

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 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, and wherein the 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.

[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

[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. 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. 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. 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. 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.

[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

What is claimed is:

1. A polypeptide comprising at least one VHH domain that binds OX40, wherein the 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, and wherein the 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.
2. The polypeptide of claim 1, wherein the VHH domain is humanized.
- ~~3. The polypeptide of claim 1 or claim 2, wherein the VHH domain comprises a framework 2 (FR2) comprising the amino acid sequence of SEQ ID NO: 22.~~
- ~~4. The polypeptide of any one of claims 1 to 3, wherein the 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.~~
- ~~5.3.~~ The polypeptide of ~~any one of claims 1 to 4~~ or claim 2, wherein the VHH domain comprises the amino acid sequence of SEQ ID NO: 9.
- ~~6.4.~~ The polypeptide of any one of claims 1 to ~~53~~, comprising two VHH domains.
- ~~7.5.~~ The polypeptide of any one of claims 1 to ~~53~~, comprising three VHH domains.
- ~~8.6.~~ The polypeptide of any one of claims 1 to ~~53~~, comprising four VHH domains.
- ~~9.7.~~ The polypeptide of any one of claims 1 to ~~86~~, wherein the polypeptide comprises at least one binding domain that binds a second antigen other than OX40.
- ~~10.8.~~ The polypeptide of claim ~~97~~, wherein the second antigen is selected from PD-1, PD-L1, and 41BB.
- ~~11.9.~~ The polypeptide of claim ~~108~~, wherein the at least one binding domain that binds a second antigen is an antagonist or an agonist.
- ~~12.10.~~ The polypeptide of any one of claims ~~53~~ to ~~86~~, wherein each VHH domain binds OX40.
- ~~13.11.~~ The polypeptide of claim ~~1210~~, 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.
- ~~14.12.~~ The polypeptide of claim ~~1311~~, wherein each VHH domain comprises a framework 2 (FR2) comprising the amino acid sequence of SEQ ID NO: 22.
- ~~15.13.~~ The polypeptide of claim ~~1311~~ or claim ~~1412~~, 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.

~~16.14.~~ The polypeptide of any one of claims ~~13.11~~ to ~~15.13~~, wherein each VHH domain comprises the amino acid sequence of SEQ ID NO: 9.

~~17.15.~~ The polypeptide of any one of claims 1 to ~~16.14~~, wherein the polypeptide comprises an Fc domain.

~~18.16.~~ The polypeptide of claim ~~17.15~~, wherein the Fc domain comprises an amino acid sequence selected from SEQ ID NOs: 25 and 26.

~~19.17.~~ The polypeptide of any one of claims 1 to ~~7.5~~, wherein the polypeptide comprises the amino acid sequence of SEQ ID NO: 14.

~~20.18.~~ The polypeptide of any one of claims 1 to ~~7.5~~ and ~~17.15~~, wherein the polypeptide comprises the amino acid sequence of SEQ ID NO: 15.

~~21.19.~~ A polypeptide that binds OX40 comprising the amino acid sequence of SEQ ID NO: 15.

~~22.20.~~ A polypeptide that binds OX40 consisting of the amino acid sequence of SEQ ID NO: 15.

~~23.21.~~ The polypeptide of any one of claims 1 to ~~22.20~~, which forms a dimer under physiological conditions.

~~24.22.~~ The polypeptide of any one of claims 1 to ~~23.21~~, wherein the polypeptide increases CD4⁺ and/or CD8⁺ T cell proliferation *in vitro* and/or *in vivo*.

~~25.23.~~ The polypeptide of claim ~~24.22~~, wherein the polypeptide increases CD4⁺ and/or CD8⁺ T cell proliferation in the presence of Treg cells.

~~26.24.~~ The polypeptide of claim ~~24.22~~ or claim ~~25.23~~, 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.

~~27.25.~~ The polypeptide of any one of claims 1 to ~~26.24~~, wherein the polypeptide increases CD25 expression on CD4⁺ and/or CD8⁺ T cells *in vitro* and/or *in vivo*.

~~28.26.~~ The polypeptide of claim ~~27.25~~, 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.

~~29.27.~~ The polypeptide of any one of claims 1 to ~~28.26~~, wherein the polypeptide increases CD71 expression on CD4⁺ and/or CD8⁺ T cells *in vitro* and/or *in vivo*.

~~30.28.~~ The polypeptide of claim ~~29.27~~, 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.

~~31.29.~~ The polypeptide of any one of claims 1 to ~~30.28~~ wherein the polypeptide increases NFκB signaling in CD4⁺ and/or CD8⁺ T cells *in vitro* and/or *in vivo*.

~~32.30.~~ The polypeptide of claim ~~31.29~~, 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.

~~33.31.~~ The polypeptide of any one of claims 1 to ~~3230~~, wherein the polypeptide increases IFN γ expression in CD4⁺ and/or CD8⁺ T cells *in vitro* and/or *in vivo*.

~~34.32.~~ The polypeptide of claim ~~3331~~, wherein the polypeptide increases IFN γ 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.

~~35.33.~~ The polypeptide of any one of claims ~~2625~~ to ~~3432~~, wherein the polypeptide increases the expression in the presence of Treg cells.

~~36.34.~~ The polypeptide of any one of claims ~~2422~~ to ~~3533~~, 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.

~~37.35.~~ The polypeptide of any one of claims 1 to ~~3634~~, which is an agonist of OX40 biological activity.

~~38.36.~~ The polypeptide of any one of claims 1 to ~~3735~~, wherein the OX40 is human OX40.

~~39.37.~~ The polypeptide of any one of claims 1 to ~~3836~~, 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.

~~40.38.~~ The polypeptide of any one of claims 1 to ~~3937~~, 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.

~~41.39.~~ A pharmaceutical composition comprising the polypeptide of any one of claims ~~4~~ to ~~4038~~ and a pharmaceutically acceptable carrier.

~~42.40.~~ An isolated nucleic acid that encodes the polypeptide of any one of claims 1 to ~~4238~~.

~~43.41.~~ A vector comprising the nucleic acid of claim ~~4240~~.

~~44.42.~~ A host cell comprising the nucleic acid of claim ~~4240~~ or the vector of claim ~~4341~~.

~~45.43.~~ A host cell that expresses the polypeptide of any one of claims 1 to ~~4038~~.

~~46.44.~~ The host cell of claim ~~4442~~ or claim ~~4543~~, which secretes the polypeptide of any one of claims 1 to ~~4038~~.

~~47.45.~~ The host cell of any one of claims ~~4442~~ to ~~4644~~, which is a primary human cell.

~~48.46.~~ The host cell of claim ~~4745~~, which is a primary human T cell.

~~49.47.~~ The host cell of any one of claims ~~4442~~ to ~~4846~~, which is a chimeric antigen receptor (CAR)-T cell.

~~50-48.~~ A method of producing the polypeptide of any one of claims 1 to ~~40-38~~ comprising incubating the host cell of any one of claims ~~44-42~~ to ~~46-44~~ under conditions suitable for expression of the polypeptide.

~~51-49.~~ The method of claim ~~50-48~~, further comprising isolating the polypeptide.

~~52-50.~~ 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 ~~40-38~~.

~~53-51.~~ 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 ~~40-38~~.

~~54-52.~~ 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 ~~40-38~~.

~~55-53.~~ An *in vitro* method of increasing NFκB signaling in CD4⁺ and/or CD8⁺ T cells comprising contacting T cells with the polypeptide of anyone of claims 1 to ~~40-38~~.

~~56-54.~~ An *in vitro* method of increasing IFNγ expression in CD4⁺ and/or CD8⁺ T cells comprising contacting T cells with the polypeptide of anyone of claims 1 to ~~40-38~~.

~~57-55.~~ The method of any one of claims ~~52-50~~ to ~~56-54~~, wherein the CD4⁺ and/or CD8⁺ T cells are in the presence of Treg cells.

~~58-56.~~ The method of any one of claims ~~52-50~~ to ~~57-55~~, wherein the increase is at least 1.5-fold, at least 2-fold, at least 3-fold, or by at least 5-fold.

~~59-57.~~ Use of the polypeptide of any one of claims ~~1-4~~ to ~~40-38~~, the pharmaceutical composition of claim ~~41-39~~, or the host cell of any one of claims ~~47-45~~ to ~~49-47~~ in the manufacture of a medicament for treating cancer.

~~60-58.~~ The use of claim ~~59-57~~, 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.

~~61-59.~~ The use of claim ~~59-57~~ or ~~60-58~~, wherein the medicament is for administration with an additional therapeutic agent ~~is to be administered~~.

~~62-60.~~ The use of claim ~~61-59~~, wherein the additional therapeutic agent is an anti-cancer agent.

~~63-61.~~ The use of claim ~~62-60~~, wherein the anti-cancer agent is selected from a chemotherapeutic agent, an anti-cancer biologic, radiation therapy, CAR-T therapy, and an oncolytic virus.

~~64-62.~~ The use of any one of claims ~~61-59~~ to ~~63-61~~, wherein the additional therapeutic agent is an anti-cancer biologic.

~~65-63.~~ The use of claim ~~64-62~~, wherein the anti-cancer biologic is an agent that inhibits PD-1 and/or PD-L1.

~~66-64.~~ The use of claim ~~65-63~~, 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.

~~67-65.~~ The use of claim ~~64-62~~, wherein the anti-cancer biologic is an agent that inhibits VISTA, gpNMB, B7H3, B7H4, HHLA2, CD73, CTLA4, or TIGIT.

~~68-66.~~ The use of any one of claims ~~64-62~~ to ~~67-65~~, wherein the anti-cancer agent is an antibody.

~~69-67.~~ The use of claim ~~64-62~~, wherein the anti-cancer biologic is a cytokine.

~~70.—~~ ~~The use of claim 64, wherein the anti-cancer agent is CAR-T therapy.~~

~~71-68.~~ The use of claim ~~64-62~~, wherein the anti-cancer agent is an oncolytic virus.

~~72-69.~~ The use of any one of claims ~~59-57~~ to ~~71-68~~, further comprising tumor resection, CAR-T therapy, and/or radiation therapy.

~~73-70.~~ Use of the polypeptide of any one of claims ~~1-4~~ to ~~40-38~~ in the manufacture of a medicament for increasing CD4⁺ and/or CD8⁺ T cell proliferation in a subject with cancer.

~~74-71.~~ Use of the polypeptide of any one of claims ~~1-4~~ to ~~40-38~~ in the manufacture of a medicament for increasing CD25 expression on CD4⁺ and/or CD8⁺ T cells in a subject with cancer.

~~75-72.~~ Use of the polypeptide of any one of claims ~~1-4~~ to ~~40-38~~ in the manufacture of a medicament for increasing CD71 expression on CD4⁺ and/or CD8⁺ T cells in a subject with cancer.

~~76~~73. Use of the polypeptide of any one of claims ~~1-4~~ to ~~40-38~~ in the manufacture of a medicament for increasing NFκB signaling in CD4⁺ and/or CD8⁺ T cells in a subject with cancer.

~~77~~74. Use of the polypeptide of any one of claims ~~1-4~~ to ~~40-38~~ in the manufacture of a medicament for increasing IFNγ expression in CD4⁺ and/or CD8⁺ T cells in a subject with cancer.

~~78~~75. The use of any one of claims ~~73-70~~ to ~~77~~74, wherein the CD4⁺ and/or CD8⁺ T cells are in the presence of Treg cells.

~~79~~76. The use of any one of claims ~~73-70~~ to ~~78~~75, wherein the increase is at least 1.5-fold, at least 2-fold, at least 3-fold, or by at least 5-fold.