

WE CLAIM

1. A tetravalent binding antibody molecule comprising:

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

(b) a bivalent low-density lipoprotein receptor-related protein (LRP) binding domain, and

5 (c) a bivalent Frizzled (FZD) binding domain,

wherein the LRP binding domain is attached to one terminus of the Fc domain and the FZD binding domain is attached to the other end of the Fc domain,

wherein the LRP binding domain is selected from the group consisting of: a diabody that binds LRP5, a diabody that binds LRP6, two scFv that bind LRP5, two scFv that bind LRP6, two
10 Fab that bind LRP5, and two Fab that bind LRP6 and combinations thereof; and

wherein the FZD binding domain is selected from the group consisting of: a diabody that binds one or more FZD, two scFv that bind one or more FZD, two Fab that bind one or more FZD

wherein the FZD is a Frizzled Class Receptor 1 (FZD1), Frizzled Class Receptor 2 (FZD2), Frizzled Class Receptor 3 (FZD3), Frizzled Class Receptor 5 (FZD4), Frizzled Class Receptor 5
15 (FZD5), Frizzled Class Receptor 6 (FZD6), Frizzled Class Receptor 7 (FZD7), Frizzled Class Receptor 8 (FZD8), Frizzled Class Receptor 9 (FZD9), or Frizzled Class Receptor 10 (FZD10)

wherein when the LRP binding domain binds only LRP5, the FZD binding domain binds a FZD selected from the group consisting of FZD1, FZD2, FZD3, FZD5, FZD6, FZD7, FZD8, FZD9, FZD10, and combinations thereof.

20 2. ~~The tetravalent binding antibody molecule of claim 1, wherein~~

~~(a) the LRP binding domain diabody is attached to the N-terminal of the Fc domain, and~~

~~(b) the FZD binding domain is attached to the C-terminal of the Fc domain.~~

23. The tetravalent binding antibody molecule of claim 12, wherein the LRP binding domain diabody is attached to the N-terminal of the Fc domain, and the FZD binding domain is attached to the C-terminal of the Fc domain and wherein

(a) the LRP binding domain diabody is attached to the N-terminal of the Fc domain via a
5 VL or VH of the diabody, and

(b) the FZD binding domain comprises two FZD binding Fab fused to the C-terminal of the Fc, wherein each Fab is attached to the Fc domain via a heavy or light chain variable domain (VH or VL) of the Fab linked to the CH3 domain of the Fc domain.

4. ~~The tetravalent binding antibody molecule of claim 1, wherein the FZD binding domain is attached to the N-terminal of the Fc domain and the LRP binding domain is attached to the C-terminal of the Fc domain.~~

35. The tetravalent binding antibody molecule of claim 14, wherein the FZD binding domain is attached to the N-terminal of the Fc domain and the LRP binding domain is attached to the C-terminal of the Fc domain and wherein

15 (a) the FZD binding domain comprises two Fabs that bind the FZD, wherein each Fab is attached to N-terminal of the Fc domain via a heavy or light chain variable domain (VH or VL) of the Fab linked to the CH2 domain of the Fc domain, and

(b) the LRP binding domain comprises a diabody or two scFv that bind LRP5 and/or LRP6, wherein the diabody or two scFv are attached to the C-terminal of the Fc domain via a
20 VL or VH of the diabody or scFv linked to the CH3 of the Fc domain.

46. The tetravalent binding antibody molecule of claim 1, wherein the LRP binding domain and/or the FZD binding domain is bispecific.

7. ~~The tetravalent binding antibody molecule of claim 6 wherein the LRP binding domain binds to LRP5 and LRP6.~~

25 8. ~~The tetravalent binding antibody molecule of claim 7 wherein the LRP binding domain binds to a Wnt3A site (E3E4 domains) on LRP5 and a Wnt3A site (E3E4 domains) on LRP6.~~

59. The tetravalent binding antibody molecule of claim 1, wherein

(a) the diabody of the LRP binding domain binds LRP5 and comprises heavy chain complementary determining regions CDR-H1, CDR-H2 and CDR-H3 and light chain complementary determining regions CDR-L1, CDR-L2 and CDR-L3, of an antibody of Table 3 or Table 6, or

(b) the diabody of the LRP binding domain binds LRP6 and comprises heavy chain complementary determining regions CDR-H1, CDR-H2 and CDR-H3 and light chain complementary determining regions CDR-L1, CDR-L2 and CDR-L3 of an antibody of Table 4 or Table 6, or

(c) the diabody of the LRP binding domain binds LRP5 and LRP6, the diabody comprising CDR-H1, CDR-H2 and CDR-H3 and CDR-L1, CDR-L2 and CDR-L3 of an antibody of Table 3 or Table 6 and CDR-H1, CDR-H2 and CDR-H3 and CDR-L1, CDR-L2 and CDR-L3 of an antibody of Table 4 or Table 6.

610. The tetravalent binding antibody molecule of claim 1 wherein the Fc domain or fragment thereof is in a knob-in-hole configuration.

711. The tetravalent binding antibody molecule of claim 1, wherein the Fc domain lacks one or more effector functions.

812. The tetravalent binding antibody molecule of claim 1, wherein the LRP binding domain and FZD binding domain are each attached to the Fc domain by a linker.

~~13. The tetravalent binding antibody molecule of claim 12, wherein the linker comprises 1 to 100, 1 to 50, 1-30, 1-25, 1-10, 1-6 amino acids, 1-5 amino acids, or 2-4 amino acids.~~

~~14. The tetravalent binding antibody molecule of claim 1, wherein the LRP binding domain binds LRP6 or binds LRP5 and LRP6, and the FZD binding domain binds a FZD selected from the group consisting of: FZD1, FZD2, FZD3, FZD4, FZD5, FZD6, FZD7, FZD8, FZD9, and FZD10.~~

915. The tetravalent binding antibody molecule of claim 114, wherein the FZD binding domain binds FZD4 or FZD5 and the LRP binding diabody binds to a Wnt3A site on LRP5 and a Wnt 3A site on LRP6.

16. ~~The tetravalent binding antibody molecule of claim 15, wherein the FZD binding domain comprises two Fabs that bind FZD4 or FZD5 or multiple FZD.~~

1017. The tetravalent binding antibody molecule of claim 116, wherein the FZD-binding Fab comprise light chain complementary determining regions CDR-L1, CDR-L2 and CDR-L3 and heavy chain CDRs, CDR-H1, CDR-H2 and CDR-H3 of an antibody of Table 1, Table 2 or Table 6.

18. ~~The tetravalent binding antibody molecule of claim 15, wherein the LRP binding diabody binds to a Wnt3A site on LRP5 and a Wnt 3A site on LRP6.~~

19. ~~The tetravalent binding antibody molecule of claim 1, wherein~~

(a) ~~the diabody of the LRP binding domain binds LRP5 comprises heavy chain complementary determining regions CDR H1, CDR H2 and CDR H3 and light chain complementary determining regions CDR L1, CDR L2 and CDR L3, of an antibody of Table 3 or Table 6, or~~

(b) ~~the diabody of the LRP binding domain binds LRP6 and comprises heavy chain complementary determining regions CDR H1, CDR H2 and CDR H3 and light chain complementary determining regions CDR L1, CDR L2 and CDR L3 of an antibody of Table 4 or Table 6, or~~

(c) ~~the diabody of the LRP binding domain binds LRP5 and LRP6, the diabody comprising~~

(i) ~~CDR H1, CDR H2 and CDR H3 and CDR L1, CDR L2 and CDR L3 of an antibody of Table 3 or Table 6, and~~

(ii) ~~CDR H1, CDR H2 and CDR H3 and CDR L1, CDR L2 and CDR L3 of an antibody of Table 4 or Table 6.~~

~~1120~~. The tetravalent binding antibody molecule of claim ~~23~~ comprising,

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

(1) a peptide comprising a heavy chain variable domain (VH) that binds LRP5
and/or LRP6 and a light chain variable domain (VL) that binds LRP5 and/or
LRP6

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

(3) a VH that binds FZD4, FZD5, or FZD4 and/or FZD5 and one or more other
FZD, and

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

wherein

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

the VH that binds LRP6 comprises the CDR-H1, CDR-H2 and CDR-H3 of an
antibody of Table 4 or Table 6, and

(b) the VL that binds LRP5 comprises light chain CDRs (CDR-L1, CDR-L2 and CDR-
L3), of an antibody of Table 3 or Table 6, or

the VL that binds LRP6 comprises the CDR-L1, CDR-L2 and CDR-L3 of an antibody
of Table 4 or Table 6, and

(c) the VH that binds FZD4, FZD5, or FZD4 and/or FZD5 and one or more other FZD
comprises the heavy chain CDRs (CDR-H1, CDR-H2 and CDR-H3), of an antibody of
Table 1, Table 2 or Table 6,

and

(d) a third and fourth light chain monomer each comprising from N terminus to C terminus
a VL that binds FZD4, FZD5, or FZD4 and/or FZD5 and one or more other FZD and

a constant light chain domain 1 (CL1), the VL that binds FZD4, FZD5, or FZD4 and/or FZD5 and one or more other FZD comprises the light chain CDRs (CDR-L1, CDR-L2 and CDR-L3), of an antibody of Table 1, Table 2 or Table 6,

wherein the first and second heavy chain monomer dimerize via their Fc regions and the VL and VH that bind LRP5 or LRP6 of the first monomer pair with the VH and VL that bind LRP5 or LRP6 of the second monomer forming a bivalent diabody that binds LRP5 or LRP6, and

the CL1 and VLs that bind FZD4, FZD5, or FZD4 and/or FZD5 and one or more other FZD of the third and fourth light chain monomers pair with the CH1 and VHs that bind FZD4, FZD5, or FZD4 and/or FZD5 and one or more other FZD of the first and second heavy chain monomers forming two Fabs that bind FZD4, FZD5, or FZD4 and/or FZD5 and one or more other FZD, wherein the diabody forms the N-terminal bivalent LRP5 or LRP6 binding domain and the two Fab form the C-terminal bivalent FZD4, FZD5, or FZD4 and/or FZD5 and one or more other FZD binding domain.

1221. The tetravalent binding antibody molecule of claim 1120, wherein the LRP binding diabody is bispecific, wherein

the CDRs of the VHs that bind LRP5 or LRP6 of the first heavy chain monomer and the CDRs of the VHs that bind LRP5 or LRP6 of the second heavy chain monomer are non-identical, and

the CDRs of the VLs that bind LRP5 and LRP6 of the first heavy chain monomer and the CDRs of the VLs that bind LRP5 and LRP6 second heavy chain monomer are non-identical.

1322. The tetravalent binding antibody molecule of claim 1120, wherein the first and second monomers dimerize via a knob-in-hole configuration of the Fc regions or fragments thereof.

1423. The tetravalent binding antibody molecule of claim 1120, wherein the Fc domain lacks one or more effector functions.

1524. The tetravalent binding antibody molecule of claim 1120, wherein the peptide of (a)(1) is linked to the Fc region by a first peptide linker, and the Fc region is linked to the VH that binds

FZD4 or FZD5 or to multiple FZD (pan-FZD) by a second peptide linker and the VH that binds FZD4 or FZD5 or to multiple FZD (pan-FZD) is linked to the CH1 by a third peptide linker, and the VL that binds FZD4 or FZD5 or to multiple FZD (pan-FZD) is linked to the CL1 domain by a peptide linker.

5 ~~25. The tetravalent binding antibody molecule of claim 24, wherein one or more of the second, third or fourth peptide linker comprises 1 to 100, 1 to 50, 1-30, 1-25, 1-10 or 1-5 amino acids.~~

~~26. The tetravalent binding antibody molecule of claim 20, wherein in each first and second heavy chain monomer, the VH that binds to LRP5 or LRP6 is linked to the VL that binds to LRP5 or LRP6 by a peptide linker.~~

10 ~~27. The tetravalent binding antibody molecule of claim 26, wherein the peptide linker comprises 1-6 amino acids, 1-5 amino acids, or 2-4 amino acids.~~

~~28. The tetravalent binding antibody molecule of claim 20 wherein the tetravalent binding antibody molecule does not comprise a FZD binding domain comprising the CDRs of the FZD-binding antibody 5044 in combination with the Wnt co-receptor binding domain comprising the~~
 15 ~~CDRs of LRP6-binding antibody 2542 and/or antibody 2539.~~

1629. The tetravalent binding antibody molecule of claim 1120, wherein the tetravalent binding antibody molecule comprises

(a) a first heavy chain comprising the amino acid sequence of the hole heavy chain construct of FZD5-LRP6 of Table 11, a second heavy chain comprising the amino acid sequence of the knob heavy chain construct of FZD5-LRP6 of Table 11 and a light chain comprising the amino acid sequence of the light chain construct of LRP6 of Table 11 wherein the amino acid sequence of the CDRs are the amino acid sequence of the CDRs of ANT59, or

(b) a first heavy chain comprising the amino acid sequence of the hole heavy chain construct of pFZD-LRP6 of Table 11, a second heavy chain comprising the amino acid a knob heavy chain construct of pFZD-LRP6 of Table 11 and a light chain comprising the amino acid sequence of the light chain construct of pFZD-LRP6 of Table 11 wherein

the amino acid sequence of the CDRs are the amino acid sequence of the CDRs of ANT9.

1730. A tetravalent binding antibody molecule having an N-terminal FZD4 or FZD5 binding domain comprising two FZD4-binding or two FZD5-binding Fabs and a C-terminal LRP binding domain comprising two LRP binding scFvs, the tetravalent binding antibody molecule comprising

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

(i) a first heavy chain variable domain (VH) that binds FZD 4 or 5

(ii) a heavy chain constant region domain 1 (CH1 domain)

10 (ii) an Fc region or fragment thereof comprising a constant heavy chain domain 3 (CH3 domain),

(iv) a peptide comprising a VH that binds LRP5 or LRP6, and a first light chain variable domain (VL) that binds LRP5 or LRP6, and

15 (b) a third and fourth light chain monomer each monomer comprising from N terminus to C terminus a second VL that binds the FZD and a constant light chain domain 1 (CL1 domain),

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

20 wherein the first VH that binds FZD4 or FZD5 and CH1 domain of the first and second monomer pairs with the second VL and CL1 domain of the third and fourth monomers forming two Fabs that bind FZD4 or FZD5, and the second VH and first VL of each heavy chain monomer pair to form a scFv that binds LRP5 or LRP6,

25 wherein the LRP binding scFvs form a C-terminal LRP binding domain of the tetravalent binding antibody molecule and the FZD4 or FZD5 binding Fabs form an N-terminus FZD4 or FZD5-binding domain of the tetravalent binding antibody molecule,

wherein when the LRP binding domain binds only LRP5, the FZD binding domain binds FZD5.

1831. The tetravalent binding antibody molecule of claim 17-30, wherein

the VH that binds FZD4 or FZD5 comprises the heavy chain CDRs (CDR-H1, CDR-H2 and CDR-H3), of an antibody of Table 1, Table 2 or Table 6,

the VH that binds LRP5 comprises heavy chain CDRs (CDR-H1, CDR-H2 and CDR-H3), of an antibody of Table 3 or Table 6, or the VH that binds LRP6 comprises the CDR-H1, CDR-H2 and CDR-H3 of an antibody of Table 4, and

the VL that binds LRP5 comprises light chain CDRs (CDR-L1, CDR-L2 and CDR-L3), of an antibody of Table 3 or Table 6, or the VL that binds LRP6 comprises the CDR-L1, CDR-L2 and CDR-L3 of an antibody of Table 4.

1932. A tetravalent binding antibody molecule comprising an N-terminal LRP binding diabody and a C-terminal domain comprising two FZD4-binding or FZD5-binding scFv, the tetravalent binding antibody molecule comprising

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

(a) a first peptide, said first peptide comprising a first heavy chain variable domain (VH) and a first light chain variable (VL) domain that bind LRP5 or LRP6,

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

(c) a second peptide, said second peptide comprising a second VL and a second VH that bind a FZD4 or FZD5,

wherein the first and second monomers dimerize via the Fc regions or fragments thereof, the first VH and VL of each monomer pairs with the first VH and VL of the other monomer forming a diabody that binds LRP5 or LRP6 and the second VL and VH of each monomer pair to form a scFvs that bind FZD4 or FZD5,

wherein the LRP binding diabody forms the N-terminal LRP binding domain of the tetravalent binding antibody molecule and the two FZD4-binding or FZD5-binding scFvs form the C-terminal FZD-binding domain of the tetravalent binding antibody molecule

wherein when the LRP binding domain binds only LRP5, the FZD binding domain binds FZD5.

2033. The tetravalent binding antibody molecule of claim 19-32, wherein

the VH that binds LRP5 comprises heavy chain CDRs (CDR-H1, CDR-H2 and CDR-H3), of an antibody of Table 3 or Table 6, or the VH that binds LRP6 comprises the CDR-H1, CDR-H2 and CDR-H3 of an antibody of Table 4,

the VL that binds LRP5 comprises light chain CDRs (CDR-L1, CDR-L2 and CDR-L3), of an antibody of Table 3 or Table 6, or the VL that binds LRP6 comprises the CDR-L1, CDR-L2 and CDR-L3 of an antibody of Table 4, and

the VH that binds FZD comprises the heavy chain CDRs (CDR-H1, CDR-H2 and CDR-H3), of an antibody of Table 1, Table 2 or Table 6.

2134. A tetravalent binding antibody molecule in a Diabody-Fc-scFv format comprising an Fc domain, a LRP5/6 co-receptor binding domain including a bispecific diabody that binds at least two sites on the co-receptor,

wherein the diabody is attached to the amino terminus of the Fc domain and a FZD binding domain is attached to the carboxy terminus of the Fc domain comprising two scFv fragments each binding FZD wherein the scFv is specific for FZD5, or pan-specific binding to FZD5 and one or more other FZD.

2235. A tetravalent binding antibody molecule in an IgG-diabody format having an Fc domain, a FZD binding domain that comprises of two Fab fragments attached to the N-terminus of the Fc domain, each Fab binding to an FZD5, or FZD4 and FZD5 and a LRP5/6 co-receptor binding domain attached to the C- terminus of the Fc domain that is composed of a diabody that binds at

least two sites on the co-receptor LRP5/6, wherein the Fabs are specific for FZD5 or pan-specific, binding to FZD5 and one or more FZD.

2336. A tetravalent binding antibody molecule in an IgG-Diabody format comprising an Fc domain, an N-terminal binding domain for a FZD, wherein the FZD-binding Fabs bind FZD5 or FZD5 and at least one other FZD and a C-terminal binding domain for a LRP5/6 co-receptor and including a LRP5/6 coreceptor-binding diabody.

2437. The tetravalent binding antibody molecule of claim 2336, comprising:

a first and second heavy chain monomer, wherein each heavy chain monomer includes a single-chain polypeptide from N-terminus to C-terminus:

(a) a heavy chain variable domain (VH) that binds FZD5 or FZD4 and FZD5, linked to

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

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

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

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

~~38. The tetravalent binding antibody molecule of any one of claims 1 to 37, wherein the tetravalent binding antibody molecule comprises~~

~~(a) a first heavy chain comprising the amino acid sequence of the hole heavy chain construct of SEQ ID NO: 986, a second heavy chain comprising the amino acid a knob heavy chain construct of SEQ ID NO: 987 and a light chain comprising the amino acid sequence of the light chain construct of SEQ ID NO: 992 wherein the amino acid sequence of the CDRs are the amino acid sequence of the CDRs of ANT80; or~~

(b) ~~a first heavy chain comprising the amino acid sequence of the hole heavy chain construct of SEQ ID NO: 988, a second heavy chain comprising the amino acid a knob heavy chain construct of SEQ ID NO: 989 and a light chain comprising the amino acid sequence of the light chain construct of SEQ ID NO: 992 wherein the amino acid sequence of the CDRs are the amino acid sequence of the CDRs of ANT 98; or~~

(c) ~~a first heavy chain comprising the amino acid sequence of the hole heavy chain construct of SEQ ID NO: 990, a second heavy chain comprising the amino acid a knob heavy chain construct of SEQ ID NO: 991 and a light chain comprising the amino acid sequence of the light chain construct of SEQ ID NO: 992 wherein the amino acid sequence of the CDRs are the amino acid sequence of the CDRs of ANT99; or~~

~~(d) a first heavy chain comprising the amino acid sequence of the hole heavy chain construct selected from the group consisting of SEQ ID NO: 986, SEQ ID NO: 988, and SEQ ID NO: 990; a second heavy chain comprising the amino acid of a knob heavy chain construct selected from the group consisting of SEQ ID NO: 987, SEQ ID NO: 989, and SEQ ID NO: 991; and a light chain comprising the amino acid sequence of the light chain construct of SEQ ID NO: 992.~~

2539. A pharmaceutical composition comprising a tetravalent binding antibody molecule of any one claims 1 to 2438 and a pharmaceutically acceptable carrier.

~~40. A method for promoting endothelial cell barrier functions in a tissue comprising administering an effective amount of a tetravalent binding antibody molecule of any one of claims 1 to 38 to a tissue.~~

~~41. The method of claim 40, wherein the tissue is brain, kidney or eye tissue.~~

~~42. The method of claim 41, wherein the tetravalent binding antibody molecule is administered to eye tissue by intravitreal injection.~~

~~43. A method for treating inflammatory bowel disease in a subject in need thereof comprising administering an effective amount of a tetravalent binding molecule of any one of claims 1 to 38 to said subject.~~

~~44. A method for increasing retinal or brain endothelial cell barrier functions, decreasing endothelial cell permeability, enhancing or restoring blood retina and blood brain barrier maintenance in a subject in need thereof comprising contacting an endothelial cell comprising an FZD4 receptor and an LRP5 and/or LRP6 in a subject in need thereof with an effective amount of a tetravalent binding antibody molecule of any one of claim 1 to 38.~~

~~45. The method of claim 43 or 44, wherein the tetravalent binding antibody molecule is administered to the subject in need thereof by injection, topically, or orally.~~

~~46. The method of claim 43 or 44, wherein the tetravalent binding antibody molecule is administered subcutaneously, intravenously, intraperitoneally, intrathecally, intravitreously or intraocularly.—~~