGCGACATCGCGTGGAGTGGAGAGCAATGGCAGCCGCGAGAACACTACAAGACCAAGCTCCCGTCTGCTGGACTCCGACGGCTCTTCTTCTGTACAGCAAGCTCACCGTGGACAAGCCGCTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGATGAGGCTCTGCACAACCACTACACGCAGAAAGCTCTCCCTGTCTCGGGTAAAGCGGCAGCGAGACTCCCGGACCTCAGAGTCCGCCACACCCGAAAGTGGTGGCGAGAGGTTCAAGCTGGTGGAGTCTGGCGGTGGCTGTGTCAGCCAGGGGCTCACTCCGTTGTCTGTGCACTTATTATGGCTACTATTATGCCGATAGCGTCAAGGGCCGTTTCACTATAAGCGCAGACATCAAAAACACAGCTACCTACAAATGAACAGCTTAAAGAGCTGAGGACACTGCCGTCTTCTCGGGTGGAGGTGGCAGTGATCCAGATGACCCAGTCCCGGAGCTCCCTGTCCGCCTGTGGCGATAGGGTCAACCTACCTGCCGTGCCAGTCCGCTGTCCAGCGCTGTAGCCCTGGTATCAACAGAAACCAAGTCCGAAAGCTTCTGATTCGAGTCCCTCTCTGCTGTGAGTCCCTTCTGCTGAGTCCGCGACGGATTTCACTCTGAGTCCGCTTCTGCTGAGTCCGCTTCTGCTGAGTCCGCGACGGTACCAAGGTGGAGATCAAA	21	EVQLVESGGGLVQPGGSLRLCAASGFMISYYIHWRQAPGKGLWEWVASVSSSYTYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYCARSSYAWAIDYWGQGTILVTVSSGGGSDIQMTQSPSSLSASVGDRTITCRASQVSSAVAWYQQKPGKAPKLLIYSASSLYSGVPSRFSRSGTDFTLTISLQPEDFATYYCQSGWWGVSLLTFGGGTKEIKLEKDKHTKVEPKTSKTHTCPPCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTSKAGQPRPMVFDLPPSREEMTKNQVSLWCMVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMEALHNHYTQKSLSLSPGKSGSETPGTSEATPESGGGEVQLVESGGGLVQPGGSLRLCAASGFMISYSSIHWRQAPGKLEWVAYISSYGYTYADSVKGRFTISA DTSKNTAYLQMNSLRAEDTAVYCARAHYFPWAGAMDYWGQGTILVTVSSGGGSDIQMTQSPSSLSASVGDRTITCRASQVSSAVAWYQQKPGKAPKLLIYSASSLYSGVPSRFSRSGTDFTLTISLQPEDFATYYCQYYWPIITFGQGTKEIK	22

Table 1A

[illegible]

Table 1A

ID	DNA	SEQ ID	Protein	SEQ ID
5063-knob-2539-2542	GAGGTTGAGTCTGGAGTCTGGCGTGGCTGGTGCAGCCAGGGGCTCACTCCGTTTGCTGTGCGAGCTTCTGGCTTCAACA TCTCTTATATATATCACTGGTGGTGCAGGCCGCTGAGGCTGGAATGGTGGTGCATCTATTTATCTTCTTCTGGCTATA CTTATTATGCCGATAGCGTCAAGGCCGTTTCACTATAAGCGCAGACATCAAAAACACAGCTACTACAAATGAACAGCTTA AGAGCTGAGGACACTGCCGCTATTATTGTCTGCTCTTTCTACTGGCTATGGACTACTGGGGTCAAGAACCTGGTCAC CGTCTCTCGGGTGGAGTGGCAGTGATATCAGATGACCCAGCTCCCTGCTCGGCTCTGTGGCGATAGGGTCACC ATCACCTGCCGTGCCAGTCACTCGTGTCCAGCGCTGTAGCTGTGATCAACAGAAACAGGAAAGCTCCGAAGCTTCTGATTTA CTCGGATCCAGCTCTACTCTGGAGTCCCTTCTCGCTTCTGTAGCGCTTCCGGACGGATTTCACCTGACCATCAGCAGCTCT GCAGCGGAAGACTTCGCAACTTATTACTGTGCAACTTCTACCTGTCTACCTGTCGACAGGGTACCAAGTGGAGA TCAAACTCGAGgacaaactacacAAAGTGGAGCCCAAAACTTCTgataagaccatactTGCACACCGTGCAGCACCTGAACCTCTG GGGGACCGTCAGTCTTCTTCTCCCAAAACCAAGGACACCTCATGATCTCCGGACCCCTGAGGTACATGCGTGTGGT GGAGCTGAGCCACGAAGACCTGAGGTCAAGTTCACTGGTACGTGGAGGCTGCAATATGCCAAGACAAAGCCGCG CGAGGAGCAGTACAAACAGCAGTACCGTGTGTCAGCGTCTCACCGTCTGCACAGGAGTGGCTGAATGGCAAGGAGTACAA GTGCAAGGTCTCCAAAGCCCTCCAGCCCATCGAGAAACCATCTCAAAGCCAAAGGGAGCCCGAGAACCAATGGTG TTTGACCTGCCCATCCCGGAGGAGATGACCAAGACCAAGTCAAGCTGTGGTGCATGGTCAAGGCTCTATCCAGCGACA TCGCGTGGAGTGGAGAGCAATGGCGAGCCGAGAACCACTACAAGACCGCTCCGCTGCTGAGCTCCGAGCTCTTCTT CCTGTACAGCAAGCTCACCGTGGACAGAGCCGCTGGCAGAGGGGAAAGTCTTCTCATGCTCGGTGATGATGAGGCTCTGCAC AACCACTACAGCAGAGAGCTCTCCCTGTCTCCGGTAAAGCGCAGGAGCTCCCGGACCTCAGAGTCGCCACACCCG AAAGTGGTGGCGGAGAGGTTACGCTGGTGGAGTCTGGCGGTGGCTGTGTCAGCCAGGGGCTCACTCCGTTTGTCTGTGCGAG CTTCTGGCTTCAACATCTTATCTTCTATCCACTGGTGGTCAAGGCGCTGAGGCGCTGGAATGGGTGCAATATTTCTT CTTATTGGCTATACCTATTATGCCGATAGCTCAAGGCCGTTTCACTATAAGCGCAGACACATCAAAAACACAGCTACCTAC AAATGAACAGCTTAAGAGCTGAGGACACTGCCGCTATTATTGTCTCGGCTCAATATCTCCGTTGGCTGGTGTATGGACTAC TGGGTCAAGGAACCTGGTCAACGCTCTCTCGGTGGAGTGGCAGTGATATCAGATGACCCAGTCCCGAGCTCCCTGTCCG CCTCTGTGGCGATAGGTCAACATCACCTGCCGTGCCAGTCAGTCCGCTGTCCAGCGCTGTAGCCTGGTATCAACAGAACCAAG AAAGGCTCCGAAGCTTCTGATTACTCGGCATCCAGCTCTACTCTGGAGTCCCTTCTCGTCTCTGTAGCCGTTCCGGGACGGA TTTCACTCTGACCATCAGCAGTCTGCAGCGGAAGACTTCGCAACTTATTACTGTGAGCAATACTACTGGCCGATCAGGTTCCGGACA GGGTACCAAGGTGGAGATCAAA	25	EVQLVESGGGLVQPGGSLRLCAASGFNIS YYIHVVROAPGKGLEWVASVPSGYYTY ADSVKGRFTISADTSKNTAYLQMNSRAED TAVYYCARSPYWAMDYWGQGLTVTVSS GGGSDIQMTQSPSSLSASVGDRTVITCRA SOSVSAVAVYQQKPKAPKLLIYSASSLY SGVPSRFSGRSGTDFTLTISLQPEDFATY YQQSYAAYLFTFGQGTKEIKLEDKHTK VEPKTSDKTHITCPPAPPELLGGPSVFLHPP KPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVV SVTLVHQQDWLNGKEYKCKVSNKALPAPIE KTSKAKGQPREPMVFDLPSPREEMTKNQ VSLWCMVKGFYPSDIAVEWESNGQPENN YKTTTPVLDSDGSFFLYSKLTVDKSRWQQG NVFCSVMHEALHNHYTQKSLSPGKSG SETPTGSEATPESGGGEVQLVESGGGLVQ PGGSLRSCAASGFNISYSIHVVROAPGK GLEWVASVPSGYYTYADSVKGRFTISADT SKNTAYLQMNSRAEDTAVYYCARAHYFP WAGAMDYWGQGLTVTVSSGGGSDIQ MTQSPSSLSASVGDRTVITCRASQSVSAV AWYQQKPKAPKLLIYSASSLYSGVPSRFS GSRSGTDFTLTISLQPEDFATYCCQYYW PITFGQGKVEIK	26

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5063- hole- 0539- 0542	GAGGTT CAGCTGGTGAGTCTGGCGGTGGCTGGTGCAGCAGGGGGCTCACTCGGTTTGCTCTGTCAGCTTCTGGCTTCAACA TCTCTATTATATACCACTGGGTGCTGAGCGCCGGTGAAGCGCTGGAATGGTTGCATCTATTATCTCTTCTTGCGCTATA CTTATTAGCCGATAGCTCAAGGCGGTTTCACTATAAGCGAGACACTCAAACACACAGCTCACTCAAAATGAACACGCTTA AGAGCTGAGGACGCTGCCGTCTATTGTCTCTCTTCTACTGGGCTATGAGACTCTGGGTCAAGAACCCCTGGTCA CGTCTCTGGGTGGAGTGGCAGTGATATCCAGATGACCCAGTCCCGAGCTCCCTGTCGCCCTCTGTGGCGATAGGGTCACC ATACATCGCGTGCAGTCAGTCGCTGCAGCGCTGAGCTGGTATCAACAGAACAGGAAAGCTCGGAAGCTCTGATTTA CTCGGCATCAGCCCTCTACTCGGAGTCCCTCTCGCTCTCTGGTAGCGGTTCCGGGCGGATTTCACTCGACCATCAGCAGTCT GCAGCGGAAGACTTCGCAACTTATTACTGTGCAAGCACTTACGCTCTTACCTGTTCCAGCTTCGACAGGGTACCAAGTGGAGA TCAAA+825CTCGAGgagaaactacacaAAAGTTGAGCCCAATCTTCTgataagaccataaTTGCCACCGTGCCACGACTGAACTC CTGGGGGACCGTCAAGTCTTCTCTTCCCCCAAAACCAGAGCACCTCATGATCTCCCGACCCCTGAGGTCACATGCTGGTGGT GGTGAGCTGAGCACGAGACCTTGAGGTCAAGTTCACCTGATGTCGAGCGGCTGGAGTGCATAATGCCAAGACAAAGCC GCGGAGGAGCAGTCAACAGCAGCTACCGTGTGGTCAGCGTCTCACTCGTCTGCACAGGACTGGCTGAATGGCAAGGAGTA CAAGTGCAAGGTTCCAAACAAAGCCCTCCAGCCCCATCGAGAAACCATCTCAAAGCGCAAGGACGAGCCCGAGAACACAG GTGTACACCTGCCCCCACTCGGGAGCTGATACGACCAACAGCTGAGCTGAGCTGCGCGCTCAAGAGCTTCTATCCAGCG ACATCGCGTGGAGTGGAGAGCAATGGGACGCGGAGAACCACTACAAGCACCGCTCCCGTCTGCTGGAATCCGACGCTCT TCTTCTGCTGAGCAAGTCACTGAGTGAACAGAGCAGGTGGCAGCGGGAACGCTTCTCATGCTCGTGATGATGAGGCTCT GCACACCACTACAGCAGAAAGCTCTCCTGTCTCCGGGTAAAGCGGACGAGACTCCCGGAGCTCAGAGTCGCGCCACA CCGAAAGTGGTGGCGAGAGGTTACGTTGGAGTCTGGCGGTGGCTGGTGCAGCCAGGGGCTCACTCCGTTTGTCTGT TGACTTCTGGCTTCAACATCTCTTCTATTATATCACTGGGTGGTGGTGAAGCGCTGAAGTGGGTGATGATCTAT TATCTCTTATGGCTATCTTCTATGCGATAGCTCAAGGGCGGTTCACTATAAGCGGACACATCCAAAACACAGCCTA CTTACAAATGAACAGCTTAAGAGCTGAGGACACTCGGCTCTATTATTGTCTGCGACCTGTCGGATCCAAAACACCGTACTTCTC TGTTGGGCTATGAGTACTGGGTCAAGGAACCTTGGTCAACGCTCTCTCGGTGGAGGTGGCAGTGATATCCAGATGACCCAG TCCCCAGCTCCCTGTCCGCTCTGGGGCATAGGGTACCATCACTCGCGTCCGACGTCAGTCGCTGACGCGCTGAGCCTG GTATCAACAGAAACCCAGAAAGCTCCGAAGCTTCTGATTTTACTCGGCATCCAGCCTCTACTCTGGAGTCCCTTCTCGCTCTCGG TAGCCGTTCCGGGACGGATTTACTCTGACCATCAGCAGTCTGCAGCGGGAAGACTTCGCAACTTATTACTGTCAAGCAATCTCTT GGGCTCGGTTACGTTCCGACAGGGTACCAAGGTGGAGTCAAA	EVQLVESGGGLVQPGGSLRLSCAASGFNIS YYIHVVRRQAPGKGLWEVWASVPSGYTY ADSVKGRFTSADTSKNTAYLQMNSLRYS TAVYKCARSFYAMDYWGQGLTLTVSS GGGSDIQMTQSPSSLSASVGRVITTCRA SQSVSAVAMYQQKPKAPKLLIYSSASSLY SGVPSRFSGSRSGDFTLTLSLQPEDFATY YCOQSYAAYLFTFGQGTQKVEIKLEDKTHTK VEPKSDKTHNCPCPAPPELLGGPSVFLP PKPKDITLMISRTPEVTCVVDVSHEDPEVK FNWYDGVVEVHNAKTKPREEQYNSTYRV VSVLT/LHQDWLNGKEVKCVSNKALPAP IEKTSKAGKQPREPQYVTLPIRELMTSNQ VSLCAVKGFPSDI/VSEWESGQPENNY KTTPTVLDSDGSFSLVTVDKSRWQQG NVFSCSVMHLEALHHYTKLSLSPGKSG SETPTGESATPESGGGEVQLVESGGGLVQ PGGSLRLSCAASGFMISYIHVVRRQAPGK GLEWVASVSYGYTSADVSKGRFTSADT SKNTAYLQMNSLRADTSVYQKARTVGRS KKPYFSGWAMDYWGQGLTLTVSSGGGG SDIQMTQSPSSLSASVGRVITTCRASQSV SSAVAMYQQKPKAPKLLIYSSASSLYSGVP SRFSGRSGDFTLTLSLQPEDFATYTCQQ YSWGPFTFGGQTKVEIK	27	28

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5080- knob- 0539- 2542	GAGGTTGAGCTGGTGAGTCTGGCGGTGGCTGGTGAGCGGAGGCGCTCACTCGGTTTGCTGTGAGCTTCTGGCTTCAACA TCTCTATTATTTATGCACTGGGTGCTGAGGCCCGGCTGGAATGGTTGCATCTATTCTTCTATTATAGCTCTA CTTCTTATAGGCTCAAGGCGGTTTGCACTAAGCGATAGCAACCTCAAAACACACAGCTCAAAATGAACAGCTTA AGAGCTGAGGACAGCTGCGCTATTATTGCTGCTCTGATACCGGGTATGAACTCTGGGCTCAAGGACCCCTGGTCACCG TCTCTCGGTGGAGTGGCAGTGATATCAGATGACCCAGTCCCGAGCTCCTGTCCGCTCTGTGGCGATAGGGTACCACT CACTGCGGTGCCAGTCACTGGTGTGAGCGCTGAGCTGGTATCAACAGAAACAGGAAAGCTCCGAAGCTTCTGATTTACT CGGATCCAGCTCTACTCTGGAGTCCCTCTCGCTTCTGCTGAGCGGATTTCCGCTGACCATCAGCAGTCTGC AGCCGGAAGACTTCGCACTTATTACTGTACAGCAATTTGGTCTTACCCGATCAGCTTGGACAGGGTACCAAGGTGGAGATCAA ACTCGAAGaaaactcacaaaAAGTGGAGCCCAAAACTTTgataagaccatactTGCCACCGTgCCCCAGCACCTGAACCTCTGGGG GGACCGTCAGTCTCTCTTCCCCCAAAACCAAGACACCTCATGATCTCCGAGCCCTGAGGTACATGCGTGGTGGTGA CGTGAGCCCAAGAACCTTGAGTCAAGTTCAACTGGTACGTGACGCGGTGGAGTGCTAATGCCAAGACAAGCCGCGGA GGAGCAGTTACAACAGCAGCTACCGTGTGCTGAGCTCTACCGTCTGCACAGGACTGGCTGAATGGCAAGGAGTACAAGTGC AAGGTCTCAAAAGCCCTCCAGACCCCATCGAGAAACCATCTCAAAGCAAGGCGAGCCCGAGAACCAATGGTGTGTTG ACCTGCCCATCCGCGAGGAGTGAACCAAGAACCAAGTCAAGCTGTGCTGATGCTCAAGGGCTTCTATCCACAGCAGTCCG CTGTGGAGTGGAGCAATGGGACGCGAGAACACTACAGACACCGCTCCCGTGTGAGTCCGACGGCTCTCTTCTCTCTG TACAGAAAGCTCAACGCTGGACAAGACCGCTGGCAGCAGGGAAGCTCTTCTATGCTCCGTGATGATGAGGCTCTGCACAAC ACTACGCAAGAACGCTCTCCGTCTCGGGTAAAGCGGACGAGACTCCGGAAGCTCAGAGTCCGCCACACCCGAAAG TGGTGGCGAGAGGTTGAGCTGGTGGAGTCTGGCGTGGCTGGTGCAGCGAGGGCTCACTCCGTTTGTCTGTGCAGCTCT GGCTTCAACATCTTATTCTTATCCACTGGGTGCTGAGCGCCCGGTGAAGGCCCTGGAATGGTTGCATATATTCTTCTTAT TATGGCTATTATTATGCCGATAGCTCAAGGCGGTTTCACTAATAGCGAGACAGCTCAAAAACACAGCTACCTACAAAT GAACAGCTTAAGAGCTGAGGACACTCCGCTCTATTATGTGCTCGGCTCTTACTTCCGCTGGGCTGTGCTATGGACTACTGGG GTCAAGGAACCTTGTCAACGCTCTCTCGGTGGAGTGGCAGTGATATCCAGATGACCCAGTCCCGAGCTCTCTGCGCTCT GTGGCGATAGGTCACCATCACTGCCGTGCCAGTCACTGGTGTCCAGCGCTGATCGCTGGTATCAACAGAAACACGAAAG CTCCGAAGCTTCTGATTACTCGGATCAAGCTCTACTCTGGAGTCCCTTCTCGCTTCTGTGATGCCGTTCCGGACGGAATTC CTCTGACCATCAGCAGTCTGCAGCGGAGACTTCGCAACTTATTACTGTGAGCAATACTACTGCCGATCATCGTTCGACAGGGT ACCAAGGTGGAGATCAA	29	EVQLVESGGGLVQPGGSLRLSCAASGFNIS YISMHWVROAPGKGLWEVASISYSYST YAVYKGRFTISADTSKNTAYLQMNSLRAE DTAVYKARFWYPGMDYWGQGTITLVSS GGGSDIQMTQSPSSLSASVGRVITTCRA SQSVSAVAVYQQKPKAPKLIIYSASSLY SGVPSRFSGSRSGDFTLTISLQPEDFATY YCOQHWSPYITFGQGTKEIKLEKHTIKV EPKTSDKHTICPPCAPPELLGGPSVFLPPK PKDTLMISRTPEVTCVVVDVSHEDPEVKFN WYDVGVEVHNAKTKPREEQYNSTYRVVS VLTVLHQDWLNGKEYCKVSNKALPAPIE KTISSAKGQPREPMVFDLPPSRREMTKNQ VSLWCMVKGFYPDSIAVEVESNGQPENN YKTTTPPVLDSDGSFELYSLTVDSKSPQQG NVFSCSVMHHEALHNHYTQKSLSLPGKSG SETPTGSEATPESGGGVQLVESGGGLVQ PGGSLRLSCAASGFINYSIIHWVROAPGK GLEWVAYISSYGYTYADSVKGRFTISADT SKNTAYLQMNSLRAEDTAVYKCARAHYFP WAGAMDYWGQGTITLVSSGGGSDIQ MTQSPSSLSASVGRVITTCRASQSVSAV AWYQQKPKAPKLIIYSASSLYSGVPSRF GSRSGDFTLTISLQPEDFATYCCQQYWW PITFGQGTKEIK	30

Table 1A

D	DNA	SEQ ID	Protein	SEQ ID
5080- hole- 2539- 2542	GAGGTTGAGTCTGGCGGTGGCTGGTGCAGCAGGGGGCTCACTCCGTTTGCTCTGTGAGCTTCTGGCTTCAACA TCTCTATTATTTATGACCTGGGTGCTGAGGCCCGGGTAAAGGCTGGTATGCTATTTCTTCTATTATAGCTCTA CTTCTATGCGATAGCTCAAGGCGGTTTGCATATTAAGCGACACCTCAAAACACACAGCTCAAAATGAACAGCTTA AGAGCTGAGGACAGCTGCGCTATTATTGCTGCTATTCGATACCGGGTATGAACTCTGGGGTCAAGGAACCTGGTCACCG TCTCTCGGTGGAGTGGCAGTGATATCAGATGACCCAGTCCCGAGCTCCTGTCCGCTCTGTGGCGATAGGGTACCACT CACTGCCGTGCCAGTCACTCGGTGTCAGCGCTGAGCTGGTATCAACAGAAACAGGAAGCTCCGAAGCTTCTGATTTACT CGGCACTCAGCTCTACTCTGGAGTCCCTTCTCGTCTCTGGTACCGTTCGGGACGGATTTTCACTCTGACCATCAGCAGTCTGC AGCGGAAGACTTCGCAACTTATTAATGTGCAGCAACTTGGTCTTACCCGATCAGCTTCCGACAGGGTACCAAGGTGGAGATCAA A+B28CTGAGgacaaaatacacAAAAGTTGAGCCCAAATCTTCTgataagaccataaTTCGCCACCGTCCGACGACCTGAACCTCTG GGGGACCGTCAAGTCTTCTTCCCCCAAACCCAAAGACACCTCATGATCTCCGACACCCCTGAGTCACTACGCTGGTGGT GGAGCTGAGCCACGAAGACCTTGAGTCAAGTTCAACTGGTACGTGAGCGGTGGAGGTGCATAATGCCAAGACAAGCCGCG GGAGAGCAGTACACAGCAGCTACCGTGTGGTCAGCGTCTCACCGTCTGCACAGGACTGGCTGAATGCCAAGGAGTACAA GTCAAGGTCTCAACAAAGCCCTCCACGCCCATCGAAAAACCATCTCCAAAGCAAGGACGAGCCCGAGAACCCACAGGTG TACACCTCCCCCAATCCGGGAGTGATGACCGAGAACCACTACAGCAACCGCTCCCGTGTGGAGTCTTATCCCGAGCAT CGCGTGGAGTGGGAGCAATGGGACGCGGAGCAACTACAGACACGCTCCCGTGTGGACTTCCGACGCTCTCTTCTC CTGTGAGCAAGCTCACCGTGAGCAAGCAGGTGGCAGCAGGGAAACGTCTTCTATCTCCGTGATGCATGAGGCTCTGCACA ACCACTACGACGAAGACGCTCTCCGTCTCCGGGTAAAGCGGACGAGACTCCGGGACCTCAGAGTCCGCCACACCCGA AAGTGGTGGCGAGAGGTTCAAGTGGTGGAGTCTGGCGGTGGCTGGTGACGACGGGGCTCACTCCGTTTGTCTGTGCAGC TTCTGGCTCAACATCTTCTTATTATATCCATGGGTGAGCGGCCCGGGTAAAGGCTTGAATGGGTTCATCTATTATTC TCTTATGGCTATCTTATGCGCATAGCTCAAGGGCGGTTTCACTATAAGCGACACACATCCAAAACACAGCTACCTACA AATGAACAGCTTAAGAGCTGAGGACACTCCGCTCTATTATGTGCTCGCACTGTTGTTGATCCAAAACACCGTACTTCTCTGGTT GGGCTATGGAATCTGGGTCAAGGAACCTGTGTCACCGTCTCTCCGGTGGAGTGGCAGTGATATCCAGATGACCCAGTCCCC GAGCTCTCTGCCCTCTGTGGGGATAGGGTACCACTACCTGCCGTGCCAGTCACTCCGTGTCCAGCGCTGAGCTCTGGTATC AACAAGAACGAGAAAGCTCCGAAGCTTCTGATTACTCGGCATCAGCTCTACTCTGGAGTCCCTTCTCGCTTCTCTGTTAGCC GTTCCGGGACGGAATTCATCTGACCATCAGAGTCTGAGCCGGAAGACTTCGCAACTTATTACTGTGACGAATACTCTTGGGGT CCGTTTCAAGTTCGGACAGGGTACCAAGGTGGAGATCAAA	31	EVQLVESGGGLVQPGGSLRLSCAASGFNIS YISMHWVRQAPGKGLWVASISYSST YADVYKGRFTISADTSKNITAYLQMNLSRAE DTAVYKGRFAYWPGMDYWGQGTITLVSS GGGSDIQMTQSPSSLSASVGDRTITTCRA SQSVSAVAWYQQKPKAPKLLYSASSLY SGVPSRFSGSRGTDFTLTISLQPEDFATY YCOQHWSYPIITFGQGTQVEIKDKHTKV EPKSSDKTHNCPAPPELLGGPSVFLPPK PKDTLMISRTPEVTCVVDVSHEDPEVKFN WYDGVVEVHNAKTPREEQYNSTYRVVS VLTVLHQDWLNGKEYCKVSNKALPAPIE KTISSAKGQPREPQVYTLPPRELMTSNQV SLSCAVKGYPSDIAEVESWGQPPENNYK TTPPVLDSDGFVLSKLTVDKSRVQQGN VFCSVMHEALHNHYTQKSLSLPGKSGSE TPGTSEATPESGGEVQLVESGGGLVQP GGSLRLSCAASGFNISYIHWVRQAPGKG LEWVASISYSYGTYSADSVKGRFTISADTS KNITAYLQMNLSRAEDTAVYVCARTVRGSK KPYFSGWAMDYWGQGTITLVSSGGGGS DIQMTQSPSSLSASVGDRTITTCRASQSV SAVAWYQQKPKAPKLLYSASSLYSGVPS RFSGSRGTDFTLTISLQPEDFATYVCQY SWGPFTHFGQGTKEIK	32

Table 1A

ID	DNA	SEQ ID	Protein	SEQ ID
5081- knob- 2539- 2542	GAGGTTGAGCTGGTGGAGTCTGGCGGTGGCTGGTGCAGCCAGGGGCTCACTCCGTTTGCTGTGCTGTGGCTTCTGGCTTCAACC TCTCTTATATATATCACTGGGTGGCTGAGCCCGGGTAAAGGCTGGAATGGGTGGCTATATATATATATATCTGTGGCTATA CTTATATGCCGATAGCGTCAAGGGCGTTTCACTATAAGCGGAGACATCAAAAACAGACCTACTACAAATGAACAGCTTA AGAGCTGAGGACACTGCCGTCTATATTTGCTCGCTCTTTTGGCTTTTACTACTGGGTCAAGGAACCTGGTCAAC GTCTCTCGGTGGAGGTGGAGTATATCCAGATGACCCAGTCCCGGAGCTCCCTGTCCGCTCTGTGGCGGATAGGGTCAACA TCACTGCGGTGCCAGTCACTGCTGTCAGCGCTAGCTGTATCAACAGAAACAGGAAGCTCCGAAGCTTCTGATTTAC TCGGCATCCAGCTCTACTGGAGTCCCTTCTCGCTTCTGTGGTAGCCGTTCCGGGACGGAATTCCTCTGACCATCAGCAGTCTG CAGCCGGAAGACTTCGCAACTTATTAAGTGTCAAGGTGGTGGGTCCGTTACGTTCCGACAGGTACCAAGTGGAGATCA AACTCGAGgacaaaactcacacAAAGTGGAGCCCAAACTTCTlgaagaagaccataactTlGCCCAACCGTGGCCAGCACCCTGAACCTCTGGGG GGACCGTCACTCTCTCTTCCCCCAAAACCAAGGACACCTCATGATCTCCCGGACCCCTGAGGTACATGCGTGGTGGTGGG CGTGAGCCACGAAGACCTGAGTCAAGTCAAGTGGTACGTTGAGCGGTGGAGGTGCATAATGCCAAGACAAAGCCGCGCA GGAGCAGTACAACAGACGACGTACCGTGTGGTCAAGCTCTCACCGTCTGACCCAGGACTGGCTGAATGGCAAGGAGTACAAGTGC AAGTCTCCCAAAAGCCCTCCAGCCCCCATCGAGAAACCATCTCAAGCCAAAGGGGAGCCCGGAGAACCAATGGTGTGTTG ACCTGCCCATCCGGGAGGAGATGACCAAGAACCCAGGTCAAGCTGTGGTGCATGGTCAAGGGCTTCTATCCAGCGACATCGC CGTGGAGTGGGAGAGCAATGGGAGCCGGAGAACTACAAGACCAAGCTCCCGTGTGAGCTCCGAGCTCCGAGCTCTTCTTCTG TACAGCAAGCTACCGTGGACAAGAGCCGCTGGCAGAGGGGAGCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACC ACTACAGCAGAAGAGCTCTCCCTGTCTCCGGGTAAAGCGGACGAGACTCCCGGACCTCAGAGTCCGACACCCCGAAAG TGGTGGCGGAGAGGTTACGCTGGTGGATCTGGCGGTGGCTGGTGCAGCCCGGGCTCACTCCGTTTGTCTGTGCGAGCTTCT GGCTTCAACATCTTATCTATCCACTGGGTGGTCAAGCCCCGGGTAAAGGGCTGGAATGGGTGCATATATTTCTTCTAT TATGGCTATATATATGCCGATAGCGTCAAGGGCCGTTTCACTATAAGCGCAGACATCAAAAACACAGCCTACCTACAAAT GAACAGCTTAAGAGCTGAGGACACTGCCGTCTATTTGTCTCGCTCAATTAATCTCCGCTGGCTGGTGTGCTATGGACTACTGGG GTCAAGGAACCTGTGCTCCGCTGGGTGGAGGTGGCAGTATATCCAGATGACCCAGTCCCGGAGCTCCCTGTCCGCTCT GTGGCGGATAGGTCAACATCACTGCCGTGCCAGTCACTGCTGAGCGCTGTAGCCTGGTATCAACAGAAACAGGAAAG CTCCGAAGCTTCTGATTTACTCGGCATCCAGCCTCTACTCTGAGTCCCTTCTCGCTTCTGCTGAGCGTTCCGGGACGGAATTTCA CTTGACCATCAGCAGTCTGCAGCCGGAAGACTTCGCAACTTATTAAGTGTGAGCAATTAAGTGTGAGCGGATCAGCTTCGAGACGGGT ACCAAGGTGGAGATCAAA	33	EVQLVESGGGLVQPGGSLRLCAASGFNLS YYMHWVROAPGKGLWVASVSYGYT YYADSVKGRFTISADTSKNITAYLQMNSLRA EDTAVYYCARSSFAWAFDWYWGQGLTVTV SSGGGSDIQMTQSPSSLSASVGDRTITC RASQSVSAVAWYQQKPGKAPKLLIYSASS LYSGVPSRFSGSRGTDFTLTISLQPEDFAT YYCQQGGWGPFTFGQGTKEIKLEDKTHT KVEPKTSDKTHTCPPCPAPELLGGPSVFLFP PKPKDTLMISRTPEVTCVVDVSHEDPEVK FNWYVDGVEVHNAKTKPREEQYNSTYRV VSVLTVLHQDWLNGKEYKCKVSNKALPAP IEKTSKAGGQPREPMVFDLPSPREEMTKN QVSLWCMVKGFYPSDIAVEWESNGQEN NYKTTTPVLDSDGSFSLYSLTVDKSRWQQ GNVFCSVNMEALHNHYTQKSLSLPGKS GSETPTGTSATPESGGGVQLVESGGGLV QPGGSLRLCAASGFNISYSSIHWWVROAPG KGLWVAIYSSYYGTYADSVKGRFTISAD TSKNITAYLQMNSLRAEDTAVYYCARAHYF PWAGAMDYWGQGLTVTVSSGGGGSDIQ MTQSPSSLSASVGDRTITCRASQSVSAV AWYQQKPGKAPKLLIYSASSLYSGVPSRF GSRGTDFTLTISLQPEDFATYYCQQYYW PITFGQGTKEIK	34

Table 1A

ID	DNA	SEQ ID	Protein	SEQ ID
5081- hole- 2539- 2542	GAGGTTACGCTGGTGAGATCTGGCGGTGGCTGGTGTGACGCGAGGGGGCTCACTCCGTTTGTCTGTGACGCTTCTGGCTTCAACC TCTCTATTATTATATGACGTGGCTGAGGCCCGGCTGAAGGCGCTGGAATGGGTGCATCTATTATTCTATTCTGCGCTATA CTTATTATCCGATAGCTCAAGGCGCTTACTATAAGGCACACATCAAAACACACTACCTACCTCAAAATGAACAGCCTTA AGAGCTGAGGACACTGCCGCTATTATTGTCTCTCTTCCGCTTGTGACTACTGCTGGGTCAAGGAACCACTGCTACCC GTCTCTGGGTGAGGTGGCAGTGATATCAGATGACCGACTCCCGAGCTCCTCTGCGCCTCTGTGGCGATAGGGTGACCA TCACCTGCCGTGCAGTACGCTGTCCAGGCTGTAGCTGTGATCAACAAGAACAGAAAGCTCCGAAGCTTCTGATTAC TCGGCATCCAGCCTCTACTCTGAGTCCCTTCTGCTTCTGTGTAGCGGCTCCGGGACGGATTTCATCTGACCATTACGACGCTG CAGCGGAAGACTTCGAACTTATTATGTGTGACGAGGCTGGTTGGGGTCCGTTTCAGCTTCGGACAGGTTACCAAGCTGGAGATCA AA+B31CTCGAGgacaaatacacAAAGTTGAGCCCAATCTTCTgataagaccataaTTGCCACCGTCCACAGCCTGAACCTCTG GGGGAGCTGAGTCTTCTCTTCCCCAAAACCAAGGACACCTCATGATCTCCGGACCCCTGAGTGCACATGCGTGGTGGT GGAGCTGAGCCACGAAGACCTTGAGTCAAGTTCACTGTTAGTGGAGCGGCTGGAGGTGCATAATGCCAAGACAACAGCCGCG GGAGGAGCACTACACAGCACGTACCGTGTGTGAGCGTCTCACCGTCTGCACAGGACTGGCTGAATGCCAAGGAGTACAA GTGCAAGGTCTCAACAAAGCCCTCCAGCCCTCATGAGAAACCATCTCCAAAGCAAGAGGAGCCCGGAGAACACACAGGTG TACACCTGCCCAATCCGGGAGCTGATCAGCAGCAACAGGTGACGTGCGCGGTCAAGAGCTTCTATCCAGCGACAT CGCGTGGAGTGGGAGAGCAATGGCAGCGGAGAACCACTACAGACAGCCTCCCGTGTGAGCTCGACGCTCTCTCTTC CTGTGAGCAAGCTCACCGTGGACAAGAGCAGGTGGACAGGGAAACGTTCTTCATGCTCCGTGATGCATGAGGCTCTGCACA ACCACTACGACGAGAAGCTCTCCGTCTCCGGGTAAAAGCGGAGCGAGACTCCGGGACCTCAGAGTCGCGCACACCCGA AAGTGGTGGCGGAGAGTTACGCTGGTGGAGTCTGGCGTGGCTGGTGACGCCAGGGGCTCACTCCGTTTGTCTGTGACG TTCTGGCTTCAACATCTTATTATATCCATGGGTGGCTCAGGCCCGGGTAAAGGCCTGGAAATGGGTTCATCTATTATTC TCTTATGGCTACTTCTTATGCCATAGCTCAAGGGCGTTTCACTAAGCGACACATCAACAAACAGCCTACTTCTGTT AATGAACAGCTTAAGAGCTGAGACACTCCGCTCTATTATTGTCTCGCACTTCTGTGGATCCAAAACCGTACTTCTCGTT GGGCTATGGACTACTGGGTCAAGGAACCTTGTCACCGTCTCTCGGGTGGAGTGGCAGTGATATCCAGATGACCCAGTCCCC GAGCTCCTGTCCGCTCTGTGGCGATAGGGTACCACTACCTGCCGTGCCAGTCCGTTGTCCAGCGCTGTAGCCTGGTATC AACAAGAACAGGAAAGCTCCGAAGCTCTGATTACTCGGCATCAGCCTCACTCTGAGTCCCTTCTCGTCTCTGTTAGCC GTTCCGGGACGGAATTCATCTGACCATCAGCAGTCTGCGCGGGAAGACTTCGCAACTTATTACTGTGACGAATACTCTTGGGT CCGTTACGTTCCGACAGGGTACCAAGGTGGAGATCAA	EVQLVESGGGLVQPGGSLRLSCAASGFLNLS YYMHWRVQAPGKGLWEVAVSYVSGYT YVAVYKGRFTSADTSKNTAYLQMNLSRA EDTAVYKRRSFAWAFDYGWGQSLTV SSGGGSDIQMTQSPSSLSASVGDRTITC RASQSVSAVAVYQKPGKAPKLIIYSASS LYSGVPSRFSGRSDTDFLTITSLQPEDFAT YYCQQGGWGPFTFGGQTKVKEIKLDTHT KVEPKSDKTHNCPCPAPELLGGPSVFLF PKPKDTLIMISRTPEVTCVVDVSHEDPEV KFNWYVDGEVHNAKTKPREEQYNSTYR VWSVLTVLHQDWLNGKEYCKVKSNKALPA PIEKTISKAKGQPREPQVYTLPIRELMTSN QVLSCAVKGFYPSPDIAVEWESNGQPENN YKTTTPPVDSMHVSKLTQDKSRWQQ GNVFSQVLMHEALHNHYTKSLSPGKS GSETPTSESATPESGGEVQLVPSGGLV QPGGSLRLSCAASGFLNISYYIHVVROAPG KGLWEVAVSYVSGYTAVDSVKGRTISAD TSKNTAYLQMNLSRAEDTAVYVCARTVG SKKPYFSGWAMDYWGQGLTVTVSSGG GSDIQMTQSPSSLSASVGDRTITCRASQS VSSAVAVYQKPGKAPKLIIYSASSLYSGV PSRFSGRSGTDFLTITSLQPEDFATYYCQ QYSWGVPFTFGGQTKVEIK	35	36

Table 1A

ID	DNA	SEQ ID	Protein	SEQ ID
2928- knob- 2539- 2542	GAGGTTGAGCTGGAGTCTGGCGGTGGCTGGTGCAGCCAGGGGCTCACTCCGTTTGTCTGTGCGAGCTTCTGGCTTCAACA TCTCTTATTTCTTATCCACTGGGTGCGTCAAGCCCGGTAAGGCTGGAATGGTGTGCATCTATTATCTCTTATAGCTCTA CTTATTATGCCGATAGCGTCAAGGGCGTTTCACTATAAGCGCAGACATCCAAAAACAGCCTACTCTACAAATGAACAGCTTA AGAGCTGAGGACACTGCCGTCTATTATTGTCTGCTACTAGCTATGGACTACTGGGTCAAGGAACCTGGTCAACCGTCTCTCTC GGGTGGAGGTGGCAGTGATATCCAGATGACCCAGTCCCGAGCTCTGTCGCTCTGTGGCGATAGGGTCAACCTCACCTGCG CGTGGCAGTCACTCCGTGTCCAGCGCTGTAGCTGTGGTATCAACAGAAAGCTCCGAAGCTTCTGATTACTCGGCGATC CAGCCTCTACTCTGGAGTCCCTCTCTGCTGTAGCCGTTCGGGACGGATTTCACCTGACCATCAGAGTCTGCAGCCGGA AGACTTCGCAACTTATTACTGTGAGCAAGCTTCTACTACCCGATCACGTTCCGACAGGTTACCAAGGTGGAGATCAAACTCGAG acaaactcacaaAAAGTGGAGCCCAAACTTCTGataagaccataactTGCACCCGTCGCCAGCCTGAACCTCTGGGGGACCGTC AGTCTTCTCTTCCCCCAAAACCAAGGACACCTCTATGATCTCCGGACCCCTGAGGTACATGCGTGGTGTGGAGTGAAGCC ACGAAGACCTGAGGTCAAGTTCAACTGGTACGTGGACGGCTGGAGGTGCATATGCCAGACAAAGCCGCGAGGAGCAAT ACAACAGCAGTACCGTGTGGTCAAGCTCTCACCCTGTCACCGAGTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTC CAACAAAGCCCTCCAGCCCCATCGAGAAACCATCTCAAAGCCAAAGGAGCCCGAGAACCAATGGTGTGTTGACCTGCCCT CATCCCGGAGGAGATGACAAAGAACAGGTCAAGCTGTGGTCAAGGGCTCTATCCAGCGACATCGCGTGGAGT GGGAGAGCAATGGGCAAGCCGGAGAACAACTACAAGACCAAGCTCCCGTGTGGACTCCGAGCGCTCTTCTTCTGTACAGCAA GCTCACGCTGGACAAGAGCCGCTGGCAGCAGGGGAACGTCTTCTATGCTCCGTGATGAGGCTCTGCACAAACCACTACACG CAGAAGAGCTCTCCCTGTCTCCGGTAAAGCGGACGAGACTCCCGGACCTCAGAGTCCGACACCCCAAGTGGTGGC GGAGAGGTTCAAGTGGTGGAGTCTGGCGTGGCTGTGTCAGCCAGGGGCTCACTCCGTTTGTCTGTGCACTTCTGGCTTCA ACATCTTATCTTCTATCCACTGGGTGCGTCAAGCCCGGGTAAGGGCTGGAATGGGTGCATATTTCTTCTTATTATGGCT ATACTTATGCGGATAGCGTCAAGGGCGTTTCACTATAAGCGCAGACATCAAAAAACACAGCCTACCTACAAATGAACAGC TTAAGAGCTGAGGACACTGCCGTCTATTATTGTCTCGGCTCAATTCTCCGTGGGCTGGTGTCTATGGACTACTGGGTCAAGG AACCTGGTCACCGTCTCTCGGGTGGAGTGGCAGTGATATCCAGATGACCCAGTCCCGAGCTCCCTGTCCGCTCTGTGGGC GATAGGGTCAACCATCACTGCCGTGCCAGTCACTCCGTGTCCAGCGCTGTAGCTGGTATCAACAGAAACCCAGGAAAGCTCCGA AGCTTCTGATTACTCGGCATCCAGCTCTACTCTGGAGTCCCTCTCTGCTGTGAGCGCTTCCGAGGAGGATTTCACTCTGA CCATCAGAGTCTGCAGCCGAAGACTTCGCAACTTATTACTGTAGCAATACTACTGGCCGATCAGCTTGGAGAGGGTACCAAG GTGGAGATCAAA	37	EVQLVESGGGLVQPGGSLRLCAASGFNIS YSSHWVRQAPGKGLWVASIYPSYSTYY ADSVKGRFTISADTSKNTAYLQMNSLRAED TAVYYCARYYAMDYWGQGLTLTVSSGGG GSDIQMTQSPSSLSASVGDRTITTCRASQS VSSAVAWYQQKPKAPKLLIYSASSLYSGV PSRFSGSRSGTDFLTITSSLPQEDFATYYCQ QAFYYPIITFGQGTKEIKLEDKTHITKVEPKT SDKTHITCPPCPAPELLGGPSVFLPPPKKDT LMISRTPEVTCVVDVSHEDPEVKFNWYV DGEVHNAKTKPREEQYNSTYRVWSLTV LHQDWLNGKEYKCKVSNKALPAPIETISK AKGQPREPMVFDLPPSREEMTKNQVSLW CMVKGFPYSDIAVEWESNGQPENNYKTTTP PVLDSGSHFLYSLKTLVDKSRWQQGNVFS CSVMHEALHNHYTQKSLSLSPKSGSETP GTSESATPESGGGEVQLVESGGGLVQPGG SLRLCAASGFNISYSSHWVRQAPGKGL WVAYISSYGYTYADSVKGRFTISADTSK NTAYLQMNSLRAEDTAVYYCARAHYFPW AGAMDYWGQGLTLTVSSGGGSDIQMT QSPSSLSASVGDRTITTCRASQSVSSAVAW YQKPKGAPKLLIYSASSLYSGVPSRFSGSR SGTDFLTITSSLPQEDFATYYCQYYWPIITF GQGTKEIK	38

Table 1A

ID	DNA	SEQ ID	Protein	SEQ ID
2928- hole- 2539- 2542	GAGGTTCAGCTGGTGGAGTCTGGCGGTGGCTGGTGCAGCCAGGGGCTCACTCCGTTTGTCTGTGCAGCTTCTGGCTTCAACA TCTCTTATTCTTATCCACTGGGTGCTCAGGCCCGGTAAGGCTGGAATGGTTGCATCTATTATCTCTTATAGCTCTA CTTATTATGCCGATAGCGTCAAGGCCGTTTCACTATAAGCGCAGACATCCAAAAACACAGCTACTACAAATGAACAGCTTA AGAGCTGAGGACACTGCCGTCTATTATTGTCTGCTACTAGCTATGGACTACTGGGTCAAGGAACCTGGTCAACCGTCTCCTC GGGTGGAGGTGGCAGTGATATCAGATGACCCAGTCCCGAGCTCCTGTCGCTCTGTGGCGATAGGGTCAACCATCACCTGC CGTGCCAGTCACTCCGTGTCAGCGCTGTAGCTGGTATCAACAGAAACAGGAAAGCTCCGAAGCTTCTGATTTACTCGGCATC CAGCCTCTACTCTGGAGTCCCTTCTGCTTCTGTAGCCGTTCCGGAGCGGATTTCACTCTGACCATCAGAGTCTGCAGCCGGA AGACTTCGCAACTTATTACTGTGAGCAAGCTTCTACTACCCGATCACGTTCCGACAGGGTACCAAGGTGGAGATCAAA+B33CTC GAGgacaaactcacataAAAGTTGAGCCCAATCTTCTgataagaccataatTGCCACCCTGCCAGCACCTGAACTCTGGGGGAC GTGAGTCTTCTCTTCCCCCAAAACCAAGGACACCTCATGATCTCCGGACCCCTGAGGTACATGCGTGGTGGTGGACGTGA GCCACGAAGACCTGAGGTCAAGTTCACTGTGACGTGGAGGTGCAATAATGCCAAGACAAGCCGCGGGAGGAG CAGTACAACAGCACGTACCGTGTGGTCAAGCTCTCACCGTCTGACCCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGG TCTCCAACAAAGCCCTCCAGCCCATCGAAAAACCATCTCAAAGCCAAAGGGCAGCCCGGAGAAACACAGGTGTACACCTG CCCCAATCCGGAGCTGATGACGACCAACAGGTGAGCTGAGCTGCGCCGTCAAAGGCTTCTATCCAGCGACATCGCCGTGG AGTGGGAGAGCAATGGGACCGGAGAACAACTACAAGACCAAGCTCCGCTGCTGGACTCCGACGGCTCTTCTTCTCTGTGAG CAAGCTCACCTGGACAGAGGAGTGGCAGCAGGGGAAAGCTTCTCTATGCTCCGTGATGATGAGGTCTGCAACACCACTAC ACGCAAGAGCCTCTCCCTGCTCCGGGTAAAGCGGCAGCGAGACTCCCGGACCTCAGAGTCCGCCACACCCGAAAGTGGTG GCGGAGAGGTTGAGCTGTGGAGTCTGGCGTGGCTGGTGAGCGAGGGGCTCACTCGTTTGTCTGTGAGCTTCTGGCTTCTGGCTT CAACATCTCTTATTATATCACTGGTGGTCAAGCCCGGTAAGGCGCTGGAATGGTGGTATTTATTTCTTCTTATGG CTATCTCTTATGCCGATAGCGTCAAGGCCGTTTCACTATAAGCGCAGACACATCCAAAAACACAGCTACCTACAAATGAACA GCTTAAGAGCTGAGGACACTGCCGTCTATTATTGTCTGCACTGTTCTGGATCCAAAAACCGTACTTCTCTGGTTGGGCTATG GACTACTGGGTCAAGGAACCTGGTCAACCTCTCTCGGTGGAGTGGAGTGATATCCAGATGACCCAGTCCCGAGCTCCC TGTCCGCTCTGTGGCGATAGGTCACCATCACCTGCCGTGCCAGTCACTCCGTGTCAGCGCTGAGCTGGTATCAACAGAAA CCAGAAAAGCTCCGAAGCTTCTGATTTACTCGGATCCAGCTCTACTGAGTCCCTTCTGCTTCTCTGAGCCGTTCCGGG ACGGAATTCCTGACCATCAGAGTCTGACGCCGGAAGACTTCGCAACTTATCTGTGAGCAATACTCTGGGGTCCGTTTCTAC GTTCCGACAGGGTACCAAGGTGGAGATCAAA	39		40
			EVQLVESGGGLVQPGGSLRLSCAASGFNIS YSSIHVWRQAPGKGLWVASIYPSYSTYY ADSVKGRFTISADTSKNTAYLQMNSLRAED TAVYYCARYAMDYWGQGLTLTVSSGGG GSDIQMTQSPSSLSASVGDRTITCRASQS VSSAVAWYQQKPKAPKLLIYSASSLYSGV PSRFSGSRSGTDFLTLSISLQPEDFATYYCQ QAFYYPITFGQGTKEIKLEDKTHITKVEPKS SDKTHNCPCPAPELLGGPSVFLFPPPKPD TLMISRTPEVTCVVVDVSHEDPEVKFNWY VDGVEVHNAKTKPREEQYNSTYRVVSVLT VLIHQDWLNGKEYCKVSNKALPAPIEKTIS KAKGQPREPQVYTLPPRIELMTSNQVSLSC AVKGFYPSDIAVEWESNGQPENNYKTTTP VLDSGDFFLVSKLTVDKSRWQQGNVFC SYMHEALHNYTKSLSLSPGKSGSETPGT SESATPESGGGEVQLVESGGGLVQPGGSL RLSCAASGFNISYIHWVRQAPGKGLW VASIYSSGYTSYADSVKGRFTISADTSKNT AYLQMNSLRAEDTAVYYCARTVIRGSKPY PSGWAMDYWGQGLTLTVSSGGGSDIQ MTQSPSSLSASVGDRTITCRASQSVSAV AWYQQKPKAPKLLIYSASSLYSGVPSRFS GSRSGTDFLTLSISLQPEDFATYYCQYSW GPFTFGQGTKEIK	

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ID	DNA	SEQ ID	Protein	SEQ ID
5019-knob-2459-2460	GAGGTTACAGCTGGTGGAGTCTGGCGGTGGCTGGTGCAGCCAGGGGCTCACTCCGTTTGTCTGTGCAGCTTCTGGCTTCAACA TCGGTTCTTCTATCACTGGGTGGTGCAGCCCGGGTAAAGGCTGGAATGGGTGGTGTGATCTATTATTTCTGTTTGGCTCTTA CTTCTTATGCCGATAGCGTCAAGGGCGTTTCACTATAAGCGCAGACATCCAAAAACAGACCTACTACAAATGAACAGCTTA AGAGCTGAGGACACTGCCGTCTATTATTGTCTCGCTACCAATTCCTGTTTGTCTTGTGACTACTGGGTCAAGGAACCTT GGTCACCGTCTCCTCGGGTGGAGGTGGCAGTGATATCCAGATGACCCAGTCCCGAGCTCCCTGTCCGCTCTGTGGCGGATAGG GTCAACATCACCTGCCGTGCCAGTCACTGTCAGCGCTGTAGCCTGTATCAACAGAAACAGGAAAGCTCCGAAGCTTCT GATTACTCGGCATCAGCCTTACTCTGGAGTCCCTTCTCGTGTAGCCGTTCCGGGACGGAATTCACCTGACCATCAG CAGTCTGCAGCGGAAGACTTCGCAACTTATTACTGTGCAAGGTGTTTACCTGTTCCAGTCCGACAGGGTACCAAGGTGGAG ATCAAACTCGAGgataaaactcacacaAAAGTGGAGcccaaaCTTCTgataagaccctactTgccaccCGTgccacAGCACTGAACCTCT GGGGGACCGTCAGTCTTCTTCCCCCAAAACCAAGGACACCTCATGATCTCCGGACCCCTGAGGTACATGCGTGGTGG TGGACGTGAGCCACGAAGACCTGAGGTCAAGTTCACTGGTACGTGGACGGGTGAGGTGCATAATGCCAAGACAAAGCCGC GCCAGGAGCAGTACAACAGCAGTACCGTGTGTGTCAGCGTCTCACCGTCTGCACACGAGACTGGCTGAATGGCAAGGAGTACA AGTGCAAGGTCTCAACAAAGCCTCCAGCCCCATCGAGAAACCATCTCAAGCAAAAGGGCAGCCCCGAGAACCAATGGT GTTTGACCTGCCCATCCGGAGGAGATGACCAAGAACCAAGTCAAGCTGAGTGGTGCATGGTCAAGGCTTCTATCCAGCGAC ATCGCGTGGAGTGGGAGAGCAATGGCAGCCGGAGAACCACTACAAGACCAAGCTCCCGTCTGCTGGACTCCGACGCTCTTCT TCTGTACAGCAAGCTCACCGTGGACAAAGCCGCTGGCAGCAGGGAAACGTCTTCTATGCTCCGTGATGATGAGGCTCTGCA CAACCACTACACGCAGAAAGCCTCTCCCTGTCTCGGGTAAAGCGGCAGCGAGACTCCCGGACCTCAGAGTCCGCCACACCC GAAAGTGGTGGCGGAGAGTTCAAGCTGGTGGAGTCTGGCGGTGGCTGTGACAGCCAGGGGCTCACTCCGTTTGTCTGTGCA GCTTCTGGCTTCAACTTTTCTTCTTCTATACACTGGGTGCTAGGCCCGGGTAAAGGCTGGAATGGGTGCACTATTCT TCTTCTATGGCTACTATTATGCCGATAGCGTCAAGGGCGCTTCTACTATAAGCGCAGACATCCAAAAACACAGCCTACCTA CAAAAGAACGCTTAAGAGCTGAGGACACTGCCGTCTATTATTGTCTCGCGGTGGTCTGGTGTCTTCTATTACGGTTTCTGTTAC TACTCTTGGTGGGCTTGGACTACTGGGTCAAGAACCTGTCACCGTCTCTCGGGTGGAGGTGGCAGTATATCCAGATGA CCAGTCCCGAGCTCCCTGTCCGCTCTGTGGCGATAGGTCACCATCACTGCCGTGCCAGTCAGTCCGCTGTCAGCGCTGA GCCGTGTATCAACAGAAACAGGAAAGCTCCGAAGCTTCTGATTACTGGCATCCAGCTCTACTCTGGAGTCCCTTCTCGCTTC TCTGGTAGCCGTTCCGGGACGGAATTCACCTGACCATCAGCAGTCTGCAGCCGGAAGACTTCGCAACTTATTACTGTACGCAAGC TCTTACGCTCCGATCACGTTCCGACAGGGTACCAAGGTGGAGATCAAA	41	EVQLVESGGGLVQPGGSLRLCAASGFNIG SSSIHWVROAPGKGLWVASVAFSTSY ADSVKGRFTISADTSKNTAYLQMNSLRAED TAVYYCARYHPFGFALDYWGQGLTVTVS SGGGSDIQMTQSPSSLSASVGRVTITCR ASQSVSSAVAWYQQKPKAPKLLIYSASSL YSGVPSRFSGRSGTDFLTISLQPEDFAT YYCQQQVYLFTFGQGTKEIKLEDKTHKV EPKTSKTHITCCPPCAPPELLGGPSVFLFPPK PKDTLMISRTPEVTCVVVDVSHEDPEVKFN WYVDGVEVHNAKTKPREEQYNSTYRVVS VLTVLHQDWLNGKEYKCKVSNKALPAPIE KTSKAKGQPREPMVFDLPPSREEMTKNQ VSLWCMVKGFYPSDIAVEWESNGQPENN YKTTTPVLDSDGSFELYSLTVDKSRWQQG NVFCSVMHEALHNHYTKSLSPGKSG SETPTGSEATPESGGGEVQLVESGGGLVQ PGGSLRLCAASGFNFSSTSIHWVROAPGK GLEWVASISSYGYTYADSVKGRFTISADT SKNTAYLQMNSLRAEDTAVYYCARGSGV SHYGSVYYSWAWLDYWGQGLTVTVSSGG GGSDIQMTQSPSSLSASVGRVTITCRASQ SVSSAVAWYQQKPKAPKLLIYSASSL VPSRFSGRSGTDFLTISLQPEDFATYYC QQASYAPITFGGQTKVEIK*	42

Table 1A

ID	DNA	SEQ ID	Protein	SEQ ID
5019- hole- 2459- 2460	GAGGTTACGTTGGAGTCTGGCGGTGGCTGGTGCAGCGAGGGGCTCACTCCGTTTGCTCTGTCAGCTTCTGGCTTCAACA TCGGTTCTTCTATTCACATGGGTGGTCAAGGCCCGGTGAAGTGAGTGGTGCATCTATTTATTCGCTTTGGCTCTA CTTCTATCGCGTAGCTCAAGGCCGTTTCACTATAGCGCAGACATCCAAACAGCACTACTCAAAATGAACACCTTA AGCTTACGAGGACAGCTGCCGTCTATTGTCTGCTACCACTTCCGTTCCGTTTCTTTGGACTCTGGGTCAAGAACCTT GGTCACCGTCTCTCGGGTGGAGTGGCAGTGATATCAGATGACCCAGTCCCGAGCTCCCTGTCGCCCTCTGTGGCGATAGG GTCAACATCACTGCCGTGCAGTCGTCTCCAGGCTGTAGCTGGTATCAACAGAAACAGAAAGCTCCGAAGCTTCT GATTTACTCGGCATTCAGCCCTACTCTGAGTCCCTTCTCGCTTCTGTTAGCGCTTCCGGACGGATTCTACTCTGACCATCAG CAGTCTGAGCCCGAAGACTCGCAACTATTATTCATGTGCAGCAAGGTGTTTACCTGTTCCGACAGGTTACCAAGGTGGAG ATCAAACTCGAGagcaaaactcacabaAAAGTTAGGCCAAATCTTCTgataagaccataaTtGCCACCGTCCCAACCTGAACTCT GGGGGACCGTCACTTCTCTCCCAACCAACCAAGGACACCTCTAGTATCTCCGGACCTCTGAGTGCATGCGTGGTGG TGACGTGAGCCACGAAGACCTGAGTGCAGTTCAACTGTTAGTGACGGCTGGAGTGCATATGCAAGACAAAGCCGC GGGAGGACAGTACAACAGCAGTACCTGTGGTCAGCTCTCACCTCTGCACAGACTGGCTGAATGGCAAGGAGTACA AGTCAAGGTCTTCAACAAAGCCCTCCAGCCCTCATCGAAAAACCATCTCAAAGCCAAAGGAGCCGAGCCGAGAACCCAGGT GTACACCTGCCCAATCCGGGAGTGTATGACACGACCAAGTCAAGTCAAGTGGCGCTCAAGAGCTTCTATCCAGGCAC ATCGCGTGGAGTGGAGAGATGGCAGCGGAGAACCACTACAAGCCACGCCCTCCGCTGCTGGACTCCGAGCTCCCTCT TCCTGTGAGCAAGTCAACGTGGACAGAGCAGAGTGGCAGCGGGAAGCTCTCTCATGTCCGTGATGCATGAGGCTCTGCA CAACACTACGCGAGAAGCTCTCCTGTCTCCGGTAAAGGGCAGCAGACTCCGGGACCTCAGAGTCCGCCACACC GAAAGTGGTGGCGAGAGTTACGCTGGTGGAGTCTGGCGTGGCTGGTGCAGCCAGGGGCTCACTCCGTTTGTCTGTGCA GCTTCTGGCTTCAACCTCTCTATTATATATGACACTGGTGGTGCAGGCCCGGTGAAGGCTGGAATGGTGTGATCTATTAT TCTCTTATGCGTATACCTATTATGCGATAGGCTCAAGGCCGTTTCACTATAGCGCAGACATCCAAACAGCAAGCTACCTA CAAAATGAACGCTTAAGACTGAGGACATGCCGCTATTATTGTCTGCTGGTCTCATGTTCTGCTGCTTACTCTGTTATGGAC TACTGGGTCAAGGAACCTGTCAACCTCTCTCGGTGGAGTGGCAGTGATATCCAGATGACCAAGTCCCGAGCTCCCTGT CCGCTCTGTGGCGATAGGTTACCATCACTCCGTGCCAGTCACTCCGTTCAGCGCTGAGCTGGTATCAACAGAAACCA GGAAAGCTCCGAAGCTCTGATTACTCGGCATCCAGCTCTACTCTGAGTCCCTTCTCGCTTCTGAGCCGTTCCGGGACG GATTTCACTCGACCATCAGCAGTCTGAGCCGGAAGACTTCGCAACTTATTCTGTGCAAGCAATCTTCTATTCTGTATCAGCTTCG GACAGGTTACCAAGTTGGAGTCAAA	43	EVQLVESGGGLVQPGGSLRLSCAASGFGNIG SSSIHWVRRQAPGKGLWEWVAISYSAFSTSY ADSKGRFTTSADTSKNTAYLQMNLSRAED TAVVYCARYHFFPEGLDYLWGQGLTLVTS SGGGSDIQMTQSPSSLSASVGRVITTCR ASQSVSAVAVYQQKPGKAPKLLIYASLL YSGVPSRFSGSRSGTDFLTLTISLQPEDFAT YYCQQGVYLTFFGQGTKVEIKLEDKTHKV EPKSDKTHNCPCPAPPELLGGPSVFLPPK PKDTLMISRTPEVTCVVDVSHEDPEVKFN WYVDGVEVHNAKTKPREEQYNSTYRVWS VLTVLHQDLWNLNGKEYCKVSNKALPAPIE KITISKAKGQPREPQVYTLPPRELMTSNQV SLSCAVKGYPISDIAEVESWGQPPENYK TTPPVLDSDGSFHLVSKLTVDKSRVQQGN VFCSVMHEALHNHYTQKSLSLSPKSGSE TPGTSEATPESGGEVQLVESGGLVQIP GGSLRLSCAASGFGNLSYYMHVWRQAPG KGLWEVAISYSSGYTYTADSVKGRFTISAD TSKNTAYLQMNLSRAEDTAVVYCARWISH VSGHYSGMDYWGQGLTLVTSSGGGSDI QMTQSPSSLSASVGRVITTCRASQSVSA VAVYQQKPGKAPKLLIYASLLSYGVPSRF SGSRSGTDFLTLTISLQPEDFATYYCQSSY SLITFGQGTKEIK*	44

Table 1B

ID	N- fusion VH (VH1)	SEQ ID NO:	SEQ ID NO:	N- fusion VL (VL2)	SEQ ID NO:	SEQ ID NO:	Fc fusion	C- fusion VH (VH3)	SEQ ID NO:	SEQ ID NO:	VH-VL linker	C- fusion VL (VL4)	SEQ ID NO:
5019- knob- 2539- 2542	5019	45	47	5019	46	48	49	50	GATATCCAGATG ACCCAGTCCCCG AGT	GGTGGAG GGTGGC AGT	2542	50	GATATCCAGATG ACCCAGTCCCCG AGT AGTCTCCTGTCC GCCTCTGTGGCG GATAGGGTCACC ATCACCTGCCGT GCCAGTCAGTCC GTGTCCAGCGCT GTAGCTGGTAT CAACAGAAACCA GGAAAAAGCTCCG AAGCTTCTGATT TACTCGGCATCC AGCCTCTACTCT GGAGTCCCTTCT CGCTTCTCTGGT AGCCGTTCCGGG ACGGATTTCAT CTGACCATCAGC AGTCTGCAGCCG GAAGACTTCGCA ACTTATTACTGTC AGCAATACTACT GGCCGATCAGT TGGACAGGGTA CCAAGTGGAG ATCAATGA

Table 1B

ID	N- fusion VH (VH1)	SEQ ID NO:	SEQ ID NO:	N- fusion VL (VL2)	SEQ ID NO:	SEQ ID NO:	Fc fusion	C- fusion VH (VH3)	SEQ ID NO:	SEQ ID NO:	VH-VL linker	C- fusion VL (VL4)	SEQ ID NO:	
5019- hole- 2539- 2542	5019	45	47	5019	46	47	GAGGTTTCAGCTGG TGGAGTCTGGCGG TGGCCTGGTGCAG CCAGGGGGCTCAC TCCGTTTGTCTGT GCAGCTTCTGGCT TCAACATCGGTTCT TCTTCTATCCACTG GGTGGGTACAGGCC CCGGGTAAAGGCC TGGAAATGGGTTGC ATCTATTATCTG CTTTTGGCTCTACT TCTTATGCGGATA GCGTCAAGGCCG TTTCACTATAAGCG CAGACATCCAA AAACACAGCCTAC CTACAAATGAACA GCTTAAGAGCTGA GGACACTGGCGTC TATTATTGCTCG CTACCATTTCCGT TCGGTTTTTGTG GACTACTGGGGTC AAGGAACCTCGT CACCCTCTCTCG	GATATCCAGATG ACCCAGTCCCGGA GCTCCTGTCGCG CTCTGTGGCGCA TAGGGTCAACATC ACCTGCGGTGCCA GTCAAGTCCGTGTC CAGCGCTGTAGC CTGGTATCAACAG AAACCAAGAAA GCTCCGAAGCTTC TGATTTACTGGC ATCAGCCTCTAC TCTGAGTCCCTT CTGCTTCTCTGG TAGCGTTCCGG GACGGATTCACT CTGACCATCAGCA GTCTGACGCCG AAGACTTCGAAC TTATTACTGTACG CAAGGTGTTTACC TGTTCAAGTTCCG ACAGGGTACCAA GGTGGAGATCAA A	CTCGAGaataaactcacataAAGTTGAGCC CAAAATCTTCTgataagaaccataaTTCGCCAAC GTGCCAGACCTGAACCTCTCTGGGGGAC CGTCAAGTCTCTCTCTCCCGCCAAACCCCA AGGACACCTCATGATCTCCGGACCCCTG AGGTCAATCGTGGTGGTGGAGCTGAGC CACGAAGACCTGAGGTCAAGTTCACTG GTACGTGGAGCGGTGGAGGTGCATAATG CCAAGACAAAGCCGGGAGGAGCAGTA CAACAGCAGTACCGTGTGGTCAGCGTCC TCACCGTCTGCACAGGACTGGCTGAAT GGCAAGAGTACAAGTGCAAGGTCTCCAA CAAGCCCTCCAGCCCTCATCGAGAAA CCATCTCAAGCCAAAGGGCAGCCCGGA GAACACAGGTGTACACCTGCCCAAT CCGGGAGCTGATGACACAGCAACAGGTCA GCTGAGCTGCCCGCTCAAGGCTTCTAT CCAGCGACATCGCGTGGAGTGGAGA GCAATGGGACGCCGGAGAACAACTACAA GACACGCTCCCGTGTGGACTCCGAGC GCTCTTCTTCTCTGTGAGCAAGCTCACCG TGGACAAGACAGGTGGCAGCAGGGGAA CGTCTCTCATGCTCCGTGATGATGAGGC TCTGCACAACCACTACAGCAGAGAGCC TCTCCTGTCTCCGGGTAAAGCGGAGC GAGACTCCCGGGACCTCAGAGTCCGCCAC ACCCGAAAAGTGGTGGCGGA	2542	53	46	2539	52
		GAGGTTTCAGCTGG GAGTCTGGCGGTGG CTGGTGGCAGCGAGG GGCTCACTCCGTTGT CCTGTGAGCCAGGG CTTCAACATCTCTTCT ATTATATCACTGGGT GGTCAAGGCCCGGG TAAGGGCTGGAAATG GGTCAAGGCCCGGG TCTCTATGGCTATA CTTCTATGGCTATG CGTCAAGGCCGTTTC ACTAAGCGGAGAC ACATCAAAACACAG CCTACTACAAATGAA CAGCTTAAGAGCTGA GGACACTGCCGTCTAT TATTGTCTGCAGCTG TTCTGGGATCCAAAA ACCGTACTTCTCTGT TGGGCTATGGACTACT GGGGTCAAGGAACCC TGGTCAAGGTTCTCTC G	46	2539	52	46	2539	52						
		GATATCCAGATG ACCCAGTCCCGG AGT												

Table 1B

ID	N- fusion VH (VH1)	SEQ ID NO:	N- fusion VL (VL2)	SEQ ID NO:	SEQ ID NO:	SEQ ID NO:	Fc fusion	C- fusion VH (VH3)	SEQ ID NO:	SEQ ID NO:	VH-VL linker	C- fusion VL (VL4)	SEQ ID NO:						
5019- Fc- 2539	5019	45	5019	46	47	48	GAGGTTCACTGG TGGAGTCTGGCGG TGGCTGTGTGCAG CCAGGGGCTCAC TCCGTTTGTCTGT GCACTTCTGGCT TCAACATCGGTTCT TCTTCTATCACTG GGTGGGTACAGCC CCGGGTAAAGGCC TGGAAATGGGTTGC ATCTATTATTCTG CTTTTGGCTTACT TCTTATGCCGATA GCGTCAAGGGCGG TTTCACTATAAGCG CAGACATATCCAA AAACACAGCTAC CTACAAATGAAAC GCTTAAGAGCTGA GGAACTGCGGTC TATTATTGTGCTCG CTACATTTCCCGT TCGGTTTTGCTTTG GACTACTGGGGTC AAGGAACCTGGT CACCCTCTCTCG	GGTGGG GGTGGC AGT	GATATCCAGATG ACCCAGTCCCGGA CTCTCTGTCCGC CTCTGTGGGCGA TAGGGTCACCATC ACCTGCGGTGCCA GTCAAGTCTGGTGC CAGCGCTGTAGC CTGGTATCAACAG AAACACAGAAAA GCTCCGAAGCTTC TGATTACTTGGC ATCCGACGCTTAC TCTGGAGTCCCTT CTCGCTTCTCTGG TAGCCGTTCCGG GAGGATTTACT CTGACCATCAGCA GTCTGCAGCCGG AAGACTTTCGAAC TTATTACTGTAG CAAGGTGTTTACC TGTTCACTTCGG ACAGGGTACCAA GGTGGAGATCAA A	51	CTCGAGGacacaaactcacacAAAGTTGAGCC CAAAATCTTCTTgataagaaccatactgccaccgtg ccagacctgaactcttgggggagaccgtcagttctctt cttccccaaaccccaaggagacacccctgaatctccc ggacccctgagggtcacatgctgttgggtggagcgtgagc caggaagaccttgaggtaagtcaacttggtaegtggga cggcgtggagggtgataatgccaagacaaagccgcgag gaaggacagtaacacagcacgtaccgttggtaegtcagc tcctcacgcttctccaccaggaactggcgaatggc-aag gggtacaagtcgaaggtcttccaacaagccctctccag cccccatcagagaacacctctccaagcacaaggagca ggcccgagaactcacaggtgtacacccctgccccatccc gggagagagatgaacgaagaccaggtcagctgacttg cc-tggcacaaggcttctatcccgagcagatcgc-gtgg agtcacagctcccgctgcttggacttcga-cggctcttct tccttcacagcagctcacccgtggacaaagacaggtgg cagcagagggaacgtttcttctgcttccgtgagcaaga ggctctgacacacacacacagcagagagacgtcttccc tgcttccgggttaaaAGCGCAGCGAGACTCCC GGGACCTCAGAGTCCGCCACACCCGAAAG TGGTGGCGGA	2539	49	GAGGTTTCAGCTGGTG GAGTCTGGCGGTGGC CTGGTGACGCGAGGG GGCTCACTCCGTTTGT CCTGTGCAGCTTCTGG CTTCAACATCTCTTATT CTTCTATCCACTGGT GGTCAAGGCCCGGG TAAGGGCTGGAATG GGTTGCATATATTCT TCTTATTATGGCTATA CTTATTATGCCGATAG CGTCAAGGGCGCTTTC ACTATAAGCGGAGAC ACATCAAAACACAG CCTACTACAAATGAA CAGCTTAAGAGCTGA GGACACTGGCGTCTAT TATTGTCTGGCGTCT ATTACTTCCGTGGGC TGGTGTCTATGGACTAC TGGGTCAAGGAACC CG	2539	46	GGTGGG GGTGGC AGT	52	GATATCCAGATG ACCCAGTCCCGG AGTCTCCTGTCC GCCTCTGTGGGC GATAGGGTCACC ATCACCTGCCGT GCCAGTCAAGTCC GTGTCCAGCGCT GTAGCCTGGTAT CAACAGAAACCA GGAAGAGCTCCG AAGCTTCTGATT TACTCGGCATCC AGCCTCTACTCT GGAGTCCCTTCT CGCTTCTCTGGT AGCCGTTCGCGG ACGGAATTCACCT CTGACCATCAGC AGTCTGCAGCCG GAAGACTTCGCA ACTTATTACTGTC AGCAATACTCTT GGGGTCCGTTCA GGTTCGGACAGG GTACCAAGGTGG AGATCAAAATGA

Table 1B

ID	N- fusion VH (VH1)	SEQ ID NO:	N- fusion VL (VL2)	SEQ ID NO:	SEQ ID NO:	Fc fusion	C- fusion VH (VH3)	SEQ ID NO:	SEQ ID NO:	VH-VL linker	C- fusion VL (VL4)	SEQ ID NO:
5019- Fc- 2542	5019	45	5019	47	51	53	2542	53	46	2542	50	
		TGGAAGTCTGGCGG TGGCGTGTGGAG CCAGGGGCTCAC TCCGTTTGTCTGT GCAACTCTGGCT TCAACATCGCTTCT TCTCTATCCACTG GGTGGCTGAGCGT CCGGGTAAAGGCC TGGAAATGGGTTC ATCATTTATTTCT CTTTTGTCTTACT TCTTATGCGGATA GCGTCAAGGCGCG TTTCACTATAAAGG CAGACATAACAA AAACACAGCTTAC CTACAAATGAACA GCTTAAGAGCTGA GGACACTTCGCTC TATTATGTGCTCG CTACCAATTCTCCG TCGGTTTTGCTTGG AAGGAACCTTGGT CACCCTCTCTCG	GATATCCAGATG ACCCAGTCCCCGA GCTCCTGTCCGC CTCTGTGGCGA TAGGGTCACCATC ACCTGCCGTGCCA GTCACTCCGTGTC CAGCGCTGTAGC CTGGTATCAACAG AAACCAGGAAAA GCTCCGAAGCTTC TGATTTACTGGC ATCCAGCTCTTAC TCTGGAGTCCCTT CTCGCTTCTGG TAGCCGTTCCGG GACGAGTTTCACT CTGACCATCAGCA GTCTGCAGCCGG AAGACTTCGCAAC TTATTATGTGCTG CAAGGTGTTTACC TGTTCAAGTTCGG ACAGGGTACCAA GGTGGAGATCAA A	CTCGAGcacaacactcacacAAAGTTGAGCC CAAATCTTCTgataagaccataactgccaccgtg ccagacactgaactctggggagacgcgtcagctcttctt cttcccccaaaacccaagagacacccctcagctctccc ggacccctgaggtaacatgcgtgggtggagcgtgagac cacgaagaccctgaggtaagttcaactggtaegtggga cgcggtggaggtgataatgccaagacaaagccgcagg gagagacagtaacaacagcagcgtaccgtgtgtagcgg tcctcacgtcttcgaccagagactggcgaatggcgaag gggtacaagtgcaaggctctccaacaagccctccacag cccccatcagagaaccattctcaaaagcacaagaggca gccccgagaatcacaggggtatcacctccgcccccatccc gggagagagatgaacaaagacacaggtcagctgactctg ccggcacaaggcttctatcccgagcgcacatcgcgctgtt agtgaggagagcaatgggcagcgcaggagaacaactaca agtgaggagagcaatgggtctctcgtcgtgtagcaga ggctctgcaacacacacacagcagaagagcctctccc tgctccgggttaaaAGCGCAGCGAGACTCCC GGGACTCTCAGAGTCCGCCACACCCGAAAG TGGTGGCGGA	GAGGTTCAGCTGGTG GAGTCTGGCGGTGGC CTGGTGAGCCAGGG GGCTCACTCCGTTGT CCTGTGCAGCTCTGG CTTCAACATCTCTTCT ATTATCTCACTGGGT GGTCACTCTATTAT TAAGGGCTGGAAAT TCTTCTATGGCTATA CTTCTTATGCCGATAG CGTCAAGGGCGCTTTC ACTATAAGCGGAGAC ACATCCAAAACACAG CCTACTACAAATGAA CAGCTTAAGAGCTGA GGACACTGCCGTCTAT TATTGTCTGCAGCTG TTCGTGGATCCAAAA ACCGTACTTCTCTGT TGGGCTATGGACTACT GGGGTCAAGGAACCC TGGTCAACCGTCTCTC G	GGTGGG GGTGGC AGT	GATATCCAGATG ACCCAGTCCCCG AGCTCCTGTCC GGCTCTGTGGGC GATAGGGTCACC ATCACCTGCCGT GCCAGTCAGTCC GTGTCCAGCGCT GTAGCCTGGTAT CAACAGAAACCA GAAAAAGCTCCG AAGCTTCTGATT TACTCGGCATCC AGCCTCTACTCT GGAGTCCCTTCT CGCTTCTCTGGT AGCCGTTCCGGG ACGGATTTCAT CTGACCATCAGC AGTCTGCAGCCG GAAGACTTCGCA ACTTATTACTGTC AGCAATACTACT GGCCGATCAGCT TCGGACAGGGTA CCAAGGTGGAG ATCAAAATGA					

Table 1B

ID	N- fusion VH (VH1)	SEQ ID NO:	SEQ ID NO:	N- fusion VL (VL2)	SEQ ID NO:	SEQ ID NO:	Fc fusion	C- fusion VH (VH3)	SEQ ID NO:	SEQ ID NO:	VH-VL linker	C- fusion VL (VL4)	SEQ ID NO:	SEQ ID NO:				
5038- knob- 2539- 2542	5038	54	55	5038	46	GGTGA GGTGC AGT	GAGGTTTCAGCTGG TGGAGTCTGGCGG TGGCCTGGTGCAG CCAGGGGGCTCAC TCCGTTTGTCTGT GCAGCTCTGGCT TCAACATCTCTTAT TATTATGCACTG GGTGGCTCAGGCC CCGGGTAAAGGCC TGGAAATGGGTTGC ATCTATTATCTT ATTATGGCTACT TATTATGCCGATA GGCTCAAGGGCGG TTTCACTATAAGCG CAGACATATCAA AAACACAGCTAC CTACAAATGAACA GCTTAAGAGCTGA GGACACTGGCGTC TATTATGTGCTCG CTCTCTTTCTCT GGGCTATGGACTA CTGGGTCAGGA ACCTGGTCAACG TCTCTCG	46	GGTGA GGTGC AGT	5038	48	GATATCCAGATG ACCCAGTCCCGGA GCTCTCTGTCCGC TCTGTGGGCGCA CCAGGGTCCACATC ACCTGCCGTGCCA GTCAAGTCCGTGTC CAGCGCTGTAGC CTGGTATCAACAG CAACAGAGACCTGAGGTCAAGTTCAACTG GTCAGTGGAGGGGTGGAGGTGCATAATG CCAAGACAAAGCCGGCGAGGAGCAGTA CAACGACGTACCTGTGTGTCAGCGTCC TCACCGTCTGCACAGGACTGGCTGAAT GGCAAGGAGTACAAGTGCAAGGTCTCCAA CAAGCCCTCCAGCCCCATCGAGAAA CCATCTCAAAGCCAAAGGGAGCCCCGA GAAACCAATGGTGTGTGACCTGCCCGCTCA CGGAGGAGATGACCAAGAACACAGGTCA GCCGTGGTGCATGTCAGGGGCTTCTAT CCACGCGACATCCGCTGGAGTGGGAGA GCAATGGGAGCGGGAGAACAACTACAA GACACGCGCTCCGTGTGGAATCCGACG GCTCTCTTCTGTACAGCAAGCTCACCG TGGACAAGAGCCGCTGGCAGCAGGGGAA CGTCTTCTCATGCTCCGTGATGTCATGGC TCTGCACAACCACTACACGACAGAGGCC TCTCCCTGTCTCCGGGTAAAAGCGGAGC GAGACTCCCGGACCTCAGAGTCCGCCAC ACCCGAAAGTGGTGGCGGA	49	GAGGTTTCAGCTGGT GAGTCTGGCGGTGGC CTGGTGCAGCCAGGG GGCTCACTCCGTTTGT CCTGTGAGCTTCTGG CTTCAACATCTCTTAT CTTCTATCCACTGGT GGCTCAGGCCCGGG TAAGGGCTGGAATG GGTGCATATATTTCT TCTATTATGGCTATA CTTATTATGCCGATG CGTCAAGGGCGTTTC ACTATAAGCGGAC ACATCAAAACACAG CCTACCTACAAATGAA CAGCTTAAGAGCTGA GGACACTGGCTCTAT TATTGTCTGGCGTC ATTACTCCCGTGGC TGGTGTATGGACTAC TGGGTCAAGGAACC CG	46	GGTGA GGTGC AGT	50	GATATCCAGATG ACCCAGTCCCGG AGCTCTCTGTCC GCCTCTGTGGCG GATAGGTCAAC ATCACCTGCCGT GCCAGTCAGTCC GTGTCCAGCGCT GTAGCTGGTAT CAACAGAAACA GGAAAAGCTCCG AAGCTTCTGATT TACTCGCATCC AGCTCTACTCT GGAGTCCCTCT CGCTCTCTGGT AGCCGTTCCGGG ACGGAATTCAC CTGACCATCAGC AGTCTGCAGCCG GAAGACTTCGCA ACTTATTACTGTC AGCAATACTACT GGCCGATCACGT TCGGACAGGGTA CCAAGGTGGAG ATCAAAATGA

Table 1B

ID	N- fusion VH (VH1)	SEQ ID NO:	SEQ ID NO:	N- fusion VL (VL2)	SEQ ID NO:	Fc fusion	C- fusion VH (VH3)	SEQ ID NO:	SEQ ID NO:	VH-VL linker	C- fusion VL (VL4)	SEQ ID NO:
5038- hole- 2539- 2542	5038	54	55	5038	55	56	56	53	46	GGTGGG AGT	2539	52
		TGGAGTCTGGCGG TGGCCTGGTGCAG CCAGGGGCTCAC TCCGTTTGTCTGT GCAGCTCTGGCT TCAACATCTCTTAT TATTATGCACTG GGTGGCTCAGGCC CCGGTAAAGGCC TGGAAATGGGTTGC ATCTATTATCTT ATTATGGCTATCT TATTATGCCGATA TTCCTACTAAGCG CAGACATCCAA AAACACAGCCTAC CTACAAATGAACA GCTTAAGAGCTGA GGACACTGGCGTC TATTATGTGCTCG CTCTCTTTCTCTT GGGCTATGGACTA CTGGGGTCAAGGA ACCTGGGTCAACG TCTCTCG	GATATCCAGATG ACCCAGTCCCGG GCTCCTGTCGCG CTCTGTGGCGGA TAGGGTCAACATC ACCTGCCGTGCCA GTCAAGTCCGTGTC CAGGCTGTAGC CTGGTATCAACAG AAACCAAGAAAA GCTCCGAAGCTTC TGATTTACTCGGC ATCCAGCCTCTAC TCTGGAGTCCCTT CTGCTTCTCTGG TAGCGTTCCGG GACGGATTCACT CTGACCATCAGCA GTCTGCAGCCGG AAGACTTCGCAAC TTATTAAGTCTAG CAACATCCGTGGT CTGGTGGTTACCT GATCAGTTTCGG ACAGGGTACCAA GGTGGAGATCAA A	CTCGAGgagaaaaactcacacaaaAGTTGAGCC CAAAATCTTCTgataagagccataaatGCCCAAC GTGCCAGACACTGAATCTCTGGGGGGAC CGTCAGTCTTCTCTCTCCCGCCCAAAACCCA AGGACACCTCATGATCTCCCGGACCCCTG AGGTCACTGCGTGGTGGTGGAGCTGAGC CACGAAGACCTGAGGTCAAGTTCACTG GTACGTGGAGGGGTGGAGGTGCATAATG CCAAGACAAAGCCGGGAGGAGCAGTA CAACAGCAGTACCTGTGTGTGCAGCTCC TCACCGTCTGCACAGGACTGGCTGAAT GGCAAGGAGTACAAGTGCAAGGTCTCCAA CAAGCCCTCCAGCCCCCATCGAGAAAA CCATCTCCAAAGCCAAAGGGCAGCCCCGA GAACACAGGTGTACACCTGCCCCCAAT CCGGGAGCTGATGACCAAGCAACCAAGTCA GCTGAGCTGCCCGCTCAAGGCTTCTAT CCACGCAATCGCCGTGGAGTGGGAGA GCAATGGGACGCCGGAGAACAACTACAA GACACGCTCCCGTGTGGACTCCGACG GCTCTTCTCTCGTGAAGCAAGCTCACCG TGGACAAGACAGGTGGCAGCAGGGGAA CGTCTTCTCATGCTCCGTGATGATGAGGC TCTGCACACCACTACACGACAGAGAGCC TCTCCTGTCTCCGGGTAAAAGCGGCAGC GAGACTCCCGGAGCCTCAGATCCGCCAC ACCCGAAAAAGTGGTGGCGGA	5242	53	GAGGTTGAGTGGTG GAGTCTGGCGGTGGC CTGGTGAGCAGGG GGCTCACTCCGTTTGT CCTGTGCAGTCTCTGG CTTCAACATCTCTCTT ATTATATCACTGGGT GGTTCATCTATTAT TAAGGGCTGGAAATG GGTCAAGGCCCGGG TAAGGGCTGGAAATG TCTCTATGGCTATA CTTCTATGGCTATG CGTCAAGGCCGTTTC ACTAAGGCGAGAC ACATCAAAACACAG CCTACCTACAAATGAA CAGCTTAAGCTGA GGACACTGCCGTCTAT TATTGTCTCGCACTG TTCGTGGATCCAAAA ACCGTACTTCTCTGT TGGGCTATGGACTACT GGGGTCAAGGAACCC TGGTCAACCGTCTCTC G	46	GGTGGG AGT	2539	52	GATATCCAGATG ACCCAGTCCCGG AGCTCCTGTCC GCCTCTGTGGGC GATAGGGTCACC ATCACCTGCCGT GCCAGTCAGTCC GTGTCCAGCGCT GTAGCCTGGTAT CAACAGAAACCA GGAAAGCTCCG AAGCTTCTGATT TACTCGCATCC AGCCTCTACTCT GGAGTCCCTTCT CGCTTCTCTGGT AGCCGTTCCGGG ACGGATTCACT CTGACCATCAGC AGTCTGCAGCCG GAAGACTTCGCA ACTTATTACTGTC AGCAATACTCTT GGGGTCCGTTC GGTTCGACAGG AGATCAAAATGA

Table 1B

ID	N- fusion VH (VH1)	SEQ ID NO:	SEQ ID NO:	N- fusion VL (VL2)	SEQ ID NO:	SEQ ID NO:	Fc fusion	C- fusion VH (VH3)	SEQ ID NO:	SEQ ID NO:	VH-VL linker	C- fusion VL (VL4)	SEQ ID NO:	SEQ ID NO:
5044- knob- 2539- 2542	5044	57	58	5044	46	50	CTCGAGGcacaactcacacAAAGTGGAGCC CAAAACTTCTgataagaccataactTGCCCAACC GTGCCACAGCTGAACCTCTCTGGGGGAC CGTCACTCTCTCTCTCCCGCAAAACCCA AGGACACCTCATGATCTCCGGACCCCTG AGGTCAATGGGTGGTGGAGTGAGCG GTACGTGGAGCGGTGGAGGTGCATAATG CCAAGACAAAGCCGGCGAGAGCAGTA CAACAGCAGCTACCGTGGTGAGCGTCC TCACGTCTGCACAGGACTGGCTGAAT GGCAAGGAGTACAAGTGCAAGGTCTCCAA CAAGCCCTCCGAGCCCCATCGAGAAA CCATCTCAAAGCCAAAGGGGAGCCCCGA GAACCAATGGTGTGTGACCTGCCCGCTCC CGGAGGAGATGACCAAGAACACAGGTCA GCCTGTGGTGCATGTCAAGGGCTTCTAT CCACGCACTCCCGTGGAGTGGGAGA GCAATGGGAGCGGAGAACAACTACAA GACCAAGCTCCCGTGGTGGACTCCGACG GGCTCTCTCTCTGTACAGCAAGCTCACC TGGCAAGAGCCGCTGGCAGCAGGGGAA CGTCTTCTCATGTCTGTGATGCATGAGGC TCTGCAACAACCTACACAGCAGAAGGCC TCTCCTCTGTCTCCGGGTAAAAGCGGAGC GAGACTCCCGGAGCTCAGAGTCCGCCAC ACCCGAAAAAGTGGTGGCGGA	2539	49	46	GGTGGG GGTGGC AGT	2542	50	GATATCCAGATG ACCCAGTCCCCG AGCTCTCTGTC GCCTCTGTGGC GATAGGTCAACC ATCACCTGCGCT GCCAGTCAGTCC GTGTCCAGCGCT GTAGCTGGTAT CAACAGAACTCCG GAAAAGCTCCG AAGCTTCTGATT TACTCGCATCC AGCTCTACTCT GGAGTCCCTCT CGCTTCTCTGT AGCCGTTCCGGG ACGGAATTCAC CTGACCATCAGC AGTCTGCAGCCG GAAGACTTCGCA ACTTATTACTGTC AGCAATACTACT GGCCGATCAGT TCGGACAGGGTA CCAAGGTGGAG ATCAAAATGA

Table 1B

ID	N- fusion VH (VH1)	SEQ ID NO:	SEQ ID NO:	N- fusion VL (VL2)	SEQ ID NO:	SEQ ID NO:	Fc fusion	C- fusion VH (VH3)	SEQ ID NO:	SEQ ID NO:	VH-VL linker	C- fusion VL (VL4)	SEQ ID NO:		
5044- hole- 2539- 2542	5044	57	58	5044	46	56	CTCGAGGcaaaactcacacaAAAGTTGAGCC CAAATCTTCTTgataagaccataaTGTCCCAACC GTGCCAGCACTGAACCTCTCTGGGGGAC GGTCAAGTCTCTCTCTCCCGCAAAACCCA AGGACACCTCATGATCTCCGGACCCCTG AGGTCAATGGTGGTGGTGGAGTGAGGC GTACGTGGAGCGGCTGGAGGTGCAATAATG CCAAGACAAAGCCGGGAGGAGCAGTAA CAACAGCACTGACCGTGGTGAGCGTCC TCACGTCTGCACAGGACTGGCTGAAT GGCAAGGAGTACAAGTGCAAGGTCTCCAA CAAGCCCTCCCAAGCCCCATCGAGAAA CCATCTCAAGCCAAAGGGCAGCCCCGA GAACACAGGTGTACACCTGCCCCCAAT CCGGGAGCTGATGACCAAGCAACAGGTCA GGCTGAGCTGGCCGCTCAAAGGCTTCTAT CCAGCGACATCCCGTGGAGTGGAGA GCAATGGGACGCCGAGAACAACTACAA GACCAAGCTCCCGTGTGGACTCCGACG GCTCTCTTCTCTGTGAGCAAGCTCACCG TGGACAAGACAGGTGGCAGCAGGGGAA CGTCTTCTCATGCTCGTGTGATGAGGC TCTGCAACAACCACTACAGCAGAGAGCC TCTCCTCTGTCTCCGGGTAAAGTCGGCAGC GAGAACTCCCGGACCTCAGAGTCCGCCAC ACCCGAAAAGTGGTGGCGGA	2542	53	GAGTTCAGCTGGTG GAGTCTGGCGGTGGC CTGGTGCAAGCCAGGG GGCTCACTCCGTTTGT CCTGTGCAGCTCTGG CTTCAACATCTCTTCTT ATTATATCACTGGGT TAAGGGCTGGAAATG GGTTCATCTATTAT TCTCTTATGGCTATA CTTCTATGGCTGATT CGTCAAGGGCGGCTTTC ACTATAAGCGGAC ACATCAAAACACAG CCTACTACAATGAA CAGCTTAAGAGCTGA GGACACTGCCGTCTAT TATTGTCTGCAGCTG TTCGTGGATCCAAAA ACCGTACTTCTCTGT TGGGCTATGGACTACT GGGGTCAAGGAACCC TGGTCACCGTCTCTC G	46	GGTGGG GGTGGC AGT	2539	52	GATATCCAGATG ACCCAGTCCCCG AGCTCCTGTGCC GCCTCTGTGGGC GATAGGTCAAC ATCACCTGCCGT GCCAGTCAGTCC GTGTCCAGCGCT GTAGCTGGTAT CAACAGAAACCA GGAAAAGCTCCG AAGCTTCTGATT TACTCGGCATCC AGCCTCTACTCT GGAGTCCCTTCT CGCTTCTCTGGT AGCCGTTCCGGG ACGGATTCACT CTGACCATCAGC AGTCTGCAGCCG GAAGACTTCGCA ACTTATCTACTCT AGCAATACTCTT GGGGTCCGTTCA CGTTCGGAGAG GTACCAAGGTGG AGATCAAAATGA

Table 1B

ID	N- fusion VH (VH1)	SEQ ID NO:	SEQ ID NO:	N- fusion VL (VL2)	SEQ ID NO:	SEQ ID NO:	Fc fusion	C- fusion VH (VH3)	SEQ ID NO:	SEQ ID NO:	VH-VL linker	C- fusion VL (VL4)	SEQ ID NO:	SEQ ID NO:
5048- knob- 2539- 2542	5048	59	60	5048	46	50	CTCGAGgacaaactcacacAAAGTGGAGCC CAAAAGTCTGtatagagccataactTGCCCAACC GTGCCACAGCTGAACCTCTCTGGGGGAC CGTCACTCTCTCTTCCCGCAAAACCCA AGGACACCTCATGATCTCCGGACCCCTG AGGTCAATGGTGGTGGTGGAGCTGAGCC GTACGTGGAGCGGCTGGAGGTGCATAATG CCAAGACAAAGCCGCGAGAGCAGTA CAACAGCAGCTACCGTGGTGAGCGTCC TCACGTCTGCACAGGACTGGCTGAAT GGCAAGGAGTACAAGTGCAAGGTCTCCAA CAAGCCCTCCGAGCCCCATCGAGAAA CCATCTCAAAGCCAAAGGGCAGCCCCGA GAACCAATGGTGTGTGACCTGCCCACTCC CGGAGGAGATGACCAAGAACACAGGTCA GCTGTGGTGCATGTGCAAGGGCTTCTAT CCACGACATCCCGCTGGAGTGGGAGA GCAATGGGAGCGGAGAACAACTACAA GACCAAGCTCCCGTGGTGGACTCCGACG GGCTCTTCTCTGTACAGCAAGCTCACCG TGGACAAGCCGCTGGCAGCAGGGGAA CGTCTCTCATGTCTGTGATGCACTAGGC TCTGCACAACCACTACAGCAGAGAGCC TCTCCTGTCTCCGGGTAAAAGCGGAGC GAGACTCCCGGAGCCTCAGAGTCCGCCAC ACCCGAAAGTGGTGGCGGA	2539	49	46	GGTGGG GGTGGC AGT	2542	50	GATATCCAGATG ACCCAGTCCCCG AGCTCCTGTCC GCCTCTGTGGC GATAGGGTCACC ATCACCTGCCGT GCCAGTCAGTCC GTGTCCAGCGCT GTAGCCTGGTAT CAACAGAACTCA GGAAAAGCTCCG AAGCTTCTGATT TACTCGCATCC AGCCTCTACTCT GGAGTCCCTCT CGCTTCTCTGGT AGCCGTTCCGGG ACGGATTCTACT CTGACCATCAGC AGTCTGCAGCCG GAAGACTTCGCA ACTTATTACTGTC AGCAATACTACT GGCCGATCAGT TCGGACAGGGTA CCAAGGTGGAG ATCAAAATGA

Table 1B

ID	N- fusion VH (VH1)	SEQ ID NO:	SEQ ID NO:	N- fusion VL (VL2)	SEQ ID NO:	SEQ ID NO:	Fc fusion	C- fusion VH (VH3)	SEQ ID NO:	SEQ ID NO:	VH-VL linker	C- fusion VL (VL4)	SEQ ID NO:	SEQ ID NO:
5048- hole- 2539- 2542	5048	59	60	5048	46	56	CTCGAGgacaaaactcacacAAAGTTGAGCC CAAAATCTTCTgataagaccataaTTCGCCACCC GTGCCAGCACTGAACCTCTCTGGGGGAC CGTCAGTCTCTCTCTCCGCCCAAAACCCA AGGACACCTCATGATCTCCGGACCCCTG AGGTCACTGGTGGTGGTGGAGCTGAGCC GTACGTGGAGCGGCTGGAGGTGCATAATG CCAAGACAAAGCCGGGAGGAGCAGTAA CAACAGCAGCTACCGTGGTGCAGCGTCC TCACGCTCTGCACAGCACTGGCTGAAT GGCAAGGAGTACAAGTGCAAGGTCTCCAA CAAGCCCTCCCAAGCCCCATCGAGAAAA CCATCTCCAAGCCAAAGGGCAGCCCCGA GAACACAGGTGTACACCTGCCCCCAAT CCGGGAGCTGATGACACAGCAACCAAGTCA GCTGAGCTCGCCGCTCAAGGCTTCTAT CCACGCAATCGCCGTGGAGTGGGAGA GCAATGGGACGCCGGAGAACAACTACAA GACACGCTCCCGTGTGGACTCCGACG GCTCTCTTCTCTGTGAGCAAGCTCACCG TGGACAAGAGCAGGTGGCAGCAGGGGAA CGTCTCTCATGCTCCGTGATGATGAGGC TCTGCACAACCACTACAGCAGAGAGCC TCTCCTCTGTCTCCGGGTAAAAGCGGAGC GAGACTCCCGGAGCCTCAGAGTCCGCCAC ACCCGAAAAAGTGGTGGCGGA	2542	53	GAGGTTTCAGCTGGTG GAGTCTGGCGGTGGC CTGGTGAGCCAGGG GGCTCACTCCGTTTGT CCTGTGCAGCTTCTGG CTTCAACATCTCTCTT ATTATATCACTGGGT GGTCAAGGCCCGGG TAAGGGCTGGAAATG GGTGGCATCTATTAT TCTCTTATGGCTATA CTTCTATGGCGATAG CGTCAAGGCCGTTTC ACTAAGCGCAGAC ACATCAAAACACAG CCTACTACAATGAA CAGCTTAAGAGCTGA GGACACTGCCGTCTAT TATTGTCTGCAGCTG TTCTGGGATCCAAAA ACCGTACTTCTCTGT TGGGCTATGGACTACT GGGGTCAAGGAACCC TGGTCAACGCTCTCTC G	46	GGTGGG GGTGGC AGT	2539	GATATCCAGATG ACCCAGTCCCCG AGCTCCTGTCC GCCTCTGTGGC GATAGGTCACC ATCACAGTCCT GGAAGAGCTCCG AAGCTTCTGATT TACTCGCATCC AGCCTCTACTCT GGAGTCCCTTCT CGCTTCTCTGT AGCCGTTCCGGG ACGGATTCTACT CTGACCATCAGC AGTCTGCAGCCG GAAGACTTCGCA ACTTATTACTGTC AGCAATACTCTT GGGGTCCGTTCA GGTTCGACAGG GTACCAAGGTGG AGATCAAAATGA

Table 1B

ID	N- fusion VH (VH1)	SEQ ID NO:	SEQ ID NO:	N- fusion VL (VL2)	SEQ ID NO:	SEQ ID NO:	Fc fusion	C- fusion VH (VH3)	SEQ ID NO:	SEQ ID NO:	VH-VL linker	C- fusion VL (VL4)	SEQ ID NO:	SEQ ID NO:	
5062- knob- 2539- 2542	5062	61	62	5062	46	48	CTCGAGgcaaaactcacacAAAGTGGAGCC CAAAAATCTCTgataagaccataactTGCCCAACC GTGCCACAGCTGAACCTCTCTGGGGGAC CGTCAGTCTCTCTCTCCGCCAAAAACCA AGGACACCTCATGATCTCCGGACCCCTG AGGTCAATCGGTGGTGGTGGAGCTGAGC GTACGTGGAGCGGTGGAGGTGCATAATG CCAAGACAAAGCCGCGAGAGCAGTA CAACAGCAGCTACCGTGGTGAGCGTCC TCACGCTCTGCACAGAGACTGGCTGAAT GGCAAGAGAGTACAAGTGCAAGGTCTCCAA CAAGCCCTCCGAGCCCCATCGAGAAA CCATCTCAAGCCAAAGGGCAGCCCCGA GAAACCAATGGTGTGTGACCTGCCCTCC CGGAGAGAGATGACCAAGAACACAGGTCA GCTGTGGTGTGATGTCAAGGGCTTCTAT CCACGCGACATCCCGTGGAGTGGGAGA GCAATGGGACGCGGAGAACAACTACAA GACCAAGCTCCCGTGGACTCCGACG GCTCTCTCTCTGTACAGCAAGCTCACCG TGGACAAGAGCCGCTGGCAGCAGGGAA CGTCTTCTCATGCTCCGTGATGATGAGGC TCTGCACAACCACTACAGCAGAGAGCC TCTCCTGTCTCCGGGTAAAAGCGGAGC GAGACTCCCGGACCTCAGAGTCCGCCAC ACCCGAAAAAGTGGTGGCGGA	2539	49	GAGGTTGAGCTGGTG GAGTCTGGCGGTGGC CTGGTGCAAGCCAGGG GGCTCACTCCGTTTGT CTCTGCAGCTTCTGG CTTCAACATCTCTATT CTTCTATCCACTGATT GCGTCAGGCCCGGG TAAGGCGCTGGAATG GGTTCATATTTCT TCTATTATGGCTATA CTTATTATGCCGATT CGTCAAGGGCCGTTTC CTATATAGCGGAGC ACATCAAAAACACAG CCTACTCAAACTGAA CAGCTTAAGAGCTGA GGACATCGCTCTAT TATTGTCTGGCGTCT ATTACTCCCGTGGC TGGTGCTATGCACTAC TGGGGTCAAGGAACC CTGGGTCAACCGTCTCT CG	46	GGTGGG GGTGGC AGT	2542	50	GATATCCAGATG ACCCAGTCCCCG AGCTCCTGTGCC GCCTCTGTGGG GATAGGGTCACC ATCACCTGCCGT GCCAGTCAGTCC GTGTCCAGCGCT GTAGCCTGGTAT CAACAGAAACCA GGAAAAAGCTCCG AAGCTTCTGATT TACTCGCATCC AGCCTCTACTCT GGAGTCCCTCT CGCTTCTCTGGT AGCCGTTCCGGG ACGGATTTCAT CTGACCATCAGC AGTCTGCAGCCG GAAGACTTCGCA ACTTATTACTGTC AGCAATACTACT GGCCGATCAGT TCGGACAGGGTA CCAAGGTGGAG ATCAAAATGA

Table 1B

ID	N- fusion VH (VH1)	SEQ ID NO:	SEQ ID NO:	N- fusion VL (VL2)	SEQ ID NO:	SEQ ID NO:	Fc fusion	C- fusion VH (VH3)	SEQ ID NO:	SEQ ID NO:	VH-VL linker	C- fusion VL (VL4)	SEQ ID NO:			
5062- hole- 2539- 2542	5062	61	62	5062	48	62	GATATCCAGATG ACCCAGTCCCGGA GCTCCTGTCCGC CTCTGTGGCGA TAGGGTCAACATC ACCTGCGGTGCCA GTCAAGTCCGTGTC CAGGCGTGTAGC CTGGTATCAACAG AAACAGAAAA GCTCCGAAGCTTC TGATTTACTTGGC ATCCAGCTCTAC TCTGGAGTCCCTT CTCGCTTCTCTGG TAGCCGTTCCGG GACGATTTCACT CTGACCATCAGCA GTCTGACGCCGG AAGACTTCGAAC TTATTACTGTAG CAATCTGGTTGGT GGGGTGTTCCTCT GATCAGTTCGG ACAGGGTACCAA GGTGGAGATCAA A	56	CTCGAGcacaactcacataAAGTTGAGCC CAAACTTCTTgataagaccataatTGCCCAAC GTGCCAGCACTGAACCTCTCGGGGGAC CGTCAGTCTCTCTTCCCGCCAAACCCA AGGACACCTCATGATCTCCGGACCCCTG AGGTCACTGCGTGGTGGTGGAGCTGAGC GACCAAGACCTGAGGTCAAGTTCAACTG GTACGTGGAGCGGTGGAGGTGCATAATG CCAAGACAAAGCCGGGAGGAGCAGTA CAACAGCACTACCGTGTGTGTCAGCGTCC TCACGTCTGCACAGGACTGGCTGAAT GGCAAGAGTACAAGTGCAAGGTCTCCAA CAAGCCCTCCAGCCCATCGAGAAA CCATCTCAAGCCAAAGGGCAGCCCGA GACCAAGAGTGTACACCTGCCCCCAAT CCGGGAGCTGATGACCAAGCAACAGGTCA GCTGAGCTGGCGCTCAAAGGCTTCTAT CCAGCGACATCCCGTGGAGTGGAGA GCAATGGGACGCGGAGAACAACTACAA GACCAAGCTCCCGTGTGGACTCCGACG GCTCTTCTTCTCTGTGAGCAAGCTCACCG TGGACAAGACAGGTGGCAGCAGGGGAA CGTCTTCTCATGCTCGTGTGATGATGAGGC TCTGCAACAACCACTACAGCAGAGAGCC TCTCCTCTGTCTCCGGGTAAAGCGCGAGC GAGACTCCCGGACCTCAGAGTCCGCCAC ACCCGAAAGTGGTGGCGGA	53	GAGGTTCAGCTGGTG GAGTCTGGCGGTGGC CTGGTGACAGCAGGG GGCTCACTCCGTTTGT CCTGTGACGCTCTGG CTTCAACATCTCTTCTT ATTATATCACTGGGT GGTTCATCTATTAT TAAGGGCTGGAAATG GGTTCATCTATTAT TCTCTTATTGGCTATA CTTCTTATGGCTATG CGTCAAGGCCGTTTC ACTATAAGCGAGAC ACATCAAAACACAG CCTACTACAATGAA CAGCTTAAGAGCTGA GGACACTGCCGTCTAT TATTGTCTGCAGCTG TTCTGGATTCACAAA ACCGTACTTCTCTGT TGGGCTATGGACTACT GGGGTCAAGGAACCC TGGTCAACGCTCTCTC G	46	GGTGGAG GGTGGC AGT	2539	52	GATATCCAGATG ACCCAGTCCCGG AGCTCCTGTGCC GCCTCTGTGGCG GATAGGTCAAC ATCACCTGCCGT GCCAGTCAGTCC GTGTCCAGCGCT GTAGCCTGGTAT CAACAGAAACCA GGAAAAAGCTCCG AAGCTTCTGATT TACTCGGCATCC AGCCTCTACTCT GGAGTCCCTCT CGCTTCTCTGGT AGCCGTTCCGGG ACGGAATTCAGT CTGACCATCAGC AGTCTGCAGCCG GAAGACTTCGCA ACTTATTACTGTC AGCAATACTCTT GGGGTCCGTTCA GGGTTCGAGAGG GTACCAAGGTGG AGATCAAAATGA

Table 1B

ID	N- fusion VH (VH1)	SEQ ID NO:	SEQ ID NO:	N- fusion VL (VL2)	SEQ ID NO:	Fc fusion	C- fusion VH (VH3)	SEQ ID NO:	SEQ ID NO:	VH-VL linker	C- fusion VL (VL4)	SEQ ID NO:
5063- knob- 2539- 2542	5063	63	64	5063	46	GAGGTTGAGCTGG TGGAGTCTGGCGG TGGCTGTGTCAG CCAGGGGCTCAC TCCGTTTGTCTGT GCAGCTCTGGCT TCAACATCTCTTAT TATTATCCACTG GGTGGCTCAGGCC CCGGTAAGGGCC TGGATGGGTTGC ATCTATTATCTT CTTCTGGCTACT TATTATCCGATA GGCTCAAGGCCG TTTCACTATAAGCG CAGACATCCAA AAACACAGCTAC CTACAAATGAACA GCTTAAGAGCTGA GGACACTGGCTG TATTATTGCTCG CTCTCTTTCTACT GGCTATGGACTA CTGGGGTCAAGGA ACCTGGTCAACCG TCTCTCG	48	CTCGAGgagaaaaactcacacaaaAGTGGAGCC CAAAACTTCTgataagaccataactTGCCCAACC GTGCCAGACACTGAACCTCTGGGGGGAC CGTCAGTCTTCTCTCTCCGCCCAAAACCCA AGGACACCTCATGATCTCCGGGACCCCTG AGGTCACTGCGTGGTGGTGGAGCTGAGC CACGAAGACCTGAGGTCAAGTTCACTG GTACGTGGAGGGGTGGAGGTGCATAATG CCAAGACAAAGCCGGCGAGGAGCAGTA CAACGACGTACCTGTGTGTGTCAGCTCC TCACCGTCTGCACAGGACTGGCTGAAT GGCAAGGAGTACAAGTGCAAGGTCTCCAA CAAGCCCTCCAGCCCCCATCGAGAAAA CCATCTCCAAAGCCAAAGGGGAGCCCCGA GAACCAATGGTGTGTGACCTGCCCCCTCC CGGAGGAGATGACCAAGAACACAGGTCA GCTGTGGTGTGATGTCAGGGCTTCTAT CCACGCAATCGCGGTGGAGTGGGAGA GCAATGGGAGCGCGGAGAACAACTACAA GACCAAGCTCCCGCTGGACTCCGAGC GGCTCTTCTCTGTACAGCAAGCTCACCG TGGACAGAGCGCTGGCAGCAGGGGAA CGTCTCTCATGCTCCGTGATGATGAGGC TCTGCACCAACACTACACGACAGAGGCC TCTCCCTGTCTCCGGGTAAAAGCGGAGC GAGACTCCGGGACCTCAGAGTCCGCCAC ACCCGAAAAAGTGGTGGCGGA	49	GAGGTTGAGCTGGTG GAGTCTGGCGGTGGC CTGGTGAGCAGGG GGCTCACTCCGTTTGT CCTGTGAGCTTCTGG CTTCAACATCTCTTAT CTTCTATCCACTGGGT GGCTCAGGCCCGGG TAAGGGCTGGAAATG GGTGCATATATTTCT TCTATTATGGCTATA CTTATTATGCCATAG CGTCAAGGCCGTTTC ACTATAAGCGCAGAC ACATCAAAACACAG CCTACCTACAAATGAA CAGCTTAAGAGCTGA TATTGTCTCGCGCTC ATTACTTCCGTGGGC TGGTGTCTATGGACTAC TGGGTCAAGGAACC CG	50	GATATCCAGATG ACCCAGTCCCG AGCTCTCTGTCC GCCTCTGTGGGC GATAGGGTCACC ATCACCTGCCGT GCCAGTCAGTCC GTGTCCAGCGCT GTAGCTGGTAT CAACAGAAACCA GGAAAAGCTCCG AAGCTTCTGATT TACTCGCATCC AGCTCTACTCT GGAGTCCCTTCT CGCTTCTCTGT AGCCGTTCCGGG ACGGATTCTACT CTGACCATCAGC AGTCTGCAGCCG GAAGACTTCGCA ACTTATTACTGTC AGCAATACTACT GGCCGATCAGT TCGGACAGGGTA CCAAGGTGGAG ATCAAAATGA

Table 1B

ID	N- fusion VH (VH1)	SEQ ID NO:	SEQ ID NO:	N- fusion VL (VL2)	SEQ ID NO:	SEQ ID NO:	Fc fusion	C- fusion VH (VH3)	SEQ ID NO:	SEQ ID NO:	VH-VL linker	C- fusion VL (VL4)	SEQ ID NO:
5063- hole- 2539- 2542	5063	63	64	5063	46	56	CTCGAGGacaaactcacacAAAGTTGAGCC CAAACTCTCTgataagaccataaTGGCCACCC GTGCCAGACCTGAATCTCTCTGGGGGAGC CGTCAGTCTTCTCTCTCCCGCCAAACCCCA AGGACACCTCATGATCTCCGGACCCCTG AGGTCACTGGTGGTGGTGAGCTGAGCC GTACGTGGAGCGGCTGGAGGTGCATAATG CCAAGACAAAGCCGGGAGGAGCAGTGA CAACAGCAGCTACCGGTGGAGTCAAGCTCC TCACGCTCTGCACAGGACTGGCTGAAT GGCAAGGAGTACAAGTGCAAGGTCTCCAA CAAGCCCTCCAGGCCCATCGAGAAA CCATCTCCAAGCCAAAGGGCAGCCCCGA GAACACAGGTGTACACCTGCCCCCAAT CCGGGAGCTGATGACCAAGCAACCAAGTCA GCTGAGCTCGCCGCTCAAGGCTTCTAT CCACGCAATCGCCGTGGAGTGGGAGA GCAATGGGACGCCGGAGAACAACTACAA GACACGCTCCCGTGTGGACTCCGACG GCTCTCTTCTCTGTGAGCAAGCTCACCG TGGACAAGACAGGTGGCAGCAGGGGAA CGTCTTCTCATGCTCGTGATGATGAGGC TCTGCACAACCACTACACGACAGAAGGCC TCTCCTGTCTCCGGGTAAAAGCGGAGC GAGACTCCCGGAGCTCAGAGTCCGCCAC ACCCGAAAAAGTGGTGGCGGA	2542	53	GAGGTTTCAGCTGGTG GAGTCTGGCGGTGGC CTGGTGAGCAGCGGG GGCTCACTCCGTTTGT CCTGTGCAGCTCTGG CTTCAACATCTCTCTT ATTATATCACTGGGT GGTCAAGGCCCGGG TAAGGGCTGGAAATG GGTGCATCTATTAT TCTCTATGGCTATA CTTCTATGCCGATAG CGTCAAGGCCGTTTC ACTAAGCGGAGAC ACATCAAAAAACACAG CCTACTACAAATGAA CAGCTTAAGAGCTGA GGACACTGCCGTCTAT TATTGTCTGCAGCTG TTCGTGGATCCAAAA ACCGTACTTCTCTGT TGGGCTATGGACTACT GGGGTCAAGGAACCC TGGTCAACCGTCTCTC G	52	GATATCCAGATG ACCCAGTCCCCG AGCTCCTGTCC GCCTCTGTGGC GATAGGTCACC ATCACAGAGCTCC GGAAAGCTCCG AAGCTTCTGATT TACTCGCATCC AGCCTCTACTCT GGAGTCCCTTCT CGCTTCTCTGGT AGCCGTTCCGGG ACGGATTCTACT CTGACCATCAGC AGTCTGCAGCCG GAAGACTTCGCA ACTTATTACTGTC AGCAATACTCTT GGGGTCCGTTCA GGGTTCGACAGG GTACCAAGGTGG AGATCAAAATGA	

Table 1B

ID	N- fusion VH (VH1)	SEQ ID NO:	SEQ ID NO:	N- fusion VL (VL2)	SEQ ID NO:	Fc fusion	C- fusion VH (VH3)	SEQ ID NO:	SEQ ID NO:	VH-VL linker	C- fusion VL (VL4)	SEQ ID NO:
5080- knob- 2539- 2542	5080	65	66	5080	46	GAGGTTGAGCTGG TGGAGTCTGGCGG TGGCCTGGTGCAG CCAGGGGCTCAC TCCGTTTGCTGT GCAGCTTCTGGCT TCAACATCTCTTAT TATCTATGACTG GGTGGCTCAGGCC CCGGTAAAGGCC TGGAAATGGGTTC ATCTATTCTCTT ATTATGCTTACT TCTTATGCGGATA GCGTCAAGGCCG TTTCATATAAGCG CAGACATCCAA AAACACAGCCTAC CTACAAATGAACA GCTTAAGAGCTGA GGACACTGCGGTC TATTATTGCTCG CTCTGGTACCG GGTATGGACTACT GGGGTCAAGGAAC CCTGGTCCCGTC TCCTCG	46	GGTGA GGTGC AGT	5080	SEQ ID NO:	GATATCCAGATG ACCCAGTCCCG AGCTCCTGTCC GCCTCTGTGGC GATAGGTCAAC ATCACCCTGCGT GCCAGTCAGTCC GTGTCCAGCGCT GTAGCTGGTAT CAACAGAAACCA GGAAGAGCTCCG AAGCTTCTGATT TACTCGCATCC AGCCTCTACTCT GGAGTCCCTTCT CGCTTCTCTGT AGCCGTTCCGGG ACGGATTCTACT CTGACCATCAGC AGTCTGCAGCCG GAAGACTTCGCA ACTTATTACTGTC AGCAATACTACT GGCCGATCAGT TCGGACAGGGTA CCAAGGTGGAG ATCAATGA	50
		48	49	2539	46	CTCGAGgagaaaaactcacataAAAGTGGAGCC CAAAACCTTCTgataagaccataactTGCCCAAC GTGCCAGACACTGAACCTCTGGGGGAGC CGTCAGTCTTCTCTCTCCGCCCAAAACCA AGGACACCTCATGATCTCCCGGACCCCTG AGGTCACTGCGTGGTGGTGGAGCTGAGC CACGAAGACCTGAGGTCAAGTTCACTG GTACGTGGAGCGGTGGAGGTGCATAATG CCAAGACAAAGCCGCGGAGGACAGTA CAACGACGTACCTGTGTGTGTCAGCGTCC TCACCGTCTGCACAGAGACTGGCTGAAT GGCAAGGAGTACAAGTGCAAGGTCTCCAA CAAAGCCTCCAGCCCCCATCGAGAAAA CCATCTCCAAAGCAAAGGGGAGCCCCGA GAACCAATGGTGTGTGACCTGCCCGCTCC CGGAGGAGATGACCAAGAACACAGGTCA GCTGTGGTGTGATGTCAGGGCTTCTAT CCCAGCGACATCGCGGTGGAGTGGAGA GCAATGGGACGCGGAGAACAACTACAA GACCAGCTCCCGCTGGACTCCGAGC GCTCTTCTCTGTGTACAGCAAGCTCAGC TGGACAGAGCGCTGGCAGCAGGGGAA CGTCTCTCATGCTCCGTGATGATGAGGC TCTGCACCAACACTACACGACAGAGGCC TCTCCTGTCTCCGGTAAAGCGGAGC GAGACTCCCGGAGCCTCAGAGTCCGCCAC ACCCGAAAAGTGGTGGCGGA	48	GGTGA GGTGC AGT	2539	SEQ ID NO:	GAGGTTGAGCTGGT GAGTCTGGCGGTGGC CTGGTGCAGCAGG GGCTCACTCCGTTGT CCTGTGCAGCTCTGG CTTCACATCTCTTAT CTCTATCCACTGGT GCGTCAGGCCCGGG TAAGGGCTGGAAATG GGTGCAATATTTCT TCTTATTGGCTATA CTATTATGCCATAG CGTCAAGGCCGTTTC ACTAAGGCGAGAC ACATCAAAACACAG CCTACCTACAAATGA CAGCTTAAGCTGA GGACACTGCCGTCTAT TATTGTCTCGGCTC ATTACTTCCGTGGC TGGTGTCTATGGATCC TGGGTCAAGGAACC CTGGTCAACGCTCTCT CG	46

Table 1B

ID	N- fusion VH (VH1)	SEQ ID NO:	SEQ ID NO:	N- fusion VL (VL2)	SEQ ID NO:	SEQ ID NO:	Fc fusion	C- fusion VH (VH3)	SEQ ID NO:	SEQ ID NO:	VH-VL linker	C- fusion VL (VL4)	SEQ ID NO:	SEQ ID NO:	
5080- hole- 2539- 2542	5080	65	66	5080	66	GATATCCAGATG ACCCAGTCCCCGA GCTCCTGTCCGC CTCTGTGGCGA TAGGGTCACCATC ACCTGCGGTGCCA GTCAAGTCCGTGTC CAGGCGCTGTAGC CTGGTATCAACAG AAACAGGAAAA GCTCCGAAGCTTC TGATTTACTGGC ATCTATTCTCTT ATTATGCTCTACT TCTTATGCCGATA GGCTCAAGGGCGG TTTCACTATAAGGG CAGACACTCAAA AAACAGAGCTAC CTACAAATGAACA TATTAAAGACTGA GGACACTGCGTC TATTATTGTGCTG CTTCTGTGACCGG GGTATGGACTACT GGGGTCAAGGAAC CTGTGGTCAACGTC TCCTCG	56	CTCGAGcacaacctcacacaAAAGTTGAGCC CAAAATCTTCTgataagaccataatTGCCCAACC GTGCCAGCACTGAACCTCTCCGGGGAC CGTCAGTCTTCTCTTCCCGCCAAAAACCA AGGACACCTCATGATCTCCGGACCCCTG AGGTCAATGCGTGGTGGTGGAGTGAGGC GTACGTGGAGCGGCTGGAGGTGCATAATG CCAAGACAAAGCCGGGAGGAGCAGTGA CAACAGCAGTACCGTGTGTGTCAGCGTCC TCACGTCTGCACAGCACTGGCTGAAT GGCAAGAGTACAAGTGCAAGGTCTCCAA CAAAGCCCTCCAGCCCCCATCGAGAAA CCATCTCAAAGCAAGGGGAGCCCCGA GAACACAGGTGTACACCTCTGCCCAAT CCGGGAGCTGATGACCAAGCAACAGGTCA GCTGAGCTGGCCGCTCAAGGCTTCTAT CCACGCAATCGCCGTGGAGTGGGAGA GCAATGGGACGCGGAGAACAACTACAA GACCACTGCTCCGCTGGACTCCGACG GCTCTTCTTCTCGTGAGCAAGCTCACCG TGGACAAGAGCAGGTGGCAGCAGGGGAA CGTCTCTCATGCTCCGTGATGATGAGGC TCTGCACAACCACTACAGCAGAGAGCC TCTCCCTGTCTCCGGGTAAAAGCGGAGC GAGACTCCCGGACCTCAGAGTCCGCCAC ACCCGAAAAAGTGGTGGCGGA	53	GAGGTTTCAGCTGGTG GAGTCTGGCGGTGGC CTGGTGGCGCAGCGGG GGCTCACTCGTTTGT CCTGTGCAAGCTCTTGG CTTCAACATCTCTCTT ATTATATCCACTGGT GGCTCAAGGCCCGGG TAAGGGCTGGAATG GGTGCATCTATTAT TCTTCTATGGCTATA CTTCTATGCGGATAG CGTCAAGGGCGGTTTC ACTATAGCGAGAC ACATCAAAAACACAG CCTACTCAAAATGAA CAGCTTAAGAGCTGA GGACACTGCGCTAT TATTGTGCTGCACTG TTCGTGGATCCAAAA ACCGTACTTCTGTGT TGGGCTATGGACTACT GGGGTCAAGGAACCC TGGTCAAGGCTCTCTC G	46	GGTGGG GGTGGC AGT	2539	52	GATATCCAGATG ACCCAGTCCCCG AGCTCCTGTGCC GCCTCTGTGGCG GATAGGTCAAC ATCACCTGCCGT GCCAGTCAGTCC GTGTCCAGCGCT GTAGCTGGTAT CAACAGAAATCA GGAAAAAGCTCCG AAGCTTCTGATT TACTCGGCATCC AGCTCTACTCT GGAGTCCCTTCT CGCTTCTCTGGT AGCCGTTCGCGG ACGGATTTCAT CTGACCATCAGC AGTCTGCAGCCG GAAGACTTCGCA ACTTATTACTGTC AGCAATACTCTT GGGGTCCGTTC GGGTTCGAGAGG GTACCAAGGTGG AGATCAAAATGA

Table 1B

ID	N- fusion VH (VH1)	SEQ ID NO:	SEQ ID NO:	N- fusion VL (VL2)	SEQ ID NO:	SEQ ID NO:	Fc fusion	C- fusion VH (VH3)	SEQ ID NO:	SEQ ID NO:	VH-VL linker	C- fusion VL (VL4)	SEQ ID NO:					
5081- knob- 2539- 2542	5081	67	68	5081	46	68	GATATCCAGATG ACCCAGTCCCCGA GCTCCTGTCCGC CTCTGTGGCGA TAGGGTCACCATC ACCTGCCGTGCCA GTCAAGTCCGTGTC CAGCGCTGTAGC CTGGTATCAACAG AAACAGGAAAA GCTCCGAAGCTTC TGATTTACTGGC ATCTATTATCTT ATTCTGGCTACT TATTATGCCGATA TTCCTCTAAGCG TTCCTATAAGGG CAGACATATCAA AAACACAGCTAC CTACAAATGAACA GCTTAAGAGCTGA GGACACTGGCGTC TATTATTGCTCGT CTCTCTTTGCTT GGGCTTTTGACTA CTGGGGTCAAGGA ACCTGGTCAACG TCTCTCTG	GAGGTTCACTGG TGAAGTCTGGCGG TGGCCTGGTGCAG CCAGGGGGCTCAC TCCGTTTGTCTGT GCAGCTTCTGGCT TCAACTCTCTTAT TATTATGCACTG GGTGGCTCAGGCC CCGGGTAAAGGCC TGGAAATGGGTTGC ATCTATTATCTT ATTCTGGCTACT TATTATGCCGATA TTCCTCTAAGCG TTCCTATAAGGG CAGACATATCAA AAACACAGCTAC CTACAAATGAACA GCTTAAGAGCTGA GGACACTGGCGTC TATTATTGCTCGT CTCTCTTTGCTT GGGCTTTTGACTA CTGGGGTCAAGGA ACCTGGTCAACG TCTCTCTG	5081	GGTGGG GGTGGC AGT	2542	50	GATATCCAGATG ACCCAGTCCCCG AGCTCCTGTCC GCCTCTGTGGC GATAGGTCAAC ATCACCTGCCGT GCCAGTCAGTCC GTGTCCAGCGCT GTAGCCTGGTAT CAACAGAAACCA GGAAAAGCTCCG AAGCTTCTGATT TACTCGCATCC AGCCTCTACTCT GGAGTCCCTTCT CGCTTCTCTGGT AGCCGTTCCGGG ACGGATTTCAT CTGACCATCAGC AGTCTGCAGCCG GAAGACTTCGCA ACTTATTACTGTC AGCAATACTACT GGCCGATCAGT TCGGACAGGGTA CCAAGGTGGAG ATCAAAATGA	46	GGTGGG GGTGGC AGT	2542	50	GATATCCAGATG ACCCAGTCCCCG AGCTCCTGTCC GCCTCTGTGGC GATAGGTCAAC ATCACCTGCCGT GCCAGTCAGTCC GTGTCCAGCGCT GTAGCCTGGTAT CAACAGAAACCA GGAAAAGCTCCG AAGCTTCTGATT TACTCGCATCC AGCCTCTACTCT GGAGTCCCTTCT CGCTTCTCTGGT AGCCGTTCCGGG ACGGATTTCAT CTGACCATCAGC AGTCTGCAGCCG GAAGACTTCGCA ACTTATTACTGTC AGCAATACTACT GGCCGATCAGT TCGGACAGGGTA CCAAGGTGGAG ATCAAAATGA

Table 1B

ID	N- fusion VH (VH1)	SEQ ID NO:	SEQ ID NO:	N- fusion VL (VL2)	SEQ ID NO:	SEQ ID NO:	Fc fusion	C- fusion VH (VH3)	SEQ ID NO:	SEQ ID NO:	VH-VL linker	C- fusion VL (VL4)	SEQ ID NO:				
5081- hole- 2539- 2542	5081	67	68	5081	46	56	CTCGAGcacaactcacataAAGTTGAGCC CAAAATCTTCTgataagaccataaTGTCCCAAC GTGCCAGCACTGAACCTCTCTGGGGGAC CGTCAGTCTTCTCTTCCCCCAAAACCA AGGACACCTCATGATCTCCGGACCCCTG AGGTCAATGGTGGTGGTGGAGCTGAGC GTACGTGGAGCGGTGGAGGTGCATAATG CCAAGACAAAGCCGGGAGGAGCAGTA CAACAGCACTACCGTGGTGCAGCGTCC TCACGTCTGCACAGGACTGGCTGAAT GGCAAGAGTACAAGTGCAAGGTCTCCAA CAAGCCCTCCCAAGCCCCATCGAAAA CCATCTCAAGCCAAAGGGCAGCCCCGA GAACACAGGTGTACACCTGCCCCCAAT CCGGGAGCTGATGACACAGCAACCAAGTCA GCTGAGCTCGCCGCTCAAAGGCTTCTAT CCACGCAATCGCCGTGGAGTGGGAGA GCAATGGGACGCCGAGAACAACTACAA GACACGCTCCCGTGTGGACTCCGACG GCTCTTCTTCTCGTGAGCAAGCTCACCG TGGACAAGACAGGTGGCAGCAGGGGAA CGTCTCTCATGCTCGTGTGATGATGAGGC TCTGCACAACCACTACAGCAGAGAGCC TCTCCTCTGTCTCCGGGTAAAGCGCGAGC GAGACTCCCGGACCTCAGAGTCCGCCAC ACCCGAAAAGTGGTGGCGGA	2542	53	GAGGTTGAGCTGGTG GAGTCTGGCGGTGGC CTGGTGAGCAGCGGG GGCTCACTCCGTTTGT CCTGTGAGCTTCTGG CTTCAACATCTCTTCTT ATTATATCACTGGGT TAAGGGCTGGAATG GGTTCATCTATTAT TCTCTTATGGCTATA CTTCTATGCCGATAG CGTCAAGGCCGTTTC ACTATAAGCGGAC ACATCAAAACACAG CCTACTACAATGAA CAGCTTAAGAGCTGA GGACACTGCCGTCTAT TATTGTCTGCAGCTG TTCGTGGATCCAAAA ACCGTACTTCTCTGT TGGGCTATGGACTACT GGGGTCAAGGAACCC TGGTCAACGCTCTCTC G	46	GGTGGG GGTGGC AGT	2539	52	GATATCCAGATG ACCCAGTCCCCGA GCTCCTGTCCGC CTCTGTGGCGGA TAGGGTCAACATC ACCTGCCGTGCCA GTCAAGTCCGTGTC CAGGCTGTAGC CTGGTATCAACAG AAACAGGAAAA GCTCCGAAGCTTC TGATTTACTGGC ATCCAGCCTCTAC TCTGGAGTCCCTT CTGCTTCTCTGG TAGCCGTTCCGG GACGGAATTTACT CTGACCATCAGCA GTCTGACGCCGG AAGACTTCGAAC TTATTACTGTAG CAAGTGGTGG GGTCCGTTCACTG TCGGACAGGGTA CCAAGTGGAGA TCAAA	52	GATATCCAGATG ACCCAGTCCCCG AGCTCCTGTCC GGCTCTGTGGG GATAGGCTCAC ATCACCTGCGGT GCCAGCTAGTCC GTGTCCAGCGCT TAGCTCTGGTAT CAACAGAAACA GGAAAAAGCTCCG AAGCTTCTGAT TACTGGCGATCC AGCTCTACTCT GGAGTCCCTCT CGCTTCTTGGT AGCGCTTCGGG ACGGATTCTACT CTGACCATCAGC GTGACCATCAGC AGTCTGACGCG AGTCTGACGCG GAAGACTTGGCA ACTTATACTGTCT AGCAATACTCTT GGGGTCCGTTCA CGTTCGGACAG GTACCAAGGTGG AGATCAATGA

Table 1B

ID	N- fusion VH (VH1)	SEQ ID NO:	SEQ ID NO:	N- fusion VL (VL2)	SEQ ID NO:	SEQ ID NO:	Fc fusion	C- fusion VH (VH3)	SEQ ID NO:	SEQ ID NO:	VH-VL linker	C- fusion VL (VL4)	SEQ ID NO:
2928- hole- 2539- 2542	2928	69	70	2928	46	70	GATATCCAGATG ACCCAGTCCCGGA GCTCCTGTCCGC CTCTGTGGCGGA TAGGGTCAACATC ACCTGCCGTGCCA GTCAAGTCCGTGTC CAGCGCTGTAGC CTGGTATCAACAG AAACCAAGAAAA GCTCCGAAGCTTC TGATTTACTTGGC ATCCAGCCTCTAC TCTGGAGTCCCTT CTCGCTTCTCTGG TAGCCGTTCCGG GACGGAATTTACT CTGACCATCAGCA GTCTGACGCCGG AAGACTTCGAAC TTATTACTGTACG CAAGCTTTCTACT ACCCGATCAGTT CGGACAGGGTAC CAAGGTGGAGAT CAAA	2928	GGTGGAG GGTGGC AGT	2539	52	GATATCCAGATG ACCCAGTCCCGG AGCTCCTGTCC GCCTCTGTGGGC GATAGGGTCACC ATCACCTGCCGT GCCAGTCAGTCC GTGTCCAGCGCT GTAGCCTGGTAT CAACAGAAACCA GGAAAAGCTCCG AAGCTTCTGATT TACTCGGCATCC AGCCTCTACTCT GGAGTCCCTTCT CGCTTCTCTGGT AGCCGTTCGCGG ACGGATTCTACT CTGACCATCAGC AGTCTGCAGCCG GAAGACTTCGCA ACTTATTACTGTC AGCAATACTCTT GGGGTCCGTTCA CGTTCGGACAGG GTACCAAGGTGG AGATCAAAATGA	

Table 1B

ID	N- fusion VH (VH1)	SEQ ID NO:	SEQ ID NO:	N- fusion VL (VL2)	SEQ ID NO:	SEQ ID NO:	Fc fusion	C- fusion VH (VH3)	SEQ ID NO:	SEQ ID NO:	VH-VL linker	C- fusion VL (VL4)	SEQ ID NO:
5019- knob- 2459- 2460	5019	45	GAGGTTCAAGTGG TGGAGTCTGGCGG TGGCCTGGTGCAG CCAGGGGCTCAC TCCGTTTGTCTGT GCAGCTTCTGGCT TCAACATCGGTTCT TCTTCTATCCACTG GGTGGCTCAGGCC CCGGGTAAAGGCC TGGAAATGGGTTGC ATCTATTATCTG CTTTTGCCTCTACT TCTTATGCCGATA GCGTCAAGGGCGG TTTCACTATAAGCG CAGACACTCAAA AAACACAGCCTAC CTACAAATGAACA GCTTAAAGAGCTGA GGACACTGCGGTC TATTATTGCTCGT CTACCATTTCCGTT TCGGTTTTGCTTTG GACTACTGGGGTC AAGGAACCTCGGT CACCGTCTCTCG	46	GGTGGAG GGTGGC AGT	47	GATATCCAGATG ACCCAGTCCCCGA GCTCCTGTCCGC CTCTGTGGCGGA TAGGGTCAACATC ACCTGCGGTGCCA GTCAAGTCCGTGTC CAGGCTGTAGC CTGGTATCAACAG AAACAGGAAAA GCTCCGAAGCTTC TGATTTACTGGC ATCAAGCCTCTAC TCTGAGTCCCTT CTGCTTCTCTGG TAGCCGTTCCGG GACGGAATTCAT CTGACCATCAGCA GTCTGCAGCCGG AAGACTTCGAAC TTATTACTGTACG CAAGGTGTTTACC TGTTCAAGTTCCG ACAGGGTACCAA GGTGGAGATCAA A	48	CTCGAGgacaaactcacataAAAGTGAAGCC CAAAAATTTCTgataagagccataactTGCCCAACC GTGCCAGACCTGAACCTCTCGGGGGAC CGTCAGTCTCTCTTCCCGCCAAACCCA AGGACACCTCATGATCTCCGGACCCCTG AGGTCAATGGTGGTGGTGGAGCTGAGC GTACGTGGAGCGGCTGGAGGTGCATAATG CCAAGACAAAGCCGGCGAGAGCAGTA CAACGACGTAACCTGTGTGTCAGCGTCC TCACGCTCTGCACAGAGACTGGCTGAAT GGCAAGGAGTACAAGTGCAAGGTCTCCAA CAAGCCCTCCAGCCCCCTATCGAGAAA CCATCTCAAAGCCAAAGGGGAGCCCCGA GAACCAATGGTGTGTGACCTGCCCTCCCTC CGGAGGAGATGACCAAGAACACAGGTCA GCTGTGGTGCATGTGCAAGGGCTTCTAT CCACGCAATCGCGGTGGAGTGGGAGA GCAATGGGAGCGGAGAACAACTACAA GACCACGCTCCCGTGTGGACTCCGAGC GCTCTCTTCTCTGTACAGCAAGCTCACCG TGGACAAGAGCCGCTGGCAGCAGGGGAA CGTCTTCTCATGCTCCGTGATGATGAGGC TCTGCAACAACCTACACGACAGAGGCC TCTCCTGTCTCCGGGTAAAGCGGCGAGC GAGACTCCCGGAGCCTCAGAGTCCGCCAC ACCCGAAAAAGTGGTGGCGGA	71	GAGGTTCAAGTGGTG GAGTCTGGCGGTGGC CTGGTGACAGCAGGG GGCTCACTCCGTTGT CCTGTGAGCTTCTGG CTTCAACATTTCTTCT CTTCTATCACTGGGT GGCTCAGGCCCGGG TAAGGGCTGGAATG GGTGGCATCTATTCT TCTCTATGGCTATA CTTATTATGCCATAG CGTCAAGGGCGTTTC ACTATAAGCGGAC ACATCAAAACACAG CCTACTCAAAATGAA CAGCTTAAGAGCTGA GGACACTGCCGTCTAT TATTGTCTCGGGTG GTTCTGGTGTCTTCA TTACGGTCTGTTTAC TACTCTGGTGGGCTT TGGACTACTGGGGTC AAGGAACCTGGTCA CCGTCTCTCTCG	72	GATATCCAGATG ACCCAGTCCCCG AGCTCCTGTCC GCCTCTGTGGC GATAGGTCAACC ATCACCTGCCGT GCCAGTCAGTCC GTAGCCTGGTAT CAACAGAAACCA GGAAAAAGCTCCG AAGCTTCTGATT TACTCGCATCC AGCCTCTACTCT GGAGTCCCTTCT CGCTTCTCTGT AGCCGTTCCGGG ACGGAATTCAC CTGACCATCAGC AGTCTGCAGCCG GAAGACTTCGCA ACTTATTACTGTC AGCAAGCTTCTT ACGCTCCGATCA CGTTCGAGCAGG GTACCAAGGTGG AGATCAA

Table 1B

D	N- fusion VH (VH1)	SEQ ID NO:	SEQ ID NO:	VH-VL linker	N- fusion VL (VL2)	SEQ ID NO:	SEQ ID NO:	Fc fusion	C- fusion VH (VH3)	SEQ ID NO:	SEQ ID NO:	VH-VL linker	C- fusion VL (VL4)	SEQ ID NO:	SEQ ID NO:
5019- hole- 42459- 2460	5019	45	GAGGTTTCAGCTGG TGGAGTCTGGCGG TGGCTCTGGTGCA CCAGGGGGCTAC TCCGTTTGTCTGT GCAGCTCTGGCT TCACATCGGTTCT TCTTCTATCACTG GGTGGCTGACGCC CCGGTAAGGGCC TGGAAATGGGTTG ATCTATTATCTG CTTTTGCTCTACT TCTTATGCGGATA GGCTCAAGGGCCG TTGACATATAAGG TTCACATCTCAA AAACACAGCCTAC CTACAATGAACA GCTTAAGAGCTGA GGCACTGGCGCT TATTATTGTCTG CTACATTTCGGT TCCGTTTGTCTTTG GACTACTGGGGTC AAGGAACCCCTGGT CACGCTCTCTCG	46	GGTGGA GGTGGC AGT	5019	47	GATATCCAGATG ACCCAGTCCCCA GTCTGCTTCGCG TCTGTGGCGA TAGGGTACCAATC ACCTGCCGTGCCA GTGAGTCTGTAGC TCTTCTATCACTG GGTGGCTGACGCC CCGGTAAGGGCC TGGAAATGGGTTG ATCTATTATCTG CTTTTGCTCTACT TCTTATGCGGATA GGCTCAAGGGCCG TTGACATATAAGG TTCACATCTCAA AAACACAGCCTAC CTACAATGAACA GCTTAAGAGCTGA GGCACTGGCGCT TATTATTGTCTG CTACATTTCGGT TCCGTTTGTCTTTG GACTACTGGGGTC AAGGAACCCCTGGT CACGCTCTCTCG	46	GGTGGA GGTGGC AGT	5019	73	GAGGTTTCAGCTGGT GAGTCTGGCGTGG CTGGTGACCCAGG GGCTACTCCGTTTG CCGTGACGCTTCGG CTTCAACCTCTCTATT ATTATATGCATGGGT GGCTGAGGCCCGG TAAGGGCTGGAATG GGTTCATCTATTAT TCTTCTATGGCTATA CTTATATGGCGATG CTCTAAGGGCGTTTC ACTATAAGCGACAG ACATCCAAAACACAG CCCTACTCAAAATGAA CAGCTTAAGAGCTGA GGACACTGCCGTCTAT TATTGTCTCGCTGGT CTCATGTTTCTGGTCA TTACTCTGTATGGAC TACTGGGGTCAAGGA ACCTGGTACCCTGCT CCTCG	74	GATATCCAGATG ACCCAGTCCCCG AGCTCCCTCTGC GGCTCTGTGGCG GATAGGGTCACC ATCACCTGCCGT GCCAGTCACTC GTGTCCAGCGCT GTAGCCTGGTAT CAACAGAAACA GGAAAAGCTCCG AAGCTCTCTGATT TACTCGCATCC AGSCCTCTACT GGAGTCCCTCT CGCTCTCTGGT AGCCGTTCCGGG AGGGAITTCAC CTGACCATCAGC AGTCTGGAGCG GAAGACTTCGA ACCTATTACTCTC AGCAATCTCTT ATTCTGTATCA CGTTCGACAGG GTACCAAGGTGG AGATCAA

TABLE 2

Antibody No.	Alternate name	FZD recognized	Co-Receptor recognized
2746	F ⁶	FZD6	
2747	F ^{6.1}	FZD6	
2864	F ^{P1}	FZD1, FZD2, FZD4, FZD5, FZD7, FZD8	
2870	F ^{P2}	FZD1, FZD2, FZD4, FZD5, FZD7, FZD8	
2876	F ^{2.1}	FZD2	
2886	F ^{2/7}	FZD2, FZD7(?)	
2890	F ²	FZD2	
2928	F ⁵	FZD5	
2939	F ^{P3}	FZD1, FZD2, FZD4, FZD5, FZD7, FZD8	
2969	F ^{9/10}	FZD9, FZD10	
2974	F ^{9/10.1}	FZD9, FZD10	
5019	F ^P	FZD1, FZD2, FZD4, FZD5, FZD7, FZD8	
5027	F ⁴	FZD4	
5038	F ^{4.1}	FZD4	
5044	F ^{4.4}	FZD4	
5048	F ^{4.7}	FZD4	
5056	F ^{P4}	FZD1, FZD2, FZD4, FZD6, FZD7, FZD8	
5062	F ^{4.2}	FZD4	
5063	F ^{4.5}	FZD4	
5067	F ^{P4}	FZD1, FZD2, FZD4, FZD5, FZD7, FZD8	
5075	F ^{P5}	FZD1, FZD2, FZD4, FZD5, FZD7, FZD8	
5076	F ^{P7}	FZD1, FZD2, FZD4, FZD5, FZD7, FZD8	
5080	F ^{4.3}	FZD4	
5081	F ^{4.6}	FZD4	
12735	F ⁷	FZD1, FZD2, FZD4, FZD5, FZD7, FZD8	
2459	L5 ¹		LRP5-W1
2460	L5 ³		LRP5-W3
2539	L6 ³		LRP6-W3
2540			LRP6-W3
2542	L6 ¹		LRP6-W1

TABLE 3

Nomenclature	Description
5019-2539 Bi-IgG	A bispecific immunoglobulin with a first binding domain for FZD derived from antibody 5019 and a second binding domain for the Wnt3a binding site on LRP6, derived from antibody 2539, wherein both binding domains are on the same side of the Fc domain
5019-2542 Bi-IgG	A bispecific immunoglobulin with a first binding domain for FZD derived from antibody 5019 and a second binding domain for the Wnt1 binding site on LRP6, derived from antibody 2542, wherein both binding domains are on the same side of the Fc domain
5019-2539-K/H (FZD/LRP6-W3)	A bispecific diabody comprising an Fc domain in a knob in hole configuration and one binding domain comprising a binding site for FZD derived from antibody 5019 and a binding site for the Wnt3a binding site on LRP6 that is derived from antibody 2539
5019-2542-K/H (FZD/LRP6-W1)	A bispecific diabody comprising an Fc domain in a knob in hole configuration and one binding domain having a binding site for FZD derived from antibody 5019 and a binding site for the Wnt1 binding site on LRP6 that is derived from antibody 2542
5019-Fc-2539	A tetravalent binding molecule comprising an Fc domain and a binding domain for FZD and a binding domain for LRP6, wherein the binding domain for FZD is in a diabody configuration that is bivalent, monospecific and derived from antibody 5019, and the binding domain for LRP6 is in a diabody configuration that is bivalent and monospecific for binding the Wnt 3a binding site and derived from antibody 2539
5019-Fc-2542	A tetravalent binding molecule comprising an Fc domain and a binding domain for FZD and a binding domain for LRP6, wherein the binding domain for FZD is in a diabody configuration that is bivalent, monospecific and derived from antibody 5019, and the binding domain for LRP6 is in a diabody configuration that is bivalent and monospecific for binding the Wnt 1 binding site and derived from antibody 2542
5019Ag 5019-K/H- 2539-2542	A tetravalent binding molecule comprising an Fc domain and a binding domain for FZD and a binding domain for LRP6, wherein the FC domain is in a knob in hole configuration, the binding domain for FZD is in a diabody configuration that is bivalent, monospecific and derived from antibody 5019, and the binding domain for LRP6 is in a diabody configuration that is bivalent and bispecific binding the Wnt1 and Wnt 3a binding sites on LRP6 and is derived from antibodies 2542 and 2539
5019-K/H- 2539-MBP	A tetravalent binding molecule comprising an Fc domain and a binding domain for FZD and a binding domain comprising a binding site for LRP6 and a Maltose Binding Protein (MBP), wherein the FC domain is in a knob in hole configuration, the binding domain for FZD is in a diabody configuration that is bivalent, monospecific and

	derived from antibody 5019, and the binding domain having a binding site for LRP6 is in a diabody configuration having a binding site for LRP6 derived from antibody 2539 and a binding site for the Maltose Binding Protein.
5038Ag 5038-K/H- 2539-2542	A tetravalent binding molecule comprising an Fc domain and a binding domain for a FZD and a binding domain for LRP6, wherein the FC domain is in a knob in hole configuration, the binding domain for the FZD is in a diabody configuration that is bivalent, monospecific, and derived from antibody 5039, and the binding domain for LRP6 is in a diabody configuration that is bivalent and bispecific binding the Wnt1 and Wnt 3a binding sites on LRP6 and is derived from antibodies 2542 and 2539
5044Ag 5044-K/H- 2539-2542	A tetravalent binding molecule comprising an Fc domain and a binding domain for a FZD and a binding domain for LRP6, wherein the FC domain is in a knob in hole configuration, the binding domain for the FZD is in a diabody configuration that is bivalent, monospecific and derived from antibody 5044, and the binding domain for LRP6 is in a diabody configuration that is bivalent and bispecific binding the Wnt1 and Wnt 3a binding sites on LRP6 and is derived from antibodies 2542 and 2539
5048Ag 5048-K/H- 2539-2542	A tetravalent binding molecule comprising an Fc domain and a binding domain for a FZD and a binding domain for LRP6, wherein the FC domain is in a knob in hole configuration, the binding domain for the FZD is in a diabody configuration that is bivalent, monospecific and derived from antibody 50448, and the binding domain for LRP6 is in a diabody configuration that is bivalent and bispecific binding the Wnt1 and Wnt 3a binding sites on LRP6 and is derived from antibodies 2542 and 2539
5063Ag 5063-K/H- 2539-2542	A tetravalent binding molecule comprising an Fc domain and a binding domain for a FZD and a binding domain for LRP6, wherein the FC domain is in a knob in hole configuration, the binding domain for the FZD is in a diabody configuration that is bivalent, monospecific and derived from antibody 5063, and the binding domain for LRP6 is in a diabody configuration that is bivalent and bispecific binding the Wnt1 and Wnt 3a binding sites on LRP6 and is derived from antibodies 2542 and 2539
5080Ag 5080-K/H- 2539-2542	A tetravalent binding molecule comprising an Fc domain and a binding domain for a FZD and a binding domain for LRP6, wherein the FC domain is in a knob in hole configuration, the binding domain for the FZD is in a diabody configuration that is bivalent, monospecific and derived from antibody 5080, and the binding domain for LRP6 is in a diabody configuration that is bivalent and bispecific binding the Wnt1 and Wnt 3a binding sites on LRP6 and is derived from antibodies 2542 and 2539
5081Ag 5081-K/H- 2539-2542	A tetravalent binding molecule comprising an Fc domain and a binding domain for FZD and a binding domain for LRP6, wherein the FC domain is in a knob in hole configuration, the binding domain for FZD is in a diabody configuration that is

	bivalent, monospecific and derived from antibody 5081, and the binding domain for LRP6 is in a diabody configuration that is bivalent and bispecific binding the Wnt1 and Wnt 3a binding sites on LRP6 and is derived from antibodies 2542 and 2539
2876Ag	A tetravalent binding molecule comprising an Fc domain and a binding domain for a FZD and a binding domain for LRP6, wherein the Fc domain is in a knob in hole configuration, the binding domain for the FZD is in a diabody configuration that is bivalent, monospecific and derived from antibody 2876, and the binding domain for LRP6 is in a diabody configuration that is bivalent and bispecific binding the Wnt1 and Wnt 3a binding sites on LRP6 and is derived from antibodies 2542 and 2539
2890Ag	A tetravalent binding molecule comprising an Fc domain and a binding domain for a FZD and a binding domain for LRP6, wherein the FC domain is in a knob in hole configuration, the binding domain for the FZD is in a diabody configuration that is bivalent, monospecific, and derived from antibody 2890, and the binding domain for LRP6 is in a diabody configuration that is bivalent and bispecific binding the Wnt1 and Wnt 3a binding sites on LRP6 and is derived from antibodies 2542 and 2539
2886Ag	A tetravalent binding molecule comprising an Fc domain and a binding domain for a FZD and a binding domain for LRP6, wherein the FC domain is in a knob in hole configuration, the binding domain for the FZD is in a diabody configuration that is bivalent and derived from antibody 2886, and the binding domain for LRP6 is in a diabody configuration that is bivalent and bispecific binding the Wnt1 and Wnt 3a binding sites on LRP6 and is derived from antibodies 2542 and 2539
2747Ag	A tetravalent binding molecule comprising an Fc domain and a binding domain for a FZD and a binding domain for LRP6, wherein the FC domain is in a knob in hole configuration, the binding domain for the FZD is in a diabody configuration that is bivalent, derived from antibody 2747, and the binding domain for LRP6 is in a diabody configuration that is bivalent and bispecific binding the Wnt1 and Wnt 3a binding sites on LRP6 and is derived from antibodies 2542 and 2539
2969Ag	A tetravalent binding molecule comprising an Fc domain and a binding domain for a FZD and a binding domain for LRP6, wherein the FC domain is in a knob in hole configuration, the binding domain for the FZD is in a diabody configuration that is bivalent, derived from antibody 2969, and the binding domain for LRP6 is in a diabody configuration that is bivalent and bispecific binding the Wnt1 and Wnt 3a binding sites on LRP6 and is derived from antibodies 2542 and 2539
2974Ag	A tetravalent binding molecule comprising an Fc domain and a binding domain for a FZD and a binding domain for LRP6, wherein the FC domain is in a knob in hole configuration, the binding domain for the FZD is in a diabody configuration that is

	bivalent, derived from antibody 2074, and the binding domain for LRP6 is in a diabody configuration that is bivalent and bispecific binding the Wnt1 and Wnt 3a binding sites on LRP6 and is derived from antibodies 2542 and 2539
Homodiabody 2539-Fc	A diabody comprising an Fc domain and two binding sites for the Wnt3a binding site on LRP6 that is derived from antibody 2539
Homodiabody 2542-Fc	A diabody comprising an Fc domain and two binding sites for the Wnt1 binding site on LRP6 that is derived from antibody 2542
FP+P-L61+3	A tetravalent binding molecule comprising an Fc domain and a binding domain for FZD and a binding domain for LRP6, wherein the FC domain is in a knob in hole configuration, wherein the binding domain for FZD is in a diabody configuration that is bivalent, monospecific and derived from antibody 5019, and the binding domain for LRP6 is in a diabody configuration that is bivalent and bispecific binding the Wnt1 and Wnt 3a binding sites on LRP6 and is derived from antibodies 2542 and 2539
FP*+P*-L61+3	A tetravalent binding molecule comprising an Fc domain and a binding domain for FZD and a binding domain for LRP6, wherein the FC domain is in a knob in hole configuration, wherein the binding domain for FZD is in a scFv configuration such that the binding domain is bivalent, monospecific, and the scFv is derived from antibody 5019, and the binding domain for LRP6 is in a diabody configuration bivalent and bispecific binding the Wnt1 and Wnt 3a binding sites on LRP6 and is derived from antibodies 2542 and 2539
FP+P-L61*+3*	A tetravalent binding molecule comprising an Fc domain and a binding domain for FZD and a binding domain for LRP6, wherein the FC domain is in a knob in hole configuration, wherein the binding domain for FZD is a diabody configuration that is bivalent and monospecific and derived from antibody 5019, and the binding domain for LRP6 is in a scFv configuration that is bispecific for binding the Wnt1 and Wnt 3a binding sites on LRP6 and the scFv is derived from antibodies 2542 and 2539
FP*+P*-L61*+3*	A tetravalent binding molecule comprising an Fc domain and a binding domain for FZD and a binding domain for LRP6, wherein the FC domain is in a knob in hole configuration, wherein the binding domain for FZD is in a scFv configuration that is bivalent, monospecific, and derived from antibody 5019, and the binding domain for LRP6 is in a scFv configuration that is bivalent and bispecific for binding to the Wnt1 and Wnt 3a binding sites on LRP6 and is derived from antibodies 2542 and 2539