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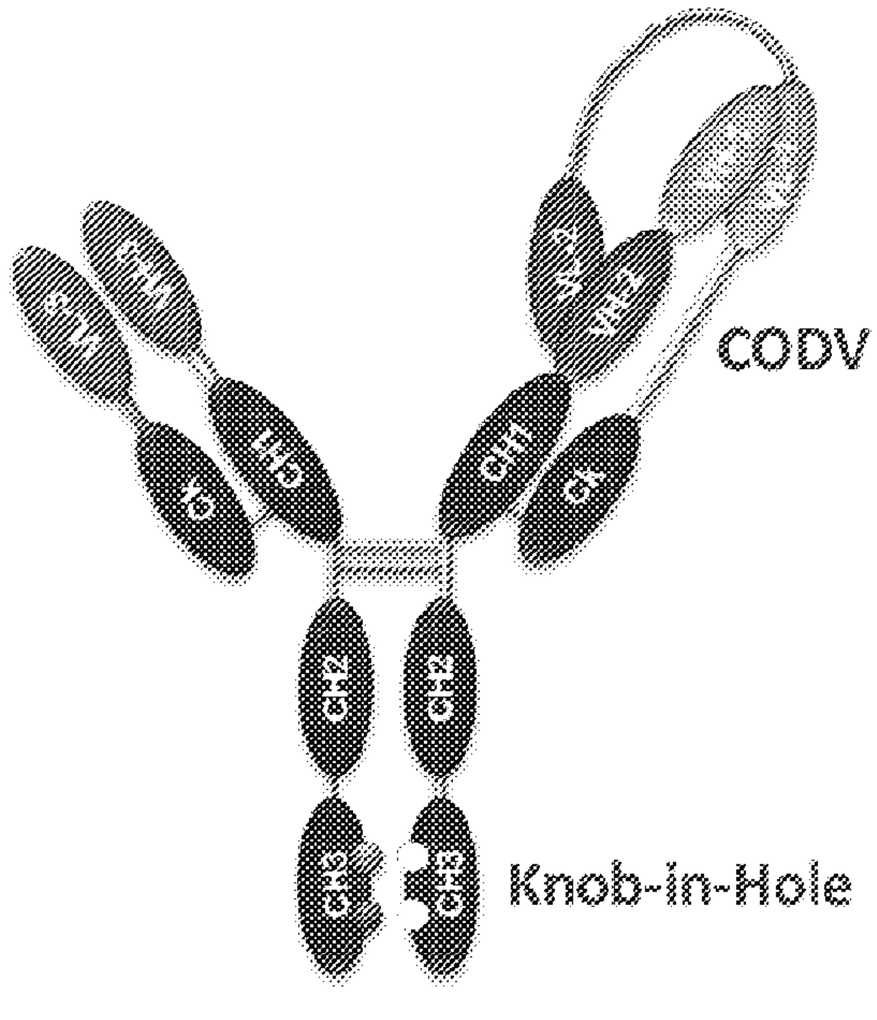
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(54) Titre : PROTEINES DE LIAISON TRISPECIFIQUES ET/OU TRIVALENTES
(54) Title: TRISPECIFIC AND/OR TRIVALENT BINDING PROTEINS

FIG. 1A



(57) Abrégé/Abstract:
The disclosure provides trispecific and/or trivalent binding proteins comprising four polypeptide chains that form three antigen binding sites that specifically bind one or more target proteins, wherein a first pair of polypeptides forming the binding protein possess dual variable domains having a cross-over orientation and wherein a second pair of polypeptides forming the binding protein possess a single variable domain. The disclosure also provides methods for making trispecific and/or trivalent binding proteins and uses of such binding proteins.

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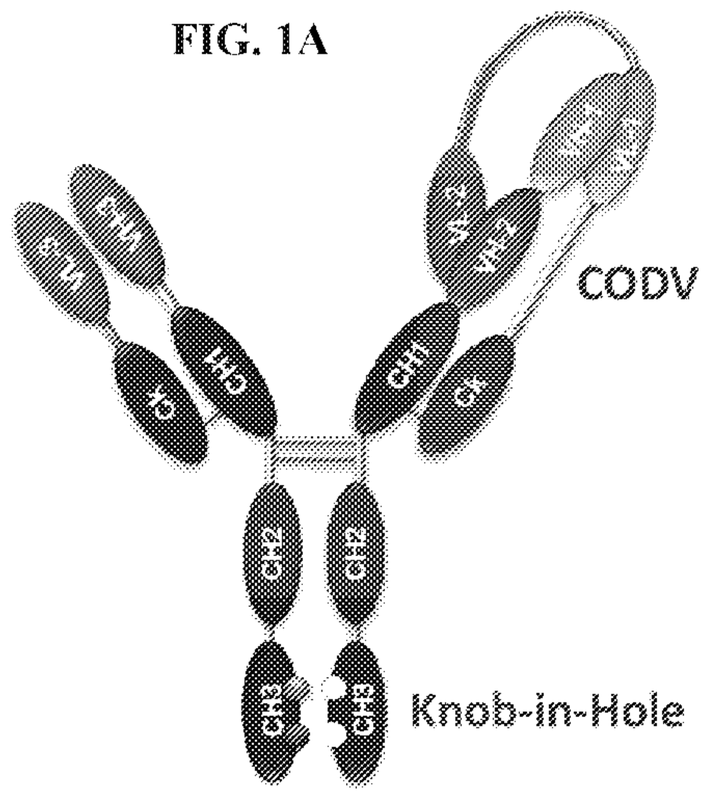
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(54) Title: TRISPECIFIC AND/OR TRIVALENT BINDING PROTEINS

FIG. 1A



(57) Abstract: The disclosure provides trispecific and/or trivalent binding proteins comprising four polypeptide chains that form three antigen binding sites that specifically bind one or more target proteins, wherein a first pair of polypeptides forming the binding protein possess dual variable domains having a cross-over orientation and wherein a second pair of polypeptides forming the binding protein possess a single variable domain. The disclosure also provides methods for making trispecific and/or trivalent binding proteins and uses of such binding proteins.

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JUMBO APPLICATIONS/PATENTS

THIS SECTION OF THE APPLICATION/PATENT CONTAINS MORE THAN ONE VOLUME

THIS IS VOLUME 1 OF 2
CONTAINING PAGES 1 TO 153

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TRISPECIFIC AND/OR TRIVALENT BINDING PROTEINS

CROSS REFERENCES TO RELATED APPLICATIONS

[0001] This application claims the priority benefit of U.S. Provisional Application Serial No. 62/322,036, filed April 13, 2016; U.S. Provisional Application Serial No. 62/331,191, filed May 3, 2016; U.S. Provisional Application Serial No. 62/412,187, filed October 24, 2016; and EP Application No. EP17305298.6, filed March 17, 2017; which are incorporated herein by reference in their entirety.

SUBMISSION OF SEQUENCE LISTING ON ASCII TEXT FILE

[0002] The content of the following submission on ASCII text file is incorporated herein by reference in its entirety: a computer readable form (CRF) of the Sequence Listing (file name: 183952027140SEQLISTING.txt, date recorded: April 11, 2017, size: 200 KB).

FIELD OF THE INVENTION

[0003] The disclosure relates to trispecific and/or trivalent binding proteins comprising four polypeptide chains that form three antigen binding sites that specifically bind one or more target proteins, wherein a first pair of polypeptides forming the binding protein possess dual variable domains having a cross-over orientation and wherein a second pair of polypeptides forming the binding protein possess a single variable domain. The disclosure also relates to methods for making trispecific and/or trivalent binding proteins and uses of such binding proteins.

BACKGROUND

[0004] Monoclonal antibody based biotherapeutics have become an important avenue for new drug development. Monoclonal antibody technology offers specific targeting, precise signaling delivery and/or payload to specific cell population, and provides long lasting biological effect through its Fc functions. Efforts in antibody engineering have allowed developing bispecific antibodies combining the specificities of two monoclonal antibodies for various biological applications, expanding the scope of antibody drug development. Newly discovered neutralizing antibodies with improved breadth and

potency may provide more options for developing biotherapeutics to treat complexed diseases such as cancer, arthritis, and/or inflammatory disorders.

BRIEF SUMMARY

[0005] Provided herein are multispecific binding proteins (*e.g.*, antibodies) that form three antigen binding sites. These binding proteins can specifically bind one, two, or three antigen targets or target proteins.

[0006] In one embodiment, the disclosure provides a binding protein comprising four polypeptide chains that form three antigen binding sites that specifically bind one or more antigen targets or target proteins, wherein a first polypeptide chain has a structure represented by the formula:

$$V_{L2}\text{-}L_1\text{-}V_{L1}\text{-}L_2\text{-}C_L \qquad [I]$$

and a second polypeptide chain has a structure represented by the formula:

$$V_{H1}\text{-}L_3\text{-}V_{H2}\text{-}L_4\text{-}C_{H1} \qquad [II]$$

and a third polypeptide chain has a structure represented by the formula:

$$V_{H3}\text{-}C_{H1} \qquad [III]$$

and a fourth polypeptide chain has a structure represented by the formula:

$$V_{L3}\text{-}C_L \qquad [IV]$$

wherein:

V_{L1} is a first immunoglobulin light chain variable domain;

V_{L2} is a second immunoglobulin light chain variable domain;

V_{L3} is a third immunoglobulin light chain variable domain;

V_{H1} is a first immunoglobulin heavy chain variable domain;

V_{H2} is a second immunoglobulin heavy chain variable domain;

V_{H3} is a third immunoglobulin heavy chain variable domain;

C_L is an immunoglobulin light chain constant domain;

C_{H1} is an immunoglobulin C_{H1} heavy chain constant domain; and

L_1, L_2, L_3 and L_4 are amino acid linkers;

and wherein the polypeptide of formula I and the polypeptide of formula II form a cross-over light chain-heavy chain pair.

[0007] In some embodiments, the second and/or third polypeptide chain further comprises an Fc region linked to C_{H1}, the Fc region comprising an immunoglobulin hinge region and C_{H2} and C_{H3} immunoglobulin heavy chain constant domains.

[0008] In another embodiment, the disclosure provides a binding protein comprising four polypeptide chains that form three antigen binding sites that specifically bind one or more target proteins, wherein a first polypeptide chain comprises a structure represented by the formula:

$$V_{L2}\text{-}L_1\text{-}V_{L1}\text{-}L_2\text{-}C_L \qquad [I]$$

and a second polypeptide chain comprises a structure represented by the formula:

$$V_{H1}\text{-}L_3\text{-}V_{H2}\text{-}L_4\text{-}C_{H1}\text{-hinge-}C_{H2}\text{-}C_{H3} \qquad [II]$$

and a third polypeptide chain comprises a structure represented by the formula:

$$V_{H3}\text{-}C_{H1}\text{-hinge-}C_{H2}\text{-}C_{H3} \qquad [III]$$

and a fourth polypeptide chain comprises a structure represented by the formula:

$$V_{L3}\text{-}C_L \qquad [IV]$$

wherein:

V_{L1} is a first immunoglobulin light chain variable domain;

V_{L2} is a second immunoglobulin light chain variable domain;

V_{L3} is a third immunoglobulin light chain variable domain;

V_{H1} is a first immunoglobulin heavy chain variable domain;

V_{H2} is a second immunoglobulin heavy chain variable domain;

V_{H3} is a third immunoglobulin heavy chain variable domain;

C_L is an immunoglobulin light chain constant domain;

C_{H1} is an immunoglobulin C_{H1} heavy chain constant domain;

C_{H2} is an immunoglobulin C_{H2} heavy chain constant domain;

C_{H3} is an immunoglobulin C_{H3} heavy chain constant domain;

hinge is an immunoglobulin hinge region connecting the C_{H1} and C_{H2} domains; and

L_1, L_2, L_3 and L_4 are amino acid linkers;

and wherein the polypeptide of formula I and the polypeptide of formula II form a cross-over light chain-heavy chain pair.

[0009] In some embodiments, the binding protein is trispecific and capable of specifically binding three different antigen targets. In some embodiments, the binding protein is trivalent but bispecific and capable of specifically binding three antigen targets, two of them being identical. In some embodiments, the binding protein of the present disclosure is trivalent but monopecific and capable of specifically binding three antigen targets, all of them being identical. In some embodiments, the binding protein is capable of inhibiting the function of one or more target proteins. In some embodiments, the binding protein is trispecific and capable of specifically binding three different antigen targets.

[0010] In some embodiments, a binding protein of the present disclosure comprises one, two, or three antigen binding sites that specifically bind a target protein selected from A2AR, APRIL, ATPDase, BAFF, BAFFR, BCMA, BlyS, BTK, BTLA, B7DC, B7H1, B7H4 (also known as VTCN1), B7H5, B7H6, B7H7, B7RP1, B7-4, C3, C5, CCL2 (also known as MCP-1), CCL3 (also known as MIP-1a), CCL4 (also known as MIP-1b), CCL5 (also known as RANTES), CCL7 (also known as MCP-3), CCL8 (also known as mcp-2), CCL11 (also known as eotaxin), CCL15 (also known as MIP-1d), CCL17 (also known as TARC), CCL19 (also known as MIP-3b), CCL20 (also known as MIP-3a), CCL21 (also known as MIP-2), CCL24 (also known as MPIF-2/eotaxin-2), CCL25 (also known as TECK), CCL26 (also known as eotaxin-3), CCR3, CCR4, CD3, CD19, CD20, CD23 (also known as FCER2, a receptor for IgE), CD24, CD27, CD28, CD38, CD39, CD40, CD70, CD80 (also known as B7-1), CD86 (also known as B7-2), CD122, CD137 (also known as 41BB), CD137L, CD152 (also known as CTLA4), CD154 (also known as CD40L), CD160, CD272, CD273 (also known as PDL2), CD274 (also known as PDL1), CD275 (also known as B7H2), CD276 (also known as B7H3), CD278 (also known as ICOS), CD279 (also known as PD-1), CDH1 (also known as E-cadherin), chitinase, CLEC9, CLEC91, CRTH2, CSF-1 (also known as M-CSF), CSF-2 (also known as GM-CSF), CSF-3 (also known as GCSF), CX3CL1 (also known as SCYD1), CXCL12 (also known as SDF1), CXCL13, CXCR3, DNGR-1, ectonucleoside triphosphate diphosphohydrolase 1, EGFR, ENTPD1, FCER1A, FCER1, FLAP, FOLH1, Gi24, GITR, GITRL, GM-CSF, Her2, HHLA2, HMGB1, HVEM, ICOSLG, IDO, IFNα, IgE, IGF1R, IL2Rbeta, IL1, IL1A, IL1B, IL1F10, IL2, IL4, IL4Ra, IL5, IL5R, IL6, IL7, IL7Ra, IL8, IL9, IL9R, IL10, rhIL10, IL12, IL13, IL13Ra1, IL13Ra2, IL15, IL17, IL17Rb (also known as a receptor for IL25), IL18, IL22, IL23, IL25, IL27, IL33, IL35, ITGB4 (also known as b4 integrin), ITK, KIR, LAG3, LAMP1, leptin, LPFS2, MHC class II, NCR3LG1, NKG2D, NTPDase-1, OX40, OX40L, PD-1H, platelet receptor, PROM1, S152, SISP1, SLC, SPG64, ST2 (also known as a receptor for IL33), STEAP2, Syk kinase, TACI, TDO, T14, TIGIT, TIM3, TLR, TLR2, TLR4, TLR5, TLR9, TMEF1, TNFa, TNFRSF7, Tp55, TREM1, TSLP (also known as a co-receptor for IL7Ra), TSLPR, TWEAK, VEGF, VISTA, Vstm3, WUCAM, and XCR1 (also known as GPR5/CCXCR1). In some embodiments, one or more of the above antigen targets are human antigen targets. In some embodiments, the binding protein of the present disclosure is trispecific and capable of specifically binding three different antigen targets selected from the above list. In some embodiments, the binding protein of the present disclosure is trivalent but bispecific and capable of specifically binding three antigen targets

selected from the above list, two of them being identical. In some embodiments, the binding protein of the present disclosure is trivalent but monopecific and capable of specifically binding three antigen targets selected from the above list, all of them being identical. In some embodiments, the binding protein specifically binds three target proteins that correspond to two target proteins on T cells and to one tumor target protein. In some embodiments, one of said target proteins on T cells is CD3. In some embodiments, one of said target proteins on T cells is CD28. In some embodiments, said tumor target protein is CD38. In some embodiments, the binding protein specifically binds three target proteins that correspond to two target proteins on T cells and to one target protein selected from the group consisting of A2AR, APRIL, ATPDase, BAFF, BAFFR, BCMA, BlyS, BTK, BTLA, B7DC, B7H1, B7H4, B7H5, B7H6, B7H7, B7RP1, B7-4, C3, C5, CCL2, CCL3, CCL4, CCL5, CCL7, CCL8, CCL11, CCL15, CCL17, CCL19, CCL20, CCL21, CCL24, CCL25, CCL26, CCR3, CCR4, CD3, CD19, CD20, CD23, CD24, CD27, CD28, CD38, CD39, CD40, CD70, CD80, CD86, CD122, CD137, CD137L, CD152, CD154, CD160, CD272, CD273, CD274, CD275, CD276, CD278, CD279, CDH1, chitinase, CLEC9, CLEC91, CRTH2, CSF-1, CSF-2, CSF-3, CX3CL1, CXCL12, CXCL13, CXCR3, DNGR-1, ectonucleoside triphosphate diphosphohydrolase 1, EGFR, ENTPD1, FCER1A, FCER1, FLAP, FOLH1, Gi24, GITR, GITRL, GM-CSF, Her2, HHLA2, HMGB1, HVEM, ICOSLG, IDO, IFNα, IgE, IGF1R, IL2Rbeta, IL1, IL1A, IL1B, IL1F10, IL2, IL4, IL4Ra, IL5, IL5R, IL6, IL7, IL7Ra, IL8, IL9, IL9R, IL10, rhIL10, IL12, IL13, IL13Ra1, IL13Ra2, IL15, IL17, IL17Rb, IL18, IL22, IL23, IL25, IL27, IL33, IL35, ITGB4, ITK, KIR, LAG3, LAMP1, leptin, LPFS2, MHC class II, NCR3LG1, NKG2D, NTPDase-1, OX40, OX40L, PD-1H, platelet receptor, PROM1, S152, SISP1, SLC, SPG64, ST2, STEAP2, Syk kinase, TACI, TDO, T14, TIGIT, TIM3, TLR, TLR2, TLR4, TLR5, TLR9, TMEF1, TNFa, TNFRSF7, Tp55, TREM1, TSLP, TSLPR, TWEAK, VEGF, VISTA, Vstm3, WUCAM, and XCR1.

[0011] In another embodiment, the disclosure provides a binding protein comprising four polypeptide chains that form three antigen binding sites, wherein a first polypeptide chain has a structure represented by the formula:

$$V_{L2}\text{-}L_1\text{-}V_{L1}\text{-}L_2\text{-}C_L \qquad [I]$$

and a second polypeptide chain has a structure represented by the formula:

$$V_{H1}\text{-}L_3\text{-}V_{H2}\text{-}L_4\text{-}C_{H1} \qquad [II]$$

and a third polypeptide chain has a structure represented by the formula:

$$V_{H3}\text{-}C_{H1} \qquad [III]$$

and a fourth polypeptide chain has a structure represented by the formula:

V_{L3}-C_L [IV]

wherein:

V_{L1} is a first immunoglobulin light chain variable domain;

V_{L2} is a second immunoglobulin light chain variable domain;

V_{L3} is a third immunoglobulin light chain variable domain;

V_{H1} is a first immunoglobulin heavy chain variable domain;

V_{H2} is a second immunoglobulin heavy chain variable domain;

V_{H3} is a third immunoglobulin heavy chain variable domain;

C_L is an immunoglobulin light chain constant domain;

C_{H1} is an immunoglobulin C_{H1} heavy chain constant domain; and

L_1, L_2, L_3 and L_4 are amino acid linkers;

wherein the polypeptide of formula I and the polypeptide of formula II form a cross-over light chain-heavy chain pair;

wherein:

(a) V_{L1}, V_{L2} and V_{L3} are each independently a variable domain derived from an amino acid sequence as set forth in any one of SEQ ID NOs: 2, 4, 10, 14, 18, 22, 115;

(b) V_{L1}, V_{L2} and V_{L3} each independently comprise light chain complementarity determining regions of a variable domain comprising an amino acid sequence as set forth in any one of SEQ ID NOs: 43-59, 123-125;

(c) V_{L1}, V_{L2} and V_{L3} each independently comprise a variable domain sequence as set forth in any one of SEQ ID NOs:151, 153, 155, 157, 159, 161, 163, 165, and 167;

(d) V_{L1}, V_{L2} and V_{L3} each independently comprise light chain complementarity determining regions comprising an amino acid sequence as set forth in any one of SEQ ID NOs: 43-59, 123-125, 138-140, and 149; or

(e) V_{L1}, V_{L2} and V_{L3} each independently comprise light chain complementarity determining regions and/or a variable domain sequence shown in Tables 2-5; and

wherein:

(a) V_{H1}, V_{H2}, and V_{H3} are each independently a variable domain derived from an amino acid sequence as set forth in any one of SEQ ID NOs: 1, 3, 9, 13, 17, 21, 114;

(b) V_{H1}, V_{H2} and V_{H3} each independently comprise heavy chain complementarity determining regions of a variable domain comprising an amino acid sequence as set forth in any one of SEQ ID NOs: 25-42, 120-122;

(c) V_{H1}, V_{H2}, and V_{H3} each independently comprise a variable domain sequence as set forth in any one of SEQ ID NOs:150, 152, 154, 156, 158, 160, 162, 164, and 166;

(d) V_{H1}, V_{H2} and V_{H3} each independently comprise heavy chain complementarity determining regions comprising an amino acid sequence as set forth in any one of SEQ ID NOs: 25-42, 120-122, and 126-128; or

(e) V_{H1}, V_{H2} and V_{H3} each independently comprise heavy chain complementarity determining regions and/or a variable domain sequence shown in Tables 2-5.

[0012] In some embodiments, the second and/or third polypeptide chain further comprises an Fc region linked to C_{H1}, the Fc region comprising an immunoglobulin hinge region and C_{H2} and C_{H3} immunoglobulin heavy chain constant domains.

[0013] In another embodiment, the disclosure provides a binding protein comprising four polypeptide chains that form three antigen binding sites, wherein a first polypeptide chain comprises a structure represented by the formula:

$$V_{L2}\text{-}L_1\text{-}V_{L1}\text{-}L_2\text{-}C_L \qquad [I]$$

and a second polypeptide chain comprises a structure represented by the formula:

$$V_{H1}\text{-}L_3\text{-}V_{H2}\text{-}L_4\text{-}C_{H1}\text{-hinge-}C_{H2}\text{-}C_{H3} \qquad [II]$$

and a third polypeptide chain comprises a structure represented by the formula:

$$V_{H3}\text{-}C_{H1}\text{-hinge-}C_{H2}\text{-}C_{H3} \qquad [III]$$

and a fourth polypeptide chain comprises a structure represented by the formula:

$$V_{L3}\text{-}C_L \qquad [IV]$$

wherein:

V_{L1} is a first immunoglobulin light chain variable domain;

V_{L2} is a second immunoglobulin light chain variable domain;

V_{L3} is a third immunoglobulin light chain variable domain;

V_{H1} is a first immunoglobulin heavy chain variable domain;

V_{H2} is a second immunoglobulin heavy chain variable domain;

V_{H3} is a third immunoglobulin heavy chain variable domain;

C_L is an immunoglobulin light chain constant domain;

C_{H1} is an immunoglobulin C_{H1} heavy chain constant domain;

C_{H2} is an immunoglobulin C_{H2} heavy chain constant domain;

C_{H3} is an immunoglobulin C_{H3} heavy chain constant domain;

hinge is an immunoglobulin hinge region connecting the C_{H1} and C_{H2} domains; and

L_1, L_2, L_3 and L_4 are amino acid linkers;

wherein the polypeptide of formula I and the polypeptide of formula II form a cross-over light chain-heavy chain pair;

wherein:

(a) V_{L1}, V_{L2} and V_{L3} are each independently a variable domain derived from an amino acid sequence as set forth in any one of SEQ ID NOs: 2, 4, 10, 14, 18, 22, 115;

(b) V_{L1}, V_{L2} and V_{L3} each independently comprise light chain complementarity determining regions of a variable domain comprising an amino acid sequence as set forth in any one of SEQ ID NOs: 43-59, 123-125;

(c) V_{L1}, V_{L2} and V_{L3} each independently comprise a variable domain sequence as set forth in any one of SEQ ID NOs:151, 153, 155, 157, 159, 161, 163, 165, and 167;

(d) V_{L1}, V_{L2} and V_{L3} each independently comprise light chain complementarity determining regions comprising an amino acid sequence as set forth in any one of SEQ ID NOs: 43-59, 123-125, 138-140, and 149; or

(e) V_{L1}, V_{L2} and V_{L3} each independently comprise light chain complementarity determining regions and/or a variable domain sequence shown in Tables 2-5;

wherein:

(a) V_{H1}, V_{H2}, and V_{H3} are each independently a variable domain derived from an amino acid sequence as set forth in any one of SEQ ID NOs: 1, 3, 9, 13, 17, 21, 114;

(b) V_{H1}, V_{H2} and V_{H3} each independently comprise heavy chain complementarity determining regions of a variable domain comprising an amino acid sequence as set forth in any one of SEQ ID NOs: 25-42, 120-122;

(c) V_{H1}, V_{H2}, and V_{H3} each independently comprise a variable domain sequence as set forth in any one of SEQ ID NOs:150, 152, 154, 156, 158, 160, 162, 164, and 166;

(d) V_{H1}, V_{H2} and V_{H3} each independently comprise heavy chain complementarity determining regions comprising an amino acid sequence as set forth in any one of SEQ ID NOs: 25-42, 120-122, and 126-128; or

(e) V_{H1}, V_{H2} and V_{H3} each independently comprise heavy chain complementarity determining regions and/or a variable domain sequence shown in Tables 2-5.

[0014] In some embodiments, V_{L1}, V_{L2} and V_{L3} each independently comprise a variable domain sequence as set forth in any one of SEQ ID NOs:151, 153, 155, 157, 159, 161, 163, 165, and 167; and V_{H1}, V_{H2}, and V_{H3} each independently comprise a variable domain sequence as set forth in any one of SEQ ID NOs:150, 152, 154, 156, 158, 160, 162, 164, and 166. In some embodiments, V_{L1}, V_{L2} and V_{L3} each independently comprise light chain complementarity determining regions comprising an amino acid sequence as set forth in

any one of SEQ ID NOs: 43-59, 123-125, 138-140, and 149; and (d) V_{H1}, V_{H2} and V_{H3} each independently comprise heavy chain complementarity determining regions comprising an amino acid sequence as set forth in any one of SEQ ID NOs: 25-42, 120-122, and 126-128.

[0015] In some embodiments of any of the binding proteins described herein, (a) V_{H1} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:28, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:29, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:30; V_{L1} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:46, a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 47, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:48; V_{H2} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:34, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:35, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:36; V_{L2} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:52, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:53, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:54; V_{H3} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:25, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:26, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:27; and V_{L3} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:43, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:44, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:45; (b) V_{H1} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:31, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:32, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:33; V_{L1} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:49, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:50, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:51; V_{H2} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:34, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:35, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:36; V_{L2} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:52, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:53, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:54; V_{H3} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:25, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:26, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:27; and V_{L3} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:43, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:44, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:45; (c)

V_{H1} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:28, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:29, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:30; V_{L1} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:46, a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 47, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:48; V_{H2} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:34, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:35, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:36; V_{L2} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:52, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:53, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:54; V_{H3} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:37, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:38, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:39; and V_{L3} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:55, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:56, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:57; (d) V_{H1} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:31, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:32, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:33; V_{L1} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:49, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:50, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:51; V_{H2} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:34, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:35, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:36; V_{L2} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:52, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:53, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:54; V_{H3} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:37, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:38, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:39; and V_{L3} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:55, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:56, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:57; (e) V_{H1} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:28, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:29, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:30; V_{L1} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:46, a CDR-L2 comprising the amino acid sequence of SEQ

ID NO: 47, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:48; V_{H2} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:34, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:35, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:36; V_{L2} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:52, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:53, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:54; V_{H3} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:40, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:41, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:42; and V_{L3} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:58, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:44, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:59; (f) V_{H1} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:31, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:32, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:33; V_{L1} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:49, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:50, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:51; V_{H2} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:34, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:35, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:36; V_{L2} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:52, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:53, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:54; V_{H3} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:40, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:41, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:42; and V_{L3} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:58, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:44, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:59; (g) V_{H1} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:28, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:29, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:30; V_{L1} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:46, a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 47, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:48; V_{H2} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:34, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:35, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:36; V_{L2} comprises a CDR-L1 comprising the amino

acid sequence of SEQ ID NO:52, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:53, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:54; V_{H3} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:126, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:127, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:128; and V_{L3} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:138, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:139, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:140; (h) V_{H1} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:31, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:32, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:33; V_{L1} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:49, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:50, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:51; V_{H2} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:34, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:35, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:36; V_{L2} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:52, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:53, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:54; V_{H3} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:126, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:127, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:128; and V_{L3} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:138, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:139, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:140; (i) V_{H1} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:28, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:29, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:30; V_{L1} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:46, a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 47, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:48; V_{H2} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:34, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:35, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:36; V_{L2} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:52, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:53, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:54; V_{H3} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:120, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:121, and a CDR-

H3 comprising the amino acid sequence of SEQ ID NO:122; and V_{L3} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:123, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:124, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:125; or (j) V_{H1} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:31, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:32, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:33; V_{L1} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:49, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:50, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:51; V_{H2} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:34, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:35, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:36; V_{L2} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:52, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:53, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:54; V_{H3} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:120, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:121, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:122; and V_{L3} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:123, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:124, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:125. In some embodiments, (a) V_{H1} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:34, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:35, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:36; V_{L1} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:52, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:53, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:54; V_{H2} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:25, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:26, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:27; V_{L2} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:43, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:44, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:45; V_{H3} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:25, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:26, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:27; and V_{L3} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:43, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:44, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:45.

[0016] In some embodiments, the binding protein comprises one antigen binding site that specifically binds a T-cell surface protein and another antigen binding site that specifically binds an antigen target, *e.g.*, a tumor target protein. In some embodiments, the binding protein comprises an antigen binding site that specifically binds CD3, an antigen binding site that specifically binds CD28, and an antigen binding site that specifically binds a tumor target protein selected from the group consisting of CD19, CD20, CD38, Her2, and LAMP1. In some embodiments, V_{H1} and V_{L1} form a first antigen binding site that specifically binds human CD3, V_{H2} and V_{L2} form a second antigen binding site that specifically binds human CD28, and V_{H3} and V_{L3} form a third antigen binding site that specifically binds a human tumor target protein. In some embodiments, V_{H1} and V_{L1} form a first antigen binding site that specifically binds human CD28, V_{H2} and V_{L2} form a second antigen binding site that specifically binds human CD3, and V_{H3} and V_{L3} form a third binding site that specifically binds a human tumor target protein. In some embodiments, the antigen binding site specifically binds a human tumor target protein selected from the group consisting of CD19, CD20, CD38, Her2, and LAMP1. In some embodiments, the antigen binding site that specifically binds CD3 comprises: (a) a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 152 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO: 153; or (b) a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 154 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO: 155. In some embodiments, the antigen binding site that specifically binds CD3 comprises six CDRs, or a heavy chain and a light chain variable domain, shown in Tables 2-5. In some embodiments, the antigen binding site that specifically binds CD28 comprises: (a) a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 160 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO: 161; or (b) a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 162 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO: 163. In some embodiments, the antigen binding site that specifically binds CD28 comprises six CDRs, or a heavy chain and a light chain variable domain, shown in Tables 2-5. In some embodiments, the antigen binding site that specifically binds a tumor target protein comprises: (a) a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 156 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO: 157; (b) a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 158 and a light chain variable domain comprising the amino acid sequence of

SEQ ID NO: 159; (c) a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 164 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO: 165; (d) a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 150 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO: 151; or (e) a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 166 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO: 167. In some embodiments, the antigen binding site that specifically binds a tumor target protein comprises six CDRs, or a heavy chain and a light chain variable domain, shown in Tables 2-5. In some embodiments, the antigen binding site that specifically binds a tumor target protein comprises six CDRs, or a heavy chain and a light chain variable domain, of an anti-Her2, anti-CD19, anti-CD20, anti-CD38, or anti-LAMP1 binding domain shown in Tables 2-5.

[0017] In another embodiment, the disclosure provides a binding protein comprising four polypeptide chains that form three antigen binding sites, wherein a first polypeptide chain has a structure represented by the formula:

$$V_{L2}\text{-}L_1\text{-}V_{L1}\text{-}L_2\text{-}C_L \qquad [I]$$

and a second polypeptide chain has a structure represented by the formula:

$$V_{H1}\text{-}L_3\text{-}V_{H2}\text{-}L_4\text{-}C_{H1} \qquad [II]$$

and a third polypeptide chain has a structure represented by the formula:

$$V_{H3}\text{-}C_{H1} \qquad [III]$$

and a fourth polypeptide chain has a structure represented by the formula:

$$V_{L3}\text{-}C_L \qquad [IV]$$

wherein:

V_{L1} is a first immunoglobulin light chain variable domain;

V_{L2} is a second immunoglobulin light chain variable domain;

V_{L3} is a third immunoglobulin light chain variable domain;

V_{H1} is a first immunoglobulin heavy chain variable domain;

V_{H2} is a second immunoglobulin heavy chain variable domain;

V_{H3} is a third immunoglobulin heavy chain variable domain;

C_L is an immunoglobulin light chain constant domain;

C_{H1} is an immunoglobulin C_{H1} heavy chain constant domain; and

L_1, L_2, L_3 and L_4 are amino acid linkers;

wherein the polypeptide of formula I and the polypeptide of formula II form a cross-over light chain-heavy chain pair;

wherein:

(a) V_{L1}, V_{L2} and V_{L3} are each independently a variable domain derived from an amino acid sequence as set forth in any one of SEQ ID NOs: 61, 63, 69, 71, 74, 76, 82, 86, 88, 94; or

(b) V_{L1}, V_{L2} and V_{L3} each independently comprise light chain complementarity determining regions of a variable domain of at least one amino acid sequence set forth in any one of SEQ ID NOs: 61, 63, 69, 71, 74, 76, 82, 86, 88, 94;

(c) V_{L1}, V_{L2} and V_{L3} each independently comprise a variable domain sequence as set forth in any one of SEQ ID NOs:169, 171, and 173;

(d) V_{L1}, V_{L2} and V_{L3} each independently comprise light chain complementarity determining regions comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 141-147, 178, and 179; or

(e) V_{L1}, V_{L2} and V_{L3} each independently comprise light chain complementarity determining regions and/or a variable domain sequence shown in Tables 2-5; and

wherein:

(a) V_{H1}, V_{H2}, and V_{H3} are each independently a variable domain derived from an amino acid sequence as set forth in any one of SEQ ID NOs: 60, 62, 68, 73, 75, 81, 85, 87, 93; or

(b) V_{H1}, V_{H2}, and V_{H3} each independently comprise heavy chain complementarity determining regions of a variable domain of at least one amino acid sequence set forth in any one of in any one of SEQ ID NOs: 60, 62, 68, 73, 75, 81, 85, 87, 93;

(c) V_{H1}, V_{H2}, and V_{H3} each independently comprise a variable domain sequence as set forth in any one of SEQ ID NOs:168, 170, and 172;

(d) V_{H1}, V_{H2} and V_{H3} each independently comprise heavy chain complementarity determining regions comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 129-137; or

(e) V_{H1}, V_{H2} and V_{H3} each independently comprise heavy chain complementarity determining regions and/or a variable domain sequence shown in Tables 2-5.

[0018] In some embodiments, the second and/or third polypeptide chain further comprises an Fc region linked to C_{H1}, the Fc region comprising an immunoglobulin hinge region and C_{H2} and C_{H3} immunoglobulin heavy chain constant domains.

[0019] In another embodiment, the disclosure provides a binding protein comprising four polypeptide chains that form three antigen binding sites, wherein a first polypeptide chain comprises a structure represented by the formula:

$$V_{L2}\text{-}L_1\text{-}V_{L1}\text{-}L_2\text{-}C_L \qquad [I]$$

and a second polypeptide chain comprises a structure represented by the formula:

$$V_{H1}\text{-}L_3\text{-}V_{H2}\text{-}L_4\text{-}C_{H1}\text{-hinge-}C_{H2}\text{-}C_{H3} \quad [II]$$

and a third polypeptide chain comprises a structure represented by the formula:

$$V_{H3}\text{-}C_{H1}\text{-hinge-}C_{H2}\text{-}C_{H3} \quad [III]$$

and a fourth polypeptide chain comprises a structure represented by the formula:

$$V_{L3}\text{-}C_L \quad [IV]$$

wherein:

V_{L1} is a first immunoglobulin light chain variable domain;

V_{L2} is a second immunoglobulin light chain variable domain;

V_{L3} is a third immunoglobulin light chain variable domain;

V_{H1} is a first immunoglobulin heavy chain variable domain;

V_{H2} is a second immunoglobulin heavy chain variable domain;

V_{H3} is a third immunoglobulin heavy chain variable domain;

C_L is an immunoglobulin light chain constant domain;

C_{H1} is an immunoglobulin C_{H1} heavy chain constant domain;

C_{H2} is an immunoglobulin C_{H2} heavy chain constant domain;

C_{H3} is an immunoglobulin C_{H3} heavy chain constant domain;

hinge is an immunoglobulin hinge region connecting the C_{H1} and C_{H2} domains; and

L_1, L_2, L_3 and L_4 are amino acid linkers;

wherein the polypeptide of formula I and the polypeptide of formula II form a cross-over light chain-heavy chain pair;

wherein:

(a) V_{L1}, V_{L2} and V_{L3} are each independently a variable domain derived from an amino acid sequence as set forth in any one of SEQ ID NOs: 61, 63, 69, 71, 74, 76, 82, 86, 88, 94;

(b) V_{L1}, V_{L2} and V_{L3} each independently comprise light chain complementarity determining regions of a variable domain of at least one amino acid sequence set forth in any one of SEQ ID NOs: 61, 63, 69, 71, 74, 76, 82, 86, 88, 94;

(c) V_{L1}, V_{L2} and V_{L3} each independently comprise a variable domain sequence as set forth in any one of SEQ ID NOs:169, 171, and 173;

(d) V_{L1}, V_{L2} and V_{L3} each independently comprise light chain complementarity determining regions comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 141-147, 178, and 179; or

(e) V_{L1}, V_{L2} and V_{L3} each independently comprise light chain complementarity determining regions and/or a variable domain sequence shown in Tables 2-5;

wherein:

(a) V_{H1}, V_{H2}, and V_{H3} are each independently a variable domain derived from an amino acid sequence as set forth in any one of SEQ ID NOs: 60, 62, 68, 73, 75, 81, 85, 87, 93;

(b) V_{H1}, V_{H2}, and V_{H3} each independently comprise heavy chain complementarity determining regions of a variable domain of at least one amino acid sequence set forth in any one of in any one of SEQ ID NOs: 60, 62, 68, 73, 75, 81, 85, 87, 93;

(c) V_{H1}, V_{H2}, and V_{H3} each independently comprise a variable domain sequence as set forth in any one of SEQ ID NOs:168, 170, and 172;

(d) V_{H1}, V_{H2} and V_{H3} each independently comprise heavy chain complementarity determining regions comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 129-137;

(e) V_{H1}, V_{H2} and V_{H3} each independently comprise heavy chain complementarity determining regions and/or a variable domain sequence shown in Tables 2-5.

[0020] In some embodiments, V_{L1}, V_{L2} and V_{L3} each independently comprise a variable domain sequence as set forth in any one of SEQ ID NOs:169, 171, and 173; and V_{H1}, V_{H2}, and V_{H3} each independently comprise a variable domain sequence as set forth in any one of SEQ ID NOs:168, 170, and 172. In some embodiments, V_{L1}, V_{L2} and V_{L3} each independently comprise light chain complementarity determining regions comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 141-147, 178, and 179; and V_{H1}, V_{H2} and V_{H3} each independently comprise heavy chain complementarity determining regions comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 129-137.

[0021] In some embodiments of any of the binding proteins described herein, (a) V_{H1} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:132, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:133, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:134; V_{L1} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:143, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:179, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:144; V_{H2} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:135, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:136, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:137; V_{L2} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:145, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:146, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:147; V_{H3} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:129, a CDR-H2

comprising the amino acid sequence of SEQ ID NO:130, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:131; and V_{L3} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:141, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:178, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:142; (b) V_{H1} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:135, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:136, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:137; V_{L1} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:145, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:146, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:147; V_{H2} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:132, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:133, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:134; V_{L2} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:143, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:179, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:144; V_{H3} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:129, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:130, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:131; and V_{L3} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:141, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:178, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:142; (c) V_{H1} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:132, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:133, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:134; V_{L1} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:143, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:179, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:144; V_{H2} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:129, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:130, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:131; V_{L2} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:141, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:178, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:142; V_{H3} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:135, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:136, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:137; and V_{L3} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:145, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:146, and a

CDR-L3 comprising the amino acid sequence of SEQ ID NO:147; (d) V_{H1} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:129, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:130, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:131; V_{L1} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:141, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:178, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:142; V_{H2} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:132, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:133, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:134; V_{L2} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:143, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:179, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:144; V_{H3} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:135, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:136, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:137; and V_{L3} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:145, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:146, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:147; (e) V_{H1} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:135, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:136, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:137; V_{L1} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:145, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:146, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:147; V_{H2} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:129, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:130, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:131; V_{L2} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:141, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:178, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:142; V_{H3} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:132, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:133, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:134; and V_{L3} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:143, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:179, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:144; (f) V_{H1} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:129, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:130, and a CDR-H3 comprising the amino acid sequence of SEQ ID

NO:131; V_{L1} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:141, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:178, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:142; V_{H2} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:135, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:136, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:137; V_{L2} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:145, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:146, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:147; V_{H3} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:132, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:133, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:134; and V_{L3} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:143, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:179, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:144; (g) V_{H1} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:135, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:136, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:137; V_{L1} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:145, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:146, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:147; V_{H2} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:132, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:133, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:134; V_{L2} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:143, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:179, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:144; V_{H3} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:135, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:136, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:137; and V_{L3} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:145, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:146, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:147; (h) V_{H1} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:135, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:136, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:137; V_{L1} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:145, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:146, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:147; V_{H2} comprises a CDR-H1 comprising the amino acid sequence of SEQ

ID NO:132, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:133, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:134; V_{L2} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:143, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:179, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:144; V_{H3} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:132, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:133, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:134; and V_{L3} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:143, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:179, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:144; (i) V_{H1} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:132, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:133, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:134; V_{L1} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:143, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:179, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:144; V_{H2} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:135, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:136, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:137; V_{L2} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:145, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:146, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:147; V_{H3} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:135, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:136, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:137; and V_{L3} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:145, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:146, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:147; or (j) V_{H1} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:132, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:133, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:134; V_{L1} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:143, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:179, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:144; V_{H2} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:135, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:136, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:137; V_{L2} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:145, a CDR-L2 comprising the amino acid sequence of SEQ

ID NO:146, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:147; V_{H3} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:132, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:133, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:134; and V_{L3} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:143, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:179, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:144. In some embodiments, one or more of V_{H1}, V_{L1}, V_{H2}, V_{L2}, V_{H3}, and V_{L3} comprises one, two, or three CDR sequences of an antibody shown in Tables 2-5.

[0022] In some embodiments, the binding protein comprises three antigen binding sites, where one, two, or three of the antigen binding site(s) specifically bind(s) a cytokine target protein selected from the group consisting of IL-4, IL-13 and TNFa. In some embodiments, (a)V_{H1} and V_{L1} form a first antigen binding site that specifically binds human TNFa, V_{H2} and V_{L2} form an antigen binding site that specifically binds human IL13, and V_{H3} and V_{L3} form an antigen binding site that specifically binds human IL4; (b) V_{H1} and V_{L1} form a first antigen binding site that specifically binds human TNFa, V_{H2} and V_{L2} form a second antigen binding site that specifically binds human IL4, and V_{H3} and V_{L3} form a third antigen binding site that specifically binds human IL13; (c) V_{H1} and V_{L1} form a first antigen binding site that specifically binds human IL4, V_{H2} and V_{L2} form a second antigen binding site that specifically binds human TNFa, and V_{H3} and V_{L3} form a third antigen binding site that specifically binds human IL13; (d) V_{H1} and V_{L1} form a first antigen binding site that specifically binds human IL4, V_{H2} and V_{L2} form a second antigen binding site that specifically binds human IL13, and V_{H3} and V_{L3} form a third antigen binding site that specifically binds human TNFa; (e) V_{H1} and V_{L1} form a first antigen binding site that specifically binds human IL13, V_{H2} and V_{L2} form a second antigen binding site that specifically binds human IL4, and V_{H3} and V_{L3} form a third antigen binding site that specifically binds human TNFa; or (f) V_{H1} and V_{L1} form a first antigen binding site that specifically binds human IL13, V_{H2} and V_{L2} form a second antigen binding site that specifically binds human TNFa, and V_{H3} and V_{L3} form a third antigen binding site that specifically binds human IL4. In some embodiments, the antigen binding site that specifically binds human TNFa comprises a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:168 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO:169. In some embodiments, the antigen binding site that specifically binds human IL4 comprises a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:170 and a light chain variable domain comprising the

amino acid sequence of SEQ ID NO:171. In some embodiments, the antigen binding site that specifically binds human IL13 comprises a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:172 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO:173.

[0023] In some embodiments of any of the binding proteins described herein, the second and/or third polypeptide chain further comprises an Fc region linked to C_{H1}, the Fc region comprising an immunoglobulin hinge region and C_{H2} and C_{H3} immunoglobulin heavy chain constant domains. In some embodiments, at least one of L_1, L_2, L_3 or L_4 is independently 0 amino acids in length. In some embodiments, L_1, L_2, L_3 or L_4 are each independently at least one amino acid in length. In some embodiments, the binding protein is trispecific and capable of specifically binding three different antigen targets. In some embodiments, the binding protein is trispecific and capable of specifically binding three different antigen targets. In some embodiments, the binding protein is capable of inhibiting the function of one or more target proteins.

[0024] In some embodiments of any of the binding proteins described herein, at least one of L_1, L_2, L_3 or L_4 is independently 0 amino acids in length. In some embodiments, L_1, L_2, L_3 or L_4 are each independently at least one amino acid in length. In some embodiments, one, two, three, or all four of L_1, L_2, L_3 and L_4 are between 0 and 15 amino acids in length. In some embodiments, at least two of L_1, L_2, L_3 and L_4 are between 1 and 15 amino acids in length. In some embodiments, (a) L_1, L_2, L_3 and L_4 each independently are zero amino acids in length or comprise a sequence selected from the group consisting of GGGGSGGGGS (SEQ ID NO:104), GGGGSGGGGSGGGGS (SEQ ID NO:105), S, RT, TKGPS (SEQ ID NO:106), GQPKAAP (SEQ ID NO: 175), and GGSGSSGSGG (SEQ ID NO:148); or (b) L_1, L_2, L_3 and L_4 each independently comprise a sequence selected from the group consisting of GGGGSGGGGS (SEQ ID NO:104), GGGGSGGGGSGGGGS (SEQ ID NO:105), S, RT, TKGPS (SEQ ID NO:106), GQPKAAP (SEQ ID NO: 175), and GGSGSSGSGG (SEQ ID NO:148). In some embodiments, L_1 comprises the sequence GQPKAAP (SEQ ID NO: 175), L_2 comprises the sequence TKGPS (SEQ ID NO:106), L_3 comprises the sequence S, and L_4 comprises the sequence RT; L_1 comprises the sequence GGGGSGGGGS (SEQ ID NO:104), L_2 comprises the sequence GGGGSGGGGS (SEQ ID NO:104), L_3 is 0 amino acids in length, and L_4 is 0 amino acids in length; L_1 comprises the sequence GGSGSSGSGG (SEQ ID NO:148), L_2 comprises the sequence GGSGSSGSGG (SEQ ID NO:148), L_3 is 0 amino acids in length, and L_4 is 0 amino acids in length; or L_1 comprises the sequence GGGGSGGGGSGGGGS (SEQ ID NO:105), L_2 is 0 amino acids in

length, L_3 comprises the sequence GGGGSGGGGSGGGGS (SEQ ID NO:105), and L_4 is 0 amino acids in length.

[0025] In some embodiments of any of the binding proteins described herein, the second polypeptide chain further comprises a first Fc region linked to C_{H1}, the first Fc region comprising an immunoglobulin hinge region and C_{H2} and C_{H3} immunoglobulin heavy chain constant domains, wherein the first Fc region comprises amino acid substitutions at positions corresponding to positions 354 and 366 of human IgG1 or IgG4 according to EU Index, wherein the amino acid substitutions are S354C and T366W; and wherein the third polypeptide chain further comprises a second Fc region linked to C_{H1}, the second Fc region comprising an immunoglobulin hinge region and C_{H2} and C_{H3} immunoglobulin heavy chain constant domains, wherein the second Fc region comprises amino acid substitutions at positions corresponding to positions 349, 366, 368, and 407 of human IgG1 or IgG4 according to EU Index, wherein the amino acid substitutions are Y349C, T366S, L368A, and Y407V. In some embodiments, the second polypeptide chain further comprises a first Fc region linked to C_{H1}, the first Fc region comprising an immunoglobulin hinge region and C_{H2} and C_{H3} immunoglobulin heavy chain constant domains, wherein the first Fc region comprises amino acid substitutions at positions corresponding to positions 349, 366, 368, and 407 of human IgG1 or IgG4 according to EU Index, wherein the amino acid substitutions are Y349C, T366S, L368A, and Y407V; and wherein the third polypeptide chain further comprises a second Fc region linked to C_{H1}, the second Fc region comprising an immunoglobulin hinge region and C_{H2} and C_{H3} immunoglobulin heavy chain constant domains, wherein the second Fc region comprises amino acid substitutions at positions corresponding to positions 354 and 366 of human IgG1 or IgG4 according to EU Index, wherein the amino acid substitutions are S354C and T366W. In some embodiments, the second polypeptide chain further comprises a first Fc region linked to C_{H1}, the first Fc region comprising an immunoglobulin hinge region and C_{H2} and C_{H3} immunoglobulin heavy chain constant domains, and wherein the third polypeptide chain further comprises a second Fc region linked to C_{H1}, the second Fc region comprising an immunoglobulin hinge region and C_{H2} and C_{H3} immunoglobulin heavy chain constant domains; wherein the first and/or second Fc regions comprise amino acid substitutions at positions corresponding to positions 428 and 434 of human IgG1 or IgG4 according to EU Index, wherein the amino acid substitutions are M428L and N434S. In some embodiments, the C_{H3} domain of the second polypeptide chain comprises amino acid substitutions at positions corresponding to positions 354 and 366 of human IgG1 or IgG4

according to EU Index, wherein the amino acid substitutions are S354C and T366W; and wherein the C_{H3} domain of the third polypeptide chain comprises amino acid substitutions at positions corresponding to positions 349, 366, 368, and 407 of human IgG1 or IgG4 according to EU Index, wherein the amino acid substitutions are Y349C, T366S, L368A, and Y407V. In some embodiments, the C_{H3} domain of the second polypeptide chain comprises amino acid substitutions at positions corresponding to positions 349, 366, 368, and 407 of human IgG1 or IgG4 according to EU Index, wherein the amino acid substitutions are Y349C, T366S, L368A, and Y407V; and wherein the C_{H3} domain of the third polypeptide chain comprises amino acid substitutions at positions corresponding to positions 354 and 366 of human IgG1 or IgG4 according to EU Index, wherein the amino acid substitutions are S354C and T366W. In some embodiments, the C_{H3} domains of the second and the third polypeptide chains both comprise amino acid substitutions at positions corresponding to positions 428 and 434 of human IgG1 or IgG4 according to EU Index, wherein the amino acid substitutions are M428L and N434S. In some embodiments, the second polypeptide chain further comprises a first Fc region linked to C_{H1}, the first Fc region comprising an immunoglobulin hinge region and C_{H2} and C_{H3} immunoglobulin heavy chain constant domains; wherein the third polypeptide chain further comprises a second Fc region linked to C_{H1}, the second Fc region comprising an immunoglobulin hinge region and C_{H2} and C_{H3} immunoglobulin heavy chain constant domains; and wherein only one of the first and the second Fc regions comprises amino acid substitutions at positions corresponding to positions 435 and 436 of human IgG1 or IgG4 according to EU Index, wherein the amino acid substitutions are H435R and Y436F. In some embodiments, the C_{H3} domains of the second and the third polypeptide chains are human IgG1 C_{H3} domains, and wherein only one of the C_{H3} domains comprises amino acid substitutions at positions corresponding to positions 435 and 436 of human IgG1 or IgG4 according to EU Index, wherein the amino acid substitutions are H435R and Y436F. In some embodiments, the second polypeptide chain further comprises a first Fc region linked to C_{H1}, the first Fc region comprising an immunoglobulin hinge region and C_{H2} and C_{H3} immunoglobulin heavy chain constant domains; wherein the third polypeptide chain further comprises a second Fc region linked to C_{H1}, the second Fc region comprising an immunoglobulin hinge region and C_{H2} and C_{H3} immunoglobulin heavy chain constant domains; wherein the first and/or second Fc regions are human IgG4 Fc regions; and wherein the first and the second Fc regions each comprise amino acid substitutions at positions corresponding to positions 228 and 409 of human IgG4 according to EU Index, wherein the amino acid substitutions

are S228P and R409K. In some embodiments, the C_{H3} domains of the second and the third polypeptide chains are human IgG4 C_{H3} domains, and wherein the C_{H3} domains each comprise amino acid substitutions at positions corresponding to positions 228 and 409 of human IgG4 according to EU Index, wherein the amino acid substitutions are S228P and R409K. In some embodiments, the second polypeptide chain further comprises a first Fc region linked to C_{H1}, the first Fc region comprising an immunoglobulin hinge region and C_{H2} and C_{H3} immunoglobulin heavy chain constant domains; wherein the third polypeptide chain further comprises a second Fc region linked to C_{H1}, the second Fc region comprising an immunoglobulin hinge region and C_{H2} and C_{H3} immunoglobulin heavy chain constant domains; wherein the first and/or second Fc regions are human IgG4 Fc regions; and wherein the first and the second Fc regions each comprise amino acid substitutions at positions corresponding to positions 234 and 235 of human IgG4 according to EU Index, wherein the amino acid substitutions are F234A and L235A. In some embodiments, the C_{H3} domains of the second and the third polypeptide chains are human IgG4 C_{H3} domains, and wherein the C_{H3} domains each comprise amino acid substitutions at positions corresponding to positions 234 and 235 of human IgG4 according to EU Index, wherein the amino acid substitutions are F234A and L235A. In some embodiments, the second polypeptide chain further comprises a first Fc region linked to C_{H1}, the first Fc region comprising an immunoglobulin hinge region and C_{H2} and C_{H3} immunoglobulin heavy chain constant domains; wherein the third polypeptide chain further comprises a second Fc region linked to C_{H1}, the second Fc region comprising an immunoglobulin hinge region and C_{H2} and C_{H3} immunoglobulin heavy chain constant domains; wherein the first and/or second Fc regions are human IgG1 Fc regions; and wherein the first and the second Fc regions each comprise amino acid substitutions at positions corresponding to positions 234 and 235 of human IgG1 according to EU Index, wherein the amino acid substitutions are L234A and L235A. In some embodiments, the C_{H3} domains of the second and the third polypeptide chains are human IgG1 C_{H3} domains, and wherein the C_{H3} domains each comprise amino acid substitutions at positions corresponding to positions 234 and 235 of human IgG1 according to EU Index, wherein the amino acid substitutions are L234A and L235A. In some embodiments, the first and/or second Fc regions are human IgG1 Fc regions. In some embodiments, the first and/or second Fc regions are human IgG4 Fc regions.

[0026] In some embodiments of any of the binding proteins described herein, the C_L domain of the first polypeptide chain is a human kappa C_L domain, and the C_L domain of the fourth polypeptide chain is a human lambda C_L domain; or the C_L domain of the first

polypeptide chain is a human lambda C_L domain, and the C_L domain of the fourth polypeptide chain is a human kappa C_L domain. In some embodiments, the first polypeptide chain comprises a lambda C_L domain; wherein the C_{H3} domain of the second polypeptide chain comprises amino acid substitutions at positions corresponding to positions 354 and 366 of human IgG1 or IgG4 according to EU Index, wherein the amino acid substitutions are S354C and T366W; wherein the C_{H3} domain of the third polypeptide chain comprises amino acid substitutions at positions corresponding to positions 349, 366, 368, 407, 435, and 436 of human IgG1 or IgG4 according to EU Index, wherein the amino acid substitutions are Y349C, T366S, L368A, Y407V, H435R, and Y436F; and wherein the fourth polypeptide chain comprises a kappa C_L domain. In some embodiments, the first polypeptide chain comprises a lambda C_L domain; wherein the C_{H3} domain of the third polypeptide chain comprises amino acid substitutions at positions corresponding to positions 354 and 366 of human IgG1 or IgG4 according to EU Index, wherein the amino acid substitutions are S354C and T366W; wherein the C_{H3} domain of the second polypeptide chain comprises amino acid substitutions at positions corresponding to positions 349, 366, 368, 407, 435, and 436 of human IgG1 or IgG4 according to EU Index, wherein the amino acid substitutions are Y349C, T366S, L368A, Y407V, H435R, and Y436F; and wherein the fourth polypeptide chain comprises a kappa C_L domain. In some embodiments, the first polypeptide chain comprises a lambda C_L domain; wherein the C_{H3} domain of the second polypeptide chain comprises amino acid substitutions at positions corresponding to positions 354, 366, 435, and 436 of human IgG1 or IgG4 according to EU Index, wherein the amino acid substitutions are S354C, T366W, H435R, and Y436F; wherein the C_{H3} domain of the third polypeptide chain comprises amino acid substitutions at positions corresponding to positions 349, 366, 368, and 407 of human IgG1 or IgG4 according to EU Index, wherein the amino acid substitutions are Y349C, T366S, L368A, and Y407V; and wherein the fourth polypeptide chain comprises a kappa C_L domain. In some embodiments, the first polypeptide chain comprises a kappa C_L domain; wherein the C_{H3} domain of the second polypeptide chain comprises amino acid substitutions at positions corresponding to positions 354 and 366 of human IgG1 or IgG4 according to EU Index, wherein the amino acid substitutions are S354C and T366W; wherein the C_{H3} domain of the third polypeptide chain comprises amino acid substitutions at positions corresponding to positions 349, 366, 368, 407, 435, and 436 of human IgG1 or IgG4 according to EU Index, wherein the amino acid substitutions are Y349C, T366S, L368A, Y407V, H435R, and Y436F; and wherein the fourth polypeptide chain comprises a lambda C_L domain. In some

embodiments, second and/or third polypeptide chain comprise a human IgG1 or IgG4 Fc region.

[0027] In another embodiment, the disclosure provides a binding protein comprising a first polypeptide chain, a second polypeptide chain, a third polypeptide chain and a fourth polypeptide chain wherein:

(a) the first polypeptide chain comprises the amino acid sequence of SEQ ID NO: 4 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 4; the second polypeptide chain comprises the amino acid sequence of SEQ ID NO: 3 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 3; the third polypeptide chain comprises the amino acid sequence of SEQ ID NO: 1 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 1; and the fourth polypeptide chain comprises the amino acid sequence of SEQ ID NO: 2 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 2;

(b) the first polypeptide chain comprises the amino acid sequence of SEQ ID NO: 10 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 10; the second polypeptide chain comprises the amino acid sequence of SEQ ID NO: 9 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 9; the third polypeptide chain comprises the amino acid sequence of SEQ ID NO: 1 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 1; and the fourth polypeptide chain comprises the amino acid sequence of SEQ ID NO: 2 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 2;

(c) the first polypeptide chain comprises the amino acid sequence of SEQ ID NO: 4 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 4; the second polypeptide chain comprises the amino acid sequence of SEQ ID NO: 3 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 3; the third polypeptide chain comprises the amino acid sequence of SEQ ID NO: 13 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 13; and the fourth polypeptide chain comprises the amino acid sequence of SEQ ID NO: 14 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 14;

(d) the first polypeptide chain comprises the amino acid sequence of SEQ ID NO: 10 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ

ID NO: 10; the second polypeptide chain comprises the amino acid sequence of SEQ ID NO: 9 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 9; the third polypeptide chain comprises the amino acid sequence of SEQ ID NO: 13 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 13; and the fourth polypeptide chain comprises the amino acid sequence of SEQ ID NO: 14 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 14;

(e) the first polypeptide chain comprises the amino acid sequence of SEQ ID NO: 4 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 4; the second polypeptide chain comprises the amino acid sequence of SEQ ID NO: 3 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 3; the third polypeptide chain comprises the amino acid sequence of SEQ ID NO: 17 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 17; and the fourth polypeptide chain comprises the amino acid sequence of SEQ ID NO: 18 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 18

(f) the first polypeptide chain comprises the amino acid sequence of SEQ ID NO: 10 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 10; the second polypeptide chain comprises the amino acid sequence of SEQ ID NO: 9 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 9; the third polypeptide chain comprises the amino acid sequence of SEQ ID NO: 17 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 17; and the fourth polypeptide chain comprises the amino acid sequence of SEQ ID NO: 18 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 18;

(g) the first polypeptide chain comprises the amino acid sequence of SEQ ID NO: 4 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 4; the second polypeptide chain comprises the amino acid sequence of SEQ ID NO: 3 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 3; the third polypeptide chain comprises the amino acid sequence of SEQ ID NO: 21 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 21; and the fourth polypeptide chain comprises the amino acid sequence of SEQ ID NO: 22 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 22;

(h) the first polypeptide chain comprises the amino acid sequence of SEQ ID NO: 10 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 10; the second polypeptide chain comprises the amino acid sequence of SEQ ID NO: 9 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 9; the third polypeptide chain comprises the amino acid sequence of SEQ ID NO: 21 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 21; and the fourth polypeptide chain comprises the amino acid sequence of SEQ ID NO: 22 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 22;

(i) the first polypeptide chain comprises the amino acid sequence of SEQ ID NO: 63 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 63; the second polypeptide chain comprises the amino acid sequence of SEQ ID NO: 62 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 62; the third polypeptide chain comprises the amino acid sequence of SEQ ID NO: 60 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 60; and the fourth polypeptide chain comprises the amino acid sequence of SEQ ID NO: 61 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 61;

(j) the first polypeptide chain comprises the amino acid sequence of SEQ ID NO: 69 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 69; the second polypeptide chain comprises the amino acid sequence of SEQ ID NO: 68 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 68; the third polypeptide chain comprises the amino acid sequence of SEQ ID NO: 60 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 60; and the fourth polypeptide chain comprises the amino acid sequence of SEQ ID NO: 61 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 61;

(k) the first polypeptide chain comprises the amino acid sequence of SEQ ID NO: 69 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 69; the second polypeptide chain comprises the amino acid sequence of SEQ ID NO: 68 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 68; the third polypeptide chain comprises the amino acid sequence of SEQ ID NO: 60 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 60; and the fourth polypeptide chain comprises the amino acid sequence of SEQ

ID NO: 71 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 71;

(l) the first polypeptide chain comprises the amino acid sequence of SEQ ID NO: 76 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 76; the second polypeptide chain comprises the amino acid sequence of SEQ ID NO: 75 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 75; the third polypeptide chain comprises the amino acid sequence of SEQ ID NO: 73 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 73; and the fourth polypeptide chain comprises the amino acid sequence of SEQ ID NO: 74 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 74;

(m) the first polypeptide chain comprises the amino acid sequence of SEQ ID NO: 82 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 82; the second polypeptide chain comprises the amino acid sequence of SEQ ID NO: 81 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO:81; the third polypeptide chain comprises the amino acid sequence of SEQ ID NO: 73 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 73; and the fourth polypeptide chain comprises the amino acid sequence of SEQ ID NO: 74 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 74;

(n) the first polypeptide chain comprises the amino acid sequence of SEQ ID NO: 88 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO:88; the second polypeptide chain comprises the amino acid sequence of SEQ ID NO: 87 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 87; the third polypeptide chain comprises the amino acid sequence of SEQ ID NO: 85 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 85; and the fourth polypeptide chain comprises the amino acid sequence of SEQ ID NO: 86 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 86;

(o) the first polypeptide chain comprises the amino acid sequence of SEQ ID NO: 94 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 94; the second polypeptide chain comprises the amino acid sequence of SEQ ID NO: 93 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 93; the third polypeptide chain comprises the amino acid sequence of SEQ ID

NO: 85 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 85; and the fourth polypeptide chain comprises the amino acid sequence of SEQ ID NO: 86 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 86;

(p) the first polypeptide chain comprises the amino acid sequence of SEQ ID NO: 69 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 69; the second polypeptide chain comprises the amino acid sequence of SEQ ID NO: 68 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 68; the third polypeptide chain comprises the amino acid sequence of SEQ ID NO: 73 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 73; and the fourth polypeptide chain comprises the amino acid sequence of SEQ ID NO: 74 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 74;

(q) the first polypeptide chain comprises the amino acid sequence of SEQ ID NO: 69 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 69; the second polypeptide chain comprises the amino acid sequence of SEQ ID NO: 68 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 68; the third polypeptide chain comprises the amino acid sequence of SEQ ID NO: 85 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 85; and the fourth polypeptide chain comprises the amino acid sequence of SEQ ID NO: 86 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 86;

(r) the first polypeptide chain comprises the amino acid sequence of SEQ ID NO: 63 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 63; the second polypeptide chain comprises the amino acid sequence of SEQ ID NO: 62 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 62; the third polypeptide chain comprises the amino acid sequence of SEQ ID NO: 73 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 73; and the fourth polypeptide chain comprises the amino acid sequence of SEQ ID NO: 74 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 74;

(s) the first polypeptide chain comprises the amino acid sequence of SEQ ID NO: 63 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 63; the second polypeptide chain comprises the amino acid sequence of SEQ ID NO:

62 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 62; the third polypeptide chain comprises the amino acid sequence of SEQ ID NO: 85 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 85; and the fourth polypeptide chain comprises the amino acid sequence of SEQ ID NO: 86 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 86;

(t) the first polypeptide chain comprises the amino acid sequence of SEQ ID NO: 4 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 4; the second polypeptide chain comprises the amino acid sequence of SEQ ID NO: 3 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 3; the third polypeptide chain comprises the amino acid sequence of SEQ ID NO: 114 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 114; and the fourth polypeptide chain comprises the amino acid sequence of SEQ ID NO: 115 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 115; or

(u) the first polypeptide chain comprises the amino acid sequence of SEQ ID NO: 10 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 10; the second polypeptide chain comprises the amino acid sequence of SEQ ID NO: 9 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 9; the third polypeptide chain comprises the amino acid sequence of SEQ ID NO: 114 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 114; and the fourth polypeptide chain comprises the amino acid sequence of SEQ ID NO: 115 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 115.

[0028] In another embodiment, the disclosure provides an isolated nucleic acid molecule comprising a nucleotide sequence encoding the binding protein or polypeptide thereof according to any of the above embodiments. In another embodiment, the disclosure provides an expression vector comprising the nucleic acid molecule according to one of the above embodiments. In another embodiment, the disclosure provides an isolated host cell comprising the nucleic acid molecule according to any of the above embodiments. In another embodiment, the disclosure provides an isolated host cell comprising the expression vector according to any of the above embodiments. In some embodiments, the isolated host cell is a mammalian cell or an insect cell. In one embodiment, the disclosure provides a vector system comprising one or more vectors encoding a first, second, third, and fourth

polypeptide chain of a binding protein according to any of the above embodiments. In some embodiments, the vector system comprises a first vector encoding the first polypeptide chain of the binding protein, a second vector encoding the second polypeptide chain of the binding protein, a third vector encoding the third polypeptide chain of the binding protein, and a fourth vector encoding the fourth polypeptide chain of the binding protein. In some embodiments, the vector system comprises a first vector encoding the first and second polypeptide chains of the binding protein, and a second vector encoding the third and fourth polypeptide chains of the binding protein. In some embodiments, the one or more vectors are expression vectors. In one embodiment, the disclosure provides an isolated host cell comprising the vector system according to any of the above embodiments. In one embodiment, the disclosure provides a method of producing a binding protein, the method comprising: a) culturing a host cell according to any of the above embodiments under conditions such that the host cell expresses the binding protein; and b) isolating the binding protein from the host cell. In one embodiment, the disclosure provides a pharmaceutical composition comprising the binding protein according to any of the above embodiments and a pharmaceutically acceptable carrier.

[0029] In another embodiment, the disclosure provides a method of preventing and/or treating cancer in a patient comprising administering to the patient a therapeutically effective amount of at least one binding protein or pharmaceutical composition according to any of the above embodiments. In another embodiment, the disclosure provides a binding protein or pharmaceutical composition according to any of the above embodiments for use in preventing and/or treating cancer in a patient. In another embodiment, the disclosure provides a binding protein according to any of the above embodiments for the manufacture of a medicament for preventing and/or treating cancer in a patient. In some embodiments, the binding protein comprises one antigen binding site that specifically binds a T-cell surface protein and another antigen binding site that specifically binds a tumor target protein. In some embodiments, the binding protein comprises an antigen binding site that specifically binds CD3, an antigen binding site that specifically binds CD28, and an antigen binding site that specifically binds a tumor target protein selected from the group consisting of CD19, CD20, CD38, Her2, and LAMP1. In some embodiments, the at least one binding protein is co-administered with a chemotherapeutic agent. In some embodiments, the patient is a human. In some embodiments, the binding protein is capable of inhibiting the function of one or more target proteins selected from the group consisting of A2AR, APRIL, ATPDase, BAFF, BAFFR, BCMA, BlyS, BTK, BTLA, B7DC, B7H1, B7H4,

B7H5, B7H6, B7H7, B7RP1, B7-4, C3, C5, CCL2, CCL3, CCL4, CCL5, CCL7, CCL8, CCL11, CCL15, CCL17, CCL19, CCL20, CCL21, CCL24, CCL25, CCL26, CCR3, CCR4, CD3, CD19, CD20, CD23, CD24, CD27, CD28, CD38, CD39, CD40, CD70, CD80, CD86, CD122, CD137, CD137L, CD152, CD154, CD160, CD272, CD273, CD274, CD275, CD276, CD278, CD279, CDH1, chitinase, CLEC9, CLEC91, CRTH2, CSF-1, CSF-2, CSF-3, CX3CL1, CXCL12, CXCL13, CXCR3, DNGR-1, ectonucleoside triphosphate diphosphohydrolase 1, EGFR, ENTPD1, FCER1A, FCER1, FLAP, FOLH1, Gi24, GITR, GITRL, GM-CSF, Her2, HHLA2, HMGB1, HVEM, ICOSLG, IDO, IFNα, IgE, IGF1R, IL2Rbeta, IL1, IL1A, IL1B, IL1F10, IL2, IL4, IL4Ra, IL5, IL5R, IL6, IL7, IL7Ra, IL8, IL9, IL9R, IL10, rhIL10, IL12, IL13, IL13Ra1, IL13Ra2, IL15, IL17, IL17Rb, IL18, IL22, IL23, IL25, IL27, IL33, IL35, ITGB4, ITK, KIR, LAG3, LAMP1, leptin, LPFS2, MHC class II, NCR3LG1, NKG2D, NTPDase-1, OX40, OX40L, PD-1H, platelet receptor, PROM1, S152, SISP1, SLC, SPG64, ST2, STEAP2, Syk kinase, TACI, TDO, T14, TIGIT, TIM3, TLR, TLR2, TLR4, TLR5, TLR9, TMEF1, TNFa, TNFRSF7, Tp55, TREM1, TSLP, TSLPR, TWEAK, VEGF, VISTA, Vstm3, WUCAM, and XCR1.

[0030] In another embodiment, the disclosure provides a method of preventing and/or treating an inflammatory disease or disorder in a patient comprising administering to the patient a therapeutically effective amount of at least one binding protein or pharmaceutical composition according to any of the above embodiments. In another embodiment, the disclosure provides a binding protein or pharmaceutical composition according to any of the above embodiments for use in preventing and/or treating an inflammatory disease or disorder in a patient. In another embodiment, the disclosure provides a binding protein according to any of the above embodiments for the manufacture of a medicament for preventing and/or treating an inflammatory disease or disorder in a patient. In some embodiments, the binding protein comprises three antigen binding sites that each specifically bind a cytokine target protein selected from the group consisting of IL-4, IL-13 and TNFa. In some embodiments, two of the three binding sites specifically bind a cytokine target protein selected from the group consisting of IL-4, IL-13 and TNFa. In some embodiments, the at least one binding protein is co-administered with an anti-inflammatory agent. In some embodiments, the patient is a human. In some embodiments, the binding protein is capable of inhibiting the function of one or more target proteins selected from the group consisting of A2AR, APRIL, ATPDase, BAFF, BAFFR, BCMA, BlyS, BTK, BTLA, B7DC, B7H1, B7H4, B7H5, B7H6, B7H7, B7RP1, B7-4, C3, C5, CCL2, CCL3, CCL4, CCL5, CCL7, CCL8, CCL11, CCL15, CCL17, CCL19, CCL20,

CCL21, CCL24, CCL25, CCL26, CCR3, CCR4, CD3, CD19, CD20, CD23, CD24, CD27, CD28, CD38, CD39, CD40, CD70, CD80, CD86, CD122, CD137, CD137L, CD152, CD154, CD160, CD272, CD273, CD274, CD275, CD276, CD278, CD279, CDH1, chitinase, CLEC9, CLEC91, CRTH2, CSF-1, CSF-2, CSF-3, CX3CL1, CXCL12, CXCL13, CXCR3, DNGR-1, ectonucleoside triphosphate diphosphohydrolase 1, EGFR, ENTPD1, FCER1A, FCER1, FLAP, FOLH1, Gi24, GITR, GITRL, GM-CSF, Her2, HHLA2, HMGB1, HVEM, ICOSLG, IDO, IFNα, IgE, IGF1R, IL2Rbeta, IL1, IL1A, IL1B, IL1F10, IL2, IL4, IL4Ra, IL5, IL5R, IL6, IL7, IL7Ra, IL8, IL9, IL9R, IL10, rhIL10, IL12, IL13, IL13Ra1, IL13Ra2, IL15, IL17, IL17Rb, IL18, IL22, IL23, IL25, IL27, IL33, IL35, ITGB4, ITK, KIR, LAG3, LAMP1, leptin, LPFS2, MHC class II, NCR3LG1, NKG2D, NTPDase-1, OX40, OX40L, PD-1H, platelet receptor, PROM1, S152, SISP1, SLC, SPG64, ST2, STEAP2, Syk kinase, TACI, TDO, T14, TIGIT, TIM3, TLR, TLR2, TLR4, TLR5, TLR9, TMEF1, TNFa, TNFRSF7, Tp55, TREM1, TSLP, TSLPR, TWEAK, VEGF, VISTA, Vstm3, WUCAM, and XCR1.

[0031] In another embodiment, the disclosure provides a method of purifying a binding protein produced by a host cell, comprising:

(a) producing in a host cell a binding protein comprising four polypeptide chains that form three antigen binding sites that specifically bind one or more antigen targets or target proteins, wherein a first polypeptide chain comprises a structure represented by the formula:

$$V_{L2}\text{-}L_1\text{-}V_{L1}\text{-}L_2\text{-}C_L \qquad [I]$$

and a second polypeptide chain comprises a structure represented by the formula:

$$V_{H1}\text{-}L_3\text{-}V_{H2}\text{-}L_4\text{-}C_{H1}\text{-hinge-}C_{H2}\text{-}C_{H3} \qquad [II]$$

and a third polypeptide chain comprises a structure represented by the formula:

$$V_{H3}\text{-}C_{H1} \qquad [III]$$

and a fourth polypeptide chain comprises a structure represented by the formula:

$$V_{L3}\text{-}C_L \qquad [IV]$$

V_{L1} is a first immunoglobulin light chain variable domain;

V_{L2} is a second immunoglobulin light chain variable domain;

V_{L3} is a third immunoglobulin light chain variable domain;

V_{H1} is a first immunoglobulin heavy chain variable domain;

V_{H2} is a second immunoglobulin heavy chain variable domain;

V_{H3} is a third immunoglobulin heavy chain variable domain;

C_L is an immunoglobulin light chain constant domain;

C_{H1} is an immunoglobulin C_{H1} heavy chain constant domain;

C_{H2} is an immunoglobulin C_{H2} heavy chain constant domain;

C_{H3} is an immunoglobulin C_{H3} heavy chain constant domain;

hinge is an immunoglobulin hinge region connecting the C_{H1} and C_{H2} domains; and

L_1, L_2, L_3 and L_4 are amino acid linkers;

wherein the polypeptide of formula I and the polypeptide of formula II form a cross-over light chain-heavy chain pair; wherein only one of the C_{H3} domain of the second polypeptide chain and the C_{H3} domain of the third polypeptide chain comprises amino acid substitutions at positions corresponding to positions 435 and 436 of human IgG1 or IgG4 according to EU Index, wherein the amino acid substitutions are H435R and Y436F;

(b) contacting the binding protein produced in (a) with Protein A; and

(c) eluting the binding protein from Protein A under conditions suitable for isolating the binding protein away from binding proteins comprising either 0 or 2 C_{H3} domains comprising the amino acid substitutions are H435R and Y436F.

[0032] In some embodiments, the C_L domain of the first polypeptide chain is a human kappa C_L domain, and the C_L domain of the fourth polypeptide chain is a human lambda C_L domain; or the C_L domain of the first polypeptide chain is a human lambda C_L domain, and the C_L domain of the fourth polypeptide chain is a human kappa C_L domain, and the method further comprises: (d) contacting the binding protein eluted in (c) with a kappa light chain affinity medium; and (e) eluting the binding protein from the kappa light chain affinity medium under conditions suitable for isolating the binding protein away from binding proteins comprising only lambda C_L domains. In some embodiments, the method further comprises, after (e), (f) contacting the binding protein eluted in (e) with a lambda light chain affinity medium; and (g) eluting the binding protein from the lambda light chain affinity medium under conditions suitable for isolating the binding protein away from binding proteins comprising only kappa C_L domains. In some embodiments, the C_L domain of the first polypeptide chain is a human kappa C_L domain, and the C_L domain of the fourth polypeptide chain is a human lambda C_L domain, and the C_L domain of the fourth polypeptide chain is a human kappa C_L domain, and the method further comprises: (d) contacting the binding protein eluted in (c) with a lambda light chain affinity medium; and (e) eluting the binding protein from the lambda light chain affinity medium under conditions suitable for isolating the binding protein away from binding proteins comprising only kappa C_L domains. In some embodiments, the method further comprises, after (e), (f) contacting the binding

protein eluted in (e) with a kappa light chain affinity medium; and (g) eluting the binding protein from the kappa light chain affinity medium under conditions suitable for isolating the binding protein away from binding proteins comprising only lambda C_L domains. In some embodiments, the first polypeptide chain comprises a lambda C_L domain; wherein the C_{H3} domain of the second polypeptide chain comprises amino acid substitutions at positions corresponding to positions 354 and 366 of human IgG1 according to EU Index, wherein the amino acid substitutions are S354C and T366W; wherein the C_{H3} domain of the third polypeptide chain comprises amino acid substitutions at positions corresponding to positions 349, 366, 368, 407, 435, and 436 of human IgG1 according to EU Index, wherein the amino acid substitutions are Y349C, T366S, L368A, Y407V, H435R, and Y436F; and wherein the fourth polypeptide chain comprises a kappa C_L domain. In some embodiments, the binding protein is detected in one or more of (c) and (e) using hydrophobic interaction chromatography (HIC). In some embodiments, the C_{H3} domains and/or Fc regions of the second and the third polypeptide chains are human IgG1 or IgG4 C_{H3} domains and/or Fc regions.

[0033] It is to be understood that one, some, or all of the properties of the various embodiments described herein may be combined to form other embodiments of the present invention. These and other aspects of the invention will become apparent to one of skill in the art. These and other embodiments of the invention are further described by the detailed description that follows.

BRIEF DESCRIPTION OF THE DRAWINGS

[0034] **FIGS. 1A-1C** show schematic representations of trispecific binding proteins comprising four polypeptide chains that form three antigen binding sites that specifically binds three target proteins, wherein a first pair of polypeptides possess dual variable domains having a cross-over orientation (VH1-VH2 and VL2-VL1) forming two antigen binding sites, and wherein a second pair of polypeptides possess a single variable domain (VH3 and VL3) forming a single antigen binding site. **FIG. 1A** shows a trispecific binding protein comprising a "knobs-into-holes" mutation, where the knob is on the second pair of polypeptides with a single variable domain. **FIG. 1B** shows a trispecific binding protein comprising a "knobs-into-holes" mutation, where the knob is on the first pair of polypeptides having the cross-over orientation. **FIG. 1C** shows the orientation of variable domains on the polypeptide chains, and the knob/hole orientation for the binding proteins shown in Tables 1-3. "Heavy chain A" (*e.g.*, a third polypeptide chain of the present

disclosure) indicates the variable domain of heavy chain A. "Light chain A" (*e.g.*, a fourth polypeptide chain of the present disclosure) indicates the variable domain of light chain A. "Heavy chain B" (*e.g.*, a second polypeptide chain of the present disclosure) indicates variable domain 1 and variable domain 2 of heavy chain B. "Light chain B" (*e.g.*, a first polypeptide chain of the present disclosure) indicates variable domain 1 and variable domain 2 of light chain B.

[0035] **FIG. 2** shows the results of an ELISA assay determining the binding of an anti-Her2 x CD28 x CD3 IgG4 trispecific antibody (Binding Protein 1), or isotype control antibody, to human CD3, CD28 and Her2. The bound antibodies were detected using a horseradish peroxidase (HRP)-conjugated anti-Fab secondary antibody.

[0036] **FIGS. 3A-3C** show the results of antibody-mediated specific killing of $Her2^+$ breast cancer cells using an anti-Her2 x CD28 x CD3 IgG4 trispecific antibody (referred to herein as "Binding protein 1"), an anti-CD28 x CD3 IgG4 bispecific antibody (huCD28 x CD3), an anti-Her2 IgG1 antibody, or a control antibody (human-IgG1), using human PBMC at the E:T=10. **FIG. 3A** shows the results of trispecific antibody-mediated specific killing of ZR-75-1 cells. **FIG. 3B** shows the results of trispecific antibody-mediated specific killing of AU565 cells. **FIG. 3C** shows the results of FACS analysis determining the cell surface expression of the indicated markers on ZR-75-1 and AU565 cells.

[0037] **FIGS. 4 & 5** show the results of antibody-mediated specific killing of $Her2^+$ breast cancer cells using an anti-Her2 x CD28 x CD3 IgG4 trispecific antibody (Binding protein 1), an anti-CD28 x CD3 IgG4 bispecific antibody (huCD28 x CD3), an anti-Her2 IgG1 antibody, or a control antibody (human-IgG1). **FIG. 4** shows the results of antibody-mediated specific killing of ZR-75-1 cells using human peripheral blood mononuclear cells (PBMCs) from donor KP45926 at E:T=10. **FIG. 5** shows the results of antibody-mediated specific killing of AU565 cells using human PBMCs from donor KP45944 at E:T=10. **FIGS. 4 & 5** confirm similar cell killing results as shown in **FIGS. 3A-3C** using PBMCs from a different donor.

[0038] **FIGS. 6A & 6B** show the activation ($CD69^+$) and proliferation of human T cells treated with anti-Her2 x CD28 x CD3 IgG4 trispecific binding protein ($HER2/CD28_{sup}xCD3_{mid}$; referred to herein as "Binding Protein 1"), anti-Her2 x CD28 x CD3 IgG4 trispecific binding protein lacking the anti-CD28 binding domain ($HER2/\Delta CD28_{sup}xCD3_{mid}$), anti-Her2 x CD28 x CD3 IgG4 trispecific binding protein lacking the anti-CD3 binding domain ($HER2/CD28_{sup}x\Delta CD3_{mid}$), anti-Her2 x CD28 x CD3 IgG4 trispecific binding protein lacking both anti-CD28 and anti-CD3 binding domains

(HER2/Δ($CD28_{sup}$x$\Delta CD3_{mid}$)), control anti-CD3 monoclonal antibody, or control IgG4 antibody. **FIG. 6A** shows the activation ($CD69^+$) of human $CD4^+$ T cells from three donors. **FIG. 6B** shows the activation ($CD69^+$) of human $CD8^+$ T cells from three donors. $CD28_{sup}$: anti-CD28 superagonist antibody. $CD3_{mid}$: anti-CD3 antibody.

[0039] **FIGS. 7A-C** show IL-2, NFκB, and nuclear factor of activated T-cells (NFAT) pathway activation via anti-CD3 and anti-CD28 signaling, as measured by luciferase assay using human Jurkat T cells with an IL-2 promoter-luciferase construct (**FIG. 7A**), an NFκB promoter-luciferase construct (**FIG. 7B**), or an NFAT promoter-luciferase construct (**FIG. 7C**). Antibodies tested were those described above in reference to **FIGS. 6A-6D**.

[0040] **FIG. 8** shows the results of an ELISA assay determining binding of an anti-CD19 x CD28 x CD3 IgG4 trispecific antibody (referred to herein as "Binding Protein 3"), or isotype control antibody, to CD3, CD28, and CD19. The bound antibodies were detected using a horseradish peroxidase (HRP)-conjugated anti-Fab secondary antibody.

[0041] **FIGS. 9A-9N** show the results of antibody-mediated specific killing of $CD19^+$ human GCB lymphoma cells using an anti-CD19 x CD28 x CD3 IgG4 trispecific antibody (referred to herein as "Binding Protein 3"), or the indicated controls, using human PBMC as effector cells at E:T=10. **FIG. 9A** shows the results of antibody-mediated specific killing of OCI-LY19 cells. **FIG. 9B** shows the results of FACS analysis determining the cell surface expression of the indicated markers on OCI-LY19 cells. **FIG. 9C** shows the results of antibody-mediated specific killing of OCI-LY19 cells using PBMCs from donor KP48572 at E:T=10. **FIG. 9D** shows the results of antibody-mediated specific killing of OCI-LY19 cells using PBMCs from donor KP48573 at E:T=10. **FIG. 9E** shows the results of antibody-mediated specific killing of human lymphoma KARPASS-422 cells using PBMCs from donor KP48572 at E:T=10. **FIG. 9F** shows the results of antibody-mediated specific killing of KARPASS-422 cells using PBMCs from donor KP48573 at E:T=10. **FIG. 9G** shows the results of antibody-mediated specific killing of human chronic B cell leukemia MeC1 cells using PBMCs from donor KP48572 at E:T=10. **FIG. 9H** shows the results of antibody-mediated specific killing of human multiple myeloma RPMI8226 cells using PBMCs from donor KP48775 at E:T=10. **FIG. 9I** shows the results of antibody-mediated specific killing of human Burkitt's lymphoma Raji cells using PBMCs from donor KP48572 at E:T=10. **FIG. 9J** shows the results of antibody-mediated specific killing of Human diffuse large B-cell lymphoma HBL1 cells using PBMCs from donor KP48775 at E:T=10. **FIG. 9K** shows the results of antibody-mediated specific killing of Large cell lymphoma SUDHL8 cells using PBMCs from donor KP48572 at E:T=10. **FIG.**

9L shows the results of antibody-mediated specific killing of SUDHL8 cells using PBMCs from donor KP48573 at E:T=10. **FIG. 9M** shows the results of antibody-mediated specific killing of human B cell lymphoma ARH77 cells using PBMCs from donor KP48775 at E:T=10. **FIG. 9N** shows the results of antibody-mediated specific killing of OCI-Ly3 cells using PBMCs from donor KP48775 at E:T=10.

[0042] **FIG. 10** shows the results of an ELISA assay determining binding of an anti-CD38 x CD28 x CD3 IgG4 trispecific antibody (Binding Protein 5), or isotype control antibody, to CD3, CD28 and CD38. The bound antibodies were detected using a horseradish peroxidase (HRP)-conjugated anti-Fab secondary antibody.

[0043] **FIGS. 11A-11D** show the results of antibody-mediated specific killing of $CD38^+$ human multiple myeloma cancer cells using an anti-CD38 x CD28 x CD3 IgG4 trispecific antibody (Binding protein 5), an anti-CD28 x CD3 IgG4 bispecific antibody (huCD28 x CD3), an anti-CD38 IgG1 antibody, or a control antibody (human-IgG1). **FIG. 11A** shows the results of antibody-mediated specific killing of MOLP-8 cells using human PBMC as effector cells at E:T=10. **FIG. 11B** shows the results of antibody-mediated specific killing of RPMI-8226 cells using human PBMC as effector cells at E:T=10. **FIG. 11C** shows the results of antibody-mediated specific killing of KMS-12-BM cells using human PBMC as effector cells at E:T=10. **FIG. 11D** shows the results of FACS analysis determining the cell surface expression of the indicated markers on MOLP-8, RPMI-8226, and KMS-12-BM cells.

[0044] **FIGS. 12A-12D** show the results of antibody-mediated specific killing of $CD38^+$ human multiple myeloma cancer cells using an anti-CD38 x CD28 x CD3 IgG4 trispecific antibody (Binding protein 5), an anti-CD28 x CD3 IgG4 bispecific antibody (huCD28 x CD3), an anti-CD38 IgG1 antibody, or a control antibody (human-IgG1), using human PBMC as effector cells at E:T=10 . **FIG. 12A** shows the results of antibody-mediated specific killing of NCI-H929 cells. **FIG. 12B** shows the results of antibody-mediated specific killing of MM.1S cells. **FIG. 12C** shows the results of antibody-mediated specific killing of MM.1R cells. **FIG. 12D** shows the results of FACS analysis determining the cell surface expression of the indicated markers on NCI-H929, MM.1S, and MM.1R cells.

[0045] **FIGS. 13A-13D** show the results of antibody-mediated specific killing of $CD38^+$ human multiple myeloma cancer cells using an anti-CD38 x CD28 x CD3 IgG4 trispecific antibody (Binding protein 5), an anti-CD28 x CD3 IgG4 bispecific antibody (huCD28 x CD3), an anti-CD38 IgG1 antibody, or a control antibody (human-IgG1), using

human PBMC as effector cells at E:T=10. **FIG. 13A** shows the results of antibody-mediated specific killing of OPM-2 cells. **FIG. 13B** shows the results of antibody-mediated specific killing of KMS-26 cells. **FIG. 13C** shows the results of antibody-mediated specific killing of U266 cells. **FIG. 13D** shows the results of FACS analysis determining the cell surface expression of the indicated markers on OPM-2, KMS-26, and U226 cells.

[0046] **FIGS. 14A-14C** show the results of antibody-mediated specific killing of $CD38^+$ human lymphoma cancer cells using an anti-CD38 x CD28 x CD3 IgG4 trispecific antibody (Binding protein 5), an anti-CD28 x CD3 IgG4 bispecific antibody (huCD28 x CD3), an anti-CD38 IgG1 antibody, or a control antibody (human-IgG1), using human PBMC as effector cells at E:T=10. **FIG. 14A** shows the results of antibody-mediated specific killing of SUDHL-8 cells. **FIG. 14B** shows the results of antibody-mediated specific killing of OCI-LY19 cells. **FIG. 14C** shows the results of FACS analysis determining the cell surface expression of the indicated markers on SUDHL-8 and OCI-LY19 cells.

[0047] **FIGS. 15A-15D** show the results of antibody-mediated specific killing of $CD38^+$ ALL cancer cells using an anti-CD38 x CD28 x CD3 IgG4 trispecific antibody (Binding protein 5), an anti-CD28 x CD3 IgG4 bispecific antibody (huCD28 x CD3), an anti-CD38 IgG1 antibody, or a control antibody (human-IgG1), using human PBMC as effector cells at E:T=10. **FIG. 15A** shows the results of antibody-mediated specific killing of KOPN-8 cells. **FIG. 15B** shows the results of antibody-mediated specific killing of HAL-1 cells. **FIG. 15C** shows the results of antibody-mediated specific killing of CCRF-SB cells. **FIG. 15D** shows the results of FACS analysis determining the cell surface expression of the indicated markers on KOPN-8, HAL-1, and CCRF-SB cells.

[0048] **FIG. 16** shows the results of antibody-mediated specific killing of $CD38^+$ myeloma cancer cells using anti-CD38 x CD28 x CD3 IgG4 trispecific antibodies (referred to herein as "Binding protein 5" and "Binding Protein 6," depending on the specific anti-CD28 binding domain used), an anti-CD28 x CD3 IgG4 bispecific antibody (huCD28 x CD3), an anti-CD38 IgG1 antibody, a control anti-CD38 IgG1 antibody, or a control antibody (human-IgG1), using PBMCs from donor PK45926 at E:T=10.

[0049] **FIGS. 17A & 17B** show IL-2, NFκB, and nuclear factor of activated T-cells (NFAT) pathway activation via anti-CD3 and anti-CD28 signaling, as measured by luciferase assay using human Jurkat T cells with an IL-2 promoter-luciferase construct (**FIG. 17A**) or an NFAT promoter-luciferase construct (**FIG. 17B**). Antibodies tested were anti-CD38 x CD28 x CD3 IgG4 trispecific IgG4 antibody (Binding Protein 5, labeled as

"Tri-Ab"), anti-CD38 x CD28 x CD3 IgG4 trispecific IgG4 antibody lacking the CD28 binding domain (labeled as "Tri-Ab (ΔCD28)), anti-CD38 x CD28 x CD3 IgG4 trispecific IgG4 antibody lacking the CD3 binding domain (labeled as "Tri-Ab (ΔCD3)), and anti-CD38 x CD28 x CD3 IgG4 trispecific IgG4 antibody lacking the CD3 and CD28 binding domains (labeled as "Tri-Ab (ΔCD28x ΔCD3)). Luciferase assays were performed in duplicate for each of the indicated Tri-Abs.

[0050] **FIGS. 18A-18E** show the results of a dose escalation toxicity study using the anti-Her2 x CD28 x CD3 IgG4 trispecific antibody (referred to herein as "Binding protein 1") in non-human primates (dose escalating from 0.1, 0.5, 2.5, 5, 10, to 100 μg/kg; animals labeled as "409" and "410"). **FIG. 18A** shows the results of circulating $CD4^+$ T cells percentage in each animal, 6 hours post administering the anti-Her2 x CD28 x CD3 trispecific antibody. **FIG. 18B** shows the results of circulating $CD8^+$ T cells percentage in each animal, 6 hours post administering the anti-Her2 x CD28 x CD3 trispecific antibody. **FIG. 18C** shows the results of the activation ($CD69^+$) of circulating $CD4^+$ T cells 6 hours post dosing. **FIG. 18D** shows the results of the activation ($CD69^+$) of circulating $CD8^+$ T cells 6 hours post dosing. **FIG. 18E** shows the inflammatory cytokine release observed 6 hours post administering the anti-Her2 x CD28 x CD3 trispecific antibody at each dosing.

[0051] **FIGS. 19A-B** show the *in vivo* anti-tumor activity of the anti-Her2 x CD28 x CD3 IgG4 trispecific antibody (referred to herein as "Binding protein 1") in the $CD34^+$ umbilical cord blood cell humanized NSG mouse model implanted with BT474 cells. **FIG. 19A** shows the change in body weight of mice treated with the indicated concentrations of the anti-Her2 x CD28 x CD3 trispecific binding protein or PBS control. **FIG. 19B** shows the change in tumor volume in mice treated with the indicated concentrations of the anti-Her2 x CD28 x CD3 trispecific binding protein or PBS control.

[0052] **FIGS. 20A-20H** show the *in vivo* anti-tumor activity of the anti-Her2 x CD28 x CD3 IgG4 trispecific antibody (referred to herein as "Binding protein 1") in the human PBMCs humanized NSG mouse model implanted with BT474 cells. **FIG. 20A** shows the effect of administering the indicated concentrations of the anti-Her2 x CD28 x CD3 trispecific binding protein, the indicated concentrations of Herceptin, or vehicle control, on the body weight of the mice. **FIG. 20B** shows the dose-dependent anti-tumor activity of the anti-Her2 x CD28 x CD3 trispecific binding protein, Herceptin or indicated controls, as in individual mice. **FIG. 20C** shows the average tumor volume in the mice after administration of the indicated concentrations of the anti-Her2 x CD28 x CD3 trispecific binding protein or PBS control. **FIG. 20D** shows the average tumor volume in the mice

after administration of the indicated concentrations of Herceptin or PBS control. **FIG. 20E** shows bar graphs of the average tumor volume at day 34 in the mice after administration of the indicated concentrations of the anti-Her2 x CD28 x CD3 trispecific binding protein, the indicated concentrations of Herceptin, or PBS control. **FIG. 20F** shows the average tumor weight at day 34 in the mice after administration of the indicated concentrations of the anti-Her2 x CD28 x CD3 trispecific binding protein, the indicated concentrations of Herceptin, or PBS control. **FIG. 20G** shows the human CD45+, CD3+, CD4+, CD8+ cells in the blood of the mice at the end of the study. **FIG. 20H** shows the human CD45+, CD3+, CD4+, CD8+ cells in the spleens of the mice at the end of the study.

[0053] **FIGS. 21A-F** show the results of a dose escalation toxicity study using the anti-CD38 x CD28 x CD3 IgG4 trispecific antibody (Binding protein 5) in non-human primates (dose escalating from 0.1, 0.5, 2.5, 5, 10, to 100 μg/kg). **FIG. 21A** shows T cell activation ($CD69^+$) (line graph) and proliferation (bar graph) of circulating $CD4^+$ T cells after administration of the anti-CD38 x CD28 x CD3 trispecific antibody. **FIG. 21B** shows T cell activation ($CD69^+$) (line graph) and proliferation (bar graph) of circulating $CD8^+$ T cells after administration of the anti-CD38 x CD28 x CD3 trispecific antibody. **FIG. 21C** shows IL6 release in animals receiving the anti-CD38 x CD28 x CD3 trispecific antibody 6 hours post each dosing by individual animal. **FIG. 21D** shows IL10 release in animals receiving the anti-CD38 x CD28 x CD3 trispecific antibody 6 hours post each dosing by individual animal. **FIG. 21E** shows TNFα release in animals receiving the anti-CD38 x CD28 x CD3 trispecific antibody. **FIG. 21F** shows IFNγ release in animals receiving the anti-CD38 x CD28 x CD3 trispecific antibody 6 hours post each dosing by individual animal.

[0054] **FIGS. 22A-22C** show the *in vivo* anti-tumor activity of the anti-CD38 x CD28 x CD3 IgG4 trispecific antibody (Binding protein 5) in the CD34+ umbilical cord blood cells humanized NSG mouse model implanted with RPMI-8226 multiple myeloma cells transduced with CD38 and PD-L1. As a pilot study, this experiment determined the working dose range for the Binding protein 5. **FIG. 22A** shows the *in vivo* tumor growth curve in groups of the indicated concentrations of the anti-CD38 x CD28 x CD3 trispecific binding protein or controls. **FIG. 22B** shows tumor infiltrating human $CD8^+$ T cells in mice administered the anti-CD38 x CD28 x CD3 trispecific binding protein or the indicated controls. **FIG. 22C** shows tumor infiltrating human $CD4^+$ T cells in mice administered the anti-CD38 x CD28 x CD3 trispecific binding protein or the indicated controls.

[0055] **FIGS. 23A-23D** show the *in vivo* activity of the anti-CD38 x CD28 x CD3 IgG4 trispecific antibody (Binding protein 5) in the CD34+ umbilical cord blood cells humanized

NSG mouse model implanted with RPMI-8226 cells transduced with CD38 and PD-L1.

FIG. 23A shows the change in body weight of mice treated with the indicated concentrations of the anti-CD38 x CD28 x CD3 trispecific binding protein or PBS control.

FIG. 23B shows the change in tumor volume in mice treated with the indicated concentrations of the anti-CD38 x CD28 x CD3 trispecific binding protein or PBS control.

FIG. 23C shows the tumor volumes in each group at Day 19. The tumor volumes in all treated groups showed marked reduction, which are statistically different form the PBS control group. **FIG. 23D** shows the serum concentration of inflammatory cytokines IFN-g, TNF, and IL-2 in mice four hours after the first dose of the indicated concentrations of the anti-CD38 x CD28 x CD3 trispecific binding protein or PBS control.

[0056] **FIGS. 24 & 25** show the *in vivo* activation of T cells in the CD34+ umbilical cord blood cells humanized NSG mouse model by administering an anti-CD38 x CD28 x CD3 IgG4 trispecific antibody (Binding protein 5; triangles), an anti-CD28 x CD3 IgG4 bispecific antibody (squares), or an anti-CD28 IgG4 antibody (circles) by determining the increase in the percentage of $CD69^+$ T cells. **FIG. 24** shows the *in vivo* activation of $CD4^+$ T cells. **FIG. 25** shows the *in vivo* activation of $CD8^+$ T cells.

[0057] **FIGS. 26A-26C** show the *in vivo* activation of T cells in the CD34+ umbilical cord blood cells humanized NSG mouse model by administering an anti-CD38 x CD28 x CD3 IgG4 trispecific antibody (Binding protein 5; triangles), an anti-CD28 x CD3 IgG4 bispecific antibody (squares), or an anti-CD28 IgG4 antibody (circles) by determining the serum levels of inflammatory cytokines. **FIG. 26A** shows the serum levels of IL-2. **FIG. 26B** shows the serum levels of TNF. **FIG. 26C** shows the serum levels of IFN-γ.

[0058] **FIGS. 27A & 27B** show the purification of the indicated proteins by size exclusion chromatography. **FIG. 27A** shows the purification of Binding Proteins 9-15 by size exclusion chromatography. **FIG. 27B** shows the purification of Binding Proteins 16-19 by size exclusion chromatography.

[0059] **FIG. 28A** depicts the trispecific binding protein used in experiments for optimizing a purification scheme and configuration of optional binding protein features (*e.g.*, kappa/lambda light chains, knob/hole mutations, and H435R/Y436F mutations).

[0060] **FIG. 28B** shows each of the configurations tested.

[0061] **FIG. 29** shows representative chromatograms from analytical hydrophobic interaction chromatography (HIC), demonstrating that trispecific binding proteins were distinguishable from mispaired species.

[0062] **FIGS. 30A & 30B** show the successful purification of a binding protein with lambda light chain for CODV arm, kappa light chain for Fab arm, knob mutations on CODV arm, hole mutations on Fab arm, and RF mutations on Fab arm by Protein A followed by KappaSelect (GE Healthcare) purification steps. Successful purification of binding protein from mispaired species was demonstrated by hydrophobic interaction chromatography (HIC; **FIG. 30A**) and SDS-PAGE (**FIG. 30B**).

DETAILED DESCRIPTION

[0063] The disclosure provides trispecific and/or trivalent binding proteins comprising four polypeptide chains that form three antigen binding sites that specifically bind to one or more target proteins, wherein a first pair of polypeptides forming the binding protein possess dual variable domains having a cross-over orientation and wherein a second pair of polypeptides forming the binding protein possess a single variable domain.

General Definitions

[0064] As utilized in accordance with the present disclosure, the following terms, unless otherwise indicated, shall be understood to have the following meanings. Unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular.

[0065] The term "polynucleotide" as used herein refers to single-stranded or double-stranded nucleic acid polymers of at least 10 nucleotides in length. In certain embodiments, the nucleotides comprising the polynucleotide can be ribonucleotides or deoxyribonucleotides or a modified form of either type of nucleotide. Such modifications include base modifications such as bromuridine, ribose modifications such as arabinoside and 2',3'-dideoxyribose, and internucleotide linkage modifications such as phosphorothioate, phosphorodithioate, phosphoroselenoate, phosphorodiselenoate, phosphoroanilothioate, phoshoraniladate and phosphoroamidate. The term "polynucleotide" specifically includes single-stranded and double-stranded forms of DNA.

[0066] An "isolated polynucleotide" is a polynucleotide of genomic, cDNA, or synthetic origin or some combination thereof, which: (1) is not associated with all or a portion of a polynucleotide in which the isolated polynucleotide is found in nature, (2) is linked to a polynucleotide to which it is not linked in nature, or (3) does not occur in nature as part of a larger sequence.

[0067] An "isolated polypeptide" is one that: (1) is free of at least some other polypeptides with which it would normally be found, (2) is essentially free of other

polypeptides from the same source, *e.g.*, from the same species, (3) is expressed by a cell from a different species, (4) has been separated from at least about 50 percent of polynucleotides, lipids, carbohydrates, or other materials with which it is associated in nature, (5) is not associated (by covalent or noncovalent interaction) with portions of a polypeptide with which the "isolated polypeptide" is associated in nature, (6) is operably associated (by covalent or noncovalent interaction) with a polypeptide with which it is not associated in nature, or (7) does not occur in nature. Such an isolated polypeptide can be encoded by genomic DNA, cDNA, mRNA or other RNA, of synthetic origin, or any combination thereof. Preferably, the isolated polypeptide is substantially free from polypeptides or other contaminants that are found in its natural environment that would interfere with its use (therapeutic, diagnostic, prophylactic, research or otherwise).

[0068] Naturally occurring antibodies typically comprise a tetramer. Each such tetramer is typically composed of two identical pairs of polypeptide chains, each pair having one full-length "light" chain (typically having a molecular weight of about 25 kDa) and one full-length "heavy" chain (typically having a molecular weight of about 50-70 kDa). The terms "heavy chain" and "light chain" as used herein refer to any immunoglobulin polypeptide having sufficient variable domain sequence to confer specificity for a target antigen. The amino-terminal portion of each light and heavy chain typically includes a variable domain of about 100 to 110 or more amino acids that typically is responsible for antigen recognition. The carboxy-terminal portion of each chain typically defines a constant domain responsible for effector function. Thus, in a naturally occurring antibody, a full-length heavy chain immunoglobulin polypeptide includes a variable domain (V_H) and three constant domains (C_{H1}, C_{H2}, and C_{H3}), wherein the V_H domain is at the amino-terminus of the polypeptide and the C_{H3} domain is at the carboxyl-terminus, and a full-length light chain immunoglobulin polypeptide includes a variable domain (V_L) and a constant domain (C_L), wherein the V_L domain is at the amino-terminus of the polypeptide and the C_L domain is at the carboxyl-terminus.

[0069] Human light chains are typically classified as kappa and lambda light chains, and human heavy chains are typically classified as mu, delta, gamma, alpha, or epsilon, and define the antibody's isotype as IgM, IgD, IgG, IgA, and IgE, respectively. IgG has several subclasses, including, but not limited to, IgG1, IgG2, IgG3, and IgG4. IgM has subclasses including, but not limited to, IgM1 and IgM2. IgA is similarly subdivided into subclasses including, but not limited to, IgA1 and IgA2. Within full-length light and heavy chains, the variable and constant domains typically are joined by a "J" region of about 12 or more

amino acids, with the heavy chain also including a "D" region of about 10 more amino acids. *See, e.g.*, FUNDAMENTAL IMMUNOLOGY (Paul, W., ed., Raven Press, 2nd ed., 1989), which is incorporated by reference in its entirety for all purposes. The variable regions of each light/heavy chain pair typically form an antigen binding site. The variable domains of naturally occurring antibodies typically exhibit the same general structure of relatively conserved framework regions (FR) joined by three hypervariable regions, also called complementarity determining regions or CDRs. The CDRs from the two chains of each pair typically are aligned by the framework regions, which may enable binding to a specific epitope. From the amino-terminus to the carboxyl-terminus, both light and heavy chain variable domains typically comprise the domains FR1, CDR1, FR2, CDR2, FR3, CDR3, and FR4.

[0070] The term "CDR set" refers to a group of three CDRs that occur in a single variable region capable of binding the antigen. The exact boundaries of these CDRs have been defined differently according to different systems. The system described by Kabat (Kabat *et al.*, SEQUENCES OF PROTEINS OF IMMUNOLOGICAL INTEREST (National Institutes of Health, Bethesda, Md. (1987) and (1991)) not only provides an unambiguous residue numbering system applicable to any variable region of an antibody, but also provides precise residue boundaries defining the three CDRs. These CDRs may be referred to as Kabat CDRs. Chothia and coworkers (Chothia and Lesk, 1987, *J. Mol. Biol.* 196: 901-17; Chothia *et al.*, 1989, *Nature* 342: 877-83) found that certain sub-portions within Kabat CDRs adopt nearly identical peptide backbone conformations, despite having great diversity at the level of amino acid sequence. These sub-portions were designated as L1, L2, and L3 or H1, H2, and H3 where the "L" and the "H" designates the light chain and the heavy chain regions, respectively. These regions may be referred to as Chothia CDRs, which have boundaries that overlap with Kabat CDRs. Other boundaries defining CDRs overlapping with the Kabat CDRs have been described by Padlan, 1995, *FASEB J.* 9: 133-39; MacCallum, 1996, *J. Mol. Biol.* 262(5): 732-45; and Lefranc, 2003, *Dev. Comp. Immunol.* 27: 55-77. Still other CDR boundary definitions may not strictly follow one of the herein systems, but will nonetheless overlap with the Kabat CDRs, although they may be shortened or lengthened in light of prediction or experimental findings that particular residues or groups of residues or even entire CDRs do not significantly impact antigen binding. The methods used herein may utilize CDRs defined according to any of these systems, although certain embodiments use Kabat or Chothia defined CDRs. Identification of predicted CDRs using the amino acid sequence is well known in the field, such as in

Martin, A.C. "Protein sequence and structure analysis of antibody variable domains," *In Antibody Engineering*, Vol. 2. Kontermann R., Dübel S., eds. Springer-Verlag, Berlin, p. 33–51 (2010). The amino acid sequence of the heavy and/or light chain variable domain may be also inspected to identify the sequences of the CDRs by other conventional methods, *e.g.*, by comparison to known amino acid sequences of other heavy and light chain variable regions to determine the regions of sequence hypervariability. The numbered sequences may be aligned by eye, or by employing an alignment program such as one of the CLUSTAL suite of programs, as described in Thompson, 1994, *Nucleic Acids Res.* 22: 4673-80. Molecular models are conventionally used to correctly delineate framework and CDR regions and thus correct the sequence-based assignments.

[0071] The term "Fc" as used herein refers to a molecule comprising the sequence of a non-antigen-binding fragment resulting from digestion of an antibody or produced by other means, whether in monomeric or multimeric form, and can contain the hinge region. The original immunoglobulin source of the native Fc is preferably of human origin and can be any of the immunoglobulins, although IgG1 and IgG2 are preferred. Fc molecules are made up of monomeric polypeptides that can be linked into dimeric or multimeric forms by covalent (*i.e.*, disulfide bonds) and non-covalent association. The number of intermolecular disulfide bonds between monomeric subunits of native Fc molecules ranges from 1 to 4 depending on class (*e.g.*, IgG, IgA, and IgE) or subclass (*e.g.*, IgG1, IgG2, IgG3, IgA1, and IgGA2). One example of a Fc is a disulfide-bonded dimer resulting from papain digestion of an IgG. The term "native Fc" as used herein is generic to the monomeric, dimeric, and multimeric forms.

[0072] A F(ab) fragment typically includes one light chain and the V_H and C_{H1} domains of one heavy chain, wherein the V_H-C_{H1} heavy chain portion of the F(ab) fragment cannot form a disulfide bond with another heavy chain polypeptide. As used herein, a F(ab) fragment can also include one light chain containing two variable domains separated by an amino acid linker and one heavy chain containing two variable domains separated by an amino acid linker and a C_{H1} domain.

[0073] A F(ab') fragment typically includes one light chain and a portion of one heavy chain that contains more of the constant region (between the C_{H1} and C_{H2} domains), such that an interchain disulfide bond can be formed between two heavy chains to form a $F(ab')_2$ molecule.

[0074] The term "binding protein" as used herein refers to a non-naturally occurring (or recombinant or engineered) molecule that specifically binds to at least one target antigen,

and which comprises four polypeptide chains that form at least three antigen binding sites, wherein a first polypeptide chain has a structure represented by the formula:

$$V_{L2}\text{-}L_1\text{-}V_{L1}\text{-}L_2\text{-}C_L \qquad [I]$$

and a second polypeptide chain has a structure represented by the formula:

$$V_{H1}\text{-}L_3\text{-}V_{H2}\text{-}L_4\text{-}C_{H1} \qquad [II]$$

and a third polypeptide chain has a structure represented by the formula:

$$V_{H3}\text{-}C_{H1} \qquad [III]$$

and a fourth polypeptide chain has a structure represented by the formula:

$$V_{L3}\text{-}C_L \qquad [IV]$$

wherein:

V_{L1} is a first immunoglobulin light chain variable domain;

V_{L2} is a second immunoglobulin light chain variable domain;

V_{L3} is a third immunoglobulin light chain variable domain;

V_{H1} is a first immunoglobulin heavy chain variable domain;

V_{H2} is a second immunoglobulin heavy chain variable domain;

V_{H3} is a third immunoglobulin heavy chain variable domain;

C_L is an immunoglobulin light chain constant domain;

C_{H1} is an immunoglobulin C_{H1} heavy chain constant domain; and

L_1, L_2, L_3 and L_4 are amino acid linkers;

and wherein the polypeptide of formula I and the polypeptide of formula II form a cross-over light chain-heavy chain pair.

[0075] A "recombinant" molecule is one that has been prepared, expressed, created, or isolated by recombinant means.

[0076] One embodiment of the disclosure provides binding proteins having biological and immunological specificity to between one and three target antigens. Another embodiment of the disclosure provides nucleic acid molecules comprising nucleotide sequences encoding polypeptide chains that form such binding proteins. Another embodiment of the disclosure provides expression vectors comprising nucleic acid molecules comprising nucleotide sequences encoding polypeptide chains that form such binding proteins. Yet another embodiment of the disclosure provides host cells that express such binding proteins (*i.e.*, comprising nucleic acid molecules or vectors encoding polypeptide chains that form such binding proteins).

[0077] The term "swapability" as used herein refers to the interchangeability of variable domains within the binding protein format and with retention of folding and ultimate binding affinity. "Full swapability" refers to the ability to swap the order of both V_{H1} and V_{H2} domains, and therefore the order of V_{L1} and V_{L2} domains, in the polypeptide chain of formula I or the polypeptide chain of formula II (*i.e.*, to reverse the order) while maintaining full functionality of the binding protein as evidenced by the retention of binding affinity. Furthermore, it should be noted that the designations V_H and V_L refer only to the domain's location on a particular protein chain in the final format. For example, V_{H1} and V_{H2} could be derived from V_{L1} and V_{L2} domains in parent antibodies and placed into the V_{H1} and V_{H2} positions in the binding protein. Likewise, V_{L1} and V_{L2} could be derived from V_{H1} and V_{H2} domains in parent antibodies and placed in the V_{H1} and V_{H2} positions in the binding protein. Thus, the V_H and V_L designations refer to the present location and not the original location in a parent antibody. V_H and V_L domains are therefore "swappable."

[0078] The term "antigen" or "target antigen" or "antigen target" as used herein refers to a molecule or a portion of a molecule that is capable of being bound by a binding protein, and additionally is capable of being used in an animal to produce antibodies capable of binding to an epitope of that antigen. A target antigen may have one or more epitopes. With respect to each target antigen recognized by a binding protein, the binding protein is capable of competing with an intact antibody that recognizes the target antigen.

[0079] The term "Her2" refers to human epidermal growth factor receptor 2 which is a member of the epidermal growth factor receptor family.

[0080] "CD3" is cluster of differentiation factor 3 polypeptide and is a T-cell surface protein that is typically part of the T cell receptor (TCR) complex.

[0081] "CD28" is cluster of differentiation 28 polypeptide and is a T-cell surface protein that provides co-stimulatory signals for T-cell activation and survival.

[0082] "CD19" is cluster of differentiation 19 polypeptide and is located on B-cells.

[0083] "CD20" is cluster of differentiation 20 polypeptide and is an activated-glycosylated phosphoprotein expressed on the surface of B-cells.

[0084] "CD38" is cluster of differentiation 38 polypeptide and is a glycoprotein found on the surface of many immune cells.

[0085] "LAMP1" is lysosomal-associated membrane protein 1.

[0086] "IL-4" is interleukin 4 and is a cytokine that induces differentiation of naïve helper T cells.

[0087] "IL-13" is interleukin 13 and is a cytokine secreted by many cell types such as T-cells.

[0088] "TNFa" is tumor necrosis factor alpha and is a cytokine involved in systematic inflammation.

[0089] The term "T-cell engager" refers to binding proteins directed to a host's immune system, more specifically the T cells' cytotoxic activity as well as directed to a tumor target protein.

[0090] The term "monospecific binding protein" refers to a binding protein that specifically binds to one antigen target.

[0091] The term "monovalent binding protein" refers to a binding protein that has one antigen binding site.

[0092] The term "bispecific binding protein" refers to a binding protein that specifically binds to two different antigen targets.

[0093] The term "bivalent binding protein" refers to a binding protein that has two binding sites.

[0094] The term "trispecific binding protein" refers to a binding protein that specifically binds to three different antigen targets.

[0095] The term "trivalent binding protein" refers to a binding protein that has three binding sites. In particular embodiments the trivalent binding protein can bind to one antigen target. In other embodiments, the trivalent binding protein can bind to two antigen targets. In other embodiments, the trivalent binding protein can bind to three antigen targets.

[0096] An "isolated" binding protein is one that has been identified and separated and/or recovered from a component of its natural environment. Contaminant components of its natural environment are materials that would interfere with diagnostic or therapeutic uses for the binding protein, and may include enzymes, hormones, and other proteinaceous or non-proteinaceous solutes. In some embodiments, the binding protein will be purified: (1) to greater than 95% by weight of antibody as determined by the Lowry method, and most preferably more than 99% by weight, (2) to a degree sufficient to obtain at least 15 residues of N-terminal or internal amino acid sequence by use of a spinning cup sequenator, or (3) to homogeneity by SDS-PAGE under reducing or nonreducing conditions using Coomassie blue or, preferably, silver stain. Isolated binding proteins include the binding protein *in situ* within recombinant cells since at least one component of the binding protein's natural environment will not be present.

[0097] The terms "substantially pure" or "substantially purified" as used herein refer to a compound or species that is the predominant species present (*i.e.*, on a molar basis it is more abundant than any other individual species in the composition). In some embodiments, a substantially purified fraction is a composition wherein the species comprises at least about 50% (on a molar basis) of all macromolecular species present. In other embodiments, a substantially pure composition will comprise more than about 80%, 85%, 90%, 95%, or 99% of all macromolar species present in the composition. In still other embodiments, the 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.

[0098] A "neutralizing" binding protein as used herein refers to a molecule that is able to block or substantially reduce an effector function of a target antigen to which it binds. As used herein, "substantially reduce" means at least about 60%, preferably at least about 70%, more preferably at least about 75%, even more preferably at least about 80%, still more preferably at least about 85%, most preferably at least about 90% reduction of an effector function of the target antigen.

[0099] The term "epitope" includes any determinant, preferably a polypeptide determinant, capable of specifically binding to an immunoglobulin or T-cell receptor. In certain embodiments, epitope determinants include chemically active surface groupings of molecules such as amino acids, sugar side chains, phosphoryl groups, or sulfonyl groups, and, in certain embodiments, may have specific three-dimensional structural characteristics and/or specific charge characteristics. An epitope is a region of an antigen that is bound by an antibody or binding protein. In certain embodiments, a binding protein is said to specifically bind an antigen when it preferentially recognizes its target antigen in a complex mixture of proteins and/or macromolecules. In some embodiments, a binding protein is said to specifically bind an antigen when the equilibrium dissociation constant is $\leq 10^{-8}$ M, more preferably when the equilibrium dissociation constant is $\leq 10^{-9}$ M, and most preferably when the dissociation constant is $\leq 10^{-10}$ M.

[0100] The dissociation constant (K_D) of a binding protein can be determined, for example, by surface plasmon resonance. Generally, surface plasmon resonance analysis measures real-time binding interactions between ligand (a target antigen on a biosensor matrix) and analyte (a binding protein in solution) by surface plasmon resonance (SPR) using the BIAcore system (Pharmacia Biosensor; Piscataway, NJ). Surface plasmon analysis can also be performed by immobilizing the analyte (binding protein on a biosensor matrix) and

presenting the ligand (target antigen). The term "K_D," as used herein refers to the dissociation constant of the interaction between a particular binding protein and a target antigen.

[0101] The term "specifically binds" as used herein refers to the ability of a binding protein or an antigen-binding fragment thereof to bind to an antigen containing an epitope with an Kd of at least about 1×10^{-6} M, 1×10^{-7} M, 1×10^{-8} M, 1×10^{-9} M, 1×10^{-10} M, 1×10^{-11} M, 1×10^{-12} M, or more, and/or to bind to an epitope with an affinity that is at least two-fold greater than its affinity for a nonspecific antigen.

[0102] The term "linker" as used herein refers to one or more amino acid residues inserted between immunoglobulin domains to provide sufficient mobility for the domains of the light and heavy chains to fold into cross over dual variable region immunoglobulins. A linker is inserted at the transition between variable domains or between variable and constant domains, respectively, at the sequence level. The transition between domains can be identified because the approximate size of the immunoglobulin domains are well understood. The precise location of a domain transition can be determined by locating peptide stretches that do not form secondary structural elements such as beta-sheets or alpha-helices as demonstrated by experimental data or as can be assumed by techniques of modeling or secondary structure prediction. The linkers described herein are referred to as L_1, which is located on the light chain between the C-terminus of the V_{L2} and the N-terminus of the V_{L1} domain; and L_2, which is located on the light chain between the C-terminus of the V_{L1} and the N-terminus of the C_L domain. The heavy chain linkers are known as L_3, which is located between the C-terminus of the V_{H1} and the N-terminus of the V_{H2} domain; and L_4, which is located between the C-terminus of the V_{H2} and the N-terminus of the C_{H1} domain.

[0103] The term "vector" as used herein refers to any molecule (*e.g.*, nucleic acid, plasmid, or virus) that is used to transfer coding information to a host cell. The term "vector" includes a nucleic acid molecule that is capable of transporting another nucleic acid to which it has been linked. One type of vector is a "plasmid," which refers to a circular double-stranded DNA molecule into which additional DNA segments may be inserted. Another type of vector is a viral vector, wherein additional DNA segments may be inserted into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (*e.g.*, bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (*e.g.*, non-episomal mammalian vectors) can be integrated into the genome of a host cell upon introduction into the host cell and thereby are replicated along with the host genome. In addition, certain vectors are capable of directing

the expression of genes to which they are operatively linked. Such vectors are referred to herein as "recombinant expression vectors" (or simply, "expression vectors"). In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. The terms "plasmid" and "vector" may be used interchangeably herein, as a plasmid is the most commonly used form of vector. However, the disclosure is intended to include other forms of expression vectors, such as viral vectors (*e.g.*, replication defective retroviruses, adenoviruses, and adeno-associated viruses), which serve equivalent functions.

[0104] The phrase "recombinant host cell" (or "host cell") as used herein refers to a cell into which a recombinant expression vector has been introduced. A recombinant host cell or host cell is intended to refer not only to the particular subject cell, but also to the progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but such cells are still included within the scope of the term "host cell" as used herein. A wide variety of host cell expression systems can be used to express the binding proteins, including bacterial, yeast, baculoviral, and mammalian expression systems (as well as phage display expression systems). An example of a suitable bacterial expression vector is pUC19. To express a binding protein recombinantly, a host cell is transformed or transfected with one or more recombinant expression vectors carrying DNA fragments encoding the polypeptide chains of the binding protein such that the polypeptide chains are expressed in the host cell and, preferably, secreted into the medium in which the host cells are cultured, from which medium the binding protein can be recovered.

[0105] The term "transformation" as used herein refers to a change in a cell's genetic characteristics, and a cell has been transformed when it has been modified to contain a new DNA. For example, a cell is transformed where it is genetically modified from its native state. Following transformation, the transforming DNA may recombine with that of the cell by physically integrating into a chromosome of the cell, or may be maintained transiently as an episomal element without being replicated, or may replicate independently as a plasmid. A cell is considered to have been stably transformed when the DNA is replicated with the division of the cell. The term "transfection" as used herein refers to the uptake of foreign or exogenous DNA by a cell, and a cell has been "transfected" when the exogenous DNA has been introduced inside the cell membrane. A number of transfection techniques are well known in the art. Such techniques can be used to introduce one or more exogenous DNA molecules into suitable host cells.

[0106] The term "naturally occurring" as used herein and applied to an object refers to the fact that the object can be found in nature and has not been manipulated by man. For example, a polynucleotide or polypeptide 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 is naturally-occurring. Similarly, "non-naturally occurring" as used herein refers to an object that is not found in nature or that has been structurally modified or synthesized by man.

[0107] As used herein, the twenty conventional amino acids and their abbreviations follow conventional usage. Stereoisomers (*e.g.*, D-amino acids) of the twenty conventional amino acids; unnatural amino acids and analogs such as α-, α-disubstituted amino acids, N-alkyl amino acids, lactic acid, and other unconventional amino acids may also be suitable components for the polypeptide chains of the binding proteins. 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 carboxyl-terminal direction, in accordance with standard usage and convention.

[0108] Naturally occurring residues may be divided into classes based on common side chain properties:

(1) hydrophobic: Met, Ala, Val, Leu, Ile, Phe, Trp, Tyr, Pro;

(2) polar hydrophilic: Arg, Asn, Asp, Gln, Glu, His, Lys, Ser, Thr ;

(3) aliphatic: Ala, Gly, Ile, Leu, Val, Pro;

(4) aliphatic hydrophobic: Ala, Ile, Leu, Val, Pro;

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

(6) acidic: Asp, Glu;

(7) basic: His, Lys, Arg;

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

(9) aromatic: His, Trp, Tyr, Phe; and

(10) aromatic hydrophobic: Phe, Trp, Tyr.

[0109] Conservative amino acid substitutions may involve exchange of a member of one of these classes with another member of the same class. Non-conservative substitutions may involve the exchange of a member of one of these classes for a member from another class.

[0110] A skilled artisan will be able to determine suitable variants of the polypeptide chains of the binding proteins using well-known techniques. For example, one skilled in the art may identify suitable areas of a polypeptide chain that may be changed without destroying activity by targeting regions not believed to be important for activity. Alternatively, one skilled in the art can identify residues and portions of the molecules that are conserved among similar polypeptides. In addition, even areas that may be important for biological activity or for structure may be subject to conservative amino acid substitutions without destroying the biological activity or without adversely affecting the polypeptide structure.

[0111] The term "patient" as used herein includes human and animal subjects.

[0112] The terms "treatment" or "treat" as used herein refer to both therapeutic treatment and prophylactic or preventative measures. Those in need of treatment include those having a disorder as well as those prone to have the disorder or those in which the disorder is to be prevented. In particular embodiments, binding proteins can be used to treat humans with cancer, or humans susceptible to cancer, or ameliorate cancer in a human subject. The binding proteins can also be used to prevent cancer in a human patient. In particular embodiments, the cancer is multiple myeloma, acute lymphoblastic leukemia, chronic lymphocytic leukemia, acute myeloid leukemia, lymphoma, breast cancer such as Her2+ breast cancer, germinal center B-cell lympohoma or B-cell acute lymphoblastic leukemia, In other embodiments, the binding proteins can be used to treat humans with inflammatory disorders, or humans susceptible to inflammatory disorders, or ameliorate inflammatory disorders in a human subject.

[0113] The terms "pharmaceutical composition" or "therapeutic composition" as used herein refer to a compound or composition capable of inducing a desired therapeutic effect when properly administered to a patient.

[0114] The term "pharmaceutically acceptable carrier" or "physiologically acceptable carrier" as used herein refers to one or more formulation materials suitable for accomplishing or enhancing the delivery of a binding protein.

[0115] The terms "effective amount" and "therapeutically effective amount" when used in reference to a pharmaceutical composition comprising one or more binding proteins refer to an amount or dosage sufficient to produce a desired therapeutic result. More specifically, a therapeutically effective amount is an amount of a binding protein sufficient to inhibit, for some period of time, one or more of the clinically defined pathological processes associated with the condition being treated. The effective amount may vary depending on the specific binding protein that is being used, and also depends on a variety of factors and conditions

related to the patient being treated and the severity of the disorder. For example, if the binding protein is to be administered *in vivo*, factors such as the age, weight, and health of the patient as well as dose response curves and toxicity data obtained in preclinical animal work would be among those factors considered. The determination of an effective amount or therapeutically effective amount of a given pharmaceutical composition is well within the ability of those skilled in the art.

[0116] One embodiment of the disclosure provides a pharmaceutical composition comprising a pharmaceutically acceptable carrier and a therapeutically effective amount of a binding protein.

Trispecific and/or Trivalent Binding Proteins

[0117] In one embodiment, the binding protein of the disclosure is a trispecific and/or trivalent binding protein comprising four polypeptide chains that form three antigen binding sites that specifically bind one or more (*e.g.*, three) different antigen targets or target proteins, wherein a first polypeptide chain comprises a structure represented by the formula:

$$V_{L2}\text{-}L_1\text{-}V_{L1}\text{-}L_2\text{-}C_L \qquad [I]$$

and a second polypeptide chain comprises a structure represented by the formula:

$$V_{H1}\text{-}L_3\text{-}V_{H2}\text{-}L_4\text{-}C_{H1} \qquad [II]$$

and a third polypeptide chain comprises a structure represented by the formula:

$$V_{H3}\text{-}C_{H1} \qquad [III]$$

and a fourth polypeptide chain comprises a structure represented by the formula:

$$V_{L3}\text{-}C_L \qquad [IV]$$

wherein:

V_{L1} is a first immunoglobulin light chain variable domain;

V_{L2} is a second immunoglobulin light chain variable domain;

V_{L3} is a third immunoglobulin light chain variable domain;

V_{H1} is a first immunoglobulin heavy chain variable domain;

V_{H2} is a second immunoglobulin heavy chain variable domain;

V_{H3} is a third immunoglobulin heavy chain variable domain;

C_L is an immunoglobulin light chain constant domain;

C_{H1} is an immunoglobulin C_{H1} heavy chain constant domain; and

L_1, L_2, L_3 and L_4 are amino acid linkers;

and wherein the polypeptide of formula I and the polypeptide of formula II form a cross-over light chain-heavy chain pair.

[0118] In one embodiment, the binding protein of the disclosure is a trispecific and/or trivalent binding protein comprising four polypeptide chains that form three antigen binding sites that specifically bind one or more (*e.g.*, three) antigen targets or target proteins, wherein a first polypeptide chain comprises a structure represented by the formula:

$$V_{L2}\text{-}L_1\text{-}V_{L1}\text{-}L_2\text{-}C_L \qquad [I]$$

and a second polypeptide chain comprises a structure represented by the formula:

$$V_{H1}\text{-}L_3\text{-}V_{H2}\text{-}L_4\text{-}C_{H1} \qquad [II]$$

and a third polypeptide chain comprises a structure represented by the formula:

$$V_{H3}\text{-}C_{H1} \qquad [III]$$

and a fourth polypeptide chain comprises a structure represented by the formula:

$$V_{L3}\text{-}C_L \qquad [IV]$$

wherein:

V_{L1} is a first immunoglobulin light chain variable domain;

V_{L2} is a second immunoglobulin light chain variable domain;

V_{L3} is a third immunoglobulin light chain variable domain;

V_{H1} is a first immunoglobulin heavy chain variable domain;

V_{H2} is a second immunoglobulin heavy chain variable domain;

V_{H3} is a third immunoglobulin heavy chain variable domain;

C_L is an immunoglobulin light chain constant domain;

C_{H1} is an immunoglobulin C_{H1} heavy chain constant domain; and

L_1, L_2, L_3 and L_4 are amino acid linkers;

and wherein the polypeptide of formula I and the polypeptide of formula II form a cross-over light chain-heavy chain pair.

[0119] In one embodiment, the binding protein of the disclosure is a trispecific and/or trivalent binding protein comprising four polypeptide chains that form three antigen binding sites that specifically bind one or more (*e.g.*, three) different antigen targets or target proteins, wherein a first polypeptide chain comprises a structure represented by the formula:

$$V_{L2}\text{-}L_1\text{-}V_{L1}\text{-}L_2\text{-}C_L \qquad [I]$$

and a second polypeptide chain comprises a structure represented by the formula:

$$V_{H1}\text{-}L_3\text{-}V_{H2}\text{-}L_4\text{-}C_{H1}\text{-hinge-}C_{H2}\text{-}C_{H3} \qquad [II]$$

and a third polypeptide chain comprises a structure represented by the formula:

$$V_{H3}\text{-}\ C_{H1}\text{-hinge-}C_{H2}\text{-}C_{H3} \qquad [III]$$

and a fourth polypeptide chain comprises a structure represented by the formula:

$$V_{L3}\text{-}\ C_L \qquad [IV]$$

wherein:

V_{L1} is a first immunoglobulin light chain variable domain;

V_{L2} is a second immunoglobulin light chain variable domain;

V_{L3} is a third immunoglobulin light chain variable domain;

V_{H1} is a first immunoglobulin heavy chain variable domain;

V_{H2} is a second immunoglobulin heavy chain variable domain;

V_{H3} is a third immunoglobulin heavy chain variable domain;

C_L is an immunoglobulin light chain constant domain;

C_{H1} is an immunoglobulin C_{H1} heavy chain constant domain;

C_{H2} is an immunoglobulin C_{H2} heavy chain constant domain;

C_{H3} is an immunoglobulin C_{H3} heavy chain constant domain;

hinge is an immunoglobulin hinge region connecting the C_{H1} and C_{H2} domains; and L_1, L_2, L_3 and L_4 are amino acid linkers;

and wherein the polypeptide of formula I and the polypeptide of formula II form a cross-over light chain-heavy chain pair.

[0120] In one embodiment, the binding protein of the disclosure is a trispecific and/or trivalent binding protein comprising four polypeptide chains that form three antigen binding sites that specifically bind one or more (*e.g.*, three) antigen targets or target proteins, wherein a first polypeptide chain comprises a structure represented by the formula:

$$V_{L2}\text{-}L_1\text{-}V_{L1}\text{-}L_2\text{-}C_L \qquad [I]$$

and a second polypeptide chain comprises a structure represented by the formula:

$$V_{H1}\text{-}L_3\text{-}V_{H2}\text{-}L_4\text{-}C_{H1}\text{-hinge-}C_{H2}\text{-}C_{H3} \qquad [II]$$

and a third polypeptide chain comprises a structure represented by the formula:

$$V_{H3}\text{-}\ C_{H1}\text{-hinge-}C_{H2}\text{-}C_{H3} \qquad [III]$$

and a fourth polypeptide chain comprises a structure represented by the formula:

$$V_{L3}\text{-}\ C_L \qquad [IV]$$

wherein:

V_{L1} is a first immunoglobulin light chain variable domain;

V_{L2} is a second immunoglobulin light chain variable domain;

V_{L3} is a third immunoglobulin light chain variable domain;

V_{H1} is a first immunoglobulin heavy chain variable domain;

V_{H2} is a second immunoglobulin heavy chain variable domain;

V_{H3} is a third immunoglobulin heavy chain variable domain;

C_L is an immunoglobulin light chain constant domain;

C_{H1} is an immunoglobulin C_{H1} heavy chain constant domain;

C_{H2} is an immunoglobulin C_{H2} heavy chain constant domain;

C_{H3} is an immunoglobulin C_{H3} heavy chain constant domain;

hinge is an immunoglobulin hinge region connecting the C_{H1} and C_{H2} domains; and L_1, L_2, L_3 and L_4 are amino acid linkers;

and wherein the polypeptide of formula I and the polypeptide of formula II form a cross-over light chain-heavy chain pair.

[0121] In some embodiments, the first polypeptide chain and the second polypeptide chain have a cross-over orientation that forms two distinct antigen binding sites. In some embodiments, the VH1 and VL1 form a binding pair and form the first antigen binding site. In some embodiments, the VH2 and VL2 form a binding pair and form the second antigen binding site. In some embodiments, the third polypeptide and the fourth polypeptide form a third antigen binding site. In some embodiments, the VH3 and VL3 form a binding pair and form the third antigen binding site.

[0122] In one embodiment, the binding protein of the disclosure is a trispecific and/or trivalent binding protein comprising four polypeptide chains that form three antigen binding sites that specifically bind one or more (*e.g.*, three) antigen targets or target proteins, wherein a first polypeptide chain comprises a structure represented by the formula:

$$V_{D1}\text{-}L_1\text{-}V_{D2}\text{-}L_2\text{-}C_L \qquad [I]$$

and a second polypeptide chain comprises a structure represented by the formula:

$$V_{D3}\text{-}L_3\text{-}V_{D4}\text{-}L_4\text{-}C_{H1}\text{-hinge-}C_{H2}\text{-}C_{H3} \qquad [II]$$

and a third polypeptide chain comprises a structure represented by the formula:

$$V_{H3}\text{-}\ C_{H1}\text{-hinge-}C_{H2}\text{-}C_{H3} \qquad [III]$$

and a fourth polypeptide chain comprises a structure represented by the formula:

$$V_{L3}\text{-}\ C_L \qquad [IV]$$

wherein:

V_{D1} is a variable domain of heavy or light chain of a first immunoglobulin;

V_{D2} is a variable domain of heavy or light chain of a second immunoglobulin;

V_{D3} is a variable domain of heavy or light chain of a third immunoglobulin;

V_{D4} is a variable domain of heavy or light chain of a fourth immunoglobulin;

V_{H3} is an immunoglobulin heavy chain variable domain;

V_{L3} is an immunoglobulin light chain variable domain;

C_L is an immunoglobulin light chain constant domain;

C_{H1} is an immunoglobulin C_{H1} heavy chain constant domain;

C_{H2} is an immunoglobulin C_{H2} heavy chain constant domain;

C_{H3} is an immunoglobulin C_{H3} heavy chain constant domain;

hinge is an immunoglobulin hinge region connecting the C_{H1} and C_{H2} domains; and

L_1, L_2, L_3 and L_4 are amino acid linkers;

and wherein the polypeptide of formula I and the polypeptide of formula II form a cross-over light chain-heavy chain pair.

[0123] In some embodiments, the binding protein of the disclosure comprises three antigen binding sites that specifically bind one, two, or three antigen targets or target proteins. In some embodiments, the binding protein binds three antigen targets. In some embodiments, the binding protein binds three different antigen targets. In some embodiments, two of the antigen binding sites bind the same antigen target. In those embodiments, the binding protein comprises the same binding domains twice, or different binding domains, and/or specifically binds different antigens or epitopes on the same antigen target. In some embodiments, three of the antigen binding sites bind the same antigen target. In those embodiments, the binding protein comprises the same binding domains three times, or different binding domains, and/or specifically binds different antigens or epitopes on the same antigen target.

[0124] In some embodiments, V_{L1}, V_{L2} and V_{L3} are each independently a variable domain derived from an amino acid sequence as set forth in any one of SEQ ID NOs: 2, 4, 10, 14, 18, 22 or 115; and V_{H1}, V_{H2} and V_{H3}, are each independently a variable domain derived from an amino acid sequence as set forth in any one of SEQ ID NOs: 1, 3, 9, 13, 17, 21 or 114. In other embodiments, V_{L1}, V_{L2} and V_{L3} are each independently a variable domain derived from an amino acid sequence as set forth in any one of SEQ ID NOs: 61, 63, 69, 71, 74, 76, 82, 86, 88 or 94; and V_{H1}, V_{H2} and V_{H3}, are each independently a variable domain derived from an amino acid sequence as set forth in any one of SEQ ID NOs: 60, 62, 68, 73, 75, 81, 85, 87 or 93. In other embodiments, V_{L1}, V_{L2} and V_{L3} each independently comprise light chain complementarity determining regions of a variable domain comprising an amino acid sequence as set forth in any one of SEQ ID NOs: 43-59, 123-125; and V_{H1}, V_{H2} and V_{H3} each independently comprise heavy chain complementarity determining regions of a variable domain comprising an amino acid sequence as set forth in any one of SEQ ID NOs: 25-42, 120-122. In other embodiments, V_{L1}, V_{L2} and V_{L3} each independently comprise light chain complementarity determining regions of a variable domain comprising an amino acid sequence as set forth in any one of SEQ ID NOs: 61, 63, 69, 71, 74, 76, 82, 86, 88 or 94; and V_{H1}, V_{H2} and V_{H3} each independently comprise heavy chain complementarity determining

regions of a variable domain comprising an amino acid sequence as set forth in any one of SEQ ID NOs: 60, 62, 68, 73, 75, 81, 85, 87 or 93. In some embodiments, V_{H1}, V_{H2} and V_{H3} each independently comprise heavy chain complementarity determining regions and/or a variable domain sequence shown in Tables 2-5.

[0125] In some embodiments, V_{L1}, V_{L2} and V_{L3} each independently comprise a variable domain sequence as set forth in any one of SEQ ID NOs:169, 171, and 173; and/or V_{H1}, V_{H2}, and V_{H3} each independently comprise a variable domain sequence as set forth in any one of SEQ ID NOs:168, 170, and 172. In some embodiments, V_{L1}, V_{L2} and V_{L3} each independently comprise light chain complementarity determining regions comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 141-147, 178, and 179; and/or V_{H1}, V_{H2} and V_{H3} each independently comprise heavy chain complementarity determining regions comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 129-137. In some embodiments, V_{L1}, V_{L2} and V_{L3} each independently comprise a variable domain sequence as set forth in any one of SEQ ID NOs:151, 153, 155, 157, 159, 161, 163, 165, and 167; and/or V_{H1}, V_{H2}, and V_{H3} each independently comprise a variable domain sequence as set forth in any one of SEQ ID NOs:150, 152, 154, 156, 158, 160, 162, 164, and 166. In some embodiments, V_{L1}, V_{L2} and V_{L3} each independently comprise light chain complementarity determining regions comprising an amino acid sequence as set forth in any one of SEQ ID NOs: 43-59, 123-125, 138-140, and 149; and/or V_{H1}, V_{H2} and V_{H3} each independently comprise heavy chain complementarity determining regions of a variable domain comprising an amino acid sequence as set forth in any one of SEQ ID NOs: 25-42, 120-122, and 126-128. In some embodiments, V_{L1}, V_{L2} and V_{L3} each independently comprise light chain complementarity determining regions and/or a variable domain sequence shown in Tables 2-5.

[0126] In particular embodiments, the order of the V_{H1} and V_{H2} domains, and therefore the order of V_{L1} and V_{L2} domains, in the polypeptide chain of formula I or the polypeptide chain of formula II (*i.e.*, to reverse the order) are swapped.

[0127] In some embodiments, the first polypeptide chain comprises the amino acid sequence of SEQ ID NO: 4 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 4 optionally comprising CDRs that are 100% identical to the CDRs of the polypeptide chain of SEQ ID NO: 4; the second polypeptide chain comprises the amino acid sequence of SEQ ID NO: 3 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 3 optionally comprising CDRs that are 100% identical to the CDRs of the polypeptide chain of SEQ ID NO: 3; the

third polypeptide chain comprises the amino acid sequence of SEQ ID NO: 1 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 1 optionally comprising CDRs that are 100% identical to the CDRs of the polypeptide chain of SEQ ID NO: 1; and the fourth polypeptide chain comprises the amino acid sequence of SEQ ID NO: 2 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 2 optionally comprising CDRs that are 100% identical to the CDRs of the polypeptide chain of SEQ ID NO: 2.

[0128] In some embodiments, the first polypeptide chain comprises the amino acid sequence of SEQ ID NO: 10 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 10 optionally comprising CDRs that are 100% identical to the CDRs of the polypeptide chain of SEQ ID NO: 10; the second polypeptide chain comprises the amino acid sequence of SEQ ID NO: 9 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 9 optionally comprising CDRs that are 100% identical to the CDRs of the polypeptide chain of SEQ ID NO: 9; the third polypeptide chain comprises the amino acid sequence of SEQ ID NO: 1 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 1 optionally comprising CDRs that are 100% identical to the CDRs of the polypeptide chain of SEQ ID NO: 1; and the fourth polypeptide chain comprises the amino acid sequence of SEQ ID NO: 2 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 2 optionally comprising CDRs that are 100% identical to the CDRs of the polypeptide chain of SEQ ID NO: 2.

[0129] In some embodiments, the first polypeptide chain comprises the amino acid sequence of SEQ ID NO: 4 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 4 optionally comprising CDRs that are 100% identical to the CDRs of the polypeptide chain of SEQ ID NO: 4; the second polypeptide chain comprises the amino acid sequence of SEQ ID NO: 3 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 3 optionally comprising CDRs that are 100% identical to the CDRs of the polypeptide chain of SEQ ID NO: 3; the third polypeptide chain comprises the amino acid sequence of SEQ ID NO: 13 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 13 optionally comprising CDRs that are 100% identical to the CDRs of the polypeptide chain of SEQ ID NO: 13; and the fourth polypeptide chain comprises the amino acid sequence of SEQ ID NO: 14 or an amino acid sequence that is at least 95% identical to the amino acid

sequence of SEQ ID NO: 14 optionally comprising CDRs that are 100% identical to the CDRs of the polypeptide chain of SEQ ID NO: 14.

[0130] In some embodiments, the first polypeptide chain comprises the amino acid sequence of SEQ ID NO: 10 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 10 optionally comprising CDRs that are 100% identical to the CDRs of the polypeptide chain of SEQ ID NO: 10; the second polypeptide chain comprises the amino acid sequence of SEQ ID NO: 9 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 9 optionally comprising CDRs that are 100% identical to the CDRs of the polypeptide chain of SEQ ID NO: 9; the third polypeptide chain comprises the amino acid sequence of SEQ ID NO: 13 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 13 optionally comprising CDRs that are 100% identical to the CDRs of the polypeptide chain of SEQ ID NO: 13; and the fourth polypeptide chain comprises the amino acid sequence of SEQ ID NO: 14 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 14 optionally comprising CDRs that are 100% identical to the CDRs of the polypeptide chain of SEQ ID NO: 14.

[0131] In some embodiments, the first polypeptide chain comprises the amino acid sequence of SEQ ID NO: 4 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 4 optionally comprising CDRs that are 100% identical to the CDRs of the polypeptide chain of SEQ ID NO: 4; the second polypeptide chain comprises the amino acid sequence of SEQ ID NO: 3 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 3 optionally comprising CDRs that are 100% identical to the CDRs of the polypeptide chain of SEQ ID NO: 3; the third polypeptide chain comprises the amino acid sequence of SEQ ID NO: 17 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 17 optionally comprising CDRs that are 100% identical to the CDRs of the polypeptide chain of SEQ ID NO: 17; and the fourth polypeptide chain comprises the amino acid sequence of SEQ ID NO: 18 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 18 optionally comprising CDRs that are 100% identical to the CDRs of the polypeptide chain of SEQ ID NO: 18.

[0132] In some embodiments, the first polypeptide chain comprises the amino acid sequence of SEQ ID NO: 10 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 10 optionally comprising CDRs that are 100% identical to the CDRs of the polypeptide chain of SEQ ID NO: 10; the second polypeptide chain

comprises the amino acid sequence of SEQ ID NO: 9 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 9 optionally comprising CDRs that are 100% identical to the CDRs of the polypeptide chain of SEQ ID NO: 9; the third polypeptide chain comprises the amino acid sequence of SEQ ID NO: 17 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 17 optionally comprising CDRs that are 100% identical to the CDRs of the polypeptide chain of SEQ ID NO: 17; and the fourth polypeptide chain comprises the amino acid sequence of SEQ ID NO: 18 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 18 optionally comprising CDRs that are 100% identical to the CDRs of the polypeptide chain of SEQ ID NO: 18.

[0133] In some embodiments, the first polypeptide chain comprises the amino acid sequence of SEQ ID NO: 4 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 4 optionally comprising CDRs that are 100% identical to the CDRs of the polypeptide chain of SEQ ID NO: 4; the second polypeptide chain comprises the amino acid sequence of SEQ ID NO: 3 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 3 optionally comprising CDRs that are 100% identical to the CDRs of the polypeptide chain of SEQ ID NO: 3; the third polypeptide chain comprises the amino acid sequence of SEQ ID NO: 21 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 21 optionally comprising CDRs that are 100% identical to the CDRs of the polypeptide chain of SEQ ID NO: 21; and the fourth polypeptide chain comprises the amino acid sequence of SEQ ID NO: 22 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 22 optionally comprising CDRs that are 100% identical to the CDRs of the polypeptide chain of SEQ ID NO: 22.

[0134] In some embodiments, the first polypeptide chain comprises the amino acid sequence of SEQ ID NO: 10 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 10 optionally comprising CDRs that are 100% identical to the CDRs of the polypeptide chain of SEQ ID NO: 10; the second polypeptide chain comprises the amino acid sequence of SEQ ID NO: 9 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 9 optionally comprising CDRs that are 100% identical to the CDRs of the polypeptide chain of SEQ ID NO: 9; the third polypeptide chain comprises the amino acid sequence of SEQ ID NO: 21 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 21 optionally comprising CDRs that are 100% identical to the CDRs of the polypeptide chain of

SEQ ID NO: 21; and the fourth polypeptide chain comprises the amino acid sequence of SEQ ID NO: 22 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 22 optionally comprising CDRs that are 100% identical to the CDRs of the polypeptide chain of SEQ ID NO: 22.

[0135] In some embodiments, the first polypeptide chain comprises the amino acid sequence of SEQ ID NO: 63 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 63 optionally comprising CDRs that are 100% identical to the CDRs of the polypeptide chain of SEQ ID NO: 63; the second polypeptide chain comprises the amino acid sequence of SEQ ID NO: 62 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 62 optionally comprising CDRs that are 100% identical to the CDRs of the polypeptide chain of SEQ ID NO: 62; the third polypeptide chain comprises the amino acid sequence of SEQ ID NO: 60 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 60 optionally comprising CDRs that are 100% identical to the CDRs of the polypeptide chain of SEQ ID NO: 60; and the fourth polypeptide chain comprises the amino acid sequence of SEQ ID NO: 61 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 61 optionally comprising CDRs that are 100% identical to the CDRs of the polypeptide chain of SEQ ID NO: 61.

[0136] In some embodiments, the first polypeptide chain comprises the amino acid sequence of SEQ ID NO: 69 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 69 optionally comprising CDRs that are 100% identical to the CDRs of the polypeptide chain of SEQ ID NO: 69; the second polypeptide chain comprises the amino acid sequence of SEQ ID NO: 68 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 68 optionally comprising CDRs that are 100% identical to the CDRs of the polypeptide chain of SEQ ID NO: 68; the third polypeptide chain comprises the amino acid sequence of SEQ ID NO: 60 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 60 optionally comprising CDRs that are 100% identical to the CDRs of the polypeptide chain of SEQ ID NO: 60; and the fourth polypeptide chain comprises the amino acid sequence of SEQ ID NO: 61 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 61 optionally comprising CDRs that are 100% identical to the CDRs of the polypeptide chain of SEQ ID NO: 61.

[0137] In some embodiments, the first polypeptide chain comprises the amino acid sequence of SEQ ID NO: 69 or an amino acid sequence that is at least 95% identical to the

amino acid sequence of SEQ ID NO: 69 optionally comprising CDRs that are 100% identical to the CDRs of the polypeptide chain of SEQ ID NO: 69; the second polypeptide chain comprises the amino acid sequence of SEQ ID NO: 68 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 68 optionally comprising CDRs that are 100% identical to the CDRs of the polypeptide chain of SEQ ID NO: 68; the third polypeptide chain comprises the amino acid sequence of SEQ ID NO: 60 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 60 optionally comprising CDRs that are 100% identical to the CDRs of the polypeptide chain of SEQ ID NO: 60; and the fourth polypeptide chain comprises the amino acid sequence of SEQ ID NO: 71 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 71 optionally comprising CDRs that are 100% identical to the CDRs of the polypeptide chain of SEQ ID NO: 71.

[0138] In some embodiments, the first polypeptide chain comprises the amino acid sequence of SEQ ID NO: 76 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 76 optionally comprising CDRs that are 100% identical to the CDRs of the polypeptide chain of SEQ ID NO: 76; the second polypeptide chain comprises the amino acid sequence of SEQ ID NO: 75 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 75 optionally comprising CDRs that are 100% identical to the CDRs of the polypeptide chain of SEQ ID NO: 75; the third polypeptide chain comprises the amino acid sequence of SEQ ID NO: 73 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 73 optionally comprising CDRs that are 100% identical to the CDRs of the polypeptide chain of SEQ ID NO: 73; and the fourth polypeptide chain comprises the amino acid sequence of SEQ ID NO: 74 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 74 optionally comprising CDRs that are 100% identical to the CDRs of the polypeptide chain of SEQ ID NO: 74.

[0139] In some embodiments, the first polypeptide chain comprises the amino acid sequence of SEQ ID NO: 82 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 82 optionally comprising CDRs that are 100% identical to the CDRs of the polypeptide chain of SEQ ID NO: 82; the second polypeptide chain comprises the amino acid sequence of SEQ ID NO: 81 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO:81 optionally comprising CDRs that are 100% identical to the CDRs of the polypeptide chain of SEQ ID NO: 81; the third polypeptide chain comprises the amino acid sequence of SEQ ID NO: 73 or an amino

acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 73 optionally comprising CDRs that are 100% identical to the CDRs of the polypeptide chain of SEQ ID NO: 73; and the fourth polypeptide chain comprises the amino acid sequence of SEQ ID NO: 74 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 74 optionally comprising CDRs that are 100% identical to the CDRs of the polypeptide chain of SEQ ID NO: 74.

[0140] In some embodiments, the first polypeptide chain comprises the amino acid sequence of SEQ ID NO: 88 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 88 optionally comprising CDRs that are 100% identical to the CDRs of the polypeptide chain of SEQ ID NO: 88; the second polypeptide chain comprises the amino acid sequence of SEQ ID NO: 87 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 87 optionally comprising CDRs that are 100% identical to the CDRs of the polypeptide chain of SEQ ID NO: 87; the third polypeptide chain comprises the amino acid sequence of SEQ ID NO: 85 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 85 optionally comprising CDRs that are 100% identical to the CDRs of the polypeptide chain of SEQ ID NO: 85; and the fourth polypeptide chain comprises the amino acid sequence of SEQ ID NO: 86 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 86 optionally comprising CDRs that are 100% identical to the CDRs of the polypeptide chain of SEQ ID NO: 86.

[0141] In some embodiments, the first polypeptide chain comprises the amino acid sequence of SEQ ID NO: 94 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 94 optionally comprising CDRs that are 100% identical to the CDRs of the polypeptide chain of SEQ ID NO: 94; the second polypeptide chain comprises the amino acid sequence of SEQ ID NO: 93 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 93 optionally comprising CDRs that are 100% identical to the CDRs of the polypeptide chain of SEQ ID NO: 93; the third polypeptide chain comprises the amino acid sequence of SEQ ID NO: 85 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 85 optionally comprising CDRs that are 100% identical to the CDRs of the polypeptide chain of SEQ ID NO: 85; and the fourth polypeptide chain comprises the amino acid sequence of SEQ ID NO: 86 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 86 optionally comprising CDRs that are 100% identical to the CDRs of the polypeptide chain of SEQ ID NO: 86.

[0142] In some embodiments, the first polypeptide chain comprises the amino acid sequence of SEQ ID NO: 69 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 69 optionally comprising CDRs that are 100% identical to the CDRs of the polypeptide chain of SEQ ID NO: 69; the second polypeptide chain comprises the amino acid sequence of SEQ ID NO: 68 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 68 optionally comprising CDRs that are 100% identical to the CDRs of the polypeptide chain of SEQ ID NO: 68; the third polypeptide chain comprises the amino acid sequence of SEQ ID NO: 73 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 73 optionally comprising CDRs that are 100% identical to the CDRs of the polypeptide chain of SEQ ID NO: 73; and the fourth polypeptide chain comprises the amino acid sequence of SEQ ID NO: 74 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 74 optionally comprising CDRs that are 100% identical to the CDRs of the polypeptide chain of SEQ ID NO: 74.

[0143] In some embodiments, the first polypeptide chain comprises the amino acid sequence of SEQ ID NO: 69 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 69 optionally comprising CDRs that are 100% identical to the CDRs of the polypeptide chain of SEQ ID NO: 69; the second polypeptide chain comprises the amino acid sequence of SEQ ID NO: 68 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 68 optionally comprising CDRs that are 100% identical to the CDRs of the polypeptide chain of SEQ ID NO: 68; the third polypeptide chain comprises the amino acid sequence of SEQ ID NO: 85 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 85 optionally comprising CDRs that are 100% identical to the CDRs of the polypeptide chain of SEQ ID NO: 85; and the fourth polypeptide chain comprises the amino acid sequence of SEQ ID NO: 86 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 86 optionally comprising CDRs that are 100% identical to the CDRs of the polypeptide chain of SEQ ID NO: 86.

[0144] In some embodiments, the first polypeptide chain comprises the amino acid sequence of SEQ ID NO: 63 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 63 optionally comprising CDRs that are 100% identical to the CDRs of the polypeptide chain of SEQ ID NO: 63; the second polypeptide chain comprises the amino acid sequence of SEQ ID NO: 62 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 62 optionally comprising

CDRs that are 100% identical to the CDRs of the polypeptide chain of SEQ ID NO: 62; the third polypeptide chain comprises the amino acid sequence of SEQ ID NO: 73 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 73 optionally comprising CDRs that are 100% identical to the CDRs of the polypeptide chain of SEQ ID NO: 73; and the fourth polypeptide chain comprises the amino acid sequence of SEQ ID NO: 74 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 74 optionally comprising CDRs that are 100% identical to the CDRs of the polypeptide chain of SEQ ID NO: 74.

[0145] In some embodiments, the first polypeptide chain comprises the amino acid sequence of SEQ ID NO: 63 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 63 optionally comprising CDRs that are 100% identical to the CDRs of the polypeptide chain of SEQ ID NO: 63; the second polypeptide chain comprises the amino acid sequence of SEQ ID NO: 62 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 62 optionally comprising CDRs that are 100% identical to the CDRs of the polypeptide chain of SEQ ID NO: 62; the third polypeptide chain comprises the amino acid sequence of SEQ ID NO: 85 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 85 optionally comprising CDRs that are 100% identical to the CDRs of the polypeptide chain of SEQ ID NO: 85; and the fourth polypeptide chain comprises the amino acid sequence of SEQ ID NO: 86 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 86 optionally comprising CDRs that are 100% identical to the CDRs of the polypeptide chain of SEQ ID NO: 86.

[0146] In some embodiments, the first polypeptide chain comprises the amino acid sequence of SEQ ID NO: 4 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 4 optionally comprising CDRs that are 100% identical to the CDRs of the polypeptide chain of SEQ ID NO: 4; the second polypeptide chain comprises the amino acid sequence of SEQ ID NO: 3 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 3 optionally comprising CDRs that are 100% identical to the CDRs of the polypeptide chain of SEQ ID NO: 3; the third polypeptide chain comprises the amino acid sequence of SEQ ID NO: 114 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 114 optionally comprising CDRs that are 100% identical to the CDRs of the polypeptide chain of SEQ ID NO: 114; and the fourth polypeptide chain comprises the amino acid sequence of SEQ ID NO: 115 or an amino acid sequence that is at least 95% identical to the amino acid

sequence of SEQ ID NO: 115 optionally comprising CDRs that are 100% identical to the CDRs of the polypeptide chain of SEQ ID NO: 115.

[0147] In some embodiments, the first polypeptide chain comprises the amino acid sequence of SEQ ID NO: 10 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 10 optionally comprising CDRs that are 100% identical to the CDRs of the polypeptide chain of SEQ ID NO: 10; the second polypeptide chain comprises the amino acid sequence of SEQ ID NO: 9 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 9 optionally comprising CDRs that are 100% identical to the CDRs of the polypeptide chain of SEQ ID NO: 9; the third polypeptide chain comprises the amino acid sequence of SEQ ID NO: 114 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 114 optionally comprising CDRs that are 100% identical to the CDRs of the polypeptide chain of SEQ ID NO: 114; and the fourth polypeptide chain comprises the amino acid sequence of SEQ ID NO: 115 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 115 optionally comprising CDRs that are 100% identical to the CDRs of the polypeptide chain of SEQ ID NO: 115.

[0148] In other embodiments, the binding protein of the disclosure is a trispecific and/or trivalent binding protein comprising four polypeptide chains that form three antigen binding sites that specifically bind one or more (*e.g.*, three) different target proteins, wherein a first polypeptide chain has a structure represented by the formula:

$$V_{L2}\text{-}L_1\text{-}V_{L1}\text{-}L_2\text{-}C_L \qquad [I]$$

and a second polypeptide chain has a structure represented by the formula:

$$V_{H1}\text{-}L_3\text{-}V_{H2}\text{-}L_4\text{-}C_{H1}\text{-hinge-}C_{H2}\text{-}C_{H3}\text{ (hole)} \qquad [II]$$

and a third polypeptide chain has a structure represented by the formula:

$$V_{H3}\text{-}C_{H1}\text{-hinge-}C_{H2}\text{-}C_{H3}\text{ (knob)} \qquad [III]$$

and a fourth polypeptide chain has a structure represented by the formula:

$$V_{L3}\text{-}C_L \qquad [IV]$$

wherein:

V_{L1} is a first immunoglobulin light chain variable domain;

V_{L2} is a second immunoglobulin light chain variable domain;

V_{L3} is a third immunoglobulin light chain variable domain;

V_{H1} is a first immunoglobulin heavy chain variable domain;

V_{H2} is a second immunoglobulin heavy chain variable domain;

V_{H3} is a third immunoglobulin heavy chain variable domain;

C_L is an immunoglobulin light chain constant domain;

C_{H1} is an immunoglobulin C_{H1} heavy chain constant domain; and

L_1, L_2, L_3 and L_4 are amino acid linkers;

and wherein the polypeptide of formula I and the polypeptide of formula II form a cross-over light chain-heavy chain pair.

[0149] In other embodiments, the binding protein of the disclosure is a trispecific and/or trivalent binding protein comprising four polypeptide chains that form three antigen binding sites that specifically bind one or more (*e.g.*, three) target proteins, wherein a first polypeptide chain has a structure represented by the formula:

$$V_{L2}\text{-}L_1\text{-}V_{L1}\text{-}L_2\text{-}C_L \qquad [I]$$

and a second polypeptide chain has a structure represented by the formula:

$$V_{H1}\text{-}L_3\text{-}V_{H2}\text{-}L_4\text{-}C_{H1}\text{-hinge-}C_{H2}\text{-}C_{H3}\text{ (hole)} \qquad [II]$$

and a third polypeptide chain has a structure represented by the formula:

$$V_{H3}\text{-}C_{H1}\text{-hinge-}C_{H2}\text{-}C_{H3}\text{ (knob)} \qquad [III]$$

and a fourth polypeptide chain has a structure represented by the formula:

$$V_{L3}\text{-}C_L \qquad [IV]$$

wherein:

V_{L1} is a first immunoglobulin light chain variable domain;

V_{L2} is a second immunoglobulin light chain variable domain;

V_{L3} is a third immunoglobulin light chain variable domain;

V_{H1} is a first immunoglobulin heavy chain variable domain;

V_{H2} is a second immunoglobulin heavy chain variable domain;

V_{H3} is a third immunoglobulin heavy chain variable domain;

C_L is an immunoglobulin light chain constant domain;

C_{H1} is an immunoglobulin C_{H1} heavy chain constant domain; and

L_1, L_2, L_3 and L_4 are amino acid linkers;

and wherein the polypeptide of formula I and the polypeptide of formula II form a cross-over light chain-heavy chain pair.

[0150] In some embodiments, the first polypeptide chain and the second polypeptide chain have a cross-over orientation that forms two distinct antigen binding sites. In some embodiments, the VH1 and VL1 form a binding pair and form the first antigen binding site. In some embodiments, the VH2 and VL2 form a binding pair and form the second antigen binding site. In some embodiments, the third polypeptide and the fourth polypeptide form a third antigen binding site. In some embodiments, the VH3 and VL3 form a binding pair and

form the third antigen binding site. In some embodiments, the second polypeptide chain and the third polypeptide chain comprise one or more modifications. In some embodiments, the second polypeptide chain and the third polypeptide chain of a binding protein are different, *e.g.*, having different C_{H1}, C_{H2}, and/or C_{H3} domain(s) (such as those including a modification described herein). In some embodiments, the first polypeptide chain and the fourth polypeptide chain comprise one or more modifications. In some embodiments, the first polypeptide chain and the fourth polypeptide chain of a binding protein are different, *e.g.*, having different C_L domains (such as those including a modification described herein, and/or lambda vs. kapp C_L domains).

[0151] In some embodiments, a binding protein of the present disclosure comprises an antigen binding site comprising a heavy chain variable domain comprising an amino acid sequence that is at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO:150, optionally comprising CDRs that are 100% identical to the CDRs of the polypeptide chain of SEQ ID NO: 150, and/or a light chain variable domain comprising an amino acid sequence that is at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO:151, optionally comprising CDRs that are 100% identical to the CDRs of the polypeptide chain of SEQ ID NO: 151. In some embodiments, a binding protein of the present disclosure comprises an antigen binding site comprising a heavy chain variable domain comprising an amino acid sequence that is at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO:152, optionally comprising CDRs that are 100% identical to the CDRs of the polypeptide chain of SEQ ID NO: 152, and/or a light chain variable domain comprising an amino acid sequence that is at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO:153, optionally comprising CDRs that are 100% identical to the CDRs of the polypeptide chain of SEQ ID NO: 153. In some embodiments, a binding protein of the present disclosure comprises an antigen binding site comprising a heavy chain variable domain comprising an amino acid sequence that is at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO:154, optionally comprising CDRs that are 100% identical to the CDRs of the polypeptide chain of SEQ ID NO: 154, and/or a light chain variable domain comprising an amino acid sequence that is at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO:155, optionally comprising CDRs that are 100% identical to the CDRs of the polypeptide chain of SEQ ID NO: 155. In some embodiments, a binding protein of the present disclosure comprises an antigen binding site comprising a heavy chain variable domain comprising an

amino acid sequence that is at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO:156, optionally comprising CDRs that are 100% identical to the CDRs of the polypeptide chain of SEQ ID NO: 156, and/or a light chain variable domain comprising an amino acid sequence that is at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO:157, optionally comprising CDRs that are 100% identical to the CDRs of the polypeptide chain of SEQ ID NO: 157. In some embodiments, a binding protein of the present disclosure comprises an antigen binding site comprising a heavy chain variable domain comprising an amino acid sequence that is at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO:158, optionally comprising CDRs that are 100% identical to the CDRs of the polypeptide chain of SEQ ID NO: 158, and/or a light chain variable domain comprising an amino acid sequence that is at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO:159, optionally comprising CDRs that are 100% identical to the CDRs of the polypeptide chain of SEQ ID NO: 159. In some embodiments, a binding protein of the present disclosure comprises an antigen binding site comprising a heavy chain variable domain comprising an amino acid sequence that is at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO:160, optionally comprising CDRs that are 100% identical to the CDRs of the polypeptide chain of SEQ ID NO: 160, and/or a light chain variable domain comprising an amino acid sequence that is at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO:161, optionally comprising CDRs that are 100% identical to the CDRs of the polypeptide chain of SEQ ID NO: 161. In some embodiments, a binding protein of the present disclosure comprises an antigen binding site comprising a heavy chain variable domain comprising an amino acid sequence that is at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO:162, optionally comprising CDRs that are 100% identical to the CDRs of the polypeptide chain of SEQ ID NO: 162, and/or a light chain variable domain comprising an amino acid sequence that is at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO:163, optionally comprising CDRs that are 100% identical to the CDRs of the polypeptide chain of SEQ ID NO: 163. In some embodiments, a binding protein of the present disclosure comprises an antigen binding site comprising a heavy chain variable domain comprising an amino acid sequence that is at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO:164, optionally comprising CDRs that are 100% identical to the CDRs of the polypeptide chain of SEQ ID NO: 164, and/or a light chain variable domain comprising an amino acid

sequence that is at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO:165, optionally comprising CDRs that are 100% identical to the CDRs of the polypeptide chain of SEQ ID NO: 165. In some embodiments, a binding protein of the present disclosure comprises an antigen binding site comprising a heavy chain variable domain comprising an amino acid sequence that is at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO:166, optionally comprising CDRs that are 100% identical to the CDRs of the polypeptide chain of SEQ ID NO: 166, and/or a light chain variable domain comprising an amino acid sequence that is at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO:167, optionally comprising CDRs that are 100% identical to the CDRs of the polypeptide chain of SEQ ID NO: 167. In some embodiments, a binding protein of the present disclosure comprises an antigen binding site comprising a heavy chain variable domain comprising an amino acid sequence that is at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO:168, optionally comprising CDRs that are 100% identical to the CDRs of the polypeptide chain of SEQ ID NO: 168, and/or a light chain variable domain comprising an amino acid sequence that is at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO:169, optionally comprising CDRs that are 100% identical to the CDRs of the polypeptide chain of SEQ ID NO: 169. In some embodiments, a binding protein of the present disclosure comprises an antigen binding site comprising a heavy chain variable domain comprising an amino acid sequence that is at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO:170, optionally comprising CDRs that are 100% identical to the CDRs of the polypeptide chain of SEQ ID NO: 170, and/or a light chain variable domain comprising an amino acid sequence that is at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO:171, optionally comprising CDRs that are 100% identical to the CDRs of the polypeptide chain of SEQ ID NO: 171. In some embodiments, a binding protein of the present disclosure comprises an antigen binding site comprising a heavy chain variable domain comprising an amino acid sequence that is at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO:172, optionally comprising CDRs that are 100% identical to the CDRs of the polypeptide chain of SEQ ID NO: 172, and/or a light chain variable domain comprising an amino acid sequence that is at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO:173, optionally comprising CDRs that are 100% identical to the CDRs of the polypeptide chain of SEQ ID NO: 173.

[0152] In some embodiments, a binding protein of the present disclosure binds to one, two, or three antigen targets with an equilibrium dissociation constant (K_D) that is less than or equal to 1μM, 500nM, 100nM, 50nM, 10nM, 5nM, or 1nM. Exemplary assays for determining K_D are known in the art. For example, in some embodiments, K_D is determined by measuring binding kinetics at between 0°C and 37°C *e.g.*, at 0°C, 4°C, 25°C, or 37°C) using the techniques described in Example 1 (*e.g.*, SPR or ELISA).

[0153] In some embodiments, a binding protein of the present disclosure activates CD4 and/or CD8 T cells *in vitro* and/or induces antibody-mediated *in vitro* cell killing of a cell expressing one or more antigen targets of one or more binding domains of the binding protein. Exemplary *in vitro* cell killing and T cell activation assays are known in the art. For example, in some embodiments, *in vitro* cell killing and/or T cell activation is assayed using the techniques described in Example 1.

[0154] In some embodiments, a binding protein of the present disclosure specifically binds to, and/or blocks signaling mediated by, one or more cytokines. Exemplary cytokine release assays are known in the art. For example, in some embodiments, cytokine release is assayed using the techniques described in Example 1.

[0155] In some embodiments, a binding protein of the present disclosure comprises a first antigen binding site that specifically binds a target protein on T cells, a second antigen binding site that specifically binds a target protein on T cells, and a third antigen binding site that specifically binds an antigen target or target protein. In some embodiments, a binding protein of the present disclosure comprises a first antigen binding site that specifically binds a target protein on T cells, a second antigen binding site that specifically binds a target protein on T cells, and a third antigen binding site that specifically binds a tumor target protein. In some embodiments, a binding protein of the present disclosure comprises a first antigen binding site that specifically binds a target protein on T cells, a second antigen binding site that specifically binds a target protein on T cells, and a third antigen binding site that specifically binds a human tumor target protein. In some some embodiments, the first and second antigen binding sites specifically bind a tumor target protein for instance selected from CD3 and CD28, respectively. In some some embodiments, the first and second antigen binding sites specifically bind a tumor target protein for instance selected from CD28 and CD3, respectively. In some embodiments, the third antigen binding site specifically binds CD19, CD20, CD38, Her2, or LAMP1. Further examples of such targets and target proteins are provided *infra*.

[0156] In some embodiments, a binding protein of the present disclosure comprises a first antigen binding site that specifically binds CD3, a second antigen binding site that specifically binds CD28, and a third antigen binding site that specifically binds an antigen target or target protein. In some embodiments, a binding protein of the present disclosure comprises a first antigen binding site that specifically binds CD28, a second antigen binding site that specifically binds CD3, and a third antigen binding site that specifically binds an antigen target or target protein. Further examples of such antigen targets or target proteins are provided *infra*. In some embodiments, a binding protein of the present disclosure comprises a first antigen binding site that specifically binds CD3, a second antigen binding site that specifically binds CD28, and a third antigen binding site that specifically binds a tumor target protein. In some embodiments, a binding protein of the present disclosure comprises a first antigen binding site that specifically binds human CD3, a second antigen binding site that specifically binds human CD28, and a third antigen binding site that specifically binds a human tumor target protein. In some embodiments, a binding protein of the present disclosure comprises a first antigen binding site that specifically binds CD28, a second antigen binding site that specifically binds CD3, and a third antigen binding site that specifically binds a tumor target protein. In some embodiments, a binding protein of the present disclosure comprises a first antigen binding site that specifically binds human CD28, a second antigen binding site that specifically binds human CD3, and a third antigen binding site that specifically binds a human tumor target protein. In some embodiments, the third antigen binding site specifically binds CD19, CD20, CD38, Her2, or LAMP1. Further examples of such tumor antigen targets or tumor target proteins are provided *infra*.

[0157] In some embodiments, the antigen binding site that specifically binds CD3 comprises a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 152 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO: 153; or a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 154 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO: 155. Additional VH, VL, and/or CDR sequences of antibodies that specifically bind CD3 suitable for use in any of the binding proteins described herein may be found in International Publication No. WO2016/116626, which is incorporated by reference herein in its entirety. In some embodiments, the antigen binding site that specifically binds CD3 comprises six CDRs, or a heavy chain and a light chain variable domain, shown in Tables 2-5. In some embodiments, the antigen binding site that specifically binds CD3 comprises (i) three heavy chain CDRs of SEQ ID Nos. 34, 35 and 36, respectively, and three light chain

CDRs of SEQ ID Nos. 52, 53 and 54, respectively; or (ii) three heavy chain CDRs of SEQ ID Nos. 34, 35 and 36, respectively, and three light chain CDRs of SEQ ID Nos. 149, 53 and 54, respectively. In some embodiments, the antigen binding site that specifically binds CD3 is part of a polypeptide chain comprising the amino acid sequence of SEQ ID NO:3 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO:3 optionally comprising CDRs that are 100% identical to the CDRs of the polypeptide chain of SEQ ID NO:3. In some embodiments, the antigen binding site that specifically binds CD3 is part of a polypeptide chain comprising the amino acid sequence of SEQ ID NO:4 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO:4 optionally comprising CDRs that are 100% identical to the CDRs of the polypeptide chain of SEQ ID NO:4.

[0158] In some embodiments, the antigen binding site that specifically binds CD28 comprises a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 160 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO: 161; or a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 162 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO: 163. In some embodiments, the antigen binding site that specifically binds CD28 comprises six CDRs, or a heavy chain and a light chain variable domain, shown in Tables 2-5. In some embodiments, the antigen binding site that specifically binds CD28 comprises (i) three heavy chain CDRs of SEQ ID Nos. 28, 29 and 30, respectively, and three light chain CDRs of SEQ ID Nos. 46, 47 and 48, respectively; or (ii) three heavy chain CDRs of SEQ ID Nos. 31, 32 and 33, respectively, and three light chain CDRs of SEQ ID Nos. 49, 50 and 51, respectively. In some embodiments, the antigen binding site that specifically binds CD28 is part of a polypeptide chain comprising the amino acid sequence of SEQ ID NO:3 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO:3 optionally comprising CDRs that are 100% identical to the CDRs of the polypeptide chain of SEQ ID NO:3. In some embodiments, the antigen binding site that specifically binds CD28 is part of a polypeptide chain comprising the amino acid sequence of SEQ ID NO:4 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO:4 optionally comprising CDRs that are 100% identical to the CDRs of the polypeptide chain of SEQ ID NO:4.

[0159] In some embodiments, a binding protein of the present disclosure comprises a first antigen binding site that specifically binds CD3, a second antigen binding site that specifically binds CD28, and a third antigen binding site that specifically binds CD38, or a

first antigen binding site that specifically binds CD28, a second antigen binding site that specifically binds CD3, and a third antigen binding site that specifically binds CD38, wherein:

- the antigen binding site specifically binding CD3 comprises (i) three heavy chain CDRs of SEQ ID Nos. 34, 35 and 36, respectively, and three light chain CDRs of SEQ ID Nos. 52, 53 and 54, respectively; or (ii) three heavy chain CDRs of SEQ ID Nos. 34, 35 and 36, respectively, and three light chain CDRs of SEQ ID Nos. 149, 53 and 54, respectively; and
- the antigen binding site specifically binding CD28 comprises (i) three heavy chain CDRs of SEQ ID Nos. 28, 29 and 30, respectively, and three light chain CDRs of SEQ ID Nos. 46, 47 and 48, respectively; or (ii) three heavy chain CDRs of SEQ ID Nos. 31, 32 and 33, respectively, and three light chain CDRs of SEQ ID Nos. 49, 50 and 51, respectively; and
- the antigen binding site specifically binding CD38 comprises (i) three heavy chain CDRs of SEQ ID Nos. 40, 41 and 42, respectively, and three light chain CDRs of SEQ ID Nos. 58, 44 and 59, respectively.

[0160] In some embodiments, the antigen binding site that specifically binds a tumor target protein comprises a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 156 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO: 157; a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 158 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO: 159; a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 164 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO: 165; a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 150 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO: 151; or a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 166 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO: 167. In some embodiments, the antigen binding site that specifically binds a tumor target protein comprises six CDRs, or a heavy chain and a light chain variable domain, shown in Tables 2-5. In some embodiments, the antigen binding site that specifically binds a tumor target protein comprises six CDRs of an anti-Her2, anti-CD19, anti-CD20, anti-CD38, or anti-LAMP1 binding domain shown in Tables 2-5.

[0161] In some embodiments, a binding protein of the present disclosure comprises four polypeptide chains that form three antigen binding sites, wherein a first polypeptide chain comprises a structure represented by the formula:

$$V_{L2}\text{-}L_1\text{-}V_{L1}\text{-}L_2\text{-}C_L \qquad [I]$$

and a second polypeptide chain comprises a structure represented by the formula:

$$V_{H1}\text{-}L_3\text{-}V_{H2}\text{-}L_4\text{-}C_{H1}\text{-hinge-}C_{H2}\text{-}C_{H3} \qquad [II]$$

and a third polypeptide chain comprises a structure represented by the formula:

$$V_{H3}\text{-}C_{H1}\text{-hinge-}C_{H2}\text{-}C_{H3} \qquad [III]$$

and a fourth polypeptide chain comprises a structure represented by the formula:

$$V_{L3}\text{-}C_L \qquad [IV]$$

wherein:

V_{L1} is a first immunoglobulin light chain variable domain;

V_{L2} is a second immunoglobulin light chain variable domain;

V_{L3} is a third immunoglobulin light chain variable domain;

V_{H1} is a first immunoglobulin heavy chain variable domain;

V_{H2} is a second immunoglobulin heavy chain variable domain;

V_{H3} is a third immunoglobulin heavy chain variable domain;

C_L is an immunoglobulin light chain constant domain;

C_{H1} is an immunoglobulin C_{H1} heavy chain constant domain;

C_{H2} is an immunoglobulin C_{H2} heavy chain constant domain;

C_{H3} is an immunoglobulin C_{H3} heavy chain constant domain;

hinge is an immunoglobulin hinge region connecting the C_{H1} and C_{H2} domains; and

L_1, L_2, L_3 and L_4 are amino acid linkers;

wherein the polypeptide of formula I and the polypeptide of formula II form a cross-over light chain-heavy chain pair;

wherein:

V_{L1}, V_{L2} and V_{L3} each independently comprise a variable domain sequence as set forth in any one of SEQ ID NOs:169, 171, and 173; and

wherein:

V_{H1}, V_{H2}, and V_{H3} each independently comprise a variable domain sequence as set forth in any one of SEQ ID NOs:168, 170, and 172.

[0162] In some embodiments, a binding protein of the present disclosure comprises four polypeptide chains that form three antigen binding sites, wherein a first polypeptide chain comprises a structure represented by the formula:

V_{L2}-L_1-V_{L1}-L_2-C_L [I]

and a second polypeptide chain comprises a structure represented by the formula:

V_{H1}-L_3-V_{H2}-L_4-C_{H1}-hinge-C_{H2}-C_{H3} [II]

and a third polypeptide chain comprises a structure represented by the formula:

V_{H3}-C_{H1}-hinge-C_{H2}-C_{H3} [III]

and a fourth polypeptide chain comprises a structure represented by the formula:

V_{L3}-C_L [IV]

wherein:

V_{L1} is a first immunoglobulin light chain variable domain;

V_{L2} is a second immunoglobulin light chain variable domain;

V_{L3} is a third immunoglobulin light chain variable domain;

V_{H1} is a first immunoglobulin heavy chain variable domain;

V_{H2} is a second immunoglobulin heavy chain variable domain;

V_{H3} is a third immunoglobulin heavy chain variable domain;

C_L is an immunoglobulin light chain constant domain;

C_{H1} is an immunoglobulin C_{H1} heavy chain constant domain;

C_{H2} is an immunoglobulin C_{H2} heavy chain constant domain;

C_{H3} is an immunoglobulin C_{H3} heavy chain constant domain;

hinge is an immunoglobulin hinge region connecting the C_{H1} and C_{H2} domains; and

L_1, L_2, L_3 and L_4 are amino acid linkers;

wherein the polypeptide of formula I and the polypeptide of formula II form a cross-over light chain-heavy chain pair;

wherein:

V_{L1}, V_{L2} and V_{L3} each independently comprise light chain complementarity determining regions comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 141-147, 178, and 179; and

wherein:

V_{H1}, V_{H2} and V_{H3} each independently comprise heavy chain complementarity determining regions comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 129-137.

[0163] In some embodiments, a binding protein of the present disclosure comprises four polypeptide chains that form three antigen binding sites, wherein a first polypeptide chain comprises a structure represented by the formula:

V_{L2}-L_1-V_{L1}-L_2-C_L [I]

and a second polypeptide chain comprises a structure represented by the formula:

$$V_{H1}\text{-}L_3\text{-}V_{H2}\text{-}L_4\text{-}C_{H1}\text{-hinge-}C_{H2}\text{-}C_{H3} \quad [II]$$

and a third polypeptide chain comprises a structure represented by the formula:

$$V_{H3}\text{-}C_{H1}\text{-hinge-}C_{H2}\text{-}C_{H3} \quad [III]$$

and a fourth polypeptide chain comprises a structure represented by the formula:

$$V_{L3}\text{-}C_L \quad [IV]$$

wherein:

V_{L1} is a first immunoglobulin light chain variable domain;

V_{L2} is a second immunoglobulin light chain variable domain;

V_{L3} is a third immunoglobulin light chain variable domain;

V_{H1} is a first immunoglobulin heavy chain variable domain;

V_{H2} is a second immunoglobulin heavy chain variable domain;

V_{H3} is a third immunoglobulin heavy chain variable domain;

C_L is an immunoglobulin light chain constant domain;

C_{H1} is an immunoglobulin C_{H1} heavy chain constant domain;

C_{H2} is an immunoglobulin C_{H2} heavy chain constant domain;

C_{H3} is an immunoglobulin C_{H3} heavy chain constant domain;

hinge is an immunoglobulin hinge region connecting the C_{H1} and C_{H2} domains; and

L_1, L_2, L_3 and L_4 are amino acid linkers;

wherein the polypeptide of formula I and the polypeptide of formula II form a cross-over light chain-heavy chain pair;

wherein:

V_{L1}, V_{L2} and V_{L3} each independently comprise a variable domain sequence as set forth in any one of SEQ ID NOs:151, 153, 155, 157, 159, 161, 163, 165, and 167; and

wherein:

V_{H1}, V_{H2}, and V_{H3} each independently comprise a variable domain sequence as set forth in any one of SEQ ID NOs:150, 152, 154, 156, 158, 160, 162, 164, and 166.

[0164] In some embodiments, a binding protein of the present disclosure comprises four polypeptide chains that form three antigen binding sites, wherein a first polypeptide chain comprises a structure represented by the formula:

$$V_{L2}\text{-}L_1\text{-}V_{L1}\text{-}L_2\text{-}C_L \quad [I]$$

and a second polypeptide chain comprises a structure represented by the formula:

$$V_{H1}\text{-}L_3\text{-}V_{H2}\text{-}L_4\text{-}C_{H1}\text{-hinge-}C_{H2}\text{-}C_{H3} \quad [II]$$

and a third polypeptide chain comprises a structure represented by the formula:

$$V_{H3}\text{-}C_{H1}\text{-hinge-}C_{H2}\text{-}C_{H3} \quad [III]$$

and a fourth polypeptide chain comprises a structure represented by the formula:

$$V_{L3}\text{-}C_{L} \quad [IV]$$

wherein:

V_{L1} is a first immunoglobulin light chain variable domain;

V_{L2} is a second immunoglobulin light chain variable domain;

V_{L3} is a third immunoglobulin light chain variable domain;

V_{H1} is a first immunoglobulin heavy chain variable domain;

V_{H2} is a second immunoglobulin heavy chain variable domain;

V_{H3} is a third immunoglobulin heavy chain variable domain;

C_L is an immunoglobulin light chain constant domain;

C_{H1} is an immunoglobulin C_{H1} heavy chain constant domain;

C_{H2} is an immunoglobulin C_{H2} heavy chain constant domain;

C_{H3} is an immunoglobulin C_{H3} heavy chain constant domain;

hinge is an immunoglobulin hinge region connecting the C_{H1} and C_{H2} domains; and

L_1, L_2, L_3 and L_4 are amino acid linkers;

wherein the polypeptide of formula I and the polypeptide of formula II form a cross-over light chain-heavy chain pair;

wherein:

V_{L1}, V_{L2} and V_{L3} each independently comprise light chain complementarity determining regions comprising an amino acid sequence as set forth in any one of SEQ ID NOs: 43-59, 123-125, 138-140, and 149; and

wherein:

V_{H1}, V_{H2} and V_{H3} each independently comprise heavy chain complementarity determining regions comprising an amino acid sequence as set forth in any one of SEQ ID NOs: 25-42, 120-122, and 126-128.

[0165] In some embodiments, a binding protein of the present disclosure comprises an antigen binding site that specifically binds CD3, an antigen binding site that specifically binds CD28, and an antigen binding site that specifically binds an antigen target other than CD3 or CD28. In some embodiments, a binding protein of the present disclosure comprises an antigen binding site that specifically binds human CD3, an antigen binding site that specifically binds human CD28, and an antigen binding site that specifically binds a human antigen target other than CD3 or CD28. In some embodiments, a binding protein of the present disclosure comprises (a) an antigen binding site that specifically binds CD3, wherein

the antigen binding site that specifically binds CD3 comprises (i) a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 152 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO: 153, (ii) a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 154 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO: 155, (iii) a heavy chain variable domain comprising a CDR-H1 comprising the amino acid sequence of SEQ ID NO:34, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:35, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:36, and a light chain variable domain comprising a CDR-L1 comprising the amino acid sequence of SEQ ID NO:52, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:53, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:54, or (iv) a heavy chain variable domain comprising a CDR-H1 comprising the amino acid sequence of SEQ ID NO:34, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:35, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:36, and a light chain variable domain comprising a CDR-L1 comprising the amino acid sequence of SEQ ID NO:149, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:53, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:54; (b) an antigen binding site that specifically binds CD28, wherein the antigen binding site that specifically binds CD28 comprises (i) a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 160 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO: 161, (ii) a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 162 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO: 163, (iii) a heavy chain variable domain comprising a CDR-H1 comprising the amino acid sequence of SEQ ID NO:28, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:29, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:30, and a light chain variable domain comprising a CDR-L1 comprising the amino acid sequence of SEQ ID NO:46, a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 47, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:48, or (iv) a heavy chain variable domain comprising a CDR-H1 comprising the amino acid sequence of SEQ ID NO:31, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:32, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:33, and a light chain variable domain comprising a CDR-L1 comprising the amino acid sequence of SEQ ID NO:49, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:50, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:51; and (c) an antigen binding site that specifically binds an antigen target other than CD3 or CD28. In some embodiments, a binding protein of the present disclosure comprises a

first polypeptide chain comprising the amino acid sequence of SEQ ID NO:4 or 10, a second polypeptide chain comprising the amino acid sequence of SEQ ID NO:3 or 9, and a third and a fourth polypeptide chain, wherein the third and fourth polypeptide chains form an antigen binding domain that specifically binds an antigen target other than CD3 or CD28. In some embodiments, the antigen binding site that specifically binds an antigen target other than CD3 or CD28 binds an antigen target selected from A2AR, APRIL, ATPDase, BAFF, BAFFR, BCMA, BlyS, BTK, BTLA, B7DC, B7H1, B7H4 (also known as VTCN1), B7H5, B7H6, B7H7, B7RP1, B7-4, C3, C5, CCL2 (also known as MCP-1), CCL3 (also known as MIP-1a), CCL4 (also known as MIP-1b), CCL5 (also known as RANTES), CCL7 (also known as MCP-3), CCL8 (also known as mcp-2), CCL11 (also known as eotaxin), CCL15 (also known as MIP-1d), CCL17 (also known as TARC), CCL19 (also known as MIP-3b), CCL20 (also known as MIP-3a), CCL21 (also known as MIP-2), CCL24 (also known as MPIF-2/eotaxin-2), CCL25 (also known as TECK), CCL26 (also known as eotaxin-3), CCR3, CCR4, CD19, CD20, CD23 (also known as FCER2, a receptor for IgE), CD24, CD27, CD38, CD39, CD40, CD70, CD80 (also known as B7-1), CD86 (also known as B7-2), CD122, CD137 (also known as 41BB), CD137L, CD152 (also known as CTLA4), CD154 (also known as CD40L), CD160, CD272, CD273 (also known as PDL2), CD274 (also known as PDL1), CD275 (also known as B7H2), CD276 (also known as B7H3), CD278 (also known as ICOS), CD279 (also known as PD-1), CDH1 (also known as E-cadherin), chitinase, CLEC9, CLEC91, CRTH2, CSF-1 (also known as M-CSF), CSF-2 (also known as GM-CSF), CSF-3 (also known as GCSF), CX3CL1 (also known as SCYD1), CXCL12 (also known as SDF1), CXCL13, CXCR3, DNGR-1, ectonucleoside triphosphate diphosphohydrolase 1, EGFR, ENTPD1, FCER1A, FCER1, FLAP, FOLH1, Gi24, GITR, GITRL, GM-CSF, Her2, HHLA2, HMGB1, HVEM, ICOSLG, IDO, IFNα, IgE, IGF1R, IL2Rbeta, IL1, IL1A, IL1B, IL1F10, IL2, IL4, IL4Ra, IL5, IL5R, IL6, IL7, IL7Ra, IL8, IL9, IL9R, IL10, rhIL10, IL12, IL13, IL13Ra1, IL13Ra2, IL15, IL17, IL17Rb (also known as a receptor for IL25), IL18, IL22, IL23, IL25, IL27, IL33, IL35, ITGB4 (also known as b4 integrin), ITK, KIR, LAG3, LAMP1, leptin, LPFS2, MHC class II, NCR3LG1, NKG2D, NTPDase-1, OX40, OX40L, PD-1H, platelet receptor, PROM1, S152, SISP1, SLC, SPG64, ST2 (also known as a receptor for IL33), STEAP2, Syk kinase, TACI, TDO, T14, TIGIT, TIM3, TLR, TLR2, TLR4, TLR5, TLR9, TMEF1, TNFa, TNFRSF7, Tp55, TREM1, TSLP (also known as a co-receptor for IL7Ra), TSLPR, TWEAK, VEGF, VISTA, Vstm3, WUCAM, and XCR1 (also known as GPR5/CCXCR1).

[0166] In some embodiments, V_{H1} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:28, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:29, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:30; V_{L1} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:46, a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 47, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:48; V_{H2} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:34, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:35, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:36; V_{L2} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:52, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:53, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:54; V_{H3} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:25, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:26, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:27; and V_{L3} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:43, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:44, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:45.

[0167] In some embodiments, V_{H1} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:34, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:35, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:36; V_{L1} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:52, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:53, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:54; V_{H2} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:25, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:26, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:27; V_{L2} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:43, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:44, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:45; V_{H3} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:25, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:26, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:27; and V_{L3} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:43, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:44, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:45.

[0168] In some embodiments, V_{H1} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:31, a CDR-H2 comprising the amino acid sequence of SEQ ID

NO:32, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:33; V_{L1} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:49, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:50, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:51; V_{H2} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:34, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:35, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:36; V_{L2} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:52, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:53, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:54; V_{H3} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:25, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:26, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:27; and V_{L3} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:43, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:44, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:45.

[0169] In some embodiments, V_{H1} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:28, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:29, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:30; V_{L1} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:46, a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 47, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:48; V_{H2} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:34, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:35, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:36; V_{L2} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:52, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:53, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:54; V_{H3} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:37, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:38, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:39; and V_{L3} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:55, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:56, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:57.

[0170] In some embodiments, V_{H1} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:31, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:32, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:33; V_{L1} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:49, a CDR-L2

comprising the amino acid sequence of SEQ ID NO:50, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:51; V_{H2} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:34, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:35, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:36; V_{L2} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:52, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:53, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:54; V_{H3} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:37, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:38, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:39; and V_{L3} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:55, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:56, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:57.

[0171] In some embodiments, V_{H1} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:28, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:29, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:30; V_{L1} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:46, a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 47, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:48; V_{H2} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:34, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:35, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:36; V_{L2} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:52, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:53, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:54; V_{H3} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:40, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:41, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:42; and V_{L3} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:58, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:44, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:59.

[0172] In some embodiments, V_{H1} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:31, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:32, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:33; V_{L1} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:49, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:50, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:51; V_{H2} comprises a CDR-H1 comprising the amino acid

sequence of SEQ ID NO:34, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:35, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:36; V_{L2} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:52, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:53, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:54; V_{H3} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:40, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:41, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:42; and V_{L3} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:58, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:44, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:59.

[0173] In some embodiments, V_{H1} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:28, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:29, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:30; V_{L1} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:46, a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 47, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:48; V_{H2} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:34, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:35, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:36; V_{L2} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:52, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:53, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:54; V_{H3} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:126, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:127, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:128; and V_{L3} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:138, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:139, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:140.

[0174] In some embodiments, V_{H1} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:31, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:32, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:33; V_{L1} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:49, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:50, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:51; V_{H2} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:34, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:35, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:36; V_{L2}

comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:52, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:53, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:54; V_{H3} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:126, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:127, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:128; and V_{L3} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:138, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:139, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:140.

[0175] In some embodiments, V_{H1} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:132, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:133, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:134; V_{L1} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:143, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:179, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:144; V_{H2} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:135, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:136, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:137; V_{L2} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:145, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:146, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:147; V_{H3} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:129, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:130, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:131; and V_{L3} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:141, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:178, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:142.

[0176] In some embodiments, V_{H1} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:135, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:136, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:137; V_{L1} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:145, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:146, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:147; V_{H2} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:132, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:133, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:134; V_{L2} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:143, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:179, and a CDR-L3 comprising the

amino acid sequence of SEQ ID NO:144; V_{H3} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:129, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:130, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:131; and V_{L3} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:141, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:178, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:142.

[0177] In some embodiments, V_{H1} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:135, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:136, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:137; V_{L1} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:145, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:146, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:147; V_{H2} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:132, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:133, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:134; V_{L2} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:143, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:179, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:144; V_{H3} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:129, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:130, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:131; and V_{L3} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:141, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:178, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:142.

[0178] In some embodiments, V_{H1} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:132, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:133, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:134; V_{L1} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:143, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:179, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:144; V_{H2} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:129, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:130, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:131; V_{L2} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:141, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:178, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:142; V_{H3} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:135, a CDR-H2 comprising the amino acid sequence of SEQ

ID NO:136, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:137; and V_{L3} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:145, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:146, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:147.

[0179] In some embodiments, V_{H1} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:129, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:130, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:131; V_{L1} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:141, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:178, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:142; V_{H2} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:132, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:133, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:134; V_{L2} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:143, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:179, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:144; V_{H3} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:135, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:136, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:137; and V_{L3} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:145, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:146, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:147.

[0180] In some embodiments, V_{H1} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:135, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:136, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:137; V_{L1} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:145, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:146, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:147; V_{H2} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:129, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:130, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:131; V_{L2} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:141, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:178, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:142; V_{H3} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:132, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:133, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:134; and V_{L3} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:143, a CDR-L2

comprising the amino acid sequence of SEQ ID NO:179, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:144.

[0181] In some embodiments, V_{H1} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:129, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:130, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:131; V_{L1} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:141, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:178, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:142; V_{H2} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:135, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:136, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:137; V_{L2} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:145, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:146, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:147; V_{H3} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:132, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:133, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:134; and V_{L3} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:143, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:179, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:144.

[0182] In some embodiments, V_{H1} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:135, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:136, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:137; V_{L1} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:145, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:146, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:147; V_{H2} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:132, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:133, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:134; V_{L2} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:143, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:179, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:144; V_{H3} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:135, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:136, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:137; and V_{L3} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:145, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:146, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:147.

[0183] In some embodiments, V_{H1} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:135, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:136, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:137; V_{L1} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:145, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:146, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:147; V_{H2} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:132, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:133, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:134; V_{L2} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:143, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:179, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:144; V_{H3} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:132, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:133, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:134; and V_{L3} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:143, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:179, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:144.

[0184] In some embodiments, V_{H1} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:132, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:133, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:134; V_{L1} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:143, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:179, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:144; V_{H2} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:135, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:136, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:137; V_{L2} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:145, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:146, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:147; V_{H3} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:135, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:136, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:137; and V_{L3} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:145, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:146, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:147.

[0185] In some embodiments, V_{H1} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:132, a CDR-H2 comprising the amino acid sequence of SEQ ID

NO:133, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:134; V_{L1} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:143, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:179, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:144; V_{H2} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:135, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:136, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:137; V_{L2} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:145, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:146, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:147; V_{H3} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:132, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:133, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:134; and V_{L3} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:143, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:179, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:144.

[0186] In some embodiments, V_{H1} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:28, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:29, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:30; V_{L1} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:46, a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 47, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:48; V_{H2} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:34, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:35, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:36; V_{L2} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:52, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:53, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:54; V_{H3} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:120, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:121, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:122; and V_{L3} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:123, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:124, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:125.

[0187] In some embodiments, V_{H1} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:31, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:32, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:33; V_{L1} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:49, a CDR-L2

comprising the amino acid sequence of SEQ ID NO:50, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:51; V_{H2} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:34, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:35, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:36; V_{L2} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:52, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:53, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:54; V_{H3} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:120, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:121, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:122; and V_{L3} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:123, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:124, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:125.

Antigen targets

[0188] In some embodiments, a binding protein of the present disclosure binds one or more (*e.g.*, one, two, or three) of the following antigen targets or target proteins: A2AR, APRIL, ATPDase, BAFF, BAFFR, BCMA, BlyS, BTK, BTLA, B7DC, B7H1, B7H4 (also known as VTCN1), B7H5, B7H6, B7H7, B7RP1, B7-4, C3, C5, CCL2 (also known as MCP-1), CCL3 (also known as MIP-1a), CCL4 (also known as MIP-1b), CCL5 (also known as RANTES), CCL7 (also known as MCP-3), CCL8 (also known as mcp-2), CCL11 (also known as eotaxin), CCL15 (also known as MIP-1d), CCL17 (also known as TARC), CCL19 (also known as MIP-3b), CCL20 (also known as MIP-3a), CCL21 (also known as MIP-2), CCL24 (also known as MPIF-2/eotaxin-2), CCL25 (also known as TECK), CCL26 (also known as eotaxin-3), CCR3, CCR4, CD3, CD19, CD20, CD23 (also known as FCER2, a receptor for IgE), CD24, CD27, CD28, CD38, CD39, CD40, CD70, CD80 (also known as B7-1), CD86 (also known as B7-2), CD122, CD137 (also known as 41BB), CD137L, CD152 (also known as CTLA4), CD154 (also known as CD40L), CD160, CD272, CD273 (also known as PDL2), CD274 (also known as PDL1), CD275 (also known as B7H2), CD276 (also known as B7H3), CD278 (also known as ICOS), CD279 (also known as PD-1), CDH1 (also known as E-cadherin), chitinase, CLEC9, CLEC91, CRTH2, CSF-1 (also known as M-CSF), CSF-2 (also known as GM-CSF), CSF-3 (also known as GCSF), CX3CL1 (also known as SCYD1), CXCL12 (also known as SDF1), CXCL13, CXCR3, DNGR-1, ectonucleoside triphosphate diphosphohydrolase 1, EGFR, ENTPD1, FCER1A, FCER1, FLAP, FOLH1, Gi24, GITR, GITRL, GM-CSF, Her2, HHLA2, HMGB1, HVEM, ICOSLG, IDO, IFNα, IgE, IGF1R, IL2Rbeta, IL1, IL1A, IL1B, IL1F10, IL2, IL4, IL4Ra, IL5, IL5R, IL6, IL7, IL7Ra,

IL8, IL9, IL9R, IL10, rhIL10, IL12, IL13, IL13Ra1, IL13Ra2, IL15, IL17, IL17Rb (also known as a receptor for IL25), IL18, IL22, IL23, IL25, IL27, IL33, IL35, ITGB4 (also known as b4 integrin), ITK, KIR, LAG3, LAMP1, leptin, LPFS2, MHC class II, NCR3LG1, NKG2D, NTPDase-1, OX40, OX40L, PD-1H, platelet receptor, PROM1, S152, SISP1, SLC, SPG64, ST2 (also known as a receptor for IL33), STEAP2, Syk kinase, TACI, TDO, T14, TIGIT, TIM3, TLR, TLR2, TLR4, TLR5, TLR9, TMEF1, TNFa, TNFRSF7, Tp55, TREM1, TSLP (also known as a co-receptor for IL7Ra), TSLPR, TWEAK, VEGF, VISTA, Vstm3, WUCAM, and XCR1 (also known as GPR5/CCXCR1). In some embodiments, one or more of the above antigen targets are human antigen targets.

[0189] In one embodiment, the binding proteins specifically bind to one or more tumor antigen targets (*e.g.*, target proteins). In other embodiments, the binding proteins specifically bind to one or more tumor target protein and one or more target protein on a T-cell including a T cell receptor complex. These T-cell engager binding proteins are capable of recruiting T cells transiently to target cells and, at the same time, activating the cytolytic activity of the T cells. Examples of target proteins on T cells include but are not limited to CD3 and CD28, among others. Further examples of such antigen targets or target proteins are provided *supra*. In some embodiments, the trispecific binding proteins may be generated by combining the antigen binding domains of two or more monospecific antibodies (parent antibodies) into one antibody. In some embodiments, a binding protein of the present disclosure binds one or more (*e.g.*, one, two, or three) of the following antigen targets: CD3, CD19, CD20, CD28, CD38, Her2, LAMP1, IL-4, IL-13 and TNFa.

[0190] In some embodiments of the disclosure, the trivalent binding protein is capable of binding three antigen targets. In some embodiments of the disclosure, the trivalent binding protein is capable of binding three different antigen targets. In one embodiment, the binding protein is trispecific and one light chain-heavy chain pair is capable of binding two different antigen targets or epitopes and one light chain-heavy chain pair is capable of binding one antigen target or epitope. In another embodiment, the binding protein is capable of binding three tumor antigen targets. In another embodiment, the binding protein is capable of binding three different tumor antigen targets. In other embodiments, the binding protein is capable of inhibiting the function of one or more of the antigen targets.

[0191] In some embodiments, a binding protein of the present disclosure binds one or more tumor target proteins. In some embodiments, the binding protein is capable of specifically binding three epitopes on a single tumor target protein. In some embodiments, the binding protein is capable of specifically binding three different epitopes on a single

tumor target protein. In some embodiments, the binding protein is capable of binding two different epitopes on a first tumor target protein, and one epitope on a second tumor target protein. In some embodiments, the first and second tumor target proteins are different. In some embodiments, the binding protein is capable of specifically binding three different tumor target proteins.

[0192] In some embodiments, a binding protein of the present disclosure binds one or more cytokine target proteins. In some embodiments, the binding protein is capable of specifically binding three epitopes on a single cytokine target protein. In some embodiments, the binding protein is capable of specifically binding three different epitopes on a single cytokine target protein. In some embodiments, the binding protein is capable of binding two different epitopes on a first cytokine target protein, and one epitope on a second cytokine target protein. In some embodiments, the first and second cytokine target proteins are different. In some embodiments, the binding protein is capable of specifically binding three different cytokine target proteins. In some embodiments, the one or more cytokine target proteins are one or more of IL-4, IL-13 and/or TNFa. Further examples of cytokine target proteins are provided *infra*.

[0193] In some embodiments, a binding protein of the present disclosure binds one or more tumor target proteins and one or more T cell target proteins. In some embodiments, the binding protein is capable of specifically binding one tumor target protein and two different epitopes on a single T cell target protein. In some embodiments, the binding protein is capable of specifically binding one tumor target protein and two different T cell target proteins (e.g., CD28 and CD3). In some embodiments, the binding protein is capable of specifically binding one T cell target protein and two different epitopes on a single tumor target protein. In some embodiments, the binding protein is capable of specifically binding one T cell target protein and two different tumor target proteins. In some embodiments, the first and second polypeptide chains of the binding protein form two antigen binding sites that specifically target two T cell target proteins, and the third and fourth polypeptide chains of the binding protein form an antigen binding site that specifically binds a tumor target protein. In some embodiments, the first and second polypeptide chains of the binding protein form two antigen binding sites that specifically target two tumor target proteins, and the third and fourth polypeptide chains of the binding protein form an antigen binding site that specifically binds a T cell target protein. In some embodiments, the one or more tumor target proteins are one or more of CD3, CD19, CD20, CD28, CD38, Her2, LAMP1, IL-4, IL-13 and/or TNFa. In some embodiments, the one or more T cell target proteins are one or more of CD3 and

CD28. Further examples of tumor target proteins and T cell target proteins are provided *supra*.

[0194] In some embodiments, a binding protein of the present disclosure binds, independently of each other, same or different, one, two or three antigen targets or target proteins, selected from cytokine target proteins, tumor target antigens or tumor target proteins, T cell target proteins, immune checkpoint inhibitors, immune checkpoint modulators, immune checkpoint costimulatory molecules, and/or target molecules on the surface of an immune cell. In some embodiments, a binding protein of the present disclosure is trivalent but bispecific and capable of specifically binding twice to the same antigen targets or target proteins. In some embodiments, a binding protein of the present disclosure is capable of specifically binding two different epitopes on a single cytokine target proteins, tumor target antigens or tumor target proteins, T cell target proteins, immune checkpoint inhibitors, immune checkpoint modulators, immune checkpoint costimulatory molecules, and/or target molecules on the surface of an immune cell.. Further examples of such antigen targets or target proteins are provided *supra*.

[0195] The binding proteins of the disclosure may be prepared using domains or sequences obtained or derived from any human or non-human antibody, including, for example, human, murine, or humanized antibodies.

Linkers

[0196] In some embodiments, the linkers L_1, L_2, L_3 and L_4 range from no amino acids (length=0) to about 100 amino acids long, or less than 100, 50, 40, 30, 20, or 15 amino acids or less. The linkers can also be 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acids long. L_1, L_2, L_3 and L_4 in one binding protein may all have the same amino acid sequence or may all have different amino acid sequences.

[0197] Examples of suitable linkers include a single glycine (Gly) residue; a diglycine peptide (Gly-Gly); a tripeptide (Gly-Gly-Gly); a peptide with four glycine residues (Gly-Gly-Gly-Gly; SEQ ID NO: 98); a peptide with five glycine residues (Gly-Gly-Gly-Gly-Gly; SEQ ID NO: 99); a peptide with six glycine residues (Gly-Gly-Gly-Gly-Gly-Gly; SEQ ID NO: 100); a peptide with seven glycine residues (Gly-Gly-Gly-Gly-Gly-Gly-Gly; SEQ ID NO: 101); a peptide with eight glycine residues (Gly-Gly-Gly-Gly-Gly-Gly-Gly-Gly; SEQ ID NO: 102). Other combinations of amino acid residues may be used such as the peptide Gly-Gly-Gly-Gly-Ser (SEQ ID NO: 103), the peptide Gly-Gly-Gly-Gly-Ser-Gly-Gly-Gly-Gly-Ser (SEQ ID NO: 104), the peptide Gly-Gly-Gly-Gly-Ser-Gly-Gly-Gly-Gly-Ser-Gly-Gly-Gly-Gly-Ser (SEQ ID NO: 105), and the peptide Gly-Gly-Ser-Gly-Ser-Ser-Gly-Ser-Gly-Gly

(SEQ ID NO:148). Other suitable linkers include a single Ser, and Val residue; the dipeptide Arg-Thr, Gln-Pro, Ser-Ser, Thr-Lys, and Ser-Leu; Thr-Lys-Gly-Pro-Ser (SEQ ID NO: 106), Thr-Val-Ala-Ala-Pro (SEQ ID NO: 107), Gln-Pro-Lys-Ala-Ala (SEQ ID NO: 108), Gln-Arg-Ile-Glu-Gly (SEQ ID NO: 109); Ala-Ser-Thr-Lys-Gly-Pro-Ser (SEQ ID NO: 110), Arg-Thr-Val-Ala-Ala-Pro-Ser (SEQ ID NO:111), Gly-Gln-Pro-Lys-Ala-Ala-Pro (SEQ ID NO:112), and His-Ile-Asp-Ser-Pro-Asn-Lys (SEQ ID NO:113). The examples listed above are not intended to limit the scope of the disclosure in any way, and linkers comprising randomly selected amino acids selected from the group consisting of valine, leucine, isoleucine, serine, threonine, lysine, arginine, histidine, aspartate, glutamate, asparagine, glutamine, glycine, and proline have been shown to be suitable in the binding proteins. For additional descriptions of linker sequences, *see, e.g.,* WO2012135345.

[0198] The identity and sequence of amino acid residues in the linker may vary depending on the type of secondary structural element necessary to achieve in the linker. For example, glycine, serine, and alanine are best for linkers having maximum flexibility. Some combination of glycine, proline, threonine, and serine are useful if a more rigid and extended linker is necessary. Any amino acid residue may be considered as a linker in combination with other amino acid residues to construct larger peptide linkers as necessary depending on the desired properties.

[0199] In some embodiments, the length of L_1 is at least twice the length of L_3. In some embodiments, the length of L_2 is at least twice the length of L_4. In some embodiments, the length of L_1 is at least twice the length of L_3, and the length of L_2 is at least twice the length of L_4. In some embodiments, L_1 is 3 to 12 amino acid residues in length, L_2 is 3 to 14 amino acid residues in length, L_3 is 1 to 8 amino acid residues in length, and L_4 is 1 to 3 amino acid residues in length. In some embodiments, L_1 is 5 to 10 amino acid residues in length, L_2 is 5 to 8 amino acid residues in length, L_3 is 1 to 5 amino acid residues in length, and L_4 is 1 to 2 amino acid residues in length. In some embodiments, L_1 is 7 amino acid residues in length, L_2 is 5 amino acid residues in length, L_3 is 1 amino acid residue in length, and L_4 is 2 amino acid residues in length. In some embodiments, L_1 is 10 amino acid residues in length, L_2 is 10 amino acid residues in length, L_3 is 0 amino acid residue in length, and L_4 is 0 amino acid residues in length. In some embodiments, L_1, L_2, L_3, and L_4 each have an independently selected length from 0 to 15 amino acids (*e.g.*, 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 amino acids), wherein at least two of the linkers have a length of 1 to 15 amino acids (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 amino acids). In some embodiments, L_1, L_2, L_3, and L_4 are each 0 amino acids in length.

[0200] In some embodiments, L_1, L_2, L_3, and/or L_4 comprise the sequence Asp-Lys-Thr-His-Thr (SEQ ID NO: 525). In some embodiments, L_1 comprises the sequence Asp-Lys-Thr-His-Thr (SEQ ID NO: 525). In some embodiments, L_3 comprises the sequence Asp-Lys-Thr-His-Thr (SEQ ID NO: 525).

[0201] In some embodiments, L_1, L_2, L_3, and/or L_4 comprise a sequence derived from a naturally occurring sequence at the junction between an antibody variable domain and an antibody constant domain (*e.g.*, as described in WO2012/135345). For example, in some embodiments, the linker comprises a sequence found at the transition between an endogenous V_H and C_{H1} domain, or between an endogenous V_L and C_L domain (*e.g.*, kappa or lambda). In some embodiments, the linker comprises a sequence found at the transition between an endogenous human V_H and C_{H1} domain, or between an endogenous human V_L and C_L domain (*e.g.*, human kappa or lambda).

[0202] In some embodiments, L_1, L_2, L_3, and/or L_4 comprise the sequence Gly-Gln-Pro-Lys-Ala-Ala-Pro (SEQ ID NO: 175). In some embodiments, L_1 comprises the sequence Gly-Gln-Pro-Lys-Ala-Ala-Pro (SEQ ID NO: 175). In some embodiments, L_1 comprises the sequence Gly-Gln-Pro-Lys-Ala-Ala-Pro (SEQ ID NO: 175), L_2 comprises the sequence Thr-Lys-Gly-Pro-Ser-Arg (SEQ ID NO: 176), L_3 comprises the sequence Ser, and L_4 comprises the sequence Arg-Thr. In some embodiments, L_3 comprises the sequence Gly-Gln-Pro-Lys-Ala-Ala-Pro (SEQ ID NO: 175). In some embodiments, L_1 comprises the sequence Ser, L_2 comprises the sequence Arg-Thr, L_3 comprises the sequence Gly-Gln-Pro-Lys-Ala-Ala-Pro (SEQ ID NO: 175) and L_4 comprises the sequence Thr-Lys-Gly-Pro-Ser-Arg (SEQ ID NO: 176).

[0203] In some embodiments, L_1, L_2, L_3 and L_4 each independently comprise a sequence selected from $(GGGGS)_n$ (wherein n is an integer between 0 and 5; SEQ ID NO:174), GGGGSGGGGS (SEQ ID NO:104), GGGGSGGGGSGGGGS (SEQ ID NO:105), S, RT, TKGPS (SEQ ID NO:106), GQPKAAP (SEQ ID NO: 175), and GGSGSSGSGG (SEQ ID NO:148). In some embodiments, L_1 comprises the sequence GQPKAAP (SEQ ID NO: 175), L_2 comprises the sequence TKGPS (SEQ ID NO:106), L_3 comprises the sequence S, and L_4 comprises the sequence RT. In some embodiments, L_1 comprises the sequence GGGGSGGGGS (SEQ ID NO:104), L_2 comprises the sequence GGGGSGGGGS (SEQ ID NO:104), L_3 is 0 amino acids in length, and L_4 is 0 amino acids in length. In some embodiments, L_1 comprises the sequence GGSGSSGSGG (SEQ ID NO:148), L_2 comprises the sequence GGSGSSGSGG (SEQ ID NO:148), L_3 is 0 amino acids in length, and L_4 is 0 amino acids in length. In some embodiments, L_1 comprises the sequence

GGGGSGGGGSGGGGS (SEQ ID NO:105), L_2 is 0 amino acids in length, L_3 comprises the sequence GGGGSGGGGSGGGGS (SEQ ID NO:105), and L_4 is 0 amino acids in length. In some embodiments, L_1 and L_2 are zero amino acids in length, and L_3 and L_4 each comprise an independently selected sequence selected from $(GGGGS)_n$ (wherein n is an integer between 0 and 5; SEQ ID NO:174), GGGGSGGGGS (SEQ ID NO:104), GGGGSGGGGSGGGGS (SEQ ID NO:105), S, RT, TKGPS (SEQ ID NO:106), GQPKAAP (SEQ ID NO: 175), and GGSGSSGSGG (SEQ ID NO:148). In some embodiments, L_3 and L_4 are zero amino acids in length, and L_1 and L_2 each comprise an independently selected sequence selected from $(GGGGS)_n$ (wherein n is an integer between 0 and 5; SEQ ID NO:174), GGGGSGGGGS (SEQ ID NO:104), GGGGSGGGGSGGGGS (SEQ ID NO:105), S, RT, TKGPS (SEQ ID NO:106), GQPKAAP (SEQ ID NO: 175), and GGSGSSGSGG (SEQ ID NO:148).

Fc regions and constant domains

[0204] In some embodiments, a binding protein of the present disclosure comprises a second polypeptide chain further comprising an Fc region linked to CH1, the Fc region comprising an immunoglobulin hinge region and CH2 and CH3 immunoglobulin heavy chain constant domains. In some embodiments, a binding protein of the present disclosure comprises a third polypeptide chain further comprising an Fc region linked to CH1, the Fc region comprising an immunoglobulin hinge region and CH2 and CH3 immunoglobulin heavy chain constant domains. In some embodiments, a binding protein of the present disclosure comprises a second polypeptide chain further comprising an Fc region linked to CH1, the Fc region comprising an immunoglobulin hinge region and CH2 and CH3 immunoglobulin heavy chain constant domains, and a third polypeptide chain further comprising an Fc region linked to CH1, the Fc region comprising an immunoglobulin hinge region and CH2 and CH3 immunoglobulin heavy chain constant domains.

[0205] In some embodiments, a binding protein of the present disclosure includes one or two Fc variants. The term "Fc variant" as used herein refers to a molecule or sequence that is modified from a native Fc but still comprises a binding site for the salvage receptor, FcRn (neonatal Fc receptor). Exemplary Fc variants, and their interaction with the salvage receptor, are known in the art. Thus, the term "Fc variant" can comprise a molecule or sequence that is humanized from a non-human native Fc. Furthermore, a native Fc comprises regions that can be removed because they provide structural features or biological activity that are not required for the antibody-like binding proteins of the invention. Thus, the term "Fc variant" comprises a molecule or sequence that lacks one or more native Fc sites or residues, or in which one or more Fc sites or residues has be modified, that affect or are involved in: (1)

disulfide bond formation, (2) incompatibility with a selected host cell, (3) N-terminal heterogeneity upon expression in a selected host cell, (4) glycosylation, (5) interaction with complement, (6) binding to an Fc receptor other than a salvage receptor, or (7) antibody-dependent cellular cytotoxicity (ADCC).

[0206] To improve the yields of the binding proteins, the C_{H3} domains can be altered by the "knob-into-holes" technology which is described in detail with several examples in, for example, International Publication No. WO 96/027011, Ridgway *et al.*, 1996, *Protein Eng.* 9: 617-21; and Merchant *et al.*, 1998, *Nat. Biotechnol.* 16: 677-81. Specifically, the interaction surfaces of the two C_{H3} domains are altered to increase the heterodimerisation of both heavy chains containing these two C_{H3} domains. Each of the two C_{H3} domains (of the two heavy chains) can be the "knob," while the other is the "hole." The introduction of a disulfide bridge further stabilizes the heterodimers (Merchant *et al.*, 1998; Atwell *et al.*, 1997, *J. Mol. Biol.* 270: 26-35) and increases the yield. In particular embodiments, the knob is on the second pair of polypeptides with a single variable domain. In other embodiments, the knob is on the first pair of polypeptides having the cross-over orientation. In yet other embodiments, the C_{H3} domains do not include a knob in hole.

[0207] In some embodiments, a binding protein of the present disclosure comprises a "knob" mutation on the second polypeptide chain and a "hole" mutation on the third polypeptide chain. In some embodiments, a binding protein of the present disclosure comprises a "knob" mutation on the third polypeptide chain and a "hole" mutation on the second polypeptide chain. In some embodiments, the "knob" mutation comprises substitution(s) at positions corresponding to positions 354 and/or 366 of human IgG1 or IgG4 according to EU Index. In some embodiments, the amino acid substitutions are S354C, T366W, T366Y, S354C and T366W, or S354C and T366Y. In some embodiments, the "knob" mutation comprises substitutions at positions corresponding to positions 354 and 366 of human IgG1 or IgG4 according to EU Index. In some embodiments, the amino acid substitutions are S354C and T366W. In some embodiments, the "hole" mutation comprises substitution(s) at positions corresponding to positions 407 and, optionally, 349, 366, and/or 368 and of human IgG1 or IgG4 according to EU Index. In some embodiments, the amino acid substitutions are Y407V or Y407T and optionally Y349C, T366S, and/or L368A. In some embodiments, the "hole" mutation comprises substitutions at positions corresponding to positions 349, 366, 368, and 407 of human IgG1 or IgG4 according to EU Index. In some embodiments, the amino acid substitutions are Y349C, T366S, L368A, and Y407V.

[0208] In some embodiments, the second polypeptide chain further comprises a first Fc region linked to CH1, the first Fc region comprising an immunoglobulin hinge region and CH2 and CH3 immunoglobulin heavy chain constant domains, wherein the first Fc region comprises amino acid substitution(s) at positions corresponding to positions 366 and optionally 354 of human IgG1 or IgG4 according to EU Index, wherein the amino acid substitutions are T366W or T366Y and optionally S354C; and wherein the third polypeptide chain further comprises a second Fc region linked to CH1, the second Fc region comprising an immunoglobulin hinge region and CH2 and CH3 immunoglobulin heavy chain constant domains, wherein the second Fc region comprises amino acid substitution(s) at positions corresponding to positions 407 and optionally 349, 366, and/or 368 and of human IgG1 or IgG4 according to EU Index, wherein the amino acid substitutions are Y407V or Y407T and optionally Y349C, T366S, and/or L368A.

[0209] In some embodiments, the second polypeptide chain further comprises a first Fc region linked to CH1, the first Fc region comprising an immunoglobulin hinge region and CH2 and CH3 immunoglobulin heavy chain constant domains, wherein the first Fc region comprises amino acid substitution(s) at positions corresponding to positions 407 and optionally 349, 366, and/or 368 and of human IgG1 or IgG4 according to EU Index, wherein the amino acid substitutions are Y407V or Y407T and optionally Y349C, T366S, and/or L368A; and wherein the third polypeptide chain further comprises a second Fc region linked to CH1, the second Fc region comprising an immunoglobulin hinge region and CH2 and CH3 immunoglobulin heavy chain constant domains, wherein the second Fc region comprises amino acid substitution(s) at positions corresponding to positions 366 and optionally 354 of human IgG1 or IgG4 according to EU Index, wherein the amino acid substitutions are T366W or T366Y and optionally S354C.

[0210] In some embodiments, the second polypeptide chain further comprises a first Fc region linked to CH1, the first Fc region comprising an immunoglobulin hinge region and CH2 and CH3 immunoglobulin heavy chain constant domains, wherein the first Fc region comprises amino acid substitution at position corresponding to position 366 of human IgG1 or IgG4 according to EU Index, wherein the amino acid substitution is T366W; and wherein the third polypeptide chain further comprises a second Fc region linked to CH1, the second Fc region comprising an immunoglobulin hinge region and CH2 and CH3 immunoglobulin heavy chain constant domains, wherein the second Fc region comprises amino acid substitution(s) at positions corresponding to positions 366, 368, and/or 407 and of human

IgG1 or IgG4 according to EU Index, wherein the amino acid substitutions are T366S, L368A, and/or Y407V.

[0211] In some embodiments, the second polypeptide chain further comprises a first Fc region linked to CH1, the first Fc region comprising an immunoglobulin hinge region and CH2 and CH3 immunoglobulin heavy chain constant domains, wherein the first Fc region comprises amino acid substitution(s) at positions corresponding to positions 366, 368, and/or 407 and of human IgG1 or IgG4 according to EU Index, wherein the amino acid substitutions are T366S, L368A, and/or Y407V; and wherein the third polypeptide chain further comprises a second Fc region linked to CH1, the second Fc region comprising an immunoglobulin hinge region and CH2 and CH3 immunoglobulin heavy chain constant domains, wherein the second Fc region comprises amino acid substitution at position corresponding to position 366 of human IgG1 or IgG4 according to EU Index, wherein the amino acid substitution is T366W.

[0212] In some embodiments, the second polypeptide chain further comprises a first Fc region linked to CH1, the first Fc region comprising an immunoglobulin hinge region and CH2 and CH3 immunoglobulin heavy chain constant domains, wherein the first Fc region comprises amino acid substitutions at positions corresponding to positions 354 and 366 of human IgG1 or IgG4 according to EU Index, wherein the amino acid substitutions are S354C and T366W; and wherein the third polypeptide chain further comprises a second Fc region linked to CH1, the second Fc region comprising an immunoglobulin hinge region and CH2 and CH3 immunoglobulin heavy chain constant domains, wherein the second Fc region comprises amino acid substitutions at positions corresponding to positions 349, 366, 368, and 407 of human IgG1 or IgG4 according to EU Index, wherein the amino acid substitutions are Y349C, T366S, L368A, and Y407V. In some embodiments, the second polypeptide chain further comprises a first Fc region linked to CH1, the first Fc region comprising an immunoglobulin hinge region and CH2 and CH3 immunoglobulin heavy chain constant domains, wherein the first Fc region comprises amino acid substitutions at positions corresponding to positions 349, 366, 368, and 407 of human IgG1 or IgG4 according to EU Index, wherein the amino acid substitutions are Y349C, T366S, L368A, and Y407V; and wherein the third polypeptide chain further comprises a second Fc region linked to CH1, the second Fc region comprising an immunoglobulin hinge region and CH2 and CH3 immunoglobulin heavy chain constant domains, wherein the second Fc region comprises amino acid substitutions at positions corresponding to positions 354 and 366 of human IgG1 or IgG4 according to EU Index, wherein the amino acid substitutions are S354C and T366W.

In some embodiments, the first and/or second Fc regions are human IgG1 Fc regions. In some embodiments, the first and/or second Fc regions are human IgG4 Fc regions.

[0213] In some embodiments, a binding protein of the present disclosure comprises one or more mutations to improve serum half-life (See e.g., Hinton, P.R. et al. (2006) J. Immunol. 176(1):346-56). In some embodiments, the mutation comprises substitutions at positions corresponding to positions 428 and 434 of human IgG1 or IgG4 according to EU Index, wherein the amino acid substitutions are M428L and N434S. In some embodiments, the binding protein comprises a second polypeptide chain further comprising a first Fc region linked to CH1, the first Fc region comprising an immunoglobulin hinge region and CH2 and CH3 immunoglobulin heavy chain constant domains, and a third polypeptide chain further comprising a second Fc region linked to CH1, the second Fc region comprising an immunoglobulin hinge region and CH2 and CH3 immunoglobulin heavy chain constant domains, wherein the first and/or second Fc regions comprise amino acid substitutions at positions corresponding to positions 428 and 434 of human IgG1 or IgG4 according to EU Index, wherein the amino acid substitutions are M428L and N434S. In some embodiments, a binding protein of the present disclosure comprises knob and hole mutations and one or more mutations to improve serum half-life. In some embodiments, the first and/or second Fc regions are human IgG1 Fc regions. In some embodiments, the first and/or second Fc regions are human IgG4 Fc regions.

[0214] In some embodiments, a binding protein of the present disclosure comprises one or more mutations to improve stability, *e.g.*, of the hinge region and/or dimer interface of IgG4 (*See e.g.*, Spiess, C. *et al.* (2013) *J. Biol. Chem.* 288:26583-26593). In some embodiments, the mutation comprises substitutions at positions corresponding to positions 228 and 409 of human IgG4 according to EU Index, wherein the amino acid substitutions are S228P and R409K. In some embodiments, the binding protein comprises a second polypeptide chain further comprising a first Fc region linked to C_{H1}, the first Fc region comprising an immunoglobulin hinge region and C_{H2} and C_{H3} immunoglobulin heavy chain constant domains, and a third polypeptide chain further comprising a second Fc region linked to C_{H1}, the second Fc region comprising an immunoglobulin hinge region and C_{H2} and C_{H3} immunoglobulin heavy chain constant domains; wherein the first and second Fc regions are human IgG4 Fc regions; and wherein the first and the second Fc regions each comprise amino acid substitutions at positions corresponding to positions 228 and 409 of human IgG4 according to EU Index, wherein the amino acid substitutions are S228P and R409K. In some embodiments, a binding protein of the present disclosure comprises knob and hole mutations

and one or more mutations to improve stability. In some embodiments, the first and/or second Fc regions are human IgG4 Fc regions.

[0215] In some embodiments, a binding protein of the present disclosure comprises one or more mutations to improve purification, *e.g.*, by modulating the affinity for a purification reagent. For example, it is known that heterodimeric binding proteins can be selectively purified away from their homodimeric forms if one of the two Fc regions of the heterodimeric form contains mutation(s) that reduce or eliminate binding to Protein A, because the heterodimeric form will have an intermediate affinity for Protein A-based purification than either homodimeric form and can be selectively eluted from Protein A, *e.g.*, by use of a different pH (*See e.g.*, Smith, E.J. *et al.* (2015) *Sci. Rep.* 5:17943). In some embodiments, the mutation comprises substitutions at positions corresponding to positions 435 and 436 of human IgG1 or IgG4 according to EU Index, wherein the amino acid substitutions are H435R and Y436F. In some embodiments, the binding protein comprises a second polypeptide chain further comprising a first Fc region linked to C_{H1}, the first Fc region comprising an immunoglobulin hinge region and C_{H2} and C_{H3} immunoglobulin heavy chain constant domains, and a third polypeptide chain further comprising a second Fc region linked to C_{H1}, the second Fc region comprising an immunoglobulin hinge region and C_{H2} and C_{H3} immunoglobulin heavy chain constant domains; and wherein only one of the first and the second Fc regions comprises amino acid substitutions at positions corresponding to positions 435 and 436 of human IgG1 or IgG4 according to EU Index, wherein the amino acid substitutions are H435R and Y436F. In some embodiments, a binding protein of the present disclosure comprises knob and hole mutations and one or more mutations to improve purification. In some embodiments, the first and/or second Fc regions are human IgG1 Fc regions. In some embodiments, the first and/or second Fc regions are human IgG4 Fc regions.

[0216] In some embodiments, a binding protein of the present disclosure comprises one or more mutations to reduce effector function, *e.g.*, Fc receptor-mediated antibody-dependent cellular phagocytosis (ADCP), complement-dependent cytotoxicity (CDC), and/or antibody-dependent cellular cytotoxicity (ADCC). In some embodiments, the second polypeptide chain further comprises a first Fc region linked to C_{H1}, the first Fc region comprising an immunoglobulin hinge region and C_{H2} and C_{H3} immunoglobulin heavy chain constant domains; wherein the third polypeptide chain further comprises a second Fc region linked to C_{H1}, the second Fc region comprising an immunoglobulin hinge region and C_{H2} and C_{H3} immunoglobulin heavy chain constant domains; wherein the first and second Fc regions are

human IgG1 Fc regions; and wherein the first and the second Fc regions each comprise amino acid substitutions at positions corresponding to positions 234 and 235 of human IgG1 according to EU Index, wherein the amino acid substitutions are L234A and L235A. In some embodiments, the Fc regions of the second and the third polypeptide chains are human IgG1 Fc regions, and wherein the Fc regions each comprise amino acid substitutions at positions corresponding to positions 234 and 235 of human IgG1 according to EU Index, wherein the amino acid substitutions are L234A and L235A. In some embodiments, the second polypeptide chain further comprises a first Fc region linked to C_{H1}, the first Fc region comprising an immunoglobulin hinge region and C_{H2} and C_{H3} immunoglobulin heavy chain constant domains; wherein the third polypeptide chain further comprises a second Fc region linked to C_{H1}, the second Fc region comprising an immunoglobulin hinge region and C_{H2} and C_{H3} immunoglobulin heavy chain constant domains; wherein the first and second Fc regions are human IgG1 Fc regions; and wherein the first and the second Fc regions each comprise amino acid substitutions at positions corresponding to positions 234, 235, 329 of human IgG1 according to EU Index, wherein the amino acid substitutions are L234A, L235A, and P329A. In some embodiments, the Fc regions of the second and the third polypeptide chains are human IgG1 Fc regions, and wherein the Fc regions each comprise amino acid substitutions at positions corresponding to positions 234, 235, and 329 of human IgG1 according to EU Index, wherein the amino acid substitutions are L234A, L235A, and P329A. In some embodiments, the mutation comprises substitutions at positions corresponding to positions 234 and 235 of human IgG4 according to EU Index, wherein the amino acid substitutions are F234A and L235A. In some embodiments, the binding protein comprises a second polypeptide chain further comprising a first Fc region linked to C_{H1}, the first Fc region comprising an immunoglobulin hinge region and C_{H2} and C_{H3} immunoglobulin heavy chain constant domains, and a third polypeptide chain further comprising a second Fc region linked to C_{H1}, the second Fc region comprising an immunoglobulin hinge region and C_{H2} and C_{H3} immunoglobulin heavy chain constant domains; and wherein the first and the second Fc regions each comprise amino acid substitutions at positions corresponding to positions 234 and 235 of human IgG4 according to EU Index, wherein the amino acid substitutions are F234A and L235A. In some embodiments, a binding protein of the present disclosure comprises knob and hole mutations and one or more mutations to reduce effector function. In some embodiments, the first and/or second Fc regions are human IgG1 Fc regions. In some embodiments, the first and/or second Fc regions are human IgG4 Fc regions. For further

description of Fc mutations at position 329, *see, e.g.*, Shields, R.L. *et al.* (2001) *J. Biol. Chem.* 276:6591-6604 and WO1999051642.

[0217] In some embodiments, the types of mutations described *supra* can be combined in any order or combination. For example, a binding protein of the present disclosure can comprise two or more of the "knob" and "hole" mutations, the one or more mutations to improve serum half-life, the one or more mutations to improve IgG4 stability, the one or more mutations to improve purification, and/or the one or more mutations to reduce effector function described *supra*.

[0218] In certain embodiments, a binding protein of the present disclosure comprises: a first polypeptide chain that comprises a lambda C_L domain; a C_{H3} domain of a second polypeptide chain that comprises amino acid substitutions at positions corresponding to positions 354 and 366 of human IgG1 according to EU Index, wherein the amino acid substitutions are S354C and T366W; a C_{H3} domain of a third polypeptide chain that comprises amino acid substitutions at positions corresponding to positions 349, 366, 368, 407, 435, and 436 of human IgG1 according to EU Index, wherein the amino acid substitutions are Y349C, T366S, L368A, Y407V, H435R, and Y436F; and a fourth polypeptide chain that comprises a kappa C_L domain. In some embodiments, the first polypeptide chain comprises a lambda C_L domain; wherein the C_{H3} domain of the third polypeptide chain comprises amino acid substitutions at positions corresponding to positions 354 and 366 of human IgG1 according to EU Index, wherein the amino acid substitutions are S354C and T366W; wherein the C_{H3} domain of the second polypeptide chain comprises amino acid substitutions at positions corresponding to positions 349, 366, 368, 407, 435, and 436 of human IgG1 according to EU Index, wherein the amino acid substitutions are Y349C, T366S, L368A, Y407V, H435R, and Y436F; and wherein the fourth polypeptide chain comprises a kappa C_L domain. In some embodiments, the first polypeptide chain comprises a lambda C_L domain; wherein the C_{H3} domain of the second polypeptide chain comprises amino acid substitutions at positions corresponding to positions 354, 366, 435, and 436 of human IgG1 according to EU Index, wherein the amino acid substitutions are S354C, T366W, H435R, and Y436F; wherein the C_{H3} domain of the third polypeptide chain comprises amino acid substitutions at positions corresponding to positions 349, 366, 368, and 407 of human IgG1 according to EU Index, wherein the amino acid substitutions are Y349C, T366S, L368A, and Y407V; and wherein the fourth polypeptide chain comprises a kappa C_L domain. In some embodiments, the first polypeptide chain comprises a kappa C_L domain; wherein the C_{H3} domain of the second polypeptide chain comprises amino acid substitutions

at positions corresponding to positions 354 and 366 of human IgG1 according to EU Index, wherein the amino acid substitutions are S354C and T366W; wherein the C_{H3} domain of the third polypeptide chain comprises amino acid substitutions at positions corresponding to positions 349, 366, 368, 407, 435, and 436 of human IgG1 according to EU Index, wherein the amino acid substitutions are Y349C, T366S, L368A, Y407V, H435R, and Y436F; and wherein the fourth polypeptide chain comprises a lambda C_L domain.

[0219] In some embodiments, a binding protein of the present disclosure is purified by protein A affinity chromatography, kappa light chain affinity chromatography (*e.g.*, using a KappaSelect resin according to manufacturer's instructions; GE Healthcare), and optionally lambda light chain affinity chromatography (*e.g.*, using a LambdaFabSelect resin according to manufacturer's instructions; GE Healthcare). In some embodiments, a binding protein of the present disclosure is purified by Protein A affinity chromatography, lambda light chain affinity chromatography (*e.g.*, using a LambdaFabSelect resin according to manufacturer's instructions; GE Healthcare), and optionally kappa light chain affinity chromatography (*e.g.*, using a KappaSelect resin according to manufacturer's instructions; GE Healthcare). In some embodiments, the binding protein comprises two Fc regions, each comprising a C_{H3} domain, and only one of the C_{H3} domains comprises amino acid substitutions at positions corresponding to positions 435 and 436 of human IgG1 or IgG4 according to EU Index, wherein the amino acid substitutions are H435R and Y436F. In some embodiments, a binding protein of the present disclosure is purified by protein A affinity chromatography, then kappa light chain affinity chromatography (*e.g.*, using a KappaSelect resin according to manufacturer's instructions; GE Healthcare), then optionally lambda light chain affinity chromatography (*e.g.*, using a LambdaFabSelect resin according to manufacturer's instructions; GE Healthcare) in sequence. In some embodiments, a binding protein of the present disclosure is purified by Protein A affinity chromatography, then lambda light chain affinity chromatography (*e.g.*, using a LambdaFabSelect resin according to manufacturer's instructions; GE Healthcare), then optionally kappa light chain affinity chromatography (*e.g.*, using a KappaSelect resin according to manufacturer's instructions; GE Healthcare) in sequence. For example, in some embodiments, the binding protein is contacted with Protein A, eluted from Protein A under conditions suitable for isolating the binding protein away from binding proteins comprising either 0 or 2 C_{H3} domains comprising the amino acid substitutions are H435R and Y436F, contacted with a kappa light chain affinity medium (*e.g.*, as used in the KappaSelect resin; GE Healthcare), and eluted from the kappa light chain affinity medium under conditions suitable for isolating the binding protein away from binding

proteins comprising only lambda C_L domains (*e.g.*, according to manufacturer's instructions). Conditions suitable for the Protein A elution are known in the art, including without limitation a stepwise elution gradient from pH4.5-2.8. In some embodiments, Protein A or a Protein A variant useful for protein purification is employed. In some embodiments, the Protein A is attached to a substrate or resin, *e.g.*, as part of a chromatography medium. In some embodiments, after elution from the kappa light chain affinity medium, the binding protein is contacted with a lambda light chain affinity medium (*e.g.*, as used in the LambdaFabSelect resin; GE Healthcare), and eluted from the lambda light chain affinity medium under conditions suitable for isolating the binding protein away from binding proteins comprising only kappa C_L domains (*e.g.*, according to manufacturer's instructions). In some embodiments, a binding protein of the present disclosure is detected using HIC chromatography. In some embodiments, the binding protein comprises: a first polypeptide chain that comprises a lambda C_L domain; a C_{H3} domain of a second polypeptide chain that comprises amino acid substitutions at positions corresponding to positions 354 and 366 of human IgG1 or IgG4according to EU Index, wherein the amino acid substitutions are S354C and T366W; a C_{H3} domain of a third polypeptide chain that comprises amino acid substitutions at positions corresponding to positions 349, 366, 368, 407, 435, and 436 of human IgG1 or IgG4according to EU Index, wherein the amino acid substitutions are Y349C, T366S, L368A, Y407V, H435R, and Y436F; and a fourth polypeptide chain that comprises a kappa C_L domain. In some embodiments, the binding protein is produced by a host cell. In some embodiments, the binding protein is purified from a cell culture medium or host cell extract. In some embodiments, the binding proteins are secreted by a host cell or produced and extracted from a host cell (*e.g.*, before being contacted with Protein A). In some embodiments, the binding protein is in a cell culture medium or host cell extract when contacted with Protein A. In some embodiments, the binding protein is purified away from other binding proteins, polypeptides, and/or other cellular components.

[0220] In some embodiments, CH1, CH2, CH3 and CL of the trispecific binding proteins described herein may comprise any of CH1, CH2, CH3 and CL sequences of binding proteins 1-53.

Nucleic acids

[0221] Standard recombinant DNA methodologies are used to construct the polynucleotides that encode the polypeptides which form the binding proteins, incorporate these polynucleotides into recombinant expression vectors, and introduce such vectors into host cells. *See e.g.*, Sambrook *et al.*, 2001, MOLECULAR CLONING: A LABORATORY MANUAL

(Cold Spring Harbor Laboratory Press, 3rd ed.). Enzymatic reactions and purification techniques may be performed according to manufacturer's specifications, as commonly accomplished in the art, or as described herein. Unless specific definitions are provided, the nomenclature 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. Similarly, conventional techniques may be used for chemical syntheses, chemical analyses, pharmaceutical preparation, formulation, delivery, and treatment of patients.

[0222] Other aspects of the present disclosure relate to isolated nucleic acid molecules comprising a nucleotide sequence encoding any of the binding proteins described herein. In some embodiments, the isolated nucleic acid is operably linked to a heterologous promoter to direct transcription of the binding protein-coding nucleic acid sequence. A promoter may refer to nucleic acid control sequences which direct transcription of a nucleic acid. A first nucleic acid sequence is operably linked to a second nucleic acid sequence when the first nucleic acid sequence is placed in a functional relationship with the second nucleic acid sequence. For instance, a promoter is operably linked to a coding sequence of a binding protein if the promoter affects the transcription or expression of the coding sequence. Examples of promoters may include, but are not limited to, promoters obtained from the genomes of viruses (such as polyoma virus, fowlpox virus, adenovirus (such as Adenovirus 2), bovine papilloma virus, avian sarcoma virus, cytomegalovirus, a retrovirus, hepatitis-B virus, Simian Virus 40 (SV40), and the like), from heterologous eukaryotic promoters (such as the actin promoter, an immunoglobulin promoter, from heat-shock promoters, and the like), the CAG-promoter (Niwa et al., Gene 108(2):193-9, 1991), the phosphoglycerate kinase (PGK)-promoter, a tetracycline-inducible promoter (Masui et al., Nucleic Acids Res. 33:e43, 2005), the lac system, the trp system, the tac system, the trc system, major operator and promoter regions of phage lambda, the promoter for 3-phosphoglycerate kinase, the promoters of yeast acid phosphatase, and the promoter of the yeast alpha-mating factors. Polynucleotides encoding binding proteins of the present disclosure may be under the control of a constitutive promoter, an inducible promoter, or any other suitable promoter described herein or other suitable promoter that will be readily recognized by one skilled in the art.

[0223] In some embodiments, the isolated nucleic acid is incorporated into a vector. In some embodiments, the vector is an expression vector. Expression vectors may include one or more regulatory sequences operatively linked to the polynucleotide to be expressed. The term "regulatory sequence" includes promoters, enhancers and other expression control

elements (*e.g.*, polyadenylation signals). Examples of suitable enhancers may include, but are not limited to, enhancer sequences from mammalian genes (such as globin, elastase, albumin, α-fetoprotein, insulin and the like), and enhancer sequences from a eukaryotic cell virus (such as SV40 enhancer on the late side of the replication origin (bp 100-270), the cytomegalovirus early promoter enhancer, the polyoma enhancer on the late side of the replication origin, adenovirus enhancers, and the like). Examples of suitable vectors may include, for example, plasmids, cosmids, episomes, transposons, and viral vectors (*e.g.*, adenoviral, vaccinia viral, Sindbis-viral, measles, herpes viral, lentiviral, retroviral, adeno-associated viral vectors, etc.). Expression vectors can be used to transfect host cells, such as, for example, bacterial cells, yeast cells, insect cells, and mammalian cells. Biologically functional viral and plasmid DNA vectors capable of expression and replication in a host are known in the art, and can be used to transfect any cell of interest.

[0224] Other aspects of the present disclosure relate to a vector system comprising one or more vectors encoding a first, second, third, and fourth polypeptide chain of any of the binding proteins described herein. In some embodiments, the vector system comprises a first vector encoding the first polypeptide chain of the binding protein, a second vector encoding the second polypeptide chain of the binding protein, a third vector encoding the third polypeptide chain of the binding protein, and a fourth vector encoding the fourth polypeptide chain of the binding protein. In some embodiments, the vector system comprises a first vector encoding the first and second polypeptide chains of the binding protein, and a second vector encoding the third and fourth polypeptide chains of the binding protein. In some embodiments, the vector system comprises a first vector encoding the first and third polypeptide chains of the binding protein, and a second vector encoding the second and fourth polypeptide chains of the binding protein. In some embodiments, the vector system comprises a first vector encoding the first and fourth polypeptide chains of the binding protein, and a second vector encoding the second and third polypeptide chains of the binding protein. In some embodiments, the vector system comprises a first vector encoding the first, second, third, and fourth polypeptide chains of the binding protein. The one or more vectors of the vector system may be any of the vectors described herein. In some embodiments, the one or more vectors are expression vectors.

Isolated host cells

[0225] Other aspects of the present disclosure relate to an isolated host cell comprising one or more isolated polynucleotides, vectors, and/or vector systems described herein. In some embodiments, the host cell is a bacterial cell (*e.g.*, an *E. coli* cell). In some

embodiments, the host cell is a yeast cell (*e.g.*, an *S. cerevisiae* cell). In some embodiments, the host cell is an insect cell. Examples of insect host cells may include, for example, Drosophila cells (*e.g.*, S2 cells), Trichoplusia ni cells (*e.g.*, High Five™ cells), and Spodoptera frugiperda cells (*e.g.*, Sf21 or Sf9 cells). In some embodiments, the host cell is a mammalian cell. Examples of mammalian host cells may include, for example, human embryonic kidney cells (*e.g.*, 293 or 293 cells subcloned for growth in suspension culture), Expi293TM cells, CHO cells, baby hamster kidney cells (*e.g.*, BHK, ATCC CCL 10), mouse sertoli cells (*e.g.*, TM4 cells), monkey kidney cells (*e.g.*, CV1 ATCC CCL 70), African green monkey kidney cells (*e.g.*, VERO-76, ATCC CRL-1587), human cervical carcinoma cells (*e.g.*, HELA, ATCC CCL 2), canine kidney cells (*e.g.*, MDCK, ATCC CCL 34), buffalo rat liver cells (*e.g.*, BRL 3A, ATCC CRL 1442), human lung cells (*e.g.*, W138, ATCC CCL 75), human liver cells (*e.g.*, Hep G2, HB 8065), mouse mammary tumor cells (*e.g.*, MMT 060562, ATCC CCL51), TRI cells, MRC 5 cells, FS4 cells, a human hepatoma line (*e.g.*, Hep G2), and myeloma cells (*e.g.*, NS0 and Sp2/0 cells).

[0226] Other aspects of the present disclosure relate to a method of producing any of the binding proteins described herein. In some embodiments, the method includes a) culturing a host cell (*e.g.*, any of the host cells described herein) comprising an isolated nucleic acid, vector, and/or vector system (*e.g.*, any of the isolated nucleic acids, vectors, and/or vector systems described herein) under conditions such that the host cell expresses the binding protein; and b) isolating the binding protein from the host cell. Methods of culturing host cells under conditions to express a protein are well known to one of ordinary skill in the art. Methods of isolating proteins from cultured host cells are well known to one of ordinary skill in the art, including, for example, by affinity chromatography (*e.g.*, two step affinity chromatography comprising protein A affinity chromatography followed by size exclusion chromatography).

Uses For Binding Proteins

[0227] The binding proteins can be employed in any known assay method, such as competitive binding assays, direct and indirect sandwich assays, and immunoprecipitation assays for the detection and quantitation of one or more target antigens. The binding proteins will bind the one or more target antigens with an affinity that is appropriate for the assay method being employed.

[0228] For diagnostic applications, in certain embodiments, binding proteins can be labeled with a detectable moiety. The detectable moiety can be any one that is capable of producing, either directly or indirectly, a detectable signal. For example, the detectable

moiety can be a radioisotope, such as ^{3}H, ^{14}C, ^{32}P, ^{35}S, ^{125}I, ^{99}Tc, ^{111}In, or ^{67}Ga; a fluorescent or chemiluminescent compound, such as fluorescein isothiocyanate, rhodamine, or luciferin; or an enzyme, such as alkaline phosphatase, β-galactosidase, or horseradish peroxidase.

[0229] The binding proteins are also useful for *in vivo* imaging. A binding protein labeled with a detectable moiety can be administered to an animal, preferably into the bloodstream, and the presence and location of the labeled antibody in the host assayed. The binding protein can be labeled with any moiety that is detectable in an animal, whether by nuclear magnetic resonance, radiology, or other detection means known in the art.

[0230] The binding proteins can also be used for cell activation, tumor targeting, neutralization of cytokine activities, neutralization of viral infection, combination of multiple signaling events, to treat cancer, arthritis, and/or inflammatory disorders. For example, in some embodiments, a binding protein specifically binds one, two, or three antigen targets selected from A2AR, APRIL, ATPDase, BAFF, BAFFR, BCMA, BlyS, BTK, BTLA, B7DC, B7H1, B7H4 (also known as VTCN1), B7H5, B7H6, B7H7, B7RP1, B7-4, C3, C5, CCL2 (also known as MCP-1), CCL3 (also known as MIP-1a), CCL4 (also known as MIP-1b), CCL5 (also known as RANTES), CCL7 (also known as MCP-3), CCL8 (also known as mcp-2), CCL11 (also known as eotaxin), CCL15 (also known as MIP-1d), CCL17 (also known as TARC), CCL19 (also known as MIP-3b), CCL20 (also known as MIP-3a), CCL21 (also known as MIP-2), CCL24 (also known as MPIF-2/eotaxin-2), CCL25 (also known as TECK), CCL26 (also known as eotaxin-3), CCR3, CCR4, CD3, CD19, CD20, CD23 (also known as FCER2, a receptor for IgE), CD24, CD27, CD28, CD38, CD39, CD40, CD70, CD80 (also known as B7-1), CD86 (also known as B7-2), CD122, CD137 (also known as 41BB), CD137L, CD152 (also known as CTLA4), CD154 (also known as CD40L), CD160, CD272, CD273 (also known as PDL2), CD274 (also known as PDL1), CD275 (also known as B7H2), CD276 (also known as B7H3), CD278 (also known as ICOS), CD279 (also known as PD-1), CDH1 (also known as E-cadherin), chitinase, CLEC9, CLEC91, CRTH2, CSF-1 (also known as M-CSF), CSF-2 (also known as GM-CSF), CSF-3 (also known as GCSF), CX3CL1 (also known as SCYD1), CXCL12 (also known as SDF1), CXCL13, CXCR3, DNGR-1, ectonucleoside triphosphate diphosphohydrolase 1, EGFR, ENTPD1, FCER1A, FCER1, FLAP, FOLH1, Gi24, GITR, GITRL, GM-CSF, Her2, HHLA2, HMGB1, HVEM, ICOSLG, IDO, IFNα, IgE, IGF1R, IL2Rbeta, IL1, IL1A, IL1B, IL1F10, IL2, IL4, IL4Ra, IL5, IL5R, IL6, IL7, IL7Ra, IL8, IL9, IL9R, IL10, rhIL10, IL12, IL13, IL13Ra1, IL13Ra2, IL15, IL17, IL17Rb (also known as a receptor for IL25), IL18, IL22, IL23, IL25, IL27, IL33, IL35, ITGB4 (also known as b4 integrin), ITK, KIR, LAG3, LAMP1, leptin, LPFS2, MHC

class II, NCR3LG1, NKG2D, NTPDase-1, OX40, OX40L, PD-1H, platelet receptor, PROM1, S152, SISP1, SLC, SPG64, ST2 (also known as a receptor for IL33), STEAP2, Syk kinase, TACI, TDO, T14, TIGIT, TIM3, TLR, TLR2, TLR4, TLR5, TLR9, TMEF1, TNFa, TNFRSF7, Tp55, TREM1, TSLP (also known as a co-receptor for IL7Ra), TSLPR, TWEAK, VEGF, VISTA, Vstm3, WUCAM, and XCR1 (also known as GPR5/CCXCR1). In some embodiments, one or more of the above antigen targets are human antigen targets.

[0231] In some embodiments, a binding protein of the present disclosure is adminstered to a patient in need thereof for the treatment or prevention of cancer. For example, in some embodiments, the binding protein comprises one antigen binding site that specifically binds a T-cell surface protein and another antigen binding site that specifically binds a tumor target protein (*e.g.*, two antigen binding sites that specifically bind T-cell surface proteins and one antigen binding site that specifically binds a tumor target protein, or two antigen binding sites that specifically bind tumor target proteins and one antigen binding site that specifically binds a T-cell surface protein). In certain embodiments, the binding protein comprises an antigen binding site that specifically binds CD3, an antigen binding site that specifically binds CD28, and an antigen binding site that specifically binds a tumor target protein selected from CD19, CD20, CD38, Her2, and LAMP1. In some embodiments, the binding protein is co-administered with a chemotherapeutic agent. In some embodiments, the patient is a human.

[0232] In some embodiments, a binding protein of the present disclosure is adminstered to a patient in need thereof for the treatment or prevention of an inflammatory disease or disorder. In some embodiments, the binding protein comprises three antigen binding sites that each specifically bind a cytokine target protein selected from IL-4, IL-13 and TNFa. In some embodiments, the binding protein is co-administered with an anti-inflammatory agent. In some embodiments, the patient is a human.

[0233] The disclosure also relates to a kit comprising a binding protein and other reagents useful for detecting target antigen levels in biological samples. Such reagents can include a detectable label, blocking serum, positive and negative control samples, and detection reagents. In some embodiments, the kit comprises a composition comprising any binding protein, polynucleotide, vector, vector system, and/or host cell described herein. In some embodiments, the kit comprises a container and a label or package insert on or associated with the container. Suitable containers include, for example, bottles, vials, syringes, IV solution bags, etc. The containers may be formed from a variety of materials such as glass or plastic. The container holds a composition which is by itself or combined with another composition effective for treating, preventing and/or diagnosing a condition and may have a

sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). In some embodiments, the label or package insert indicates that the composition is used for preventing, diagnosing, and/or treating the condition of choice. Alternatively, or additionally, the article of manufacture or kit may further comprise a second (or third) container comprising a pharmaceutically-acceptable buffer, such as bacteriostatic water for injection (BWFI), phosphate-buffered saline, Ringer's solution and dextrose solution. It may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, and syringes.

Binding Protein Therapeutic Compositions and Administration Thereof

[0234] Therapeutic or pharmaceutical compositions comprising binding proteins are within the scope of the disclosure. Such therapeutic or pharmaceutical compositions can comprise a therapeutically effective amount of a binding protein, or binding protein-drug conjugate, in admixture with a pharmaceutically or physiologically acceptable formulation agent selected for suitability with the mode of administration.

[0235] Acceptable formulation materials preferably are nontoxic to recipients at the dosages and concentrations employed.

[0236] The pharmaceutical composition can contain formulation materials for modifying, maintaining, or preserving, for example, the pH, osmolarity, viscosity, clarity, color, isotonicity, odor, sterility, stability, rate of dissolution or release, adsorption, or penetration of the composition. Suitable formulation materials include, but are not limited to, amino acids (such as glycine, glutamine, asparagine, arginine, or lysine), antimicrobials, antioxidants (such as ascorbic acid, sodium sulfite, or sodium hydrogen-sulfite), buffers (such as borate, bicarbonate, Tris-HCl, citrates, phosphates, or other organic acids), bulking agents (such as mannitol or glycine), chelating agents (such as ethylenediamine tetraacetic acid (EDTA)), complexing agents (such as caffeine, polyvinylpyrrolidone, beta-cyclodextrin, or hydroxypropyl-beta-cyclodextrin), fillers, monosaccharides, disaccharides, and other carbohydrates (such as glucose, mannose, or dextrins), proteins (such as serum albumin, gelatin, or immunoglobulins), coloring, flavoring and diluting agents, emulsifying agents, hydrophilic polymers (such as polyvinylpyrrolidone), low molecular weight polypeptides, salt-forming counterions (such as sodium), preservatives (such as benzalkonium chloride, benzoic acid, salicylic acid, thimerosal, phenethyl alcohol, methylparaben, propylparaben, chlorhexidine, sorbic acid, or hydrogen peroxide), solvents (such as glycerin, propylene glycol, or polyethylene glycol), sugar alcohols (such as mannitol or sorbitol), suspending

agents, surfactants or wetting agents (such as pluronics; PEG; sorbitan esters; polysorbates such as polysorbate 20 or polysorbate 80; triton; tromethamine; lecithin; cholesterol or tyloxapal), stability enhancing agents (such as sucrose or sorbitol), tonicity enhancing agents (such as alkali metal halides – preferably sodium or potassium chloride – or mannitol sorbitol), delivery vehicles, diluents, excipients and/or pharmaceutical adjuvants (*see, e.g.*, REMINGTON'S PHARMACEUTICAL SCIENCES (18th Ed., A.R. Gennaro, ed., Mack Publishing Company 1990), and subsequent editions of the same, incorporated herein by reference for any purpose).

[0237] The optimal pharmaceutical composition will be determined by a skilled artisan depending upon, for example, the intended route of administration, delivery format, and desired dosage. Such compositions can influence the physical state, stability, rate of *in vivo* release, and rate of *in vivo* clearance of the binding protein.

[0238] The primary vehicle or carrier in a pharmaceutical composition can be either aqueous or non-aqueous in nature. For example, a suitable vehicle or carrier for injection can be water, physiological saline solution, or artificial cerebrospinal fluid, possibly supplemented with other materials common in compositions for parenteral administration. Neutral buffered saline or saline mixed with serum albumin are further exemplary vehicles. Other exemplary pharmaceutical compositions comprise Tris buffer of about pH 7.0-8.5, or acetate buffer of about pH 4.0-5.5, which can further include sorbitol or a suitable substitute. In one embodiment of the disclosure, binding protein compositions can be prepared for storage by mixing the selected composition having the desired degree of purity with optional formulation agents in the form of a lyophilized cake or an aqueous solution. Further, the binding protein can be formulated as a lyophilizate using appropriate excipients such as sucrose.

[0239] The pharmaceutical compositions of the disclosure can be selected for parenteral delivery or subcutaneous. Alternatively, the compositions can be selected for inhalation or for delivery through the digestive tract, such as orally. The preparation of such pharmaceutically acceptable compositions is within the skill of the art.

[0240] The formulation components are present in concentrations that are acceptable to the site of administration. For example, buffers are used to maintain the composition at physiological pH or at a slightly lower pH, typically within a pH range of from about 5 to about 8.

[0241] When parenteral administration is contemplated, the therapeutic compositions for use can be in the form of a pyrogen-free, parenterally acceptable, aqueous solution

comprising the desired binding protein in a pharmaceutically acceptable vehicle. A particularly suitable vehicle for parenteral injection is sterile distilled water in which a binding protein is formulated as a sterile, isotonic solution, properly preserved. Yet another preparation can involve the formulation of the desired molecule with an agent, such as injectable microspheres, bio-erodible particles, polymeric compounds (such as polylactic acid or polyglycolic acid), beads, or liposomes, that provides for the controlled or sustained release of the product which can then be delivered via a depot injection. Hyaluronic acid can also be used, and this can have the effect of promoting sustained duration in the circulation. Other suitable means for the introduction of the desired molecule include implantable drug delivery devices.

[0242] In one embodiment, a pharmaceutical composition can be formulated for inhalation. For example, a binding protein can be formulated as a dry powder for inhalation. Binding protein inhalation solutions can also be formulated with a propellant for aerosol delivery. In yet another embodiment, solutions can be nebulized.

[0243] It is also contemplated that certain formulations can be administered orally. In one embodiment of the disclosure, binding proteins that are administered in this fashion can be formulated with or without those carriers customarily used in the compounding of solid dosage forms such as tablets and capsules. For example, a capsule can be designed to release the active portion of the formulation at the point in the gastrointestinal tract when bioavailability is maximized and pre-systemic degradation is minimized. Additional agents can be included to facilitate absorption of the binding protein. Diluents, flavorings, low melting point waxes, vegetable oils, lubricants, suspending agents, tablet disintegrating agents, and binders can also be employed.

[0244] Another pharmaceutical composition can involve an effective quantity of binding proteins in a mixture with non-toxic excipients that are suitable for the manufacture of tablets. By dissolving the tablets in sterile water, or another appropriate vehicle, solutions can be prepared in unit-dose form. Suitable excipients include, but are not limited to, inert diluents, such as calcium carbonate, sodium carbonate or bicarbonate, lactose, or calcium phosphate; or binding agents, such as starch, gelatin, or acacia; or lubricating agents such as magnesium stearate, stearic acid, or talc.

[0245] Additional pharmaceutical compositions of the disclosure will be evident to those skilled in the art, including formulations involving binding proteins in sustained- or controlled-delivery formulations. Techniques for formulating a variety of other sustained- or controlled-delivery means, such as liposome carriers, bio-erodible microparticles or porous

beads and depot injections, are also known to those skilled in the art. Additional examples of sustained-release preparations include semipermeable polymer matrices in the form of shaped articles, *e.g.* films, or microcapsules. Sustained release matrices can include polyesters, hydrogels, polylactides, copolymers of L-glutamic acid and gamma ethyl-L-glutamate, poly(2-hydroxyethyl-methacrylate), ethylene vinyl acetate, or poly-D(-)-3-hydroxybutyric acid. Sustained-release compositions can also include liposomes, which can be prepared by any of several methods known in the art.

[0246] Pharmaceutical compositions to be used for *in vivo* administration typically must be sterile. This can be accomplished by filtration through sterile filtration membranes. Where the composition is lyophilized, sterilization using this method can be conducted either prior to, or following, lyophilization and reconstitution. The composition for parenteral administration can be stored in lyophilized form or in a 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.

[0247] Once the pharmaceutical composition has been formulated, it can be stored in sterile vials as a solution, suspension, gel, emulsion, solid, or as a dehydrated or lyophilized powder. Such formulations can be stored either in a ready-to-use form or in a form (*e.g.*, lyophilized) requiring reconstitution prior to administration.

[0248] The disclosure also encompasses kits for producing a single-dose administration unit. The kits can each contain both a first container having a dried protein and a second container having an aqueous formulation. Also included within the scope of this disclosure are kits containing single and multi-chambered pre-filled syringes (*e.g.*, liquid syringes and lyosyringes).

[0249] The effective amount of a binding protein pharmaceutical composition to be employed therapeutically will depend, for example, upon the therapeutic context and objectives. One skilled in the art will appreciate that the appropriate dosage levels for treatment will thus vary depending, in part, upon the molecule delivered, the indication for which the binding protein is being used, the route of administration, and the size (body weight, body surface, or organ size) and condition (the age and general health) of the patient. Accordingly, the clinician can titer the dosage and modify the route of administration to obtain the optimal therapeutic effect.

[0250] Dosing frequency will depend upon the pharmacokinetic parameters of the binding protein in the formulation being used. Typically, a clinician will administer the

composition until a dosage is reached that achieves the desired effect. The composition can therefore be administered as a single dose, as two or more doses (which may or may not contain the same amount of the desired molecule) over time, or as a continuous infusion via an implantation device or catheter. Further refinement of the appropriate dosage is routinely made by those of ordinary skill in the art and is within the ambit of tasks routinely performed by them. Appropriate dosages can be ascertained through use of appropriate dose-response data.

[0251] The route of administration of the pharmaceutical composition is in accord with known methods, *e.g.*, orally; through injection by intravenous, intraperitoneal, intracerebral (intraparenchymal), intracerebroventricular, intramuscular, intraocular, intraarterial, intraportal, or intralesional routes; by sustained release systems; or by implantation devices. Where desired, the compositions can be administered by bolus injection or continuously by infusion, or by implantation device.

[0252] The composition can also be administered locally via implantation of a membrane, sponge, or other appropriate material onto which the desired molecule has been absorbed or encapsulated. Where an implantation device is used, the device can be implanted into any suitable tissue or organ, and delivery of the desired molecule can be via diffusion, timed-release bolus, or continuous administration.

[0253] In some embodiments, the present disclosure relates to a method of preventing and/or treating a proliferative disease or disorder (*e.g.*, cancer). In some embodiments, the method comprises administering to a patient a therapeutically effective amount of at least one of the binding proteins described herein. In some embodiments, the patient is a human. In some embodiments, the at least one binding protein is administered in combination with one or more anti-cancer therapies (*e.g.*, any anti-cancer therapy known in the art). In some embodiments, the at least one binding protein is administered before the one or more anti-cancer therapies. In some embodiments, the at least one binding protein is administered concurrently with the one or more anti-cancer therapies. In some embodiments, the at least one binding protein is administered after the one or more anti-retroviral therapies.

[0254] In some embodiments, the present disclosure relates to a method of preventing and/or treating an inflammatory disease or disorder (*e.g.*, cancer). In some embodiments, the method comprises administering to a patient a therapeutically effective amount of at least one of the binding proteins described herein. In some embodiments, the patient is a human. In some embodiments, the at least one binding protein is administered in combination with one or more anti-inflammatory therapies (*e.g.*, any anti-inflammatory therapy known in the art).

In some embodiments, the at least one binding protein is administered before the one or more anti-inflammatory therapies. In some embodiments, the at least one binding protein is administered concurrently with the one or more anti-inflammatory therapies. In some embodiments, the at least one binding protein is administered after the one or more anti-inflammatory therapies.

[0255] Without limiting the present disclosure, a number of embodiments of the present disclosure are described below for purpose of illustration.

[0256] Item 1: A binding protein comprising four polypeptide chains that form three antigen binding sites that specifically bind one or more target proteins, wherein a first polypeptide chain comprises a structure represented by the formula:

$$V_{L2}\text{-}L_1\text{-}V_{L1}\text{-}L_2\text{-}C_L \qquad [I]$$

and a second polypeptide chain comprises a structure represented by the formula:

$$V_{H1}\text{-}L_3\text{-}V_{H2}\text{-}L_4\text{-}C_{H1} \qquad [II]$$

and a third polypeptide chain comprises a structure represented by the formula:

$$V_{H3}\text{-}C_{H1} \qquad [III]$$

and a fourth polypeptide chain comprises a structure represented by the formula:

$$V_{L3}\text{-}C_L \qquad [IV]$$

wherein:

V_{L1} is a first immunoglobulin light chain variable domain;

V_{L2} is a second immunoglobulin light chain variable domain;

V_{L3} is a third immunoglobulin light chain variable domain;

V_{H1} is a first immunoglobulin heavy chain variable domain;

V_{H2} is a second immunoglobulin heavy chain variable domain;

V_{H3} is a third immunoglobulin heavy chain variable domain;

C_L is an immunoglobulin light chain constant domain;

C_{H1} is an immunoglobulin C_{H1} heavy chain constant domain; and

L_1, L_2, L_3 and L_4 are amino acid linkers;

and wherein the polypeptide of formula I and the polypeptide of formula II form a cross-over light chain-heavy chain pair.

[0257] Item 2: A binding protein comprising four polypeptide chains that form three antigen binding sites that specifically bind one or more target proteins, wherein a first polypeptide chain comprises a structure represented by the formula:

$$V_{L2}\text{-}L_1\text{-}V_{L1}\text{-}L_2\text{-}C_L \qquad [I]$$

and a second polypeptide chain comprises a structure represented by the formula:

$$V_{H1}\text{-}L_3\text{-}V_{H2}\text{-}L_4\text{-}C_{H1}\text{-hinge-}C_{H2}\text{-}C_{H3} \quad [II]$$

and a third polypeptide chain comprises a structure represented by the formula:

$$V_{H3}\text{-}C_{H1}\text{-hinge-}C_{H2}\text{-}C_{H3} \quad [III]$$

and a fourth polypeptide chain comprises a structure represented by the formula:

$$V_{L3}\text{-}C_L \quad [IV]$$

wherein:

V_{L1} is a first immunoglobulin light chain variable domain;

V_{L2} is a second immunoglobulin light chain variable domain;

V_{L3} is a third immunoglobulin light chain variable domain;

V_{H1} is a first immunoglobulin heavy chain variable domain;

V_{H2} is a second immunoglobulin heavy chain variable domain;

V_{H3} is a third immunoglobulin heavy chain variable domain;

C_L is an immunoglobulin light chain constant domain;

C_{H1} is an immunoglobulin C_{H1} heavy chain constant domain;

C_{H2} is an immunoglobulin C_{H2} heavy chain constant domain;

C_{H3} is an immunoglobulin C_{H3} heavy chain constant domain;

hinge is an immunoglobulin hinge region connecting the C_{H1} and C_{H2} domains; and

L_1, L_2, L_3 and L_4 are amino acid linkers;

and wherein the polypeptide of formula I and the polypeptide of formula II form a cross-over light chain-heavy chain pair.

[0258] Item 3: The binding protein of item 1, wherein the second and/or the third polypeptide chain further comprises an Fc region linked to C_{H1}, the Fc region comprising an immunoglobulin hinge region and C_{H2} and C_{H3} immunoglobulin heavy chain constant domains.

[0259] Item 4: The binding protein of any one of items 1-3, wherein at least one of L_1, L_2, L_3 or L_4 is independently 0 amino acids in length.

[0260] Item 5: The binding protein of any one of items 1-3, wherein L_1, L_2, L_3 or L_4 are each independently at least one amino acid in length.

[0261] Item 6: The binding protein of any one of items 1-3 and 5, wherein (a) L_1, L_2, L_3 and L_4 each independently are zero amino acids in length or comprise a sequence selected from the group consisting of GGGGSGGGGS (SEQ ID NO:104), GGGGSGGGGSGGGGS (SEQ ID NO:105), S, RT, TKGPS (SEQ ID NO:106), GQPKAAP (SEQ ID NO: 175), and GGSGSSGSGG (SEQ ID NO:148); or (b) L_1, L_2, L_3 and L_4 each independently comprise a sequence selected from the group consisting of GGGGSGGGGS (SEQ ID NO:104),

GGGGSGGGGSGGGGS (SEQ ID NO:105), S, RT, TKGPS (SEQ ID NO:106), GQPKAAP (SEQ ID NO: 175), and GGSGSSGSGG (SEQ ID NO:148).

[0262] Item 7: The binding protein of any one of items 1-5, wherein

(a) L_1 comprises the sequence GQPKAAP (SEQ ID NO: 175), L_2 comprises the sequence TKGPS (SEQ ID NO:106), L_3 comprises the sequence S, and L_4 comprises the sequence RT;

(b) L_1 comprises the sequence GGGGSGGGGS (SEQ ID NO:104), L_2 comprises the sequence GGGGSGGGGS (SEQ ID NO:104), L_3 is 0 amino acids in length, and L_4 is 0 amino acids in length;

(c) L_1 comprises the sequence GGSGSSGSGG (SEQ ID NO:148), L_2 comprises the sequence GGSGSSGSGG (SEQ ID NO:148), L_3 is 0 amino acids in length, and L_4 is 0 amino acids in length; or

(d) L_1 comprises the sequence GGGGSGGGGSGGGGS (SEQ ID NO:105), L_2 is 0 amino acids in length, L_3 comprises the sequence GGGGSGGGGSGGGGS (SEQ ID NO:105), and L_4 is 0 amino acids in length.

[0263] Item 8: The binding protein of any one of items 1-7, wherein the binding protein is trispecific and capable of specifically binding three different antigen targets.

[0264] Item 9: The binding protein of any one of items 1-8, wherein the binding protein specifically binds three target proteins that correspond to two target proteins on T cells and to one tumor target protein.

[0265] Item 10: The binding protein of item 9, wherein one of said target proteins on T cells is CD3.

[0266] Item 11: The binding protein of item 9 or item 10, wherein one of said target proteins on T cells is CD28.

[0267] Item 12: The binding protein of any one of items 9-11, wherein said tumor target protein is CD38.

[0268] Item 13: The binding protein of any one of items 1-8, wherein the binding protein specifically binds three target proteins that correspond to two target proteins on T cells and to one target protein selected from the group consisting of A2AR, APRIL, ATPDase, BAFF, BAFFR, BCMA, BlyS, BTK, BTLA, B7DC, B7H1, B7H4, B7H5, B7H6, B7H7, B7RP1, B7-4, C3, C5, CCL2, CCL3, CCL4, CCL5, CCL7, CCL8, CCL11, CCL15, CCL17, CCL19, CCL20, CCL21, CCL24, CCL25, CCL26, CCR3, CCR4, CD3, CD19, CD20, CD23, CD24, CD27, CD28, CD38, CD39, CD40, CD70, CD80, CD86, CD122, CD137, CD137L, CD152, CD154, CD160, CD272, CD273, CD274, CD275, CD276,

CD278, CD279, CDH1, chitinase, CLEC9, CLEC91, CRTH2, CSF-1, CSF-2, CSF-3, CX3CL1, CXCL12, CXCL13, CXCR3, DNGR-1, ectonucleoside triphosphate diphosphohydrolase 1, EGFR, ENTPD1, FCER1A, FCER1, FLAP, FOLH1, Gi24, GITR, GITRL, GM-CSF, Her2, HHLA2, HMGB1, HVEM, ICOSLG, IDO, IFNα, IgE, IGF1R, IL2Rbeta, IL1, IL1A, IL1B, IL1F10, IL2, IL4, IL4Ra, IL5, IL5R, IL6, IL7, IL7Ra, IL8, IL9, IL9R, IL10, rhIL10, IL12, IL13, IL13Ra1, IL13Ra2, IL15, IL17, IL17Rb, IL18, IL22, IL23, IL25, IL27, IL33, IL35, ITGB4, ITK, KIR, LAG3, LAMP1, leptin, LPFS2, MHC class II, NCR3LG1, NKG2D, NTPDase-1, OX40, OX40L, PD-1H, platelet receptor, PROM1, S152, SISP1, SLC, SPG64, ST2, STEAP2, Syk kinase, TACI, TDO, T14, TIGIT, TIM3, TLR, TLR2, TLR4, TLR5, TLR9, TMEF1, TNFa, TNFRSF7, Tp55, TREM1, TSLP, TSLPR, TWEAK, VEGF, VISTA, Vstm3, WUCAM, and XCR1.

[0269] Item 14: The binding protein of any one of items 1-13, wherein the binding protein is capable of inhibiting the function of one or more target proteins.

[0270] Item 15: The binding protein of item 14, wherein the one or more target proteins are selected from the group consisting of A2AR, APRIL, ATPDase, BAFF, BAFFR, BCMA, BlyS, BTK, BTLA, B7DC, B7H1, B7H4, B7H5, B7H6, B7H7, B7RP1, B7-4, C3, C5, CCL2, CCL3, CCL4, CCL5, CCL7, CCL8, CCL11, CCL15, CCL17, CCL19, CCL20, CCL21, CCL24, CCL25, CCL26, CCR3, CCR4, CD3, CD19, CD20, CD23, CD24, CD27, CD28, CD38, CD39, CD40, CD70, CD80, CD86, CD122, CD137, CD137L, CD152, CD154, CD160, CD272, CD273, CD274, CD275, CD276, CD278, CD279, CDH1, chitinase, CLEC9, CLEC91, CRTH2, CSF-1, CSF-2, CSF-3, CX3CL1, CXCL12, CXCL13, CXCR3, DNGR-1, ectonucleoside triphosphate diphosphohydrolase 1, EGFR, ENTPD1, FCER1A, FCER1, FLAP, FOLH1, Gi24, GITR, GITRL, GM-CSF, Her2, HHLA2, HMGB1, HVEM, ICOSLG, IDO, IFNα, IgE, IGF1R, IL2Rbeta, IL1, IL1A, IL1B, IL1F10, IL2, IL4, IL4Ra, IL5, IL5R, IL6, IL7, IL7Ra, IL8, IL9, IL9R, IL10, rhIL10, IL12, IL13, IL13Ra1, IL13Ra2, IL15, IL17, IL17Rb, IL18, IL22, IL23, IL25, IL27, IL33, IL35, ITGB4, ITK, KIR, LAG3, LAMP1, leptin, LPFS2, MHC class II, NCR3LG1, NKG2D, NTPDase-1, OX40, OX40L, PD-1H, platelet receptor, PROM1, S152, SISP1, SLC, SPG64, ST2, STEAP2, Syk kinase, TACI, TDO, T14, TIGIT, TIM3, TLR, TLR2, TLR4, TLR5, TLR9, TMEF1, TNFa, TNFRSF7, Tp55, TREM1, TSLP, TSLPR, TWEAK, VEGF, VISTA, Vstm3, WUCAM, and XCR1.

[0271] Item 16: A binding protein comprising four polypeptide chains that form three antigen binding sites, wherein a first polypeptide chain comprises a structure represented by the formula:

$$V_{L2}\text{-}L_1\text{-}V_{L1}\text{-}L_2\text{-}C_L \qquad [I]$$

and a second polypeptide chain comprises a structure represented by the formula:

$$V_{H1}\text{-}L_3\text{-}V_{H2}\text{-}L_4\text{-}C_{H1} \qquad [II]$$

and a third polypeptide chain comprises a structure represented by the formula:

$$V_{H3}\text{-}C_{H1} \qquad [III]$$

and a fourth polypeptide chain comprises a structure represented by the formula:

$$V_{L3}\text{-}C_L \qquad [IV]$$

wherein:

V_{L1} is a first immunoglobulin light chain variable domain;

V_{L2} is a second immunoglobulin light chain variable domain;

V_{L3} is a third immunoglobulin light chain variable domain;

V_{H1} is a first immunoglobulin heavy chain variable domain;

V_{H2} is a second immunoglobulin heavy chain variable domain;

V_{H3} is a third immunoglobulin heavy chain variable domain;

C_L is an immunoglobulin light chain constant domain;

C_{H1} is an immunoglobulin C_{H1} heavy chain constant domain; and

L_1, L_2, L_3 and L_4 are amino acid linkers;

wherein the polypeptide of formula I and the polypeptide of formula II form a cross-over light chain-heavy chain pair;

wherein:

(a) V_{L1}, V_{L2} and V_{L3} each independently comprise a variable domain sequence as set forth in any one of SEQ ID NOs:151, 153, 155, 157, 159, 161, 163, 165, and 167; or

(b) V_{L1}, V_{L2} and V_{L3} each independently comprise light chain complementarity determining regions comprising an amino acid sequence as set forth in any one of SEQ ID NOs: 43-59, 123-125, 138-140, and 149; and

wherein:

(a) V_{H1}, V_{H2}, and V_{H3} each independently comprise a variable domain sequence as set forth in any one of SEQ ID NOs:150, 152, 154, 156, 158, 160, 162, 164, and 166; or

(b) V_{H1}, V_{H2} and V_{H3} each independently comprise heavy chain complementarity determining regions comprising an amino acid sequence as set forth in any one of SEQ ID NOs: 25-42, 120-122, and 126-128.

[0272] Item 17: A binding protein comprising four polypeptide chains that form three antigen binding sites, wherein a first polypeptide chain comprises a structure represented by the formula:

$$V_{L2}\text{-}L_1\text{-}V_{L1}\text{-}L_2\text{-}C_L \qquad [I]$$

and a second polypeptide chain comprises a structure represented by the formula:

$$V_{H1}\text{-}L_3\text{-}V_{H2}\text{-}L_4\text{-}C_{H1}\text{-hinge-}C_{H2}\text{-}C_{H3} \quad [II]$$

and a third polypeptide chain comprises a structure represented by the formula:

$$V_{H3}\text{-}C_{H1}\text{-hinge-}C_{H2}\text{-}C_{H3} \quad [III]$$

and a fourth polypeptide chain comprises a structure represented by the formula:

$$V_{L3}\text{-}C_L \quad [IV]$$

wherein:

V_{L1} is a first immunoglobulin light chain variable domain;

V_{L2} is a second immunoglobulin light chain variable domain;

V_{L3} is a third immunoglobulin light chain variable domain;

V_{H1} is a first immunoglobulin heavy chain variable domain;

V_{H2} is a second immunoglobulin heavy chain variable domain;

V_{H3} is a third immunoglobulin heavy chain variable domain;

C_L is an immunoglobulin light chain constant domain;

C_{H1} is an immunoglobulin C_{H1} heavy chain constant domain;

C_{H2} is an immunoglobulin C_{H2} heavy chain constant domain;

C_{H3} is an immunoglobulin C_{H3} heavy chain constant domain;

hinge is an immunoglobulin hinge region connecting the C_{H1} and C_{H2} domains; and

L_1, L_2, L_3 and L_4 are amino acid linkers;

wherein the polypeptide of formula I and the polypeptide of formula II form a cross-over light chain-heavy chain pair;

wherein:

(a) V_{L1}, V_{L2} and V_{L3} each independently comprise a variable domain sequence as set forth in any one of SEQ ID NOs:151, 153, 155, 157, 159, 161, 163, 165, and 167; or

(b) V_{L1}, V_{L2} and V_{L3} each independently comprise light chain complementarity determining regions comprising an amino acid sequence as set forth in any one of SEQ ID NOs: 43-59, 123-125, 138-140, and 149; and

wherein:

(a) V_{H1}, V_{H2}, and V_{H3} each independently comprise a variable domain sequence as set forth in any one of SEQ ID NOs:150, 152, 154, 156, 158, 160, 162, 164, and 166; or

(b) V_{H1}, V_{H2} and V_{H3} each independently comprise heavy chain complementarity determining regions comprising an amino acid sequence as set forth in any one of SEQ ID NOs: 25-42, 120-122, and 126-128.

[0273] Item 18: The binding protein of item 16, wherein the second and/or the third polypeptide chain further comprises an Fc region linked to C_{H1}, the Fc region comprising an immunoglobulin hinge region and C_{H2} and C_{H3} immunoglobulin heavy chain constant domains.

[0274] Item 19: The binding protein of any one of items 16-18, wherein at least one of L_1, L_2, L_3 or L_4, is independently 0 amino acids in length.

[0275] Item 20: The binding protein of any one of items 16-18, wherein L_1, L_2, L_3 or L_4 are each independently at least one amino acid in length.

[0276] Item 21: The binding protein of any one of items 16-18 and 20, wherein (a) L_1, L_2, L_3 and L_4 each independently are zero amino acids in length or comprise a sequence selected from the group consisting of GGGGSGGGGS (SEQ ID NO:104), GGGGSGGGGSGGGGS (SEQ ID NO:105), S, RT, TKGPS (SEQ ID NO:106), GQPKAAP (SEQ ID NO: 175), and GGSGSSGSGG (SEQ ID NO:148); or (b) L_1, L_2, L_3 and L_4 each independently comprise a sequence selected from the group consisting of GGGGSGGGGS (SEQ ID NO:104), GGGGSGGGGSGGGGS (SEQ ID NO:105), S, RT, TKGPS (SEQ ID NO:106), GQPKAAP (SEQ ID NO: 175), and GGSGSSGSGG (SEQ ID NO:148).

[0277] Item 22: The binding protein of any one of items 16-20, wherein:

(a) L_1 comprises the sequence GQPKAAP (SEQ ID NO: 175), L_2 comprises the sequence TKGPS (SEQ ID NO:106), L_3 comprises the sequence S, and L_4 comprises the sequence RT;

(b) L_1 comprises the sequence GGGGSGGGGS (SEQ ID NO:104), L_2 comprises the sequence GGGGSGGGGS (SEQ ID NO:104), L_3 is 0 amino acids in length, and L_4 is 0 amino acids in length;

(c) L_1 comprises the sequence GGSGSSGSGG (SEQ ID NO:148), L_2 comprises the sequence GGSGSSGSGG (SEQ ID NO:148), L_3 is 0 amino acids in length, and L_4 is 0 amino acids in length; or

(d) L_1 comprises the sequence GGGGSGGGGSGGGGS (SEQ ID NO:105), L_2 is 0 amino acids in length, L_3 comprises the sequence GGGGSGGGGSGGGGS (SEQ ID NO:105), and L_4 is 0 amino acids in length.

[0278] Item 23: The binding protein of any one of items 16-22, wherein:

(a) V_{H1} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:28, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:29, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:30; V_{L1} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:46, a CDR-L2 comprising the amino

acid sequence of SEQ ID NO: 47, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:48; V_{H2} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:34, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:35, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:36; V_{L2} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:52, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:53, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:54; V_{H3} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:25, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:26, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:27; and V_{L3} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:43, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:44, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:45;

(b) V_{H1} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:31, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:32, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:33; V_{L1} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:49, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:50, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:51; V_{H2} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:34, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:35, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:36; V_{L2} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:52, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:53, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:54; V_{H3} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:25, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:26, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:27; and V_{L3} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:43, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:44, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:45;

(c) V_{H1} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:28, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:29, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:30; V_{L1} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:46, a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 47, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:48; V_{H2} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID

NO:34, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:35, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:36; V_{L2} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:52, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:53, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:54; V_{H3} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:37, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:38, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:39; and V_{L3} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:55, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:56, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:57;

(d) V_{H1} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:31, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:32, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:33; V_{L1} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:49, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:50, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:51; V_{H2} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:34, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:35, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:36; V_{L2} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:52, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:53, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:54; V_{H3} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:37, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:38, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:39; and V_{L3} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:55, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:56, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:57;

(e) V_{H1} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:28, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:29, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:30; V_{L1} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:46, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:179, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:48; V_{H2} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:34, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:35, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:36; V_{L2} comprises a CDR-L1

comprising the amino acid sequence of SEQ ID NO:52, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:53, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:54; V_{H3} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:40, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:41, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:42; and V_{L3} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:58, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:44, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:59;

(f) V_{H1} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:31, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:32, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:33; V_{L1} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:49, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:50, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:51; V_{H2} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:34, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:35, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:36; V_{L2} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:52, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:53, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:54; V_{H3} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:40, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:41, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:42; and V_{L3} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:58, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:44, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:59;

(g) V_{H1} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:28, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:29, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:30; V_{L1} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:46, a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 47, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:48; V_{H2} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:34, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:35, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:36; V_{L2} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:52, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:53, and a CDR-L3 comprising the amino acid sequence of

SEQ ID NO:54; V_{H3} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:126, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:127, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:128; and V_{L3} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:138, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:139, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:140;

(h) V_{H1} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:31, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:32, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:33; V_{L1} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:49, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:50, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:51; V_{H2} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:34, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:35, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:36; V_{L2} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:52, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:53, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:54; V_{H3} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:126, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:127, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:128; and V_{L3} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:138, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:139, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:140;

(i) V_{H1} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:28, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:29, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:30; V_{L1} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:46, a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 47, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:48; V_{H2} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:34, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:35, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:36; V_{L2} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:52, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:53, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:54; V_{H3} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:120, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:121, and a CDR-

H3 comprising the amino acid sequence of SEQ ID NO:122; and V_{L3} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:123, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:124, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:125; or

(j) V_{H1} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:31, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:32, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:33; V_{L1} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:49, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:50, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:51; V_{H2} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:34, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:35, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:36; V_{L2} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:52, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:53, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:54; V_{H3} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:120, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:121, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:122; and V_{L3} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:123, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:124, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:125.

[0279] Item 24: A binding protein comprising four polypeptide chains that form three antigen binding sites, wherein a first polypeptide chain comprises a structure represented by the formula:

$$V_{L2}\text{-}L_1\text{-}V_{L1}\text{-}L_2\text{-}C_L \qquad [I]$$

and a second polypeptide chain comprises a structure represented by the formula:

$$V_{H1}\text{-}L_3\text{-}V_{H2}\text{-}L_4\text{-}C_{H1} \qquad [II]$$

and a third polypeptide chain comprises a structure represented by the formula:

$$V_{H3}\text{-}C_{H1} \qquad [III]$$

and a fourth polypeptide chain comprises a structure represented by the formula:

$$V_{L3}\text{-}C_L \qquad [IV]$$

wherein:

V_{L1} is a first immunoglobulin light chain variable domain;

V_{L2} is a second immunoglobulin light chain variable domain;

V_{L3} is a third immunoglobulin light chain variable domain;

V_{H1} is a first immunoglobulin heavy chain variable domain;

V_{H2} is a second immunoglobulin heavy chain variable domain;

V_{H3} is a third immunoglobulin heavy chain variable domain;

C_L is an immunoglobulin light chain constant domain;

C_{H1} is an immunoglobulin C_{H1} heavy chain constant domain; and

L_1, L_2, L_3 and L_4 are amino acid linkers;

wherein the polypeptide of formula I and the polypeptide of formula II form a cross-over light chain-heavy chain pair;

wherein:

(a) V_{L1}, V_{L2} and V_{L3} each independently comprise a variable domain sequence as set forth in any one of SEQ ID NOs:169, 171, and 173; or

(b) V_{L1}, V_{L2} and V_{L3} each independently comprise light chain complementarity determining regions comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 141-147, 178, and 179;

wherein:

(a) V_{H1}, V_{H2}, and V_{H3} each independently comprise a variable domain sequence as set forth in any one of SEQ ID NOs:168, 170, and 172; or

(b) V_{H1}, V_{H2} and V_{H3} each independently comprise heavy chain complementarity determining regions comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 129-137.

[0280] Item 25: A binding protein comprising four polypeptide chains that form three antigen binding sites, wherein a first polypeptide chain comprises a structure represented by the formula:

$$V_{L2}\text{-}L_1\text{-}V_{L1}\text{-}L_2\text{-}C_L \qquad [I]$$

and a second polypeptide chain comprises a structure represented by the formula:

$$V_{H1}\text{-}L_3\text{-}V_{H2}\text{-}L_4\text{-}C_{H1}\text{-hinge-}C_{H2}\text{-}C_{H3} \qquad [II]$$

and a third polypeptide chain comprises a structure represented by the formula:

$$V_{H3}\text{-}C_{H1}\text{-hinge-}C_{H2}\text{-}C_{H3} \qquad [III]$$

and a fourth polypeptide chain comprises a structure represented by the formula:

$$V_{L3}\text{-}C_L \qquad [IV]$$

wherein:

V_{L1} is a first immunoglobulin light chain variable domain;

V_{L2} is a second immunoglobulin light chain variable domain;

V_{L3} is a third immunoglobulin light chain variable domain;

V_{H1} is a first immunoglobulin heavy chain variable domain;

V_{H2} is a second immunoglobulin heavy chain variable domain;

V_{H3} is a third immunoglobulin heavy chain variable domain;

C_L is an immunoglobulin light chain constant domain;

C_{H1} is an immunoglobulin C_{H1} heavy chain constant domain;

C_{H2} is an immunoglobulin C_{H2} heavy chain constant domain;

C_{H3} is an immunoglobulin C_{H3} heavy chain constant domain;

hinge is an immunoglobulin hinge region connecting the C_{H1} and C_{H2} domains; and

L_1, L_2, L_3 and L_4 are amino acid linkers;

wherein the polypeptide of formula I and the polypeptide of formula II form a cross-over light chain-heavy chain pair;

wherein:

(a) V_{L1}, V_{L2} and V_{L3} each independently comprise a variable domain sequence as set forth in any one of SEQ ID NOs:169, 171, and 173; or

(b) V_{L1}, V_{L2} and V_{L3} each independently comprise light chain complementarity determining regions comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 141-147, 178, and 179;

wherein:

(a) V_{H1}, V_{H2}, and V_{H3} each independently comprise a variable domain sequence as set forth in any one of SEQ ID NOs:168, 170, and 172; or

(b) V_{H1}, V_{H2} and V_{H3} each independently comprise heavy chain complementarity determining regions comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 129-137.

[0281] Item 26: The binding protein of item 24, wherein the second and/or the third polypeptide chain further comprises an Fc region linked to C_{H1}, the Fc region comprising an immunoglobulin hinge region and C_{H2} and C_{H3} immunoglobulin heavy chain constant domains.

[0282] Item 27: The binding protein of any one of items 24-26, wherein at least one of L_1, L_2, L_3 and L_4, is independently 0 amino acids in length.

[0283] Item 28: The binding protein of any one of items 24-26, wherein L_1, L_2, L_3 and L_4 are each independently at least one amino acid in length.

[0284] Item 29: The binding protein of any one of items 24-26 and 28, wherein (a) L_1, L_2, L_3 and L_4 each independently are zero amino acids in length or comprise a sequence selected from the group consisting of GGGGSGGGGS (SEQ ID NO:104), GGGGSGGGGSGGGGS (SEQ ID NO:105), S, RT, TKGPS (SEQ ID NO:106), GQPKAAP

(SEQ ID NO: 175), and GGSGSSGSGG (SEQ ID NO:148); or (b) L_1, L_2, L_3 and L_4 each independently comprise a sequence selected from the group consisting of GGGGSGGGGS (SEQ ID NO:104), GGGGSGGGGSGGGGS (SEQ ID NO:105), S, RT, TKGPS (SEQ ID NO:106), GQPKAAP (SEQ ID NO: 175), and GGSGSSGSGG (SEQ ID NO:148).

[0285] Item 30: The binding protein of any one of items 24-28, wherein:

(a) L_1 comprises the sequence GQPKAAP (SEQ ID NO: 175), L_2 comprises the sequence TKGPS (SEQ ID NO:106), L_3 comprises the sequence S, and L_4 comprises the sequence RT;

(b) L_1 comprises the sequence GGGGSGGGGS (SEQ ID NO:104), L_2 comprises the sequence GGGGSGGGGS (SEQ ID NO:104), L_3 is 0 amino acids in length, and L_4 is 0 amino acids in length;

(c) L_1 comprises the sequence GGSGSSGSGG (SEQ ID NO:148), L_2 comprises the sequence GGSGSSGSGG (SEQ ID NO:148), L_3 is 0 amino acids in length, and L_4 is 0 amino acids in length; or

(d) L_1 comprises the sequence GGGGSGGGGSGGGGS (SEQ ID NO:105), L_2 is 0 amino acids in length, L_3 comprises the sequence GGGGSGGGGSGGGGS (SEQ ID NO:105), and L_4 is 0 amino acids in length.

[0286] Item 31: The binding protein of any one of items 24-30, wherein:

(a) V_{H1} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:132, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:133, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:134; V_{L1} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:143, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:179, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:144; V_{H2} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:135, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:136, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:137; V_{L2} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:145, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:146, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:147; V_{H3} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:129, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:130, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:131; and V_{L3} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:141, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:178, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:142;

(b) V_{H1} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:135, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:136, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:137; V_{L1} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:145, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:146, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:147; V_{H2} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:132, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:133, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:134; V_{L2} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:143, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:47, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:144; V_{H3} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:129, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:130, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:131; and V_{L3} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:141, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:178, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:142;

(c) V_{H1} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:132, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:133, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:134; V_{L1} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:143, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:179, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:144; V_{H2} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:129, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:130, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:131; V_{L2} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:141, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:178, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:142; V_{H3} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:135, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:136, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:137; and V_{L3} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:145, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:146, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:147;

(d) V_{H1} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:129, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:130, and a CDR-

H3 comprising the amino acid sequence of SEQ ID NO:131; V_{L1} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:141, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:178, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:142; V_{H2} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:132, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:133, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:134; V_{L2} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:143, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:179, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:144; V_{H3} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:135, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:136, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:137; and V_{L3} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:145, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:146, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:147;

(e) V_{H1} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:135, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:136, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:137; V_{L1} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:145, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:146, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:147; V_{H2} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:129, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:130, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:131; V_{L2} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:141, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:178, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:142; V_{H3} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:132, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:133, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:134; and V_{L3} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:143, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:179, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:144;

(f) V_{H1} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:129, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:130, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:131; V_{L1} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:141, a CDR-L2 comprising the amino

acid sequence of SEQ ID NO:178, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:142; V_{H2} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:135, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:136, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:137; V_{L2} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:145, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:146, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:147; V_{H3} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:132, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:133, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:134; and V_{L3} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:143, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:179, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:144;

(g) V_{H1} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:135, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:136, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:137; V_{L1} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:145, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:146, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:147; V_{H2} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:132, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:133, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:134; V_{L2} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:143, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:179, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:144; V_{H3} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:135, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:136, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:137; and V_{L3} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:145, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:146, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:147;

(h) V_{H1} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:135, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:136, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:137; V_{L1} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:145, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:146, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:147; V_{H2} comprises a CDR-H1 comprising the amino acid sequence of SEQ

ID NO:132, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:133, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:134; V_{L2} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:143, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:179, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:144; V_{H3} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:132, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:133, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:134; and V_{L3} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:143, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:179, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:144;

(i) V_{H1} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:132, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:133, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:134; V_{L1} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:143, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:179, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:144; V_{H2} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:135, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:136, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:137; V_{L2} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:145, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:146, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:147; V_{H3} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:135, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:136, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:137; and V_{L3} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:145, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:146, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:147; or

(j) V_{H1} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:132, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:133, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:134; V_{L1} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:143, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:179, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:144; V_{H2} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:135, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:136, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:137; V_{L2} comprises a CDR-L1

comprising the amino acid sequence of SEQ ID NO:145, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:146, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:147; V_{H3} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:132, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:133, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:134; and V_{L3} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:143, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:179, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:144.

[0287] Item 32: The binding protein of any one of items 1, 3-16, 18-24, and 26-31, wherein the second polypeptide chain further comprises a first Fc region linked to C_{H1}, the first Fc region comprising an immunoglobulin hinge region and C_{H2} and C_{H3} immunoglobulin heavy chain constant domains, wherein the first Fc region comprises amino acid substitutions at positions corresponding to positions 354 and 366 of human IgG1 or IgG4 according to EU Index, wherein the amino acid substitutions are S354C and T366W; and wherein the third polypeptide chain further comprises a second Fc region linked to C_{H1}, the second Fc region comprising an immunoglobulin hinge region and C_{H2} and C_{H3} immunoglobulin heavy chain constant domains, wherein the second Fc region comprises amino acid substitutions at positions corresponding to positions 349, 366, 368, and 407 of human IgG1 or IgG4 according to EU Index, wherein the amino acid substitutions are Y349C, T366S, L368A, and Y407V.

[0288] Item 33: The binding protein of any one of items 1, 3-16, 18-24, and 26-31, wherein the second polypeptide chain further comprises a first Fc region linked to C_{H1}, the first Fc region comprising an immunoglobulin hinge region and C_{H2} and C_{H3} immunoglobulin heavy chain constant domains, wherein the first Fc region comprises amino acid substitutions at positions corresponding to positions 349, 366, 368, and 407 of human IgG1 or IgG4 according to EU Index, wherein the amino acid substitutions are Y349C, T366S, L368A, and Y407V; and wherein the third polypeptide chain further comprises a second Fc region linked to C_{H1}, the second Fc region comprising an immunoglobulin hinge region and C_{H2} and C_{H3} immunoglobulin heavy chain constant domains, wherein the second Fc region comprises amino acid substitutions at positions corresponding to positions 354 and 366 of human IgG1 or IgG4 according to EU Index, wherein the amino acid substitutions are S354C and T366W.

[0289] Item 34: The binding protein of any one of items 1, 3-16, 18-24, and 26-33, wherein the second polypeptide chain further comprises a first Fc region linked to C_{H1}, the first Fc region comprising an immunoglobulin hinge region and C_{H2} and C_{H3} immunoglobulin

heavy chain constant domains, and wherein the third polypeptide chain further comprises a second Fc region linked to C_{H1}, the second Fc region comprising an immunoglobulin hinge region and C_{H2} and C_{H3} immunoglobulin heavy chain constant domains; wherein the first and second Fc regions comprise amino acid substitutions at positions corresponding to positions 428 and 434 of human IgG1 or IgG4 according to EU Index, wherein the amino acid substitutions are M428L and N434S.

[0290] Item 35: The binding protein of any one of items 2, 4-15, 17, 19-23, 25, and 27-31, wherein the C_{H3} domain of the second polypeptide chain comprises amino acid substitutions at positions corresponding to positions 354 and 366 of human IgG1 or IgG4 according to EU Index, wherein the amino acid substitutions are S354C and T366W; and wherein the C_{H3} domain of the third polypeptide chain comprises amino acid substitutions at positions corresponding to positions 349, 366, 368, and 407 of human IgG1 or IgG4 according to EU Index, wherein the amino acid substitutions are Y349C, T366S, L368A, and Y407V.

[0291] Item 36: The binding protein of any one of items 2, 4-15, 17, 19-23, 25, and 27-31, wherein the C_{H3} domain of the second polypeptide chain comprises amino acid substitutions at positions corresponding to positions 349, 366, 368, and 407 of human IgG1 or IgG4 according to EU Index, wherein the amino acid substitutions are Y349C, T366S, L368A, and Y407V; and wherein the C_{H3} domain of the third polypeptide chain comprises amino acid substitutions at positions corresponding to positions 354 and 366 of human IgG1 or IgG4 according to EU Index, wherein the amino acid substitutions are S354C and T366W.

[0292] Item 37: The binding protein of any one of items 2, 4-15, 17, 19-23, 25, 27-31, 35, and 36, wherein the C_{H3} domains of the second and the third polypeptide chains both comprise amino acid substitutions at positions corresponding to positions 428 and 434 of human IgG1 or IgG4 according to EU Index, wherein the amino acid substitutions are M428L and N434S

[0293] Item 38: The binding protein of any one of items 1, 3-16, 18-24, and 26-34, wherein the second polypeptide chain further comprises a first Fc region linked to C_{H1}, the first Fc region comprising an immunoglobulin hinge region and C_{H2} and C_{H3} immunoglobulin heavy chain constant domains; wherein the third polypeptide chain further comprises a second Fc region linked to C_{H1}, the second Fc region comprising an immunoglobulin hinge region and C_{H2} and C_{H3} immunoglobulin heavy chain constant domains; wherein the first and second Fc regions are human IgG1 or IgG4 Fc regions; and wherein only one of the first and the second Fc regions comprises amino acid substitutions at positions corresponding to

positions 435 and 436 of human IgG1 or IgG4 according to EU Index, wherein the amino acid substitutions are H435R and Y436F.

[0294] Item 39: The binding protein of any one of items 2, 4-15, 17, 19-23, 25, 27-31, and 35-37, wherein the C_{H3} domains of the second and the third polypeptide chains are human IgG1 or IgG4 C_{H3} domains, and wherein only one of the C_{H3} domains comprises amino acid substitutions at positions corresponding to positions 435 and 436 of human IgG1 or IgG4 according to EU Index, wherein the amino acid substitutions are H435R and Y436F.

[0295] Item 40: The binding protein of any one of items 1, 3-16, 18-24, 26-34, and 38, wherein the second polypeptide chain further comprises a first Fc region linked to C_{H1}, the first Fc region comprising an immunoglobulin hinge region and C_{H2} and C_{H3} immunoglobulin heavy chain constant domains; wherein the third polypeptide chain further comprises a second Fc region linked to C_{H1}, the second Fc region comprising an immunoglobulin hinge region and C_{H2} and C_{H3} immunoglobulin heavy chain constant domains; wherein the first and second Fc regions are human IgG4 Fc regions; and wherein the first and the second Fc regions each comprise amino acid substitutions at positions corresponding to positions 228 and 409 of human IgG4 according to EU Index, wherein the amino acid substitutions are S228P and R409K.

[0296] Item 41: The binding protein of any one of items 2, 4-15, 17, 19-23, 25, 27-31, 35-37, and 39, wherein the C_{H3} domains of the second and the third polypeptide chains are human IgG4 C_{H3} domains, and wherein the C_{H3} domains each comprise amino acid substitutions at positions corresponding to positions 228 and 409 of human IgG4 according to EU Index, wherein the amino acid substitutions are S228P and R409K.

[0297] Item 42: The binding protein of any one of items 1, 3-16, 18-24, 26-34, 38, and 40, wherein the second polypeptide chain further comprises a first Fc region linked to C_{H1}, the first Fc region comprising an immunoglobulin hinge region and C_{H2} and C_{H3} immunoglobulin heavy chain constant domains; wherein the third polypeptide chain further comprises a second Fc region linked to C_{H1}, the second Fc region comprising an immunoglobulin hinge region and C_{H2} and C_{H3} immunoglobulin heavy chain constant domains; wherein the first and second Fc regions are human IgG4 Fc regions; and wherein the first and the second Fc regions each comprise amino acid substitutions at positions corresponding to positions 234 and 235 of human IgG4 according to EU Index, wherein the amino acid substitutions are F234A and L235A.

[0298] Item 43: The binding protein of any one of items 2, 4-15, 17, 19-23, 25, 27-31, 35-37, 39, and 41, wherein the C_{H3} domains of the second and the third polypeptide chains

are human IgG4 C_{H3} domains, and wherein the C_{H3} domains each comprise amino acid substitutions at positions corresponding to positions 234 and 235 of human IgG4 according to EU Index, wherein the amino acid substitutions are F234A and L235A.

[0299] Item 44: The binding protein of any one of items 1, 3-16, 18-24, 26-34, 38, and 40, wherein the second polypeptide chain further comprises a first Fc region linked to C_{H1}, the first Fc region comprising an immunoglobulin hinge region and C_{H2} and C_{H3} immunoglobulin heavy chain constant domains; wherein the third polypeptide chain further comprises a second Fc region linked to C_{H1}, the second Fc region comprising an immunoglobulin hinge region and C_{H2} and C_{H3} immunoglobulin heavy chain constant domains; wherein the first and second Fc regions are human IgG1 Fc regions; and wherein the first and the second Fc regions each comprise amino acid substitutions at positions corresponding to positions 234 and 235 of human IgG4 according to EU Index, wherein the amino acid substitutions are L234A and L235A.

[0300] Item 45: The binding protein of any one of items 2, 4-15, 17, 19-23, 25, 27-31, 35-37, 39, and 41, wherein the C_{H3} domains of the second and the third polypeptide chains are human IgG1 C_{H3} domains, and wherein the C_{H3} domains each comprise amino acid substitutions at positions corresponding to positions 234 and 235 of human IgG4 according to EU Index, wherein the amino acid substitutions are L234A and L235A.

[0301] Item 46: The binding protein of any one of items 2, 4-15, 17, 19-23, 25, 27-31, 35-37, 39, 41, 43, and 45, wherein VH1 and VL1 form a first antigen binding site that specifically binds human CD3, wherein VH2 and VL2 form a second antigen binding site that specifically binds human CD28, and wherein VH3 and VL3 form a third antigen binding site that specifically binds a human tumor target protein.

[0302] Item 47: The binding protein of any one of items 2, 4-15, 17, 19-23, 25, 27-31, 35-37, 39, 41, 43, and 45, wherein VH1 and VL1 form a first antigen binding site that specifically binds human CD28, wherein VH2 and VL2 form a second antigen binding site that specifically binds human CD3, and wherein VH3 and VL3 form a third antigen binding site that specifically binds a human tumor target protein.

[0303] Item 48: The binding protein of item 46 or item 47, wherein the third antigen binding site specifically binds a human tumor target protein selected from the group consisting of CD19, CD20, CD38, Her2, and LAMP1.

[0304] Item 49: The binding protein of any one of items 46-48, wherein the antigen binding site that specifically binds CD3 comprises:

(a) a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 152 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO: 153; or

(b) a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 154 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO: 155.

[0305] Item 50: The binding protein of any one of items 46-49, wherein the antigen binding site that specifically binds CD28 comprises:

(a) a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 160 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO: 161; or

(b) a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 162 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO: 163.

[0306] Item 51: The binding protein of any one of items 46-50, wherein the antigen binding site that specifically binds a tumor target protein comprises:

(a) a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 156 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO: 157;

(b) a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 158 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO: 159;

(c) a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 164 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO: 165;

(d) a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 150 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO: 151; or

(e) a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 166 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO: 167.

[0307] Item 52: The binding protein of any one of items 2, 17, 35-37, 39, 41, 43, and 45, wherein:

(a)VH1 and VL1 form a first antigen binding site that specifically binds human TNFa, VH2 and VL2 form a second antigen binding site that specifically binds human IL13, and VH3 and VL3 form a third antigen binding site that specifically binds human IL4;

(b) VH1 and VL1 form a first antigen binding site that specifically binds human TNFa, VH2 and VL2 form a second antigen binding site that specifically binds human IL4, and VH3 and VL3 form a third antigen binding site that specifically binds human IL13;

(c) VH1 and VL1 form a first antigen binding site that specifically binds human IL4, VH2 and VL2 form a second antigen binding site that specifically binds human TNFa, and VH3 and VL3 form a third antigen binding site that specifically binds human IL13;

(d) VH1 and VL1 form a first antigen binding site that specifically binds human IL4, VH2 and VL2 form a second antigen binding site that specifically binds human IL13, and VH3 and VL3 form a third antigen binding site that specifically binds human TNFa;

(e) VH1 and VL1 form a first antigen binding site that specifically binds human IL13, VH2 and VL2 form a second antigen binding site that specifically binds human IL4, and VH3 and VL3 form a third antigen binding site that specifically binds human TNFa; or

(f) VH1 and VL1 form a first antigen binding site that specifically binds human IL13, VH2 and VL2 form a second antigen binding site that specifically binds human TNFa, and VH3 and VL3 form a third antigen binding site that specifically binds human IL4.

[0308] Item 53: The binding protein of item 52, wherein the antigen binding site that specifically binds human TNFa comprises a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:168 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO:169.

[0309] Item 54: The binding protein of item 52 or item 53, wherein the antigen binding site that specifically binds human IL4 comprises a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:170 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO:171.

[0310] Item 55: The binding protein of any one of items 52-54, wherein the antigen binding site that specifically binds human IL13 comprises a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:172 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO:173.

[0311] Item 56: The binding protein of any one of items 1-55, wherein:

(a) the C_L domain of the first polypeptide chain is a human kappa C_L domain, and the C_L domain of the fourth polypeptide chain is a human lambda C_L domain; or

(b) the C_L domain of the first polypeptide chain is a human lambda C_L domain, and the C_L domain of the fourth polypeptide chain is a human kappa C_L domain.

[0312] Item 57: The binding protein of any one of items 2, 17, 25, 35-37, 39, 41, and 43-55, wherein the first polypeptide chain comprises a lambda C_L domain; wherein the C_{H3} domain of the second polypeptide chain comprises amino acid substitutions at positions corresponding to positions 354 and 366 of human IgG1 according to EU Index, wherein the amino acid substitutions are S354C and T366W; wherein the C_{H3} domain of the third polypeptide chain comprises amino acid substitutions at positions corresponding to positions 349, 366, 368, 407, 435, and 436 of human IgG1 according to EU Index, wherein the amino acid substitutions are Y349C, T366S, L368A, Y407V, H435R, and Y436F; and wherein the fourth polypeptide chain comprises a kappa C_L domain.

[0313] Item 58: A binding protein comprising a first polypeptide chain, a second polypeptide chain, a third polypeptide chain and a fourth polypeptide chain wherein:

(a) the first polypeptide chain comprises the amino acid sequence of SEQ ID NO: 4 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 4; the second polypeptide chain comprises the amino acid sequence of SEQ ID NO: 3 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 3; the third polypeptide chain comprises the amino acid sequence of SEQ ID NO: 1 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 1; and the fourth polypeptide chain comprises the amino acid sequence of SEQ ID NO: 2 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 2;

(b) the first polypeptide chain comprises the amino acid sequence of SEQ ID NO: 10 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 10; the second polypeptide chain comprises the amino acid sequence of SEQ ID NO: 9 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 9; the third polypeptide chain comprises the amino acid sequence of SEQ ID NO: 1 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 1; and the fourth polypeptide chain comprises the amino acid sequence of SEQ ID NO: 2 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 2;

(c) the first polypeptide chain comprises the amino acid sequence of SEQ ID NO: 4 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 4; the second polypeptide chain comprises the amino acid sequence of SEQ ID NO: 3

or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 3; the third polypeptide chain comprises the amino acid sequence of SEQ ID NO: 13 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 13; and the fourth polypeptide chain comprises the amino acid sequence of SEQ ID NO: 14 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 14;

(d) the first polypeptide chain comprises the amino acid sequence of SEQ ID NO: 10 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 10; the second polypeptide chain comprises the amino acid sequence of SEQ ID NO: 9 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 9; the third polypeptide chain comprises the amino acid sequence of SEQ ID NO: 13 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 13; and the fourth polypeptide chain comprises the amino acid sequence of SEQ ID NO: 14 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 14;

(e) the first polypeptide chain comprises the amino acid sequence of SEQ ID NO: 4 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 4; the second polypeptide chain comprises the amino acid sequence of SEQ ID NO: 3 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 3; the third polypeptide chain comprises the amino acid sequence of SEQ ID NO: 17 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 17; and the fourth polypeptide chain comprises the amino acid sequence of SEQ ID NO: 18 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 18;

(f) the first polypeptide chain comprises the amino acid sequence of SEQ ID NO: 10 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 10; the second polypeptide chain comprises the amino acid sequence of SEQ ID NO: 9 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 9; the third polypeptide chain comprises the amino acid sequence of SEQ ID NO: 17 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 17; and the fourth polypeptide chain comprises the amino acid sequence of SEQ ID NO: 18 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 18;

(g) the first polypeptide chain comprises the amino acid sequence of SEQ ID NO: 4 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 4; the second polypeptide chain comprises the amino acid sequence of SEQ ID NO: 3 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 3; the third polypeptide chain comprises the amino acid sequence of SEQ ID NO: 21 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 21; and the fourth polypeptide chain comprises the amino acid sequence of SEQ ID NO: 22 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 22;

(h) the first polypeptide chain comprises the amino acid sequence of SEQ ID NO: 10 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 10; the second polypeptide chain comprises the amino acid sequence of SEQ ID NO: 9 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 9; the third polypeptide chain comprises the amino acid sequence of SEQ ID NO: 21 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 21; and the fourth polypeptide chain comprises the amino acid sequence of SEQ ID NO: 22 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 22;

(i) the first polypeptide chain comprises the amino acid sequence of SEQ ID NO: 63 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 63; the second polypeptide chain comprises the amino acid sequence of SEQ ID NO: 62 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 62; the third polypeptide chain comprises the amino acid sequence of SEQ ID NO: 60 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 60; and the fourth polypeptide chain comprises the amino acid sequence of SEQ ID NO: 61 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 61;

(j) the first polypeptide chain comprises the amino acid sequence of SEQ ID NO: 69 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 69; the second polypeptide chain comprises the amino acid sequence of SEQ ID NO: 68 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 68; the third polypeptide chain comprises the amino acid sequence of SEQ ID NO: 60 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 60; and the fourth polypeptide chain comprises the amino acid sequence of SEQ

ID NO: 61 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 61;

(k) the first polypeptide chain comprises the amino acid sequence of SEQ ID NO: 69 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 69; the second polypeptide chain comprises the amino acid sequence of SEQ ID NO: 68 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 68; the third polypeptide chain comprises the amino acid sequence of SEQ ID NO: 60 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 60; and the fourth polypeptide chain comprises the amino acid sequence of SEQ ID NO: 71 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 71;

(l) the first polypeptide chain comprises the amino acid sequence of SEQ ID NO: 76 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 76; the second polypeptide chain comprises the amino acid sequence of SEQ ID NO: 75 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 75; the third polypeptide chain comprises the amino acid sequence of SEQ ID NO: 73 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 73; and the fourth polypeptide chain comprises the amino acid sequence of SEQ ID NO: 74 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 74;

(m) the first polypeptide chain comprises the amino acid sequence of SEQ ID NO: 82 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 82; the second polypeptide chain comprises the amino acid sequence of SEQ ID NO: 81 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO:81; the third polypeptide chain comprises the amino acid sequence of SEQ ID NO: 73 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 73; and the fourth polypeptide chain comprises the amino acid sequence of SEQ ID NO: 74 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 74;

(n) the first polypeptide chain comprises the amino acid sequence of SEQ ID NO: 88 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO:88; the second polypeptide chain comprises the amino acid sequence of SEQ ID NO: 87 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 87; the third polypeptide chain comprises the amino acid sequence of SEQ ID

NO: 85 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 85; and the fourth polypeptide chain comprises the amino acid sequence of SEQ ID NO: 86 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 86;

(o) the first polypeptide chain comprises the amino acid sequence of SEQ ID NO: 94 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 94; the second polypeptide chain comprises the amino acid sequence of SEQ ID NO: 93 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 93; the third polypeptide chain comprises the amino acid sequence of SEQ ID NO: 85 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 85; and the fourth polypeptide chain comprises the amino acid sequence of SEQ ID NO: 86 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 86;

(p) the first polypeptide chain comprises the amino acid sequence of SEQ ID NO: 69 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 69; the second polypeptide chain comprises the amino acid sequence of SEQ ID NO: 68 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 68; the third polypeptide chain comprises the amino acid sequence of SEQ ID NO: 73 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 73; and the fourth polypeptide chain comprises the amino acid sequence of SEQ ID NO: 74 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 74;

(q) the first polypeptide chain comprises the amino acid sequence of SEQ ID NO: 69 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 69; the second polypeptide chain comprises the amino acid sequence of SEQ ID NO: 68 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 68; the third polypeptide chain comprises the amino acid sequence of SEQ ID NO: 85 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 85; and the fourth polypeptide chain comprises the amino acid sequence of SEQ ID NO: 86 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 86;

(r) the first polypeptide chain comprises the amino acid sequence of SEQ ID NO: 63 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 63; the second polypeptide chain comprises the amino acid sequence of SEQ ID

DEMANDE OU BREVET VOLUMINEUX

LA PRÉSENTE PARTIE DE CETTE DEMANDE OU CE BREVET COMPREND PLUS D'UN TOME.

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NOTE POUR LE TOME / VOLUME NOTE:

CLAIMS

What is claimed is:

1. A binding protein comprising four polypeptide chains that form three antigen binding sites that specifically bind one or more target proteins, wherein a first polypeptide chain comprises a structure represented by the formula:

$$V_{L2}\text{-}L_1\text{-}V_{L1}\text{-}L_2\text{-}C_L \qquad [I]$$

and a second polypeptide chain comprises a structure represented by the formula:

$$V_{H1}\text{-}L_3\text{-}V_{H2}\text{-}L_4\text{-}C_{H1} \qquad [II]$$

and a third polypeptide chain comprises a structure represented by the formula:

$$V_{H3}\text{-}C_{H1} \qquad [III]$$

and a fourth polypeptide chain comprises a structure represented by the formula:

$$V_{L3}\text{-}C_L \qquad [IV]$$

wherein:

V_{L1} is a first immunoglobulin light chain variable domain;

V_{L2} is a second immunoglobulin light chain variable domain;

V_{L3} is a third immunoglobulin light chain variable domain;

V_{H1} is a first immunoglobulin heavy chain variable domain;

V_{H2} is a second immunoglobulin heavy chain variable domain;

V_{H3} is a third immunoglobulin heavy chain variable domain;

C_L is an immunoglobulin light chain constant domain;

C_{H1} is an immunoglobulin C_{H1} heavy chain constant domain; and

L_1, L_2, L_3 and L_4 are amino acid linkers;

and wherein the polypeptide of formula I and the polypeptide of formula II form a cross-over light chain-heavy chain pair.

2. A binding protein comprising four polypeptide chains that form three antigen binding sites that specifically bind one or more target proteins, wherein a first polypeptide chain comprises a structure represented by the formula:

$$V_{L2}\text{-}L_1\text{-}V_{L1}\text{-}L_2\text{-}C_L \qquad [I]$$

and a second polypeptide chain comprises a structure represented by the formula:

$$V_{H1}\text{-}L_3\text{-}V_{H2}\text{-}L_4\text{-}C_{H1}\text{-hinge-}C_{H2}\text{-}C_{H3} \quad [II]$$

and a third polypeptide chain comprises a structure represented by the formula:

$$V_{H3}\text{-}C_{H1}\text{-hinge-}C_{H2}\text{-}C_{H3} \quad [III]$$

and a fourth polypeptide chain comprises a structure represented by the formula:

$$V_{L3}\text{-}C_L \quad [IV]$$

wherein:

V_{L1} is a first immunoglobulin light chain variable domain;

V_{L2} is a second immunoglobulin light chain variable domain;

V_{L3} is a third immunoglobulin light chain variable domain;

V_{H1} is a first immunoglobulin heavy chain variable domain;

V_{H2} is a second immunoglobulin heavy chain variable domain;

V_{H3} is a third immunoglobulin heavy chain variable domain;

C_L is an immunoglobulin light chain constant domain;

C_{H1} is an immunoglobulin C_{H1} heavy chain constant domain;

C_{H2} is an immunoglobulin C_{H2} heavy chain constant domain;

C_{H3} is an immunoglobulin C_{H3} heavy chain constant domain;

hinge is an immunoglobulin hinge region connecting the C_{H1} and C_{H2} domains; and

L_1, L_2, L_3 and L_4 are amino acid linkers;

and wherein the polypeptide of formula I and the polypeptide of formula II form a cross-over light chain-heavy chain pair.

3. The binding protein of claim 1, wherein the second and/or the third polypeptide chain further comprises an Fc region linked to C_{H1}, the Fc region comprising an immunoglobulin hinge region and C_{H2} and C_{H3} immunoglobulin heavy chain constant domains.

4. The binding protein of claim 1 or claim 2, wherein at least one of L_1, L_2, L_3 or L_4 is independently 0 amino acids in length.

5. The binding protein of claim 1 or claim 2, wherein L_1, L_2, L_3 or L_4 are each independently at least one amino acid in length.

6. The binding protein of claim 1 or claim 2, wherein (a) L_1, L_2, L_3 and L_4 each independently are zero amino acids in length or comprise a sequence selected from the group consisting of GGGGSGGGGS (SEQ ID NO:104), GGGGSGGGGSGGGGS (SEQ ID NO:105), S, RT, TKGPS (SEQ ID NO:106), GQPKAAP (SEQ ID NO: 175), and GGSGSSGSGG (SEQ ID NO:148); or (b) L_1, L_2, L_3 and L_4 each independently comprise a sequence selected from the group consisting of GGGGSGGGGS (SEQ ID NO:104), GGGGSGGGGSGGGGS (SEQ ID NO:105), S, RT, TKGPS (SEQ ID NO:106), GQPKAAP (SEQ ID NO: 175), and GGSGSSGSGG (SEQ ID NO:148).

7. The binding protein of claim 1 or claim 2, wherein

(a) L_1 comprises the sequence GQPKAAP (SEQ ID NO: 175), L_2 comprises the sequence TKGPS (SEQ ID NO:106), L_3 comprises the sequence S, and L_4 comprises the sequence RT;

(b) L_1 comprises the sequence GGGGSGGGGS (SEQ ID NO:104), L_2 comprises the sequence GGGGSGGGGS (SEQ ID NO:104), L_3 is 0 amino acids in length, and L_4 is 0 amino acids in length;

(c) L_1 comprises the sequence GGSGSSGSGG (SEQ ID NO:148), L_2 comprises the sequence GGSGSSGSGG (SEQ ID NO:148), L_3 is 0 amino acids in length, and L_4 is 0 amino acids in length; or

(d) L_1 comprises the sequence GGGGSGGGGSGGGGS (SEQ ID NO:105), L_2 is 0 amino acids in length, L_3 comprises the sequence GGGGSGGGGSGGGGS (SEQ ID NO:105), and L_4 is 0 amino acids in length.

8. The binding protein of claim 1 or claim 2, wherein the binding protein is trispecific and capable of specifically binding three different antigen targets.

9. The binding protein of any one of claims 1-8, wherein the binding protein specifically binds three target proteins that correspond to two target proteins on T cells and to one tumor target protein.

10. The binding protein of claim 9, wherein one of said target proteins on T cells is CD3.

11. The binding protein of claim 9 or claim 10, wherein one of said target proteins on T cells is CD28.

12. The binding protein of any one of claims 9-11, wherein said tumor target protein is CD38.

13. The binding protein of any one of claims 1-8, wherein the binding protein specifically binds three target proteins that correspond to two target proteins on T cells and to one target protein selected from the group consisting of A2AR, APRIL, ATPDase, BAFF, BAFFR, BCMA, BlyS, BTK, BTLA, B7DC, B7H1, B7H4, B7H5, B7H6, B7H7, B7RP1, B7-4, C3, C5, CCL2, CCL3, CCL4, CCL5, CCL7, CCL8, CCL11, CCL15, CCL17, CCL19, CCL20, CCL21, CCL24, CCL25, CCL26, CCR3, CCR4, CD3, CD19, CD20, CD23, CD24, CD27, CD28, CD38, CD39, CD40, CD70, CD80, CD86, CD122, CD137, CD137L, CD152, CD154, CD160, CD272, CD273, CD274, CD275, CD276, CD278, CD279, CDH1, chitinase, CLEC9, CLEC91, CRTH2, CSF-1, CSF-2, CSF-3, CX3CL1, CXCL12, CXCL13, CXCR3, DNGR-1, ectonucleoside triphosphate diphosphohydrolase 1, EGFR, ENTPD1, FCER1A, FCER1, FLAP, FOLH1, Gi24, GITR, GITRL, GM-CSF, Her2, HHLA2, HMGB1, HVEM, ICOSLG, IDO, IFNα, IgE, IGF1R, IL2Rbeta, IL1, IL1A, IL1B, IL1F10, IL2, IL4, IL4Ra, IL5, IL5R, IL6, IL7, IL7Ra, IL8, IL9, IL9R, IL10, rhIL10, IL12, IL13, IL13Ra1, IL13Ra2, IL15, IL17, IL17Rb, IL18, IL22, IL23, IL25, IL27, IL33, IL35, ITGB4, ITK, KIR, LAG3, LAMP1, leptin, LPFS2, MHC class II, NCR3LG1, NKG2D, NTPDase-1, OX40, OX40L, PD-1H, platelet receptor, PROM1, S152, SISP1, SLC, SPG64, ST2, STEAP2, Syk kinase, TACI, TDO, T14, TIGIT, TIM3, TLR, TLR2, TLR4, TLR5, TLR9, TMEF1, TNFa, TNFRSF7, Tp55, TREM1, TSLP, TSLPR, TWEAK, VEGF, VISTA, Vstm3, WUCAM, and XCR1.

14. The binding protein of claim 1 or claim 2, wherein the binding protein is capable of inhibiting the function of one or more target proteins.

15. The binding protein of claim 14, wherein the one or more target proteins are selected from the group consisting of A2AR, APRIL, ATPDase, BAFF, BAFFR, BCMA, BlyS, BTK, BTLA, B7DC, B7H1, B7H4, B7H5, B7H6, B7H7, B7RP1, B7-4, C3, C5, CCL2, CCL3,

CCL4, CCL5, CCL7, CCL8, CCL11, CCL15, CCL17, CCL19, CCL20, CCL21, CCL24, CCL25, CCL26, CCR3, CCR4, CD3, CD19, CD20, CD23, CD24, CD27, CD28, CD38, CD39, CD40, CD70, CD80, CD86, CD122, CD137, CD137L, CD152, CD154, CD160, CD272, CD273, CD274, CD275, CD276, CD278, CD279, CDH1, chitinase, CLEC9, CLEC91, CRTH2, CSF-1, CSF-2, CSF-3, CX3CL1, CXCL12, CXCL13, CXCR3, DNGR-1, ectonucleoside triphosphate diphosphohydrolase 1, EGFR, ENTPD1, FCER1A, FCER1, FLAP, FOLH1, Gi24, GITR, GITRL, GM-CSF, Her2, HHLA2, HMGB1, HVEM, ICOSLG, IDO, IFNα, IgE, IGF1R, IL2Rbeta, IL1, IL1A, IL1B, IL1F10, IL2, IL4, IL4Ra, IL5, IL5R, IL6, IL7, IL7Ra, IL8, IL9, IL9R, IL10, rhIL10, IL12, IL13, IL13Ra1, IL13Ra2, IL15, IL17, IL17Rb, IL18, IL22, IL23, IL25, IL27, IL33, IL35, ITGB4, ITK, KIR, LAG3, LAMP1, leptin, LPFS2, MHC class II, NCR3LG1, NKG2D, NTPDase-1, OX40, OX40L, PD-1H, platelet receptor, PROM1, S152, SISP1, SLC, SPG64, ST2, STEAP2, Syk kinase, TACI, TDO, T14, TIGIT, TIM3, TLR, TLR2, TLR4, TLR5, TLR9, TMEF1, TNFa, TNFRSF7, Tp55, TREM1, TSLP, TSLPR, TWEAK, VEGF, VISTA, Vstm3, WUCAM, and XCR1.

16. A binding protein comprising four polypeptide chains that form three antigen binding sites, wherein a first polypeptide chain comprises a structure represented by the formula:

$$V_{L2}\text{-}L_1\text{-}V_{L1}\text{-}L_2\text{-}C_L \qquad [I]$$

and a second polypeptide chain comprises a structure represented by the formula:

$$V_{H1}\text{-}L_3\text{-}V_{H2}\text{-}L_4\text{-}C_{H1} \qquad [II]$$

and a third polypeptide chain comprises a structure represented by the formula:

$$V_{H3}\text{-}C_{H1} \qquad [III]$$

and a fourth polypeptide chain comprises a structure represented by the formula:

$$V_{L3}\text{-}C_L \qquad [IV]$$

wherein:

V_{L1} is a first immunoglobulin light chain variable domain;

V_{L2} is a second immunoglobulin light chain variable domain;

V_{L3} is a third immunoglobulin light chain variable domain;

V_{H1} is a first immunoglobulin heavy chain variable domain;

V_{H2} is a second immunoglobulin heavy chain variable domain;

V_{H3} is a third immunoglobulin heavy chain variable domain;

C_L is an immunoglobulin light chain constant domain;

C_{H1} is an immunoglobulin C_{H1} heavy chain constant domain; and

L_1, L_2, L_3 and L_4 are amino acid linkers;

wherein the polypeptide of formula I and the polypeptide of formula II form a cross-over light chain-heavy chain pair;

wherein:

(a) V_{L1}, V_{L2} and V_{L3} each independently comprise a variable domain sequence as set forth in any one of SEQ ID NOs:151, 153, 155, 157, 159, 161, 163, 165, and 167; or

(b) V_{L1}, V_{L2} and V_{L3} each independently comprise light chain complementarity determining regions comprising an amino acid sequence as set forth in any one of SEQ ID NOs: 43-59, 123-125, 138-140, and 149; and

wherein:

(a) V_{H1}, V_{H2}, and V_{H3} each independently comprise a variable domain sequence as set forth in any one of SEQ ID NOs:150, 152, 154, 156, 158, 160, 162, 164, and 166; or

(b) V_{H1}, V_{H2} and V_{H3} each independently comprise heavy chain complementarity determining regions comprising an amino acid sequence as set forth in any one of SEQ ID NOs: 25-42, 120-122, and 126-128.

17. A binding protein comprising four polypeptide chains that form three antigen binding sites, wherein a first polypeptide chain comprises a structure represented by the formula:

$$V_{L2}\text{-}L_1\text{-}V_{L1}\text{-}L_2\text{-}C_L \qquad [I]$$

and a second polypeptide chain comprises a structure represented by the formula:

$$V_{H1}\text{-}L_3\text{-}V_{H2}\text{-}L_4\text{-}C_{H1}\text{-hinge-}C_{H2}\text{-}C_{H3} \qquad [II]$$

and a third polypeptide chain comprises a structure represented by the formula:

$$V_{H3}\text{-}C_{H1}\text{-hinge-}C_{H2}\text{-}C_{H3} \qquad [III]$$

and a fourth polypeptide chain comprises a structure represented by the formula:

$$V_{L3}\text{-}C_L \qquad [IV]$$

wherein:

V_{L1} is a first immunoglobulin light chain variable domain;

V_{L2} is a second immunoglobulin light chain variable domain;

V_{L3} is a third immunoglobulin light chain variable domain;

V_{H1} is a first immunoglobulin heavy chain variable domain;

V_{H2} is a second immunoglobulin heavy chain variable domain;

V_{H3} is a third immunoglobulin heavy chain variable domain;

C_L is an immunoglobulin light chain constant domain;

C_{H1} is an immunoglobulin C_{H1} heavy chain constant domain;

C_{H2} is an immunoglobulin C_{H2} heavy chain constant domain;

C_{H3} is an immunoglobulin C_{H3} heavy chain constant domain;

hinge is an immunoglobulin hinge region connecting the C_{H1} and C_{H2} domains; and

L_1, L_2, L_3 and L_4 are amino acid linkers;

wherein the polypeptide of formula I and the polypeptide of formula II form a cross-over light chain-heavy chain pair;

wherein:

(a) V_{L1}, V_{L2} and V_{L3} each independently comprise a variable domain sequence as set forth in any one of SEQ ID NOs:151, 153, 155, 157, 159, 161, 163, 165, and 167; or

(b) V_{L1}, V_{L2} and V_{L3} each independently comprise light chain complementarity determining regions comprising an amino acid sequence as set forth in any one of SEQ ID NOs: 43-59, 123-125, 138-140, and 149; and

wherein:

(a) V_{H1}, V_{H2}, and V_{H3} each independently comprise a variable domain sequence as set forth in any one of SEQ ID NOs:150, 152, 154, 156, 158, 160, 162, 164, and 166; or

(b) V_{H1}, V_{H2} and V_{H3} each independently comprise heavy chain complementarity determining regions comprising an amino acid sequence as set forth in any one of SEQ ID NOs: 25-42, 120-122, and 126-128.

18. The binding protein of claim 16, wherein the second and/or the third polypeptide chain further comprises an Fc region linked to C_{H1}, the Fc region comprising an immunoglobulin hinge region and C_{H2} and C_{H3} immunoglobulin heavy chain constant domains.

19. The binding protein of claim 16 or claim 17, wherein at least one of L_1, L_2, L_3 or L_4, is independently 0 amino acids in length.

20. The binding protein of claim 16 or claim 17, wherein L_1, L_2, L_3 or L_4 are each independently at least one amino acid in length.

21. The binding protein of claim 16 or claim 17, wherein (a) L_1, L_2, L_3 and L_4 each independently are zero amino acids in length or comprise a sequence selected from the group consisting of GGGGSGGGGS (SEQ ID NO:104), GGGGSGGGGSGGGGS (SEQ ID NO:105), S, RT, TKGPS (SEQ ID NO:106), GQPKAAP (SEQ ID NO: 175), and GGSGSSGSGG (SEQ ID NO:148); or (b) L_1, L_2, L_3 and L_4 each independently comprise a sequence selected from the group consisting of GGGGSGGGGS (SEQ ID NO:104), GGGGSGGGGSGGGGS (SEQ ID NO:105), S, RT, TKGPS (SEQ ID NO:106), GQPKAAP (SEQ ID NO: 175), and GGSGSSGSGG (SEQ ID NO:148).

22. The binding protein of claim 16 or claim 17, wherein:

(a) L_1 comprises the sequence GQPKAAP (SEQ ID NO: 175), L_2 comprises the sequence TKGPS (SEQ ID NO:106), L_3 comprises the sequence S, and L_4 comprises the sequence RT;

(b) L_1 comprises the sequence GGGGSGGGGS (SEQ ID NO:104), L_2 comprises the sequence GGGGSGGGGS (SEQ ID NO:104), L_3 is 0 amino acids in length, and L_4 is 0 amino acids in length;

(c) L_1 comprises the sequence GGSGSSGSGG (SEQ ID NO:148), L_2 comprises the sequence GGSGSSGSGG (SEQ ID NO:148), L_3 is 0 amino acids in length, and L_4 is 0 amino acids in length; or

(d) L_1 comprises the sequence GGGGSGGGGSGGGGS (SEQ ID NO:105), L_2 is 0 amino acids in length, L_3 comprises the sequence GGGGSGGGGSGGGGS (SEQ ID NO:105), and L_4 is 0 amino acids in length.

23. The binding protein of claim 16 or claim 17, wherein:

(a) V_{H1} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:28, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:29, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:30; V_{L1} comprises a CDR-L1 comprising the amino

acid sequence of SEQ ID NO:46, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:47, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:48; V_{H2} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:34, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:35, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:36; V_{L2} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:52, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:53, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:54; V_{H3} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:25, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:26, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:27; and V_{L3} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:43, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:44, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:45;

(b) V_{H1} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:31, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:32, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:33; V_{L1} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:49, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:50, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:51; V_{H2} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:34, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:35, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:36; V_{L2} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:52, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:53, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:54; V_{H3} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:25, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:26, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:27; and V_{L3} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:43, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:44, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:45;

(c) V_{H1} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:28, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:29, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:30; V_{L1} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:46, a CDR-L2 comprising the amino acid sequence of SEQ ID

NO:47, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:48; V_{H2} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:34, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:35, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:36; V_{L2} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:52, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:53, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:54; V_{H3} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:37, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:38, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:39; and V_{L3} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:55, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:56, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:57;

(d) V_{H1} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:31, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:32, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:33; V_{L1} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:49, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:50, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:51; V_{H2} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:34, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:35, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:36; V_{L2} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:52, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:53, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:54; V_{H3} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:37, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:38, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:39; and V_{L3} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:55, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:56, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:57;

(e) V_{H1} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:28, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:29, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:30; V_{L1} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:46, a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 47, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:48; V_{H2} comprises

a CDR-H1 comprising the amino acid sequence of SEQ ID NO:34, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:35, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:36; V_{L2} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:52, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:53, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:54; V_{H3} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:40, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:41, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:42; and V_{L3} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:58, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:44, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:59;

(f) V_{H1} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:31, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:32, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:33; V_{L1} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:49, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:50, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:51; V_{H2} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:34, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:35, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:36; V_{L2} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:52, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:53, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:54; V_{H3} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:40, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:41, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:42; and V_{L3} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:58, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:44, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:59;

(g) V_{H1} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:28, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:29, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:30; V_{L1} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:46, a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 47, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:48; V_{H2} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:34, a CDR-H2 comprising the

amino acid sequence of SEQ ID NO:35, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:36; V_{L2} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:52, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:53, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:54; V_{H3} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:126, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:127, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:128; and V_{L3} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:138, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:139, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:140;

(h) V_{H1} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:31, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:32, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:33; V_{L1} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:49, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:50, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:51; V_{H2} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:34, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:35, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:36; V_{L2} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:52, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:53, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:54; V_{H3} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:126, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:127, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:128; and V_{L3} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:138, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:139, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:140;

(i) V_{H1} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:28, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:29, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:30; V_{L1} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:46, a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 47, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:48; V_{H2} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:34, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:35, and a CDR-H3 comprising the amino acid sequence of

SEQ ID NO:36; V_{L2} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:52, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:53, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:54; V_{H3} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:120, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:121, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:122; and V_{L3} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:123, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:124, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:125; or

(j) V_{H1} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:31, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:32, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:33; V_{L1} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:49, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:50, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:51; V_{H2} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:34, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:35, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:36; V_{L2} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:52, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:53, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:54; V_{H3} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:120, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:121, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:122; and V_{L3} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:123, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:124, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:125.

24. A binding protein comprising four polypeptide chains that form three antigen binding sites, wherein a first polypeptide chain comprises a structure represented by the formula:

$$V_{L2}\text{-}L_1\text{-}V_{L1}\text{-}L_2\text{-}C_L \qquad [I]$$

and a second polypeptide chain comprises a structure represented by the formula:

$$V_{H1}\text{-}L_3\text{-}V_{H2}\text{-}L_4\text{-}C_{H1} \qquad [II]$$

and a third polypeptide chain comprises a structure represented by the formula:

$$V_{H3}\text{-}C_{H1} \qquad [III]$$

and a fourth polypeptide chain comprises a structure represented by the formula:

$$V_{L3}\text{-}C_L \qquad [IV]$$

wherein:

V_{L1} is a first immunoglobulin light chain variable domain;

V_{L2} is a second immunoglobulin light chain variable domain;

V_{L3} is a third immunoglobulin light chain variable domain;

V_{H1} is a first immunoglobulin heavy chain variable domain;

V_{H2} is a second immunoglobulin heavy chain variable domain;

V_{H3} is a third immunoglobulin heavy chain variable domain;

C_L is an immunoglobulin light chain constant domain;

C_{H1} is an immunoglobulin C_{H1} heavy chain constant domain; and

L_1, L_2, L_3 and L_4 are amino acid linkers;

wherein the polypeptide of formula I and the polypeptide of formula II form a cross-over light chain-heavy chain pair;

wherein:

(a) V_{L1}, V_{L2} and V_{L3} each independently comprise a variable domain sequence as set forth in any one of SEQ ID NOs:169, 171, and 173; or

(b) V_{L1}, V_{L2} and V_{L3} each independently comprise light chain complementarity determining regions comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 141-147, 178, and 179;

wherein:

(a) V_{H1}, V_{H2}, and V_{H3} each independently comprise a variable domain sequence as set forth in any one of SEQ ID NOs:168, 170, and 172; or

(b) V_{H1}, V_{H2} and V_{H3} each independently comprise heavy chain complementarity determining regions comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 129-137.

25. A binding protein comprising four polypeptide chains that form three antigen binding sites, wherein a first polypeptide chain comprises a structure represented by the formula:

$$V_{L2}\text{-}L_1\text{-}V_{L1}\text{-}L_2\text{-}C_L \qquad [I]$$

and a second polypeptide chain comprises a structure represented by the formula:

$$V_{H1}\text{-}L_3\text{-}V_{H2}\text{-}L_4\text{-}C_{H1}\text{-hinge-}C_{H2}\text{-}C_{H3} \quad [II]$$

and a third polypeptide chain comprises a structure represented by the formula:

$$V_{H3}\text{-}C_{H1}\text{-hinge-}C_{H2}\text{-}C_{H3} \quad [III]$$

and a fourth polypeptide chain comprises a structure represented by the formula:

$$V_{L3}\text{-}C_L \quad [IV]$$

wherein:

V_{L1} is a first immunoglobulin light chain variable domain;

V_{L2} is a second immunoglobulin light chain variable domain;

V_{L3} is a third immunoglobulin light chain variable domain;

V_{H1} is a first immunoglobulin heavy chain variable domain;

V_{H2} is a second immunoglobulin heavy chain variable domain;

V_{H3} is a third immunoglobulin heavy chain variable domain;

C_L is an immunoglobulin light chain constant domain;

C_{H1} is an immunoglobulin C_{H1} heavy chain constant domain;

C_{H2} is an immunoglobulin C_{H2} heavy chain constant domain;

C_{H3} is an immunoglobulin C_{H3} heavy chain constant domain;

hinge is an immunoglobulin hinge region connecting the C_{H1} and C_{H2} domains; and

L_1, L_2, L_3 and L_4 are amino acid linkers;

wherein the polypeptide of formula I and the polypeptide of formula II form a cross-over light chain-heavy chain pair;

wherein:

(a) V_{L1}, V_{L2} and V_{L3} each independently comprise a variable domain sequence as set forth in any one of SEQ ID NOs:169, 171, and 173; or

(b) V_{L1}, V_{L2} and V_{L3} each independently comprise light chain complementarity determining regions comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 141-147, 178, and 179;

wherein:

(a) V_{H1}, V_{H2}, and V_{H3} each independently comprise a variable domain sequence as set forth in any one of SEQ ID NOs:168, 170, and 172; or

(b) V_{H1}, V_{H2} and V_{H3} each independently comprise heavy chain complementarity determining regions comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 129-137.

26. The binding protein of claim 24, wherein the second and/or the third polypeptide chain further comprises an Fc region linked to C_{H1}, the Fc region comprising an immunoglobulin hinge region and C_{H2} and C_{H3} immunoglobulin heavy chain constant domains.

27. The binding protein of claim 24 or claim 25, wherein at least one of L_1, L_2, L_3 and L_4, is independently 0 amino acids in length.

28. The binding protein of claim 24 or claim 25, wherein L_1, L_2, L_3 and L_4 are each independently at least one amino acid in length.

29. The binding protein of claim 24 or claim 25, wherein (a) L_1, L_2, L_3 and L_4 each independently are zero amino acids in length or comprise a sequence selected from the group consisting of GGGGSGGGGS (SEQ ID NO:104), GGGGSGGGGSGGGGS (SEQ ID NO:105), S, RT, TKGPS (SEQ ID NO:106), GQPKAAP (SEQ ID NO: 175), and GGSGSSGSGG (SEQ ID NO:148); or (b) L_1, L_2, L_3 and L_4 each independently comprise a sequence selected from the group consisting of GGGGSGGGGS (SEQ ID NO:104), GGGGSGGGGSGGGGS (SEQ ID NO:105), S, RT, TKGPS (SEQ ID NO:106), GQPKAAP (SEQ ID NO: 175), and GGSGSSGSGG (SEQ ID NO:148).

30. The binding protein of claim 24 or claim 25, wherein:

(a) L_1 comprises the sequence GQPKAAP (SEQ ID NO: 175), L_2 comprises the sequence TKGPS (SEQ ID NO:106), L_3 comprises the sequence S, and L_4 comprises the sequence RT;

(b) L_1 comprises the sequence GGGGSGGGGS (SEQ ID NO:104), L_2 comprises the sequence GGGGSGGGGS (SEQ ID NO:104), L_3 is 0 amino acids in length, and L_4 is 0 amino acids in length;

(c) L_1 comprises the sequence GGSGSSGSGG (SEQ ID NO:148), L_2 comprises the sequence GGSGSSGSGG (SEQ ID NO:148), L_3 is 0 amino acids in length, and L_4 is 0 amino acids in length; or

(d) L_1 comprises the sequence GGGGSGGGGSGGGGS (SEQ ID NO:105), L_2 is 0 amino acids in length, L_3 comprises the sequence GGGGSGGGGSGGGGS (SEQ ID NO:105), and L_4 is 0 amino acids in length.

31. The binding protein of claim 24 or claim 25, wherein:

(a) V_{H1} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:132, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:133, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:134; V_{L1} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:143, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:179, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:144; V_{H2} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:135, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:136, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:137; V_{L2} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:145, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:146, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:147; V_{H3} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:129, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:130, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:131; and V_{L3} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:141, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:178, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:142;

(b) V_{H1} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:135, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:136, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:137; V_{L1} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:145, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:146, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:147; V_{H2} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:132, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:133, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:134; V_{L2} comprises a CDR-L1 comprising the amino acid

sequence of SEQ ID NO:143, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:47, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:144; V_{H3} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:129, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:130, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:131; and V_{L3} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:141, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:178, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:142;

(c) V_{H1} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:132, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:133, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:134; V_{L1} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:143, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:179, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:144; V_{H2} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:129, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:130, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:131; V_{L2} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:141, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:178, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:142; V_{H3} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:135, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:136, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:137; and V_{L3} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:145, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:146, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:147;

(d) V_{H1} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:129, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:130, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:131; V_{L1} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:141, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:178, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:142; V_{H2} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:132, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:133, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:134; V_{L2} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:143, a CDR-L2 comprising the amino acid sequence of SEQ ID

NO:179, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:144; V_{H3} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:135, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:136, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:137; and V_{L3} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:145, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:146, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:147;

(e) V_{H1} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:135, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:136, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:137; V_{L1} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:145, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:146, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:147; V_{H2} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:129, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:130, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:131; V_{L2} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:141, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:178, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:142; V_{H3} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:132, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:133, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:134; and V_{L3} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:143, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:179, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:144;

(f) V_{H1} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:129, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:130, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:131; V_{L1} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:141, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:178, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:142; V_{H2} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:135, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:136, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:137; V_{L2} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:145, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:146, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:147; V_{H3}

comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:132, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:133, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:134; and V_{L3} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:143, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:179, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:144;

(g) V_{H1} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:135, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:136, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:137; V_{L1} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:145, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:146, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:147; V_{H2} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:132, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:133, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:134; V_{L2} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:143, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:179, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:144; V_{H3} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:135, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:136, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:137; and V_{L3} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:145, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:146, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:147;

(h) V_{H1} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:135, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:136, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:137; V_{L1} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:145, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:146, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:147; V_{H2} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:132, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:133, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:134; V_{L2} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:143, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:179, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:144; V_{H3} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:132, a CDR-H2

comprising the amino acid sequence of SEQ ID NO:133, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:134; and V_{L3} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:143, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:179, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:144;

(i) V_{H1} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:132, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:133, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:134; V_{L1} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:143, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:179, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:144; V_{H2} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:135, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:136, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:137; V_{L2} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:145, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:146, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:147; V_{H3} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:135, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:136, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:137; and V_{L3} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:145, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:146, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:147; or

(j) V_{H1} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:132, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:133, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:134; V_{L1} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:143, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:179, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:144; V_{H2} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:135, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:136, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:137; V_{L2} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:145, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:146, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:147; V_{H3} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:132, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:133, and a CDR-H3 comprising the amino

acid sequence of SEQ ID NO:134; and V_{L3} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:143, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:179, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:144.

32. The binding protein of any one of claims 1, 16, and 24, wherein the second polypeptide chain further comprises a first Fc region linked to C_{H1}, the first Fc region comprising an immunoglobulin hinge region and C_{H2} and C_{H3} immunoglobulin heavy chain constant domains, wherein the first Fc region comprises amino acid substitutions at positions corresponding to positions 354 and 366 of human IgG1 or IgG4 according to EU Index, wherein the amino acid substitutions are S354C and T366W; and wherein the third polypeptide chain further comprises a second Fc region linked to C_{H1}, the second Fc region comprising an immunoglobulin hinge region and C_{H2} and C_{H3} immunoglobulin heavy chain constant domains, wherein the second Fc region comprises amino acid substitutions at positions corresponding to positions 349, 366, 368, and 407 of human IgG1 or IgG4 according to EU Index, wherein the amino acid substitutions are Y349C, T366S, L368A, and Y407V.

33. The binding protein of any one of claims 1, 16, and 24, wherein the second polypeptide chain further comprises a first Fc region linked to C_{H1}, the first Fc region comprising an immunoglobulin hinge region and C_{H2} and C_{H3} immunoglobulin heavy chain constant domains, wherein the first Fc region comprises amino acid substitutions at positions corresponding to positions 349, 366, 368, and 407 of human IgG1 or IgG4 according to EU Index, wherein the amino acid substitutions are Y349C, T366S, L368A, and Y407V; and wherein the third polypeptide chain further comprises a second Fc region linked to C_{H1}, the second Fc region comprising an immunoglobulin hinge region and C_{H2} and C_{H3} immunoglobulin heavy chain constant domains, wherein the second Fc region comprises amino acid substitutions at positions corresponding to positions 354 and 366 of human IgG1 or IgG4 according to EU Index, wherein the amino acid substitutions are S354C and T366W.

34. The binding protein of any one of claims 1, 16, 24, 32, and 33, wherein the second polypeptide chain further comprises a first Fc region linked to C_{H1}, the first Fc region comprising an immunoglobulin hinge region and C_{H2} and C_{H3} immunoglobulin heavy chain constant domains, and wherein the third polypeptide chain further comprises a second Fc

region linked to C_{H1}, the second Fc region comprising an immunoglobulin hinge region and C_{H2} and C_{H3} immunoglobulin heavy chain constant domains; wherein the first and second Fc regions comprise amino acid substitutions at positions corresponding to positions 428 and 434 of human IgG1 or IgG4 according to EU Index, wherein the amino acid substitutions are M428L and N434S.

35. The binding protein of any one of claims 2, 17, and 25, wherein the C_{H3} domain of the second polypeptide chain comprises amino acid substitutions at positions corresponding to positions 354 and 366 of human IgG1 or IgG4 according to EU Index, wherein the amino acid substitutions are S354C and T366W; and wherein the C_{H3} domain of the third polypeptide chain comprises amino acid substitutions at positions corresponding to positions 349, 366, 368, and 407 of human IgG1 or IgG4 according to EU Index, wherein the amino acid substitutions are Y349C, T366S, L368A, and Y407V.

36. The binding protein of any one of claims 2, 17, and 25, wherein the C_{H3} domain of the second polypeptide chain comprises amino acid substitutions at positions corresponding to positions 349, 366, 368, and 407 of human IgG1 or IgG4 according to EU Index, wherein the amino acid substitutions are Y349C, T366S, L368A, and Y407V; and wherein the C_{H3} domain of the third polypeptide chain comprises amino acid substitutions at positions corresponding to positions 354 and 366 of human IgG1 or IgG4 according to EU Index, wherein the amino acid substitutions are S354C and T366W.

37. The binding protein of any one of claims 2, 17, 25, 35, and 36, wherein the C_{H3} domains of the second and the third polypeptide chains both comprise amino acid substitutions at positions corresponding to positions 428 and 434 of human IgG1 or IgG4 according to EU Index, wherein the amino acid substitutions are M428L and N434S.

38. The binding protein of any one of claims 1, 16, 24, and 32-34, wherein the second polypeptide chain further comprises a first Fc region linked to C_{H1}, the first Fc region comprising an immunoglobulin hinge region and C_{H2} and C_{H3} immunoglobulin heavy chain constant domains; wherein the third polypeptide chain further comprises a second Fc region linked to C_{H1}, the second Fc region comprising an immunoglobulin hinge region and C_{H2} and

C_{H3} immunoglobulin heavy chain constant domains; wherein the first and second Fc regions are human IgG1 or IgG4 Fc regions; and wherein only one of the first and the second Fc regions comprises amino acid substitutions at positions corresponding to positions 435 and 436 of human IgG1 or IgG4 according to EU Index, wherein the amino acid substitutions are H435R and Y436F.

39. The binding protein of any one of claims 2, 17, 25, and 35-37, wherein the C_{H3} domains of the second and the third polypeptide chains are human IgG1 or IgG4 C_{H3} domains, and wherein only one of the C_{H3} domains comprises amino acid substitutions at positions corresponding to positions 435 and 436 of human IgG1 or IgG4 according to EU Index, wherein the amino acid substitutions are H435R and Y436F.

40. The binding protein of any one of claims 1, 16, 24, 32-34, and 38, wherein the second polypeptide chain further comprises a first Fc region linked to C_{H1}, the first Fc region comprising an immunoglobulin hinge region and C_{H2} and C_{H3} immunoglobulin heavy chain constant domains; wherein the third polypeptide chain further comprises a second Fc region linked to C_{H1}, the second Fc region comprising an immunoglobulin hinge region and C_{H2} and C_{H3} immunoglobulin heavy chain constant domains; wherein the first and second Fc regions are human IgG4 Fc regions; and wherein the first and the second Fc regions each comprise amino acid substitutions at positions corresponding to positions 228 and 409 of human IgG4 according to EU Index, wherein the amino acid substitutions are S228P and R409K.

41. The binding protein of any one of claims 2, 17, 25, 35-37, and 39, wherein the C_{H3} domains of the second and the third polypeptide chains are human IgG4 C_{H3} domains, and wherein the C_{H3} domains each comprise amino acid substitutions at positions corresponding to positions 228 and 409 of human IgG4 according to EU Index, wherein the amino acid substitutions are S228P and R409K.

42. The binding protein of any one of claims 1, 16, 24, 32-34, 38, and 40, wherein the second polypeptide chain further comprises a first Fc region linked to C_{H1}, the first Fc region comprising an immunoglobulin hinge region and C_{H2} and C_{H3} immunoglobulin heavy chain constant domains; wherein the third polypeptide chain further comprises a second Fc region

linked to C_{H1}, the second Fc region comprising an immunoglobulin hinge region and C_{H2} and C_{H3} immunoglobulin heavy chain constant domains; wherein the first and second Fc regions are human IgG4 Fc regions; and wherein the first and the second Fc regions each comprise amino acid substitutions at positions corresponding to positions 234 and 235 of human IgG4 according to EU Index, wherein the amino acid substitutions are F234A and L235A.

43. The binding protein of any one of claims 2, 17, 25, 35-37, 39, and 41, wherein the C_{H3} domains of the second and the third polypeptide chains are human IgG4 C_{H3} domains, and wherein the C_{H3} domains each comprise amino acid substitutions at positions corresponding to positions 234 and 235 of human IgG4 according to EU Index, wherein the amino acid substitutions are F234A and L235A.

44. The binding protein of any one of claims 1, 16, 24, 32-34, 38, and 40, wherein the second polypeptide chain further comprises a first Fc region linked to C_{H1}, the first Fc region comprising an immunoglobulin hinge region and C_{H2} and C_{H3} immunoglobulin heavy chain constant domains; wherein the third polypeptide chain further comprises a second Fc region linked to C_{H1}, the second Fc region comprising an immunoglobulin hinge region and C_{H2} and C_{H3} immunoglobulin heavy chain constant domains; wherein the first and second Fc regions are human IgG1 Fc regions; and wherein the first and the second Fc regions each comprise amino acid substitutions at positions corresponding to positions 234 and 235 of human IgG4 according to EU Index, wherein the amino acid substitutions are L234A and L235A.

45. The binding protein of any one of claims 2, 17, 25, 35-37, 39, and 41, wherein the C_{H3} domains of the second and the third polypeptide chains are human IgG1 C_{H3} domains, and wherein the C_{H3} domains each comprise amino acid substitutions at positions corresponding to positions 234 and 235 of human IgG4 according to EU Index, wherein the amino acid substitutions are L234A and L235A.

46. The binding protein of any one of claims 2, 17, 35-37, 39, 41, 43, and 45, wherein V_{H1} and V_{L1} form a first antigen binding site that specifically binds human CD3, wherein V_{H2} and V_{L2} form a second antigen binding site that specifically binds human CD28, and wherein V_{H3} and V_{L3} form a third antigen binding site that specifically binds a human tumor target protein.

47. The binding protein of any one of claims 2, 17, 35-37, 39, 41, 43, and 45, wherein V_{H1} and V_{L1} form a first antigen binding site that specifically binds human CD28, wherein V_{H2} and V_{L2} form a second antigen binding site that specifically binds human CD3, and wherein V_{H3} and V_{L3} form a third antigen binding site that specifically binds a human tumor target protein.

48. The binding protein of claim 46 or claim 47, wherein the third antigen binding site specifically binds a human tumor target protein selected from the group consisting of CD19, CD20, CD38, Her2, and LAMP1.

49. The binding protein of any one of claims 46-48, wherein the antigen binding site that specifically binds CD3 comprises:

(a) a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 152 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO: 153; or

(b) a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 154 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO: 155.

50. The binding protein of any one of claims 46-49, wherein the antigen binding site that specifically binds CD28 comprises:

(a) a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 160 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO: 161; or

(b) a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 162 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO: 163.

51. The binding protein of any one of claims 46-50, wherein the antigen binding site that specifically binds a tumor target protein comprises:

(a) a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 156 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO: 157;

(b) a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 158 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO: 159;

(c) a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 164 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO: 165;

(d) a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 150 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO: 151; or

(e) a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 166 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO: 167.

52. The binding protein of any one of claims 2, 17, 35-37, 39, 41, 43, and 45, wherein:

(a)V_{H1} and V_{L1} form a first antigen binding site that specifically binds human TNFa, V_{H2} and V_{L2} form a second antigen binding site that specifically binds human IL13, and V_{H3} and V_{L3} form a third antigen binding site that specifically binds human IL4;

(b) V_{H1} and V_{L1} form a first antigen binding site that specifically binds human TNFa, V_{H2} and V_{L2} form a second antigen binding site that specifically binds human IL4, and V_{H3} and V_{L3} form a third antigen binding site that specifically binds human IL13;

(c) V_{H1} and V_{L1} form a first antigen binding site that specifically binds human IL4, V_{H2} and V_{L2} form a second antigen binding site that specifically binds human TNFa, and V_{H3} and V_{L3} form a third antigen binding site that specifically binds human IL13;

(d) V_{H1} and V_{L1} form a first antigen binding site that specifically binds human IL4, V_{H2} and V_{L2} form a second antigen binding site that specifically binds human IL13, and V_{H3} and V_{L3} form a third antigen binding site that specifically binds human TNFa;

(e) V_{H1} and V_{L1} form a first antigen binding site that specifically binds human IL13, V_{H2} and V_{L2} form a second antigen binding site that specifically binds human IL4, and V_{H3} and V_{L3} form a third antigen binding site that specifically binds human TNFa; or

(f) V_{H1} and V_{L1} form a first antigen binding site that specifically binds human IL13, V_{H2} and V_{L2} form a second antigen binding site that specifically binds human TNFa, and V_{H3} and V_{L3} form a third antigen binding site that specifically binds human IL4.

53. The binding protein of claim 52, wherein the antigen binding site that specifically binds human TNFa comprises a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:168 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO:169.

54. The binding protein of claim 52 or claim 53, wherein the antigen binding site that specifically binds human IL4 comprises a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:170 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO:171.

55. The binding protein of any one of claims 52-54, wherein the antigen binding site that specifically binds human IL13 comprises a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:172 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO:173.

56. The binding protein of any one of claims 1-55, wherein:

(a) the C_L domain of the first polypeptide chain is a human kappa C_L domain, and the C_L domain of the fourth polypeptide chain is a human lambda C_L domain; or

(b) the C_L domain of the first polypeptide chain is a human lambda C_L domain, and the C_L domain of the fourth polypeptide chain is a human kappa C_L domain.

57. The binding protein of any one of claims 2, 17, 25, 35-37, 39, 41, and 43-55, wherein the first polypeptide chain comprises a lambda C_L domain; wherein the C_{H3} domain of the second polypeptide chain comprises amino acid substitutions at positions corresponding to positions 354 and 366 of human IgG1 according to EU Index, wherein the amino acid substitutions are S354C and T366W; wherein the C_{H3} domain of the third polypeptide chain comprises amino acid substitutions at positions corresponding to positions 349, 366, 368, 407, 435, and 436 of human IgG1 according to EU Index, wherein the amino acid substitutions are Y349C, T366S, L368A, Y407V, H435R, and Y436F; and wherein the fourth polypeptide chain comprises a kappa C_L domain.

58. A binding protein comprising a first polypeptide chain, a second polypeptide chain, a third polypeptide chain and a fourth polypeptide chain wherein:

(a) the first polypeptide chain comprises the amino acid sequence of SEQ ID NO: 4 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 4; the second polypeptide chain comprises the amino acid sequence of SEQ ID NO: 3 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 3; the third polypeptide chain comprises the amino acid sequence of SEQ ID NO: 1 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 1; and the fourth polypeptide chain comprises the amino acid sequence of SEQ ID NO: 2 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 2;

(b) the first polypeptide chain comprises the amino acid sequence of SEQ ID NO: 10 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 10; the second polypeptide chain comprises the amino acid sequence of SEQ ID NO: 9 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 9; the third polypeptide chain comprises the amino acid sequence of SEQ ID NO: 1 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 1; and the fourth polypeptide chain comprises the amino acid sequence of SEQ ID NO: 2 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 2;

(c) the first polypeptide chain comprises the amino acid sequence of SEQ ID NO: 4 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 4; the second polypeptide chain comprises the amino acid sequence of SEQ ID NO: 3 or an

amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 3; the third polypeptide chain comprises the amino acid sequence of SEQ ID NO: 13 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 13; and the fourth polypeptide chain comprises the amino acid sequence of SEQ ID NO: 14 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 14;

(d) the first polypeptide chain comprises the amino acid sequence of SEQ ID NO: 10 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 10; the second polypeptide chain comprises the amino acid sequence of SEQ ID NO: 9 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 9; the third polypeptide chain comprises the amino acid sequence of SEQ ID NO: 13 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 13; and the fourth polypeptide chain comprises the amino acid sequence of SEQ ID NO: 14 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 14;

(e) the first polypeptide chain comprises the amino acid sequence of SEQ ID NO: 4 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 4; the second polypeptide chain comprises the amino acid sequence of SEQ ID NO: 3 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 3; the third polypeptide chain comprises the amino acid sequence of SEQ ID NO: 17 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 17; and the fourth polypeptide chain comprises the amino acid sequence of SEQ ID NO: 18 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 18;

(f) the first polypeptide chain comprises the amino acid sequence of SEQ ID NO: 10 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 10; the second polypeptide chain comprises the amino acid sequence of SEQ ID NO: 9 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 9; the third polypeptide chain comprises the amino acid sequence of SEQ ID NO: 17 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 17; and the fourth polypeptide chain comprises the amino acid sequence of SEQ ID NO: 18 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 18;

(g) the first polypeptide chain comprises the amino acid sequence of SEQ ID NO: 4 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID

NO: 4; the second polypeptide chain comprises the amino acid sequence of SEQ ID NO: 3 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 3; the third polypeptide chain comprises the amino acid sequence of SEQ ID NO: 21 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 21; and the fourth polypeptide chain comprises the amino acid sequence of SEQ ID NO: 22 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 22;

(h) the first polypeptide chain comprises the amino acid sequence of SEQ ID NO: 10 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 10; the second polypeptide chain comprises the amino acid sequence of SEQ ID NO: 9 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 9; the third polypeptide chain comprises the amino acid sequence of SEQ ID NO: 21 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 21; and the fourth polypeptide chain comprises the amino acid sequence of SEQ ID NO: 22 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 22;

(i) the first polypeptide chain comprises the amino acid sequence of SEQ ID NO: 63 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 63; the second polypeptide chain comprises the amino acid sequence of SEQ ID NO: 62 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 62; the third polypeptide chain comprises the amino acid sequence of SEQ ID NO: 60 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 60; and the fourth polypeptide chain comprises the amino acid sequence of SEQ ID NO: 61 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 61;

(j) the first polypeptide chain comprises the amino acid sequence of SEQ ID NO: 69 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 69; the second polypeptide chain comprises the amino acid sequence of SEQ ID NO: 68 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 68; the third polypeptide chain comprises the amino acid sequence of SEQ ID NO: 60 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 60; and the fourth polypeptide chain comprises the amino acid sequence of SEQ ID NO: 61 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 61;

(k) the first polypeptide chain comprises the amino acid sequence of SEQ ID NO: 69 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 69; the second polypeptide chain comprises the amino acid sequence of SEQ ID NO: 68 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 68; the third polypeptide chain comprises the amino acid sequence of SEQ ID NO: 60 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 60; and the fourth polypeptide chain comprises the amino acid sequence of SEQ ID NO: 71 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 71;

(l) the first polypeptide chain comprises the amino acid sequence of SEQ ID NO: 76 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 76; the second polypeptide chain comprises the amino acid sequence of SEQ ID NO: 75 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 75; the third polypeptide chain comprises the amino acid sequence of SEQ ID NO: 73 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 73; and the fourth polypeptide chain comprises the amino acid sequence of SEQ ID NO: 74 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 74;

(m) the first polypeptide chain comprises the amino acid sequence of SEQ ID NO: 82 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 82; the second polypeptide chain comprises the amino acid sequence of SEQ ID NO: 81 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO:81; the third polypeptide chain comprises the amino acid sequence of SEQ ID NO: 73 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 73; and the fourth polypeptide chain comprises the amino acid sequence of SEQ ID NO: 74 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 74;

(n) the first polypeptide chain comprises the amino acid sequence of SEQ ID NO: 88 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO:88; the second polypeptide chain comprises the amino acid sequence of SEQ ID NO: 87 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 87; the third polypeptide chain comprises the amino acid sequence of SEQ ID NO: 85 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 85;

and the fourth polypeptide chain comprises the amino acid sequence of SEQ ID NO: 86 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 86;

(o) the first polypeptide chain comprises the amino acid sequence of SEQ ID NO: 94 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 94; the second polypeptide chain comprises the amino acid sequence of SEQ ID NO: 93 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 93; the third polypeptide chain comprises the amino acid sequence of SEQ ID NO: 85 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 85; and the fourth polypeptide chain comprises the amino acid sequence of SEQ ID NO: 86 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 86;

(p) the first polypeptide chain comprises the amino acid sequence of SEQ ID NO: 69 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 69; the second polypeptide chain comprises the amino acid sequence of SEQ ID NO: 68 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 68; the third polypeptide chain comprises the amino acid sequence of SEQ ID NO: 73 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 73; and the fourth polypeptide chain comprises the amino acid sequence of SEQ ID NO: 74 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 74;

(q) the first polypeptide chain comprises the amino acid sequence of SEQ ID NO: 69 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 69; the second polypeptide chain comprises the amino acid sequence of SEQ ID NO: 68 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 68; the third polypeptide chain comprises the amino acid sequence of SEQ ID NO: 85 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 85; and the fourth polypeptide chain comprises the amino acid sequence of SEQ ID NO: 86 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 86;

(r) the first polypeptide chain comprises the amino acid sequence of SEQ ID NO: 63 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 63; the second polypeptide chain comprises the amino acid sequence of SEQ ID NO: 62 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 62; the third polypeptide chain comprises the amino acid sequence of SEQ ID NO: 73 or an

amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 73; and the fourth polypeptide chain comprises the amino acid sequence of SEQ ID NO: 74 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 74;

(s) the first polypeptide chain comprises the amino acid sequence of SEQ ID NO: 63 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 63; the second polypeptide chain comprises the amino acid sequence of SEQ ID NO: 62 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 62; the third polypeptide chain comprises the amino acid sequence of SEQ ID NO: 85 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 85; and the fourth polypeptide chain comprises the amino acid sequence of SEQ ID NO: 86 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 86;

(t) the first polypeptide chain comprises the amino acid sequence of SEQ ID NO: 4 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 4; the second polypeptide chain comprises the amino acid sequence of SEQ ID NO: 3 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 3; the third polypeptide chain comprises the amino acid sequence of SEQ ID NO: 114 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 114; and the fourth polypeptide chain comprises the amino acid sequence of SEQ ID NO: 115 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 115; or

(u) the first polypeptide chain comprises the amino acid sequence of SEQ ID NO: 10 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 10; the second polypeptide chain comprises the amino acid sequence of SEQ ID NO: 9 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 9; the third polypeptide chain comprises the amino acid sequence of SEQ ID NO: 114 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 114; and the fourth polypeptide chain comprises the amino acid sequence of SEQ ID NO: 115 or an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 115.

59. An isolated nucleic acid molecule comprising a nucleotide sequence encoding the binding protein of any one of claims 1-58.

60. An expression vector comprising the nucleic acid molecule of claim 59.

61. An isolated host cell comprising the nucleic acid molecule of claim 59.

62. An isolated host cell comprising the expression vector of claim 60.

63. The isolated host cell of claim 61 or claim 62, wherein the host cell is a mammalian cell or an insect cell.

64. A pharmaceutical composition comprising the binding protein of any one of claims 1-58 and a pharmaceutically acceptable carrier.

65. A method of preventing and/or treating cancer in a patient comprising administering to the patient a therapeutically effective amount of at least one binding protein of any one of claims 1-23 and 32-58 or the pharmaceutical composition of claim 64.

66. The method of claim 65, wherein the binding protein comprises one antigen binding site that specifically binds a T-cell surface protein and another antigen binding site that specifically binds a tumor target protein.

67. The method of claim 66, wherein the binding protein comprises a first antigen binding site that specifically binds CD3, a second antigen binding site that specifically binds CD28, and a third antigen binding site that specifically binds a tumor target protein selected from the group consisting of CD19, CD20, CD38, Her2, and LAMP1.

68. The method of any one of claims 65-67, wherein the at least one binding protein is co-administered with a chemotherapeutic agent.

69. A method of preventing and/or treating an inflammatory disease or disorder in a patient comprising administering to the patient a therapeutically effective amount of at least one binding protein of any one of claims 1-15, 24-45, and 52-58 or the pharmaceutical composition of claim 64.

70. The method of claim 69, wherein the binding protein comprises three antigen binding sites that each specifically bind a cytokine target protein selected from the group consisting of IL-4, IL-13 and TNFa.

71. The method of claim 69 or claim 70, wherein the at least one binding protein is co-administered with an anti-inflammatory agent.

72. The method of any one of claims 65-71, wherein the patient is a human.

73. The method of claim 65 or claim 69, wherein the binding protein is capable of inhibiting the function of one or more target proteins selected from the group consisting of A2AR, APRIL, ATPDase, BAFF, BAFFR, BCMA, BlyS, BTK, BTLA, B7DC, B7H1, B7H4, B7H5, B7H6, B7H7, B7RP1, B7-4, C3, C5, CCL2, CCL3, CCL4, CCL5, CCL7, CCL8, CCL11, CCL15, CCL17, CCL19, CCL20, CCL21, CCL24, CCL25, CCL26, CCR3, CCR4, CD3, CD19, CD20, CD23, CD24, CD27, CD28, CD38, CD39, CD40, CD70, CD80, CD86, CD122, CD137, CD137L, CD152, CD154, CD160, CD272, CD273, CD274, CD275, CD276, CD278, CD279, CDH1, chitinase, CLEC9, CLEC91, CRTH2, CSF-1, CSF-2, CSF-3, CX3CL1, CXCL12, CXCL13, CXCR3, DNGR-1, ectonucleoside triphosphate diphosphohydrolase 1, EGFR, ENTPD1, FCER1A, FCER1, FLAP, FOLH1, Gi24, GITR, GITRL, GM-CSF, Her2, HHLA2, HMGB1, HVEM, ICOSLG, IDO, IFNα, IgE, IGF1R, IL2Rbeta, IL1, IL1A, IL1B, IL1F10, IL2, IL4, IL4Ra, IL5, IL5R, IL6, IL7, IL7Ra, IL8, IL9, IL9R, IL10, rhIL10, IL12, IL13, IL13Ra1, IL13Ra2, IL15, IL17, IL17Rb, IL18, IL22, IL23, IL25, IL27, IL33, IL35, ITGB4, ITK, KIR, LAG3, LAMP1, leptin, LPFS2, MHC class II, NCR3LG1, NKG2D, NTPDase-1, OX40, OX40L, PD-1H, platelet receptor, PROM1, S152, SISP1, SLC, SPG64, ST2, STEAP2, Syk kinase, TACI, TDO, T14, TIGIT, TIM3, TLR, TLR2, TLR4, TLR5, TLR9, TMEF1, TNFa, TNFRSF7, Tp55, TREM1, TSLP, TSLPR, TWEAK, VEGF, VISTA, Vstm3, WUCAM, and XCR1.

74. The binding protein of any one of claims 1-23 and 32-58 or the composition of claim 64 for use in preventing and/or treating cancer in a patient.

75. The binding protein for use or the composition for use of claim 74, wherein the binding protein comprises one antigen binding site that specifically binds a T-cell surface protein and another antigen binding site that specifically binds a tumor target protein.

76. The binding protein for use or the composition for use of claim 75, wherein the binding protein comprises a first antigen binding site that specifically binds CD3, a second antigen binding site that specifically binds CD28, and a third antigen binding site that specifically binds a tumor target protein selected from the group consisting of CD19, CD20, CD38, Her2, and LAMP1.

77. The binding protein for use or the composition for use of any one of claims 74-76, wherein the binding protein is co-administered with a chemotherapeutic agent.

78. The binding protein of any one of claims 1-15, 24-45, and 52-58 or the pharmaceutical composition of claim 64 for use in preventing and/or treating an inflammatory disease or disorder in a patient.

79. The binding protein for use or the composition for use of claim 78, wherein the binding protein comprises three antigen binding sites that each specifically bind a cytokine target protein selected from the group consisting of IL-4, IL-13 and TNFa.

80. The binding protein for use or the composition for use of claim 78 or claim 79, wherein the binding protein is co-administered with an anti-inflammatory agent.

81. The binding protein for use or the composition for use of any one of claims 74-80, wherein the patient is a human.

82. The binding protein for use or the composition for use of claim 74 or claim 78, wherein the binding protein is capable of inhibiting the function of one or more target proteins selected from the group consisting of A2AR, APRIL, ATPDase, BAFF, BAFFR, BCMA, BlyS, BTK, BTLA, B7DC, B7H1, B7H4, B7H5, B7H6, B7H7, B7RP1, B7-4, C3, C5, CCL2, CCL3, CCL4, CCL5, CCL7, CCL8, CCL11, CCL15, CCL17, CCL19, CCL20, CCL21, CCL24, CCL25, CCL26, CCR3, CCR4, CD3, CD19, CD20, CD23, CD24, CD27, CD28, CD38, CD39, CD40, CD70, CD80, CD86, CD122, CD137, CD137L, CD152, CD154, CD160, CD272,

CD273, CD274, CD275, CD276, CD278, CD279, CDH1, chitinase, CLEC9, CLEC91, CRTH2, CSF-1, CSF-2, CSF-3, CX3CL1, CXCL12, CXCL13, CXCR3, DNGR-1, ectonucleoside triphosphate diphosphohydrolase 1, EGFR, ENTPD1, FCER1A, FCER1, FLAP, FOLH1, Gi24, GITR, GITRL, GM-CSF, Her2, HHLA2, HMGB1, HVEM, ICOSLG, IDO, IFNα, IgE, IGF1R, IL2Rbeta, IL1, IL1A, IL1B, IL1F10, IL2, IL4, IL4Ra, IL5, IL5R, IL6, IL7, IL7Ra, IL8, IL9, IL9R, IL10, rhIL10, IL12, IL13, IL13Ra1, IL13Ra2, IL15, IL17, IL17Rb, IL18, IL22, IL23, IL25, IL27, IL33, IL35, ITGB4, ITK, KIR, LAG3, LAMP1, leptin, LPFS2, MHC class II, NCR3LG1, NKG2D, NTPDase-1, OX40, OX40L, PD-1H, platelet receptor, PROM1, S152, SISP1, SLC, SPG64, ST2, STEAP2, Syk kinase, TACI, TDO, T14, TIGIT, TIM3, TLR, TLR2, TLR4, TLR5, TLR9, TMEF1, TNFa, TNFRSF7, Tp55, TREM1, TSLP, TSLPR, TWEAK, VEGF, VISTA, Vstm3, WUCAM, and XCR1.

83. A method of purifying a binding protein produced by a host cell, comprising:

(a) producing the binding protein of any one of claims 2, 17, 25, 35-37, 41, 43, 45, and 46-55 in a host cell, wherein only one of the C_{H3} domain of the second polypeptide chain and the C_{H3} domain of the third polypeptide chain comprises amino acid substitutions at positions corresponding to positions 435 and 436 of human IgG1 or IgG4 according to EU Index, wherein the amino acid substitutions are H435R and Y436F;

(b) contacting the binding protein produced in (a) with Protein A; and

(c) eluting the binding protein from Protein A under conditions suitable for isolating the binding protein away from binding proteins comprising either 0 or 2 C_{H3} domains comprising the amino acid substitutions are H435R and Y436F.

84. The method of claim 83, wherein the C_L domain of the first polypeptide chain is a human kappa C_L domain, and the C_L domain of the fourth polypeptide chain is a human lambda C_L domain; or the C_L domain of the first polypeptide chain is a human lambda C_L domain, and the C_L domain of the fourth polypeptide chain is a human kappa C_L domain, and the method further comprises:

(d) contacting the binding protein eluted in (c) with a kappa light chain affinity medium; and

(e) eluting the binding protein from the kappa light chain affinity medium under conditions suitable for isolating the binding protein away from binding proteins comprising only lambda C_L domains.

85. The method of claim 84, further comprising, after (e):

(f) contacting the binding protein eluted in (e) with a lambda light chain affinity medium; and

(g) eluting the binding protein from the lambda light chain affinity medium under conditions suitable for isolating the binding protein away from binding proteins comprising only kappa C_L domains.

86. The method of claim 83, wherein the C_L domain of the first polypeptide chain is a human kappa C_L domain, and the C_L domain of the fourth polypeptide chain is a human lambda C_L domain; or the C_L domain of the first polypeptide chain is a human lambda C_L domain, and the C_L domain of the fourth polypeptide chain is a human kappa C_L domain, and the method further comprises:

(d) contacting the binding protein eluted in (c) with a lambda light chain affinity medium; and

(e) eluting the binding protein from the lambda light chain affinity medium under conditions suitable for isolating the binding protein away from binding proteins comprising only kappa C_L domains.

87. The method of claim 86, further comprising, after (e):

(f) contacting the binding protein eluted in (e) with a kappa light chain affinity medium; and

(g) eluting the binding protein from the kappa light chain affinity medium under conditions suitable for isolating the binding protein away from binding proteins comprising only lambda C_L domains.

88. The method of any one of claims 83-87, wherein the first polypeptide chain comprises a lambda C_L domain; wherein the C_{H3} domain of the second polypeptide chain comprises amino

acid substitutions at positions corresponding to positions 354 and 366 of human IgG1 according to EU Index, wherein the amino acid substitutions are S354C and T366W; wherein the C_{H3} domain of the third polypeptide chain comprises amino acid substitutions at positions corresponding to positions 349, 366, 368, 407, 435, and 436 of human IgG1 according to EU Index, wherein the amino acid substitutions are Y349C, T366S, L368A, Y407V, H435R, and Y436F; and wherein the fourth polypeptide chain comprises a kappa C_L domain.

89. The method of any one of claims 83-88, wherein the binding protein is detected in one or more of (c) and (e) using hydrophobic interaction chromatography (HIC).

90. The method of any one of claims 83-89, wherein the C_{H3} domains of the second and the third polypeptide chains are human IgG1 or IgG4 C_{H3} domains.

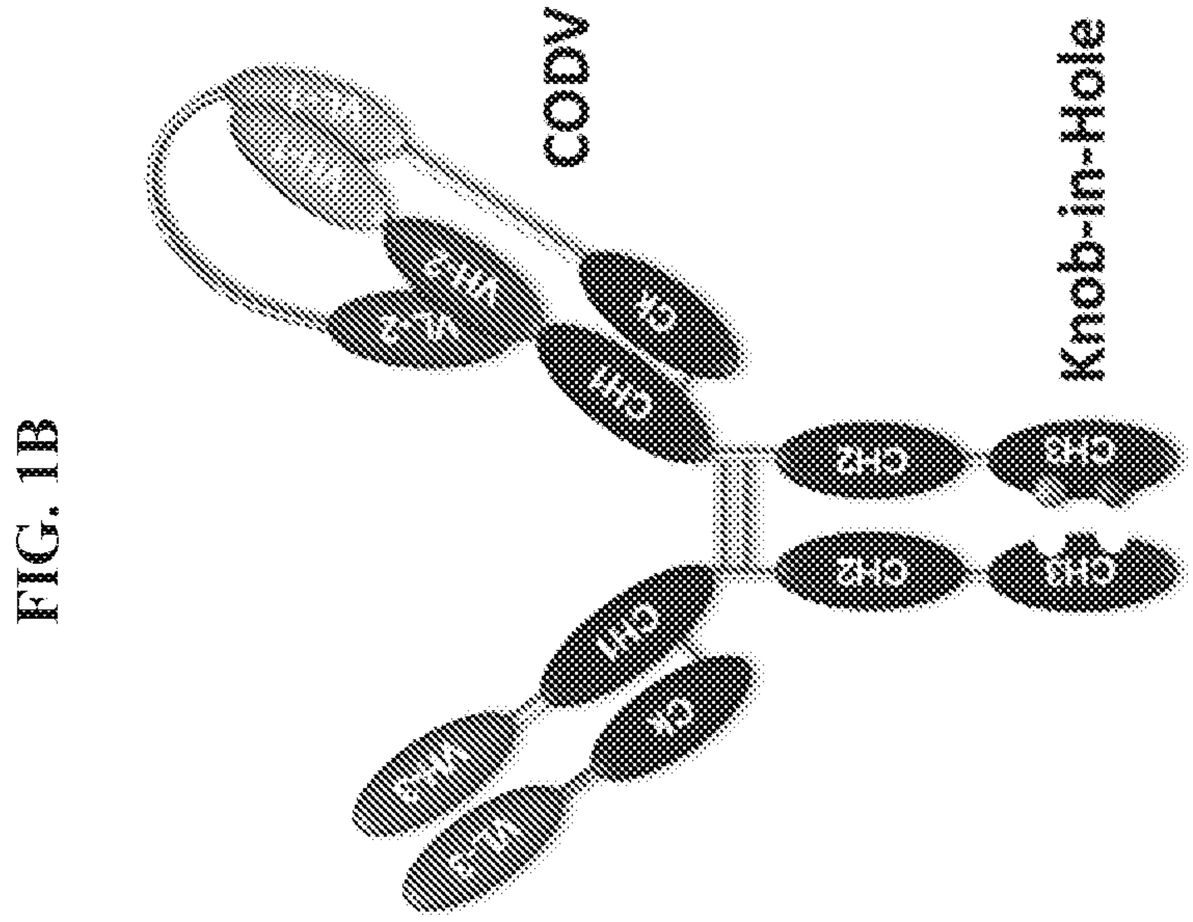

CODV
Knob-in-Hole
VH-2
VL-2
CH1
CL
VH
VL
CH2
CH3

FIG. 1B

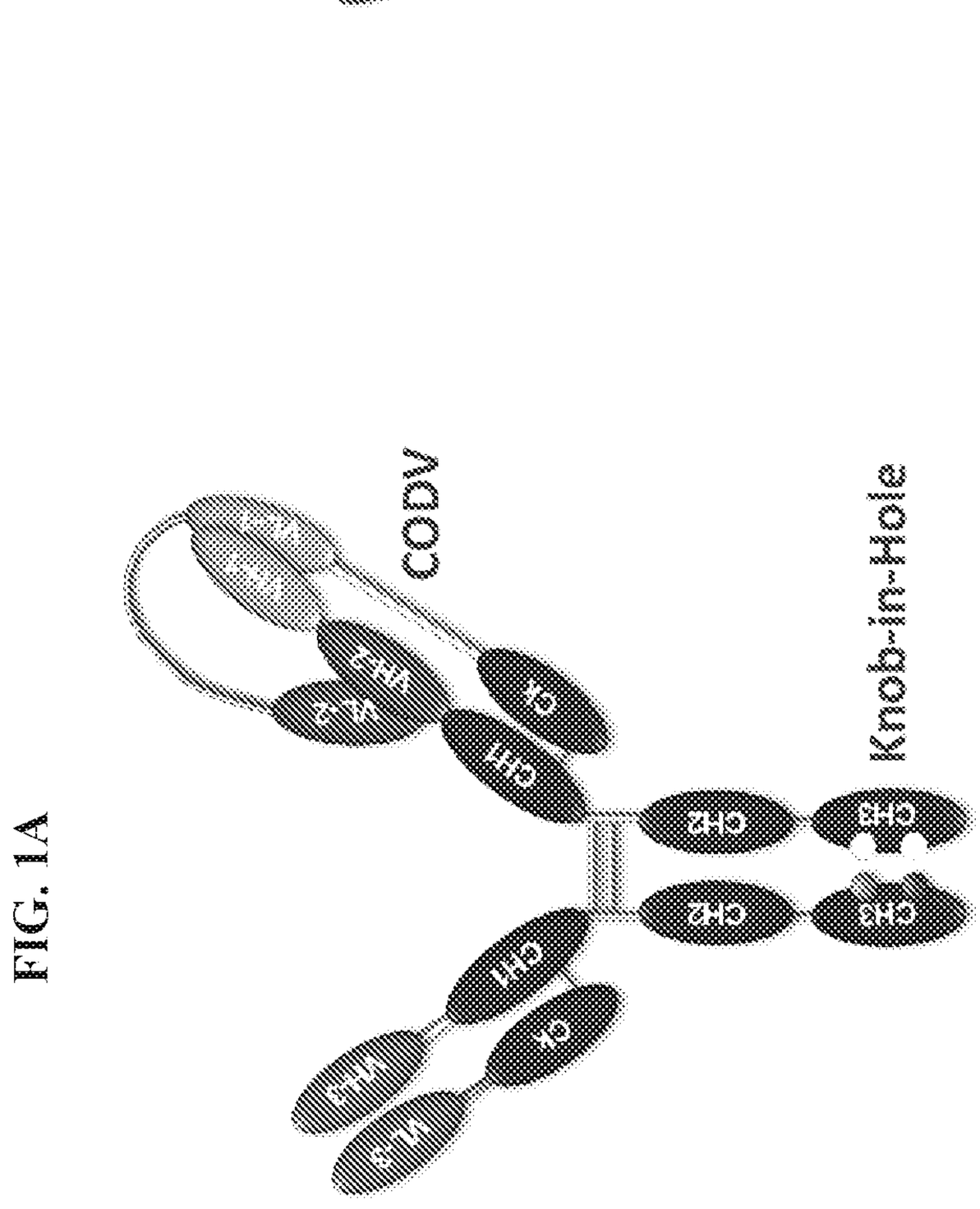

CODV
Knob-in-Hole
VH-2
VL-2
CH1
CL
VH
VL
CH2
CH3

FIG. 1A

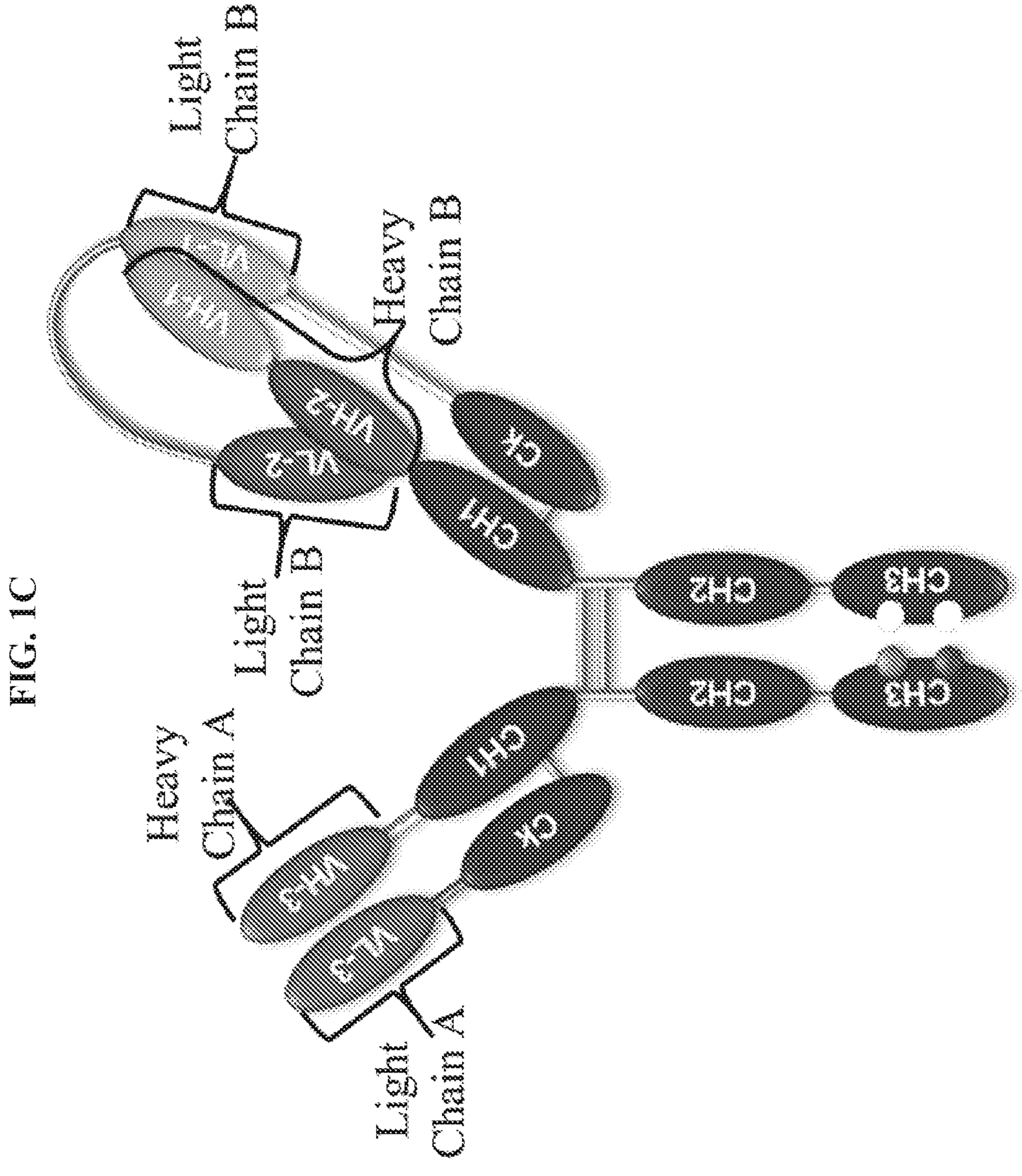

Light Chain A
Heavy Chain A
Light Chain B
Heavy Chain B
VL-A
VH-A
VL-B
VH-B
CL
CH1
CH2
CH3
CL
CH1
CH2
CH3
VL-A
VH-A
VL-B
VH-B
Light Chain A
Heavy Chain A
Light Chain B
Heavy Chain B

FIG. 1C

FIG. 2

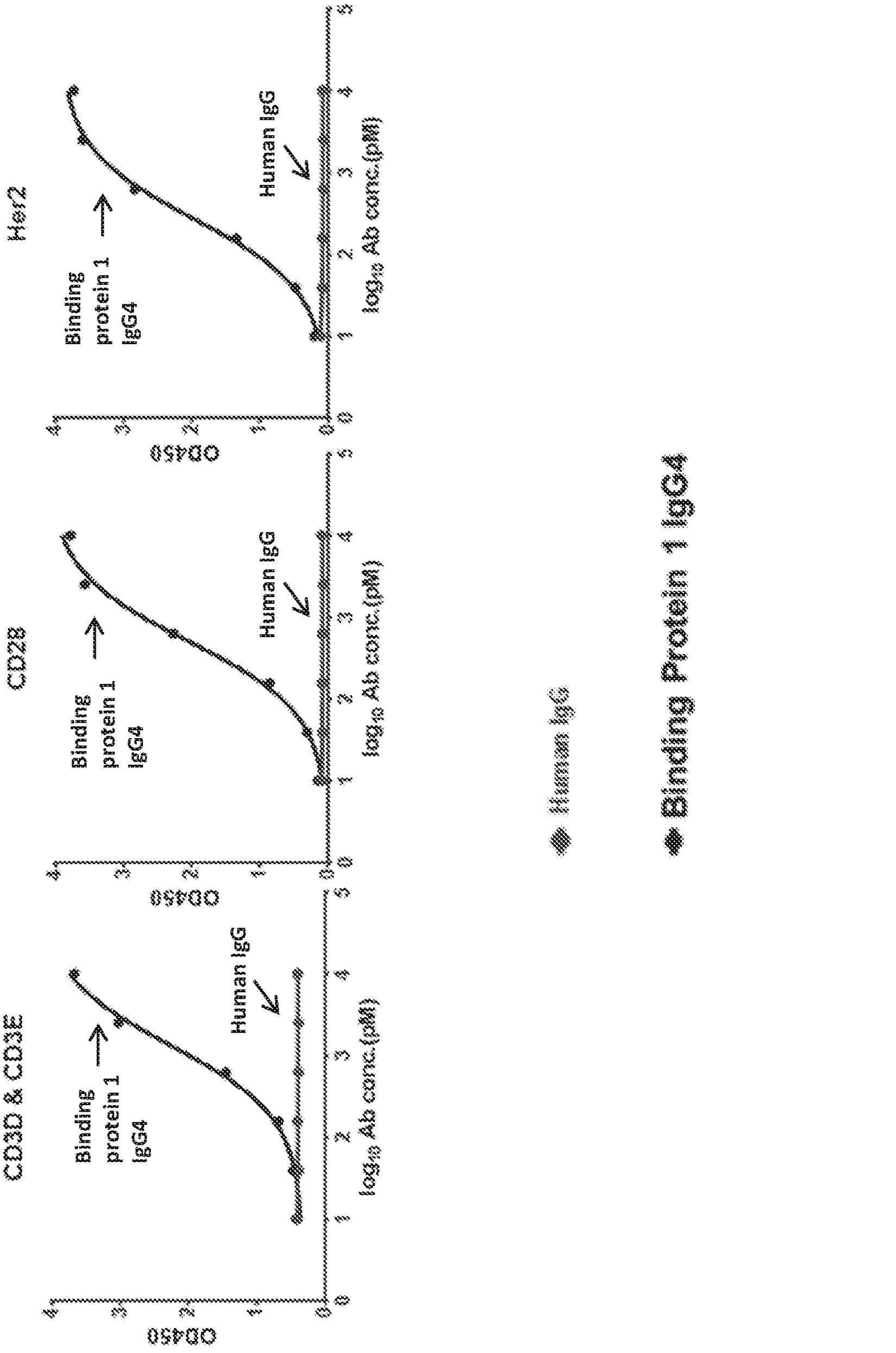

Her2
CD28
CD3D & CD3E
Binding protein 1 IgG4
Human IgG
OD450
log10 Ab conc.(pM)
Human IgG
Binding Protein 1 IgG4

FIG. 3A

ZR-75-1

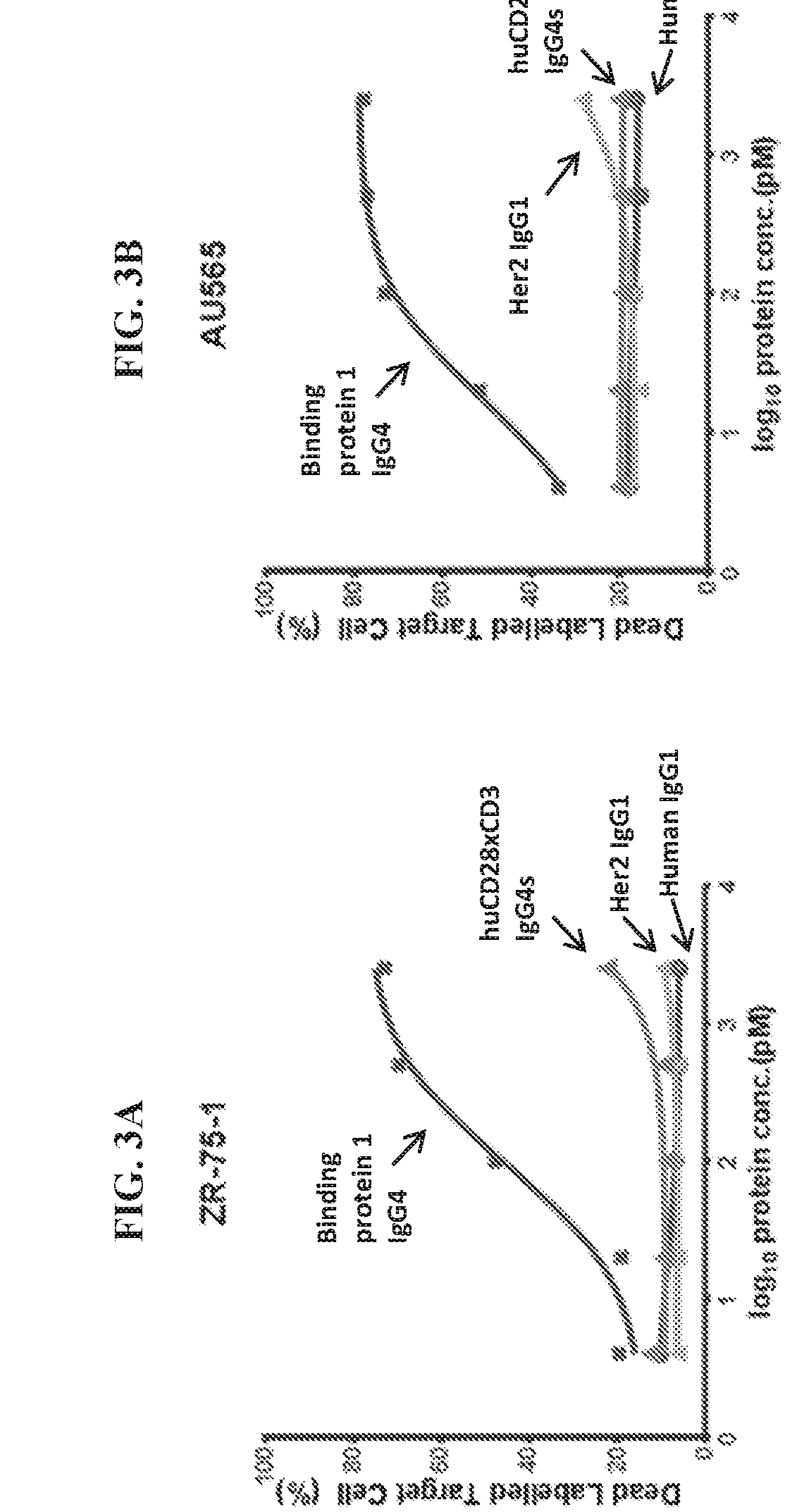


FIG. 3B

AU565

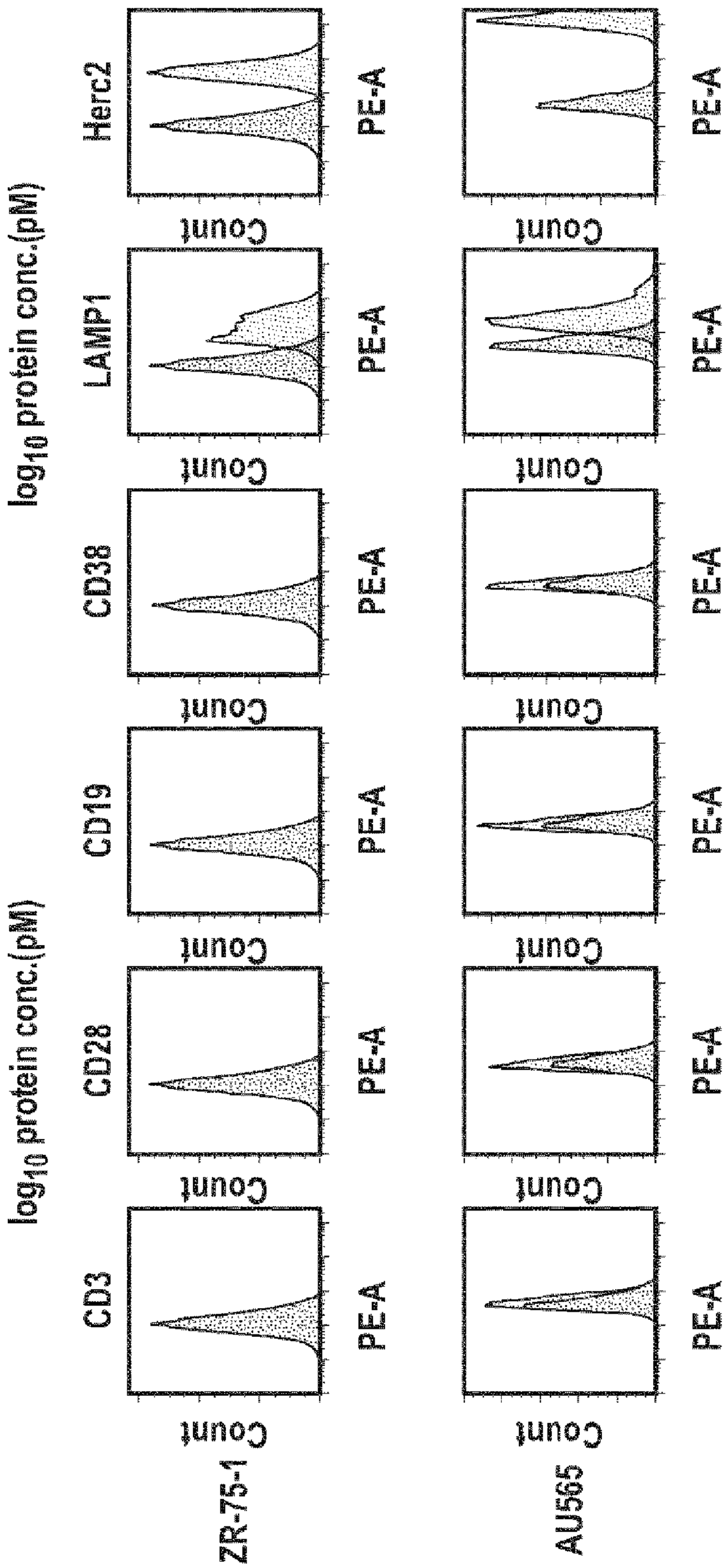

log10 protein conc.(pM)
Herc2
LAMP1
CD38
CD19
CD28
CD3
ZR-75-1
AU565
Count
PE-A

FIG. 3C

FIG. 5

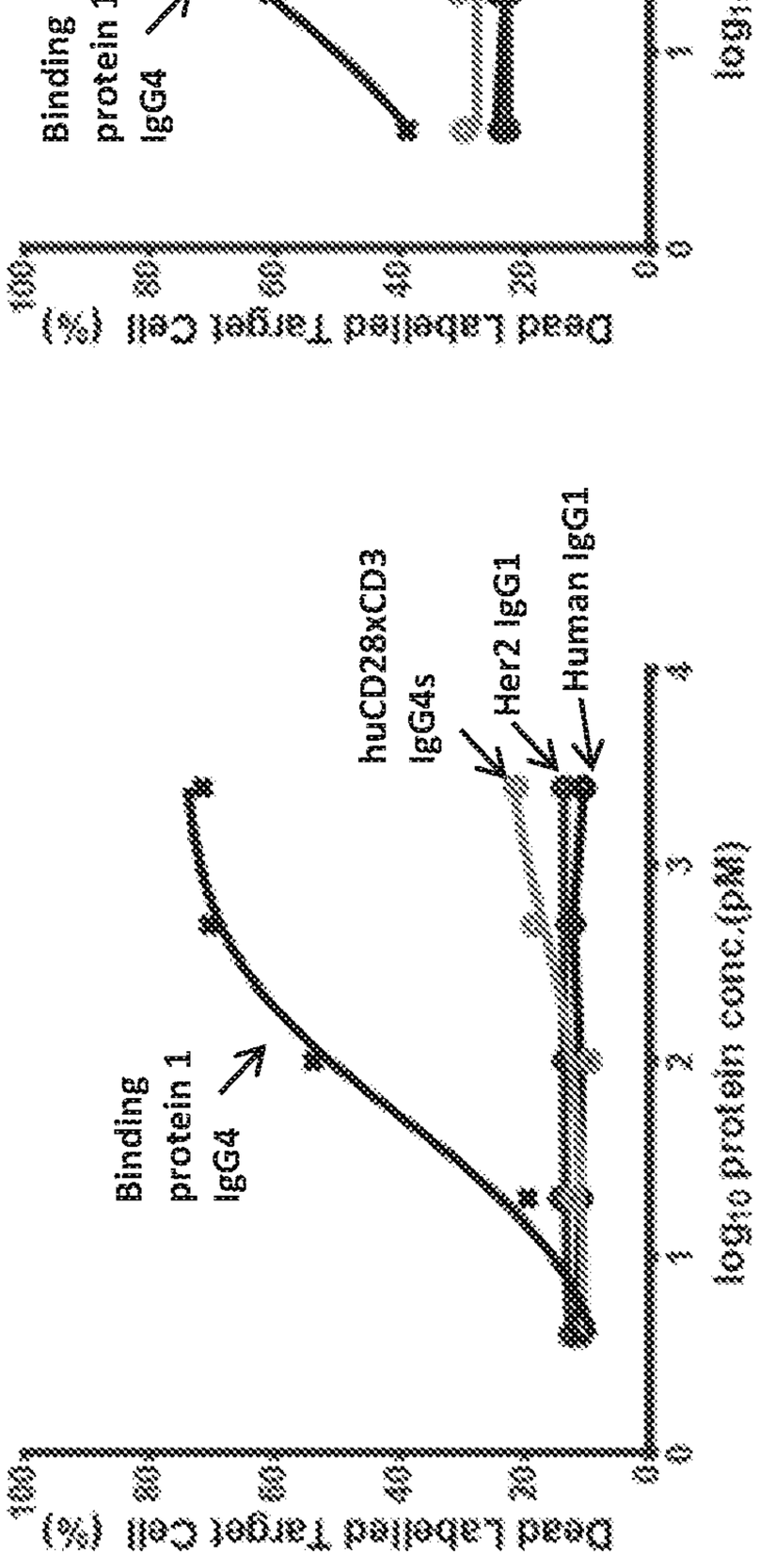

AU565/CD28xCD3
Donor KP45944
Dead Labelled Target Cell (%)
100
80
60
40
20
0
0
1
2
3
log10 protein conc.(pM)
Binding protein 1 IgG4
huCD28xCD3 IgG4s
Her2 IgG1
Human IgG1

FIG. 4

ZR-75-1/CD28xCD3
Donor KP45926
Dead Labelled Target Cell (%)
100
80
60
40
20
0
0
1
2
3
log10 protein conc.(pM)
Binding protein 1 IgG4
huCD28xCD3 IgG4s
Her2 IgG1
Human IgG1

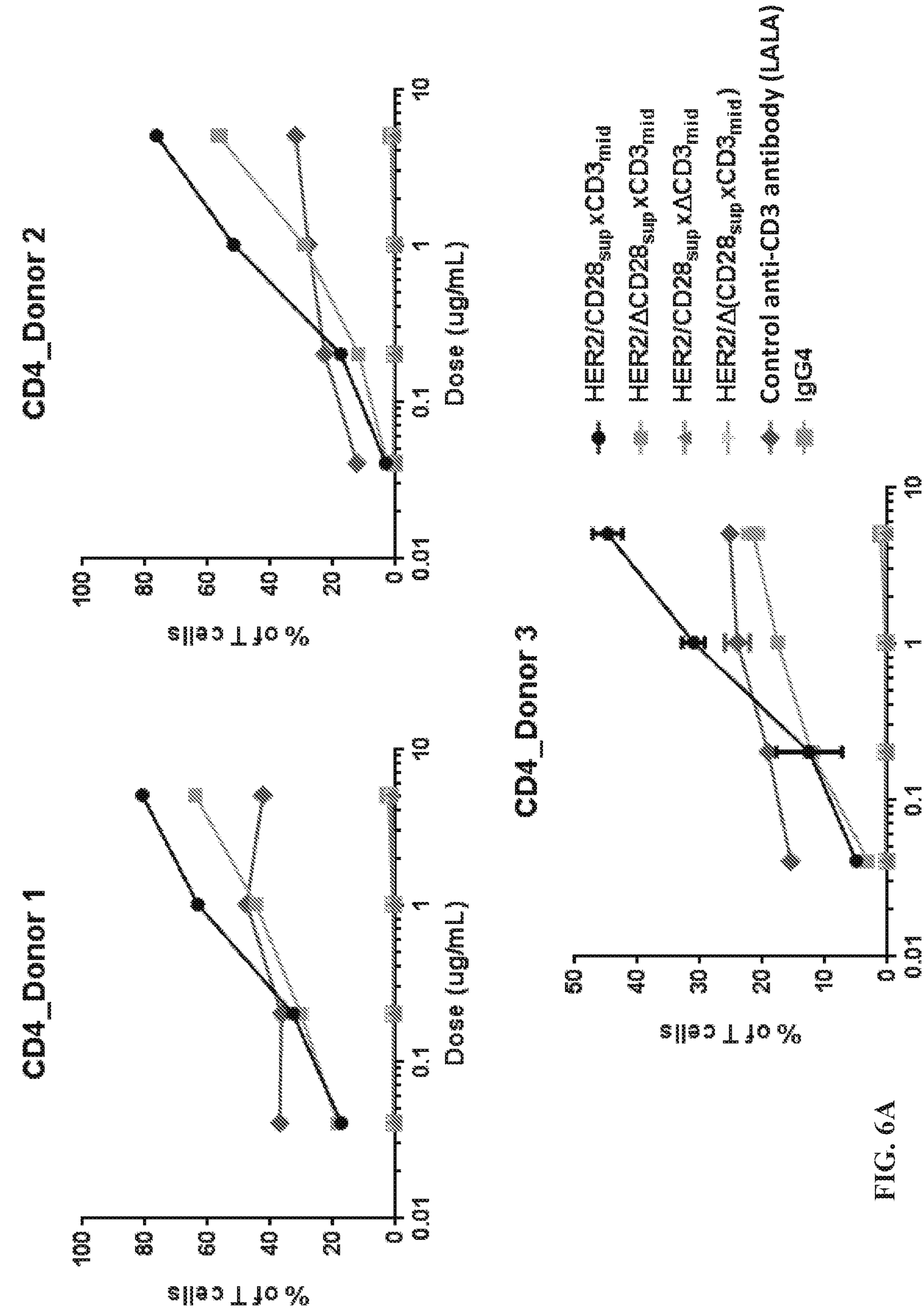

CD4_Donor 2
% of T cells
Dose (ug/mL)
HER2/CD28sup xCD3mid
HER2/ΔCD28sup xCD3mid
HER2/CD28sup xΔCD3mid
HER2/Δ(CD28sup xCD3mid
Control anti-CD3 antibody (LALA)
IgG4
CD4_Donor 1
CD4_Donor 3

FIG. 6A

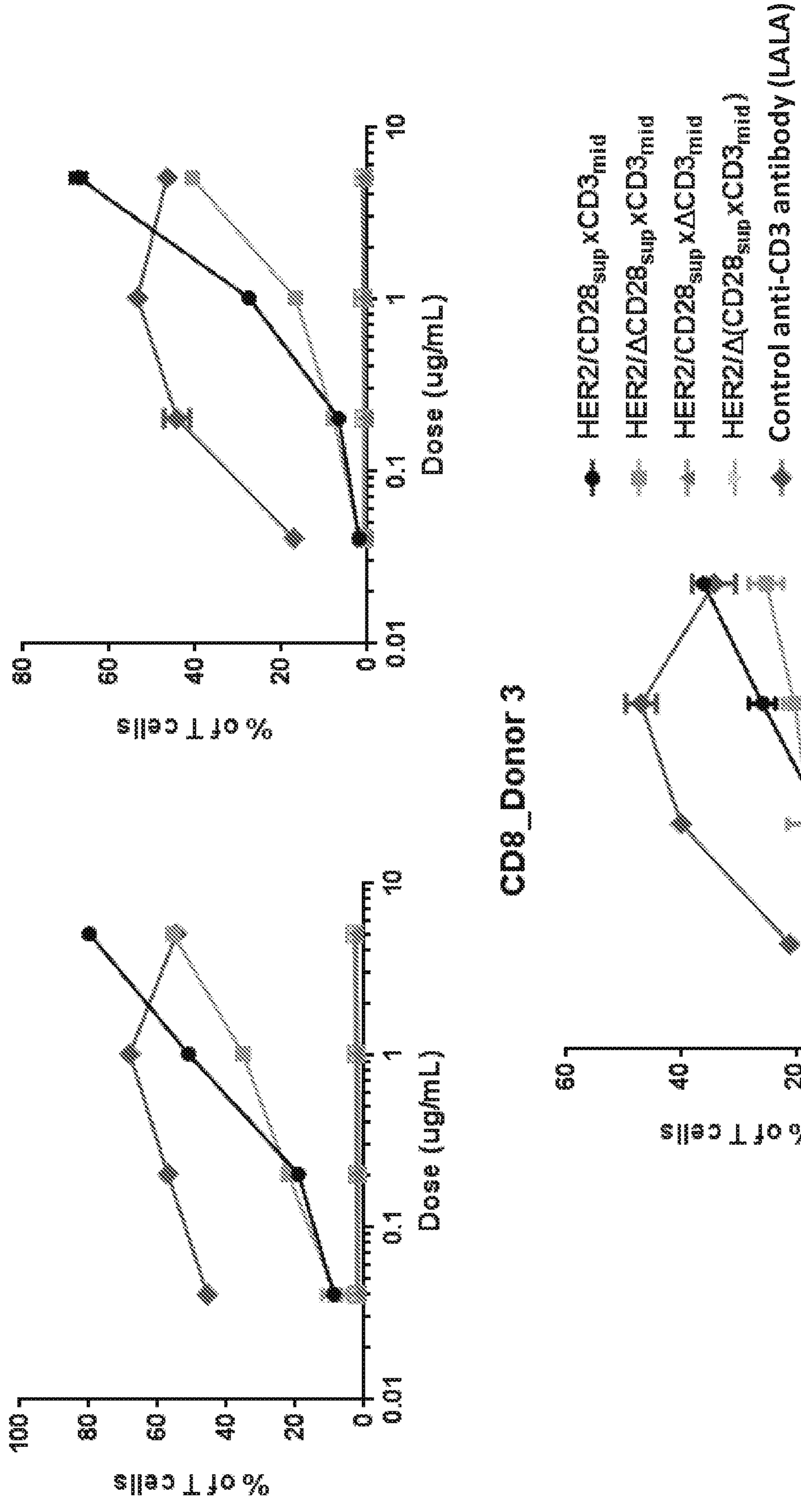

CD8_Donor 2
% of T cells
Dose (ug/mL)
0.01
0.1
1
10
0
20
40
60
80
HER2/CD28sup xCD3mid
HER2/ΔCD28sup xCD3mid
HER2/CD28sup xΔCD3mid
HER2/Δ(CD28sup xCD3mid)
Control anti-CD3 antibody (LALA)
IgG4
CD8_Donor 3
CD8_Donor 1
100

FIG. 6B

FIG. 7A

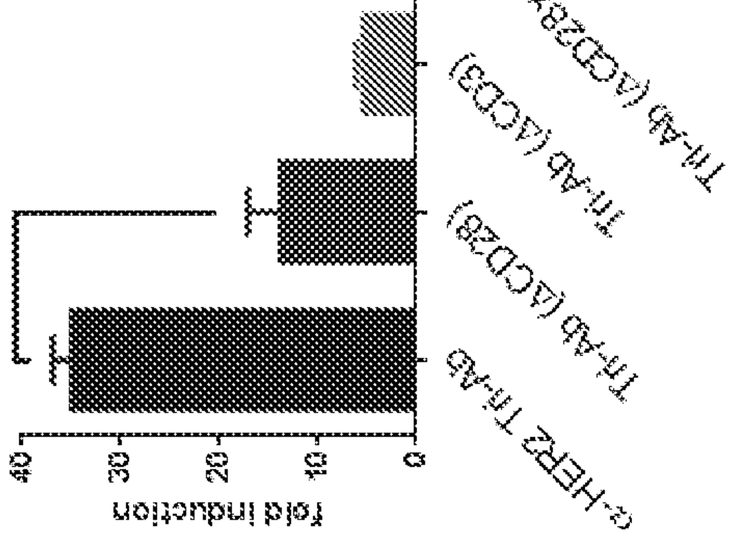


FIG. 7B

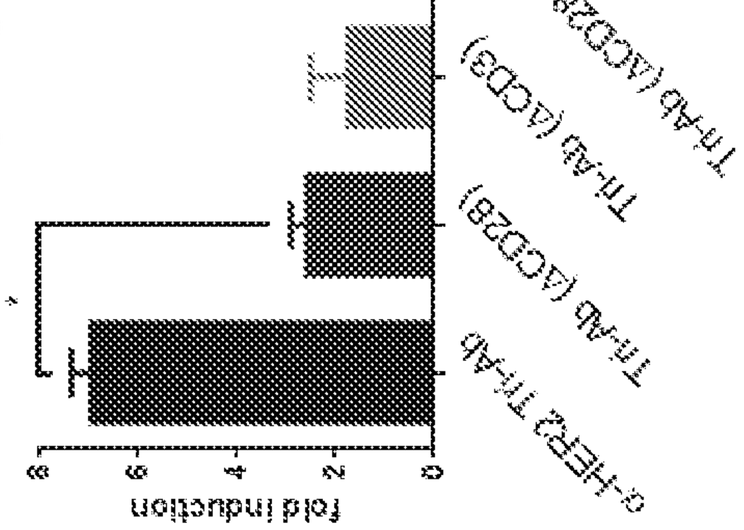


FIG. 7C

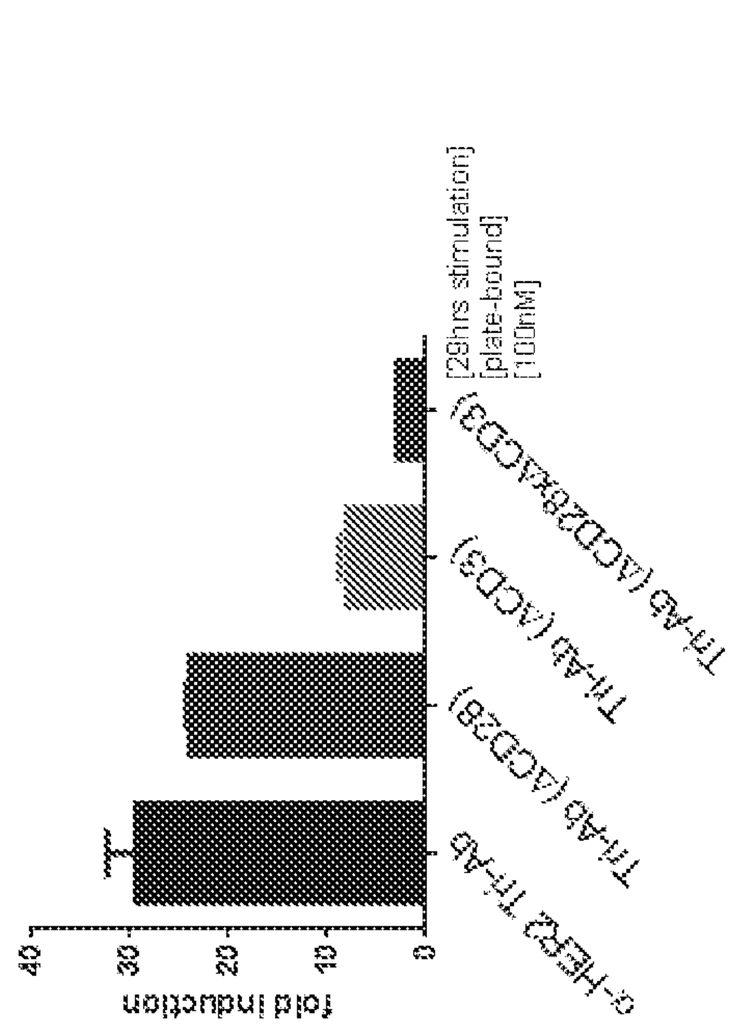
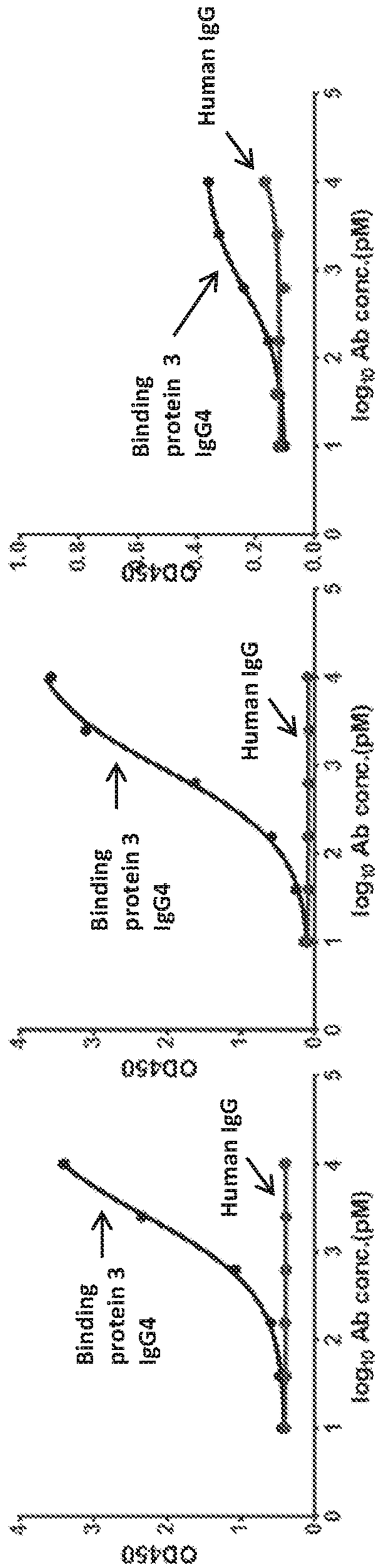


FIG. 8


CD18
Human IgG
Binding protein 3 IgG4
log10 Ab conc.(pM)
OD450
CD28
CD3D & CD3E

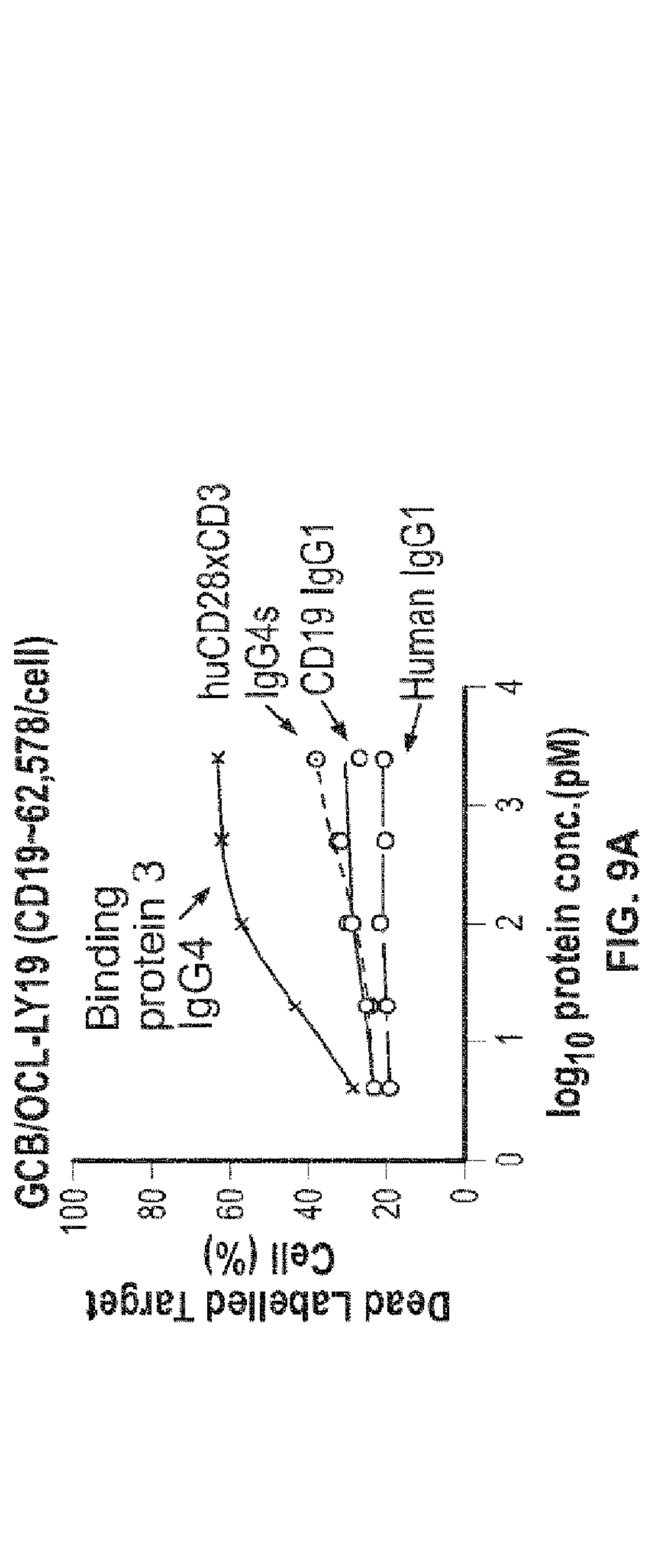

GCB/OCL-LY19 (CD19~62,578/cell)
Bead Labelled Target Cell (%)
100
80
60
40
20
0
0
1
2
3
4
log10 protein conc.(pM)
Binding protein 3 IgG4
huCD28xCD3
IgG4s
CD19 IgG1
Human IgG1

FIG. 9A

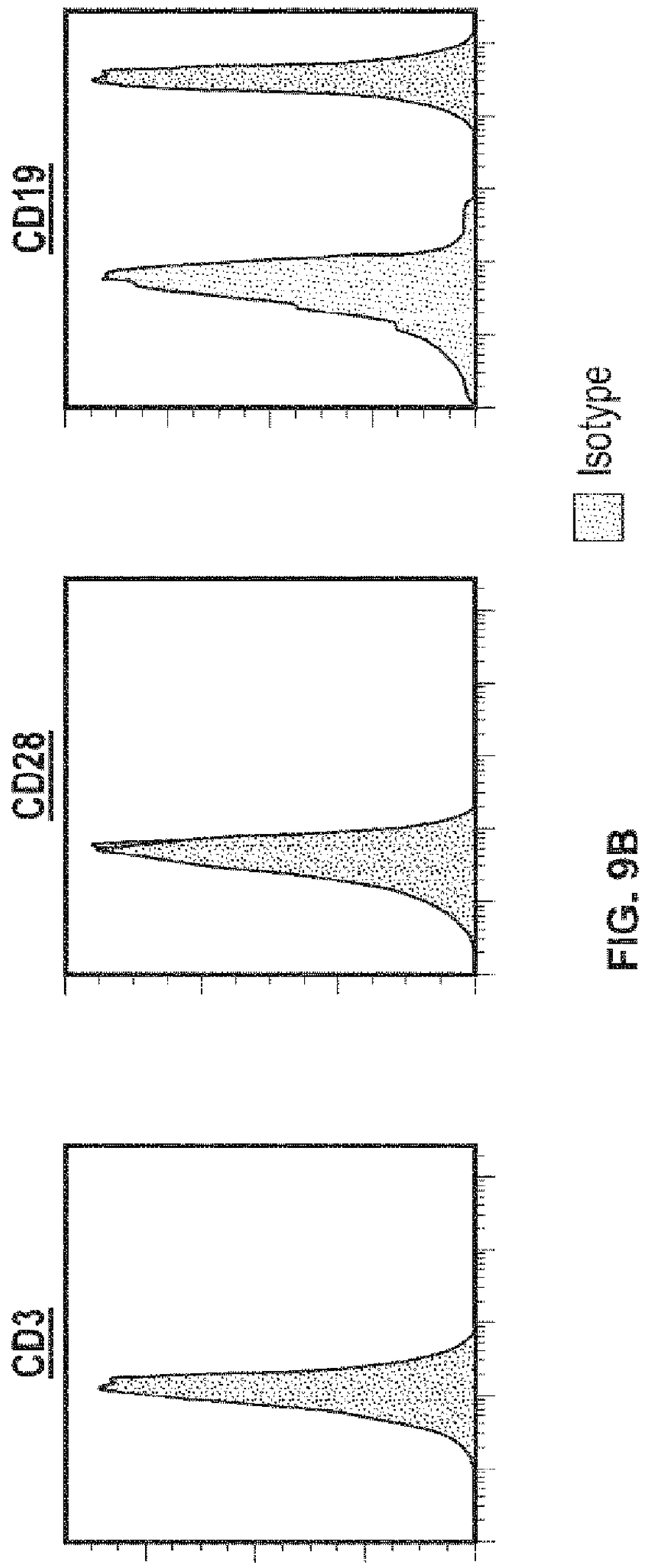

CD28
CD19
CD3
OCI-LY19
Isotype

FIG. 9B

FIG. 9C

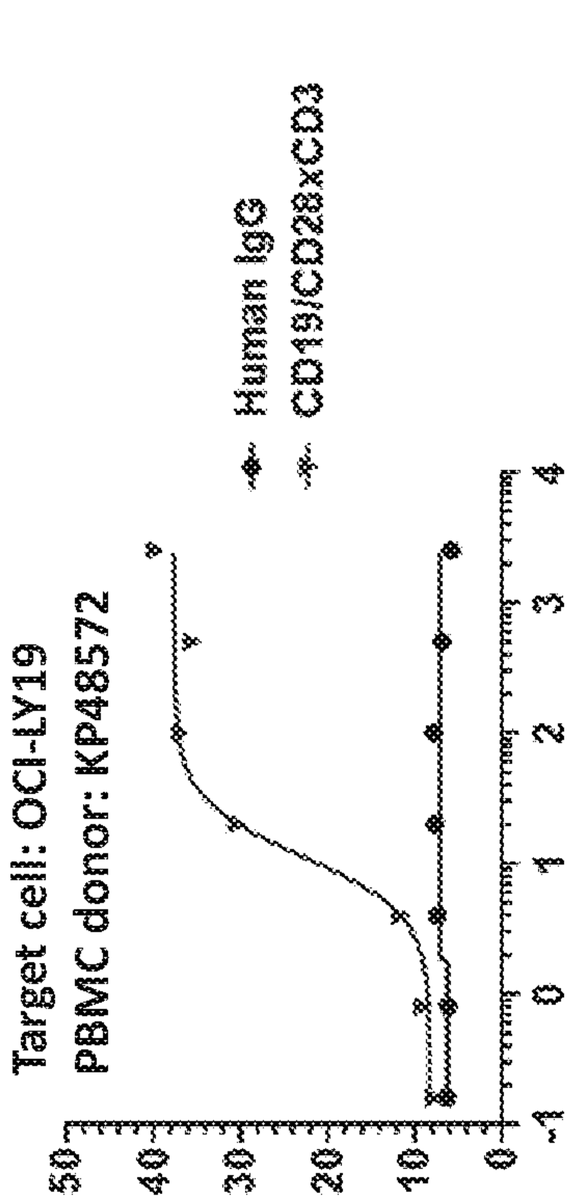


FIG. 9D

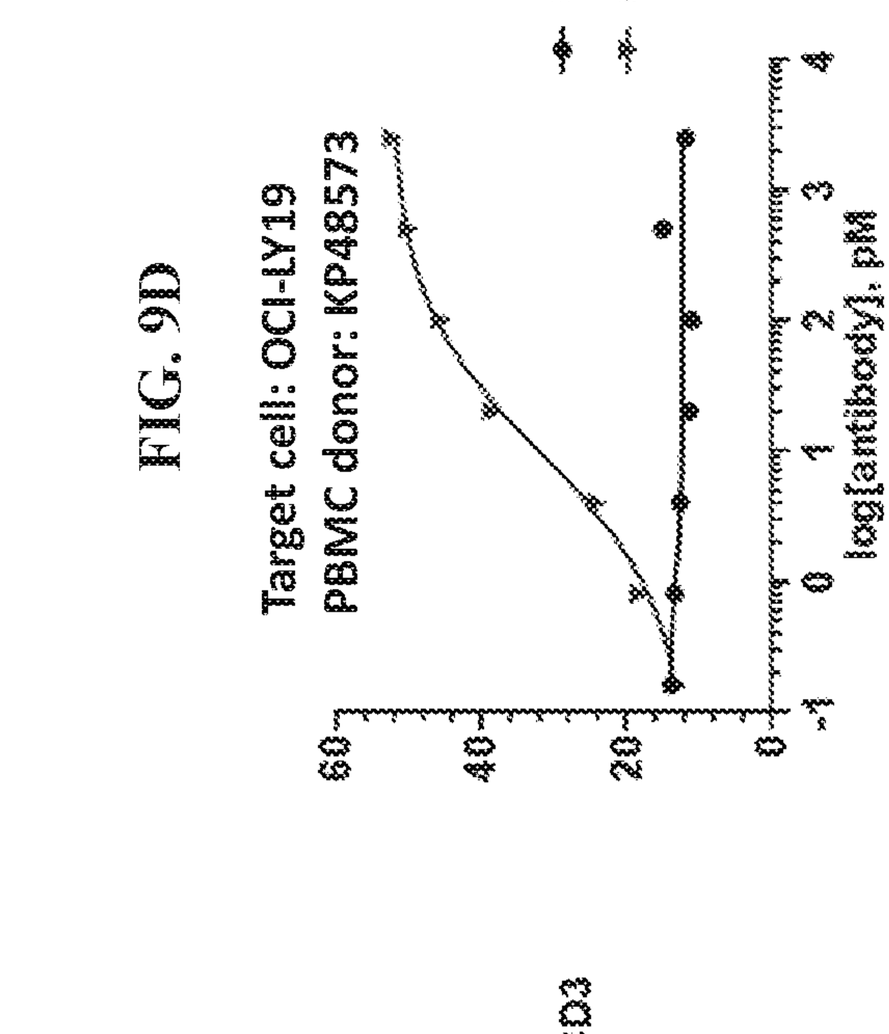


FIG. 9E

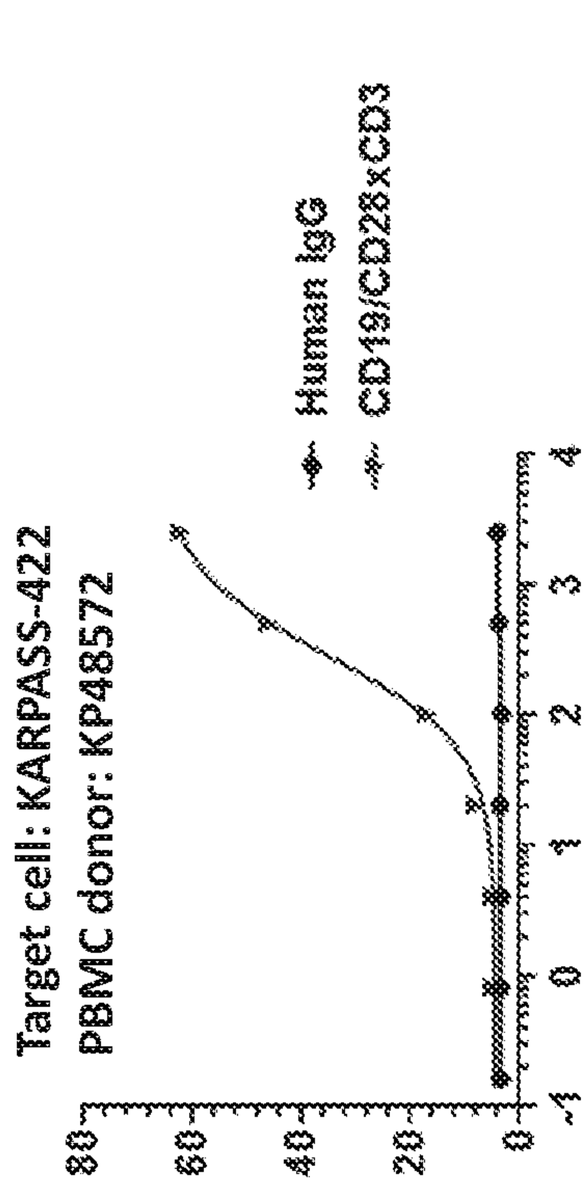


FIG. 9F

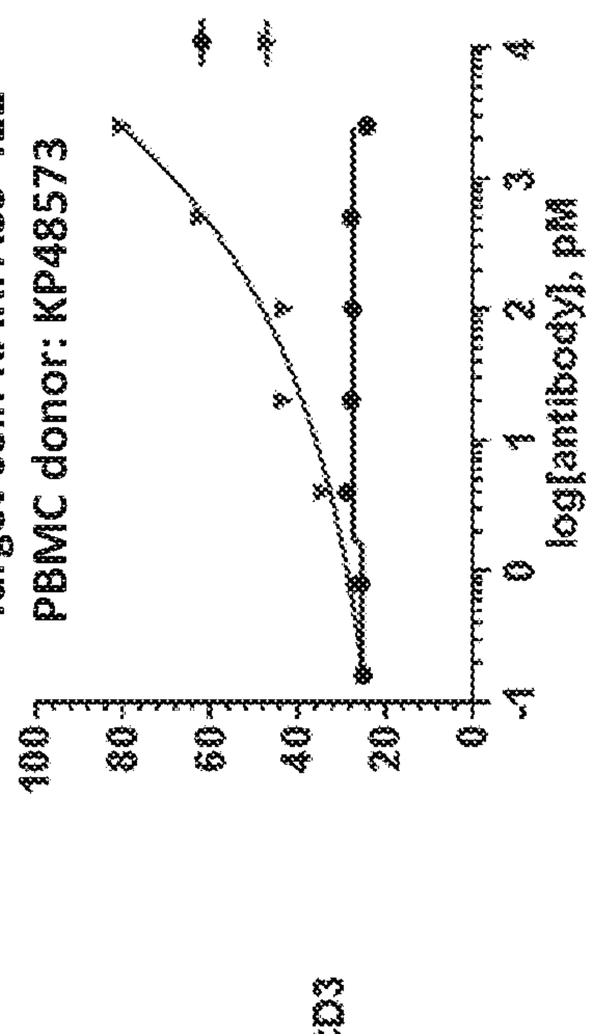


FIG. 9H

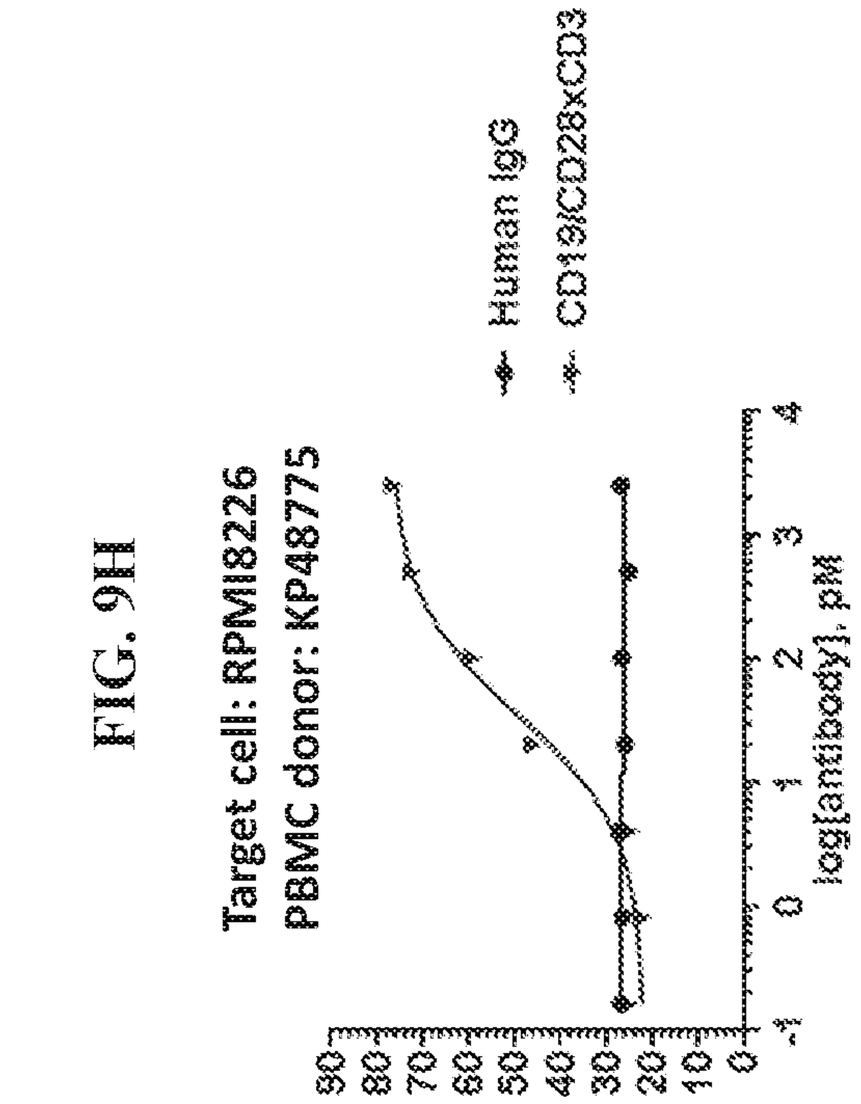


FIG. 9J

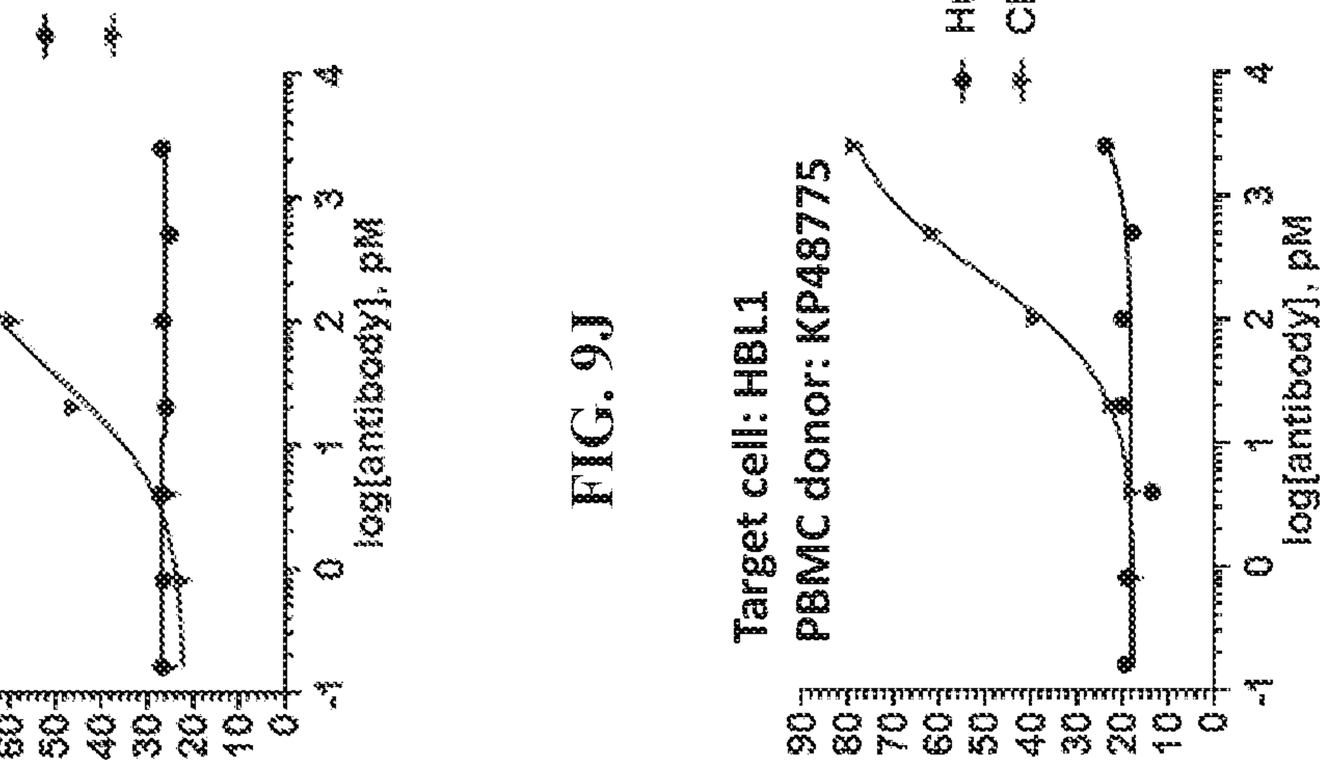


FIG. 9G

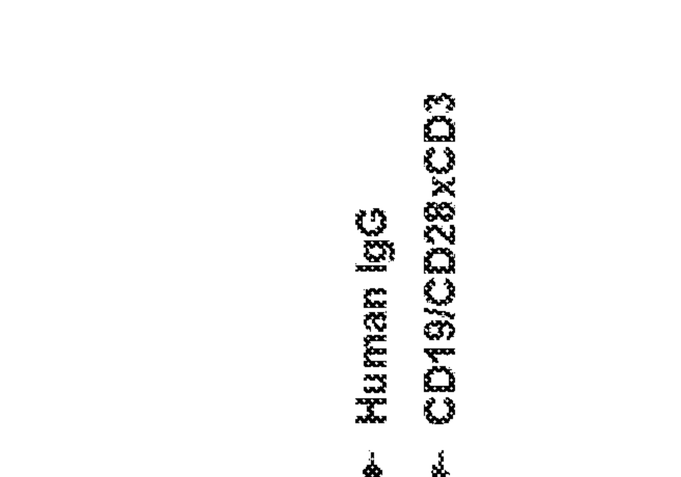


FIG. 9I

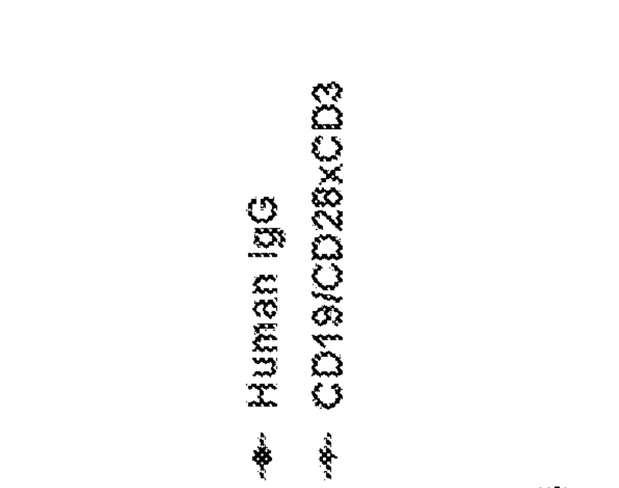


FIG. 9L

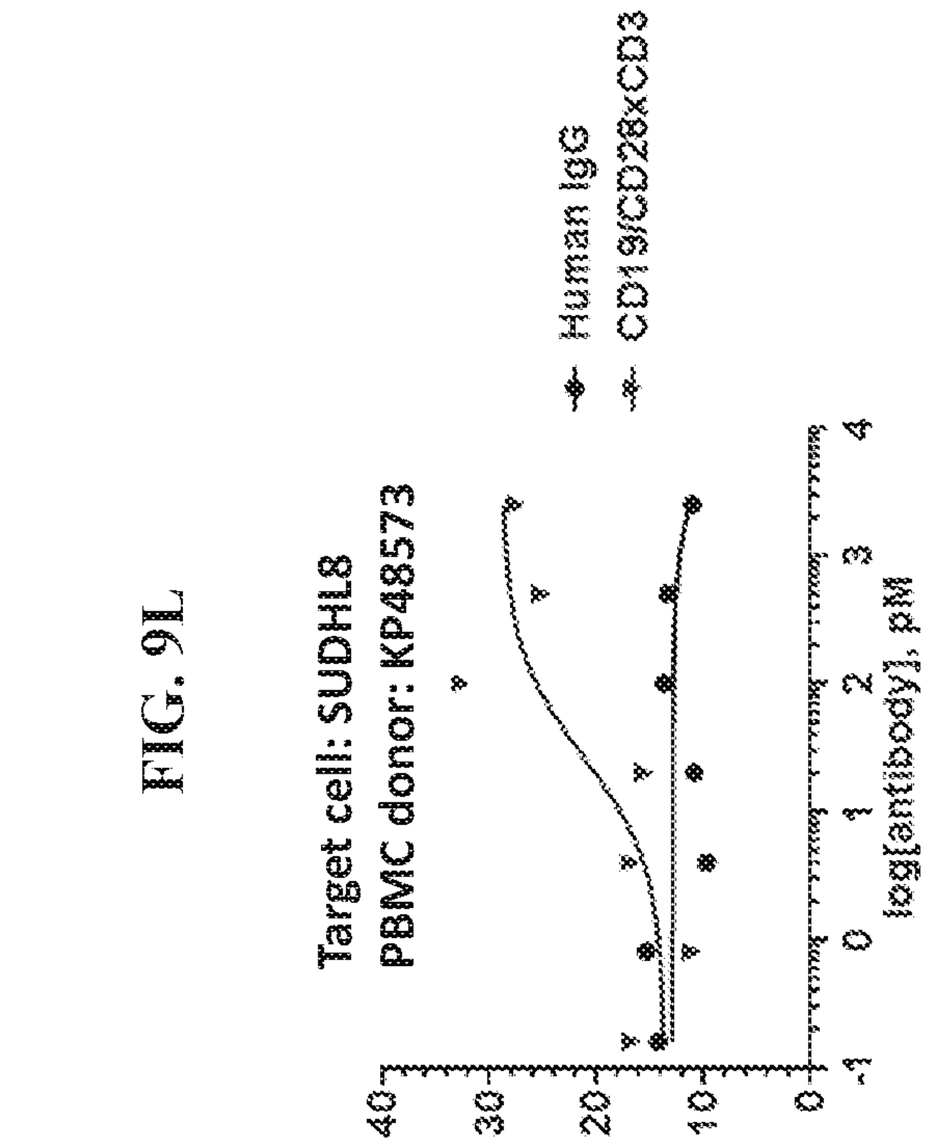


FIG. 9N

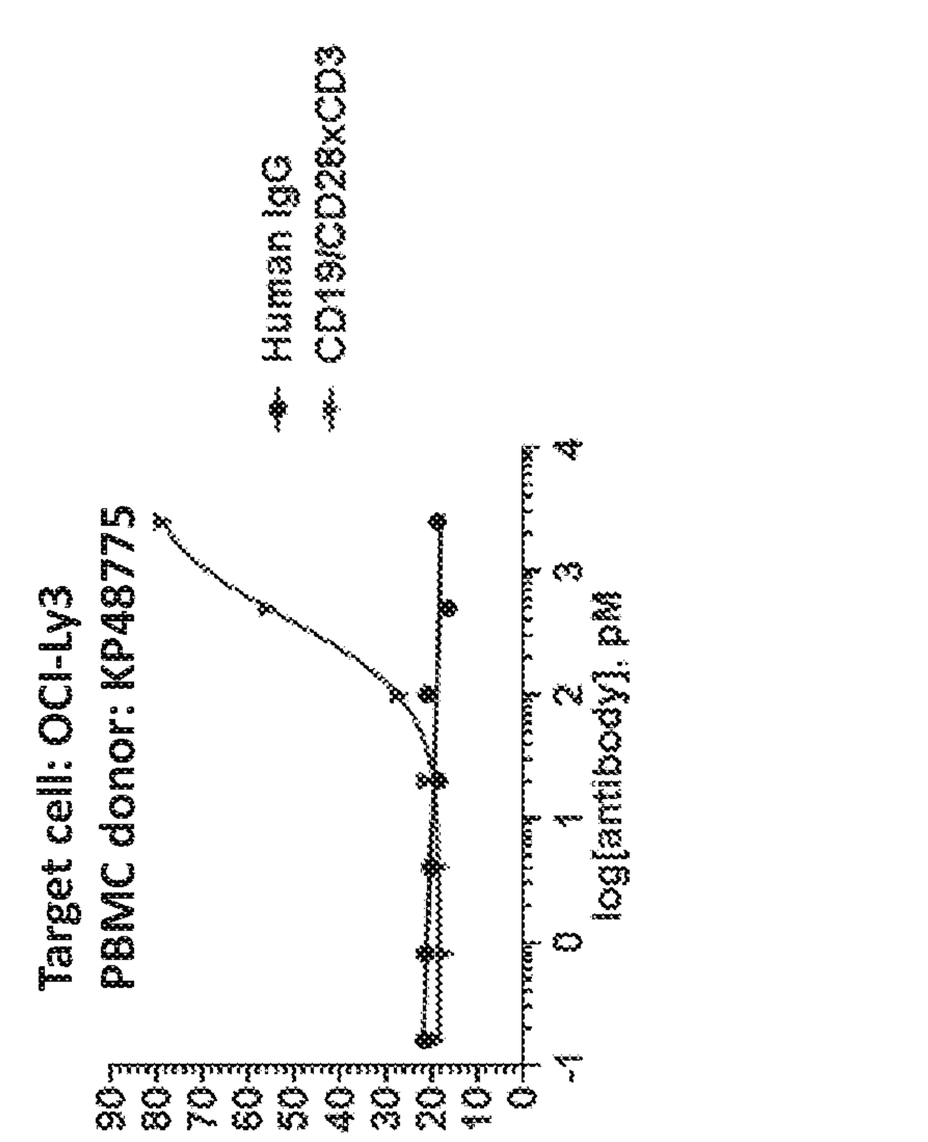


FIG. 9K

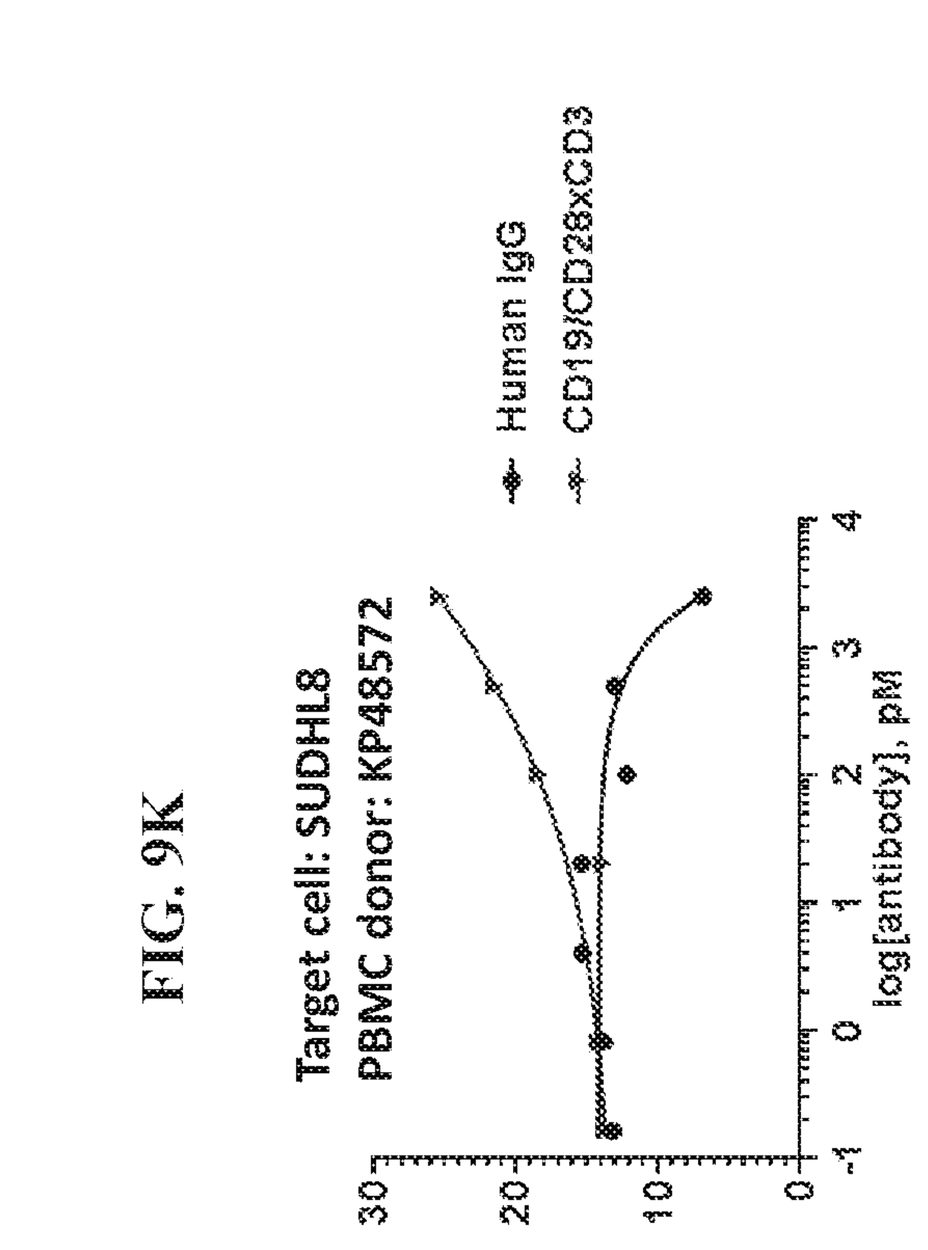
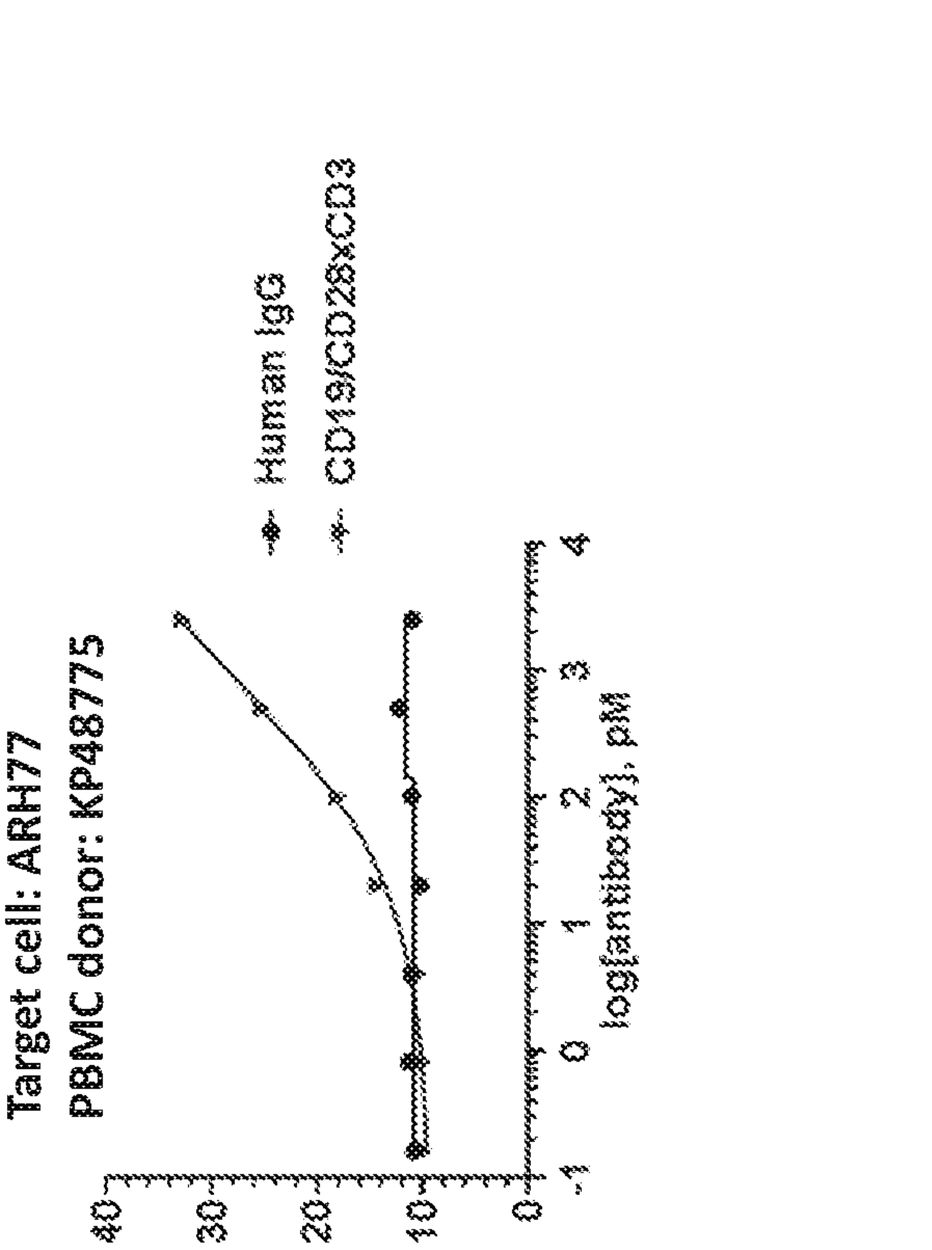


FIG. 9M



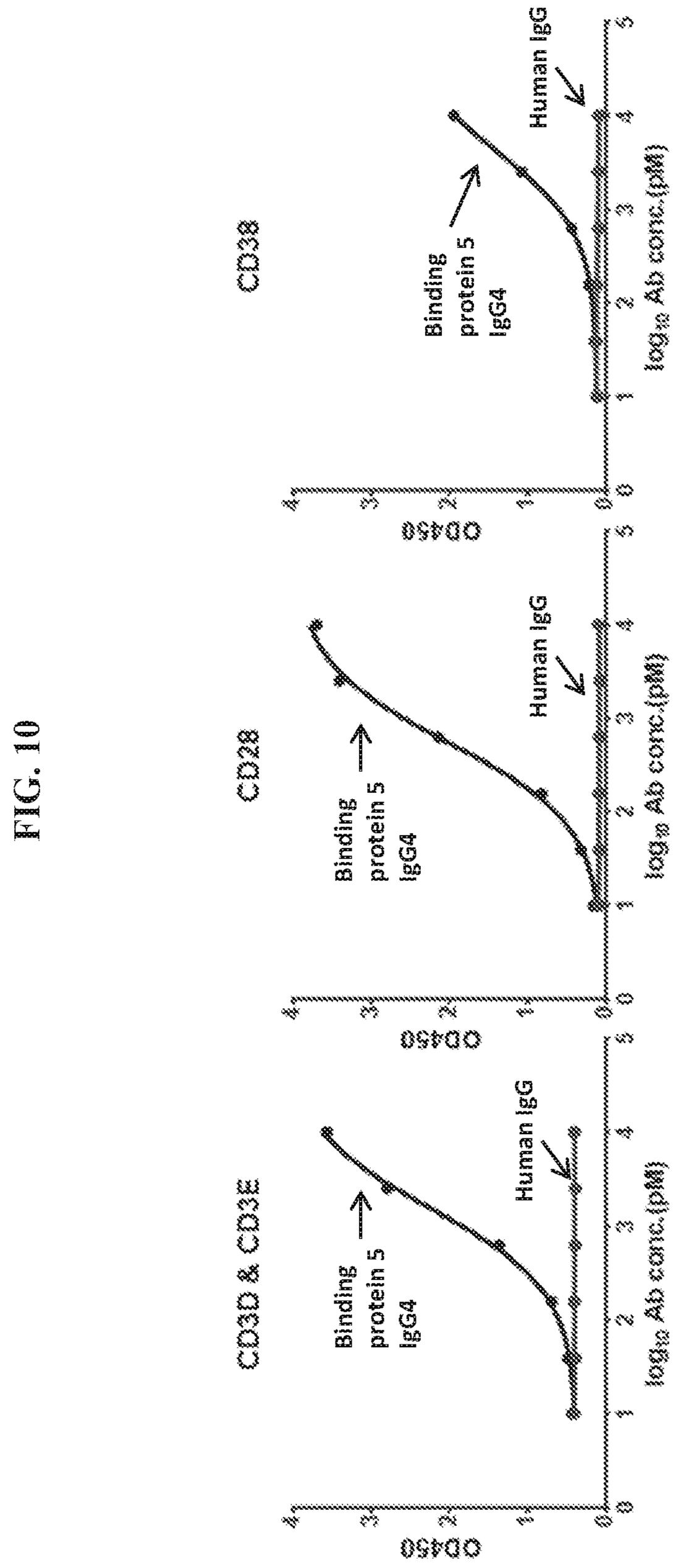

CD38
CD28
CD3D & CD3E
Binding protein 5 IgG4
Human IgG
OD450
log10 Ab conc.(pM)
Binding Protein 5 IgG4
Human IgG

FIG. 10

FIG. 11A

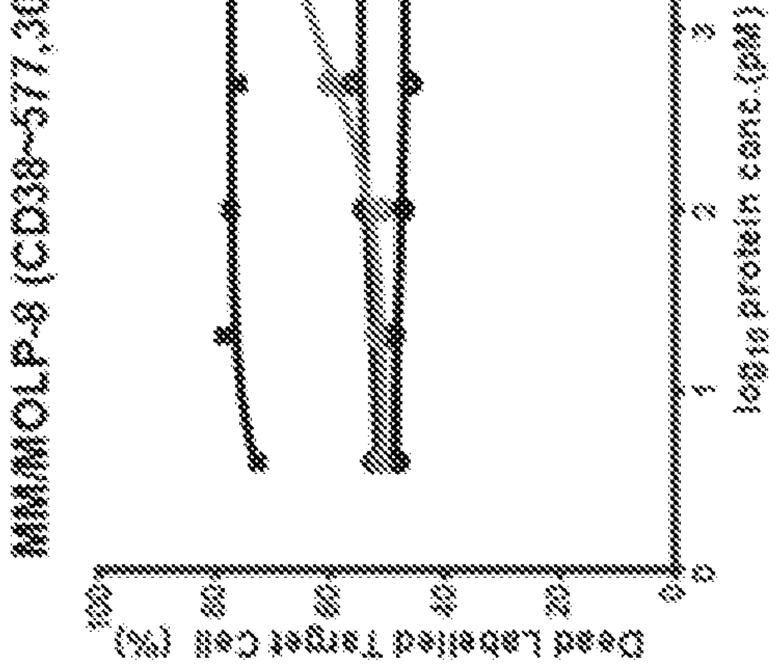


FIG. 11B

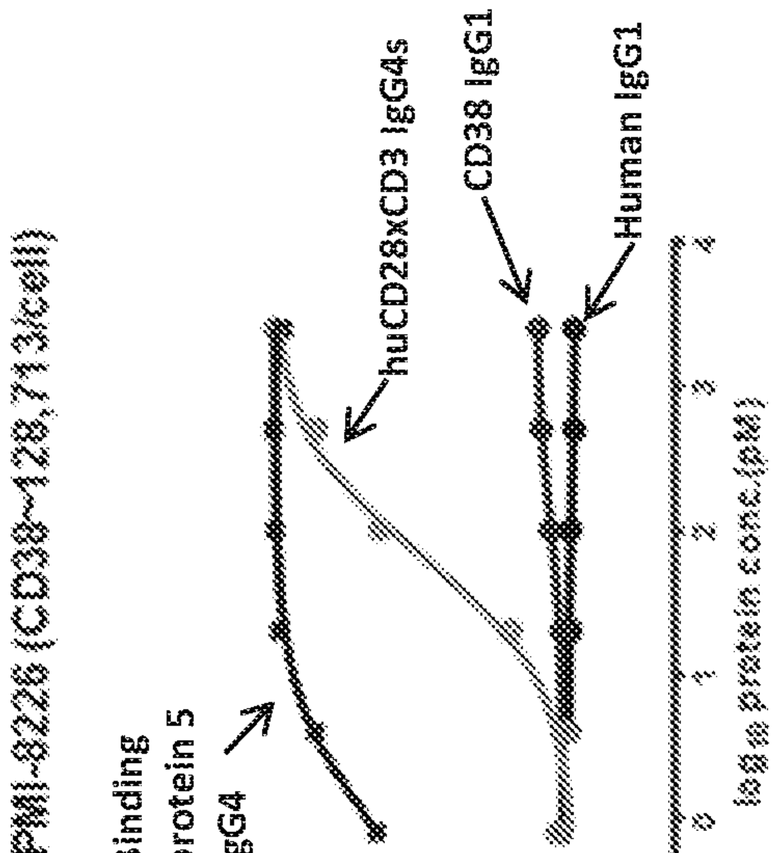
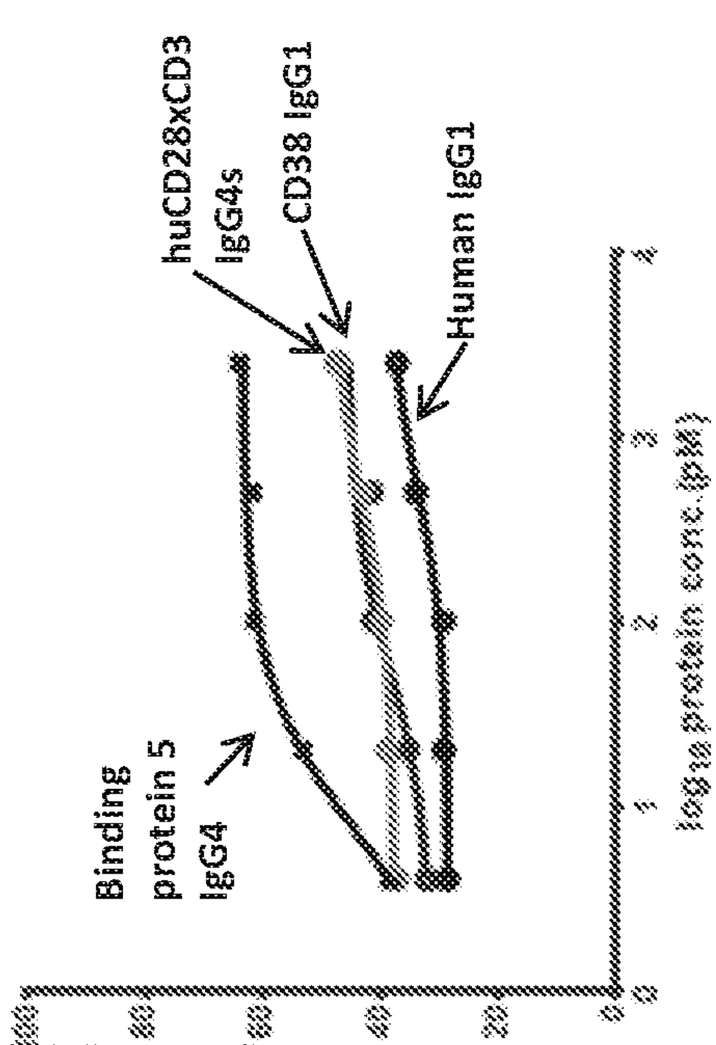


FIG. 11C



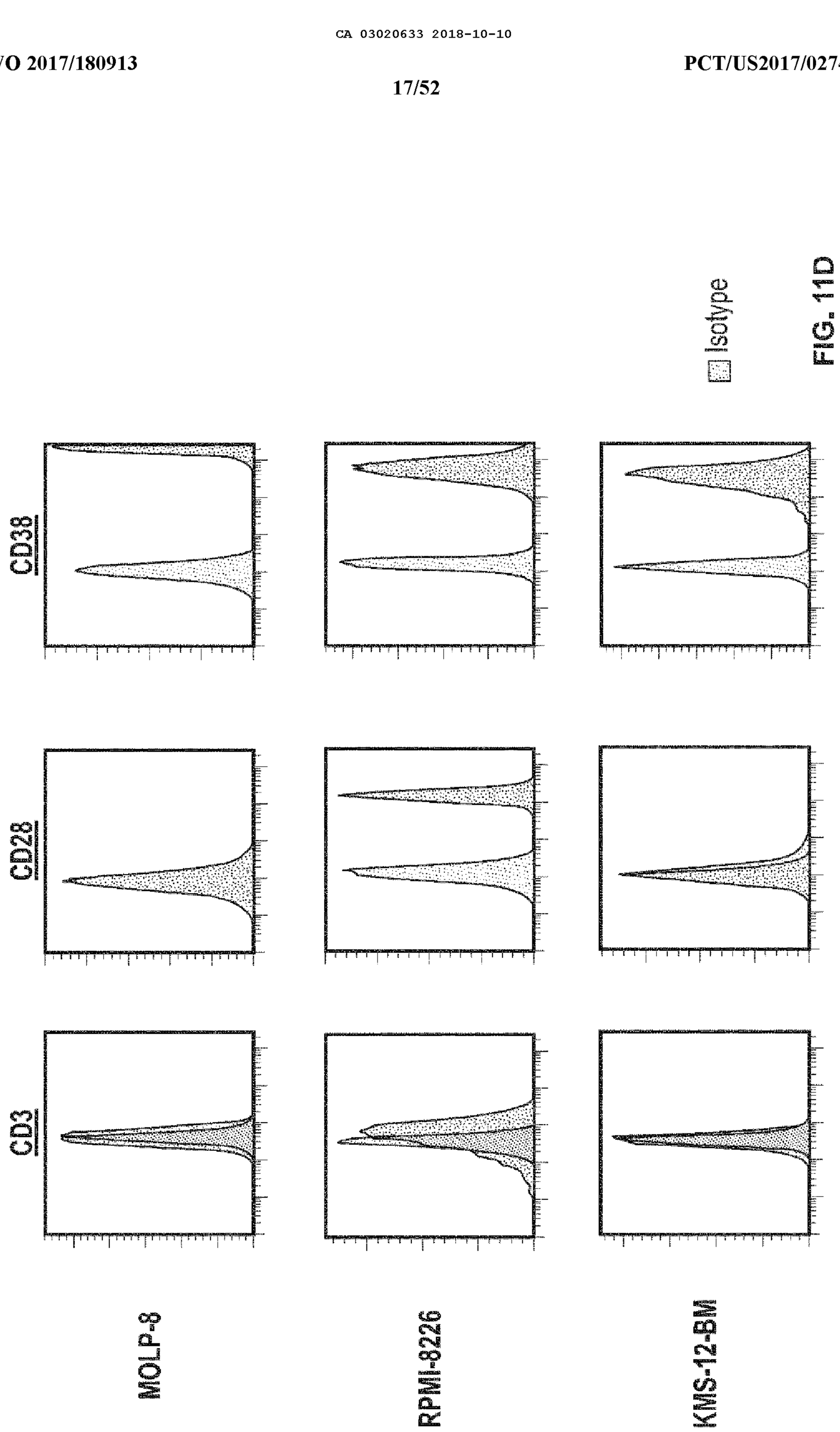


FIG. 11D

FIG. 12A

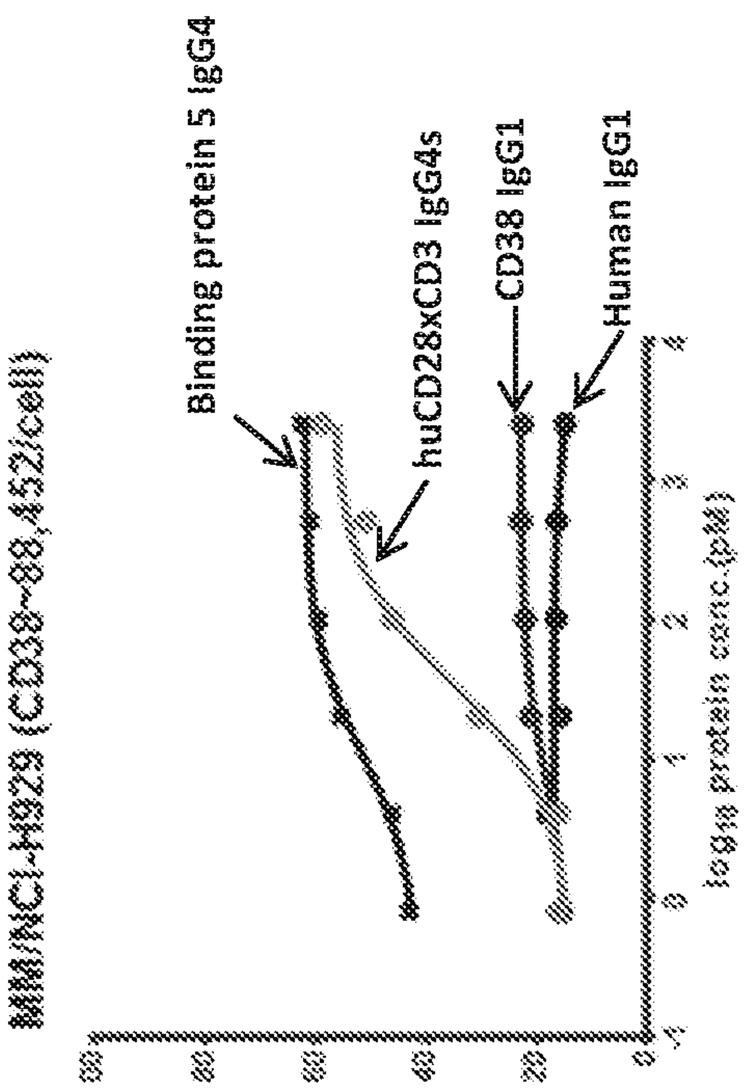


FIG. 12B

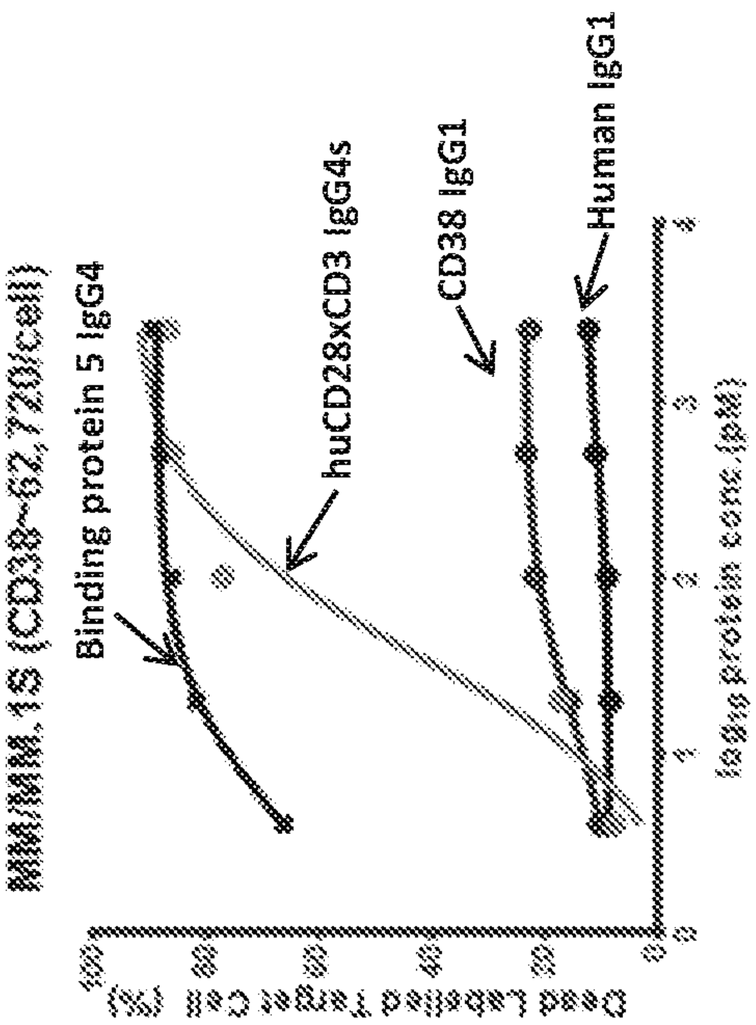


FIG. 12C

FIG. 12D

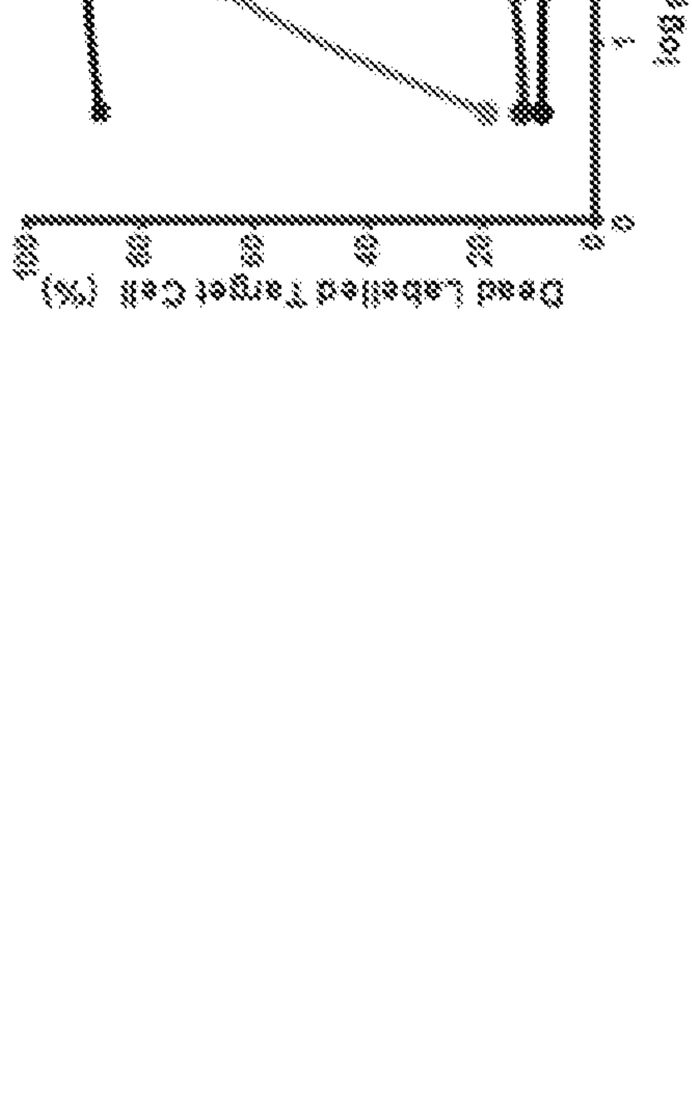


FIG. 12D

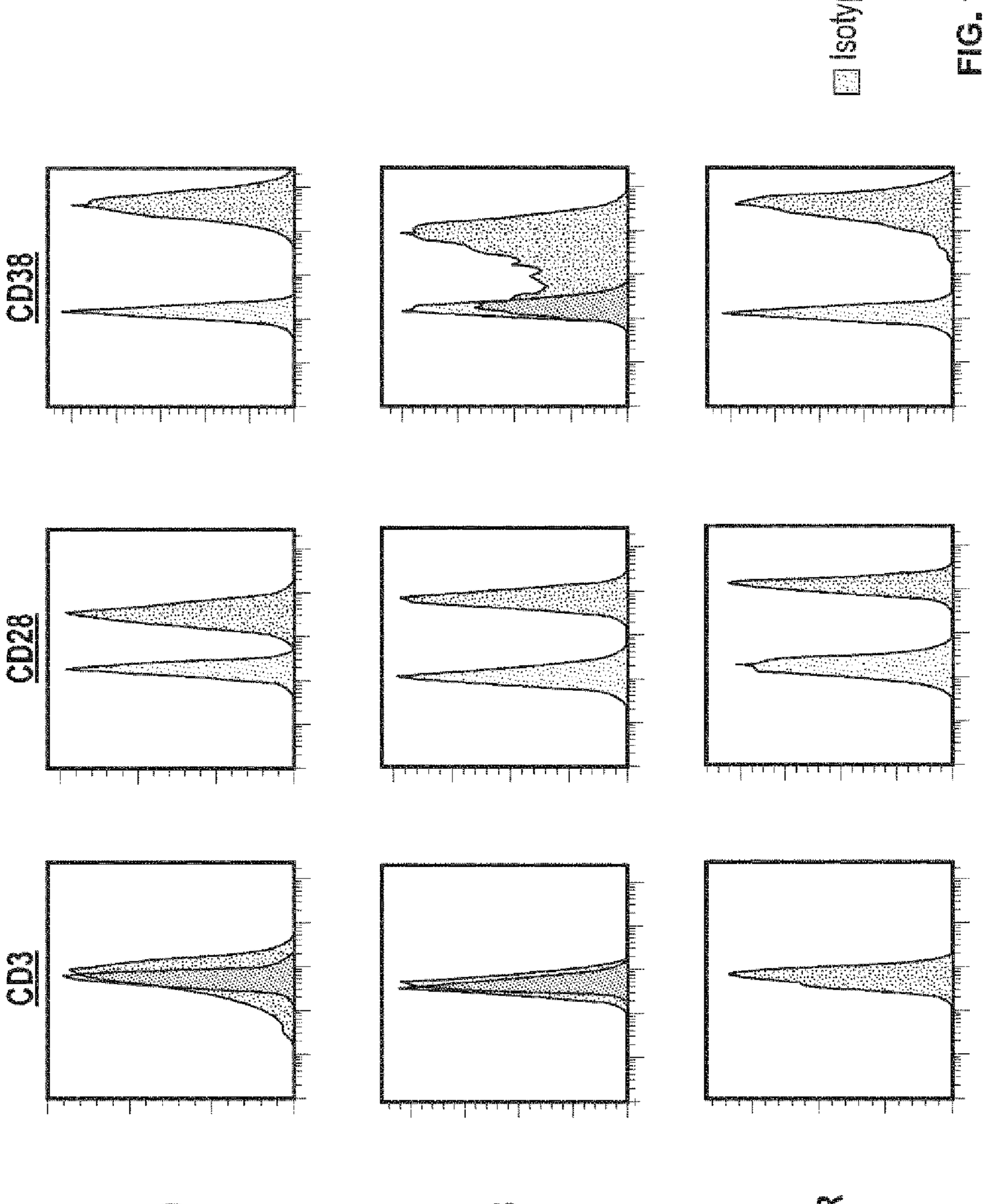


FIG. 13A

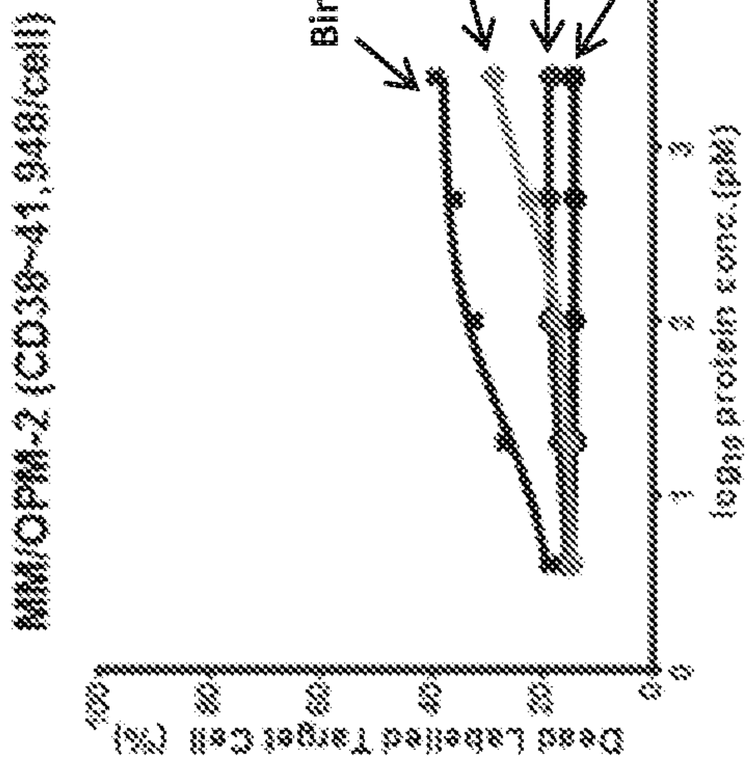


FIG. 13B

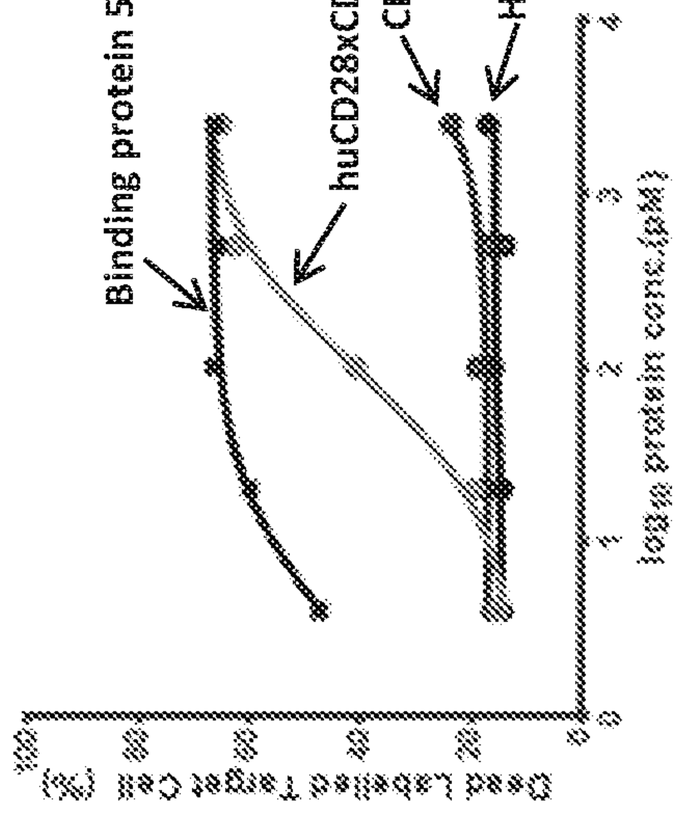


FIG. 13C

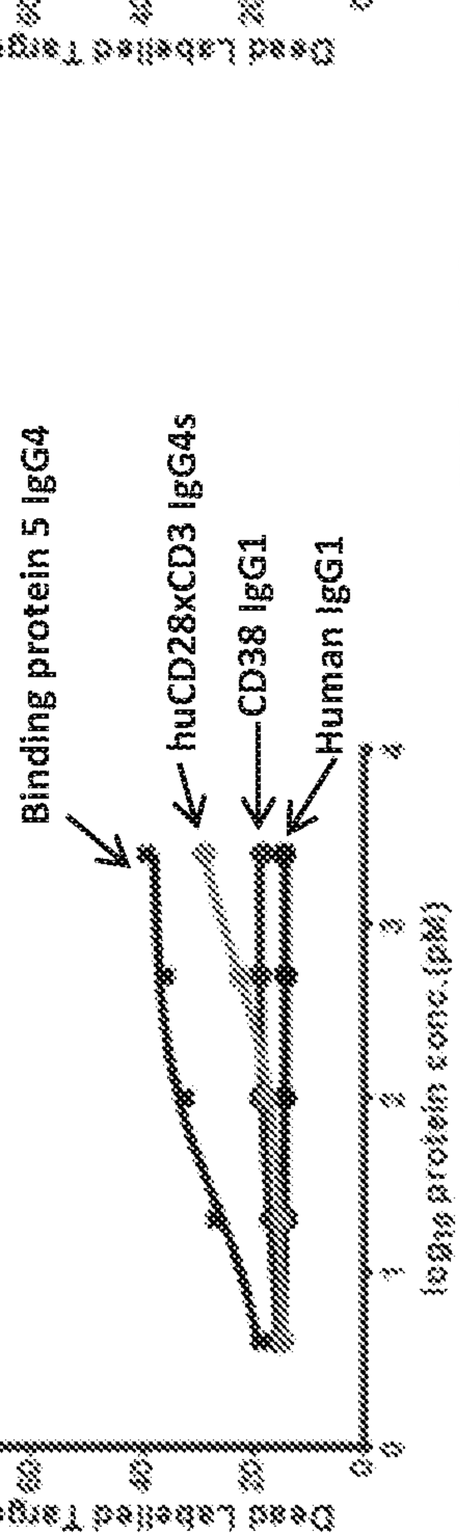
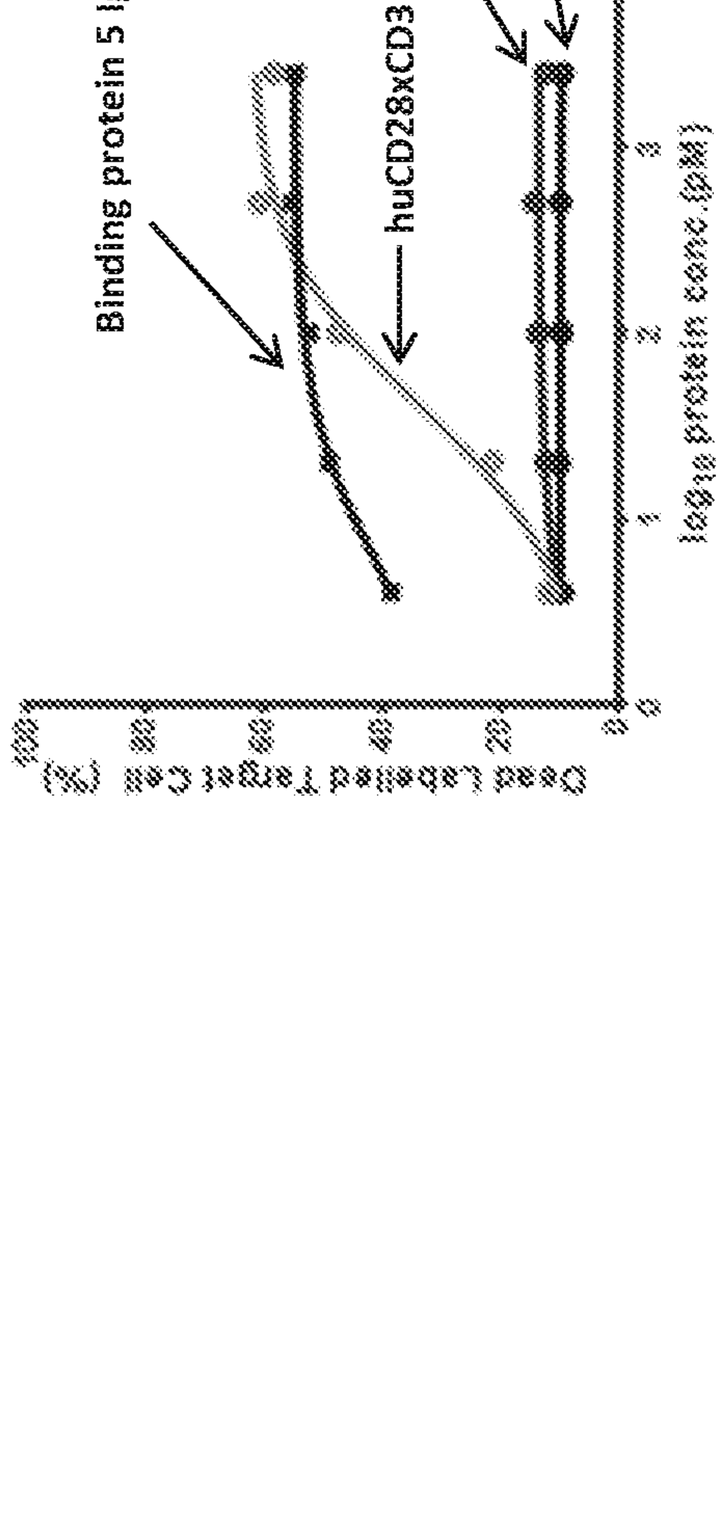


FIG. 13D



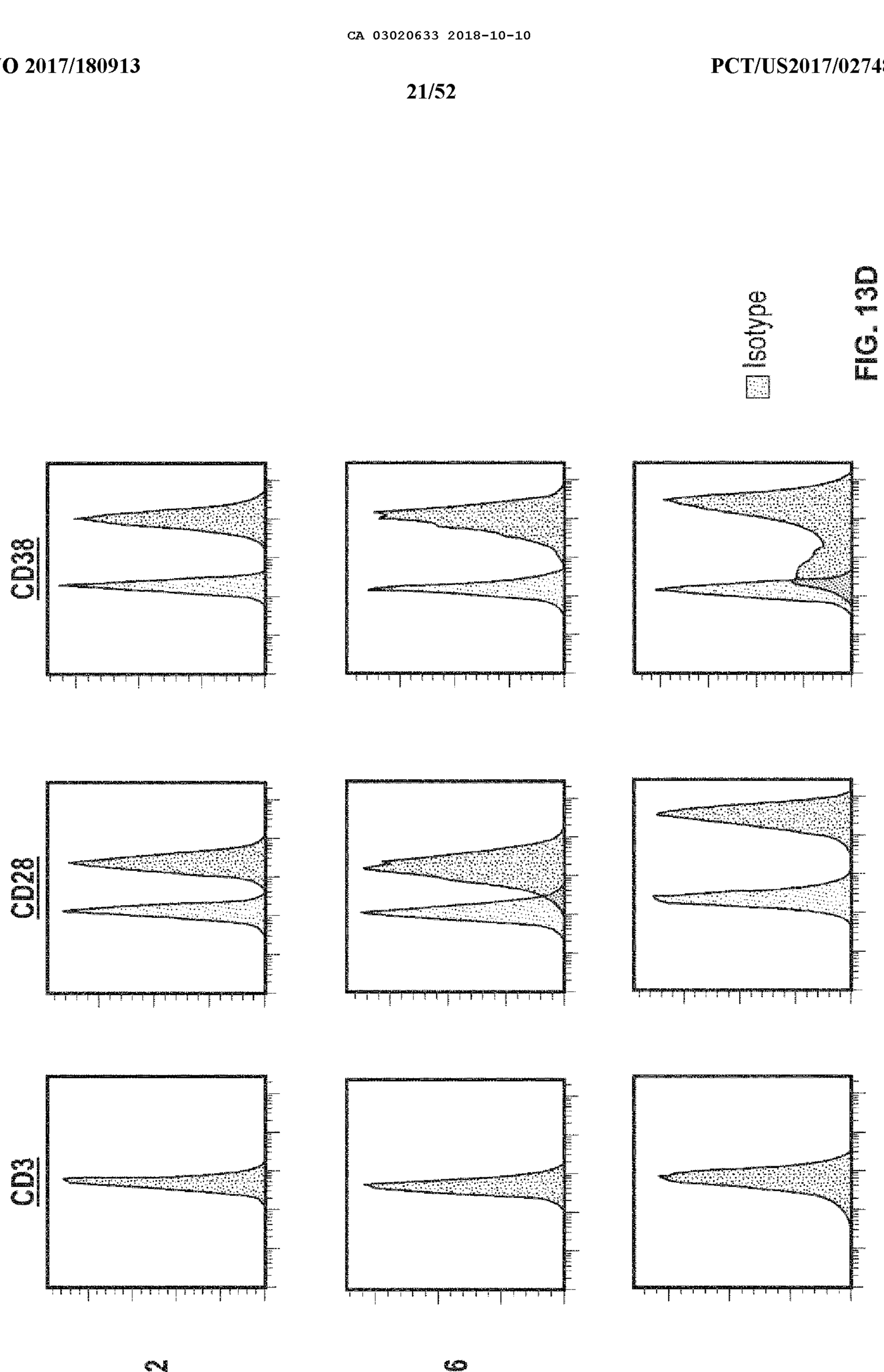


FIG. 13D

FIG. 14A

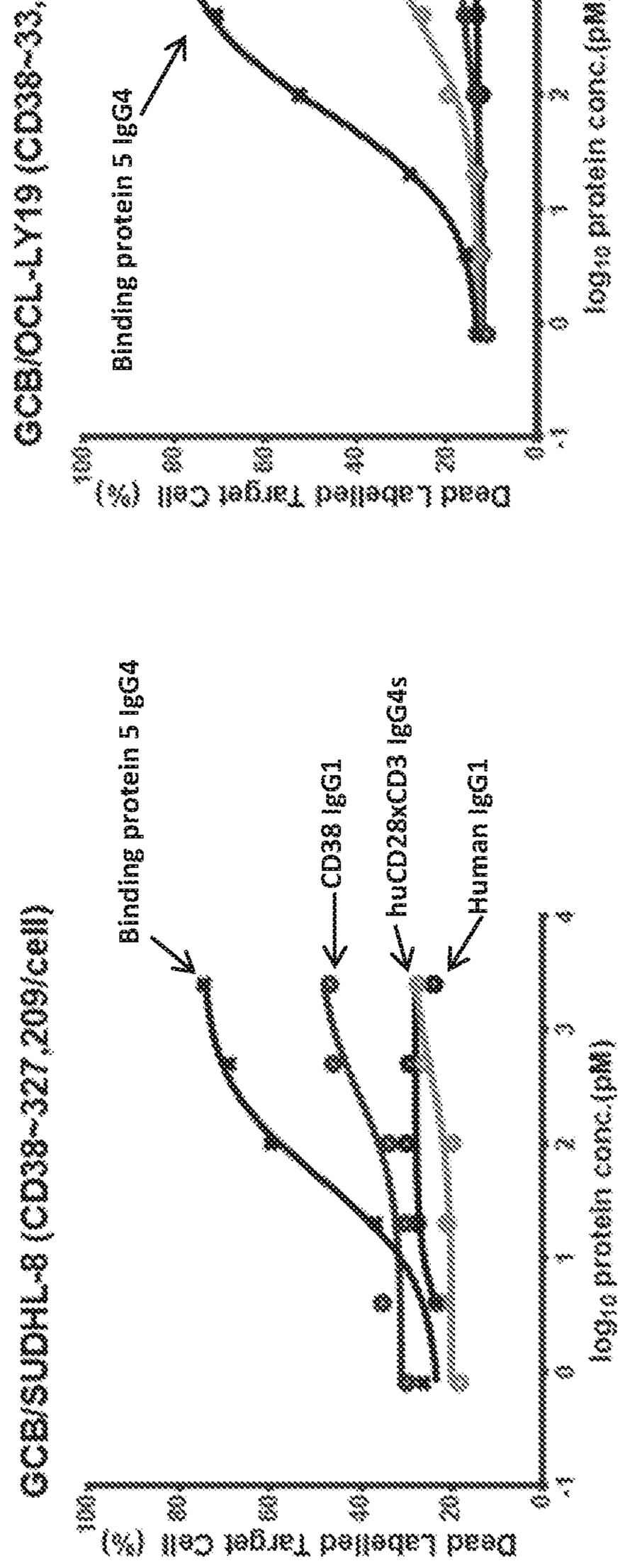


FIG. 14B

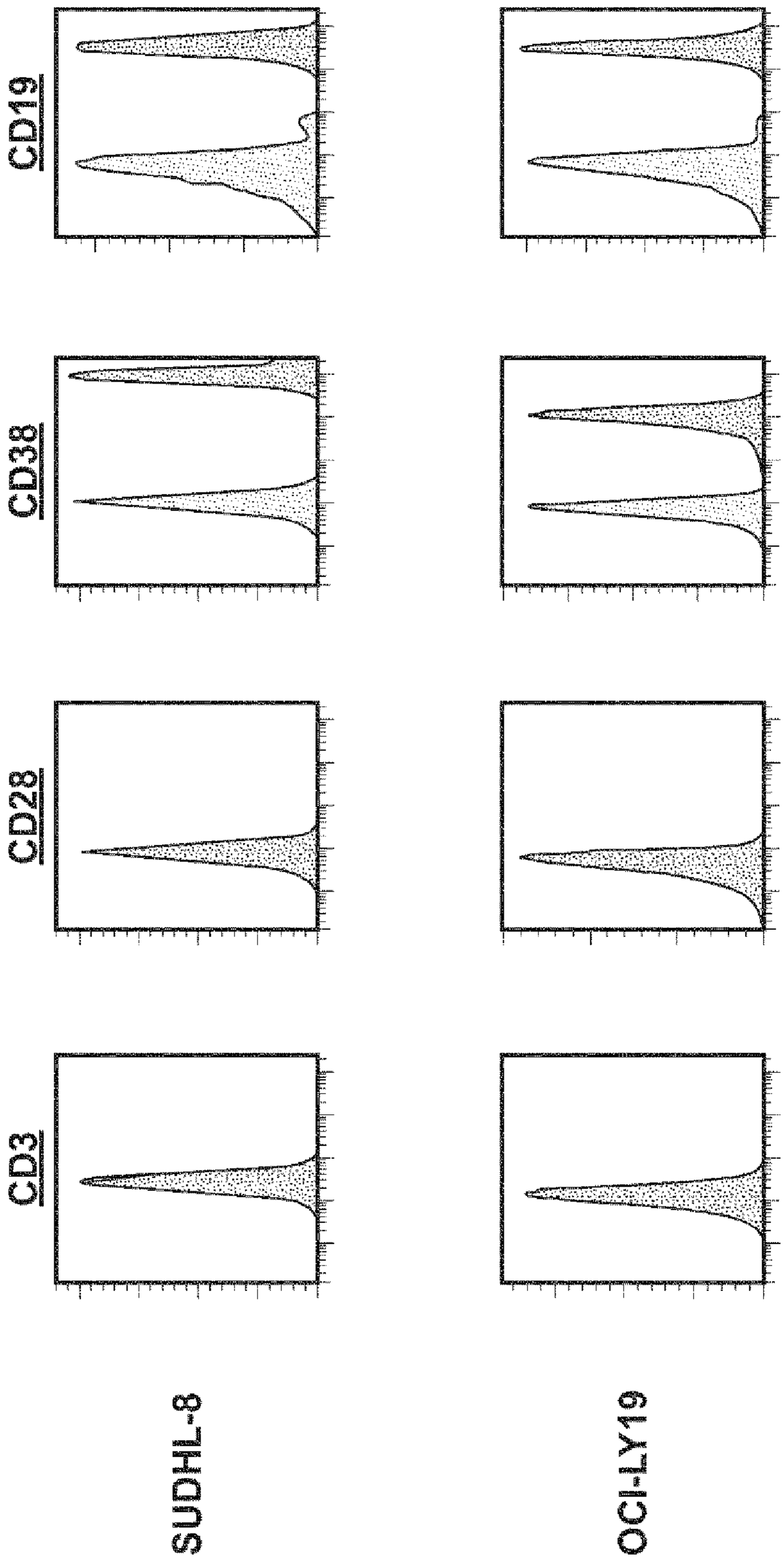


FIG. 14C

FIG. 15A

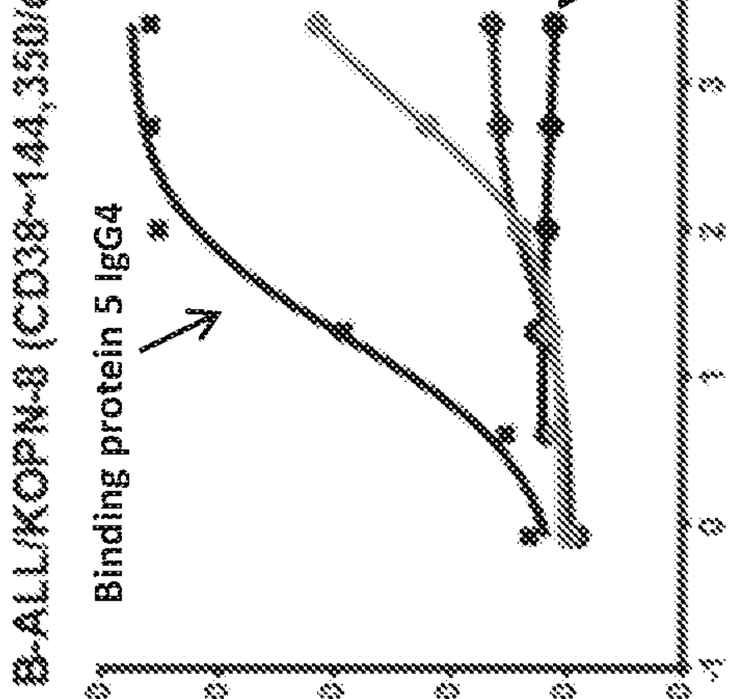


FIG. 15B

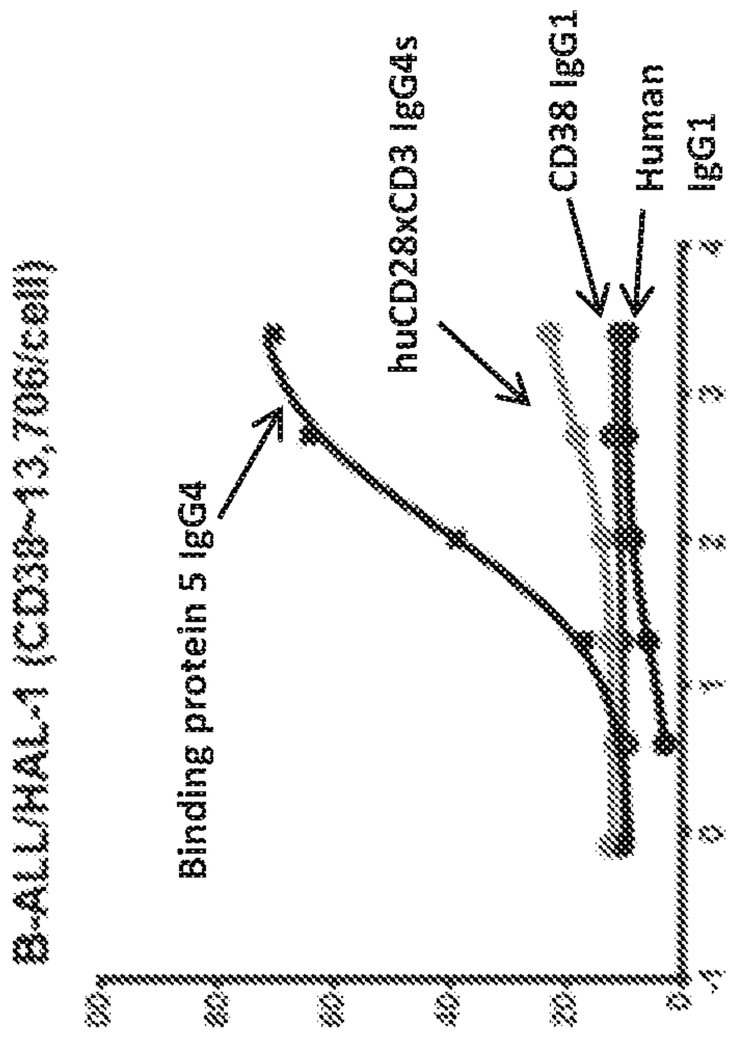
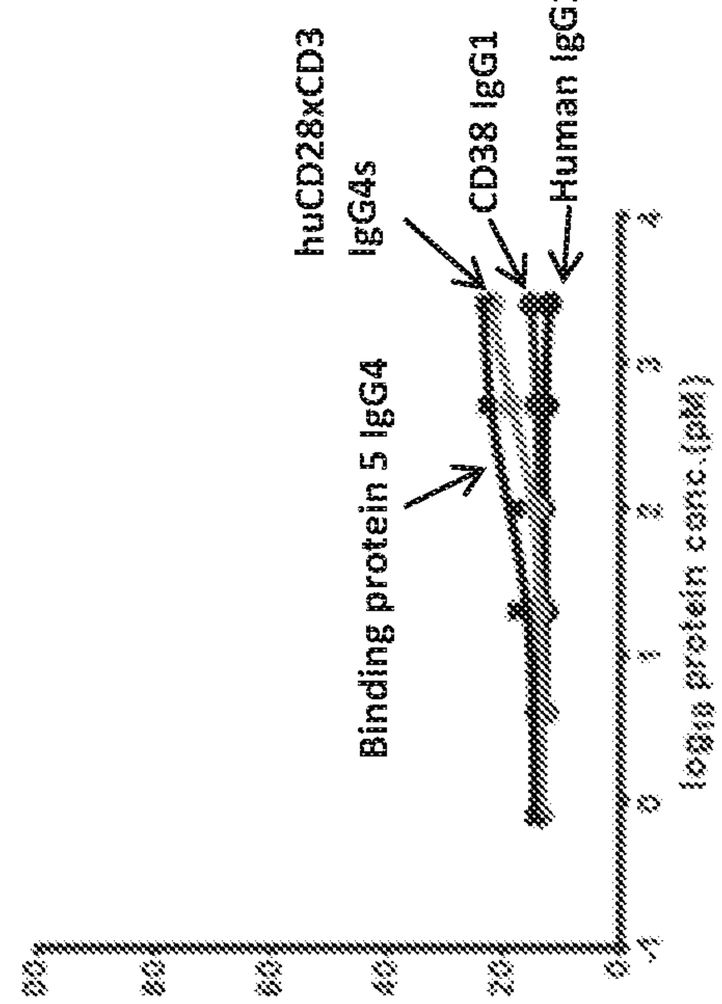


FIG. 15C



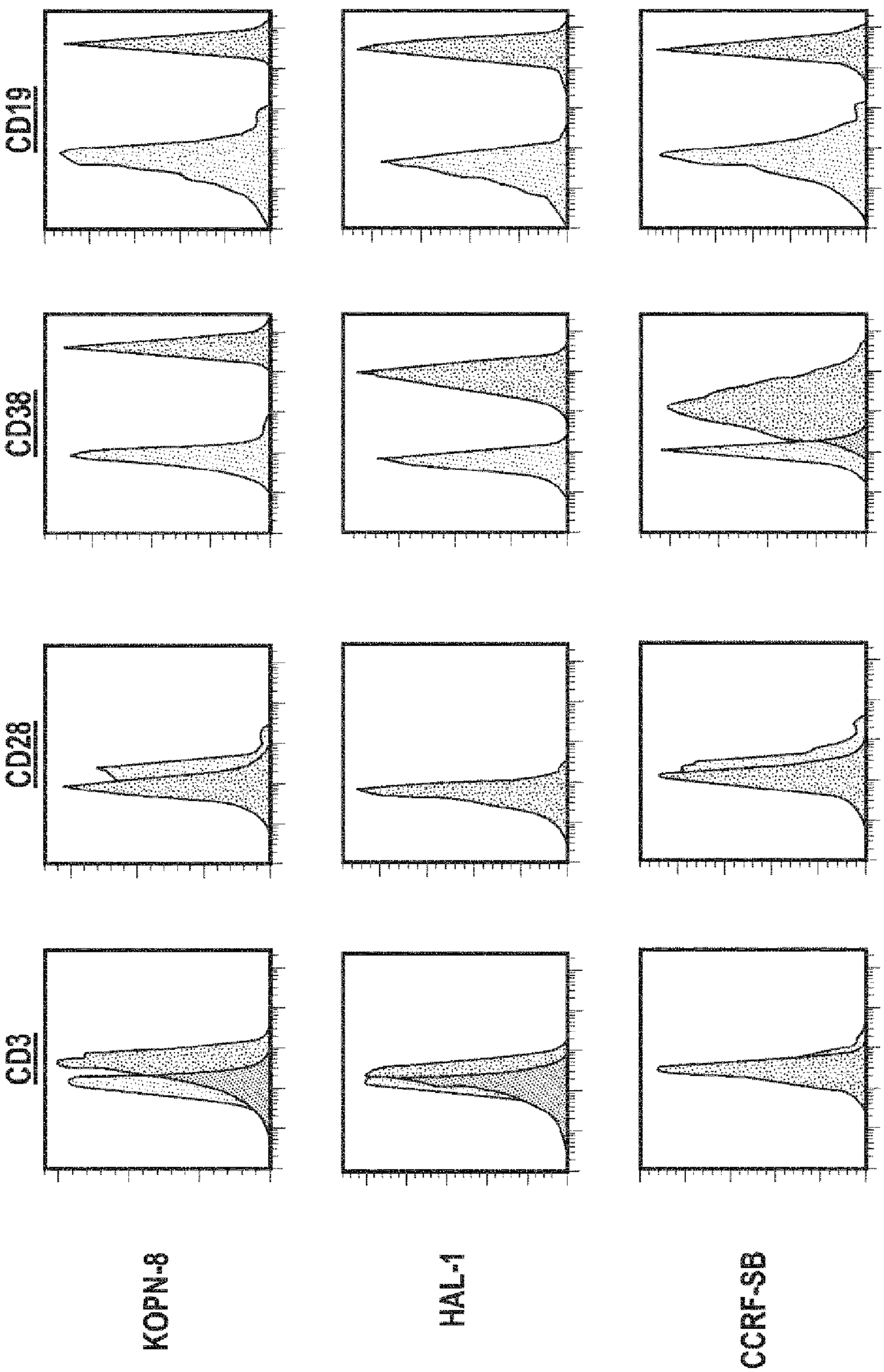


FIG. 15D

FIG. 16

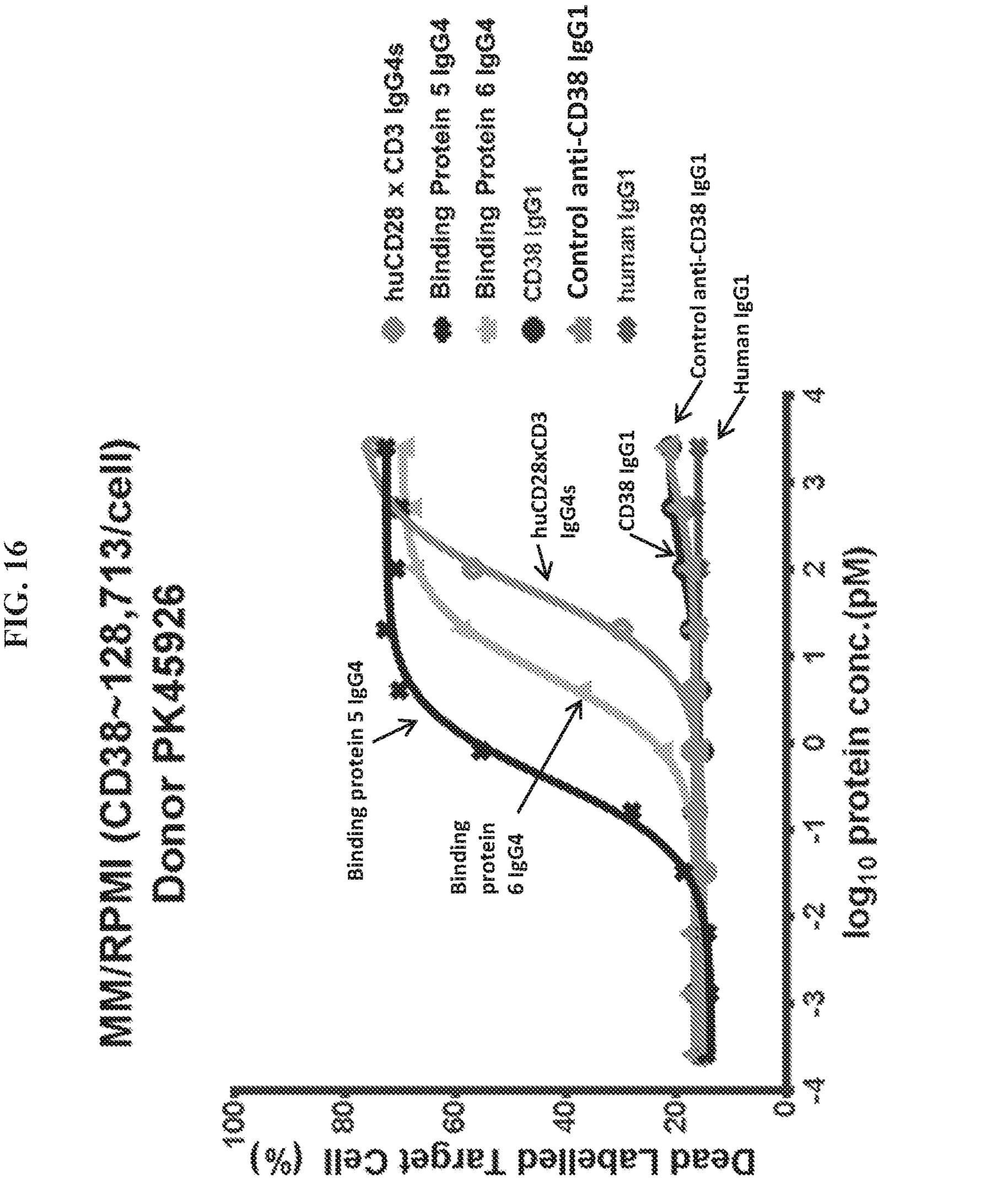

MM/RPMI (CD38~128,713/cell)
Donor PK45926
huCD28 x CD3 IgG4s
Binding Protein 5 IgG4
Binding Protein 6 IgG4
CD38 IgG1
Control anti-CD38 IgG1
human IgG1
huCD28xCD3 IgG4s
CD38 IgG1
Control anti-CD38 IgG1
Human IgG1
Binding protein 5 IgG4
Binding protein 6 IgG4
Dead Labelled Target Cell (%)
log10 protein conc.(pM)
100
80
60
40
20
0
-4
-3
-2
-1
0
1
2
3
4

FIG. 17B

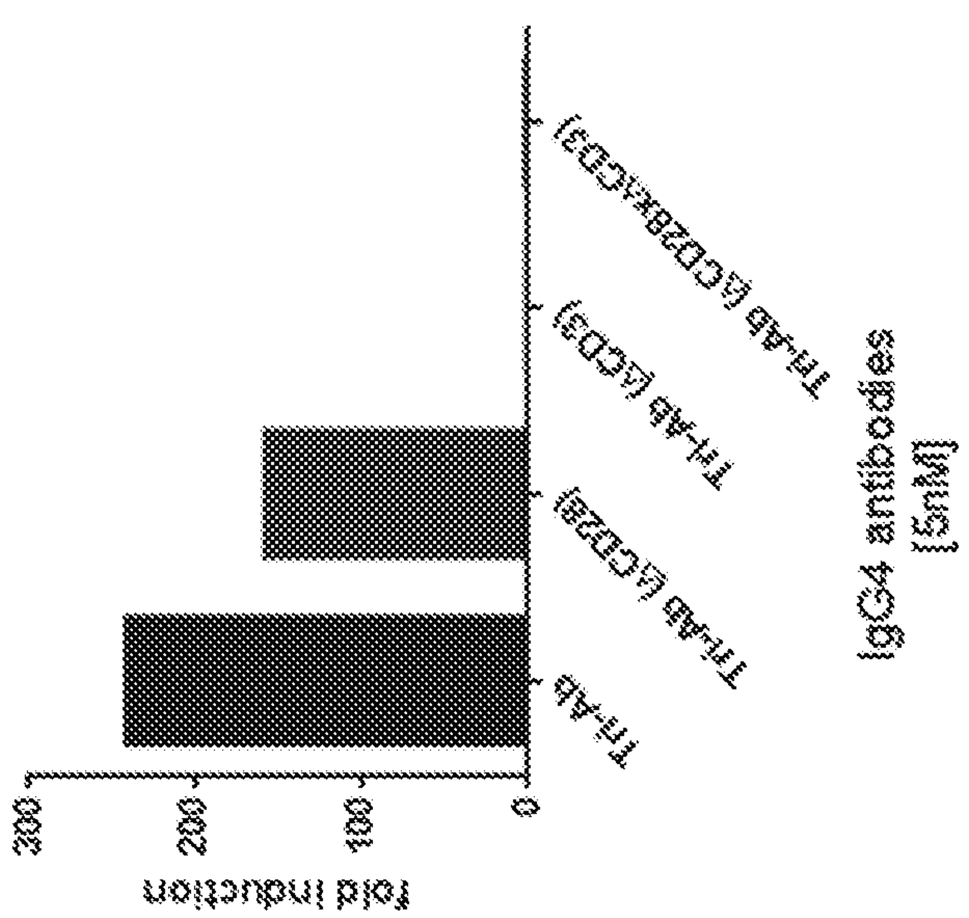


FIG. 17A

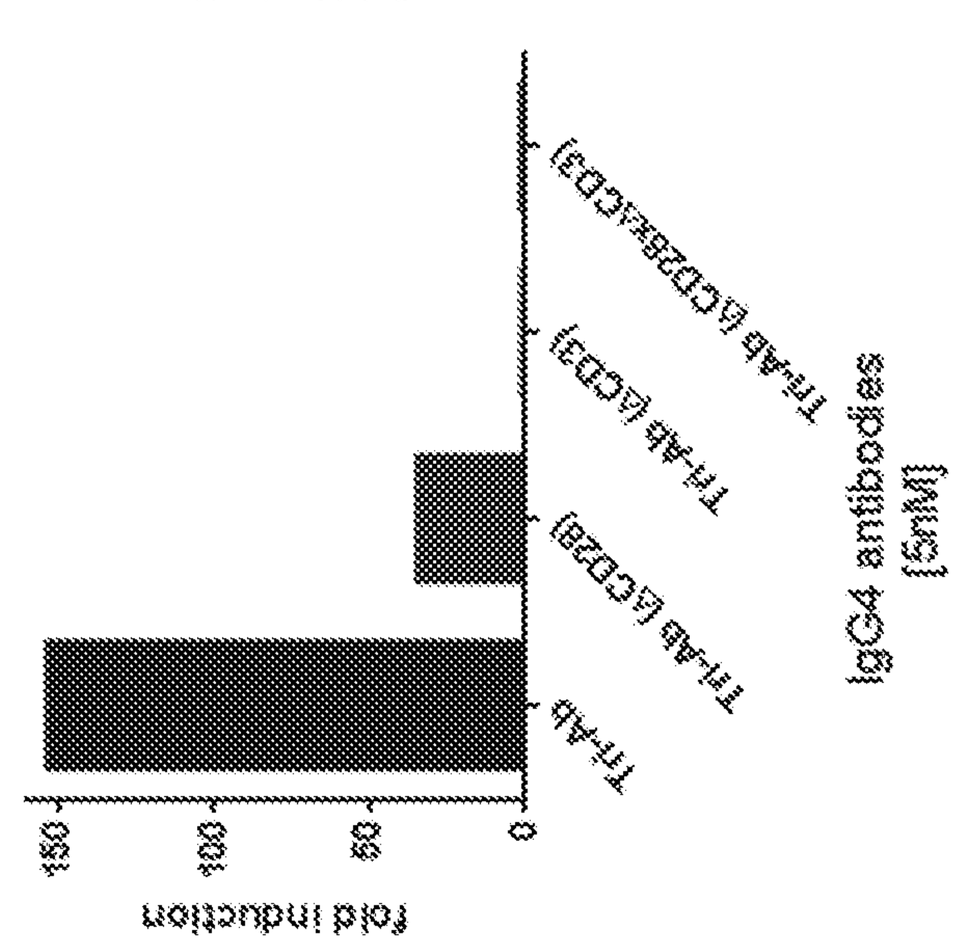


FIG. 18



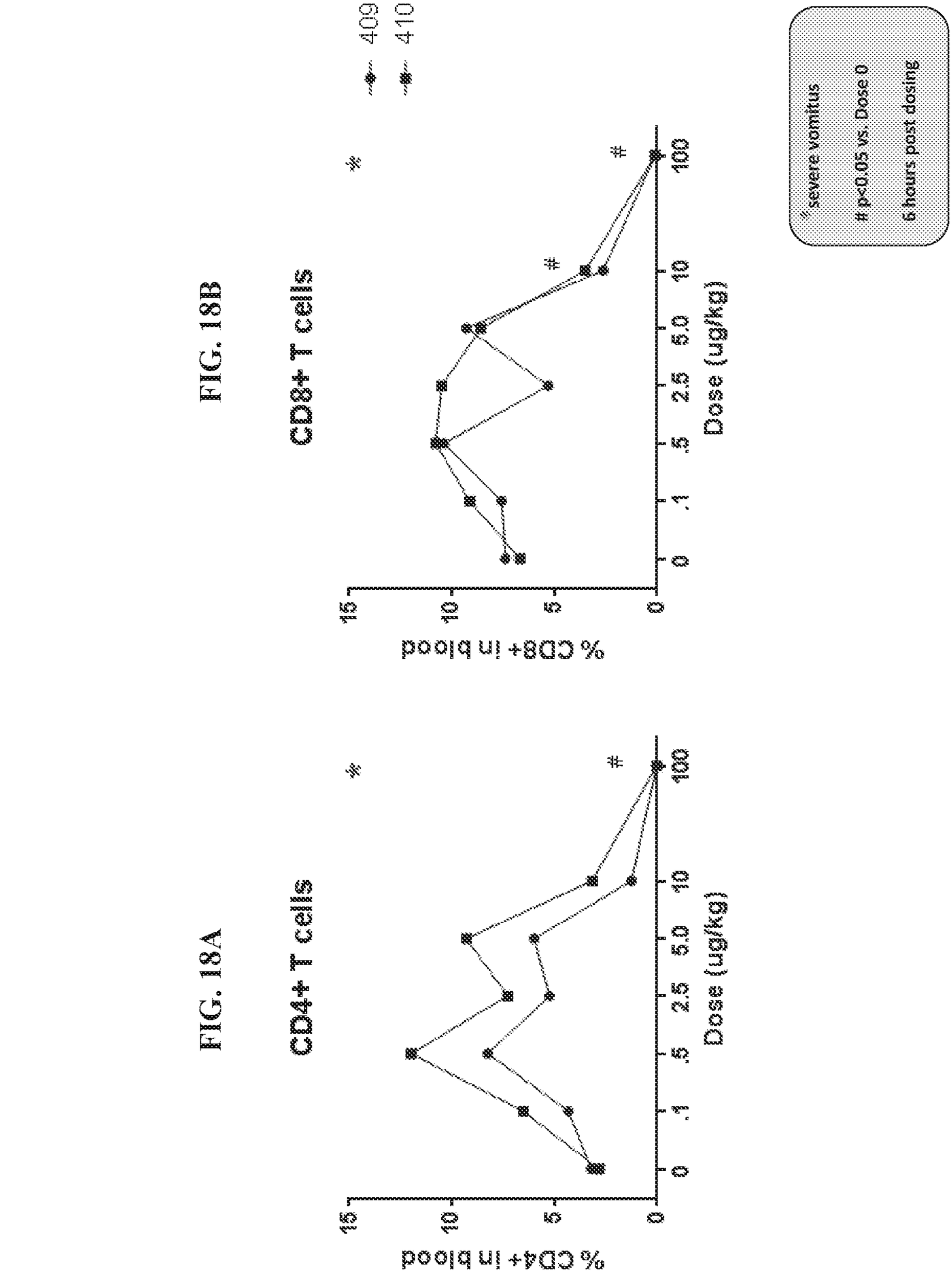


FIG. 18B

FIG. 18A

FIG. 18D

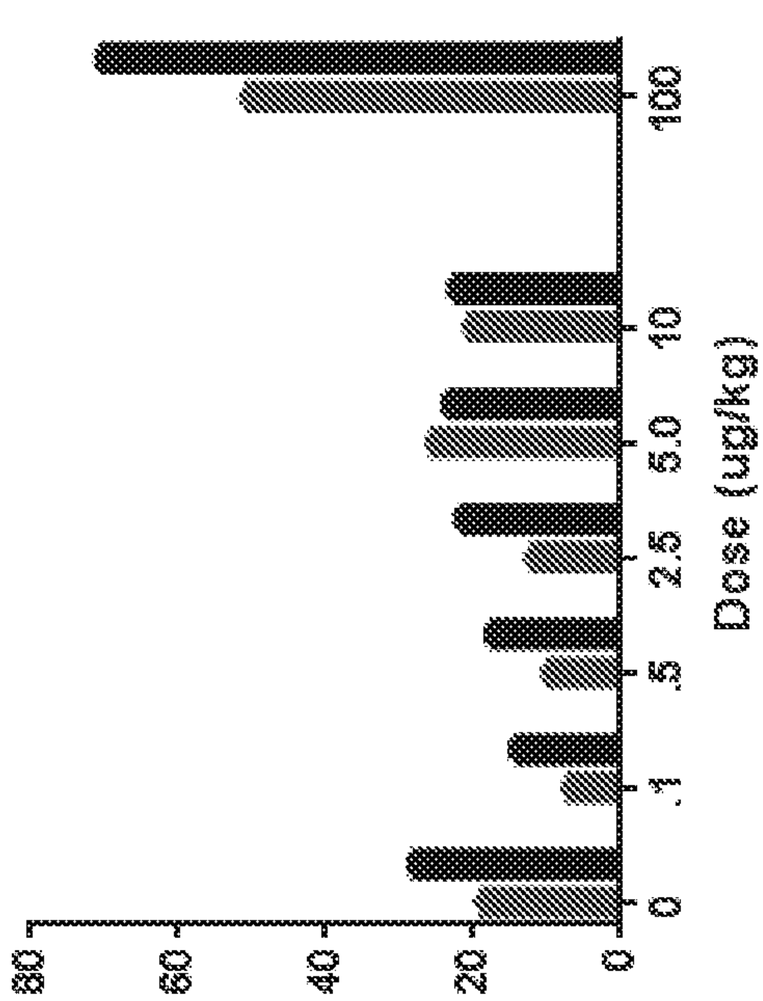
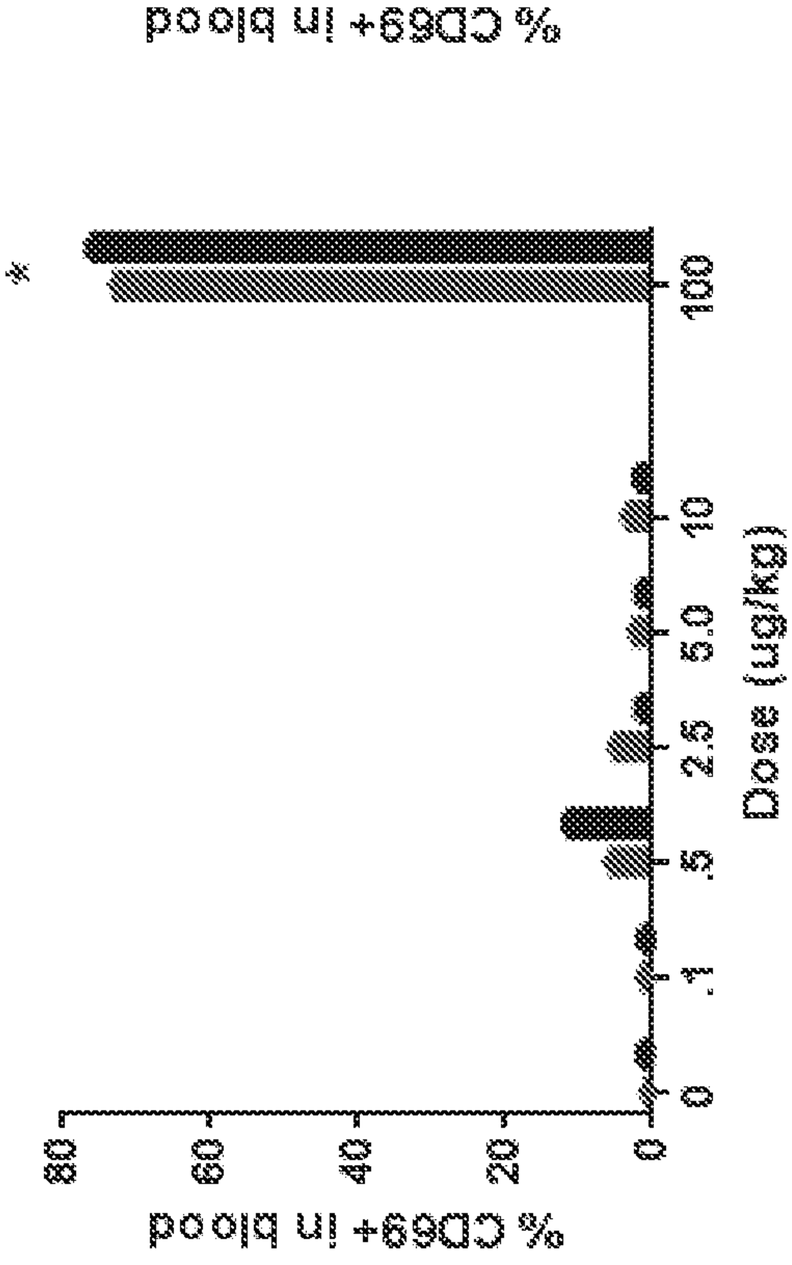


FIG. 18C



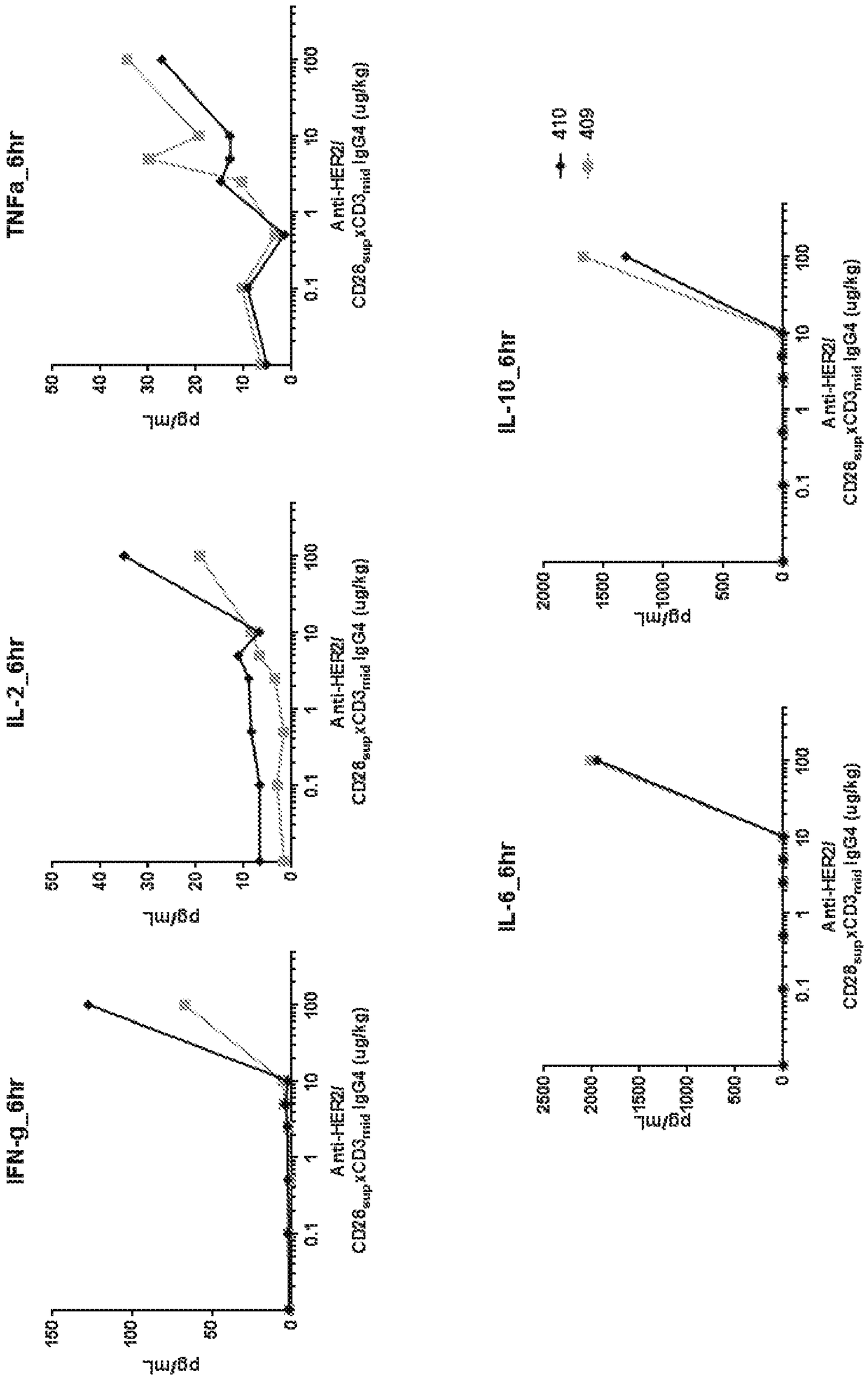

TNFa_6hr
IL-10_6hr
IL-2_6hr
IL-6_6hr
IFN-g_6hr
pg/mL
Anti-HER2/CD28$_{sup}$xCD3$_{mid}$ IgG4 (ug/kg)
410
409

FIG. 18E

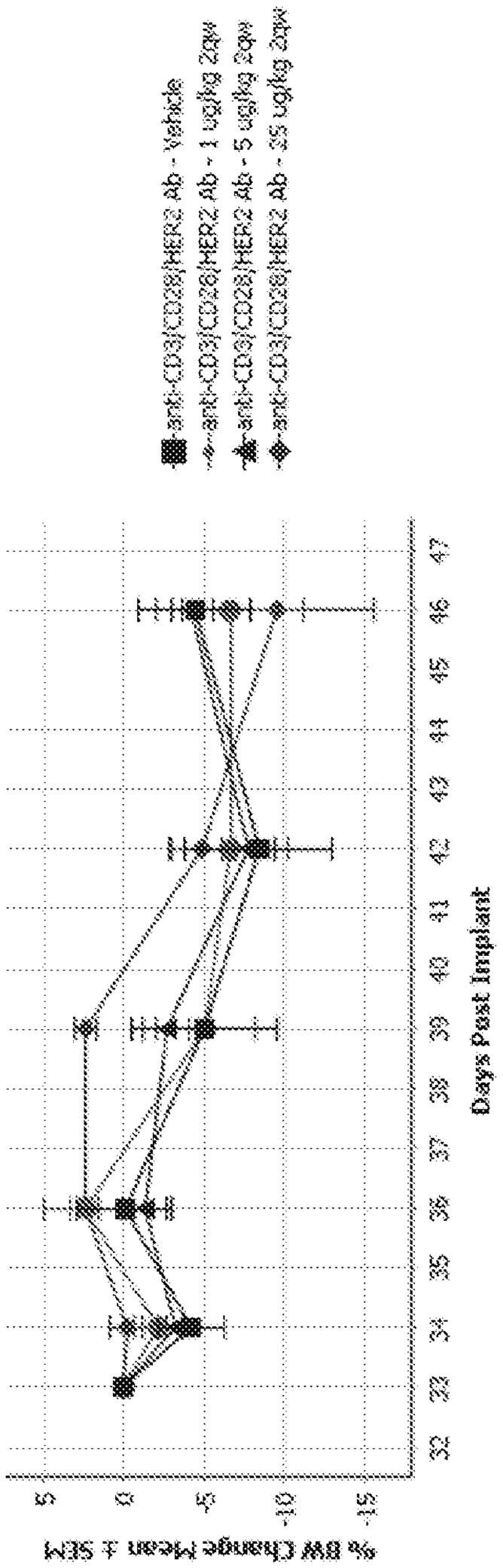


FIG. 19A

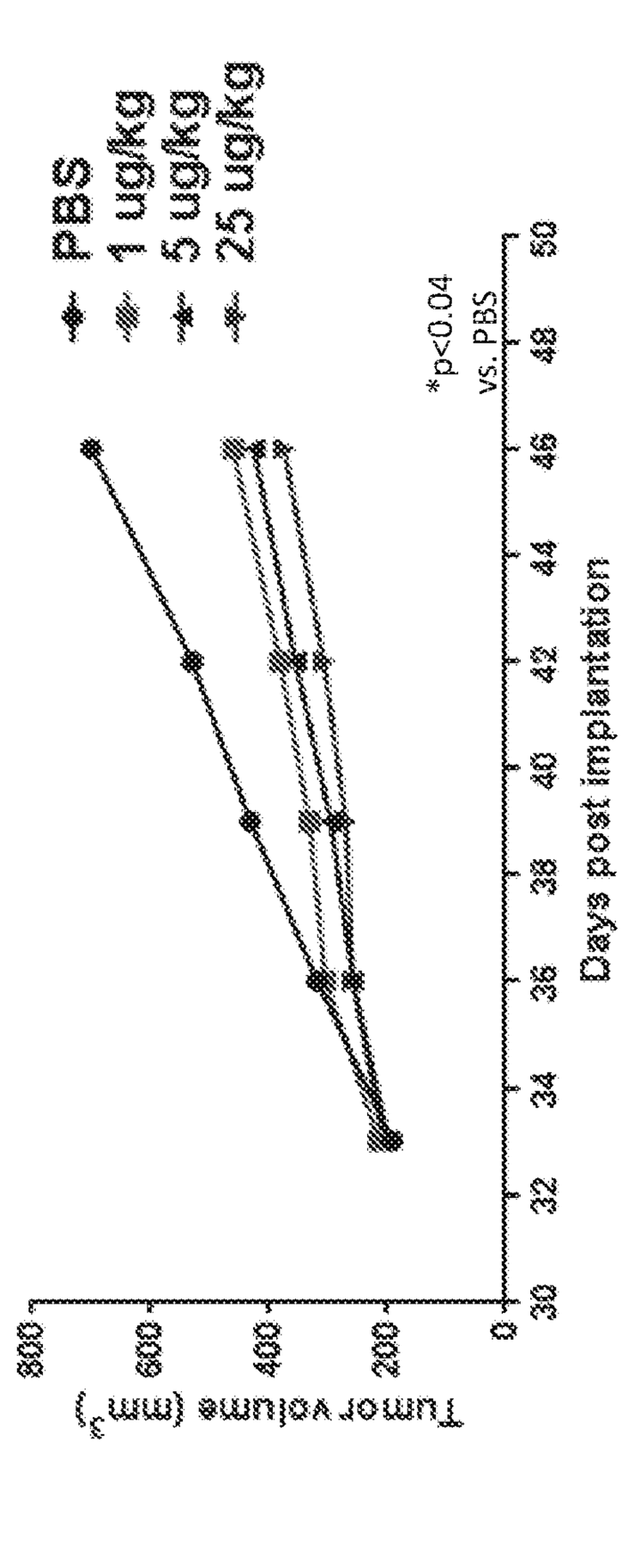
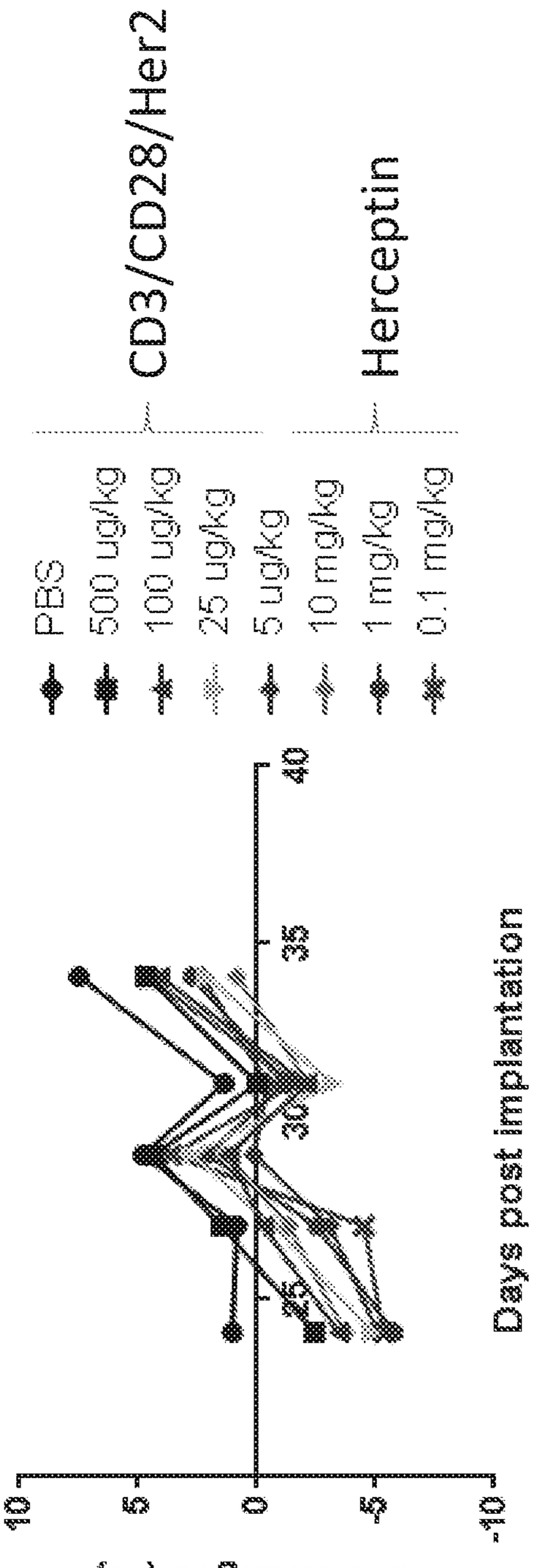


FIG. 19B

FIG. 20A


BW
Days post implantation
BW changes (%)
25
30
35
40
-20
-15
-10
-5
0
5
PBS
500 ug/kg
100 ug/kg
25 ug/kg
5 ug/kg
10 mg/kg
1 mg/kg
0.1 mg/kg
CD3/CD28/Her2
Herceptin

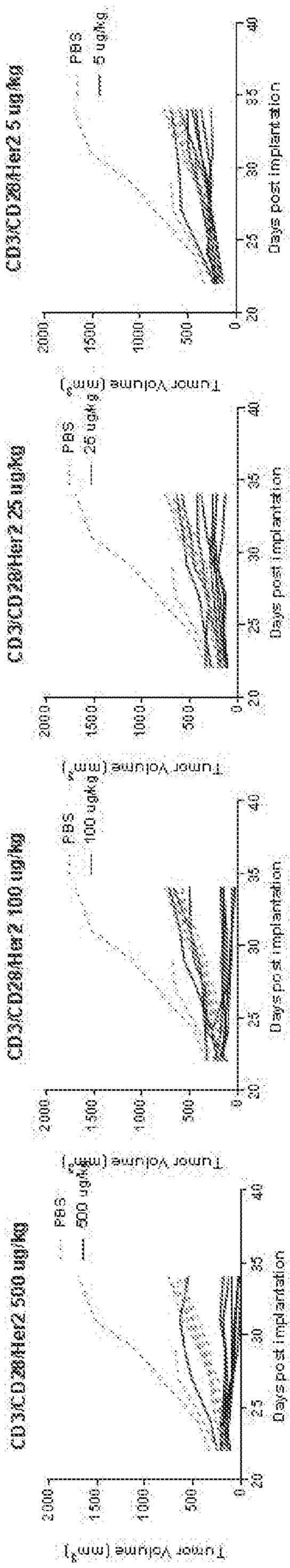

CD3xCD28xHer2 5 ug/kg
PBS
5 ug/kg
Tumor Volume (mm³)
Days post implantation
CD3xCD28xHer2 25 ug/kg
PBS
25 ug/kg
Tumor Volume (mm³)
Days post implantation
CD3xCD28xHer2 100 ug/kg
PBS
100 ug/kg
Tumor Volume (mm³)
Days post implantation
CD3xCD28xHer2 500 ug/kg
PBS
500 ug/kg
Tumor Volume (mm³)
Days post implantation
Herceptin 0.1 mg/kg
PBS
0.1 mg/kg
Tumor Volume (mm³)
Days post implantation
Herceptin 1 mg/kg
PBS
1 mg/kg
Tumor Volume (mm³)
Days post implantation
Herceptin 10 mg/kg
PBS
10 mg/kg
Tumor Volume (mm³)
Days post implantation

FIG. 20B

FIG. 20C

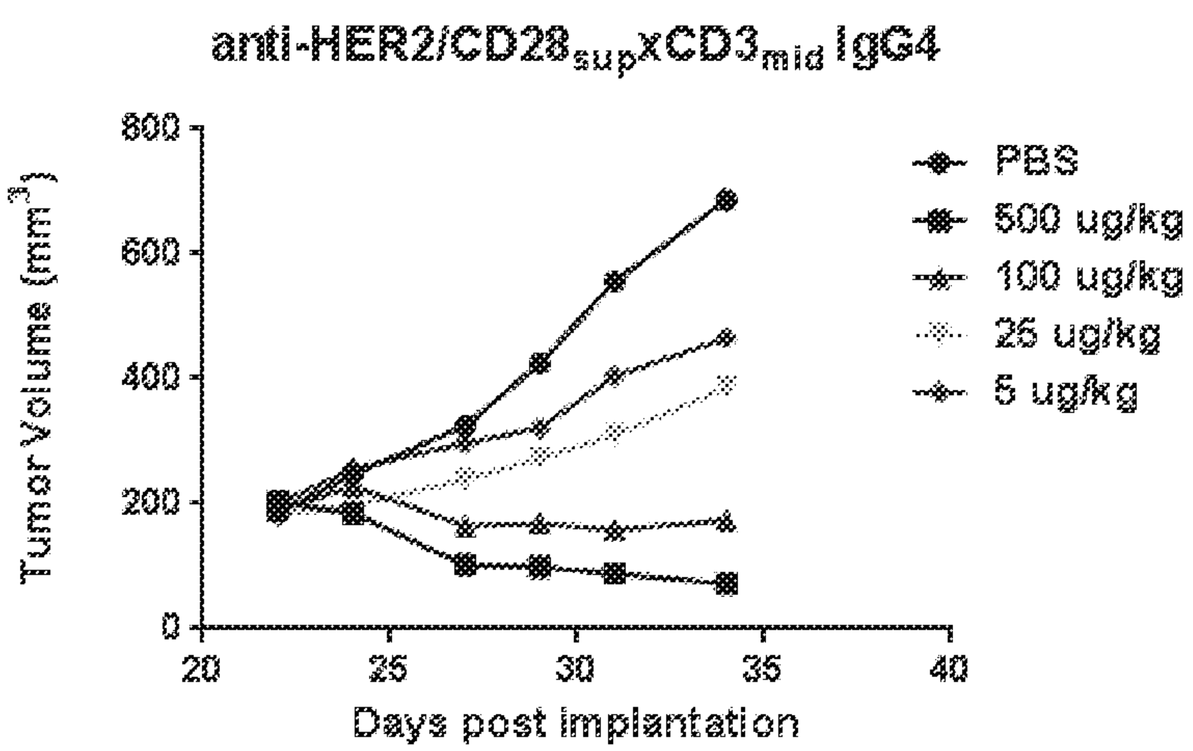


FIG. 20D

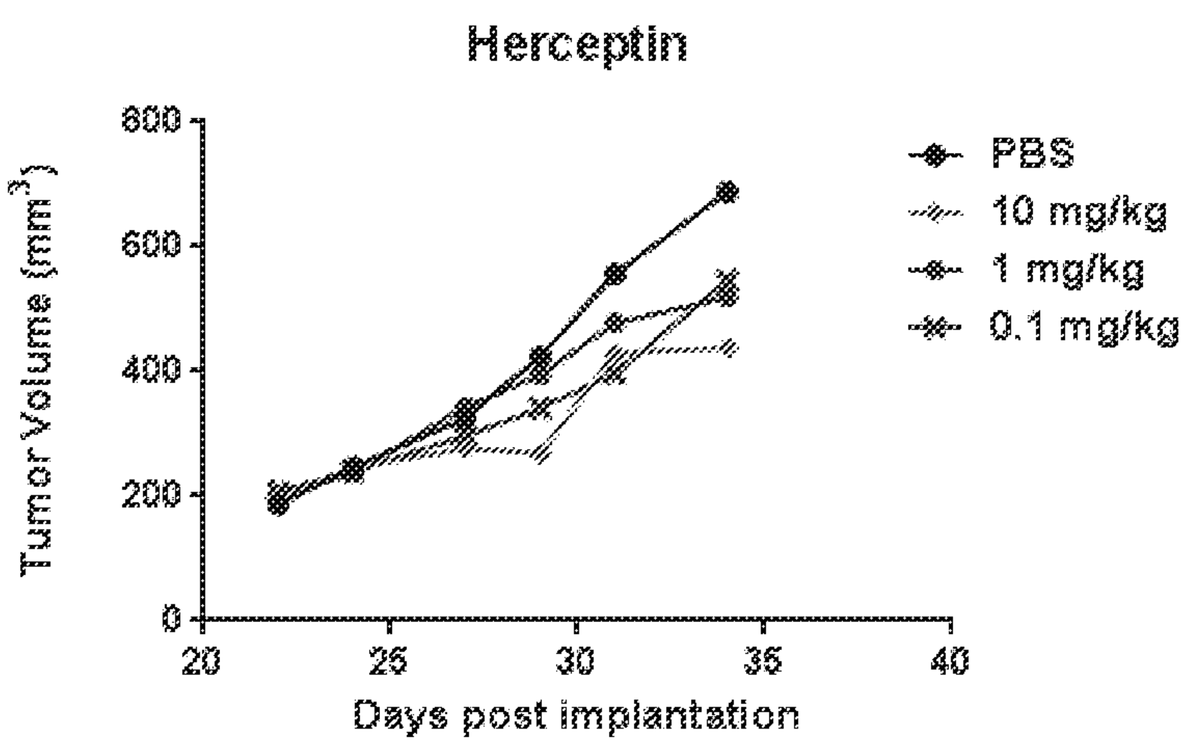


FIG. 20E

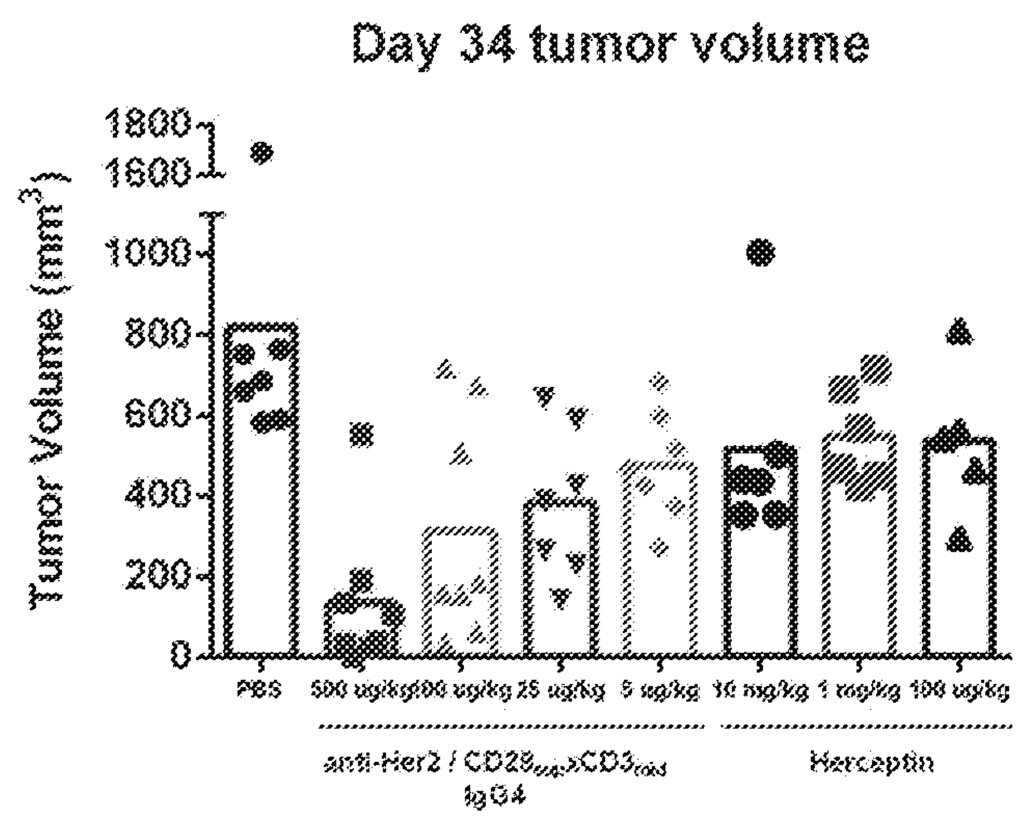
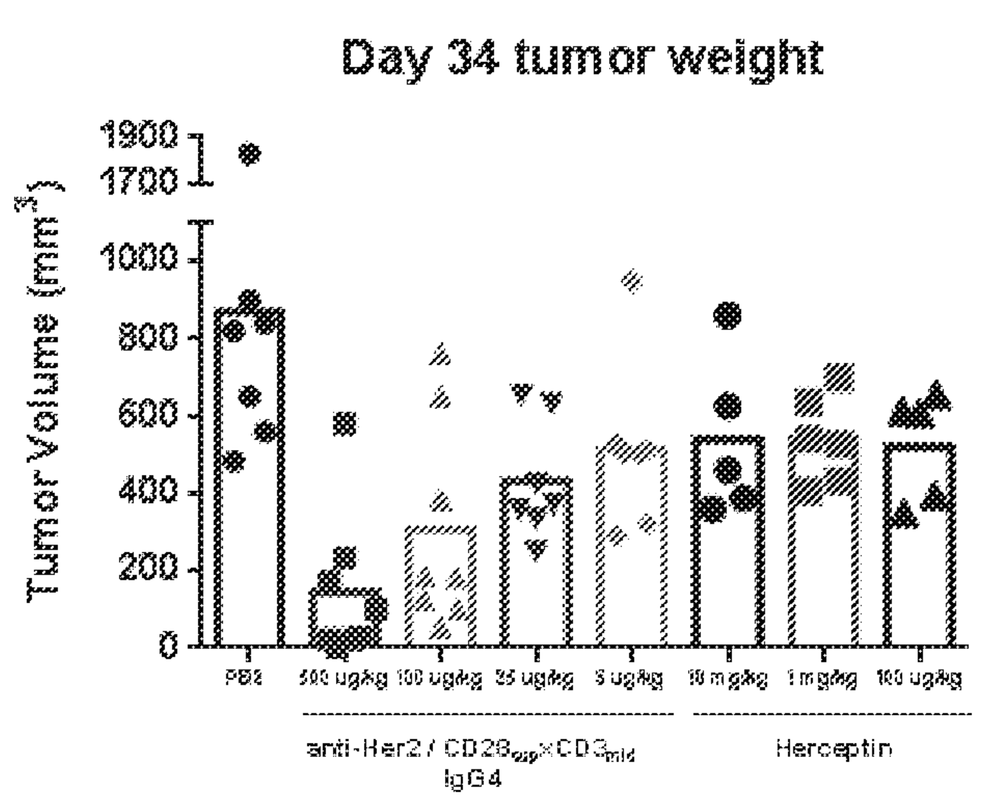


FIG. 20F



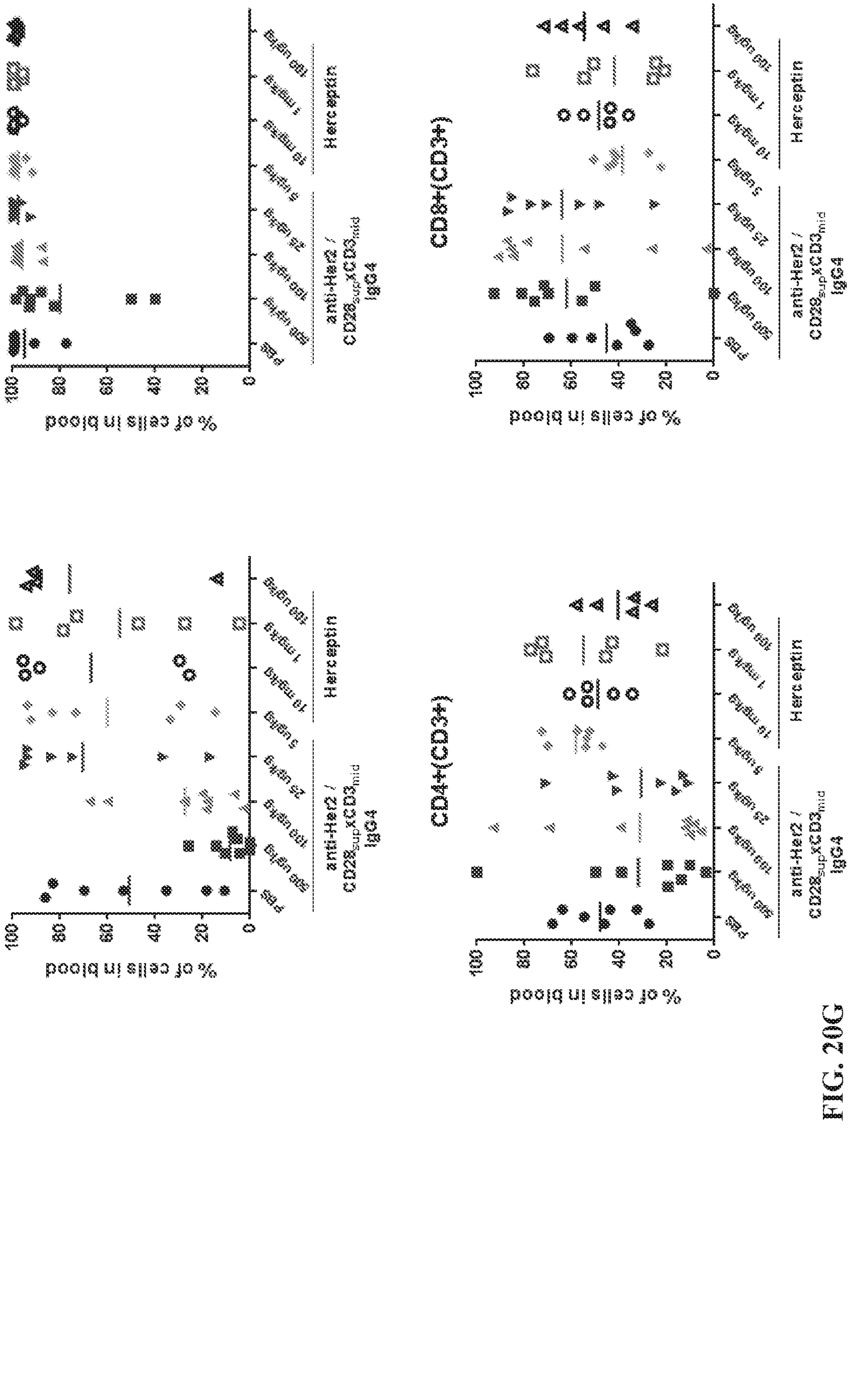

CD3+(hCD45+)
% of cells in blood
100
80
60
40
20
0
epcam cdh1
1 megDa
10 megDa
5 megDa
2 megDa
1 megDa
500 kDa
250 kDa
150 kDa
anti-Her2 / CD28supxCD3mid IgG4
Herceptin
CD8+(CD3+)
CD4+(CD3+)
human CD45

FIG. 20G

CD3+(hCD45+)
% of cells in spleen
100
80
60
40
20
0
Herceptin
anti-Her2 / CD28supxCD3mid IgG4
CD8+(CD3+)
CD4+(CD3+)
human CD45

FIG. 20H

FIG. 21A

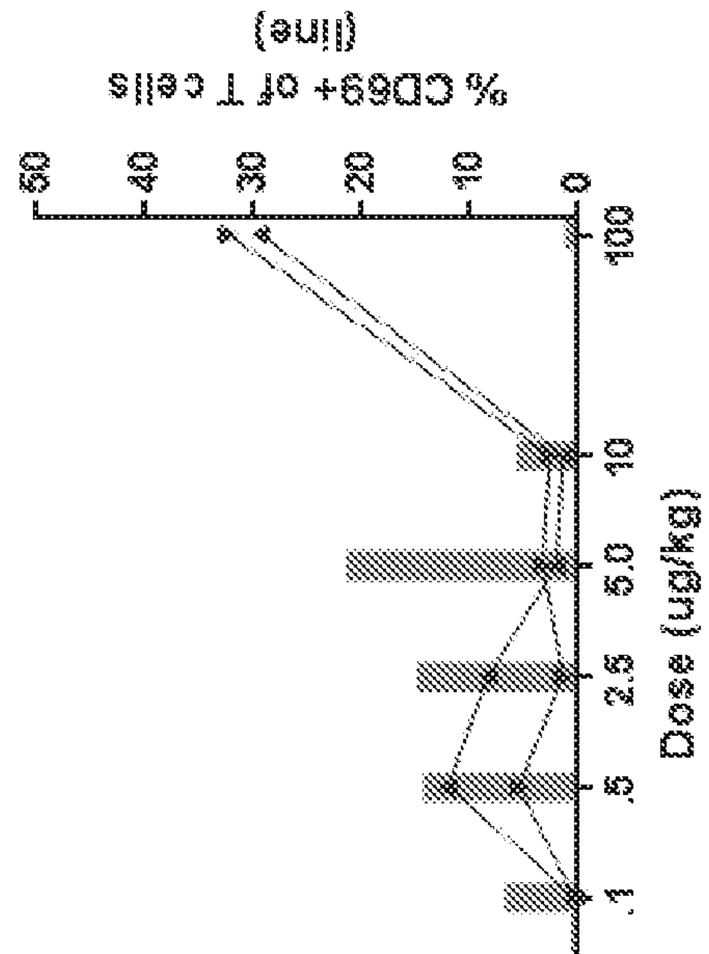
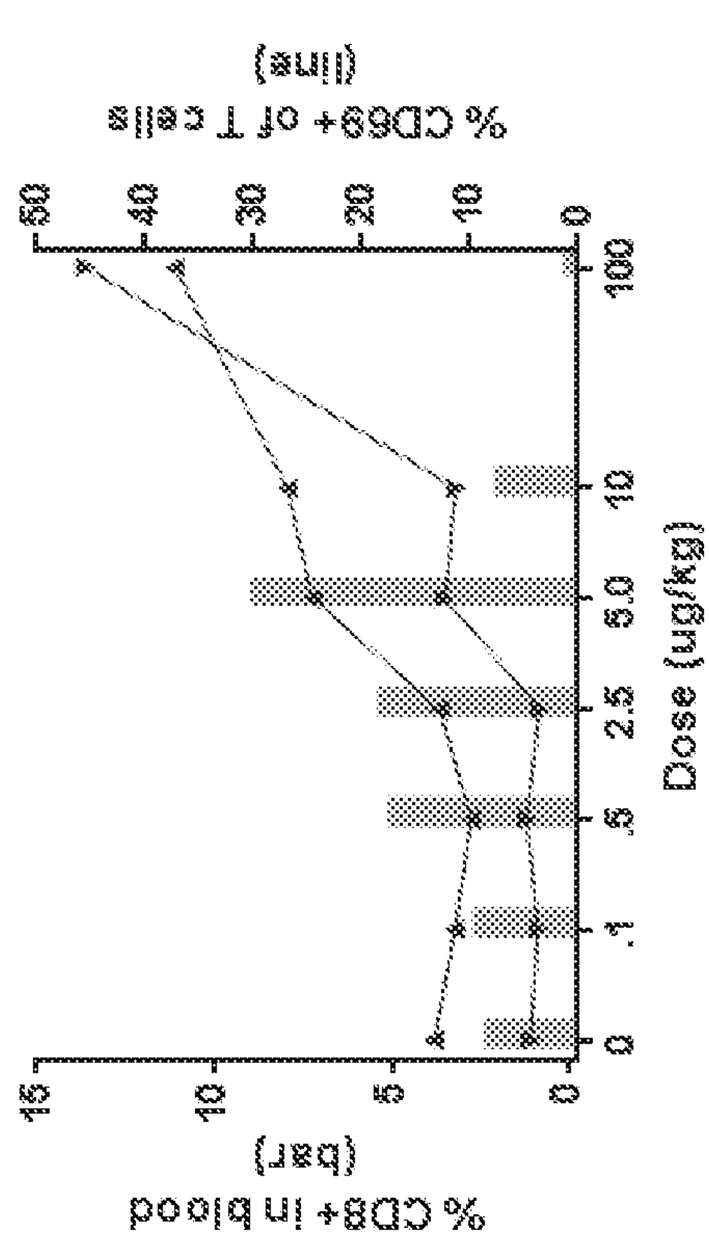


FIG. 21B



"Serious adverse event (vomiting). Animals recovered 24 hrs later.

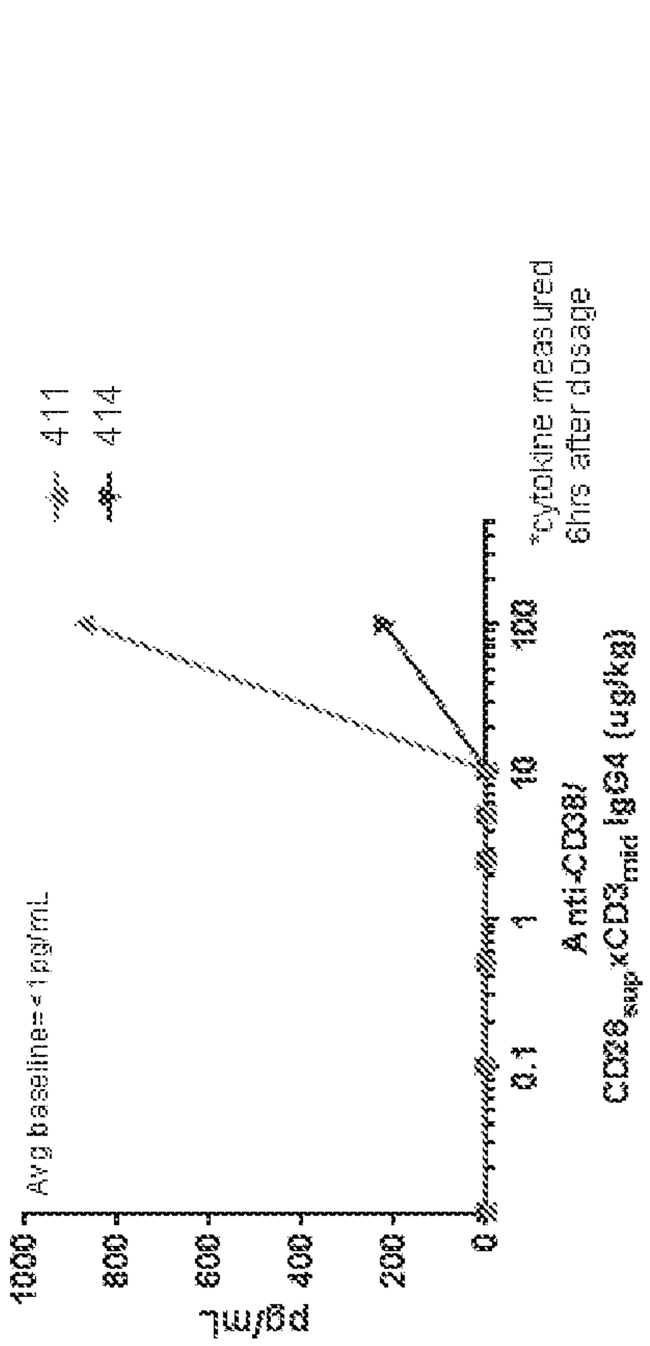


FIG. 21C

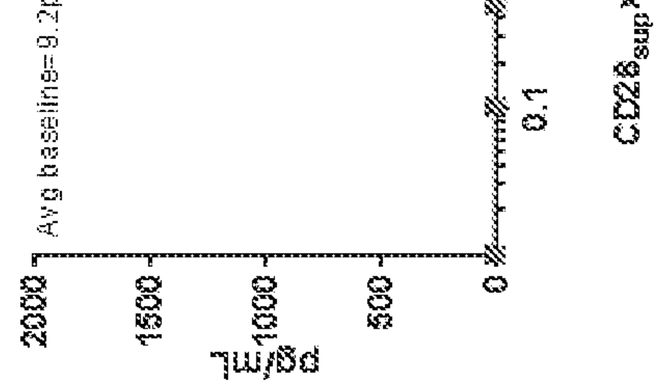


FIG. 21D

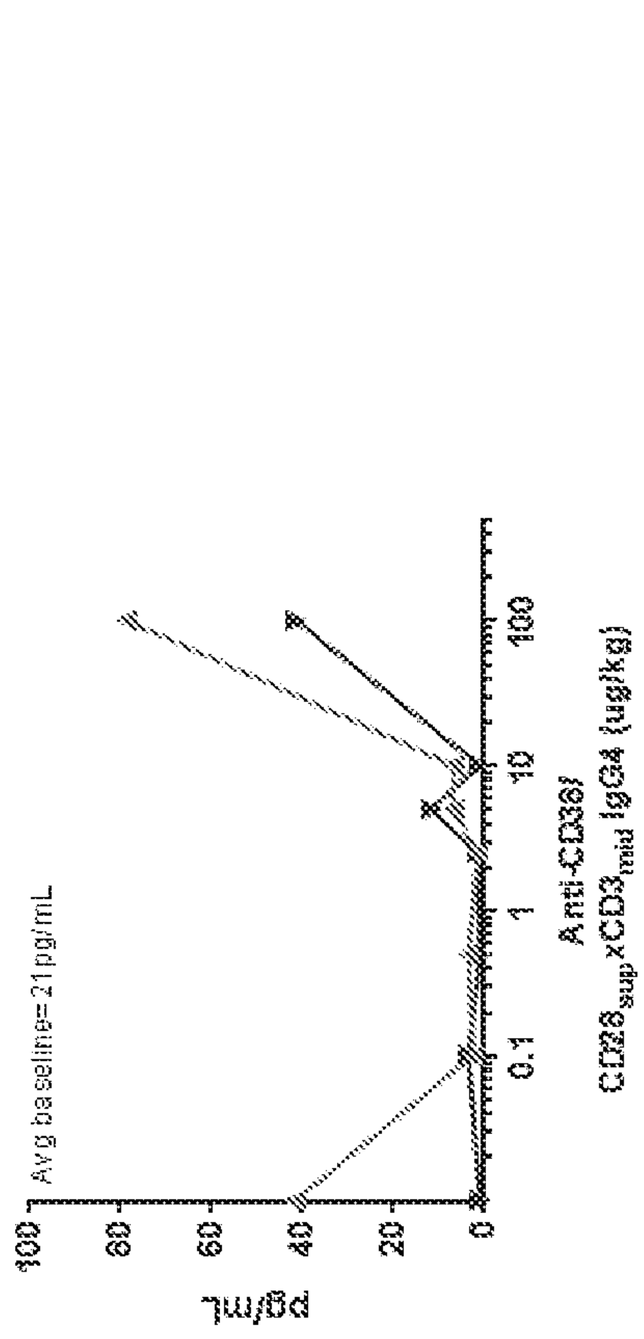


FIG. 21E

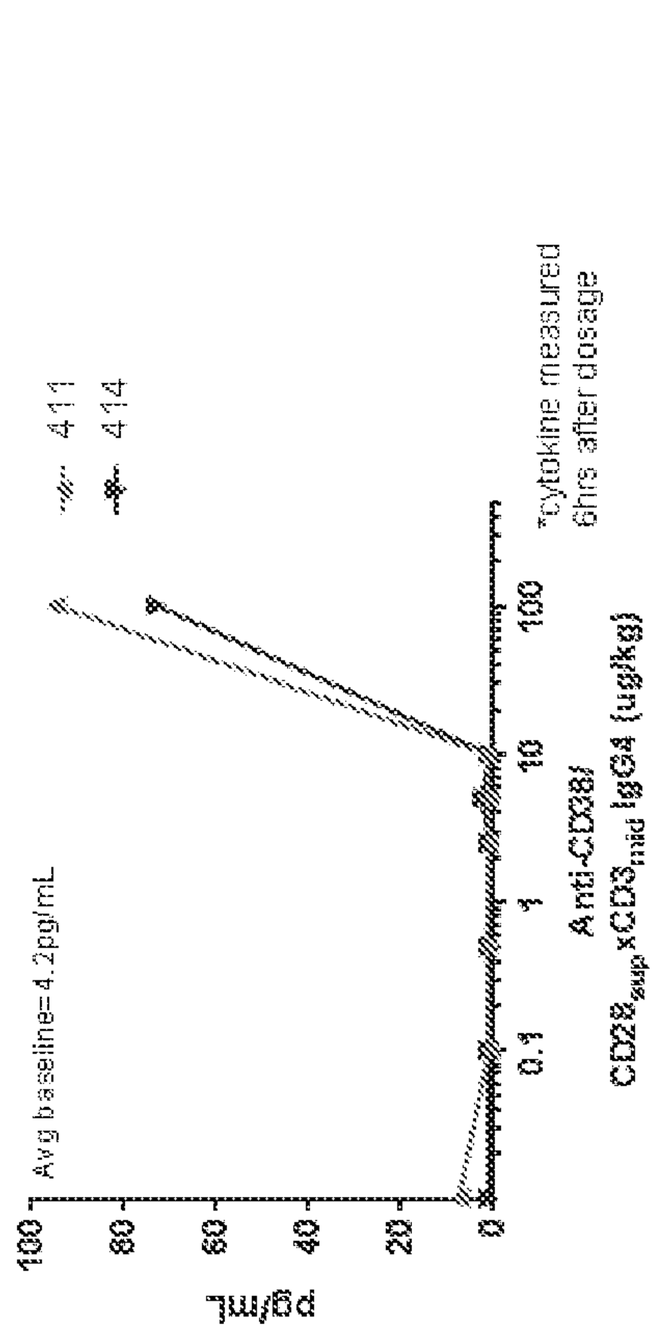
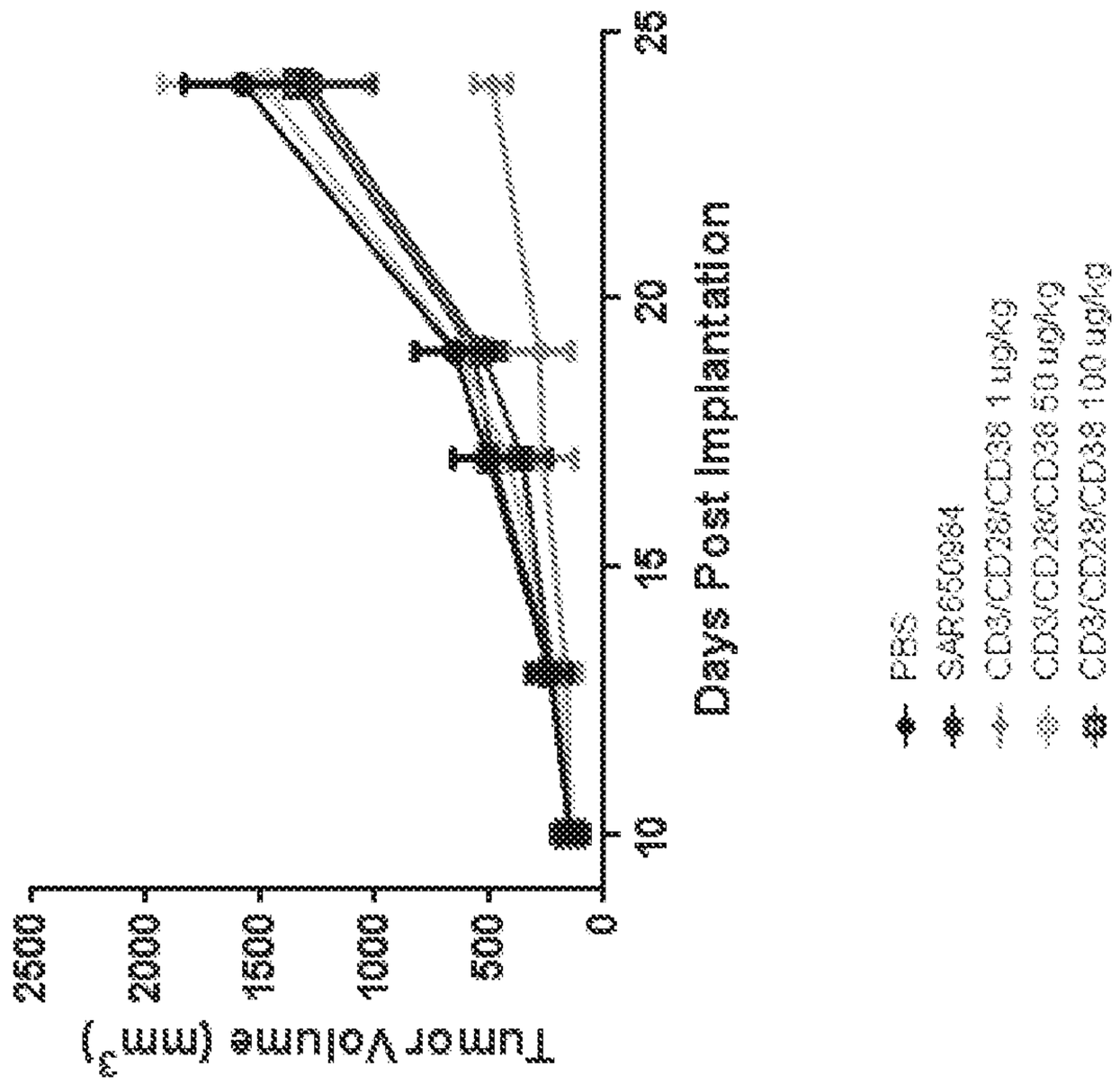


FIG. 21F

FIG. 22A


Tumor Volume (mm^3)
2500
2000
1500
1000
500
0
5
10
15
20
25
Days Post Implantation
PBS
SAR650984
CD3/CD28/CD38 1 ug/kg
CD3/CD28/CD38 50 ug/kg
CD3/CD28/CD38 100 ug/kg

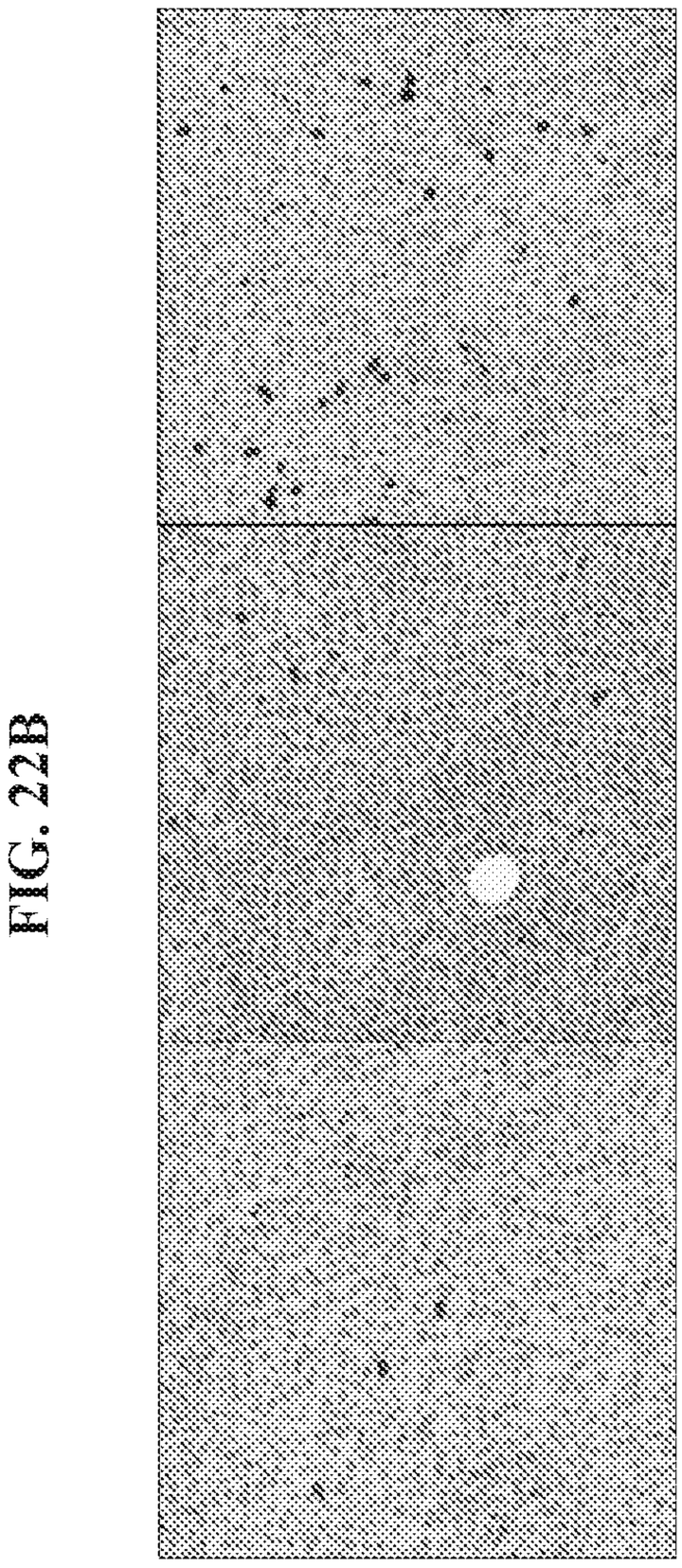


FIG. 22B

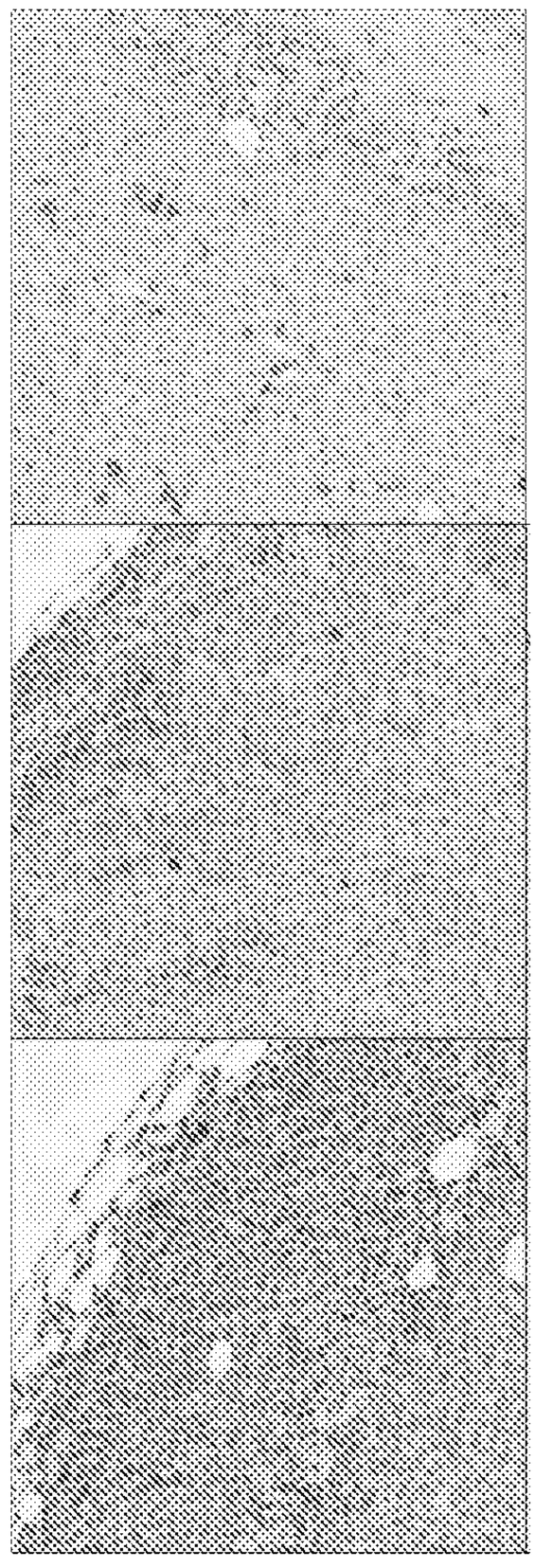


FIG. 22C

FIG. 23A

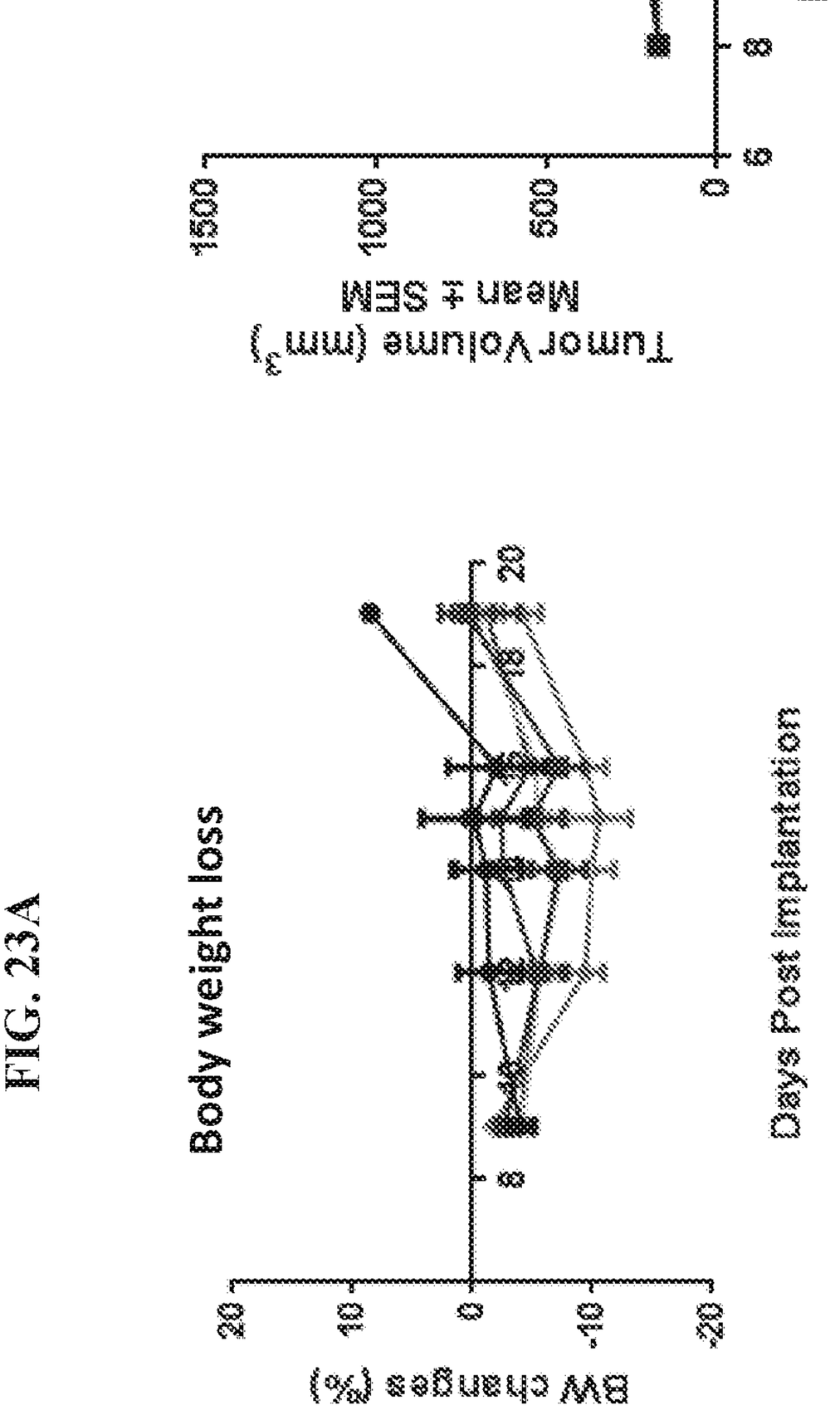
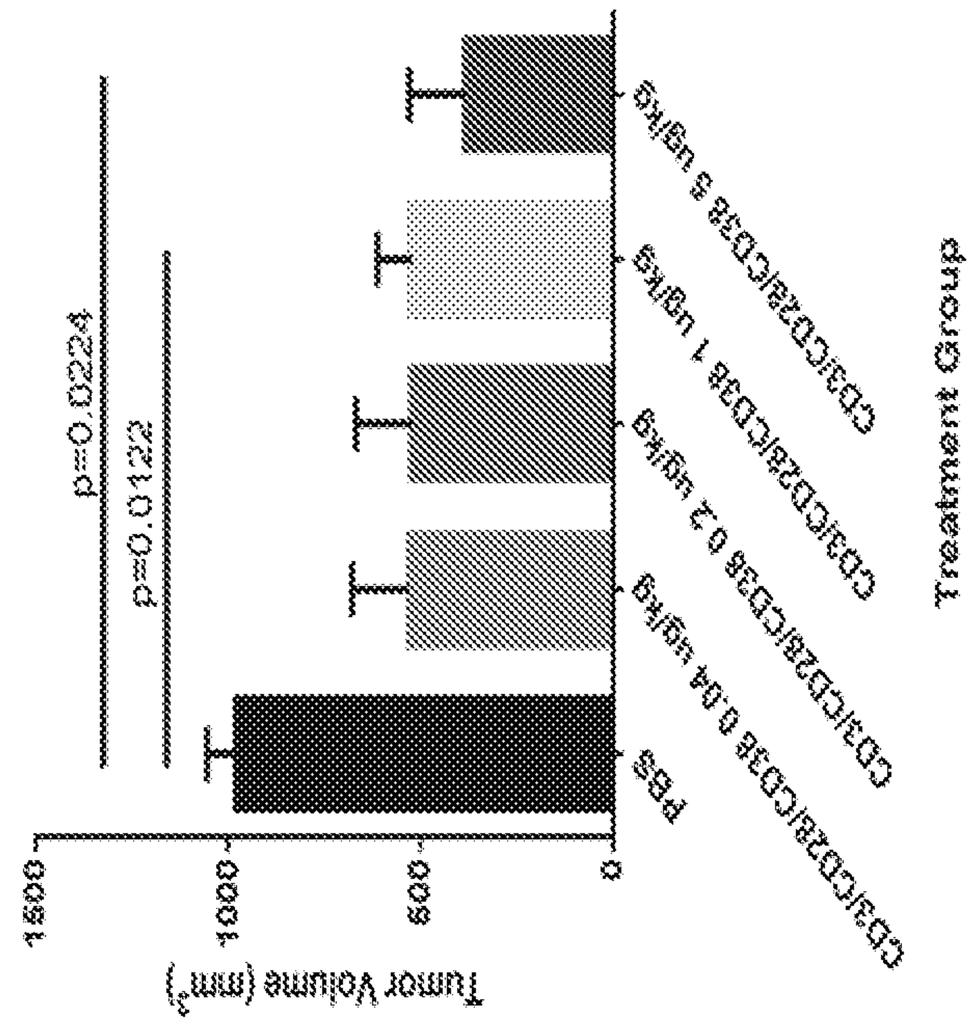


FIG. 23B

FIG. 23C


Day19_ALLGroup
p=0.0224
p=0.0122
p=0.0
Tumor Volume (mm³)
1500
1000
500
0
PBS
CD3/CD28/CD3ε 0.04 ug/kg
CD3/CD28/CD3ε 0.2 ug/kg
CD3/CD28/CD3ε 1 ug/kg
CD3/CD28/CD3ε 5 ug/kg
Treatment Group

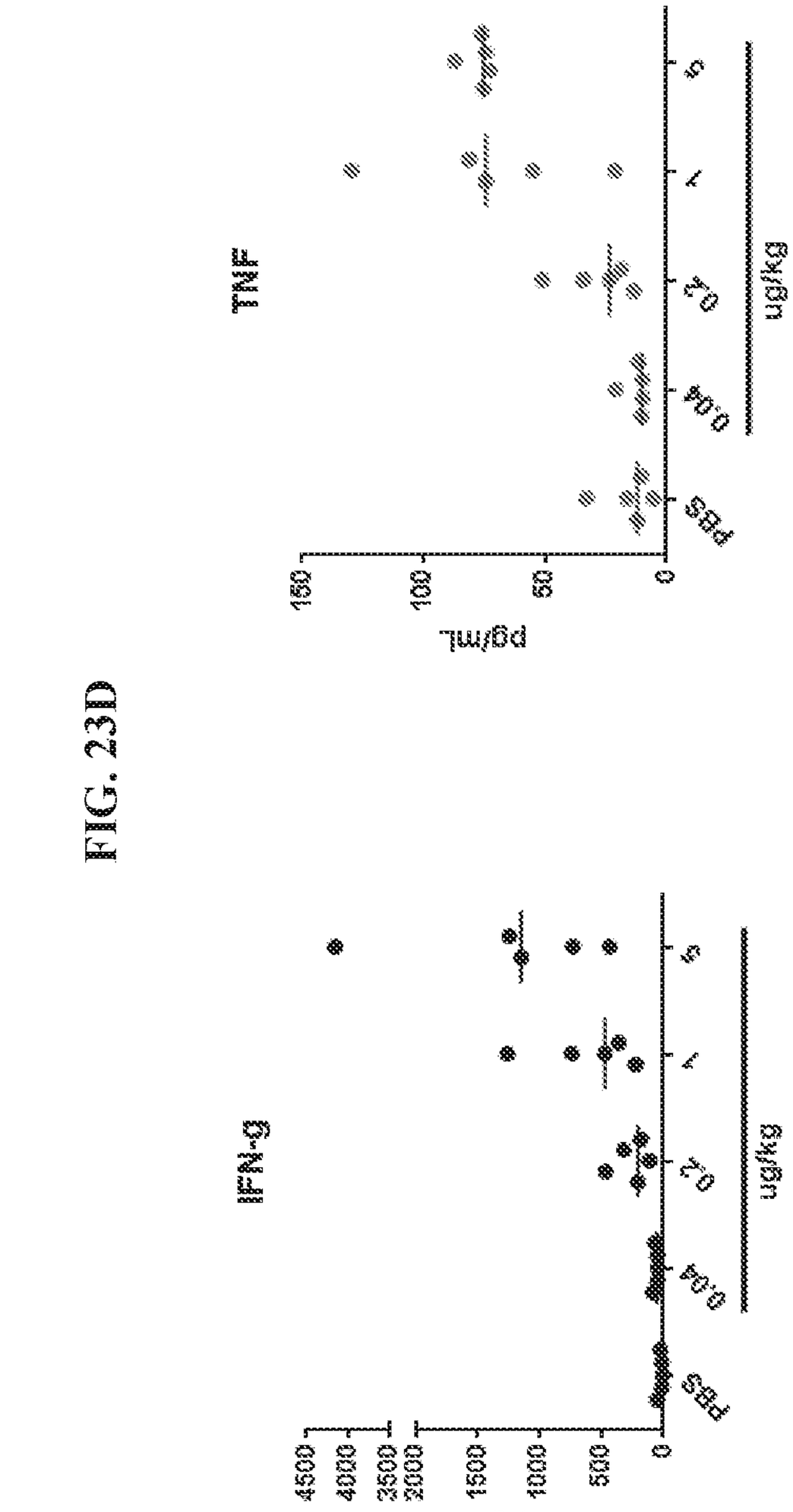

IL-2
pg/mL
3000
2000
1000
0
PBS
0.04
0.2
1
5
ug/kg
TNF
pg/mL
150
100
50
0
PBS
0.04
0.2
1
5
ug/kg
IFN-g
pg/mL
4500
4000
3500
2000
1500
1000
500
0
PBS
0.04
0.2
1
5
ug/kg

FIG. 23D

FIG. 24

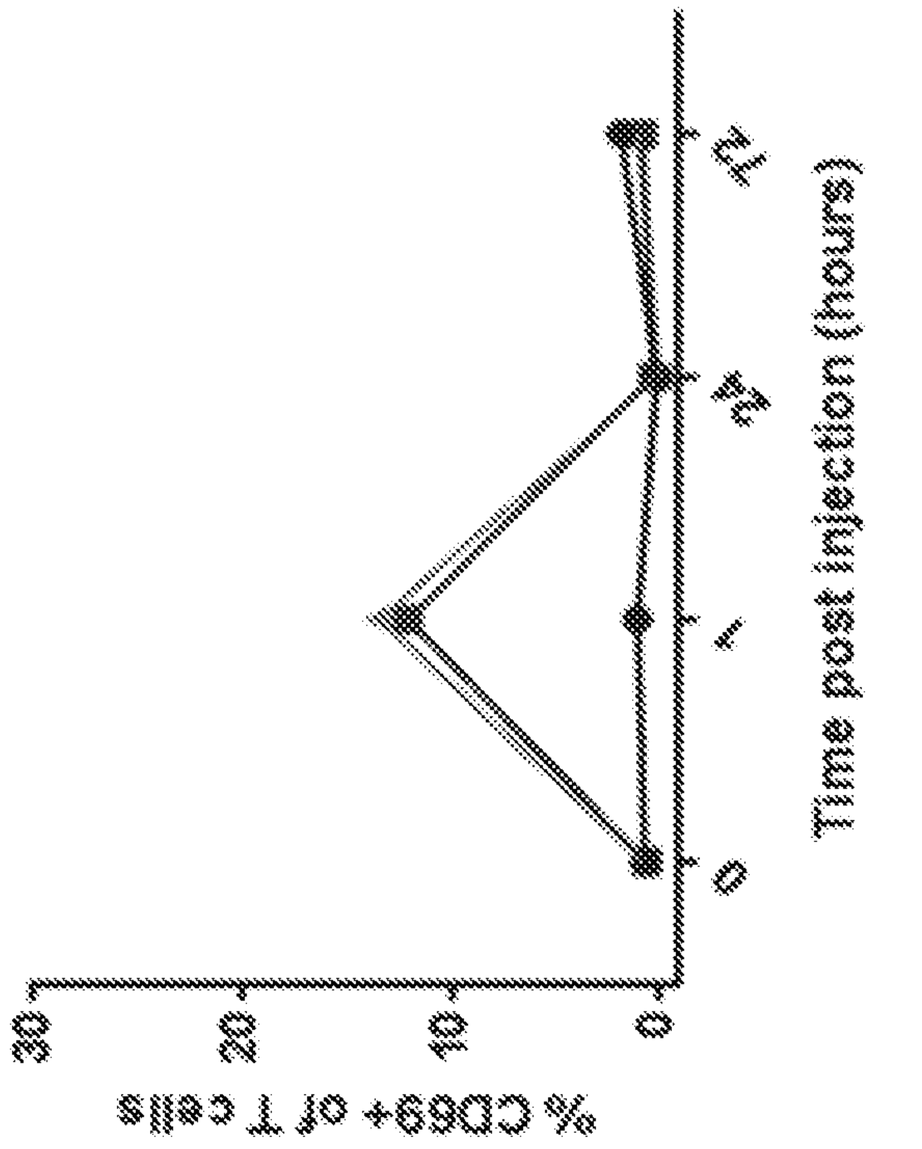

CD4+ T cells
% CD69+ of T cells
0
20
40
60
0
24
48
72
Time post injection (hours)
CD38SB 19xCD28sup
CD28sup xCD3mid IgG4
CD28sup IgG4

FIG. 25

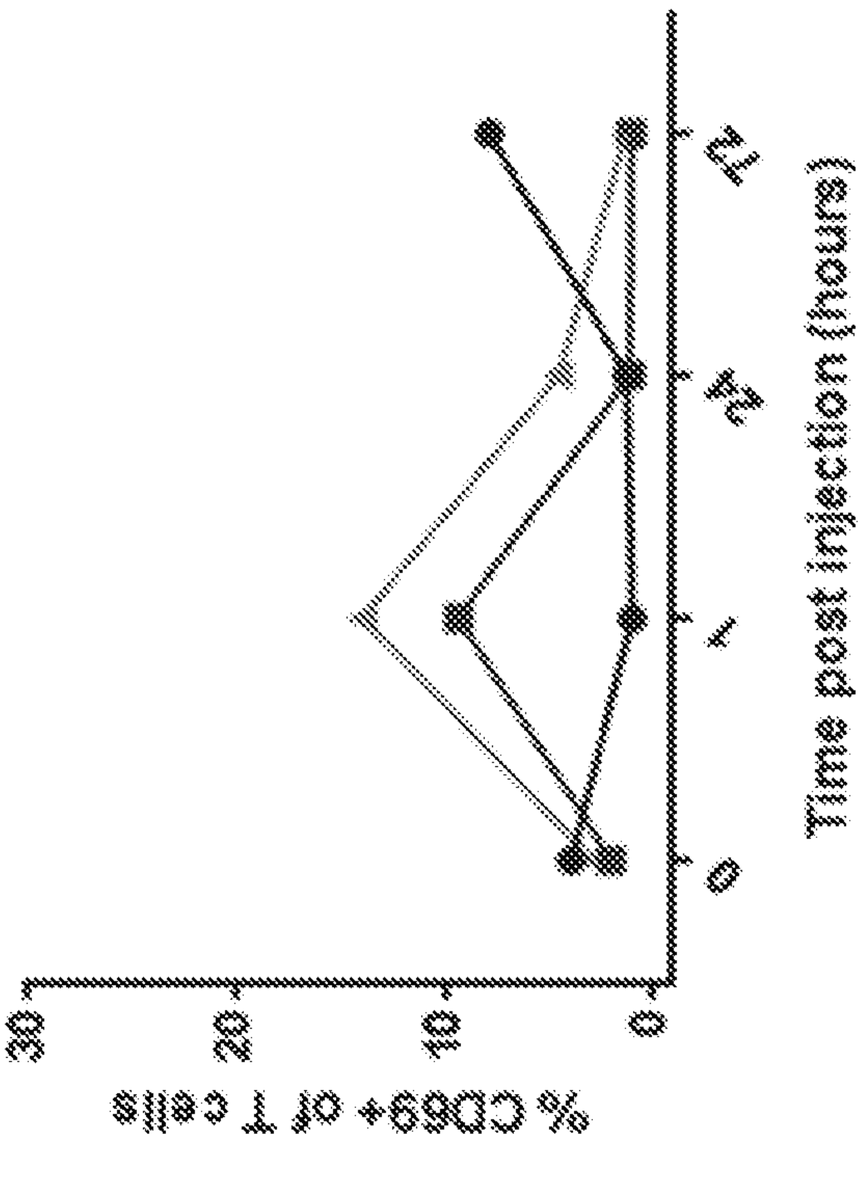

CD8+ T cells
% CD69+ of T cells
0
20
40
60
0
24
48
72
Time post injection (hours)
CD38SB 19xCD28sup
CD28sup xCD3mid IgG4
CD28sup IgG4

FIG. 26A

IL-2

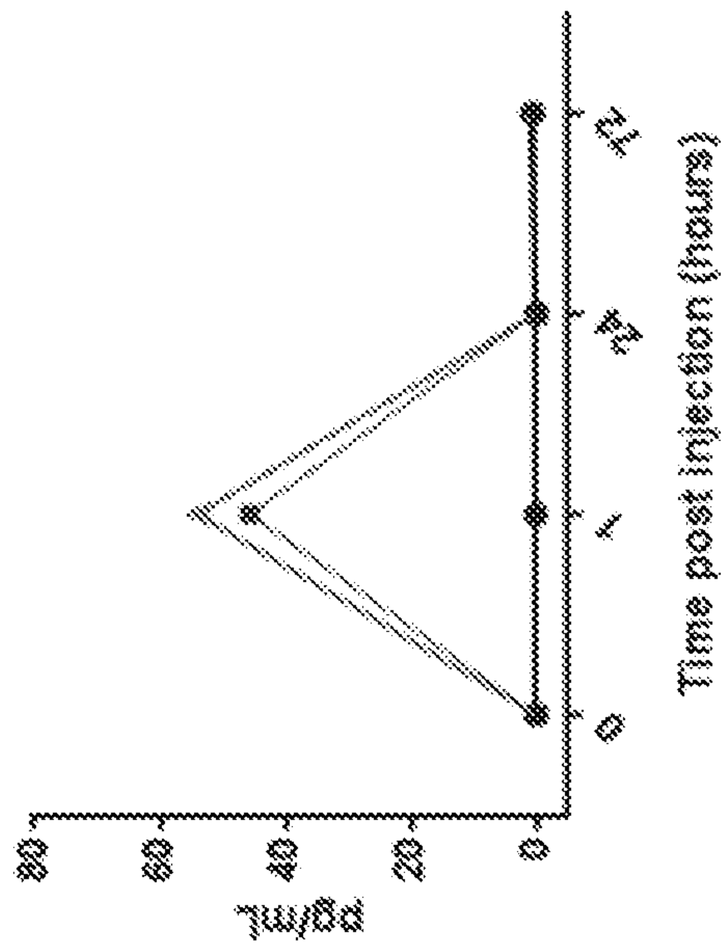


FIG. 26B

TNF

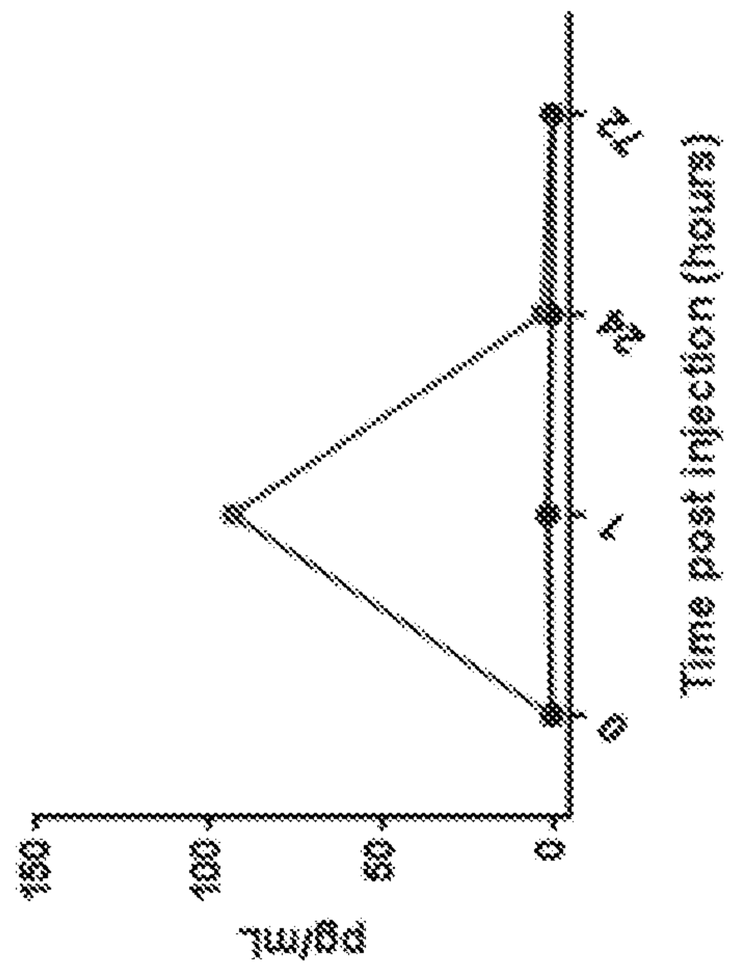
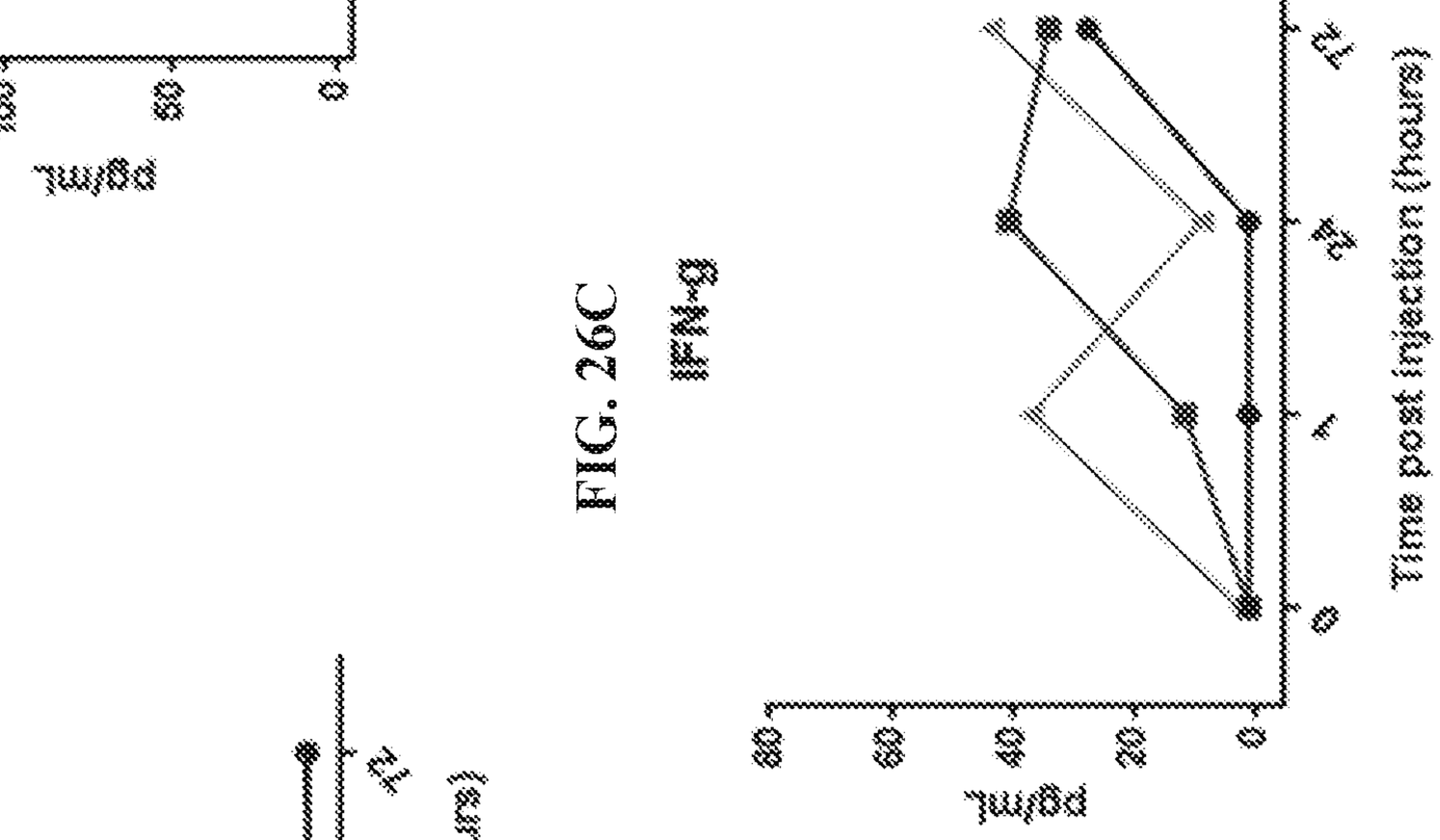


FIG. 26C

IFN-g



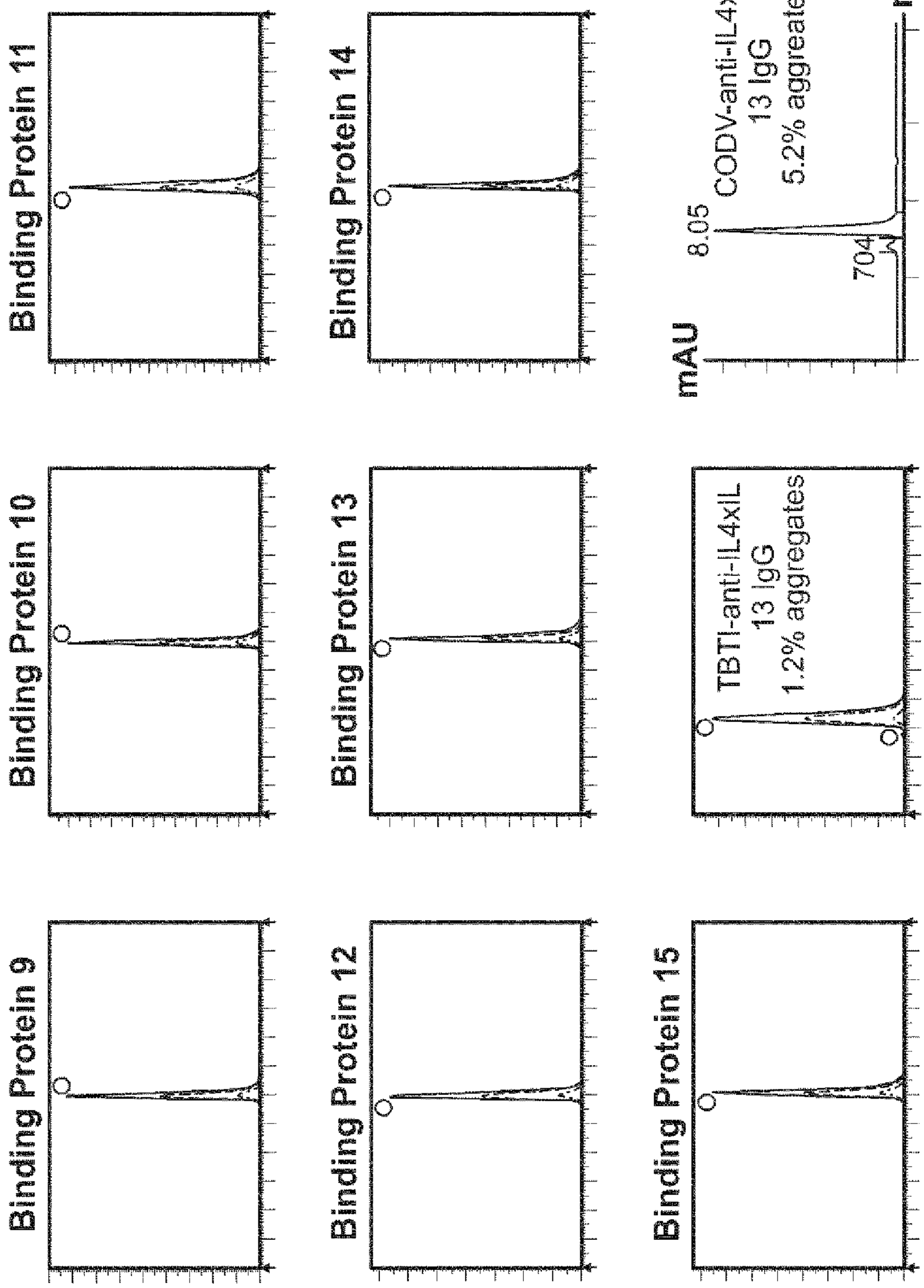

Binding Protein 11
Binding Protein 14
mAU
8.05
CODV-anti-IL4xIL
13 IgG
5.2% aggreates
704
min
Binding Protein 10
Binding Protein 13
TBTI-anti-IL4xIL
13 IgG
1.2% aggregates
Binding Protein 9
Binding Protein 12
Binding Protein 15

FIG. 27A

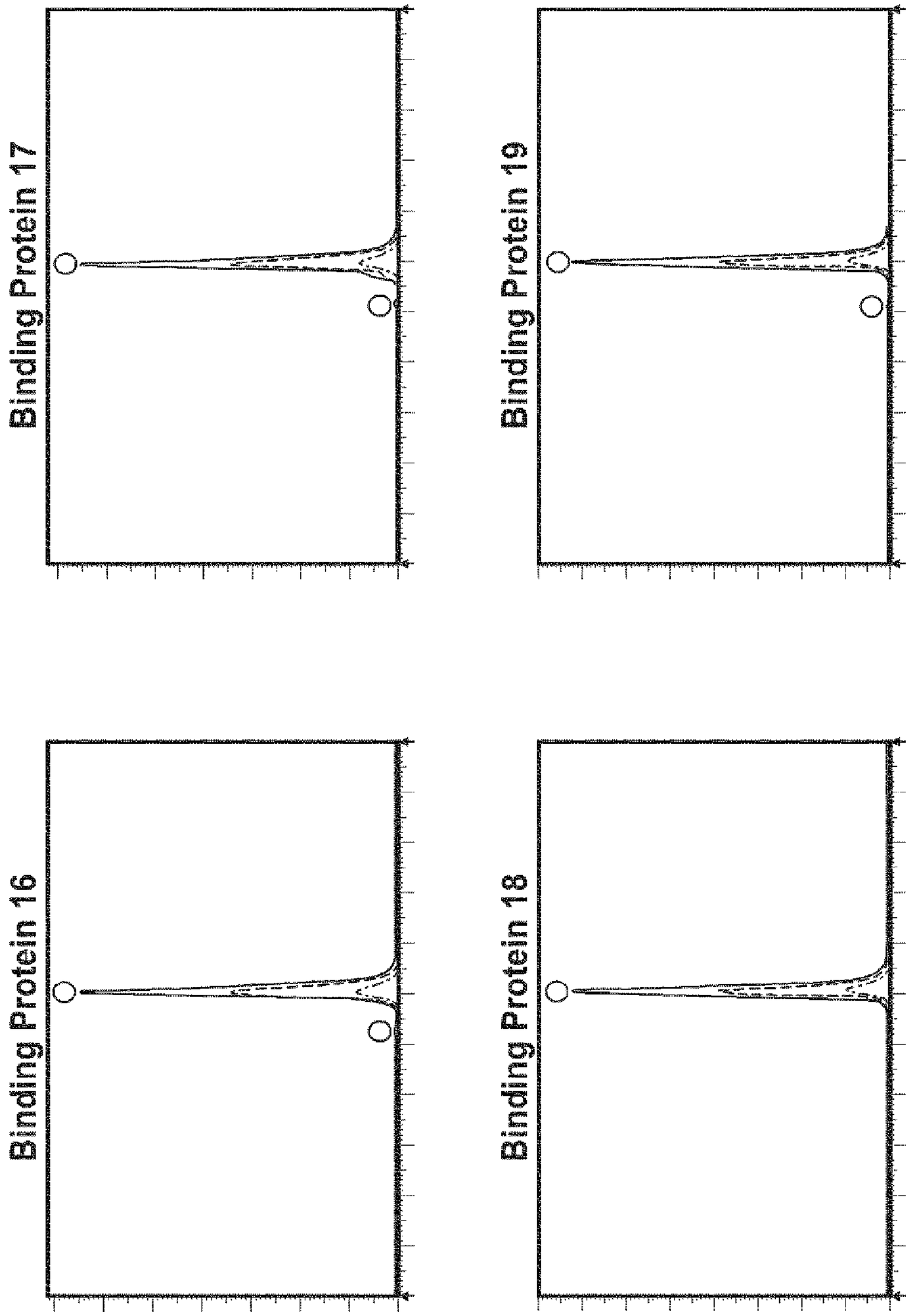

Binding Protein 17
Binding Protein 19
Binding Protein 16
Binding Protein 18

FIG. 27B

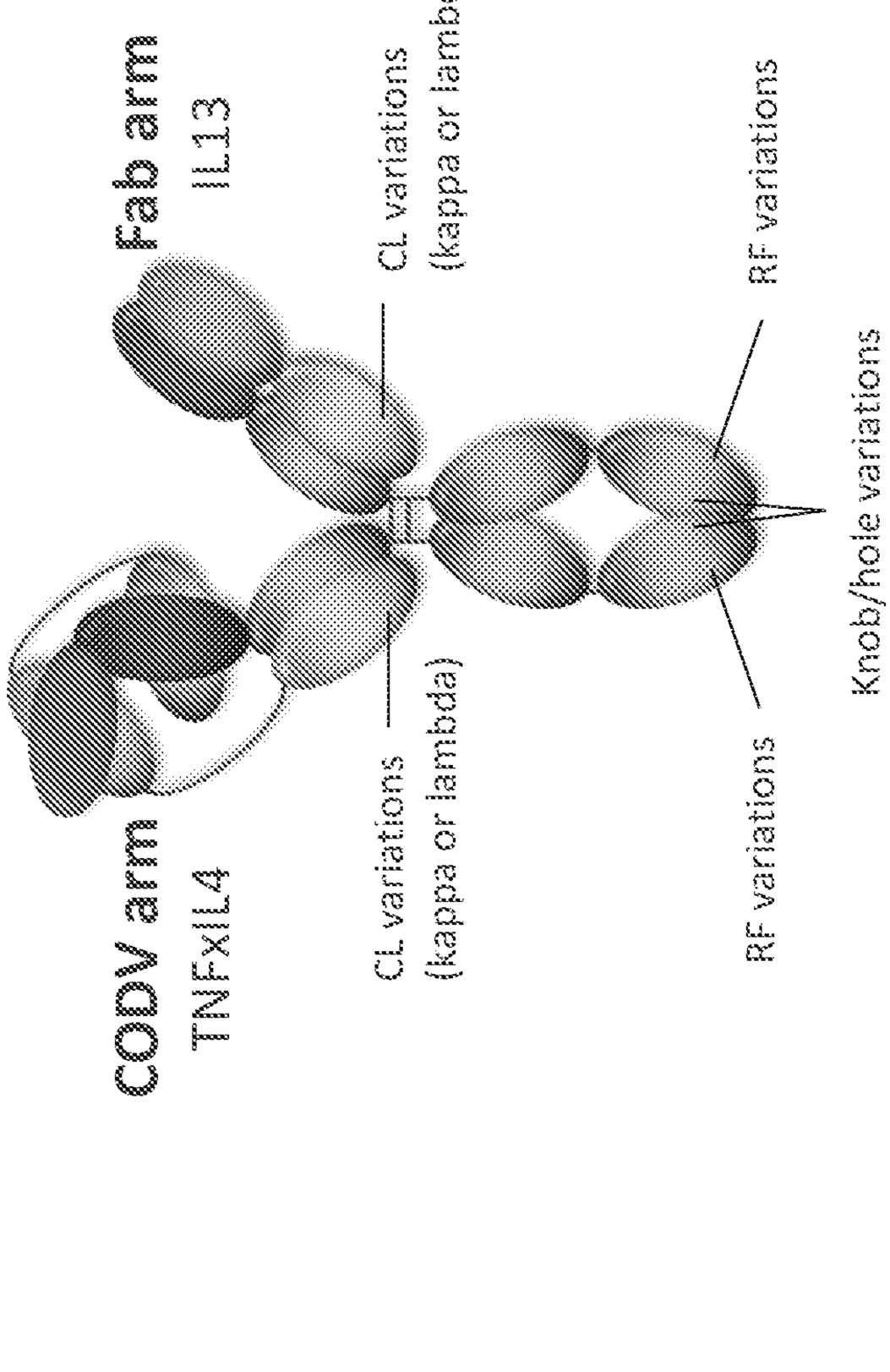

Fab arm
IL13
CL variations
(kappa or lambda)
RF variations
Knob/hole variations
CODV arm
TNFxIL4
CL variations
(kappa or lambda)
RF variations

FIG. 28A

FIG. 28B

CODV-arm	Fab-arm	CODV-arm	Fab-arm	RF mutation
Kappa	Kappa	Hole	Knob	no RF
Kappa	Kappa	Hole	Knob	Hole
Kappa	Kappa	Hole	Knob	Knob
Kappa	lambda	Knob	Hole	no RF
Kappa	lambda	Knob	Hole	Hole
Kappa	lambda	Knob	Hole	Knob
lambda	Kappa	Knob	Hole	no RF
lambda	Kappa	Knob	Hole	Hole
lambda	Kappa	Knob	Hole	Knob
Kappa	Kappa	Knob	Hole	no RF
Kappa	Kappa	Knob	Hole	Hole
Kappa	Kappa	Knob	Hole	Knob
Kappa	lambda	Hole	Knob	no RF
Kappa	lambda	Hole	Knob	Hole
Kappa	lambda	Hole	Knob	Knob
lambda	Kappa	Hole	Knob	no RF
lambda	Kappa	Hole	Knob	Hole
lambda	Kappa	Hole	Knob	Knob

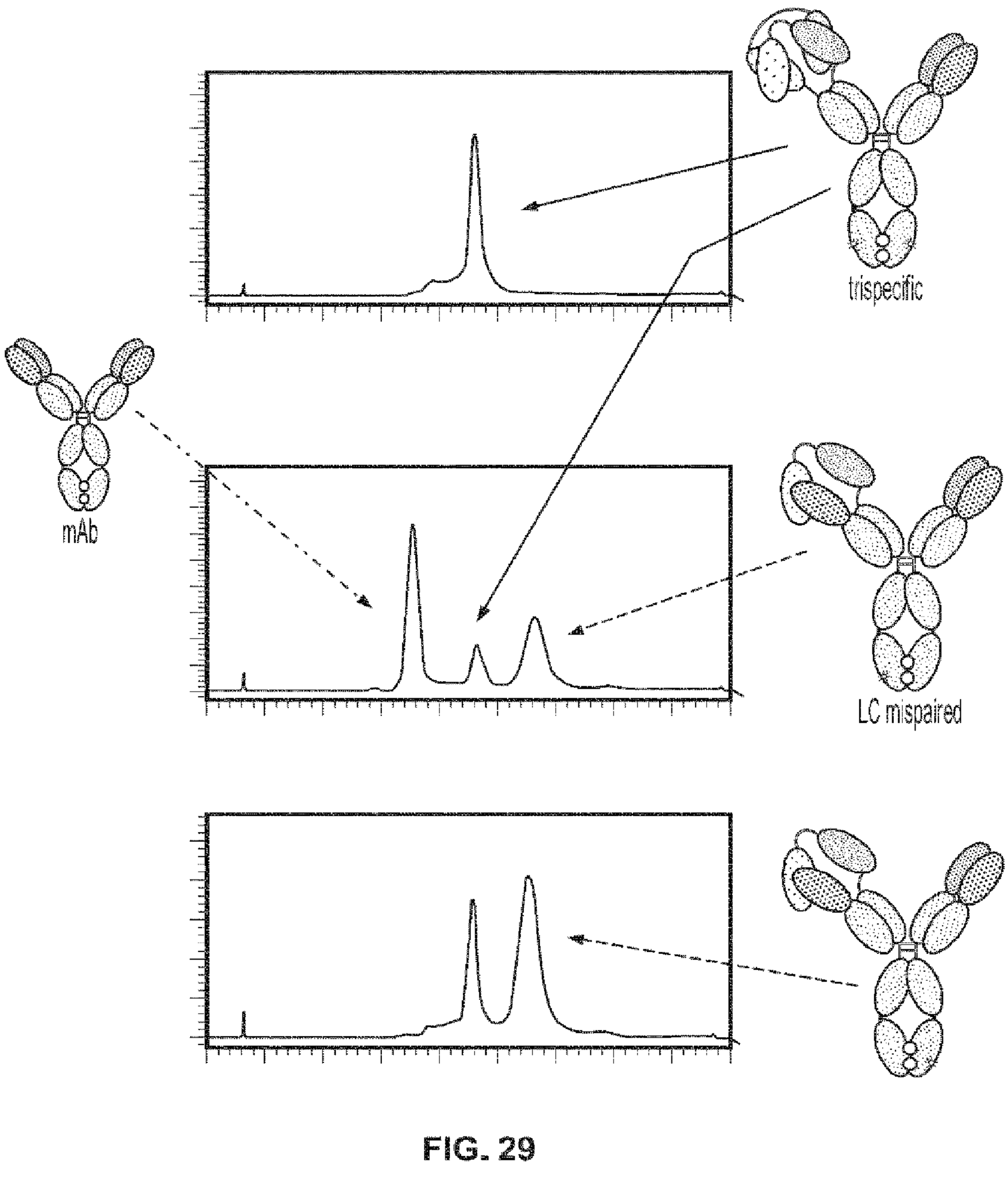


FIG. 29

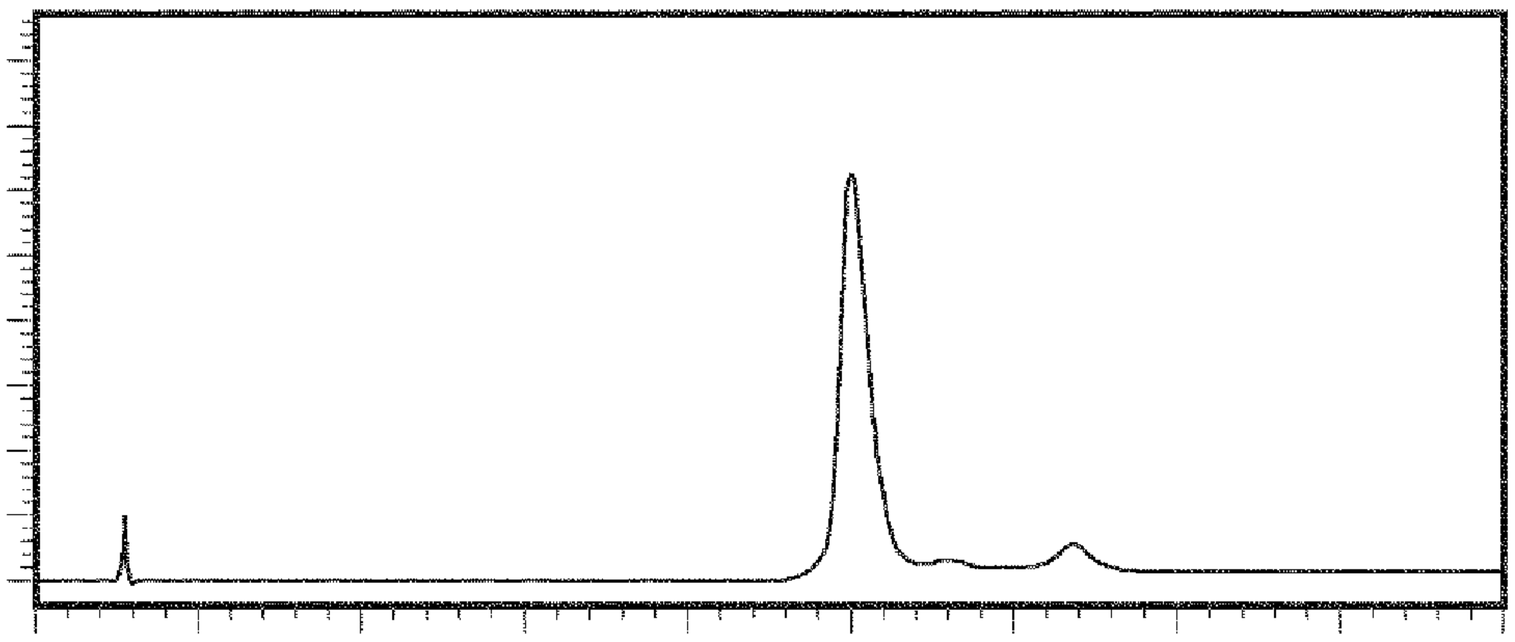


FIG. 30A

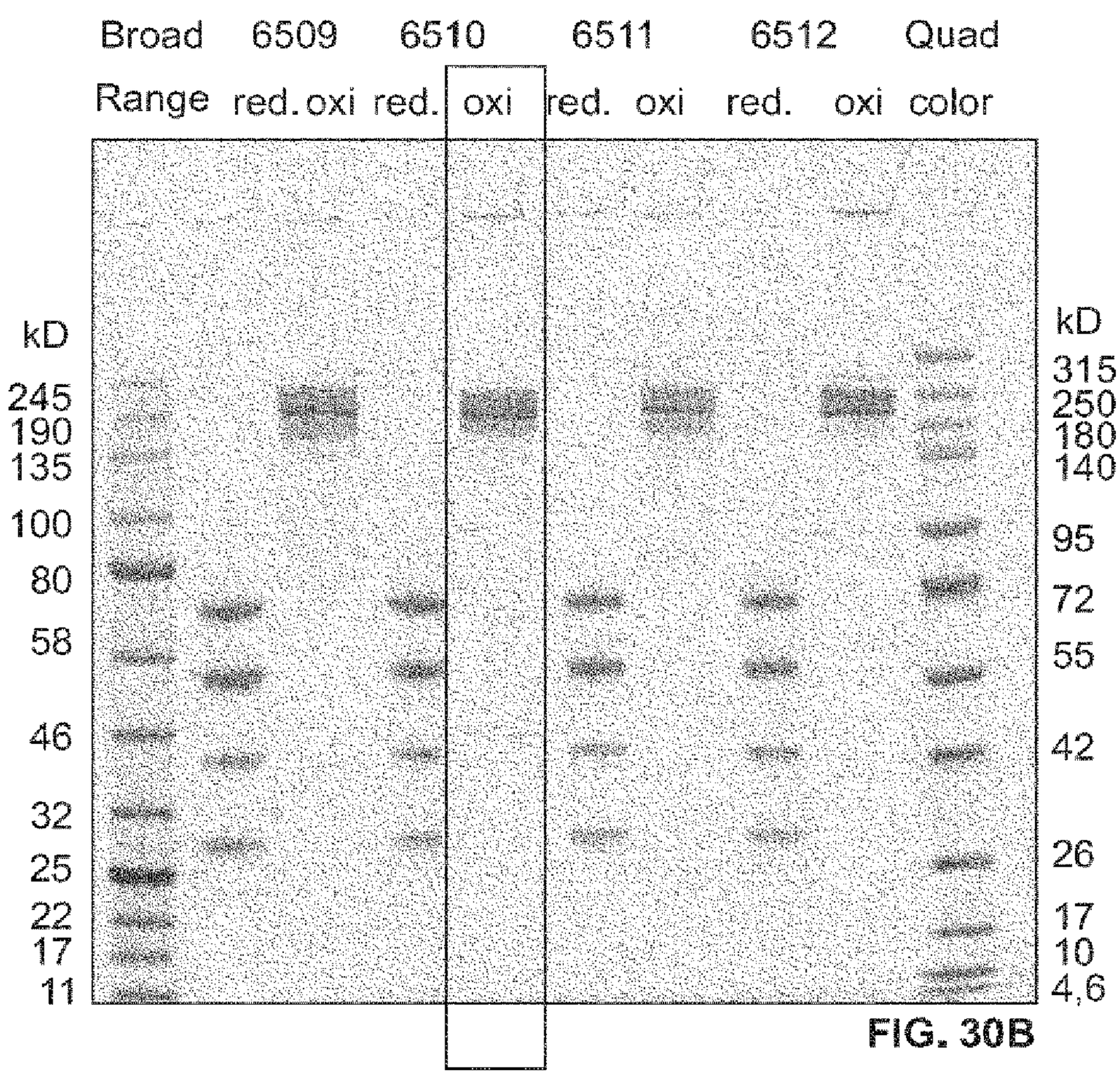


FIG. 30B

FIG. 1A

