

comprising the amino acid sequence of SEQ ID NO: 23. In some examples, the VHH domain comprises the amino acid sequence of SEQ ID NO: 9.

[0006a] In one aspect, the present disclosure refers to a polypeptide comprising at least one VHH domain that binds OX40, wherein the VHH domain comprises the amino acid sequence of SEQ ID NO: 9.

[0007] In some examples, the polypeptide comprises two VHH domains. In some examples, the polypeptide comprises three VHH domains. In some examples, the polypeptide comprises four VHH domains. In some examples, the polypeptide comprises at least one binding domain that binds a second antigen other than OX40. In some such examples, the second antigen is selected from PD-1, PD-L1, and 41BB. In some examples, the at least one binding domain that binds a second antigen is an antagonist or an agonist. In some examples, the at least one binding domain that binds a second antigen is a VHH domain.

[0008] In some examples, each VHH domain binds OX40. In some examples, each VHH domain comprises a CDR1 comprising the amino acid sequence of SEQ ID NO: 10, a CDR2 comprising the amino acid sequence of SEQ ID NO: 11, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 12. In some examples, each VHH domain comprises a framework 2 (FR2) comprising the amino acid sequence of SEQ ID NO: 22. In some examples, each VHH domain comprises a FR2 comprising the amino acid sequence of SEQ ID NO: 22 and a FR3 comprising the amino acid sequence of SEQ ID NO: 23. In some examples, each VHH domain comprises the amino acid sequence of SEQ ID NO: 9.

[0009] In some examples, the polypeptide comprises an Fc domain. In some examples, the Fc domain comprises an amino acid sequence selected from SEQ ID NOs: 25 and 26. In some examples, the polypeptide comprises the amino acid sequence of SEQ ID NO: 14. In some examples, the polypeptide comprises the amino acid sequence of SEQ ID NO: 15. In another aspect, the present disclosure refers to a polypeptide that binds OX40 comprising the amino acid sequence of SEQ ID NO: 15. In another aspect, the present disclosure refers to a polypeptide that binds OX40 consisting of the amino acid sequence of SEQ ID NO: 15.

[0010] In various examples, the polypeptide provided herein forms a dimer under physiological conditions. In some such examples, the polypeptide comprises an Fc domain.

[0011] In some examples, a polypeptide provided herein increases CD4⁺ and/or CD8⁺ T cell proliferation *in vitro* and/or *in vivo*. In some examples, the polypeptide increases CD4⁺ and/or CD8⁺ T cell proliferation in the presence of Treg cells. In some examples, the polypeptide increases CD4⁺ and/or CD8⁺ T cell proliferation *in vitro* by at least 1.5-fold or by at least 2-fold. In some examples, the polypeptide increases CD4⁺ and/or CD8⁺ T cell proliferation *in vivo* by at least 1.5-fold or by at least 2-fold.

[0012] In some examples, the polypeptide increases CD25 expression on CD4⁺ and/or CD8⁺ T cells *in vitro* and/or *in vivo*. In some examples, the polypeptide increases CD25

expression on CD4⁺ and/or CD8⁺ T cells *in vitro* by at least 1.5-fold or by at least 2-fold. In some examples, the polypeptide increases CD25 expression on CD4⁺ and/or CD8⁺ T cells *in vivo* by at least 1.5-fold or by at least 2-fold.

[0013] In some examples, the polypeptide increases CD71 expression on CD4⁺ and/or CD8⁺ T cells *in vitro* and/or *in vivo*. In some examples, the polypeptide increases CD71 expression on CD4⁺ and/or CD8⁺ T cells *in vitro* by at least 1.5-fold or by at least 2-fold. In some examples, the polypeptide increases CD71 expression on CD4⁺ and/or CD8⁺ T cells *in vivo* by at least 1.5-fold or by at least 2-fold.

[0014] In some examples, the polypeptide increases NFκB signaling in CD4⁺ and/or CD8⁺ T cells *in vitro* and/or *in vivo*. In some examples, the polypeptide increases NFκB signaling in CD4⁺ and/or CD8⁺ T cells *in vitro* by at least 1.5-fold, at least 2-fold, at least 3-fold, or by at least 5-fold. In some examples, the polypeptide increases NFκB signaling in CD4⁺ and/or CD8⁺ T cells *in vivo* by at least 1.5-fold, at least 2-fold, at least 3-fold, or by at least 5-fold.

[0015] In some examples, the polypeptide increases IFNγ expression in CD4⁺ and/or CD8⁺ T cells *in vitro* and/or *in vivo*. In some examples, the polypeptide increases IFNγ expression in CD4⁺ and/or CD8⁺ T cells *in vitro* by at least 1.5-fold, at least 2-fold, at least 3-fold, or by at least 5-fold. In some examples, the polypeptide increases IFNγ expression in CD4⁺ and/or CD8⁺ T cells *in vivo* by at least 1.5-fold, at least 2-fold, at least 3-fold, or by at least 5-fold.

[0016] In various examples, the polypeptide increases the expression of CD25, CD71, and/or IFNγ, and/or increases NFκB signaling in the presence of Treg cells. In various examples, the increase is determined as an average of results from T cells of at least five or at least ten different healthy human donors.

[0017] In various examples, the polypeptide comprising at least one VHH domain that binds OX40 provided herein is an agonist of OX40 biological activity. In some examples, the OX40 is human OX40. In some examples, the polypeptide binds human OX40 with an affinity (K_D) of less than 10 nM, less than 5 nM, less than 2 nM, or less than 1 nM. In some examples, the polypeptide binds cynomolgus monkey OX40 with an affinity (K_D) of less than 10 nM, less than 5 nM, less than 2 nM, or less than 1 nM.

[0018] In some examples, pharmaceutical compositions are provided, comprising a polypeptide comprising at least one VHH domain that binds OX40 provided herein and a pharmaceutically acceptable carrier.

[0018a] In another aspect, the present disclosure refers to a pharmaceutical composition comprising the polypeptide as disclosed herein and a pharmaceutically acceptable carrier, wherein the polypeptide comprises at least two VHH domains that bind OX40.

[0019] In some examples, an isolated nucleic acid is provided that encodes a polypeptide comprising at least one VHH domain that binds OX40 provided herein. In some examples,

[0021a] In another aspect, the present disclosure refers to an *in vitro* method of increasing CD4⁺ and/or CD8⁺ T cell proliferation comprising contacting T cells with the polypeptide as disclosed herein. In another aspect, the present disclosure refers to an *in vitro* method of increasing CD25 expression on CD4⁺ and/or CD8⁺ T cells comprising contacting T cells with the polypeptide as disclosed herein. In another aspect, the present disclosure refers to an *in vitro* method of increasing CD71 expression on CD4⁺ and/or CD8⁺ T cells comprising contacting T cells with the polypeptide as disclosed herein. In another aspect, the present disclosure refers to an *in vitro* method of increasing NFκB signaling in CD4⁺ and/or CD8⁺ T cells comprising contacting T cells with the polypeptide as disclosed herein. In another aspect, the present disclosure refers to an *in vitro* method of increasing IFNγ expression in CD4⁺ and/or CD8⁺ T cells comprising contacting T cells with the polypeptide as disclosed herein.

[0021b] In another aspect, the present disclosure refers to use of the polypeptide as disclosed herein in the manufacture of a medicament for increasing CD4⁺ and/or CD8⁺ T cell proliferation in a subject with cancer, wherein the polypeptide comprises at least two VHH domains that bind OX40. In another aspect, the present disclosure refers to use of the polypeptide as disclosed herein in the manufacture of a medicament for increasing CD25 expression on CD4⁺ and/or CD8⁺ T cells in a subject with cancer, wherein the polypeptide comprises at least two VHH domains that bind OX40. In another aspect, the present disclosure refers to use of the polypeptide as disclosed herein in the manufacture of a medicament for increasing CD71 expression on CD4⁺ and/or CD8⁺ T cells in a subject with cancer, wherein the polypeptide comprises at least two VHH domains that bind OX40. In another aspect, the present disclosure refers to use of the polypeptide as disclosed herein in the manufacture of a medicament for increasing NFκB signaling in CD4⁺ and/or CD8⁺ T cells in a subject with cancer, wherein the polypeptide comprises at least two VHH domains that bind OX40. In another aspect, the present disclosure refers to use of the polypeptide as disclosed herein in the manufacture of a medicament for increasing IFNγ expression in CD4⁺ and/or CD8⁺ T cells in a subject with cancer, wherein the polypeptide comprises at least two VHH domains that bind OX40.

[0022] In some examples, methods of treating cancer are provided, comprising administering to a subject with cancer a pharmaceutically effective amount of a polypeptide comprising at least one VHH domain that binds OX40 provided herein. In some examples, methods of treating cancer are provided, comprising administering to a subject with cancer a pharmaceutically effective amount of a host cell that expresses the polypeptide comprising at least one VHH domain that binds OX40 provided herein. In some examples, the host cell secretes the OX40-binding polypeptide. In some examples, the host cell expresses the OX40-binding polypeptide on the surface. In some examples, the cancer is selected from basal cell carcinoma, biliary tract cancer; bladder cancer; bone cancer; brain and central nervous system cancer; breast cancer; cancer of the peritoneum; cervical cancer; choriocarcinoma; colon

and rectum cancer; connective tissue cancer; cancer of the digestive system; endometrial cancer; esophageal cancer; eye cancer; cancer of the head and neck; gastric cancer; gastrointestinal cancer; glioblastoma; hepatic carcinoma; hepatoma; intra-epithelial neoplasm; kidney or renal cancer; larynx cancer; liver cancer; lung cancer; small-cell lung cancer; non-small cell lung cancer; adenocarcinoma of the lung; squamous carcinoma of the lung; melanoma; myeloma; neuroblastoma; oral cavity cancer; ovarian cancer; pancreatic cancer; prostate cancer; retinoblastoma; rhabdomyosarcoma; rectal cancer; cancer of the respiratory system; salivary gland carcinoma; sarcoma; skin cancer; squamous cell cancer; stomach cancer; testicular cancer; thyroid cancer; uterine or endometrial cancer; cancer of the urinary system; vulval cancer; lymphoma; Hodgkin's lymphoma; non-Hodgkin's lymphoma; B-cell lymphoma; low grade/follicular non-Hodgkin's lymphoma (NHL); small lymphocytic (SL) NHL; intermediate grade/follicular NHL; intermediate grade diffuse NHL; high grade immunoblastic NHL; high grade lymphoblastic NHL; high grade small non-cleaved cell NHL; bulky disease NHL; mantle cell lymphoma; AIDS-related lymphoma; Waldenstrom's macroglobulinemia; chronic lymphocytic leukemia (CLL); acute lymphoblastic leukemia (ALL); Hairy cell leukemia; and chronic myeloblastic leukemia.

[0022a] In another aspect, the present disclosure refers to use of the polypeptide as disclosed herein, the pharmaceutical composition as disclosed herein, or the host cell as disclosed herein in the manufacture of a medicament for treating cancer, wherein the polypeptide comprises at least two VHH domains that bind OX40.

[0023] In some examples, the method of treating cancer further comprises administering an additional therapeutic agent. In some examples, the additional therapeutic agent is an anti-cancer agent. In some examples, the anti-cancer agent is selected from a chemotherapeutic agent, an anti-cancer biologic, radiation therapy, CAR-T therapy, and an oncolytic virus. In some examples, the additional therapeutic agent is an anti-cancer biologic. In some examples, the anti-cancer biologic is an agent that inhibits PD-1 and/or PD-L1. In some examples, the anti-cancer biologic is selected from nivolumab, pidilizumab, pembrolizumab, durvalumab, atezolizumab, avelumab, AMP-224, BMS-936559, AMP-514, MDX-1105, TSR-042, STI-A1010, and STI-A1110. In some examples, the anti-cancer biologic is an agent that inhibits VISTA, gpNMB, B7H3, B7H4, HHLA2, CD73, CTLA4, or TIGIT. In some examples, the anti-cancer biologic is an antibody. In some examples, the anti-cancer biologic is a cytokine. In some examples, the anti-cancer agent is CAR-T therapy. In some examples, the anti-cancer agent is an oncolytic virus. In some examples, a method of treating cancer provided herein further comprises tumor resection and/or radiation therapy.

BRIEF DESCRIPTION OF THE FIGURES

[0024] FIG. 1 shows nonspecific binding of 1D10v1-Fc and 1D10v6-Fc to untransfected HEK293 cells.

What is claimed is:

1. A polypeptide comprising at least one VHH domain that binds OX40, wherein the VHH domain comprises the amino acid sequence of SEQ ID NO: 9.
2. The polypeptide of claim 1, comprising two VHH domains.
3. The polypeptide of claim 1, comprising three VHH domains.
4. The polypeptide of claim 1, comprising four VHH domains.
5. The polypeptide of any one of claims 1 to 4, wherein the polypeptide comprises at least one binding domain that binds a second antigen other than OX40.
6. The polypeptide of claim 5, wherein the second antigen is selected from PD-1, PD-L1, and 41BB.
7. The polypeptide of claim 6, wherein the at least one binding domain that binds a second antigen is an antagonist or an agonist.
8. The polypeptide of any one of claims 1 to 4, wherein each VHH domain binds OX40.
9. The polypeptide of claim 8, wherein each VHH domain comprises a CDR1 comprising the amino acid sequence of SEQ ID NO: 10, a CDR2 comprising the amino acid sequence of SEQ ID NO: 11, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 12.
10. The polypeptide of claim 9, wherein each VHH domain comprises a framework 2 (FR2) comprising the amino acid sequence of SEQ ID NO: 22.
11. The polypeptide of claim 9 or claim 10, wherein each VHH domain comprises a FR2 comprising the amino acid sequence of SEQ ID NO: 22 and a FR3 comprising the amino acid sequence of SEQ ID NO: 23.
12. The polypeptide of any one of claims 9 to 11, wherein each VHH domain comprises the amino acid sequence of SEQ ID NO: 9.
13. The polypeptide of any one of claims 1 to 12, wherein the polypeptide comprises an Fc domain.
14. The polypeptide of claim 13, wherein the Fc domain comprises an amino acid sequence selected from SEQ ID NOs: 25 and 26.
15. The polypeptide of claim 1, wherein the polypeptide comprises the amino acid sequence of SEQ ID NO: 14.
16. The polypeptide of claim 1, wherein the polypeptide comprises the amino acid sequence of SEQ ID NO: 15.
17. A polypeptide that binds OX40 comprising the amino acid sequence of SEQ ID NO: 15.

18. A polypeptide that binds OX40 consisting of the amino acid sequence of SEQ ID NO: 15.

19. The polypeptide of any one of claims 13, 14, and 16 to 18, which forms a dimer under physiological conditions.

20. The polypeptide of any one of claims 1 to 19, wherein the polypeptide increases CD4⁺ and/or CD8⁺ T cell proliferation *in vitro* and/or *in vivo*.

21. The polypeptide of claim 20, wherein the polypeptide increases CD4⁺ and/or CD8⁺ T cell proliferation in the presence of Treg cells.

22. The polypeptide of claim 20 or claim 21, wherein the polypeptide increases CD4⁺ and/or CD8⁺ T cell proliferation *in vitro* by at least 1.5-fold or by at least 2-fold.

23. The polypeptide of any one of claims 1 to 22, wherein the polypeptide increases CD25 expression on CD4⁺ and/or CD8⁺ T cells *in vitro* and/or *in vivo*.

24. The polypeptide of claim 23, wherein the polypeptide increases CD25 expression on CD4⁺ and/or CD8⁺ T cells *in vitro* by at least 1.5-fold or by at least 2-fold.

25. The polypeptide of any one of claims 1 to 24, wherein the polypeptide increases CD71 expression on CD4⁺ and/or CD8⁺ T cells *in vitro* and/or *in vivo*.

26. The polypeptide of claim 25, wherein the polypeptide increases CD71 expression on CD4⁺ and/or CD8⁺ T cells *in vitro* by at least 1.5-fold or by at least 2-fold.

27. The polypeptide of any one of claims 1 to 26 wherein the polypeptide increases NFκB signaling in CD4⁺ and/or CD8⁺ T cells *in vitro* and/or *in vivo*.

28. The polypeptide of claim 27, wherein the polypeptide increases NFκB signaling in CD4⁺ and/or CD8⁺ T cells *in vitro* by at least 1.5-fold, at least 2-fold, at least 3-fold, or by at least 5-fold.

29. The polypeptide of any one of claims 1 to 28, wherein the polypeptide increases IFNγ expression in CD4⁺ and/or CD8⁺ T cells *in vitro* and/or *in vivo*.

30. The polypeptide of claim 29, wherein the polypeptide increases IFNγ expression in CD4⁺ and/or CD8⁺ T cells *in vitro* by at least 1.5-fold, at least 2-fold, at least 3-fold, or by at least 5-fold.

31. The polypeptide of any one of claims 23 to 30, wherein the polypeptide increases the expression in the presence of Treg cells.

32. The polypeptide of any one of claims 20 to 31, wherein the increase is determined as an average of results from T cells of at least five or at least ten different healthy human donors.

33. The polypeptide of any one of claims 1 to 32, which is an agonist of OX40 biological activity.

34. The polypeptide of any one of claims 1 to 33, wherein the OX40 is human OX40.
35. The polypeptide of any one of claims 1 to 34, wherein the polypeptide binds human OX40 with an affinity (K_D) of less than 10 nM, less than 5 nM, less than 2 nM, or less than 1 nM.
36. The polypeptide of any one of claims 1 to 35, wherein the polypeptide binds cynomolgus monkey OX40 with an affinity (K_D) of less than 10 nM, less than 5 nM, less than 2 nM, or less than 1 nM.
37. A pharmaceutical composition comprising the polypeptide of any one of claims 1 to 36 and a pharmaceutically acceptable carrier, wherein the polypeptide comprises at least two VHH domains that bind OX40.
38. An isolated nucleic acid that encodes the polypeptide of any one of claims 1 to 36.
39. A vector comprising the nucleic acid of claim 38.
40. A host cell comprising the nucleic acid of claim 38 or the vector of claim 39.
41. A host cell that expresses the polypeptide of any one of claims 1 to 36.
42. The host cell of claim 40 or claim 41, which secretes the polypeptide of any one of claims 1 to 36.
43. The host cell of any one of claims 40 to 42, which is a primary human cell.
44. The host cell of claim 43, which is a primary human T cell.
45. The host cell of any one of claims 40 to 44, which is a chimeric antigen receptor (CAR)-T cell.
46. A method of producing the polypeptide of any one of claims 1 to 36 comprising incubating the host cell of any one of claims 40 to 42 under conditions suitable for expression of the polypeptide.
47. The method of claim 46, further comprising isolating the polypeptide.
48. An *in vitro* method of increasing CD4⁺ and/or CD8⁺ T cell proliferation comprising contacting T cells with the polypeptide of any one of claims 1 to 36.
49. An *in vitro* method of increasing CD25 expression on CD4⁺ and/or CD8⁺ T cells comprising contacting T cells with the polypeptide of any one of claims 1 to 36.
50. An *in vitro* method of increasing CD71 expression on CD4⁺ and/or CD8⁺ T cells comprising contacting T cells with the polypeptide of any one of claims 1 to 36.
51. An *in vitro* method of increasing NF κ B signaling in CD4⁺ and/or CD8⁺ T cells comprising contacting T cells with the polypeptide of anyone of claims 1 to 36.
52. An *in vitro* method of increasing IFN γ expression in CD4⁺ and/or CD8⁺ T cells comprising contacting T cells with the polypeptide of anyone of claims 1 to 36.

53. The method of any one of claims 48 to 52, wherein the CD4⁺ and/or CD8⁺ T cells are in the presence of Treg cells.

54. The method of any one of claims 48 to 53, wherein the increase is at least 1.5-fold, at least 2-fold, at least 3-fold, or by at least 5-fold.

55. Use of the polypeptide of any one of claims 1-36, the pharmaceutical composition of claim 37, or the host cell of any one of claims 43 to 45 in the manufacture of a medicament for treating cancer, wherein the polypeptide comprises at least two VHH domains that bind OX40.

56. The use of claim 55, wherein the cancer is selected from basal cell carcinoma, biliary tract cancer; bladder cancer; bone cancer; brain and central nervous system cancer; breast cancer; cancer of the peritoneum; cervical cancer; choriocarcinoma; colon and rectum cancer; connective tissue cancer; cancer of the digestive system; endometrial cancer; esophageal cancer; eye cancer; cancer of the head and neck; gastric cancer; gastrointestinal cancer; glioblastoma; hepatic carcinoma; hepatoma; intra-epithelial neoplasm; kidney or renal cancer; larynx cancer; liver cancer; lung cancer; small-cell lung cancer; non-small cell lung cancer; adenocarcinoma of the lung; squamous carcinoma of the lung; melanoma; myeloma; neuroblastoma; oral cavity cancer; ovarian cancer; pancreatic cancer; prostate cancer; retinoblastoma; rhabdomyosarcoma; rectal cancer; cancer of the respiratory system; salivary gland carcinoma; sarcoma; skin cancer; squamous cell cancer; stomach cancer; testicular cancer; thyroid cancer; uterine or endometrial cancer; cancer of the urinary system; vulval cancer; lymphoma; Hodgkin's lymphoma; non-Hodgkin's lymphoma; B-cell lymphoma; low grade/follicular non-Hodgkin's lymphoma (NHL); small lymphocytic (SL) NHL; intermediate grade/follicular NHL; intermediate grade diffuse NHL; high grade immunoblastic NHL; high grade lymphoblastic NHL; high grade small non-cleaved cell NHL; bulky disease NHL; mantle cell lymphoma; AIDS-related lymphoma; Waldenstrom's macroglobulinemia; chronic lymphocytic leukemia (CLL); acute lymphoblastic leukemia (ALL); Hairy cell leukemia; and chronic myeloblastic leukemia.

57. The use of claim 55 or 56, wherein the medicament is for administration with an additional therapeutic agent.

58. The use of claim 57, wherein the additional therapeutic agent is an anti-cancer agent.

59. The use of claim 58, wherein the anti-cancer agent is selected from a chemotherapeutic agent, an anti-cancer biologic, radiation therapy, and an oncolytic virus.

60. The use of any one of claims 57 to 59, wherein the additional therapeutic agent is an anti-cancer biologic.

61. The use of claim 60, wherein the anti-cancer biologic is an agent that inhibits PD-1 and/or PD-L1.
62. The use of claim 61, wherein the anti-cancer biologic is selected from nivolumab, pidilizumab, pembrolizumab, durvalumab, atezolizumab, avelumab, AMP-224, BMS-936559, AMP-514, MDX-1105, TSR-042, STI-A1010, and STI-A1110.
63. The use of claim 60, wherein the anti-cancer biologic is an agent that inhibits VISTA, gpNMB, B7H3, B7H4, HHLA2, CD73, CTLA4, or TIGIT.
64. The use of any one of claims 60 to 63, wherein the anti-cancer agent is an antibody.
65. The use of claim 60, wherein the anti-cancer biologic is a cytokine.
66. The use of claim 60, wherein the anti-cancer agent is an oncolytic virus.
67. The use of any one of claims 55 to 66, further comprising tumor resection, CAR-T therapy, and/or radiation therapy.
68. Use of the polypeptide of any one of claims 1 to 36 in the manufacture of a medicament for increasing CD4⁺ and/or CD8⁺ T cell proliferation in a subject with cancer, wherein the polypeptide comprises at least two VHH domains that bind OX40.
69. Use of the polypeptide of any one of claims 1 to 36 in the manufacture of a medicament for increasing CD25 expression on CD4⁺ and/or CD8⁺ T cells in a subject with cancer, wherein the polypeptide comprises at least two VHH domains that bind OX40.
70. Use of the polypeptide of any one of claims 1 to 36 in the manufacture of a medicament for increasing CD71 expression on CD4⁺ and/or CD8⁺ T cells in a subject with cancer, wherein the polypeptide comprises at least two VHH domains that bind OX40.
71. Use of the polypeptide of any one of claims 1 to 36 in the manufacture of a medicament for increasing NFκB signaling in CD4⁺ and/or CD8⁺ T cells in a subject with cancer, wherein the polypeptide comprises at least two VHH domains that bind OX40.
72. Use of the polypeptide of any one of claims 1 to 36 in the manufacture of a medicament for increasing IFNγ expression in CD4⁺ and/or CD8⁺ T cells in a subject with cancer, wherein the polypeptide comprises at least two VHH domains that bind OX40.
73. The use of any one of claims 68 to 72, wherein the CD4⁺ and/or CD8⁺ T cells are in the presence of Treg cells.
74. The use of any one of claims 68 to 73, wherein the increase is at least 1.5-fold, at least 2-fold, at least 3-fold, or by at least 5-fold.