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(54) **MULTISPECIFIC POLYPEPTIDE
CONSTRUCTS HAVING CONSTRAINED CD3
BINDING AND RELATED METHODS AND
USES**

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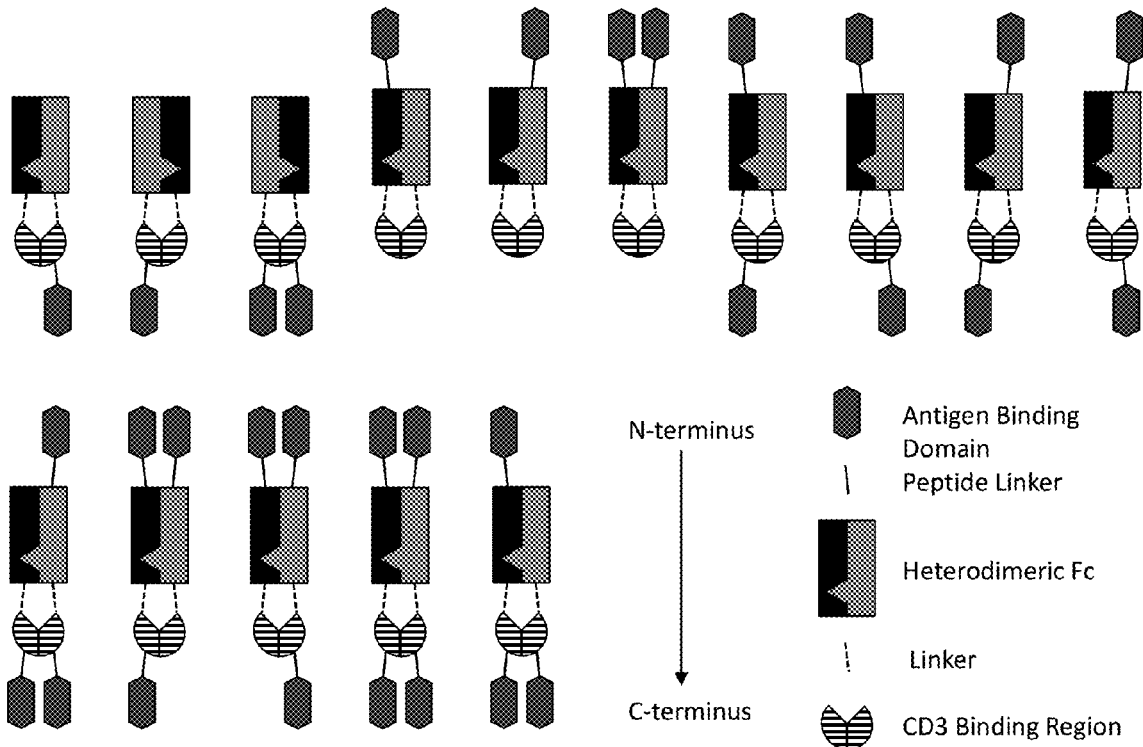
(52) **U.S. Cl.**
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(2013.01); *C07K 1/14* (2013.01); *C07K*
2317/75 (2013.01); *C07K 2317/31* (2013.01);
C07K 2317/732 (2013.01); *C07K 2317/734*
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2317/624 (2013.01); *C07K 2317/64* (2013.01);
C07K 2317/66 (2013.01); *C07K 2317/569*
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2317/522 (2013.01); *A61P 35/00* (2018.01)

(57)

ABSTRACT

The invention relates generally to multispecific polypeptides having constrained CD3 binding. In some embodiments, components of the multispecific polypeptides are connected by a non-cleavable linker. Also provided are methods of making and using these multispecific polypeptides in a variety of therapeutic, diagnostic and prophylactic indications.

Specification includes a Sequence Listing.



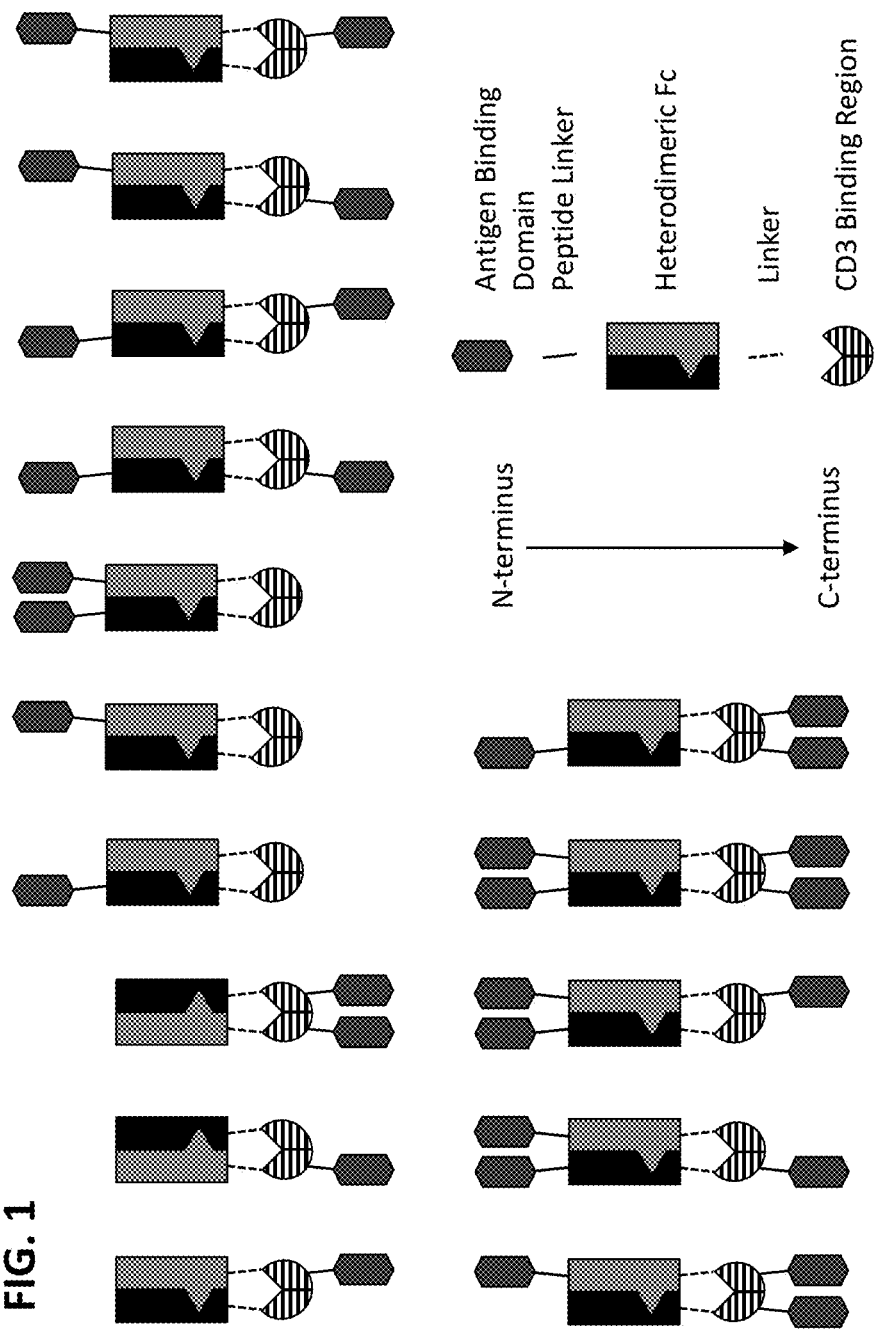


FIG. 1

FIG. 2A

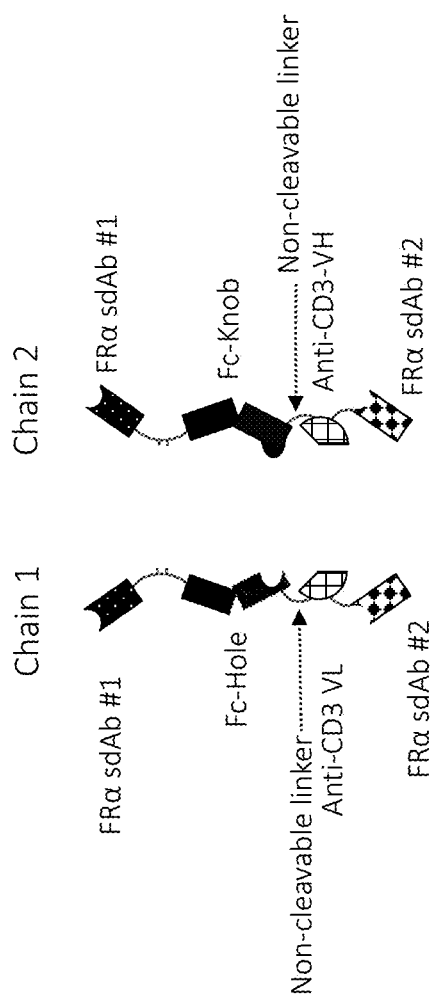


FIG. 2B

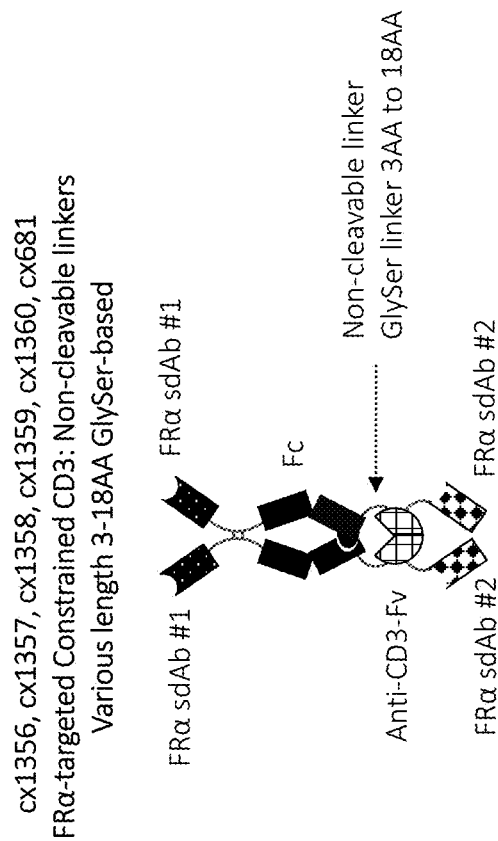


FIG. 3A

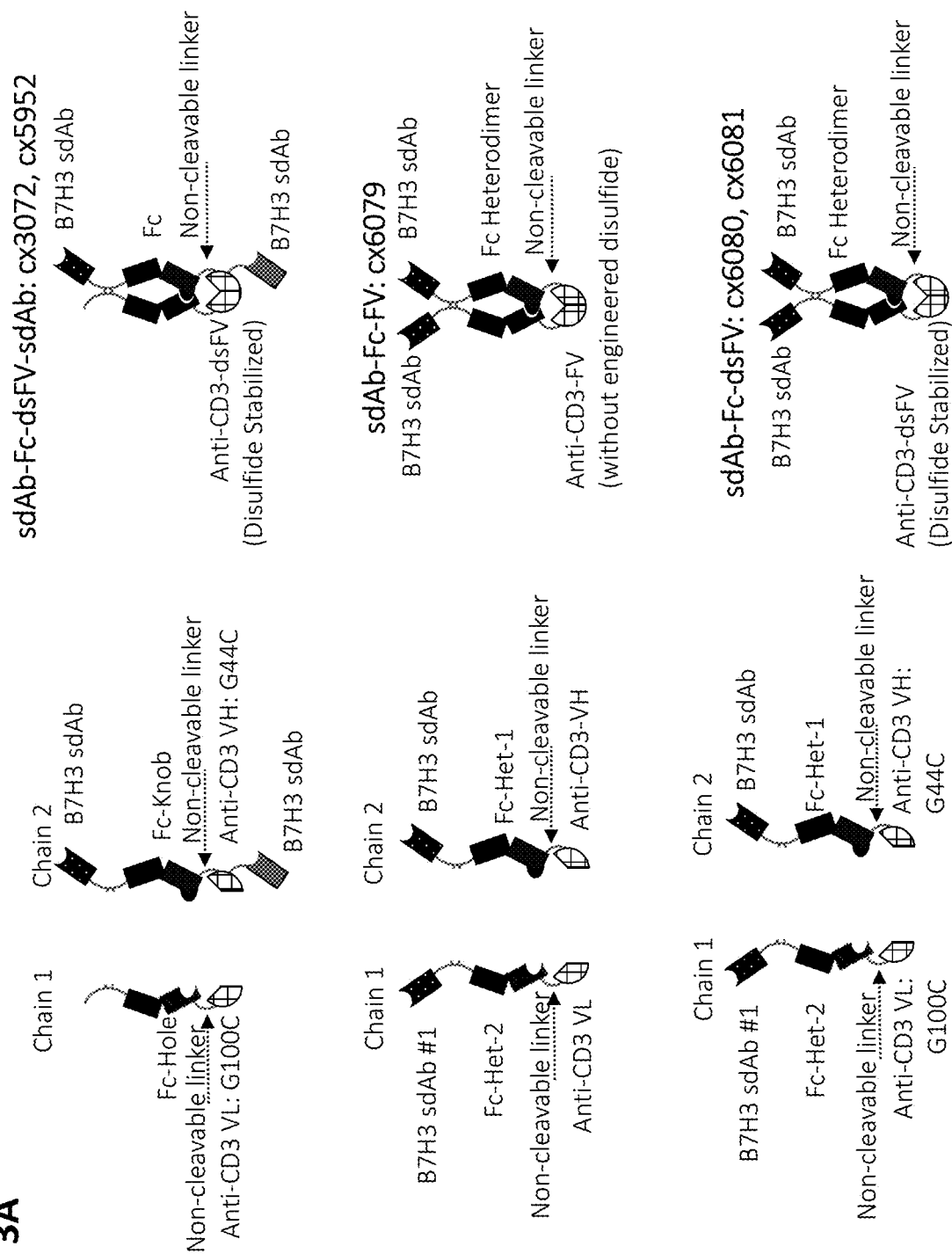


FIG. 3B

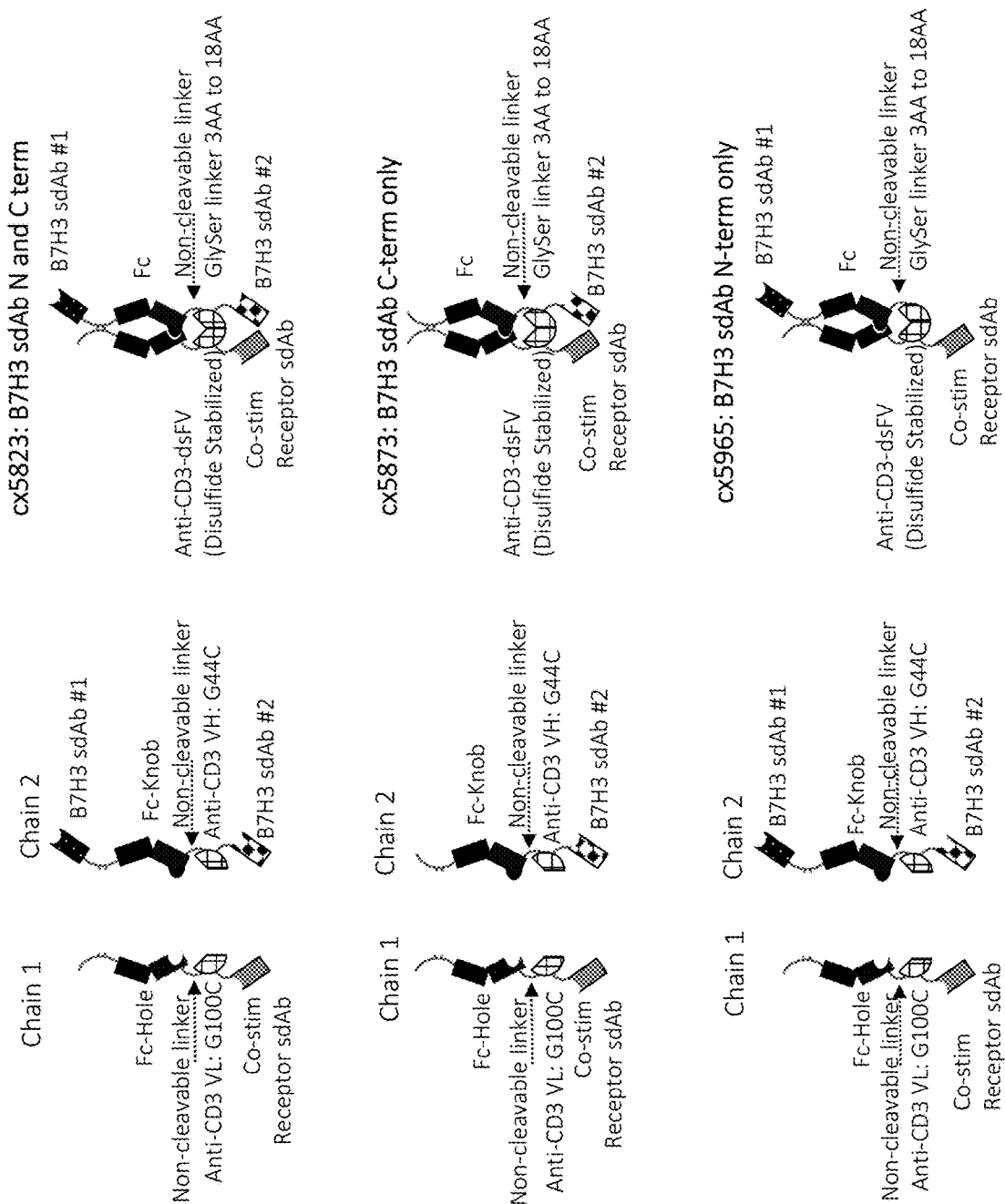
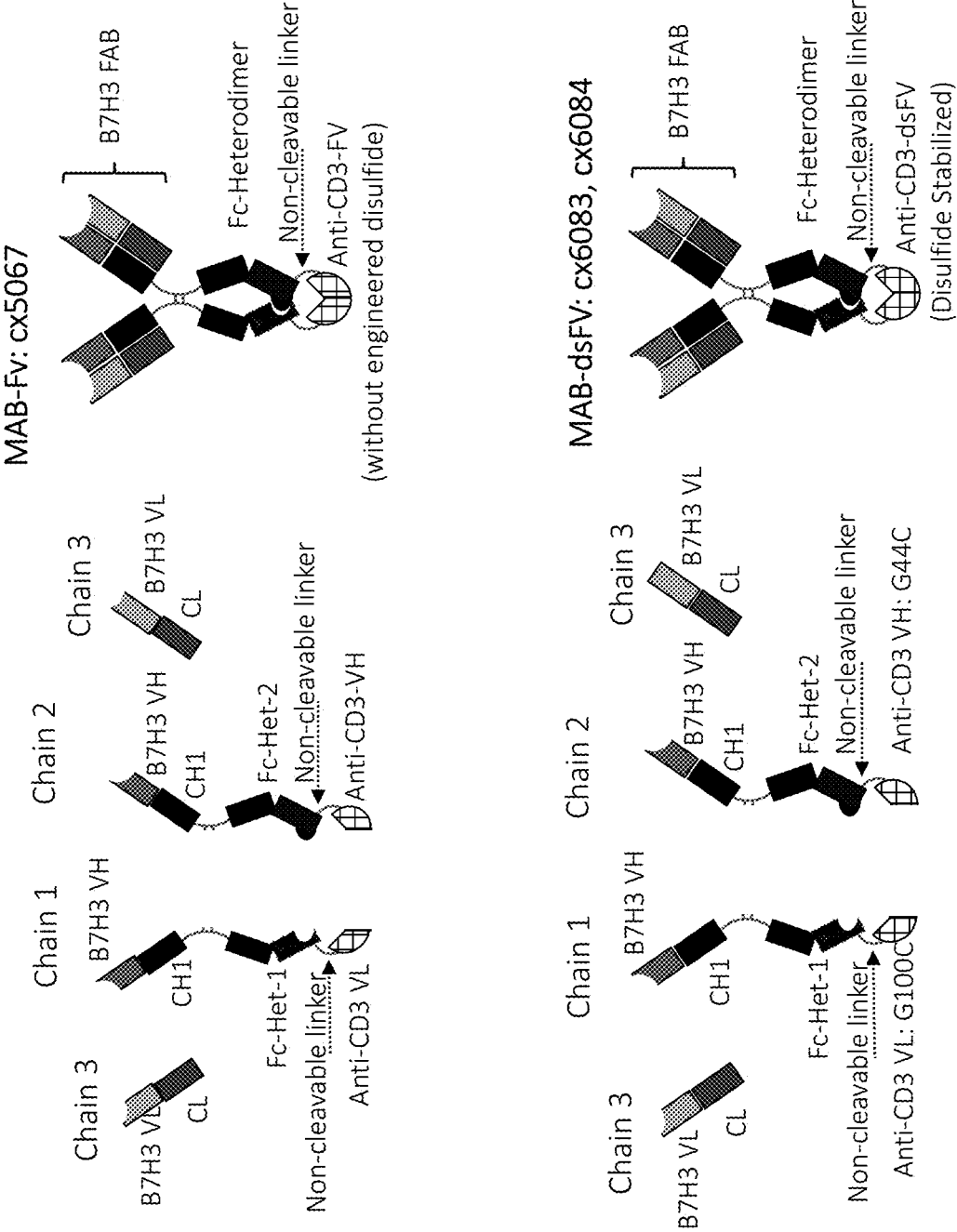


FIG. 3C



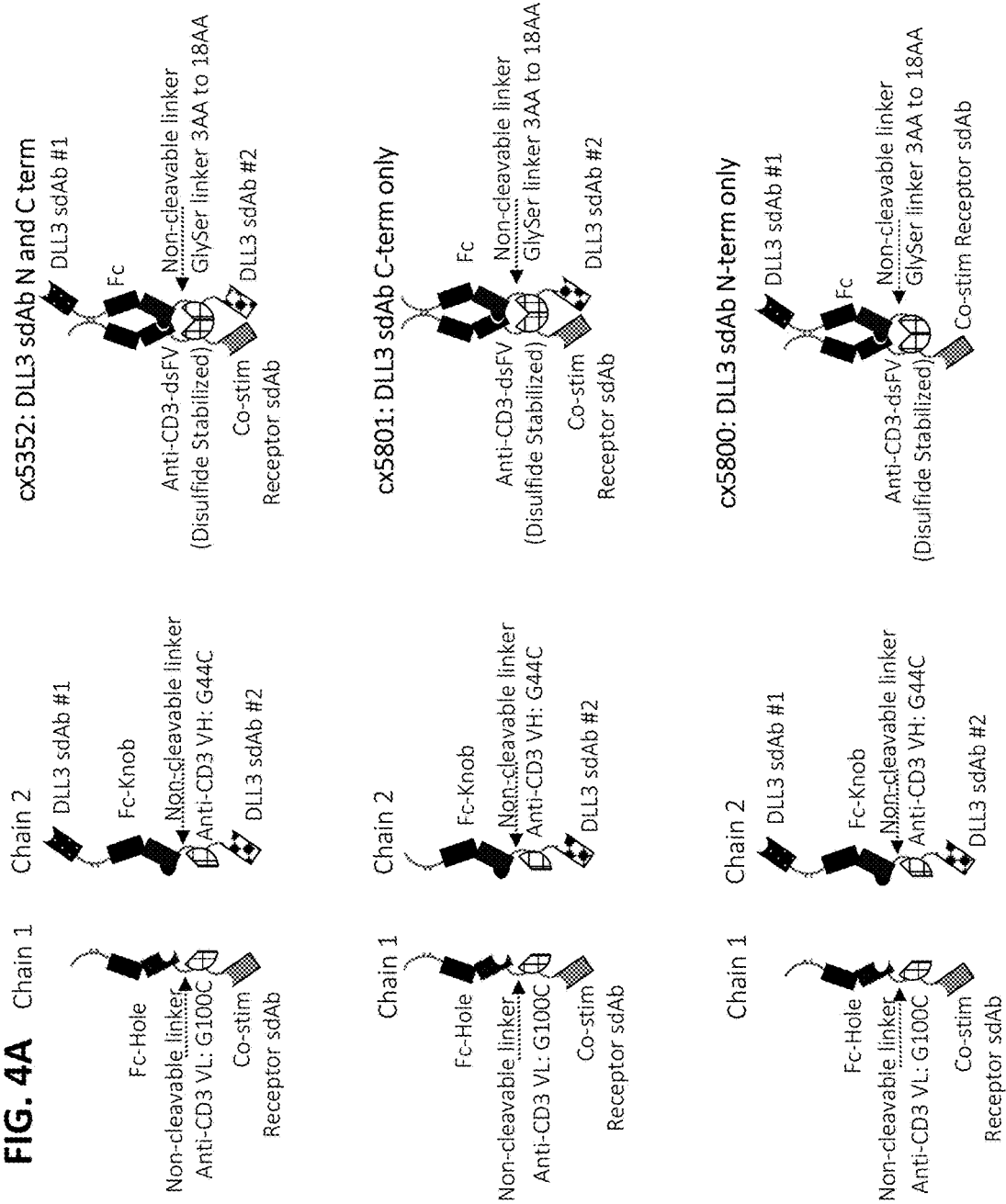
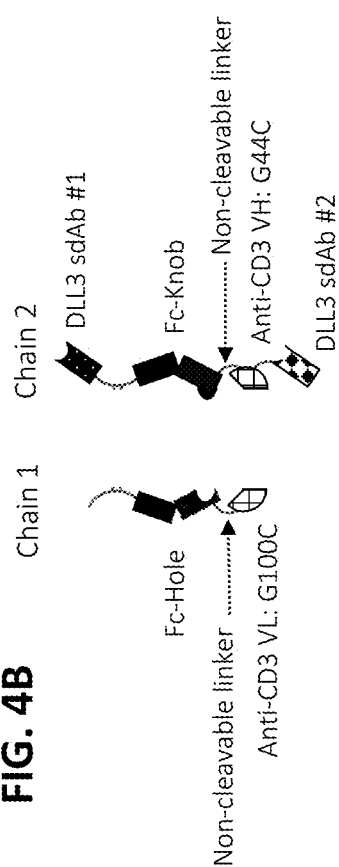


FIG. 4B



cx5499: DLL3 sdAb N and C term

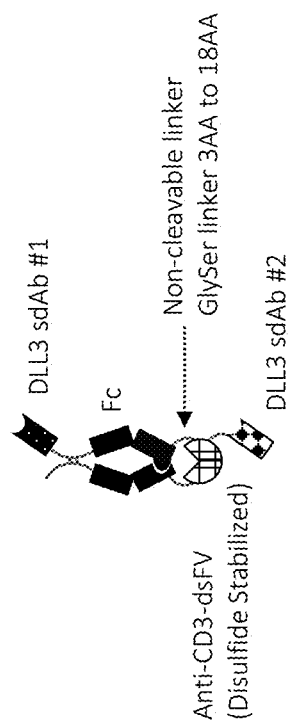


FIG. 5A

Binding to OVCAR5 cells

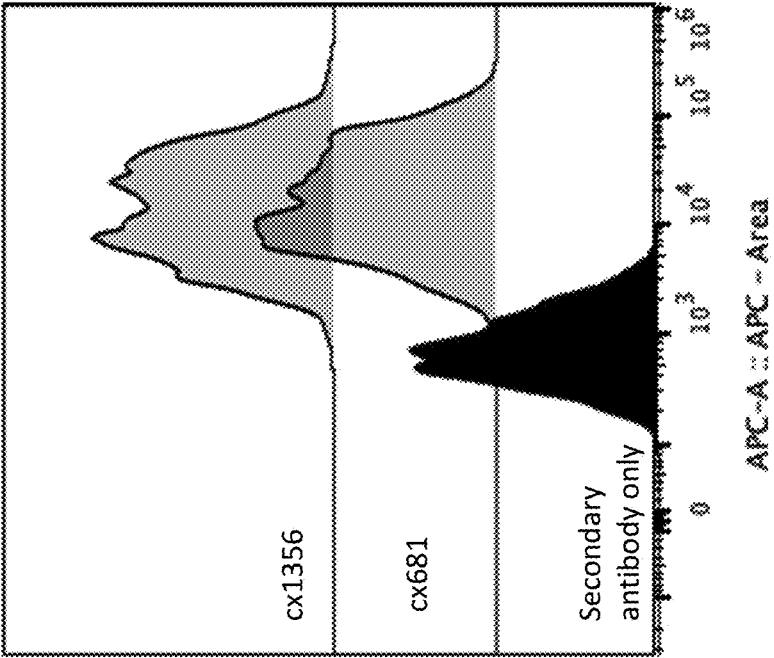
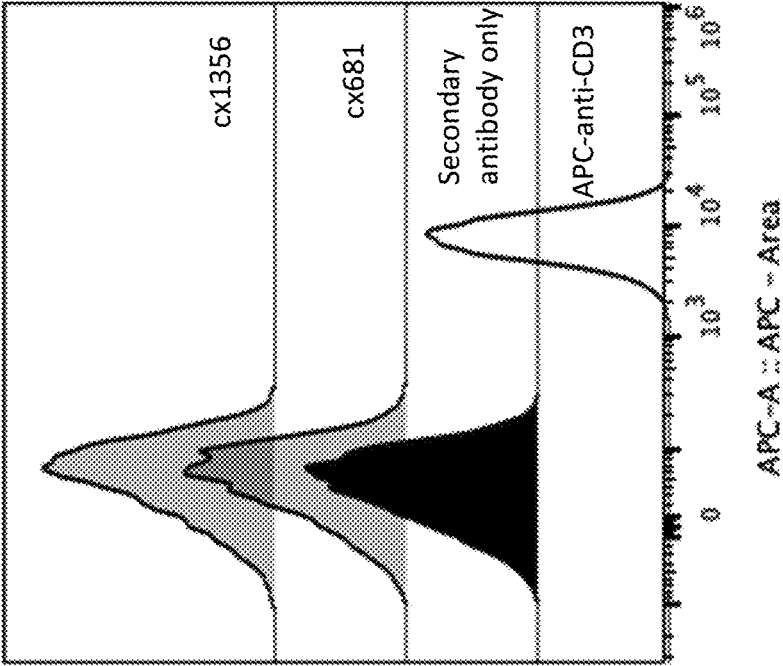


FIG. 5B

Binding to T cells



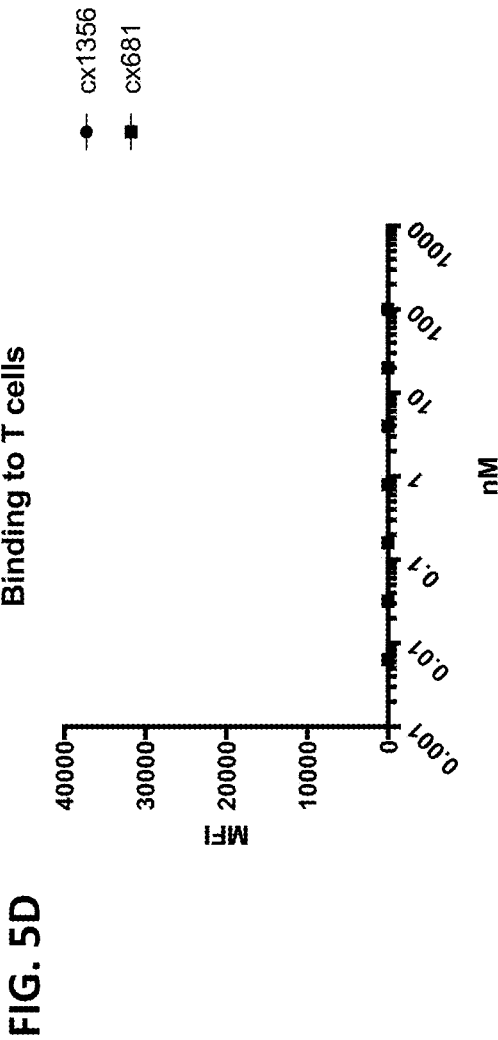
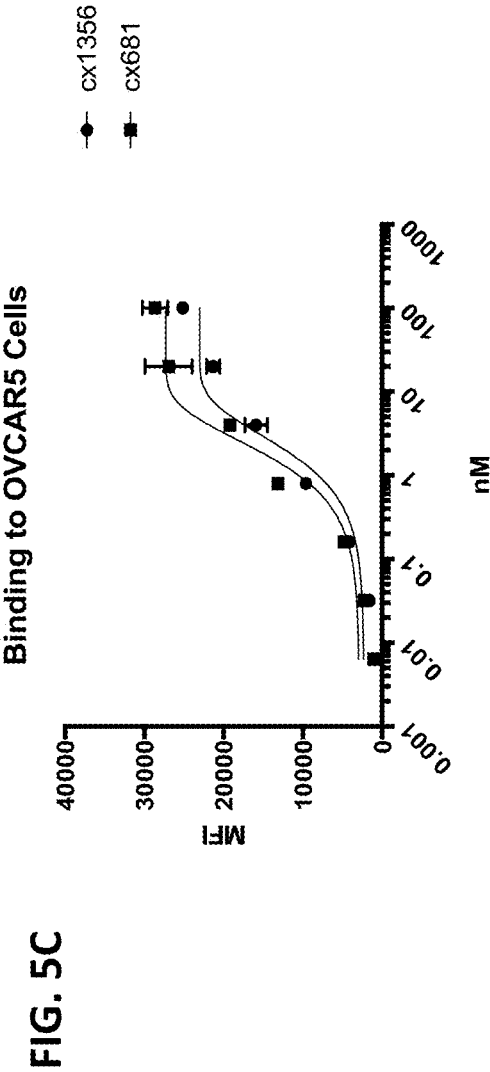


FIG. 6A

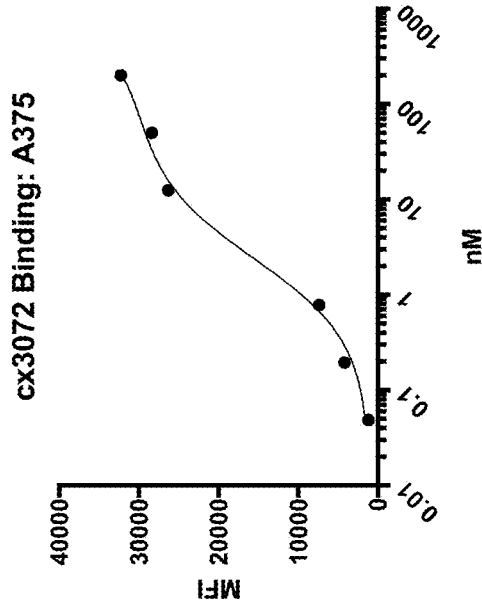
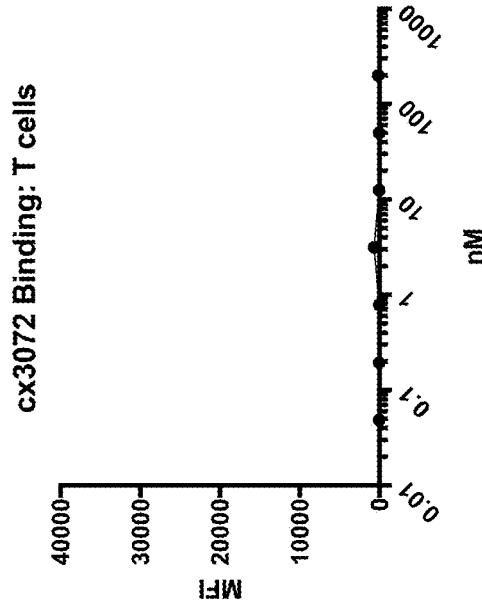
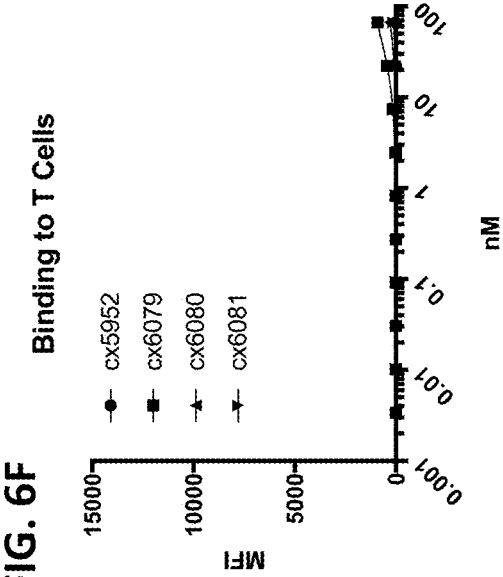
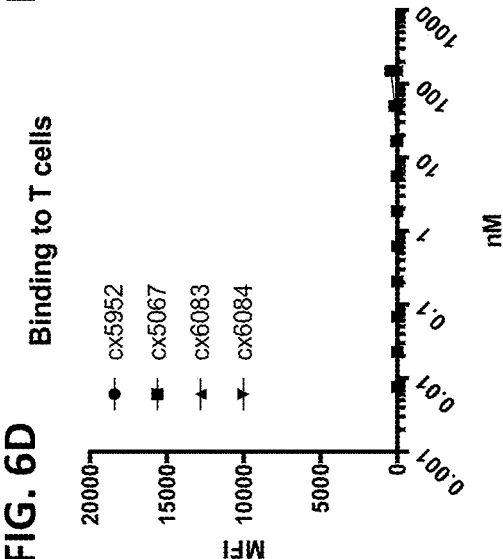
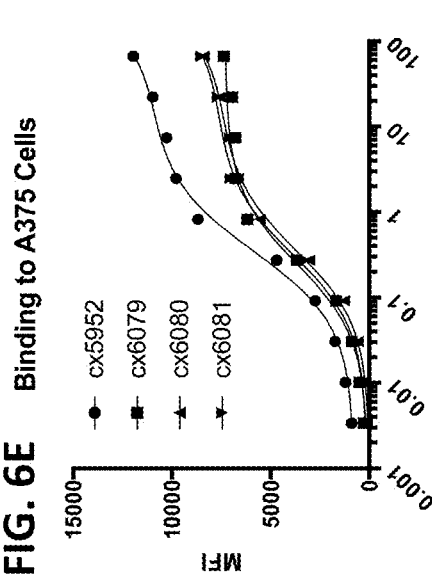
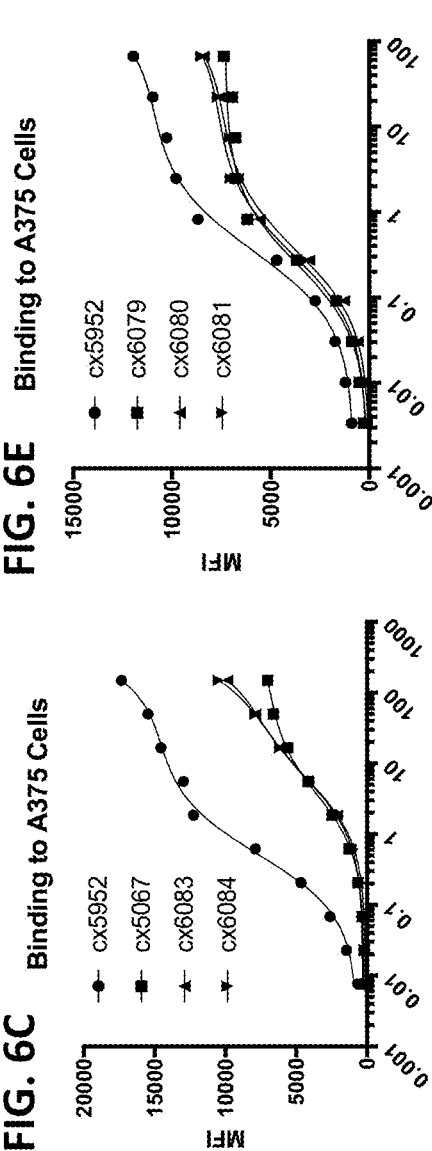
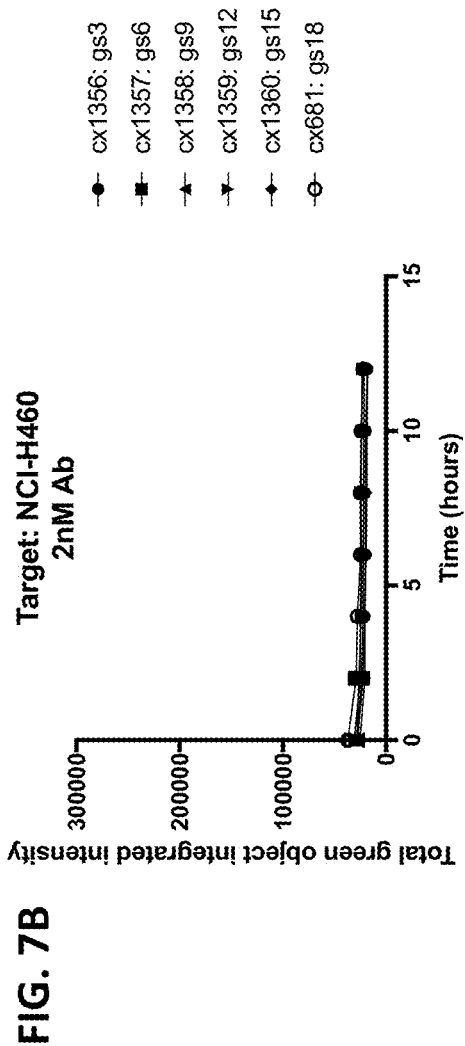
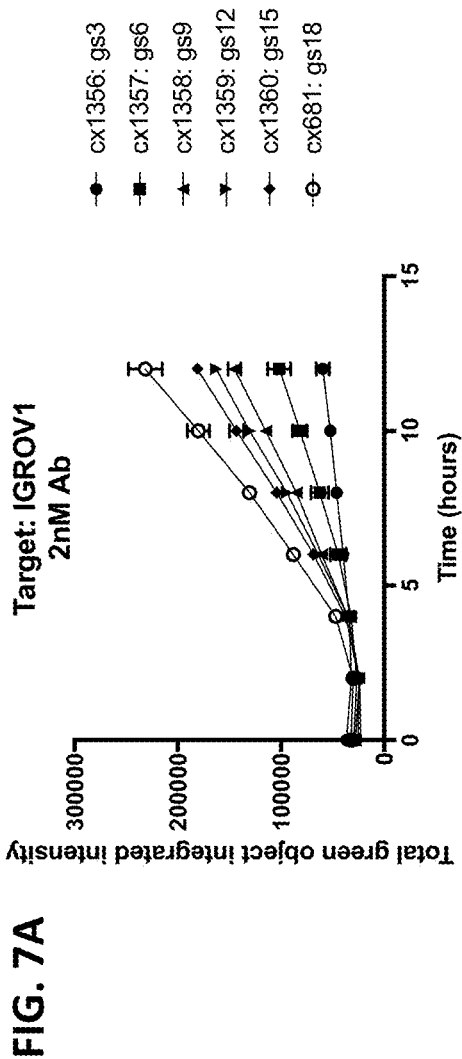
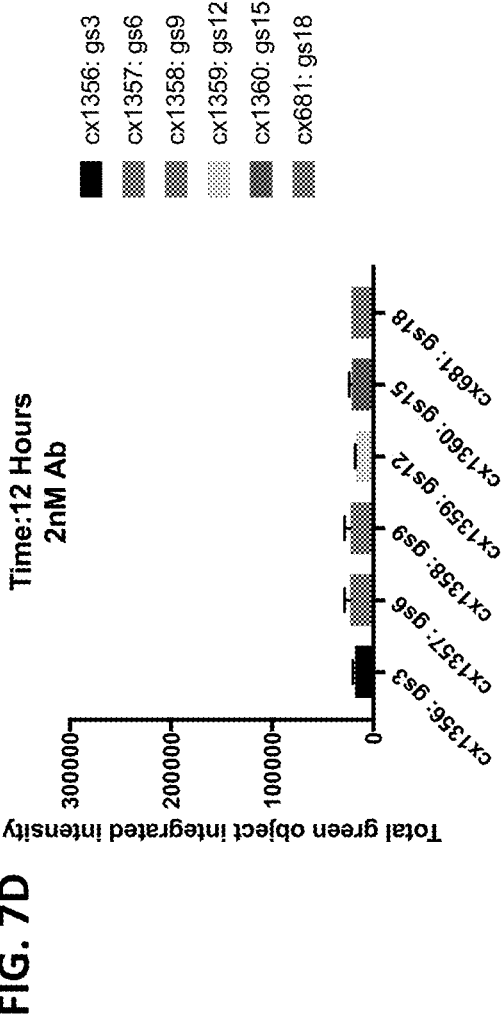
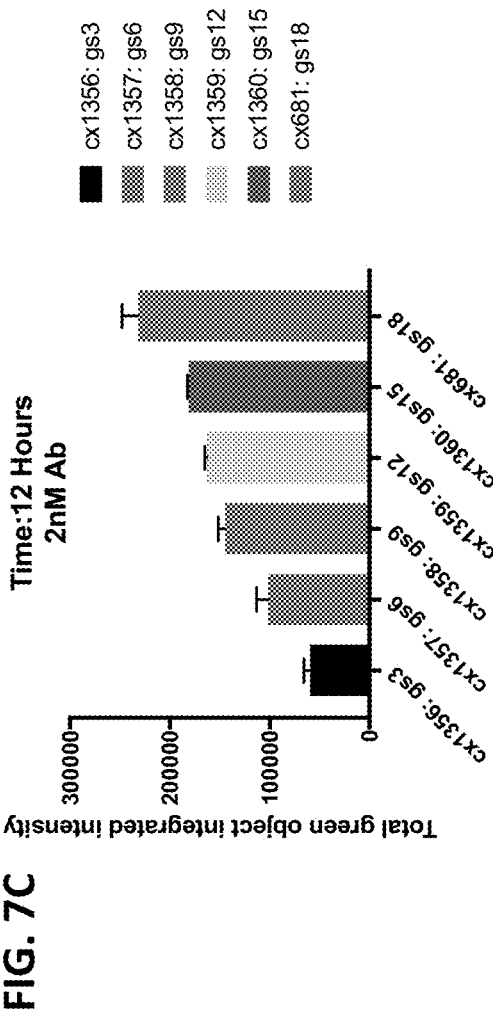


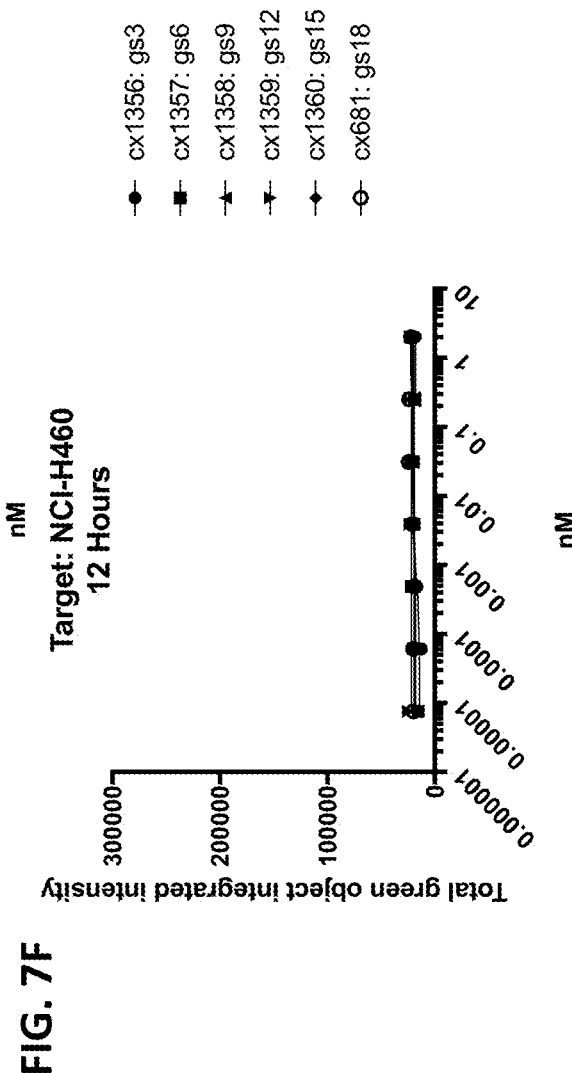
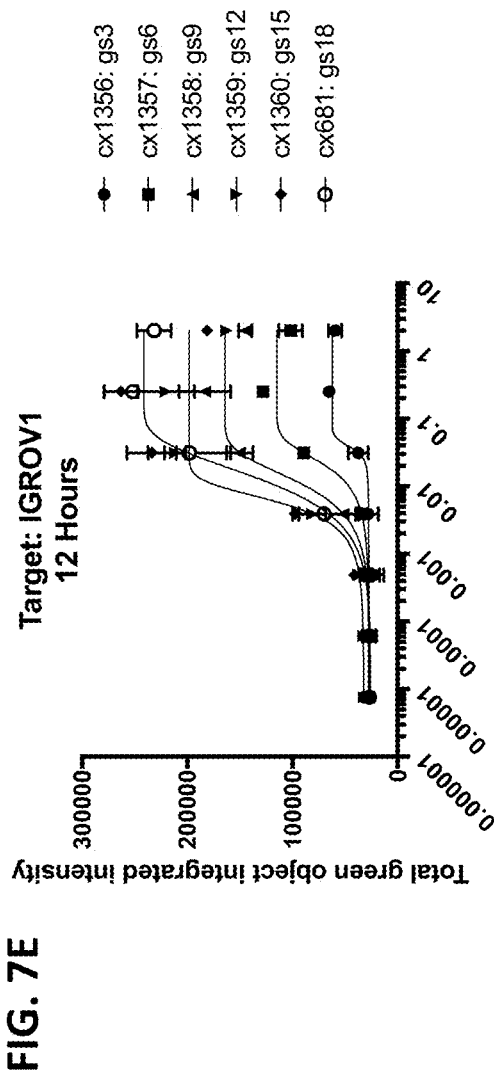
FIG. 6B











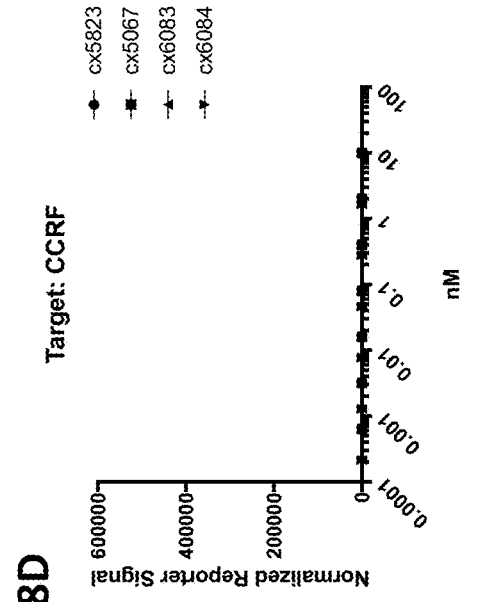
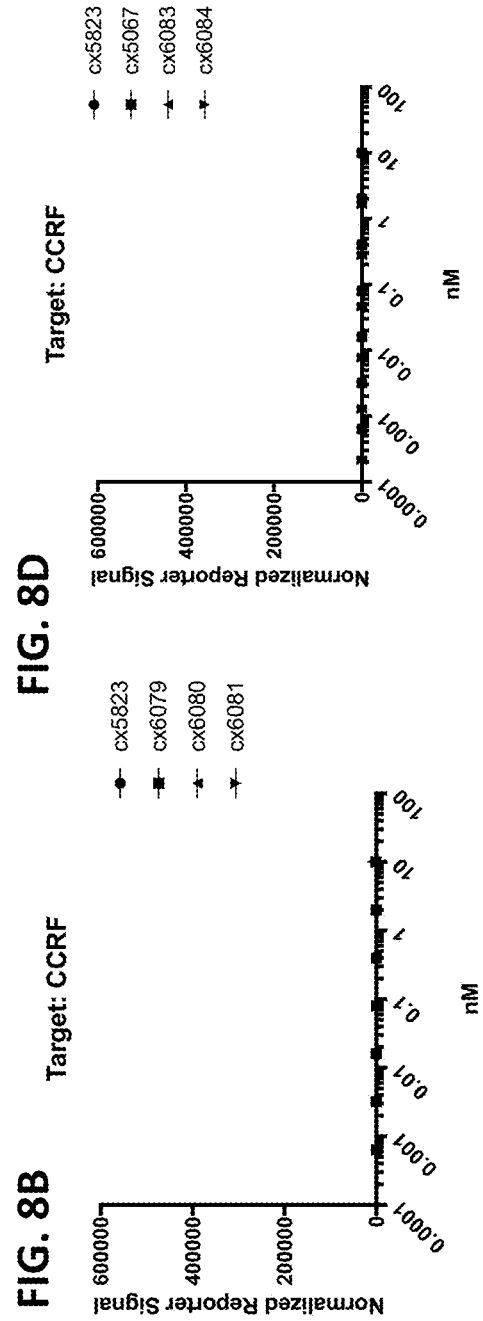
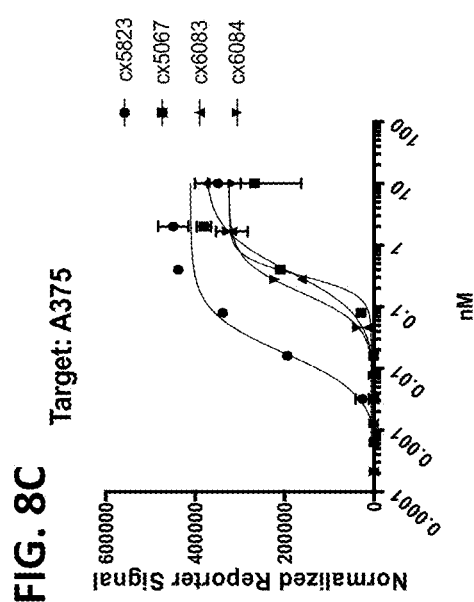
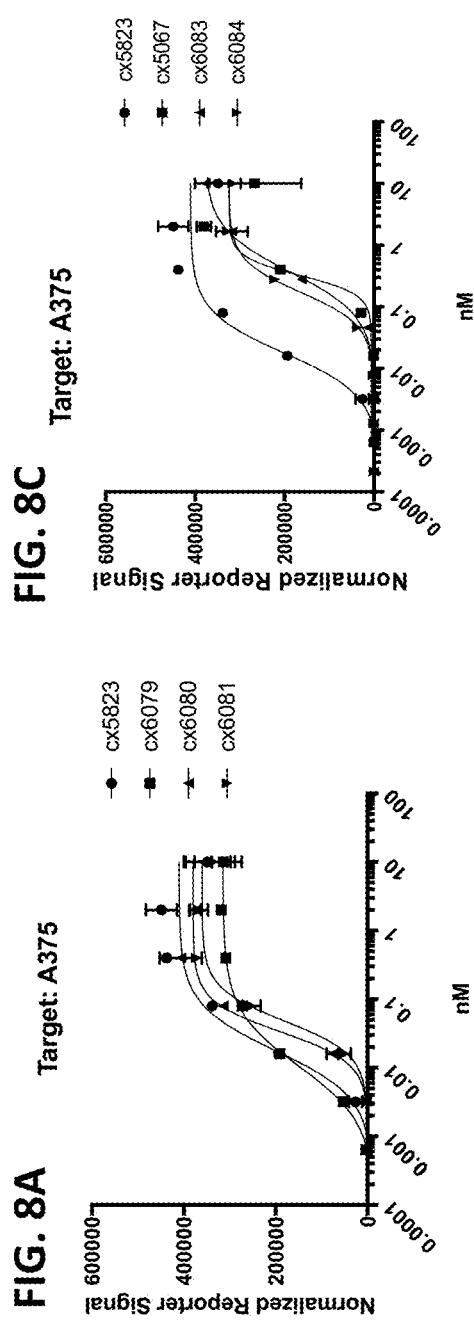


FIG. 9A

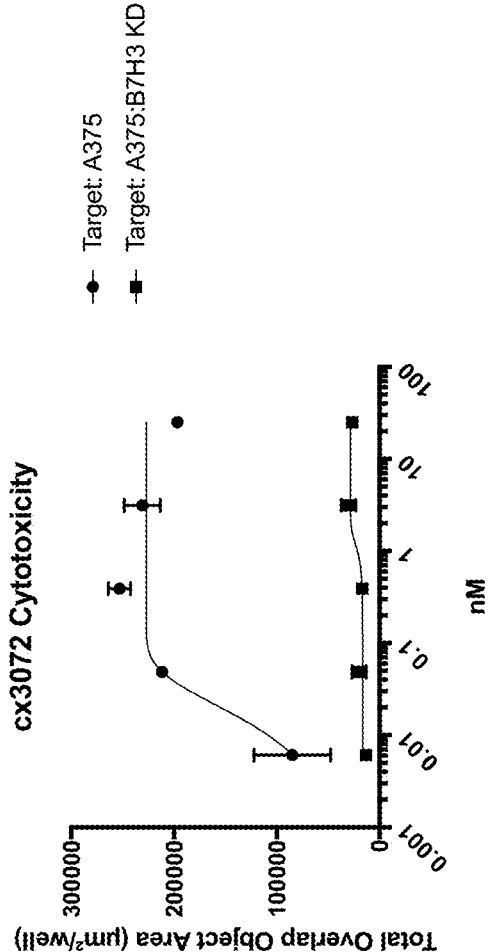
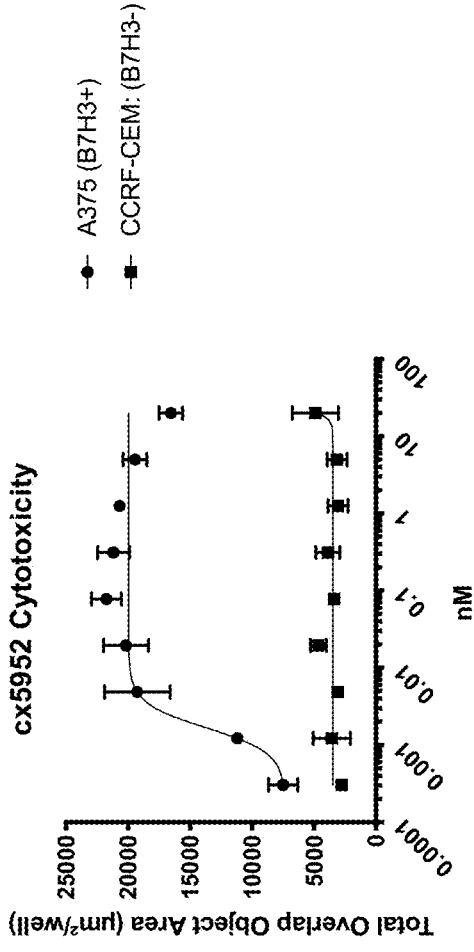


FIG. 9B



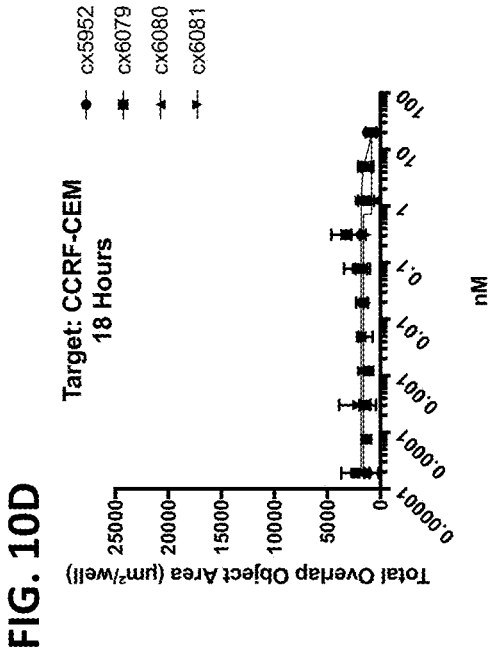
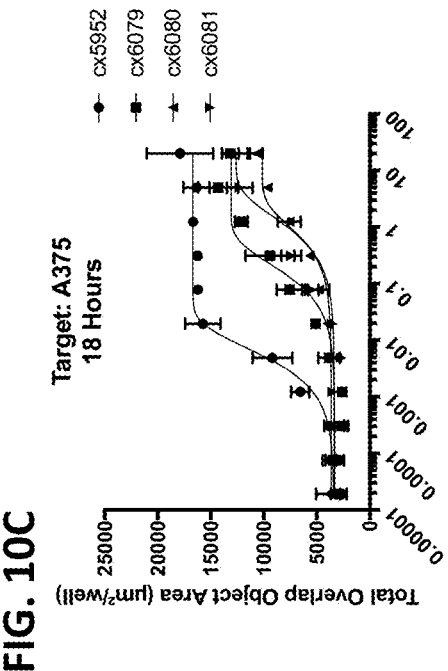
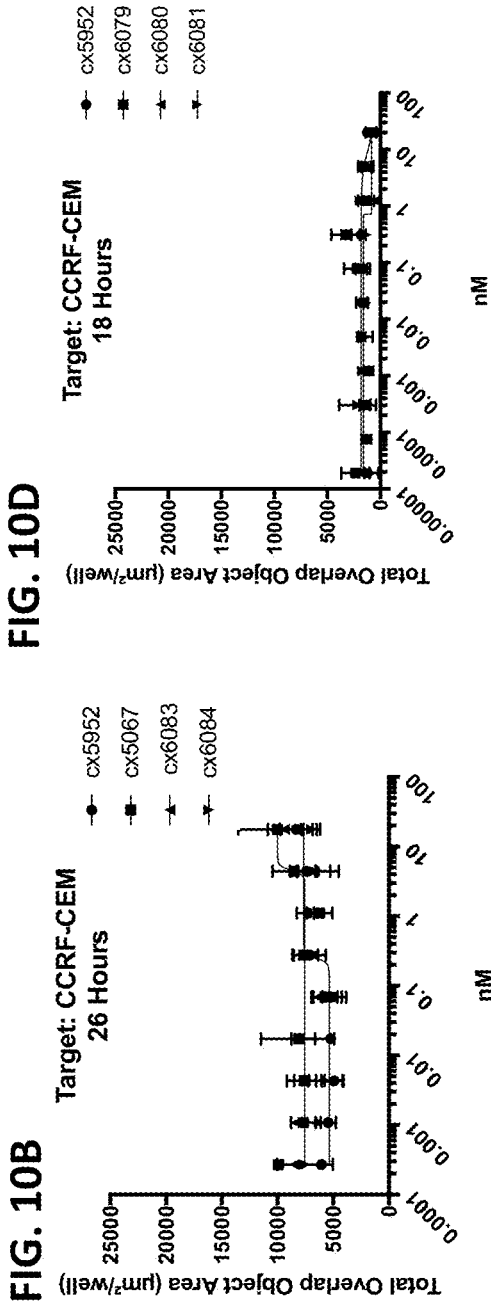
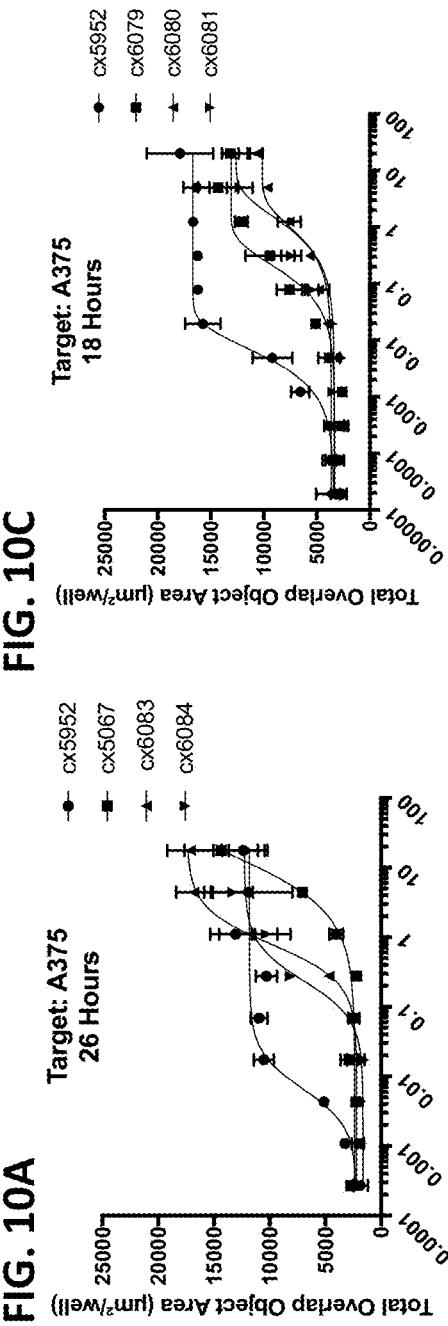


FIG. 11A

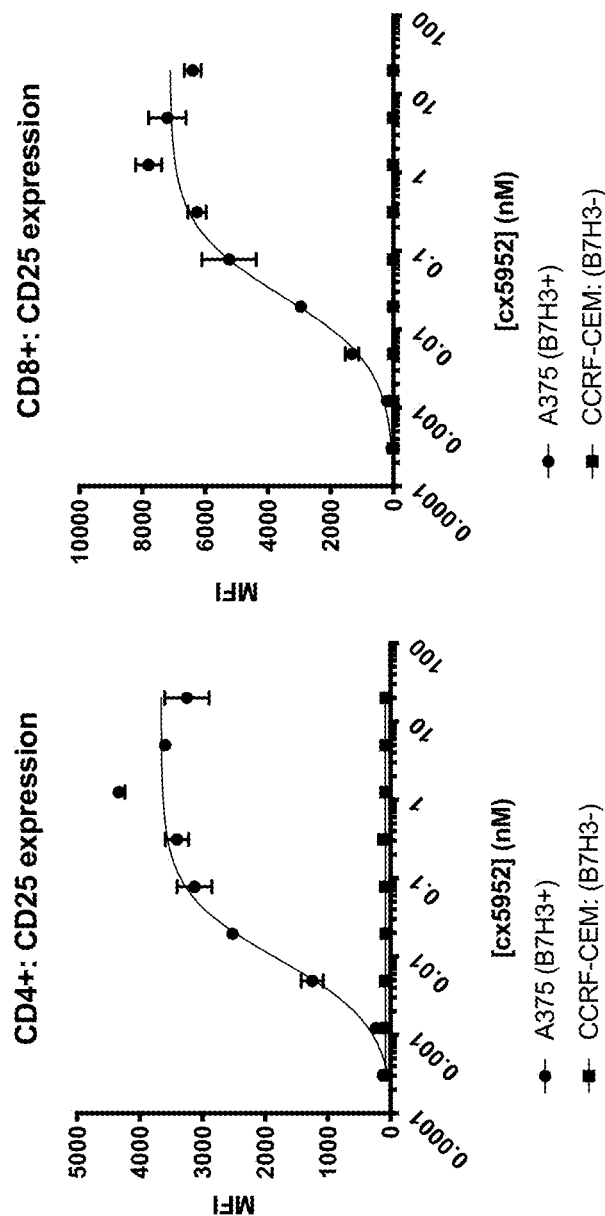


FIG. 11B

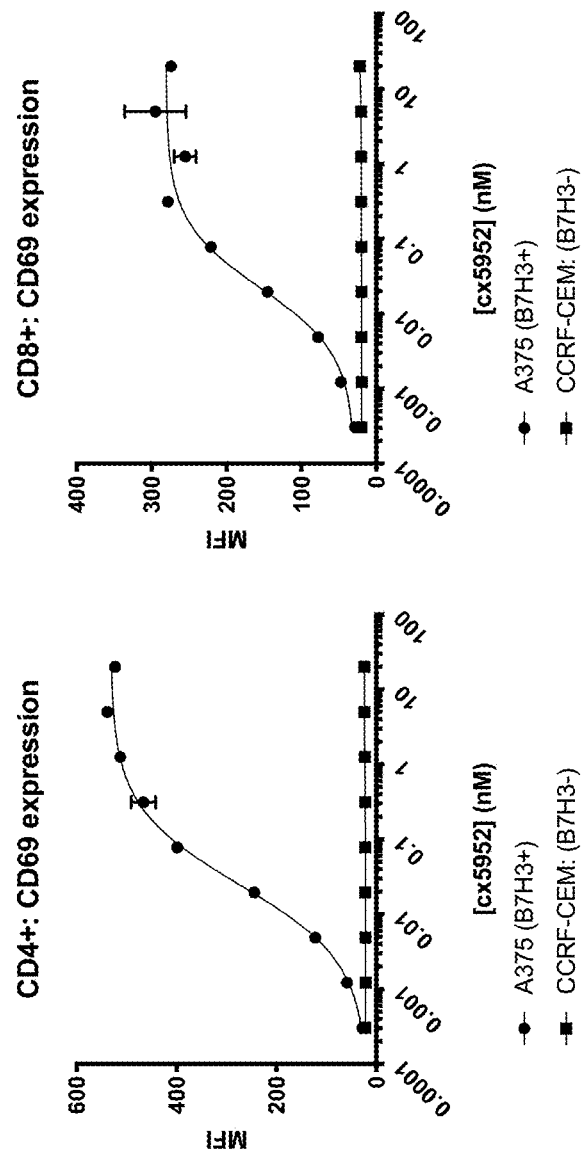
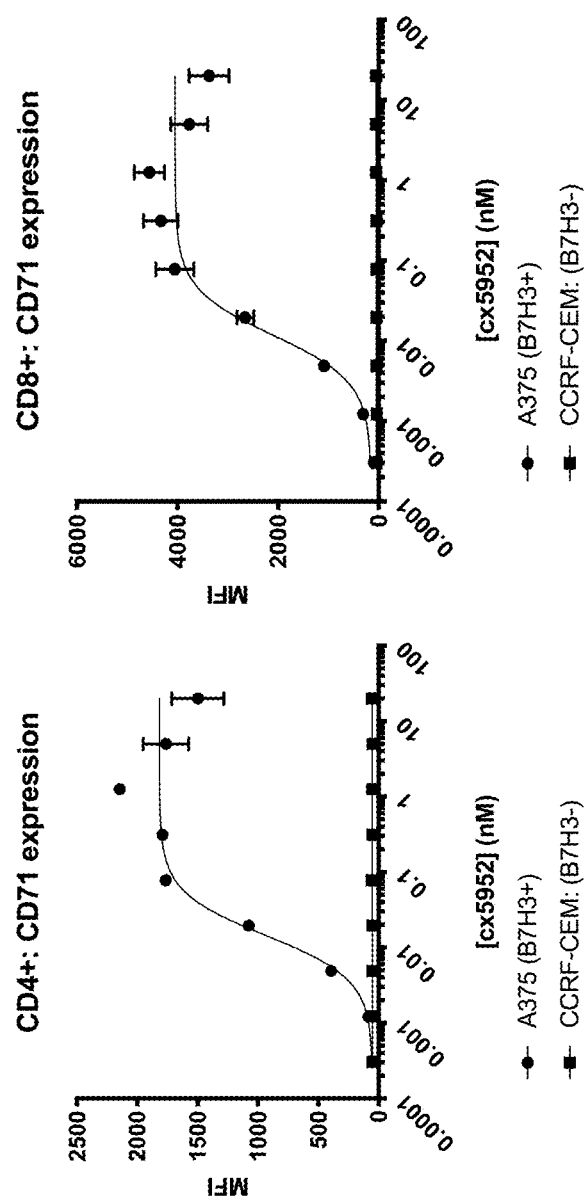
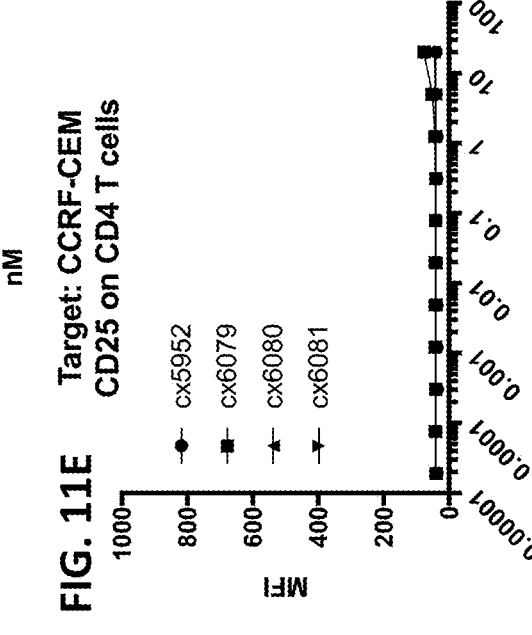
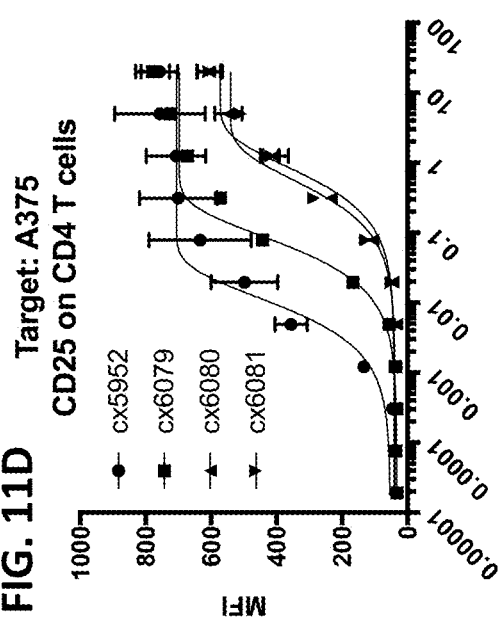
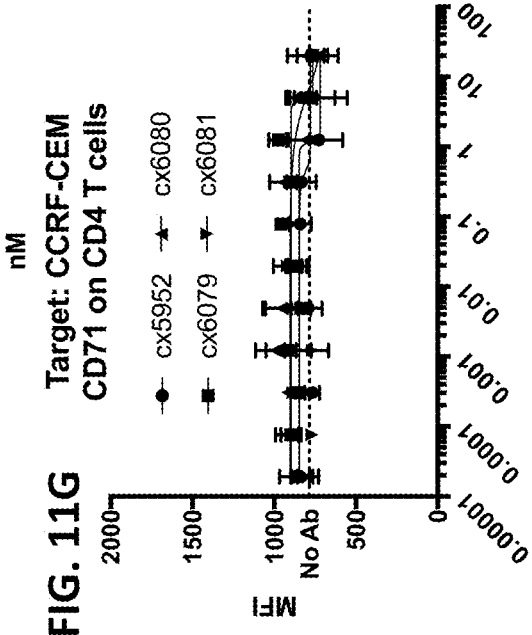
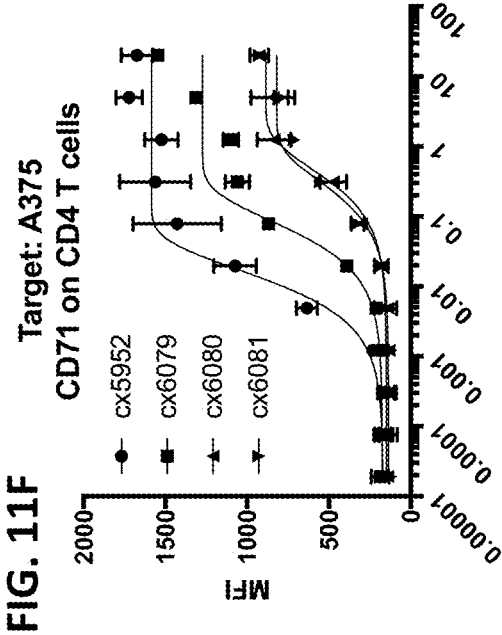
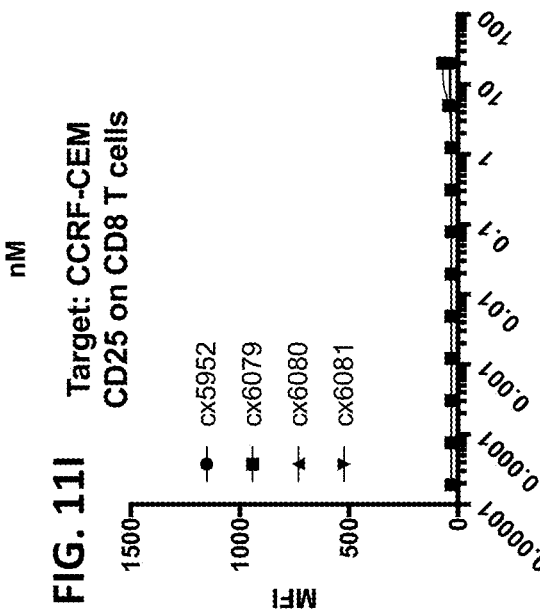
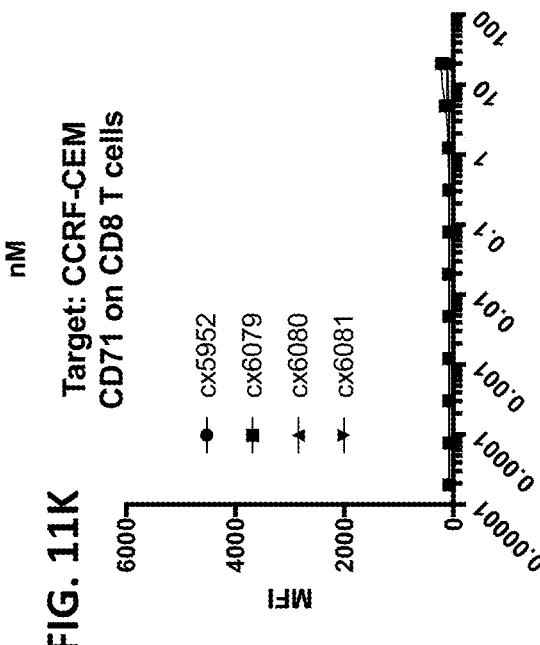
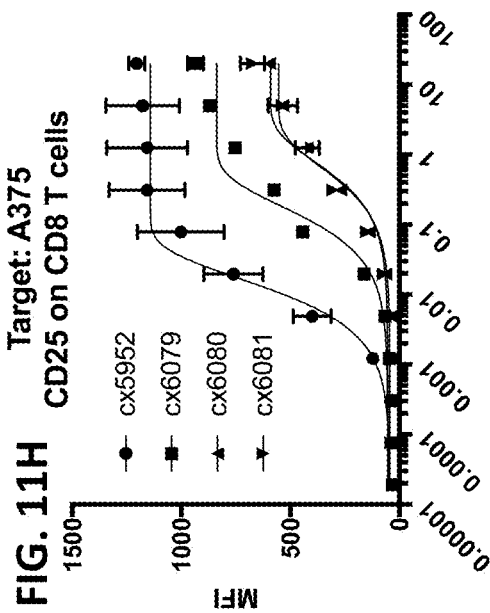
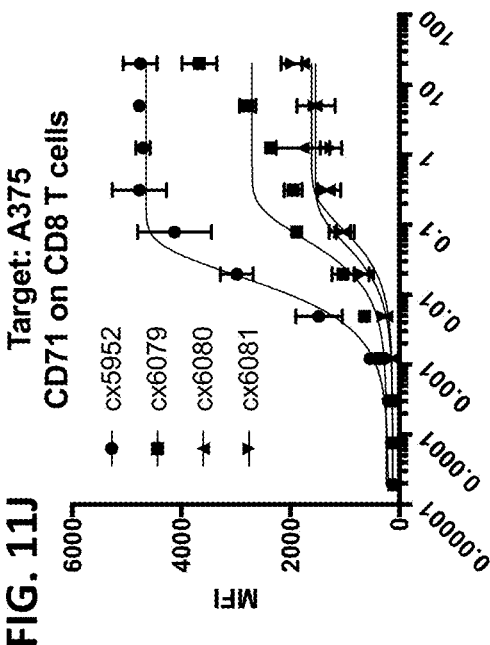
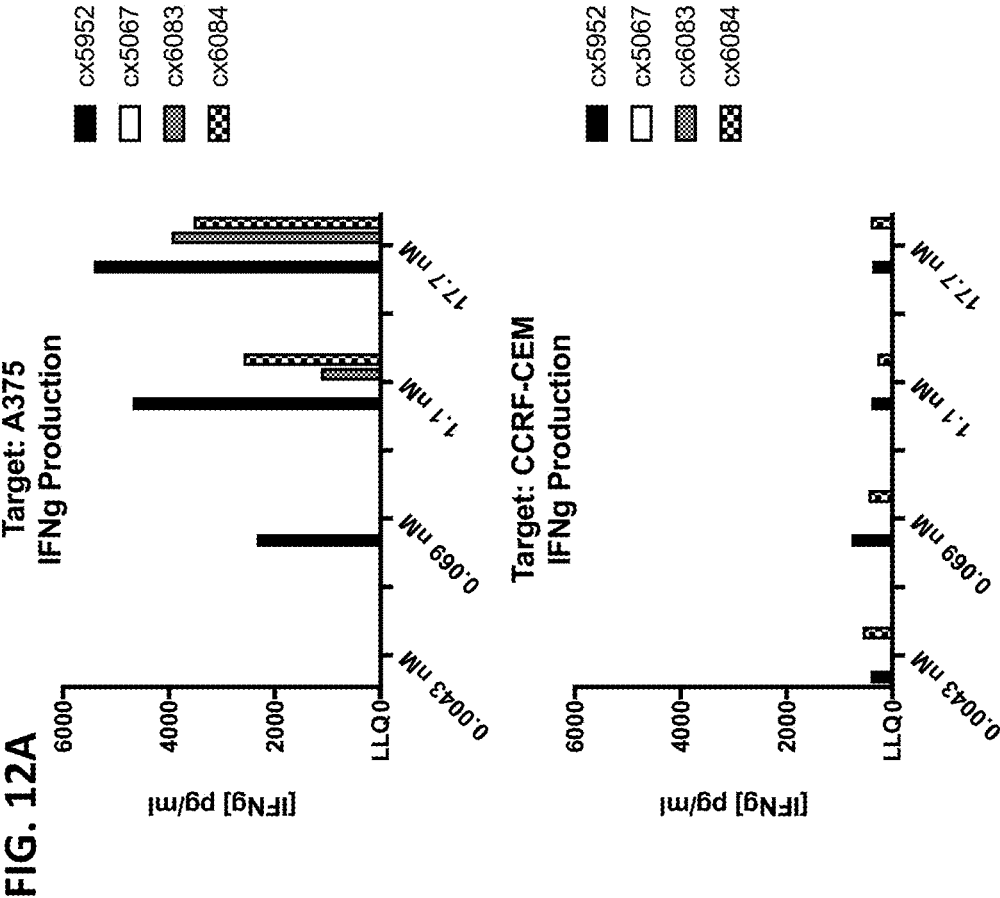


FIG. 11C









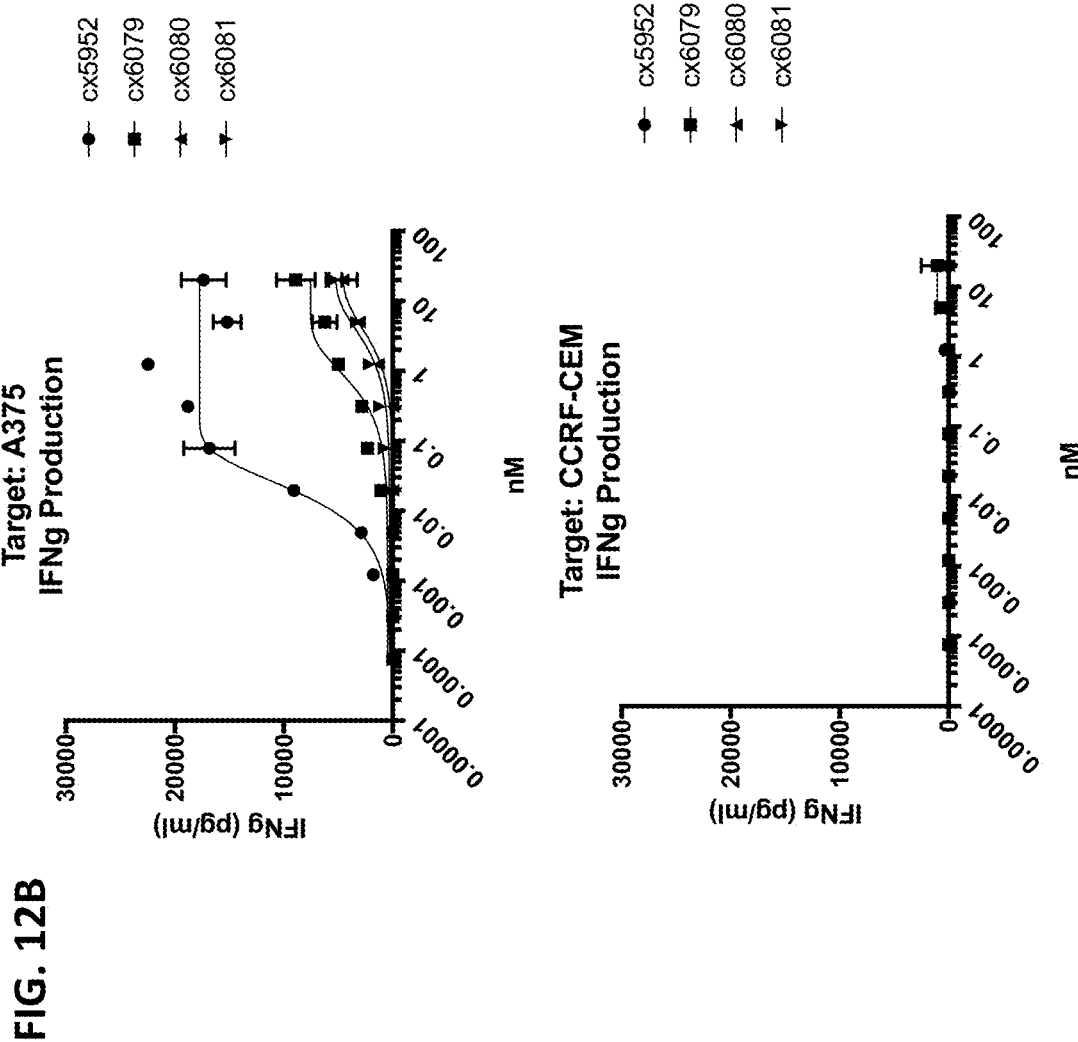


FIG. 13A

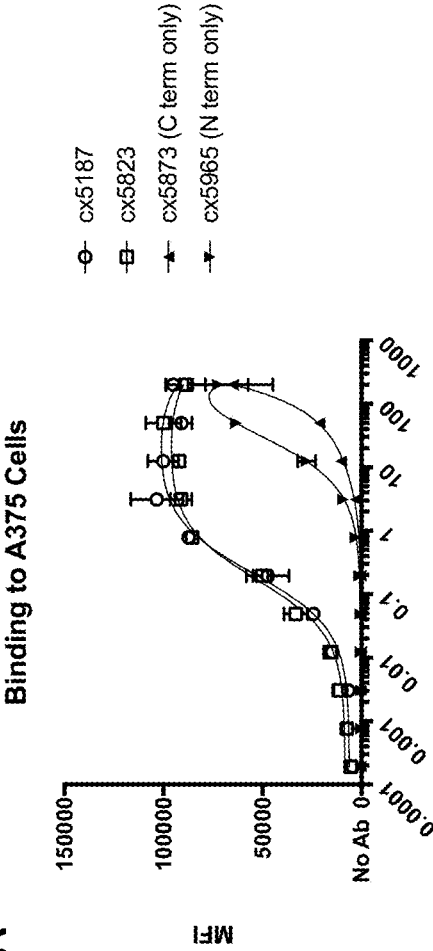
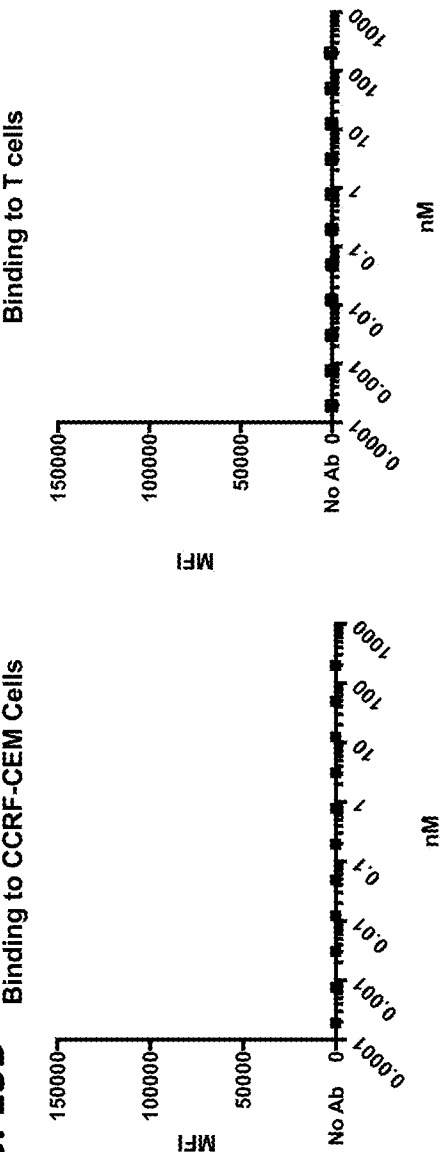


FIG. 13B



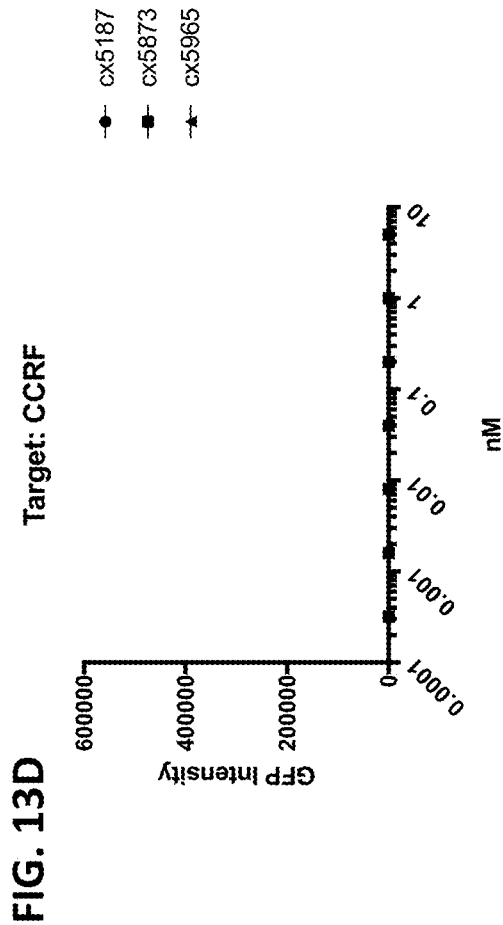
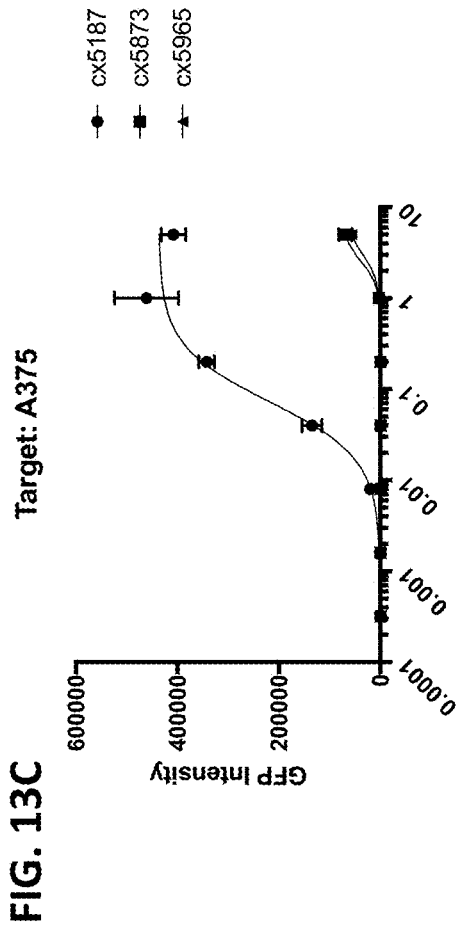


FIG. 14A

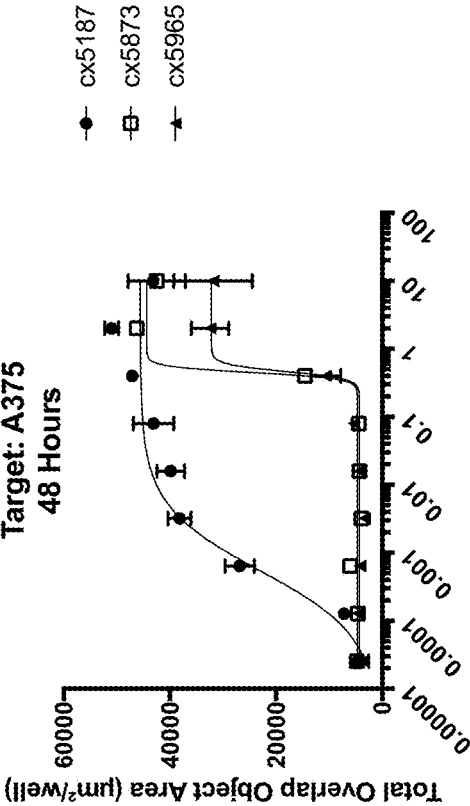
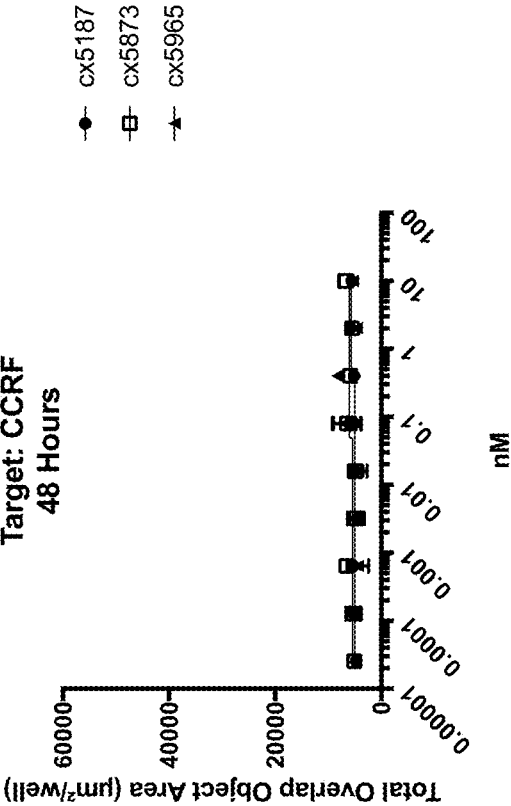
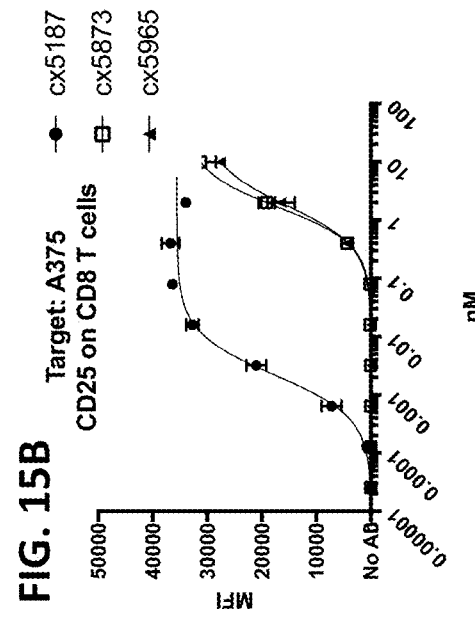
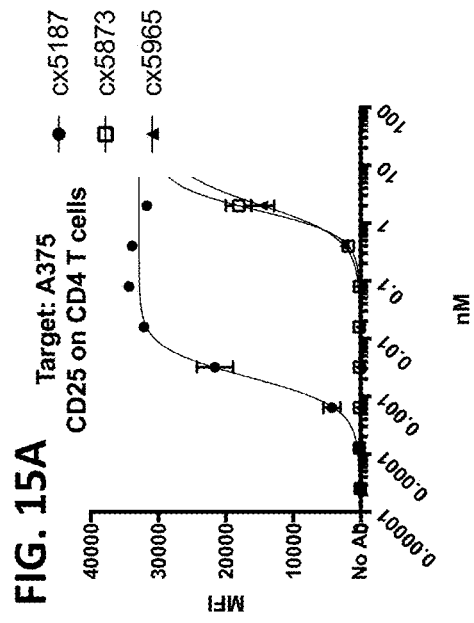
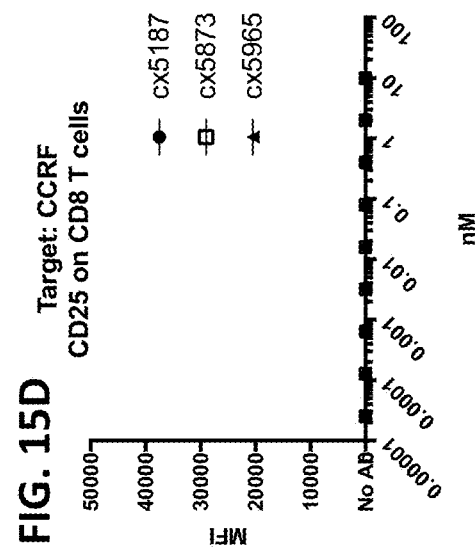
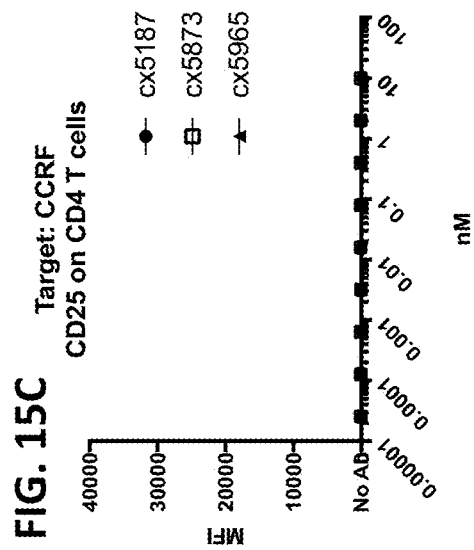
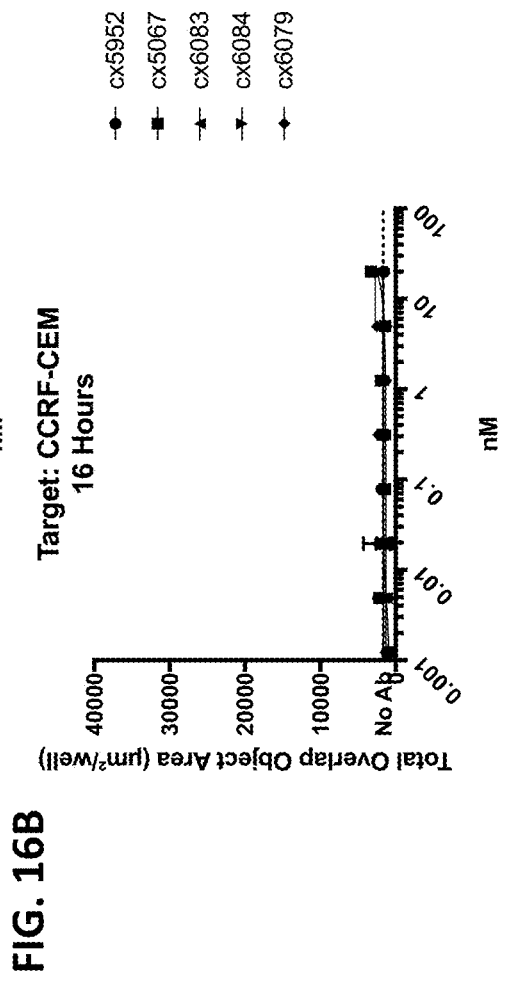
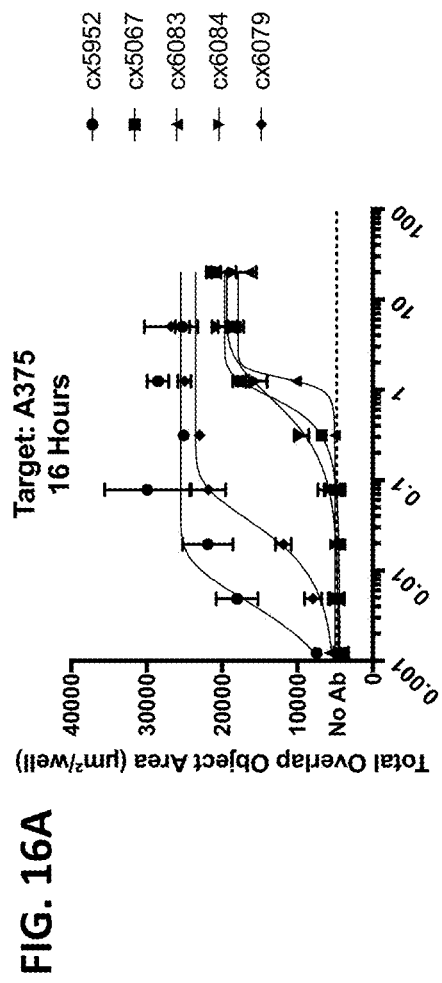
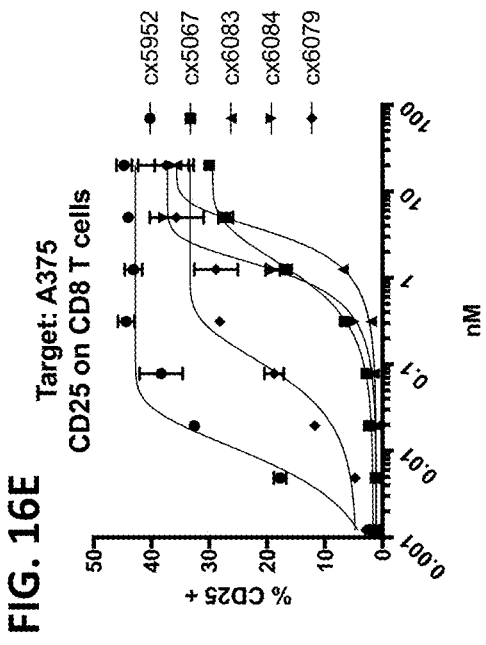
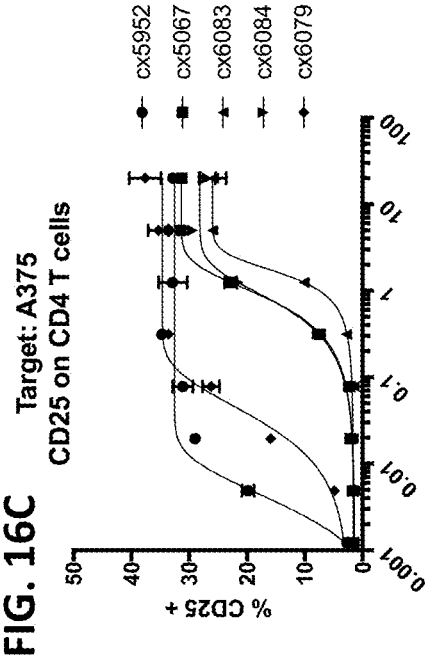
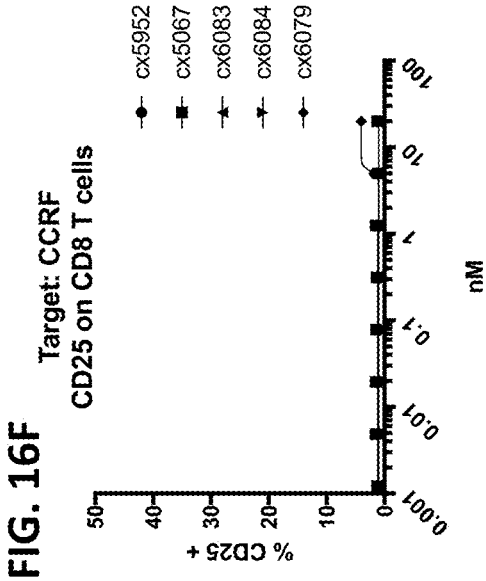
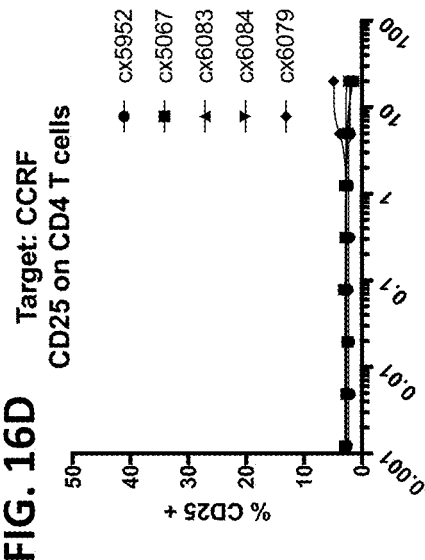


FIG. 14B









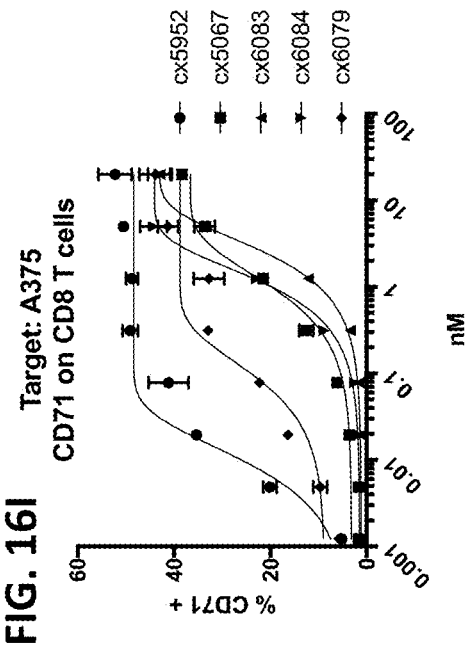
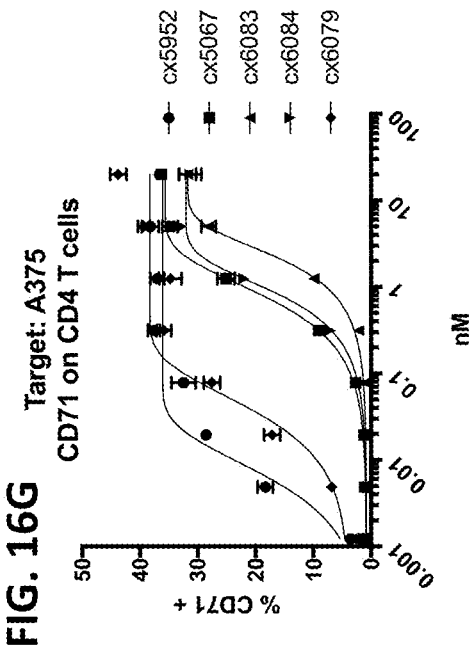
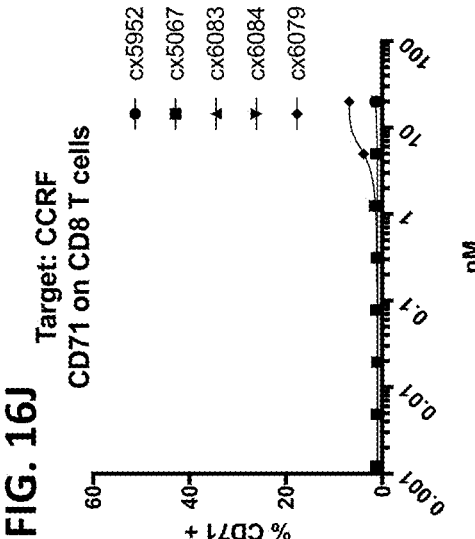
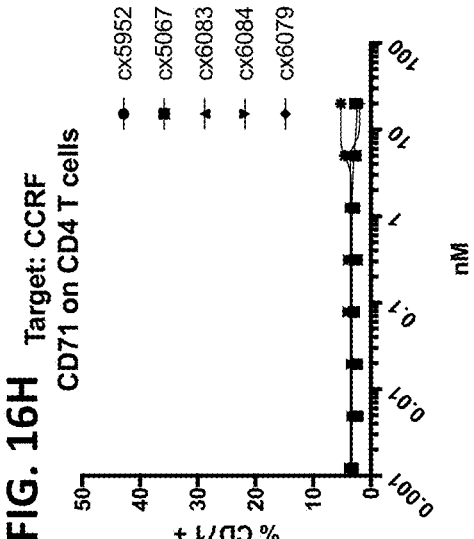


FIG. 16K

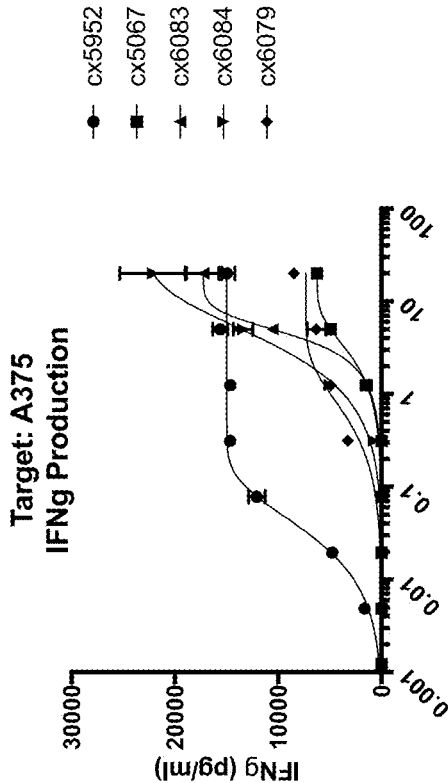
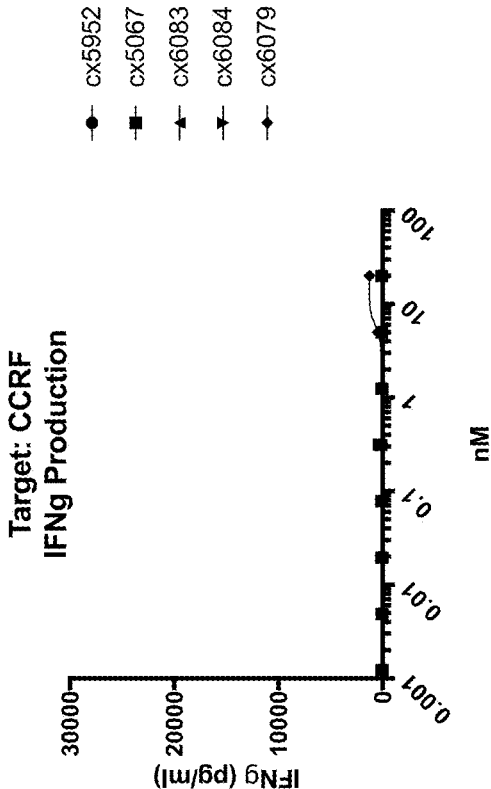


FIG. 16L



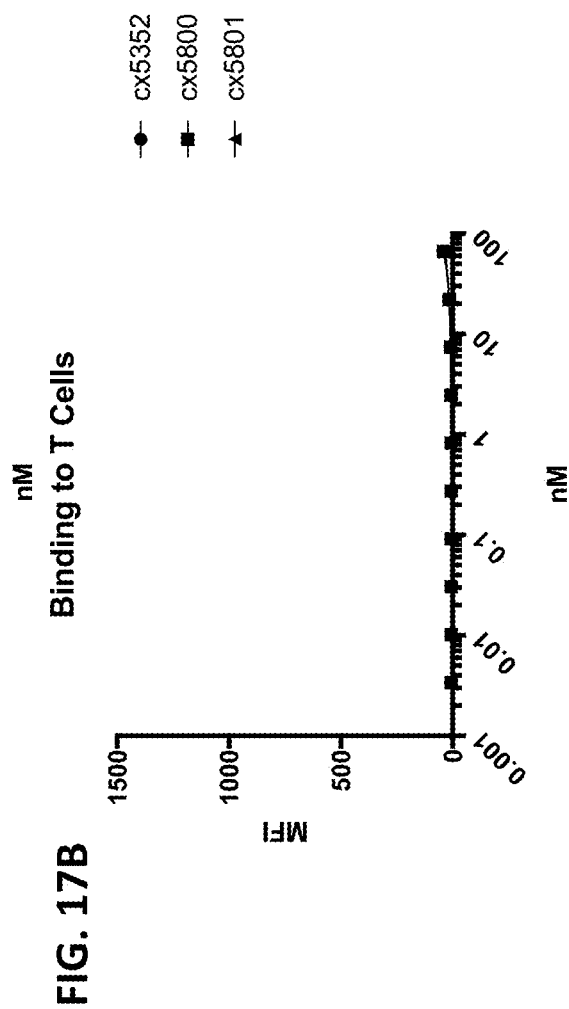
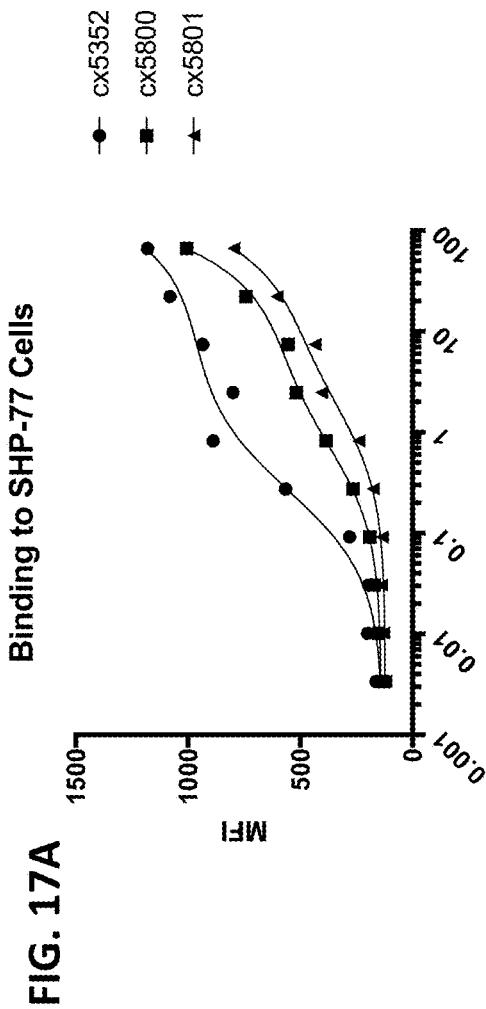


FIG. 17C

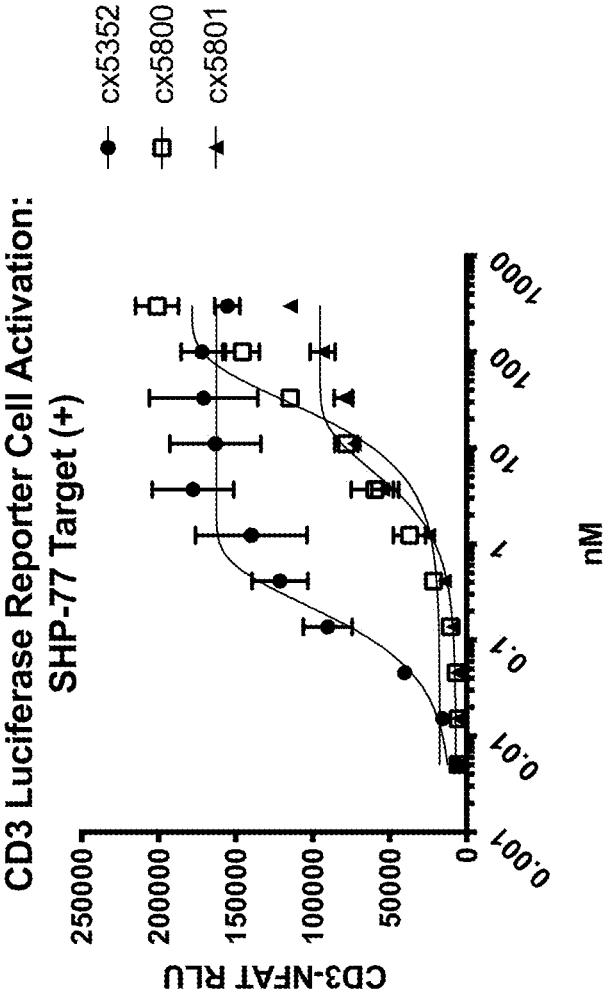


FIG. 18A

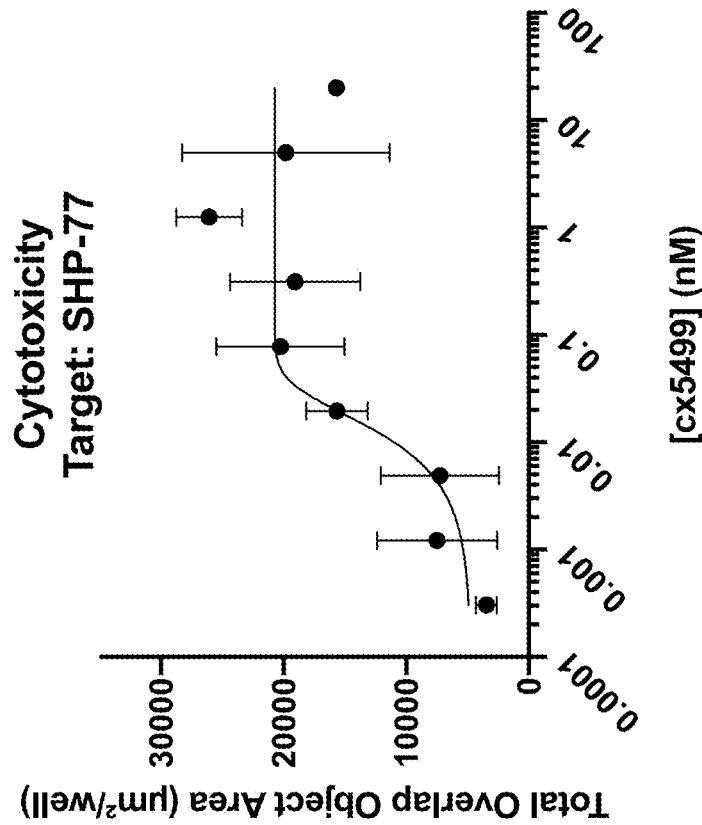


FIG. 18B CD25 Expression on CD4 T-cells

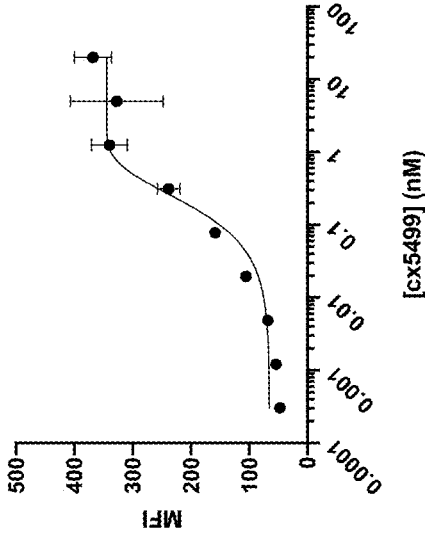


FIG. 18C CD69 Expression on CD4 T-cells

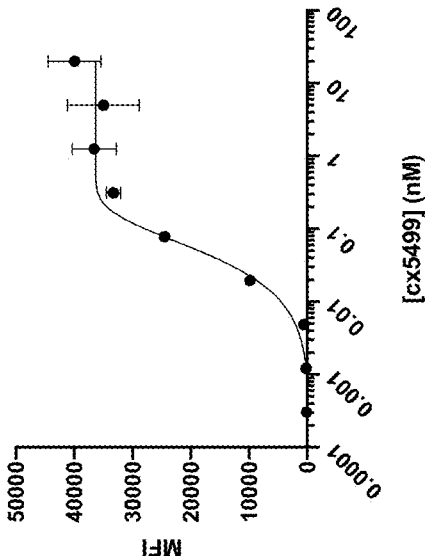


FIG. 18D CD25 Expression on CD8 T-cells

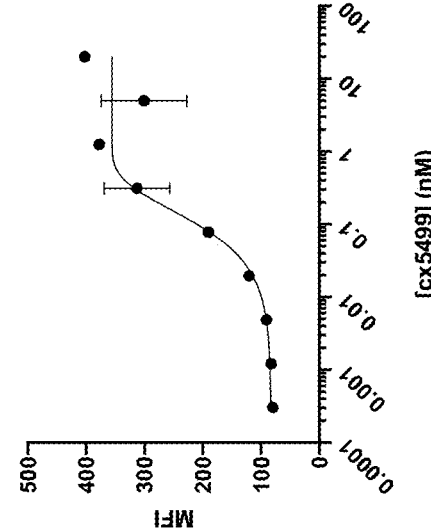
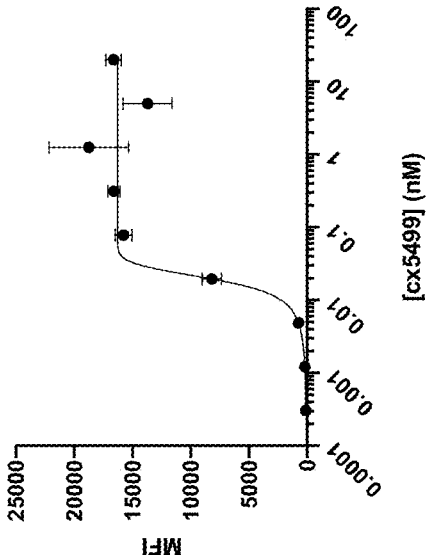


FIG. 18E CD69 Expression on CD8 T-cells



MULTISPECIFIC POLYPEPTIDE CONSTRUCTS HAVING CONSTRAINED CD3 BINDING AND RELATED METHODS AND USES

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. provisional application 62/656,331, filed Apr. 11, 2018, entitled “MULTISPECIFIC POLYPEPTIDE CONSTRUCTS HAVING CONSTRAINED CD3 BINDING AND RELATED METHODS AND USES,” 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 744952000200SeqList.TXT, created Apr. 10, 2019 which is 212 kilobytes in size. The information in the electronic format of the Sequence Listing is incorporated by reference in its entirety.

FIELD

[0003] The invention relates generally to multispecific polypeptides having constrained CD3 binding. In some embodiments, components of the multispecific polypeptides are connected by a non-cleavable linker. Also provided are methods of making and using these multispecific polypeptides in a variety of therapeutic, diagnostic and prophylactic indications.

BACKGROUND

[0004] Therapeutic antibodies that cause target cell depletion generally rely on effector functions mediated via interaction with Fc-gamma-receptors (FcγRs) and complement proteins. Effector cells expressing FcγRs are predominately those of the innate immune system. T-cells are not direct effector cells involved in antibody mediated target cell depletion.

[0005] CD3 (Cluster of Differentiation 3) T-cell co-receptor is a multimeric protein composed of four distinct polypeptide chains, referred to as the ε, γ, δ, and ζ chains. The CD3 complex serves as the signaling module of the T cell receptor that associates non-covalently with the antigen-binding α/β chains of T cell receptor (TCR).

[0006] Because direct engagement of CD3 results in T-cell activation, it is a desirable target for a variety of therapeutic and/or diagnostic indications. Accordingly, there exists a need for antibodies and therapeutics that target the CD3/TCR pathway.

SUMMARY

[0007] The present disclosure provides multispecific polypeptide constructs that exhibit constrained CD3 binding. In some embodiments, the multispecific polypeptide construct is composed of a first component comprising an immunoglobulin Fc region and a second component comprising a CD3-binding region, wherein the first and second components are coupled by a linker, such as a non-cleavable linker, wherein the Fc region is positioned N-terminal to the CD3-binding region; and one or both of the first and second

components comprises an antigen binding domain that binds a tumor associated antigen (TAA). In some embodiments, the CD3-binding region binds CD3 (CD3ε). In some embodiments, the antigen binding 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. In some embodiments, the first component comprises a first antigen binding domain and the second component comprises a second antigen binding domain, wherein each of the antigen binding domains bind a tumor associated antigen (TAA). In some cases, the first antigen binding domain is positioned at the amino terminus of the multispecific construct and the second antigen binding domain is positioned at the carboxy terminus of the multispecific construct. In some embodiments, the first antigen binding 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. In particular embodiments of provided multispecific polypeptide constructs, at least one antigen binding domain is positioned carboxy-terminally relative to the CD3 binding region of the multispecific polypeptide construct.

[0008] Provided herein is a multispecific polypeptide construct, wherein the multispecific construct comprises in order, from N-terminus to C-terminus: a first antigen binding domain that binds to a tumor-associated antigen (TAA); an immunoglobulin Fc region; a linker, such as a non-cleavable linker, a CD3 binding region that binds CD3 (CD3ε); and a second antigen binding domain that binds a tumor-associated antigen (TAA). Also provided is a multispecific polypeptide construct, wherein the multispecific construct comprises in order, from N-terminus to C-terminus: an immunoglobulin Fc region; a linker, such as a non-cleavable linker; a CD3 binding region that binds CD3 (CD3ε); and an antigen binding domain that binds a tumor-associated antigen (TAA). Provided is a multispecific polypeptide construct, wherein the multispecific construct comprises in order, from N-terminus to C-terminus: an antigen binding domain that binds to a tumor-associated antigen (TAA); an immunoglobulin Fc region; a linker, such as a non-cleavable linker; and a CD3 binding region that binds CD3 (CD3ε).

[0009] In some of any of the provided embodiments, the linker is a non-cleavable linker. In some embodiments, the linker is a linker that does not contain a substrate recognition site specific to cleavage by a protease.

[0010] In some of any of the provided embodiments, the positioning of the Fc region N-terminal to the CD3 binding region reduces or prevents the ability of the CD3 binding region to bind CD3. In some embodiments, the first component (component #1) and the second component (component #2) of the multispecific polypeptide constructs are linked and binding to CD3 is disallowed, unless the antigen binding domain(s) is bound to its cognate antigen. In some embodiments, component #1 contains at least one antigen binding domain and an Fc region. In some embodiments, component #2 contains at least a CD3 binding region domain and an antigen binding domain, the former of which is capable of binding CD3 (when the multispecific construct is bound to antigen recognized by the antigen binding domain or domains of component #1 or component #2). Thus, linkage of the CD3 binding region to the Fc region as described ensures that the multispecific polypeptide constructs do not bind or otherwise engage CD3 unless the antigen binding domain(s) is bound to its cognate antigen.

This is advantageous as it prevents systemic binding of the CD3 binding region to T-cells and instead focuses the CD3 binding region's ability to bind to site of antigen expression. This is beneficial as it diminishes or eliminates a major binding sink of peripheral T-cells, potentially allowing more favorable distribution and localization at site of antigen expression, e.g., tumor cells or the tumor microenvironment.

[0011] When the antigen binding domain(s) is bound to its cognate antigen, the multispecific polypeptide construct, via component #2, is capable of forming an immune synapse between an antigen-expressing cell and a T-cell. This co-engagement mediates antigen dependent T-cell activation, cytotoxicity, cytokine release, degranulation and proliferation. In some embodiments, the multispecific polypeptide constructs are capable of interacting with FcγRs and mediating innate immune effector functions, for example antibody dependent cellular cytotoxicity (ADCC) and antibody dependent cellular phagocytosis (ADCP). In some embodiments, the multispecific polypeptide constructs are capable of interacting with complement proteins, namely C1q, and mediating complement-dependent cytotoxicity.

[0012] In some embodiments, the cognate antigen recognized by the antigen binding domain(s) of a provided multispecific polypeptide construct is a tumor associated antigen (TAA).

[0013] Thus, among the provided embodiments, the multispecific polypeptide construct is composed of a first component comprising an immunoglobulin Fc region and a second component comprising a CD3-binding region, wherein the first and second components are coupled by a linker, such as a non-cleavable linker, wherein the Fc region is positioned N-terminal to the CD3-binding region; and one or both of the first and second components comprises an antigen binding domain that binds a tumor associated antigen (TAA). In some embodiments, the CD3-binding region binds CD3 (CD3ε). In some embodiments, the antigen binding 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. In some embodiments, the first component comprises a first antigen binding domain and the second component comprises a second antigen binding domain, wherein each of the antigen binding domains bind a tumor associated antigen (TAA). In some cases, the first antigen binding domain is positioned at the amino terminus of the multispecific construct and the second antigen binding domain is positioned at the carboxy terminus of the multispecific construct. In some embodiments, the first antigen binding 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. In particular embodiments of provided multispecific polypeptide constructs, at least one antigen binding domain is positioned carboxy-terminally relative to the CD3 binding region of the multispecific polypeptide construct.

[0014] In some embodiments, the CD3 binding region is an antibody or an antigen binding fragment. In particular embodiments, the antibody or antigen binding fragment is a two chain polypeptide containing a variable heavy (VH) and a variable light (VL) chain. In some embodiments, the antibody or antigen-binding fragment is an Fv. In particular embodiments, the Fv is a disulfide-stabilized Fv (dsFv) containing an interchain disulfide bond between the VH and VL chains.

[0015] Provided herein is a multispecific polypeptide construct comprising a first component comprising an immunoglobulin Fc region and a second component comprising a CD3-binding region, wherein the CD3 binding region is an anti-CD3 antibody or antigen binding fragment that is an Fv antibody fragment comprising a variable heavy chain region (VH) and a variable light chain region (VL); the Fc is a heterodimeric Fc comprising a first Fc polypeptide and a second Fc polypeptide and the VH and VL of 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 non-cleavable linker, wherein the Fc region is positioned N-terminal to the CD3-binding region; and the first component comprises a first antigen binding domain and the second component comprises a second antigen binding domain, wherein each of the antigen binding domains bind a tumor associated antigen (TAA). In some embodiments, the CD3-binding region binds CD3 (CD3ε). In some embodiments, the first antigen binding domain is positioned amino-terminally relative to the Fc region of the multispecific construct and the second antigen binding domain is positioned carboxy-terminally relative to the CD3 binding region of the multispecific construct. In some embodiments, the multispecific construct comprises in order, from N-terminus to C-terminus: the first antigen binding domain that binds to a tumor-associated antigen (TAA); the immunoglobulin Fc region; the non-cleavable linker; the CD3 binding region, wherein the CD3 binding region binds CD3 (CD3ε); and the second antigen binding domain that binds a tumor-associated antigen (TAA).

[0016] Provided herein is a multispecific polypeptide construct comprising a first component comprising an immunoglobulin Fc region and a second component comprising a CD3-binding region, wherein: the CD3 binding region is an anti-CD3 antibody or antigen binding fragment that is a disulfide-stabilized Fv antibody fragment (dsFv) comprising a variable heavy chain (VH) and a variable light chain (VL); the Fc is a heterodimeric Fc comprising a first Fc polypeptide and a second Fc polypeptide and the VH and VL of 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 non-cleavable linker, wherein the Fc region is positioned N-terminal to the CD3-binding region; and one or both of the first and second components comprises an antigen binding domain that binds a tumor associated antigen (TAA). In particular embodiments of provided multispecific polypeptide constructs, at least one antigen binding domain is positioned carboxy-terminally relative to the CD3 binding region of the multispecific polypeptide construct. In some embodiments, the CD3-binding region binds CD3 (CD3ε).

[0017] Provided herein is a multispecific polypeptide construct comprising a first component comprising an immunoglobulin Fc region and a second component comprising a CD3-binding region, wherein: the CD3 binding region is an anti-CD3 antibody or antigen binding fragment that is an Fv antibody fragment comprising a variable heavy chain (VH) and a variable light chain (VL); the Fc is a heterodimeric Fc comprising a first Fc polypeptide and a second Fc polypeptide and the VH and VL of 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 non-cleavable linker, wherein the Fc region is positioned N-terminal to the CD3-binding region; and one or

both of the first and second components comprises an antigen binding domain that binds a tumor associated antigen (TAA), wherein the antigen-binding domain is a single chain antibody fragment, such as an sdAb or an scFv. In particular embodiments of provided multispecific polypeptide constructs, at least one antigen binding domain is positioned carboxy-terminally relative to the CD3 binding region of the multispecific polypeptide construct. In some embodiments, the CD3-binding region binds CD3 (CD3 ϵ).

[0018] In embodiments provided herein, the multispecific construct comprises in order, from N-terminus to C-terminus: the first antigen binding domain that binds to a tumor-associated antigen (TAA); the immunoglobulin Fc region; the non-cleavable linker; the CD3 binding region, wherein the CD3 binding region binds CD3 (CD3 ϵ); and the second antigen binding domain that binds a tumor-associated antigen (TAA).

[0019] In embodiments provided herein, the multispecific construct comprises in order, from N-terminus to C-terminus: the immunoglobulin Fc region; the non-cleavable linker; the CD3 binding region, wherein the CD3 binding region binds CD3 (CD3 ϵ); and an antigen binding domain that binds a tumor-associated antigen (TAA).

[0020] In embodiments provided herein, the multispecific construct comprises in order, from N-terminus to C-terminus: the antigen binding domain that binds to a tumor-associated antigen (TAA); the immunoglobulin Fc region; the non-cleavable linker; and the CD3 binding region, wherein the CD3 binding region binds CD3 (CD3 ϵ).

[0021] Provided herein is a multispecific polypeptide construct, wherein the multispecific construct comprises in order, from N-terminus to C-terminus: a first antigen binding domain that binds to a tumor-associated antigen (TAA); an immunoglobulin Fc region; a linker, such as a non-cleavable linker; a CD3 binding region that binds CD3 (CD3 ϵ); and a second antigen binding domain that binds a tumor-associated antigen (TAA). Also provided is a multispecific polypeptide construct, wherein the multispecific construct comprises in order, from N-terminus to C-terminus: an immunoglobulin Fc region; a linker, such as a non-cleavable linker; a CD3 binding region that binds CD3 (CD3 ϵ); and an antigen binding domain that binds a tumor-associated antigen (TAA). Provided is a multispecific polypeptide construct, wherein the multispecific construct comprises in order, from N-terminus to C-terminus: an antigen binding domain that binds to a tumor-associated antigen (TAA); an immunoglobulin Fc region; a linker, such as a non-cleavable linker; and a CD3 binding region that binds CD3 (CD3 ϵ).

[0022] In some aspects, the antigen binding domain, or independently each of the antigen binding domains, is selected from an antibody or antigen binding fragment, a natural cognate binding partner, an Anticalin (engineered lipocalin), a Darpin, a Fynomer, a Centyrin (engineered fibronectin III domain), a cystine-knot domain, an Affilin, an Affibody, or an engineered CH3 domain. In some embodiments, the natural cognate binding partner comprises an extracellular domain or binding fragment thereof of the native cognate binding partner of the TAA, or a variant thereof that exhibits binding activity to the TAA.

[0023] In some embodiments, the antigen-binding domain(s) includes one or more copies of an antibody or an antigen-binding fragment thereof. In some embodiments, the antigen-binding domain(s) 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 antigen-binding domain(s) include one or more copies of 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 camelid heavy chain only antibodies. 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.

[0024] In some embodiments, the one or more antigen binding domains independently bind an antigen that is a tumor associated antigen (TAA). In some examples, the antigen binding domain, or independently each of the antigen binding domains, binds to a tumor antigen selected from among 1-92-LFA-3, 5T4, 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 (FRa), GAL3ST1, G-CSF, G-CSFR, GD2, GTR, 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 (FcεRI), 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, PDGF-Ralpha, PDGFRbeta, PD-1, PD-L1, PD-L2, Phosphatidylserine, 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.

[0025] In some embodiments, the Fc region is a homodimeric Fc region. In some embodiments, the immunoglobulin Fc region of the first component is an IgG isotype selected

from the group consisting of IgG1 isotype, IgG2 isotype, IgG3 isotype, and IgG4 subclass. In some examples, the Fc region is an Fc region of a human IgG1, a human IgG2, a human IgG3, or a human IgG4, or is an immunologically active fragment thereof. In some embodiments, the Fc region comprises a polypeptide comprising the amino acid sequence set forth in SEQ ID NO: 1 or a sequence of amino acids that has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% sequence identity to SEQ ID NO:1. In some cases, the Fc region comprises a polypeptide comprising the amino acid sequence set forth in SEQ ID NO: 2 or a sequence of amino acids that has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% sequence identity to SEQ ID NO:2. In some of any such embodiments, the Fc region comprises a polypeptide comprising the amino acid sequence set forth in SEQ ID NO: 4 or a sequence of amino acids that has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% sequence identity to SEQ ID NO:4. In some examples, the Fc region comprises a polypeptide comprising the amino acid sequence set forth in SEQ ID NO: 5 or a sequence of amino acids that has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% sequence identity to SEQ ID NO:5. In some examples, the Fc region comprises a polypeptide comprising the amino acid sequence set forth in SEQ ID NO: 6 or a sequence of amino acids that has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% sequence identity to SEQ ID NO:6.

[0026] In some embodiments, the immunoglobulin Fc region is a polypeptide comprising an amino acid sequence that is derived from an amino acid sequence selected from the group consisting of SEQ ID NOS: 1-6.

[0027] In some embodiments, the immunoglobulin Fc region is a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 1-6. In some embodiments, the immunoglobulin Fc region is a polypeptide comprising an amino acid 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 an amino acid sequence selected from the group consisting of SEQ ID NOS: 1-6.

[0028] In some embodiments, the Fc region is a heterodimeric Fc region.

[0029] In some embodiments, the Fc region is a heterodimer containing a first Fc polypeptide and a second Fc polypeptide wherein one or both of the first and second Fc polypeptides of the heterodimeric Fc region are a variant Fc polypeptide comprising at least one modification to induce heterodimerization compared to an Fc region of human IgG1, human IgG2 or human IgG4. In some embodiments, the at least one modification is in or compared to an Fc region of human IgG1. In some embodiments, the at least one modification is in or compared to the Fc polypeptide set forth in SEQ ID NO:1 or an immunologically active fragment thereof. In some cases, one or both 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:1 or an immunologically active fragment thereof. In some embodiments, each of the Fc polypeptides of the heterodimeric Fc independently comprise at least one amino acid modification. In some cases, the at least one modification is selected from a steric modification(s), a knob-into-hole modification(s), a charge mutation(s) to increase electrostatic complementarity

of the polypeptides, a modification(s) to alter the isoelectric point (pI variant), or combinations thereof.

[0030] In some examples, the amino acid modification is a charge mutation to increase electrostatic complementarity of the polypeptides. In some embodiments, the first and/or second Fc polypeptides 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. In some embodiments, the first or second 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. In some embodiments, at least the first or second Fc polypeptides each 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. In some embodiments, the first and second Fc polypeptides each 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.

[0031] In some examples, the amino acid modification is a knob-into-hole modification.

[0032] In some 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 T366W. In some cases, 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 polypeptide is at one of a position Ser354 and Y349 and the modification of the second Fc polypeptide is at the other of the position Ser354 and Y349. In some embodiments, the first Fc polypeptide comprises the modifications T366W/S354C and the second Fc polypeptide comprises the modifications T366S/L368A/Y407V/Y349C. In some embodiments, the first Fc polypeptide comprises the modifications L368D/K370S and the second Fc polypeptide comprises the modifications S364K/E357Q.

[0033] In some embodiments, the first Fc polypeptide comprises the modifications L368D/K370S and the second Fc polypeptide comprises the modifications S364K/E357Q.

[0034] In some embodiments, at least one of the first and second polypeptide comprises the modifications Q295E/N384D/Q418E/N421D.

[0035] In some embodiments, one of the first or second Fc polypeptide of the heterodimeric Fc further comprises a modification at residue Ile253. In some instances, the modification is Ile253Arg. In some embodiments, one of the first or second Fc polypeptide of the heterodimeric Fc further comprises a modification at residue His435. In some instances, the modification is His435Arg.

[0036] In some embodiments, the Fc region, such as the first and/or second Fc polypeptide comprises a polypeptide that lacks Lys447.

[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:82, 86 or 201, and the second polypeptide of the heterodimeric Fc comprises the sequence of amino acids set forth in any of SEQ ID NOS:83, 87, 90, 92, 202 or 205. In some embodi-

ments, the first Fc polypeptide and the second Fc polypeptide comprises sequences selected from the group consisting of SEQ ID NOS: 82 and 83, respectively; SEQ ID NOS: 86 and 87, respectively; SEQ ID NOS: 201 and 202, respectively; SEQ ID NOS: 82 and 90, respectively; SEQ ID NOS: 86 and 92, respectively; and SEQ ID NOS: 201 and 205, respectively.

[0038] In some embodiments, the immunoglobulin Fc region is a polypeptide comprising an amino acid sequence that is derived from an amino acid sequence selected from the group consisting of SEQ ID NOS: 1-6 comprising one or modifications. In some embodiments, the immunoglobulin Fc region is a polypeptide comprising an amino acid sequence that is derived from an amino acid sequence selected from the group consisting of SEQ ID NOS: 1-6 comprising one or modifications to prevent glycosylation, to alter Fc receptor interactions, to reduce Fc receptor binding, to enhance the interaction with CD32A, to reduce the complement protein C1q binding, to extend the half-life, to enhance FcRn binding, to alter antibody-dependent cellular cytotoxicity (ADCC) and/or complement-dependent cytotoxicity (CDC), to induce heterodimerization, to prevent dimerization, to stabilize the homodimerization at the CH3:CH3 interface, and combinations thereof.

[0039] In some embodiments, modifications within the Fc region reduce binding to Fc-receptor-gamma receptors while having minimal impact on binding to the neonatal Fc receptor (FcRn). In some embodiments, the mutated or modified Fc polypeptide includes the following mutations: Met252Tyr and Met428Leu or Met252Tyr and Met428Val (M252Y, M428L, or M252Y, M428V) using the Kabat numbering system.

[0040] In some embodiments, the Fc region comprises a polypeptide comprising at least one modification to enhance FcRn binding. In some examples, 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 particular embodiments, the modification is at position Met252 and at position Met428. In some cases, the modification is Met252Y and Met428L. In some cases, the modification is Met252Y and Met428V.

[0041] In some embodiments, the first polypeptide of the heterodimeric Fc comprises the sequence of amino acids set forth in any of SEQ ID NOS: 94, 96 or 207, and the second polypeptide of the heterodimeric Fc comprises the sequence of amino acids set forth in any of SEQ ID NOS: 98, 100, or 209. In some embodiments, the first Fc polypeptide and the second Fc polypeptide comprises sequences selected from the group consisting of SEQ ID NOS: 94 and 98, respectively; SEQ ID NOS: 96 and 100, respectively; and SEQ ID NOS: 207 and 209, respectively.

[0042] In some embodiments, the Fc region comprises a polypeptide comprising at least one modification to enhance FcγR binding. In some cases, the modification is modification at Ser239 or Ile332. In some embodiments, the glycosylation of the Fc region is modified to enhance FcγR binding as compared to an unmodified Fc region. In some examples, the Fc region lacks or has reduced fucose content.

[0043] In some 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 embodiments, the one or more amino acid modification is deletion of one or more of Glu233, Leu234 or Leu235.

[0044] In some embodiments, the first polypeptide of the heterodimeric Fc comprises the sequence of amino acids set forth in any of SEQ ID NOS: 82, 86, 94 or 96, and the second polypeptide of the heterodimeric Fc comprises the sequence of amino acids set forth in any of SEQ ID NOS: 83, 87, 90, 92, 98 or 100. In some 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. In some aspects, the first polypeptide of the heterodimeric Fc comprises the sequence of amino acids set forth in any of SEQ ID NOS: 84, 88, 95 or 97 and the second polypeptide of the heterodimeric Fc comprises the sequence of amino acids set forth in any of SEQ ID NOS: 85, 89, 91, 93, 99 or 101.

[0045] In some embodiments, the first polypeptide of the heterodimeric Fc comprises the sequence of amino acids set forth in any of SEQ ID NOS: 84, 88, 95, 97, 203 or 208 and the second polypeptide of the heterodimeric Fc comprises the sequence of amino acids set forth in any of SEQ ID NOS: 85, 89, 91, 93, 99, 101, 204, 206 or 210. In some embodiments, the first Fc polypeptide and the second Fc polypeptide comprises sequences selected from the group consisting of SEQ ID NOS: 84 and 85, respectively; SEQ ID NOS: 88 and 89, respectively; SEQ ID NOS: 203 and 204, respectively; SEQ ID NOS: 95 and 99, respectively; SEQ ID NOS: 97 and 101, respectively; SEQ ID NOS: 208 and 210, respectively; SEQ ID NOS: 84 and 91, respectively; SEQ ID NOS: 88 and 93, respectively; and SEQ ID NOS: 203 and 206, respectively.

[0046] In some embodiments, the CD3 binding region is an anti-CD3 antibody or antigen-binding fragment. In some embodiments, the anti-CD3 antibody or antigen binding fragment comprises a variable heavy chain region (VH) and a variable light chain region (VL). In some of any such embodiments, the CD3 binding region is monovalent.

[0047] In some 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 embodiments, the Fc is a heterodimeric Fc and the VH and VL that comprise the anti-CD3 antibody or antigen binding fragment are linked to opposite polypeptides of the heterodimeric Fc.

[0048] In some embodiments, the CD3 binding region is not able to, or is not substantially able to, bind or engage CD3 unless at least one of the antigen binding domains is bound to its TAA. In some aspects, the CD3 binding region is not able to, or is not substantially able, to bind or engage CD3 unless at least two of the antigen binding domains is bound to their TAA(s).

[0049] In some embodiments, the multispecific polypeptide construct contains a linker that is a polypeptide linker. In some embodiments, the linker is a polypeptide of up to 25 amino acids in length. In some cases, the linker is a polypeptide of 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 a polypeptide that is 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 amino acids in length.

[0050] In some embodiments, the linker is 3 to 18 amino acids in length. In some embodiments, the linker is 12 to 18 amino acids in length. In some embodiments, the linker is 15 to 18 amino acids in length. In some embodiments, the linker is a polypeptide that is 18 amino acids in length.

[0051] In some embodiments, the non-cleavable linker does not contain a substrate recognition site that is specifically recognized for cleavage by a protease. In some embodiments, the protease is produced by an immune effector cell, by a tumor, or by cells present in the tumor microenvironment. In some 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 embodiments, the protease is selected from among matrilysin, a matrix metalloprotease (MMP), granzyme B, and combinations thereof. In some embodiments, the protease is granzyme B.

[0052] In some embodiments, the linker comprises the amino acid sequence GS, GGS, GGGGS (SEQ ID NO:149), GGGGGS (SEQ ID NO:135) and combinations thereof. In some embodiments, the linker comprises the amino acid sequence (GGS)_n, wherein n is 1 to 10. In some embodiments, the linker comprises the amino acid sequence (GGGGS)_n (SEQ ID NO: 173), wherein n is 1 to 10. In some embodiments, the linker comprises (GGGGGS)_n (SEQ ID NO:172), wherein n is 1 to 4.

[0053] In some embodiments, the linker is or comprises GGS. In some embodiments, the linker is or comprises GGGGS (SEQ ID NO: 149). In some embodiments, the linker is or comprises GGGGGS (SEQ ID NO: 135). In some embodiments, the linker is or comprises GGS GGS (“(GGS)₂”) (SEQ ID NO: 10). In some embodiments, the linker is or comprises GGS GGS GGS (“(GGS)₃”) (SEQ ID NO: 11). In some embodiments, the linker is or comprises GGS GGS GGS GGS (“(GGS)₄”) (SEQ ID NO: 12). In some embodiments, the linker is or comprises GGS GGS GGS GGS GGS (“(GGS)₅”) (SEQ ID NO: 13). In some embodiments, the linker is or comprises GGGGGSGGGGGSGGGGGGS (SEQ ID NO: 119). In some embodiments, the linker is or comprises GGS GGGGGSGGGGGSGGGGGGS (SEQ ID NO: 147). In some embodiments, the linker is or comprises GGGGGSGGGGGSGGGGGGS (SEQ ID NO:170). In some embodiments, the linker is or comprises GGGGG (SEQ ID NO:192).

[0054] In some embodiments, the antigen binding domain and the immunoglobulin Fc region of the first component (which in some cases is the first antigen binding domain) are operably linked via one or more further amino acid linkers (referred to herein as an intra-component linker). The intra-component peptide linker of the first component (also called LP1) can be a peptide linker such as any as described in

Section 11.3. The intra-component linker present in the first component, i.e. linking the Fc region and an antigen binding domain, 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, these intra-component linkers are composed predominately of the amino acids Glycine and Serine, denoted as GS-linkers herein. In some embodiments, the GS-linker comprises an amino acid sequence selected from the group consisting of GGS GGS, i.e., (GGS)₂ (SEQ ID NO: 10); GGS GGS GGS, i.e., (GGS)₃ (SEQ ID NO: 11); GGS GGS GGS GGS, i.e., (GGS)₄ (SEQ ID NO: 12); and GGS GGS GGS GGS GGS, i.e., (GGS)₅ (SEQ ID NO: 13).

[0055] In some embodiments, 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 domain of the anti-CD3 antibody or antigen binding fragment thereof; and (ii) a second polypeptide comprising the second Fc polypeptide of the heterodimeric Fc region, the linker and the VL domain of the anti-CD3 antibody or antigen binding fragment thereof, wherein one or both of the first and second polypeptide comprise at least one antigen-binding domain that binds to a tumor associated antigen (TAA). In some embodiments, the VH of the anti-CD3 antibody or antigen-binding fragment is on the same polypeptide as the at least one antigen-binding domain that binds to a tumor associated antigen (TAA). In some embodiments, the polypeptide comprising the VL of the anti-CD3 antibody or antigen-binding fragment does not contain the at least one antigen-binding domain that binds to a tumor associated antigen (TAA). In particular embodiments of provided multispecific polypeptide constructs, at least one antigen binding domain is positioned carboxy-terminally relative to the CD3 binding region of the multispecific polypeptide construct.

[0056] In some embodiments, the second component includes one or more copies of the CD3 binding region.

[0057] In some embodiments, the CD3 binding region is an anti-CD3 antibody or antigen-binding fragment that includes one or more copies of an antibody or an antigen-binding fragment thereof that is able to bind or engage CD3, such as CD3ε. In some embodiments, the anti-CD3 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.

[0058] 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).

[0059] In some embodiments, the anti-CD3ε Fv antibody fragment includes an amino acid sequence selected from the group of SEQ ID NO: 32-81, 191, 196-200, 211, and 212. In some embodiments, the anti-CD3ε Fv antibody fragment includes 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: 32-81, 191, 196-200, 211, and 212. In some embodiments, the anti-CD3ε Fv antibody fragment includes a combination of an amino acid sequence selected from the group of SEQ ID NO: 32-62, 196-198, and 211 and an amino acid sequence selected from the group consisting of SEQ ID NO: 63-81, 191, 199, 200, and 212. In some embodiments, the anti-CD3ε Fv antibody fragment includes a combination of 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: 32-62, 196-198, and 211 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: 63-81, 191, 199, 200, and 212.

[0060] In some embodiments, the anti-CD3 ϵ Fv antibody fragment is a disulfide stabilized anti-CD3 binding Fv fragment (dsFv).

[0061] In some embodiments, the first component includes one or more copies of an antigen-binding domain. In certain embodiments, the first component contains at least two antigen binding domains, such as two antigen binding domains. In some embodiments, the at least two antigen binding domains of the first component bind to the same TAA. In some cases, the at least two antigen binding domains of the first component bind to different epitopes of the same TAA. In some instances, the at least two antigen binding domains of the first component bind to the same epitope of the same TAA. In some embodiments, the at least two antigen binding domain of the first component bind to different TAAs.

[0062] In some embodiments, the second component includes one or more copies of an antigen-binding domain. In certain embodiments, the second component contains at least two antigen binding domains, such as two antigen binding domains. In some embodiments, the at least two antigen binding domains of the second component bind to the same TAA. In some cases, the at least two antigen binding domains of the second component bind to different epitopes of the same TAA. In some instances, the at least two antigen binding domains of the second component binds to a same epitope of the same TAA. In some embodiments, the at least two antigen binding domains of the second component bind to different TAAs.

[0063] In some embodiments, the first component contains a first antigen binding domain and the antigen binding domain of the second component is a second antigen binding domain. In some embodiments, the multispecific antigen binding domain comprises at least a first antigen binding domain and a second antigen binding domain, wherein the first antigen binding domain and second antigen binding domain bind to the same TAA. In some cases, the first antigen binding domain and the second antigen binding domain bind different epitopes of the same TAA. In some instances, the first antigen binding domain and the second antigen binding domain bind the same epitope of the same TAA. In some embodiments, the multispecific antigen binding domain comprises at least a first antigen binding domain and a second antigen binding domain wherein the first antigen binding domain and the second antigen binding domain bind different TAAs.

[0064] In some embodiments, the antigen binding domain of the second component (which in some cases is the second antigen binding domain) and the CD3 binding region are operably linked via one or more further amino acid linkers (referred to herein as an intra-component linker). The intra-component peptide linker of the second component (also called LP2) can be a peptide linker such as any as described in Section 11.3. The intra-component linker of present in the second component, i.e. linking the CD3 binding region and an antigen binding domain, 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 intra-component linker of the second component is composed predominately of the amino acids Glycine and Serine, denoted as GS-linkers herein. 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: 10); GGSGGSGGS, i.e., (GGS)₃ (SEQ ID NO: 11); GGSGGSGGSGGS, i.e., (GGS)₄ (SEQ ID NO: 12); and GGSGGSGGSGGSGGS, i.e., (GGS)₅ (SEQ ID NO: 13).

[0065] Provided herein is a multispecific polypeptide construct, the multispecific polypeptide construct comprising a first component comprising a heterodimeric Fc region 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 or antigen-binding fragment; and one or both of the first and second components comprises an antigen binding domain that binds a tumor associated antigen (TAA).

[0066] In some embodiments, the linker is a polypeptide of up to 50 amino acids in length. In some embodiments, the linker is a polypeptide of up to 25 amino acids in length. In some embodiments, the linker is a polypeptide of up to 15 amino acids in length.

[0067] In any of the provide embodiments, the one or more antigen binding domain that binds TAA results in monovalent, bivalent, trivalent, or tetravalent binding to the TAA. In some embodiments, the one or more antigen binding domains that bind TAA independently are selected from an sdAb, an scFv or a Fab. In some embodiments, the one or more antigen binding domains that binds a TAA is a single chain molecule, such as a single chain antibody fragment containing a VH and a VL, for example an sdAb or an scFv. In some embodiments the one or more antigen binding domains that binds a TAA is a sdAb, such as a V_HH or a V_H_{NAR}. In some embodiments, at least one of the antigen binding domains is a Fab containing a first chain comprising a VH-CH1 (Fd) and a second chain comprising a VL-CL.

[0068] In some embodiments, the antigen binding domain that binds the TAA is attached to the VH of the anti-CD3 binding domain. In some embodiments, the antigen binding domain that binds the TAA is attached to the same side (e.g., knob or hole) of the heterodimeric Fc to which the VH of the anti-CD3 binding domain is attached. In some embodiments, the antigen binding domain that binds the TAA is a sdAb attached to the VH of the anti-CD3 binding domain. In some embodiments, the antigen binding domain that binds the TAA is a sdAb attached to same side (e.g., knob or hole) of the heterodimeric Fc domain to which the VH of the anti-CD3 binding domain is attached. In some embodiments, the antigen binding domain that binds the TAA is a V_HH or a V_H_{NAR} that is attached to the VH of the anti-CD3 binding domain. In some embodiments, the antigen binding domain that binds the TAA is a V_HH or a V_H_{NAR} that is attached to the same side (e.g., knob or hole) of the heterodimeric Fc domain to which the VH of the anti-CD3 binding domain is attached. In some embodiments, the antigen binding domain that binds the TAA is a V_HH attached to the VH of the anti-CD3 binding domain. In some

embodiments, the antigen binding domain that binds the TAA is a V_HH attached to the same side (e.g., knob or hole) of the heterodimeric Fc domain to which the VH of the anti-CD3 binding domain is attached. In some embodiments, the antigen binding domain that binds the TAA is a VH_{NAR} attached to the VH of the anti-CD3 binding domain. In some embodiments, the antigen binding domain that binds the TAA is a VH_{NAR} attached to the same side (e.g., knob or hole) or the Fc domain to which the VH of the anti-CD3 binding domain is attached.

[0069] In some embodiments, 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 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 and the VL domain of the anti-CD3 antibody or antigen binding fragment, wherein one or both of the first and second polypeptide comprise at least one antigen-binding domain that binds to a tumor associated antigen (TAA). In some instances, only one of the first or second polypeptide comprises the at least one antigen-binding domain that binds a TAA.

[0070] In some embodiments, the at least one of the antigen binding domain(s) is a Fab. In some embodiments, the multispecific polypeptide construct comprises: (i) a first polypeptide comprising the first Fc polypeptide of the heterodimeric Fc region, the linker and the VH domain of the anti-CD3 antibody or antigen binding fragment; (ii) a second polypeptide comprising the second Fc polypeptide of the heterodimeric Fc region, the linker and the VL domain of the anti-CD3 antibody or antigen binding fragment, and (iii) a third polypeptide comprising a VH-CH1 (Fd) or VL-CL of a Fab antibody fragment that binds to a tumor-associated antigen, wherein the first and/or second polypeptide further comprises the other of the VH-CH1 (Fd) or VL-CL of the Fab antibody fragment. In some cases, only one of the first or second polypeptide comprises the other of the VH-CH1 (Fd) or VL-CL of the Fab antibody fragment. In some embodiments, both the first or second polypeptide comprises the other of the VH-CH1 (Fd) or VL-CL of the Fab antibody fragment. In some cases, the other of the VH-CH1 (Fd) or VL-CL of the Fab antibody fragment is positioned amino-terminally relative to the Fc region and/or carboxy-terminally relative to the CD3 binding region of one of the first or second polypeptide of the multispecific polypeptide construct. In some embodiments, the other of the VH-CH1 (Fd) or VL-CL of the Fab antibody fragment is positioned amino-terminally relative to the Fc region of the first polypeptide or second polypeptide and carboxy-terminally relative to the CD3 binding region of the other of the first or second polypeptide.

[0071] In some embodiments, the at least one antigen binding domain is positioned amino-terminally relative to the Fc region and/or is positioned carboxy-terminally relative to the CD3 binding region of one of the first or second polypeptide of the multispecific polypeptide construct. In some cases, the at least one antigen binding domain is positioned amino-terminally relative to the Fc region of the multispecific construct and the second antigen binding domain is positioned carboxy-terminally relative to the CD3 binding region of the multispecific construct. In particular embodiments of provided multispecific polypeptide constructs, at least one antigen binding domain is positioned

carboxy-terminally relative to the CD3 binding region of the multispecific polypeptide construct. In some embodiments, the at least one antigen binding domain is a sdAb. In some embodiments the at least one antigen binding domain that is a sdAb is positioned carboxy-terminally to the CD3 binding region of the multispecific construct. In some embodiments the at least one antigen binding domain that is a sdAb is positioned amino-terminally to the Fc region of the multispecific construct. In some embodiments the at least one antigen binding domain is a V_HH . In some embodiments, the at least one antigen binding domain that is a V_HH is positioned carboxy-terminally to the CD3 binding region of the multispecific construct. In some embodiments the at least one antigen binding domain that is a V_HH is positioned amino-terminally to the Fc region of the multispecific construct.

[0072] In some embodiments, the multispecific polypeptide construct comprises a first linking peptide (LP1) between the first antigen binding domain and the immunoglobulin Fc polypeptide region (Fc region). In some embodiments, the multispecific polypeptide construct comprises a second linking peptide (LP2) between the anti-CD3 binding domain (CD3 binding region) and the second antigen binding domain. In some embodiments, the multispecific polypeptide construct comprises a first linking peptide (LP1) between the first antigen binding domain and the immunoglobulin Fc polypeptide region (Fc region) and a second linking peptide (LP2) between the anti-CD3 binding domain (CD3 binding region) and the second antigen binding domain.

[0073] In some embodiments, the multispecific polypeptide construct has the structural arrangement from N-terminus to C-terminus as follows: first antigen binding domain-LP1-immunoglobulin Fc polypeptide linker region (Fc region)-linker-anti-CD3 binding domain-LP2-second antigen binding domain. In some embodiments, the multispecific polypeptide construct has the structural arrangement from N-terminus to C-terminus as follows: second antigen binding domain-LP2-immunoglobulin Fc polypeptide linker region (Fc region)-linker-anti-CD3 binding domain (CD3 binding region)-LP1-first antigen binding domain.

[0074] In some embodiments, the two linking peptides LP1 and LP2 are not identical to each other. In some cases, LP1 or LP2 is independently a peptide of about 1 to 20 amino acids in length. In some examples, LP1 or LP2 independently comprise a peptide that is or comprises any Gly-Ser linker as set forth in SEQ ID NOs: 10-13, 119, 135, 147, 149.

[0075] In some embodiments, the multispecific construct is a construct having any of the structural arrangement shown in FIG. 1. In some embodiments, the construct is a bispecific construct that has a structural arrangement from N-terminus to C-terminus as follows. The N-terminal end of the bispecific construct includes a first antigen binding domain that binds a tumor associated antigen (TAA). The first binding domain binds a first epitope on the TAA target. Coupled to the first antigen binding domain is a central immunoglobulin Fc polypeptide region that regulates FcγR interactions and/or FcRn interaction. In some embodiments, the central immunoglobulin Fc polypeptide region is heterodimeric. The immunoglobulin Fc polypeptide region is coupled to a linker located at a position C-terminal to the end of the immunoglobulin Fc polypeptide region. The linker is attached to an anti-CD3 binding sequence located C-termi-

nal to the Fc region, which, in some cases, is at the distal end of the second component. The C-terminus of the bispecific construct includes a second antigen binding domain that binds a TAA. In some embodiments, the second antigen binding domain binds the same TAA as the first antigen binding domain located within the first component. In some embodiments, the second antigen binding domain binds a second epitope on the TAA, wherein the second epitope is non-competitive with the first epitope on the TAA. In some embodiments, the second antigen binding domain binds a distinct TAA from that of the first antigen binding domain.

[0076] In some of any of the provided embodiments, the anti-CD3 antibody or antigen binding fragment is an Fv antibody fragment. In some embodiments, the Fv antibody fragment comprises a disulfide stabilized anti-CD3 binding Fv fragment (dsFv). In some embodiments, the anti-CD3 binding sequence is an Fv antibody fragment that is engineered to include a disulfide linkage between the variable heavy chain (VH) and variable light chain (VL) regions, thereby producing a disulfide stabilized anti-CD3 binding Fv fragment (dsFv). In some embodiments, the VH and VL domains that comprise the anti-CD3 Fv are operably linked to opposite members of a heterodimeric Fc region. In these embodiments, the anti-CD3 Fv binds CD3 in a monovalent fashion. In aspects as provided, the anti-CD3 dsFv does not engage CD3 unless the multispecific polypeptide construct is bound to a cognate antigen.

[0077] In some embodiments, each of the first antigen binding domain and the second antigen binding domain of the bispecific construct includes one or more copies of an antibody or an antigen-binding fragment thereof. In some embodiments, each of the first antigen binding domain and the second antigen binding domain of the bispecific construct 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 antigen binding domain, or independently each of the antigen binding domains, 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 each of the first antigen binding domain and the second antigen binding domain of the bispecific construct includes one or more copies of 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}5 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.

[0078] In some embodiments, the antibody or antigen-binding fragment is an sdAb. In some cases, the sdAb is a human or humanized sdAb. In some aspects, the sdAb is V_HH, V_{NAR}, an engineered VH domain or an engineered VK domain. In some examples, the antibody or antigen-binding

fragment thereof is an scFv. In some cases, the antibody or antigen-binding fragment thereof is a Fab.

[0079] 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: 16); a VH CD2 comprising the amino acid sequence RIRSKYNNYATYYADSVKD (SEQ ID NO: 17); a VH CDR3 comprising the amino acid sequence HGNTGNSYVSWFAY (SEQ ID NO: 18), a VL CDR1 comprising the amino acid sequence RSSTGAVTTSNYAN (SEQ ID NO: 19); a VL CDR2 comprising the amino acid sequence GTNKRAP (SEQ ID NO: 20); and a VL CDR3 comprising the amino acid sequence ALWYSNLWV (SEQ ID NO: 21).

[0080] 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 GFTFNTYAMN (SEQ ID NO: 211); a VH CDR2 comprising the amino acid sequence RIRSKYNNYATY (SEQ ID NO: 212); a VH CDR3 comprising the amino acid sequence HGNTGNSYVSWFAY (SEQ ID NO: 18), a VL CDR1 comprising the amino acid sequence RSSTGAVTTSNYAN (SEQ ID NO: 19); a VL CDR2 comprising the amino acid sequence GTNKRAP (SEQ ID NO: 20); and a VL CDR3 comprising the amino acid sequence ALWYSNLWV (SEQ ID NO: 21).

[0081] In some of any of the provided embodiments, the anti-CD3 antibody or antigen-binding fragment comprises a VH CDR1 sequence that includes at least the amino acid sequence GFTFNTYAMN (SEQ ID NO: 211); a VH CDR2 sequence that includes at least the amino acid sequence RIRSKYNNYATY (SEQ ID NO: 212); a VH CDR3 sequence that includes at least the amino acid sequence HGNTGNSYVSWFAY (SEQ ID NO: 18), a VL CDR1 sequence that includes at least the amino acid sequence GSSTGAVTTSNYAN (SEQ ID NO: 229); a VL CDR2 sequence that includes at least the amino acid sequence GTNKRAP (SEQ ID NO: 230); and a VL CDR3 sequence that includes at least the amino acid sequence ALWYSNLHWV (SEQ ID NO: 225).

[0082] 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: 14, 32-62, 196-198, and 211 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: 14, 32-62, 196-198, and 211; and a VL having the amino acid sequence of any of SEQ ID NOS: 15, 63-81, 191, 199, 200, and 212 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: 15, 63-81, 191, 199, 200, and 212.

[0083] In some embodiments, the anti-CD3 antibody or antigen-binding fragment is an Fv. In some embodiments, the anti-CD3 Fv comprises: a VH having the amino acid sequence of any of SEQ ID NOS: 14, 32-43, 45-47, 48, 196 and 211 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: 14, 32-43, 45-47, 48, 196 and 211; and a VL having the amino acid sequence of any of SEQ ID NOS: 15, 63, 65-71, 73, 75, 77, and 199 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: 15, 63, 65-71, 73, 75, 77, and 199. In some cases, the anti-CD3 dsFv comprises the amino acid sequence of SEQ ID NO: 14 and the amino acid sequence of SEQ ID NO: 15.

In other cases, the anti-CD3 Fv comprises the amino acid sequence of SEQ ID NO: 196 and the amino acid sequence of SEQ ID NO: 199.

[0084] In some embodiments, the VH and VL chain regions of the CD3 binding domain each independently comprise at least one amino acid modification. In some embodiments, the at least one amino acid modification of the VH and VL chain regions of the CD3 binding domain increase the stability of the CD3 binding domain. In some embodiments, the at least one amino acid modification of the VH and VL chain regions of the CD3 binding domain increase the ability of the CD3 binding domain to bind CD3. In some embodiments, the at least one amino acid modification of the VH and VL chain regions of the CD3 binding domain increase the stability of the CD3 binding domain by creating a disulfide linkage between the VH and VL chain regions.

[0085] In some embodiments, the CD3 binding region has a disulfide stabilized linkage between the VH and VL regions. In some embodiments, the anti-CD3 antibody or antigen-binding fragment is disulfide stabilized Fv (dsFv). 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. In some embodiments, the disulfide stabilized anti-CD3 Fv comprises an anti-CD3 VH with the mutation G44C and an anti-CD3 VL with the mutation G100C 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.

[0086] In some embodiments, the anti-CD3 dsFv comprises: a VH having the amino acid sequence of any of SEQ ID NOS: 44, 49-62, 197 and 198 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: 44, 49-62, 197 and 198; and a VL having the amino acid sequence of any of SEQ ID NOS: 64, 72, 74, 76, 78-81, 191, 200 and 212 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: 64, 72, 74, 76, 78-81, 191, 200 and 212. In some cases, the anti-CD3 dsFv comprises the amino acid sequence of SEQ ID NO: 44 and the amino acid sequence of SEQ ID NO: 72. In some embodiments, the anti-CD3 dsFv comprises the amino acid sequence of SEQ ID NO: 198 and the amino acid sequence of SEQ ID NO: 200. In some embodiments, the anti-CD3 dsFv comprises the amino acid sequence of SEQ ID NO: 197 and the amino acid sequence of SEQ ID NO: 200.

[0087] In some embodiments, the multispecific construct also includes an agent conjugated to the multispecific construct. In some embodiments, the agent is a therapeutic agent. In some embodiments, the agent is a detectable moiety. In some embodiments, the detectable moiety is a diagnostic agent. In some embodiments, the agent is conjugated to the multispecific construct via a linker. In some embodiments, the linker is a non-cleavable linker.

[0088] In some embodiments, the multispecific construct described herein is used in conjunction with one or more additional agents or a combination of additional agents. Suitable additional agents include current pharmaceutical and/or surgical therapies for an intended application, such as, for example, cancer. For example, the multispecific

construct can be used in conjunction with an additional chemotherapeutic or anti-neoplastic agent.

[0089] In some embodiments, the multispecific construct and additional agent are formulated into a single therapeutic composition, and the multispecific construct and additional agent are administered simultaneously. In some embodiments, the multispecific construct and additional agent are separate from each other, e.g., each is formulated into a separate therapeutic composition, and the multispecific construct and the additional agent are administered simultaneously, or the multispecific construct and the additional agent are administered at different times during a treatment regimen. For example, the multispecific construct is administered prior to the administration of the additional agent, the multispecific construct is administered subsequent to the administration of the additional agent, or the multispecific construct and the additional agent are administered in an alternating fashion. As described herein, the multispecific construct and additional agent are administered in single doses or in multiple doses.

[0090] In some embodiments, the multispecific construct naturally contains one or more disulfide bonds. In some embodiments, the multispecific construct can be engineered to include one or more disulfide bonds.

[0091] The disclosure also provides an isolated nucleic acid molecule or polynucleotide encoding at least a portion of a multispecific construct described herein and/or one or more nucleic acid molecules encoding a multispecific construct described herein, such as for example, at least a first nucleic acid encoding at least a portion of the first component of the multispecific construct and a second nucleic acid encoding at least a portion of the second component of the multispecific construct, as well as vectors that include these isolated nucleic acid sequences.

[0092] Among the provided embodiments is a polynucleotide(s) encoding any of the provided multispecific polypeptide constructs. Also provided is a polynucleotide encoding a polypeptide chain of any of the provided multispecific polypeptide constructs. Further provided is a polynucleotide, comprising a first nucleic acid sequence encoding a first polypeptide of any of the provided multispecific constructs 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 cases, the first nucleic acid sequence and second nucleic acid sequence are operably linked to the same promoter. In some embodiments, the multispecific polypeptide construct comprises a third polypeptide chain, and the polynucleotide further comprises a third nucleic acid encoding the third polypeptide of the multispecific construct. In some embodiments, the third nucleic acid is separated from the first and/or second polypeptide by an internal ribosome entry site (IRES), or a nucleic acid encoding a self-cleaving peptide or a peptide that causes ribosome skipping and/or the third nucleic acid sequence is operably linked to the same promoter as the first and/or second nucleic acid sequence. In some examples, 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 (SEQ ID NOS: 159-164, or encoded by the sequence set forth in SEQ ID NO: 165)

[0093] Provided herein is a vector comprising any of the provided polynucleotides. In some embodiments, the vector is an expression vector. In some examples, the vector is a viral vector or a eukaryotic vector, optionally wherein the eukaryotic vector is a mammalian vector.

[0094] Provided is a cell, comprising any of the provided polynucleotides or vectors. In some cases, the cell is recombinant or isolated. In some examples, the cell is a mammalian cell. In some examples, the cell is a HEK293 or CHO cell.

[0095] The disclosure provides methods of producing a multispecific construct by culturing a cell under conditions that lead to expression of the multispecific construct, wherein the cell comprises such a nucleic acid molecule(s). In some embodiments, the cell comprises such a vector.

[0096] Provided herein is a method of producing a multispecific polypeptide construct, the method comprising introducing into a cell any of the provided polynucleotides or vectors and culturing the cell under conditions to that lead to expression of the multispecific construct to produce the multispecific polypeptide construct. Also provided is a method of producing a multispecific polypeptide construct, the method comprising culturing any of the provided cells under conditions in which the multispecific polypeptide is expressed or produced by the cell. In some cases, the cell is a mammalian cell. In some examples, the cell is a HEK293 or CHO cell. In some embodiments, the method further includes isolating or purifying the multispecific polypeptide construct from the cell. In some cases, the multispecific polypeptide construct is a heterodimer.

[0097] Provided herein is a multispecific polypeptide construct produced by any of the provided methods.

[0098] Provided herein is a method of stimulating or inducing an immune response, the method comprising contacting a target cell and a T cell with the any of the provided multispecific polypeptide constructs or pharmaceutical compositions, said target cell expressing a tumor associated antigen recognized by the multispecific polypeptide construct. In some embodiments, the target cell is a tumor cell expressing the tumor associated antigen (TAA).

[0099] In some embodiments, the contacting is carried out ex vivo or in vitro. In some embodiments, the contacting is carried out in vivo in a subject.

[0100] Provided is a method of stimulating or inducing an immune response in a subject, the method comprising administering, to a subject in need thereof, a therapeutically effective amount of any of the provided multispecific conjugates or pharmaceutical compositions. In some cases, the method increases cell-mediated immunity. In some embodiments, the method increases T-cell activity. In some embodiments, the method increases cytolytic T-cell (CTL) activity. In some examples, the immune response is increased against a tumor or cancer. In some embodiments, the method treats a disease or condition in the subject.

[0101] The present disclosure also provides methods of treating, preventing, delaying the progression of or otherwise ameliorating a symptom of one or more pathologies or alleviating a symptom associated with such pathologies, by administering a multispecific polypeptide construct of the disclosure to a subject in which such treatment or prevention is desired. 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 multispecific conjugates or

pharmaceutical compositions. In some embodiments, the disease or condition is a tumor or a cancer.

[0102] In some embodiments of any of the provided method, the subject, such as the subject to be treated is, e.g., human or other mammal. In some embodiments of any of the provided method, the subject is a human. In some embodiments, the subject is a non-human mammal, such as a non-human primate, companion animal (e.g., cat, dog, horse), farm animal, work animal, or zoo animal. In some embodiments, the subject is a rodent.

[0103] A multispecific polypeptide construct of the disclosure used in any of the embodiments of these methods and uses can be administered at any stage of the disease. For example, such a multispecific polypeptide construct can be administered to a patient suffering cancer of any stage, from early to metastatic. The terms subject and patient are used interchangeably herein.

[0104] A multispecific polypeptide construct of the disclosure used in any of the embodiments of these methods and uses can be used in a treatment regimen comprising neoadjuvant therapy.

[0105] A multispecific polypeptide construct of the disclosure used in any of the embodiments of these methods and uses can be administered either alone or in combination with one or more additional agents, including small molecule inhibitors, other antibody-based therapies, polypeptide or peptide-based therapies, nucleic acid-based therapies and/or other biologics. In some embodiments, a multispecific polypeptide construct is administered in combination with one or more additional agents such as, by way of non-limiting example, a chemotherapeutic agent, such as an alkylating agent, an anti-metabolite, an anti-microtubule agent, a topoisomerase inhibitor, a cytotoxic antibiotic, and any other nucleic acid damaging agent. In some embodiments, the additional agent is a taxane, such as paclitaxel (e.g., Abraxane®). In some embodiments, the additional agent is an anti-metabolite, such as gemcitabine. In some embodiments, the additional agent is an alkylating agent, such as platinum-based chemotherapy, such as carboplatin or cisplatin. In some embodiments, the additional agent is a targeted agent, such as a kinase inhibitor, e.g., sorafenib or erlotinib. In some embodiments, the additional agent is a targeted agent, such as another antibody, e.g., a monoclonal antibody (e.g., bevacizumab), a bispecific antibody, or a multispecific antibody. In some embodiments, the additional agent is a proteasome inhibitor, such as bortezomib or carfilzomib. In some embodiments, the additional agent is an immune modulating agent, such as lenolidomide or IL-2. In some embodiments, the additional agent is radiation. In some embodiments, the additional agent is an agent considered standard of care by those skilled in the art. In some embodiments, the additional agent is a chemotherapeutic agent well known to those skilled in the art. In some embodiments, the multispecific polypeptide construct and the additional agent(s) are formulated in a single composition. In some embodiments, the multispecific polypeptide construct and the additional agent(s) are administered as two or more separate compositions. In some embodiments, the multispecific polypeptide construct and the additional agent(s) are administered simultaneously. In some embodiments, the multispecific polypeptide construct and the additional agent(s) are administered sequentially.

[0106] In some embodiments, the additional agent(s) is a chemotherapeutic agent, such as a chemotherapeutic agent

selected from the group consisting of docetaxel, paclitaxel, abraxane (i.e., albumin-conjugated paclitaxel), doxorubicin, oxaliplatin, carboplatin, cisplatin, irinotecan, and gemcitabine.

[0107] In some embodiments, the additional agent(s) is a checkpoint inhibitor, a kinase inhibitor, an agent targeting inhibitors in the tumor microenvironment, and/or a T cell or NK agonist. In some embodiments, the additional agent(s) is radiation therapy, alone or in combination with another additional agent(s) such as a chemotherapeutic or anti-neoplastic agent. In some embodiments, the additional agent(s) is a vaccine, an oncovirus, and/or a DC-activating agent such as, by way of non-limiting example, a toll-like receptor (TLR) agonist and/or α -CD40. In some embodiments, the additional agent(s) is a tumor-targeted antibody designed to kill the tumor via ADCC or via direct conjugation to a toxin (e.g., an antibody drug conjugate (ADC)).

[0108] In some embodiments, the checkpoint inhibitor is an inhibitor of a target selected from the group consisting of CTLA-4, LAG-3, PD-1, PDL1, TIGIT, TIM-3, B7H3, B7H4, and Vista. In some embodiments, the kinase inhibitor is selected from the group consisting of B-RAFi, MEKi, and Btk inhibitors, such as ibrutinib. In some embodiments, the kinase inhibitor is crizotinib. In some embodiments, the tumor microenvironment inhibitor is selected from the group consisting of an IDO inhibitor, an α -CSF1R inhibitor, an α -CCR4 inhibitor, a TGF-beta, a myeloid-derived suppressor cell, or a T-regulatory cell. In some embodiments, the agonist is selected from the group consisting of OX40, GITR, CD137, CD28, ICOS, CD27, and HVEM. In some embodiments, the checkpoint inhibitor is an antibody that binds a target selected from CTLA-4, PD-1, and/or PD-L1. In some embodiments, the checkpoint inhibitor is an anti-CTLA4 antibody, an anti-PD-1 antibody, and an anti-PD-L1 antibody, and/or combinations thereof. In some embodiments, the checkpoint inhibitor is an anti-CTLA4 antibody such as, e.g., Yervoy™. In some embodiments, the checkpoint inhibitor is an anti-PD-1 antibody such as, e.g., Opdivo™ and/or Keytruda™.

[0109] In some embodiments, the inhibitor is a CTLA-4 inhibitor. In some embodiments, the inhibitor is a LAG-3 inhibitor. In some embodiments, the inhibitor is a PD-1 inhibitor. In some embodiments, the inhibitor is a PDL1 inhibitor. In some embodiments, the inhibitor is a TIGIT inhibitor. In some embodiments, the inhibitor is a TIM-3 inhibitor. In some embodiments, the inhibitor is a B7H3 inhibitor. In some embodiments, the inhibitor is a B7H4 inhibitor. In some embodiments, the inhibitor is a Vista inhibitor. In some embodiments, the inhibitor is a B-RAFi inhibitor. In some embodiments, the inhibitor is a MEKi inhibitor. In some embodiments, the inhibitor is a Btk inhibitor. In some embodiments, the inhibitor is ibrutinib. In some embodiments, the inhibitor is crizotinib. In some embodiments, the inhibitor is an IDO inhibitor. In some embodiments, the inhibitor is an α -CSF1R inhibitor. In some embodiments, the inhibitor is an α -CCR4 inhibitor. In some embodiments, the inhibitor is a TGF-beta. In some embodiments, the inhibitor is a myeloid-derived suppressor cell. In some embodiments, the inhibitor is a T-regulatory cell.

[0110] In some embodiments, the agonist is OX40. In some embodiments, the agonist is GITR. In some embodiments, the agonist is CD137. In some embodiments, the agonist is CD28. In some embodiments, the agonist is ICOS.

In some embodiments, the agonist is CD27. In some embodiments, the agonist is HVEM.

[0111] In some embodiments, the multispecific polypeptide construct is administered during and/or after treatment in combination with one or more additional agents such as, for example, a chemotherapeutic agent, an anti-inflammatory agent, and/or an immunosuppressive agent. In some embodiments, the multispecific polypeptide construct and the additional agent are formulated into a single therapeutic composition, and the multispecific polypeptide construct and additional agent are administered simultaneously. Alternatively, the multispecific polypeptide construct and additional agent are separate from each other, e.g., each is formulated into a separate therapeutic composition, and the multispecific polypeptide construct and the additional agent are administered simultaneously, or the multispecific polypeptide construct and the additional agent are administered at different times during a treatment regimen. For example, the multispecific polypeptide construct is administered prior to the administration of the additional agent, the multispecific polypeptide construct is administered subsequent to the administration of the additional agent, or the multispecific polypeptide construct and the additional agent are administered in an alternating fashion. As described herein, the multispecific polypeptide construct and additional agent are administered in single doses or in multiple doses.

[0112] In some embodiments, the multispecific polypeptide construct and the additional agent(s) are administered simultaneously. For example, the multispecific polypeptide construct and the additional agent(s) can be formulated in a single composition or administered as two or more separate compositions. In some embodiments, the multispecific polypeptide construct and the additional agent(s) are administered sequentially, or the multispecific polypeptide construct and the additional agent are administered at different times during a treatment regimen.

[0113] In addition to the elements described above, the multispecific polypeptide construct can contain additional elements such as, for example, amino acid sequence N- or C-terminal of the multispecific polypeptide construct. For example, a multispecific polypeptide construct can include a targeting moiety to facilitate delivery to a cell or tissue of interest. Multispecific polypeptide construct can be conjugated to an agent, such as a therapeutic agent, a detectable moiety or a diagnostic agent. Examples of agents are disclosed herein.

[0114] The multispecific polypeptide construct can also include any of the conjugated agents, linkers and other components described herein in conjunction with a multispecific polypeptide construct of the disclosure.

[0115] The disclosure also pertains to immunoconjugates comprising a multispecific polypeptide construct conjugated to a cytotoxic agent such as 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). Suitable cytotoxic agents for use in targeting diseased T cells such as in a T cell-derived lymphoma include, for example, dolastatins and derivatives thereof (e.g. auristatin E, AFP, MMAD, MMAF, MMAE). In some embodiments, the agent is a dolastatin. In some embodiments, the agent is an auristatin or derivative thereof. In some embodiments, the agent is a maytansinoid or maytansinoid derivative. In some embodiments, the agent is DM1 or DM4. In some embodiments, the agent is a duo-

carmycin or derivative thereof. In some embodiments, the agent is a calicheamicin or derivative thereof. In some embodiments, the agent is a pyrrolobenzodiazepine.

[0116] In some embodiments, the linker between the multispecific polypeptide construct and the cytotoxic agent is cleavable. In some embodiments, the linker is non-cleavable. In some embodiments, two or more linkers are present. The two or more linkers are all the same, e.g., cleavable or non-cleavable, or the two or more linkers are different, e.g., at least one cleavable and at least one non-cleavable.

[0117] The multispecific polypeptide constructs and conjugates thereof are useful in methods for treating a variety of disorders and/or diseases. Non-limiting examples of disease include: all types of cancers (breast, lung, colorectal, prostate, melanomas, head and neck, pancreatic, etc.), rheumatoid arthritis, Crohn's disease, 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 non-limiting example, renal cell carcinoma; and/or skin cancer, such as by way of non-limiting 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.

[0118] Also provided is a pharmaceutical composition comprising any of the multispecific polypeptide constructs provided herein and a pharmaceutically acceptable carrier. In some cases, the pharmaceutical composition is sterile. Pharmaceutical compositions according to the disclosure can include a multispecific polypeptide construct of the disclosure and a carrier. These pharmaceutical compositions can be included in kits, such as, for example, diagnostic kits.

[0119] One skilled in the art will appreciate that the antibodies of the disclosure have a variety of uses. For example, the proteins of the disclosure are used as therapeutic agents for a variety of disorders. The antibodies of the disclosure are also used as reagents in diagnostic kits or as diagnostic tools, or these antibodies can be used in competition assays to generate therapeutic reagents.

BRIEF DESCRIPTION OF DRAWINGS

[0120] FIG. 1 is a schematic of the basic components of the multispecific polypeptide constructs of the present disclosure having constrained CD3 binding. The antigen binding domain(s) are positioned at the amino and/or carboxy termini. The Fc region, such as a heterodimeric Fc region, is positioned N-terminal to the CD3 binding region. This positioning of the Fc in close proximity to the CD3 binding

region obstructs CD3 binding. The linker can be a non-cleavable linker as provided herein.

[0121] FIGS. 2A and B is a schematic of various FR α -targeting constrained CD3 constructs composed of two polypeptides, Chain 1 and Chain 2. As shown in FIG. 2A, Chain 1 contains a FR α sdAb (antigen binding domain), linked to a heterodimeric Fc "hole," linked via a non-cleavable linker (ranging from 3 amino acids in cx1356 to 18 amino acids in cx681) to anti-CD3 VL domain, linked to a second FR α sdAb; and Chain 2 contains a FR α sdAb, linked to a complementary heterodimeric Fc "knob," linked via the same non-cleavable linker as above to anti-CD3 VH domain, linked to second FR α sdAb. When co-expressed the CD3 binding domain is properly assembled via the association of the VL:VH on the hole and knob, respectively (FIG. 2B). An exemplary anti-CD3 is a disulfide-stabilized Fv (dsFv) containing a variable light (VL) chain with the mutation G100C and a variable heavy (VH) chain with a mutation G44C.

[0122] FIG. 3A is a schematic of various B7H3-targeting constrained CD3 constructs composed of two polypeptides, Chain 1 and Chain 2. Chain 1 contains either a heterodimeric Fc "hole," linked via a non-cleavable linker to an anti-CD3 VL domain modified at G100C (top); a B7H3-targeting sdAb linked to a heterodimeric Fc "hole," linked via a non-cleavable linker to an anti-CD3 VL domain (middle); or a B7H3-targeting sdAb linked to a heterodimeric Fc "hole," linked via a non-cleavable linker to an anti-CD3 VL domain modified at G100C (bottom). Chain 2 contains either a B7H3-targeted sdAb, linked to a complementary heterodimeric Fc "knob," linked via the linker as above to an anti-CD3 VH domain modified at G44C linked to second B7H3 sdAb (top); a B7H3-targeted sdAb, linked to a complementary heterodimeric Fc "knob," linked via the linker as above to an anti-CD3 VH domain (middle); or a B7H3-targeted sdAb, linked to a complementary heterodimeric Fc "knob," linked via the linker as above to an anti-CD3 VH domain modified by G44C (bottom). When co-expressed the CD3 binding domain is properly assembled via the association of the VL:VH on the hole and knob, respectively. Where denoted the VH:VL interaction is stabilized by an engineered disulfide bond between the modified residues G44C in the VH domain and G100C in the VL domain.

[0123] FIG. 3B is a schematic of various B7H3-targeting constrained CD3 constructs composed of two polypeptides, Chain 1 and Chain 2. Chain 1 contains a heterodimeric Fc "hole," linked via a non-cleavable linker to an anti-CD3 VL domain modified at G100C linked to a co-stimulatory receptor targeting sdAb. Chain 2 contains either a B7H3-targeted sdAb, linked to a complementary heterodimeric Fc "knob," linked via the linker as above to an anti-CD3 VH domain modified at G44C linked to second B7H3-targeted sdAb (top); a heterodimeric Fc "knob," linked via the linker as above to an anti-CD3 VH domain modified at G44C linked to a B7H3-targeted sdAb (middle); or a B7H3-targeted sdAb, linked to a complementary heterodimeric Fc "knob," linked via the linker as above to an anti-CD3 VH domain modified by G44C (bottom). When co-expressed the CD3 binding domain is properly assembled via the association of the VL:VH on the hole and knob, respectively. VH:VL interaction is stabilized by an engineered disulfide bond between the modified residues G44C in the VH domain and G100C in the VL domain. The resulting constructs are engage B7H3 either in bivalent (top) or monovalent (middle)

and bottom) manner. All the constructs herein express contain a co-stimulatory receptor targeting sdAb.

[0124] FIG. 3C is a schematic of various B7H3-targeting constrained CD3 constructs composed of three polypeptides, Chain 1, Chain 2 and Chain 3, wherein the B7H3 targeting domain is a FAB. Chain 1 contains a B7H3-targeting VH, an IgG Constant Heavy 1 (CH1) linked via a hinge to a first member of a heterodimeric Fc (Fc-Het-1), linked via the linker as above to an anti-CD3 VL domain that either lacks (top) or contains the modification of G100C (bottom). Chain 2 contains a B7H3-targeting VH, an IgG Constant Heavy 1 (CH1) linked via a hinge to a second member of a heterodimeric Fc (Fc-Het-2), linked via the linker as above to an anti-CD3 VH domain that either lacks (top) or contains the modification of G44C (bottom). Chain 3 contains a complementary B7H3-targeting VL domain linked to human Ig Constant Light (CL) region. When co-expressed the CD3 binding domain is properly assembled via the association of the VL:VH on the complementary heterodimeric Fc regions. Where denoted the VH:VL interaction is stabilized by an engineered disulfide bond between the modified residues G44C in the VH domain and G100C in the VL domain.

[0125] FIG. 4A is a schematic of various DLL3-targeting constrained CD3 constructs composed of two polypeptides, Chain 1 and Chain 2. Chain 1 contains a heterodimeric Fc “hole”, linked via a non-cleavable linker to an anti-CD3 VL domain modified at G100C linked to a co-stimulatory receptor targeting sdAb. Chain 2 contains either a DLL3-targeted sdAb, linked to a complementary heterodimeric Fc “knob”, linked via the linker as above to an anti-CD3 VH domain modified at G44C linked to second DLL3-targeted sdAb (top); a heterodimeric Fc “knob”, linked via the linker as above to an anti-CD3 VH domain modified at G44C linked to a DLL3-targeted sdAb (middle); or a DLL3-targeted sdAb, linked to a complementary heterodimeric Fc “knob”, linked via the linker as above to an anti-CD3 VH domain modified by G44C (bottom). When co-expressed the CD3 binding domain is properly assembled via the association of the VL:VH on the hole and knob, respectively. VH:VL interaction is stabilized by an engineered disulfide bond between the modified residues G44C in the VH domain and G100C in the VL domain. The resulting constructs are engage DLL3 either in bivalent (top) or monovalent (middle and bottom) manner. All the constructs herein express contain a co-stimulatory receptor targeting sdAb.

[0126] FIG. 4B is a schematic of a DLL3-targeting constrained CD3 construct, cx5499 composed of two polypeptides, Chain 1 and Chain 2. cx5499 is identical to cx5352 shown in FIG. 4A (top), but lacking co-stimulatory receptor targeting sdAb on the C-terminus of Chain 1. When co-expressed the CD3 binding domain is properly assembled via the association of the VL:VH on the hole and knob, respectively. VH:VL interaction is stabilized by an engineered disulfide bond between the modified residues G44C in the VH domain and G100C in the VL domain. The resulting constructs are engage DLL3 either in bivalent (top) or monovalent (middle and bottom) manner.

[0127] FIG. 5A-D depict cellular binding by representative FR α -targeting constrained CD3 engaging constructs, cx1356 and cx681. FIG. 5A and FIG. 5C show binding to Ovar5 cells (a FR α positive ovarian cancer cell line). FIG. 5B and FIG. 5D depict the lack of binding to T-cells. FIG. 5A and FIG. 5B display histograms of the normalized cell counts vs fluorescence at 100 nM of each construct. 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 cx1356 and cx681 are shown in the gray shaded traces in FIG. 5A and FIG. 5B. The full titration of each construct on the various cell types are shown in FIG. 5C and FIG. 5D.

[0128] FIG. 6A-F depict cellular binding by representative B7H3-targeting constrained CD3 engaging constructs. FIGS. 6A, C, and E show binding to A375 cells (a B7H3 positive human melanoma cell line). FIGS. 6B, D, and F show the lack of binding to isolated T cells.

[0129] FIG. 7A-F depict the impact of linker length on the capacity to agonize CD3 in the presence of FR α positive IGROV1 cells (FIG. 7A, 7C, 7E), or FR α negative NCI-H460 cells (FIG. 7B, 7D, 7F). FIG. 7A-B show the kinetics of CD3 signaling by 2 nM of various constructs on antigen positive and negative cells, respectively. FIG. 7C-D show the magnitude of CD3 agonizing capacity by 2 nM of various constructs on antigen positive and negative cells, respectively. FIG. 7E-F show the potency of CD3 agonizing capacity of various constructs with differing linker lengths on antigen positive and negative cells, respectively. A Jurkat CD3 NFAT-GFP reporter cell line was used to assess CD3 signaling. Constrained CD3 binding proteins only effectively engage and cluster CD3 on T cells when bound to a second antigen on target cells.

[0130] FIG. 8A-D depict the ability of representative B7H3-targeting constrained CD3 engaging constructs to agonize CD3 in a target dependent manner. FIG. 8A and FIG. 8C depict the capacity to mediate CD3 signaling in the presence of B7H3 positive A375 cells, while FIG. 8B and FIG. 8D show the inability to mediate CD3 signaling in the presence of B7H3 negative CCRF-CEM cells. A Jurkat CD3 NFAT-GFP reporter cell line was used to assess CD3 agonism.

[0131] FIG. 9A depicts the ability of a representative B7H3-targeting constrained CD3 engaging construct (cx3072) to induce T-cell mediated cytotoxicity in a target dependent manner. Target cells were labeled with cytoID red label and dying cells were visualized by addition of Caspase 3/7 green reagent. Cytotoxicity was assessed by determining the overlap area of red target cells and green dying cells. A B7H3 negative A375 cell line, generated by CRISPR technology was used to test antigen specific T-cell mediated cytotoxicity. cx3072 was unable to elicit T-cell mediated cytotoxicity of these B7H3 deficient cells.

[0132] FIG. 9B shows the ability of cx5952 to induce T-cell mediated cytotoxicity in the presence of B7H3 positive A375 cells, but not in the presence of B7H3 negative CCRF-CEM cells.

[0133] FIGS. 10A and 10B depict the ability of representative B7H3-targeting constrained CD3 engaging constructs to induce T-cell mediated cytotoxicity in a target dependent manner. FIG. 10A depicts the capacity of these constructs to induce T-cell mediated cytotoxicity in the presence of B7H3 positive A375 cells, while FIG. 10B depicts the capacity of these constructs to induce T-cell mediated cytotoxicity in the presence of B7H3 negative CCRF-CEM cells. Cytotoxicity was assessed by determining the overlap area of red target cells and green dying cells.

[0134] FIGS. 10C and 10D depict the ability of representative B7H3-targeting constrained CD3 engaging constructs to induce T-cell mediated cytotoxicity in a target dependent manner. FIG. 10C depicts the capacity of these constructs to

induce T-cell mediated cytotoxicity in the presence of B7H3 positive A375 cells, while FIG. 10C depicts the capacity of these constructs to induce T-cell mediated cytotoxicity in the presence of B7H3 negative CCRF-CEM cells. Cytotoxicity was assessed by determining the overlap area of red target cells and green dying cells.

[0135] FIGS. 11A-C depict the ability of a representative B7H3-targeting constrained CD3 engaging construct (cx5952) to induce T-cell mediated T-cell activation in a target dependent manner. T-cell activation of CD4+ or CD8+ T cells was assessed by expression of the T cell activation markers CD25 (FIG. 11A), CD69 (FIG. 11B), and CD71 (FIG. 11C).

[0136] FIGS. 11D-11K depict the ability of representative B7H3-targeting constrained CD3 engaging constructs to induce T-cell activation in a target dependent manner. B7H3-target-dependent CD4+ T-cell activation is shown by expression of the T cell activation markers CD25 (FIG. 11D) and CD71 (FIG. 11F). B7H3-target-dependent CD8+ T-cell activation is shown by expression of the T cell activation markers CD25 (FIG. 11H) and CD71 (FIG. 11J). T-cell activation was not observed in the absence of B7H3 positive cells, based on T cell activation marker CD25 as shown in CD4+ T cells (FIG. 11E) or CD8+ T cells (FIG. 11I) or based on T cell activation marker CD71 as shown in CD4+ T cells (FIG. 11G) or CD8+ T cells (FIG. 11K).

[0137] FIG. 12A depicts the ability of representative B7H3-targeting constrained CD3 engaging constructs to induce IFN γ production in a target dependent manner. FIG. 12A shows the production of IFN γ from T-cells cultured with B7H3 positive A375 cells and in the presence of B7H3 negative CCRF-CEM cells in the presence of the representative B7H3-targeting CD3 engaging constructs.

[0138] FIG. 12B depicts the ability of representative B7H3-targeting constrained CD3 engaging constructs to induce IFN γ production in a target dependent manner. FIG. 12B shows the production of IFN γ from T-cells cultured with B7H3 positive A375 cells and in the presence of B7H3 negative CCRF-CEM cells in the presence of the representative B7H3-targeting CD3 engaging constructs.

[0139] FIGS. 13A and 13B depict cellular binding of representative B7H3-targeting constrained CD3 engaging constructs. cx5187 and cx5823 each contain two B7H3 binding domains while complex cx5873 and cx5965 each contain one B7H3 binding domain. FIG. 13A shows binding to B7H3 positive A375 cells. FIG. 13B shows the lack of binding to B7H3 negative CCRF-CEM cells and isolated T-cells.

[0140] FIG. 13C and FIG. 13D depict the ability of representative B7H3-targeting constrained CD3 engaging constructs to agonize CD3 in a target dependent manner. FIG. 13C shows that engaging B7H3 positive A375 cells with a construct that is bivalent and bi-epitopic to B7H3 (cx5187) induced more potent CD3 signaling than constructs that are monovalent to B7H3 (cx5873 and cx5965). FIG. 13D shows the lack of activation of T-cells in the presence of B7H3 negative CCRF-CEM cells. A Jurkat CD3 NFAT-GFP reporter cell line was used to assess CD3 agonism.

[0141] FIG. 14A and FIG. 14B depict the ability of representative B7H3-targeting constrained CD3 engaging constructs to induce T-cell mediated cytotoxicity in a target dependent manner. FIG. 14A shows that targeting B7H3 positive A375 cells with a construct that is bivalent and

bi-epitopic to B7H3 (cx5187) induced more potent T-cell mediated cytotoxicity than constructs that are monovalent to B7H3 (cx5873 and cx5965). FIG. 14B depicts the lack of T-cell mediated cytotoxicity against B7H3 negative CCRF-CEM cells.

[0142] FIG. 15A-D depict the ability of representative B7H3-targeting constrained CD3 engaging molecules to activate T-cells in the presence of B7H3 positive A375 cells, but not in the presence of B7H3 negative CCRF-CEM cells. FIGS. 15A and 15B show that targeting B7H3 positive A375 cells with a construct that is bivalent and bi-epitopic to B7H3 (cx5187), induced more potent CD25 expression on CD4+ and CD8+ T-cells than constructs that are monovalent to B7H3 (cx5873 and cx5965). FIGS. 15C and 15D show the lack of CD25 expression on CD4+ and CD8+ T-cells in the presence of B7H3 negative CCRF-CEM cells.

[0143] FIGS. 16A and 16B demonstrate the ability of representative B7H3-targeting constrained CD3 engaging constructs to elicit T-cell mediated cytotoxicity in the presence of B7H3-positive A375 cells (FIG. 16A) but not in the presence of CCRF-CEM B7H3-negative cells (FIG. 16B).

[0144] FIGS. 16C-16J demonstrate the ability of representative B7H3-targeting constrained CD3 engaging constructs to elicit T cell activation in the presence of B7H3-positive A375 cells but not in the presence of CCRF-CEM B7H3-negative cells, as assessed by: expression of CD25 on CD4+ T cells (FIGS. 16C and 16D, respectively), CD25 expression on CD8+ T cells (FIGS. 16E and 16F, respectively), CD71 expression on CD4+ T cells (FIGS. 16G and 16H, respectively), CD71 expression on CD8+ T cells (FIGS. 16I and 16J, respectively).

[0145] FIGS. 16K and 16L demonstrate the ability of representative B7H3-targeting constrained CD3 engaging constructs to elicit T cell cytokine production in the presence of B7H3-positive A375 cells (FIG. 16K) but not in the presence of CCRF-CEM B7H3-negative cells (FIG. 16L).

[0146] FIGS. 17A and 17B demonstrate that representative monovalent (cx5800 and cx5801) and bivalent (cx5352) DLL3-targeting constrained CD3 engaging constructs bound to a DLL3 expressing cell line, SHP-77 (FIG. 17A), but not to isolated T-cells (FIG. 17B). Binding was assessed by flow cytometry.

[0147] FIG. 17C depicts the ability of representative DLL3-targeting constrained CD3 engaging constructs to agonize CD3 signaling in the presence of DLL3 positive SHP-77 cells. Engaging DLL3 positive cells with a construct that is bivalent and bi-epitopic to DLL3 (cx5352) induced more potent T-cell activation than constructs that are monovalent to DLL3 (cx5800 and cx5801). A Jurkat CD3 NFAT-Luciferase reporter cell line was used to assess CD3 signaling.

[0148] FIG. 18A-18E demonstrate the ability of a representative DLL3-targeting constrained CD3 engaging construct, cx5499 to elicit T-cell mediated cytotoxicity and T-cell activation in the presence of DLL3-positive SHP-77 cells. FIG. 18A demonstrates the ability of the representative DLL3-targeting constrained CD3 engaging constructs to elicit T-cell mediated cytotoxicity in the presence of DLL3-positive SHP-77 cells. FIGS. 18C-18D demonstrate the ability of representative DLL3-targeting constrained CD3 engaging constructs to elicit T cell activation in the presence of DLL3-positive SHP-77 cells, as assessed by: expression of CD25 on CD4+ T cells (FIG. 18B), CD69 expression on

CD4+ T cells (FIG. 18C), CD25 expression on CD8+ T cells (FIG. 18D) and CD69 expression on CD8+ T cells (FIG. 18E).

DETAILED DESCRIPTION

[0149] The present disclosure provides constrained T-cell engaging fusion proteins in the form of multispecific polypeptide constructs that bind at least CD3 and a second antigen. The multispecific polypeptide constructs provided herein include at least a first component that includes one or more copies of an antigen-binding domain that binds an antigen operably linked to 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 region, 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 some embodiments, the antigen is a tumor associated antigen (TAA). In some embodiments, the linker is a non-cleavable linker. In some embodiments, the linker does not contain a substrate recognition site that is specifically recognized by a protease, such as a protease that is granzyme B, an MMP or matriptase.

[0150] The provided 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 antigen binding domain(s) is positioned on the amino-terminal (N-term) region of the multispecific polypeptide construct. In some embodiments, the antigen binding domain(s) is positioned on the carboxy-terminal (C-term) region of the multispecific polypeptide construct. In some embodiments, the antigen binding domain(s) is positioned on both the N- and C-terminal regions of the multispecific polypeptide construct. Various configurations of a multispecific polypeptide construct as provided herein are shown in FIG. 1.

[0151] The provided multispecific polypeptide constructs exhibit constrained T-cell engaging activity because such constructs only substantially bind to CD3 once an antigen is bound via the antigen-binding domain. This is exemplified in the Examples and Figures provided herein, which demonstrate the ability of constrained CD3-engaging proteins to efficiently bind TAA positive cells, while having little to no binding of T cells. This unique property allows constrained CD3-engaging proteins to distribute to sites where TAA is present without binding to peripheral T cells. This format is distinct from other CD3 engaging multispecific constructs, in that constitutive CD3 binding is disallowed or eliminated, providing a significant benefit by avoiding peripheral T-cell binding and permitting superior distribution to the site(s) where antigen is present as recognized by the antigen binding domain. Furthermore, other CD3 engaging constructs mediate antigen-dependent T-cell activation. However, the multispecific polypeptide constructs provided herein mediate both antigen dependent T-cell binding and activation.

[0152] The constrained T-cell engaging activity of the provided multispecific polypeptide constructs is due, in some aspects, to the positioning of the Fc region N-terminal to the CD3-binding region. In some embodiments, such positioning reduces, attenuates, dampens and/or prevents

CD3 binding by the CD3 binding region. In the absence of antigen binding by the antigen binding domain, the multispecific polypeptide constructs provided herein demonstrate reduced or eliminated CD3 binding and T-cell activating capacity. In some embodiments, in the presence of an antigen binding event mediated by the antigen binding domain(s) of the multispecific polypeptide constructs, the capacity to bind CD3 by the CD3 binding region is greatly enhanced. In some embodiments, in the presence of an antigen binding event mediated by the antigen binding domains(s) of the multispecific polypeptide constructs, the capacity to activate T-cells is greatly enhanced. Engagement of its cognate antigen by the antigen binding domain(s) within the multispecific polypeptide construct leads to subsequent T-cell engagement and mediates antigen-dependent T-cell activation, such as cytotoxicity, cytokine release, degranulation and proliferation. In some embodiments, the provided multispecific polypeptide constructs can be used to increase an immune response, such as to enhance T-cell activity, including cytolytic (or cytotoxic) T-cell activity. The modulation of the immune response can, in some aspects, treat a disease or condition in a subject.

[0153] In some embodiments, the one or more antigen binding domains bind an antigen on a tumor cell or a cell of the tumor microenvironment. In some aspects, the provided multispecific polypeptide constructs can be used to increase immune responses, such as T-cell activity, e.g. cytotoxicity activity, against a tumor or cancer. In some embodiments, the provided multispecific polypeptide constructs can be used to treat a tumor or cancer in the subject.

[0154] In some embodiments, the multispecific polypeptide constructs of the disclosure ensure that there will be no binding of T-cells via CD3 in peripheral blood, as the CD3 binding region of these constructs is constrained or otherwise blocked and/or inhibited by the presence of the Fc region. Thus, the multispecific polypeptide constructs of the disclosure provide a number of advantages. In some aspects, these constructs limit the sink effect caused by binding all T-cells. In some aspects, these constructs reduce systemic toxicity.

[0155] In some embodiments, the provided multispecific polypeptide constructs of the disclosure allow for controlled biodistribution to a desired site in a subject, such as, for example, a site of tumor-associated antigen (TAA) expression. Sites of TAA expression include, for example, tumor and the surrounding tumor microenvironment.

[0156] In some embodiments, the multispecific polypeptide constructs of the disclosure exhibit specificity for CD3 and one or more other antigen. In some embodiments, the multispecific polypeptide constructs can contain more than one antigen binding domain able to bind one or more TAA, such as 2, 3 or 4 antigen binding domains, see e.g. FIG. 1. In some embodiments, the one or more antigen binding domains bind the same antigen. In some embodiments, the multispecific polypeptide constructs include more than one antigen binding domains that bind distinct epitopes on the same antigen. In some embodiments, the multispecific polypeptide constructs include more than one antigen binding domains that bind one or more distinct antigens. In some embodiments, the multispecific polypeptide constructs include more than one antigen binding domains that bind distinct epitopes on the same antigens as well as include additional antigen binding domains that bind to one or more distinct antigens. In some aspects, the provided multispecific

polypeptide constructs are bispecific polypeptide constructs, such that they are able to bind to CD3 and to another antigen, such as a TAA, via binding of the antigen-binding domain of the multispecific polypeptide construct. In some examples, the provided multispecific polypeptide constructs are bispecific polypeptide constructs that provide multivalent engagement of one or more TAA through the use of a first antigen-binding domain and a second antigen-binding domain. For example, in some embodiments, the bispecific polypeptide constructions include a first antigen-binding single domain antibody (sdAb) and a second antigen-binding sdAb.

[0157] In some embodiments, the 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), such that the CD3 binding is constrained and T-cell interaction is obviated, 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.

[0158] 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, such as any as described.

[0159] In some embodiments, the Fc region is a homodimeric Fc region. In some embodiments, the Fc region is a heterodimeric Fc region. In some embodiments, the Fc region is a monomeric Fc region. In some embodiments, the Fc region of the multispecific polypeptide constructs are capable of interacting with FcγRs and mediating innate immune effector functions, for example, antibody dependent cellular toxicity (ADCC) and antibody dependent cellular phagocytosis (ADCP). In some embodiments, the Fc region of the multispecific polypeptide constructs are capable of interacting with complement proteins, namely C1q, and mediating complement dependent cytotoxicity. Thus, in some aspects, the multispecific polypeptide constructs of the disclosure allow for multiple immune effector mechanisms, including innate immune effectors and T-cells.

[0160] In some embodiments, the multispecific polypeptide constructs of the disclosure allow for T-cell and NK cell mediated cytotoxicity to occur simultaneously. In some cases, such activity can occur in a multispecific polypeptide construct in which is contained a first antigen binding domain, e.g., a first anti-TAA antigen binding domain, and a second antigen binding domain, e.g., a second anti-TAA antigen binding domain, that can target distinct and/or non-competing epitopes on a given TAA.

[0161] It is contemplated that the constrained CD3 engaging constructs are amenable for use with any TAA-binding domain, allowing better therapeutic exposure within the tumor or tumor-microenvironment by avoiding interactions with peripheral T-cells and mediating potent TAA-dependent T-cell cytotoxicity. In some embodiments, the second portion or component contains a CD3 binding region that is monovalent to CD3, such that there will be no activation of T-cell unless there is TAA present.

[0162] In some aspects, the multispecific polypeptide constructs of the disclosure provide a number of advantages over current bispecific therapeutics. The multispecific polypeptide constructs of the disclosure are smaller than a

conventional therapeutic antibody, e.g., 150 kDa vs. 125 kDa, which will allow for better target, e.g. tumor, penetration. In some aspects, the size of the entire multispecific polypeptide construct provides long half-life for the construct. In some aspects, the multispecific polypeptide constructs of the disclosure exhibit reduced systemic toxicity or toxicity of any area outside the tumor and/or tumor microenvironment, since CD3 binding by the CD3 binding region depends on TAA engagement before CD3 engagement will occur.

[0163] All publications and patent documents cited herein are incorporated herein by reference as if each such publication or document was specifically and individually indicated to be incorporated herein by reference. Citation of publications and patent documents is not intended as an admission that any is pertinent prior art, nor does it constitute any admission as to the contents or date of the same. The invention having now been described by way of written description, those of skill in the art will recognize that the invention can be practiced in a variety of embodiments and that the foregoing description and examples below are for purposes of illustration and not limitation of the claims that follow.

I. DEFINITIONS

[0164] 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. The term “a” entity or “an” entity refers to one or more of that entity. For example, a compound refers to one or more compounds. As such, the terms “a”, “an”, “one or more” and “at least one” can be used interchangeably. Further, unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular. Generally, nomenclatures utilized in connection with, and techniques of, cell and tissue culture, molecular biology, and protein and oligo- or polynucleotide chemistry and hybridization described herein are those well-known and commonly used in the art. Standard techniques are used for recombinant DNA, oligonucleotide synthesis, and tissue culture and transformation (e.g., electroporation, lipofection). Enzymatic reactions and purification techniques are performed according to manufacturer’s specifications or as commonly accomplished in the art or as described herein. The foregoing techniques and procedures are generally performed according to conventional methods well known in the art and as described in various general and more specific references that are cited and discussed throughout the present specification. See e.g., Sambrook et al. *Molecular Cloning: A Laboratory Manual* (2d ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989)). The nomenclatures utilized in connection with, and the laboratory procedures and techniques of, analytical chemistry, synthetic organic chemistry, and medicinal and pharmaceutical chemistry described herein are those well-known and commonly used in the art. Standard techniques are used for chemical syntheses, chemical analyses, pharmaceutical preparation, formulation, and delivery, and treatment of patients.

[0165] As utilized in accordance with the present disclosure, the following terms, unless otherwise indicated, shall be understood to have the following meanings:

[0166] As used herein, the term “antibody” refers to immunoglobulin molecules and antigen-binding portions of

immunoglobulin (Ig) molecules, i.e., molecules that contain an antigen binding site that specifically binds (immunoreacts with) an antigen. By “specifically bind” or “immunoreacts with” or “immunospecifically bind” is meant that the antibody reacts with one or more antigenic determinants of the desired antigen and does not react with other polypeptides or binds at much lower affinity ($K_d > 10^{-6}$). Antibodies include, but are not limited to, polyclonal, monoclonal, chimeric, fully human, domain antibody, single chain, Fab, and $F(ab')_2$ fragments, Fvs, scFvs, and a Fab expression library. Typically, an “antigen-binding fragment” contains at least one CDR of an immunoglobulin heavy and/or light chain that binds to at least one epitope of the antigen of interest. In this regard, an antigen-binding fragment may comprise 1, 2, 3, 4, 5, or all 6 CDRs of a variable heavy chain (VH) and variable light chain (VL) sequence from antibodies that bind the antigen, such as generally six CDRs for an antibody containing a VH and a VL (“CDR1,” “CDR2” and “CDR3” for each of a heavy and light chain), or three CDRs for an antibody containing a single variable domain. Antigen binding fragments include single domain antibodies, such as those only containing a VH or only containing a VL, including, for example, V_{H^H} , V_{NAR} , engineered V_H or V_K domains.

[0167] The basic antibody structural unit is known to comprise a tetramer. Each tetramer is composed of two identical pairs of polypeptide chains, each pair having one “light” (about 25 kDa) and one “heavy” chain (about 50-70 kDa). The amino-terminal portion of each chain includes a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. The carboxy-terminal portion of each chain defines a constant region primarily responsible for effector function. In general, antibody molecules obtained from humans relate to any of the classes IgG, IgM, IgA, IgE and IgD, which differ from one another by the nature of the heavy chain present in the molecule. Certain classes have subclasses as well, such as IgG₁, IgG₂, IgG₃, IgG₄, and others. Furthermore, in humans, the light chain may be a kappa chain or a lambda chain.

[0168] The term “monoclonal antibody” (mAb) or “monoclonal antibody composition”, as used herein, refers to a population of antibody molecules that contain only one molecular species of antibody molecule consisting of a unique light chain gene product and a unique heavy chain gene product. In particular, the complementarity determining regions (CDRs) of the monoclonal antibody are identical in all the molecules of the population. MAbs contain an antigen binding site capable of immunoreacting with a particular epitope of the antigen characterized by a unique binding affinity for it.

[0169] The term “antigen-binding site” or “binding portion” refers to the part of the immunoglobulin molecule that participates in antigen binding. The antigen binding site is formed by amino acid residues of the N-terminal variable (“V”) regions of the heavy (“H”) and light (“L”) chains. Three highly divergent stretches within the V regions of the heavy and light chains, referred to as “hypervariable regions,” are interspersed between more conserved flanking stretches known as “framework regions,” or “FRs”. Thus, the term “FR” refers to amino acid sequences that are naturally found between, and adjacent to, hypervariable regions in immunoglobulins. In an antibody molecule, the three hypervariable regions of a light chain and the three hypervariable regions of a heavy chain are disposed relative

to each other in three dimensional space to form an antigen-binding surface. The antigen-binding surface is complementary to the three-dimensional surface of a bound antigen, and the three hypervariable regions of each of the heavy and light chains are referred to as “complementarity-determining regions,” or “CDRs.” The assignment of amino acids to each domain is in accordance with the definitions of Kabat Sequences of Proteins of Immunological Interest (National Institutes of Health, Bethesda, Md. (1987 and 1991)), or Chothia & Lesk J. Mol. Biol. 196:901-917 (1987), Chothia et al. Nature 342:878-883 (1989).

[0170] As used herein, the term “epitope” includes any specific portion of an antigen targeted by an antibody, antibody fragment or other binding domain. The term “epitope” includes any protein region to which specific binding is directed. The term “epitope” includes any protein determinant capable of specific binding to an immunoglobulin or T-cell receptor. Epitopic determinants usually consist of chemically active surface groupings of molecules such as amino acids or sugar side chains and usually have specific three dimensional structural characteristics, as well as specific charge characteristics. For example, antibodies may be raised against N-terminal, central, or C-terminal peptides of a polypeptide. In addition, antibodies may be raised against linear or discontinuous epitopes of a polypeptide. An antibody is said to specifically bind an antigen when the dissociation constant is $\leq 1 \mu\text{M}$; for example, in some embodiments $\leq 100 \text{ nM}$ and in some embodiments, $\leq 10 \text{ nM}$ and does not display binding to other proteins either closely related or distinct.

[0171] As used herein, the terms “specific binding,” “immunological binding,” and “immunological binding properties” refer to the non-covalent interactions of the type that occur between an immunoglobulin molecule and an antigen for which the immunoglobulin is specific. The strength, or affinity of immunological binding interactions can be expressed in terms of the dissociation constant (K_d) of the interaction, wherein a smaller K_d represents a greater affinity. Immunological binding properties of selected polypeptides can be quantified using methods well known in the art. One such method entails measuring the rates of antigen-binding site/antigen complex formation and dissociation, wherein those rates depend on the concentrations of the complex partners, the affinity of the interaction, and geometric parameters that equally influence the rate in both directions. Thus, both the “on rate constant” (K_m) and the “off rate constant” (K_{off}) can be determined by calculation of the concentrations and the actual rates of association and dissociation. (See Nature 361:186-87 (1993)). The ratio of K_{off}/K_{on} enables the cancellation of all parameters not related to affinity and is equal to the dissociation constant K_d . (See, generally, Davies et al. (1990) Annual Rev Biochem 59:439-473). An antibody of the present disclosure is said to specifically bind to EGFR, when the binding constant (K_d) is $\leq 1 \mu\text{M}$, for example, in some embodiments $\leq 100 \text{ nM}$, in some embodiments 10 nM , and in some embodiments $\leq 100 \mu\text{M}$ to about $1 \mu\text{M}$, as measured by assays such as radioligand binding assays or similar assays known to those skilled in the art.

[0172] 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 the “isolated polynucleotide” (1) is not associated with all or a portion of a polynucleotide in which the

“isolated polynucleotide” is 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. Polynucleotides in accordance with the disclosure include the nucleic acid molecules encoding the heavy chain immunoglobulin molecules shown herein, and nucleic acid molecules encoding the light chain immunoglobulin molecules shown herein.

[0173] The term “isolated protein” referred to herein means a protein of cDNA, recombinant RNA, or synthetic origin or some combination thereof, which by virtue of its origin, or source of derivation, the “isolated protein” (1) is not associated with proteins found in nature, (2) is free of other proteins from the same source, e.g., free of murine proteins, (3) is expressed by a cell from a different species, or (4) does not occur in nature.

[0174] The term “polypeptide” is used herein as a generic term to refer to native protein, fragments, or analogs of a polypeptide sequence. Hence, native protein fragments, and analogs are species of the polypeptide genus. Polypeptides in accordance with the disclosure comprise the heavy chain immunoglobulin molecules shown herein, and the light chain immunoglobulin molecules shown herein, as well as antibody molecules formed by combinations comprising the heavy chain immunoglobulin molecules with light chain immunoglobulin molecules, such as kappa light chain immunoglobulin molecules, and vice versa, as well as fragments and analogs thereof.

[0175] The term “naturally-occurring” as used herein as applied to an object refers to the fact that an object can be found in nature. For example, a polypeptide or polynucleotide sequence that is present in an organism (including viruses) that can be isolated from a source in nature and that has not been intentionally modified by man in the laboratory or otherwise is naturally-occurring.

[0176] 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.

[0177] The term “control sequence” as used herein refers to polynucleotide sequences that are necessary to effect the expression and processing of coding sequences to which they are ligated. The nature of such control sequences differs depending upon the host organism in prokaryotes, such control sequences generally include promoter, ribosomal binding site, and transcription termination sequence in eukaryotes, generally, such control sequences include promoters and transcription termination sequence. The term “control sequences” is intended to include, at a minimum, all components whose presence is essential for expression and processing, and can also include additional components whose presence is advantageous, for example, leader sequences and fusion partner sequences. The term “polynucleotide” as referred to herein means nucleotides of at least 10 bases in length, either ribonucleotides or deoxynucleotides or a modified form of either type of nucleotide. The term includes single and double stranded forms of DNA.

[0178] The term “oligonucleotide” referred to herein includes naturally occurring, and modified nucleotides linked together by naturally occurring, and non-naturally

occurring oligonucleotide linkages. Oligonucleotides are a polynucleotide subset generally comprising a length of 200 bases or fewer. In some embodiments, oligonucleotides are 10 to 60 bases in length, for example, in some embodiments, 12, 13, 14, 15, 16, 17, 18, 19, or 20 to 40 bases in length. Oligonucleotides are usually single stranded, e.g., for probes, although oligonucleotides may be double stranded, e.g., for use in the construction of a gene mutant. Oligonucleotides of the disclosure are either sense or antisense oligonucleotides.

[0179] The term “naturally occurring nucleotides” referred to herein includes deoxyribonucleotides and ribonucleotides. The term “modified nucleotides” referred to herein includes nucleotides with modified or substituted sugar groups and the like. The term “oligonucleotide linkages” referred to herein includes oligonucleotide linkages such as phosphorothioate, phosphorodithioate, phosphoroserine, phosphorodiselenoate, phosphoroanilothioate, phosphoraniladate, phosphoromidate, and the like. See e.g., LaPlanche et al. *Nucl. Acids Res.* 14:9081 (1986); Stec et al. *J. Am. Chem. Soc.* 106:6077 (1984), Stein et al. *Nucl. Acids Res.* 16:3209 (1988), Zon et al. *Anti Cancer Drug Design* 6:539 (1991); Zon et al. *Oligonucleotides and Analogues: A Practical Approach*, pp. 87-108 (F. Eckstein, Ed., Oxford University Press, Oxford England (1991)); Stec et al. U.S. Pat. No. 5,151,510; Uhlmann and Peyman *Chemical Reviews* 90:543 (1990). An oligonucleotide can include a label for detection, if desired.

[0180] As used herein, the twenty conventional amino acids and their abbreviations follow conventional usage. See *Immunology—A Synthesis* (2nd Edition, E. S. Golub and D. R. Gren, Eds., Sinauer Associates, Sunderland, Mass. (1991)). Stereoisomers (e.g., D-amino acids) of the twenty conventional amino acids, unnatural amino acids such as α -, α -disubstituted amino acids, N-alkyl amino acids, lactic acid, and other unconventional amino acids may also be suitable components for polypeptides of the present disclosure. Examples of unconventional amino acids include: 4-hydroxyproline, γ -carboxyglutamate, ϵ -N,N,N-trimethyllysine, ϵ -N-acetyllysine, O-phosphoserine, N-acetylserine, N-formylmethionine, 3-methylhistidine, 5-hydroxylysine, σ -N-methylarginine, and other similar amino acids and imino acids (e.g., 4-hydroxyproline). In the polypeptide notation used herein, the left-hand direction is the amino terminal direction and the right-hand direction is the carboxy-terminal direction, in accordance with standard usage and convention.

[0181] Similarly, unless specified otherwise, the left-hand end of single-stranded polynucleotide sequences is the 5' end and the left-hand direction of double-stranded polynucleotide sequences is referred to as the 5' direction. The direction of 5' to 3' addition of nascent RNA transcripts is referred to as the transcription direction sequence regions on the DNA strand having the same sequence as the RNA and that are 5' to the 5' end of the RNA transcript are referred to as “upstream sequences”, sequence regions on the DNA strand having the same sequence as the RNA and that are 3' to the 3' end of the RNA transcript are referred to as “downstream sequences”.

[0182] As applied to polypeptides, the term “substantial identity” means that two peptide sequences, when optimally aligned, such as by the programs GAP or BESTFIT using default gap weights, share at least 80 percent sequence identity, for example, in some embodiments, at least 90

percent sequence identity, in some embodiments, at least 95 percent sequence identity, and in some embodiments, at least 99 percent sequence identity.

[0183] In some embodiments, residue positions that are not identical differ by conservative amino acid substitutions.

[0184] As discussed herein, minor variations in the amino acid sequences of antibodies or immunoglobulin molecules are contemplated as being encompassed by the present disclosure, providing that the variations in the amino acid sequence maintain at least 75%, for example, in some embodiments, at least 80%, 90%, 95%, and in some embodiments 99%. In particular, conservative amino acid replacements are contemplated. Conservative replacements are those that take place within a family of amino acids that are related in their side chains. Genetically encoded amino acids are generally divided into families: (1) acidic amino acids are aspartate, glutamate; (2) basic amino acids are lysine, arginine, histidine; (3) non-polar amino acids are alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan, and (4) uncharged polar amino acids are glycine, asparagine, glutamine, cysteine, serine, threonine, tyrosine. The hydrophilic amino acids include arginine, asparagine, aspartate, glutamine, glutamate, histidine, lysine, serine, and threonine. The hydrophobic amino acids include alanine, cysteine, isoleucine, leucine, methionine, phenylalanine, proline, tryptophan, tyrosine and valine. Other families of amino acids include (i) serine and threonine, which are the aliphatic-hydroxy family; (ii) asparagine and glutamine, which are the amide containing family; (iii) alanine, valine, leucine and isoleucine, which are the aliphatic family; and (iv) phenylalanine, tryptophan, and tyrosine, which are the aromatic family. For example, it is reasonable to expect that an isolated replacement of a leucine with an isoleucine or valine, an aspartate with a glutamate, a threonine with a serine, or a similar replacement of an amino acid with a structurally related amino acid will not have a major effect on the binding or properties of the resulting molecule, especially if the replacement does not involve an amino acid within a framework site. Whether an amino acid change results in a functional peptide can readily be determined by assaying the specific activity of the polypeptide derivative. Assays are described in detail herein. Fragments or analogs of antibodies or immunoglobulin molecules can be readily prepared by those of ordinary skill in the art. In some embodiments, amino- and carboxy-termini of fragments or analogs occur near boundaries of functional domains. Structural and functional domains can be identified by comparison of the nucleotide and/or amino acid sequence data to public or proprietary sequence databases. Computerized comparison methods are used to identify sequence motifs or predicted protein conformation domains that occur in other proteins of known structure and/or function. Methods to identify protein sequences that fold into a known three-dimensional structure are known. Bowie et al. *Science* 253:164 (1991). Thus, the foregoing examples demonstrate that those of skill in the art can recognize sequence motifs and structural conformations that may be used to define structural and functional domains in accordance with the disclosure.

[0185] In some embodiments, amino acid substitutions are those that: (1) reduce susceptibility to proteolysis, (2) reduce susceptibility to oxidation, (3) alter binding affinity for forming protein complexes, (4) alter binding affinities, and (4) confer or modify other physicochemical or functional

properties of such analogs. Analogs can include various mutants of a sequence other than the naturally-occurring peptide sequence. For example, single or multiple amino acid substitutions (for example, conservative amino acid substitutions) may be made in the naturally-occurring sequence (for example, in the portion of the polypeptide outside the domain(s) forming intermolecular contacts. A conservative amino acid substitution should not substantially change the structural characteristics of the parent sequence (e.g., a replacement amino acid should not tend to break a helix that occurs in the parent sequence, or disrupt other types of secondary structure that characterizes the parent sequence). Examples of art-recognized polypeptide secondary and tertiary structures are described in *Proteins, Structures and Molecular Principles* (Creighton, Ed., W. H. Freeman and Company, New York (1984)); *Introduction to Protein Structure* (C. Branden and J. Tooze, eds., Garland Publishing, New York, N.Y. (1991)); and Thornton et al. *Nature* 354:105 (1991).

[0186] The term “polypeptide fragment” as used herein refers to a polypeptide that has an amino terminal and/or carboxy-terminal deletion and/or one or more internal deletion(s), but where the remaining amino acid sequence is identical to the corresponding positions in the naturally-occurring sequence deduced, for example, from a full length cDNA sequence. Fragments typically are at least 5, 6, 8 or 10 amino acids long, for example, in some embodiments, at least 14 amino acids long, in some embodiments, at least 20 amino acids long, usually at least 50 amino acids long, and in some embodiments, at least 70 amino acids long. The term “analog” as used herein refers to polypeptides that are comprised of a segment of at least 25 amino acids that has substantial identity to a portion of a deduced amino acid sequence and that has specific binding to EGFR, under suitable binding conditions. Typically, polypeptide analogs comprise a conservative amino acid substitution (or addition or deletion) with respect to the naturally-occurring sequence. Analogs typically are at least 20 amino acids long, for example, in some embodiments, at least 50 amino acids long or longer, and can often be as long as a full-length naturally-occurring polypeptide.

[0187] The term “agent” is used herein to denote a chemical compound, a mixture of chemical compounds, a biological macromolecule, or an extract made from biological materials.

[0188] As used herein, the terms “label” or “labeled” refers to incorporation of a detectable marker, e.g., by incorporation of a radiolabeled amino acid or attachment to a polypeptide of biotinyl moieties that can be detected by marked avidin (e.g., streptavidin containing a fluorescent marker or enzymatic activity that can be detected by optical or calorimetric methods). In certain situations, the label or marker can also be therapeutic. Various methods of labeling polypeptides and glycoproteins are known in the art and may be used. Examples of labels for polypeptides include, but are not limited to, the following: radioisotopes or radionuclides (e.g., ^3H , ^{14}C , ^{15}N , ^{35}S , ^{90}Y , ^{99}Tc , ^{111}In , ^{125}I , ^{131}I), fluorescent labels (e.g., a fluorophore, rhodamine, lanthanide phosphors), enzymatic labels (e.g., horseradish peroxidase, p-galactosidase, luciferase, alkaline phosphatase), chemiluminescent, biotinyl groups, predetermined polypeptide epitopes recognized by a secondary reporter (e.g., leucine zipper pair sequences, binding sites for secondary antibodies, metal binding domains, epitope tags). In some

embodiments, labels are attached by spacer arms of various lengths to reduce potential steric hindrance. The term “pharmaceutical agent or drug” as used herein refers to a chemical compound or composition capable of inducing a desired therapeutic effect when properly administered to a patient.

[0189] 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.

[0190] The term “pharmaceutical composition” refers to 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., multispecific polypeptide construct) and a carrier, excipient, or diluent. The carrier, excipient, or diluent is typically a pharmaceutically acceptable carrier, excipient or diluent, respectively.

[0191] The terms “treating,” “treatment,” or “therapy” of a disease or disorder as used herein mean slowing, stopping or reversing the disease or disorders progression, as evidenced by decreasing, cessation or elimination of either clinical or diagnostic symptoms, by administration of a pharmaceutical composition of the disclosure either alone or in combination with another compound as described herein. “Treating,” “treatment,” or “therapy” also means a decrease in the severity of symptoms in an acute or chronic disease or disorder or a decrease in the relapse rate. 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). “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.

[0192] The terms “effective amount” or “therapeutically effective amount” refer to a quantity and/or concentration of a composition 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.

[0193] 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.

[0194] 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.

[0195] The term patient includes human and veterinary subjects.

[0196] Other chemistry terms herein are used according to conventional usage in the art, as exemplified by The McGraw-Hill Dictionary of Chemical Terms (Parker, S., Ed., McGraw-Hill, San Francisco (1985)).

[0197] 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”.

II. MULTISPECIFIC POLYPEPTIDE CONSTRUCTS

[0198] Provided herein is a multispecific polypeptide construct containing a first component containing an immunoglobulin Fc region and a second component comprising a CD3-binding region, wherein the first and second components are coupled by a linker, wherein the Fc region is positioned N-terminal to the CD3-binding region; and one or both of the first and second components comprises an antigen binding domain that binds a tumor associated antigen (TAA). In some embodiments, the linker is a non-cleavable linker. In some embodiments, the linker does not contain a substrate recognition that is specifically recognized for cleavage by a protease.

[0199] In some embodiments, the multispecific polypeptide construct contains in order, from N-terminus to C-terminus: an immunoglobulin Fc region; a linker; a CD3 binding region that binds CD3 (CD3 ϵ); and an antigen binding domain that binds a tumor-associated antigen (TAA). In some embodiments, the multispecific polypeptide construct contains in order, from N-terminus to C-terminus: an antigen binding domain that binds to a tumor-associated antigen (TAA); an immunoglobulin Fc region; a linker; and a CD3 binding region that binds CD3 (CD3 ϵ). In some embodiments, the multispecific polypeptide construct contains at least a first antigen binding domain that binds a TAA and a second antigen binding domain that binds a TAA. In some embodiments, the multispecific polypeptide construct contains, in order, from N-terminus to C-terminus: a first antigen binding domain that binds to a tumor-associated antigen (TAA); an immunoglobulin Fc region; a linker; a CD3 binding region that binds CD3 (CD3 ϵ); and a second antigen binding domain that binds a tumor-associated antigen (TAA).

[0200] Each of the components of the multispecific polypeptide constructs of the disclosure is described in more detail below.

[0201] 1. Anti-CD3 Binding Domains:

[0202] The multispecific polypeptide constructs of the disclosure include one or more copies of an anti-CD3 binding domain. The anti-CD3 binding domains of the disclosure activate T cells via engagement of CD3 ϵ 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 CD3 ϵ on T cells by partially or completely modulating, e.g., agonizing, stimulating, activating or otherwise augmenting CD3-mediated T cell activation.

[0203] In preferred embodiments, the anti-CD3 binding domains of the disclosure specifically bind the epsilon chain of CD3, also known as CD3 ϵ . The anti-CD3 ϵ binding domains of the disclosure activate T cells via engagement of CD3 ϵ on the T cells. The anti-CD3 ϵ binding domains of the disclosure include monoclonal antibodies, such as, for example, mammalian monoclonal antibodies, primate monoclonal antibodies, fully human monoclonal antibodies, as well as humanized monoclonal antibodies and chimeric antibodies, as well as antigen-binding fragments thereof. In some embodiments, the anti-CD3 ϵ binding domain includes one or more copies of an antibody or an antigen-binding fragment thereof.

[0204] In some embodiments, the anti-CD3 ϵ binding domain includes a combination of a VH CDR1 sequence, a VH CDR2 sequence, and a VH CDR3 sequence, wherein at least one of the VH CDR1 sequence, the VH CDR2 sequence, and the VH CDR3 sequence is selected from a VH CDR1 sequence that includes at least the amino acid sequence TYAMN (SEQ ID NO: 16); a VH CDR2 sequence that includes at least the amino acid sequence RIRSKYNNYATYYADSVKD (SEQ ID NO: 17); and a VH CDR3 sequence that includes at least the amino acid sequence HGNFGNSYVSWFAY (SEQ ID NO: 18). In some embodiments, the anti-CD3 ϵ binding domain includes a combination of a VH CDR1 sequence, a VH CDR2 sequence, and a VH CDR3 sequence, wherein at least one of the VH CDR1 sequence, the VH CDR2 sequence, and the VH CDR3 sequence is selected from a VH CDR1 sequence that includes at least the amino acid sequence GFTFNTYAMN (SEQ ID NO: 211); a VH CDR2 sequence that includes at least the amino acid sequence RIRSKYNNYATY (SEQ ID NO: 212); and a VH CDR3 sequence that includes at least the amino acid sequence HGNFGNSYVSWFAY (SEQ ID NO: 18).

[0205] In some embodiments, the anti-CD3 ϵ binding domain includes a combination of a VL CDR1 sequence, a VL CDR2 sequence, and a VL CDR3 sequence, wherein at least one of the VL CDR1 sequence, the VL CDR2 sequence, and the VL CDR3 sequence is selected from a VL CDR1 sequence that includes at least the amino acid sequence RSSTGAVTTSNYAN (SEQ ID NO: 19); a VL CDR2 sequence that includes at least the amino acid sequence GTNKRAP (SEQ ID NO: 20); and a VL CDR3 sequence that includes at least the amino acid sequence ALWYSNLWV (SEQ ID NO: 21).

[0206] In some embodiments, the anti-CD3 ϵ binding domain includes a VH CDR1 sequence that includes at least the amino acid sequence TYAMN (SEQ ID NO: 16); a VH CDR2 sequence that includes at least the amino acid sequence RIRSKYNNYATYYADSVKD (SEQ ID NO: 17); a VH CDR3 sequence that includes at least the amino acid sequence HGNFGNSYVSWFAY (SEQ ID NO: 18), a VL CDR1 sequence that includes at least the amino acid sequence RSSTGAVTTSNYAN (SEQ ID NO: 19); a VL

CDR2 sequence that includes at least the amino acid sequence GTNKRAP (SEQ ID NO: 20); and a VL CDR3 sequence that includes at least the amino acid sequence ALWYSNLWV (SEQ ID NO: 21).

[0207] In some embodiments, the anti-CD3 ϵ binding domain includes a VH CDR1 sequence that includes at least the amino acid sequence GFTFNTYAMN (SEQ ID NO: 211); a VH CDR2 sequence that includes at least the amino acid sequence RIRSKYNNYATY (SEQ ID NO: 212); a VH CDR3 sequence that includes at least the amino acid sequence HGNFGNSYVSWFAY (SEQ ID NO: 18), a VL CDR1 sequence that includes at least the amino acid sequence RSSTGAVTTSNYAN (SEQ ID NO: 19); a VL CDR2 sequence that includes at least the amino acid sequence GTNKRAP (SEQ ID NO: 20); and a VL CDR3 sequence that includes at least the amino acid sequence ALWYSNLWV (SEQ ID NO: 21).

[0208] In some embodiments, the anti-CD3 ϵ binding domain includes a VH CDR1 sequence that includes at least the amino acid sequence GFTFNTYAMN (SEQ ID NO: 211); a VH CDR2 sequence that includes at least the amino acid sequence RIRSKYNNYATY (SEQ ID NO: 212); a VH CDR3 sequence that includes at least the amino acid sequence HGNFGNSYVSWFAY (SEQ ID NO: 18), a VL CDR1 sequence that includes at least the amino acid sequence GSSTGAVTTSNYAN (SEQ ID NO: 229); a VL CDR2 sequence that includes at least the amino acid sequence GTNKRAP (SEQ ID NO: 230); and a VL CDR3 sequence that includes at least the amino acid sequence ALWYSNLWV (SEQ ID NO: 225).

[0209] In some embodiments, the anti-CD3 ϵ binding domain includes a combination of a VH CDR1 sequence, a VH CDR2 sequence, and a VH CDR3 sequence, wherein at least one of the VH CDR1 sequence, the VH CDR2 sequence, and the VH CDR3 sequence is selected from a VH CDR1 sequence that includes a 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: 16); a VH CDR2 sequence that includes a 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: 17); and a VH CDR3 sequence that includes a 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: 18).

[0210] In some embodiments, the anti-CD3 ϵ binding domain includes a combination of a VH CDR1 sequence, a VH CDR2 sequence, and a VH CDR3 sequence, wherein at least one of the VH CDR1 sequence, the VH CDR2 sequence, and the VH CDR3 sequence is selected from a VH CDR1 sequence that includes a 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: 211); a VH CDR2 sequence that includes a 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: 212); and a VH CDR3 sequence that includes a 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: 18).

[0211] In some embodiments, the anti-CD3 ϵ binding domain includes a combination of a VL CDR1 sequence, a

VL CDR2 sequence, and a VL CDR3 sequence, wherein at least one of the VL CDR1 sequence, the VL CDR2 sequence, and the VL CDR3 sequence is selected from a VL CDR1 sequence that includes a 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: 19); a VL CDR2 sequence that includes a 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: 20); and a VL CDR3 sequence that includes a 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: 21).

[0212] In some embodiments, the anti-CD3 ϵ 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: 16); 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: 17); 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: 18), 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: 19); 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: 20); 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: 21).

[0213] In some embodiments, the anti-CD3 ϵ 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: 211); 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: 212); 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: 18), 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: 19); 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: 20); 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: 21).

[0214] In some embodiments, the anti-CD3 ϵ 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: 211); a VH CDR2 sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to amino acid sequence RIRSKYNNYATY (SEQ ID NO: 212); 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: 18), 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: 229); 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: 230); 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: 225).

[0215] In some embodiments, the anti-CD3 ϵ binding domain includes a VH CDR1 sequence that includes at least the amino acid sequence GFTSTYAMN (SEQ ID NO: 227); a VH CDR2 sequence that includes at least the amino acid sequence RIRSKYNNYATY (SEQ ID NO: 228); a VH CDR3 sequence that includes at least the amino acid sequence HGNFGDSYVSWFAY (SEQ ID NO: 224), a VL CDR1 sequence that includes at least the amino acid sequence GSSTGAVTTSNYAN (SEQ ID NO: 229); a VL CDR2 sequence that includes at least the amino acid sequence GTNKRAP (SEQ ID NO: 230); and a VL CDR3 sequence that includes at least the amino acid sequence ALWYSNHWV (SEQ ID NO: 225).

[0216] In some embodiments, the anti-CD3 ϵ 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: 227); 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: 228); 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: 224), 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: 229); 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: 230); 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: 225).

[0217] In some embodiments, the anti-CD3 ϵ binding domain includes a CDR3 that includes at least amino acids VLWYSNRWV (SEQ ID NO: 226). In some embodiments, the anti-CD3 ϵ 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: 226).

[0218] In some embodiments, the anti-CD3 ϵ 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 CD3 ϵ (referred to herein as an anti-CD3 ϵ Fv fragment). In some embodiments, the anti-CD3 ϵ 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.

[0219] 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).

[0220] 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 11.2. 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.

[0221] In some embodiments, the anti-CD3 ϵ Fv antibody fragment includes an amino acid sequence selected from the group of SEQ ID NO: 32-81. In some embodiments, the anti-CD3 ϵ Fv antibody fragment includes 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: 32-81. In some embodiments, the anti-CD3 ϵ Fv antibody fragment includes a combination of an amino acid sequence selected from the group of SEQ ID NO: 32-62 and an amino acid sequence selected from the group consisting of SEQ ID NO: 63-81. In some embodiments, the anti-CD3 ϵ Fv antibody fragment includes a combination of 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: 32-62 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: 63-81 an amino acid sequence.

[0222] In some embodiments, the anti-CD3 ϵ 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: 32-81. In some embodiments, the anti-CD3 ϵ binding domain thereof includes a combination of a heavy chain variable region amino acid sequence selected from the group of SEQ ID NO: 32-62 and a light chain variable region amino acid sequence comprising an amino acid sequence selected from the group of SEQ ID NO: 63-81.

[0223] In some embodiments, the anti-CD3 ϵ 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-CD3 ϵ 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: 14, 15, 32-81, 191, 196-200, 211, and 212. In some embodiments, the anti-CD3 ϵ 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: 14, 15, 32-81, 191, 196-200, 211, and 212. In some embodiments, the anti-CD3 ϵ 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: 14, 32-62, 196-198, and 211 and light chain variable amino acid sequence selected from the group consisting of SEQ ID NO: 15, 63-81, 191, 199, 200, and 212. In some embodiments, the anti-CD3 ϵ 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: 14, 32-62, 196-198, and 211 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: 15, 63-81, 191, 199, 200, and 212.

[0224] In some embodiments, the anti-CD3 ϵ 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: 32-81, 191, 196-200, 211, and 212. In some embodiments, the anti-CD3 ϵ binding domain thereof includes a combination of a heavy chain variable region amino acid sequence selected from the group of SEQ ID NO: 32-62, 196-198, and 211 and a light chain variable region amino acid sequence comprising an amino acid sequence selected from the group of SEQ ID NO: 63-81, 191, 199, 200, and 212.

[0225] In some embodiments, the anti-CD3 ϵ Fv antibody fragment includes a combination of 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: 14, 32-43, 45-47, 48, 196 and 211 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: 15, 63, 65-71, 73, 75, 77, and 199. In some embodiments, the anti-CD3 ϵ Fv antibody fragment includes a combination of an amino acid sequence selected from the group of SEQ ID NO: 14, 32-43, 45-47, 48, 196 and 211 and an amino acid sequence selected from the group consisting of SEQ ID NO: 15, 63, 65-71, 73, 75, 77, and 199.

[0226] In some embodiments, the anti-CD3 ϵ 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: 14. In some embodiments, the anti-CD3 ϵ 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: 15. In some embodiments, the anti-CD3 ϵ 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: 14 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: 15. In some embodiments, the anti-CD3 ϵ binding domain thereof includes a variable heavy chain (VH) comprising the amino acid sequence of SEQ ID NO: 14. In some embodiments, the

anti-CD3 ϵ binding domain includes a variable light chain (VL) comprising the amino acid sequence of SEQ ID NO: 15. In some embodiments, the anti-CD3 ϵ binding domain thereof includes a variable heavy chain (VH) comprising the amino acid sequence of SEQ ID NO: 14 and a variable light chain (VL) comprising the amino acid sequence of SEQ ID NO: 15.

[0227] In some embodiments, the anti-CD3 ϵ 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: 196. In some embodiments, the anti-CD3 ϵ 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: 199. In some embodiments, the anti-CD3 ϵ 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: 196 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: 199. In some embodiments, the anti-CD3 ϵ binding domain thereof includes a variable heavy chain (VH) comprising the amino acid sequence of SEQ ID NO: 196. In some embodiments, the anti-CD3 ϵ binding domain includes a variable light chain (VL) comprising the amino acid sequence of SEQ ID NO: 199. In some embodiments, the anti-CD3 ϵ binding domain thereof includes a variable heavy chain (VH) comprising the amino acid sequence of SEQ ID NO: 196 and a variable light chain (VL) comprising the amino acid sequence of SEQ ID NO: 199.

[0228] In particular embodiments, the Fv is a disulfide stabilized Fv fragment (dsFv) in which 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.

[0229] In some embodiments, the anti-CD3 ϵ Fv antibody fragment includes a combination of 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: 44, 49-62, 197 and 198 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: 64, 72, 74, 76, 78-81, 191, 200 and 212. 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. In some embodiments, the anti-CD3 ϵ Fv antibody fragment includes a combination

of an amino acid sequence selected from the group of SEQ ID NO: 44, 49-62, 197 and 198 and an amino acid sequence selected from the group consisting of SEQ ID NO: 64, 72, 74, 76, 78-81, 191, 200 and 212.

[0230] In some embodiments, the anti-CD3 ϵ 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: 44. In some embodiments, the anti-CD3 ϵ 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: 72. In some embodiments, the anti-CD3 ϵ 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: 44 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: 72. 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. In some embodiments, the anti-CD3 ϵ binding domain thereof includes a variable heavy chain (VH) comprising the amino acid sequence of SEQ ID NO: 44. In some embodiments, the anti-CD3 ϵ binding domain includes a variable light chain (VL) comprising the amino acid sequence of SEQ ID NO: 72. In some embodiments, the anti-CD3 ϵ binding domain thereof includes a variable heavy chain (VH) comprising the amino acid sequence of SEQ ID NO: 44 and a variable light chain (VL) comprising the amino acid sequence of SEQ ID NO: 72.

[0231] In some embodiments, the anti-CD3 ϵ 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: 198. In some embodiments, the anti-CD3 ϵ 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: 200. In some embodiments, the anti-CD3 ϵ 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: 198 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: 200. 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. In some embodiments, the anti-CD3 ϵ binding domain thereof includes a variable heavy chain (VH) comprising the amino acid sequence of SEQ ID NO: 198. In some embodiments, the anti-CD3 ϵ binding domain includes a variable light chain (VL) comprising the amino acid sequence of SEQ ID NO: 200. In some embodiments, the anti-CD3 ϵ binding domain thereof includes a variable heavy chain (VH) comprising the amino acid sequence of SEQ ID NO: 198 and a

variable light chain (VL) comprising the amino acid sequence of SEQ ID NO: 200.

[0232] In some embodiments, the anti-CD3 ϵ 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: 197. In some embodiments, the anti-CD3 ϵ 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: 200. In some embodiments, the anti-CD3 ϵ 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: 197 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: 200. 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. In some embodiments, the anti-CD3 ϵ binding domain thereof includes a variable heavy chain (VH) comprising the amino acid sequence of SEQ ID NO: 197. In some embodiments, the anti-CD3 ϵ binding domain includes a variable light chain (VL) comprising the amino acid sequence of SEQ ID NO: 200. In some embodiments, the anti-CD3 ϵ binding domain thereof includes a variable heavy chain (VH) comprising the amino acid sequence of SEQ ID NO: 197 and a variable light chain (VL) comprising the amino acid sequence of SEQ ID NO: 200.

[0233] 2. Immunoglobulin Fc Polypeptides:

[0234] The first component of the multispecific polypeptide constructs of the disclosure includes 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. In some embodiments, the Fc region is a human Fc. In some embodiments, the immunoglobulin Fc region is a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 1-6. In some embodiments, the immunoglobulin Fc region contains an Fc chain that is an immunologically active fragment of any of SEQ ID Nos: 1-6. In some embodiments, the immunoglobulin Fc region contains an Fc polypeptide chain 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 any of SEQ ID NOs: 1-6 or an immunologically active fragment thereof.

[0235] In some embodiments, the multispecific polypeptide construct is a dimer formed by polypeptides, each containing an Fc. In some specific embodiments, identical or substantially identical polypeptides will be dimerized to create a homodimer. In some embodiments, the dimer is a homodimer in which the two polypeptides of the multispecific polypeptide construct are the same. In other cases, 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. Exemplary modifications to promote heterodimerization are known, including any as described below.

[0236] 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, such functions can be altered, such as reduced or enhanced, in an Fc for use with the provided multispecific polypeptide constructs.

[0237] 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. Thus, 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 and the Fc region linked to the TAA-antigen binding 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.

[0238] In some embodiments, the Fc region includes an Fc polypeptide that is mutated or modified to alter one or more effector functions. 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_IgH-Gnber.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).

[0239] In some embodiments, provided multispecific polypeptide constructs that contain an Fc region that exhibits reduced effector functions, may be a desirable candidate for applications in which constrained 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 Fc γ R binding (hence likely lacking ADCC activity), but retains FcRn binding ability. The primary cells for mediating ADCC, NK cells, express Fc γ RIII only, whereas monocytes express Fc γ RI, Fc γ RII and Fc γ RIII. 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, ACTITM non-radioactive cytotoxicity assay for flow cytometry (CellTechnology, Inc. Mountain View, Calif.; and CytoTox 96TM 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)).

[0240] 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:

(SEQ ID NO: 1)

PAP¹LT¹GPS VFLPPKPKD TLMISRTPEV TCVVDVSHE DPEVKFNWYV

DGVEVHNAKT KPREEQ¹NST YRVVSVLTVL HQDWLNGKEY KCKVSNKALP

APIEKTISKA KGQPREPQVY TLPPSRDELT KNQVSLTCLV KGFYPSDIAV

EWESNGQPEN NYKTPPVLD SDGSFPLYSK LTVDKSRWQQ GNVFSCSVMH

EALHNHYTQK SL¹SLSPGK

[0241] 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: 1.

[0242] In some embodiments, an IgG1 Fc polypeptide or a variant thereof such as any described below can be made in a G1 ml or G1 m3 allotype. In some embodiments, the Fc region can contain amino acids of the human G1 ml allotype, such as residues containing Asp (D) and Leu (L) at positions 356 and 358, e.g. as set forth in SEQ ID NO:1. In some cases, an Fc polypeptide can contain amino acid substitutions E356D and M358L to reconstitute residues of allotype G1 ml. 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: 194 and 195. In some cases, an Fc polypeptide can contain amino acid substitutions D356E and L358M to reconstitute residues of

allotype G1 m3. In some embodiments, the human IgG1 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, the contents of each of which are hereby incorporated by reference in their entireties.

[0243] In some embodiments, the Fc region, such as the human IgG1 Fc region is modified to enhance ADCC activity or CDC activity. 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.

[0244] In some embodiments, the human IgG1 Fc region fusion proteins of the present disclosure lack or have 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. In some embodiments,

the human IgG1 Fc region is modified at amino acid Asn297 (Boxed, Kabat Numbering) to prevent glycosylation of the fusion protein, e.g., Asn297Ala (N297A) or Asn297Asp (N297D).

[0245] 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. 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) or Ala327 (A327). For example, Leu 234Ala (L234A), Leu235Ala (L235A), Asp265Asn (D265N), Asp270Asn (D270N), Ser298Asn (S298N), Asn297Ala (N297A), Asn325Glu (N325E) or Ala327Ser (A327S). In some embodiments, the Fc region of the fusion protein is modified at amino acid Leu235 (Boxed in SEQ ID NO:1 above, 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 (Boxed in SEQ ID NO:1 above, Kabat Numbering) to alter Fc receptor interactions, e.g., Leu234Ala (L234A). In some embodiments, the Fc region of the fusion protein is altered at both amino acid 234 and 235, e.g., Leu234Ala and Leu235Ala (L234A/L235A) or Leu234Val and Leu235Ala (L234V/L235A). 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).

[0246] 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, 1 *Biol Chem* Vol. 281(33) 23514-23524), Met428Leu and Asn434Ser (M428L, N434S) (Zalevsky et al 2010 *Nature Biotech*, Vol. 28(2) 157-159) (EU index of Kabat et al 1991 *Sequences of Proteins of Immunological Interest*). In some embodiments, the mutated or modified Fc polypeptide includes the following mutations: Met252Tyr and Met428Leu or Met252Tyr and Met428Val (M252Y, M428L, or M252Y, M428V) using the Kabat numbering system.

[0247] 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 these embodiments, Fc deletion of these three amino acids reduces the complement protein C1q binding.

binding: Glu233 (E233), Leu234 (L234), or Leu235 (L235). The one or more mutations can include E233P, L234V and/or L235A.

[0249] In some embodiments, the Fc region of the fusion protein is altered at Gly236 (boxed in SEQ ID NO:1 above) to reduce Fc receptor binding. For example, wherein Gly236 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).

[0250] 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 or E233P/L234V/L235A/G236del.

[0251] In some embodiments, the human IgG1 Fc region lacks Lys447 (EU index of Kabat et al 1991 *Sequences of Proteins of Immunological Interest*).

[0252] 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: 2.

[0253] 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:

(SEQ ID NO: 3)
PAPPVAGPSV FLFPPKPKDT LMISRTPEVT CVVVDVSHED PEVQFNWYVD
GVEVHNAKTK PREEQINSTF RVVSVLTVVH QDWLNGKEYK CKVSNKGLPA
PIEKTISKTK GQPREPQVYT LPPSREEMTK NQVSLTCLVK GFYPSDISVE
WESNGQPENN YKTTTPMPLDS DGSFFLYSKL TVDKSRWQQG NVPSCSVMH
ALHNHYTQKS LSLSPGK

(SEQ ID NO: 2)
PAPGGPSVFL FPPKPKDTLM ISRTPEVTCV VVDVSHEDPE
VKFNWYVDGV EVHNAKTKPR EEQYNSTYRV VSVLTVLHQD
WLNGKEYKCK VSNKALPAPI EKTISKAKGQ PREPQVYTLF
PSRDELTKNQ VSLTCLVKG F YPSDIAVEWE SNGQPENNYK
TTPPVLDSDG SFFLYSKLTV DKSRWQQGNV FSCSVMH
HNHYTQKSLS LSPGK

[0248] In some embodiments, the Fc region is mutated in one or more of the following positions to reduce Fc receptor

[0254] 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: 3.

[0255] In some embodiments, the human IgG2 Fc region is modified at amino acid Asn297 (Boxed, to prevent to glycosylation of the antibody, e.g., Asn297Ala (N297A) or Asn297Asp (N297D). In some embodiments, the human IgG2 Fc region lacks Lys447 (EU index of Kabat et al 1991 *Sequences of Proteins of Immunological Interest*).

[0256] 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:

(SEQ ID NO: 4)
PAPELLGGPS VFLFPPKPKD TLMISRTPEV TCVVVDVSHE DPEVQFKWYV
DGVEVHNAKT KPREEQINST FRVSVLTVL HQDWLNGKEY KCKVSNKALP

-continued

APIEKTISK T KGQPREPQVY TLPPSREEMT KNQVSLTCLV KGFYPSDIAV
 EWESSGQPEN NYNTTPPMLD SDGSFFLYSK LTVDKSRWQQ GNIFSCSVMH
 EALHNFTQK SLSLSPGK

[0257] 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: 4.

[0258] In some embodiments, the human IgG3 Fc region is modified at amino acid Asn297 (Boxed, 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 lacks Lys447 (EU index of Kabat et al 1991 *Sequences of Proteins of Immunological Interest*).

[0259] 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:

(SEQ ID NO: 5)
 PAPELLGGPS VFLPPPKPKD TLMISRTPEV TCVVVDVSHE DPEVKFNWYV
 DGVEVHNAKT KPREEQNST YRVVSVLTVL HQDWLNGKEY KCKVSNKGLP
 SSIEKTISKA KGQPREPQVY TLPPSQEEMT KNQVSLTCLV KGFYPSDIAV
 EWESNGQPEN NYKTTPPVLD SDGSFFLYSR LTVDKSRWQE GNVFSCSVMH
 EALHNHYTQK SLSLSLGK

[0260] 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: 5.

[0261] 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:

(SEQ ID NO: 6)
 PAPELLGGPS VFLPPPKPKD TLMISRTPEV TCVVVDVSQE DPEVQFNWYV
 DGVEVHNAKT KPREEQNST YRVVSVLTVL HQDWLNGKEY KCKVSNKGLP
 SSIEKTISKA KGQPREPQVY TLPPSQEEMT KNQVSLTCLV KGFYPSDIAV
 EWESNGQPEN NYKTTPPVLD SDGSFFLYSR LTVDKSRWQE GNVFSCSVMH
 EALHNHYTQK SLSLSLGK

[0262] 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: 6.

[0263] In other 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 (Boxed, Kabat Numbering) to prevent to glycosylation of the antibody, e.g., Asn297Ala (N297A) or Asn297Asp (N297D). In some embodiments, the human IgG4 Fc region lacks Lys447 (EU index of Kabat et al 1991 *Sequences of Proteins of Immunological Interest*).

[0264] In some embodiments, the human IgG Fc region is modified to stabilize the homodimerization at the CH3:CH3 interface by introducing two disulfide bonds by changing Ser354 to Cys (S354C) and Tyr349 to Cys (Y349C) (S354C/Y349C).

[0265] In particular embodiments of multispecific polypeptide constructs provided herein, the human IgG Fc region is modified to induce heterodimerization. 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. U.S. Pat. Nos. 5,731,168, 7,183,076, 9,701,759, 9,605,084, and 9,650,446. 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.

[0266] 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.

[0267] 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 1.

TABLE 1

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

[0268] 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.

[0269] In some embodiments, a first Fc polypeptide that is modified to contain protuberance (hole) 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 posi-

tionable 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.

[0270] 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.

[0271] 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.

[0272] 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).

[0273] 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: 201 or 207 and the second Fc polypeptide is selected from an Fc polypeptide comprising the sequence set forth in SEQ ID NO: 202, 205 or 209. In some embodiments, the first Fc polypeptide is or comprises the sequence of amino acids set forth in any of SEQ ID NOS: 82, 86, 94 or 96 and the second Fc polypeptide is or comprises the sequence of amino acids set forth in any of SEQ ID NOS: 83, 87, 90, 92, 98 or 100.

[0274] 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: 201 and a second Fc polypeptide that is or comprises SEQ ID NO: 202 or 205. In some embodiments, the first Fc polypeptide is or comprises the sequence set forth in SEQ ID NO: 82 and the second Fc polypeptide is or comprises the sequence set forth in SEQ ID NO: 83 or 90. In some embodiments, the first Fc polypeptide is or comprises the sequence set forth in SEQ ID NO: 86 and the second Fc polypeptide is or comprises the sequence set forth in SEQ ID NO: 87 or 92. The first and second Fc polypeptide can be formatted on either polypeptide chain of the construct.

[0275] 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: 203 or 208 and the second Fc polypeptide is selected from an Fc polypeptide comprising the sequence set forth in SEQ ID NO: 204, 206 or 210. In some embodiments, the first Fc polypeptide is or comprises the sequence of amino acids set forth in any of SEQ ID NOS: 84, 88, 95 or 97 and the second Fc polypeptide is or comprises the sequence of amino acids set forth in any of SEQ ID NOS: 85, 89, 91, 93, 99 or 101.

[0276] In particular examples, the first Fc polypeptide is or comprises the sequence set forth in SEQ ID NOS: 203 and a second Fc polypeptide that is or comprises SEQ ID NO: 204 or 206. In some embodiments, the first Fc polypeptide is or comprises the sequence set forth in SEQ ID NO: 84 and the second Fc polypeptide is or comprises the sequence set forth in SEQ ID NO: 85 or 91. In some embodiments, the first Fc polypeptide is or comprises the sequence set forth in SEQ ID NO: 88 and the second Fc polypeptide is or comprises the sequence set forth in SEQ ID NO: 89 or 93. The first and second Fc polypeptide can be formatted on either polypeptide chain of the construct.

[0277] 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:207 and the second Fc polypeptide is or comprises the sequence set forth in SEQ ID NO:209. In some embodiments, the first Fc polypeptide is or comprises the sequence set forth in SEQ ID NO:94 and the second Fc polypeptide is or comprises the sequence set forth in SEQ ID NO: 98. In some embodiments, the first Fc polypeptide is or comprises the sequence set forth in SEQ ID NO:96 and the second Fc polypeptide is or comprises the sequence set forth in SEQ ID NO: 100. In other examples, the first Fc polypeptide is or comprises the sequence set forth in SEQ ID NO:208 and the second Fc polypeptide is or comprises the sequence set forth in SEQ ID NO:210. In some embodiments, the first Fc polypeptide is or comprises the sequence set forth in SEQ ID NO:95 and the second Fc polypeptide is or comprises the sequence set forth in SEQ ID NO: 99. In some embodiments, the first Fc polypeptide is or comprises the sequence set forth in SEQ ID NO:97 and the second Fc polypeptide is or comprises the sequence set forth in SEQ ID NO: 101. The first and second Fc polypeptide can be formatted on either polypeptide chain of the construct.

[0278] 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.

[0279] 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 embodiment, a first Fc polypeptide can contain mutations D221E/P228E/L368E and a second Fc polypeptide can contain mutations D221R/P228R/K409R. In another embodiment, a first Fc polypeptide can contain mutations C220E/P228E/368E and a second Fc polypeptide can contain mutations C220R/E224R/P228R/K409R.

[0280] 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.

[0281] In some embodiments, a combination of steric heterodimerization variants (e.g. knob and hole) and pI or charge pair variants can be used.

[0282] 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 heterodimerization comprise the sequences set forth in SEQ ID NOS:194 and 195. The first and second Fc polypeptide can be formatted on either polypeptide chain of the construct.

[0283] The resulting 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.

[0284] 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, Jendeborg et al. (Jendeborg 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 IgG1 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.

[0285] 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 may be 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.

[0286] 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.

[0287] In some embodiments, one Fc polypeptide of a heterodimeric Fc comprises the sequence of amino acids set forth in any of SEQ ID NOS:201 (e.g. SEQ ID NO:82), 86, 207 (e.g. SEQ ID NO:94), or 96, and the other Fc polypeptide of the heterodimeric Fc contains the sequence of amino acids set forth in any of SEQ ID NOS:201 (e.g. SEQ ID NO:83), 87, 205 (e.g. SEQ ID NO:90), 92, 209 (e.g. SEQ ID NO:98), or 100. In some embodiments, one Fc polypeptide of a heterodimeric Fc comprises the sequence of amino acids set forth in any of SEQ ID NOS: 203 (e.g. SEQ ID NO:84), 88, 208 (e.g. SEQ ID NO:95), or 97 and the other Fc polypeptide of the heterodimeric Fc comprises the sequence of amino acids set forth in any of SEQ ID NOS: 204 (e.g. SEQ ID NO:85), 89, 206 (e.g. SEQ ID NO:91), 93, 210 (e.g. SEQ ID NO:99), or 101.

[0288] 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.

[0289] 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) or Ala327 (A327). For example, Leu 234Ala (L234A), Leu235Ala (L235A), Asp265Asn (D265N), Asp270Asn (D270N), Ser298Asn (S298N), Asn297Ala (N297A), Asn325Glu (N325E) or Ala327Ser (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).

[0290] 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: 7), 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: 8).

[0291] 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: 9). In some embodiments, the fusion protein contains linker polypeptides. In other embodiments, the fusion protein contains linker and hinge polypeptides.

[0292] 3. Linkers

[0293] The provided 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 a non-cleavable linker. In some embodiments, the linker does not contain a substrate recognition site that is specifically recognized for cleavage by the protease. Thus, linkers in the provided multispecific polypeptide constructs do not include an amino acid sequence that can serve as a substrate for a protease, such as an extracellular protease. For example, the non-cleavable linker does not include a cleavage sequence containing at least one peptide bond which lies within a cleavable peptide sequence of a protease.

[0294] 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. Because the provided multispecific polypeptide constructs are multimers, such as dimers, the provided constructs include a linker joining the first Fc polypeptide and a first domain (e.g. VH) of the CD3 binding region of the first polypeptide and the second Fc polypeptide and second domain (e.g. VL) of the CD3 binding region of the second polypeptide. Typically, the linkers present in the first and second polypeptides of the 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.

[0295] 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. Nos. 5,258,498; 5,525,491; 5,525,491, 6,132,992).

[0296] 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 embodiment, the linker is a polypeptide linker. The polypeptide linker can be a flexible linker or a rigid linker or a combination of both.

[0297] 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.

[0298] In certain aspects, the longer the linker length, the greater the CD3 binding when the multispecific polypeptide conjugate is bound 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.

[0299] 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.

[0300] 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:171), wherein n is 0 to 10. In particular embodiments, the linker contains the sequence (GGGGS)_n (SEQ ID NO: 173), 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:172), 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.

[0301] In some embodiments, the linker is (in one-letter amino acid code): GGS, GGGGS (SEQ ID NO: 149), or GGGGGS (SEQ ID NO: 135). In some embodiments, the

GS-linker comprises an amino acid sequence of GGS₂GS, i.e., (GGS)₂ (SEQ ID NO: 10); GGS₂GS₂GS, i.e., (GGS)₃ (SEQ ID NO: 11); GGS₂GS₂GS₂GS, i.e., (GGS)₄ (SEQ ID NO: 12); GGS₂GS₂GS₂GS₂GS, i.e., (GGS)₅ (SEQ ID NO: 13); GGS₂GS₂GS₂GS₂GS₂GS, i.e., (GGS)₆ (SEQ ID NO: 14); GGS₂GS₂GS₂GS₂GS₂GS₂GS, i.e., (GGS)₇ (SEQ ID NO: 15); GGS₂GS₂GS₂GS₂GS₂GS₂GS₂GS, i.e., (GGS)₈ (SEQ ID NO: 16); GGS₂GS₂GS₂GS₂GS₂GS₂GS₂GS₂GS, i.e., (GGS)₉ (SEQ ID NO: 17); GGS₂GS₂GS₂GS₂GS₂GS₂GS₂GS₂GS₂GS, i.e., (GGS)₁₀ (SEQ ID NO: 18); GGS₂GS₂GS₂GS₂GS₂GS₂GS₂GS₂GS₂GS₂GS, i.e., (GGS)₁₁ (SEQ ID NO: 19); GGS₂GS₂GS₂GS₂GS₂GS₂GS₂GS₂GS₂GS₂GS₂GS, i.e., (GGS)₁₂ (SEQ ID NO: 20); GGS₂GS₂GS₂GS₂GS₂GS₂GS₂GS₂GS₂GS₂GS₂GS₂GS, i.e., (GGS)₁₃ (SEQ ID NO: 21); GGS₂GS₂GS₂GS₂GS₂GS₂GS₂GS₂GS₂GS₂GS₂GS₂GS₂GS, i.e., (GGS)₁₄ (SEQ ID NO: 22); GGS₂GS₂GS₂GS₂GS₂GS₂GS₂GS₂GS₂GS₂GS₂GS₂GS₂GS₂GS, i.e., (GGS)₁₅ (SEQ ID NO: 23); GGS₂GS₂GS₂GS₂GS₂GS₂GS₂GS₂GS₂GS₂GS₂GS₂GS₂GS₂GS₂GS, i.e., (GGS)₁₆ (SEQ ID NO: 24); GGS₂GS₂GS₂GS₂GS₂GS₂GS₂GS₂GS₂GS₂GS₂GS₂GS₂GS₂GS₂GS₂GS, i.e., (GGS)₁₇ (SEQ ID NO: 25); GGS₂GS₂GS₂GS₂GS₂GS₂GS₂GS₂GS₂GS₂GS₂GS₂GS₂GS₂GS₂GS₂GS₂GS, i.e., (GGS)₁₈ (SEQ ID NO: 26); GGS₂GS₂GS₂GS₂GS₂GS₂GS₂GS₂GS₂GS₂GS₂GS₂GS₂GS₂GS₂GS₂GS₂GS₂GS, i.e., (GGS)₁₉ (SEQ ID NO: 27); GGS₂GS₂GS₂GS₂GS₂GS₂GS₂GS₂GS₂GS₂GS₂GS₂GS₂GS₂GS₂GS₂GS₂GS₂GS, i.e., (GGS)₂₀ (SEQ ID NO: 28). In some embodiments, the linker is GGGG (SEQ ID NO: 103). In some embodiments, the linker is GGGGG (SEQ ID NO: 192). In some of any of the above examples, serine can be replaced with alanine (e.g., (Gly4Ala) or (Gly3Ala)).

[0302] In some embodiments, the linker includes a peptide linker having the amino acid sequence Gly_xXaa-Gly_y-Xaa-Gly_z (SEQ ID NO: 174), 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-Gly-Xaa-Gly-Gly-Gly (SEQ ID NO: 175), wherein each Xaa is selected as above.

[0303] In some embodiments, the linker is serine-rich linkers based on the repetition of a (SSSSG)_n (SEQ ID NO: 185) motif where n is at least 1, though n can be 2, 3, 4, 5, 6, 7, 8 and 9.

[0304] 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.

[0305] 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: 177).

[0306] 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: 178), wherein n is 2 to 20, preferably 4 to 10, including but not limited to, AS-(AP)_n-GT (SEQ ID NO: 179) or AS-(EAAAK)_n-GT (SEQ ID NO: 180), 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: 181), (PGGGS)_n (SEQ ID NO: 182), (AGGGS)_n (SEQ ID NO: 183) or GGS-(EGKSSGSGSESKST)_n-GGS (SEQ ID NO: 184), wherein n is 2 to 20. In some embodiments, the linker is SSSASASSA (SEQ ID NO: 186), GSPGSPG (SEQ ID NO: 187), or ATTTGSSPGPT (SEQ ID NO: 176). 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.

[0307] In some embodiments, the linker is not a cleavable linker (used interchangeably with non-cleavable linker). In some embodiments, the linker is not 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.

[0308] In some embodiments, the linker does not contain 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.

[0309] 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. Typically, a protease 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 sequence, 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 non-cleavable linker, or a linker that does not contain a substrate recognition site that is specifically recognized for cleavage by a protease, is a linker that, if cleaved at all, is cleaved by a protease at a rate of less than $1 \times 10^4 \text{ M}^{-1} \text{ S}^{-1}$, or less than $5 \times 10^3 \text{ M}^{-1} \text{ S}^{-1}$, less than $1 \times 10^3 \text{ M}^{-1} \text{ S}^{-1}$, or less than $1 \times 10^2 \text{ M}^{-1} \text{ S}^{-1}$ or less.

[0310] In some embodiments, the linkers in the multispecific constructs provided herein do not contain a substrate recognition site for a protease that include, for example, matrix metalloproteases (MMP), cysteine proteases, serine

proteases and plasmin activators. In particular embodiments, the linker does not contain a substrate recognition site for a protease that 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.

[0311] In some embodiments, the linker does not contain a substrate recognition site that is specifically recognized by one or more of the following enzymes or proteases: ADAMS, ADAMTS, e.g. ADAMS; ADAMS; ADAM10; ADAM12; ADAM15; ADAM17/TACE; ADAMDEC1; ADAMTS1; ADAMTS4; ADAMTSS; 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; Otu-bain-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, MMP1, 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, Guanidinobenzoate, HtrA1, Human Neutrophil Elastase, Lactoferrin, Marapsin, NS3/4A, PACE4, Plasmin, PSA, tPA, Thrombin, Trypsin, uPA; Type II Transmembrane Serine Proteases (TTSPs), e.g., DESC1, DPP-4, FAP, Hepsin, Matrilysin-2, Matrilysin, TMPRSS2, TMPRSS3, or TMPRSS4; and any combination thereof. In some embodiments, the linker does not contain a substrate recognition site that is specifically recognized by granzyme B, a matrilysin or an MMP, such as MMP-2.

[0312] In some embodiments, the linker does not comprise an amino acid that is a substrate for Granzyme B. In some embodiments, the linker does not contain an amino acid sequence having the general formula P4 P3 P2 P1↓P1' (SEQ ID NO: 150), 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, the linker does not contain an amino acid sequence having the general formula P4 P3 P2 P1↓P1' (SEQ ID NO: 151), 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.

[0313] In some embodiments, the linker does not contain the amino acid sequence LEAD (SEQ ID NO: 22), LEPD (SEQ ID NO: 142), or LEAE (SEQ ID NO: 143). In some embodiments, the linker does not contain the amino acid sequence IEPDI (SEQ ID NO: 136), LEADT (SEQ ID NO: 137), IEPDG (SEQ ID NO: 138), IEPDV (SEQ ID NO: 139), IEPDS (SEQ ID NO: 140), IEPDT (SEQ ID NO: 141), IEPDP (SEQ ID NO: 144), LEPDG (SEQ ID NO: 152) or LEADG (SEQ ID NO: 153).

[0314] In some embodiments, the linker does not comprise an amino acid that is a substrate for matrilysin. In some embodiments, the linker does not comprise the sequence P1QAR↓(A/V) (SEQ ID NO: 154), wherein P1 is any amino acid. In some embodiments, the linker does not comprise the sequence RQAR(A/V) (SEQ ID NO: 155). In some

embodiments, the linker does not comprise the amino acid sequence RQAR (SEQ ID NO: 23). In some embodiments, the linker does not comprise the amino acid sequence RQARV (SEQ ID NO: 156)

[0315] In some embodiments, the linker does not comprise 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 linker does not contain a sequence having the general formula P3 P2 P1↓P1' (SEQ ID NO: 157), 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 linker does not contain the general formula P3 P2 P1↓P1' (SEQ ID NO: 158), wherein P3 is P; P2 is Q or D; P1 is A or N; and P1' is L or I. In some embodiments, the linker does not comprise the amino acid sequence PAGL (SEQ ID NO: 24).

[0316] In some embodiments, the linker is not a linker comprising the amino acid sequence set forth as TGLEAD-GSPAGLGRQARVG (SEQ ID NO: 25); TGLEAD-GSRQARVGPAGLG (SEQ ID NO: 26); TGSPAGLEAD-GSRQARVGS (SEQ ID NO: 27); TGPAGLGLGADGSRQARVG (SEQ ID NO: 28); TGRQARVGLGADGSPAGLG (SEQ ID NO: 29); TGSRQARVGPAGLEADGS (SEQ ID NO: 30); and TGPAGLGRQARVGLGADGS (SEQ ID NO: 31); GPAGLGLGPDGSRQARVG (SEQ ID NO: 104); GSGGGGGIEPDIGGSGGS (SEQ ID NO: 105); GSGGGGGLEADTGGSGGS (SEQ ID NO: 106); GSIEPDIGS (SEQ ID NO: 107); GSLEADTGS (SEQ ID NO: 108); GSGGGGGIEPDGGSGGS (SEQ ID NO: 109); GSGGGGGIEPDVGGSGGS (SEQ ID NO: 110); GSGGGGGIEPDGSGSGGS (SEQ ID NO: 111); GSGGGGGIEPDTGGSGGS (SEQ ID NO: 112); GGG-SLEPDGSGGS (SEQ ID NO: 113); and GPAGLGLGADGSRQARVG (SEQ ID NO: 114), GGGGGGGSGSGSGGS (SEQ ID NO: 115); GSSAGSEAGSGQAGVGS (SEQ ID NO: 116); GSGGGGGLEAGSGSGGS (SEQ ID NO: 117); GSGGGGGIEPDGSGSGGS (SEQ ID NO: 118); TGGSGGGGGIEPDIGGSGGS (SEQ ID NO: 148).

[0317] 4. Antigen Binding Domains:

[0318] The multispecific polypeptide constructs of the present disclosure include at least one antigen binding domain, such as at least a first antigen binding domain and a second antigen binding domain. In some aspects, the antigen binding domain, or independently each of the antigen binding domains, is selected from an antibody or antigen binding fragment, a natural cognate binding partner, an Anticalin (engineered lipocalin), a Darpin, a Fynommer, a Centyrin (engineered fibronectin III domain), a cystine-knot domain, an Affilin, an Affibody, or an engineered CH3 domain. In some embodiments, the natural cognate binding partner comprises an extracellular domain or binding fragment thereof of the native cognate binding partner of the TAA, or a variant thereof that exhibits binding activity to the TAA.

[0319] In some embodiments, a TAA is a counter-structure that is present primarily on tumor cells of a mammalian subject but generally not found on normal cells of the mammalian subject. A tumor specific antigen need not be exclusive to tumor cells but the percentage of cells of a particular mammal that have the tumor associated antigen is sufficiently high or the levels of the tumor associated antigen on the surface of the tumor are sufficiently high such that it can be targeted by anti-tumor therapeutics, such as multi-

specific polypeptide constructs as provided, and provide prevention or treatment of the mammal from the effects of the tumor. In some embodiments, in a random statistical sample of cells from a mammal with a tumor, at least 50% of the cells displaying a TAA are cancerous. In other embodiments, at least 60%, 70%, 80%, 85%, 90%, 95%, or 99% of the cells displaying a TAA are cancerous.

[0320] In some embodiments, the antigen binding domain, or independently each of the antigen binding domains, such as the first antigen-binding domain and the second antigen binding domains, includes one or more copies of an antibody or an antigen-binding fragment thereof. In some embodiments, the antigen binding domain, or independently each of the antigen binding domains, 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 antigen binding domain, or independently each of the antigen binding domains, such as the first antigen-binding domain and the second antigen binding domains, 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. In some embodiments, each of the first antigen-binding domain and the second antigen binding domains includes one or more single domain antibody (sdAb) fragments, for example V_HH , V_{NAR} , engineered V_H or V_K domains. V_HH s 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.

[0321] 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 at least one sdAb or an scFv that binds a TAA. In some embodiments, the at least one scFv or sdAb that binds a TAA 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 TAA, 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 scFvs or sdAbs that bind to a TAA, positioned 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 three scFv or sdAb, in which two are positioned amino-terminally relative to the Fc region or carboxy-terminally relative to the CD3 binding region, and the third is positioned at the other end of the multispecific polypeptide construct.

[0322] 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 tumor-associated antigen; 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, the same or different scFv or sdAb that binds to a tumor-associated antigen. The scFv or sdAb that binds to a TAA 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. In some embodiments, the antigen binding domain, or independently each of the antigen binding domains, of the multispecific polypeptide constructs contains VH and VL sequences assembled as FABs or scFvs. In some embodiments, the antigen binding domain, or independently each of the antigen binding domains, of the multispecific polypeptide constructs contains binding domains as single domain antibodies (sdAbs).

[0323] In some embodiments, the antigen binding domain or independently each of the antigen binding domains, such as the first antigen-binding domain and the second antigen binding domains, contains more than one chain. 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 VH and VL sequences assembled as FABs.

[0324] 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 TAA. 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) and VL-CL, that binds to a TAA, 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 TAA, 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.

[0325] 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 tumor-associated antigen; 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 tumor-associated antigen, and a third polypeptide comprising the other of the VH-CH1 (Fd) or VL-CL of the Fab antibody fragment that binds to the TAA.

[0326] In some embodiments, the antigen binding domain, or independently each of the antigen binding domains, is or includes an extracellular domain or binding fragment thereof

of the native cognate binding partner of the TAA, or a variant thereof that exhibits binding activity to the TAA.

[0327] In some embodiments, each of the antigen binding domains, such as each of the first antigen-binding domain and the second antigen binding domains, bind the same antigen. In some embodiments, each of the first antigen-binding domain and the second antigen binding domains bind a different antigen. In some embodiments, each of the antigen binding domains, such as each of the first antigen-binding domain and the second antigen binding domains, bind the same tumor associated antigen (TAA). In some embodiments, each of the antigen binding domains, such as each of the first antigen-binding domain and the second antigen binding domains, bind a different TAA. In some embodiments, each of the antigen binding domains, such as each of the first antigen-binding domain and the second antigen binding domains, bind a different epitope on the same TAA. In some embodiments, each of the antigen binding domains, such as each of the first antigen-binding domain and the second antigen binding domains, bind the same epitope on the same TAA.

[0328] In some embodiments, the antigen binding domains results in monovalent, bivalent, trivalent, or tetra-valent 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).

[0329] In some embodiments, the TAA is selected from the group consisting of 1-92-LFA-3, 5T4, Alpha-4 integrin, Alpha-V integrin, alpha4beta1 integrin, alpha4beta7 integrin, AGR2, Anti-Lewis-Y, Apelin J receptor, APRIL, B7-H3, B7-H4, BAF, 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 (FRa), 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 (FcεRI), 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, Nicas-

trin, Notch Receptors, Notch 1, Notch 2, Notch 3, Notch 4, NOV, OSM-R, OX-40, PAR2, PDGF-AA, PDGF-BB, PDGF-Ralpha, PDGFRbeta, PD-1, PD-L1, PD-L2, Phosphatidylserine, 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.

[0330] In some embodiments, at least one antigen binding domain, or independently each antigen binding domain, binds the tumor associated antigen (TAA) folate receptor alpha (FRa). For example, the antigen binding domain contains the binding domain as an sdAb that binds FRα. Exemplary FRα-binding sdAbs are set forth in SEQ ID NOS: 120, 121, and 122.

[0331] In some embodiments, at least one antigen binding domain, or independently each antigen binding domain, binds the tumor associated antigen (TAA) cMET. For example, the antigen binding domain contains the binding domain as a sdAb that binds cMET. An exemplary cMET-binding sdAb is set forth in SEQ ID NO: 123 (U.S. Pat. No. 9,346,884).

[0332] In some embodiments, at least one antigen binding domain, or independently each antigen binding domain, binds the tumor associated antigen (TAA) B7H3. For example, the antigen binding domain contains the binding domain as an scFv that binds B7H3. An exemplary B7H3-binding scFv is set forth in SEQ ID NO: 124. In some embodiments, the antigen binding domain is a sdAb, such as a VHH. Exemplary B7H3-binding sdAbs are set forth in any of SEQ ID NOS: 214-218. In some embodiments, the antigen binding domain is or contains a Fab antibody fragment comprising a VH-CH1 (Fd) and LC. An exemplary B7H3 Fd is set forth in SEQ ID NO: 127 and an exemplary B7H3 LC is set forth in SEQ ID NO: 128 (PCT Publication No. WO2017/030926).

[0333] In some embodiments, at least one antigen binding domain, or independently each antigen binding domain, binds the tumor associated antigen (TAA) CD20. In some embodiments, such an antigen-binding domain contains a VH set forth in SEQ ID NO: 189 and a VL set forth in SEQ ID NO: 190 or a sequence that exhibits at least at or about 85%, 90%, 95%, 96%, 97%, 98%, 98%, or 99% sequence identity to SEQ ID NO: 189 or SEQ ID NO:190. For example, the antigen binding domain contains the binding domain as an scFv that binds CD20. Exemplary CD20-binding scFvs are set forth in SEQ ID NO: 125 and 213 (U.S. Pub. No. US 2005/0123546).

[0334] In some embodiments, at least one antigen binding domain, or independently each antigen binding domain, binds the tumor associated antigen (TAA) DLL3. For example, the antigen binding domain contains the binding domain as an scFv that binds DLL3. Exemplary DLL3-binding scFv is set forth in SEQ ID NO: 126 and 188 (U.S. Pub. No. US 2017/0037130). In some embodiments, the antigen binding domain is a sdAb, such as a VHH. Exemplary DLL3-binding sdAbs are set forth in any of SEQ ID NO: 219 or SEQ ID NO:220. In some embodiments, the antigen binding domain is or contains a Fab antibody fragment comprising a Fd and LC that binds DLL3. An

exemplary DLL3 Fd is set forth in SEQ ID NO: 133 and an exemplary DLL3 LC is set forth in SEQ ID NO: 134 (U.S. Pat. No. 8,044,178).

[0335] In some embodiments, at least one antigen binding domain, or independently each antigen binding domain, binds the tumor associated antigen (TAA) 5T4. An exemplary 5T4 Fd is set forth in SEQ ID NO: 129 and an exemplary 5T4 LC is set forth in SEQ ID NO: 130. In some embodiments, the antibody binding domain comprises a VH-CH1 (Fd) or VL-CL as set forth in SEQ ID NOS: 167 and 168 (U.S. Pat. No. 8,044,178).

[0336] In some embodiments, at least one antigen binding domain, or independently each antigen binding domain, binds the tumor associated antigen (TAA) gpNMB. In some embodiments, the antigen binding domain is or contains a Fab fragment comprising a Fd and LC chain. An exemplary gpNMB Fd is set forth in SEQ ID NO: 131 and an exemplary gpNMB LC is set forth in SEQ ID NO: 132.

[0337] In some embodiments, the antigen binding 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 Section 11.3, although generally peptides linking the antigen binding domain or domains is not a cleavable linker.

[0338] In some embodiments, the multispecific polypeptide construct comprises a first linking peptide (LP1) between the first antigen binding domain 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 antigen binding domain. In some embodiments, the multispecific polypeptide construct comprises a first linking peptide (LP1) between the first antigen binding domain and the Fc region and a second linking peptide (LP2) between the CD3 binding region and the second antigen binding domain. In some aspects, the multispecific polypeptide construct has the structural arrangement from N-terminus to C-terminus as follows: first antigen binding domain-LP1-Fc region-linker-CD3 binding region-LP2-second antigen binding domain. In some embodiments, the two linking peptides are not identical to each other.

[0339] In some embodiments, the 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: 10-13, 119, 135, 147, 149 or GGS.

III. PHARMACEUTICAL COMPOSITION

[0340] Provided herein are compositions of any of the provided multispecific polypeptide constructs. It will be appreciated that administration of therapeutic entities in accordance with the disclosure will be administered with suitable carriers, excipients, and other agents that are incorporated into formulations to provide improved transfer, delivery, tolerance, and the like. A multitude of appropriate formulations can be found in the formulary known to all pharmaceutical chemists: Remington's Pharmaceutical Sciences (15th ed., Mack Publishing Company, Easton, Pa. (1975)), particularly Chapter 87 by Blaug, Seymour, therein. These formulations include, for example, powders, pastes, ointments, jellies, waxes, oils, lipids, lipid (cationic or anionic) containing vesicles (such as Lipofectin™), DNA

conjugates, anhydrous absorption pastes, oil-in-water and water-in-oil emulsions, emulsions carbowax (polyethylene glycols of various molecular weights), semi-solid gels, and semi-solid mixtures containing carbowax. Any of the foregoing mixtures may be appropriate in treatments and therapies in accordance with the present disclosure, provided that the active ingredient in the formulation is not inactivated by the formulation and the formulation is physiologically compatible and tolerable with the route of administration. See also Baldrick P. "Pharmaceutical excipient development: the need for preclinical guidance." *Regul. Toxicol Pharmacol.* 32(2):210-8 (2000), Wang W. "Lyophilization and development of solid protein pharmaceuticals." *Int. J. Pharm.* 203 (1-2):1-60 (2000), Charman WN "Lipids, lipophilic drugs, and oral drug delivery-some emerging concepts." *J Pharm Sci.* 89(8):967-78 (2000), Powell et al. "Compendium of excipients for parenteral formulations" *PDA J Pharm Sci Technol.* 52:238-311 (1998) and the citations therein for additional information related to formulations, excipients and carriers well known to pharmaceutical chemists.

[0341] In some embodiments, the multispecific polypeptide constructs, conjugated multispecific polypeptide constructs, and compositions thereof—referred to collectively herein as the Therapeutic(s) and derivatives, fragments, analogs and homologs thereof, can be incorporated into pharmaceutical compositions suitable for administration. Principles and considerations involved in preparing such compositions, as well as guidance in the choice of components are provided, for example, in Remington's Pharmaceutical Sciences: The Science And Practice Of Pharmacy 19th ed. (Alfonso R. Gennaro, et al., editors) Mack Pub. Co., Easton, Pa.: 1995; Drug Absorption Enhancement: Concepts, Possibilities, Limitations, And Trends, Harwood Academic Publishers, Langhorne, Pa., 1994; and Peptide And Protein Drug Delivery (Advances In Parenteral Sciences, Vol. 4), 1991, M. Dekker, New York.

[0342] Such compositions typically comprise the multispecific polypeptide construct or a conjugated thereof and a pharmaceutically acceptable carrier. Where a multispecific polypeptide construct includes a fragment of an antibody, the smallest fragment of the antibody that specifically binds to the target protein can be used. For example, based upon the variable-region sequences of an antibody, peptide molecules can be designed that retain the ability of the antibody to bind the target protein sequence. Such peptides can be synthesized chemically and/or produced by recombinant DNA technology. (See, e.g., Marasco et al., *Proc. Natl. Acad. Sci. USA*, 90: 7889-7893 (1993)).

[0343] 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.

[0344] The formulations to be used for in vivo administration must be sterile. This is readily accomplished by filtration through sterile filtration membranes.

[0345] 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, 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 bisulfate; 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.

[0346] 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 suitable 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 that delays absorption, for example, aluminum monostearate and gelatin.

[0347] 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. Where the composition is lyophilized, sterilization using this method may be conducted either prior to or following lyophilization and reconstitution. The composition for parenteral administration may be stored in lyophilized form or in solution. In addition, parenteral compositions generally are placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle.

[0348] In some embodiments, the pharmaceutical composition is administered to a subject through any route, including orally, transdermally, by inhalation, intravenously, intra-arterially, intramuscularly, direct application to a wound site, application to a surgical site, intraperitoneally, by suppository, subcutaneously, intradermally, transcutaneously, by nebulization, intrapleurally, intraventricularly, intra-articularly, intraocularly, or intraspinally.

[0349] 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.

[0350] For administration by inhalation, the multispecific polypeptide construct are delivered in the form of an aerosol spray from pressured container or dispenser that contains a suitable propellant, e.g., a gas such as carbon dioxide, or a nebulizer.

[0351] 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.

[0352] 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.

[0353] In one embodiment, the Therapeutics are prepared with carriers that will protect the compound against rapid elimination from the body, such as sustained/controlled release formulations, 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.

[0354] For example, the Therapeutics can be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacrylate) microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles, and nano-capsules) or in macroemulsions.

[0355] In some embodiments, the pharmaceutical composition comprises a pharmaceutically-acceptable excipient, for example a filler, binder, coating, preservative, lubricant, flavoring agent, sweetening agent, coloring agent, a solvent, a buffering agent, a chelating agent, or stabilizer. Examples of pharmaceutically-acceptable fillers include cellulose, dibasic calcium phosphate, calcium carbonate, microcrystalline cellulose, sucrose, lactose, glucose, mannitol, sorbitol, maltol, pregelatinized starch, corn starch, or potato starch. Examples of pharmaceutically-acceptable binders include polyvinylpyrrolidone, starch, lactose, xylitol, sorbitol, maltitol, gelatin, sucrose, polyethylene glycol, methyl cellulose, or cellulose. Examples of pharmaceutically-acceptable coatings include hydroxypropyl methylcellulose (HPMC), shellac, corn protein zein, or gelatin. Examples of pharmaceutically-acceptable disintegrants include polyvinylpyrrolidone, carboxymethyl cellulose, or sodium starch glycolate. Examples of pharmaceutically-acceptable lubricants include polyethylene glycol, magnesium stearate, or stearic acid. Examples of pharmaceutically-acceptable preservatives include methyl parabens, ethyl parabens, propyl paraben, benzoic acid, or sorbic acid. Examples of pharmaceutically-acceptable sweetening agents include sucrose, saccharine, aspartame, or sorbitol. Examples of pharmaceutically-acceptable buffering agents include carbonates, citrates, gluconates, acetates, phosphates, or tartrates.

[0356] Sustained-release preparations can be prepared. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the antibody, which matrices are in the form of shaped articles, e.g., films, or microcapsules. In some embodiments, the pharmaceutical composition further comprises an agent for the controlled or sustained release of the product, such as injectable microspheres, bio-erodible particles, polymeric compounds (polylactic acid, polyglycolic acid), beads, or liposomes. Examples of sustained-release matrices include polyesters, hydrogels (for example, poly (2-hydroxyethyl-methacrylate), or poly(vinylalcohol)), polylactides (U.S. Pat. No. 3,773,919), copolymers of L-glutamic acid and γ ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such as the LUPRON DEPOT™ (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), and poly-D-(-)-3-hydroxybutyric acid. While polymers such as ethylene-vinyl acetate and lactic acid-glycolic acid enable release of molecules for over 100 days, certain hydrogels release proteins for shorter time periods.

[0357] The materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) and 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. Pat. No. 4,522,811.

[0358] 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.

[0359] Further provided are kits comprising the pharmaceutical compositions (or articles of manufacture) described herein. The pharmaceutical compositions can be included in a container, pack, or dispenser together with instructions for administration. The kits described herein may also include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, syringes, and package inserts with instructions for performing any methods described herein.

[0360] The formulation can also contain more than one multispecific polypeptide construct as necessary for the particular indication being treated, for example, those with complementary activities that do not adversely affect each other. In some embodiments, or in addition, the composition can comprise an agent that enhances its function, such as, for example, a cytotoxic agent, cytokine, chemotherapeutic agent, or growth-inhibitory agent. Such molecules are suitably present in combination in amounts that are effective for the purpose intended.

[0361] In some embodiments, the dosage of the pharmaceutical composition is a single dose or a repeated dose. In some embodiments, the doses are given to a subject once per day, twice per day, three times per day, or four or more times per day. In some embodiments, about 1 or more (such as about 2 or more, about 3 or more, about 4 or more, about 5 or more, about 6 or more, or about 7 or more) doses are given in a week. In some embodiments, multiple doses are given over the course of days, weeks, months, or years. In some embodiments, a course of treatment is about 1 or more doses (such as about 2 or more doses, about 3 or more doses, about 4 or more doses, about 5 or more doses, about 7 or more doses, about 10 or more doses, about 15 or more doses, about 25 or more doses, about 40 or more doses, about 50 or more doses, or about 100 or more doses).

[0362] In some embodiments, the pharmaceutical composition is administered to a subject. Generally, dosages and routes of administration of the pharmaceutical composition are determined according to the size and condition of the subject, according to standard pharmaceutical practice. For example, the therapeutically effective dose can be estimated initially either in cell culture assays or in animal models such as mice, rats, rabbits, dogs, pigs, or monkeys. An animal model may also be used to determine the appropriate concentration range and route of administration. Such information can then be used to determine useful doses and routes for administration in humans. The exact dosage will be determined in light of factors related to the subject requiring

treatment. Dosage and administration are adjusted to provide sufficient levels of the active compound or to maintain the desired effect. Factors that may be taken into account include the severity of the disease state, the general health of the subject, the age, weight, and gender of the subject, time and frequency of administration, drug combination(s), reaction sensitivities, and response to therapy. 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.

IV. METHODS OF USE AND THERAPEUTIC ADMINISTRATION

[0363] Also provided are methods for using and uses of the multispecific polypeptide constructs. Such methods and uses include therapeutic methods and uses, for example, involving administration of the molecules 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 and/or composition is administered in an effective amount to effect treatment of the disease or disorder. Uses include uses of the multispecific polypeptide constructs 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 multispecific polypeptide constructs, 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.

[0364] In one embodiment, a multispecific polypeptide construct 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. A multispecific polypeptide construct is administered to the subject. A multispecific polypeptide construct is administered to the subject and will generally have an effect due to its binding with the target(s).

[0365] In some embodiments, provided herein is a method of modulating an immune response in a subject by administering a therapeutically effective amount of any of the provided multispecific conjugates or pharmaceutical compositions. 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.

[0366] Administration of the multispecific polypeptide construct may activate innate immune cells via engagement of FcγRs through the Fc-region of the multispecific polypeptide construct. Administration of the multispecific polypeptide construct may agonize, stimulate, activate, and/or augment innate immune cell effector functions, including ADCC, cytokine release, degranulation and/or ADCC. In examples where a multispecific polypeptide construct contains a cleavable linker, administration of the multispecific polypeptide construct may activate T-cell once the linker(s)

joining the first and second component is cleaved by a protease thereby allowing the anti-CD3 binding portion to bind CD3ε on the T cells. Administration of the multispecific polypeptide construct may agonize, stimulate, activate, and/or augment CD3-mediated T cell activation, cytotoxicity, cytokine release and/or proliferation.

[0367] 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 multispecific conjugates or pharmaceutical compositions. 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.

[0368] In some embodiments, the pharmaceutical composition 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.

[0369] Non-limiting examples of disease include: all types of cancers (breast, lung, colorectal, prostate, melanomas, head and neck, pancreatic, etc.), rheumatoid arthritis, Crohn's disease, 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.

[0370] A therapeutically effective amount of a multispecific polypeptide construct of the disclosure relates generally to the amount needed to achieve a therapeutic objective. As noted above, this may be a binding interaction between the multispecific polypeptide construct and its target antigen(s) that, in certain cases, agonize, stimulate, activate, and/or augment FcγR-mediated innate immune cell activation or CD3-mediated T cell activation. The amount required to be administered will furthermore depend on the binding affinity of the multispecific polypeptide construct for its specific antigen(s), and will also depend on the rate at which an administered multispecific polypeptide construct is depleted from the free volume other subject to which it is administered. Common ranges for therapeutically effective dosing of a multispecific polypeptide construct 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 dosing of a multispecific polypeptide construct of the disclosure 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.

[0371] Efficaciousness of treatment is determined in association with any known method for diagnosing or treating the particular disorder. Methods for the screening of multispecific polypeptide construct 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 multispecific polypeptide constructs 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 pepsan 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.

[0372] The multispecific polypeptide construct are also useful in a variety of diagnostic and prophylactic formulations. In one embodiment, a multispecific polypeptide construct 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.

[0373] In another embodiment of the disclosure, a multispecific polypeptide construct is administered to human individuals diagnosed with a clinical indication associated with one or more of the aforementioned disorders. Upon diagnosis, a multispecific polypeptide construct is administered to mitigate or reverse the effects of the clinical indication.

[0374] Combination Therapies

[0375] In some embodiments, the multispecific polypeptide constructs, conjugated multispecific polypeptide constructs, and compositions thereof—referred to collectively herein as the Therapeutic(s)—are administered in conjunction with one or more additional agents, or a combination of additional agents. Suitable additional agents include current pharmaceutical and/or surgical therapies for an intended application. For example, the Therapeutic(s) can be used in conjunction with an additional chemotherapeutic or anti-neoplastic agent. For example, the Therapeutic(s) and additional agent are formulated into a single therapeutic composition, and the Therapeutic(s) and additional agent are administered simultaneously. In some embodiments, the Therapeutic(s) and additional agent are separate from each other, e.g., each is formulated into a separate therapeutic composition, and the Therapeutic(s) and the additional agent are administered simultaneously, or the Therapeutic(s) and the additional agent are administered at different times during a treatment regimen. For example, the Therapeutic(s) is administered prior to the administration of the additional agent, the Therapeutic(s) is administered subsequent to the administration of the additional agent, or the Therapeutic(s) and the additional agent are administered in an alternating fashion. As described herein, the Therapeutic(s) and additional agent are administered in single doses or in multiple doses. In some embodiments, the additional agent is coupled or otherwise attached to the Therapeutic(s). Suitable addi-

tional agents are selected according to the purpose of the intended application (i.e., killing, prevention of cell proliferation, hormone therapy or gene therapy). Such agents may include but is not limited to, for example, pharmaceutical agents, toxins, fragments of toxins, alkylating agents, enzymes, antibiotics, antimetabolites, antiproliferative agents, hormones, neurotransmitters, DNA, RNA, siRNA, oligonucleotides, antisense RNA, aptamers, diagnostics, radiopaque dyes, radioactive isotopes, fluorogenic compounds, magnetic labels, nanoparticles, marker compounds, lectins, compounds that alter cell membrane permeability, photochemical compounds, small molecules, liposomes, micelles, gene therapy vectors, viral vectors, and the like. Finally, combinations of agents or combinations of different classes of agents may be used.

[0376] In one embodiment, the multispecific polypeptide constructs are administered in combination therapy, i.e., combined with other agents, e.g., therapeutic agents, that are useful for treating pathological conditions or disorders, such as autoimmune disorders and inflammatory diseases. The term “in combination” in this context means that the agents are given substantially contemporaneously, either simultaneously or sequentially. If given sequentially, at the onset of administration of the second compound, the first of the two compounds is still detectable at effective concentrations at the site of treatment.

[0377] For example, the combination therapy can include one or more multispecific polypeptide constructs of the disclosure co-formulated with, and/or co-administered with, one or more additional therapeutic agents, e.g., one or more cytokine and growth factor inhibitors, immunosuppressants, anti-inflammatory agents, metabolic inhibitors, enzyme inhibitors, and/or cytotoxic or cytostatic agents, as described in more detail below. Furthermore, one or more multispecific polypeptide constructs described herein may be used in combination with two or more of the therapeutic agents described herein. Such combination therapies may advantageously utilize lower dosages of the administered therapeutic agents, thus avoiding possible toxicities or complications associated with the various monotherapies.

[0378] In other embodiments, one or more multispecific polypeptide constructs of the disclosure can be co-formulated with, and/or co-administered with, one or more anti-inflammatory drugs, immunosuppressants, or metabolic or enzymatic inhibitors. Nonlimiting examples of the drugs or inhibitors that can be used in combination with the antibodies described herein, include, but are not limited to, one or more of: nonsteroidal anti-inflammatory drug(s) (NSAIDs), e.g., ibuprofen, tenidap, naproxen, meloxicam, piroxicam, diclofenac, and indomethacin; sulfasalazine; corticosteroids such as prednisolone; cytokine suppressive anti-inflammatory drug(s) (CSAIDs); inhibitors of nucleotide biosynthesis, e.g., inhibitors of purine biosynthesis, folate antagonists (e.g., methotrexate (N-[4-[(2,4-diamino-6-pteridiny) methyl] methylamino] benzoyl]-L-glutamic acid); and inhibitors of pyrimidine biosynthesis, e.g., dihydroorotate dehydrogenase (DHODH) inhibitors. Suitable therapeutic agents for use in combination with the antibodies of the disclosure include NSAIDs, CSAIDs, (DHODH) inhibitors (e.g., leflunomide), and folate antagonists (e.g., methotrexate).

[0379] Examples of additional inhibitors include one or more of: corticosteroids (oral, inhaled and local injection); immunosuppressants, e.g., cyclosporin, tacrolimus (FK-

506); and mTOR inhibitors, e.g., sirolimus (rapamycin—RAPAMUNE™ or rapamycin derivatives, e.g., soluble rapamycin derivatives (e.g., ester rapamycin derivatives, e.g., CCI-779); agents that interfere with signaling by proinflammatory cytokines such as TNF α or IL-1 (e.g. IRAK, NIK, IKK, p38 or MAP kinase inhibitors); COX2 inhibitors, e.g., celecoxib, rofecoxib, and variants thereof; phosphodiesterase inhibitors, e.g., R973401 (phosphodiesterase Type IV inhibitor); phospholipase inhibitors, e.g., inhibitors of cytosolic phospholipase 2 (cPLA2) (e.g., trifluoromethyl ketone analogs); inhibitors of vascular endothelial cell growth factor or growth factor receptor, e.g., VEGF inhibitor and/or VEGF-R inhibitor; and inhibitors of angiogenesis. Suitable therapeutic agents for use in combination with the antibodies of the disclosure are immunosuppressants, e.g., cyclosporin, tacrolimus (FK-506); mTOR inhibitors, e.g., sirolimus (rapamycin) or rapamycin derivatives, e.g., soluble rapamycin derivatives (e.g., ester rapamycin derivatives, e.g., CCI-779); COX2 inhibitors, e.g., celecoxib and variants thereof; and phospholipase inhibitors, e.g., inhibitors of cytosolic phospholipase 2 (cPLA2), e.g., trifluoromethyl ketone analogs. Additional examples of therapeutic agents that can be combined with a multispecific polypeptide construct include one or more of: 6-mercaptopurines (6-MP); azathioprine sulphasalazine; mesalazine; olsalazine; chloroquine/hydroxychloroquine (PLAQUENIL®); pencillamine; aurothiomalate (intramuscular and oral); azathioprine; coichicine; beta-2 adrenoreceptor agonists (salbutamol, terbutaline, salmeterol); xanthines (theophylline, aminophylline); cromoglycate; nedocromil; ketotifen; ipratropium and oxitropium; mycophenolate mofetil; adenosine agonists; antithrombotic agents; complement inhibitors; and adrenergic agents.

V. EXEMPLARY EMBODIMENTS

[0380] Among the provided embodiments are:

[0381] 1. A multispecific polypeptide construct, the multispecific polypeptide construct comprising a first component comprising an immunoglobulin Fc region and a second component comprising a CD3-binding region, wherein:

[0382] the first and second components are coupled by a non-cleavable linker, wherein the Fc region is positioned N-terminal to the CD3-binding region; and

[0383] one or both of the first and second components comprises an antigen binding domain that binds a tumor associated antigen (TAA).

[0384] 2. The multispecific polypeptide construct of embodiment 1, wherein the CD3-binding region binds CD3 (CD3 ϵ).

[0385] 3. The multispecific construct of embodiment 1 or embodiment 2, wherein the antigen binding 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.

[0386] 4. The multispecific polypeptide construct of any of embodiments 1-3, wherein the first component comprises a first antigen binding domain and the second component comprises a second antigen binding domain, wherein each of the antigen binding domains bind a tumor associated antigen (TAA).

[0387] 5. The multispecific polypeptide construct of embodiment 4, wherein the first antigen binding domain is positioned amino-terminally relative to the Fc region of the multispecific construct and the second antigen binding

domain is positioned carboxy-terminally relative to the CD3 binding region of the multispecific construct.

[0388] 6. A multispecific polypeptide construct, wherein the multispecific construct comprises in order, from N-terminus to C-terminus:

[0389] a first antigen binding domain that binds to a tumor-associated antigen (TAA);

[0390] an immunoglobulin Fc region;

[0391] a non-cleavable linker;

[0392] a CD3 binding region that binds CD3 (CD3 ϵ); and

[0393] a second antigen binding domain that binds a tumor-associated antigen (TAA).

[0394] 7. A multispecific polypeptide construct, wherein the multispecific construct comprises in order, from N-terminus to C-terminus:

[0395] an immunoglobulin Fc region;

[0396] a non-cleavable linker;

[0397] a CD3 binding region that binds CD3 (CD3 ϵ); and

[0398] an antigen binding domain that binds a tumor-associated antigen (TAA).

[0399] 8. A multispecific polypeptide construct, wherein the multispecific construct comprises in order, from N-terminus to C-terminus:

[0400] an antigen binding domain that binds to a tumor-associated antigen (TAA);

[0401] an immunoglobulin Fc region;

[0402] a non-cleavable linker; and

[0403] a CD3 binding region that binds CD3 (CD3 ϵ).

[0404] 9. The multispecific polypeptide construct of any of embodiments 1-8, wherein the Fc region is a homodimeric Fc region.

[0405] 10. The multispecific polypeptide construct of any of embodiments 1-9, wherein the Fc region is an Fc region of a human IgG1, a human IgG2, a human IgG3, or a human IgG4, or is an immunologically active fragment thereof.

[0406] 11. The multispecific polypeptide construct of any of embodiments 1-10, wherein the Fc region comprises a polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 1 or a sequence of amino acids that has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% sequence identity to SEQ ID NO:1.

[0407] 12. The multispecific polypeptide construct of any of embodiments 1-10, wherein the Fc region comprises a polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 2 or a sequence of amino acids that has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% sequence identity to SEQ ID NO:2;

[0408] the Fc region comprises a polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 4 or a sequence of amino acids that has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% sequence identity to SEQ ID NO:4; or

[0409] the Fc region comprises a polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 5 or a sequence of amino acids that has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% sequence identity to SEQ ID NO:5.

[0410] 13. The multispecific polypeptide construct of any of embodiments 1-6, 9 and 12, wherein the Fc region is a heterodimeric Fc region.

[0411] 14. The multispecific polypeptide construct of embodiment 13, wherein one or both 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:1 or an immunologically active fragment thereof.

[0412] 15. The multispecific polypeptide construct of embodiment 14, wherein each of the Fc polypeptides of the heterodimeric Fc independently comprise at least one amino acid modification.

[0413] 16. The multispecific polypeptide construct of embodiment 15, wherein each of the Fc polypeptides of the heterodimeric Fc comprise a knob-into-hole modification or comprise a charge mutation to increase electrostatic complementarity of the polypeptides.

[0414] 17. The multispecific polypeptide construct of embodiment 16, wherein the amino acid modification is a knob-into-hole modification.

[0415] 18. The multispecific polypeptide construct of any of embodiments 13-17, wherein 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 T366W.

[0416] 19. The multispecific polypeptide construct of embodiment 18, wherein 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 Y349 and the modification of the second Fc polypeptide is at the other of the position Ser354 and Y349.

[0417] 20. The multispecific polypeptide construct of embodiment 16, wherein the amino acid modification is a charge mutation to increase electrostatic complementarity of the polypeptides.

[0418] 21. The multispecific polypeptide construct of any of embodiments 13-16 and 20, wherein 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.

[0419] 22. The multispecific polypeptide construct of any of embodiments 14-21, wherein one of the first or second Fc polypeptide of the heterodimeric Fc further comprises a modification at residue Ile253.

[0420] 23. The multispecific polypeptide construct of embodiment 22, wherein the modification is Ile253Arg.

[0421] 24. The multispecific polypeptide construct of any of embodiments 14-23, wherein one of the first or second Fc polypeptide of the heterodimeric Fc further comprises a modification at residue His435.

[0422] 25. The multispecific polypeptide construct of embodiment 24, wherein the modification is His435Arg.

[0423] 26. The multispecific polypeptide construct of any of embodiments 1-25, wherein the Fc region comprises a polypeptide that lacks Lys447.

[0424] 27. The multispecific polypeptide construct of any of embodiments 1-26, wherein the Fc region comprises a polypeptide comprising at least one modification to enhance FcRn binding.

[0425] 28. The multispecific polypeptide construct of embodiment 27, wherein the modification is at a position selected from the group consisting of Met252, Ser254, Thr256, Met428, Asn434, and combinations thereof.

[0426] 29. The multispecific polypeptide construct of embodiment 28, wherein the modification is at a position

selected from the group consisting of Met252Y, Ser254T, Thr256E, Met428L, Met428V, Asn434S, and combinations thereof.

[0427] 30. The multispecific polypeptide construct of embodiment 28, wherein the modification is at position Met252 and at position Met428.

[0428] 31. The multispecific polypeptide construct of embodiment 30, wherein the modification is Met252Y and Met428L.

[0429] 32. The multispecific polypeptide construct of embodiment 30, wherein the modification is Met252Y and Met428V.

[0430] 33. The multispecific polypeptide construct of any of embodiments 13-32, wherein the first polypeptide of the heterodimeric Fc comprises the sequence of amino acids set forth in any of SEQ ID NOS:82, 86, 94 or 96, and the second polypeptide of the heterodimeric Fc comprises the sequence of amino acids set forth in any of SEQ ID NOS:83, 87, 90, 92, 98 or 100.

[0431] 34. The multispecific polypeptide construct of any of embodiments 1-33, 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 or C1q.

[0432] 35. The multispecific polypeptide construct of embodiment 34, wherein the one or more amino acid modification is deletion of one or more of Glu233, Leu234 or Leu235.

[0433] 36. The multispecific polypeptide construct of any of embodiments 13-32, 34 and 35, wherein the first polypeptide of the heterodimeric Fc comprises the sequence of amino acids set forth in any of SEQ ID NOS: 84, 88, 95 or 97 and the second polypeptide of the heterodimeric Fc comprises the sequence of amino acids set forth in any of SEQ ID NOS: 85, 89, 91, 93, 99 or 101.

[0434] 37. The multispecific polypeptide construct of any of embodiments 1-32, wherein the Fc region comprises a polypeptide comprising at least one modification to enhance FcγR binding.

[0435] 38. The multispecific polypeptide construct of embodiment 37, wherein the modification is modification at Ser239 or Ile332.

[0436] 39. The multispecific polypeptide construct of any of embodiments 1-32 and 37, wherein the glycosylation of the Fc region is modified to enhance FcγR binding as compared to an unmodified Fc region.

[0437] 40. The multispecific polypeptide construct of embodiment 39, wherein the Fc region lacks or has reduced fucose content.

[0438] 41. The multispecific polypeptide construct of any of embodiments 1-40, wherein the CD3 binding region is an anti-CD3 antibody or antigen-binding fragment.

[0439] 42. The multispecific polypeptide construct of embodiment 41, wherein the anti-CD3 antibody or antigen binding fragment comprises a variable heavy chain region (VH) and a variable light chain region (VL).

[0440] 43. The multispecific polypeptide construct of any of embodiments 1-42, wherein the CD3 binding region is monovalent.

[0441] 44. The multispecific polypeptide construct of any of embodiments 41-43, wherein the anti-CD3 antibody or antigen binding fragment is not a single chain antibody, optionally is not a single chain variable fragment (scFv).

[0442] 45. The multispecific polypeptide construct of embodiment 42 or embodiment 44, wherein the Fc is a heterodimeric Fc and the VH and VL that comprise the anti-CD3 antibody or antigen binding fragment are linked to opposite polypeptides of the heterodimeric Fc.

[0443] 46. The multispecific polypeptide construct of any of embodiments 1-45, wherein the CD3 binding region is not able to, or is not substantially able to, bind or engage CD3 unless at least one of the antigen binding domain is bound to its TAA.

[0444] 47. The multispecific polypeptide construct of any of embodiments 1-46, wherein the CD3 binding region is not able to, or is not substantially able, to bind or engage CD3 unless at least two of the antigen binding domain is bound to its TAA.

[0445] 48. The multispecific polypeptide construct of any of embodiments 1-47, wherein the linker is a polypeptide linker.

[0446] 49. The multispecific polypeptide construct of embodiment 48, wherein the linker is a polypeptide of up to 25 amino acids in length.

[0447] 50. The multispecific polypeptide construct of embodiment 48 or embodiment 49, wherein the linker is a polypeptide of 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.

[0448] 51. The multispecific polypeptide construct of any of embodiments 48-50, wherein the linker is a polypeptide that is 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 amino acids in length.

[0449] 52. The multispecific polypeptide construct of any of embodiments 48-51, wherein the linker is a polypeptide that is 3 to 18 amino acids in length.

[0450] 53. The multispecific polypeptide construct of any of embodiments 48-51, wherein the linker is a polypeptide that is 12 to 18 amino acids in length.

[0451] 54. The multispecific polypeptide construction of any of embodiments 48-51, wherein the linker is a polypeptide that is 15 to 18 amino acids in length.

[0452] 55. A multispecific polypeptide construct, the multispecific polypeptide construct comprising a first component comprising a heterodimeric Fc region 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:

[0453] the VH and VL that comprise the anti-CD3 antibody or antigen binding fragment are linked to opposite polypeptides of the heterodimeric Fc;

[0454] the first and second components are coupled by a non-cleavable linker, wherein the heterodimeric Fc region is positioned N-terminal to the anti-CD3 antibody; and

[0455] one or both of the first and second components comprises an antigen binding domain that binds a tumor associated antigen (TAA).

[0456] 56. The multispecific polypeptide construct of any of embodiments 52-55, wherein the non-cleavable linker does not contain a substrate recognition site that is specifically recognized for cleavage by a protease.

[0457] 57. The multispecific polypeptide construct of embodiment 56, wherein the protease is produced by an immune effector cell, by a tumor, or by cells present in the tumor microenvironment.

[0458] 58. The multispecific polypeptide construct of embodiment 57, 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.

[0459] 59. The multispecific polypeptide construct of any of embodiments 56-58, wherein the protease is selected from among matriptase, a matrix metalloprotease (MMP), Granzyme B, and combinations thereof.

[0460] 60. The multispecific polypeptide construct of embodiment 59, wherein the protease is granzyme B.

[0461] 61. The multispecific polypeptide construct of any of embodiments 1-60, wherein the non-cleavable linker comprises GS, GGS, GGGGS (SEQ ID NO:149), GGGGGS (SEQ ID NO:135) and combinations thereof.

[0462] 62. The multispecific polypeptide construct of any of embodiments 1-61, wherein the non-cleavable linker comprises (GGS)_n, wherein n is 1 to 10.

[0463] 63. The multispecific polypeptide construct of any of embodiments 1-62, wherein the non-cleavable linker comprises (GGGGS)_n (SEQ ID NO: 173), wherein n is 1 to 10.

[0464] 64. The multispecific polypeptide construct of any of embodiments 1-62, wherein the non-cleavable linker comprises (GGGGGS)_n (SEQ ID NO:172), wherein n is 1 to 4.

[0465] 65. The multispecific polypeptide construct of any of embodiments 1-64, wherein the non-cleavable linker comprises GGS.

[0466] 66. The multispecific polypeptide construct of any of embodiments 1-64, wherein the non-cleavable linker comprises GGGGS (SEQ ID NO: 149).

[0467] 67. The multispecific polypeptide construct of any of embodiments 1-64, wherein the non-cleavable linker comprises GGGGGS (SEQ ID NO: 135).

[0468] 68. The multispecific polypeptide construct of any of embodiments 1-64, wherein the non-cleavable linker comprises (GGS)₂ (SEQ ID NO: 10).

[0469] 69. The multispecific polypeptide construct of any of embodiments 1-64, wherein the non-cleavable linker comprises GGSGGSGGS (SEQ ID NO: 11).

[0470] 70. The multispecific polypeptide construct of any of embodiments 1-64, wherein the non-cleavable linker comprises GSGSGSGSGGS (SEQ ID NO: 12).

[0471] 71. The multispecific polypeptide construct of any of embodiments 1-64, wherein the non-cleavable linker comprises GSGSGSGSGSGSGGS (SEQ ID NO: 13).

[0472] 72. The multispecific polypeptide construct of any of embodiments 1-64, wherein the non-cleavable linker comprises GGGGSGGGGSGGGGGS (SEQ ID NO: 119).

[0473] 73. The multispecific polypeptide construct of any of embodiments 1-64, wherein the non-cleavable linker comprises GGSGGGSGGGGSGGGGS (SEQ ID NO: 147).

[0474] 74. The multispecific polypeptide construct of any of embodiments 1-64, wherein the non-cleavable linker comprises and GGGGSGGGGSGGGGS (SEQ ID NO:170).

[0475] 75. The multispecific polypeptide construct of any of embodiments 45-74, 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 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 and the VL domain of the anti-CD3 antibody or antigen binding fragment, wherein one or both of the first and second polypeptide comprise at least one antigen-binding domain that binds to a tumor associated antigen (TAA).

[0476] 76. The multispecific polypeptide construct of any of embodiments 1-75, wherein one or more antigen binding domain that binds TAA results in monovalent, bivalent, trivalent, or tetravalent binding to the TAA.

[0477] 77. The multispecific polypeptide construct of embodiment 75, wherein only one of the first or second polypeptide comprises the at least one antigen-binding domain that binds a TAA.

[0478] 78. The multispecific polypeptide construct of embodiment 75 or embodiment 77, wherein the at least one antigen binding domain is positioned amino-terminally relative to the Fc region and/or is positioned carboxy-terminally relative to the CD3 binding region of one of the first or second polypeptide of the multispecific polypeptide construct.

[0479] 79. The multispecific polypeptide construct of embodiment 75 or embodiment 77, wherein the at least one antigen binding domain is positioned amino-terminally relative to the Fc region of the multispecific construct and the second antigen binding domain is positioned carboxy-terminally relative to the CD3 binding region of the multispecific construct.

[0480] 80. The multispecific polypeptide construct of any of embodiments 1-79, wherein the antigen binding domain, or independently each of the antigen binding domains, comprises an extracellular domain or binding fragment thereof of the native cognate binding partner of the TAA, or a variant thereof that exhibits binding activity to the TAA.

[0481] 81. The multispecific polypeptide construct of any of embodiments 1-79, wherein the antigen binding domain, or independently each of the antigen binding domains, 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.

[0482] 82. The multispecific polypeptide construct of embodiment 81, wherein the antibody or antigen-binding fragment thereof is a Fv, a scFv, a Fab, a single domain antibody (sdAb), a VNAR, or a VHH.

[0483] 83. The multispecific polypeptide construct of embodiment 81 or embodiment 82, wherein the antibody or antigen-binding fragment is an sdAb.

[0484] 84. The multispecific polypeptide construct of embodiment 83, wherein the sdAb is a human or humanized sdAb.

[0485] 85. The multispecific polypeptide construct of embodiment 83 or embodiment 84, wherein the sdAb is VHH, VNAR, an engineered VH domain or an engineered VK domain.

[0486] 86. The multispecific polypeptide construct of embodiment 81 or embodiment 82, wherein the antibody or antigen-binding fragment thereof is an scFv.

[0487] 87. The multispecific polypeptide construct of embodiment 81 or embodiment 82, wherein the antibody or antigen-binding fragment thereof is a Fab.

[0488] 88. The multispecific polypeptide construct of embodiment 87, wherein the multispecific polypeptide construct comprises:

[0489] (i) a first polypeptide comprising the first Fc polypeptide of the heterodimeric Fc region, the linker and the VH domain of the anti-CD3 antibody or antigen binding fragment;

[0490] (ii) a second polypeptide comprising the second Fc polypeptide of the heterodimeric Fc region, the linker and the VL domain of the anti-CD3 antibody or antigen binding fragment, and

[0491] (iii) a third polypeptide comprising a VH-CH1 (Fd) or VL-CL of a Fab antibody fragment that binds to a tumor-associated antigen, wherein the first and/or second polypeptide further comprises the other of the VH-CH1 (Fd) or VL-CL of the Fab antibody fragment.

[0492] 89. The multispecific polypeptide construct of embodiment 88, wherein only one of the first or second polypeptide comprises the other of the VH-CH1 (Fd) or VL-CL of the Fab antibody fragment.

[0493] 90. The multispecific polypeptide construct of embodiment 89, wherein both the first or second polypeptide comprises the other of the VH-CH1 (Fd) or VL-CL of the Fab antibody fragment.

[0494] 91. The multispecific polypeptide construct of embodiment 89 or embodiment 90, wherein the other of the VH-CH1 (Fd) or VL-CL of the Fab antibody fragment is positioned amino-terminally relative to the Fc region and/or at the carboxy-terminally relative to the CD3 binding region of one of the first or second polypeptide of the multispecific polypeptide construct.

[0495] 92. The multispecific polypeptide construct of any of embodiments 89-91, wherein the other of the VH-CH1 (Fd) or VL-CL of the Fab antibody fragment is positioned amino-terminally relative to the Fc region of the first polypeptide or second polypeptide and at the carboxy-terminally relative to the CD3 binding region of the other of the first or second polypeptide.

[0496] 93. The multispecific polypeptide construct of any of embodiments 1-92, wherein the antigen binding domain, or independently each of the antigen binding domains, binds to a tumor antigen selected from among 1-92-LFA-3, 5T4, 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 (FR α), GAL3ST1, G-CSF, G-CSFR, GD2, GTR, GLUT1, GLUT4, GM-CSF, GM-CSFR, GP IIb/IIIa receptors, Gp130, GPIIB/IIIA, GPNMB, GRP78, HER2/neu, HER3, HER4, HGF, hGH, HVEM, Hyaluronidase, ICOS, IFN α , IFN β , IFN γ , IgE, IgE Receptor (Fc ϵ R1), 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, Nicas-trin, Notch Receptors, Notch 1, Notch 2, Notch 3, Notch 4, NOV, OSM-R, OX-40, PAR2, PDGF-AA, PDGF-BB, PDGFR α , PDGFR β , PD-1, PD-L1, PD-L2, Phosphatidylserine, P1GF, PSCA, PSMA, PSGR, RAAG12, RAGE, SLC44A4, Sphingosine 1 Phosphate, STEAP1, STEAP2, TAG-72, TAPA1, TEM-8, TGF β , TIGIT, TIM-3, TLR2, TLR4, TLR6, TLR7, TLR8, TLR9, TMEM31, TNF α , 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.

[0497] 94. The multispecific polypeptide construct of any of embodiments 1-93, wherein multispecific antigen binding domain comprises at least a first antigen binding domain and a second antigen binding domain, wherein the first antigen binding domain and second antigen binding domain bind to the same TAA.

[0498] 95. The multispecific polypeptide construct of embodiment 94, wherein the first antigen binding domain and the second antigen binding domain binds a different epitope of the same TAA.

[0499] 96. The multispecific polypeptide construct of embodiment 94, wherein the first antigen binding domain and the second antigen binding domain binds the same epitope of the same TAA.

[0500] 97. The multispecific polypeptide construct of any of embodiments 1-96, wherein multispecific antigen binding domain comprises at least a first antigen binding domain and a second antigen binding domain wherein the first antigen binding domain and the second antigen binding domain bind a different TAA.

[0501] 98. The multispecific polypeptide construct of any of embodiments 5-97, wherein the multispecific polypeptide construct comprises a first linking peptide (LP1) between the first antigen binding domain and the Fc region.

[0502] 99. The multispecific polypeptide construct of any of embodiments 5-98, wherein the multispecific polypeptide construct comprises a second linking peptide (LP2) between the CD3 binding region and the second antigen binding domain.

[0503] 100. The multispecific polypeptide construct of any of embodiments 5-99, wherein the multispecific polypeptide

construct comprises a first linking peptide (LP1) between the first antigen binding domain and the Fc region and a second linking peptide (LP2) between the CD3 binding region and the second antigen binding domain, and wherein the multispecific polypeptide construct has the structural arrangement from N-terminus to C-terminus as follows: first antigen binding domain-LP1-Fc region-non-cleavable linker-CD3 binding region-LP2-second antigen binding domain.

[0504] 101. The multispecific polypeptide construct of embodiment 100, wherein the two linking peptides are identical to each other.

[0505] 102. The multispecific polypeptide construct of embodiment 100, wherein the two linking peptides are not identical to each other.

[0506] 103. The multispecific polypeptide construct of any of embodiments 98-102, wherein LP1 or LP2 is independently a peptide of about 1 to 20 amino acids in length.

[0507] 104. The multispecific polypeptide of embodiment 103, wherein LP1 or LP2 independently comprise a peptide that is or comprises any Gly-Ser linker as set forth in SEQ ID NOS: 10-13, 119, 135, 147, 149 or GGS.

[0508] 105. The multispecific polypeptide construct of any of embodiments 41-104, wherein the anti-CD3 antibody or antigen binding fragment is an Fv antibody fragment.

[0509] 106. The multispecific polypeptide construct of embodiment 105, wherein the Fv antibody fragment comprises a disulfide stabilized anti-CD3 binding Fv fragment (dsFv).

[0510] 107. The multispecific polypeptide construct of any of embodiments 41-106, wherein:

[0511] the anti-CD3 antibody or antigen-binding fragment comprises a VH CDR1 comprising the amino acid sequence TYAMN (SEQ ID NO: 16) or SEQ ID NO:211; a VH CD2 comprising the amino acid sequence RIRSKYNNYATYY-ADSVKD (SEQ ID NO: 17) or SEQ ID NO:212; a VH CDR3 comprising the amino acid sequence HGNFGN-SYVSWEAY (SEQ ID NO: 18), a VL CDR1 comprising the amino acid sequence RSSTGAVTTSNYAN (SEQ ID NO: 19); a VL CDR2 comprising the amino acid sequence GTNKRAP (SEQ ID NO: 20); and a VL CDR3 comprising the amino acid sequence ALWYSNLWV (SEQ ID NO: 21); or the anti-CD3 antibody or antigen-binding fragment comprises a VH CDR1 sequence that includes at least the amino acid sequence GFTFNTYAMN (SEQ ID NO: 211); a VH CDR2 sequence that includes at least the amino acid sequence RIRSKYNNYATY (SEQ ID NO: 212); a VH CDR3 sequence that includes at least the amino acid sequence HGNFGNSYVSWEAY (SEQ ID NO: 18), a VL CDR1 sequence that includes at least the amino acid sequence GSSTGAVTTSNYAN (SEQ ID NO: 229); a VL CDR2 sequence that includes at least the amino acid sequence GTNKRAP (SEQ ID NO: 230); and a VL CDR3 sequence that includes at least the amino acid sequence ALWYSNHWV (SEQ ID NO: 225).

[0512] 108. The multispecific polypeptide construct of embodiment 106 or embodiment 107, wherein the anti-CD3 dsFv comprises:

[0513] a VH having the amino acid sequence of any of SEQ ID NOS: 14 and 32-62 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: 14 and 32-62; and

[0514] a VL having the amino acid sequence of any of SEQ ID NOS: 15 and 63-81 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: 14 and 32-62.

[0515] 109. The multispecific polypeptide construct of any of embodiments 106-108, wherein the anti-CD3 dsFv comprises the amino acid sequence of SEQ ID NO: 14 and the amino acid sequence of SEQ ID NO: 15.

[0516] 110. The multispecific polypeptide construct of any of embodiments 102-104, wherein the anti-CD3 dsFv comprises the amino acid sequence of SEQ ID NO: 44 and the amino acid sequence of SEQ ID NO: 72.

[0517] 111. The multispecific polypeptide construct of any of embodiments 1-109, wherein the multispecific polypeptide construct is conjugated to an agent.

[0518] 112. The multispecific polypeptide construct of embodiment 111, wherein the agent is a therapeutic agent, an antineoplastic agent, a toxin or fragment thereof, a detectable moiety or a diagnostic agent.

[0519] 113. The multispecific polypeptide construct of embodiment 112, wherein the agent is conjugated to the multispecific polypeptide construct via a linker.

[0520] 114. A polynucleotide(s) encoding the multispecific polypeptide constructs of any of embodiments 1-113.

[0521] 115. A polynucleotide encoding a polypeptide chain of any of the multispecific polypeptide constructs of any of embodiments 1-113.

[0522] 116. A polynucleotide, comprising a first nucleic acid sequence encoding a first polypeptide of a multispecific construct of any of embodiments 1-115 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.

[0523] 117. The polynucleotide of embodiment 116, wherein the first nucleic acid sequence and second nucleic acid sequence are operably linked to the same promoter.

[0524] 118. The polynucleotide of embodiment 116 or embodiment 117, wherein the multispecific polypeptide construct comprises a third polypeptide chain, and the polynucleotide further comprises a third nucleic acid encoding the third polypeptide of the multispecific construct.

[0525] 119. The polynucleotide of embodiment 118, wherein the third nucleic acid is separated from the first and/or second polypeptide by an internal ribosome entry site (IRES), or a nucleic acid encoding a self-cleaving peptide or a peptide that causes ribosome skipping and/or the third nucleic acid sequence is operably linked to the same promoter as the first and/or second nucleic acid sequence.

[0526] 120. The polynucleotide of any of embodiments 116-119, 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.

[0527] 121. A vector, comprising the polynucleotide of any of embodiments 114-120.

[0528] 122. The vector of embodiment 121 that is an expression vector.

[0529] 123. The vector of embodiment 121 or 122 that is a viral vector or a eukaryotic vector, optionally wherein the eukaryotic vector is a mammalian vector.

[0530] 124. A cell, comprising polynucleotide or polynucleotides of any of embodiments 114-120, or a vector or vectors of any of embodiments 121-123.

[0531] 125. The cell of embodiment 124, wherein the cell is recombinant or isolated.

[0532] 126. The cell of embodiment 125, wherein the cell is a mammalian cell.

[0533] 127. The cell of embodiment 126, wherein the cell is a HEK293 or CHO cell.

[0534] 128. A method of producing a multispecific polypeptide construct, the method comprising introducing into a cell a polynucleotide or polynucleotides of any of embodiments 114-120 or a vector or vectors of any of embodiments 121-123 and culturing the cell under conditions to produce the multispecific polypeptide construct.

[0535] 129. A method of producing a multispecific polypeptide construct, the method comprising culturing the cell of any of embodiments 124-127 under conditions in which the multispecific polypeptide is produced by the cell.

[0536] 130. The cell of embodiment 128 or 129, wherein the cell is a mammalian cell.

[0537] 131. The cell of embodiment 130, wherein the cell is a HEK293 or CHO cell.

[0538] 132. The method of embodiment 128 or embodiment 129, further comprising isolating or purifying the multispecific polypeptide construct from the cell.

[0539] 133. The method of any of embodiments 128-132, wherein the multispecific polypeptide construct is a heterodimer.

[0540] 134. A multispecific polypeptide construct produced by the method of any of embodiments 128-133.

[0541] 135. A pharmaceutical composition comprising the multispecific polypeptide construct of any of embodiments 1-113 or embodiment 134 and a pharmaceutically acceptable carrier.

[0542] 136. The pharmaceutical composition of embodiment 135 that is sterile.

[0543] 137. A method of stimulating or inducing an immune response, the method comprising contacting a target cell and a T cell with the multispecific polypeptide construct of any of embodiments 1-113 or embodiment 134 or the pharmaceutical composition of embodiments 109 or embodiment 110, said target cell expressing a tumor associated antigen recognized by the multispecific polypeptide construct.

[0544] 138. The method of embodiment 137, wherein the target cell is a tumor cell expressing the tumor associated antigen (TAA).

[0545] 139. The method of embodiment 137 or embodiment 138, wherein the contacting is carried out ex vivo or in vitro.

[0546] 140. The method of any of embodiments 137-149, wherein the contacting is carried out in vivo in a subject.

[0547] 141. A method of stimulating or inducing an immune response in a subject, the method comprising administering, to a subject in need thereof, a therapeutically effective amount of the multispecific conjugate of any of embodiments 1-113 or embodiment 134 or the pharmaceutical composition of embodiments 109 or embodiment 110.

[0548] 142. The method of any of embodiments 137-141, which increases cell-mediated immunity.

[0549] 143. The method of any of embodiments 137-142, which increases T-cell activity.

[0550] 144. The method of any of embodiments 137-143, which increases cytolytic T-cell (CTL) activity.

[0551] 145. The method of any of embodiments 137-144, wherein the immune response is increased against a tumor or cancer.

[0552] 146. The method of any of embodiments 137-145, wherein the method treats a disease or condition in the subject.

[0553] 147. 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 multispecific conjugate of any of embodiments 1-113 or the pharmaceutical composition of embodiments 135 or embodiment 136.

[0554] 148. The method of embodiment 146 or embodiment 147, wherein the disease or condition is a tumor or a cancer.

[0555] 149. The method of any of embodiments 140-148, wherein said subject is a human.

VI. EXAMPLES

[0556] The following examples are included for illustrative purposes only and are not intended to limit the scope of the invention.

Example 1: Method of Producing Multispecific Constructs With Constrained CD3 Binding

[0557] Example 1 describes the generation and expression of multispecific polypeptide constructs containing a CD3 binding region that exhibits constrained CD3 binding. The multispecific constructs were generated in various configurations, as shown in FIG. 1, FIGS. 2A-2B, FIGS. 3A-3C and FIGS. 4A-4B, 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 one or more antigen binding domains that binds a tumor associated antigen (TAA) positioned amino-terminally relative to the Fc region and/or carboxy-terminally relative to the CD3 binding region of the multispecific polypeptide construct.

[0558] A. Design and Generation of Constructs

[0559] 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 either a disulfide-stabilized (dsFv) antibody (anti-CD3 VH with the mutation G44C and VL with the mutation G100C) or contained a non-disulfide stabilized Fv antibody, as set forth in Tables E1.1 and E1.2. Various exemplary Fc polypeptide pairs to facilitate heterodimerization of the polypeptide chains were used as set forth in Tables E1.1 and Table E1.2. One or both of the polypeptide chains additionally encoded one or more TAA antigen binding domain amino-terminal to the Fc domain and/or carboxy-terminal to the CD3 binding region, in various configurations. Similar constructs can be generated using other heterodimeric Fc configurations, including other knob-into-hole configurations, such as any

as described; other CD3-binding regions, including other anti-CD3 antibodies, including dsFv or other monovalent fragments; or other TAA antigen-binding fragments, such as scFv, sdAb or Fab formats can also be used.

[0560] Among generated constructs, the non-cleavable linker included linkers ranging from 3-18 amino acids in size. Examples of non-cleavable linkers used in exemplary generated molecules were GGS (e.g. contained in exemplary construct cx1356), GSGSGS (SEQ ID NO:10, contained in exemplary construct cx1357), GSGSGSGS (SEQ ID NO:11, contained in exemplary construct cx1358), GSGSGSGSGS (SEQ ID NO:12, contained in exemplary construct cx1359), GSGSGSGSGSGS (SEQ ID NO:13, contained in exemplary construct cx1360), and GGGGGSGGGSGSGSGS (SEQ ID NO:119, contained in exemplary construct cx5823 and cx5952) or GSGGGSGSGSGSGSGS (SEQ ID NO: 147, contained in exemplary construct cx681).

[0561] Any antigen binding domain that binds to a TAA can be employed in the provided multispecific polypeptide constructs. Exemplary generated proteins contained an antigen binding domain that binds Folate Receptor Alpha (FR α), B7H3 (CD276), or Delta-like 3 (DLL3). The antigen-binding domain can include single chain fragments (e.g. sdAb or scFv) or two chain antigen-binding fragments (Fabs). When the TAA was provided as a single chain fragment, e.g. sdAb or scFv, the TAA antigen binding domain was linked at the N-terminus to one or both polypeptide chains of the Fc heterodimer (e.g. hole and/or knob) by a peptide linker, e.g. PGGGG (SEQ ID NO:102) and/or was linked at the C-terminus to one or both domains (e.g. VH and/or VL) of the CD3 binding region by a peptide linker, e.g. GGGG (SEQ ID NO:103). Other similar peptide linkers can be employed. When the TAA was provided as a Fab antigen-binding fragment the construct was composed of a VH and CH1 linked directly to one or both Fc polypeptides without a linker, as well as a light chain

composed of a VL and CL. These TAA binding Fabs can be located on the amino- or carboxy-terminus of the heterodimeric Fc.

[0562] Multispecific polypeptide constructs were generated containing 1, 2, 3 or 4 TAA antigen binding domain, such as to provide for monovalent, bivalent, trivalent, or tetravalent binding, respectively. In some cases, the TAA antigen binding domains were the same (mono-epitopic). In some cases, the TAA antigen binding domains were different, such that the generated multispecific polypeptide constructs exhibited specificity for at least two different TAAs, to different epitopes of the same TAA (bi-epitopic) or the same epitopes of the same TAA (mono-epitopic).

[0563] Among the generated proteins were constructs in which the TAA antigen binding domains were composed as single domain antibodies (sdAbs). Polynucleotides were generated to encode polypeptide chains of exemplary multispecific polypeptide constructs containing non-cleavable linkers. These included constructs designated cx1356, cx1357, cx1358, cx1359, cx1360, and cx681, targeting FR α as depicted in FIG. 2B; cx3072, cx5952, cx6079, cx6080, cx6081, cx5823, cx5873, and cx5965, targeting B7H3 as depicted in FIGS. 3A and 3B; and cx5352, cx5499, cx5800, and cx5801 targeting DLL3 as depicted in FIGS. 4A and 4B. Notably, some constructs were generated wherein the VH domain of the dsFv anti-CD3 antibody and the sdAb were both linked to the same side (e.g. hole or knob side) of the Fc heterodimer (e.g. cx3072 and cx5952, shown in FIG. 3A). Constructs were engineered without a disulfide stabilized Fv or were engineered with a disulfide linkage stabilizing the VH and VL domains of the anti-CD3 antibody. Some of the exemplary constructs generated additionally contained a sdAb (containing a CDR1, a CDR2 and a CDR3 set forth in SEQ ID Nos: 221, 222 and 223, respectively) targeting 4-1BB co-stimulatory receptor (e.g. cx5823, cx5873, cx5965, cx5352, cx5801, cx5800). A list of exemplary constrained CD3 binding constructs having sdAb TAA domains is given below in Table E1.1.

TABLE E1.1

Exemplary Constrained CD3 engaging constructs							
Construct ID	Chain	N-term sdAb (Target)	Fc	Linker	CD3 Binding Domain	C-term sdAb (Target)	Disulfide Stabilized
cx681	1	FR α sdAb1 (SEQ ID NO: 120)	IgG1-Knob (SEQ ID NO: 82, 86 or 201)	GSGGGSGSGSGS (SEQ ID NO: 147)	VH34 (SEQ ID NO: 198)	FR α sdAb2 (SEQ ID NO: 121)	yes
	2	FR α sdAb1 (SEQ ID NO: 120)	IgG1-Hole (SEQ ID NO: 83, 87 or 202)	GSGGGSGSGSGS (SEQ ID NO: 147)	VL21 (SEQ ID NO: 200)	FR α sdAb2 (SEQ ID NO: 121)	
cx1356	1	FR α sdAb1 (SEQ ID NO: 120)	IgG1-Knob (SEQ ID NO: 82, 86 or 201)	GGS	VH34 (SEQ ID NO: 198)	FR α sdAb2 (SEQ ID NO: 121)	yes
	2	FR α sdAb1 (SEQ ID NO: 120)	IgG1-Hole (SEQ ID NO: 83, 87 or 202)	GGS	VL21 (SEQ ID NO: 200)	FR α sdAb2 (SEQ ID NO: 121)	
cx1357	1	FR α sdAb1 (SEQ ID NO: 120)	IgG1-Knob (SEQ ID NO: 82, 86 or 201)	GSGSGS (SEQ ID NO: 10)	VH34 (SEQ ID NO: 198)	FR α sdAb2 (SEQ ID NO: 121)	yes

TABLE E1.1-continued

Exemplary Constrained CD3 engaging constructs						
Construct ID	Chain	N-term sdAb (Target)	Fc	Linker	CD3 Binding Domain	C-term sdAb (Target) Disulfide Stabilized
cx1358	2	FRα sdAb1 (SEQ ID NO: 120)	IgG1-Hole (SEQ ID NO: 83, 87 or 202)	GGSGGS (SEQ ID NO: 10)	VL21 (SEQ ID NO: 200)	FRα sdAb2 (SEQ ID NO: 121)
	1	FRα sdAb1 (SEQ ID NO: 120)	IgG1-Knob (SEQ ID NO: 82, 86 or 201)	GGSGGSGGS (SEQ ID NO: 11)	VH34 (SEQ ID NO: 198)	FRα sdAb2 (SEQ ID NO: 121) yes
	2	FRα sdAb1 (SEQ ID NO: 120)	IgG1-Hole (SEQ ID NO: 83, 87 or 202)	GGSGGSGGS (SEQ ID NO: 11)	VL21 (SEQ ID NO: 200)	FRα sdAb2 (SEQ ID NO: 121)
cx1359	1	FRα sdAb1 (SEQ ID NO: 120)	IgG1-Knob (SEQ ID NO: 82, 86 or 201)	GGSGGSGGSGGS (SEQ ID NO: 12)	VH34 (SEQ ID NO: 198)	FRα sdAb2 (SEQ ID NO: 121) yes
	2	FRα sdAb1 (SEQ ID NO: 120)	IgG1-Hole (SEQ ID NO: 83, 87 or 202)	GGSGGSGGSGGS (SEQ ID NO: 12)	VL21 (SEQ ID NO: 200)	FRα sdAb2 (SEQ ID NO: 121)
cx1360	1	FRα sdAb1 (SEQ ID NO: 120)	IgG1-Knob (SEQ ID NO: 82, 86 or 201)	GGSGGSGGSGGSG (SEQ ID NO: 13)	VH34 (SEQ ID NO: 198)	FRα sdAb2 (SEQ ID NO: 121) yes
	2	FRα sdAb1 (SEQ ID NO: 120)	IgG1-Hole (SEQ ID NO: 83, 87 or 202)	GGSGGSGGSGGSG (SEQ ID NO: 13)	VL21 (SEQ ID NO: 200)	FRα sdAb2 (SEQ ID NO: 121)
cx5823	1	B7H3 sdAb4 (SEQ ID NO: 214)	xELL-Knob (SEQ ID NO: 88)	GGGGSGGGGGSG (SEQ ID NO: 119)	VH13 (SEQ ID NO: 44)	B7H3 sdAb5 (SEQ ID NO: 216) yes
	2	None	xELL-Hole (SEQ ID NO: 93)	GGGGSGGGGGSG (SEQ ID NO: 119)	VL10 (SEQ ID NO: 72)	Co-stim Receptor sdAb
cx5952	1	B7H3 sdAb4 (SEQ ID NO: 214)	xELL-Knob (SEQ ID NO: 88)	GGGGSGGGGGSG (SEQ ID NO: 119)	VH13 (SEQ ID NO: 44)	B7H3 sdAb5 (SEQ ID NO: 216) yes
	2	None	xELL-Hole (SEQ ID NO: 93)	GGGGSGGGGGSG (SEQ ID NO: 119)	VL10 (SEQ ID NO: 72)	None
cx6079	1	B7H3 sdAb4 (SEQ ID NO: 214)	Fc-Het-1 (SEQ ID NO: 194)	GGGGSGGGSGGG (SEQ ID NO: 170)	VH32 (SEQ ID NO: 196)	None no
	2	B7H3 sdAb4 (SEQ ID NO: 214)	Fc-Het-2 (SEQ ID NO: 195)	GGGGSGGGSGGG (SEQ ID NO: 170)	VL20 (SEQ ID NO: 199)	None
cx6080	1	B7H3 sdAb4 (SEQ ID NO: 214)	Fc-Het-1 (SEQ ID NO: 194)	GGGGSGGGSGGG (SEQ ID NO: 170)	VH33 (SEQ ID NO: 197)	None yes
	2	B7H3 sdAb4 (SEQ ID NO: 214)	Fc-Het-2 (SEQ ID NO: 195)	GGGGSGGGSGGG (SEQ ID NO: 170)	VL21 (SEQ ID NO: 200)	None
cx6081	1	B7H3 sdAb4 (SEQ ID NO: 214)	Fc-Het-1 (SEQ ID NO: 194)	GGGGSGGGSGGG (SEQ ID NO: 170)	VH13 (SEQ ID NO: 44)	None yes

TABLE E1.1-continued

Exemplary Constrained CD3 engaging constructs							
Construct ID	Chain	N-term sdAb (Target)	Fc	Linker	CD3 Binding Domain	C-term sdAb (Target)	Disulfide Stabilized
	2	B7H3 sdAb4 (SEQ ID NO: 214)	Fc-Het-2 (SEQ ID NO: 195)	GGGGSGGGSGGG GS (SEQ ID NO: 170)	VL10 (SEQ ID NO: 72)	None	
cx5841	1	B7H3 sdAb4 (SEQ ID NO: 214)	xELL-Knob (SEQ ID NO: 84 or 203)	GGGGSGGGGGSG GGGGS (SEQ ID NO: 119)	VH13 (SEQ ID NO: 44)	B7H3 sdAb3 (SEQ ID NO: 217)	yes
	2	None	xELL-Hole (SEQ ID NO: 91, 93 or 206)	GGGGSGGGGGSG GGGGS (SEQ ID NO: 119)	VL10 (SEQ ID NO: 72)	Co-stim Receptor sdAb	
cx5187	1	B7H3 sdAb4 (SEQ ID NO: 214)	xELL-Knob (SEQ ID NO: 84, 88 or 203)	GGGGSGGGGGSG GGGGS (SEQ ID NO: 119)	VH13 (SEQ ID NO: 44)	B7H3 sdAb3 (SEQ ID NO: 217)	yes
	2	None	xELL-Hole (SEQ ID NO: 91, 93 or 206)	GGGGSGGGGGSG GGGGS (SEQ ID NO: 119)	VL10 (SEQ ID NO: 72)	Co-stim Receptor sdAb	
cx5499	1	DLL3 sdAb1 (SEQ ID NO: 219)	xELL-Knob (SEQ ID NO: 84, 88 or 203)	GGGGSGGGGGSG GGGGS (SEQ ID NO: 119)	VH13 (SEQ ID NO: 44)	DLL3 sdAb2 (SEQ ID NO: 220)	yes
	2	None	xELL-Hole (SEQ ID NO: 91, 93 or 206)	GGGGSGGGGGSG GGGGS (SEQ ID NO: 119)	VL10 (SEQ ID NO: 72)	None	
cx3072	1	B7H3 sdAb2 (SEQ ID NO: 215)	IgG1-Knob (SEQ ID NO: 82, 86 or 201)	GGGGSGGGGGSG GGGGS (SEQ ID NO: 119)	VH13 (SEQ ID NO: 44)	B7H3 sdAb1 (SEQ ID NO: 218)	yes
	2	None	IgG1-Knob (SEQ ID NO: 83, 87 or 202)	GGGGSGGGGGSG GGGGS (SEQ ID NO: 119)	VL10 (SEQ ID NO: 72)	None	
cx5873	1	None	xELL-Knob (SEQ ID NO: 84, 88 or 203)	GGGGSGGGGGSG GGGGS (SEQ ID NO: 119)	VH13 (SEQ ID NO: 44)	B7H3 sdAb3 (SEQ ID NO: 217)	yes
	2	None	xELL-Hole (SEQ ID NO: 91, 93 or 206)	GGGGSGGGGGSG GGGGS (SEQ ID NO: 119)	VL10 (SEQ ID NO: 72)	Co-stim Receptor sdAb	
cx5965	1	B7H3 sdAb4 (SEQ ID NO: 214)	xELL-Knob (SEQ ID NO: 84, 88 or 203)	GGGGSGGGGGSG GGGGS (SEQ ID NO: 119)	VH13 (SEQ ID NO: 44)	none	yes
	2	None	xELL-Hole (SEQ ID NO: 91, 93 or 206)	GGGGSGGGGGSG GGGGS (SEQ ID NO: 119)	VL10 (SEQ ID NO: 72)	Co-stim Receptor sdAb	
cx5352	1	DLL3 sdAb1 (SEQ ID NO: 219)	xELL-Knob (SEQ ID NO: 84, 88 or 203)	GGGGSGGGGGSG GGGGS (SEQ ID NO: 119)	VH13 (SEQ ID NO: 44)	DLL3 sdAb2 (SEQ ID NO: 220)	yes
	2	None	xELL-Hole (SEQ ID NO: 91, 93 or 206)	GGGGSGGGGGSG GGGGS (SEQ ID NO: 119)	VL10 (SEQ ID NO: 72)	Co-stim Receptor sdAb	

TABLE E1.1-continued

Exemplary Constrained CD3 engaging constructs							
Construct ID	Chain	N-term sdAb (Target)	Fc	Linker	CD3 Binding Domain	C-term sdAb (Target)	Disulfide Stabilized
cx5800	1	DLL3 sdAb1 (SEQ ID NO: 219)	xELL-Knob (SEQ ID NO: 84, 88 or 203)	GGGGSGGGGGSG GGGGS (SEQ ID NO: 119)	VH13 (SEQ ID NO: 44)	None	yes
	2	None	xELL-Hole (SEQ ID NO: 91, 93 or 206)	GGGGSGGGGGSG GGGGS (SEQ ID NO: 119)	VL10 (SEQ ID NO: 72)	Co-stim Receptor sdAb	
cx5801	1	None	xELL-Knob (SEQ ID NO: 84, 88 or 203)	GGGGSGGGGGSG GGGGS (SEQ ID NO: 119)	VH13 (SEQ ID NO: 44)	DLL3 sdAb2 (SEQ ID NO: 220)	yes
	2	None	xELL-Hole (SEQ ID NO: 91, 93 or 206)	GGGGSGGGGGSG GGGGS (SEQ ID NO: 119)	VL10 (SEQ ID NO: 72)	Co-stim Receptor sdAb	

[0564] Exemplary generated constructs also included constructs in which the TAA antigen binding domains were composed as a Fab (designated MAB formats). In this example, an anti-B7H3 Fab was used composed of a heavy

disulfide linkage stabilizing the VH and VL domains of the anti-CD3 antibody (anti-CD3 VH with the mutation G44C and VL with the mutation G100C). A list of exemplary Fab constructs is provided below in Table E1.2.

TABLE E1.2

Exemplary B7H3-targeted constrained CD3 engaging constructs							
Construct ID	Chain	N-term Domain	Fc	Linker	CD3 Binding Domain	C-term sdAb (Target)	Disulfide Stabilized
cx5067	1	B7H3-Fab (SEQ ID NOs: 127, 128)	Fc-Het-1 (SEQ ID NO: 194)	GGGGSGGGSGGGG S (SEQ ID NO: 170)	VH32 (SEQ ID NO: 196)	None	no
	2	B7H3-Fab (SEQ ID NOs: 127, 128)	Fc-Het-2 (SEQ ID NO: 195)	GGGGSGGGSGGGG S (SEQ ID NO: 170)	VL20 (SEQ ID NO: 199)	None	
cx6083	1	B7H3-Fab (SEQ ID NOs: 127, 128)	Fc-Het-1 (SEQ ID NO: 194)	GGGGSGGGSGGGG S (SEQ ID NO: 170)	VH33 (SEQ ID NO: 197)	None	yes
	2	B7H3-Fab (SEQ ID NOs: 127, 128)	Fc-Het-2 (SEQ ID NO: 195)	GGGGSGGGSGGGG S (SEQ ID NO: 170)	VL21 (SEQ ID NO: 200)	None	
cx6084	1	B7H3-Fab (SEQ ID NOs: 127, 128)	Fc-Het-1 (SEQ ID NO: 194)	GGGGSGGGSGGGG S (SEQ ID NO: 170)	VH13 (SEQ ID NO: 44)	None	yes
	2	B7H3-Fab (SEQ ID NOs: 127, 128)	Fc-Het-2 (SEQ ID NO: 195)	GGGGSGGGSGGGG S (SEQ ID NO: 170)	VL10 (SEQ ID NO: 72)	None	

chain Fd set forth in SEQ ID NO:127 and a light chain set forth in SEQ ID NO:128. Polynucleotides were generated to encode polypeptide chains of exemplary multispecific polypeptide constructs containing non-cleavable linkers. These included cx5067, cx6083, and cx6084, as depicted in FIG. 3C. Constructs were engineered without a disulfide stabilized anti-CD3 antibody Fv or were engineered with a

[0565] B. Expression and Purification of Generated Constructs

[0566] Separate plasmids encoding each chain of the 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 col-

lected after 3-14 days, and purified by protein A chromatography, followed by either preparative size exclusion chromatography (SEC) or flow-through hydrophobic interaction chromatography (HIC). In some cases, heterodimeric protein was enriched for during purification due to a mutation designed into one chain of the heterodimeric Fc at position I253R or H435R (e.g. in the hole-Fc) such that it did not bind protein A, and thus homodimers of I253R or H435R were not purified. The second chromatography step by 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.

[0567] The method favored production of heterodimeric multispecific polypeptide constructs, containing properly paired species of heterodimeric Fc and the anti-CD3 Fv (e.g. disulfide stabilized anti-CD3 Fv). 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.

Example 2: Assessment of Binding of Constrained CD3 Binding Constructs to Cancer Cells and Primary T Cells by Flow Cytometry

[0568] 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.

[0569] A. FR-Alpha Binding

[0570] Binding of exemplary multispecific polypeptide constructs of the disclosure containing an antigen-binding domain directed against Folate Receptor Alpha (FR α) to CD3 on the surface of primary T cells and to FR α expressing cells (Ovar-5) was assessed by flow cytometry. The tumor antigen binding domains of the tested constructs bind the Folate Receptor Alpha (FR α), which is not expressed on the primary T cells. The tested constructs included cx1356 and cx681, containing a non-cleavable linker of 3 amino acids in cx1356 or a non-cleavable linker of 18 amino acids in cx681 (see FIG. 2B and Table E1.1).

[0571] For the studies described in FIGS. 5A and 5B, 100 nM of each construct, cx1356 or cx681 was used and assessed for binding to either the Ovar-5 cells or primary T-cells. An anti-CD3 antibody was included as a control. A titration of the constrained CD3 engaging constructs on either FR α expressing cells (Ovar-5; FIG. 5C) or isolated primary T-cells (FR α negative; FIG. 5D), was conducted to assess binding. Bound constructs were detected using fluorophore-conjugated anti-human IgG Fc secondary antibody.

[0572] The tested FR α -targeting constructs with various linkers between the Fc and the component of the CD3 binding domains were found to bind FR α expressing cells (Ovar-5) (FIGS. 5A and 5C), but lacked the capacity to bind T-cells (FIGS. 5B and 5D). These results are consistent with a finding that binding to CD3 on T cells in isolation is constrained in the provided formats.

[0573] B. B7H3 Binding

[0574] Binding of exemplary multispecific constructs containing an antigen-binding domain directed against B7H3 were assessed for binding to B7H3 positive A375 tumor cells or primary T-cells. The constructs were generated containing antigen-binding domain(s) that were either

sdAbs or a FABs, and that were positioned either only N-terminal to the Fc or both N-terminal to the Fc and C-terminal to the anti-CD3 binding domain (see FIGS. 3A and 3C and Table E1.1). Among the various formats of constructs tested included: sdAb-Fc-dsFV-sdAb (cx3072, cx5952) sdAb-Fc-FV (cx6079), sdAb-Fc-dsFV (cx6080, cx6081), MAB-FV (cx5067) and MAB-dsFV (cx6083, cx6084), where the FV represents the anti-CD3 binding domain composed of VH and VL domain pairs and “ds” notes disulfide stabilized via an engineered interdomain disulfide bond.

[0575] FIGS. 6A-F demonstrate that these constructs are capable of binding to B7H3 but not T-cells in isolation. Binding was assessed as described above via flow cytometry using a fluorophore-conjugated anti-human Fc secondary antibody. cx3072 bound to A375 cells with high affinity (FIG. 6A) but not to isolated T-cells (FIG. 6B). The tested sdAb-Fc-dsFV-sdAb (cx5952) displayed higher binding affinity compared to a FAB containing MAB-FV (cx5067) and MAB-dsFV constructs (cx6083, cx6084) (FIG. 6C). The B7H3-targeting sdAb containing constructs (cx5952, cx6079, cx6080, and cx6081) bound to B7H3 positive cells with similar affinities (depicted in FIG. 6E), with cx5952 displaying higher maximal binding. cx6079, cx6080 and cx6081 contain two identical B7H3-targeting sdAbs, whereas cx5952, and cx3072 contain two distinct B7H3-targeting sdAb that bind different epitopes. The MAB-FV, cx5067, contains two identical B7H3-targeting FAB domains. Notably none of the exemplary B7H3-targeted constrained CD3 engaging constructs bound isolated primary human T-cells, as depicted in FIGS. 6B, 6D, and 6F. These results further support that binding to CD3 on T cells in isolation is constrained in the provided formats.

Example 3: Assessment of CD3 Signaling Activity in Co-Cultures with Antigen-Expressing Target Cells and Impact of Linker Length on Activity

[0576] The effect of various length linkers between the Fc and the component domains (VH and VL) that comprise the CD3 binding region on T-cell activating capacity was tested using a Jurkat reporter assay. The CD3 reporter cells were developed from Jurkat cells that naturally express CD3 and were engineered to express NFAT-driven green fluorescence protein (GFP). Agonism of CD3 results in NFAT signaling and production of green fluorescence.

[0577] Antigen targeting constrained CD3 engaging constructs were titrated onto co-cultures of target cells and Jurkat CD3 reporter cells. In this assay, target cells included either IGROV1 (FR α positive) or NCI-460 (FR α negative). For reporter assays utilizing adherent antigen expressing 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 GFP expression as the total integrated green object in the well.

[0578] FR α -targeting constrained CD3 engaging constructs, generated as described in Example 1 containing GlySer-based linkers of varying lengths as listed in Table E3 were used in these assays.

TABLE E3

Tested Linker Lengths	
SEQ ID NO	Linker
—	gs3: GGS
10	gs6: GGS GGS
11	gs9: GGS GGS GGS
12	gs12: GGS GGS GGS GGS
13	gs15: GGS GGS GGS GGS GGS
119	gs18: GGS GGS GGS GGS GGS GGS
147	gs18: GGS GGS GGS GGS GGS GGS GGS

[0579] As shown in FIGS. 7A-7F, the T-cell activating activity as determined by fluorescent intensity was dependent on co-culture with FR α -antigen expressing target cells. The length of the linker and T-cell activating capacity were positively correlated. T-cell activating capacity was shown to directly relate to linker length, indicating shorter linkers restrict CD3 binding to a greater extent (see FIGS. 7A, 7C and 7E). Importantly, T-cell engagement of the constructs is dependent on TAA-binding, as these constructs did not demonstrate a T-cell binding capacity in isolation (e.g. solution form when unbound to target TAA) as shown above in Example 2. Further, no observable fluorescence was measured in co-cultures with FR α negative cells (FIGS. 7B, 7D and 7E). Together, these constructs displayed restricted or substantially reduced binding to CD3, yet were capable of activating T-cells in a target dependent manner.

Example 4: Assessment of T Cell Activating Activity of B7H3-Targeted Constrained CD3 Binding Constructs

[0580] Constructs containing either a B7H3-targeted sdAb or a Fab as the tumor-associated antigen-binding domain were assessed for T-cell activating activity in a T cell reporter assay and in a T cell cytotoxicity assay. Activity of B7H3-targeted constrained CD3 engaging constructs that were formatted with an anti-B7H3 sdAb (e.g. cx5823, cx6079, cx6080, cx6081, cx3072 and cx5952) or anti-B7H3 MAB constructs formatted with a Fab (e.g. cx5067, cx6083 or cx6084) as the antigen-binding domain(s) were assessed (see FIGS. 3A-3C and Table E1.1). All tested constructs, except cx5067 and cx6079, contained a disulfide-stabilized anti-CD3 Fv (dsFv) containing an interchain disulfide bond created by the modification of anti-CD3 VH G44C paired with VL G100C. The anti-CD3 Fv of cx5067, designated MAB-Fv, was not disulfide-stabilized.

[0581] A. T cell Reporter Activity

[0582] The NFAT-GFP CD3 Jurkat reporter described in Example 3 was used to compare the CD3 agonistic properties of B7H3-targeted constrained CD3 engaging constructs when co-cultured in the presence of B7H3-positive cells (A375) or non-target CCRF-CEM cells that naturally lack B7H3 expression. In this assay, anti-B7H3 sdAb constructs, cx5823, cx6079, cx6080 and cx6081, or anti-B7H3 Fabs constructs, cx5067, cx6083 and cx6084, were used as the B7H3-targeting domains. As shown in FIG. 8A, the constructs containing B7H3-targeted sdAb displayed similar potencies of antigen-dependent CD3 activation. As shown in

FIG. 8C the exemplary cx5823 construct containing B7H3-targeted sdAbs was found to be superior at mediating antigen-dependent CD3 activation compared to the constructs containing B7H3-targeted Fabs. Although cx5823 is formatted with a binding domain for a costimulatory receptor, it is unlikely that this component contributed to the difference in results, since Jurkat T cells do not express the costimulatory receptor. None of the constructs demonstrated activity against the B7H3-negative CCRF-CEM cells (FIGS. 8B and 8D).

[0583] B. Cytotoxicity

[0584] To further assess activity of the molecules, exemplary B7H3-targeted constructs cx3072 and cx5952 (each formatted as sdAb-dsFv), cx6083 and cx6084 (MAB-dsFv), cx5067 (MAB-Fv), cx6079 (sdAb-Fv), and cx6080 and cx6081 (sdAb-dsFv) were tested in a T-cell-mediated cytotoxicity assay. Target cells included the B7H3 positive cell line, A375, and either modified A375 cells, wherein B7H3 gene was disrupted by CRISPR (A375:B7H3 KD), or CCRF-CEM cells that naturally lacked B7H3 expression. Target cells were seeded at 1.0×10^4 cells per well, allowed to settle at room temperature for uniform distribution, and incubated for several hours at 37° C. Primary T cells were negatively enriched from PBMCs isolated from healthy human donor leukopaks and added at a 10:1 T cell-to-target cell ratio. Green caspase-3/7 reagent was added, which fluorescently labels nuclear DNA of cells undergoing apoptosis was added. Multispecific constructs with constrained CD3 engaging activity 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.

[0585] As shown in FIGS. 9A and 9B, exemplary constructs cx3072 and cx5952 containing sdAb B7H3-targeted antigen-binding domains induced potent T-cell-mediated cytotoxicity of B7H3 positive (A375) but not B7H3 negative cell lines.

[0586] When compared to exemplary B7H3-targeting constrained CD3 engagers with Fab B7H3-targeting domains (cx5067, cx6083 and cx6084), the exemplary cx5952 sdAb B7H3-targeting constrained CD3 engager mediated enhanced target-dependent T-cell cytotoxicity (FIG. 10A). No measurable T cell cytotoxicity was observed against the B7H3 negative cell line CCRF-CEM for any of the tested constructs, consistent with the capacity to potentially induce antigen-dependent T-cell activation (FIG. 10B). Of the constructs tested, representative MAB-dsFV constructs cx6084 and cx6083 contained the engineered disulfide, whereas the representative MAB-FV construct cx5067 lacked this stabilizing modification. Notably cx6083 and cx5067 are identical with the exception of the presence (cx6083) or absence (cx5067) of the engineered disulfide within the anti-CD3 FV domain (depicted in FIG. 3C). The engineered disulfide was created by the modification of G44C within VH and G100C within VL. As shown in FIG. 10A, cx6083 displayed superior potency in mediating target-dependent T-cell cytotoxicity compared to cx5067, suggesting that the incorporation of the inter-domain disulfide is beneficial in T-cell mediated cytotoxicity, likely by enhancing proper association of the VH and VL domains that comprise the anti-CD3 FV.

[0587] When compared to other exemplary B7H3-targeting constrained CD3 engagers with sdAb B7H3-targeting domains (cx6079, cx6080 and cx6081), the exemplary

cx5952 sdAb B7H3-targeting constrained CD3 engager mediated enhanced target-dependent T-cell cytotoxicity (FIG. 10C). No measurable T cell cytotoxicity was observed against the B7H3 negative cell line CCRF-CEM for any of the tested constructs, consistent with the capacity to potentially induce antigen-dependent T-cell activation (FIG. 10D). Of the constructs tested, sdAb-dsFV constructs cx5952, cx6080 and cx6081 contained the engineered disulfide linkage, whereas the sdAb-FV construct cx6079 lacked this stabilizing modification. The engineered disulfide was created by the modification of G44C within VH and G100C within VL. Notably, cx5952 was engineered to have two distinct B7H3 targeting domains, one located at the amino terminal and one located at the carboxy terminal. cx6079, cx6080, and cx6081 were engineered to have two identical B7H3 targeting domains, both located at the amino terminal (see FIG. 3A).

[0588] C. T Cell Modulation

[0589] To further assess T cell modulation, exemplary multispecific CD3 constrained binding constructs was assessed by monitoring the ability of the constructs to modulate T cell activation markers. To assess T cell activation, suspension cells from T cell cytotoxicity assays above, involving culture of T cells with B7H3 positive (A375) or B7H3 negative cell lines (CCRF-CEM) in the presence of an exemplary B7H3-targeted constrained CD3 engaging construct, cx5952, were collected. Cells were 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.

[0590] Results are shown for CD25 expression (FIG. 11A), CD69 expression (FIG. 11B) and CD71 expression (FIG. 11C) on CD4+ and CD8+ T cells following the co-culture with B7H3 positive (A375) or B7H3 negative cell lines (CCRF-CEM) in the presence of cx5952. The results showed that cx5952 mediated a dose-dependent B7H3-dependent T-cell activation via CD3 binding, as evidenced by increased expression of CD25, CD69 and CD71 in CD4+ and CD8+ T cells.

[0591] When compared to the other exemplary B7H3-targeting constrained CD3 engagers with sdAb B7H3-targeting domains (cx6079, cx6080 and cx6081), the exemplary cx5952 sdAb B7H3-targeting constrained CD3 engager mediated increased T cell activation as evidenced by increased expression of CD25 in CD4+ T cells (FIG. 11D) and in CD8+ T cells (FIG. 11I) and increased expression of CD71 in CD4+ T cells (FIG. 11F) and in CD8+ T cells (FIG. 11J). Increased expression of the surface markers on T cells was not observed in the presence of the B7H3-targeting constrained CD3 engager constructs in cultures with B7H3 negative cell lines (FIGS. 11E and 11G for CD4+ T cells and FIGS. 11I and 11K for CD8+ T cells).

[0592] D. T Cell Cytokine Production

[0593] Supernatants from T cell cytotoxicity tumor cell co-culture assays, involving co-culture of T cells with B7H3 positive, A375 or negative, CCRF-CEM cells in the presence of cx5952, cx6083, cx6084 or cx5067, were analyzed for IFN γ content by sandwich ELISA. 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. 12A, the representative sdAb-Fc-dsFV-sdAb construct, cx5952, was superior to the tested B7H3-targeted FAB containing constructs, cx6083, cx6084 and cx5067 at eliciting target-dependent cytokine release from activated T-cells. Importantly, the MAB-dsFV constructs, cx6083 and cx6084 were superior to the MAB-FV construct, cx5067, demonstrating the importance of interdomain disulfide stabilizing modification for enhancing T-cell function.

[0594] When compared to the other exemplary B7H3-targeting constrained CD3 engagers with sdAb B7H3-targeting domains (cx6079, cx6080 and cx6081), the exemplary cx5952 sdAb B7H3-targeting constrained CD3 engager mediated substantially increased production of IFN γ in the presence of B7H3-target cells T cells but not in cultures with B7H3 negative cell lines (FIG. 12B)

[0595] E. Summary

[0596] These observations further support that the antigen-targeted constrained CD3 format provided herein lack or exhibit reduced T-cell binding in isolation while maintaining potent B7H3-dependent T-cell cytotoxicity inducing capacities. Without wishing to be bound by theory, together these results show that utilization of antigen targeted sAbs instead of a Fabs may reduce the immune synapse distance between the TAA expressing tumor cell and the CD3 expressing T-cells and enhance T cell activity and cytotoxicity. Notably, it was found that the inclusion of an interchain disulfide bond created by the modification of anti-CD3 VH G44C paired with VL G100C greatly enhanced the activity of constrained CD3 engaging constructs. Further, the more potent B7H3-dependent T cell activity by cx5952 compared to other sdAb B7H3-targeting domain constructs suggests that the positioning of the B7H3-targeting sdAb C-terminal to the anti-CD3 binding domain or the fact that cx5952 binds two distinct epitopes on B7H3 whereas the other constructs tested bind to a single epitope in a bivalent manner, contributed to this enhanced activity.

Example 5: Assessment of CD3-Constrained Multispecific Constructs Containing Single or Multiple B7H3-Binding Targeting Domains

[0597] Activity of constructs containing a monovalent sdAb antigen-binding domain (positioned at either the N or C-terminus) was compared to activity of dual binding (bivalent) constructs that contained antigen-targeting sAbs positioned at both the N and C-termini. Binding was assessed substantially as described in Example 2 and T cell activity was assessed in the Jurkat reporter assay and T cell cytotoxicity assays substantially as described in Examples 3 and 4.

[0598] A. Binding

[0599] As shown in FIG. 13A the bivalent B7H3-targeting constrained CD3 engaging constructs, cx5187 and cx5823, displayed higher affinity binding to B7H3 positive A375 cells, compared to the monovalent versions, cx5873 and cx5965. None of these constructs displayed any detectable binding to B7H3 negative CCRF-CEM cells or isolated T-cells (FIG. 13B).

[0600] B. T Cell Reporter Activity

[0601] B7H3 Antigen-dependent CD3 agonistic capacities of antigen-targeted constrained CD3 engaging constructs that engage the antigen in a monovalent or bivalent manner were assessed using CD3-NFAT Jurkat reporter cells, in an

assay substantially as described above. As shown in FIG. 13C, substantially increased fluorescence reporter activity was observed in the presence of the exemplary bivalent B7H3-targeted construct cx5187 compared to reporter activity for the exemplary monovalent constructs cs5873 and cx5965. No reporter activity was observed when constructs were incubated with Jurkat reporter cells co-cultured with B7H3-negative CCRF target cells (FIG. 13D).

[0602] C. Cytotoxic Activity

[0603] Cytotoxicity of B7H3-targeted CD3 constrained binding constructs was assessed against a melanoma cell line, A375, and a T-cell acute lymphoblastic leukemia cell line, CCRF-CEM, which were used as B7H3 positive and negative cell lines, respectively. Cytotoxicity was assessed substantially as described in Example 4. As shown in FIG. 14A an exemplary bivalent B7H3-targeted constrained CD3 engaging construct, cx5187, displayed enhanced target-dependent T-cell mediated cytotoxicity compared to the monovalent versions of the constructs, cx5873 and cx5965. In these assays, no cytotoxicity was observed in the absence of B7H3 expression of the target cells, as shown in FIG. 14B wherein the CCRF-CEM cells were used as target cells.

[0604] D. T Cell Modulation

[0605] T cell modulation was assessed by monitoring expression of CD25, substantially as described in Example 4, in suspension cells from T cell cytotoxicity assays above, involving culture of T cells with B7H3 positive (A375) or B7H3 negative cell lines (CCRF-CEM) in the presence of cx5187, cx5873 or cx5965. As shown in FIGS. 15A and 15B, an exemplary bivalent B7H3-targeted constrained CD3 engaging construct, cx5187, displayed enhanced target-dependent T-cell mediated activation compared to the monovalent versions of the constructs, cx5873 and cx5965, as evidenced by enhanced potency of CD25 upregulation on CD4 and CD8 T-cells. In these assays, no T-cell activation was observed in the absence of B7H3 expression of the target cells, as shown in FIGS. 15C and 15D, wherein the CCRF-CEM cells were used as target cells. These results demonstrated that the B7H3-targeting constrained CD3 engaging constructs induced potent antigen-dependent activation of both CD4 and CD8 T-cells.

[0606] D. Summary

[0607] Together, these results demonstrate that bivalent antigen-targeted constrained CD3 engaging constructs displayed superior antigen-dependent CD3 binding and activity than the monovalent antigen-targeted constrained CD3 engaging constructs. These results are consistent with a finding that constructs containing dual antigen-binding domains positioned at both the N and C-termini have superior binding and T cell activity than monovalent constructs containing only a single monovalent antigen-binding domain. Furthermore, without wishing to be bound by theory, positioning one of the sdAbs C-terminal to the CD3 binding domain may form a more optimal immune synapse compared to constructs wherein the sdAbs are only positioned N-terminal to the Fc as the latter may increase the immune synapse distance.

Example 6: Assessment of a CD3-Constrained Multispecific Constructs Containing B7H3-Targeting sdAb and Fab Domains

[0608] Constructs containing either B7H3-targeted sdAb (s) or a Fab as the tumor-associated antigen-binding domain were assessed for T-cell activating activity. Activity of

B7H3-targeted constrained CD3 engaging constructs that were formatted with anti-B7H3 sdAbs (e.g. cx5952 and cx6079) or anti-B7H3 MAB constructs formatted with a Fab (e.g. cx5067, cx6083 or cx6084) as the antigen-binding domain(s) were assessed (see FIGS. 3A and 3C and Table E1.1 and E1.2). All tested constructs, except cx6079 and cx5067, contained a disulfide-stabilized anti-CD3 Fv (dsFv) containing an interchain disulfide bond created by the modification of anti-CD3 VH G44C paired with VL G100C. The anti-CD3 Fv of cx5067, designated MAB-Fv, and the anti-CD3 Fv of cx6079, designated sdAb-Fc-Fv, were not disulfide-stabilized. Additionally, cx5952 was engineered to contain two distinct B7H3-targeting sdAb domains, with one located N-terminal to the Fc domain and one located C-terminal to the CD3-binding domain. By contrast, cx6079 was engineered to contain two identical B7H3-targeting sdAb domains, both located N-terminal to the Fc domain. The Fvs of all three Fab constructs were engineered to be N-terminal to the Fc domain.

[0609] A. Cytotoxicity

[0610] Cytotoxicity of B7H3-targeted CD3 constrained binding constructs was assessed substantially as described in Example 4. Cytotoxicity was assessed against a melanoma cell line, A375, and a T-cell acute lymphoblastic leukemia cell line, CCRF-CEM, which were used as B7H3 positive and negative cell lines, respectively. As shown in FIG. 16A the exemplary constrained CD3 engaging constructs formatted with B7H3-targeting sdAbs, cx5952 and cx6079, were superior at eliciting antigen-dependent T-cell cytotoxicity compared to the anti-B7H3 MAB constructs formatted with a Fab, cx5067, cx6083, and cx6084. Notably, cx5952 was more potent than cx6079, suggesting the positioning of the B7H3-targeting sdAb C-terminal to the anti-CD3 binding domain and/or the stabilization of the anti-CD3 FV via engineered disulfide contributed to this enhanced activity. In these assays, no cytotoxicity was observed in the presence of B7H3-negative CRF-CEM cells target cells, as shown in FIG. 16B.

[0611] B. T Cell Modulation

[0612] To further assess T cell modulation, exemplary multispecific CD3 constrained binding constructs were assessed by monitoring the ability of the constructs to modulate T cell activation markers, substantially as described in Example 4. To assess T cell activation, suspension cells from T cell cytotoxicity assays above, involving culture of T cells with B7H3 positive (A375) or B7H3 negative cell lines (CCRF-CEM) in the presence of an exemplary B7H3-targeted constrained CD3 engaging constructs, were collected. Tested constructs included anti-B7H3 constructs formatted with sdAbs (e.g. cx5952 and cx6079) and anti-B7H3 constructs formatted with a Fab (e.g. cx5067, cx6083 and cx6084).

[0613] Cells were stained with a live/dead stain and fluorophore-conjugated anti-CD4, anti-CD8, anti-CD25 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 or CD71 or percent CD25- or CD71-positive.

[0614] Results are shown for CD25 expression (FIG. 16C-F) and CD71 expression (FIG. 16G-J) on CD4+ and CD8+ T cells following the co-culture with B7H3 positive (A375) or B7H3 negative cell lines (CCRF-CEM) in the presence of the described constructs. The results showed that cx5952 mediated a dose-dependent B7H3-dependent T-cell

activation via CD3 binding, as evidenced by increased expression of CD25 and CD71 in CD4+ and CD8+ T cells. cx5952 was the most potent over other B7H3-targeted constrained CD3 engaging constructs and inducing T-dependent T-cell activation.

[0615] C. T Cell Cytokine Production

[0616] Supernatants from T cell cytotoxicity tumor cell co-culture assays, involving co-culture of T cells with B7H3 positive, A375 or negative, CCRF-CEM cells in the presence of cx5952, cx6079, cx6083, cx6084 or cx5067, were analyzed for IFN γ content by sandwich ELISA. 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. 16K, the representative sdAb-Fc-dsFv-sdAb construct, cx5952, was superior to the tested B7H3-targeted FAB containing constructs, cx6083, cx6084 and cx5067 at eliciting target-dependent cytokine release from activated T-cells. This is consistent with the finding from the antigen dependent cytotoxicity and activation assays. Importantly, the MAB-dsFv constructs, cx6083 and cx6084 were superior to the MAB-Fv construct, cx5067, demonstrating the importance of interdomain disulfide stabilizing modification for enhancing T-cell function.

[0617] D. Summary

[0618] Together, these results demonstrate that constrained anti-CD3 constructs formatted with anti-B7H3 sdAb binding domains were superior at eliciting antigen-dependent T-cell cytotoxicity compared to the anti-B7H3 MAB constructs formatted with a Fab B7H3 binding domain. Further, that cx5952 was more potent than cx6079 suggested that the positioning of the B7H3-targeting sdAb C-terminal to the CD3 binding domain, the stabilization of the anti-CD3 Fv via engineered disulfide, or both, contribute to enhanced activity. Without wishing to be bound by theory, positioning one of the sdAbs C-terminal to the CD3 binding domain may form a more optimal immune synapse compared to constructs wherein the sdAbs are only positioned N-terminal to the Fc as the latter may increase the immune synapse distance.

Example 7: Assessment of CD3-Constrained Multispecific Constructs Containing Single or Multiple Antigen-Binding DLL3-Targeting Domains

[0619] This example describes the assessment and characterization of exemplary generated DLL3-targeted constrained CD3 engaging constructs in human primary T cell in vitro assays.

[0620] Binding and activity of DLL3-targeted constrained CD3 engaging constructs that were formatted with an anti-DLL3 sdAb (e.g. cx5352, cx5800, cx5801, and cx5499) as the antigen-binding domain(s) were assessed (see FIGS. 4A-4B and Table E1.1). All tested constructs contained a disulfide-stabilized anti-CD3 Fv (dsFv) containing an inter-chain disulfide bond created by the modification of anti-CD3 VH G44C paired with VL G100C. Further, the DLL3-targeted constructs were engineered to contain a co-stimulatory receptor sdAb C-terminal to the CD3 dsFv, except for cx5499, which did not contain this co-stimulatory receptor sdAb domain.

[0621] A. Binding

[0622] Binding was assessed substantially as described in Example 2. As shown in FIG. 17A the bivalent DLL3-targeting constrained CD3 engaging constructs, cx5352 displayed higher affinity binding to DLL3 positive, SHP-77 cells compared to the monovalent versions, cx5800 and cx5801. None of the constructs tested displayed binding to DLL3-negative primary T cells, as depicted in FIG. 17B. These binding assays were conducted by flow cytometry, wherein bound constructs were detected using a fluorophore-conjugated anti-human IgG Fc secondary antibody.

[0623] A. T Cell Reporter Activity

[0624] T cell activity was assessed in a reporter assay substantially as described in Example 2, except that Jurkat cells expressing NFAT-driven Luciferase were used and luciferase activity was monitored. NFAT-driven Luciferase CD3 Jurkat reporter cells were co-cultured with SHP-77 (DLL3-positive) target cells in the presence of monovalent and bivalent constructs containing antigen-binding domains against the DLL3 antigen (see FIG. 4A). Specifically, as shown in FIG. 17C, the exemplary bivalent construct cx5352 induced substantially greater luciferase activity in this assay compared to the exemplary monovalent constructs cx5800 and cx5801. These results are consistent with results observed with B7H3-targeted constructs, thereby indicating that the activity of the constructs is not specific to a particular target antigen.

[0625] C. Cytotoxicity

[0626] Cytotoxicity of cx5499, a DLL3-targeted CD3 constrained binding construct formatted with two distinct sdAb binding domains located at its amino and carboxy termini, was assessed against a DLL3 expressing cell line, SHP-77, using an assay substantially as described in Example 4. As shown in FIG. 18A, cx5499 induced potent T-cell mediated cytotoxicity directed toward the SHP-77 cell line.

[0627] D. T Cell Modulation

[0628] To further assess T cell modulation, exemplary multispecific CD3 constrained binding constructs were assessed by monitoring the ability of the constructs to modulate T cell activation markers. To assess T cell activation, suspension cells from T cell cytotoxicity assays above, involving culture of T cells with DLL3 positive SHP-77 cells in the presence of cx5499, in the presence of an exemplary DLL3-targeted constrained CD3 engaging constructs, were collected. Cells were stained with a live/dead stain and fluorophore-conjugated anti-CD4, anti-CD8, anti-CD25 and/or anti-CD69 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 or CD69 or percent CD25- or CD69-positive.

[0629] FIG. 18B and FIG. 18D depict results for CD25 expression on CD4 T cells or CD8 T cells, respectively, upon culture of T cells with DLL3 positive, SHP-77 cells, in the presence of an exemplary DLL3-targeted constrained CD3 engaging construct, cx5499. FIG. 18C and FIG. 18E depict results for CD69 expression on CD4 cells or CD8 T cells, respectively, upon culture of T cells with DLL3 positive, SHP-77 cells, in the presence of an exemplary DLL3-targeted constrained CD3 engaging construct, cx5499. The results showed that cx5499 mediated a dose-dependent DLL3-dependent T-cell activation via CD3 binding, as evidenced by increased expression of CD25 and CD69 on CD4+ and CD8+ T cells.

[0630] E. Summary

[0631] Together, these results demonstrate that constrained anti-CD3 constructs formatted with anti-DLL3 sdAb binding domains are capable of binding to a DLL3-expressing cell line, SHP-77, and eliciting antigen-dependent T-cell cytotoxicity and activation. This result is consistent with a finding that the constrained CD3 engaging constructs of the disclosure have broad applicability to

specifically target numerous tumor antigens and elicit T-cell cytotoxicity and activation against target-expressing cells.

[0632] While the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the disclosure, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.

SEQUENCE TABLE						
SEQ ID NO	SEQUENCE					DESCRIPTION
1	PAPELLGGPS VFLFPPKPKD TLMISRTPEV TCVVVDVSHE DPEVKFNWYV DGVEVHNAKT KPREEQYNST YRVVSVLTVL HQDWLNGKEY KCKVSNKALP APIEKTISKA KGQPREPQVY TLPPSRDEL TKNQVSLTCLV KGFYPSDIAV EWESNGQPEN NYKTPPVLD SDGSFFLYSK LTVDKSRWQQ GNVFSCSVMH EALHNHYTQK SLSLSPGK					IgG1 Fc
2	PAPGGPSVFL FPPKPKDTLM ISRTPEVTCV VVDVSHEDPE VKFNWYVDGV EVHNAKT KPR EEQYNSTYRV VSVLTVLHQD WLNGKEYCK VSNKALPAPI EKTISKAKGQ PREPQVYTL PPSRDELTKNQ VSLTCLVKGF YPSDIAVEWE SNGQPENNYK TTPPVLDSDG SFFLYSKLTV DKSRWQQGNV FSCSVMEAL HNHYTQKSLS LSPGK					xELL Fc
3	PAPPVAGPSV FLFPPKPKDT LMISRTPEVT CVVVDVSHED PEVQFNWYVD GVEVHNAKT KPREEQFNSTF RVVSVLTVVH QDWLNGKEY CKVSNKGLPA PIEKTISKTK GQPREPQVYT LPPSREEMTK NQVSLTCLVK GFYPSDISVE WESNGQPENN YKTPPMLDS DGSFFLYSKL TVDKSRWQQG NVFSCSVME ALHNHYTQKS LSLSPGK					IgG2 Fc
4	PAPELLGGPS VFLFPPKPKD TLMISRTPEV TCVVVDVSHE DPEVQFKWYV DGVEVHNAKT KPREEQYNST FRVSVLTVL HQDWLNGKEY KCKVSNKALP APIEKTISKTK KGQPREPQVY TLPPSREEMTK NQVSLTCLV KGFYPSDIAV EWESSGQPEN NYNTTPPMLD SDGSFFLYSK LTVDKSRWQQ GNIFSCSVMH EALHNRTQK SLSLSPGK					IgG3 Fc
5	PAPEFLGGPS VFLFPPKPKD TLMISRTPEV TCVVVDVSQE DPEVQFNWYV DGVEVHNAKT KPREEQFNST YRVVSVLTVL HQDWLNGKEY KCKVSNKGLP SSIEKTISKA KGQPREPQVY TLPPSQEEMTK NQVSLTCLV KGFYPSDIAV EWESNGQPEN NYKTPPVLD SDGSFFLYSR LTVDKSRWQE GNVFSCSVMH EALHNHYTQK SLSLSLGK					IgG4 Fc
6	PAPELLGGPS VFLFPPKPKD TLMISRTPEV TCVVVDVSQE DPEVQFNWYV DGVEVHNAKT KPREEQFNST YRVVSVLTVL HQDWLNGKEY KCKVSNKGLP SSIEKTISKA KGQPREPQVY TLPPSQEEMTK NQVSLTCLV KGFYPSDIAV EWESNGQPEN NYKTPPVLD SDGSFFLYSR LTVDKSRWQE GNVFSCSVMH EALHNHYTQK SLSLSLGK					IgG4 Fc
7	EPKSSDKTHTCPPC					Hinge
8	DKTHTCPPC					Hinge
9	ESKYGPPCPPC					Hinge
10	GGSGGS					(GGG) ₂
11	GGSGGSGGS					(GGG) ₃
12	GGSGGSGGSGGS					(GGG) ₄
13	GGSGGSGGSGGSGGS					(GGG) ₅
14	EVQLVESGGGLVQPKGSLKLSCAASGFTFNTYAMNWVRQAPGKGLEWVA RIRSKYNNYATYYADSVKDRFTISRDDQSILYLQMNNLKTEDTAMYYCVR HGNFGNSYVSWFAYWGQGLTVTSA					anti-CD3 VH
15	QAVVTQESALTTSPGETVTLTCSRSTGAVTTSNYANWVQEKPDHLFTGLIG GTNKRAPGVPARFSGSLIGDKAALTITGAQIEDEAIYFCALWYSNLWVFGG GTKLTVL					anti-CD3 VL
16	TYAMN					anti-CD3 VH CDR1

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SEQUENCE TABLE		
SEQ ID NO	SEQUENCE	DESCRIPTION
17	RIRSKYNNYATYYADSVKD	anti-CD3 VH CDR2
18	HGNFGNSYVSWFAY	anti-CD3 VH CDR3
19	RSSTGAVTTSNYAN	anti-CD3 VL CDR1
20	GTNKRAP	anti-CD3 VL CDR2
21	ALWYSNLWV	anti-CD3 VL CDR3
22	LEAD	Granzyme B substrate
23	RQAR	Granzyme B substrate
24	PAGL	MMP substrate
25	TGLEADGSPAGLGRQARVG	Linker
26	TGLEADGSRQARVGPAGLG	Linker
27	TGSPAGLEADGSRQARVGS	Linker
28	TGPAGLGLEADGSRQARVG	Linker
29	TGRQARVGLEADGSPAGLG	Linker
30	TGSRQARVGPAGLEADGS	Linker
31	TGPAGLGSRQARVGLEADGS	Linker
32	EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPGKGLEWVGR RIRSKYNNYATYYADSVKDRFTISRDDSKNSLYLQMNSLKTEDTAVYYCVRH GNFGNSYVSWFAYWGQGLTLTVSS	anti-CD3 VH1
33	EVKLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVA RIRSKYNNYATYYADSVKDRFTISRDDSKNSLYLQMNNLKTEDTAMYYCVRH HGNFGNSYVSWFAYWGQGLTLTVSS	anti-CD3 VH2
34	EVKLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVAR IRSKYNNYATYYADSVKDRFTISRDDSKNSLYLQMNNLKIEDTAMYYCVRH GNFGNSYVSWFAYWGQGLTLTVSS	anti-CD3 VH3
35	EVKLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVAR IRSKYNNYATYYADSVKDRFTISRDDSKNSLYLQMNSLKIEDTAMYYCVRH GNFGNSYVSWFAYWGQGLTLTVSS	anti-CD3 VH4
36	EVKLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVAR IRSKYNNYATYYADSVKDRFTISRDDSKNSLYLQMNSLKIEDTAMYYCVRH GNFGNSYVSWFAYWGQGLTLTVSS	anti-CD3 VHS
37	EVQLLESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPGKGLEWVSRI RSKYNNYATYYADSVKGRFTISRDDSKNTLYLQMNSLRAEDTAVYYCVRH GNFGNSYVSWFAYWGQGLTLTVSS	anti-CD3 VH6
38	EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMSWVRQAPGKGLEWVGR IRSKYNNYATYYADSVKGRFTISRDDSKNTLYLQMNSLRAEDTAVYYCVRH GNFGDSYVSWFAYWGQGLTLTVSS	anti-CD3 VH7
39	EVQLVESGGGLVQPGGSLRLSCAASGFTFNKYAMNWVRQAPGKGLEWVA RIRSKYNNYATYYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCV RHGNFGNSYISYWAYWGQGLTLTVS	anti-CD3 VH8

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SEQUENCE TABLE		
SEQ ID NO	SEQUENCE	DESCRIPTION
40	EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVA RIRSKYNNYATYYADSVKGRFTISRDDSKNTLYLQMNSLRAEDTAVYYCVR HGNFGNSYVSWFAYWGQGTITVTVSS	anti-CD3 VH9
41	EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVA RIRSKYNNYATYYADSVKGRFTISRDDSKNTLYLQMNSLRAEDTAVYYCVR HGNFGNSYVSWFAYWGQGTITVTVSS	anti-CD3 VH10
42	EVQLVESGGGLVQPGLSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVA RIRSKYNNYATYYADSVKDRFTISRDDSKNTLYLQMNSLRAEDTAVYYCVR HGNFGNSYVSWFAYWGQGTITVTVSS	anti-CD3 VH11
43	EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVA RIRSKYNNYATYYADSVKGRFTISRDDAKNTLYLQMNSLRAEDTAVYYCVR HGNFGNSYVSWFAYWGQGTITVTVKP	anti-CD3 VH12
44	EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKCLEWVAR IRSKYNNYATYYADSVKGRFTISRDDAKNTLYLQMSSLRAEDTAVYYCVRH GNFGNSYVSWFAYWGQGTITVTVKP	anti-CD3 VH13
45	EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVA RIRSKYNNYATYYADSVKGRFTISRDDAKNTLYLQMSSLRAEDTAVYYCVR HGNFGNSYVSWFAYWGCGTLTVTKP	anti-CD3 VH14
46	EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVAR IRSKYNNYATYYADSVKGRFTISRDDAKNTLYLQMSSLRAEDTAVYYCVRH GNFGNSYVSWFAYWGQGTITVTVSS	anti-CD3 VH15
47	EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVS IRSKYNNYATYYADSVKGRFTISRDDAKNTLYLQMSSLRAEDTAVYYCVRH GNFGNSYVSWFAYWGQGTITVTVSS	anti-CD3 VH16
48	EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVS IRSKYNNYATYYADSVKGRFTISRDDAKNTLYLQMSSLRAEDTAVYYCVRH GNFGNSYVSWFAYWGQGTITVTVSS	anti-CD3 VH17
49	EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKCLEWVAR IRSKYNNYATYYADSVKGRFTISRDDAKNTLYLQMSSLRAEDTAVYYCVRH GNFGNSYVSWFAYWGQGTITVTVSS	anti-CD3 VH18
50	EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKCLEWVS IRSKYNNYATYYADSVKGRFTISRDDAKNTLYLQMSSLRAEDTAVYYCVRH GNFGNSYVSWFAYWGQGTITVTVSS	anti-CD3 VH19
51	EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKCLEWVSRI RSKYNNYATYYADSVKGRFTISRDDAKNTLYLQMSSLRAEDTAVYYCVRH GNFGNSYVSWFAYWGQGTITVTVSS	anti-CD3 VH20
52	EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKCLEWVGR IRSKYNNYATYYADSVKDRFTISRDDSKNTLYLQMNSLRAEDTAVYYCVRH GNFGNSYVSWFAYWGQGTITVTVSS	anti-CD3 VH21
53	EVKLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKCLEWVAR IRSKYNNYATYYADSVKDRFTISRDDSKNTLYLQMNSLRAEDTAVYYCVR HGNFGNSYVSWFAYWGQGTITVTVSS	anti-CD3 VH22
54	EVKLVESGGGLVQPKGRSLRLSCAASGFTFNTYAMNWVRQAPGKCLEWVAR IRSKYNNYATYYADSVKDRFTISRDDSKNTLYLQMNSLRAEDTAVYYCVRH GNFGNSYVSWFAYWGQGTITVTVSS	anti-CD3 VH23
55	EVKLVESGGGLVQPKGRSLRLSCAASGFTFNTYAMNWVRQAPGKCLEWVAR IRSKYNNYATYYADSVKDRFTISRDDSKNTLYLQMNSLRAEDTAVYYCVRH GNFGNSYVSWFAYWGQGTITVTVSS	anti-CD3 VH24
56	EVKLVESGGGLVQPKGRSLRLSCAASGFTFNTYAMNWVRQAPGKCLEWVAR IRSKYNNYATYYADSVKDRFTISRDDSKNTLYLQMNSLRAEDTAVYYCVRH GNFGNSYVSWFAYWGQGTITVTVSS	anti-CD3 VH25

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SEQUENCE TABLE		
SEQ ID NO	SEQUENCE	DESCRIPTION
57	EVQLLESGGGLVQPGGSLRLSCAASGFTFSTYAMNWRQAPGKCLEWVSRI RSKYNNYATYYADSVKGRFTISRDDSKNTLYLQMNSLRAEDTAVYYCVRH GNFGNSYVSWFAYWGQGLTVTVSS	anti-CD3 VH26
58	EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMSWVRQAPGKCLEWVGRI RSKYNNYATYYADSVKGRFTISRDDSKNTLYLQMNSLRAEDTAVYYCVRH GNFGDSYVSWFAYWGQGLTVTVSS	anti-CD3 VH27
59	EVQLVESGGGLVQPGGSLRLSCAASGFTFNKYAMNWRQAPGKCLEWVA RIRSKYNNYATYYADSVKDRFTISRDDSKNTAYLQMNMLKTEDTAVYYCV RHGNFGNSYISYWAYWGQGLTVTVS	anti-CD3 VH28
60	EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWRQAPGKCLEWVAR IRSKYNNYATYYADSVKGRFTISRDDSKNTLYLQMNSLRAEDTAVYYCVRH GNFGNSYVSWFAYWGQGTTVTVSS	anti-CD3 VH29
61	EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWRQAPGKCLEWVAR IRSKYNNYATYYADSVKGRFTISRDDSKNTLYLQMNSLRAEDTAVYYCVRH GNFGNSYVSYFAYWGQGTTVTVSS	anti-CD3 VH30
62	EVQLVESGGGLVQPKGSLRLSCAASGFTFNTYAMNWRQAPGKCLEWVA RIRSKYNNYATYYADSVKDRFTISRDDSQSILYLQMNMLKTEDTAMYYCVR HGNFGNSYVSWFAYWGQGLTVTVSS	anti-CD3 VH31
63	QAVVTQESALTTSPGETVTLTCSRSTGAVTTSNYANWVQEKPDHLFTGLIG GTNKRAPGVPARFSGSLIGDKAALTITGAQIEDEAIYFCALWYSNLWVFGG GTKLTVL	anti-CD3 VL1
64	QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQKPGKSPRGLIG GTNKRAPGVPARFSGSLLGGKAALTISGAQPEDEADYYCALWYSNHWVFG CGTKLEIK	anti-CD3 VL2
65	QAVVTQEPSLTVSPGGTVTLTCRSSTGAVTTSNYANWVQKPGQAPRGLIG GTNKRAPWTPARFSGSLLGGKAALTITGAQAEDEADYYCALWYSNLWVFG GGTKLTVL	anti-CD3 VL3
66	QAVVTQEPSFSVSPGGTVTLTCRSSTGAVTTSNYANWVQTPGQAFRGLIG GTNKRAPGVPARFSGSLIGDKAALTITGAQADDESIYFCALWYSNLWVFGG GTKLTVL	anti-CD3 VL4
67	QAVVTQEPSFSVSPGGTVTLTCRSSTGAVTTSNYANWVQTPGQAFRGLIG GTNKRAPGVPARFSGSILGNKAALTITGAQADDESIYFCALWYSNLWVFGG GTKLTVL	anti-CD3 VL5
68	QAVVTQEPSFSVSPGGTVTLTCRSSTGAVTTSNYANWVQTPGQAFRGLIG GTNKRAPGVPARFSGSILGNKAALTITGAQADDESYYCALWYSNLWVFG GGTKLTVL	anti-CD3 VL6
69	QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQEKPGQAFRGLIG GTNKRAPGTPARFSGSLLGGKAALTLSGAQPEDEAEYYCALWYSNLWVFG GGTKLTVL	anti-CD3 VL7
70	QTVVTQEPSLTVSPGGTVTLTCGSSTGAVTSGNYPNWVQKPGQAPRGLIG GTKFLAPGTPARFSGSLLGGKAALTLSGVQPEDEAEYYCVLWYSNRWVFG GGTKLTVL	anti-CD3 VL8
71	QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQKPGQAFRGLIG GTNKRAPGVPARFSGSLLGGKAALTISGAQPEDEADYYCALWYSNHWVFG GGTKLEIK	anti-CD3 VL9
72	QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQKPGQAFRGLIG GTNKRAPGVPARFSGSLLGGKAALTISGAQPEDEADYYCALWYSNHWVFG CGTKLEIK	anti-CD3 VL10
73	QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQKPGQCPRGLIG GTNKRAPGVPARFSGSLLGGKAALTISGAQPEDEADYYCALWYSNHWVFG EGTKLEIK	anti-CD3 VL11

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SEQUENCE TABLE		
SEQ ID NO	SEQUENCE	DESCRIPTION
74	QAVVTQESALTTSPGETVTTLTCRSSTGAVTTSNYANWVQEKPDHLFTGLIG GTNKRAPGVPARFSGSLIGDKAALTITGAQIEDEAIYFCALWYSNLWVFGC GTKLTVL	anti-CD3 VL12
75	QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQKPGKSPRGLIG GTNKRAPGVPARFSGSLLGGKAALTISGAQPEDEADYYCALWYSNHWVFG GGTKLEIK	anti-CD3 VL13
76	QAVVTQEPSLTVSPGGTVTLTCRSSTGAVTTSNYANWVQKPGQAPRGLIG GTNKRAPWTPARFSGSLLGGKAALTITGAQAEDEADYYCALWYSNLWVFG CGTKLTVL	anti-CD3 VL14
77	QAVVTQEPSFSVSPGGTVTLTCRSSTGAVTTSNYANWVQQTGQAFRGLIG GTNKRAPGVPARFSGSLIGDKAALTITGAQADDESIFYCALWYSNLWVFG GTKLTVL	anti-CD3 VL15
78	QAVVTQEPSFSVSPGGTVTLTCRSSTGAVTTSNYANWVQQTGQAFRGLIG GTNKRAPGVPARFSGSILGNKAALTITGAQADDESIFYCALWYSNLWVFG GTKLTVL	anti-CD3 VL16
79	QAVVTQEPSFSVSPGGTVTLTCRSSTGAVTTSNYANWVQQTGQAFRGLIG GTNKRAPGVPARFSGSILGNKAALTITGAQADDESYYCALWYSNLWVFG CGTKLTVL	anti-CD3 VL17
80	QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQEKPGQAFRGLIG GTNKRAPGTPARFSGSLLGGKAALTLSGAQPEDEAEYYCALWYSNLWVFG CGTKLTVL	anti-CD3 VL18
81	QTVVTQEPSLTVSPGGTVTLTCGSSTGAVTSGNYPNWVQKPGQAPRGLIG GTKFLAPGTPARFSGSLLGGKAALTLSGVQPEDEAEYYCVLWYSNRWVFG CGTKLTVL	anti-CD3 VL19
82	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEV KFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCK VSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYP SDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFC SVMHEALHNHYTQKSLSLSP	Knob Fc
83	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPE VKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCK KVSNAKALPAPIEKTISKAKGQPREPQVCTLPSPRDELTKNQVSLCAVKGFYP SDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFC SVMHEALHNHYTQKSLSLSP	Hole Fc
84	DKTHTCPPCPAPGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NFWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVS NKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYP SDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFC SVMHEALHNHYTQKSLSLSP	Knob Fc
85	DKTHTCPPCPAPGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NFWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVS NKALPAPIEKTISKAKGQPREPQVCTLPSPRDELTKNQVSLCAVKGFYP SDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFC SVMHEALHNHYTQKSLSLSP	Hole Fc
86	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEV KFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCK VSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYP SDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFC SVMHEALHNHYTQKSLSLSP	Knob Fc
87	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPE VKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCK KVSNAKALPAPIEKTISKAKGQPREPQVCTLPSPRDELTKNQVSLCAVKGFYP SDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFC SVMHEALHNHYTQKSLSLSP	Hole Fc
88	DKTHTCPPCPAPGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NFWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVS	Knob Fc

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SEQUENCE TABLE		
SEQ ID NO	SEQUENCE	DESCRIPTION
	NKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSD IAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSCV MHEALHNHYTQKSLSLSPG	
89	DKTHTCPPCPAPGGPSVFLFPPKPKDTLMRSRTPEVTCVVVDVSHEDPEVKF NWKYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKV NKALPAPIEKTISKAKGQPREPQVCTLPSPRDELTKNQVSLSCAVKGFYPSDI AVEWESNGQPENNYKTTTPVLDSDGSFFLVSKLTVDKSRWQQGNVFCSCV MHEALHNHYTQKSLSLSPG	Hole Fc
90	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEV KFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCK VSNKALPAPIEKTISKAKGQPREPQVCTLPSPRDELTKNQVSLSCAVKGFYPS DIAVEWESNGQPENNYKTTTPVLDSDGSFFLVSKLTVDKSRWQQGNVFCSC VMHEALHNRYTQKSLSLSPT	Hole Fc
91	DKTHTCPPCPAPGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWKYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKV NKALPAPIEKTISKAKGQPREPQVCTLPSPRDELTKNQVSLSCAVKGFYPSDI AVEWESNGQPENNYKTTTPVLDSDGSFFLVSKLTVDKSRWQQGNVFCSCV MHEALHNRYTQKSLSLSPT	Hole Fc
92	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEV KFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCK VSNKALPAPIEKTISKAKGQPREPQVCTLPSPRDELTKNQVSLSCAVKGFYPS DIAVEWESNGQPENNYKTTTPVLDSDGSFFLVSKLTVDKSRWQQGNVFCSC VMHEALHNRYTQKSLSLSPG	Hole Fc
93	DKTHTCPPCPAPGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWKYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKV NKALPAPIEKTISKAKGQPREPQVCTLPSPRDELTKNQVSLSCAVKGFYPSDI AVEWESNGQPENNYKTTTPVLDSDGSFFLVSKLTVDKSRWQQGNVFCSCV MHEALHNRYTQKSLSLSPG	Hole Fc
94	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLYISRTPEVTCVVVDVSHEDPEV KFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCK VSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFY SDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFC SVVHEALHNHYTQKSLSLSPT	Knob Fc
95	DKTHTCPPCPAPGGPSVFLFPPKPKDTLYISRTPEVTCVVVDVSHEDPEVKFN WYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSN KALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDI AVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSCV HEALHNHYTQKSLSLSPT	Knob Fc
96	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLYISRTPEVTCVVVDVSHEDPEV KFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCK VSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFY SDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFC SVVHEALHNHYTQKSLSLSPG	Knob Fc
97	DKTHTCPPCPAPGGPSVFLFPPKPKDTLYISRTPEVTCVVVDVSHEDPEVKFN WYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSN KALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDI AVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSCV HEALHNHYTQKSLSLSPG	Knob Fc
98	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLYISRTPEVTCVVVDVSHEDPEV KFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCK VSNKALPAPIEKTISKAKGQPREPQVCTLPSPRDELTKNQVSLSCAVKGFYPS DIAVEWESNGQPENNYKTTTPVLDSDGSFFLVSKLTVDKSRWQQGNVFCSC VVHEALHNRYTQKSLSLSPT	Hole Fc
99	DKTHTCPPCPAPGGPSVFLFPPKPKDTLYISRTPEVTCVVVDVSHEDPEVKFN WYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSN KALPAPIEKTISKAKGQPREPQVCTLPSPRDELTKNQVSLSCAVKGFYPSDIA VEWESNGQPENNYKTTTPVLDSDGSFFLVSKLTVDKSRWQQGNVFCSCV HEALHNRYTQKSLSLSPT	Hole Fc

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SEQUENCE TABLE		
SEQ ID NO	SEQUENCE	DESCRIPTION
100	DKTHTCPCPCAPPELLGGPSVFLFPPKPKDTLYISRTPEVTCVVVDVSHEDPEV KFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCK VSNKALPAPIEKTISKAKGQPREPQVCTLPSPSRDELTKNQVSLSCAVKGFYP SDIAVEWESNGQPENNYKTTPVLDSDGSFFLVSKLTVDKSRWQQGNVFS CSVVHEALHNRYTQKSLSLSPG	Hole Fc
101	DKTHTCPCPCAPGGPSVFLFPPKPKDTLYISRTPEVTCVVVDVSHEDPEVKFN WYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSN KALPAPIEKTISKAKGQPREPQVCTLPSPSRDELTKNQVSLSCAVKGFYP SDIAVEWESNGQPENNYKTTPVLDSDGSFFLVSKLTVDKSRWQQGNVFS CSVVHEALHNRYTQKSLSLSPG	Hole Fc
102	PGGGG	Peptide Linker
103	GGGG	Peptide Linker
104	GPAGLGLEPDGSRQARVG	Linker
105	GGSGGGGIEPDIGSGGS	Linker
106	GGSGGGGLEADTGGSGGS	Linker
107	GSIEPDIGS	Linker
108	GSLEADTGS	Linker
109	GGSGGGGIEPDGGSGGS	Linker
110	GGSGGGGIEPDVGGSGGS	Linker
111	GGSGGGGIEPDSGGSGGS	Linker
112	GGSGGGGIEPDGGSGGS	Linker
113	GGGSLEPDGSGS	Linker
114	GPAGLGLEADGSRQARVG	Linker
115	GGEGGGGSGSGSGGS	Linker
116	GSSAGSEAGSGQAGVGS	Linker
117	GGSGGGGLEAEGSGSGGS	Linker
118	GGSGGGGIEPDGGSGGS	Linker
119	GGGGGSGGGGSGGGGS	Linker
120	QLQLQESGGGLVQPGGSLRLSCAASGFTLDNYAIGWFRQAPGKEREGVSCIS SSDGSTYYADSVKGRFTISRDNKGTIVYLLMNSLKPEDTAVYYCATELVPA CTYSNGRGPLDGM DYWGKGTQVTVKP	FR alpha sdAb
121	EVQLVESGGGEVQPGGSLRLSCAASGIFSIDATAWYRQAPGKQRELVAIITS SGSTNYPEVSVKGRFTISRDNKNTVYLMNSLRAEDTAVYYCNAITRYGGS TYDFWGQGTLLVTVKP	FR alpha sdAb
122	EVQPGGSLRLSCAASETFGVVFTLGWYRQAPGKGREFVARVTGTDVTDYA ESVKGRFTISSDFARNTVYLMNSLRAEDTAVYYCNTGAYWGQGTLLVTVK P	FR alpha sdAb
123	EVQLVESGGGLVQPGGSLRLSCAASGFILDYIAIGWFRQAPGKEREGVLCID ASDDITYADSVKGRFTISRDNKNTVYLMNSLKPEDTGYYCATPIGLSS SCLLEYDIDYWGQGTLLVTVKP	cMET sdAb
124	EVQLVESGGGLVQPGGSLRLSCAASGFTFSFGMHVWRQAPGKLEWVAY ISSDSSAIYYADTVKGRFTISRDNKNSLYLMNSLRDEDTAVYYCGRGREN IYYGSRLDYWGQGTITVTVSSSGGGGSGGGGSGGGSDIQLTQSPSFLSASV GDRVTITCKASQNVDTNVAWYQQKPKGKAPKALIIYSASYRSGVPSRFS GSGSGTDFTLTISLQPEDFATYYCQQYNYPFTFGQGTKLEIK	B7H3 scFv

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SEQUENCE TABLE		
SEQ ID NO	SEQUENCE	DESCRIPTION
125	QVQLVQSGAEVKKPGSSVKVSCKASGYAFSYSWINWVRQAPGQGLEWMG RIFPGDGDITDYNKFKGRVTITADKSTSTAYMELSSLRSEDVAVYYCARNV FDGYWLVYWGGTTLVTVSGSGGGSGGGGTGGGSDIVMTQTPLSLPVT GEPASISCRSSKSLHLSNGITYLYWYLQKPGQSPQLLIYQMSNLVSGVPDRFS GSGSGTDFTLKISRVEAEDVGVYYCAQNLELPYTFGGGTKVEIK	CD20 scFv
126	QVQLQESGPGLVKPSSETLSLTCTVSGGSISSYYWSWIRQPPGKGLEWIGYVY YSGTTNYPNPSLKSRVTISVDTSKNQFSLKLSVTAADTAVYYCASIATVGFY FDYWGQGTTLVTVSSGGGGSGGGSGGGGSEIVLTQSPGTLSPGERVTLSC RASQRVNNYLAWYQQRPGQAPRLLIYGASSRATGIPDRFSGSGSGTDFTLT ISRLEPEDFAVYYCQYDRSPLTFGGGTKLEIK	DLL3 scFv
127	EVQLVESGGGLVQPGGSLRLSCAASGFTFSSFGMHVWRQAPGKGLEWVAY ISSDSSAIYYADTVKGRFTISRDNAKNSLYLQMNSLRDEDVAVYYCGRGREN IYYGSRLDYWGQGTTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDY FPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNV NHKPSNTKVDKKVEPKSC	B7H3 Fd
128	DIQLTQSPSFLSASVGRDVTITCKASQNVDTNVAWYQKPKGKAPKALIYSAS YRYSQVPSRFSGSGSGTDFTLTISLQPEDFATYYCQYNNYPPTFGQGTKLE IKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSG NSQESVTEQDSKDSSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSF NRRGEC	B7H3 LC
129	EVQLVESGGGLVQPGKSLRLSCAASGFTFNTYAMNWRQAPGKGLEWVA RIRSKSNYATYYADSVKDRFTISRDDSQSMYLYLQMNLLKTEDTAMYYCV RQWDYDVRAMNYWGQGTSTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGC LVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSC	5T4 Fd
130	DIVMTQSHIFMSTSVGDRVSIITCKASQDVTAVAWYQKPKGQSPKLLIYWA STRLTGVDPDRFTGSGSGTDFTLTISNVQSEDLADYFCQYSSYPYTFGGGTK LEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQ SGNSQESVTEQDSKDSSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTK SFNRRGEC	5T4 LC
131	QVQLQESGPGLVKPSQTLSTCTVSGGSISSFNYWSWIRHHPGKGLEWIGY IYYSGSTYSNPSLKSRVTISVDTSKNQFSLTLSSVTAADTAVYYCARGYNWN YFDYWGQGTTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPV TVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHK SNTKVDKKVEPKSC	gpNMB Fd
132	EIVMTQSPATLSVSPGERATLSCRASQSVNDNLVWYQKPGQAPRLLIYGAS TRATGIPARFSGSGSGTEFTLTISLQSEDFAVYYCQYNNWPPWTFGGGTK VEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQ SGNSQESVTEQDSKDSSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTK SFNRRGEC	gpNMB LC
133	QIQLVQSGPELKKPGETVKISCKASGYFTFTNYGMNWKQAPGKGLKWA WINTYTGEPTYADDFKGRFAFSLETASTASLQIINLKNEDTATYFCARIGDS SPSDYWGQGTTLTVSSSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFP EPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVN HKPSNTKVDKKVEPKSC	DLL3 Fd
134	SIVMTQTPKFLLVASAGDRVTITCKASQSVSNDVVWYQKPKGQSPKLLIYYAS NRYTGVDPDRFAGSGYGTDFSTISTVQAEDLAVYFCQQDYTSPTWTFGGGTK LEIRRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQ SGNSQESVTEQDSKDSSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTK SFNRRGEC	DLL3 LC
135	GGGGGS	Peptide Linker
136	IEPDI	Linker
137	LEADT	Linker
138	IEPDG	Linker
139	IEPDV	Linker
140	IEPDS	Linker

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SEQUENCE TABLE		
SEQ ID NO	SEQUENCE	DESCRIPTION
141	IEPDT	Linker
142	LEPD	Linker
143	LEAE	Linker
144	IEPDP	Linker
145	QAVVTQEPSTLTVSPGGTVTLTCSRSTGAVTTSNYANWVQQKPGQAPRGLIG GTNKRAPWTPARFSGSLGGAALITGAQAEDEADYYCALWYNLWVFG GGTKLTVLGGGGSGGGGEVQLVESGGGLVQPGGSLRLSCAASGFTFSSFGM HWVRQAPGKGLEWVAYISDSSAIYYADTVKGRFTISRDNKNSLYLQMNS LRDEDTAVYYCGRGRENIIYGSRLDYWGQGTITVTVSSGGCGGGKVAALKE KVAALKEKVAALKEKVAALKE	Second Polypeptide Chain of B7-H3 x CD3 Bispecific DART-A Diabody
146	DKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPE VKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDNLNGKEYKC KVSINKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLSCAVKGFY PSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLVSKLTVDKSRWQQGNVFS CSVMHEALHNRYTQKSLSLSPGK	Third Polypeptide Chain of B7-H3 x CD3 Bispecific DART-A Diabody
147	GGSGGGSGGGSGGGGS	Linker
148	TGGSGGGGIEPDIGSGGS	Linker
149	GGGGS	Linker
150	X ₁ X ₂ X ₃ X ₄ X ₅ (P ₄ P ₃ P ₂ P ₁ ↓ P ₁ ') X ₁ = I, L, Y, M, F, V, or A; (P ₄ = I, L, Y, M, F, V, or A) X ₂ = A, G, S, V, E, D, Q, N, or Y; (P ₃ = A, G, S, V, E, D, Q, N, or Y) X ₃ = H, P, A, V, G, S, or T; (P ₂ = H, P, A, V, G, S, or T) X ₄ = D or E; (P ₁ = D or E) X ₅ = I, L, Y, M, F, V, T, S, G or A (P ₁ ' = I, L, Y, M, F, V, T, S, G or A)	Linker consensus
151	X ₁ E X ₃ D X ₅ (P ₄ P ₃ P ₂ P ₁ ↓ P ₁ ') X ₁ = I or L; (P ₄ = I or L) (P ₃ = E) X ₃ = P or A; (P ₂ = P or A) X ₅ = I, V, T, S, or G (P ₁ ' = I, V, T, S, or G)	Linker consensus
152	LEPDG	Linker
153	LEADG	Linker
154	X ₁ QARX ₅ (P ₁ QAR↓(A/V)) X ₁ = any amino acid; (P ₁ is any amino acid) X ₅ = A or V	Linker consensus
155	RQARX ₅ (RQAR(A/V)) X ₅ = A or V	Linker consensus
156	RQARV	Linker
157	X ₁ X ₂ X ₃ X ₄ (P ₃ P ₂ P ₁ ↓ P ₁ ') X ₁ = P, V or A; (P ₃ = P, V or A) X ₂ = Q or D; (P ₂ = Q or D) X ₃ = A or N; (P ₁ = A or N) X ₄ = L, I or M (P ₁ ' = L, I or M)	Linker consensus
158	PX ₂ X ₃ X ₄ (P ₃ P ₂ P ₁ ↓ P ₁ ') (P ₃ = P) X ₂ = Q or D; (P ₂ = Q or D) X ₃ = A or N; (P ₁ = A or N) X ₄ = L or I (P ₁ ' = L or I)	Linker consensus
159	GSATNFSLLKQAGDVEENPGP	P2A

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SEQUENCE TABLE		
SEQ ID NO	SEQUENCE	DESCRIPTION
160	ATNFSLLKQAGDVEENPGP	P2A
161	QCTNYALLKLAGDVESNPGP	E2A
162	VKQTLNFDLLKLAGDVESNPGP	F2A
163	EGRGSLTTCGDVEENPGP	T2A
164	LEGGGEGRGSLTTCGDVEENPGPR	T2A
165	GGATCTGGAGCAACAACTTCTCACTACTCAACAAGCAGGTGACGTGG AGGAGAATCCCGGACCC	P2A DNA
166	GSPAGLEADGSRQARVGS	Linker
167	EVQLVESGGGL VQPKGSLKLS CAASGFTFNT YAMNWVRQAP GKGLEWVARI RSKSNNYATY YADSVKDRFT ISRDDSQSML YLQMNNLKTE DTAMYXCVRQ WDYDVRAMNY WGQGTSVTVS S	anti-5T4 VH
168	DIVMTQSHIF MSTSVGDRVS ITCKASQDVD TAVAWYQQKP GQSPKLLIYW ASTRLTGVPD RFTGSGSGTD FTLTISNVQS EDLADYFCQQ YSSYPYTFGG GTKLEIK	anti-5T4 VL
169	DIQLTQSPSF LSASVGDRVT ITCKASQNVN TNVAWYQQKP GKAPKALIYS ASYRYSGVPS RFSGSGSGTD FTLTISSLQP EDFATYYCQQ YNNYPFTFGQ GTKLEIKGGG SGGGGEVQLV ESGGGLVQPG GSLRLSCAAS GFTTFSTYAMN WVRQAPGKGL EWVGRIRSKY NNYATYYADS VKDRFTISRD DSKNSLYLQM NSLKTEDTAV YYCVRHGNFG NSYVSWFAYW GQGTLLVTSS GCGGGGEVAA LEKEVAALEK EVAALEKEVA ALEKGGGDKT HTCPPCPAPE AAGGPSVFLF PPKPKDTLMI SRTPEVTCVV VDVSHEDPEV KFNWYVDGVE VHNAKTKPRE EQYNSTYRVV SVLTVLHQDW LNGKEYKCKV SNKALPAPIE KTISKAKGQP REPQVYTLPP SREEMTKNQV SLWCLVKGFY PSDIAVEWES NGQPENNYKT TPPVLDSDGS FFLYSKLTVD KSRWQQGNVF SCSVMHEALH NHYTQKSLSL SPGK	First Polypeptide Chain of B7-H3 x CD3 Bispecific DART-A Diabody
170	GGGGSGGGSGGGGS	Linker
171	GGG(GGS)n wherein n is 0 to 10	Linker
172	(GGGGGS)n wherein n is 1 to 4	Linker
173	(GGGGGS)n wherein n is 1 to 10	Linker
174	Gly _x Xaa-Gly _y -Xaa-Gly _z 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
175	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
176	ATTTGSSPGPT	Linker
177	GGGGG-C-GGGGG	Linker
178	(EAAAK)n n = 2-20	Linker
179	AS-(AP)n-GT n = 2-20	Linker
180	AS-(EAAAK)n-GT n = 2-20	Linker

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SEQUENCE TABLE		
SEQ ID NO	SEQUENCE	DESCRIPTION
181	(GGGA) n n = 2-20	Linker
182	(PGGS) n n = 2-20	Linker
183	(AGGS) n n = 2-20	Linker
184	GG- (EGKSSGSGSESKST) n-GGS n = 2-20	Linker
185	(SSSSG) n n = 1-9	Linker
186	SSSASASSA	Linker
187	GSPGSPG	Linker
188	QVQLQESGPG LVKPSETLSL TCTVSGGIS SYWWSWIRQP PGKGLEWIGY VYYSGTTNYN PSLKSRVTIS VDTSKNQFSL KLSSVTAADT AVYYCASIAY TGFYFDYWQ GTLVTVSSGG GSGGGGSGG GSEIVLTQS PGTLSLSPGE RVTLSCRASQ RVNNNYLAWY QQRPGQAPRL LIYGASSRAT GIPDRFSGSG SGTDFTLTIS RLEPEDFAVY YCQQYDRSPL TFGGGTKLEI K	DLL3 scFv
189	QVQLVQSGAE VKKPGSSVKV SCKASGYAFS YSWINWVRQA PGQGLEWMGR IFPGDGDYD NGKFKGRVTI TADKSTSTAY MELSSLRSED TAVYYCARNV FDGYWLVYWG QGTLVTVSS	CD20 VH
190	DIVMTQTPLS LPVTPGEPAS ISCRSSKSL HSNIGITYLYW YLQKPGQSPQ LLIYQMSNLV SGVPDRFSGS GSGTDFTLKI SRVEAEDVGV YYCAQNLLEP YTFGGGTKVE IKRTV	CD20 VL
191	QAVVTQEPSTLVSPGGTVTLTCSSTGAVTTSNYANWVQKPGQAPRGLIG GTNKRAPGVPARFSGSLGGKAALTI SGAQPEDEADYYCALWYSNHWFVFG CGTKLTVL	anti-CD3 VL35 (CON)
192	GGGGG	linker
193	GGGGSGGGSGGGGS	linker
194	DKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVKHEDPEV KFNWYVDGVEVHNAKTKPREEEYNSTYRVVSVLTVLHQDWLNGKEYKCK VSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCDVSGGFYPS DIAVEWESDGPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGDVFSFS VMHEALHNHYTQKSLSLSPGK	Fc-Het-1
195	DKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVKHEDPEV KFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCK VSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPS SDIAVEWESNGQENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSFS SVMHEALHNHYTQKSLSLSPGK	Fc-Het-2
196	EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPGKLEWVGR IRSKYNNYATYYADSVKGRFTISRDDSKNTLYLQMNSLRAEDTAVYYCVRH GNFGDSYVSWFAYWGQGLVTVSS	CD3 -VH32
197	EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPGKLEWVGR IRSKYNNYATYYADSVKGRFTISRDDSKNTLYLQMNSLRAEDTAVYYCVRH GNFGDSYVSWFAYWGQGLVTVSS	CD3 -VH33
198	EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPGKLEWVAR IRSKYNNYATYYADTVKGRFTISRDDAKNTLYLQMNSLRAEDTAVYYCVR HGNFGDSYVSWFAYWGQGLVTV	CD3 -VH34
199	QAVVTQEPSTLVSPGGTVTLTCSSTGAVTTSNYANWVQKPGKSPRGLIG GTNKRAPGVPARFSGSLGGKAALTI SGAQPEDEADYYCALWYSNHWFVFG GGTKLTVL	CD3 -VL20

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SEQUENCE TABLE		
SEQ ID NO	SEQUENCE	DESCRIPTION
200	QAVVTQEPSTLVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGKSPRGLIG GTNKRAPGVPARFSGSLLGGKAALTISGAQPEDEADYYCALWYSNHWWFG CGTKLTVL	CD3-VL21
201	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEV KFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCK VSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYP SDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSLKLTVDKSRWQQGNVFSC SVMHEALHNHYTQKSLSLSP	Knob Fc
202	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMRSRTPEVTCVVVDVSHEDPE VKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKC KVSNAKALPAPIEKTISKAKGQPREPQVCTLPSPRDELTKNQVSLSCAVKGFYP SDIAVEWESNGQPENNYKTTTPVLDSDGSFFLVSLKLTVDKSRWQQGNVFSC SVMHEALHNHYTQKSLSLSP	Hole Fc
203	DKTHTCPPCPAPEGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWKYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVS NKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSD IAVEWESNGQPENNYKTTTPVLDSDGSFFLYSLKLTVDKSRWQQGNVFSCSV MHEALHNHYTQKSLSLSP	Knob Fc
204	DKTHTCPPCPAPEGPSVFLFPPKPKDTLMRSRTPEVTCVVVDVSHEDPEVKF NWKYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVS NKALPAPIEKTISKAKGQPREPQVCTLPSPRDELTKNQVSLSCAVKGFYPSDI AVEWESNGQPENNYKTTTPVLDSDGSFFLVSLKLTVDKSRWQQGNVFSCSV MHEALHNHYTQKSLSLSP	Hole Fc
205	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEV KFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCK VSNKALPAPIEKTISKAKGQPREPQVCTLPSPRDELTKNQVSLSCAVKGFYPS DIAVEWESNGQPENNYKTTTPVLDSDGSFFLVSLKLTVDKSRWQQGNVFSCS VMHEALHNRYTQKSLSLSP	Hole Fc
206	DKTHTCPPCPAPEGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWKYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVS NKALPAPIEKTISKAKGQPREPQVCTLPSPRDELTKNQVSLSCAVKGFYPSDI AVEWESNGQPENNYKTTTPVLDSDGSFFLVSLKLTVDKSRWQQGNVFSCSV MHEALHNRYTQKSLSLSP	Hole Fc
207	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLYISRTPEVTCVVVDVSHEDPEV KFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCK VSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYP SDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSLKLTVDKSRWQQGNVFSC SVVHEALHNHYTQKSLSLSP	Knob Fc
208	DKTHTCPPCPAPEGPSVFLFPPKPKDTLYISRTPEVTCVVVDVSHEDPEVKFN WYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSN KALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDI AVEWESNGQPENNYKTTTPVLDSDGSFFLYSLKLTVDKSRWQQGNVFSCSVV HEALHNHYTQKSLSLSP	Knob Fc
209	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLYISRTPEVTCVVVDVSHEDPEV KFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCK VSNKALPAPIEKTISKAKGQPREPQVCTLPSPRDELTKNQVSLSCAVKGFYPS DIAVEWESNGQPENNYKTTTPVLDSDGSFFLVSLKLTVDKSRWQQGNVFSCS VVHEALHNRYTQKSLSLSP	Hole Fc
210	DKTHTCPPCPAPEGPSVFLFPPKPKDTLYISRTPEVTCVVVDVSHEDPEVKFN WYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSN KALPAPIEKTISKAKGQPREPQVCTLPSPRDELTKNQVSLSCAVKGFYPSDIA VEWESNGQPENNYKTTTPVLDSDGSFFLVSLKLTVDKSRWQQGNVFSCSVH EALHNRYTQKSLSLSP	Hole Fc
211	GFTFNTYAMN	anti-CD3 VH CDR1
212	RIRSKYNNYATY	anti-CD3 VH CDR2

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SEQUENCE TABLE		
SEQ ID NO	SEQUENCE	DESCRIPTION
213	QVQLVQSGAE VKKPGSSVKV SCKASGYAFS YSWINWVRQA PGQGLEWMGR IFPGDGD TDY NGKFKGRVTI TADKSTSTAY MELSSLRSED TAVYYCARNV FDGYWLVYWG QGTLVTVSSG GGGSGGGGSG GGGSDIVMTQ TPLSLPVTGP EPASISCRSS KSLLSHNGIT YLYWYLQKPG QSPQLLIYQM SNLVSGVPDR FSGSGSGTDF TLKISRVEAE DVGVIYCAQN LELPYTFGGG TKVEIK	CD20 scFv
214	EVQLVESGGGEVQPGGSLRLSCAASGFTFSSYHMSWFRQAPGKLEWVSTIYSSG TGTFYAESVKGRFTISRDNAKNTLYLQMSSLRPEDTAVYYCATSGPVRGWGPRSQGT LVTVKP	B7H3 sdAb B7h3 hz1A5v51
215	EVQLVESGGGEVQPGGSLRLSCAASGFTFSSYHMSWFRQAPGKQREPVATS HHGGTTNYAGSVKGRFTISRDNAKNTVYLQMNTLRAEDTAVYYCKADHG YQGRGYWGQGT LVTVKP	sdAb B7H3 hz58E05v27
216	EVQLVESGGGEVQPGGSLRLSCAASGFTFSSYHMSWFRQAPGKQREL VATSHHGGT TNYAGSVKGRFTISRDNAKNTVYLQMNTLRAEDTAVYYCKADHG YQGRGYWGQGT LVTVKP	sdAb B7H3 hz58E05v55
217	EVQLVESGGGEVQPGGSLRLSCAASGFTFSSYHMSWFRQAPGKQREPVATS HHGGTTNYAGSVKGRFTISRDNAKNTVYLQMNTLRAEDTAVYYCKADHG YQGRGYWGQGT LVTVKP	sdAb B7H3 hz58E05v48
218	EVQLVESGGGEVQPGGSLRLSCAPSEITFSTYTMGWFRQAPGKEREFAVAV NWGGGSKYYAESVKGRFTISRDNAKNTVYLQMSSLRAEDTAVYYCAAGG AYSGPYDTRQYTYWGQGT LVTVKPGG	sdAb B7H3 hz57B04v24
219	EVQLVESGGGEVQPGGSLRLSCAASGSIF SINAMGWYRQAPGKQREL VAGF TGDNTIYAESVKGRFTISRDNAKNTVYLQMSSLRAEDTAVYYCAADVQLF SRDYEFYWGQGT LVTVKP	sdAb DLL3 hz10D9v7
220	EVQLVESGGGEVQPGGSLRLSCGPSEIITSDKSMGWVRQAPGKQRLVAGIS NVGSTNYAQSVKGRFTISRDNAKNTVYLQMSSLRAEDTAVYYCYARDFEN EYWGQGT LVTVKP	sdAb DLL3 hz8E7v16
221	GFSFSINAMG	41BB CDR1
222	AIESGRNTV	41BB CDR2
223	LKGNRVVSPSVAY	41BB CDR3
224	HGNFGDSYVSWFAY	CD3 -VH7, VH33 CDR3
225	ALWYSNHVV	CD3 -VL2, VL21 CDR3
226	VLWYSNRVW	CD3 -VL8 CDR3
227	GFTFSTYAMN	CD3 VH33 CDR1
228	RIRSKYNNYATY	CD3 VH33 CDR1
229	GSSTGAVTTSNYAN	CD3 VL21 CDR1
230	GTNKRAP	CD3 VL21 CDR2

SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 230

<210> SEQ ID NO 1

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<211> LENGTH: 218
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: IgG1 Fc

<400> SEQUENCE: 1
Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro
1      5      10      15
Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys
20     25     30
Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp
35     40     45
Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu
50     55     60
Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu
65     70     75     80
His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn
85     90     95
Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly
100    105    110
Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu
115    120    125
Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr
130    135    140
Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn
145    150    155    160
Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe
165    170    175
Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn
180    185    190
Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr
195    200    205
Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
210    215

<210> SEQ ID NO 2
<211> LENGTH: 215
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: xELL Fc

<400> SEQUENCE: 2
Pro Ala Pro Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys
1      5      10      15
Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val
20     25     30
Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp
35     40     45
Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr
50     55     60
Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp
65     70     75     80
Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu

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85	90	95
Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg		
100	105	110
Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys		
115	120	125
Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp		
130	135	140
Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys		
145	150	155
Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser		
165	170	175
Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser		
180	185	190
Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser		
195	200	205
Leu Ser Leu Ser Pro Gly Lys		
210	215	

<210> SEQ ID NO 3
 <211> LENGTH: 217
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: IgG2 Fc

<400> SEQUENCE: 3

Pro Ala Pro Pro Val Ala Gly Pro Ser Val Phe Leu Phe Pro Pro Lys		
1	5	10
Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val		
20	25	30
Val Val Asp Val Ser His Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr		
35	40	45
Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu		
50	55	60
Gln Phe Asn Ser Thr Phe Arg Val Val Ser Val Leu Thr Val Val His		
65	70	75
Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys		
85	90	95
Gly Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Thr Lys Gly Gln		
100	105	110
Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met		
115	120	125
Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro		
130	135	140
Ser Asp Ile Ser Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn		
145	150	155
Tyr Lys Thr Thr Pro Pro Met Leu Asp Ser Asp Gly Ser Phe Phe Leu		
165	170	175
Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val		
180	185	190
Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln		
195	200	205
Lys Ser Leu Ser Leu Ser Pro Gly Lys		

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210	215
<210> SEQ ID NO 4	
<211> LENGTH: 218	
<212> TYPE: PRT	
<213> ORGANISM: Artificial Sequence	
<220> FEATURE:	
<223> OTHER INFORMATION: IgG3 Fc	
<400> SEQUENCE: 4	
Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro	
1 5 10 15	
Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys	
20 25 30	
Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Gln Phe Lys Trp	
35 40 45	
Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu	
50 55 60	
Glu Gln Tyr Asn Ser Thr Phe Arg Val Val Ser Val Leu Thr Val Leu	
65 70 75 80	
His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn	
85 90 95	
Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Thr Lys Gly	
100 105 110	
Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu	
115 120 125	
Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr	
130 135 140	
Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Ser Gly Gln Pro Glu Asn	
145 150 155 160	
Asn Tyr Asn Thr Thr Pro Pro Met Leu Asp Ser Asp Gly Ser Phe Phe	
165 170 175	
Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn	
180 185 190	
Ile Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn Arg Phe Thr	
195 200 205	
Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys	
210 215	

<210> SEQ ID NO 5
 <211> LENGTH: 218
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: IgG4 Fc

<400> SEQUENCE: 5

Pro Ala Pro Glu Phe Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro	
1 5 10 15	
Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys	
20 25 30	
Val Val Val Asp Val Ser Gln Glu Asp Pro Glu Val Gln Phe Asn Trp	
35 40 45	
Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu	
50 55 60	

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Glu	Gln	Phe	Asn	Ser	Thr	Tyr	Arg	Val	Val	Ser	Val	Leu	Thr	Val	Leu
65					70					75					80
His	Gln	Asp	Trp	Leu	Asn	Gly	Lys	Glu	Tyr	Lys	Cys	Lys	Val	Ser	Asn
			85						90					95	
Lys	Gly	Leu	Pro	Ser	Ser	Ile	Glu	Lys	Thr	Ile	Ser	Lys	Ala	Lys	Gly
		100						105					110		
Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr	Thr	Leu	Pro	Pro	Ser	Gln	Glu	Glu
		115					120					125			
Met	Thr	Lys	Asn	Gln	Val	Ser	Leu	Thr	Cys	Leu	Val	Lys	Gly	Phe	Tyr
	130					135					140				
Pro	Ser	Asp	Ile	Ala	Val	Glu	Trp	Glu	Ser	Asn	Gly	Gln	Pro	Glu	Asn
145					150					155					160
Asn	Tyr	Lys	Thr	Thr	Pro	Pro	Val	Leu	Asp	Ser	Asp	Gly	Ser	Phe	Phe
			165						170					175	
Leu	Tyr	Ser	Arg	Leu	Thr	Val	Asp	Lys	Ser	Arg	Trp	Gln	Glu	Gly	Asn
			180					185					190		
Val	Phe	Ser	Cys	Ser	Val	Met	His	Glu	Ala	Leu	His	Asn	His	Tyr	Thr
		195					200					205			
Gln	Lys	Ser	Leu	Ser	Leu	Ser	Leu	Gly	Lys						
	210					215									

<210> SEQ ID NO 6

<211> LENGTH: 218

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: IgG4 Fc

<400> SEQUENCE: 6

Pro	Ala	Pro	Glu	Leu	Leu	Gly	Gly	Pro	Ser	Val	Phe	Leu	Phe	Pro	Pro
1				5					10					15	
Lys	Pro	Lys	Asp	Thr	Leu	Met	Ile	Ser	Arg	Thr	Pro	Glu	Val	Thr	Cys
			20					25					30		
Val	Val	Val	Asp	Val	Ser	Gln	Glu	Asp	Pro	Glu	Val	Gln	Phe	Asn	Trp
			35				40					45			
Tyr	Val	Asp	Gly	Val	Glu	Val	His	Asn	Ala	Lys	Thr	Lys	Pro	Arg	Glu
	50					55					60				
Glu	Gln	Phe	Asn	Ser	Thr	Tyr	Arg	Val	Val	Ser	Val	Leu	Thr	Val	Leu
65					70					75					80
His	Gln	Asp	Trp	Leu	Asn	Gly	Lys	Glu	Tyr	Lys	Cys	Lys	Val	Ser	Asn
			85						90					95	
Lys	Gly	Leu	Pro	Ser	Ser	Ile	Glu	Lys	Thr	Ile	Ser	Lys	Ala	Lys	Gly
		100						105					110		
Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr	Thr	Leu	Pro	Pro	Ser	Gln	Glu	Glu
		115					120					125			
Met	Thr	Lys	Asn	Gln	Val	Ser	Leu	Thr	Cys	Leu	Val	Lys	Gly	Phe	Tyr
	130					135					140				
Pro	Ser	Asp	Ile	Ala	Val	Glu	Trp	Glu	Ser	Asn	Gly	Gln	Pro	Glu	Asn
145					150					155					160
Asn	Tyr	Lys	Thr	Thr	Pro	Pro	Val	Leu	Asp	Ser	Asp	Gly	Ser	Phe	Phe
			165						170					175	
Leu	Tyr	Ser	Arg	Leu	Thr	Val	Asp	Lys	Ser	Arg	Trp	Gln	Glu	Gly	Asn
			180					185					190		

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Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr
195 200 205

Gln Lys Ser Leu Ser Leu Ser Leu Gly Lys
210 215

<210> SEQ ID NO 7
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Hinge

<400> SEQUENCE: 7

Glu Pro Lys Ser Ser Asp Lys Thr His Thr Cys Pro Pro Cys
1 5 10

<210> SEQ ID NO 8
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Hinge

<400> SEQUENCE: 8

Asp Lys Thr His Thr Cys Pro Pro Cys
1 5

<210> SEQ ID NO 9
<211> LENGTH: 11
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Hinge

<400> SEQUENCE: 9

Glu Ser Lys Tyr Gly Pro Pro Cys Pro Pro Cys
1 5 10

<210> SEQ ID NO 10
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic - Linker(GGS)2

<400> SEQUENCE: 10

Gly Gly Ser Gly Gly Ser
1 5

<210> SEQ ID NO 11
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic - Linker (GGS)3

<400> SEQUENCE: 11

Gly Gly Ser Gly Gly Ser Gly Gly Ser
1 5

<210> SEQ ID NO 12
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence

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<220> FEATURE:

<223> OTHER INFORMATION: Synthetic - Linker (GGS)4

<400> SEQUENCE: 12

Gly Gly Ser Gly Gly Ser Gly Gly Ser Gly Gly Ser
1 5 10

<210> SEQ ID NO 13

<211> LENGTH: 15

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic - Linker (GGS)5

<400> SEQUENCE: 13

Gly Gly Ser Gly Gly Ser Gly Gly Ser Gly Gly Ser Gly Gly Ser
1 5 10 15

<210> SEQ ID NO 14

<211> LENGTH: 125

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic - anti-CD3 VH

<400> SEQUENCE: 14

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Lys Gly
1 5 10 15
Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asn Thr Tyr
20 25 30
Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ala Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp
50 55 60
Ser Val Lys Asp Arg Phe Thr Ile Ser Arg Asp Asp Ser Gln Ser Ile
65 70 75 80
Leu Tyr Leu Gln Met Asn Asn Leu Lys Thr Glu Asp Thr Ala Met Tyr
85 90 95
Tyr Cys Val Arg His Gly Asn Phe Gly Asn Ser Tyr Val Ser Trp Phe
100 105 110
Ala Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ala
115 120 125

<210> SEQ ID NO 15

<211> LENGTH: 109

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: anti-CD3 VL

<400> SEQUENCE: 15

Gln Ala Val Val Thr Gln Glu Ser Ala Leu Thr Thr Ser Pro Gly Glu
1 5 10 15
Thr Val Thr Leu Thr Cys Arg Ser Ser Thr Gly Ala Val Thr Thr Ser
20 25 30
Asn Tyr Ala Asn Trp Val Gln Glu Lys Pro Asp His Leu Phe Thr Gly
35 40 45
Leu Ile Gly Gly Thr Asn Lys Arg Ala Pro Gly Val Pro Ala Arg Phe
50 55 60

-continued

Ser Gly Ser Leu Ile Gly Asp Lys Ala Ala Leu Thr Ile Thr Gly Ala
65 70 75 80

Gln Thr Glu Asp Glu Ala Ile Tyr Phe Cys Ala Leu Trp Tyr Ser Asn
85 90 95

Leu Trp Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu
100 105

<210> SEQ ID NO 16
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: anti-CD3 VH CDR1

<400> SEQUENCE: 16

Thr Tyr Ala Met Asn
1 5

<210> SEQ ID NO 17
<211> LENGTH: 19
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: anti-CD3 VH CDR2

<400> SEQUENCE: 17

Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp Ser
1 5 10 15

Val Lys Asp

<210> SEQ ID NO 18
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: anti-CD3 VH CDR3

<400> SEQUENCE: 18

His Gly Asn Phe Gly Asn Ser Tyr Val Ser Trp Phe Ala Tyr
1 5 10

<210> SEQ ID NO 19
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: anti-CD3 VL CDR1

<400> SEQUENCE: 19

Arg Ser Ser Thr Gly Ala Val Thr Thr Ser Asn Tyr Ala Asn
1 5 10

<210> SEQ ID NO 20
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: anti-CD3 VL CDR2

<400> SEQUENCE: 20

Gly Thr Asn Lys Arg Ala Pro
1 5

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<210> SEQ ID NO 21
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: anti-CD3 VL CDR3

<400> SEQUENCE: 21

Ala Leu Trp Tyr Ser Asn Leu Trp Val
1 5

<210> SEQ ID NO 22
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Granzyme B substrate

<400> SEQUENCE: 22

Leu Glu Ala Asp
1

<210> SEQ ID NO 23
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Granzyme B substrate

<400> SEQUENCE: 23

Arg Gln Ala Arg
1

<210> SEQ ID NO 24
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: MMP substrate

<400> SEQUENCE: 24

Pro Ala Gly Leu
1

<210> SEQ ID NO 25
<211> LENGTH: 19
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Linker

<400> SEQUENCE: 25

Thr Gly Leu Glu Ala Asp Gly Ser Pro Ala Gly Leu Gly Arg Gln Ala
1 5 10 15

Arg Val Gly

<210> SEQ ID NO 26
<211> LENGTH: 19
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Linker

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<400> SEQUENCE: 26

Thr Gly Leu Glu Ala Asp Gly Ser Arg Gln Ala Arg Val Gly Pro Ala
1 5 10 15

Gly Leu Gly

<210> SEQ ID NO 27

<211> LENGTH: 19

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Linker

<400> SEQUENCE: 27

Thr Gly Ser Pro Ala Gly Leu Glu Ala Asp Gly Ser Arg Gln Ala Arg
1 5 10 15

Val Gly Ser

<210> SEQ ID NO 28

<211> LENGTH: 19

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Linker

<400> SEQUENCE: 28

Thr Gly Pro Ala Gly Leu Gly Leu Glu Ala Asp Gly Ser Arg Gln Ala
1 5 10 15

Arg Val Gly

<210> SEQ ID NO 29

<211> LENGTH: 19

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Linker

<400> SEQUENCE: 29

Thr Gly Arg Gln Ala Arg Val Gly Leu Glu Ala Asp Gly Ser Pro Ala
1 5 10 15

Gly Leu Gly

<210> SEQ ID NO 30

<211> LENGTH: 18

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Linker

<400> SEQUENCE: 30

Thr Gly Ser Arg Gln Ala Arg Val Gly Pro Ala Gly Leu Glu Ala Asp
1 5 10 15

Gly Ser

<210> SEQ ID NO 31

<211> LENGTH: 20

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Linker

<400> SEQUENCE: 31

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Thr Gly Pro Ala Gly Leu Gly Ser Arg Gln Ala Arg Val Gly Leu Glu
1 5 10 15

Ala Asp Gly Ser
20

<210> SEQ ID NO 32
<211> LENGTH: 125
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: anti-CD3 VH1

<400> SEQUENCE: 32

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Thr Tyr
20 25 30

Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Gly Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp
50 55 60

Ser Val Lys Asp Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Ser
65 70 75 80

Leu Tyr Leu Gln Met Asn Ser Leu Lys Thr Glu Asp Thr Ala Val Tyr
85 90 95

Tyr Cys Val Arg His Gly Asn Phe Gly Asn Ser Tyr Val Ser Trp Phe
100 105 110

Ala Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
115 120 125

<210> SEQ ID NO 33
<211> LENGTH: 125
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: anti-CD3 VH2

<400> SEQUENCE: 33

Glu Val Lys Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asn Thr Tyr
20 25 30

Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ala Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp
50 55 60

Ser Val Lys Asp Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Ser Ser
65 70 75 80

Leu Tyr Leu Gln Met Asn Asn Leu Lys Thr Glu Asp Thr Ala Met Tyr
85 90 95

Tyr Cys Val Arg His Gly Asn Phe Gly Asn Ser Tyr Val Ser Trp Phe
100 105 110

Ala Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
115 120 125

-continued

<210> SEQ ID NO 34
<211> LENGTH: 125
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: anti-CD3 VH3

<400> SEQUENCE: 34

Glu Val Lys Leu Val Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Arg
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asn Thr Tyr
20 25 30
Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ala Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp
50 55 60
Ser Val Lys Asp Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Ser Ile
65 70 75 80
Leu Tyr Leu Gln Met Asn Asn Leu Lys Thr Glu Asp Thr Ala Met Tyr
85 90 95
Tyr Cys Val Arg His Gly Asn Phe Gly Asn Ser Tyr Val Ser Trp Phe
100 105 110
Ala Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
115 120 125

<210> SEQ ID NO 35
<211> LENGTH: 125
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: anti-CD3 VH4

<400> SEQUENCE: 35

Glu Val Lys Leu Val Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Arg
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asn Thr Tyr
20 25 30
Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ala Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp
50 55 60
Ser Val Lys Asp Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Ser Ile
65 70 75 80
Leu Tyr Leu Gln Met Asn Ser Leu Lys Thr Glu Asp Thr Ala Met Tyr
85 90 95
Tyr Cys Val Arg His Gly Asn Phe Gly Asn Ser Tyr Val Ser Trp Phe
100 105 110
Ala Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
115 120 125

<210> SEQ ID NO 36
<211> LENGTH: 125
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: anti-CD3 VH5

<400> SEQUENCE: 36

-continued

Glu Val Lys Leu Val Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Arg
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asn Thr Tyr
20 25 30
Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ala Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp
50 55 60
Ser Val Lys Asp Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Ser Ile
65 70 75 80
Leu Tyr Leu Gln Met Asn Ser Leu Lys Thr Glu Asp Thr Ala Met Tyr
85 90 95
Tyr Cys Val Arg His Gly Asn Phe Gly Asn Ser Tyr Val Ser Trp Phe
100 105 110
Ala Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
115 120 125

<210> SEQ ID NO 37
<211> LENGTH: 125
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: anti-CD3 VH6

<400> SEQUENCE: 37

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Thr Tyr
20 25 30
Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ser Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp
50 55 60
Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr
65 70 75 80
Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr
85 90 95
Tyr Cys Val Arg His Gly Asn Phe Gly Asn Ser Tyr Val Ser Trp Phe
100 105 110
Ala Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
115 120 125

<210> SEQ ID NO 38
<211> LENGTH: 125
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: anti-CD3 VH7

<400> SEQUENCE: 38

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Thr Tyr
20 25 30
Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val

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35	40	45
Gly Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp		
50	55	60
Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr		
65	70	75 80
Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr		
	85	90 95
Tyr Cys Val Arg His Gly Asn Phe Gly Asp Ser Tyr Val Ser Trp Phe		
	100	105 110
Ala Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser		
	115	120 125

<210> SEQ ID NO 39
 <211> LENGTH: 124
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: anti-CD3 VH8

<400> SEQUENCE: 39

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asn Lys Tyr
20 25 30
Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ala Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp
50 55 60
Ser Val Lys Asp Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr
65 70 75 80
Ala Tyr Leu Gln Met Asn Asn Leu Lys Thr Glu Asp Thr Ala Val Tyr
85 90 95
Tyr Cys Val Arg His Gly Asn Phe Gly Asn Ser Tyr Ile Ser Tyr Trp
100 105 110
Ala Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser
115 120

<210> SEQ ID NO 40
 <211> LENGTH: 125
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: anti-CD3 VH9

<400> SEQUENCE: 40

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asn Thr Tyr
20 25 30
Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ala Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp
50 55 60
Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr
65 70 75 80

Leu	Tyr	Leu	Gln	Met	Asn	Ser	Leu	Arg	Ala	Glu	Asp	Thr	Ala	Val	Tyr
				85					90					95	
Tyr	Cys	Val	Arg	His	Gly	Asn	Phe	Gly	Asn	Ser	Tyr	Val	Ser	Trp	Phe
			100					105					110		
Ala	Tyr	Trp	Gly	Gln	Gly	Thr	Thr	Val	Thr	Val	Ser	Ser			
		115					120				125				

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<210> SEQ ID NO 41
<211> LENGTH: 125
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: anti-CD3 VH10
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<400> SEQUENCE: 41

Glu	Val	Gln	Leu	Val	Glu	Ser	Gly	Gly	Gly	Leu	Val	Gln	Pro	Gly	Gly
1				5					10					15	
Ser	Leu	Arg	Leu	Ser	Cys	Ala	Ala	Ser	Gly	Phe	Thr	Phe	Asn	Thr	Tyr
			20					25					30		
Ala	Met	Asn	Trp	Val	Arg	Gln	Ala	Pro	Gly	Lys	Gly	Leu	Glu	Trp	Val
		35				40						45			
Ala	Arg	Ile	Arg	Ser	Lys	Tyr	Asn	Asn	Tyr	Ala	Thr	Tyr	Tyr	Ala	Asp
50					55						60				
Ser	Val	Lys	Gly	Arg	Phe	Thr	Ile	Ser	Arg	Asp	Asp	Ser	Lys	Asn	Thr
65				70					75					80	
Leu	Tyr	Leu	Gln	Met	Asn	Ser	Leu	Arg	Ala	Glu	Asp	Thr	Ala	Val	Tyr
			85					90					95		
Tyr	Cys	Val	Arg	His	Gly	Asn	Phe	Gly	Asn	Ser	Tyr	Val	Ser	Tyr	Phe
			100					105					110		
Ala	Tyr	Trp	Gly	Gln	Gly	Thr	Thr	Val	Thr	Val	Ser	Ser			
		115				120					125				

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<210> SEQ ID NO 42
<211> LENGTH: 125
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: anti-CD3 VH11
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<400> SEQUENCE: 42

Glu	Val	Gln	Leu	Val	Glu	Ser	Gly	Gly	Gly	Leu	Val	Gln	Pro	Lys	Gly
1				5					10					15	
Ser	Leu	Lys	Leu	Ser	Cys	Ala	Ala	Ser	Gly	Phe	Thr	Phe	Asn	Thr	Tyr
			20					25					30		
Ala	Met	Asn	Trp	Val	Arg	Gln	Ala	Pro	Gly	Lys	Gly	Leu	Glu	Trp	Val
		35					40					45			
Ala	Arg	Ile	Arg	Ser	Lys	Tyr	Asn	Asn	Tyr	Ala	Thr	Tyr	Tyr	Ala	Asp
	50					55					60				
Ser	Val	Lys	Asp	Arg	Phe	Thr	Ile	Ser	Arg	Asp	Asp	Ser	Gln	Ser	Ile
65					70					75					80
Leu	Tyr	Leu	Gln	Met	Asn	Asn	Leu	Lys	Thr	Glu	Asp	Thr	Ala	Met	Tyr
				85					90					95	
Tyr	Cys	Val	Arg	His	Gly	Asn	Phe	Gly	Asn	Ser	Tyr	Val	Ser	Trp	Phe
			100					105					110		
Ala	Tyr	Trp	Gly	Gln	Gly	Thr	Leu	Val	Thr	Val	Ser	Ser			
		115					120					125			

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<210> SEQ ID NO 43
<211> LENGTH: 125
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: anti-CD3 VH12

<400> SEQUENCE: 43

Glu	Val	Gln	Leu	Val	Glu	Ser	Gly	Gly	Gly	Leu	Val	Gln	Pro	Gly	Gly
1				5					10					15	
Ser	Leu	Arg	Leu	Ser	Cys	Ala	Ala	Ser	Gly	Phe	Thr	Phe	Asn	Thr	Tyr
			20					25					30		
Ala	Met	Asn	Trp	Val	Arg	Gln	Ala	Pro	Gly	Lys	Gly	Leu	Glu	Trp	Val
		35				40						45			
Ala	Arg	Ile	Arg	Ser	Lys	Tyr	Asn	Asn	Tyr	Ala	Thr	Tyr	Tyr	Ala	Asp
	50					55				60					
Ser	Val	Lys	Gly	Arg	Phe	Thr	Ile	Ser	Arg	Asp	Asp	Ala	Lys	Asn	Thr
65				70					75					80	
Leu	Tyr	Leu	Gln	Met	Ser	Ser	Leu	Arg	Ala	Glu	Asp	Thr	Ala	Val	Tyr
			85					90						95	
Tyr	Cys	Val	Arg	His	Gly	Asn	Phe	Gly	Asn	Ser	Tyr	Val	Ser	Trp	Phe
		100					105						110		
Ala	Tyr	Trp	Gly	Gln	Gly	Thr	Leu	Val	Thr	Val	Lys	Pro			
		115					120					125			

<210> SEQ ID NO 44
<211> LENGTH: 125
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: anti-CD3 VH13

<400> SEQUENCE: 44

Glu	Val	Gln	Leu	Val	Glu	Ser	Gly	Gly	Gly	Leu	Val	Gln	Pro	Gly	Gly
1				5					10					15	
Ser	Leu	Arg	Leu	Ser	Cys	Ala	Ala	Ser	Gly	Phe	Thr	Phe	Asn	Thr	Tyr
			20					25					30		
Ala	Met	Asn	Trp	Val	Arg	Gln	Ala	Pro	Gly	Lys	Cys	Leu	Glu	Trp	Val
		35				40						45			
Ala	Arg	Ile	Arg	Ser	Lys	Tyr	Asn	Asn	Tyr	Ala	Thr	Tyr	Tyr	Ala	Asp
	50					55				60					
Ser	Val	Lys	Gly	Arg	Phe	Thr	Ile	Ser	Arg	Asp	Asp	Ala	Lys	Asn	Thr
65				70					75					80	
Leu	Tyr	Leu	Gln	Met	Ser	Ser	Leu	Arg	Ala	Glu	Asp	Thr	Ala	Val	Tyr
			85					90						95	
Tyr	Cys	Val	Arg	His	Gly	Asn	Phe	Gly	Asn	Ser	Tyr	Val	Ser	Trp	Phe
		100					105						110		
Ala	Tyr	Trp	Gly	Gln	Gly	Thr	Leu	Val	Thr	Val	Lys	Pro			
		115					120					125			

<210> SEQ ID NO 45
<211> LENGTH: 125
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: anti-CD3 VH14

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<400> SEQUENCE: 45

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asn Thr Tyr
20 25 30
Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ala Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp
50 55 60
Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ala Lys Asn Thr
65 70 75 80
Leu Tyr Leu Gln Met Ser Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr
85 90 95
Tyr Cys Val Arg His Gly Asn Phe Gly Asn Ser Tyr Val Ser Trp Phe
100 105 110
Ala Tyr Trp Gly Cys Gly Thr Leu Val Thr Val Lys Pro
115 120 125

<210> SEQ ID NO 46

<211> LENGTH: 125

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: anti-CD3 VH15

<400> SEQUENCE: 46

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Thr Tyr
20 25 30
Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ala Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp
50 55 60
Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ala Lys Asn Thr
65 70 75 80
Leu Tyr Leu Gln Met Ser Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr
85 90 95
Tyr Cys Val Arg His Gly Asn Phe Gly Asn Ser Tyr Val Ser Trp Phe
100 105 110
Ala Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
115 120 125

<210> SEQ ID NO 47

<211> LENGTH: 125

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: anti-CD3 VH16

<400> SEQUENCE: 47

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asn Thr Tyr
20 25 30

-continued

Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45

Ser Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp
 50 55 60

Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ala Lys Asn Thr
 65 70 75 80

Leu Tyr Leu Gln Met Ser Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr
 85 90 95

Tyr Cys Val Arg His Gly Asn Phe Gly Asn Ser Tyr Val Ser Trp Phe
 100 105 110

Ala Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
 115 120 125

<210> SEQ ID NO 48
 <211> LENGTH: 125
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: anti-CD3 VH17

<400> SEQUENCE: 48

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Thr Tyr
 20 25 30

Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45

Ser Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp
 50 55 60

Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ala Lys Asn Thr
 65 70 75 80

Leu Tyr Leu Gln Met Ser Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr
 85 90 95

Tyr Cys Val Arg His Gly Asn Phe Gly Asn Ser Tyr Val Ser Trp Phe
 100 105 110

Ala Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
 115 120 125

<210> SEQ ID NO 49
 <211> LENGTH: 125
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: anti-CD3 VH18

<400> SEQUENCE: 49

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Thr Tyr
 20 25 30

Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Cys Leu Glu Trp Val
 35 40 45

Ala Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp
 50 55 60

Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ala Lys Asn Thr

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65	70	75	80
Leu Tyr Leu Gln Met Ser Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr			
	85	90	95
Tyr Cys Val Arg His Gly Asn Phe Gly Asn Ser Tyr Val Ser Trp Phe			
	100	105	110
Ala Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser			
	115	120	125

<210> SEQ ID NO 50
 <211> LENGTH: 125
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: anti-CD3 VH19

<400> SEQUENCE: 50

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly			
1	5	10	15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asn Thr Tyr			
	20	25	30
Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Cys Leu Glu Trp Val			
	35	40	45
Ser Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp			
	50	55	60
Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ala Lys Asn Thr			
65	70	75	80
Leu Tyr Leu Gln Met Ser Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr			
	85	90	95
Tyr Cys Val Arg His Gly Asn Phe Gly Asn Ser Tyr Val Ser Trp Phe			
	100	105	110
Ala Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser			
	115	120	125

<210> SEQ ID NO 51
 <211> LENGTH: 125
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: anti-CD3 VH20

<400> SEQUENCE: 51

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly			
1	5	10	15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Thr Tyr			
	20	25	30
Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Cys Leu Glu Trp Val			
	35	40	45
Ser Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp			
	50	55	60
Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ala Lys Asn Thr			
65	70	75	80
Leu Tyr Leu Gln Met Ser Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr			
	85	90	95
Tyr Cys Val Arg His Gly Asn Phe Gly Asn Ser Tyr Val Ser Trp Phe			
	100	105	110

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Ala	Tyr	Trp	Gly	Gln	Gly	Thr	Leu	Val	Thr	Val	Ser	Ser
		115					120				125	

<210> SEQ ID NO 52
<211> LENGTH: 125
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: anti-CD3 VH21

<400> SEQUENCE: 52

Glu	Val	Gln	Leu	Val	Glu	Ser	Gly	Gly	Gly	Leu	Val	Gln	Pro	Gly	Gly
1			5					10					15		
Ser	Leu	Arg	Leu	Ser	Cys	Ala	Ala	Ser	Gly	Phe	Thr	Phe	Ser	Thr	Tyr
		20						25					30		
Ala	Met	Asn	Trp	Val	Arg	Gln	Ala	Pro	Gly	Lys	Cys	Leu	Glu	Trp	Val
		35					40					45			
Gly	Arg	Ile	Arg	Ser	Lys	Tyr	Asn	Asn	Tyr	Ala	Thr	Tyr	Tyr	Ala	Asp
	50					55				60					
Ser	Val	Lys	Asp	Arg	Phe	Thr	Ile	Ser	Arg	Asp	Asp	Ser	Lys	Asn	Ser
65				70					75					80	
Leu	Tyr	Leu	Gln	Met	Asn	Ser	Leu	Lys	Thr	Glu	Asp	Thr	Ala	Val	Tyr
			85					90						95	
Tyr	Cys	Val	Arg	His	Gly	Asn	Phe	Gly	Asn	Ser	Tyr	Val	Ser	Trp	Phe
			100					105					110		
Ala	Tyr	Trp	Gly	Gln	Gly	Thr	Leu	Val	Thr	Val	Ser	Ser			
		115					120				125				

<210> SEQ ID NO 53
<211> LENGTH: 125
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: anti-CD3 VH22

<400> SEQUENCE: 53

Glu	Val	Lys	Leu	Val	Glu	Ser	Gly	Gly	Gly	Leu	Val	Gln	Pro	Gly	Gly
1			5					10					15		
Ser	Leu	Arg	Leu	Ser	Cys	Ala	Ala	Ser	Gly	Phe	Thr	Phe	Asn	Thr	Tyr
		20						25					30		
Ala	Met	Asn	Trp	Val	Arg	Gln	Ala	Pro	Gly	Lys	Cys	Leu	Glu	Trp	Val
		35					40					45			
Ala	Arg	Ile	Arg	Ser	Lys	Tyr	Asn	Asn	Tyr	Ala	Thr	Tyr	Tyr	Ala	Asp
	50					55				60					
Ser	Val	Lys	Asp	Arg	Phe	Thr	Ile	Ser	Arg	Asp	Asp	Ser	Lys	Ser	Ser
65				70					75					80	
Leu	Tyr	Leu	Gln	Met	Asn	Asn	Leu	Lys	Thr	Glu	Asp	Thr	Ala	Met	Tyr
			85					90						95	
Tyr	Cys	Val	Arg	His	Gly	Asn	Phe	Gly	Asn	Ser	Tyr	Val	Ser	Trp	Phe
			100					105					110		
Ala	Tyr	Trp	Gly	Gln	Gly	Thr	Leu	Val	Thr	Val	Ser	Ser			
		115					120				125				

<210> SEQ ID NO 54
<211> LENGTH: 125
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence

-continued

<220> FEATURE:

<223> OTHER INFORMATION: anti-CD3 VH23

<400> SEQUENCE: 54

Glu Val Lys Leu Val Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Arg
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asn Thr Tyr
20 25 30
Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Cys Leu Glu Trp Val
35 40 45
Ala Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp
50 55 60
Ser Val Lys Asp Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Ser Ile
65 70 75 80
Leu Tyr Leu Gln Met Asn Asn Leu Lys Thr Glu Asp Thr Ala Met Tyr
85 90 95
Tyr Cys Val Arg His Gly Asn Phe Gly Asn Ser Tyr Val Ser Trp Phe
100 105 110
Ala Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
115 120 125

<210> SEQ ID NO 55

<211> LENGTH: 125

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: anti-CD3 VH24

<400> SEQUENCE: 55

Glu Val Lys Leu Val Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Arg
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asn Thr Tyr
20 25 30
Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Cys Leu Glu Trp Val
35 40 45
Ala Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp
50 55 60
Ser Val Lys Asp Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Ser Ile
65 70 75 80
Leu Tyr Leu Gln Met Asn Ser Leu Lys Thr Glu Asp Thr Ala Met Tyr
85 90 95
Tyr Cys Val Arg His Gly Asn Phe Gly Asn Ser Tyr Val Ser Trp Phe
100 105 110
Ala Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
115 120 125

<210> SEQ ID NO 56

<211> LENGTH: 125

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: anti-CD3 VH25

<400> SEQUENCE: 56

Glu Val Lys Leu Val Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Arg
1 5 10 15

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Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asn Thr Tyr
      20                25                30

Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Cys Leu Glu Trp Val
      35                40                45

Ala Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp
      50                55                60

Ser Val Lys Asp Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Ser Ile
      65                70                75                80

Leu Tyr Leu Gln Met Asn Ser Leu Lys Thr Glu Asp Thr Ala Met Tyr
      85                90                95

Tyr Cys Val Arg His Gly Asn Phe Gly Asn Ser Tyr Val Ser Trp Phe
      100               105               110

Ala Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
      115                120                125

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<210> SEQ ID NO 57
<211> LENGTH: 125
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: anti-CD3 VH26

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<400> SEQUENCE: 57

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Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1      5      10      15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Thr Tyr
      20                25                30

Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Cys Leu Glu Trp Val
      35                40                45

Ser Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp
      50                55                60

Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr
      65                70                75                80

Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr
      85                90                95

Tyr Cys Val Arg His Gly Asn Phe Gly Asn Ser Tyr Val Ser Trp Phe
      100               105               110

Ala Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
      115                120                125

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<210> SEQ ID NO 58
<211> LENGTH: 125
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: anti-CD3 VH27

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<400> SEQUENCE: 58

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Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1      5      10      15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Thr Tyr
      20                25                30

Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Cys Leu Glu Trp Val
      35                40                45

Gly Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp
      50                55                60

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Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr
65                               70                               75                               80

Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr
                               85                               90                               95

Tyr Cys Val Arg His Gly Asn Phe Gly Asp Ser Tyr Val Ser Trp Phe
                               100                              105                              110

Ala Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
                               115                              120                              125

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<210> SEQ ID NO 59
<211> LENGTH: 124
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: anti-CD3 VH28

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<400> SEQUENCE: 59

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Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1           5           10           15

Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asn Lys Tyr
          20          25          30

Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Cys Leu Glu Trp Val
          35          40          45

Ala Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp
          50          55          60

Ser Val Lys Asp Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr
65                               70                               75                               80

Ala Tyr Leu Gln Met Asn Asn Leu Lys Thr Glu Asp Thr Ala Val Tyr
          85          90          95

Tyr Cys Val Arg His Gly Asn Phe Gly Asn Ser Tyr Ile Ser Tyr Trp
          100         105         110

Ala Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser
          115         120

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<210> SEQ ID NO 60
<211> LENGTH: 125
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: anti-CD3 VH29

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<400> SEQUENCE: 60

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Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1           5           10           15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asn Thr Tyr
          20          25          30

Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Cys Leu Glu Trp Val
          35          40          45

Ala Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp
          50          55          60

Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr
65                               70                               75                               80

Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr
          85          90          95

Tyr Cys Val Arg His Gly Asn Phe Gly Asn Ser Tyr Val Ser Trp Phe

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100	105	110
Ala Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser		
115	120	125

<210> SEQ ID NO 61
<211> LENGTH: 125
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: anti-CD3 VH30

<400> SEQUENCE: 61

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly		
1	5	10
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asn Thr Tyr		
20	25	30
Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Cys Leu Glu Trp Val		
35	40	45
Ala Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp		
50	55	60
Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr		
65	70	75
Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr		
85	90	95
Tyr Cys Val Arg His Gly Asn Phe Gly Asn Ser Tyr Val Ser Tyr Phe		
100	105	110
Ala Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser		
115	120	125

<210> SEQ ID NO 62
<211> LENGTH: 125
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: anti-CD3 VH31

<400> SEQUENCE: 62

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Lys Gly		
1	5	10
Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asn Thr Tyr		
20	25	30
Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Cys Leu Glu Trp Val		
35	40	45
Ala Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp		
50	55	60
Ser Val Lys Asp Arg Phe Thr Ile Ser Arg Asp Asp Ser Gln Ser Ile		
65	70	75
Leu Tyr Leu Gln Met Asn Asn Leu Lys Thr Glu Asp Thr Ala Met Tyr		
85	90	95
Tyr Cys Val Arg His Gly Asn Phe Gly Asn Ser Tyr Val Ser Trp Phe		
100	105	110
Ala Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser		
115	120	125

<210> SEQ ID NO 63
<211> LENGTH: 109

-continued

<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: anti-CD3 VL1

<400> SEQUENCE: 63

Gln Ala Val Val Thr Gln Glu Ser Ala Leu Thr Thr Ser Pro Gly Glu
1 5 10 15
Thr Val Thr Leu Thr Cys Arg Ser Ser Thr Gly Ala Val Thr Thr Ser
20 25 30
Asn Tyr Ala Asn Trp Val Gln Glu Lys Pro Asp His Leu Phe Thr Gly
35 40 45
Leu Ile Gly Gly Thr Asn Lys Arg Ala Pro Gly Val Pro Ala Arg Phe
50 55 60
Ser Gly Ser Leu Ile Gly Asp Lys Ala Ala Leu Thr Ile Thr Gly Ala
65 70 75 80
Gln Thr Glu Asp Glu Ala Ile Tyr Phe Cys Ala Leu Trp Tyr Ser Asn
85 90 95
Leu Trp Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu
100 105

<210> SEQ ID NO 64
<211> LENGTH: 109
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: anti-CD3 VL2

<400> SEQUENCE: 64

Gln Ala Val Val Thr Gln Glu Pro Ser Leu Thr Val Ser Pro Gly Gly
1 5 10 15
Thr Val Thr Leu Thr Cys Gly Ser Ser Thr Gly Ala Val Thr Thr Ser
20 25 30
Asn Tyr Ala Asn Trp Val Gln Gln Lys Pro Gly Lys Ser Pro Arg Gly
35 40 45
Leu Ile Gly Gly Thr Asn Lys Arg Ala Pro Gly Val Pro Ala Arg Phe
50 55 60
Ser Gly Ser Leu Leu Gly Gly Lys Ala Ala Leu Thr Ile Ser Gly Ala
65 70 75 80
Gln Pro Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Leu Trp Tyr Ser Asn
85 90 95
His Trp Val Phe Gly Cys Gly Thr Lys Leu Glu Ile Lys
100 105

<210> SEQ ID NO 65
<211> LENGTH: 109
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: anti-CD3 VL3

<400> SEQUENCE: 65

Gln Ala Val Val Thr Gln Glu Pro Ser Leu Thr Val Ser Pro Gly Gly
1 5 10 15
Thr Val Thr Leu Thr Cys Arg Ser Ser Thr Gly Ala Val Thr Thr Ser
20 25 30
Asn Tyr Ala Asn Trp Val Gln Gln Lys Pro Gly Gln Ala Pro Arg Gly

-continued

35	40	45
Leu Ile Gly Gly Thr Asn Lys Arg Ala Pro Trp Thr Pro Ala Arg Phe		
50	55	60
Ser Gly Ser Leu Leu Gly Gly Lys Ala Ala Leu Thr Ile Thr Gly Ala		
65	70	75
Gln Ala Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Leu Trp Tyr Ser Asn		
	85	90
Leu Trp Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu		
100	105	

<210> SEQ ID NO 66
 <211> LENGTH: 109
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: anti-CD3 VL4

<400> SEQUENCE: 66

Gln Ala Val Val Thr Gln Glu Pro Ser Phe Ser Val Ser Pro Gly Gly		
1	5	10
Thr Val Thr Leu Thr Cys Arg Ser Ser Thr Gly Ala Val Thr Thr Ser		
	20	25
Asn Tyr Ala Asn Trp Val Gln Gln Thr Pro Gly Gln Ala Phe Arg Gly		
	35	40
Leu Ile Gly Gly Thr Asn Lys Arg Ala Pro Gly Val Pro Ala Arg Phe		
50	55	60
Ser Gly Ser Leu Ile Gly Asp Lys Ala Ala Leu Thr Ile Thr Gly Ala		
65	70	75
Gln Ala Asp Asp Glu Ser Ile Tyr Phe Cys Ala Leu Trp Tyr Ser Asn		
	85	90
Leu Trp Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu		
100	105	

<210> SEQ ID NO 67
 <211> LENGTH: 109
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: anti-CD3 VL5

<400> SEQUENCE: 67

Gln Ala Val Val Thr Gln Glu Pro Ser Phe Ser Val Ser Pro Gly Gly		
1	5	10
Thr Val Thr Leu Thr Cys Arg Ser Ser Thr Gly Ala Val Thr Thr Ser		
	20	25
Asn Tyr Ala Asn Trp Val Gln Gln Thr Pro Gly Gln Ala Phe Arg Gly		
	35	40
Leu Ile Gly Gly Thr Asn Lys Arg Ala Pro Gly Val Pro Ala Arg Phe		
50	55	60
Ser Gly Ser Ile Leu Gly Asn Lys Ala Ala Leu Thr Ile Thr Gly Ala		
65	70	75
Gln Ala Asp Asp Glu Ser Ile Tyr Phe Cys Ala Leu Trp Tyr Ser Asn		
	85	90
Leu Trp Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu		
100	105	

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<210> SEQ ID NO 68
<211> LENGTH: 109
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: anti-CD3 VL6

<400> SEQUENCE: 68

Gln Ala Val Val Thr Gln Glu Pro Ser Phe Ser Val Ser Pro Gly Gly
1 5 10 15
Thr Val Thr Leu Thr Cys Arg Ser Ser Thr Gly Ala Val Thr Thr Ser
20 25 30
Asn Tyr Ala Asn Trp Val Gln Gln Thr Pro Gly Gln Ala Phe Arg Gly
35 40 45
Leu Ile Gly Gly Thr Asn Lys Arg Ala Pro Gly Val Pro Ala Arg Phe
50 55 60
Ser Gly Ser Ile Leu Gly Asn Lys Ala Ala Leu Thr Ile Thr Gly Ala
65 70 75 80
Gln Ala Asp Asp Glu Ser Asp Tyr Tyr Cys Ala Leu Trp Tyr Ser Asn
85 90 95
Leu Trp Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu
100 105

<210> SEQ ID NO 69
<211> LENGTH: 109
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: anti-CD3 VL7

<400> SEQUENCE: 69

Gln Ala Val Val Thr Gln Glu Pro Ser Leu Thr Val Ser Pro Gly Gly
1 5 10 15
Thr Val Thr Leu Thr Cys Gly Ser Ser Thr Gly Ala Val Thr Thr Ser
20 25 30
Asn Tyr Ala Asn Trp Val Gln Glu Lys Pro Gly Gln Ala Phe Arg Gly
35 40 45
Leu Ile Gly Gly Thr Asn Lys Arg Ala Pro Gly Thr Pro Ala Arg Phe
50 55 60
Ser Gly Ser Leu Leu Gly Gly Lys Ala Ala Leu Thr Leu Ser Gly Ala
65 70 75 80
Gln Pro Glu Asp Glu Ala Glu Tyr Tyr Cys Ala Leu Trp Tyr Ser Asn
85 90 95
Leu Trp Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu
100 105

<210> SEQ ID NO 70
<211> LENGTH: 109
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: anti-CD3 VL8

<400> SEQUENCE: 70

Gln Thr Val Val Thr Gln Glu Pro Ser Leu Thr Val Ser Pro Gly Gly
1 5 10 15
Thr Val Thr Leu Thr Cys Gly Ser Ser Thr Gly Ala Val Thr Ser Gly

-continued

20					25					30					
Asn	Tyr	Pro	Asn	Trp	Val	Gln	Gln	Lys	Pro	Gly	Gln	Ala	Pro	Arg	Gly
	35						40					45			
Leu	Ile	Gly	Gly	Thr	Lys	Phe	Leu	Ala	Pro	Gly	Thr	Pro	Ala	Arg	Phe
	50					55					60				
Ser	Gly	Ser	Leu	Leu	Gly	Gly	Lys	Ala	Ala	Leu	Thr	Leu	Ser	Gly	Val
	65				70					75				80	
Gln	Pro	Glu	Asp	Glu	Ala	Glu	Tyr	Tyr	Cys	Val	Leu	Trp	Tyr	Ser	Asn
			85						90					95	
Arg	Trp	Val	Phe	Gly	Gly	Gly	Thr	Lys	Leu	Thr	Val	Leu			
		100						105							

<210> SEQ ID NO 71
 <211> LENGTH: 109
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: anti-CD3 VL9

<400> SEQUENCE: 71

Gln	Ala	Val	Val	Thr	Gln	Glu	Pro	Ser	Leu	Thr	Val	Ser	Pro	Gly	Gly
1				5					10					15	
Thr	Val	Thr	Leu	Thr	Cys	Gly	Ser	Ser	Thr	Gly	Ala	Val	Thr	Thr	Ser
			20				25					30			
Asn	Tyr	Ala	Asn	Trp	Val	Gln	Gln	Lys	Pro	Gly	Gln	Ala	Phe	Arg	Gly
	35						40					45			
Leu	Ile	Gly	Gly	Thr	Asn	Lys	Arg	Ala	Pro	Gly	Val	Pro	Ala	Arg	Phe
	50					55					60				
Ser	Gly	Ser	Leu	Leu	Gly	Gly	Lys	Ala	Ala	Leu	Thr	Ile	Ser	Gly	Ala
	65				70					75				80	
Gln	Pro	Glu	Asp	Glu	Ala	Asp	Tyr	Tyr	Cys	Ala	Leu	Trp	Tyr	Ser	Asn
			85						90					95	
His	Trp	Val	Phe	Gly	Gly	Gly	Thr	Lys	Leu	Glu	Ile	Lys			
		100						105							

<210> SEQ ID NO 72
 <211> LENGTH: 109
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: anti-CD3 VL10

<400> SEQUENCE: 72

Gln	Ala	Val	Val	Thr	Gln	Glu	Pro	Ser	Leu	Thr	Val	Ser	Pro	Gly	Gly
1				5					10					15	
Thr	Val	Thr	Leu	Thr	Cys	Gly	Ser	Ser	Thr	Gly	Ala	Val	Thr	Thr	Ser
			20				25					30			
Asn	Tyr	Ala	Asn	Trp	Val	Gln	Gln	Lys	Pro	Gly	Gln	Ala	Phe	Arg	Gly
	35						40					45			
Leu	Ile	Gly	Gly	Thr	Asn	Lys	Arg	Ala	Pro	Gly	Val	Pro	Ala	Arg	Phe
	50					55					60				
Ser	Gly	Ser	Leu	Leu	Gly	Gly	Lys	Ala	Ala	Leu	Thr	Ile	Ser	Gly	Ala
	65				70					75				80	
Gln	Pro	Glu	Asp	Glu	Ala	Asp	Tyr	Tyr	Cys	Ala	Leu	Trp	Tyr	Ser	Asn
			85						90					95	

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His Trp Val Phe Gly Cys Gly Thr Lys Leu Glu Ile Lys
100 105

<210> SEQ ID NO 73
<211> LENGTH: 109
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: anti-CD3 VL11

<400> SEQUENCE: 73

Gln Ala Val Val Thr Gln Glu Pro Ser Leu Thr Val Ser Pro Gly Gly
1 5 10 15
Thr Val Thr Leu Thr Cys Gly Ser Ser Thr Gly Ala Val Thr Thr Ser
20 25 30
Asn Tyr Ala Asn Trp Val Gln Gln Lys Pro Gly Gln Cys Phe Arg Gly
35 40 45
Leu Ile Gly Gly Thr Asn Lys Arg Ala Pro Gly Val Pro Ala Arg Phe
50 55 60
Ser Gly Ser Leu Leu Gly Gly Lys Ala Ala Leu Thr Ile Ser Gly Ala
65 70 75 80
Gln Pro Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Leu Trp Tyr Ser Asn
85 90 95
His Trp Val Phe Gly Glu Gly Thr Lys Leu Glu Ile Lys
100 105

<210> SEQ ID NO 74
<211> LENGTH: 109
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: anti-CD3 VL12

<400> SEQUENCE: 74

Gln Ala Val Val Thr Gln Glu Ser Ala Leu Thr Thr Ser Pro Gly Glu
1 5 10 15
Thr Val Thr Leu Thr Cys Arg Ser Ser Thr Gly Ala Val Thr Thr Ser
20 25 30
Asn Tyr Ala Asn Trp Val Gln Glu Lys Pro Asp His Leu Phe Thr Gly
35 40 45
Leu Ile Gly Gly Thr Asn Lys Arg Ala Pro Gly Val Pro Ala Arg Phe
50 55 60
Ser Gly Ser Leu Ile Gly Asp Lys Ala Ala Leu Thr Ile Thr Gly Ala
65 70 75 80
Gln Thr Glu Asp Glu Ala Ile Tyr Phe Cys Ala Leu Trp Tyr Ser Asn
85 90 95
Leu Trp Val Phe Gly Cys Gly Thr Lys Leu Thr Val Leu
100 105

<210> SEQ ID NO 75
<211> LENGTH: 109
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: anti-CD3 VL13

<400> SEQUENCE: 75

Gln Ala Val Val Thr Gln Glu Pro Ser Leu Thr Val Ser Pro Gly Gly

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1	5	10	15
Thr Val Thr Leu Thr Cys Gly Ser Ser Thr Gly Ala Val Thr Thr Ser	20	25	30
Asn Tyr Ala Asn Trp Val Gln Gln Lys Pro Gly Lys Ser Pro Arg Gly	35	40	45
Leu Ile Gly Gly Thr Asn Lys Arg Ala Pro Gly Val Pro Ala Arg Phe	50	55	60
Ser Gly Ser Leu Leu Gly Gly Lys Ala Ala Leu Thr Ile Ser Gly Ala	65	70	75
Gln Pro Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Leu Trp Tyr Ser Asn	85	90	95
His Trp Val Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys	100	105	

<210> SEQ ID NO 76
 <211> LENGTH: 109
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: anti-CD3 VL14

<400> SEQUENCE: 76

Gln Ala Val Val Thr Gln Glu Pro Ser Leu Thr Val Ser Pro Gly Gly	1	5	10	15
Thr Val Thr Leu Thr Cys Arg Ser Ser Thr Gly Ala Val Thr Thr Ser	20	25	30	
Asn Tyr Ala Asn Trp Val Gln Gln Lys Pro Gly Gln Ala Pro Arg Gly	35	40	45	
Leu Ile Gly Gly Thr Asn Lys Arg Ala Pro Trp Thr Pro Ala Arg Phe	50	55	60	
Ser Gly Ser Leu Leu Gly Gly Lys Ala Ala Leu Thr Ile Thr Gly Ala	65	70	75	80
Gln Ala Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Leu Trp Tyr Ser Asn	85	90	95	
Leu Trp Val Phe Gly Cys Gly Thr Lys Leu Thr Val Leu	100	105		

<210> SEQ ID NO 77
 <211> LENGTH: 109
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: anti-CD3 VL15

<400> SEQUENCE: 77

Gln Ala Val Val Thr Gln Glu Pro Ser Phe Ser Val Ser Pro Gly Gly	1	5	10	15
Thr Val Thr Leu Thr Cys Arg Ser Ser Thr Gly Ala Val Thr Thr Ser	20	25	30	
Asn Tyr Ala Asn Trp Val Gln Gln Thr Pro Gly Gln Ala Phe Arg Gly	35	40	45	
Leu Ile Gly Gly Thr Asn Lys Arg Ala Pro Gly Val Pro Ala Arg Phe	50	55	60	
Ser Gly Ser Leu Ile Gly Asp Lys Ala Ala Leu Thr Ile Thr Gly Ala	65	70	75	80

-continued

Gln Ala Asp Asp Glu Ser Ile Tyr Phe Cys Ala Leu Trp Tyr Ser Asn
85 90 95

Leu Trp Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu
100 105

<210> SEQ ID NO 78
<211> LENGTH: 109
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: anti-CD3 VL16

<400> SEQUENCE: 78

Gln Ala Val Val Thr Gln Glu Pro Ser Phe Ser Val Ser Pro Gly Gly
1 5 10 15

Thr Val Thr Leu Thr Cys Arg Ser Ser Thr Gly Ala Val Thr Thr Ser
20 25 30

Asn Tyr Ala Asn Trp Val Gln Gln Thr Pro Gly Gln Ala Phe Arg Gly
35 40 45

Leu Ile Gly Gly Thr Asn Lys Arg Ala Pro Gly Val Pro Ala Arg Phe
50 55 60

Ser Gly Ser Ile Leu Gly Asn Lys Ala Ala Leu Thr Ile Thr Gly Ala
65 70 75 80

Gln Ala Asp Asp Glu Ser Ile Tyr Phe Cys Ala Leu Trp Tyr Ser Asn
85 90 95

Leu Trp Val Phe Gly Cys Gly Thr Lys Leu Thr Val Leu
100 105

<210> SEQ ID NO 79
<211> LENGTH: 109
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: anti-CD3 VL17

<400> SEQUENCE: 79

Gln Ala Val Val Thr Gln Glu Pro Ser Phe Ser Val Ser Pro Gly Gly
1 5 10 15

Thr Val Thr Leu Thr Cys Arg Ser Ser Thr Gly Ala Val Thr Thr Ser
20 25 30

Asn Tyr Ala Asn Trp Val Gln Gln Thr Pro Gly Gln Ala Phe Arg Gly
35 40 45

Leu Ile Gly Gly Thr Asn Lys Arg Ala Pro Gly Val Pro Ala Arg Phe
50 55 60

Ser Gly Ser Ile Leu Gly Asn Lys Ala Ala Leu Thr Ile Thr Gly Ala
65 70 75 80

Gln Ala Asp Asp Glu Ser Asp Tyr Tyr Cys Ala Leu Trp Tyr Ser Asn
85 90 95

Leu Trp Val Phe Gly Cys Gly Thr Lys Leu Thr Val Leu
100 105

<210> SEQ ID NO 80
<211> LENGTH: 109
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: anti-CD3 VL18

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<400> SEQUENCE: 80

Gln Ala Val Val Thr Gln Glu Pro Ser Leu Thr Val Ser Pro Gly Gly
 1 5 10 15
 Thr Val Thr Leu Thr Cys Gly Ser Ser Thr Gly Ala Val Thr Thr Ser
 20 25 30
 Asn Tyr Ala Asn Trp Val Gln Glu Lys Pro Gly Gln Ala Phe Arg Gly
 35 40 45
 Leu Ile Gly Gly Thr Asn Lys Arg Ala Pro Gly Thr Pro Ala Arg Phe
 50 55 60
 Ser Gly Ser Leu Leu Gly Gly Lys Ala Ala Leu Thr Leu Ser Gly Ala
 65 70 75 80
 Gln Pro Glu Asp Glu Ala Glu Tyr Tyr Cys Ala Leu Trp Tyr Ser Asn
 85 90 95
 Leu Trp Val Phe Gly Cys Gly Thr Lys Leu Thr Val Leu
 100 105

<210> SEQ ID NO 81

<211> LENGTH: 109

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: anti-CD3 VL19

<400> SEQUENCE: 81

Gln Thr Val Val Thr Gln Glu Pro Ser Leu Thr Val Ser Pro Gly Gly
 1 5 10 15
 Thr Val Thr Leu Thr Cys Gly Ser Ser Thr Gly Ala Val Thr Ser Gly
 20 25 30
 Asn Tyr Pro Asn Trp Val Gln Gln Lys Pro Gly Gln Ala Pro Arg Gly
 35 40 45
 Leu Ile Gly Gly Thr Lys Phe Leu Ala Pro Gly Thr Pro Ala Arg Phe
 50 55 60
 Ser Gly Ser Leu Leu Gly Gly Lys Ala Ala Leu Thr Leu Ser Gly Val
 65 70 75 80
 Gln Pro Glu Asp Glu Ala Glu Tyr Tyr Cys Val Leu Trp Tyr Ser Asn
 85 90 95
 Arg Trp Val Phe Gly Cys Gly Thr Lys Leu Thr Val Leu
 100 105

<210> SEQ ID NO 82

<211> LENGTH: 226

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Knob Fc

<400> SEQUENCE: 82

Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly
 1 5 10 15
 Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met
 20 25 30
 Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
 35 40 45
 Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val
 50 55 60

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His	Asn	Ala	Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	Tyr	Asn	Ser	Thr	Tyr
65					70					75					80
Arg	Val	Val	Ser	Val	Leu	Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly
			85					90					95		
Lys	Glu	Tyr	Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala	Leu	Pro	Ala	Pro	Ile
			100					105					110		
Glu	Lys	Thr	Ile	Ser	Lys	Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val
		115					120					125			
Tyr	Thr	Leu	Pro	Pro	Cys	Arg	Asp	Glu	Leu	Thr	Lys	Asn	Gln	Val	Ser
	130					135					140				
Leu	Trp	Cys	Leu	Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu
145					150					155					160
Trp	Glu	Ser	Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro
			165					170						175	
Val	Leu	Asp	Ser	Asp	Gly	Ser	Phe	Phe	Leu	Tyr	Ser	Lys	Leu	Thr	Val
		180						185					190		
Asp	Lys	Ser	Arg	Trp	Gln	Gln	Gly	Asn	Val	Phe	Ser	Cys	Ser	Val	Met
		195					200					205			
His	Glu	Ala	Leu	His	Asn	His	Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser
	210					215					220				
Pro	Thr														
225															

<210> SEQ ID NO 83
 <211> LENGTH: 226
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Hole Fc

<400> SEQUENCE: 83

Asp	Lys	Thr	His	Thr	Cys	Pro	Pro	Cys	Pro	Ala	Pro	Glu	Leu	Leu	Gly
1				5					10					15	
Gly	Pro	Ser	Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met
			20					25					30		
Arg	Ser	Arg	Thr	Pro	Glu	Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser	His
		35					40					45			
Glu	Asp	Pro	Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val
	50					55					60				
His	Asn	Ala	Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	Tyr	Asn	Ser	Thr	Tyr
65					70					75					80
Arg	Val	Val	Ser	Val	Leu	Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly
			85					90						95	
Lys	Glu	Tyr	Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala	Leu	Pro	Ala	Pro	Ile
			100					105					110		
Glu	Lys	Thr	Ile	Ser	Lys	Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val
		115					120					125			
Cys	Thr	Leu	Pro	Pro	Ser	Arg	Asp	Glu	Leu	Thr	Lys	Asn	Gln	Val	Ser
	130					135					140				
Leu	Ser	Cys	Ala	Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu
145					150					155					160
Trp	Glu	Ser	Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro
			165					170						175	

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Val	Leu	Asp	Ser	Asp	Gly	Ser	Phe	Phe	Leu	Val	Ser	Lys	Leu	Thr	Val
		180						185					190		
Asp	Lys	Ser	Arg	Trp	Gln	Gln	Gly	Asn	Val	Phe	Ser	Cys	Ser	Val	Met
		195					200					205			
His	Glu	Ala	Leu	His	Asn	His	Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser
	210					215					220				
Pro	Thr														
225															

<210> SEQ ID NO 84
<211> LENGTH: 223
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Knob Fc

<400> SEQUENCE: 84

Asp	Lys	Thr	His	Thr	Cys	Pro	Pro	Cys	Pro	Ala	Pro	Gly	Gly	Pro	Ser
1				5					10					15	
Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met	Ile	Ser	Arg
		20						25					30		
Thr	Pro	Glu	Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser	His	Glu	Asp	Pro
		35				40						45			
Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val	His	Asn	Ala
	50					55					60				
Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	Tyr	Asn	Ser	Thr	Tyr	Arg	Val	Val
65					70					75				80	
Ser	Val	Leu	Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly	Lys	Glu	Tyr
		85						90						95	
Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala	Leu	Pro	Ala	Pro	Ile	Glu	Lys	Thr
		100						105					110		
Ile	Ser	Lys	Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr	Thr	Leu
	115					120						125			
Pro	Pro	Cys	Arg	Asp	Glu	Leu	Thr	Lys	Asn	Gln	Val	Ser	Leu	Trp	Cys
	130					135					140				
Leu	Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu	Trp	Glu	Ser
145					150					155				160	
Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro	Val	Leu	Asp
			165						170					175	
Ser	Asp	Gly	Ser	Phe	Phe	Leu	Tyr	Ser	Lys	Leu	Thr	Val	Asp	Lys	Ser
		180						185					190		
Arg	Trp	Gln	Gln	Gly	Asn	Val	Phe	Ser	Cys	Ser	Val	Met	His	Glu	Ala
		195					200					205			
Leu	His	Asn	His	Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser	Pro	Thr	
	210					215					220				

<210> SEQ ID NO 85
<211> LENGTH: 223
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Hole Fc

<400> SEQUENCE: 85

Asp	Lys	Thr	His	Thr	Cys	Pro	Pro	Cys	Pro	Ala	Pro	Gly	Gly	Pro	Ser
1				5					10					15	

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Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met	Arg	Ser	Arg
		20						25					30		
Thr	Pro	Glu	Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser	His	Glu	Asp	Pro
		35					40					45			
Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val	His	Asn	Ala
	50					55					60				
Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	Tyr	Asn	Ser	Thr	Tyr	Arg	Val	Val
	65				70					75				80	
Ser	Val	Leu	Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly	Lys	Glu	Tyr
			85					90					95		
Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala	Leu	Pro	Ala	Pro	Ile	Glu	Lys	Thr
			100					105					110		
Ile	Ser	Lys	Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	Cys	Thr	Leu
		115				120					125				
Pro	Pro	Ser	Arg	Asp	Glu	Leu	Thr	Lys	Asn	Gln	Val	Ser	Leu	Ser	Cys
	130					135					140				
Ala	Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu	Trp	Glu	Ser
	145				150					155				160	
Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro	Val	Leu	Asp
			165					170						175	
Ser	Asp	Gly	Ser	Phe	Phe	Leu	Val	Ser	Lys	Leu	Thr	Val	Asp	Lys	Ser
		180						185					190		
Arg	Trp	Gln	Gln	Gly	Asn	Val	Phe	Ser	Cys	Ser	Val	Met	His	Glu	Ala
		195				200					205				
Leu	His	Asn	His	Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser	Pro	Thr	
	210					215					220				

<210> SEQ ID NO 86

<211> LENGTH: 226

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Knob Fc

<400> SEQUENCE: 86

Asp	Lys	Thr	His	Thr	Cys	Pro	Pro	Cys	Pro	Ala	Pro	Glu	Leu	Leu	Gly
1			5					10					15		
Gly	Pro	Ser	Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met
		20					25					30			
Ile	Ser	Arg	Thr	Pro	Glu	Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser	His
	35					40					45				
Glu	Asp	Pro	Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val
	50				55					60					
His	Asn	Ala	Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	Tyr	Asn	Ser	Thr	Tyr
	65				70					75				80	
Arg	Val	Val	Ser	Val	Leu	Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly
			85					90					95		
Lys	Glu	Tyr	Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala	Leu	Pro	Ala	Pro	Ile
			100					105					110		
Glu	Lys	Thr	Ile	Ser	Lys	Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val
	115					120					125				
Tyr	Thr	Leu	Pro	Pro	Cys	Arg	Asp	Glu	Leu	Thr	Lys	Asn	Gln	Val	Ser
	130					135					140				

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Leu Trp Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
145          150          155          160

Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro
          165          170          175

Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val
          180          185          190

Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met
          195          200          205

His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser
          210          215          220

Pro Gly
225

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<210> SEQ ID NO 87
<211> LENGTH: 226
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Hole Fc

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<400> SEQUENCE: 87

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Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly
1      5      10      15

Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met
20     25     30

Arg Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
35     40     45

Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val
50     55     60

His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr
65     70     75     80

Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly
85     90     95

Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile
100    105    110

Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val
115    120    125

Cys Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser
130    135    140

Leu Ser Cys Ala Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
145    150    155    160

Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro
165    170    175

Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Val Ser Lys Leu Thr Val
180    185    190

Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met
195    200    205

His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser
210    215    220

Pro Gly
225

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<210> SEQ ID NO 88

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<211> LENGTH: 223
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Knob Fc

<400> SEQUENCE: 88
Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Gly Gly Pro Ser
1      5      10      15
Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg
      20      25      30
Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro
      35      40      45
Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala
50      55      60
Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val
65      70      75      80
Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr
      85      90      95
Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr
      100     105     110
Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu
      115     120     125
Pro Pro Cys Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Trp Cys
      130     135     140
Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser
      145     150     155     160
Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp
      165     170     175
Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser
      180     185     190
Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala
      195     200     205
Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
      210     215     220

<210> SEQ ID NO 89
<211> LENGTH: 223
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Hole Fc

<400> SEQUENCE: 89
Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Gly Gly Pro Ser
1      5      10      15
Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Arg Ser Arg
      20      25      30
Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro
      35      40      45
Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala
50      55      60
Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val
65      70      75      80
Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr

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85					90					95					
Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala	Leu	Pro	Ala	Pro	Ile	Glu	Lys	Thr
			100					105					110		
Ile	Ser	Lys	Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	Cys	Thr	Leu
		115					120					125			
Pro	Pro	Ser	Arg	Asp	Glu	Leu	Thr	Lys	Asn	Gln	Val	Ser	Leu	Ser	Cys
		130					135					140			
Ala	Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu	Trp	Glu	Ser
					150							155			160
Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro	Val	Leu	Asp
					165										175
Ser	Asp	Gly	Ser	Phe	Phe	Leu	Val	Ser	Lys	Leu	Thr	Val	Asp	Lys	Ser
			180						185					190	
Arg	Trp	Gln	Gln	Gly	Asn	Val	Phe	Ser	Cys	Ser	Val	Met	His	Glu	Ala
			195						200					205	
Leu	His	Asn	His	Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser	Pro	Gly	
		210					215						220		

<210> SEQ ID NO 90
 <211> LENGTH: 226
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Hole Fc

<400> SEQUENCE: 90

Asp	Lys	Thr	His	Thr	Cys	Pro	Pro	Cys	Pro	Ala	Pro	Glu	Leu	Leu	Gly
1				5					10					15	
Gly	Pro	Ser	Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met
			20					25					30		
Ile	Ser	Arg	Thr	Pro	Glu	Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser	His
		35					40					45			
Glu	Asp	Pro	Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val
		50				55					60				
His	Asn	Ala	Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	Tyr	Asn	Ser	Thr	Tyr
		65			70					75					80
Arg	Val	Val	Ser	Val	Leu	Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly
			85					90						95	
Lys	Glu	Tyr	Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala	Leu	Pro	Ala	Pro	Ile
			100					105						110	
Glu	Lys	Thr	Ile	Ser	Lys	Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val
		115						120					125		
Cys	Thr	Leu	Pro	Pro	Ser	Arg	Asp	Glu	Leu	Thr	Lys	Asn	Gln	Val	Ser
		130				135						140			
Leu	Ser	Cys	Ala	Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu
		145			150					155					160
Trp	Glu	Ser	Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro
			165					170						175	
Val	Leu	Asp	Ser	Asp	Gly	Ser	Phe	Phe	Leu	Val	Ser	Lys	Leu	Thr	Val
			180					185					190		
Asp	Lys	Ser	Arg	Trp	Gln	Gln	Gly	Asn	Val	Phe	Ser	Cys	Ser	Val	Met
		195					200					205			
His	Glu	Ala	Leu	His	Asn	Arg	Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser

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210	215	220
Pro Thr		
225		
<210> SEQ ID NO 91 <211> LENGTH: 223 <212> TYPE: PRT <213> ORGANISM: Artificial Sequence <220> FEATURE: <223> OTHER INFORMATION: Hole Fc <400> SEQUENCE: 91		
Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Gly Gly Pro Ser		
1	5	10 15
Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg		
	20	25 30
Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro		
	35	40 45
Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala		
	50	55 60
Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val		
	65	70 75 80
Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr		
	85	90 95
Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr		
	100	105 110
Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Cys Thr Leu		
	115	120 125
Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Ser Cys		
	130	135 140
Ala Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser		
	145	150 155 160
Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp		
	165	170 175
Ser Asp Gly Ser Phe Phe Leu Val Ser Lys Leu Thr Val Asp Lys Ser		
	180	185 190
Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala		
	195	200 205
Leu His Asn Arg Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Thr		
	210	215 220
<210> SEQ ID NO 92 <211> LENGTH: 226 <212> TYPE: PRT <213> ORGANISM: Artificial Sequence <220> FEATURE: <223> OTHER INFORMATION: Hole Fc <400> SEQUENCE: 92		
Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly		
1	5	10 15
Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met		
	20	25 30
Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His		
	35	40 45

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Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val
 50 55 60
 His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr
 65 70 75 80
 Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly
 85 90 95
 Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile
 100 105 110
 Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val
 115 120 125
 Cys Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser
 130 135 140
 Leu Ser Cys Ala Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
 145 150 155 160
 Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro
 165 170 175
 Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Val Ser Lys Leu Thr Val
 180 185 190
 Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met
 195 200 205
 His Glu Ala Leu His Asn Arg Tyr Thr Gln Lys Ser Leu Ser Leu Ser
 210 215 220
 Pro Gly
 225

<210> SEQ ID NO 93
 <211> LENGTH: 223
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Hole Fc

<400> SEQUENCE: 93

Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Gly Gly Pro Ser
 1 5 10 15
 Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg
 20 25 30
 Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro
 35 40 45
 Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala
 50 55 60
 Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val
 65 70 75 80
 Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr
 85 90 95
 Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr
 100 105 110
 Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Cys Thr Leu
 115 120 125
 Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Ser Cys
 130 135 140
 Ala Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser
 145 150 155 160

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Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro	Val	Leu	Asp
				165					170					175	
Ser	Asp	Gly	Ser	Phe	Phe	Leu	Val	Ser	Lys	Leu	Thr	Val	Asp	Lys	Ser
			180					185					190		
Arg	Trp	Gln	Gln	Gly	Asn	Val	Phe	Ser	Cys	Ser	Val	Met	His	Glu	Ala
		195					200					205			
Leu	His	Asn	Arg	Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser	Pro	Gly	
	210					215					220				

<210> SEQ ID NO 94
 <211> LENGTH: 226
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Knob Fc

<400> SEQUENCE: 94

Asp	Lys	Thr	His	Thr	Cys	Pro	Pro	Cys	Pro	Ala	Pro	Glu	Leu	Leu	Gly
1				5				10					15		
Gly	Pro	Ser	Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu	Tyr
			20					25					30		
Ile	Ser	Arg	Thr	Pro	Glu	Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser	His
		35					40					45			
Glu	Asp	Pro	Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val
	50					55					60				
His	Asn	Ala	Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	Tyr	Asn	Ser	Thr	Tyr
65					70					75				80	
Arg	Val	Val	Ser	Val	Leu	Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly
			85					90						95	
Lys	Glu	Tyr	Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala	Leu	Pro	Ala	Pro	Ile
			100					105					110		
Glu	Lys	Thr	Ile	Ser	Lys	Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val
		115				120						125			
Tyr	Thr	Leu	Pro	Pro	Cys	Arg	Asp	Glu	Leu	Thr	Lys	Asn	Gln	Val	Ser
	130					135					140				
Leu	Trp	Cys	Leu	Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu
145					150					155				160	
Trp	Glu	Ser	Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro
			165					170						175	
Val	Leu	Asp	Ser	Asp	Gly	Ser	Phe	Phe	Leu	Tyr	Ser	Lys	Leu	Thr	Val
		180					185						190		
Asp	Lys	Ser	Arg	Trp	Gln	Gln	Gly	Asn	Val	Phe	Ser	Cys	Ser	Val	Val
		195					200					205			
His	Glu	Ala	Leu	His	Asn	His	Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser
	210					215					220				
Pro	Thr														
225															

<210> SEQ ID NO 95
 <211> LENGTH: 223
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Knob Fc

<400> SEQUENCE: 95

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Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Gly Gly Pro Ser
1 5 10 15
Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Tyr Ile Ser Arg
20 25 30
Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro
35 40 45
Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala
50 55 60
Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val
65 70 75 80
Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr
85 90 95
Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr
100 105 110
Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu
115 120 125
Pro Pro Cys Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Trp Cys
130 135 140
Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser
145 150 155 160
Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp
165 170 175
Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser
180 185 190
Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Val His Glu Ala
195 200 205
Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Thr
210 215 220

<210> SEQ ID NO 96

<211> LENGTH: 226

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Knob Fc

<400> SEQUENCE: 96

Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly
1 5 10 15
Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Tyr
20 25 30
Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
35 40 45
Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val
50 55 60
His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr
65 70 75 80
Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly
85 90 95
Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile
100 105 110
Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val
115 120 125

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Tyr Thr Leu Pro Pro Cys Arg Asp Glu Leu Thr Lys Asn Gln Val Ser
 130 135 140
 Leu Trp Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
 145 150 155 160
 Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro
 165 170 175
 Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val
 180 185 190
 Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Val
 195 200 205
 His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser
 210 215 220
 Pro Gly
 225

<210> SEQ ID NO 97
 <211> LENGTH: 223
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Knob Fc

<400> SEQUENCE: 97

Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Gly Gly Pro Ser
 1 5 10 15
 Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Tyr Ile Ser Arg
 20 25 30
 Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro
 35 40 45
 Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala
 50 55 60
 Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val
 65 70 75 80
 Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr
 85 90 95
 Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr
 100 105 110
 Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu
 115 120 125
 Pro Pro Cys Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Trp Cys
 130 135 140
 Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser
 145 150 155 160
 Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp
 165 170 175
 Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser
 180 185 190
 Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Val His Glu Ala
 195 200 205
 Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
 210 215 220

<210> SEQ ID NO 98

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<211> LENGTH: 226
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Hole Fc

<400> SEQUENCE: 98
Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly
1      5      10      15
Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Tyr
      20      25      30
Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
      35      40      45
Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val
      50      55      60
His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr
      65      70      75      80
Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly
      85      90      95
Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile
      100     105     110
Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val
      115     120     125
Cys Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser
      130     135     140
Leu Ser Cys Ala Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
      145     150     155     160
Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro
      165     170     175
Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Val Ser Lys Leu Thr Val
      180     185     190
Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Val
      195     200     205
His Glu Ala Leu His Asn Arg Tyr Thr Gln Lys Ser Leu Ser Leu Ser
      210     215     220

Pro Thr
225

<210> SEQ ID NO 99
<211> LENGTH: 223
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Hole Fc

<400> SEQUENCE: 99
Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Gly Gly Pro Ser
1      5      10      15
Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Tyr Ile Ser Arg
      20      25      30
Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro
      35      40      45
Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala
      50      55      60
Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val

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65	70	75	80
Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr	85	90	95
Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr	100	105	110
Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Cys Thr Leu	115	120	125
Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Ser Cys	130	135	140
Ala Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser	145	150	155
Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp	165	170	175
Ser Asp Gly Ser Phe Phe Leu Val Ser Lys Leu Thr Val Asp Lys Ser	180	185	190
Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Val His Glu Ala	195	200	205
Leu His Asn Arg Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Thr	210	215	220

<210> SEQ ID NO 100
 <211> LENGTH: 226
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Hole Fc

 <400> SEQUENCE: 100

Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly	1	5	10	15
Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Tyr	20	25	30	
Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His	35	40	45	
Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val	50	55	60	
His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr	65	70	75	80
Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly	85	90	95	
Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile	100	105	110	
Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val	115	120	125	
Cys Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser	130	135	140	
Leu Ser Cys Ala Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu	145	150	155	160
Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro	165	170	175	
Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Val Ser Lys Leu Thr Val	180	185	190	
Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Val				

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195	200	205
His Glu Ala Leu His Asn Arg Tyr Thr Gln Lys Ser Leu Ser Leu Ser		
210	215	220

Pro Gly
225

<210> SEQ ID NO 101
 <211> LENGTH: 223
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Hole Fc

<400> SEQUENCE: 101

Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Gly Gly Pro Ser		
1	5	10 15
Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Tyr Ile Ser Arg		
20	25	30
Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro		
35	40	45
Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala		
50	55	60
Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val		
65	70	75 80
Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr		
85	90	95
Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr		
100	105	110
Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Cys Thr Leu		
115	120	125
Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Ser Cys		
130	135	140
Ala Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser		
145	150	155 160
Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp		
165	170	175
Ser Asp Gly Ser Phe Phe Leu Val Ser Lys Leu Thr Val Asp Lys Ser		
180	185	190
Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Val His Glu Ala		
195	200	205
Leu His Asn Arg Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly		
210	215	220

<210> SEQ ID NO 102
 <211> LENGTH: 5
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Peptide Linker

<400> SEQUENCE: 102

Pro Gly Gly Gly Gly
1 5

<210> SEQ ID NO 103
 <211> LENGTH: 4

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<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Peptide Linker

<400> SEQUENCE: 103

Gly Gly Gly Gly
1

<210> SEQ ID NO 104
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Linker

<400> SEQUENCE: 104

Gly Pro Ala Gly Leu Gly Leu Glu Pro Asp Gly Ser Arg Gln Ala Arg
1 5 10 15

Val Gly

<210> SEQ ID NO 105
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Linker

<400> SEQUENCE: 105

Gly Gly Ser Gly Gly Gly Gly Ile Glu Pro Asp Ile Gly Gly Ser Gly
1 5 10 15

Gly Ser

<210> SEQ ID NO 106
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Linker

<400> SEQUENCE: 106

Gly Gly Ser Gly Gly Gly Gly Leu Glu Ala Asp Thr Gly Gly Ser Gly
1 5 10 15

Gly Ser

<210> SEQ ID NO 107
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Linker

<400> SEQUENCE: 107

Gly Ser Ile Glu Pro Asp Ile Gly Ser
1 5

<210> SEQ ID NO 108
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Linker

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<400> SEQUENCE: 108

Gly Ser Leu Glu Ala Asp Thr Gly Ser
1 5

<210> SEQ ID NO 109

<211> LENGTH: 18

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Linker

<400> SEQUENCE: 109

Gly Gly Ser Gly Gly Gly Gly Ile Glu Pro Asp Gly Gly Gly Ser Gly
1 5 10 15

Gly Ser

<210> SEQ ID NO 110

<211> LENGTH: 18

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Linker

<400> SEQUENCE: 110

Gly Gly Ser Gly Gly Gly Gly Ile Glu Pro Asp Val Gly Gly Ser Gly
1 5 10 15

Gly Ser

<210> SEQ ID NO 111

<211> LENGTH: 18

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Linker

<400> SEQUENCE: 111

Gly Gly Ser Gly Gly Gly Gly Ile Glu Pro Asp Ser Gly Gly Ser Gly
1 5 10 15

Gly Ser

<210> SEQ ID NO 112

<211> LENGTH: 18

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Linker

<400> SEQUENCE: 112

Gly Gly Ser Gly Gly Gly Gly Ile Glu Pro Asp Thr Gly Gly Ser Gly
1 5 10 15

Gly Ser

<210> SEQ ID NO 113

<211> LENGTH: 12

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Linker

<400> SEQUENCE: 113

Gly Gly Gly Ser Leu Glu Pro Asp Gly Ser Gly Ser

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1	5	10
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<210> SEQ ID NO 114
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Linker

<400> SEQUENCE: 114

Gly Pro Ala Gly Leu Gly Leu Glu Ala Asp Gly Ser Arg Gln Ala Arg
1 5 10 15

Val Gly

<210> SEQ ID NO 115
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Linker

<400> SEQUENCE: 115

Gly Gly Glu Gly Gly Gly Gly Ser Gly Gly Ser Gly Gly Gly Ser
1 5 10 15

<210> SEQ ID NO 116
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Linker

<400> SEQUENCE: 116

Gly Ser Ser Ala Gly Ser Glu Ala Gly Gly Ser Gly Gln Ala Gly Val
1 5 10 15

Gly Ser

<210> SEQ ID NO 117
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Linker

<400> SEQUENCE: 117

Gly Gly Ser Gly Gly Gly Gly Leu Glu Ala Glu Gly Ser Gly Gly Gly
1 5 10 15

Gly Ser

<210> SEQ ID NO 118
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Linker

<400> SEQUENCE: 118

Gly Gly Ser Gly Gly Gly Gly Ile Glu Pro Asp Pro Gly Gly Ser Gly
1 5 10 15

Gly Ser

-continued

<210> SEQ ID NO 119

<211> LENGTH: 18

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Linker

<400> SEQUENCE: 119

Gly Gly Gly Gly Gly Ser Gly Gly Gly Gly Gly Ser Gly Gly Gly Gly
1 5 10 15

Gly Ser

<210> SEQ ID NO 120

<211> LENGTH: 129

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: FR alpha sdAb

<400> SEQUENCE: 120

Gln Leu Gln Leu Gln Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Leu Asp Asn Tyr
20 25 30

Ala Ile Gly Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Glu Gly Val
35 40 45

Ser Cys Ile Ser Ser Ser Asp Gly Ser Thr Tyr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asn Asn Ala Lys Gly Thr Val Tyr
65 70 75 80

Leu Leu Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Thr Glu Leu Val Pro Ala Cys Thr Tyr Ser Asn Gly Arg Gly Pro
100 105 110

Leu Asp Gly Met Asp Tyr Trp Gly Lys Gly Thr Gln Val Thr Val Lys
115 120 125

Pro

<210> SEQ ID NO 121

<211> LENGTH: 119

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: FR alpha sdAb

<400> SEQUENCE: 121

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Glu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Ser Ile Phe Ser Ile Asp
20 25 30

Ala Thr Ala Trp Tyr Arg Gln Ala Pro Gly Lys Gln Arg Glu Leu Val
35 40 45

Ala Ile Ile Thr Ser Ser Gly Ser Thr Asn Tyr Pro Glu Ser Val Lys
50 55 60

Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Val Tyr Leu
65 70 75 80

Gln Met Ser Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Asn

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85	90	95
Ala Ile Thr Arg Tyr Gly Gly Ser Thr Tyr Asp Phe Trp Gly Gln Gly		
100	105	110
Thr Leu Val Thr Val Lys Pro		
115		

<210> SEQ ID NO 122
 <211> LENGTH: 101
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: FR alpha sdAb

<400> SEQUENCE: 122

Glu Val Gln Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Glu		
1	5	10 15
Thr Phe Gly Val Val Phe Thr Leu Gly Trp Tyr Arg Gln Ala Pro Gly		
20	25	30
Lys Gly Arg Glu Phe Val Ala Arg Val Thr Gly Thr Asp Thr Val Asp		
35	40	45
Tyr Ala Glu Ser Val Lys Gly Arg Phe Thr Ile Ser Ser Asp Phe Ala		
50	55	60
Arg Asn Thr Val Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr		
65	70	75 80
Ala Val Tyr Tyr Cys Asn Thr Gly Ala Tyr Trp Gly Gln Gly Thr Leu		
85	90	95
Val Thr Val Lys Pro		
100		

<210> SEQ ID NO 123
 <211> LENGTH: 125
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: cMET sdAb

<400> SEQUENCE: 123

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly		
1	5	10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Ile Leu Asp Tyr Tyr		
20	25	30
Ala Ile Gly Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Glu Gly Val		
35	40	45
Leu Cys Ile Asp Ala Ser Asp Asp Ile Thr Tyr Tyr Ala Asp Ser Val		
50	55	60
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Val Tyr		
65	70	75 80
Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Gly Val Tyr Tyr Cys		
85	90	95
Ala Thr Pro Ile Gly Leu Ser Ser Ser Cys Leu Leu Glu Tyr Asp Tyr		
100	105	110
Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Lys Pro		
115	120	125

<210> SEQ ID NO 124
 <211> LENGTH: 245

-continued

<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: B7H3 scFv

<400> SEQUENCE: 124

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Phe
20 25 30
Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ala Tyr Ile Ser Ser Asp Ser Ser Ala Ile Tyr Tyr Ala Asp Thr Val
50 55 60
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
65 70 75 80
Leu Gln Met Asn Ser Leu Arg Asp Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Gly Arg Gly Arg Glu Asn Ile Tyr Tyr Gly Ser Arg Leu Asp Tyr Trp
100 105 110
Gly Gln Gly Thr Thr Val Thr Val Ser Ser Ser Gly Gly Gly Gly Ser
115 120 125
Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Asp Ile Gln Leu Thr Gln
130 135 140
Ser Pro Ser Phe Leu Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr
145 150 155 160
Cys Lys Ala Ser Gln Asn Val Asp Thr Asn Val Ala Trp Tyr Gln Gln
165 170 175
Lys Pro Gly Lys Ala Pro Lys Ala Leu Ile Tyr Ser Ala Ser Tyr Arg
180 185 190
Tyr Ser Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp
195 200 205
Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr
210 215 220
Tyr Cys Gln Gln Tyr Asn Asn Tyr Pro Phe Thr Phe Gly Gln Gly Thr
225 230 235 240
Lys Leu Glu Ile Lys
245

<210> SEQ ID NO 125
<211> LENGTH: 247
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: CD20 scFv

<400> SEQUENCE: 125

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
1 5 10 15
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Ala Phe Ser Tyr Ser
20 25 30
Trp Ile Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45
Gly Arg Ile Phe Pro Gly Asp Gly Asp Thr Asp Tyr Asn Gly Lys Phe
50 55 60

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Lys Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Ser Thr Ala Tyr
 65 70 75 80
 Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95
 Ala Arg Asn Val Phe Asp Gly Tyr Trp Leu Val Tyr Trp Gly Gln Gly
 100 105 110
 Thr Leu Val Thr Val Ser Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly
 115 120 125
 Gly Thr Gly Gly Gly Gly Ser Asp Ile Val Met Thr Gln Thr Pro Leu
 130 135 140
 Ser Leu Pro Val Thr Pro Gly Glu Pro Ala Ser Ile Ser Cys Arg Ser
 145 150 155 160
 Ser Lys Ser Leu Leu His Ser Asn Gly Ile Thr Tyr Leu Tyr Trp Tyr
 165 170 175
 Leu Gln Lys Pro Gly Gln Ser Pro Gln Leu Leu Ile Tyr Gln Met Ser
 180 185 190
 Asn Leu Val Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly
 195 200 205
 Thr Asp Phe Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Val Gly
 210 215 220
 Val Tyr Tyr Cys Ala Gln Asn Leu Glu Leu Pro Tyr Thr Phe Gly Gly
 225 230 235 240
 Gly Thr Lys Val Glu Ile Lys
 245

<210> SEQ ID NO 126
 <211> LENGTH: 241
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: DLL3 scFv

<400> SEQUENCE: 126

Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Lys Pro Ser Glu
 1 5 10 15
 Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Gly Ser Ile Ser Ser Tyr
 20 25 30
 Tyr Trp Ser Trp Ile Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Ile
 35 40 45
 Gly Tyr Val Tyr Tyr Ser Gly Thr Thr Asn Tyr Asn Pro Ser Leu Lys
 50 55 60
 Ser Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn Gln Phe Ser Leu
 65 70 75 80
 Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys Ala
 85 90 95
 Ser Ile Ala Val Thr Gly Phe Tyr Phe Asp Tyr Trp Gly Gln Gly Thr
 100 105 110
 Leu Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
 115 120 125
 Gly Gly Gly Gly Ser Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu
 130 135 140
 Ser Leu Ser Pro Gly Glu Arg Val Thr Leu Ser Cys Arg Ala Ser Gln
 145 150 155 160

-continued

Arg Val Asn Asn Asn Tyr Leu Ala Trp Tyr Gln Gln Arg Pro Gly Gln
 165 170 175

Ala Pro Arg Leu Leu Ile Tyr Gly Ala Ser Ser Arg Ala Thr Gly Ile
 180 185 190

Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr
 195 200 205

Ile Ser Arg Leu Glu Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln
 210 215 220

Tyr Asp Arg Ser Pro Leu Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile
 225 230 235 240

Lys

<210> SEQ ID NO 127
 <211> LENGTH: 225
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: B7H3 Fd

<400> SEQUENCE: 127

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Phe
 20 25 30

Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45

Ala Tyr Ile Ser Ser Asp Ser Ser Ala Ile Tyr Tyr Ala Asp Thr Val
 50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
 65 70 75 80

Leu Gln Met Asn Ser Leu Arg Asp Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95

Gly Arg Gly Arg Glu Asn Ile Tyr Tyr Gly Ser Arg Leu Asp Tyr Trp
 100 105 110

Gly Gln Gly Thr Thr Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro
 115 120 125

Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr
 130 135 140

Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr
 145 150 155 160

Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro
 165 170 175

Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr
 180 185 190

Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn
 195 200 205

His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser
 210 215 220

Cys
 225

<210> SEQ ID NO 128
 <211> LENGTH: 214

-continued

<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: B7H3 LC

<400> SEQUENCE: 128

Asp Ile Gln Leu Thr Gln Ser Pro Ser Phe Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Lys Ala Ser Gln Asn Val Asp Thr Asn
20 25 30

Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Ala Leu Ile
35 40 45

Tyr Ser Ala Ser Tyr Arg Tyr Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Asn Asn Tyr Pro Phe
85 90 95

Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg Thr Val Ala Ala
100 105 110

Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
115 120 125

Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
130 135 140

Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
145 150 155 160

Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
165 170 175

Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
180 185 190

Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
195 200 205

Phe Asn Arg Gly Glu Cys
210

<210> SEQ ID NO 129
<211> LENGTH: 225
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 5T4 Fd

<400> SEQUENCE: 129

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Lys Gly
1 5 10 15

Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asn Thr Tyr
20 25 30

Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ala Arg Ile Arg Ser Lys Ser Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp
50 55 60

Ser Val Lys Asp Arg Phe Thr Ile Ser Arg Asp Asp Ser Gln Ser Met
65 70 75 80

Leu Tyr Leu Gln Met Asn Asn Leu Lys Thr Glu Asp Thr Ala Met Tyr
85 90 95

-continued

Tyr Cys Val Arg Gln Trp Asp Tyr Asp Val Arg Ala Met Asn Tyr Trp
 100 105 110
 Gly Gln Gly Thr Ser Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro
 115 120 125
 Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr
 130 135 140
 Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr
 145 150 155 160
 Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro
 165 170 175
 Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr
 180 185 190
 Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn
 195 200 205
 His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser
 210 215 220
 Cys
 225

<210> SEQ ID NO 130
 <211> LENGTH: 214
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: 5T4 LC

<400> SEQUENCE: 130

Asp Ile Val Met Thr Gln Ser His Ile Phe Met Ser Thr Ser Val Gly
 1 5 10 15
 Asp Arg Val Ser Ile Thr Cys Lys Ala Ser Gln Asp Val Asp Thr Ala
 20 25 30
 Val Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ser Pro Lys Leu Leu Ile
 35 40 45
 Tyr Trp Ala Ser Thr Arg Leu Thr Gly Val Pro Asp Arg Phe Thr Gly
 50 55 60
 Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Asn Val Gln Ser
 65 70 75 80
 Glu Asp Leu Ala Asp Tyr Phe Cys Gln Gln Tyr Ser Ser Tyr Pro Tyr
 85 90 95
 Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg Thr Val Ala Ala
 100 105 110
 Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
 115 120 125
 Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
 130 135 140
 Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
 145 150 155 160
 Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
 165 170 175
 Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
 180 185 190
 Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
 195 200 205

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Phe Asn Arg Gly Glu Cys
210

<210> SEQ ID NO 131
<211> LENGTH: 222
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: gpNMB Fd

<400> SEQUENCE: 131

Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Lys Pro Ser Gln
1 5 10 15
Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Gly Ser Ile Ser Ser Phe
20 25 30
Asn Tyr Tyr Trp Ser Trp Ile Arg His His Pro Gly Lys Gly Leu Glu
35 40 45
Trp Ile Gly Tyr Ile Tyr Tyr Ser Gly Ser Thr Tyr Ser Asn Pro Ser
50 55 60
Leu Lys Ser Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn Gln Phe
65 70 75 80
Ser Leu Thr Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr
85 90 95
Cys Ala Arg Gly Tyr Asn Trp Asn Tyr Phe Asp Tyr Trp Gly Gln Gly
100 105 110
Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe
115 120 125
Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu
130 135 140
Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp
145 150 155 160
Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu
165 170 175
Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser
180 185 190
Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro
195 200 205
Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys
210 215 220

<210> SEQ ID NO 132
<211> LENGTH: 215
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: gpNMB LC

<400> SEQUENCE: 132

Glu Ile Val Met Thr Gln Ser Pro Ala Thr Leu Ser Val Ser Pro Gly
1 5 10 15
Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Asp Asn Asn
20 25 30
Leu Val Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile
35 40 45
Tyr Gly Ala Ser Thr Arg Ala Thr Gly Ile Pro Ala Arg Phe Ser Gly

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50	55	60
Ser Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Ser		
65	70	75 80
Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Tyr Asn Asn Trp Pro Pro		
	85	90 95
Trp Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala		
	100	105 110
Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser		
	115	120 125
Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu		
	130	135 140
Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser		
	145	150 155 160
Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu		
	165	170 175
Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val		
	180	185 190
Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys		
	195	200 205
Ser Phe Asn Arg Gly Glu Cys		
	210	215

<210> SEQ ID NO 133

<211> LENGTH: 223

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: DLL3 Fd

<400> SEQUENCE: 133

Gln Ile Gln Leu Val Gln Ser Gly Pro Glu Leu Lys Lys Pro Gly Glu		
1	5	10 15
Thr Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn Tyr		
	20	25 30
Gly Met Asn Trp Val Lys Gln Ala Pro Gly Lys Gly Leu Lys Trp Met		
	35	40 45
Ala Trp Ile Asn Thr Tyr Thr Gly Glu Pro Thr Tyr Ala Asp Asp Phe		
	50	55 60
Lys Gly Arg Phe Ala Phe Ser Leu Glu Thr Ser Ala Ser Thr Ala Ser		
	65	70 75 80
Leu Gln Ile Ile Asn Leu Lys Asn Glu Asp Thr Ala Thr Tyr Phe Cys		
	85	90 95
Ala Arg Ile Gly Asp Ser Ser Pro Ser Asp Tyr Trp Gly Gln Gly Thr		
	100	105 110
Thr Leu Thr Val Ser Ser Ser Ser Ala Ser Thr Lys Gly Pro Ser Val		
	115	120 125
Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala		
	130	135 140
Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser		
	145	150 155 160
Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val		
	165	170 175
Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro		

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180	185	190
Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys		
195	200	205
Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys		
210	215	220

<210> SEQ ID NO 134
 <211> LENGTH: 214
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: DLL3 LC

<400> SEQUENCE: 134

Ser	Ile	Val	Met	Thr	Gln	Thr	Pro	Lys	Phe	Leu	Leu	Val	Ser	Ala	Gly
1				5					10					15	
Asp	Arg	Val	Thr	Ile	Thr	Cys	Lys	Ala	Ser	Gln	Ser	Val	Ser	Asn	Asp
		20					25						30		
Val	Val	Trp	Tyr	Gln	Gln	Lys	Pro	Gly	Gln	Ser	Pro	Lys	Leu	Leu	Ile
		35				40						45			
Tyr	Tyr	Ala	Ser	Asn	Arg	Tyr	Thr	Gly	Val	Pro	Asp	Arg	Phe	Ala	Gly
	50				55					60					
Ser	Gly	Tyr	Gly	Thr	Asp	Phe	Ser	Phe	Thr	Ile	Ser	Thr	Val	Gln	Ala
65				70					75					80	
Glu	Asp	Leu	Ala	Val	Tyr	Phe	Cys	Gln	Gln	Asp	Tyr	Thr	Ser	Pro	Trp
			85					90						95	
Thr	Phe	Gly	Gly	Gly	Thr	Lys	Leu	Glu	Ile	Arg	Arg	Thr	Val	Ala	Ala
		100					105						110		
Pro	Ser	Val	Phe	Ile	Phe	Pro	Pro	Ser	Asp	Glu	Gln	Leu	Lys	Ser	Gly
		115				120						125			
Thr	Ala	Ser	Val	Val	Cys	Leu	Leu	Asn	Asn	Phe	Tyr	Pro	Arg	Glu	Ala
	130				135						140				
Lys	Val	Gln	Trp	Lys	Val	Asp	Asn	Ala	Leu	Gln	Ser	Gly	Asn	Ser	Gln
145				150					155					160	
Glu	Ser	Val	Thr	Glu	Gln	Asp	Ser	Lys	Asp	Ser	Thr	Tyr	Ser	Leu	Ser
			165					170						175	
Ser	Thr	Leu	Thr	Leu	Ser	Lys	Ala	Asp	Tyr	Glu	Lys	His	Lys	Val	Tyr
		180				185						190			
Ala	Cys	Glu	Val	Thr	His	Gln	Gly	Leu	Ser	Ser	Pro	Val	Thr	Lys	Ser
	195					200						205			
Phe	Asn	Arg	Gly	Glu	Cys										
210															

<210> SEQ ID NO 135
 <211> LENGTH: 6
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Peptide linker

<400> SEQUENCE: 135

Gly	Gly	Gly	Gly	Gly	Ser
1				5	

<210> SEQ ID NO 136
 <211> LENGTH: 5

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<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Linker

<400> SEQUENCE: 136

Ile Glu Pro Asp Ile
1 5

<210> SEQ ID NO 137
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Linker

<400> SEQUENCE: 137

Leu Glu Ala Asp Thr
1 5

<210> SEQ ID NO 138
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Linker

<400> SEQUENCE: 138

Ile Glu Pro Asp Gly
1 5

<210> SEQ ID NO 139
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Linker

<400> SEQUENCE: 139

Ile Glu Pro Asp Val
1 5

<210> SEQ ID NO 140
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Linker

<400> SEQUENCE: 140

Ile Glu Pro Asp Ser
1 5

<210> SEQ ID NO 141
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Linker

<400> SEQUENCE: 141

Ile Glu Pro Asp Thr
1 5

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<210> SEQ ID NO 142
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Linker

<400> SEQUENCE: 142

Leu Glu Pro Asp
1

<210> SEQ ID NO 143
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Linker

<400> SEQUENCE: 143

Leu Glu Ala Glu
1

<210> SEQ ID NO 144
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Linker

<400> SEQUENCE: 144

Ile Glu Pro Asp Pro
1 5

<210> SEQ ID NO 145
<211> LENGTH: 274
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Second Polypeptide Chain of B7-H3 x CD3
Bispecific DART-A Diabody

<400> SEQUENCE: 145

Gln Ala Val Val Thr Gln Glu Pro Ser Leu Thr Val Ser Pro Gly Gly
1 5 10 15
Thr Val Thr Leu Thr Cys Arg Ser Ser Thr Gly Ala Val Thr Thr Ser
20 25 30
Asn Tyr Ala Asn Trp Val Gln Gln Lys Pro Gly Gln Ala Pro Arg Gly
35 40 45
Leu Ile Gly Gly Thr Asn Lys Arg Ala Pro Trp Thr Pro Ala Arg Phe
50 55 60
Ser Gly Ser Leu Leu Gly Gly Lys Ala Ala Leu Thr Ile Thr Gly Ala
65 70 75 80
Gln Ala Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Leu Trp Tyr Ser Asn
85 90 95
Leu Trp Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly Gly Gly
100 105 110
Gly Ser Gly Gly Gly Gly Glu Val Gln Leu Val Glu Ser Gly Gly Gly
115 120 125
Leu Val Gln Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly
130 135 140

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Phe	Thr	Phe	Ser	Ser	Phe	Gly	Met	His	Trp	Val	Arg	Gln	Ala	Pro	Gly
145					150					155					160
Lys	Gly	Leu	Glu	Trp	Val	Ala	Tyr	Ile	Ser	Ser	Asp	Ser	Ser	Ala	Ile
			165						170					175	
Tyr	Tyr	Ala	Asp	Thr	Val	Lys	Gly	Arg	Phe	Thr	Ile	Ser	Arg	Asp	Asn
		180						185					190		
Ala	Lys	Asn	Ser	Leu	Tyr	Leu	Gln	Met	Asn	Ser	Leu	Arg	Asp	Glu	Asp
		195					200					205			
Thr	Ala	Val	Tyr	Tyr	Cys	Gly	Arg	Gly	Arg	Glu	Asn	Ile	Tyr	Tyr	Gly
	210					215					220				
Ser	Arg	Leu	Asp	Tyr	Trp	Gly	Gln	Gly	Thr	Thr	Val	Thr	Val	Ser	Ser
225					230					235					240
Gly	Gly	Cys	Gly	Gly	Gly	Lys	Val	Ala	Ala	Leu	Lys	Glu	Lys	Val	Ala
			245						250					255	
Ala	Leu	Lys	Glu	Lys	Val	Ala	Ala	Leu	Lys	Glu	Lys	Val	Ala	Ala	Leu
		260						265					270		

Lys Glu

<210> SEQ ID NO 146
 <211> LENGTH: 227
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Third Polypeptide Chain of B7-H3 x CD3
 Bispecific DART-A Diabody

<400> SEQUENCE: 146

Asp	Lys	Thr	His	Thr	Cys	Pro	Pro	Cys	Pro	Ala	Pro	Glu	Ala	Ala	Gly
1				5				10						15	
Gly	Pro	Ser	Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met
			20					25					30		
Ile	Ser	Arg	Thr	Pro	Glu	Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser	His
		35					40					45			
Glu	Asp	Pro	Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val
	50					55					60				
His	Asn	Ala	Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	Tyr	Asn	Ser	Thr	Tyr
65					70					75					80
Arg	Val	Val	Ser	Val	Leu	Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly
			85					90						95	
Lys	Glu	Tyr	Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala	Leu	Pro	Ala	Pro	Ile
		100						105					110		
Glu	Lys	Thr	Ile	Ser	Lys	Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val
		115					120					125			
Tyr	Thr	Leu	Pro	Pro	Ser	Arg	Glu	Glu	Met	Thr	Lys	Asn	Gln	Val	Ser
		130				135						140			
Leu	Ser	Cys	Ala	Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu
145					150					155					160
Trp	Glu	Ser	Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro
			165					170						175	
Val	Leu	Asp	Ser	Asp	Gly	Ser	Phe	Phe	Leu	Val	Ser	Lys	Leu	Thr	Val
		180						185					190		
Asp	Lys	Ser	Arg	Trp	Gln	Gln	Gly	Asn	Val	Phe	Ser	Cys	Ser	Val	Met
		195					200					205			

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His	Glu	Ala	Leu	His	Asn	Arg	Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser
210					215						220				

Pro Gly Lys
225

<210> SEQ ID NO 147
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Linker

<400> SEQUENCE: 147

Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly
1			5					10					15		

Gly Ser

<210> SEQ ID NO 148
<211> LENGTH: 19
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Linker

<400> SEQUENCE: 148

Thr	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ile	Glu	Pro	Asp	Ile	Gly	Gly	Ser
1			5					10					15		

Gly Gly Ser

<210> SEQ ID NO 149
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Linker

<400> SEQUENCE: 149

Gly	Gly	Gly	Gly	Ser
1			5	

<210> SEQ ID NO 150
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Linker consensus
<220> FEATURE:
<221> NAME/KEY: VARIANT
<222> LOCATION: (1)..(1)
<223> OTHER INFORMATION: X1= I, L, Y, M, F, V, or A
<220> FEATURE:
<221> NAME/KEY: VARIANT
<222> LOCATION: (2)..(2)
<223> OTHER INFORMATION: X2 = A, G, S, V, E, D, Q, N, or Y
<220> FEATURE:
<221> NAME/KEY: VARIANT
<222> LOCATION: (3)..(3)
<223> OTHER INFORMATION: X3 = H, P, A, V, G, S, or T
<220> FEATURE:
<221> NAME/KEY: VARIANT
<222> LOCATION: (4)..(4)
<223> OTHER INFORMATION: X4 = D or E
<220> FEATURE:
<221> NAME/KEY: VARIANT
<222> LOCATION: (5)..(5)
<223> OTHER INFORMATION: X5 = I, L, Y, M, F, V, T, S, G or A

-continued

<400> SEQUENCE: 150

Xaa Xaa Xaa Xaa Xaa
1 5

<210> SEQ ID NO 151

<211> LENGTH: 5

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Linker consensus

<220> FEATURE:

<221> NAME/KEY: VARIANT

<222> LOCATION: (1)..(1)

<223> OTHER INFORMATION: X1 = I or L

<220> FEATURE:

<221> NAME/KEY: VARIANT

<222> LOCATION: (3)..(3)

<223> OTHER INFORMATION: X3 = P or A

<220> FEATURE:

<221> NAME/KEY: VARIANT

<222> LOCATION: (5)..(5)

<223> OTHER INFORMATION: X5 = I, V, T, S, or G

<400> SEQUENCE: 151

Xaa Glu Xaa Asp Xaa
1 5

<210> SEQ ID NO 152

<211> LENGTH: 5

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Linker

<400> SEQUENCE: 152

Leu Glu Pro Asp Gly
1 5

<210> SEQ ID NO 153

<211> LENGTH: 5

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Linker

<400> SEQUENCE: 153

Leu Glu Ala Asp Gly
1 5

<210> SEQ ID NO 154

<211> LENGTH: 5

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Linker consensus

<220> FEATURE:

<221> NAME/KEY: VARIANT

<222> LOCATION: (1)..(1)

<223> OTHER INFORMATION: X1 = any amino acid

<220> FEATURE:

<221> NAME/KEY: VARIANT

<222> LOCATION: (5)..(5)

<223> OTHER INFORMATION: X5 = A or V

<400> SEQUENCE: 154

Xaa Gln Ala Arg Xaa

-continued

1 5

<210> SEQ ID NO 155
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Linker consensus
<220> FEATURE:
<221> NAME/KEY: VARIANT
<222> LOCATION: (5)..(5)
<223> OTHER INFORMATION: X5 = A or V

<400> SEQUENCE: 155

Arg Gln Ala Arg Xaa
1 5

<210> SEQ ID NO 156
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Linker

<400> SEQUENCE: 156

Arg Gln Ala Arg Val
1 5

<210> SEQ ID NO 157
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Linker consensus
<220> FEATURE:
<221> NAME/KEY: VARIANT
<222> LOCATION: (1)..(1)
<223> OTHER INFORMATION: X1 = P, V or A
<220> FEATURE:
<221> NAME/KEY: VARIANT
<222> LOCATION: (2)..(2)
<223> OTHER INFORMATION: X2 = Q or D
<220> FEATURE:
<221> NAME/KEY: VARIANT
<222> LOCATION: (3)..(3)
<223> OTHER INFORMATION: X3 = A or N
<220> FEATURE:
<221> NAME/KEY: VARIANT
<222> LOCATION: (4)..(4)
<223> OTHER INFORMATION: X4 = L, I or M

<400> SEQUENCE: 157

Xaa Xaa Xaa Xaa
1

<210> SEQ ID NO 158
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Linker consensus
<220> FEATURE:
<221> NAME/KEY: VARIANT
<222> LOCATION: (2)..(2)
<223> OTHER INFORMATION: X2 = Q or D
<220> FEATURE:
<221> NAME/KEY: VARIANT
<222> LOCATION: (3)..(3)
<223> OTHER INFORMATION: X3 = A or N

-continued

<220> FEATURE:
<221> NAME/KEY: VARIANT
<222> LOCATION: (4)..(4)
<223> OTHER INFORMATION: X4 = L or I

<400> SEQUENCE: 158

Pro Xaa Xaa Xaa
1

<210> SEQ ID NO 159
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: P2A

<400> SEQUENCE: 159

Gly Ser Gly Ala Thr Asn Phe Ser Leu Leu Lys Gln Ala Gly Asp Val
1 5 10 15
Glu Glu Asn Pro Gly Pro
20

<210> SEQ ID NO 160
<211> LENGTH: 19
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: P2A

<400> SEQUENCE: 160

Ala Thr Asn Phe Ser Leu Leu Lys Gln Ala Gly Asp Val Glu Glu Asn
1 5 10 15
Pro Gly Pro

<210> SEQ ID NO 161
<211> LENGTH: 20
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: E2A

<400> SEQUENCE: 161

Gln Cys Thr Asn Tyr Ala Leu Leu Lys Leu Ala Gly Asp Val Glu Ser
1 5 10 15
Asn Pro Gly Pro
20

<210> SEQ ID NO 162
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: F2A

<400> SEQUENCE: 162

Val Lys Gln Thr Leu Asn Phe Asp Leu Leu Lys Leu Ala Gly Asp Val
1 5 10 15
Glu Ser Asn Pro Gly Pro
20

<210> SEQ ID NO 163
<211> LENGTH: 18

-continued

<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: T2A

<400> SEQUENCE: 163

Glu Gly Arg Gly Ser Leu Leu Thr Cys Gly Asp Val Glu Glu Asn Pro
1 5 10 15

Gly Pro

<210> SEQ ID NO 164
<211> LENGTH: 24
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: T2A

<400> SEQUENCE: 164

Leu Glu Gly Gly Gly Glu Gly Arg Gly Ser Leu Leu Thr Cys Gly Asp
1 5 10 15

Val Glu Glu Asn Pro Gly Pro Arg
20

<210> SEQ ID NO 165
<211> LENGTH: 66
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: P2A

<400> SEQUENCE: 165

ggatctggag caacaaactt ctactactc aaacaagcag gtgacgtgga ggagaatccc 60

ggacccc 66

<210> SEQ ID NO 166
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Linker

<400> SEQUENCE: 166

Gly Ser Pro Ala Gly Leu Glu Ala Asp Gly Ser Arg Gln Ala Arg Val
1 5 10 15

Gly Ser

<210> SEQ ID NO 167
<211> LENGTH: 122
<212> TYPE: PRT
<213> ORGANISM: Mus musculus
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: anti-5T4 VH
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (97)..(97)
<223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid

<400> SEQUENCE: 167

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Lys Gly
1 5 10 15

Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asn Thr Tyr

-continued

20	25	30
Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val		
35	40	45
Ala Arg Ile Arg Ser Lys Ser Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp		
50	55	60
Ser Val Lys Asp Arg Phe Thr Ile Ser Arg Asp Asp Ser Gln Ser Met		
65	70	80
Leu Tyr Leu Gln Met Asn Asn Leu Lys Thr Glu Asp Thr Ala Met Tyr		
85	90	95
Xaa Cys Val Arg Gln Trp Asp Tyr Asp Val Arg Ala Met Asn Tyr Trp		
100	105	110
Gly Gln Gly Thr Ser Val Thr Val Ser Ser		
115	120	

<210> SEQ ID NO 168
 <211> LENGTH: 107
 <212> TYPE: PRT
 <213> ORGANISM: Mus musculus
 <220> FEATURE:
 <221> NAME/KEY: MISC_FEATURE
 <223> OTHER INFORMATION: anti-5T4 VL

<400> SEQUENCE: 168

Asp Ile Val Met Thr Gln Ser His Ile Phe Met Ser Thr Ser Val Gly
1 5 10 15
Asp Arg Val Ser Ile Thr Cys Lys Ala Ser Gln Asp Val Asp Thr Ala
20 25 30
Val Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ser Pro Lys Leu Leu Ile
35 40 45
Tyr Trp Ala Ser Thr Arg Leu Thr Gly Val Pro Asp Arg Phe Thr Gly
50 55 60
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Asn Val Gln Ser
65 70 75 80
Glu Asp Leu Ala Asp Tyr Phe Cys Gln Gln Tyr Ser Ser Tyr Pro Tyr
85 90 95
Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
100 105

<210> SEQ ID NO 169
 <211> LENGTH: 504
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: First Polypeptide Chain of B7-H3 x CD3
 Bispecific DART-A Diabody

<400> SEQUENCE: 169

Asp Ile Gln Leu Thr Gln Ser Pro Ser Phe Leu Ser Ala Ser Val Gly
1 5 10 15
Asp Arg Val Thr Ile Thr Cys Lys Ala Ser Gln Asn Val Asp Thr Asn
20 25 30
Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Ala Leu Ile
35 40 45
Tyr Ser Ala Ser Tyr Arg Tyr Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro

-continued

65	70	75	80
Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Asn Asn Tyr Pro Phe	85	90	95
Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Gly Gly Gly Ser Gly	100	105	110
Gly Gly Gly Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln	115	120	125
Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe	130	135	140
Ser Thr Tyr Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu	145	150	155
Glu Trp Val Gly Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr	165	170	175
Tyr Ala Asp Ser Val Lys Asp Arg Phe Thr Ile Ser Arg Asp Asp Ser	180	185	190
Lys Asn Ser Leu Tyr Leu Gln Met Asn Ser Leu Lys Thr Glu Asp Thr	195	200	205
Ala Val Tyr Tyr Cys Val Arg His Gly Asn Phe Gly Asn Ser Tyr Val	210	215	220
Ser Trp Phe Ala Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser	225	230	235
Gly Gly Cys Gly Gly Gly Glu Val Ala Ala Leu Glu Lys Glu Val Ala	245	250	255
Ala Leu Glu Lys Glu Val Ala Ala Leu Glu Lys Glu Val Ala Ala Leu	260	265	270
Glu Lys Gly Gly Gly Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala	275	280	285
Pro Glu Ala Ala Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro	290	295	300
Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val	305	310	315
Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val	325	330	335
Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln	340	345	350
Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln	355	360	365
Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala	370	375	380
Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro	385	390	395
Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr	405	410	415
Lys Asn Gln Val Ser Leu Trp Cys Leu Val Lys Gly Phe Tyr Pro Ser	420	425	430
Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr	435	440	445
Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr	450	455	460
Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe	465	470	475
			480

-continued

Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys
485 490 495

Ser Leu Ser Leu Ser Pro Gly Lys
500

<210> SEQ ID NO 170
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Linker

<400> SEQUENCE: 170

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
1 5 10 15

<210> SEQ ID NO 171
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Linker
<220> FEATURE:
<221> NAME/KEY: REPEAT
<222> LOCATION: (4) .. (6)
<223> OTHER INFORMATION: repeated 0 to 10 times

<400> SEQUENCE: 171

Gly Gly Ser Gly Gly Ser
1 5

<210> SEQ ID NO 172
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Linker
<220> FEATURE:
<221> NAME/KEY: REPEAT
<222> LOCATION: (1) .. (6)
<223> OTHER INFORMATION: repeated 1 to 4 times

<400> SEQUENCE: 172

Gly Gly Gly Gly Gly Ser
1 5

<210> SEQ ID NO 173
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Linker
<220> FEATURE:
<221> NAME/KEY: REPEAT
<222> LOCATION: (1) .. (5)
<223> OTHER INFORMATION: Repeated 1 to 10 times
<220> FEATURE:
<221> NAME/KEY: REPEAT
<222> LOCATION: (1) .. (5)
<223> OTHER INFORMATION: Repeated 1 to 5 times

<400> SEQUENCE: 173

Gly Gly Gly Gly Ser
1 5

-continued

<210> SEQ ID NO 174
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Linker
<220> FEATURE:
<221> NAME/KEY: REPEAT
<222> LOCATION: (1)..(1)
<223> OTHER INFORMATION: Repeated 1 to 5 times
<220> FEATURE:
<221> NAME/KEY: VARIANT
<222> LOCATION: (2)..(2)
<223> OTHER INFORMATION: X2= A, V, L, I, M,F, W, P, G, S, T, C, Y, N,Q,
K, R, H, D, or E
<220> FEATURE:
<221> NAME/KEY: REPEAT
<222> LOCATION: (3)..(3)
<223> OTHER INFORMATION: Repeated 1 to 5 times
<220> FEATURE:
<221> NAME/KEY: VARIANT
<222> LOCATION: (4)..(4)
<223> OTHER INFORMATION: X4 = A, V, L, I, M,F, W, P, G, S, T, C, Y, N,Q,
K, R, H, D, or E
<220> FEATURE:
<221> NAME/KEY: REPEAT
<222> LOCATION: (5)..(5)
<223> OTHER INFORMATION: Repeated 1 to 5 times

<400> SEQUENCE: 174

Gly Xaa Gly Xaa Gly
1 5

<210> SEQ ID NO 175
<211> LENGTH: 11
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Linker
<220> FEATURE:
<221> NAME/KEY: VARIANT
<222> LOCATION: (4)..(4)
<223> OTHER INFORMATION: X4 = A, V, L, I, M,F, W, P, G, S, T, C, Y, N,Q,
K, R, H, D, or E
<220> FEATURE:
<221> NAME/KEY: VARIANT
<222> LOCATION: (8)..(8)
<223> OTHER INFORMATION: X8 = A, V, L, I, M,F, W, P, G, S, T, C, Y, N,Q,
K, R, H, D, or E

<400> SEQUENCE: 175

Gly Gly Gly Xaa Gly Gly Gly Xaa Gly Gly Gly
1 5 10

<210> SEQ ID NO 176
<211> LENGTH: 11
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Linker

<400> SEQUENCE: 176

Ala Thr Thr Thr Gly Ser Ser Pro Gly Pro Thr
1 5 10

<210> SEQ ID NO 177
<211> LENGTH: 11
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:

-continued

<223> OTHER INFORMATION: Linker

<400> SEQUENCE: 177

Gly Gly Gly Gly Gly Cys Gly Gly Gly Gly Gly
1 5 10

<210> SEQ ID NO 178

<211> LENGTH: 5

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Linker

<220> FEATURE:

<221> NAME/KEY: REPEAT

<222> LOCATION: (1)..(5)

<223> OTHER INFORMATION: Repeated 2 to 20 times

<400> SEQUENCE: 178

Glu Ala Ala Ala Lys
1 5

<210> SEQ ID NO 179

<211> LENGTH: 6

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Linker

<220> FEATURE:

<221> NAME/KEY: REPEAT

<222> LOCATION: (3)..(4)

<223> OTHER INFORMATION: Repeated 2 to 20 times

<400> SEQUENCE: 179

Ala Ser Ala Pro Gly Thr
1 5

<210> SEQ ID NO 180

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Linker

<220> FEATURE:

<221> NAME/KEY: REPEAT

<222> LOCATION: (3)..(7)

<223> OTHER INFORMATION: Repeated 2 to 20 times

<400> SEQUENCE: 180

Ala Ser Glu Ala Ala Ala Lys Gly Thr
1 5

<210> SEQ ID NO 181

<211> LENGTH: 5

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Linker

<220> FEATURE:

<221> NAME/KEY: REPEAT

<222> LOCATION: (1)..(5)

<223> OTHER INFORMATION: Repeated 2 to 20 times

<400> SEQUENCE: 181

Gly Gly Gly Gly Ala
1 5

-continued

<210> SEQ ID NO 182
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Linker
<220> FEATURE:
<221> NAME/KEY: REPEAT
<222> LOCATION: (1)..(5)
<223> OTHER INFORMATION: Repeated 2 to 20 times

<400> SEQUENCE: 182

Pro Gly Gly Gly Ser
1 5

<210> SEQ ID NO 183
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Linker
<220> FEATURE:
<221> NAME/KEY: REPEAT
<222> LOCATION: (1)..(5)
<223> OTHER INFORMATION: Repeated 2 to 20 times

<400> SEQUENCE: 183

Ala Gly Gly Gly Ser
1 5

<210> SEQ ID NO 184
<211> LENGTH: 20
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Linker
<220> FEATURE:
<221> NAME/KEY: REPEAT
<222> LOCATION: (4)..(17)
<223> OTHER INFORMATION: Repeated 2 to 20 times

<400> SEQUENCE: 184

Gly Gly Ser Glu Gly Lys Ser Ser Gly Ser Gly Ser Glu Ser Lys Ser
1 5 10 15

Thr Gly Gly Ser
20

<210> SEQ ID NO 185
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Linker
<220> FEATURE:
<221> NAME/KEY: REPEAT
<222> LOCATION: (1)..(5)
<223> OTHER INFORMATION: Repeated 1 to 9 times

<400> SEQUENCE: 185

Ser Ser Ser Ser Gly
1 5

<210> SEQ ID NO 186
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:

-continued

<223> OTHER INFORMATION: Linker

<400> SEQUENCE: 186

Ser Ser Ser Ala Ser Ala Ser Ser Ala
1 5

<210> SEQ ID NO 187

<211> LENGTH: 7

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Linker

<400> SEQUENCE: 187

Gly Ser Pro Gly Ser Pro Gly
1 5

<210> SEQ ID NO 188

<211> LENGTH: 241

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: DLL3 scFv

<400> SEQUENCE: 188

Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Lys Pro Ser Glu
1 5 10 15

Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Gly Ser Ile Ser Ser Tyr
20 25 30

Tyr Trp Ser Trp Ile Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Ile
35 40 45

Gly Tyr Val Tyr Tyr Ser Gly Thr Thr Asn Tyr Asn Pro Ser Leu Lys
50 55 60

Ser Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn Gln Phe Ser Leu
65 70 75 80

Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys Ala
85 90 95

Ser Ile Ala Val Thr Gly Phe Tyr Phe Asp Tyr Trp Gly Gln Gly Thr
100 105 110

Leu Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
115 120 125

Gly Gly Gly Gly Ser Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu
130 135 140

Ser Leu Ser Pro Gly Glu Arg Val Thr Leu Ser Cys Arg Ala Ser Gln
145 150 155 160

Arg Val Asn Asn Asn Tyr Leu Ala Trp Tyr Gln Gln Arg Pro Gly Gln
165 170 175

Ala Pro Arg Leu Leu Ile Tyr Gly Ala Ser Ser Arg Ala Thr Gly Ile
180 185 190

Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr
195 200 205

Ile Ser Arg Leu Glu Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln
210 215 220

Tyr Asp Arg Ser Pro Leu Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile
225 230 235 240

Lys

-continued

<210> SEQ ID NO 189
<211> LENGTH: 119
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: CD20 VH

<400> SEQUENCE: 189

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
1 5 10 15
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Ala Phe Ser Tyr Ser
20 25 30
Trp Ile Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45
Gly Arg Ile Phe Pro Gly Asp Gly Asp Thr Asp Tyr Asn Gly Lys Phe
50 55 60
Lys Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Ser Thr Ala Tyr
65 70 75 80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Ala Arg Asn Val Phe Asp Gly Tyr Trp Leu Val Tyr Trp Gly Gln Gly
100 105 110
Thr Leu Val Thr Val Ser Ser
115

<210> SEQ ID NO 190
<211> LENGTH: 115
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: CD20 VL

<400> SEQUENCE: 190

Asp Ile Val Met Thr Gln Thr Pro Leu Ser Leu Pro Val Thr Pro Gly
1 5 10 15
Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Lys Ser Leu Leu His Ser
20 25 30
Asn Gly Ile Thr Tyr Leu Tyr Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45
Pro Gln Leu Leu Ile Tyr Gln Met Ser Asn Leu Val Ser Gly Val Pro
50 55 60
Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80
Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Ala Gln Asn
85 90 95
Leu Glu Leu Pro Tyr Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105 110
Arg Thr Val
115

<210> SEQ ID NO 191
<211> LENGTH: 109
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: anti-CD3 VL35 (CON)

-continued

<400> SEQUENCE: 191

Gln Ala Val Val Thr Gln Glu Pro Ser Leu Thr Val Ser Pro Gly Gly
1 5 10 15
Thr Val Thr Leu Thr Cys Gly Ser Ser Thr Gly Ala Val Thr Thr Ser
20 25 30
Asn Tyr Ala Asn Trp Val Gln Gln Lys Pro Gly Gln Ala Phe Arg Gly
35 40 45
Leu Ile Gly Gly Thr Asn Lys Arg Ala Pro Gly Val Pro Ala Arg Phe
50 55 60
Ser Gly Ser Leu Leu Gly Gly Lys Ala Ala Leu Thr Ile Ser Gly Ala
65 70 75 80
Gln Pro Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Leu Trp Tyr Ser Asn
85 90 95
His Trp Val Phe Gly Cys Gly Thr Lys Leu Thr Val Leu
100 105

<210> SEQ ID NO 192

<211> LENGTH: 5

<212> TYPE: PRT

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: linker

<400> SEQUENCE: 192

Gly Gly Gly Gly Gly
1 5

<210> SEQ ID NO 193

<211> LENGTH: 15

<212> TYPE: PRT

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: linker

<400> SEQUENCE: 193

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
1 5 10 15

<210> SEQ ID NO 194

<211> LENGTH: 226

<212> TYPE: PRT

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: Fc-Het-1

<400> SEQUENCE: 194

Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly
1 5 10 15
Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
20 25 30
Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Lys His Glu
35 40 45
Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
50 55 60
Asn Ala Lys Thr Lys Pro Arg Glu Glu Glu Tyr Asn Ser Thr Tyr Arg
65 70 75 80
Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys

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85	90	95
Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu		
100	105	110
Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr		
115	120	125
Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu		
130	135	140
Thr Cys Asp Val Ser Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp		
145	150	155
Glu Ser Asp Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val		
165	170	175
Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp		
180	185	190
Lys Ser Arg Trp Glu Gln Gly Asp Val Phe Ser Cys Ser Val Met His		
195	200	205
Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro		
210	215	220
Gly Lys		
225		

<210> SEQ ID NO 195

<211> LENGTH: 226

<212> TYPE: PRT

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: Fc-Het-2

<400> SEQUENCE: 195

Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly		
1	5	15
Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile		
20	25	30
Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Lys His Glu		
35	40	45
Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His		
50	55	60
Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg		
65	70	75
Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys		
85	90	95
Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu		
100	105	110
Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr		
115	120	125
Thr Leu Pro Pro Ser Arg Glu Gln Met Thr Lys Asn Gln Val Lys Leu		
130	135	140
Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp		
145	150	155
Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val		
165	170	175
Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp		
180	185	190
Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His		

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195	200	205
Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro		
210	215	220

Gly Lys
225

<210> SEQ ID NO 196
 <211> LENGTH: 125
 <212> TYPE: PRT
 <213> ORGANISM: artificial sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: CD3-VH32

<400> SEQUENCE: 196

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly		
1	5	10
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Thr Tyr		
20	25	30
Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val		
35	40	45
Gly Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp		
50	55	60
Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr		
65	70	75
Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr		
85	90	95
Tyr Cys Val Arg His Gly Asn Phe Gly Asp Ser Tyr Val Ser Trp Phe		
100	105	110
Ala Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser		
115	120	125

<210> SEQ ID NO 197
 <211> LENGTH: 125
 <212> TYPE: PRT
 <213> ORGANISM: artificial sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: CD3-VH33

<400> SEQUENCE: 197

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly		
1	5	10
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Thr Tyr		
20	25	30
Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Cys Leu Glu Trp Val		
35	40	45
Gly Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp		
50	55	60
Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr		
65	70	75
Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr		
85	90	95
Tyr Cys Val Arg His Gly Asn Phe Gly Asp Ser Tyr Val Ser Trp Phe		
100	105	110
Ala Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser		
115	120	125

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<210> SEQ ID NO 198
<211> LENGTH: 123
<212> TYPE: PRT
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: CD3-VH34

<400> SEQUENCE: 198

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Thr Tyr
20 25 30
Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Cys Leu Glu Trp Val
35 40 45
Ala Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp
50 55 60
Thr Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ala Lys Asn Thr
65 70 75 80
Leu Tyr Leu Gln Met Ser Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr
85 90 95
Tyr Cys Val Arg His Gly Asn Phe Gly Asp Ser Tyr Val Ser Trp Phe
100 105 110
Ala Tyr Trp Gly Gln Gly Thr Leu Val Thr Val
115 120

<210> SEQ ID NO 199
<211> LENGTH: 109
<212> TYPE: PRT
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: CD3-VL20

<400> SEQUENCE: 199

Gln Ala Val Val Thr Gln Glu Pro Ser Leu Thr Val Ser Pro Gly Gly
1 5 10 15
Thr Val Thr Leu Thr Cys Gly Ser Ser Thr Gly Ala Val Thr Thr Ser
20 25 30
Asn Tyr Ala Asn Trp Val Gln Gln Lys Pro Gly Lys Ser Pro Arg Gly
35 40 45
Leu Ile Gly Gly Thr Asn Lys Arg Ala Pro Gly Val Pro Ala Arg Phe
50 55 60
Ser Gly Ser Leu Leu Gly Gly Lys Ala Ala Leu Thr Ile Ser Gly Ala
65 70 75 80
Gln Pro Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Leu Trp Tyr Ser Asn
85 90 95
His Trp Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu
100 105

<210> SEQ ID NO 200
<211> LENGTH: 109
<212> TYPE: PRT
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: CD3-VL21

<400> SEQUENCE: 200

Gln Ala Val Val Thr Gln Glu Pro Ser Leu Thr Val Ser Pro Gly Gly

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1	5	10	15
Thr Val Thr Leu Thr Cys Gly Ser Ser Thr Gly Ala Val Thr Thr Ser	20	25	30
Asn Tyr Ala Asn Trp Val Gln Gln Lys Pro Gly Lys Ser Pro Arg Gly	35	40	45
Leu Ile Gly Gly Thr Asn Lys Arg Ala Pro Gly Val Pro Ala Arg Phe	50	55	60
Ser Gly Ser Leu Leu Gly Gly Lys Ala Ala Leu Thr Ile Ser Gly Ala	65	70	75
Gln Pro Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Leu Trp Tyr Ser Asn	85	90	95
His Trp Val Phe Gly Cys Gly Thr Lys Leu Thr Val Leu	100	105	

<210> SEQ ID NO 201

<211> LENGTH: 225

<212> TYPE: PRT

<213> ORGANISM: artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: Knob Fc

<400> SEQUENCE: 201

Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly	1	5	10	15
Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met	20	25	30	
Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His	35	40	45	
Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val	50	55	60	
His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr	65	70	75	80
Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly	85	90	95	
Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile	100	105	110	
Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val	115	120	125	
Tyr Thr Leu Pro Pro Cys Arg Asp Glu Leu Thr Lys Asn Gln Val Ser	130	135	140	
Leu Trp Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu	145	150	155	160
Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro	165	170	175	
Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val	180	185	190	
Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met	195	200	205	
His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser	210	215	220	

Pro
225

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<210> SEQ ID NO 202
<211> LENGTH: 225
<212> TYPE: PRT
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Hole Fc

<400> SEQUENCE: 202

Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly
1 5 10 15

Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met
20 25 30

Arg Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
35 40 45

Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val
50 55 60

His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr
65 70 75 80

Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly
85 90 95

Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile
100 105 110

Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val
115 120 125

Cys Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser
130 135 140

Leu Ser Cys Ala Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
145 150 155 160

Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro
165 170 175

Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Val Ser Lys Leu Thr Val
180 185 190

Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met
195 200 205

His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser
210 215 220

Pro
225

<210> SEQ ID NO 203
<211> LENGTH: 222
<212> TYPE: PRT
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Knob Fc

<400> SEQUENCE: 203

Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Gly Gly Pro Ser
1 5 10 15

Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg
20 25 30

Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro
35 40 45

Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala
50 55 60

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Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	Tyr	Asn	Ser	Thr	Tyr	Arg	Val	Val		
65					70				75						80		
Ser	Val	Leu	Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly	Lys	Glu	Tyr		
			85					90						95			
Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala	Leu	Pro	Ala	Pro	Ile	Glu	Lys	Thr		
			100					105					110				
Ile	Ser	Lys	Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr	Thr	Leu		
		115				120						125					
Pro	Pro	Cys	Arg	Asp	Glu	Leu	Thr	Lys	Asn	Gln	Val	Ser	Leu	Trp	Cys		
		130				135					140						
Leu	Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu	Trp	Glu	Ser		
145					150					155					160		
Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro	Val	Leu	Asp		
			165					170						175			
Ser	Asp	Gly	Ser	Phe	Phe	Leu	Tyr	Ser	Lys	Leu	Thr	Val	Asp	Lys	Ser		
			180					185					190				
Arg	Trp	Gln	Gln	Gly	Asn	Val	Phe	Ser	Cys	Ser	Val	Met	His	Glu	Ala		
		195					200					205					
Leu	His	Asn	His	Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser	Pro				
	210					215					220						

<210> SEQ ID NO 204

<211> LENGTH: 222

<212> TYPE: PRT

<213> ORGANISM: artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: Hole Fc

<400> SEQUENCE: 204

Asp	Lys	Thr	His	Thr	Cys	Pro	Pro	Cys	Pro	Ala	Pro	Gly	Gly	Pro	Ser		
1				5				10						15			
Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met	Arg	Ser	Arg		
			20					25					30				
Thr	Pro	Glu	Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser	His	Glu	Asp	Pro		
			35				40					45					
Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val	His	Asn	Ala		
			50			55					60						
Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	Tyr	Asn	Ser	Thr	Tyr	Arg	Val	Val		
65				70					75					80			
Ser	Val	Leu	Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly	Lys	Glu	Tyr		
			85					90						95			
Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala	Leu	Pro	Ala	Pro	Ile	Glu	Lys	Thr		
			100					105					110				
Ile	Ser	Lys	Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	Cys	Thr	Leu		
		115				120						125					
Pro	Pro	Ser	Arg	Asp	Glu	Leu	Thr	Lys	Asn	Gln	Val	Ser	Leu	Ser	Cys		
		130				135					140						
Ala	Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu	Trp	Glu	Ser		
145					150					155					160		
Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro	Val	Leu	Asp		
			165					170						175			
Ser	Asp	Gly	Ser	Phe	Phe	Leu	Val	Ser	Lys	Leu	Thr	Val	Asp	Lys	Ser		
			180					185					190				

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Arg	Trp	Gln	Gln	Gly	Asn	Val	Phe	Ser	Cys	Ser	Val	Met	His	Glu	Ala
		195					200					205			

Leu	His	Asn	His	Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser	Pro
210						215					220		

<210> SEQ ID NO 205
 <211> LENGTH: 225
 <212> TYPE: PRT
 <213> ORGANISM: artificial sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Hole Fc

<400> SEQUENCE: 205

Asp	Lys	Thr	His	Thr	Cys	Pro	Pro	Cys	Pro	Ala	Pro	Glu	Leu	Leu	Gly
1				5					10					15	
Gly	Pro	Ser	Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met
			20					25					30		
Ile	Ser	Arg	Thr	Pro	Glu	Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser	His
		35					40					45			
Glu	Asp	Pro	Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val
	50					55					60				
His	Asn	Ala	Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	Tyr	Asn	Ser	Thr	Tyr
65					70					75				80	
Arg	Val	Val	Ser	Val	Leu	Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly
			85					90						95	
Lys	Glu	Tyr	Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala	Leu	Pro	Ala	Pro	Ile
			100					105					110		
Glu	Lys	Thr	Ile	Ser	Lys	Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val
			115				120					125			
Cys	Thr	Leu	Pro	Pro	Ser	Arg	Asp	Glu	Leu	Thr	Lys	Asn	Gln	Val	Ser
	130					135					140				
Leu	Ser	Cys	Ala	Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu
145					150				155					160	
Trp	Glu	Ser	Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro
			165					170						175	
Val	Leu	Asp	Ser	Asp	Gly	Ser	Phe	Phe	Leu	Val	Ser	Lys	Leu	Thr	Val
		180					185						190		
Asp	Lys	Ser	Arg	Trp	Gln	Gln	Gly	Asn	Val	Phe	Ser	Cys	Ser	Val	Met
		195					200					205			
His	Glu	Ala	Leu	His	Asn	Arg	Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser
210						215						220			
Pro															
225															

<210> SEQ ID NO 206
 <211> LENGTH: 222
 <212> TYPE: PRT
 <213> ORGANISM: artificial sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Hole Fc

<400> SEQUENCE: 206

Asp	Lys	Thr	His	Thr	Cys	Pro	Pro	Cys	Pro	Ala	Pro	Gly	Gly	Pro	Ser
1				5					10					15	
Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met	Ile	Ser	Arg
		20						25					30		

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Thr	Pro	Glu	Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser	His	Glu	Asp	Pro
	35					40						45			
Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val	His	Asn	Ala
	50					55					60				
Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	Tyr	Asn	Ser	Thr	Tyr	Arg	Val	Val
	65				70					75				80	
Ser	Val	Leu	Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly	Lys	Glu	Tyr
			85						90					95	
Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala	Leu	Pro	Ala	Pro	Ile	Glu	Lys	Thr
			100						105					110	
Ile	Ser	Lys	Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	Cys	Thr	Leu
			115				120					125			
Pro	Pro	Ser	Arg	Asp	Glu	Leu	Thr	Lys	Asn	Gln	Val	Ser	Leu	Ser	Cys
	130					135					140				
Ala	Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu	Trp	Glu	Ser
	145				150					155				160	
Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro	Val	Leu	Asp
				165					170					175	
Ser	Asp	Gly	Ser	Phe	Phe	Leu	Val	Ser	Lys	Leu	Thr	Val	Asp	Lys	Ser
			180						185				190		
Arg	Trp	Gln	Gln	Gly	Asn	Val	Phe	Ser	Cys	Ser	Val	Met	His	Glu	Ala
			195				200					205			
Leu	His	Asn	Arg	Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser	Pro		
	210					215					220				

<210> SEQ ID NO 207

<211> LENGTH: 225

<212> TYPE: PRT

<213> ORGANISM: artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: Knob Fc

<400> SEQUENCE: 207

Asp	Lys	Thr	His	Thr	Cys	Pro	Pro	Cys	Pro	Ala	Pro	Glu	Leu	Leu	Gly
1			5						10					15	
Gly	Pro	Ser	Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu	Tyr
		20					25						30		
Ile	Ser	Arg	Thr	Pro	Glu	Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser	His
	35					40						45			
Glu	Asp	Pro	Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val
	50				55						60				
His	Asn	Ala	Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	Tyr	Asn	Ser	Thr	Tyr
	65				70					75				80	
Arg	Val	Val	Ser	Val	Leu	Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly
			85					90						95	
Lys	Glu	Tyr	Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala	Leu	Pro	Ala	Pro	Ile
			100					105					110		
Glu	Lys	Thr	Ile	Ser	Lys	Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val
			115					120				125			
Tyr	Thr	Leu	Pro	Pro	Cys	Arg	Asp	Glu	Leu	Thr	Lys	Asn	Gln	Val	Ser
	130					135					140				
Leu	Trp	Cys	Leu	Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu
	145				150					155				160	

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Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro
165 170 175
Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val
180 185 190
Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Val
195 200 205
His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser
210 215 220
Pro
225

<210> SEQ ID NO 208
<211> LENGTH: 222
<212> TYPE: PRT
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Knob Fc

<400> SEQUENCE: 208

Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Gly Gly Pro Ser
1 5 10 15
Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Tyr Ile Ser Arg
20 25 30
Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro
35 40 45
Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala
50 55 60
Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val
65 70 75 80
Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr
85 90 95
Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr
100 105 110
Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu
115 120 125
Pro Pro Cys Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Trp Cys
130 135 140
Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser
145 150 155 160
Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp
165 170 175
Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser
180 185 190
Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Val His Glu Ala
195 200 205
Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
210 215 220

<210> SEQ ID NO 209
<211> LENGTH: 225
<212> TYPE: PRT
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Hole Fc

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<400> SEQUENCE: 209

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Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly
1      5      10      15
Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Tyr
20      25      30
Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
35      40      45
Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val
50      55      60
His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr
65      70      75      80
Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly
85      90      95
Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile
100     105     110
Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val
115     120     125
Cys Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser
130     135     140
Leu Ser Cys Ala Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
145     150     155     160
Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro
165     170     175
Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Val Ser Lys Leu Thr Val
180     185     190
Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Val
195     200     205
His Glu Ala Leu His Asn Arg Tyr Thr Gln Lys Ser Leu Ser Leu Ser
210     215     220
Pro
225

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<210> SEQ ID NO 210

<211> LENGTH: 222

<212> TYPE: PRT

<213> ORGANISM: artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: Hole Fc

<400> SEQUENCE: 210

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Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Gly Gly Pro Ser
1      5      10      15
Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Tyr Ile Ser Arg
20      25      30
Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro
35      40      45
Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala
50      55      60
Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val
65      70      75      80
Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr
85      90      95
Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr

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100	105	110
Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Cys Thr Leu		
115	120	125
Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Ser Cys		
130	135	140
Ala Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser		
145	150	155
Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp		
165	170	175
Ser Asp Gly Ser Phe Phe Leu Val Ser Lys Leu Thr Val Asp Lys Ser		
180	185	190
Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Val His Glu Ala		
195	200	205
Leu His Asn Arg Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro		
210	215	220

<210> SEQ ID NO 211
 <211> LENGTH: 10
 <212> TYPE: PRT
 <213> ORGANISM: artificial sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: anti-CD3 VH CDR1

<400> SEQUENCE: 211

Gly Phe Thr Phe Asn Thr Tyr Ala Met Asn
1 5 10

<210> SEQ ID NO 212
 <211> LENGTH: 12
 <212> TYPE: PRT
 <213> ORGANISM: artificial sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: anti-CD3 VH CDR2

<400> SEQUENCE: 212

Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr
1 5 10

<210> SEQ ID NO 213
 <211> LENGTH: 246
 <212> TYPE: PRT
 <213> ORGANISM: Artificial sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: CD20 scFv

<400> SEQUENCE: 213

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
1 5 10 15
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Ala Phe Ser Tyr Ser
20 25 30
Trp Ile Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45
Gly Arg Ile Phe Pro Gly Asp Gly Asp Thr Asp Tyr Asn Gly Lys Phe
50 55 60
Lys Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Ser Thr Ala Tyr
65 70 75 80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

-continued

Ala Arg Asn Val Phe Asp Gly Tyr Trp Leu Val Tyr Trp Gly Gln Gly
 100 105 110

Thr Leu Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly
 115 120 125

Ser Gly Gly Gly Gly Ser Asp Ile Val Met Thr Gln Thr Pro Leu Ser
 130 135 140

Leu Pro Val Thr Pro Gly Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser
 145 150 155 160

Lys Ser Leu Leu His Ser Asn Gly Ile Thr Tyr Leu Tyr Trp Tyr Leu
 165 170 175

Gln Lys Pro Gly Gln Ser Pro Gln Leu Leu Ile Tyr Gln Met Ser Asn
 180 185 190

Leu Val Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr
 195 200 205

Asp Phe Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Val Gly Val
 210 215 220

Tyr Tyr Cys Ala Gln Asn Leu Glu Leu Pro Tyr Thr Phe Gly Gly Gly
 225 230 235 240

Thr Lys Val Glu Ile Lys
 245

<210> SEQ ID NO 214
 <211> LENGTH: 118
 <212> TYPE: PRT
 <213> ORGANISM: Artificial sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: B7H3 sdAb B7h3 hz1A5v51

<400> SEQUENCE: 214

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Glu Val Gln Pro Gly Gly
 1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Ser Phe Ser Ser Asn
 20 25 30

Val Met Met Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45

Ser Thr Ile Tyr Ser Ser Gly Thr Gly Thr Phe Tyr Ala Glu Ser Val
 50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
 65 70 75 80

Leu Gln Met Ser Ser Leu Arg Pro Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95

Ala Thr Ser Gly Pro Val Arg Gly Trp Gly Pro Arg Ser Gln Gly Thr
 100 105 110

Leu Val Thr Val Lys Pro
 115

<210> SEQ ID NO 215
 <211> LENGTH: 117
 <212> TYPE: PRT
 <213> ORGANISM: artificial sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: sdAb B7H3 hz58E05v27

<400> SEQUENCE: 215

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Glu Val Gln Pro Gly Gly

-continued

1	5	10	15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Ser Thr Phe Ser Ser Tyr	20	25	30
His Met Ser Trp Phe Arg Gln Ala Pro Gly Lys Gln Arg Glu Pro Val	35	40	45
Ala Thr Ser His His Gly Gly Thr Thr Asn Tyr Ala Gly Ser Val Lys	50	55	60
Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Val Tyr Leu	65	70	75
Gln Met Asn Thr Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Lys	85	90	95
Ala Asp His Gly Tyr Gln Gly Arg Gly Tyr Trp Gly Gln Gly Thr Leu	100	105	110
Val Thr Val Lys Pro	115		

<210> SEQ ID NO 216
 <211> LENGTH: 117
 <212> TYPE: PRT
 <213> ORGANISM: artificial sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: sdAb B7H3 hz58E05v55

<400> SEQUENCE: 216

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Glu Val Gln Pro Gly Gly	1	5	10	15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr	20	25	30	
His Met Ser Trp Phe Arg Gln Ala Pro Gly Lys Gln Arg Glu Leu Val	35	40	45	
Ala Thr Ser His His Gly Gly Thr Thr Asn Tyr Ala Gly Ser Val Lys	50	55	60	
Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Val Tyr Leu	65	70	75	80
Gln Met Asn Thr Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Lys	85	90	95	
Ala Asp His Gly Tyr Gln Gly Arg Gly Tyr Trp Gly Gln Gly Thr Leu	100	105	110	
Val Thr Val Lys Pro	115			

<210> SEQ ID NO 217
 <211> LENGTH: 117
 <212> TYPE: PRT
 <213> ORGANISM: artificial sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: sdAb B7H3
 hz58E05v48

<400> SEQUENCE: 217

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Glu Val Gln Pro Gly Gly	1	5	10	15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr	20	25	30	
His Met Ser Trp Phe Arg Gln Ala Pro Gly Lys Gln Arg Glu Pro Val	35	40	45	

-continued

Ala Thr Ser His His Gly Gly Thr Thr Asn Tyr Ala Gly Ser Val Lys
 50 55 60
 Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Val Tyr Leu
 65 70 75 80
 Gln Met Asn Thr Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Lys
 85 90 95
 Ala Asp His Gly Tyr Gln Gly Arg Gly Tyr Trp Gly Gln Gly Thr Leu
 100 105 110
 Val Thr Val Lys Pro
 115

<210> SEQ ID NO 218
 <211> LENGTH: 127
 <212> TYPE: PRT
 <213> ORGANISM: artificial sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: sdAb B7H3 hz57B04v24

<400> SEQUENCE: 218

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Glu Val Gln Pro Gly Gly
 1 5 10 15
 Ser Leu Arg Leu Ser Cys Ala Pro Ser Glu Arg Thr Phe Ser Thr Tyr
 20 25 30
 Thr Met Gly Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Glu Phe Val
 35 40 45
 Ala Val Val Asn Trp Gly Gly Gly Ser Lys Tyr Tyr Ala Glu Ser Val
 50 55 60
 Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Val Tyr
 65 70 75 80
 Leu Gln Met Ser Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95
 Ala Ala Gly Gly Ala Tyr Ser Gly Pro Tyr Tyr Asp Thr Arg Gln Tyr
 100 105 110
 Thr Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Lys Pro Gly Gly
 115 120 125

<210> SEQ ID NO 219
 <211> LENGTH: 120
 <212> TYPE: PRT
 <213> ORGANISM: artificial sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: sdAb DLL3 hz10D9v7

<400> SEQUENCE: 219

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Glu Val Gln Pro Gly Gly
 1 5 10 15
 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Ser Ile Phe Ser Ile Asn
 20 25 30
 Ala Met Gly Trp Tyr Arg Gln Ala Pro Gly Lys Gln Arg Glu Leu Val
 35 40 45
 Ala Gly Phe Thr Gly Asp Thr Asn Thr Ile Tyr Ala Glu Ser Val Lys
 50 55 60
 Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Val Tyr Leu
 65 70 75 80
 Gln Met Ser Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala

-continued

85	90	95
Ala Asp Val Gln Leu Phe Ser Arg Asp Tyr Glu Phe Tyr Trp Gly Gln		
100	105	110
Gly Thr Leu Val Thr Val Lys Pro		
115	120	

<210> SEQ ID NO 220
<211> LENGTH: 115
<212> TYPE: PRT
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: sdAb DLL3 hz8E7v16

<400> SEQUENCE: 220

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Glu Val Gln Pro Gly Gly		
1	5	10
Ser Leu Arg Leu Ser Cys Gly Pro Ser Glu Ile Ile Thr Ser Asp Lys		
20	25	30
Ser Met Gly Trp Val Arg Gln Ala Pro Gly Lys Gln Arg Asn Leu Val		
35	40	45
Ala Gly Ile Ser Asn Val Gly Ser Thr Asn Tyr Ala Gln Ser Val Lys		
50	55	60
Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Val Tyr Leu		
65	70	75
Gln Met Ser Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Tyr		
85	90	95
Ala Arg Asp Phe Glu Asn Glu Tyr Trp Gly Gln Gly Thr Leu Val Thr		
100	105	110
Val Lys Pro		
115		

<210> SEQ ID NO 221
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: 41BB CDR1

<400> SEQUENCE: 221

Gly Phe Ser Phe Ser Ile Asn Ala Met Gly	
1	10

<210> SEQ ID NO 222
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: 41BB CDR2

<400> SEQUENCE: 222

Ala Ile Glu Ser Gly Arg Asn Thr Val	
1	5

<210> SEQ ID NO 223
<211> LENGTH: 13
<212> TYPE: PRT
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: 41BB CDR3

-continued

<400> SEQUENCE: 223

Leu Lys Gly Asn Arg Val Val Ser Pro Ser Val Ala Tyr
1 5 10

<210> SEQ ID NO 224

<211> LENGTH: 14

<212> TYPE: PRT

<213> ORGANISM: artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: CD3-VH7, VH33 CDR3

<400> SEQUENCE: 224

His Gly Asn Phe Gly Asp Ser Tyr Val Ser Trp Phe Ala Tyr
1 5 10

<210> SEQ ID NO 225

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: CD3-VL2, VL21 CDR3

<400> SEQUENCE: 225

Ala Leu Trp Tyr Ser Asn His Trp Val
1 5

<210> SEQ ID NO 226

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: CD3-VL8 CDR3

<400> SEQUENCE: 226

Val Leu Trp Tyr Ser Asn Arg Trp Val
1 5

<210> SEQ ID NO 227

<211> LENGTH: 10

<212> TYPE: PRT

<213> ORGANISM: artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: CD3 VH33 CDR1

<400> SEQUENCE: 227

Gly Phe Thr Phe Ser Thr Tyr Ala Met Asn
1 5 10

<210> SEQ ID NO 228

<211> LENGTH: 12

<212> TYPE: PRT

<213> ORGANISM: artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: CD3 VH33 CDR2

<400> SEQUENCE: 228

Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr
1 5 10

<210> SEQ ID NO 229

<211> LENGTH: 14

<212> TYPE: PRT

<213> ORGANISM: artificial sequence

<220> FEATURE:

-continued

<223> OTHER INFORMATION: CD3 VL21 CDR1

<400> SEQUENCE: 229

Gly Ser Ser Thr Gly Ala Val Thr Thr Ser Asn Tyr Ala Asn
 1 5 10

<210> SEQ ID NO 230

<211> LENGTH: 7

<212> TYPE: PRT

<213> ORGANISM: artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: CD3 VL21 CDR2

<400> SEQUENCE: 230

Gly Thr Asn Lys Arg Ala Pro
 1 5

What is claimed:

1. A multispecific polypeptide construct, the multispecific polypeptide construct comprising a first component comprising an immunoglobulin Fc region and a second component comprising a CD3-binding region, wherein:

the CD3 binding region is an anti-CD3 antibody or antigen binding fragment that is an Fv antibody fragment comprising a variable heavy chain region (VH) and a variable light chain region (VL);

the Fc is a heterodimeric Fc comprising a first Fc polypeptide and a second Fc polypeptide and the VH and VL of 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 non-cleavable linker, wherein the Fc region is positioned N-terminal to the CD3-binding region; and

the first component comprises a first antigen binding domain and the second component comprises a second antigen binding domain, wherein each of the antigen binding domains bind a tumor associated antigen (TAA).

2. (canceled)

3. The multispecific polypeptide construct of claim 1, wherein the first antigen binding domain is positioned amino-terminally relative to the Fc region of the multispecific polypeptide construct and the second antigen binding domain is positioned carboxy-terminally relative to the CD3 binding region of the multispecific polypeptide construct.

4. The multispecific polypeptide construct of claim 1, wherein the multispecific polypeptide construct comprises in order, from N-terminus to C-terminus:

the first antigen binding domain that binds to a tumor-associated antigen (TAA);

the immunoglobulin Fc region;

the non-cleavable linker;

the CD3 binding region, wherein the CD3 binding region binds CD3 (CD3ε); and

the second antigen binding domain that binds a tumor-associated antigen (TAA).

5. A multispecific polypeptide construct, the multispecific polypeptide construct comprising a first component comprising an immunoglobulin Fc region and a second component comprising a CD3-binding region, wherein:

the CD3 binding region is an anti-CD3 antibody or antigen binding fragment that is a disulfide-stabilized Fv antibody fragment (dsFv) comprising a variable heavy chain (VH) and a variable light chain (VL);

the Fc is a heterodimeric Fc comprising a first Fc polypeptide and a second Fc polypeptide and the VH and VL of 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 non-cleavable linker, wherein the Fc region is positioned N-terminal to the CD3-binding region; and

one or both of the first and second components comprises an antigen binding domain that binds a tumor associated antigen (TAA).

6. A multispecific polypeptide construct, the multispecific polypeptide construct comprising a first component comprising an immunoglobulin Fc region and a second component comprising a CD3-binding region, wherein:

the CD3 binding region is an anti-CD3 antibody or antigen binding fragment that is an Fv antibody fragment comprising a variable heavy chain (VH) and a variable light chain (VL);

the Fc is a heterodimeric Fc comprising a first Fc polypeptide and a second Fc polypeptide and the VH and VL of 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 non-cleavable linker, wherein the Fc region is positioned N-terminal to the CD3-binding region; and

one or both of the first and second components comprises an antigen binding domain that binds a tumor associated antigen (TAA), wherein the antigen-binding domain is a single chain antibody fragment.

7. The multispecific polypeptide construct of claim 6, wherein the single chain antibody fragment is a single domain antibody or is a single chain variable fragment (scFv).

8. (canceled)

9. The multispecific polypeptide construct of claim 5, wherein the multispecific polypeptide construct is selected from:

(A) a multispecific polypeptide construct that comprises in order, from N-terminus to C-terminus:
the first antigen binding domain that binds to a tumor-associated antigen (TAA);
the immunoglobulin Fc region;
the non-cleavable linker;
the CD3 binding region, wherein the CD3 binding region binds CD3 (CD3 ϵ); and
the second antigen binding domain that binds a tumor-associated antigen (TAA); or

(B) a multispecific polypeptide construct that comprises in order, from N-terminus to C-terminus:
the immunoglobulin Fc region;
the non-cleavable linker;
the CD3 binding region, wherein the CD3 binding region binds CD3 (CD3 ϵ); and
an antigen binding domain that binds a tumor-associated antigen (TAA); or

(C) a multispecific polypeptide construct that comprises in order, from N-terminus to C-terminus:
the antigen binding domain that binds to a tumor-associated antigen (TAA);
the immunoglobulin Fc region;
the non-cleavable linker; and
the CD3 binding region, wherein the CD3 binding region binds CD3 (CD3 ϵ).

10. The multispecific polypeptide construct of claim **6**, wherein the multispecific polypeptide construct is selected from:

(A) a multispecific polypeptide construct that comprises in order, from N-terminus to C-terminus:
the first antigen binding domain that binds to a tumor-associated antigen (TAA);
the immunoglobulin Fc region;
the non-cleavable linker;
the CD3 binding region, wherein the CD3 binding region binds CD3 (CD3 ϵ); and
the second antigen binding domain that binds a tumor-associated antigen (TAA); or

(B) a multispecific polypeptide construct that comprises in order, from N-terminus to C-terminus:
the immunoglobulin Fc region;
the non-cleavable linker;
the CD3 binding region, wherein the CD3 binding region binds CD3 (CD3 ϵ); and
an antigen binding domain that binds a tumor-associated antigen (TAA); or

(C) a multispecific polypeptide construct that comprises in order, from N-terminus to C-terminus:
the antigen binding domain that binds to a tumor-associated antigen (TAA);
the immunoglobulin Fc region;
the non-cleavable linker; and
the CD3 binding region, wherein the CD3 binding region binds CD3 (CD3 ϵ).

11-14. (canceled)

15. The multispecific polypeptide construct of claim **6**, wherein the heterodimeric Fc region comprises at least one modification selected from among the following: a steric modification(s), a knob-into-hole modification(s), a charge mutation(s) to increase electrostatic complementarity of the polypeptides, a modification(s) to alter the isoelectric point (pI variant), or combinations thereof.

16-35. (canceled)

36. The multispecific polypeptide construct of claim **6**, wherein the linker is a polypeptide linker.

37. (canceled)

38. The multispecific polypeptide construct of claim **36**, wherein the linker is a polypeptide of from or from about 2 to 24 amino acids.

39-43. (canceled)

44. The multispecific polypeptide construct of claim **6**, wherein the non-cleavable linker comprises:

(GGS) $_n$, wherein n is 1 to 10;

(GGGGS) $_n$ (SEQ ID NO: 173), wherein n is 1 to 10; or

(GGGGGS) $_n$ (SEQ ID NO: 172), wherein n is 1 to 4.

45. (canceled)

46. (canceled)

47. The multispecific polypeptide construct of claim **6**, wherein the non-cleavable linker is or comprises an amino acid sequence selected from among

GGS;	(SEQ ID NO: 149)
<u>GGGGS</u> ;	(SEQ ID NO: 135)
<u>GGGGGS</u> ;	(SEQ ID NO: 10)
(GGS) $_2$;	(SEQ ID NO: 11)
<u>GGSGGSGGS</u> ;	(SEQ ID NO: 12)
<u>GGSGGSGGSGGS</u> ;	(SEQ ID NO: 13)
<u>GGSGGSGGSGGSGGS</u> ;	(SEQ ID NO: 119)
GGGGSGGGSGGGSGGGGS;	(SEQ ID NO: 147)
<u>GGSGGGSGGGSGGGSGGGGS</u> ;	(SEQ ID NO: 170)
and	
<u>GGGGSGGGSGGGSGGGGS</u> .	

48-56. (canceled)

57. The multispecific polypeptide construct of claim **6**, 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 domain of the anti-CD3 antibody or antigen binding fragment thereof; and (ii) a second polypeptide comprising the second Fc polypeptide of the heterodimeric Fc region, the linker and the VL domain of the anti-CD3 antibody or antigen binding fragment thereof, wherein one or both of the first and second polypeptide comprise at least one antigen-binding domain that binds to a tumor associated antigen (TAA).

58. The multispecific polypeptide construct of claim **6**, wherein the VH of the anti-CD3 antibody or antigen-binding fragment is on the same polypeptide as the at least one antigen-binding domain that binds to a tumor associated antigen (TAA).

59. The multispecific polypeptide construct of claim **58**, wherein the polypeptide comprising the VL of the anti-CD3

antibody or antigen-binding fragment does not contain the at least one antigen-binding domain that binds to a tumor associated antigen (TAA).

60. (canceled)

61. (canceled)

62. The multispecific polypeptide construct of claim 6, wherein only one of the first and second polypeptide comprises the at least one antigen-binding domain that binds a TAA.

63. The multispecific polypeptide construct of claim 6, wherein:

the at least one antigen binding domain is positioned amino-terminally relative to the Fc region and/or is positioned carboxy-terminally relative to the CD3 binding region of one of the first or second polypeptide of the multispecific polypeptide construct; or

the at least one antigen binding domain comprises a first and a second antigen binding domain and the first antigen binding domain is positioned amino-terminally relative to the Fc region of the multispecific polypeptide construct and the second antigen binding domain is positioned carboxy-terminally relative to the CD3 binding region of the multispecific polypeptide construct.

64. (canceled)

65. The multispecific polypeptide construct of claim 1, wherein the antigen binding domain, or independently each of the antigen binding domains:

comprises an extracellular domain or binding fragment thereof of the native cognate binding partner of the TAA, or a variant thereof that exhibits binding activity to the TAA; or

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.

66. (canceled)

67. The multispecific polypeptide construct of claim 65, wherein the antibody or antigen-binding fragment thereof is a Fv, a scFv, a Fab, or a single domain antibody (sdAb).

68-77. (canceled)

78. The multispecific polypeptide construct of claim 6, wherein the antigen binding domain, or independently each of the antigen binding domains, binds to a tumor antigen selected from among 1-92-LFA-3, 5T4, 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 (FR α), 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, IFN α , IFN β , IFN γ , IgE, IgE Receptor (Fc ϵ RI), 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, PDGFR α , PDGFR β , PD-1, PD-L1, PD-L2, Phosphatidylserine, P1GF, PSCA, PSMA, PSGR, RAAG12, RAGE, SLC44A4, Sphingosine 1 Phosphate, STEAP1, STEAP2, TAG-72, TAPA1, TEM-8, TGF β , TIGIT, TIM-3, TLR2, TLR4, TLR6, TLR7, TLR8, TLR9, TMEM31, TNF α , 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.

79. The multispecific polypeptide construct of any of claim 6, wherein the multispecific polypeptide construct comprises:

at least a first antigen binding domain and a second antigen binding domain, wherein the first antigen binding domain and second antigen binding domain bind to the same TAA;

at least a first antigen binding domain and a second antigen binding domain wherein the first antigen binding domain and the second antigen binding domain binds different epitopes of the same TAA;

at least a first antigen binding domain and a second antigen binding domain wherein the first antigen binding domain and the second antigen binding domain bind the same epitope of the same TAA; or

at least a first antigen binding domain and a second antigen binding domain wherein the first antigen binding domain and the second antigen binding domain bind a different TAA.

80-86. (canceled)

87. The multispecific polypeptide construct of claim 6, wherein the Fv antibody fragment comprises a disulfide stabilized anti-CD3 binding Fv fragment (dsFv).

88-91. (canceled)

92. The multispecific polypeptide construct of claim 6, wherein the multispecific polypeptide construct is conjugated to an agent selected from among the following: a therapeutic agent, an antineoplastic agent, a toxin or fragment thereof, a detectable moiety, and a diagnostic agent.

93. (canceled)

94. (canceled)

95. A polynucleotide(s) encoding the multispecific polypeptide constructs of claim 6.

96-101. (canceled)

102. A vector, comprising the polynucleotide of claim 95.

103. (canceled)

104. (canceled)

105. A cell, comprising the polynucleotide or polynucleotides of claim 95.

106-108. (canceled)

109. A method of producing a multispecific polypeptide construct, the method comprising introducing into a cell the polynucleotide or polynucleotides of claim **95** and culturing the cell under conditions to produce the multispecific polypeptide construct.

110-113. (canceled)

114. A pharmaceutical composition comprising the multispecific polypeptide construct of claim **6** and a pharmaceutically acceptable carrier.

115. (canceled)

116. A method of stimulating or inducing an immune response, the method comprising contacting a target cell and a T cell with the multispecific polypeptide construct of claim **6**, said target cell expressing a tumor associated antigen recognized by the multispecific polypeptide construct.

117-125. (canceled)

126. 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 multispecific polypeptide construct of claim **6**.

127. The method of claim **126**, wherein the disease or condition is a tumor or a cancer.

128. (canceled)

129. The multispecific polypeptide construct of claim **1**, wherein the Fv antibody fragment comprises a disulfide stabilized anti-CD3 binding Fv fragment (dsFv).

130. The multispecific polypeptide construct of claim **5**, wherein the antigen binding domain, or independently each of the antigen binding domains:

comprises an extracellular domain or binding fragment thereof of the native cognate binding partner of the TAA, or a variant thereof that exhibits binding activity to the TAA; or

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.

131. The multispecific polypeptide construct of claim **1**, wherein the non-cleavable linker is or comprises an amino acid sequence selected from among GGS; GGGGS (SEQ ID NO: 149); GGGGGS (SEQ ID NO: 135); (GGS)₂ (SEQ ID NO: 10); GGSGGSGGS (SEQ ID NO: 11); GGSGGSGGSGGS (SEQ ID NO: 12); GGSGGSGGSGGSGGS (SEQ ID NO: 13); GGGGGSGGGGSGGGGGS (SEQ ID NO: 119); GGSGGGGSGGGGSGGGGGS (SEQ ID NO: 147); and GGGGSGGGGSGGGGGS (SEQ ID NO:170).

132. The multispecific polypeptide construct of claim **5**, wherein the non-cleavable linker is or comprises an amino acid sequence selected from among GGS; GGGGS (SEQ ID

NO: 149); GGGGGS (SEQ ID NO: 135); (GGS)₂ (SEQ ID NO: 10); GGSGGSGGS (SEQ ID NO: 11); GGSGGSGGSGGS (SEQ ID NO: 12); GGSGGSGGSGGSGGS (SEQ ID NO: 13); GGGGGSGGGGSGGGGGS (SEQ ID NO: 119); GGSGGGGSGGGGSGGGGGS (SEQ ID NO: 147); and GGGGSGGGGSGGGGGS (SEQ ID NO:170).

133. A polynucleotide(s) encoding the multispecific polypeptide constructs of claim **1**.

134. A cell, comprising the polynucleotide or polynucleotides of claim **133**.

135. A method of producing a multispecific polypeptide construct, the method comprising introducing into a cell the polynucleotide or polynucleotides of claim **133** and culturing the cell under conditions to produce the multispecific polypeptide construct.

136. A pharmaceutical composition comprising the multispecific polypeptide construct of claim **1** and a pharmaceutically acceptable carrier.

137. A method of stimulating or inducing an immune response, the method comprising contacting a target cell and a T cell with the multispecific polypeptide construct of claim **1**, said target cell expressing a tumor associated antigen recognized by the multispecific polypeptide construct.

138. 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 multispecific polypeptide construct-of claim **1**.

139. A polynucleotide(s) encoding the multispecific polypeptide constructs of claim **5**.

140. A cell, comprising the polynucleotide or polynucleotides of claim **139**.

141. A method of producing a multispecific polypeptide construct, the method comprising introducing into a cell the polynucleotide or polynucleotides of claim **139** and culturing the cell under conditions to produce the multispecific polypeptide construct.

142. A pharmaceutical composition comprising the multispecific polypeptide construct of claim **5** and a pharmaceutically acceptable carrier.

143. A method of stimulating or inducing an immune response, the method comprising contacting a target cell and a T cell with the multispecific polypeptide construct of claim **5**, said target cell expressing a tumor associated antigen recognized by the multispecific polypeptide construct.

144. 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 multispecific polypeptide construct-of claim **5**.

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