

~~WHAT IS CLAIMED:~~CLAIMS

1. A heterodimeric protein comprising a first Fc polypeptide and a second Fc polypeptide, wherein:

- a) the first Fc polypeptide comprises aspartate, glutamate, and tryptophan at amino acid positions 239, 332, and 366, respectively; and
- b) the second Fc polypeptide comprises aspartate, serine, alanine, and valine at amino acid positions 239, 366, 368, and 407, respectively, but does not comprise glutamate at amino acid position 332,

wherein the amino acid positions are numbered according to the EU index.

2. The heterodimeric protein of claim 1, wherein:

- a) the first Fc polypeptide further comprises leucine at amino acid position 330; and/or
- b) the second Fc polypeptide does not comprise leucine at amino acid position 330,

wherein the amino acid positions are numbered according to the EU index.

~~3. The heterodimeric protein of claim 1 or 2, wherein the second Fc polypeptide does not comprise leucine at amino acid position 330, wherein the amino acid positions are numbered according to the EU index.~~

~~4.3.~~ The heterodimeric protein of ~~any one of claims 1-3~~claim 1 or 2, wherein the first Fc polypeptide comprises a first antigen-binding moiety and/or the second Fc polypeptide comprises a second antigen-binding moiety.

~~5.4.~~ The heterodimeric protein of claim ~~43~~43, wherein the first and/or second antigen-binding moiety comprises an antibody variable domain, an extracellular domain of a cell surface receptor, a soluble T cell receptor, or a ligand, optionally wherein:

a) the first and/or second antigen-binding moiety comprises a VH, a VL, a VHH, a VH/VL pair, an scFv, a diabody, and/or a Fab;

b) the cell surface receptor is a tumor necrosis factor superfamily receptor, a vascular endothelial growth factor receptor, or a transforming growth factor receptor; or

c) the ligand is a hormone or growth factor;

wherein the first and second antigen-binding moieties specifically bind to the same or different target molecules.

~~6. — The heterodimeric protein of claim 5, wherein the first and/or second antigen binding moiety comprises a VH, a VL, a VHH, a VH/VL pair, an scFv, a diabody, and/or a Fab.~~

~~7. — The heterodimeric protein of claim 5, wherein the cell surface receptor is a tumor necrosis factor superfamily receptor, a vascular endothelial growth factor receptor, or a transforming growth factor receptor.~~

~~8. — The heterodimeric protein of claim 5, wherein the ligand is a hormone or growth factor.~~

~~9. — The heterodimeric protein of any one of claims 4-8, wherein the first and second antigen binding moieties specifically bind to the same or different target molecules.~~

~~10.5. — The heterodimeric protein of any one of claims 1-49, wherein the first and/or second Fc polypeptide comprises a CH1 domain, hinge region, CH2 domain and/or CH3 domain of human IgG₁, IgG₂, IgG₃, IgG₄, IgA₁, or IgA₂.~~

~~11.6. — The heterodimeric protein of claim 5-10, wherein the first and/or second Fc polypeptide comprises an antibody heavy chain, optionally wherein:~~

~~a) the antibody heavy chain lacks a CH1 domain and/or a portion of a hinge region; or~~

~~b) the antibody heavy chain is a full-length antibody heavy chain,~~

~~optionally wherein the antibody heavy chain is a human IgG₁, IgG₂, IgG₃, IgG₄, IgA₁, or IgA₂ heavy chain;~~

~~optionally wherein the first and/or second Fc polypeptide further comprises an antibody light chain, optionally wherein the antibody light chain is a human kappa or lambda light chain.~~

~~12. — The heterodimeric protein of claim 11, wherein the antibody heavy chain lacks a CH1 domain and/or a portion of a hinge region.~~

~~13. — The heterodimeric protein of claim 11, wherein the antibody heavy chain is a full length antibody heavy chain.~~

~~14. — The heterodimeric protein of any one of claims 11–13, wherein the antibody heavy chain is a human IgG₁, IgG₂, IgG₃, IgG₄, IgA₁, or IgA₂ heavy chain.~~

~~15. — The heterodimeric protein of any of claims 11–14, wherein the first and/or second Fc polypeptide further comprises an antibody light chain.~~

~~16. — The heterodimeric protein of claim 15, wherein the antibody light chain is a human kappa or lambda light chain.~~

~~17.7. — The heterodimeric protein of any one of claims 1–6 and 9–14, wherein:~~

~~a) the first Fc polypeptide comprises a first half-antibody comprising a first antibody heavy chain and a first antibody light chain; and/or~~

~~b) the second Fc polypeptide comprises a second half-antibody comprising a second antibody heavy chain and a second antibody light chain; or~~

~~c) the first Fc polypeptide comprises a first half-antibody comprising a first antibody heavy chain and a first antibody light chain, and the second Fc polypeptide comprises a second half-antibody comprising a second antibody heavy chain and a second antibody light chain;~~

~~optionally wherein the first and second half-antibodies bind to different target molecules or to different regions of the same target molecule.~~

~~18. — The heterodimeric protein of any one of claims 1–6 and 9–15, wherein:~~

~~a) the first Fc polypeptide comprises a first half-antibody comprising a first antibody heavy chain and a first antibody light chain; and~~

~~b) the second Fc polypeptide comprises a second half-antibody comprising a second antibody heavy chain and a second antibody light chain.~~

~~19. The heterodimeric protein of claim 17 or 18, wherein the first and second half antibodies bind to different target molecules or to different regions of the same target molecule.~~

~~20.8. The heterodimeric protein of any one of claims 1-711 and 13-19, wherein:~~

~~a) the first Fc polypeptide comprises an amino acid sequence that is at least 75% identical (optionally at least 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% identical) to an amino acid sequence selected from the group consisting of SEQ ID NO: 1-3, 6-7, 10-11, 24-27, 48-51, and 62-65; and/or~~

~~b) the second Fc polypeptide comprises an amino acid sequence that is at least 75% identical (optionally at least 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% identical) to an amino acid sequence selected from the group consisting of SEQ ID NO: 1-3, 5, 12-13, 36-37, 60-61, and 66-67; or~~

~~c) the first Fc polypeptide comprises an amino acid sequence that is at least 75% identical (optionally at least 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% identical) to an amino acid sequence selected from the group consisting of SEQ ID NO: 1-3, 6-7, 10-11, 24-27, 48-51, and 62-65; and the second Fc polypeptide comprises an amino acid sequence that is at least 75% identical (optionally at least 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% identical) to an amino acid sequence selected from the group consisting of SEQ ID NO: 1-3, 5, 12-13, 36-37, 60-61, and 66-67.~~

~~21. The heterodimeric protein of claim 20, wherein the first Fc polypeptide comprises an amino acid sequence that is at least 75% identical (optionally at least 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% identical) to an amino acid sequence selected from the group consisting of SEQ ID NO: 1-3, 6-7, 10-11, 24-27, 48-51, and 62-65; and the second Fc polypeptide comprises an amino acid sequence that is at least 75% identical (optionally at least 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% identical) to an amino acid sequence selected from the group consisting of SEQ ID NO: 1-3, 5, 12-13, 36-37, 60-61, and 66-67.~~

~~22.9.~~ The heterodimeric protein of any one of claims ~~1-811 and 13-21~~, wherein the first Fc polypeptide and second Fc polypeptide comprise amino acid sequences that are at least 75% identical (optionally at least 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% identical) to the amino acid sequences of SEQ ID NOs: 24 and 36; 24 and 37; 25 and 36; 25 and 37; 26 and 36; 26 and 37; 27 and 36; 27 and 37; 48 and 60; 48 and 61; 49 and 60; 49 and 61; 50 and 60; 50 and 61; 51 and 60; 51 and 61; 62 and 66; 62 and 67; 63 and 66; 63 and 67; 64 and 66; 64 and 67; 65 and 66; or 65 and 67, respectively.

~~23.10.~~ The heterodimeric protein of any one of claims ~~1-922~~, wherein the first Fc polypeptide and the second Fc polypeptide comprises the amino acid sequences set forth in SEQ ID NOs: 24 and 36; 24 and 37; 25 and 36; 25 and 37; 26 and 36; 26 and 37; 27 and 36; 27 and 37; 48 and 60; 48 and 61; 49 and 60; 49 and 61; 50 and 60; 50 and 61; 51 and 60; 51 and 61; 62 and 66; 62 and 67; 63 and 66; 63 and 67; 64 and 66; 64 and 67; 65 and 66; or 65 and 67, respectively~~51 and the second Fc polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 61.~~

~~24.~~ The heterodimeric protein of any one of claims ~~1-22~~, wherein the first Fc polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 50 and the second Fc polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 60.

~~25.~~ The heterodimeric protein of any one ~~any one~~ of claims ~~1-22~~, wherein the first Fc polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 51 and the second Fc polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 60.

~~26.~~ The heterodimeric protein of any one ~~any one~~ of claims ~~1-22~~, wherein the first Fc polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 50 and the second Fc polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 61.

~~27.~~ The heterodimeric protein of any one of claims ~~2-22~~, wherein the first Fc polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 49 and the second Fc polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 61.

28. — The heterodimeric protein of any one of claims 2-22, wherein the first Fc polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 48 and the second Fc polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 60.

29. — The heterodimeric protein of any one of claims 2-22, wherein the first Fc polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 49 and the second Fc polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 60.

30. — The heterodimeric protein of any one of claims 2-22, wherein the first Fc polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 48 and the second Fc polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 61.

31. — The heterodimeric protein of any one of claims 1-22, wherein the first Fc polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 65 and the second Fc polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 67.

32. — The heterodimeric protein of any one of claims 1-22, wherein the first Fc polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 64 and the second Fc polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 66.

33. — The heterodimeric protein of any one of claims 1-22, wherein the first Fc polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 65 and the second Fc polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 66.

34. — The heterodimeric protein of any one of claims 1-22, wherein the first Fc polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 64 and the second Fc polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 67.

35. — The heterodimeric protein of any one of claims 2-22, wherein the first Fc polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 63 and the second Fc polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 67.

36. — ~~The heterodimeric protein of any one of claims 2-22, wherein the first Fe polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 62 and the second Fe polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 66.~~

37. — ~~The heterodimeric protein of any one of claims 2-22, wherein the first Fe polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 63 and the second Fe polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 66.~~

38. — ~~The heterodimeric protein of any one any one of claims 2-22, wherein the first Fe polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 62 and the second Fe polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 67.~~

39. — ~~The heterodimeric protein of any one of claims 1-11 and 13-22, wherein the first Fe polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 27 and the second Fe polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 37.~~

40. — ~~The heterodimeric protein of any one of claims 1-11 and 13-22, wherein the first Fe polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 26 and the second Fe polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 36.~~

41. — ~~The heterodimeric protein of any one of claims 1-11 and 13-22, wherein the first Fe polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 27 and the second Fe polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 36.~~

42. — ~~The heterodimeric protein of any one of claims 1-11 and 13-22, wherein the first Fe polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 26 and the second Fe polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 37.~~

~~43. The heterodimeric protein of any one of claims 2-11 and 13-22, wherein the first Fc polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 25 and the second Fc polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 37.~~

~~44. The heterodimeric protein of any one of claims 2-11 and 13-22, wherein the first Fc polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 24 and the second Fc polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 36.~~

~~45. The heterodimeric protein of any one of claims 2-11 and 13-22, wherein the first Fc polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 25 and the second Fc polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 36.~~

~~46. The heterodimeric protein of any one of claims 2-11 and 13-22, wherein the first Fc polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 24 and the second Fc polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 37.~~

~~47.11.~~ A pharmaceutical composition comprising the heterodimeric protein of any one of the preceding claims and a pharmaceutically acceptable carrier or excipient.

~~48.12.~~ An isolated polynucleotide encoding the first and the second Fc polypeptides of the heterodimeric protein of any one of the preceding claims.

~~49.13.~~ A vector comprising the polynucleotide of claim ~~48.12.~~

~~50.14.~~ A recombinant host cell comprising:

- a) the polynucleotide of claim ~~48.12.~~;
- b) the vector of claim ~~49.13.~~;
- c) a first polynucleotide encoding the first Fc polypeptide of the heterodimeric protein of any one of the preceding claims, and a second polynucleotide encoding the second Fc polypeptide of the heterodimeric protein of any one of the preceding claims;

- d) a first vector comprising a first polynucleotide encoding the first Fc polypeptide of the heterodimeric protein of any one of the preceding claims, and a second vector comprising a second polynucleotide encoding the second Fc polypeptide of the heterodimeric protein of any one of the preceding claims;
- e) a first polynucleotide encoding the first antibody heavy chain of the heterodimeric protein of claim ~~187~~, and a second polynucleotide encoding the second antibody heavy chain of the heterodimeric protein of claim ~~187~~, and optionally a third polynucleotide encoding the first antibody light chain of the heterodimeric protein of claim ~~187~~, and/or a fourth polynucleotide encoding the second antibody light chain of the heterodimeric protein of claim ~~187~~; or
- f) a first vector comprising a first polynucleotide encoding the first antibody heavy chain of the heterodimeric protein of claim ~~187~~, and a second vector comprising a second polynucleotide encoding the second antibody heavy chain of the heterodimeric protein of claim ~~187~~, and optionally a third vector comprising a third polynucleotide encoding the first antibody light chain of the heterodimeric protein of claim ~~187~~, and/or a fourth vector comprising a fourth polynucleotide encoding the second antibody light chain of the heterodimeric protein of claim ~~187~~.

~~54.15.~~ A method of producing a heterodimeric protein, the method comprising:

(i) expressing in a cell:

- a) a first polynucleotide encoding the first Fc polypeptide of the heterodimeric protein of any one of claims 1-~~46~~10; and
- b) a second polynucleotide encoding the second Fc polypeptide of the heterodimeric protein of any one of claims 1-~~1046~~; or

(ii) expressing in a cell:

- a) a first polynucleotide encoding the first antibody heavy chain of the heterodimeric protein of claim 7;
- b) a second polynucleotide encoding the second antibody heavy chain of the heterodimeric protein of claim 7;
- c) a third polynucleotide encoding the first antibody light chain of the heterodimeric protein of claim 7; and

d) a fourth polynucleotide encoding the second antibody light chain of the heterodimeric protein of claim 7,

under conditions whereby the heterodimeric protein is produced.

~~52. A method of producing a heterodimeric protein, the method comprising expressing in a cell:~~

- ~~a) a first polynucleotide encoding the first antibody heavy chain of the heterodimeric protein of claim 18;~~
- ~~b) a second polynucleotide encoding the second antibody heavy chain of the heterodimeric protein of claim 18;~~
- ~~c) a third polynucleotide encoding the first antibody light chain of the heterodimeric protein of claim 18; and~~
- ~~d) a fourth polynucleotide encoding the second antibody light chain of the heterodimeric protein of claim 18;~~

~~under conditions whereby the heterodimeric protein is produced.~~

~~53.16.~~ A method of producing a heterodimeric protein, the method comprising:

- a) expressing in a first cell a first polynucleotide encoding the first Fc polypeptide of the heterodimeric protein of any one of claims 1-~~46~~10, under conditions whereby the first Fc polypeptide is produced;
- b) expressing in a second cell a second polynucleotide encoding the second Fc polypeptide of the heterodimeric protein of any one of claims 1-~~46~~10, under conditions whereby the second Fc polypeptide is produced; and
- c) contacting the first and the second Fc polypeptides produced in steps (a) and (b), under conditions whereby the first Fc polypeptide and the second Fc polypeptide heterodimerize to produce the heterodimeric protein.

~~54.17.~~ A method of producing a heterodimeric protein, the method comprising:

- a) expressing in a first cell a first polynucleotide encoding the first antibody heavy chain of the heterodimeric protein of claim ~~18~~7 and second polynucleotide encoding the

- first antibody light chain of the heterodimeric protein of claim ~~18~~7, under conditions whereby the first Fc polypeptide is produced;
- b) expressing in a second cell a third polynucleotide encoding the second antibody heavy chain of the heterodimeric protein of claim ~~18~~7 and fourth polynucleotide encoding the second antibody light chain of the heterodimeric protein of claim ~~18~~7, under conditions whereby the second Fc polypeptide is produced; and
- c) contacting the first and the second Fc polypeptides produced in steps (a) and (b), under conditions whereby the first Fc polypeptide and the second Fc polypeptide heterodimerize to produce the heterodimeric protein.

~~55~~18. A method of producing a heterodimeric protein, the method comprising contacting the first Fc polypeptide and the second Fc polypeptide of the heterodimeric protein of any one claims 1-~~46~~10 under conditions whereby the first Fc polypeptide and the second Fc polypeptide heterodimerize to produce the heterodimeric protein.