

## CLAIMS

What is claimed is:

1. A multispecific antigen binding protein (MABP) comprising:
  - (a) a first antigen binding portion comprising a heavy chain variable domain ( $V_H$ ) and a light chain variable domain ( $V_L$ ), wherein the  $V_H$  and  $V_L$  together form an antigen-binding site that specifically binds a first epitope,
  - (b) a second antigen binding portion comprising a first single-domain antibody (sdAb) that specifically binds a second epitope, and
  - (c) a third antigen binding portion comprising a second single-domain antibody (sdAb) that specifically binds a third epitope,wherein the first antigen binding portion, the second antigen binding portion, and the third antigen binding portion are fused to each other.
2. The MABP of claim 1, wherein the MABP is a trispecific antigen binding protein.
3. The MABP of claim 1 or 2, wherein the first antigen binding portion is a full-length antibody consisting of two heavy chains and two light chains.
4. The MABP of claim 1 or 2, wherein the first antigen binding portion is an antibody fragment comprising a heavy chain comprising the  $V_H$  and a light chain comprising the  $V_L$ .
5. The MABP of any one of claims 1-4, wherein the first sdAb and/or the second sdAb is a  $V_HH$ .
6. The MABP of claim 5, wherein the MABP comprises:
  - (1) a first polypeptide comprising from the N-terminus to the C-terminus: the first sdAb and a heavy chain of the first antigen binding portion; and
  - (2) a second polypeptide comprising from the N-terminus to the C-terminus: the second sdAb and a light chain of the first antigen binding portion.
7. The MABP of claim 5, wherein the MABP comprises:
  - (1) a first polypeptide comprising from the N-terminus to the C-terminus: a heavy chain of the first antigen binding portion and the first sdAb; and
  - (2) a second polypeptide comprising from the N-terminus to the C-terminus: the second sdAb and a light chain of the first antigen binding portion.
8. The MABP of claim 5, wherein the MABP comprises:
  - (1) a first polypeptide comprising from the N-terminus to the C-terminus: the first sdAb and a heavy chain of the first antigen binding portion; and
  - (2) a second polypeptide comprising from the N-terminus to the C-terminus: a light chain of the first antigen binding portion and the second sdAb.
9. The MABP of claim 5, wherein the MABP comprises:
  - (1) a first polypeptide comprising from the N-terminus to the C-terminus: a heavy chain of the first antigen binding portion and the first sdAb; and

- (2) a second polypeptide comprising from the N-terminus to the C-terminus: a light chain of the first antigen binding portion and the second sdAb.
10. The MABP of claim 5, wherein the MABP comprises:
- (1) a first polypeptide comprising from the N-terminus to the C-terminus: the first sdAb, a heavy chain of the first antigen binding portion, and the second sdAb; and
- (2) a second polypeptide comprising from the N-terminus to the C-terminus: a light chain of the first antigen binding portion.
11. The MABP of claim 5, wherein the MABP comprises:
- (1) a first polypeptide comprising from the N-terminus to the C-terminus: a heavy chain of the first antigen binding portion; and
- (2) a second polypeptide comprising from the N-terminus to the C-terminus: the first sdAb, a light chain of the first antigen binding portion and the second sdAb.
12. The MABP of claim 5, wherein the MABP comprises:
- (1) a first polypeptide comprising from the N-terminus to the C-terminus: the first sdAb, the second sdAb, and a heavy chain of the first antigen binding portion; and
- (2) a second polypeptide comprising from the N-terminus to the C-terminus: a light chain of the first antigen binding portion.
13. The MABP of claim 5, wherein the MABP comprises:
- (1) a first polypeptide comprising from the N-terminus to the C-terminus: a heavy chain of the first antigen binding portion, the first sdAb, and the second sdAb; and
- (2) a second polypeptide comprising from the N-terminus to the C-terminus: a light chain of the first antigen binding portion.
14. The MABP of claim 5, wherein the MABP comprises:
- (1) a first polypeptide comprising from the N-terminus to the C-terminus: a heavy chain of the first antigen binding portion; and
- (2) a second polypeptide comprising from the N-terminus to the C-terminus: the first sdAb, the second sdAb, and a light chain of the first antigen binding portion.
15. The MABP of claim 5, wherein the MABP comprises:
- (1) a first polypeptide comprising from the N-terminus to the C-terminus: a heavy chain of the first antigen binding portion; and
- (2) a second polypeptide comprising from the N-terminus to the C-terminus: a light chain of the first antigen binding portion, the first sdAb, and the second sdAb.
16. The MABP of any one of claims 6-15, wherein the MABP comprises two chains of the first polypeptide and two chains of the second polypeptide.
17. The MABP of any one of claims 1-16, wherein the first epitope, the second epitope and/or the third epitope is from an immune checkpoint molecule.

18. The MABP of claim 17, wherein the immune checkpoint molecule is selected from the group consisting of PD-1, PD-L1, PD-L2, CTLA-4, B7-H3, TIM-3, LAG-3, TIGIT, VISTA, ICOS, 4-1BB, OX40, GITR, and CD40.
19. The MABP of claim 18, wherein the first antigen binding portion is an anti-PD-1 antibody or antigen binding fragment thereof.
20. The MABP of claim 19, wherein the anti-PD-1 antibody is derived from pembrolizumab.
21. The MABP of any one of claims 17-20, wherein the second antigen binding portion comprises an anti-TIGIT sdAb.
22. The MABP of claim 21, wherein the anti-TIGIT sdAb comprises the amino acid sequence of SEQ ID NO: 31, or a variant thereof comprising up to about 3 amino acid substitutions.
23. The MABP of any one of claims 17-21, wherein the third antigen binding portion comprises an anti-LAG-3 sdAb.
24. The MABP of claim 23, wherein the anti-LAG-3 sdAb comprises the amino acid sequence of SEQ ID NO: 32, or a variant thereof comprising up to about 3 amino acid substitutions.
25. The MABP of any one of claims 17-24, wherein the MABP comprises:
  - (1) a first polypeptide comprising the amino acid sequence of any one of SEQ ID NOs: 16, 18, 20, 23, 25, 26, 28, or a variant thereof comprising up to about 5 amino acid substitutions; and
  - (2) a second polypeptide comprising the amino acid sequence of any one of SEQ ID NOs: 15, 17, 19, 21, 22, 24, 27, or a variant thereof comprising up to about 5 amino acid substitutions.
26. The MABP of any one of claims 1-16, wherein the first epitope, the second epitope and/or the third epitope is from a tumor antigen or a cell surface antigen of an immune effector cell.
27. The MABP of claim 26, wherein the first epitope, the second epitope and/or the third epitope is a tumor antigen selected from the group consisting of HER2, BRAF, EGFR, VEGFR2, CD20, RANKL, CD38, and CD52.
28. The MABP of claim 27, wherein the first antigen binding portion is an anti-HER2 antibody or antigen binding fragment thereof.
29. The MABP of claim 28, wherein the anti-HER2 antibody is derived from trastuzumab.
30. The MABP of any one of claims 26-29, wherein the second antigen binding portion comprises an anti-CD3 sdAb.
31. The MABP of any one of claims 26-30, wherein the third antigen binding portion comprises an anti-EGFR sdAb.
32. The MABP of any one of claims 1-16, wherein the first epitope, the second epitope, and/or the third epitope is from a pro-inflammatory molecule.
33. The MABP of claim 32, wherein the pro-inflammatory molecule is selected from the group consisting of IL-1 $\beta$ , TNF- $\alpha$ , IL-5, IL-6, IL-6R, IL-17A, IL-17F and eotaxin-1.

34. The MABP of claim 33, wherein the first antigen binding portion is an anti-TNF- $\alpha$  antibody or antigen binding fragment thereof.
35. The MABP of claim 34, wherein the anti-TNF- $\alpha$  antibody is derived from adalimumab.
36. The MABP of any one of claims 32-35, wherein the second antigen binding portion comprises an anti-IL-17A sdAb.
37. The MABP of claim 36, wherein the third antigen binding portion comprises an anti-IL-17F sdAb.
38. The MABP of any one of claims 1-16, wherein the first epitope, the second epitope and/or the third epitope is from an angiogenic factor.
39. The MABP of claim 38, wherein the first antigen binding portion is an anti-Ang2 antibody or antigen binding fragment thereof.
40. The MABP of claim 39, wherein the anti-Ang2 antibody is derived from LC10.
41. The MABP of any one of claims 38-40, wherein the second antigen binding portion comprises an anti-VEGF sdAb.
42. The MABP of any one of claims 38-41, wherein the second antigen binding portion comprises an anti-DLL4 sdAb.
43. The MABP of any one of claims 1-42, wherein the first antigen binding portion comprises a human, humanized or chimeric antibody or antigen binding fragment thereof.
44. The MABP of any one of claims 1-43, wherein the first antigen binding portion comprises an Fc region.
45. The MABP of claim 44, wherein the Fc region is an IgG1 Fc.
46. The MABP of claim 44, wherein the Fc region is an IgG4 Fc having an S228P mutation.
47. The MABP of any one of claims 1-46, wherein the first antigen binding portion, the second antigen binding portion and/or the third antigen binding portion are fused to each other via a peptide linker.
48. The MABP of claim 47, wherein the peptide linker is no more than about 30 amino acids long.
49. The MABP of claim 48, wherein the peptide linker comprises any one of the amino acid sequences of SEQ ID NOs: 1-7.
50. The MABP of any one of claims 1-49, wherein the first sdAb and/or the second sdAb is a camelid, humanized, or human sdAb.
51. The MABP of any one of claims 1-50, wherein the multispecific antigen binding protein has an aggregation onset temperature of at least about 55°C.
52. The MABP of any one of claims 1-50, wherein the multispecific antigen binding protein is stable for at least about one week at 25 °C at a concentration of at least about 50 mg/mL.
53. The MABP of any one of claims 1-50, wherein the multispecific antigen binding protein is stable after at least about 5 freeze-thaw cycles at a concentration of at least 50 mg/mL.

54. A pharmaceutical composition comprising the MABP of any one of claims 1-53 and a pharmaceutically acceptable carrier.
55. A method of treating a disease in an individual, comprising administering to the individual an effective amount of the pharmaceutical composition of claim 54.
56. The method of claim 55, wherein the disease is a cancer.
57. The method of claim 56, wherein the cancer is selected from the group consisting of breast cancer, renal cancer, melanoma, lung cancer, glioblastoma, head and neck cancer, prostate cancer, ovarian carcinoma, bladder carcinoma, and lymphoma.
58. The method of claim 55, wherein the disease is an inflammatory or autoimmune disease.
59. The method of claim 58, wherein the inflammatory or autoimmune disease is selected from the group consisting of arthritis, colitis, psoriasis, severe asthma, and moderate to severe Crohn's disease.