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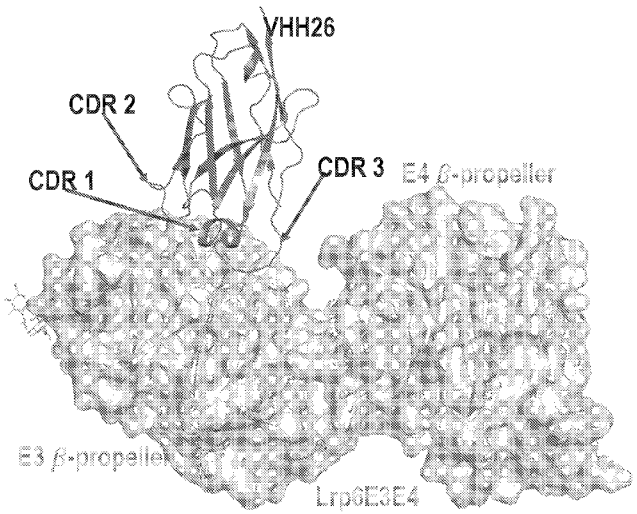
(54) Title: ANTI-LRP5/6 ANTIBODIES AND METHODS OF USE

Figure 1



(57) Abstract: The present invention provides anti-LRP5/6 monoclonal antibodies and related compositions, which may be used in any of a variety of therapeutic methods for treating diseases and disorders associated with Wnt pathway signaling.

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ANTI-LRP5/6 ANTIBODIES AND METHODS OF USE

Cross Reference to Related Applications

This application claims priority to U.S. Provisional Application No. 62/607,879, filed December 19, 2017, and US Provisional Application No. 62/680,515, filed June 4, 2018, both of which are incorporated by reference herein in their entireties.

Statement Regarding Sequence Listing

The Sequence Listing associated with this application is provided in text format in lieu of a paper copy, and is hereby incorporated by reference into the specification. The name of the text file containing the Sequence Listing is SRZN_005_02WO_ST25.txt. The text file is 181 KB, was created on December 19, 2018, and is being submitted electronically via EFS-Web.

BACKGROUND

Technical Field

The present invention relates generally to anti-LRP5/6 antibodies, compositions and methods of using same. Such antibodies are useful, for example, in methods of modulating Wnt signaling pathways.

Description of the Related Art

Wnt ("Wingless-related integration site" or "Wingless and Int-1" or "Wingless-Int") ligands and their signals play key roles in the control of development, homeostasis and regeneration of many essential organs and tissues, including bone, liver, skin, stomach, intestine, kidney, central nervous system, mammary gland, taste bud, ovary, cochlea and many other tissues (reviewed, e.g., by Clevers, Loh, and Nusse, 2014; 346:1table 1B8012). Modulation of Wnt signaling pathways has potential for treatment of degenerative diseases and tissue injuries.

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

BRIEF SUMMARY

An isolated antibody, or an antigen-binding fragment thereof, that binds to one or more LRP5 or LRP6 receptor, comprising a sequence comprising: CDRH1, CDRH2 and CDRH3 sequences set forth for any of the antibodies of Table 1A; and/or CDRL1, CDRL2 and CDRL3 sequences set forth for any of the antibodies of Table 1A, or a variant of said antibody, or antigen-binding fragment thereof, comprising one or more amino acid modifications, wherein said variant comprises less than 8 amino acid substitutions in said CDR sequences. In particular embodiments, the isolated antibody, or antigen-binding fragment thereof comprises a heavy chain variable region comprising an amino acid sequence having at least 90% identity to the amino acid sequence set forth in any of SEQ ID NOs:1-24 or a heavy chain variable region comprising the amino acid sequence set forth in any of SEQ ID NOs:1-24.

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

In certain embodiments, any of the antibodies, or antigen-binding fragments thereof, are a fusion protein. In certain embodiments, the antibody, or antigen-binding fragment thereof, is fused to a polypeptide sequence that binds one or more Fzd receptors. In certain embodiments, the polypeptide sequence that binds one or more Fzd receptors is an antibody, or an antigen-binding fragment thereof, that binds to one or more Fzd receptors.

In certain embodiments, any of the isolated antibodies, or antigen-binding fragments thereof, disclosed herein binds to LRP5, LRP6, or both LRP5 and LRP6.

In a related embodiment, the disclosure provides an isolated antibody, or an antigen-binding fragment thereof, that competes with any of the antibodies disclosed herein for binding to LRP5 or LRP6.

In particular embodiments, any of the antibodies, or antigen-binding fragments thereof, bind to LRP5 or LRP6 with a KD of 50 μM or lower.

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

In a further related embodiment, the present disclosure provides an isolated polynucleotide encoding an antibody, or antigen-binding fragment thereof, disclosed herein. In certain embodiments, the present disclosure provides an expression vector comprising the isolated polynucleotide and an isolated host cell comprising the expression vector.

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

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

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

In another embodiment, the present disclosure includes a method for treating a subject having a disease or disorder associated with reduced Wnt signaling, comprising administering to the subject an effective amount of a pharmaceutical composition comprising an isolated antibody, or antigen-binding fragment thereof, disclosed herein that is an agonist of a Wnt signaling pathway. In particular embodiments, the disease or disorder is selected from the group consisting of: bone fractures, stress fractures, vertebral compression fractures, osteoarthritis, osteoporosis, osteoporotic fractures, non-union fractures, delayed union fractures, spinal fusion, pre-operative optimization for spine surgeries, osteonecrosis, osseointegration of implants or orthopedic devices, osteogenesis imperfecta, bone grafts, tendon repair, tendon-bone integration, tooth growth and regeneration, maxillofacial surgery, dental implantation, periodontal diseases, maxillofacial reconstruction, osteonecrosis of the jaw, hip or femoral head, avascular necrosis, alopecia, hearing loss, vestibular hypofunction, macular degeneration, age-related macular degeneration (AMD), vitreoretinopathy, retinopathy, diabetic retinopathy, diseases of retinal degeneration, Fuchs' dystrophy, cornea diseases, stroke, traumatic brain injury, Alzheimer's disease, multiple sclerosis, muscular dystrophy, muscle atrophy in sarcopenia and cachexia, diseases affecting blood brain barrier (BBB), spinal cord injuries, spinal cord diseases, oral mucositis, short bowel syndrome, inflammatory bowel diseases (IBD),

metabolic syndrome, diabetes, dyslipidemia, pancreatitis, exocrine pancreatic insufficiency, wound healing, diabetic foot ulcers, pressure sores, venous leg ulcers, epidermolysis bullosa, dermal hypoplasia, myocardial infarction, coronary artery disease, heart failure, hematopoietic cell disorders, immunodeficiencies, graft versus host diseases, acute kidney injuries, chronic kidney diseases, chronic obstructive pulmonary diseases (COPD), idiopathic pulmonary fibrosis, acute liver failure of all causes, acute liver failure drug-induced, alcoholic liver diseases, chronic liver failure of all causes, cirrhosis, liver fibrosis of all causes, portal hypertension, chronic liver insufficiency of all causes, nonalcoholic steatohepatitis (NASH), nonalcoholic fatty liver disease (NAFLD) (fatty liver), alcoholic hepatitis, hepatitis C virus-induced liver diseases (HCV), hepatitis B virus-induced liver diseases (HBV), other viral hepatitis (e.g., hepatitis A virus-induced liver diseases (HAV) and hepatitis D virus-induced liver diseases (HDV)), primary biliary cirrhosis, autoimmune hepatitis, livery surgery, liver injury, liver transplantation, “small for size” syndrome in liver surgery and transplantation, congenital liver disease and disorders, any other liver disorder or detect resulting from genetic diseases, degeneration, aging, drugs, or injuries.

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

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows a graphic representation of the crystal structure of LRP6E3E4:VHH26 binding complex. LRP6E3E4 is represented as transparent grey and VHH26 is darker grey. Positions of the CDR loops of VHH26 are marked. Glycans on the surface of LRP6E3E4 are shown as sticks representations.

Figure 2 shows a graphic representation of the crystal structure of LRP6E3E4:VHH36 binding complex. LRP6E3E4 is shown as transparent grey and VHH36 is darker grey. Positions of the CDR loops of VHH36 are marked. Glycans on the surface of LRP6E3E4 are shown as sticks representations.

Figures 3A shows Wnt activation using the A375 Wnt reporter assay. Figure 3B shows Wnt report activation in HEK293 cells with added Rspo. Clones tested in both assays are Wnt surrogate molecule comprising the Fzd binder 18R5 scFv combined with LRP VHH or sdAb binders. FLAG and His tags were attached to the 18R5:LRP surrogate molecule.

DETAILED DESCRIPTION

The present disclosure relates to antibodies and antigen-binding fragments thereof the specifically bind to LRP5 and/or LRP6, in particular antibodies having specific LRP receptor specificity and functional properties. One embodiment of the invention encompasses specific humanized antibodies and fragments thereof capable of binding to LRP5 and/or LRP6 and modulating downstream Wnt pathway signaling and biological effects. For convenience, the term “anti-LRP5/6” is used to refer collectively to antibodies and antigen-binding fragments thereof that bind to either or both of LRP5 and/or LRP6.

Embodiments of the invention pertain to the use of anti-LRP5/6 antibodies or antigen-binding fragments thereof for the diagnosis, assessment and treatment of diseases and disorders associated with Wnt signaling pathways. In certain embodiments, the subject antibodies are used in the treatment or prevention of diseases and disorders associated with aberrant

(e.g., increased or decreased) Wnt signaling, or for which either decreased or increased Wnt signaling would provide a therapeutic benefit.

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

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

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

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

Standard techniques may be used for recombinant DNA, oligonucleotide synthesis, and tissue culture and transformation (*e.g.*, electroporation, lipofection). Enzymatic reactions and purification techniques may be performed according to manufacturer's specifications or as commonly

accomplished in the art or as described herein. These and related techniques and procedures may be generally performed according to conventional methods well known in the art and as described in various general and more specific references that are cited and discussed throughout the present specification. Unless specific definitions are provided, the nomenclature utilized in connection with, and the laboratory procedures and techniques of, molecular biology, analytical chemistry, synthetic organic chemistry, and medicinal and pharmaceutical chemistry described herein are those well-known and commonly used in the art. Standard techniques may be used for recombinant technology, molecular biological, microbiological, chemical syntheses, chemical analyses, pharmaceutical preparation, formulation, and delivery, and treatment of patients.

Embodiments of the present invention relate to antibodies that bind to LRP5 and/or LRP6. Sequences of illustrative antibodies, or antigen-binding fragments, or complementarity determining regions (CDRs) thereof, are set forth in SEQ ID NOs:1-24.

As is well known in the art, an antibody is an immunoglobulin molecule capable of specific binding to a target, such as a carbohydrate, polynucleotide, lipid, polypeptide, etc., through at least one epitope recognition site, located in the variable region of the immunoglobulin molecule. As used herein, the term encompasses not only intact polyclonal or monoclonal antibodies, but also fragments thereof (such as dAb, Fab, Fab', $F(ab')_2$, Fv), single chain (scFv), VHH or sdAbs (also known as nanobodies), synthetic variants thereof, naturally occurring variants, fusion proteins comprising an antibody portion with an antigen-binding fragment of the required specificity, humanized antibodies, chimeric antibodies, and any other modified configuration of the immunoglobulin molecule that comprises an antigen-binding site or fragment (epitope recognition site) of the required specificity. "Diabodies", multivalent or multispecific fragments constructed by gene fusion (WO94/13804; P. Holliger et al., Proc. Natl. Acad. Sci. USA 90 6444-6448, 1993) are also a particular form of antibody contemplated herein. Minibodies

comprising a scFv joined to a CH3 domain are also included herein (S. Hu et al., Cancer Res., 56, 3055-3061, 1996). See *e.g.*, Ward, E. S. *et al.*, Nature 341, 544-546 (1989); Bird et al., Science, 242, 423-426, 1988; Huston et al., PNAS USA, 85, 5879-5883, 1988); PCT/US92/09965; WO94/13804; P. Holliger et al., Proc. Natl. Acad. Sci. USA 90 6444-6448, 1993; Y. Reiter et al., Nature Biotech, 14, 1239-1245, 1996; S. Hu et al., Cancer Res., 56, 3055-3061, 1996.

The term "antigen-binding fragment" as used herein refers to a polypeptide fragment that contains at least one CDR of an immunoglobulin heavy and/or light chains that binds to the antigen of interest, in particular to LRP5 and/or LRP6. In this regard, an antigen-binding fragment of the herein described antibodies may comprise 1, 2, 3, 4, 5, or all 6 CDRs of a VH and VL sequence set forth herein from antibodies that bind LRP5 and/or LRP6. An antigen-binding fragment of the LRP5/6-specific antibodies described herein is capable of binding to LRP5 and/or LRP6. In certain embodiments, an antigen-binding fragment or an antibody comprising an antigen-binding fragment, increases Wnt signaling events. In certain embodiments, the antibody or antigen-binding fragment binds specifically to and/or modulates the biological activity of the human Wnt signaling pathway. In certain embodiments, the antibody or antigen-binding fragment thereof increases or decreases Wnt signaling.

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

The term "epitope" includes any determinant, preferably a polypeptide determinant, capable of specific binding to an immunoglobulin or T-cell receptor. An epitope is a region of an antigen that is bound by an antibody. In certain embodiments, epitope determinants include chemically active surface groupings of molecules such as amino acids, sugar side chains, phosphoryl or sulfonyl, and may in certain embodiments have specific three-dimensional structural characteristics, and/or specific charge characteristics. In certain embodiments, an antibody is said to specifically bind an antigen when it preferentially recognizes its target antigen in a complex mixture of proteins and/or macromolecules. An antibody is said to specifically bind an antigen when the equilibrium dissociation constant is $\leq 10^{-7}$ or 10^{-8} M. In some embodiments, the equilibrium dissociation constant may be $\leq 10^{-9}$ M or $\leq 10^{-10}$ M.

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

As used herein, the term "FR set" refers to the four flanking amino acid sequences which frame the CDRs of a CDR set of a heavy or light chain V region. Some FR residues may contact bound antigen; however, FRs are

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

The structures and locations of immunoglobulin variable domains may be determined by reference to Kabat, E. A. et al., Sequences of Proteins of Immunological Interest. 4th Edition. US Department of Health and Human Services. 1987, and updates thereof, now available on the Internet (immuno.bme.nwu.edu). Alternatively, CDRs may be determined by using "IMGT®, the international ImMunoGeneTics information system® available at http://www.imgt.org (see, e.g., Lefranc, M.-P. et al. (1999) *Nucleic Acids Res.*, 27:209-212; Ruiz, M. et al. (2000) *Nucleic Acids Res.*, 28:219-221; Lefranc, M.-P. (2001) *Nucleic Acids Res.*, 29:207-209; Lefranc, M.-P. (2003) *Nucleic Acids Res.*, 31:307-310; Lefranc, M.-P. et al. (2004) *In Silico* Biol., 5, 0006 [Epub], 5:45-60 (2005)]; Lefranc, M.-P. et al. (2005) *Nucleic Acids Res.*, 33:D593-597; Lefranc, M.-P. et al. (2009) *Nucleic Acids Res.*, 37:D1006-1012; Lefranc, M.-P. et al. (2015) *Nucleic Acids Res.*, 43:D413-422).

A “monoclonal antibody" refers to a homogeneous antibody population wherein the monoclonal antibody is comprised of amino acids (naturally occurring and non-naturally occurring) that are involved in the selective binding of an epitope. Monoclonal antibodies are highly specific, being directed against a single epitope. The term "monoclonal antibody" encompasses not only intact monoclonal antibodies and full-length monoclonal

antibodies, but also fragments thereof (such as Fab, Fab', $F(ab')_2$, Fv), single chain (scFv), variants thereof, fusion proteins comprising an antigen-binding portion, humanized monoclonal antibodies, chimeric monoclonal antibodies, and any other modified configuration of the immunoglobulin molecule that comprises an antigen-binding fragment (epitope recognition site) of the required specificity and the ability to bind to an epitope. It is not intended to be limited as regards the source of the antibody or the manner in which it is made (*e.g.*, by hybridoma, phage selection, recombinant expression, transgenic animals, etc.). The term includes whole immunoglobulins as well as the fragments etc. described above under the definition of "antibody".

The proteolytic enzyme papain preferentially cleaves IgG molecules to yield several fragments, two of which (the F(ab) fragments) each comprise a covalent heterodimer that includes an intact antigen-binding site. The enzyme pepsin is able to cleave IgG molecules to provide several fragments, including the $F(ab')_2$ fragment which comprises both antigen-binding sites. An Fv fragment for use according to certain embodiments of the present invention can be produced by preferential proteolytic cleavage of an IgM, and on rare occasions of an IgG or IgA immunoglobulin molecule. Fv fragments are, however, more commonly derived using recombinant techniques known in the art. The Fv fragment includes a non-covalent $V_H::V_L$ heterodimer including an antigen-binding site which retains much of the antigen recognition and binding capabilities of the native antibody molecule. Inbar *et al.* (1972) *Proc. Nat. Acad. Sci. USA 69*:2659-2662; Hochman *et al.* (1976) *Biochem 15*:2706-2710; and Ehrlich *et al.* (1980) *Biochem 19*:4091-4096.

In certain embodiments, single chain Fv or scFV antibodies are contemplated. For example, Kappa bodies (Ill *et al.*, *Prot. Eng*. 10: 949-57 (1997); minibodies (Martin *et al.*, *EMBO J* 13: 5305-9 (1994); diabodies (Holliger *et al.*, *PNAS* 90: 6444-8 (1993); or Janusins (Traunecker *et al.*, *EMBO J* 10: 3655-59 (1991) and Traunecker *et al., Int. J. Cancer Suppl*. 7: 51-52 (1992), may be prepared using standard molecular biology techniques following the teachings of the present application with regard to selecting antibodies

having the desired specificity. In still other embodiments, bispecific or chimeric antibodies may be made that encompass the ligands of the present disclosure. For example, a chimeric antibody may comprise CDRs and framework regions from different antibodies, while bispecific antibodies may be generated that bind specifically to LRP5 and/or LRP6 through one binding domain and to a second molecule through a second binding domain. These antibodies may be produced through recombinant molecular biological techniques or may be physically conjugated together.

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

In certain embodiments, an LRP5/6 binding antibody as described herein is in the form of a diabody. Diabodies are multimers of polypeptides, each polypeptide comprising a first domain comprising a binding region of an immunoglobulin light chain and a second domain comprising a binding region of an immunoglobulin heavy chain, the two domains being linked (*e.g.* by a peptide linker) but unable to associate with each other to form an antigen binding site: antigen binding sites are formed by the association of the first domain of one polypeptide within the multimer with the second domain of another polypeptide within the multimer (WO94/13804).

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

Where bispecific antibodies are to be used, these may be conventional bispecific antibodies, which can be manufactured in a variety of

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

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

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

conventional antibodies. The small size of the UniBody® can be a great benefit when treating some forms of cancer, allowing for better distribution of the molecule over larger solid tumors and potentially increasing efficacy.

In certain embodiments, the antibodies of the present disclosure may take the form of a VHH or sdAb. VHH or sdAb are encoded by single genes and are efficiently produced in almost all prokaryotic and eukaryotic hosts *e.g. E. coli* (see *e.g.* U.S. Pat. No. 6,765,087), molds (for example *Aspergillus* or *Trichoderma*) and yeast (for example *Saccharomyces, Kluyvermyces, Hansenula* or *Pichia* (see *e.g.* U.S. Pat. No. 6,838,254). The production process is scalable and multi-kilogram quantities of VHH or sdAb have been produced. VHH or sdAbs may be formulated as a ready-to-use solution having a long shelf life. The Nanoclone® method (see, e.g., WO 06/079372) is a proprietary method for generating VHH or sdAbs against a desired target, based on automated high-throughput selection of B-cells.

In certain embodiments, the anti-LRP5/6 antibodies or antigen-binding fragments thereof as disclosed herein are humanized. This refers to a chimeric molecule, generally prepared using recombinant techniques, having an antigen-binding site derived from an immunoglobulin from a non-human species and the remaining immunoglobulin structure of the molecule based upon the structure and/or sequence of a human immunoglobulin. The antigen-binding site may comprise either complete variable domains fused onto constant domains or only the CDRs grafted onto appropriate framework regions in the variable domains. Epitope binding sites may be wild type or modified by one or more amino acid substitutions. This eliminates the constant region as an immunogen in human individuals, but the possibility of an immune response to the foreign variable region remains (LoBuglio, A. F. *et al.*, (1989) *Proc Natl Acad Sci USA* 86:4220-4224; Queen *et al.*, *PNAS* (1988) 86:10029-10033; Riechmann *et al.*, *Nature* (1988) 332:323-327). Illustrative methods for humanization of the anti-LRP5/6 antibodies disclosed herein include the methods described in U.S. patent no. 7,462,697.

Another approach focuses not only on providing human-derived constant regions, but modifying the variable regions as well so as to reshape them as closely as possible to human form. It is known that the variable regions of both heavy and light chains contain three complementarity-determining regions (CDRs) which vary in response to the epitopes in question and determine binding capability, flanked by four framework regions (FRs) which are relatively conserved in a given species and which putatively provide a scaffolding for the CDRs. When nonhuman antibodies are prepared with respect to a particular epitope, the variable regions can be "reshaped" or "humanized" by grafting CDRs derived from nonhuman antibody on the FRs present in the human antibody to be modified. Application of this approach to various antibodies has been reported by Sato, K., *et al.*, (1993) *Cancer Res* 53:851-856. Riechmann, L., *et al.*, (1988) *Nature* 332:323-327; Verhoeyen, M., *et al.*, (1988) *Science* 239:1534-1536; Kettleborough, C. A., *et al.*, (1991) *Protein Engineering* 4:773-3783; Maeda, H., *et al.*, (1991) *Human Antibodies Hybridoma* 2:124-134; Gorman, S. D., *et al.*, (1991) *Proc Natl Acad Sci USA* 88:4181-4185; Tempest, P. R., *et al.*, (1991) *Bio/Technology* 9:266-271; Co, M. S., *et al.*, (1991) *Proc Natl Acad Sci USA* 88:2869-2873; Carter, P., *et al.*, (1992) *Proc Natl Acad Sci USA* 89:4285-4289; and Co, M. S. *et al.*, (1992) *J Immunol* 148:1149-1154. In some embodiments, humanized antibodies preserve all CDR sequences (for example, a humanized mouse antibody which contains all six CDRs from the mouse antibodies). In other embodiments, humanized antibodies have one or more CDRs (one, two, three, four, five, six) which are altered with respect to the original antibody, which are also termed one or more CDRs "derived from" one or more CDRs from the original antibody.

In certain embodiments, the antibodies of the present disclosure may be chimeric antibodies. In this regard, a chimeric antibody is comprised of an antigen-binding fragment of an anti-LRP5/6 antibody operably linked or otherwise fused to a heterologous Fc portion of a different antibody. In certain embodiments, the heterologous Fc domain is of human origin. In other embodiments, the heterologous Fc domain may be from a different Ig class

from the parent antibody, including IgA (including subclasses IgA1 and IgA2), IgD, IgE, IgG (including subclasses IgG1, IgG2, IgG3, and IgG4), and IgM. In further embodiments, the heterologous Fc domain may be comprised of CH2 and CH3 domains from one or more of the different Ig classes. As noted above with regard to humanized antibodies, the anti-LRP5/6 antigen-binding fragment of a chimeric antibody may comprise only one or more of the CDRs of the antibodies described herein (*e.g.*, 1, 2, 3, 4, 5, or 6 CDRs of the antibodies described herein), or may comprise an entire variable domain (VL, VH or both).

In certain embodiments, the Fc region of an antibody or fragment thereof may be derived from any of a variety of different Fcs, including but not limited to, a wild-type or modified IgG1, IgG2, IgG3, IgG4 or other isotype, e.g., wild-type or modified human IgG1, human IgG2, human IgG3, human IgG4, human IgG4Pro (comprising a mutation in core hinge region that prevents the formation of IgG4 half molecules), human IgA, human IgE, human IgM, or the modified IgG1 referred to as IgG1 LALAPG. The L235A, P329G (LALA-PG) variant has been shown to eliminate complement binding and fixation as well as Fc-γ dependent antibody-dependent cell-mediated cytotoxity (ADCC) in both murine IgG2a and human IgG1. In particular embodiments of any of the IgG disclosed herein, the IgG comprises one or more of the following amino acid substitutions: N297G, N297A, N297E, L234A, L235A, or P236G.

In certain embodiments, antibodies or antigen-binding fragments thereof disclosed herein include fusion proteins, e.g., Wnt signaling pathway agonist fusion proteins, also referred to herein as “Wnt surrogates.” Wnt surrogates of the present invention are usually biologically active in binding to a cognate Frizzled receptor, and in activation of Wnt signaling, i.e., the surrogate is a Wnt agonist. The term "Wnt agonist activity" refers to the ability of an agonist to mimic the effect or activity of a Wnt protein binding to a frizzled protein. The ability of the agonists of the invention to mimic the activity of Wnt can be confirmed by a number of assays. The agonists of the invention typically initiate a reaction or activity that is similar to or the same as that initiated by the receptor's natural ligand. In particular, the agonists of the invention enhance the

canonical Wnt/β-catenin signaling pathway. As used herein, the term "enhances" refers to a measurable increase in the level of Wnt/β-catenin signaling compared with the level in the absence of an agonist of the invention.

In particular embodiments, a Wnt signaling pathway agonist fusion protein (or Wnt surrogate) comprises an anti-LRP5/6 antibody, or antigen-binding fragment thereof, disclosed herein fused to a polypeptide that specifically binds to one or more Frizzled (Fzd) receptors. In particular embodiments, the polypeptide that specifically binds to one or more Fzd receptor is an antibody or antigen-binding fragment thereof. If certain embodiments, it is an antibody or antigen-binding fragment thereof disclosed in the U.S. provisional patent application no. 62/607,877, titled, "Anti-Frizzled antibodies and Methods of Use," Attorney docket number SRZN-004/00US, filed on December 19, 2017, which is incorporated herein by reference in its entirety.

In certain embodiment, the Fzd binding domain may be selected from any domain that binds Fzd at high affinity, e.g., a KD of at least about 1 x 10^{-7} M, at least about 1 x 10^{-8} M, at least about 1 x 10^{-9} M, or at least about 1 x 10^{-10} M. Suitable Fzd binding domains include, without limitation, de novo designed Fzd binding proteins, antibody derived binding proteins, e.g. scFv, Fab, etc. and other portions of antibodies that specifically bind to one or more Fzd proteins; VHH or sdAb derived binding domains; knottin-based engineered scaffolds; norrin and engineered binding fragments derived therefrom, naturally occurring Fzd binding domains, and the like. A Fzd binding domain may be affinity selected to enhance binding to a desired Fzd protein or plurality of Fzd proteins, e.g. to provide tissue selectivity.

In some embodiments the Fzd binding domain binds to one, two, three, four, five or more different frizzled proteins, e.g., one or more of human frizzled proteins Fzd1, Fzd2, Fzd3, Fzd4, Fzd5, Fzd6, Fzd7, Fzd8, Fzd9, Fzd10. In some embodiments, the Fzd binding domain binds to Fzd1, Fzd2, Fzd5, Fzd7 and Fzd8. In other embodiments the Fzd binding domain is selective for one or more frizzled protein of interest, e.g. having a specificity for

the one or more desired frizzled protein of at least 10-fold, 25-fold, 50-fold, 100-fold, 200-fold or more relative to other frizzled proteins.

In certain embodiments, the Fzd binding domain comprises the six CDR regions of the pan specific frizzled antibody OMP-18R5 (vantictumab). In certain embodiments, the Fzd binding domain is an scFv comprising the six CDR regions of the pan-specific frizzled antibody OMP-18R5 (vantictumab). See, for example, U.S. Patent no. 8507442, herein specifically incorporated by reference. For example, the CDR sequences of OMP-18R5 include a heavy chain CDR1 comprising GFTFSHYTLS (SEQ ID NO:25), a heavy chain CDR2 comprising VISGDGSYTYYADSVKG (SEQ ID NO:26), and a heavy chain CDR3 comprising NFIKYVFAN (SEQ ID NO:27), and (ii) a light chain CDR1 comprising SGDKLGKKYAS (SEQ ID NO:28) or SGDNIGSFYVH (SEQ ID NO:31), a light chain CDR2 comprising EKDNRPSG (SEQ ID NO:29) or DKSNRPSG (SEQ ID NO:32), and a light chain CDR3 comprising SSFAGNSLE (SEQ ID NO:30) or QSYANTLSL (SEQ ID NO:33). In particular embodiments, the frizzled binding domain is an antibody or derivative thereof, including without limitation scFv, minibodies, VHH or sdAbs and various antibody mimetics comprising any of these CDR sequences. In certain embodiments, these CDR sequences comprise one or more amino acid modifications.

In other embodiments, the Fzd binding domain comprises a variable region sequence, or the CDRs thereof, from any of a number of frizzled specific antibodies, which are known in the art and are commercially available, or can be generated de novo. Any of the frizzled polypeptides can be used as an immunogen or in screening assays to develop an antibody. Non-limiting examples of frizzled binding domains include antibodies available from Biolegend, e.g. Clone CH3A4A7 specific for human frizzled 4 (CD344); Clone W3C4E11 specific for human Fz9 (CD349); antibodies available from Abcam, e.g. ab64636 specific for Fz7; ab83042 specific for human Fz4; ab77379 specific for human Fz7; ab75235 specific for human Fz8; ab102956 specific for human Fz9; and the like. Other examples of suitable antibodies are described

in, inter alia, US Patent application 20140105917; US Patent application 20130230521; US Patent application 20080267955; US Patent application 20080038272; US Patent application 20030044409; etc., each herein specifically incorporated by reference.

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

The anti-LRP5/6 antibody, or antigen binding fragment thereof, and the Fzd binding domain may be directly joined, or may be separated by a linker, e.g. a polypeptide linker, or a non-peptidic linker, etc. The region of the Wnt surrogate that binds one or more Fzd receptor and the region of the Wnt surrogate that binds LRP5 and/or LRP6 may be contiguous or separated by a linker, e.g. a polypeptide linker, or a non-peptidic linker, *etc.* The length of the linker, and therefore the spacing between the binding domains can be used to modulate the signal strength, and can be selected depending on the desired use of the Wnt surrogate. The enforced distance between binding domains can vary, but in certain embodiments may be less than about 100 angstroms, less than about 90 angstroms, less than about 80 angstroms, less than about 70 angstroms, less than about 60 angstroms, or less than about 50 angstroms. In some embodiments the linker is a rigid linker, in other embodiments the linker is a flexible linker. Where the linker is a peptide linker, it may be from about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 21, 22, 23, 24, 25, 26, 27, 28, 29, 30 or more amino acids in length, and is of sufficient length and amino acid composition to enforce the distance between binding domains. In

some embodiments, the linker comprises or consists of one or more glycine and/or serine residues.

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

In certain embodiments, a Wnt surrogate direct activates canonical Wnt signaling through binding to one or more Fzd proteins and to LRP5 and or LRP6, particularly by binding to these proteins on a cell surface, e.g. the surface of a human cell. The direct activation of Wnt signaling by a Wnt surrogate is in contrast to potentiation of Wnt signaling, which enhances activity only when native Wnt proteins are present.

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

In certain embodiments, an antibody or antigen-binding fragment thereof disclosed herein inhibits Wnt pathway signaling. In particular embodiments, binding of an anti-LRP5/6 antibody or antigen-binding fragment thereof blocks or inhibits the binding of endogenous Wnt to one or more LRP5/6 receptor on a cell surface, thus reducing or inhibiting Wnt signaling.

Various methods are known in the art for measuring the level of canonical Wnt/β-catenin signaling. These include, but are not limited to assays that measure: Wnt/β-catenin target gene expression; TCF reporter gene expression; β-catenin stabilization; LRP phosphorylation; Axin translocation

from cytoplasm to cell membrane and binding to LRP. The canonical Wnt/β-catenin signaling pathway ultimately leads to changes in gene expression through the transcription factors TCF7, TCF7L1, TCF7L2 and LEF. The transcriptional response to Wnt activation has been characterized in a number of cells and tissues. As such, global transcriptional profiling by methods well known in the art can be used to assess Wnt/β-catenin signaling activation or inhibition.

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

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

The recruitment of dephosphorylated β-catenin to the membrane, stabilization and phosphorylation status of β-catenin, and translocation of β-catenin to the nucleus (Klapholz- Brown Z et al., PLoS One. 2(9) e945, 2007), in some cases mediated by complex formation with TCF transcription factors and TNIK are key steps in the Wnt signaling pathway. Stabilization is mediated

by Disheveled family proteins that inhibit the "destruction" complex so that degradation of intracellular β-catenin is reduced, and translocation of β-catenin to the nucleus follows thereafter. Therefore, measuring the level and location of β-catenin in a cell is a good reflection of the level of Wnt/β-catenin signaling. A non-limiting example of such an assay is the "BioImage β-Catenin Redistribution Assay" (Thermo Scientific) which provides recombinant U20S cells that stably express human β-catenin fused to the C-terminus of enhanced green fluorescent protein (EGFP). Imaging and analysis is performed with a fluorescence microscope or HCS platform allowing the levels and distribution of EGFP-β-catenin to be visualized.

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

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

In certain embodiments, a Wnt signaling pathway antagonist or inhibitor inhibits or decreases canonical Wnt pathway signaling, e.g., β-catenin signaling, by at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 75%, 80%, 90%,

95%, or 100%, as compared to the β-catenin signaling observed in the presence of a neutral substance or negative control as measured in an assay described above, for example as measured in the TOPFlash assay. A positive control may be included in these assays.

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

For example, human native sequence Wnt proteins of interest in the present application include the following: Wnt-1 (GenBank Accession No. NM_005430); Wnt-2 (GenBank Accession No. NM_003391); Wnt-2B (Wnt-13) (GenBank Accession No. NM_004185 (isoform 1), NM_024494.2 (isoform 2)), Wnt-3 (RefSeq.: NM_030753), Wnt3a (GenBank Accession No. NM_033131), Wnt-4 (GenBank Accession No. NM_030761), Wnt-5A (GenBank Accession No. NM_003392), Wnt-5B (GenBank Accession No. NM_032642), Wnt-6 (GenBank Accession No. NM_006522), Wnt-7A (GenBank Accession No. NM_004625), Wnt- 7B (GenBank Accession No. NM_058238), Wnt-8A (GenBank Accession No. NM_058244), Wnt-8B (GenBank Accession No. NM_003393), Wnt-9A (Wnt- 14) (GenBank Accession No. NM_003395), Wnt-9B (Wnt-15) (GenBank Accession No. NM_003396), Wnt-1 OA (GenBank Accession No. NM_025216), Wnt-10B (GenBank Accession No. NM_003394), Wnt-11 (GenBank Accession No. NM_004626), Wnt- 16 (GenBank Accession No. NM_016087)). Although each member has varying degrees of sequence identity with the family, all encode small (i.e., 39-46 kD), acylated, palmitoylated, secreted glycoproteins that contain 23-24 conserved cysteine residues whose spacing is highly conserved (McMahon, A P et al., Trends Genet. 1992; 8: 236-242; Miller, J R. Genome Biol. 2002; 3(1): 3001.1-3001.15). Other native sequence Wnt polypeptides of interest include orthologs of the above from any mammal, including domestic and farm animals, and zoo,

laboratory or pet animals, such as dogs, cats, cattle, horses, sheep, pigs, goats, rabbits, rats, mice, frogs, zebra fish, fruit fly, worm, etc.

"Wnt pathway signaling" or "Wnt signaling" is used herein to refer to the mechanism by which a biologically active Wnt exerts its effects upon a cell to modulate a cell's activity. Wnt proteins modulate cell activity by binding to Wnt receptors, including proteins from the Frizzled (Fzd) family of proteins, proteins from the ROR family of proteins, the proteins LRP5 and LRP6 from the LRP family of proteins, the protein FRL1/crypto, and the protein Derailed/Ryk. Once activated by Wnt binding, the Wnt receptor(s) will activate one or more intracellular signaling cascades. These include the canonical Wnt signaling pathway; the Wnt/planar cell polarity (Wnt/PCP) pathway; the Wnt-calcium (Wnt/Ca^{2+}) pathway (Giles, RH et al. (2003) Biochim Biophys Acta 1653, 1-24; Peifer, M. et al. (1994) Development 120: 369-380; Papkoff, J. et al (1996) Mol. Cell Biol. 16: 2128-2134; Veeman, M. T. et al. (2003) Dev. Cell 5: 367-377); and other Wnt signaling pathways as is well known in the art.

For example, activation of the canonical Wnt signaling pathway results in the inhibition of phosphorylation of the intracellular protein β-catenin, leading to an accumulation of β- catenin in the cytosol and its subsequent translocation to the nucleus where it interacts with transcription factors, e.g. TCF/LEF, to activate target genes. Activation of the Wnt/PCP pathway activates RhoA, c-Jun N-terminal kinase (JNK), and nemo-like kinase (NLK) signaling cascades to control such biological processes as tissue polarity and cell movement. Activation of the Wnt/Ca^{2+} by, for example, binding of Wnt-4, Wnt-5A or Wnt-11, elicits an intracellular release of calcium ions, which activates calcium sensitive enzymes like protein kinase C (PKC), calcium-calmodulin dependent kinase II (CamKII) or calcineurin (CaCN). By assaying for activity of the above signaling pathways, the biological activity of an antibody or antigen-binding fragment thereof, e.g., a Wnt surrogate, can be readily determined.

In certain embodiments, functional properties of anti-LRP5/6 antibodies and antigen-binding fragments thereof may be assessed using a variety of methods known to the skilled person, including e.g., affinity/binding

assays (for example, surface plasmon resonance, competitive inhibition assays), cytotoxicity assays, cell viability assays, cell proliferation or differentiation assays in response to a Wnt, cancer cell and/or tumor growth inhibition using *in vitro* or *in vivo* models, including but not limited to any described herein. Other assays may test the ability of antibodies described herein to block normal Wnt/LRP5/6-mediated responses. The antibodies and antigen-binding fragments thereof described herein may also be tested for effects on LRP5/6 receptor internalization, *in vitro* and *in vivo* efficacy, *etc.* Such assays may be performed using well-established protocols known to the skilled person (see *e.g.*, Current Protocols in Molecular Biology (Greene Publ. Assoc. Inc. & John Wiley & Sons, Inc., NY, NY); Current Protocols in Immunology (Edited by: John E. Coligan, Ada M. Kruisbeek, David H. Margulies, Ethan M. Shevach, Warren Strober 2001 John Wiley & Sons, NY, NY); or commercially available kits.

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

Marks et al. (*Bio/Technology*, 1992, 10:779-783) describe methods of producing repertoires of antibody variable domains in which consensus primers directed at or adjacent to the 5' end of the variable domain area are used in conjunction with consensus primers to the third framework region of human VH genes to provide a repertoire of VH variable domains lacking a CDR3. Marks et al further describe how this repertoire may be combined with a CDR3 of a particular antibody. Using analogous techniques, the CDR3-derived sequences of the presently described antibodies may be shuffled with repertoires of VH or VL domains lacking a CDR3, and the shuffled complete VH or VL domains combined with a cognate VL or VH domain to provide an antibody or antigen-binding fragment thereof that binds LRP5 and/or

LRP6. The repertoire may then be displayed in a suitable host system such as the phage display system of WO92/01047 so that suitable antibodies or antigen-binding fragments thereof may be selected. A repertoire may consist of at least from about 10^4 individual members and upwards by several orders of magnitude, for example, to about from 10^6 to 10^8 or 10^{10} or more members. Analogous shuffling or combinatorial techniques are also disclosed by Stemmer (Nature, 1994, 370:389-391), who describes the technique in relation to a β-lactamase gene but observes that the approach may be used for the generation of antibodies.

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

In certain embodiments, a specific VH and/or VL of the antibodies described herein may be used to screen a library of the complementary variable domain to identify antibodies with desirable properties, such as increased affinity for LRP5 and/or LRP6. Such methods are described, for example, in Portolano *et al.*, J. Immunol. (1993) 150:880-887; Clarkson *et al.*, Nature (1991) 352:624-628.

Other methods may also be used to mix and match CDRs to identify antibodies having desired binding activity, such as binding to LRP5 and/or LRP6. For example: Klimka *et al.*, *British Journal of Cancer* (2000) 83: 252-260, describe a screening process using a mouse VL and a human VH library with CDR3 and FR4 retained from the mouse VH. After obtaining antibodies, the VH was screened against a human VL library to obtain antibodies that bound antigen. Beiboer *et al.*, J. Mol. Biol. (2000) 296:833-849

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

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

Also disclosed herein is a method for obtaining an antibody antigen binding domain specific for LRP5 and/or LRP6 antigen, the method comprising providing by way of addition, deletion, substitution or insertion of one or more amino acids in the amino acid sequence of a VH domain set out herein a VH domain which is an amino acid sequence variant of the VH domain, optionally combining the VH domain thus provided with one or more VL domains, and testing the VH domain or VH/VL combination or combinations to identify a specific binding member or an antibody antigen binding domain specific for LRP5 and/or LRP6 and optionally with one or more desired properties. The VL domains may have an amino acid sequence which is substantially as set out herein. An analogous method may be employed in which one or more sequence variants of a VL domain disclosed herein are combined with one or more VH domains.

In particular embodiments, anti-LRP5/6 antibodies, and antigen-binding fragments thereof, are water soluble. By "water soluble" it is meant a composition that is soluble in aqueous buffers in the absence of detergent, usually soluble at a concentration that provides a biologically effective dose of the polypeptide. Compositions that are water soluble form a substantially homogenous composition that has a specific activity that is at least about 5% that of the starting material from which it was purified, usually at least about 10%, 20%, or 30% that of the starting material, more usually about 40%, 50%,

or 60% that of the starting material, and may be about 50%, about 90% or greater. Anti-LRP5/6 antibodies and antigen-binding fragments thereof, including Wnt surrogates, of the present invention typically form a substantially homogeneous aqueous solution at concentrations of at least 25 μM and higher, e.g. at least 25 μM, 40 μM, or 50 μM, usually at least 60 μM, 70 μM, 80 μM, or 90 μM, sometimes as much as 100 μM, 120 μM, or 150 μM. In other words, compositions of the present invention typically form a substantially homogeneous aqueous solution at concentrations of about 0.1 mg/ml, about 0.5 mg/ml, of about 1 mg/ml or more.

An epitope that "specifically binds" or "preferentially binds" (used interchangeably herein) to an antibody or a polypeptide is a term well understood in the art, and methods to determine such specific or preferential binding are also well known in the art. A molecule is said to exhibit "specific binding" or "preferential binding" if it reacts or associates more frequently, more rapidly, with greater duration and/or with greater affinity with a particular cell or substance than it does with alternative cells or substances. An antibody "specifically binds" or "preferentially binds" to a target if it binds with greater affinity, avidity, more readily, and/or with greater duration than it binds to other substances. For example, an antibody that specifically or preferentially binds to LRP5 is an antibody that binds LRP5 with greater affinity, avidity, more readily, and/or with greater duration than it binds to LRP6 or non-LRP5/6 proteins. It is also understood by reading this definition that, for example, an antibody (or moiety or epitope) that specifically or preferentially binds to a first target may or may not specifically or preferentially bind to a second target. As such, "specific binding" or "preferential binding" does not necessarily require (although it can include) exclusive binding. Generally, but not necessarily, reference to binding means preferential binding.

Immunological binding generally refers to the non-covalent interactions of the type which occur between an immunoglobulin molecule and an antigen for which the immunoglobulin is specific, for example by way of illustration and not limitation, as a result of electrostatic, ionic, hydrophilic and/or

hydrophobic attractions or repulsion, steric forces, hydrogen bonding, van der Waals forces, and other interactions. The strength, or affinity of immunological binding interactions can be expressed in terms of the dissociation constant (K_d) of the interaction, wherein a smaller K_d represents a greater affinity. Immunological binding properties of selected polypeptides can be quantified using methods well known in the art. One such method entails measuring the rates of antigen-binding site/antigen complex formation and dissociation, wherein those rates depend on the concentrations of the complex partners, the affinity of the interaction, and on geometric parameters that equally influence the rate in both directions. Thus, both the "on rate constant" (K_{on}) and the "off rate constant" (K_{off}) can be determined by calculation of the concentrations and the actual rates of association and dissociation. The ratio of K_{off} /K_{on} enables cancellation of all parameters not related to affinity, and is thus equal to the dissociation constant K_d. See, generally, Davies *et al.* (1990) *Annual Rev. Biochem. 59*:439-473.

In certain embodiment, the anti-LRP5/6 antibodies bind LRP5 and/or LRP6 with a K_D of less than or equal to about 1 x 10^{-4} M, less than or equal to about 1 x 10^{-5} M, less than or equal to about 1 x 10^{-6} M, less than or equal to about 1 x 10^{-7} M, less than or equal to about 1 x 10^{-8} M, less than or equal to about 1 x 10^{-9} M, or at least about 1 x 10^{-10} M. In certain embodiments, the anti-LRP5/6 antibodies described herein bind LRP5 and/or LRP6 with a K_D of less than about 10,000 nM, less than about 1000 nM, less than about 100 nM, less than about 10 nM, less than about 1 nM or less than about 0.1 nM, and in some embodiments, the antibodies may have even higher affinity for one or more Fzd receptor. In certain embodiments, the anti-LRP5/6 antibodies described herein have an affinity K_D of about 100, 150, 155, 160, 170, 175, 180, 185, 190, 191, 192, 193, 194, 195, 196, 197, 198 or 199 picomolar, and in some embodiments, the antibodies may have even higher affinity for LRP5 and/or LRP6.

The term "immunologically active", with reference to an epitope being or "remaining immunologically active", refers to the ability of an antibody

(*e.g.*, anti-LRP5/6 antibody) to bind to the epitope under different conditions, for example, after the epitope has been subjected to reducing and denaturing conditions.

An antibody or antigen-binding fragment thereof according to certain preferred embodiments of the present application may be one that competes for binding to LRP5 and/or LRP6 with any antibody described herein which both (i) specifically binds to the antigen and (ii) comprises a VH and/or VL domain disclosed herein, or comprises a VH CDR3 disclosed herein, or a variant of any of these. Competition between antibodies may be assayed easily *in vitro*, for example using ELISA and/or by tagging a specific reporter molecule to one antibody which can be detected in the presence of other untagged antibodies, to enable identification of specific antibodies which bind the same epitope or an overlapping epitope. Thus, there is provided herein a specific antibody or antigen-binding fragment thereof, comprising a human antibody antigen-binding site which competes with an antibody described herein that binds to LRP5 and/or LRP6.

In this regard, as used herein, the terms “competes with”, "inhibits binding" and "blocks binding" (*e.g.*, referring to inhibition/blocking of binding of a Wnt to LRP5 and/or LRP6 or referring to inhibition/blocking of binding of an anti-LRP5/6 antibody to LRP5 and/or LRP6) are used interchangeably and encompass both partial and complete inhibition/blocking. The inhibition/blocking of a Wnt to LRP5 and/or LRP6 preferably reduces or alters the normal level or type of cell signaling that occurs when the Wnt binds to the LRP5 and/or LRP6 without inhibition or blocking. Inhibition and blocking are also intended to include any measurable decrease in the binding of a Wnt to LRP5 and/or LRP6 when in contact with an anti-LRP5/6 antibody as disclosed herein as compared to the ligand not in contact with an anti-LRP5/6 antibody, *e.g.*, the blocking of the Wnt to LRP5 and/or LRP6 by at least about 10%, 20%, 30%, 40%, 50%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%.

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

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

release of inflammation mediators, B cell activation, endocytosis, phagocytosis, and cytotoxic attack.

The ability to mediate cytotoxic and phagocytic effector functions is a potential mechanism by which antibodies destroy targeted cells. The cell-mediated reaction wherein nonspecific cytotoxic cells that express FcγRs recognize bound antibody on a target cell and subsequently cause lysis of the target cell is referred to as antibody dependent cell-mediated cytotoxicity (ADCC) (Raghavan *et al.*, 1996, Annu Rev Cell Dev Biol 12:181-220; Ghetie *et al.*, 2000, Annu Rev Immunol 18:739-766; Ravetch *et al.*, 2001, Annu Rev Immunol 19:275-290). The cell-mediated reaction wherein nonspecific cytotoxic cells that express FcγRs recognize bound antibody on a target cell and subsequently cause phagocytosis of the target cell is referred to as antibody dependent cell-mediated phagocytosis (ADCP). All FcγRs bind the same region on Fc, at the N-terminal end of the Cg2 (CH2) domain and the preceding hinge. This interaction is well characterized structurally (Sondermann *et al.*, 2001, J Mol Biol 309:737-749), and several structures of the human Fc bound to the extracellular domain of human FcγRIIIb have been solved (pdb accession code 1E4K) (Sondermann *et al.*, 2000, Nature 406:267-273.) (pdb accession codes 1IIS and 1IIX) (Radaev *et al.*, 2001, J Biol Chem 276:16469-16477.)

The different IgG subclasses have different affinities for the FcγRs, with IgG1 and IgG3 typically binding substantially better to the receptors than IgG2 and IgG4 (Jefferis *et al.*, 2002, Immunol Lett 82:57-65). All FcγRs bind the same region on IgG Fc, yet with different affinities: the high affinity binder FcγRI has a K_d for IgG1 of 10^{-8} M^{-1}, whereas the low affinity receptors FcγRII and FcγRIII generally bind at 10^{-6} and 10^{-5} respectively. The extracellular domains of FcγRIIIa and FcγRIIIb are 96% identical; however, FcγRIIIb does not have an intracellular signaling domain. Furthermore, whereas FcγRI, FcγRIIa/c, and FcγRIIIa are positive regulators of immune complex-triggered activation, characterized by having an intracellular domain that has an immunoreceptor tyrosine-based activation motif (ITAM), FcγRIIb

has an immunoreceptor tyrosine-based inhibition motif (ITIM) and is therefore inhibitory. Thus the former are referred to as activation receptors, and FcγRIIb is referred to as an inhibitory receptor. The receptors also differ in expression pattern and levels on different immune cells. Yet another level of complexity is the existence of a number of FcγR polymorphisms in the human proteome. A particularly relevant polymorphism with clinical significance is V158/F158 FcγRIIIa. Human IgG1 binds with greater affinity to the V158 allotype than to the F158 allotype. This difference in affinity, and presumably its effect on ADCC and/or ADCP, has been shown to be a significant determinant of the efficacy of the anti-CD20 antibody rituximab (Rituxan®, a registered trademark of IDEC Pharmaceuticals Corporation). Patients with the V158 allotype respond favorably to rituximab treatment; however, patients with the lower affinity F158 allotype respond poorly (Cartron *et al.*, 2002, Blood 99:754-758). Approximately 10-20% of humans are V158/V158 homozygous, 45% are V158/F158 heterozygous, and 35-45% of humans are F158/F158 homozygous (Lehrnbecher *et al.*, 1999, Blood 94:4220-4232; Cartron *et al.*, 2002, Blood 99:754-758). Thus 80-90% of humans are poor responders, that is, they have at least one allele of the F158 FcγRIIIa.

The Fc region is also involved in activation of the complement cascade. In the classical complement pathway, C1 binds with its C1q subunits to Fc fragments of IgG or IgM, which has formed a complex with antigen(s). In certain embodiments of the invention, modifications to the Fc region comprise modifications that alter (either enhance or decrease) the ability of an ***-specific antibody as described herein to activate the complement system (see *e.g.*, U.S. Patent 7,740,847). To assess complement activation, a complement-dependent cytotoxicity (CDC) assay may be performed (See, *e.g.*, Gazzano-Santoro *et al.*, J. Immunol. Methods, 202:163 (1996)).

Thus in certain embodiments, the present invention provides anti-LRP5/6 antibodies having a modified Fc region with altered functional properties, such as reduced or enhanced CDC, ADCC, or ADCP activity, or enhanced binding affinity for a specific FcγR or increased serum half-life. Other

modified Fc regions contemplated herein are described, for example, in issued U.S. Patents 7,317,091; 7,657,380; 7,662,925; 6,538,124; 6,528,624; 7,297,775; 7,364,731; Published U.S. Applications US2009092599; US20080131435; US20080138344; and published International Applications WO2006/105338; WO2004/063351; WO2006/088494; WO2007/024249.

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

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

In another contemplated embodiment, a LRP5/6-specific antibody as described herein may be conjugated or operably linked to another therapeutic compound, referred to herein as a conjugate. The conjugate may be a cytotoxic agent, a chemotherapeutic agent, a cytokine, an anti-angiogenic agent, a tyrosine kinase inhibitor, a toxin, a radioisotope, or other therapeutically active agent. Chemotherapeutic agents, cytokines, anti-angiogenic agents, tyrosine kinase inhibitors, and other therapeutic agents have been described above, and all of these aforementioned therapeutic agents may find use as antibody conjugates.

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

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

The present invention further provides in certain embodiments an isolated nucleic acid encoding an antibody or antigen-binding fragment thereof as described herein, for instance, a nucleic acid which codes for a CDR or VH or VL domain as described herein. Nucleic acids include DNA and RNA.

These and related embodiments may include polynucleotides encoding antibodies that bind LRP5 and/or LRP6 as described herein. The term "isolated polynucleotide" as used herein shall mean a polynucleotide of genomic, cDNA, or synthetic origin or some combination thereof, which by virtue of its origin the isolated polynucleotide (1) is not associated with all or a portion of a polynucleotide in which the isolated polynucleotide is found in nature, (2) is linked to a polynucleotide to which it is not linked in nature, or (3) does not occur in nature as part of a larger sequence.

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

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

The term "polynucleotide" as referred to herein means single-stranded or double-stranded nucleic acid polymers. In certain embodiments, the nucleotides comprising the polynucleotide can be ribonucleotides or deoxyribonucleotides or a modified form of either type of nucleotide. Said modifications include base modifications such as bromouridine, ribose

modifications such as arabinoside and 2',3'-dideoxyribose and internucleotide linkage modifications such as phosphorothioate, phosphorodithioate, phosphoroselenoate, phosphorodiselenoate, phosphoroanilothioate, phoshoraniladate and phosphoroamidate. The term "polynucleotide" specifically includes single and double stranded forms of DNA.

The term "naturally occurring nucleotides" includes deoxyribonucleotides and ribonucleotides. The term "modified nucleotides" includes nucleotides with modified or substituted sugar groups and the like.

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

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

As will be understood by those skilled in the art, polynucleotides may include genomic sequences, extra-genomic and plasmid-encoded sequences and smaller engineered gene segments that express, or may be

adapted to express, proteins, polypeptides, peptides and the like. Such segments may be naturally isolated, or modified synthetically by the skilled person.

As will be also recognized by the skilled artisan, polynucleotides may be single-stranded (coding or antisense) or double-stranded, and may be DNA (genomic, cDNA or synthetic) or RNA molecules. RNA molecules may include HnRNA molecules, which contain introns and correspond to a DNA molecule in a one-to-one manner, and mRNA molecules, which do not contain introns. Additional coding or non-coding sequences may, but need not, be present within a polynucleotide according to the present disclosure, and a polynucleotide may, but need not, be linked to other molecules and/or support materials. Polynucleotides may comprise a native sequence or may comprise a sequence that encodes a variant or derivative of such a sequence.

Therefore, according to these and related embodiments, the present disclosure also provides polynucleotides encoding the anti-LRP5/6 antibodies and antigen-binding fragments thereof described herein. In certain embodiments, polynucleotides are provided that comprise some or all of a polynucleotide sequence encoding an antibody or antigen-binding fragment thereof as described herein and complements of such polynucleotides.

It will be appreciated by those of ordinary skill in the art that, as a result of the degeneracy of the genetic code, there are many nucleotide sequences that encodes an antibody as described herein. Some of these polynucleotides bear minimal sequence identity to the nucleotide sequence of the native or original polynucleotide sequence that encode antibodies that bind to LRP5 and/or LRP6. Nonetheless, polynucleotides that vary due to differences in codon usage are expressly contemplated by the present disclosure. In certain embodiments, sequences that have been codon-optimized for mammalian expression are specifically contemplated.

Therefore, in another embodiment of the invention, a mutagenesis approach, such as site-specific mutagenesis, may be employed for the preparation of variants and/or derivatives of the antibodies described herein.

By this approach, specific modifications in a polypeptide sequence can be made through mutagenesis of the underlying polynucleotides that encode them. These techniques provide a straightforward approach to prepare and test sequence variants, for example, incorporating one or more of the foregoing considerations, by introducing one or more nucleotide sequence changes into the polynucleotide.

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

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

As will be appreciated by those of skill in the art, site-specific mutagenesis techniques have often employed a phage vector that exists in both a single stranded and double stranded form. Typical vectors useful in site-directed mutagenesis include vectors such as the M13 phage. These phages

are readily commercially available and their use is generally well-known to those skilled in the art. Double-stranded plasmids are also routinely employed in site directed mutagenesis that eliminates the step of transferring the gene of interest from a plasmid to a phage.

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

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

Antibodies or antigen-binding fragments thereof as provided herein, and encoding nucleic acid molecules and vectors, may be isolated and/or purified, *e.g.* from their natural environment, in substantially pure or homogeneous form, or, in the case of nucleic acid, free or substantially free of nucleic acid or genes of origin other than the sequence encoding a polypeptide with the desired function. Nucleic acid may comprise DNA or RNA and may be

wholly or partially synthetic. Reference to a nucleotide sequence as set out herein encompasses a DNA molecule with the specified sequence, and encompasses a RNA molecule with the specified sequence in which U is substituted for T, unless context requires otherwise.

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

The expression of antibodies and antigen-binding fragments in prokaryotic cells such as *E. coli* is well established in the art. For a review, see for example Pluckthun, A. Bio/Technology 9: 545-551 (1991). Expression in eukaryotic cells in culture is also available to those skilled in the art as an option for production of antibodies or antigen-binding fragments thereof, see recent reviews, for example Ref, M. E. (1993) Curr. Opinion Biotech. 4: 573-576; Trill J. J. *et al.* (1995) Curr. Opinion Biotech 6: 553-560.

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

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

The present invention also provides, in certain embodiments, a method which comprises using a construct as stated above in an expression system in order to express a particular polypeptide such as an LRP5- or LRP6-specific antibody as described herein. The term "transduction" is used to refer to the transfer of genes from one bacterium to another, usually by a phage. "Transduction" also refers to the acquisition and transfer of eukaryotic cellular sequences by retroviruses. The term "transfection" is used to refer to the uptake of foreign or exogenous DNA by a cell, and a cell has been "transfected" when the exogenous DNA has been introduced inside the cell membrane. A number of transfection techniques are well known in the art and are disclosed herein. See, *e.g.*, Graham *et al.*, 1973, Virology 52:456; Sambrook *et al.*, 2001,

MOLECULAR CLONING, A LABORATORY MANUAL, Cold Spring Harbor Laboratories; Davis *et al.*, 1986, BASIC METHODS IN MOLECULAR BIOLOGY, Elsevier; and Chu *et al.*, 1981, Gene 13:197. Such techniques can be used to introduce one or more exogenous DNA moieties into suitable host cells.

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

The terms "polypeptide" "protein" and "peptide" and "glycoprotein" are used interchangeably and mean a polymer of amino acids not limited to any particular length. The term does not exclude modifications such as myristylation, sulfation, glycosylation, phosphorylation and addition or deletion of signal sequences. The terms "polypeptide" or "protein" means one or more chains of amino acids, wherein each chain comprises amino acids covalently linked by peptide bonds, and wherein said polypeptide or protein can comprise a plurality of chains non-covalently and/or covalently linked together by peptide bonds, having the sequence of native proteins, that is, proteins produced by naturally-occurring and specifically non-recombinant cells, or genetically-engineered or recombinant cells, and comprise molecules having the amino

acid sequence of the native protein, or molecules having deletions from, additions to, and/or substitutions of one or more amino acids of the native sequence. The terms "polypeptide" and "protein" specifically encompass the antibodies that bind to LRP and/or LRP6 of the present disclosure, or sequences that have deletions from, additions to, and/or substitutions of one or more amino acid of an anti-LRP5 or anti-LRP6 antibody. Thus, a "polypeptide" or a "protein" can comprise one (termed "a monomer") or a plurality (termed "a multimer") of amino acid chains.

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

Amino acid sequence modification(s) of the antibodies described herein are contemplated. For example, it may be desirable to improve the binding affinity and/or other biological properties of the antibody. For example, amino acid sequence variants of an antibody may be prepared by introducing appropriate nucleotide changes into a polynucleotide that encodes the antibody, or a chain thereof, or by peptide synthesis. Such modifications include, for example, deletions from, and/or insertions into and/or substitutions

of, residues within the amino acid sequences of the antibody. Any combination of deletion, insertion, and substitution may be made to arrive at the final antibody, provided that the final construct possesses the desired characteristics (*e.g.*, high affinity binding to LRP5 and/or LRP6). The amino acid changes also may alter post-translational processes of the antibody, such as changing the number or position of glycosylation sites. Any of the variations and modifications described above for polypeptides of the present invention may be included in antibodies of the present invention.

The present disclosure provides variants of the antibodies disclosed herein. In certain embodiments, such variant antibodies or antigen-binding fragments, or CDRs thereof, bind to LRP5 and/or LRP6 at least about 50%, at least about 70%, and in certain embodiments, at least about 90% as well as an antibody sequence specifically set forth herein. In further embodiments, such variant antibodies or antigen-binding fragments, or CDRs thereof, bind to LRP5 and/or LRP6 with greater affinity than the antibodies set forth herein, for example, that bind quantitatively at least about 105%, 106%, 107%, 108%, 109%, or 110% as well as an antibody sequence specifically set forth herein.

In particular embodiments, the antibody or antigen-binding fragment thereof, e.g., a Fab, scFv, VHH or sdAb, or Wnt surrogate, may comprise: a) a heavy chain variable region comprising: i. a CDR1 region that is identical in amino acid sequence to the heavy chain CDR1 region of a selected antibody described herein; ii. a CDR2 region that is identical in amino acid sequence to the heavy chain CDR2 region of the selected antibody; and iii. a CDR3 region that is identical in amino acid sequence to the heavy chain CDR3 region of the selected antibody; and/or b) a light chain variable domain comprising: i. a CDR1 region that is identical in amino acid sequence to the light chain CDR1 region of the selected antibody; ii. a CDR2 region that is identical in amino acid sequence to the light chain CDR2 region of the selected antibody; and iii. a CDR3 region that is identical in amino acid sequence to the light chain CDR3 region of the selected antibody; wherein the antibody

specifically binds a selected target (e.g., LRP5 and/or LRP6). In a further embodiment, the antibody, or antigen-binding fragment thereof, is a variant antibody wherein the variant comprises a heavy and light chain identical to the selected antibody except for up to 8, 9, 10, 11, 12, 13, 14, 15, or more amino acid substitutions in the CDR regions of the VH and VL regions. In this regard, there may be 1, 2, 3, 4, 5, 6, 7, 8, or in certain embodiments, 9, 10, 11, 12, 13, 14, 15 more amino acid substitutions in the CDR regions of the selected antibody. Substitutions may be in CDRs either in the VH and/or the VL regions. (See *e.g.*, Muller, 1998, Structure 6:1153-1167).

In particular embodiments, the antibody or antigen-binding fragment thereof, e.g., a Fab, scFv, VHH or sdAb, or Wnt surrogate, may comprise one or more, two or more, three or more, four or more, five or more, or six the CDRs identified in Table 1A for any particular antibody. In certain embodiments, the antibody or antigen-binding fragment thereof comprises a CDRH1 comprising or consisting of any of SEQ ID NOs:34-172; a CDRH2 comprising or consisting of any of SEQ ID NOs:173-312; a CDRH3 comprising or consisting of any of SEQ ID NOs: 313-485; a CDRL1 comprising or consisting of any of SEQ ID NOs: 486-524; a CDRL2 comprising or consisting of any of SEQ ID NOs: 525-556; and/or a CDRL3 comprising or consisting of any of SEQ ID NOs: 557-607.

In particular embodiments, a subject antibody, e.g., a Fab, scFv, VHH or sdAb, or Wnt surrogate, may have: a) a heavy chain variable region having an amino acid sequence that is at least 80% identical, at least 95% identical, at least 90%, at least 95% or at least 98% or 99% identical, to the heavy chain variable region of an anti-LRP5/6 antibody described herein; and/or b) a light chain variable region having an amino acid sequence that is at least 80% identical, at least 85%, at least 90%, at least 95% or at least 98% or 99% identical, to the light chain variable region of an anti-LRP5/6 antibody described herein. The amino acid sequence of illustrative heavy and/or light chain regions are set forth in SEQ ID NOs: 1-24.

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

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

In particular embodiments, the disclosure provides antibodies or antigen-binding fragments thereof that bind to the E3 E4 region of LRP6. In particular embodiments, they bind to the E3 β-propeller region of LRP6. In certain embodiments, they bind to a region of LRP6 that comprises or consists of amino acid residues 637-878, where the amino acid sequence and numbering is consist with that described in the Examples. In certain embodiments, they bind to an epitope within the region of LRP6 comprising amino acids 637-878. In certain embodiments, the antibody or antigen-binding fragment thereof contacts the LRP6 at any or all of the contact points disclosed in Table 3. In one embodiment, the core interaction-site or epitope on LRP6 (inter-atomic distances between Lrp6E3E4 and VHH26 less than or equal to 5.0 Å) includes: Arg639, Ala640, Lys622, Glu663, Ile681, Ser682, Lys684, Asp705, Tyr706, Glu708, Thr724, Gly725, Arg751, Try767, Gly768, Gly769, Arg792, Leu810, Asp811, His834, Phe836, Trp850, Ser851, Arg853, Asp874, Tyr875, and Met877 of LRP6. In another embodiment, the core interaction-site (inter-atomic distances between Lrp6E3E4 and VHH36 less than or equal to 5.0 Å) includes: Glu663, Ser665, Ile681, Tyr706, Glu708, Thr724, Ser749, Arg751, Trp767, Gly768, Arg792, Leu810, Asn813, Pro833, His834, Phe836, Trp850, Ser851, Arg853, Asp874, Try875, and Met877 of LRP6.

In another embodiment of invention, the anti-LRP5/6 antibodies and humanized versions thereof are derived from rabbit monoclonal antibodies, and in particular are generated using RabMAb® technology. These antibodies are advantageous as they require minimal sequence modifications, thereby facilitating retention of functional properties after humanization using mutational lineage guided (MLG) humanization technology (see e.g., U.S. Patent No. 7,462,697). Thus, illustrative methods for making the anti-*** antibodies of the present disclosure include the RabMab® rabbit monoclonal antibody technology described, for example, in U.S. Patents 5,675,063 and 7,429,487. In this regard, in certain embodiments, the anti-LRP5/6 antibodies of the

disclosure are produced in rabbits. In particular embodiments, a rabbit-derived immortal B-lymphocyte capable of fusion with a rabbit splenocyte is used to produce a hybrid cell that produces an antibody. The immortal B-lymphocyte does not detectably express endogenous immunoglobulin heavy chain and may contain, in certain embodiments, an altered immunoglobulin heavy chain-encoding gene.

Compositions

Pharmaceutical compositions comprising an anti-LRP5/6 antibody or antigen-binding fragment thereof, e.g., a Wnt surrogate, described herein and one or more pharmaceutically acceptable diluent, carrier, or excipient are also disclosed. In particular embodiments, the pharmaceutical composition further comprises one or more Wnt polypeptides or Norrin polypeptides.

In further embodiments, pharmaceutical compositions comprising a polynucleotide comprising a nucleic acid sequence encoding an anti-LRP5/6 antibody or antigen-binding fragment thereof, e.g., a Wnt surrogate, described herein and one or more pharmaceutically acceptable diluent, carrier, or excipient are also disclosed. In particular embodiments, the pharmaceutical composition further comprises one or more polynucleotides comprising a nucleic acid sequence encoding a Wnt polypeptide or Norrin polypeptide. In certain embodiments, the polynucleotides are DNA or mRNA, e.g., a modified mRNA. In particular embodiments, the polynucleotides are modified mRNAs further comprising a 5' cap sequence and/or a 3' tailing sequence, e.g., a polyA tail. In other embodiments, the polynucleotides are expression cassettes comprising a promoter operatively linked to the coding sequences. In certain embodiments, the nucleic acid sequence encoding the anti-LRP5/6 antibody or antigen-binding fragment thereof, e.g., a Wnt surrogate, and the nucleic acid sequence encoding the Wnt polypeptide or Norrin polypeptide are present in the same polynucleotide.

In further embodiments, pharmaceutical compositions comprising an expression vector, e.g., a viral vector, comprising a polynucleotide comprising a nucleic acid sequence encoding an anti-LRP5/6 antibody or

antigen-binding fragment thereof, e.g., a Wnt surrogate, described herein and one or more pharmaceutically acceptable diluent, carrier, or excipient are also disclosed. In particular embodiments, the pharmaceutical composition further comprises an expression vector, e.g., a viral vector, comprising a polynucleotide comprising a nucleic acid sequence encoding a Wnt polypeptide or Norrin polypeptide. In certain embodiments, the nucleic acid sequence encoding the anti-LRP5/6 antibody or antigen-binding fragment thereof, e.g., a Wnt surrogate, and the nucleic acid sequence encoding the Wnt polypeptide or Norrin polypeptide are present in the same polynucleotide, e.g., expression cassette.

The present invention further contemplates a pharmaceutical composition comprising a cell comprising an expression vector comprising a polynucleotide comprising a promoter operatively linked to a nucleic acid encoding an anti-LRP5/6 antibody or antigen-binding fragment thereof described herein and one or more pharmaceutically acceptable diluent, carrier, or excipient. In particular embodiments, the pharmaceutical composition further comprises a cell comprising an expression vector comprising a polynucleotide comprising a promoter operatively linked to a nucleic acid sequence encoding a Wnt polypeptide or a Norrin polypeptide. In certain embodiments, the nucleic acid sequence encoding the anti-LRP5/6 antibody or antigen-binding fragment thereof, e.g., a Wnt surrogate, and the nucleic acid sequence encoding the Wnt polypeptide or Norrin polypeptide are present in the same polynucleotide, e.g., expression cassette and/or in the same cell. In particular embodiments, the cell is a heterologous cell or an autologous cell obtained from the subject to be treated. In particular embodiments, the cell is a stem cell, e.g., an adipose-derived stem cell or a hematopoietic stem cell.

The present disclosure contemplates pharmaceutical compositions comprising a first molecule for delivery of anti-LRP5/6 antibody or antigen-binding fragment thereof, e.g., a Wnt surrogate, as a first active agent and a second molecule for delivery of a Wnt polypeptide or Norrin polypeptide. The first and second molecule may be the same type of molecule or different

types of molecules. For example, in certain embodiments, the first and second molecule may each be independently selected from the following types of molecules: polypeptides, small organic molecules, nucleic acids encoding the first or second active agent (optionally DNA or mRNA, optionally modified RNA), vectors comprising a nucleic acid sequence encoding the first or second active agent (optionally expression vectors or viral vectors), and cells comprising a nucleic acid sequence encoding the first or second active agent (optionally an expression cassette).

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

Pharmaceutical compositions may further include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous administration, suitable carriers include physiological saline, bacteriostatic

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

Sterile solutions can be prepared by incorporating the anti-LRP5/6 antibody or antigen-binding fragment thereof (or encoding polynucleotide or cell comprising the same) in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, methods of preparation are vacuum drying and freeze-drying that yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

In one embodiment, the pharmaceutical compositions are prepared with carriers that will protect the antibody or antigen-binding fragment thereof against rapid elimination from the body, such as a controlled release

formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. The materials can also be obtained commercially. Liposomal suspensions can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art.

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

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

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

The present invention includes pharmaceutically acceptable salts of the anti-LRP5/6 antibodies or antigen-binding fragments thereof, e.g., Wnt surrogates, described herein. The term “pharmaceutically acceptable salt” refers to physiologically and pharmaceutically acceptable salts of the

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

Pharmaceutically acceptable base addition salts are formed with metals or amines, such as alkali and alkaline earth metals or organic amines. Metals used as cations comprise sodium, potassium, magnesium, calcium, and the like. Amines comprise N-N'-dibenzylethylenediamine, chloroprocaine, choline, diethanolamine, dicyclohexylamine, ethylenediamine, N-methylglucamine, and procaine (see, for example, Berge et al., "Pharmaceutical Salts," J. Pharma Sci., 1977, 66, 119). The base addition salts of said acidic compounds are prepared by contacting the free acid form with a sufficient amount of the desired base to produce the salt in the conventional manner. The free acid form may be regenerated by contacting the salt form with an acid and isolating the free acid in the conventional manner. The free acid forms differ from their respective salt forms somewhat in certain physical properties such as solubility in polar solvents, but otherwise the salts are equivalent to their respective free acid for purposes of the present invention.

In some embodiments, the pharmaceutical composition provided herein comprise a therapeutically effective amount of an anti-LRP5/6 antibody or antigen-binding fragment thereof, e.g., a Wnt surrogate, described herein in admixture with a pharmaceutically acceptable carrier, diluent and/or excipient, for example saline, phosphate buffered saline, phosphate and amino acids, polymers, polyols, sugar, buffers, preservatives and other proteins. Exemplary amino acids, polymers and sugars and the like are octylphenoxy polyethoxy

ethanol compounds, polyethylene glycol monostearate compounds, polyoxyethylene sorbitan fatty acid esters, sucrose, fructose, dextrose, maltose, glucose, mannitol, dextran, sorbitol, inositol, galactitol, xylitol, lactose, trehalose, bovine or human serum albumin, citrate, acetate, Ringer's and Hank's solutions, cysteine, arginine, carnitine, alanine, glycine, lysine, valine, leucine, polyvinylpyrrolidone, polyethylene and glycol. Preferably, this formulation is stable for at least six months at 4º C.

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

Methods of Use

The present disclosure also provides methods for using the LRP5/6-specific antibodies, antigen-binding fragments thereof, e.g., Wnt surrogates, disclosed herein, e.g., to modulate a Wnt signaling pathway, e.g., to increase or decrease Wnt signaling, and the administration of Fzd-specific antibodies, antigen-binding fragments thereof, and Wnt surrogates disclosed herein in a variety of therapeutic settings. Provided herein are methods of treatment using the antibodies that bind one or more Fzd receptors or antigen-binding fragments thereof. In one embodiment, an antibody, or antigen-binding fragment thereof, of the present invention is provided to a subject having a disease involving inappropriate or deregulated Wnt signaling, e.g., increased or reduced Wnt signaling.

Increasing Wnt Pathway Signaling and Related Therapeutic Methods

In certain embodiments, an anti-LRP5/6 antibody or antigen-binding fragment thereof, e.g., a Wnt surrogate, may be used to increase Wnt signaling in a tissue or cell. Thus, in some aspects, the present invention provides a method for increasing Wnt signaling or enhancing Wnt signaling in a tissue or cell, comprising contacting the tissue or cell with an effective amount

of a an anti-LRP5/6 antibody or antigen-binding fragment thereof, e.g., a Wnt surrogate, disclosed herein, wherein the anti-LRP5/6 antibody or antigen-binding fragment thereof is a Wnt signaling pathway agonist. In some embodiments, contacting occurs in vitro, ex vivo, or in vivo. In particular embodiments, the cell is a cultured cell, and the contacting occurs in vitro. In certain embodiments, the method comprises further contacting the tissue or cell with one or more Wnt polypeptides or Norrin polypeptides.

In related aspects, the present invention provides a method for increasing Wnt signaling in a tissue or cell, comprising contacting the tissue or cell with an effective amount of a polynucleotide comprising an anti-LRP5/6 antibody or antigen-binding fragment thereof, e.g., a Wnt surrogate, of the present invention. In certain embodiments, the target tissue or cell is also contacted with a polynucleotide comprising a nucleic acid sequence that encodes a Wnt polypeptide or a Norrin polypeptide. In certain embodiments, the polynucleotides are DNA or mRNA, e.g., a modified mRNA. In particular embodiments, the polynucleotides are modified mRNAs further comprising a 5' cap sequence and/or a 3' tailing sequence, e.g., a polyA tail. In other embodiments, the polynucleotides are expression cassettes comprising a promoter operatively linked to the coding sequences. In certain embodiments, the nucleic acid sequence encoding the anti-LRP5/6 antibody or antigen-binding fragment thereof, e.g., a Wnt surrogate, and the nucleic acid sequence encoding the Wnt polypeptide or Norrin polypeptide are present in the same polynucleotide.

In related aspects, the present invention provides a method for increasing Wnt signaling in a tissue or cell, comprising contacting the tissue or cell with an effective amount of a vector comprising a nucleic acid sequence encoding an anti-LRP5/6 antibody or antigen-binding fragment thereof, e.g., a Wnt surrogate. In certain embodiments, the tissue or cell is also contacted with a vector comprising a nucleic acid sequence that encodes a Wnt polypeptide or a Norrin polypeptide. In certain embodiments, the vector is an expression vector, and may comprise a promoter operatively linked to the nucleic acid sequence. In

particular embodiments, the vector is a viral vector. In certain embodiments, the nucleic acid sequence encoding the anti-LRP5/6 antibody or antigen-binding fragment thereof, e.g., a Wnt surrogate, and the nucleic acid sequence encoding the Wnt polypeptide or Norrin polypeptide are present in the same vector, e.g., in the same expression cassette.

In related aspects, the present invention provides a method for increasing Wnt signaling in a tissue, comprising contacting the tissue with an effective amount of a cell comprising a nucleic acid sequence encoding an anti-LRP5/6 antibody or antigen-binding fragment thereof, e.g., a Wnt surrogate, of the present invention. In certain embodiments, the tissue is also contacted with a cell comprising a nucleic acid sequence that encodes a Wnt polypeptide or Norrin polypeptide. In certain embodiments, the nucleic acid sequence encoding the anti-LRP5/6 antibody or antigen-binding fragment thereof, e.g., a Wnt surrogate, and the nucleic acid sequence encoding the Wnt polypeptide or Norrin polypeptide are present in the same cell. In particular embodiments, the cell is a heterologous cell or an autologous cell obtained from the subject to be treated. In certain embodiments, the cell was transduced with a vector comprising an expression cassette encoding the anti-LRP5/6 antibody or antigen-binding fragment thereof, e.g., a Wnt surrogate, or the Wnt polypeptide or Norrin polypeptide. In particular embodiments, the cell is a stem cell, e.g., an adipose-derived stem cell or a hematopoietic stem cell.

Anti-LRP5/6 antibodies and antigen-binding fragments thereof, e.g., Wnt surrogates, may be used in to treat a disease, disorder or condition, for example, by increasing Wnt signaling in a targeted cell, tissue or organ. Thus, in some aspects, the present invention provides a method for treating a disease or condition in a subject in need thereof, e.g., a disease or disorder associated with reduced Wnt signaling, or for which increased Wnt signaling would provide a therapeutic benefit, comprising contacting the subject with an effective amount of a composition of the present disclosure. In particular embodiments, the composition is a pharmaceutical composition comprising any of: an anti-LRP5/6 antibody or antigen-binding fragment thereof, e.g., a Wnt

surrogate; a polynucleotide comprising a nucleic acid sequence encoding an anti-LRP5/6 antibody or antigen-binding fragment thereof, e.g., a Wnt surrogate,, e.g., a DNA or mRNA, optionally a modified mRNA; a vector comprising a nucleic acid sequence encoding an anti-LRP5/6 antibody or antigen-binding fragment thereof, e.g., a Wnt surrogate,, e.g., an expression vector or viral vector; or a cell comprising a nucleic acid sequence encoding an anti-LRP5/6 antibody or antigen-binding fragment thereof, e.g., a Wnt surrogate, e.g., a cell transduced with an expression vector or viral vector encoding an anti-LRP5/6 antibody or antigen-binding fragment thereof, e.g., a Wnt surrogate. In particular embodiments, the disease or condition is a pathological disease or disorder, or an injury, e.g., an injury resulting from a wound. In certain embodiments, the wound may be the result of another therapeutic treatment. In certain embodiments, the disease or condition comprises impaired tissue repair, healing or regeneration, or would benefit from increased tissue repair, healing or regeneration. In some embodiments, contacting occurs in vivo, i.e., the subject composition is administered to a subject.

In certain embodiments, the method comprises further contacting the subject with a pharmaceutical composition comprising one or more Wnt polypeptides or Norrin polypeptides. The present disclosure contemplates contacting a subject with a first molecule for delivery of an anti-LRP5/6 antibody or antigen-binding fragment thereof, e.g., a Wnt surrogate, as a first active agent and a second molecule for delivery of a Wnt polypeptide or Norrin polypeptide. The first and second molecule may be the same type of molecule or different types of molecules. For example, in certain embodiments, the first and second molecule may each be independently selected from the following types of molecules: polypeptides, small organic molecules, nucleic acids encoding the first or second active agent (optionally DNA or mRNA, optionally modified RNA), vectors comprising a nucleic acid sequence encoding the first or second active agent (optionally expression vectors or viral vectors), and cells

comprising a nucleic acid sequence encoding the first or second active agent (optionally an expression cassette).

In related aspects, the present invention provides a method for treating a disease or condition, e.g., a disease or disorder associated with reduced Wnt signaling, or for which increased Wnt signaling would provide a therapeutic benefit, comprising contacting a subject in need thereof with a pharmaceutical composition comprising an effective amount of a polynucleotide comprising a nucleic acid sequence encoding an anti-LRP5/6 antibody or antigen-binding fragment thereof, e.g., a Wnt surrogate, disclosed herein. In certain embodiments, the subject is also contacted with a pharmaceutical composition comprising an effective amount of a polynucleotide comprising a nucleic acid sequence that encodes a Wnt polypeptide or a Norrin polypeptide. In certain embodiments, the polynucleotides are DNA or mRNA, e.g., a modified mRNA. In particular embodiments, the polynucleotides are modified mRNAs further comprising a 5' cap sequence and/or a 3' tailing sequence, e.g., a polyA tail. In other embodiments, the polynucleotides are expression cassettes comprising a promoter operatively linked to the coding sequences. In certain embodiments, the nucleic acid sequence encoding the anti-LRP5/6 antibody or antigen-binding fragment thereof, e.g., a Wnt surrogate, and the nucleic acid sequence encoding the Wnt polypeptide or Norrin polypeptide are present in the same polynucleotide.

In related aspects, the present invention provides a method for treating a disease or condition, e.g., a disease or disorder associated with reduced Wnt signaling, or for which increased Wnt signaling would provide a therapeutic benefit, comprising contacting a subject in need thereof with a pharmaceutical composition comprising an effective amount of a vector comprising a nucleic acid sequence encoding an anti-LRP5/6 antibody or antigen-binding fragment thereof, e.g., a Wnt surrogate. In certain embodiments, the subject is also contacted with a pharmaceutical composition comprising an effective amount of a vector comprising a nucleic acid sequence that encodes a Wnt polypeptide or a Norrin polypeptide. In certain

embodiments, the vector is an expression vector, and may comprise a promoter operatively linked to the nucleic acid sequence. In particular embodiments, the vector is a viral vector. In certain embodiments, the nucleic acid sequence encoding the anti-LRP5/6 antibody or antigen-binding fragment thereof, e.g., a Wnt surrogate, and the nucleic acid sequence encoding the Wnt polypeptide or Norrin polypeptide are present in the same vector, e.g., in the same expression cassette.

In related aspects, the present invention provides a method for treating a disease or condition, e.g., a disease or disorder associated with reduced Wnt signaling, or for which increased Wnt signaling would provide a therapeutic benefit, comprising contacting a subject in need thereof with a pharmaceutical composition comprising an effective amount of a cell comprising a nucleic acid sequence encoding an anti-LRP5/6 antibody or antigen-binding fragment thereof, e.g., a Wnt surrogate. In certain embodiments, the subject is also contacted with a cell comprising a nucleic acid sequence that encodes a Wnt polypeptide or a Norrin polypeptide. In certain embodiments, the nucleic acid sequence encoding the anti-LRP5/6 antibody or antigen-binding fragment thereof, e.g., a Wnt surrogate, and the nucleic acid sequence encoding the Wnt polypeptide or Norrin polypeptide are present in the same cell. In particular embodiments, the cell is a heterologous cell or an autologous cell obtained from the subject to be treated. In certain embodiments, the cell was transduced with a vector comprising an expression cassette encoding the anti-LRP5/6 antibody or antigen-binding fragment thereof, e.g., a Wnt surrogate, or the Wnt polypeptide or Norrin polypeptide. In particular embodiments, the cell is a stem cell, e.g., an adipose-derived stem cell or a hematopoietic stem cell.

Wnt signaling plays key roles in the developmental process and maintenance of stem cells. Reactivation of Wnt signals is associated with regeneration and repair of most tissues after injuries and diseases. Anti-LRP5/6 antibody or antigen-binding fragment thereof, e.g., a Wnt surrogate, molecules are expected to provide benefit of healing and tissue repair in response to

injuries and diseases. Causes of tissue damage and loss include but are not limited to aging, degeneration, hereditary conditions, infection and inflammation, traumatic injuries, toxins/metabolic-induced toxicities, or other pathological conditions. Wnt signals and enhancers of Wnt signals have been shown to activate adult, tissue-resident stem cells. In some embodiments, the compounds of the invention are administered for use in treating diseased or damaged tissue, for use in tissue regeneration and for use in cell growth and proliferation, and/or for use in tissue engineering.

For example, compositions of the present invention may be used to promote or increase bone growth or regeneration, bone grafting, healing of bone fractures, treatment of osteoporosis and osteoporotic fractures, spinal fusion, spinal cord injuries, including vertebral compression fractures, pre-operative spinal surgery optimization, osseointegration of orthopedic devices, tendon-bone integration, tooth growth and regeneration, dental implantation, periodontal diseases, maxillofacial reconstruction, and osteonecrosis of the jaw. They may also be used in the treatment of alopecia; enhancing regeneration of sensory organs, e.g. treatment of hearing loss, including regeneration of inner and outer auditory hair cells treatment of vestibular hypofunction, treatment of macular degeneration, treatment of retinopathies, including vitreoretinopathy, diabetic retinopathy, other diseases of retinal degeneration, Fuchs' dystrophy, other cornea disease, etc.; treatment of stroke, traumatic brain injury, Alzheimer's disease, multiple sclerosis, muscular dystrophy, muscle atrophy as a result of sarcopenia or cachexia, and other conditions affecting the degeneration or integrity of the blood brain barrier. The compositions of this invention may also be used in treatment of oral mucositis, treatment of short bowel syndrome, inflammatory bowel diseases (IBD), including Crohn's disease (CD) and ulcerative colitis (UC), in particular CD with fistula formation, other gastrointestinal disorders; treatment of metabolic syndrome, dyslipidemia, treatment of diabetes, treatment of pancreatitis, conditions where exocrine or endocrine pancreas tissues are damaged; conditions where enhanced epidermal regeneration is desired, e.g., epidermal wound healing, treatment of

diabetic foot ulcers, syndromes involving tooth, nail, or dermal hypoplasia, etc., conditions where angiogenesis is beneficial; treatment of myocardial infarction, coronary artery disease, heart failure; enhanced growth of hematopoietic cells, e.g. enhancement of hematopoietic stem cell transplants from bone marrow, mobilized peripheral blood, treatment of immunodeficiencies, graft versus host diseases, etc.; treatment of acute kidney injuries, chronic kidney diseases; treatment of lung diseases, chronic obstructive pulmonary diseases (COPD), pulmonary fibrosis, including idiopathic pulmonary fibrosis, enhanced regeneration of lung tissues. The compositions of the present invention may also be used in enhanced regeneration of liver cells, e.g. liver regeneration, treatment of cirrhosis, enhancement of liver transplantations, treatment of acute liver failure, treatment of chronic liver diseases with hepatitis C or B virus infection or post-antiviral drug therapies, alcoholic liver diseases, alcoholic hepatitis, non-alcoholic liver diseases with steatosis or steatohepatitis, and the like. The compositions of this invention may treat diseases and disorders including, without limitation, conditions in which regenerative cell growth is desired.

Human genetics involving loss-of-function or gain-of-function mutations in Wnt signaling components show strong evidence supporting enhancing Wnt signals for bone growth. Conditions in which enhanced bone growth is desired may include, without limitation, fractures, grafts, ingrowth around prosthetic devices, osteoporosis, osteoporotic fractures, spinal fusion, vertebral compression fractures, pre-operative optimization for spinal surgeries, osteonecrosis of the jaw, dental implantation, periodontal diseases, maxillofacial reconstruction, and the like. An anti-LRP5/6 antibody or antigen-binding fragment thereof, e.g., a Wnt surrogate, enhances and promotes Wnt signals which are critical in promoting bone regeneration. Methods for regeneration of bone tissues benefit from administration of the compounds of the invention, which can be systemic or localized. In some embodiments, bone marrow cells are exposed to molecules of the invention, such that stem cells within that marrow become activated.

In some embodiments, bone regeneration is enhanced by contacting a responsive cell population, e.g. bone marrow, bone progenitor cells, bone stem cells, etc. with an effective dose of an anti-LRP5/6 antibody or antigen-binding fragment thereof, e.g., a Wnt surrogate, disclosed herein. Methods for regeneration of bone tissues benefit from administration of the anti-LRP5/6 antibody or antigen-binding fragment thereof, e.g., a Wnt surrogate disclosed herein, which can be systemic or localized. In some such embodiments, the contacting is performed in vivo. In other such embodiments, the contacting is performed ex vivo. The molecule may be localized to the site of action, e.g. by loading onto a matrix, which is optionally biodegradable, and optionally provides for a sustained release of the active agent. Matrix carriers include, without limitation, absorbable collagen sponges, ceramics, hydrogels, polymeric microspheres, nanoparticles, bone cements, and the like.

Compositions comprising one or more anti-LRP5/6 antibody or antigen-binding fragment thereof, e.g., a Wnt surrogate, disclosed herein can be used for the in vivo treatment of skeletal tissue deficiencies. By "skeletal tissue deficiency", it is meant a deficiency in bone or other skeletal connective tissue at any site where it is desired to restore the bone or connective tissue, no matter how the deficiency originated, e.g. whether as a result of surgical intervention, removal of tumor, ulceration, implant, fracture, or other traumatic or degenerative conditions. The compositions of the present invention can be used as part of a regimen for restoring cartilage function to a connective tissue, for the repair of defects or lesions in cartilage tissue such as degenerative wear and arthritis, trauma to the tissue, displacement of torn meniscus, meniscectomy, a luxation of a joint by a torn ligament, malalignment of joints, bone fracture, or by hereditary disease.

An anti-LRP5/6 antibody or antigen-binding fragment thereof, e.g., a Wnt surrogate, may also be used for treatment of periodontal diseases. Periodontal diseases are a leading cause of tooth loss and are linked to multiple systemic conditions. In some embodiments, tooth or underlying bone regeneration is enhanced by contacting a responsive cell population. In some

such embodiments, the contacting is performed in vivo. In other such embodiments, the contacting is performed ex vivo, with subsequent implantation of the activated stem or progenitor cells. The molecule may be localized to the site of action, e.g. by loading onto a matrix, which is optionally biodegradable, and optionally provides for a sustained release of the active agent. Matrix carriers include, without limitation, absorbable collagen sponges, ceramics, hydrogels, bone cements, polymeric microspheres, nanoparticles, and the like.

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

An anti-LRP5/6 antibody or antigen-binding fragment thereof, e.g., a Wnt surrogate, may also be used in regeneration of retinal tissue. In the adult mammalian retina, Muller glia cells are capable of regenerating retinal cells, including photoreceptors, for example after neurotoxic injury in vivo. Wnt signaling and enhancers of Wnt signals can promote proliferation of Muller glia-derived retinal progenitors after damage or during degeneration. The compositions of the invention may also be used in the regeneration of tissues and other cell types in the eye. For examples age-related macular degeneration (AMD), other retina degenerative diseases, cornea diseases, Fuchs' dystrophy, vitreoretinopathy, hereditary diseases, etc. can benefit from the compositions of the present inventions. AMD is characterized by progressively decreased central vision and visual acuity. Fuchs' dystrophy is characterized by

progressive loss of cornea endothelial cells. Wnt signal and enhancing of Wnt signal can promote regeneration of cornea endothelium, retina epithelium, etc. in the eye tissue. In other embodiments, compositions of the present invention can be used, for example, in an infusion; in a matrix or other depot system; or other topical application to the eye for retinal regeneration and treatment of macular degeneration.

Specific populations of proliferating cells for homeostatic renewal of hepatocytes have been identified through lineage tracing studies, for example Axin2-positive cells in peri-central region. Lineage tracing studies also identified additional potential liver progenitor cells, including but not limited to Lgr-positive cells. The self-renewing liver cells and other populations of potential progenitor cells, including Lgr5-positive and Axin2-positive cells, are identified to be capable of regeneration responding to Wnt signals and/or R-spondins following injuries. Numerous preclinical models of acute liver injury and failure and chronic liver diseases showed recovery and regeneration of hepatocytes benefit from enhancing Wnt signals. The compositions of this invention may be used in treatment of acute liver failure, acute alcoholic liver injuries, treatment of chronic liver diseases with hepatitis C or B virus infection or post-antiviral drug therapies, chronic alcoholic liver diseases, alcoholic hepatitis, non-alcoholic fatty liver diseases and non-alcoholic steatohepatitis (NASH), treatment of cirrhosis and severe chronic liver diseases of all causes, and enhanced regeneration of liver cells. Methods for regeneration of liver tissue benefit from administration of the compounds of the invention, which can be systemic or localized. These include, but are not limited to, methods of systemic administration and methods of localized administration e.g. by injection into the liver tissue, by injection into veins or blood vessels leading into the liver, by implantation of a sustained release formulation, and the like.

Wnt signals play an important role in regeneration of various epithelial tissues. Various epidermal conditions benefit from treatment with the compounds of the present invention. Mucositis occurs when there is a breakdown of the rapidly divided epithelial cells lining the gastro-intestinal tract,

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

In addition to skin and gastrointestinal tract, Wnt signals and enhancement and promotion of Wnt signals also play an important role in repair and regeneration of tissues including pancreas, kidney, and lung in preclinical models. An anti-LRP5/6 antibody or antigen-binding fragment thereof, e.g., a Wnt surrogate, may benefit various disease conditions involving exocrine and endocrine pancreas, kidney, or lung. The anti-LRP5/6 antibody or antigen-binding fragment thereof, e.g., a Wnt surrogate, may be used in treatment of metabolic syndrome; treatment of diabetes, treatment of acute or chronic pancreatitis, exocrine pancreatic insufficiency, treatment of acute kidney injuries, chronic kidney diseases, treatment of lung diseases, including but not limited to chronic obstructive pulmonary diseases (COPD), pulmonary fibrosis, in particular idiopathic pulmonary fibrosis (IPF), and other conditions that cause loss of lung epithelial tissues. Methods for regeneration of these tissues benefit from administration of the compounds of the invention, which can be systemic or localized.

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

Stroke, traumatic brain injury, Alzheimer's disease, multiple sclerosis and other conditions affecting the blood brain barrier (BBB) may be treated with an anti-LRP5/6 antibody or antigen-binding fragment thereof, e.g., a Wnt surrogate. Angiogenesis is critical to ensure the supply of oxygen and nutrients to many tissues throughout the body, and is especially important for the CNS as the neural tissue is extremely sensitive to hypoxia and ischemia. CNS endothelial cells which form the BBB differ from endothelial cells in non-neural tissue, in that they are highly polarized cells held together by tight junctions and express specific transporters. Wnt signaling regulates CNS vessel formation and/or function. Conditions in which the BBB is compromised can benefit from administration of the compounds of the invention, which can be systemic or localized e.g. by direct injection, intrathecal administration, implantation of sustained release formulations, and the like. In addition, Wnt signal is actively involved in neurogenesis and plays a role of neuroprotection following injury. The compositions of the present invention may also be used in treatment of spinal cord injuries, other spinal cord diseases, stroke, traumatic brain injuries, etc.

Wnt signals also play a role in angiogenesis. An anti-LRP5/6 antibody or antigen-binding fragment thereof, e.g., a Wnt surrogate, may benefit conditions where angiogenesis is beneficial, treatment of myocardial infarction, coronary artery disease, heart failure, diabetic retinopathy, etc., and conditions from hereditary diseases. Methods for regeneration of these tissues benefit

from administration of the compounds of the invention, which can be systemic or localized.

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

Reducing Wnt Pathway Signaling and Related Therapeutic Methods

In certain embodiments, an anti-LRP5/6 antibody or antigen-binding fragment thereof, may be used to decrease or inhibit Wnt signaling in a tissue or cell. Thus, in some aspects, the present invention provides a method for decreasing Wnt signaling or inhibiting Wnt signaling in a tissue or cell, comprising contacting the tissue or cell with an effective amount of an anti-LRP5/6 antibody, or antigen-binding fragment thereof, disclosed herein, wherein the anti-LRP5/6 antibody or antigen-binding fragment thereof is a Wnt signaling pathway antagonist or inhibitor. In some embodiments, contacting occurs in vitro, ex vivo, or in vivo. In particular embodiments, the cell is a cultured cell, and the contacting occurs in vitro.

In related aspects, the present invention provides a method for decreasing or inhibiting Wnt signaling in a tissue or cell, comprising contacting the tissue or cell with an effective amount of a polynucleotide comprising an anti-LRP5/6 antibody or antigen-binding fragment thereof, of the present invention, wherein the anti-LRP5/6 antibody or antigen-binding fragment thereof is a Wnt signaling pathway antagonist or inhibitor. In certain embodiments, the polynucleotides are DNA or mRNA, e.g., a modified mRNA. In particular embodiments, the polynucleotides are modified mRNAs further comprising a 5' cap sequence and/or a 3' tailing sequence, e.g., a polyA tail. In other

embodiments, the polynucleotides are expression cassettes comprising a promoter operatively linked to the coding sequences

In related aspects, the present invention provides a method for decreasing or inhibiting Wnt signaling in a tissue or cell, comprising contacting the tissue or cell with an effective amount of a vector comprising a nucleic acid sequence encoding an anti-LRP5/6 antibody or antigen-binding fragment thereof, wherein the anti-LRP5/6 antibody or antigen-binding fragment thereof is a Wnt signaling pathway antagonist or inhibitor. In certain embodiments, the vector is an expression vector, and may comprise a promoter operatively linked to the nucleic acid sequence. In particular embodiments, the vector is a viral vector.

In related aspects, the present invention provides a method for decreasing or inhibiting Wnt signaling in a tissue, comprising contacting the tissue with an effective amount of a cell comprising a nucleic acid sequence encoding an anti-LRP5/6 antibody or antigen-binding fragment thereof, wherein the anti-LRP5/6 antibody or antigen-binding fragment thereof is a Wnt signaling pathway antagonist or inhibitor. In particular embodiments, the cell is a heterologous cell or an autologous cell obtained from the subject to be treated. In certain embodiments, the cell was transduced with a vector comprising an expression cassette encoding the anti-LRP5/6 antibody or antigen-binding fragment thereof, wherein the anti-LRP5/6 antibody or antigen-binding fragment thereof is a Wnt signaling pathway antagonist or inhibitor. In particular embodiments, the cell is a stem cell, e.g., an adipose-derived stem cell or a hematopoietic stem cell.

Anti-LRP5/6 antibodies and antigen-binding fragments thereof, wherein the anti-LRP5/6 antibody or antigen-binding fragment thereof is a Wnt signaling pathway antagonist or inhibitor, may be used in to treat a disease, disorder or condition, for example, by decreasing or inhibiting Wnt signaling in a cell, tissue or organ. Thus, in some aspects, the present invention provides a method for treating a disease or condition in a subject in need thereof, e.g., a disease or disorder associated with increased or deregulated Wnt signaling, or

for which decreased Wnt signaling would provide a therapeutic benefit, comprising contacting the subject with an effective amount of a composition comprising an anti-LRP5/6 antibody or antigen-binding fragment thereof, wherein the anti-LRP5/6 antibody or antigen-binding fragment thereof is a Wnt signaling pathway antagonist or inhibitor. In particular embodiments, the composition is a pharmaceutical composition comprising any of: an anti-LRP5/6 antibody or antigen-binding fragment thereof; a polynucleotide comprising a nucleic acid sequence encoding an anti-LRP5/6 antibody or antigen-binding fragment thereof, e.g., a DNA or mRNA, optionally a modified mRNA; a vector comprising a nucleic acid sequence encoding an anti-LRP5/6 antibody or antigen-binding fragment thereof, e.g., an expression vector or viral vector; or a cell comprising a nucleic acid sequence encoding an anti-LRP5/6 antibody or antigen-binding fragment thereof, e.g., a cell transduced with an expression vector or viral vector encoding an anti-LRP5/6 antibody or antigen-binding fragment thereof. In particular embodiments, the disease or condition is a pathological disease or disorder, or an injury. In some embodiments, contacting occurs in vivo, i.e., the subject composition is administered to a subject.

In related aspects, the present invention provides a method for treating a disease or condition, e.g., a disease or disorder associated with increased Wnt signaling, or for which reduced Wnt signaling would provide a therapeutic benefit, comprising contacting a subject in need thereof with a pharmaceutical composition comprising an effective amount of a polynucleotide comprising a nucleic acid sequence encoding an anti-LRP5/6 antibody or antigen-binding fragment thereof, wherein the antibody or antigen-binding fragment thereof is a Wnt signaling pathway antagonist or inhibitor, disclosed herein. In certain embodiments, the polynucleotides are DNA or mRNA, e.g., a modified mRNA. In particular embodiments, the polynucleotides are modified mRNAs further comprising a 5' cap sequence and/or a 3' tailing sequence, e.g., a polyA tail. In other embodiments, the polynucleotides are expression cassettes comprising a promoter operatively linked to the coding sequences

In related aspects, the present invention provides a method for treating a disease or condition, e.g., a disease or disorder associated with increased Wnt signaling, or for which decreased Wnt signaling would provide a therapeutic benefit, comprising contacting a subject in need thereof with a pharmaceutical composition comprising an effective amount of a vector comprising a nucleic acid sequence encoding an anti-LRP5/6 antibody or antigen-binding fragment thereof, wherein the antibody or antigen-binding fragment thereof is a Wnt signaling pathway antagonist or inhibitor. In certain embodiments, the vector is an expression vector, and may comprise a promoter operatively linked to the nucleic acid sequence. In particular embodiments, the vector is a viral vector.

In related aspects, the present invention provides a method for treating a disease or condition, e.g., a disease or disorder associated with increased Wnt signaling, or for which decreased Wnt signaling would provide a therapeutic benefit, comprising contacting a subject in need thereof with a pharmaceutical composition comprising an effective amount of a cell comprising a nucleic acid sequence encoding an anti-LRP5/6 antibody or antigen-binding fragment thereof, wherein the antibody or antigen-binding fragment thereof is a Wnt signaling pathway antagonist or inhibitor. In particular embodiments, the cell is a heterologous cell or an autologous cell obtained from the subject to be treated. In certain embodiments, the cell was transduced with a vector comprising an expression cassette encoding the anti-LRP5/6 antibody or antigen-binding fragment thereof. In particular embodiments, the cell is a stem cell, e.g., an adipose-derived stem cell or a hematopoietic stem cell.

In certain embodiments, methods of treating or preventing diseases or disorders in a subject in need thereof, by providing to the subject an effective amount of an anti-LRP5/6 antibody, or an antigen-binding fragment thereof, wherein the antibody or the antigen-binding fragment thereof is an inhibitor of a Wnt signaling pathway, may be used to treat a cancer or tumor, e.g., a solid or liquid tumor. Examples of cancers and tumors that may be treated include, but are not limited to: colon tumors (e.g. colon cancer or

adenoma), stomach tumors (e.g., stomach cancer), small intestine tumors (e.g., small intestinal cancer), liver tumors (e.g., liver cancer), pancreas tumors (e.g., pancreatic cancer), lung tumors (e.g., lung cancer), ovary tumors (e.g., ovarian cancer), kidney (e.g., kidney cancer), brain tumors (e.g., brain cancer), spinal cord tumors (e.g., spinal cord cancer), skin tumors (e.g., skin cancer or melanoma), head and neck tumors (e.g., head and neck cancer), gastointestinal tract tumors (e.g., gastrointestinal cancer, esophageal cancer, oral mucosa cancer, tongue cancer, stomach cancer, intestinal cancer, colon cancer), breast tumors (e.g., breast cancer), prostate tumors (e.g., prostate cancer), bone tumors (e.g., bone cancer), vascular tumors, Wilms tumor, leukemina/lymphoma, soft tissue tumors (e.g., soft tissue sarcoma or synovial sarcoma) and metastatic cancers, etc.

In certain embodiments, methods of treating or preventing diseases or disorders in a subject in need thereof, by providing to the subject an effective amount of an anti-LRP5/6 antibody, or an antigen-binding fragment thereof, wherein the antibody or the antigen-binding fragment thereof is an inhibitor of a Wnt signaling pathway, may be used to treat degenerative diseases. Examples of degenerative diseases that may be treated include, but are not limited to osteoarthritis, cartilage degeneration, sports injuries (e.g., cartilage injury), retinopathy, atherosclerosis, neurodegenerative disorders, and vascular disorders e.g. vasculitis, conditions with abnormal angiogenesis.

In certain embodiments, methods of treating or preventing diseases or disorders in a subject in need thereof, by providing to the subject an effective amount of an anti-LRP5/6 antibody, or an antigen-binding fragment thereof, wherein the antibody or the antigen-binding fragment thereof is an inhibitor of a Wnt signaling pathway, may be used to treat fibrosis. Examples of fibrosis that may be treated include, but are not limited to, lung fibrosis (including but not limited to COPD. idiopathic pulmonary fibrosis), kidney fibrosis (e.g. end stage renal failure), liver fibrosis, congenital liver storage diseases, and cardiac fibrosis.

In certain embodiments, methods of treating or preventing diseases or disorders in a subject in need thereof, by providing to the subject an effective amount of an anti-LRP5/6 antibody, or an antigen-binding fragment thereof, wherein the antibody or the antigen-binding fragment thereof is an inhibitor of a Wnt signaling pathway, may be used to treat heart failure, e.g., congestive heart failure, systolic heart failure, heart failure with preserved ejection fraction, or coronary artery disease.

In certain embodiments, methods of treating or preventing diseases or disorders in a subject in need thereof, by providing to the subject an effective amount of an anti-LRP5/6 antibody, or an antigen-binding fragment thereof, wherein the antibody or the antigen-binding fragment thereof is an inhibitor of a Wnt signaling pathway, may be used to treat heterotopic ossification, osteopetrosis, or congenital high bone mass disorders.

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

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

The dose and dosage regimen may depend upon a variety of factors readily determined by a physician, such as the nature of the disease or disorder, the characteristics of the subject, and the subject's history. In

particular embodiments, the amount of anti-LRP5/6 antibody or antigen-binding fragment thereof, e.g., a Wnt surrogate, administered or provided to the subject is in the range of about 0.01 mg/kg to about 50 mg/kg, 0.1 mg/kg to about 500 mg/kg, or about 0.1 mg/kg to about 50 mg/kg of the subject's body weight.

The terms "treatment", "treating" and the like are used herein to generally mean obtaining a desired pharmacologic and/or physiologic effect. The effect may be prophylactic in terms of completely or partially preventing a disease or symptom thereof, e.g. reducing the likelihood that the disease or symptom thereof occurs in the subject, and/or may be therapeutic in terms of a partial or complete cure for a disease and/or adverse effect attributable to the disease. "Treatment" as used herein covers any treatment of a disease in a mammal, and includes: (a) preventing the disease from occurring in a subject which may be predisposed to the disease but has not yet been diagnosed as having it; (b) inhibiting the disease, i.e., arresting its development; or (c) relieving the disease, i.e., causing regression of the disease. The therapeutic agent (e.g., anti-LRP5/6 antibody or antigen-binding fragment thereof) may be administered before, during or after the onset of disease or injury. The treatment of ongoing disease, where the treatment stabilizes or reduces the undesirable clinical symptoms of the patient, is of particular interest. Such treatment is desirably performed prior to complete loss of function in the affected tissues. The subject therapy will desirably be administered during the symptomatic stage of the disease, and in some cases after the symptomatic stage of the disease. In some embodiments, the subject method results in a therapeutic benefit, e.g., preventing the development of a disorder, halting the progression of a disorder, reversing the progression of a disorder, etc. In some embodiments, the subject method comprises the step of detecting that a therapeutic benefit has been achieved. The ordinarily skilled artisan will appreciate that such measures of therapeutic efficacy will be applicable to the particular disease being modified, and will recognize the appropriate detection methods to use to measure therapeutic efficacy.

Promoting Cell, Tissue and Organoid Growth and Related Methods

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

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

Wnt signaling is a key component of stem cell culture. For example, the stem cell culture media as described in WO2010/090513, WO2012/014076, Sato et al., 2011 (GASTROENTEROLOGY 201 1; 141: 1762-1772) and Sato et al., 2009 (Nature 459, 262-5). The Wnt surrogate molecules disclosed herein are suitable alternatives to Rspondin for use in these stem cell culture media, or may be combined with Rspondin.

Accordingly, in one embodiment, the disclosure provides a method for enhancing the proliferation of stem cells comprising contacting stem cells with one or more Wnt surrogate molecules disclosed herein. In one embodiment, the disclosure provides a cell culture medium comprising one or more Wnt surrogate molecules disclosed herein. In some embodiments, the cell culture medium may be any cell culture medium already known in the art that normally comprises Wnt or Rspondin, but wherein the Wnt or Rspondin is

replaced (wholly or partially) or supplemented by Wnt surrogate molecule(s) disclosed herein. For example, the culture medium may be as described in as described in WO2010/090513, WO2012/014076, Sato et al., 2011 (GASTROENTEROLOGY 201 1; 141: 1762-1772) and Sato et al., 2009 (Nature 459, 262-5), which are hereby incorporated by reference in their entirety.

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

These methods of enhancing proliferation of stem cells can be used to grow new organoids and tissues from stem cells, as for example described in WO2010/090513 WO2012/014076, Sato et al., 201 1

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

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

Diagnostic and Related Methods

Other embodiments of the present invention relate, in part, to diagnostic applications for detecting the presence of cells or tissues expressing LRP5 and/or LRP6. Thus, the present disclosure provides methods of detecting LRP5 and/or LRP6 in a sample, such as detection of cells or tissues expressing LRP5 or LRP6. Such methods can be applied in a variety of known detection formats, including, but not limited to immunohistochemistry (IHC), immunocytochemistry (ICC), *in situ* hybridization (ISH), whole-mount *in situ* hybridization (WISH), fluorescent DNA *in situ* hybridization (FISH), flow cytometry, enzyme immuno-assay (EIA), and enzyme linked immuno-assay (ELISA). In particular embodiments, a method comprises contacting a tissue or cell, e.g., obtained from a subject, with an antibody or antigen-binding fragment thereof disclosed herein, and then determining an amount of binding of the antibody or antigen-binding fragment thereof to the tissue or cell, thus determining the presence of or an amount of the LRP5 and/or LRP6 receptor(s) in the tissue or cell.

ISH is a type of hybridization that uses a labeled complementary DNA or RNA strand (*i.e.*, primary binding agent) to localize a specific DNA or RNA sequence in a portion or section of a cell or tissue (*in situ*), or if the tissue is small enough, the entire tissue (whole mount ISH). One having ordinary skill in the art would appreciate that this is distinct from immunohistochemistry, which localizes proteins in tissue sections using an antibody as a primary

binding agent. DNA ISH can be used on genomic DNA to determine the structure of chromosomes. Fluorescent DNA ISH (FISH) can, for example, be used in medical diagnostics to assess chromosomal integrity. RNA ISH (hybridization histochemistry) is used to measure and localize mRNAs and other transcripts within tissue sections or whole mounts.

In various embodiments, the antibodies and antigen-binding fragments thereof described herein are conjugated to a detectable label that may be detected directly or indirectly. In this regard, an antibody "conjugate" refers to an anti-LRP5/6 antibody or antigen-binding fragment thereof that is covalently linked to a detectable label. In the present invention, DNA probes, RNA probes, monoclonal antibodies, antigen-binding fragments thereof, and antibody derivatives thereof, such as a single-chain-variable-fragment antibody or an epitope tagged antibody, may all be covalently linked to a detectable label. In “direct detection”, only one detectable antibody is used, *i.e.*, a primary detectable antibody. Thus, direct detection means that the antibody that is conjugated to a detectable label may be detected, per se, without the need for the addition of a second antibody (secondary antibody).

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

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

secondary antibody or binding agent to the primary detectable antibody. Examples of primary detectable binding agents or antibodies requiring addition of a secondary binding agent or antibody include enzymatic detectable binding agents and hapten detectable binding agents or antibodies.

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

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

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

Examples of polymer particle labels include micro particles or latex particles of polystyrene, PMMA or silica, which can be embedded with fluorescent dyes, or polymer micelles or capsules which contain dyes, enzymes or substrates.

Examples of metal particle labels include gold particles and coated gold particles, which can be converted by silver stains. Examples of haptens include DNP, fluorescein isothiocyanate (FITC), biotin, and digoxigenin. Examples of enzymatic labels include horseradish peroxidase

(HRP), alkaline phosphatase (ALP or AP), β-galactosidase (GAL), glucose-6-phosphate dehydrogenase, β-N-acetylglucosamimidase, β-glucuronidase, invertase, Xanthine Oxidase, firefly luciferase and glucose oxidase (GO). Examples of commonly used substrates for horseradishperoxidase include 3,3'-diaminobenzidine (DAB), diaminobenzidine with nickel enhancement, 3-amino-9-ethylcarbazole (AEC), Benzidine dihydrochloride (BDHC), Hanker-Yates reagent (HYR), Indophane blue (IB), tetramethylbenzidine (TMB), 4-chloro-1-naphtol (CN), .alpha.-naphtol pyronin (.alpha.-NP), o-dianisidine (OD), 5-bromo-4-chloro-3-indolylphosp- hate (BCIP), Nitro blue tetrazolium (NBT), 2-(p-iodophenyl)-3-p-nitropheny- l-5-phenyl tetrazolium chloride (INT), tetranitro blue tetrazolium (TNBT), 5-bromo-4-chloro-3-indoxyl-beta-D-galactoside/ferro-ferricyanide (BCIG/FF).

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

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

Detectable labels may be linked to the antibodies described herein or to any other molecule that specifically binds to a biological marker of interest, *e.g.*, an antibody, a nucleic acid probe, or a polymer. Furthermore, one of ordinary skill in the art would appreciate that detectable labels can also be conjugated to second, and/or third, and/or fourth, and/or fifth binding agents or antibodies, etc. Moreover, the skilled artisan would appreciate that each additional binding agent or antibody used to characterize a biological marker of

interest may serve as a signal amplification step. The biological marker may be detected visually using, *e.g.*, light microscopy, fluorescent microscopy, electron microscopy where the detectable substance is for example a dye, a colloidal gold particle, a luminescent reagent. Visually detectable substances bound to a biological marker may also be detected using a spectrophotometer. Where the detectable substance is a radioactive isotope detection can be visually by autoradiography, or non-visually using a scintillation counter. See, *e.g.*, Larsson, 1988, Immunocytochemistry: Theory and Practice, (CRC Press, Boca Raton, Fla.); Methods in Molecular Biology, vol. 80 1998, John D. Pound (ed.) (Humana Press, Totowa, N.J.).

The invention further provides kits for detecting one or more LRP receptor or cells or tissues expressing one or more LRP receptors in a sample, wherein the kits contain at least one antibody, polypeptide, polynucleotide, vector or host cell as described herein. In certain embodiments, a kit may comprise buffers, enzymes, labels, substrates, beads or other surfaces to which the antibodies of the invention are attached, and the like, and instructions for use.

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

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

EXAMPLES

EXAMPLE 1

CHARACTERIZATION OF ANTI-LRP5/6 ANTIBODIES

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

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

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

Protein concentrations were determined using a NanoDrop Spectrophotometer (Thermo Scientific) by the direct UV A280 method. The relationship of absorbance to protein concentration is linear based on Beer-Lamber equation, $A = \varepsilon l c$; A is the absorbance value, ε is the wavelength-dependent extinction coefficient, l is the path length in centimeters, and c is the protein concentration. The experimental extinction coefficients of all produced proteins were estimated by their amino acid sequences.

Binding kinetics of antibody fragments to LRP5 extracellular domain (LRP5 ECD) and/or LRP6 extracellular domain (LRP6 ECD) protein targets was determined by bio-layer interferometry (BLI) using Octet Red 96 (PALL ForteBio, Fremont, CA) instruments at 30° C, 1000 rpm with streptavidin (SA) biosensors. C-terminal biotinylated LRP ECD recombinant protein was diluted to 20 nM in the running buffer (PBS, 0.05% Tween-20, 0.5% BSA, pH 7.2) and captured to the SA biosensor until coupling length reached 0.2 nm. Following capture of the LRP5 or LRP6, the SA biosensor with captured biotinylated-LRP5 or biotinylated LRP6 was dipped into wells containing the relevant antibody fragment at 7 different concentrations (0, 1.37, 4.12, 12.4, 37, 111.1, 333.3, 1000 nM) in running buffer, plus a well with only running buffer as a reference channel. K_D was determined by global fitting, 1:1 binding model according to manufacturer recommended settings.

Binding measurements were also performed by surface plasmon resonance on a BIAcore T100 (GE Healthcare, Pittsburgh, PA) and all proteins purified on SEC prior to experiments. Biotinylated Lrp6 E3E4 was coupled at a low density to streptavidin on a SA sensor chip (GE Healthcare, Pittsburgh, PA). An unrelated biotinylated protein was captured at equivalent coupling density to the control flow cells. Increasing concentrations of scFv-Nab fusion molecules (e.g., 18R5 scFv- LRP6 binding Nabs) were flown over the chip in 1xHBS-P (GE Healthcare, Pittsburgh, PA) containing 0.5 % BSA at 40 µl/ml. The chip surface was regenerated after each injection with 2 M MgCl2 in HBS-P for 60 seconds. Curves were reference-subtracted and all data were

analyzed using the Biacore T100 evaluation software version 2.0 with a 1:1 Langmuir binding model to determine the KD values.

Table 1A provides the heavy chain CDRs (CDRH1, CDRH2, and CDRH3) and light chain CDRs (CDRL1, CDRL2, and CDRL3) for the indicated antibody clones, and indicates the initial LRP5 or LRP6 that the antibody fragment was shown to bind. The Abgenesis software from Distributed Bio was used to map the specificity determining regions (SDRs) shown below, which include the Kabat definition of CDRs (Padlan et al. *FASEB J*. 9, 133-139 (1995).

Where light chain CDRs are not provided, the antibody fragment did not comprise a light chain.

Table 1A: Clone IDs and CDR sequences

Clone ID	Confirmed Binding	CDRH1	CDRH1 SEQ ID	CDRH2	CDRH2 SEQ ID	CDRH3	CDRH3 SEQ ID	CDRL1	CDRL1 SEQ ID	CDRL2	CDRL2 SEQ ID	CDRL3	CDRL3 SEQ ID
001S-C08	LRP6e1e2	YTISNYYIH	171	GMINPSGGSTTYA	249	CAIVRGKKWYFDLW	330	RASQYISNYLN	515	AASSLQS	529	CQQSYITPLTF	578
001S-C10	LRP6e1e2	RTFGTYPNG	121	AAISWGGRTAYA	188	CYARTVIGGFGAFRAHW	483						
001S-D10	LRP6e1e2	RTFSRYAMA	131	AAIRWSGGGTYYA	177	CAASMEAMNSLRVNKERYYQSW	324						
001S-E10	LRP6e1e2	LTFSNAAMA	102	AAISRSGANTAYS	184	CTLVNEIKTWW	469						
001S-F10	LRP6e1e2	RTFSSYAMA	134	AAIKWSGTNTYYA	173	CAASMEAMNSLRVNKERYYQSW	324						
001S-G10	LRP6e1e2	RTFSRYVMG	133	AAITWRGGSTYYA	194	CATGPNSIY	433						
001S-A11	LRP6e1e2	RTFGNYDMG	119	AGIRWSGSTLYA	197	CYARTVIGGFGAFRAHW	483						
001S-B11	LRP6e1e2	RRFTTYGMG	112	AAVTWRSGSTYYA	196	CAAGSTVVAEFNYW	320						
001S-C11	LRP6e1e2	SISSFNTMG	148	AVITTGGDTSYS	228	CNKVNAITKL	459						
001S-E11	LRP6e1e2	RTLSRYSMG	140	AAISRSGDRIYYS	185	CTLVNEIKTWW	469						
001S-F11	LRP6e1e2	RTFSSYAMS	135	AVIGRSGGIKYYA	224	CATRRPFNSYNTEQSYDSW	434						
001S-G11	LRP6e1e2	SIFRLGTMY	144	ASIGKSGSTNYA	207	CKQHPNGYR	446						
001S-H11	LRP6e1e2	RTLSSFAMG	141	ATISRSGGNTYYA	220	CNLREWNNSGAGYW	460						
001S-A12	LRP6e1e2	IAFRYYDMG	96	AAITWNGRSSDYA	192	CAAVFTGRFYGRPPREKYDYW	326						
001S-B12	LRP6e1e2	RLLSYYALA	111	AAISRNGDKSHYS	182	CTLVNEIKTWW	469						

Clone ID	Confirmed Binding	CDRH1	CDRH1 SEQ ID	CDRH2	CDRH2 SEQ ID	CDRH3	CDRH3 SEQ ID	CDRL1	CDRL1 SEQ ID	CDRL2	CDRL2 SEQ ID	CDRL3	CDRL3 SEQ ID
001S-C12	LRP6e1e2	RTFSNYAVG	130	AAISRFGGSTYYV	181	CAADRIENYLGRYDPSEYEYW	317						
001S-D12	LRP6e1e2	RTFSRYAMG	132	GAISRSGNNTYYA	231	CTLVNEIKTWW	469						
001S-F12	LRP6e1e2	RTFRSYTMG	126	AAISGSGGSTTYA	178	CNADIKTTTYSPLRNYW	449						
008S-B01	LRP5	TIFSINTMG	153	ATMTSGGNTNYA	222	CYRRQWASSWGARNYEYW	484						
008S-C01	LRP5	NINSIETLG	106	ANMRGGGYMKYA	204	CHGRDYGSNAPQYW	442						
008S-D01	LRP5	NINSIETLG	106	ANMRGGGYMKYA	204	CYVKLRDDDYVYR	485						
008S-E01	LRP5	NINSIETLG	106	ANMRGGGYMKYA	204	CNAVTYNGYTIR	457						
008S-G01	LRP5	NINSIETLG	106	ANMRGGGYMKYA	204	CYARTQRMGVVNSYW	482						
008S-A02	LRP5	NINSIETLG	106	ANMRGGGYMKYA	204	CNAVTFGGNTIR	455						
008S-C02	LRP5	NINSIETLG	106	ANMRGGGYMKYA	204	CNAVTYDGY	456						
008S-D02	LRP5	NINSIETLG	106	ANMRGGGYMKYA	204	CAAQFRNDYGLRYQSTNNYW	322						
008S-E02	LRP5	NINSIETLG	106	ANMRGGGYMKYA	204	CNANYRGNRYW	453						
009S-C01	LRP6e3e4	GSFSGYYWT	82	GEINHSGATNYN	232	CVRYAWPEFDHW	478	RASQRVSNYLN	505	AASSLQG	528	CQQSYSVPYTF	596
009S-B02	LRP6e3e4	GSLSGYYWS	83	GEINHSGSTNYN	233	CVRYAWPEFDHW	478	RASQSISNYLN	506	AASSLQS	529	CQQSYSLPLTF	588
009S-C02	LRP6e3e4	GSFSDYYWS	81	GEINHSGSTNYN	233	CVRYAWPEFDHW	478	RASQSISNYLN	506	AASSLQS	529	CQQSYSMPLTF	589

Clone ID	Confirmed Binding	CDRH1	CDRH1 SEQ ID	CDRH2	CDRH2 SEQ ID	CDRH3	CDRH3 SEQ ID	CDRL1	CDRL1 SEQ ID	CDRL2	CDRL2 SEQ ID	CDRL3	CDRL3 SEQ ID
009S-D02	LRP6e3e4	GTFSSYAIS	90	GGIIPIFGTANYA	236	CVYGRDFDYW	479	SGSSSNVGNNYVS	523	DNDKRPS	541	CESWDSSLSSEVF	557
010S-A02	LRP6e1e2	HTFSSYAMG	95	AAISQSGYVRYYA	179	CKIYGLNGQPLGSW	444						
010S-B02	LRP6e1e2	RTFNSGTMG	123	AAITWRGGITYYA	193	CNADGYSWDGRSGRRLELW	448						
010S-D02	LRP6e1e2	RTFSSYAVG	136	AAISYSGGSTKYA	190	CAASVYISRRDSDYGYW	325						
010S-E02	LRP6e1e2	LSSGRPFSSYVMG	100	AAISWSGGSTKYA	189	CKLQVRPIGYSSAYSRNYW	445						
010S-F02	LRP6e1e2	RSFNSYVIG	114	AAIRWSGDNTYYA	176	CAASMEAMNSLRVNKERYYQSW	324						
009S-E02	LRP6e1e2	RRFTTYGMG	112	AAVTWRSGSTYYA	196	CAAGSTVVAEFNYW	320						
009S-F02	LRP6e1e2	RTFSYYAMG	138	AAISRSGGIYYA	186	CNTVRPLWAW	462						
009S-G02	LRP6e1e2	SIFSIYAMG	147	AVITSGGKTVYA	227	CYADSRSSWYDEYLEHW	480						
009S-H02	LRP6e1e2	SIVRSLPMA	149	ATINDAQRYYA	215	CNTSPYMHDVW	461						
009S-A03	LRP6e1e2	RTFSVYGVG	137	AAVSASGGYTWYA	195	CKAAPRWGGATAYW	443						
010S-G02	LRP6e1e2	SIVRSLPMA	149	ATINDAQRYYA	215	CNTSPYMHDVW	461						
010S-A03	LRP6e1e2	RTFRRYAMG	125	ATISASGGNTAYA	219	CNAPAWLYDDDYW	454						
009S-B03	LRP6e1e2	RTFSNYAVG	130	AAISRFGGSTYYV	181	CAADRIENYLGRYYDPSEYEYW	317						
010S-B03	LRP6e1e2	RTFSNYAVG	130	AAISRFGGSTYYA	180	CHAKQLRNGQMYTYW	440						

Clone ID	Confirmed Binding	CDRH1	CDRH1 SEQ ID	CDRH2	CDRH2 SEQ ID	CDRH3	CDRH3 SEQ ID	CDRL1	CDRL1 SEQ ID	CDRL2	CDRL2 SEQ ID	CDRL3	CDRL3 SEQ ID
010S-D03	LRP6e1e2	ISSVYGMG	97	AAIQWSADNTFYA	175	CAARTSGGLFHYRRSDHWDTW	323						
009S-C03	LRP6e1e2	LPFSRYAMA	98	AGMSGEGRNTKYR	201	CSSRGYW	466						
009S-D03	LRP6e1e2	SIFSDGAMG	145	AVISGGRTGYA	225	CNTYPFPIYKKGYPFW	463						
009S-E03	LRP6e1e2	RRFTTYGMG	112	AAVTWRSGSTYYA	196	CAAGSTVVAEFNYW	320						
009S-F03	LRP6e1e2	RTFSSYAMS	135	AVIGRSGGIKYYA	224	CATRRPFNSYNTEQSYDSW	434						
010S-E03	LRP6e1e2	RSVSIYPMG	117	AAINWSGDSTKYA	174	CNAVVVGLSRRIDNIW	458						
010S-F03	LRP6e1e2	RTFSRYVMG	133	AAITWRGGSTYYA	194	CATGPNSIY	433						
009S-G03	LRP6e1e2	RSVSSYNMG	118	AAISRRGGIIEYG	183	CHAVENILGRFVDYW	441						
009S-H03	LRP6e1e2	SIFSINTMG	146	AVITSGGKTVYA	227	CYADSRSSWYDEYLEHW	480						
009S-A04	LRP6e1e2	RTLSAYDMG	139	GGIRWSGGTTLYP	240	CYARTVIGGFGAFRAHW	483						
009S-B04	LRP6e3e4	SIFMINTMA	143	ATIRPVVSETTYA	216	CNAKRPWGTRDEYW	452						
010S-G03	LRP6e3e4	RSFNSYTTT	113	AAIRGSSGSTFYA	175	CNAASTVTAWPYYGPDYW	447						
009S-C04	LRP6e3e4	FRFSISTMG	40	AYITGGGRTMDG	230	CNAFVRSDFDRYYDYW	451						
009S-D04	LRP6e3e4	TIVSIYRIN	154	AGITSSGRTIYA	200	CNAASTVTAWPYYGPDYW	447						
010S-H03	LRP6e3e4	RIFSIYDMG	110	SGIRWSGGTSYA	283	CSSRGYW	466						

Clone ID	Confirmed Binding	CDRH1	CDRH1 SEQ ID	CDRH2	CDRH2 SEQ ID	CDRH3	CDRH3 SEQ ID	CDRL1	CDRL1 SEQ ID	CDRL2	CDRL2 SEQ ID	CDRL3	CDRL3 SEQ ID
009S-E04	LRP6e3e4	RIFAIYDIA	107	AMIRPVVTEI DYA	203	CNAKRPWGSRD EYW	452						
010S-A04	LRP6e3e4	SLFSFNAV G	151	ASISSGGRTN YA	210	CSKGGVYGGTYV PDSW	465						
009S-F04	LRP6e3e4	RSLSSFAM G	116	ARISRGDGYT DEA	206	CAAVQAVIGGTL TTAYDYW	327						
010S-B04	LRP6e3e4	RVLSYYAM A	142	AGITRGGATT YYS	199	CAAGPNWSTRN REYDYW	319						
009S-G04	LRP6e3e4	GTFSRYHM G	88	SAITWSGGRT YYA	282	CALTWAPTPTNR RSDYAYW	349						
009S-H04	LRP6e3e4	RIFAIYDMA	108	ATIRPVVSETT YA	216	CNAKRPWGTRD EYW	452						
010S-C04	LRP6e3e4	SLFSFNAM G	150	ASISSGSRTNY A	211	CSKGGVYGGTYV PDSW	465						
010S-D04	LRP6e3e4	RIFAIYDIA	107	ATIRPVVTQID YA	218	CNAKRPWGSRD EYW	452						
010S-E04	LRP6e3e4	RTFGSDVM G	120	ALTGWGDGS TTYYE	202	CAAARRSGTYDIG QYLRESAYVFW	313						
010S-F04	LRP6e3e4	RTFSRYAM G	132	AAITRSGSNT YYA	191	CAADPRGVTLPR ATAYEYW	316						
009S-A05	LRP6e3e4	RTFSDYSM G	128	AGISWIADNR YYA	198	CTAGRSRYLYGSS LNGPYDYW	467						
010S-G04	LRP6e3e4	VIFALYDIA	155	ATIRPVVTETD YA	217	CNAKRPWGSRD EYW	452						
010S-H04	LRP6e3e4	RSFSDFFM G	115	ATISWSGSSA NYE	221	CAAAYSYSQYGSS YSYW	314						
010S-A05	LRP6e3e4	LSFSSYAM G	99	AAISRSGVSTY YA	187	CAAKFGVLATTES RHDYW	321						
010S-C05	LRP6e3e4	RTFNIDDM G	122	ASIRWSGQSP YYA	208	CNAETYSGNTIW	450						

Clone ID	Confirmed Binding	CDRH1	CDRH1 SEQ ID	CDRH2	CDRH2 SEQ ID	CDRH3	CDRH3 SEQ ID	CDRL1	CDRL1 SEQ ID	CDRL2	CDRL2 SEQ ID	CDRL3	CDRL3 SEQ ID
010S-D05	LRP6e3e4	RTFSDYSMA	127	AGISWIADNRYYA	198	CAGDRSRYLYGDSLRGPYGYW	329						
010S-E05	LRP6e3e4	SVFTTFAKG	152	ASITASSDRTFYA	213	CAAYSTFNTDVASMKPDYW	328						
010S-F05	LRP6e3e4	RIFSIYDIA	109	ATIRPVVTETDYA	217	CNAKRPWGSRDEYW	452						
013S-G04	LRP6e3e4	RIFAIYDIA	107	ATIRPVVSETTYA	216	CNAKRPWGTRDEYW	452						
013S-H04	LRP6e3e4	RTFSMYDMG	129	ASIRWSSGNTWYA	209	CYANIYYTRRAPEEYW	481						
013S-A05	LRP6e3e4	RTFNTYAMG	124	ASVSWRYDRTYYT	214	CAADTNWRAGPRVGIDEYAYW	318						
013S-B05	LRP6e3e4	FAFSTTAMS	36	STINPGGLSKSYA	298	CTKGGIQ	468						
013S-C05	LRP6e3e4	NIFPIDDMS	105	ATVTSGGRINYA	223	CNVDRTLYGKYKEYW	464						
013S-D05	LRP6e3e4	RIFSIYDMG	110	SGIRWSGGTSYA	283	CGSRGYW	439						
013S-E05	LRP6e3e4	YTFTYRYLH	170	GGIIPIFGTADYA	235	CARDWELYGMDVW	376	RASQSISSYLN	510	AASSLQS	529	CQQSYSTPLTF	592
013S-F05	LRP6e3e4	GTFSSYAIS	90	GIINPSGGSTSYA	248	CARAGYYDSSGYYAFDIW	358	RASQSISSYLN	510	AASSLQS	529	CQQSYSTPLTF	592
013S-G05	LRP6e3e4	YTFTYRYLH	170	GGVIPIFGTADYA	242	CASDIVVDDAFDTW	422	RASQDISNYLN	495	AASTLQS	532	CQQGNSFPYTF	570
010S-G06	LRP6e3e4	FSFETYGMS	42	SGISGSGGRTHYA	285	CARDLDYW	369	QASQDISNYLN	495	AASSLQS	529	CQQSYRIHWTF	581
009S-B05	LRP6e3e4	FTFDAYAMH	47	STLSGDANNAYYA	304	CARGGSGWSNYYGMDVW	395	RASQSISSYLN	510	AASSLQS	529	CQQSYSTPLTF	592
009S-C05	LRP6e3e4	YTFTYRYLH	170	GRIIPVLKITNYA	255	CAVVDDAFDIW	438						

Clone ID	Confirmed Binding	CDRH1	CDRH1 SEQ ID	CDRH2	CDRH2 SEQ ID	CDRH3	CDRH3 SEQ ID	CDRL1	CDRL1 SEQ ID	CDRL2	CDRL2 SEQ ID	CDRL3	CDRL3 SEQ ID
009S-D05	LRP6e3e4	FTLRNHWLS	78	SAISGSGGSTYYA	280	CATRTGYSYGFNFWAFDIW	435	RASQSISSYLN	510	AASSLQS	529	CQQSYSTPLTF	592
009S-E05	LRP6e3e4	YTFTNNFMH	165	GHVDPGDGETIYA	243	CARDWGIAAAGDYYYGMDVW	377	RASQGINSYLA	497	DAKGLHP	533	CQQSYSAPLSF	584
009S-F05	LRP6e3e4	FTFDDYGMS	48	SAIGTGGGTYYA	277	CARLGSYGSPYYYYGMDVW	415	RASQSISSYLN	510	AASSLQS	529	CQQSYSTPLTF	592
009S-G05	LRP6e3e4	FTFSDYYMS	55	SGVSWNGSRTHYA	292	CAKDSGLV	337	QASQDISNYLN	495	AASTLQR	531	CQQSYSAPLTF	585
009S-C06	LRP6e3e4	YTFASYDIH	160	GWMNPNSGNTGYA	272	CARATGSGWYTDLGYW	359	RASRNINRYLN	516	AASSLLS	527	CQQSYNVPFTF	580
009S-D06	LRP6e3e4	FTFSSHSTH	60	STISDTNSGTYYA	299	CAKAQATGWSGYYTFDYW	331	RASQSISSYLN	510	AASSLQS	529	CQQSYSTPLTF	592
009S-E06	LRP6e3e4	FTFTDYGLH	74	AVISYGGSNKYYA	226	CASGYSYGLYYYGMDVW	425	RASQSISSYLN	510	AASSLQS	529	CQQSYSTPLTF	592
009S-F06	LRP6e3e4	YTFTYRYLH	170	GGIIPIFGTANYA	236	CATEAALDAFDIW	432	RASQSISSYLN	510	AASSLQS	529	CQQSYSTPLTF	592
009S-G06	LRP6e3e4	YIFTDYYMH	158	GWINPNSGGTNYA	270	CARDFLGSTGDYW	365	RASQNIGLYLN	504	DASSLQR	540	CQQSYSTPYTF	594
009S-H06	LRP6e3e4	FTFSSSAMH	61	SAIGTGGSTYYA	277	CAKGGDYFYYYGMDVW	340	RASQSISSYLN	510	AASSLQS	529	CQQSYSTPLTF	592
009S-A07	LRP6e3e4	YTFTYRYLH	170	GGIIPIFGTANYA	236	CATAYGSSSLNIDYW	429	RASQSISSYLN	510	AASSLQS	529	CQQSYSTPLTF	592
009S-B07	LRP6e3e4	YTFTGYYMH	164	GWINPNSGGTNYA	270	CVKDGGSFPLAYAFDIW	476	RASQSISSYLN	510	AASSLQS	529	CQQSYSTPLTF	592
009S-D07	LRP6e3e4	FPFRYYGMS	38	ARIGWNGGSIVYA	205	CARDYSDRSGIDYW	378	RSSQSLLHSNGYNYLD	519	LGSNRAS	550	CMQATQFPLTF	564
009S-F07	LRP6e3e4	GTFSSYAIS	90	GIINPSGGSTSYA	248	CARAAGNFWSGYYTFDYW	353	RASQSISSYLN	510	AASSLQS	529	CQQSYSTPLTF	592
009S-G07	LRP6e3e4	YTFTYRYLH	170	GGIIPIFGTANYA	236	CARGSYGMDVW	407	RASQGISNYLA	499	DASNLET	534	CLQDFSFPWTF	558

Clone ID	Confirmed Binding	CDRH1	CDRH1 SEQ ID	CDRH2	CDRH2 SEQ ID	CDRH3	CDRH3 SEQ ID	CDRL1	CDRL1 SEQ ID	CDRL2	CDRL2 SEQ ID	CDRL3	CDRL3 SEQ ID
009S-H07	LRP6e3e4	YTFTGYYMH	164	GWMNPNSGNTGYA	272	CASSVVPAGPAGVYAFDIW	426	RASQSISSYLN	510	AASSLQS	529	CQQSYSTPLTF	592
009S-A08	LRP6e3e4	GTFSSHAIN	89	GWISANNGNTDYA	271	CARDQDYGDYGWYYYGMDVW	372	RASQGISNYLA	499	GSSTLQS	546	CQQTYSIPPTF	599
011S-C01	LRP6e3e4	LTFTSHGMS	103	SYVSDSGSSVYYA	311	CARHPGSFGGYSYAWYYYYGMDVW	413	RASQSISSYLN	510	AASSLQS	529	CQQSYSTPLTF	592
009S-C08	LRP6e3e4	FSFNTFGIH	43	AVISYDGSNKYYA	226	CAKSIAAAGTGYYGMDVW	346	RASQSISSYLN	510	AASSLQS	529	CQQSYSTPLTF	592
009S-D08	LRP6e3e4	YTFTSYDIN	168	GGIIPIFGTANYA	236	CARGPYYFDYW	401	RASQGISNNLN	498	DASSLES	539	CLQHNSYPFTF	561
011S-F01	LRP6e3e4	FSFSDYYMS	45	SGISESGGRTYYA	284	CASAADFDYW	420	RASQDISNYLN	495	AASSLQS	529	CLQDYSYPRTF	559
009S-E08	LRP6e3e4	YGFTGYYIH	157	GWMNPNSGNTGYA	272	CARGYGDYDLW	410	QASQDISNYLN	495	DASSLES	539	CQQSYRYPTF	583
009S-F08	LRP6e3e4	DTFANYGFS	34	GXVNAGNGNTTYA	273	CAKGWLDFDYW	345	QASQDISNYLN	495	DASSLES	539	CQQSYSTSITF	595
009S-G08	LRP6e3e4	FTFSDFAMT	53	SYISGDSGYTNYA	306	CARLGSYPGPYYYYMDVW	416	RASQSISSYLN	510	AASSLQS	529	CQQSYSTPLTF	592
009S-H08	LRP6e3e4	YTFTDYFMN	162	GIINPSGDSTRFA	245	CARDDGLGGMDVW	362	QASQDISNYLA	494	AASSLQS	529	CQQSYSTPLTF	592
009S-A09	LRP6e3e4	YTFTYRYLH	170	GRIIPILGSTNYA	254	CTTDLWDYW	475	QASQGITNYLN	495	AASSLQS	529	CLQDYTDPFTF	560
011S-F02	LRP6e3e4	FTFSTYGMH	71	SSISVSSGTTHYA	297	CARGGSGSYYYAFDIW	394	RASQSISSYLN	510	AASSLQS	529	CQQSYSTPLTF	592
011S-G02	LRP6e3e4	YTFTSYAMN	167	GGIIPIFGTANYA	236	CARDASGGSTGWYYFDSW	361	RASQGISSYLA	503	AASSLQS	529	CQQAYSFPWTF	568
011S-A03	LRP6e3e4	FTFSSYWMH	67	STISGSGGRTYYA	300	CATSPYGVFTLDYW	436	RASQSISSYLN	510	AASSLQS	529	CQQSYSTPLTF	592
011S-C03	LRP6e3e4	YTFSYRYLH	161	GGIIPIFGTANYA	236	CASTVTTDAFDIW	427	QASQDISNYLN	495	DASSLES	539	CQQSYSFPPFTF	586

Clone ID	Confirmed Binding	CDRH1	CDRH1 SEQ ID	CDRH2	CDRH2 SEQ ID	CDRH3	CDRH3 SEQ ID	CDRL1	CDRL1 SEQ ID	CDRL2	CDRL2 SEQ ID	CDRL3	CDRL3 SEQ ID
011S-D03	LRP6e3e4	FSFDDYGMS	41	SVISSGGTIYYA	305	CARHLSSGYLSYYGMDVW	412	RASQSISSYLA	509	AASTLQS	532	CQQSYSTPLTF	592
011S-F03	LRP6e3e4	FTFSSYAMS	64	SAISGSGGSTYYA	280	CAKGGRDGYKGYFDYW	342	KSSQSVLYTTTNRNHIA	492	WASSRKS	554	CQQYYSTPYTF	607
011S-C04	LRP6e1e2	GTFNSNAIS	85	GWMNPNSGNTGYA	272	CARDYYGSGSYNYGMDVW	379	GASQSVPRNSLA	486	GASQRAT	543	CQQYHNWPPEYTF	602
011S-D04	LRP6e1e2	YTFTSYDIN	168	GIINPSGGSTSYA	248	CAREAYYYYGMDVW	381	RASQSISSYLN	510	AASSLQS	529	CQQSYSTPLTF	592
011S-H04	LRP6e1e2	YIFTDYYMH	158	GRIIPILGRANYA	252	CARGGYSTLDYW	396	QASQDISNYLN	495	AASTLQS	532	CQQSFSTPRTF	574
008S-F02	LRP5	YTFTNYCMH	166	GIINPSDGSTSHA	244	CAKDMVHLIVALAIDYW	336	RSSQSLLHSDGYTYLY	518	TLSYRAS	553	CMQALEALFTF	562
010S-C06	LRP6e1e2	FTFNSYSMD	50	SSISPRGGSTYYA	295	CAPYYYDKSAKPLRSYFDHW	352	RASQSISSYLN	510	AASSLQS	529	CQQSYSTPLTF	592
010S-E06	LRP6e3e4	LTVSSNYMS	104	SGISWNSGSIGYA	289	CARGSDCSGGSCYYSFDYW	404	RASQSISSYLN	510	AASSLQS	529	CQQSYSTPLTF	592
010S-F06	LRP6e3e4	FTFSSWMH	62	SAIGTGGGTYYA	277	CAREVAVKDYYYYMDVW	386	RASQSISSYLN	510	AASSLQS	529	CQQSYSTPLTF	592
010S-H06	LRP6e3e4	YTFTSYDIN	168	GRIIPILGRTNYA	253	CAREERGATGRAFDIW	383	RASQSISSYLN	510	AASSLQS	529	CQQSYSTPLTF	592
010S-A07	LRP6e3e4	FTFSSYAMH	63	ASISSTSGSKYYA	212	CAKTYYDFWSGYYTFDYW	347	RASQSISSYLN	510	AASSLQS	529	CQQSYSTPLTF	592
010S-B07	LRP6e3e4	FTFSDYYMS	55	SMISYNGGRAFYA	293	CARGNPYYFDYW	399	RASQSISSYLN	510	AASSLQS	529	CQQSYSTPLTF	592
010S-C07	LRP6e3e4	FTFSKTDMH	56	STITTDSRGTYYA	303	CAKGGDYYYYYGMDVW	341	RASQSISSYLN	510	AASSLQS	529	CQQSYSTPLTF	592
010S-D07	LRP6e3e4	YTFTYRYLH	170	GGIIPIFGTANYA	236	CANGLEDAYAFDIW	350	RASQSISSYLN	510	AASSLQS	529	CQQSYSTPLTF	592
009S-D05	LRP6e3e4	FTLRNHWLS	78	SAISGSGGSTYYA	280	CATRTGYSYGFNFWAFDIW	435	RASQSISSYLN	510	AASSLQS	529	CQQSYSTPLTF	592

Clone ID	Confirmed Binding	CDRH1	CDRH1 SEQ ID	CDRH2	CDRH2 SEQ ID	CDRH3	CDRH3 SEQ ID	CDRL1	CDRL1 SEQ ID	CDRL2	CDRL2 SEQ ID	CDRL3	CDRL3 SEQ ID
009S-E05	LRP6e3e4	YTFTNNFMH	165	GHVDPGDGETIYA	243	CARDWGIAAAGDYYYGMDVW	377	RASQGINSYLA	497	DAKGLHP	533	CQQSYSAPLSF	584
010S-E07	LRP6e3e4	YTFTYRYLH	170	GGIIPIFGTANYA	236	CAKDDFSLYGMDVW	332	RASQSISSYLN	510	AASSLQS	529	CQQSYSTPLTF	592
009S-F05	LRP6e3e4	FTFDDYGMS	48	SAIGTGGGTYYA	277	CARLGSYGSPYYYYGMDVW	415	RASQSISSYLN	510	AASSLQS	529	CQQSYSTPLTF	592
010S-F07	LRP6e3e4	YTFTYRYLH	170	GGIIPIFGTANYA	236	CARLDYGETEGNGDW	414	RASQSVYSNLA	514	DTSNRAT	542	CQQYNNWPPITF	603
010S-G07	LRP6e3e4	FTFSSYAMH	63	STISGSGGSTYYA	301	CARAGYGRYYYGMDVW	356	RVSQGISSYLN	520	AASSLQS	529	CQQTYTIPFTF	600
009S-G05	LRP6e3e4	FTFSDYYMS	55	SGVSWNGSRTHYA	292	CAKDSGLV	337	QASQDISNYLN	495	AASTLQR	531	CQQSYSAPLTF	585
010S-H07	LRP6e3e4	YTFTYRYLH	170	GGIIPIFGTANYA	236	CARDDSMGAFDIW	363	QASQDISNYLN	495	GTSNLQS	547	CQQSYSTPYTF	594
010S-A08	LRP6e3e4	HTFLTYDIN	94	GRITPRLGIANYA	257	CASYFGVMDVW	428	RASQSISSYLN	510	AASSLQS	529	CQQSYSTPLTF	592
009S-A07	LRP6e3e4	YTFTYRYLH	170	GGIIPIFGTANYA	236	CATAYGSSSLNIDYW	429	RASQSISSYLN	510	AASSLQS	529	CQQSYSTPLTF	592
009S-B07	LRP6e3e4	YTFTGYYMH	164	GWINPNSGGTNYA	270	CVKDGGSFPLAYAFDIW	476	RASQSISSYLN	510	AASSLQS	529	CQQSYSTPLTF	592
009S-B06	LRP6e3e4	YTFTYRYLH	170	GGIIPIFGTANYA	236	CAPALTDAGSFDYW	351	RVSQSISSYLN	521	AASSLQS	529	CQQSYSTPLTF	592
010S-B08	LRP6e3e4	YTFTYRYLH	170	GGIIPVFGTADYA	238	CARDREQQILDYW	373	RASQGISNNLN	498	DASNLET	534	CQQSYTSRLTF	597
010S-C08	LRP6e3e4	FTFSTFGMH	69	STITSSGGSTYYA	302	CARAGIAAAPGSRNYYGMDVW	354	RASQSISSYLN	510	AASSLQS	529	CQQSYSTPLTF	592
009S-C06	LRP6e3e4	YTFASYDIH	160	GWMNPNSGNTGYA	272	CARATGSGWYTDLGYW	359	RASRNINRYLN	516	AASSLLS	527	CQQSYNVPFTF	580
009S-D06	LRP6e3e4	FTFSSHSTH	60	STISDTNSGTYYA	299	CAKAQATGWSGYYTFDYW	331	RASQSISSYLN	510	AASSLQS	529	CQQSYSTPLTF	592

Clone ID	Confirmed Binding	CDRH1	CDRH1 SEQ ID	CDRH2	CDRH2 SEQ ID	CDRH3	CDRH3 SEQ ID	CDRL1	CDRL1 SEQ ID	CDRL2	CDRL2 SEQ ID	CDRL3	CDRL3 SEQ ID
010S-D08	LRP6e3e4	FTFSSSWMH	62	SAIGTGGGTYYA	277	CAKEDYDSSGYYYYYFQHW	339	RASQSISSYLN	510	AASSLQS	529	CQQSYSTPLTF	592
009S-E06	LRP6e3e4	FTFTDYGLH	74	AVISYGGSNKYYA	226	CASGYSYGLYYYGMDVW	425	RASQSISSYLN	510	AASSLQS	529	CQQSYSTPLTF	592
010S-E08	LRP6e3e4	YSFTRTDMH	159	GYISAYTGHTSYA	274	CARDLGGTADYW	370	RASQSISSYLN	510	ZASSLQS	555	CQQSYSTPLTF	592
010S-F08	LRP6e3e4	LTFDDHAMH	101	SYISSSGRTIFYA	308	CVRGDSGWGILYYVMDVW	477	RASQSISSYLN	510	AASSLQS	529	CQQSYSTPLTF	592
009S-F06	LRP6e3e4	YTFTYRYLH	170	GGIIPIFGTANYA	236	CATEAALDAFDIW	432	RASQSISSYLN	510	AASSLQS	529	CQQSYSTPLTF	592
010S-G08	LRP6e3e4	YIFTDYYMH	158	GGFDPEDGETIYA	234	CARGGGPNEHDYYFDYW	392	RASQSVRSSDLA	512	GSSSRAT	545	CQQYGRSPRYSF	601
010S-H08	LRP6e3e4	FTFZNAWMS	77	SGISGSGGSTYYA	286	CARGRGKKNYYYGMDVW	402	RASQSISSYLN	510	AASSLQS	529	CQQSYSTPLTF	592
010S-A09	LRP6e3e4	FTFSTYYMS	73	SGISWNGGKTHYV	287	CARGGDFDYW	390	QASQDIANYLN	493	AASSLQS	529	CQQSYSTPYTF	594
010S-B09	LRP6e3e4	GTFSSYAIS	90	GWINPNSGDTNYA	260	CARGEQWLVWGFDPW	389	RASQSISRYLN	508	KASSLES	549	CQQSYDSPWTF	577
009S-G06	LRP6e3e4	YIFTDYYMH	158	GWINPNSGGTNYA	270	CARDFLGSTGDYW	365	RASQNIGLYLN	504	DASSLQR	540	CQQSYSTPYTF	594
010S-C09	LRP6e3e4	YTFTYRYLH	170	GGIIPIFGTANYA	236	CARDEVEGGMDVW	364	RASQSISSYLN	510	AASSLQS	529	CQQSYSTPLTF	592
009S-H06	LRP6e3e4	FTFSSSAMH	61	SAIGTGGSTYYA	277	CAKGGDYFYYYYGMDVW	340	RASQSISSYLN	510	AASSLQS	529	CQQSYSTPLTF	592
010S-D09	LRP6e3e4	GTFSSYTIS	91	GGIVPAYRRANYA	241	CAKGGYELDYW	344	QASQDISNYLN	495	AASSLQS	529	CQQIHSYPLTF	573
010S-E09	LRP6e3e4	GDLSIYTIN	80	GWINAGNGNTTYA	259	CARGGDSSGYYYYAFDIW	391	RASQSISSYLN	510	AASSLQS	529	CQQSYSTPLTF	592
009S-A07	LRP6e3e4	YTFTYRYLH	170	GGIIPIFGTANYA	236	CATAYGSSSLNIDYW	429	RASQSISSYLN	510	AASSLQS	529	CQQSYSTPLTF	592

Clone ID	Confirmed Binding	CDRH1	CDRH1 SEQ ID	CDRH2	CDRH2 SEQ ID	CDRH3	CDRH3 SEQ ID	CDRL1	CDRL1 SEQ ID	CDRL2	CDRL2 SEQ ID	CDRL3	CDRL3 SEQ ID
009S-B07	LRP6e3e4	YTFTGYYMH	164	GWINPNSGGTNYA	270	CVKDGGSFPLAYAFDIW	476	RASQSISSYLN	510	AASSLQS	529	CQQSYSTPLTF	592
009S-D08	LRP6e3e4	YTFTSYDIN	168	GGIIPIFGTANYA	236	CARGPYYFDYW	401	RASQGISNNLN	498	DASSLES	539	CLQHNSYPFTF	561
010S-F09	LRP6e3e4	FTFDEYAMH	49	STISGSGGSTYYA	301	CASAKNDFWSGYFAFDYW	421	RASQSISSYLN	510	AASSLQS	529	CQQSYSTPLTF	592
010S-G09	LRP6e3e4	GTFNTHTIT	86	GWMNPNSGNTGYA	272	CARGNLDFDYW	398	QASQDISNYLN	495	DASNLET	534	CQQSYSTPLTF	592
010S-H09	LRP6e3e4	FTFSDHYMS	54	SAISSGSDRTYYA	281	CARYSGYDFDYW	419	RASQGISNYLN	500	AASTLQS	532	CQQGYGTPPMF	571
010S-A10	LRP6e3e4	FSFSSYSMN	46	SYISSSSSTIYYA	309	CARGSGYYGPGYYGMDVW	406	RASQSISSYLN	510	AASSLQS	529	CQQSYSTPLTF	592
009S-D07	LRP6e3e4	FPFRYYGMS	38	ARIGWNGGSIVYA	205	CARDYSDRSGIDYW	378	RSSQSLLHSNGYNYLD	519	LGSNRAS	550	CMQATQFPLTF	564
010S-B10	LRP6e3e4	FAFKDYYMT	35	SAIGAGGGTYYA	275	CARESALYSSSWYYYYGMDVW	385	RASQSISSYLN	510	GTSSLHT	548	CQQANSFPFTF	566
010S-C10	LRP6e3e4	FTFSSYAMS	64	SAISGSGGSTYYA	280	CAKGGRDGYKGYFDYW	342	KSSQSILSSSSNRDSLA	491	WASSRKS	554	CQQYYNIPYSF	605
009S-E07	LRP6e3e4	YTFTGYYIH	163	ZHVDPEDGETIYA	312	CARGPAAIGILGWFDPW	400	RASQSISSYLN	510	AASSLQS	529	CQQSYSTPLTF	592
010S-D10	LRP6e3e4	YIFTDYYMH	158	GWMNPNSGNTGYA	272	CARTLSGYSSSWYVFDYW	418	RASQSISSYLN	510	AASSLQS	529	CQQSYSTPLTF	592
010S-E10	LRP6e3e4	FTFSSYSMN	66	SGISWNSGTTGYS	290	CARDHSSGWRHYFDYW	367	RASQSISSYLN	510	AASSLQS	529	CQQSYSTPLTF	592
010S-F10	LRP6e3e4	FTFSNSDMN	57	SYISGNSGYTNYA	307	CASGSYYSDFDYW	424	RASQSISNYLN	506	AASTLES	530	CQQANSFPPTF	566
010S-G10	LRP6e3e4	GTFSSYAIS	90	GRINPNGGGTIYA	256	CAREGGYYFDYW	384	RASQGISNYLA	499	AASSLQS	529	CQQSYSTPWTF	593
009S-F07	LRP6e3e4	GTFSSYAIS	90	GIINPSGGSTSYA	248	CARAAGNFWSGYYTFDYW	353	RASQSISSYLN	510	AASSLQS	529	CQQSYSTPLTF	592

Clone ID	Confirmed Binding	CDRH1	CDRH1 SEQ ID	CDRH2	CDRH2 SEQ ID	CDRH3	CDRH3 SEQ ID	CDRL1	CDRL1 SEQ ID	CDRL2	CDRL2 SEQ ID	CDRL3	CDRL3 SEQ ID
009S-G07	LRP6e3e4	YTFTYRYLH	170	GGIIPIFGTANYA	236	CARGSYGMDVW	407	RASQGISNYLA	499	DASNLET	534	CLQDFSFPWTF	558
010S-H10	LRP6e3e4	YTFTSYYMH	169	GWINPNSGGTNYA	270	CAREAAEIPVGAFDIW	380	KSSHSLLYSSDNKNYLA	490	WSSTRES	554	CQQYYSTPQTF	606
010S-A11	LRP6e3e4	FTFSNSDMN	57	SYISGNSGYTNYA	307	CASGSYYSDFDYW	424	RASQSIZNYLN	511	ZASTLES	556	CQQANSFPPTF	566
010S-B11	LRP6e3e4	FTFRNYAIH	51	SAIGTGGDTYYA	276	CARDGGIRDFDYW	366	QASQDISNYLN	495	AASTLQS	532	CQQSYSTPLTF	592
010S-C11	LRP6e3e4	YTFTYRYLH	170	GGIIPIFGTANYA	236	CAADDLGLELHYW	315	RASQSISSYLN	510	AASSLQS	529	CQQSYSTPLTF	592
009S-H07	LRP6e3e4	YTFTGYYMH	164	GWMNPNSGNTGYA	272	CASSVVPAGPAGVYAFDIW	426	RASQSISSYLN	510	AASSLQS	529	CQQSYSTPLTF	592
009S-A08	LRP6e3e4	GTFSSHAIN	89	GWISANNGNTDYA	271	CARDQDYGDYGWYYGMDVW	372	RASQGISNYLA	499	GSSTLQS	546	CQQTYSIPPTF	599
010S-D11	LRP6e3e4	YTFTYRYLH	170	GGIIPVFGTANYA	239	CATDEYSSSYAFDIW	430	RASQSVSSNLA	513	GASTRAT	544	CQQFDRSPLTF	569
010S-E11	LRP6e3e4	FTFSAHGMH	52	SGISESGGSTYYA	284	CARGRGYSYGYYAFDIW	403	RASQSISSYLN	510	AASSLQS	529	CQQSYSTPLTF	592
010S-F11	LRP6e3e4	YTFTYRYLH	170	GGIIPIFGTANYA	236	CARDSDWGVVDPW	374	RASQSISSYLN	510	AASSLQS	529	CQQSYSTPLTF	592
010S-G11	LRP6e3e4	YTFTYRYLH	170	GRIIPVLKITNYA	255	CAVVDDAFDIW	438	RASQSISSYLN	510	AASSLQS	529	CQQSYSTPLTF	592
010S-H11	LRP6e3e4	YTFTYRYLH	170	GGIIPIFGTANYA	236	CAKDGTDGRFDPW	333	RASQDISSYLA	496	SASTLQS	552	CQQSNSFPYTF	575
009S-B08	LRP6e3e4	FTFTSSAVQ	76	GWINAGNGNTTYA	259	CARRGGDVTVPAAYYAMDVW	417	RASQSISSYLN	510	ZASSLQS	555	CQQSYSTPLTF	592
010S-A12	LRP6e3e4	VTFSRYPIS	156	GGIIPIFGTANYA	236	CAKDSGNYGYYGMDVW	338	RASQSISSYLN	510	AASSLQS	529	CQQSYSTPLTF	592
010S-B12	LRP6e3e4	FTFSSYDMH	65	SGITSNGGATYYA	291	CARGTTGKGYYYGMDVW	408	RASQSISSYLN	510	AASSLQS	529	CQQSYSTPLTF	592

Clone ID	Confirmed Binding	CDRH1	CDRH1 SEQ ID	CDRH2	CDRH2 SEQ ID	CDRH3	CDRH3 SEQ ID	CDRL1	CDRL1 SEQ ID	CDRL2	CDRL2 SEQ ID	CDRL3	CDRL3 SEQ ID
010S-C12	LRP6e3e4	FTFSNYWIH	58	SAIGTGGGTYYA	277	CTTAGYKAARRSVYPRIFNFDYW	472	RASQSISSYLN	510	AASSLQS	529	CQQSYSTPLTF	592
010S-D12	LRP6e3e4	YTFTYRYLH	170	GRIIPIFGTANYA	250	CAREEGVGGMDVW	382	RPSQSIGSWLA	517	DASNLQS	535	CQQSSSTPYTF	576
010S-E12	LRP6e3e4	FTFSSYAMH	63	SAIGAGGGTYYA	275	CARGVSSGYYYYYGMDVW	409	RASQSISSYLN	510	AASSLQS	529	CQQSYSTPLTF	592
010S-F12	LRP6e3e4	FTVSSNYMS	79	SAIGTGGGTYYA	277	CARAGTNWGGWYFDLW	355	RASQGISRDLA	501	AASTLQS	532	CQQSYSPPFTF	590
010S-G12	LRP6e3e4	FALSGYYMS	37	SSISSSSTYIRYA	296	CATVTGYSSAGAFDIW	437	RASQSISSYLN	510	AASSLQS	529	CQQSYSTPLTF	592
011S-A01	LRP6e3e4	FTFSTHAFH	70	SAIRGSGERTYYA	278	CARDLRNWGSPYWYFDLW	371	RASQSISSYLN	510	AASSLQS	529	CQQSYSTPLTF	592
011S-B01	LRP6e3e4	GTFSHYTIS	87	GWINAGNGNTKYS	258	CAKGGSLDMDVW	343	RASQGISNYLA	499	AASSLHS	526	CQQSYRTPLTF	582
011S-C01	LRP6e3e4	LTFTSHGMS	103	SYVSDSGSSVYYA	311	CARHPGSFGGYSYAWYYYYGMDVW	413	RASQSISSYLN	510	AASSLQS	529	CQQSYSTPLTF	592
011S-D01	LRP6e3e4	GTISDYTVS	93	GIINPSGGSTSYA	248	CARGYYDFDYW	411	RASQGISNYLA	499	AASSLQS	529	CQQSYSTPLTF	592
009S-C08	LRP6e3e4	FSFNTFGIH	43	AVISYDGSNKYYA	226	CAKSIAAAGTGYYGMDVW	346	RASQSISSYLN	510	AASSLQS	529	CQQSYSTPLTF	592
011S-E01	LRP6e3e4	FPFZYYSMN	39	SAISGRDGRTYYA	279	CAKDLGIQLPDYYFDYW	334	RASQGISSALA	502	AASTLQS	532	CQQSYSSPPTF	591
009S-D08	LRP6e3e4	YTFTSYDIN	168	GGIIPIFGTANYA	236	CARGPYYFDYW	401	RASQGISNNLN	498	DASSLES	539	CLQHNSYPFTF	561
011S-F01	LRP6e3e4	FSFSDYYMS	45	SGISESGGRTYYA	284	CASAADFDYW	420	RASQDISNYLN	495	AASSLQS	529	CLQDYSYPRTF	559
009S-E08	LRP6e3e4	YGFTGYYIH	157	GWMNPNSGNTGYA	272	CARGYGDYDLW	410	QASQDISNYLN	495	DASSLES	539	CQQSYRYPTF	583
009S-F08	LRP6e3e4	DTFANYGFS	34	GXVNAGNGNTTYA	273	CAKGWLDFDYW	345	QASQDISNYLN	495	DASSLES	539	CQQSYSTSITF	595

Clone ID	Confirmed Binding	CDRH1	CDRH1 SEQ ID	CDRH2	CDRH2 SEQ ID	CDRH3	CDRH3 SEQ ID	CDRL1	CDRL1 SEQ ID	CDRL2	CDRL2 SEQ ID	CDRL3	CDRL3 SEQ ID
011S-G01	LRP6e3e4	YTFTYRYLH	170	GGIIPLFGTANYA	237	CTTDDYGDQYGMDVW	474	RASQSISSYLN	510	AASSLQS	529	CQQSYSTPLTF	592
011S-H01	LRP6e3e4	YTFTYRYLH	170	GGIIPIFGTANYA	236	CTTDDYGDLTHLDYW	473	RASQSISSYLN	510	AASSLQS	529	CQQSYSTPLTF	592
011S-A02	LRP6e3e4	GTFSSYAIS	90	GWMNPNSGNTGYA	272	CARDKGYAFDIW	368	RSSQSLLHSNGYNYLD	519	AASSLQS	529	CMQALQTPITF	563
011S-B02	LRP6e3e4	YSFTRTDMH	159	GYISAYTGHTSYA	274	CARDLGGTADYW	370	RZSQSZSZYLN	522	AASSLQS	529	CQQSYSTPLTF	592
011S-C02	LRP6e3e4	FTFSTYSMN	72	SGISWNSGRIGYA	288	CARDVGAFDIW	375	QASQDISNYLN	495	AASILQS	525	CQQSYSIPFTF	587
009S-G08	LRP6e3e4	FTFSDFAMT	53	SYISGDSGYTNYA	306	CARLGSYPGPYYYYMDVW	416	RASQSISSYLN	510	AASSLQS	529	CQQSYSTPLTF	592
011S-D02	LRP6e3e4	FTFSSYAMS	64	SSISGSGGVTYYA	294	CARGGNTYYYYYGMDVW	393	RASQSISSYLN	510	AASSLQS	529	CQQSYSTPLTF	592
009S-H08	LRP6e3e4	YTFTDYFMN	162	GIINPSGDSTRFA	245	CARDDGLGGMDVW	362	QASQDISNYLA	494	AASSLQS	529	CQQSYSTPLTF	592
011S-E02	LRP6e3e4	YTFTYRYLH	170	GGIIPIFGTANYA	236	CATDYGDYYYGMDVW	431	RASQSISSYLN	510	AASSLQS	529	CQQSYSTPLTF	592
009S-A09	LRP6e3e4	YTFTYRYLH	170	GRIIPILGSTNYA	254	CTTDLWDYW	475	QASQGITNYLN	495	AASSLQS	529	CLQDYTDPFTF	560
011S-F02	LRP6e3e4	FTFSTYGMH	71	SSISVSSGTTHYA	297	CARGGSGSYYYAFDIW	394	RASQSISSYLN	510	AASSLQS	529	CQQSYSTPLTF	592
011S-G02	LRP6e3e4	YTFTSYAMN	167	GGIIPIFGTANYA	236	CARDASGGSTGWYYFDSW	361	RASQGISSYLA	503	AASSLQS	529	CQQAYSFPWTF	568
011S-H02	LRP6e3e4	YTFTNNFMH	165	GIINPSGGSTSYA	248	CARGLYKRYSYGYGMDVW	397	RASQSISSYLN	510	AASSLQS	529	CQQSYSTPLTF	592
009S-B09	LRP6e3e4	FSFNTYAMN	44	AVTSYDGGKKNYA	229	CARDAGGDYDYW	360	QASQDISNYLN	495	AASSLQS	529	CQQSYNTPRTF	579
009S-C09	LRP6e3e4	GTFHTYGLS	84	GGIIPIFGTANYA	236	CARGSGWSGLDYW	405	QASQDISNYLN	495	DASNLET	534	CQQSYTTPFTF	598

Clone ID	Confirmed Binding	CDRH1	CDRH1 SEQ ID	CDRH2	CDRH2 SEQ ID	CDRH3	CDRH3 SEQ ID	CDRL1	CDRL1 SEQ ID	CDRL2	CDRL2 SEQ ID	CDRL3	CDRL3 SEQ ID
011S-A03	LRP6e3e4	FTFSSYWMH	67	STISGSGGRTYA	300	CATSPYGVFTLDYW	436	RASQSISSYLN	510	AASSLQS	529	CQQSYSTPLTF	592
011S-B03	LRP6e3e4	GTFSZYAIS	92	GIINPSGGSTNYA	247	CARAGYWSGYGYYGMDVW	357	RASQSISSYLN	510	AASSLQS	529	CQQSYSTPLTF	592
011S-C03	LRP6e3e4	YTFSYRYLH	161	GGIIPIFGTANYA	236	CASTVTTDAFDIW	427	QASQDISNYLN	495	DASSLES	539	CQQSYSFPPFTF	586
011S-D03	LRP6e3e4	FSFDDYGMS	41	SVISSGGTIYYA	305	CARHLSSGYLSYYGMDVW	412	RASQSISSYLA	509	AASTLQS	532	CQQSYSTPLTF	592
009S-F09	LRP6e3e4	YSFTRTDMH	159	GYISAYTGHTSYA	274	CARDLGGTADYW	370	RASQSISSYLN	510	AASSLQS	529	CQQSYSTPLTF	592
011S-E03	LRP6e3e4	FTFSSYAMS	64	SAISGSGGSTYYA	280	CAKGGRDGYKGYFDYW	342	KSSHSLLSTSTNRNQLA	489	WASSRKS	554	CQQYNNWPYTF	604
009S-G09	LRP6e3e4	FTFSRHSMN	59	SYSSGNSGYTNYA	310	CARGDLEFDYW	388	RASQGISNYLA	499	SASSLQS	551	CQQGYNTPRTF	572
011S-F03	LRP6e3e4	FTFSSYAMS	64	SAISGSGGSTYYA	280	CAKGGRDGYKGYFDYW	342	KSSQSVLYTTTNRNHIA	492	WASSRKS	554	CQQYYSTPYTF	607
009S-H09	LRP6e3e4	FTFSSYAMS	64	SAISGSGGSTYYA	280	CAKGGRDGYKGYFDYW	342	KSSHSLLSTSTNRNHLA	488	WASSRKS	554	CQQYYNIPYSF	605
011S-G03	LRP6e3e4	YTFTYRYLH	170	GRIIPIHGIANYA	251	CAREYSYGYFRYW	387	RASQGISSYLA	503	DASNLET	534	CQQANSLFTF	567
009S-A10	LRP6e3e4	FTFTSSAMQ	75	GIINPSGGSTIYA	246	CASGDTYDLYSLDVW	423	RASQSISRWLA	507	AASSLQS	529	CQQAYSFPWTF	568
009S-B10	LRP6e3e4	YIFTDYYMH	158	GWINAGNGNTTYA	259	CAKVASGWSWPFDIW	348	RASQSISSYLN	510	AASSLQS	529	CQQSYSTPLTF	592
011S-B04	LRP6e1e2	YTFTSYDIN	168	GIINPSGGSTSYA	248	CTREHSYYYGMDVW	470	RASQSISSYLN	510	AASSLQS	529	CQQSYSTPLTF	592
011S-C04	LRP6e1e2	GTFNSNAIS	85	GWMNPNSGNTGYA	272	CARDYYGSGSYNYGMDVW	379	GASQSVPRNSLA	486	GASQRAT	543	CQQYHNWPPEYTF	602
011S-D04	LRP6e1e2	YTFTSYDIN	168	GIINPSGGSTSYA	248	CAREAYYYYGMDVW	381	RASQSISSYLN	510	AASSLQS	529	CQQSYSTPLTF	592

Clone ID	Confirmed Binding	CDRH1	CDRH1 SEQ ID	CDRH2	CDRH2 SEQ ID	CDRH3	CDRH3 SEQ ID	CDRL1	CDRL1 SEQ ID	CDRL2	CDRL2 SEQ ID	CDRL3	CDRL3 SEQ ID
011S-E04	LRP6e1e2	FTFSSZZMH	68	SAIGTGGGTZYA	277	CAKDLGRAAAGSMDVW	335	WASQSVRGNYVA	524	DASNRAA	536	CQHRSNWPLTF	565
011S-F04	LRP6e1e2	YIFTDYYMH	158	GRIIPILGRANYA	252	CARGGYSTLDYW	396	HGSQDISNYLN	487	DASNRQS	538	CQQSFSTPRTF	574
011S-H04	LRP6e1e2	YIFTDYYMH	158	GRIIPILGRANYA	252	CARGGYSTLDYW	396	QASQDISNYLN	495	AASTLQS	532	CQQSFSTPRTF	574
011S-A05	LRP6e1e2	FTFSSYAMH	63	SAIGTGGGTYYA	277	CAKDLGRAAAGSMDVW	335	WASQSVRGNYVA	524	DASNRAG	537	CQHRSNWPLTF	565
011S-B05	LRP6e1e2	YZFTDYYMH	172	GWMNPNSGNTGYA	272	CTRVAWGLDYW	471	RASQSISSYLN	510	AASSLQS	529	CQQSYSTPLTF	592
011S-C05	LRP6e1e2	FTFSSYAMH	63	SAIGTGGGTYYA	277	CAKDLGRAAAGSMDVW	335	WASQSVRGNYVA	524	DASNRAA	536	CQHRSNWPLTF	565

Table 1B provides the sequence identifier number of the antibody heavy chain fragment (HC) for illustrative clones having only a heavy chain region, and their binding characteristics. In certain embodiments, the LRP5/6 binding domain is an Fab or was derived from an Fab, so Table 1B includes VH and CH1 sequence, but not CH2 or CH3 sequences. In certain embodiments, the LRP5/6 binding domain is a VHH or sdAb or was derived from a VHH or sdAb, so Table 2B includes the VHH domain. K_D data as determined by Octet BLI is shown for various Fzd receptors. The "Confirmed Binding" column indicates binding results from Octet BLI binding or BIAcore SPR. Blank entries denote that the binding to the specific Fzd receptor has not yet been determined. As shown in Table 1B, Octet BLI or BIAcore SPR sensorgrams for anti-LRP5/6 antibody fragments demonstrated a range of affinities for LRP5 and/or LRP6.

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

Clone ID	HC Seq ID NO	BLI or SPR Confirmed Binding	Kd (nM)
001S-F11	1	LRP6e1e2	*
009S-G02	2	LRP6e1e2	*
009S-A03	3	LRP6e1e2	*
009S-D03	4	LRP6e1e2	*
009S-F03	5	LRP6e1e2	*
009S-H03	6	LRP6e1e2	*
009S-A04	7	LRP6e1e2	*
009S-B04	8	LRP6e3e4	*
009S-D04	9	LRP6e3e4	**
009S-E04	10	LRP6e3e4	*
009S-F04	11	LRP6e3e4	**
009S-G04	12	LRP6e3e4	*
009S-H04	13	LRP6e3e4	*
009S-A05	14	LRP6e3e4	**
013S-G04	15	LRP6e3e4	*
013S-H04	16	LRP6e3e4	*

013S-C05	17	LRP6e3e4	*
013S-D05	18	LRP6e3e4	*
013S-G04	19	LRP6e3e4	*
013S-H04	20	LRP6e3e4	*
013S-A05	21	LRP6e3e4	**
013S-C05	22	LRP6e3e4	*
013S-D05	23	LRP6e3e4	*
008S-D01	24	LRP5	

* Indicates < 500 nM; ** indicates > 500 nM

Table 1C provides additional binding characteristics for certain clones, including relative binding affinities to LRP6E1E2 and LRP6E3E4 domains. The entry of "n.b." indicates no binding.

Table 1C. LRP6 Binding Characteristics of sub-set of clones

Antigen	Clone ID	Octet confirmed binding	LRP6e1e2	LRP6e3e4
LRP6e1e2	010S-E02	LRP6e1e2	*	
LRP6e1e2	009S-G02	LRP6e1e2	*	n.b.
LRP6e1e2	009S-A03	LRP6e1e2	*	**
LRP6e1e2	010S-B03	LRP6e1e2	**	
LRP6e1e2	009S-D03	LRP6e1e2	*	n.b.
LRP6e1e2	009S-F03	LRP6e1e2	*	n.b.
LRP6e1e2	009S-H03	LRP6e1e2, e3e4	*	*
LRP6e1e2	009S-A04	LRP6e1e2	*	n.b.
LRP6e3e4	009S-B04	LRP6e3e4	n.b.	*
LRP6e3e4	009S-D04	LRP6e1e2, e3e4	*	*
LRP6e3e4	009S-E04	LRP6e3e4		*
LRP6e3e4	009S-F04	LRP6e3e4		**
LRP6e3e4	009S-G04	LRP6e3e4	n.b.	*
LRP6e3e4	009S-H04	LRP6e3e4	n.b.	*

LRP6e3e4	009S-A05	LRP6e3e4		**
LRP6e3e4	010S-G04	Lrp6e3e4		*
LRP6e3e4	010S-E05	LRP6e3e4		*
LRP6e3e4	013S-G04	LRP6e3e4	n.b.	*
LRP6e3e4	013S-H04	LRP6e3e4	n.b.	*
LRP6e3e4	013S-C05	LRP6e3e4	n.b.	*
LRP6e3e4	013S-D05	LRP6e3e4	n.b.	*
LRP6e3e4	009S-B05	LRP6e3e4		*
LRP6e3e4	010S-A07	LRP6e3e4		*
LRP6e3e4	009S-D05	LRP6e3e4		
LRP6e3e4	010S-E07	LRP6e3e4		*
LRP6e3e4	009S-F05	LRP6e3e4		*
LRP6e3e4	010S-G07	LRP6e3e4		*
LRP6e3e4	009S-G05	LRP6e3e4		
LRP6e3e4	010S-A08	LRP6e3e4		*
LRP6e3e4	009S-D06	LRP6e3e4		*
LRP6e3e4	010S-F08	Lrp6e3e4		*
LRP6e3e4	010S-A09	LRP6e3e4		**
LRP6e3e4	010S-D09	LRP6e3e4		**
LRP6e3e4	009S-D08	LRP6e3e4		**
LRP6e3e4	010S-D10	LRP6e3e4		**
LRP6e3e4	010S-D11	LRP6e3e4		*
LRP6e3e4	010S-F11	LRP6e3e4		*
LRP6e3e4	010S-G11	LRP6e3e4		*
LRP6e3e4	010S-B12	LRP6e3e4		**
LRP6e3e4	010S-D12	LRP6e3e4	n.b.	**
LRP6e3e4	010S-F12	LRP6e3e4		*
LRP6e3e4	011S-B01	LRP6e3e4		*
LRP6e3e4	011S-D01	LRP6e3e4		**
LRP6e3e4	011S-H01	LRP6e3e4		*

LRP6e3e4	011S-B02	LRP6e3e4		*
LRP6e3e4	011S-G02	LRP6e3e4		*
LRP6e3e4	011S-B03	LRP6e3e4		*
LRP6e3e4	011S-C03	LRP6e3e4		**
LRP6e3e4	009S-F09	LRP6e3e4	n.b.	*
LRP6e3e4	009S-G09	LRP6e3e4		*
LRP6e1e2	011S-B05	LRP6e1e2	*	
LRP6e1e2	011S-C05	LRP6e1e2	*	

* Indicates < 500 nM; ** indicates > 500 nM

EXAMPLE 2

ALANINE-SCANNING MUTATION OF AN ANTI-LRP6 ANTIBODY FRAGMENT

One antibody fragment, 009S-E04, was selected for alanine-scanning mutagenesis of CDRs, and the respective LRP6 binding affinities of the various mutants were determined by Octet BLI as described in Example 1. As shown in Table 2, a large number of mutants bound LRP6 with a similar affinity as the wild type antibody fragment, demonstrating that the LRP6 antibodies and antigen-binding fragments thereof can tolerate amino acid modifications within the CDRs.

Table 2. 009S-E04 alanine-scanning mutant CDR sequences and K_D (nM) to biotinylated-LRP6 CRD as determined by Octet BLI.

Clone ID	Kd (nM)	CDRH1	SEQ ID No.	CDRH2	SEQ ID No.	CDRH3	SEQ ID No.
WT	*	RIFAIYDIA	107	AMIRPVVTEIDYA	203	CNAKRPWGSRDEYW	608
K1A	*	RIFAIYDIA	107	AMIRPVVTEIDYA	203	CNAARPWGSRDEYW	609
R2A	*	RIFAIYDIA	107	AMIRPVVTEIDYA	203	CNAKAPWGSRDEYW	610
P3A	*	RIFAIYDIA	107	AMIRPVVTEIDYA	203	CNAKRAWGSRDEYW	611
W4A	*	RIFAIYDIA	107	AMIRPVVTEIDYA	203	CNAKRPAGSRDEYW	612
G5A	*	RIFAIYDIA	107	AMIRPVVTEIDYA	203	CNAKRPWASRDEYW	613
S6A	*	RIFAIYDIA	107	AMIRPVVTEIDYA	203	CNAKRPWGARDEYW	614
R7A	*	RIFAIYDIA	107	AMIRPVVTEIDYA	203	CNAKRPWGSADEYW	615

D8A	*	RIFAIYDIA	107	AMIRPVVTEIDYA	203	CNAKRPWGSRAEYW	616
E9A	*	RIFAIYDIA	107	AMIRPVVTEIDYA	203	CNAKRPWGSRDAYW	617
Y10A	*	RIFAIYDIA	107	AMIRPVVTEIDYA	203	CNAKRPWGSRDEAW	618

* Indicates < 100 nM

EXAMPLE 3

CHARACTERIZATION OF A 18R5SCFV-LRP VHH WNT SURROGATE FUSION PROTEIN IN 293 AND A375 WNT DEPENDENT REPORTER ASSAYS

The ability of Wnt surroate fusion proteins comprising an scFv region that binds to one or more Fzd and a VHH or sdAb (or single domain antibody, Nab) region that binds to LRP5 and/or LRP6 was demonstrated. The Fzd binder, 18R5, in scFv format, was fused to various Lrp5 and Lrp6 binders disclosed herein. The LRP binders were Nab and were fused to the C-terminus of 18R5 scFv with a 6-amino acid linker. The fusion proteins containing a C-terminal poly-His-tag were expressed in expi293 cells according to the manufacturer's protocol, and purified from the conditioned media using complete His-tag purification resin, and Superdex 200 10/300 GL equilibrated in HBS (10 mM HEPES, pH 7.3, 150 mM NaCl). Fractions containing monomeric fusions were pooled and concentrated.

The fusion proteins were tested for their ability to induce Wnt pathway signaling using Wnt dependent reporter assays in 293 and A375 cell lines under the following conditions. 10,000 A375 and HEK293 Wnt reporter cells, stably transfected with the STF Wnt reporter plasmid or a variant thereof, were seeded in triplicate for each condition in 96-well plates, and stimulated with the fusion proteins for 16–20 h in the presence or absence of 25 nM Fc-Rspo2. The conditions tested including various concentrations of each fusion protein ranging from 10^{-2} nM to 10^{3} nM. After washing cells with PBS, cells in each well were lysed in 30 µl passive lysis buffer (Promega). 10 µl per well of lysate was assayed using the Firefly Luciferase Assay kit (Promega).

The results demonstrated that a number of the Wnt surrogates tested activated the Wnt signaling pathway in a concentration dependent manner, and this was further enhanced by treatment with Fc-Rspo2 (data not shown).

EXAMPLE 4

CRYSTAL STRUCTURES OF LRP6E3E4:BINDER COMPLEXES

Lrp6, Low-density lipoprotein receptor-related protein 6, is a 1613 amino acid containing, single-pass membrane protein that plays a critical role in Wnt-mediated activation of ß-catenin signaling. Lrp6 and related Lrp5 shares 70% sequence identity between them. Extracellular regions of Lrp6 and Lrp5 contains four ß-propeller domains referred to here as E1, E2, E3, and E4 domains. In this session, we describe structures of Lrp6E3E4 domains (residues 631 to 1246 of uniprot entry O75581 (https://www.uniprot.org/uniprot/O75581). Sequence Lrp6E3E4 construct, used for structural studies, containing biotin acceptor peptide (BAP) and an eight-Histidine motif at their C-terminus is as follows:

LRP6E3E4_O75581_631-1246:

EAFLLFSRRADIRRISLETNNNNVAIPLTGVKEASALDFDVTDNRIYWT
DISLKTISRAFMNGSALEHVVEFGLDYPEGMAVDWLGKNLYWADTGTNRIEVSKLD
GQHRQVLVWKDLDSPRALALDPAEGFMYWTEWGGKPKIDRAAMDGSERTTLVPN
VGRANGLTIDYAKRRLYWTDLDTNLIESSNMLGLNREVIADDLPHPFGLTQYQDYIY
WTDWSRRSIERANKTSGQNRTIIQGHLDYVMDILVFHSSRQSGWNECASSNGHCS
HLCLAVPVGGFVCGCPAHYSLNADNRTCSAPTTFLLFSQKSAINRMVIDEQQSPDII
LPIHSLRNVRAIDYDPLDKQLYWIDSRQNMIRKAQEDGSQGFTVVVSSVPSQNLEIQ
PYDLSIDIYSRYIYWTCEATNVINVTRLDGRSVGVVLKGEQDRPRAVVVNPEKGYM
YFTNLQERSPKIERAALDGTEREVLFFSGLSKPIALALDSRLGKLFWADSDLRRIESS
DLSGANRIVLEDSNILQPVGLTVFENWLYWIDKQQQMIEKIDMTGREGRTKVQARIA
QLSDIHAVKELNLQEYRQHPCAQDNGGCSHICLVKGDGTTRCSCPMHLVLLQDEL
SCGEPPSGSGGLNDIFEAQKIEWHEGSGSHHHHHHHH (SEQ ID NO:619)

EXAMPLE 5

EXPRESSION AND PURIFICATION OF LRP6E3E4 FOR STRUCTURAL STUDIES

A stable-cell line expressing Lrp6E3E4 domain was generated in Expi293 cells with G418 selection. For large-scale expression, a frozen vial Expi293 Cells expressing Lrp6E3E4 was thawed into 20mL of Expi293 media (Thermofisher).

Cells were monitored for viability and expended on alternative days until density of ~3.0 to 4.0 x10^6 cell/mL was reached at desired volumes, typically 6 to 10L. At this stage, cells treated with 2mM valparoic acid, allowed to grow continuously to higher density, and media was harvested by centrifugation after ~48 hours. Fzd CRD_Xtal proteins were purified from media by incubation with HisComplete resin (1mL per L of culture; Roche) pre-equilibrated in PBS (50 mM sodium di-hydrogen phosphate pH 8.0, 300 mM NaCl), and eluted with 250 mM imidazole. Elutions were concentrated to 5mL, and further polished on a HiLoad 16/600 Superdex 200 pg column (GE Life Sciences) pre-equilibrated with HBS (20mM HEPES pH 7.4, and 150mM sodium chloride). Fractions near main peak was further analyzed by SDS-Polyacrylamide Gel Electrophoresis (SDS-PAGE) to confirm the content. SDS-PAGE was performed using Tris-HCl 4-15% gel (Bio-Rad, Hercules, CA) under both non-reducing conditions. The samples were prepared in Laemmli sample buffer and heated at 100□C for 5 min. Fractions containing LRP6E3E4 were concentrated to ~2mg/mL and frozen in the presence of 10% glycerol for storage at -80C until further use. Protein concentrations were determined using a NanoDrop Spectrophotometer (Thermo Scientific) by the direct UV A280 method. The relationship of absorbance to protein concentration is linear based on Beer-Lamber equation, $A = \varepsilon l c$; A is the absorbance value, ε is the wavelength- dependent extinction coefficient, l is the path length in centimeters, and c is the protein concentration. The extinction coefficients of all produced proteins were estimated by their amino acid sequences.

EXAMPLE 6

EXPRESSION AND PURIFICATION OF VHH OR sdAb OR FAB BINDERS

Plasmids expressing light-chain and heavy chain (with hexa-histidine tag at its C-terminus) in the case of Fab or that expressing VHH binders were transfected for expression in Expi293 cells, typically at 1000mL scale, following the standard protocols from the manufacturer (Thermofisher). After 4 days of continuous cell growth, media were harvested by centrifugation, and bound to Complete-His resin (2.5mL per 1L culture; Roche) pre-equilibrated in PBS, and eluted under gravity-flow using 250mM imidazole in PBS. Elutions containing Fab binders were

concentrated to ~5mL, and further polished on a HiLoad 16/600 Superdex 200 pg column (GE Life Sciences) column pre-equilibrated with HBS. Fractions near the main peak were further analyzed by SDS-Polyacrylamide Gel Electrophoresis (SDS-PAGE) to confirm the content. SDS-PAGE was performed using Tris-HCl 4-15% gel (Bio-Rad, Hercules, CA) under both non-reducing conditions. The samples were prepared in Laemmli sample buffer and heated at 100□C for 5 min. Fractions containing Fab or VHH binders were concentrated to ~3 mg/mL and frozen in the presence of 10% glycerol for storage at -80C until further use. Protein concentrations were determined using a NanoDrop Spectrophotometer (Thermo Scientific) by the direct UV A280 method. The relationship of absorbance to protein concentration is linear based on Beer-Lamber equation, $A = \varepsilon l c$; A is the absorbance value, ε is the wavelength- dependent extinction coefficient, l is the path length in centimeters, and c is the protein concentration. The extinction coefficients of all produced proteins were estimated by their amino acid sequences.

EXAMPLE 7

LRP6E3E4:VHH / FAB COMPLEX FORMATION, CRYSTALLIZATION, AND STRUCTURE DETERMINATION

Purified Lrp6E3E4 and VHH / Fab binders were mixed at 1.1:1 molar ratio (little excess of the smaller molecular weight protein), and incubated with carboxy-peptidase A and B at a w/w ratio of 100:1 for over-night at 4°C. Complex formation was confirmed by observation of a single-major peak on SuperdexS200 Increase (10/300 GL) column pre-equilibrated in HBS. Fractions containing complexes were further checked by SDS-PAGE, and concentrated to 10 to 25mg/mL for crystallization screens. Initial crystallization screen, using commercially available MCSG1, MCSG2, MCSG3, MCSG4, PACT (Molecular Dimensions), PEGs I, and PEGs II (Qiagen) screen, and optimization by grid-screens or microseed matrix screen [MMS; Microseed matrix screening for optimization in protein crystallization: what have we learned? D'Arcy, A., Bergfors, T., Cowan-Jacob S.W., and Marshd, M. Acta Cryst. F70, 1117-1126 (2014)]. were performed using Mosquito (TTP LabTech) liquid handler, and equilibrated at 18°C inside an EchoTherm incubator (Torrey

Pines Scientific). 96-well plate crystal screening experiments were periodically monitored manually via a DiscoveryV20 stereomicroscope (Zeiss), and crystals were frozen for data collection by plunging into liquid nitrogen in the presence of various cryo-protectants (typically 15 to 30% v/v of glycerol or ethyleneglycol or 1.1 to 2.5 M sodium malanote pH 7.0). X-ray diffraction datasets were collected at the Berkeley Center for Structural Biology at the Advanced Light Source (ALS), Berkeley CA, and processed with XDS [Kabsch, W. XDS. Acta Cryst. D66, 125-132 (2010)], and xdsme [Legrand, P. XDSME: XDS Made Easier (2017) GitHub repository, https://github.com/legrandp/xdsme DOI 10.5281/zenodo.837885] programs. Structure of Lrp6E3E4:VHH / Fab complexes were determined by molecular replacement method using Phaser [Phaser crystallographic software. A.J. McCoy, R.W. Grosse-Kunstleve, P.D. Adams, M.D. Winn, L.C. Storoni, and R.J. Read. J Appl Crystallogr 40, 658-674 (2007)] with published structures Lrp6E3E4 [PDB code: 4A0P; Chen S, Bubeck D, MacDonald BT, Liang WX, Mao JH, Malinauskas T, Llorca O, Aricescu AR, Siebold C, He X, Jones EY. Dev. Cell 21 848-61 (2011)] and a VHH or sdAb [PDB code: 6B20, chain E; Gulati S, Jin H, Masuho I, Orban T, Cai Y, Pardon E, Martemyanov KA, Kiser PD, Stewart PL, Ford CP, Steyaert J, Palczewski K. Nat Commun 9 1996 (2018)] , followed by refinement and validation by MolProbity as implemented in Phenix [PHENIX: a comprehensive Python-based system for macromolecular structure solution. P.D. Adams, P.V. Afonine, G. Bunkoczi, V.B. Chen, I.W. Davis, N. Echols, J.J. Headd, L.W. Hung, G.J. Kapral, R.W. Grosse-Kunstleve, A.J. McCoy, N.W. Moriarty, R. Oeffner, R.J. Read, D.C. Richardson, J.S. Richardson, T.C. Terwilliger, and P.H. Zwart. Acta Cryst. D66, 213-221 (2010); MolProbity: all-atom structure validation for macromolecular crystallography. V.B. Chen, W.B. Arendall, J.J. Headd, D.A. Keedy, R.M. Immormino, G.J. Kapral, L.W. Murray, J.S. Richardson, and D.C. Richardson. Acta Cryst. D66, 12-21 (2010)]. Crystallography models were manually inspected and built using COOT [Features and development of Coot. P. Emsley, B. Lohkamp, W.G. Scott, and K.. Cowtan. Acta Cryst. D66, 486-501 (2010)]. Analyses of refined crystal structures, and image creations were performed using MOE (CCG) and PyMol (Schrodinger).

EXAMPLE 8

STRUCTURE OF LRP6E3E4:VHH26 COMPLEX

Sequence of the VHH26 (009S-E04):

DVQLVESGGGLVQAGGSLRLACAGSGRIFAIYDIAWYRHPPGNQREL VAMIRPVVTEIDYADSVKGRFTISRNNAMKTVYLQMNNLKPEDTAVYYCNAKRPWG SRDEYWGQGTQVTVSSGSGSGHHHHHH (SEQ ID NO:620)

Diffraction quality crystals of Lrp6E3E4:VHH26 (concentration = 12.4 mg/mL) were obtained by MMS in the PACT screen, G8 condition containing 0.2 M sodium sufate, 0.1 M bis-Tris-propane pH 7.5, and 20 % (v/v) PEG3350. Crystal was cryo-protected using 27% glycerol in well-solution. Lrp6E3E4:VHH26 complex crystallized in the *P*3121 space group (a=b=136.64 Å and c=104.70 Å) with one complex molecule per asymmetric unit. Structure of Lrp6E3E4:VHH26 complex was determined at a resolution of 2.40 Å, and refined to R_{cryst} and R_{free} factors of 18.5% and 23.0%, respectively.

Overall structure of Lrp6E3E4:VHH26 complex shown in Figure 3.1(A), which reveals that the epitope on Lrp6E3E4 for VHH26 (Figure 4.1(B)) localized within the E3 ß-propeller of Lrp6. Structure of the complex allow us to identify epitope of Lrp6E3E4 for VHH26 with the following residues of Lrp6E3E4 defining the core interaction-site (inter-atomic distances between Lrp6E3E4 and VHH26 less than or equal to 5.0 Å):

Arg639, Ala640, Lys622, Glu663, Ile681, Ser682, Lys684, Asp705, Tyr706, Glu708, Thr724, Gly725, Arg751, Trp767, Gly768, Gly769, Arg792, Leu810, Asp811, His834, Phe836, Trp850, Ser851, Arg853, Asp874, Tyr875, and Met877.

In addition, following residues on Lrp6E3E4 could be identified as immediate-interaction site (inter-atomic distances between Lrp6E3E4 and VHH26 greater than 5.0 Å and less than or equal to 8.0 Å):

Arg638, Asp641, Val661, Ala664, Ser665, Asp680, Leu683, Thr685, Leu704, Pro707, Asp723, Thr726, Asn727, Asp748, Ser749, Pro750, Lys770, Pro771, Gly791, Asn794, Asp809, Thr812, Asn813, Pro833, Pro835, Asp849, and Arg852.

Structure of Lrp6E3E4:VHH26 complex also allow us to identify following residues on VHH26 as core-interaction site (inter-atomic distances between Lrp6E3E4 and VHH26 less than or equal to 5.0 Å):

Gly26, Arg27, Phe29, Ala30, Ile31, Tyr32, Arg52, Pro53, Val54, Val55, Glu57, Asn74, Ala75, Lys77, Arg100, Pro101, Trp102, Gly103, Ser104, Arg105 Asp106, and Tyr108.

Structure of Lrp6E3E4:VHH26 complex also allow us to identify following residues on VHH26 as core-interaction site (inter-atomic distances between Lrp6E3E4 and VHH26 greater than 5.0 Å and less than or equal to 8.0 Å):

Gly24, Ser25, Ile28, Asp33, Met50, Ile51, Thr56, Arg72, Asn73, Met76, Lys99, and Glu107.

EXAMPLE 9

STRUCTURE OF LRP6E3E4:VHH36 COMPLEX

Sequence of the VHH36 (013S-D05):

QVKLEESGGGLVQAGGSLRLSCAASGRIFSIYDMGWFRQAPGKERE FVSGIRWSGGTSYADSVKGRFTISKDNAKNTIYLQMNNLKAEDTAVYYCGSRGYW GQGTLVTVSSGSGSGHHHHHH (SEQ ID NO:621)

Diffraction quality crystals of Lrp6E3E4:VHH36 (concentration = 14.0 mg/mL) were obtained by grid-screen optimization in a condition containing 1.6 M ammonium sulfate and 0.1 M Tris pH 8.2. Crystal was cryo-protected using 1.1 M sodium malonate pH 7.0 in 1.6M ammonium sulfate and Tris pH 9.0. Lrp6E3E4:VHH36 complex crystallized in the $P6_5$ space group (a=b=180.61 Å and c=98.63 Å) with one complex molecule per asymmetric unit. Structure of Lrp6E3E4:VHH36 complex was determined at a resolution of 2.70 Å, and refined to R_{cryst} and R_{free} factors of 18.6% and 22.8%, respectively.

Overall structure of Lrp6E3E4:VHH36 complex shown in Figure 3.1(A), which reveals that the epitope on Lrp6E3E4 for VHH36 (Figure 4.1(B) localized within the E3 ß-propeller of Lrp6. Structure of the complex allow us to identify epitope of Lrp6E3E4 for VHH36 with the following residues of Lrp6E3E4 defining the

core interaction-site (inter-atomic distances between Lrp6E3E4 and VHH36 less than or equal to 5.0 Å):

Glu663, Ser665, Ile681, Tyr706, Glu708, Thr724, Ser749, Arg751, Trp767, Gly768, Arg792, Leu810, Asn813, Pro833, His834, Phe836, Trp850, Ser851, Arg853, Asp874, Tyr875, and Met877.

In addition, following residues on Lrp6E3E4 could be identified as immediate-interaction site (inter-atomic distances between Lrp6E3E4 and VHH36 greater than 5.0 Å and less than or equal to 8.0 Å):

Ser637, Arg638, Arg639, Lys662, Ala664, Ala666, Thr679, Asp680, Ser682, Lys684, Pro707, Gly725, Asn727, Asp748, Pro750, Glu766, Gly769, Pro771, Asn794, Thr808, Asp809, Asp811, Thr812, Leu814, Leu832, Pro835, Asp849, Arg852, His872, Leu873, Val876, and Asp878.

Structure of Lrp6E3E4:VHH36 complex also allow us to identify following residues on VHH36 as core-interaction site (inter-atomic distances between Lrp6E3E4 and VHH36 less than or equal to 5.0 Å):

Gln1 (modified as pyroglutamate), Val2, Lys3, Ala24, Ser25, Gly26, Arg27, Ile28, Ser30, Ile31, Tyr32, Trp53, Asn73, Asn76, Arg98, and Tyr100.

Structure of Lrp6E3E4:VHH36 complex also allow us to identify following residues on VHH36 as core-interaction site (inter-atomic distances between Lrp6E3E4 and VHH36 greater than 5.0 Å and less than or equal to 8.0 Å):

Leu4, Ala23, Phe29, Asp33, Arg52, Ser54, Lys71, Asp72, Ala74, Lys75, Ser97, and Gly99.

Table 3. Summary of Binding Characteristics of Antigen Binding proteins of LRP as determined by co-crystal structures. In the cases of multi-specific binders, corresponding residues of related LRP5 are listed, using amino-acid single-letter codes, in addition to the structurally determined epitope information on LRP for which crystal structures were determined.

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

Antigen Binding Protein	Antigen	Interaction site on LRP6 (<5 angstroms)	Interaction site on LRP6 (5-8 angstroms)
VHH26 (009S-E04)	LRP6E3E4	Arg639, Ala640, Lys662, Glu663, Ile681, Ser682, Lys684, Asp705, Tyr706, Glu708, Thr724, Gly725, Arg751, Trp767, Gly768, Gly769, Arg792, Leu810, Asp811, His834, Phe836, Trp850, Ser851, Arg853, Asp874, Tyr875, Met877.	Arg638, Asp641, Val661, Ala664, Ser665, Asp680, Leu683, Thr685, Leu704, Pro707, Asp723, Thr726, Asn727, Asp748, Ser749, Pro750, Lys770, Pro771, Gly791, Asn794, Asp809, Thr812, Asn813, Pro833, Pro835, Asp849, Arg852.
	Corresponding residues on LPR5	Arg652, Ala653, Lys675, Glu676, Val694, Ser695, Lys697, Asp718, Tyr719, Glu721, Thr737, Gly738, R764, Trp780, Gly781, Gly782, Arg805, Leu823, Asp824, His847, Phe849, Trp863, Asn864, His866, Asp887, Phe888, M890.	Ser651, Ala654, Val674, Ala677, Ser678, Asp693, Leu696, Thr698, Leu717, Pro720, Asp736, Thr739, Asn740, Asp761, Asn762, Pro763, Lys783, Pro784, Gly804, Asn807, Asp822, Thr825, Asn826, Pro846, Pro848, Asp862, Leu865.
VHH36 (013S-D05)	LRP6E3E4	Glu663, Ser665, Ile681, Tyr706, Glu708, Thr724, Ser749, Arg751, Trp767, Gly768, Arg792, Leu810, Asn813, Pro833, His834, Phe836, Trp850, Ser851, Arg853, Asp874, Try875, Met877.	Ser637, Arg638, Arg639, Lys662, Ala664, Ala666, Thr679, Asp680, Ser682, Lys684, Pro707, Gly725, Asn727, Asp748, Pro750, Glu766, Gly769, Pro771, Asn794, Thr808, Asp809, Asp811, Thr812, Leu814, Leu832, Pro835, Asp849, Arg852, His872, Leu873, Val876, Asp878.
	Corresponding residues on LPR5	Glu676, Ser678, Val694, Tyr719, Glu721, Thr737, Asn762, Arg764, Trp780, Gly781, Arg805, Leu823, Asn826, Pro846, His847, Phe849, Trp863, Asn864, His866, Asp887, Phe888, M890.	Thr650, Ser651, Arg652, Lys675, Ala677, Ala679, Thr692, Asp693, Ser695, Lys697, Pro720, Gly738, Asn740, Asp761, Pro763, Glu779, Gly782, Pro784, Asn807, Thr821, Asp822, Asp824, Thr825, Met827, Leu845, Pro848, Asp862, Leu865, His885, Leu885, Val889, Asp891.

These and other changes can be made to the embodiments in light of the above-detailed description. In general, in the following claims, the terms used should not be construed to limit the claims to the specific embodiments disclosed in the specification and the claims, but should be construed to include all possible embodiments along with the full scope of equivalents to which such claims are entitled. Accordingly, the claims are not limited by the disclosure.

CLAIMS

What is claimed is:

1. An isolated antibody, or an antigen-binding fragment thereof, that binds to one or more LRP5 or LRP6 receptor, comprising a sequence comprising:

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

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

(iii) CDRH1, CDRH2, CDRH3, CDRL1, CDRL2, and CDRL3 sequences set forth for any of the antibodies of Table 2

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

2. The isolated antibody, or antigen-binding fragment thereof, of claim 1, comprising a heavy chain variable region comprising an amino acid sequence having at least 90% identity to the amino acid sequence set forth in any of SEQ ID NOs:1-24.

3. The isolated antibody, or antigen-binding fragment thereof, of claim 2, comprising a heavy chain variable region comprising the amino acid sequence set forth in any of SEQ ID NOs:1-24.

4. The isolated antibody, or antigen-binding fragment thereof, of any of claims 1-3, wherein the antibody, or antigen-binding fragment thereof, is humanized.

5. The isolated antibody, or antigen-binding fragment thereof, of any of claims 1-5, wherein the antibody, or antigen-binding fragment thereof, is a single chain antibody, a scFv, a univalent antibody lacking a hinge region, a VHH or sdAb, or a minibody.

6. The isolated antibody, or antigen-binding fragment thereof, of claim 5, wherein the antibody, or antigen-binding fragment thereof, is a VHH VHH or sdAb.

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

8. The isolated antibody, or antigen-binding fragment thereof, of any of claims 1-7, wherein the antibody, or antigen-binding fragment thereof, is a fusion protein.

9. The isolated antibody, or antigen-binding fragment thereof, of claim 8, wherein the antibody, or antigen-binding fragment thereof, is fused to a polypeptide sequence that binds one or more Frizzled (Fzd) receptors.

10. The isolated antibody, or antigen-binding fragment thereof, of claim 9, wherein the polypeptide sequence that binds one or more Fzd receptors is an antibody, or an antigen-binding fragment thereof, that binds to one or more Fzd receptor.

11. The isolated antibody, or antigen-binding fragment thereof, of any of claims 1-10, wherein the antibody, or antigen-binding fragment thereof, binds to LRP5.

12. The isolated antibody, or antigen-binding fragment thereof, of claim 11, wherein the antibody, or antigen-binding fragment thereof, binds to LRP6

13. The isolated antibody, or antigen-binding fragment thereof, of claim 12, wherein the antibody, or antigen-binding fragment thereof, binds to: LRP5 and LRP6.

14. An isolated antibody, or an antigen-binding fragment thereof, that competes with the antibody of any of claims 1-13 for binding to a human LRP5 or LRP6.

15. An isolated antibody, or antigen-binding fragment thereof, of any of claims 1-14, that binds to the LRP5 or LRP6 with a KD of 50 μM or lower.

16. The isolated antibody, or antigen-binding fragment thereof, of any of claims 1-15, which modulates a Wnt signaling pathway in a cell, optionally a mammalian cell.

17. The isolated antibody, or antigen-binding fragment thereof, of claim 16, which increases signaling via the Wnt signaling pathway in the cell.

18. The isolated antibody, or antigen-binding fragment thereof, of claim 16, which decreases signaling via the Wnt signaling pathway in the cell.

19. The isolated antibody, or antigen-binding fragment thereof, of any of claims 16-18, wherein the Wnt signaling pathway is a canonical Wnt signaling pathway.

20. The isolated antibody, or antigen-binding fragment thereof, of any of claims 16-18, wherein the Wnt signaling pathway is a non-canonical Wnt signaling pathway

21. An isolated polynucleotide encoding the isolated antibody, or antigen-binding fragment thereof, according to any of claims 1-20.

22. An expression vector comprising the isolated polynucleotide of claim 22.

23. An isolated host cell comprising the expression vector of claim 22.

24. A pharmaceutical composition comprising a physiologically acceptable excipient, diluent, or carrier, and a therapeutically effective amount of the isolated antibody, or antigen-binding fragment thereof, according to any of claims 1-20 or 33.

25. A method for agonizing a Wnt signaling pathway in a cell, comprising contacting the cell with the isolated antibody, or antigen-binding fragment thereof, according to claim 17.

26. The method of claim 24, wherein the antibody, or antigen-binding fragment thereof, is a fusion protein comprising a polypeptide sequence that binds one or more frizzled (Fzd) receptors.

27. A method for inhibiting a Wnt signaling pathway in a cell, comprising contacting the cell with the isolated antibody, or antigen-binding fragment thereof, according to claim 18.

28. A method for treating a subject having a disease or disorder associated with reduced Wnt signaling, comprising administering to the subject an effective amount of the pharmaceutical composition of claim 24, wherein the isolated antibody, or antigen-binding fragment thereof is an agonist of a Wnt signaling pathway.

29. The method of claim 28, wherein the disease or disorder is selected from the group consisting of: bone fractures, stress fractures, vertebral compression fractures, osteoarthritis, osteoporosis, osteoporotic fractures, non-union fractures, delayed union fractures, spinal fusion, pre-operative optimization for spine surgeries, osteonecrosis, osseointegration of implants or orthopedic devices, osteogenesis imperfecta, bone grafts, tendon repair, tendon-bone integration, tooth growth and regeneration, maxillofacial surgery, dental implantation, periodontal diseases, maxillofacial reconstruction, osteonecrosis of the jaw, hip or femoral head, avascular necrosis,

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

30. A method for treating a subject having a disease or disorder associated with increased or enhanced Wnt signaling, comprising administering to the subject an effective amount of the pharmaceutical composition of claim 24, wherein the isolated antibody, or antigen-binding fragment thereof is an inhibitor of a Wnt signaling pathway.

31. The method of claim 29, wherein the disease or disorder is selected from the group consisting of: tumors and cancers, degenerative disorders, fibrosis of any organ or tissue, heart failure, coronary artery disease, heterotopic ossification, osteopetrosis, and congenital high bone mass disorders.

32. An isolated antibody, or an antigen-binding fragment thereof, that binds to a LRP6 receptor, wherein the antibody or antigen-binding fragment thereof binds to the E3 β-propeller region of the LRP6 receptor or a corresponding region of the LRP5 receptor.

33. An isolated antibody, or an antigen-binding fragment thereof, that binds to an LRP6 receptor, wherein the antibody or antigen-binding fragment thereof binds to one or more epitopes within the region of the LRP6 receptor comprising or consisting of amino acid residues 637-878 or a corresponding region of the LRP5 receptor.

34. An isolated antibody, or an antigen-binding fragment thereof, that binds one or more LRP receptor, wherein the antibody or antigen-binding fragment thereof contacts the LRP receptor with a distance of less than 5 angstroms at any of the sets of amino acid residues indicated in Table 3.

Figure 1

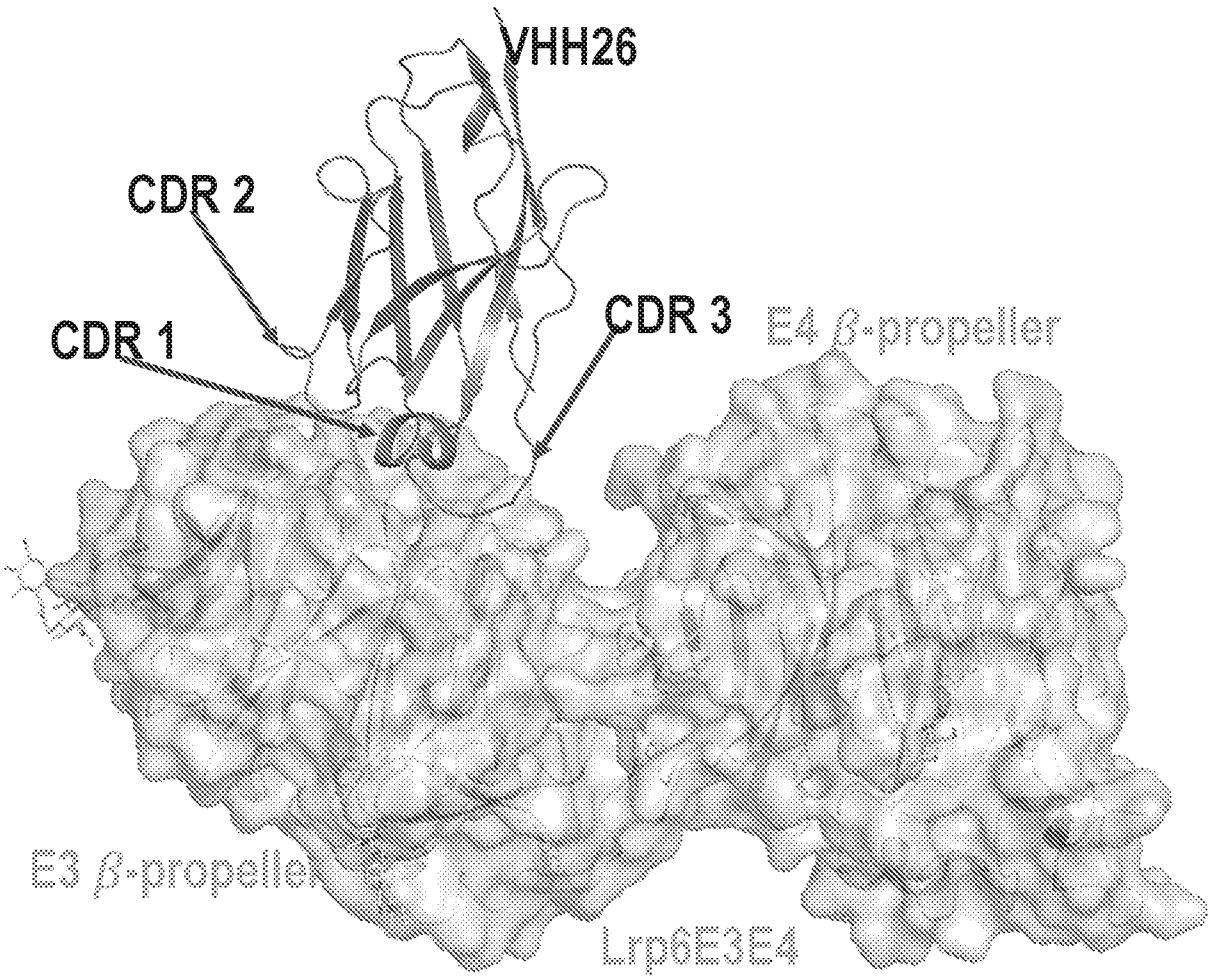
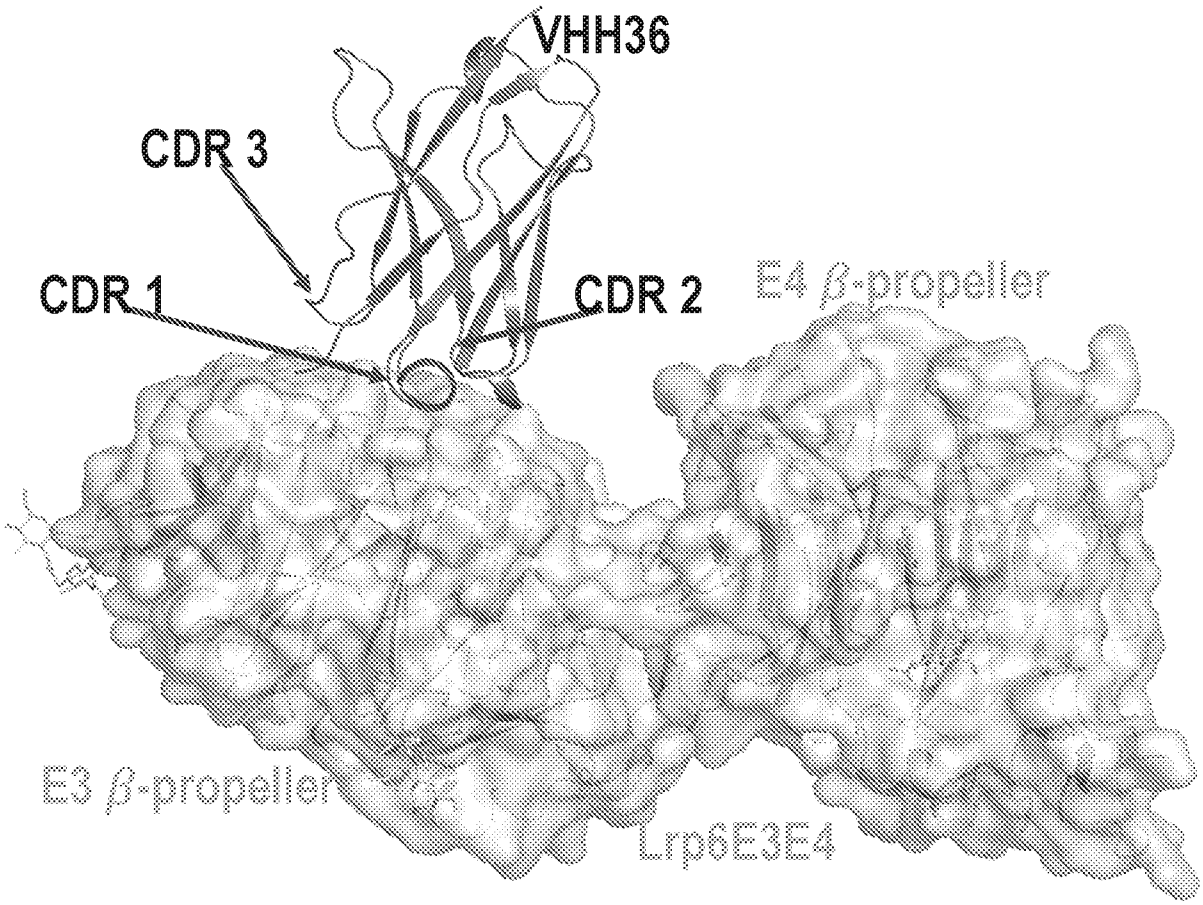


Figure 2



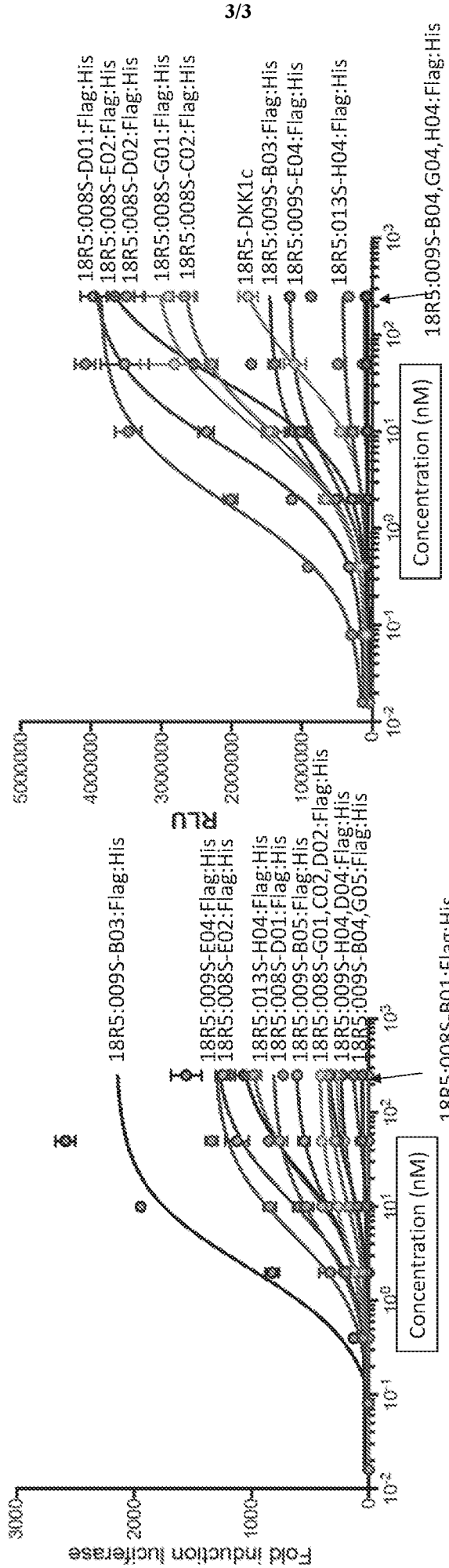

A375 Wnt reporter activation
Fold induction luciferase
Concentration (nM)
18R5:008S-B01:Flag:His
18R5:009S-E02,F02,G02,H02,A03,C03,E03,F03,G03,H03,A04,C04,G04,F04,A05,D05,E05,F05:Flag:His
HEK293 Wnt reporter activation with Rspo
RLU
18R5:009S-B03:Flag:His
18R5:009S-E04:Flag:His
18R5:008S-E02:Flag:His
18R5:013S-H04:Flag:His
18R5:008S-D01:Flag:His
18R5:009S-B05:Flag:His
18R5:008S-G01,C02,D02:Flag:His
18R5:009S-H04,D04:Flag:His
18R5:009S-B04,G05:Flag:His
18R5:009S-B04,G04,H04:Flag:His
18R5-DKK1c
18R5:008S-D01:Flag:His
18R5:008S-E02:Flag:His
18R5:008S-D02:Flag:His
18R5:008S-G01:Flag:His
18R5:008S-C02:Flag:His
18R5:009S-B03:Flag:His
18R5:009S-E04:Flag:His
18R5:013S-H04:Flag:His

Fig. 3

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 18/66620

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 39/395, A61K 48/00, A61P 1/04 (2019.01)
CPC - A61K 39/3955, C07K 16/468

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
See Search History Document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
See Search History Document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
See Search History Document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- A	US 2013/0058934 A1 (CONG et al.) 7 March 2013 (07.03.2013) para [0007-0008], [0170] [0175], [0473]	32-34 --- 1, 4/1, 7
A	US 2012/0237523 A1 (MASCOLA et al.) 20 September 2012 (20.09.2012) para [0152], SEQ ID NO: 1205	1, 4/1, 7
A	WO 2010/021697 A2 (BELOUSKI et al.) 25 February 2010 (25.02.2010) Claim 9, SEQ ID NO: 47	1, 4/1, 7
A	US 2015/0010560 A1 (AMGEN Inc.) 8 January 2015 Table 4B; SEQ ID NO: 128	1, 4/1, 7

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
11 March 2019	01 MAY 2019
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 18/66620

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 5, 6, 8-31
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

----------Continued on Supplemental Sheet----------

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1, 4/1, 7, 32-34

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 18/66620

Continuation of Box No. III Observations where unity of invention is lacking:

Group I+, claims claims 1-4, 7 and 32-34, drawn to an isolated antibody, or an antigen-binding fragment thereof, that binds to one or more LRP receptor. The anti-LRP antibody will be searched to the extent that the CDRH1, CDRH2 and CDRH3 sequences encompass amino acid sequences SEQ ID Nos: 171, 249 and 330, respectively. It is believed that claims 1, 4(in part), 7 and 32-34 encompass this first named invention (note, none of SEQ ID Nos: 1-24 in claims 2 and 3 comprise SEQ ID NO: 171), and thus these claims will be searched without fee to the extent that the CDRH1, CDRH2 and CDRH3 sequences encompass SEQ ID Nos: 171, 249 and 330. Additional CDRH1, CDRH2 and CDRH3 sequence(s) will be searched upon the payment of additional fees. Applicants must specify the claims that encompass any additionally elected CDRH1, CDRH2 and CDRH3 sequence(s). Applicants must further indicate, if applicable, the claims which encompass the first named invention, if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched. An exemplary election would be an anti-LRP antibody comprising CDRL1, CDRL2 and CDRL3 sequences SEQ ID Nos: 515, 529 and 578, respectively, (claims 1, 4(in part), 7 and 32-34 (note, none of SEQ ID Nos: 1-24 in claims 2 and 3 comprise SEQ ID NO: 515).

The inventions listed as Group I+ do not relate to a single special technical feature under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Special technical features

The inventions of Group I+ each include the special technical feature of a unique amino acid sequence. Each amino acid sequence encodes a unique peptide, and is considered a distinct technical feature.

No technical features are shared between the anti-LRP amino acid sequences of Group I+ and, accordingly, this group lacks unity a priori.

Additionally, even if Group I+ were considered to share the technical features of including: an isolated antibody, or an antigen-binding fragment thereof, that binds to one or more LRP5 or LRP6 receptor, comprising a sequence comprising: CDRH1, CDRH2 and CDRH3 sequences, and/or CDRL 1, CDRL2 and CDRL3 sequences;

wherein the antibody or antigen-binding fragment thereof binds to the E3 beta-propeller region of the LRP6 receptor or a corresponding region of the LRP5 receptor;

wherein the antibody or antigen-binding fragment thereof binds to one or more epitopes within the region of the LRP6 receptor comprising or consisting of amino acid residues 637-878 or a corresponding region of the LRP5 receptor; and

wherein the antibody or antigen-binding fragment thereof contacts the LRP receptor with a distance of less than 5 angstroms at any of the sets of amino acid residues indicated in Table 3.

However, these shared technical features were previously taught by US 2013/0058934 A1 to Cong et al. (hereinafter "Cong").

Cong teaches an isolated antibody, or an antigen-binding fragment thereof, that binds to a LRP6 receptor, wherein the antibody or antigen-binding fragment thereof binds to the E3 Beta-propeller region of the LRP6 receptor or a corresponding region of the LRP5 receptor (para [0007] - "The invention provides LRP6 antibodies (e.g., monovalent, bivalent) and methods of making LRP6 antibodies that inhibit or enhance the canonical Wnt signaling pathway. The LRP6 antibodies of the invention bind to distinct LRP6 Beta-propeller regions."; para [0008] - "Accordingly, in one aspect the invention pertains to an isolated bivalent antibody or a bivalent fragment thereof to a low density lipoprotein-related protein 6 (LRP6)").

Cong further teaches the antibody or antigen-binding fragment thereof binds to one or more epitopes within the region of the LRP6 receptor comprising or consisting of amino acid residues 637-878 or a corresponding region of the LRP5 receptor (para [0175] - "The extracellular domain of LRP6 is defined by amino acid residues 19 to 1246 and contains four Beta-propeller domains at amino acid residues 43-324, 352-627, 654-929, and 957-1250, which correspond to Beta-propeller regions 1, 2, 3 and 4, respectively."; para [0007] - "Propeller 3 antibodies bind to the Beta-propeller 3 domain and block Propeller3-dependent Wnts such as Wnt3a and Wnt3."; para [0008] - "Accordingly, in one aspect the invention pertains to an isolated bivalent antibody or a bivalent fragment thereof to a low density lipoprotein-related protein 6 (LRP6)").

Cong further teaches the antibody or antigen-binding fragment thereof contacts the LRP receptor with a distance of less than 5 angstroms at any of the sets of amino acid residues indicated in Table 3 (Note, applicant Table 3 comprises in part LRP6 residues Lys662, Glu663, Ile681, Trp850, Ser851, Arg853; para [0007] - "Propeller 3 antibodies bind to the Beta-propeller 3 domain"; [0170] "Additional characterization of the Propeller 3 domain of LRP6 identified residues in this domain responsible for interaction with the antibodies. Antibody binding sites within YWTD-EGF region of Propeller 3 were identified...and correspond to a concave surface between blade 1 and 6 of Propeller 3 domain"; [0471]-[0473] "To identify antibody binding sites within YWTD-EGF region of Propeller 3...Thus the binding assay data are in good agreement with the binding interface as mapped by HDx MS suggesting the residues R638, W850, S851, R852, and R853 participate directly in the epitope").

As the technical features were known in the art at the time of the invention, they cannot be considered special technical features that would otherwise unify the groups.

Therefore, Group I+ inventions lack unity under PCT Rule 13 because they do not share the same or corresponding special technical feature.

NOTE, continuation of Item 4 above: claims 5, 6, 8-31 are held unsearchable because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).