

CLAIMS

1. A bispecific fusion protein, wherein the fusion protein has a structure in which a second binding domain is inserted in the hinge region of a full-length immunoglobulin G (IgG) through an optional peptide linker, and the Fab region of the IgG is a first binding domain; after inserting the second binding domain, the heavy chain of the IgG is the heavy chain of the fusion protein, and the light chain of the IgG is the light chain of the fusion protein; the second binding domain is selected from a human receptor or ligand, a fragment of the receptor or ligand, and a fragment combination of the receptor or ligand; wherein the receptor or ligand forms a dimerization or multimerization structure in a natural signaling pathway to activate or inhibit the signaling pathway, and the second binding domain and the first binding domain target different targets.

2. The fusion protein according to claim 1, wherein the first binding domain targets and binds to an immune checkpoint molecule or a tumor antigen; preferably, -

~~3. The fusion protein according to claim 2, wherein~~ the immune checkpoint molecule includes PD-1, PD-L1, CTLA-4, LAG-3, FGL1, TIM-3, Galectin-9, TIGIT, CD155 and CD47; the tumor antigen includes Claudin 18.2, HER-2, Mesothelin, BCMA, SSTR2, GPRC5D, PSMA, FCRH5, CD33, CD123, CD20, A33, CEA, CD28, DLL3, EGFR, VEGFR, VEGFR2, VEGF-A, Nectin-4, FGFR, C-met, RANKL, PDGF, PDGFR, PDGFR α , DLL4, Ang-1 and Ang-2; preferably, -

~~4. The fusion protein according to claim 3, wherein~~ the second binding domain targets and binds to human TGF- β , CTLA-4, VEGF, LAG3, CD27, 4-1BB, OX40, CD47, FGL1, TLT-2, CD28, HGF, CSF1, CXCL1, CXCL2, CXCL3, CXCL5, CXCL6, CXCL7, CXCL8, CXCL9, CXCL10, CXCL12, GITR, EGF and ICOSL; preferably, -

~~5. The fusion protein according to claim 4, wherein~~ the receptor or ligand is human TGF- β receptor (TGF- β R), CD80, CD86, VEGFR, VEGF-trap, FGL1, CD70, 4-1BBL, OX40L, SIRP α , B7-H3, C-

met, CSF1R, CXCR2, CXCR3, CXCR4, GITRL, EGFR and ICOS.

36. The fusion protein according to claim 24, wherein the first binding domain and the second binding domain are selected from the following combinations:

(1) the first binding domain targets and binds to PD-L1, and the second binding domain targets and binds to human TGF- β , VEGF, FGL1, CD47, CD155, HGF, CSF1, CXCL1, CXCL2, CXCL3, CXCL5, CXCL6, CXCL7, CXCL8, CXCL9, CXCL10, CXCL12, EGF or ICOSL; or

(2) the first binding domain targets and binds to PD-1, and the second binding domain targets and binds to human CTLA-4, VEGF, HGF, EGF, CD28, LAG3, CD27, 4-1BB or OX40; or

(3) the first binding domain targets and binds to CTLA-4, and the second binding domain targets and binds to human CD28, VEGF, HGF, EGF, LAG3, CD27, 4-1BB or OX40; or

(4) the first binding domain targets and binds to LAG-3, and the second binding domain targets and binds to human CD28, VEGF, HGF, EGF, CTLA-4, CD27, 4-1BB or OX40; or

(5) the first binding domain targets and binds to FGL1, and the second binding domain targets and binds to human TGF- β , VEGF, CD47, CD155, HGF, CSF1, CXCL1, CXCL2, CXCL3, CXCL5, CXCL6, CXCL7, CXCL8, CXCL9, CXCL10, CXCL12, EGF or ICOSL; or

(6) the first binding domain targets and binds to TIM-3, and the second binding domain targets and binds to human VEGF, HGF, EGF, LAG3, CD27, 4-1BB or OX40; or

(7) the first binding domain targets and binds to Galectin-9, and the second binding domain targets and binds to human TGF- β , VEGF, CD47, CD155, HGF, CSF1, CXCL1, CXCL2, CXCL3, CXCL5, CXCL6, CXCL7, CXCL8, CXCL9, CXCL10, CXCL12, EGF or ICOSL; or

(8) the first binding domain targets and binds to TIGIT, and the second binding domain targets and binds to human TGF- β , HGF, EGF or VEGF; or

(9) the first binding domain targets and binds to CD155, and the second binding domain targets and binds to human TGF- β , VEGF, HGF, EGF, CD47 or CD155; or

(10) the first binding domain targets and binds to Claudin 18.2, HER-2, mesothelin, BCMA, SSTR2, GPRC5D, PSMA, FCRH5, CD33, CD123, CD20, A33, CEA, CD28, DLL3, EGFR,

VEGFR, VEGFR2, VEGF-A, Nectin-4, FGFR, C-met, RANKL, PDGF, PDGFR, PDGFR α , DLL-4, Ang-1 or Ang-2, and the second binding domain targets and binds to human CTLA-4, TGF- β , VEGF, FGL1, LAG3, 4-1BB, OX40, CD27, CD28, CD47, CD155, HGF, CSF1, CXCL1, CXCL2, CXCL3, CXCL5, CXCL6, CXCL7, CXCL8, CXCL9, CXCL10, CXCL12, EGF or ICOSL; or

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~~7. The fusion protein according to claim 6, wherein the first binding domain and the receptor or ligand are selected from the following combinations:~~

(~~11~~) the first binding domain targets and binds to PD-L1, and the receptor or ligand is selected from TGF- β R2, VEGFR, LAG-3, SIRP α , TIGIT, C-MET, CSF1R, CXCR2, CXCR3, CXCR4, EGFR or ICOS; or

(~~12~~) the first binding domain targets and binds to PD-1, and the receptor or ligand is selected from human CD80, CD86, VEGFR, c-MET, EGFR, FGL1, CD70, 4-1BBL or OX40L; or

(~~13~~) the first binding domain targets and binds to CTLA-4, and the receptor or ligand is selected from human CD80, CD86, VEGFR, c-MET, EGFR, FGL1, CD70, 4-1BBL or OX40L; or

(~~14~~) the first binding domain targets and binds to LAG-3, and the receptor or ligand is selected from human VEGFR, c-MET, EGFR, CD80, CD86, CD70, 4-1BBL or OX40L; or

(~~15~~) the first binding domain targets and binds to FGL1, and the receptor or ligand is selected from human TGF- β R2, VEGFR, SIRP α , TIGIT, c-MET, CSF1R, CXCR2, CXCR3, CXCR4, EGFR or ICOS; or

(~~16~~) the first binding domain targets and binds to TIM-3, and the receptor or ligand is selected from human VEGFR, c-MET, EGFR, FGL1, CD70, 4-1BBL or OX40L; or

(~~17~~) the first binding domain targets and binds to Galectin-9, and the receptor or ligand is selected from human TGF- β R2, VEGFR, SIRP α , TIGIT, c-MET, CSF1R, CXCR2, CXCR3, CXCR4, EGFR or ICOS; or

(~~18~~) the first binding domain targets and binds to TIGIT, and the receptor or ligand is selected from human TGF- β R2, c-MET, EGFR or VEGFR; or

(19) the first binding domain targets and binds to CD155, and the receptor or ligand is selected from human TGF- β RII, VEGFR, c-MET, EGFR, SIRP α or TIGIT; or

(~~1~~20) the first binding domain targets and binds to Claudin 18.2, HER-2, mesothelin, BCMA, SSTR2, GPRC5D, PSMA, FCRH5, CD33, CD123, CD20, A33, CEA, CD28, DLL3, EGFR, VEGFR, VEGFR2, Nectin-4, FGFR, c-MET, RANKL, PDGF, PDGFR, PDGFR α , DLL4, Ang-1 or Ang-2, and the receptor or ligand is selected from human CTLA-4, CD80, CD86, TGF- β RII, VEGFR, FGL1, LAG3, 4-1BB, OX40, CD27, CD28, CD70, c-MET, SIRP α , TIGIT, CSF1R, CXCR2, CXCR3, CXCR4, EGFR or ICOS.

~~4~~8. The fusion protein according to any one of claims 1-~~3~~7, wherein the fragment of the receptor or ligand comprises a fragment of the extracellular region and a fragment of the binding domain of the receptor or ligand.

~~5~~9. The fusion protein according to claim ~~3~~7, wherein the first binding domain targets and binds to PD-L1; the second binding domain is a fragment of human TGF- β RII; or the first binding domain targets and binds to PD-1, and the second binding domain is selected from human CD80 ECD, CD80 IgV region, human CD80 IgVIgC, VEGFR1 ECD, VEGFR2 ECD or a combination of the second extracellular region of VEGFR1 and the third extracellular region of VEGFR2; or the first binding domain targets and binds to Claudin 18.2, HER-2 or EGFR, and the second binding domain is selected from human CD80 ECD, CD80 IgV region, human CD80 IgVIgC, VEGFR1 ECD, VEGFR2 ECD or a combination of the second extracellular region of VEGFR1 and the third extracellular region of VEGFR2.

~~6~~40. The fusion protein according to claim ~~5~~9, wherein the second binding domain is selected from:

(1) human TGF- β RII comprising the sequence as shown in SEQ ID NO: 31, 131 or 132, or a sequence having an identity of greater than 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% compared with the sequence as shown in SEQ ID NO: 31, 131 or 132, or an amino acid sequence having 1,

2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acid substitutions or deletions on the sequence as shown in SEQ ID NO: 31, 131 or 132; or

(2) CD80 ECD comprising the sequence as shown in SEQ ID NO: 32 or 133, or a sequence having an identity of greater than 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% compared with the sequence as shown in SEQ ID NO: 32 or 133, or an amino acid sequence having 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acid substitutions or deletions on the sequence as shown in SEQ ID NO: 32 or 133; or

(3) a combination of the second extracellular region of VEGFR1 and the third extracellular region of VEGFR2, comprising the sequence as shown in SEQ ID NO: 33, or a sequence having an identity of greater than 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% compared with the sequence as shown in SEQ ID NO: 33, or an amino acid sequence having 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acid substitutions or deletions on the sequence as shown in SEQ ID NO: 33.

~~744.~~ The fusion protein according to claim ~~59~~ or ~~640~~, wherein the first binding domain targets and binds to PD-L1, and the CDRs of the heavy chain variable region and/or the CDRs of the light chain variable region in the Fab of the IgG have the same CDR sequence as the antibody defined by the following sequence, or have 1-2 amino acid substitutions on the CDRs of the antibody defined by the following sequence, wherein the antibody is defined as follows:

(1) the amino acid sequence of the heavy chain variable region is shown in SEQ ID NO: 66; and/or

(2) the amino acid sequence of the light chain variable region is shown in SEQ ID NO: 67; or preferably,-

~~12. The fusion protein according to claim 11, wherein~~ the CDRs of the heavy chain variable region and/or the CDRs of the light chain variable region in the Fab of the IgG are as follows:

(~~43~~) for the heavy chain variable region, the amino acid sequence of CDR1 is selected from SEQ ID NOs: 1-5 and amino acid sequences having 1 or 2 amino acid substitutions on SEQ ID NOs: 1-5; the amino acid sequence of CDR2 is selected from SEQ ID NOs: 6-10 and amino acid

sequences having 1 or 2 amino acid substitutions on SEQ ID NOs: 6-10; the amino acid sequence of CDR3 is selected from SEQ ID NOs: 11-15 and amino acid sequences having 1 or 2 amino acid substitutions on SEQ ID NOs: 11-15; and/or

(~~24~~) for the light chain variable region, the amino acid sequence of CDR1 is selected from SEQ ID NOs: 16-20 and amino acid sequences having 1 or 2 amino acid substitutions on SEQ ID NOs: 16-20; the amino acid sequence of CDR2 is selected from SEQ ID NOs: 21-25 and amino acid sequences having 1 or 2 amino acid substitutions on SEQ ID NOs: 21-25; the amino acid sequence of CDR3 is selected from SEQ ID NOs: 26-30 and amino acid sequences having 1 or 2 amino acid substitutions on SEQ ID NOs: 26-30; or preferably,

~~13. The fusion protein according to claim 12, wherein:~~

(~~15~~) the amino acid sequences of CDRs 1-3 of the heavy chain variable region are SEQ ID NOs: 1, 6, 11, or an amino acid sequence having 1 or 2 amino acid substitutions on SEQ ID NOs: 1, 6, 11; and/or an amino acid sequence of CDRs 1-3 of the light chain variable region are SEQ ID NOs: 16, 21, 26, or an amino acid sequence having 1 or 2 amino acid substitutions on SEQ ID NOs: 16, 21, 26; or

(~~26~~) the amino acid sequences of CDRs 1-3 of the heavy chain variable region are SEQ ID NOs: 2, 7, 12, or an amino acid sequence having 1 or 2 amino acid substitutions on SEQ ID NOs: 2, 7, 12; and/or an amino acid sequence of CDRs 1-3 of the light chain variable region are SEQ ID NOs: 17, 22, 27, or an amino acid sequence having 1 or 2 amino acid substitutions on SEQ ID NOs: 17, 22, 27; or

(~~37~~) the amino acid sequences of CDRs 1-3 of the heavy chain variable region are SEQ ID NOs: 3, 8, 13, or an amino acid sequence having 1 or 2 amino acid substitutions on SEQ ID NOs: 3, 8, 13; and/or an amino acid sequence of CDRs 1-3 of the light chain variable region are SEQ ID NOs: 18, 23, 28, or an amino acid sequence having 1 or 2 amino acid substitutions on SEQ ID NOs: 18, 23, 28; or

(~~48~~) the amino acid sequences of CDRs 1-3 of the heavy chain variable region are SEQ ID NOs: 4, 9, 14, or an amino acid sequence having 1 or 2 amino acid substitutions on SEQ ID NOs:

4, 9, 14; and/or an amino acid sequence of CDRs 1-3 of the light chain variable region are SEQ ID NOs: 19, 24, 29, or an amino acid sequence having 1 or 2 amino acid substitutions on SEQ ID NOs: 19, 24, 29; or

(~~5~~9) the amino acid sequences of CDRs 1-3 of the heavy chain variable region are SEQ ID NOs: 5, 10, 15, or an amino acid sequence having 1 or 2 amino acid substitutions on SEQ ID NOs: 5, 10, 15; and/or an amino acid sequence of CDRs 1-3 of the light chain variable region are SEQ ID NOs: 20, 25, 30, or an amino acid sequence having 1 or 2 amino acid substitutions on SEQ ID NOs: 20, 25, 30.

~~14. The fusion protein according to claim 13, wherein the amino acid sequences of CDRs 1-3 of the heavy chain variable region are SEQ ID NOs: 3, 8, 13; and/or an amino acid sequence of CDRs 1-3 of the light chain variable region are SEQ ID NOs: 18, 23, 28.~~

~~8~~15. The fusion protein according to claim ~~7~~14, wherein:

(1) the heavy chain variable region has a sequence as shown in SEQ ID NO: 66, or a sequence that has the same CDRs 1-3 as SEQ ID NO: 66 and has an identity of greater than 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% compared with SEQ ID NO: 66; and/or

(2) the heavy chain variable region has a sequence as shown in SEQ ID NO: 67, or a sequence that has the same CDRs 1-3 as SEQ ID NO: 67 and has an identity of greater than 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% compared with SEQ ID NO: 67; or preferably,

~~16. The fusion protein according to claim 15, wherein:~~

(~~1~~3) the heavy chain of the fusion protein has an amino acid sequence as shown in SEQ ID NO: 72, or a sequence having an identity of greater than 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% compared with SEQ ID NO: 72;

(~~2~~4) the light chain of the fusion protein has an amino acid sequence as shown in SEQ ID NO: 73, or a sequence having an identity of greater than 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%

compared with SEQ ID NO: 73; or preferably,-

~~17. The fusion protein according to claim 16, wherein:~~

(~~15~~) the amino acid sequence of the heavy chain of the fusion protein has 1-15 amino acid site mutations or has an alternative peptide linker compared with SEQ ID NO: 72;

(~~26~~) the amino acid sequence of the light chain of the fusion protein has 1-10 amino acid site mutations compared with SEQ ID NO: 73.

~~18. The fusion protein according to claim 59, wherein the first binding domain targets and binds to PD-1, and the CDRs of the heavy chain variable region and/or the CDRs of the light chain variable region in the Fab of the IgG have the same CDR sequence as the antibody defined by the following sequence, or have 1-2 amino acid substitutions on the CDRs of the antibody defined by the following sequence, wherein the antibody is defined as follows:~~

(1) the amino acid sequence of the heavy chain variable region is shown in SEQ ID NO: 64; and/or

(2) the amino acid sequence of the light chain variable region is shown in SEQ ID NO: 65; or preferably,-

~~19. The fusion protein according to claim 18, wherein the CDRs of the heavy chain variable region and/or the CDRs of the light chain variable region in the Fab of the IgG are as follows:~~

(~~43~~) for the heavy chain variable region, the amino acid sequence of CDR1 is selected from SEQ ID NOs: 34-38 and an amino acid sequence having 1 or 2 amino acid substitutions on SEQ ID NOs: 34-38; the amino acid sequence of CDR2 is selected from SEQ ID NOs: 39-43 and an amino acid sequence having 1 or 2 amino acid substitutions on SEQ ID NOs: 39-43; the amino acid sequence of CDR3 is selected from SEQ ID NOs: 44-48 and an amino acid sequence having 1 or 2 amino acid substitutions on SEQ ID NOs: 44-48; and/or

(~~24~~) for the light chain variable region, the amino acid sequence of CDR1 is selected from

SEQ ID NOs: 49-53 and an amino acid sequence having 1 or 2 amino acid substitutions on SEQ ID NOs: 49-53; the amino acid sequence of CDR2 is selected from SEQ ID NOs: 54-58 and an amino acid sequence having 1 or 2 amino acid substitutions on SEQ ID NOs: 54-58; the amino acid sequence of CDR3 is selected from SEQ ID NOs: 59-63 and an amino acid sequence having 1 or 2 amino acid substitutions on SEQ ID NOs: 59-63; or preferably,

~~20. The fusion protein according to claim 19,~~ wherein:

(~~15~~) the amino acid sequences of CDRs 1-3 of the heavy chain variable region are SEQ ID NOs: 34, 39, 44, or an amino acid sequence having 1 or 2 amino acid substitutions on SEQ ID NOs: 34, 39, 44; and/or an amino acid sequence of CDRs 1-3 of the light chain variable region are SEQ ID NOs: 49, 54, 59, or an amino acid sequence having 1 or 2 amino acid substitutions on SEQ ID NOs: 49, 54, 59; or

(~~26~~) the amino acid sequences of CDRs 1-3 of the heavy chain variable region are SEQ ID NOs: 35, 40, 45, or an amino acid sequence having 1 or 2 amino acid substitutions on SEQ ID NOs: 35, 40, 45; and/or an amino acid sequence of CDRs 1-3 of the light chain variable region are SEQ ID NOs: 50, 55, 60, or an amino acid sequence having 1 or 2 amino acid substitutions on SEQ ID NOs: 50, 55, 60; or

(~~37~~) the amino acid sequences of CDRs 1-3 of the heavy chain variable region are SEQ ID NOs: 36, 41, 46, or an amino acid sequence having 1 or 2 amino acid substitutions on SEQ ID NOs: 36, 41, 46; and/or an amino acid sequence of CDRs 1-3 of the light chain variable region are SEQ ID NOs: 51, 56, 61, or an amino acid sequence having 1 or 2 amino acid substitutions on SEQ ID NOs: 51, 56, 61; or

(~~48~~) the amino acid sequences of CDRs 1-3 of the heavy chain variable region are SEQ ID NOs: 37, 42, 47, or an amino acid sequence having 1 or 2 amino acid substitutions on SEQ ID NOs: 37, 42, 47; and/or an amino acid sequence of CDRs 1-3 of the light chain variable region are SEQ ID NOs: 52, 57, 62, or an amino acid sequence having 1 or 2 amino acid substitutions on SEQ ID NOs: 52, 57, 62; or

(~~59~~) the amino acid sequences of CDRs 1-3 of the heavy chain variable region are SEQ ID

NOs: 38, 43, 48, or an amino acid sequence having 1 or 2 amino acid substitutions on SEQ ID NOs: 38, 43, 48; and/or an amino acid sequence of CDRs 1-3 of the light chain variable region are SEQ ID NOs: 53, 58, 63, or an amino acid sequence having 1 or 2 amino acid substitutions on SEQ ID NOs: 53, 58, 63.

~~1024.~~ The fusion protein according to claim ~~920~~, wherein :__

(1) the amino acid sequences of CDRs 1-3 of the heavy chain variable region are SEQ ID NOs: 36, 41, 46; and/or

(2) an amino acid sequence of CDRs 1-3 of the light chain variable region are SEQ ID NOs: 51, 56, 61; or preferably,-

~~22.~~ ~~The fusion protein according to claim 21~~, wherein:

~~(43)~~ the heavy chain variable region has a sequence as shown in SEQ ID NO: 64, or a sequence that has the same CDRs 1-3 as SEQ ID NO: 64 and has an identity of greater than 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% compared with SEQ ID NO: 64; and/or

~~(24)~~ the light chain variable region has a sequence as shown in SEQ ID NO: 65, or a sequence that has the same CDRs 1-3 as SEQ ID NO: 65 and has an identity of greater than 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% compared with SEQ ID NO: 65; or preferably,-

~~23.~~ ~~The fusion protein according to claim 22~~, wherein:

~~(45)~~ the heavy chain of the fusion protein has an amino acid sequence as shown in SEQ ID NO: 68, or a sequence having an identity of greater than 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% compared with SEQ ID NO: 68;

~~(26)~~ the light chain of the fusion protein has an amino acid sequence as shown in SEQ ID NO: 69, or a sequence having an identity of greater than 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% compared with SEQ ID NO: 69; or preferably,-

~~24. The fusion protein according to claim 23, wherein:~~

~~(17)~~ the amino acid sequence of the heavy chain of the fusion protein has 1-15 amino acid site mutations or has an alternative peptide linker compared with SEQ ID NO: 68;

~~(28)~~ the amino acid sequence of the light chain of the fusion protein has 1-10 amino acid site mutations compared with SEQ ID NO: 69; or preferably,-

~~25. The fusion protein according to claim 22, wherein:~~

~~(19)~~ the heavy chain of the fusion protein has an amino acid sequence as shown in SEQ ID NO: 70, or a sequence having an identity of greater than 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% compared with SEQ ID NO: 70;

~~(210)~~ the light chain of the fusion protein has an amino acid sequence as shown in SEQ ID NO: 71, or a sequence having an identity of greater than 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% compared with SEQ ID NO: 71; or preferably,-

~~26. The fusion protein according to claim 25, wherein:~~

~~(111)~~ the amino acid sequence of the heavy chain of the fusion protein has 1-15 amino acid site mutations or has an alternative peptide linker compared with SEQ ID NO: 70;

~~(212)~~ the amino acid sequence of the light chain of the fusion protein has 1-10 amino acid site mutations compared with SEQ ID NO: 71.

~~1127.~~ The fusion protein according to any one of claims 1-~~640~~, wherein the IgG is Atezolizumab, Avelumab, Durvalumab, Nivolumab, Pembrolizumab, Cemiplimab or Ipilimumab.

~~1228.~~ The fusion protein according to any one of claims 1-~~1127~~, wherein the C-terminus or/and N-terminus of the second binding domain has a peptide linker, and the peptide linker consists

of 2-30 amino acids; or preferably,-

~~29. The fusion protein of claim 28, wherein the peptide linker is:~~

- (1) (GGGGS)_n; or
- (2) AKTTPKLEEGEFSEAR (SEQ ID NO: 80); or
- (3) AKTTPKLEEGEFSEARV (SEQ ID NO: 81); or
- (4) AKTTPKLGG (SEQ ID NO: 82); or
- (5) SAKTTPKLGG (SEQ ID NO: 83); or
- (6) SAKTTP (SEQ ID NO: 84); or
- (7) RADAAP (SEQ ID NO: 85); or
- (8) RADAAPTVS (SEQ ID NO: 86); or
- (9) RADAAAAGGPGS (SEQ ID NO: 87); or
- (10) RADAAAAA (SEQ ID NO: 88); or
- (11) SAKTTPKLEEGEFSEARV (SEQ ID NO: 89); or
- (12) ADAAP (SEQ ID NO: 90); or
- (13) DAAPTVSIFPP (SEQ ID NO: 91); or
- (14) TVAAP (SEQ ID NO: 92); or
- (15) TVAAPSVFIFPP (SEQ ID NO: 93); or
- (16) QPKAAP (SEQ ID NO: 94); or
- (17) QPKAAPSVTLFPP (SEQ ID NO: 95); or
- (18) AKTTPP (SEQ ID NO: 96); or
- (19) AKTTPPSVTPLAP (SEQ ID NO: 97); or
- (20) AKTTAP (SEQ ID NO: 98); or
- (21) AKTTAPSVYPLAP (SEQ ID NO: 99); or

- (22) ASTKGP (SEQ ID NO: 100); or
- (23) ASTKGPSVFPLAP (SEQ ID NO: 101); or
- (24) GENKVEYAPALMALS (SEQ ID NO: 102); or
- (25) GPAKELTPLKEAKVS (SEQ ID NO: 103); or
- (26) GHEAAVMQVQYPAS (SEQ ID NO: 104); or
- (27) GGGGSGGGGSGGGGSA (SEQ ID NO: 105),
- wherein n is equal to 1, 2, 3 or 4.

~~30. The fusion protein according to any one of claims 1-29, wherein the size of the inserted second binding domain does not exceed 300 amino acids.~~

~~31. The fusion protein according to any one of claims 1-30, wherein the insertion site of the second binding domain is located in a middle front part of the hinge region, and the insertion site does not affect the formation of disulfide bonds of the immunoglobulin.~~

~~32. The fusion protein according to claim 31, wherein the middle front part of the hinge region refers to the part before 231A.~~

~~33. The fusion protein according to any one of claims 1-32, wherein part of the amino acids in the hinge region before and after the insertion site are substituted or deleted.~~

~~34. The fusion protein according to claim 33, wherein the hinge region contains D221G and/or C220V mutations.~~

~~35. The fusion protein according to any one of claims 1-34, wherein the IgG is selected from~~

mammalian IgG, humanized IgG, and human IgG, and the mammal includes mouse, rat and rabbit; preferably, the IgG is IgG1, IgG2, IgG3 or IgG4.

~~36. The fusion protein according to any one of claims 1-35, wherein the Fc region of the fusion protein is aglycosylated or deglycosylated, or has reduced fucosylation or is afucosylated.~~

1337. An isolated polynucleotide encoding the fusion protein according to any one of claims 1-1236.

1438. A nucleic acid construct comprising the polynucleotide according to claim 1337, preferably the nucleic acid construct is a vector.

1539. A host cell comprising the polynucleotide according to claim 1337 or the nucleic acid construct or the vector according to claim 1438, preferably the cell is a prokaryotic cell, eukaryotic cell, yeast cell, mammal cell, E. coli cell or CHO cell, NS0 cell, Sp2/0 cell, or BHK cell.

1640. A pharmaceutical composition comprising the fusion protein according to any one of claims 1-1236 and a pharmaceutically acceptable carrier.

~~41. A method for treating tumor or cancer, comprising the step of administering the fusion protein according to any one of claims 1-36 or the pharmaceutical composition according to claim 40 to a subject in need of a treatment or relief.~~

1742. ~~Use of~~ The fusion protein according to any one of claims 1-1236, the polynucleotide according to claim 1337, the nucleic acid construct or the vector according to claim 1438, or the pharmaceutical composition according to claim 1640 ~~in the manufacture of a medicament for use~~

in the treatment or prevention of a tumor or cancer; preferably, -

~~43. The use according to claim 42, wherein the tumor or cancer includes a solid tumor or a non-solid tumor.~~

~~44. A diagnostic kit comprising the fusion protein according to any one of claims 1-36.~~

~~1845.~~ A method of manufacturing the fusion protein according to any one of claims 1-~~1236~~, comprising culturing the host cell according to claim ~~1539~~ under a condition allowing the expression of the nucleic acid construct according to claim ~~1438~~, and recovering the produced fusion protein from the culture.