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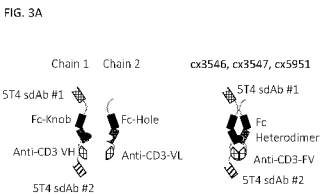
(54) Title: 5T4 SINGLE DOMAIN ANTIBODIES AND THERAPEUTIC COMPOSITIONS THEREOF



(57) Abstract: Provided herein are binding polypeptides that specifically bind 5T4. More specifically, provided herein are fusion proteins, including multivalent and/or multispecific constructs and chimeric antigen receptors, that bind 5T4. Also provided are pharmaceutical compositions containing the polypeptides, nucleic acid molecules encoding the polypeptides and vectors and cells thereof, and methods of use and uses of the provided 5T4 binding polypeptides for treating diseases and conditions, such as cancer.

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[Continued on next page]

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5T4 SINGLE DOMAIN ANTIBODIES AND THERAPEUTIC COMPOSITIONS THEREOF

Cross-Reference to Related Applications

[0001] This application claims priority to U.S. provisional applications: 62/744,631, filed October 11, 2018, entitled "5T4 SINGLE DOMAIN ANTIBODIES AND THERAPEUTIC COMPOSITIONS THEREOF"; and 62/877,824, filed July 23, 2019, entitled "5T4 SINGLE DOMAIN ANTIBODIES AND THERAPEUTIC COMPOSITIONS THEREOF" the contents of which are incorporated by reference in their entirety for all purposes.

Incorporation by Reference of Sequence Listing

[0002] The present application is being filed along with a Sequence Listing in electronic format. The Sequence Listing is provided as a file entitled 744952000540SeqList.TXT, created October 8, 2019 which is 368 kilobytes in size. The information in the electronic format of the Sequence Listing is incorporated by reference in its entirety.

Field

[0003] This disclosure generally provides binding polypeptides that specifically bind 5T4. More specifically, the disclosure relates to fusion proteins, including multivalent and/or multispecific constructs and chimeric antigen receptors, that bind at least 5T4. The disclosure also provides nucleic acid molecules encoding the polypeptides and vectors and cells thereof, and methods of use and uses of the provided 5T4 binding polypeptides for treating diseases and conditions, such as cancer.

Background

[0004] 5T4, also known as trophoblast glycoprotein (TPBG), is a transmembrane glycoprotein expressed on the surface of a wide variety of tumor cells, and its expression has been associated with poor prognosis in a number of cancers. The expression of 5T4 on a variety of cancers in humans, combined with its rare expression in normal adult tissues, makes 5T4 a desirable therapeutic target. Improved therapeutic molecules and agents targeting 5T4 are needed. Provided herein are embodiments that meet such needs.

Summary

[0005] Provided herein is a 5T4-binding polypeptide construct, comprising at least one heavy chain only variable domain (5T4 VHH domain)that specifically binds 5T4 and one or more additional binding domain that binds to a target other than 5T4. In some embodiments, the at least one 5T4 VHH domain

comprises a complementarity determining region 1 (CDR1) comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 86-87 and 288-292, 296, and 297; a complementarity determining region 2 (CDR2) comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 88-99, 298, and 299; and a complementarity determining region 3 (CDR3) comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 100-102, 300, 301, and 303.

[0006] Provided herein is a 5T4-binding construct, comprising at least one heavy chain only variable domain (5T4 VHH domain) comprising a complementarity determining region 1 (CDR1) comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 86-87 and 288-292, 296, and 297; a complementarity determining region 2 (CDR2) comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 88-99, 298, and 299; and a complementarity determining region 3 (CDR3) comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 100-102, 300, 301, and 303.

[0007] In some of any of the provided embodiments, the 5T4 is a human 5T4. In some embodiments, the at least one 5T4 VHH domain is humanized. In some embodiments, the 5T4 has the sequence set forth in SEQ ID NO: 244 or a mature form thereof lacking the signal sequence.

[0008] In some of any of the provided embodiments,the one or more additional binding domains binds to an activating receptor on an immune cell. In some of any of the provided embodiments, the immune cell is a T cell. In some of any of the provided embodiments, the activating receptor is CD3 (CD3ε). In some examples, the 5T4-binding polypeptide construct is bispecific for 5T4 and CD3. In some embodiments, the immune cell is a Natural Killer (NK) cell.

[0009] In some of any of the provided embodiments, the activating receptor is CD16 (CD16a). In some examples, the 5T4-binding polypeptide construct is bispecific for 5T4 and CD16a.

[0010] In some of any of the provided embodiments, the one or more additional binding domain binds to a cytokine receptor.

[0011] In some of any of the provided embodiments, the one or more additional binding domain comprises an antibody or antigen-binding fragment thereof. In some embodiments, the one or more additional binding domain is monovalent. In some embodiments, the antibody or antigen-binding fragment thereof is an Fv, a disulfide-stabilized Fv (dsFv), scFv, a Fab, a single domain antibody (sdAb), a VNAR, or a VHH. In some embodiments, the single domain antibody (sdAb) is a VNAR, or a VHH. In some embodiments, a single domain antibody (sdAb) is a camelid VHH. In some embodiments, a single domain antibody (sdAb) is a humanized form of a camelid VHH.

[0012] In some of any of the provided embodiments, the one or more additional binding domain is a cytokine or is a truncated fragment or variant thereof capable of binding to the cytokine receptor. In some aspects, the cytokine is an interferon, or is a truncated fragment or variant of an interferon. In some examples, the interferon is a type I interferon or a type II interferon, is a truncated fragment or variant of a type I interferon or is a truncated fragment or variant of a type II interferon. In some embodiments, the

type I interferon is an IFN-alpha or an IFN-beta or is a truncated fragment or variant thereof; or the type II interferon is an IFN-gamma or is a truncated fragment or variant thereof.

[0013] In some of any of the provided embodiments, the polypeptide comprises an immunoglobulin Fc region. In some of any of the provided embodiments, the polypeptide comprises an immunoglobulin Fc region that links the at least one single domain antibody and the one or more additional binding domain. In some of any of the provided embodiments, the 5T4-binding polypeptide construct is a dimer. In some of any of the provided embodiments, the Fc region is a homodimeric Fc region. In some of any of the provided embodiments, the Fc region comprises the sequence of amino acids set forth in any of SEQ ID NOS: 8, 10, 11, 12 or 13, or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to any of SEQ ID NOS: 8, 10, 11, 12 or 13 and binds 5T4. In some of any of the provided embodiments, the Fc region is a human IgG1. In some of any of the provided embodiments, the Fc region comprises the sequence of amino acids set forth in SEQ ID NO:8 or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 8. In some of any of the provided embodiments, the Fc region is a heterodimeric Fc region.

[0014] In some embodiments, the Fc region exhibits effector function. In some of any of the provided embodiments, the Fc region comprises a polypeptide comprising one or more amino acid modification that reduces effector function and/or reduces binding to an effector molecule selected from an Fc gamma receptor or C1q. In some examples, the one or more amino acid modification is deletion of one or more of Glu233, Leu234 or Leu235. In some cases, the Fc region comprises the sequence of amino acids set forth in SEQ ID NO:9 or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 9.

[0015] In some of any of the provided embodiments, the at least one 5T4 VHH domain comprises the VHH domain sequence set forth in any of SEQ ID NOS: 245-287, 294, 295, 302, 360 or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to any of SEQ ID NOS: 245-287, 294, 295, 302, 360 and binds 5T4.

[0016] In some of any of the provided embodiments, the at least one 5T4 VHH domain binds to an epitope in human 5T4 but does not exhibit crossreactive binding to mouse 5T4.

[0017] In some of any of the provided embodiments, the at least one 5T4 VHH domain binds to amino acid residues between amino acids 60 and 112 of SEQ ID NO:382.

[0018] In some of any of the provided embodiments, the at least one 5T4 VHH domain binds to amino acid residues between amino acids 173 and 224 of SEQ ID NO:382.

[0019] In some of any of the provided embodiments, the at least one 5T4 VHH domain comprises the sequence set forth in (i) SEQ ID NO:245, (ii) a humanized variant of SEQ ID NO:245, or (iii) a

sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO:245 and binds 5T4. In some of any of the provided embodiments, the at least one 5T4 VHH domain comprises a CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 288 and 289; a CDR2 comprising an amino acid sequence set forth in SEQ ID NO: 88; and a CDR3 comprising an amino acid sequence set forth in SEQ ID NO: 100. In some examples, the at least one 5T4 VHH domain comprises a CDR1, CDR2 and CDR3 set forth in SEQ ID NOS: 288, 88, and 100, respectively; or SEQ ID NOS: 289, 88, and 100, respectively.

[0020] In some of any of the provided embodiments, a 5T4 VHH domain comprises the VHH domain sequence set forth in SEQ ID NO:245. In some of any of the provided embodiments, a 5T4 VHH domain comprises the VHH domain sequence set forth in SEQ ID NO:249. In some of any of the provided embodiments, a 5T4 VHH domain comprises the VHH domain sequence set forth in SEQ ID NO:254. In some of any of the provided embodiments, a 5T4 VHH domain comprises the VHH domain sequence set forth in SEQ ID NO:255. In some of any of the provided embodiments, a 5T4 VHH domain comprises the VHH domain sequence set forth in SEQ ID NO:270. In some of any of the provided embodiments, a 5T4 VHH domain comprises the VHH domain sequence set forth in SEQ ID NO:276. In some of any of the provided embodiments, a 5T4 VHH domain comprises the VHH domain sequence set forth in SEQ ID NO:287. In some of any of the provided embodiments, a 5T4 VHH domain comprises the VHH domain sequence set forth in SEQ ID NO:294. In some of any of the provided embodiments, a 5T4 VHH domain comprises the VHH domain sequence set forth in SEQ ID NO:302. In some of any of the provided embodiments, a 5T4 VHH domain comprises the VHH domain sequence set forth in SEQ ID NO:360.

[0021] In some of any of the provided embodiments, the at least one 5T4 VHH domain comprises the sequence of amino acids set forth in any one of SEQ ID NOs: 246-254 or 360 or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 246-254 or 360 and binds 5T4. In some of any of the provided embodiments, the at least one 5T4 VHH domain comprises the sequence of amino acids set forth in any one of SEQ ID NOS: 246-254 or 360. In some of any of the provided embodiments, the at least one 5T4 VHH domain comprises the sequence set forth in (i) SEQ ID NO:255, (ii) a humanized variant of SEQ ID NO: 255, or (iii) a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 255 and binds 5T4. In some of any of the provided embodiments, the at least one 5T4 VHH domain comprises a CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 86, 290-292; a CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 89-94; and a CDR3 comprising an amino acid sequence set forth in SEQ ID NO: 101. In some examples, the at least one 5T4 VHH domain comprises a CDR1, CDR2 and CDR3 set forth in SEQ

ID NOS: 290, 90, and 101, respectively; SEQ ID NOS: 290, 91, and 101, respectively; SEQ ID NOS: 290, 92, and 101, respectively; SEQ ID NOS: 290, 93, and 101, respectively; SEQ ID NOS: 290, 94, and 101, respectively; SEQ ID NOS: 291, 94, and 101, respectively; SEQ ID NOS: 292, 94, and 101, respectively; or SEQ ID NOS: 86, 94, and 101, respectively, the at least one 5T4 VHH domain comprises the sequence of amino acids set forth in any one of SEQ ID NOs: 256-275 or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 256-275 and binds 5T4. In some of any of the provided embodiments, the at least one 5T4 VHH domain comprises the sequence of amino acids set forth in any one of SEQ ID NOS: 256-275. In some of any of the provided embodiments, the at least one 5T4 VHH domain comprises the sequence set forth in (i) SEQ ID NO:276 (ii) a humanized variant of SEQ ID NO: 276, or (iii) a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 276 and binds 5T4. In some of any of the provided embodiments, the at least one 5T4 VHH domain comprises a CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 86 and 87; a CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 95-99; and a CDR3 comprising an amino acid sequence set forth in SEQ ID NO: 102. In some examples, the at least one 5T4 VHH domain comprises a CDR1, CDR2 and CDR3 set forth in SEQ ID NOS: 87, 95, and 102, respectively; SEQ ID NOS: 87, 96, and 102, respectively; SEQ ID NOS: 87, 97, and 102, respectively; SEQ ID NOS: 87, 98, and 102, respectively; SEQ ID NOS: 87, 99, and 102, respectively; or SEQ ID NOS: 86, 98, and 102, respectively.

[0022] In some of any of the provided embodiments, the at least one 5T4 VHH domain comprises the sequence of amino acids set forth in any one of SEQ ID NOs: 277-287 or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 277-287 and binds 5T4. In some of any of the provided embodiments, the at least one 5T4 VHH domain comprises the sequence of amino acids set forth in any one of SEQ ID NOS: 277-287. In some of any of the provided embodiments, the at least one 5T4 VHH domain comprises the sequence set forth in (i) SEQ ID NO:294 (ii) a humanized variant of SEQ ID NO:294, or (iii) a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO:294 and binds 5T4. In some of any of the provided embodiments, the at least one 5T4 VHH domain comprises a CDR1 comprising an amino acid sequence set forth in SEQ ID NO: 296; a CDR2 comprising an amino acid sequence set forth in SEQ ID NO: 298; and a CDR3 comprising an amino acid sequence set forth in SEQ ID NO: 300. In some examples, the at least one 5T4 VHH domain comprises a CDR3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 300, 301, and 303.

[0023] In some of any of the provided embodiments, the at least one 5T4 VHH domain comprises a CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 288,

296, and 297; and/or a CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 88, 298, and 299.

[0024] In some of any of the provided embodiments, the at least one 5T4 VHH domain comprises the sequence set forth in (i) SEQ ID NO:295 (ii) a humanized variant of SEQ ID NO:295, or (iii) a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO:295 and binds 5T4. In some of any of the provided embodiments, the at least one 5T4 VHH domain comprises a CDR1 comprising an amino acid sequence set forth in SEQ ID NO: 297; a CDR2 comprising an amino acid sequence set forth in SEQ ID NO: 299; and a CDR3 comprising an amino acid sequence set forth in SEQ ID NO: 301.

[0025] In some of any of the provided embodiments, the at least one 5T4 VHH domain comprises the sequence set forth in (i) SEQ ID NO:302 (ii) a humanized variant of SEQ ID NO: 302, or (iii) a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 302 and binds 5T4. In some of any of the provided embodiments, the at least one 5T4 VHH domain comprises a CDR1 comprising an amino acid sequence set forth in SEQ ID NO: 288; a CDR2 comprising an amino acid sequence set forth in SEQ ID NO: 88; and a CDR3 comprising an amino acid sequence set forth in SEQ ID NO: 303.

[0026] In some of any of the provided embodiments, the at least one 5T4 VHH domain comprises the sequence set forth in (i) SEQ ID NO:294, 295, or 302 (ii) a humanized variant of SEQ ID NO: 294, 295, or 302, or (iii) a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 294, 295, or 302 and binds 5T4. In some of any of the provided embodiments, the at least one 5T4 VHH domain comprises a CDR1, CDR2 and CDR3 set forth in SEQ ID NOS: 296, 298, and 300, respectively; SEQ ID NOS:297, 299, and 301, respectively; or SEQ ID NOS:288, 88, and 303, respectively.

[0027] In some of any of the provided embodiments, a 5T4 VHH domain binds to an epitope located between amino acid residues 60 and 112 of the 5T4 extracellular domain set forth in SEQ ID NO:411. In some of any of the provided embodiments, a 5T4 VHH domain binds to an epitope located between amino acid residues 173 and 224 of the 5T4 extracellular domain set forth in SEQ ID NO:412.

[0028] In some embodiments, a 5T4 VHH does not cross react with the mouse 5T4 antigen. In some embodiments, a 5T4 VHH comprising the amino acid sequence set forth in SEQ ID NO:245, or humanized variants thereof, do not cross react with the mouse 5T4 antigen. In some embodiments, a 5T4 VHH comprising the amino acid sequence set forth in SEQ ID NO:255, or humanized variants thereof, do not cross react with the mouse 5T4 antigen. In some embodiments, a 5T4 VHH comprising the amino acid sequence set forth in SEQ ID NO:276, or humanized variants thereof, do not cross react with the mouse 5T4 antigen.

[0029] In some of any of the provided embodiments, the at least one 5T4 VHH domain is set forth in SEQ ID NO:245, 249, 255, 270, 276, 294, 295 or 302. In some of any of the provided embodiments,

the at least one 5T4 VHH domain is set forth in SEQ ID NO:254 or 360. In some of any of the provided embodiments, the at least one 5T4 VHH domain is set forth in SEQ ID NO:360.

[0030] In some of any of the provided embodiments, a 5T4 VHH domain may comprise additional amino acids at its N- and/or C-terminal, such as for linkage to another amino acid sequence, such as another polypeptide. In some of any of the provided embodiments, a 5T4 VHH domain may comprise a flexible linker, such as a glycine linker or a linker composed predominately of the amino acids Glycine and Serine, denoted as GS-linkers herein. Such linkers of the present disclosure can be of various lengths, for example, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 amino acids in length. In some embodiments, the linker comprises an amino acid sequence selected from the group consisting of GGSGGS, i.e., $(GGS)_2$ (SEQ ID NO: 1); GGSGGSGGS, i.e., $(GGS)_3$ (SEQ ID NO: 2); GGSGGSGGSGGS, i.e., $(GGS)_4$ (SEQ ID NO: 3); and GGSGGSGGSGGSGGS, i.e., $(GGS)_5$ (SEQ ID NO: 4), Gly-Gly (GG), GGG, GGGG (SEQ ID NO: 5), GGGGG (SEQ ID NO: 6), and GGGGGG (SEQ ID NO: 7). In some embodiments, the linker is (GGGGS)n, wherein n is 1 to 5 (SEQ ID NO:123); (GGGGGS)n, wherein n is 1 to 4 (SEQ ID NO:124); GGGGS (SEQ ID NO:125); GGGGGS (SEQ ID NO:126); GGGGGSGGGGGSGGGGGS (SEQ ID NO:127); GGGGSGGGGSGGGGS (SEQ ID NO:128); GGSGGGGSGGGGSGGGGS (SEQ ID NO:129); or PGGGG (SEQ ID NO:327). In some embodiments, the linker is a GG linker. In some embodiments, the 5T4-binding polypeptide includes a combination of a GS-linker and a Glycine linker. In some embodiments, a 5T4 VHH domain may comprise the additional linker at its C-terminus, such as for linkage to another amino acid sequence, such as another polypeptide. In some of any of the provided embodiments, a 5T4 VHH domain may comprise the linker at its N-terminus, such as for linkage to another amino acid sequence, such as another polypeptide.

[0031] Provided herein is a multispecific polypeptide construct, comprising a first component comprising a heterodimeric Fc region comprising a first Fc polypeptide and a second Fc polypeptide and a second component comprising an anti-CD3 antibody or antigen-binding fragment comprising a variable heavy chain region (VH) and a variable light chain region (VL), wherein the VH and VL that comprise the anti-CD3 antibody or antigen binding fragment are linked to opposite polypeptides of the heterodimeric Fc; the first and second components are coupled by a linker, wherein the heterodimeric Fc region is positioned N-terminal to the anti-CD3 antibody; and one or both of the first and second components comprises at least one antigen binding domain comprising a heavy chain only variable domain that specifically binds 5T4 (5T4 VHH domain).In some embodiments, the multispecific polypeptide construct comprises at least a first polypeptide comprising the first Fc polypeptide of the heterodimeric Fc region, the linker and the VH or VL domain of the anti-CD3 antibody or antigen binding fragment; and a second polypeptide comprising the second Fc polypeptide of the heterodimeric Fc region, the linker, optionally the same linker as present in the first polypeptide, and the other of the

VH or VL domain of the anti-CD3 antibody or antigen binding fragment, wherein one or both of the first and second polypeptide comprise the at least one 5T4 VHH domain.

[0032] In some of any of the provided embodiments, one or both of the first and second Fc polypeptides of the heterodimeric Fc region comprises at least one modification to induce heterodimerization compared to a polypeptide of a homodimeric Fc region, optionally compared to the Fc polypeptide set forth in SEQ ID NO: 8 or an immunologically active fragment thereof. In some embodiments, each of the first and second Fc polypeptides of the heterodimeric Fc independently comprise at least one amino acid modification. In some embodiments,each of the first and second Fc polypeptides of the heterodimeric Fc comprise a knob-into-hole modification or comprise a charge mutation to increase electrostatic complementarity of the polypeptides. In some examples, the amino acid modification is a knob-into-hole modification.

[0033] In some of any of the provided embodiments, the first Fc polypeptide of the heterodimeric Fc comprises the modification selected from among Thr366Ser, Leu368Ala, Tyr407Val, and combinations thereof and the second Fc polypeptide of the heterodimeric Fc comprises the modification Thr366Trp. In some of any of the provided embodiments, the first and second Fc polypeptides further comprises a modification of a non-cysteine residue to a cysteine residue, wherein the modification of the first polypeptide is at one of the position Ser354 and Tyr349 and the modification of the second Fc polypeptide is at the other of the position Ser354 and Tyr349. In some of any of the provided embodiments, the VL domain of the anti-CD3 antibody or antigen binding fragment is linked to the first Fc polypeptide of the heterodimeric Fc and the VH domain of the anti-CD3 antibody or antigen binding fragment is linked to the second Fc polypeptide of the heterodimeric Fc.

[0034] In some of any of the provided embodiments, the amino acid modification is a charge mutation to increase electrostatic complementarity of the polypeptides. In some of any of the provided embodiments, the first and/or second Fc polypeptides or each of the first and second Fc polypeptide comprise a modification in complementary positions, wherein the modification is replacement with an amino acid having an opposite charge to the complementary amino acid of the other polypeptide.

[0035] In some of any of the provided embodiments, one of the first or second Fc polypeptide of the heterodimeric Fc further comprises a modification at residue Ile253. In some examples, the modification is Ile253Arg. In some of any of the provided embodiments, one of the first or second Fc polypeptide of the heterodimeric Fc further comprises a modification at residue His435. In some examples, the modification is His435Arg. In some of any of the provided embodiments, the Fc region comprises a polypeptide that lacks Lys447.

[0036] In some of any of the provided embodiments, the Fc region comprises a polypeptide comprising at least one modification to enhance FcRn binding. In some embodiments, the modification is at a position selected from the group consisting of Met252, Ser254, Thr256, Met428, Asn434, and combinations thereof. In some cases, the modification is at a position selected from the group consisting

of Met252Y, Ser254T, Thr256E, Met428L, Met428V, Asn434S, and combinations thereof. In some examples, the modification is at position Met252 and at position Met428. In some embodiments, the modification is Met252Y and Met428L. In some cases, the modification is Met252Y and Met428V.

[0037] In some of any of the provided embodiments, the first polypeptide of the heterodimeric Fc comprises the sequence of amino acids set forth in any of SEQ ID NOS:103, 107, 115 or 117, and the second polypeptide of the heterodimeric Fc comprises the sequence of amino acids set forth in any of SEQ ID NOS:104, 108, 111, 113, 119 or 121.

[0038] In some embodiments, the first Fc polypeptide of the heterodimeric Fc region comprises the sequence of amino acids set forth in any of SEQ ID NOS:103, 107, 115, 117, 328, or 334 and the second Fc polypeptide of the heterodimeric Fc region comprises the sequence of amino acids set forth in any of SEQ ID NOS:104, 108, 111, 113, 119, 121, 329, 332, or 336.

[0039] In some of any of the provided embodiments, the Fc region comprises a polypeptide comprising at least one amino acid modification that reduces effector function and/or reduces binding to an effector molecule selected from an Fc gamma receptor or C1q. In some examples, the one or more amino acid modification is deletion of one or more of Glu233, Leu234 or Leu235.

[0040] In some of any of the provided embodiments, the first polypeptide of the heterodimeric Fc comprises the sequence of amino acids set forth in any of SEQ ID NOS: 105, 109, 116 or 118 and the second polypeptide of the heterodimeric Fc comprises the sequence of amino acids set forth in any of SEQ ID NOS: 106, 110, 112, 114, 120 or 122.

[0041] In some of any of the provided embodiments, the first Fc polypeptide of the heterodimeric Fc region comprises the sequence of amino acids set forth in any of SEQ ID NOS: 105, 109, 116, 118, 330, or 335 and the second Fc polypeptide of the heterodimeric Fc region comprises the sequence of amino acids set forth in any of SEQ ID NOS: 106, 110, 112, 114, 120, 122, 331, 333, or 337.

[0042] In some of any of the provided embodiments, the anti-CD3 antibody or antigen binding fragment is monovalent. In some of any of the provided embodiments, the anti-CD3 antibody or antigen binding fragment is not a single chain antibody, optionally is not a single chain variable fragment (scFv). In some of any of the provided embodiments, the anti-CD3 antibody or antigen binding fragment is an Fv antibody fragment. In some examples, the Fv antibody fragment comprises a disulfide stabilized anti-CD3 binding Fv fragment (dsFv).

[0043] In some of any of the provided embodiments, the anti-CD3 antibody or antigen-binding fragment comprises a VH CDR1 comprising the amino acid sequence TYAMN (SEQ ID NO: 29); a VH CD2 comprising the amino acid sequence RIRSKYNNYATYYADSVKD (SEQ ID NO: 30); a VH CDR3 comprising the amino acid sequence HGNFGNSYVSWFAY (SEQ ID NO: 31), a VL CDR1 comprising the amino acid sequence RSSTGAVTTSNYAN (SEQ ID NO: 32); a VL CDR2 comprising the amino acid sequence GTNKRAP (SEQ ID NO: 33); and a VL CDR3 comprising the amino acid sequence ALWYSNLWV (SEQ ID NO: 34). In some of any of the provided embodiments, the anti-CD3

antibody or antigen-binding fragment comprises a VH having the amino acid sequence of any of SEQ ID NOS: 27, 35-65, 341, 343, and 358 or a sequence that exhibits at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to any of SEQ ID NOS: 27, 35-65, 341, 343, and 358 and binds CD3; and a VL having the amino acid sequence of any of SEQ ID NOS: 28, 66-84, 293, 340, and 342 or a sequence that exhibits at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to any of SEQ ID NOS: 28, 66-84, 293, 340, and 342 and binds CD3. In some of any of the provided embodiments, the anti-CD3 antibody or antigen-binding fragment comprises a VH having the amino acid sequence of any of SEQ ID NOS: 27, 35-65, 341, 343, 358, 388, 389, 392, 393, or a sequence that exhibits at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to any of SEQ ID NOS: 27, 35-65, 341, 343, 358, 388, 389, 392, 393, and binds CD3; and a VL having the amino acid sequence of any of SEQ ID NOS: 28, 66-84, 293, 340, 342, 390, 391, 394, 395, or a sequence that exhibits at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to any of SEQ ID NOS: 28, 66-84, 293, 340, 342, 390, 391, 394, 395, and binds CD3. Any of the above VH and VL sequences can be combined in any combination in an anti-CD3 antibody or antigen-binding fragment in the provided constructs herein.

[0044] In some of any of the provided embodiments, the at least one antigen binding domain binds to an epitope in human 5T4 but does not exhibit crossreactive binding to mouse 5T4.

[0045] In some of any of the provided embodiments, the at least one antigen binding domain binds to amino acid residues between amino acids 60 and 112 of SEQ ID NO:382.

[0046] In some of any of the provided embodiments, the at least one antigen binding domain binds to amino acid residues between amino acids 173 and 224 of SEQ ID NO:382.

[0047] In some of any of the provided embodiments, the anti-CD3 antibody or antigen-binding fragment comprises the amino acid sequence of SEQ ID NO: 47 and the amino acid sequence of SEQ ID NO: 75. In some examples, the anti-CD3 antibody or antigen-binding fragment comprises the amino acid sequence of SEQ ID NO: 47 and the amino acid sequence of SEQ ID NO: 293. In some examples, the anti-CD3 antibody or antigen-binding fragment comprises the amino acid sequence of SEQ ID NO: 341 and the amino acid sequence of SEQ ID NO: 342.

[0048] In some of any of the provided embodiments, the at least one 5T4 single domain antibody is positioned amino-terminally relative to the Fc region and/or carboxy-terminally relative to the CD3 binding region of the multispecific polypeptide construct. In some of any of the provided embodiments, the multispecific polypeptide construct comprises a first 5T4 VHH domain that specifically bind 5T4 and a second 5T4 VHH domain that specifically binds 5T4. In some of any of the provided embodiments, the first or second 5T4 VHH domain is positioned amino-terminally relative to the Fc region of the multispecific construct and the other of the first or second 5T4 VHH domain is positioned carboxy-terminally relative to the CD3 binding region of the multispecific construct.

[0049] In some of any of the provided embodiments, the first polypeptide comprises in order of N-terminus to C-terminus a first 5T4 VHH domain that binds 5T4, a first polypeptide comprising the first Fc polypeptide of the heterodimeric Fc region, the linker, the VH or VL domain of the anti-CD3 antibody or antigen binding fragment and a second 5T4 VHH domain that binds 5T4; and the second polypeptide comprises in order of N-terminus to C-terminus the second Fc polypeptide of the heterodimeric Fc region, the linker, optionally the same linker as present in the first polypeptide, and the other of the VH or VL domain of the anti-CD3 antibody or antigen binding fragment.

[0050] In some of any of the provided embodiments, the first and second 5T4 VHH domain are the same. In some of any of the provided embodiments, the first and second 5T4 VHH domain are different. In some of any of the provided embodiments, the first and second 5T4 VHH domain bind a distinct or non-overlapping epitope of 5T4 and/or do not compete for binding to 5T4. In some of any of the provided embodiments, a 5T4 VHH domain binds to an epitope located between amino acid residues 60 and 112 of the 5T4 extracellular domain corresponding to residues set forth in SEQ ID NO:382. In some of any of the provided embodiments, a 5T4 VHH domain binds to an epitope located between amino acid residues 173 and 224 of the 5T4 extracellular domain corresponding to residues set forth in SEQ ID NO:382.

[0051] In some of any of the provided embodiments, the first and second 5T4 VHH domains bind to the same or an overlapping epitope of the 5T4 extracellular domain.In some of any of the provided embodiments, the first and second 5T4 VHH domains both bind an epitope located between amino acid residues 60 and 112 of the 5T4 extracellular domain corresponding to residues set forth in SEQ ID NO: 382. In some of any of the provided embodiments, the first and second 5T4 VHH domains both bind an epitope located between amino acid residues 173 and 224 of the 5T4 extracellular domain corresponding to residues set forth in SEQ ID NO:382.

[0052] In some of any of the provided embodiments, the first and second 5T4 VHH domains bind to different epitopes of the 5T4 extracellular domain. In some of any of the provided embodiments, the first 5T4 VHH domain binds an epitope located between amino acid residues 60 and 112 of the 5T4 extracellular domain corresponding to residues set forth in SEQ ID NO:382, and the second 5T4 VHH domain binds an epitope located between amino acid residues 173 and 224 of the 5T4 extracellular domain corresponding to residues set forth in SEQ ID NO:382.

[0053] In some of any of the provided embodiments, the first VHH domain or sdAb comprises the amino acid sequence set forth in any one of SEQ ID NOS: 245-254, 295, 302, a humanized variant thereof, or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to any of SEQ ID NOS: 245-254, 295, 302, and binds 5T4; and the second VHH domain or sdAb comprises the amino acid sequence set forth in any one of SEQ ID NOS: 255-287, 294, a humanized variant thereof, or a sequence of amino acids that

exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to any of SEQ ID NOS: 255-287, 294 and binds 5T4.

[0054] In some of any of the provided embodiments, the first VHH domain or sdAb comprises the amino acid sequence set forth in any one of SEQ ID NOS: 245-254, 295, 302, 360, a humanized variant thereof, or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to any of SEQ ID NOS: 245-254, 295, 302, 360, and binds 5T4; and the second VHH domain or sdAb comprises the amino acid sequence set forth in any one of SEQ ID NOS: 255-287, 294, a humanized variant thereof, or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to any of SEQ ID NOS: 255-287, 294 and binds 5T4.

[0055] In some of any of the provided embodiments, the first and second antibody (5T4 VHH domain) are selected from SEQ ID NO: 245 and SEQ ID NO: 294, respectively. In some of any of the provided embodiments, the first and second 5T4 VHH domains are selected from SEQ ID NO: 245 and SEQ ID NO: 276, respectively. In some of any of the provided embodiments, the first and second 5T4 VHH domain are selected from SEQ ID NO: 245 and SEQ ID NO: 255, respectively. In some of any of the provided embodiments, the first and second 5T4 VHH domain are selected from SEQ ID NO: 245 and SEQ ID NO: 294, respectively. In some of any of the provided embodiments, the first and second 5T4 VHH domain are selected from SEQ ID NO: 295 and SEQ ID NO: 294, respectively. In some of any of the provided embodiments, the first and second 5T4 VHH domain are selected from SEQ ID NO: 249 and SEQ ID NO: 270, respectively. In some of any of the provided embodiments, the first and second 5T4 VHH domain are selected from SEQ ID NO: 254 and SEQ ID NO: 287; respectively. In some of any of the provided embodiments, the first and second 5T4 VHH domain are selected from SEQ ID NO: 302 and SEQ ID NO: 302, respectively. In some of any of the provided embodiments, the first and second 5T4 VHH domain are selected fromSEQ ID NO: 360 and SEQ ID NO: 287, respectively.In some of any of the provided embodiments, the at least one 5T4 VHH domain, or each of the first and second 5T4 VHH domain, independently comprises the VHH domain sequence set forth in any of SEQ ID NOS: 245-287, 294, 295, 302, or 360, or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to any of SEQ ID NOS: 245-287, 294, 295, 302, or 360 and binds 5T4.

[0056] In some of any of the provided embodiments, the at least one 5T4 VHH domain, or each of the first and second 5T4 VHH domain, independently comprises the VHH domain sequence set forth in SEQ ID NO: 360, or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 360 and binds 5T4.

[0057] In some of any of the provided embodiments, the at least one 5T4 VHH domain, or each of the first and second 5T4 VHH domain, independently comprises the sequence set forth in (i) SEQ ID

NO: 245, (ii) a humanized variant of SEQ ID NO: 245, or (iii) a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 245, and binds 5T4. In some of any of the provided embodiments, the at least one 5T4 VHH domain, or each of the first and second 5T4 VHH domain, independently comprises a CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 86-87 and 288-292, 296, and 297; a CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 88-99, 298, and 299; and a CDR3 comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 100-102, 300, 301, and 303. In some of any of the provided embodiments, the at least one 5T4 VHH domain, or each of the first and second 5T4 VHH domain, independently comprises a CDR1, CDR2 and CDR3 set forth in SEQ ID NOS: 288, 88, and 100, respectively; SEQ ID NOS: 289, 88, and 100, respectively; SEQ ID NOS: 290, 90, and 101, respectively; SEQ ID NOS: 290, 91, and 101, respectively; SEQ ID NOS: 290, 92, and 101, respectively; SEQ ID NOS: 290, 93, and 101, respectively; SEQ ID NOS: 290, 94, and 101, respectively; SEQ ID NOS: 291, 94, and 101, respectively; SEQ ID NOS: 292, 94, and 101, respectively; SEQ ID NOS: 86, 94, and 101, respectively; SEQ ID NOS: 87, 95, and 102, respectively; SEQ ID NOS: 87, 96, and 102, respectively; SEQ ID NOS: 87, 97, and 102, respectively; SEQ ID NOS: 87, 98, and 102, respectively; SEQ ID NOS: 87, 99, and 102, respectively; SEQ ID NOS: 86, 98, and 102, respectively; SEQ ID NOS: 296, 298, and 300, respectively; SEQ ID NOS: 297, 299, and 301, respectively; or SEQ ID NOS: 288, 88, and 303, respectively.

[0058] In some of any of the provided embodiments, the at least one 5T4 VHH domain, or each of the first and second 5T4 VHH domain, independently comprises the sequence of amino acids set forth in any one of SEQ ID NOS: 246-254 or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NOS: 246-254, and binds 5T4. In some of any of the provided embodiments, the at least one 5T4 VHH domain, or each of the first and second 5T4 VHH domain, independently comprises the sequence of amino acids set forth in any one of SEQ ID NOS: 246-254.

[0059] In some of any of the provided embodiments, the at least one 5T4 VHH domain, or each of the first and second 5T4 VHH domain, independently comprises the sequence of amino acids set forth in SEQ ID NO: 360 or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 360, and binds 5T4. In some of any of the provided embodiments, the at least one 5T4 VHH domain, or each of the first and second 5T4 VHH domain, independently comprises the sequence of amino acids set forth in SEQ ID NO: 360.

[0060] In some of any of the provided embodiments, the at least one 5T4 VHH domain, or each of the first and second 5T4 VHH domain, independently comprises the sequence set forth in (i) SEQ ID NO: 255, (ii) a humanized variant of SEQ ID NO: 255, or (iii) a sequence of amino acids that exhibits at

least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 255, and binds 5T4. In some of any of the provided embodiments, the at least one 5T4 VHH domain, or each of the first and second 5T4 VHH domain, independently comprises a CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 86, 290-292; a CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 89-94; and a CDR3 comprising an amino acid sequence set forth in SEQ ID NO: 101. In some examples, the at least one 5T4 VHH domain, or each of the first and second 5T4 VHH domain, independently comprises a CDR1, CDR2 and CDR3 set forth in SEQ ID NOS: 290, 90, and 101, respectively; SEQ ID NOS: 290, 91, and 101, respectively; SEQ ID NOS: 290, 92, and 101, respectively; SEQ ID NOS: 290, 93, and 101, respectively; SEQ ID NOS: 290, 94, and 101, respectively; SEQ ID NOS: 291, 94, and 101, respectively; SEQ ID NOS: 292, 94, and 101, respectively; or SEQ ID NOS: 86, 94, and 101, respectively.

[0061] In some of any of the provided embodiments, the at least one 5T4 VHH domain, or each of the first and second 5T4 VHH domain, independently comprises the sequence of amino acids set forth in any one of SEQ ID NOs: 256-275 or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 256-275, and binds 5T4. In some of any of the provided embodiments, the at least one 5T4 VHH domain, or each of the first and second 5T4 VHH domain, independently comprises the sequence of amino acids set forth in any one of SEQ ID NOS: 256-275. In some examples, the at least one 5T4 VHH domain, or each of the first and second 5T4 VHH domain, independently comprises the sequence set forth in (i) SEQ ID NO: 276 (ii) a humanized variant of SEQ ID NO: 276, or (iii) a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 276, and binds 5T4. In some of any of the provided embodiments, the at least one 5T4 VHH domain, or each of the first and second sdAb, independently comprises a CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 86 and 87; a CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 95-99; and a CDR3 comprising an amino acid sequence set forth in SEQ ID NO: 102. In some of any of the provided embodiments, the at least one 5T4 VHH domain, or each of the first and second 5T4 VHH domain, independently comprises a CDR1, CDR2 and CDR3 set forth in SEQ ID NOS: 87, 95, and 102, respectively; SEQ ID NOS: 87, 96, and 102, respectively; SEQ ID NOS: 87, 97, and 102, respectively; SEQ ID NOS: 87, 98, and 102, respectively; SEQ ID NOS: 87, 99, and 102, respectively; or SEQ ID NOS: 86, 98, and 102, respectively.

In some of any of the provided embodiments, the at least one 5T4 VHH domain, or each of the first and second 5T4 VHH domain, independently comprises the sequence of amino acids set forth in any one of SEQ ID NOs: 277-287 or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 277-287,

and binds 5T4. In some of any of the provided embodiments, the at least one 5T4 VHH domain, or each of the first and second 5T4 VHH domain, independently comprises the sequence of amino acids set forth in any one of SEQ ID NOS: 277-287. In some of any of the provided embodiments, the at least one 5T4 VHH domain, or each of the first and second 5T4 VHH domain, independently comprises the sequence set forth in (i) SEQ ID NO: 294 (ii) a humanized variant of SEQ ID NO: 294, or (iii) a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 294, and binds 5T4. In some of any of the provided embodiments, the at least one 5T4 VHH domain, or each of the first and second 5T4 VHH domain, independently comprises a CDR1 comprising an amino acid sequence set forth in SEQ ID NO: 296; a CDR2 comprising an amino acid sequence set forth in SEQ ID NO: 298; and a CDR3 comprising an amino acid sequence set forth in SEQ ID NO: 300.

[0062] In some of any of the provided embodiments, the at least one 5T4 VHH domain, or each of the first and second 5T4 VHH domain, independently comprises the sequence set forth in (i) SEQ ID NO: 295 (ii) a humanized variant of SEQ ID NO: 295, or (iii) a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 295, and binds 5T4. In some of any of the provided embodiments, the at least one 5T4 VHH domain, or each of the first and second 5T4 VHH domain, independently comprises a CDR1 comprising an amino acid sequence set forth in SEQ ID NO: 297; a CDR2 comprising an amino acid sequence set forth in SEQ ID NO: 299; and a CDR3 comprising an amino acid sequence set forth in SEQ ID NO: 301. In some of any of the provided embodiments, the at least one 5T4 VHH domain, or each of the first and second 5T4 VHH domain, independently comprises the sequence set forth in (i) SEQ ID NO: 302 (ii) a humanized variant of SEQ ID NO: 302, or (iii) a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 302, and binds 5T4. In some of any of the provided embodiments, the at least one 5T4 VHH domain, or each of the first and second 5T4 VHH domain, independently comprises a CDR1 comprising an amino acid sequence set forth in SEQ ID NO: 288; a CDR2 comprising an amino acid sequence set forth in SEQ ID NO: 88; and a CDR3 comprising an amino acid sequence set forth in SEQ ID NO: 303.

[0063] In some of any of the provided embodiments, the at least one 5T4 VHH domain, or each of the first and second sdAb, independently comprises the sequence set forth in (i) SEQ ID NO: 294, 295, or 302 (ii) a humanized variant of SEQ ID NO: 294, 295, or 302, or (iii) a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 294, 295, or 302, and binds 5T4.

[0064] In some of any of the provided embodiments, the at least one 5T4 VHH domain, or each of the first and second 5T4 VHH domain, independently comprises a CDR1, CDR2 and CDR3 set forth in

SEQ ID NOS: 296, 298, and 300, respectively; SEQ ID NOS:297, 299, and 301, respectively; or SEQ ID NOS:288, 88, and 303, respectively.

[0065] In some of any of the provided embodiments, the at least one 5T4 VHH domain, or each of the first and second 5T4 VHH domain, independently is set forth in SEQ ID NO: 245, 249, 255, 270, 276, 294, 295 or 302.

[0066] In some of any of the provided embodiments, the at least one 5T4 VHH domain, or each of the first and second 5T4 VHH domain, independently is set forth in SEQ ID NO: 245, 249, 254, 255, 270, 276, 287, 294, 295, 302, or 360.

[0067] In some of any of the provided embodiments, one or both of the first and second components comprises at least one co-stimulatory receptor binding region (CRBR) that binds a co-stimulatory receptor. In some of any of the provided embodiments, the at least one co-stimulatory receptor binding region (CRBR) is positioned amino-terminally relative to the Fc region and/or carboxy-terminally relative to the CD3 binding region of the multispecific polypeptide construct. In some embodiments, the multispecific polypeptide construct comprises only one co-stimulatory receptor binding region (CRBR). In some of any of the provided embodiments, the at least one co-stimulatory receptor binding region (CRBR) is positioned amino-terminally relative to the Fc region of the multispecific polypeptide construct. In some of any of the provided embodiments, the at least one co-stimulatory receptor binding region (CRBR) is positioned carboxy-terminally relative to the CD3 binding region of the multispecific polypeptide construct

[0068] In some of any of the provided embodiments, the first polypeptide comprises in order of N-terminus to C-terminus a first 5T4 VHH domain that binds 5T4, a first polypeptide comprising the first Fc polypeptide of the heterodimeric Fc region, the linker, the VH or VL domain of the anti-CD3 antibody or antigen binding fragment and a second 5T4 VHH domain that binds 5T4; and the second polypeptide comprises the CRBR and comprises in order of N-terminus to C-terminus the second Fc polypeptide of the heterodimeric Fc region, the linker, optionally the same linker as present in the first polypeptide, the other of the VH or VL domain of the anti-CD3 antibody or antigen binding fragment, wherein the CRBR is positioned amino-terminally relative to the Fc region or carboxy-terminally relative to the anti-CD3 antibody or antigen binding fragment of the second polypeptide.

[0069] In some of any of the provided embodiments, the at least one co-stimulatory receptor binding region (CRBR) is or comprises the extracellular domain or binding fragment thereof of the native cognate binding partner of the co-stimulatory receptor, or a variant thereof that exhibits binding activity to the co-stimulatory receptor. In some of any of the provided embodiments, the at least one co-stimulatory receptor binding region (CRBR) is an antibody or antigen-binding fragment thereof selected from the group consisting of a Fab fragment, a F(ab')2 fragment, an Fv fragment, a scFv, a scAb, a dAb, a single domain heavy chain antibody, and a single domain light chain antibody. In some examples, the antibody or antigen-binding fragment thereof is a Fv, a scFv, a Fab, a single domain antibody (sdAb), a

VNAR, or a VHH. In some cases, the antibody or antigen-binding fragment is an sdAb. In some embodiments, the sdAb is a human or humanized sdAb. In some embodiments, the sdAb is a VNAR or a VHH.

[0070] In some of any of the provided embodiments, the at least one co-stimulatory receptor binding region (CRBR) binds a co-stimulatory receptor selected from among 41BB (CD137), OX40 (CD134), CD27, glucocorticoid-induced TNFR-related protein (GITR), CD28, ICOS, CD40, B-cell activating factor receptor (BAFF-R), B-cell maturation antigen (BCMA), Transmembrane activator and CAML interactor (TACI), and NKG2D. In some of any of the provided embodiments, the at least one co-stimulatory receptor binding region (CRBR) binds a co-stimulatory receptor selected from among 41BB (CD137), OX40 (CD134), and glucocorticoid-induced TNFR-related protein (GITR). In some of any of the provided embodiments, the at least one co-stimulatory receptor binding region (CRBR) comprises the sequence of amino acids set forth in SEQ ID NO:210 or a sequence that has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the sequence set forth in SEQ ID NO:210 and binds 4-1BB. In some of any of the provided embodiments, the at least one co-stimulatory receptor binding region (CRBR) comprises the sequence of amino acids set forth in SEQ ID NO:210 binds 4-1BB.

[0071] In some of any of the provided embodiments, one or both of the first and second components comprises at least one inhibitory receptor binding region (IRBR) that binds an inhibitory receptor. In some of any of the provided embodiments, the at least one inhibitory receptor binding region (IRBR) is positioned amino-terminally relative to the Fc region and/or carboxy-terminally relative to the CD3 binding region of the multispecific polypeptide construct. In some of any of the provided embodiments, the multispecific polypeptide construct comprises only one inhibitory receptor binding region (IRBR).

[0072] In some of any of the provided embodiments, the first polypeptide comprises in order of N-terminus to C-terminus a first 5T4 VHH domain that binds 5T4, a first polypeptide comprising the first Fc polypeptide of the heterodimeric Fc region, the linker, the VH or VL domain of the anti-CD3 antibody or antigen binding fragment and a second 5T4 VHH domain that binds 5T4; and the second polypeptide comprises the IRBR and comprises in order of N-terminus to C-terminus the second Fc polypeptide of the heterodimeric Fc region, the linker, optionally the same linker as present in the first polypeptide, the other of the VH or VL domain of the anti-CD3 antibody or antigen binding fragment, wherein the IRBR is positioned amino-terminally relative to the Fc region or carboxy-terminally relative to the anti-CD3 antibody or antigen-binding fragment of the second polypeptide.

[0073] In some of any of the provided embodiments, the at least one IRBR is or comprises the extracellular domain or binding fragment thereof of the native cognate binding partner of the inhibitory receptor, or a variant thereof that exhibits binding activity to the inhibitory receptor. In some of any of the provided embodiments, the at least one IRBR is an antibody or antigen-binding fragment thereof selected from the group consisting of a Fab fragment, a F(ab')2 fragment, an Fv fragment, a scFv, a scAb,

a dAb, a single domain heavy chain antibody, and a single domain light chain antibody. In some embodiments,the antibody or antigen-binding fragment thereof is a Fv, a scFv, a Fab, a single domain antibody (sdAb), a VNAR, or a VHH. In some cases, the antibody or antigen-binding fragment is an sdAb. In some embodiments, the sdAb is a human or humanized sdAb.

[0074] In some of any of the provided embodiments, the at least one IRBR binds a inhibitory receptor selected from among PD-1, CTLA-4, TIGIT, VISTA and TIM3. In some examples, the at least one IRBR binds PD-1.

[0075] In some of any of the provided embodiments, the first polypeptide comprises in order of N-terminus to C-terminus a first 5T4 VHH domain that binds 5T4, a first polypeptide comprising the first Fc polypeptide of the heterodimeric Fc region, the linker, the VH or VL domain of the anti-CD3 antibody or antigen binding fragment and a second 5T4 VHH domain that binds 5T4; and the second polypeptide comprises in order of N-terminus to C-terminus one of the IRBR or the CRBR, the second Fc polypeptide of the heterodimeric Fc region, the linker, optionally the same linker as present in the first polypeptide, the other of the VH or VL domain of the anti-CD3 antibody or antigen binding fragment, and the other of the CRBR or IRBR.

[0076] In some of any of the provided embodiments, the linker is a peptide or polypeptide linker, optionally wherein the linker is 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 amino acids in length. In some of any of the provided embodiments, the linker is a non-cleavable linker. In some of any of the provided embodiments, the non-cleavable linker is or comprises GG. In some of any of the provided embodiments, the non-cleavable linker comprises GS, GGS, GGGGS (SEQ ID NO:125), GGGGGS (SEQ ID NO:126) and combinations thereof. In some of any of the provided embodiments, the linker is or comprises the sequence GGGGGSGGGGGSGGGGGS (SEQ ID NO:127). In some of any of the provided embodiments, the linker is a cleavable linker. In some of any of the provided embodiments, the cleavable linker is a polypeptide that functions as a substrate for a protease.

[0077] In some of any of the provided embodiments, the protease is produced by an immune effector cell, by a tumor, or by cells present in the tumor microenvironment. In some of any of the provided embodiments, the protease is produced by an immune effector cell and the immune effector cell is an activated T cell, a natural killer (NK) cell, or an NK T cell. In some examples, the protease is selected from among matriptase, a matrix metalloprotease (MMP), granzyme B, and combinations thereof. In some cases, the protease is granzyme B.

[0078] In some of any of the provided embodiments, the cleavable linker comprises the amino acid sequence GGSGGGG**IEPD**IGGSGGS (SEQ ID NO:171).

[0079] Provided herein is an isolated single domain antibody that binds 5T4, comprising a complementarity determining region 1 (CDR1) comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 86-87 and 288-292, 296, and 297; a complementarity determining region 2 (CDR2) comprising an amino acid sequence selected from the group consisting of SEQ ID NO:

88-99, 298, and 299; and a complementarity determining region 3 (CDR3) comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 100-102, 300, 301, and 303. In some embodiments, the isolated single domain antibody comprises the amino acid sequence set forth in any of SEQ ID NOS: 245-287, 294, 295, 302, or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to any of SEQ ID NOS: 245-287, 294, 295, 302, and binds 5T4.

[0080] In some of any of the provided embodiments, the isolated single domain antibody binds to an epitope in human 5T4 but does not exhibit crossreactive binding to mouse 5T4.

[0081] In some of any of the provided embodiments, the isolated single domain antibody binds to amino acid residues between amino acids 60 and 112 of SEQ ID NO:382.

[0082] In some of any of the provided embodiments, the isolated single domain antibody binds to amino acid residues between amino acids 173 and 224 of SEQ ID NO:382.

[0083] Provided herein is an isolated single domain antibody that binds 5T4, comprising a complementarity determining region 1 (CDR1) comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 86-87 and 288-292, 296, and 297; a complementarity determining region 2 (CDR2) comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 88-99, 298, and 299; and a complementarity determining region 3 (CDR3) comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 100-102, 300, 301, and 303. In some embodiments, the isolated single domain antibody comprises the amino acid sequence set forth in any of SEQ ID NOS: 245-287, 294, 295, 302, 360, or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to any of SEQ ID NOS: 245-287, 294, 295, 302, 360, and binds 5T4.

[0084] In some of any of the provided embodiments, the single domain antibody comprises the sequence set forth in (i) SEQ ID NO: 245, (ii) a humanized variant of SEQ ID NO: 245, or (iii) a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 245, and binds 5T4. In some of any of the provided embodiments, the sdAb comprises a CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 288 and 289; a CDR2 comprising an amino acid sequence set forth in SEQ ID NO: 88; and a CDR3 comprising an amino acid sequence set forth in SEQ ID NO: 100. In some of any of the provided embodiments, the sdAb comprises a CDR1, CDR2 and CDR3 set forth in SEQ ID NOS: 288, 88, and 100, respectively; or SEQ ID NOS: 289, 88, and 100, respectively. In some of any of the provided embodiments, the sdAb comprises the sequence of amino acids set forth in any one of SEQ ID NOs: 246-254 or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 246-254, and binds 5T4. In some of any of the provided embodiments, the sdAb comprises the sequence of amino acids set forth in any one of SEQ ID NOS: 246-254.

[0085] In some of any of the provided embodiments, the single domain antibody comprises the sequence set forth in (i) SEQ ID NO: 245, (ii) a humanized variant of SEQ ID NO: 245, or (iii) a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 245, and binds 5T4. In some of any of the provided embodiments, the sdAb comprises a CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 288 and 289; a CDR2 comprising an amino acid sequence set forth in SEQ ID NO: 88; and a CDR3 comprising an amino acid sequence set forth in SEQ ID NO: 100. In some of any of the provided embodiments, the sdAb comprises a CDR1, CDR2 and CDR3 set forth in SEQ ID NOS: 288, 88, and 100, respectively; or SEQ ID NOS: 289, 88, and 100, respectively. In some of any of the provided embodiments, the sdAb comprises the sequence of amino acids set forth in any one of SEQ ID NOs: 246-254 or 360 or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 246-254, and binds 5T4. In some of any of the provided embodiments, the sdAb comprises the sequence of amino acids set forth in any one of SEQ ID NOS: 246-254 or 360.

[0086] In some of any of the provided embodiments, the sdAb comprises the sequence set forth in (i) SEQ ID NO: 255, (ii) a humanized variant of SEQ ID NO: 255, or (iii) a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 255, and binds 5T4. In some of any of the provided embodiments, the sdAb comprises a CDR1 comprising an amino acid sequence set forth in SEQ ID NO: 86, 290-292; a CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 89-94; and a CDR3 comprising an amino acid sequence set forth in SEQ ID NO: 101. In some of any of the provided embodiments, the sdAb comprises a CDR1, CDR2 and CDR3 set forth in SEQ ID NOS: 290, 90, and 101, respectively; SEQ ID NOS: 290, 91, and 101, respectively; SEQ ID NOS: 290, 92, and 101, respectively; SEQ ID NOS: 290, 93, and 101, respectively; SEQ ID NOS: 290, 94, and 101, respectively; SEQ ID NOS: 291, 94, and 101, respectively; SEQ ID NOS: 292, 94, and 101, respectively; or SEQ ID NOS: 86, 94, and 101, respectively.

[0087] In some of any of the provided embodiments, the sdAb comprises the sequence of amino acids set forth in any one of SEQ ID NOs: 256-275 or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 256-275, and binds 5T4. In some of any of the provided embodiments, the sdAb comprises the sequence of amino acids set forth in any one of SEQ ID NOS: 256-275. In some of any of the provided embodiments, the sdAb comprises the sequence set forth in (i) SEQ ID NO: 276 (ii) a humanized variant of SEQ ID NO: 276, or (iii) a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 276, and binds 5T4. In some of any of the provided embodiments, the sdAb comprises a CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 86 and 87;

a CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 95-99; and a CDR3 comprising an amino acid sequence set forth in SEQ ID NO: 102. In some examples, the sdAb comprises a CDR1, CDR2 and CDR3 set forth in SEQ ID NOS: 87, 95, and 102, respectively; SEQ ID NOS: 87, 96, and 102, respectively; SEQ ID NOS: 87, 97, and 102, respectively; SEQ ID NOS: 87, 98, and 102, respectively; SEQ ID NOS: 87, 99, and 102, respectively; or SEQ ID NOS: 86, 98, and 102, respectively.

[0088] In some of any of the provided embodiments, the sdAb comprises the sequence of amino acids set forth in any one of SEQ ID NOs: 277-287 or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 277-287, and binds 5T4. In some of any of the provided embodiments, the at least one 5T4 VHH domain comprises the sequence of amino acids set forth in any one of SEQ ID NOS: 277-287.

[0089] In some of any of the provided embodiments, the sdAb comprises the sequence set forth in (i) SEQ ID NO: 294 (ii) a humanized variant of SEQ ID NO: 294, or (iii) a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 294, and binds 5T4. In some of any of the provided embodiments, the sdAb comprises a CDR1 comprising an amino acid sequence set forth in SEQ ID NO: 296; a CDR2 comprising an amino acid sequence set forth in SEQ ID NO: 298; and a CDR3 comprising an amino acid sequence set forth in SEQ ID NO: 300.

[0090] In some of any of the provided embodiments, the sdAb comprises a CDR3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 300, 301, and 303. In some of any of the provided embodiments, the sdAb comprises a CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 288, 296, and 297; and/or a CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 88, 298, and 299.

[0091] In some of any of the provided embodiments, the sdAb comprises the sequence set forth in (i) SEQ ID NO:295 (ii) a humanized variant of SEQ ID NO:295, or (iii) a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO:295, and binds 5T4. In some of any of the provided embodiments, the sdAb comprises a CDR1 comprising an amino acid sequence set forth in SEQ ID NO: 297; a CDR2 comprising an amino acid sequence set forth in SEQ ID NO: 299; and a CDR3 comprising an amino acid sequence set forth in SEQ ID NO: 301. In some of any of the provided embodiments, the sdAb comprises the sequence set forth in (i) SEQ ID NO:302 (ii) a humanized variant of SEQ ID NO:302, or (iii) a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO:302, and binds 5T4. In some of any of the provided embodiments, the sdAb comprises a CDR1 comprising an amino acid sequence set forth in SEQ ID NO: 288; a CDR2 comprising an amino acid sequence set forth in SEQ ID NO: 88; and

a CDR3 comprising an amino acid sequence set forth in SEQ ID NO: 303. In some of any of the provided embodiments, the sdAb comprises the sequence set forth in (i) SEQ ID NO: 294, 295, or 302 (ii) a humanized variant of SEQ ID NO: 294, 295, or 302, or (iii) a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 294, 295, or 302, and binds 5T4. In some of any of the provided embodiments, the at least one 5T4 VHH domain comprises a CDR1, CDR2 and CDR3 set forth in SEQ ID NOS: 296, 298, and 300, respectively; SEQ ID NOS:297, 299, and 301, respectively; or SEQ ID NOS:288, 88, and 303, respectively.

[0092] Provided herein is a polynucleotide(s) encoding any of the 5T4-binding polypeptide provided herein.

[0093] Provided herein is a polynucleotide(s) encoding any of the multispecific polypeptide constructs provided herein.

[0094] Provided herein is a polynucleotide, comprising a first nucleic acid sequence encoding a first polypeptide of any of the multispecific constructs provided herein and a second nucleic acid sequence encoding a second polypeptide of the multispecific construct, wherein the first and second nucleic acid sequence are separated by an internal ribosome entry site (IRES), or a nucleic acid encoding a self-cleaving peptide or a peptide that causes ribosome skipping. In some embodiments, the first nucleic acid sequence and second nucleic acid sequence are operably linked to the same promoter.

[0095] In some of any of the provided embodiments, the nucleic acid encoding a self-cleaving peptide or a peptide that causes ribosome skipping is selected from a T2A, a P2A, a E2A or a F2A.

[0096] Provided herein is a polynucleotide encoding any of the provided single domain antibodies.

[0097] Provided herein is a vector comprising any of the provided polynucleotides. In some of any of the provided embodiments, the vector is an expression vector. In some of any of the provided embodiments, the vector is a viral vector or a eukaryotic vector, optionally wherein the eukaryotic vector is a mammalian vector.

[0098] Provided herein is a cell comprising any of the provided polynucleotide or polynucleotides or any of the provided vector or vectors. In some embodiments, the cell is recombinant or isolated. In some embodiments, the cell is a mammalian cell.

[0099] Provided herein is a method of producing a polypeptide, the method comprising introducing into a cell any of the provided polynucleotide or polynucleotides or any of the provided vector or vectors of and culturing the cell under conditions to produce the multispecific polypeptide construct. In some of any of the provided embodiments, the method further comprises isolating or purifying the polypeptide from the cell.

[0100] Provided herein is a polypeptide produced by any of the provided methods.

[0101] Provided herein is an engineered immune cell, comprising a chimeric antigen receptor comprising an extracellular domain comprising any of the provided single domain antibodies, a

transmembrane domain; and an intracellular signaling domain. In some embodiments, the cell is a lymphocyte. In some of any of the provided embodiments, the cell is a T cell or a natural killer (NK) cell. In some of any of the provided embodiments, the intracellular signaling domain comprises an immunoreceptor tyrosine-based activation motif (ITAM) signaling domain. In some of any of the provided embodiments, the intracellular signaling domain is or comprises a CD3zeta signaling domain, optionally a human CD3zeta signaling domain. In some of any of the provided embodiments, the intracellular signaling domain further comprises a signaling domain of a costimulatory molecule. In some of any of the provided embodiments, the costimulatory molecule is CD28, ICOS, 41BB or OX40, optionally a human CD28, a human ICOS, a human 41BB or a human OX40.

[0102] Provided herein is a pharmaceutical composition comprising any of provided 5T4-binding polypeptides, multispecific polypeptide constructs, single domain antibodies or engineered immune cells. In some of any of the provided embodiments, the pharmaceutical composition includes a pharmaceutically acceptable carrier. In some embodiments, the pharmaceutical composition is sterile.

[0103] Provided herein is a method of stimulating or inducing an immune response in a subject, the method comprising administering, to a subject in need thereof, any of the provided 5T4-binding polypeptides, multispecific polypeptide constructs, single domain antibodies or engineered immune cells or a pharmaceutical compositions. In some embodiments, the immune response is increased against a tumor or cancer, optionally a tumor or a cancer that expresses 5T4. In some embodiments, the method treats a disease or condition in the subject.

[0104] Provided herein is a method of treating a disease or condition in a subject, the method comprising administering, to a subject in need thereof, a therapeutically effective amount of any of the provided 5T4-binding polypeptides, multispecific polypeptide constructs, single domain antibodies or engineered immune cells or a pharmaceutical compositions. In some of any of the provided embodiments, the disease or condition is a tumor or a cancer. In some of any of the provided embodiments, said subject is a human.

Brief Description of the Drawings

[0105] **FIGS. 1A-B** depict the ability of various single domain antibodies targeting 5T4 to bind cell surface 5T4. Binding was assessed by flow cytometry on HEK-293 freestyle cells endogenously expressing 5T4.

[0106] **FIGS. 2A-2F** depict the ability of sdAbs and humanized variants thereof targeting 5T4 to bind cell surface 5T4 on T47D cells. Binding was assessed by flow cytometry on the 5T4 positive cell line T47D. **FIGS. 2A-2C** show binding of 12E9 and humanized variants thereof on T47D. **FIGS. 2D-2E** show binding of 14B5 and humanized variants thereof on T47D. **FIG. 2F** shows binding of 16G10 and humanized variants thereof on T47D. Herein the 5T4 sdAbs were operably linked to a human Fc.

[0107] **FIGS. 3A-E** depict representative 5T4-targeted constrained CD3 engagers without (FIGS. 3A, 3C, 3E) and with a 41BB binding domain (FIGS. 3B, 3D). cx3546 and cx3547 have the same 5T4-targeting sdAb (12E9) positioned at the N-termini of one chain of the heterodimer and distinct 5T4-targeting sdAbs (14B5 or 16G10, respectively) positioned at the C-termini of one chain of the heterodimer. cx3499 and cx3497 are identical to cx3546 and cx3547, respectively, but have a 41BB-targeting sdAb positioned at the C-termini of the opposite chain of the heterodimer. Each of cx3546, cx3547, cx3499 and cx3497 contain a cleavable linker between the Fc region and the CD3 binding region. cx5185 and cx5951 are the same but the former includes a 4-1BB targeting sdAb positioned C-terminally to the CD3 binding region; both contain a non-cleavable linker between the Fc region and the CD3 binding region and contain humanized versions of the 5T4-targeting sdAbs, hz12E9v9 and hz16G10v11, positioned at the N- and C-termini of the Fc heterodimer. These representative 5T4-targeted constrained CD3 engagers display bivalent binding to 5T4, and in constructs containing a 41BB binding domain, display monovalent binding to 41BB.

[0108] **FIGS. 4A-4M** depict the binding of various 5T4-target constrained CD3 engaging constructs to the 5T4 positive Ovcar-5 cell line (top left) and the lack of binding to primary human T-cells (top right). The bottom panel shows a titration of the 5T4-target constrained CD3 engaging constructs to compare binding to Ovcar-5 and primary human T-cells. Binding was assessed by flow cytometry using an anti-human IgG APC secondary antibody. The histograms display the normalized cell counts vs fluorescence at 200 nM of each construct.

[0109] **FIGS. 5A-5F** depict the ability of 5T4-targeted constrained CD3 engaging constructs to elicit 5T4-dependent T-cell activation. A Jurkat CD3 NFAT-GFP reporter cell line was used to monitor T-cell activation. Ovcar-5 cells (**FIGS. 5A, 5C, 5E**) were used as antigen-positive cells and CCRF-CEM cells (**FIGS. 5B, 5D, 5F**) were used as antigen-negative cells.

[0110] **FIGS. 6A** and **6B** depict the ability of 5T4-targeted constrained CD3 engaging constructs to mediate antigen specific T-cell cytotoxicity on a 5T4 positive cell line, Ovcar-5 (**FIG. 6A**), and the lack of cytotoxicity directed toward a 5T4 negative cell line, CCRF-CEM (**FIG. 6B**).

[0111] **FIGS. 7A-7D** depict the ability of 5T4-targeted constrained CD3 engaging constructs to mediate antigen specific T-cell activation on CD4 T cells (**FIG. 7A-7B**) and CD8 T cells (**FIG. 7C-7D**) as assessed by flow cytometry by analyzing the activation marker CD25. A 5T4 positive cell line, Ovcar-5, or a 5T4 negative cell line, CCRF-CEM, was used to assess cell activation mediated by exemplary 5T4-targeted constrained CD3 engaging constructs.

[0112] **FIGS. 8A-8D** depict the ability of 5T4-targeted constrained CD3 engaging constructs to mediate antigen specific T-cell activation on CD4 T cells (**FIG. 8A-8B**) and CD8 T cells (**FIG. 8C-8D**) as assessed by flow cytometry by analyzing the activation marker CD69. A 5T4 positive cell line, Ovcar-5, or a 5T4 negative cell line, CCRF-CEM, was used to assess cell activation mediated by exemplary 5T4-targeted constrained CD3 engaging constructs.

[0113] **FIGS. 9A-9D** depict the ability of 5T4-targeted constrained CD3 engaging constructs to mediate antigen specific T-cell activation on CD4 T cells (**FIG. 9A-9B**) and CD8 T cells (**FIG. 9C-9D**) as assessed by flow cytometry by analyzing the activation marker CD71. A 5T4 positive cell line, Ovcar-5, or a 5T4 negative cell line, CCRF-CEM, was used to assess cell activation mediated by exemplary 5T4-targeted constrained CD3 engaging constructs.

[0114] **FIGS. 10A-10C** show the ability of 5T4-targeted constrained CD3 engaging constructs to elicit IFNgamma (**FIG. 10A** and **10B**) or TNFalpha (**FIG. 10C**) production from T cells in an antigen-dependent manner. Cytokine production was monitored using an ELISA method (**FIG. 10A**) or FluoroSpot assay (**FIG. 10B** and **10C**).

[0115] **FIG. 11A** shows the ability of 5T4 sdAbs, 12E9 and 4D3, formatted as sdAb-Fcs, to mediate NK cell activation as assessed by CD107a expression by flow cytometry. **FIGS. 11B** to **11D** depict results of a CD16 reporter activation assay, a surrogate for ADCC activity, using a Jurkat reporter cell line engineered to stably express CD16a with an NFAT-driven luciferase reporter gene. **FIG. 11B** depicts relative luminescence units (RLU) of the luciferase reporter in the presence or absence of 5T4+ cells and treated with 50 nM of the indicated sdAb-Fc or hzsdAb-Fc. **FIG. 11C** and **11D** depict RLU of the luciferase reporter in the presence (**FIG. 11C**) or absence (**FIG. 11D**) of 5T4+ cells and treated with a titration of the indicated hzsdAb-Fc.

[0116] **FIGS. 12A-12D** depict cellular binding by representative 5T4-targeting constrained CD3 engaging constructs, cx3497 (with a 41BB binding domain) and cx3547 (without a 41BB binding domain). **FIG. 12A** and **FIG. 12B** show binding to Ovcar-5 cells (a 5T4 positive melanoma cell line). **FIG. 12C** and **FIG. 12D** depict binding to T cells and show the lack of binding to T cells in isolation by the tested constructs. **FIG. 12A** and **FIG. 12C** display histograms of the normalized cell counts vs. fluorescence at 200 nM of each construct. The full titration of each construct on the various cell types are shown in **FIG. 12B** and **FIG. 12D**. Shown in **FIGS. 12A** and **12C**, the secondary anti-human APC antibody only control is shown in the filled black trace, while the positive control anti-CD3 binding is shown in the open trace, and cx3547 and cx3497 are shown in the gray shaded traces.

[0117] **FIGS. 13A** and **13B** depict the kinetics of T-cell cytotoxicity mediated by representative 5T4-targeting constrained CD3 engaging constructs, cx3497 (with a 41BB binding domain) and cx3547 (without a 41BB binding domain) toward a 5T4 positive cell line, Ovcar-5 (**FIG. 13A**), and a 5T4 negative cell line, CCRF-CEM (**FIG. 13B**). Total overlap area is representative of double positive: cleaved caspase-3/7 substrate in fluorescently labeled target cells. The initial cytotoxicity observed on the antigen negative cell line is likely mediated by MHC-mismatch between the target cells and the T-cells. Notably only, cx3497 containing a 41BB binding domain was capable of inducing cytotoxicity and the kinetics are consistent with that of 41BB upregulation following TCR-signaling.

[0118] **FIG. 14** shows a comparison of IFNγ production by T cells treated with representative 5T4-targeted constrained CD3 engaging constructs with a 41BB binding domain, cx3499 and cx3497, and

without a 41BB binding domain, cx3546 and cx3547 in the presence of 5T4-positive Ovcar-5 cells and 5T4-negative CCRF-CEM cells. Cytokine production was monitored by ELISA. cx3546 and cx3499 contain the same 5T4-targeting sdAbs, and cx3547 and cx3497 contain the same 5T4-targeting sdAbs. Thus, the only difference between these sets of constructs is the addition of the 41BB binding domain. 5T4-targeted constrained CD3 engaging constructs incorporating a 41BB binding domain display enhanced capacity to induce IFNγ production from T cells in an antigen-dependent manner.

[0119] **FIGS. 15A and 15B** depict the potency of T-cell-mediated cytotoxicity driven by exemplary 5T4-targeted constrained CD3 engaging constructs with a 41BB binding domain, cx5185, and without a 41BB binding domain, cx5951. A titration range of 200 nM to 3.1 pM of the CD3 engaging constructs on the 5T4-positive Ovcar5 cell line is shown in **FIG. 15A** and the 5T4-negative CCRF-CEM cell line shown in **FIG. 15B**.

[0120] **FIG. 16A** and **FIG. 16B** depict T cell activation as assessed by CD25 expression on CD4 (**FIG. 16A**) and CD8 (**FIG. 16B**) cells following incubation with representative 5T4-targeted constrained CD3 engaging constructs with a 41BB binding domain, cx5185, and without a 41BB binding domain, cx5951, in the presence of 5T4 positive cells, A375, Ovcar-5,and SHP-77. T-cell activation was assessed by flow cytometery monitoring cell surface expression of CD25.

[0121] **FIG. 17** shows a comparison of IFNγ production by T-cells treated with a titration of representative 5T4-targeted constrained CD3 engaging constructs with a 41BB binding domain, cx5185, and without a 41BB binding domain, cx5951, in the presence of 5T4 positive cell lines, A375, SHP-77, and Ovcar5.

[0122] **FIG. 18** depicts T cell proliferation on CD4 T cells following incubation with representative 5T4-targeted constrained CD3 engaging constructs with a 41BB binding domain, cx5185, and without a 41BB binding domain, cx5951, in the presence of 5T4 positive cell lines, A375, Ovcar-5, and SHP-77. T-cell proliferation was monitored via dilution of CellTrace™ dye. The results depict the percent of proliferating cells as monitored by dilution of CellTrace™ Violet dye.

[0123] **FIG. 19** depicts T cell proliferation on CD8 T cells following incubation with representative 5T4-targeted constrained CD3 engaging constructs with a 41BB binding domain, cx5185, and without a 41BB binding domain, cx5951, in the presence of 5T4 positive cell lines, A375, Ovcar-5, and SHP-77. T-cell proliferation was monitored via dilution of CellTrace™ dye. The results depict the percent of proliferating cells as monitored by dilution of CellTrace™ Violet dye.

[0124] **FIG. 20** depicts assessment of mitochondrial function in CD4 T-cells following incubation with representative 5T4-targeted constrained CD3 engaging constructs with a 41BB binding domain, cx5185, and without a 41BB binding domain, cx5951, in the presence of 5T4 positive cells, A375, Ovcar-5,and SHP-77. Mitochondrial function was assessed by flow cytometery using MitoTracker Green, a fluorescent mitochondria-selective probe that accumulates in active mitochondria.

[0125] **FIG. 21** depicts assessment of mitochondrial function in CD8 T-cells following incubation with representative 5T4-targeted constrained CD3 engaging constructs with a 41BB binding domain, cx5185, and without a 41BB binding domain, cx5951, in the presence of 5T4 positive cells, A375, Ovcar-5,and SHP-77. Mitochondrial function was assessed by flow cytometery using MitoTracker Green, a fluorescent mitochondria-selective probe that accumulates in active mitochondria.

[0126] **FIG. 22** shows the ability of the 5T4-targeted constrained CD3 engaging construct with a 41BB binding domain, cx5185, but not the same construct lacking a 41BB binding domain, cx5951, to mediate 41BB signaling. 41BB signaling was monitored using a Jurkat 41BB NFkB-luciferase reporter cell and recombinant plate bound 5T4 as the source of the antigen.

[0127] **FIG. 23A-B** depict exemplary TAA-targeted constrained CD3 engagers with a co-stimulatory receptor binding region (CRBR). The constructs have an antigen-targeting sdAb positioned at the N and C-termini of one chain of the heterodimer, the Fc knob, and have a co-stimulatory receptor binding region (CRBR) positioned at the C-termini of the opposite chain of the heterodimer, the Fc hole, but have the VH and VL of the CD3 binding Fv positioned on opposite sides with respect to each other.

[0128] **FIG. 24** depict results of T cell reporter assays for exemplary constructs described in **FIG. 23A-B**. **FIGS. 24A** and **24B** depict mean fluorescence intensity (MFI) of the GFP reporter when the TAA-positive cell line A375 or the TAA-negative cell line CCRF-CEM, respectively, was co-cultured with Jurkat CD3 NFAT-GFP reporter cells. **FIGS. 24C** and **24D** depict relative luminescent units (RLU) of the luciferase reporter when the TAA-positive cell line A375 or the TAA-negative cell line CCRF-CEM, respectively, were co-cultured with Jurkat CD3 NFAT-Luciferase reporter cells.

[0129] **FIG. 25** is a schematic of various hybrid mouse/human 5T4 constructs used for epitope mapping. Regions of the human 5T4 extracellular domain (ECD) were grafted in place of the corresponding regions of the mouse 5T4 full length construct. The sdAbs are able to bind human 5T4 but not mouse 5T4. Binding of a sdAb to a hybrid mouse/human 5T4 indicated that the epitope bound by the sdAb was in the grafted region.

[0130] **FIG. 26A** depicts the binding of 12E9v9 to the various hybrid mouse/human 5T4 constructs, demonstrating the epitope recognized by this sdAb likely resides between amino acid residues 60 and 112 (SEQ ID NO:411). **FIG. 26B** depicts the binding of 16G10v11 to the various hybrid mouse/human 5T4 constructs, demonstrating the eptitope recoginized by this sdAb likely resides between amino acid residues 173 and 224 (SEQ ID NO:412). **FIG. 26C** depicts the binding of 14B5v17 to the various hybrid mouse/human 5T4 constructs, demonstrating the epitope recognized by this sdAb likely resides between amino acid residues 173 and 224(SEQ ID NO:412). The Hu and Mu denote the full length human and mouse 5T4 extracellular domain constructs, respectively.

[0131] **FIGS. 27A-27B** depict the ability of 5T4-targeted constrained CD3 engaging constructs to elicit 5T4-dependent T-cell activation. A Jurkat CD3 NFAT-GFP reporter cell line was used to monitor

T-cell activation. SKOV-3 (**FIG. 27A**) cells were used as antigen-positive cells and CCRF-CEM (**FIG. 27B**) were used as antigen-negative cells.

Detailed Description

[0132] Provided herein are polypeptides that specifically bind to 5T4, hereinafter also called 5T4-binding polypeptides. In some embodiments, the provided binding polypeptides comprise at least one VHH domain that binds 5T4. In some embodiments, a 5T4-binding polypeptide provided herein comprises one, two, three, four, five, six, seven, or eight VHH domains that each individually binds 5T4. In some embodiments, a 5T4-binding polypeptide provided herein comprises one, two, three, or four VHH domains that bind 5T4. In some embodiments, the 5T4-binding polypeptides are monospecific. In some embodiments, the 5T4-binding polypeptides are multispecific. For example, provided 5T4-binding polypeptides include polypeptides that may comprise at least one VHH domain that binds 5T4 and one or more additional binding domains, such as one or more additional VHH domains, that bind one or more target proteins other than 5T4.

[0133] In some embodiments, a 5T4-binding polypeptide comprises at least one VHH domain that binds 5T4 and an Fc domain. In some embodiments, a 5T4-binding polypeptide provided herein comprises one, two, three, or four VHH domains that bind 5T4 and an Fc domain. In some embodiments, an Fc domain mediates dimerization of the 5T4-binding polypeptide at physiological conditions such that a dimer is formed that doubles the number of 5T4 binding sites. For example, a 5T4-binding polypeptide comprising three VHH domains that bind 5T4 and an Fc region is trivalent as a monomer, but at physiological conditions, the Fc region may mediate dimerization, such that the 5T4-binding polypeptide exists as a hexavalent dimer under such conditions.

[0134] 5T4 oncofetal antigen (also known as trophoblast glycoprotein, TPBG; 5T4 oncofetal trophoblast glycoprotein; and Wnt-activated inhibitory factor 1, WAIF1) is a 72 kDa glycoprotein identified by a murine monoclonal antibody produced by a hybridoma from splenocytes of mice immunized with syncytiotrophoblast microvillous membrane glycoproteins. 5T4 is expressed on the surface of a wide variety of tumor cells and tumor vasculature including, but not limited to, renal cell carcinoma, head and heck squamous cell carcinoma, colorectal carcinoma, ovarian carcinoma and gastric carcinoma. Transduction of the 5T4 cDNA into cell lines enhances cell motility and reduces cell–cell contacts suggesting that it may be mechanistically involved in the malignant phenotype (Carsberg et al., 1996, Int J Cancer. 68 1:84–92). In addition, upregulation of 5T4 expression is a marker of loss of pluripotency in the early differentiation of embryonic stem (ES) cells and forms an integrated component of an epithelial-mesenchymal transition, a process important during both embryonic development and metastatic spread of epithelial tumors (Southgate et al., 2010, PLoS One. 5(4):e9982). 5T4 expression is very limited in normal tissue but is widespread in transformed cell lines as well as malignant tumors throughout their development (Hole & Stern, 1988, Br. J. Cancer, 57, 239-246; Southall et al., 1990, Br.

J. Cancer, 61, 89-95.; Jones et al., 1990, Br. J. Cancer, 61, 96-100.; Starzynska et al. 1992, Br. J. Cancer 66: 867-869). In addition, a high level of 5T4 expression on tumor tissue correlated with advanced tumor stage and poorer survival in colorectal carcinoma (Starzynska et al., 1992, Br. J. Cancer 66: 867-869), gastric carcinoma (Starzynska et al., 1998, Eur J Gastroenterol Hepatol. 10(6):479-84) , ovarian carcinoma (Wrigley et al., 1995, Int J Gynecol Cancer 5(4):269-274), head and neck squamous cell carcinoma (Kerk et al., 2017, Clin Cancer Res. 23(10): 2516–2527.) and acute lymphoblastic leukemia (McGinn et al., 2017, Haematologica, 102: 1075-1084).

[0135] An exemplary sequence of human 5T4 is set forth as follows:

MPGGCSRGPAAGDGRLRLARLALVLLGWVSSSSPTSSASSFSSSAPFLAS
AVSAQPPLPDQCPALCECSEAARTVKCVNRNLTEVPTDLPAYVRNLFLTG
NQLAVLPAGAFARRPPLAELAALNLSGSRLDEVRAGAFEHLPSLRQLDLS
HNPLADLSPFAFSGSNASVSAPSPLVELILNHIVPPEDERQNRSFEGMVV
AALLAGRALQGLRRLELASNHFLYLPRDVLAQLPSLRHLDLSNNSLVSLT
YVSFRNLTHLESLHLEDNALKVLHNGTLAELQGLPHIRVFLDNNPWVCDC
HMADMVTWLKETEVVQGKDRLTCAYPEKMRNRVLLELNSADLDCDPILPP
SLQTSYVFLGIVLALIGAIFLLVLYLNRKGIKKWMHNIRDACRDHMEGYH
YRYEINADPRLTNLSSNSDV (SEQ ID NO:244, signal sequence underlined)

[0136] In some embodiments, a provided 5T4 binding polypeptides bind to the extracellular domain of human 5T4, such as to a region or epitope within the sequence of amino acids set forth in SEQ ID NO:382. In some aspects, a provided 5T4 binding polypeptide binds to a contiguous sequence of amino acids in the extracellular domain of human 5T4 set forth in SEQ ID NO:382. In some embodiments, the epitope is a linear epitope.

[0137] In some embodiments, provided are 5T4 binding polypeptides that contain at least a first 5T4 VHH domain sequence and a second 5T4 VHH domain sequence in which both bind to a region or epitope within the sequence of amino acids set forth in SEQ ID NO:382. In some aspects, each of the first 5T4 VHH domain sequence and a second 5T4 VHH domain sequence binds to a contiguous sequence of amino acids in the extracellular domain of human 5T4 set forth in SEQ ID NO:382. In some embodiments, the epitope is a linear epitope. In some embodiments, the first 5T4 VHH domain sequence and the second 5T4 VHH domain sequence bind to the same or an overlapping epitope in the 5T4 extracellular domain. In some embodiments, the first 5T4 VHH domain sequence and the second 5T4 VHH domain sequence bind to different epitopes in the 5T4 extracellular domain. In some embodiments, the first 5T4 VHH domain binds to an epitope located within amino acids 60-112, inclusive, of the human 5T4 extracellular domain (given by SEQ ID NO:411) and the second 5T4 VHH domain binds to an epitope located within amino acids 173-224, inclusive, of the human 5T4 extracellular domain (given by SEQ ID NO:412). In some embodiments, the first 5T4 VHH domain comprises the amino acid sequence set forth in SEQ ID NO:245 or a humanized variant thereof, and the second 5T4 VHH domain comprises the amino acid sequence set forth in SEQ ID NO:276 or a humanized variant thereof. In some embodiments, the first 5T4 VHH domain comprises the amino acid sequence set forth in SEQ ID

NO:360 and the second 5T4 VHH domain comprises the amino acid sequence set forth in SEQ ID NO:287.

[0138] In some embodiments, a 5T4 VHH does not cross react with the mouse 5T4 antigen. In some embodiments, a 5T4 VHH comprising the amino acid sequence set forth in SEQ ID NO:245, or humanized variants thereof, do not cross react with the mouse 5T4 antigen. In some embodiments, a 5T4 VHH comprising the amino acid sequence set forth in SEQ ID NO:255, or humanized variants thereof, do not cross react with the mouse 5T4 antigen. In some embodiments, a 5T4 VHH comprising the amino acid sequence set forth in SEQ ID NO:276, or humanized variants thereof, do not cross react with the mouse 5T4 antigen.

[0139] In some embodiments, provided are multispecific polypeptide constructs that contain at least a first 5T4 VHH domain and a second 5T4 VHH domain. In some embodiments, provided are multispecific polypeptide constructs that contain a first 5T4 VHH domain comprising the amino acid sequence set forth in SEQ ID NO:360, and a second 5T4 VHH domain comprising the amino acid sequence set forth in SEQ ID NO:360. In some embodiments, provided are multispecific polypeptide constructs that contain a first 5T4 VHH domain comprising the amino acid sequence set forth in SEQ ID NO:287 and a second 5T4 VHH domain comprising the amino acid sequence set forth in SEQ ID NO:287. In some embodiments, provided are multispecific polypeptide constructs that contain at least a first 5T4 VHH domain comprising the amino acid sequence set forth in SEQ ID NO:360, or a humanized variant thereof, and a second 5T4 VHH domain comprising the amino acid sequence set forth in SEQ ID NO:287, or a humanized variant thereof.

[0140] In some cases, the provided 5T4 binding polypeptides directly block or inhibit activity of 5T4, which, in some aspects, can be used as a therapeutic to inhibit or reduce tumor cell growth or survival.

[0141] A variety of 5T4 polypeptide binding formats are provided. In some examples, 5T4 binding polypeptides include 5T4 VHH-Fc polypeptides. In some embodiments, the Fc is an Fc that exhibits immune effector activity, such as one or more effector functions such as antibody-dependent cellular cytotoxicity (ADCC), antibody-dependent cellular phagocytosis (ADCP) and/or complement-dependent cytotoxicity (CDC).

[0142] In some embodiments, the provided 5T4-binding polypeptides can be used to stimulate an immune response in a subject, which, in some aspects, treats a disease or disorder, such as a cancer, in the subject. In some aspects, a 5T4-binding polypeptide provided herein, such as a 5T4-Fc, can bind to 5T4-expressing tumor cells and induce an active immune response against the tumor cells expressing 5T4. In some cases, the active immune response can cause the death of the cancerous cells (e.g., antibody binding to cancer cells inducing apoptotic cell death), or inhibit the growth (e.g., block cells cycle progression) of the cancerous cells. In other cases, a 5T4-binding polypeptide provided herein, such as a 5T4 VHH- Fc, can bind to cancerous cells and antibody dependent cellular cytotoxicity (ADCC) can

eliminate cancerous cells to which the 5T4-binding polypeptide binds. In some cases, provided 5T4 VHH-binding polypeptides can also activate both cellular and humoral immune responses and recruit more natural killer cells or increased production of cytokines (e.g., IL-2, IFN-gamma, IL-12, TNF-alpha, TNF-beta, etc.) that further activate an individual's immune system to destroy cancerous cells. In yet another embodiment, 5T4 binding polypeptides, such as 5T4 VHH-Fc, can bind to cancerous cells, and macrophages or other phagocytic cell can opsonize the cancerous cells, such as via CDC or ADCP processes.

[0143] In other aspects, also provided herein are VHH-binding polypeptides that exhibit multispecific binding. In some cases, the binding polypeptides include polypeptides that exhibit dual affinity for 5T4 and a T cell antigen, such as CD3. In some aspects, such dual affinity molecules are capable of engaging or activating T cells at the site of a tumor upon binding of tumor-expressed 5T4. In particular, among such molecules provided herein are molecules that exhibit constrained CD3 binding. Also provided herein are engineered cells, such as engineered T cells, that express a chimeric antigen receptor containing a 5T4 binding polypeptide.

[0144] All publications, including patent documents, scientific articles and databases, referred to in this application are incorporated by reference in their entirety for all purposes to the same extent as if each individual publication were individually incorporated by reference. If a definition set forth herein is contrary to or otherwise inconsistent with a definition set forth in the patents, applications, published applications and other publications that are herein incorporated by reference, the definition set forth herein prevails over the definition that is incorporated herein by reference.

[0145] The techniques and procedures described or referenced herein are generally well understood and commonly employed using conventional methodology by those skilled in the art, such as, for example, the widely utilized methodologies described in Sambrook *et al.*, Molecular Cloning: A Laboratory Manual 3rd. edition (2001) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. CURRENT PROTOCOLS IN MOLECULAR BIOLOGY (F. M. Ausubel, *et al. eds.*, (2003)); the series METHODS IN ENZYMOLOGY (Academic Press, Inc.): PCR 2: A PRACTICAL APPROACH (M. J. MacPherson, B. D. Hames and G. R. Taylor *eds.* (1995)), Harlow and Lane, *eds.* (1988) ANTIBODIES, A LABORATORY MANUAL, and ANIMAL CELL CULTURE (R. I. Freshney, *ed.* (1987)); Oligonucleotide Synthesis (M. J. Gait, ed., 1984); Methods in Molecular Biology, Humana Press; Cell Biology: A Laboratory Notebook (J. E. Cellis, *ed.*, 1998) Academic Press; Animal Cell Culture (R. I. Freshney), *ed.*, 1987); Introduction to Cell and Tissue Culture (J. P. Mather and P. E. Roberts, 1998) Plenum Press; Cell and Tissue Culture Laboratory Procedures (A. Doyle, J. B. Griffiths, and D. G. Newell, eds., 1993-8) J. Wiley and Sons; Handbook of Experimental Immunology (D. M. Weir and C. C. Blackwell, *eds.*); Gene Transfer Vectors for Mammalian Cells (J. M. Miller and M. P. Calos, *eds.*, 1987); PCR: The Polymerase Chain Reaction, (Mullis *et al.*, *eds.*, 1994); Current Protocols in Immunology (J. E. Coligan *et al.*, *eds.*, 1991); Short Protocols in Molecular Biology (Wiley and Sons, 1999);

Immunobiology (C. A. Janeway and P. Travers, 1997); Antibodies (P. Finch, 1997); Antibodies: A Practical Approach (D. Catty., *ed.*, IRL Press, 1988-1989); Monoclonal Antibodies: A Practical Approach (P. Shepherd and C. Dean, *eds.*, Oxford University Press, 2000); Using Antibodies: A Laboratory Manual (E. Harlow and D. Lane (Cold Spring Harbor Laboratory Press, 1999); The Antibodies (M. Zanetti and J. D. Capra, *eds.*, Harwood Academic Publishers, 1995); and Cancer: Principles and Practice of Oncology (V. T. DeVita *et al.*, *eds.*, J.B. Lippincott Company, 1993); and updated versions thereof.

[0146] The section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described.

I. DEFINITIONS

[0147] Unless otherwise defined, scientific and technical terms used in connection with the present disclosure shall have the meanings that are commonly understood by those of ordinary skill in the art. Further, unless otherwise required by context or expressly indicated, singular terms shall include pluralities and plural terms shall include the singular. For any conflict in definitions between various sources or references, the definition provided herein will control.

[0148] It is understood that embodiments of the invention described herein include "consisting" and/or "consisting essentially of" embodiments. As used herein, the singular form "a", "an", and "the" includes plural references unless indicated otherwise. Use of the term "or" herein is not meant to imply that alternatives are mutually exclusive.

[0149] In this application, the use of "or" means "and/or" unless expressly stated or understood by one skilled in the art. In the context of a multiple dependent claim, the use of "or" refers back to more than one preceding independent or dependent claim.

[0150] The term "about" as used herein refers to the usual error range for the respective value readily known to the skilled person in this technical field. Reference to "about" a value or parameter herein includes (and describes) embodiments that are directed to that value or parameter *per se*. For example, description referring to "about X" includes description of "X".

[0151] The terms "nucleic acid molecule", "nucleic acid" and "polynucleotide" may be used interchangeably, and refer to a polymer of nucleotides. Such polymers of nucleotides may contain natural and/or non-natural nucleotides, and include, but are not limited to, DNA, RNA, and PNA. "Nucleic acid sequence" refers to the linear sequence of nucleotides comprised in the nucleic acid molecule or polynucleotide.

[0152] The term "isolated polynucleotide" as used herein shall mean a polynucleotide of genomic, cDNA, or synthetic origin or some combination thereof, which by virtue of its origin (1) is not associated with all or a portion of a polynucleotide found in nature, (2) is operably linked to a polynucleotide that it is not linked to in nature, or (3) does not occur in nature as part of a larger sequence.

[0153] The terms "polypeptide" and "protein" are used interchangeably to refer to a polymer of amino acid residues, and are not limited to a minimum length. Such polymers of amino acid residues may contain natural or non-natural amino acid residues, and include, but are not limited to, peptides, oligopeptides, dimers, trimers, and multimers of amino acid residues. Both full-length proteins and fragments thereof are encompassed by the definition. The terms also include post-expression modifications of the polypeptide, for example, glycosylation, sialylation, acetylation, phosphorylation, and the like. Furthermore, for purposes of the present disclosure, a "polypeptide" refers to a protein which includes modifications, such as deletions, additions, and substitutions (generally conservative in nature), to the native sequence, as long as the protein maintains the desired activity. These modifications may be deliberate, as through site-directed mutagenesis, or may be accidental, such as through mutations of hosts which produce the proteins or errors due to PCR amplification.

[0154] The term "isolated protein" referred to herein means that a subject protein (1) is free of at least some other proteins with which it would typically be found in nature, (2) is essentially free of other proteins from the same source, *e.g.*, from the same species, (3) is expressed by a cell from a different species, (4) has been separated from at least about 50 percent of polynucleotides, lipids, carbohydrates, or other materials with which it is associated in nature, (5) is not associated (by covalent or noncovalent interaction) with portions of a protein with which the "isolated protein" is associated in nature, (6) is operably associated (by covalent or noncovalent interaction) with a polypeptide with which it is not associated in nature, or (7) does not occur in nature. Such an isolated protein can be encoded by genomic DNA, cDNA, mRNA or other RNA, of may be of synthetic origin, or any combination thereof. In certain embodiments, the isolated protein is substantially pure or substantially free from proteins or polypeptides or other contaminants that are found in its natural environment that would interfere with its use (therapeutic, diagnostic, prophylactic, research or otherwise).

[0155] As used herein, "substantially pure" means an object species is the predominant species present (*i.e.,* on a molar basis it is more abundant than any other individual species in the composition), and a substantially purified fraction is a composition wherein the object species comprises at least about 50 percent (on a molar basis) of all macromolecular species present. Generally, a substantially pure composition will comprise more than about 80 percent of all macromolecular species present in the composition, for example, in some embodiments, more than about 85%, 90%, 95%, and 99%. In some embodiments, the object species is purified to essential homogeneity (contaminant species cannot be detected in the composition by conventional detection methods) wherein the composition consists essentially of a single macromolecular species.

[0156] The term "operably linked" as used herein refers to positions of components so described are in a relationship permitting them to function in their intended manner. A control sequence "operably linked" to a coding sequence is ligated in such a way that expression of the coding sequence is achieved under conditions compatible with the control sequences.

[0157] The term "specifically binds" to an antigen or epitope is a term that is well understood in the art, and methods to determine such specific binding are also well known in the art. A molecule is said to exhibit "specific binding" or "preferential binding" if it reacts or associates more frequently, more rapidly, with greater duration and/or with greater affinity with a particular cell or substance than it does with alternative cells or substances. A single-domain antibody (sdAb) or VHH-containing polypeptide "specifically binds" or "preferentially binds" to a target if it binds with greater affinity, avidity, more readily, and/or with greater duration than it binds to other substances. For example, a sdAb or VHH-containing polypeptide that specifically or preferentially binds to a 5T4 epitope is a sdAb or VHH-containing polypeptide that binds this epitope with greater affinity, avidity, more readily, and/or with greater duration than it binds to other 5T4 epitopes or non-5T4 epitopes. It is also understood by reading this definition that; for example, a sdAb or VHH-containing polypeptide that specifically or preferentially binds to a first target may or may not specifically or preferentially bind to a second target. As such, "specific binding" or "preferential binding" does not necessarily require (although it can include) exclusive binding. Generally, but not necessarily, reference to binding means preferential binding. "Specificity" refers to the ability of a binding protein to selectively bind an antigen.

[0158] As used herein, the term "epitope" refers to a site on a target molecule (for example, an antigen, such as a protein, nucleic acid, carbohydrate or lipid) to which an antigen-binding molecule (for example, a sdAb or VHH-containing polypeptide) binds. Epitopes often include a chemically active surface grouping of molecules such as amino acids, polypeptides or sugar side chains and have specific three-dimensional structural characteristics as well as specific charge characteristics. Epitopes can be formed both from contiguous and/or juxtaposed noncontiguous residues (for example, amino acids, nucleotides, sugars, lipid moiety) of the target molecule. Epitopes formed from contiguous residues (for example, amino acids, nucleotides, sugars, lipid moiety) typically are retained on exposure to denaturing solvents whereas epitopes formed by tertiary folding typically are lost on treatment with denaturing solvents. An epitope may include but is not limited to at least 3, at least 5 or 8-10 residues (for example, amino acids or nucleotides). In some embodiments, an epitope is less than 20 residues (for example, amino acids or nucleotides) in length, less than 15 residues or less than 12 residues. Two antibodies may bind the same epitope within an antigen if they exhibit competitive binding for the antigen. In some embodiments, an epitope can be identified by a certain minimal distance to a CDR residue on the antigen-binding molecule. In some embodiments, an epitope can be identified by the above distance, and further limited to those residues involved in a bond (for example, a hydrogen bond) between a residue of the antigen-binding molecule and an antigen residue. An epitope can be identified by various scans as well, for example an alanine or arginine scan can indicate one or more residues that the antigen-binding molecule can interact with. Unless explicitly denoted, a set of residues as an epitope does not exclude other residues from being part of the epitope for a particular antigen-binding molecule. Rather, the presence of such a set designates a minimal series (or set of species) of epitopes. Thus, in some

embodiments, a set of residues identified as an epitope designates a minimal epitope of relevance for the antigen, rather than an exclusive list of residues for an epitope on an antigen.

[0159] A "nonlinear epitope" or "conformational epitope" comprises noncontiguous polypeptides, amino acids and/or sugars within the antigenic protein to which an antigen-binding molecule specific to the epitope binds. In some embodiments, at least one of the residues will be noncontiguous with the other noted residues of the epitope; however, one or more of the residues can also be contiguous with the other residues.

[0160] A "linear epitope" comprises contiguous polypeptides, amino acids and/or sugars within the antigenic protein to which an antigen-binding molecule specific to the epitope binds. It is noted that, in some embodiments, not every one of the residues within the linear epitope need be directly bound (or involved in a bond) by the antigen-binding molecule. In some embodiments, linear epitopes can be from immunizations with a peptide that effectively consisted of the sequence of the linear epitope, or from structural sections of a protein that are relatively isolated from the remainder of the protein (such that the antigen-binding molecule can interact, at least primarily), just with that sequence section.

[0161] The terms "antibody" and "antigen-binding molecule" are used interchangeably in the broadest sense and encompass various polypeptides that comprise antibody-like antigen-binding domains, including but not limited to conventional antibodies (typically comprising at least one heavy chain and at least one light chain), single-domain antibodies (sdAbs, comprising just one chain, which is typically similar to a heavy chain), VHH-containing polypeptides (polypeptides comprising at least one heavy chain only antibody variable domain, or VHH), and fragments of any of the foregoing so long as they exhibit the desired antigen-binding activity. In some embodiments, an antibody comprises a dimerization domain. Such dimerization domains include, but are not limited to, heavy chain constant domains (comprising CH1, hinge, CH2, and CH3, where CH1 typically pairs with a light chain constant domain, CL, while the hinge mediates dimerization) and Fc domains (comprising hinge, CH2, and CH3, where the hinge mediates dimerization).

[0162] The term antibody also includes, but is not limited to, chimeric antibodies, humanized antibodies, and antibodies of various species such as camelid (including llama), shark, mouse, human, cynomolgus monkey, *etc.*

[0163] The term "variable region" or "variable domain" refers to the domain of an antibody heavy or light chain that is involved in binding the antibody to antigen. The variable regions of the heavy chain and light chain (V_H and V_L, respectively) of a native antibody generally have similar structures, with each domain comprising four conserved framework regions (FRs) and three CDRs. (See, *e.g.*, Kindt *et al.* Kuby Immunology, 6th ed., W.H. Freeman and Co., page 91 (2007). A single V_H or V_L domain may be sufficient to confer antigen-binding specificity, e.g. a single domain antibody, such as a VHH. Furthermore, antibodies that bind a particular antigen may be isolated using a V_H or V_L domain from an

antibody that binds the antigen to screen a library of complementary V_L or V_H domains, respectively. See, *e.g.*, Portolano *et al.*, J. Immunol. 150:880-887 (1993); Clarkson *et al.*, Nature 352:624-628 (1991).

[0164] An "antibody fragment" or "antigen-binding fragment" refers to a molecule other than a conventional or intact antibody that comprises a portion of an conventional or intact antibody containing at least a variable region that binds an antigen. Examples of antibody fragments include but are not limited to Fv, single chain Fvs (sdFvs), Fab, Fab', Fab'-SH, $F(ab')_2$; diabodies; linear antibodies; an single-domain antibodies comprising only the V_H region (VHH).

[0165] As used herein, "monovalent" with reference to a binding molecule refers to binding molecules that have a single antigen recognition site that is specific for a target antigen. Examples of monovalent binding molecules include, for example, a monovalent antibody fragment, a proteinaceous binding molecule with antibody-like binding properties or an MHC molecule. Examples of monovalent antibody fragments include, but are not limited to, a Fab fragment, an Fv fragment, and a single- chain Fv fragment (scFv).

[0166] The terms "single domain antibody", "sdAb," "VHH" are used interchangeably herein to refer to an antibody having a single monomeric domain antigen binding/recognition domain. Such antibodies include a camelid antibody or shark antibody. In some embodiments, a VHH comprises three CDRs and four framework regions, designated FR1, CDR1, FR2, CDR2, FR3, CDR3, and FR4. In some embodiments, a VHH may be truncated at the N-terminus or C-terminus such that it comprise only a partial FR1 and/or FR4, or lacks one or both of those framework regions, so long as the VHH substantially maintains antigen binding and specificity.

[0167] The term "VHH-containing polypeptide" refers to a polypeptide that comprises at least one VHH domain. In some embodiments, a VHH polypeptide comprises two, three, or four or more VHH domains, wherein each VHH domain may be the same or different. In some embodiments, a VHH-containing polypeptide comprises an Fc domain. In some such embodiments, the VHH polypeptide may form a dimer. Nonlimiting structures of VHH-containing polypeptides include VHH_1-Fc, VHH_1-VHH_2-Fc, and VHH_1-VHH_2-VHH_3-Fc, wherein VHH_1, VHH_2, and VHH_3 may be the same or different. In some embodiments of such structures, one VHH may be connected to another VHH by a linker, or one VHH may be connected to the Fc by a linker. In some such embodiments, the linker comprises 1-20 amino acids, preferably 1-20 amino acids predominantly composed of glycine and, optionally, serine. In some embodiments, when a VHH-containing polypeptide comprises an Fc, it forms a dimer. Thus, the structure VHH_1-VHH_2-Fc, if it forms a dimer, is considered to be tetravalent (i.e., the dimer has four VHH domains). Similarly, the structure VHH_1-VHH_2-VHH_3-Fc, if it forms a dimer, is considered to be hexavalent (i.e., the dimer has six VHH domains).

[0168] As used herein, a 5T4-binding polypeptide is a polypeptide or protein that specifically binds 5T4. Typically, a 5T4-binding polypeptide herein is a VHH-containing polypeptide containing at least one VHH domain that binds 5T4. A 5T4-binding polypeptide includes conjugates, including fusion

proteins. A 5T4-binding polypeptide includes fusion proteins, including those containing an Fc domain. In some embodiments, a 5T4-binding polypeptide contains two, three, or four or more VHH domains that each specifically bind to 5T4, wherein each VHH domain may be the same or different. In some embodiments, a 5T4-binidng polypeptide is multivalent. In some embodiments, a 5T4-binding polypeptide is multispecific. In some cases, a 5T4-binding polypeptide may contain one or more additional domains that bind to one or more further or additional antigens other than 5T4.

[0169] The term "monoclonal antibody" refers to an antibody (including an sdAb or VHH-containing polypeptide) of a substantially homogeneous population of antibodies, that is, the individual antibodies comprising the population are identical except for possible naturally-occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigenic site. Furthermore, in contrast to polyclonal antibody preparations, which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen. Thus, a sample of monoclonal antibodies can bind to the same epitope on the antigen. The modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies may be made by the hybridoma method first described by Kohler and Milstein, 1975, Nature 256:495, or may be made by recombinant DNA methods such as described in U.S. Pat. No. 4,816,567. The monoclonal antibodies may also be isolated from phage libraries generated using the techniques described in McCafferty *et al.*, 1990, Nature 348:552-554, for example.

[0170] The term "CDR" denotes a complementarity determining region as defined by at least one manner of identification to one of skill in the art. The precise amino acid sequence boundaries of a given CDR or FR can be readily determined using any of a number of well-known schemes, including those described by Kabat *et al.* (1991), "Sequences of Proteins of Immunological Interest," 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD ("Kabat" numbering scheme); Al-Lazikani *et al.*, (1997) JMB 273,927-948 ("Chothia" numbering scheme); MacCallum *et al.*, J. Mol. Biol. 262:732-745 (1996), "Antibody-antigen interactions: Contact analysis and binding site topography," J. Mol. Biol. 262, 732-745." ("Contact" numbering scheme); Lefranc MP *et al.*, "IMGT unique numbering for immunoglobulin and T cell receptor variable domains and Ig superfamily V-like domains," Dev Comp Immunol, 2003 Jan;27(1):55-77 ("IMGT" numbering scheme); Honegger A and Plückthun A, "Yet another numbering scheme for immunoglobulin variable domains: an automatic modeling and analysis tool," J Mol Biol, 2001 Jun 8;309(3):657-70, ("Aho" numbering scheme); and Martin *et al.*, "Modeling antibody hypervariable loops: a combined algorithm," PNAS, 1989, 86(23):9268-9272, ("AbM" numbering scheme).

[0171] The boundaries of a given CDR or FR may vary depending on the scheme used for identification. For example, the Kabat scheme is based on structural alignments, while the Chothia

scheme is based on structural information. Numbering for both the Kabat and Chothia schemes is based upon the most common antibody region sequence lengths, with insertions accommodated by insertion letters, for example, "30a," and deletions appearing in some antibodies. The two schemes place certain insertions and deletions ("indels") at different positions, resulting in differential numbering. The Contact scheme is based on analysis of complex crystal structures and is similar in many respects to the Chothia numbering scheme. The AbM scheme is a compromise between Kabat and Chothia definitions based on that used by Oxford Molecular's AbM antibody modeling software.

[0172] In some embodiments, CDRs can be defined in accordance with any of the Chothia numbering schemes, the Kabat numbering scheme, a combination of Kabat and Chothia, the AbM definition, and/or the contact definition. A VHH comprises three CDRs, designated CDR1, CDR2, and CDR3. Table 1, below, lists exemplary position boundaries of CDR-H1, CDR-H2, CDR-H3 as identified by Kabat, Chothia, AbM, and Contact schemes, respectively. For CDR-H1, residue numbering is listed using both the Kabat and Chothia numbering schemes. FRs are located between CDRs, for example, with FR-H1 located before CDR-H1, FR-H2 located between CDR-H1 and CDR-H2, FR-H3 located between CDR-H2 and CDR-H3 and so forth. It is noted that because the shown Kabat numbering scheme places insertions at H35A and H35B, the end of the Chothia CDR-H1 loop when numbered using the shown Kabat numbering convention varies between H32 and H34, depending on the length of the loop.

Table 1. Boundaries of CDRs according to various numbering schemes.

CDR	Kabat	Chothia	AbM	Contact
CDR-H1 (Kabat Numbering[1])	H31--H35B	H26--H32..34	H26--H35B	H30--H35B
CDR-H1 (Chothia Numbering[2])	H31--H35	H26--H32	H26--H35	H30--H35
CDR-H2	H50--H65	H52--H56	H50--H58	H47--H58
CDR-H3	H95--H102	H95--H102	H95--H102	H93--H101

1 - Kabat *et al.* (1991), "Sequences of Proteins of Immunological Interest," 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD
2 - Al-Lazikani *et al.*, (1997) JMB 273,927-948

[0173] Thus, unless otherwise specified, a "CDR" or "complementary determining region," or individual specified CDRs (*e.g.*, CDR-H1, CDR-H2, CDR-H3), of a given antibody or region thereof, such as a variable region thereof, should be understood to encompass a (or the specific) complementary determining region as defined by any of the aforementioned schemes. For example, where it is stated that a particular CDR (*e.g.*, a CDR-H3) contains the amino acid sequence of a corresponding CDR in a given VHH amino acid sequence, it is understood that such a CDR has a sequence of the corresponding CDR (*e.g.*, CDR-H3) within the VHH, as defined by any of the aforementioned schemes. In some embodiments, specific CDR sequences are specified. Exemplary CDR sequences of provided antibodies are described using various numbering schemes (see *e.g.* Table 1), although it is understood that a

provided antibody can include CDRs as described according to any of the other aforementioned numbering schemes or other numbering schemes known to a skilled artisan.

[0174] As used herein, "conjugate," "conjugation" or grammatical variations thereof refers the joining or linking together of two or more compounds resulting in the formation of another compound, by any joining or linking methods known in the art. It can also refer to a compound which is generated by the joining or linking together two or more compounds. For example, a VHH domain linked directly or indirectly to one or more chemical moieties or polypeptide is an exemplary conjugate. Such conjugates include fusion proteins, those produced by chemical conjugates and those produced by any other methods.

[0175] An immunoglobulin Fc fusion ("Fc-fusion"), such as VHH-Fc, is a molecule comprising one or more VHH domains operably linked to an Fc region of an immunoglobulin. An immunoglobulin Fc region may be linked indirectly or directly to one or more VHH domains. Various linkers are known in the art and can optionally be used to link an Fc to a fusion partner to generate an Fc-fusion. In some such embodiments, the linker comprises 1-20 amino acids, preferably 1-20 amino acids predominantly composed of glycine and, optionally, serine. Fc-fusions of identical species can be dimerized to form Fc-fusion homodimers, or using non-identical species to form Fc-fusion heterodimers. In some embodiments, the Fc is a mammalian Fc such as human Fc.

[0176] The term "heavy chain constant region" as used herein refers to a region comprising at least three heavy chain constant domains, C_H1, hinge, C_H2, and C_H3. Of course, non-function-altering deletions and alterations within the domains are encompassed within the scope of the term "heavy chain constant region," unless designated otherwise. Nonlimiting exemplary heavy chain constant regions include γ, δ, and α. Nonlimiting exemplary heavy chain constant regions also include ε and μ. Each heavy constant region corresponds to an antibody isotype. For example, an antibody comprising a γ constant region is an IgG antibody, an antibody comprising a δ constant region is an IgD antibody, and an antibody comprising an α constant region is an IgA antibody. Further, an antibody comprising a μ constant region is an IgM antibody, and an antibody comprising an ε constant region is an IgE antibody. Certain isotypes can be further subdivided into subclasses. For example, IgG antibodies include, but are not limited to, IgG1 (comprising a γ_1 constant region), IgG2 (comprising a γ_2 constant region), IgG3 (comprising a γ_3 constant region), and IgG4 (comprising a γ_4 constant region) antibodies; IgA antibodies include, but are not limited to, IgA1 (comprising an α_1 constant region) and IgA2 (comprising an α_2 constant region) antibodies; and IgM antibodies include, but are not limited to, IgM1 and IgM2.

[0177] A "Fc region" as used herein refers to a portion of a heavy chain constant region comprising CH2 and CH3. In some embodiments, an Fc region comprises a hinge, CH2, and CH3. In various embodiments, when an Fc region comprises a hinge, the hinge mediates dimerization between two Fc-containing polypeptides. An Fc region may be of any antibody heavy chain constant region isotype discussed herein. In some embodiments, an Fc region is an IgG1, IgG2, IgG3, or IgG4.

[0178] A "functional Fc region" possesses an "effector function" of a native sequence Fc region. Exemplary "effector functions" include Fc receptor binding; Clq binding and complement dependent cytotoxicity (CDC); Fc receptor binding; antibody-dependent cell-mediated cytotoxicity (ADCC); phagocytosis; down regulation of cell surface receptors (for example B-cell receptor); and B-cell activation, etc. Such effector functions generally require the Fc region to be combined with a binding domain (for example, an antibody variable domain) and can be assessed using various assays.

[0179] A "native sequence Fc region" comprises an amino acid sequence identical to the amino acid sequence of an Fc region found in nature. Native sequence human Fc regions include a native sequence human IgG1 Fc region (non-A and A allotypes); native sequence human IgG2 Fc region; native sequence human IgG3 Fc region; and native sequence human IgG4 Fc region as well as naturally occurring variants thereof.

[0180] A "variant Fc region" comprises an amino acid sequence which differs from that of a native sequence Fc region by virtue of at least one amino acid modification. In some embodiments, a "variant Fc region" comprises an amino acid sequence which differs from that of a native sequence Fc region by virtue of at least one amino acid modification, yet retains at least one effector function of the native sequence Fc region. In some embodiments, the variant Fc region has at least one amino acid substitution compared to a native sequence Fc region or to the Fc region of a parent polypeptide, for example, from about one to about ten amino acid substitutions, and preferably, from about one to about five amino acid substitutions in a native sequence Fc region or in the Fc region of the parent polypeptide. In some embodiments, the variant Fc region herein will possess at least about 80% sequence identity with a native sequence Fc region and/or with an Fc region of a parent polypeptide, at least about 90% sequence identity therewith, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity therewith.

[0181] In general, the numbering of the residues in an immunoglobulin heavy chain or portion thereof, such as an Fc region, is that of the EU index as in Kabat *et al.*, *Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991). The "EU index as in Kabat" refers to the residue numbering of the human IgG1 EU antibody.

[0182] "Fc receptor" or "FcR" describes a receptor that binds to the Fc region of an antibody. In some embodiments, an FcγR is a native human FcR. In some embodiments, an FcR is one which binds an IgG antibody (a gamma receptor) and includes receptors of the FcγRI, FcγRII, and FcγRIII subclasses, including allelic variants and alternatively spliced forms of those receptors. FcγRII receptors include FcγRIIA (an "activating receptor") and FcγRIIB (an "inhibiting receptor"), which have similar amino acid sequences that differ primarily in the cytoplasmic domains thereof. Activating receptor FcγRIIA contains an immunoreceptor tyrosine-based activation motif (ITAM) in its cytoplasmic domain Inhibiting receptor FcγRIIB contains an immunoreceptor tyrosine-based inhibition motif (ITIM) in its cytoplasmic domain. (*See*, for example, Daeron, Annu. Rev. Immunol. 15:203-234 (1997)). FcRs are reviewed, for

example, in Ravetch and Kinet, *Annu. Rev. Immunol* 9:457-92 (1991); Capel *et al.*, *Immunomethods* 4:25-34 (1994); and de Haas *et al.*, *J. Lab. Clin. Med.* 126:330-41 (1995). Other FcRs, including those to be identified in the future, are encompassed by the term "FcR" herein. For example, the term "Fc receptor" or "FcR" also includes the neonatal receptor, FcRn, which is responsible for the transfer of maternal IgGs to the fetus (Guyer *et al.*, *J. Immunol.* 117:587 (1976) and Kim *et al.*, *J. Immunol.* 24:249 (1994)) and regulation of homeostasis of immunoglobulins. Methods of measuring binding to FcRn are known (see, for example, Ghetie and Ward, *Immunol. Today* 18(12):592-598 (1997); Ghetie *et al.*, *Nature Biotechnology*, 15(7):637-640 (1997); Hinton *et al.*, *J. Biol. Chem.* 279(8):6213-6216 (2004); WO 2004/92219 (Hinton *et al.*).

[0183] An "acceptor human framework" as used herein is a framework comprising the amino acid sequence of a heavy chain variable domain (V_H) framework derived from a human immunoglobulin framework or a human consensus framework, as discussed herein. An acceptor human framework derived from a human immunoglobulin framework or a human consensus framework can comprise the same amino acid sequence thereof, or it can contain amino acid sequence changes. In some embodiments, the number of amino acid changes are fewer than 10, or fewer than 9, or fewer than 8, or fewer than 7, or fewer than 6, or fewer than 5, or fewer than 4, or fewer than 3, across all of the human frameworks in a single antigen binding domain, such as a VHH.

[0184] As used herein, a "chimeric antigen receptor" or "CAR" refers to an engineered receptor, which introduces an antigen specificity, via an antigen binding domain, onto cells to which it is engineered (for example T cells such as naive T cells, central memory T cells, effector memory T cells or combination thereof) thus combining the antigen binding properties of the antigen binding domain with the T cell activity (e.g. lytic capacity and self renewal) of T cells. A CAR typically includes an extracellular antigen-binding domain (ectodomain), a transmembrane domain and an intracellular signaling domain. The intracellular signaling domain generally contains at least one ITAM signaling domain, e.g. derived from CD3zeta, and optionally at least one costimulatory signaling domain, e.g. derived from CD28 or 4-1BB. In a CAR provided herein, a VHH domain forms the antigen binding domain and is located at the extracellular side when expressed in a cell.

[0185] "Affinity" refers to the strength of the sum total of noncovalent interactions between a single binding site of a molecule (for example, an antibody or VHH-containing polypeptide) and its binding partner (for example, an antigen). The affinity or the apparent affinity of a molecule X for its partner Y can generally be represented by the dissociation constant (K_D) or the $K_{D\text{-apparent}}$, respectively. Affinity can be measured by common methods known in the art (such as, for example, ELISA K_D, KinExA, flow cytometry, and/or surface plasmon resonance devices), including those described herein. Such methods include, but are not limited to, methods involving BIAcore®, Octet®, or flow cytometry.

[0186] The term "K_D", as used herein, refers to the equilibrium dissociation constant of an antigen-binding molecule/antigen interaction. When the term "K_D" is used herein, it includes K_D and $K_{D\text{-apparent}}$.

[0187] In some embodiments, the K_D of the antigen-binding molecule is measured by flow cytometry using an antigen-expressing cell line and fitting the mean fluorescence measured at each antibody concentration to a non-linear one-site binding equation (Prism Software graphpad). In some such embodiments, the K_D is $K_{D\text{-apparent}}$.

[0188] The term "biological activity" refers to any one or more biological properties of a molecule (whether present naturally as found in vivo, or provided or enabled by recombinant means). Biological properties include, but are not limited to, binding a ligand, inducing or increasing cell proliferation (such as T cell proliferation), and inducing or increasing expression of cytokines.

[0189] An "affinity matured" VHH-containing polypeptide refers to a VHH-containing polypeptide with one or more alterations in one or more CDRs compared to a parent VHH-containing polypeptide that does not possess such alterations, such alterations resulting in an improvement in the affinity of the VHH-containing polypeptide for antigen.

[0190] A "humanized VHH" as used herein refers to a VHH in which one or more framework regions have been substantially replaced with human framework regions. In some instances, certain framework region (FR) residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, the humanized VHH can comprise residues that are found neither in the original VHH nor in the human framework sequences, but are included to further refine and optimize VHH or VHH-containing polypeptide performance. In some embodiments, a humanized VHH-containing polypeptide comprises a human Fc region. As will be appreciated, a humanized sequence can be identified by its primary sequence and does not necessarily denote the process by which the antibody was created.

[0191] The term "substantially similar" or "substantially the same," as used herein, denotes a sufficiently high degree of similarity between two or more numeric values such that one of skill in the art would consider the difference between the two or more values to be of little or no biological and/or statistical significance within the context of the biological characteristic measured by said value. In some embodiments the two or more substantially similar values differ by no more than about any one of 5%, 10%, 15%, 20%, 25%, or 50%.

[0192] A polypeptide "variant" means a biologically active polypeptide having at least about 80% amino acid sequence identity with the native sequence polypeptide after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Such variants include, for instance, polypeptides wherein one or more amino acid residues are added, or deleted, at the N- or C-terminus of the polypeptide. In some embodiments, a variant will have at least about 80% amino acid sequence identity. In some embodiments, a variant will have at least about 90% amino acid sequence identity. In some embodiments, a variant will have at least about 95% amino acid sequence identity with the native sequence polypeptide.

[0193] As used herein, "percent (%) amino acid sequence identity" and "homology" with respect to a peptide, polypeptide or antibody sequence are defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the specific peptide or polypeptide sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN or MEGALIGNTM (DNASTAR) software. Those skilled in the art can determine appropriate parameters for measuring alignment, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared.

[0194] An amino acid substitution may include but are not limited to the replacement of one amino acid in a polypeptide with another amino acid. Exemplary substitutions are shown in Table 2. Amino acid substitutions may be introduced into an antibody of interest and the products screened for a desired activity, for example, retained/improved antigen binding, decreased immunogenicity, or improved ADCC or CDC.

Table 2

Original Residue	**Exemplary Substitutions**
Ala (A)	Val; Leu; Ile
Arg (R)	Lys; Gln; Asn
Asn (N)	Gln; His; Asp, Lys; Arg
Asp (D)	Glu; Asn
Cys (C)	Ser; Ala
Gln (Q)	Asn; Glu
Glu (E)	Asp; Gln
Gly (G)	Ala
His (H)	Asn; Gln; Lys; Arg
Ile (I)	Leu; Val; Met; Ala; Phe; Norleucine
Leu (L)	Norleucine; Ile; Val; Met; Ala; Phe
Lys (K)	Arg; Gln; Asn
Met (M)	Leu; Phe; Ile
Phe (F)	Trp; Leu; Val; Ile; Ala; Tyr
Pro (P)	Ala
Ser (S)	Thr
Thr (T)	Val; Ser
Trp (W)	Tyr; Phe
Tyr (Y)	Trp; Phe; Thr; Ser
Val (V)	Ile; Leu; Met; Phe; Ala; Norleucine

[0195] Amino acids may be grouped according to common side-chain properties:

(1) hydrophobic: Norleucine, Met, Ala, Val, Leu, Ile;

(2) neutral hydrophilic: Cys, Ser, Thr, Asn, Gln;

(3) acidic: Asp, Glu;

(4) basic: His, Lys, Arg;

(5) residues that influence chain orientation: Gly, Pro;

(6) aromatic: Trp, Tyr, Phe.

[0196] Non-conservative substitutions will entail exchanging a member of one of these classes for another class.

[0197] The term "vector" is used to describe a polynucleotide that can be engineered to contain a cloned polynucleotide or polynucleotides that can be propagated in a host cell. A vector can include one or more of the following elements: an origin of replication, one or more regulatory sequences (such as, for example, promoters and/or enhancers) that regulate the expression of the polypeptide of interest, and/or one or more selectable marker genes (such as, for example, antibiotic resistance genes and genes that can be used in colorimetric assays, for example, β-galactosidase). The term "expression vector" refers to a vector that is used to express a polypeptide of interest in a host cell.

[0198] A "host cell" refers to a cell that may be or has been a recipient of a vector or isolated polynucleotide. Host cells may be prokaryotic cells or eukaryotic cells. Exemplary eukaryotic cells include mammalian cells, such as primate or non-primate animal cells; fungal cells, such as yeast; plant cells; and insect cells. Nonlimiting exemplary mammalian cells include, but are not limited to, NSO cells, PER.C6® cells (Crucell), and 293 and CHO cells, and their derivatives, such as 293-6E, CHO-DG44, CHO-K1, CHO-S, and CHO-DS cells. Host cells include progeny of a single host cell, and the progeny may not necessarily be completely identical (in morphology or in genomic DNA complement) to the original parent cell due to natural, accidental, or deliberate mutation. A host cell includes cells transfected in vivo with a polynucleotide(s) a provided herein.

[0199] The term "isolated" as used herein refers to a molecule that has been separated from at least some of the components with which it is typically found in nature or produced. For example, a polypeptide is referred to as "isolated" when it is separated from at least some of the components of the cell in which it was produced. Where a polypeptide is secreted by a cell after expression, physically separating the supernatant containing the polypeptide from the cell that produced it is considered to be "isolating" the polypeptide. Similarly, a polynucleotide is referred to as "isolated" when it is not part of the larger polynucleotide (such as, for example, genomic DNA or mitochondrial DNA, in the case of a DNA polynucleotide) in which it is typically found in nature, or is separated from at least some of the components of the cell in which it was produced, for example, in the case of an RNA polynucleotide. Thus, a DNA polynucleotide that is contained in a vector inside a host cell may be referred to as "isolated".

[0200] The terms "individual" and "subject" are used interchangeably herein to refer to an animal; for example a mammal. The term patient includes human and veterinary subjects. In some embodiments, methods of treating mammals, including, but not limited to, humans, rodents, simians, felines, canines, equines, bovines, porcines, ovines, caprines, mammalian laboratory animals, mammalian farm animals, mammalian sport animals, and mammalian pets, are provided. The subject can be male or female and can be any suitable age, including infant, juvenile, adolescent, adult, and geriatric subjects. In some examples, an "individual" or "subject" refers to an individual or subject in need of treatment for a disease or disorder. In some embodiments, the subject to receive the treatment can be a patient, designating the fact that the subject has been identified as having a disorder of relevance to the treatment, or being at adequate risk of contracting the disorder. In particular embodiments, the subject is a human, such as a human patient.

[0201] A "disease" or "disorder" as used herein refers to a condition where treatment is needed and/or desired.

[0202] The term "tumor cell", "cancer cell", "cancer", "tumor", and/or "neoplasm", unless otherwise designated, are used herein interchangeably and refer to a cell (or cells) exhibiting an uncontrolled growth and/or abnormal increased cell survival and/or inhibition of apoptosis which interferes with the normal functioning of bodily organs and systems. Included in this definition are benign and malignant cancers, polyps, hyperplasia, as well as dormant tumors or micrometastases.

[0203] The terms "cancer" and "tumor" encompass solid and hematological/lymphatic cancers and also encompass malignant, pre-malignant, and benign growth, such as dysplasia. Also, included in this definition are cells having abnormal proliferation that is not impeded (*e.g.* immune evasion and immune escape mechanisms) by the immune system (*e.g.* virus infected cells). Exemplary cancers include, but are not limited to: 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 (including gastrointestinal cancer); glioblastoma; hepatic carcinoma; hepatoma; intra-epithelial neoplasm; kidney or renal cancer; larynx cancer; leukemia; liver cancer; lung cancer (*e.g.*, small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung, and squamous carcinoma of the lung); melanoma; myeloma; neuroblastoma; oral cavity cancer (lip, tongue, mouth, and pharynx); 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 including Hodgkin's and non-Hodgkin's lymphoma, as well as B-cell lymphoma (including 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; and Waldenstrom's Macroglobulinemia; chronic lymphocytic leukemia (CLL); acute lymphoblastic leukemia (ALL); Hairy cell leukemia; chronic myeloblastic leukemia; as well as other carcinomas and sarcomas; and post-transplant lymphoproliferative disorder (PTLD), as well as abnormal vascular proliferation associated with phakomatoses, edema (such as that associated with brain tumors), and Meigs' syndrome.

[0204] The term "non-tumor cell" as used herein refers to a normal cells or tissue. Exemplary non-tumor cells include, but are not limited to: T-cells, B-cells, natural killer (NK) cells, natural killer T (NKT) cells, dendritic cells, monocytes, macrophages, epithelial cells, fibroblasts, hepatocytes, interstitial kidney cells, fibroblast-like synoviocytes, osteoblasts, and cells located in the breast, skeletal muscle, pancreas, stomach, ovary, small intestines, placenta, uterus, testis, kidney, lung, heart, brain, liver, prostate, colon, lymphoid organs, bone, and bone-derived mesenchymal stem cells. The term "a cell or tissue located in the periphery" as used herein refers to non-tumor cells not located near tumor cells and/or within the tumor microenvironment.

[0205] The term "cells or tissue within the tumor microenvironment" as used herein refers to the cells, molecules, extracellular matrix and/or blood vessels that surround and/or feed a tumor cell. Exemplary cells or tissue within the tumor microenvironment include, but are not limited to: tumor vasculature; tumor-infiltrating lymphocytes; fibroblast reticular cells; endothelial progenitor cells (EPC); cancer-associated fibroblasts; pericytes; other stromal cells; components of the extracellular matrix (ECM); dendritic cells; antigen presenting cells; T-cells; regulatory T-cells (Treg cells); macrophages; neutrophils; myeloid-derived suppressor cells (MDSCs) and other immune cells located proximal to a tumor. Methods for identifying tumor cells, and/or cells/tissues located within the tumor microenvironment are well known in the art, as described herein, below.

[0206] In some embodiments, an "increase" or "decrease" refers to a statistically significant increase or decrease, respectively. As will be clear to the skilled person, "modulating" can also involve effecting a change (which can either be an increase or a decrease) in affinity, avidity, specificity and/or selectivity of a target or antigen, for one or more of its ligands, binding partners, partners for association into a homomultimeric or heteromultimeric form, or substrates; effecting a change (which can either be an increase or a decrease) in the sensitivity of the target or antigen for one or more conditions in the medium or surroundings in which the target or antigen is present (such as pH, ion strength, the presence of co-factors, *etc.*); and/or cellular proliferation or cytokine production, compared to the same conditions but without the presence of a test agent. This can be determined in any suitable manner and/or using any suitable assay known *per se* or described herein, depending on the target involved.

[0207] As used herein, "an immune response" is meant to encompass cellular and/or humoral immune responses that are sufficient to inhibit or prevent onset or ameliorate the symptoms of disease

(for example, cancer or cancer metastasis). "An immune response" can encompass aspects of both the innate and adaptive immune systems.

[0208] As used herein, the terms "treating," "treatment," or "therapy" of a disease, disorder or condition is an approach for obtaining beneficial or desired clinical results. "Treatment" as used herein, covers any administration or application of a therapeutic for disease in a mammal, including a human. For purposes of this disclosure, beneficial or desired clinical results include, but are not limited to, any one or more of: alleviation of one or more symptoms, diminishment of extent of disease, preventing or delaying spread (for example, metastasis, for example metastasis to the lung or to the lymph node) of disease, preventing or delaying recurrence of disease, delay or slowing of disease progression, amelioration of the disease state, inhibiting the disease or progression of the disease, inhibiting or slowing the disease or its progression, arresting its development, and remission (whether partial or total). Also encompassed by "treatment" is a reduction of pathological consequence of a proliferative disease. The methods provided herein contemplate any one or more of these aspects of treatment. In-line with the above, the term treatment does not require one-hundred percent removal of all aspects of the disorder.

[0209] As used herein in the context of cancer, the terms "treatment" or, "inhibit," "inhibiting" or "inhibition" of cancer refers to at least one of: a statistically significant decrease in the rate of tumor growth, a cessation of tumor growth, or a reduction in the size, mass, metabolic activity, or volume of the tumor, as measured by standard criteria such as, but not limited to, the Response Evaluation Criteria for Solid Tumors (RECIST), or a statistically significant increase in progression free survival (PFS) or overall survival (OS).

[0210] "Ameliorating" means a lessening or improvement of one or more symptoms as compared to not administering a therapeutic agent. "Ameliorating" also includes shortening or reduction in duration of a symptom.

[0211] "Preventing," "prophylaxis," or "prevention" of a disease or disorder refers to administration of a pharmaceutical composition, either alone or in combination with another compound, to prevent the occurrence or onset of a disease or disorder or some or all of the symptoms of a disease or disorder or to lessen the likelihood of the onset of a disease or disorder.

[0212] The terms "inhibition" or "inhibit" refer to a decrease or cessation of any phenotypic characteristic or to the decrease or cessation in the incidence, degree, or likelihood of that characteristic. To "reduce" or "inhibit" is to decrease, reduce or arrest an activity, function, and/or amount as compared to a reference. In some embodiments, by "reduce" or "inhibit" is meant the ability to cause an overall decrease of 10% or greater. In some embodiments, by "reduce" or "inhibit" is meant the ability to cause an overall decrease of 50% or greater. In some embodiments, by "reduce" or "inhibit" is meant the ability to cause an overall decrease of 75%, 85%, 90%, 95%, or greater. In some embodiments, the amount noted above is inhibited or decreased over a period of time, relative to a control over the same period of time.

[0213] As used herein, "delaying development of a disease" means to defer, hinder, slow, retard, stabilize, suppress and/or postpone development of the disease (such as cancer). This delay can be of varying lengths of time, depending on the history of the disease and/or individual being treated. As is evident to one skilled in the art, a sufficient or significant delay can, in effect, encompass prevention, in that the individual does not develop the disease. For example, a late stage cancer, such as development of metastasis, may be delayed.

[0214] "Preventing," as used herein, includes providing prophylaxis with respect to the occurrence or recurrence of a disease in a subject that may be predisposed to the disease but has not yet been diagnosed with the disease. Unless otherwise specified, the terms "reduce", "inhibit", or "prevent" do not denote or require complete prevention over all time, but just over the time period being measured.

[0215] The term "anti-cancer agent" is used herein in its broadest sense to refer to agents that are used in the treatment of one or more cancers. Exemplary classes of such agents in include, but are not limited to, chemotherapeutic agents, anti-cancer biologics (such as cytokines, receptor extracellular domain-Fc fusions, and antibodies), radiation therapy, CAR-T therapy, therapeutic oligonucleotides (such as antisense oligonucleotides and siRNAs) and oncolytic viruses.

[0216] The term "biological sample" means a quantity of a substance from a living thing or formerly living thing. Such substances include, but are not limited to, blood, (for example, whole blood), plasma, serum, urine, amniotic fluid, synovial fluid, endothelial cells, leukocytes, monocytes, other cells, organs, tissues, bone marrow, lymph nodes and spleen.

[0217] The term "control" or "reference" refers to a composition known to not contain an analyte ("negative control") or to contain an analyte ("positive control"). A positive control can comprise a known concentration of analyte.

[0218] The terms "effective amount" or "therapeutically effective amount" refer to a quantity and/or concentration of a composition containing an active ingredient (e.g. sdAb or VHH-containing polypeptide) that when administered into a patient either alone (i.e., as a monotherapy) or in combination with additional therapeutic agents, yields a statistically significant decrease in disease progression as, for example, by ameliorating or eliminating symptoms and/or the cause of the disease. An effective amount may be an amount that relieves, lessens, or alleviates at least one symptom or biological response or effect associated with a disease or disorder, prevents progression of the disease or disorder, or improves physical functioning of the patient. A therapeutically effective amount of a composition containing an active agent may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the active agent to elicit a desired response in the individual. A therapeutically effective amount is also one in which any toxic or detrimental effects of the active agent are outweighed by the therapeutically beneficial effects. A therapeutically effective amount may be delivered in one or more administrations. A therapeutically effective amount refers to an amount

effective, at dosages and for periods of time necessary, to achieve the desired therapeutic and/or prophylactic result.

[0219] As used herein, a composition refers to any mixture of two or more products, substances, or compounds, including cells. It may be a solution, a suspension, liquid, powder, a paste, aqueous, non-aqueous or any combination thereof.

[0220] The terms "pharmaceutical formulation" and "pharmaceutical composition" refer to a preparation which is in such form as to permit the biological activity of the active ingredient(s) to be effective, and which contains no additional components which are unacceptably toxic to a subject to which the formulation would be administered. Hence, it is a composition suitable for pharmaceutical use in a mammalian subject, often a human. A pharmaceutical composition typically comprises an effective amount of an active agent (e.g., sdAb or VHH-containing polypeptide) and a carrier, excipient, or diluent. The carrier, excipient, or diluent is typically a pharmaceutically acceptable carrier, excipient or diluent, respectively. Such formulations may be sterile.

[0221] A "pharmaceutically acceptable carrier" refers to a non-toxic solid, semisolid, or liquid filler, diluent, encapsulating material, formulation auxiliary, or carrier conventional in the art for use with a therapeutic agent that together comprise a "pharmaceutical composition" for administration to a subject. A pharmaceutically acceptable carrier is non-toxic to recipients at the dosages and concentrations employed and are compatible with other ingredients of the formulation. The pharmaceutically acceptable carrier is appropriate for the formulation employed.

[0222] Administration "in combination with" one or more further therapeutic agents includes simultaneous (concurrent) and sequential administration in any order.

[0223] The term "concurrently" is used herein to refer to administration of two or more therapeutic agents, where at least part of the administration overlaps in time, or where the administration of one therapeutic agent falls within a short period of time relative to administration of the other therapeutic agent, or wherein the therapeutic effect of both agents overlap for at least a period of time.

[0224] The term "sequentially" is used herein to refer to administration of two or more therapeutic agents that does not overlap in time, or wherein the therapeutic effects of the agents do not overlap.

[0225] As used herein, "in conjunction with" refers to administration of one treatment modality in addition to another treatment modality. As such, "in conjunction with" refers to administration of one treatment modality before, during, or after administration of the other treatment modality to the individual.

[0226] The term "package insert" is used to refer to instructions customarily included in commercial packages of therapeutic products, that contain information about the indications, usage, dosage, administration, combination therapy, contain dications and/or warnings concerning the use of such therapeutic products.

[0227] An "article of manufacture" is any manufacture (for example, a package or container) or kit comprising at least one reagent, for example, a medicament for treatment of a disease or disorder (for example, cancer), or a probe for specifically detecting a biomarker described herein. In some embodiments, the manufacture or kit is promoted, distributed, or sold as a unit for performing the methods described herein.

[0228] The terms "label" and "detectable label" mean a moiety attached, for example, to an antibody or antigen to render a reaction (for example, binding) between the members of the specific binding pair, detectable. The labeled member of the specific binding pair is referred to as "detectably labeled." Thus, the term "labeled binding protein" refers to a protein with a label incorporated that provides for the identification of the binding protein. In some embodiments, the label is a detectable marker that can produce a signal that is detectable by visual or instrumental means, for example, incorporation of a radiolabeled amino acid or attachment to a polypeptide of biotinyl moieties that can be detected by marked avidin (for example, streptavidin containing a fluorescent marker or enzymatic activity that can be detected by optical or colorimetric methods). Examples of labels for polypeptides include, but are not limited to, the following: radioisotopes or radionuclides (for example, ^{3}H, ^{14}C, ^{35}S, ^{90}Y, ^{99}Tc, ^{111}In, ^{125}I, ^{131}I, ^{177}Lu, ^{166}Ho, or ^{153}Sm); chromogens, fluorescent labels (for example, FITC, rhodamine, lanthanide phosphors), enzymatic labels (for example, horseradish peroxidase, luciferase, alkaline phosphatase); chemiluminescent markers; biotinyl groups; predetermined polypeptide epitopes recognized by a secondary reporter (for example, leucine zipper pair sequences, binding sites for secondary antibodies, metal binding domains, epitope tags); and magnetic agents, such as gadolinium chelates. Representative examples of labels commonly employed for immunoassays include moieties that produce light, for example, acridinium compounds, and moieties that produce fluorescence, for example, fluorescein. In this regard, the moiety itself may not be detectably labeled but may become detectable upon reaction with yet another moiety.

II. VHH DOMAINS BINDING 5T4

[0229] Provided herein are 5T4-binding polypeptides that are VHH-containing polypeptides containing at least one VHH domain that specifically binds to 5T4. In some embodiments, the VHH domain binds human 5T4. In some of any of the provided embodiments, the VHH domain binds 5T4 having the sequence set forth in SEQ ID NO: 244 or a mature form thereof lacking the signal sequence.

[0230] In some embodiments, the VHH-containing polypeptides incorporate multiple copies of a VHH domain provided herein. In such embodiments, the VHH-containing polypeptide may incorporate multiple copies of the same VHH domain. In some embodiments, the VHH-containing polypeptides may incorporate multiple copies of a VHH domain that are different but that recognize the same epitope on 5T4.

[0231] In some embodiments, a 5T4 VHH does not cross react with the mouse 5T4 antigen. In some embodiments, a 5T4 VHH comprising the amino acid sequence set forth in SEQ ID NO:245, or humanized variants thereof, do not cross react with the mouse 5T4 antigen. In some embodiments, a 5T4 VHH comprising the amino acid sequence set forth in SEQ ID NO:255, or humanized variants thereof, do not cross react with the mouse 5T4 antigen. In some embodiments, a 5T4 VHH comprising the amino acid sequence set forth in SEQ ID NO:276, or humanized variants thereof, do not cross react with the mouse 5T4 antigen.In some embodiments, a provided 5T4 VHH domain binds to an epitope located between amino acid residues 60 and 112 of the 5T4 extracellular domain corresponding to amino acid residues set forth in SEQ ID NO:382. In some embodiments, a 5T4 VHH domain having the amino acid sequence set forth in SEQ ID NO:360 (12E9v9) binds to an epitope located between amino acid residues 60 and 112 of the 5T4 extracellular domain corresponding to amino acid residues set forth in SEQ ID NO:382.

[0232] In some embodiments, a 5T4 VHH domain binds to an epitope located between amino acid residues 173 and 224 of the 5T4 extracellular domain corresponding to amino acid residues set forth in SEQ ID NO:382. In some embodiments, a 5T4 VHH domain having the amino acid sequence set forth in SEQ ID NOS:272 or 287 (16G10v11 or 14B5v17) binds to an epitope located between amino acid residues 60 and 112 of the 5T4 extracellular domain corresponding to amino acid residues set forth in SEQ ID NO:382.

[0233] The VHH-containing polypeptides can be formatted in a variety of formats, including any as described in Section III below.

[0234] Single domain antibodies are antibodies whose complementary determining regions are part of a single domain polypeptide. Examples include, but are not limited to, heavy chain antibodies, antibodies naturally devoid of light chains, single domain antibodies derived from conventional 4-chain antibodies, engineered antibodies and single domain scaffolds other than those derived from antibodies. Single domain antibodies may be derived from any species including, but not limited to mouse, human, camel, llama, alpaca, vicuna, guanaco, shark, goat, rabbit, and/or bovine. In some embodiments, a single domain antibody as used herein is a naturally occurring single domain antibody known as heavy chain antibody devoid of light chains. For clarity reasons, this variable domain derived from a heavy chain antibody naturally devoid of light chain is known herein as a VHH to distinguish it from the conventional VH of four chain immunoglobulins. Such a VHH molecule can be derived from antibodies raised in Camelidae species, for example in camel, llama, dromedary, alpaca, vicuna and guanaco. Other species besides Camelidae may produce heavy chain antibodies naturally devoid of light chain; such VHHs are within the scope of the disclosure.

[0235] A VHH domain is an antibody fragment that is a single monomeric variable antibody domain that is able to bind selectively to a specific antigen. With a molecular weight of only 12-15 kDa, VHH domains (also called single-domain antibodies) are much smaller than common antibodies (150-160 kDa)

which are composed of two heavy protein chains and two light chains, and even smaller than Fab fragments (~50 kDa, one light chain and half a heavy chain) and single-chain variable fragments (~25 kDa, two variable domains, one from a light and one from a heavy chain).

[0236] Methods for the screening of VHH domains, including VHH-binding polypeptides, that possess the desired specificity for 5T4 include, but are not limited to, enzyme linked immunosorbent assay (ELISA), enzymatic assays, flow cytometry, and other immunologically mediated techniques known within the art.

[0237] Among the provided VHH domains provided herein are 5T4 VHH (llama-derived) and humanized sequences, such as any described below.

[0238] In some embodiments, a VHH domain that binds 5T4 may be humanized. Humanized antibodies (such as VHH-containing polypeptides) are useful as therapeutic molecules because humanized antibodies reduce or eliminate the human immune response to non-human antibodies, which can result in an immune response to an antibody therapeutic, and decreased effectiveness of the therapeutic. Generally, a humanized antibody comprises one or more variable domains in which CDRs, (or portions thereof) are derived from a non-human antibody, and FRs (or portions thereof) are derived from human antibody sequences. A humanized antibody optionally will also comprise at least a portion of a human constant region. In some embodiments, some FR residues in a humanized antibody are substituted with corresponding residues from a non-human antibody (for example, the antibody from which the CDR residues are derived), for example, to restore or improve antibody specificity or affinity.

[0239] Humanized antibodies and methods of making them are reviewed, for example, in Almagro and Fransson, (2008) *Front. Biosci.* 13: 1619-1633, and are further described, for example, in Riechmann *et al.*, (1988) *Nature* 332:323-329; Queen *et al.*, (1989) *Proc. Natl Acad. Sci. USA* 86: 10029-10033; US Patent Nos. 5, 821,337, 7,527,791, 6,982,321, and 7,087,409; Kashmiri *et al.*, (2005) *Methods* 36:25-34; Padlan, (1991) *Mol. Immunol.* 28:489-498 (describing "resurfacing"); Dall'Acqua *et al.*, (2005) *Methods* 36:43-60 (describing "FR shuffling"); and Osbourn *et al.*, (2005) *Methods* 36:61-68 and Klimka *et al.*, (2000) *Br. J. Cancer*, 83:252-260 (describing the "guided selection" approach to FR shuffling).

[0240] Human framework regions that can be used for humanization include but are not limited to: framework regions selected using the "best-fit" method (see, for example, Sims *et al.* (1993) *J. Immunol.* 151 :2296); framework regions derived from the consensus sequence of human antibodies of a particular subgroup of heavy chain variable regions (see, for example, Carter *et al.* (1992) *Proc. Natl. Acad. Sci. USA*, 89:4285; and Presta *et al.* (1993) *J. Immunol*, 151:2623); human mature (somatically mutated) framework regions or human germline framework regions (see, for example, Almagro and Fransson, (2008) *Front. Biosci.* 13:1619-1633); and framework regions derived from screening FR libraries (see, for example, Baca *et al.*, (1997) *J. Biol. Chem.* 272: 10678-10684 and Rosok *et al.*, (1996) *J. Biol. Chem.* 271 :22611-22618). Typically, the FR regions of a VHH are replaced with human FR regions to make a humanized VHH. In some embodiments, certain FR residues of the human FR are replaced in order to

improve one or more properties of the humanized VHH. VHH domains with such replaced residues are still referred to herein as "humanized."

[0241] Provided herein is a VHH domain that binds 5T4 in which the VHH domain comprises a CDR1, CDR2 and CDR3 contained in a VHH amino acid sequences selected from any of SEQ ID NO:245-287, 294, 295, 302, or 360 or an amino acid sequence that has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the VHH region amino acid selected from any one of SEQ ID NOs: 245-287, 294, 295, 302, or 360. In some embodiments, a 5T4 VHH domain provided herein contains a CDR1 set forth in any one of SEQ ID NOS: 288-292, 86-87, 296, 297, a CDR2 set forth in any one of SEQ ID NOS: 88-99, 298, 299 and a CDR3 set forth in any one of SEQ ID NOS: 100-102, 300, 301, 303. Among the provided 5T4 VHH domain has the amino acid sequence set forth in any of SEQ ID NOS: 245-287, 294, 295, 302, or 360, or an amino acid sequence that has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the V_HH region amino acid selected from any one of SEQ ID NOs: 245-287, 294, 295, 302, or 360. In some embodiments, the 5T4 VHH domain has the sequence of amino acids set forth in any one of SEQ ID NOS: 245-287, 294, 295, 302, or 360.

[0242] In some embodiments, a 5T4 VHH domain provided herein contains a CDR1, CDR2, CDR3 contained in a VHH domain set forth in SEQ ID NO:245, or an amino acid sequence that has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the VHH region amino acid selected set forth in SEQ ID NO: 245. In some embodiments, the 5T4 VHH domain has the amino acid sequence set forth in SEQ ID NO: 245 or an amino acid sequence that has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the amino acid selected set forth in SEQ ID NO: 245. In some embodiments, the 5T4 VHH domain is a humanized variant of the amino acid sequence set forth in SEQ ID NO: 245.

[0243] In some embodiments, a 5T4 VHH domain provided herein contains a CDR1 set forth in SEQ ID NOS: 288 or 289, a CDR2 set forth in SEQ ID NO: 88 and a CDR3 set forth in SEQ ID NO: 100.

[0244] In some embodiments, the 5T4 VHH domain provided herein contains a CDR1, CDR2, and CDR3 set forth in SEQ ID NOs: 288, 88, and 100, respectively. In some embodiments, the 5T4 VHH domain provided herein contains a CDR1, CDR2 and CDR3 set forth in SEQ ID NOs: 289, 88, and 100, respectively.

[0245] In some aspects, a VHH domain that binds 5T4 comprises a CDR1, CDR2 and CDR3 contained in a VHH amino acid sequences selected from any of SEQ ID NO:246-254 , or an amino acid sequence that has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the V_HH region amino acid selected from any one of SEQ ID NOs: 246-254.

[0246] In some aspects, a VHH domain that binds 5T4 comprises a CDR1, CDR2 and CDR3 contained in a VHH amino acid sequences selected from any of SEQ ID NO:246-254 and 360 , or an

amino acid sequence that has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the V_HH region amino acid selected from any one of SEQ ID NOs: 246-254 and 360.

[0247] In some cases, the provided 5T4 VHH domain is a humanized variant that has the amino acid sequence set forth in any of SEQ ID NOS: 246-254 and 360 or an amino acid sequence that has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the VHH region amino acid selected from any one of SEQ ID NOs: 246-254. In some embodimetns, the 5T4 humanized VHH domain has the sequence of amino acids set forth in any one of SEQ ID NOS: 246-254.

[0248] In some cases, the provided 5T4 VHH domain is a humanized variant that has the amino acid sequence set forth in any of SEQ ID NOS: 246-254 and 360 or an amino acid sequence that has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the VHH region amino acid selected from any one of SEQ ID NOs: 246-254 and 360. In some embodimetns, the 5T4 humanized VHH domain has the sequence of amino acids set forth in any one of SEQ ID NOS: 246-254 and 360.

[0249] In some embodiments, a 5T4 VHH domain provided herein contains a CDR1, CDR2, CDR3 contained in a VHH domain set forth in SEQ ID NO:255, or an amino acid sequence that has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the VHH region amino acid selected set forth in SEQ ID NO: 255. In some embodiments, the 5T4 VHH domain has the amino acid sequence set forth in SEQ ID NO: 255 or an amino acid sequence that has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the amino acid selected set forth in SEQ ID NO: 255. In some embodiments, the 5T4 VHH domain is a humanized variant of the amino acid sequence set forth in SEQ ID NO: 255.

[0250] In some embodiments, a 5T4 VHH domain provided herein contains a CDR1 set forth in any one of SEQ ID NOs: 86, 290-292, a CDR2 set forth in any one of SEQ ID NOs: 89-94 and a CDR3 set forth in SEQ ID NO: 101.

[0251] In some embodiments, the 5T4 VHH domain provided herein contains a CDR1, CDR2, and CDR3 set forth in SEQ ID NOs: 290, 89, and 101, respectively. In some embodiments, the 5T4 VHH domain provided herein contains a CDR1, CDR2, and CDR3 set forth in SEQ ID NOs: 290, 90, and 101, respectively. In some embodiments, the 5T4 VHH domain provided herein contains a CDR1, CDR2, and CDR3 set forth in SEQ ID NOs: 290, 91, and 101, respectively. In some embodiments, the 5T4 VHH domain provided herein contains a CDR1, CDR2, and CDR3 set forth in SEQ ID NOs: 290, 92, and 101, respectively. In some embodiments, the 5T4 VHH domain provided herein contains a CDR1, CDR2, and CDR3 set forth in SEQ ID NOs: 290, 93, and 101, respectively. In some embodiments, the 5T4 VHH domain provided herein contains a CDR1, CDR2, and CDR3 set forth in SEQ ID NOs: 290, 94, and 101, respectively. In some embodiments, the 5T4 VHH domain provided herein contains a CDR1, CDR2, and CDR3 set forth in SEQ ID NOs: 291, 94, and 101, respectively. In some embodiments, the 5T4 VHH domain provided herein contains a CDR1, CDR2, and CDR3 set forth in SEQ ID NOs: 292, 94, and 101,

respectively. In some embodiments, the 5T4 VHH domain provided herein contains a CDR1, CDR2, and CDR3 set forth in SEQ ID NOs: 86, 94, and 101, respectively.

[0252] In some aspects, a VHH domain that binds 5T4 comprises a CDR1, CDR2 and CDR3 contained in a VHH amino acid sequences selected from any of SEQ ID NO:256-275, or an amino acid sequence that has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the VHH region amino acid selected from any one of SEQ ID NOs: 256-275.

[0253] In some cases, the provided 5T4 VHH domain is a humanized variant that has the amino acid sequence set forth in any of SEQ ID NOS: 256-275 or an amino acid sequence that has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the VHH region amino acid selected from any one of SEQ ID NOs: 256-275. In some embodimetns, the 5T4 humaized VHH domain has the sequence of amino acids set forth in any one of SEQ ID NOS: 256-275.

[0254] In some embodiments, a 5T4 VHH domain provided herein contains a CDR1, CDR2, CDR3 contained in a VHH domain set forth in SEQ ID NO:276, or an amino acid sequence that has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the VHH region amino acid selected set forth in SEQ ID NO: 276. In some embodiments, the 5T4 VHH domain has the amino acid sequence set forth in SEQ ID NO: 276 or an amino acid sequence that has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the amino acid selected set forth in SEQ ID NO: 276. In some embodiments, the 5T4 VHH domain is a humanized variant of the amino acid sequence set forth in SEQ ID NO: 276.

[0255] In some embodiments, a 5T4 VHH domain provided herein contains a CDR1 set forth in any one of SEQ ID NOs: 86 or 87, a CDR2 set forth in SEQ ID NO: 95-99 and a CDR3 set forth in SEQ ID NO:102.

[0256] In some embodiments, the 5T4 VHH domain provided herein contains a CDR1, CDR2, and CDR3 set forth in SEQ ID NOs: 87, 95, 102, respectively. In some embodiments, the 5T4 VHH domain provided herein contains a CDR1, CDR2, and CDR3 set forth in SEQ ID NOs: 87, 96, 102, respectively. In some embodiments, the 5T4 VHH domain provided herein contains a CDR1, CDR2, and CDR3 set forth in SEQ ID NOs: 87, 97, 102, respectively. In some embodiments, the 5T4 VHH domain provided herein contains a CDR1, CDR2, and CDR3 set forth in SEQ ID NOs: 87, 98, 102, respectively. In some embodiments, the 5T4 VHH domain provided herein contains a CDR1, CDR2, and CDR3 set forth in SEQ ID NOs: 87, 99, 102, respectively. In some embodiments, the 5T4 VHH domain provided herein contains a CDR1, CDR2, and CDR3 set forth in SEQ ID NOs: 86, 98, 102, respectively.

[0257] In some aspects, a VHH domain that binds 5T4 comprises a CDR1, CDR2 and CDR3 contained in a VHH amino acid sequences selected from any of SEQ ID NO:277-287, or an amino acid sequence that has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the V_HH region amino acid selected from any one of SEQ ID NOs: 277-287.

[0258] In some cases, the provided 5T4 VHH domain is a humanized variant that has the amino acid sequence set forth in any of SEQ ID NOS: 277-287 or an amino acid sequence that has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the V_HH region amino acid selected from any one of SEQ ID NOs: 277-287. In some embodimetns, the 5T4 humanized VHH domain has the sequence of amino acids set forth in any one of SEQ ID NOS: 277-287.

[0259] In some embodiments, a 5T4 VHH domain provided herein contains a CDR1, CDR2, CDR3 contained in a VHH domain set forth in SEQ ID NO: 294, or an amino acid sequence that has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the VHH region amino acid selected set forth in SEQ ID NO: 294. In some embodiments, the 5T4 VHH domain has the amino acid sequence set forth in SEQ ID NO: 294 or an amino acid sequence that has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the amino acid selected set forth in SEQ ID NO: 294. In some embodiments, the 5T4 VHH domain is a humanized variant of the amino acid sequence set forth in SEQ ID NO: 294.

[0260] In some embodiments, a 5T4 VHH domain provided herein contains a CDR1 set forth in SEQ ID NO: 296, a CDR2 set forth in SEQ ID NO: 298 and a CDR3 set forth in SEQ ID NO:.300.

[0261] In some embodiments, the 5T4 VHH domain provided herein contains a CDR1, CDR2, and CDR3 set forth in SEQ ID NO: 296, 298, 300, respectively.

[0262] In some embodiments, a 5T4 VHH domain provided herein contains a CDR1, CDR2, CDR3 contained in a VHH domain set forth in SEQ ID NO: 295, or an amino acid sequence that has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the VHH region amino acid selected set forth in SEQ ID NO: 295. In some embodiments, the 5T4 VHH domain has the amino acid sequence set forth in SEQ ID NO: 295 or an amino acid sequence that has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the amino acid selected set forth in SEQ ID NO: 295. In some embodiments, the 5T4 VHH domain is a humanized variant of the amino acid sequence set forth in SEQ ID NO: 295.

[0263] In some embodiments, a 5T4 VHH domain provided herein contains a CDR1 set forth in SEQ ID NO: 297, a CDR2 set forth in SEQ ID NO: 299 and a CDR3 set forth in SEQ ID NO:.301.

[0264] In some embodiments, the 5T4 VHH domain provided herein contains a CDR1, CDR2, and CDR3 set forth in SEQ ID NO: 297, 299, 301, respectively.

[0265] In some embodiments, a 5T4 VHH domain provided herein contains a CDR1, CDR2, CDR3 contained in a VHH domain set forth in SEQ ID NO: 302, or an amino acid sequence that has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the VHH region amino acid selected set forth in SEQ ID NO: 302. In some embodiments, the 5T4 VHH domain has the amino acid sequence set forth in SEQ ID NO: 302 or an amino acid sequence that has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the amino acid selected set forth in SEQ

ID NO: 302. In some embodiments, the 5T4 VHH domain is a humanized variant of the amino acid sequence set forth in SEQ ID NO: 302.

[0266] In some embodiments, a 5T4 VHH domain provided herein contains a CDR1 set forth in SEQ ID NO: 288, a CDR2 set forth in SEQ ID NO: 88 and a CDR3 set forth in SEQ ID NO:.303.

[0267] In some embodiments, the 5T4 VHH domain provided herein contains a CDR1, CDR2, and CDR3 set forth in SEQ ID NO: 288, 88, 303, respectively.

III. FUSION PROTEINS AND CONJUGATES CONTAINING 5T4-BINDING POLYPEPTIDES

[0268] Provided herein are fusion proteins and conjugates containing 5T4-binding polypeptides containing at least one VHH domain that specifically binds 5T4 linked, directly or indirectly, to one or more additional domains or moieties. In some embodiments, the fusion protein or conjugate of the present disclosure is composed of a single polypeptide. In other embodiments, the fusion protein or conjugate of the present disclosure is composed of more than one polypeptide. In some embodiments, the 5T4-binding polypeptide of the present disclosure incorporates at least one VHH domain that specifically binds 5T4. In some aspects, the 5T4-binding polypeptide is multivalent. In some embodiments, the 5T4-binding polypeptides include two or more copies of a VHH domain that specifically binds 5T4, for example, three or more, four or more, five or more, or six or more copies of a VHH domain that specifically binds 5T4. In certain aspects, the 5T4-binding polypeptide is multispecific. For example, in some cases, the one or more additional domain may be one or more additional binding domain that binds to one or more further antigen or protein.

[0269] In some embodiments, the 5T4-binding polypeptides of the present disclosure include two or more polypeptide sequences that are operably linked via amino acid linkers. In some embodiments, these linkers are composed predominately of the amino acids Glycine and Serine, denoted as GS-linkers herein. The GS-linkers of the fusion proteins of the present disclosure can be of various lengths, for example, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 amino acids in length. In some embodiments, the GS-linker comprises an amino acid sequence selected from the group consisting of GGSGGS, i.e., $(GGS)_2$ (SEQ ID NO: 1); GGSGGSGGS, i.e., $(GGS)_3$ (SEQ ID NO: 2); GGSGGSGGSGGS, i.e., $(GGS)_4$ (SEQ ID NO: 3); and GGSGGSGGSGGSGGS, i.e., $(GGS)_5$ (SEQ ID NO: 4). In some embodiments, the linker is a flexible linker comprising Glycine residues, such as, by way of non-limiting example, GG, GGG, GGGG (SEQ ID NO: 5), GGGGG (SEQ ID NO: 6), and GGGGGG (SEQ ID NO: 7). In some embodiments, the linker is(GGGGS)n, wherein n is 1 to 5 (SEQ ID NO:123); (GGGGGS)n, wherein n is 1 to 4 (SEQ ID NO:124); GGGGS (SEQ ID NO:125); GGGGGS (SEQ ID NO:126); GGGGGSGGGGGSGGGGGS (SEQ ID NO:127); GGGGSGGGGSGGGGS (SEQ ID NO:128); GGSGGGGSGGGGSGGGGS (SEQ ID NO:129); or PGGGG (SEQ ID NO:327). In some embodiments, the 5T4-binding polypeptide includes a combination of a GS-linker and a Glycine linker.

A. Fc Fusions

[0270] Provided herein is a 5T4-binding polypeptide that is a fusion protein containing at least one VHH domain that binds 5T4 provided herein and an Fc domain. In some embodiments, a 5T4-binding polypeptide provided herein comprises one, two, three, or four VHH domains that bind 5T4 and an Fc domain.

[0271] In some embodiments, incorporation of an immunoglobulin Fc region into the fusion protein can, in some aspects, be composed of two polypeptides that together form a dimer. In some embodiments, an Fc domain mediates dimerization of the 5T4-binding polypeptide at physiological conditions, such as when expressed from a cell, such that a dimer is formed that doubles the number of 5T4 binding sites. For example, a 5T4-binding polypeptide comprising three VHH domains that bind 5T4 and an Fc region is trivalent as a monomer, but the Fc region may mediate dimerization, such that the 5T4-binding polypeptide exists as a hexavalent dimer under such conditions. In some embodiments, a 5T4 VHH domain is fused to an IgG Fc region and in these embodiments,the fusion protein is bivalent having two 5T4 VHH domains per molecule. In some embodiments, two 5T4 binding domains (2x) are fused to an IgG Fc region and in these embodiments, the fusion protein is tetravalent having four 5T4 VHH domains per molecule. In some embodiments, three 5T4 VHH domain (3x) are fused to an IgG Fc region and in these embodiments, the fusion protein is hexavalent having six 5T4 VHH domains per molecule.

[0272] In some embodiments, the multivalent 5T4-binding polypeptide is bivalent. In some embodiments, the bivalent 5T4-binding polypeptide of the disclosure includes two copies of a 5T4-binding polypeptide having the following structure: (5T4 VHH)-Linker-Fc. In some embodiments, the multivalent 5T4-binding polypeptide is tetravalent. In some embodiments, the tetravalent 5T4-binding polypeptide of the disclosure includes two copies of a 5T4-polypeptide having the following structure: (5T4 VHH)-Linker-(5T4 VHH)-Linker- Fc. In some embodiments, the multivalent 5T4-binding polypeptide is hexavalent. In some embodiments, the hexavalent 5T4-binding polypeptide of the disclosure includes two copies of a 5T4-binding polypeptide having the following structure: (5T4 VHH)-Linker-(5T4 VHH)-Linker-(5T4 VHH)-Linker- Fc.

[0273] In some cases, the CH3 domain of the Fc region can be used as homodimerization domain, such that the resulting fusion protein is formed from two identical polypeptides. In other cases, the CH3 dimer interface region of the Fc region can be mutated so as to enable heterodimerization. For example, a heterodimerization domain can be incorporated into the fusion protein such that the construct is an asymmetric fusion protein.

[0274] In any of the provided embodiments, a 5T4 VHH domain can be any as described above. In come embodiments, the 5T4 VHH domain is a humanized VHH domain that binds 5T4.

[0275] In various embodiments, an Fc domain included in a 5T4-bindng polypeptide is a human Fc domain, or is derived from a human Fc domain. In some embodiments, the fusion protein contains an

immunoglobulin Fc region. In some embodiments, the immunoglobulin Fc region is an IgG isotype selected from the group consisting of IgG1 isotype, IgG2 isotype, IgG3 isotype, and IgG4 subclass.

[0276] In some embodiments, the immunoglobulin Fc region or immunologically active fragment thereof is an IgG isotype. For example, the immunoglobulin Fc region of the fusion protein is of human IgG1 isotype, having an amino acid sequence:

PAPELLGGPS VFLFPPKPKD TLMISRTPEV TCVVVDVSHE DPEVKFNWYV DGVEVHNAKT KPREEQYNST YRVVSVLTVL HQDWLNGKEY KCKVSNKALP APIEKTISKA KGQPREPQVY TLPPSRDELT KNQVSLTCLV KGFYPSDIAV EWESNGQPEN NYKTTPPVLD SDGSFFLYSK LTVDKSRWQQ GNVFSCSVMH EALHNHYTQK SLSLSPGK (SEQ ID NO: 8)

[0277] In some embodiments, the immunoglobulin Fc region or immunologically active fragment thereof comprises a human IgG1 polypeptide sequence that is at least 50%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO: 8.

[0278] In some embodiments where the fusion protein of the disclosure includes an Fc polypeptide, the Fc polypeptide is mutated or modified. In some cases, the mutations include one or more amino acid substitutions to reduce an effector function of the Fc polypeptide. Various examples of mutations to Fc polypeptides to alter, such as reduce, effector function are known, including any as described below. In some embodiments, reference to amino acid substitutions in an Fc region is by EU numbering by Kabat (also called Kabat numbering) unless described with reference to a specific SEQ ID NO. EU numbering is known and is according to the most recently updated IMGT Scientific Chart (IMGT®, the international ImMunoGeneTics information system®, http://www.imgt.org/IMGTScientificChart/Numbering/Hu_IGHGnber.html (created: 17 May 2001, last updated: 10 Jan 2013) and the EU index as reported in Kabat, E.A. et al. Sequences of Proteins of Immunological interest. 5th ed. US Department of Health and Human Services, NIH publication No. 91-3242 (1991).

[0279] In some embodiments, an Fc region that exhibits reduced effector functions may be a desirable candidate for applications in which 5T4 or CD3 binding is desired yet certain effector functions (such as CDC and ADCC) are unnecessary or deleterious. *In vitro* and/or *in vivo* cytotoxicity assays can be conducted to confirm the reduction/depletion of CDC and/or ADCC activities. For example, Fc receptor (FcR) binding assays can be conducted to ensure that the multispecific polypeptide constructs and/or cleaved components thereof lack FcγR binding (hence likely lacking ADCC activity), but retain FcRn binding ability. The primary cells for mediating ADCC, NK cells, express FcγRIII only, whereas monocytes express FcγRI, FcγRII and FcγRIII. Non-limiting examples of *in vitro* assays to assess ADCC activity of a molecule of interest is described in U.S. Pat. No. 5,500,362 (see, *e.g.,* Hellstrom, I. *et al.*

Proc. Nat'l Acad. Sci. USA 83:7059-7063 (1986)) and Hellstrom, I *et al.*, *Proc. Nat'l Acad. Sci. USA* 82:1499-1502 (1985); U.S. Pat. No. 5,821,337 (see Bruggemann, M. *et al.*, *J. Exp. Med.* 166:1351-1361 (1987)). Alternatively, non-radioactive assay methods may be employed (see, for example, ACTI™ non-radioactive cytotoxicity assay for flow cytometry (CellTechnology, Inc. Mountain View, Calif.; and CytoTox 96™ non-radioactive cytotoxicity assay (Promega, Madison, Wis.). Useful effector cells for such assays include peripheral blood mononuclear cells (PBMC) and Natural Killer (NK) cells. Alternatively, or additionally, ADCC activity of the molecule of interest may be assessed *in vivo*, *e.g.*, in an animal model such as that disclosed in Clynes *et al. Proc. Nat'l Acad. Sci. USA* 95:652-656 (1998). C1q binding assays may also be carried out to confirm that the multispecific polypeptide construct or cleaved components thereof is unable to bind C1q and hence lacks CDC activity. See, *e.g.*, C1q and C3c binding ELISA in WO 2006/029879 and WO 2005/100402. To assess complement activation, a CDC assay may be performed (see, for example, Gazzano-Santoro *et al.*, *J. Immunol. Methods* 202:163 (1996); Cragg, M. S. *et al.*, *Blood* 101:1045-1052 (2003); and Cragg, M. S. and M. J. Glennie, *Blood* 103:2738-2743 (2004)). FcRn binding and *in vivo* clearance/half-life determinations can also be performed using methods known in the art (see, *e.g.*, Petkova, S. B. *et al.*, *Int'l. Immunol.* 18(12):1759-1769 (2006)).

[0280] In some embodiments, the human IgG Fc region is modified to alter antibody-dependent cellular cytotoxicity (ADCC) and/or complement-dependent cytotoxicity (CDC), e.g., the amino acid modifications described in Natsume et al., 2008 Cancer Res, 68(10): 3863-72; Idusogie et al., 2001 J Immunol, 166(4): 2571-5; Moore et al., 2010 mAbs, 2(2): 181-189; Lazar et al., 2006 PNAS, 103(11): 4005–4010, Shields et al., 2001 JBC, 276(9): 6591–6604; Stavenhagen et al., 2007 Cancer Res, 67(18): 8882-8890; Stavenhagen et al., 2008 Advan. Enzyme Regul., 48: 152-164; Alegre et al, 1992 J Immunol, 148: 3461-3468; Reviewed in Kaneko and Niwa, 2011 Biodrugs, 25(1):1-11.

[0281] Examples of mutations that enhance ADCC include modification at Ser239 and Ile332, for example Ser239Asp and Ile332Glu (S239D, I332E). Examples of mutations that enhance CDC include modifications at Lys326 and Glu333. In some embodiments, the Fc region is modified at one or both of these positions, for example Lys326Ala and/or Glu333Ala (K326A and E333A) using the Kabat numbering system.

[0282] In some embodiments, the Fc region of the fusion protein is altered at one or more of the following positions to reduce Fc receptor binding: Leu 234 (L234), Leu235 (L235), Asp265 (D265), Asp270 (D270), Ser298 (S298), Asn297 (N297), Asn325 (N325), Ala327 (A327) or Pro329 (P329). For example, Leu 234Ala (L234A), Leu235Ala (L235A), Leu235Glu (L235E), Asp265Asn (D265N), Asp265Ala (D265A), Asp270Asn (D270N), Ser298Asn (S298N), Asn297Ala (N297A), Pro329Ala (P329A) or Pro239Gly (P329G), Asn325Glu (N325E) orAla327Ser (A327S). In preferred embodiments, modifications within the Fc region reduce binding to Fc-receptor-gamma receptors while have minimal impact on binding to the neonatal Fc receptor (FcRn).

[0283] In some embodiments, the human IgG1 Fc region is modified at amino acid Asn297 (Kabat Numbering) to prevent glycosylation of the fusion protein, e.g., Asn297Ala (N297A) or Asn297Asp (N297D). In some embodiments, the Fc region of the fusion protein is modified at amino acid Leu235 (Kabat Numbering) to alter Fc receptor interactions, e.g., Leu235Glu (L235E) or Leu235Ala (L235A). In some embodiments, the Fc region of the fusion protein is modified at amino acid Leu234 (Kabat Numbering) to alter Fc receptor interactions, e.g., Leu234Ala (L234A). In some embodiments, the Fc region of the fusion protein is modified at amino acid Leu234 (Kabat Numbering) to alter Fc receptor interactions, e.g., Leu235Glu (L235E). In some embodiments, the Fc region of the fusion protein is altered at both amino acids 234 and 235, e.g., Leu234Ala and Leu235Ala (L234A/L235A) or Leu234Val and Leu235Ala (L234V/L235A). In some embodiments, the Fc region of the fusion protein is altered at amino acids at 234, 235, and 297, e.g., Leu234Ala, Leu235Ala, Asn297Ala (L234A/L235A/N297A). In some embodiments, the Fc region of the fusion protein is altered at amino acids at 234, 235, and 329, e.g., Leu234Ala, Leu235Ala, Pro239Ala (L234A/L235A/P329A). In some embodiments, the Fc region of the fusion protein is modified at amino acid Asp265 (Kabat Numbering) to alter Fc receptor interactions, e.g Asp265Ala (D265A). In some embodiments, the Fc region of the fusion protein is modified at amino acid Pro329 (Kabat Numbering) to alter Fc receptor interactions, e.g Pro329Ala (P329A) or Pro329Gly (P329G). In some embodiments, the Fc region of the fusion protein is altered at both amino acids 265 and 329, e.g., Asp265Ala and Pro329Ala (D265A/P329A) or Asp265Ala and Pro329Gly (D265A/P329G). In some embodiments, the Fc region of the fusion protein is altered at amino acids at 234, 235, and 265, e.g., Leu234Ala, Leu235Ala, Asp265Ala (L234A/L235A/D265A). In some embodiments, the Fc region of the fusion protein is altered at amino acids at 234, 235, and 329, e.g., Leu234Ala, Leu235Ala, Pro329Gly (L234A/L235A/P329G). In some embodiments, the Fc region of the fusion protein is altered at amino acids at 234, 235, 265 and 329, e.g., Leu234Ala, Leu235Ala, Asp265Ala, Pro329Gly (L234A/L235A/D265A/P329G). In some embodiments, the Fc region of the fusion protein is altered at Gly235 to reduce Fc receptor binding. For example, wherein Gly235 is deleted from the fusion protein. In some embodiments, the human IgG1 Fc region is modified at amino acid Gly236 to enhance the interaction with CD32A, e.g., Gly236Ala (G236A). In some embodiments, the human IgG1 Fc region lacks Lys447 (EU index of Kabat et al 1991 Sequences of Proteins of Immunological Interest).

[0284] In some embodiments, the Fc region of the fusion protein is lacking an amino acid at one or more of the following positions to reduce Fc receptor binding: Glu233 (E233), Leu234 (L234), or Leu235 (L235).). In some embodiments, the Fc region of the fusion protein is lacking an amino acid at one or more of the following positions Glu233 (E233), Leu234 (L234), or Leu235 (L235) and is modified at one or more of the Asp265 (D265), Asn297 (N297), or Pro329 (P329) to reduce Fc receptor binding. For example, an Fc region included in a 5T4-bindng polypeptide is derived from a human Fc domain, and comprises a three amino acid deletion in the lower hinge corresponding to IgG1 E233,

L234, and L235. In some aspects, such Fc polypeptides do not engage FcγRs and thus are referred to as "effector silent" or "effector null." For example, Fc deletion of these three amino acids reduces the complement protein C1q binding. In some embodiments, a polypeptide with an Fc region with Fc deletion of these three amino acids retains binding to FcRn and therefore has extended half-life and transcytosis associated with FcRn mediated recycling. Such a modified Fc region is referred to as "Fc xELL" or "Fc deletion" and has the following amino acid sequence:

PAPGGPSVFL FPPKPKDTLM ISRTPEVTCV VVDVSHEDPE VKFNWYVDGV EVHNAKTKPR EEQYNSTYRV VSVLTVLHQD WLNGKEYKCK VSNKALPAPI EKTISKAKGQ PREPQVYTLP PSRDELTKNQ VSLTCLVKGF YPSDIAVEWE SNGQPENNYK TTPPVLDSDG SFFLYSKLTV DKSRWQQGNV FSCSVMHEAL HNHYTQKSLS LSPGK (SEQ ID NO: 9)

[0285] In some embodiments, the immunoglobulin Fc region or immunologically active fragment thereof comprises a human IgG1 polypeptide sequence that is at least 50%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO: 9.

[0286] In some embodiments, the human IgG Fc region is modified to enhance FcRn binding. Examples of Fc mutations that enhance binding to FcRn are Met252Tyr, Ser254Thr, Thr256Glu (M252Y, S254T, T256E, respectively) (Kabat numbering, Dall'Acqua et al 2006, J. Biol Chem Vol. 281(33) 23514–23524), Met428Leu and Asn434Ser (M428L, N434S) (Zalevsky et al 2010 Nature Biotech, Vol. 28(2) 157-159), or Met252Ile, Thr256Asp, Met428Leu (M252I, T256D, M428L, respectively), (EU index of Kabat et al 1991 Sequences of Proteins of Immunological Interest).

[0287] In some embodiments, the Fc domain included in a 5T4-bindng polypeptide is derived from a human Fc domain and comprises mutations M252Y and M428V, herein referred to as "Fc-YV". In some embodiments, the mutated or modified Fc polypeptide includes the following mutations: M252Y and M428L using the Kabat numbering system. In some embodiments, such mutations enhance binding to FcRn at the acidic pH of the endosome (near 6.5), while losing detectable binding at neutral pH (about 7.2), allowing for enhanced FcRn mediated recycling and extended half-life.

[0288] In some embodiments, the Fc domain included in a 5T4-binding polypeptide is derived from a human Fc domain and comprises mutations to induce heterodimerization. In some embodiments, such mutations include those referred to as "knob" and "hole" mutations. For example, having an amino acid modification within the CH3 domain at Thr366, which when replaced with a more bulky amino acid, e.g., Try (T366W), is able to preferentially pair with a second CH3 domain having amino acid modifications to less bulky amino acids at positions Thr366, Leu368, and Tyr407, e.g., Ser, Ala and Val, respectively (T366S/L368A/Y407V). In some embodiments, the "knob" Fc domain comprises the mutation T366W. In some embodiments, the "hole" Fc domain comprises mutations T366S, L368A, and

Y407V. Heterodimerization via CH3 modifications can be further stabilized by the introduction of a disulfide bond, for example by changing Ser354 to Cys (S354C) and Y349 to Cys (Y349C) on opposite CH3 domains (Reviewed in Carter, 2001 Journal of Immunological Methods, 248: 7–15). In some embodiments, Fc domains used for heterodimerization comprise additional mutations, such as the mutation S354C on a first member of a heterodimeric Fc pair that forms an asymmetric disulfide with a corresponding mutation Y349C on the second member of a heterodimeric Fc pair. In some embodiments, one member of a heterodimeric Fc pair comprises the modification H435R or H435K to prevent protein A binding while maintaining FcRn binding. In some embodiments, one member of a heterodimeric Fc pair comprises the modification H435R or H435K, while the second member of the heterodimeric Fc pair is not modified at H435. In various embodiments, the hole Fc domain comprises the modification H435R or H435K (referred to as "hole-R" in some instances when the modification is H435R), while the knob Fc domain does not. In some instances, the hole-R mutation improves purification of the heterodimer over homodimeric hole Fc domains that may be present.

[0289] In some embodiments, the human IgG Fc region is modified to prevent dimerization. In these embodiments, the fusion proteins of the present disclosure are monomeric. For example modification at residue Thr366 to a charged residue, e.g. Thr366Lys, Thr366Arg, Thr366Asp, or Thr366Glu (T366K, T366R, T366D, or T366E, respectively), prevents CH3-CH3 dimerization.

[0290] In some embodiments, the immunoglobulin Fc region or immunologically active fragment of the fusion protein is of human IgG2 isotype, having an amino acid sequence:

PAPPVAGPSV FLFPPKPKDT LMISRTPEVT CVVVDVSHED PEVQFNWYVD GVEVHNAKTK PREEQFNSTF RVVSVLTVVH QDWLNGKEYK CKVSNKGLPA PIEKTISKTK GQPREPQVYT LPPSREEMTK NQVSLTCLVK GFYPSDISVE WESNGQPENN YKTTPPMLDS DGSFFLYSKL TVDKSRWQQG NVFSCSVMHE ALHNHYTQKS LSLSPGK (SEQ ID NO: 10)

[0291] In some embodiments, the fusion or immunologically active fragment thereof comprises a human IgG2 polypeptide sequence that is at least 50%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO: 10.

[0292] In some embodiments, the human IgG2 Fc region is modified at amino acid Asn297 (e.g. to prevent to glycosylation of the antibody, e.g., Asn297Ala (N297A) or Asn297Asp (N297D). In some embodiments, the human IgG2 Fc region is lacks Lys447 (EU index of Kabat et al 1991 Sequences of Proteins of Immunological Interest).

[0293] In some embodiments, the immunoglobulin Fc region or immunologically active fragment of the fusion protein is of human IgG3 isotype, having an amino acid sequence:

PAPELLGGPS VFLFPPKPKD TLMISRTPEV TCVVVDVSHE DPEVQFKWYV DGVEVHNAKT KPREEQYNST FRVVSVLTVL HQDWLNGKEY KCKVSNKALP APIEKTISKT KGQPREPQVY TLPPSREEMT KNQVSLTCLV KGFYPSDIAV EWESSGQPEN NYNTTPPMLD SDGSFFLYSK LTVDKSRWQQ GNIFSCSVMH EALHNRFTQK SLSLSPGK (SEQ ID NO: 11)

[0294] In some embodiments, the antibody or immunologically active fragment thereof comprises a human IgG3 polypeptide sequence that is at least 50%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO: 11.

[0295] In some embodiments, the human IgG3 Fc region is modified at amino acid Asn297 (Kabat Numbering) to prevent to glycosylation of the antibody, e.g., Asn297Ala (N297A) or Asn297Asp (N297D). In some embodiments, the human IgG3 Fc region is modified at amino acid 435 to extend the half-life, e.g., Arg435His (R435H). In some embodiments, the human IgG3 Fc region is lacks Lys447 (EU index of Kabat et al 1991 Sequences of Proteins of Immunological Interest).

[0296] In some embodiments, the immunoglobulin Fc region or immunologically active fragment of the fusion protein is of human IgG4 isotype, having an amino acid sequence:

PAPEFLGGPS VFLFPPKPKD TLMISRTPEV TCVVVDVSQE DPEVQFNWYV DGVEVHNAKT KPREEQFNST YRVVSVLTVL HQDWLNGKEY KCKVSNKGLP SSIEKTISKA KGQPREPQVY TLPPSQEEMT KNQVSLTCLV KGFYPSDIAV EWESNGQPEN NYKTTPPVLD SDGSFFLYSR LTVDKSRWQE GNVFSCSVMH EALHNHYTQK SLSLSLGK (SEQ ID NO: 12)

[0297] In some embodiments, the antibody or immunologically active fragment thereof comprises a human IgG4 polypeptide sequence that is at least 50%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO: 12.

[0298] In some embodiments, the immunoglobulin Fc region or immunologically active fragment of the fusion protein is of human IgG4 isotype, having an amino acid sequence:

PAPELLGGPS VFLFPPKPKD TLMISRTPEV TCVVVDVSQE DPEVQFNWYV DGVEVHNAKT KPREEQFNST YRVVSVLTVL HQDWLNGKEY KCKVSNKGLP SSIEKTISKA KGQPREPQVY TLPPSQEEMT KNQVSLTCLV KGFYPSDIAV EWESNGQPEN NYKTTPPVLD SDGSFFLYSR LTVDKSRWQE GNVFSCSVMH EALHNHYTQK SLSLSLGK (SEQ ID NO: 13)

[0299] In some embodiments, the antibody or immunologically active fragment thereof comprises a human IgG4 polypeptide sequence that is at least 50%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO: 13.

[0300] In some embodiments, the human IgG4 Fc region is modified at amino acid 235 to alter Fc receptor interactions, e.g., Leu235Glu (L235E). In some embodiments, the human IgG4 Fc region is modified at amino acid Asn297 (Kabat Numbering) to prevent to glycosylation of the antibody, e.g., Asn297Ala (N297A) or Asn297Asp (N297D). In some embodiments, the human IgG4 Fc region is lacks Lys447 (EU index of Kabat et al 1991 Sequences of Proteins of Immunological Interest).

[0301] In some embodiments, the fusion protein contains a polypeptide derived from an immunoglobulin hinge region. The hinge region can be selected from any of the human IgG subclasses. For example, the fusion protein may contain a modified IgG1 hinge having the sequence of EPKSSDKTHTCPPC (SEQ ID NO: 14), where in the Cys220 that forms a disulfide with the C-terminal cysteine of the light chain is mutated to serine, e.g., Cys220Ser (C220S). In other embodiments, the fusion protein contains a truncated hinge having a sequence DKTHTCPPC (SEQ ID NO: 15).

[0302] In some embodiments, the fusion protein has a modified hinge from IgG4, which is modified to prevent or reduce strand exchange, e.g., Ser228Pro (S228P), having the sequence ESKYGPPCPPC (SEQ ID NO: 16). In some embodiments, the fusion protein contains linker polypeptides. In other embodiments, the fusion protein contains linker and hinge polypeptides.

[0303] In some embodiments, the Fc region lacks or has reduced Fucose attached to the N-linked glycan-chain at N297. There are numerous ways to prevent fucosylation, including but not limited to production in a FUT8 deficient cell line; addition inhibitors to the mammalian cell culture media, for example Castanospermine; and metabolic engineering of the production cell line.

[0304] In some embodiments, the Fc region is engineered to eliminate recognition by pre-existing antibodies found in humans. In some embodiments, VHH-containing polypeptides of the present disclosure are modified by mutation of position Leu11, for example Leu11Glu (L11E) or Leu11Lys (L11K). In other embodiments, single domain antibodies of the present disclosure are modified by changes in carboxy-terminal region, for example the terminal sequence has the sequence GQGTLVTVKPGG (SEQ ID NO: 17) or GQGTLVTVEPGG (SEQ ID NO: 18) or modification thereof. In some embodiments, the VHH-containing polypeptides of the present disclosure are modified by mutation of position 11 and by changes in carboxy-terminal region.

[0305] In some embodiments, the one or more polypeptides of the fusion proteins of the present disclosure are operably linked via amino acid linkers. In some embodiments, these linkers are composed predominately of the amino acids Glycine and Serine, denoted as GS-linkers herein. In some embodiments, these linkers are composed predominately of the amino acids Glycine, denoted as glycine linkers herein. The GS-linkers or glycine linkers of the fusion proteins of the present disclosure can be of various lengths, for example, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 amino acids in length.

[0306] In some embodiments, the GS-linker comprises an amino acid sequence selected from the group consisting of GGSGGS, i.e., $(GGS)_2$ (SEQ ID NO: 1); GGSGGSGGS, i.e., $(GGS)_3$ (SEQ ID NO: 2); GGSGGSGGSGGS, i.e., $(GGS)_4$ (SEQ ID NO: 3); and GGSGGSGGSGGSGGS, i.e., $(GGS)_5$

(SEQ ID NO: 4). In some embodiments, the linker is a flexible linker is a glycine linker comprising Glycine residues, such as, by way of non-limiting example, GG, GGG, GGGG (SEQ ID NO: 5), GGGGG (SEQ ID NO: 6), and GGGGGG (SEQ ID NO: 7). In some embodiments, the linker is(GGGGS)n, wherein n is 1 to 5 (SEQ ID NO:123); (GGGGGS)n, wherein n is 1 to 4 (SEQ ID NO:124); GGGGS (SEQ ID NO:125); GGGGGS (SEQ ID NO:126); GGGGGSGGGGGSGGGGGS (SEQ ID NO:127); GGGGSGGGGSGGGGS (SEQ ID NO:128); GGSGGGGSGGGGSGGGGS (SEQ ID NO:129); or PGGGG (SEQ ID NO:327). In some embodiments, the fusion proteins can include a combination of a GS-linker and a Glycine linker.

B. Conjugates

[0307] Provided herein are conjugates containing at least one VHH domain that specifically binds 5T4 provided herein and one or more further moiety. The further moiety can be a therapeutic agent, such as a cytotoxic agent, or can be a detection agent. In some embodiments, the moiety can be a targeting moiety, a small molecule drug (non-polypeptide drug of less than 500 Daltons molar mass), a toxin, a cytostatic agent, a cytotoxic agent, an immunosuppressive agent, a radioactive agent suitable for diagnostic purposes, a radioactive metal ion for therapeutic purposes, a prodrug-activating enzyme, an agent that increases biological half-life, or a diagnostic or detectable agent.

[0308] In some embodiments, the conjugate is an antibody drug conjugate (ADC, also called immunoconjugates) containing one or more 5T4 VHH domain provided herein conjugated to a therapeutic agent, which is either cytotoxic, cytostatic or otherwise provides some therapeutic benefit. In some embodiments, the cytotoxic agent is a chemotherapeutic agent, a drug, a growth inhibitory agent, a toxin (e.g., an enzymatically active toxin of bacterial, fungal, plant, or animal origin, or fragments thereof), or a radioactive isotope (i.e., a radioconjugate). In some embodiments, provided antibody drug conjugates of the present disclosure allow targeted-delivery of the drug moiety to tumors. In some cases, this can result in targeted killing of the tumor cell.

[0309] In some embodiments, there is provided a 5T4-binding conjugate comprising at least one 5T4 VHH domain provided herein conjugated with a therapeutic agent. In some embodiments, the therapeutic agent includes, for example, daunomycin, doxorubicin, methotrexate, and vindesine (Rowland et al., Cancer Immunol. Immunother. 21:183-187, 1986). In some embodiments, the therapeutic agent has an intracellular activity. In some embodiments, the 5T4-binding conjugate is internalized and the therapeutic agent is a cytotoxin that blocks the protein synthesis of the cell, therein leading to cell death. In some embodiments, the therapeutic agent is a cytotoxin comprising a polypeptide having ribosome-inactivating activity including, for example, gelonin, bouganin, saporin, ricin, ricin A chain, bryodin, diphtheria toxin, restrictocin, Pseudomonas exotoxin A and variants thereof. In some embodiments, where the therapeutic agent is a cytotoxin comprising a polypeptide having a ribosome-

inactivating activity, the 5T4-binding conjugate must be internalized upon binding to the target cell in order for the protein to be cytotoxic to the cells.

[0310] In some embodiments, there is provided a 5T4-binding conjugate comprising at least one 5T4 VHH domain provided herein conjugated with a toxin. In some embodiments, the toxin includes, for example, bacterial toxins such as diphtheria toxin, plant toxins such as ricin, small molecule toxins such as geldanamycin (Mandler et al., J. Nat. Cancer Inst. 92(19):1573-1581 (2000); Mandler et al., Bioorganic & Med. Chem. Letters 10:1025- 1028 (2000); Mandler et al., Bioconjugate Chem. 13:786-791 (2002)), maytansinoids (EP 1391213; Liu et al., Proc. Natl. Acad. Sci. USA 93:8618-8623 (1996)), and calicheamicin (Lode et al., Cancer Res. 58:2928 (1998); Hinman et al., Cancer Res. 53:3336-3342 (1993)). The toxins may exert their cytotoxic and cytostatic effects by mechanisms including tubulin binding, DNA binding, or topoisomerase inhibition.

[0311] In some embodiments, there is provided a 5T4-binding conjugate comprising at least one 5T4 VHH domain provided herein conjugated with a label, which can generate a detectable signal, indirectly or directly. These 5T4-binding conjugates can be used for research or diagnostic applications, such as for the in vivo detection of cancer. The label is preferably capable of producing, either directly or indirectly, a detectable signal. For example, the label may be radio-opaque or a radioisotope, such as 3H, 14C, 32P, 35S, 123I, 125I, 131I; a fluorescent (fluorophore) or chemiluminescent (chromophore) compound, such as fluorescein isothiocyanate, rhodamine or luciferin; an enzyme, such as alkaline phosphatase, β-galactosidase or horseradish peroxidase; an imaging agent; or a metal ion. In some embodiments, the label is a radioactive atom for scintigraphic studies, for example 99Tc or 123I, or a spin label for nuclear magnetic resonance (NMR) imaging (also known as magnetic resonance imaging, MRI), such as zirconium-89, iodine-123, iodine-131, indium-111, fluorine-19, carbon-13, nitrogen-15, oxygen-17, gadolinium, manganese or iron. Zirconium-89 may be complexed to various metal chelating agents and conjugated to antibodies, e.g., for PET imaging (WO 2011/056983).

[0312] The 5T4-binding conjugates may be prepared using any methods known in the art. See, e.g., WO 2009/067800, WO 2011/133886, and U.S. Patent Application Publication No. 2014322129, incorporated by reference herein in their entirety.

[0313] In some embodiments, the attachment can be covalent or non-covalent, e.g., via a biotin-streptavidin non-covalent interaction. In some embodiments, 1, 2, 3, 4, 5 or more moieties, which can be the same or different, are conjugated, linked or fused to a 5T4 VHH domain to form a 5T4-binding conjugate. In some embodiments, such moieties can be attached to the VHH domain using various molecular biological or chemical conjugation and linkage methods known in the art and described below. In some embodiments, linkers such as peptide linkers, cleavable linkers, non-cleavable linkers or linkers that aid in the conjugation reaction, can be used to link or conjugate the effector moieties to the variant polypeptide or immunomodulatory protein.

[0314] In some embodiments, a 5T4 VHH domain is conjugated to one or more moieties, e.g. about 1 to about 20 drug moieties per VHH, through a linker (L). In some embodiments, the 5T4-binding conjugate comprises the following components: (VHH domain), $(L)_q$ and $(moiety)_m$, wherein the VHH domain is any of the described VHH domains capable of specifically binding 5T4 as described; L is a linker for linking the protein or polypeptide to the moiety; m is at least 1; q is 0 or more; and the resulting 5T4-binding conjugate binds to 5T4. In particular embodiments, m is 1 to 4 and q is 0 to 8.

[0315] The linker may be composed of one or more linker components. For covalent attachment of the antibody and the drug moiety the linker typically has two reactive functional groups, i.e. bivalency in a reactive sense. Bivalent linker reagents which are useful to attach two or more functional or biologically active moieties, such as peptides, nucleic acids, drugs, toxins, antibodies, haptens, and reporter groups are known, and methods have been described their resulting conjugates (Hermanson, G. T. (1996) Bioconjugate Techniques; Academic Press: New York, p 234-242).

[0316] Exemplary linker components include 6-maleimidocaproyl ("MC"), maleimidopropanoyl ("MP"), valine-citrulline ("val-cit"), a alanine-phenylalanine ("ala-phe"), p-aminobenzyloxycarbonyl ("PAB"), N-Succinimidyl 4-(2-pyridylthio)pentanoate ("SPP"), N-Succinimidyl 4-(N-maleimidomethyl)cyclohexane-I carboxylate ("SMCC"), and N-Succinimidyl (4-iodo-acetyl)aminobenzoate ("SIAB").

[0317] In some embodiments, the linker may comprise amino acid residues. Exemplary amino acid linker components include a dipeptide, a tripeptide, a tetrapeptide or a pentapeptide. Exemplary dipeptides include: valine-citrulline (vc or val-cit), alanine-phenylalanine (af or ala-phe). Exemplary tripeptides include: glycine-valine-citrulline (gly-val-cit) and glycine-glycine-glycine (gly-gly-gly). Amino acid residues which comprise an amino acid linker component include those occurring naturally, as well as minor amino acids and non-naturally occurring amino acid analogs, such as citrulline. Amino acid linker components can be designed and optimized in their selectivity for enzymatic cleavage by a particular enzymes, for example, a tumor-associated protease, cathepsin B, C and D, at a plasmin protease.

[0318] Conjugates of a VHH domain and cytotoxic agent can be made using a variety of bifunctional protein-coupling agents such as N-succinimidyl-3-(2-pyridyldithiol) propionate (SPDP), iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCl), active esters (such as disuccinimidyl substrate), aldehydes (such as glutaraldehyde), bis-azido compounds (such as bis(p-azidobenzoyl) hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as toluene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene).

[0319] The antibody drug conjugate can be prepared by a variety of methods, such as organic chemistry reactions, conditions, and reagents known to those skilled in the art. In one embodiments, methods include: (1) reaction of a nucleophilic group of a VHH domain with a bivalent linker reagent, to

form VHH-L, via a covalent bond, followed by reaction with a drug moiety D; and (2) reaction of a nucleophilic group of a drug moiety with a bivalent linker reagent, to form D-L, via a covalent bond, followed by reaction with the nucleophilic group of a VHH domain.

[0320] Nucleophilic groups on antibodies, including VHH domains, include, but are not limited to: (i) N-terminal amine groups, (ii) side chain amine groups, e.g. lysine, (iii) side chain thiol groups, e.g. cysteine, and (iv) sugar hydroxyl or amino groups where the antibody is glycosylated. Amine, thiol, and hydroxyl groups are nucleophilic and capable of reacting to form covalent bonds with electrophilic groups on linker moieties and linker reagents including: (i) active esters such as NHS esters, HOBt esters, haloformates, and acid halides; (ii) alkyl and benzyl halides such as haloacetamides; (iii) aldehydes, ketones, carboxyl, and maleimide groups. Additional nucleophilic groups can be introduced into antibodies through the reaction of lysines with 2-iminothiolane (Traut's reagent) resulting in conversion of an amine into a thiol. Reactive thiol groups may be introduced into the antibody (or fragment thereof) by introducing one, two, three, four, or more cysteine residues (e.g., preparing mutant antibodies comprising one or more non-native cysteine amino acid residues).

[0321] Conjugates, such as antibody drug conjugates, may also be produced by modification of an antibody, such as a VHH domain, to introduce electrophilic moieties, which can react with nucleophilic substituents on the linker reagent or drug. The sugars of glycosylated antibodies may be oxidized, e.g., with periodate oxidizing reagents, to form aldehyde or ketone groups which may lead with the amine group of linker reagents or drug moieties. The resulting imine Schiff base groups may form a stable linkage, or may be reduced, e.g., by borohydride reagents to form stable amine linkages. In one embodiment, reaction of the carbohydrate portion of a glycosylated antibody with either glactose oxidase or sodium meta-periodate may yield carbonyl (aldehyde and ketone) groups in the protein that can react with appropriate groups on the drug (Hermanson, Bioconjugate Techniques). In another embodiment, proteins containing N-terminal serine or threonine residues can react with sodium meta-periodate, resulting in production of an aldehyde in place of the first amino acid. Such aldehyde can be reacted with a drug moiety or linker nucleophile.

[0322] Likewise, nucleophilic groups on a drug moiety include, but are not limited to: amine, thiol, hydroxyl, hydrazide, oxime, hydrazine, thiosemicarbazone, hydrazine carboxylate, and arylhydrazide groups capable of reacting to form covalent bonds with electrophilic groups on linker moieties and linker reagents including: (i) active esters such as NHS esters, HOBi esters, haloformates, and acid halides; (ii) alkyl and benzyl halides such as haloacetamides; (iii) aldehydes, ketones, carboxyl, and maleimide groups.

[0323] Alternatively, a fusion protein containing a VHH domain and cytotoxic agent may be made, e.g., by recombinant techniques or peptide synthesis. The length of DNA may comprise respective regions encoding the two portions of the conjugate either adjacent one another or separated by a region encoding a linker peptide which does not destroy the desired properties of the conjugate.

C. Multispecific Formats

[0324] Provided herein are 5T4-binding polypeptides that are multispecific containing at least one VHH domain that binds 5T4 and one or more additional binding domains. Typically, the one or more additional domains bind to a second antigen or protein other than 5T4. In some embodiments, the one or more additional domain is an antibody or antigen-binding fragment specific for the second antigen or protein. In some embodiments, the additional domain is a VHH domain.

[0325] In some embodiments, a multispecific 5T4-binding polypeptide comprises at least one VHH domain that binds 5T4 and at least one additional binding domain that binds a second antigen or protein. In some embodiments, this second antigen is a tumor associated antigen (TAA) or tumor microenvironment associated antigen (TMEAA). In some embodiments, this second antigen is an immunomodulatory antigen, wherein said antigen is involved with enhancing or dampening a signaling pathway in an immune cell. In some embodiments, this second antigen is a tumor associated antigen (TAA) or tumor microenvironment associated antigen (TMEAA). In some embodiments, this second antigen is an immunomodulatory antigen, wherein said antigen is involved with enhancing or dampening a signaling pathway in an immune cell.

[0326] In some cases, a multispecific 5T4-binding polypeptide can further contain an Fc domain, such as any described above. In some embodiments, a multispecific 5T4-binding polypeptide provided herein at least one VHH domains that bind 5T4, at least one additional binding domain that binds a second antigen or protein, and an Fc domain. In some embodiments, an Fc domain mediates dimerization of the multispecific 5T4-binding polypeptide at physiological conditions such that a dimer is formed that doubles the number of binding sites for 5T4 and for the additional antigen or protein.

[0327] Non-limiting exemplary multispecific 5T4-binding polypeptides are described below.

1. Bispecific T cells Engager

[0328] In some embodiments, the 5T4-binding polypeptide is a bispecific construct that is or comprises at least one 5T4 VHH domain provided herein and at least one additional binding molecule capable of binding to a surface molecule expressed on a T cell. In some embodiments, the surface molecule is an activating component of a T cell, such as a component of the T cell receptor complex. In particular aspects, the surface molecule is an activating T cell antigen that is expressed on a T cell and is capable of inducing T cell activation upon interaction with an antigen binding molecule. For example, in some aspects, interaction of an antigen binding molecule with an activating T cell antigen may induce T cell activation by triggering the signaling cascade of the T cell receptor complex. Suitable assays to measure T cell activation are known, and include any assay to measure or assess proliferation, differentiation, cytokine secretion, cytotoxic activity and/or expression of one or more activation marker. In some embodiments, the simultaneous or near simultaneous binding of such a 5T4-binding polypeptide to both of its targets, 5T4 expressed on target cell and a T cell molecule expressed on a T cell, e.g.

activating T cell antigen, can result in a temporary interaction between the target cell and T cell, thereby resulting in activation, *e.g.* cytotoxic activity, of the T cell and subsequent lysis of the target cell.

[0329] In some embodiments, the T surface molecule, such as activating T cell antigen, is CD3 or is CD2. Specifically, a provided bispecific 5T4-binding polypeptide is capable of specifically binding an activating T cell antigen expressed on a human T cell, such as human CD3 or human CD3. In particular aspects, the additional binding domain that is specific to the activating T cell antigen (e.g. CD3 or CD2) is an antibody or antigen-binding fragment. In some embodiments, a 5T4-binding polypeptide can be a bispecific antibody T cell-engager containing at least one 5T4 VHH domain that specifically binds to 5T4 and an additional binding molecule that is an antibody or antigen-binding fragment specific for an activating component of a T cell (*e.g.* a T cell surface molecule, *e.g.* CD3 or CD2).

[0330] Among bispecific antibody T cell-engagers are bispecific T cell engager (BiTE) molecules, which contain tandem scFv molecules fused by a flexible linker (see e.g. Nagorsen and Bauerle, Exp Cell Res 317, 1255-1260 (2011); tandem scFv molecules fused to each other via, e.g. a flexible linker, and that further contain an Fc domain composed of a first and a second subunit capable of stable association (WO2013026837); diabodies and derivatives thereof, including tandem diabodies (Holliger et al, Prot Eng 9, 299-305 (1996); Kipriyanov et al, J Mol Biol 293, 41-66 (1999)); dual affinity retargeting (DART) molecules that can include the diabody format with a C-terminal disulfide bridge; or triomabs that include whole hybrid mouse/rat IgG molecules (Seimetz et al, Cancer Treat Rev 36, 458-467 (2010). Similar formats of any of the above molecules can be generated using any of the 5T4 VHH domains provided herein.

[0331] In some embodiments, the additional binding domain specific to an activating T cell antigen is an antigen-binding fragment selected from a Fab fragment, a $F(ab')_2$ fragment, an Fv fragment, a scFv, disulfide stabilized Fv fragment (dsFv), a scAb, a dAb, a single domain heavy chain antibody (VHH), or a single domain light chain antibody. In some embodiments, the additional binding domain is monovalent for binding the activating T cell antigen, such as CD2 or CD3.

[0332] In some embodiments, the additional binding domain is capable of binding to CD3 or a CD3 complex. A CD3 complex is a complex of at least five membrane-bound polypeptides in mature T-lymphocytes that are non-covalently associated with one another and with the T-cell receptor. The CD3 complex includes the gamma, delta, epsilon, zeta, and eta chains (also referred to as subunits). In some embodiments, the additional binding molecule is an antibody or antigen-binding fragment capable of specifically binding to CD3 or a CD3 complex, also called a CD3-binding domain. In some embodiments, the CD3-binding domain capable of binding CD3 or a CD3 complex includes one or more copies of an anti-CD3 Fab fragment, an anti-CD3 $F(ab')_2$ fragment, an anti-CD3 Fv fragment, an anti-CD3 scFv, an anti-CD3 dsFv, an anti-CD3 scAb, an anti-CD3 dAb, an anti-CD3 single domain heavy chain antibody (VHH), and an anti-CD3 single domain light chain antibody. In some embodiments, the anti-CD3 binding domain is monovalent for binding CD3.

[0333] In some cases, the CD3-binding domain recognizes the CD3ε-chain. In some embodiments, the anti-CD3ε binding domain includes one or more copies of an anti-CD3ε Fab fragment, an anti-CD3ε $F(ab')_2$ fragment, an anti-CD3ε Fv fragment, an anti-CD3ε scFv, an anti-CD3ε dsFv, an anti-CD3ε scAb, an anti-CD3ε dAb, an anti-CD3ε single domain heavy chain antibody (VHH), and an anti-CD3ε single domain light chain antibody. In some embodiments, the anti-CD3ε binding domain is monovalent for binding CD3ε.

[0334] Exemplary monoclonal antibodies against CD3 or a CD3 complex include, but are not limited to, OKT3, SP34, UCHT1 or 64.1, or an antigen-binding fragment thereof (See e.g., June, et al., J. Immunol. 136:3945-3952 (1986); Yang, et al., J. Immunol. 137:1097-1100 (1986); and Hayward, et al., Immunol. 64:87-92 (1988)). In some aspects, clustering of CD3 on T cells, e.g., by immobilized or cell-localized or tethered anti-CD3-antibodies, leads to T cell activation similar to the engagement of the T cell receptor but independent from its clone typical specificity. In one embodiment, the CD3-binding domain monovalently and specifically binds a CD3 antigen, and is derived from OKT3 (ORTHOCLONE-OKT3™ (muromonab-CD3); humanized OKT3 (U.S. Pat. No. 7,635,475 and published international application No. WO2005040220); SP34 (Pessano et ai. The EMBO Journal. 4: 337-344, 1985); humanized variant of SP34 (WO2015001085); Teplizumab™ (MGA031, Eli Lilly); an anti-CD3 binding molecule described in US2011/0275787; UCHT1 (Pollard et al. 1987 J Histochem Cytochem. 35(11):1329-38; WO2000041474); NI0401 (WO2007/033230); visilizumab (US Patent No. 5,834,597); BC-3 (Anasetti et al., Transplantation 54: 844 (1992); H2C (described in PCT publication no. WO2008/119567); V9 (described in Rodrigues et al., Int J Cancer Suppl 7, 45-50 (1992) and U.S. Pat. No. 6,054,297)). Other anti-CD3 antibodies also can be used in the constructs provided herein, including any described in International published PCT application Nos. WO199404679, WO2008119567, WO2015095392, WO2016204966, WO2019133761; published patent application Nos. US20170369563, US20180194842, US20180355038; U.S. Patent Nos. 7,728,114, 7,381,803, 7,994,289.

[0335] In some embodiments, the CD3-binding domain contains a variable heavy (VH) chain set forth in SEQ ID NO:19 and/or a variable light chain set forth in SEQ ID NO:20, or VH and/or VL sequences having at least 60%, 70%, 80%, 90%, 95%, 97%, 98%, 99% identity with these sequences and specifically binds CD3. In some embodiments, the CD3-binding domain contains a CDRH1, CDRH2 and CDRH3 of the variable heavy (VH) chain set forth in SEQ ID NO:19 and a CDRL1, CDRL2 and CDRL3 variable light chain set forth in SEQ ID NO:20. In some cases, the CD3-binding region comprisies a humanized version of the VH sequence set forth in SEQ ID NO:19 and a humanized version of the VL sequence set forth in SEQ ID NO:20. In some embodiments a CD3-binding region can contain a humanized OKT3 derived VH domain sequence set forth in any one of SEQ ID NOs 21, 22, 23 and/or a VL domain sequence set forth in any one of SEQ ID NOs 24, 25, 26, or VH and/or VL sequences having at least 60%, 70%, 80%, 90%, 95%, 97%, 98%, 99% identity with these sequences and specifically binds CD3. In some embodiments, the CD3-binding domain is a Fab, scFv, Fv or dsFv, in

which is contained any combination of the above VH and VL sequence, particularly any combination of a VH sequence set forth in any of SEQ ID NOS: 21, 22, 23 and a VL sequence set forth in any of SEQ ID NOS: 24, 25, 26.In some embodiments, the anti-CD3ε binding domain includes a VH CDR1 sequence that includes at least the amino acid sequence TYAMN (SEQ ID NO: 29); a VH CD2 sequence that includes at least the amino acid sequence RIRSKYNNYATYYADSVKD (SEQ ID NO: 30); a VH CDR3 sequence that includes at least the amino acid sequence HGNFGNSYVSWFAY (SEQ ID NO: 31), a VL CDR1 sequence that includes at least the amino acid sequence RSSTGAVTTSNYAN (SEQ ID NO: 32); a VL CDR2 sequence that includes at least the amino acid sequence GTNKRAP (SEQ ID NO: 33); and a VL CDR3 sequence that includes at least the amino acid sequence ALWYSNLWV (SEQ ID NO: 34). In some embodiments, the CD3-binding domain is a Fab, scFv, Fv or dsFv, in which is contained a VH CDR1 sequence that includes at least the amino acid sequence TYAMN (SEQ ID NO: 29); a VH CD2 sequence that includes at least the amino acid sequence RIRSKYNNYATYYADSVKD (SEQ ID NO: 30); a VH CDR3 sequence that includes at least the amino acid sequence HGNFGNSYVSWFAY (SEQ ID NO: 31), a VL CDR1 sequence that includes at least the amino acid sequence RSSTGAVTTSNYAN (SEQ ID NO: 32); a VL CDR2 sequence that includes at least the amino acid sequence GTNKRAP (SEQ ID NO: 33); and a VL CDR3 sequence that includes at least the amino acid sequence ALWYSNLWV (SEQ ID NO: 34).

[0336] In some embodiments, the CD3-binding domain contains a variable heavy (VH) chain set forth in SEQ ID NO:27 and/or a variable light chain set forth in SEQ ID NO:28, or VH and/or VL sequences having at least 60%, 70%, 80%, 90%, 95%, 97%, 98%, 99% identity with these sequences and specifically binds to CD3. In some embodiments, the CD3-binding domain contains a CDRH1, CDRH2 and CDRH3 of the variable heavy (VH) chain set forth in SEQ ID NO:27 and a CDRL1, CDRL2 and CDRL3 variable light chain set forth in SEQ ID NO:28. In some embodiments, the CD3-binding domain contains a CDRH1, CDRH2 and CDRH3 set forth in SEQ ID NOs:29, 30 and 31, respectively and a CDRL1, CDRL2 and CDRL3 variable light chain set forth in SEQ ID NO:32, 33, and 34, respectively. In some cases, the CD3-binding region comprises a humanized version of the VH sequence set forth in SEQ ID NO:27 and a humanized version of the VL sequence set forth in SEQ ID NO:28. In some embodiments a CD3-binding region can contain a humanized VH domain sequence set forth in any one of SEQ ID NOs 27, 35-65, 341, 343, or 358 and/or a VL domain sequence set forth in any one of SEQ ID NOs:28, 66-84, 293, 340, or 342, or VH and/or VL sequences having at least 60%, 70%, 80%, 90%, 95%, 97%, 98%, 99% identity with these sequences and specifically binds to CD3. In some embodiments a CD3-binding region can contain a humanized VH domain sequence set forth in any one of SEQ ID NOs 27, 35-65, 341, 343, 358, 388, 389, 392, 393 and/or a VL domain sequence set forth in any one of SEQ ID NOs:28, 66-84, 293, 340, 342, 390, 391, 394, 395, or VH and/or VL sequences having at least 60%, 70%, 80%, 90%, 95%, 97%, 98%, 99% identity with these sequences and specifically binds to CD3. In some embodiments, the anti-CD3ε binding domain thereof includes a

variable heavy chain (VH) comprising the amino acid sequence of SEQ ID NO: 47 and a variable light chain (VL) comprising the amino acid sequence of SEQ ID NO: 75.

[0337] In some embodiments, the CD3-binding domain is a Fab, scFv, Fv or dsFv, in which is contained any combination of the above VH and VL sequence, particularly any combination of a VH sequence set forth in any of SEQ ID NOS: 27, 35-65, 341, 343, or 358 and a VL sequence set forth in any of SEQ ID NOS: 28, 66-84, 293, 340, or 342. In some embodiments, the anti-CD3 binding domain is a Fab, scFv, Fv or dsFv, in which is contained a variable heavy chain (VH) comprising the amino acid sequence of SEQ ID NO: 47 and a variable light chain (VL) comprising the amino acid sequence of SEQ ID NO: 75.

[0338] In some embodiments, the CD3-binding domain contains a variable heavy (VH) chain set forth in any one of SEQ ID NO:388, 389, 392, or 393. In some embodiments, the CD3-binding domain contains a variable light (VL) chain set forth in any one of SEQ ID NO:390, 391, 394, or 395.

[0339] The provided bispecific constructs can be formatted in any of a number of formats containing the at least one 5T4 VHH domain and the at least one additional domain specific to an activating T cell antigen, such as a CD3-binding domain.

[0340] In one embodiment, the bispecific construct is a bispecific single-domain antibody-linked Fab (S-Fab) containing at least one 5T4 VHH domain as described linked, directly or indirectly to a Fab antigen binding fragment specific to a T cell activating antigen, e.g. CD3, such as an anti-CD3 Fab. The Fab against a T cell activating antigen, e.g. anti-CD3 Fab, can contain any of the VH and VL sequences as described. In some embodiments, the 5T4 VHH domain is linked to the C-terminus of the VH or VL chain of an anti-CD3 Fab. In some embodiments, the S-Fab can be further modified, such as by conjugation with polyethylene glycol (PEG), N-(2-hydroxypropyl) methacrylamide (HPMA) copolymers, proteins (such as albumin), polyglutamic acid or PASylation (Pan et al. (2018) International Jounral of Nanomedicine, 2018:3189-3201).

[0341] In another embodiment, the bispecific construct is a scFv-single domain antibody in which the construct contains at least one 5T4 VHH as described linked, directly or indirectly, to an scFv containing a VH and a VL of an antigen binding domain specific to a T cell activating antigen, e.g. CD3. The scFv against a T cell activating antigen, e.g. anti-CD3 scFv, can contain any of the VH and VL sequences as described. In some embodiments, the VHH domain and the scFv are connected by a linker, such as a peptide linker. In some embodiments, the peptide linker can be a peptide linker as described herein. In some embodiments, the VHH domain and the scFv are each connected, optionally through a hinge region or a linker (e.g. peptide linker), to an Fc region, such as an N-terminus of an Fc region. The Fc region can be any described herein, such as a human Fc region or a variant thereof, e.g. a human IgG1 Fc region or a variant thereof. In particular examples, the Fc region is formed by variant Fc domains, e.g. variant human IgG1 domains, that are mutated or modified to promote heterodimerization in which different polypeptides can be dimerized to yield a heterodimer.

[0342] In a further embodiment, the CD3-binding domain is a single domain antibody, such as is a VHH domain that specifically binds to CD3. Single domain antibodies, including VHH domains that bind to CD3 are known, see e.g. published U.S. patent application No. US20160280795. In some embodiments, the CD3-binding domain is an anti-CD3 VHH set forth in SEQ ID NO:85, or a sequence that exhibits at least 60%, 70%, 80%, 90%, 95%, 97%, 98%, 99% identity with SEQ ID NO:85 and specifically binds to CD3. In such aspects, a bispecific construct provided herein can include at least one 5T4 VHH domain and at least one CD3 VHH domain. For formatting the constructs, in some cases, each VHH domain is connected, optionally through a hinge region or linker (e.g. peptide linker), to an Fc region, such as an N-terminus of an Fc region. The Fc region can be any described herein, such as a human Fc region or a variant thereof, e.g. a human IgG1 Fc region or a variant thereof. In particular examples, the Fc region is formed by variant Fc domains, e.g. variant human IgG1 domains, that are mutated or modified to promote heterodimerization in which different polypeptides can be dimerized to yield a heterodimer.

[0343] In the above embodiments, exemplary modifications of an Fc region to promote heterodimerization are known, including any as described below, e.g. Table 3. In some embodiments, one Fc polypeptide of a heterodimeric Fc comprises the sequence of amino acids set forth in any of SEQ ID NOS:328 (e.g. SEQ ID NO:103 or 107), 334 (e.g. SEQ ID NO:115 or 117), and the other Fc polypeptide of the heterodimeric Fc contains the sequence of amino acids set forth in any of SEQ ID NOS:329 (e.g. SEQ ID NO:104 or 108), 332 (e.g. SEQ ID NO:111 or 113), 336 (e.g. SEQ ID NO:119 or 121). In some embodiments, one Fc polypeptide of a heterodimeric Fc comprises the sequence of amino acids set forth in any of SEQ ID NOS: 330 (e.g. SEQ ID NO:105 or 109), 335 (e.g. SEQ ID NO:116 or 118) and the other Fc polypeptide of the heterodimeric Fc comprises the sequence of amino acids set forth in any of SEQ ID NOS: 331 (e.g. SEQ ID NO:106 or 110), 333 (e.g. SEQ ID NO:112 or 114), 337 (e.g. SEQ ID NO:120 or 122).

2. Constrained CD3 Multispecific Construct

[0344] In some embodiments, the 5T4-binding polypeptide is a multispecific polypeptide construct that is a constrained T-cell engaging fusion protein. In particular aspects, the constrained multispecific constructs provided herein bind an activating T cell antigen, such as a CD3, and 5T4. The constrained multispecific polypeptide constructs provided herein include at least a first component that includes an immunoglobulin Fc region, a second component that includes one or more copies of at least a binding domain that binds CD3 (referred to herein as an anti-CD3 binding domain or a CD3 binding domain, which are terms that are used interchangeably herein), and a linker, such as a polypeptide linker, that joins the first component and the second component. In the provided multispecific polypeptide constructs, one or both of the first and second components contain at least one 5T4 VHH domain, which, when engaged upon binding to antigen, render the constrained CD3 binding region substantially able to bind CD3. **FIGS. 3A-3E** depict exemplary formats of a constrained multispecific construct.

[0345] In some embodiments, the constrained multispecific polypeptide constructs provided herein exist in two states in terms of capacity to bind CD3 and subsequently activate T-cells: (1) the "inactive" state occurs when there is no binding of any or all of the antigen binding domain(s) to 5T4, such that the CD3 binding is constrained and T-cell interaction is obviated or reduced, and (2) the "active" state occurs upon antigen binding by any or all of the antigen binding domain(s), such that the CD3 binding region is able to bind CD3 and the T-cell interaction is allowed.

[0346] In some embodiments, the Fc region is linked to the CD3 binding domain via a linker or linkers. In some embodiments, the Fc region is linked to the CD3 binding region via a non-cleavable linker or linkers. In some embodiments, the Fc region is linked to the CD3 binding region via a cleavable linker or an otherwise labile linker or linkers. In some embodiments, cleavable linker is a linker that can be specifically cleaved in the presence of a protease. In some aspects, enhanced CD3 binding occurs following cleavage of the cleavable linker. In some such aspects, the "active" state can be further amplified via several mechanisms, including via cleavage of the linker joining the CD3 binding region and the Fc region. In some embodiments, the cleavable linker is a linker that contains a substrate recognition site for a protease. In some embodiments, wherein the Fc region and the CD3 binding region are linked by a cleavable linker, enhanced CD3 binding may occur following cleavage within the linker(s).

[0347] Further, in aspects wherein the Fc region and the CD3 binding region are operably linked by a cleavable linker, cleavage of the linker(s) between the Fc region and the CD3 binding region may separate the constrained multispecific polypeptide constructs into a first and second component. Depending on the composition of the constrained multispecific polypeptide construct, the first and second component may have distinct functionalities. In some embodiments, the Fc region is a region that exhibits one or more effector functions, such as ADCC, CDC or ADCP functions. In such examples, the constrained multispecific polypeptide constructs of the disclosure can be used to produce a self-amplifying system. For example, in some aspects, the incorporation of a protease cleavable linker between the Fc and the components of the CD3 binding domain enables for amplification of the T-cell activating capacity by allowing full exposure of the CD3 binding domain. Depending on the specific linker included, the amplification step can be mediated by tumor associated proteases or by granzymes released following antigen dependent-T-cell activation. If a tumor protease cleavable linker is included the amplification is mediated by the tumor or tumor-microenvironment. Whereas, if a granzyme B cleavable linker is included the amplification may be self-mediated by T-cells following antigen-dependent activation. Furthermore, in cases wherein an effector enabled Fc is included in the construct, amplification may be mediated by granzymes released from NK cell that occurs through an ADCC mechanism.

[0348] The provided constrained multispecific polypeptide constructs include a configuration in which the first component containing the Fc region is N-terminal to the second component containing the

CD3 binding region. In such an embodiment, the first and second components are joined via a linker that is C-terminal to the end of the Fc region. In some embodiments, the at least one 5T4 VHH domain is positioned on the amino-terminal (N-term) region of the multispecific polypeptide construct. In some embodiments, the at least one 5T4 VHH domain is positioned on the carboxy-terminal (C-term) region of the multispecific polypeptide construct. In some embodiments, the constrained multispecific polypeptide construct contains at least two 5T4 VHH domains that are positioned on both the N- and C-terminal regions of the multispecific polypeptide construct.

[0349] In some embodiments, the constrained multispecific polypeptide construct is a dimer, in which dimerization is formed by covalent or non-covalent interactions between two polypeptide chains. In some embodiments, the two polypeptide chains are covalently bonded to each other by, for example, interchain disulfide bonds. In some embodiments, the Fc region mediates dimerization via interchain disulfide bonds. In particular embodiments, a constrained multispecific polypeptide construct contains a heterodimeric Fc region in which, in some cases, the polypeptide chains of the multispecific polypeptide construct are different (heterodimer). In particular examples of a heterodimeric multispecific polypeptide construct, the CD3-binding region is a two chain polypeptide containing a VH and a VL chain, such as is an Fv antibody fragment containing the VH and VL. In some embodiments, the Fv antibody fragment includes a disulfide stabilized anti-CD3 binding Fv fragment (dsFv).

[0350] In particular embodiments, the Fv is a disulfide stabilized Fv fragment (dsFv) in which the the V_H-V_L heterodimer is stabilized by an interchain disulfide bond. In some embodiments, the interchain disulfide bond is engineered by mutation of position in framework positions of the VH and/or VL chain. In some embodiments, the VH chain contains the mutation G44C and the VL chain contains the mutation G100C, each by kabat numbering. In some embodiments, the disulfide stabilized anti-CD3 Fv comprises an anti-CD3 VH with the mutation at position 105 to Cys and an anti-CD3 VL with the mutation position 43 to Cys by Kabat numbering.

[0351] In some embodiments, a constrained multispecific polypeptide construct is formed from or includes two polypeptides, including a first polypeptide comprising a first Fc polypeptide of a heterodimeric Fc region, a linker (e.g. cleavable or non-cleavable linker), a VH domain of an anti-CD3 antibody or antigen binding fragment (e.g. Fv); and a second polypeptide comprising a second Fc polypeptide of the heterodimeric Fc region, the linker (e.g. the cleavable linker or non-cleavable), a VL domain of the anti-CD3 antibody or antigen binding fragment (e.g. Fv). In some embodiments, the first polypeptide contains one or two VHH domains that bind to 5T4. In some embodiments, the second polypeptide contains one or two VHH domains that bind to 5T4. In some embodiments, a constrained multispecific polypeptide construct contains at least two 5T4 VHH domains. In some cases, at least one 5T4 VHH domain is located N-terminally to the Fc polypeptide and at least one 5T4 VHH domain is located C-terminally to the chain of the CD3-binding region.

[0352] In some embodiments, the first polypeptide or second polypeptide or both the first and second polypeptide further include a co-stimulatory receptor binding region (CRBR) that binds a co-stimulatory receptor. In some embodiments, the CRBR of the first and/or second polypeptide can be located N-terminally to the Fc polypeptide and/or C-terminally to the chain of the CD3-binding region.

[0353] In some embodiments, a constrained multispecific polypeptide construct contains at least two VHH domains that bind 5T4 and at least one co-stimulatory receptor binding region (CRBR) that binds a co-stimulatory receptor. In some embodiments, a constrained multispecific polypeptide construct contains (1) a first polypeptide comprising in order of N-terminus to C-terminus: a first 5T4 VHH domain, the first Fc polypeptide of a heterodimeric Fc region, a linker (e.g. a cleavable or non-cleavable linker), a chain (e.g. VH or VL) of an anti-CD3 antibody or antigen binding fragment (e.g. Fv or dsFv), and a second 5T4 VHH domain; and (2) a second polypeptide comprising in order of N-terminus to C-terminus: the second Fc polypeptide of the heterodimeric Fc region, the same linker (e.g. same cleavable linker), the other chain (other of the VH or VL) of the anti-CD3 antibody or antigen binding fragment, and a co-stimulatory receptor binding region (CRBR) that binds a co-stimulatory receptor.

[0354] In some embodiments, the first polypeptide or second polypeptide or both the first and second polypeptide further include an inhibitory receptor binding region (IRBR) that binds an inhibitory receptor. In some embodiments, the IRBR of the first and/or second polypeptide can be located N-terminally to the Fc polypeptide and/or C-terminally to the chain of the CD3-binding region.

[0355] In some embodiments, a constrained multispecific polypeptide construct contains at least two VHH domains that bind 5T4 and at least one inhibitory receptor binding region (IRBR) that binds an inhibitory receptor. In some embodiments, a constrained multispecific polypeptide construct contains (1) a first polypeptide comprising in order of N-terminus to C-terminus: a first 5T4 VHH domain, the first Fc polypeptide of a heterodimeric Fc region, a linker (e.g. a cleavable or non-cleavable linker), a chain (e.g. VH or VL) of an anti-CD3 antibody or antigen binding fragment (e.g. Fv or dsFv), and a second 5T4 VHH domain; and (2) a second polypeptide comprising in order of N-terminus to C-terminus: the second Fc polypeptide of the heterodimeric Fc region, the same linker (e.g. same cleavable or non-cleavable linker), the other chain (other of the VH or VL) of the anti-CD3 antibody or antigen binding fragment, and an inhibitory receptor binding region (IRBR) that binds an inhibitory receptor.

[0356] In some embodiments, at least one of the first polypeptide or second polypeptide further include a co-stimulatory receptor binding region (CRBR) that binds a co-stimulatory receptor and at least one of the first polypeptide or second polypeptide further includes an inhibitory receptor binding region (IRBR) that binds an inhibitory receptor. In some embodiments, the CRBR of the first and/or second polypeptide can be located N-terminally to the Fc polypeptide and/or C-terminally to the chain of the CD3-binding region. In some embodiments, the IRBR of the first and/or second polypeptide can be located N-terminally to the Fc polypeptide and/or C-terminally to the chain of the CD3-binding region.

[0357] In some embodiments, a constrained multispecific polypeptide construct contains at least two VHH domains that bind 5T4, a co-stimulatory receptor binding region (CRBR) that binds a co-stimulatory receptor and an inhibitory receptor binding region (IRBR) that binds an inhibitory receptor. In some embodiments, a constrained multispecific polypeptide construct contains (1) a first polypeptide comprising in order of N-terminus to C-terminus: a first 5T4 VHH domain, the first Fc polypeptide of a heterodimeric Fc region, a linker (e.g. a cleavable linker), a chain (e.g. VH or VL) of an anti-CD3 antibody or antigen binding fragment (e.g. Fv or dsFv), and a second 5T4 VHH domain; and (2) a second polypeptide comprising in order of N-terminus to C-terminus: one of an IRBR or CRBR, the second Fc polypeptide of the heterodimeric Fc region, the same linker (e.g. same cleavable linker), the other chain (other of the VH or VL) of the anti-CD3 antibody or antigen binding fragment, and the other of the IRBR or CRBR.

[0358] Each of the components of the multispecific polypeptide constructs of the disclosure is described in more detail below.

a. 5T4 VHH antigen binding domain

[0359] A constrained multispecific polypeptide construct of the disclosure includes at least one 5T4 VHH domain from among any provided herein. In some embodiments, the 5T4 VHH domain comprises the sequence of amino acids set forth in any of SEQ ID NOS: 245-287, 294-295, 302. In some embodiments, the 5T4 VHH domain comprises the sequence of amino acids set forth in any of SEQ ID NO: 245-287, 294-295, 302, 360. In some embodiments, the 5T4 VHH domain comprises the sequence of amino acids set forth in SEQ ID NO: 360.

[0360] In particular embodiments, a constrained multispecific polypeptide construct contains at least two 5T4 domain. In some cases, at least one 5T4 VHH domain is positioned amino terminally relative to an Fc polypeptide of the heterodimeric Fc and at least one 5T4 VHH domain is positioned carboxy-terminally relative to VH or VL chain of the CD3 binding region.

[0361] In aspects of a constrained multispecific polypeptide construct containing at least two or containing two 5T4 VHH domains, each of the 5T4 VHH domains can bind to the same or an overlapping epitope on 5T4.

[0362] In aspects of a constrained multispecific polypeptide construct containing at least two or containing two 5T4 VHH domains, each of the 5T4 VHH domains can bind to a different or a non-overlapping epitope on 5T4. In some embodiments, the first and second 5T4 sdAb bind a distinct or non-overlapping epitope of 5T4 and/or do not compete for binding to 5T4.

[0363] In some embodiments, a 5T4 VHH does not cross react with the mouse 5T4 antigen. In some embodiments, a 5T4 VHH comprising the amino acid sequence set forth in SEQ ID NO:245, or humanized variants thereof, do not cross react with the mouse 5T4 antigen. In some embodiments, a 5T4 VHH comprising the amino acid sequence set forth in SEQ ID NO:255, or humanized variants thereof, do not cross react with the mouse 5T4 antigen. In some embodiments, a 5T4 VHH comprising the amino

acid sequence set forth in SEQ ID NO:276, or humanized variants thereof, do not cross react with the mouse 5T4 antigen.

[0364] In some examples, the first sdAb comprises the amino acid sequence set forth in any one of SEQ ID NOS: 245-254, 295, 302, 360 a humanized variant thereof, or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to any of SEQ ID NOS: 245-254, 295, 302, 360 and binds 5T4; and the second sdAb comprises the amino acid sequence set forth in any one of SEQ ID NOS: 255-287, 294, 302, a humanized variant thereof, or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to any of SEQ ID NOS: 255-287, 294 and binds 5T4.

[0365] In some embodiments, the first sdAb (first 5T4 VHH domain) comprises the amino acid sequence set forth in SEQ ID NO: 245 or a humanized variant set forth in any of SEQ ID NOS: 246-254, 295, or302 ; and the second sdAb (second 5T4 VHH domain) comprises the amino acid sequence set forth in SEQ ID NO:255 or a humanized variant set forth in any of SEQ ID NOS: 256-275, SEQ ID NO:276 or a humanized variant thereof set forth in any of SEQ ID NOS: 277-287, 294, or 302.

[0366] In some embodiments, the first sdAb (first 5T4 VHH domain) comprises the amino acid sequence set forth in SEQ ID NO: 245 or a humanized variant set forth in any of SEQ ID NOS: 246-254, 295, 302 or 360; and the second sdAb (second 5T4 VHH domain) comprises the amino acid sequence set forth in SEQ ID NO:255 or a humanized variant set forth in any of SEQ ID NOS: 256-275, SEQ ID NO:276 or a humanized variant thereof set forth in any of SEQ ID NOS: 277-287, 294, or 302.

[0367]

[0368] In some embodiments, the first sdAb (first 5T4 VHH domain) and the second sdAb (second 5T4 VHH domain) have the amino acid sequences set forth in SEQ ID NO: 245 and SEQ ID NO:294. In some embodiments, the first sdAb (first 5T4 VHH domain) and the second sdAb (second 5T4 VHH domain) have the amino acid sequences set forth in SEQ ID NO: 245 and SEQ ID NO:276. In some embodiments, the first sdAb (first 5T4 VHH domain) and the second sdAb (second 5T4 VHH domain) have the amino acid sequences set forth in SEQ ID NO: 245 and SEQ ID NO:255. In some embodiments, the first sdAb (first 5T4 VHH domain) and the second sdAb (second 5T4 VHH domain) have the amino acid sequences set forth in SEQ ID NO:245 and SEQ ID NO: 295. In some embodiments, the first sdAb (first 5T4 VHH domain) and the second sdAb (second 5T4 VHH domain) have the amino acid sequences set forth in SEQ ID NO: 295 and SEQ ID NO:294. In some embodiments, the first sdAb (first 5T4 VHH domain) and the second sdAb (second 5T4 VHH domain) have the amino acid sequences set forth in SEQ ID NO: 249 and SEQ ID NO:270. In some embodiments, the first sdAb (first 5T4 VHH domain) and the second sdAb (second 5T4 VHH domain) have the amino acid sequences set forth in SEQ ID NO: 254 and SEQ ID NO:287. In some embodiments, the first sdAb (first 5T4 VHH domain) and the second sdAb (second 5T4 VHH domain) have the amino acid sequences set forth in

SEQ ID NO:302 and SEQ ID NO:302. In some embodiments, the first sdAb (first 5T4 VHH domain) and the second sdAb (second 5T4 VHH domain) have the amino acid sequences set forth in SEQ ID NO: 360 and SEQ ID NO:287.

[0369] In some embodiments, a constrained multispecific polypeptide construct contains at least one 5T4 VHH domains, such as any provided herein, and at least one further antigen binding domain specific to another tumor associated antigen (TAA). In some embodiments, the at least one further antigen binding domain includes one or more copies of an antibody or an antigen-binding fragment thereof selected from the group consisting of a Fab fragment, a $F(ab')_2$ fragment, an Fv fragment, a scFv, a scAb, a dAb, a single domain heavy chain antibody, and a single domain light chain antibody. In particular embodiments, the further TAA antigen binding domain is a single chain antibody. In some examples, the single chain is an scFv, a scAb, a single domain heavy chain antibody, or a single domain light chain antibody. For example, in some cases, the further TAA antigen binding domain includes one or more single domain antibody (sdAb) fragments, for example V_HH, V_{NAR}, engineered V_H or V_K domains. V_HHs can be generated from natural camelid heavy chain only antibodies, genetically modified rodents that produce heavy chain only antibodies, or naïve/synthetic camelid or humanized camelid single domain antibody libraries. $V_{NAR}s$ can be generated from cartilaginous fish heavy chain only antibodies. Various methods have been implemented to generate monomeric sdAbs from conventionally heterodimeric V_H and V_K domains, including interface engineering and selection of specific germline families.

[0370] In some embodiments, the further TAA is selected from the group consisting of 1-92-LFA-3, Alpha-4 integrin, Alpha-V integrin, alpha4beta1 integrin, alpha4beta7 integrin, AGR2, Anti-Lewis-Y, Apelin J receptor, APRIL, B7-H3, B7-H4, BAFF, BTLA, C5 complement, C-242, CA9, CA19-9, (Lewis a), Carbonic anhydrase 9, CD2, CD3, CD6, CD9, CD11a, CD19, CD20, CD22, CD24, CD25, CD27, CD28, CD30, CD33, CD38, CD40, CD40L, CD41, CD44, CD44v6, CD47, CD51, CD52, CD56, CD64, CD70, CD71, CD74, CD80, CD81, CD86, CD95, CD117, CD123, CD125, CD132, (IL-2RG), CD133, CD137, CD138, CD166, CD172A, CD248, CDH6, CEACAM5 (CEA), CEACAM6 (NCA-90), CLAUDIN-3, CLAUDIN-4, cMet, Collagen, Cripto, CSFR, CSFR-1, CTLA-4, CTGF, CXCL10, CXCL13, CXCR1, CXCR2, CXCR4, CYR61, DL44, DLK1, DLL3, DLL4, DPP-4, DSG1, EDA, EDB, EGFR, EGFRviii, Endothelin B receptor (ETBR), ENPP3, EpCAM, EPHA2, EPHB2, ERBB3, F protein of RSV, FAP, FGF-2, FGF8, FGFR1, FGFR2, FGFR3, FGFR4, FLT-3, Folate receptor alpha (FRα), GAL3ST1, G-CSF, G-CSFR, GD2, GITR, GLUT1, GLUT4, GM-CSF, GM-CSFR, GP IIb/IIIa receptors, Gp130, GPIIB/IIIA, GPNMB, GRP78, HER2/neu, HER3, HER4, HGF, hGH, HVEM, Hyaluronidase, ICOS, IFNalpha, IFNbeta, IFNgamma, IgE, IgE Receptor (FceRI), IGF, IGF1R, IL1B, IL1R, IL2, IL11, IL12, IL12p40, IL-12R, IL-12Rbeta1, IL13, IL13R, IL15, IL17, IL18, IL21, IL23, IL23R, IL27/IL27R (wsx1), IL29, IL-31R, IL31/IL31R, IL2R, IL4, IL4R, IL6, IL6R, Insulin Receptor, Jagged Ligands, Jagged 1, Jagged 2, KISS1-R, LAG-3, LIF-R, Lewis X, LIGHT, LRP4, LRRC26,

Ly6G6D, LyPD1, MCSP, Mesothelin, MRP4, MUC1, Mucin-16 (MUC16, CA-125), Na/K ATPase, NGF, Nicastrin, Notch Receptors, Notch 1, Notch 2, Notch 3, Notch 4, NOV, OSM-R, OX-40, PAR2, PDGF-AA, PDGF-BB, PDGFRalpha, PDGFRbeta, PD-1, PD-L1, PD-L2, Phosphatidyl-serine, P1GF, PSCA, PSMA, PSGR, RAAG12, RAGE, SLC44A4, Sphingosine 1 Phosphate, STEAP1, STEAP2, TAG-72, TAPA1, TEM-8, TGFbeta, TIGIT, TIM-3, TLR2, TLR4, TLR6, TLR7, TLR8, TLR9, TMEM31, TNFalpha, TNFR, TNFRS12A, TRAIL-R1, TRAIL-R2, Transferrin, Transferrin receptor, TRK-A, TRK-B, uPAR, VAP1, VCAM-1, VEGF, VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGFR1, VEGFR2, VEGFR3, VISTA, WISP-1, WISP-2, and WISP-3.

[0371] In some embodiments, the antigen binding domain, such as a 5T4 VHH domain, is linked, directly or indirectly via a linker, to the Fc region and/or to the CD3 binding region. In some embodiments, linkage is via a linker. In some embodiments, the linker is a linking peptide (LP), which can include any flexible or rigid linker as described. In some embodiments, the linker is selected from the group consisting of GGSGGS, i.e., $(GGS)_2$ (SEQ ID NO: 1); GGSGGSGGS, i.e., $(GGS)_3$ (SEQ ID NO: 2); GGSGGSGGSGGS, i.e., $(GGS)_4$ (SEQ ID NO: 3); and GGSGGSGGSGGSGGS, i.e., $(GGS)_5$ (SEQ ID NO: 4). In some embodiments, the linker is a flexible linker comprising Glycine residues, such as, by way of non-limiting example, GG, GGG, GGGG (SEQ ID NO: 5), GGGGG (SEQ ID NO: 6), and GGGGGG (SEQ ID NO: 7). In some embodiments, the linker is(GGGGS)n, wherein n is 1 to 5 (SEQ ID NO:123); (GGGGGS)n, wherein n is 1 to 4 (SEQ ID NO:124); GGGGS (SEQ ID NO:125); GGGGGS (SEQ ID NO:126); GGGGGSGGGGGSGGGGGS (SEQ ID NO:127); GGGGSGGGGSGGGGS (SEQ ID NO:128); GGSGGGGSGGGGSGGGGS (SEQ ID NO:129); or PGGGG (SEQ ID NO:327). In some embodiments, the linker includes a combination of a GS-linker and a Glycine linker.

b. Fc region

[0372] A constrained multispecific polypeptide construct includes an immunoglobulin Fc region. Generally, the constrained multispecific polypeptide construct is a dimer formed by polypeptides, each containing an Fc. The Fc polypeptide can be any as set forth above. In particular embodiments, the Fc region is formed by Fc domains that are mutated or modified to promote heterodimerization in which different polypeptides can be dimerized to yield a heterodimer. Thus, in some embodiments, the dimer is a heterodimer in which two polypeptide chains of the multispecific polypeptide construct are different.

[0373] Various methods are known for promoting heterodimerization of complementary Fc polypeptides, see e.g. Ridgway et al, Protein Eng. 9:617-621 (1996); Merchant et al, Nat. Biotechnol. 16(7): 677-81 (1998); Moore et al. (2011) MAbs, 3:546-57; Von Kreudenstein et al. MAbs, (2013) 5:646-54; Gunasekaran et al. (2010) J. Biol. Chem., 285:19637-46; Leaver-Fay et al. (2016) Structure, 24:641-51; Ha et al. (2016) Frontiers in Immunology, 7:1; Davis et al. (2010) Protein Eng Des Sel, 23:195-202; published international PCT Appl. No. WO 1998/050431, WO 2009/089004, WO2011143545 WO 2014/067011, WO 2012/058768, WO2018027025; published U.S. patent Appl. No. US20140363426, US20150307628, US20180016354, US20150239991; and U.S. patent Nos.

US5731168, US7183076, US9701759, US9605084, and US9650446. Methods to promote heterodimerization of Fc chains include mutagenesis of the Fc region, such as by including a set of "knob-into-hole" mutations or including mutations to effect electrostatic steering of the Fc to favor attractive interactions among different polypeptide chains. For example, in some embodiments, the Fc polypeptides of a heterodimer includes a mutation to alter charge polarity across the Fc dimer interface such that coexpression of electrostatically matched Fc chains support favorable attractive interactions thereby promoting desired Fc heterodimer formation, whereas unfavorable repulsive charge interactions suppress unwanted Fc homodimer formation (Guneskaran et al. (2010) JBC, 285: 19637-19646). When co-expressed in a cell, association between the chains is possible but the chains do not substantially self-associate due to charge repulsion. Other strategies for generating a heterodimeric Fc include mixing human IgG and IgA CH3 domain segments to create a complementary CH3 heterodimer, which is referred to as a SEED Fc.

[0374] Methods and variants for heterodimerization also include those described in published international PCT App. WO2014/145806, including "knobs and holes" mutations (also called "skew" variants), mutations that relate to "electrostatic steering" or "charge pairs," and pI variants. Heterodimeric variants also include any as described in U.S. published Appl. No. US2012/0149876 or US2018/011883.

[0375] In some embodiments, to promote heterodimerization both polypeptides of the Fc heterodimer contain paired or complementary amino acid modifications. Exemplary paired amino acid modification of polypeptides of an Fc fusion are set forth in **Table 3**.

Table 3: Paired amino acids of Heterodimeric Fc

First Fc polypeptide	Second Fc Polypeptide
T366W	T366S/L368W/Y407V
T366W/S354C	T366S/L368A/Y407V/Y349C
S364H/F405A	Y349T/Y349F
T350V/L351Y/F405A/Y407V	T350V/T366L/K392L/T394W
K360D/D399M/Y407A	E345R/Q347R/T366V/K409V
K409D/K392D	D399K/E356K
K360E/K409W	Q347R/D399V/F405T
L360E/K409W/Y349C	Q347R/399V/F405T/S354C
K370E/K409W	E357N/D399V/F405T

[0376] In some embodiments, modifications include introduction of a protuberance (knob) into a first Fc polypeptide and a cavity (hole) into a second Fc polypeptide such that the protuberance is positionable in the cavity to promote complexing of the first and second Fc-containing polypeptides. Amino acids targeted for replacement and/or modification to create protuberances or cavities in a polypeptide are typically interface amino acids that interact or contact with one or more amino acids in the interface of a second polypeptide.

[0377] In some embodiments, a first Fc polypeptide that is modified to contain protuberance (knob) amino acids include replacement of a native or original amino acid with an amino acid that has at least

one side chain which projects from the interface of the first Fc polypeptide and is therefore positionable in a compensatory cavity (hole) in an adjacent interface of a second polypeptide. Most often, the replacement amino acid is one which has a larger side chain volume than the original amino acid residue. One of skill in the art knows how to determine and/or assess the properties of amino acid residues to identify those that are ideal replacement amino acids to create a protuberance. In some embodiments, the replacement residues for the formation of a protuberance are naturally occurring amino acid residues and include, for example, arginine (R), phenylalanine (F), tyrosine (Y), or tryptophan (W). In some examples, the original residue identified for replacement is an amino acid residue that has a small side chain such as, for example, alanine, asparagine, aspartic acid, glycine, serine, threonine, or valine.

[0378] In some embodiments, a second Fc polypeptide that is modified to contain a cavity (hole) is one that includes replacement of a native or original amino acid with an amino acid that has at least one side chain that is recessed from the interface of the second polypeptide and thus is able to accommodate a corresponding protuberance from the interface of a first polypeptide. Most often, the replacement amino acid is one which has a smaller side chain volume than the original amino acid residue. One of skill in the art knows how to determine and/or assess the properties of amino acid residues to identify those that are ideal replacement residues for the formation of a cavity. Generally, the replacement residues for the formation of a cavity are naturally occurring amino acids and include, for example, alanine (A), serine (S), threonine (T) and valine (V). In some examples, the original amino acid identified for replacement is an amino acid that has a large side chain such as, for example, tyrosine, arginine, phenylalanine, or tryptophan.

[0379] The CH3 interface of human IgG1, for example, involves sixteen residues on each domain located on four anti-parallel β-strands which buries 1090 Å2 from each surface (see e.g., Deisenhofer et al. (1981) Biochemistry, 20:2361-2370; Miller et al., (1990) J Mol. Biol., 216, 965-973; Ridgway et al., (1996) Prot. Engin., 9: 617-621; U.S. Pat. No. 5,731,168). Modifications of a CH3 domain to create protuberances or cavities are described, for example, in U.S. Pat. No. 5,731,168; International Patent Applications WO98/50431 and WO 2005/063816; and Ridgway et al., (1996) Prot. Engin., 9: 617-621. In some examples, modifications of a CH3 domain to create protuberances or cavities are typically targeted to residues located on the two central anti-parallel β-strands. The aim is to minimize the risk that the protuberances which are created can be accommodated by protruding into the surrounding solvent rather than being accommodated by a compensatory cavity in the partner CH3 domain.

[0380] For example, in some embodiments the heterodimeric Fc includes a polypeptide having an amino acid modification within the CH3 domain at Thr366, which when replaced with a more bulky amino acid, e.g., Try (T366W), is able to preferentially pair with a second CH3 domain having amino acid modifications to less bulky amino acids at positions Thr366, Leu368, and Tyr407, e.g., Ser, Ala and Val, respectively (T366S/L368A/Y407V). Heterodimerization via CH3 modifications can be further stabilized by the introduction of a disulfide bond, for example by changing Ser354 to Cys (S354C) and

Tyr349 to Cys (Y349C) on opposite CH3 domains (Reviewed in Carter, 2001 Journal of Immunological Methods, 248: 7–15).

[0381] In particular embodiments, a multispecific polypeptide construct contains a first and second Fc able to mediate Fc heterodimerization contains a first Fc polypeptide containing mutations T366W and S354C and a second Fc polypeptide containing mutations T366S, L368A, Y407V and Y349C. In some embodiments, the first Fc polypeptide is selected from an Fc polypeptide comprising the sequence set forth in SEQ ID NO: 328 or 334 and the second Fc polypeptide is selected from an Fc polypeptide comprising the sequence set forth in SEQ ID NO: 329, 332 or 336. In some embodiments, the first Fc polypeptide is or comprises the sequence of amino acids set forth in any of SEQ ID NOS: 103, 107, 115 or 117 and the second Fc polypeptide is or comprises the sequence of amino acids set forth in any of SEQ ID NOS: 104, 108, 111, 113, 119 or 121.

[0382] In some embodiments, the Fc polypeptide exhibits features providing Fc-mediated effector functions. In particular examples, the first Fc polypeptide is or comprises the sequence set forth in SEQ ID NOs:328 and a second Fc polypeptide that is or comprises SEQ ID NO: 329 or 332. In some embodiments, the first Fc polypeptide is or comprises the sequence set forth in SEQ ID NO: 103 and the second Fc polypeptide is or comprises the sequence set forth in SEQ ID NO: 104 or 111. In some embodiments, the first Fc polypeptide is or comprises the sequence set forth in SEQ ID NO: 107 and the second Fc polypeptide is or comprises the sequence set forth in SEQ ID NO: 108 or 113. The first and second Fc polypeptide can be formatted on either polypeptide chain of the construct.

[0383] In some embodiments, one or both of the first and second Fc polypeptides can further include one or more amino acid mutations to further reduce one or more Fc effector functions, such as reduced Fc receptor binding. Exemplary mutations to reduce Fc effector functions include any as described. In some embodiments, the modification can be a deletion of one or more positions Glu233 (E233), Leu234 (L234), or Leu235 (L235), such as a deletion of amino acids Glu233 (E233), Leu234 (L234), and Leu235 (L235). In some embodiments, the first Fc polypeptide is selected from an Fc polypeptide comprising the sequence set forth in SEQ ID NO: 330 or 335 and the second Fc polypeptide is selected from an Fc polypeptide comprising the sequence set forth in SEQ ID NO: 331,333 or 337. In some embodiments, the first Fc polypeptide is or comprises the sequence of amino acids set forth in any of SEQ ID NOS: 105, 109, 116, or 118 and the second Fc polypeptide is or comprises the sequence of amino acids set forth in any of SEQ ID NOS: 106, 110, 112, 114, 120 or 122.

[0384] In particular examples, the first Fc polypeptide is or comprises the sequence set forth in SEQ ID NOs:330 and a second Fc polypeptide that is or comprises SEQ ID NO: 331 or 333. In some embodiments, the first Fc polypeptide is or comprises the sequence set forth in SEQ ID NO: 105 and the second Fc polypeptide is or comprises the sequence set forth in SEQ ID NO: 106 or 112. In some embodiments, the first Fc polypeptide is or comprises the sequence set forth in SEQ ID NO: 109 and the

second Fc polypeptide is or comprises the sequence set forth in SEQ ID NO: 110 or 114. The first and second Fc polypeptide can be formatted on either polypeptide chain of the construct.

[0385] In some embodiments, the first Fc polypeptide or second Fc polypeptide further includes mutations M252Y and/or M428V. In particular examples, the first Fc polypeptide is or comprises the sequence set forth in SEQ ID NO:334 and the second Fc polypeptide is or comprises the sequence set forth in SEQ ID NO:336. In some embodiments, the first Fc polypeptide is or comprises the sequence set forth in SEQ ID NO:115 and the second Fc polypeptide is or comprises the sequence set forth in SEQ ID NO: 119. In some embodiments, the first Fc polypeptide is or comprises the sequence set forth in SEQ ID NO:117 and the second Fc polypeptide is or comprises the sequence set forth in SEQ ID NO: 121. In other examples, the first Fc polypeptide is or comprises the sequence set forth in SEQ ID NO:335 and the second Fc polypeptide is or comprises the sequence set forth in SEQ ID NO:337. In some embodiments, the first Fc polypeptide is or comprises the sequence set forth in SEQ ID NO:116 and the second Fc polypeptide is or comprises the sequence set forth in SEQ ID NO: 120. In some embodiments, the first Fc polypeptide is or comprises the sequence set forth in SEQ ID NO:118 and the second Fc polypeptide is or comprises the sequence set forth in SEQ ID NO: 122. The first and second Fc polypeptide can be formatted on either polypeptide chain of the construct.

[0386] Additional examples of variants that can facilitate the promotion of heterodimers are any combination or pair of steric variants (e.g. skew variants) of a first Fc polypeptide and a second Fc polypeptide from among: S364K/E357Q and L368D/K370S; L368D/K370S and S364K; L368E/K370S and S364K; T411T/E360E/Q362E and D401K; L368D/K370S and S364K/E357L, K370S and S364K/E357Q and T366S/L368A/Y407V and T366W or 366S/L368A/Y407V/Y349C and T366W/S354C), where each pair represents mutations in the first Fc polypeptide and second Fc polypeptide. In particular embodiments, a provided construct contains a first and second Fc polypeptide containing the pair of mutations L368D/K370S and S364K and E357Q.

[0387] An additional mechanism that can be used in the generation of heterodimers is sometimes referred to as "electrostatic steering" as described in Gunasekaran et al., J. Biol. Chem. 285(25):19637 (2010). This is sometimes referred to herein as "charge pairs". In this embodiment, electrostatics are used to skew the formation towards heterodimerization. As those in the art will appreciate, these may also have an effect on pI, and thus on purification, and thus could in some cases also be considered pI variants. However, as these were generated to force heterodimerization and were not used as purification tools, they are classified as "steric variants". In one embodiments, a first Fc polypeptide can contain mutations D221E/P228E/L368E and a second Fc polypeptide can contain mutations D221R/P228R/K409R. In another embodiments, a first Fc polypeptide can contain mutations C220E/P228E/368E and a second Fc polypeptide can contain mutations C220R/E224R/P228R/K409R.

[0388] In some embodiments, heterodimerization can be facilitated by pI variants. In some aspects, a pI variant can include those that increase the pI of the protein (basic changes). In other aspects, the pI

variant can include those that decrease the pI of the protein (acidic changes). In some cases, all combinations of these variants can be done, including combinations in which one Fc polypeptide may be wild type, or a variant that does not display a significantly different pI from wild-type, and the other Fc polypeptide can be either more basic or more acidic. Alternatively, each Fc polypeptide can be changed, one to more basic and one to more acidic. In some embodiments, at least one Fc polypeptide is a negative pI variant Fc containing mutations Q295E/N384D/Q418E/N421D.

[0389] In some embodiments, a combination of steric heterodimerization variants (e.g. knob and hole) and pI or charge pair variants can be used.

[0390] In particular embodiments, the provided constructs contains (a)a first Fc polypeptide comprising the skew variants S364K/E357Q; and b) a second Fc polypeptide containing skew variants L368D/K370S and the pI variants N208D/Q295E/N384D/Q418E/N421D. In some embodiments, one or both of the first and second polypeptide can contain further mutations to reduce Fc effector activity, such as the exemplary mutations E233P/L234V/L235A/G236del/S267K. An example of such a first Fc polypeptide and a second Fc polypeptide able to mediate Fc heterodimeriztion comprise the sequences set forth in SEQ ID NOs:338 and 339. The first and second Fc polypeptide can be formatted on either polypeptide chain of the construct.

[0391] The resulting constrained multispecific polypeptide constructs can be purified by any suitable method such as, for example, by affinity chromatography over Protein A or Protein G columns. Where two nucleic acid molecules encoding different polypeptides are transformed into cells, formation of homo- and heterodimers will occur. Conditions for expression can be adjusted so that heterodimer formation is favored over homodimer formation.

[0392] Techniques for recovery of heterodimers from homodimers based on a differential affinity of the heterodimers for an affinity reagent are known. In some aspects, such techniques include designing a heterodimer so that one of the Fc polypeptide chains does not bind to the affinity reagent protein A. In some cases, one of the polypeptide chain can contain one or more amino acid substitution to abrogate or reduce affinity for the protein A reagent in one of the polypeptides of the Fc heterodimer, see e.g. WO2017134440, WO2010151792, Jendeberg et al. (Jendeberg et al., (1997) J. Immunol. Methods, 201(1): 25-34. In some of these embodiments, the Fc region may be modified at the protein-A binding site on one member of the heterodimer so as to prevent protein-A binding and thereby enable more efficient purification of the heterodimeric fusion protein. An exemplary modification within this binding site is Ile253, for example Ile253Arg (I253R). In some embodiments, the modification may be H435R or H435R/Y436F. In some embodiments, an Fc polypeptide of an Fc heterodimer can contain a modification so that it is capable of binding protein A but not protein G (pA+/pG-). Exemplary pA+/pG- amino acid modifications include an Fc containing serine at position 428, serine at position 434 and optionally histidine at position 436, with reference to human IgGl or comprising these residues at the corresponding positions in human IgG 2, 3, or 4. In some aspects, such amino acid modifications in one

IgG Fc polypeptide at positions 428, 434 and optionally 436 reduces or prevents the binding of protein G, enhancing the purification of the protein.

[0393] In some embodiments, any of such modifications to confer differential affinity to an affinity reagent can be combined with any one or more other amino acid modifications described above. For example, the I253R modification maybe combined with either the T366S/L368A/Y407V modifications or with the T366W modifications. The T366S/L368A/Y407V modified Fc is capable of forming homodimers as there is no steric occlusion of the dimerization interface as there is in the case of the T336W modified Fc. Therefore, in some embodiments, the I253R modification is combined with the T366S/L368A/Y407V modified Fc to disallow purification any homodimeric Fc that may have formed. Similar modifications can be employed by combining T366S/L368A/Y407V and H453R.

[0394] In some embodiments, the Fc regions of the heterodimeric molecule additionally can contain one or more other Fc mutation, such as any described above. In some embodiments, the heterodimer molecule contains an Fc region with a mutation that reduces effector function. In some embodiments, the Fc region is altered to provide reduced Fc-mediated effector functions, such as via reduced Fc receptor binding, e.g. binding to FcγR binding but generally not FcRn binding.

[0395] In some embodiments, the Fc region is mutated in one or more of the following positions to reduce Fc receptor binding: Glu233 (E233), Leu234 (L234), or Leu235 (L235). The one or more mutations can include E233P, L234V and/or L235A.

[0396] In particular embodiments, the mutations of the Fc region to reduce Fc effector function, e.g. via reducing Fc receptor binding to FcγR, include mutations from among any of G236R/L328R, E233P/L234V/L235A/G236del/S239K, E233P/L234V/L235A/G236del/S267K, E233P/L234V/L235A/G236del/S239K/A327G, E233P/L234V/L235A/G236del/S267K/A327G, E233P/L234V/L235A/G236del, D265A/P329A, D265A/P329G, D265A/N297A, L234V/L235A/D265A, L234V/L235A/N297A, L234V/L235A/P329A, or L234V/L235A/P329G. In some embodiments, one Fc polypeptide of a heterodimeric Fc comprises the sequence of amino acids set forth in any of SEQ ID NOS:328 (e.g. SEQ ID NO:103 or 107), 334 (e.g. SEQ ID NO:115 or 117), and the other Fc polypeptide of the heterodimeric Fc contains the sequence of amino acids set forth in any of SEQ ID NOS: 329 (e.g. SEQ ID NO:104 or 108), 332 (e.g. SEQ ID NO:111 or 113), 336 (e.g. SEQ ID NO:119 or 121). In some embodiments, one Fc polypeptide of a heterodimeric Fc comprises the sequence of amino acids set forth in any of SEQ ID NOS: 330 (e.g. SEQ ID NO:105 or 109), 335 (e.g. SEQ ID NO:116 or 118) and the other Fc polypeptide of the heterodimeric Fc comprises the sequence of amino acids set forth in any of SEQ ID NOS: 331 (e.g. SEQ ID NO:106 or 110), 333 (e.g. SEQ ID NO:112 or 114), 337 (SEQ ID NO:120 or 122).

[0397] In some embodiments, the Fc region of the provided multispecific polypeptide constructs exhibit one or more effector functions. In some cases, the Fc region is capable of providing Fc-mediated effector functions, such as for example, ADCC (*e.g.*, release of granzyme B by NK cells), ADCP, and/or

CDC. In general, the Fc region is responsible for effector functions, such as complement-dependent cytotoxicity (CDC) and antibody-dependent cell cytotoxicity (ADCC), in addition to the antigen-binding capacity, which is the main function of immunoglobulins. Additionally, the FcRn sequence present in the Fc region plays the role of regulating the IgG level in serum by increasing the *in vivo* half-life by conjugation to an *in vivo* FcRn receptor. In some embodiments in which the multispecific polypeptide constructs contain a cleavable linker, cleavage of the linker can produce two components that each have biological activity: the CD3-binding region that is able to bind and engage CD3 on a T cell, which, in some aspects, also can contain a CRBR for inducing a costimulatory signal on the T cell and/or an IRBR for inducing an inhibitory signal on the T cell; and the Fc region linked to the 5T4 VHH domain that can exhibit target-specific effector function. In particular embodiments provided herein, the multispecific polypeptide constructs contain a non-cleavable linker and may, in some aspects, not exhibit an independent Fc-mediated effector function.

[0398] In some embodiments, the Fc region includes an Fc polypeptide that is mutated or modified to alter one or more effector functions. Thus, in some cases, effector functions such as on or more of ADCC, ADCP and/or CDC can be altered, such as reduced or enhanced, in an Fc for use with the provided constrained multispecific polypeptide constructs. Exemplary mutations to reduce effector function include any as described above.

[0399] In some embodiments, an IgG1 Fc polypeptide or a variant thereof such as any described below can be made in a G1 m1 or G1 m3 allotype. In some embodiments, the Fc region can contain amino acids of the human G1 m1 allotype, such as residues containing Asp (D) and Leu (L) at positions 356 and 358, e.g. as set forth in SEQ ID NO:8. In some cases, an Fc polypeptide can contain amino acid substitutions E356D and M358L to reconstitute residues of allotype G1 m1. In other embodiments, the Fc region can contain amino acids of the human G1 m3 allotype, such as residues Glu (E) and Met (M) at positions 356 and 358 by EU numbering, e.g. as set forth in SEQ ID NOS: 338 and 339. In some cases, an Fc polypeptide can contain amino acid substitutions D356E and L358M to reconstitute residues of allotype G1 m3.

c. CD3 binding domain

[0400] A constrained multispecific polypeptide construct includes one or more copies of an antiCD3 binding domain. The anti-CD3 binding domains of the disclosure activate T cells via engagement of CD3 or a member of the CD3 complex on the T cells. In preferred embodiments, the anti-CD3 binding domains of the disclosure specifically bind the epsilon chain of CD3, also known as CD3ε. The anti-CD3ε binding domains of the disclosure activate T cells via engagement of CD3ε on the T cells. The anti-CD3 binding domains of the disclosure agonize, stimulate, activate, and/or otherwise augment CD3-mediated T cell activation. Biological activities of CD3 include, for example, T cell activation and other signaling through interaction between CD3 and the antigen-binding subunits of the T-Cell Receptor

(TCR). For example, the anti-CD3 binding domains of the disclosure completely or partially activate T cells via engagement of CD3ε on T cells by partially or completely modulating, *e.g.*, agonizing, stimulating, activating or otherwise augmenting CD3-mediated T cell activation.

[0401] The CD3 binding domain can be any as described above. In particular embodiments, the CD3 binding domain is an Fv antibody fragment that binds CD3ε (referred to herein as an anti-CD3ε Fv fragment). In some embodiments, the anti-CD3ε Fv antibody fragment is a disulfide stabilized anti-CD3 binding Fv fragment (dsFv). In some embodiments, the anti-CD3 binding domain is monovalent for binding CD3.

[0402] In some embodiments, the CD3 binding region is an Fv antibody fragment containing a variable heavy chain (Hv, also called VH) and variable light chain (Lv, also called VL), such as any as described. In aspects of such embodiments, the immunoglobulin Fc region is a heterodimeric Fc region containing two different Fc polypeptides capable of heterodimeric association between both polypeptides of the Fc heterodimer, such as any as described. In such embodiments, the variable heavy chain (VH) and variable light chain (VL) of the CD3 binding region are linked on opposite chains of the heterodimeric Fc.

[0403] In some embodiments, the CD3 binding region is an Fv or dsFv of SP34 (Pessano et ai. The EMBO Journal. 4: 337-344, 1985) or of a humanized variant of SP34 (WO2015001085).

[0404] In some embodiments, the anti-CD3ε binding domain thereof is an Fv, such as a dsFv fragment, that includes a combination of heavy chain variable amino acid sequence and a light chain variable amino acid sequence. In some embodiments, the CD3-binding domain is an Fv or dsFv fragment in which is contained a VH CDR1 sequence that includes at least the amino acid sequence TYAMN (SEQ ID NO: 29); a VH CDR2 sequence that includes at least the amino acid sequence RIRSKYNNYATYYADSVKD (SEQ ID NO: 30); a VH CDR3 sequence that includes at least the amino acid sequence HGNFGNSYVSWFAY (SEQ ID NO: 31), a VL CDR1 sequence that includes at least the amino acid sequence RSSTGAVTTSNYAN (SEQ ID NO: 32); a VL CDR2 sequence that includes at least the amino acid sequence GTNKRAP (SEQ ID NO: 33); and a VL CDR3 sequence that includes at least the amino acid sequence ALWYSNLWV (SEQ ID NO: 34).

[0405] In some embodiments, the anti-CD3ε binding domain includes a VH CDR1 sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence TYAMN (SEQ ID NO: 29); a VH CDR2 sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence RIRSKYNNYATYYADSVKD (SEQ ID NO: 30); a VH CDR3 sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence HGNFGNSYVSWFAY (SEQ ID NO: 31), a VL CDR1 sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence RSSTGAVTTSNYAN (SEQ ID NO: 32); a VL CDR2 sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%,

98%, 99% or more identical to the amino acid sequence GTNKRAP (SEQ ID NO: 33); and a VL CDR3 sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence ALWYSNLWV (SEQ ID NO: 34).

[0406] In some embodiments, the anti-CD3ε binding domain includes a VH CDR1 sequence that includes at least the amino acid sequence GFTFNTYAMN (SEQ ID NO: 350); a VH CDR2 sequence that includes at least the amino acid sequence RIRSKYNNYATY (SEQ ID NO: 351); a VH CDR3 sequence that includes at least the amino acid sequence HGNFGNSYVSWFAY (SEQ ID NO:31), a VL CDR1 sequence that includes at least the amino acid sequence RSSTGAVTTSNYAN (SEQ ID NO: 32); a VL CDR2 sequence that includes at least the amino acid sequence GTNKRAP (SEQ ID NO: 33); and a VL CDR3 sequence that includes at least the amino acid sequence ALWYSNLWV (SEQ ID NO:34).

[0407] In some embodiments, the anti-CD3ε binding domain includes a VH CDR1 sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence GFTFNTYAMN (SEQ ID NO: 350); a VH CDR2 sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence RIRSKYNNYATY (SEQ ID NO: 351); a VH CDR3 sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence HGNFGNSYVSWFAY (SEQ ID NO: 31), a VL CDR1 sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence RSSTGAVTTSNYAN (SEQ ID NO: 32); a VL CDR2 sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence GTNKRAP (SEQ ID NO: 33); and a VL CDR3 sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence ALWYSNLWV (SEQ ID NO: 34).

[0408] In some embodiments, the anti-CD3ε binding domain includes a VH CDR1 sequence that includes at least the amino acid sequence GFTFNTYAMN (SEQ ID NO:350); a VH CDR2 sequence that includes at least the amino acid sequence RIRSKYNNYATY (SEQ ID NO: 351); a VH CDR3 sequence that includes at least the amino acid sequence HGNFGNSYVSWFAY (SEQ ID NO: 31), a VL CDR1 sequence that includes at least the amino acid sequence GSSTGAVTTSNYAN (SEQ ID NO: 357); a VL CDR2 sequence that includes at least the amino acid sequence GTNKRAP (SEQ ID NO: 33); and a VL CDR3 sequence that includes at least the amino acid sequence ALWYSNHWV (SEQ ID NO: 353).

[0409] In some embodiments, the anti-CD3ε binding domain includes a VH CDR1 sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence GFTFNTYAMN (SEQ ID NO: 350); a VH CDR2 sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence RIRSKYNNYATY (SEQ ID NO: 351); a VH CDR3 sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence HGNFGNSYVSWFAY (SEQ ID NO: 31), a VL CDR1 sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more

identical to the amino acid sequence GSSTGAVTTSNYAN (SEQ ID NO: 357); a VL CDR2 sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence GTNKRAP (SEQ ID NO: 33); and a VL CDR3 sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence ALWYSNHWV (SEQ ID NO: 353).

[0410] In some embodiments, the anti-CD3ε binding domain includes a VH CDR1 sequence that includes at least the amino acid sequence GFTFSTYAMN (SEQ ID NO: 355); a VH CDR2 sequence that includes at least the amino acid sequence RIRSKYNNYATY (SEQ ID NO: 356); a VH CDR3 sequence that includes at least the amino acid sequence HGNFGDSYVSWFAY (SEQ ID NO: 352), a VL CDR1 sequence that includes at least the amino acid sequence GSSTGAVTTSNYAN (SEQ ID NO: 357); a VL CDR2 sequence that includes at least the amino acid sequence GTNKRAP (SEQ ID NO: 33); and a VL CDR3 sequence that includes at least the amino acid sequence ALWYSNHWV (SEQ ID NO: 353).

[0411] In some embodiments, the anti-CD3ε binding domain includes a VH CDR1 sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence GFTFSTYAMN (SEQ ID NO: 355); a VH CDR2 sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence RIRSKYNNYATY (SEQ ID NO: 356); a VH CDR3 sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence HGNFGDSYVSWFAY (SEQ ID NO: 352), a VL CDR1 sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence GSSTGAVTTSNYAN (SEQ ID NO: 357); a VL CDR2 sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence GTNKRAP (SEQ ID NO: 33); and a VL CDR3 sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence ALWYSNHWV (SEQ ID NO: 353).

[0412] In some embodiments, the anti-CD3ε binding domain includes a CDR3 that includes at least amino acids VLWYSNRWV (SEQ ID NO:354). In some embodiments, the anti-CD3ε binding domain includes a CDR3 that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acids VLWYSNRWV (SEQ ID NO:354).

[0413] In some embodiments, the anti-CD3ε binding domain includes one or more copies of an antibody or an antigen-binding fragment thereof selected from the group consisting of a Fab fragment, a $F(ab')_2$ fragment, an Fv fragment, a scFv, a scAb, a dAb, a single domain heavy chain antibody, and a single domain light chain antibody. In some embodiments, the anti-CD3 binding domain includes an Fv antibody fragment that binds CD3ε (referred to herein as an anti-CD3ε Fv fragment). In some embodiments, the anti-CD3ε Fv antibody fragment is a disulfide stabilized anti-CD3 binding Fv fragment (dsFv). In some embodiments, the anti-CD3 binding domain is monovalent for binding CD3.

[0414] In some embodiments, the CD3 binding region is not a single chain antibody. For example, in some aspects, the CD3 binding region is not a single chain variable fragment (scFv).

[0415] In some embodiments, the CD3 binding region is an Fv antibody fragment containing a variable heavy chain (Hv, also called VH) and variable light chain (Lv, also called VL), such as any as described. In aspects of such embodiments, the immunoglobulin Fc region is a heterodimeric Fc region containing two different Fc polypeptides capable of heterodimeric association between both polypeptides of the Fc heterodimer, such as any as described in Section III.C.2.b. In such embodiments, the variable heavy chain (VH) and variable light chain (VL) of the CD3 binding region are linked on opposite chains of the heterodimeric Fc.

[0416] In some embodiments, the anti-CD3ε binding domain thereof is an Fv fragment that includes a combination of heavy chain variable amino acid sequence and a light chain variable amino acid sequence. In some embodiments, the anti-CD3ε binding domain thereof is an Fv fragment that includes a combination of heavy chain variable amino acid sequence and a light chain variable amino acid sequence comprising an amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to an amino acid sequence selected from the group consisting of SEQ ID NOs: 27-28, 35-84, 293, 340-343, and 358. In some embodiments, the anti-CD3ε binding domain thereof is an Fv fragment that includes a combination of heavy chain variable amino acid sequence and a light chain variable amino acid sequence comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 27-28, 35-84, 293, 340-343, and 358.

[0417] In some embodiments, the anti-CD3ε binding domain thereof is an Fv fragment that includes a combination of heavy chain variable amino acid sequence selected from the group of SEQ ID NO: 27, 35-65, 341, 343, and 358 and light chain variable amino acid sequence selected from the group consisting of SEQ ID NO: 28, 66-84, 293, 340, and 342. In some embodiments, the anti-CD3ε binding domain thereof is an Fv fragment that includes a combination of heavy chain variable amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to an amino acid sequence selected from the group consisting of SEQ ID NO: 27, 35-65, 341, 343, and 358 and a light chain variable amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to an amino acid sequence selected from the group consisting of SEQ ID NO: 28, 66-84, 293, 340, and 342.

[0418] In some embodiments, the anti-CD3ε binding domain thereof includes a combination of a heavy chain variable region amino acid sequence and a light chain variable region amino acid sequence comprising an amino acid sequence selected from the group of SEQ ID NO: 27-28, 35-84, 293, 340-343, and 358. In some embodiments, the anti-CD3ε binding domain thereof includes a combination of a heavy chain variable region amino acid sequence selected from the group of SEQ ID NO: 27, 35-65, 341, 343, and 358 and a light chain variable region amino acid sequence comprising an amino acid sequence selected from the group of SEQ ID NO: 28,66-84, 293, 340, and 342. In some embodiments, the anti-

CD3ε Fv antibody fragment includes a combination of a heavy chain variable amino acid sequence selected from the group of SEQ ID NO: 27, 35-65, 341, 343, and 358 and a light chain variable amino acid sequence selected from the group consisting of SEQ ID NO: 28, 66-84, 293, 340, 342.In some embodiments, the anti-CD3ε Fv antibody fragment includes a combination of a heavy chain variable amino acid sequence selected from the group of SEQ ID NO: 27, 35-65, 341, 343, 358, 388, 389, 392, 393 and a light chain variable amino acid sequence selected from the group consisting of SEQ ID NO: 28, 66-84, 293, 340, 342, 390, 391, 394, 395.

[0419] In some embodiments, the anti-CD3ε binding domain thereof includes a variable heavy chain (VH) comprising an amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence of SEQ ID NO:27. In some embodiments, the anti-CD3ε binding domain includes a variable light chain (VL) comprising an amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence of SEQ ID NO: 28. In some embodiments, the anti-CD3ε binding domain thereof includes a variable heavy chain (VH) comprising an amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence of SEQ ID NO: 27 and a variable light chain (VL) comprising an amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence of SEQ ID NO: 28. In some embodiments, the anti-CD3ε binding domain thereof includes a variable heavy chain (VH) comprising the amino acid sequence of SEQ ID NO: 27. In some embodiments, the anti-CD3ε binding domain includes a variable light chain (VL) comprising the amino acid sequence of SEQ ID NO: 28. In some embodiments, the anti-CD3ε binding domain thereof includes a variable heavy chain (VH) comprising the amino acid sequence of SEQ ID NO: 27 and a variable light chain (VL) comprising the amino acid sequence of SEQ ID NO: 28.

[0420] In some embodiments, the anti-CD3ε binding domain thereof includes a variable heavy chain (VH) comprising an amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence of SEQ ID NO: 341. In some embodiments, the anti-CD3ε binding domain includes a variable light chain (VL) comprising an amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence of SEQ ID NO: 342. In some embodiments, the anti-CD3ε binding domain thereof includes a variable heavy chain (VH) comprising an amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence of SEQ ID NO: 341 and a variable light chain (VL) comprising an amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence of SEQ ID NO: 342. In some embodiments, the anti-CD3ε binding domain thereof includes a variable heavy chain (VH) comprising the amino acid sequence of SEQ ID NO: 341. In some embodiments, the anti-CD3ε binding domain includes a variable light chain (VL) comprising the amino acid sequence of SEQ ID

NO: 342. In some embodiments, the anti-CD3ε binding domain thereof includes a variable heavy chain (VH) comprising the amino acid sequence of SEQ ID NO: 341 and a variable light chain (VL) comprising the amino acid sequence of SEQ ID NO: 342.

[0421] In particular embodiments, the Fv is a disulfide stabilized Fv fragment (dsFv) in which the the V_H-V_L heterodimer is stabilized by an interchain disulfide bond. In some embodiments, the interchain disulfide bond is engineered by mutation of position in framework positions of the VH and/or VL chain. In some embodiments, the disulfide stabilized anti-CD3 Fv comprises an anti-CD3 VH with the mutation 44 to Cys and an anti-CD3 VL with the mutation 100 to Cys by Kabat numbering. For example, in some embodiments, the VH chain contains the mutation G44C and the VL chain contains the mutation G100C, each by kabat numbering. In some embodiments, the disulfide stabilized anti-CD3 Fv comprises an anti-CD3 VH with the mutation at position 105 to Cys and an anti-CD3 VL with the mutation position 43 to Cys by Kabat numbering.

[0422] In some embodiments, the anti-CD3ε binding domain thereof is an Fv, such as a dsFv fragment, that includes a heavy chain variable amino acid sequence selected from the group of SEQ ID NO: 35-65 and a light chain variable amino acid sequence selected from the group consisting of SEQ ID NO: 66-84, 293. In some embodiments, the anti-CD3ε binding domain thereof is an Fv, such as a dsFv fragment, that includes a heavy chain variable amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to an amino acid sequence selected from the group consisting of SEQ ID NO: 35-65 and an amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to an amino acid sequence selected from the group consisting of SEQ ID NO: 66-84, 293 an amino acid sequence. In some embodiments, the anti-CD3 binding domain is an Fv or dsFv, in which is contained a variable heavy chain (VH) comprising the amino acid sequence of SEQ ID NO: 47 and a variable light chain (VL) comprising the amino acid sequence of SEQ ID NO: 293.

[0423] In some embodiments, the anti-CD3ε binding domain thereof is an Fv fragment that includes a combination of heavy chain variable amino acid sequence and a light chain variable amino acid sequence. In some embodiments, the anti-CD3ε binding domain thereof is an Fv fragment that includes a combination of heavy chain variable amino acid sequence and a light chain variable amino acid sequence comprising an amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to an amino acid sequence selected from the group consisting of SEQ ID NOs: 27-28, 35-84, 293, 340-343, and 358. In some embodiments, the anti-CD3ε binding domain thereof is an Fv fragment that includes a combination of heavy chain variable amino acid sequence and a light chain variable amino acid sequence comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 27-28, 35-84, 293, 340-343, and 358.

[0424] In some embodiments, the anti-CD3ε binding domain thereof is an Fv fragment that includes a combination of heavy chain variable amino acid sequence selected from the group of SEQ ID NO: 27,

35-65, 341, 343, and 358 and light chain variable amino acid sequence selected from the group consisting of SEQ ID NO: 28, 66-84, 293, 340, and 342. In some embodiments, the anti-CD3ε binding domain thereof is an Fv fragment that includes a combination of heavy chain variable amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to an amino acid sequence selected from the group consisting of SEQ ID NO: 27, 35-65, 341, 343, and 358 and a light chain variable amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to an amino acid sequence selected from the group consisting of SEQ ID NO: 28, 66-84, 293, 340, and 342.

[0425] In some embodiments, the anti-CD3ε binding domain thereof includes a combination of a heavy chain variable region amino acid sequence and a light chain variable region amino acid sequence comprising an amino acid sequence selected from the group of SEQ ID NO: 27-28, 35-84, 293, 340-343, and 358. In some embodiments, the anti-CD3ε binding domain thereof includes a combination of a heavy chain variable region amino acid sequence selected from the group of SEQ ID NO: 27, 35-65, 341, 343, and 358 and a light chain variable region amino acid sequence comprising an amino acid sequence selected from the group of SEQ ID NO: 28,66-84, 293, 340, and 342. In some embodiments, the anti-CD3ε Fv antibody fragment includes a combination of a heavy chain variable amino acid sequence selected from the group of SEQ ID NO: 27, 35-65, 341, 343, and 358 and a light chain variable amino acid sequence selected from the group consisting of SEQ ID NO: 28, 66-84, 293, 340, 342.

[0426] In some embodiments, the anti-CD3ε binding domain thereof includes a variable heavy chain (VH) comprising an amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence of SEQ ID NO:27. In some embodiments, the anti-CD3ε binding domain includes a variable light chain (VL) comprising an amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence of SEQ ID NO: 28. In some embodiments, the anti-CD3ε binding domain thereof includes a variable heavy chain (VH) comprising an amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence of SEQ ID NO: 27 and a variable light chain (VL) comprising an amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence of SEQ ID NO: 28. In some embodiments, the anti-CD3ε binding domain thereof includes a variable heavy chain (VH) comprising the amino acid sequence of SEQ ID NO: 27. In some embodiments, the anti-CD3ε binding domain includes a variable light chain (VL) comprising the amino acid sequence of SEQ ID NO: 28. In some embodiments, the anti-CD3ε binding domain thereof includes a variable heavy chain (VH) comprising the amino acid sequence of SEQ ID NO: 27 and a variable light chain (VL) comprising the amino acid sequence of SEQ ID NO: 28.

[0427] In some embodiments, the anti-CD3ε binding domain thereof includes a variable heavy chain (VH) comprising an amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%,

97%, 98%, 99% or more identical to the amino acid sequence of SEQ ID NO: 341. In some embodiments, the anti-CD3ε binding domain includes a variable light chain (VL) comprising an amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence of SEQ ID NO: 342. In some embodiments, the anti-CD3ε binding domain thereof includes a variable heavy chain (VH) comprising an amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence of SEQ ID NO: 341 and a variable light chain (VL) comprising an amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence of SEQ ID NO: 342. In some embodiments, the anti-CD3ε binding domain thereof includes a variable heavy chain (VH) comprising the amino acid sequence of SEQ ID NO: 341. In some embodiments, the anti-CD3ε binding domain includes a variable light chain (VL) comprising the amino acid sequence of SEQ ID NO: 342. In some embodiments, the anti-CD3ε binding domain thereof includes a variable heavy chain (VH) comprising the amino acid sequence of SEQ ID NO: 341 and a variable light chain (VL) comprising the amino acid sequence of SEQ ID NO: 342.

[0428] In particular embodiments, the Fv is a disulfide stabilized Fv fragment (dsFv) in which the the V_H-V_L heterodimer is stabilized by an interchain disulfide bond. In some embodiments, the interchain disulfide bond is engineered by mutation of position in framework positions of the VH and/or VL chain. In some embodiments, the disulfide stabilized anti-CD3 Fv comprises an anti-CD3 VH with the mutation 44 to Cys and an anti-CD3 VL with the mutation 100 to Cys by Kabat numbering. For example, in some embodiments, the VH chain contains the mutation G44C and the VL chain contains the mutation G100C, each by kabat numbering. In some embodiments, the disulfide stabilized anti-CD3 Fv comprises an anti-CD3 VH with the mutation at position 105 to Cys and an anti-CD3 VL with the mutation position 43 to Cys by Kabat numbering.

[0429] In some embodiments, the anti-CD3ε binding domain thereof includes a combination of a heavy chain variable region amino acid sequence and a light chain variable region amino acid sequence comprising an amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to an amino acid sequence selected from the group consisting of SEQ ID NO: 67, 75, 77, 79, 81-84, 293, and 340. In some embodiments, the anti-CD3ε binding domain thereof includes a combination of a heavy chain variable region amino acid sequence and a light chain variable region amino acid sequence comprising an amino acid sequence selected from the group of SEQ ID NO: 47, 52-65, 67, 75, 77, 79, 81-84, 293, 340, 343, and 358. In some embodiments, the anti-CD3ε Fv antibody fragment includes a combination of a heavy chain variable amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to a light chain variable amino acid sequence selected from the group consisting of SEQ ID NO: 47 and 52-65, 343, and 358 and an amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to an amino acid sequence selected from the group consisting of SEQ ID NO: 67, 75, 77, 79, 81-84, 293,

and 340. In some embodiments, the anti-CD3ε Fv antibody fragment includes a combination of a heavy chain variable amino acid sequence selected from the group of SEQ ID NO: 47, 52-65, 343, and 358 and a light chain variable amino acid sequence selected from the group consisting of SEQ ID NO: 67, 75, 77, 79, 81-84, 293, and 340. In some of any such embodiments, the anti-CD3 Fv is a dsFv that has a VH chain containing the mutation G44C and a VL chain containing the mutation G100C, each by kabat numbering.

[0430] In some embodiments, the anti-CD3ε binding domain thereof includes a variable heavy chain (VH) comprising an amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence of SEQ ID NO: 47. In some embodiments, the anti-CD3ε binding domain includes a variable light chain (VL) comprising an amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence of SEQ ID NO: 75. In some embodiments, the anti-CD3ε binding domain thereof includes a variable heavy chain (VH) comprising an amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence of SEQ ID NO: 47 and a variable light chain (VL) comprising an amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence of SEQ ID NO: 75. In some embodiments, the anti-CD3ε binding domain thereof includes a variable heavy chain (VH) comprising the amino acid sequence of SEQ ID NO: 47. In some embodiments, the anti-CD3ε binding domain includes a variable light chain (VL) comprising the amino acid sequence of SEQ ID NO: 75. In some embodiments, the anti-CD3ε binding domain thereof includes a variable heavy chain (VH) comprising the amino acid sequence of SEQ ID NO: 47 and a variable light chain (VL) comprising the amino acid sequence of SEQ ID NO: 75. In some of any such embodiments, the anti-CD3 Fv is a dsFv that has a VH chain containing the mutation G44C and a VL chain containing the mutation G100C, each by kabat numbering.

[0431] In some embodiments, the anti-CD3ε binding domain thereof includes a variable heavy chain (VH) comprising an amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence of SEQ ID NO: 343. In some embodiments, the anti-CD3ε binding domain includes a variable light chain (VL) comprising an amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence of SEQ ID NO: 340. In some embodiments, the anti-CD3ε binding domain thereof includes a variable heavy chain (VH) comprising an amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence of SEQ ID NO: 343 and a variable light chain (VL) comprising an amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence of SEQ ID NO: 340. In some embodiments, the anti-CD3ε binding domain thereof includes a variable heavy chain (VH) comprising the amino acid sequence of SEQ ID NO: 343. In some embodiments, the anti-CD3ε binding domain

includes a variable light chain (VL) comprising the amino acid sequence of SEQ ID NO: 340. In some embodiments, the anti-CD3ε binding domain thereof includes a variable heavy chain (VH) comprising the amino acid sequence of SEQ ID NO: 343 and a variable light chain (VL) comprising the amino acid sequence of SEQ ID NO: 340. In some of any such embodiments, the anti-CD3 Fv is a dsFv that has a VH chain containing the mutation G44C and a VL chain containing the mutation G100C, each by kabat numbering.

[0432] In some embodiments, the anti-CD3ε binding domain thereof includes a variable heavy chain (VH) comprising an amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence of SEQ ID NO: 358. In some embodiments, the anti-CD3ε binding domain includes a variable light chain (VL) comprising an amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence of SEQ ID NO: 340. In some embodiments, the anti-CD3ε binding domain thereof includes a variable heavy chain (VH) comprising an amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence of SEQ ID NO: 358 and a variable light chain (VL) comprising an amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence of SEQ ID NO: 340. In some embodiments, the anti-CD3ε binding domain thereof includes a variable heavy chain (VH) comprising the amino acid sequence of SEQ ID NO: 358. In some embodiments, the anti-CD3ε binding domain includes a variable light chain (VL) comprising the amino acid sequence of SEQ ID NO: 340. In some embodiments, the anti-CD3ε binding domain thereof includes a variable heavy chain (VH) comprising the amino acid sequence of SEQ ID NO:358 and a variable light chain (VL) comprising the amino acid sequence of SEQ ID NO: 340. In some of any such embodiments, the anti-CD3 Fv is a dsFv that has a VH chain containing the mutation G44C and a VL chain containing the mutation G100C, each by kabat numbering.

d. Linker

[0433] A constrained multispecific polypeptide constructs contain a linker that joins or couples the first component containing the immunoglobulin Fc region and the second component containing the CD3 binding region. In some embodiments, the linker is positioned at the end of the C-terminal region of the Fc region, such that the Fc region is N-terminal to the CD3 binding region. It is understood that because the provided constrained multispecific polypeptide constructs are multimers, such as dimers containing a first and second polypeptide that together form the first and second component, the provided constructs include a linker joining the Fc portion and the CD3 binding region of the first and a linker joining the Fc portion and the CD3 binding region of the second polypeptide. In some embodiments, the first polypeptide includes a first Fc polypeptide of a heterodimeric Fc region, a linker, and a first domain (e.g. VH) of a CD3 binding region, and the second polypeptide includes a second Fc polypeptide of the heterodimeric Fc region, a linker and second domain (e.g. VL) of the CD3 binding region. Typically, the

linkers present in the first and second polypeptides of the constrained multispecific polypeptide construct are the same. Thus, in some embodiments, each domain of the CD3 binding domain is linked via a linker, such as the same linker, to opposite polypeptides of the Fc, such as heterodimeric Fc.

[0434] Various polypeptide linkers for use in fusion proteins are known (see e.g. Chen et al. (2013) Adv. Drug. Deliv. 65:1357-1369; and International PCT publication No. WO 2014/099997, WO2000/24884; U.S. Pat. No. 5,258,498; U.S. Pat. No. 5,525,491; U.S. Pat. No. 5,525,491, U.S. Pat. No. 6,132,992).

[0435] In some embodiments, the linker is chosen so that, when the CD3 binding region is joined to the Fc region of the multispecific polypeptide conjugate, the CD3 binding region is constrained and not able to, or not substantially able to, bind or engage CD3 on the surface of a cell, e.g. T cell, upon contact of the multispecific polypeptide construct with the cell. Various assays can be employed to assess binding or engagement of CD3 by the multispecific polypeptide construct, including assays to assess T cell binding, NFAT activation using a reporter system, cytolytic T cell activity, cytokine production and/or expression of T cell activation markers. Exemplary assays are shown in the provided Examples. Typically, the linker also is one that ensures correct folding of the polypeptide construct, does not exhibit a charge that would be inconsistent with the activity or function of the linked polypeptides or form bonds or other interactions with amino acid residues in one or more of the domains that would impede or alter activity of the linked polypeptides. In some embodiments, the linker is a polypeptide linker. The polypeptide linker can be a flexible linker or a rigid linker or a combination of both. In some aspects, the linker is a short, medium or long linker. In some embodiments, the linker is up to 40 amino acids in length. In some embodiments, the linker is up to 25 amino acids in length. In some embodiments, the linker is at least or is at least about 2 amino acids in length. In some aspects, a suitable length is, e.g., a length of at least one and typically fewer than about 40 amino acid residues, such as 2-25 amino acid residues, 5-20 amino acid residues, 5-15 amino acid residues, 8-12 amino acid . In some embodiments, the linker is from or from about 2 to 24 amino acids, 2 to 20 amino acids, 2 to 18 amino acids, 2 to 14 amino acids, 2 to 12 amino acids, 2 to 10 amino acids, 2 to 8 amino acids, 2 to 6 amino acids, 6 to 24 amino acids, 6 to 20 amino acids, 6 to 18 amino acids, 6 to 14 amino acids, 6 to 12 amino acids, 6 to 10 amino acids, 6 to 8 amino acids, 8 to 24 amino acids, 8 to 20 amino acids, 8 to 18 amino acids, 8 to 14 amino acids, 8 to 12 amino acids, 8 to 10 amino acids, 10 to 24 amino acids, 10 to 20 amino acids, 10 to 18 amino acids, 10 to 14 amino acids, 10 to 12 amino acids, 12 to 24 amino acids, 12 to 20 amino acids, 12 to 18 amino acids, 12 to 14 amino acids, 14 to 24 amino acids, 14 to 20 amino acids, 14 to 18 amino acids, 18 to 24 amino acids, 18 to 20 amino acids or 20 to 24 amino acids. In some embodiments, the linker is 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 amino acids in length.

[0436] In certain aspects, the longer the linker length, the greater the CD3 binding when the multispecific polypeptide conjugate is bounds to its antigen, e.g. TAA. Thus, in some aspects, the linker is greater than 12 amino acids in length, such as greater than 13, 14, 15, 16, 17 or 18 amino acids in

length. In some embodiments, the linker is 12 to 40 amino acids in length, 12 to 30 amino acids, 12 to 24 amino acids, 12 to 18 acids, 12 to 15 amino acids, 15 to 40 amino acids, 15 to 30 amino acids, 15 to 24 amino acids, 15 to 18 amino acids, 18 to 40 amino acids, 18 to 30 amino acids, 18 to 24 amino acids, 24 to 40 amino acids, 24 to 30 amino acids or 30 to 40 amino acids.

[0437] The linkers can be naturally-occurring, synthetic or a combination of both. Particularly suitable linker polypeptides predominantly include amino acid residues selected from Glycine (Gly), Serine (Ser), Alanine (Ala), and Threonine (Thr). For example, the linker may contain at least 75% (calculated on the basis of the total number of residues present in the peptide linker), such as at least 80%, at least 85%, or at least 90% of amino acid residues selected from Gly, Ser, Ala, and Thr. The linker may also consist of Gly, Ser, Ala and/or Thr residues only. In some embodiments, the linker contains 1-25 glycine residues, 5-20 glycine residues, 5-15 glycine residues, or 8-12 glycine residues. In some aspects, suitable peptide linkers typically contain at least 50% glycine residues, such as at least 75% glycine residues. In some embodiments, a peptide linker comprises glycine residues only. In some embodiments, a peptide linker comprises glycine and serine residues only.

[0438] In some embodiments, these linkers are composed predominately of the amino acids Glycine and Serine, denoted as GS-linkers herein. In some embodiments, the linker contains (GGS)n, wherein n is 1 to 10, such as 1 to 5, for example 1 to 3, such as GGS(GGS)n (SEQ ID NO:363), wherein n is 0 to 10. In particular embodiments, the linker contains the sequence (GGGGS)n (SEQ ID NO: 123), wherein n is 1 to 10 or n is 1 to 5, such as 1 to 3. In further embodiments, the linker contains (GGGGGS)n (SEQ ID NO:124), wherein n is 1 to 4, such as 1 to 3. The linker can include combinations of any of the above, such as repeats of 2, 3, 4, or 5 GS, GGS, GGGGS, and/or GGGGGS linkers may be combined. In some embodiments, such a linker is 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18 or 19 amino acids in length.

[0439] In some embodiments, the linker is (in one-letter amino acid code): GGS, GGGGS (SEQ ID NO: 125), GGGGGS (SEQ ID NO: 126), or GGGGSGGGGSGGGGS (SEQ ID NO:346). In some embodiments, the GS-linker comprises an amino acid sequence of GGSGGS, *i.e.*, $(GGS)_2$ (SEQ ID NO: 1); GGSGGSGGS, *i.e.*, $(GGS)_3$ (SEQ ID NO: 2); GGSGGSGGSGGS, *i.e.*, $(GGS)_4$ (SEQ ID NO: 3); GGSGGSGGSGGSGGS, *i.e.*, $(GGS)_5$ (SEQ ID NO: 4); GGGGGSGGGGGSGGGGGS, *i.e.*, $(G5S)_3$ (SEQ ID NO: 127), GGSGGGGSGGGGSGGGGS (SEQ ID NO: 129) and GGGGSGGGGSGGGGS (SEQ ID NO:128). In some embodiments, the linker is GGGG (SEQ ID NO:5). In some of any of the above examples, serine can be replaced with alanine (e.g., (Gly4Ala) or (Gly3Ala)). In some embodiments, the linker is GGGGG (SEQ ID NO:6). In some embodiments, the linker is PGGGG (SEQ ID NO:327).

[0440] In some embodiments, the linker includes a peptide linker having the amino acid sequence $Gly_xXaa\text{-}Gly_y\text{-}Xaa\text{-}Gly_z$ (SEQ ID NO:130), wherein each Xaa is independently selected from Alanine (Ala), Valine (Val), Leucine (Leu), Isoleucine (Ile), Methionine (Met), Phenylalanine (Phe), Tryptophan

(Trp), Proline (Pro), Glycine (Gly), Serine (Ser), Threonine (Thr), Cysteine (Cys), Tyrosine (Tyr), Asparagine (Asn), Glutamine (Gln), Lysine (Lys), Arginine (Arg), Histidine (His), Aspartate (Asp), and Glutamate (Glu), and wherein x, y, and z are each integers in the range from 1-5. In some embodiments, each Xaa is independently selected from the group consisting of Ser, Ala, and Thr. In a specific variation, each of x, y, and z is equal to 3 (thereby yielding a peptide linker having the amino acid sequence Gly-Gly-Gly-Xaa-Gly-Gly-Gly-Xaa-Gly-Gly-Gly (SEQ ID NO:131), wherein each Xaa is selected as above.

[0441] In some embodiments, the linker is serine-rich linkers based on the repetition of a (SSSSG)n (SEQ ID NO:132) motif where n is at least 1, though y can be 2, 3, 4, 5, 6, 7, 8 and 9.

[0442] In some cases, it may be desirable to provide some rigidity into the peptide linker. This may be accomplished by including proline residues in the amino acid sequence of the peptide linker. Thus, in some embodiments, a linker comprises at least one proline residue in the amino acid sequence of the peptide linker. For example, a peptide linker can have an amino acid sequence wherein at least 25% (e.g., at least 50% or at least 75%) of the amino acid residues are proline residues. In one particular embodiment, the peptide linker comprises proline residues only.

[0443] In some aspects, a peptide linker comprises at least one cysteine residue, such as one cysteine residue. For example, in some embodiments, a linker comprises at least one cysteine residue and amino acid residues selected from the group consisting of Gly, Ser, Ala, and Thr. In some such embodiments, a linker comprises glycine residues and cysteine residues, such as glycine residues and cysteine residues only. Typically, only one cysteine residue will be included per peptide linker. One example of a specific linker comprising a cysteine residue includes a peptide linker having the amino acid sequence Gly_m-Cys-Gly_n, wherein n and m are each integers from 1-12, e.g., from 3-9, from 4-8, or from 4-7. In a specific variation, such a peptide linker has the amino acid sequence GGGGG-C-GGGGG (SEQ ID NO: 133).

[0444] In some embodiments, the linker of the fusion protein is a structured or constrained linker. In particular embodiments, the structured linker contains the sequence (AP)n or (EAAAK)n (SEQ ID NO:134), wherein n is 2 to 20, preferably 4 to 10, including but not limited to, AS-(AP)n-GT (SEQ ID NO:135) or AS-(EAAAK)n-GT (SEQ ID NO:136), wherein n is 2 to 20, such as 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15. In other embodiments, the linker comprises the sequences (GGGGA)n (SEQ ID NO:137), (PGGGS)n (SEQ ID NO:138), (AGGGS)n (SEQ ID NO:139) or GGS-(EGKSSGSGSESKST)n-GGS (SEQ ID NO:140, wherein n is 2 to 20), (ADAAP)n (SEQ ID NO:396, wherein n is 2 to 20), (ADAAP)n-G (SEQ ID NO:397, wherein n is 2 to 20), (GEPQG)n (SEQ ID NO:398, wherein n is 2 to 20), (GEPQG)n-G (SEQ ID NO:399, wherein n is 2 to 20), (AGGEP)n (SEQ ID NO:400, wherein n is 2 to 20), (AGGEP)n-G (SEQ ID NO:401, wherein n is 2 to 20), (AGSEP)n (SEQ ID NO:402, wherein n is 2 to 20), (AGSEP)n-G (SEQ ID NO:403, wherein n is 2 to 20), (GGGEQ)n (SEQ ID NO:404, wherein n is 2 to 20), or (GGGEQ)n-G (SEQ ID NO:405, wherein n is 2 to 20). In some embodiments, the linker is SSSASASSA (SEQ ID NO:141), GSPGSPG (SEQ ID

NO:142), ATTTGSSPGPT (SEQ ID NO:143), ADAAPADAAPG (SEQ ID NO:406), GEPQGGEPQGG (SEQ ID NO:407), AGGEPAGGEPG (SEQ ID NO:408), AGSEPAGSEPG (SEQ ID NO:409), or GGGEQGGGEQG (SEQ ID NO:410). In some embodiments, such linkers, by virtue of their structure, may be more resistant to proteolytic degradation, thereby offering an advantage when injected in vivo. In some embodiments, such linkers are negatively charged and may be better suited for dampening the binding of the CD3 binding domain to CD3.

[0445] In some embodiments, the linker is not a cleavable linker, also called non-cleavable linker. In some embodiments, the linker is not a cleavable by a protease. In some embodiments, a linker that is not a cleavable linker or that is not cleavable by a protease is one that is generally stable for in vivo delivery or recombinant production. In some aspects, a linker that is not cleavable by a protease includes those that do not contain at least one peptide bond which preferably lies within a cleavable peptide sequence or recognition site of a protease. In particular embodiments, a non-cleavable linker is not a target substrate for a protease, such that it is not preferentially or specifically cleaved by a protease compared to a linker that contains a substrate recognition site for the same protease.

[0446] In some embodiments, the linker does not contains a substrate recognition site or cleavage site for a particular protease, which is the sequence recognized by the active site of a protease that is cleaved by a protease. Typically, for example, for a serine protease, a cleavage sequence is made up of the P1-P4 and P1′-P4′ amino acids in a substrate, where cleavage occurs after the P1 position. Typically, a cleavage sequence for a serine protease is six residues in length to match the extended substrate specificity of many proteases, but can be longer or shorter depending upon the protease. Typically, the linker does not include a P1-P1′ scissile bond sequence that is recognized by a protease. In some aspects, a non-cleavable linker or a linker that does not contain a substrate recognition site that is specifically recognized for cleavage by a protease is one whose cleavage by a protease is substantially less than cleavage of a target substrate of the protease.

[0447] In some embodiments, the linker is a cleavable linker. In some aspects, a cleavable linker is a linker, such as any described above, that further includes a sequence that is a substrate for a protease due to the presence of at least one bond that can be broken under physiological conditions. In some cases, a cleavable linker is susceptible to or sensitive to cleavage under specific conditions that exist in vivo, such as following exposure to an extracellular protease, including those present in cellular environments in vivo. In some cases, the protease may be present in a particular physiological microenvironment, such as the tumor microenvironment, thereby restricting the sites at which cleavage may occur.

[0448] A protease typically exhibits specificity or preference for cleavage of a particular target substrate compared to another non-target substrate. Such a degree of specificity can be determined based on the rate constant of cleavage of a sequence, e.g. linker, which is a measure of preference of a protease for its substrate and the efficiency of the enzyme. Any method to determine the rate of increase of

cleavage over time in the presence of various concentrations of substrate can be used to calculate the specificity constant. For example, a substrate is linked to a fluorogenic moiety, which is released upon cleavage by a protease. By determining the rate of cleavage at different protease concentrations the specificity constant for cleavage (k_{cat}/K_m) can be determined for a particular protease towards a particular linker. In some embodiments, a cleavable linker is a linker that is capable of being specifically cleaved by a protease at a rate of about at least 1×10^4 $M^{-1}S^{-1}$, or at least 5×10^4 $M^{-1}S$, at least 10×10^4 $M^{-1}S$. at least 10×10^5 $M^{-1}S$ or more.

[0449] In some embodiments, a constrained multispecific polypeptide constructs of the disclosure include a cleavable linker that joins the first and second components. In some embodiments, the cleavable linker includes an amino acid sequence that can serve as a substrate for a protease, usually an extracellular protease. For example, the cleavable linker may include a cleavage sequence containing at least one peptide bond which preferably lies within a cleavable peptide sequence of a protease. Suitable proteases include, for example, matrix metalloproteases (MMP), cysteine proteases, serine proteases and plasmin activators, which are formed or activated in intensified manner in diseases such as rheumatoid arthritis or cancer, leading to excessive tissue degradation, inflammations and metastasis. In particular embodiments, the protease is a protease that is produced by a tumor, an activated immune effector cell (e.g. a T cell or a NK cell), or a cell in a tumor microenvironment. In some embodiments, the protease is a granzyme B, a matriptase or an MMP, such as MMP-2.

[0450] The cleavable linker may be selected based on a protease that is produced by a tumor that is in proximity to cells that express the target and/or produced by a tumor that is co-localized in tissue with the desired target of the multispecific polypeptide constructs. There are reports in the literature of increased levels of proteases having known substrates in a number of cancers, *e.g.*, solid tumors. See, *e.g.*, La Rocca et al, (2004) British J. of Cancer 90(7): 1414-1421.

[0451] In some embodiments, the cleavable linker that joins the first and second component of a constrained multispecific polypeptide construct is cleaved by a protease produced by an immune effector cell that is activated by one of the components. For example, multispecific polypeptide constructs that encompass an effector enabled or enhanced IgG Fc region are capable of eliciting ADCC when engaged with the target antigen. Central to ADCC is the release of granzyme B and perforin from the effector cells, namely NK cells and cytotoxic T-cells. Upon release, granzyme B enters the target cell in a perforin dependent manner wherein it mediates apoptosis. Importantly, granzyme B is active within the extracellular synapse between the effector cell and the target cell. In some embodiments, the cleavable linker that joins the first and second component multispecific polypeptide construct is cleaved by granzyme B. Granzyme B is released during effector cell activation mediated by one of the components of the multispecific polypeptide construct. In some embodiments, granzyme B and other proteases can be produced by immune effector cells, including activated T cells or NK cells. In some embodiments, activation of T cells by CD3 engagement upon binding of a TAA by a multispecific polypeptide

construct may release such proteases, which then can cleave a specific cleavable linker thereby potentiating or increasing activity of the CD3 binding molecule to engage CD3. In some embodiments, the cleavage can amplify or increase the activity achieved by the multispecific construct when bound to TAA in an uncleaved state.

[0452] Exemplary substrates include but are not limited to substrates cleavable by one or more of the following enzymes or proteases: ADAMS, ADAMTS, *e.g.* ADAM8; ADAM9; ADAM10; ADAM12; ADAM15; ADAM17/TACE; ADAMDEC1; ADAMTS1; ADAMTS4; ADAMTS5; aspartate proteases, *e.g.*, BACE or Renin; aspartic cathepsins, *e.g.*, Cathepsin D or Cathepsin E; Caspases, *e.g.*, Caspase 1, Caspase 2, Caspase 3, Caspase 4, Caspase 5, Caspase 6, Caspase 7, Caspase 8, Caspase 9, Caspase 10, or Caspase 14; cysteine cathepsins, *e.g.*, Cathepsin B, Cathepsin C, Cathepsin K, Cathepsin L, Cathepsin S, Cathepsin V/L2, Cathepsin X/Z/P; Cysteine proteinases, *e.g.*, Cruzipain; Legumain; Otubain-2; KLKs, *e.g.*, KLK4, KLK5, KLK6, KLK7, KLK8, KLK10, KLK11, KLK13, or KLK14; Metallo proteinases, *e.g.*, Meprin; Neprilysin; PSMA; BMP-1; MMPs, *e.g.*, MMP1, MMP2, MMP3, MMP7, MMP8, MMP9, MMP10, MMP11, MMP12, MMP13, MMP14, MMP15, MMP16, MMP17, MMP19, MMP20, MMP23, MMP24, MMP26, or MMP27, serine proteases, *e.g.*, activated protein C, Cathepsin A, Cathepsin G, Chymase, coagulation factor proteases (*e.g.*, FVIIa, FIXa, FXa, FXIa, FXIIa), Elastase, granzyme B, Guanidinobenzoatase, HtrA1, Human Neutrophil Elastase, Lactoferrin, Marapsin, NS3/4A, PACE4, Plasmin, PSA, tPA, Thrombin, Tryptase, uPA; Type II Transmembrane Serine Proteases (TTSPs), *e.g.*, DESC1, DPP-4, FAP, Hepsin, Matriptase-2, Matriptase, TMPRSS2, TMPRSS3, or TMPRSS4; and any combination thereof.

[0453] In some embodiments, the cleavable linker is cleaved by multiple proteases, *e.g.*, 2 or more proteases, 3 or more proteases, 4 or more proteases, and so on.

[0454] In some embodiments, the cleavable linker is selected for use with a specific protease, for example a protease that is known to be produced by a tumor that is in proximity to cells that express the target and/or produced by a tumor that is co-localized with the target of the multispecific polypeptide construct.

[0455] In some embodiments, the cleavable linker contains a substrate recognition site or cleavage site for a particular protease, which is the sequence recognized by the active site of a protease that is cleaved by a protease. Typically, for example, for a serine protease, a cleavage sequence is made up of the P1-P4 and P1′-P4′ amino acids in a substrate, where cleavage occurs after the P1 position. Typically, a cleavage sequence for a serine protease is six residues in length to match the extended substrate specificity of many proteases, but can be longer or shorter depending upon the protease. Typically, the cleavable linker includes a P1-P1′ scissile bond sequence that is recognized by a protease. In some aspects, the cleavable linker is engineered to introduce a peptide bond able to be cleaved by a specific protease, for example by introducing a substrate recognition site sequence or cleavage sequence of the protease.

[0456] In some embodiments, the cleavable linker includes a combination of two or more substrate sequences. In some embodiments, each substrate sequence is cleaved by the same protease. In some embodiments, at least two of the substrate sequences are cleaved by different proteases. In some embodiments, the cleavable linker comprises an amino acid that is a substrate for granzyme B. In some embodiments, a granzyme B cleavable linker contains an amino acid sequence having the general formula P4 P3 P2 P1 ↓ P1' (SEQ ID NO: 144), wherein P4 is amino acid I, L, Y, M, F, V, or A; P3 is amino acid A, G, S, V, E, D, Q, N, or Y; P2 is amino acid H, P, A, V, G, S, or T; P1 is amino acid D or E; and P1' is amino acid I, L, Y, M, F, V, T, S, G or A. In some embodiments, a granzyme B cleavable linker contains an amino acid sequence having the general formula P4 P3 P2 P1 ↓ P1' (SEQ ID NO: 145), wherein P4 is amino acid I or L; P3 is amino acid E; P2 is amino acid P or A; P1 is amino acid D; and P1' is amino acid I, V, T, S, or G.

[0457] In some embodiments, the substrate for granzyme B comprises the amino acid sequence LEAD (SEQ ID NO: 146), LEPD (SEQ ID NO: 147), or LEAE (SEQ ID NO:148). In some embodiments, the cleavable linker contains the amino acid sequence the cleavable linker comprises the amino acid sequence IEPDI (SEQ ID NO:149), LEPDG (SEQ ID NO:150), LEADT (SEQ ID NO:151), IEPDG (SEQ ID NO:152), IEPDV (SEQ ID NO:153), IEPDS (SEQ ID NO:154), IEPDT (SEQ ID NO:155), IEPDP (SEQ ID NO:361), IEPDG (SEQ ID NO:152) or LEADG (e.g., SEQ ID NO:144).

[0458] In some embodiments, the cleavable linker comprises an amino acid that is a substrate for matriptase. In some embodiments, the cleavable linker comprises the sequence P1QAR↓(A/V) (SEQ ID NO: 156), wherein P1 is any amino acid. In some embodiments, the cleavable linker comprises the sequence RQAR(A/V) (SEQ ID NO: 157). In some embodiments, the substrate for matriptase comprises the amino acid sequence RQAR (SEQ ID NO: 158). In some embodiments, the cleavable linker comprises the amino acid sequence RQARV (SEQ ID NO: 159).

[0459] In some embodiments, the cleavable linker comprises an amino acid that is a substrate for one or more matrix metalloproteases (MMPs). In some embodiments, the MMP is MMP-2. In some embodiments, the cleavable linker contains. the general formula P3 P2 P1 ↓ P1' (SEQ ID NO: 160), wherein P3 is P, V or A; P2 is Q or D; P1 is A or N; and P1' is L, I or M. In some embodiments, the cleavable linker contains the general formula P3 P2 P1 ↓ P1' (SEQ ID NO: 161), wherein P3 is P; P2 is Q or D; P1 is A or N; and P1' is L or I. In some embodiments, the substrate for MMP comprises the amino acid sequence PAGL (SEQ ID NO: 162).

[0460] In some embodiments, the cleavable linker comprises a combination of an amino acid sequence that is a substrate for granzyme B and an amino acid sequence that is a substrate for matriptase. In some embodiments, the cleavable linker comprises a combination of the amino acid sequence LEAD (SEQ ID NO: 146) and the amino acid sequence RQAR (SEQ ID NO: 158).

[0461] In some embodiments, the cleavable linker comprises a combination of an amino acid sequence that is a substrate for granzyme B and an amino acid sequence that is a substrate for MMP. In

some embodiments, the cleavable linker comprises a combination of the amino acid sequence LEAD (SEQ ID NO: 146) and the amino acid sequence PAGL (SEQ ID NO: 162).

[0462] In some embodiments, the cleavable linker comprises a combination of an amino acid sequence that is a substrate for matriptase and an amino acid sequence that is a substrate for MMP. In some embodiments, the cleavable linker comprises a combination of the amino acid sequence RQAR (SEQ ID NO: 158) and the amino acid sequence PAGL (SEQ ID NO: 162).

[0463] In some embodiments, the cleavable linker comprises a combination of an amino acid sequence that is a substrate for granzyme B, an amino acid sequence that is a substrate for matriptase, and an amino acid sequence that is a substrate for MMP. In some embodiments, the cleavable linker comprises a combination of an amino acid sequence that is a substrate for granzyme B and an amino acid sequence that is a substrate for MMP. In some embodiments, the cleavable linker comprises a combination of the amino acid sequence LEAD (SEQ ID NO: 146), the amino acid sequence RQAR (SEQ ID NO: 158), and the amino acid sequence PAGL (SEQ ID NO: 162).

[0464] The cleavable linker can include any known linkers. Examples of cleavable linkers are described in Be'liveau et al. (2009) FEBS Journal, 276; U.S. published application Nos. US20160194399; US20150079088; US20170204139; US20160289324; US20160122425; US20150087810; US20170081397; U.S. Patent No. US9644016.

[0465] In some embodiments, the cleavable linker comprises an amino acid sequence selected from the group consisting of TGLEADGSPAGLGRQARVG (SEQ ID NO: 163); TGLEADGSRQARVGPAGLG (SEQ ID NO: 164); TGSPAGLEADGSRQARVGS (SEQ ID NO: 162); TGPAGLGLEADGSRQARVG (SEQ ID NO: 166); TGRQARVGLEADGSPAGLG (SEQ ID NO: 167); TGSRQARVGPAGLEADGS (SEQ ID NO: 168); and TGPAGLGSRQARVGLEADGS (SEQ ID NO:169); GPAGLGLEPDGSRQARVG (SEQ ID NO: 170); GGSGGGGIEPDIGGSGGS (SEQ ID NO: 171); GGSGGGGLEADTGGSGGS (SEQ ID NO: 172); GSIEPDIGS (SEQ ID NO: 173); GSLEADTGS (SEQ ID NO: 174); GGSGGGGIEPDGGGSGGS (SEQ ID NO: 175); GGSGGGGIEPDVGGSGGS (SEQ ID NO: 176); GGSGGGGIEPDSGGSGGS (SEQ ID NO: 177); GGSGGGGIEPDTGGSGGS (SEQ ID NO: 178); GGGSLEPDGSGS (SEQ ID NO: 179); and GPAGLGLEADGSRQARVG (SEQ ID NO: 180), GGEGGGGSGGSGGGS (SEQ ID NO: 181); GSSAGSEAGGSGQAGVGS (SEQ ID NO: 182); GGSGGGGLEAEGSGGGGS (SEQ ID NO: 183); GGSGGGGIEPDPGGSGGS(SEQ ID NO: 184); TGGSGGGGIEPDIGGSGGS (SEQ ID NO: 185).

e. Costimulatory Binding Domain

[0466] The multispecific polypeptide constructs of the present disclosure include one or more co-stimulatory receptor binding region (CRBR) that binds a costimulatory receptor. In some embodiments, the one or more CRBR of the provided multispecific polypeptide constructs binds a co-stimulatory receptor expressed on T cells. In some embodiments, the co-stimulatory receptor is upregulated, induced, or expressed on the surface of an activated T cell. In some aspects, the CRBR binds a co-

stimulatory receptor and stimulates the co-stimulatory receptor. In some embodiments, agonistic binding of the co-stimulatory receptor to the CRBR of the multispecific polypeptide induces downstream signaling in the T cell to potentiate or enhance T cell activation or functionalities following engagement of CD3. In some embodiments, the CRBR, or independently each of the CRBRs, is an antibody or antigen binding fragment, a natural cognate binding partner of the co-stimulatory receptor, an Anticalin (engineered lipocalin), a Darpin, a Fynomer, a Centyrin (engineered fibroneticin III domain), a cystine-knot domain, an Affilin, an Affibody, or an engineered CH3 domain.

[0467] In some embodiments, the CRBR, or independently each of the CRBRs, such as the first CRBR and the second CRBRs, includes one or more copies of an antibody or an antigen-binding fragment thereof. In some embodiments, the CRBR or independently each of the CRBRs, such as the first antigen-binding domain and the second CRBRs, includes one or more copies of an antibody or an antigen-binding fragment thereof selected from the group consisting of a Fab fragment, a $F(ab')_2$ fragment, an Fv fragment, a scFv, a scAb, a dAb, a single domain heavy chain antibody, and a single domain light chain antibody.

[0468] In some embodiments, the CRBR, or independently each of the CRBRs, such as the first CRBR and the second CRBRs, is a single chain antibody. In some examples, the single chain is an scFv, a scAb, a single domain heavy chain antibody, or a single domain light chain antibody.

[0469] In some embodiments, the CRBR, or independently each of the CRBRs, such as the first CRBR and the second CRBR, includes one or more single domain antibody (sdAb) fragments, for example V_HH, V_{NAR}, engineered V_H or V_K domains. V_HHs can be generated from natural camelid heavy chain only antibodies, genetically modified rodents that produce heavy chain only antibodies, or naïve/synthetic camelid or humanized camelid single domain antibody libraries. $V_{NAR}s$ can be generated from cartilaginous fish heavy chain only antibodies. Various methods have been implemented to generate monomeric sdAbs from conventionally heterodimeric V_H and V_K domains, including interface engineering and selection of specific germline families.

[0470] In some embodiments, the CRBR, or independently each of the CRBRs such as the first CRBR and/or the second CRBR, of the multispecific polypeptide constructs contains at least one sdAb or an scFv that binds a costimulatory receptor. In some embodiments, the at least one scFv or sdAb that binds a costimulatory receptor is positioned amino-terminally relative to the Fc region and/or carboxy-terminally relative to the CD3 binding region of the multispecific polypeptide construct. In some embodiments, the multispecific polypeptide construct contains only one scFv or sdAb that binds to a costimulatory receptor, which can be positioned either amino-terminally relative to the Fc region and/or carboxy-terminally relative to the CD3 binding region. In some embodiments, the multispecific polypeptide construct contains two scFv or sdAb that bind to a costimulatory receptor, positioned amino-terminally relative to the Fc region and/or carboxy-terminally relative to the CD3 binding region.

[0471] In some embodiments, the multispecific polypeptide construct is formed from or includes two polypeptides, including a first polypeptide comprising a first Fc polypeptide of a heterodimeric Fc region, a linker, a VH domain of an anti-CD3 antibody or antigen binding fragment (e.g. Fv), and an scFv or sdAb that binds to a costimulatory receptor; and a second polypeptide comprising a second Fc polypeptide of the heterodimeric Fc region, the linker, a VL domain of the anti-CD3 antibody or antigen binding fragment (e.g. Fv) and, optionally, another, the same or different, scFv or sdAb that binds to a costimulatory receptor. The scFv or sdAb that binds the costimulatory receptor can be positioned amino terminally relative to an Fc polypeptide of the heterodimeric Fc and/or carboxy-terminally relative to a VH or VL chain of the CD3 binding region. At least one of the first and/or second polypeptide of the multispecific polypeptide construct also includes an antigen binding domain that binds a TAA or a chain thereof as described in Section II.4. In some embodiments, the antigen binding domain that binds a TAA is a scFv or sdAb and is included as part of the first and/or second polypeptide of the multispecific polypeptide construct. In some embodiments, the antigen binding domain that binds a TAA is a Fab, and the multispecific polypeptide construct is additionally formed from a third polypeptide where at least the first and second polypeptide include a chain of the Fab that binds TAA (e.g. VH-CH1 or VL-CL of a Fab) and the third polypeptide contains the other chain of the Fab that binds TAA (e.g. the other of VH-CH1 or VL-CL of a Fab).

[0472] In some embodiments, the CRBR or independently each of the CRBRs, such as the first CRBR and/or the second CRBRs, contains more than one chain. In some embodiments, the CRBR or independently each of the CRBRs, such as the first CRBR and/or the second CRBRs, of the multispecific polypeptide constructs contains VH and VL sequences assembled as FABs.

[0473] In some embodiments, the antigen binding domain or independently each of the antigen binding domains, such as the first antigen-binding domain and/or the second antigen binding domains, of the multispecific polypeptide constructs contains a VH-CH1 (Fd) and a VL-CL of a Fab antibody that binds a costimulatory receptor. In some embodiments, the Fab antibody containing a VH-CH1 (Fd) and a VL-CL is positioned amino-terminally relative to the Fc region and/or carboxy-terminally relative to the CD3 binding region of the multispecific polypeptide construct. In some embodiments, the multispecific polypeptide construct contains only one Fab antibody, containing a VH-CH1 (Fd) or VL-CL, that binds to a costimulatory receptor, which can be positioned either amino-terminally relative to the Fc region and/or carboxy-terminally relative to the CD3 binding region. In some embodiments, the multispecific polypeptide construct contains two Fab antibody fragments, each containing a VH-CH1 (Fd) and VL-CL, that binds to a costimulatory receptor, in which one is positioned amino-terminally relative to the Fc region and the other is positioned carboxy-terminally relative to the CD3 binding region.

[0474] In some embodiments, the multispecific polypeptide construct is formed from or includes three or more polypeptides, including a first polypeptide comprising a first Fc polypeptide of a

heterodimeric Fc region, a linker and a VH-CH1 (Fd) or VL-CL of a Fab antibody fragment that binds to a costimulatory receptor; a second polypeptide comprising a second Fc polypeptide of the heterodimeric Fc region, the linker and, optionally, the same VH-CH1 (Fd) or VL-CL of the Fab antibody fragment that binds to a costimulatory receptor, and a third polypeptide comprising the other of the VH-CH1 (Fd) or VL-CL of the Fab antibody fragment that binds to the costimulatory receptor. The first, second and/or third polypeptide of the multispecific polypeptide construct also can include a 5T4 VHH domain, such as any as described.

[0475] In some embodiments, the CRBR, or independently each of the CRBRs, is or includes a natural (native) cognate binding partner of the co-stimulatory receptor (e.g. a natural ligand), or a variant thereof that exhibits binding activity to the co-stimulatory receptor.

[0476] In some embodiments, the one or more CRBR of the provided multispecific polypeptide constructs bind a co-stimulatory receptor expressed on T cells. In some embodiments, there are more than one CRBR that binds to a costimulatory receptor and each of the CRBRs, such as the first CRBR and the second CRBR, bind the same co-stimulatory receptor. In some embodiments, each of the CRBRs, such as the first CRBR and the CRBRs, bind a different co-stimulatory receptor. In some embodiments, each of the CRBRs, such as the first CRBR and the second CRBR bind a different epitope on the same co-stimulatory receptor. In some embodiments, each of the CRBRs, such as the first antigen-CRBR and the CRBR, bind the same epitope on the same co-stimulatory receptor.

[0477] In some embodiments, the CRBR, or independently each of the CRBRs that binds a co-stimulatory receptor results in monovalent, bivalent, trivalent, or tetravalent binding to the co-stimulatory receptor.

[0478] In some embodiments, the antigen binding domains results in monovalent, bivalent, trivalent, or tetravalent binding to the TAA. In some embodiments, bivalent binding to the TAA comprises two antigen binding domains that bind the same epitope of the same antigen (e.g. mono-epitopic). In some embodiments, bivalent binding to the TAA comprises two antigen binding domains that bind different epitopes of the same antigen (e.g. bi-epitopic). In some embodiments, monovalent binding to the TAA comprises one antigen binding domain that binds one epitope of the antigen (e.g.mono-epitopic).

[0479] In some embodiments, the co-stimulatory receptor is expressed on T cells, such as primary T cells obtained from a subject. In some embodiments, the co-stimulatory receptor is expressed on human T cells, such as primary human T cells obtained from a human subject.

[0480] In some embodiments, the co-stimulatory receptor is a member of the tumor necrosis factor (TNF) receptor family. In some embodiments, the costimulatory receptor is a member of the immunoglobulin superfamily (IgSF). In some embodiments, the costimulatory receptor is a member of the B7 family of receptors.

[0481] In some embodiments, the co-stimulatory receptor is selected from the group consisting of 41BB (CD137), OX40 (CD134), CD27, glucocorticoid-induced TNFR-related protein (GITR), CD28, ICOS, CD40, B-cell activating factor receptor (BAFF-R), B-cell maturation antigen (BCMA), Transmembrane activator and CAML interactor (TACI), and NKG2D. In some embodiments, the co-stimulatory receptor is selected from 41BB, OX40, GITR, ICOS, or CD28. In some embodiments, the co-stimulatory receptor is selected from 41BB, OX40, or GITR.

[0482] In some embodiments, the costimulatory receptor is 41BB. In some embodiments, the costimulatory receptor is OX40. In some embodiments, the costimulatory receptor is GITR. In some embodiments, the costimulatory receptor is ICOS. In some embodiments, the costimulatory receptor is CD28.

[0483] In some embodiments, the CRBR of the multispecific polypeptide is or comprises an agonistic binding molecule to the co-stimulatory receptor. The CRBR can bind to the co-stimulatory receptor and initiate, induce, or stimulate a reaction or activity that is similar to or the same as that initiated, induced, or stimulated by the receptor's natural ligand. In some aspects, the binding of the CRBR to the co-stimulatory receptor induces or stimulates a downstream signal that is more than 5%, more than 10%, more than 20%, more than 30%, more than 40%, more than 50%, more than 60%, more than 70%, more than 80%, more than 90%, or more than 100% of the signal that is initiated, induced, or stimulated by the receptor's natural ligand.

[0484] In some embodiments, the one or more CRBR is an antibody or fragment thereof that binds to the co-stimulatory receptor 41BB (CD137), OX40 (CD134), CD27, glucocorticoid-induced TNFR-related protein (GITR), CD28, ICOS, CD40, B-cell activating factor receptor (BAFF-R), B-cell maturation antigen (BCMA). In some embodiments, the one or more CRBR is an antibody or fragment thereof that binds to the co-stimulatory receptor 41BB, OX40, GITR, ICOS, or CD28. In some embodiments, the one or more CRBR is an antibody or fragment thereof that binds to the co-stimulatory receptor 41BB, OX40, or GITR. Exemplary polypeptides for binding 41BB, OX40 and GITR are described in PCT publication. No. WO2017123650, WO2017123673, and WO2017015623, respectively. In some embodiments, the one or more CRBR is a single domain antibody (sdAb) that binds the co-stimulatory receptor, such as those described in PCT publication. No. WO2017123650, WO2017123673, and WO2017015623.

[0485] In some examples, the co-stimulatory receptor binding region (CRBR) binds or comprises a natural cognate binding partner of 41BB (CD137), OX40 (CD134), CD27, glucocorticoid-induced TNFR-related protein (GITR), CD28, ICOS, CD40, B-cell activating factor receptor (BAFF-R), B-cell maturation antigen (BCMA), Transmembrane activator and CAML interactor (TACI), NKG2D. In some embodiments, the natural cognate binding partner is selected from 41BB ligand (41BBL), OX40L (CD252), CD70, GITR Ligand/TNFSF18, CD80 (B7-1), CD86 (B7-2), ICOS Ligand (ICOSL), CD154

(CD40L), B-cell activating factor (BAFF), A proliferation-inducing ligand (APRIL), NKG2D ligands, or a functional fragment thereof.

[0486] In some embodiments, the co-stimulatory receptor binding region (CRBR) is an antibody or antigen binding fragment that binds 41BB. In particular examples, the CRBR that binds 4-1BB is a single domain antibody. In some embodiments, the sdAb contains a CDR1 GFSFSINAMG (set forth in SEQ ID NO:347), a CDR2 AIESGRNTV (set forth in SEQ ID NO:348) and a CDR3 LKGNRVVSPSVAY (set forth in SEQ ID NO: 349). Examples of sdAb that target 41BB are described in PCT publication. No. WO2017123650.

[0487] Exemplary sequences of CRBRs are set forth in **Table 4**.

[0488] In some embodiments, at least one CRBR, or independently each CRBR, binds the co-stimulatory receptor 41BB. In some examples, the CRBR is or contains an antibody or antigen binding fragment specific to or that binds 41BB, such as a sdAb or fragments containing a VH and VL (e.g. scFv). In some embodiments, at least one CRBR, or independently each CRBR, is a natural ligand of 41BB or is a functional binding fragment thereof. In some embodiments, a 41BB-binding CRBR is a functional fragment of 41BB ligand (41BBL) containing the extracellular domain or a truncated portion thereof, such as corresponding to amino acids 50-254 of UniProt No. P41273, e.g. as set forth in SEQ ID NO:186, or a truncated portion or fragment thereof set forth in any of SEQ ID NOS:202-209. Exemplary 41BB-binding CRBRs are set forth in any of SEQ ID NOS: 186-210 and 359. In some embodiments, at least one CRBR, or independently each CRBR, is an anticalin set forth in any of SEQ ID NOS:193-201. In some embodiments, a sdAb, such as a VHH, contains a CDR1, a CDR2, and a CDR3 having a sequence set forth in SEQ ID NO:347, 348, and 349, respectively. A 41BB-binding CRBR, such as a sdAb, can include the sequence set forth in SEQ ID NO:210. A 41BB-binding CRBR, such as a sdAb, can include the sequence set forth in SEQ ID NO:359. In some embodiments, the 4-1BB-binding domain contains an antigen binding antibody fragment containing a VH and a VL, such as a single chain fragment in which the VH and VL are separated by a linker, for example an scFv. In some embodiments, the 41BB binding CRBR contains a VH set forth in any of SEQ ID NOS: 187, 189 and 191, and a VL set forth in any of SEQ ID NO: 188, 190, or 192. The CRBRs, or independently each CRBR, in a provided multispecific polypeptide construct can have at least 85%, 85%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to any of the foregoing SEQ ID Nos and bind 41BB.

[0489] In some embodiments, at least one CRBR, or independently each CRBR, binds the co-stimulatory receptor OX40. In some examples, the CRBR is or contains an antibody or antigen binding fragment specific to or that binds OX40, such as a sdAb or fragments containing a VH and VL (e.g. scFv). In some embodiments, at least on CRBR, or independently each CRBR, is a natural ligand of OX40 or is a functional binding fragment thereof. Exemplary of such OX40-binding CRBRs are set forth in any of SEQ ID NOS: 211-220. In some embodiments, the OX40- binding CRBR contains an VH

set forth in any of SEQ ID NOS: 216 and 218, and a VL set forth in any of SEQ ID NO: 217 and 219. The CRBRs, or independently each CRBR, in a provided multispecific polypeptide construct can have at least 85%, 85%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to any of the foregoing SEQ ID Nos and bind OX40.

[0490] In some embodiments, at least one CRBR, or independently each CRBR, binds the co-stimulatory receptor GITR. In some examples, the CRBR is or contains an antibody or antigen binding fragment specific to or that binds GITR, such as a sdAb or fragments containing a VH and VL (e.g. scFv). In some embodiments, at least one CRBR, or independently each CRBR, is a natural ligand of GITR or is a functional binding fragment thereof. Exemplary of such GITR-binding CRBRs are set forth in any of SEQ ID NOS: 221-230. In some embodiments, the GITR binding CRBR contains a VH set forth in any of SEQ ID NOS: 222, 224, 226, and 228 and a VL set forth in any of SEQ ID NO: 223, 225, 227, and 229. The CRBRs, or independently each CRBR, in a provided multispecific polypeptide construct can have at least 85%, 85%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to any of the foregoing SEQ ID Nos and bind GITR.

[0491] In some embodiments, at least one CRBR, or independently each CRBR, binds the co-stimulatory receptor CD27. In some examples, the CRBR is or contains an antibody or antigen binding fragment specific to or that binds CD27, such as a sdAb or fragments containing a VH and VL (e.g. scFv). In some embodiments, at least one CRBR, or independently each CRBR, is a natural ligand of CD27 or is a functional binding fragment thereof. Exemplary of such CD27-binding CRBRs are set forth in any of SEQ ID NOS: 231. In some embodiments, the CD27 binding CRBR contains a VH set forth SEQ ID NO: 232 and a VL set forth in SEQ ID NO: 233. The CRBRs, or independently each CRBR, in a provided multispecific polypeptide construct can have at least 85%, 85%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to any of the foregoing SEQ ID Nos and bind CD27.

[0492] In some embodiments, at least one CRBR, or independently each CRBR, binds the co-stimulatory receptor ICOS. In some examples, the CRBR is or contains an antibody or antigen binding fragment specific to or that binds ICOS, such as a sdAb or fragments containing a VH and VL (e.g. scFv). In some embodiments, at least one CRBR, or independently each CRBR, is a natural ligand of ICOS or is a functional binding fragment thereof. An exemplary ICOS-binding CRBR sequence is set forth in SEQ ID NO: 234.

[0493] In some embodiments, at least one CRBR, or independently each CRBR, binds the co-stimulatory receptor CD28. In some examples, the CRBR is or contains an antibody or antigen binding fragment specific to or that binds CD28, such as a sdAb or fragments containing a VH and VL (e.g. scFv). In some embodiments, at least one CRBR, or independently each CRBR, is a natural ligand of CD28 or is a functional binding fragment thereof. An exemplary CD28-binding CRBR sequence is set forth in SEQ ID NO: 235.

TABLE 4: Exemplary CRBR Sequences			
CRBR	**Format**	**Reference**	**SEQ ID NO**
41BB binding CRBR Sequences			
41BBL	Natural Ligand	UniProt accession no. P41273	186
PF-05082566	VH	US 2012/0237498 (SEQ ID NO: 43)	187
	VL	US 2012/0237498 (SEQ ID NO: 45)	188
BMS663513	VH	WO 2005/035584 (SEQ ID NO: 9)	189
	VL	WO 2005/035584 (SEQ ID NO: 6)	190
MSB7	VH	US 2017/0226215 (SEQ ID NO: 138)	191
	VL	US 2017/0226215 (SEQ ID NO: 28)	192
41BB Anticalin	Anticalin	WO 2016/177762 (SEQ ID NO: 12)	193
41BB Anticalin	Anticalin	WO 2016/177762 (SEQ ID NO: 13)	194
41BB Anticalin	Anticalin	WO 2016/177762 (SEQ ID NO: 14)	195
41BB Anticalin	Anticalin	WO 2016/177762 (SEQ ID NO: 15)	196
41BB Anticalin	Anticalin	WO 2016/177762 (SEQ ID NO: 16)	197
41BB Anticalin	Anticalin	WO 2016/177762 (SEQ ID NO: 17)	198
41BB Anticalin	Anticalin	WO 2016/177762 (SEQ ID NO: 18)	199
41BB Anticalin	Anticalin	WO 2016/177762 SEQ ID NO: 19)	200
41BB Anticalin	Anticalin	WO 2016/177762 (SEQ ID NO: 20)	201
71-254 of human 41BBL	41BB ligand	WO 2017/167672 (SEQ ID NO: 3)	202
85-254 of human 41BBL	41BB ligand	WO 2017/167672 (SEQ ID NO: 4)	203
80-254 of human 41BBL	41BB ligand	WO 2017/167672 (SEQ ID NO: 5)	204
52-254 of human 4lBBL	41BB ligand	WO 2017/167672 (SEQ ID NO: 6)	205
71-248 of human 41BBL	41BB ligand	WO 2017/167672 (SEQ ID NO: 7)	206
85-248 of human 41BBL	41BB ligand	WO 2017/167672 (SEQ ID NO: 8)	207
80-248 of human 41BBL	41BB ligand	WO 2017/167672 (SEQ ID NO: 9)	208
52-248 of human 41BBL	41BB ligand	WO 2017/167672 (SEQ ID NO: 10)	209
41BB sdAb	sdAb	US 2017/0198050	210
41BB sdAb	sdAb		359
OX40-binding CRBR Sequences			
OX40 ligand	Natural Ligand	UniProt accession no. P23510	211
OX40 ligand	Natural Ligand	US 7,959,925 (SEQ ID NO: 2)	212
human OX40L: 51-183	Natural Ligand	WO 2017/167672 (SEQ ID NO: 11)	213
Human Ox40L: 51-183 N90D	Natural Ligand	WO 2017/167672 (SEQ ID NO: 12)	214
Human OX40L: 52-183	Natural Ligand	WO 2017/167672 (SEQ ID NO: 13)	215
1A07	VH	US 2015/0307617 (SEQ ID NO: 56)	216

	VL	US 2015/0307617 (SEQ ID NO: 59)	217
1949	VH	WO 2016/179517 (SEQ ID NO: 16)	218
	VL	WO 2016/179517	219
1D10v1	sdAb	US 9,006,399	220
GITR-binding CRBR Sequences			
GITR ligand	Natural Ligand	UniProt no. Q9UNG2	221
36E5	VH	US 2014/0348841 (SEQ ID NO: 104)	222
	VL	US 2014/0348841 (SEQ ID NO: 105)	223
TRX-518	VH	US 2013/0183321 (SEQ ID NO: 54)	224
	VL	US 2013/0183321 (SEQ ID NO: 44)	225
5H7v2	VH	US 2015/0064204 (SEQ ID NO: 282)	226
	VL	US 2015/0064204 (SEQ ID NO: 134)	227
41G5v2	VH	US 2015/0064204 (SEQ ID NO: 312)	228
	VL	US 2015/0064204 (SEQ ID NO: 124)	229
C06v3	sdAb	US 2017/0022284 (SEQ ID NO: 59)	230
CD27-binding CRBR Sequences			
CD70-ECD	Natural Ligand	UniProt no. P32970	231
1F5	VH	US 2011/0274685	232
	VL	US 2011/0274685	233
CD28-binding CRBR Sequences			
CD28 sdAb	sdAb		235
ICOS-binding CRBR Sequences			
ICOS sdAb	sdAb		234

[0494] In some embodiments, the one or more CRBR is linked, directly or indirectly via a linker, to the Fc region and/or to the CD3 binding region. In some embodiments, linkage is via a linker. In some embodiments, the linker is a linking peptide (LP), which can include any flexible or rigid linker as described herein, although generally the peptide linking the CRBR or regions is not a cleavable liker.

[0495] In some embodiments, the multispecific polypeptide construct comprises a linking peptide (LP) between the CRBR and the Fc region. In some embodiments, the multispecific polypeptide construct comprises a linking peptide (LP) between the CD3 binding region and the CRBR.

f. Inhibitory Receptor Binding Regions (IRBR)

[0496] The multispecific polypeptide constructs of the present disclosure include one or more inhibitor receptor binding region (IRBR) that binds an inhibitory receptor. In some embodiments, the one or more IRBR of the provided multispecific polypeptide constructs bind an inhibitory receptor expressed on T cells. In some embodiments, the inhibitory receptor is upregulated, induced, or expressed

on the surface of an activated T cell. In some aspects, the IRBR blocks an interaction between the inhibitory receptor and its ligand, thereby reducing, suppressing or decreasing an inhibitory signal in the cell to which the IRBR binds, e.g. T cell. In some embodiments, the IRBR, or independently each of the IRBRs, is an antibody or antigen binding fragment, a natural cognate binding partner of the co-stimulatory receptor, an Anticalin (engineered lipocalin), a Darpin, a Fynomer, a Centyrin (engineered fibroneticin III domain), a cystine-knot domain, an Affilin, an Affibody, or an engineered CH3 domain.

[0497] In some embodiments, the IRBR, or independently each of the IRBRs, such as the first IRBR and the second IRBR, includes one or more copies of an antibody or an antigen-binding fragment thereof. In some embodiments, the IRBR or independently each of the IRBRs, such as the first IRBR and the second IRBR, includes one or more copies of an antibody or an antigen-binding fragment thereof selected from the group consisting of a Fab fragment, a $F(ab')_2$ fragment, an Fv fragment, a scFv, a scAb, a dAb, a single domain heavy chain antibody, and a single domain light chain antibody.

[0498] In some embodiments, the IRBR, or independently each of the IRBRs, such as the first IRBR and the second IRBR, is a single chain antibody. In some examples, the single chain is an scFv, a scAb, a single domain heavy chain antibody, or a single domain light chain antibody.

[0499] In some embodiments, the IRBR, or independently each of the IRBRs, such as the first IRBR and the second IRBR, includes one or more single domain antibody (sdAb) fragments, for example V_HH, V_{NAR}, engineered V_H or V_K domains. V_HHs can be generated from natural camelid heavy chain only antibodies, genetically modified rodents that produce heavy chain only antibodies, or naïve/synthetic camelid or humanized camelid single domain antibody libraries. $V_{NAR}s$ can be generated from cartilaginous fish heavy chain only antibodies. Various methods have been implemented to generate monomeric sdAbs from conventionally heterodimeric V_H and V_K domains, including interface engineering and selection of specific germline families.

[0500] In some embodiments, the IRBR, or independently each of the IRBRs such as the first IRBR and/or the second IRBR, of the multispecific polypeptide constructs contains at least one sdAb or an scFv that binds an inhibitory receptor. In some embodiments, the at least one scFv or sdAb that binds an inhibitory receptor is positioned amino-terminally relative to the Fc region and/or carboxy-terminally relative to the CD3 binding region of the multispecific polypeptide construct. In some embodiments, the multispecific polypeptide construct contains only one scFv or sdAb that binds to an inhibitory receptor, which can be positioned either amino-terminally relative to the Fc region and/or carboxy-terminally relative to the CD3 binding region. In some embodiments, the multispecific polypeptide construct contains two scFv or sdAb that bind to an inhibitory receptor, positioned amino-terminally relative to the Fc region and/or carboxy-terminally relative to the CD3 binding region.

[0501] In some embodiments, the multispecific polypeptide construct is formed from or includes two polypeptides, including a first polypeptide comprising a first Fc polypeptide of a heterodimeric Fc region, a linker, a VH domain of an anti-CD3 antibody or antigen binding fragment (e.g. Fv), and an

scFv or sdAb that binds to an inhibitory receptor; and a second polypeptide comprising a second Fc polypeptide of the heterodimeric Fc region, the linker, a VL domain of the anti-CD3 antibody or antigen binding fragment (e.g. Fv) and, optionally, another, the same or different, scFv or sdAb that binds to an inhibitory receptor. The scFv or sdAb that binds the inhibitory receptor can be positioned amino terminally relative to an Fc polypeptide of the heterodimeric Fc and/or carboxy-terminally relative to a VH or VL chain of the CD3 binding region. At least one of the first and/or second polypeptide of the multispecific polypeptide construct also includes an antigen binding domain that binds a TAA or a chain thereof as described in Section II.4. In some embodiments, the antigen binding domain that binds a TAA is a scFv or sdAb and is included as part of the first and/or second polypeptide of the multispecific polypeptide construct. In some embodiments, the antigen binding domain that binds a TAA is a Fab, and the multispecific polypeptide construct is additionally formed from a third polypeptide where at least the first and second polypeptide include a chain of the Fab that binds TAA (e.g. VH-CH1 or VL-CL of a Fab) and the third polypeptide contains the other chain of the Fab that binds TAA (e.g. the other of VH-CH1 or VL-CL of a Fab).

[0502] In some embodiments, the multispecific polypeptide construct is formed from or includes two polypeptides, including a first polypeptide comprising in order: a first antigen binding domain specific for a TAA, a first Fc polypeptide of a heterodimeric Fc region, a linker, a VH domain of an anti-CD3 antibody or antigen binding fragment (e.g. Fv), and a second antigen binding domain specific for a TAA; and a second polypeptide containing the IRBR and comprising in order: a second Fc polypeptide of the heterodimeric Fc region, the linker, a VL domain of the anti-CD3 antibody or antigen binding fragment (e.g. Fv) , wherein the IRBR is positioned amino terminally to the Fc region and/or C-terminally to the CD3 binding region. In some embodiments, the IRBR is positioned on the second polypeptide carboxy-terminally to the CD3 binding region. In some embodiments, the IRBR is positioned on the second polypeptide amino-terminally to the Fc region. In some embodiments, the IRBR is positioned amino terminally to the Fc region and C-terminally to the CD3 binding region. In some embodiments, the first and second antigen binding domain is specific to a TAA are the same. In some embodiments, the first and second antigen binding domain is specific to a TAA are different. In some embodiments, the first antigen binding domain and the second antigen binding domain bind a different TAA. In some embodiments, the first antigen binding domain and the second antigen binding domain bind a distinct or non-overlapping epitope of the same TAA and/or compete for binding to the same TAA.

[0503] In some embodiments, the IRBR or independently each of the IRBRs, such as the first IRBR and/or the second IRBR, contains more than one chain. In some embodiments, the IRBR or independently each of the IRBRs, such as the first IRBR and/or the second IRBR, of the multispecific polypeptide constructs contains VH and VL sequences assembled as FABs.

[0504] In some embodiments, the antigen binding domain or independently each of the antigen binding domains, such as the first antigen-binding domain and/or the second antigen binding domains, of the multispecific polypeptide constructs contains a VH-CH1 (Fd) and a VL-CL of a Fab antibody that binds an inhibitory receptor. In some embodiments, the Fab antibody containing a VH-CH1 (Fd) and a VL-CL is positioned amino-terminally relative to the Fc region and/or carboxy-terminally relative to the CD3 binding region of the multispecific polypeptide construct. In some embodiments, the multispecific polypeptide construct contains only one Fab antibody, containing a VH-CH1 (Fd) or VL-CL, that binds to an inhibitory receptor, which can be positioned either amino-terminally relative to the Fc region and/or carboxy-terminally relative to the CD3 binding region. In some embodiments, the multispecific polypeptide construct contains two Fab antibody fragments, each containing a VH-CH1 (Fd) and VL-CL, that binds to an inhibitoryreceptor, in which one is positioned amino-terminally relative to the Fc region and the other is positioned carboxy-terminally relative to the CD3 binding region.

[0505] In some embodiments, the multispecific polypeptide construct is formed from or includes three or more polypeptides, including a first polypeptide comprising a first Fc polypeptide of a heterodimeric Fc region, a linker and a VH-CH1 (Fd) or VL-CL of a Fab antibody fragment that binds to an inhibitory receptor; a second polypeptide comprising a second Fc polypeptide of the heterodimeric Fc region, the linker and, optionally, the same VH-CH1 (Fd) or VL-CL of the Fab antibody fragment that binds to a inhibitory receptor, and a third polypeptide comprising the other of the VH-CH1 (Fd) or VL-CL of the Fab antibody fragment that binds to the inhibitory receptor. The first, second and/or third polypeptide of the multispecific polypeptide construct also can include an antigen binding domain that binds a TAA or a chain thereof as described in Section II.4. In some embodiments, the antigen binding domain that binds a TAA is a scFv or sdAb and is included as part of the first and/or second polypeptide of the multispecific polypeptide construct. In some embodiments, the antigen binding domain that binds a TAA is a Fab, and the multispecific polypeptide construct is additionally formed from a fourth polypeptide where at least a first and second polypeptide includes a chain of the Fab that binds TAA (e.g. VH-CH1 or VL-CL of a Fab) and the fourth polypeptide contains the other chain of the Fab that binds TAA (e.g. the other of VH-CH1 or VL-CL of a Fab).

[0506] In some embodiments, the IRBR, or independently each of the IRBRs, is or includes a natural (native) cognate binding partner of the inhibitor receptor (e.g. a natural ligand), or a variant thereof that exhibits binding activity to the inhibitory receptor.

[0507] In some embodiments, the one or more IRBR of the provided multispecific polypeptide constructs binds a inhibitory receptor expressed on T cells. In some embodiments, there are more than one IRBR that binds to an inhibitory receptor and each of the IRBRs, such as the first IRBR and the second IRBR, bind the same co-stimulatory receptor. In some embodiments, each of the IRBRs, such as the first IRBR and the second IRBR, binds a different inhibitory receptor. In some embodiments, each of the IRBRs, such as the first IRBR and the second IRBR binds a different epitope on the same inhibitory

receptor. In some embodiments, each of the IRBRs, such as the first IRBR and the second IRBR, binds the same epitope on the same inhibitory receptor.

[0508] In some embodiments, the IRBR, or independently each of the IRBRs that binds a inhibitory receptor results in monovalent, bivalent, trivalent, or tetravalent binding to the inhibitory receptor.

[0509] In some embodiments, the inhibitory receptor is expressed on T cells, such as primary T cells of a subject. In some embodiments, the inhibitory receptor is expressed on human T cells, such as primary human T cells of a human subject.

[0510] In some embodiments, the inhibitory receptor is a member of the tumor necrosis factor (TNF) receptor family. In some embodiments, the inhibitory receptor is a member of the immunoglobulin superfamily (IgSF).

[0511] In some embodiments, the inhibitory receptor is Programmed cell death protein 1 (PD-1), cytotoxic T-lymphocyte associated protein 4 (CTLA-4), T cell immunoreceptor with Ig and ITIM domains (TIGIT), V-domain immunoglobulin suppressor of T cell activation (VISTA), T cell immunoglobulin and mucin-domain containing-3 (TIM3), or lymphocyte activation gene 3 (LAG3). In some embodiments, the one or more IRBR is an antibody or fragment thereof that binds to the inhibitor receptor PD-1, CTLA-4, TIGIT, VISTA, TIM3 or LAG3. In particular embodiments, the antibody or antigen-binding fragment is humanized or is human.

[0512] In some examples, the inhibitory receptor binding region (IRBR) binds or comprises a natural cognate binding partner of PD-1, CTLA-4, TIGIT, VISTA, or TIM3. In some embodiments, the natural cognate binding partner is selected from PD-L1, PD-L2, CD80, CD86, CD155, CD112, or VSIG-3/IGSF11, or a functional fragment thereof.

[0513] In some examples, the IRBR contains an antibody fragment, such as an scFv, that contains a variable light (VL) chain and a variable heavy (VH) chain of an antibody that that binds an inhibitory receptor, such as PD-1, CTLA-4, TIGIT, VISTA, or TIM3. In some examples, the IRBR contains a single domain antibody or a VHH domain that specifically binds an inhibitory receptor, such as a PD-1, CTLA-4, TIGIT, VISTA, or TIM3, see e.g. described in PCT publication No. WO2018068695 or WO2018068201.

[0514] In some embodiments, the inhibitory receptor is PD-1. In some embodiments, the one or more IRBR is an antibody fragment that binds to PD-1.

[0515] In some embodiments, the IRBR is or contains a VHH domain that binds PD-1 comprising a CDR1, CDR2 and CDR3 contained in a VHH amino acid sequences selected from any of SEQ ID NO: 304-320, 326, or 364-381 or an amino acid sequence that has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the VHH region amino acid selected from any one of SEQ ID NOs: 304-320, 326 or 364-381 and binds PD-1.

[0516] In some embodiments, the IRBR is or contains a VHH domain that contains a CDR1, CDR2, CDR3 contained in a VHH domain set forth in SEQ ID NO: 326, or an amino acid sequence that has at

least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the VHH region amino acid selected set forth in SEQ ID NO: 326 and that binds PD-1. In some embodiments, the IRBR is or contains a VHH domain that has the amino acid sequence set forth in SEQ ID NO: 326 or an amino acid sequence that has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the amino acid selected set forth in SEQ ID NO: 326 and that binds PD-1. In some embodiments, IRBR is or contains a VHH domain that is a humanized variant of the amino acid sequence set forth in SEQ ID NO: 326.In some embodiments, an IRBR that binds PD-1 has a VHH domain that comprises a CDR1 set forth in any one of SEQ ID NOS: 321, 322 or 323, a CDR2 set forth in SEQ ID NO: 324 and a CDR3 set forth in SEQ ID NO: 325.

[0517] In some embodiments, an IRBR that binds PD-1 has a VHH domain that contains a CDR1, CDR2, and CDR3 set forth in SEQ ID NOs: 322, 324, and 325, respectively. In some embodiments, an IRBR that binds PD-1 has a VHH domain that contains a CDR1, CDR2 and CDR3 set forth in SEQ ID NOs: 321, 324, and 325, respectively. In some embodiments, the an IRBR that binds PD-1 has a VHH domain that contains a CDR1, CDR2 and CDR3 set forth in SEQ ID NOs: 323, 324, and 325, respectively.

[0518] In some aspects, the IRBR is or contains a VHH domain that contains a CDR1, CDR2 and CDR3 contained in a VHH amino acid sequence selected from any of SEQ ID NO:304-320, or an amino acid sequence that has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the V_HH region amino acid selected from any one of SEQ ID NOs: 304-320 and that binds PD-1.

[0519] In some cases, the IRBR contains a VHH domain that is a humanized variant that has the amino acid sequence set forth in any of SEQ ID NOS: 304-320 or an amino acid sequence that has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the VHH region amino acid selected from any one of SEQ ID NOs: 304-320 and that binds PD-1. In some embodimetns, the IRBR is or contains a VHH domain seqeunce that is a humanized VHH domain having the sequence of amino acids set forth in any one of SEQ ID NOS: 304-320.

[0520] In some embodiments, the IRBR is or contains a VHH domain that contains a CDR1, CDR2, CDR3 contained in a VHH domain set forth in SEQ ID NO: 364, or an amino acid sequence that has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the VHH region amino acid selected set forth in SEQ ID NO: 364 and that binds PD-1. In some embodiments, the IRBR is or contains a VHH domain that has the amino acid sequence set forth in SEQ ID NO: 364 or an amino acid sequence that has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the amino acid selected set forth in SEQ ID NO: 364 and that binds PD-1. In some embodiments, IRBR is or contains a VHH domain that is a humanized variant of the amino acid sequence set forth in SEQ ID NO: 364.

[0521] In some embodiments, an IRBR that binds PD-1 has a VHH domain that comprises a CDR1 set forth in any one of SEQ ID NOS: 321, 322 or 323, a CDR2 set forth in SEQ ID NO: 324 and a CDR3 set forth in SEQ ID NO: 325.

[0522] In some embodiments, an IRBR that binds PD-1 has a VHH domain that contains a CDR1, CDR2, and CDR3 set forth in SEQ ID NOs: 322, 324, and 325, respectively. In some embodiments, an IRBR that binds PD-1 has a VHH domain that contains a CDR1, CDR2 and CDR3 set forth in SEQ ID NOs: 321, 324, and 325, respectively. In some embodiments, the an IRBR that binds PD-1 has a VHH domain that contains a CDR1, CDR2 and CDR3 set forth in SEQ ID NOs: 323, 324, and 325, respectively.

[0523] In some aspects, the IRBR is or contains a VHH domain that contains a CDR1, CDR2 and CDR3 contained in a VHH amino acid sequence selected from any of SEQ ID NO:365-381, or an amino acid sequence that has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the V_HH region amino acid selected from any one of SEQ ID NOs: 365-381 and that binds PD-1.

[0524] In some cases, the IRBR contains a VHH domain that is a humanized variant that has the amino acid sequence set forth in any of SEQ ID NOS:365-381 or an amino acid sequence that has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the VHH region amino acid selected from any one of SEQ ID NOs:365-381 and that binds PD-1. In some embodimetns, the IRBR is or contains a VHH domain seqeunce that is a humanized VHH domain having the sequence of amino acids set forth in any one of SEQ ID NOS:365-381.

[0525] In some embodiments, the one or more IRBR is linked, directly or indirectly via a linker, to the Fc region and/or to the CD3 binding region. In some embodiments, linkage is via a linker. In some embodiments, the linker is a linking peptide (LP), which can include any flexible or rigid linker as described, such as in Section II.3, although generally the peptide linking the IRBR or regions is not a cleavable liker.

[0526] In some embodiments, the multispecific polypeptide construct comprises a linking peptide (LP) between the IRBR and the Fc region. In some embodiments, the multispecific polypeptide construct comprises a linking peptide (LP) between the CD3 binding region and the IRBR.

[0527] In some embodiments, the multispecific polypeptide construct comprises more than one IRBR. In some embodiments, the multispecific polypeptide construct comprises a first linking peptide (LP1) between the first IRBR and the Fc region. In some embodiments, the multispecific polypeptide construct comprises a second linking peptide (LP2) between the CD3 binding region and the second IRBR. In some embodiments, the multispecific polypeptide construct comprises a first linking peptide (LP1) between the first IRBR and the Fc region and a second linking peptide (LP2) between the CD3 binding region and the second IRBR. In some aspects, the multispecific polypeptide construct has the structural arrangement from N-terminus to C-terminus as follows: IRBR and/or antigen binding domain –

LP1- Fc region –linker –CD3 binding region – LP2 – IRBR and/or antigen binding domain. In some embodiments, the two linking peptides are not identical to each other.

[0528] In some embodiments, the LP (e.g., LP1 or LP2) is independently a peptide of about 1 to 20 amino acids in length. In some embodiments, the LP1 or LP2 is independently a peptide that is or comprises any Gly-Ser linker as set forth in SEQ ID NOs: 1-4, 125-127, 129 or GGS.

[0529] In some embodiments, the multispecific polypeptide construct contains both a CRBR and an IRBR. In some embodiments, one of the CRBR or IRBR is positioned amino-terminally relative to the Fc region and the other of the CRBR or IRBR is positioned carboxy-terminally relative to the CD3 binding region of the multispecific polypeptide construct. In some embodiments, the CRBR and IRBR are present on different polypeptide of a heterodimeric multispecific polypeptide construct, in which at least one of the polypeptides also contains the at least one antigen binding domain specific to a TAA. In some embodiments, the CRBR and IRBR are present on the same polypeptide (first polypeptide) of a heterodimeric multispecific polypeptide construct and the at least one antigen binding domain specific to a TAA is on the other (or second) polypeptide of the heterodimeric multispecific polypeptide construct.

[0530] In some embodiments, the multispecific polypeptide construct is formed from or includes two polypeptides. In some aspects, the first polypeptide comprises in order: a first antigen binding domain specific for a TAA, a first Fc polypeptide of a heterodimeric Fc region, a linker, a VH domain of an anti-CD3 antibody or antigen binding fragment (e.g. Fv), and a second antigen binding domain specific for a TAA; and a second polypeptide comprising in order: one of the IRBR or CRBR, a second Fc polypeptide of the heterodimeric Fc region, the linker, a VL domain of the anti-CD3 antibody or antigen binding fragment (e.g. Fv) , and the other of the IRBR or CRBR. In some embodiments, the IRBR is positioned on the second polypeptide carboxy-terminally to the CD3 binding region and the CRBR is positioned on the second polypeptide amino-terminally to the Fc region. In some embodiments, the IRBR is positioned on the second polypeptide amino -terminally to the Fc region and the CRBR is positioned on the second polypeptide carboxy-terminally to the CD3 binding region. In some embodiments, the first and second antigen binding domain is specific to a TAA are the same. In some embodiments, the first and second antigen binding domain is specific to a TAA are different. In some embodiments, the first antigen binding domain and the second antigen binding domain bind a different TAA. In some embodiments, the first antigen binding domain and the second antigen binding domain bind a distinct or non-overlapping epitope of the same TAA and/or compete for binding to the same TAA.

3. NK Recruitment

[0531] In some embodiments, the 5T4-binding polypeptide is a bispecific construct that is or comprises at least one 5T4 VHH domain provided herein and at least one additional binding molecule capable of binding to a surface molecule expressed on a Natural Killer (NK) cells and/or recruiting NK

cells. In particular aspects, the multispecific construct is bispecific for 5T4 and the NK cell surface molecule. In some embodiments, the surface molecule is CD16 (FcγRIII). Specifically, a provided bispecific 5T4-binding polypeptide is capable of specifically binding an NK activating receptor expressed on a human NK cells cell, such as human CD16a.

[0532] CD16, a low affinity receptor for the Fc portion of some IgGs known to be involved in antibody-dependent cellular cytotoxicity (ADCC), is the best-characterized membrane receptor responsible for triggering of target cell lysis by NK cells (Mandelboim et al., 1999, PNAS 96:5640-5644). Generally, a large majority (approximately 90%) of human NK cells express CD56 at low density (CD56dim) and FcγRIII (CD16) at a high level (Cooper et al., 2001, Trends Immunol. 22:633-640). Human FcγRIII exists as two isoforms, CD16a (FcγRIIIA) and CD16b (FcγRIIIB), that share 96% sequence identity in their extracellular immunoglobulin-binding regions (van de Winkel and Capel, 1993, Immunol. Today 14(5):215-221). In particular embodiments, the additional binding molecule is capable of specifically binding CD16a.

[0533] CD16a is expressed on macrophages, mast cells, and NK cells as a transmembrane receptor. On NK cells, the alpha chain of CD16a associates with the immunoreceptor tyrosine-based activation motif (ITAM) containing FcεRI γ-chain and/or the T-cell receptor (TCR)/CD3 ζ-chain to mediate signaling (Wirthmueller et al., 1992, J. Exp. Med. 175:1381-1390). The interaction of CD16a with different combinations of homo- and hetero-dimers of the γ and ζ chains has been observed in NK cells, indicating the ability to mediate signaling via different signaling pathways via variations of the CD16a complex in NK cells (Anderson et al., 1990, PNAS 87(6):2274-2278; Ackerly et al., 1992, Int. J. Cancer Suppl. 7:11-14). FcγR-expressing effector cells have been shown to be involved in destroying tumor cells via ADCC. For example, engagement of CD16a, such as with an agonist binding molecule capable of specifically binding CD16a can result in activating of NK cells expressing CD16a, thereby eliciting a biological response, in particular a signaling response. In some cases, the binding molecule is capable of triggering cell killing, in a manner analogous to antibody-dependent cellular cytotoxicity (ADCC), by virtue of its binding to such cells.

[0534] In particular examples, 5T4-binding polypeptides include bispecific molecules that can specifically bind to 5T4 and to CD16a may target NK cells to cells bearing such antigen, so that the cell bearing the antigen may be eradicated via NK cell mediated cell killing. For example, a binding molecule that specifically binds 5T4 expressed on a tumor cell may target NK-cells to the tumor cell. In some cases, activation of the NK cell caused by the binding molecule binding to CD16a can lead to killing of the tumor cells.

[0535] In some embodiments, the additional binding domain specific to an activating NK cell receptor, such as CD16a, is an antigen-binding fragment selected from a Fab fragment, a $F(ab')_2$ fragment, an Fv fragment, a scFv, disulfide stabilized Fv fragment (dsFv), a scAb, a dAb, a single domain heavy chain antibody (VHH), or a single domain light chain antibody. In some embodiments,

the additional binding domain is monovalent for binding the activating T NK cell receptor, such as CD16a.

[0536] In some cases, the additional binding domain recognizes CD3-binding domain recognizes CD16a. In some embodiments, the anti-CD16a binding domain includes one or more copies of an anti-CD16a Fab fragment, an anti-CD16a $F(ab')_2$ fragment, an anti-CD16a Fv fragment, an anti-CD16a scFv, an anti-CD16a dsFv, an anti-CD16a scAb, an anti-CD16a dAb, an anti-CD16a single domain heavy chain antibody (VHH), and an anti-CD16a single domain light chain antibody. In some embodiments, the anti-CD16a binding domain is monovalent for binding CD16a. In some embodiments, the BH73-binding polypeptide is a bispecific construct that binds BH73 and agonizes the activity of CD16a.

[0537] Antibodies and antigen-binding fragments thereof specific for CD16a are known and include, for example, NM3E2 (McCall et al. (1999) Mol. Immunol., 36:433-045. Other anti-CD16a antibodies also can be used in the constructs provided herein, including any described in published U.S. patent application No. US10160280795; U.S. Patent No. 9,701,750; Behar et al. (2008) Protein Eng Des Sel. 21:1-10; Arndt et al., (1999) Blood 94:2562-2568. In particular examples, the anti-CD16a is an anti-CD16a scFv. In some embodiments, the anti-CD16a is an anti-CD16a antibody included in a TandAb molecule (see e.g. Reush et al. (2014) Mabs, 6:727-738). In some apects, the anti-CD16a is an anti-CD16a or antigen binding fragment, such as an scFv, described in U.S. Patent No. 9,035,026.

[0538] The provided bispecific constructs can be formatted in any of a number of formats containing the at least one 5T4 VHH domain and the at least one additional domain specific to an activating NK cell receptor, such as a CD16a-binding domain.

[0539] In one embodiment, the bispecific construct is a bispecific single-domain antibody-linked Fab (S-Fab) containing at least one 5T4 VHH domain as described linked, directly or indirectly to a Fab antigen binding fragment specific to an NK cell activating receptor, e.g. CD16a, such as an anti-CD16a Fab. In some embodiments, the 5T4 VHH domain is linked to the C-terminus of the VH or VL chain of an anti-C16a Fab. In some embodiments, the S-Fab can be further modified, such as by conjugation with polyethylene glycol (PEG), N-(2-hydroxypropyl) methacrylamide (HPMA) copolymers, proteins (such as albumin), polyglutamic acid or PASylation (Pan et al. (2018) International Jounral of Nanomedicine, 2018:3189-3201).

[0540] In another embodiment, the bispecific construct is a scFv-single domain antibody in which the construct contains at least one 5T4 VHH as described linked, directly or indirectly, to an scFv containing a VH and a VL of an antigen binding domain specific to an NK cell activating receptor, e.g. CD16a. The scFv against an NK cell activating receptor, e.g. anti-CD16a scFv, can contain any of the VH and VL sequences as described. In some embodiments, the VHH domain and the scFv are connected by a linker, such as a peptide linker. In some embodiments, the peptide linker can be a peptide linker as described herein. In some embodiments, the VHH domain and the scFv are each connected, optionally

through a hinge region or a linker (e.g. peptide linker), to an Fc region, such as an N-terminus of an Fc region. The Fc region can be any described herein, such as a human Fc region or a variant thereof, e.g. a human IgG1 Fc region or a variant thereof. In particular examples, the Fc region is formed by variant Fc domains, e.g. variant human IgG1 domains, that are mutated or modified to promote heterodimerization in which different polypeptides can be dimerized to yield a heterodimer.

[0541] In a further embodiment, the antigen binding domain specific to an NK cell activating receptor, e.g. CD16a, is a single domain antibody, such as is a VHH domain that specifically binds to CD16a. Single domain antibodies, including VHH domains that bind to CD16a are known, see e.g. published U.S. patent application No. US20160280795. In such aspects, a bispecific construct provided herein can include at least one 5T4 VHH domain and at least one CD16a VHH domain. For formatting the constructs, in some cases, each VHH domain is connected, optionally through a hinge region or linker (e.g. peptide linker), to an Fc region, such as an N-terminus of an Fc region. The Fc region can be any described herein, such as a human Fc region or a variant thereof, e.g. a human IgG1 Fc region or a variant thereof. In particular examples, the Fc region is formed by variant Fc domains, e.g. variant human IgG1 domains, that are mutated or modified to promote heterodimerization in which different polypeptides can be dimerized to yield a heterodimer.

[0542] In the above embodiments, exemplary modifications of an Fc region to promote heterodimerization are known, including any as described below, e.g. Table 3. In some embodiments, one Fc polypeptide of a heterodimeric Fc comprises the sequence of amino acids set forth in any of SEQ ID NOS: 328 (e.g. SEQ ID NO:103 or 107), 334 (e.g. SEQ ID NO:115 or 117), and the other Fc polypeptide of the heterodimeric Fc contains the sequence of amino acids set forth in any of SEQ ID NOS: 329 (e.g. SEQ ID NO:104 or 108), 332 (e.g. SEQ ID NO:111 or 113), 336 (e.g. SEQ ID NO:119 or 121). In some embodiments, one Fc polypeptide of a heterodimeric Fc comprises the sequence of amino acids set forth in any of SEQ ID NOS: 330 (e.g. SEQ ID NO:105 or 109), 335 (e.g. SEQ ID NO:116 or 118) and the other Fc polypeptide of the heterodimeric Fc comprises the sequence of amino acids set forth in any of SEQ ID NOS: 331 (e.g. SEQ ID NO:106 or 110), 333 (e.g. SEQ ID NO:112 or 114), 337 (e.g. SEQ ID NO:120 or 122).

4. Cytokine fusion and/or cytokine receptor targeting

[0543] In some embodiments, the 5T4-binding polypeptide is a multispecific polypeptide construct that is a cytokine-antibody fusion protein (also called a 5T4 VHH-cytokine fusion). In some aspects, at least one 5T4 VHH domain provided herein is linked, directly or indirectly, to at least one cytokine, such as to an interferon. In particular embodiments, the cytokine is an interferon capable of exhibiting anti-proliferative activity, apoptotic activity and/or anti-viral activity. In some embodiments, the interferon of a 5T4 VHH-cytokine fusion provided herein is capable of binding to a receptor composed of IFNAR1 and/or 2. Any of a variety of assays can be used to assess the effect of such fusion proteins on binding IFNAR1 and/or 2, reducing or decreasing the growth rather and/or proliferation rate of a cancer cell,

reducing tumor size, eliminating tumors or inducing the death of a cancer cell (e.g. via apoptosis). Such assays in include in vitro assays with various cancer cell lines known to express 5T4 or in vivo assays employing animal tumor models.

[0544] In some embodiments, the interferon is a type I interferon, such as a human type I interferon or a variant thereof. In some aspects, the human type I interferon is a variant that is a truncated human type I interferon or a human mutant type I interferon. In some embodiments, the type I interferon or variant thereof is a wild-type human IFN-alpha (IFN-alpha; alpha2 and natural higher affinity variants such as alpha 14), interferon beta (IFN-beta) as well as mutants and/or truncated forms thereof. In some embodiments, the interferon is a type II interferon, such as a human type II interferon or a variant thereof. In some aspects, the human type II interferon is a variant that is a truncated human type II interferon or a human mutant type II interferon. In some embodiments, the type II interferon or variant thereof is a wild-type human interferon gamma (IFN-gamma) as well as mutants and/or truncated forms thereof. In some embodiments, the provided cytokine-antibody fusion proteins can be used to inhibit the growth and/or proliferation of target cells (e.g. cancer cells) that express or overexpress 5T4.

[0545] In some embodiments, the 5T4 VHH-cytokine fusion protein is similar in format to any as described in International PCT published application No. WO2014194100; U.S. Patent No. 9,803,021; Valedkarimi et al. (2017) Biomed Pharmacother., 95:731-742; or Young et al. (2014) Semin Oncol., 41:623-636.

[0546] In particular embodiments, the interferon, e.g. a type I interferon, such as a human type I interferon (e.g. IFN-alpha, IFN-beta, or IFN-gamma) is one that possesses the endogenous binding affinity and/or activity of the native or wild-type interferon, preferably at a level of at least 60%, or of at least or at least about 80%, such as at least 90%, 95%, 98%, 99%, 100%, or a level greater than the native wild-type interferon (in its isolated form).

[0547] Interferons and interferon mutants are a well known and well characterized group of cytokines (see e.g., WO 2002/095067; WO 2002/079249; WO 2002/101048; WO 2002/095067; WO 2002/083733; WO 2002/086156; WO 2002/083733; WO 2003/000896; WO 2002/101048; WO 2002/079249; WO 2003/000896; WO 2004/022593; WO2004/022747; WO 2003/023032; WO 2004/022593 and also in Kim et al. (2003) Cancer Lett. 189(2): 183-188; Hussain et a/. (2000) J. Interferon Cytokine Res. 20(9): 763-768; Hussain et al. (1998) J. Interferon Cytokine Res. 18(7): 469-477; Nyman et al. (1988) Biochem. J. 329 (Pt 2): 295-302; Golovleva et al. (1997) J. Interferon Cytokine Res. 17(10): 637-645; Hussain et al. (1997) J. Interferon Cytokine Res. 17(9): 559-566; Golovleva et al. (1997) Hum. Hered. 47(4): 185-188; Kita et al. (1991) J. Interferon Cytokine Res. 17(3): 135-140; Golovleva et al. (1996) Am. J. Hum. Genet. 59(3): 570-578; Hussain et al. (1996) J. Interferon Cytokine Res. 16(7): 523-529; Linge et al. (1995) Biochim Biophys Acta. Any of such can be used in the provided cytokine-antibody fusion proteins.

[0548] In some embodiments, the interferon is a human type I interferon. Alleles of the human interferon family of genes/proteins are known, see e.g. Pestka (10983) Arch Biochem Biophys., 221:1-37; Diaz et al. (1994) Genomics, 22:540-52; Pestka (1986) Meth. Enzymol, 199: 3-4; and Krause et al. (2000) J. Biol. Chem., 275:22995-3004.

[0549] In some embodiments, the interferon is a full-length IFN-alpha (e.g. human IFN-alpha), a full-length IFN-beta (e.g. human IFN-beta) or a full-length IFN-gamma (e.g. human IFN-gamma). In some embodiments, the interferon is a biologically active truncated IFN-alpha (e.g. human IFN-alpha), a biologically active truncated IFN-beta (e.g. human IFN-beta) or a biologically active truncated IFN-gamma (e.g. human IFN-gamma). In some embodiments, a biologically active truncated interferon contains a contiguous sequence of amino acids of a wild-type or native interferon that is truncated at the N- and/or C-terminus and comprises a length that is at least or at least about 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90% or more the length of the native or wild-type interferon. Any of a variety of standard assays for assessing biological activity of an interferon can be used. For example, IFN-alpha activity can be assayed by measuring antiviral activity against a particular test virus. Kits for assaying for IFN-alpha activity are commercially available (see, e.g., ILITE™ alphabeta kit by Neutekbio, Ireland). In some aspects, the IFN-alpha is an IFN-a2a (e.g. Acc. No. CAA23805), IFN-a-c (Acc. No. P01566), IFN-a-d (Acc. No. AAB59403); IFNa-5 (Acc. No. CAA26702); IFNa-6 (Acc. No. AA26704); IFNa-4 (Acc. No. NP_066546); IFNa-4b (Acc. No. CAA26701); IFNa-I (Acc. No. AAA52725); IFNa-J (Acc. No. CAA23792); IFNa-H (Acc. No. CAA23794); IFNa-F (Acc. No. AAA52718); IFNa-7 (Acc. No. CAA26903), or is a biologically active fragment thereof. In some aspects, the IFN-beta is IFN-beta set forth in Acc. No. AAC41702 or is a biologically active fragment thereof. In some aspects, the IFN-gamma is IFN-gamma set forth in Acc. No. P01579 or is a biologically active fragment thereof.

[0550] In some embodiments, a provided 5T4 VHH-cytokine fusion contains a variant or mutant interferon alpha 2 (IFNa2) is contemplated. Certain mutants include a mutation of the His at position 57, and/or the E at position 58, and/or the Q at position 61. In certain embodiments the mutants include the mutation H57Y, and/or E58N, and/or Q61S. In certain embodiments the mutants include a mutated IFNa2 having the mutations H57Y, E58N, and Q61S (YNS) (see, e.g., Kalie et al. (2007) J. Biol. Chem., 282: 11602-11611). In other embodiments mutants include a mutation of the His at position 57, and/or the E at position 58, and/or the Q at position 61 to A (alanine). In certain embodiments the mutants include a mutated IFNa2 having the mutations H57A, E58A, and Q61A (HEQ) (see, e.g., Jaitin et al. (2006) Mo/. Cellular Biol, 26(5): 1888-1897). In certain embodiments the mutant interferon comprises a mutation of His at position 57 to A, Y, or M, and/or a mutation of E at position 58 to A, or N, or D, or L, and/or a mutation of Q at position 61 to A, or S, or L, or D. [0244] In certain embodiments mutant include mutants of interferon alpha 8 (IFN-a8), such as variants with amino acid replacement corresponding to R145 to V, I, or L, and/or A146 to N, or S, and/or M149 to Y, e.g.

R145V/A146N/M149Y), R145I,/A146S/M149Y or R145L/A146S/M149Y (see, e.g,. Yamamoto et.al,. (2009) J. Interferon & cytokine Res, 29: 161-170.

[0551] In some embodiments, a provided 5T4 VHH-cytokine fusion contains a mutant or variant IFN-beta containing a serine substituted for the naturally occurring cysteine at amino acid 17 (see, e.g., Hawkins et al. (1985) Cancer Res., 45, 5914-5920).

[0552] In some embodiments, a provided 5T4 VHH-cytokine fusion contains a truncated interferon. In one embodiment, a truncated interferon includes a human IFN-alpha with deletions of up to the first 15 amino-terminal amino acid residues and/or up to the last 10-13 carboxyl-terminal amino acid residues, which has been shown to retain activity of the native or wild-type human IFN-alpha (see e.g. Ackerman (1984) Proc. Natl. Acad. Sci, USA, 81: 1045-1047). In some embodiments, a truncated human IFN-alpha has 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 or 13 carboxyl terminal amino acid residues deleted and/or 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 amino terminal amino acid residues deleted.

[0553] In some embodiments, a provided 5T4 VHH-cyokine fusion contains a truncated interferon, such as described in published U.S. patent appl. No. US2009/0025106. In some embodiments, a provided 5T4 VHH-cytokine fusion contains a truncated IFN-gamma containing N- and/or C-terminal deletions, such as described in Lundell et al. (1991) Protein Neg., 4:335-341; Pan et al. (1987) Eur. J. Biochem., 166:145-149.

[0554] In some embodiments, the interferon, e.g. human interferon, is a mutant interferon that is resistant to proteolysis compared to the unmodified, typically wild-type protein, see e.g. U.S. Patent No. 7,998,469; U.S. Patent No. 8,052,964; U.S. Patent No. 4,832,959 U.S. Patent NO. 6,120,762; WO1992/008737; and EP219781.

[0555] In aspects of the provided 5T4 VHH-cytokine fusion proteins, the antibody and the cytokine, e.g. interferon, are attached directly or are attached indirectly via a linker, such as a peptide linker. The attachment can be to the N- or C-terminus of the VHH domain, so long as the attachment does not interfere with binding of the antibody to 5T4. Any linker, e.g. peptide linker, described herein can be used. In some embodiments, the linker is a GS-linker that comprises an amino acid sequence selected from the group consisting of GGSGGS, i.e., $(GGS)_2$ (SEQ ID NO: 1); GGSGGSGGS, i.e., $(GGS)_3$ (SEQ ID NO: 2); GGSGGSGGSGGS, i.e., $(GGS)_4$ (SEQ ID NO: 3); and GGSGGSGGSGGSGGS, i.e., $(GGS)_5$ (SEQ ID NO: 4). In some embodiments, the linker is(GGGGS)n, wherein n is 1 to 5 (SEQ ID NO:123); (GGGGGS)n, wherein n is 1 to 4 (SEQ ID NO:124); GGGGS (SEQ ID NO:125); GGGGGS (SEQ ID NO:126); GGGGGSGGGGGSGGGGGS (SEQ ID NO:127); GGGGSGGGGSGGGGS (SEQ ID NO:128); GGSGGGGSGGGGSGGGGS (SEQ ID NO:129). In some embodiments, the linker is a flexible linker comprising Glycine residues, such as, by way of non-limiting example, GG, GGG, GGGG (SEQ ID NO: 5), GGGGG (SEQ ID NO: 6), GGGGGG (SEQ ID NO: 7), and PGGGG (SEQ ID NO:327). In some embodiments, the fusion proteins can include a combination of a GS-linker and a Glycine linker.

D. Chimeric Receptors and Engineered Cells

[0556] Provided herein are chimeric antigen receptors (CARs) having an extracellular domain comprising one or more of the 5T4 VHH domains provided herein, such as any of the sequences of a 5T4 VHH domain provided herein. CAR constructs provided herein include an extracellular domain containing the one or more 5T4 VHH, a transmembrane domain and an intracellular signaling region. The one or more 5T4 VHH domain which form the antigen binding unit of the CAR "binds" or is "capable of binding", i.e. targets, 5T4 with sufficient affinity such the CAR is useful in therapy in targeting a cell or tissue expressing 5T4.

[0557] CARs are synthetic receptors typically containing an extracellular targeting/binding moiety that is associated with one or more signaling domains in a single fusion molecule, and that is expressed on the surface of a cell, such as a T cell. Thus, CARs combine antigen-specificity and T cell activating properties in a single fusion molecule. First generation CARs typically included the cytoplasmic region of the CD3zeta or Fc l receptor γ chain as their signaling domain. First generation CARs have been tested in phase I clinical studies in patients with ovarian cancer, renal cancer, lymphoma, and neuroblastoma, where they have induced modest responses (reviewed in Sadelain et al., Curr Opin Immunol, 21 (2): 215-223, 2009). Second generation CARs, which contain the signalling domains of a costimulatory molecule, such as CD28, and CD3zeta, provide dual signalling to direct combined activating and co-stimulatory signals. Third generation CARs are more complex with three or more signaling domains (reviewed in Sadelain et al., Cancer Discovery (3), 388-398, 2013 and Dotti et al, Immuno. Rev, 257 (1), 1-36, 2014).

[0558] In some embodiments, a provided CAR contains at least one antigen binding domain comprising a 5T4 VHH domain that targets or is capable of specifically binding 5T4. In some embodiments, the CAR contains at least two antigen binding domains (where at least one comprises a 5T4 VHH domain) which target one or more antigen. In one embodiment, the antigen binding domain of a CAR comprises two or at least two 5T4 VHH domains that are specific for 5T4, thus providing a bivalent binding molecule. In one embodiment, the antigen binding domain comprises two or at least two 5T4 VHH domains that are specific for 5T4, but bind to different epitopes on said antigen. In such cases, the antigen binding domain comprises a first 5T4 VHH domain that binds to a first epitope of 5T4 and a second VHH domain that binds to a second epitope of 5T4. The epitopes may be overlapping. Thus, in some embodiments, the antigen binding domain is biparatopic and the CAR is a biparatopic CAR. In yet another embodiment, the antigen binding domain comprises two 5T4 VHH domains that are specific for 5T4 and bind to the same epitopes on 5T4.

[0559] The transmembrane domain of a CAR provided herein is a domain that typically crosses or is capable of crossing or spanning the plasma membrane and is connected, directly or indirectly (e.g. via a spacer, such as an immunoglobulin hinge sequence) to the extracellular antigen binding domain and the endoplasmic portion containing the intracellular signaling domain. In one embodiment, the transmembrane domain of the CAR is a transmembrane region of a transmembrane protein (for example

Type I transmembrane proteins), an artificial hydrophobic sequence or a combination thereof. In one embodiment, the transmembrane domain comprises the CD3zeta domain or CD28 transmembrane domain. Other transmembrane domains will be apparent to those of skill in the art and may be used in connection with embodiments of a CAR provided herein.

[0560] The intracellular signaling region of a CAR provided herein contains one or more intracellular signaling domain that transmits a signal to a T cell upon engagement of the antigen binding domain of the CAR, such as upon binding antigen. In some embodiments, the intracellular region contains an intracellular signaling domain that is or contains an ITAM signaling domain. Exemplary intracellular signaling domains include, for example, a signaling domain derived from ζ chain of the T-cell receptor complex or any of its homologs (e.g., η chain, FcsRIy and β chains, MB 1 (Iga) chain, B29 (Ig) chain, etc.), human CD3zeta chain, CD3 polypeptides (Δ, δ and ε), syk family tyrosine kinases (Syk, ZAP 70, etc.), src family tyrosine kinases (Lck, Fyn, Lyn, etc.) and other molecules involved in T-cell transduction, such as CD2, CD5, OX40 and CD28. In particular embodiments, the intracellular signaling region contains an intracellular signaling domain derived from the human CD3 zeta chain.

[0561] In some embodiments, the endodomain comprises at CD3-zeta signaling domain. In some embodiments, the CD3-zeta signaling domain comprises the sequence of amino acids set forth in SEQ ID NO: 236 or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% or more sequence identity to SEQ ID NO: 236 and retains the activity of T cell signaling.

[0562] In some embodiments, the intracellular signaling region of a CAR can further contain an intracellular signaling domain derived from a costimulatory molecule. In such examples, such a signaling domain may enhance CAR-T cell activity, such as via enhancement of proliferation, survival and/or development of memory cells, after antigen specific engagement, for example, compared to a CAR that only contains an ITAM containing signaling domain, e.g. CD3 zeta. In some embodiments, the co-stimulatory domain is a functional signaling domain obtained from a protein selected from: CD28, CD137 (4-IBB), CD134 (OX40), DapIO, CD27, CD2, CD5, ICAM-1 , LFA- 1 (CD1 la/CD18), Lck, TNFR-I, TNFR-II, Fas, CD30, CD40 or combinations thereof. In particular embodiments, the costimulatory signaling domain is derived or obtained from a human protein. In some aspects, the costimulatory signaling domain is derived or obtained from human CD28 or human CD137 (4-IBB).

[0563] In some embodiments, the costimulatory signaling domain is a derived from CD28 or 4-1BB and comprises the sequence of amino acids set forth in any of SEQ ID NOS: 237-240 or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% or more sequence identity to SEQ ID NO: 237-240 and retains the activity of T cell costimulatory signaling.

[0564] In particular embodiments, the CAR further comprises a hinge or spacer region which connects the extracellular antigen binding domain and the transmembrane domain. This hinge or spacer

region can be used to achieve different lengths and flexibility of the resulting CAR. Examples of the hinge or spacer region that can be used include, but are not limited to, Fc fragments of antibodies or fragments or derivatives thereof, hinge regions of antibodies, or fragments or derivatives thereof, C_H2 regions of antibodies, C_H3 regions of antibodies, artificial spacer sequences, for example peptide sequences, or combinations thereof. Other hinge or spacer region will be apparent to those of skill in the art and may be used. In one embodiment, the hinge is an lgG4 hinge or a CD8A hinge.

[0565] In some embodiments, the spacer and transmembrane domain are the hinge and transmembrane domain derived from CD8, such as having an exemplary sequence set forth in SEQ ID NO: 241-243 or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO: 241-243.

[0566] Also provided herein is an isolated nucleic acid construct comprising at least one nucleic acid encoding a CAR as provided herein. In some aspects, the construct is an expression vector for expression of the CAR in a cell. The expression vector may be a viral vector. Viral vector technology is well known in the art and is described, for example, in Sambrook et al. (Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, New York, 2013). A number of viral based systems have been developed for gene transfer into mammalian cells. For example, retroviruses such as, adenovirus vectors are used. In one embodiment, a lentivirus vector is used.

[0567] In a further aspect, also provided is an isolated cell or cell population comprising one or more nucleic acid construct as described above. Also provided is an isolated cell or cell population that has been genetically modified to express a CAR provided herein. Thus, provided herein are genetically engineered cells which comprise, such as stably express, a CAR provided herein. In one embodiment, the cell is selected from the group consisting of a T cell, a Natural Killer (NK) cell, a cytotoxic T lymphocyte (CTL), a regulatory T cell, hematopoietic stem cells and/or pluripotent embryonic/induced stem cells. In some cases, the cell is a T cell, such as a CD4 and/or CD8 T cell. In some embodiments, the cells are autologous to the subject. For example, in some embodiments, T cells may be isolated from a patient (also called primary T cells) for engineering, e.g. transfection or transduction, with a CAR nucleic acid construct.

[0568] In an exemplary example, primary T-cells can be purified *ex vivo* (CD4 cells or CD8 cells or both) and stimulated with a TCR/CD28 agonists, such as anti-CD3/anti-CD28 coated beads. After a 2 or 3 day activation process, a recombinant expression vector encoding the CAR can be stably introduced into the primary T cells through standard lentiviral or retroviral transduction protocols or plasmid electroporation strategies. Cells can be monitored for CAR expression by, for example, flow cytometry using anti-epitope tag or antibodies that cross-react with native parental molecule. T-cells that express the CAR can be enriched through sorting with anti-epitope tag antibodies or enriched for high or low expression depending on the application.

[0569] The CAR engineered T-cells can be assayed for appropriate function by a variety of means. In some cases, *in vitro* cytotoxicity, proliferation, or cytokine assays (e.g., IFN-gamma expression) can be used to assess the function of engineered T-cells. Exemplary standard endpoints are percent lysis of a tumor line, proliferation of the engineered T-cell, or IFN-gamma protein expression in culture supernatant. In some cases, the ability to stimulate activation of T cells upon stimulation of the CAR, e.g. via antigen, can be assessed, such as by monitoring expression of activation markers such as CD69, CD44, or CD62L, proliferation and/or cytokine production.

[0570] Also provided herein are methods for the prevention and/or treatment of a disease or condition in a subject, such as a cancer, that includes administering to a subject engineered cells comprising a CAR provided herein. Generally, the subject is in need of treatment for the disease or condition. pharmaceutically active amount of a cell and/or of a pharmaceutical composition of the invention.

IV. POLYPEPTIDE EXPRESSION AND PRODUCTION

[0571] Nucleic acid molecules comprising polynucleotides that encode any of the provided sdAb and 5T4-binding polypeptides are provided. In some embodiments, the provided nucleic acid sequences and particularly DNA sequences encode fusion proteins as provided herein. In any of the foregoing embodiments, the nucleic acid molecule may also encode a leader sequence that directs secretion of the 5T4-binding polypeptide, which leader sequence is typically cleaved such that it is not present in the secreted polypeptide. The leader sequence may be a native heavy chain (or VHH) leader sequence, or may be another heterologous leader sequence.

[0572] Nucleic acid molecules can be constructed using recombinant DNA techniques conventional in the art. In some embodiments, a nucleic acid molecule is an expression vector that is suitable for expression in a selected host cell.

[0573] Vectors comprising nucleic acids that encode the 5T4-binding polypeptides described herein are provided. Such vectors include, but are not limited to, DNA vectors, phage vectors, viral vectors, retroviral vectors, *etc.* In some embodiments, a vector is selected that is optimized for expression of polypeptides in a desired cell type, such as CHO or CHO-derived cells, or in NSO cells. Exemplary such vectors are described, for example, in Running Deer *et al.*, *Biotechnol. Prog.* 20:880-889 (2004).

[0574] In particular, a DNA vector that encodes a desired 5T4-binding polypeptides, such as a fusion protein, can be used to facilitate the methods of preparing the 5T4-binding polypeptides described herein and to obtain significant quantities. The DNA sequence can be inserted into an appropriate expression vector, i.e., a vector which contains the necessary elements for the transcription and translation of the inserted protein-coding sequence. A variety of host-vector systems may be utilized to express the protein-coding sequence. These include mammalian cell systems infected with virus (e.g., vaccinia virus, adenovirus, etc.); insect cell systems infected with virus (e.g., baculovirus);

microorganisms such as yeast containing yeast vectors, or bacteria transformed with bacteriophage DNA, plasmid DNA or cosmid DNA. Depending on the host-vector system utilized, any one of a number of suitable transcription and translation elements may be used.

[0575] The disclosure also provides methods of producing a 5T4-binding polypeptides by culturing a cell under conditions that lead to expression of the polypeptide, wherein the cell comprises an isolated nucleic acid molecule encoding a 5T4-binding polypeptide described herein, and/or vectors that include these isolated nucleic acid sequences.

[0576] In some embodiments, a 5T4-binding polypeptide may be expressed in prokaryotic cells, such as bacterial cells; or in eukaryotic cells, such as fungal cells (such as yeast), plant cells, insect cells, and mammalian cells. Such expression may be carried out, for example, according to procedures known in the art. Exemplary eukaryotic cells that may be used to express polypeptides include, but are not limited to, COS cells, including COS 7 cells; 293 cells, including 293-6E cells; CHO cells, including CHO-S, DG44. Lec13 CHO cells, and FUT8 CHO cells; PER.C6® cells (Crucell); and NSO cells. In some embodiments, the 5T4-binding polypeptides may be expressed in yeast. *See, e.g.*, U.S. Publication No. US 2006/0270045 A1. In some embodiments, a particular eukaryotic host cell is selected based on its ability to make desired post-translational modifications to the polypeptide. For example, in some embodiments, CHO cells produce polypeptides that have a higher level of sialylation than the same polypeptide produced in 293 cells.

[0577] Introduction of one or more nucleic acids (such as vectors) into a desired host cell may be accomplished by any method, including but not limited to, calcium phosphate transfection, DEAE-dextran mediated transfection, cationic lipid-mediated transfection, electroporation, transduction, infection, *etc.* Nonlimiting exemplary methods are described, for example, in Sambrook *et al.*, Molecular Cloning, A Laboratory Manual, 3rd ed. Cold Spring Harbor Laboratory Press (2001). Nucleic acids may be transiently or stably transfected in the desired host cells, according to any suitable method.

[0578] Host cells comprising any of the nucleic acids or vectors described herein are also provided. In some embodiments, a host cell that expresses a 5T4-binding polypeptide described herein is provided. The 5T4-binding polypeptides expressed in host cells can be purified by any suitable method. Such methods include, but are not limited to, the use of affinity matrices or hydrophobic interaction chromatography. Suitable affinity ligands include the ROR1 ECD and agents that bind Fc regions. For example, a Protein A, Protein G, Protein A/G, or an antibody affinity column may be used to bind the Fc region and to purify a 5T4-binding polypeptide that comprises an Fc region. Hydrophobic interactive chromatography, for example, a butyl or phenyl column, may also suitable for purifying some polypeptides such as antibodies. Ion exchange chromatography (for example anion exchange chromatography and/or cation exchange chromatography) may also suitable for purifying some polypeptides such as antibodies. Mixed-mode chromatography (for example reversed phase/anion exchange, reversed phase/cation exchange, hydrophilic interaction/anion exchange, hydrophilic

interaction/cation exchange, *etc.*) may also suitable for purifying some polypeptides such as antibodies. Many methods of purifying polypeptides are known in the art.

[0579] In some embodiments, the 5T4-binding polypeptide is produced in a cell-free system. Nonlimiting exemplary cell-free systems are described, for example, in Sitaraman *et al.*, *Methods Mol. Biol.* 498: 229-44 (2009); Spirin, *Trends Biotechnol.* 22: 538-45 (2004); Endo *et al.*, *Biotechnol. Adv.* 21: 695-713 (2003).

[0580] In some embodiments, 5T4-binding polypeptides prepared by the methods described above are provided. In some embodiments, the 5T4-binding polypeptide is prepared in a host cell. In some embodiments, the 5T4-binding polypeptide is prepared in a cell-free system. In some embodiments, the 5T4-binding polypeptide is purified. In some embodiments, a cell culture media comprising a 5T4-binding polypeptide is provided.

[0581] In some embodiments, compositions comprising antibodies prepared by the methods described above are provided. In some embodiments, the composition comprises a 5T4-binding polypeptide prepared in a host cell. In some embodiments, the composition comprises a 5T4-binding polypeptide prepared in a cell-free system. In some embodiments, the composition comprises a purified 5T4-binding polypeptide.

V. PHARMACEUTICAL COMPOSITIONS AND FORMULATIONS

[0582] Provided herein are pharmaceutical compositions containing any of the 5T4-binding polypeptides provided herein or engineered cells expressing the same. In some embodiments, 5T4-binding polypeptides, such as fusion proteins of the disclosure (also referred to herein as "active compounds"), and derivatives, fragments, analogs and homologs thereof, can be incorporated into pharmaceutical compositions suitable for administration. In some embodiments, engineered cells expressing a chimeric receptor, such as a chimeric antigen receptor, containing a 5T4-binding polypeptide provided herein can be incorporated into pharmaceutical compositions suitable for administration.

[0583] Such compositions typically contain a pharmaceutically acceptable carrier. As used herein, the term "pharmaceutically acceptable carrier" is intended to include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. Suitable carriers are described in the most recent edition of Remington's Pharmaceutical Sciences, a standard reference text in the field, which is incorporated herein by reference. Suitable examples of such carriers or diluents include, but are not limited to, water, saline, ringer's solutions, dextrose solution, and 5% human serum albumin. Liposomes and non-aqueous vehicles such as fixed oils may also be used. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is

incompatible with the active compound, use thereof in the compositions is contemplated. Supplementary active compounds can also be incorporated into the compositions.

[0584] A pharmaceutical composition of the disclosure is formulated to be compatible with its intended route of administration. Examples of routes of administration include parenteral, *e.g.*, intravenous, intradermal, subcutaneous, intratumoral, oral (*e.g.*, inhalation), transdermal (*i.e.,* topical), transmucosal, and rectal administration. Solutions or suspensions used for parenteral, intradermal, or subcutaneous application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid (EDTA); buffers such as acetates, citrates or phosphates, and agents for the adjustment of tonicity such as sodium chloride or dextrose. The pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

[0585] Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, CREMOPHOR EL™ (BASF, Parsippany, N.J.) or phosphate buffered saline (PBS). In all cases, the composition must be sterile and should be fluid to the extent that easy syringeability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as manitol, sorbitol, sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate and gelatin.

[0586] Sterile injectable solutions can be prepared by incorporating the active compound in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, methods of preparation are vacuum drying and freeze-drying that yields a powder of

the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

[0587] Oral compositions generally include an inert diluent or an edible carrier. They can be enclosed in gelatin capsules or compressed into tablets. For the purpose of oral therapeutic administration, the active compound can be incorporated with excipients and used in the form of tablets, troches, or capsules. Oral compositions can also be prepared using a fluid carrier for use as a mouthwash, wherein the compound in the fluid carrier is applied orally and swished and expectorated or swallowed. Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the composition. The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring.

[0588] For administration by inhalation, the compounds are delivered in the form of an aerosol spray from pressured container or dispenser which contains a suitable propellant, *e.g.*, a gas such as carbon dioxide, or a nebulizer.

[0589] Systemic administration can also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration, detergents, bile salts, and fusidic acid derivatives. Transmucosal administration can be accomplished through the use of nasal sprays or suppositories. For transdermal administration, the active compounds are formulated into ointments, salves, gels, or creams as generally known in the art.

[0590] The compounds can also be prepared in the form of suppositories (*e.g.*, with conventional suppository bases such as cocoa butter and other glycerides) or retention enemas for rectal delivery.

[0591] In one embodiment, the active compounds are prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. The materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art, for example, as described in U.S. Patent No. 4,522,811.

[0592] It is especially advantageous to formulate oral or parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subject to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the disclosure are dictated by and directly dependent on the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and the limitations inherent in the art of compounding such an active compound for the treatment of individuals.

[0593] The pharmaceutical compositions can be included in a kit, container, pack, or dispenser together with instructions for administration. These pharmaceutical compositions can be included in diagnostic kits with instructions for use.

[0594] Pharmaceutical compositions are administered in an amount effective for treatment or prophylaxis of the specific indication. The therapeutically effective amount is typically dependent on the weight of the subject being treated, his or her physical or health condition, the extensiveness of the condition to be treated, or the age of the subject being treated. In some embodiments, the pharmaceutical composition may be administered in an amount in the range of about 50 μg/kg body weight to about 50 mg/kg body weight per dose. In some embodiments, the pharmaceutical composition may be administered in an amount in the range of about 100 μg/kg body weight to about 50 mg/kg body weight per dose. In some embodiments, the pharmaceutical composition may be administered in an amount in the range of about 100 μg/kg body weight to about 20 mg/kg body weight per dose. In some embodiments, the pharmaceutical composition may be administered in an amount in the range of about 0.5 mg/kg body weight to about 20 mg/kg body weight per dose.

[0595] In some embodiments, the pharmaceutical composition may be administered in an amount in the range of about 10 mg to about 1,000 mg per dose. In some embodiments, the pharmaceutical composition may be administered in an amount in the range of about 20 mg to about 500 mg per dose. In some embodiments, the pharmaceutical composition may be administered in an amount in the range of about 20 mg to about 300 mg per dose. In some embodiments, the pharmaceutical composition may be administered in an amount in the range of about 20 mg to about 200 mg per dose.

[0596] The pharmaceutical composition may be administered as needed to subjects. In some embodiments, an effective dose of the pharmaceutical composition is administered to a subject one or more times. In various embodiments, an effective dose of the pharmaceutical composition is administered to the subject once a month, less than once a month, such as, for example, every two months, every three months, or every six months. In other embodiments, an effective dose of the pharmaceutical composition is administered more than once a month, such as, for example, every two weeks, every week, twice per week, three times per week, daily, or multiple times per day. An effective dose of the pharmaceutical composition is administered to the subject at least once. In some

embodiments, the effective dose of the pharmaceutical composition may be administered multiple times, including for periods of at least a month, at least six months, or at least a year. In some embodiments, the pharmaceutical composition is administered to a subject as-needed to alleviate one or more symptoms of a condition.

VI. METHODS OF TREATMENT AND USES

[0597] The 5T4-binding polypeptides or engineered cells expressing the same described herein are useful in a variety of therapeutic, diagnostic and prophylactic indications. For example, the 5T4-binding polypeptides or engineered cells are useful in treating a variety of diseases and disorders in a subject. Such methods and uses include therapeutic methods and uses, for example, involving administration of the molecules or engineered cells, or compositions containing the same, to a subject having a disease, condition, or disorder, such as a tumor or cancer. In some embodiments, the molecule ore engineered cell is administered in an effective amount to effect treatment of the disease or disorder. Uses include uses of molecules containing the 5T4-binding polypeptides or engineered cells in such methods and treatments, and in the preparation of a medicament in order to carry out such therapeutic methods. In some embodiments, the methods are carried out by administering the 5T4-binding polypeptides or engineered cells, or compositions comprising the same, to the subject having or suspected of having the disease or condition. In some embodiments, the methods thereby treat the disease or condition or disorder in the subject.

[0598] In one embodiment, a 5T4-binding polypeptide or engineered cell of the disclosure may be used as therapeutic agents. Such agents will generally be employed to diagnose, prognose, monitor, treat, alleviate, and/or prevent a disease or pathology in a subject. A therapeutic regimen is carried out by identifying a subject, *e.g.*, a human patient or other mammal suffering from (or at risk of developing) a disorder using standard methods. In some cases, a subject is selected that is known, suspected or that has been identified as having a tumor expressing 5T4. A 5T4-binding polypeptide or engineered cell is administered to the subject. A 5T4-binding polypeptide or engineered cell is administered to the subject and will generally have an effect due to its binding with the target(s).

[0599] In some embodiments, a provided 5T4 polypeptide multi-specific polypeptide construct or engineered cell is capable of modulating, e.g. increasing, an immune response when administered to a subject, such as by engagement of CD3 and/or a CD3 signal in a cell. In some embodiments, provided herein is a method of modulating an immune response in a subject by administering a therapeutically effective amount of a provided multispecific construction or engineered cell, or pharmaceutical compositions thereof. In some embodiments, the method of modulating an immune response increases or enhances an immune response in a subject. For example, the increase or enhanced response may be an increase in cell-mediated immunity. In some examples, the method increases T-cell activity, such as

cytolytic T-cell (CTL) activity. In some embodiments, the modulated (e.g., increased) immune response is against a tumor or cancer.

[0600] In some embodiments, administration of a 5T4-binding polypeptide, such as an 5T4-Fc fusion protein or a multispecific construction containing an Fc region, may activate innate immune cells via engagement of FcγRs through the Fc-region of the multispecific polypeptide construct. Administration of such multispecific polypeptide constructs may agonize, stimulate, activate, and/or augment innate immune cell effector functions, including ADCC, cytokine release, degranulation and/or ADCP. In the case of a constrained multispecific polypeptide construct, administration of such multispecific polypeptide constructs may activate T-cells once the linker(s) joining the first and second component is cleaved by a protease and/or upon binding of 5T4 on a target cell (e.g. tumor cell), thereby allowing the anti-CD3 binding portion to bind CD3ε on the T cells. In some cases, administration of the multispecific polypeptide constructs may agonize, stimulate, activate, and/or augment CD3-mediated T cell activation, cytotoxicity, cytokine release and/or proliferation.

[0601] In some embodiments, the provided methods are for treating a disease or condition in a subject by administering a therapeutically effective amount of any of the provided 5T4-binding polypeptides or engineered cells or pharmaceutical compositions thereof. In some embodiments, the disease or condition is a tumor or a cancer. Generally, alleviation or treatment of a disease or disorder involves the lessening of one or more symptoms or medical problems associated with the disease or disorder. For example, in the case of cancer, the therapeutically effective amount of the drug can accomplish one or a combination of the following: reduce the number of cancer cells; reduce the tumor size; inhibit (*i.e.*, to decrease to some extent and/or stop) cancer cell infiltration into peripheral organs; inhibit tumor metastasis; inhibit, to some extent, tumor growth; and/or relieve to some extent one or more of the symptoms associated with the cancer. In some embodiments, a composition of this disclosure can be used to prevent the onset or reoccurrence of the disease or disorder in a subject, *e.g.*, a human or other mammal, such as a non-human primate, companion animal (*e.g.*, cat, dog, horse), farm animal, work animal, or zoo animal. The terms subject and patient are used interchangeably herein.

[0602] In some embodiments, the 5T4-binding polypeptides or engineered cells, or pharmaceutical compositions thereof, can be used to inhibit growth of mammalian cancer cells (such as human cancer cells). A method of treating cancer can include administering an effective amount of any of the pharmaceutical compositions described herein to a subject with cancer. The effective amount of the pharmaceutical composition can be administered to inhibit, halt, or reverse progression of cancers. Human cancer cells can be treated *in vivo*, or *ex vivo*. In *ex vivo* treatment of a human patient, tissue or fluids containing cancer cells are treated outside the body and then the tissue or fluids are reintroduced back into the patient. In some embodiments, the cancer is treated in a human patient in vivo by administration of the therapeutic composition into the patient.

[0603] Non-liming examples of disease include: all types of cancers (breast, lung, colorectal, prostate, melanomas, head and neck, pancreatic, etc.), rheumatoid arthritis, Crohn's disuse, SLE, cardiovascular damage, ischemia, etc. For example, indications would include leukemias, including T-cell acute lymphoblastic leukemia (T-ALL), lymphoblastic diseases including multiple myeloma, and solid tumors, including lung, colorectal, prostate, pancreatic, and breast, including triple negative breast cancer. For example, indications include bone disease or metastasis in cancer, regardless of primary tumor origin; breast cancer, including by way of non-limiting example, ER/PR+ breast cancer, Her2+ breast cancer, triple-negative breast cancer; colorectal cancer; endometrial cancer; gastric cancer; glioblastoma; head and neck cancer, such as esophageal cancer; lung cancer, such as by way of non-limiting example, non-small cell lung cancer; multiple myeloma ovarian cancer; pancreatic cancer; prostate cancer; sarcoma, such as osteosarcoma; renal cancer, such as by way of nonlimiting example, renal cell carcinoma; and/or skin cancer, such as by way of nonlimiting example, squamous cell cancer, basal cell carcinoma, or melanoma. In some embodiments, the cancer is a squamous cell cancer. In some embodiments, the cancer is a skin squamous cell carcinoma. In some embodiments, the cancer is an esophageal squamous cell carcinoma. In some embodiments, the cancer is a head and neck squamous cell carcinoma. In some embodiments, the cancer is a lung squamous cell carcinoma.

[0604] In some embodiments, the 5T4-binding polypeptides or engineered cells, or pharmaceutical compositions thereof, or are useful in treating, alleviating a symptom of, ameliorating and/or delaying the progression of a cancer or other neoplastic condition. In some embodiments, the cancer is bladder cancer, breast cancer, uterine/cervical cancer, ovarian cancer, prostate cancer, testicular cancer, esophageal cancer, gastrointestinal cancer, pancreatic cancer, colorectal cancer, colon cancer, kidney cancer, head and neck cancer, lung cancer, stomach cancer, germ cell cancer, bone cancer, liver cancer, thyroid cancer, skin cancer, neoplasm of the central nervous system, lymphoma, leukemia, myeloma, sarcoma, and virus-related cancer. In certain embodiments, the cancer is a metastatic cancer, refractory cancer, or recurrent cancer.

[0605] In some embodiments, a therapeutically effective amount of a 5T4-binding polypeptide, such as a fusion protein or multispecific polypeptide construct, of the disclosure relates generally to the amount needed to achieve a therapeutic objective. Typically, precise amount of the compositions of the present disclosure to be administered can be determined by a physician with consideration of individual differences in age, weight, tumor size, extent of infection or metastasis, and condition of the patient (subject).

[0606] In some embodiments, a therapeutically effective dose may be, by way of nonlimiting example, from about 0.01 μg/kg body weight to about 10 mg/kg body weight. In some embodiments, the therapeutically effective dose may be, by way of nonlimiting example, from about 0.01 mg/kg body weight to about 5-10 mg/kg body weight. Common dosing frequencies may range, for example, from twice daily to once a week.

[0607] In some embodiments, a therapeutic amount of an engineered cell composition of the present disclosure is administered. It can generally be stated that a pharmaceutical composition comprising engineered cells, e.g., T cells, as described herein may be administered at a dosage of 10^4 to 10^9 cells/kg body weight, such as 10^5 to 10^6 cells/kg body weight, including all integer values within those ranges. Engineered cell compositions, such as T cell compositions, may also be administered multiple times at these dosages. The cells can be administered by using infusion techniques that are commonly known in immunotherapy (see, e.g., Rosenberg et al, New Eng. J. of Med. 319: 1676, 1988). The optimal dosage and treatment regime for a particular patient can readily be determined by one skilled in the art of medicine by monitoring the patient for signs of disease and adjusting the treatment accordingly.

[0608] Efficaciousness of treatment is determined in association with any known method for diagnosing or treating the particular disorder. Methods for the screening of 5T4-binding polypeptides, or engineered cells containing the same, that possess the desired specificity include, but are not limited to, enzyme linked immunosorbent assay (ELISA) and other immunologically mediated techniques known within the art. A variety of means are known for determining if administration of the provided 5T4-binding polypeptides or engineered cells sufficiently modulates immunological activity by eliminating, sequestering, or inactivating immune cells mediating or capable of mediating an undesired immune response; inducing, generating, or turning on immune cells that mediate or are capable of mediating a protective immune response; changing the physical or functional properties of immune cells; or a combination of these effects. Examples of measurements of the modulation of immunological activity include, but are not limited to, examination of the presence or absence of immune cell populations (using flow cytometry, immunohistochemistry, histology, electron microscopy, polymerase chain reaction (PCR)); measurement of the functional capacity of immune cells including ability or resistance to proliferate or divide in response to a signal (such as using T-cell proliferation assays and pepscan analysis based on 3H-thymidine incorporation following stimulation with anti-CD3 antibody, anti-T-cell receptor antibody, anti-CD28 antibody, calcium ionophores, PMA (phorbol 12-myristate 13-acetate) antigen presenting cells loaded with a peptide or protein antigen; B cell proliferation assays); measurement of the ability to kill or lyse other cells (such as cytotoxic T cell assays); measurements of the cytokines, chemokines, cell surface molecules, antibodies and other products of the cells (e.g., by flow cytometry, enzyme-linked immunosorbent assays, Western blot analysis, protein microarray analysis, immunoprecipitation analysis); measurement of biochemical markers of activation of immune cells or signaling pathways within immune cells (e.g., Western blot and immunoprecipitation analysis of tyrosine, serine or threonine phosphorylation, polypeptide cleavage, and formation or dissociation of protein complexes; protein array analysis; DNA transcriptional, profiling using DNA arrays or subtractive hybridization); measurements of cell death by apoptosis, necrosis, or other mechanisms (e.g., annexin V staining, TUNEL assays, gel electrophoresis to measure DNA laddering, histology; fluorogenic caspase assays, Western blot analysis of caspase substrates); measurement of the genes,

proteins, and other molecules produced by immune cells (e.g., Northern blot analysis, polymerase chain reaction, DNA microarrays, protein microarrays, 2- dimensional gel electrophoresis, Western blot analysis, enzyme linked immunosorbent assays, flow cytometry); and measurement of clinical symptoms or outcomes such as improvement of autoimmune, neurodegenerative, and other diseases involving self-proteins or self-polypeptides (clinical scores, requirements for use of additional therapies, functional status, imaging studies) for example, by measuring relapse rate or disease severity.

[0609] The provided 5T4-binding polypeptides are also useful in a variety of diagnostic and prophylactic formulations. In one embodiment, a 5T4-binding polypeptide is administered to patients that are at risk of developing one or more of the aforementioned disorders. A patient's or organ's predisposition to one or more of the disorders can be determined using genotypic, serological or biochemical markers.

[0610] In another embodiment of the disclosure, a 5T4-binding polypeptide or engineered cell is administered to human individuals diagnosed with a clinical indication associated with one or more of the aforementioned disorders. Upon diagnosis, such a therapeutic agent is administered to mitigate or reverse the effects of the clinical indication.

Combination Therapy

[0611] 5T4-binding polypeptides or engineered cells of the present disclosure can be administered alone or in combination with other modes of treatment, such as other anti-cancer agents. They can be provided before, substantially contemporaneous with, or after other modes of treatment (i.e., concurrently or sequentially). In some embodiments, the method of treatment described herein can further include administering: radiation therapy, chemotherapy, vaccination, targeted tumor therapy, CAR-T therapy, oncolytic virus therapy, cancer immunotherapy, cytokine therapy, surgical resection, chromatin modification, ablation, cryotherapy, an antisense agent against a tumor target, a siRNA agent against a tumor target, a microRNA agent against a tumor target or an anti-cancer/tumor agent, or a biologic, such as an antibody, cytokine, or receptor extracellular domain-Fc fusion.

[0612] In some embodiments, a 5T4-binding polypeptide provided herein is given concurrently with one or more chemotherapeutic agent, CAR-T (chimeric antigen receptor T-cell) therapy, oncolytic virus therapy, cytokine therapy, and/or agents that target other checkpoint molecules, such as VISTA, gpNMB, B7H4, HHLA2, CD73, CTLA4, TIGIT, etc.

[0613] In some embodiments, the 5T4-binding polypeptide or engineered cells of the present disclosure is used in combination with other anti-tumor agents, such as anti-HER-2 antibodies, anti-CD20 antibodies, an epidermal growth factor receptor (EGFR) antagonist (*e.g.*, a tyrosine kinase inhibitor), HER1/EGFR inhibitor (*e.g.*, erlotinib (TARCEVA®), platelet derived growth factor inhibitors (*e.g.*, GLEEVEC® (Imatinib Mesylate)), a COX-2 inhibitor (*e.g.*, celecoxib), interferons, CTLA4 inhibitors (*e.g.*, anti-CTLA antibody ipilimumab (YERVOY®)), PD-1 inhibitors (*e.g.*, anti-PD1 antibodies, BMS-936558), PDL1 inhibitors (*e.g.*, anti-PDL1 antibodies, MPDL3280A), PDL2 inhibitors

(*e.g.*, anti-PDL2 antibodies), cytokines, antagonists (*e.g.*, neutralizing antibodies) that bind to one or more of the following targets ErbB2, ErbB3, ErbB4, PDGFR-beta, BlyS, APRIL, BCMA, PD-1, PDL1, PDL2, CTLA4, or VEGF receptor(s), TRAIL/Apo2, and other bioactive and organic chemical agents, *etc.*

[0614] In some embodiments, a 5T4-binding polypeptide or engineered cell provided herein is given concurrently with a PD-1/PD-L1 therapy. Examples of PD-1 / PD-L1 therapy include nivolumab (BMS); pidilizumab (CureTech, CT-011), pembrolizumab (Merck); durvalumab (Medimmune/AstraZeneca); atezolizumab (Genentech/Roche); avelumab (Pfizer); AMP-224 (Amplimmune); BMS-936559; AMP-514 (Amplimmune); MDX-1105 (Merck); TSR-042 (Tesaro/AnaptysBio, ANB-011); STI-A1010 (Sorrento Therapeutics); STI-A1110 (Sorrento Therapeutics); and other agents that are directed against programmed death-1 (PD-1) or programmed death ligand 1 (PD-L1).

[0615] In some embodiments, the 5T4-binding polypeptide or engineered cell of the present disclosure may be used in combination with a chemotherapeutic agent. Examples of chemotherapeutic agents include, but are not limited to, alkylating agents such as thiotepa and CYTOXAN® cyclosphosphamide; alkyl sulfonates such as busulfan, improsulfan and piposulfan; aziridines such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines including altretamine, triethylenemelamine, trietylenephosphoramide, triethiylenethiophosphoramide and trimethylolomelamine; acetogenins (especially bullatacin and bullatacinone); a camptothecin (including the synthetic analogue topotecan); bryostatin; callystatin; CC-1065 (including its adozelesin, carzelesin and bizelesin synthetic analogues); cryptophycins (particularly cryptophycin 1 and cryptophycin 8); dolastatin; duocarmycin (including the synthetic analogues, KW-2189 and CB1-TM1); eleutherobin; pancratistatin; a sarcodictyin; spongistatin; nitrogen mustards such as chlorambucil, chlornaphazine, cholophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard; nitrosureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, and ranimnustine; antibiotics such as the enediyne antibiotics (*e.g.*, calicheamicin, especially calicheamicin gamma1I and calicheamicin omegaI1 (*see, e.g.*, Agnew, *Chem Intl. Ed. Engl.*, 33: 183-186 (1994)); dynemicin, including dynemicin A; bisphosphonates, such as clodronate; an esperamicin; as well as neocarzinostatin chromophore and related chromoprotein enediyne antiobiotic chromophores), aclacinomysins, actinomycin, authramycin, azaserine, bleomycins, cactinomycin, carabicin, carminomycin, carzinophilin, chromomycinis, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, ADRIAMYCIN® doxorubicin (including morpholino-doxorubicin, cyanomorpholino-doxorubicin, 2-pyrrolino-doxorubicin and deoxydoxorubicin), epirubicin, esorubicin, idarubicin, marcellomycin, mitomycins such as mitomycin C, mycophenolic acid, nogalamycin, olivomycins, peplomycin, potfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin; anti-metabolites such as methotrexate and 5-fluorouracil (5-FU); folic acid analogues such as denopterin, methotrexate,

pteropterin, trimetrexate; purine analogs such as fludarabine, 6-mercaptopurine, thiamiprine, thioguanine; pyrimidine analogs such as ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, floxuridine; androgens such as calusterone, dromostanolone propionate, epitiostanol, mepitiostane, testolactone; anti-adrenals such as aminoglutethimide, mitotane, trilostane; folic acid replenisher such as frolinic acid; aceglatone; aldophosphamide glycoside; aminolevulinic acid; eniluracil; amsacrine; bestrabucil; bisantrene; edatraxate; defofamine; demecolcine; diaziquone; elfornithine; elliptinium acetate; an epothilone; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidainine; maytansinoids such as maytansine and ansamitocins; mitoguazone; mitoxantrone; mopidanmol; nitraerine; pentostatin; phenamet; pirarubicin; losoxantrone; podophyllinic acid; 2-ethylhydrazide; procarbazine; PSK® polysaccharide complex (JHS Natural Products, Eugene, OR); razoxane; rhizoxin; sizofiran; spirogermanium; tenuazonic acid; triaziquone; 2,2',2"-trichlorotriethylamine; trichothecenes (especially T-2 toxin, verracurin A, roridin A and anguidine); urethan; vindesine; dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside ("Ara-C"); cyclophosphamide; thiotepa; taxoids, *e.g.*, TAXOL® paclitaxel (Bristol- Myers Squibb Oncology, Princeton, N.J.), ABRAXANE® Cremophor-free, albumin-engineered nanoparticle formulation of paclitaxel (American Pharmaceutical Partners, Schaumberg, Illinois), and TAXOTERE® doxetaxel (Rhône- Poulenc Rorer, Antony, France); chloranbucil; GEMZAR® gemcitabine; 6-thioguanine; mercaptopurine; methotrexate; platinum analogs such as cisplatin, oxaliplatin and carboplatin; vinblastine; platinum; etoposide (VP-16); ifosfamide; mitoxantrone; vincristine; NAVELBINE® vinorelbine; novantrone; teniposide; edatrexate; daunomycin; aminopterin; xeloda; ibandronate; irinotecan (Camptosar, CPT-11) (including the treatment regimen of irinotecan with 5-FU and leucovorin); topoisomerase inhibitor RFS 2000; difluorometlhylornithine (DMFO); retinoids such as retinoic acid; capecitabine; combretastatin; leucovorin (LV); oxaliplatin, including the oxaliplatin treatment regimen (FOLFOX); inhibitors of PKC-alpha, Raf, H-Ras, EGFR (*e.g.*, erlotinib (TARCEVA®)) and VEGF-A that reduce cell proliferation and pharmaceutically acceptable salts, acids or derivatives of any of the above.

[0616] Further nonlimiting exemplary chemotherapeutic agents include anti-hormonal agents that act to regulate or inhibit hormone action on cancers such as anti-estrogens and selective estrogen receptor modulators (SERMs), including, for example, tamoxifen (including NOLVADEX® tamoxifen), raloxifene, droloxifene, 4-hydroxytamoxifen, trioxifene, keoxifene, LY117018, onapristone, and FARESTON® toremifene; aromatase inhibitors that inhibit the enzyme aromatase, which regulates estrogen production in the adrenal glands, such as, for example, 4(5)-imidazoles, aminoglutethimide, MEGASE® megestrol acetate, AROMASIN® exemestane, formestanie, fadrozole, RIVISOR® vorozole, FEMARA® letrozole, and ARIMIDEX® anastrozole; and anti-androgens such as flutamide, nilutamide, bicalutamide, leuprolide, and goserelin; as well as troxacitabine (a 1,3-dioxolane nucleoside cytosine analog); antisense oligonucleotides, particularly those which inhibit expression of genes in signaling

pathways implicated in abherant cell proliferation, such as, for example, PKC-alpha, Ralf and H-Ras; ribozymes such as a VEGF expression inhibitor (*e.g.*, ANGIOZYME® ribozyme) and a HER2 expression inhibitor; vaccines such as gene therapy vaccines, for example, ALLOVECTIN® vaccine, LEUVECTIN® vaccine, and VAXID® vaccine; PROLEUKIN® (aldesleukin) rIL-2; LURTOTECAN® topoisomerase 1 inhibitor; ABARELIX® GnRH agoninst; and pharmaceutically acceptable salts, acids or derivatives of any of the above.

[0617] In some embodiments, the 5T4-binding polypeptide and the additional agent are formulated into a single therapeutic composition, and the 5T4-binding polypeptide and additional agent are administered simultaneously. Alternatively, the 5T4-binding polypeptide or engineered cell and the additional agent are separate from each other, e.g., each is formulated into a separate therapeutic composition, and the 5T4-binding polypeptide or engineered cell and the additional agent are administered simultaneously, or the 5T4-binding polypeptide or engineered cell and the additional agent are administered at different times during a treatment regimen. For example, the 5T4-binding polypeptide or engineered cell is administered prior to the administration of the additional agent, the 5T4-binding polypeptide or engineered cell is administered subsequent to the administration of the additional agent, or the 5T4-binding polypeptide or engineered cell and the additional agent are administered in an alternating fashion. The 5T4-binding polypeptide and additional agent may be administered in single doses or in multiple doses.

[0618] In some embodiments, the 5T4-binding polypeptide or engineered cell and the additional agent(s) are administered simultaneously. For example, the 5T4-binding polypeptide and the additional agent(s) can be formulated in a single composition or administered as two or more separate compositions. In some embodiments, the 5T4-binding polypeptide or engineered cell and the additional agent(s) are administered sequentially, or the 5T4-binding polypeptide or engineered cell and the additional agent are administered at different times during a treatment regimen.

VII. EXEMPLARY EMBODIMENTS

[0619] Among the provided embodiments are:

1. A 5T4-binding polypeptide construct, comprising at least one heavy chain only variable domain (5T4 VHH domain) that specifically binds 5T4 and one or more additional binding domain that binds to a target other than 5T4.

2. The 5T4-binding polypeptide construct of embodiment 1, wherein the at least one 5T4 VHH domain comprises a complementarity determining region 1 (CDR1) comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 86-87 and 288-292, 296, and 297; a complementarity determining region 2 (CDR2) comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 88-99, 298, and 299; and a complementarity determining region 3

(CDR3) comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 100-102, 300, 301, and 303.

3. A 5T4-binding polypeptide construct, comprising at least one heavy chain only variable domain (5T4 VHH domain) comprising a complementarity determining region 1 (CDR1) comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 86-87 and 288-292, 296, and 297; a complementarity determining region 2 (CDR2) comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 88-99, 298, and 299; and a complementarity determining region 3 (CDR3) comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 100-102, 300, 301, and 303.

4. The 5T4-binding polypeptide construct of any of embodiments 1-3, wherein the 5T4 is a human 5T4.

5. The 5T4-binding polypeptide construct of any of embodiments 1-4, wherein the at least one 5T4 VHH domain is humanized.

6. The 5T4-binding polypeptide construct of any of embodiments 1, 2, 4 and 5, wherein the one or more additional binding domains binds to an activating receptor on an immune cell.

7. The 5T4-binding polypeptide construct of embodiment 6, wherein the immune cell is a T cell.

8. The 5T4-binding polypeptide construct of embodiment 6 or embodiment 7, wherein the activating receptor is CD3 (CD3ε).

9. The 5T4-binding polypeptide construct of embodiment 8 that is bispecific for 5T4 and CD3.

10. The 5T4-binding polypeptide construct of embodiment 6, wherein the immune cell is a Natural Killer (NK) cell.

11. The 5T4-binding polypeptide construct of embodiment 6 or embodiment 10, wherein the activating receptor is CD16 (CD16a).

12. The 5T4-binding polypeptide construct of embodiment 11 that is bispecific for 5T4 and CD16a.

13. The 5T4-binding polypeptide construct of any of embodiments 1, 2, 4 and 5, wherein the one or more additional binding domain binds to a cytokine receptor.

14. The 5T4-binding polypeptide construct of any of embodiments 1, 2 and 4-13, wherein the one or more additional binding domain comprises an antibody or antigen-binding fragment thereof.

15. The 5T4-binding polypeptide construct of any of embodiments 1, 2 and 4-14, wherein the one or more additional binding domain is monovalent.

16. The 5T4-binding polypeptide construct of embodiment 14 or embodiment 15, wherein the antibody or antigen-binding fragment thereof is an Fv, a disulfide-stabilized Fv (dsFv), scFv, a Fab, a single domain antibody (sdAb).

17. The 5T4-binding polypeptide construct of embodiment 13, wherein the one or more additional binding domain is a cytokine or is a truncated fragment or variant thereof capable of binding to the cytokine receptor.

18. The 5T4-binding polypeptide construct of embodiment 17, wherein the cytokine is an interferon, or is a truncated fragment or variant of thereof.

19. The 5T4-binding polypeptide construct of embodiment 18, wherein the interferon is a type I interferon or a type II interferon, is a truncated fragment or variant of a type I interferon or is a truncated fragment or variant of a type II interferon.

20. The 5T4-binding polypeptide construct of embodiment 19, wherein:

the type I interferon is an IFN-alpha or an IFN-beta or is a truncated fragment or variant thereof; or

the type II interferon is an IFN-gamma or is a truncated fragment or variant thereof.

21. The 5T4-binding polypeptide construct of any of embodiments 1-20, wherein the polypeptide construct comprises an immunoglobulin Fc region.

22. The 5T4-binding polypeptide construct of any of embodiments 1, 2 and 4-21, wherein the polypeptide comprises an immunoglobulin Fc region that links the at least one 5T4 VHH domain and the one or more additional binding domain.

23. The 5T4-binding polypeptide construct of any of embodiments 1-22 that is a dimer.

24. The 5T4-binding polypeptide construct of any of embodiments 21-23, wherein the Fc region is a homodimeric Fc region.

25. The 5T4-binding polypeptide construct of any of embodiments 21-24, wherein the Fc region comprises the sequence of amino acids set forth in any of SEQ ID NOS: 8, 10, 11, 12 or 13, or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to any of SEQ ID NOS: 8, 10, 11, 12 or 13.

26. The 5T4-binding polypeptide construct of any of embodiments 21-25, wherein the Fc region is a human IgG1.

27. The 5T4-binding polypeptide construct of embodiment 26, wherein the Fc region comprises the sequence of amino acids set forth in SEQ ID NO:8 or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 8.

28. The 5T4-binding polypeptide construct of any of embodiments 21-23, wherein the Fc region is a heterodimeric Fc region.

29. The 5T4-binding polypeptide construct of any of embodiments 21-28, wherein the Fc region exhibits effector function.

30. The 5T4-binding polypeptide construct of any of embodiments 21-29, wherein the Fc region comprises a polypeptide comprising one or more amino acid modification that reduces effector function and/or reduces binding to an effector molecule selected from an Fc gamma receptor or C1q.

31. The 5T4-binding polypeptide construct of embodiment 30, wherein the one or more amino acid modification is deletion of one or more of Glu233, Leu234 or Leu235.

32. The 5T4-binding polypeptide construct of embodiment 30 or embodiment 31, wherein the Fc region comprises the sequence of amino acids set forth in SEQ ID NO:9 or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 9.

33. The 5T4-binding polypeptide construct of any of embodiments 1-32, wherein the at least one 5T4 VHH domain comprises the sequence set forth in any of SEQ ID NOS: 245-253, 255-287, 294, 295, 302, and 360 or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to any of SEQ ID NOS: 245-253, 255-287, 294, 295, 302, and 360 and binds 5T4.

34. The 5T4-binding polypeptide construct of any of embodiments 1-33, wherein the at least one 5T4 VHH domain binds to an epitope in human 5T4 but does not exhibit crossreactive binding to mouse 5T4.

35. The 5T4-binding polypeptide construct of any of embodiments 1-34, wherein the at least one 5T4 VHH domain binds to amino acid residues between amino acids 60 and 112 of SEQ ID NO:382.

36. The 5T4-binding polypeptide construct of any of embodiments 1-35, wherein the at least one 5T4 VHH domain binds to amino acid residues between amino acids 173 and 224 of SEQ ID NO:382.

37. The 5T4-binding polypeptide construct of any of embodiments 1-36, wherein the at least one 5T4 VHH domain comprises the sequence set forth in (i) SEQ ID NO:245, (ii) a humanized variant of SEQ ID NO:245, or (iii) a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO:245 and binds 5T4.

38. The 5T4-binding polypeptide construct of any of embodiments 1-37, wherein the at least one 5T4 VHH domain comprises a CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 288 and 289; a CDR2 comprising an amino acid sequence set forth in SEQ ID NO: 88; and a CDR3 comprising an amino acid sequence set forth in SEQ ID NO: 100.

39. The 5T4-binding polypeptide construct of any of embodiments 1-38, wherein the at least one 5T4 VHH domain comprises a CDR1, CDR2 and CDR3 set forth in SEQ ID NOS: 288, 88, and 100, respectively; or SEQ ID NOS: 289, 88, and 100, respectively.

40. The 5T4-binding polypeptide construct of any of embodiments 1-39, wherein the at least one 5T4 VHH domain comprises the sequence of amino acids set forth in any one of SEQ ID NOs: 246-

253 and 360 or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 246-253 and 360 and binds 5T4.

41. The 5T4-binding polypeptide construct of embodiments 1-40, wherein the at least one 5T4 VHH domain comprises the sequence of amino acids set forth in any one of SEQ ID NOS: 246-253 and 360.

42. The 5T4-binding polypeptide construct of any of embodiments 1-36, wherein the at least one 5T4 VHH domain comprises the sequence set forth in (i) SEQ ID NO:255, (ii) a humanized variant of SEQ ID NO: 255, or (iii) a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 255 and binds 5T4.

43. The 5T4-binding polypeptide construct of any of embodiments 1-36 and 42, wherein the at least one 5T4 VHH domain comprises a CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 86, 290-292; a CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 89-94; and a CDR3 comprising an amino acid sequence set forth in SEQ ID NO: 101.

44. The 5T4-binding polypeptide construct of any of embodiments 1-36, 42 and 43, wherein the at least one 5T4 VHH domain comprises a CDR1, CDR2 and CDR3 set forth in SEQ ID NOS: 290, 90, and 101, respectively; SEQ ID NOS: 290, 91, and 101, respectively; SEQ ID NOS: 290, 92, and 101, respectively; SEQ ID NOS: 290, 93, and 101, respectively; SEQ ID NOS: 290, 94, and 101, respectively; SEQ ID NOS: 291, 94, and 101, respectively; SEQ ID NOS: 292, 94, and 101, respectively; or SEQ ID NOS: 86, 94, and 101, respectively.

45. The 5T4-binding polypeptide construct of any of embodiments 1-36 and 42-44, wherein the at least one 5T4 VHH domain comprises the sequence of amino acids set forth in any one of SEQ ID NOs: 256-275 or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 256-275 and binds 5T4.

46. The 5T4-binding polypeptide construct of embodiments 1-36 and 43-45, wherein the at least one 5T4 VHH domain comprises the sequence of amino acids set forth in any one of SEQ ID NOS: 256-275.

47. The 5T4-binding polypeptide construct of any of embodiments 1-36, wherein the at least one 5T4 VHH domain comprises the sequence set forth in (i) SEQ ID NO:276 (ii) a humanized variant of SEQ ID NO: 276, or (iii) a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 276 and binds 5T4.

48. The 5T4-binding polypeptide constructof any of embodiments 1-33 and 47, wherein the at least one 5T4 VHH domain comprises a CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 86 and 87; a CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 95-99; and a CDR3 comprising an amino acid sequence set forth in SEQ ID NO: 102.

49. The 5T4-binding polypeptide construct of any of embodiments 1-36, 47 and 48, wherein the at least one 5T4 VHH domain comprises a CDR1, CDR2 and CDR3 set forth in SEQ ID NOS: 87, 95, and 102, respectively; SEQ ID NOS: 87, 96, and 102, respectively; SEQ ID NOS: 87, 97, and 102, respectively; SEQ ID NOS: 87, 98, and 102, respectively; SEQ ID NOS: 87, 99, and 102, respectively; or SEQ ID NOS: 86, 98, and 102, respectively.

50. The 5T4-binding polypeptide construct of any of embodiments 1-36 and 47-49, wherein the at least one 5T4 VHH domain comprises the sequence of amino acids set forth in any one of SEQ ID NOs: 277-287 or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 277-287 and binds 5T4.

51. The 5T4-binding polypeptide construct of embodiments 1-36 and 47-50, wherein the at least one 5T4 VHH domain comprises the sequence of amino acids set forth in any one of SEQ ID NOS: 277-287.

52. The 5T4-binding polypeptide construct of any of embodiments 1-36, wherein the at least one 5T4 VHH domain comprises the sequence set forth in (i) SEQ ID NO:294 (ii) a humanized variant of SEQ ID NO:294, or (iii) a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO:294 and binds 5T4.

53. The 5T4-binding polypeptide construct of any of embodiments 1-36 and 52, wherein the at least one 5T4 VHH domain comprises a CDR1 comprising an amino acid sequence set forth in SEQ ID NO: 296; a CDR2 comprising an amino acid sequence set forth in SEQ ID NO: 298; and a CDR3 comprising an amino acid sequence set forth in SEQ ID NO: 300.

54. The 5T4-binding polypeptide construct of any of embodiments 1-36 and 52, wherein the at least one 5T4 VHH domain comprises a CDR3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 300, 301, and 303.

55. The 5T4-binding polypeptide construct of any of embodiments 1-36 and 52, wherein the at least one 5T4 VHH domain comprises:

a CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 288, 296, and 297; and/or

a CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 88, 298, and 299.

56. The 5T4-binding polypeptide construct of any of embodiments 1-36, wherein the at least one 5T4 VHH domain comprises the sequence set forth in (i) SEQ ID NO:295 (ii) a humanized variant of SEQ ID NO:295, or (iii) a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO:295 and binds 5T4.

57. The 5T4-binding polypeptide construct of any of embodiments 1-36 and 56, wherein the at least one 5T4 VHH domain comprises a CDR1 comprising an amino acid sequence set forth in SEQ ID NO: 297; a CDR2 comprising an amino acid sequence set forth in SEQ ID NO: 299; and a CDR3 comprising an amino acid sequence set forth in SEQ ID NO: 301.

58. The 5T4-binding polypeptide construct of any of embodiments 1-36, wherein the at least one 5T4 VHH domain comprises the sequence set forth in (i) SEQ ID NO:302 (ii) a humanized variant of SEQ ID NO: 302, or (iii) a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 302 and binds 5T4.

59. The 5T4-binding polypeptide construct of any of embodiments 1-36 and 56, wherein the at least one 5T4 VHH domain comprises a CDR1 comprising an amino acid sequence set forth in SEQ ID NO: 288; a CDR2 comprising an amino acid sequence set forth in SEQ ID NO: 88; and a CDR3 comprising an amino acid sequence set forth in SEQ ID NO: 303.

60. The 5T4-binding polypeptide construct of any of embodiments 1-36, wherein the at least one 5T4 VHH domain comprises the sequence set forth in (i) SEQ ID NO:294, 295, or 302 (ii) a humanized variant of SEQ ID NO: 294, 295, or 302, or (iii) a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 294, 295, or 302 and binds 5T4.

61. The 5T4-binding polypeptide construct of any of embodiments 1-36 and 60, wherein the at least one 5T4 VHH domain comprises a CDR1, CDR2 and CDR3 set forth in SEQ ID NOS: 296, 298, and 300, respectively; SEQ ID NOS:297, 299, and 301, respectively; or SEQ ID NOS:288, 88, and 303, respectively.

62. The 5T4-binding polypeptide construct of any of embodiments 1-36, 60 and 61, wherein the at least one 5T4 VHH domain is set forth in SEQ ID NO:245, 249, 255, 270, 276, 294, 295 or 302.

63. A multispecific polypeptide construct, comprising: (a) a first component comprising a heterodimeric Fc region comprising a first Fc polypeptide and a second Fc polypeptide and (b) a second component comprising an anti-CD3 antibody or antigen-binding fragment comprising a variable heavy chain region (VH) and a variable light chain region (VL), wherein:

the VH and VL that comprise the anti-CD3 antibody or antigen binding fragment are linked to opposite polypeptides of the heterodimeric Fc;

the first and second components are coupled by a linker, wherein the heterodimeric Fc region is positioned N-terminal to the anti-CD3 antibody; and

one or both of the first and second components comprises at least one heavy chain only variable domain (5T4 VHH domain).

64. The multispecific polypeptide construct of embodiment 63, wherein the multispecific polypeptide construct comprises at least (i) a first polypeptide comprising the first Fc polypeptide of the heterodimeric Fc region, the linker and the VH or VL domain of the anti-CD3 antibody or antigen binding fragment; and (ii) a second polypeptide comprising the second Fc polypeptide of the heterodimeric Fc region, the linker, optionally the same linker as present in the first polypeptide, and the other of the VH or VL domain of the anti-CD3 antibody or antigen binding fragment,

wherein one or both of the first and second polypeptide comprise the at least one 5T4 VHH domain.

65. The multispecific polypeptide construct of embodiment 63 or embodiment 64, wherein one or both of the first and second Fc polypeptides of the heterodimeric Fc region comprises at least one modification to induce heterodimerization compared to a polypeptide of a homodimeric Fc region, optionally compared to the Fc polypeptide set forth in SEQ ID NO: 8 or an immunologically active fragment thereof.

66. The multispecific polypeptide construct of embodiment 65, wherein each of the first and second Fc polypeptides of the heterodimeric Fc region independently comprise at least one amino acid modification.

67. The multispecific polypeptide construct of embodiment 65 or 66, wherein each of the first and second Fc polypeptides of the heterodimeric Fc region comprise a knob-into-hole modification or comprise a charge mutation to increase electrostatic complementarity of the polypeptides.

68. The multispecific polypeptide construct of embodiment 65-67, wherein the amino acid modification is a knob-into-hole modification.

69. The multispecific polypeptide construct of any of embodiments 63-68, wherein the first Fc polypeptide of the heterodimeric Fc region comprises the modification selected from among Thr366Ser, Leu368Ala, Tyr407Val, and combinations thereof and the second Fc polypeptide of the heterodimeric Fc region comprises the modification Thr366Trp.

70. The multispecific polypeptide of embodiment 69, wherein the first and second Fc polypeptides further comprise a modification of a non-cysteine residue to a cysteine residue, wherein the modification of the first Fc polypeptide is at one of the position Ser354 and Tyr349 and the modification of the second Fc polypeptide is at the other of the position Ser354 and Tyr349.

71. The multispecific polypeptide construct of any of embodiments 65-67, wherein the amino acid modification is a charge mutation to increase electrostatic complementarity of the polypeptides.

72. The multispecific polypeptide construct of any of embodiments 63-67 and 71, wherein the first and/or second Fc polypeptides or each of the first and second Fc polypeptide comprise an amino acid modification in complementary positions, wherein the modification is replacement with an amino acid having an opposite charge to the complementary amino acid of the other polypeptide.

73. The multispecific polypeptide construct of any of embodiments 63-72, wherein one of the first or second Fc polypeptide of the heterodimeric Fc region further comprises a modification at residue Ile253.

74. The multispecific polypeptide construct of embodiment 73, wherein the modification is Ile253Arg.

75. The multispecific polypeptide construct of any of embodiments 63-74, wherein one of the first or second Fc polypeptide of the heterodimeric Fc region further comprises a modification at residue His435.

76. The multispecific polypeptide construct of embodiment 75, wherein the modification is His435Arg.

77. The multispecific polypeptide construct of any of embodiments 63-76, wherein the Fc region comprises a polypeptide that lacks Lys447.

78. The multispecific polypeptide construct of any of embodiments 63-77, wherein the Fc region comprises a polypeptide comprising at least one modification to enhance FcRn binding.

79. The multispecific polypeptide construct of embodiment 78, wherein the at least one modification is at a position selected from the group consisting of Met252, Ser254, Thr256, Met428, Asn434, and combinations thereof.

80. The multispecific polypeptide construct of embodiment 78or embodiment 79, wherein the at least one modification is selected from the group consisting of Met252Y, Ser254T, Thr256E, Met428L, Met428V, Asn434S, and combinations thereof.

81. The multispecific polypeptide construct of any of embodiments 78-80, wherein the at least one modification is at position Met252 and at position Met428.

82. The multispecific polypeptide construct of any of embodiments 78-81, wherein the at least one modification is Met252Y and Met428L.

83. The multispecific polypeptide construct of any of embodiments 78-81, wherein the at least one modification is Met252Y and Met428V.

84. The multispecific polypeptide construct of any of embodiments 63-83, wherein the first Fc polypeptide of the heterodimeric Fc region comprises the sequence of amino acids set forth in any of SEQ ID NOS:103, 107, 115, 117, 328, or 334 and the second Fc polypeptide of the heterodimeric Fc region comprises the sequence of amino acids set forth in any of SEQ ID NOS:104, 108, 111, 113, 119, 121, 329, 332, or 336.

85. The polypeptide construct of any of embodiments 21-84, wherein the Fc region comprises a polypeptide comprising at least one amino acid modification that reduces effector function and/or reduces binding to an effector molecule selected from an Fc gamma receptor and C1q.

86. The multispecific polypeptide construct of embodiment 85, wherein the at least one amino acid modification is deletion of one or more of Glu233, Leu234 and Leu235.

87. The multispecific polypeptide construct of any of embodiments 63-86, wherein the first Fc polypeptide of the heterodimeric Fc region comprises the sequence of amino acids set forth in any of SEQ ID NOS: 105, 109, 116, 118, 330, or 335 and the second Fc polypeptide of the heterodimeric Fc region comprises the sequence of amino acids set forth in any of SEQ ID NOS: 106, 110, 112, 114, 120, 122, 331, 333, or 337.

88. The multispecific polypeptide construct of any of embodiment 63-87, wherein the anti-CD3 antibody or antigen binding fragment is monovalent.

89. The multispecific polypeptide construct of any of embodiments 63-88, wherein the anti-CD3 antibody or antigen binding fragment is not a single chain antibody, optionally is not a single chain variable fragment (scFv).

90. The multispecific polypeptide construct of any of embodiments 63-89, wherein the anti-CD3 antibody or antigen binding fragment is an Fv antibody fragment.

91. The multispecific polypeptide construct of embodiment 90, wherein the Fv antibody fragment comprises a disulfide stabilized anti-CD3 binding Fv fragment (dsFv).

92. The multispecific polypeptide construct of 63-91, wherein the anti-CD3 antibody or antigen-binding fragment comprises a VH CDR1 comprising the amino acid sequence TYAMN (SEQ ID NO: 29); a VH CDR2 comprising the amino acid sequence RIRSKYNNYATYYADSVKD (SEQ ID NO: 30); a VH CDR3 comprising the amino acid sequence HGNFGNSYVSWFAY (SEQ ID NO: 31), a VL CDR1 comprising the amino acid sequence RSSTGAVTTSNYAN (SEQ ID NO: 32); a VL CDR2 comprising the amino acid sequence GTNKRAP (SEQ ID NO: 33); and a VL CDR3 comprising the amino acid sequence ALWYSNLWV (SEQ ID NO: 34).

93. The multispecific polypeptide construct of any of embodiments 63-92, wherein the anti-CD3 antibody or antigen-binding fragment comprises:

a VH having the amino acid sequence of any of SEQ ID NOS: 27, 35-65, 341, 343, and 358 or a sequence that exhibits at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to any of SEQ ID NOS: 27, 35-65, 341, 343, and 358 and binds CD3; and

a VL having the amino acid sequence of any of SEQ ID NOS: 28, 66-84, 293, 340, and 342 or a sequence that exhibits at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to any of SEQ ID NOS: 28, 66-84, 293, 340, and 342 and 293 and binds CD3.

94. The multispecific polypeptide construct of any of embodiments 63-93, wherein the anti-CD3 antibody or antigen-binding fragment comprises the amino acid sequence of SEQ ID NO: 47 and the amino acid sequence of SEQ ID NO: 75.

95. The multispecific polypeptide construct of any of embodiments 63-93, wherein the anti-CD3 antibody or antigen-binding fragment comprises the amino acid sequence of SEQ ID NO: 47 and the amino acid sequence of SEQ ID NO: 293.

96. The multispecific polypeptide construct of any of embodiment 63-92, wherein the at least one 5T4 VHH domain is positioned amino-terminally relative to the Fc region and/or carboxy-terminally relative to the CD3 binding region of the multispecific polypeptide construct.

97. The multispecific polypeptide construct of any of embodiments 63-96, wherein the multispecific polypeptide construct comprises a first 5T4 VHH domain that specifically binds 5T4 and a second 5T4 VHH domain that specifically binds 5T4.

98. The multispecific polypeptide construct of embodiment 97, wherein the first or second 5T4 VHH domain is positioned amino-terminally relative to the Fc region of the multispecific construct and the other of the first or second 5T4 VHH domain is positioned carboxy-terminally relative to the CD3 binding region of the multispecific construct.

99. The multispecific polypeptide construct of embodiment 97 or embodiment 98, wherein

the first component comprises in order of N-terminus to C-terminus a first 5T4 VHH domain that binds 5T4, the first Fc polypeptide of the heterodimeric Fc region, the linker, the VH or VL domain of the anti-CD3 antibody or antigen binding fragment and a second 5T4 VHH domain that binds 5T4; and

the second component comprises in order of N-terminus to C-terminus the second Fc polypeptide of the heterodimeric Fc region, the linker, optionally the same linker as present in the first component, and the other of the VH or VL domain of the anti-CD3 antibody or antigen binding fragment.

100. The multispecific polypeptide construct of any of embodiments 97-99, wherein the first and second 5T4 VHH domain are the same.

101. The multispecific polypeptide construct of any of embodiments 97-99, wherein the first and second 5T4 VHH domain are different.

102. The multispecific polypeptide construct of embodiment 101, wherein the first and second 5T4 VHH domain bind a distinct or non-overlapping epitope of 5T4 and/or do not compete for binding to 5T4.

103. The multispecific polypeptide construct of embodiment 102, wherein:

the first 5T4 VHH domain comprises the amino acid sequence set forth in any one of SEQ ID NOS: 245-253, 295, 302, and 360 a humanized variant thereof, or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to any of SEQ ID NOS: 245-253, 295, 302, and 360 and binds 5T4; and

the second 5T4 VHH domain comprises the amino acid sequence set forth in any one of SEQ ID NOS: 255-287, 294, 302, a humanized variant thereof, or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to any of SEQ ID NOS: 255-287, 294, 302, and binds 5T4.

104. The multispecific polypeptide construct of embodiment 102, wherein:

the first and second 5T4 VHH domains are selected from SEQ ID NO: 245 and SEQ ID NO: 294; SEQ ID NO: 245 and SEQ ID NO: 276; SEQ ID NO: 245 and SEQ ID NO: 255; SEQ ID NO:245 and SEQ ID NO:295; SEQ ID NO: 295 and SEQ ID NO: 294; SEQ ID NO: 249 and SEQ ID NO: 270; SEQ ID NO:302 and SEQ ID NO:302; or SEQ ID NO: 360 and SEQ ID NO: 287.

105. The multispecific polypeptide construct of any of embodiments 63-104, wherein the at least one 5T4 VHH domain, or each of the first and second 5T4 VHH domain, independently comprises the VHH domain sequence set forth in any of SEQ ID NOS: 245-253, 255-287, 294, 295, 302, and 360 or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to any of SEQ ID NOS: 245-253, 255-287, 294, 295, 302, and 360 and binds 5T4.

106. The multispecific polypeptide construct of any of embodiments 63-105, wherein the at least one 5T4 VHH domain binds to an epitope in human 5T4 but does not exhibit crossreactive binding to mouse 5T4.

107. The multispecific polypeptide construct of any of embodiments 63-106, wherein the at least one 5T4 VHH domain binds to amino acid residues between amino acids 60 and 112 of SEQ ID NO:382.

108. The multispecific polypeptide construct of any of embodiments 63-107, wherein the at least one 5T4 VHH domain binds to amino acid residues between amino acids 173 and 224 of SEQ ID NO:382.

109. The multispecific polypeptide construct of any of embodiments 63-108, wherein the at least one 5T4 VHH domain, or each of the first and second 5T4 VHH domain, independently comprises the sequence set forth in (i) SEQ ID NO: 245, (ii) a humanized variant of SEQ ID NO: 245, or (iii) a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 245, and binds 5T4.

110. The multispecific polypeptide construct of any of embodiments 63-109, wherein the at least one 5T4 VHH domain, or each of the first and second 5T4 VHH domain, independently comprises a CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 86-87 and 288-292, 296, and 297; a CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 88-99, 298, and 299; and a CDR3 comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 100-102, 300, 301, and 303.

111. The multispecific construct of any of embodiments 63-110, wherein the at least one 5T4 VHH domain, or each of the first and second 5T4 VHH domain, independently comprises a CDR1, CDR2 and CDR3 set forth in SEQ ID NOS: 288, 88, and 100, respectively; SEQ ID NOS: 289, 88, and 100, respectively; SEQ ID NOS: 290, 89, and 101, respectively; SEQ ID NOS: 290, 90, and 101, respectively; SEQ ID NOS: 290, 91, and 101, respectively; SEQ ID NOS: 290, 92, and 101, respectively; SEQ ID NOS: 290, 93, and 101, respectively; SEQ ID NOS: 290, 94, and 101, respectively; SEQ ID NOS: 291, 94, and 101, respectively; SEQ ID NOS: 292, 94, and 101, respectively; SEQ ID NOS: 86, 94, and 101, respectively; SEQ ID NOS: 87, 95, and 102, respectively; SEQ ID NOS: 87, 96, and 102, respectively; SEQ ID NOS: 87, 97, and 102, respectively; SEQ ID NOS: 87, 98, and 102, respectively; SEQ ID NOS: 87, 99, and 102, respectively; SEQ ID NOS: 86, 98, and 102, respectively; SEQ ID NOS: 296, 298, and 300, respectively; SEQ ID NOS: 297, 299, and 301, respectively; or SEQ ID NOS: 288, 88, and 303, respectively.

112. The multispecific polypeptide construct of any of embodiments 63-111, wherein the at least one 5T4 VHH domain, or each of the first and second 5T4 VHH domain, independently comprises the sequence of amino acids set forth in any one of SEQ ID NOS: 246-253 and 360 or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NOS: 246-253 and 360, and binds 5T4.

113. The multispecific polypeptide construct of any of embodiments 63-112, wherein the at least one 5T4 VHH domain, or each of the first and second 5T4 VHH domain, independently comprises the sequence of amino acids set forth in any one of SEQ ID NOS: 246-253 and 360.

114. The multispecific polypeptide construct of any of embodiments 63-108, wherein the at least one 5T4 VHH domain, or each of the first and second 5T4 VHH domain, independently comprises the sequence set forth in (i) SEQ ID NO: 255, (ii) a humanized variant of SEQ ID NO: 255, or (iii) a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 255, and binds 5T4.

115. The multispecific polypeptide construct of any of embodiments 63-108 and 114, wherein the at least one 5T4 VHH domain, or each of the first and second 5T4 VHH domain, independently comprises a CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 86, 290-292; a CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 89-94; and a CDR3 comprising an amino acid sequence set forth in SEQ ID NO: 101.

116. The multispecific polypeptide construct of any of embodiments 63-108, 114 and 115, wherein the at least one 5T4 VHH domain, or each of the first and second 5T4 VHH domain, independently comprises a CDR1, CDR2 and CDR3 set forth in SEQ ID NOS: 290, 89, and 101, respectively; SEQ ID NOS: 290, 90, and 101, respectively; SEQ ID NOS: 290, 91, and 101, respectively; SEQ ID NOS: 290, 92, and 101, respectively; SEQ ID NOS: 290, 93, and 101, respectively; SEQ ID

NOS: 290, 94, and 101, respectively; SEQ ID NOS: 291, 94, and 101, respectively; SEQ ID NOS: 292, 94, and 101, respectively; or SEQ ID NOS: 86, 94, and 101, respectively.

117. The multispecific polypeptide construct of any of embodiments 63-108 and 114-116, wherein the at least one 5T4 VHH domain, or each of the first and second 5T4 VHH domain, independently comprises the sequence of amino acids set forth in any one of SEQ ID NOs: 256-275 or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 256-275, and binds 5T4.

118. The multispecific polypeptide construct of embodiments 63-108 and 114-117, wherein the at least one 5T4 VHH domain, or each of the first and second 5T4 VHH domain, independently comprises the sequence of amino acids set forth in any one of SEQ ID NOS: 256-275.

119. The multispecific polypeptide construct of any of embodiments 63-108, wherein the at least one 5T4 VHH domain, or each of the first and second 5T4 VHH domain, independently comprises the sequence set forth in (i) SEQ ID NO: 276 (ii) a humanized variant of SEQ ID NO: 276, or (iii) a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 276, and binds 5T4.

120. The multispecific polypeptide of any of embodiments 63-108 and 119, wherein the at least one 5T4 VHH domain, or each of the first and second 5T4 VHH domain, independently comprises a CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 86 and 87; a CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 95-99; and a CDR3 comprising an amino acid sequence set forth in SEQ ID NO: 102.

121. The multispecific polypeptide construct of any of embodiments 63-108, 119 and 120, wherein the at least one 5T4 VHH domain, or each of the first and second 5T4 VHH domain, independently comprises a CDR1, CDR2 and CDR3 set forth in SEQ ID NOS: 87, 95, and 102, respectively; SEQ ID NOS: 87, 96, and 102, respectively; SEQ ID NOS: 87, 97, and 102, respectively; SEQ ID NOS: 87, 98, and 102, respectively; SEQ ID NOS: 87, 99, and 102, respectively; or SEQ ID NOS: 86, 98, and 102, respectively.

122. The multispecific polypeptide construct of any of embodiments 63-108 and 119-121, wherein the at least one 5T4 VHH domain, or each of the first and second 5T4 VHH domain, independently comprises the sequence of amino acids set forth in any one of SEQ ID NOs: 277-287 or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 277-287, and binds 5T4.

123. The multispecific polypeptide construct of embodiments 63-108 and 119-122, wherein the at least one 5T4 VHH domain, or each of the first and second 5T4 VHH domain, independently comprises the sequence of amino acids set forth in any one of SEQ ID NOS: 277-287.

124. The multispecific polypeptide construct of any of embodiments 63-108, wherein the at least one 5T4 VHH domain, or each of the first and second 5T4 VHH domain, independently comprises

the sequence set forth in (i) SEQ ID NO: 294 (ii) a humanized variant of SEQ ID NO: 294, or (iii) a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 294, and binds 5T4.

125. The multispecific polypeptide construct of any of embodiments 63-108 and 124, wherein the at least one 5T4 VHH domain, or each of the first and second 5T4 VHH domain, independently comprises a CDR1 comprising an amino acid sequence set forth in SEQ ID NO: 296; a CDR2 comprising an amino acid sequence set forth in SEQ ID NO: 298; and a CDR3 comprising an amino acid sequence set forth in SEQ ID NO: 300.

126. The multispecific polypeptide construct of any of embodiments 63-106, wherein the at least one 5T4 VHH domain, or each of the first and second 5T4 VHH domain, independently comprises the sequence set forth in (i) SEQ ID NO: 295 (ii) a humanized variant of SEQ ID NO: 295, or (iii) a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 295, and binds 5T4.

127. The multispecific polypeptide construct of any of embodiments 63-106 and 126, wherein the at least one 5T4 VHH domain, or each of the first and second 5T4 VHH domain, independently comprises a CDR1 comprising an amino acid sequence set forth in SEQ ID NO: 297; a CDR2 comprising an amino acid sequence set forth in SEQ ID NO: 299; and a CDR3 comprising an amino acid sequence set forth in SEQ ID NO: 301.

128. The multispecific polypeptide construct of any of embodiments 63-106, wherein the at least one 5T4 VHH domain, or each of the first and second 5T4 VHH domain, independently comprises the sequence set forth in (i) SEQ ID NO: 302 (ii) a humanized variant of SEQ ID NO: 302, or (iii) a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 302, and binds 5T4.

129. The multispecific polypeptide construct of any of embodiments 63-106 and 126, wherein the at least one 5T4 VHH domain, or each of the first and second 5T4 VHH domain, independently comprises a CDR1 comprising an amino acid sequence set forth in SEQ ID NO: 288; a CDR2 comprising an amino acid sequence set forth in SEQ ID NO: 88; and a CDR3 comprising an amino acid sequence set forth in SEQ ID NO: 303.

130. The multispecific polypeptide construct of any of embodiments 63-106, wherein the at least one 5T4 VHH domain, or each of the first and second 5T4 VHH domain, independently comprises the sequence set forth in (i) SEQ ID NO: 294, 295, or 302 (ii) a humanized variant of SEQ ID NO: 294, 295, or 302, or (iii) a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 294, 295, or 302, and binds 5T4.

131. The multispecific polypeptide construct of any of embodiments 63-106 and 130, wherein the at least one 5T4 VHH domain, or each of the first and second 5T4 VHH domain, independently

comprises a CDR1, CDR2 and CDR3 set forth in SEQ ID NOS: 296, 298, and 300, respectively; SEQ ID NOS:297, 299, and 301, respectively; or SEQ ID NOS:288, 88, and 303, respectively.

132. The multispecific polypeptide construct of any of embodiments 63-106, 130 and 131, wherein the at least one 5T4 VHH domain, or each of the first and second 5T4 VHH domain, independently is set forth in SEQ ID NO: 245, 249, 255, 270, 276, 294, 295, 302, or 360.

133. The multispecific polypeptide construct of any of embodiments 63-132, wherein one or both of the first and second components comprises at least one co-stimulatory receptor binding region (CRBR) that binds a co-stimulatory receptor.

134. The multispecific polypeptide construct of embodiment 133, wherein the at least one co-stimulatory receptor binding region (CRBR) is positioned amino-terminally relative to the Fc region and/or carboxy-terminally relative to the CD3 binding region of the multispecific polypeptide construct.

135. The multispecific polypeptide construct of embodiment 133 or embodiment 134, wherein the multispecific polypeptide construct comprises only one co-stimulatory receptor binding region (CRBR).

136. The multispecific polypeptide construct of any of embodiments 133-135, wherein the multispecific polypeptide construct comprises two co-stimulatory receptor binding region (CRBR), optionally which are the same or different.

137. The multispecific polypeptide construct of any of embodiments 133-136, wherein the at least one co-stimulatory receptor binding region (CRBR) is or comprises the extracellular domain or binding fragment thereof of the native cognate binding partner of the co-stimulatory receptor, or a variant thereof that exhibits binding activity to the co-stimulatory receptor.

138. The multispecific polypeptide construct of any of embodiments 133-136, wherein the at least one co-stimulatory receptor binding region (CRBR) is an antibody or antigen-binding fragment thereof selected from the group consisting of a Fab fragment, a F(ab')2 fragment, an Fv fragment, a scFv, a scAb, a dAb, a single domain heavy chain antibody, and a single domain light chain antibody.

139. The multispecific polypeptide construct of embodiment 138, wherein the antibody or antigen-binding fragment thereof is a Fv, a scFv, a Fab, or a single domain antibody (sdAb).

140. The multispecific polypeptide construct of embodiment 138 or embodiment 139, wherein the antibody or antigen-binding fragment is an sdAb.

141. The multispecific polypeptide construct of embodiment 140, wherein the sdAb is a human or humanized sdAb.

142. The multispecific polypeptide construct of any of embodiments 133-141, wherein the at least one co-stimulatory receptor binding region (CRBR) binds a co-stimulatory receptor selected from among 41BB (CD137), OX40 (CD134), CD27, glucocorticoid-induced TNFR-related protein (GITR), CD28, ICOS, CD40, B-cell activating factor receptor (BAFF-R), B-cell maturation antigen (BCMA), Transmembrane activator and CAML interactor (TACI), and NKG2D.

143. The multispecific polypeptide construct of any of embodiments 133-142, wherein the at least one co-stimulatory receptor binding region (CRBR) binds a co-stimulatory receptor selected from among 41BB (CD137), OX40 (CD134), and glucocorticoid-induced TNFR-related protein (GITR).

144. The multispecific polypeptide construct of any of embodiments 133-143, wherein the at least one co-stimulatory receptor binding region (CRBR) comprises the sequence of amino acids set forth in SEQ ID NO:210 or a sequence that has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the sequence set forth in SEQ ID NO:210 and binds 4-1BB.

145. The multispecific polypeptide construct of any of embodiments 63-144, wherein one or both of the first and second components comprises at least one inhibitory receptor binding region (IRBR) that binds an inhibitory receptor.

146. The multispecific polypeptide construct of embodiment 145, wherein the at least one inhibitory receptor binding region (IRBR) is positioned amino-terminally relative to the Fc region and/or carboxy-terminally relative to the CD3 binding region of the multispecific polypeptide construct.

147. The multispecific polypeptide construct of embodiment 145 or embodiment 146, wherein the multispecific polypeptide construct comprises only one inhibitory receptor binding region (IRBR).

148. The multispecific polypeptide construct of any of embodiments 145-147, wherein:

the first component comprises in order of N-terminus to C-terminus a first 5T4 VHH domain that binds 5T4, the first Fc polypeptide of the heterodimeric Fc region, the linker, the VH or VL domain of the anti-CD3 antibody or antigen binding fragment and a second 5T4 VHH domain that binds 5T4; and

the second component comprises the IRBR and comprises in order of N-terminus to C-terminus the second Fc polypeptide of the heterodimeric Fc region, the linker, optionally the same linker as present in the first component, the other of the VH or VL domain of the anti-CD3 antibody or antigen binding fragment, wherein the IRBR is positioned amino-terminally relative to the Fc region and/or carboxy-terminally relative to the anti-CD3 antibody or antigen-binding fragment of the second component.

149. The multispecific polypeptide construct of any of embodiments 145-148, wherein the at least one IRBR is or comprises the extracellular domain or binding fragment thereof of the native cognate binding partner of the inhibitory receptor, or a variant thereof that exhibits binding activity to the inhibitory receptor.

150. The multispecific polypeptide construct of any of embodiments 139-142, wherein the at least one IRBR is an antibody or antigen-binding fragment thereof selected from the group consisting of a Fab fragment, a F(ab')2 fragment, an Fv fragment, a scFv, a scAb, a dAb, a single domain heavy chain antibody, and a single domain light chain antibody.

151. The multispecific polypeptide construct of embodiment 150, wherein the antibody or antigen-binding fragment thereof is a Fv, a scFv, a Fab, a single domain antibody (sdAb).

152. The multispecific polypeptide construct of embodiment 150 or embodiment 151, wherein the antibody or antigen-binding fragment is an sdAb.

153. The multispecific polypeptide construct of embodiment 152, wherein the sdAb is a human or humanized sdAb.

153. The multispecific polypeptide construct of any of embodiments 145-153, wherein the at least one IRBR binds a inhibitory receptor selected from among PD-1, CTLA-4, TIGIT, VISTA and TIM3.

155. The multispecific polypeptide construct of any of embodiments 145-154, wherein the at least one IRBR binds PD-1.

156. The multispecific polypeptide construct of any of embodiments 145-155, wherein:

the first component comprises in order of N-terminus to C-terminus a first 5T4 VHH domain that binds 5T4, the first Fc polypeptide of the heterodimeric Fc region, the linker, the VH or VL domain of the anti-CD3 antibody or antigen binding fragment and a second 5T4 VHH domain that binds 5T4; and

the second polypeptide comprises in order of N-terminus to C-terminus one of the IRBR or the CRBR, the second Fc polypeptide of the heterodimeric Fc region, the linker, optionally the same linker as present in the first component, the other of the VH or VL domain of the anti-CD3 antibody or antigen binding fragment, and the other of the CRBR or IRBR.

157. The multispecific polypeptide construct of any of embodiments 63-156, wherein the linker is a peptide or polypeptide linker, optionally wherein the linker is 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 amino acids in length

158. The multispecific polypeptide construct of any of embodiments 63-157, wherein the linker is a non-cleavable linker

159. The multispecific polypeptide construct of embodiment 158, wherein the non-cleavable linker is or comprises GG

160. The multispecific polypeptide construct of embodiment 158, wherein the non-cleavable linker comprises GS, GGS, GGGGS (SEQ ID NO:125), GGGGGS (SEQ ID NO:126) or combinations thereof.

161. The multispecific polypeptide construct of any of embodiments 63-158, wherein the linker is or comprises the sequence GGGGGSGGGGGSGGGGGS (SEQ ID NO:127)

162. The multispecific polypeptide construct of any of embodiments 63-157, wherein the linker is a cleavable linker.

163. The multispecific polypeptide construct of embodiment 162, wherein the cleavable linker is a polypeptide that functions as a substrate for a protease.

164. The multispecific polypeptide construct of embodiment 163, wherein the protease is produced by an immune effector cell, by a tumor cell, or by cells present in the tumor microenvironment.

165. The multispecific polypeptide construct of embodiment 163 or embodiment 164, wherein the protease is produced by an immune effector cell and the immune effector cell is an activated T cell, a natural killer (NK) cell, or an NK T cell.

166. The multispecific polypeptide construct of any of embodiments 163-165, wherein the protease is selected from among matriptase, a matrix metalloprotease (MMP), granzyme B, and combinations thereof.

167. The multispecific polypeptide construct of embodiment 166, wherein the protease is granzyme B.

168. The multispecific polypeptide construct of any of embodiments 163-167, wherein the cleavable linker comprises the amino acid sequence GGSGGGGIEPDIGGSGGS (SEQ ID NO:171).

169. An isolated single domain antibody (sdAb) that binds 5T4, comprising a complementarity determining region 1 (CDR1) comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 86-87 and 288-292, 296, and 297; a complementarity determining region 2 (CDR2) comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 88-99, 298, and 299; and a complementarity determining region 3 (CDR3) comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 100-102, 300, 301, and 303.

170. The isolated single domain antibody (sdAb) of embodiment 169, wherein the at least one 5T4 VHH domain binds to an epitope in human 5T4 but does not exhibit crossreactive binding to mouse 5T4.

171. The isolated single domain antibody (sdAb) of embodiment 169 or 170, wherein the at least one 5T4 VHH domain binds to amino acid residues between amino acids 60 and 112 of SEQ ID NO:382.

172. The isolated single domain antibody (sdAb) of any of embodiments 169-171, wherein the at least one 5T4 VHH domain binds to amino acid residues between amino acids 173 and 224 of SEQ ID NO:382

173. The isolated single domain antibody of embodiment 169, comprising the amino acid sequence set forth in any of SEQ ID NOS: 245-253, 255-287, 294, 295, 302, and 360 or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to any of SEQ ID NOS: 245-253, 255-287, 294, 295, 302, and 360 and binds 5T4.

174. The isolated single domain antibody of any of embodiments 169-173, wherein the single domain antibody comprises the sequence set forth in (i) SEQ ID NO: 245, (ii) a humanized variant of SEQ ID NO: 245, or (iii) a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 245, and binds 5T4.

175. The isolated single domain antibody of any of embodiments 169-174, wherein the sdAb comprises a CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 288 and 289; a CDR2 comprising an amino acid sequence set forth in SEQ ID NO: 88; and a CDR3 comprising an amino acid sequence set forth in SEQ ID NO: 100.

176. The isolated single domain antibody of any of embodiments 169-175, wherein the sdAb comprises a CDR1, CDR2 and CDR3 set forth in SEQ ID NOS: 288, 88, and 100, respectively; or SEQ ID NOS: 289, 88, and 100, respectively.

177. The isolated single domain antibody of any of embodiments 169-176, wherein the sdAb comprises the sequence of amino acids set forth in any one of SEQ ID NOs: 246-253 and 360 or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 246-253 and 360, and binds 5T4.

178. The isolated single domain antibody of any of embodiments 169-177, wherein the sdAb comprises the sequence of amino acids set forth in any one of SEQ ID NOS: 246-253 and 360.

179. The isolated single domain antibody of any of embodiments 169-173, wherein the sdAb comprises the sequence set forth in (i) SEQ ID NO: 255, (ii) a humanized variant of SEQ ID NO: 255, or (iii) a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 255, and binds 5T4.

180. The isolated single domain antibody of any of embodiments 169-173 and 179, wherein the sdAb comprises a CDR1 comprising an amino acid sequence set forth in SEQ ID NO: 86, 290-292; a CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 89-94; and a CDR3 comprising an amino acid sequence set forth in SEQ ID NO: 101.

181. The isolated single domain antibody of any of embodiments 169-173, 179, and 180, wherein the sdAb comprises a CDR1, CDR2 and CDR3 set forth in SEQ ID NOS: 290, 89, and 101, respectively; SEQ ID NOS: 290, 90, and 101, respectively; SEQ ID NOS: 290, 91, and 101, respectively; SEQ ID NOS: 290, 92, and 101, respectively; SEQ ID NOS: 290, 93, and 101, respectively; SEQ ID NOS: 290, 94, and 101, respectively; SEQ ID NOS: 291, 94, and 101, respectively; SEQ ID NOS: 292, 94, and 101, respectively; or SEQ ID NOS: 86, 94, and 101, respectively.

182. The isolated single domain antibody of any of embodiments 169-173 and 179-181, wherein the sdAb comprises the sequence of amino acids set forth in any one of SEQ ID NOs: 256-275 or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 256-275, and binds 5T4.

183. The isolated single domain antibody of any of embodiments 169-173 and 179-182, wherein the sdAb comprises the sequence of amino acids set forth in any one of SEQ ID NOS: 256-275.

184. The isolated single domain antibody of any of embodiments 169-173, wherein the sdAb comprises the sequence set forth in (i) SEQ ID NO: 276 (ii) a humanized variant of SEQ ID NO: 276, or

(iii) a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 276, and binds 5T4.

185. The isolated single domain antibody of any of embodiments 169-173 or embodiment 184, wherein the sdAb comprises a CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 86 and 87; a CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 95-99; and a CDR3 comprising an amino acid sequence set forth in SEQ ID NO: 102.

186. The isolated single domain antibody of any of embodiments 169-173, 184 and 185, wherein the sdAb comprises a CDR1, CDR2 and CDR3 set forth in SEQ ID NOS: 87, 95, and 102, respectively; SEQ ID NOS: 87, 96, and 102, respectively; SEQ ID NOS: 87, 97, and 102, respectively; SEQ ID NOS: 87, 98, and 102, respectively; SEQ ID NOS: 87, 99, and 102, respectively; or SEQ ID NOS: 86, 98, and 102, respectively.

187. The isolated single domain antibody of any of embodiments 169-173 and 184-186, wherein the sdAb comprises the sequence of amino acids set forth in any one of SEQ ID NOs: 277-287 or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 277-287, and binds 5T4.

188. The isolated single domain antibody of any of embodiments 169-173 and 184-187, wherein the sdAb comprises the sequence of amino acids set forth in any one of SEQ ID NOS: 277-287.

189. The isolated single domain antibody of any of embodiments 169-173, wherein the sdAb comprises the sequence set forth in (i) SEQ ID NO: 294 (ii) a humanized variant of SEQ ID NO: 294, or (iii) a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 294, and binds 5T4.

190. The isolated single domain antibody of embodiment 169-173 or 189, wherein the sdAb comprises a CDR1 comprising an amino acid sequence set forth in SEQ ID NO: 296; a CDR2 comprising an amino acid sequence set forth in SEQ ID NO: 298; and a CDR3 comprising an amino acid sequence set forth in SEQ ID NO: 300.

191. The isolated single domain antibody of embodiment 169-173 or 189, wherein the sdAb comprises a CDR3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 300, 301, and 303.

192. The isolated single domain antibody of embodiment 169-173 or 189, wherein the sdAb comprises:

a CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 288, 296, and 297; and/or

a CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 88, 298, and 299.

193. The isolated single domain antibody of any of embodiments 169-173, wherein the sdAb comprises the sequence set forth in (i) SEQ ID NO:295 (ii) a humanized variant of SEQ ID NO:295, or (iii) a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO:295, and binds 5T4.

194. The isolated single domain antibody of any of embodiments 169-173 and 193, wherein the sdAb comprises a CDR1 comprising an amino acid sequence set forth in SEQ ID NO: 297; a CDR2 comprising an amino acid sequence set forth in SEQ ID NO: 299; and a CDR3 comprising an amino acid sequence set forth in SEQ ID NO: 301.

195. The isolated single domain antibody of any of embodiments 169-173, wherein the sdAb comprises the sequence set forth in (i) SEQ ID NO:302 (ii) a humanized variant of SEQ ID NO:302, or (iii) a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO:302, and binds 5T4.

196. The isolated single domain antibody of any of embodiments 169-173 and 193, wherein the sdAb comprises a CDR1 comprising an amino acid sequence set forth in SEQ ID NO: 288; a CDR2 comprising an amino acid sequence set forth in SEQ ID NO: 88; and a CDR3 comprising an amino acid sequence set forth in SEQ ID NO: 303.

197. The isolated single domain antibody of any of embodiments 169-173, wherein the sdAb comprises the sequence set forth in (i) SEQ ID NO: 294, 295, or 302 (ii) a humanized variant of SEQ ID NO: 294, 295, or 302, or (iii) a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 294, 295, or 302, and binds 5T4.

198. The isolated single domain antibody of any of embodiments 169-173 and 197, wherein the sdAb comprises a CDR1, CDR2 and CDR3 set forth in SEQ ID NOS: 296, 298, and 300, respectively; SEQ ID NOS:297, 299, and 301, respectively; or SEQ ID NOS:288, 88, and 303, respectively.

199. A polynucleotide(s) encoding the 5T4-binding polypeptide construct of any of embodiments 1-62.

200. A polynucleotide(s) encoding the multispecific polypeptide construct of any of embodiments 63-168.

201. A polynucleotide, comprising a first nucleic acid sequence encoding a first polypeptide of a multispecific construct of any of embodiments 63-168 and a second nucleic acid sequence encoding a second polypeptide of the multispecific construct, wherein the first and second nucleic acid sequence are separated by an internal ribosome entry site (IRES), or a nucleic acid encoding a self-cleaving peptide or a peptide that causes ribosome skipping.

202. The polynucleotide of embodiment 201, wherein the first nucleic acid sequence and second nucleic acid sequence are operably linked to the same promoter.

203. The polynucleotide of embodiment 201 or 202, wherein the nucleic acid encoding a self-cleaving peptide or a peptide that causes ribosome skipping is selected from a T2A, a P2A, a E2A or a F2A.

204. A polynucleotide encoding the single domain antibody of any of embodiments 169-198.

205. A vector, comprising the polynucleotide of any of embodiments 199-204.

206. The vector of embodiment 205 that is an expression vector.

207. The vector of embodiment 205 or embodiment 206 that is a viral vector or a eukaryotic vector, optionally wherein the eukaryotic vector is a mammalian vector.

208. A cell, comprising polynucleotide or polynucleotides of-any of embodiments 199-204, or a vector or vectors of any of embodiments 205-207.

209. The cell of embodiment 208, wherein the cell is recombinant or isolated.

210. The cell of embodiment 209, wherein the cell is a mammalian cell.

211. A method of producing a polypeptide, the method comprising introducing into a cell a polynucleotide or polynucleotides of any of embodiments 199-204 or a vector or vectors of any of embodiments 205-207 and culturing the cell under conditions to produce the multispecific polypeptide construct.

212. The method of embodiment 211, further comprising isolating or purifying the polypeptide from the cell.

213. A polypeptide produced by the method of embodiment 211 or embodiment 212.

214. An engineered immune cell, comprising a chimeric antigen receptor comprising:

an extracellular domain comprising the single domain antibody of any of embodiments 169-198;

a transmembrane domain; and

an intracellular signaling domain.

215. The engineered immune cell of embodiment 214, wherein the cell is a lymphocyte.

216. The engineered immune cell of embodiment 214 or embodiment 215, wherein the cell is a T cell or a natural killer (NK) cell.

217. The engineered immune cell of any of embodiments 214-216, wherein the intracellular signaling domain comprises an immunoreceptor tyrosine-based activation motif (ITAM) signaling domain.

218. The engineered immune cell of any of embodiments 214-217, wherein the intracellular signaling domain is or comprises a CD3zeta signaling domain, optionally a human CD3zeta signaling domain.

219. The engineered immune cell of embodiment 214-218, wherein the intracellular signaling domain further comprises a signaling domain of a costimulatory molecule.

220. The engineered immune cell of embodiment 219, wherein the costimulatory molecule is CD28, ICOS, 41BB or OX40, optionally a human CD28, a human ICOS, a human 41BB or a human OX40.

221. A pharmaceutical composition comprising the 5T4-binding polypeptide construct of any of embodiments 1-62, the multispecific polypeptide construct of any of embodiments 63-168, the single domain antibody of any of embodiments 169-198 or the engineered immune cell of any of embodiments 214-220.

222. The pharmaceutical composition of embodiment 221, comprising a pharmaceutically acceptable carrier.

223. The pharmaceutical composition of embodiment 221 or embodiment 222 that is sterile.

224. A method of stimulating or inducing an immune response in a subject, the method comprising administering, to a subject in need thereof, the 5T4-binding polypeptide construct of any of embodiments 1-62, the multispecific polypeptide construct of any of embodiments 63-168, the single domain antibody of any of embodiments 169-198 or the engineered immune cell of any of embodiments 214-220 or a pharmaceutical composition of embodiment 221-223.

225. The method of embodiment 224, wherein the immune response is increased against a tumor or cancer, optionally a tumor or a cancer that expresses 5T4.

226. The method of embodiment 224 or embodiment 225, wherein the method treats a disease or condition in the subject.

227. A method of treating a disease or condition in a subject, the method comprising administering, to a subject in need thereof, a therapeutically effective amount of the 5T4-binding polypeptide construct of any of embodiments 1-59, the multispecific polypeptide construct of any of embodiments 60-161, the single domain antibody of any of embodiments 162-188 or the engineered immune cell of any of embodiments 214-220 or a pharmaceutical composition of embodiment 221-223.

228. The method of embodiment 226 or embodiment 227, wherein the disease or condition is a tumor or a cancer.

229. The method of any of embodiments 224-228, wherein said subject is a human.

VIII. EXAMPLES

[0620] The following examples are included for illustrative purposes only and are not intended to limit the scope of the invention.

Example 1: Generation of 5T4 sdAb

[0621] Single domain antibodies targeting human 5T4 were generated via immunization of llamas and alpaca. Llamas and alpacas were immunized with a recombinant version of the human 5T4

extracellular domain (ECD; amino acids 32-355 of human 5T4, e.g. UniProt No. Q13641) set forth in SEQ ID NO:362 and as follows:

SSPTSSASSFSSSAPFLASAVSAQPPLPDQCPALCECSEAARTVKCVNRNLTEVPTDLPAYVRN
LFLTGNQLAVLPAGAFARRPPLAELAALNLSGSRLDEVRAGAFEHLPSLRQLDLSHNPLADLS
PFAFSGSNASVSAPSPLVELILNHIVPPEDERQNRSFEGMVVAALLAGRALQGLRRLELASNH
FLYLPRDVLAQLPSLRHLDLSNNSLVSLTYVSFRNLTHLESLHLEDNALKVLHNGTLAELQGL
PHIRVFLDNNPWVCDCHMADMVTWLKETEVVQGKDRLTCAYPEKMRNRVLLELNSADLD
CDPILPPSLQTS

[0622] Following the development of specific anti-5T4 antibody titers, llama/alpaca peripheral blood mononuclear cells (PBMCs) were isolated from 500 mL of blood from the immunized animal and total mRNA was isolated using the Qiagen RNeasy Maxi Kit and subsequently converted to first strand cDNA using Thermo Superscript IV Reverse Transcriptase and oligo-dT priming. Single domain antibody (sdAb; also called VHH) sequences were specifically amplified via PCR using the cDNA as template and cloned into a yeast surface display vector as sdAb-Fc-AGA2 fusion proteins. The Fc was a human IgG1 Fc (set forth in SEQ ID NO:8).

[0623] Yeast libraries displaying these sdAbs were enriched using recombinant forms of the 5T4 ECD via magnetic bead isolation followed by fluorescence activated cell sorting (FACS). Sorted yeast were plated out and isolated colonies were picked into 96-well blocks and grown in media that switched the expression from surface displayed sdAb-Fc to secretion into the media. Supernatants from the 96-well yeast secretion cultures were applied to Ovcar-5 (5T4 positive) or CCRF-CEM cells (5T4 negative), washed, treated with fluorophore labelled anti-human Fc secondary antibody, and analyzed by 96-well flow cytometry.

[0624] Binders to 5T4 positive cells and not to 5T4 negative cells were cloned into mammalian expression vectors as sdAb-Fcs, expressed by transient transfection in HEK293 freestyle cells (293F cells) or CHO cells using polyethylenimine. Recombinant protein secreted into the supernatant was collected after 3-7 days and purified by protein A chromatography.

[0625] Exemplary identified sdAbs are set forth in **Table E1A**. In some cases, the sdAbs can include a flexible linker (e.g. GG) for linkage to another polypeptide, such as an Fc or another sdAb.

Table E1A: 5T4 sdAbs

Clone name	CDR1	SEQ ID NO	CDR2	SEQ ID NO	CDR3	SEQ ID NO	VHH SEQ ID NO
L12E9	RRPFSSKTMA	288	AVRWIGGATR	88	GQAWGTKFTDYSD	100	245
L14B5	ERPFGTYAMG	290	AVSRNGGASQ	89	RSAAYSRSSEVYTGKDEYYY	101	255
L16G10	GRPFSSSAMG	87	AVSRNGGSSY	95	RSAAYSRSSETYTEKHDYTY	102	276

Table E1A: 5T4 sdAbs							
Clone name	**CDR1**	**SEQ ID NO**	**CDR2**	**SEQ ID NO**	**CDR3**	**SEQ ID NO**	**VHH SEQ ID NO**
7E1	GRTRSLRTMA	296	AISWRSDSTY	298	GGGWLATTPDEYTY	300	294
14F4	GVTWNSYTMA	297	AIRWTVDTTY	299	GRKWPKADDY	301	295
4D3	RRPFSSKTMA	288	AVRWIGGATR	88	GQTWGTKFTDYSD	303	302

Example 2: Binding of sdAb to 5T4 expressing cells by flow cytometry

[0626] Specificity and relative affinity were assessed for purified sdAb-Fcs on 5T4-expressing cells. Binding of 5T4-sdAb-Fc fusion proteins was assessed by flow cytometry using 5T4-expressing cells. A titration series of the fusion protein was incubated with the 5T4-expressing cell lines (approximately 2.5 x 10^4 to 5 x 10^4 cells/well) for 30 minutes at 4 degrees Celsius in FACS Buffer (PBS 1% BSA, 0.1% NaN_3 pH 7.4) in 96 well plates. Following three wash steps in FACS buffer, an APC-conjugated anti-human Fcγ specific secondary antibody (Jackson ImmunoResearch) was added and incubated for 30 minutes at 4 degrees Celsius. Following three additional wash steps in FACS buffer bound antibody was detected via flow cytometry (IQue Intellicyte).

[0627] **FIGS. 1A-1B** set forth results for exemplary 5T4 sdAbs, namely 4D3 (SEQ ID NO: 302), 12E9 (SEQ ID NO: 245), 7E1 (SEQ ID NO: 294), 14B5 (SEQ ID NO: 255), 16G10 (SEQ ID NO: 276), 14F4 (SEQ ID NO: 295).

[0628] An epitope binning assay for binding to 5T4 were carried out using exemplary generated sdAbs to test antibodies pairwise for blocking of another's binding to the epitope of an antigen. Results are set forth in **Table E1B**:

Table E1B: Epitope Binning	
12E9	BIN1
7E1	BIN2
14B5	BIN2
16G10	BIN2
4D3	BIN1
14F4	BIN1

Example 3: Humanization of camelid derived 5T4 sdAb

[0629] Exemplary camelid derived 5T4 sdAbs, 12E9, 14B5 and 16G10, were humanized using the human VH3-23 germline as scaffold. Camelid residues that contribute to solubility, specificity, stability and/or affinity remained unmodified. In addition, all humanized variant contained the modification of Leu11Glu (L11E) and the carboxy-terminal modifications of Ser112Lys (S112K) and Ser113Pro (S113P) as these are known prevent or reduce the recognition of pre-existing ADA directed toward sdAbs (as described in US20160207981).

[0630] Table E2 sets forth exemplary 5T4 sdAbs humanized variants.

Table E2: 5T4 sdAbs Humanized Variants							
Clone name	**CDR1**	**SEQ ID NO**	**CDR2**	**SEQ ID NO**	**CDR3**	**SEQ ID NO**	**VHH SEQ ID NO**
L12E9 Humanized Variants							
hz12E9v1	RRPFSSKTMA	288	AVRWIGGATR	88	GQAWGTKFTDYSD	100	246
hz12E9v2	RRPFSSKTMA	288	AVRWIGGATR	88	GQAWGTKFTDYSD	100	247
hz12E9v3	RRPFSSKTMA	288	AVRWIGGATR	88	GQAWGTKFTDYSD	100	248
hz12E9v4	RRPFSSKTMA	288	AVRWIGGATR	88	GQAWGTKFTDYSD	100	249
hz12E9v5	RRPFSSKTMA	288	AVRWIGGATR	88	GQAWGTKFTDYSD	100	250
hz12E9v6	RRPFSSKTMA	288	AVRWIGGATR	88	GQAWGTKFTDYSD	100	251
hz12E9v7	RRPFSSKTMA	288	AVRWIGGATR	88	GQAWGTKFTDYSD	100	252
hz12E9v8	RRPFSSKTMA	288	AVRWIGGATR	88	GQAWGTKFTDYSD	100	253
hz12E9v9	GRPFSSKTMA	289	AVRWIGGATR	88	GQAWGTKFTDYSD	100	254 360
L14B5 Humanized Variants							
hz14B5v1	ERPFGTYAMG	290	AVSRNGGASQ	89	RSAAYSRSSEVYTGKDE YYY	101	256
hz14B5v2	ERPFGTYAMG	290	AVSRNGGASQ	89	RSAAYSRSSEVYTGKDE YYY	101	257
hz14B5v3	ERPFGTYAMG	290	AVSRNGGASQ	89	RSAAYSRSSEVYTGKDE YYY	101	258
hz14B5v4	ERPFGTYAMG	290	AVSRNGGASQ	89	RSAAYSRSSEVYTGKDE YYY	101	259
hz14B5v5	ERPFGTYAMG	290	AVSRNAGASQ	90	RSAAYSRSSEVYTGKDE YYY	101	260
hz14B5v6	ERPFGTYAMG	290	AVSRNTGASQ	91	RSAAYSRSSEVYTGKDE YYY	101	261
hz14B5v7	ERPFGTYAMG	290	AVSRQGGASQ	92	RSAAYSRSSEVYTGKDE YYY	101	262
hz14B5v8	ERPFGTYAMG	290	AVSRGGGASQ	93	RSAAYSRSSEVYTGKDE YYY	101	263
hz14B5v9	ERPFGTYAMG	290	AVSRNGGASQ	89	RSAAYSRSSEVYTGKDE YYY	101	264
hz14B5v10	ERPFGTYAMG	290	AVSRNGGASQ	89	RSAAYSRSSEVYTGKDE YYY	101	265
hz14B5v11	ERPFGTYAMG	290	AVSRNAGASQ	90	RSAAYSRSSEVYTGKDE YYY	101	266
hz14B5v12	ERPFGTYAMG	290	AVSRNAGASQ	90	RSAAYSRSSEVYTGKDE YYY	101	267
hz14B5v13	ERPFGTYAMG	290	AVSRNAGASQ	90	RSAAYSRSSEVYTGKDE YYY	101	268
hz14B5v14	ERPFGTYAMG	290	AVSRNAGASQ	90	RSAAYSRSSEVYTGKDE YYY	101	269
hz14B5v15	ERPFGTYAMG	290	AVSRNAGASY	94	RSAAYSRSSEVYTGKDE YYY	101	270
hz14B5v16	ERPFGTYAMG	290	AVSRNAGASQ	90	RSAAYSRSSEVYTGKDE YYY	101	271
hz14B5v17	ERPFGTYAMG	290	AVSRNAGASY	94	RSAAYSRSSEVYTGKDE YYY	101	272

Table E2: 5T4 sdAbs Humanized Variants							
Clone name	**CDR1**	**SEQ ID NO**	**CDR2**	**SEQ ID NO**	**CDR3**	**SEQ ID NO**	**VHH SEQ ID NO**
hz14B5v18	ERPFSSYAMG	291	AVSRNAGASY	94	RSAAYSRSSEVYTGKDEYYY	101	273
hz14B5v19	GRPFGTYAMG	292	AVSRNAGASY	94	RSAAYSRSSEVYTGKDEYYY	101	274
hz14B5v20	GRPFSSYAMG	86	AVSRNAGASY	94	RSAAYSRSSEVYTGKDEYYY	101	275
L16G10 Humanized Variants							
hz16G10v1	GRPFSSSAMG	87	AVSRNGGSSY	95	RSAAYSRSSETYTEKHDYTY	102	277
hz16G10v2	GRPFSSSAMG	87	AVSRNGGSSY	95	RSAAYSRSSETYTEKHDYTY	102	278
hz16G10v3	GRPFSSSAMG	87	AVSRNGGSSY	95	RSAAYSRSSETYTEKHDYTY	102	279
hz16G10v4	GRPFSSSAMG	87	AVSRQGGSSY	96	RSAAYSRSSETYTEKHDYTY	102	280
hz16G10v5	GRPFSSSAMG	87	AVSRGGGSSY	97	RSAAYSRSSETYTEKHDYTY	102	281
hz16G10v6	GRPFSSSAMG	87	AVSRNAGSSY	98	RSAAYSRSSETYTEKHDYTY	102	282
hz16G10v7	GRPFSSSAMG	87	AVSRNTGSSY	99	RSAAYSRSSETYTEKHDYTY	102	283
hz16G10v8	GRPFSSSAMG	87	AVSRNGGSSY	95	RSAAYSRSSETYTEKHDYTY	102	284
hz16G10v9	GRPFSSSAMG	87	AVSRNGGSSY	95	RSAAYSRSSETYTEKHDYTY	102	285
hz16G10v10	GRPFSSSAMG	87	AVSRNAGSSY	98	RSAAYSRSSETYTEKHDYTY	102	286
hz16G10v11	GRPFSSYAMG	86	AVSRNAGSSY	98	RSAAYSRSSETYTEKHDYTY	102	287

[0631] Humanized variant of the 5T4 sdAbs were tested for their ability to bind 5T4 expressing cells substantially as described in Example 2, and binding was compared to the parental sdAb. 5T4 expressing T47D cells were used in these studies. Results are shown in **FIGS. 2A-2F**, which confirm binding of the humanized variants. In some cases, binding was increased compared to the parental sdAb.

Example 4: Method of producing 5T4-targeted constrained CD3 binding proteins

[0632] Multispecific polypeptide constructs were generated containing a disulfide stabilized anti-CD3 Fv binding region that exhibits constrained CD3 binding, a heterodimeric Fc domain, and one or more 5T4 sdAb described above positioned amino-terminally relative to the Fc region and/or carboxy-terminally relative to the CD3 binding region. The multispecific constructs were generated in various configurations, as shown in **FIG. 3A-3E**. In some cases, the constrained CD3 engaging constructs

contained at least one co-stimulatory receptor binding region (CRBR) positioned amino-terminally relative to the Fc region and/or carboxy-terminally relative to the CD3 binding region.

[0633] In the exemplary constructs, polynucleotides encoding at least a first polypeptide chain and a second polypeptide chain of the heterodimeric multispecific polypeptide construct were generated and cloned into a plasmid for expression. The first polypeptide chain generally included in order, from the N-terminus to C-terminus, an Fc hole polypeptide (e.g. set forth in SEQ ID NO:112, or in some cases SEQ ID NO:114); a cleavable or a non-cleavable linker, such as one containing one or more substrate recognition sites for a protease; and a variable light (VL) domain of a dsFv anti-CD3 antibody (e.g. set forth in SEQ ID NO:75). The second polypeptide chain generally included in order, from the N-terminus to C-terminus, an Fc knob polypeptide (e.g. set forth in SEQ ID NO: 105, or in some cases SEQ ID NO:109); the same cleavable linker or the same non-cleavable linker as the first polypeptide chain; and a variable heavy domain of a dsFv anti-CD3 antibody (e.g. set forth in SEQ ID NO:47). The constructs were generated with the exemplary non-cleavable linker, GGGGGSGGGGGSGGGGGS (SEQ ID NO:127), or the exemplary cleavable linker, GGSGGGG**IEPD**IGGSGGS (SEQ ID NO:171) containing a substrate recognition site for granzyme B. One or both of the polypeptide chains additionally encoded one or more 5T4 sdAb amino terminal to the Fc domain and/or carboxy terminal to the CD3 binding region, and/or a co-stimulatory receptor binding domain amino terminal to the Fc domain and/or carboxy terminal to the CD3 binding region, in various configurations.

[0634] Separate plasmids encoding each chain of a heterodimeric constrained CD3 binding protein were transiently transfected at an equimolar ratio into mammalian cells (either HEK293 or CHO) using polyethylenimine. Recombinant protein secreted into the supernatant was collected after 3-7 days, and purified by protein A chromatography, followed by either preparative size exclusion chromatography (SEC) or flow-through hydrophobic interaction chromatography (HIC). Heterodimeric protein was selectively purified owing to a mutation designed into one chain of the heterodimeric Fc at position I253R or H435R (usually the hole-Fc) such that it did not bind protein A. The second chromatography step on SEC (AKTA with Superdex-200 resin) or FT-HIC (AKTA with butyl/phenyl sepharose) was used to remove undesired cross-paired species containing two heterodimeric Fcs that were more hydrophobic and twice the expected molecular weight.

[0635] The method favored production of heterodimeric multispecific polypeptide constructs, containing properly paired species of heterodimeric Fc and the disulfide stabilized anti-CD3 Fv as described (e.g. anti-CD3 VH with the mutation G44C as set forth in SEQ ID NO: 47 and VL with the mutation G100C as set forth in SEQ ID NO: 293). Purified heterodimeric constrained CD3 binding protein was stable and did not accumulate cross-paired species upon prolonged incubation at 4°C or increased protein concentration.

[0636] **Table E3** sets forth exemplary generated constrained multispecific constructs. The 5T4 binding domains of cx3315 are FAB positioned N and C-terminally within the constrained CD3 engaging construct.

Table E3: 5T4 VHH Constrained Multispecific Constructs

Construct #	Chain	N-term sdAb (Target) (SEQ ID NO)	Fc	Linker	CD3 Binding Domain	C-term sdAb (Target) (SEQ ID NO)
cx3253	1	12E9 (5T4) (245)	hFc-Knob	IEPDI	Con1 VH	7E1 (5T4) (294)
	2	-	hFc-Hole	IEPDI	Con1 VL	RH3v5-1 (41BB) (210)
cx3497	1	12E9 (5T4) (245)	hFc-Knob	IEPDI	Con1 VH	16G10 (5T4) (276)
	2	-	hFc-Hole	IEPDI	Con1 VL	RH3v5-1 (41BB) (210)
cx3499	1	12E9 (5T4) (245)	hFc-Knob	IEPDI	Con1 VH	14B5 (5T4) (255)
	2	-	hFc-Hole	IEPDI	Con1 VL	RH3v5-1 (41BB) (210)
cx4224	1	14F4 (5T4) (295)	hFc-Knob	IEPDI	Con1 VH	7E1 (5T4) (294)
	2	-	hFc-Hole	IEPDI	Con1 VL	RH3v5-1 (41BB) (210)
cx3262	1	12E9 (5T4) (245)	hFc-Knob	IEPDI	Con1 VH	7E1 (5T4) (294)
	2	-	hFc-Hole	IEPDI	Con1 VL	-
cx4910	1	hz12E9v4 (5T4) (249)	xELL-Knob	(G5S)3	Con1 VH	hz14B5v15 (5T4) (270)
	2	-	xELL-Hole	(G5S)3	Con1 VL	RH3v5-1 (41BB) (210)
cx4912	1	hz14B5v15 (5T4) (270)	xELL-Knob	(G5S)3	Con1 VH	hz12E9v4 (5T4) (249)
	2	-	xELL-Hole	(G5S)3	Con1 VL	RH3v5-1 (41BB) (210)
cx4911	1	hz12E9v4 (5T4) (249)	xELL-Knob	(G5S)3	Con1 VH	hz14B5v15 (5T4) (270)
	2	-	xELL-Hole	(G5S)3	Con1 VL	-
cx4913	1	hz14B5v15 (5T4) (270)	xELL-Knob	(G5S)3	Con1 VH	hz12E9v4 (5T4) (249)
	2	-	xELL-Hole	(G5S)3	Con1 VL	-
cx3546	1	12E9 (5T4) (245)	hFc-Knob	IEPDI	Con1 VH	14B5 (5T4) (255)
	2	-	hFc-Hole	IEPDI	Con1 VL	-
cx3547	1	12E9 (5T4) (245)	hFc-Knob	IEPDI	Con1 VH	16G10 (5T4) (276)
	2	-	hFc-Hole	IEPDI	Con1 VL	-
cx3264	1	4D3 (5T4) (302)	hFc-Knob	IEPDI	Con1 VH	4D3 (5T4) (302)
	2	-	hFc-Hole	IEPDI	Con2 VL	RH3v5-1 (41BB) (210)

cx3265	1	4D3 (5T4) (302)	hFc-Knob	IEPDI	Con1 VH	4D3 (5T4) (302)
	2	-	hFc-Hole	IEPDI	Con1 VL	-
cx5185	1	hz12E9v9 (5T4) (360)	xELL-Knob	(G5S)3	Con1 VH	hz16G10v11(5T4) (287)
	2	-	xELL-Hole	(G5S)3	Con1 VL	RH3v5-1 (41BB) (210)
cx7859	1	hz12E9v9 (5T4) (360)	xELL-Knob	(G5S)3	Con1 VH	hz12E9v9 (5T4) (360)
	2	-	xELL-Hole	(G5S)3	Con1 VL	RH3v5-1 (41BB) (210)
cx7860	1	hz16G10v11(5T4) (287)	xELL-Knob	(G5S)3	Con1 VH	hz16G10v11(5T4) (287)
	2	-	xELL-Hole	(G5S)3	Con1 VL	RH3v5-1 (41BB) (210)

Example 5: Comparison of Binding to Isolated Primary T-cells vs. 5T4-Expressing Cancer Cells

[0637] Binding of exemplary 5T4-targeting constrained CD3 engaging constructs set forth in **Table E3** to CD3 on the surface of primary T cells and 5T4 expressing cells (Ovcar-5) was assessed by flow cytometry. The T cells were primary T-cells that were negatively enriched from PBMCs isolated from healthy human donor leukopaks. Bound construct was detected with fluorophore-conjugated secondary antibodies specific for the human Fc (anti-human IgG APC secondary antibody) and binding was measured by flow cytometry. Cells incubated with secondary antibody only served as negative controls. Cells incubated with secondary antibody only served as negative controls.

The results, from flow cytometry histograms displaying normalized cell counts versus fluorescence at 200 nM of each construct, are shown in **FIG. 4A** (cx3253), **FIG. 4B** (cx3264), **FIG. 4C** (cx3497), **FIG. 4D** (cx3499), **FIG. 4E** (cx3547), **FIG. 4F** (cx3546), **FIG. 4G** (cx4224) , **FIG. 4H** (cx4913), **FIG. 4I** (cx3265), **FIG. 4J** (cx4912), **FIG. 4K** (cx3262), **FIG. 4L** (cx4911), and **FIG. 4M** (cx4910). As shown, the representative 5T4-targeting constrained CD3 engagers were found to bind Ovcar-5cells expressing 5T4 (top left and bottom panels of each of the figures). However, as shown in the top right and bottom panels of each of the figures, the same constructs were not able to bind to T cells in isolation.

[0638] **Table E4 s**ummarizes the affinity of exemplary molecules for 5T4 or CD3, as determined from flow cytometry, in these studies.

Table E4: Construct Binding Affinity

Construct #	Affinity 5T4	Affinity CD3
cx3253	0.120 nM	>200 nM
cx3497	0.646 nM	>200 nM
cx3499	0.653 nM	>200 nM
cx4224	0.871 nM	>200 nM
cx3262	0.116 nM	>200 nM

cx4910	0.575 nM	>200 nM
cx4912	0.119 nM	>200 nM
cx4911	0.259 nM	>200 nM
cx4913	0.348 nM	>200 nM
cx3546	0.436 nM	>200 nM
cx3547	0.419 nM	>200 nM
cx3264	0.145 nM	>200 nM
cx3265	0.156 nM	>200 nM

Example 6: Assessment of 5T4-dependent CD3 reporter T cell activation using a reporter assay

[0639] This example describes assessment of the ability of various representative 5T4-targeting constrained CD3 engaging constructs to activate a CD3 NFAT reporter Jurkat cell line in co-culture with 5T4-expressing Ovcar-5 cells or a 5T4 negative cell line, CCFR-CEM. In the reporter assay, engagement of CD3 in the Jurkat cells results in NFAT signaling and production of green fluorescence. These assays were used to demonstrate that while T-cell binding via the CD3-binding domain is restricted or inhibited on isolated T-cells (as shown in Example 5), once the 5T4-targeted constrained CD3 engaging constructs provided herein are bound to a cognate antigen they are capable of engaging T-cells and mediating T-cell activation.

[0640] Antigen targeting constrained CD3 engaging constructs were titrated onto co-cultures of 5T4-expressing target cells, Ovcar-5, and engineered Jurkat cells that expressed NFAT-driven green fluorescence protein (GFP). For reporter assays utilizing adherent target cells, target cells were seeded, allowed to settle at room temperature for uniform distribution, and incubated for several hours at 37°C to permit adherence prior to addition of reporter cells and antigen targeting constrained CD3 engaging constructs. Assay plates were serially imaged using an IncuCyte ZOOM system and CD3 reporter cell activation was determined by measuring total green object integrated intensity. As shown, assessed 5T4-targeted constrained CD3 engaging constructs induced reporter activity in cultures containing 5T4 positive cells **(FIG. 5A, 5C, and 5E)**, but no measurable reporter activity was observed when T cells were cultured with 5T4 negative cell lines (CCRF-CEMs) **(FIG. 5B, 5D, and 5F)**.

Example 7: Assessment of functional activity

[0641] This Example describes the assessment and characterization of exemplary generated 5T4-target constrained CD3 engaging constructs in human primary T cell *in vitro* assays.

A. T cell-mediated cytotoxicity

[0642] Target cells were fluorescently labeled with CytoID red. For cytotoxicity assays utilizing adherent target cells Ovcar-5 or CCRF-CEM, target cells were seeded, allowed to settle at room temperature for uniform distribution, and incubated for several hours at 37°C to permit adherence prior to

addition of other assay components. Primary T cells were negatively enriched from PBMCs isolated from healthy human donor leukopaks and added at a 10:1-40:1 T cell-to-target cell ratio. Green caspase-3/7 reagent was added, which fluorescently labeled nuclear DNA of cells undergoing apoptosis. Antibodies were titrated onto the co-culture and assay plates were serially imaged using an IncuCyte ZOOM system. Target cell death was determined by measuring total red/green overlap object area.

[0643] As shown in **FIG. 6A**, assessed 5T4-targeted constrained CD3 engaging constructs induced potent T-cell-mediated cytotoxicity of 5T4 positive (Ovcar-5) cell line. As shown in **FIG. 6B**, no measurable T cell cytotoxicity was observed against a 5T4 negative cell line (CCRF-CEM), consistent with the capacity to potently induce antigen-dependent T-cell activation. These observations support that the antigen-targeted constrained CD3 format provided herein compared to other CD3 engaging formats, lack or exhibit reduced T-cell binding in isolation while maintaining potent 5T4-dependent T-cell cytotoxicity inducing capacities.

B. T cell activation

[0644] To assess T cell activation, suspension cells from T cell-mediated cytotoxicity assays were collected and stained with a live/dead stain and fluorophore-conjugated anti-CD4, anti-CD8, anti-CD25, anti-CD69, and/or anti-CD71 antibodies. Cells were analyzed using a SONY SA3800 spectral analyzer and CD4+ or CD8+ T cell activation was determined by measuring expression levels of CD25, CD69 or CD71 or percent CD25-, CD69- or CD71-positive.

[0645] **FIGS. 7A-7B** and **FIGS. 7C-7D** depict results for CD25 expression on CD4 T cells and CD8 T cells, respectively, upon culture of T cells with 5T4 positive (Ovcar-5) or 5T4 negative (CCRF-CEM) cell lines in the presence of exemplary constructs. **FIGS. 8A-8B** and **FIGS. 8C-8D** depict results for CD69 expression on CD4 cells and CD8 T cells, respectively, upon culture of T cells with a 5T4 positive (Ovcar-5) or a 5T4 negative (CCRF-CEM) cell line in the presence of exemplary constructs. **FIGS. 9A-9B** and **FIGS. 9C-9D** depict results for CD71 expression on CD4 T cells and CD8 T cells, respectively, upon culture of T cells with a 5T4 positive (Ovcar-5) or a 5T4 negative (CCRF-CEM) cell line in the presence of exemplary constructs. The results showed that the exemplary assessed 5T4-targeting constrained CD3 engaging constructs mediated a dose-dependent 5T4-dependent T-cell activation via CD3 binding, as evidenced by increased expression of CD25, CD69 and CD71 in CD4+ and CD8+ T cells.

[0646] Thus, the results demonstrated that the 5T4-targeting constrained CD3 engaging constructs of the present invention induced potent antigen-dependent activation of both CD4 and CD8 T-cells.

C. T cell cytokine production (ELISA)

[0647] Supernatants from T cell-mediated cytotoxicity assays were analyzed for IFNγ content by sandwich ELISA (BioLegend, USA). The manufacturer's instructions were followed and a standard curve was generated from which cytokine concentration values of supernatant samples were interpolated. Samples that had absorbance values below the lower limit of detection were assigned a cytokine

concentration equal to half that of the lowest standard concentration. As shown in **FIG. 10A**, exemplary assessed 5T4-targeting constrained CD3 engaging constructs elicited IFNγ production by T-cells with 5T4 positive (Ovcar-5) and no measurable IFNγ production was observed with a 5T4 negative cell line (CCRF-CEM).

D. T cell cytokine production (FluoroSpot)

[0648] FluoroSpot membranes were coated with IFNγ and TNFα capture antibodies overnight at 4°C. Membranes were washed with PBS and antibody titrations, target cells, and PBMCs or purified T cells negatively enriched from PBMCs were added. Cells were seeded at a 1:10 ratio of target cell to effector cell. Assay plates were incubated for ~24 h at 37°C and membranes were prepared according to the manufacturer's (C.T.L.) instructions. Membranes were imaged using a CTL-ImmunoSpot S6 Universal Analyzer. Cytokine spot count was measured using uniform exposure time and intensity settings among assay wells. **FIG. 10B** (IFNγ) and **FIG. 10C** (TNFα) depict the ability of exemplary 5T4-targeted constrained CD3 engaging constructs to elicit cytokine production from PBMCs or T cells in 5T4-dependent manner.

Example 8: Assessment of NK Cell Activation

A. Activation of primary NK cells

[0649] To assess the ability of sdAb-IgG1-Fc to activate NK cells in the presence of 5T4-positive cells, co-cultures of A549 cells (2.5 x 10^3 cells/well) and PBMCs (1.55 x 10^6 cells/well; 2.5 x10^4 NK cells/well) were treated with antibody titrations for 4 hours at 37 degrees Celsius. Cells were stained with FITC-anti-CD56 and APC-anti-CD107a. NK activation was determined by measuring CD107a levels on CD56+ cells by flow cytometry. As shown in **FIG. 11A**, 12E9 and 4D3 formatted as sdAb-Fcs are capable of eliciting target dependent NK cell activation.

B. ADCC reporter activation

[0650] To assess the Fc effector function of exemplary 5T4-targeted sdAbs, a Jurkat reporter cell line engineered to stably express CD16a with an NFAT-driven luciferase reporter gene was used. Jurkat reporter cells were seeded (approximately 6 x 10^4 cells/well) in the presence or absence of 5T4-expressing cells (T47D; approximately 3 x 10^4 cells/well). 50 nanomolar of antibody was added to the cells and assay plates were incubated at 37 degrees Celsius for six hours, with a final assay volume 75 microliters. Assay plates were equilibrated to room temperature, 75 microliters of Bio-Glo was added to sample wells, and assay plates were incubated at room temperature for 10 minutes. 100 microliter aliquots were transferred to white 96-well plates and luminescence was measured using a Clariostar microplate reader. **FIG. 11B** depicts the ability of 12E9-Fc, 4F7-Fc, 4F11-Fc, hz12E9v9-Fc, and hz16G10v11-Fc to activate CD16 reporter cells in an antigen-dependent manner. **FIGS. 11C** and **11D** depict the ability of cx7884 (hz12E9v9-Fc) and cx7885 (hz16G10v11-Fc) to activate CD16 reporter cells in an antigen- and dose-dependent manner.

Example 9: Comparison of 5T4-Targeting CD3 Engaging Constructs

A. Binding to cancer cells and primary T cells

[0651] This Example describes studies assessing binding of exemplary constructs to T cells or to cancer cells. These studies were carried out in single cultures containing either only the T cells or only the cancer cells in isolation from each other.

[0652] Binding of exemplary CD3 engaging constructs of the disclosure to CD3 on the surface of primary T cells and to 5T4-expressing Ovcar5 cells was assessed. Primary T cells were negatively enriched from PBMCs isolated from healthy human donor leukopaks. Bound constructs were detected with fluorophore-conjugated secondary antibodies and binding was measured by flow cytometry. Cells incubated with secondary antibody only served as negative controls.

[0653] Exemplary 5T4-targeting constrained CD3 engaging constructs were assessed. As shown in Table E3, cx3497 incorporates a 41BB binding domain as a co-stimulatory receptor binding region (CRBR), whereas cx3547 did not incorporate a CRBR. The exemplary cx3497 construct contained a sdAb (containing a CDR1, a CDR2, and a CDR3 set forth in SEQ ID NOS:347, 348, and 349, respectively; e.g. set forth in SEQ ID NO:210) targeting a 41BB co-stimulatory receptor as a CRBR. Single domain antibodies were incorporated as the 5T4 binding domains of both constructs as well as the 41BB binding domain of cx3497. As shown in **FIGS. 12A** and **12B,** both constructs displayed binding to a 5T4 expressing cell, Ovcar-5. The constructs, however, were unable to bind T-cells in isolation (**FIGS. 12C** and **12D**).

B. T cell-mediated cytotoxicity

[0654] This Example describes the assessment and characterization of the tested constrained CD3 engaging constructs in human primary T cell *in vitro* assays.

[0655] Target cells were fluorescently labeled with CytoID red. For adherent target cells, target cells were seeded, allowed to settle at room temperature for uniform distribution, and incubated for several hours at 37°C to permit adherence prior to addition of other assay components. Primary T cells were negatively enriched from PBMCs isolated from healthy human donor leukopaks and added at a 10:1-40:1 T cell-to-target cell ratio. Green caspase-3/7 reagent was added, which fluorescently labeled nuclear DNA of cells undergoing apoptosis. Antibodies were titrated onto the co-culture and assay plates were serially imaged using an IncuCyte ZOOM system. Target cell death was determined by measuring total red/green overlap object area (as shown in **FIGS. 13A** and **13B**). Herein target cells are labeled as red with the CytoID red, while apoptosis was monitored with the green fluorescent caspase-3/7 substrate, thus apoptotic target cells those that are dual labeled red and green.

[0656] In an exemplary assay, the multispecifc construct that were tested included the 5T4-targeted constrained CD3 construct with a 41BB-binding costimulatory receptor binding region (cx3497). As a control, a corresponding 5T4-targeted constrained CD3 construct without the costimulatory receptor binding region also was tested (cx3547). The target cells were 5T4-positive Ovcar5 cells or 5T4-

negative CCRF cells. As shown in **FIGS. 13A** and **13B**, a representative 5T4-targeted constrained CD3 engaging construct, cx3547, lacked the capacity to mediate antigen specific cytotoxicity, whereas the addition of a 41BB binding domain induced specific T-cell cytotoxicity toward a 5T4 expressing cell line, Ovcar-5, but not toward a 5T4 negative cell line, CCRF-CEM. Notably, T-cell mediated cytotoxicity mediated by cx3497 was not observed until approximately 40hrs, which is consistent with the kinetics of 41BB upregulation following TCR signaling.

[0657] These results show that the addition of the co-stimulatory receptor binding region targeting a costimulatory receptor, such as 41BB, enhanced the potency of T-cell mediated cytotoxicity over the construct that lacked a 41BB binding domain. These observations support that the antigen-targeted constrained CD3 format with additional co-stimulatory capacity provided herein compared to other CD3 engaging formats, display enhanced potency of mediated cytotoxicity, without substantially binding T cells absent antigen engagement.

C. T cell cytokine production (ELISA)

[0658] Supernatants from T cell-mediated cytotoxicity assays, described above, were analyzed for IFNγ content by sandwich ELISA (BioLegend, USA). The manufacturer's instructions were followed and a standard curve was generated from which cytokine concentration values of supernatant samples were interpolated. Samples that had absorbance values below the lower limit of detection were assigned a cytokine concentration equal to half that of the lowest standard concentration.

[0659] **FIG. 14** shows that a representative 5T4-targeted constrained CD3 engaging constructs were observed to elicit enhanced IFNγ production by T cells in an antigen dependent manner when a 41BB binding domain was incorporated into the constructs (cx3499 and cx3497 vs. cx3546 and cx3547).

Example 10: Generation and Assessment of CD3-constrained Multispecific Constructs Containing Antigen-Binding 5T4-Targeting Domain with or without a Costimulatory Binding Region

[0660] Additional multispecific constructs were generated to contain a heterodimeric Fc region of an immunoglobulin coupled by a linker (e.g. a non-cleavable linker) to the CD3 binding region, and antigen binding domains that binds the 5T4 tumor associated antigen (TAA) positioned amino-terminally relative to the Fc region and carboxy-terminally relative to the CD3 binding region of the multispecific polypeptide construct. Constructs were generated with or without a 4-1BB targeting sdAb as a CRBR and T cell activity was compared in various assays.

A. Design and Generation of Constructs

[0661] Exemplary multispecific constructs were generated with formats as depicted in **FIG. 3A-E**. Polynucleotides encoding at least a first polypeptide chain and a second polypeptide chain of the heterodimeric multispecific polypeptide construct were generated and cloned into a plasmid for expression. The first polypeptide chain generally included in order, from the N-terminus to C-terminus, a

first Fc polypeptide (e.g. an Fc hole polypeptide); a non-cleavable linker; and a variable light (VL) domain of an anti-CD3 antibody. The second polypeptide chain generally included in order, from the N-terminus to C-terminus, a second Fc polypeptide (e.g. an Fc knob polypeptide); the same non-cleavable linker as the first polypeptide chain; and a variable heavy (VH) domain of an anti-CD3 antibody. The anti-CD3 antibody included a disulfide- stabilized (dsFv) antibody (anti-CD3 VH with the mutation G44C and VL with the mutation G100C), as set forth in Table E5. One of the polypeptide chains additionally encoded two 5T4 antigen binding domains, one amino-terminal to the Fc domain and one carboxy-terminal to the CD3 binding region. The exemplary construct cx5951 was generated without a CRBR, whereas the construct cx5185 contained a 4-1BB antigen binding domain (e.g. sdAb) as a CRBR positioned carboxy-terminally relative to the CD3 binding region, e.g. a sdAb (containing a CDR1, a CDR2 and a CDR3 set forth in SEQ ID NO: 347, 348, and 349, respectively; e.g. set forth in SEQ ID NO:210).

[0662] Components of the exemplary constrained CD3 binding constructs having 5T4-targeting sdAb domains is given below in **Table E5**. The constructs were expressed and purified substantially as described in Example 4.

Table E5: 5T4 VHH Constrained Multispecific Constructs

Construct	Chain	N-term sdAb (Target)	Fc	Linker	CD3 Binding Domain	C-term sdAb (Target)	Disulfide Stabilized
cx5951	1	5T4 sdAb hz12E9v09 (360)	xELL-Knob (105)	GGGGGSGGGGGSGGGGGS (127)	VH13 (47)	5T4 sdAb hz16G10v11 (287)	yes
	2	None	hxELL-Hole (106)	GGGGGSGGGGGSGGGGGS (127)	VL10 (75)	None	
cx5185	1	5T4 sdAb hz12E9v09 (360)	xELL-Knob (105)	GGGGGSGGGGGSGGGGGS (127)	VH13 (47)	5T4 sdAb hz16G10v11 (287)	yes
	2	None	xELL-Hole (112)	GGGGGSGGGGGSGGGGGS (127)	VL10 (75)	4-1BB sdAb RH3v5-1 (210)	

B. T Cell Activity

[0663] Activity of the constructs described above to engage CD3 in various assays was compared.

1. Cytotoxic Activity

[0664] Cytotoxic activity towards target cells was assessed in the presence of exemplary 5T4-targeted constructs with a 41BB-binding costimulatory receptor binding region (cx5185) or without the CRBR (cx5951). For cytotoxicity assays, Ovcar-5 cells expressing 5T4 or control CCRF-CEM cells that do not express 5T4 were used as target cells and were seeded, allowed to settle at room temperature for uniform distribution, and incubated for several hours at 37 degrees Celsius to permit adherence prior to addition of other assay components. Primary T cells were negatively enriched from PBMCs isolated from

healthy human donor leukopaks and added at a 10:1-40:1 T cell-to-target cell ratio. Herein target cells were labeled as red with the CytoID red, while apoptosis was monitored with the green fluorescent caspase-3/7 substrate; thus apoptotic target cells are those that are dual labeled red and green. Assay plates were serially imaged using an IncuCyte ZOOM system. Target cell death was determined by measuring total red/green overlap object area.

[0665] As shown in **FIG. 15A**, after 48 hours, a marked difference in potency of T-cell mediated target cell cytotoxicity against 5T4-expressing target cells was observed with the 5T4-targeted constrained CD3 engaging construct with a 41BB-binding costimulatory receptor binding region, cx5185, compared to a the same construct lacking the 41BB-binding costimulatory receptor binding region, cx5951. Cytotoxic activity against non-target cells was not observed (**FIG. 15B**).

[0666] These observations support that the antigen-dependent constrained CD3 format with the additional co-stimulatory capacity provided herein, display enhanced potency of mediated cytotoxicity compared to other CD3 engaging formats.

2. T cell activation

[0667] To assess T cell activation mediated by exemplary 5T4-targeted constrained CD3 engaging constructs, cx5185 and cx5951 were incubated in a co-culture of T-cells and 5T4-expressing target cells, either A375 cells, Ovcar-5 cells, or SHP-77 cells. To assess T cell activation, cells were collected and stained with a live/dead stain and fluorophore-conjugated anti-CD4, anti-CD8, and/or anti-CD25 antibodies. Cells were analyzed using a SONY SA3800 spectral analyzer and CD4+ or CD8+ T cell activation was determined by measuring expression levels of CD25. T-cell activation was assessed by CD25 expression on CD4 and CD8 populations. T cell activation, as measured by expression of CD25, was evident in CD4 (**FIG. 16A**) and CD8 (**FIG. 16B**) T cells that had been incubated with 5T4-expressing target cells in the presence of the exemplary constructs. As shown in **FIGS. 16A-B**, the 5T4-targeted constrained CD3 engaging construct incorporating the 41BB binding domain (cx5185) displayed enhanced activating capacity toward both CD4 and CD8 T-cells compared to the similar construct lacking the 41BB binding domain, cx5951.

3. T cell cytokine production (ELISA)

[0668] The impact of the incorporation of the 41BB binding domain into an exemplary 5T4-targeted constrained CD3 engaging construct on T-cell mediated IFNγ production was assessed using various 5T4-expressing cell lines, A375, SHP-77, and Ovcar5. After co-culture of T cells and 5T4-expressing target cells, supernatants were analyzed for IFNγ content by sandwich ELISA (BioLegend, USA). The manufacturer's instructions were followed and a standard curve was generated from which cytokine concentration values of supernatant samples were interpolated. Samples that had absorbance values below the lower limit of detection were assigned a cytokine concentration equal to half that of the lowest standard concentration. As shown in **FIG. 17**, the 5T4-targeted constrained CD3 engaging construct

incorporating the 41BB binding domain, cx5185, displayed enhanced IFNγ production compared to the similar construct lacking the 41BB binding domain, cx5951.

4. T Cell Proliferation

[0669] T-cell proliferation was assessed by measuring the dilution of CellTrace™ Violet dye (Thermo Fisher Scientific) in labeled CD4+ or CD8+ T cells by flow cytometry. T cells were negatively enriched from PBMCs and labeled with CellTrace™ Violet according to the manufacturer's protocol. 5T4-targeted constrained CD3 engaging constructs were titrated onto co-cultures of labeled T cells and 5T4-expressing cells A375, Ovcar-5, and SHP-77, and assay plates were incubated at 37 degrees C for five days. Cells were stained with the viability dye propidium iodide as well as fluorophore-conjugated anti-CD4 and anti-CD8 antibodies and analyzed using a SONY SA3800 spectral analyzer. Percent proliferated CD4+ or CD8+ T cells was determined by gating on the appropriate viable T cell subpopulation and measuring the percentage of cells with CellTrace™ Violet intensities lower than that of T cells from untreated co-cultures.

[0670] The 5T4-targeted constrained CD3 engaging construct incorporating the 41BB binding domain, cx5185, enhanced the proliferation of both CD4 (**FIG. 18**) and CD8 (**FIG. 19**) T-cells compared to the similar construct lacking the 41BB binding domain, cx5951.

5. T Cell Mitochondrial Assessment

[0671] 41BB signaling has been suggested to enhance mitochondrial function. Mitochodrial function can be monitored using the mitochondrial-selective fluorescent probe MitoTracker Green (Thermo Fisher Scientific), which accumulates in active mitochondria. To assess mitochondrial function of T cells, T cells were co-cultured for five days with 5T4-expressing cell lines A375, Ovcar-5, and SHP-77, in the presence of exemplary 5T4-targeted constrained CD3 engaging constructs. MitoTracker Green was added at a final cell staining concentration of 100 nM as well as the viability dye propidium iodide and fluorophore-conjugated anti-CD4 and anti-CD8 antibodies, and cells were analyzed using a SONY SA3800 spectral analyzer. Median MitoTracker Green fluorescent intensity of CD4+ or CD8+ T cells was determined by gating on the appropriate viable T cell subpopulation. The 5T4-targeted constrained CD3 engaging construct incorporating the 41BB binding domain, cx5185, enhanced mitochondrial function of both CD4 (**FIG. 20**) and CD8 (**FIG. 21**) T-cells compared to the similar construct lacking the 41BB binding domain, cx5951.

6. T Cell Reporter Assay

[0672] The capacity of constrained CD3 engaging constructs containing 5T4-targeted sdAbs to mediate specific agonism of the 41BB co-stimulatory signaling pathway was also assessed. A Jurkat 41BB NFκB-Luciferase reporter cell line was used to test exemplary 5T4-targeting constrained CD3 engaging constructs with either no co-stimulatory receptor binding domain (cx5951) or a 41BB binding domain (cx5185). Recombinant plate bound 5T4 was used as the source of the antigen As shown in **FIG.**

22, cx5185 incorporating the 41BB binding domain was found to induce specific agonism of the targeted co-stimulatory receptor.

C. Summary

[0673] Together, these results demonstrate that CD3 engaging constructs containing 5T4-targeting sdAb domains, with and without a CRBR, are capable of antigen-dependent activation of T cells. Notably, the 5T4-targeted constrained CD3 engaging construct incorporating a 41BB binding domain displayed superior antigen-dependent and activity than the 5T4-targeted constrained CD3 engaging construct without a 41BB binding domain.

Example 11: Comparison of Orientation of CD3 Binding Region in CD3-constrained Multispecific Constructs Containing Antigen-Targeting Domains

A. Design and Generation of Constructs

[0674] Multispecific polypeptide constructs were generated as shown in **FIGS. 23A-B**, to contain a heterodimeric Fc region of an immunoglobulin coupled by a linker (e.g. a non-cleavable linker) to the CD3 binding region, an antigen binding domain (e.g. sdAb) as a CRBR positioned carboxy-terminally relative to the CD3 binding region, and dual antigen binding domains that bind a tumor associated antigen (TAA) positioned amino-terminally relative to the Fc region and carboxy-terminally relative to the CD3 binding region of the multispecific polypeptide construct.

[0675] Polynucleotides encoding at least a first polypeptide chain and a second polypeptide chain of the heterodimeric multispecific polypeptide construct were generated and cloned into a plasmid for expression. The first polypeptide chain generally included in order, from the N-terminus to C-terminus, a first Fc polypeptide (e.g. an Fc hole polypeptide); a non-cleavable linker; a variable light (VL; **FIG. 23B**) or variable heavy (VH; **FIG. 23A**) domain of an anti-CD3 antibody; and an antigen-binding domain (e.g. sdAb) as a CRBR. The second polypeptide chain generally included in order, from the N-terminus to C-terminus, a first antigen-binding domain (e.g. sdAb #1), a second Fc polypeptide (e.g. an Fc knob polypeptide); the same linker as the first polypeptide chain; the other of the variable heavy (VH) or variable light (VL) domain of an anti-CD3 antibody; and a second antigen-binding domain (e.g. sdAb #2). The anti-CD3 antibody included a disulfide-stabilized (dsFv) antibody (anti-CD3 VH with the mutation G44C and VL with the mutation G100C).

[0676] Notably, as shown in **FIGS. 23A-B**, the orientation of the anti-CD3 VH and anti-CD3 VL of the CD3 Fv were positioned differently relative to the Fc knob or Fc hole of the heterodimeric Fc region. As shown in **FIG. 23A**, the construct was generated in which the first polypeptide of the heterodimeric construct had the VL of CD3 Fv positioned C-terminal to the Fc knob and antigen-binding domains on the extreme N and C-termini and the second polypepide of the heterodimeric construct had the VH of CD3 Fv positioned C-terminal to the Fc Hole and a CRBR sdAb on the extreme C-termini. In contrast, **FIG. 23B** depicts an exemplary construct in which the first polypeptide of the heterodimeric construct

had the VH of CD3 Fv positioned C-terminal to the Fc knob and antigen-binding domains on the extreme N and C-termini and the second polypepide of the heterodimeric construct had the VL of CD3 Fv positioned C-terminal to the Fc Hole and a CRBR sdAb on the extreme C-termini. Some exemplary constructs generated contained a sdAb (containing a CDR1, a CDR2, and a CDR3 set forth in SEQ ID NOS:347, 348, and 349, respectively; e.g. set forth in SEQ ID NO:210) targeting a 41BB co-stimulatory receptor as a CRBR.

[0677] The constructs were expressed as purified substantially as described in Example 4.

B. T Cell Reporter Activity

[0678] To compare CD3 engagement, the exemplary constructs were tested in an antigen-dependent CD3 reporter assay by assessing their ability to activate a CD3 NFAT reporter Jurkat cell line in a co-culture with target antigen-expressing cells. Activation was assessed by monitoring either green fluorescent or luciferase reporter signal in Jurkat reporter cells.

[0679] Antigen targeting constrained CD3 engaging constructs were titrated onto co-cultures of either A375 cells expressing the target antigen or control CCRF-CEM cells not expressing the target antigen, and engineered Jurkat cells that express NFAT-driven green fluorescence protein (GFP). Engagement of CD3 results in NFAT signaling and production of green fluorescence. For reporter assays utilizing adherent target cells, target cells were seeded, allowed to settle at room temperature for uniform distribution, and incubated for several hours at 37 degrees Celsius to permit adherence prior to addition of reporter cells and antigen targeting constrained CD3 engaging constructs. Assay plates were serially imaged using an IncuCyte ZOOM system and CD3 reporter cell activation was determined by measuring total green object integrated intensity.

[0680] As shown in **FIG. 24A,** the exemplary antigen-targeted constrained CD3 engaging constructs exhibited capacity to mediate target antigen specific T-cell activation when incubated in reporter T cell co-cultures in the presence of antigen-expressing target cells. Reporter activity, however, was not observed in co-cultures with cells not expressing target antigen (**FIG. 24B**). Notably, the construct with the Knob-VH; Hole-VL format displayed enhanced T cell activation compared to the construct with the Knob-VL; Hole-VH format.

[0681] In a similar assay, the same antigen targeting constrained CD3 engaging constructs were titrated onto co-cultures of either A375 cells expressing target antigen or control CCRF-CEM cells not expressing target antigen, and engineered Jurkat cells that express NFAT-driven luciferase. As shown in **FIG. 24C**, the exemplary antigen-targeted constrained CD3 engaging constructs exhibited capacity to mediate target antigen specific T-cell activation when incubated in reporter T cell co-cultures in the presence of antigen-expressing target cells. Again, reporter activity, was not observed in co-cultures with cells not expressing target antigen (**FIG. 24D**). As in the GFP reporter assay, the construct with the Knob-VH; Hole-VL format displayed enhanced T cell activation compared to the construct with the Knob-VL; Hole-VH format.

[0682] These results are consistent with an observation that enhanced CD3 engagement and activity is observed when the components of the CD3 Fv are oriented so that the VH and VL are positioned C-terminally to the Fc Knob and Fc Hole regions, respectively.

Example 12: 5T4 sdAb Epitope Determination

A. Epitope Determination

[0683] The 5T4 targeting sdAbs, 12E9 (SEQ ID NO:245), 16G10 (SEQ ID NO:276) and 14B5 (SEQ ID NO:255) and humanized variants thereof do not cross react with the mouse 5T4 antigen. In order to determine the location of the epitope recognized by these sdAbs, various mouse:human chimeric 5T4 constructs were generated, whenin various portions of the murine extracellular domain of 5T4 were replaced with that of human 5T4. The constructs were generated by grafting a nucleic acid sequence encoding the specific region of human 5T4 into the cognate region of mouse 5T4 in a mammalian expression plasmid encoding the full 5T4 coding region (extracellular domain, transmembrane domain, intracellular domain) fused to an intracellular Citrine tag. The constructs tested included a fully human construct ("Hu"; SEQ ID NO:382), a fully murine construct ("Mu"; SEQ ID NO:383), chimeric hmc5T4.1 (SEQ ID NO:384); chimeric hmc5T4.2 (SEQ ID NO:385), chimeric hmc5T4.3 (SEQ ID NO:386), and chimeric hmc5T4.4 (SEQ ID NO:387) (**FIG. 25**). CHO cells were transiently transfected with these constructs to enable cell surface expression, and binding was monitored by flow cytometry. As shown in **FIGS. 26A-26C**, 12E9v9 (SEQ ID NO:360) recognized an epitope that resides between amino acid residues 60 and 112 (SEQ ID NO:411), while 16G10v11 (SEQ ID NO:287) and 14B5v17 (SEQ ID NO:272) both recoginized epitope(s) that reside between amino acid residues 173 and 224 (SEQ ID NO:412).

B. Binding to 5T4 Expressing Cells

[0684] Exemplary generated 5T4-targeting constrained CD3 engaging constructs were assessed for their ability to activate a CD3 NFAT reporter Jurkat cell line in co-cultures with 5T4-expressing SKOV3 cells, or 5T4 negative CCFR-CEM cells. All three of the constructs tested contained two 5T4-targeted sdAbs. One of the constructs (cx5185) contained two 5T4-targeted sdAbs (hz12E9v9 and hz16G10v11) that bound distinct epitopes, while the other two constructs (cx7859 and cx7860) each contained two 5T4-targeting sdAbs (either hz12E9v9 or hz16G10v11, respectively) against the same epitope.

[0685] In the reporter assay, engagement of CD3 in the Jurkat cells results in NFAT signaling and production of green fluorescence. Antigen targeting constrained CD3 engaging constructs were titrated onto co-cultures of 5T4-expressing SKOV 3 target cells and engineered Jurkat cells that expressed NFAT-driven green fluorescence protein (GFP). Target cells were seeded, allowed to settle at room temperature for uniform distribution, and incubated for several hours at 37°C to permit adherence prior to addition of reporter cells and antigen targeting constrained CD3 engaging constructs. CD3 reporter cell activation was determined as described in Example 6.

[0686] As shown, assessed 5T4-targeted constrained CD3 engaging constructs induced reporter activity in cultures containing 5T4 positive cells **(FIG. 27A)**, but no measurable reporter activity was observed when T cells were cultured with 5T4 negative cells (CCRF-CEMs) **(FIG. 27B)**. As shown in **FIG. 27A**, the construct containing two 5T4-targeted sdAbs that bound distinct epitopes (cx5185) induced greater activation of the reporter cells than constructs that were also bivalent for 5T4, but only bound one of the two epitopes (cx7859 and cx7860).

[0687] The present invention is not intended to be limited in scope to the particular disclosed embodiments, which are provided, for example, to illustrate various aspects of the invention. Various modifications to the compositions and methods described will become apparent from the description and teachings herein. Such variations may be practiced without departing from the true scope and spirit of the disclosure and are intended to fall within the scope of the present disclosure.

Sequences

#	SEQUENCE	ANNOTATION
1	GGSGGS	(GGS)2 linker
2	GGSGGSGGS	(GGS)3 linker
3	GGSGGSGGSGGS	(GGS)4 linker
4	GGSGGSGGSGGSGGS	(GGS)5 linker
5	GGGG	glycine linker
6	GGGGG	glycine linker
7	GGGGGG	glycine linker
8	PAPELLGGPS VFLFPPKPKD TLMISRTPEV TCVVVDVSHE DPEVKFNWYV DGVEVHNAKT KPREEQYNST YRVVSVLTVL HQDWLNGKEY KCKVSNKALP APIEKTISKA KGQPREPQVY TLPPSRDELT KNQVSLTCLV KGFYPSDIAV EWESNGQPEN NYKTTPPVLD SDGSFFLYSK LTVDKSRWQQ GNVFSCSVMH EALHNHYTQK SLSLSPGK	human IgG1 Fc
9	PAPGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLN GKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDEL TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDS DGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLS LSPGK	Fc xELL
10	PAPPVAGPSV FLFPPKPKDT LMISRTPEVT CVVVDVSHED PEVQFNWYVD GVEVHNAKTK PREEQFNSTF RVVSVLTVVH QDWLNGKEYK CKVSNKGLPA PIEKTISKTK GQPREPQVYT LPPSREEMTK NQVSLTCLVK GFYPSDISVE WESNGQPENN YKTTPPMLDS DGSFFLYSKL TVDKSRWQQG NVFSCSVMHE ALHNHYTQKS LSLSPGK	human IgG2 Fc
11	PAPELLGGPS VFLFPPKPKD TLMISRTPEV TCVVVDVSHE DPEVQFKWYV DGVEVHNAKT KPREEQYNST FRVVSVLTVL HQDWLNGKEY KCKVSNKALP APIEKTISKT KGQPREPQVY TLPPSREEMT KNQVSLTCLV KGFYPSDIAV EWESSGQPEN NYNTTPPMLD SDGSFFLYSK LTVDKSRWQQ GNIFSCSVMH EALHNRFTQK SLSLSPGK	human IgG3 Fc
12	PAPEFLGGPS VFLFPPKPKD TLMISRTPEV TCVVVDVSQE DPEVQFNWYV DGVEVHNAKT KPREEQFNST YRVVSVLTVL HQDWLNGKEY KCKVSNKGLP SSIEKTISKA KGQPREPQVY TLPPSQEEMT KNQVSLTCLV KGFYPSDIAV EWESNGQPEN NYKTTPPVLD SDGSFFLYSR LTVDKSRWQE GNVFSCSVMH EALHNHYTQK SLSLSLGK	human IgG4 Fc
13	PAPELLGGPS VFLFPPKPKD TLMISRTPEV TCVVVDVSQE DPEVQFNWYV DGVEVHNAKT KPREEQFNST YRVVSVLTVL HQDWLNGKEY KCKVSNKGLP SSIEKTISKA KGQPREPQVY TLPPSQEEMT KNQVSLTCLV KGFYPSDIAV EWESNGQPEN NYKTTPPVLD SDGSFFLYSR LTVDKSRWQE GNVFSCSVMH EALHNHYTQK SLSLSLGK	human IgG4 Fc
14	EPKSSDKTHTCPPC	modified IgG1 hinge
15	DKTHTCPPC	truncated IgG1 hinge
16	ESKYGPPCPPC	modified IgG4 hinge
17	GQGTLVTVKPGG	carboxy-terminal sequence

#	SEQUENCE	ANNOTATION
18	GQGTLVTVEPGG	carboxy-terminal sequence
19	VQLVQSGGG VVQPGRSLRL SCKASGYTFT RYTMHWVRQA PGKGLEWIGYINPSRGYTNY NQKVKDRFTI SRDNSKNTAF LQMDSLRPED TGVYFCARYYDDHYCLDYWG QGTPVTVSS	OKT3 VH
20	DIQMTQSPSS LSASVGDRVT ITCSASSSVS YMNWYQQTPG KAPKRWIYDTSKLASGVPSR FSGSGSGTDY TFTISSLQPE DIATYYCQQW SSNPFTFGQGTKLQIT	OKT3 VL
21	QVQLVQSGGG VVQPGRSLRL SCKASGYTFT RYTMHWVRQA PGKGLEWIGYINPSRGYTNY NQKVKDRFTI SRDNSKNTAF LQMDSLRPED TGVYFCARYYDDHYSLDYWG QGTPVTVSS	OKT3 humanized VH
22	DVQLVQSGAE VKKPGASVKV SCKASGYTFT RYTMHWVRQA PGQGLEWIGYINPSRGYTNY ADSVKGRFTI TTDKSTSTAY MELSSLRSED TATYYCARYYDDHYCLDYWG QGTTVTVSS	OKT3 humanized VH
23	QVQLVQSGAE LKKPGASVKV SCKASGYTFT RYTMHWVRQA PGQCLEWMGYINPSRGYTNY NQKFKDKATL TADKSTSTAY MELRSLRSDD TAVYYCARYYDDHYSLDYWG QGTLVTVSS	OKT3 humanized VH
24	QIVLTQSPAI MSASPGEKVT MTCSASSSVS YMNWYQQKSG TSPKRWIYDTSKLASGVPAH FRGSGSGTSY SLTISGMEAE DAATYYCQQW SSNPFTFGSGTKLEIN	OKT3 humanized VL
25	DIQMTQSPSS LSASVGDRVT ITCRASQSVS YMNWYQQKPG KAPKRWIYDTSKVASGVPAR FSGSGSGTDY SLTINSLEAE DAATYYCQQW SSNPLTFGGGTKVEIK	OKT3 humanized VL
26	DIQLTQSPSI LSASVGDRVT ITCRASSSVS YMNWYQQKPG KAPKRWIYDTSKVASGVPYR FSGSGSGTEY TLTISSMQPE DFATYYCQQW SSNPLTFGCGTKVEIKRT	OKT3 humanized VL
27	EVQLVESGGGLVQPKGSLKLSCAASGFTFNTYAMNWVRQAP GKGLEWVARIRSKYNNYATYYADSVKDRFTISRDDSQSILYL QMNNLKTEDTAMYYCVRHGNFGNSYVSWFAYWGQGTLVTV SA	anti-CD3 Hv
28	QAVVTQESALTTSPGETVTLTCRSSTGAVTTSNYANWVQEKP DHLFTGLIGGTNKRAPGVPARFSGSLIGDKAALTITGAQTEDE AIYFCALWYSNLWVFGGGTKLTVL	anti-CD3 Lv
29	TYAMN	anti-CD3 VH CDR1
30	RIRSKYNNYATYYADSVKD	anti-CD3 VH CDR2
31	HGNFGNSYVSWFAY	anti-CD3 VH CDR3
32	RSSTGAVTTSNYAN	anti-CD3 VL CDR1
33	GTNKRAP	anti-CD3 VL CDR2
34	ALWYSNLWV	anti-CD3 VL CDR3
35	EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPG KGLEWVGRIRSKYNNYATYYADSVKDRFTISRDDSKNSLYLQ MNSLKTEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS	anti-CD3 VH1
36	EVKLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAP GKGLEWVARIRSKYNNYATYYADSVKDRFTISRDDSKSSLYL	anti-CD3 VH2

#	SEQUENCE	ANNOTATION
	QMNNLKTEDTAMYYCVRHGNFGNSYVSWFAYWGQGTLVTV SS	
37	EVKLVESGGGLVKPGRSLRLSCAASGFTFNTYAMNWVRQAP GKGLEWVARIRSKYNNYATYYADSVKDRFTISRDDSKSILYL QMNNLKTEDTAMYYCVRHGNFGNSYVSWFAYWGQGTLVTV SS	anti-CD3 VH3
38	EVKLVESGGGLVKPGRSLRLSCAASGFTFNTYAMNWVRQAP GKGLEWVARIRSKYNNYATYYADSVKDRFTISRDDSKSILYL QMNSLKTEDTAMYYCVRHGNFGNSYVSWFAYWGQGTLVTV SS	anti-CD3 VH4
39	EVKLVESGGGLVKPGRSLRLSCAASGFTFNTYAMNWVRQAP GKGLEWVARIRSKYNNYATYYADSVKDRFTISRDDSKSILYL QMNSLKTEDTAMYYCVRHGNFGNSYVSWFAYWGQGTLVTV SS	anti-CD3 VH5
40	EVQLLESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPG KGLEWVSRIRSKYNNYATYYADSVKGRFTISRDDSKNTLYLQ MNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVS S	anti-CD3 VH6
41	EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMSWVRQAPG KGLEWVGRIRSKYNNYATYYADSVKGRFTISRDDSKNTLYLQ MNSLRAEDTAVYYCVRHGNFGDSYVSWFAYWGQGTLVTVS S	anti-CD3 VH7
42	EVQLVESGGGLVQPGGSLKLSCAASGFTFNKYAMNWVRQAP GKGLEWVARIRSKYNNYATYYADSVKDRFTISRDDSKNTAYL QMNNLKTEDTAVYYCVRHGNFGNSYISYWAYWGQGTLVTV S	anti-CD3 VH8
43	EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAP GKGLEWVARIRSKYNNYATYYADSVKGRFTISRDDSKNTLYL QMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTTVTV SS	anti-CD3 VH9
44	EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAP GKGLEWVARIRSKYNNYATYYADSVKGRFTISRDDSKNTLYL QMNSLRAEDTAVYYCVRHGNFGNSYVSYFAYWGQGTTVTVS S	anti-CD3 VH10
45	EVQLVESGGGLVQPKGSLKLSCAASGFTFNTYAMNWVRQAP GKGLEWVARIRSKYNNYATYYADSVKDRFTISRDDSQSILYL QMNNLKTEDTAMYYCVRHGNFGNSYVSWFAYWGQGTLVTV SS	anti-CD3 VH11
46	EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAP GKGLEWVARIRSKYNNYATYYADSVKGRFTISRDDAKNTLYL QMSSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTV KP	anti-CD3 VH12
47	EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAP GKCLEWVARIRSKYNNYATYYADSVKGRFTISRDDAKNTLYL QMSSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTV KP	anti-CD3 VH13
48	EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAP GKGLEWVARIRSKYNNYATYYADSVKGRFTISRDDAKNTLYL QMSSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGCGTLVTV KP	anti-CD3 VH14
49	EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPG KGLEWVARIRSKYNNYATYYADSVKGRFTISRDDAKNTLYLQ MSSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS	anti-CD3 VH15

#	SEQUENCE	ANNOTATION
50	EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAP GKGLEWVSRIRSKYNNYATYYADSVKGRFTISRDDAKNTLYL QMSSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTV SS	anti-CD3 VH16
51	EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPG KGLEWVSRIRSKYNNYATYYADSVKGRFTISRDDAKNTLYLQ MSSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS	anti-CD3 VH17
52	EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPG KCLEWVARIRSKYNNYATYYADSVKGRFTISRDDAKNTLYLQ MSSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS	anti-CD3 VH18
53	EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAP GKCLEWVSRIRSKYNNYATYYADSVKGRFTISRDDAKNTLYL QMSSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTV SS	anti-CD3 VH19
54	EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPG KCLEWVSRIRSKYNNYATYYADSVKGRFTISRDDAKNTLYLQ MSSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS	anti-CD3 VH20
55	EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPG KCLEWVGRIRSKYNNYATYYADSVKDRFTISRDDSKNSLYLQ MNSLKTEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS	anti-CD3 VH21
56	EVKLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAP GKCLEWVARIRSKYNNYATYYADSVKDRFTISRDDSKSSLYL QMNNLKTEDTAMYYCVRHGNFGNSYVSWFAYWGQGTLVTV SS	anti-CD3 VH22
57	EVKLVESGGGLVKPGRSLRLSCAASGFTFNTYAMNWVRQAP GKCLEWVARIRSKYNNYATYYADSVKDRFTISRDDSKSILYLQ MNNLKTEDTAMYYCVRHGNFGNSYVSWFAYWGQGTLVTVS S	anti-CD3 VH23
58	EVKLVESGGGLVKPGRSLRLSCAASGFTFNTYAMNWVRQAP GKCLEWVARIRSKYNNYATYYADSVKDRFTISRDDSKSILYLQ MNSLKTEDTAMYYCVRHGNFGNSYVSWFAYWGQGTLVTVS S	anti-CD3 VH24
59	EVKLVESGGGLVKPGRSLRLSCAASGFTFNTYAMNWVRQAP GKCLEWVARIRSKYNNYATYYADSVKDRFTISRDDSKSILYLQ MNSLKTEDTAMYYCVRHGNFGNSYVSWFAYWGQGTLVTVS S	anti-CD3 VH25
60	EVQLLESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPG KCLEWVSRIRSKYNNYATYYADSVKGRFTISRDDSKNTLYLQ MNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVS S	anti-CD3 VH26
61	EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMSWVRQAPG KCLEWVGRIRSKYNNYATYYADSVKGRFTISRDDSKNTLYLQ MNSLRAEDTAVYYCVRHGNFGDSYVSWFAYWGQGTLVTVS S	anti-CD3 VH27
62	EVQLVESGGGLVQPGGSLKLSCAASGFTFNKYAMNWVRQAP GKCLEWVARIRSKYNNYATYYADSVKDRFTISRDDSKNTAYL QMNNLKTEDTAVYYCVRHGNFGNSYISYWAYWGQGTLVTV S	anti-CD3 VH28
63	EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAP GKCLEWVARIRSKYNNYATYYADSVKGRFTISRDDSKNTLYL QMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTTVTV SS	anti-CD3 VH29

#	SEQUENCE	ANNOTATION
64	EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAP GKCLEWVARIRSKYNNYATYYADSVKGRFTISRDDSKNTLYL QMNSLRAEDTAVYYCVRHGNFGNSYVSYFAYWGQGTTVTVS S	anti-CD3 VH30
65	EVQLVESGGGLVQPKGSLKLSCAASGFTFNTYAMNWVRQAP GKCLEWVARIRSKYNNYATYYADSVKDRFTISRDDSQSILYLQ MNNLKTEDTAMYYCVRHGNFGNSYVSWFAYWGQGTLVTVS S	anti-CD3 VH31
66	QAVVTQESALTTSPGETVTLTCRSSTGAVTTSNYANWVQEKP DHLFTGLIGGTNKRAPGVPARFSGSLIGDKAALTITGAQTEDE AIYFCALWYSNLWVFGGGTKLTVL	anti-CD3 VL1
67	QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKP GKSPRGLIGGTNKRAPGVPARFSGSLLGGKAALTISGAQPEDE ADYYCALWYSNHWVFGCGTKLEIK	anti-CD3 VL2
68	QAVVTQEPSLTVSPGGTVTLTCRSSTGAVTTSNYANWVQQKP GQAPRGLIGGTNKRAPWTPARFSGSLLGGKAALTITGAQAED EADYYCALWYSNLWVFGGGTKLTVL	anti-CD3 VL3
69	QAVVTQEPSFSVSPGGTVTLTCRSSTGAVTTSNYANWVQQTP GQAFRGLIGGTNKRAPGVPARFSGSLIGDKAALTITGAQADDE SIYFCALWYSNLWVFGGGTKLTVL	anti-CD3 VL4
70	QAVVTQEPSFSVSPGGTVTLTCRSSTGAVTTSNYANWVQQTP GQAFRGLIGGTNKRAPGVPARFSGSILGNKAALTITGAQADDE SIYFCALWYSNLWVFGGGTKLTVL	anti-CD3 VL5
71	QAVVTQEPSFSVSPGGTVTLTCRSSTGAVTTSNYANWVQQTP GQAFRGLIGGTNKRAPGVPARFSGSILGNKAALTITGAQADDE SDYYCALWYSNLWVFGGGTKLTVL	anti-CD3 VL6
72	QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQEKP GQAFRGLIGGTNKRAPGTPARFSGSLLGGKAALTLSGAQPEDE AEYYCALWYSNLWVFGGGTKLTVL	anti-CD3 VL7
73	QTVVTQEPSLTVSPGGTVTLTCGSSTGAVTSGNYPNWVQQKP GQAPRGLIGGTKFLAPGTPARFSGSLLGGKAALTLSGVQPEDE AEYYCVLWYSNRWVFGGGTKLTVL	anti-CD3 VL8
74	QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKP GQAFRGLIGGTNKRAPGVPARFSGSLLGGKAALTISGAQPEDE ADYYCALWYSNHWVFGGGTKLEIK	anti-CD3 VL9
75	QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKP GQAFRGLIGGTNKRAPGVPARFSGSLLGGKAALTISGAQPEDE ADYYCALWYSNHWVFGCGTKLEIK	anti-CD3 VL10
76	QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKP GQCFRGLIGGTNKRAPGVPARFSGSLLGGKAALTISGAQPEDE ADYYCALWYSNHWVFGEGTKLEIK	anti-CD3 VL11
77	QAVVTQESALTTSPGETVTLTCRSSTGAVTTSNYANWVQEKP DHLFTGLIGGTNKRAPGVPARFSGSLIGDKAALTITGAQTEDE AIYFCALWYSNLWVFGCGTKLTVL	anti-CD3 VL12
78	QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKP GKSPRGLIGGTNKRAPGVPARFSGSLLGGKAALTISGAQPEDE ADYYCALWYSNHWVFGGGTKLEIK	anti-CD3 VL13
79	QAVVTQEPSLTVSPGGTVTLTCRSSTGAVTTSNYANWVQQKP GQAPRGLIGGTNKRAPWTPARFSGSLLGGKAALTITGAQAED EADYYCALWYSNLWVFGCGTKLTVL	anti-CD3 VL14
80	QAVVTQEPSFSVSPGGTVTLTCRSSTGAVTTSNYANWVQQTP GQAFRGLIGGTNKRAPGVPARFSGSLIGDKAALTITGAQADDE SIYFCALWYSNLWVFGGGTKLTVL	anti-CD3 VL15

#	SEQUENCE	ANNOTATION
81	QAVVTQEPSFSVSPGGTVTLTCRSSTGAVTTSNYANWVQQTP GQAFRGLIGGTNKRAPGVPARFSGSILGNKAALTITGAQADDE SIYFCALWYSNLWVFGCGTKLTVL	anti-CD3 VL16
82	QAVVTQEPSFSVSPGGTVTLTCRSSTGAVTTSNYANWVQQTP GQAFRGLIGGTNKRAPGVPARFSGSILGNKAALTITGAQADDE SDYYCALWYSNLWVFGCGTKLTVL	anti-CD3 VL17
83	QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQEKP GQAFRGLIGGTNKRAPGTPARFSGSLLGGKAALTLSGAQPEDE AEYYCALWYSNLWVFGCGTKLTVL	anti-CD3 VL18
84	QTVVTQEPSLTVSPGGTVTLTCGSSTGAVTSGNYPNWVQQKP GQAPRGLIGGTKFLAPGTPARFSGSLLGGKAALTLSGVQPEDE AEYYCVLWYSNRWVFGCGTKLTVL	anti-CD3 VL19
85	QVQLQESGGGLVQAGGSLRLSCAASGRTFSNYHMGWFRQAP GKERELVAAISGSGGSTYYTDSVKGRFTISRNNAKNTMSLQM SNLKPEDTGVYYCTTPTEKGSSIDYWGQGTQVTVSSGRYPYD VPDY	anti-CD3 VHH
86	GRPFSSYAMG	5T4 CDR-H1
87	GRPFSSSAMG	5T4 CDR-H1
88	AVRWIGGATR	5T4 CDR-H2
89	AVSRNGGASQ	5T4 CDR-H2
90	AVSRNAGASQ	5T4 CDR-H2
91	AVSRNTGASQ	5T4 CDR-H2
92	AVSRQGGASQ	5T4 CDR-H2
93	AVSRGGGASQ	5T4 CDR-H2
94	AVSRNAGASY	5T4 CDR-H2
95	AVSRNGGSSY	5T4 CDR-H2
96	AVSRQGGSSY	5T4 CDR-H2
97	AVSRGGGSSY	5T4 CDR-H2
98	AVSRNAGSSY	5T4 CDR-H2
99	AVSRNTGSSY	5T4 CDR-H2
100	GQAWGTKFTDYSD	5T4 CDR-H3
101	RSAAYSRSSEVYTGKDEYYY	5T4 CDR-H3
102	RSAAYSRSSETYTEKHDYTY	5T4 CDR-H3
103	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVV DVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSV LTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQ VYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESNGQPEN NYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEA LHNHYTQKSLSLSPT	Knob Fc
104	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMRSRTPEVTCVV VDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVS VLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP QVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPEN NYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEA LHNHYTQKSLSLSPT	Hole Fc
105	DKTHTCPPCPAPGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTV LHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYT LPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYK TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHN HYTQKSLSLSPT	Knob Fc

#	SEQUENCE	ANNOTATION
106	DKTHTCPPCPAPGGPSVFLFPPKPKDTLMRSRTPEVTCVVVDV SHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLT VLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVC TLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYK TTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHN HYTQKSLSLSPT	Hole Fc
107	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVV DVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSV LTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQ VYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESNGQPEN NYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEA LHNHYTQKSLSLSPG	Knob Fc
108	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMRSRTPEVTCVV VDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVS VLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP QVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPEN NYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEA LHNHYTQKSLSLSPG	Hole Fc
109	DKTHTCPPCPAPGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTV LHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYT LPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYK TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHN HYTQKSLSLSPG	Knob Fc
110	DKTHTCPPCPAPGGPSVFLFPPKPKDTLMRSRTPEVTCVVVDV SHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLT VLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVC TLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYK TTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHN HYTQKSLSLSPG	Hole Fc
111	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVV DVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSV LTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQ VCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEAL HNRYTQKSLSLSPT	Hole Fc
112	DKTHTCPPCPAPGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTV LHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCT LPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKT TPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNR YTQKSLSLSPT	Hole Fc
113	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVV DVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSV LTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQ VCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEAL HNRYTQKSLSLSPG	Hole Fc
114	DKTHTCPPCPAPGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTV LHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCT LPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKT	Hole Fc

#	SEQUENCE	ANNOTATION
	TPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNR YTQKSLSLSPG	
115	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLYISRTPEVTCVVV DVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSV LTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQ VYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESNGQPEN NYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVVHEA LHNHYTQKSLSLSPT	Knob Fc
116	DKTHTCPPCPAPGGPSVFLFPPKPKDTLYISRTPEVTCVVVDVS HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTV LHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYT LPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYK TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVVHEALHN HYTQKSLSLSPT	Knob Fc
117	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLYISRTPEVTCVVV DVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSV LTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQ VYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESNGQPEN NYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVVHEA LHNHYTQKSLSLSPG	Knob Fc
118	DKTHTCPPCPAPGGPSVFLFPPKPKDTLYISRTPEVTCVVVDVS HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTV LHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYT LPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYK TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVVHEALHN HYTQKSLSLSPG	Knob Fc
119	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLYISRTPEVTCVVV DVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSV LTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQ VCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVVHEAL HNRYTQKSLSLSPT	Hole Fc
120	DKTHTCPPCPAPGGPSVFLFPPKPKDTLYISRTPEVTCVVVDVS HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTV LHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCT LPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKT TPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVVHEALHNR YTQKSLSLSPT	Hole Fc
121	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLYISRTPEVTCVVV DVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSV LTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQ VCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVVHEAL HNRYTQKSLSLSPG	Hole Fc
122	DKTHTCPPCPAPGGPSVFLFPPKPKDTLYISRTPEVTCVVVDVS HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTV LHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCT LPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKT TPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVVHEALHNR YTQKSLSLSPG	Hole Fc
123	(GGGGS)n, wherein n is 1 to 5	Linker
124	(GGGGGS)n, wherein n is 1 to 4	linker
125	GGGGS	Linker

#	SEQUENCE	ANNOTATION
126	GGGGGS	Linker
127	GGGGGSGGGGGSGGGGGS	Linker
128	GGGGSGGGGSGGGGS	Linker
129	GGSGGGGSGGGGSGGGGS	Linker
130	GlyxXaa-Glyy-Xaa-Glyz Xaa is independently selected from A, V, L, I, M,F, W, P, G, S, T, C, Y, N,Q, K, R, H, D, or E x, y, and z are each integers in the range from 1-5	Linker
131	Gly-Gly-Gly-Xaa-Gly-Gly-Gly-Xaa-Gly-Gly-Gly Xaa is independently selected from A, V, L, I, M,F, W, P, G, S, T, C, Y, N,Q, K, R, H, D, or E	Linker
132	(SSSSG)n n=1-9	Linker
133	GGGGG-C-GGGGG	Linker
134	(EAAAK)n n=2-20	Linker
135	AS-(AP)n-GT n=2-20	Linker
136	AS-(EAAAK)n-GT n=2-20	Linker
137	(GGGGA)n n=2-20	Linker
138	(PGGGS)n n=2-20	Linker
139	(AGGGS)n n=2-20	Linker
140	GGS-(EGKSSGSGSESKST)n-GGS n=2-20	Linker
141	SSSASASSA	Linker
142	GSPGSPG	Linker
143	ATTTGSSPGPT	Linker
144	X1 X2 X3 X4 X5 (P4 P3 P2 P1 ↓ P1') X1= I, L, Y, M, F, V, or A; (P4 = I, L, Y, M, F, V, or A) X2 = A, G, S, V, E, D, Q, N, or Y; (P3 = A, G, S, V, E, D, Q, N, or Y) X3 = H, P, A, V, G, S, or T; (P2 = H, P, A, V, G, S, or T) X4 = D or E; (P1 = D or E) X5 = I, L, Y, M, F, V, T, S, G or A (P1' = I, L, Y, M, F, V, T, S, G or A)	Linker consensus
145	X1 E X3 D X5 (P4 P3 P2 P1 ↓ P1') X1 = I or L; (P4 = I or L) (P3 = E) X3 = P or A; (P2 = P or A) X5 = I, V, T, S, or G (P1' = I, V, T, S, or G)	Linker consensus
146	LEAD	granzyme B substrate
147	LEPD	Linker
148	LEAE	Linker
149	IEPDI	Linker
150	LEPDG	Linker
151	LEADT	Linker
152	IEPDG	Linker

#	SEQUENCE	ANNOTATION
153	IEPDV	Linker
154	IEPDS	Linker
155	IEPDT	Linker
156	X1QARX5 (P1QAR↓(A/V)) X1 = any amino acid; (P1 is any amino acid) X5 = A or V	Linker consensus
157	RQARX5 (RQAR(A/V)) X5 = A or V	Linker
158	RQAR	matriptase substrate
159	RQARV	linker
160	X1X2 X3 X4 (P3 P2 P1 ↓ P1') X1 = P, V or A; (P3 = P, V or A) X2 = Q or D; (P2 = Q or D) X3 = A or N; (P1 = A or N) X4 = L, I or M (P1' = L, I or M)	Linker consensus
161	PX2X3X4 (P3 P2 P1 ↓ P1') (P3 = P) X2 = Q or D; (P2 = Q or D) X3 = A or N; (P1 is A or N) X4 = L or I (P1' is L or I)	Linker consensus
162	PAGL	MMP substrate
163	TGLEADGSPAGLGRQARVG	Linker
164	TGLEADGSRQARVGPAGLG	Linker
165	TGSPAGLEADGSRQARVGS	Linker
166	TGPAGLGLEADGSRQARVG	Linker
167	TGRQARVGLEADGSPAGLG	Linker
168	TGSRQARVGPAGLEADGS	Linker
169	TGPAGLGSRQARVGLEADGS	Linker
170	GPAGLGLEPDGSRQARVG	Linker
171	GGSGGGGIEPDIGGSGGS	Linker
172	GGSGGGGLEADTGGSGGS	Linker
173	GSIEPDIGS	Linker
174	GSLEADTGS	Linker
175	GGSGGGGIEPDGGGSGGS	Linker
176	GGSGGGGIEPDVGGSGGS	Linker
177	GGSGGGGIEPDSGGSGGS	Linker
178	GGSGGGGIEPDTGGSGGS	Linker
179	GGGSLEPDGSGS	Linker
180	GPAGLGLEADGSRQARVG	Linker
181	GGEGGGGSGGSGGGS	Linker
182	GSSAGSEAGGSGQAGVGS	Linker
183	GGSGGGGLEAEGSGGGGS	Linker
184	GGSGGGGIEPDPGGSGGS	Linker
185	TGGSGGGGIEPDIGGSGGS	Linker
186	ACPWAVSGARASPGSAASPRLREGPELSPDDPAGLLDLRQGM FAQLVAQNVLLIDGPLSWYSDPGLAGVSLTGGLSYKEDTKEL VVAKAGVYYVFFQLELRRVVAGEGSGSVSLALHLQPLRSAAG AAALALTVDLPPASSEANSAFGFQGRLLHLSAGQRLGVHLHT EARARHAWQLTQGATVLGLFRVTPEIPAGLPSPRSE	41BBL

#	SEQUENCE	ANNOTATION
187	EVQLVQSGAEVKKPGESLRISCKGSGYSFSTYWISWVRQMPG KGLEWMGKIYPGDSYTNYSPSFQGQVTISADKSISTAYLQWSS LKASDTAMYYCARGYGIFDYWGQGTLVTVSS	41BB VH
188	SYELTQPPSVSVSPGQTASITCSGDNIGDQYAHWYQQKPGQSP VLVIYQDKNRPSGIPERFSGSNSGNTATLTISGTQAMDEADYY CATYTGFGSLAVFGGGTKLTVL	41BB VL
189	QVQLQQWGAGLLKPSETLSLTCAVYGGSFSGYYWSWIRQSPE KGLEWIGEINHGGYVTYNPSLESRVTISVDTSKNQFSLKLSSVT AADTAVYYCARDYGPGNYDWYFDLWGRGTLVTVSS	41BB VH
190	EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQA PRLLIYDASNRATGIPARFSGSGSGTDFTLTISSLEPEDFAVYYC QQRSNWPPALTFGGGTKVEIK	41BB VL
191	QMQLVQSGAEVKKPGASVKVSCKASGYSFSGYYMHWVRQA PGQGLEWMGWVNPMSGGTNYAQKFQGRVTITRDTSASTAY MELSSLRSEDTAVYYCAREGMAMRLELDKWGQGTLVTVSS	41BB VH
192	SYELTQPPSVSVAPGKTARITCGGNNIGSKSVHWYQQKPGQAP VLVIYYDSDRPSGIPERFSGSNSGNTATLTISRVEAGDEADYYC QVWDSSSVVFGGGTQLTVL	41BB VL
193	QDSTSDLIPAPPLSKVPLQQNFQDNQFHGKWYVVGQAGNIKL REDKDPNKMMATIYELKEDKSYNVTGVTFDDKKCTYAISTFV PGSQPGEFTLGKIKSFPGHTSSLVRVVSTNYNQHAMVFFKFVF QNREEFYITLYGRTKELTSELKENFIRFSKSLGLPENHIVFPVPI DQCIDG	41BB Anticalin
194	QDSTSDLIPAPPLSKVPLQQNFQDNQFHGKWYVVGQAGNIRL REDKDPIKMMATIYELKEDKSYDVTMVKFDDKKCMYDIWTF VPGSQPGEFTLGKIKSFPGHTSSLVRVVSTNYNQHAMVFFKFV FQNREEFYITLYGRTKELTSELKENFIRFSKSLGLPENHIVFPVPI DQCIDG	41BB Anticalin
195	QDSTSDLIPAPPLSKVPLQQNFQDNQFHGKWYVVGQAGNIRL REDKDPNKMMATIYELKEDKSYDVTAVAFDDKKCTYDIWTF VPGSQPGEFTLGKIKSFPGHTSSLVRVVSTNYNQHAMVFFKFV FQNREEFYITLYGRTKELTSELKENFIRFSKSLGLPENHIVFPVPI DQCIDG	41BB Anticalin
196	QDSTSDLIPAPPLSKVPLQQNFQDNQFHGKWYVVGQAGNIKL REDKDPNKMMATIYELKEDKSYDVTAVAFDDKKCTYDIWTF VPGSQPGEFTLGKIKSFPGHTSSLVRVVSTNYNQHAMVFFKFV FQNREEFYITLYGRTKELTSELKENFIRFSKSLGLPENHIVFPVPI DQCIDG	41BB Anticalin
197	QDSTSDLIPA PPLSKVPLQQ NFQDNQFHGK WYVVGQAGNI KLREDSKMMA TIYELKEDKS YDVTGVSFDD KKCTYAIMTF VPGSQPGEFT LGKIKSFPGH TSSLVRVVST NYNQHAMVFF KFVFQNREEF YITLYGRTKE LTSELKENFI RFSKSLGLPE NHIVFPVPID QCIDG	41BB Anticalin
198	QDSTSDLIPA PPLSKVPLQQ NFQDNQFHGK WYVVGQAGNI KLREDKDPVK MMATIYELKE DKSYDVTGVT FDDKKCRYDI STFVPGSQPG EFTFGKIKSF PGHTSSLVRV VSTNYNQHAM VFFKFVFQNR EEFYITLYGR TKELTSELKE NFIRFSKSLG LPENHIVFPV PIDQCIDG	41BB Anticalin
199	QDSTSDLIPAPPLSKVPLQQNFQDNQFHGKWYVVGQAGNIRL REDKDPHKMMATIYELKEDKSYDVTGVTFDDKKCTYAISTFV PGSQPGEFTLGKIKSFPGHTSSLVRVVSTNYNQHAMVFFKFVF QNREEFYITLYGRTKELTSELKENFIRFSKSLGLPENHIVFPVPI DQCIDG	41BB Anticalin

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200	QDSTSDLIPAPPLSKVPLQQNFQDNQFHGKWYVVGQAGNIKL REDKDPNKMMATIYELKEDKSYDVTGVTFDDKKCTYAISTLV PGSQPGEFTFGKIKSFPGHTSSLVRVVSTNYNQHAMVFFKFVF QNREEFYITLYGRTKELTSELKENFIRFSKSLGLPENHIVFPVPI DQCIDG	41BB Anticalin
201	QDSTSDLIPAPPLSKVPLQQNFQDNQFHGKWYVVGQAGNIRL REDKDPSKMMATIYELKEDKSYDVTAVTFDDKKCNYAISTFV PGSQPGEFTLGKIKSFPGHTSSLVRVVSTNYNQHAMVFFKFVF QNREEFYITLYGRTKELTSELKENFIRFSKSLGLPENHIVFPVPI DQCIDG	41BB Anticalin
202	REGPELSPDDPAGLLDLRQGMFAQLVAQNVLLIDGPLSWYSD PGLAGVSLTGGLSYKEDTKELVVAKAGVYYVFFQLELRRVVA GEGSGSVSLALHLQPLRSAAGAAALALTVDLPPASSEARNSAF GFQGRLLHLSAGQRLGVHLHTEARARHAWQLTQGATVLGLF RVTPEIPAGLPSPRSE	71-254 of human 41BBL
203	LDLRQGMFAQLVAQNVLLIDGPLSWYSDPGLAGVSLTGGLSY KEDTKELVVAKAGVYYVFFQLELRRVVAGEGSGSVSLALHLQ PLRSAAGAAALALTVDLPPASSEARNSAFGFQGRLLHLSAGQR LGVHLHTEARARHAWQLTQGATVLGLFRVTPEIPAGLPSPRSE	85-254 of human 41BBL
204	DPAGLLDLRQGMFAQLVAQNVLLIDGPLSWYSDPGLAGVSLT GGLSYKEDTKELVVAKAGVYYVFFQLELRRVVAGEGSGSVSL ALHLQPLRSAAGAAALALTVDLPPASSEARNSAFGFQGRLLHL SAGQRLGVHLHTEARARHAWQLTQGATVLGLFRVTPEIPAGL PSPRSE	80-254 of human 41BBL
205	PWAVSGARASPGSAASPRLREGPELSPDDPAGLLDLRQGMFA QLVAQNVLLIDGPLSWYSDPGLAGVSLTGGLSYKEDTKELVV AKAGVYYVFFQLELRRVVAGEGSGSVSLALHLQPLRSAAGAA ALALTVDLPPASSEARNSAFGFQGRLLHLSAGQRLGVHLHTE ARARHAWQLTQGATVLGLFRVTPEIPAGLPSPRSE	52-254 of human 4-lBBL
206	REGPELSPDDPAGLLDLRQGMFAQLVAQNVLLIDGPLSWYSD PGLAGVSLTGGLSYKEDTKELVVAKAGVYYVFFQLELRRVVA GEGSGSVSLALHLQPLRSAAGAAALALTVDLPPASSEARNSAF GFQGRLLHLSAGQRLGVHLHTEARARHAWQLTQGATVLGLF RVTPEIPAGL	71-248 of human 41BBL
207	LDLRQGMFAQLVAQNVLLIDGPLSWYSDPGLAGVSLTGGLSY KEDTKELVVAKAGVYYVFFQLELRRVVAGEGSGSVSLALHLQ PLRSAAGAAALALTVDLPPASSEARNSAFGFQGRLLHLSAGQR LGVHLHTEARARHAWQLTQGATVLGLFRVTPEIPAGL	85-248 of human 41BBL
208	DPAGLLDLRQGMFAQLVAQNVLLIDGPLSWYSDPGLAGVSLT GGLSYKEDTKELVVAKAGVYYVFFQLELRRVVAGEGSGSVSL ALHLQPLRSAAGAAALALTVDLPPASSEARNSAFGFQGRLLHL SAGQRLGVHLHTEARARHAWQLTQGATVLGLFRVTPEIPAGL	80-248 of human 41BBL
209	PWAVSGARASPGSAASPRLREGPELSPDDPAGLLDLRQGMFA QLVAQNVLLIDGPLSWYSDPGLAGVSLTGGLSYKEDTKELVV AKAGVYYVFFQLELRRVVAGEGSGSVSLALHLQPLRSAAGAA ALALTVDLPPASSEARNSAFGFQGRLLHLSAGQRLGVHLHTE ARARHAWQLTQGATVLGLFRVTPEIPAGL	52-248 of human 41BBL
210	EVQLLESGGGEVQPGGSLRLSCAASGFSFSINAMGWYRQAPG KRREFVAAIESGRNTVYAESVKGRFTISRDNAKNTVYLQMSSL RAEDTAVYYCGLLKGNRVVSPSVAYWGQGTLVTVKP	41BB sdAb
211	QVSHRYPRIQSIKVQFTEYKKEKGFILTSQKEDEIMKVQNNSVI INCDGFYLISLKGYFSQEVNISLHYQKDEEPLFQLKKVRSVNSL	OX40 ligand

#	SEQUENCE	ANNOTATION
	MVASLTYKDKVYLNVTTDNTSLDDFHVNGGELILIHQNPGEF CVL	
212	QVSHRYPRFQ SIKVQFTEYK KEKGFILTSQ KEDEIMKVQN NSVIINCDGF YLISLKGYFS QEVNISLHYQ KDEEPLFQLK KVRSVNSLMV ASLTYKDKVY LNVTTDNTSL DDFHVNGGEL ILIHQNPGEFCVL	OX40 ligand
213	QVSHRYPRIQSIKVQFTEYKKEKGFILTSQKEDEIMKVQNNSVI INCDGFYLISLKGYFSQEVNISLHYQKDEEPLFQLKKVRSVNSL MVASLTYKDKVYLNVTTDNTSLDDFHVNGGELILIHQNPGEF CVL	OX40 ligand
214	QVSHRYPRIQ SIKVQFTEYK KEKGFILTSQ KEDEIMKVQN NSVIINCDGF YLISLKGYFS QEVNISLHYQ KDEEPLFQLK KVRSVNSLMV ASLTYKDKVY LNVTTDNTSL DDFHVNGGEL ILIHQNPGEF CVL	OX40 ligand
215	VSHRYPRIQSIKVQFTEYKKEKGFILTSQKEDEIMKVQNNSVII NCDGFYLISLKGYFSQEVNISLHYQKDEEPLFQLKKVRSVNSL MVASLTYKDKVYLNVTTDNTSLDDFHVNGGELILIHQNPGEF CVL	OX40 ligand
216	EVQLVQSGAEVKKPGASVKVSCKASGYTFTDSYMSWVRQAP GQGLEWIGDMYPDNGDSSYNQKFRERVTITRDTSTSTAYLELS SLRSEDTAVYYCVLAPRWYFSVWGQGTLVTVSS	OX40 VH
217	DIQMTQSPSSLSASVGDRVTITCRASQDISNYLNWYQQKPGKA PKLLIYYTSRLRSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYC QQGHTLPPTFGQGTKVEIKRT	OX40 VL
218	EVQLVESGGGLVQPGGSLKLSCAASGFTFSGSAMHWVRQASG KGLEWVGRIRSKANSYATAYAASVKGRFTISRDDSKNTAYLQ MNSLKTEDTAVYYCTSGIYDSSGYDYWGQGTLVTVSS	OX40 VH
219	DIVMTQSPLSLPVTPGEPASISCRSSQSLLHSNGYNYLDWYLQ KPGQSPQLLIYLGSNRASGVPDRFSGSGSGTDFTLKISRVEAED VGVYYCMQALQTPLTFGGGTKVEIK	OX40 VL
220	EVQLLESGGGEVQPGGSLRLSCAASGFTFSDAFMYWVRQAPG KGLEWVSSISNRGLKTAYAESVKGRFTISRDNAKNTLYLQMSS LRAEDTAVYYCSRDVDGDFRGQGTLVTVKP	OX40 sdAb
221	QLETAKEPCMAKFGPLPSKWQMASSEPPCVNKVSDWKLEILQ NGLYLIYGQVAPNANYNDVAPFEVRLYKNKDMIQTLTNKSKI QNVGGTYELHVGDTIDLIFNSEHQVLKNNTYWGIILLANPQFIS	GITR ligand
222	QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYAMSWVRQAPG KGLEWVASISSGGTTYYPDSVKGRFTISRDNSKNTLYLQMNSL RAEDTAVYYCARVGGYYDSMDYWGQGTLVTVSS	GITR VH
223	EIVLTQSPGTLSLSPGERATLSCRASESVDNYGVSFMNWYQQK PGQAPRLLIYAASNQGSGIPDRFSGSGSGTDFTLTISRLEPEDFA VYYCQQTKEVTWTFGQGTKVEIK	GITR VL
224	QVTLRESGPALVKPTQTLTLTCTFSGFSLSTSGMGVGWIRQPP GKALEWLAHIWWDDDKYYQPSLKSRLTISKDTSKNQVVLTM TNMDPVDTATYYCARTRRYFPFAYWGQGTLVTVSS	GITR VH
225	EIVMTQSPATLSVSPGERATLSCKASQNVGTNVAWYQQKPGQ APRLLIYSASYRYSGIPARFSGSGSGTEFTLTISSLQSEDFAVYY CQQYNTDPLTFGGGTKVEIK	GITR VL
226	QVQLQESGPGLVKPSETLSLTCTVSGGSISSGGYFWSWIRQPPG KGLEWIGYIYYSGTTYYNPSLKSRVTISIDTSKNQFSLKLSSVT AADTAVYYCARDLFYYDTSGPRGFDPWGQGTLVTVSS	GITR VH

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227	EIVLTQSPGTLSLSPGERATLSCRASQTVSSNYLAWYQQKPGQAPRLLIYGSSTRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYDSSPWTFGQGTKVEIK	GITR VL
228	QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLEWVAVIWYPGSNKYYAESVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARGGELGRYYYYGMDVWGQGTTVTVSS	GITR VH
229	DIQMTQSPSSLSASVGDRVTVTCRASQGIRNDLGWYQQKPGKAPKRLIYAASSLQSGVPSRFSGSGSGTEFTLTISSLQPEDFATYYCLQHNNYPWTFGQGTKVDIK	GITR VL
230	EVQLLESGGGEVQPGGSLRLSCAASGSVFSIDAMGWYRQAPGKQRELVAVLSGISSAKYAASAPGRFTISRDNAKNTVYLQMSSLRAEDTAVYYCYADVSTGWGRDAHGYWGQGTLVTV	GITR sdAb
231	MPEEGSGCSVRRRPYGCVLRAALVPLVAGLVICLVVCIQRFAQAQQQLPLESLGWDVAELQLNHTGPQQDPRLYWQGGPALGRSFLHGPELDKGQLRIHRDGIYMVHIQVTLAICSSTTASRHHPTTLAVGICSPASRSISLLRLSFHQGCTIASQRLTPLARGDTLCTNLTGTLLPSRNTDETFFGVQWVRP	UniProt No. P32970, CD70-ECD residues 39-193 (underline)
232	QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYDMHWVRQAPGKGLEWVAVIWYDGSNKYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARGSGNWGFFDYWGQGTLVTVSS	CD70 VH
233	DIQMTQSPSSLSASVGDRVTITCRASQGISRWLAWYQQKPEKAPKSLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYNTYPRTFGQGTKVEIK	CD70 VL
234	QVQLQQSGGGLVQPGGSLRLSCAASGSIFSINGMGWYRQAPGKERELVAGLTSGGSVTNYADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCRAEIFTRTGENYYGMDYWGKGTQVTVKP	ICOS sdAb
235	EVQLVESGGGEVQPGGSLRLSCAASGRMFSNYAMGWFRQAPGKEREFVAAINYRRDAADYAESVKGRFTISRDNAKNTVYLQMNSLRAEDTAVYYCGFTYAGWASSRRDDYNYWGQGTLVTVKP	CD28 sdAb
236	RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR	CD3zeta signaling domain
237	KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL	4-1BB-derived costimulatory domain
238	SKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRS	CD28-derived costimulatory domain
239	RSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRS	CD28-derived costimulatory domain 2
240	FWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRS	CD28-derived costimulatory domain 3
241	KPTTTPAPRPPTPAPTIASQPLSLRPEASRPAAGGAVHTRGLDFASDIYIWAPLAGTCGVLLLSLVITLYC	CD8-derived hinge and transmembrane domain
242	AKPTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVIT	CD8-derived hinge and

#	SEQUENCE	ANNOTATION
		transmembrane domain
243	KPTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDF ACDIYIWAPLAGTCGVLLLSLVIT	CD8 hinge and transmembrane domain
244	MPGGCSRGPAAGDGRLRLARLALVLLGWVSSSSPTSSASSFSS SAPFLASAVSAQPPLPDQCPALCECSEAARTVKCVNRNLTEVP TDLPAYVRNLFLTGNQLAVLPAGAFARRPPLAELAALNLSGSR LDEVRAGAFEHLPSLRQLDLSHNPLADLSPFAFSGSNASVSAPS PLVELILNHIVPPEDERQNRSFEGMVVAALLAGRALQGLRRLE LASNHFLYLPRDVLAQLPSLRHLDLSNNSLVSLTYVSFRNLTH LESLHLEDNALKVLHNGTLAELQGLPHIRVFLDNNPWVCDCH MADMVTWLKETEVVQGKDRLTCAYPEKMRNRVLLELNSAD LDCDPILPPSLQTSYVFLGIVLALIGAIFLLVLYLNRKGIKKWM HNIRDACRDHMEGYHYRYEINADPRLTNLSSNSDV	5T4
245	QVQLVQSGGGLVQAGGSLRLSCAASRRPFSSKTMAWFRQTPG KEREFVAAVRWIGGATRYTDSVKGRFSISKDNAINTVYLQMN SLKSEDTAVYYCAAGQAWGTKFTDYSDWGQGTQVTVKP	L12E9
246	EVQLVESGGGEVQPGGSLRLSCAASRRPFSSKTMAWFRQAPG KGREFVAAVRWIGGATRYAESVKGRFTISRDNAKNTLYLQMS SLRAEDTAVYYCAAGQAWGTKFTDYSDWGQGTLVTVKP	hz12E9v1
247	EVQLVESGGGEVQPGGSLRLSCAASRRPFSSKTMAWFRQAPG KEREFVAAVRWIGGATRYAESVKGRFTISRDNAKNTLYLQMS SLRAEDTAVYYCAAGQAWGTKFTDYSDWGQGTLVTVKP	hz12E9v2
248	EVQLVESGGGEVQPGGSLRLSCAASRRPFSSKTMAWFRQAPG KEREFVAAVRWIGGATRYAESVKGRFTISRDNAKNTVYLQMS SLRAEDTAVYYCAAGQAWGTKFTDYSDWGQGTLVTVKP	hz12E9v3
249	EVQLVESGGGEVQPGGSLRLSCAASRRPFSSKTMAWFRQAPG KEREFVAAVRWIGGATRYTESVKGRFTISRDNAKNTVYLQMS SLRAEDTAVYYCAAGQAWGTKFTDYSDWGQGTLVTVKP	hz12E9v4
250	EVQLVESGGGEVQPGGSLRLSCAASRRPFSSKTMAWFRQAPG KEREFVAAVRWIGGATRYTESVKGRFTISRDNAKNTLYLQMS SLRAEDTAVYYCAAGQAWGTKFTDYSDWGQGTLVTVKP	hz12E9v5
251	EVQLVESGGGEVQAGGSLRLSCAASRRPFSSKTMAWFRQTPG KEREFVAAVRWIGGATRYAESVKGRFTISRDNAKNTVYLQMS SLRAEDTAVYYCAAGQAWGTKFTDYSDWGQGTLVTVKP	hz12E9v6
252	EVQLVESGGGEVQPGGSLRLSCAASRRPFSSKTMAWFRQAPG KEREFVAAVRWIGGATRYTDSVKGRFSISKDNAKNTVYLQMS SLRAEDTAVYYCAAGQAWGTKFTDYSDWGQGTLVTVKP	hz12E9v7
253	EVQLVESGGGEVQPGGSLRLSCAASRRPFSSKTMAWFRQAPG KEREFVAAVRWIGGATRYAESVKGRFTISRDNAINTVYLQMN SLKSEDTAVYYCAAGQAWGTKFTDYSDWGQGTLVTVKP	hz12E9v8
254	EVQLVESGGGEVQPGGSLRLSCAASGRPFSSKTMAWFRQAPG KEREFVAAVRWIGGATRYTESVKGRFTISRDNAKNTVYLQMS SLRAEDTAVYYCAAGQAWGTKFTDYSDWGQGTLVTVKPGG	hz12E9v9
255	QVQLVQSGGGLVQAGDSLTLSCAVSERPFGTYAMGWFRQAP GRERDLVAAVSRNGGASQYGDSVKGRFSISRDNIKNTMYLQM NSLKPEDTAVYYCAARSAAYSRSSEVYTGKDEYYYWGQGTQ VTVKP	L14B5
256	EVQLVESGGGEVQPGGSLRLSCAASERPFGTYAMGWFRQAPG KGRDLVSAVSRNGGASQYAESVKGRFTISRDNAKNTLYLQMS SLRAEDTAVYYCAARSAAYSRSSEVYTGKDEYYYWGQGTLV TVKP	hz14B5v1

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257	EVQLVESGGGEVQPGGSLRLSCAASERPFGTYAMGWFRQAPG KERDLVAAVSRNGGASQYAESVKGRFTISRDNAKNTLYLQMS SLRAEDTAVYYCAARSAAYSRSSEVYTGKDEYYYWGQGTLV TVKP	hz14B5v2
258	EVQLVESGGGEVQPGGSLRLSCAASERPFGTYAMGWFRQAPG KERDLVAAVSRNGGASQYAESVKGRFTISRDNAKNTMYLQM SSLRAEDTAVYYCAARSAAYSRSSEVYTGKDEYYYWGQGTL VTVKP	hz14B5v3
259	EVQLVESGGGEVQPGGSLRLSCAASERPFGTYAMGWFRQAPG KERDLVAAVSRNGGASQYAESVKGRFTISRDNAKNTVYLQM SSLRAEDTAVYYCAARSAAYSRSSEVYTGKDEYYYWGQGTL VTVKP	hz14B5v4
260	EVQLVESGGGEVQPGGSLRLSCAASERPFGTYAMGWFRQAPG KERDLVAAVSRNAGASQYAESVKGRFTISRDNAKNTMYLQM SSLRAEDTAVYYCAARSAAYSRSSEVYTGKDEYYYWGQGTL VTVKP	hz14B5v5
261	EVQLVESGGGEVQPGGSLRLSCAASERPFGTYAMGWFRQAPG KERDLVAAVSRNTGASQYAESVKGRFTISRDNAKNTMYLQM SSLRAEDTAVYYCAARSAAYSRSSEVYTGKDEYYYWGQGTL VTVKP	hz14B5v6
262	EVQLVESGGGEVQPGGSLRLSCAASERPFGTYAMGWFRQAPG KERDLVAAVSRQGGASQYAESVKGRFTISRDNAKNTMYLQM SSLRAEDTAVYYCAARSAAYSRSSEVYTGKDEYYYWGQGTL VTVKP	hz14B5v7
263	EVQLVESGGGEVQPGGSLRLSCAASERPFGTYAMGWFRQAPG KERDLVAAVSRGGGASQYAESVKGRFTISRDNAKNTMYLQM SSLRAEDTAVYYCAARSAAYSRSSEVYTGKDEYYYWGQGTL VTVKP	hz14B5v8
264	EVQLVESGGGEVQPGGSLRLSCAASERPFGTYAMGWFRQAPG KERDLVAAVSRNGGASQYGDSVKGRFTISRDNAKNTMYLQM SSLRAEDTAVYYCAARSAAYSRSSEVYTGKDEYYYWGQGTL VTVKP	hz14B5v9
265	EVQLVESGGGEVQPGGSLRLSCAASERPFGTYAMGWFRQAPG KERDLVAAVSRNGGASQYGESVKGRFTISRDNAKNTMYLQM SSLRAEDTAVYYCAARSAAYSRSSEVYTGKDEYYYWGQGTL VTVKP	hz14B5v10
266	EVQLVESGGGEVQAGDSLTLSCAVSERPFGTYAMGWFRQAP GRERDLVAAVSRNAGASQYAESVKGRFTISRDNAKNTMYLQ MSSLRAEDTAVYYCAARSAAYSRSSEVYTGKDEYYYWGQGT LVTVKP	hz14B5v11
267	EVQLVESGGGEVQPGGSLRLSCAASERPFGTYAMGWFRQAPG KERDLVAAVSRNAGASQYGESVKGRFSISRDNIKNTMYLQMS SLKPEDTAVYYCAARSAAYSRSSEVYTGKDEYYYWGQGTLV TVKP	hz14B5v12
268	EVQLVESGGGEVQPGGSLRLSCAASERPFGTYAMGWFRQAPG KERETVAAVSRNAGASQYAESVKGRFTISRDNAKNTMYLQM SSLRAEDTAVYYCAARSAAYSRSSEVYTGKDEYYYWGQGTL VTVKP	hz14B5v13
269	EVQLVESGGGEVQPGGSLRLSCAASERPFGTYAMGWFRQAPG KEREFVAAVSRNAGASQYAESVKGRFTISRDNAKNTMYLQM SSLRAEDTAVYYCAARSAAYSRSSEVYTGKDEYYYWGQGTL VTVKP	hz14B5v14

#	SEQUENCE	ANNOTATION
270	EVQLVESGGGEVQPGGSLRLSCAASERPFGTYAMGWFRQAPG KERDLVAAVSRNAGASYYAESVKGRFTISRDNAKNTMYLQM SSLRAEDTAVYYCAARSAAYSRSSEVYTGKDEYYYWGQGTL VTVKP	hz14B5v15
271	EVQLVESGGGEVQPGGSLRLSCAASERPFGTYAMGWFRQAPG KERDLVAAVSRNAGASQYGEFVKGRFTISRDNAKNTMYLQM SSLRAEDTAVYYCAARSAAYSRSSEVYTGKDEYYYWGQGTL VTVKP	hz14B5v16
272	EVQLVESGGGEVQPGGSLRLSCAASERPFGTYAMGWFRQAPG KERDLVAAVSRNAGASYYAESVKGRFTISRDNAKNTVYLQM SSLRAEDTAVYYCAARSAAYSRSSEVYTGKDEYYYWGQGTL VTVKP	hz14B5v17
273	EVQLVESGGGEVQPGGSLRLSCAASERPFSSYAMGWFRQAPG KERDLVAAVSRNAGASYYAESVKGRFTISRDNAKNTVYLQM SSLRAEDTAVYYCAARSAAYSRSSEVYTGKDEYYYWGQGTL VTVKP	hz14B5v18
274	EVQLVESGGGEVQPGGSLRLSCAASGRPFGTYAMGWFRQAP GKERDLVAAVSRNAGASYYAESVKGRFTISRDNAKNTVYLQ MSSLRAEDTAVYYCAARSAAYSRSSEVYTGKDEYYYWGQGT LVTVKP	hz14B5v19
275	EVQLVESGGGEVQPGGSLRLSCAASGRPFSSYAMGWFRQAPG KERDLVAAVSRNAGASYYAESVKGRFTISRDNAKNTVYLQM SSLRAEDTAVYYCAARSAAYSRSSEVYTGKDEYYYWGQGTL VTVKP	hz14B5v20
276	QVQLLQSGGGLVQAGGSLILSCAVSGRPFSSSAMGWFRQAPG KERETVAAVSRNGGSSYYADFVKGRFTISRDNDKNTVYLQM NSLKPEDTAVYYCAARSAAYSRSSETYTEKHDYTYWGQGTQ VTVKP	L16G10
277	EVQLVESGGGEVQPGGSLRLSCAASGRPFSSSAMGWFRQAPG KGRETVSAVSRNGGSSYYAESVKGRFTISRDNAKNTLYLQMS SLRAEDTAVYYCAARSAAYSRSSETYTEKHDYTYWGQGTLV TVKP	hz16G10v1
278	EVQLVESGGGEVQPGGSLRLSCAASGRPFSSSAMGWFRQAPG KERETVAAVSRNGGSSYYAESVKGRFTISRDNAKNTLYLQMS SLRAEDTAVYYCAARSAAYSRSSETYTEKHDYTYWGQGTLV TVKP	hz16G10v2
279	EVQLVESGGGEVQPGGSLRLSCAASGRPFSSSAMGWFRQAPG KERETVAAVSRNGGSSYYAESVKGRFTISRDNAKNTVYLQMS SLRAEDTAVYYCAARSAAYSRSSETYTEKHDYTYWGQGTLV TVKP	hz16G10v3
280	EVQLVESGGGEVQPGGSLRLSCAASGRPFSSSAMGWFRQAPG KERETVAAVSRQGGSSYYAESVKGRFTISRDNAKNTVYLQMS SLRAEDTAVYYCAARSAAYSRSSETYTEKHDYTYWGQGTLV TVKP	hz16G10v4
281	EVQLVESGGGEVQPGGSLRLSCAASGRPFSSSAMGWFRQAPG KERETVAAVSRGGGSSYYAESVKGRFTISRDNAKNTVYLQMS SLRAEDTAVYYCAARSAAYSRSSETYTEKHDYTYWGQGTLV TVKP	hz16G10v5
282	EVQLVESGGGEVQPGGSLRLSCAASGRPFSSSAMGWFRQAPG KERETVAAVSRNAGSSYYAESVKGRFTISRDNAKNTVYLQMS SLRAEDTAVYYCAARSAAYSRSSETYTEKHDYTYWGQGTLV TVKP	hz16G10v6

#	SEQUENCE	ANNOTATION
283	EVQLVESGGGEVQPGGSLRLSCAASGRPFSSSAMGWFRQAPG KERETVAAVSRNTGSSYYAESVKGRFTISRDNAKNTVYLQMS SLRAEDTAVYYCAARSAAYSRSSETYTEKHDYTYWGQGTLV TVKP	hz16G10v7
284	EVQLVESGGGEVQPGGSLRLSCAASGRPFSSSAMGWFRQAPG KERETVAAVSRNGGSSYYADFVKGRFTISRDNAKNTVYLQMS SLRAEDTAVYYCAARSAAYSRSSETYTEKHDYTYWGQGTLV TVKP	hz16G10v8
285	EVQLVESGGGEVQPGGSLRLSCAASGRPFSSSAMGWFRQAPG KERETVAAVSRNGGSSYYAESVKGRFTISRDNDKNTVYLQMS SLRAEDTAVYYCAARSAAYSRSSETYTEKHDYTYWGQGTLV TVKP	hz16G10v9
286	EVQLVESGGGEVQPGGSLRLSCAASGRPFSSSAMGWFRQAPG KERETVAAVSRNAGSSYYADFVKGRFTISRDNAKNTVYLQMS SLRAEDTAVYYCAARSAAYSRSSETYTEKHDYTYWGQGTLV TVKP	hz16G10v10
287	EVQLVESGGGEVQPGGSLRLSCAASGRPFSSYAMGWFRQAPG KERETVAAVSRNAGSSYYAESVKGRFTISRDNAKNTVYLQMS SLRAEDTAVYYCAARSAAYSRSSETYTEKHDYTYWGQGTLV TVKP	hz16G10v11
288	RRPFSSKTMA	5T4CDR-H1
289	GRPFSSKTMA	5T4 CDR-H1
290	ERPFGTYAMG	5T4 CDR-H1
291	ERPFSSYAMG	5T4 CDR-H1
292	GRPFGTYAMG	5T4 CDR-H1
293	QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKP GQAFRGLIGGTNKRAPGVPARFSGSLLGGKAALTISGAQPEDE ADYYCALWYSNHWVFGCGTKLTVL	anti-CD3 VL (CON)
294	QVQLVQSGGGLVQAGASLRLSCVASGRTRSLRTMAWFRQAP GKERIFVAAISWRSDSTYYADSVKGRFTISRDNAKNTAYLQM NTLKPEDTAVYYCAAGGGWLATTPDEYTYWGQGTLVTVKP	7E1
295	QVQLQESGGGLMQAGDSLRLSCVVSGVTWNSYTMAWFRQA PGKEREFVAAIRWTVDTTYYADFVKGRFTISRDYAKKTMYLQ MNNLKPEDAAVYYCAVGRKWPKADDYWGQGTLVTVKP	14F4
296	GRTRSLRTMA	5T4 CDR-H1
297	GVTWNSYTMA	5T4 CDR-H1
298	AISWRSDSTY	5T4 CDR-H2
299	AIRWTVDTTY	5T4 CDR-H2
300	GGGWLATTPDEYTY	5T4 CDR-H3
301	GRKWPKADDY	5T4 CDR-H3
302	QVQLQESGGGLVQAGGSLRLSCAASRRPFSSKTMAWFRQTPG KEREFVAAVRWIGGATRYTDSVKGRFSISKDNAINTVYLQTNS LKSEDTAVYYCAAGQTWGTKFTDYSDWGQGTLVTVKP	4D3
303	GQTWGTKFTDYSD	5T4 CDR-H3
304	EVQLVESGGGEVQPGGSLRLSCAASGSMTGANTMGWYRQAP GKGRDLVSLIGNYVTHYAESVKGRFTISRDNAKNTLYLQMSS LRAEDTAVYYCYLYTDNLGTSWGQGTLVTVKPGG	hz18H10v1
305	EVQLVESGGGEVQPGGSLRLSCAASGSMTGANTMGWYRQAP GKQRDLVSLIGNYVTHYAESVKGRFTISRDNAKNTLYLQMSS LRAEDTAVYYCYLYTDNLGTSWGQGTLVTVKPGG	hz18H10v2

#	SEQUENCE	ANNOTATION
306	EVQLVESGGGEVQPGGSLRLSCAASGSMTGANTMGWYRQAP GKQRDLVSLIGNYVTHYAESVKGRFTISRDNAKNTVYLQMSS LRAEDTAVYYCYLYTDNLGTSWGQGTLVTVKPGG	hz18H10v3
307	EVQLVESGGGEVQPGGSLRLSCAASGSMTGANTMGWYRQAP GKQRDLVALIGNYVTHYAESVKGRFTISRDNAKNTVYLQMSS LRAEDTAVYYCYLYTDNLGTSWGQGTLVTVKPGG	hz18H10v4
308	EVQLVESGGGEVQPGGSLRLSCAASGSMTGANTMGWYRQAP GKQRDLVALIGNYVTHYAESVKGRFTISRENAKNTVYLQMSS LRAEDTAVYYCYLYTDNLGTSWGQGTLVTVKPGG	hz18H10v5
309	EVQLVESGGGEVQPGGSLRLSCAASGSVTGANTMGWYRQAP GKQRDLVALIGNYVTHYAESVKGRFTISRDNAKNTVYLQMSS LRAEDTAVYYCYLYTDNLGTSWGQGTLVTVKPGG	hz18H10v6
310	EVQLVESGGGEVQPGGSLRLSCAASGSITGANTMGWYRQAPG KQRDLVALIGNYVTHYAESVKGRFTISRDNAKNTVYLQMSSL RAEDTAVYYCYLYTDNLGTSWGQGTLVTVKPGG	hz18H10v7
311	EVQLVESGGGEVQPGGSLRLSCAASGSVTGANTMGWYRQAP GKQRDLVSLIGNYVTHYAESVKGRFTISRDNAKNTLYLQMSS LRAEDTAVYYCYLYTDNLGTSWGQGTLVTVKPGG	hz18H10v8
312	EVQLVESGGGEVQPGGSLRLSCAASGSVTGANTMGWYRQAP GKQRDLVSLIGNYVTHYAESVKGRFTISRDNAKNTVYLQMSS LRAEDTAVYYCYLYTDNLGTSWGQGTLVTVKPGG	hz18H10v9
313	EVQLVESGGGEVQPGGSLRLSCAASGSMTGANTMGWYRQAP GKQRDLVSLIGNYVTHYAESVKGRFTISRENAKNTVYLQMSS LRAEDTAVYYCYLYTDNLGTSWGQGTLVTVKPGG	hz18H10v10
314	EVQLVESGGGEVQPGGSLRLSCAASGSVTGANTMGWYRQAP GKQRDLVSLIGNYVTHYAESVKGRFTISRENAKNTVYLQMSS LRAEDTAVYYCYLYTDNLGTSWGQGTLVTVKPGG	hz18H10v11
315	EVQLVESGGGEVQPGGSLRLSCAASGSVTGANTMGWYRQAP GKQRDLVALIGNYVTHYAESVKGRFTISRENAKNTVYLQMSS LRAEDTAVYYCYLYTDNLGTSWGQGTLVTVKPGG	hz18H10v12
316	EVQLVESGGGEVQPGGSLRLSCAASGSMTGANTMGWYRQAP GKQRELVALIGNYVTHYAESVKGRFTISRENAKNTVYLQMSS LRAEDTAVYYCYLYTDNLGTSWGQGTLVTVKPGG	hz18H10v13
317	EVQLVESGGGEVQPGGSLRLSCAASGSVTGANTMGWYRQAP GKQRDLVALIGNYVTHYAESVKGRFTISRDNAKNTLYLQMSS LRAEDTAVYYCYLYTDNLGTSWGQGTLVTVKPGG	hz18H10v14
318	EVQLVESGGGEVQPGGSLRLSCAASGSITGANTMGWYRQAPG KQRDLVALIGNYVTHYAESVKGRFTISRDNAKNTLYLQMSSL RAEDTAVYYCYLYTDNLGTSWGQGTLVTVKPGG	hz18H10v15
319	EVQLVESGGGEVQPGGSLRLSCAASGSITGANTMGWYRQAPG KQRDLVALIGNYVTHYAESVKGRFTISRENAKNTVYLQMSSL RAEDTAVYYCYLYTDNLGTSWGQGTLVTVKPGG	hz18H10v16
320	EVQLVESGGGEVQPGGSLRLSCAASGSITGANTMGWYRQAPG KQRDLVALIGNYVTHYAESVKGRFTISRDNAKNTVYLQMNSL RAEDTAVYYCYLYTDNLGTSWGQGTLVTVKPGG	hz18H10v17
321	GSMTGANTMG	CDR1
322	GSVTGANTMG	CDR1
323	GSITGANTMG	CDR1
324	LIGNYVTH	CDR2
325	YTDNLGTS	CDR3

#	SEQUENCE	ANNOTATION
326	QVQLVQSGGGLVQPGGSLRLSCVASGSMTGANTMGWYRQAP GKQRDLVALIGNYHYADSVKGRFTISRENAKNTVILQMNSLN PEDTAVYYCYLYTDNLGTSWGQGTLVTVKPGG	18H10
327	PGGGG	Linker
328	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVV DVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSV LTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQ VYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESNGQPEN NYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEA LHNHYTQKSLSLSP	Knob Fc
329	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMRSRTPEVTCVV VDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVS VLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP QVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPEN NYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEA LHNHYTQKSLSLSP	Hole Fc
330	DKTHTCPPCPAPGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTV LHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYT LPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYK TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHN HYTQKSLSLSP	Knob Fc
331	DKTHTCPPCPAPGGPSVFLFPPKPKDTLMRSRTPEVTCVVVDV SHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLT VLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVC TLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYK TTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHN HYTQKSLSLSP	Hole Fc
332	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVV DVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSV LTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQ VCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEAL HNRYTQKSLSLSP	Hole Fc
333	DKTHTCPPCPAPGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTV LHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCT LPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKT TPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNR YTQKSLSLSP	Hole Fc
334	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLYISRTPEVTCVVV DVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSV LTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQ VYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESNGQPEN NYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVVHEA LHNHYTQKSLSLSP	Knob Fc
335	DKTHTCPPCPAPGGPSVFLFPPKPKDTLYISRTPEVTCVVVDVS HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTV LHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYT LPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYK TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVVHEALHN HYTQKSLSLSP	Knob Fc

#	SEQUENCE	ANNOTATION
336	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLYISRTPEVTCVVV DVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSV LTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQ VCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVVHEAL HNRYTQKSLSLSP	Hole Fc
337	DKTHTCPPCPAPGGPSVFLFPPKPKDTLYISRTPEVTCVVVDVS HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTV LHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCT LPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKT TPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVVHEALHNR YTQKSLSLSP	Hole Fc
338	DKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVD VKHEDPEVKFNWYVDGVEVHNAKTKPREEEYNSTYRVVSVL TVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQV YTLPPSREEMTKNQVSLTCDVSGFYPSDIAVEWESDGQPENNY KTTPPVLDSDGSFFLYSKLTVDKSRWEQGDVFSCSVMHEALH NHYTQKSLSLSPGK	Fc-Het-1
339	DKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVD VKHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVL TVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQV YTLPPSREQMTKNQVKLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEAL HNHYTQKSLSLSPGK	Fc-Het-2
340	QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKP GKSPRGLIGGTNKRAPGVPARFSGSLLGGKAALTISGAQPEDE ADYYCALWYSNHWVFGCGTKLTVL	anti-CD3 VL21
341	EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPG KGLEWVGRIRSKYNNYATYYADSVKGRFTISRDDSKNTLYLQ MNSLRAEDTAVYYCVRHGNFGDSYVSWFAYWGQGTLVTVS S	anti-CD3 VH32
342	QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKP GKSPRGLIGGTNKRAPGVPARFSGSLLGGKAALTISGAQPEDE ADYYCALWYSNHWVFGGGTKLTVL	anti-CD3 VL20
343	EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPG KCLEWVARIRSKYNNYATYYADTVKGRFTISRDDAKNTLYLQ MSSLRAEDTAVYYCVRHGNFGDSYVSWFAYWGQGTLVTV	anti-CD3 VH34
344	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVV DVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSV LTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQ VYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESNGQPEN NYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEA LHNHYTQKSLSLSP	IgG1 Knob
345	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMRSRTPEVTCVV VDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVS VLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP QVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPEN NYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEA LHNHYTQKSLSLSP	IgG1 Knob
346	GGGGSGGGGSGGGGS	Linker
347	GFSFSINAMG	41BB CDR1
348	AIESGRNTV	41BB CDR2
349	LKGNRVVSPSVAY	41BB CDR3

#	SEQUENCE	ANNOTATION
350	GFTFNTYAMN	anti-CD3 VH CDR1
351	RIRSKYNNYATY	anti-CD3 VH CDR2
352	HGNFGDSYVSWFAY	anti-CD3 VH CDR3
353	ALWYSNHWV	anti-CD3 VL CDR3
354	VLWYSNRWV	anti-CD3 VL CDR3
355	GFTFSTYAMN	anti-CD3 VH CDR1
356	RIRSKYNNYATY	anti-CD3 VH CDR2
357	GSSTGAVTTSNYAN	anti-CD3 VL CDR1
358	EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPGKCLEWVGRIRSKYNNYATYYADSVKGRFTISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGDSYVSWFAYWGQGTLVTVSS	anti-CD3 VH33
359	EVQLVESGGGEVQPGGSLRLSCAASGFSFSINAMGWYRQAPGKRREFVAAIESGRNTVYAESVKGRFTISRDNAKNTVYLQMSSLRAEDTAVYYCGLLKGNRVVSPSVAYWGQGTLVTVKP	41BB sdAb
360	EVQLVESGGGEVQPGGSLRLSCAASGRPFSSKTMAWFRQAPGKEREFVAAVRWIGGATRYTESVKGRFTISRDNAKNTVYLQMSSLRAEDTAVYYCAAGQAWGTKFTDYSDWGQGTLVTVKP	hz12E9v09
361	IEPDP	Linker
362	SSPTSSASSFSSSAPFLASAVSAQPPLPDQCPALCECSEAARTVKCVNRNLTEVPTDLPAYVRNLFLTGNQLAVLPAGAFARRPPLAELAALNLSGSRLDEVRAGAFEHLPSLRQLDLSHNPLADLSPFAFSGSNASVSAPSPLVELILNHIVPPEDERQNRSFEGMVVAALLAGRALQGLRRLELASNHFLYLPRDVLAQLPSLRHLDLSNNSLVSLTYVSFRNLTHLESLHLEDNALKVLHNGTLAELQGLPHIRVFLDNNPWVCDCHMADMVTWLKETEVVQGKDRLTCAYPEKMRNRVLLELNSADLDCDPILPPSLQTS	5T4 ECD, amino acids 32-355 of human 5T4 (UniProt No. Q13641)
363	GGS(GGS)n, where n=0 to 10	linker
364	QVQLVQSGGGLVQPGGSLRLSCVASGSMTGANTMGWYRQAPGKQRDLVALIGNYHYADSVKGRFTISRENAKNTVILQMNSLNPEDTAVYYCYLYTDNLGTSWGQGTLVTVKP	18H10
365	EVQLVESGGGEVQPGGSLRLSCAASGSMTGANTMGWYRQAPGKGRDLVSLIGNYVTHYAESVKGRFTISRDNAKNTLYLQMSSLRAEDTAVYYCYLYTDNLGTSWGQGTLVTVKP	hz18H10v1
366	EVQLVESGGGEVQPGGSLRLSCAASGSMTGANTMGWYRQAPGKQRDLVSLIGNYVTHYAESVKGRFTISRDNAKNTLYLQMSSLRAEDTAVYYCYLYTDNLGTSWGQGTLVTVKP	hz18H10v2
367	EVQLVESGGGEVQPGGSLRLSCAASGSMTGANTMGWYRQAPGKQRDLVSLIGNYVTHYAESVKGRFTISRDNAKNTVYLQMSSLRAEDTAVYYCYLYTDNLGTSWGQGTLVTVKP	hz18H10v3
368	EVQLVESGGGEVQPGGSLRLSCAASGSMTGANTMGWYRQAPGKQRDLVALIGNYVTHYAESVKGRFTISRDNAKNTVYLQMSSLRAEDTAVYYCYLYTDNLGTSWGQGTLVTVKP	hz18H10v4

#	SEQUENCE	ANNOTATION
369	EVQLVESGGGEVQPGGSLRLSCAASGSMTGANTMGWYRQAP GKQRDLVALIGNYVTHYAESVKGRFTISRENAKNTVYLQMSS LRAEDTAVYYCYLYTDNLGTSWGQGTLVTVKP	hz18H10v5
370	EVQLVESGGGEVQPGGSLRLSCAASGSVTGANTMGWYRQAP GKQRDLVALIGNYVTHYAESVKGRFTISRDNAKNTVYLQMSS LRAEDTAVYYCYLYTDNLGTSWGQGTLVTVKP	hz18H10v6
371	EVQLVESGGGEVQPGGSLRLSCAASGSITGANTMGWYRQAPG KQRDLVALIGNYVTHYAESVKGRFTISRDNAKNTVYLQMSSL RAEDTAVYYCYLYTDNLGTSWGQGTLVTVKP	hz18H10v7
372	EVQLVESGGGEVQPGGSLRLSCAASGSVTGANTMGWYRQAP GKQRDLVSLIGNYVTHYAESVKGRFTISRDNAKNTLYLQMSS LRAEDTAVYYCYLYTDNLGTSWGQGTLVTVKP	hz18H10v8
373	EVQLVESGGGEVQPGGSLRLSCAASGSVTGANTMGWYRQAP GKQRDLVSLIGNYVTHYAESVKGRFTISRDNAKNTVYLQMSS LRAEDTAVYYCYLYTDNLGTSWGQGTLVTVKP	hz18H10v9
374	EVQLVESGGGEVQPGGSLRLSCAASGSMTGANTMGWYRQAP GKQRDLVSLIGNYVTHYAESVKGRFTISRENAKNTVYLQMSS LRAEDTAVYYCYLYTDNLGTSWGQGTLVTVKP	hz18H10v10
375	EVQLVESGGGEVQPGGSLRLSCAASGSVTGANTMGWYRQAP GKQRDLVSLIGNYVTHYAESVKGRFTISRENAKNTVYLQMSS LRAEDTAVYYCYLYTDNLGTSWGQGTLVTVKP	hz18H10v11
376	EVQLVESGGGEVQPGGSLRLSCAASGSVTGANTMGWYRQAP GKQRDLVALIGNYVTHYAESVKGRFTISRENAKNTVYLQMSS LRAEDTAVYYCYLYTDNLGTSWGQGTLVTVKP	hz18H10v12
377	EVQLVESGGGEVQPGGSLRLSCAASGSMTGANTMGWYRQAP GKQRELVALIGNYVTHYAESVKGRFTISRENAKNTVYLQMSS LRAEDTAVYYCYLYTDNLGTSWGQGTLVTVKP	hz18H10v13
378	EVQLVESGGGEVQPGGSLRLSCAASGSVTGANTMGWYRQAP GKQRDLVALIGNYVTHYAESVKGRFTISRDNAKNTLYLQMSS LRAEDTAVYYCYLYTDNLGTSWGQGTLVTVKP	hz18H10v14
379	EVQLVESGGGEVQPGGSLRLSCAASGSITGANTMGWYRQAPG KQRDLVALIGNYVTHYAESVKGRFTISRDNAKNTLYLQMSSL RAEDTAVYYCYLYTDNLGTSWGQGTLVTVKP	hz18H10v15
380	EVQLVESGGGEVQPGGSLRLSCAASGSITGANTMGWYRQAPG KQRDLVALIGNYVTHYAESVKGRFTISRENAKNTVYLQMSSL RAEDTAVYYCYLYTDNLGTSWGQGTLVTVKP	hz18H10v16
381	EVQLVESGGGEVQPGGSLRLSCAASGSITGANTMGWYRQAPG KQRDLVALIGNYVTHYAESVKGRFTISRDNAKNTVYLQMNSL RAEDTAVYYCYLYTDNLGTSWGQGTLVTVKP	hz18H10v17
382	SSPTSSASSFSSSAPFLASAVSAQPPLPDQCPALCECSEAARTVK CVNRNLTEVPTDLPAYVRNLFLTGNQLAVLPAGAFARRPPLA ELAALNLSGSRLDEVRAGAFEHLPSLRQLDLSHNPLADLSPFA FSGSNASVSAPSPLVELILNHIVPPEDERQNRSFEGMVVAALLA GRALQGLRRLELASNHFLYLPRDVLAQLPSLRHLDLSNNSLVS LTYVSFRNLTHLESLHLEDNALKVLHNGTLAELQGLPHIRVFL DNNPWVCDCHMADMVTWLKETEVVQGKDRLTCAYPEKMR NRVLLELNSADLDCDPILPPSLQTSYVFLGIVLALIGAIFLLVLY LNRKGIKKWMHNIRDACRDHMEGYHYRYEINADPRLTNLSS NSDV	human 5T4 construct
383	SAPSSSVPSSSTSPAAFLASGSAQPPPAERCPAACECSEAARTV KCVNRNLLEVPADLPPYVRNLFLTGNQMTVLPAGAFARQPPL ADLEALNLSGNHLKEVCAGAFEHLPGLRRLDLSHNPLTNLSAF	murine 5T4 construct

#	SEQUENCE	ANNOTATION
	AFAGSNASVSAPSPLEELILNHIVPPEDQRQNGSFEGMVAFEG MVAAALRSGLALRGLTRLELASNHFLFLPRDLLAQLPSLRYLD LRNNSLVSLTYASFRNLTHLESLHLEDNALKVLHNSTLAEWH GLAHVKVFLDNNPWVCDCYMADMVAWLKETEVVPDKARLT CAFPEKMRNRGLLDLNSSDLDCDAVLPQSLQTSYVFLGIVLAL IGAIFLLVLYLNRKGIKKWMHNIRDACRDHMEGYHYRYEINA DPRLTNLSSNSDV	
384	SSPTSSASSFSSSAPFLASAVSAQPPLPDQCPALCECSEAARTVK CVNRNLTEVPTDLPAYVRNLFLTGNQLAVLPAGAFARRPPLA ELAALNLSGSRLDEVRAGAFEHLPSLRQLDLSHNPLADLSPFA FSGSNASVSAPSPLVELILNHIVPPEDERQNRSFEGMVVAALLS GLALRGLTRLELASNHFLFLPRDLLAQLPSLRYLDLRNNSLVS LTYASFRNLTHLESLHLEDNALKVLHNSTLAEWHGLAHVKVF LDNNPWVCDCYMADMVAWLKETEVVPDKARLTCAFPEKMR NRGLLDLNSSDLDCDAVLPQSLQTSYVFLGIVLALIGAIFLLVL YLNRKGIKKWMHNIRDACRDHMEGYHYRYEINADPRLTNLS SNSDV	hmc5T4.1 (human residues underlined)
385	SAPSSSVPSSSTSPAAFLASGSAQPPPAERCPAACECSEAARTV KCVNRNLLEVPADLPPYVRNLFLTGNQMTVLPAGAFARQPPL ADLEALNLSGNHLKEVCAGAFEHLPSLRQLDLSHNPLADLSPF AFSGSNASVSAPSPLVELILNHIVPPEDERQNRSFEGMVVAALL AGRALQGLRRLELASNHFLYLPRDVLAQLPSLRHLDLSNNSLV SLTYVSFRNLTHLESLHLEDNALKVLHNSTLAEWHGLAHVKV FLDNNPWVCDCYMADMVAWLKETEVVPDKARLTCAFPEKM RNRGLLDLNSSDLDCDAVLPQSLQTSYVFLGIVLALIGAIFLLV LYLNRKGIKKWMHNIRDACRDHMEGYHYRYEINADPRLTNL SSNSDV	hmc5T4.2 (human residues underlined)
386	SSPTSSASSFSSSAPFLASAVSAQPPLPDQCPALCECSEAARTVK CVNRNLTEVPTDLPAYVRNLFLTGNQMTVLPAGAFARQPPLA DLEALNLSGNHLKEVCAGAFEHLPGLRRLDLSHNPLTNLSAFA FAGSNASVSAPSPLEELILNHIVPPEDQRQNGSFEGMVAFEGM VAAALRSGLALRGLTRLELASNHFLFLPRDLLAQLPSLRYLDL RNNSLVSLTYASFRNLTHLESLHLEDNALKVLHNSTLAEWHG LAHVKVFLDNNPWVCDCYMADMVAWLKETEVVPDKARLTC AFPEKMRNRGLLDLNSSDLDCDAVLPQSLQTSYVFLGIVLALI GAIFLLVLYLNRKGIKKWMHNIRDACRDHMEGYHYRYEINA DPRLTNLSSNSDV	hmc5T4.3 (human residues underlined)
387	SAPSSSVPSSSTSPAAFLASGSAQPPPAERCPAACECSEAARTV KCVNRNLLEVPADLPPYVRNLFLTGNQMTVLPAGAFARQPPL ADLEALNLSGNHLKEVCAGAFEHLPGLRRLDLSHNPLTNLSAF AFAGSNASVSAPSPLEELILNHIVPPEDQRQNGSFEGMVAFEG MVAAALRSGLALRGLTRLELASNHFLFLPRDLLAQLPSLRYLD LRNNSLVSLTYASFRNLTHLESLHLEDNALKVLHNSTLAEWH GLAHVKVFLDNNPWVCDCYMADMVTWLKETEVVQGKDRLT CAYPEKMRNRVLLELNSADLDCDPILPPSLQTSYVFLGIVLALI GAIFLLVLYLNRKGIKKWMHNIRDACRDHMEGYHYRYEINA DPRLTNLSSNSDV	hmc5T4.4 (human residues underlined)
388	EVQLVESGGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQAP GKGLEWVSGISWNSGSIGYADSVKGFTISRDNAKNSLYLQMN SLRAEDTALYYCAKDSRGYGDYRLGGAYWGQGTLVTVSS	Anti-CD3 VH 312557
389	EVQLVESGGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQAP GKCLEWVSGISWNSGSIGYADSVKGFTISRDNAKNSLYLQMN SLRAEDTALYYCAKDSRGYGDYRLGGAYWGQGTLVTVSS	Anti-CD3 VH 312557 G44C

#	SEQUENCE	ANNOTATION
390	EIVMTQSPATLSVSPGERATLSCRASQSVSSNLAWYQQKPGQA PRLLIYGASTRATGIPARFSGSGSGTEFTLTISSLQSEDFAVYYC QQYNNWPWTFGQGTKVEIK	Anti-CD3 VL 312557
391	EIVMTQSPATLSVSPGERATLSCRASQSVSSNLAWYQQKPGQA PRLLIYGASTRATGIPARFSGSGSGTEFTLTISSLQSEDFAVYYC QQYNNWPWTFGCGTKVEIK	Anti-CD3 VL 312557 Q100C
392	EVQLVESGGGLVQPGRSLRLSCVASGFTFDDYSMHWVRQAP GKGLEWVSGISWNSGSKDYADSVKGRFTISRDNAKNSLYLQM NSLRAEDTALYYCAKYGSGYGKFYHYGLDVWGQGTTVTVSS	CD3-VH-G
393	EVQLVESGGGLVQPGRSLRLSCVASGFTFDDYSMHWVRQAP GKCLEWVSGISWNSGSKDYADSVKGRFTISRDNAKNSLYLQM NSLRAEDTALYYCAKYGSGYGKFYHYGLDVWGQGTTVTVSS	CD3-VH-G
394	DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKA PKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYC QQSYSTPPITFGQGTRLEIK	V_{K1}-39Jκ5
395	DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKA PKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYC QQSYSTPPITFGCGTRLEIK	V_{K1}-39Jκ5 Q100C
396	(ADAAP)n n=2-20	linker
397	(ADAAP)n-G n=2-20	linker
398	(GEPQG)n n=2-20	linker
399	(GEPQG)n-G n=2-20	linker
400	(AGGEP)n n=2-20	linker
401	(AGGEP)n-G n=2-20	linker
402	(AGSEP)n n=2-20	linker
403	(AGSEP)n-G n=2-20	linker
404	(GGGEQ)n n=2-20	linker
405	(GGGEQ)n-G n=2-20	linker
406	ADAAPADAAPG	linker
407	GEPQGGEPQGG	linker
408	AGGEPAGGEPG	linker
409	AGSEPAGSEPG	linker
410	GGGEQGGGEQG	linker
411	AYVRNLFLTGNQLAVLPAGAFARRPPLAELAALNLSGSRLDE VRAGAFEHLPS	Amino acids 60-112 of human 5T4 ECD
412	LAGRALQGLRRLELASNHFLYLPRDVLAQLPSLRHLDLSNNSL VSLTYVSFR	Amino acids 173-224 of human 5T4 ECD

Claims

1. A 5T4-binding polypeptide construct, comprising at least one heavy chain only variable domain (5T4 VHH domain) that specifically binds 5T4 and one or more additional binding domain that binds to a target other than 5T4.

2. The 5T4-binding polypeptide construct of claim 1, wherein the at least one 5T4 VHH domain comprises a complementarity determining region 1 (CDR1) comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 86-87 and 288-292, 296, and 297; a complementarity determining region 2 (CDR2) comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 88-99, 298, and 299; and a complementarity determining region 3 (CDR3) comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 100-102, 300, 301, and 303.

3. A 5T4-binding polypeptide construct, comprising at least one heavy chain only variable domain (5T4 VHH domain) comprising a complementarity determining region 1 (CDR1) comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 86-87 and 288-292, 296, and 297; a complementarity determining region 2 (CDR2) comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 88-99, 298, and 299; and a complementarity determining region 3 (CDR3) comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 100-102, 300, 301, and 303.

4. The 5T4-binding polypeptide construct of any of claims 1-3, wherein the 5T4 is a human 5T4.

5. The 5T4-binding polypeptide construct of any of claims 1-4, wherein the at least one 5T4 VHH domain is humanized.

6. The 5T4-binding polypeptide construct of any of claims 1, 2, 4 and 5, wherein the one or more additional binding domains binds to an activating receptor on an immune cell.

7. The 5T4-binding polypeptide construct of claim 6, wherein the immune cell is a T cell.

8. The 5T4-binding polypeptide construct of claim 6 or claim 7, wherein the activating receptor is CD3 (CD3ε).

9. The 5T4-binding polypeptide construct of claim 8 that is bispecific for 5T4 and CD3.

10. The 5T4-binding polypeptide construct of claim 6, wherein the immune cell is a Natural Killer (NK) cell.

11. The 5T4-binding polypeptide construct of claim 6 or claim 10, wherein the activating receptor is CD16 (CD16a).

12. The 5T4-binding polypeptide construct of claim 11 that is bispecific for 5T4 and CD16a.

13. The 5T4-binding polypeptide construct of any of claims 1, 2, 4 and 5, wherein the one or more additional binding domain binds to a cytokine receptor.

14. The 5T4-binding polypeptide construct of any of claims 1, 2 and 4-13, wherein the one or more additional binding domain comprises an antibody or antigen-binding fragment thereof.

15. The 5T4-binding polypeptide construct of any of claims 1, 2 and 4-14, wherein the one or more additional binding domain is monovalent.

16. The 5T4-binding polypeptide construct of claim 14 or claim 15, wherein the antibody or antigen-binding fragment thereof is an Fv, a disulfide-stabilized Fv (dsFv), scFv, a Fab, a single domain antibody (sdAb).

17. The 5T4-binding polypeptide construct of claim 13, wherein the one or more additional binding domain is a cytokine or is a truncated fragment or variant thereof capable of binding to the cytokine receptor.

18. The 5T4-binding polypeptide construct of claim 17, wherein the cytokine is an interferon, or is a truncated fragment or variant of thereof.

19. The 5T4-binding polypeptide construct of claim 18, wherein the interferon is a type I interferon or a type II interferon, is a truncated fragment or variant of a type I interferon or is a truncated fragment or variant of a type II interferon.

20. The 5T4-binding polypeptide construct of claim 19, wherein:

the type I interferon is an IFN-alpha or an IFN-beta or is a truncated fragment or variant thereof; or

the type II interferon is an IFN-gamma or is a truncated fragment or variant thereof.

21. The 5T4-binding polypeptide construct of any of claims 1-20, wherein the polypeptide construct comprises an immunoglobulin Fc region.

22. The 5T4-binding polypeptide construct of any of claims 1, 2 and 4-21, wherein the polypeptide comprises an immunoglobulin Fc region that links the at least one 5T4 VHH domain and the one or more additional binding domain.

23. The 5T4-binding polypeptide construct of any of claims 1-22 that is a dimer.

24. The 5T4-binding polypeptide construct of any of claims 21-23, wherein the Fc region is a homodimeric Fc region.

25. The 5T4-binding polypeptide construct of any of claims 21-24, wherein the Fc region comprises the sequence of amino acids set forth in any of SEQ ID NOS: 8, 10, 11, 12 or 13, or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to any of SEQ ID NOS: 8, 10, 11, 12 or 13.

26. The 5T4-binding polypeptide construct of any of claims 21-25, wherein the Fc region is a human IgG1.

27. The 5T4-binding polypeptide construct of claim 26, wherein the Fc region comprises the sequence of amino acids set forth in SEQ ID NO:8 or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 8.

28. The 5T4-binding polypeptide construct of any of claims 21-23, wherein the Fc region is a heterodimeric Fc region.

29. The 5T4-binding polypeptide construct of any of claims 21-28, wherein the Fc region exhibits effector function.

30. The 5T4-binding polypeptide construct of any of claims 21-29, wherein the Fc region comprises a polypeptide comprising one or more amino acid modification that reduces effector function and/or reduces binding to an effector molecule selected from an Fc gamma receptor or C1q.

31. The 5T4-binding polypeptide construct of claim 30, wherein the one or more amino acid modification is deletion of one or more of Glu233, Leu234 or Leu235.

32. The 5T4-binding polypeptide construct of claim 30 or claim 31, wherein the Fc region comprises the sequence of amino acids set forth in SEQ ID NO:9 or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 9.

33. The 5T4-binding polypeptide construct of any of claims 1-32, wherein the at least one 5T4 VHH domain comprises the sequence set forth in any of SEQ ID NOS: 245-253, 255-287, 294, 295, 302, and 360 or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to any of SEQ ID NOS: 245-253, 255-287, 294, 295, 302, and 360 and binds 5T4.

34. The 5T4-binding polypeptide construct of any of claims 1-33, wherein the at least one 5T4 VHH domain binds to an epitope in human 5T4 but does not exhibit crossreactive binding to mouse 5T4.

35. The 5T4-binding polypeptide construct of any of claims 1-34, wherein the at least one 5T4 VHH domain binds to amino acid residues between amino acids 60 and 112 of SEQ ID NO:382.

36. The 5T4-binding polypeptide construct of any of claims 1-35, wherein the at least one 5T4 VHH domain binds to amino acid residues between amino acids 173 and 224 of SEQ ID NO:382.

37. The 5T4-binding polypeptide construct of any of claims 1-36, wherein the at least one 5T4 VHH domain comprises the sequence set forth in (i) SEQ ID NO:245, (ii) a humanized variant of SEQ ID NO:245, or (iii) a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO:245 and binds 5T4.

38. The 5T4-binding polypeptide construct of any of claims 1-37, wherein the at least one 5T4 VHH domain comprises a CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 288 and 289; a CDR2 comprising an amino acid sequence set forth in SEQ ID NO: 88; and a CDR3 comprising an amino acid sequence set forth in SEQ ID NO: 100.

39. The 5T4-binding polypeptide construct of any of claims 1-38, wherein the at least one 5T4 VHH domain comprises a CDR1, CDR2 and CDR3 set forth in SEQ ID NOS: 288, 88, and 100, respectively; or SEQ ID NOS: 289, 88, and 100, respectively.

40. The 5T4-binding polypeptide construct of any of claims 1-39, wherein the at least one 5T4 VHH domain comprises the sequence of amino acids set forth in any one of SEQ ID NOs: 246-253 and 360 or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 246-253 and 360 and binds 5T4.

41. The 5T4-binding polypeptide construct of claims 1-40, wherein the at least one 5T4 VHH domain comprises the sequence of amino acids set forth in any one of SEQ ID NOS: 246-253 and 360.

42. The 5T4-binding polypeptide construct of any of claims 1-36, wherein the at least one 5T4 VHH domain comprises the sequence set forth in (i) SEQ ID NO:255, (ii) a humanized variant of SEQ ID NO: 255, or (iii) a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 255 and binds 5T4.

43. The 5T4-binding polypeptide construct of any of claims 1-36 and 42, wherein the at least one 5T4 VHH domain comprises a CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 86, 290-292; a CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 89-94; and a CDR3 comprising an amino acid sequence set forth in SEQ ID NO: 101.

44. The 5T4-binding polypeptide construct of any of claims 1-36, 42 and 43, wherein the at least one 5T4 VHH domain comprises a CDR1, CDR2 and CDR3 set forth in SEQ ID NOS: 290, 90, and 101, respectively; SEQ ID NOS: 290, 91, and 101, respectively; SEQ ID NOS: 290, 92, and 101, respectively; SEQ ID NOS: 290, 93, and 101, respectively; SEQ ID NOS: 290, 94, and 101, respectively; SEQ ID NOS: 291, 94, and 101, respectively; SEQ ID NOS: 292, 94, and 101, respectively; or SEQ ID NOS: 86, 94, and 101, respectively.

45. The 5T4-binding polypeptide construct of any of claims 1-36 and 42-44, wherein the at least one 5T4 VHH domain comprises the sequence of amino acids set forth in any one of SEQ ID NOs:

256-275 or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 256-275 and binds 5T4.

46. The 5T4-binding polypeptide construct of claims 1-36 and 43-45, wherein the at least one 5T4 VHH domain comprises the sequence of amino acids set forth in any one of SEQ ID NOS: 256-275.

47. The 5T4-binding polypeptide construct of any of claims 1-36, wherein the at least one 5T4 VHH domain comprises the sequence set forth in (i) SEQ ID NO:276 (ii) a humanized variant of SEQ ID NO: 276, or (iii) a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 276 and binds 5T4.

48. The 5T4-binding polypeptide constructof any of claims 1-33 and 47, wherein the at least one 5T4 VHH domain comprises a CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 86 and 87; a CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 95-99; and a CDR3 comprising an amino acid sequence set forth in SEQ ID NO: 102.

49. The 5T4-binding polypeptide construct of any of claims 1-36, 47 and 48, wherein the at least one 5T4 VHH domain comprises a CDR1, CDR2 and CDR3 set forth in SEQ ID NOS: 87, 95, and 102, respectively; SEQ ID NOS: 87, 96, and 102, respectively; SEQ ID NOS: 87, 97, and 102, respectively; SEQ ID NOS: 87, 98, and 102, respectively; SEQ ID NOS: 87, 99, and 102, respectively; or SEQ ID NOS: 86, 98, and 102, respectively.

50. The 5T4-binding polypeptide construct of any of claims 1-36 and 47-49, wherein the at least one 5T4 VHH domain comprises the sequence of amino acids set forth in any one of SEQ ID NOs: 277-287 or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 277-287 and binds 5T4.

51. The 5T4-binding polypeptide construct of claims 1-36 and 47-50, wherein the at least one 5T4 VHH domain comprises the sequence of amino acids set forth in any one of SEQ ID NOS: 277-287.

52. The 5T4-binding polypeptide construct of any of claims 1-36, wherein the at least one 5T4 VHH domain comprises the sequence set forth in (i) SEQ ID NO:294 (ii) a humanized variant of

SEQ ID NO:294, or (iii) a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO:294 and binds 5T4.

53. The 5T4-binding polypeptide construct of any of claims 1-36 and 52, wherein the at least one 5T4 VHH domain comprises a CDR1 comprising an amino acid sequence set forth in SEQ ID NO: 296; a CDR2 comprising an amino acid sequence set forth in SEQ ID NO: 298; and a CDR3 comprising an amino acid sequence set forth in SEQ ID NO: 300.

54. The 5T4-binding polypeptide construct of any of claims 1-36 and 52, wherein the at least one 5T4 VHH domain comprises a CDR3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 300, 301, and 303.

55. The 5T4-binding polypeptide construct of any of claims 1-36 and 52, wherein the at least one 5T4 VHH domain comprises:

a CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 288, 296, and 297; and/or

a CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 88, 298, and 299.

56. The 5T4-binding polypeptide construct of any of claims 1-36, wherein the at least one 5T4 VHH domain comprises the sequence set forth in (i) SEQ ID NO:295 (ii) a humanized variant of SEQ ID NO:295, or (iii) a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO:295 and binds 5T4.

57. The 5T4-binding polypeptide construct of any of claims 1-36 and 56, wherein the at least one 5T4 VHH domain comprises a CDR1 comprising an amino acid sequence set forth in SEQ ID NO: 297; a CDR2 comprising an amino acid sequence set forth in SEQ ID NO: 299; and a CDR3 comprising an amino acid sequence set forth in SEQ ID NO: 301.

58. The 5T4-binding polypeptide construct of any of claims 1-36, wherein the at least one 5T4 VHH domain comprises the sequence set forth in (i) SEQ ID NO:302 (ii) a humanized variant of SEQ ID NO: 302, or (iii) a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 302 and binds 5T4.

59. The 5T4-binding polypeptide construct of any of claims 1-36 and 56, wherein the at least one 5T4 VHH domain comprises a CDR1 comprising an amino acid sequence set forth in SEQ ID NO: 288; a CDR2 comprising an amino acid sequence set forth in SEQ ID NO: 88; and a CDR3 comprising an amino acid sequence set forth in SEQ ID NO: 303.

60. The 5T4-binding polypeptide construct of any of claims 1-36, wherein the at least one 5T4 VHH domain comprises the sequence set forth in (i) SEQ ID NO:294, 295, or 302 (ii) a humanized variant of SEQ ID NO: 294, 295, or 302, or (iii) a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 294, 295, or 302 and binds 5T4.

61. The 5T4-binding polypeptide construct of any of claims 1-36 and 60, wherein the at least one 5T4 VHH domain comprises a CDR1, CDR2 and CDR3 set forth in SEQ ID NOS: 296, 298, and 300, respectively; SEQ ID NOS:297, 299, and 301, respectively; or SEQ ID NOS:288, 88, and 303, respectively.

62. The 5T4-binding polypeptide construct of any of claims 1-36, 60 and 61, wherein the at least one 5T4 VHH domain is set forth in SEQ ID NO:245, 249, 255, 270, 276, 294, 295 or 302.

63. A multispecific polypeptide construct, comprising: (a) a first component comprising a heterodimeric Fc region comprising a first Fc polypeptide and a second Fc polypeptide and (b) a second component comprising an anti-CD3 antibody or antigen-binding fragment comprising a variable heavy chain region (VH) and a variable light chain region (VL), wherein:

the VH and VL that comprise the anti-CD3 antibody or antigen binding fragment are linked to opposite polypeptides of the heterodimeric Fc;

the first and second components are coupled by a linker, wherein the heterodimeric Fc region is positioned N-terminal to the anti-CD3 antibody; and

one or both of the first and second components comprises at least one heavy chain only variable domain (5T4 VHH domain).

64. The multispecific polypeptide construct of claim 63, wherein the multispecific polypeptide construct comprises at least (i) a first polypeptide comprising the first Fc polypeptide of the heterodimeric Fc region, the linker and the VH or VL domain of the anti-CD3 antibody or antigen binding fragment; and (ii) a second polypeptide comprising the second Fc polypeptide of the

heterodimeric Fc region, the linker, optionally the same linker as present in the first polypeptide, and the other of the VH or VL domain of the anti-CD3 antibody or antigen binding fragment,

wherein one or both of the first and second polypeptide comprise the at least one 5T4 VHH domain.

65. The multispecific polypeptide construct of claim 63 or claim 64, wherein one or both of the first and second Fc polypeptides of the heterodimeric Fc region comprises at least one modification to induce heterodimerization compared to a polypeptide of a homodimeric Fc region, optionally compared to the Fc polypeptide set forth in SEQ ID NO: 8 or an immunologically active fragment thereof.

66. The multispecific polypeptide construct of claim 65, wherein each of the first and second Fc polypeptides of the heterodimeric Fc region independently comprise at least one amino acid modification.

67. The multispecific polypeptide construct of claim 65 or 66, wherein each of the first and second Fc polypeptides of the heterodimeric Fc region comprise a knob-into-hole modification or comprise a charge mutation to increase electrostatic complementarity of the polypeptides.

68. The multispecific polypeptide construct of claim 65-67, wherein the amino acid modification is a knob-into-hole modification.

69. The multispecific polypeptide construct of any of claims 63-68, wherein the first Fc polypeptide of the heterodimeric Fc region comprises the modification selected from among Thr366Ser, Leu368Ala, Tyr407Val, and combinations thereof and the second Fc polypeptide of the heterodimeric Fc region comprises the modification Thr366Trp.

70. The multispecific polypeptide of claim 69, wherein the first and second Fc polypeptides further comprise a modification of a non-cysteine residue to a cysteine residue, wherein the modification of the first Fc polypeptide is at one of the position Ser354 and Tyr349 and the modification of the second Fc polypeptide is at the other of the position Ser354 and Tyr349.

71. The multispecific polypeptide construct of any of claims 65-67, wherein the amino acid modification is a charge mutation to increase electrostatic complementarity of the polypeptides.

72. The multispecific polypeptide construct of any of claims 63-67 and 71, wherein the first and/or second Fc polypeptides or each of the first and second Fc polypeptide comprise an amino acid

modification in complementary positions, wherein the modification is replacement with an amino acid having an opposite charge to the complementary amino acid of the other polypeptide.

73. The multispecific polypeptide construct of any of claims 63-72, wherein one of the first or second Fc polypeptide of the heterodimeric Fc region further comprises a modification at residue Ile253.

74. The multispecific polypeptide construct of claim 73, wherein the modification is Ile253Arg.

75. The multispecific polypeptide construct of any of claims 63-74, wherein one of the first or second Fc polypeptide of the heterodimeric Fc region further comprises a modification at residue His435.

76. The multispecific polypeptide construct of claim 75, wherein the modification is His435Arg.

77. The multispecific polypeptide construct of any of claims 63-76, wherein the Fc region comprises a polypeptide that lacks Lys447.

78. The multispecific polypeptide construct of any of claims 63-77, wherein the Fc region comprises a polypeptide comprising at least one modification to enhance FcRn binding.

79. The multispecific polypeptide construct of claim 78, wherein the at least one modification is at a position selected from the group consisting of Met252, Ser254, Thr256, Met428, Asn434, and combinations thereof.

80. The multispecific polypeptide construct of claim 78or claim 79, wherein the at least one modification is selected from the group consisting of Met252Y, Ser254T, Thr256E, Met428L, Met428V, Asn434S, and combinations thereof.

81. The multispecific polypeptide construct of any of claims 78-80, wherein the at least one modification is at position Met252 and at position Met428.

82. The multispecific polypeptide construct of any of claims 78-81, wherein the at least one modification is Met252Y and Met428L.

83. The multispecific polypeptide construct of any of claims 78-81, wherein the at least one modification is Met252Y and Met428V.

84. The multispecific polypeptide construct of any of claims 63-83, wherein the first Fc polypeptide of the heterodimeric Fc region comprises the sequence of amino acids set forth in any of SEQ ID NOS:103, 107, 115, 117, 328, or 334 and the second Fc polypeptide of the heterodimeric Fc region comprises the sequence of amino acids set forth in any of SEQ ID NOS:104, 108, 111, 113, 119, 121, 329, 332, or 336.

85. The polypeptide construct of any of claims 21-84, wherein the Fc region comprises a polypeptide comprising at least one amino acid modification that reduces effector function and/or reduces binding to an effector molecule selected from an Fc gamma receptor and C1q.

86. The multispecific polypeptide construct of claim 85, wherein the at least one amino acid modification is deletion of one or more of Glu233, Leu234 and Leu235.

87. The multispecific polypeptide construct of any of claims 63-86, wherein the first Fc polypeptide of the heterodimeric Fc region comprises the sequence of amino acids set forth in any of SEQ ID NOS: 105, 109, 116, 118, 330, or 335 and the second Fc polypeptide of the heterodimeric Fc region comprises the sequence of amino acids set forth in any of SEQ ID NOS: 106, 110, 112, 114, 120, 122, 331, 333, or 337.

88. The multispecific polypeptide construct of any of claim 63-87, wherein the anti-CD3 antibody or antigen binding fragment is monovalent.

89. The multispecific polypeptide construct of any of claims 63-88, wherein the anti-CD3 antibody or antigen binding fragment is not a single chain antibody, optionally is not a single chain variable fragment (scFv).

90. The multispecific polypeptide construct of any of claims 63-89, wherein the anti-CD3 antibody or antigen binding fragment is an Fv antibody fragment.

91. The multispecific polypeptide construct of claim 90, wherein the Fv antibody fragment comprises a disulfide stabilized anti-CD3 binding Fv fragment (dsFv).

92. The multispecific polypeptide construct of 63-91, wherein the anti-CD3 antibody or antigen-binding fragment comprises a VH CDR1 comprising the amino acid sequence TYAMN (SEQ ID NO: 29); a VH CDR2 comprising the amino acid sequence RIRSKYNNYATYYADSVKD (SEQ ID NO: 30); a VH CDR3 comprising the amino acid sequence HGNFGNSYVSWFAY (SEQ ID NO: 31), a VL CDR1 comprising the amino acid sequence RSSTGAVTTSNYAN (SEQ ID NO: 32); a VL CDR2 comprising the amino acid sequence GTNKRAP (SEQ ID NO: 33); and a VL CDR3 comprising the amino acid sequence ALWYSNLWV (SEQ ID NO: 34).

93. The multispecific polypeptide construct of any of claims 63-92, wherein the anti-CD3 antibody or antigen-binding fragment comprises:

a VH having the amino acid sequence of any of SEQ ID NOS: 27, 35-65, 341, 343, and 358 or a sequence that exhibits at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to any of SEQ ID NOS: 27, 35-65, 341, 343, and 358 and binds CD3; and

a VL having the amino acid sequence of any of SEQ ID NOS: 28, 66-84, 293, 340, and 342 or a sequence that exhibits at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to any of SEQ ID NOS: 28, 66-84, 293, 340, and 342 and 293 and binds CD3.

94. The multispecific polypeptide construct of any of claims 63-93, wherein the anti-CD3 antibody or antigen-binding fragment comprises the amino acid sequence of SEQ ID NO: 47 and the amino acid sequence of SEQ ID NO: 75.

95. The multispecific polypeptide construct of any of claims 63-93, wherein the anti-CD3 antibody or antigen-binding fragment comprises the amino acid sequence of SEQ ID NO: 47 and the amino acid sequence of SEQ ID NO: 293.

96. The multispecific polypeptide construct of any of claim 63-92, wherein the at least one 5T4 VHH domain is positioned amino-terminally relative to the Fc region and/or carboxy-terminally relative to the CD3 binding region of the multispecific polypeptide construct.

97. The multispecific polypeptide construct of any of claims 63-96, wherein the multispecific polypeptide construct comprises a first 5T4 VHH domain that specifically binds 5T4 and a second 5T4 VHH domain that specifically binds 5T4.

98. The multispecific polypeptide construct of claim 97, wherein the first or second 5T4 VHH domain is positioned amino-terminally relative to the Fc region of the multispecific construct and

the other of the first or second 5T4 VHH domain is positioned carboxy-terminally relative to the CD3 binding region of the multispecific construct.

99. The multispecific polypeptide construct of claim 97 or claim 98, wherein

the first component comprises in order of N-terminus to C-terminus a first 5T4 VHH domain that binds 5T4, the first Fc polypeptide of the heterodimeric Fc region, the linker, the VH or VL domain of the anti-CD3 antibody or antigen binding fragment and a second 5T4 VHH domain that binds 5T4; and

the second component comprises in order of N-terminus to C-terminus the second Fc polypeptide of the heterodimeric Fc region, the linker, optionally the same linker as present in the first component, and the other of the VH or VL domain of the anti-CD3 antibody or antigen binding fragment.

100. The multispecific polypeptide construct of any of claims 97-99, wherein the first and second 5T4 VHH domain are the same.

101. The multispecific polypeptide construct of any of claims 97-99, wherein the first and second 5T4 VHH domain are different.

102. The multispecific polypeptide construct of claim 101, wherein the first and second 5T4 VHH domain bind a distinct or non-overlapping epitope of 5T4 and/or do not compete for binding to 5T4.

103. The multispecific polypeptide construct of claim 102, wherein:

the first 5T4 VHH domain comprises the amino acid sequence set forth in any one of SEQ ID NOS: 245-253, 295, 302, and 360 a humanized variant thereof, or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to any of SEQ ID NOS: 245-253, 295, 302, and 360 and binds 5T4; and

the second 5T4 VHH domain comprises the amino acid sequence set forth in any one of SEQ ID NOS: 255-287, 294, 302, a humanized variant thereof, or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to any of SEQ ID NOS: 255-287, 294, 302, and binds 5T4.

104. The multispecific polypeptide construct of claim 102, wherein:

the first and second 5T4 VHH domains are selected from SEQ ID NO: 245 and SEQ ID NO: 294; SEQ ID NO: 245 and SEQ ID NO: 276; SEQ ID NO: 245 and SEQ ID NO: 255; SEQ ID NO:245 and SEQ ID NO:295; SEQ ID NO: 295 and SEQ ID NO: 294; SEQ ID NO: 249 and SEQ ID NO: 270; SEQ ID NO:302 and SEQ ID NO:302; or SEQ ID NO: 360 and SEQ ID NO: 287.

105. The multispecific polypeptide construct of any of claims 63-104, wherein the at least one 5T4 VHH domain, or each of the first and second 5T4 VHH domain, independently comprises the VHH domain sequence set forth in any of SEQ ID NOS: 245-253, 255-287, 294, 295, 302, and 360 or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to any of SEQ ID NOS: 245-253, 255-287, 294, 295, 302, and 360 and binds 5T4.

106. The multispecific polypeptide construct of any of claims 63-105, wherein the at least one 5T4 VHH domain binds to an epitope in human 5T4 but does not exhibit crossreactive binding to mouse 5T4.

107. The multispecific polypeptide construct of any of claims 63-106, wherein the at least one 5T4 VHH domain binds to amino acid residues between amino acids 60 and 112 of SEQ ID NO:382.

108. The multispecific polypeptide construct of any of claims 63-107, wherein the at least one 5T4 VHH domain binds to amino acid residues between amino acids 173 and 224 of SEQ ID NO:382.

109. The multispecific polypeptide construct of any of claims 63-108, wherein the at least one 5T4 VHH domain, or each of the first and second 5T4 VHH domain, independently comprises the sequence set forth in (i) SEQ ID NO: 245, (ii) a humanized variant of SEQ ID NO: 245, or (iii) a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 245, and binds 5T4.

110. The multispecific polypeptide construct of any of claims 63-109, wherein the at least one 5T4 VHH domain, or each of the first and second 5T4 VHH domain, independently comprises a CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 86-87 and 288-292, 296, and 297; a CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 88-99, 298, and 299; and a CDR3 comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 100-102, 300, 301, and 303.

111. The multispecific construct of any of claims 63-110, wherein the at least one 5T4 VHH domain, or each of the first and second 5T4 VHH domain, independently comprises a CDR1, CDR2 and CDR3 set forth in SEQ ID NOS: 288, 88, and 100, respectively; SEQ ID NOS: 289, 88, and 100, respectively; SEQ ID NOS: 290, 89, and 101, respectively; SEQ ID NOS: 290, 90, and 101, respectively;

SEQ ID NOS: 290, 91, and 101, respectively; SEQ ID NOS: 290, 92, and 101, respectively; SEQ ID NOS: 290, 93, and 101, respectively; SEQ ID NOS: 290, 94, and 101, respectively; SEQ ID NOS: 291, 94, and 101, respectively; SEQ ID NOS: 292, 94, and 101, respectively; SEQ ID NOS: 86, 94, and 101, respectively; SEQ ID NOS: 87, 95, and 102, respectively; SEQ ID NOS: 87, 96, and 102, respectively; SEQ ID NOS: 87, 97, and 102, respectively; SEQ ID NOS: 87, 98, and 102, respectively; SEQ ID NOS: 87, 99, and 102, respectively; SEQ ID NOS: 86, 98, and 102, respectively; SEQ ID NOS: 296, 298, and 300, respectively; SEQ ID NOS: 297, 299, and 301, respectively; or SEQ ID NOS: 288, 88, and 303, respectively.

112. The multispecific polypeptide construct of any of claims 63-111, wherein the at least one 5T4 VHH domain, or each of the first and second 5T4 VHH domain, independently comprises the sequence of amino acids set forth in any one of SEQ ID NOS: 246-253 and 360 or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NOS: 246-253 and 360, and binds 5T4.

113. The multispecific polypeptide construct of any of claims 63-112, wherein the at least one 5T4 VHH domain, or each of the first and second 5T4 VHH domain, independently comprises the sequence of amino acids set forth in any one of SEQ ID NOS: 246-253 and 360.

114. The multispecific polypeptide construct of any of claims 63-108, wherein the at least one 5T4 VHH domain, or each of the first and second 5T4 VHH domain, independently comprises the sequence set forth in (i) SEQ ID NO: 255, (ii) a humanized variant of SEQ ID NO: 255, or (iii) a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 255, and binds 5T4.

115. The multispecific polypeptide construct of any of claims 63-108 and 114, wherein the at least one 5T4 VHH domain, or each of the first and second 5T4 VHH domain, independently comprises a CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 86, 290-292; a CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 89-94; and a CDR3 comprising an amino acid sequence set forth in SEQ ID NO: 101.

116. The multispecific polypeptide construct of any of claims 63-108, 114 and 115, wherein the at least one 5T4 VHH domain, or each of the first and second 5T4 VHH domain, independently comprises a CDR1, CDR2 and CDR3 set forth in SEQ ID NOS: 290, 89, and 101, respectively; SEQ ID NOS: 290, 90, and 101, respectively; SEQ ID NOS: 290, 91, and 101, respectively; SEQ ID NOS: 290, 92, and 101, respectively; SEQ ID NOS: 290, 93, and 101, respectively; SEQ ID NOS: 290, 94, and 101,

respectively; SEQ ID NOS: 291, 94, and 101, respectively; SEQ ID NOS: 292, 94, and 101, respectively; or SEQ ID NOS: 86, 94, and 101, respectively.

117. The multispecific polypeptide construct of any of claims 63-108 and 114-116, wherein the at least one 5T4 VHH domain, or each of the first and second 5T4 VHH domain, independently comprises the sequence of amino acids set forth in any one of SEQ ID NOs: 256-275 or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 256-275, and binds 5T4.

118. The multispecific polypeptide construct of claims 63-108 and 114-117, wherein the at least one 5T4 VHH domain, or each of the first and second 5T4 VHH domain, independently comprises the sequence of amino acids set forth in any one of SEQ ID NOS: 256-275.

119. The multispecific polypeptide construct of any of claims 63-108, wherein the at least one 5T4 VHH domain, or each of the first and second 5T4 VHH domain, independently comprises the sequence set forth in (i) SEQ ID NO: 276 (ii) a humanized variant of SEQ ID NO: 276, or (iii) a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 276, and binds 5T4.

120. The multispecific polypeptide of any of claims 63-108 and 119, wherein the at least one 5T4 VHH domain, or each of the first and second 5T4 VHH domain, independently comprises a CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 86 and 87; a CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 95-99; and a CDR3 comprising an amino acid sequence set forth in SEQ ID NO: 102.

121. The multispecific polypeptide construct of any of claims 63-108, 119 and 120, wherein the at least one 5T4 VHH domain, or each of the first and second 5T4 VHH domain, independently comprises a CDR1, CDR2 and CDR3 set forth in SEQ ID NOS: 87, 95, and 102, respectively; SEQ ID NOS: 87, 96, and 102, respectively; SEQ ID NOS: 87, 97, and 102, respectively; SEQ ID NOS: 87, 98, and 102, respectively; SEQ ID NOS: 87, 99, and 102, respectively; or SEQ ID NOS: 86, 98, and 102, respectively.

122. The multispecific polypeptide construct of any of claims 63-108 and 119-121, wherein the at least one 5T4 VHH domain, or each of the first and second 5T4 VHH domain, independently comprises the sequence of amino acids set forth in any one of SEQ ID NOs: 277-287 or a sequence of

amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 277-287, and binds 5T4.

123. The multispecific polypeptide construct of claims 63-108 and 119-122, wherein the at least one 5T4 VHH domain, or each of the first and second 5T4 VHH domain, independently comprises the sequence of amino acids set forth in any one of SEQ ID NOS: 277-287.

124. The multispecific polypeptide construct of any of claims 63-108, wherein the at least one 5T4 VHH domain, or each of the first and second 5T4 VHH domain, independently comprises the sequence set forth in (i) SEQ ID NO: 294 (ii) a humanized variant of SEQ ID NO: 294, or (iii) a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 294, and binds 5T4.

125. The multispecific polypeptide construct of any of claims 63-108 and 124, wherein the at least one 5T4 VHH domain, or each of the first and second 5T4 VHH domain, independently comprises a CDR1 comprising an amino acid sequence set forth in SEQ ID NO: 296; a CDR2 comprising an amino acid sequence set forth in SEQ ID NO: 298; and a CDR3 comprising an amino acid sequence set forth in SEQ ID NO: 300.

126. The multispecific polypeptide construct of any of claims 63-106, wherein the at least one 5T4 VHH domain, or each of the first and second 5T4 VHH domain, independently comprises the sequence set forth in (i) SEQ ID NO: 295 (ii) a humanized variant of SEQ ID NO: 295, or (iii) a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 295, and binds 5T4.

127. The multispecific polypeptide construct of any of claims 63-106 and 126, wherein the at least one 5T4 VHH domain, or each of the first and second 5T4 VHH domain, independently comprises a CDR1 comprising an amino acid sequence set forth in SEQ ID NO: 297; a CDR2 comprising an amino acid sequence set forth in SEQ ID NO: 299; and a CDR3 comprising an amino acid sequence set forth in SEQ ID NO: 301.

128. The multispecific polypeptide construct of any of claims 63-106, wherein the at least one 5T4 VHH domain, or each of the first and second 5T4 VHH domain, independently comprises the sequence set forth in (i) SEQ ID NO: 302 (ii) a humanized variant of SEQ ID NO: 302, or (iii) a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 302, and binds 5T4.

129. The multispecific polypeptide construct of any of claims 63-106 and 126, wherein the at least one 5T4 VHH domain, or each of the first and second 5T4 VHH domain, independently comprises a CDR1 comprising an amino acid sequence set forth in SEQ ID NO: 288; a CDR2 comprising an amino acid sequence set forth in SEQ ID NO: 88; and a CDR3 comprising an amino acid sequence set forth in SEQ ID NO: 303.

130. The multispecific polypeptide construct of any of claims 63-106, wherein the at least one 5T4 VHH domain, or each of the first and second 5T4 VHH domain, independently comprises the sequence set forth in (i) SEQ ID NO: 294, 295, or 302 (ii) a humanized variant of SEQ ID NO: 294, 295, or 302, or (iii) a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 294, 295, or 302, and binds 5T4.

131. The multispecific polypeptide construct of any of claims 63-106 and 130, wherein the at least one 5T4 VHH domain, or each of the first and second 5T4 VHH domain, independently comprises a CDR1, CDR2 and CDR3 set forth in SEQ ID NOS: 296, 298, and 300, respectively; SEQ ID NOS:297, 299, and 301, respectively; or SEQ ID NOS:288, 88, and 303, respectively.

132. The multispecific polypeptide construct of any of claims 63-106, 130 and 131, wherein the at least one 5T4 VHH domain, or each of the first and second 5T4 VHH domain, independently is set forth in SEQ ID NO: 245, 249, 255, 270, 276, 294, 295, 302, or 360.

133. The multispecific polypeptide construct of any of claims 63-132, wherein one or both of the first and second components comprises at least one co-stimulatory receptor binding region (CRBR) that binds a co-stimulatory receptor.

134. The multispecific polypeptide construct of claim 133, wherein the at least one co-stimulatory receptor binding region (CRBR) is positioned amino-terminally relative to the Fc region and/or carboxy-terminally relative to the CD3 binding region of the multispecific polypeptide construct.

135. The multispecific polypeptide construct of claim 133 or claim 134, wherein the multispecific polypeptide construct comprises only one co-stimulatory receptor binding region (CRBR).

136. The multispecific polypeptide construct of any of claims 133-135, wherein the multispecific polypeptide construct comprises two co-stimulatory receptor binding region (CRBR), optionally which are the same or different.

137. The multispecific polypeptide construct of any of claims 133-136, wherein the at least one co-stimulatory receptor binding region (CRBR) is or comprises the extracellular domain or binding fragment thereof of the native cognate binding partner of the co-stimulatory receptor, or a variant thereof that exhibits binding activity to the co-stimulatory receptor.

138. The multispecific polypeptide construct of any of claims 133-136, wherein the at least one co-stimulatory receptor binding region (CRBR) is an antibody or antigen-binding fragment thereof selected from the group consisting of a Fab fragment, a F(ab')2 fragment, an Fv fragment, a scFv, a scAb, a dAb, a single domain heavy chain antibody, and a single domain light chain antibody.

139. The multispecific polypeptide construct of claim 138, wherein the antibody or antigen-binding fragment thereof is a Fv, a scFv, a Fab, or a single domain antibody (sdAb).

140. The multispecific polypeptide construct of claim 138 or claim 139, wherein the antibody or antigen-binding fragment is an sdAb.

141. The multispecific polypeptide construct of claim 140, wherein the sdAb is a human or humanized sdAb.

142. The multispecific polypeptide construct of any of claims 133-141, wherein the at least one co-stimulatory receptor binding region (CRBR) binds a co-stimulatory receptor selected from among 41BB (CD137), OX40 (CD134), CD27, glucocorticoid-induced TNFR-related protein (GITR), CD28, ICOS, CD40, B-cell activating factor receptor (BAFF-R), B-cell maturation antigen (BCMA), Transmembrane activator and CAML interactor (TACI), and NKG2D.

143. The multispecific polypeptide construct of any of claims 133-142, wherein the at least one co-stimulatory receptor binding region (CRBR) binds a co-stimulatory receptor selected from among 41BB (CD137), OX40 (CD134), and glucocorticoid-induced TNFR-related protein (GITR).

144. The multispecific polypeptide construct of any of claims 133-143, wherein the at least one co-stimulatory receptor binding region (CRBR) comprises the sequence of amino acids set forth in SEQ ID NO:210 or a sequence that has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%,

95%, 96%, 97%, 98%, or 99% sequence identity to the sequence set forth in SEQ ID NO:210 and binds 4-1BB.

145. The multispecific polypeptide construct of any of claims 63-144, wherein one or both of the first and second components comprises at least one inhibitory receptor binding region (IRBR) that binds an inhibitory receptor.

146. The multispecific polypeptide construct of claim 145, wherein the at least one inhibitory receptor binding region (IRBR) is positioned amino-terminally relative to the Fc region and/or carboxy-terminally relative to the CD3 binding region of the multispecific polypeptide construct.

147. The multispecific polypeptide construct of claim 145 or claim 146, wherein the multispecific polypeptide construct comprises only one inhibitory receptor binding region (IRBR).

148. The multispecific polypeptide construct of any of claims 145-147, wherein:

the first component comprises in order of N-terminus to C-terminus a first 5T4 VHH domain that binds 5T4, the first Fc polypeptide of the heterodimeric Fc region, the linker, the VH or VL domain of the anti-CD3 antibody or antigen binding fragment and a second 5T4 VHH domain that binds 5T4; and

the second component comprises the IRBR and comprises in order of N-terminus to C-terminus the second Fc polypeptide of the heterodimeric Fc region, the linker, optionally the same linker as present in the first component, the other of the VH or VL domain of the anti-CD3 antibody or antigen binding fragment, wherein the IRBR is positioned amino-terminally relative to the Fc region and/or carboxy-terminally relative to the anti-CD3 antibody or antigen-binding fragment of the second component.

149. The multispecific polypeptide construct of any of claims 145-148, wherein the at least one IRBR is or comprises the extracellular domain or binding fragment thereof of the native cognate binding partner of the inhibitory receptor, or a variant thereof that exhibits binding activity to the inhibitory receptor.

150. The multispecific polypeptide construct of any of claims 139-142, wherein the at least one IRBR is an antibody or antigen-binding fragment thereof selected from the group consisting of a Fab fragment, a F(ab')2 fragment, an Fv fragment, a scFv, a scAb, a dAb, a single domain heavy chain antibody, and a single domain light chain antibody.

151. The multispecific polypeptide construct of claim 150, wherein the antibody or antigen-binding fragment thereof is a Fv, a scFv, a Fab, a single domain antibody (sdAb).

152. The multispecific polypeptide construct of claim 150 or claim 151, wherein the antibody or antigen-binding fragment is an sdAb.

153. The multispecific polypeptide construct of claim 152, wherein the sdAb is a human or humanized sdAb.

153. The multispecific polypeptide construct of any of claims 145-153, wherein the at least one IRBR binds a inhibitory receptor selected from among PD-1, CTLA-4, TIGIT, VISTA and TIM3.

155. The multispecific polypeptide construct of any of claims 145-154, wherein the at least one IRBR binds PD-1.

156. The multispecific polypeptide construct of any of claims 145-155, wherein:

the first component comprises in order of N-terminus to C-terminus a first 5T4 VHH domain that binds 5T4, the first Fc polypeptide of the heterodimeric Fc region, the linker, the VH or VL domain of the anti-CD3 antibody or antigen binding fragment and a second 5T4 VHH domain that binds 5T4; and

the second polypeptide comprises in order of N-terminus to C-terminus one of the IRBR or the CRBR, the second Fc polypeptide of the heterodimeric Fc region, the linker, optionally the same linker as present in the first component, the other of the VH or VL domain of the anti-CD3 antibody or antigen binding fragment, and the other of the CRBR or IRBR.

157. The multispecific polypeptide construct of any of claims 63-156, wherein the linker is a peptide or polypeptide linker, optionally wherein the linker is 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 amino acids in length.

158. The multispecific polypeptide construct of any of claims 63-157, wherein the linker is a non-cleavable linker.

159. The multispecific polypeptide construct of claim 158, wherein the non-cleavable linker is or comprises GG.

160. The multispecific polypeptide construct of claim 158, wherein the non-cleavable linker comprises GS, GGS, GGGGS (SEQ ID NO:125), GGGGGS (SEQ ID NO:126) or combinations thereof.

161. The multispecific polypeptide construct of any of claims 63-158, wherein the linker is or comprises the sequence GGGGGSGGGGGSGGGGGS (SEQ ID NO:127).

162. The multispecific polypeptide construct of any of claims 63-157, wherein the linker is a cleavable linker.

163. The multispecific polypeptide construct of claim 162, wherein the cleavable linker is a polypeptide that functions as a substrate for a protease.

164. The multispecific polypeptide construct of claim 163, wherein the protease is produced by an immune effector cell, by a tumor cell, or by cells present in the tumor microenvironment.

165. The multispecific polypeptide construct of claim 163 or claim 164, wherein the protease is produced by an immune effector cell and the immune effector cell is an activated T cell, a natural killer (NK) cell, or an NK T cell.

166. The multispecific polypeptide construct of any of claims 163-165, wherein the protease is selected from among matriptase, a matrix metalloprotease (MMP), granzyme B, and combinations thereof.

167. The multispecific polypeptide construct of claim 166, wherein the protease is granzyme B.

168. The multispecific polypeptide construct of any of claims 163-167, wherein the cleavable linker comprises the amino acid sequence GGSGGGGIEPDIGGSGGS (SEQ ID NO:171).

169. An isolated single domain antibody (sdAb) that binds 5T4, comprising a complementarity determining region 1 (CDR1) comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 86-87 and 288-292, 296, and 297; a complementarity determining region 2 (CDR2) comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 88-99, 298, and 299; and a complementarity determining region 3 (CDR3) comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 100-102, 300, 301, and 303.

170. The isolated single domain antibody (sdAb) of claim 169, wherein the at least one 5T4 VHH domain binds to an epitope in human 5T4 but does not exhibit crossreactive binding to mouse 5T4.

171. The isolated single domain antibody (sdAb) of claim 169 or 170, wherein the at least one 5T4 VHH domain binds to amino acid residues between amino acids 60 and 112 of SEQ ID NO:382.

172. The isolated single domain antibody (sdAb) of any of claims 169-171, wherein the at least one 5T4 VHH domain binds to amino acid residues between amino acids 173 and 224 of SEQ ID NO:382.

173. The isolated single domain antibody of claim 169, comprising the amino acid sequence set forth in any of SEQ ID NOS: 245-253, 255-287, 294, 295, 302, and 360 or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to any of SEQ ID NOS: 245-253, 255-287, 294, 295, 302, and 360 and binds 5T4.

174. The isolated single domain antibody of any of claims 169-173, wherein the single domain antibody comprises the sequence set forth in (i) SEQ ID NO: 245, (ii) a humanized variant of SEQ ID NO: 245, or (iii) a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 245, and binds 5T4.

175. The isolated single domain antibody of any of claims 169-174, wherein the sdAb comprises a CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 288 and 289; a CDR2 comprising an amino acid sequence set forth in SEQ ID NO: 88; and a CDR3 comprising an amino acid sequence set forth in SEQ ID NO: 100.

176. The isolated single domain antibody of any of claims 169-175, wherein the sdAb comprises a CDR1, CDR2 and CDR3 set forth in SEQ ID NOS: 288, 88, and 100, respectively; or SEQ ID NOS: 289, 88, and 100, respectively.

177. The isolated single domain antibody of any of claims 169-176, wherein the sdAb comprises the sequence of amino acids set forth in any one of SEQ ID NOs: 246-253 and 360 or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 246-253 and 360, and binds 5T4.

178. The isolated single domain antibody of any of claims 169-177, wherein the sdAb comprises the sequence of amino acids set forth in any one of SEQ ID NOS: 246-253 and 360.

179. The isolated single domain antibody of any of claims 169-173, wherein the sdAb comprises the sequence set forth in (i) SEQ ID NO: 255, (ii) a humanized variant of SEQ ID NO: 255, or (iii) a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 255, and binds 5T4.

180. The isolated single domain antibody of any of claims 169-173 and 179, wherein the sdAb comprises a CDR1 comprising an amino acid sequence set forth in SEQ ID NO: 86, 290-292; a CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 89-94; and a CDR3 comprising an amino acid sequence set forth in SEQ ID NO: 101.

181. The isolated single domain antibody of any of claims 169-173, 179, and 180, wherein the sdAb comprises a CDR1, CDR2 and CDR3 set forth in SEQ ID NOS: 290, 89, and 101, respectively; SEQ ID NOS: 290, 90, and 101, respectively; SEQ ID NOS: 290, 91, and 101, respectively; SEQ ID NOS: 290, 92, and 101, respectively; SEQ ID NOS: 290, 93, and 101, respectively; SEQ ID NOS: 290, 94, and 101, respectively; SEQ ID NOS: 291, 94, and 101, respectively; SEQ ID NOS: 292, 94, and 101, respectively; or SEQ ID NOS: 86, 94, and 101, respectively.

182. The isolated single domain antibody of any of claims 169-173 and 179-181, wherein the sdAb comprises the sequence of amino acids set forth in any one of SEQ ID NOs: 256-275 or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 256-275, and binds 5T4.

183. The isolated single domain antibody of any of claims 169-173 and 179-182, wherein the sdAb comprises the sequence of amino acids set forth in any one of SEQ ID NOS: 256-275.

184. The isolated single domain antibody of any of claims 169-173, wherein the sdAb comprises the sequence set forth in (i) SEQ ID NO: 276 (ii) a humanized variant of SEQ ID NO: 276, or (iii) a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 276, and binds 5T4.

185. The isolated single domain antibody of any of claims 169-173 or claim 184, wherein the sdAb comprises a CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 86 and 87; a CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 95-99; and a CDR3 comprising an amino acid sequence set forth in SEQ ID NO: 102.

186. The isolated single domain antibody of any of claims 169-173, 184 and 185, wherein the sdAb comprises a CDR1, CDR2 and CDR3 set forth in SEQ ID NOS: 87, 95, and 102, respectively; SEQ ID NOS: 87, 96, and 102, respectively; SEQ ID NOS: 87, 97, and 102, respectively; SEQ ID NOS: 87, 98, and 102, respectively; SEQ ID NOS: 87, 99, and 102, respectively; or SEQ ID NOS: 86, 98, and 102, respectively.

187. The isolated single domain antibody of any of claims 169-173 and 184-186, wherein the sdAb comprises the sequence of amino acids set forth in any one of SEQ ID NOs: 277-287 or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 277-287, and binds 5T4.

188. The isolated single domain antibody of any of claims 169-173 and 184-187, wherein the sdAb comprises the sequence of amino acids set forth in any one of SEQ ID NOS: 277-287.

189. The isolated single domain antibody of any of claims 169-173, wherein the sdAb comprises the sequence set forth in (i) SEQ ID NO: 294 (ii) a humanized variant of SEQ ID NO: 294, or (iii) a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 294, and binds 5T4.

190. The isolated single domain antibody of claim 169-173 or 189, wherein the sdAb comprises a CDR1 comprising an amino acid sequence set forth in SEQ ID NO: 296; a CDR2 comprising an amino acid sequence set forth in SEQ ID NO: 298; and a CDR3 comprising an amino acid sequence set forth in SEQ ID NO: 300.

191. The isolated single domain antibody of claim 169-173 or 189, wherein the sdAb comprises a CDR3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 300, 301, and 303.

192. The isolated single domain antibody of claim 169-173 or 189, wherein the sdAb comprises:

a CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 288, 296, and 297; and/or

a CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 88, 298, and 299.

193. The isolated single domain antibody of any of claims 169-173, wherein the sdAb comprises the sequence set forth in (i) SEQ ID NO:295 (ii) a humanized variant of SEQ ID NO:295, or (iii) a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO:295, and binds 5T4.

194. The isolated single domain antibody of any of claims 169-173 and 193, wherein the sdAb comprises a CDR1 comprising an amino acid sequence set forth in SEQ ID NO: 297; a CDR2 comprising an amino acid sequence set forth in SEQ ID NO: 299; and a CDR3 comprising an amino acid sequence set forth in SEQ ID NO: 301.

195. The isolated single domain antibody of any of claims 169-173, wherein the sdAb comprises the sequence set forth in (i) SEQ ID NO:302 (ii) a humanized variant of SEQ ID NO:302, or (iii) a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO:302, and binds 5T4.

196. The isolated single domain antibody of any of claims 169-173 and 193, wherein the sdAb comprises a CDR1 comprising an amino acid sequence set forth in SEQ ID NO: 288; a CDR2 comprising an amino acid sequence set forth in SEQ ID NO: 88; and a CDR3 comprising an amino acid sequence set forth in SEQ ID NO: 303.

197. The isolated single domain antibody of any of claims 169-173, wherein the sdAb comprises the sequence set forth in (i) SEQ ID NO: 294, 295, or 302 (ii) a humanized variant of SEQ ID NO: 294, 295, or 302, or (iii) a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 294, 295, or 302, and binds 5T4.

198. The isolated single domain antibody of any of claims 169-173 and 197, wherein the sdAb comprises a CDR1, CDR2 and CDR3 set forth in SEQ ID NOS: 296, 298, and 300, respectively; SEQ ID NOS:297, 299, and 301, respectively; or SEQ ID NOS:288, 88, and 303, respectively.

199. A polynucleotide(s) encoding the 5T4-binding polypeptide construct of any of claims 1-62.

200. A polynucleotide(s) encoding the multispecific polypeptide construct of any of claims 63-168.

201. A polynucleotide, comprising a first nucleic acid sequence encoding a first polypeptide of a multispecific construct of any of claims 63-168 and a second nucleic acid sequence encoding a second polypeptide of the multispecific construct, wherein the first and second nucleic acid sequence are separated by an internal ribosome entry site (IRES), or a nucleic acid encoding a self-cleaving peptide or a peptide that causes ribosome skipping.

202. The polynucleotide of claim 201, wherein the first nucleic acid sequence and second nucleic acid sequence are operably linked to the same promoter.

203. The polynucleotide of claim 201 or 202, wherein the nucleic acid encoding a self-cleaving peptide or a peptide that causes ribosome skipping is selected from a T2A, a P2A, a E2A or a F2A.

204. A polynucleotide encoding the single domain antibody of any of claims 169-198.

205. A vector, comprising the polynucleotide of any of claims 199-204.

206. The vector of claim 205 that is an expression vector.

207. The vector of claim 205 or claim 206 that is a viral vector or a eukaryotic vector, optionally wherein the eukaryotic vector is a mammalian vector.

208. A cell, comprising polynucleotide or polynucleotides of-any of claims 199-204, or a vector or vectors of any of claims 205-207.

209. The cell of claim 208, wherein the cell is recombinant or isolated.

210. The cell of claim 209, wherein the cell is a mammalian cell.

211. A method of producing a polypeptide, the method comprising introducing into a cell a polynucleotide or polynucleotides of any of claims 199-204 or a vector or vectors of any of claims 205-207 and culturing the cell under conditions to produce the multispecific polypeptide construct.

212. The method of claim 211, further comprising isolating or purifying the polypeptide from the cell.

213. A polypeptide produced by the method of claim 211 or claim 212.

214. An engineered immune cell, comprising a chimeric antigen receptor comprising:
an extracellular domain comprising the single domain antibody of any of claims 169-198;
a transmembrane domain; and
an intracellular signaling domain.

215. The engineered immune cell of claim 214, wherein the cell is a lymphocyte.

216. The engineered immune cell of claim 214 or claim 215, wherein the cell is a T cell or a natural killer (NK) cell.

217. The engineered immune cell of any of claims 214-216, wherein the intracellular signaling domain comprises an immunoreceptor tyrosine-based activation motif (ITAM) signaling domain.

218. The engineered immune cell of any of claims 214-217, wherein the intracellular signaling domain is or comprises a CD3zeta signaling domain, optionally a human CD3zeta signaling domain.

219. The engineered immune cell of claim 214-218, wherein the intracellular signaling domain further comprises a signaling domain of a costimulatory molecule.

220. The engineered immune cell of claim 219, wherein the costimulatory molecule is CD28, ICOS, 41BB or OX40, optionally a human CD28, a human ICOS, a human 41BB or a human OX40.

221. A pharmaceutical composition comprising the 5T4-binding polypeptide construct of any of claims 1-62, the multispecific polypeptide construct of any of claims 63-168, the single domain antibody of any of claims 169-198 or the engineered immune cell of any of claims 214-220.

222. The pharmaceutical composition of claim 221, comprising a pharmaceutically acceptable carrier.

223. The pharmaceutical composition of claim 221 or claim 222 that is sterile.

224. A method of stimulating or inducing an immune response in a subject, the method comprising administering, to a subject in need thereof, the 5T4-binding polypeptide construct of any of claims 1-62, the multispecific polypeptide construct of any of claims 63-168, the single domain antibody of any of claims 169-198 or the engineered immune cell of any of claims 214-220 or a pharmaceutical composition of claim 221-223.

225. The method of claim 224, wherein the immune response is increased against a tumor or cancer, optionally a tumor or a cancer that expresses 5T4.

226. The method of claim 224 or claim 225, wherein the method treats a disease or condition in the subject.

227. A method of treating a disease or condition in a subject, the method comprising administering, to a subject in need thereof, a therapeutically effective amount of the 5T4-binding polypeptide construct of any of claims 1-59, the multispecific polypeptide construct of any of claims 60-161, the single domain antibody of any of claims 162-188 or the engineered immune cell of any of claims 214-220 or a pharmaceutical composition of claim 221-223.

228. The method of claim 226 or claim 227, wherein the disease or condition is a tumor or a cancer.

229. The method of any of claims 224-228, wherein said subject is a human.

FIG. 1A

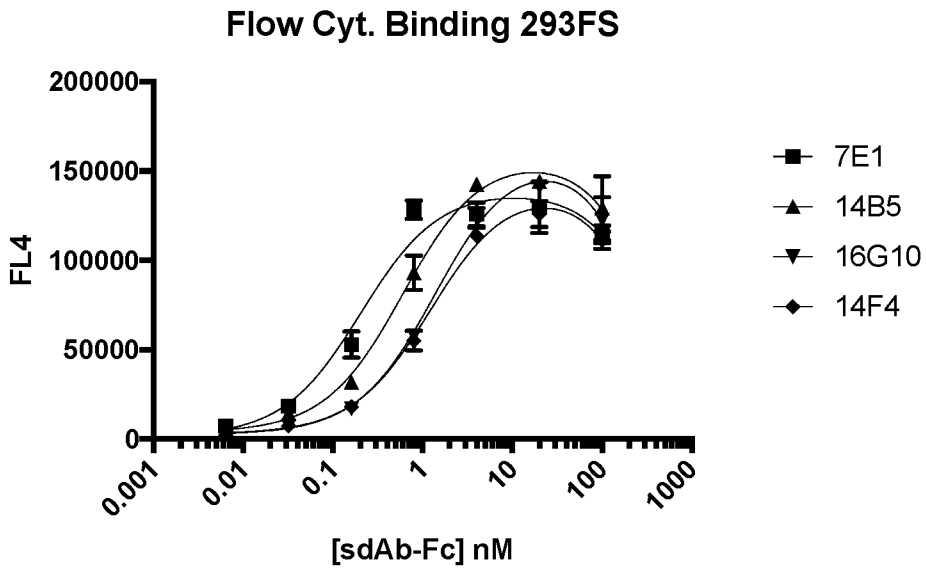

Flow Cyt. Binding 293FS
200000
150000
100000
50000
0
FL4
0.001
0.01
0.1
1
10
100
1000
[sdAb-Fc] nM
7E1
14B5
16G10
14F4

FIG. 1B

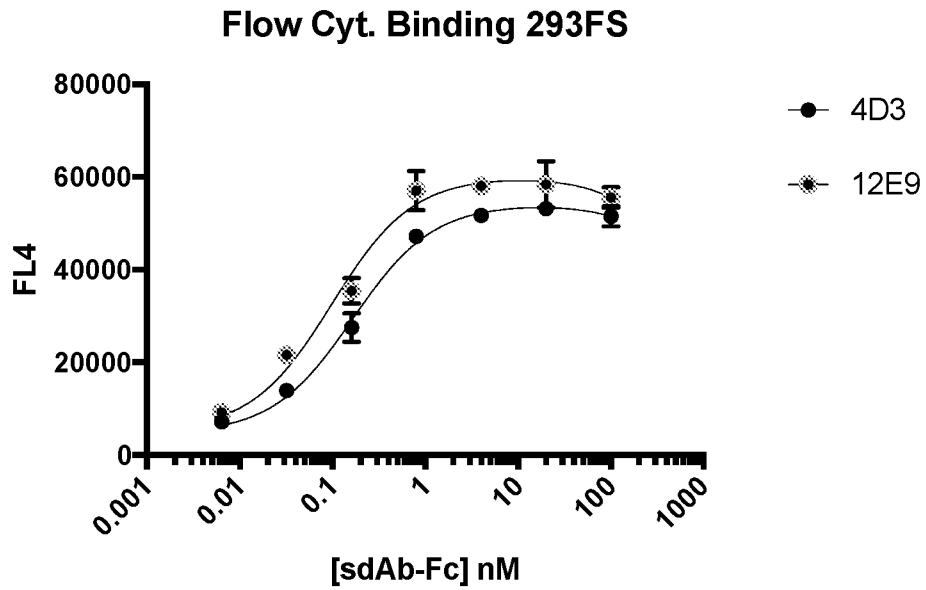

Flow Cyt. Binding 293FS
80000
60000
40000
20000
0
FL4
0.001
0.01
0.1
1
10
100
1000
[sdAb-Fc] nM
4D3
12E9

FIG. 2A

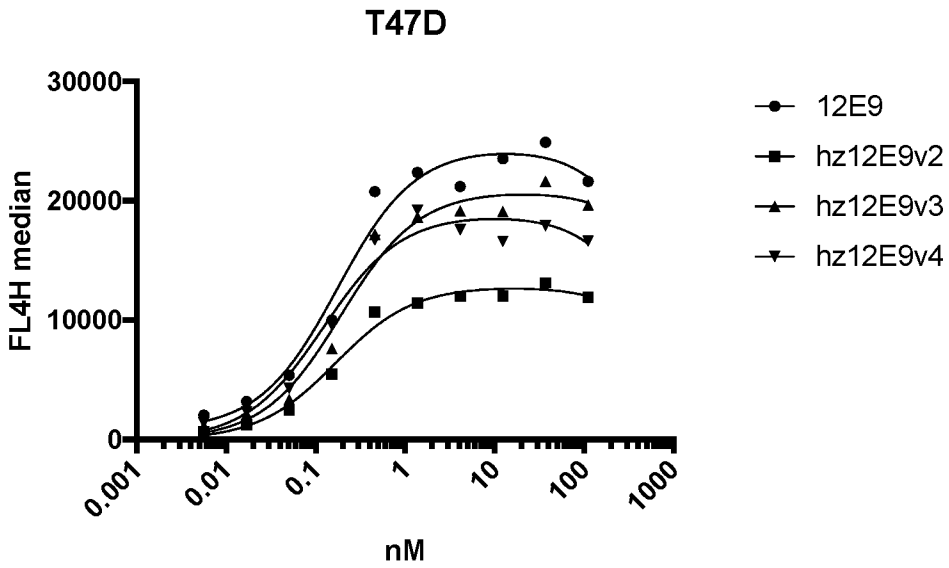

T47D
30000
20000
10000
0
FL4H median
0.001
0.01
0.1
1
10
100
1000
nM
12E9
hz12E9v2
hz12E9v3
hz12E9v4

FIG. 2B

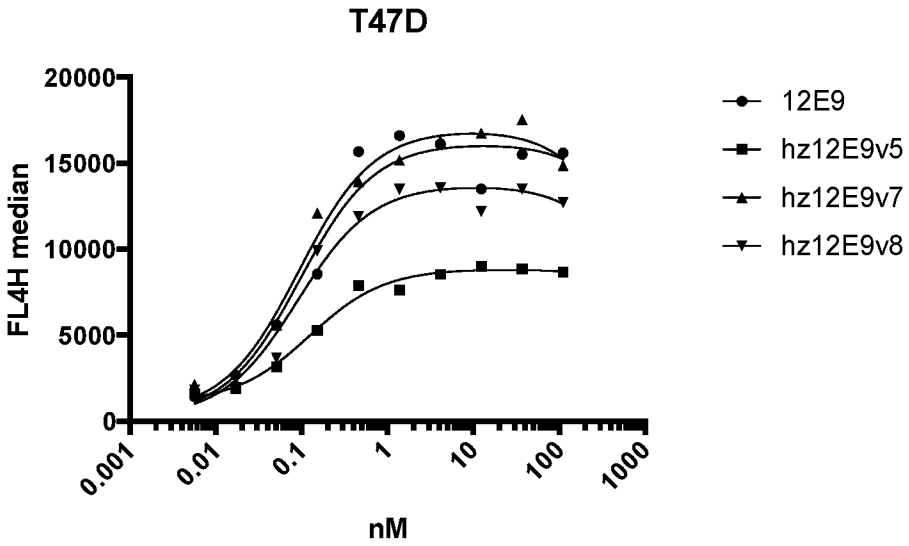

T47D
20000
15000
10000
5000
0
FL4H median
0.001
0.01
0.1
1
10
100
1000
nM
12E9
hz12E9v5
hz12E9v7
hz12E9v8

FIG. 2C

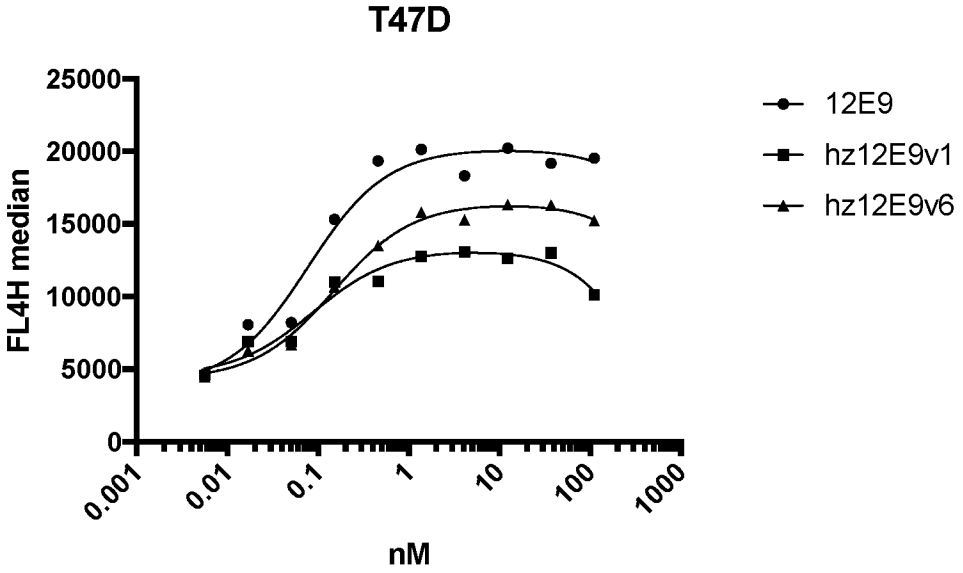

T47D
FL4H median
25000
20000
15000
10000
5000
0
0.001
0.01
0.1
1
10
100
1000
nM
12E9
hz12E9v1
hz12E9v6

FIG. 2D

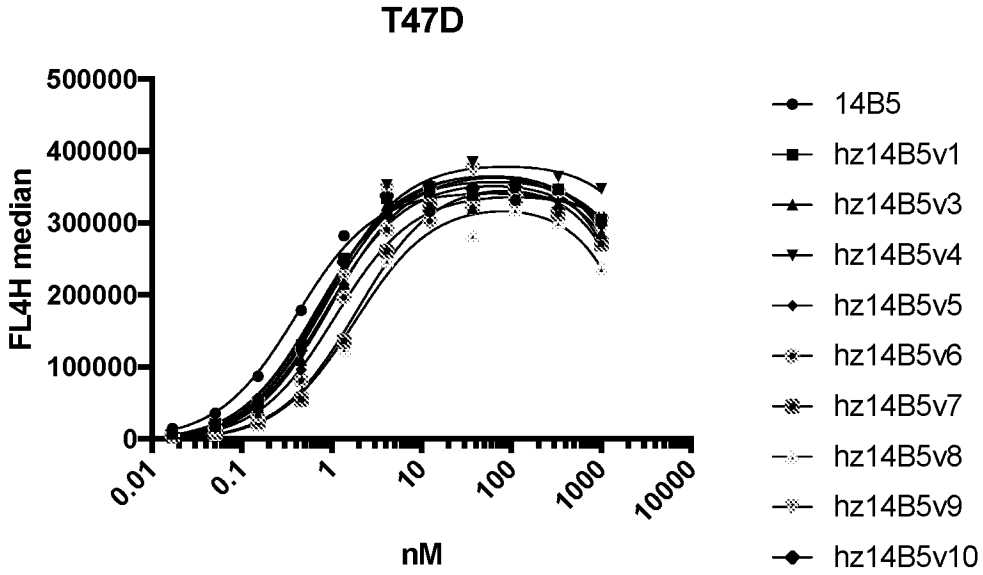

T47D
FL4H median
500000
400000
300000
200000
100000
0
0.01
0.1
1
10
100
1000
10000
nM
14B5
hz14B5v1
hz14B5v3
hz14B5v4
hz14B5v5
hz14B5v6
hz14B5v7
hz14B5v8
hz14B5v9
hz14B5v10

FIG. 2E

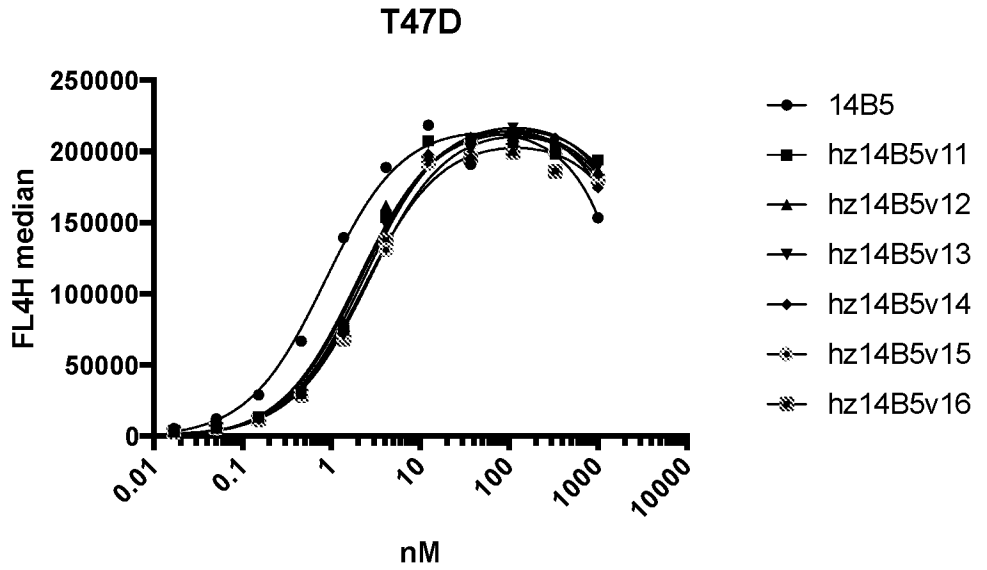

T47D
FL4H median
250000
200000
150000
100000
50000
0
0.01
0.1
1
10
100
1000
10000
nM
14B5
hz14B5v11
hz14B5v12
hz14B5v13
hz14B5v14
hz14B5v15
hz14B5v16

FIG. 2F

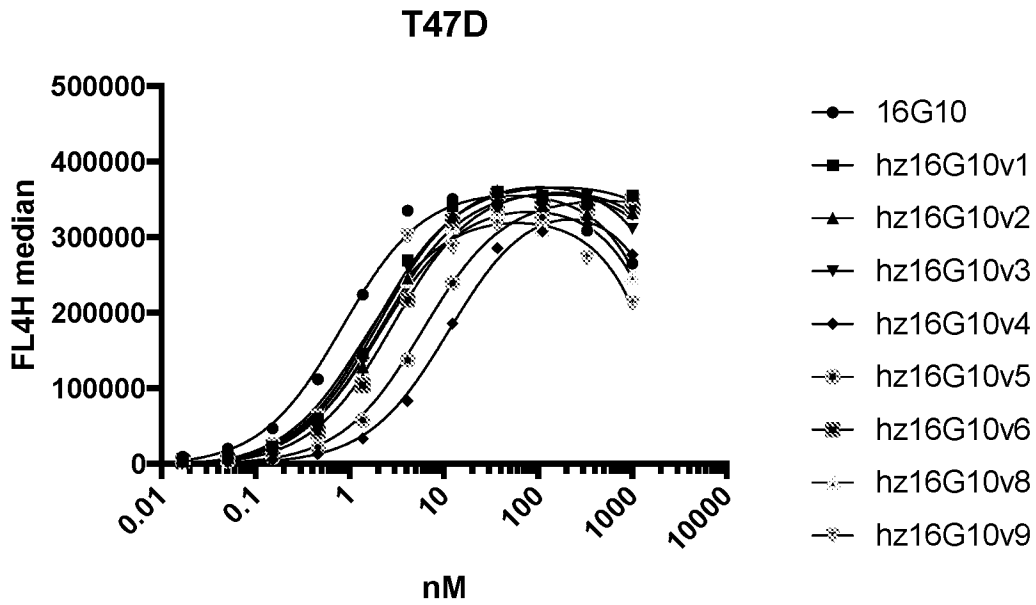

T47D
FL4H median
500000
400000
300000
200000
100000
0
0.01
0.1
1
10
100
1000
10000
nM
16G10
hz16G10v1
hz16G10v2
hz16G10v3
hz16G10v4
hz16G10v5
hz16G10v6
hz16G10v8
hz16G10v9

FIG. 3A

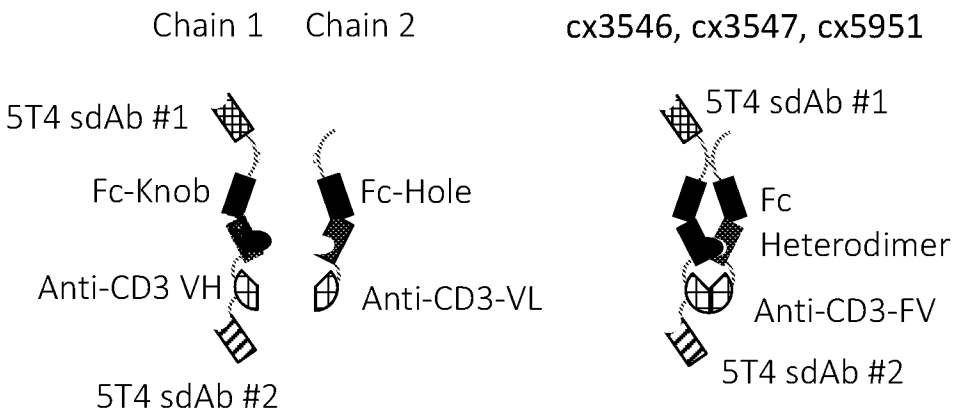

Chain 1
Chain 2
cx3546, cx3547, cx5951
5T4 sdAb #1
Fc-Knob
Anti-CD3 VH
5T4 sdAb #2
Fc-Hole
Anti-CD3-VL
5T4 sdAb #1
Fc
Heterodimer
Anti-CD3-FV
5T4 sdAb #2

FIG. 3B

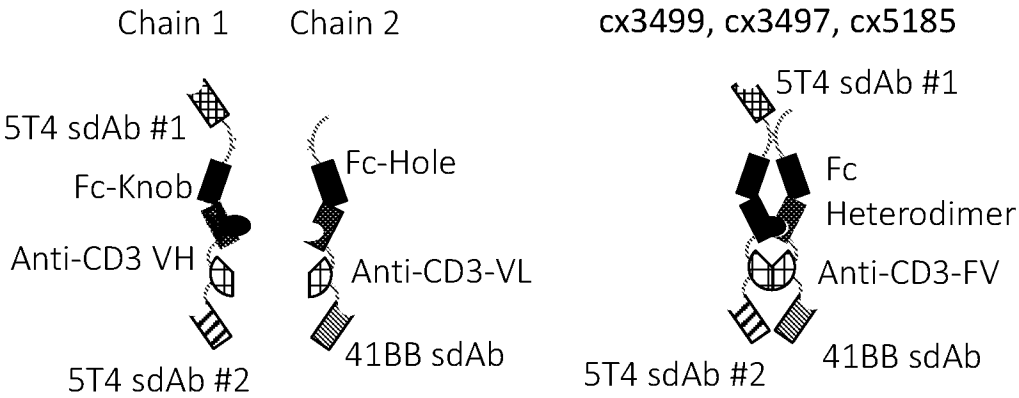

Chain 1
Chain 2
cx3499, cx3497, cx5185
5T4 sdAb #1
Fc-Knob
Anti-CD3 VH
5T4 sdAb #2
Fc-Hole
Anti-CD3-VL
41BB sdAb
5T4 sdAb #1
Fc
Heterodimer
Anti-CD3-FV
5T4 sdAb #2
41BB sdAb

FIG. 3C

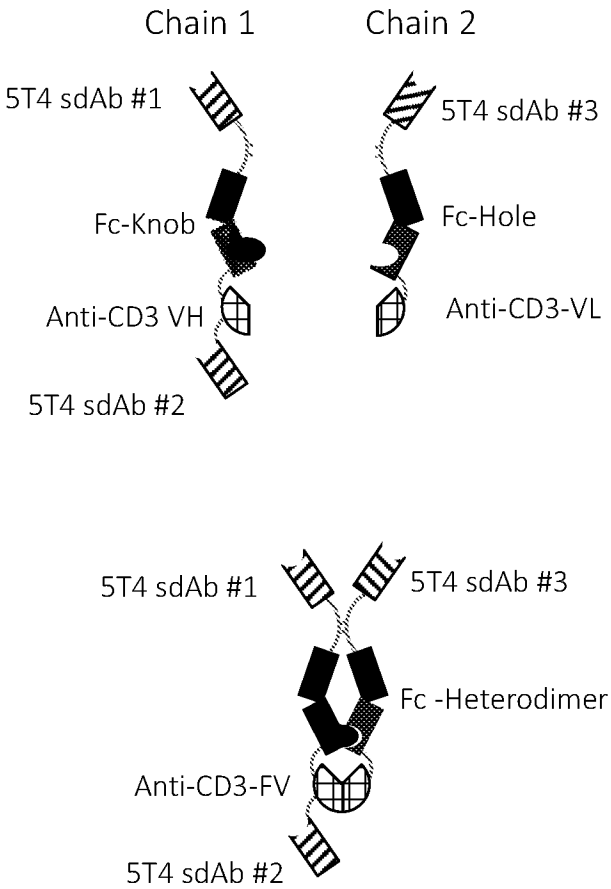

Chain 1
Chain 2
5T4 sdAb #1
5T4 sdAb #3
Fc-Knob
Fc-Hole
Anti-CD3 VH
Anti-CD3-VL
5T4 sdAb #2
5T4 sdAb #1
5T4 sdAb #3
Fc -Heterodimer
Anti-CD3-FV
5T4 sdAb #2

FIG. 3D

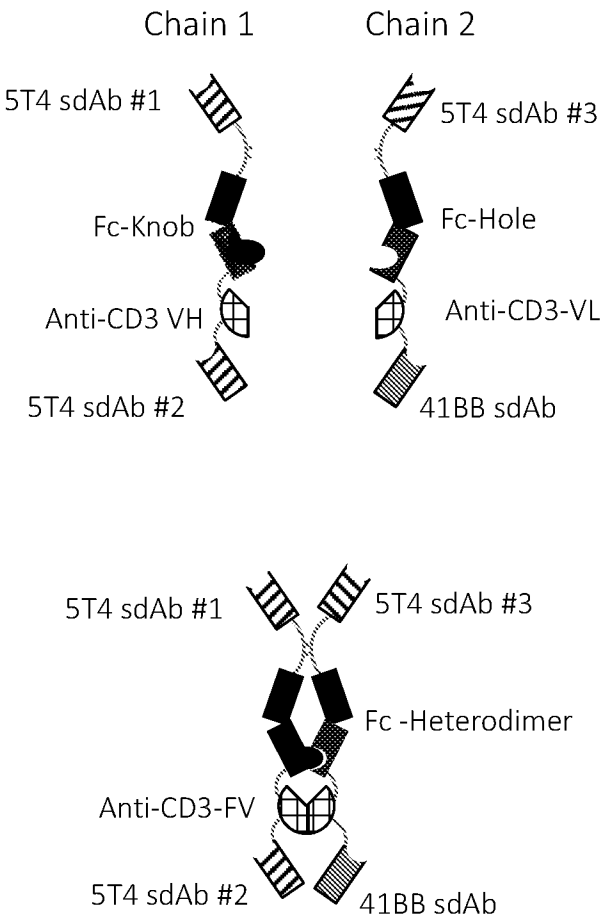

Chain 1
Chain 2
5T4 sdAb #1
5T4 sdAb #3
Fc-Knob
Fc-Hole
Anti-CD3 VH
Anti-CD3-VL
5T4 sdAb #2
41BB sdAb
5T4 sdAb #1
5T4 sdAb #3
Fc -Heterodimer
Anti-CD3-FV
5T4 sdAb #2
41BB sdAb

FIG. 3E

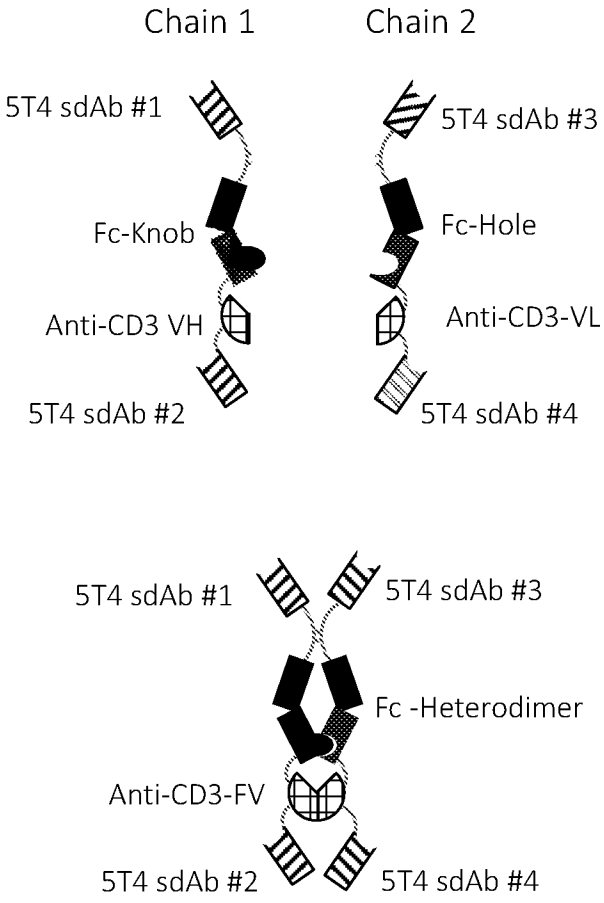
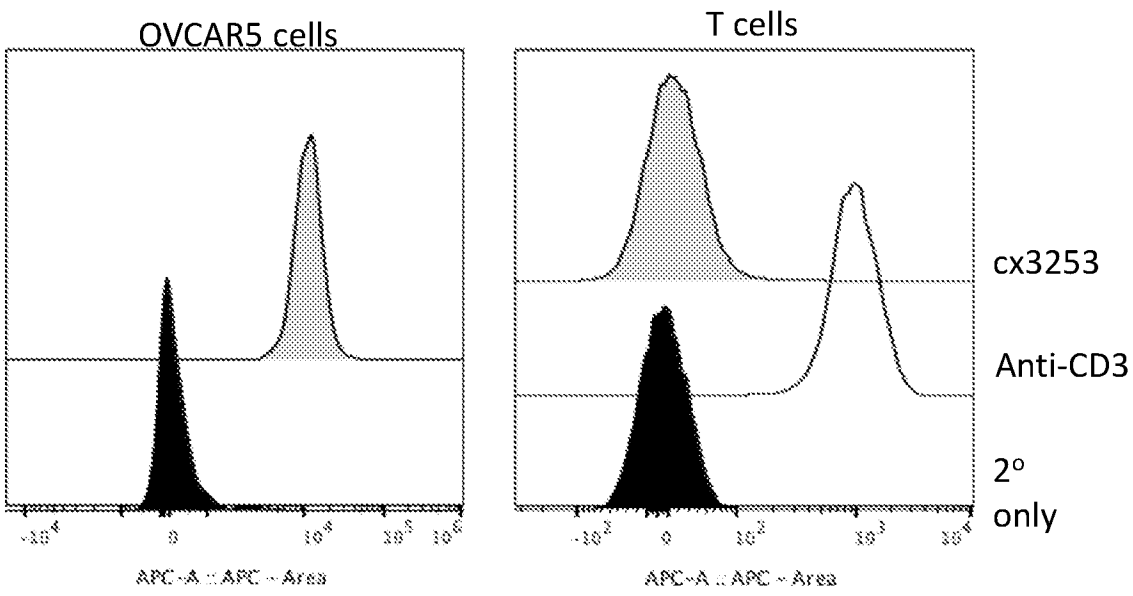

Chain 1
Chain 2
5T4 sdAb #1
5T4 sdAb #3
Fc-Knob
Fc-Hole
Anti-CD3 VH
Anti-CD3-VL
5T4 sdAb #2
5T4 sdAb #4
5T4 sdAb #1
5T4 sdAb #3
Fc -Heterodimer
Anti-CD3-FV
5T4 sdAb #2
5T4 sdAb #4

FIG. 4A


OVCAR5 cells
T cells
cx3253
Anti-CD3
2°
only
APC-A :: APC - Area
APC-A :: APC - Area

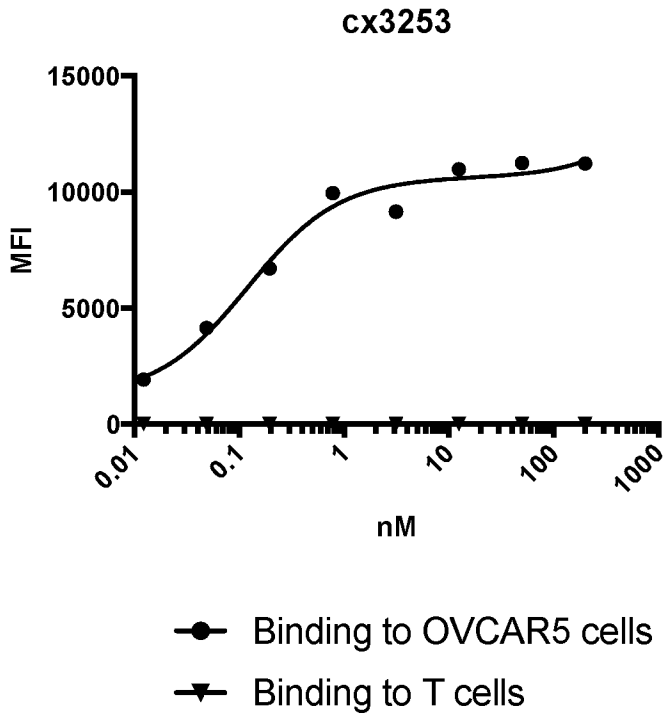

cx3253
15000
10000
5000
0
MFI
0.01
0.1
1
10
100
1000
nM
Binding to OVCAR5 cells
Binding to T cells

FIG. 4B

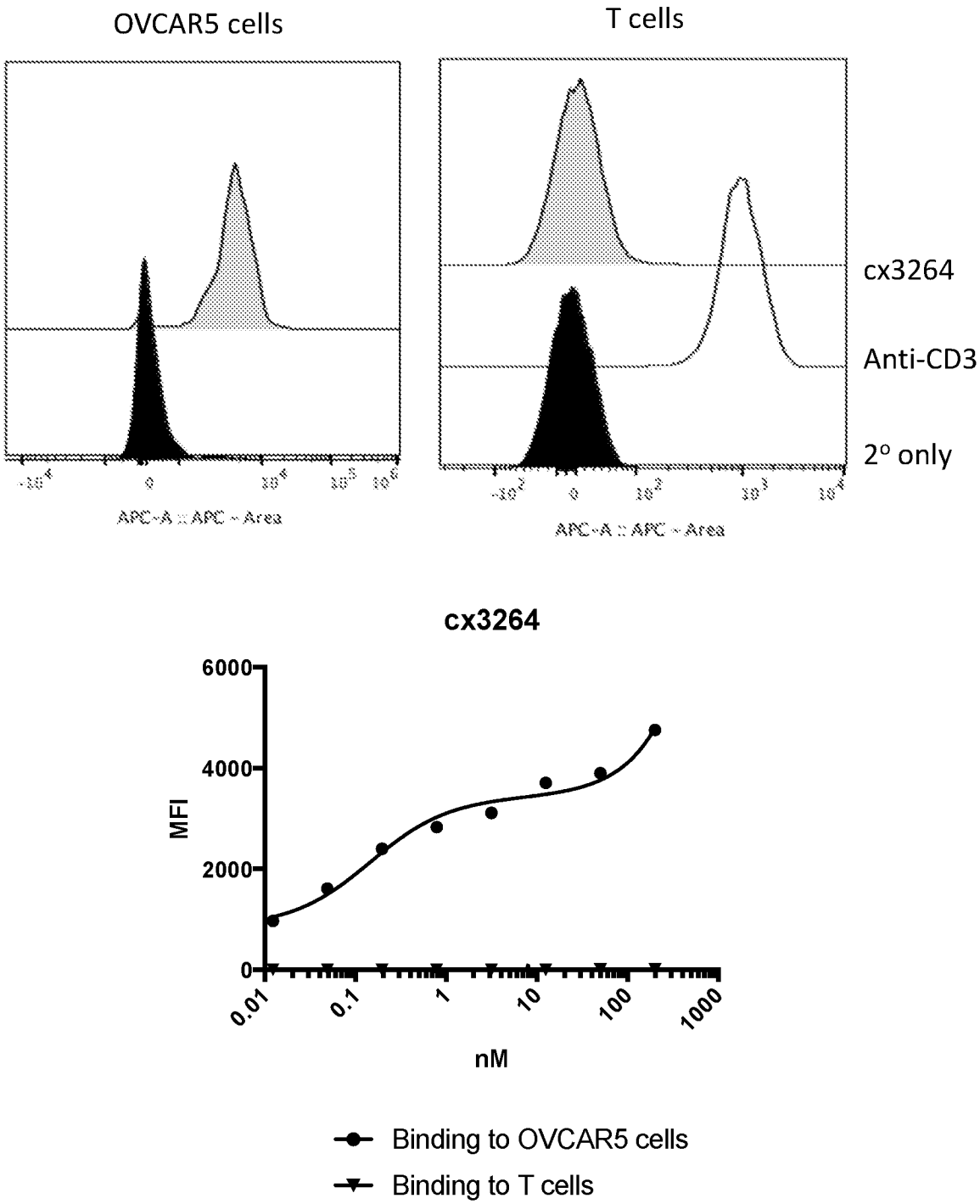
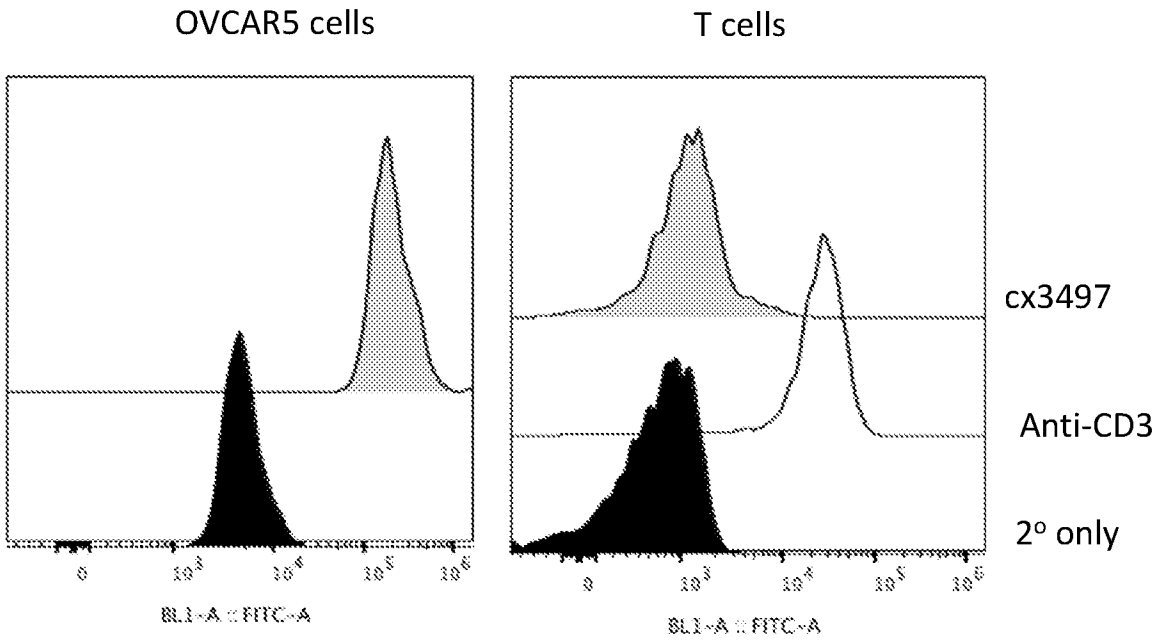

OVCAR5 cells
T cells
cx3264
Anti-CD3
2° only
APC-A :: APC - Area
APC-A :: APC - Area
cx3264
MFI
6000
4000
2000
0
0.01
0.1
1
10
100
1000
nM
Binding to OVCAR5 cells
Binding to T cells

FIG. 4C


OVCAR5 cells
T cells
cx3497
Anti-CD3
2° only
BL1-A :: FITC-A
BL1-A :: FITC-A

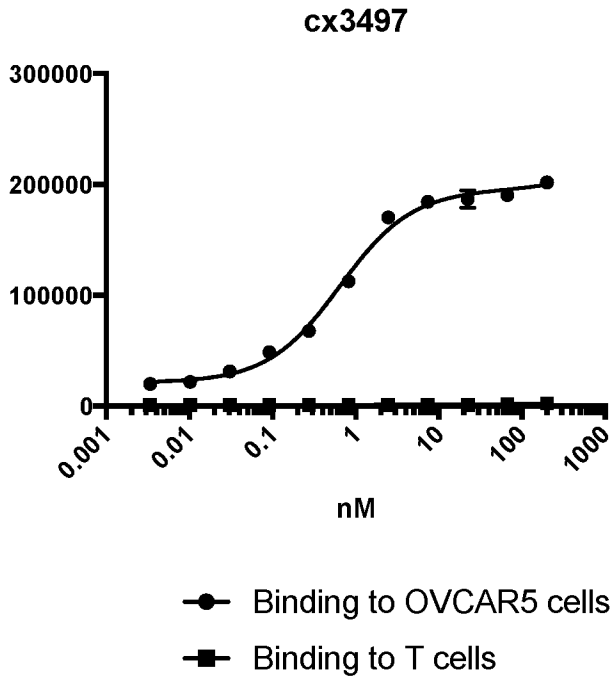

cx3497
300000
200000
100000
0
0.001
0.01
0.1
1
10
100
1000
nM
Binding to OVCAR5 cells
Binding to T cells

FIG. 4D

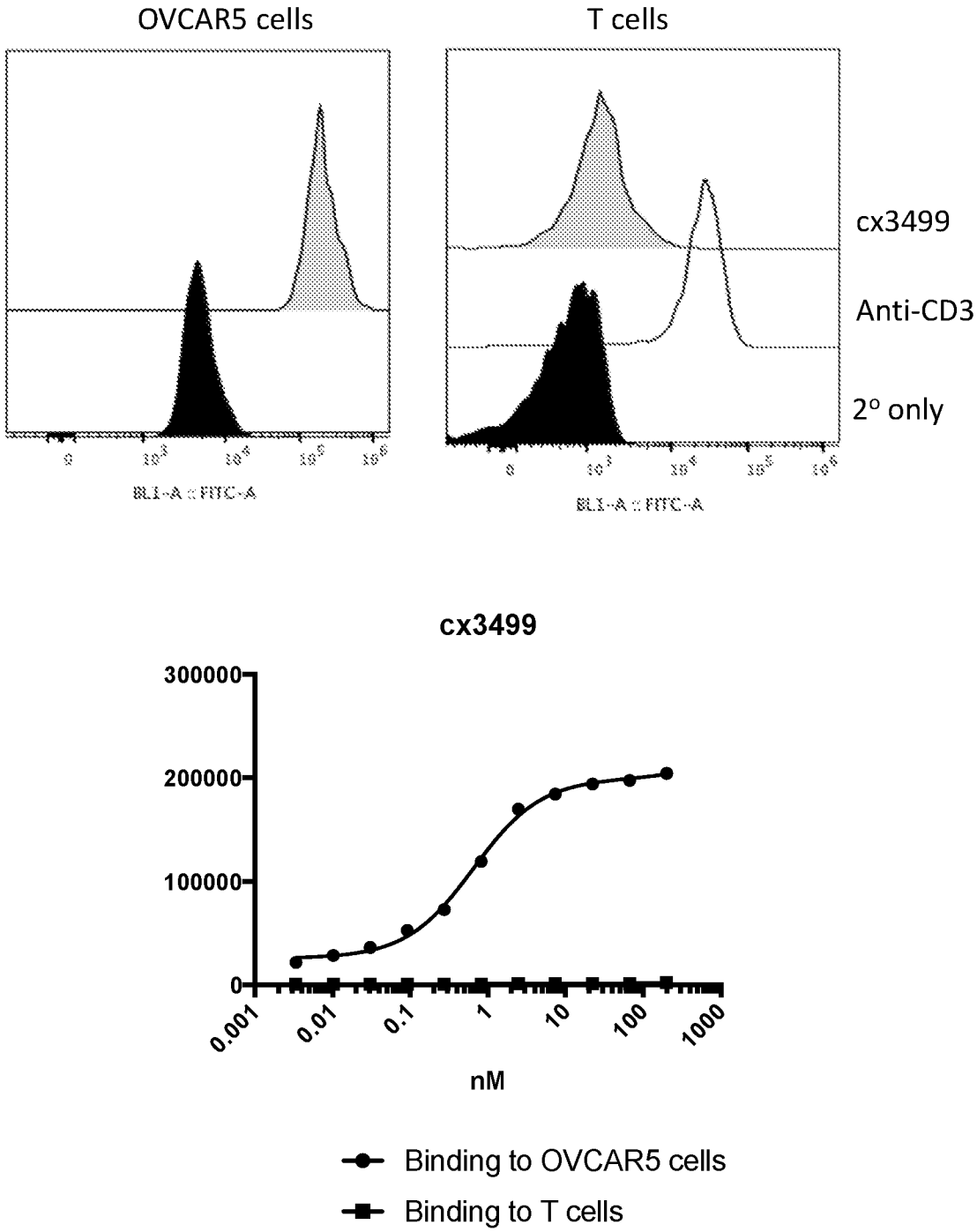

OVCAR5 cells
T cells
cx3499
Anti-CD3
2° only
cx3499
300000
200000
100000
0
0.001
0.01
0.1
1
10
100
1000
nM
Binding to OVCAR5 cells
Binding to T cells

FIG. 4E

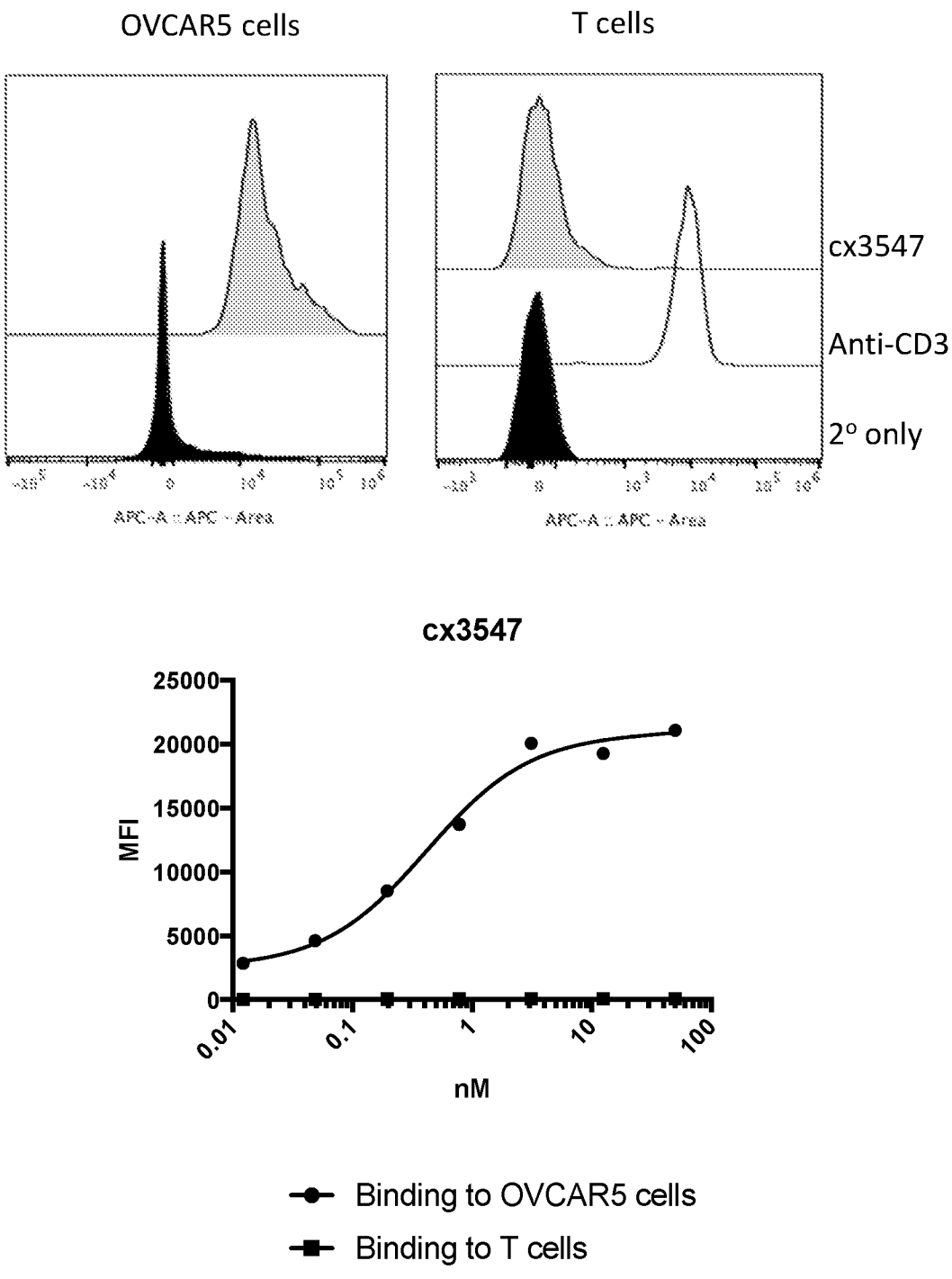

OVCAR5 cells
T cells
cx3547
Anti-CD3
2° only
APC-A :: APC - Area
APC-A :: APC - Area
cx3547
MFI
25000
20000
15000
10000
5000
0
0.01
0.1
1
10
100
nM
Binding to OVCAR5 cells
Binding to T cells

FIG. 4F

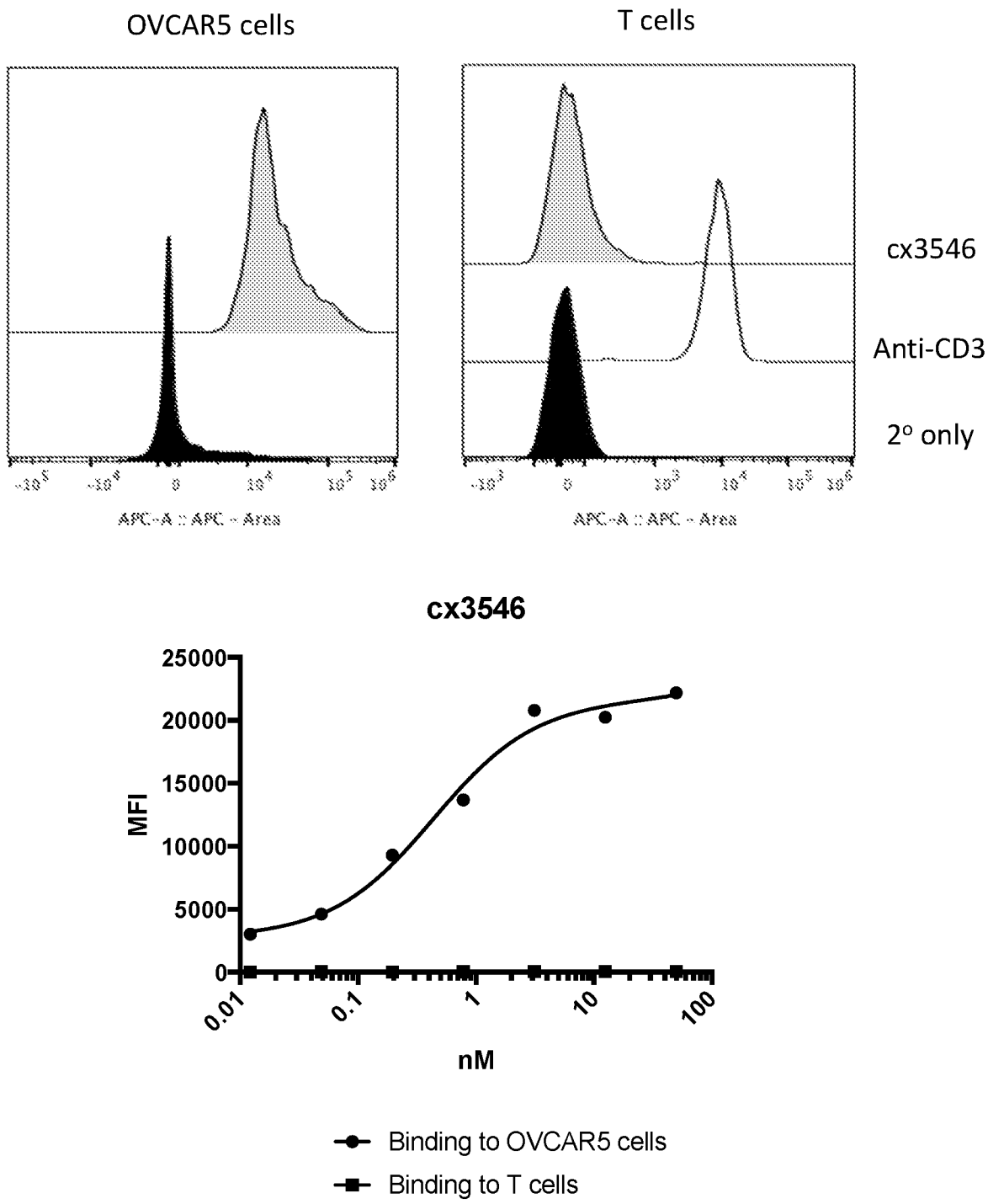

OVCAR5 cells
T cells
cx3546
Anti-CD3
2° only
APC-A :: APC - Area
APC-A :: APC - Area
cx3546
MFI
25000
20000
15000
10000
5000
0
0.01
0.1
1
10
100
nM
Binding to OVCAR5 cells
Binding to T cells

FIG. 4G

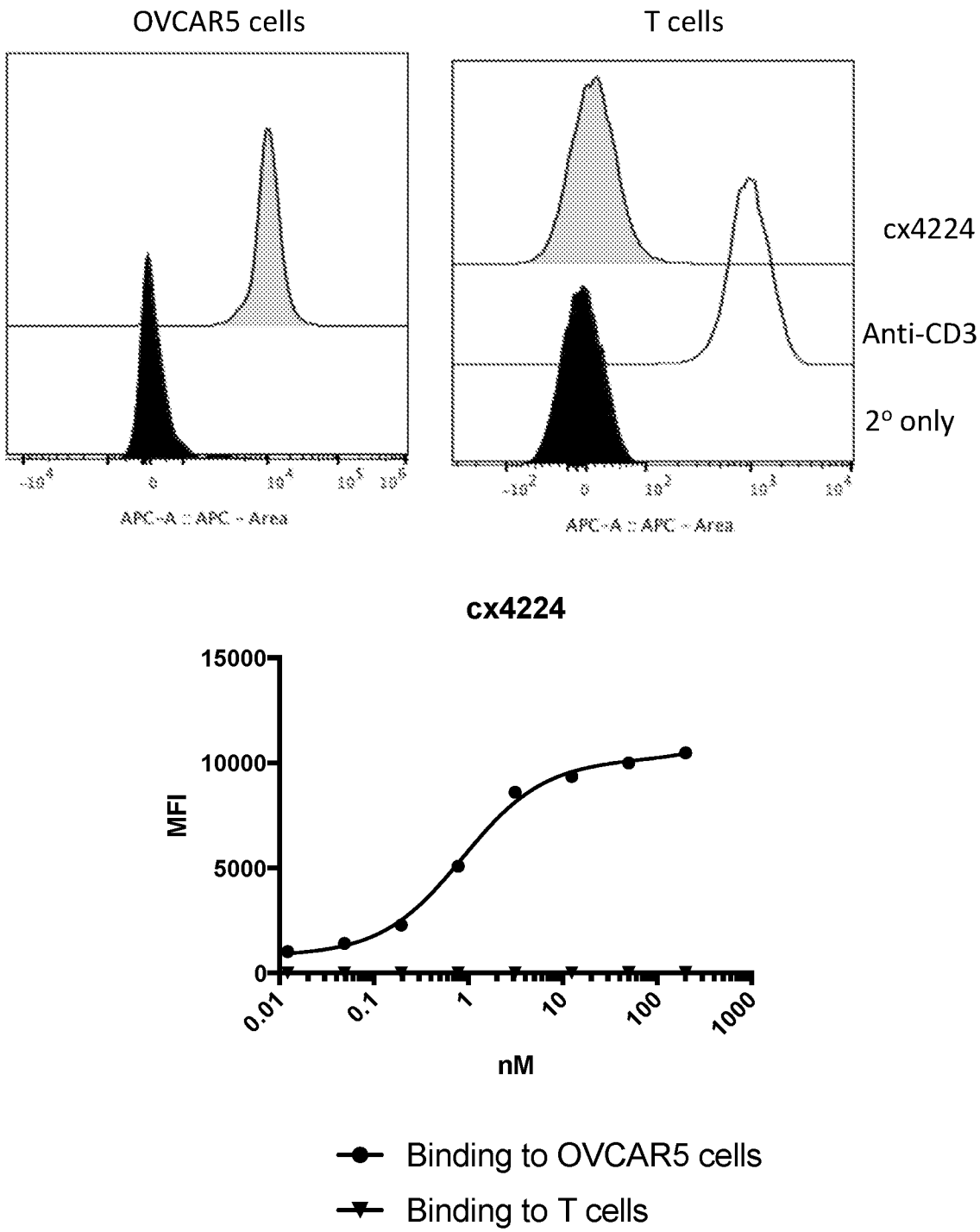
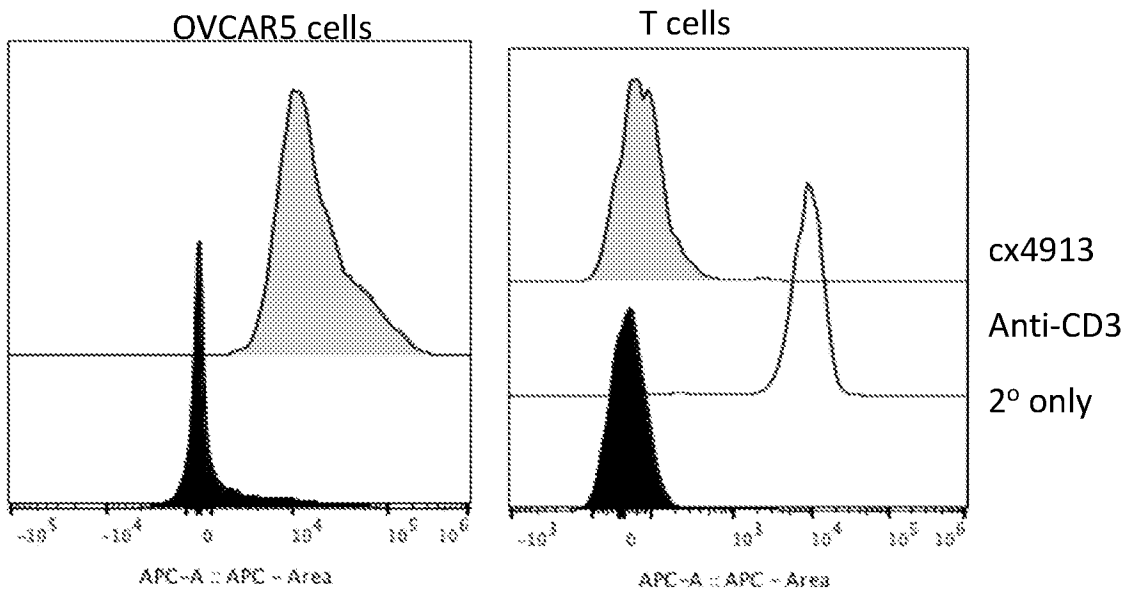

OVCAR5 cells
T cells
cx4224
Anti-CD3
2° only
APC-A :: APC - Area
APC-A :: APC - Area
cx4224
MFI
15000
10000
5000
0
0.01
0.1
1
10
100
1000
nM
Binding to OVCAR5 cells
Binding to T cells

FIG. 4H


OVCAR5 cells
T cells
cx4913
Anti-CD3
2° only
APC-A :: APC - Area
APC-A :: APC - Area

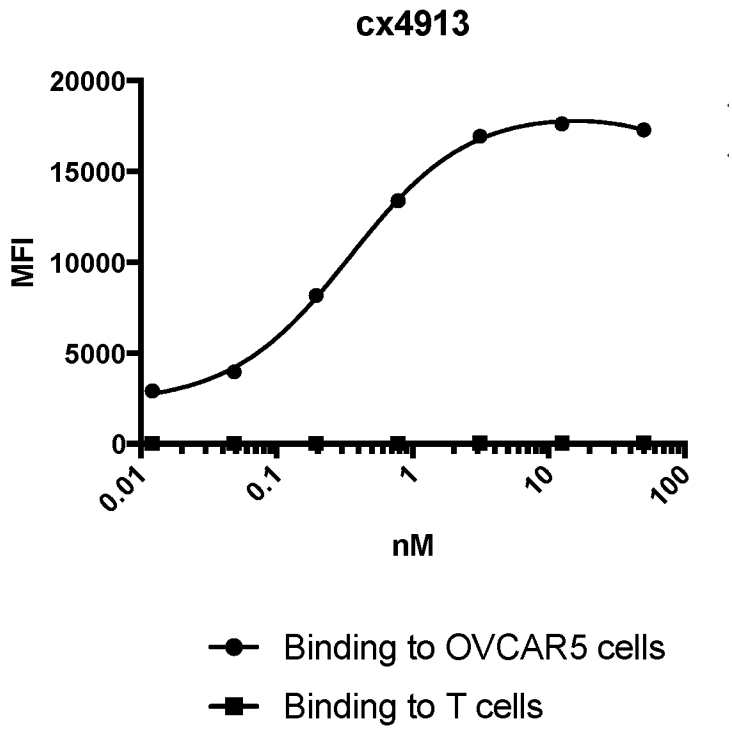
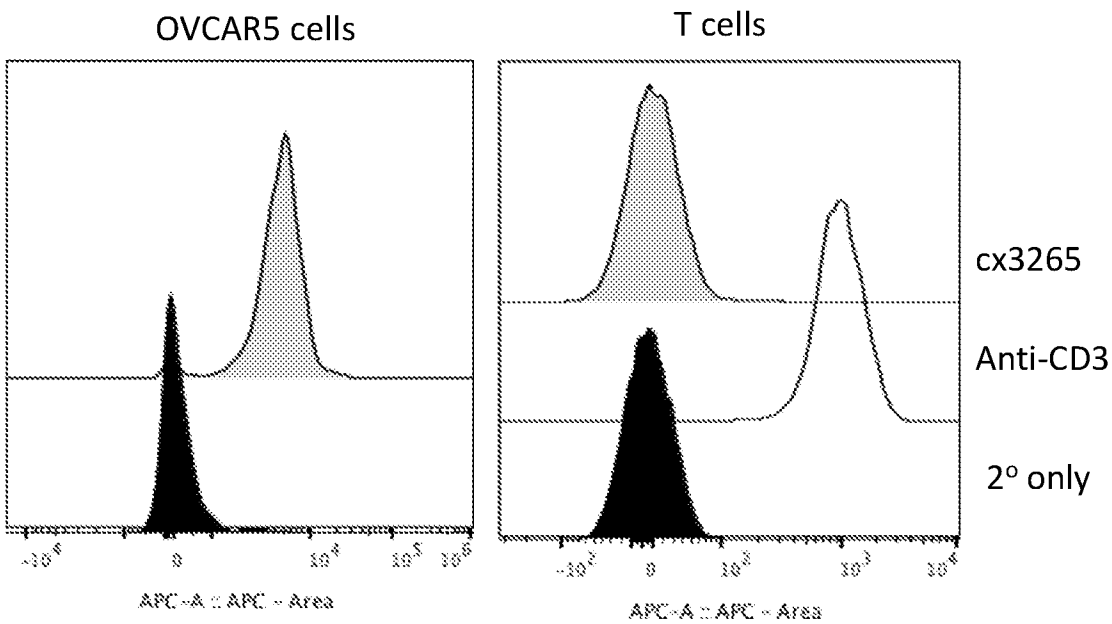

cx4913
20000
15000
10000
5000
0
MFI
0.01
0.1
1
10
100
nM
Binding to OVCAR5 cells
Binding to T cells

FIG. 4I


OVCAR5 cells
T cells
cx3265
Anti-CD3
2° only
APC-A :: APC - Area
APC-A :: APC - Area

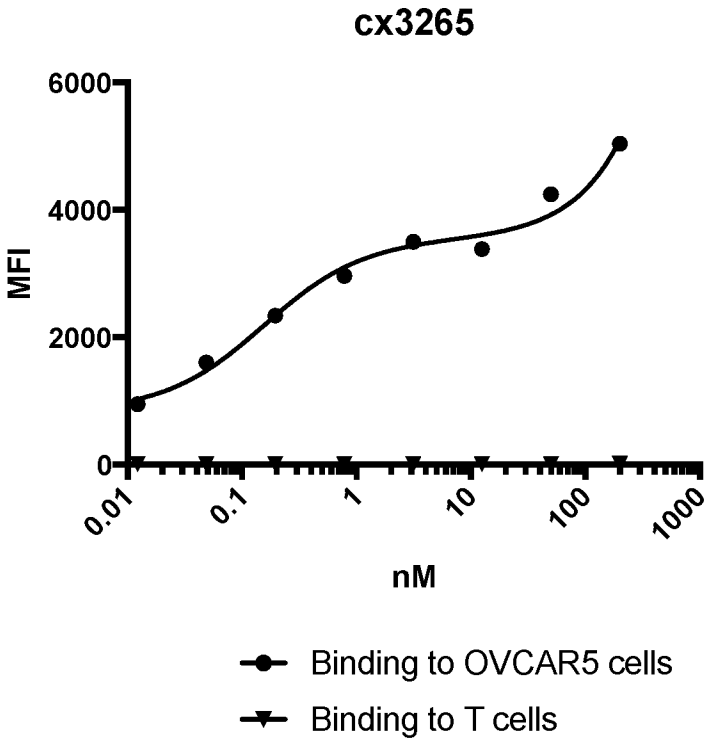

cx3265
6000
4000
2000
0
MFI
0.01
0.1
1
10
100
1000
nM
Binding to OVCAR5 cells
Binding to T cells

FIG. 4J

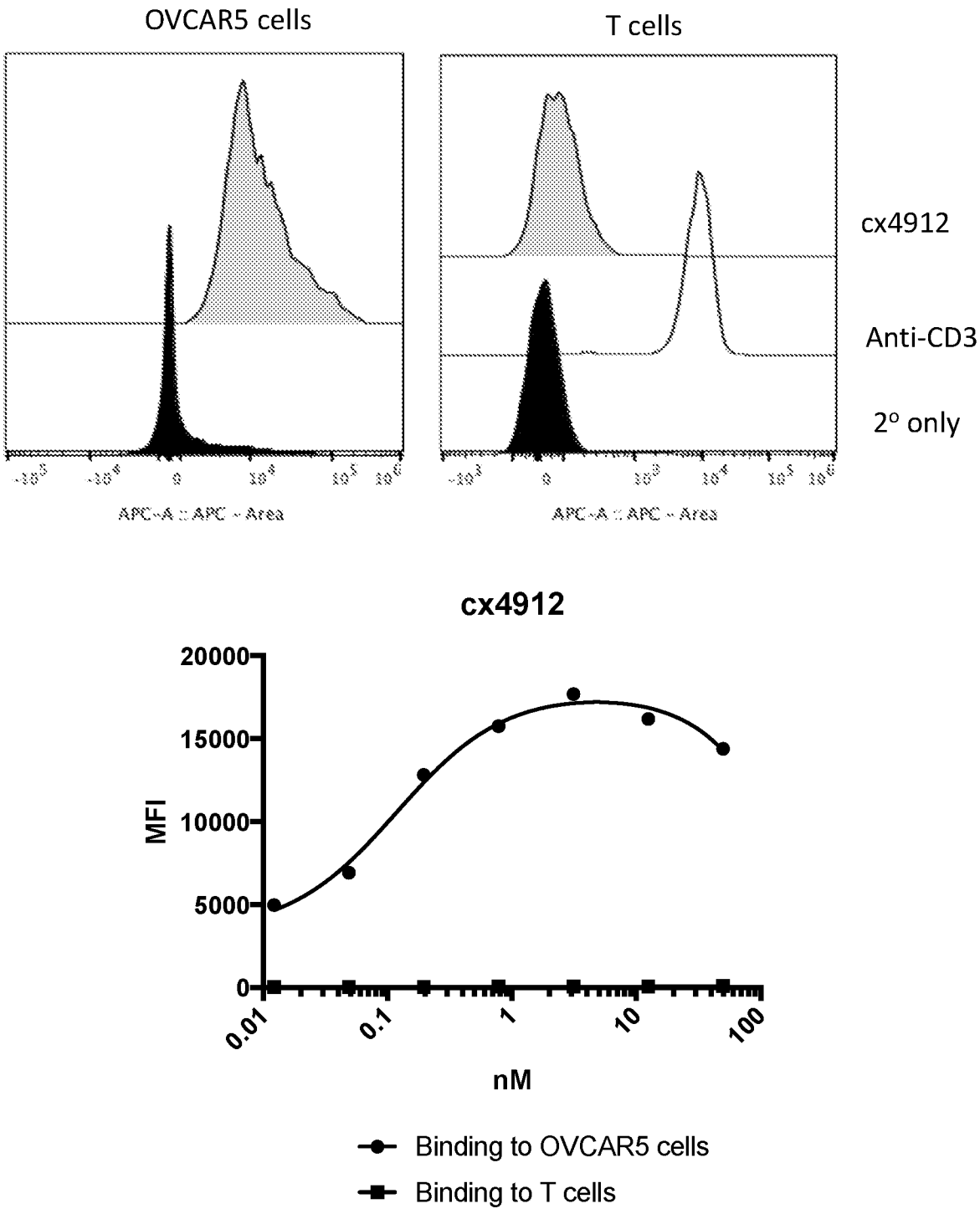
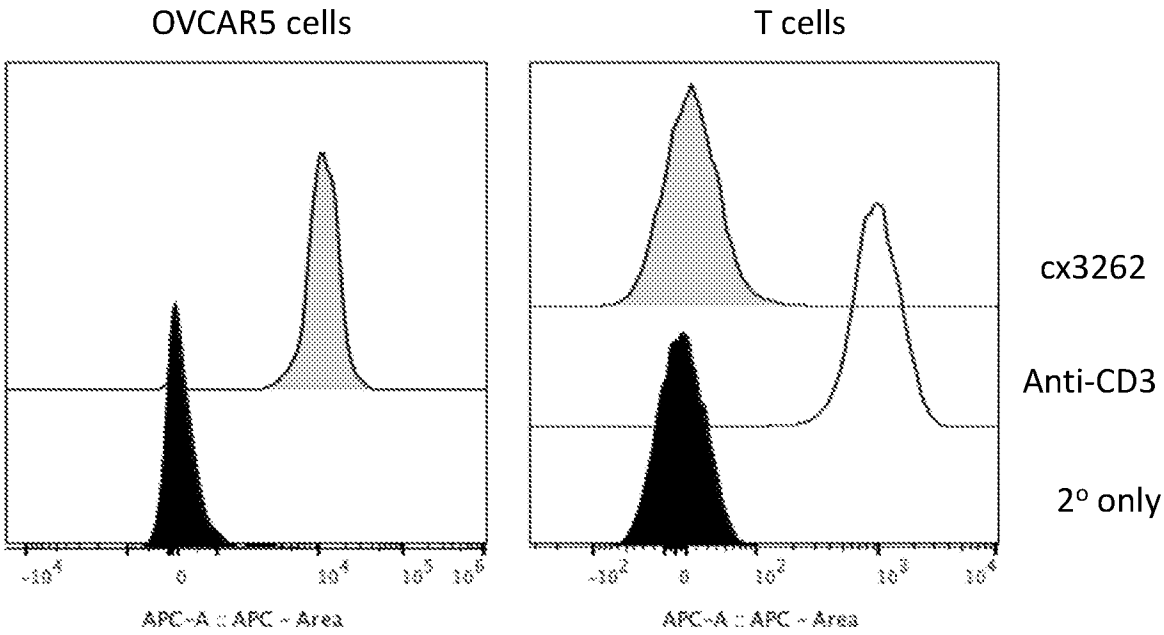

OVCAR5 cells
T cells
cx4912
Anti-CD3
2° only
APC-A :: APC - Area
APC-A :: APC - Area
cx4912
MFI
20000
15000
10000
5000
0
0.01
0.1
1
10
100
nM
Binding to OVCAR5 cells
Binding to T cells

FIG. 4K


OVCAR5 cells
T cells
cx3262
Anti-CD3
2° only
APC-A :: APC - Area
APC-A :: APC - Area

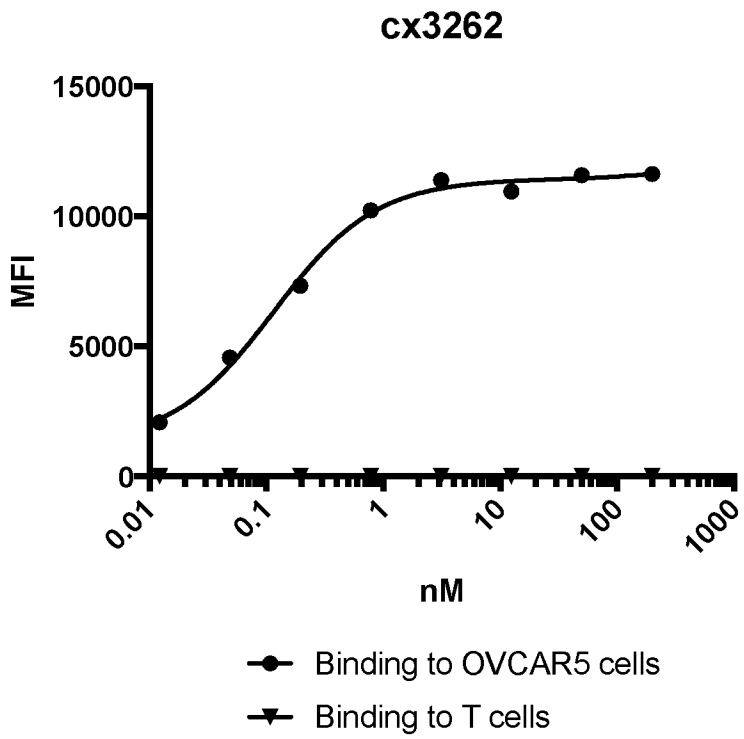
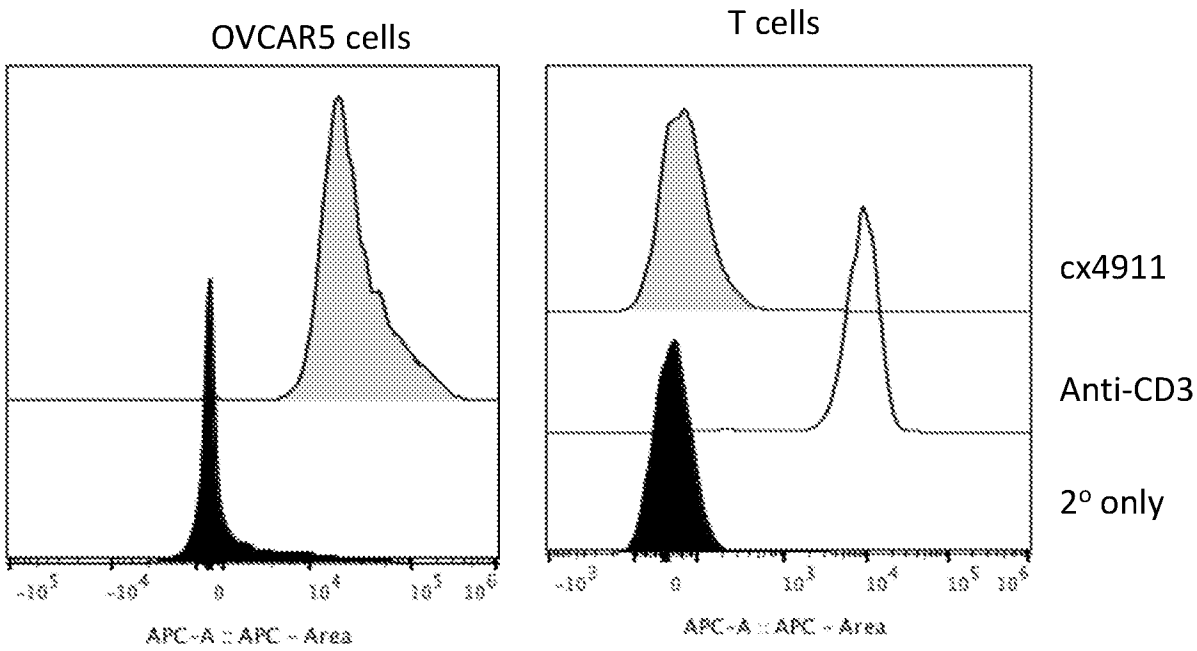

cx3262
15000
10000
5000
0
MFI
0.01
0.1
1
10
100
1000
nM
Binding to OVCAR5 cells
Binding to T cells

FIG. 4L


OVCAR5 cells
T cells
cx4911
Anti-CD3
2° only
APC-A :: APC - Area
APC-A :: APC - Area

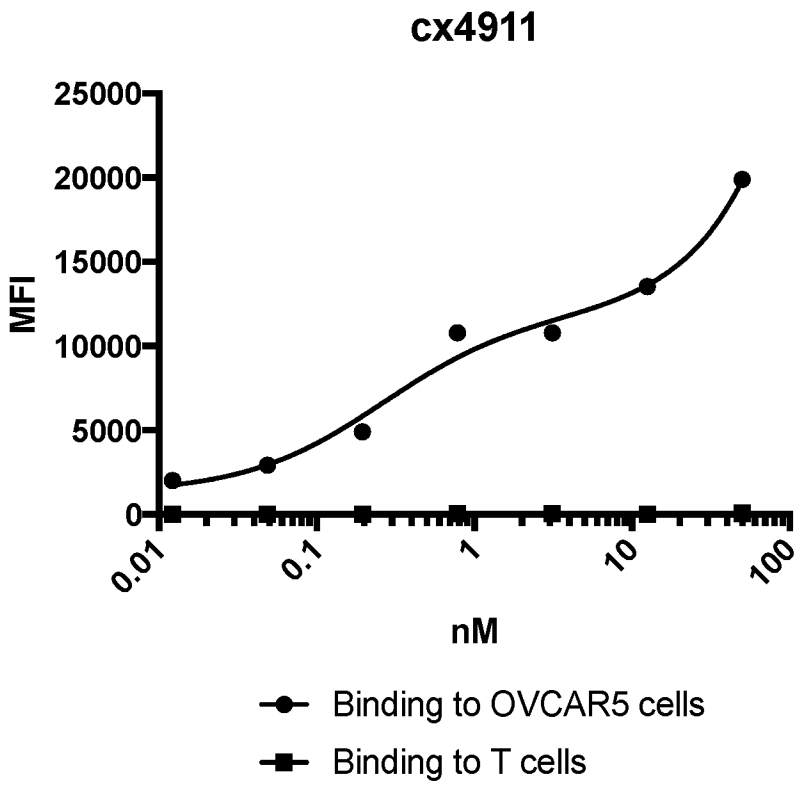
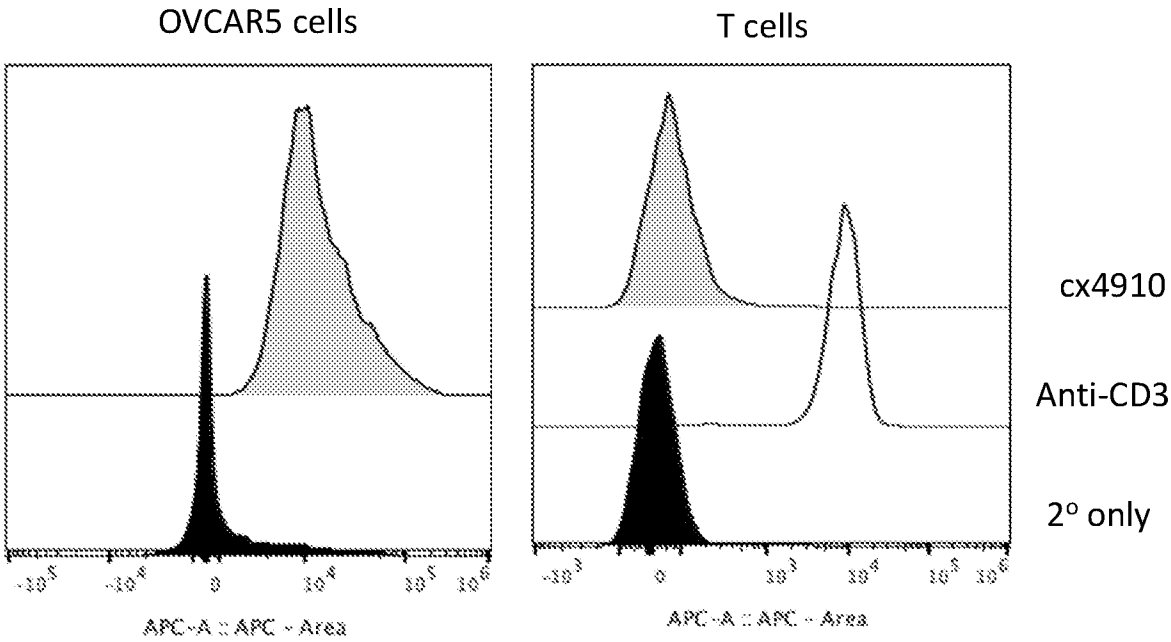

cx4911
25000
20000
15000
10000
5000
0
MFI
0.01
0.1
1
10
100
nM
Binding to OVCAR5 cells
Binding to T cells

FIG. 4M


OVCAR5 cells
T cells
cx4910
Anti-CD3
2° only
APC-A :: APC - Area
APC-A :: APC - Area

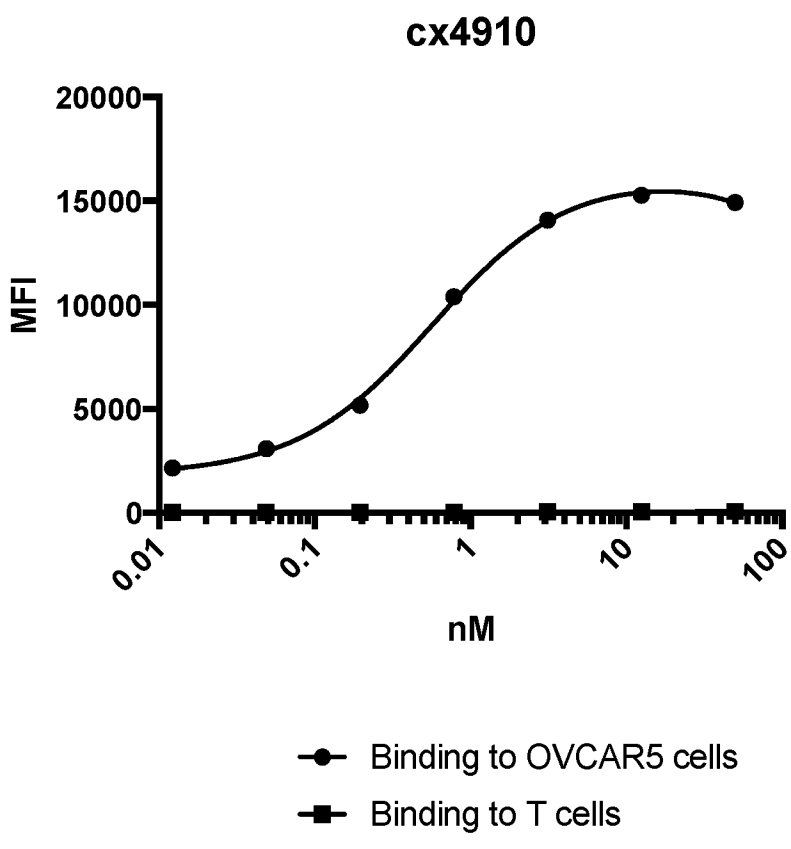

cx4910
20000
15000
10000
5000
0
MFI
0.01
0.1
1
10
100
nM
Binding to OVCAR5 cells
Binding to T cells

FIG. 5A

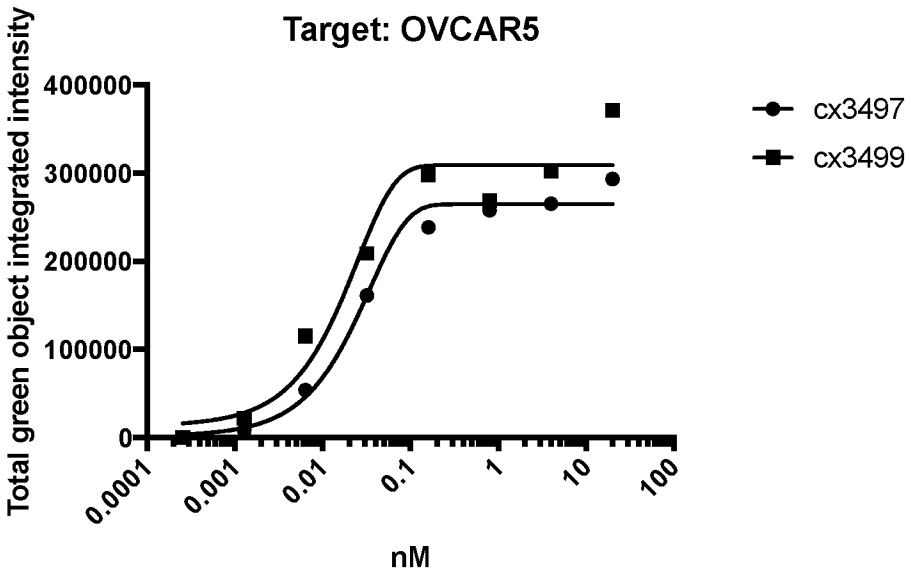

Target: OVCAR5
Total green object integrated intensity
400000
300000
200000
100000
0
0.0001
0.001
0.01
0.1
1
10
100
nM
cx3497
cx3499

FIG. 5B

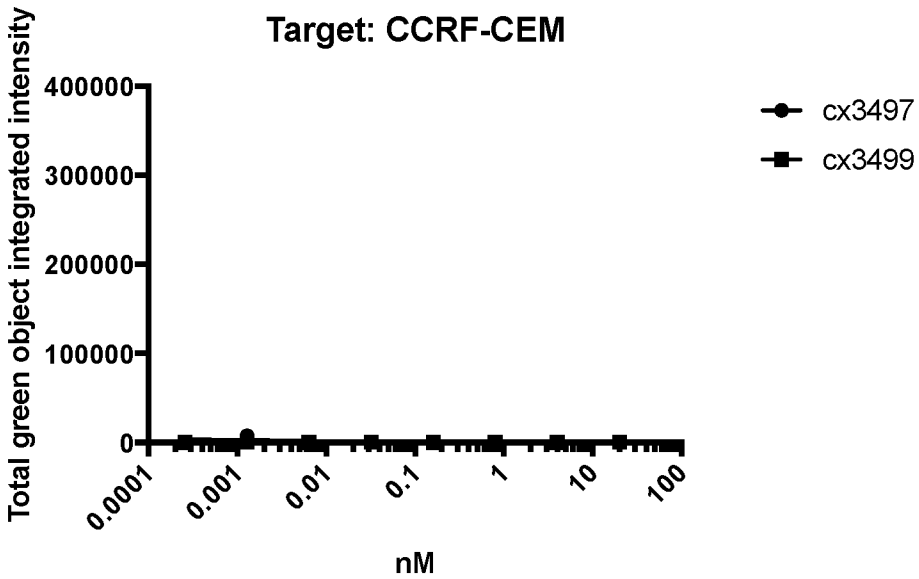

Target: CCRF-CEM
Total green object integrated intensity
400000
300000
200000
100000
0
0.0001
0.001
0.01
0.1
1
10
100
nM
cx3497
cx3499

FIG. 5C

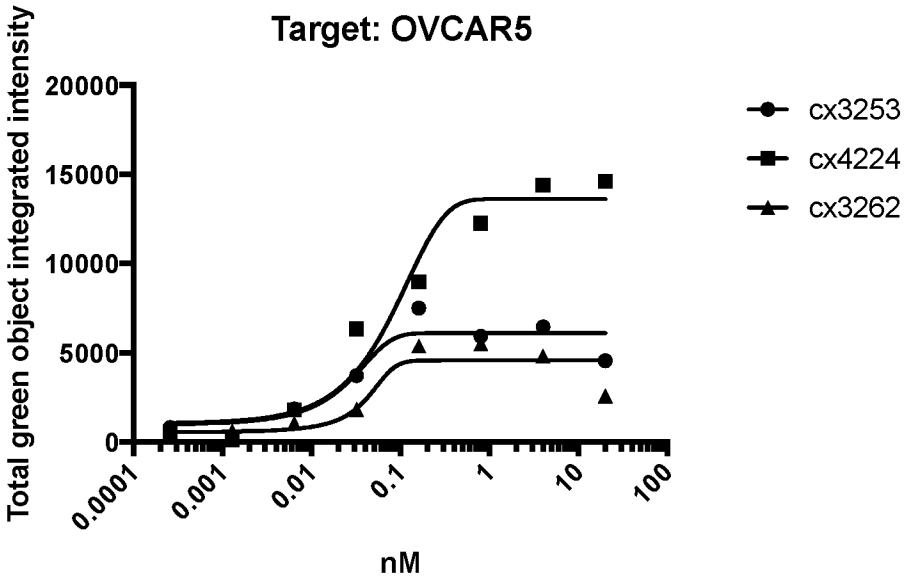

Target: OVCAR5
Total green object integrated intensity
20000
15000
10000
5000
0
0.0001
0.001
0.01
0.1
1
10
100
nM
cx3253
cx4224
cx3262

FIG. 5D

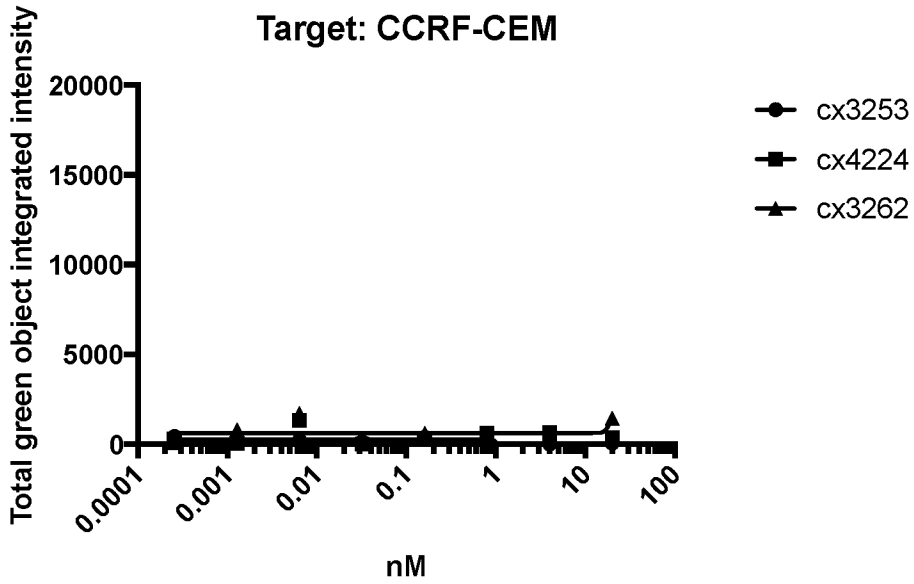

Target: CCRF-CEM
Total green object integrated intensity
20000
15000
10000
5000
0
0.0001
0.001
0.01
0.1
1
10
100
nM
cx3253
cx4224
cx3262

FIG. 5E

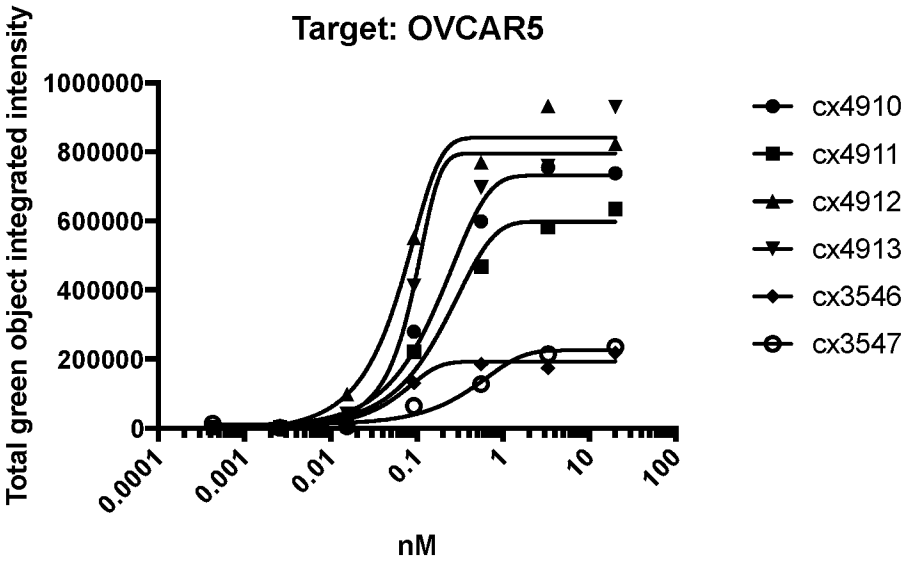

Target: OVCAR5
Total green object integrated intensity
1000000
800000
600000
400000
200000
0
0.0001
0.001
0.01
0.1
1
10
100
nM
cx4910
cx4911
cx4912
cx4913
cx3546
cx3547

FIG. 5F

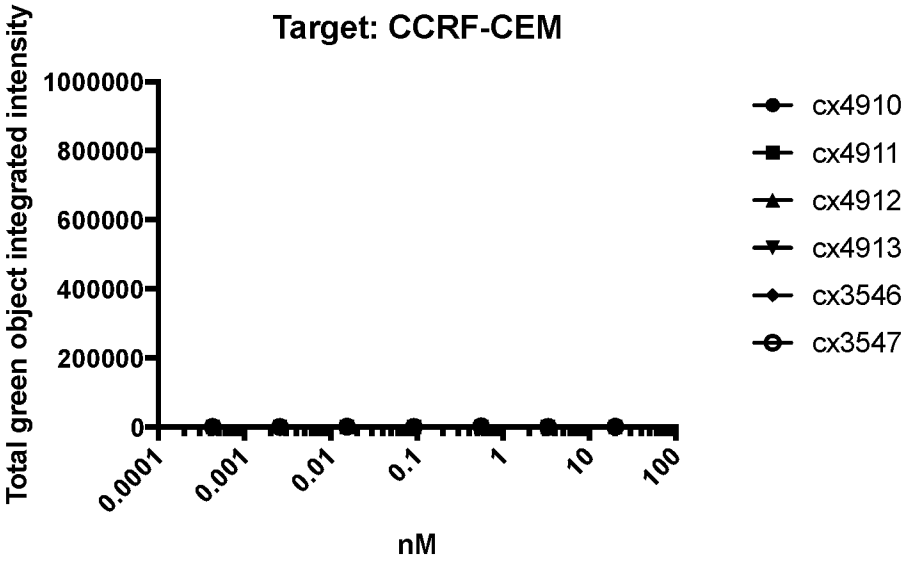

Target: CCRF-CEM
Total green object integrated intensity
1000000
800000
600000
400000
200000
0
0.0001
0.001
0.01
0.1
1
10
100
nM
cx4910
cx4911
cx4912
cx4913
cx3546
cx3547

FIG. 6A

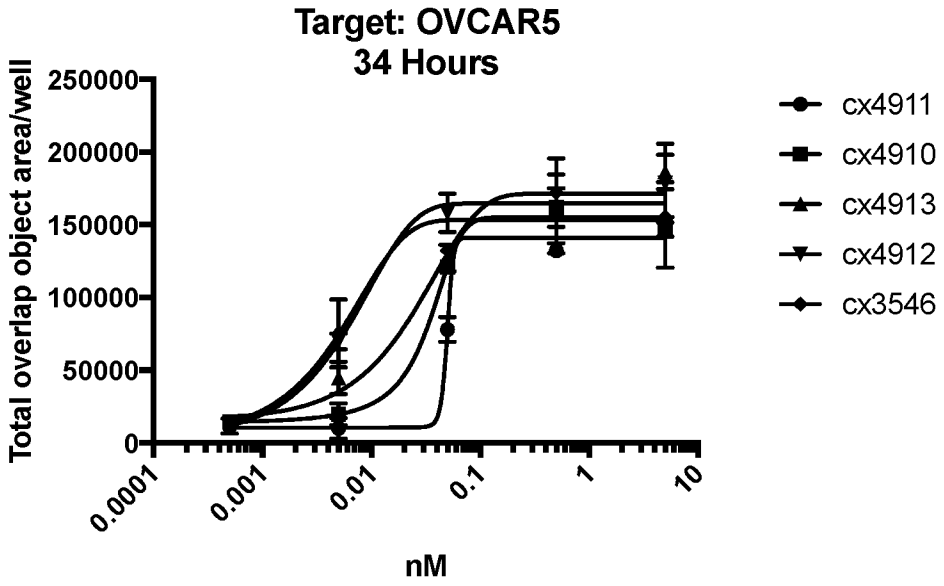

Target: OVCAR5
34 Hours
Total overlap object area/well
250000
200000
150000
100000
50000
0
0.0001
0.001
0.01
0.1
1
10
nM
cx4911
cx4910
cx4913
cx4912
cx3546

FIG. 6B

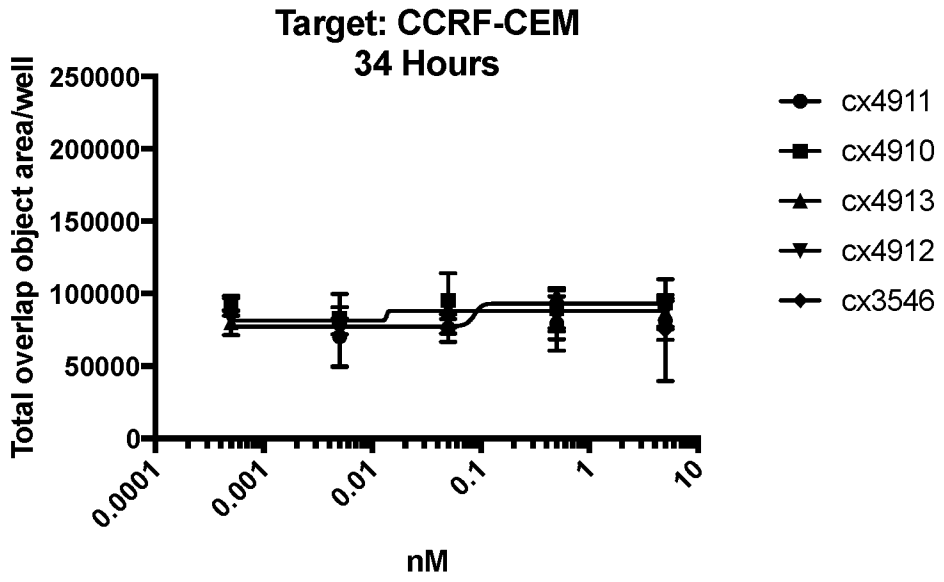

Target: CCRF-CEM
34 Hours
Total overlap object area/well
250000
200000
150000
100000
50000
0
0.0001
0.001
0.01
0.1
1
10
nM
cx4911
cx4910
cx4913
cx4912
cx3546

FIG. 7A

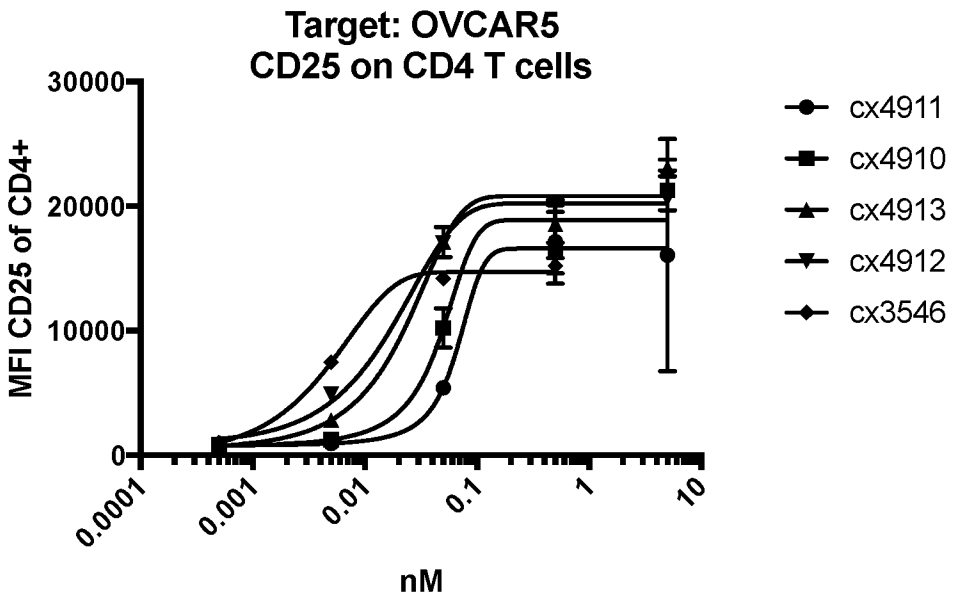

Target: OVCAR5
CD25 on CD4 T cells
MFI CD25 of CD4+
30000
20000
10000
0
0.0001
0.001
0.01
0.1
1
10
nM
cx4911
cx4910
cx4913
cx4912
cx3546

FIG. 7B

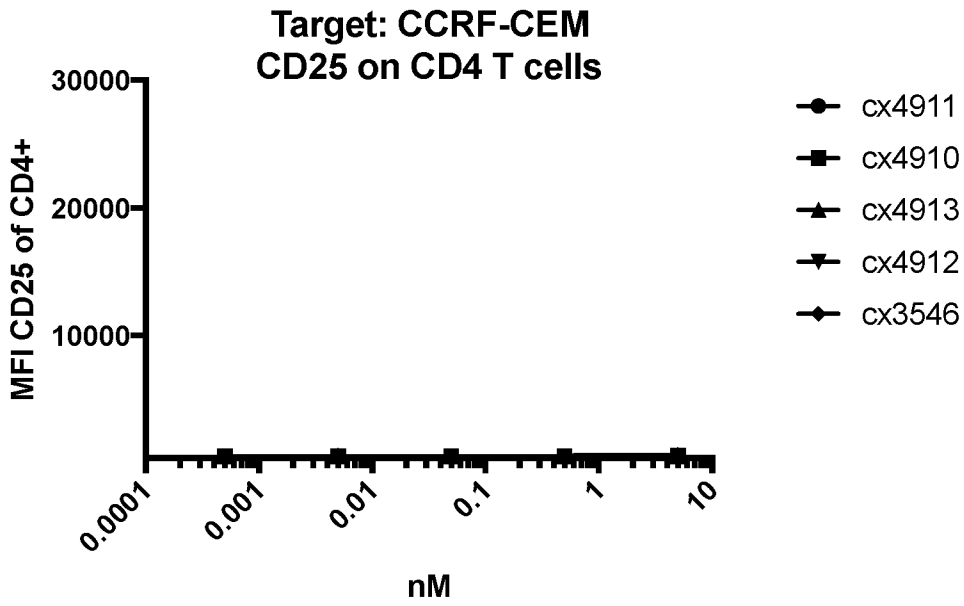

Target: CCRF-CEM
CD25 on CD4 T cells
MFI CD25 of CD4+
30000
20000
10000
0.0001
0.001
0.01
0.1
1
10
nM
cx4911
cx4910
cx4913
cx4912
cx3546

FIG. 7C

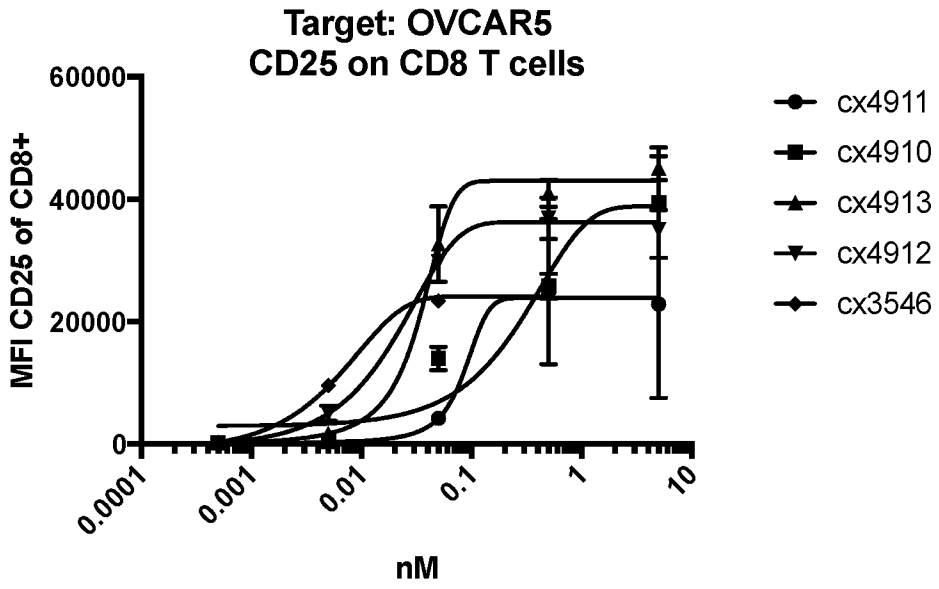

Target: OVCAR5
CD25 on CD8 T cells
MFI CD25 of CD8+
60000
40000
20000
0
0.0001
0.001
0.01
0.1
1
10
nM
cx4911
cx4910
cx4913
cx4912
cx3546

FIG. 7D

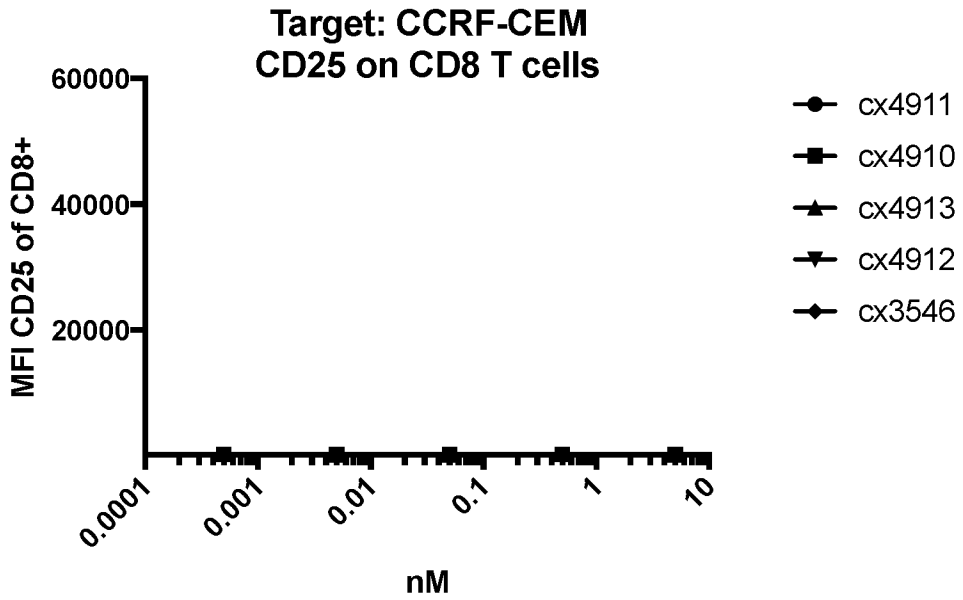

Target: CCRF-CEM
CD25 on CD8 T cells
MFI CD25 of CD8+
60000
40000
20000
0.0001
0.001
0.01
0.1
1
10
nM
cx4911
cx4910
cx4913
cx4912
cx3546

FIG. 8A

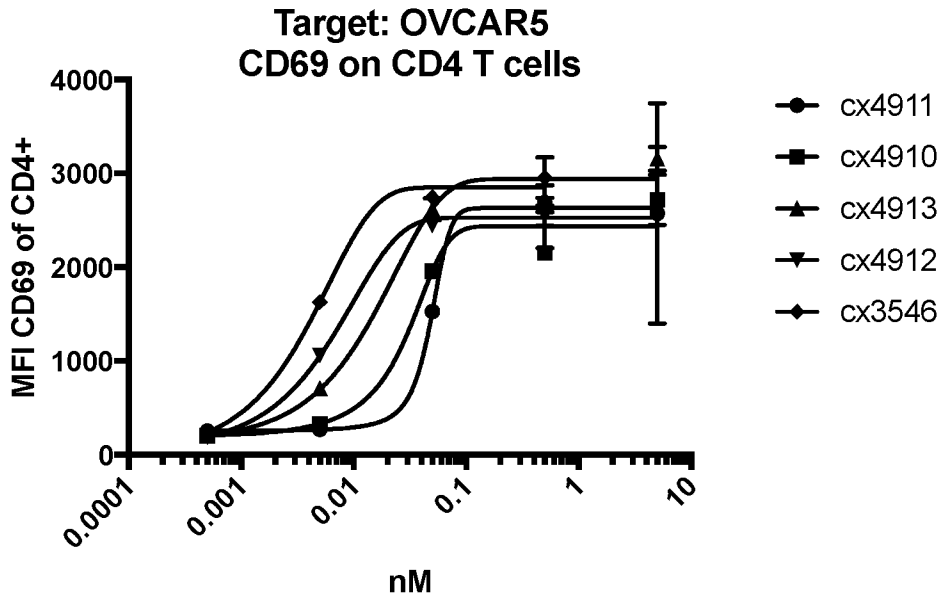

Target: OVCAR5
CD69 on CD4 T cells
MFI CD69 of CD4+
4000
3000
2000
1000
0
0.0001
0.001
0.01
0.1
1
10
nM
cx4911
cx4910
cx4913
cx4912
cx3546

FIG. 8B

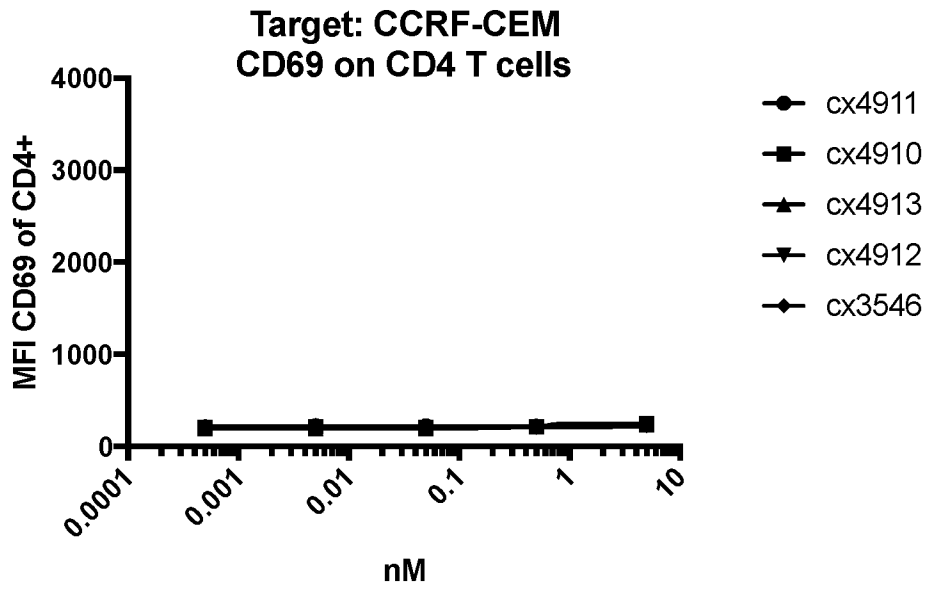

Target: CCRF-CEM
CD69 on CD4 T cells
MFI CD69 of CD4+
4000
3000
2000
1000
0
0.0001
0.001
0.01
0.1
1
10
nM
cx4911
cx4910
cx4913
cx4912
cx3546

FIG. 8C

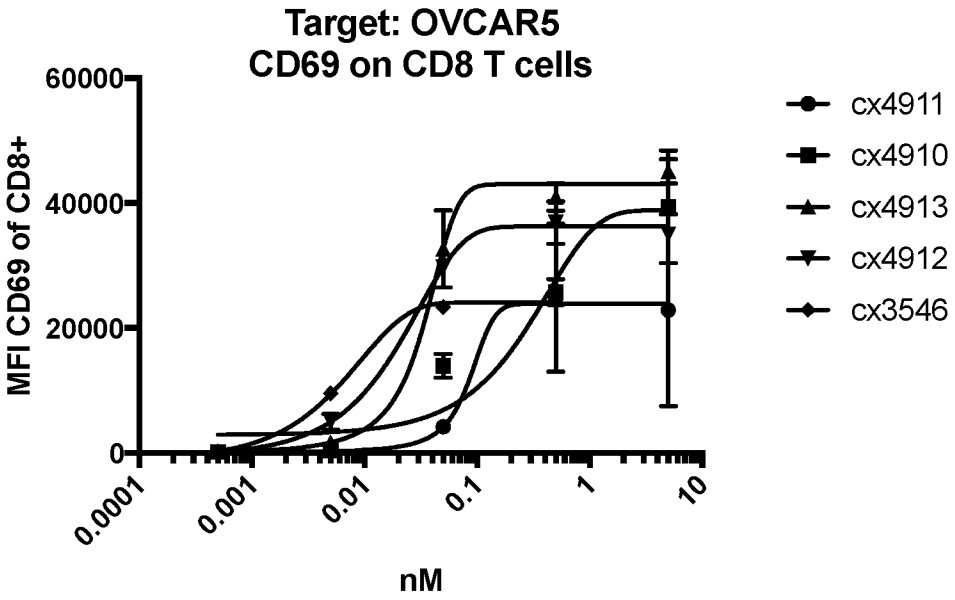

Target: OVCAR5
CD69 on CD8 T cells
MFI CD69 of CD8+
60000
40000
20000
0
0.0001
0.001
0.01
0.1
1
10
nM
cx4911
cx4910
cx4913
cx4912
cx3546

FIG. 8D

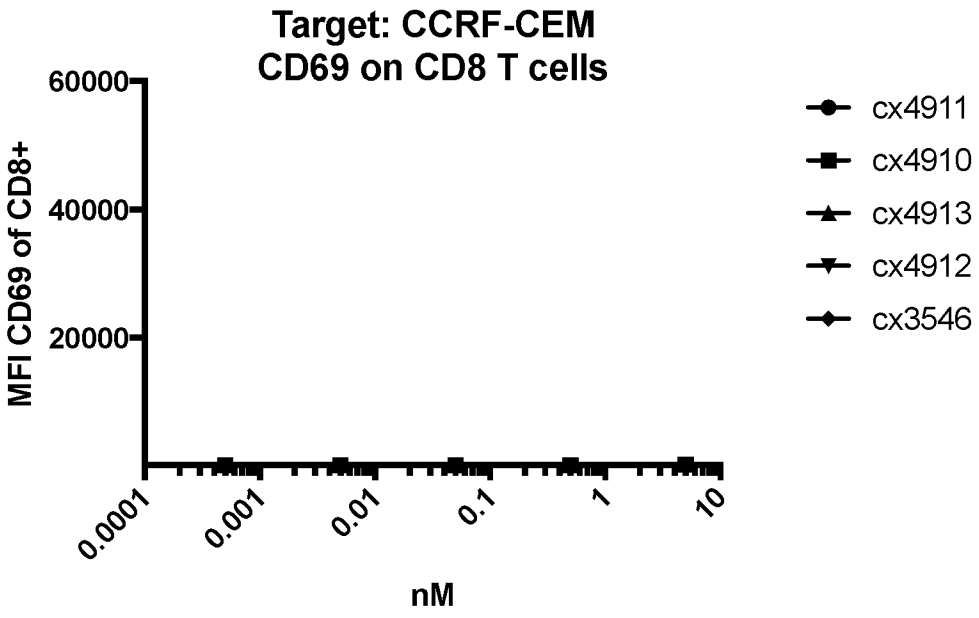

Target: CCRF-CEM
CD69 on CD8 T cells
MFI CD69 of CD8+
60000
40000
20000
0.0001
0.001
0.01
0.1
1
10
nM
cx4911
cx4910
cx4913
cx4912
cx3546

FIG. 9A

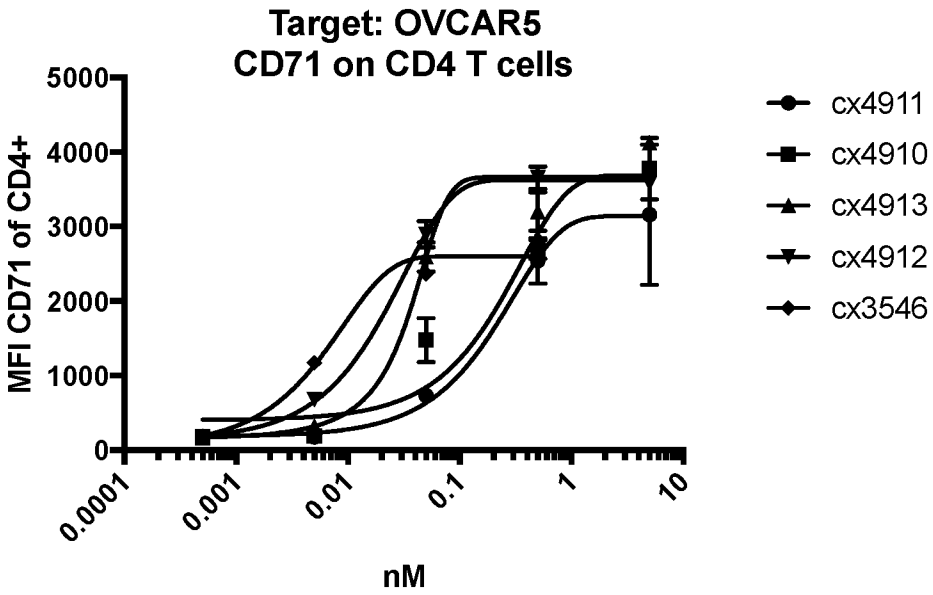

Target: OVCAR5
CD71 on CD4 T cells
MFI CD71 of CD4+
5000
4000
3000
2000
1000
0
0.0001
0.001
0.01
0.1
1
10
nM
cx4911
cx4910
cx4913
cx4912
cx3546

FIG. 9B

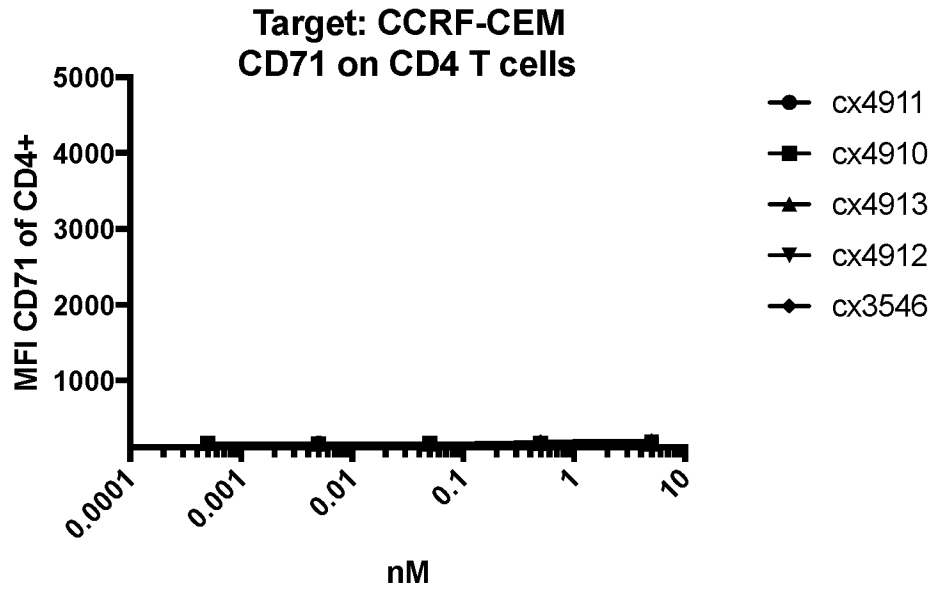

Target: CCRF-CEM
CD71 on CD4 T cells
MFI CD71 of CD4+
5000
4000
3000
2000
1000
0.0001
0.001
0.01
0.1
1
10
nM
cx4911
cx4910
cx4913
cx4912
cx3546

FIG. 9C

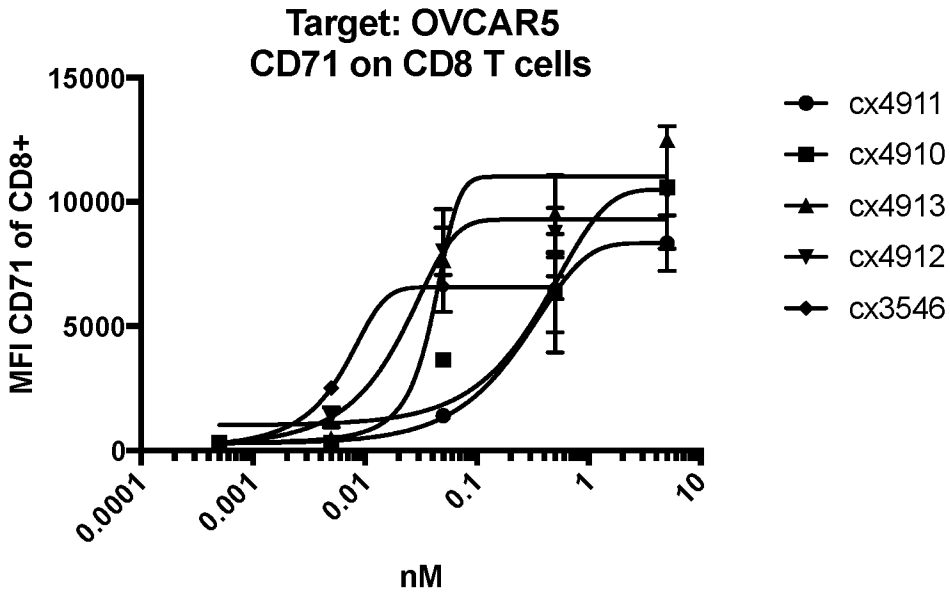

Target: OVCAR5
CD71 on CD8 T cells
MFI CD71 of CD8+
15000
10000
5000
0
0.0001
0.001
0.01
0.1
1
10
nM
cx4911
cx4910
cx4913
cx4912
cx3546

FIG. 9D

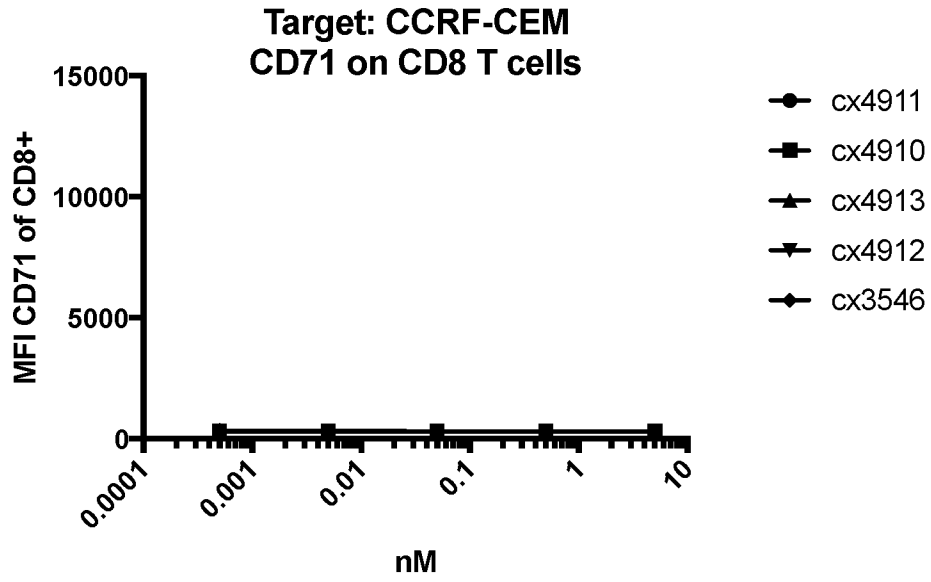

Target: CCRF-CEM
CD71 on CD8 T cells
MFI CD71 of CD8+
15000
10000
5000
0
0.0001
0.001
0.01
0.1
1
10
nM
cx4911
cx4910
cx4913
cx4912
cx3546

FIG. 10A

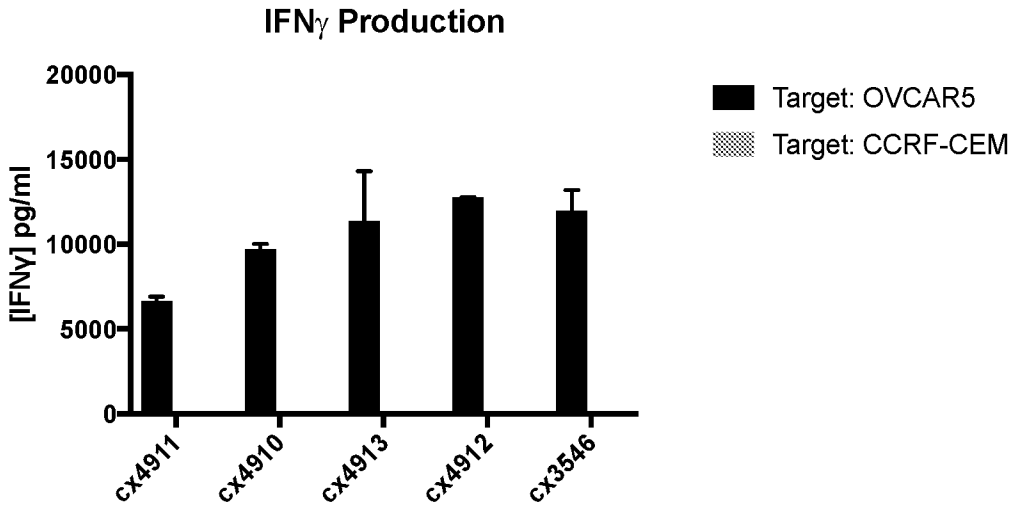


FIG. 10B

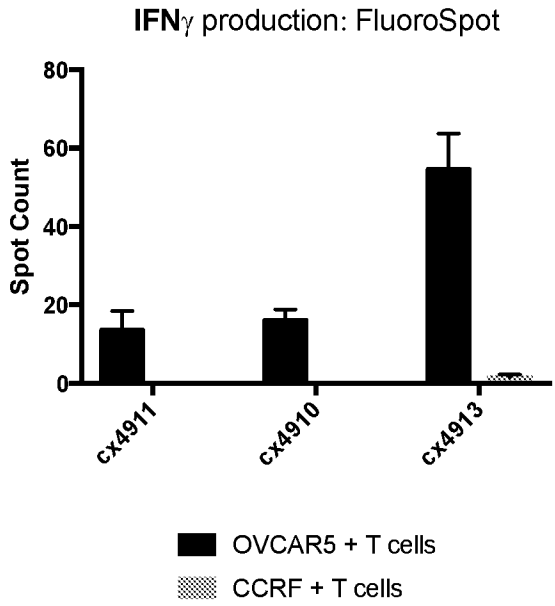


FIG. 10C

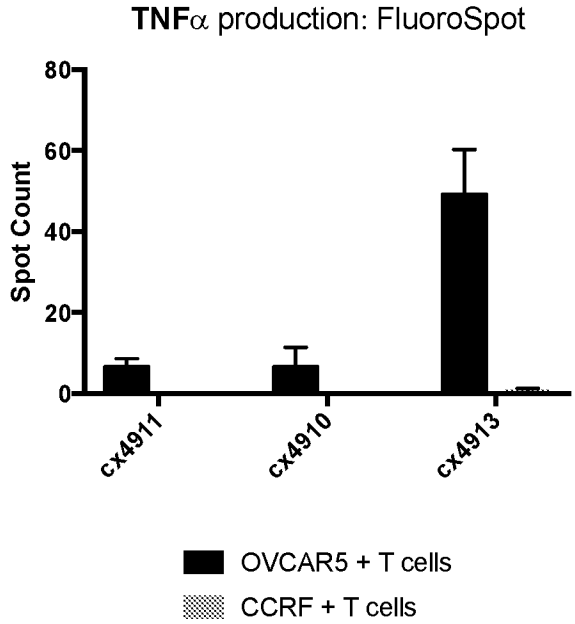


FIG. 11A

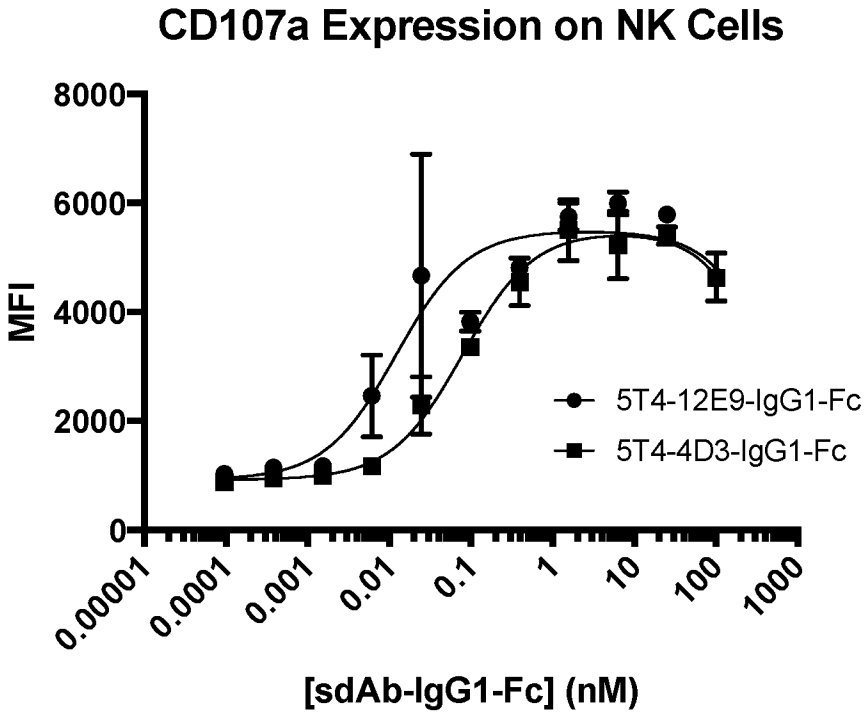


FIG. 11B

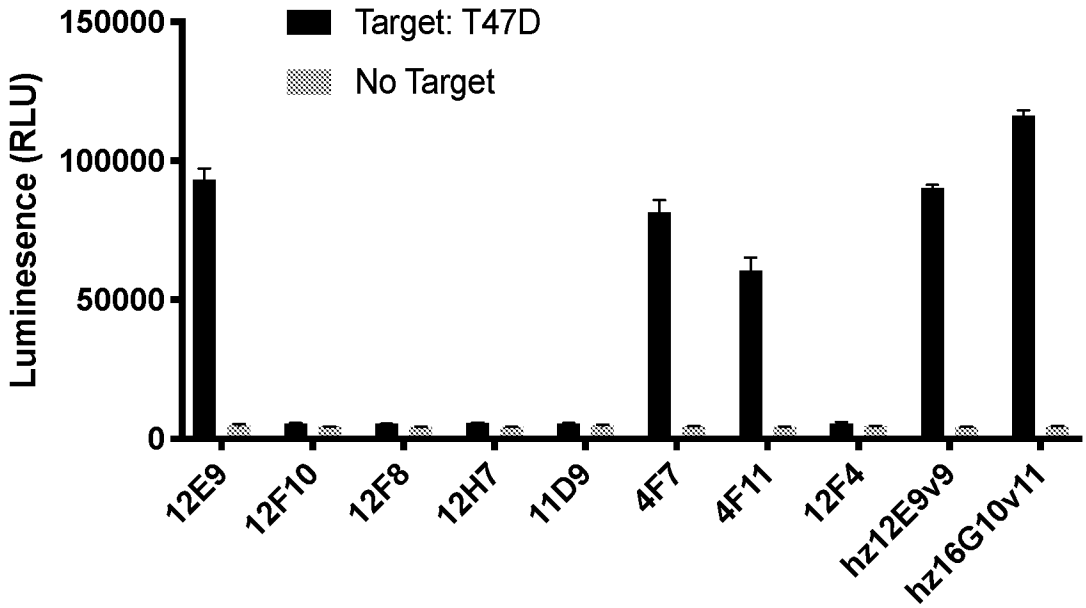


FIG. 11C

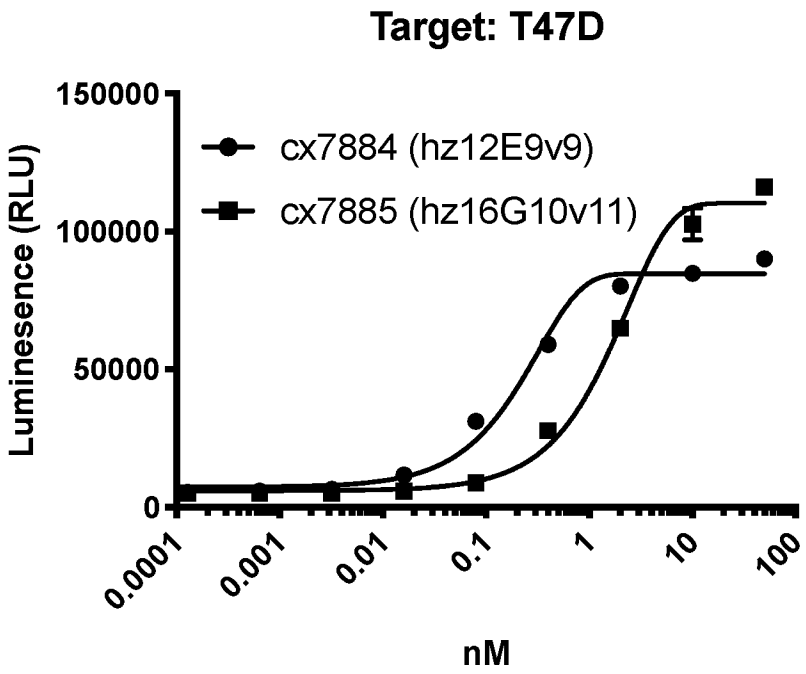


FIG. 11D

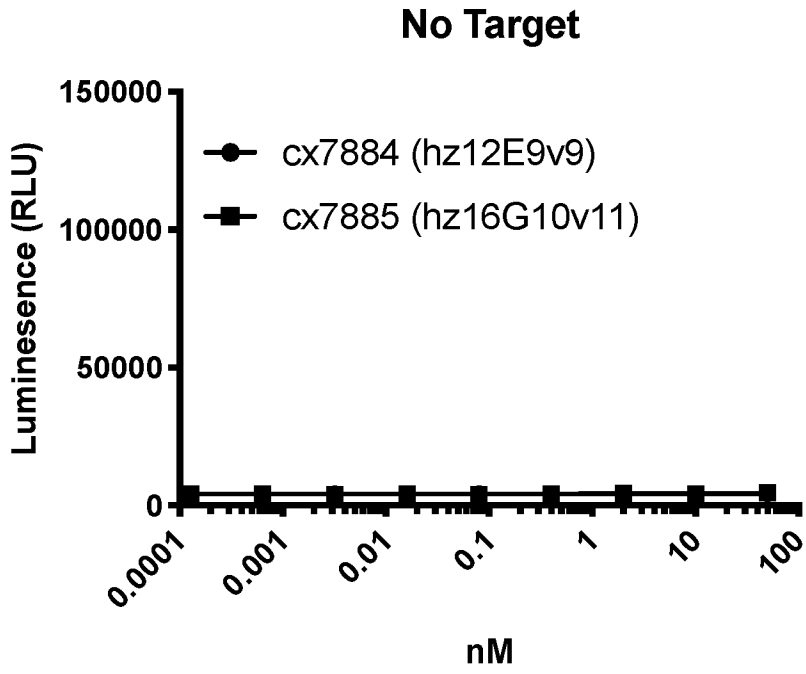


FIG. 12A

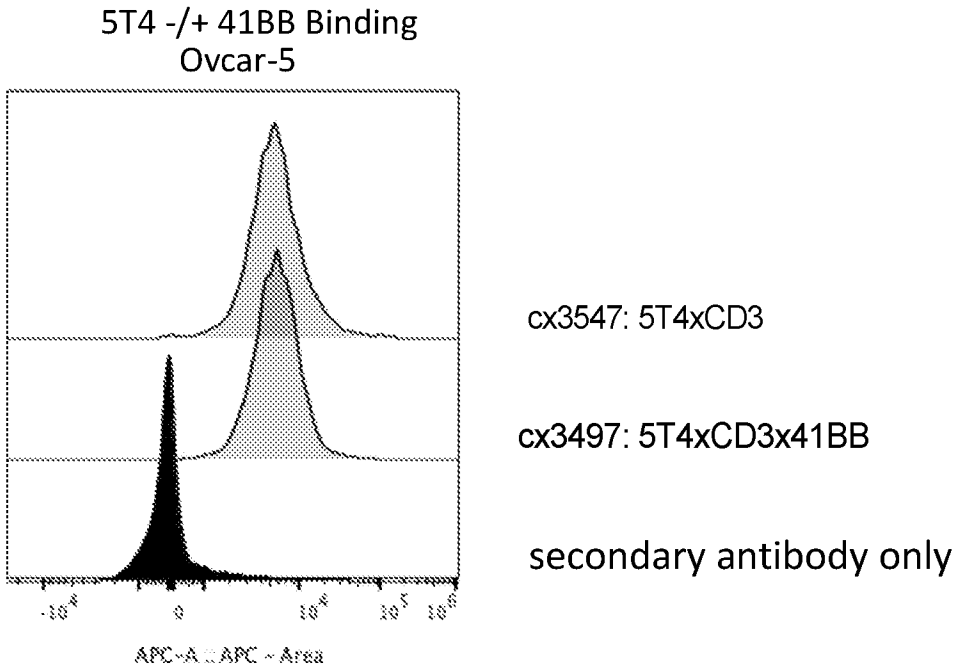


FIG. 12B

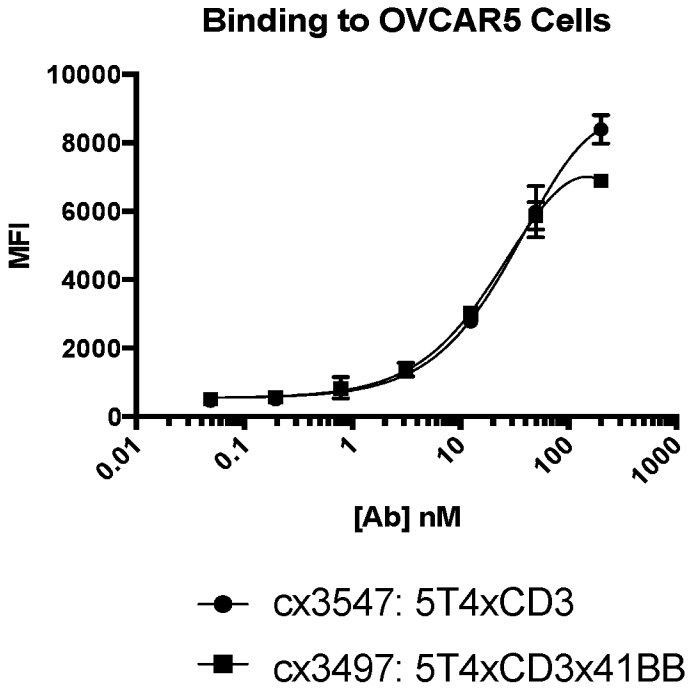


FIG. 12C

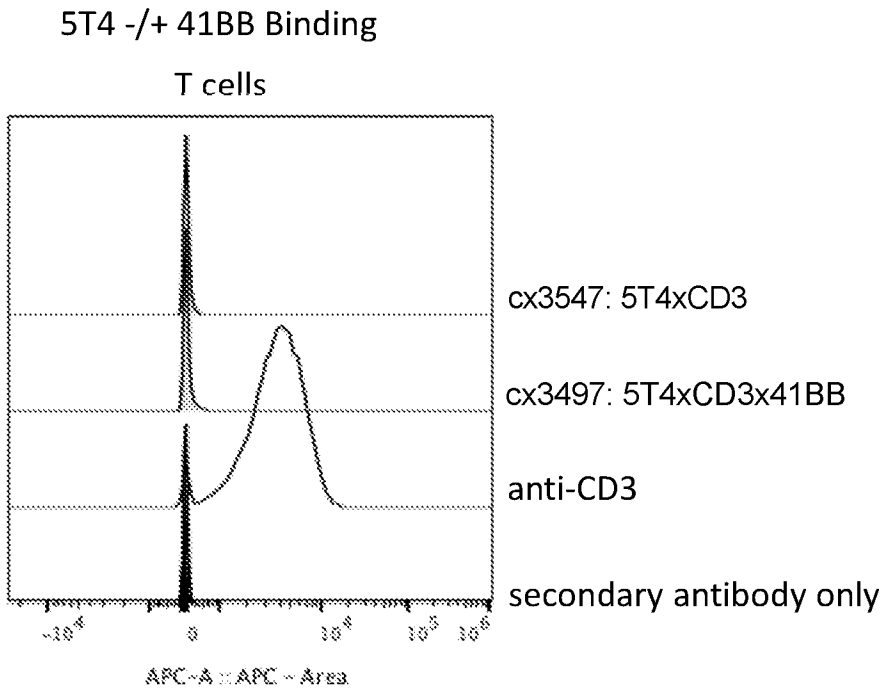


FIG. 12D

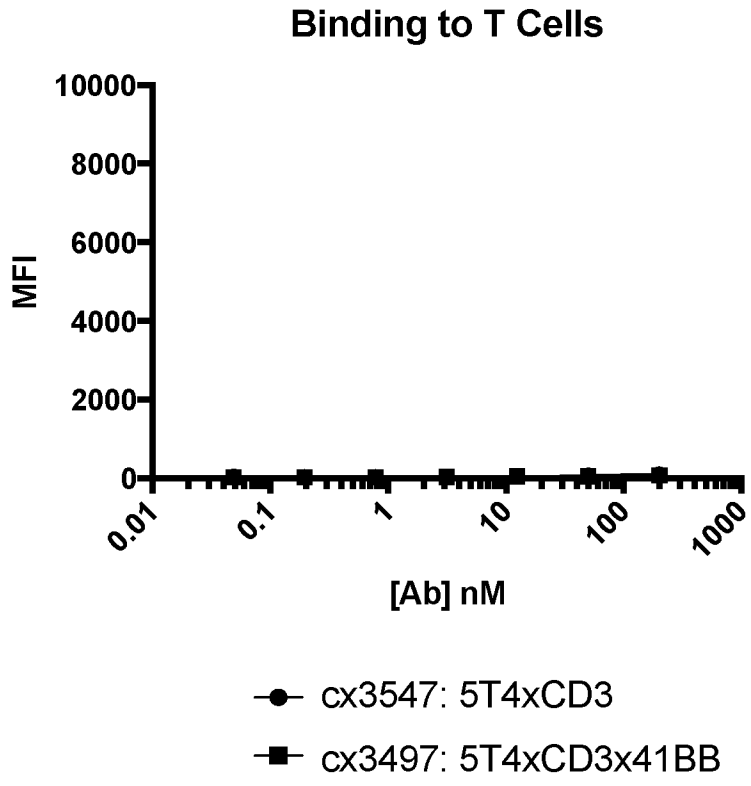


FIG. 13A

5T4 -/+ 41BB cytotox

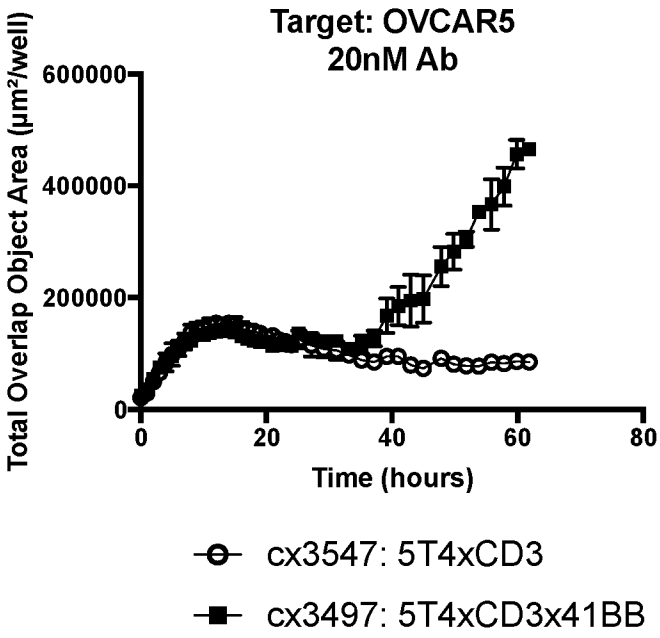


FIG. 13B

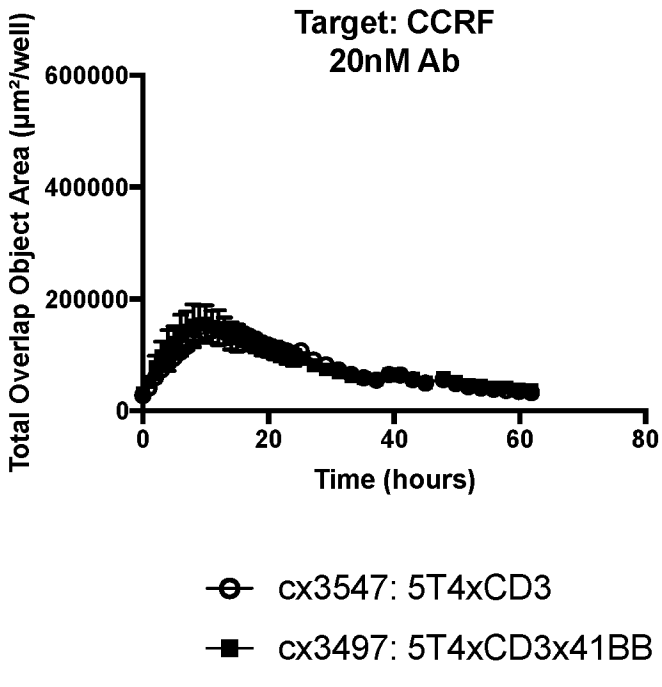


FIG. 14

5T4 -/+ 41BB INF ELISA

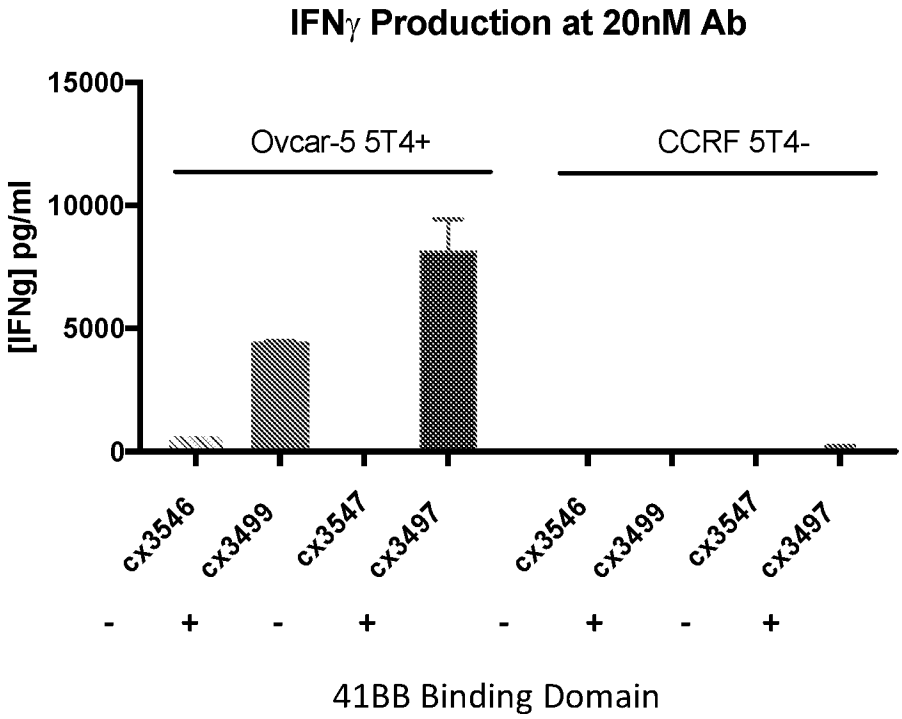


FIG. 15A

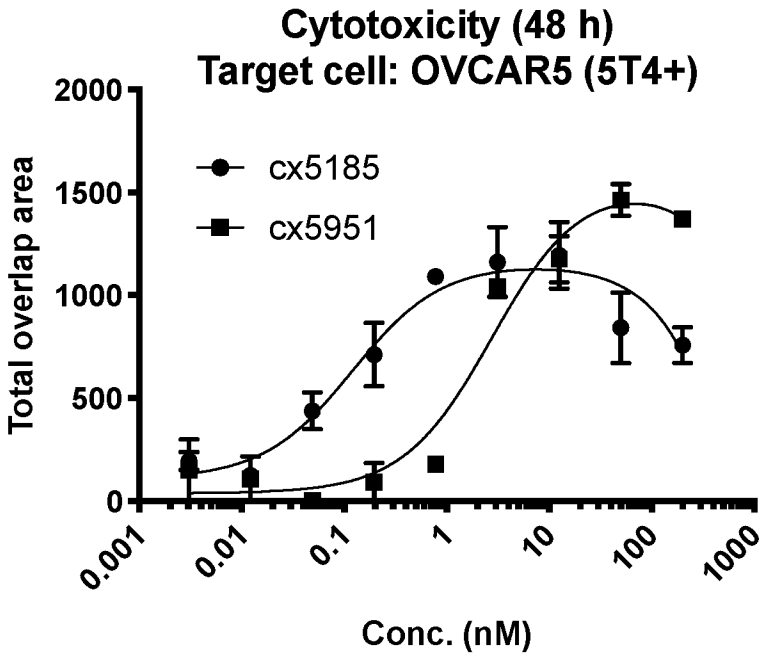


FIG. 15B

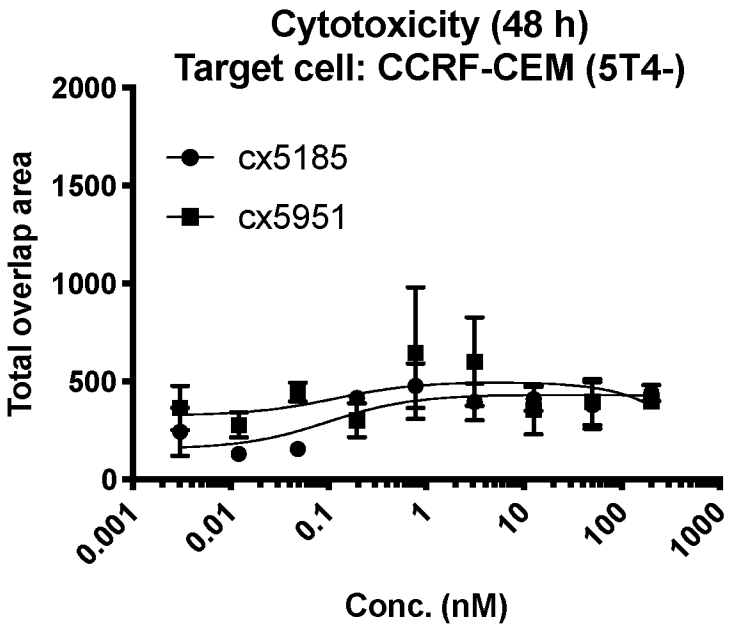


FIG. 16A

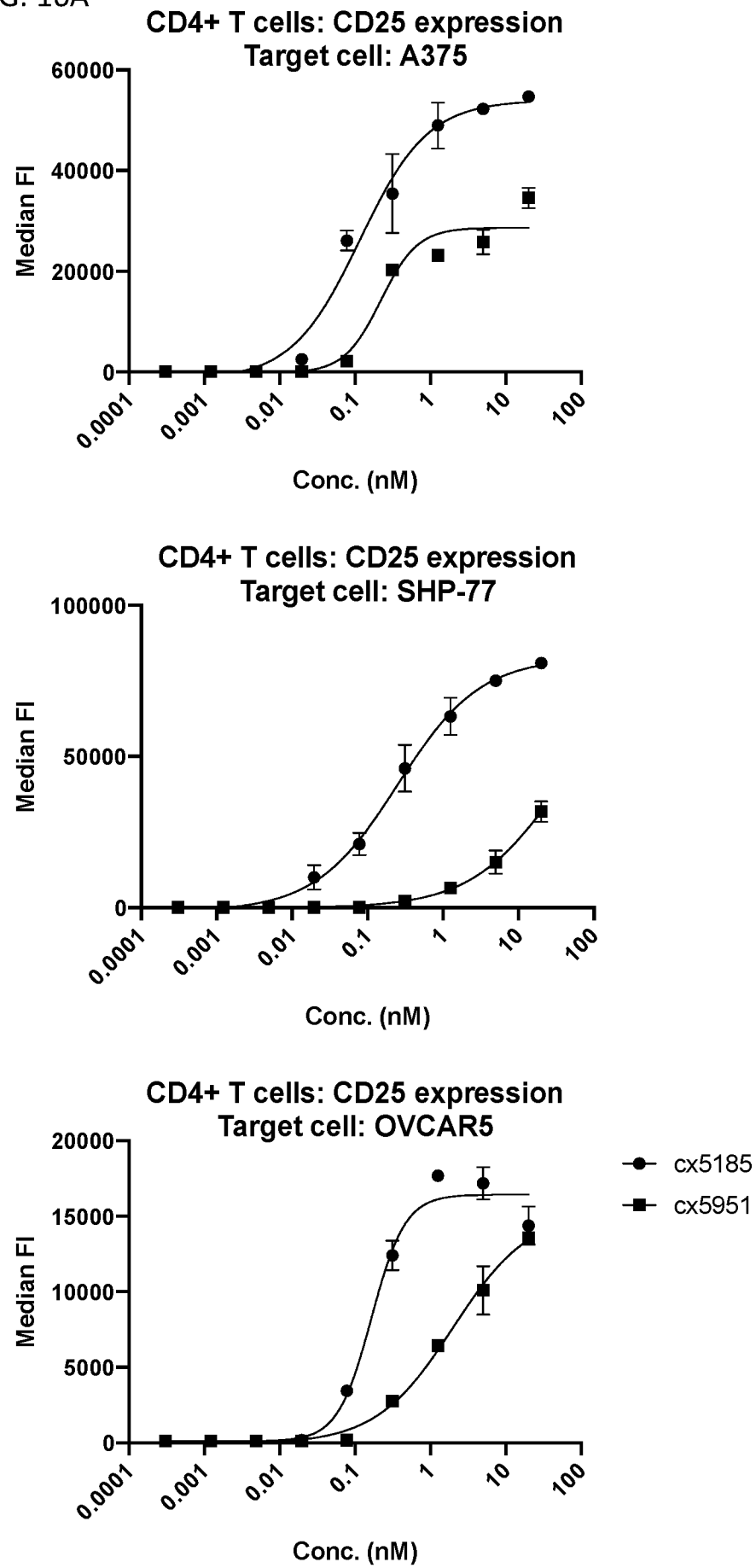

CD4+ T cells: CD25 expression
Target cell: A375
Median FI
60000
40000
20000
0
0.0001
0.001
0.01
0.1
1
10
100
Conc. (nM)
CD4+ T cells: CD25 expression
Target cell: SHP-77
Median FI
100000
50000
0
0.0001
0.001
0.01
0.1
1
10
100
Conc. (nM)
CD4+ T cells: CD25 expression
Target cell: OVCAR5
Median FI
20000
15000
10000
5000
0
0.0001
0.001
0.01
0.1
1
10
100
Conc. (nM)
cx5185
cx5951

FIG. 16B

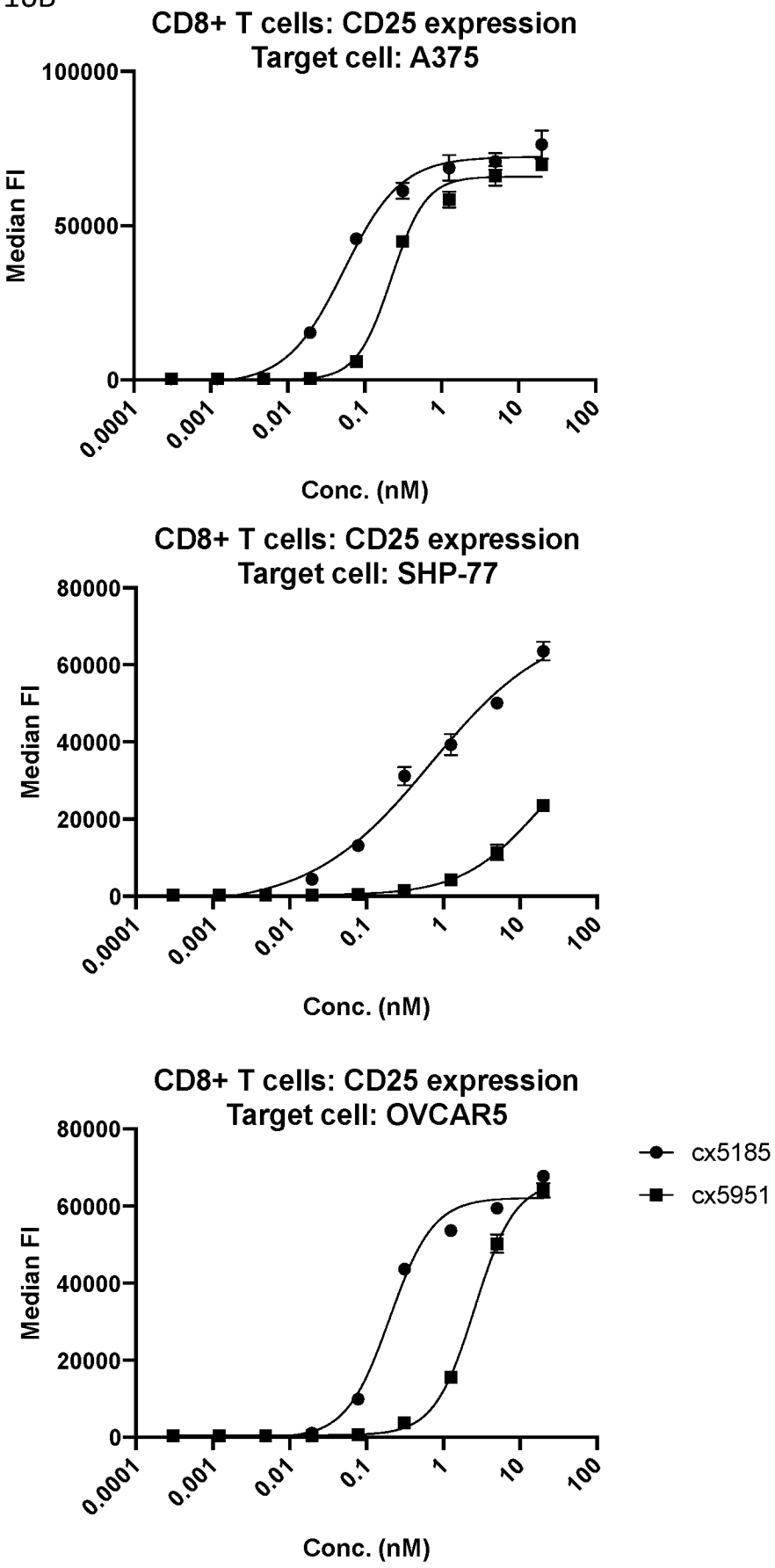

CD8+ T cells: CD25 expression
Target cell: A375
Median FI
100000
50000
0
0.0001
0.001
0.01
0.1
1
10
100
Conc. (nM)
CD8+ T cells: CD25 expression
Target cell: SHP-77
Median FI
80000
60000
40000
20000
0
Conc. (nM)
CD8+ T cells: CD25 expression
Target cell: OVCAR5
Median FI
Conc. (nM)
cx5185
cx5951

FIG. 17

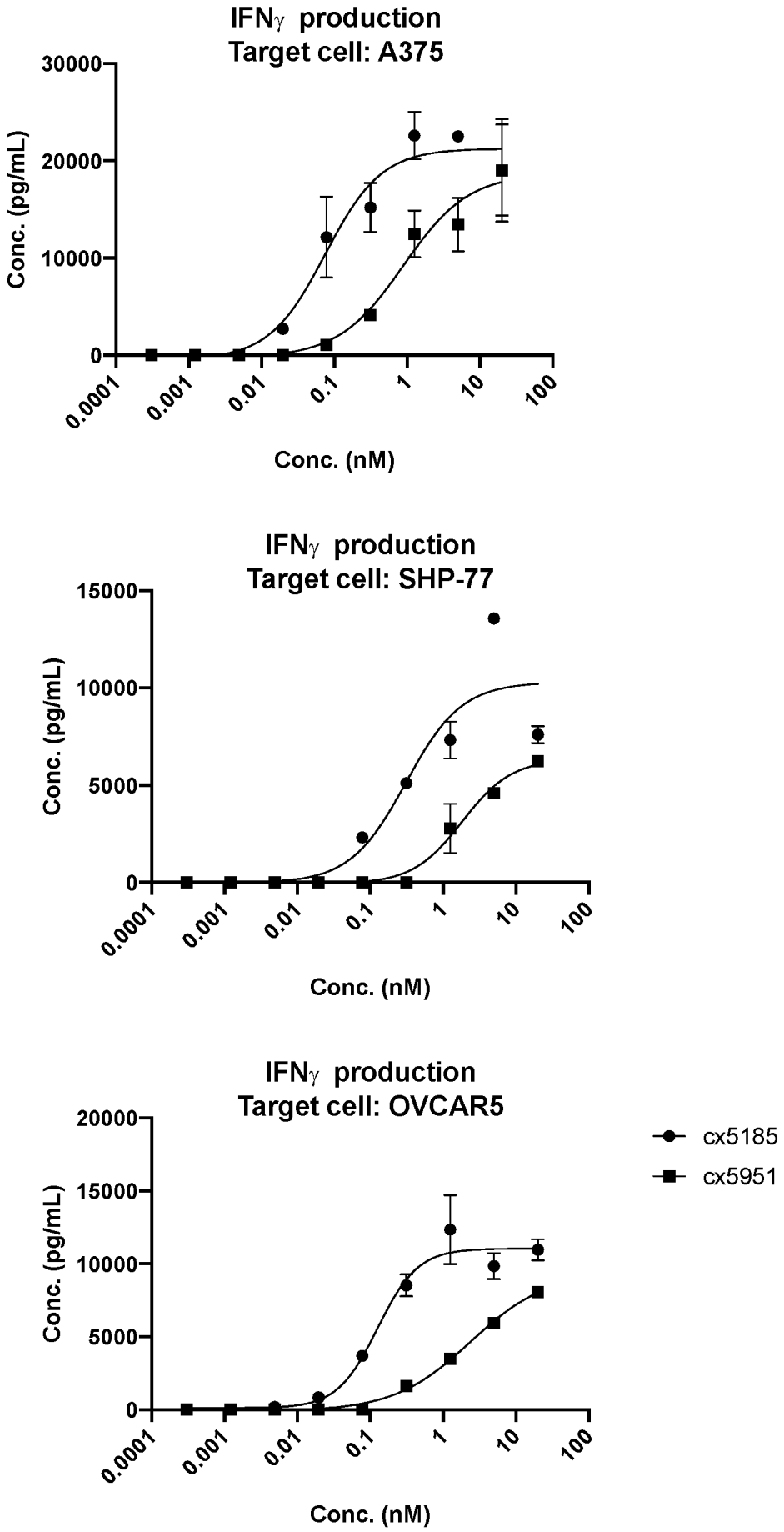

IFNγ production
Target cell: A375
Conc. (pg/mL)
30000
20000
10000
0
0.0001
0.001
0.01
0.1
1
10
100
Conc. (nM)
IFNγ production
Target cell: SHP-77
Conc. (pg/mL)
15000
10000
5000
0
Conc. (nM)
IFNγ production
Target cell: OVCAR5
Conc. (pg/mL)
20000
15000
10000
5000
0
Conc. (nM)
cx5185
cx5951

FIG. 18

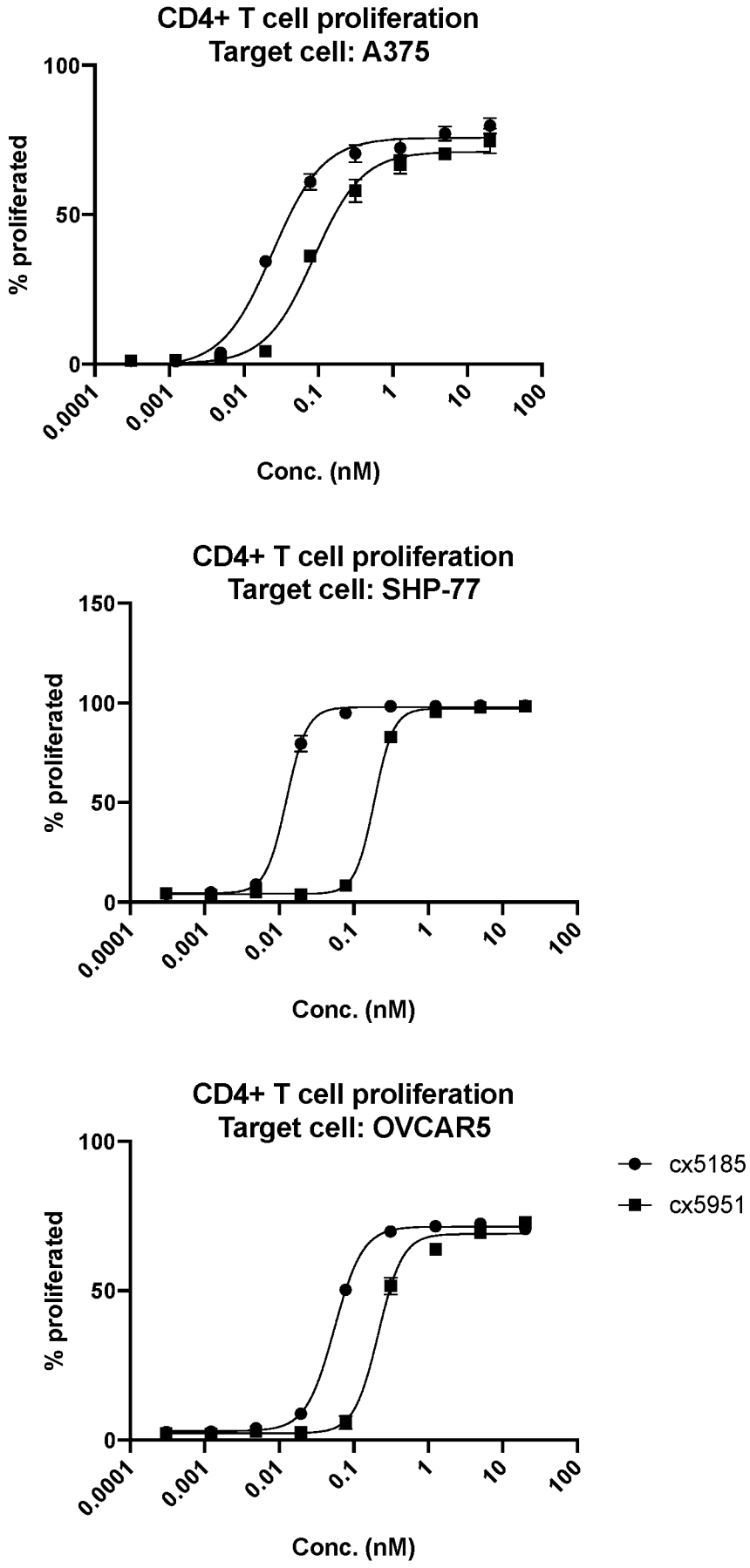

CD4+ T cell proliferation
Target cell: A375
% proliferated
100
50
0
0.0001
0.001
0.01
0.1
1
10
100
Conc. (nM)
CD4+ T cell proliferation
Target cell: SHP-77
% proliferated
150
100
50
0
0.0001
0.001
0.01
0.1
1
10
100
Conc. (nM)
CD4+ T cell proliferation
Target cell: OVCAR5
% proliferated
100
50
0
0.0001
0.001
0.01
0.1
1
10
100
Conc. (nM)
cx5185
cx5951

FIG. 19

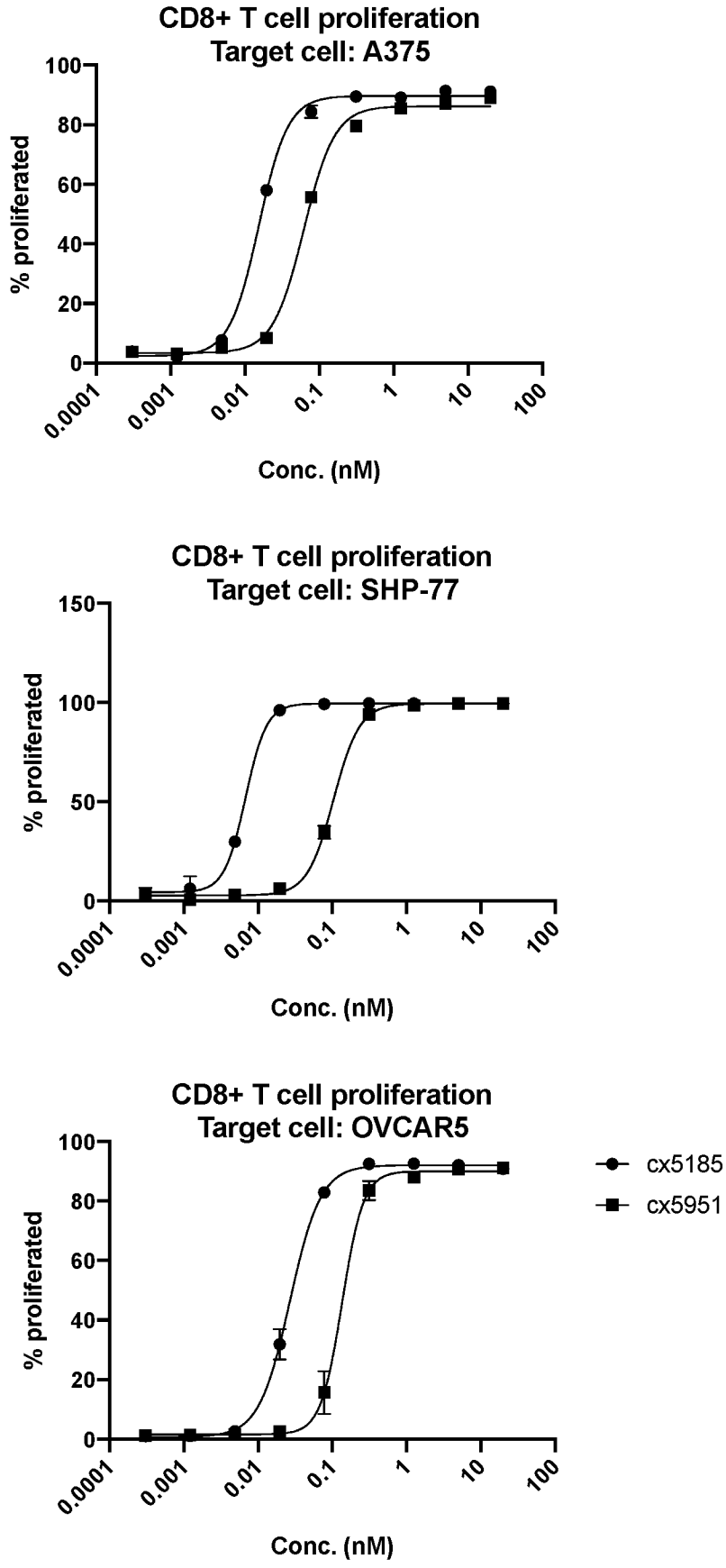
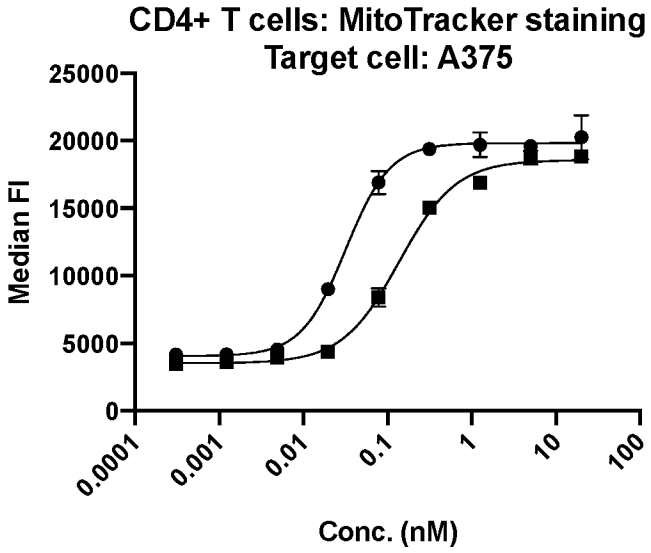
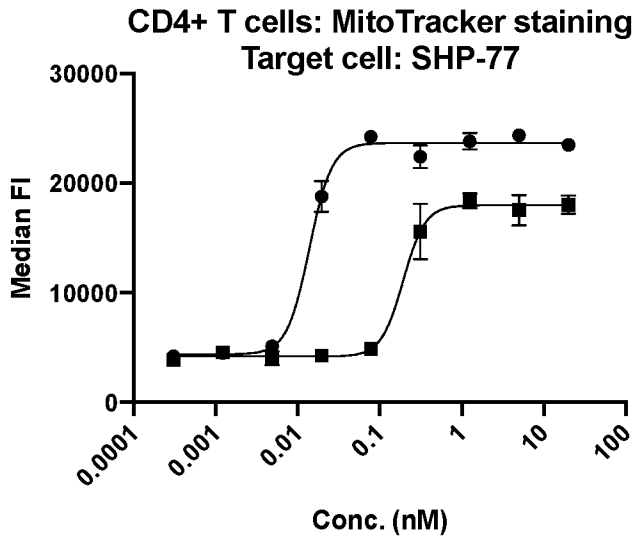

CD8+ T cell proliferation
Target cell: A375
% proliferated
100
80
60
40
20
0
0.0001
0.001
0.01
0.1
1
10
100
Conc. (nM)
CD8+ T cell proliferation
Target cell: SHP-77
% proliferated
150
100
50
0
0.0001
0.001
0.01
0.1
1
10
100
Conc. (nM)
CD8+ T cell proliferation
Target cell: OVCAR5
% proliferated
100
80
60
40
20
0
0.0001
0.001
0.01
0.1
1
10
100
Conc. (nM)
cx5185
cx5951

FIG. 20


CD4+ T cells: MitoTracker staining
Target cell: A375
Median FI
25000
20000
15000
10000
5000
0
0.0001
0.001
0.01
0.1
1
10
100
Conc. (nM)


CD4+ T cells: MitoTracker staining
Target cell: SHP-77
Median FI
30000
20000
10000
0
0.0001
0.001
0.01
0.1
1
10
100
Conc. (nM)

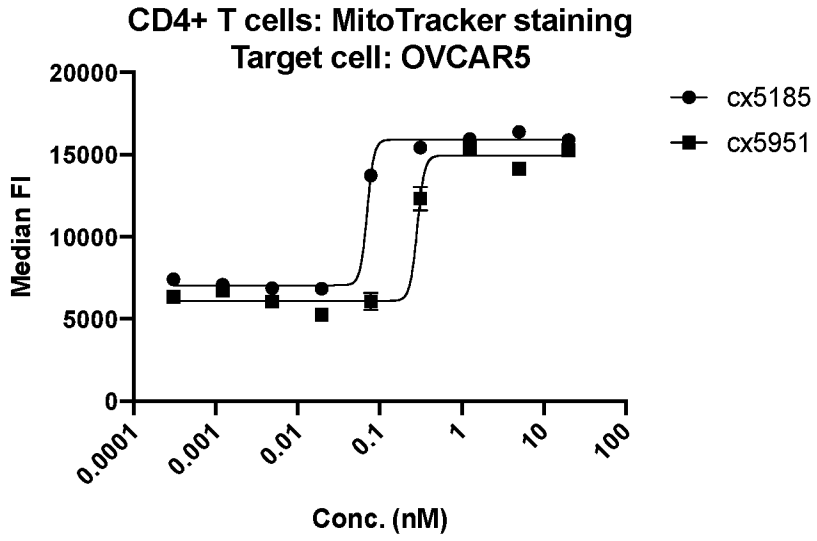

CD4+ T cells: MitoTracker staining
Target cell: OVCAR5
Median FI
20000
15000
10000
5000
0
0.0001
0.001
0.01
0.1
1
10
100
Conc. (nM)
cx5185
cx5951

FIG. 21

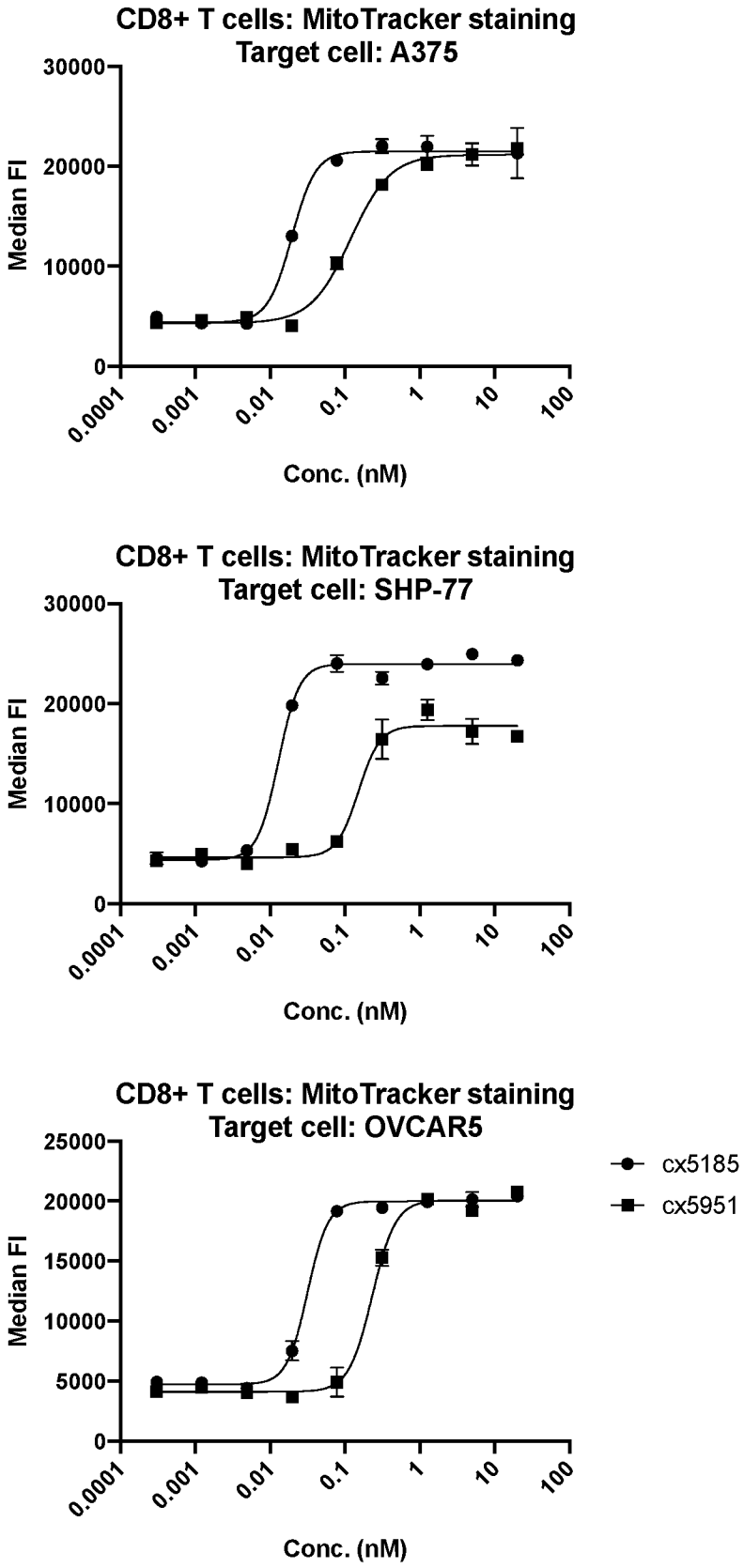

CD8+ T cells: MitoTracker staining
Target cell: A375
Median FI
30000
20000
10000
0
0.0001
0.001
0.01
0.1
1
10
100
Conc. (nM)
CD8+ T cells: MitoTracker staining
Target cell: SHP-77
Median FI
30000
20000
10000
0
0.0001
0.001
0.01
0.1
1
10
100
Conc. (nM)
CD8+ T cells: MitoTracker staining
Target cell: OVCAR5
Median FI
25000
20000
15000
10000
5000
0
0.0001
0.001
0.01
0.1
1
10
100
Conc. (nM)
cx5185
cx5951

FIG. 22

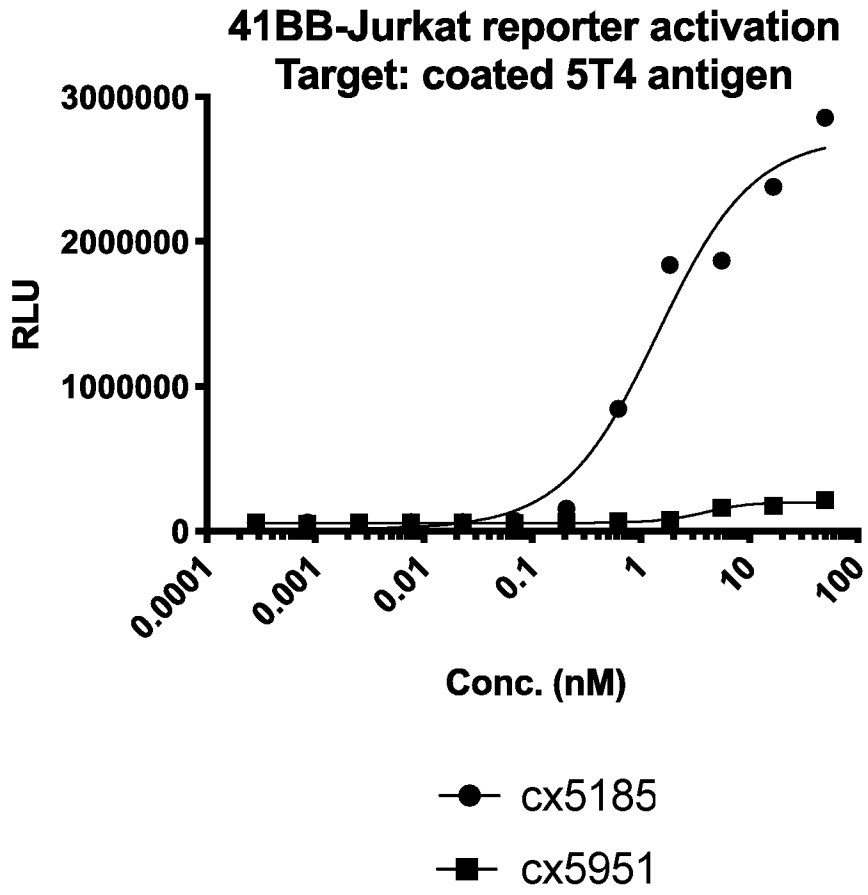

41BB-Jurkat reporter activation
Target: coated 5T4 antigen
RLU
3000000
2000000
1000000
0
0.0001
0.001
0.01
0.1
1
10
100
Conc. (nM)
cx5185
cx5951

FIG. 23A

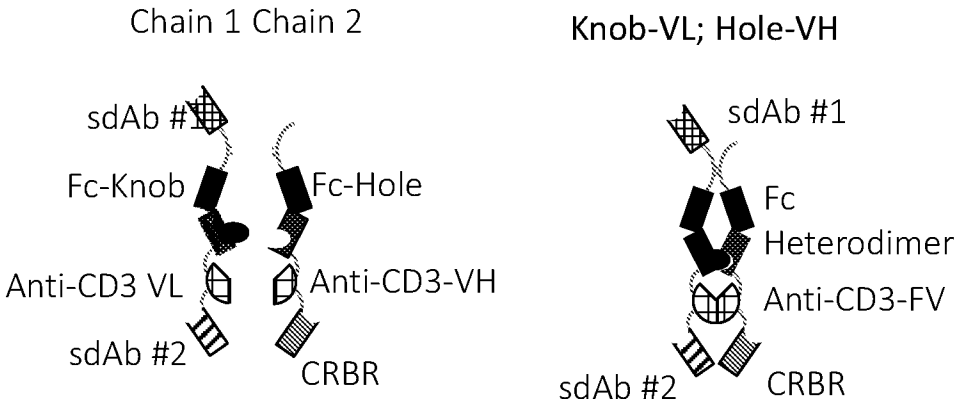


FIG. 23B

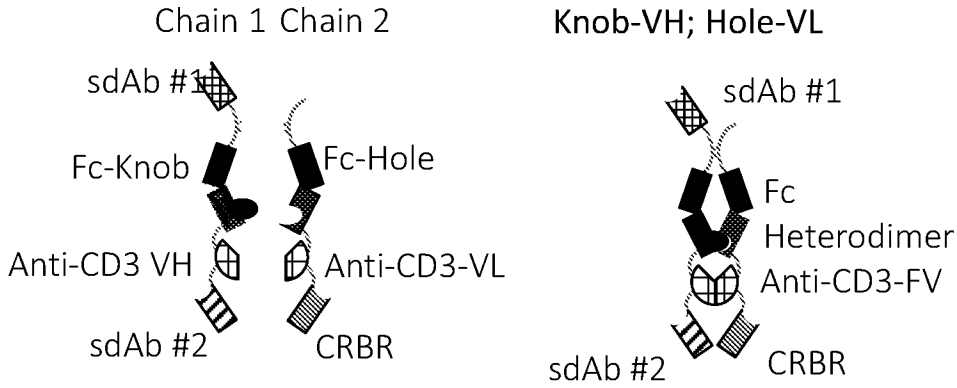


FIG. 24A

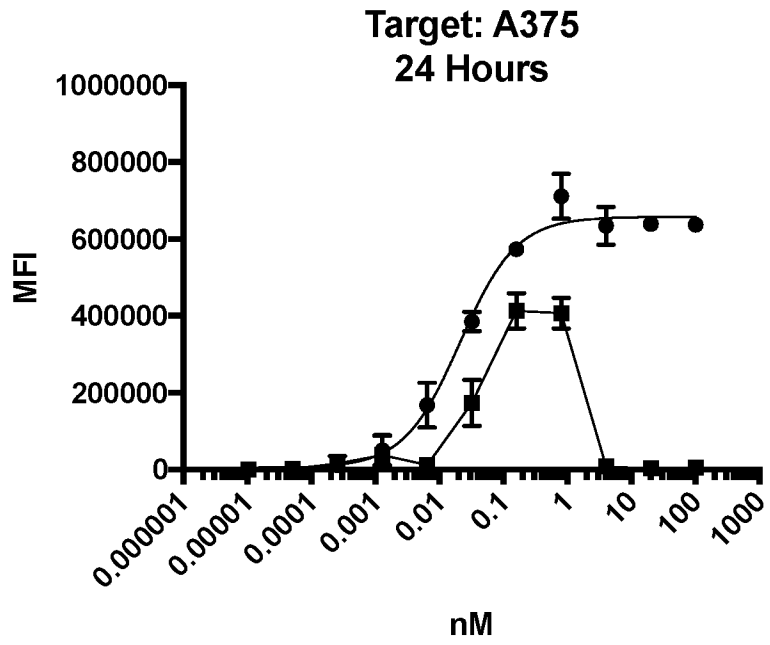


FIG. 24B

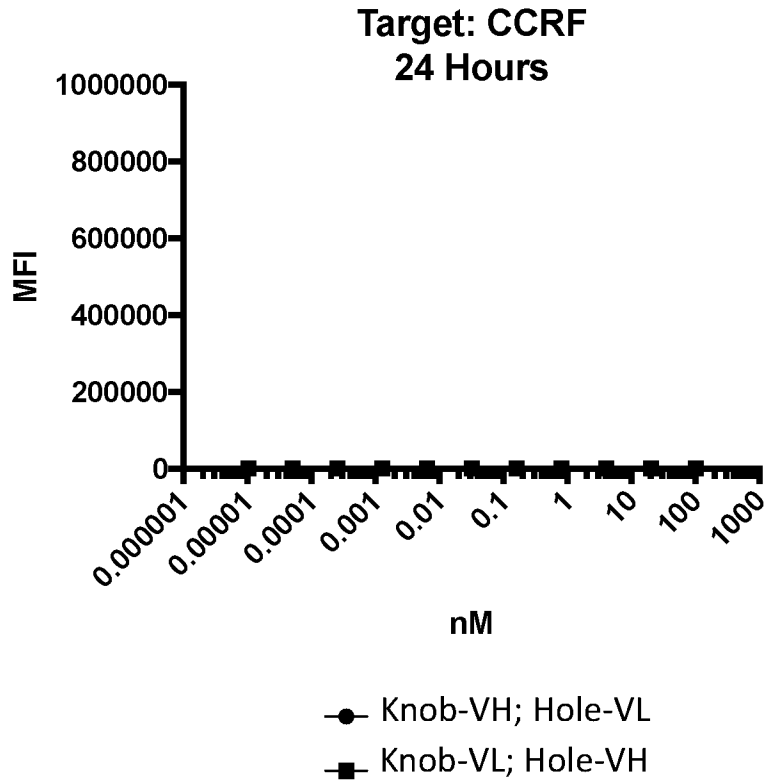


FIG. 24C

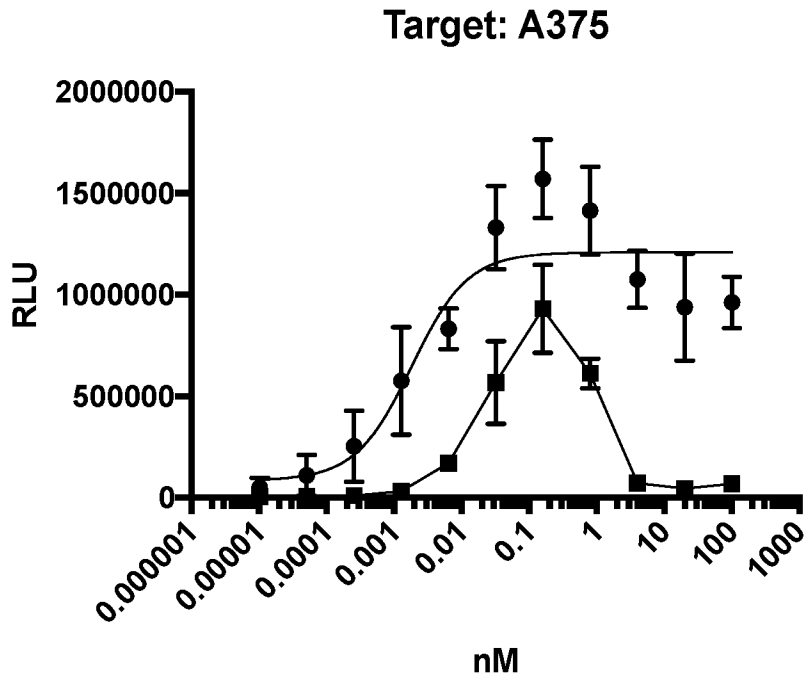


FIG. 24D

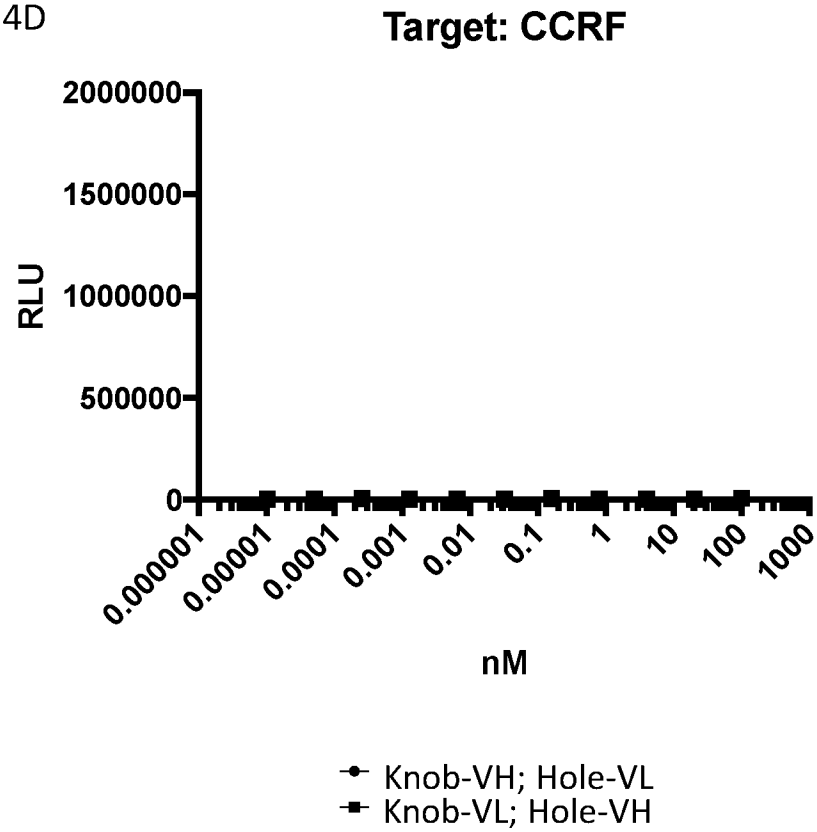


FIG. 25

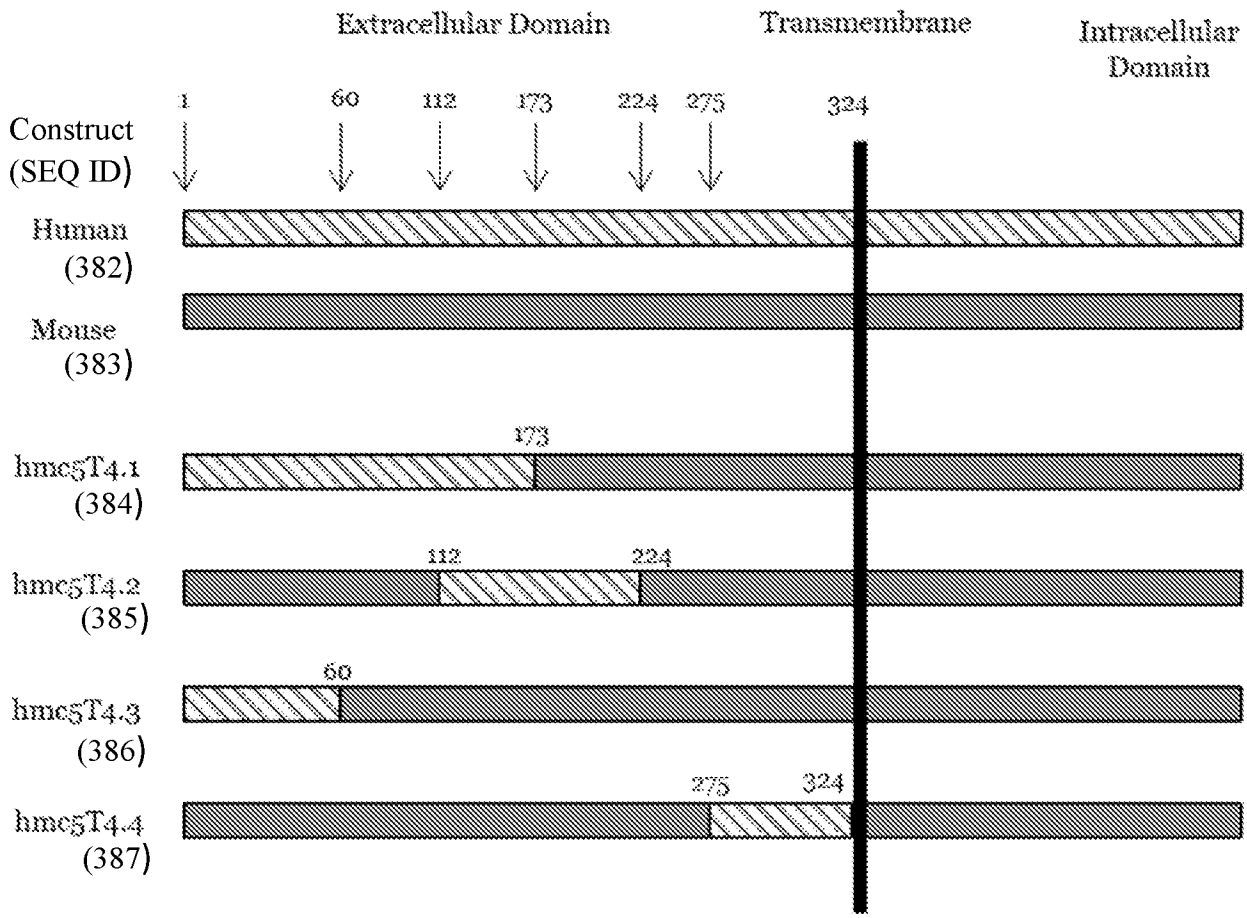

Extracellular Domain
Transmembrane
Intracellular Domain
1
60
112
173
224
275
324
Construct (SEQ ID)
Human (382)
Mouse (383)
hmc5T4.1 (384)
hmc5T4.2 (385)
hmc5T4.3 (386)
hmc5T4.4 (387)

FIG. 26A

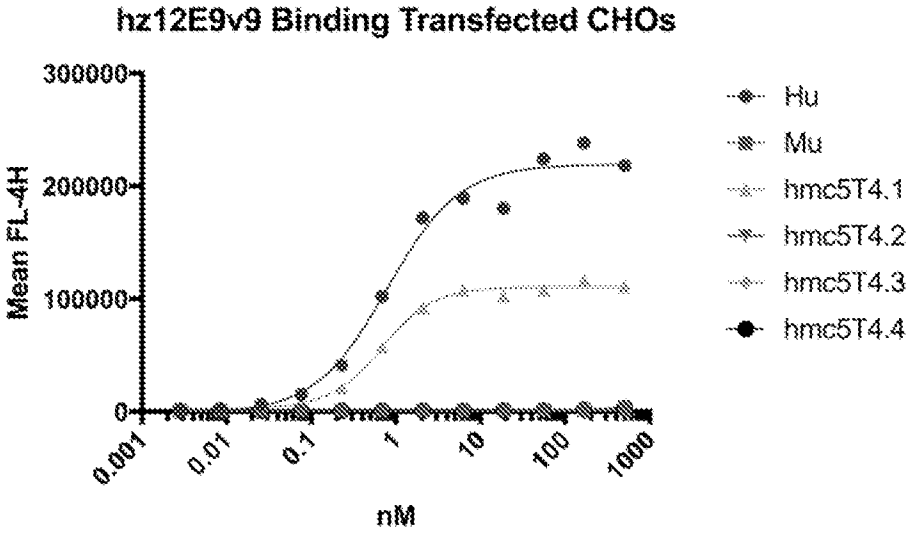


FIG. 26B

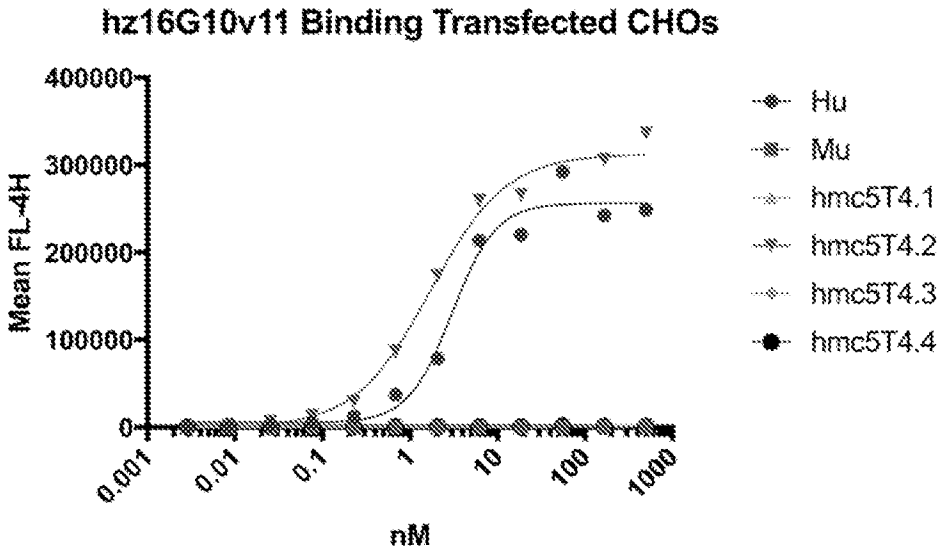


FIG. 26C

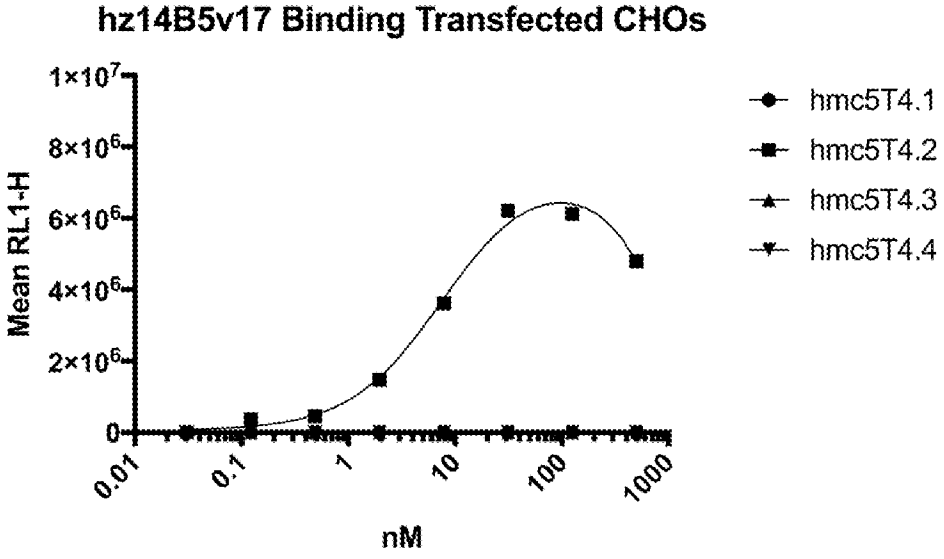


FIG. 27A

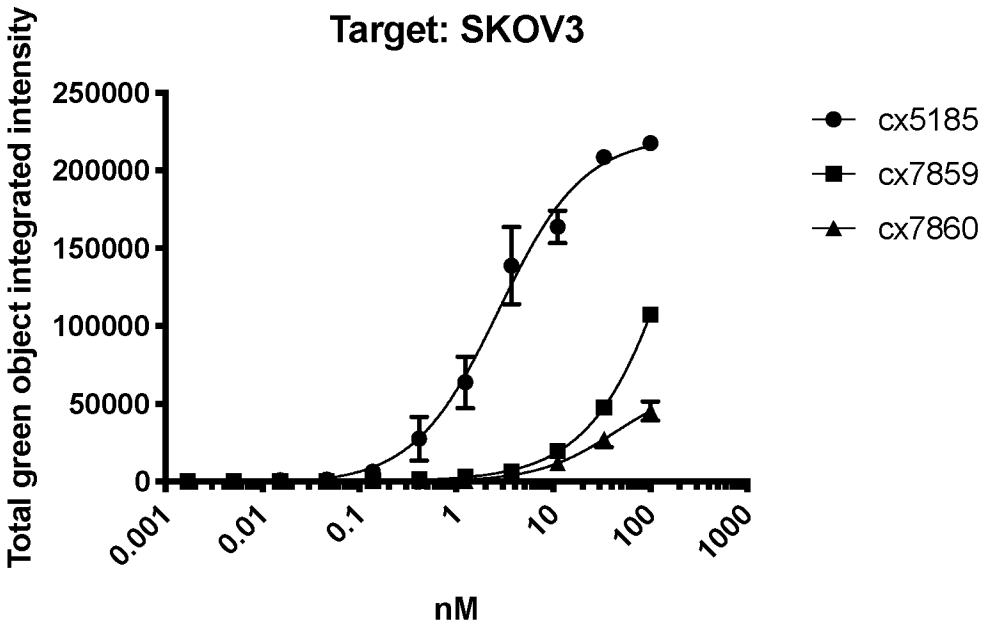
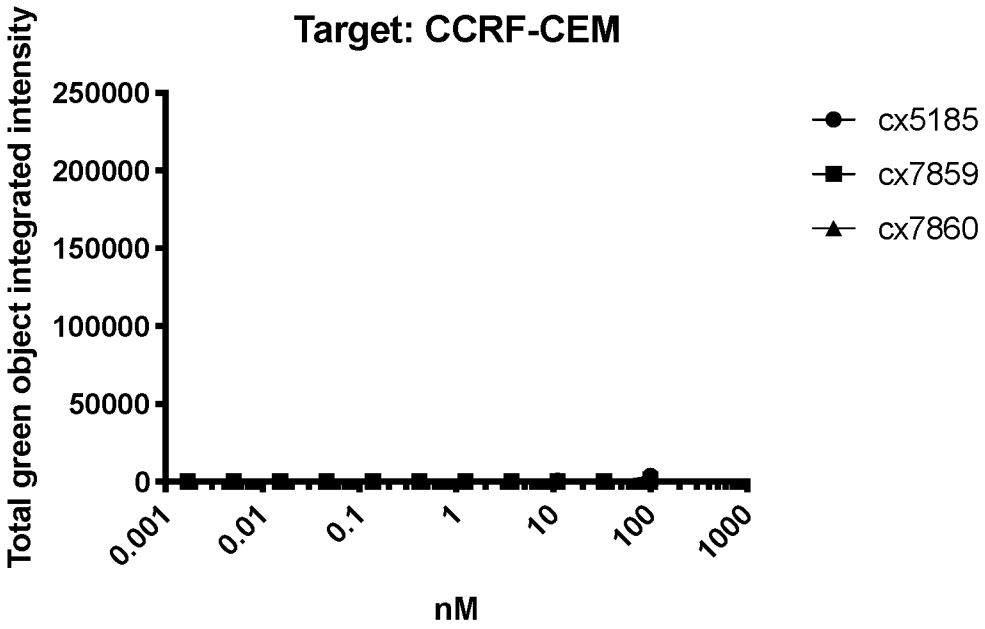


FIG. 27B



INTERNATIONAL SEARCH REPORT

International application No
PCT/US2019/055454

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61P35/00 A61K47/68 C07K16/30 C07K14/725 C07K16/28
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61P A61K C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data, BIOSIS, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CN 108 084 265 A (UNIV FUDAN) 29 May 2018 (2018-05-29)	1,4, 34-36, 199, 205-209, 211-213, 221-229
Y	paragraph [0014] - paragraph [0024] paragraph [0033] paragraph [0038] paragraph [0051] paragraph [0032]	2,3, 10-13, 17-33, 37-198, 200-204, 210, 214-220

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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
20 January 2020	03/02/2020

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Irion, Andrea

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