

TRISPECIFIC ANTI-CD38, ANTI-CD28, AND ANTI-CD3 BINDING PROTEINS AND
METHODS OF USE FOR TREATING VIRAL INFECTION

CROSS REFERENCES TO RELATED APPLICATIONS

[0001] This application claims the priority benefit of International Application No. PCT/US2018/055084, filed October 9, 2018; U.S. Provisional Application Serial No. 62/831,572, filed April 9, 2019; U.S. Provisional Application Serial No. 62/831,608, filed April 9, 2019; and EP Application No. 19306097.7, filed September 11, 2019; all of 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: 183952032140SEQLIST.TXT, date recorded: October 2, 2019, size: 144 KB).

FIELD

[0003] The disclosure relates to methods of using trispecific binding proteins comprising four polypeptide chains that form three antigen binding sites that specifically bind a CD38 polypeptide (*e.g.*, human and/or cynomolgus monkey CD38 polypeptides), a CD28 polypeptide, and a CD3 polypeptide for expanding memory T cells (*e.g.*, virus-specific memory T cells) and/or treating chronic viral infection.

BACKGROUND

[0004] As part of the human adaptive immunity, T cell immunity plays crucial role in controlling viral infection, eliminating infected cells which results in clearance of viral infection. In chronic infectious diseases such as Herpes viral infection (HSV, CMV, EBV, etc.), HIV, and HBV, viruses establish their persistence in humans by various mechanisms including immune suppression, T cell exhaustion, and latency establishment. Nevertheless, viral infection generally induces viral antigen specific immunity including antigen specific

CD8 T cells that can readily recognize infected cells for controlling or killing through cytokine release or cytotoxic T cell (CTL) mediated killing processes.

[0005] Thus, viral antigen specific T cell activation and/or amplification *in vivo* and/or *ex vivo* may provide therapeutic strategies against chronic viral infections.

BRIEF SUMMARY

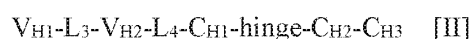
[0006] Provided herein are anti-CD38/CD28xCD3 trispecific antibodies that were developed and evaluated for their potential in activating T cells, and subsequent proliferation and/or amplification of antigen specific T cells. These trispecific Abs can effectively expand CD4 and CD8 effector and memory populations, including antigen specific CD8 T central memory and effector memory cells *in vitro*. Specifically, *in vitro* expansion of CMV, EBV, HIV-1, Influenza specific CD8 central memory and effector memory cells were demonstrated. The anti-CD38/CD28xCD3 trispecific antibodies described herein exhibited novel properties by engaging CD3/CD28/CD38, providing signaling pathways to stimulate and expand T cells, which may offer an effective strategy treating chronic infectious diseases such as HSV, CMV, EBV, HIV-1, and HBV infections.

[0007] To meet these and other needs, provided herein are binding proteins that bind a CD38 polypeptide (*e.g.*, human and cynomolgus monkey CD38 polypeptides), a CD28 polypeptide, and a CD3 polypeptide.

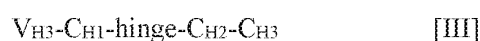
[0008] In some embodiments, provided herein is a method for expanding virus-specific memory T cells, comprising contacting a virus-specific memory T cell with a binding protein, wherein the binding protein comprises four polypeptide chains that form the three antigen binding sites, wherein a first polypeptide chain comprises a structure represented by the formula:



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



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



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



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;

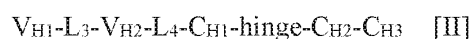
wherein the polypeptide of formula I and the polypeptide of formula II form a cross-over light chain-heavy chain pair; and

wherein V_{H1} and V_{L1} form a first antigen binding site that binds a CD28 polypeptide,
 wherein V_{H2} and V_{L2} form a second antigen binding site that binds a CD3 polypeptide, and
 wherein V_{H3} and V_{L3} form a third antigen binding site that binds a CD38 polypeptide

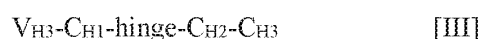
[0009] In some embodiments, provided herein is a binding protein that comprises four polypeptide chains that form the three antigen binding sites, wherein a first polypeptide chain comprises a structure represented by the formula:



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



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



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



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; and

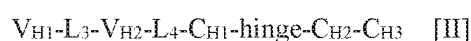
wherein V_{H1} and V_{L1} form a first antigen binding site that binds a CD28 polypeptide,
 wherein V_{H2} and V_{L2} form a second antigen binding site that binds a CD3 polypeptide, and
 wherein V_{H3} and V_{L3} form a third antigen binding site that binds a CD38 polypeptide for use in expanding virus-specific memory T cells.

[0010] In some embodiments, the virus-specific memory T cell is contacted with the binding protein *in vitro* or *ex vivo*. In some embodiments, contacting the virus-specific memory T cell with the binding protein causes activation and/or proliferation of virus-specific memory T cells.

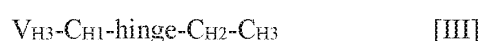
[0011] In some embodiments, provided herein is a method for expanding T cells, comprising contacting a T cell with a binding protein *in vitro* or *ex vivo*, wherein the binding protein comprises four polypeptide chains that form the three antigen binding sites, wherein a first polypeptide chain comprises a structure represented by the formula:



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



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



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



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;

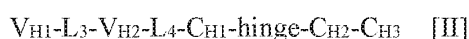
wherein the polypeptide of formula I and the polypeptide of formula II form a cross-over light chain-heavy chain pair; and

wherein V_{H1} and V_{L1} form a first antigen binding site that binds a CD28 polypeptide,
 wherein V_{H2} and V_{L2} form a second antigen binding site that binds a CD3 polypeptide, and
 wherein V_{H3} and V_{L3} form a third antigen binding site that binds a CD38 polypeptide.

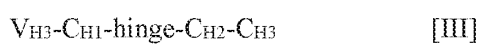
[0012] In some embodiments, provided herein is a binding protein that comprises four polypeptide chains that form the three antigen binding sites, wherein a first polypeptide chain comprises a structure represented by the formula:



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



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



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



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;

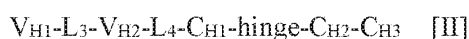
wherein the polypeptide of formula I and the polypeptide of formula II form a cross-over light chain-heavy chain pair; and
 wherein V_{H1} and V_{L1} form a first antigen binding site that binds a CD28 polypeptide,
 wherein V_{H2} and V_{L2} form a second antigen binding site that binds a CD3 polypeptide, and
 wherein V_{H3} and V_{L3} form a third antigen binding site that binds a CD38 polypeptide for use in a method for expanding T cells.

[0013] In some embodiments, the T cell is a memory T cell or an effector T cell. In some embodiments, the T cell expresses a chimeric antigen receptor (CAR) on its cell surface or comprises a polynucleotide encoding a CAR.

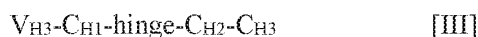
[0014] In some embodiments, provided herein is a method for treating chronic viral infection, comprising administering to an individual in need thereof an effective amount of a binding protein, wherein the binding protein comprises four polypeptide chains that form the three antigen binding sites, wherein a first polypeptide chain comprises a structure represented by the formula:



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



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



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



wherein:

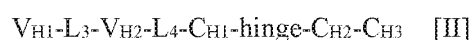
- V_{L1} is a first immunoglobulin light chain variable domain;
- V_{L2} is a second immunoglobulin light chain variable domain;
- V_{L3} is a third immunoglobulin light chain variable domain;
- V_{H1} is a first immunoglobulin heavy chain variable domain;
- V_{H2} is a second immunoglobulin heavy chain 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; and
 wherein V_{H1} and V_{L1} form a first antigen binding site that binds a CD28 polypeptide,
 wherein V_{H2} and V_{L2} form a second antigen binding site that binds a CD3 polypeptide, and
 wherein V_{H3} and V_{L3} form a third antigen binding site that binds a CD38 polypeptide.

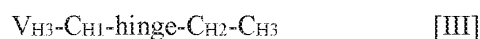
[0015] In some embodiments, provided herein is a binding protein that comprises four polypeptide chains that form the three antigen binding sites, wherein a first polypeptide chain comprises a structure represented by the formula:



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



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



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



wherein:

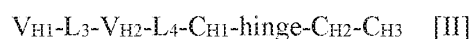
- V_{L1} is a first immunoglobulin light chain variable domain;
- V_{L2} is a second immunoglobulin light chain variable domain;
- V_{L3} is a third immunoglobulin light chain variable domain;
- V_{H1} is a first immunoglobulin heavy chain variable domain;
- V_{H2} is a second immunoglobulin heavy chain variable domain;
- V_{H3} is a third immunoglobulin heavy chain variable domain;
- CL is an immunoglobulin light chain constant domain;
- CH_1 is an immunoglobulin CH_1 heavy chain constant domain;
- CH_2 is an immunoglobulin CH_2 heavy chain constant domain;
- CH_3 is an immunoglobulin CH_3 heavy chain constant domain;
- hinge is an immunoglobulin hinge region connecting the CH_1 and CH_2 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; and wherein V_{H1} and V_{L1} form a first antigen binding site that binds a CD28 polypeptide, wherein V_{H2} and V_{L2} form a second antigen binding site that binds a CD3 polypeptide, and wherein V_{H3} and V_{L3} form a third antigen binding site that binds a CD38 polypeptide for use in a method for treating chronic viral infection, wherein said method comprises administering to an individual in need thereof an effective amount

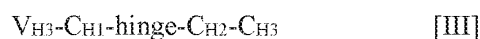
of the binding protein. In some embodiments, provided herein is a binding protein for use in a method for treating chronic viral infection, wherein said method comprises administering to an individual in need thereof an effective amount of the binding protein, wherein the binding protein comprises four polypeptide chains that form the three antigen binding sites, wherein a first polypeptide chain of the binding protein comprises a structure represented by the formula:



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



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



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



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; and

wherein V_{H1} and V_{L1} form a first antigen binding site that binds a CD28 polypeptide,

wherein V_{H2} and V_{L2} form a second antigen binding site that binds a CD3 polypeptide, and

wherein V_{H3} and V_{L3} form a third antigen binding site that binds a CD38 polypeptide.

[0016] In some embodiments, the individual is a human. In some embodiments, the binding protein is administered to the individual in pharmaceutical formulation comprising the binding protein and a pharmaceutically acceptable carrier. In some embodiments, administration of the binding protein results in activation and/or proliferation of virus-specific memory T cells in the individual.

[0017] In some embodiments that may be combined with any other embodiments described herein, the memory T cells are CD8⁺ or CD4⁺ memory T cells. In some embodiments, the memory T cells are central memory T cells (T_{CM}) or effector memory T cells (T_{EM}).

[0018] In some embodiments that may be combined with any other embodiments described herein, the CD28 polypeptide is a human CD28 polypeptide, wherein the CD3 polypeptide is a human CD3 polypeptide, and wherein the CD38 polypeptide is a human CD38 polypeptide.

[0019] In some embodiments that may be combined with any other embodiments described herein, the V_{H3} domain comprises a CDR-H1 sequence comprising the amino acid sequence of GYTFTSFN (SEQ ID NO:31), a CDR-H2 sequence comprising the amino acid sequence of IYPGNGGT (SEQ ID NO:32), and a CDR-H3 sequence comprising the amino acid sequence of ARTGGLRRAYFTY (SEQ ID NO:33), and the V_{L3} domain comprises a CDR-L1 sequence comprising the amino acid sequence of ESVDSYGNGF (SEQ ID NO:34), a CDR-L2 sequence comprising the amino acid sequence of LAS (SEQ ID NO:35), and a CDR-L3 sequence comprising the amino acid sequence of QQNKEDPWT (SEQ ID NO:36). In some embodiments, the V_{H3} domain comprises a CDR-H1 sequence comprising the amino acid sequence of GYTFTSYA (SEQ ID NO:37), a CDR-H2 sequence comprising the amino acid sequence of IYPGQGGT (SEQ ID NO:38), and a CDR-H3 sequence comprising the amino acid sequence of ARTGGLRRAYFTY (SEQ ID NO:33), and the V_{L3} domain comprises a CDR-L1 sequence comprising the amino acid sequence of QSVSSYGQGF (SEQ ID NO:39), a CDR-L2 sequence comprising the amino acid sequence of GAS (SEQ ID NO:40), and a CDR-L3 sequence comprising the amino acid sequence of QQNKEDPWT (SEQ ID NO:36). In some embodiments, the V_{H3} domain comprises a CDR-H1 sequence comprising the amino acid sequence of GFTFSSYG (SEQ ID NO:41), a CDR-H2 sequence comprising the amino acid sequence of IWYDGSNK (SEQ ID NO:42), and a CDR-H3 sequence comprising the amino acid sequence of ARMFRGAFDY (SEQ ID NO:43), and the V_{L3} domain comprises a CDR-L1 sequence comprising the amino acid sequence of QGIRND (SEQ ID NO:44), a CDR-L2

sequence comprising the amino acid sequence of AAS (SEQ ID NO:45), and a CDR-L3 sequence comprising the amino acid sequence of LQDYIYYPT (SEQ ID NO:46). In some embodiments, the V_{H3} domain comprises the amino acid sequence of

QVQLQQSGAELVRSGASVKMSCKASGYTFTSFNMHWVKETPGQGLEWIGYIYPG
 NGGTNYNQKFQKATLTADTSSSTAYMQISLTSEDSAVYFCARTGGLRRAYFTY
 WGQGTTLVTVS (SEQ ID NO:5), and the V_{L3} domain comprises the amino acid sequence of

DIVLTQSPASLAVSLGQRATISCRASESVDSYGNGFMHWYQQKPGQPPKLLIYLAS
 NLESGVPARFSGSGSRTDFTLTIDPVEADDAATYYCQQNKEDPWTFGGGTKLEIK
 (SEQ ID NO:6). In some embodiments, the V_{H3} domain comprises the amino acid sequence of

QVQLVQSGAEVVKPGASVKVSCKASGYTFTSYAMHWVKEAPGQRLEWIGYIYPG
 QGGTNYNQKFQGRATLTADTSASTAYMELSSLRSEDTAVYFCARTGGLRRAYFTY
 WGQGTTLVTVSS (SEQ ID NO:13), and the V_{L3} domain comprises the amino acid sequence of

DIVLTQSPATLSLSPGERATISCRASQSVSSYGQGMHWYQQKPGQPPRLIYGASS
 RATGIPARFSGSGSGTDFTLTISPLEDFAVYYCQQNKEDPWTFGGGTKLEIK
 (SEQ ID NO:14). In some embodiments, the V_{H3} domain comprises the amino acid sequence of

QVQLVQSGAEVVKPGASVKVSCKASGYTFTSFNMHWVKEAPGQRLEWIGYIYPG
 NGGTNYNQKFQGRATLTADTSASTAYMELSSLRSEDTAVYFCARTGGLRRAYFTY
 WGQGTTLVTVSS (SEQ ID NO:17), and the V_{L3} domain comprises the amino acid sequence of

DIVLTQSPATLSLSPGERATISCRASESVDSYGNGFMHWYQQKPGQPPRLIYLASS
 RATGIPARFSGSGSGTDFTLTISPLEDFAVYYCQQNKEDPWTFGGGTKLEIK
 (SEQ ID NO:18). In some embodiments, the V_{H3} domain comprises the amino acid sequence of

QVQLVQSGAEVVKSGASVKVSCKASGYTFTSFNMHWVKEAPGQGLEWIGYIYPG
 NGGTNYNQKFQGRATLTADTSASTAYMEISSLRSEDTAVYFCARTGGLRRAYFTY
 WGQGTTLVTVSS (SEQ ID NO:21), and the V_{L3} domain comprises the amino acid sequence of

DIVLTQSPATLSLSPGERATISCRASESVDSYGNGFMHWYQQKPGQPPRLIYLASS
 RATGIPARFSGSGSGTDFTLTISPLEDFAVYYCQQNKEDPWTFGGGTKLEIK
 (SEQ ID NO:18). In some embodiments, the V_{H3} domain comprises the amino acid

sequence of

QVQLVQSGAEVVKPGASVKMSCKASGYTFTSFNMHWVKEAPGQRLEWIGYIYPG
NGGTNYNQKFQGRATLTADTSASTAYMEISSLRSEDVAVYFCARTGGLRRAYFTY
WGQGTLVTVSS (SEQ ID NO:23), and the V_{L3} domain comprises the amino acid

sequence of

DIVLTQSPATLSLSPGERATISCRASESVDSYGNGFMHWYQQKPGQPPRLLIYLASS
RATGIPARFSGSGSGTDFTLTISPLEDFAVYYCQNKEDPWTFGGGTKLEIK
(SEQ ID NO:18). In some embodiments, the V_{H3} domain comprises the amino acid

sequence of

QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLEWVAVIWDYD
GSNKYYADSVKGRFTISGDNSKNTLYLQMNSLRAEDTAVYYCARMFRGAFDYWG
QGTLVTVSS (SEQ ID NO:9), and the V_{L3} domain comprises the amino acid sequence of
AIQMTQSPSSLSASVGDRVTITCRASQGIRNDLGWYQQKPGKAPKLLIYAASSLQS
GVPSRFSGSGSGTDFTLTISGLQPEDSATYYCLQDYIYYPTFGQGTKVEIK (SEQ ID
NO:10).

[0020] In some embodiments that may be combined with any other embodiments described herein, the V_{H1} domain comprises a CDR-H1 sequence comprising the amino acid sequence of GYTFTSY (SEQ ID NO:108), a CDR-H2 sequence comprising the amino acid sequence of IYPGNVNT (SEQ ID NO:109), and a CDR-H3 sequence comprising the amino acid sequence of TRSHYGLDWNFDV (SEQ ID NO:110), and the V_{L1} domain comprises a CDR-L1 sequence comprising the amino acid sequence of QNIYVW (SEQ ID NO:111), a CDR-L2 sequence comprising the amino acid sequence of KAS (SEQ ID NO:112), and a CDR-L3 sequence comprising the amino acid sequence of QQGQTPPY (SEQ ID NO:113). In some embodiments, the V_{H1} domain comprises a CDR-H1 sequence comprising the amino acid sequence of GFSLSDYG (SEQ ID NO:114), a CDR-H2 sequence comprising the amino acid sequence of IWAGGGT (SEQ ID NO:115), and a CDR-H3 sequence comprising the amino acid sequence of ARDKGYSYYYSMDY (SEQ ID NO:116), and the V_{L1} domain comprises a CDR-L1 sequence comprising the amino acid sequence of ESVEYYVTSL (SEQ ID NO:117), a CDR-L2 sequence comprising the amino acid sequence of AAS (SEQ ID NO:118), and a CDR-L3 sequence comprising the amino acid sequence of QQSRKVPYT (SEQ ID NO:119). In some embodiments, the V_{H1} domain comprises the amino acid sequence of QVQLVQSGAEVVKPGASVKVSKASGYTFTSYIIHWVRQAPGGGLEWIGSIYPGNVNTNYAQKFQGRATLTVDTSISTAYMELSRLRSDDTAVYYCTRSHYGLDWNFDV

WGKGTTVTVSS (SEQ ID NO:49), and the V_{L1} domain comprises the amino acid sequence of

DIQMTQSPSSLSASVGDRVTITCQASQNIYVWLNWYQQKPGKAPKLLIYKASNLHTGVPSRFSGSGSGTDFTLTISLQPEDIAITYYCQQGQTYPTFGQGTKLEIK (SEQ ID NO:50). In some embodiments, the V_{H1} domain comprises the amino acid sequence of QVQLQESGPGLVKPSQTLSTCTVSGFSLSDYGVHWVRQPPGKGLEWLGVIWAGG GTNYPNPSLKSRTISKDTSKNQVSLKLSSVTAADTAVYYCARDKGYSYYYSMDY WGQGTTVTVS (SEQ ID NO:51), and the V_{L1} domain comprises the amino acid sequence of

DIVLTQSPASLAVSPGQRATITCRASESVEYYVTSLMQWYQQKPGQPPKLLIFAASN VESGVPARFSGSGSGTDFTLTINPVEANDVANYYCQQSRKVPYTFGQGTKLEIK (SEQ ID NO:52).

[0021] In some embodiments that may be combined with any other embodiments described herein, the V_{H2} domain comprises a CDR-H1 sequence comprising the amino acid sequence of GFTFTKAW (SEQ ID NO:120), a CDR-H2 sequence comprising the amino acid sequence of IKDKSNSYAT (SEQ ID NO:121), and a CDR-H3 sequence comprising the amino acid sequence of RGVYYALSPFDY (SEQ ID NO:122), and the V_{L2} domain comprises a CDR-L1 sequence comprising the amino acid sequence of QSLVHNNANTY (SEQ ID NO:123), a CDR-L2 sequence comprising the amino acid sequence of KVS (SEQ ID NO:124), and a CDR-L3 sequence comprising the amino acid sequence of GQGTQYPFT (SEQ ID NO:125). In some embodiments, the V_{H2} domain comprises a CDR-H1 sequence comprising the amino acid sequence of GFTFTKAW (SEQ ID NO:126), a CDR-H2 sequence comprising the amino acid sequence of IKDKSNSYAT (SEQ ID NO:127), and a CDR-H3 sequence comprising the amino acid sequence of GVVYYALSPFDY (SEQ ID NO:128), and the V_{L2} domain comprises a CDR-L1 sequence comprising the amino acid sequence of QSLVHNNANTY (SEQ ID NO:129), a CDR-L2 sequence comprising the amino acid sequence of KVS (SEQ ID NO:130), and a CDR-L3 sequence comprising the amino acid sequence of GQGTQYPFT (SEQ ID NO:131). In some embodiments, the V_{H2} domain comprises the amino acid sequence of QVQLVESGGGVVQPGRSLRLSCAASGFTFTKAWMHWRQAPGKQLEWVAQIKD KSNSYATYYADSVKGRFTISRDDSKNTLYLQMNSLRAEDTAVYYCRGVYYALSPF DYWGQGTLLTVSS (SEQ ID NO:53), and the V_{L2} domain comprises the amino acid sequence of

DIVMTQTPLSLSVTPGQPASISCKSSQSLVHNNANTYLSWYLQKPGQSPQSLIYKVS

NRFSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCGQGTQYPFTFGSGTKVEIK (SEQ ID NO:54). In some embodiments, the V_{H2} domain comprises the amino acid sequence of

QVQLVESGGGVVQPGRSLRLSCAASGFTFTKAWMHWRQAPGKGLEWVAQIKD
KSNSYATYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCRGVYYALSPF
DYWGQGTLVTVSS (SEQ ID NO:84), and the V_{L2} domain comprises the amino acid sequence of

DIVMTQTPLSLSVTPGQPASISCKSSQSLVHNNGNTYLSWYLQKPGQSPQLLIYKVS
NRFSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCGQGTQYPFTFGGGTKVEIK (SEQ ID NO:85).

[0022] In some embodiments that may be combined with any other embodiments 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 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:55), GGGGSGGGSGGGGS (SEQ ID NO: 56), S, RT, TKGPS (SEQ ID NO: 57), GQPKAAP (SEQ ID NO: 58), and GSGSGSGSGG (SEQ ID NO: 59). In some embodiments, L_1 , L_2 , L_3 and L_4 each independently comprise a sequence selected from the group consisting of GGGGSGGGGS (SEQ ID NO:55), GGGGSGGGSGGGGS (SEQ ID NO:56), S, RT, TKGPS (SEQ ID NO:57), GQPKAAP (SEQ ID NO: 58), and GSGSGSGSGG (SEQ ID NO:59). In some embodiments, L_1 comprises the sequence GQPKAAP (SEQ ID NO: 58), L_2 comprises the sequence TKGPS (SEQ ID NO:57), L_3 comprises the sequence S, and L_4 comprises the sequence RT.

[0023] In some embodiments that may be combined with any other embodiments described herein, the hinge- C_{H2} - C_{H3} domains of the second and the third polypeptide chains are human IgG4 hinge- C_{H2} - C_{H3} domains, and wherein the hinge- C_{H2} - 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 hinge- C_{H2} - C_{H3} domains of the second and the third polypeptide chains are human IgG4 hinge- C_{H2} - C_{H3} domains, and wherein the hinge- C_{H2} - C_{H3} domains each comprise amino acid substitutions at positions corresponding to positions 233-236 of human IgG4 according to EU Index, wherein the amino acid substitutions are E233P, F234V, L235A, and a deletion at 236. In some embodiments, the hinge- C_{H2} - C_{H3} domains of the second and the third polypeptide chains are human IgG4 hinge- C_{H2} - C_{H3} domains, and wherein the hinge- C_{H2} - 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 hinge-CH₂-CH₃ domains of the second and the third polypeptide chains are human IgG1 hinge-CH₂-CH₃ domains, and wherein the hinge-CH₂-CH₃ domains 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 hinge-CH₂-CH₃ domains of the second and the third polypeptide chains are human IgG1 hinge-CH₂-CH₃ domains, and wherein the hinge-CH₂-CH₃ domains each comprise amino acid substitutions at positions corresponding to positions 298, 299, and 300 of human IgG1 according to EU Index, wherein the amino acid substitutions are S298N, T299A, and Y300S. In some embodiments, the hinge-CH₂-CH₃ 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 hinge-CH₂-CH₃ 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 hinge-CH₂-CH₃ 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 hinge-CH₂-CH₃ 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.

[0024] In certain embodiments, the first polypeptide chain comprises the amino acid sequence of SEQ ID NO:61, the second polypeptide chain comprises the amino acid sequence of SEQ ID NO:60, the third polypeptide chain comprises the amino acid sequence of SEQ ID NO:62, and the fourth polypeptide chain comprises the amino acid sequence of SEQ ID NO:63. In certain embodiments, the first polypeptide chain comprises the amino acid sequence of SEQ ID NO:61, the second polypeptide chain comprises the amino acid sequence of SEQ ID NO:64, the third polypeptide chain comprises the amino acid sequence of SEQ ID NO:65, and the fourth polypeptide chain comprises the amino acid sequence of SEQ ID NO:63. In certain embodiments, the first polypeptide chain comprises the amino acid sequence of SEQ ID NO:61, the second polypeptide chain comprises the amino acid

sequence of SEQ ID NO:66, the third polypeptide chain comprises the amino acid sequence of SEQ ID NO:67, and the fourth polypeptide chain comprises the amino acid sequence of SEQ ID NO:63. In certain embodiments, the first polypeptide chain comprises the amino acid sequence of SEQ ID NO:61, the second polypeptide chain comprises the amino acid sequence of SEQ ID NO:60, the third polypeptide chain comprises the amino acid sequence of SEQ ID NO:68, and the fourth polypeptide chain comprises the amino acid sequence of SEQ ID NO:69. In certain embodiments, the first polypeptide chain comprises the amino acid sequence of SEQ ID NO:61, the second polypeptide chain comprises the amino acid sequence of SEQ ID NO:64, the third polypeptide chain comprises the amino acid sequence of SEQ ID NO:70, and the fourth polypeptide chain comprises the amino acid sequence of SEQ ID NO:69. In certain embodiments, the first polypeptide chain comprises the amino acid sequence of SEQ ID NO:61, the second polypeptide chain comprises the amino acid sequence of SEQ ID NO:66, the third polypeptide chain comprises the amino acid sequence of SEQ ID NO:71, and the fourth polypeptide chain comprises the amino acid sequence of SEQ ID NO:69.

[0025] In some embodiments that may be combined with any other embodiments described herein, the virus is a human immunodeficiency virus (HIV), influenza virus, cytomegalovirus (CMV), hepatitis B virus (HBV), human papillomavirus (HPV), Epstein-barr virus (EBV), human foamy virus (HFV), herpes simplex virus 1 (HSV-1), or herpes simplex virus 2 (HSV-2).

[0026] 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

[0027] **FIG. 1** provides a schematic representation of a trispecific binding protein comprising four polypeptide chains that form three antigen binding sites that binds three target proteins: CD28, CD3, and CD38. A first pair of polypeptides possess dual variable domains having a cross-over orientation (VH1-VH2 and VL2-VL1) forming two antigen binding sites that recognize CD3 and CD28, and a second pair of polypeptides possess a single variable domain (VH3 and VL3) forming a single antigen binding site that

recognizes CD38. The trispecific binding protein shown in **FIG. 1** uses an IgG4 constant region with a “knobs-into-holes” mutation, where the knob is on the second pair of polypeptides with a single variable domain.

[0028] **FIG. 2** summarizes the binding affinities of indicated trispecific binding proteins against their cognate antigens (human CD3, CD28, and CD38) as measured by SPR.

[0029] **FIG. 3** summarizes the binding affinity of the indicated anti-CD38xanti-CD28xanti-CD3 trispecific binding proteins for human CD38, as measured by SPR or flow cytometry (FACS).

[0030] **FIGS. 4A-4D** show the characterization of *in vitro* T cell subset expansion in response to CD38^{VH1}/CD3^{mid}xCD28^{sup} trispecific antibodies. Evaluation of T cell subset expansion was performed by coating wells with 350 ng/well of the CD38 trispecific Ab in the absence of exogenous cytokines. T cell populations were measured at indicated time points. A trispecific Ab having three mutated antigen binding domains was used as negative control. Flow cytometry was used to determine central (T_{cm}) and effector memory (T_{em}) CD4 T cells (**FIG. 4A**), T helper cells (Th1, Th17, Th2) (**FIG. 4B**), central (T_{cm}) and effector memory (T_{em}) CD8 T cells (**FIG. 4C**), and cytomegalovirus (CMV) pp65-specific CD8 cells (**FIG. 4D**) as described in Example 3. Analysis of CMV-specific pp65 effector cells was performed by pentamer staining of peripheral blood mononuclear cells (PBMCs) from HLA-A2 CMV+ donors treated with the CD38 trispecific or the triple negative control antibodies.

[0031] **FIGS. 5A-5B** show the characterization of *in vitro* T cell subset expansion in PBMCs collected from CMV-infected Donor B in response to CD38^{VH1}/CD28^{sup} x CD3^{mid} trispecific antibody. T cell populations were measured at indicated time points. The triple mutant trispecific antibody was used as negative control. Flow cytometry was used to quantify CMV-specific memory CD8+ T cells (**FIG. 5A**), as well as CMV-specific central memory (T_{cm}) and effector memory (T_{em}) CD8+ T cells (**FIG. 5B**). CD38^{VH1}/CD28^{sup} x CD3^{mid} trispecific antibody activated T cells and promoted the proliferation of CMV-specific memory CD8+ T cells.

[0032] **FIGS. 6A-6B** show the characterization of *in vitro* T cell subset expansion in PBMCs collected from CMV-infected Donor C in response to CD38^{VH1}/CD28^{sup} x CD3^{mid} trispecific antibody. T cell populations were measured at indicated time points. The triple mutant trispecific antibody was used as negative control. Flow cytometry was used to quantify CMV-specific memory CD8+ T cells (**FIG. 6A**), as well as CMV-specific

central memory (T_{cm}) and effector memory (T_{em}) CD8+ T cells (**FIG. 6B**).

CD38_{VHI}/CD28_{sup} x CD3_{mid} trispecific antibody activated T cells and promoted the proliferation of CMV-specific memory CD8+ T cells.

[0033] **FIGS. 7A-7B** show the characterization of *in vitro* T cell subset expansion in PBMCs collected from Epstein-barr virus (EBV)-infected Donor A in response to CD38_{VHI}/CD28_{sup} x CD3_{mid} trispecific antibody. T cell populations were measured at indicated time points. The triple mutant trispecific antibody was used as negative control. Flow cytometry was used to quantify EBV-specific memory CD8+ T cells (**FIG. 7A**), as well as EBV-specific central memory (T_{cm}) and effector memory (T_{em}) CD8+ T cells (**FIG. 7B**). CD38_{VHI}/CD28_{sup} x CD3_{mid} trispecific antibody activated T cells and promoted the proliferation of EBV-specific memory CD8+ T cells.

[0034] **FIGS. 8A-8B** show the characterization of *in vitro* T cell subset expansion in PBMCs collected from EBV-infected Donor B in response to CD38_{VHI}/CD28_{sup} x CD3_{mid} trispecific antibody. T cell populations were measured at indicated time points. The triple mutant trispecific antibody was used as negative control. Flow cytometry was used to quantify EBV-specific memory CD8+ T cells (**FIG. 8A**), as well as EBV-specific central memory (T_{cm}) and effector memory (T_{em}) CD8+ T cells (**FIG. 8B**). CD38_{VHI}/CD28_{sup} x CD3_{mid} trispecific antibody activated T cells and promoted the proliferation of EBV-specific memory CD8+ T cells.

[0035] **FIG. 9** shows flow cytometry profiles of PBMCs from the indicated human immunodeficiency virus (HIV)-positive donors (bottom panels and top right panel) assayed for HIV Gag-specific CD8+ T cells (A*02:01 - SLYNTVATL (HIV-1 gag p17 76-84) Pentamer conjugated to PE, ProImmune) at baseline (day 0; prior to incubation with trispecific antibodies). PBMCs from an HIV-negative donor were used as negative control (top left panel). The percentages of Gag-specific CD8+ T cell population are provided and shown as inset boxes. At baseline PBMCs from HIV-positive donors contain HIV Gag-specific CD8+ T cells. Donors A-C in **FIG. 9** are the same as donors D-F shown in **FIGS. 10A-12B**.

[0036] **FIGS. 10A-10B** show the characterization of *in vitro* T cell subset expansion in PBMCs collected from HIV-positive Donor D in response to CD38_{VHI}/CD28_{sup} x CD3_{mid} trispecific antibody. T cell populations were measured at indicated time points. The triple mutant trispecific antibody was used as negative control. Flow cytometry was used to quantify HIV-specific memory CD8+ T cells (**FIG. 10A**), as well as HIV-specific central memory (T_{cm}) and effector memory (T_{em}) CD8+ T cells (**FIG. 10B**). CD38_{VHI}/CD28_{sup} x

CD3mid trispecific antibody activated T cells and promoted the proliferation of effector memory (T_{em}) CD8+ T cells.

[0037] FIGS. 11A-11B show the characterization of *in vitro* T cell subset expansion in PBMCs collected from HIV-positive Donor E in response to CD38_{VHI}/CD28_{sup} x CD3mid trispecific antibody. T cell populations were measured at indicated time points. The triple mutant trispecific antibody was used as negative control. Flow cytometry was used to quantify HIV-specific memory CD8+ T cells (FIG. 11A), as well as HIV-specific central memory (T_{cm}) and effector memory (T_{em}) CD8+ T cells (FIG. 11B). CD38_{VHI}/CD28_{sup} x CD3mid trispecific antibody activated T cells and promoted the proliferation of effector memory (T_{em}) CD8+ T cells.

[0038] FIGS. 12A-12B show the characterization of *in vitro* T cell subset expansion in PBMCs collected from HIV-positive Donor F in response to CD38_{VHI}/CD28_{sup} x CD3mid trispecific antibody. T cell populations were measured at indicated time points. The triple mutant trispecific antibody was used as negative control. Flow cytometry was used to quantify HIV-specific memory CD8+ T cells (FIG. 12A), as well as HIV-specific central memory (T_{cm}) and effector memory (T_{em}) CD8+ T cells (FIG. 12B). CD38_{VHI}/CD28_{sup} x CD3mid trispecific antibody activated T cells and promoted the proliferation of effector memory (T_{em}) CD8+ T cells.

[0039] FIGS. 13A-13B show the characterization of *in vitro* T cell subset expansion in PBMCs collected from influenza-infected Donor A in response to CD38_{VHI}/CD28_{sup} x CD3mid trispecific antibody. T cell populations were measured at indicated time points. The triple mutant trispecific antibody was used as negative control. Flow cytometry was used to quantify influenza (Flu)-specific memory CD8+ T cells (FIG. 13A), as well as Flu-specific central memory (T_{cm}) and effector memory (T_{em}) CD8+ T cells (FIG. 13B). CD38_{VHI}/CD28_{sup} x CD3mid trispecific antibody activated T cells and promoted the proliferation of T_{em} CD8+ T cells (*e.g.*, see days 7, 11) and T_{cm} CD8+ T cells (*e.g.*, see day 7).

DETAILED DESCRIPTION

[0040] The disclosure provides trispecific binding proteins comprising four polypeptide chains that form three antigen binding sites that specifically bind a CD38 polypeptide (*e.g.*, human and cynomolgus monkey CD38 polypeptides), a CD28 polypeptide, and a CD3

polypeptide, which may find use, *e.g.*, in expanding memory T cells (*e.g.*, virus-specific memory T cells) and/or treating chronic viral infection.

I. General Definitions

[0041] 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. As used in this specification and the appended claims, the singular forms “a”, “an” and “the” include plural referents unless the content clearly dictates otherwise. Thus, for example, reference to “a molecule” optionally includes a combination of two or more such molecules, and the like.

[0042] It is understood that aspects and embodiments of the present disclosure described herein include “comprising,” “consisting,” and “consisting essentially of” aspects and embodiments.

[0043] 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, phosphoraniladate and phosphoroamidate. The term “polynucleotide” specifically includes single-stranded and double-stranded forms of DNA.

[0044] 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.

[0045] 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).

[0046] 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.

[0047] 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.

[0048] 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.

[0049] In some embodiments, CDR/FR definition in an immunoglobulin light or heavy chain is to be determined based on IMGT definition (Lefranc et al. *Dev. Comp. Immunol.*, 2003, 27(1):55-77; www.imgt.org).

[0050] 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. 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, IgA2, and IgG4). 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.

[0051] 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.

[0052] 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.

[0053] 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, *e.g.*, a CD38 polypeptide of the present disclosure

[0054] A "recombinant" molecule is one that has been prepared, expressed, created, or isolated by recombinant means.

[0055] 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).

[0056] 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."

[0057] 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.

[0058] "CD38" is cluster of differentiation 38 polypeptide and is a glycoprotein found on the surface of many immune cells. In some embodiments, a binding protein of the present disclosure binds the extracellular domain of one or more CD38 polypeptide. Exemplary CD38 extracellular domain polypeptide sequences include, but are not limited

to, the extracellular domain of human CD38 (*e.g.*, as represented by SEQ ID NO:1) and the extracellular domain of cynomolgus monkey CD38 (*e.g.*, as represented by SEQ ID NO:30).

[0059] 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.

[0060] The term "monospecific binding protein" refers to a binding protein that specifically binds to one antigen target.

[0061] The term "monovalent binding protein" refers to a binding protein that has one antigen binding site.

[0062] The term "bispecific binding protein" refers to a binding protein that specifically binds to two different antigen targets. In some embodiments, a bispecific binding protein binds to two different antigens. In some embodiments, a bispecific binding protein binds to two different epitopes on the same antigen.

[0063] The term "bivalent binding protein" refers to a binding protein that has two binding sites.

[0064] The term "trispecific binding protein" refers to a binding protein that specifically binds to three different antigen targets. In some embodiments, a trispecific binding protein binds to three different antigens. In some embodiments, a trispecific binding protein binds to one, two, or three different epitopes on the same antigen.

[0065] 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.

[0066] 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.

[0067] 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 macromolecular 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.

[0068] 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.

[0069] 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.

[0070] The term "binds to" as used herein in reference to a binding protein refers to the ability of a binding protein or an antigen-binding fragment thereof to bind to an antigen containing an epitope with an K_d 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. In some embodiments, a binding protein of the present disclosure binds to two or more antigens, *e.g.*, a human and a cynomolgus monkey CD38 polypeptide.

[0071] In some embodiments, an antigen binding domain and/or binding protein of the present disclosure "cross reacts" with human and cynomolgus monkey CD38 polypeptides, *e.g.*, CD38 extracellular domains, such as SEQ ID NO:1 (human CD38 isoform A), SEQ ID NO:105 (human CD38 isoform E) and SEQ ID NO:30 (cynomolgus monkey CD38). A binding protein binding to antigen 1 (Ag1) is "cross-reactive" to antigen 2 (Ag2) when the EC_{50} s are in a similar range for both antigens. In the present application, a binding protein binding to Ag1 is cross-reactive to Ag2 when the ratio of affinity of Ag2 to affinity of Ag1 is equal or less than 10 (for instance 5, 2, 1 or 0.5), affinities being measured with the same method for both antigens.

[0072] A binding protein binding to Ag1 is "not significantly cross-reactive" to Ag2 when the affinities are very different for the two antigens. Affinity for Ag2 may not be measurable if the binding response is too low. In the present application, a binding protein binding to Ag1 is not significantly cross-reactive to Ag2, when the binding response of the binding protein to Ag2 is less than 5% of the binding response of the same binding protein to Ag1 in the same experimental setting and at the same antibody concentration. In practice, the binding protein concentration used can be the EC_{50} or the concentration required to reach the saturation plateau obtained with Ag1.

[0073] 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 L₁, 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 L₂, 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 L₃, which is located between the C-terminus of the V_{H1} and the N-terminus of the V_{H2} domain; and L₄, which is located between the C-terminus of the V_{H2} and the N-terminus of the C_{H1} domain.

[0074] 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.

[0075] 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.

[0076] 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.

[0077] 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.

[0078] 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.

[0079] 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.

[0080] 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.

[0081] 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.

[0082] The term "patient" as used herein includes human and animal subjects (*e.g.*, mammals, such as dogs, pigs, horses, cats, cows, etc.).

[0083] 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 chronic viral infection, or ameliorate chronic viral infection in a human subject.

[0084] 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.

[0085] 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.

[0086] 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.

[0087] One embodiment of the disclosure provides a pharmaceutical composition comprising a pharmaceutically acceptable carrier and a therapeutically effective amount of a binding protein.

Trispecific binding proteins

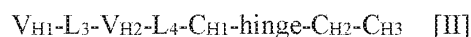
[0088] Certain aspects of the present disclosure relate to trispecific binding proteins (*e.g.*, that bind CD38, CD28, and CD3 polypeptides). Any of the CDRs or variable domains of any of the antigen binding proteins described herein may find use in a trispecific binding protein of the present disclosure.

[0089] In some embodiments, the binding protein of the disclosure is a trispecific binding protein comprising four polypeptide chains that form three antigen binding sites

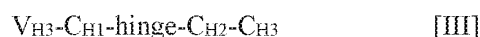
that bind one or more (*e.g.*, three) different antigen targets or target proteins. In some embodiments, a first polypeptide chain comprises a structure represented by the formula:



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



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



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



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. In some embodiments, the first polypeptide chain and the second polypeptide chain have a cross-over orientation that forms two distinct antigen binding sites.

[0090] In some embodiments, V_{H1} and V_{L1} form a first antigen binding site that binds a CD28 polypeptide, wherein V_{H2} and V_{L2} form a second antigen binding site that binds a CD3 polypeptide, and wherein V_{H3} and V_{L3} form a third antigen binding site that binds a CD38 polypeptide. In some embodiments, the CD28 polypeptide is a human CD28 polypeptide. In some embodiments, the CD3 polypeptide is a human CD3 polypeptide. In some embodiments, the CD38 polypeptide is a human CD38 polypeptide. In some

embodiments, the trispecific binding protein comprises one or more antigen binding sites described *infra*.

[0091] 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. In some embodiments, a binding protein of the present disclosure is an antibody. In some embodiments, the antibody is a monoclonal antibody. In some embodiments, the antibody is a chimeric, humanized, or human antibody.

Anti-CD38 Binding Sites

[0092] Certain aspects of the present disclosure relate to binding proteins that comprise an antigen binding site that binds a CD38 polypeptide (*e.g.*, human and cynomolgus monkey CD38 polypeptides).

[0093] In some embodiments, a binding protein or antigen-binding fragment thereof cross-reacts with human CD38 (*e.g.*, a human CD38 isoform A and/or isoform E polypeptide) and cynomolgus monkey CD38. In some embodiments, a binding protein induces apoptosis of a CD38+ cell. In some embodiments, a binding protein recruits a T cell to a CD38+ cell and optionally activates the T cell (*e.g.*, through TCR stimulation and/or costimulation).

[0094] In some embodiments, a binding site that binds CD38 comprises: an antibody heavy chain variable (VH) domain comprising a CDR-H1 sequence comprising the amino acid sequence of GYTFTSFN (SEQ ID NO:31) or GYTFTSYA (SEQ ID NO:37), a CDR-H2 sequence comprising the amino acid sequence of IYPGNGGT (SEQ ID NO:32) or IYPGQGGT (SEQ ID NO:38), and a CDR-H3 sequence comprising the amino acid sequence of ARTGGLRRAYFTY (SEQ ID NO:33); and/or an antibody light chain variable (VL) domain comprising a CDR-L1 sequence comprising the amino acid sequence of ESVDSYGNGF (SEQ ID NO:34) or QSVSSYGQGF (SEQ ID NO:39), a CDR-L2 sequence comprising the amino acid sequence of LAS (SEQ ID NO:35) or GAS (SEQ ID NO:40), and a CDR-L3 sequence comprising the amino acid sequence of QQNKEDPWT (SEQ ID NO:36). In some embodiments, a binding site that binds CD38 comprises: an antibody heavy chain variable (VH) domain comprising a CDR-H1 sequence comprising the amino acid sequence of GYTFTSFN (SEQ ID NO:31) or GYTFTSYA (SEQ ID NO:37), a CDR-H2 sequence comprising the amino acid sequence of IYPGNGGT (SEQ ID NO:32) or IYPGQGGT (SEQ ID NO:38), and a CDR-H3 sequence comprising the amino

acid sequence of ARTGGLRRAYFTY (SEQ ID NO:33); and/or an antibody light chain variable (VL) domain comprising a CDR-L1 sequence comprising the amino acid sequence of ESVDSYGNGF (SEQ ID NO:34) or QSVSSYGQG (SEQ ID NO:132), a CDR-L2 sequence comprising the amino acid sequence of LAS (SEQ ID NO:35) or GAS (SEQ ID NO:40), and a CDR-L3 sequence comprising the amino acid sequence of QQNKEDPWT (SEQ ID NO:36). In some embodiments, the binding proteins comprise 1, 2, 3, 4, 5, or 6 CDRs from an antibody VH and/or VL domain sequence of antiCD38_C2-CD38-1, antiCD38_C2-CD38-1_VH1-VL1, antiCD38_C2-CD38-1_VH3-VL3, antiCD38_C2-CD38-1_VH5-VL3, antiCD38_C2-CD38-1_VH6-VL3, CD38^{HHY1370} (may also be referred to herein as antiCD38_1370), antiCD38_C2-CD38-1_VH1-VL1xCD28supxCD3mid IgG4 FALA, antiCD38_C2-CD38-1_VH1-VL1xCD28supxCD3mid IgG1LALA P329A, antiCD38_C2-CD38-1_VH1-VL1xCD28supxCD3mid IgG1 NNSA, CD38^{HHY1370}xCD28supxCD3mid IgG4 FALA, CD38^{HHY1370}xCD28supxCD3mid IgG1LALA P329A, or CD38^{HHY1370}xCD28supxCD3mid IgG1 NNSA, as shown in Table G, H, or I.

[0095] In some embodiments, a binding site that binds CD38 comprises: an antibody heavy chain variable (VH) domain comprising a CDR-H1 sequence comprising the amino acid sequence of GYTFTSFN (SEQ ID NO:31) or GYTFTSYA (SEQ ID NO:37), a CDR-H2 sequence comprising the amino acid sequence of IYPGNGGT (SEQ ID NO:32) or IYPGQGGT (SEQ ID NO:38), and a CDR-H3 sequence comprising the amino acid sequence of ARTGGLRRAYFTY (SEQ ID NO:33); and an antibody light chain variable (VL) domain comprising a CDR-L1 sequence comprising the amino acid sequence of ESVDSYGNGF (SEQ ID NO:34) or QSVSSYGQGF (SEQ ID NO:39), a CDR-L2 sequence comprising the amino acid sequence of LAS (SEQ ID NO:35) or GAS (SEQ ID NO:40), and a CDR-L3 sequence comprising the amino acid sequence of QQNKEDPWT (SEQ ID NO:36).

[0096] In some embodiments, a binding site that binds CD38 comprises: an antibody heavy chain variable (VH) domain comprising a CDR-H1 sequence comprising the amino acid sequence of GYTFTSFN (SEQ ID NO:31), a CDR-H2 sequence comprising the amino acid sequence of IYPGNGGT (SEQ ID NO:32), and a CDR-H3 sequence comprising the amino acid sequence of ARTGGLRRAYFTY (SEQ ID NO:33); and/or an antibody light chain variable (VL) domain comprising a CDR-L1 sequence comprising the amino acid sequence of ESVDSYGNGF (SEQ ID NO:34), a CDR-L2 sequence comprising the amino acid sequence of LAS (SEQ ID NO:35), and a CDR-L3 sequence comprising the amino

acid sequence of QQNKEDPWT (SEQ ID NO:36). In some embodiments, a binding site that binds CD38 comprises: an antibody heavy chain variable (VH) domain comprising a CDR-H1 sequence comprising the amino acid sequence of GYTFTSFN (SEQ ID NO:31), a CDR-H2 sequence comprising the amino acid sequence of IYPGNGGT (SEQ ID NO:32), and a CDR-H3 sequence comprising the amino acid sequence of ARTGGLRRAYFTY (SEQ ID NO:33); and an antibody light chain variable (VL) domain comprising a CDR-L1 sequence comprising the amino acid sequence of ESVDSYGNGF (SEQ ID NO:34), a CDR-L2 sequence comprising the amino acid sequence of LAS (SEQ ID NO:35), and a CDR-L3 sequence comprising the amino acid sequence of QQNKEDPWT (SEQ ID NO:36). In other embodiments, a binding site that binds CD38 comprises: an antibody heavy chain variable (VH) domain comprising a CDR-H1 sequence comprising the amino acid sequence of GYTFTSYA (SEQ ID NO:37), a CDR-H2 sequence comprising the amino acid sequence of IYPGQGGT (SEQ ID NO:38), and a CDR-H3 sequence comprising the amino acid sequence of ARTGGLRRAYFTY (SEQ ID NO:33); and/or an antibody light chain variable (VL) domain comprising a CDR-L1 sequence comprising the amino acid sequence of QSVSSYGQGF (SEQ ID NO:39), a CDR-L2 sequence comprising the amino acid sequence of GAS (SEQ ID NO:40), and a CDR-L3 sequence comprising the amino acid sequence of QQNKEDPWT (SEQ ID NO:36). In some embodiments, a binding site that binds CD38 comprises: an antibody heavy chain variable (VH) domain comprising a CDR-H1 sequence comprising the amino acid sequence of GYTFTSYA (SEQ ID NO:37), a CDR-H2 sequence comprising the amino acid sequence of IYPGQGGT (SEQ ID NO:38), and a CDR-H3 sequence comprising the amino acid sequence of ARTGGLRRAYFTY (SEQ ID NO:33); and an antibody light chain variable (VL) domain comprising a CDR-L1 sequence comprising the amino acid sequence of QSVSSYGQGF (SEQ ID NO:39), a CDR-L2 sequence comprising the amino acid sequence of GAS (SEQ ID NO:40), and a CDR-L3 sequence comprising the amino acid sequence of QQNKEDPWT (SEQ ID NO:36).

[0097] In some embodiments, the VH domain comprises the sequence, from N-terminus to C-terminus, FR1—CDR-H1—FR2—CDR-H2—FR3—CDR-H3—FR4; where FR1 comprises the sequence QVQLVQSGAEVVKPGASVKVSCKAS (SEQ ID NO:86), QVQLVQSGAEVVKSGASVKVSCKAS (SEQ ID NO:87), or QVQLVQSGAEVVKPGASVKMSCKAS (SEQ ID NO:88); where FR2 comprises the sequence MHWVKEAPGQRLEWIGY (SEQ ID NO:90) or MHWVKEAPGQGLEWIGY (SEQ ID NO:91); where FR3 comprises the sequence

NYNQKFQGRATLTADTSASTAYMELSSLRSEDVAVYFC (SEQ ID NO:93) or NYNQKFQGRATLTADTSASTAYMEISSLRSEDVAVYFC (SEQ ID NO:94); and where FR4 comprises the sequence WGQGTLVTVSS (SEQ ID NO:96). In some embodiments, the VL domain comprises the sequence, from N-terminus to C-terminus, FR1—CDR-L1—FR2—CDR-L2—FR3—CDR-L3—FR4; where FR1 comprises the sequence DIVLTQSPATLSLSPGERATISCRAS (SEQ ID NO:97); where FR2 comprises the sequence MHWYQQKPGQPRLLIY (SEQ ID NO:99); where FR3 comprises the sequence SRATGIPARFSGSGSGTDFLTISPLEDFAVYYC (SEQ ID NO:101); and where FR4 comprises the sequence FGGGTKLEIK (SEQ ID NO:103).

[0098] In some embodiments, the VH domain comprises an amino acid sequence that is at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the amino acid sequence of SEQ ID NO:5; and/or the VL domain comprises an amino acid sequence that is at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the amino acid sequence of SEQ ID NO:6. In some embodiments, the VH domain comprises an amino acid sequence that is at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the amino acid sequence of SEQ ID NO:17; and/or the VL domain comprises an amino acid sequence that is at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the amino acid sequence of SEQ ID NO:18. In some embodiments, the VH domain comprises an amino acid sequence that is at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the amino acid sequence of SEQ ID NO:21; and/or the VL domain comprises an amino acid sequence that is at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the amino acid sequence of SEQ ID NO:18. In some embodiments, the VH domain comprises an amino acid sequence that is at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%,

at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the amino acid sequence of SEQ ID NO:23; and/or the VL domain comprises an amino acid sequence that is at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the amino acid sequence of SEQ ID NO:18. In some embodiments, the VH domain comprises an amino acid sequence that is at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the amino acid sequence of SEQ ID NO:13; and/or the VL domain comprises an amino acid sequence that is at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the amino acid sequence of SEQ ID NO:14.

[0099] In some embodiments, the VH domain comprises an amino acid sequence that is at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the amino acid sequence of SEQ ID NO:5; and the VL domain comprises an amino acid sequence that is at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the amino acid sequence of SEQ ID NO:6. In some embodiments, the VH domain comprises an amino acid sequence that is at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the amino acid sequence of SEQ ID NO:17; and the VL domain comprises an amino acid sequence that is at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the amino acid sequence of SEQ ID NO:18. In some embodiments, the VH domain comprises an amino acid sequence that is at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the amino acid sequence of SEQ ID NO:21; and the VL domain comprises an amino acid sequence that is at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%,

at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the amino acid sequence of SEQ ID NO:18. In some embodiments, the VH domain comprises an amino acid sequence that is at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the amino acid sequence of SEQ ID NO:23; and the VL domain comprises an amino acid sequence that is at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the amino acid sequence of SEQ ID NO:18. In some embodiments, the VH domain comprises an amino acid sequence that is at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the amino acid sequence of SEQ ID NO:13; and the VL domain comprises an amino acid sequence that is at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the amino acid sequence of SEQ ID NO:14.

[0100] In some embodiments, the VH domain comprises the amino acid sequence of SEQ ID NO:5; and the VL domain comprises the amino acid sequence of SEQ ID NO:6. In some embodiments, the VH domain comprises the amino acid sequence of SEQ ID NO:17; and the VL domain comprises the amino acid sequence of SEQ ID NO:18. In some embodiments, the VH domain comprises the amino acid sequence of SEQ ID NO:21; and the VL domain comprises the amino acid sequence of SEQ ID NO:18. In some embodiments, the VH domain comprises the amino acid sequence of SEQ ID NO:23; and the VL domain comprises the amino acid sequence of SEQ ID NO:18. In some embodiments, the VH domain comprises the amino acid sequence of SEQ ID NO:13; and the VL domain comprises the amino acid sequence of SEQ ID NO:14.

[0101] In some embodiments, a binding site that binds CD38 comprises: an antibody heavy chain comprising the amino acid sequence of SEQ ID NO:7 and/or an antibody light chain comprising the amino acid sequence of SEQ ID NO:8. In some embodiments, a binding protein of the present disclosure comprises an antibody heavy chain comprising the amino acid sequence of SEQ ID NO:19 and/or an antibody light chain comprising the amino acid sequence of SEQ ID NO:20. In some embodiments, a binding protein of the present disclosure comprises an antibody heavy chain comprising the amino acid sequence of SEQ

ID NO:22 and/or an antibody light chain comprising the amino acid sequence of SEQ ID NO:20. In some embodiments, a binding protein of the present disclosure comprises an antibody heavy chain comprising the amino acid sequence of SEQ ID NO:24 and/or an antibody light chain comprising the amino acid sequence of SEQ ID NO:20. In some embodiments, a binding protein of the present disclosure comprises an antibody heavy chain comprising the amino acid sequence of SEQ ID NO:15 and/or an antibody light chain comprising the amino acid sequence of SEQ ID NO:16.

[0102] In some embodiments, a binding protein of the present disclosure comprises an antibody heavy chain comprising the amino acid sequence of SEQ ID NO:7 and an antibody light chain comprising the amino acid sequence of SEQ ID NO:8. In some embodiments, a binding protein of the present disclosure comprises an antibody heavy chain comprising the amino acid sequence of SEQ ID NO:19 and an antibody light chain comprising the amino acid sequence of SEQ ID NO:20. In some embodiments, a binding protein of the present disclosure comprises an antibody heavy chain comprising the amino acid sequence of SEQ ID NO:22 and an antibody light chain comprising the amino acid sequence of SEQ ID NO:20. In some embodiments, a binding protein of the present disclosure comprises an antibody heavy chain comprising the amino acid sequence of SEQ ID NO:24 and an antibody light chain comprising the amino acid sequence of SEQ ID NO:20. In some embodiments, a binding protein of the present disclosure comprises an antibody heavy chain comprising the amino acid sequence of SEQ ID NO:15 and an antibody light chain comprising the amino acid sequence of SEQ ID NO:16.

[0103] In some embodiments, a binding site that binds CD38 comprises: an antibody heavy chain variable (VH) domain comprising a CDR-H1 sequence comprising the amino acid sequence of GFTFSSYG (SEQ ID NO:41), a CDR-H2 sequence comprising the amino acid sequence of IWYDGSNK (SEQ ID NO:42), and a CDR-H3 sequence comprising the amino acid sequence of ARMFRGAFDY (SEQ ID NO:43); and/or an antibody light chain variable (VL) domain comprising a CDR-L1 sequence comprising the amino acid sequence of QGIRND (SEQ ID NO:44), a CDR-L2 sequence comprising the amino acid sequence of AAS (SEQ ID NO:45), and a CDR-L3 sequence comprising the amino acid sequence of LQDYIYYPT (SEQ ID NO:46). In some embodiments, a binding site that binds CD38 comprises: an antibody heavy chain variable (VH) domain comprising a CDR-H1 sequence comprising the amino acid sequence of GFTFSSYG (SEQ ID NO:41), a CDR-H2 sequence comprising the amino acid sequence of IWYDGSNK (SEQ ID NO:42), and a CDR-H3 sequence comprising the amino acid sequence of ARMFRGAFDY (SEQ ID NO:43); and an

antibody light chain variable (VL) domain comprising a CDR-L1 sequence comprising the amino acid sequence of QGIRND (SEQ ID NO:44), a CDR-L2 sequence comprising the amino acid sequence of AAS (SEQ ID NO:45), and a CDR-L3 sequence comprising the amino acid sequence of LQDYIYYPT (SEQ ID NO:46).

[0104] In some embodiments, the VH domain comprises the sequence, from N-terminus to C-terminus, FR1—CDR-H1—FR2—CDR-H2—FR3—CDR-H3—FR4; where FR1 comprises the sequence QVQLVESGGGVVQPGRSLRLSCAAS (SEQ ID NO:89); where FR2 comprises the sequence MHWVRQAPGKGLEWVAV (SEQ ID NO:92); where FR3 comprises the sequence YYADSVKGRFTISGDNSKNTLYLQMNSLRAEDTAVYYC (SEQ ID NO:95); and where FR4 comprises the sequence WGQGTLVTVSS (SEQ ID NO:96). In some embodiments, the VL domain comprises the sequence, from N-terminus to C-terminus, FR1—CDR-L1—FR2—CDR-L2—FR3—CDR-L3—FR4; where FR1 comprises the sequence AIQMTQSPSSLSASVGDRVTITCRAS (SEQ ID NO:98); where FR2 comprises the sequence GWYQQKPGKAPKLLIY (SEQ ID NO:100); where FR3 comprises the sequence SLQSGVPSRFSGSGSGTDFLTISGLQPEDSATYYC (SEQ ID NO:102); and where FR4 comprises the sequence WGQGTLVTVSS (SEQ ID NO:104).

[0105] In some embodiments, the VH domain comprises an amino acid sequence that is at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the amino acid sequence of SEQ ID NO:9; and/or the VL domain comprises an amino acid sequence that is at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the amino acid sequence of SEQ ID NO:10.

[0106] In some embodiments, the VH domain comprises an amino acid sequence that is at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the amino acid sequence of SEQ ID NO:9; and the VL domain comprises an amino acid sequence that is at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the amino acid sequence of SEQ ID NO:10. In some embodiments, the VH domain comprises the amino acid sequence of SEQ ID NO:9; and the VL domain comprises the amino acid sequence of SEQ ID NO:10.

[0107] In some embodiments, a binding protein of the present disclosure comprises an antibody heavy chain comprising the amino acid sequence of SEQ ID NO:11 or an antibody light chain comprising the amino acid sequence of SEQ ID NO:12. In some embodiments, a binding protein of the present disclosure comprises an antibody heavy chain comprising the amino acid sequence of SEQ ID NO:11 and an antibody light chain comprising the amino acid sequence of SEQ ID NO:12.

Anti-CD28 Binding Sites

[0108] Certain aspects of the present disclosure relate to binding proteins that comprise an antigen binding site that binds a CD28 polypeptide (*e.g.*, a human CD28 polypeptide).

[0109] In some embodiments, a binding site that binds CD28 comprises: an antibody heavy chain variable (VH) domain comprising a CDR-H1 sequence comprising the amino acid sequence of GYTFTSYY (SEQ ID NO:108), a CDR-H2 sequence comprising the amino acid sequence of IYPGNVNT (SEQ ID NO:109), and a CDR-H3 sequence comprising the amino acid sequence of TRSHYGLDWNFDV (SEQ ID NO:110) and/or an antibody light chain variable (VL) domain comprising a CDR-L1 sequence comprising the amino acid sequence of QNIYVW (SEQ ID NO:111), a CDR-L2 sequence comprising the amino acid sequence of KAS (SEQ ID NO:112), and a CDR-L3 sequence comprising the amino acid sequence of QQGQTYPY (SEQ ID NO:113). In some embodiments, a binding site that binds CD28 comprises: an antibody heavy chain variable (VH) domain comprising a CDR-H1 sequence comprising the amino acid sequence of GYTFTSYY (SEQ ID NO:108), a CDR-H2 sequence comprising the amino acid sequence of IYPGNVNT (SEQ ID NO:109), and a CDR-H3 sequence comprising the amino acid sequence of TRSHYGLDWNFDV (SEQ ID NO:110) and an antibody light chain variable (VL) domain comprising a CDR-L1 sequence comprising the amino acid sequence of QNIYVW (SEQ ID NO:111), a CDR-L2 sequence comprising the amino acid sequence of KAS (SEQ ID NO:112), and a CDR-L3 sequence comprising the amino acid sequence of QQGQTYPY (SEQ ID NO:113).

[0110] In some embodiments, a binding site that binds CD28 comprises: an antibody heavy chain variable (VH) domain comprising a CDR-H1 sequence comprising the amino acid sequence of GFSLSDYG (SEQ ID NO:114), a CDR-H2 sequence comprising the amino acid sequence of IWAGGGT (SEQ ID NO:115), and a CDR-H3 sequence comprising the amino acid sequence of ARDKGYSYYYSMDY (SEQ ID NO:116) and/or an antibody light chain variable (VL) domain comprising a CDR-L1 sequence comprising the amino acid sequence of ESVEYYVTSL (SEQ ID NO:117), a CDR-L2 sequence comprising the amino

acid sequence of AAS (SEQ ID NO:118), and a CDR-L3 sequence comprising the amino acid sequence of QQSRKVPYT (SEQ ID NO:119). In some embodiments, a binding site that binds CD28 comprises: an antibody heavy chain variable (VH) domain comprising a CDR-H1 sequence comprising the amino acid sequence of GFSLSDYG (SEQ ID NO:114), a CDR-H2 sequence comprising the amino acid sequence of IWAGGGT (SEQ ID NO:115), and a CDR-H3 sequence comprising the amino acid sequence of ARDKGYSYYYSMDY (SEQ ID NO:116) and an antibody light chain variable (VL) domain comprising a CDR-L1 sequence comprising the amino acid sequence of ESVEYYVTSL (SEQ ID NO:117), a CDR-L2 sequence comprising the amino acid sequence of AAS (SEQ ID NO:118), and a CDR-L3 sequence comprising the amino acid sequence of QQSRKVPYT (SEQ ID NO:119).

Anti-CD3 Binding Sites

[0111] Certain aspects of the present disclosure relate to binding proteins that comprise an antigen binding site that binds a CD3 polypeptide (*e.g.*, a human CD3 polypeptide).

[0112] In some embodiments, a binding site that binds CD3 comprises: an antibody heavy chain variable (VH) domain comprising a CDR-H1 sequence comprising the amino acid sequence of GFTFTKAW (SEQ ID NO:120), a CDR-H2 sequence comprising the amino acid sequence of IKDKSNSYAT (SEQ ID NO:121), and a CDR-H3 sequence comprising the amino acid sequence of RGVYYALSPFDY (SEQ ID NO:122) and/or an antibody light chain variable (VL) domain comprising a CDR-L1 sequence comprising the amino acid sequence of QSLVHNNANTY (SEQ ID NO:123), a CDR-L2 sequence comprising the amino acid sequence of KVS (SEQ ID NO:124), and a CDR-L3 sequence comprising the amino acid sequence of GQGTQYPFT (SEQ ID NO:125). In some embodiments, a binding site that binds CD3 comprises: an antibody heavy chain variable (VH) domain comprising a CDR-H1 sequence comprising the amino acid sequence of GFTFTKAW (SEQ ID NO:120), a CDR-H2 sequence comprising the amino acid sequence of IKDKSNSYAT (SEQ ID NO:121), and a CDR-H3 sequence comprising the amino acid sequence of RGVYYALSPFDY (SEQ ID NO:122) and an antibody light chain variable (VL) domain comprising a CDR-L1 sequence comprising the amino acid sequence of QSLVHNNANTY (SEQ ID NO:123), a CDR-L2 sequence comprising the amino acid sequence of KVS (SEQ ID NO:124), and a CDR-L3 sequence comprising the amino acid sequence of GQGTQYPFT (SEQ ID NO:125).

[0113] In some embodiments, a binding site that binds CD3 comprises: an antibody heavy chain variable (VH) domain comprising a CDR-H1 sequence comprising the amino

acid sequence of GFTFTKAW (SEQ ID NO:126), a CDR-H2 sequence comprising the amino acid sequence of IKDKSNSYAT (SEQ ID NO:127), and a CDR-H3 sequence comprising the amino acid sequence of GVYYALSPFDY (SEQ ID NO:128) and/or an antibody light chain variable (VL) domain comprising a CDR-L1 sequence comprising the amino acid sequence of QSLVHNNGNTY (SEQ ID NO:129), a CDR-L2 sequence comprising the amino acid sequence of KVS (SEQ ID NO:130), and a CDR-L3 sequence comprising the amino acid sequence of GQGTQYPFT (SEQ ID NO:131).

[0114] In some embodiments of any of the trispecific binding proteins of the present disclosure, one antigen binding domain binds to a CD3 polypeptide (e.g., human CD3) and one antigen binding domain binds to a CD28 polypeptide (e.g., human CD28). In some embodiments, the V_{H1} domain comprises three CDRs from SEQ ID NOs:49 or 51 as shown in Table H, and the V_{L1} domain comprises three CDRs from SEQ ID NOs:50 or 52 as shown in Table H. In some embodiments, the V_{H2} domain comprises three CDRs from SEQ ID NOs:49 or 51 as shown in Table H, and the V_{L2} domain comprises three CDRs from SEQ ID NOs:50 or 52 as shown in Table H. In some embodiments, the V_{H1} domain comprises three CDRs from SEQ ID NOs:53 or 84 as shown in Table H, and the V_{L1} domain comprises three CDRs from SEQ ID NOs:54 or 85 as shown in Table H. In some embodiments, the V_{H2} domain comprises three CDRs from SEQ ID NOs:53 or 84 as shown in Table H, and the V_{L2} domain comprises three CDRs from SEQ ID NOs:54 or 85 as shown in Table H.

[0115] In some embodiments, the V_{H1} domain comprises the amino acid sequence of SEQ ID NO:49, the V_{L1} domain comprises the amino acid sequence of SEQ ID NO:50, the V_{H2} domain comprises the amino acid sequence of SEQ ID NO:53, and the V_{L2} domain comprises the amino acid sequence of SEQ ID NO:54. In some embodiments, the V_{H2} domain comprises the amino acid sequence of SEQ ID NO:49, the V_{L2} domain comprises the amino acid sequence of SEQ ID NO:50, the V_{H1} domain comprises the amino acid sequence of SEQ ID NO:53, and the V_{L1} domain comprises the amino acid sequence of SEQ ID NO:54. In some embodiments, the V_{H1} domain comprises the amino acid sequence of SEQ ID NO:51, the V_{L1} domain comprises the amino acid sequence of SEQ ID NO:52, the V_{H2} domain comprises the amino acid sequence of SEQ ID NO:53, and the V_{L2} domain comprises the amino acid sequence of SEQ ID NO:54. In some embodiments, the V_{H2} domain comprises the amino acid sequence of SEQ ID NO:51, the V_{L2} domain comprises the amino acid sequence of SEQ ID NO:52, the V_{H1} domain comprises the amino acid sequence of SEQ ID NO:53, and the V_{L1} domain comprises the amino acid sequence of SEQ ID NO:54.

[0116] In some embodiments, the V_{H1} domain comprises the amino acid sequence of SEQ ID NO:49, the V_{L1} domain comprises the amino acid sequence of SEQ ID NO:50, the V_{H2} domain comprises the amino acid sequence of SEQ ID NO:53, the V_{L2} domain comprises the amino acid sequence of SEQ ID NO:54, the V_{H3} domain comprises the amino acid sequence of SEQ ID NO:13, and the V_{L3} domain comprises the amino acid sequence of SEQ ID NO:14. In some embodiments, the V_{H1} domain comprises the amino acid sequence of SEQ ID NO:49, the V_{L1} domain comprises the amino acid sequence of SEQ ID NO:50, the V_{H2} domain comprises the amino acid sequence of SEQ ID NO:53, the V_{L2} domain comprises the amino acid sequence of SEQ ID NO:54, the V_{H3} domain comprises the amino acid sequence of SEQ ID NO:9, and the V_{L3} domain comprises the amino acid sequence of SEQ ID NO:10.

[0117] In certain embodiments, the first polypeptide chain comprises a polypeptide sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO:61, the second polypeptide chain comprises a polypeptide sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO:60, the third polypeptide chain comprises a polypeptide sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO:62, and the fourth polypeptide chain comprises a polypeptide sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO:63. In certain embodiments, the first polypeptide chain comprises a polypeptide sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO:61, the second polypeptide chain comprises a polypeptide sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO:64, the third polypeptide chain comprises a polypeptide sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO:65, and the fourth polypeptide chain comprises a polypeptide sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO:63. In certain embodiments, the first polypeptide chain comprises a polypeptide sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO:61, the second polypeptide chain comprises a polypeptide sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO:66, the third polypeptide chain comprises a polypeptide sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO:67, and the fourth polypeptide chain comprises a polypeptide sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO:63. In certain embodiments, the first polypeptide chain comprises a polypeptide sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO:61, the second polypeptide chain comprises a polypeptide sequence that is at least 95% identical to the amino acid sequence of SEQ ID

NO:60, the third polypeptide chain comprises a polypeptide sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO:68, and the fourth polypeptide chain comprises a polypeptide sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO:69. In certain embodiments, the first polypeptide chain comprises a polypeptide sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO:61, the second polypeptide chain comprises a polypeptide sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO:64, the third polypeptide chain comprises a polypeptide sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO:70, and the fourth polypeptide chain comprises a polypeptide sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO:69. In certain embodiments, the first polypeptide chain comprises a polypeptide sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO:61, the second polypeptide chain comprises a polypeptide sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO:66, the third polypeptide chain comprises a polypeptide sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO:71, and the fourth polypeptide chain comprises a polypeptide sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO:69.

[0118] In certain embodiments, the first polypeptide chain comprises the amino acid sequence of SEQ ID NO:61, the second polypeptide chain comprises the amino acid sequence of SEQ ID NO:60, the third polypeptide chain comprises the amino acid sequence of SEQ ID NO:62, and the fourth polypeptide chain comprises the amino acid sequence of SEQ ID NO:63. In certain embodiments, the first polypeptide chain comprises the amino acid sequence of SEQ ID NO:61, the second polypeptide chain comprises the amino acid sequence of SEQ ID NO:64, the third polypeptide chain comprises the amino acid sequence of SEQ ID NO:65, and the fourth polypeptide chain comprises the amino acid sequence of SEQ ID NO:63. In certain embodiments, the first polypeptide chain comprises the amino acid sequence of SEQ ID NO:61, the second polypeptide chain comprises the amino acid sequence of SEQ ID NO:66, the third polypeptide chain comprises the amino acid sequence of SEQ ID NO:67, and the fourth polypeptide chain comprises the amino acid sequence of SEQ ID NO:63. In certain embodiments, the first polypeptide chain comprises the amino acid sequence of SEQ ID NO:61, the second polypeptide chain comprises the amino acid sequence of SEQ ID NO:60, the third polypeptide chain comprises the amino acid sequence of SEQ ID NO:68, and the fourth polypeptide chain comprises the amino acid sequence of SEQ ID NO:69. In certain embodiments, the first polypeptide chain comprises the amino

acid sequence of SEQ ID NO:61, the second polypeptide chain comprises the amino acid sequence of SEQ ID NO:64, the third polypeptide chain comprises the amino acid sequence of SEQ ID NO:70, and the fourth polypeptide chain comprises the amino acid sequence of SEQ ID NO:69. In certain embodiments, the first polypeptide chain comprises the amino acid sequence of SEQ ID NO:61, the second polypeptide chain comprises the amino acid sequence of SEQ ID NO:66, the third polypeptide chain comprises the amino acid sequence of SEQ ID NO:71, and the fourth polypeptide chain comprises the amino acid sequence of SEQ ID NO:69.

[0119] In some embodiments, a binding protein of the present disclosure comprises 1, 2, 3, 4, 5, or 6 CDR sequences of an antibody sequence shown in Table G. In some embodiments, a binding protein of the present disclosure comprises 1, 2, 3, 4, 5, or 6 CDR sequences, a VH domain sequence, and/or a VL domain sequence of an antibody sequence shown in Table H. In some embodiments, a binding protein of the present disclosure comprises 1, 2, 3, 4, 5, or 6 CDR sequences, a VH domain sequence, and/or a VL domain sequence of an antibody sequence shown in Table I. In some embodiments, a binding protein of the present disclosure comprises 1, 2, 3, or 4 polypeptide sequences shown in Table I.

Table G. CDR sequences of anti-CD38 binding proteins.

Ab	CDR_H1	CDR_H2	CDR_H3	CDR_L1	CDR_L2	CDR_L3
antiCD38 C2- CD38-1	GYTFTSFN (SEQ ID NO:31)	IYPGNGGT (SEQ ID NO:32)	ARTGGLRRAYFTY (SEQ ID NO:33)	ESVDSYGNGF (SEQ ID NO:34)	LAS (SEQ ID NO:35)	QQNKEDPWT (SEQ ID NO:36)
antiCD38 C2- CD38- 1_VH1- VL1	GYTFTSYA (SEQ ID NO:37)	IYPGQGGT (SEQ ID NO:38)	ARTGGLRRAYFTY (SEQ ID NO:33)	QSVSSYGQGF (SEQ ID NO:39)	GAS (SEQ ID NO:40)	QQNKEDPWT (SEQ ID NO:36)
antiCD38 C2- CD38- 1_VH3- VL3	GYTFTSFN (SEQ ID NO:31)	IYPGNGGT (SEQ ID NO:32)	ARTGGLRRAYFTY (SEQ ID NO:33)	ESVDSYGNGF (SEQ ID NO:34)	LAS (SEQ ID NO:35)	QQNKEDPWT (SEQ ID NO:36)
antiCD38 C2- CD38- 1_VH5- VL3	GYTFTSFN (SEQ ID NO:31)	IYPGNGGT (SEQ ID NO:32)	ARTGGLRRAYFTY (SEQ ID NO:33)	ESVDSYGNGF (SEQ ID NO:34)	LAS (SEQ ID NO:35)	QQNKEDPWT (SEQ ID NO:36)
antiCD38 C2- CD38- 1_VH6- VL3	GYTFTSFN (SEQ ID NO:31)	IYPGNGGT (SEQ ID NO:32)	ARTGGLRRAYFTY (SEQ ID NO:33)	ESVDSYGNGF (SEQ ID NO:34)	LAS (SEQ ID NO:35)	QQNKEDPWT (SEQ ID NO:36)
CD38 ^{HHY} 1370	GFTFSSYG (SEQ ID NO:41)	IWYDGSNK (SEQ ID NO:42)	ARMFRGAFDY (SEQ ID NO:43)	QGI RND (SEQ ID NO:44)	AAS (SEQ ID NO:45)	LQDYIYYPT (SEQ ID NO:46)

Table H. Variable domain sequences of anti-CD38 and other binding proteins.

Ab	VH (protein)	VL (protein)
antiCD3 8_C2- CD38-1	QVQLQQSGAELVRSGASVKMSCK AS <u>GYTFTSF</u> NMHWVKETPGQGLE WIGYI <u>YPGN</u> GGTNYNQKFKGKAT LTADTSSTAYMQISSLTSEDSAVY FCA <u>RTGGLRRAYFTY</u> WGQGTLV TVSS (SEQ ID NO:5)	DIVLTQSPASLAVSLGQRATISC RASE <u>SVDSYGN</u> GFMHWYQQK PGOPP <u>KL</u> LIY <u>LASN</u> LESGVPARF SGSGSRDFTLTIDPVEADDAA TYYC <u>QONKEDPWTF</u> GGGTKL EIK (SEQ ID NO:6)
antiCD3 8_C2- CD38- 1_VH1- VL1	QVQLVQSGAEVVKPGASVKVSK AS <u>GYTFTSYA</u> MHWVKEAPGQRLE WIGYI <u>YPGOGG</u> TNYNQKFQGRAT LTADTSASTAYMELSSLRSED TAV YFC <u>ARTGGLRRAYFTY</u> WGQGT LTVSS (SEQ ID NO:13)	DIVLTQSPATLSLSPGERATISCR AS <u>QSVSSYGO</u> GFMHWYQQKP GQPPRLLIY <u>GASS</u> RAT GIPARFSGSGSGTDFTLTISP LEP EDFAVYYC <u>QONKEDPWTF</u> GG GTKLEIK (SEQ ID NO:14)
antiCD3 8_C2- CD38- 1_VH3- VL3	QVQLVQSGAEVVKPGASVKVSK AS <u>GYTFTSF</u> NMHWVKEAPGQRLE WIGYI <u>YPGN</u> GGTNYNQKFQGRAT LTADTSASTAYMELSSLRSED TAV YFC <u>ARTGGLRRAYFTY</u> WGQGT LTVSS (SEQ ID NO:17)	DIVLTQSPATLSLSPGERATISCR ASE <u>SVDSYGN</u> GFMHWYQQKP GQPPRLLIY <u>LASS</u> RAT GIPARFSGSGSGTDFTLTISP LEP EDFAVYYC <u>QONKEDPWTF</u> GG GTKLEIK (SEQ ID NO:18)
antiCD3 8_C2- CD38- 1_VH5- VL3	QVQLVQSGAEVVKSGASVKVSK AS <u>GYTFTSF</u> NMHWVKEAPGQGLE WIGYI <u>YPGN</u> GGTNYNQKFQGRAT LTADTSASTAYMEISSLRSED TAV YFC <u>ARTGGLRRAYFTY</u> WGQGT LTVSS (SEQ ID NO:21)	DIVLTQSPATLSLSPGERATISCR ASE <u>SVDSYGN</u> GFMHWYQQKP GQPPRLLIY <u>LASS</u> RAT GIPARFSGSGSGTDFTLTISP LEP EDFAVYYC <u>QONKEDPWTF</u> GG GTKLEIK (SEQ ID NO:18)
antiCD3 8_C2- CD38- 1_VH6- VL3	QVQLVQSGAEVVKPGASVKMSCK AS <u>GYTFTSF</u> NMHWVKEAPGQRLE WIGYI <u>YPGN</u> GGTNYNQKFQGRAT LTADTSASTAYMEISSLRSED TAV YFC <u>ARTGGLRRAYFTY</u> WGQGT LTVSS (SEQ ID NO:23)	DIVLTQSPATLSLSPGERATISCR ASE <u>SVDSYGN</u> GFMHWYQQKP GQPPRLLIY <u>LASS</u> RAT GIPARFSGSGSGTDFTLTISP LEP EDFAVYYC <u>QONKEDPWTF</u> GG GTKLEIK (SEQ ID NO:18)
CD38 ^{HH} Y1370	QVQLVESGGGVVQPGRLRLSCA AS <u>GFTFSSY</u> GMHWVRQAPGKGLE WVAVI <u>WYDGSN</u> KYYADSVKGRF TISGDN SKNTLYLQMNSLRAEDTA VYYC <u>ARMFRGA</u> FDYWGQGT LTVSS (SEQ ID NO:9)	AIQMTQSPSSLSASVGDRVITTC RAS <u>QGIRND</u> LGWYQQKPGKAP KLLIYA <u>AASS</u> LQSGVPSRFSGSGS GTDFLTITISGLQPEDSATYYC L <u>QDYIYYPT</u> FGQGTKVEIK (SEQ ID NO:10)
antiCD3 8_SB19	QVQLVQSGAEVAKPGTSVKLSCK ASGYTFTDYWMQWVKQRPQGL EWIGTIYPGDGDTGYAQKFQGKAT LTADKSSKTVYMHLSLASEDSAV YYCARGDY YGSNSLDYWGQGT SVTVSS (SEQ ID NO:47)	DIVMTQSHLSMSTSLGDPVSITC KASQDVSTVVAWYQQKPGQSP RRLIYSASYRYIGVPDRFTGSGA GTDFLTITISSVQAEDLAVYYCQ QHYSPPYTFGGGTKLEIK (SEQ ID NO:48)
Anti- CD28 ^{sup}	QVQLVQSGAEVVKPGASVKVSK AS <u>GYTFTSYI</u> HWVRQAPGQGLE WIGSI <u>YPGN</u> VNTNYAQKFQGRAT LTVDTSISTAYMELSRSLRSDDTAV	DIQMTQSPSSLSASVGDRVITTC QAS <u>QNIYVW</u> LNWYQQKPGKA PKLLIY <u>KASN</u> LHTGVPSRFSGSG SGTDFTLTITISLQPEDATYYCQ

Ab	VH (protein)	VL (protein)
	YYC <u>TRSHYGLDWNFDV</u> WGKGTT VTVSS (SEQ ID NO:49)	<u>QGQTYPTY</u> TFGQGTKLEIK (SEQ ID NO:50)
Anti- CD28 _{cvn}	QVQLQESGPGLVKPSQTLSTCTV <u>SGFSLSDYG</u> VHWVRQPPGKGLEW LGVI <u>WAGGGT</u> NYNPSLKSRTISK DTSKNQVSLKLSSVTAADTAVYY <u>CARDKGYSYYSMDY</u> WGQGTTV TVSS (SEQ ID NO:51)	DIVLTQSPASLAVSPGQRATITC RASE <u>SV EYYVTSL</u> MQWYQQKP GQPPKLLIF <u>AASN</u> VESGVPARFS SGSGSGDFTLTINPVEANDVAN YYC <u>QOSRKVPY</u> TFGQGTKLEI K (SEQ ID NO:52)
Anti- CD3 _{mid}	QVQLVESGGGVVQPGRSLRLSCA AS <u>GFTFTKAW</u> MHWVRQAPGKQL EWVAQ <u>IKDKSNSYAT</u> YYADSVKG RFTISRDDSKNTLYLQMNSLRAED TAVYYC <u>RGVYYALSPFDY</u> WGQG TLVTVSS (SEQ ID NO:53)	DIVMTQTPLSLSVTPGQPASISC KSS <u>QSLVHNNANTY</u> LSWYLQK PGQSPQSLIY <u>KVS</u> NRFSGVDPDRF SGSGSGDFTLKISRVEAEDVG VYYC <u>GOGTOYPFT</u> FGSGTKVE IK (SEQ ID NO:54)
Anti- CD3 _{low}	QVQLVESGGGVVQPGRSLRLSCA AS <u>GFTFTKAW</u> MHWVRQAPGKGL EWVAQ <u>IKDKSNSYAT</u> YYADSVKG RFTISRDN SKNTLYLQMNSLRAED TAVYYC <u>RGVYYALSPFDY</u> WGQG TLVTVSS (SEQ ID NO:84)	DIVMTQTPLSLSVTPGQPASISC KSS <u>QSLVHNNGNTY</u> LSWYLQK PGQSPQLLIY <u>KVS</u> NRFSGVDPDRF SGSGSGDFTLKISRVEAEDVG VYYC <u>GOGTOYPFT</u> FGGGTKVE IK (SEQ ID NO:85)

Note: CDR sequences are bolded and underlined in amino acid sequences above.

Table I. Full-length sequences of binding proteins.

antiCD38_C2-CD38-1_VH1-VL1xCD28supxCD3mid IgG4 FALA		
CD28supxCD3mid IgG4(hole) FALA Heavy Chain 1 <i>(e.g., a second polypeptide chain of a trispecific binding protein of the present disclosure)</i>	QVQLVQSGAEVVKPGASVKVSCASGYTF TSYYIHWVRQAPGQGLEWIGSIYPGNVNTN YAQKFQGRATLTVDTSISTAYMELSLRSD DTAVYYCTRSHYGLDWNFDVWGKGTITVT VSSSQVQLVESGGGVVQPGRSLRLSCAASG FTFTKAWMHWRQAPGKQLEWVAQIKDK SNSYATYYADSVKGRFTISRDDSKNTLYLQ MNSLRAEDTAVYYCRGVYYALSPFDYWG QGTLVTVSSRTASTKGPSVFPLAPCSRSTSE STAALGCLVKDYFPEPVTVSWNSGALTSGV HTFPAVLQSSGLYSLSSVTVPSSSLGKTKY TCNVDPHKPSNTKVDKRVESKYGPPCPPCPA PEAAGGPSVFLFPPKPKDTLMISRTPEVTCV VVDVSQEDPEVQFNWYVDGVEVHNAKTK PREEQFNSTYRVVSVLTVLHQDWLNGKEY KCKVSNKGLPSSIEKTISKAKGQPREPQVCT LPPSQEEMTKNQVSLSCAVKGFYPSDIAVE WESNGQPENNYKTTTPVLDSDGSFFLVSKL TVDKSRWQEGNVFSCSVMEALHNHYTQ KSLSLSLG	SEQ ID NO: 60
CD28supxCD3mid Light Chain 1 <i>(e.g., a first polypeptide chain of a trispecific binding protein of the present disclosure)</i>	DIVMTQTPLSLSVTPGPASISCKSSQSLVH NNANTYLSWYLQKPGQSPQSLIYKVSNRFS GVPDRFSGSGSGTDFTLKISRVEAEDVGVY YCGQGTQYPFTFGSGTKVEIKGQPKAAPDI QMTQSPSSLSASVGDRVTITCQASQNIYVW LNWYQQKPGKAPKLLIYKASNLHTGVPSRF SGSGSGTDFTLTISLQPEDATYYCQQGQT YPYTFGQGTKLEIKTKGPSRTVAAPSVFIFPP SDEQLKSGTASVVCLLNNFYPREAKVQWK VDNALQSGNSQESVTEQDSKDSSTYSLSSTL TLSKADYEKHKVYACEVTHQGLSPVTKSF NRGEC	SEQ ID NO: 61
antiCD38_C2-CD38-1_VH1-VL1 IgG4(knob) FALA Heavy Chain 2 <i>(e.g., a third polypeptide chain of a trispecific binding protein of the present disclosure)</i>	QVQLVQSGAEVVKPGASVKVSCASGYTF TSYAMHWVKEAPGQRLEWIGYIYPGQGGT NYNQKFQGRATLTADTSASTAYMELSSLRS EDTAVYFCARTGGLRRAYFTYWGQGTILVT VSSASTKGPSVFPLAPCSRSTSESTAALGCL VKDYFPEPVTVSWNSGALTSGVHTFPAVLQ SSGLYSLSSVTVPSSSLGKTKYTCNVDPHKP	SEQ ID NO: 62

protein of the present disclosure)	SNTKVDKRVESKYGPPCPPCPAPEAAGGPS VFLFPPKPKDTLMISRTPEVTCVVVDVSQED PEVQFNWYVDGVEVHNAKTKPREEQFNST YRVVSVLTVLHQDWLNGKEYKCKVSNKG LPSSIEKTISKAKGQPREPQVYTLPPCQEEM TKNQVSLWCLVKGFYPSDIAVEWESNGQP ENNYKTTPPVLDSDGSFFLYSKLTVDKSRW QEGNVFSCSVMHEALHNHYTQKSLSLSPG	
antiCD38_C2-CD38-1_VH1-VL1 Light Chain 2 (e.g., a fourth polypeptide chain of a trispecific binding protein of the present disclosure)	DIVLTQSPATLSLSPGERATISCRASQSVSSY GQGFMHWYQQKPGQPPRLLIYGASSRATGI PARFSGSGSGTDFTLTISPLEDFAVYYCQ QNKEDPWTFGGGTKLEIKRTVAAPSVEIFPP SDEQLKSGTASVVCLLNNFYPREAKVQWK VDNALQSGNSQESVTEQDSKSTYSLSSLT TLISKADYEKHKVYACEVTHQGLSSPVTKSF NRGEC	SEQ ID NO: 63
antiCD38_C2-CD38-1_VH1-VL1xCD28supxCD3mid IgG1LALA P329A		
CD28supxCD3mid IgG1(hole) LALA P329A Heavy Chain 1 (e.g., a second polypeptide chain of a trispecific binding protein of the present disclosure)	QVQLVQSGAEVVKPGASVKVSCKASGYTF TSYYIHWRQAPGQGLEWIGSIYPGNVNTN YAQKFQGRATLTVDTSISTAYMELSRLRSD DTAVYYCTRSHYGLDWNFDVWGKGTITVT VSSSQVQLVESGGGVVQPGRSLRLSCAASG FTFTKAWMHWRQAPGKQLEWVAQIKDK SNSYATYYADSVKGRFTISRDDSKNTLYLQ MNSLRAEDTAVYYCRGVYYALSPFDYWG QGTLVTVSSRTASTKGPSVFPLAPSSKSTSG GTAALGCLVKDYFPEPVTVSWNSGALTSG VHTFPAVLQSSGLYSLSSVTVTPSSSLGTQT YICNVNHNKPSNTKVDKKVEPKSCDKTHTCP PCPAPEAAGGPSVFLFPPKPKDTLMISRTPE VTCVVVDVSHEDPEVKFNWYVDGVEVHN AKTKPREEQYNSTYRVVSVLTVLHQDWLN GKEYKCKVSNKALAAPIEKTISKAKGQPRE PQVCTLPSSRDELTKNQVSLSCAVKGFYPS DIAVEWESNGQPENNYKTTPPVLDSDGSFF LVSKLTVDKSRWQQGNVFSCSVMHEALHN HYTQKSLSLSPG	SEQ ID NO: 64
CD28supxCD3mid Light Chain 1	See above.	SEQ ID NO: 61

(e.g., a first polypeptide chain of a trispecific binding protein of the present disclosure)		
antiCD38_C2-CD38-1_VH1-VL1 IgG1(knob) LALA P329A Heavy Chain 2 (e.g., a third polypeptide chain of a trispecific binding protein of the present disclosure)	QVQLVQSGAEVVKPGASVKVSCKASGYTF TSYAMHWVKEAPGQRLEWIGYIYPGQGGT NYNQKFQGRATLTADTSASTAYMELSSLRS EDTAVYFCARTGGLRRAYFTYWGQGTILVT VSSASTKGPSVFPLAPSSKSTSGGTAALGCL VKDYFPEPVTVSWNSGALTSGVHTFPAVLQ SSGLYSLSSVVTVPSSSLGTQTYICNVNHKP SNTKVDKKVEPKSCDKTHTCPPCPAPEAAG GPSVFLFPPKPKDTLMISRTPEVTCVVDVVS HEDPEVKFNWYVDGVEVHNAKTKPREEQY NSTYRVVSVLTVLHQDWLNGKEYKCKVSN KALAAPIEKTISKAKGQPREPQVYTLPPCRD ELTKNQVSLWCLVKGFYPSDIAVEWESNG QPENNYKTPPVLDSDGSFFLYSKLTVDKS RWQQGNVFSCSVMHEALHNHYTQKSLSLS PG	SEQ ID NO: 65
antiCD38_C2-CD38-1_VH1-VL1 Light Chain 2 (e.g., a fourth polypeptide chain of a trispecific binding protein of the present disclosure)	See above.	SEQ ID NO: 63
antiCD38_C2-CD38-1_VH1-VL1xCD28supxCD3mid IgG1 NNSA		
CD28supxCD3mid IgG1(hole) NNSA Heavy Chain 1 (e.g., a second polypeptide chain of a trispecific binding protein of the present disclosure)	QVQLVQSGAEVVKPGASVKVSCKASGYTF TSYIHWVRQAPGQGLEWIGSIYPGNVNTN YAKFKQGRATLTVDTSTAYMELSRLRS DTAIVYYCTRSHYGLDWNFDVWGKTTVT VSSSQVLVESGGGVVQPGRSLRLSCAASG FTFTKAWMHWRQAPGKQLEWVAQIKDK SNSYATYYADSVKGRFTISRDDSKNTLYLQ MNSLRAEDTAIVYYCRGVYYALSPFDYWG QGTILVTVSSRTASTKGPSVFPLAPSSKSTSG GTAALGCLVKDYFPEPVTVSWNSGALTSG VHTFPAVLQSSGLYSLSSVVTVPSSSLGTQT YICNVNHKPSNTKVDKKVEPKSCDKTHTCP PCPAPELLGGPSVFLFPPKPKDTLMISRTPEV	SEQ ID NO: 66

	TCVVVDVSHEDPEVKFNWYVDGVEVHNA KTKPREEQYNNASRVVSVLTVLHQDWLNG KEYKCKVSNKALPAPIEKTISKAKGQPREPQ VCTLPSSRDELTKNQVSLSCAVKGFYPSDIA VEWESNGQPENNYKTTPVLDSGDSFFLVS KLTVDKSRWQQGNVFSCSVMHEALHNHY TQKSLSLSPG	
CD28supxCD3mid Light Chain 1 (e.g., a first polypeptide chain of a trispecific binding protein of the present disclosure)	See above.	SEQ ID NO: 61
antiCD38_C2-CD38-1_VH1-VL1 IgG1(knob) NNSA Heavy Chain 2 (e.g., a third polypeptide chain of a trispecific binding protein of the present disclosure)	QVQLVQSGAEVVKPGASVKVSCKASGYTF TSYAMHWVKEAPGQRLEWIGYIYPGQGGT NYNQKFQGRATLTADTSASTAYMELSSLRS EDTAVYFCARTGGLRRAYFTYWGQGTILVT VSSASTKGPSVFPLAPSSKSTSGGTAALGCL VKDYFPEPVTVSWNSGALTSGVHTFPAVLQ SSGLYSLSVVTVPSSSLGTQTYICNVNHKP SNTKVDKKVEPKSCDKTHTCPPCPAPELLG GPSVFLFPPKPKDTLMISRTPEVTCVVVDVS HEDPEVKFNWYVDGVEVHNAKTKPREEQY NNASRVVSVLTVLHQDWLNGKEYKCKVS NKALPAPIEKTISKAKGQPREPQVYTLPPCR DELTKNQVSLWCLVKGFYPSDIAVEWESN GQPENNYKTTPVLDSGDSFFLYSKLTVDK SRWQQGNVFSCSVMHEALHNHYTQKSLSL SPG	SEQ ID NO: 67
CD38VH1 Light Chain 2 (e.g., a fourth polypeptide chain of a trispecific binding protein of the present disclosure)	See above.	SEQ ID NO: 63
CD38HHY1370XCD28supxCD3mid IgG4 FALA		
CD28supxCD3mid IgG4(hole) FALA Heavy Chain 1	See above.	SEQ ID NO: 60

(e.g., a second polypeptide chain of a trispecific binding protein of the present disclosure)		
CD28supxCD3mid Light Chain 1 (e.g., a first polypeptide chain of a trispecific binding protein of the present disclosure)	See above.	SEQ ID NO: 61
CD38^{HHY1370} IgG4(knob) FALA Heavy Chain 2 (e.g., a third polypeptide chain of a trispecific binding protein of the present disclosure)	QVQLVESGGGVVQPGRSLRLSCAASGFTFS SYGMHWVRQAPGKGLEWVAVIWDGSKN YYADSVKGRFTISGDNSKNTLYLQMNSLRA EDTAVYYCARMFRGAFDYWGQGLTVTVSS ASTKGPSVFPLAPCSRSTSESTAALGCLVKD YFPEPVTVSWNSGALTSGVHTFPAVLQSSG LYSLSSVTVPSSSLGKTYTCNVDPHKPSNT KVDKRVESKYGPPCPPCAPEAAGGPSVFL FPPKPKDTLMISRTPEVTCVVVDVSDPEV QFNWYVDGVEVHNAKTKPREEQFNSTYRV VSVLTVLHQDWLNGKEYKCKVSNKGLPSSI EKTISKAKGQPREPQVYTLPPCQEEMTKNQ VSLWCLVKGFYPSDIAVEWESNGQPENNY KTPPVLDSDGSFFLYSKLTVDKSRWQEGN VFSCSVMEALHNHYTQKSLSLGLG	SEQ ID NO: 68
CD38^{HHY1370} Light2 (e.g., a fourth polypeptide chain of a trispecific binding protein of the present disclosure)	AIQMTQSPSSLSASVGDRVTITCRASQGIRN DLGWYQQKPGKAPKLLIYAASLQSGVPSR FSGSGSGTDFTLTISGLQPEDSATYYCLQDY IYYPTFGQGTKVEIKRTVAAPSVFIFPPSDEQ LKSGTASVVCLLNNFYPREAKVQWKVDNA LQSGNSQESVTEQDSKSTYLSSTLTLSKA DYEKHKVYACEVTHQGLSSPVTKSFNRGE C	SEQ ID NO: 69
CD38^{HHY1370} xCD28supxCD3mid IgG1LALA P329A		
CD28supxCD3mid IgG1(hole) LALA P329A Heavy Chain 1 (e.g., a second polypeptide chain of a trispecific binding	See above.	SEQ ID NO: 64

protein of the present disclosure)		
CD28supxCD3mid Light Chain 1 (<i>e.g.</i> , a first polypeptide chain of a trispecific binding protein of the present disclosure)	See above.	SEQ ID NO: 61
CD38^{HHY1370} IgG1(knob) LALA P329A Heavy Chain 2 (<i>e.g.</i> , a third polypeptide chain of a trispecific binding protein of the present disclosure)	QVQLVESGGGVVQPGRSLRLSCAASGFTFS SYGMHWVRQAPGKGLEWVAVIWYDGSNK YYADSVKGRFTISGDNSKNTLYLQMNSLRA EDTAVYYCARMFRGAFDYWGQGLVTVSS ASTKGPSVFPLAPSSKSTSGGTAALGCLVK DYFPEPVTVSWNSGALTSGVHTFPAVLQSS GLYSLSSVVTVPSSSLGTQTYICNVNHKPSN TKVDKKVEPKSCDKTHTCPPCPAPEAAGGP SVFLFPPKPKDTLMISRTPEVTCVVVDVSHE DPEVKFNWYVDGVEVHNAKTKPREEQYNS TYRVVSVLTVLHQDWLNGKEYKCKVSNK ALAAPIEKTISKAKGQPREPQVYTLPPCRDE LTKNQVSLWCLVKGFYPSDIAVEWESNGQ PENNYKTPPVLDSDGSFFLYSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSLSP G	SEQ ID NO: 70
CD38^{HHY1370}Light2 (<i>e.g.</i> , a fourth polypeptide chain of a trispecific binding protein of the present disclosure)	See above.	SEQ ID NO: 69
CD38^{HHY1370}xCD28supxCD3mid IgG1 NNSA		
CD28supxCD3mid IgG1(hole) NNSA Heavy Chain 1 (<i>e.g.</i> , a second polypeptide chain of a trispecific binding protein of the present disclosure)	See above.	SEQ ID NO: 66

CD28supxCD3mid Light Chain 1 <i>(e.g., a first polypeptide chain of a trispecific binding protein of the present disclosure)</i>	See above.	SEQ ID NO: 61
CD38^{HHY1370} IgG1(knob) NNSA Heavy Chain 2 <i>(e.g., a third polypeptide chain of a trispecific binding protein of the present disclosure)</i>	QVQLVESGGGVVQPGRSLRLSCAASGFTFS SYGMHWVRQAPGKGLEWVAVIWDGSKN YYADSVKGRFTISGDNSKNTLYLQMNSLRA EDTAVYYCARMFRGAFDYWGQGLTVTVSS ASTKGPSVFPLAPSSKSTSGGTAALGCLVK DYFPEPVTVSWNSGALTSGVHTFPAVLQSS GLYSLSSVVTVPSSSLGTQTYICNVNHKPSN TKVDKKVEPKSCDKTHTCPPCPAPELLGGP SVFLFPPKPKDTLMISRTPEVTCVVVDVSHE DPEVKFNWYVDGVEVHNAKTKPREEQYN NASRVVSVLTVLHQDWLNGKEYKCKVSN KALPAPIEKTISKAKGQPREPQVYTLPPCRD ELTKNQVSLWCLVKGFYPSDIAVEWESNG QPENNYKTPPVLDSDGSFFLYSKLTVDKS RWQQGNVFSCSVMHEALHNHYTQKSLSLS PG	SEQ ID NO: 71
CD38^{HHY1370} Light2 <i>(e.g., a fourth polypeptide chain of a trispecific binding protein of the present disclosure)</i>	See above.	SEQ ID NO: 69
antiCD38_C2-CD38-1 monovalent antibody		
antiCD38_C2-CD38-1 heavy chain	QVQLQQSGAELVRSGASVKMSCKASGYTF TSFNMHWVKETPGQGLEWIGYIYPGNGGT NYNQKFKGKATLTADTSSSTAYMQISLTS EDSAVYFCARTGGLRRAYFTYWGQGLTVT VSSASTKGPSVFPLAPSSKSTSGGTAALGCL VKDYFPEPVTVSWNSGALTSGVHTFPAVLQ SSGLYSLSSVVTVPSSSLGTQTYICNVNHKP SNTKVDKKVEPKSCDKTHTCPPCPAPELLA GPDVFLFPPKPKDTLMISRTPEVTCVVVDVS HEDPEVKFNWYVDGVEVHNAKTKPREEQY NSTYRVVSVLTVLHQDWLNGKEYKCKVSN KALPLPEEKTISKAKGQPREPQVYTLPPSRD	SEQ ID NO: 7

	ELTKNQVSLTCLVKGFYPSDIAVEWESNGQ PENNYKTTTPVLDSGDSFFLYSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSLSP G	
antiCD38_C2-CD38-1 light chain	DIVLTQSPASLAIVSLGQRATISCRASESVDS YGNQFMHWYQQKPGQPPKLLIYLASNLES GVPARFSGSGSRDFTLTIDPVEADDAATY YCQQNKEDPWTFGGGKLEIKRTVAAPSVF IFPPSDEQLKSGTASVVCLLNNFYPREAKVQ WKVDNALQSGNSQESVTEQDSKSTYSLSS TLTSLKADYEKHKVYACEVTHQGLSSPVTK SFNRGEC	SEQ ID NO: 8
antiCD38_C2-CD38-1_VH1-VL1 monovalent antibody		
antiCD38_C2-CD38-1_VH1-VL1 heavy chain	QVQLVQSGAEVVKPGASVKVSCKASGYTF TSYAMHWVKEAPGQRLEWIGYIYPGQGGT NYNQKFQGRATLTADTSASTAYMELSSLRS EDTAVYFCARTGGLRRAYFTYWGQGTILVT VSSASTKGPSVFPLAPSSKSTSGGTAALGCL VKDYFPEPVTVSWNSGALTSGVHTFPAVLQ SSGLYSLSSVTVPSSSLGTQTYICNVNHKP SNTKVDKKVEPKSCDKTHTCPPCPAPELLA GPDVFLFPPKPKDTLMISRTPEVTCVVDVS HEDPEVKFNWYVDGVEVHNAKTKPREEQY NSTYRVVSVLTVLHQDWLNGKEYKCKVSN KALPLPEEKTIKAKGQPREPQVYTLPPSRD ELTKNQVSLTCLVKGFYPSDIAVEWESNGQ PENNYKTTTPVLDSGDSFFLYSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSLSP G	SEQ ID NO: 15
antiCD38_C2-CD38-1_VH1-VL1 light chain	DIVLTQSPATLSLSPGERATISCRASQSVSSY GQGFMHWYQQKPGQPPRLIYGASSRAT GIPARFSGSGSGTDFLTISPLEDFAVYYC QQNKEDPWTFGGGKLEIKRTVAAPSVF IFPPSDEQLKSGTASVVCLLNNFYPREAKVQ WKVDNALQSGNSQESVTEQDSKSTYSLSS TLTSLKADYEKHKVYACEVTHQGLSSPVTK SFNRGEC	SEQ ID NO: 16
antiCD38_C2-CD38-1_VH3-VL3 monovalent antibody		
antiCD38_C2-CD38-1_VH3-VL3 heavy chain	QVQLVQSGAEVVKPGASVKVSCKASGYTF TSFNMHWVKEAPGQRLEWIGYIYPGNNGT	SEQ ID NO: 19

	NYNQKFQGRATLTADTSASTAYMELSSLRS EDTAVYFCARTGGLRRAYFTYWGQGLVT VSSASTKGPSVFPLAPSSKSTSGGTAALGCL VKDYFPEPVTVSWNSGALTSGVHTFPAVLQ SSGLYSLSSVVTVPSSSLGTQTYICNVNHKP SNTKVDDKKVEPKSCDKTHTCPPCPAPELLA GPDVFLFPPKPKDTLMISRTPEVTCVVVDVS HEDPEVKFNWYVDGVEVHNAKTKPREEQY NSTYRVVSVLTVLHQDWLNGKEYKCKVSN KALPLPEEKTISKAKGQPREPQVYTLPPSRD ELTKNQVSLTCLVKGFYPSDIAVEWESNGQ PENNYKTPPVLDSDGSFFLYSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSLSP G	
antiCD38_C2-CD38-1_VH3-VL3 light chain	DIVLTQSPATLSLSPGERATISCRASESVDSY GNGFMHWYQQKPGQPPRLIYLASSRAT GIPARFSGSGSGTDFTLTISPLEDFAVYYC QQNKEDPWTFGGGKLEIKRTVAAPSVF IFPPSDEQLKSGTASVVCLLNNFYPREAKVQ WKVDNALQSGNSQESVTEQDSKDSSTYSLS TLTLTKADYEKHKVYACEVTHQGLSSPVTK SFNRGEC	SEQ ID NO: 20
antiCD38_C2-CD38-1_VH5-VL3 monovalent antibody		
antiCD38_C2-CD38-1_VH5-VL3 heavy chain	QVQLVQSGAEVVKSGASVKVSCKASGYTF TSFNMHWVKEAPGQGLEWIGYIYPNGGT NYNQKFQGRATLTADTSASTAYMEISSLR EDTAVYFCARTGGLRRAYFTYWGQGLVT VSSASTKGPSVFPLAPSSKSTSGGTAALGCL VKDYFPEPVTVSWNSGALTSGVHTFPAVLQ SSGLYSLSSVVTVPSSSLGTQTYICNVNHKP SNTKVDDKKVEPKSCDKTHTCPPCPAPELLA GPDVFLFPPKPKDTLMISRTPEVTCVVVDVS HEDPEVKFNWYVDGVEVHNAKTKPREEQY NSTYRVVSVLTVLHQDWLNGKEYKCKVSN KALPLPEEKTISKAKGQPREPQVYTLPPSRD ELTKNQVSLTCLVKGFYPSDIAVEWESNGQ PENNYKTPPVLDSDGSFFLYSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSLSP G	SEQ ID NO: 22
antiCD38_C2-CD38-1_VH5-VL3 light chain	DIVLTQSPATLSLSPGERATISCRASESVDSY GNGFMHWYQQKPGQPPRLIYLASSRAT	SEQ ID NO: 20

	GIPARFSGSGSGTDFTLTISPLEDFAVYYC QQNKEDPWTFGGGKLEIKRTVAAPSVF IFPPSDEQLKSGTASVVCLLNNFYPREAKVQ WKVDNALQSGNSQESVTEQDSKDSTYSLSS TLTSLKADYEKHKVYACEVTHQGLSSPVTK SFNRGEC	
antiCD38_C2-CD38-1_VH6-VL3 monovalent antibody		
antiCD38_C2-CD38-1_VH6-VL3 heavy chain	QVQLVQSGAEVVKPGASVKMSCKASGYTF TSFNMHWVKEAPGQRLEWIGYIYPGNNGT NYNQKFQGRATLTADTSASTAYMEISLRS EDTAVYFCARTGGLRRAYFTYWGQGTILVT VSSASTKGPSVFPLAPSSKSTSGGTAALGCL VKDYFPEPVTVSWNSGALTSGVHTFPAVLQ SSGLYSLSSVVTVPSSSLGTQTYICNVNHKP SNTKVDKKVEPKSCDKTHTCPPCPAPELLA GPDVFLFPPKPKDTLMISRTPEVTCVVVDVS HEDPEVKFNWYVDGVEVHNAKTKPREEQY NSTYRVVSVLTVLHQDWLNGKEYKCKVSN KALPLPEEKTISKAKGQPREPQVYTLPPSRD ELTKNQVSLTCLVKGFYPSDIAVEWESNGQ PENNYKTPPVLDSDGSFFLYSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSLSP G	SEQ ID NO: 24
antiCD38_C2-CD38-1_VH6-VL3 light chain	DIVLTQSPATLSLSPGERATISCRASEVDSY GNGFMHWYQQKPGQPRLLIYLASSRAT GIPARFSGSGSGTDFTLTISPLEDFAVYYC QQNKEDPWTFGGGKLEIKRTVAAPSVF IFPPSDEQLKSGTASVVCLLNNFYPREAKVQ WKVDNALQSGNSQESVTEQDSKDSTYSLSS TLTSLKADYEKHKVYACEVTHQGLSSPVTK SFNRGEC	SEQ ID NO: 20
CD38^{HHY1370} monovalent antibody		
CD38^{HHY1370} heavy chain	QVQLVESGGGVVQPGRSLRLSCAASGFTFS SYGMHWVRQAPGKGLEWVAVIWYDGSNK YYADSVKGRFTISGDNSKNTLYLQMNSLRA EDTAVYYCARMFRGAFDYWGQGTILVTVSS ASTKGPSVFPLAPSSKSTSGGTAALGCLVK DYFPEPVTVSWNSGALTSGVHTFPAVLQSS GLYSLSSVVTVPSSSLGTQTYICNVNHKPSN TKVDKKVEPKSCDKTHTCPPCPAPELLAGP DVFLFPPKPKDTLMISRTPEVTCVVVDVSHE	SEQ ID NO: 11

	DPEVKFNWYVDGVEVHNAKTKPREEQYNS TYRVVSVLTVLHQDWLNGKEYKCKVSNK ALPLPEEKTISKAKGQPREPQVYTLPPSRDE LTKNQVSLTCLVKGFYPSDIAVEWESNGQP ENNYKTTPPVLDSDGSFFLYSKLTVDKSRW QQGNVVFSCSVMEALHNHYTQKSLSLSPG	
CD38^{HHY1370} light chain	AIQMTQSPSSLSASVGDRVTITCRASQGIRN DLGWYQQKPGKAPKLLIYAASSLQSGVPS RFGSGSGTDFTLTISGLQPEDSATYYCLQD YIYYPTFGQGTKVEIKRTVAAPSVFIFPP SDEQLKSGTASVVCLLNNFYPREAKVQWK VDNALQSGNSQESVTEQDSKDSSTYSLSSTL TLSKADYEKHKVYACEVTHQGLSSPVTKSF NRGEC	SEQ ID NO: 12
antiCD38_SB19 monovalent antibody		
antiCD38_SB19 heavy chain	QVQLVQSGAEVAKPGTSVKLSCKASGYTFT DYWMQWVKQRPQGQGLEWIGTIYPGDGDT GYAQKFQGKATLTADKSSKTVYMHLSSLA SEDSAVYYCARGDYYGSNSLDYWGGQTSV TVSSASTKGPSVFPLAPSSKSTSGGTAALGC LVKDYFPEPVTVSWNSGALTSGVHTFPAVL QSSGLYSLSSVTVPSSSLGTQTYICNVNHK PSNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDV SHEDPEVKFNWYVDGVEVHNAKTKPREEQ YNSTYRVVSVLTVLHQDWLNGKEYKCKVS NKALPAIEKTISKAKGQPREPQVYTLPPSR DELTKNQVSLTCLVKGFYPSDIAVEWESNG QPENNYKTTPPVLDSDGSFFLYSKLTVDKS RWQQGNVVFSCSVMEALHNHYTQKSLSLS PGK	SEQ ID NO: 107
antiCD38_SB19 light chain	DIVMTQSHLSMSTSLGDPVSITCKASQDVST VVAWYQQKPGQSPRRLIYSASYRYIGVPDR FTGSGAGTDFTFTISSVQAEDLAVYYCQQH YSPPYTFGGGTKLEIKRTVAAPSVFIFPP SDEQLKSGTASVVCLLNNFYPREAKVQWK VDNALQSGNSQESVTEQDSKDSSTYSLSSTL TLSKADYEKHKVYACEVTHQGLSSPVTKSF NRGEC	SEQ ID NO: 106

Table J. Full-length polynucleotide sequences of binding proteins.

antiCD38_C2-CD38-1_VH1-VL1xCD28supxCD3mid IgG4 FALA		
CD28supxCD3mid IgG4(hole) FALA Heavy Chain 1 <i>(e.g., encoding a second polypeptide chain of a trispesific binding protein of the present disclosure)</i>	CAGGTGCAGCTGGTGCAGTCTGGCGCCGA GGTCGTGAAACCTGGCGCCTCTGTGAAGG TGTCCTGCAAGGCCAGCGGCTACACCTTT ACCAGCTACTACATCCACTGGGTGCGCCA GGCCCCCTGGACAGGGACTGGAATGGATC GGCAGCATCTACCCCGGCAACGTGAACA CCAACTACGCCCAGAAGTTCCAGGGCAG AGCCACCCTGACCGTGGACACCAGCATCA GCACCGCCTACATGGAACTGAGCCGGCTG AGAAGCGACGACACCGCCGTGTACTACT GCACCCGGTCCCCTACGGCCTGGATTGG AACTTCGACGTGTGGGGCAAGGGCACCA CCGTGACAGTGTCTAGCAGCCAGGTGCAG CTGGTGGAAATCTGGCGGCGGAGTGGTGC AGCCTGGCAGAAGCCTGAGACTGAGCTG TGCCGCCAGCGGCTTACCTTCACCAAGG CCTGGATGCACTGGGTGCGCCAGGCCCT GGAAAGCAGCTGGAATGGGTGGCCCAGA TCAAGGACAAGAGCAACAGCTACGCCAC CTACTACGCCGACAGCGTGAAGGGCCGG TTCACCATCAGCCGGGACGACAGCAAGA ACACCCTGTACCTGCAGATGAACAGCCTG CGGGCCGAGGACACCGCCGTGTACTACTG TCGGGGCGTGTACTATGCCCTGAGCCCCT TCGATTACTGGGGCCAGGGAACCCTCGTG ACCGTGTCTAGTCGGACCGCCAGCACAAA GGGCCCCATCGGTGTTCCCTCTGGCCCCTT GCAGCAGAAGCACCAGCGAATCTACAGC CGCCCTGGGCTGCCTCGTGAAGGACTACT TTCCCGAGCCCGTGACCGTGTCTGGAAC TCTGGCGCTCTGACAAGCGGCGTGACAC CTTCCAGCCGTGCTCCAGAGCAGCGGCC TGTACTCTCTGAGCAGCGTCGTGACAGTG CCCAGCAGCAGCCTGGGCACCAAGACCT ACACCTGTAACGTGGACCACAAGCCCAG CAACACCAAGGTGGACAAGCGGGTGGAA TCTAAGTACGGCCCTCCCTGCCCTCCTTG CCCAGCCCCTGAAGCTGCCGGCGGACCCT CCGTGTTCCCTGTTCCCCCAAAGCCCAAG GACACCCTGATGATCAGCCGGACCCCCGA	SEQ ID NO:72

	AGTGACCTGCGTGGTGGTGGATGTGTCCC AGGAAGATCCCGAGGTGCAGTTCAATTG GTACGTGGACGGCGTGGAAGTGCACAAC GCCAAGACCAAGCCCAGAGAGGAACAGT TCAACAGCACCTACCGGGTGGTGTCCGTG CTGACCGTGCTGCACCAGGACTGGCTGAA CGGCAAAGAGTACAAGTGCAAGGTGTCC AACAAAGGGCCTGCCCAGCTCCATCGAGA AAACCATCAGCAAGGCCAAGGGCCAGCC CCGCGAGCCTCAAGTGTGTACCCTGCCCC CTAGCCAGGAAGAGATGACCAAGAACCA GGTGTCCCTGAGCTGTGCCGTGAAAGGCT TCTACCCCAGCGACATTGCCGTGGAATGG GAGAGCAACGGCCAGCCCGAGAACAAC ACAAGACCACCCCCCTGTGCTGGACAGC GACGGCTCATTCTTCCTGGTGTCCAAGCT GACCGTGGACAAGAGCCGGTGGCAGGAA GGCAACGTGTTTCAGCTGCTCCGTGATGCA CGAGGCCCTGCACAACCACTACACCCAG AAGTCCCTGTCTCTGTCCCTGGGC	
CD28supxCD3mid Light Chain 1 <i>(e.g., encoding a first polypeptide chain of a trispecific binding protein of the present disclosure)</i>	GACATCGTGATGACCCAGACCCCCCTGAG CCTGAGCGTGACACCTGGACAGCCTGCCA GCATCAGCTGCAAGAGCAGCCAGAGCCT GGTGCACAACAACGCCAACACCTACCTG AGCTGGTATCTGCAGAAGCCCGGCCAGA GCCCCCAGTCCCTGATCTACAAGGTGTCC AACAGATTTCAGCGGCGTGCCCGACAGATT CTCCGGCAGCGGCTCTGGCACCGACTTCA CCCTGAAGATCAGCCGGGTGGAAGCCGA GGACGTGGGCGTGTACTATTGTGGCCAGG GCACCCAGTACCCCTTCACCTTTGGCAGC GGCACCAAGGTGGAAATCAAGGGCCAGC CCAAGGCCGCCCCCGACATCCAGATGACC CAGAGCCCCAGCAGCCTGTCTGCCAGCGT GGGCGACAGAGTGACCATCACCTGTTCAG GCCAGCCAGAACATCTACGTGTGGCTGAA CTGGTATCAGCAGAAGCCCGGCAAGGCC CCCAAGCTGCTGATCTACAAGGCCAGCAA CCTGCACACCGGCGTGCCCGAGCAGATTTT CTGGCAGCGGCTCCGGCACCGACTTCACC CTGACAATCAGCTCCCTGCAGCCCGAGGA CATTGCCACCTACTACTGCCAGCAGGGCC AGACCTACCCCTACACCTTTGGCCAGGGC	SEQ ID NO:73

	ACCAAGCTGGAAATCAAGACCAAGGGCC CCAGCCGTACGGTGGCCGCTCCCAGCGTG TTCATCTTCCCACCTAGCGACGAGCAGCT GAAGTCCGGCACAGCCTCTGTCTGTGTGCC TGCTGAACAACTTCTACCCCCGCGAGGCC AAAGTGCAGTGGAAGGTGGACAACGCCC TGCAGAGCGGCAACAGCCAGGAAAGCGT GACCGAGCAGGACAGCAAGGACTCCACC TACAGCCTGAGCAGCACCTGACACTGAG CAAGGCCGACTACGAGAAGCACAAAGGTG TACGCCTGCGAAGTGACCCACCAGGGCCT GTCTAGCCCCGTGACCAAGAGCTTCAACC GGGGCGAGTGT	
antiCD38_C2-CD38-1_VH1- VL1 IgG4(knob) FALA Heavy Chain 2 <i>(e.g., encoding a third polypeptide chain of a trispecific binding protein of the present disclosure)</i>	CAGGTGCAGCTGGTGCAGTCTGGCGCCGA AGTCGTGAAACCTGGCGCCTCCGTGAAGG TGTCCTGCAAGGCCAGCGGCTACACCTTT ACCAGCTACGCCATGCACTGGGTCAAAG AGGCCCCCTGGCCAGAGACTGGAATGGAT CGGCTACATCTACCCCCGCCAGGGCGGCA CCAATAACAACCAGAAGTTCCAGGGCAG AGCCACCCTGACCGCCGATACAAGCGCC AGCACCGCCTACATGGAAGTGAAGCAGCCT GCGGAGCGAGGATACCGCCGTGTACTTCT GTGCCAGAACAGGCGGCCTGAGGCGGGC CTACTTTACCTATTGGGGCCAGGGCACCC TCGTGACCGTGTCTAGCGCTAGCACAAAG GGCCCATCGGTGTTCCCTCTGGCCCCCTTG CAGCAGAAGCACCAAGCGAATCTACAGCC GCCCTGGGCTGCCTCGTGAAGGACTACTT TCCCGAGCCCGTGACCGTGTCTGGAAGT CTGGCGCTCTGACAAGCGGCGTGCACACC TTTCCAGCCGTGCTCCAGAGCAGCGGCCT GTAATCTCTGAGCAGCGTCGTGACAGTGC CCAGCAGCAGCCTGGGCACCAAGACCTA CACCTGTAACGTGGACCACAAGCCCAGC AACACCAAGGTGGACAAGCGGGTGGAAT CTAAGTACGGCCCTCCCTGCCCTCCTTGC CCAGCCCCCTGAAGCTGCCGGCGGACCCTC CGTGTTCTGTTCCTCCCAAGCCCAAGG ACACCTGATGATCAGCCGGACCCCCGAA GTGACCTGCGTGGTGGTGGATGTGTCCA GGAAGATCCCGAGGTGCAGTTCAATTGGT ACGTGGACGGCGTGGAAGTGCACAACGC	SEQ ID NO:74

	CAAGACCAAGCCCAGAGAGGAACAGTTC AACAGCACCTACCGGGTGGTGTCCGTGCT GACCGTGCTGCACCAGGACTGGCTGAAC GGCAAAGAGTACAAGTGCAAGGTGTCCA ACAAGGGCCTGCCCAGCTCCATCGAGAA AACCATCAGCAAGGCCAAGGGCCAGCCC CGCGAGCCTCAAGTGTATACCCTGCCCCC TTGCCAGGAAGAGATGACCAAGAACCAG GTGTCCCTGTGGTGTCTCGTGAAAGGCTT CTACCCAGCGACATTGCCGTGGAATGGG AGAGCAACGGCCAGCCCGAGAACAATA CAAGACCACCCCCCTGTGCTGGACAGCG ACGGCTCATTCTTCCTGTACTCCAAGCTG ACCGTGGACAAGAGCCGGTGGCAGGAAG GCAACGTGTTTCAGCTGCTCCGTGATGCAC GAGGCCCTGCACAACCACTACACCCAGA AGTCCCTGTCTCTGTCCCTGGGC	
antiCD38_C2-CD38-1_VH1-VL1 Light Chain 2 <i>(e.g., encoding a fourth polypeptide chain of a trispecific binding protein of the present disclosure)</i>	GACATCGTGCTGACACAGAGCCCTGCCAC CCTGTCTCTGAGCCCTGGCGAGAGAGCCA CCATCAGCTGTAGAGCCAGCCAGAGCGT GTCCAGCTACGGCCAGGGCTTCATGCACT GGTATCAGCAGAAGCCCGGCCAGCCCCC CAGACTGCTGATCTATGGCGCCAGCAGCA GAGCCACAGGCATCCCCGCCAGATTTTCT GGCTCTGGCAGCGGCACCGACTTCACCCT GACAATCAGCCCCCTGGAACCCGAGGAC TTCGCCGTGTACTACTGCCAGCAGAACAA AGAGGACCCCTGGACCTTCGGCGGAGGC ACCAAGCTGGAAATCAAGCGTACGGTGG CCGCTCCCAGCGTGTTTCATCTTCCCACCT AGCGACGAGCAGCTGAAGTCCGGCACAG CCTCTGTCGTGTGCCTGCTGAACAACCTC TACCCCCGCGAGGCCAAGGTGCAGTGGA AGGTGGACAATGCCCTGCAGAGCGGCAA CAGCCAGGAAAGCGTGACCGAGCAGGAC AGCAAGGACTCCACCTACAGCCTGAGCA GCACCCTGACCCTGTCCAAGGCCGATTAC GAGAAGCACAAGGTGTACGCCTGCGAAG TGACCCACCAGGGCCTGTCTAGCCCCGTG ACCAAGAGCTTCAACCGGGGCGAGTGC	SEQ ID NO:75
antiCD38_C2-CD38-1_VH1-VL1xCD28supxCD3mid IgG1LALA P329A		

CD28supxCD3mid IgG1(hole) LALA P329A Heavy Chain 1 <i>(e.g., encoding a second polypeptide chain of a trispecific binding protein of the present disclosure)</i>	CAGGTGCAGCTGGTGCAGTCTGGCGCCGA GGTCGTGAAACCTGGCGCCTCTGTGAAGG TGTCCTGCAAGGCCAGCGGCTACACCTTT ACCAGCTACTACATCCACTGGGTGCGCCA GGCCCCCTGGACAGGGACTGGAATGGATC GGCAGCATCTACCCCGGCAACGTGAACA CCAACTACGCCCAGAAGTTCCAGGGCAG AGCCACCCTGACCGTGGACACCAGCATCA GCACCGCCTACATGGAACTGAGCCGGCTG AGAAGCGACGACACCGCCGTGTACTACT GCACCCGGTCCCCTACGGCCTGGATTGG AACTTCGACGTGTGGGGCAAGGGCACCA CCGTGACAGTGTCTAGCAGCCAGGTGCAG CTGGTGGAATCTGGCGGCGGAGTGGTGC AGCCTGGCAGAAGCCTGAGACTGAGCTG TGCCGCCAGCGGCTTACCTTCACCAAGG CCTGGATGCACTGGGTGCGCCAGGCCCT GGAAAGCAGCTGGAATGGGTGGCCCAGA TCAAGGACAAGAGCAACAGCTACGCCAC CTACTACGCCGACAGCGTGAAGGGCCGG TTCACCATCAGCCGGGACGACAGCAAGA ACACCCTGTACCTGCAGATGAACAGCCTG CGGGCCGAGGACACCGCCGTGTACTACTG TCGGGGCGTGTACTATGCCCTGAGCCCCT TCGATTACTGGGGCCAGGGAACCTCGTG ACCGTGTCTAGTCGGACCGCCAGCACAAA GGGCCCCAGCGTGTTCCCTCTGGCCCCTA GCAGCAAGAGCACATCTGGCGGAACAGC CGCCCTGGGCTGCCTCGTGAAGGACTACT TTCCCGAGCCCGTGACCGTGTCTGGAAT TCTGGCGCCCTGACCAGCGGCGTGCACAC CTTCCAGCTGTGCTGCAGTCCAGCGGCC TGACAGCCTGAGCAGCGTCGTGACAGTG CCCAGCAGCTCTCTGGGCACCCAGACCTA CATCTGCAACGTGAACCACAAGCCCAGC AACACCAAGGTGGACAAGAAGGTGGAAC CCAAGAGCTGCGACAAGACCCACACCTG TCCCCCTTGTCTGCCCCGAAGCCGCCG GAGGCCCTTCCGTGTTCCCTGTTCCCCCA AAGCCCAAGGACACCCTGATGATCAGCC GGACCCCCGAAGTGACCTGCGTGGTGGTG GATGTGTCCACGAGGACCCTGAAGTGA AGTTCAATTGGTACGTGGACGGCGTGGA	SEQ ID NO:76
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	GTGCACAACGCCAAGACCAAGCCAAGAG AGGAACAGTACAACAGCACCTACCGGGT GGTGTCCGTGCTGACCGTGCTGCACCAGG ACTGGCTGAACGGCAAAGAGTACAAGTG CAAGGTGTCCAACAAGGCCCTGGCCGCCC CCATCGAGAAAACCATCAGCAAGGCCAA GGGCCAGCCCCGCGAACCCCAGGTGTGC ACACTGCCCCCAAGCAGGGACGAGCTGA CCAAGAACCAGGTGTCCCTGAGCTGTGCC GTGAAAGGCTTCTACCCCTCCGATATCGC CGTGGAATGGGAGAGCAACGGCCAGCCC GAGAACAACCTACAAGACCACCCCCCTGT GCTGGACAGCGACGGCTCATTTCTTCTGG TGTCOAAGCTGACAGTGGACAAGTCCCGG TGGCAGCAGGGCAACGTGTTTCAGCTGCTC CGTGATGCACGAGGCCCTGCACAACCACT ACACCCAGAAGTCCCTGAGCCTGAGCCCC GGC	
CD28supxCD3mid Light Chain 1 <i>(e.g., encoding a first polypeptide chain of a trispecific binding protein of the present disclosure)</i>	See above.	SEQ ID NO:73
antiCD38_C2-CD38-1_VH1-VL1 IgG1(knob) LALA P329A Heavy Chain 2 <i>(e.g., encoding a third polypeptide chain of a trispecific binding protein of the present disclosure)</i>	CAGGTGCAGCTGGTGCAGTCTGGCGCCGA AGTCGTGAAACCTGGCGCCTCCGTGAAGG TGTCTTCAAGGCCAGCGGCTACACCTTT ACCAGCTACGCCATGCACTGGGTCAAAG AGGCCCCCTGGCCAGAGACTGGAATGGAT CGGCTACATCTACCCCGGCCAGGGCGGCA CCAACTACAACCAGAAGTTCCAGGGCAG AGCCACCCTGACCGCCGATAACAAGCGCC AGCACCGCCTACATGGAACTGAGCAGCCT GCGGAGCGAGGATACCGCCGTGTACTTCT GTGCCAGAACAGGCGGCCTGAGGCGGGC CTACTTTACCTATTGGGGCCAGGGCACCC TCGTGACCGTGTCTAGCGCTAGCACAAAG GGCCCCAGCGTGTTCCCTCTGGCCCCCTAG CAGCAAGAGCACATCTGGCGGAACAGCC GCCCTGGGCTGCCTCGTGAAGGACTACTT TCCCAGAGCCCGTGACCGTGTCTTGAATT	SEQ ID NO:77

	CTGGCGCCCTGACCAGCGGCGTGCACACC TTTCCAGCTGTGCTGCAGTCCAGCGGCCT GTACAGCCTGAGCAGCGTCGTGACAGTGC CCAGCAGCTCTCTGGGCACCCAGACCTAC ATCTGCAACGTGAACCACAAGCCCAGCA ACACCAAGGTGGACAAGAAGGTGGAACC CAAGAGCTGCGACAAGACCCACACCTGT CCCCCTTGTCTGCCCCCGAAGCCGCCGG AGGCCCTTCCGTGTTCTGTTCCCCCAA AGCCCAAGGACACCCTGATGATCAGCCG GACCCCGAAGTGACCTGCGTGGTGGTGG ATGTGTCCACGAGGACCCTGAAGTGAA GTTCAATTGGTACGTGGACGGCGTGGAAG TGCACAACGCCAAGACCAAGCCAAGAGA GGAACAGTACAACAGCACCTACCGGGTG GTGTCCGTGCTGACCGTGCTGCACCAGGA CTGGCTGAACGGCAAAGAGTACAAGTGC AAGGTGTCCAACAAGGCCCTGGCCGCCCC CATCGAGAAAACCATCAGCAAGGCCAAG GGCCAGCCCCGCGAACCCCAGGTGTACA CACTGCCCCCATGCAGGGACGAGCTGACC AAGAACCAGGTGTCCCTGTGGTGTCTGGT GAAAGGCTTCTACCCCTCCGATATCGCCG TGGAATGGGAGAGCAACGGCCAGCCCGA GAACAACACTACAAGACCACCCCCCTGTGC TGGACAGCGACGGCTCATTCTTCCTGTAC TCCAAGCTGACAGTGGACAAGTCCCGGTG GCAGCAGGGCAACGTGTTTCAGCTGCTCCG TGATGCACGAGGCCCTGCACAACCACTAC ACCCAGAAGTCCCTGAGCCTGAGCCCCGG C	
antiCD38_C2-CD38-1_VH1- VL1 Light Chain 2 (e.g., encoding a fourth polypeptide chain of a trispecific binding protein of the present disclosure)	See above.	SEQ ID NO:75
antiCD38_C2-CD38-1_VH1-VL1xCD28supxCD3mid IgG1 NNSA		

CD28supxCD3mid IgG1(hole) NNSA Heavy Chain 1 <i>(e.g., encoding a second polypeptide chain of a trispecific binding protein of the present disclosure)</i>	CAGGTGCAGCTGGTGCAGTCTGGCGCCGA GGTCGTGAAACCTGGCGCCTCTGTGAAGG TGTCCTGCAAGGCCAGCGGCTACACCTTT ACCAGCTACTACATCCACTGGGTGCGCCA GGCCCCCTGGACAGGGACTGGAATGGATC GGCAGCATCTACCCCGGCAACGTGAACA CCAACTACGCCCAGAAGTTCCAGGGCAG AGCCACCCTGACCGTGGACACCAGCATCA GCACCGCCTACATGGAACTGAGCCGGCTG AGAAGCGACGACACCGCCGTGTACTACT GCACCCGGTCCCCTACGGCCTGGATTGG AACTTCGACGTGTGGGGCAAGGGCACCA CCGTGACAGTGTCTAGCAGCCAGGTGCAG CTGGTGGAATCTGGCGGCGGAGTGGTGC AGCCTGGCAGAAGCCTGAGACTGAGCTG TGCCGCCAGCGGCTTACCTTCACCAAGG CCTGGATGCACTGGGTGCGCCAGGCCCT GGAAAGCAGCTGGAATGGGTGGCCCAGA TCAAGGACAAGAGCAACAGCTACGCCAC CTACTACGCCGACAGCGTGAAGGGCCGG TTCACCATCAGCCGGGACGACAGCAAGA ACACCCTGTACCTGCAGATGAACAGCCTG CGGGCCGAGGACACCGCCGTGTACTACTG TCGGGGCGTGTACTATGCCCTGAGCCCCT TCGATTACTGGGGCCAGGGAACCTCGTG ACCGTGTCTAGTCGGACCGCCAGCACAAA GGGCCCCAGCGTGTTCCCTCTGGCCCCTA GCAGCAAGAGCACATCTGGCGGAACAGC CGCCCTGGGCTGCCTCGTGAAGGACTACT TTCCCGAGCCCGTGACCGTGTCTGGAAT TCTGGCGCCCTGACCAGCGGCGTGCACAC CTTCCAGCTGTGCTGCAGTCCAGCGGCC TGACAGCCTGAGCAGCGTCGTGACAGTG CCCAGCAGCTCTCTGGGCACCCAGACCTA CATCTGCAACGTGAACCACAAGCCCAGC AACACCAAGGTGGACAAGAAGGTGGAAC CCAAGAGCTGCGACAAGACCCACACCTG TCCCCCTTGTCTGCCCCGAACTGCTGG GAGGCCCTTCCGTGTTCCCTGTTCCCCCA AAGCCCAAGGACACCCTGATGATCAGCC GGACCCCCGAAGTGACCTGCGTGGTGGTG GATGTGTCCACGAGGACCCTGAAGTGA AGTTCAATTGGTACGTGGACGGCGTGGA	SEQ ID NO:78
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	GTGCACAACGCCAAGACCAAGCCAAGAG AGGAACAGTACAACAATGCCTCCCGGGT GGTGTCCGTGCTGACCGTGCTGCACCAGG ACTGGCTGAACGGCAAAGAGTACAAGTG CAAGGTGTCCAACAAGGCCCTGCCTGCCC CCATCGAGAAAACCATCAGCAAGGCCAA GGGCCAGCCCCGCGAACCCCAGGTGTGC AACTGCCCCCAAGCAGGGACGAGCTGA CCAAGAACCAGGTGTCCCTGAGCTGTGCC GTGAAAGGCTTCTACCCCTCCGATATCGC CGTGGAATGGGAGAGCAACGGCCAGCCC GAGAACAACCTACAAGACCACCCCCCTGT GCTGGACAGCGACGGCTCATTTCTTCTGG TGTCGAAGCTGACAGTGGACAAGTCCCGG TGGCAGCAGGGCAACGTGTTTCAGCTGCTC CGTGATGCACGAGGCCCTGCACAACCACT ACACCCAGAAGTCCCTGAGCCTGAGCCCC GGC	
CD28supxCD3mid Light Chain 1 <i>(e.g., encoding a first polypeptide chain of a trispecific binding protein of the present disclosure)</i>	See above.	SEQ ID NO:73
antiCD38_C2-CD38-1_VH1-VL1 IgG1(knob) NNSA Heavy Chain 2 <i>(e.g., encoding a third polypeptide chain of a trispecific binding protein of the present disclosure)</i>	CAGGTGCAGCTGGTGCAGTCTGGCGCCGA AGTCGTGAAACCTGGCGCCTCCGTGAAGG TGTCTTCAAGGCCAGCGGCTACACCTTT ACCAGCTACGCCATGCACTGGGTCAAAG AGGCCCCCTGGCCAGAGACTGGAATGGAT CGGCTACATCTACCCCGGCCAGGGCGGCA CCAACTACAACCAGAAGTTCCAGGGCAG AGCCACCCTGACCGCCGATAACAAGCGCC AGCACCGCCTACATGGAAGTGAAGCAGCCT GCGGAGCGAGGATACCGCCGTGTACTTCT GTGCCAGAACAGGCGGCCTGAGGCGGGC CTACTTTACCTATTGGGGCCAGGGCACCC TCGTGACCGTGTCTAGCGCTAGCACAAAG GGCCCATCGGTCTTCCCCCTGGCACCCCTC CTCCAAGAGCACCTCTGGGGGCACAGCG GCCCTGGGCTGCCTGGTCAAGGACTACTT CCCCGAACCGGTGACGGTGTCTGTGGAAGT	SEQ ID NO:79

	CAGGCGCCCTGACCAGCGGCGTGACAC CTTCCCGGCTGTCCTACAGTCCTCAGGAC TCTACTCCCTCAGCAGCGTGGTGACCGTG CCCTCCAGCAGCTTGGGCACCCAGACCTA CATCTGCAACGTGAATCACAAGCCCAGCA ACACCAAGGTGGACAAGAAAGTTGAGCC CAAATCTTGTGACAAAACCTCACACATGCC CACCGTGCCCAGCACCTGAACTCCTGGGG GGACCGTCAGTCTTCCTCTTCCCCC AAA ACCCAAGGACACCCCTCATGATCTCCCGGA CCCCTGAGGTCACATGCGTGGTGGTGGAC GTGAGCCACGAAGACCCTGAGGTCAAGT TCAACTGGTATGTTGACGGCGTGGAGGTG CATAATGCCAAGACAAAGCCGCGGGAGG AGCAGTACAACAATGCCTCCCGTGTGGTC AGCGTCCTCACCGTCCTGCACCAGGACTG GCTGAATGGCAAGGAGTACAAGTGCAAG GTCTCCAACAAAGCCCTCCAGCCCCCAT CGAGAAAACCATCTCCAAAGCCAAAGGG CAGCCCCGAGAACCACAGGTGTACACCCT GCCCCCATGCCGGGATGAGCTGACCAAG AATCAAGTCAGCCTGTGGTGCCTGGTAAA AGGCTTCTATCCCAGCGACATCGCCGTGG AGTGGGAGAGCAATGGGCAGCCGGAGAA CAACTACAAGACCACGCCTCCCGTGCTGG ACTCCGACGGCTCCTTCTTCCTCTACTCAA AACTCACCGTGGACAAGAGCAGGTGGCA GCAGGGGAACGTCTTCTCATGCTCCGTGA TGCATGAGGCTCTGCACAACCACTACACG CAGAAGAGCCTCTCCCTGTCTCCGGGT	
antiCD38_C2-CD38-1_VH1- VL1 Light Chain 2 <i>(e.g., encoding a fourth polypeptide chain of a trispecific binding protein of the present disclosure)</i>	See above.	SEQ ID NO:75
CD38_{HHY1370}XCD28supxCD3mid IgG4 FALA		
CD28supxCD3mid IgG4(hole) FALA Heavy Chain 1	See above.	SEQ ID NO:72

(e.g., encoding a second polypeptide chain of a trispecific binding protein of the present disclosure)		
CD28supxCD3mid Light Chain 1 (e.g., encoding a first polypeptide chain of a trispecific binding protein of the present disclosure)	See above.	SEQ ID NO:73
CD38^{HHY1370} IgG4(knob) FALA Heavy Chain 2 (e.g., encoding a third polypeptide chain of a trispecific binding protein of the present disclosure)	CAGGTGCAGCTGGTGGAAAGCGGCGGAG GCGTGGTGCAGCCTGGCAGGTCTCTGAGA CTGAGCTGTGCCGCCAGCGGCTTCACCTT CAGCAGCTACGGAATGCACTGGGTGCGC CAGGCCCTGGCAAAGGACTGGAATGGG TGGCCGTGATTTGGTACGACGGCAGCAAC AAGTACTACGCCGACAGCGTGAAGGGCC GGTTCACCATCAGCGGCGACAACAGCAA GAACACCCTGTACCTGCAGATGAACAGCC TGCGGGCCGAGGACACCGCCGTGTACTAC TGCGCCAGAATGTTTCAGAGGCGCCTTCGA CTACTGGGGCCAGGGCACACTCGTGACCG TGTCTAGTGCCTCGACCAAGGGCCCATCG GTGTTCCCTCTGGCCCCCTTGCAGCAGAAG CACCAGCGAATCTACAGCCGCCCTGGGCT GCCTCGTGAAGGACTACTTTCCCGAGCCC GTGACCGTGTCTTGGAACCTCTGGCGCTCT GACAAGCGGCGTGCACACCTTTCCAGCCG TGCTCCAGAGCAGCGGCCTGTACTCTCTG AGCAGCGTCGTGACAGTGCCCAGCAGCA GCCTGGGCACCAAGACCTACACCTGTAAC GTGGACCACAAGCCCAGCAACACCAAGG TGGACAAGCGGGTGGAATCTAAGTACGG CCCTCCCTGCCCTCCTTGCCCAGCCCCTG AAGCTGCCGGCGGACCCTCCGTGTTCTG TTCCCCCAAAGCCCAAGGACACCCTGAT GATCAGCCGGACCCCCGAAGTGACCTGC GTGGTGGTGGATGTGTCCCAGGAAGATCC CGAGGTGCAGTTCAATTGGTACGTGGACG GCGTGGAAGTGCACAACGCCAAGACCAA GCCAGAGAGGAACAGTTCAACAGCACC	SEQ ID NO:80

	TACCGGGTGGTGTCCGTGCTGACCGTGCT GCACCAGGACTGGCTGAACGGCAAAGAG TACAAGTGCAAGGTGTCCAACAAGGGCC TGCCCAGCTCCATCGAGAAAACCATCAGC AAGGCCAAGGGCCAGCCCCGCGAGCCTC AAGTGTATACCCTGCCCCCTTGCCAGGAA GAGATGACCAAGAACCAGGTGTCCCTGT GGTGTCTCGTGAAAGGCTTCTACCCCAGC GACATTGCCGTGGAATGGGAGAGCAACG GCCAGCCCCGAGAACAACCTACAAGACCAC CCCCCTGTGCTGGACAGCGACGGCTCAT TCTTCCTGTACTCCAAGCTGACCGTGGAC AAGAGCCGGTGGCAGGAAGGCAACGTGT TCAGCTGCTCCGTGATGCACGAGGCCCTG CACAACCACTACACCCAGAAGTCCCTGTC TCTGTCCCTGGGC	
CD38_{HHY1370} Light2 (e.g., encoding a fourth polypeptide chain of a trispecific binding protein of the present disclosure)	GCCATCCAGATGACCCAGAGCCCCAGCA GCCTGTCTGCCAGCGTGGGCGACAGAGTG ACCATCACCTGTAGAGCCAGCCAGGGCAT CCGGAACGACCTGGGCTGGTATCAGCAG AAGCCTGGCAAGGCCCCCAAGCTGCTGAT CTACGCCGCTAGCTCTCTGCAGTCCGGCG TGCCCAGCAGATTTTCTGGCAGCGGCTCC GGCACCGACTTCACCCTGACAATCTCTGG CCTGCAGCCCGAGGACAGCGCCACCTACT ACTGTCTGCAAGACTACATCTACTACCCC ACCTTCGGCCAGGGCACCAAGGTGGAAA TCAAGCGTACGGTGGCCGCTCCCAGCGTG TTCATCTTCCCACCTAGCGACGAGCAGCT GAAGTCCGGCACAGCCTCTGTCTGTGCC TGCTGAACAACCTTCTACCCCCGCGAGGCC AAAGTGCAGTGGAAGGTGGACAACGCCC TGCAGAGCGGCAACAGCCAGGAAAGCGT GACCGAGCAGGACAGCAAGGACTCCACC TACAGCCTGAGCAGCACCTGACACTGAG CAAGGCCGACTACGAGAAGCACAAAGGTG TACGCCTGCGAAGTGACCCACCAGGGCCT GTCTAGCCCCGTGACCAAGAGCTTCAACC GGGGCGAGTGT	SEQ ID NO:81
CD38_{HHY1370}xCD28supxCD3mid IgG1LALA P329A		

CD28supxCD3mid IgG1(hole) LALA P329A Heavy Chain 1 <i>(e.g., encoding a second polypeptide chain of a trispecific binding protein of the present disclosure)</i>	See above.	SEQ ID NO:76
CD28supxCD3mid Light Chain 1 <i>(e.g., encoding a first polypeptide chain of a trispecific binding protein of the present disclosure)</i>	See above.	SEQ ID NO:73
CD38^{HHY1370} IgG1(knob) LALA P329A Heavy Chain 2 <i>(e.g., encoding a third polypeptide chain of a trispecific binding protein of the present disclosure)</i>	CAGGTGCAGCTGGTGGAAAGCGGCGGAG GCGTGGTGCAGCCTGGCAGGTCTCTGAGA CTGAGCTGTGCCGCCAGCGGCTTCACCTT CAGCAGCTACGGAATGCACTGGGTGCGC CAGGCCCTGGCAAAGGACTGGAATGGG TGGCCGTGATTTGGTACGACGGCAGCAAC AAGTACTACGCCGACAGCGTGAAGGGCC GGTTCACCATCAGCGGCGACAACAGCAA GAACACCCTGTACCTGCAGATGAACAGCC TGCGGGCCGAGGACACCGCCGTGTACTAC TGCGCCAGAATGTTTCAGAGGCGCCTTCGA CTACTGGGGCCAGGGCACACTCGTGACCG TGTCTAGTGCGTCGACCAAGGGCCCCAGC GTGTTCCCTCTGGCCCCTAGCAGCAAGAG CACATCTGGCGGAACAGCCGCCCTGGGCT GCCTCGTGAAGGACTACTTTCCCGAGCCC GTGACCGTGTCTTGGAAATTCTGGCGCCCT GACCAGCGGCGTGCACACCTTTCCAGCTG TGCTGCAGTCCAGCGGCCTGTACAGCCTG AGCAGCGTCGTGACAGTGCCAGCAGCTC TCTGGGCACCCAGACCTACATCTGCAACG TGAACCACAAGCCCAGCAACACCAAGGT GGACAAGAAGGTGGAACCCAAGAGCTGC GACAAGACCCACACCTGTCCCCCTTGTC TGCCCCCGAAGCCGCCGGAGGCCCTTCCG TGTTCTGTTCCTCCCCCAAGCCCAAGGAC ACCCTGATGATCAGCCGGACCCCCGAAGT GACCTGCGTGGTGGTGGATGTGTCCCACG	SEQ ID NO:82

	AGGACCCTGAAGTGAAGTTCAATTGGTAC GTGGACGGCGTGGAAGTGCACAACGCCA AGACCAAGCCAAGAGAGGAACAGTACAA CAGCACCTACCGGGTGGTGTCCGTGCTGA CCGTGCTGCACCAGGACTGGCTGAACGGC AAAGAGTACAAGTGCAAGGTGTCCAACA AGGCCCTGGCCGCCCCCATCGAGAAAAC CATCAGCAAGGCCAAGGGCCAGCCCCGC GAACCCAGGTGTACACACTGCCCCCATG CAGGGACGAGCTGACCAAGAACCAGGTG TCCCTGTGGTGTCTGGTGAAAGGCTTCTA CCCCTCCGATATCGCCGTGGAATGGGAGA GCAACGGCCAGCCCGAGAACAACACTACAA GACCACCCCCCTGTGCTGGACAGCGACG GCTCATTCTTCCTGTACTCCAAGCTGACA GTGGACAAGTCCCGGTGGCAGCAGGGCA ACGTGTTTCAGCTGCTCCGTGATGCACGAG GCCCTGCACAACCACTACACCCAGAAGTC CCTGAGCCTGAGCCCCGGC	
CD38_{HHY1370} Light2 <i>(e.g., encoding a fourth polypeptide chain of a trispecific binding protein of the present disclosure)</i>	See above.	SEQ ID NO:81
CD38_{HHY1370}xCD28supxCD3mid IgG1 NNSA		
CD28supxCD3mid IgG1(hole) NNSA Heavy Chain 1 <i>(e.g., encoding a second polypeptide chain of a trispecific binding protein of the present disclosure)</i>	See above.	SEQ ID NO:78
CD28supxCD3mid Light Chain 1 <i>(e.g., encoding a first polypeptide chain of a trispecific binding protein of the present disclosure)</i>	See above.	SEQ ID NO:73

CD38^{HHY1370} IgG1(knob) NNSA Heavy Chain 2 <i>(e.g., encoding a third polypeptide chain of a trispecific binding protein of the present disclosure)</i>	CAGGTGCAGCTGGTGGAAAGCGGCGGAG GCGTGGTGCAGCCTGGCAGGTCTCTGAGA CTGAGCTGTGCCGCCAGCGGCTTCACCTT CAGCAGCTACGGAATGCACTGGGTGCGC CAGGCCCTGGCAAAGGACTGGAATGGG TGGCCGTGATTTGGTACGACGGCAGCAAC AAGTACTACGCCGACAGCGTGAAGGGCC GGTTCACCATCAGCGGCGACAACAGCAA GAACACCCTGTACCTGCAGATGAACAGCC TGCGGGCCGAGGACACCGCCGTGTACTAC TGCGCCAGAATGTTTACAGAGGCGCCTTCGA CTACTGGGGCCAGGGCACACTCGTGACCG TGTCTAGTGCGTCGACCAAGGGGCCATCG GTCTTCCCCCTGGCACCTCCTCCAAGAG CACCTCTGGGGGCACAGCGGCCCTGGGCT GCCTGGTCAAGGACTACTTCCCCGAACCG GTGACGGTGTCTGTGGAACCTCAGGCGCCCT GACCAGCGGCGTGCACACCTTCCCGGCTG TCCTACAGTCCTCAGGACTCTACTCCCTC AGCAGCGTGGTGACCGTGCCCTCCAGCAG CTTGGGCACCCAGACCTACATCTGCAACG TGAATCACAAGCCCAGCAACACCAAGGT GGACAAGAAAGTTGAGCCCAAATCTTGT GACAAAACCTCACACATGCCACCGTGCCC AGCACCTGAACCTCTGGGGGGACCGTCA GTCTTCCTCTTCCCCCAAACCCAAGGA CACCTCATGATCTCCCGGACCCCTGAGG TCACATGCGTGGTGGTGGACGTGAGCCAC GAAGACCCTGAGGTCAAGTTCAACTGGTA TGTTGACGGCGTGGAGGTGCATAATGCCA AGACAAAGCCGCGGGAGGAGCAGTACAA CAATGCCTCCCGTGTGGTCAGCGTCTCA CCGTCTGCACCAGGACTGGCTGAATGGC AAGGAGTACAAGTGCAAGGTCTCCAACA AAGCCCTCCCAGCCCCATCGAGAAAACC ATCTCCAAAGCCAAAGGGCAGCCCCGAG AACCACAGGTGTACACCCTGCCCCATGC CGGGATGAGCTGACCAAGAATCAAGTCA GCCTGTGGTGCCTGGTAAAAGGCTTCTAT CCCAGCGACATCGCCGTGGAGTGGGAGA GCAATGGGCAGCCGGAGAACAACTACAA GACCACGCCTCCCGTGCTGGACTCCGACG GCTCCTTCTCCTCTACTCAAACTCACCG	SEQ ID NO:83
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	TGGACAAGAGCAGGTGGCAGCAGGGGAA CGTCTTCTCATGCTCCGTGATGCATGAGG CTCTGCACAACCACTACACGCAGAAGAG CCTCTCCCTGTCTCCGGGT	
CD38_{HHY1370} Light2 (e.g., encoding a fourth polypeptide chain of a trispecific binding protein of the present disclosure)	See above.	SEQ ID NO:81

CD38 polypeptides

[0120] In some embodiments, a binding protein of the present disclosure comprises an antigen binding site that binds an extracellular domain of a human CD38 polypeptide and an extracellular domain of a cynomolgus monkey CD38 polypeptide. Exemplary assays for determining whether an antigen binding site binds an antigen are described herein and known in the art. In some embodiments, binding is determined by ELISA assay, *e.g.*, as described *infra*. In some embodiments, binding is determined by SPR assay, *e.g.*, as described *infra*. In some embodiments, binding is determined by flow cytometry assay using cells expressing a CD38 polypeptide on their cell surface, *e.g.*, as described *infra*.

[0121] In some embodiments, a binding protein of the present disclosure binds a purified polypeptide or fragment thereof comprising the amino acid sequence of SEQ ID NO:1 and/or 30 (*e.g.*, as measured by ELISA or SPR). In some embodiments, a binding protein of the present disclosure binds a polypeptide or comprising the amino acid sequence of SEQ ID NO:1 and/or 30 when expressed on the surface of a cell (*e.g.*, as measured by flow cytometry).

[0122] In some embodiments, a binding protein of the present disclosure binds to a CD38 isoform A polypeptide (*e.g.*, comprising the amino acid sequence of SEQ ID NO:1). In some embodiments, a binding protein of the present disclosure binds to a CD38 isoform E polypeptide (*e.g.*, comprising the amino acid sequence of SEQ ID NO:105 and not comprising the full amino acid sequence of SEQ ID NO:1, consisting of the amino acid sequence of SEQ ID NO:105, or consisting essentially of the amino acid sequence of SEQ ID NO:105). In some embodiments, a binding protein of the present disclosure binds to a CD38 isoform A polypeptide (*e.g.*, comprising the amino acid sequence of SEQ ID NO:1) and a CD38 isoform E polypeptide (*e.g.*, comprising the amino acid sequence of SEQ ID NO:105 and not comprising the full amino acid sequence of SEQ ID NO:1, consisting of the amino acid sequence of SEQ ID NO:105, or consisting essentially of the amino acid sequence of SEQ ID NO:105). Without wishing to be bound to theory, it is thought that binding to a CD38 isoform E polypeptide can be advantageous, *e.g.*, in targeting a binding protein of the present disclosure to cell(s) expressing a CD38 isoform E polypeptide.

Human CD38 isoform A extracellular domain polypeptide sequence

RWRQQWSGPGTTKRFETVLARCVKYTEIHPEMRHVDCQSVWDAFKGAFISKHPCN
ITEEDYQPLMKLGTQTVPCNKILLWSRIKDLAQFTQVQRDMFTLEDTLGLYADDL
TWCGEFNTSKINYQSCPDWRKDCSNNPVSVFWKTVSRRFAEAACDVVHVMLNGSR

SKIFDKNSTFGSVEVHNLQPEKVQTLEAWVIHGGREDSRDLCQDPTIKELESIISKRN
QFSCKNIYRPDKFLQCVKNPEDSSCTSEI (SEQ ID NO:1)

Human CD38 isoform E polypeptide sequence

RWRQQWSGPGTTKRFPETVLARCVKYTEIHPEMRHVDCQSVWDAFKGAFISKHPCN
ITEEDYQPLMKLGTQTVPCNKILLWSRIKDLAHQFTQVQRDMFTLEDTLLGYLADDL
TWCGEFNTSKINYQSCPDWRKDCSNNPVSFVWKTVSRRHFWECGSP (SEQ ID
NO:105)

[0123] In some embodiments, the extracellular domain of a human CD38 polypeptide comprises the amino acid sequence of SEQ ID NO:1. In some embodiments, the extracellular domain of a cynomolgus monkey CD38 polypeptide comprises the amino acid sequence of SEQ ID NO:30.

Cynomolgus monkey CD38 polypeptide sequence

RWRQQWSGSGTTSRFPETVLARCVKYTEVHPEMRHVDCQSVWDAFKGAFISKYPC
NITEEDYQPLVKLGTQTVPCNKILLWSRIKDLAHQFTQVQRDMFTLEDMLLGYLAD
DLTWCGEFNTFEINYQSCPDWRKDCSNNPVSFVWKTVSRRFAETACGVVHVMLNG
SRSKIFDKNSTFGSVEVHNLQPEKVQALEAWVIHGGREDSRDLCQDPTIKELESIISKR
NIRFFCKNIYRPDKFLQCVKNPEDSSCLSGI (SEQ ID NO:30)

Linkers

[0124] In some embodiments, the linkers L₁, L₂, L₃ and L₄ 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. L₁, L₂, L₃ and L₄ in one binding protein may all have the same amino acid sequence or may all have different amino acid sequences.

[0125] 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; a peptide with five glycine residues; a peptide with six glycine residues; a peptide with seven glycine residues; and a peptide with eight glycine residues. Other combinations of amino acid residues may be used such as the peptide GGGSGGGGS (SEQ ID NO: 55), the peptide GGGSGGGSGGGGS (SEQ ID NO: 56), the peptide TKGPS (SEQ ID NO: 57), the peptide GQPKAAP (SEQ ID NO:58), and the peptide GGSGSSGSGG (SEQ ID NO:59).

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 and International Application No. PCT/US2017/027488.

[0126] 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.

[0127] In some embodiments, at least one of L₁, L₂, L₃ or L₄ is independently 0 amino acids in length. In some embodiments, L₁, L₂, L₃ or L₄ are each independently at least one amino acid in length. In some embodiments, the length of L₁ is at least twice the length of L₃. In some embodiments, the length of L₂ is at least twice the length of L₄. In some embodiments, the length of L₁ is at least twice the length of L₃, and the length of L₂ is at least twice the length of L₄. In some embodiments, L₁ is 3 to 12 amino acid residues in length, L₂ is 3 to 14 amino acid residues in length, L₃ is 1 to 8 amino acid residues in length, and L₄ is 1 to 3 amino acid residues in length. In some embodiments, L₁ is 5 to 10 amino acid residues in length, L₂ is 5 to 8 amino acid residues in length, L₃ is 1 to 5 amino acid residues in length, and L₄ is 1 to 2 amino acid residues in length. In some embodiments, L₁ is 7 amino acid residues in length, L₂ is 5 amino acid residues in length, L₃ is 1 amino acid residue in length, and L₄ is 2 amino acid residues in length. In some embodiments, L₁ is 10 amino acid residues in length, L₂ is 10 amino acid residues in length, L₃ is 0 amino acid residue in length, and L₄ is 0 amino acid residues in length. In some embodiments, L₁, L₂, L₃, and L₄ 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, L₁, L₂, L₃, and L₄ are each 0 amino acids in length.

[0128] In some embodiments, L₁, L₂, L₃, and/or L₄ 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).

[0129] In some embodiments, L₁, L₂, L₃ and L₄ each independently are zero amino acids in length or comprise a sequence selected from the group consisting of GGGGSGGGGS (SEQ ID NO:55), GGGGSGGGGS (SEQ ID NO:56), S, RT, TKGPS (SEQ ID NO:57), GQPKAAP (SEQ ID NO: 58), and GGSGSSGSGG (SEQ ID NO:59). In some embodiments, L₁, L₂, L₃ and L₄ each independently comprise a sequence selected from the group consisting of GGGGSGGGGS (SEQ ID NO:55), GGGGSGGGGS (SEQ ID NO:56), S, RT, TKGPS (SEQ ID NO:57), GQPKAAP (SEQ ID NO: 58), and GGSGSSGSGG (SEQ ID NO:59).

[0130] In some embodiments, L₁ comprises the sequence GQPKAAP (SEQ ID NO: 58), L₂ comprises the sequence TKGPS (SEQ ID NO:57), L₃ comprises the sequence S, and L₄ comprises the sequence RT. In some embodiments, L₁ comprises the sequence GGGGSGGGGS (SEQ ID NO:55), L₂ comprises the sequence GGGGSGGGGS (SEQ ID NO:55), L₃ is 0 amino acids in length, and L₄ is 0 amino acids in length. In some embodiments, L₁ comprises the sequence GGSGSSGSGG (SEQ ID NO:59), L₂ comprises the sequence GGSGSSGSGG (SEQ ID NO:59), L₃ is 0 amino acids in length, and L₄ is 0 amino acids in length. In some embodiments, L₁ comprises the sequence GGGGSGGGGS (SEQ ID NO:56), L₂ is 0 amino acids in length, L₃ comprises the sequence GGGGSGGGGS (SEQ ID NO:56), and L₄ is 0 amino acids in length.

Fc regions and constant domains

[0131] In some embodiments, a binding protein of the present disclosure comprises a full-length antibody heavy chain or a polypeptide chain comprising an Fc region. In some embodiments, the Fc region is a human Fc region, *e.g.*, a human IgG1, IgG2, IgG3, or IgG4 Fc region. In some embodiments, the Fc region includes an antibody hinge, C_{H1}, C_{H2}, C_{H3}, and optionally C_{H4} domains. In some embodiments, the Fc region is a human IgG1 Fc region. In some embodiments, the Fc region is a human IgG4 Fc region. In some embodiments, the Fc region includes one or more of the mutations described *infra*.

[0132] 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 been 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).

[0133] In some embodiments, the Fc region comprises one or more mutations that reduce or eliminate Fc receptor binding and/or effector function of the Fc region (*e.g.*, Fc receptor-mediated antibody-dependent cellular phagocytosis (ADCP), complement-dependent cytotoxicity (CDC), and/or antibody-dependent cellular cytotoxicity (ADCC)).

[0134] In some embodiments, the Fc region is a human IgG1 Fc region comprising one or more amino acid substitutions at positions corresponding to positions 234, 235, and/or 329 of human IgG1 according to EU Index. In some embodiments, the amino acid substitutions are L234A, L235A, and/or P329A. In some embodiments, the Fc region is a human IgG1 Fc region comprising amino acid substitutions at positions corresponding to positions 298, 299, and/or 300 of human IgG1 according to EU Index. In some embodiments, the amino acid substitutions are S298N, T299A, and/or Y300S.

[0135] In some embodiments, the Fc region is a human IgG4 Fc region comprising one or more mutations that reduce or eliminate FcγI and/or FcγII binding. In some embodiments, the Fc region is a human IgG4 Fc region comprising one or more mutations that reduce or eliminate FcγI and/or FcγII binding but do not affect FcRn binding. In some embodiments, the Fc region is a human IgG4 Fc region comprising amino acid substitutions at positions corresponding to positions 228 and/or 409 of human IgG4 according to EU Index. In some embodiments, the amino acid substitutions are S228P and/or R409K. In some embodiments, the Fc region is a human IgG4 Fc region comprising amino acid substitutions at positions corresponding to positions 234 and/or 235 of human IgG4 according to EU Index. In some embodiments, the amino acid substitutions are F234A and/or L235A. In some embodiments,

the Fc region is a human IgG4 Fc region comprising amino acid substitutions at positions corresponding to positions 228, 234, 235, and/or 409 of human IgG4 according to EU Index. In some embodiments, the amino acid substitutions are S228P, F234A, L235A, and /or R409K. In some embodiments, the Fc region is a human IgG4 Fc region comprising amino acid substitutions at positions corresponding to positions 233-236 of human IgG4 according to EU Index. In some embodiments, the amino acid substitutions are E233P, F234V, L235A, and a deletion at 236. In some embodiments, the Fc region is a human IgG4 Fc region comprising amino acid mutations at substitutions corresponding to positions 228, 233-236, and/or 409 of human IgG4 according to EU Index. In some embodiments, the amino acid mutations are S228P; E233P, F234V, L235A, and a deletion at 236; and /or R409K.

[0136] 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 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; 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.

[0137] In some embodiments, one or both Fc regions are human IgG4 Fc regions comprising amino acid substitutions at positions corresponding to positions 233-236 of human IgG4 according to EU Index. In some embodiments, the amino acid substitutions are E233P, F234V, L235A, and a deletion at 236. In some embodiments, the Fc regions are human IgG4 Fc regions comprising amino acid mutations at substitutions corresponding to positions 228, 233-236, and/or 409 of human IgG4 according to EU Index. In some embodiments, the amino acid mutations are S228P, E233P, F234V, L235A, and a deletion at 236; and /or R409K. In some embodiments, one or both Fc regions are human IgG1 Fc regions comprising one or more amino acid substitutions at positions corresponding to positions 234, 235, and/or 329 of human IgG1 according to EU Index. In some embodiments, the amino acid substitutions are L234A, L235A, and/or P329A. In some embodiments, the Fc regions are human IgG1 Fc regions comprising amino acid substitutions at positions corresponding to positions 298, 299, and/or 300 of human IgG1 according to EU Index. In some embodiments, the amino acid substitutions are S298N, T299A, and/or Y300S.

[0138] To improve the yields of some binding proteins (*e.g.*, bispecific or trispecific binding proteins), the CH₃ 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 CH₃ domains are altered to increase the heterodimerisation of both heavy chains containing these two CH₃ domains. Each of the two CH₃ 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 CH₃ domains do not include a knob in hole.

[0139] In some embodiments, a binding protein of the present disclosure (*e.g.*, a trispecific binding protein) 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.

[0140] 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.

[0141] 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.

[0142] 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.

[0143] 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.

[0144] 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.

[0145] In some embodiments, the second polypeptide chain further comprises a first Fc region linked to CH1, wherein the first Fc region is a human IgG4 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 228, 354, 366, and 409 of human IgG4 according to EU Index, wherein the amino acid substitutions are S228P, S354C, T366W, and R409K; and wherein the third polypeptide chain further comprises a second Fc region linked to CH1, wherein the second Fc region is a human IgG4 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 228, 349, 366, 368, 407, and 409 of human IgG4 according to EU Index, wherein the amino acid substitutions are S228P, Y349C, T366S, L368A, Y407V, and R409K. In some embodiments, the second polypeptide chain further comprises a first Fc region linked to CH1, wherein the first Fc region is a human IgG4 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 228, 349, 366, 368, 407, and 409 of human IgG4 according to EU Index, wherein the amino acid substitutions are S228P, Y349C, T366S, L368A, Y407V, and R409K; and wherein the third polypeptide chain further comprises a second Fc region linked to CH1, wherein the second Fc region is a human IgG4 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 228, 354, 366, and 409 of human IgG4 according to EU Index, wherein the amino acid substitutions are S228P, S354C, T366W, and R409K.

[0146] In some embodiments, the second polypeptide chain further comprises a first Fc region linked to CH1, wherein the first Fc region is a human IgG4 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 234, 235, 354, and 366 of human IgG4 according to EU Index, wherein the amino acid substitutions are F234A, L235A, S354C, and T366W; and wherein the third polypeptide chain further comprises a second Fc region linked to CH1, wherein the second Fc region is a human IgG4 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 234, 235, 349, 366, 368, and 407 of human IgG4 according to EU Index, wherein the amino acid substitutions are F234A, L235A, Y349C, T366S, L368A, and Y407V. In some embodiments, the second polypeptide chain further comprises a first Fc region linked to CH1, wherein the first Fc region is a human IgG4 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 234, 235, 349, 366, 368, and 407 of human IgG4 according to EU Index, wherein the amino acid substitutions are F234A, L235A, Y349C, T366S, L368A, and Y407V; and wherein the third polypeptide chain further comprises a second Fc region linked to CH1, wherein the second Fc region is a human IgG4 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 234, 235, 354, and 366 of human IgG4 according to EU Index, wherein the amino acid substitutions are F234A, L235A, S354C, and T366W.

[0147] In some embodiments, the second polypeptide chain further comprises a first Fc region linked to CH1, wherein the first Fc region is a human IgG4 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 228, 234, 235, 354, 366, and 409 of human IgG4 according to EU Index, wherein the amino acid substitutions are S228P, F234A, L235A, S354C, T366W, and R409K; and wherein the third polypeptide chain further comprises a second Fc region linked

to CH1, wherein the second Fc region is a human IgG4 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 228, 234, 235, 349, 366, 368, 407, and 409 of human IgG4 according to EU Index, wherein the amino acid substitutions are S228P, F234A, L235A, Y349C, T366S, L368A, Y407V, and R409K. In some embodiments, the second polypeptide chain further comprises a first Fc region linked to CH1, wherein the first Fc region is a human IgG4 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 228, 234, 235, 349, 366, 368, 407, and 409 of human IgG4 according to EU Index, wherein the amino acid substitutions are S228P, F234A, L235A, Y349C, T366S, L368A, Y407V, and R409K; and wherein the third polypeptide chain further comprises a second Fc region linked to CH1, wherein the second Fc region is a human IgG4 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 228, 234, 235, 354, 366, and 409 of human IgG4 according to EU Index, wherein the amino acid substitutions are S228P, F234A, L235A, S354C, T366W, and R409K.

[0148] 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.

[0149] 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 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 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.

[0150] 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 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; 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.

[0151] 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.

[0152] 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 CH₁, the first Fc region comprising an immunoglobulin hinge region and CH₂ and CH₃ immunoglobulin heavy chain constant domains; 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 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, and 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 Fc regions of the second and the third polypeptide chains are human IgG4 Fc regions, and the 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 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.

[0153] 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.

[0154] 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*.

Nucleic acids

[0155] 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.

[0156] 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 molecules comprise a sequence that is at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NOs:60-83 and/or shown in Table J.

[0157] Certain aspects of the present disclosure relate to kits of polynucleotides. In some embodiments, one or more of the polynucleotides is a vector (*e.g.*, an expression vector). The kits may find use, *inter alia*, in producing one or more of the binding proteins described herein, *e.g.*, a trispecific binding protein of the present disclosure. In some embodiments, the kit comprises one, two, three, or four polynucleotides shown in Table J (*e.g.*, of

antiCD38_C2-CD38-1_VH1-VL1xCD28supxCD3mid IgG4 FALA, antiCD38_C2-CD38-1_VH1-VL1xCD28supxCD3mid IgG1LALA P329A, antiCD38_C2-CD38-1_VH1-VL1xCD28supxCD3mid IgG1 NNSA, CD38^{HHY1370}xCD28supxCD3mid IgG4 FALA, CD38^{HHY1370}xCD28supxCD3mid IgG1LALA P329A, or CD38^{HHY1370}CD28supxCD3mid IgG1 NNSA). In some embodiments, a kit of polynucleotides comprises: a first polynucleotide comprising the sequence of SEQ ID NO:73, a second polynucleotide comprising the sequence of SEQ ID NO:72, a third polynucleotide comprising the sequence of SEQ ID NO:74, and a fourth polynucleotide comprising the sequence of SEQ ID NO:75. In some embodiments, a kit of polynucleotides comprises: a first polynucleotide comprising the sequence of SEQ ID NO:73, a second polynucleotide comprising the sequence of SEQ ID NO:76, a third polynucleotide comprising the sequence of SEQ ID NO:77, and a fourth polynucleotide comprising the sequence of SEQ ID NO:75. In some embodiments, a kit of polynucleotides comprises: a first polynucleotide comprising the sequence of SEQ ID NO:73, a second polynucleotide comprising the sequence of SEQ ID NO:78, a third polynucleotide comprising the sequence of SEQ ID NO:79, and a fourth polynucleotide comprising the sequence of SEQ ID NO:75. In some embodiments, a kit of polynucleotides comprises: a first polynucleotide comprising the sequence of SEQ ID NO:73, a second polynucleotide comprising the sequence of SEQ ID NO:72, a third polynucleotide comprising the sequence of SEQ ID NO:80, and a fourth polynucleotide comprising the sequence of SEQ ID NO:81. In some embodiments, a kit of polynucleotides comprises: a first polynucleotide comprising the sequence of SEQ ID NO:73, a second polynucleotide comprising the sequence of SEQ ID NO:76, a third polynucleotide comprising the sequence of SEQ ID NO:82, and a fourth polynucleotide comprising the sequence of SEQ ID NO:81. In some embodiments, a kit of polynucleotides comprises: a first polynucleotide comprising the sequence of SEQ ID NO:73, a second polynucleotide comprising the sequence of SEQ ID NO:78, a third polynucleotide comprising the sequence of SEQ ID NO:83, and a fourth polynucleotide comprising the sequence of SEQ ID NO:81.

[0158] 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.

[0159] 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.

[0160] 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

[0161] Other aspects of the present disclosure relate to an isolated host cell comprising one or more isolated polynucleotides, polynucleotide kits, 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), Expi293™ 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).

Methods and Uses for binding proteins

[0162] Certain aspects of the present disclosure relate to methods for expanding virus-specific memory T cells. In some embodiments, the methods comprise contacting a virus-specific memory T cell with a binding protein of the present disclosure, *e.g.*, a trispecific binding protein that comprises a first antigen binding site that binds a CD28 polypeptide, a second antigen binding site that binds a CD3 polypeptide, and a third antigen binding site that binds a CD38 polypeptide.

[0163] In some embodiments, the virus-specific memory T cell is contacted with the binding protein *in vitro* or *ex vivo*.

[0164] In some embodiments, contacting the virus-specific memory T cell with the binding protein causes activation and/or proliferation of virus-specific memory T cells.

[0165] Other aspects of the present disclosure relate to methods for expanding T cells. In some embodiments, the methods comprise contacting a T cell with a binding protein of the present disclosure, *e.g.*, a trispecific binding protein that comprises a first antigen binding site that binds a CD28 polypeptide, a second antigen binding site that binds a CD3 polypeptide, and a third antigen binding site that binds a CD38 polypeptide.

[0166] In some embodiments, the T cell is a memory T cell or an effector T cell.

[0167] In some embodiments, the T cell expresses a chimeric antigen receptor (CAR) on its cell surface or comprises a polynucleotide encoding a CAR.

[0168] Other aspects of the present disclosure relate to methods for treating chronic viral infection, *e.g.*, in an individual in need thereof. In some embodiments, the methods comprise administering to an individual in need thereof an effective amount of a binding protein of the present disclosure, *e.g.*, a trispecific binding protein that comprises a first antigen binding site that binds a CD28 polypeptide, a second antigen binding site that binds a CD3 polypeptide, and a third antigen binding site that binds a CD38 polypeptide.

[0169] In some embodiments, the individual is a human.

[0170] In some embodiments, the binding protein is administered to the individual in pharmaceutical formulation comprising the binding protein and a pharmaceutically acceptable carrier.

[0171] In some embodiments, administration of the binding protein results in activation and/or proliferation of virus-specific memory T cells in the individual.

[0172] In any of the above methods, memory T cells can be CD8⁺ or CD4⁺ memory T cells. In any of the above methods, memory T cells can be central memory T cells (T_{CM}) or effector memory T cells (T_{EM}).

[0173] Any of the binding proteins described herein may find use in the methods of the present disclosure.

[0174] 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.

[0175] 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 ^3H , ^{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.

[0176] 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.

[0177] For clinical or research applications, in certain embodiments, binding proteins can be conjugated to a cytotoxic agent. A variety of antibodies coupled to cytotoxic agents (*i.e.*, antibody-drug conjugates) have been used to target cytotoxic payloads to specific tumor cells. Cytotoxic agents and linkers that conjugate the agents to an antibody are known in the art; *see, e.g.*, Parslow, A.C. *et al.* (2016) *Biomedicines* 4:14 and Kalim, M. *et al.* (2017) *Drug Des. Devel. Ther.* 11:2265-2276.

[0178] 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

[0179] 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. These pharmaceutical compositions may find use in any of the methods and uses described herein (*e.g., ex vivo, in vitro, and/or in vivo*).

[0180] Acceptable formulation materials preferably are nontoxic to recipients at the dosages and concentrations employed.

[0181] 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 dextrans), 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).

[0182] 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.

[0183] 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.

[0184] 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.

[0185] 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.

[0186] 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.

[0187] 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.

[0188] 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.

[0189] 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.

[0190] 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.

[0191] 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.

[0192] 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.

[0193] 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).

[0194] 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.

[0195] 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.

[0196] 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.

[0197] 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.

EXAMPLES

[0198] The Examples that follow are illustrative of specific embodiments of the disclosure, and various uses thereof. They are set forth for explanatory purposes only, and should not be construed as limiting the scope of the invention in any way.

[0199] The following terminology may be used interchangeably in the Examples and Drawings herein to refer to specific anti-CD38 antigen binding domains or antibodies:

antiCD38_C2-CD38-1_VH1-VL1 or CD38_{VH1}

antiCD38_1370 or CD38_{HHY1370}

antiCD38_SB19 or isatuximab

Example 1: Cross-reactivity and apoptosis induction of anti-CD38 antibodies

[0200] Humanized anti-CD38 variants were characterized for binding to human and cynomolgus CD38 polypeptides and for induction of apoptosis.

Materials and Methods*Binding affinity to soluble CD38 extracellular domains*

[0201] The binding properties of the anti-CD38 mAbs were evaluated using a BIAcore 2000 (BIAcore Inc., Uppsala, NJ). Briefly, a CM5 BIAcore biosensor chip was docked into the instrument and activated with 250 µL of 1:1 NHS/EDC at room temperature. A mouse anti-human Fc IgG1 (GE Healthcare #BR-1008-39) (13.5 µg/mL in 0.05M acetate buffer, pH5) was immobilized on the activated chips in flow cells 1. The immobilization was carried out at a flow rate of 5 µL/min. The chip was then blocked by injection of 55 µL of ethanolamine-HCl, pH8.5, followed by five washes with 50mM NaOH, 1M NaCl. To measure the binding of anti-CD38 mAbs to the human CD38 protein or cyno CD38 protein, antibodies were used at 2 µg/mL in BIAcore running buffer (HBS-EP). Antigens (human CD38-histag (ID2) or cyno CD38-histag (ID3)) were injected from 3 to 1000 nM. Following completion of the injection phase, dissociation was monitored in a BIAcore running buffer at the same flow rate for 360 sec. The surface was regenerated between injections using 30 µL of 50mM NaOH-1 M NaCl. Individual sensorgrams were analyzed using BIAsimulation software.

Binding affinity to human CD38-expressing pre-B cells

[0202] The binding of anti-CD38 antibodies to CD38 expressed on the surface of recombinant murine preB::300.19 cells was determined by flow cytometry. The recombinant cell line was described by J. Deckert et al. 2014 Clin. Cancer Res 20:4574-4583. Murine preB::300.19 CD38-expressing cells were coated at 40,000 cells/well on 96-well High Bind plate (MSD L15XB-3) and 100 µL/well of anti-CD38 antibodies were added for 45 min at 4°C and washed three times with PBS 1% BSA. 100 µL/well of goat anti-human IgG conjugated with Alexa488 (Jackson ImmunoResearch; # 109-545-098) was added for 45 min at 4°C and washed three times with PBS 1% BSA. Antibody binding was evaluated after centrifugation and resuspension of cells by adding 200 µl/well PBS 1% BSA and read using Guava® easyCyte™ 8HT Flow Cytometry System. Apparent KD and EC50 values were estimated using BIOST@T-BINDING and BIOST@T-SPEED software, respectively.

Recombinant CD38 proteins

[0203] Various recombinant CD38 proteins derived from isoform A with different tag and point mutations were used (SEQ ID NOs: 2, 3, 4, and 28), and a tagged version of CD38 isoform E (SEQ ID NO: 105) encompassing CD38 extracellular domain from R45-P203. The proteins were produced by transient expression in mammalian cells. Coding DNA sequences were cloned into mammalian expression plasmids under CMV enhancer/promoter and SV40 polyA signals. HEK293 cells (Invitrogen; #K9000-10) were transiently transfected with the expression plasmids using FreeStyle™ MAX 293 Expression System according to the manufacturer's instructions.

Apoptosis induction assay

[0204] Cells were incubated at 2×10^5 cells/mL in complete medium (RPMI-1640, 10% FBS, 2mM L-glutamine) with 1.5 µg/mL (10 nM) of indicated antibodies for 20 hours at 37°C with 5% CO₂. Cells were stained with AnnexinV-FITC in accordance with the manufacturer's instructions (Life Technologies). Samples were analyzed by flow cytometry on a BD FACSAria™ flow cytometer with BD FACSDiva software for acquisition control and data analysis (both BD Biosciences).

ELISA Assays

[0205] 96-well plates were coated with CD38 at 0.5 µg/well in PBS and 100 µL/well of antibodies were added to the plate. The plate was incubated at 37°C for 1h and washed five times with PBS containing 0.05% Tween-20 (PBS-T). Then, 100 µL of a 1:25,000 dilution of Anti-human IgG, conjugated with horseradish peroxidase, (Jackson Ref: 109-035-098) was added to each well. Following incubation at 37°C for 1 h in darkness, plates were washed with PBS-T five times. Antibody binding was visualized by adding TMB-H₂O₂ buffer and read at a wavelength of 450 nm. EC₅₀ values were estimated using BIOST@T-SPEED software.

Results

[0206] Binding properties of selected anti-human CD38 antibodies to soluble human CD38 and cynomolgus monkey CD38 was examined using ELISA, and surface plasmon resonance (SPR) assay using the BIAcore system (Pharmacia Biosensor; Piscataway, NJ). ELISA data were used to determine the EC₅₀ of antibody binding to human and cynomolgus monkey CD38 for humanized anti-CD38 antibodies antiCD38_C2-CD38-1_VH1-VL1, antiCD38_C2-CD38-1_VH3-VL3, antiCD38_C2-CD38-1_VH5-VL3, antiCD38_C2-CD38-1_VH6-VL3, and human anti-CD38 antibody antiCD38_1370.

[0207] The binding of the humanized anti-CD38 variants or human anti-CD38 mAb to CD38 was also evaluated using SPR assays. SPR data were used to determine the K_D and k_{off} of antibody binding to human and cynomolgus monkey CD38 for humanized anti-CD38 antibodies antiCD38_C2-CD38-1_VH1-VL1, antiCD38_C2-CD38-1_VH3-VL3, antiCD38_C2-CD38-1_VH5-VL3, antiCD38_C2-CD38-1_VH6-VL3, and human anti-CD38 antibody antiCD38_1370. The binding data, summarized in Table K, show that all the anti-CD38 mAbs bind to CD38 with similar binding characteristics.

Table K. Binding affinity of anti-CD38 mAbs to the soluble extracellular domain of humanCD38 and cynomolgusCD38 as determined by surface plasmon resonance assay.

	hCD38-his (SEQ ID NO:2)		cCD38-his (SEQ ID NO:4)	
	Kd (s-1)	KD (M)	Kd (s-1)	KD (M)
antiCD38_C2-CD38-1	2.66E-04	3.36E-10	9.85E-05	3.90E-10
antiCD38_C2-CD38-1_VH1-VL1	3.90E-04	3.32E-10	7.84E-04	3.44E-09
antiCD38_C2-CD38-1_VH3-VL3	2.83E-04	4.83E-10	1.29E-04	7.10E-10
antiCD38_C2-CD38-1_VH5-VL3	5.29E-04	8.22E-10	2.01E-04	1.14E-09
antiCD38_C2-CD38-1_VH6-VL3	3.33E-04	3.12E-10	1.25E-04	5.63E-10
antiCD38_1370	2.03E-04	1.44E-09	1.90E-04	1.38E-09

[0208] The ability of the humanized anti-CD38 variants to bind to CD38-expressing cells was assessed using the FACS-based binding assay described above. FACS data were used to determine the EC_{50} of antibody binding to human and cynomolgus monkey CD38 for humanized anti-CD38 antibodies antiCD38_C2-CD38-1_VH1-VL1, antiCD38_C2-CD38-1_VH3-VL3, antiCD38_C2-CD38-1_VH5-VL3, antiCD38_C2-CD38-1_VH6-VL3, and human anti-CD38 antibody antiCD38_1370. The binding data, set forth in Table L, shows that all humanized anti-CD38 variants exhibited similar binding affinities for cell surface CD38.

Table L. Binding affinity of anti-CD38 mAbs to CD38 expressing murine preB::300.19 cells.

	Apparent KD FACS (M)	
	hCD38-expressing cells	cCD38-expressing cells
antiCD38_C2-CD38-1	2.80E-10	2.20E-10
antiCD38_C2-CD38-1_VH1-VL1	3.30E-10	7.50E-10
antiCD38_C2-CD38-1_VH3-VL3	7.80E-10	1.31E-09
antiCD38_C2-CD38-1_VH5-VL3	5.50E-10	1.15E-09
antiCD38_C2-CD38-1_VH6-VL3	6.80E-10	1.07E-09
antiCD38_1370	2.07E-09	1.14E-09

[0209] Binding data from the ELISA, SPR, and FACS assays above are summarized in Table L2, along with sequence identity of the VH and VL domains to human V regions.

Table L2. Summary of ELISA, SPR, and FACS assays characterizing the binding of the indicated antibodies to human (Hu) and cynomolgus (Cyno) monkey CD38 polypeptides.

	INN Nomenclature	ELISA EC50 nM		SPR KD nM		FACS EC50 nM		Human V region identity	
		Hu	Cyno	Hu	Cyno	Hu	Cyno	Hu	Cyno
antiCD38_C2-CD38-1_VH1-VL1	-zumAb	0.11	0.10	0.33	3.44	0.33	0.76	84.7%	87.1%
antiCD38_C2-CD38-1_VH3-VL3	-zumAb	0.16	0.15	0.48	0.71	0.78	1.31	83.7%	83.9%
antiCD38_C2-CD38-1_VH5-VL3	-zumAb	0.16	0.17	0.82	1.14	0.55	1.15	80.6%	83.9%
antiCD38_C2-CD38-1_VH6-VL3	-zumAb	0.14	0.14	0.31	0.56	0.68	1.06	81.6%	83.9%
antiCD38_1370	-umAb	0.05	0.09	1.44	1.38	2.00	1.14	99.0%	95.8%

[0210] The ability of anti-CD38 antibodies to bind to both human CD38 isoforms A and E was also examined. For evaluating binding to CD38 isoform A and isoform E, an Enzyme-linked immunosorbent assay (ELISA) was performed by using isoform A and

isoform E proteins (prepared as described above) as capturing antigen. 96-well plates were coated with either isoform at 0.5 µg/well in PBS and 100 µL/well of antibodies were added to the plate. The plate was incubated at 37°C for 1h and washed five times with PBS containing 0.05% Tween-20 (PBS-T). Then, 100 µL of a 1:25,000 dilution of Anti-human IgG, conjugated with horseradish peroxidase, (Jackson Ref: 109-035-098) was added to each well. Following incubation at 37°C for 1 h in darkness, plates were washed with PBS-T five times. Antibody binding was visualized by adding TMB-H₂O₂ buffer and read at a wavelength of 450 nm. EC50 values were estimated using BIOST@T-SPEED software.

[0211] The binding affinity of various antibodies to CD38 isoform A (SEQ ID NO:1) and isoform E (SEQ ID NO:105) was determined, as shown in Table L3. Table M provides a comparison of binding properties for various anti-CD38 antibodies.

Table L3. Binding affinity of anti-CD38 antibodies for CD38 isoforms A and E, based on EC50 as determined by ELISA.

Antibody	CD38 isoform A EC50 (nM)	CD38 isoform E EC50 (nM)
antiCD38_C2-CD38-1	0.11 (CV 9 %)	0.08 (CV 7%)
antiCD38_C2-CD38-1_VH1-VL1	0.14 (CV 13%)	0.10 (CV 12%)
antiCD38_1370	0.47 (CV 3.7%)	0.32 (CV 5%)
antiCD38_SB19	0.10 (CV 7.1%)	No binding

Table M. Binding characteristics of various anti-CD38 antibodies.

Anti-CD38	H11 (Santa Cruz)	Daratumumab	antiCD38_SB19	antiCD38_C2-CD38-1	antiCD38_1370
Binding to huCD38 isoform A	+	+	+	+	+
Binding to huCD38 isoform E	+	-	-	+	+
Binding to cyno CD38	+	-	-	+	+

[0212] In conclusion, only antiCD38_C2-CD38-1 bound to both human and cynomolgus monkey CD38 with sub-nanomolar affinity and bound to CD38 isoforms A and E.

Example 2: Generation of trispecific anti-CD38 binding proteins

[0213] Next, binding properties of the antigen binding domains of selected anti-CD38 antibodies described in Example 1 were analyzed in the trispecific format depicted in FIG. 1.

Materials and Methods*Production and purification of trispecific binding proteins*

[0214] Trispecific binding proteins were produced by transient transfection of 4 expression plasmids into Expi293 cells using ExpiFectamine™ 293 Transfection Kit (Thermo Fisher Scientific) according to manufacturer's protocol. Briefly, 25% (w/w) of each plasmid was diluted into Opti-MEM, mixed with pre-diluted ExpiFectamine reagent for 20-30 minutes at room temperature (RT), and added into Expi293 cells (2.5×10^6 cells/ml). An optimization of transfection to determine the best ratio of plasmids was often used in order to produce the trispecific binding protein with good yield and purity.

[0215] 4-5 days post transfection, the supernatant from transfected cells was collected and filtered through 0.45 µm filter unit (Nalgene). The trispecific binding protein in the supernatant was purified using a 3-step procedure. First, protein A affinity purification was used, and the bound Ab was eluted using "IgG Elution Buffer" (Thermo Fisher Scientific). Second, product was dialyzed against PBS (pH7.4) overnight with 2 changes of PBS buffer. Any precipitate was cleared by filtration through 0.45 µm filter unit (Nalgene) before next step. Third, size-exclusion chromatography (SEC) purification (Hiload 16/600 Superdex 200pg, or Hiload 26/600 Superdex 200pg, GE Healthcare) was used to remove aggregates and different species in the prep. The fractions were analyzed on reduced and non-reduced SDS-PAGE to identify the fractions that contained the monomeric trispecific binding protein before combining them. The purified antibody can be aliquoted and stored at -80°C long term.

ELISA assays

[0216] The binding properties of the purified antibodies were analyzed either using ELISA or SPR methods. For ELISA, corresponding antigens for each binding site in the trispecific binding protein were used to coat a 96-well Immuno Plate (Nunc 439454, Thermo Fisher Scientific) overnight at 4°C using 2 µg/ml each antigen in PBS(pH7.4). The coated plate was blocked using 5% skim milk+2% BSA in PBS for one hour at RT, followed by washing with PBS+0.25% Tween 20 three times (Aqua Max 400, Molecular Devices). Serial dilution of antibodies (trispecific and control Abs) were prepared and added onto the ELISA

plates (100 μ l/well in duplicate), incubated at RT for one hour, followed by washing 5 times with PBS+0.25% Tween 20.

[0217] After washing, the HRP conjugated secondary anti-human Fab (1:5000, Cat. No. 109-035-097, Jackson ImmunoResearch Inc) was added to each well and incubated at RT for 30 minutes. After washing 5 times with PBS+0.25% Tween 20, 100 μ l of TMB Microwell Peroxidase Substrate (KPL, Gaithersburg, MD, USA) was added to each well. The reaction was terminated by adding 50 μ l 1M H₂SO₄, and OD₄₅₀ was measured using SpectraMax M5 (Molecular Devices) and analyzed using SoftMax Pro6.3 software (Molecular Devices). The final data was transferred to GraphPad Prism software (GraphPad Software, CA, USA), and plotted as shown. EC₅₀ was calculated using the same software.

[0218] ELISA assay was used to determine the binding of an anti-CD38xCD28xCD3 trispecific antibodies or isotype control antibody (human IgG4) to human CD3 (Cambridge Biologics LLC Cat#03-01-0051), CD28 (Cambridge Biologics LLC Cat#03-01-0303), and CD38 (Cambridge Biologics LLC Cat#03-01-0369). The bound antibodies were detected using a horseradish peroxidase (HRP)-conjugated anti-Fab secondary antibody (Jackson ImmunoResearch Inc #109-035-097).

Results

[0219] Anti-CD38 antigen binding domains were tested in trispecific format (anti-CD38xanti-CD28xanti-CD3) for ability to bind CD38 when other antigen binding domains are bound to their cognate ligands using SPR. For sequential binding of the three antigens to each trispecific Ab, saturating concentration (> 10 KD) of each antigen was injected for 8 min followed by 5 min dissociation. Surface regenerate was conducted by injecting 10 mM Glycine-HCl pH 2.5 for 60 sec at 30 μ l/min. Data were fitted with 1:1 kinetic binding model and analyzed using Biacore S200 Evaluation Software v 1.0. Equilibrium dissociation constant (K_D) was calculated using association rate constant (k_{on}) and dissociation rate constant (k_{off}).

[0220] This SPR-based assay showed that trispecific binding proteins were able to bind CD38 regardless of whether the CD3 and/or CD28 antigen binding domains were also bound to their cognate antigen. Kinetic parameters as measured by SPR are provided in Table M2.

Table M2. Binding of trispecific anti-CD38xanti-CD28xanti-CD3 binding proteins to 1, 2, or 3 cognate antigens.

Binding protein state prior to CD38 binding	k_a ($M^{-1}s^{-1}$)	k_d (s^{-1})	K_D (M)
No prebound	9.02E+05	1.42E-03	1.57E-09
Prebound CD3	8.35E+05	1.24E-03	1.48E-09
Prebound CD28	7.39E+05	1.32E-03	1.79E-09
Prebound CD3 then CD28	8.18E+05	1.23E-03	1.50E-09
Prebound CD28 then CD3	8.37E+05	1.23E-03	1.47E-09

[0221] These results demonstrate that all three targets can bind to the trispecific binding proteins simultaneously. Pre-binding the trispecific binding proteins with CD28, CD3, or both (in either order) did not alter binding kinetics or binding affinity to CD38.

[0222] Next, each antigen binding domain of the CD38_{SB19}CD28_{sup}CD3_{mid} trispecific binding protein was evaluated by SPR for the ability to bind cognate antigen with and without the other two antigen binding domains in saturation. Tables M3 and M4 show the results of these assays.

Table M3. Target binding without other targets present.

Target	k_a ($M^{-1}s^{-1}$)	k_d (s^{-1})	K_D (M)
CD38	8.04E+05	1.41E-03	1.75E-09
CD28	1.16E+05	3.14E-04	2.71E-09
CD3	2.90E+04	6.73E-04	2.32E-08

Table M4. Target binding with other targets in saturation.

Target	k_a ($M^{-1}s^{-1}$)	k_d (s^{-1})	K_D (M)
CD38	5.93E+05	1.44E-03	2.42E-09
CD28	1.05E+05	3.96E-04	3.77E-09
CD3	1.27E+05	2.36E-03	1.86E-08

[0223] As demonstrated in Tables M3 and M4, having two targets saturated by pre-binding with antigen did not impact the kinetics or binding affinity of the third target for

CD38 or CD28. In the case of CD3 binding, prebound CD38 and/or CD28 resulted in faster kinetics (approximately 4-fold impact on k_{on} and k_{off} values).

[0224] Anti-CD38 antigen binding domains were tested in trispecific format with two anti-CD28 antigen binding domains (super agonist, “sup,” and conventional agonist, “cyn”) and two anti-CD3 antigen binding domains (“mid” and “low”). Variable domain sequences for these antigen binding domains are provided as follows: anti-CD28_{sup}: SEQ ID NO:49 (VH) and SEQ ID NO:50 (VL); anti-CD28_{cyn}: SEQ ID NO:51 (VH) and SEQ ID NO:52 (VL); anti-CD3_{mid}: SEQ ID NO:53 (VH) and SEQ ID NO:54 (VL); anti-CD3_{low}: SEQ ID NO:84 (VH) and SEQ ID NO:85 (VL). The results of SPR assays examining binding of trispecific binding proteins are shown in **FIG. 2**. Three anti-CD38 binding domains had roughly the same binding affinity in the trispecific binding protein format as in a monospecific format. Both CD3 binding domains had approximately the same binding affinity in mono-, bi-, and trispecific formats. CD28 binding domains showed slightly lower (but still nanomolar) binding affinity in bi- or trispecific format as compared with monospecific. When the other two antigen binding domains were saturated, anti-CD38_{SB19} and anti-CD28_{sup} binding domains had similar binding affinities, compared with when the other two antigen binding domains are not bound to antigen. However, the anti-CD3_{mid} binding domain showed faster kinetics when the other two antigen binding domains were saturated. These results demonstrate that anti-CD38, anti-CD28, and anti-CD3 binding domains are compatible for use with the trispecific binding protein format.

[0225] The anti-CD38 antigen binding domains generated herein were also compared against the existing anti-CD38 antigen binding domain of antiCD38__SB19 (see SEQ ID NO:47 for VH and SEQ ID NO:48 for VL sequences, respectively). The binding of trispecific molecules to CD38 expressed on the surface of recombinant murine preB::300.19 cells was determined by flow cytometry and the corresponding anti-CD38 monovalent antibodies were assayed in parallel. The recombinant cell line was described by J. Deckert et al. 2014 Clin. Cancer Res 20:4574-4583. Murine preB::300.19 CD38-expressing cells were coated at 40,000 cells/well on 96-well High Bind plate (MSD L15XB-3) and 100 μ L/well of trispecific molecules were added for 45 min at 4°C and washed three times with PBS 1% BSA. 100 μ L/well of goat anti-human IgG conjugated with Alexa488 (Jackson ImmunoResearch; # 109-545-098) was added for 45 min at 4°C and washed three times with PBS 1% BSA. Antibody binding was evaluated after centrifugation and resuspension of cells by adding 200 μ L/well PBS 1% BSA and read using Guava® easyCyte™ 8HT Flow

Cytometry System. Apparent KD and EC50 values were estimated using BIOST@T-BINDING and BIOST@T-SPEED software, respectively.

[0226] Flow cytometry was used as described above to examine binding of antiCD38_SB19 antibody or the trispecific binding protein with the antiCD38_SB19 anti-CD38 antigen binding domain to murine pre-B cells expressing human or cynomolgus monkey CD38 polypeptide on their cell surface. The CD38xCD28^{sup}xCD3_{mid} trispecific binding protein with the antiCD38_SB19 anti-CD38 antigen binding domain bound to cells expressing human CD38 with 8-fold lower apparent affinity (EC50 = 4 nM) than antiCD38_SB19 monospecific antibody (EC50 = 0.5 nM). Neither antiCD38_SB19 monospecific antibody or the trispecific binding protein with the antiCD38_SB19 anti-CD38 antigen binding domain bound to cells expressing cynomolgus CD38.

[0227] The binding domain of humanized anti-CD38 antibody antiCD38_C2-CD38-1_VH1-VL1 was also tested in trispecific formats for binding to cells expressing human or cynomolgus CD38 polypeptides. Unlike antiCD38_SB19, CD38xCD28^{sup}xCD3_{mid} and CD38xCD28^{cvn}xCD3_{mid} trispecific binding proteins with antiCD38_C2-CD38-1_VH1-VL1 anti-CD38 antigen binding domain, as well as the antiCD38_C2-CD38-1_VH1-VL1 monospecific antibody, were able to bind both human and cynomolgus monkey CD38 polypeptides. The CD38xCD28^{cvn}xCD3_{mid} trispecific binding protein with the antiCD38_C2-CD38-1_VH1-VL1 anti-CD38 antigen binding domain bound to cells expressing human CD38 with 9-fold lower apparent affinity (EC50 = 4.4 nM) than the parental antiCD38_C2-CD38-1_VH1-VL1 antibody (EC50 = 0.5 nM). The CD38xCD28^{cvn}xCD3_{mid} trispecific binding protein with the antiCD38_C2-CD38-1_VH1-VL1 anti-CD38 antigen binding domain bound to cells expressing cynomolgus CD38 with 7.5-fold lower apparent affinity (EC50 = 7.5 nM) than the parental antiCD38_C2-CD38-1_VH1-VL1 antibody (EC50 = 1 nM). The CD38xCD28^{sup}xCD3_{mid} trispecific binding protein with the antiCD38_C2-CD38-1_VH1-VL1 anti-CD38 antigen binding domain bound to cells expressing human CD38 with a 2.5-fold lower apparent affinity (EC50 = 11 nM) than the CD38xCD28^{cvn}xCD3_{mid} trispecific binding protein with the antiCD38_C2-CD38-1_VH1-VL1 anti-CD38 antigen binding domain (EC50 = 4.4 nM).

[0228] The binding domain of humanized anti-CD38 antibody antiCD38_1370 was also compared against the antiCD38_1370 monospecific antibody for binding to cells expressing human or cynomolgus CD38 polypeptides. While the antiCD38_1370 monospecific antibody bound to cells expressing human (EC50 = 11.2 nM) or cynomolgus monkey (EC50 = 6.6 nM) CD38 polypeptides in the nM range, the CD38xCD28^{sup}xCD3_{mid} trispecific

binding protein with the antiCD38_1370 anti-CD38 antigen binding domain bound to cells expressing human or cynomolgus monkey CD38 polypeptides without saturation.

[0229] In conclusion, the affinity for CD38_{SB19}×CD28_{sup}×CD3_{mid} trispecific binding protein (antiCD38__SB19 anti-CD38 binding domain) binding to human CD38 was found to be in the same range, whether examining binding to recombinant human CD38 by SPR or to human CD38 expressed on a cell surface by flow cytometry (**FIG. 3**). Similarly, the affinity of CD38_{VH1}×CD28_{sup}×CD3_{mid/low} (antiCD38_C2-CD38-1_VH1-VL1 anti-CD38 binding domain) and CD38_{VH1}×CD28_{cvn}×CD3_{mid/low} trispecific binding proteins (antiCD38_C2-CD38-1_VH1-VL1 anti-CD38 binding domain) for binding to human CD38 was also in the same range in both assays. For CD38_{HHY1370}×CD28_{sup}×CD3_{mid} (antiCD38_1370 anti-CD38 binding domain), the K_D for binding human CD38 was determined by SPR to be 1nM, whereas no accurate EC50 value could be estimated by flow cytometry (**FIG. 3**). A summary of apparent K_D values (obtained by FACS analyses) of trispecific binding proteins with various anti-CD38 binding domains is provided in Table M5.

Table M5. Summary of apparent K_D values obtained by flow cytometry assays.

	Apparent K_D FACS (M)	
	hCD38-expressing cells	cCD38-expressing cells
Trispecific with antiCD38_C2-CD38-1_VH1-VL1 anti-CD38 binding domain	4.4 nM	7.5 nM
Trispecific with antiCD38_1370 anti-CD38 binding domain	No saturation	No saturation
Trispecific with antiCD38_SB19 anti-CD38 binding domain	4 nM	No binding
	Apparent K_D FACS (M)	
	<i>hCD38-expressing cells</i>	<i>cCD38-expressing cells</i>
antiCD38_C2-CD38-1_VH1-VL1	<i>0.5 nM</i>	<i>1 nM</i>
antiCD38_1370	<i>11.2 nM</i>	<i>6.6 nM</i>
antiCD38_SB19	<i>0.5 nM</i>	<i>No binding</i>

[0230] As expected, Δ CD38xCD28_{sup}xCD3_{mid} trispecific binding protein lacking the anti-CD38 binding domain did not bind to cells expressing human or cynomolgus monkey CD38 polypeptides. This indicates that the binding observed in this assay was specific for the CD38 antigen binding domains.

Example 3: CD38/CD3xCD28 Ab stimulates central memory CD4 and CD8, Th1 and antigen-specific responses

[0231] To determine whether the CD38/CD3xCD28 trispecific Ab could enhance cellular immune function, the phenotype of expanded T cells *in vitro* was evaluated.

Materials and Methods

[0232] Peripheral blood mononuclear cells were isolated from blood of healthy human donors collected by Research Blood Components, LLC (Boston, MA). The PBMC were added to antibody-coated plates (350 ng/well) (5×10^5 cells/mL), as previously described above, and incubated at 37°C for 3 and 7 days. The cells were collected at specific time points and analyzed by flow cytometry for T cell subsets: naïve (CCR7⁺ CD45RO⁻), Tcm (CCR7⁺ CD45RO⁺), Tem (CCR7⁻ CD45RO⁺), Tregs (CD4⁺ Foxp3⁺ CD25^{hi}). Cells were also treated with monensin (GolgiStop) (BD Biosciences, CA) for at least 6 hours before flow staining to determine intracellular cytokine expression: Th1 (CD4⁺ IFN- γ ⁺), Th2 (CD4⁺ IL-4⁺), and Th17 (CD4⁺ IL-17⁺). CMV pp65-specific CD8⁺ T cells were detected using fluorescent-conjugated pentamer restricted to the PBMC donors' HLA (A*02:01/NLVPMVATV) (ProImmune, Oxford, UK). PBMC was obtained from HemaCare (Van Nuys, CA) from donors with known CMV positive populations and HLA types. PMBC from donors negative for the restricting HLA type was used as negative control. Staining was done as per manufacturer's protocol.

Results

[0233] Human PBMCs from CMV-infected donors were incubated for 7 days with the trispecific Ab or a negative control trispecific antibody with three mutated antigen binding sites in the absence of cytokines. Analysis of the CD4 subsets revealed the greatest proliferation in the central memory pool, with a smaller increase in effector memory cells (FIG. 4A). Analysis of the CD4 subset also revealed the greatest proliferation of Th1 cells (>6-fold) compared to Th2 or Th17 cells (FIG. 4B). In the CD8 subset, there was a >150-fold increase in the central memory CD8 subset by day 7, with a lesser increase in effector memory cells (FIG. 4C). Importantly, pre-existing antigen-specific CD8 responses to CMV,

directed to the pp65 epitope in seropositive HLA-A2 donors using tetramer staining (Gratama JW, van Esser JW, et al. *Blood* **98**:1358-1364(2001)), increased >44-fold in the presence of the CD38 trispecific compared to negative control (**FIG. 4D**).

[0234] Taken together, these data indicate that the CD38 trispecific Ab stimulates Th1 function and protective CD8 memory T cell responses that are likely to enhance anti-tumor and anti-viral immunity *in vivo*.

Example 4: CD38/CD28xCD3 trispecific antibodies promote CMV-specific immune response

[0235] The activation and/or proliferation of viral antigen specific T cells could provide a therapeutic strategy against viral infections, such as infections of Cytomegalovirus (CMV). The ability of CD38/CD28xCD3 trispecific antibodies to promote activation and expansion of CMV-specific T cells was determined.

Materials and Methods

ELISA assays

[0236] Binding affinities to each target antigen by the CD38/CD28xCD3 T cell engagers were measured by ELISA. Briefly, each antigen was used to coat the 96-well Immuno Plate (Thermo Fisher Scientific) overnight at 4°C using 200 ng/well in PBS (pH7.4) of each antigen. The coated plate was blocked using 5% skim milk+2% BSA in PBS for one hour at RT, followed by washing with PBS+0.25% Tween 20 three times (Aqua Max 400, Molecular Devices). Serial dilution of antibodies (trispecific and control Abs) were prepared and added onto the ELISA plates (100 µl/well in duplicate), incubated at RT for one hour, followed by washing 5 times with PBS+0.25% Tween 20. After washing, the HRP conjugated secondary anti-human Fab (1:5000, Cat. No. 109-035-097, Jackson ImmunoResearch Inc) was added to each well and incubated at RT for 30 minutes. After washing 5 times with PBS+0.25% Tween 20, 100 µl of TMB Microwell Peroxidase Substrate (KPL, Gaithersburg, MD, USA) was added to each well. The reaction was terminated by adding 50 µl 1M H2SO4, and OD450 was measured using SpectraMax M5 (Molecular Devices) and analyzed using SoftMax Pro6.3 software (Molecular Devices). The final data was transferred to GraphPad Prism software (GraphPad Software, CA, USA), and plotted. EC50 was calculated using the same software.

SPR Assays

[0237] Human CD38-His antigens were used (Cambridge Biologics, Cambridge, MA) for full kinetic analysis. Kinetic characterization of purified antibodies was performed using SPR technology on a BIACORE 3000 (GE Healthcare). A capture assay using human IgG1 specific antibody capture and orientation of the investigated antibodies was used. For capture of Fc containing protein constructs the human antibody capture kit (GE Healthcare) was used. For capture of His-tagged antigen, anti-His antibody capture kit (GE Healthcare) was used. The capture antibody was immobilized via primary amine groups (11000 RU) on a research grade CM5 chip (GE Life Sciences) using standard procedures. The analyzed antibody was captured at a flow rate of 10 μ L/min with an adjusted RU value that would result in maximal analyte binding signal of typically 30 RU. Binding kinetics was measured against the trispecific antibodies. As assay buffer HBS EP (10 mM HEPES, pH 7.4, 150 mM NaCl, 3 mM EDTA, and 0.005 % Surfactant P20) was used at a flow rate of 30 μ L/min. Chip surfaces were regenerated with the regeneration solution of the respective capture kit. Kinetic parameters were analyzed and calculated in the BIA evaluation program package v4.1 using a flow cell without captured antibody as reference and the 1:1 Langmuir binding model with mass transfer.

In vitro T cell proliferation measurement

[0238] T cells were isolated from human Peripheral blood mononuclear cell (PBMC) donors by negative selection using a magnetic Pan T Cell Isolation Kit (Miltenyi Biotec GmbH, Germany). Antibodies were coated onto 96-well cell culture plates by preparing the antibodies in sterile PBS and dispensing 50 μ L into each well (350 ng/well). The plates were then incubated at 37°C for at least 2 hours and then washed with sterile PBS. The untouched T cells were added to the antibody-coated plates (5×10^5 cells/mL) and incubated at 37°C for multiple days. The cells were passaged with new cell culture media onto fresh antibody-coated plates on day 4. In certain experiments with 7 days incubation, only fresh medium was added w/o changing to fresh antibody-coated plate. The cells were collected at specific time points and cell numbers calculated using CountBright™ counting beads.

In vitro T cell proliferation assay and T cell subset determination

[0239] Peripheral blood mononuclear cells were isolated from blood of healthy human donors collected by Research Blood Components, LLC (Boston, MA). The PBMCs were added to antibody-coated plates (350 ng/well) (5×10^5 cells/mL), as previously described

above, and incubated at 37°C for 3 and 7 days. The cells were collected at specific time points and analyzed by flow cytometry for T cell subsets: naïve (CCR7+ CD45RO-), T_{em} (CCR7+ CD45RO+), T_{cm} (CCR7- CD45RO+), T_{regs} (CD4+ Foxp3+ CD25hi). Cells were also treated with monensin (GolgiStop) (BD Biosciences, CA) for at least 6 hours before flow staining to determine intracellular cytokine expression: Th1 (CD4+ IFN-γ+), Th2 (CD4+ IL-4+), and Th17 (CD4+ IL-17+). CMV pp65-specific, EBV BMLF-specific, Influenza A MP-specific and HIV-1 Gag-specific CD8+ T cells were detected using fluorescent-conjugated pentamer restricted to the PBMC donors' HLA/viral peptide (A*02:01/NLVPMVATV; SEQ ID NO:26), (A*02:01/GLCTLVAML; SEQ ID NO:27), (A*02:01/GILGFVFTL; SEQ ID NO:28), and (A*02:01/SLYNTVATL; SEQ ID NO:25) respectively (ProImmune, Oxford, UK). PBMC was obtained from HemaCare (Van Nuys, CA) for donors with known CMV, EBV, or Influenza A, and from BioIVT (Westbury, NY) for donors with known HIV-1 positivity and HLA types. PMBC from donors negative for the restricting HLA type was used as negative control. Staining was done as per manufacturer's protocol.

Quantification of CMV-specific T-Cells

[0240] As described above, PBMCs were isolated from blood of known CMV-infected human donors and added to plates containing the trispecific antibody or control antibody. The plates were incubated at 37°C. The cells were collected at the indicated time points and analyzed as described above.

Results

[0241] The CD38^{VH1}/CD28^{sup} x CD3^{mid} trispecific antibody activated T cells and promoted the proliferation of CMV-specific memory CD8+ T cells following incubation for up to 10 days with PBMCs isolated from CMV-infected human donor B (**FIGS. 5A-5B**) and CMV-infected human donor C (**FIGS. 6A-6B**). As shown in **FIG. 5A** (CMV Donor B) and **FIG. 6A** (CMV Donor C), the CD38^{VH1}/CD28^{sup} x CD3^{mid} trispecific antibody led to increases in CMV-specific memory CD8+ T cells (cells/μl) relative to a the triple mutant control antibody. In addition, CD38^{VH1}/CD28^{sup} x CD3^{mid} trispecific antibody increased the percentage of CMV-specific CD8+ effector memory (T_{em}) and central memory (T_{cm}) cells relative to the triple mutant control antibody (CMV Donor B, **FIG. 5B**; CMV Donor C, **FIG. 6B**).

[0242] Taken together, these data indicate that CD38/CD28xCD3 trispecific antibodies promote activation and expansion of CMV-specific T cells, such as CMV-specific CD8+ T

cells, CMV-specific effector memory (T_{em}) CD8⁺ T cells, and CMV-specific central memory (T_{cm}) CD8⁺ T cells.

Example 5: CD38/CD28xCD3 trispecific antibodies promote EBV-specific immune response

[0243] Next, the ability of CD38/CD28xCD3 trispecific antibodies to promote activation and expansion of Epstein-Barr virus (EBV)-specific T cells was determined.

Materials and Methods

Quantification of EBV-specific T-Cells

[0244] As described above, PBMCs were isolated from blood of known EBV-infected human donors and added to plates containing the trispecific antibody or control antibody. The plates were incubated at 37°C. The cells were collected at the indicated time points and analyzed as described above.

Results

[0245] The CD38^{VH1}/CD28^{sup} x CD3^{mid} trispecific antibody activated T cells and promoted the proliferation of EBV-specific memory CD8⁺ T cells following incubation for up to 11 days with PBMCs isolated from EBV-infected human donor A (**FIGS. 7A-7B**) and EBV-infected human donor B (**FIGS. 8A-8B**). As shown in **FIG. 7A** (EBV Donor A) and **FIG. 8A** (EBV Donor B), the CD38^{VH1}/CD28^{sup} x CD3^{mid} trispecific antibody led to increases in EBV-specific memory CD8⁺ T cells (cells/ μ l) relative to the triple mutant control antibody. In addition, CD38^{VH1}/CD28^{sup} x CD3^{mid} trispecific antibody increased the percentage of EBV-specific CD8⁺ T_{em} cells and T_{cm} cells relative to the triple mutant control antibody (EBV Donor A, **FIG. 7B**; EBV Donor B, **FIG. 8B**, *e.g.* see day 7).

[0246] Taken together, these data indicate that CD38/CD28xCD3 trispecific antibodies promote activation and expansion of EBV-specific T cells, such as EBV-specific CD8⁺ T cells, EBV-specific effector memory (T_{em}) CD8⁺ T cells, and EBV-specific central memory (T_{cm}) CD8⁺ T cells.

Example 6: CD38/CD28xCD3 trispecific antibodies promote HIV-specific immune response

[0247] Next, the ability of CD38/CD28xCD3 trispecific antibodies to promote activation and expansion of Human Immunodeficiency Virus (HIV)-specific T cells was determined.

Materials and Methods

Quantification of HIV-specific T-Cells

[0248] As described above, PBMCs were isolated from blood of known HIV-infected human donors and added to plates containing the trispecific antibody or control antibody. The plates were incubated at 37°C. The cells were collected at the indicated time points and analyzed as described above.

Results

[0249] On day 0 (prior to incubation with trispecific antibodies), PBMCs from HIV-positive donors exhibit HIV Gag-specific CD8 T cells (A*02:01 - SLYNTVATL (HIV-1 gag p17 76-84) Pentamer conjugated to PE, ProImmune) (**FIG. 9**). For example, three HIV-positive PBMC donors had 0.86% (HIV Donor A), 0.95% (HIV Donor B), and 2.27% (HIV Donor C) Gag-specific CD8 T cells compared to 0.33% in PBMCs from an HIV-negative donor.

[0250] Incubation of PBMCs for up to 10 days with CD38_{VH1}/CD28_{sup} x CD3_{mid} trispecific antibody activated T cells and promoted proliferation of HIV-specific T cells. As shown in **FIG. 10A** (HIV Donor D), **FIG. 11A** (HIV Donor E), and **FIG. 12A** (HIV Donor F), the CD38_{VH1}/CD28_{sup} x CD3_{mid} trispecific antibody led to increases in HIV-specific memory CD8⁺ T cells (cells/ μ l) relative to the triple mutant control antibody. In addition, CD38_{VH1}/CD28_{sup} x CD3_{mid} trispecific antibody increased the percentage of HIV-specific CD8⁺ effector memory (T_{em}) cells (*e.g.*, see days 7 and 10), and also to a lesser degree CD8⁺ central memory (T_{cm}) cells, relative to the triple mutant control antibody (HIV Donor D, **FIG. 10B**; HIV Donor E, **FIG. 11B**; HIV Donor F, **FIG. 12B**).

Example 7: CD38/CD28xCD3 trispecific antibodies promote Influenza-specific immune response

[0251] The ability of CD38/CD28xCD3 trispecific antibodies to promote activation and expansion of influenza-specific T cells was determined.

Materials and Methods

Quantification of influenza-specific T-Cells

[0252] As described above, PBMCs were isolated from blood of known influenza A-infected human donors and added to plates containing the trispecific antibody or control antibody. The plates were incubated at 37°C. The cells were collected at the indicated time points and analyzed as described above.

Results

[0253] The CD38^{VH1}/CD28^{sup} x CD3^{mid} trispecific antibody activated T cells and promoted the proliferation of influenza-specific memory CD8⁺ T cells following incubation for up to 11 days with PBMCs isolated from a known influenza-infected human donor **FIGS.13A-13B** (Influenza Donor A). As shown in **FIG. 13A**, the CD38^{VH1}/CD28^{sup} x CD3^{mid} trispecific antibody led to increases in influenza-specific memory CD8⁺ T cells (cells/ μ l) relative to the triple mutant control antibody. In addition, CD38^{VH1}/CD28^{sup} x CD3^{mid} trispecific antibody increased the percentage of influenza (Flu)-specific CD8⁺ T_{cm} cells (*e.g.*, see days 7 and 11) and T_{cm} cells (*e.g.*, see day 7) relative to the triple mutant control antibody (**FIG. 13B**).

[0254] Taken together, the data presented in Examples 1-7 demonstrate trispecific anti-CD38/CD3/CD28 antibodies stimulate potent anti-viral immunity against diverse viruses. Without wishing to be bound by theory, it is believed that CD38/CD3/CD28 trispecific antibodies can activate and promote the proliferation of T cells by engaging all three ligands on T cells. In particular, it is believed that engagement of CD3/CD28 on T cells by CD38/CD3/CD28 trispecific antibodies initiates T cell activation, proliferation, and differentiation into memory T cells. Further, without wishing to be bound by theory, it is believed that engagement of CD28 provides an advantageous co-stimulatory signal which enhances the duration and magnitude of the immune response, and promotes T cell proliferation and survival.

[0255] While the disclosure includes various embodiments, it is understood that variations and modifications will occur to those skilled in the art. Therefore, it is intended that the appended claims cover all such equivalent variations that come within the scope of the disclosure. In addition, the section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described.

[0256] Each embodiment herein described may be combined with any other embodiment or embodiments unless clearly indicated to the contrary. In particular, any feature or embodiment indicated as being preferred or advantageous may be combined with any other feature or features or embodiment or embodiments indicated as being preferred or advantageous, unless clearly indicated to the contrary.

[0257] All references cited in this application are expressly incorporated by reference herein.

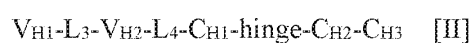
CLAIMS

What is claimed is:

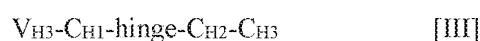
1. A method for expanding virus-specific memory T cells, comprising contacting a virus-specific memory T cell with a binding protein, wherein the binding protein comprises four polypeptide chains that form the three antigen binding sites, wherein a first polypeptide chain comprises a structure represented by the formula:



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



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



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



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;

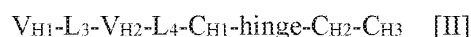
wherein the polypeptide of formula I and the polypeptide of formula II form a cross-over light chain-heavy chain pair; and

wherein V_{H1} and V_{L1} form a first antigen binding site that binds a CD28 polypeptide, wherein V_{H2} and V_{L2} form a second antigen binding site that binds a CD3 polypeptide, and wherein V_{H3} and V_{L3} form a third antigen binding site that binds a CD38 polypeptide.

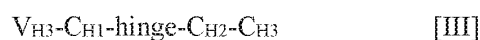
2. The method of claim 1, wherein the virus-specific memory T cell is contacted with the binding protein *in vitro* or *ex vivo*.
3. The method of claim 1 or claim 2, wherein contacting the virus-specific memory T cell with the binding protein causes activation and/or proliferation of virus-specific memory T cells.
4. A method for expanding T cells, comprising contacting a T cell with a binding protein *in vitro* or *ex vivo*, wherein the binding protein comprises four polypeptide chains that form the three antigen binding sites, wherein a first polypeptide chain comprises a structure represented by the formula:



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



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



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



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;

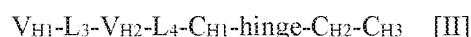
wherein the polypeptide of formula I and the polypeptide of formula II form a cross-over light chain-heavy chain pair; and

wherein V_{H1} and V_{L1} form a first antigen binding site that binds a CD28 polypeptide, wherein V_{H2} and V_{L2} form a second antigen binding site that binds a CD3 polypeptide, and wherein V_{H3} and V_{L3} form a third antigen binding site that binds a CD38 polypeptide.

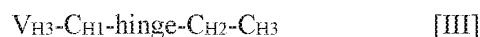
5. The method of claim 4, wherein the T cell is a memory T cell or an effector T cell.
6. The method of claim 4 or claim 5, wherein the T cell expresses a chimeric antigen receptor (CAR) on its cell surface or comprises a polynucleotide encoding a CAR.
7. A method for treating chronic viral infection, comprising administering to an individual in need thereof an effective amount of a binding protein, wherein the binding protein comprises four polypeptide chains that form the three antigen binding sites, wherein a first polypeptide chain comprises a structure represented by the formula:



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



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



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



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; and

wherein V_{H1} and V_{L1} form a first antigen binding site that binds a CD28 polypeptide, wherein V_{H2} and V_{L2} form a second antigen binding site that binds a CD3 polypeptide, and wherein V_{H3} and V_{L3} form a third antigen binding site that binds a CD38 polypeptide.

8. The method of claim 7, wherein the individual is a human.
9. The method of claim 7 or claim 8, wherein the binding protein is administered to the individual in pharmaceutical formulation comprising the binding protein and a pharmaceutically acceptable carrier.
10. The method of any one of claims 7-9, wherein administration of the binding protein results in activation and/or proliferation of virus-specific memory T cells in the individual.
11. The method of any one of claims 1-6 and 10, wherein the memory T cells are CD8+ or CD4+ memory T cells.
12. The method of any one of claims 1-6, 10, and 11, wherein the memory T cells are central memory T cells (T_{CM}) or effector memory T cells (T_{EM}).
13. The method of any one of claims 1-12, wherein the CD28 polypeptide is a human CD28 polypeptide, wherein the CD3 polypeptide is a human CD3 polypeptide, and wherein the CD38 polypeptide is a human CD38 polypeptide.
14. The method of any one of claims 1-13, wherein:

(a) the V_{H3} domain comprises a CDR-H1 sequence comprising the amino acid sequence of GYTFTSFN (SEQ ID NO:31), a CDR-H2 sequence comprising the amino acid sequence of IYPGNGGT (SEQ ID NO:32), and a CDR-H3 sequence comprising the amino acid sequence of ARTGGLRRAYFTY (SEQ ID NO:33), and the V_{L3} domain comprises a CDR-L1 sequence comprising the amino acid sequence of ESVDSYGNGF (SEQ ID NO:34), a CDR-L2 sequence comprising the amino acid sequence of LAS (SEQ ID NO:35), and a CDR-L3 sequence comprising the amino acid sequence of QQNKEDPWT (SEQ ID NO:36);

(b) the V_{H3} domain comprises a CDR-H1 sequence comprising the amino acid sequence of GYTFTSYA (SEQ ID NO:37), a CDR-H2 sequence comprising the amino acid

sequence of IYPGQGGT (SEQ ID NO:38), and a CDR-H3 sequence comprising the amino acid sequence of ARTGGLRRAYFTY (SEQ ID NO:33), and the V_{L3} domain comprises a CDR-L1 sequence comprising the amino acid sequence of QSVSSYGQGF (SEQ ID NO:39), a CDR-L2 sequence comprising the amino acid sequence of GAS (SEQ ID NO:40), and a CDR-L3 sequence comprising the amino acid sequence of QQNKEDPWT (SEQ ID NO:36); or

(c) the V_{H3} domain comprises a CDR-H1 sequence comprising the amino acid sequence of GFTFSSYG (SEQ ID NO:41), a CDR-H2 sequence comprising the amino acid sequence of IWYDGSNK (SEQ ID NO:42), and a CDR-H3 sequence comprising the amino acid sequence of ARMFRGAFDY (SEQ ID NO:43), and the V_{L3} domain comprises a CDR-L1 sequence comprising the amino acid sequence of QGIRND (SEQ ID NO:44), a CDR-L2 sequence comprising the amino acid sequence of AAS (SEQ ID NO:45), and a CDR-L3 sequence comprising the amino acid sequence of LQDYIYYPT (SEQ ID NO:46).

15. The method of claim 14, wherein:

(a) the V_{H3} domain comprises the amino acid sequence of QVQLQQSGAELVRSGASVKMSCKASGYTFTSFNMHWVKETPGQGLEWIGYIYPGN GGTNYNQKFKGKATLTADTSSSTAYMQISSLTSEDSAVYFCARTGGLRRAYFTYWG QGTLVTVS (SEQ ID NO:5), and the V_{L3} domain comprises the amino acid sequence of DIVLTQSPASLAVSLGQRATISCRASESVDSYGNGFMHWYQQKPGQPPKLLIYASN LESGVPARFSGSGSRTDFTLTIDPVEADDAATYYCQQNKEDPWTFGGGKLEIK (SEQ ID NO:6);

(b) the V_{H3} domain comprises the amino acid sequence of QVQLVQSGAEVVKPGASVKVSCKASGYTFTSYAMHWVKEAPGQRLEWIGYIYPGQ GGTNYNQKFQGRATLTADTSASTAYMELSSLRSEDTAVYFCARTGGLRRAYFTYWG QGTLVTVSS (SEQ ID NO:13), and the V_{L3} domain comprises the amino acid sequence of DIVLTQSPATLSLSPGERATISCRASQSVSSYGQGFMMHWYQQKPGQPPRLIYGASSR ATGIPARFSGSGSGTDFTLTISPLEDFAVYYCQQNKEDPWTFGGGKLEIK (SEQ ID NO:14);

(c) the V_{H3} domain comprises the amino acid sequence of QVQLVQSGAEVVKPGASVKVSCKASGYTFTSFNMHWVKEAPGQRLEWIGYIYPGN GGTNYNQKFQGRATLTADTSASTAYMELSSLRSEDTAVYFCARTGGLRRAYFTYWG

QGTLVTVSS (SEQ ID NO:17), and the V_{L3} domain comprises the amino acid sequence of DIVLTQSPATLSLSPGERATISCRASESVDSYGNGFMHWYQQKPGQPPRLIYLASSR AT GIPARFSGSGSGTDFTLTISPLEDFAVYYCQQNKEDPWTFGGGKLEIK (SEQ ID NO:18);

(d) the V_{H3} domain comprises the amino acid sequence of QVQLVQSGAEVVKSGASVKVCKASGYTFTSFNMHWVKEAPQGQLEWIGYIYPGN GGTNYNQKFQGRATLTADTSASTAYMEISSLRSEDTAVYFCARTGGLRRAYFTYWG QGTLVTVSS (SEQ ID NO:21), and the V_{L3} domain comprises the amino acid sequence of DIVLTQSPATLSLSPGERATISCRASESVDSYGNGFMHWYQQKPGQPPRLIYLASSR AT GIPARFSGSGSGTDFTLTISPLEDFAVYYCQQNKEDPWTFGGGKLEIK (SEQ ID NO:18);

(e) the V_{H3} domain comprises the amino acid sequence of QVQLVQSGAEVVKPGASVKMSCKASGYTFTSFNMHWVKEAPGQRLEWIGYIYPGN GGTNYNQKFQGRATLTADTSASTAYMEISSLRSEDTAVYFCARTGGLRRAYFTYWG QGTLVTVSS (SEQ ID NO:23), and the V_{L3} domain comprises the amino acid sequence of DIVLTQSPATLSLSPGERATISCRASESVDSYGNGFMHWYQQKPGQPPRLIYLASSR AT GIPARFSGSGSGTDFTLTISPLEDFAVYYCQQNKEDPWTFGGGKLEIK (SEQ ID NO:18); or

(f) the V_{H3} domain comprises the amino acid sequence of QVQLVESGGGVVQGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLEWVAVIWYDG SNKYYADSVKGRFTISGDN SKNTLYLQMNSLRAEDTAVYYCARMFRGAFDYWGQG TLVTVSS (SEQ ID NO:9), and the V_{L3} domain comprises the amino acid sequence of AIQMTQSPSSLSASVGDRVTITCRASQGIRNDLGWYQQKPGKAPKLLIYAASSLQSG VPSRFSGSGSGTDFTLTISGLQPEDSATYYCLQDYIYYPTFGQGKVEIK (SEQ ID NO:10).

16. The method of any one of claims 1-15, wherein:

(a) the V_{H1} domain comprises a CDR-H1 sequence comprising the amino acid sequence of GYTFTSY (SEQ ID NO:108), a CDR-H2 sequence comprising the amino acid sequence of IYPGNVNT (SEQ ID NO:109), and a CDR-H3 sequence comprising the amino acid sequence of TRSHYGLDWNFDV (SEQ ID NO:110), and the V_{L1} domain comprises a CDR-L1 sequence comprising the amino acid sequence of QNIYVW (SEQ ID NO:111), a

CDR-L2 sequence comprising the amino acid sequence of KAS (SEQ ID NO:112), and a CDR-L3 sequence comprising the amino acid sequence of QQGQTYPY (SEQ ID NO:113); or

(b) the V_{H1} domain comprises a CDR-H1 sequence comprising the amino acid sequence of GFSLSDYG (SEQ ID NO:114), a CDR-H2 sequence comprising the amino acid sequence of IWAGGGT (SEQ ID NO:115), and a CDR-H3 sequence comprising the amino acid sequence of ARDKGYSYYYSMDY (SEQ ID NO:116), and the V_{L1} domain comprises a CDR-L1 sequence comprising the amino acid sequence of ESVEYYVTSL (SEQ ID NO:117), a CDR-L2 sequence comprising the amino acid sequence of AAS (SEQ ID NO:118), and a CDR-L3 sequence comprising the amino acid sequence of QQSRKVPYT (SEQ ID NO:119).

17. The method of claim 16, wherein:

(a) the V_{H1} domain comprises the amino acid sequence of QVQLVQSGAEVVKPGASVKVSCASGYTFTSYIIHWVRQAPGGLEWIGSIYPGNVNTNYAQKFQGRATLTVDTSISTAYMELSRRLSDDTAVYYCTRSHYGLDWNFDVWGKGTTVTVSS (SEQ ID NO:49), and the V_{L1} domain comprises the amino acid sequence of DIQMTQSPSSLSASVGDRVTITCQASQNIYVWLNWYQQKPGKAPKLLIYKASNLHTGVPSRFSGSGSGTDFTLTISLQPEDATYYCQQGQTYPTYTFGQGTKLEIK (SEQ ID NO:50); or

(b) the V_{H1} domain comprises the amino acid sequence of QVQLQESGPGLVKPSQTLSTCTVSGFSLSDYGVHWVRQPPGKGLEWLGVIWAGGGTNYNPSLKSRTISKDTSKNQVSLKLSSVTAADTAVYYCARDKGYSYYYSMDYWGQGTTVTVS (SEQ ID NO:51), and the V_{L1} domain comprises the amino acid sequence of DIVLTQSPASLAVSPGQRATITCRASESVEYYVTSLMQWYQQKPGQPPKLLIFAASNVESGVPARFSGSGSGTDFTLTINPVEANDVANYYCQQSRKVPYTFGQGTKLEIK (SEQ ID NO:52).

18. The method of any one of claims 1-17, wherein:

(a) the V_{H2} domain comprises a CDR-H1 sequence comprising the amino acid sequence of GFTFTKAW (SEQ ID NO:120), a CDR-H2 sequence comprising the amino acid sequence of IKDKSNSYAT (SEQ ID NO:121), and a CDR-H3 sequence comprising the

amino acid sequence of RGVYYALSPFDY (SEQ ID NO:122), and the V_{L2} domain comprises a CDR-L1 sequence comprising the amino acid sequence of QSLVHNNANTY (SEQ ID NO:123), a CDR-L2 sequence comprising the amino acid sequence of KVS (SEQ ID NO:124), and a CDR-L3 sequence comprising the amino acid sequence of GQGTQYPFT (SEQ ID NO:125); or

(b) the V_{H2} domain comprises a CDR-H1 sequence comprising the amino acid sequence of GFTFTKAW (SEQ ID NO:126), a CDR-H2 sequence comprising the amino acid sequence of IKDKSNSYAT (SEQ ID NO:127), and a CDR-H3 sequence comprising the amino acid sequence of GVVYALSPFDY (SEQ ID NO:128), and the V_{L2} domain comprises a CDR-L1 sequence comprising the amino acid sequence of QSLVHNNGNTY (SEQ ID NO:129), a CDR-L2 sequence comprising the amino acid sequence of KVS (SEQ ID NO:130), and a CDR-L3 sequence comprising the amino acid sequence of GQGTQYPFT (SEQ ID NO:131).

19. The method of claim 18, wherein:

(a) the V_{H2} domain comprises the amino acid sequence of QVQLVESGGGVVQPGRSLRLSCAASGFTFTKAWMHWRQAPGKQLEWVAQIKDKSNSYATYYADSVKGRFTISRDDSKNTLYLQMNSLRAEDTAVYYCRGVYYALSPFDYWGQGTLLVTVSS (SEQ ID NO:53), and the V_{L2} domain comprises the amino acid sequence of DIVMTQTPLSLSVTPGPASISCKSSQSLVHNNANTYLSWYLQKPGQSPQSLIYKVSNRFSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCGQGTQYPFTFGSGTKVEIK (SEQ ID NO:54); or

(b) the V_{H2} domain comprises the amino acid sequence of QVQLVESGGGVVQPGRSLRLSCAASGFTFTKAWMHWRQAPGKGLEWVAQIKDKSNSYATYYADSVKGRFTISRDNKNTLYLQMNSLRAEDTAVYYCRGVYYALSPFDYWGQGTLLVTVSS (SEQ ID NO:84), and the V_{L2} domain comprises the amino acid sequence of DIVMTQTPLSLSVTPGPASISCKSSQSLVHNNGNTYLSWYLQKPGQSPQLLIYKVSNRFSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCGQGTQYPFTFGGGTKVEIK (SEQ ID NO:85).

20. The method of any one of claims 1-19, wherein at least one of L₁, L₂, L₃ or L₄ is independently 0 amino acids in length.
21. The method of any one of claims 1-19, wherein (a) L₁, L₂, L₃ and L₄ each independently are zero amino acids in length or comprise a sequence selected from the group consisting of GGGGSGGGGS (SEQ ID NO:55), GGGGSGGGSGGGGS (SEQ ID NO: 56), S, RT, TKGPS (SEQ ID NO: 57), GQPKAAP (SEQ ID NO: 58), and GGS GSSGSGG (SEQ ID NO: 59); or (b) L₁, L₂, L₃ and L₄ each independently comprise a sequence selected from the group consisting of GGGGSGGGGS (SEQ ID NO:55), GGGGSGGGSGGGGS (SEQ ID NO:56), S, RT, TKGPS (SEQ ID NO:57), GQPKAAP (SEQ ID NO: 58), and GGS GSSGSGG (SEQ ID NO:59).
22. The method of any one of claims 1-19, wherein L₁ comprises the sequence GQPKAAP (SEQ ID NO: 58), L₂ comprises the sequence TKGPS (SEQ ID NO:57), L₃ comprises the sequence S, and L₄ comprises the sequence RT.
23. The method of any one of claims 1-22, wherein the hinge-CH₂-CH₃ domains of the second and the third polypeptide chains are human IgG4 hinge-CH₂-CH₃ domains, and wherein the hinge-CH₂-CH₃ 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.
24. The method of any one of claims 1-22, wherein the hinge-CH₂-CH₃ domains of the second and the third polypeptide chains are human IgG4 hinge-CH₂-CH₃ domains, and wherein the hinge-CH₂-CH₃ domains each comprise amino acid substitutions at positions corresponding to positions 233-236 of human IgG4 according to EU Index, wherein the amino acid substitutions are E233P, F234V, L235A, and a deletion at 236.
25. The method of any one of claims 1-24, wherein the hinge-CH₂-CH₃ domains of the second and the third polypeptide chains are human IgG4 hinge-CH₂-CH₃ domains, and wherein the hinge-CH₂-CH₃ 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.
26. The method of any one of claims 1-22, wherein the hinge-CH₂-CH₃ domains of the second and the third polypeptide chains are human IgG1 hinge-CH₂-CH₃ domains, and

wherein the hinge-CH₂-CH₃ domains 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.

27. The method of any one of claims 1-22, wherein the hinge-CH₂-CH₃ domains of the second and the third polypeptide chains are human IgG1 hinge-CH₂-CH₃ domains, and wherein the hinge-CH₂-CH₃ domains each comprise amino acid substitutions at positions corresponding to positions 298, 299, and 300 of human IgG1 according to EU Index, wherein the amino acid substitutions are S298N, T299A, and Y300S.

28. The method of any one of claims 1-27, wherein the hinge-CH₂-CH₃ 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 hinge-CH₂-CH₃ 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.

29. The method of any one of claims 1-27, wherein the hinge-CH₂-CH₃ 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 hinge-CH₂-CH₃ 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.

30. The binding protein of claim 1, wherein:

(a) the first polypeptide chain comprises the amino acid sequence of SEQ ID NO:61, the second polypeptide chain comprises the amino acid sequence of SEQ ID NO:60, the third polypeptide chain comprises the amino acid sequence of SEQ ID NO:62, and the fourth polypeptide chain comprises the amino acid sequence of SEQ ID NO:63;

(b) the first polypeptide chain comprises the amino acid sequence of SEQ ID NO:61, the second polypeptide chain comprises the amino acid sequence of SEQ ID NO:64, the third

polypeptide chain comprises the amino acid sequence of SEQ ID NO:65, and the fourth polypeptide chain comprises the amino acid sequence of SEQ ID NO:63;

(c) the first polypeptide chain comprises the amino acid sequence of SEQ ID NO:61, the second polypeptide chain comprises the amino acid sequence of SEQ ID NO:66, the third polypeptide chain comprises the amino acid sequence of SEQ ID NO:67, and the fourth polypeptide chain comprises the amino acid sequence of SEQ ID NO:63;

(d) the first polypeptide chain comprises the amino acid sequence of SEQ ID NO:61, the second polypeptide chain comprises the amino acid sequence of SEQ ID NO:60, the third polypeptide chain comprises the amino acid sequence of SEQ ID NO:68, and the fourth polypeptide chain comprises the amino acid sequence of SEQ ID NO:69;

(e) the first polypeptide chain comprises the amino acid sequence of SEQ ID NO:61, the second polypeptide chain comprises the amino acid sequence of SEQ ID NO:64, the third polypeptide chain comprises the amino acid sequence of SEQ ID NO:70, and the fourth polypeptide chain comprises the amino acid sequence of SEQ ID NO:69; or

(f) the first polypeptide chain comprises the amino acid sequence of SEQ ID NO:61, the second polypeptide chain comprises the amino acid sequence of SEQ ID NO:66, the third polypeptide chain comprises the amino acid sequence of SEQ ID NO:71, and the fourth polypeptide chain comprises the amino acid sequence of SEQ ID NO:69.

31. The method of any one of claims 1-30, wherein the virus is a human immunodeficiency virus (HIV), influenza virus, cytomegalovirus (CMV), hepatitis B virus (HBV), human papillomavirus (HPV), Epstein-barr virus (EBV), human foamy virus (HFV), herpes simplex virus 1 (HSV-1), or herpes simplex virus 1 (HSV-2).