the retina. The resulting formation of the avascular region creates a retinal ischemic area, which results in primary damage to the retina. This ischemic condition induces the production of vascular endothelial growth factor (VEGF) and angiopoietin2 (Ang2), leading to neovascularization and vascular tuft formation. The newly generated abnormal blood vessels can be easily broken, leading to secondary damage of the retina due to exudation and hemorrhage. Disease progression of diabetic retinopathy (DR) is similar to that of FEVR and other genetic vascular malformation or insufficiency diseases. Hyperglycemia induces retinal vessel damage, leading to vaso-obliteration, ischemia, neovascularization, and hemorrhage, eventually leading to retinopathy.

[007] While genetic data has suggested the importance of Wnt signaling in establishing the proper vascular structure in the eye, whether activation of Wnt signaling post-developmentally would lead to improvement in vascular structure is unknown. Certain reports have even suggested that antagonizing rather than agonizing Wnt signaling would be beneficial in the treatment of retinopathy. Therefore, a greater understanding of retinopathy, disease progression, and Wnt signal involvement therein would be beneficial as this potentially could result in new treatments. For effective treatment of retinopathy in particular, there is a need for modulating Wnt agonist and antagonist signaling during specific stages of disease. The present invention addresses these needs and provides methods for modulating Wnt signaling agonism and antagonism during different stages of retinopathy and disease progression.

## SUMMARY

[008] The present disclosure provides multispecific antibodies or antibody fragments.

[009] A multispecific antibody or antibody fragment according to the present disclosure may comprise at least: (A) a first antigen-binding region which specifically binds to frizzled class receptor 4 (Fzd4) and comprises a first heavy chain variable domain (VH1) and a first light chain variable domain (VL1), (B) a second antigen-binding region which specifically binds to low density lipoprotein receptor-related protein 5 and/or 6 (LRP5 and/or LRP6) and comprises at least a second heavy chain variable domain (VH2).

[0010] In some examples, the VH1 comprises: (i) a heavy chain complementarity-determining region 1 (CDRH1) which has an amino acid sequence which: (i-1) has at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to the amino acid sequence of SEQ ID NO: 121 or to the CDRH1 amino acid sequence contained in SEQ ID NO: 181, optionally defined according to Kabat; (i-2) comprises or consists of the amino acid sequence of SEQ ID NO: 120, wherein $X_1$ is any amino acid, optionally S, A, Q, or N; or (i-3) comprises or consists of the amino acid sequence of SEQ ID NO: 121, 122, 123, or 124, or comprises or consists of the CDRH1 amino acid sequence contained in any one of SEQ ID NOS: 181-188, optionally defined according to Kabat; (ii) a heavy chain complementarity-determining region 2 (CDRH2) has an amino acid sequence which: (ii-1) has at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to the amino acid sequence of SEQ ID NO: 141 or to the CDRH2 amino acid sequence contained in SEQ ID NO: 181, optionally defined according to Kabat; (ii-2) comprises or consists of the amino acid sequence of SEQ ID NO: 140, wherein $X_1$ is any amino acid, optionally E, A, S, or D; or (ii-3) comprises or consists of: the amino acid sequence of any one of SEQ ID NO: 141, or 142, 143, or 144; or comprises or consists of the CDRH2 amino acid sequence contained in any one of SEQ ID NOS: 181-188, optionally defined by Kabat; and (iii) a heavy chain complementarity-determining region 3 (CDRH3) has an amino acid sequence which: (iii-1) has at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to the amino acid sequence of SEQ ID NO: 161 or to the CDRH3 amino acid sequence contained in SEQ ID NO: 181, optionally defined by Kabat; (iii-2) comprises or consists of the amino acid sequence of SEQ ID NO: 160, wherein $X_1$ is any amino acid, optionally D or any amino acid which is not S, E, A, R, T, L, H, Y, Q, or K; (iii-3) comprises or consists of the amino acid sequence of SEQ ID NO: 161, or comprises or consists of the CDRH3 amino acid sequence contained in any one of SEQ ID NOS: 181-188, optionally defined by Kabat.

[0011] In some examples, the VL1 comprises: (iv) a light chain complementarity-determining region 1 (CDRL1) which has an amino acid sequence which: (iv-1) has at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at

least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to the amino acid sequence of SEQ ID NO: 221 or to the CDRL1 amino acid sequence contained in SEQ ID NO: 281, optionally defined by Kabat; or (iv-2) comprises or consists of the amino acid sequence of SEQ ID NO: 221, or comprises or consists of the CDRL1 amino acid sequence contained in SEQ ID NO: 281, optionally defined according to Kabat; (v) a light chain complementarity-determining region 2 (CDRL2) which has an amino acid sequence which comprises or consists of: (v-1) the amino acid sequence of SEQ ID NO: 241; or (v-2) the CDRL2 amino acid sequence contained in SEQ ID NO: 281, optionally defined according to Kabat; and (vi) a light chain complementarity-determining region 3 (CDRL3) which has an amino acid sequence which comprises or consists of: (vi-1) the amino acid sequence of SEQ ID NO: 261; or (vi-2) the CDRL3 amino acid sequence contained in SEQ ID NO: 281, optionally defined according to Kabat.

[0012] In some examples, the VH2 comprises: (i) a CDRH1 which has an amino acid sequence which comprises or consists of: (i-1) the amino acid sequence of SEQ ID NO: 321; or (i-2) the CDRH1 amino acid sequence contained in any one of SEQ ID NOS: 381, 386, and 393-396, optionally defined according to IMGT or Kabat; (ii) a CDRH2 which has an amino acid sequence which: (ii-1) has at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to the amino acid sequence of SEQ ID NO: 341 or to the CDRH3 amino acid sequence contained in SEQ ID NO: 381, optionally defined according to IMGT or Kabat; (ii-2) comprises or consists of the amino acid sequence of SEQ ID NO: 341; or (ii-3) comprises or consists of the CDRH2 amino acid sequence contained in any one of SEQ ID NOS: 381, 386, and 393-396, optionally defined according to IMGT or Kabat; and (iii) a CDRH3 which has an amino acid sequence which: (iii-1) has at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to the amino acid sequence of SEQ ID NO: 361 or to the CDRH3 amino acid sequence contained in SEQ ID NO: 381, optionally defined according to IMGT or MGT or Kabat; (iii-2) comprises or consists of the amino acid sequence of SEQ ID NO: 360, wherein $X_1$, $X_2$, and $X_3$ are individually any amino acids provided that (a) $X_1$ and $X_2$ are D and E, respectively, (b) $X_1$ and $X_3$ are D and D, respectively, and/or (c) $X_2$ and $X_3$ are E and D, respectively, optionally wherein $X_1$, $X_2$, and $X_3$ are D, E, and D, respectively, D, D, and D, respectively, D, E, and E,

respectively, D, E, and S, respectively, D, E, and A, respectively, or D, E, and T, respectively; or (iii-3) comprises or consists of the amino acid sequence of any one of SEQ IDS NO: 361, 362, 363, 364, 365, and 366, or comprises or consists of the CDRH3 amino acid sequence contained in any one of SEQ ID NOS: 381, 382, 388, 393, 394, 395, and 396, optionally defined according to IMGT or Kabat.

[0012a] In one aspect, the present disclosure provides multispecific antibody or antibody fragment comprising at least:

(A) a first antigen-binding region which specifically binds to frizzled class receptor 4 (Fzd4) and comprises a first heavy chain variable domain (VH1) and a first light chain variable domain (VL1),

(B) a second antigen-binding region which specifically binds to low density lipoprotein receptor-related protein 5 and/or 6 (LRP5 and/or LRP6) and comprises at least a second heavy chain variable domain (VH2),

wherein:

(A) the VH1 comprises:

(i) a heavy chain complementarity-determining region 1 (CDRH1) which has an amino acid sequence which:

(i-1) has at least 80% identity to the amino acid sequence of SEQ ID NO: 121 or to the CDRH1 amino acid sequence contained in SEQ ID NO: 181, optionally defined according to Kabat; or

(i-2) comprises or consists of the amino acid sequence of SEQ ID NO: 121, or comprises or consists of the CDRH1 amino acid sequence contained in SEQ ID NO: 181, optionally defined according to Kabat;

(ii) a heavy chain complementarity-determining region 2 (CDRH2) which has an amino acid sequence which:

(ii-1) has at least 90% identity to the amino acid sequence of SEQ ID NO: 141 or to the CDRH2 amino acid sequence contained in SEQ ID NO: 181, optionally defined according to Kabat;

or

(ii-2) comprises or consists of: the amino acid sequence of SEQ ID NO: 141; or comprises or consists of the CDRH2 amino acid sequence contained in SEQ ID NO: 181, optionally defined by Kabat; and

(iii) a heavy chain complementarity-determining region 3 (CDRH3) which has an amino acid sequence which:

(iii-1) has at least 90% identity to the amino acid sequence of SEQ ID NO: 161 or to the CDRH3 amino acid sequence contained in SEQ ID NO: 181, optionally defined by Kabat; or

(iii-2) comprises or consists of the amino acid sequence of SEQ ID NO: 161, or comprises or consists of the CDRH3 amino acid sequence contained in SEQ ID NO: 181, optionally defined by Kabat; and

the VL1 comprises:

(iv) a light chain complementarity-determining region 1 (CDRL1) which has an amino acid sequence which:

(iv-1) has at least 90% identity to the amino acid sequence of SEQ ID NO: 221 or to the CDRL1 amino acid sequence contained in SEQ ID NO: 281, optionally defined by Kabat; or

(iv-2) comprises or consists of the amino acid sequence of SEQ ID NO: 221, or comprises or consists of the CDRL1 amino acid sequence contained in SEQ ID NO: 281, optionally defined according to Kabat;

(v) a light chain complementarity-determining region 2 (CDRL2) which has an amino acid sequence which comprises or consists of:

(v-1) the amino acid sequence of SEQ ID NO: 241; or

(v-2) the CDRL2 amino acid sequence contained in SEQ ID NO: 281, optionally defined according to Kabat; and

(vi) a light chain complementarity-determining region 3 (CDRL3) which has an amino acid sequence which comprises or consists of:

(vi-1) the amino acid sequence of SEQ ID NO: 261; or

(vi-2) the CDRL3 amino acid sequence contained in SEQ ID NO: 281, optionally defined according to Kabat; and

(B) the VH2 comprises:

(i) a CDRH1 which has an amino acid sequence which comprises or consists of:

(i-1) the amino acid sequence of SEQ ID NO: 321; or

(i-2) the CDRH1 amino acid sequence contained in SEQ ID NO: 381, optionally defined according to IMGT or Kabat;

(ii) a CDRH2 which has an amino acid sequence which:

(ii-1) has at least 90% identity to the amino acid sequence of SEQ ID NO: 341 or to the CDRH3 amino acid sequence contained in SEQ ID NO: 381, optionally defined according to IMGT or Kabat;

(ii-2) comprises or consists of the amino acid sequence of SEQ ID NO: 341; or

(ii-3) comprises or consists of the CDRH2 amino acid sequence contained in SEQ ID NO: 381, optionally defined according to IMGT or Kabat; and

(iii) a CDRH3 which has an amino acid sequence which:

(iii-1) has at least 90% identity to the amino acid sequence of SEQ ID NO: 361 or to the CDRH3 amino acid sequence contained in SEQ ID NO: 381, optionally defined according to IMGT or Kabat; or

(iii-2) comprises or consists of the amino acid sequence of any one of SEQ ID NOs: 361 and 363-366, or comprises or consists of the CDRH3 amino acid sequence contained in any one of SEQ ID NOS: 381 and 393-396, optionally defined according to IMGT or Kabat.

[0013] In some cases, the multispecific antibody or antibody fragment may comprise the VH1 described above, the VL1 described above, and the VH2 described above.

[0014] In some cases, the VH2 is, comprises, or is comprised in a nanobody.

[0015] In some cases, the multispecific antibody or antibody fragment is a bispecific antibody or antibody fragment.

[0016] In certain examples, the VH1 comprises a CDRH1, a CDRH2, and a CDRH3 having the amino acid sequences of: (i) SEQ ID NOS: 121, 141, and 161, respectively, (ii) SEQ ID NOS: 122, 142, and 161, respectively, (iii) SEQ ID NOS: 121, 142, and 161, respectively, (iv) SEQ ID NOS: 123, 142, and 161, respectively, (v) SEQ ID NOS: 124, 142, and 161, respectively, (vi) SEQ ID NOS: 122, 141, and 161, respectively, (vii) SEQ ID NOS: 122, 143, and 161, respectively,

(viii) SEQ ID NOS: 122, 144, and 161, respectively; (ix) SEQ ID NOS: 121, 143, and 161, respectively, (x) SEQ ID NOS: 121, 144, and 161, respectively, (xi) SEQ ID NOS: 123, 141, and 161, respectively, (xii) SEQ ID NOS: 123, 143, and 161, respectively, (xiii) SEQ ID NOS: 123, 144, and 161, respectively, (xiv) SEQ ID NOS: 124, 141, and 161, respectively, (xv) SEQ ID NOS: 124, 143, and 161, respectively, or (xvi) SEQ ID NOS: 124, 144, and 161, respectively.

[0017] In certain examples, the VL1 comprises a CDRL1, a CDRL2, and a CDRL3 having the amino acid sequences of SEQ ID NOS: 221, 241, and 261, respectively.

[0018] In certain examples, the VH2 comprises a CDRH1, a CDRH2, and a CDRH3 having the amino acid sequences of: (i) SEQ ID NOS: 321, 341, and 361, respectively, (ii) SEQ ID NOS: 321, 341, and 362, respectively, (iii) SEQ ID NOS: 321, 341, and 363, respectively, (iv) SEQ ID NOS: 321, 341, and 364, respectively, (v) SEQ ID NOS: 321, 341, and 365, respectively, or (vi) SEQ ID NOS: 321, 341, and 366, respectively.

[0019] In some cases, the multispecific antibody or antibody fragment may comprise the VH1 described above, the VL1 described above, and the VH2 described above.

[0020] In certain examples, the VH1 comprises: (i) the CDRH1, the CDRH2, and the CDRH3 of the VH1 comprising the amino acid sequence contained in any one of SEQ ID NOS: 181-188, optionally according to Kabat; or (ii) a CDRH1, a CDRH2, and a CDRH3 comprising the amino acid sequences of: (ii-1) SEQ ID NOS: 121, 141, and 161, respectively, (ii-2) SEQ ID NOS: 122, 142, and 161, respectively, (ii-3) SEQ ID NOS: 121, 142, and 161, respectively, (ii-4) SEQ ID NOS: 123, 142, and 161, respectively, (ii-5) SEQ ID NOS: 124, 142, and 161, respectively, (ii-6) SEQ ID NOS: 122, 141, and 161, respectively, (ii-7) SEQ ID NOS: 122, 143, and 161, respectively, or (ii-8) SEQ ID NOS: 122, 144, and 161, respectively.

[0021] In certain examples, the VL1 comprises: (i) the CDRL1, the CDRL2, and the CDRL3 of the VL1 comprising the amino acid sequence contained in SEQ ID NO: 281, optionally according to Kabat, (ii) a CDRL1, a CDRL2, and a CDRL3 comprising the amino acid sequences of SEQ ID NOS: 221, 241, and 261, respectively.

[0022] In certain examples, the VH2 comprises: (i) the CDRH1, the CDRH2, and the CDRH3 of the VH2 comprising the amino acid sequence contained in any one of SEQ ID NOS: 381, 386, 393, 394, 395, and 396, optionally according to IMGT or Kabat; or (ii) a CDRH1, a CDRH2, and a CDRH3 comprising the amino acid sequences of: (ii-1) SEQ ID NOS: 321, 341, and 361, respectively, (ii-2) SEQ ID NOS: 321, 341, and 362, respectively, (ii-3) SEQ ID NOS: 321, 341, and 363, respectively, (ii-4) SEQ ID NOS: 321, 341, and 364, respectively, (ii-5) SEQ ID NOS: 321, 341, and 365, respectively, (ii-6) SEQ ID NOS: 321, 341, and 366, respectively.

[0023] In some cases, the multispecific antibody or antibody fragment may comprise the VH1 described above, the VL1 described above, and the VH2 described above.

[0024] In particular examples, (A) the VH1 comprises: (i) the CDRH1, the CDRH2, and the CDRH3 of the VH1 comprising the amino acid sequence contained in SEQ ID NO: 181, optionally according to Kabat; or (ii) a CDRH1, a CDRH2, and a CDRH3 comprising the amino acid sequences of SEQ ID NOS: 121, 141, and 161, respectively, and the VL1 comprises: (i) the CDRL1, the CDRL2, and the CDRL3 of the VL1 comprising the amino acid sequence contained in SEQ ID NO: 281, optionally according to Kabat, or (ii) a CDRL1, a CDRL2, and a CDRL3 comprising the amino acid sequences of SEQ ID NOS: 221, 241, and 261, respectively; and (B) the VH2 comprises: (i) the CDRH1 , the CDRH2, and the CDRH3 of the VH2

comprising the amino acid sequence contained in any one of SEQ ID NO: 381, 393, 394, 395, or 396, optionally according to IMGT or Kabat; or (ii) a CDRH1, a CDRH2, and a CDRH3 comprising the amino acid sequences of SEQ ID NOS: 321, 341, and 361, respectively, SEQ ID NOS: 321, 341, and 363, respectively, SEQ ID NOS: 321, 341, and 364, respectively, SEQ ID NOS: 321, 341, and 365, or SEQ ID NOS: 321, 341, and 366, respectively.

[0025] In certain examples, the VH1 comprises: (i) a heavy chain framework region 1 (FRH1), which has an amino acid sequence which: (i-1) has at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to the amino acid sequence of SEQ ID NO: 111 or to the FRH1 amino acid sequence contained in SEQ ID NO: 181, optionally according to Kabat; (i-2) comprises or consists of the amino acid sequence of SEQ ID NO: 110, wherein $X_1$ is any amino acid, optionally Q or K; (i-3) comprises or consists of the amino acid sequence of SEQ ID NO: 111 or 112, or comprises or consists of the FRH1 amino acid sequence contained in any one of SEQ ID NOS: 181-188, optionally according to Kabat; and/or (i-4) consists of, or is derived from the FRH1 encoded by the human germline IGHV3-23*04; (ii) a heavy chain framework region 2 (FRH2), which has an amino acid sequence which: (ii-1) has at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to the amino acid sequence of SEQ ID NO: 131 or to the FRH2 amino acid sequence contained in SEQ ID NO: 181, optionally according to Kabat; (ii-2) comprises or consists of the amino acid sequence of SEQ ID NO: 130 or 131, or comprises or consists of the FRH2 amino acid sequence contained in any of SEQ ID NOS: 181-188, optionally according to Kabat; and/or (ii-3) consists of, or is derived from the FRH2 encoded by the human germline IGHV3-23*04; (iii) a heavy chain framework region 3 (FRH3), which has an amino acid sequence which: (iii-1) has at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to the amino acid sequence of SEQ ID NO: 151 or to the FRH3 amino acid sequence contained in SEQ ID NO: 181, optionally according to Kabat; (iii-2) comprises or consists of the amino acid sequence of SEQ ID NO: 150, wherein $X_1$ is any amino acid, optionally N or D, $X_2$ is any amino acid, optionally R or K, and $X_3$ is any amino acid, optionally A or T; (iii-3) comprises or

consists of the amino acid sequence of SEQ ID NO: 151 or 152, comprises or consists of or the FRH3 amino acid sequence contained in any of SEQ ID NOS: 181-188, optionally according to Kabat; and/or (iii-4) consists of, or is derived from the FRH3 encoded by the human germline IGHV3-23*04; and/or (iv) a heavy chain framework region 4 (FRH4), which has an amino acid sequence which: (iv-1) has at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to the amino acid sequence of SEQ ID NO: 171 or to the FRH4 amino acid sequence contained in SEQ ID NO: 181, optionally according to Kabat; (iv-2) comprises or consists of the amino acid sequence of SEQ ID NO: 170 or 171, or comprises or consists of the FRH4 amino acid sequence contained in any of SEQ ID NOS: 181-188, optionally according to Kabat; and/or (iv-3) consists of, or is derived from the FRH4 encoded by the human germline IGHJ4*01.

[0026] In certain examples, the VL1 comprises: (v) a light chain framework region 1 (FRL1), which has an amino acid sequence which: (v-1) has at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to the amino acid sequence of SEQ ID NO: 211 or to the FRL1 amino acid sequence contained in SEQ ID NO: 281, optionally according to Kabat; or (v-2) comprises or consists of the amino acid sequence of SEQ ID NO: 211, or comprises or consists of the FRL1 amino acid sequence contained in SEQ ID NO: 281, optionally according to Kabat; (vi) a light chain framework region 2 (FRL2), which has an amino acid sequence which: (vi-1) has at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to the amino acid sequence of SEQ ID NO: 231 or to the FRL2 amino acid sequence contained in SEQ ID NO: 281, optionally according to Kabat; or (vi-2) comprises or consists of the amino acid sequence of SEQ ID NO: 231, or comprises or consists of the FRL2 amino sequence contained in SEQ ID NO: 281, optionally according to Kabat; (vii) a light chain framework 3 (FRL3), which has an amino acid sequence which: (vii-1) has at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to the amino acid sequence of SEQ ID NO: 251 or to the FRL3 amino acid sequence contained in SEQ ID NO: 281, optionally according to Kabat; or (vii-2) comprises or consists of

the amino acid sequence of SEQ ID NO: 251, or comprises or consists of the FRL3 amino acid sequence contained in SEQ ID NO: 281, optionally according to Kabat; and/or (viii) a light chain framework 4 (FRL4), which has an amino acid sequence which: (viii-1) has at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to the amino acid sequence of SEQ ID NO: 271 or to the FRL4 amino acid sequence contained in SEQ ID NO: 281, optionally according to Kabat; or (viii-2) comprises or consists of the amino acid sequence of SEQ ID NO: 271, or comprises or consists of the FRL4 amino acid sequence contained in SEQ ID NO: 281, optionally according to Kabat.

[0027] In certain examples, the VH2 comprises: (i) a FRH1, which has an amino acid sequence which: (i-1) has at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to the amino acid sequence of SEQ ID NO: 311 or to the FRH1 amino acid sequence contained in SEQ ID NO: 381, optionally according to IMGT or Kabat; (i-2) comprises or consists of the amino acid sequence of SEQ ID NO: 310, wherein $X_1$ is any amino acid, optionally E or D, $X_2$ is any amino acid, optionally A or T, and $X_3$ is any amino acid, optionally S, optionally wherein the FRH1 does not consist of the amino acid sequence of SEQ ID NO: 310 wherein $X_1$, $X_2$, and $X_3$ are E, A, and A, respectively; (i-3) comprises or consists of the amino acid sequence of SEQ ID NO: 311 or 312, or comprises or consists of the FRH1 amino acid sequence contained in any one of SEQ ID NOS: 381, 382-386, and 393-396, optionally according to IMGT or Kabat; and/or (i-4) consists of, or is derived from the FRH1 encoded by the human germline IGHV3- 66*01; (ii) a FRH2, which has an amino acid sequence which: (ii-1) has at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to the amino acid sequence of SEQ ID NO: 331 or to the FRH2 amino acid sequence contained in SEQ ID NO: 381, optionally according to IMGT or Kabat; (ii-2) comprises or consists of the amino acid sequence of SEQ ID NO: 330, wherein $X_1$ is any amino acid, optionally Y, $X_2$ is any amino acid, optionally Q, $X_3$ is any amino acid, optionally R, $X_4$ is any amino acid, optionally L, $X_5$ is any amino acid, optionally I, and $X_6$ is any amino acid, optionally A, optionally wherein the FRH2 does not consist of the amino acid sequence of SEQ ID NO: 330 wherein $X_1$, $X_2$, $X_3$, $X_4$, $X_5$, and $X_6$ are V, G, L, W, V,

[0028] In some cases, the multispecific antibody or antibody fragment may comprise the VH1 described above, the VL1 described above, and the VH2 described above.

[0029] In certain examples, the VH1 comprises an amino acid sequence which: (i) has at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to the amino acid sequence of any one of SEQ ID NOS: 181-188; and/or (ii) comprises or consists of the amino acid sequence of SEQ ID NO: 180, wherein: $X_1$ is any amino acid, optionally Q or K; $X_2$ is any amino acid, optionally S, A, Q, or N; $X_3$ is any amino acid, optionally E, A, S, or D; $X_4$ is any amino acid, optionally N or D; $X_5$ is any amino acid, optionally R or K; $X_6$ is any amino acid, optionally E or T; and $X_7$ is any amino acid, optionally D or any amino acid which is not S, E, A, R, T, L, H, Y, Q, or K.

[0030] In certain examples, the VL1 comprises an amino acid sequence which has at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to the amino acid sequence of SEQ ID NO: 281.

[0031] In certain examples, the VH2 comprises an amino acid sequence which: (i) has at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to the amino acid sequence of any one of SEQ ID NOS: 381, 386, and 393-396; and/or (ii) comprises or consists of the amino acid sequence of SEQ ID NO: 380, wherein: $X_1$ is any amino acid, optionally E or D; $X_2$ is any amino acid such as A or T; and $X_3$ is any amino acid such as S; $X_4$ is Q; $X_5$ is any amino acid, such as Y; $X_6$ is any amino acid, such as Q or G; $X_7$ is any amino acid, such as R; $X_8$ is any amino acid, such as L or W; $X_9$ is any amino acid, such as I; and $X_{10}$ is any amino acid, such as A; $X_{11}$ is M; $X_{12}$ is M; $X_{13}$ is any amino acid, such as D or G; $X_{14}$ is any amino acid, such as L; $X_{15}$ is any amino acid, such as M; $X_{16}$ is any amino acid, such as T; $X_{17}$ is any amino acid, such as D or E; $X_{18}$ is any amino acid, such as N or S; $X_{19}$ is any amino acid, such as S or A; $X_{20}$ is any amino acid, such as M; $X_{21}$ is any amino acid, such as R or K; and $X_{22}$ is any amino acid, such as A or P; $X_{23}$, $X_{24}$, and $X_{25}$ are individually any amino acids as long as (a) $X_{23}$ and $X_{24}$ are D and E, respectively, (b) $X_{23}$ and $X_{25}$ are D and D, respectively, and/or (c) $X_{24}$ and $X_{25}$ are E and D, respectively, optionally wherein $X_{23}$, $X_{24}$, and $X_{25}$ are D, E, and D, respectively, D, D, and D, respectively, D, E, and E, respectively, D, E, and S, respectively, D, E, and A,

respectively, or D, E, and T, respectively; $X_{26}$ is any amino acid, such as R or W; and $X_{27}$ is any amino acid, such as Q or L.

[0032] In some cases, the multispecific antibody or antibody fragment may comprise the VH1 described above, the VL1 described above, and the VH2 described above.

[0033] In some particular examples, (A) the VH1 comprises an amino acid sequence which comprises or consists of the amino acid sequence of any one of SEQ ID NOS: 181-188: and/or the VL1 comprises an amino acid sequence which comprises or consists of the amino acid sequence of SEQ ID NO: 281; and/or (B) the VH2 comprises an amino acid sequence which comprises or consists of the amino acid sequence of any one of SEQ ID NOS: 381, 386, and 393-396.

[0034] In particular examples, (A) the VH1 comprises: (i) the CDRH1, the CDRH2, and the CDRH3 of the VH1 comprising the amino acid sequence contained in SEQ ID NO: 181, optionally according to Kabat; or (ii) a CDRH1, a CDRH2, and a CDRH3 which comprise the amino acid sequences of SEQ ID NOS: 121, 141, and 161, respectively, and the VL1 comprises: (i) the CDRL1, the CDRL2, and the CDRL3 of the VL1 comprising the amino acid sequence contained in SEQ ID NO: 281, optionally according to Kabat, or (ii) a CDRL1, a CDRL2, and a CDRL3 which comprise the amino acid sequences of SEQ ID NOS: 221, 241, and 261, respectively; and (B) the VH2 comprises: (i) the CDRH1, the CDRH2, and the CDRH3 of the VH2 comprising the amino acid sequence contained in SEQ ID NO: 381, optionally according to IMGT or Kabat; or (ii) a CDRH1, a CDRH2, and a CDRH3 which comprise the amino acid sequences of SEQ ID NOS: 321, 341, and 361, respectively.

[0035] In certain examples, the VH1 comprises: (i) a FRH1 which has an amino acid sequence which: (i-1) has at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to the amino acid sequence of SEQ ID NO: 111 or to the FRH1 amino acid sequence contained in SEQ ID NO: 181, optionally according to Kabat; (i-2) comprises or consists of the amino acid sequence of SEQ ID NO: 111, or comprises or consists of the FRH1 amino acid sequence contained in SEQ ID NO: 181, optionally according to Kabat; and/or (i-3) consists of, or is derived from the FRH1 encoded by the human germline IGHV3-23*04; (ii) a FRH2 which has an amino acid sequence which: (ii-1) has at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to the amino acid

sequence of SEQ ID NO: 131 or to the FRH2 amino acid sequence contained in SEQ ID NO: 181, optionally according to Kabat; (ii-2) comprises or consists of the amino acid sequence of SEQ ID NO: 131, or comprises or consists of the FRH2 amino acid sequence contained in SEQ ID NO: 181, optionally according to Kabat; and/or (ii-3) consists of, or is derived from the FRH2 encoded by the human germline IGHV3-23*04; (iii) a FRH3 which has an amino acid sequence which: (iii-1) has at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to the amino acid sequence of SEQ ID NO: 151 or to the FRH3 amino acid sequence contained in SEQ ID NO: 181, optionally according to Kabat; (iii-2) comprises or consists of the amino acid sequence of SEQ ID NO: 151, or comprises or consists of the FRH3 amino acid sequence contained in SEQ ID NO: 181, optionally according to Kabat; and/or (iii-3) consists of, or is derived from the FRH3 encoded by the human germline IGHV3-23*04; and/or (iv) a FRH4 which has an amino acid sequence which: (iv-1) has at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to the amino acid sequence of SEQ ID NO: 171 or to the FRH4 amino acid sequence contained in SEQ ID NO: 181, optionally according to Kabat; (iv-2) comprises or consists of the amino acid sequence of SEQ ID NO: 171, or comprises or consists of the FRH4 amino acid sequence contained in SEQ ID NO: 181, optionally according to Kabat; and/or (iv-3) consists of, or is derived from the FRH4 encoded by the human germline IGHJ4*01.

[0036] In certain examples, the VL1 comprises: (v) a FRL1 which has an amino acid sequence which: (v-1) has at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to the amino acid sequence of SEQ ID NO: 211 or to the FRL1 amino acid sequence contained in SEQ ID NO: 281, optionally according to Kabat; or (v-2) comprises or consists of the amino acid sequence of SEQ ID NO: 211, or comprises or consists of the FRL1 amino acid sequence contained in SEQ ID NO: 281, optionally according to Kabat; (vi) a FRL2 which has an amino acid sequence which: (vi-1) has at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to the amino acid sequence of SEQ ID NO: 231 or to the FRL2 amino acid sequence contained in SEQ

ID NO: 281, optionally according to Kabat; or (vi-2) comprises or consists of the amino acid sequence of SEQ ID NO: 231, or comprises or consists of the FRL2 amino acid sequence contained in SEQ ID NO: 281, optionally according to Kabat; (vii) a FRL3 which has an amino acid sequence which: (vii-1) has at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to the amino acid sequence of SEQ ID NO: 251 or to the FRL3 amino acid sequence contained in SEQ ID NO: 281, optionally according to Kabat; or (vii-2) comprises or consists of the amino acid sequence of SEQ ID NO: 251, or comprises or consists of the FRL3 amino acid sequence contained in SEQ ID NO: 281, optionally according to Kabat; and/or (viii) a FRL4 which has an amino acid sequence which: (viii-1) has at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to the amino acid sequence of SEQ ID NO: 271 or to the FRL4 amino acid sequence contained in SEQ ID NO: 281, optionally according to Kabat; or (viii-2) comprises or consists of the amino acid sequence of SEQ ID NO: 271, or comprises or consists of the FRL4 amino acid sequence contained in SEQ ID NO: 281, optionally according to Kabat.

[0037] In certain examples, the VH2 comprises: (i) a FRH1 which has an amino acid sequence which: (i-1) has at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to the amino acid sequence of SEQ ID NO: 311 or to the FRH1 amino acid sequence contained in SEQ ID NO: 381, optionally according to IMGT or Kabat; (i-2) comprises or consists of the amino acid sequence of SEQ ID NO: 311, or comprises or consists of the FRH1 amino acid sequence contained in SEQ ID NO: 381, optionally according to IMGT or Kabat; and/or (i-3) consists of, or is derived from the FRH1 encoded by the human germline IGHV3-66*01; (ii) a FRH2 which has an amino acid sequence which: (ii-1) has at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to the amino acid sequence of SEQ ID NO: 331 or to the FRH2 amino acid sequence contained in SEQ ID NO: 381, optionally according to IMGT or Kabat; (ii-2) comprises or consists of the amino acid sequence of SEQ ID NO: 331, or comprises or consists of the FRH2 amino acid sequence contained in SEQ ID NO:

381, optionally according to IMGT or Kabat; and/or (ii-3) consists of, or is derived from the FRH2 encoded by the human germline IGHV3- 66*01; (iii) a FRH3 which has an amino acid sequence which: (iii-1) has at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to the amino acid sequence of SEQ ID NO: 351 or to the FRH3 amino acid sequence contained in SEQ ID NO: 381, optionally according to IMGT or Kabat; (iii-2) comprises or consists of the amino acid sequence of SEQ ID NO: 351, or comprises or consists of the FRH3 amino acid sequence contained in SEQ ID NO: 381, optionally according to IMGT or Kabat; and/or (iii-3) consists of, or is derived from the FRH3 encoded by the human germline IGHV3- 66*01; and/or (iv) a FRH4 which has an amino acid sequence which: (iv-1) has at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to the amino acid sequence of SEQ ID NO: 371 or to the FRH4 amino acid sequence contained in SEQ ID NO: 381, optionally according to IMGT or Kabat; (iv-2) comprises or consists of the amino acid sequence of SEQ ID NO: 351, or comprises or consists of the FRH4 amino acid sequence contained in SEQ ID NO: 381, optionally according to IMGT or Kabat; and/or (iv-3) consists of, or is derived from the FRH4 encoded by the human germline IGHV3- 66*01.

[0038] In some cases, the multispecific antibody or antibody fragment may comprise the VH1 described above, the VL1 described above, and the VH2 described above.

[0039] In some particular examples, (A) the VH1 comprises an amino acid sequence which has at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to the amino acid sequence of any one of SEQ ID NOS: 181; and/or the VL1 comprises an amino acid sequence which at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to the amino acid sequence of SEQ ID NO: 281; and/or (B) the VH2 comprises an amino acid sequence which has at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to the amino acid sequence of SEQ ID NO: 381.

[0040] In some particular examples, (A) the VH1 comprises an amino acid sequence which comprises or consists of the amino acid sequence of SEQ ID NO:

181: and/or the VL1 comprises an amino acid sequence which comprises or consists of the amino acid sequence of SEQ ID NO: 281; and/or (B) the VH2 comprises an amino acid sequence which comprises or consists of the amino acid sequence of any of SEQ ID NOS: 381.

[0041] In particular examples, (A) the VH1 comprises an amino acid sequence which consists of the amino acid sequence of SEQ ID NO: 181: and the VL1 comprises an amino acid sequence which consists of the amino acid sequence of SEQ ID NO: 281; and (B) the VH2 comprises an amino acid sequence which consists of the amino acid sequence of SEQ ID NO: 381.

[0042] In some particular examples, a multispecific antibody or antibody fragment according to the present disclosure may comprise: (A) a first antigen-binding region which specifically binds to Fzd4 and comprises a VH1 and a VL1; and (B) a second antigen-binding region which specifically binds to LRP5 and/or LRP6 and comprises at least a VH2,

wherein: (i) the CDRH1, the CDRH2, and the CDRH3 of the VH1 are identical to those of the heavy chain variable domain (VH) having the amino acid sequence of SEQ ID NO: 181; (ii) the CDRL1, the CDRL2, and the CDRL3 of the VL1 are identical to those of the light chain variable domain (VL) having the amino acid sequence of SEQ ID NO: 281; and (iii) the CDRH1, the CDRH2, and the CDRH3 of the VH2 are identical to those of the VH having the amino acid sequence of SEQ ID NO: 381. In particular examples: (i) the VH1 comprises or consists of the amino acid sequence of SEQ ID NO: 181; (ii) the VL1 comprises or consists of the amino acid sequence of SEQ ID NO: 281; and (iii) the VH2 comprises or consists of the amino acid sequence of SEQ ID NO: 381.

[0043] In some examples, (A) the first antigen-binding region is, comprises, or is comprised in a fragment antigen-binding (Fab), a Fab' fragment, a $F(ab')_2$, a single-chain Fab (scFab), a variable fragment (Fv), a single-chain Fv (scFv), a diabody, or a triabody; and/or (B) the second antigen-binding region: (i) is, comprises, or is comprised in a nanobody or a Fd fragment; or (ii) further comprises a second light chain variable domain (VL2) and is, comprises, or is comprised in a Fab, a Fab' fragment, a $F(ab')_2$, a scFab, a Fv, a scFv, a diabody, or a triabody.

[0044] In some examples, the multispecific antibody or antibody fragment comprises one or more of the following: (a) an immunoglobulin heavy chain constant domain 1 (CH1) or a variant thereof; (b) an immunoglobulin hinge or a variant thereof; (c) an immunoglobulin heavy chain constant domain 2 (CH2) or a variant

thereof; (d) an immunoglobulin heavy chain constant domain 3 (CH3) or a variant thereof; and/or (e) an immunoglobulin light chain constant domain (CL) or a variant thereof.

[0045] In certain examples, the CH1 or a variant thereof: (a-1) is of an IgG, an IgA, an IgE, an IgD, an IgM, optionally of an IgG1, an IgG4, an IgG2, or an IgG3, further optionally of human; and/or (a-2) comprises an amino acid sequence which comprises or consists of the amino acid sequence of SEQ ID NO: 21 or 22 or which is at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or is 100% identical thereto;

[0046] In certain examples, the hinge or a variant thereof: (b-1) is of an IgG, an IgA, an IgE, an IgD, an IgM, optionally of an IgG1, an IgG4, an IgG2, or an IgG3, further optionally of human; and/or (b-2) comprises an amino acid sequence which comprises or consists of the amino acid sequence of SEQ ID NO: 31 or which is at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or is 100% identical thereto.

[0047] In certain examples, the CH2 or a variant thereof: (c-1) is of an IgG, an IgA, an IgE, an IgD, an IgM, optionally of an IgG1, an IgG4, an IgG2, or an IgG3, further optionally of human; and/or (c-2) comprises an amino acid sequence which comprises or consists of the amino acid sequence of any of SEQ ID NOS: 41-46 or which is at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or is 100% identical thereto.

[0048] In certain examples, the CH3 or a variant thereof: (d-1) is of an IgG, an IgA, an IgE, an IgD, an IgM, optionally of an IgG1, an IgG4, an IgG2, or an IgG3, further optionally of human; and/or (d-2) comprises an amino acid sequence which comprises or consists of the amino acid sequence of any of SEQ ID NOS: 51-54, 61-64, and 66-69 (optionally not including the C-terminal K) or which is at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical thereto.

[0049] In certain examples, the CL or a variant thereof: (e-1) is of a kappa light chain constant domain (CLκ), optionally human CLκ and optionally comprising an amino acid sequence which comprises or consists of the amino acid sequence of SEQ

ID NO: 11 or which is at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity thereto; and/or (e-2) is of a lambda light chain constant domain (CLλ), optionally human CLλ, and optionally comprising an amino acid sequence which comprises or consists of the amino acid sequence of SEQ ID NO: 12 or which is at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity thereto.

[0050] In some particular examples, the multispecific antibody or antibody fragment comprises the CH2 and CH3 sequences of SEQ ID NOS: 42 and 51, respectively, SEQ ID NOS: 43 and 51, respectively, SEQ ID NOS: 45 and 61, respectively, SEQ ID NO: 45 and 66, respectively, SEQ ID NOS: 46 and 61, respectively, or SEQ ID NO: 46 and 66, respectively.

[0051] In some examples, the multispecific antibody or antibody fragment comprises a fragment crystallizable (Fc) region.

[0052] In certain examples, the Fc region is of an IgG, an IgA, an IgE, an IgD, an IgM, optionally of an IgG1, an IgG4, an IgG2, or an IgG3, further optionally of human, or a variant thereof.

[0053] In certain examples, the Fc region of an IgG1 or a variant thereof may comprise one or more of the following amino acid substitutions: N297A, N297Q, D265A, L234A, L235A, C226S, C229S, P238S, E233P, L234V, G236-deleted, P238A, A327Q, A327G, P329A, P329G, K322A, L234F, L235E, P331S, T394D, A330L, P331S, F243L, R292P, Y300L, V305I, P396L, S239D, I332E, S298A, E333A, K334A, L234Y, L235Q, G236W, S239M, H268D, D270E, K326D, A330M, K334E, G236A, K326W, S239D, E333S, S267E, H268F, S324T, E345R, E430G, S440Y M428L, N434S, H310A, L328F, M252Y, I253A, S254T, T256E, H435Q, H435A, and/or any combination thereof, according to EU numbering;

[0054] In certain examples, the Fc region of an IgG2 or a variant thereof may comprise one or more of the following amino acid substitutions: P238S, V234A, G237A, H268A, H268Q, H268E, V309L, N297A, N297Q, A330S, P331S, C232S, C233S, M252Y, S254T, T256E, and/or any combination thereof, according to EU numbering.

[0055] In certain examples, the Fc region of an IgG3 or a variant thereof may comprise the amino acid substitution E235Y, according to EU numbering.

[0056] In certain examples, the Fc region of an IgG4 or a variant thereof may comprise one or more of the following amino acid substitutions: E233P, F234V, L235A, G237A, E318A, S228P, L236E, S241P, L248E, T394D, M252Y, S254T, T256E, N297A, N297Q, and/or any combination thereof, according to EU numbering.

[0057] In certain examples, the Fc region may comprise one or more amino acid substitutions that reduce an Fc effector function.

[0058] In some particular examples, the one or more amino acid substitutions are at position(s) selected from the group consisting of positions 234, 235, 236, 237, 265, 297 and 329, according to EU numbering.

[0059] In some particular examples, the one or more amino acid substitutions comprises at least the following amino acid substitutions according to EU numbering: (ii-1) L234A, L235A, and P329G; (ii-2) L234A and L235A; (ii-3) I253A, H310A, and H435Q; (ii-4) I253A, H310A, and H435A; (ii-5) D265A and P329A; (ii-6) M252Y, S254T, and T256E; and/or (ii-7) M428L and N434S. Optionally, such substitutions may be relative to a human IgG1 Fc region and/or to any of SEQ ID NOS: 71-74 (optionally not including the C-terminal K).

[0060] In some particular examples, the Fc region may comprise an amino acid sequence which comprises or consists of the amino acid sequence of any one of SEQ ID NOS: 71-74, 81-84, 86-89, 91-94, 96-99, 471-474, 476-479, 481-484, and 486-489 (optionally not including the C-terminal K) or which is at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or is 100% identical thereto.

[0061] In some examples, the multispecific antibody or antibody fragment according to the present disclosure may comprise a structure as depicted in any one of **FIGS. 24-35**, optionally any of the structures depicted in **FIG. 24**.

[0062] In some examples, the multispecific antibody or antibody fragment according to the present disclosure may comprise: (a) a first polypeptide comprising said VH1; and (b) a second polypeptide comprising said VH2 and said VL1, wherein the first and second polypeptides interact with each other, optionally via one or more disulfide bonds, permitting said VH1 and VL1 to form said first antigen-binding region.

[0063] In certain examples, (a) the first polypeptide comprises the following in the order from the N-terminus to the C-terminus: (a-1) said VH1 and (a-2) a CH1 or a variant thereof, optionally wherein the CH1 or a variant thereof: (i) is of an IgG,

optionally of an IgG1 or an IgG4, further optionally of human IgG1 or IgG4; and/or (ii) comprises an amino acid sequence which comprises or consists of the amino acid sequence of SEQ ID NO: 21 or 22 or which is at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or is 100% identical thereto; (a-3) an immunoglobulin hinge or a variant thereof, optionally wherein the hinge or a variant thereof: (i) is of an IgG, optionally of an IgG1 or an IgG4, further optionally of human IgG1 or IgG4; and/or (ii) comprising an amino acid sequence which comprises or consists of the amino acid sequence of SEQ ID NO: 31 or which is at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or is 100% identical thereto; (a-4) a CH2 or a variant thereof, optionally wherein the CH2 or a variant thereof: (i) is of an IgG, optionally of an IgG1 or an IgG4, further optionally of human IgG1 or IgG4; and/or (ii) comprises an amino acid sequence which comprises or consists of the amino acid sequence of any of SEQ ID NOS: 41-46 or which is at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or is 100% identical thereto; and (a-5) a CH3 or a variant thereof, optionally wherein the CH3 or a variant thereof: (i) is of an IgG, optionally of an IgG1 or an IgG4, further optionally of human IgG1 or IgG4; and/or (ii) comprises an amino acid sequence which comprises or consists of the amino acid sequence of any of SEQ ID NOS: 51-54, 61-64, and 66-69 (optionally not including the C-terminal K) or which is at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or is 100% identical thereto.

[0064] In certain examples, (b) the second polypeptide comprises the following in the order from the N-terminus to the C-terminus: (b-1) said VH2; (b-2) optionally a linker, which is optionally a peptide linker, further optionally a flexible linker, optionally wherein the linker: (i) comprises one or more amino acids, optionally one, two, three, four, five, six, seven, eight, nine, ten, eleven, or twelve amino acids; (ii) consists of small amino acids consisting of G, S, and/or A; (iii) comprises an amino acid sequence which comprises or consists of the amino acid sequence selected from the group consisting of SEQ ID NO: 101, SEQ ID NO: 102, SEQ ID NO: 103, SEQ ID NO: 104, SEQ ID NO: 105, G, GG, GGG, GS, SG, GGS, GSG, SGG, GSS, SGS,

and SSG; and/or (iv) comprises an amino acid sequence which comprises or consists of multiple repeats of the amino acid sequence selected from the group consisting of SEQ ID NO: 101, SEQ ID NO: 102, SEQ ID NO: 103, SEQ ID NO: 104, SEQ ID NO: 105, G, GG, GGG, GS, SG, GGS, GSG, SGG, GSS, SGS, and SSG; (b-3) said VL1; and (b-4) an CL or a variant thereof, optionally wherein the CL or a variant thereof: (i) is of a CLκ, optionally human CLκ, and optionally comprising an amino acid sequence comprising or consisting of the amino acid sequence of SEQ ID NO: 11 or having at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity thereto; or (ii) is of a CLλ, optionally human CLλ, and optionally comprising an amino acid sequence comprising or consisting of the amino acid sequence of SEQ ID NO: 12 or having at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity thereto.

[0065] In some cases, the multispecific antibody or antibody fragment may comprise both the first polypeptide described above and the second polypeptide described above.

[0066] In some particular examples, the multispecific antibody or antibody fragment comprises: two of said first polypeptides forming a dimer with each other optionally via one or more disulfide bonds; and two of said second polypeptides, wherein one of the second polypeptides is interacting with one of the first polypeptides optionally via a disulfide bond and the other of the second polypeptides is interacting with the other of the first polypeptides optionally via a disulfide bond.

[0067] In certain examples, the multispecific antibody or antibody fragment comprises a Fc region, wherein the Fc region: (I) is of an IgG, optionally of an IgG1 or an IgG4, further optionally of human, or a variant thereof; (II) comprises at least the following amino acid substitutions according to EU numbering: (ii-1) L234A, L235A, and P329G; (ii-2) L234A and L235A; (ii-3) I253A, H310A, and H435Q; (ii-4) I253A, H310A, and H435A; (ii-5) D265A and P329A; (ii-6) M252Y, S254T, and T256E; and/or (ii-7) M428L and N434S, optionally relative to a human IgG1 Fc region and/or to any of SEQ ID NOS: 71-74 (optionally not including the C-terminal K); and/or (III) comprises an amino acid sequence which comprises or consists of the amino acid sequence of any one of SEQ ID NOS: 71-74, 81-84, 86-89, 91-94, 96-99, 471-474, 476-479, 481-484, and 486-489 (optionally not including the C-terminal K)

or comprises an amino acid sequence which is at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical thereto.

[0068] In some particular examples, (a) the first polypeptide; (i) comprises an amino acid sequence which has at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NOS: 2, 7, 8, 9, 450, 451, 452, and 453 (optionally without the C-terminal K); (ii) comprises an amino acid sequence which comprises or consists of the amino acid sequence of any one of SEQ ID NOS: 402, 407, 408, 409, 460, 461, 462, and 463 (optionally without the C-terminal K), wherein: $X_1$ is any amino acid, optionally Q or K; $X_2$ is any amino acid, optionally S, A, Q, or N; $X_3$ is any amino acid, optionally E, A, S, or D; $X_4$ is any amino acid, optionally N or D; $X_5$ is any amino acid, optionally R or K; $X_6$ is any amino acid, optionally E or T; and $X_7$ is any amino acid, optionally D or any amino acid which is not S, E, A, R, T, L, H, Y, Q, or K; and/or (iii) comprises an amino acid sequence which comprises or consists of the amino acid sequence of any one of SEQ ID NOS: 2, 7, 8, 9, 450, 451, 452, and 453 (optionally without the C-terminal K).

[0069] In some particular examples, (b) the second polypeptide (i) comprises an amino acid sequence which has at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 1; and/or (ii) comprises an amino acid sequence which comprises or consists of the amino acid sequence of SEQ ID NO: 401, wherein: $X_1$ is any amino acid, optionally E or D; $X_2$ is any amino acid such as A or T; and $X_3$ is any amino acid such as S; $X_4$ is Q; $X_5$ is any amino acid, such as Y; $X_6$ is any amino acid, such as Q or G; $X_7$ is any amino acid, such as R; $X_8$ is any amino acid, such as L or W; $X_9$ is any amino acid, such as I; and $X_{10}$ is any amino acid, such as A; $X_{11}$ is M; $X_{12}$ is M; $X_{13}$ is any amino acid, such as D or G; $X_{14}$ is any amino acid, such as L; $X_{15}$ is any amino acid, such as M; $X_{16}$ is any amino acid, such as T; $X_{17}$ is any amino acid, such as D or E; $X_{18}$ is any amino acid, such as N or S; $X_{19}$ is any amino acid, such as S or A; $X_{20}$ is any amino acid, such as M; $X_{21}$ is any amino acid, such as R or K; and $X_{22}$ is any amino acid, such as A or P; $X_{23}$, $X_{24}$, and $X_{25}$ are individually any amino acids as long as (a) $X_{23}$ and $X_{24}$ are D and E, respectively, (b) $X_{23}$ and $X_{25}$ are D and D, respectively, and/or (c) $X_{24}$ and $X_{25}$ are E and D, respectively, optionally wherein $X_{23}$, $X_{24}$, and $X_{25}$ are D, E, and D,

respectively, D, D, and D, respectively, D, E, and E, respectively, D, E, and S, respectively, D, E, and A, respectively, or D, E, and T, respectively; $X_{26}$ is any amino acid, such as R or W; and $X_{27}$ is any amino acid, such as Q or L; and/or (iii) comprises an amino acid sequence which comprises or consists of the amino acid sequence of SEQ ID NO: 1.

[0070] In particular examples, (a) the first polypeptide comprises an amino acid sequence which comprises or consists of the amino acid sequence of SEQ ID NOS: 2, 7, 450, and 451 (optionally without the C-terminal K); and (b) the second polypeptide comprises an amino acid sequence which comprises or consists of the amino acid sequence of SEQ ID NO: 1.

[0071] In further particular examples, the multispecific antibody or antibody fragment comprises: two of said first polypeptides forming a dimer with each other via one or more disulfide bonds; and two of said second polypeptides, wherein one of the second polypeptides is interacting with one of the first polypeptides via a disulfide bond and the other of the second polypeptides is interacting with the other of the first polypeptides via a disulfide bond.

[0072] In further examples, a multispecific antibody or antibody fragment of according to the present disclosure may comprise: (A) two or more Fzd4- binding regions, wherein at least one of said Fzd4-binding regions is, comprises, or is comprised in said first antigen-binding region; and/or (B) two or more LRP5 and/or LRP6- binding regions, wherein at least one of said LRP5 and/or LRP6-binding regions is, comprises, or is comprised in said second antigen-binding region.

[0073] In some cases, the ratio of the number of said one or more Fzd4-binding regions and the number of said one or more LRP5 and/or LRP6-binding regions comprised in the multispecific antibody or antibody fragment may be selected from the group consisting of 2:1, 1:2, 2:2, 3:1, 1:3, 3:2, 2:3, 3:3, 4:1, 1:4, 4:2, 2:4, 4:3, 3:4, and 4:4.

[0074] In some cases, the number of said one or more Fzd4-binding regions and the number of said one or more LRP5 and/or LRP6-binding regions comprised in the multispecific antibody or antibody fragment may be: 2 and 1, respectively; 1 and 2, respectively; 2 and 2, respectively; 3 and 1, respectively; 1 and 3, respectively; 3 and 2, respectively; 2 and 3, respectively; 3 and 3, respectively; 4 and 1, respectively; 1 and 4, respectively; 4 and 2, respectively; 2 and 4, respectively; 4 and 3, respectively; 3 and 4, respectively; or 4 and 4 respectively.

[0081] In certain cases, a multispecific antibody or antibody fragment according to the present disclosure may comprise: two identical Fzd4-binding regions, each of which is, comprises, or is comprised in said first antigen-binding region; and two LRP5 and/or LRP6-binding regions, which are different from each other and (i) at least one of which is, comprises, or is comprised in or (ii) each of which is, comprises, or is comprised in said second antigen-binding region.

[0082] In certain cases, a multispecific antibody or antibody fragment according to the present disclosure may comprise: two Fzd4-binding regions, which are different from each other and (i) at least one of which is, comprises, or is comprised in or (ii) each of which is, comprises, or is comprised in said first antigen-binding region; and two LRP5 and/or LRP6-binding region, which are different from each other and (i) at least one of which is, comprises, or is comprised in or (ii) each of which is, comprises, or is comprised in said second antigen-binding region.

[0083] The present disclosure provides nucleic acids optionally wherein a nucleic acid or a combination of nucleic acids according to the present disclosure may encode any of the multispecific antibodies and antibody fragments described herein.

[0084] In some examples, the nucleic acid(s) may be DNA, cDNA, RNA, mRNA, modified mRNA, or a DNA/RNA hybrid.

[0084a] In another aspect, the present disclosure provides a nucleic acid or a combination of nucleic acids encoding the multispecific antibody or antibody fragment as described herein, optionally wherein the nucleic acid(s) is/are DNA, cDNA, RNA, mRNA, modified mRNA, or a DNA/RNA hybrid.

[0085] In some examples, a combination of nucleic acids nucleic acid or a combination of nucleic acids may comprise: (a) a first nucleic acid encoding any of the first polypeptides described herein; and (b) a second nucleic acids encoding any of the second polypeptides described herein.

[0086] The present disclosure provides vectors comprising these nucleic acids.

[0087] In some examples a vector or a combination of vectors according to the present disclosure may comprise any of the nucleic acids or any of the combinations of nucleic acids described herein.

[0088] In some examples, the vector(s) comprise(s) one or more promoters operably linked to the nucleic acid(s).

[0089] In some examples, the vector(s) is/are an expression vector.

[0090] In some examples, the vector(s) comprise(s) a plasmid, a viral vector (optionally adeno-associated viral, adenoviral, lentiviral, or retroviral), a lipid-based

vector, a self-replicating RNA vector, a virus-like particle, a polymer-based vector, and/or a nanoparticle, optionally a lipid-based nanoparticle.

[0090a] In another aspect, the present disclosure provides a vector or a combination of vectors which comprises the nucleic acid or the combination of nucleic acids as described herein, optionally wherein:

(i) the vector(s) comprise(s) one or more promoters operably linked to the nucleic acid(s);

(ii) the vector(s) is/are an expression vector; and/or

(iii) the vector(s) comprise(s) a plasmid, a viral vector (optionally adeno-associated viral, adenoviral, lentiviral, or retroviral), a lipid-based vector, a self-replicating RNA vector, a virus-like particle, a polymer-based vector, and/or a nanoparticle, optionally a lipid-based nanoparticle.

[0091] In some examples, a combination of vectors may comprise: (a) a first vector comprising a first nucleic acid encoding the first polypeptide; and (b) a second vector comprising a second nucleic acid encoding the second polypeptide.

[0092] The present disclosure provides host cells and populations of cells comprising such nucleic acids or vectors.

[0093] A host cell according to the present disclosure may comprise: (A) any of the multispecific antibodies and antibody fragments described herein; (B) any of the nucleic acids or any of the combinations of nucleic acids described herein; and/or (C) any of the vectors or any of the combinations of vectors described herein.

[0094] In some examples, the host cell is mammalian, optionally human, non-human primate, monkey, rabbit, rodent, hamster, rat, or mouse.

[0095] In some examples, the host cell is non-mammalian, optionally plant, bacterial, fungal, yeast, protozoa, or insect.

[0096] In certain examples, the host cell is: (i) a human embryonal kidney (HEK) cell, optionally a HEK293 cell, or a variant thereof, further optionally Expi293F™ cell; (ii) a CHO (Chinese Hamster Ovary) cell; (iii) an immune cell or (iv) a hybridoma.

[0096a] In another aspect, the present disclosure provides a host cell, which comprises:

(A) the multispecific antibody or antibody fragment as described herein;

(B) the nucleic acid or the combination of nucleic acids as described herein; and/or

(C) the vector or the combination of vectors as described herein,

optionally wherein the host cell is:

(i) mammalian, optionally human, non-human primate, monkey, rabbit, rodent, hamster, rat, or mouse; or

(ii) non-mammalian, optionally plant, bacterial, fungal, yeast, protozoa, or insect,

and optionally wherein the host cell is:

(i) a human embryonal kidney (HEK) cell, optionally a HEK293 cell, or a variant thereof, further optionally Expi293F™ cell;

(ii) a CHO (Chinese Hamster Ovary) cell;

(iii) an immune cell or

(iv) a hybridoma.

[0097] In another aspect, a population of cells according to the present disclosure may comprise two or more host cells described herein.

[0098] The present disclosure provides pharmaceutical compositions containing any of the foregoing. A pharmaceutical composition according to the present disclosure may comprise: (A) any of the multispecific antibodies and antibody fragments described herein; (B) any of the nucleic acids or any of the combinations of nucleic acids described herein; (C) any of the vectors or any of the combinations of vectors described herein; and/or (D) any of the host cells or the populations of cells described herein; and (II) a pharmaceutically acceptable carrier and/or excipient.

[0098a] In another aspect, the present disclosure provides a pharmaceutical composition, which comprises:

(I) (A) the multispecific antibody or antibody fragment as described herein;

(B) the nucleic acid or the combination of nucleic acids as described herein;

(C) the vector or the combination of vectors as described herein; and/or

(D) the host cell as described herein or the population of cells as described herein; and

(II) a pharmaceutically acceptable carrier and/or excipient.

[0099] The present disclosure provides *in vivo* methods, such as methods of treating a subject, methods of treating or preventing a disease, disorder, or a condition in a subject, and methods of inducing, promoting, stimulating, enhancing, and/or supporting a Wnt signaling in a target cell of a subject.

[0099a] In another aspect, the present disclosure provides a substance for use in treating a subject in need of such treatment, wherein the substance comprises an effective amount of:

(A) the multispecific antibody or antibody fragment as described herein;

(B) the nucleic acid or the combination of nucleic acids as described herein;

(C) the vector or the combination of vectors as described herein;

(D) the host cell as described herein or the population of cells as described herein; and/or

(E) the pharmaceutical composition as described herein,

optionally wherein:

(a) the subject is

(i) a mammal, optionally a human, a non-human primate, a monkey, a horse, a cow, sheep, a goat, a pig, a dog, a cat, a rabbit, a rodent, a hamster, a rat, or a mouse; or

(ii) a non-mammalian vertebrate, optionally a bird, fish, an amphibian, or a reptile;

(b) the subject comprises or has a risk of developing a disease, disorder, or a condition; and/or

(c) an additional agent, optionally an adjuvant or a therapeutic agent is to be further administered to the subject.

[0099b] In another aspect, the present disclosure provides a substance for use in treating or preventing a disease, disorder, or a condition in a subject in need of such treatment, wherein the substance comprises an effective amount of:

(A) the multispecific antibody or antibody fragment as described herein;

(B) the nucleic acid or the combination of nucleic acids as described herein;

(C) the vector or the combination of vectors as described herein;

(D) the host cell as described herein or the population of cells as described herein; and/or

(E) the pharmaceutical composition as described herein,

optionally wherein:

(a) the subject is

(i) a mammal, optionally a human, a non-human primate, a monkey, a horse, a cow, sheep, a goat, a pig, a dog, a cat, a rabbit, a rodent, a hamster, a rat, or a mouse; or

(ii) a non-mammalian vertebrate, optionally a bird, fish, an amphibian, or a reptile; and/or

(b) an additional agent, optionally an adjuvant or a therapeutic agent is to be further administered to the subject.

[00100] A method of treating a subject in need of such treatment according to the present disclosure may comprise administering to the subject an effective amount of: (A) any of the multispecific antibodies or antibody fragments described herein; (B) any of the nucleic acids or combinations of nucleic acids described herein; (C) any of the vectors or combinations of vectors described herein; (D) any of the host cells or the populations of cells described herein; and/or (E) any of the pharmaceutical compositions described herein.

[00101] In some examples, the subject comprises or has a risk of developing a disease, disorder, or a condition, e.g., because of heredity or an underlying disease or comorbidity.

[00102] In some examples, the disclosure provides methods of treating or preventing a disease, disorder, or a condition in a subject in need of such treatment which may comprise administering an effective amount of: (A) any of the multispecific antibodies or antibody fragments described herein; (B) any of the nucleic acids or combination of nucleic acids described herein; (C) any of the vectors or the combinations of vectors described herein; (D) any of the host cells or the populations of cells described herein; and/or (E) any of the pharmaceutical compositions described herein.

[00103] A method of inducing, promoting, stimulating, enhancing, and/or supporting Wnt signaling in a target cell of a subject according to the present disclosure may comprise administering to the subject an effective amount of: (A) any of the multispecific antibodies or antibody fragments described herein; (B) any of the nucleic acids or combinations of nucleic acids described herein; (C) any of the vectors or combinations of vectors described herein; (D) any of the host cells or the populations of cells described herein; and/or (E) any of the pharmaceutical compositions described herein.

[00104] In some examples, the Wnt signaling is or comprises Wnt/β-catenin signaling.

[00105] In some examples, the method promotes, stimulates, enhances, and/or supports recruitment of multiple receptor complexes each comprising (i) Fzd4 and (ii) LRP5 or LRP6.

from the group consisting of stroke, traumatic brain injury, epilepsy, Alzheimer's disease, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, Friedreich ataxia, Lewy body disease, spinal muscular atrophy, motor neuron disease, multiple sclerosis, Batten disease, and Creutzfeldt-Jakob disease.

[00115] In any of the *in vivo* methods disclosed herein, administration of the active agent may be to one or more of the subject's eyes, optionally intravitreally or via ocular drops.

[00116] In any of the *in vivo* methods disclosed herein, administration of the active agent may be effected locally, optionally to the eye, ear, nose (optionally intranasally), skin (optionally transdermally or epicutaneously), mucosa, skin, or vagina, or by inhalation.

[00117] In any of the *in vivo* methods disclosed herein, administration of the active agent may be effected parenterally administering, optionally by injection (optionally intravenous, intramuscular, subcutaneous, intradermal, intrathecal, intra-arterial, intraarticular, intraosseous, or intraperitoneal administration) or by inhalation.

[00118] In any of the *in vivo* methods disclosed herein, administration of the active agent may be effected enterally, optionally orally, sublingually, buccally, or rectally.

[00119] In any of the *in vivo* methods disclosed herein, administration of the active agent may comprise administering any of the multispecific antibodies and antibody fragments described herein to one or more of the subject's eyes at about 0.1-100000 ng per eye, about 1-100000 ng per eye, about 1-10000 ng per eye, about 10-10000 ng per eye, about 1-100 ng per eye, about 200-300 ng per eye, about 300-400 ng per eye, about 400-500 ng per eye, about 500-600 ng per eye, about 600-700 ng per eye, about 700-800 ng per eye, about 800-900 ng per eye, about 900-1000 ng per eye, about 1000-2000 ng per eye, about 2000-3000 ng per eye, about 3000-4000 ng per eye, about 4000-5000 ng per eye, about 5000-6000 ng per eye, about 6000-7000 ng per eye, about 7000-8000 ng per eye, about 8000-9000 ng per eye, about 9000-10000 ng per eye, about 10000-20000 ng per eye, about 20000-30000 ng per eye, about 30000-40000 ng per eye, about 40000-50000 ng per eye, about 50000-60000 ng per eye, about 60000-70000 ng per eye, about 70000-80000 ng per eye, about 80000-90000 ng per eye, or about 90000-100000 ng per eye.

[00120] The present disclosure provides methods of inducing, promoting, stimulating, enhancing, and/or modulating Wnt signaling in a target cell.

[00121] In some examples such a method of inducing, promoting, stimulating, enhancing, and/or supporting Wnt signaling in a target cell may comprise contacting the target cell with an effective amount of: (A) any of the multispecific antibodies or antibody fragments described herein; (B) any of the nucleic acids or combinations of nucleic acids described herein; (C) any of the vectors or combinations of vectors described herein; (D) any of the host cells or the populations of cells described herein; and/or (E) any of the pharmaceutical compositions described herein.

[00122] In some examples, the contacting occurs *in vitro*.

[00123] In some examples, the contacting occurs *ex vivo*.

[00124] In some examples, the contacting occurs *in vivo*.

[00125] In some examples, the method comprises (i) preparing a cell and/or tissue for implantation, (ii) differentiation of a stem cell, and/or (iii) preparation of an organoid.

[00126] In some examples, the method elicits or promotes bone formation, vascular formation, and/or neural formation and/or differentiation.

[00127] The present disclosure provides methods of manufacturing any of the multispecific antibodies and antibody fragments described herein.

[00128] A method of manufacturing any of the multispecific antibodies and antibody fragments described herein may comprise: (a) culturing cells comprising any of the nucleic acids or combinations of nucleic acids encoding the multispecific antibody or antibody fragment under conditions that permit the expression of said multispecific antibody or antibody fragment; and (b) harvesting and purifying the resultant multispecific antibody or antibody fragment from the cell culture from (a).

[00128a] In another aspect, the present disclosure provides a method of manufacturing the multispecific antibody or antibody fragment as described herein, comprising:

(a) culturing cells comprising the nucleic acid or the combination of nucleic acids as described herein in a condition that allows for expression of said multispecific antibody or antibody fragment, and

(b) harvesting and purifying the multispecific antibody or antibody fragment from the cell culture from (a).

[00129] The present disclosure provides methods of manufacturing any of the host cells or any of the populations of cells described herein.

[00130] A method of manufacturing any of the host cells or the populations of such cells described herein may comprise introducing any of the nucleic acids or the combinations of nucleic acids described herein and/or the vectors or combinations of vectors described herein into one or more cells.

[00131] In some examples, the introducing occurs *in vitro*.

[00132] In some examples, the introducing occurs *ex vivo*.

[00133] In some examples, the introducing occurs *in vivo*.

[00133a] In another aspect, the present disclosure provides a method of manufacturing the host cell as described herein or the population of such cells as described herein, comprising introducing the nucleic acid or the combination of nucleic acids as described herein and/or the vector or the combination of vectors as described herein into one or more cells, optionally wherein the introducing occurs *in vitro* or *ex vivo*.

[00134] Any of the multispecific antibodies or antibody fragments described herein, any of the nucleic acids or combinations of nucleic acids described herein, any of the vectors or combinations of vectors described herein, any of the host cells or populations of cells described herein, and/or pharmaceutical compositions described herein may be for use in medicine.

[00134a] In another aspect, the present disclosure provides the multispecific antibody or antibody fragment as described herein, the nucleic acid or the combination of nucleic acids as described herein, the vector or the combination of vectors as described herein, the host cell as described herein, or the population of cells as described herein, and/or the pharmaceutical composition as described herein, for use in medicine.

[00135] Any of the multispecific antibodies or antibody fragments described herein, any of the nucleic acids or combinations of nucleic acids described herein, any of the vectors or combinations of vectors described herein, any of the host cells or populations of cells described herein, and/or pharmaceutical compositions described herein may be for use in treating a disease, disorder, or condition, e.g., one involving Wnt signaling.

[00136] In some examples, the disease, disorder, or condition comprises one or more of any of the diseases described herein.

[00136a] In another aspect, the present disclosure provides the multispecific antibody or antibody fragment as described herein, the nucleic acid or the combination of nucleic acids as described herein, the vector or the combination of vectors as described herein, the host cell as described herein, or the population of cells as described herein, and/or the pharmaceutical composition as described herein, for use in treating a disease, disorder, or condition, optionally wherein the disease, disorder, or condition comprises one or more of the following:

(a) a retinopathy, optionally a retinal vascular disease (optionally caused by inhibition of vascular development or excessive angiogenesis) and/or optionally selected from the group consisting of exudative vitreoretinopathy, familiar exudative vitreoretionopathy (FEVR), retinopathy of prematurity, Norrie disease, diabetic retinopathy (DR),

diabetic macular edema, diabetic macular ischemia, age-related macular degeneration (AMD) (including wet AMD and dry AMD), retinopathy of prematurity (ROP), osteoporosis-pseudoglioma syndrome (OPPG), retinal vein occlusion, and Coats disease;

(b) a vascular disorder, optionally vascular malformation or vascular insufficiency, further optionally associated with ischemia-induced neovascularization;

(c) a bone disease or bone injury, optionally selected from the group consisting of osteoporosis (including osteoporosis pseudoglioma (OPPG) syndrome and juvenile primary osteoporosis), bone mineral density variability, and cancer-associated osteolysis;

(d) a muscle wasting disease, optionally selected from the group consisting of sarcopenia, cachexia, and muscular dystrophies; and/or

(e) a neural or neurodegenerative disease, optionally selected from the group consisting of stroke, traumatic brain injury, epilepsy, Alzheimer's disease, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, Friedreich ataxia, Lewy body disease, spinal muscular atrophy, motor neuron disease, multiple sclerosis, Batten disease, and Creutzfeldt-Jakob disease.

[00137] The present disclosure further encompasses use of any of the multispecific antibodies or antibody fragments described herein, any of the nucleic acids or combinations of nucleic acids described herein, any of the vectors or combinations of vectors described herein, any of the host cells or populations of cells described herein, and/or pharmaceutical compositions described herein for the manufacture of a medicament for treatment of a disease, disorder, or condition.

[00138] In some examples, the disease, disorder, or condition comprises one or more of any of the diseases described herein.

[00138a] In another aspect, the present disclosure provides use of the multispecific antibody or antibody fragment as described herein, the nucleic acid or the combination of nucleic acids as described herein, the vector or the combination of vectors as described herein, the host cell as described herein, or the population of cells

as described herein, and/or the pharmaceutical composition as described herein in the manufacture of a medicament for treatment of a disease, disorder, or condition, optionally wherein the disease, disorder, or condition comprises one or more of the following:

(a) a retinopathy, optionally a retinal vascular disease (optionally caused by inhibition of vascular development or excessive angiogenesis) and/or optionally selected from the group consisting of exudative vitreoretinopathy, familiar exudative vitreoretinopathy (FEVR), retinopathy of prematurity, Norrie disease, diabetic retinopathy (DR), diabetic macular edema, diabetic macular ischemia, age-related macular degeneration (AMD) (including wet AMD and dry AMD), retinopathy of prematurity (ROP), osteoporosis-pseudoglioma syndrome (OPPG), retinal vein occlusion, and Coats disease;

(b) a vascular disorder, optionally vascular malformation or vascular insufficiency, further optionally associated with ischemia-induced neovascularization;

(c) a bone disease or bone injury, optionally selected from the group consisting of osteoporosis (including osteoporosis pseudoglioma (OPPG) syndrome and juvenile primary osteoporosis), bone mineral density variability, and cancer-associated osteolysis;

(d) a muscle wasting disease, optionally selected from the group consisting of sarcopenia, cachexia, and muscular dystrophies; and/or

(e) a neural or neurodegenerative disease, optionally selected from the group consisting of stroke, traumatic brain injury, epilepsy, Alzheimer's disease, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, Friedreich ataxia, Lewy body disease, spinal muscular atrophy, motor neuron disease, multiple sclerosis, Batten disease, and Creutzfeldt-Jakob disease.

[00139] The present disclosure is based, in part, upon the use of Wnt signaling agonists and antagonists to regulate aberrant vascular formation in retinopathy indications.

[00140] The present disclosure provides methods of treating a subject suffering from a retinopathy comprising administering the subject, an engineered Wnt signaling agonist. In further examples, the engineered Wnt agonist comprises binding compositions that bind to one or more Fzd receptors and binding compositions that bind to one or more LRP receptors.

[00141] The disclosure provides an engineered Wnt signaling agonist, wherein the Wnt signaling agonist comprises: two light chains, each comprising a polypeptide sequence having at least 95% identity to SEQ ID NO: 1, and two heavy chains, each comprising a polypeptide sequence having at least 95% identity to SEQ ID NO: 2, wherein the two heavy chains are bound to each other, and each of the two

light chains is bound to a different heavy chain of the Wnt signaling agonist. In certain examples, each of the two light chains comprises a VHH domain comprising one or more of the following amino acid residue modifications: N29Q and/or hVHH3-H4 DDD (amino acids 101-103) substituted with DED. In certain examples, each of the two heavy chains comprises one or more of the following amino acid residues: a CDR1 S31, a CDR2 E62, and/or CDR3 D108.

[00142] The disclosure provides a nucleic acid comprising a sequence encoding one or more light chain and/or one or more heavy chain of the engineered Wnt signaling agonist disclosed herein, e.g., hp4SD1-03, or a fragment or variant thereof. In certain examples, the nucleic acid is an mRNA, e.g., a modified mRNA.

[00143] The disclosure provides a vector comprising a nucleic acid disclosed herein, e.g., a nucleic acid comprising a sequence encoding one or more light chain and/or one or more heavy chain of hp4SD1-03, or a fragment or variant thereof. In particular examples, the vector is an expression vector comprising a promoter operatively linked to the nucleic acid, such as, e.g., a viral vector.

[00144] The disclosure provides a host cell comprising a vector or nucleic acid disclosed herein, e.g., a vector or nucleic acid comprising a sequence encoding one or more light chain and/or one or more heavy chain of hp4SD1-03, or a fragment or variant thereof. In certain examples, the host cell is a recombinant cell. In certain examples, the host cells is a mammalian cell or an insect cell.

[00145] The disclosure provides a pharmaceutical composition comprising a pharmaceutically acceptable diluent, excipient or carrier, and:

a) an engineered Wnt signaling agonist disclosed herein;

b) a nucleic acid disclosed herein; and/or

c) a vector disclosed herein.

[00146] In certain examples, the pharmaceutical composition comprises hp4SD1-03, or a fragment or variant thereof, or the nucleic acid encodes one or more light chain and/or one or more heavy chain of hp4SD1-03, or a fragment or variant thereof, or the vector comprises a nucleic acid comprising a sequence encoding one or more light chain and/or one or more heavy chain of hp4SD1-03, or a fragment or variant thereof.

[00147] The disclosure provides a method of treating a retinopathy in a subject, comprising administering to the subject:

a) an engineered Wnt signaling agonist disclosed herein;

b) a nucleic acid disclosed herein;

c) a vector disclosed herein, and/or

d) a pharmaceutical composition disclosed herein.

[00148] In certain examples, the engineered Wnt signaling agonist is hp4SD1-03, or a fragment or variant thereof, or the pharmaceutical composition comprises hp4SD1-03, or a fragment or variant thereof, or the nucleic acid encodes one or more light chains and/or one or more heavy chains of hp4SD1-03, or a fragment or variant thereof, or the vector comprises a nucleic acid comprising a sequence encoding one or more light chains and/or one or more heavy chains of hp4SD1-03, or a fragment or variant thereof.

[00149] In certain examples of the disclosed treatment methods, the treated retinopathy is a retinal vascular disease. In certain examples, the retinal vascular disease is caused by inhibition of vascular development. In certain examples, the retinopathy is caused by excessive angiogenesis. In certain examples, the retinopathy is an early-stage retinopathy or a late-stage retinopathy. In some examples, the retinal vascular disease is selected from the group consisting of: familiar exudative vitreoretinopathy (FEVR), exudative vitreoretinopathy, Norrie disease, diabetic retinopathy (DR), diabetic macular edema, diabetic macular ischemia, age-related macular degeneration (AMD) (including wet AMD and dry AMD), retinopathy of prematurity (ROP), osteoporosis-pseudoglioma syndrome (OPPG), retinal vein occlusion, and Coats disease. The engineered Wnt signaling agonist may be administered via various routes of administration, including but not limited to systemically or locally, such as intravenously, subcutaneously, orally, topically, nasally, intravitreally, suprachoroidally, or subretinally. In certain examples, the engineered Wnt signaling agonist is administered to one or more of the subject's eyes, e.g., intravitreally.

## DESCRIPTION OF THE DRAWINGS

[00150] **FIG. 1** provides the amino acid sequences of a tetravalent, bi-specific Wnt signaling agonist, 4SD1-03_LALAPG, which comprises two heavy chains and two light chains of an anti-Fzd4 antibody, 4SD1, wherein an anti-LRP5/6 VHH domain is