



(12) **DEMANDE DE BREVET CANADIEN
CANADIAN PATENT APPLICATION**

(13) **A1**

(86) **Date de dépôt PCT/PCT Filing Date:** 2021/12/07
(87) **Date publication PCT/PCT Publication Date:** 2022/06/16
(85) **Entrée phase nationale/National Entry:** 2023/06/06
(86) **N° demande PCT/PCT Application No.:** US 2021/062233
(87) **N° publication PCT/PCT Publication No.:** 2022/125562
(30) **Priorité/Priority:** 2020/12/08 (US63/122,820)

(51) **Cl.Int./Int.Cl.** **A61K 38/00** (2006.01),
A61K 38/08 (2019.01), **A61K 47/66** (2017.01),
C07K 7/06 (2006.01), **C12N 15/62** (2006.01)
(71) **Demandeur/Applicant:**
JANUX THERAPEUTICS, INC., US
(72) **Inventeurs/Inventors:**
CAMPBELL, DAVID, US;
DIRAIMONDO, THOMAS R., US
(74) **Agent:** GOWLING WLG (CANADA) LLP

(54) **Titre : COMPOSITIONS A BASE DE PEPTIDES ET PROCEDES POUR DES DOMAINES DE LIAISON ANTI-CD3**
(54) **Title: PEPTIDE COMPOSITIONS AND METHODS FOR ANTI-CD3 BINDING DOMAINS**

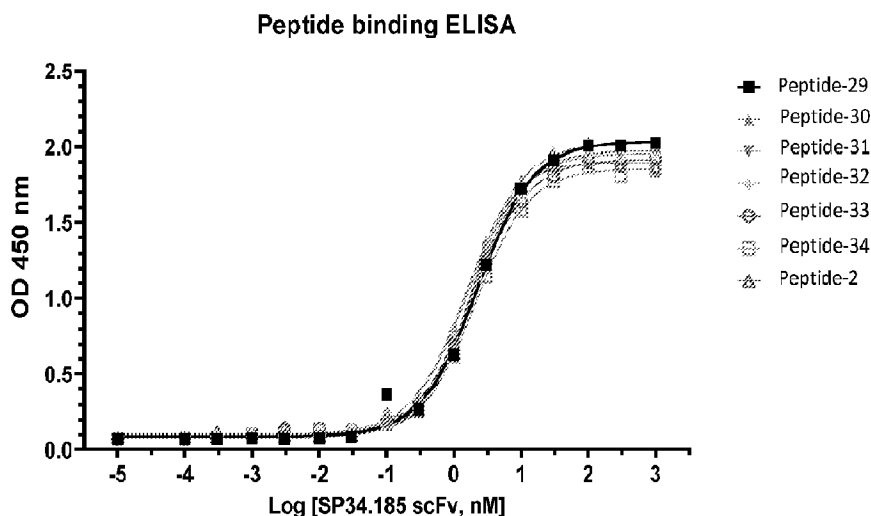


FIG. 5A

(57) **Abrégé/Abstract:**

Disclosed herein are polypeptide and polypeptide complex compositions that comprise a peptide that selectively impairs binding of an anti-CD3 binding domain to CD3 in healthy tissue but not in a disease state.

Date Submitted: 2023/06/06

CA App. No.: 3201401

Abstract:

Disclosed herein are polypeptide and polypeptide complex compositions that comprise a peptide that selectively impairs binding of an anti-CD3 binding domain to CD3 in healthy tissue but not in a disease state.

PEPTIDE COMPOSITIONS AND METHODS FOR ANTI-CD3 BINDING DOMAINS**CROSS-REFERENCE**

[0001] The present application claims the benefit of U.S. Provisional Application No. 63/122,820, filed December 8, 2020, which is incorporated herein by reference in its entirety.

SEQUENCE LISTING

[0002] The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on December 2, 2021, is named 52426-732_601_SL.txt and is 280,999 bytes in size.

SUMMARY

[0003] Disclosed herein are polypeptides or polypeptide complexes comprising a peptide that impairs binding of an anti-CD3 binding domain to CD3 wherein the peptide comprises an amino acid sequence according to Peptide-1 (GSQCLGPEWEVCPY) SEQ ID NO: 1 or Peptide-2 (VYCGPEFDES VGCM) SEQ ID NO: 2, or the peptide comprises 1, 2, or 3, amino acid substitutions, additions, or deletions relative to the amino acid sequence of Peptide-1 or Peptide-2. In some embodiments, the peptide comprises the amino acid sequence of Peptide-1. In some embodiments, the peptide comprises the amino acid sequence of Peptide-2. In some embodiments, the peptide is connected to the anti-CD3 binding domain in a configuration according to: A₁-L₁-P₁ (Formula I) wherein A₁ comprises the anti-CD3 binding domain; L₁ comprises a cleavable linker that is a substrate for a tumor specific protease; P₁ comprises the peptide that impairs binding of the anti-CD3 binding domain to CD3. In some embodiments, P₁ is connected N-terminal to the cleavable linker and A₁ is connected C-terminal to the cleavable linker. In some embodiments, P₁ is connected C-terminal to the cleavable linker and A₁ is connected N-terminal to the cleavable linker. In some embodiments, P₁ is bound to A₁ through ionic interactions, electrostatic interactions, hydrophobic interactions, Pi-stacking interactions, or H-bonding interactions, or a combination thereof. In some embodiments, A₁ is further linked to a tumor antigen binding domain (A₂). In some embodiments, the polypeptide or polypeptide complex is according to Formula Ia: P₂-L₂-A₂-A₁-L₁-P₁ wherein P₂ comprises a peptide that impairs binding of A₂ to a tumor antigen; and L₂ comprises a second cleavable linker that connects A₂ to P₂ and is a substrate for a tumor specific protease. In some embodiments, A₁ comprises an antibody or antibody fragment. In some embodiments, A₁ comprises an antibody or antibody fragment that is human or humanized. In some embodiments, L₁ is bound to N-terminus of the antibody or antibody fragment of A₁. In some embodiments, A₂ is bound to N-terminus of the antibody or antibody fragment of A₁. In some embodiments, L₁ is bound to the C-terminus of the antibody or antibody fragment of A₁. In some embodiments, A₂ is bound to the C-terminus of the antibody or antibody fragment of A₁. In some embodiments, the antibody or antibody fragment of A₁ comprises a single chain variable fragment, a single domain antibody, or a Fab fragment. In some embodiments, A₁ is the single chain variable fragment (scFv). In some embodiments, the scFv

comprises a scFv heavy chain polypeptide and a scFv light chain polypeptide. In some embodiments, A₁ is the single domain antibody. In some embodiments, the antibody or antibody fragment thereof comprises a single chain variable fragment (scFv), a heavy chain variable domain (VH domain), a light chain variable domain (VL domain), or a variable domain (VHH) of a camelid derived single domain antibody. In some embodiments, A₁ comprises an anti-CD3ε single chain variable fragment. In some embodiments, A₁ comprises an anti-CD3ε single chain variable fragment that has a KD binding of 1 μM or less to CD3 on CD3 expressing cells. In some embodiments, A₁ comprises a variable light chain and variable heavy chain each of which is capable of specifically binding to human CD3. In some embodiments, A₁ comprises the scFv comprising a scFv heavy chain variable domain and an scFv light chain variable domain comprising complementarity determining regions (CDRs): HC-CDR1, HC-CDR2, and HC-CDR3, wherein the HC-CDR1, the HC-CDR2, and the HC-CDR3 of the scFv heavy chain variable domain comprise: HC-CDR1: SEQ ID NO: 3; HC-CDR2: SEQ ID NO: 4; and HC-CDR3: SEQ ID NO: 5; and the scFv light chain variable domain comprises complementarity determining regions (CDRs): LC-CDR1, LC-CDR2, and LC-CDR3, wherein the LC-CDR1, the LC-CDR2, and the LC-CDR3 of the scFv light chain variable domain comprise: LC-CDR1: SEQ ID NO: 6; LC-CDR2: SEQ ID NO: 7; and LC-CDR3: SEQ ID NO: 8. In some embodiments, the polypeptide or polypeptide complex of formula I binds to an effector cell when L₁ is cleaved by the tumor specific protease. In some embodiments, the polypeptide or polypeptide complex of formula I binds to an effector cell when L₁ is cleaved by the tumor specific protease and A₁ binds to the effector cell. In some embodiments, the effector cell is a T cell. In some embodiments, A₁ binds to a polypeptide that is part of a TCR-CD3 complex on the effector cell. In some embodiments, the polypeptide that is part of the TCR-CD3 complex is human CD3ε. In some embodiments, A₂ comprises an antibody or antibody fragment. In some embodiments, the antibody or antibody fragment thereof of A₂ comprises a single chain variable fragment, a single domain antibody, Fab', or a Fab. In some embodiments, the antibody or antibody fragment thereof of A₂ comprises a single chain variable fragment (scFv), a heavy chain variable domain (VH domain), a light chain variable domain (VL domain), or a variable domain (VHH) of a camelid derived single domain antibody. In some embodiments, the antibody or antibody fragment thereof of A₂ is humanized or human. In some embodiments, A₂ is the Fab or Fab'. In some embodiments, the Fab or Fab' comprises (a) a Fab light chain polypeptide and (b) a Fab heavy chain polypeptide. In some embodiments, A₂ comprises an epidermal growth factor receptor (EGFR) binding domain. In some embodiments, A₂ comprises a carcinoembryonic antigen-related cell adhesion molecule CEACAM5 binding domain. In some embodiments, P₁ is further linked to a half-life extending moiety (H₁). In some embodiments, the half-life extending moiety is a single-domain antibody. In some embodiments, H₁ comprises a polymer. In some embodiments, the polymer is polyethylene glycol (PEG). In some embodiments, H₁ comprises albumin. In some embodiments, H₁ comprises an Fc domain. In some embodiments, the albumin is serum albumin. In some embodiments, the albumin is human serum albumin. In some embodiments, H₁ comprises a polypeptide, a ligand, or a small molecule. In some embodiments, the polypeptide, the ligand or the small molecule binds serum protein or a fragment thereof, a circulating immunoglobulin or a fragment thereof, or

CD35/CR1. In some embodiments, the serum protein comprises a thyroxine-binding protein, a transthyretin, a 1-acid glycoprotein, a transferrin, transferrin receptor or a transferrin-binding portion thereof, a fibrinogen, or an albumin. In some embodiments, the circulating immunoglobulin comprises IgG1, IgG2, IgG3, IgG4, sIgA, IgM or IgD. In some embodiments, the serum protein is albumin. In some embodiments, the polypeptide is an antibody. In some embodiments, the antibody comprises a single domain antibody, a single chain variable fragment, or a Fab. In some embodiments, the single domain antibody comprises a single domain antibody that binds to albumin. In some embodiments, the single domain antibody is a human antibody or humanized antibody. In some embodiments, the single domain antibody is 645gH1gL1. In some embodiments, the single domain antibody is 645dsgH5gL4. In some embodiments, the single domain antibody is 23-13-A01 -sc02. In some embodiments, the single domain antibody is A10m3 or a fragment thereof. In some embodiments, the single domain antibody is DOM7r-31. In some embodiments, the single domain antibody is Alb-1, Alb-8, or Alb-23. In some embodiments, the single domain antibody is 10G or 10GE. In some embodiments, the single domain antibody is SA21. In some embodiments, the polypeptide or polypeptide complex comprises a modified amino acid, a non-natural amino acid, a modified non-natural amino acid, or a combination thereof. In some embodiments, the modified amino acid or modified non-natural amino acid comprises a post-translational modification. In some embodiments, H₁ comprises a linking moiety (L₃) that connects H₁ to P₁. In some embodiments, L₃ is a peptide sequence having at least 5 to no more than 50 amino acids. In some embodiments, L₃ is a peptide sequence having at least 10 to no more than 30 amino acids. In some embodiments, L₃ is a peptide sequence having at least 10 amino acids. In some embodiments, L₃ is a peptide sequence having at least 18 amino acids. In some embodiments, L₃ is a peptide sequence having at least 26 amino acids. In some embodiments, L₃ has a formula selected from the group consisting of (G₂S)_n, (GS)_n, (GSGGS)_n (SEQ ID NO: 917), (GGGS)_n (SEQ ID NO: 918), (GGGGS)_n (SEQ ID NO: 919), and (GSSGGS)_n (SEQ ID NO: 920), wherein n is an integer of at least 1. In some embodiments, L₃ comprises an amino acid sequence according to (GGGGSGGGS (SEQ ID NO: 921)). In some embodiments, the Fab light chain polypeptide of A₂ is bound to a C-terminus of the single chain variable fragment (scFv) of A₁. In some embodiments, the Fab heavy chain polypeptide of A₂ is bound to a C-terminus of the single chain variable fragment (scFv) A₁. In some embodiments, the Fab light chain polypeptide of A₂ is bound to a N-terminus of the single chain variable fragment (scFv) of A₁. In some embodiments, the Fab heavy chain polypeptide of A₂ is bound to a N-terminus of the single chain variable fragment (scFv) A₁. In some embodiments, the Fab heavy chain polypeptide of A₂ is bound to the scFv heavy chain polypeptide of A₁. In some embodiments, the Fab light chain polypeptide of A₂ is bound to the scFv heavy chain polypeptide of A₁. In some embodiments, the Fab heavy chain polypeptide of A₂ is bound to the scFv light chain polypeptide of A₁. In some embodiments, the Fab light chain polypeptide of A₂ is bound to the scFv light chain polypeptide of A₁. In some embodiments, the Fab heavy chain polypeptide of A₂ is bound to the scFv heavy chain polypeptide of A₁ and L₂ is bound to the Fab light chain polypeptide of A₂. In some embodiments, the Fab light chain polypeptide of A₂ is bound to the scFv heavy chain polypeptide of A₁ and L₂ is bound to the Fab heavy chain polypeptide of A₂. In some embodiments, the Fab

heavy chain polypeptide of A₂ is bound to the scFv light chain polypeptide of A₁ and L₂ is bound to the Fab light chain polypeptide of A₂. In some embodiments, the Fab light chain polypeptide of A₂ is bound to the scFv light chain polypeptide of A₁ and L₂ is bound to the Fab heavy chain polypeptide of A₂. In some embodiments, P₂ impairs binding of A₂ to the tumor antigen. In some embodiments, P₂ is bound to A₂ through ionic interactions, electrostatic interactions, hydrophobic interactions, Pi-stacking interactions, or H-bonding interactions, or a combination thereof. In some embodiments, P₂ is bound to A₂ at or near an antigen binding site. In some embodiments, P₂ has less than 70% sequence homology to the tumor antigen. In some embodiments, P₂ comprises a peptide sequence of at least 10 amino acids in length. In some embodiments, P₂ comprises a peptide sequence of at least 10 amino acids in length and no more than 20 amino acids in length. In some embodiments, P₂ comprises a peptide sequence of at least 16 amino acids in length. In some embodiments, P₂ comprises a peptide sequence of no more than 40 amino acids in length. In some embodiments, P₂ comprises at least two cysteine amino acid residues. In some embodiments, P₂ comprises a cyclic peptide or a linear peptide. In some embodiments, P₂ comprises a cyclic peptide. In some embodiments, P₂ comprises a linear peptide. In some embodiments, L₁ is bound to N-terminus of A₁. In some embodiments, L₁ is bound to C-terminus of A₁. In some embodiments, L₂ is bound to N-terminus of A₂. In some embodiments, L₂ is bound to C-terminus of A₂. In some embodiments, L₁ or L₂ is a peptide sequence having at least 5 to no more than 50 amino acids. In some embodiments, L₁ or L₂ is a peptide sequence having at least 10 to no more than 30 amino acids. In some embodiments, L₁ or L₂ is a peptide sequence having at least 10 amino acids. In some embodiments, L₁ or L₂ is a peptide sequence having at least 18 amino acids. In some embodiments, L₁ or L₂ is a peptide sequence having at least 26 amino acids. In some embodiments, L₁ or L₂ has a formula comprising (G₂S)_n (SEQ ID NO: 922), wherein n is an integer from 1 to 3. In some embodiments, L₁ has a formula selected from the group consisting of (G₂S)_n, (GS)_n, (GSGGS)_n (SEQ ID NO: 917), (GGGS)_n (SEQ ID NO: 918), (GGGGS)_n (SEQ ID NO: 919), and (GSSGGS)_n (SEQ ID NO: 920), wherein n is an integer of at least 1. In some embodiments, P₁ becomes unbound from A₁ when L₁ is cleaved by the tumor specific protease thereby exposing A₁ to CD3. In some embodiments, P₂ becomes unbound from A₂ when L₂ is cleaved by the tumor specific protease thereby exposing A₂ to the tumor antigen. In some embodiments, the tumor specific protease is selected from the group consisting of metalloprotease, serine protease, cysteine protease, threonine protease, and aspartic protease. In some embodiments, L₁ or L₂ comprises a urokinase cleavable amino acid sequence, a matriptase cleavable amino acid sequence, a matrix metalloprotease cleavable amino acid sequence, or a legumain cleavable amino acid sequence. In some embodiments, L₁ or L₂ comprises an amino acid sequence selected from the group consisting of GGGGSLSGRSDNHGSSGT (SEQ ID NO: 923), GGGGSSGGSGGSLSGRSDNHGSSGT (SEQ ID NO: 924), ASGRSDNH (SEQ ID NO: 925), LAGRSDNH (SEQ ID NO: 926), ISSGLASGRSDNH (SEQ ID NO: 927), ISSGLLAGRSDNH (SEQ ID NO: 928), LSGRSDNH (SEQ ID NO: 929), ISSGLLSGRSDNP (SEQ ID NO: 930), ISSGLLSGRSDNH (SEQ ID NO: 931), LSGRSDNHSPLGLAGS (SEQ ID NO: 932), SPLGLAGSLSGRSDNH (SEQ ID NO: 933), SPLGLSGRSDNH (SEQ ID NO: 934), LAGRSDNHSPLGLAGS (SEQ ID NO: 935),

LSGRSDNHVPLSLKMG (SEQ ID NO: 936), and LSGRSDNHVPLSLSMG (SEQ ID NO: 937). In some embodiments, L₁ or L₂ comprises an amino acid sequence selected from the group consisting of ASGRSDNH (SEQ ID NO: 925), LAGRSDNH (SEQ ID NO: 926), ISSGLASGRSDNH (SEQ ID NO: 927), and ISSGLLAGRSDNH (SEQ ID NO: 928).

[0004] Disclosed herein are pharmaceutical compositions comprising (i) the polypeptides or polypeptide complexes of any one of the previous embodiments; and (ii) a pharmaceutically acceptable excipient.

[0005] Disclosed herein are isolated recombinant nucleic acid molecules encoding the polypeptides or polypeptide complexes of any one of the previous embodiments.

[0006] Disclosed herein are isolated polypeptides or polypeptide complexes according to Formula II: L_{1a}-P_{1a}-H_{1a} wherein: L_{1a} comprises a tumor specific protease-cleaved linking moiety that when uncleaved connects P_{1a} to an anti-CD3 binding domain that binds to CD3 and; P_{1a} comprises a peptide that impairs binding of the anti-CD3 binding domain to CD3 when L_{1a} is uncleaved wherein the peptide comprises an amino acid sequence according to Peptide-1 (GSQCLGPEWEVCPY) SEQ ID NO: 1 or Peptide-2 (VYCGPEFDESVCVM) SEQ ID NO: 2, or the peptide comprises 1, 2, or 3, amino acid substitutions, additions, or deletions relative to the amino acid sequence of Peptide-1 or Peptide-2; and H_{1a} comprises a half-life extending molecule. In some embodiments, the peptide comprises the amino acid sequence of Peptide-1. In some embodiments, the peptide comprises the amino acid sequence of Peptide-2. In some embodiments, H_{1a} comprises a polymer. In some embodiments, the polymer is polyethylene glycol (PEG). In some embodiments, H_{1a} comprises albumin. In some embodiments, H_{1a} comprises an Fc domain. In some embodiments, the albumin is serum albumin. In some embodiments, the albumin is human serum albumin. In some embodiments, H_{1a} comprises a polypeptide, a ligand, or a small molecule. In some embodiments, the polypeptide, the ligand or the small molecule binds a serum protein or a fragment thereof, a circulating immunoglobulin or a fragment thereof, or CD35/CR1. In some embodiments, the serum protein comprises a thyroxine-binding protein, a transthyretin, a 1-acid glycoprotein, a transferrin, transferrin receptor or a transferrin-binding portion thereof, a fibrinogen, or an albumin. In some embodiments, the circulating immunoglobulin molecule comprises IgG1, IgG2, IgG3, IgG4, sIgA, IgM or IgD. In some embodiments, the serum protein is albumin. In some embodiments, the polypeptide is an antibody. In some embodiments, the antibody comprises a single domain antibody, a single chain variable fragment or a Fab. In some embodiments, the antibody comprises a single domain antibody that binds to albumin. In some embodiments, the antibody is a human or humanized antibody. In some embodiments, the single domain antibody is 645gH1gL1. In some embodiments, the single domain antibody is 645dsgH5gL4. In some embodiments, the single domain antibody is 23-13-A01 -sc02. In some embodiments, the single domain antibody is A10m3 or a fragment thereof. In some embodiments, the single domain antibody is DOM7r-31. In some embodiments, the single domain antibody is DOM7h-11-15. In some embodiments, the single domain antibody is Alb-1, Alb-8, or Alb-23. In some embodiments, the single domain antibody is 10G or 10GE. In some embodiments, the single domain antibody is SA21. In some embodiments, H_{1a} comprises a

linking moiety (L_{3a}) that connects H_{1a} to P_{1a} . In some embodiments, L_{3a} is a peptide sequence having at least 5 to no more than 50 amino acids.

In some embodiments, L_{3a} is a peptide sequence having at least 10 to no more than 30 amino acids. In some embodiments, L_{3a} is a peptide sequence having at least 10 amino acids. In some embodiments, L_{3a} is a peptide sequence having at least 18 amino acids. In some embodiments, L_{3a} is a peptide sequence having at least 26 amino acids. In some embodiments, L_{3a} has a formula selected from the group consisting of $(G_2S)_n$, $(GS)_n$, $(GSGGS)_n$ (SEQ ID NO: 917), $(GGGS)_n$ (SEQ ID NO: 918), $(GGGGS)_n$ (SEQ ID NO: 919), and $(GSSGGS)_n$ (SEQ ID NO: 920), wherein n is an integer of at least 1. In some embodiments, L_{3a} comprises an amino acid sequence according to $(GGGGSGGGS)$ (SEQ ID NO: 921).

BRIEF DESCRIPTION OF THE DRAWINGS

[0007] The novel features of the invention are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present invention will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the invention are utilized, and the accompanying drawings of which:

[0008] Fig. 1A illustrates Peptide-1 peptide binding data to SP34.185 scFv by ELISA.

[0009] Fig. 1B illustrates Peptide-1 alanine scanning peptides binding data to SP34.185 scFv by ELISA.

[0010] Fig. 1C illustrates Peptide-1 alanine scanning peptides binding data to SP34.185 scFv by ELISA.

[0011] Fig. 1D illustrates Peptide-2 peptide binding data to SP34.185 scFv by ELISA.

[0012] Fig. 1E illustrates Peptide-2 alanine scanning peptides binding data to SP34.185 scFv by ELISA.

[0013] Fig. 1F illustrates Peptide-2 alanine scanning peptides binding data to SP34.185 scFv by ELISA.

[0014] Fig. 2A illustrates Peptide-1 inhibitory data of SP34.185 binding to CD3e.

[0015] Fig. 2B illustrates Peptide-1 alanine scanning peptides inhibitory data of SP34.185 binding to CD3e.

[0016] Fig. 2C illustrates Peptide-1 alanine scanning peptides inhibitory data of SP34.185 binding to CD3e.

[0017] Fig. 2D illustrates Peptide-2 inhibitory data of SP34.185 binding to CD3e.

[0018] Fig. 2E illustrates Peptide-2 alanine scanning peptides inhibitory data of SP34.185 binding to CD3e.

[0019] Fig. 2F illustrates Peptide-2 alanine scanning peptides inhibitory data of SP34.185 binding to CD3e.

[0020] Fig. 3 illustrates titration data for CD3e binding for several polypeptide complexes of this disclosure.

[0021] Fig. 4 illustrates functional tumor cell killing of PC-1, PC-2, and PC-3.

[0022] Fig. 5A illustrates peptides (Peptide-29 – Peptide-34) peptide binding data to SP34.185 scFv by ELISA. Peptide-2 is used as a positive control.

[0023] Fig. 5B illustrates peptides (Peptide-35 – Peptide-40) peptide binding data to SP34.185 scFv by ELISA. Peptide-2 is used as a positive control.

[0024] Fig. 6A illustrates peptides (Peptide-29 – Peptide-34) inhibiting binding of SP34.185 scFv to CD3 by ELISA. Peptide-2 is used as a positive control.

[0025] Fig. 6B illustrates peptides (Peptide-35 – Peptide-40) inhibiting binding of SP34.185 scFv to CD3 by ELISA. Peptide-2 is used as a positive control.

[0026] Fig. 7 illustrates the core sequence motif of Peptide-2 generated by WebLogo 3.

DETAILED DESCRIPTION

[0027] Protein-based therapies such as antibodies and bispecific or multispecific antibodies, such as T cell engagers, have proven effective for a variety of diseases and disorders. As with any therapy, there is a need to minimize off-target effects of the protein-based therapy in healthy tissue while maintaining activity of the protein-based therapy in disease tissue. One such strategy is to create an inactive form of the protein-based therapy in which a necessary binding site on the protein-based therapy is blocked with a peptide linked to the protein-based therapy, thereby preventing the protein-based therapy from binding or interacting with its cognate receptor or target antigen when in healthy tissue. For activating the protein-based therapy in the desired disease-state microenvironment, the peptide is linked to the protein-based therapy with a linker that is cleavable by a protease that is specific to the disease-state microenvironment. The peptide is then released from the protein-based therapy when in the disease-state microenvironment.

[0028] Accordingly, disclosed herein, are peptides that impair binding of anti-CD3 binding domains to cluster of differentiation 3 (CD3). The peptides as disclosed herein can be applied to a variety of antibody formats that bind to anti-CD3 to reduce the off-target effects of the antibodies in healthy tissue, while maintaining activity of the anti-CD3 antibody in a disease tissue. The peptides are attached to the anti-CD3 binding domains via protease cleavable linkers. In some embodiments, the antibodies described herein are used in a method of treating cancer. In some instances, the cancer is a solid tumor cancer. In some embodiments, the cancer is lung, breast (e.g. HER2+; ER/PR+; TNBC), cervical, ovarian, colorectal, pancreatic or gastric.

[0029] Disclosed herein are isolated polypeptides or polypeptide complexes comprising a peptide that impairs binding of an anti-CD3 binding domain to CD3 wherein the peptide comprises an amino acid sequence according to Peptide-1 (GSQCLGPEWEVCPY) SEQ ID NO: 1 or Peptide-2 (VYCGPEFDES VGCM) SEQ ID NO: 2, or the peptide comprises 1, 2, or 3, amino acid substitutions, additions, or deletions relative to the amino acid sequence of Peptide-1 or Peptide-2.

[0030] Disclosed herein are isolated polypeptides or polypeptide complexes comprising a peptide that impairs binding of an anti-CD3 binding domain to CD3 wherein the peptide comprises an amino acid sequence selected from SEQ ID NOs: 1-2 and 19-55 or the peptide comprises 1, 2, or 3, amino acid substitutions, additions, or deletions relative to an amino acid sequence selected from SEQ ID NOs: 1-2 and 19-55. In some embodiments, the peptide comprises an amino acid sequence selected from SEQ ID NOs: 1-2, and 19-55. In some embodiments, the peptide comprises the amino acid sequence of SEQ ID NO: 1. In some embodiments, the peptide comprises the amino acid sequence of SEQ ID NO: 2. In some embodiments, the peptide comprises the amino acid sequence of SEQ ID NO: 54.

[0031] Disclosed herein are isolated polypeptides or polypeptide complexes comprising an anti-CD3 binding domain that is linked to a peptide that impairs binding of the anti-CD3 binding domain to CD3 wherein the peptide comprises an amino acid sequence according to Z₁-Z₂-C- Z₄-P-Z₆-Z₇-Z₈- Z₉-Z₁₀-Z₁₁- Z₁₂-C-Z₁₄ and Z₁ is selected from D, Y, F, I, N, V, H, L, A, T, S, and P; Z₂ is selected from D, Y, L, F, I, N, A, V, H, T, and S; Z₄ is selected from G and W; Z₆ is selected from E, D, V, and P; Z₇ is selected from W, L, F, V, G, M, I, and Y; Z₈ is selected from E, D, P, and Q; Z₉ is selected from E, D, Y, V, F, W, P, L, and Q; Z₁₀ is selected from S, D, Y, T, I, F, V, N, A, P, L, and H; Z₁₁ is selected from I, Y, F, V, L, T, N, S, D, A, and H; Z₁₂ is selected from F, D, Y, L, I, V, A, N, T, P, S, and H; and Z₁₄ is selected from D, Y, N, F, I, P, V, A, T, H, L and S. In some embodiments, Z₁ is selected from D, Y, F, I, and N; Z₂ is selected from D, Y, L, F, I, and N; Z₄ is selected from G and W; Z₆ is selected from E and D; Z₇ is selected from W, L, F, and V; Z₈ is selected from E and D; Z₉ is selected from E, D, Y, and V; Z₁₀ is selected from S, D, Y, T, and I; Z₁₁ is selected from I, Y, F, V, L, and T; Z₁₂ is selected from F, D, Y, L, I, V, A, and N; and Z₁₄ is selected from D, Y, N, F, I, and P. In some embodiments, Z₁ is selected from D, Y, and F; Z₂ is selected from D, Y, L, and F; Z₄ is selected from G and W; Z₆ is selected from E and D; Z₇ is selected from W, L, and F; Z₈ is selected from E and D; Z₉ is selected from E and D; Z₁₀ is selected from S, D, and Y; Z₁₁ is selected from I, Y, and F; Z₁₂ is selected from F, D, Y, and L; and Z₁₄ is selected from D, Y, and N. In some embodiments, the peptide comprises an amino acid sequence of **Table 17**. In some embodiments, the peptide comprises an amino acid sequence according to any one of SEQ ID NOs: 44-55. In some embodiments, the peptide comprises the amino acid sequence of SEQ ID NO: 2. In some embodiments, the peptide comprises the amino acid sequence of SEQ ID NO: 54.

[0032] Disclosed herein are isolated polypeptides or polypeptide complexes comprising an anti-CD3 binding domain that is linked to a peptide that impairs binding of the anti-CD3 binding domain to CD3 wherein the peptide comprises an amino acid sequence according to U₁-U₂-C- U₄-P-U₆-U₇-U₈- U₉-U₁₀-U₁₁-U₁₂-C-U₁₄ and U₁ is selected from D, Y, F, I, N, V, H, L, A, T, S, and P; U₂ is selected from D, Y, L, F, I, N, A, V, H, T, and S; U₄ is selected from G and W; U₆ is selected from E, D, V, and P; U₇ is selected from W, L, F, V, G, M, I, and Y; U₈ is selected from E, D, P, and Q; U₉ is selected from E, D, Y, V, F, W, P, L, and Q; U₁₀ is selected from S, D, Y, T, I, F, V, N, A, P, L, and H; U₁₁ is selected from I, Y, F, V, L, T, N, S, D, A, and H; U₁₂ is selected from F, D, Y, L, I, V, A, N, T, P, S, G, and H; and U₁₄ is selected from D, Y, N, F, I, P, V, A, T, H, L, M, and S. In some embodiments, U₁ is selected from D, Y, F, I, V, and N; U₂ is selected from D, Y, L, F, I, and N; U₄ is selected from G and W; U₆ is selected from E and D; U₇ is selected from W, L, F, G, and V; U₈ is selected from E and D; U₉ is selected from E, D, Y, and V; U₁₀ is selected from S, D, Y, T, and I; U₁₁ is selected from I, Y, F, V, L, and T; U₁₂ is selected from F, D, Y, L, I, V, A, G, and N; and U₁₄ is selected from D, Y, N, F, I, M, and P. In some embodiments, U₁ is selected from D, Y, V, and F; U₂ is selected from D, Y, L, and F; U₄ is selected from G and W; U₆ is selected from E and D; U₇ is selected from W, L, G, and F; U₈ is selected from E and D; U₉ is selected from E and D; U₁₀ is selected from S, D, T, and Y; U₁₁ is selected from I, Y, V, L, and F; U₁₂ is selected from F, D, Y, G, A, and L; and U₁₄ is selected from D, Y, M, and N. In some embodiments, the peptide comprises an amino acid sequence of

Table 17. In some embodiments, the peptide comprises an amino acid sequence according to any one of SEQ ID NOs: 44-55. In some embodiments, the peptide comprises the amino acid sequence of SEQ ID NO: 2. In some embodiments, the peptide comprises the amino acid sequence of SEQ ID NO: 54.

[0033] Disclosed herein, are methods of treating cancer comprising administering to a subject in need thereof an isolated polypeptide or polypeptide complex comprising a peptide that impairs binding of an anti-CD3 binding domain to CD3 wherein the peptide comprises an amino acid sequence according to Peptide-1 (GSQCLGPEWEVCPY) SEQ ID NO: 1 or Peptide-2 (VYCGPEFDESVCGM) SEQ ID NO: 2, or the peptide comprises 1, 2, or 3, amino acid substitutions, additions, or deletions relative to the amino acid sequence of Peptide-1 or Peptide-2.

[0034] Disclosed herein, are methods of treating cancer comprising administering to a subject in need thereof an isolated polypeptide or polypeptide complex comprising a peptide that impairs binding of an anti-CD3 binding domain to CD3 wherein the peptide comprises an amino acid sequence selected from SEQ ID NOs: 1-2, and 19-55 or the peptide comprises 1, 2, or 3, amino acid substitutions, additions, or deletions relative to amino acid sequences of SEQ ID NOs: 1-2, and 19-55. In some embodiments, the peptide comprises an amino acid sequence selected from SEQ ID NOs: 1-2, and 19-55. In some embodiments, the peptide comprises the amino acid sequence of SEQ ID NO: 1. In some embodiments, the peptide comprises the amino acid sequence of SEQ ID NO: 2. In some embodiments, the peptide comprises the amino acid sequence of SEQ ID NO: 54.

[0035] Disclosed herein, are methods of treating cancer comprising administering to a subject in need thereof an isolated polypeptide or polypeptide complex comprising an anti-CD3 binding domain that is linked to a peptide that impairs binding of the anti-CD3 binding domain to CD3 wherein the peptide comprises an amino acid sequence according to Z₁-Z₂-C-Z₄-P-Z₆-Z₇-Z₈-Z₉-Z₁₀-Z₁₁-Z₁₂-C-Z₁₄ and Z₁ is selected from D, Y, F, I, N, V, H, L, A, T, S, and P; Z₂ is selected from D, Y, L, F, I, N, A, V, H, T, and S; Z₄ is selected from G and W; Z₆ is selected from E, D, V, and P; Z₇ is selected from W, L, F, V, G, M, I, and Y; Z₈ is selected from E, D, P, and Q; Z₉ is selected from E, D, Y, V, F, W, P, L, and Q; Z₁₀ is selected from S, D, Y, T, I, F, V, N, A, P, L, and H; Z₁₁ is selected from I, Y, F, V, L, T, N, S, D, A, and H; Z₁₂ is selected from F, D, Y, L, I, V, A, N, T, P, S, and H; and Z₁₄ is selected from D, Y, N, F, I, P, V, A, T, H, L and S. In some embodiments, Z₁ is selected from D, Y, F, I, and N; Z₂ is selected from D, Y, L, F, I, and N; Z₄ is selected from G and W; Z₆ is selected from E and D; Z₇ is selected from W, L, F, and V; Z₈ is selected from E and D; Z₉ is selected from E, D, Y, and V; Z₁₀ is selected from S, D, Y, T, and I; Z₁₁ is selected from I, Y, F, V, L, and T; Z₁₂ is selected from F, D, Y, L, I, V, A, and N; and Z₁₄ is selected from D, Y, N, F, I, and P. In some embodiments, Z₁ is selected from D, Y, and F; Z₂ is selected from D, Y, L, and F; Z₄ is selected from G and W; Z₆ is selected from E and D; Z₇ is selected from W, L, and F; Z₈ is selected from E and D; Z₉ is selected from E and D; Z₁₀ is selected from S, D, and Y; Z₁₁ is selected from I, Y, and F; Z₁₂ is selected from F, D, Y, and L; and Z₁₄ is selected from D, Y, and N. In some embodiments, the peptide comprises an amino acid sequence of **Table 17**. In some embodiments, the peptide comprises an amino acid sequence according to any one of SEQ ID NOs: 44-55. In some embodiments, the peptide comprises the amino acid

sequence of SEQ ID NO: 2. In some embodiments, the peptide comprises the amino acid sequence of SEQ ID NO: 54.

[0036] Disclosed herein, are methods of treating cancer comprising administering to a subject in need thereof an isolated polypeptide or polypeptide complex comprising an anti-CD3 binding domain that is linked to a peptide that impairs binding of the anti-CD3 binding domain to CD3 wherein the peptide comprises an amino acid sequence according to $U_1-U_2-C-U_4-P-U_6-U_7-U_8-U_9-U_{10}-U_{11}-U_{12}-C-U_{14}$ and U_1 is selected from D, Y, F, I, N, V, H, L, A, T, S, and P; U_2 is selected from D, Y, L, F, I, N, A, V, H, T, and S; U_4 is selected from G and W; U_6 is selected from E, D, V, and P; U_7 is selected from W, L, F, V, G, M, I, and Y; U_8 is selected from E, D, P, and Q; U_9 is selected from E, D, Y, V, F, W, P, L, and Q; U_{10} is selected from S, D, Y, T, I, F, V, N, A, P, L, and H; U_{11} is selected from I, Y, F, V, L, T, N, S, D, A, and H; U_{12} is selected from F, D, Y, L, I, V, A, N, T, P, S, G, and H; and U_{14} is selected from D, Y, N, F, I, P, V, A, T, H, L, M, and S. In some embodiments, U_1 is selected from D, Y, F, I, V, and N; U_2 is selected from D, Y, L, F, I, and N; U_4 is selected from G and W; U_6 is selected from E and D; U_7 is selected from W, L, F, G, and V; U_8 is selected from E and D; U_9 is selected from E, D, Y, and V; U_{10} is selected from S, D, Y, T, and I; U_{11} is selected from I, Y, F, V, L, and T; U_{12} is selected from F, D, Y, L, I, V, A, G, and N; and U_{14} is selected from D, Y, N, F, I, M, and P. In some embodiments, U_1 is selected from D, Y, V, and F; U_2 is selected from D, Y, L, and F; U_4 is selected from G and W; U_6 is selected from E and D; U_7 is selected from W, L, G, and F; U_8 is selected from E and D; U_9 is selected from E and D; U_{10} is selected from S, D, T, and Y; U_{11} is selected from I, Y, V, L, and F; U_{12} is selected from F, D, Y, G, A, and L; and U_{14} is selected from D, Y, M, and N. In some embodiments, the peptide comprises an amino acid sequence of **Table 17**. In some embodiments, the peptide comprises an amino acid sequence according to any one of SEQ ID NOs: 44-55. In some embodiments, the peptide comprises the amino acid sequence of SEQ ID NO: 2. In some embodiments, the peptide comprises the amino acid sequence of SEQ ID NO: 54.

[0037] In some embodiments, the peptide is connected to the anti-CD3 binding domain in a configuration according to: $A_1-L_1-P_1$ (**Formula I**) wherein A_1 comprises the anti-CD3 binding domain; L_1 comprises a cleavable linker that is a substrate for a tumor specific protease; P_1 comprises the peptide that impairs binding of the anti-CD3 binding domain to CD3. In some embodiments, the peptide is connected to the anti-CD3 binding domain in a configuration according to: $A_1-L_1-P_1$ (**Formula I**) wherein A_1 is the anti-CD3 binding domain; L_1 is a cleavable linker that is a substrate for a tumor specific protease; P_1 is the peptide that impairs binding of the anti-CD3 binding domain to CD3. In some embodiments, the peptide is connected to the anti-CD3 binding domain in a configuration comprising **Formula I**: $A_1-L_1-P_1$ (**Formula I**) wherein A_1 comprises the anti-CD3 binding domain; L_1 comprises a cleavable linker that is a substrate for a tumor specific protease; P_1 comprises the peptide that impairs binding of the anti-CD3 binding domain to CD3. In some embodiments, the peptide is connected to the anti-CD3 binding domain in a configuration comprising **Formula I**: $A_1-L_1-P_1$ (**Formula I**) wherein A_1 is the anti-CD3 binding domain; L_1 is a cleavable linker that is a substrate for a tumor specific protease; P_1 is the peptide that impairs binding of the anti-CD3 binding domain to CD3.

In some embodiments, A₁ is further linked to a tumor antigen binding domain (A₂). In some embodiments, the polypeptide or polypeptide complex is according to **Formula Ia: P₂-L₂-A₂-A₁-L₁-P₁ (Formula Ia)** wherein P₂ comprises a peptide that impairs binding of A₂ to a tumor antigen; and L₂ comprises a second cleavable linker that connects A₂ to P₂ and is a substrate for a tumor specific protease.

[0038] In some embodiments, P₁ is connected N-terminal to the cleavable linker and A₁ is connected C-terminal to the cleavable linker. In some embodiments, P₁ is connected C-terminal to the cleavable linker and A₁ is connected N-terminal to the cleavable linker. In some embodiments, P₁ is bound to A₁ through ionic interactions, electrostatic interactions, hydrophobic interactions, Pi-stacking interactions, or H-bonding interactions, or a combination thereof.

[0039] Disclosed herein are isolated polypeptides or polypeptide complexes according to **Formula II: L_{1a}-P_{1a}-H_{1a} (Formula II)** wherein: L_{1a} comprises a tumor specific protease-cleaved linking moiety that when uncleaved connects P_{1a} to an anti-CD3 binding domain that binds to CD3 and; P_{1a} comprises a peptide that impairs binding of the anti-CD3 binding domain to CD3 when L_{1a} is uncleaved wherein the peptide comprises an amino acid sequence selected from SEQ ID NOs: 1-2, 19-55 or the peptide comprises 1, 2, or 3, amino acid substitutions, additions, or deletions relative to amino acid sequences of SEQ ID NOs: 1-2, 19-55; and H_{1a} comprises a half-life extending molecule. In some embodiments, P_{1a} comprises an amino acid sequence selected from SEQ ID NOs: 1-2, and 19-55. In some embodiments, P_{1a} comprises the amino acid sequence of SEQ ID NO: 1. In some embodiments, P_{1a} comprises the amino acid sequence of SEQ ID NO: 2. In some embodiments, P_{1a} comprises the amino acid sequence of SEQ ID NO: 54.

[0040] Disclosed herein are isolated polypeptides or polypeptide complexes according to **Formula III: L_{1b}-P_{1b}-H_{1b}** wherein: L_{1b} comprises a tumor specific protease-cleaved linking moiety that when uncleaved connects P_{1b} to an anti-CD3 binding domain that binds to CD3 and; P_{1b} comprises a peptide that impairs binding of the anti-CD3 binding domain to CD3 when L_{1b} is uncleaved wherein the peptide comprises the amino acid sequence according to Z₁-Z₂-C-Z₄-P-Z₆-Z₇-Z₈-Z₉-Z₁₀-Z₁₁-Z₁₂-C-Z₁₄ and Z₁ is selected from D, Y, F, I, N, V, H, L, A, T, S, and P; Z₂ is selected from D, Y, L, F, I, N, A, V, H, T, and S; Z₄ is selected from G and W; Z₆ is selected from E, D, V, and P; Z₇ is selected from W, L, F, V, G, M, I, and Y; Z₈ is selected from E, D, P, and Q; Z₉ is selected from E, D, Y, V, F, W, P, L, and Q; Z₁₀ is selected from S, D, Y, T, I, F, V, N, A, P, L, and H; Z₁₁ is selected from I, Y, F, V, L, T, N, S, D, A, and H; Z₁₂ is selected from F, D, Y, L, I, V, A, N, T, P, S, and H; and Z₁₄ is selected from D, Y, N, F, I, P, V, A, T, H, L and S; and H_{1b} comprises a half-life extending molecule. In some embodiments, Z₁ is selected from D, Y, F, I, and N; Z₂ is selected from D, Y, L, F, I, and N; Z₄ is selected from G and W; Z₆ is selected from E and D; Z₇ is selected from W, L, F, and V; Z₈ is selected from E and D; Z₉ is selected from E, D, Y, and V; Z₁₀ is selected from S, D, Y, T, and I; Z₁₁ is selected from I, Y, F, V, L, and T; Z₁₂ is selected from F, D, Y, L, I, V, A, and N; and Z₁₄ is selected from D, Y, N, F, I, and P. In some embodiments, Z₁ is selected D, Y, and F; Z₂ is selected from D, Y, L, and F; Z₄ is selected from G and W; Z₆ is selected from E and D; Z₇ is selected from W, L, and F; Z₈ is selected from E and D; Z₉ is selected from E and D; Z₁₀ is selected from S, D, and Y; Z₁₁ is selected from I, Y, and F; Z₁₂ is selected from F, D, Y, and L; Z₁₄ is selected from D, Y, and N. In some embodiments, P_{1b}

comprises an amino acid sequence of **Table 17**. In some embodiments, P_{1b} comprises an amino acid sequences according to any one of SEQ ID NOs: 44-55. In some embodiments, P_{1b} comprises the amino acid sequences according to SEQ ID NO: 2. In some embodiments, P_{1b} comprises the amino acid sequences according to SEQ ID NO: 54.

[0041] Disclosed herein are isolated polypeptide or polypeptide complex according to **Formula IV**: L_{1c}-P_{1c}-H_{1c} wherein: L_{1c} comprises a tumor specific protease-cleaved linking moiety that when uncleaved connects P_{1c} to an anti-CD3 binding domain that binds to CD3 and; P_{1c} comprises a peptide that impairs binding of the anti-CD3 binding domain to CD3 when L_{1b} is uncleaved wherein the peptide comprises the amino acid sequence according to U₁-U₂-C-U₄-P-U₆-U₇-U₈-U₉-U₁₀-U₁₁-U₁₂-C-U₁₄ and U₁ is selected from D, Y, F, I, N, V, H, L, A, T, S, and P; U₂ is selected from D, Y, L, F, I, N, A, V, H, T, and S; U₄ is selected from G and W; U₆ is selected from E, D, V, and P; U₇ is selected from W, L, F, V, G, M, I, and Y; U₈ is selected from E, D, P, and Q; U₉ is selected from E, D, Y, V, F, W, P, L, and Q; U₁₀ is selected from S, D, Y, T, I, F, V, N, A, P, L, and H; U₁₁ is selected from I, Y, F, V, L, T, N, S, D, A, and H; U₁₂ is selected from F, D, Y, L, I, V, A, N, T, P, S, G, and H; U₁₄ is selected from D, Y, N, F, I, P, V, A, T, H, L, M, and S; and H_{1c} comprises a half-life extending molecule. In some embodiments, U₁ is selected from D, Y, F, I, V, and N; U₂ is selected from D, Y, L, F, I, and N; U₄ is selected from G and W; U₆ is selected from E and D; U₇ is selected from W, L, F, G, and V; U₈ is selected from E and D; U₉ is selected from E, D, Y, and V; U₁₀ is selected from S, D, Y, T, and I; U₁₁ is selected from I, Y, F, V, L, and T; U₁₂ is selected from F, D, Y, L, I, V, A, G, and N; U₁₄ is selected from D, Y, N, F, I, M, and P. In some embodiments, U₁ is selected from D, Y, V, and F; U₂ is selected from D, Y, L, and F; U₄ is selected from G and W; U₆ is selected from E and D; U₇ is selected from W, L, G, and F; U₈ is selected from E and D; U₉ is selected from E and D; U₁₀ is selected from S, D, T, and Y; U₁₁ is selected from I, Y, V, L, and F; U₁₂ is selected from F, D, Y, G, A, and L; U₁₄ is selected from D, Y, M, and N. In some embodiments, P_{1c} comprises an amino acid sequence of **Table 17**. In some embodiments, P_{1c} comprises an amino acid sequences according to any one of SEQ ID NOs: 44-55. In some embodiments, P_{1c} comprises the amino acid sequences according to SEQ ID NO: 2. In some embodiments, P_{1c} comprises the amino acid sequences according to SEQ ID NO: 54.

[0042] In some embodiments, the peptide (P₁, P_{1a}, P_{1b}, or P_{1c}) comprises the amino acid sequence of Peptide-1.

[0043] In some embodiments, the peptide (P₁, P_{1a}, P_{1b}, or P_{1c}) comprises the amino acid sequence of Peptide-2.

[0044] In some embodiments, the peptide (P₁, P_{1a}, P_{1b} or P_{1c}) comprises an amino acid sequence that has 1 amino acid substitution relative to Peptide-1. In some embodiments, the peptide (P₁, P_{1a}, P_{1b} or P_{1c}) comprises an amino acid sequence that has 2 amino acid substitution relative to Peptide-1. In some embodiments, the peptide (P₁, P_{1a}, P_{1b} or P_{1c}) comprises an amino acid sequence that has 3 amino acid substitution relative to Peptide-1.

[0045] In some embodiments, the peptide (P₁, P_{1a}, P_{1b} or P_{1c}) comprises an amino acid sequence that has 1 amino acid substitution relative to Peptide-2. In some embodiments, the peptide (P₁, P_{1a}, P_{1b} or P_{1c})

comprises an amino acid sequence that has 2 amino acid substitutions relative to Peptide-2. In some embodiments, the peptide (P₁, P_{1a}, P_{1b} or P_{1c}) comprises an amino acid sequence that has 3 amino acid substitutions relative to Peptide-2.

[0046] In some embodiments, the peptide (P₁, P_{1a}, P_{1b} or P_{1c}) comprises an amino acid sequence that has 1 amino acid deletion relative to Peptide-1. In some embodiments, the peptide (P₁, P_{1a}, P_{1b} or P_{1c}) comprises an amino acid sequence that has 2 amino acid deletions relative to Peptide-1. In some embodiments, the peptide (P₁, P_{1a}, P_{1b} or P_{1c}) comprises an amino acid sequence that has 3 amino acid deletions relative to Peptide-1.

[0047] In some embodiments, the peptide (P₁, P_{1a}, P_{1b} or P_{1c}) comprises an amino acid sequence that has 1 amino acid deletions relative to Peptide-2. In some embodiments, the peptide (P₁, P_{1a}, P_{1b} or P_{1c}) comprises an amino acid sequence that has 2 amino acid deletions relative to Peptide-2. In some embodiments, the peptide (P₁, P_{1a}, P_{1b} or P_{1c}) comprises an amino acid sequence that has 3 amino acid deletions relative to Peptide-2.

[0048] In some embodiments, the peptide (P₁, P_{1a}, P_{1b} or P_{1c}) comprises an amino acid sequence that has 1 amino acid additions relative to Peptide-1. In some embodiments, the peptide (P₁, P_{1a}, P_{1b} or P_{1c}) comprises an amino acid sequence that has 2 amino acid additions relative to Peptide-1. In some embodiments, the peptide (P₁, P_{1a}, P_{1b} or P_{1c}) comprises an amino acid sequence that has 3 amino acid additions relative to Peptide-1.

[0049] In some embodiments, the peptide (P₁, P_{1a}, P_{1b} or P_{1c}) comprises an amino acid sequence that has 1 amino acid addition relative to Peptide-2. In some embodiments, the peptide (P₁, P_{1a}, P_{1b} or P_{1c}) comprises an amino acid sequence that has 2 amino acid additions relative to Peptide-2. In some embodiments, the peptide (P₁, P_{1a}, P_{1b} or P_{1c}) comprises an amino acid sequence that has 3 amino acid additions relative to Peptide-2.

[0050] In some embodiments, the peptide (P₁, P_{1a}, P_{1b} or P_{1c}) comprises an amino acid sequence that has 1 amino acid addition relative to Peptide-39. In some embodiments, the peptide (P₁, P_{1a}, P_{1b} or P_{1c}) comprises an amino acid sequence that has 2 amino acid additions relative to Peptide-39. In some embodiments, the peptide (P₁, P_{1a}, P_{1b} or P_{1c}) comprises an amino acid sequence that has 3 amino acid additions relative to Peptide-39.

[0051] In some embodiments, P₁ is bound to A₁ through ionic interactions, electrostatic interactions, hydrophobic interactions, Pi-stacking interactions, or H-bonding interactions, or a combination thereof.

[0052] In some embodiments, P_{1a} is bound to the anti-CD3 binding domain through ionic interactions, electrostatic interactions, hydrophobic interactions, Pi-stacking interactions, or H-bonding interactions, or a combination thereof when L_{1a} is uncleaved.

[0053] In some embodiments, P₁, P_{1a}, P_{1b} or P_{1c} comprise a modified amino acid or non-natural amino acid, or a modified non-natural amino acid, or a combination thereof. In some embodiments, the modified amino acid or a modified non-natural amino acid comprises a post-translational modification. In some embodiments P₁, P_{1a}, P_{1b} or P_{1c} comprises a modification including, but not limited to acetylation, acylation,

ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphatidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent crosslinks, formation of cystine, formation of pyroglutamate, formylation, gamma carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination. Modifications are made anywhere to P₁, P_{1a}, P_{1b} or P_{1c} including the peptide backbone, the amino acid side chains, and the terminus.

Table 1. Amino acid sequence of Peptide-1 and Peptide-2

Construct Description	Amino Acid Sequence (N to C)	SEQ ID NO:
Peptide-1	GSQCLGPEWEVCPY	1
Peptide-2	VYCGPEFDESVCMM	2

Anti-CD3 binding domain (A₁)

[0054] In some embodiments, A₁ comprises an antibody or antibody fragment. In some embodiments, A₁ comprises an antibody or antibody fragment that is human or humanized. In some embodiments, L₁ is bound to N-terminus of the antibody or antibody fragment of A₁. In some embodiments, A₂ is bound to N-terminus of the antibody or antibody fragment of A₁. In some embodiments, L₁ is bound to the C-terminus of the antibody or antibody fragment of A₁. In some embodiments, A₂ is bound to the C-terminus of the antibody or antibody fragment of A₁. In some embodiments, the antibody or antibody fragment comprises a single chain variable fragment, a single domain antibody, or a Fab fragment. In some embodiments, A₁ is the single chain variable fragment (scFv). In some embodiments, the scFv comprises a scFv heavy chain polypeptide and a scFv light chain polypeptide. In some embodiments, A₁ is the single domain antibody. In some embodiments, the antibody or antibody fragment thereof comprises a single chain variable fragment (scFv), a heavy chain variable domain (VH domain), a light chain variable domain (VL domain), or a variable domain (VHH) of a camelid derived single domain antibody.

[0055] In some embodiments, the first target antigen is CD3. In some embodiments, A₁ comprises an anti-CD3e single chain variable fragment. In some embodiments, A₁ comprises an anti-CD3e single chain variable fragment that has a KD binding of 1 μM or less to CD3 on CD3 expressing cells. In some embodiments, A₁ comprises a variable light chain and variable heavy chain each of which is capable of specifically binding to human CD3. In some embodiments, A₁ comprises complementary determining regions (CDRs) from sp34 scFv. In some embodiments, the scFv comprises a scFv heavy chain variable domain and an scFv light chain variable domain. In some embodiments, the polypeptide or polypeptide complex of formula I binds to an effector cell when L₁ is cleaved by the tumor specific protease. In some embodiments, the polypeptide or polypeptide complex of formula I binds to an effector cell when L₁ is cleaved by the tumor specific protease and A₁ binds to the effector cell. In some embodiments, the effector

cell is a T cell. In some embodiments, A₁ binds to a polypeptide that is part of a TCR-CD3 complex on the effector cell. In some embodiments, the polypeptide that is part of the TCR-CD3 complex is human CD3ε.

[0056] In some embodiments, the scFv heavy chain variable domain comprises complementarity determining regions (CDRs): HC-CDR1, HC-CDR2, and HC-CDR3, wherein the HC-CDR1, the HC-CDR2, and the HC-CDR3 of the scFv heavy chain variable domain comprise: HC-CDR1: SEQ ID NO: 3; HC-CDR2: SEQ ID NO: 4; HC-CDR3: SEQ ID NO: 5, and wherein the CDRs comprise from 0-2 amino acid modifications in at least one of the HC-CDR1, HC-CDR2, or HC-CDR3.

[0057] In some embodiments, the scFv light chain variable domain comprises complementarity determining regions (CDRs): LC-CDR1, LC-CDR2, and LC-CDR3, wherein the LC-CDR1, the LC-CDR2, and the LC-CDR3 of the scFv light chain variable domain comprise: LC-CDR1: SEQ ID NO: 6; LC-CDR2: SEQ ID NO: 7; and LC-CDR3: SEQ ID NO: 8, and wherein the CDRs comprise from 0-2 amino acid modifications in at least one of the LC-CDR1, LC-CDR2, or LC-CDR3.

[0058] In some embodiments, the scFv heavy chain variable domain comprises complementarity determining regions (CDRs): HC-CDR1, HC-CDR2, and HC-CDR3, wherein the HC-CDR1, the HC-CDR2, and the HC-CDR3 of the scFv heavy chain variable domain comprise: HC-CDR1: SEQ ID NO: 3; HC-CDR2: SEQ ID NO: 4; HC-CDR3: SEQ ID NO: 5; and the scFv light chain variable domain comprises complementarity determining regions (CDRs): LC-CDR1, LC-CDR2, and LC-CDR3, wherein the LC-CDR1, the LC-CDR2, and the LC-CDR3 of the scFv light chain variable domain comprise: LC-CDR1: SEQ ID NO: 6; LC-CDR2: SEQ ID NO: 7; and LC-CDR3: SEQ ID NO: 8.

[0059] In some embodiments, A₁ comprises complementarity determining regions (CDRs): HC-CDR1, HC-CDR2, and HC-CDR3, wherein the HC-CDR1, the HC-CDR2, and the HC-CDR3 of A₁ comprise: HC-CDR1: SEQ ID NO: 3; HC-CDR2: SEQ ID NO: 4; HC-CDR3: SEQ ID NO: 5, and wherein the CDRs comprise from 0-2 amino acid modifications in at least one of the HC-CDR1, HC-CDR2, or HC-CDR3.

[0060] In some embodiments, A₁ comprises complementarity determining regions (CDRs): LC-CDR1, LC-CDR2, and LC-CDR3, wherein the LC-CDR1, the LC-CDR2, and the LC-CDR3 of A₁ comprise: LC-CDR1: SEQ ID NO: 6; LC-CDR2: SEQ ID NO: 7; and LC-CDR3: SEQ ID NO: 8, and wherein the CDRs comprise from 0-2 amino acid modifications in at least one of the LC-CDR1, LC-CDR2, or LC-CDR3.

[0061] In some embodiments, A₁ comprises complementarity determining regions (CDRs): HC-CDR1, HC-CDR2, and HC-CDR3, wherein the HC-CDR1, the HC-CDR2, and the HC-CDR3 of A₁ comprise: HC-CDR1: SEQ ID NO: 3; HC-CDR2: SEQ ID NO: 4; HC-CDR3: SEQ ID NO: 5; and A₁ comprises complementarity determining regions (CDRs): LC-CDR1, LC-CDR2, and LC-CDR3, wherein the LC-CDR1, the LC-CDR2, and the LC-CDR3 of A₁ comprise: LC-CDR1: SEQ ID NO: 6; LC-CDR2: SEQ ID NO: 7; and LC-CDR3: SEQ ID NO: 8.

[0062] In some embodiments, the scFv heavy chain variable domain comprises an amino acid sequence that has at least 70% sequence identity to the amino acid sequence according to SEQ ID NO: 9. In some embodiments, the scFv heavy chain variable domain comprises an amino acid sequence that has at least 80% sequence identity to the amino acid sequence according to SEQ ID NO: 9. In some embodiments, the scFv

sequence of at least 115 consecutive amino acid residues of SEQ ID NO: 9, and has at least 95% sequence identity to the at least 115 consecutive amino acid residues of SEQ ID NO: 9. In some embodiments, the scFv heavy chain variable domain comprises an amino acid sequence of at least 120 consecutive amino acid residues of SEQ ID NO: 9, and has at least 95% sequence identity to the at least 120 consecutive amino acid residues of SEQ ID NO: 9.

Table 2. Exemplary amino acid sequence of Anti-CD3 binding domain (A₁) (CDRs determined by IMGT definition)

Construct Description	Amino Acid Sequence (N to C)	SEQ ID NO:
CD3: HC: CDR1	GFTFNKYA	3
CD3: HC: CDR2	IRSKYNNYAT	4
CD3: HC: CDR3	VRHGNFGNSYISYWAY	5
CD3: LC: CDR1	TGAVTSGNY	6
CD3: LC: CDR2	GTK	7
CD3: LC: CDR3	VLWYSNRWV	8
Anti-CD3 SP34.185 scFv	EVQLVESGGGLVQPGGSLKLSCAAS <u>GFTFNK</u> <u>YAMN</u> WVRQAPGKGLEWVAR <u>IRSKYNNYAT</u> YYADSVKDRFTISRDDSKNTAYLQMNNLKTE DTAVYYC <u>VRHGNFGNSYISYWAY</u> WGQGT LTVSSGGGGSGGGGSGGGGSGTQVVTQEPSLT VSPGGTVTLTCGSST <u>TGAVTSGNY</u> PNWVQKPK GQAPRGLIG <u>GTK</u> FLAPGTPARFSGSLLGGKAA LTLSGVQPEDEAEYYC <u>VLWYSNRWV</u> FGGGT KLTVL	9

Tumor antigen binding domain (A₂)

[0064] In some embodiments, A₂ comprises an antibody or antibody fragment. In some embodiments, the antibody or antibody fragment thereof of A₂ comprises a single chain variable fragment, a single domain antibody, Fab', or a Fab. In some embodiments, the antibody or antibody fragment thereof of A₂ comprises a single chain variable fragment (scFv), a heavy chain variable domain (VH domain), a light chain variable domain (VL domain), or a variable domain (VHH) of a camelid derived single domain antibody. In some embodiments, the antibody or antibody fragment thereof of A₂ is humanized or human. In some embodiments, A₂ is the Fab or Fab'. In some embodiments, the Fab or Fab' comprises (a) a Fab light chain polypeptide and (b) a Fab heavy chain polypeptide. In some embodiments, the second target antigen comprises a tumor antigen. In some embodiments, the antibody or antibody fragment thereof comprises an epidermal growth factor receptor (EGFR) binding domain. In some embodiments, the antibody or antibody fragment thereof comprises a mesothelin binding domain. In some embodiments, A₂ comprises an amino acid sequence disclosed in Table 3 or a sequence substantially identical thereto (e.g., a sequence that has at least 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to an amino acid sequence disclosed in Table 3).

Table 3. Exemplary amino acid sequences of Anti-EGFR Fab

Construct Description	Amino Acid Sequence (N to C)	SEQ ID NO:
Anti-EGFR Fab light chain	DILLTQSPVILSVSPGERVSFSCRAS QSIGTNIH WYQQRTNGSPRLLIK YASE SISGIPSRFSGSGS GTDFTLSINSVESEDIADYYC QONNNWPTTF GAGTKLELKRTVAAPSVFIFPPSDEQLKSGTA SVVCLLNNFYPPREKVKQWKVDNALQSGNSQ ESVTEQDSKDYSLSSITLTLTKADYEKHKVY ACEVTHQGLSSPVTKSFNRGEC	10
Anti-EGFR Fab heavy chain	QVQLKQSGPGLVQPSQSLITCTVSG FSLTNY G VHWVRQSPGKGLEWLG VIW SGGNTDYNT PFTSRLSINKDNSKSKQVFFKMNSLQSQDTAIY YC ARALTYDYEFAY WGQGTLLVTVSAASTK GPSVFPLAPSSKSTSGGTAALGCLVKDYFPEP VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSV VTVPSSSLGTQTYICNVNHKPSNTKVDKKVEP KSC	11

Half-life extending molecule (H₁, H_{1a}, H_{1b}, H_{1c})

[0065] In some embodiments, P₁ is further linked to a half-life extending moiety (H₁).

[0066] In some embodiments, the half-life extending moiety is a single-domain antibody.

[0067] In some embodiments, H₁ does not block P₁ binding to CD3. In some embodiments, H_{1a} does not block the anti-CD3 binding domain from binding to the CD3. In some embodiments, H_{1b} does not block the anti-CD3 binding domain from binding to the CD3. In some embodiments, H_{1c} does not block the anti-CD3 binding domain from binding to the CD3. In some embodiments, the half-life extending molecule (H₁, H_{1a}, H_{1b}, or H_{1c}) does not have binding affinity to CD3. In some embodiments, the half-life extending molecule (H₁, H_{1a}, H_{1b}, or H_{1c}) does not have binding affinity to CD3. In some embodiments, the half-life extending molecule (H₁, H_{1a}, H_{1b}, or H_{1c}) does not shield the anti-CD3 binding domain from CD3.

[0068] In some embodiments, H₁, H_{1a}, H_{1b}, or H_{1c} comprise an amino acid sequence that has repetitive sequence motifs. In some embodiments, H₁, H_{1a}, H_{1b}, or H_{1c} comprises an amino acid sequence that has highly ordered secondary structure. “Highly ordered secondary structure,” as used in this context, means that at least about 50%, or about 70%, or about 80%, or about 90%, of amino acid residues of H₁ or H_{1a} contribute to secondary structure, as measured or determined by means, including, but not limited to, spectrophotometry (e.g. by circular dichroism spectroscopy in the “far-UV” spectral region (190-250 nm), and computer programs or algorithms, such as the Chou-Fasman algorithm and the Garnier-Osguthorpe-Robson (“GOR”) algorithm.

[0069] In some embodiments, H₁, H_{1a}, H_{1b}, or H_{1c} comprises a polymer. In some embodiments, the polymer is polyethylene glycol (PEG). In some embodiments, H₁ or H_{1a} comprises albumin. In some embodiments,

H₁ or H_{1a} comprises a Fc domain. In some embodiments, the albumin is serum albumin. In some embodiments, the albumin is human serum albumin. In some embodiments, H₁, H_{1a}, H_{1b}, or H_{1c} comprises a polypeptide, a ligand, or a small molecule. In some embodiments, the polypeptide, the ligand or the small molecule binds serum protein or a fragment thereof, a circulating immunoglobulin or a fragment thereof, or CD35/CR1. In some embodiments, the serum protein comprises a thyroxine-binding protein, a transthyretin, a l-acid glycoprotein, a transferrin, transferrin receptor or a transferrin-binding portion thereof, a fibrinogen, or an albumin. In some embodiments, the circulating immunoglobulin molecule comprises IgG1, IgG2, IgG3, IgG4, sIgA, IgM or IgD. In some embodiments, the serum protein is albumin. In some embodiments, the polypeptide is an antibody. In some embodiments, the antibody comprises a single domain antibody, a single chain variable fragment or a Fab. In some embodiments, the antibody comprises a single domain antibody. In some embodiments, the antibody comprises a single domain antibody that binds to albumin. In some embodiments, the antibody comprises a single domain antibody that binds to human serum albumin. In some embodiments, the antibody is a human or humanized antibody. In some embodiments, the antibody is selected from the group consisting of 645gH1gL1, 645dsgH5gL4, 23-13-A01 -sc02, A10m3 or a fragment thereof, DOM7r-31, DOM7h-11-15, Alb-1, Alb-8, Alb-23, 10G, 10GE, and SA21.

[0070] In some embodiments, H₁ comprises a single domain antibody. In some embodiments, H₁ comprises a single domain antibody that binds to albumin. In some embodiments, H₁ comprises a single domain antibody that binds to human serum albumin. In some embodiments, H_{1a} comprises a single domain antibody. In some embodiments, H_{1a} comprises a single domain antibody that binds to albumin. In some embodiments, H_{1a} comprises a single domain antibody that binds to human serum albumin. In some embodiments, H_{1b} comprises a single domain antibody. In some embodiments, H_{1b} comprises a single domain antibody that binds to albumin. In some embodiments, H_{1b} comprises a single domain antibody that binds to human serum albumin. In some embodiments, H_{1c} comprises a single domain antibody. In some embodiments, H_{1c} comprises a single domain antibody that binds to albumin. In some embodiments, H_{1c} comprises a single domain antibody that binds to human serum albumin.

[0071] In some embodiments, H₁, H_{1a}, H_{1b}, or H_{1c} comprise a modified amino acid or non-natural amino acid, or a modified non-natural amino acid, or a combination thereof. In some embodiments, the modified amino acid or a modified non-natural amino acid comprises a post-translational modification. In some embodiments H₁, H_{1a}, H_{1b}, or H_{1c} comprise a modification including, but not limited to acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphatidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent crosslinks, formation of cystine, formation of pyroglutamate, formylation, gamma carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination. Modifications are made anywhere to H₁, H_{1a}, H_{1b}, or H_{1c} including the peptide backbone, the

amino acid side chains, and the terminus. . In some embodiments, H₁, H_{1a}, H_{1b}, or H_{1c} comprises an amino acid sequence disclosed in **Table 4** or a sequence substantially identical thereto (e.g., a sequence that has at least 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to an amino acid sequence disclosed in Table 4).

Table 4. Exemplary amino acid sequence of half-life extending molecule (H₁, H_{1a}, H_{1b}, H_{1c})

Construct Description	Amino Acid Sequence (N to C)	SEQ ID NO:
10G single domain antibody	EVQLVESGGGLVQPGNSLRSLCAAS GFTESK FGMSWVRQAPGKGLEWVSSI SGSGRDTLYA DSVKGRFTISRDNAKTTLYLQMNSLRPEDTA VYYC TIGGSLSV SSQGTLVTVSS	12

Peptide (P₂)

[0072] In some embodiments, P₂ impairs binding of A₂ to the tumor antigen. In some embodiments, P₂ is bound to A₂ through ionic interactions, electrostatic interactions, hydrophobic interactions, Pi-stacking interactions, or H-bonding interactions, or a combination thereof. In some embodiments, P₂ is bound to A₂ at or near an antigen binding site. In some embodiments, P₂ has less than 70% sequence homology to the tumor antigen. In some embodiments, P₂ has less than 75% sequence homology to the tumor antigen. In some embodiments, P₂ has less than 80% sequence homology to the tumor antigen. In some embodiments, P₂ has less than 85% sequence homology to the tumor antigen. In some embodiments, P₂ has less than 90% sequence homology to the tumor antigen. In some embodiments, P₂ has less than 95% sequence homology to the tumor antigen. In some embodiments, P₂ has less than 98% sequence homology to the tumor antigen. In some embodiments, P₂ has less than 99% sequence homology to the tumor antigen.

[0073] In some embodiments, P₂ comprises a peptide sequence of at least 10 amino acids in length. In some embodiments, P₂ comprises a peptide sequence of at least 10 amino acids in length and no more than 20 amino acids in length. In some embodiments, P₂ comprises a peptide sequence of at least 16 amino acids in length. In some embodiments, P₂ comprises a peptide sequence of no more than 40 amino acids in length. In some embodiments, P₂ comprises at least two cysteine amino acid residues. In some embodiments, P₂ comprises a cyclic peptide or a linear peptide. In some embodiments, P₂ comprises a cyclic peptide. In some embodiments, P₂ comprises a linear peptide.

[0074] In some embodiments, P₂ comprise a modified amino acid or non-natural amino acid, or a modified non-natural amino acid, or a combination thereof. In some embodiments, the modified amino acid or a modified non-natural amino acid comprises a post-translational modification. In some embodiments P₂ comprise a modification including, but not limited to acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphatidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent crosslinks, formation of cystine, formation of pyroglutamate, formylation, gamma carboxylation,

glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination. Modifications are made anywhere to P₂ including the peptide backbone, the amino acid side chains, and the terminus.

[0075] In some embodiments, P₂ does not comprise albumin or an albumin fragment. In some embodiments, P₂ does not comprise an albumin binding domain.

Cleavable Linker (L₁ or L₂)

[0076] In some embodiments, L₁ or L₂ is a peptide sequence having at least 5 to no more than 50 amino acids. In some embodiments, L₁ or L₂ is a peptide sequence having at least 10 to no more than 30 amino acids. In some embodiments, L₁ or L₂ is a peptide sequence having at least 10 amino acids. In some embodiments, L₁ or L₂ is a peptide sequence having at least 18 amino acids. In some embodiments, L₁ or L₂ is a peptide sequence having at least 26 amino acids.

[0077] In some embodiments, L₁ has a formula selected from the group consisting of (G₂S)_n, (GS)_n, (GSGGS)_n (SEQ ID NO: 917), (GGGS)_n (SEQ ID NO: 918), (GGGGS)_n (SEQ ID NO: 919), and (GSSGGS)_n (SEQ ID NO: 920), wherein n is an integer of at least 1. In some embodiments, L₁ has a formula selected from the group consisting of (G₂S)_n, (GS)_n, (GSGGS)_n (SEQ ID NO: 917), (GGGS)_n (SEQ ID NO: 918), (GGGGS)_n (SEQ ID NO: 919), and (GSSGGS)_n (SEQ ID NO: 920), wherein n is an integer of 1. In some embodiments, L₁ has a formula selected from the group consisting of (G₂S)_n (SEQ ID NO: 922), (GS)_n (SEQ ID NO: 938), (GSGGS)_n (SEQ ID NO: 939), (GGGS)_n (SEQ ID NO: 940), (GGGGS)_n (SEQ ID NO: 941), and (GSSGGS)_n (SEQ ID NO: 942), wherein n is an integer from 1 to 3.

[0078] In some embodiments, L₁ has a formula of (G₂S)_n, wherein n is an integer of least 1. In some embodiments, L₁ has a formula of (GS)_n, wherein n is an integer of least 1. In some embodiments, L₁ has a formula of (GSGGS)_n (SEQ ID NO: 917), wherein n is an integer of least 1. In some embodiments, L₁ has a formula of (GGGS)_n (SEQ ID NO: 918), wherein n is an integer of least 1. In some embodiments, L₁ has a formula of (GGGGS)_n (SEQ ID NO: 919), wherein n is an integer of least 1. In some embodiments, L₁ has a formula of (GSSGGS)_n (SEQ ID NO: 920), wherein n is an integer of least 1.

[0079] In some embodiments, L₁ has a formula of (G₂S)_n, wherein n is an integer of 1. In some embodiments, L₁ has a formula of (GS)_n, wherein n is an integer of 1. In some embodiments, L₁ has a formula of (GSGGS)_n (SEQ ID NO: 917), wherein n is an integer of 1. In some embodiments, L₁ has a formula of (GGGS)_n (SEQ ID NO: 918), wherein n is an integer of 1. In some embodiments, L₁ has a formula of (GGGGS)_n (SEQ ID NO: 919), wherein n is an integer of 1. In some embodiments, L₁ has a formula of (GSSGGS)_n (SEQ ID NO: 920), wherein n is an integer of 1.

[0080] In some embodiments, L₁ has a formula of (G₂S)_n (SEQ ID NO: 922), wherein n is an integer from 1 to 3. In some embodiments, L₁ has a formula of (GS)_n (SEQ ID NO: 938), wherein n is an integer from 1 to 3. In some embodiments, L₁ has a formula of (GSGGS)_n (SEQ ID NO: 939), wherein n is an integer from 1 to 3. In some embodiments, L₁ has a formula of (GGGS)_n (SEQ ID NO: 940), wherein n is an integer from 1 to 3. In some embodiments, L₁ has a formula of (GGGGS)_n (SEQ ID NO: 941), wherein n is an integer from 1 to 3.

1 to 3. In some embodiments, L_1 has a formula of $(GSSGGS)_n$ (SEQ ID NO: 942), wherein n is an integer from 1 to 3.

[0081] In some embodiments, L_1 is a substrate for a tumor specific protease. In some embodiments, the tumor specific protease is selected from the group consisting of metalloprotease, serine protease, cysteine protease, threonine protease, and aspartic protease. In some embodiments, L_1 comprises a urokinase cleavable amino acid sequence, a matrilysin cleavable amino acid sequence, a matrix metalloprotease cleavable amino acid sequence, or a legumain cleavable amino acid sequence. In some embodiments, L_1 comprises an amino acid sequence selected from the group consisting of GGGGSLSGRSDNHGSSGT (SEQ ID NO: 923), GGGGSSGGSGGSGLSGRSDNHGSSGT (SEQ ID NO: 924), ASGRSDNH (SEQ ID NO: 925), LAGRSDNH (SEQ ID NO: 926), ISSGLASGRSDNH (SEQ ID NO: 927), ISSGLLAGRSDNH (SEQ ID NO: 928), LSGRSDNH (SEQ ID NO: 929), ISSGLLSGRSDNP (SEQ ID NO: 930), ISSGLLSGRSDNH (SEQ ID NO: 931), LSGRSDNHSPLGLAGS (SEQ ID NO: 932), SPLGLAGSLSGRSDNH (SEQ ID NO: 933), SPLGLSGRSDNH (SEQ ID NO: 934), LAGRSDNHSPLGLAGS (SEQ ID NO: 935), LSGRSDNHVPLSLKMG (SEQ ID NO: 936), and LSGRSDNHVPLSLSMG (SEQ ID NO: 937). In some embodiments, L_1 comprises an amino acid sequence selected from the group consisting of ASGRSDNH (SEQ ID NO: 925), LAGRSDNH (SEQ ID NO: 926), ISSGLASGRSDNH (SEQ ID NO: 927), and ISSGLLAGRSDNH (SEQ ID NO: 928).

[0082] In some embodiments, L_2 has a formula selected from the group consisting of $(G_2S)_n$, $(GS)_n$, $(GSGGS)_n$ (SEQ ID NO: 917), $(GGGS)_n$ (SEQ ID NO: 918), $(GGGGS)_n$ (SEQ ID NO: 919), and $(GSSGGS)_n$ (SEQ ID NO: 920), wherein n is an integer of at least 1. In some embodiments, L_2 has a formula selected from the group consisting of $(G_2S)_n$, $(GS)_n$, $(GSGGS)_n$ (SEQ ID NO: 917), $(GGGS)_n$ (SEQ ID NO: 918), $(GGGGS)_n$ (SEQ ID NO: 919), and $(GSSGGS)_n$ (SEQ ID NO: 920), wherein n is an integer of 1. In some embodiments, L_2 has a formula selected from the group consisting of $(G_2S)_n$ (SEQ ID NO: 922), $(GS)_n$ (SEQ ID NO: 938), $(GSGGS)_n$ (SEQ ID NO: 939), $(GGGS)_n$ (SEQ ID NO: 940), $(GGGGS)_n$ (SEQ ID NO: 941), and $(GSSGGS)_n$ (SEQ ID NO: 942), wherein n is an integer from 1 to 3.

[0083] In some embodiments, L_2 has a formula of $(G_2S)_n$ wherein n is an integer of least 1. In some embodiments, L_2 has a formula of $(GS)_n$ wherein n is an integer of least 1. In some embodiments, L_2 has a formula of $(GSGGS)_n$ (SEQ ID NO: 917), wherein n is an integer of least 1. In some embodiments, L_2 has a formula of $(GGGS)_n$ (SEQ ID NO: 918), wherein n is an integer of least 1. In some embodiments, L_2 has a formula of $(GGGGS)_n$ (SEQ ID NO: 919), wherein n is an integer of least 1. In some embodiments, L_2 has a formula of $(GSSGGS)_n$ (SEQ ID NO: 920), wherein n is an integer of least 1.

[0084] In some embodiments, L_2 has a formula of $(G_2S)_n$ wherein n is an integer of 1. In some embodiments, L_2 has a formula of $(GS)_n$ wherein n is an integer of 1. In some embodiments, L_2 has a formula of $(GSGGS)_n$ (SEQ ID NO: 917), wherein n is an integer of 1. In some embodiments, L_2 has a formula of $(GGGS)_n$ (SEQ ID NO: 918), wherein n is an integer of 1. In some embodiments, L_2 has a formula of $(GGGGS)_n$ (SEQ ID NO: 919), wherein n is an integer of 1. In some embodiments, L_2 has a formula of $(GSSGGS)_n$ (SEQ ID NO: 920), wherein n is an integer of 1.

[0085] In some embodiments, L_2 has a formula of $(G_2S)_n$ (SEQ ID NO: 922), wherein n is an integer from 1 to 3. In some embodiments, L_2 has a formula of $(GS)_n$ (SEQ ID NO: 938), wherein n is an integer from 1 to 3. In some embodiments, L_2 has a formula of $(GSGGS)_n$ (SEQ ID NO: 939), wherein n is an integer from 1 to 3. In some embodiments, L_2 has a formula of $(GGGS)_n$ (SEQ ID NO: 940), wherein n is an integer from 1 to 3. In some embodiments, L_2 has a formula of $(GGGGS)_n$ (SEQ ID NO: 941), wherein n is an integer from 1 to 3. In some embodiments, L_2 has a formula of $(GSSGGS)_n$ (SEQ ID NO: 942), wherein n is an integer from 1 to 3.

[0086] In some embodiments, L_2 is a substrate for a tumor specific protease. In some embodiments, the tumor specific protease is selected from the group consisting of metalloprotease, serine protease, cysteine protease, threonine protease, and aspartic protease. In some embodiments, L_2 comprises a urokinase cleavable amino acid sequence, a matriptase cleavable amino acid sequence, a matrix metalloprotease cleavable amino acid sequence, or a legumain cleavable amino acid sequence. In some embodiments, L_2 comprises an amino acid sequence selected from the group consisting of GGGGSLSGRSDNHGSSGT (SEQ ID NO: 923), GGGGSSGGSGGSGLSGRSDNHGSSGT (SEQ ID NO: 924), ASGRSDNH (SEQ ID NO: 925), LAGRSDNH (SEQ ID NO: 926), ISSGLASGRSDNH (SEQ ID NO: 927), ISSGLLAGRSDNH (SEQ ID NO: 928), LSGRSDNH (SEQ ID NO: 929), ISSGLLSGRSDNP (SEQ ID NO: 930), ISSGLLSGRSDNH (SEQ ID NO: 931), LSGRSDNHSPLGLAGS (SEQ ID NO: 932), SPLGLAGSLSGRSDNH (SEQ ID NO: 933), SPLGLSGRSDNH (SEQ ID NO: 934), LAGRSDNHSPLGLAGS (SEQ ID NO: 935), LSGRSDNHVPLSLKMG (SEQ ID NO: 936), and LSGRSDNHVPLSLSMG (SEQ ID NO: 937). In some embodiments, L_2 comprises an amino acid sequence selected from the group consisting of ASGRSDNH (SEQ ID NO: 925), LAGRSDNH (SEQ ID NO: 926), ISSGLASGRSDNH (SEQ ID NO: 927), ISSGLLAGRSDNH (SEQ ID NO: 928), or GGGGSGGGSGGISSGLLSGRSDNHGGGS (SEQ ID NO: 943).

[0087] In some embodiments, L_1 or L_2 comprises a modified amino acid or non-natural amino acid, or a modified non-natural amino acid, or a combination thereof. In some embodiments, the modified amino acid or a modified non-natural amino acid comprises a post-translational modification. In some embodiments, L_1 or L_2 comprises a modification including, but not limited, to acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphatidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent crosslinks, formation of cystine, formation of pyroglutamate, formylation, gamma carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination. In some embodiments, modifications are made anywhere to L_1 or L_2 including the peptide backbone, or the amino acid side chains.

Linking moiety (L_3 , L_{3a} , L_{3b} , or L_{3c})

[0088] In some embodiments, H_1 comprises a linking moiety (L_3) that connects H_1 to P_1 . In some embodiments, H_{1a} comprises a linking moiety (L_{3a}) that connects H_{1a} to P_{1a} . In some embodiments, H_{1b} comprises a linking moiety (L_{3b}) that connects H_{1b} to P_{1b} . In some embodiments, H_{1c} comprises a linking moiety (L_{3c}) that connects H_{1c} to P_{1c} .

[0089] In some embodiments, L_3 , L_{3a} , L_{3b} , or L_{3c} is a peptide sequence having at least 5 to no more than 50 amino acids. In some embodiments, L_3 , L_{3a} , L_{3b} , or L_{3c} is a peptide sequence having at least 10 to no more than 30 amino acids. In some embodiments, L_3 , L_{3a} , L_{3b} , or L_{3c} is a peptide sequence having at least 10 amino acids. In some embodiments, L_3 , L_{3a} , L_{3b} , or L_{3c} is a peptide sequence having at least 18 amino acids. In some embodiments, L_3 , L_{3a} , L_{3b} , or L_{3c} is a peptide sequence having at least 26 amino acids.

[0090] In some embodiments, L_3 , L_{3a} , L_{3b} , or L_{3c} has a formula selected from the group consisting of $(G_2S)_n$, $(GS)_n$, $(GSGGS)_n$ (SEQ ID NO: 917), $(GGGS)_n$ (SEQ ID NO: 918), $(GGGGS)_n$ (SEQ ID NO: 919), and $(GSSGGS)_n$ (SEQ ID NO: 920), wherein n is an integer of at least 1. In some embodiments, L_3 , L_{3a} , L_{3b} , or L_{3c} has a formula selected from the group consisting of $(G_2S)_n$, $(GS)_n$, $(GSGGS)_n$ (SEQ ID NO: 917), $(GGGS)_n$ (SEQ ID NO: 918), $(GGGGS)_n$ (SEQ ID NO: 919), and $(GSSGGS)_n$ (SEQ ID NO: 920), wherein n is an integer of 1. In some embodiments, L_3 , L_{3a} , L_{3b} , or L_{3c} has a formula selected from the group consisting of $(G_2S)_n$ (SEQ ID NO: 922), $(GS)_n$ (SEQ ID NO: 938), $(GSGGS)_n$ (SEQ ID NO: 939), $(GGGS)_n$ (SEQ ID NO: 940), $(GGGGS)_n$ (SEQ ID NO: 941), and $(GSSGGS)_n$ (SEQ ID NO: 942), wherein n is an integer from 1 to 3.

[0091] In some embodiments, L_3 , L_{3a} , L_{3b} , or L_{3c} has a formula of $(G_2S)_n$, wherein n is an integer of least 1. In some embodiments, L_3 , L_{3a} , L_{3b} , or L_{3c} has a formula of $(GS)_n$, wherein n is an integer of least 1. In some embodiments, L_3 , L_{3a} , L_{3b} , or L_{3c} has a formula of $(GSGGS)_n$ (SEQ ID NO: 917), wherein n is an integer of least 1. In some embodiments, L_3 , L_{3a} , L_{3b} , or L_{3c} has a formula of $(GGGS)_n$ (SEQ ID NO: 918), wherein n is an integer of least 1. In some embodiments, L_3 , L_{3a} , L_{3b} , or L_{3c} has a formula of $(GGGGS)_n$ (SEQ ID NO: 919), wherein n is an integer of least 1. In some embodiments, L_3 , L_{3a} , L_{3b} , or L_{3c} has a formula of $(GSSGGS)_n$ (SEQ ID NO: 920), wherein n is an integer of least 1.

[0092] In some embodiments, L_3 or L_{3a} has a formula of $(G_2S)_n$, wherein n is an integer of 1. In some embodiments, L_3 , L_{3a} , L_{3b} , or L_{3c} has a formula of $(GS)_n$, wherein n is an integer of 1. In some embodiments, L_3 , L_{3a} , L_{3b} , or L_{3c} has a formula of $(GSGGS)_n$ (SEQ ID NO: 917), wherein n is an integer of 1. In some embodiments, L_3 , L_{3a} , L_{3b} , or L_{3c} has a formula of $(GGGS)_n$ (SEQ ID NO: 918), wherein n is an integer of 1. In some embodiments, L_3 , L_{3a} , L_{3b} , or L_{3c} has a formula of $(GGGGS)_n$ (SEQ ID NO: 919), wherein n is an integer of 1. In some embodiments, L_3 , L_{3a} , L_{3b} , or L_{3c} has a formula of $(GSSGGS)_n$ (SEQ ID NO: 920), wherein n is an integer of 1.

[0093] In some embodiments, L_3 , L_{3a} , L_{3b} , or L_{3c} has a formula of $(G_2S)_n$ (SEQ ID NO: 922), wherein n is an integer from 1 to 3. In some embodiments, L_3 , L_{3a} , L_{3b} , or L_{3c} has a formula of $(GS)_n$ (SEQ ID NO: 938), wherein n is an integer from 1 to 3. In some embodiments, L_3 , L_{3a} , L_{3b} , or L_{3c} has a formula of $(GSGGS)_n$ (SEQ ID NO: 939), wherein n is an integer from 1 to 3. In some embodiments, L_3 , L_{3a} , L_{3b} , or L_{3c} has a formula of $(GGGS)_n$ (SEQ ID NO: 940), wherein n is an integer from 1 to 3. In some embodiments, L_3 , L_{3a} ,

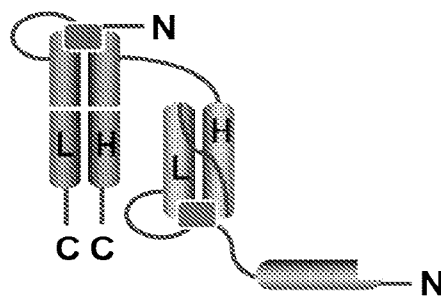
L_{3b}, or L_{3c} has a formula of (GGGGS)_n (SEQ ID NO: 941), wherein n is an integer from 1 to 3. In some embodiments, L₃, L_{3a}, L_{3b}, or L_{3c} has a formula of (GSSGGS)_n (SEQ ID NO: 942), wherein n is an integer from 1 to 3.

Configurations

[0094] In some embodiments, the Fab light chain polypeptide of A₂ is bound to a C-terminus of the single chain variable fragment (scFv) of A₁. In some embodiments, the Fab heavy chain polypeptide of A₂ is bound to a C-terminus of the single chain variable fragment (scFv) A₁. In some embodiments, the Fab light chain polypeptide of A₂ is bound to a N-terminus of the single chain variable fragment (scFv) of A₁. In some embodiments, the Fab heavy chain polypeptide of A₂ is bound to a N-terminus of the single chain variable fragment (scFv) A₁. In some embodiments, the Fab heavy chain polypeptide of A₂ is bound to the scFv heavy chain polypeptide of A₁. In some embodiments, the Fab light chain polypeptide of A₂ is bound to the scFv heavy chain polypeptide of A₁. In some embodiments, the Fab heavy chain polypeptide of A₂ is bound to the scFv light chain polypeptide of A₁. In some embodiments, the Fab light chain polypeptide of A₂ is bound to the scFv light chain polypeptide of A₁. In some embodiments, the Fab heavy chain polypeptide of A₂ is bound to the scFv heavy chain polypeptide of A₁ and L₂ is bound to the Fab light chain polypeptide of A₂.

In some embodiments, the Fab light chain polypeptide of A₂ is bound to the scFv heavy chain polypeptide of A₁ and L₂ is bound to the Fab heavy chain polypeptide of A₂. In some embodiments, the Fab heavy chain polypeptide of A₂ is bound to the scFv light chain polypeptide of A₁ and L₂ is bound to the Fab light chain polypeptide of A₂. In some embodiments, the Fab light chain polypeptide of A₂ is bound to the scFv light chain polypeptide of A₁ and L₂ is bound to the Fab heavy chain polypeptide of A₂.

[0095] Disclosed herein, in some embodiments, are polypeptides or polypeptide complexes comprising a structural arrangement according to Configuration 1:

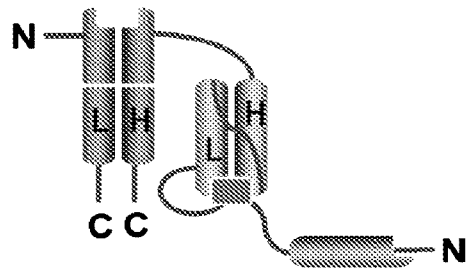


Configuration 1

wherein the polypeptide or polypeptide complex comprises a single chain variable fragment (scFv) comprising a light chain variable domain and a heavy chain variable domain, wherein the scFv is linked to a peptide (P₁) wherein the peptide comprises an amino acid sequence according to Peptide-1 (GSQCLGPEWEVCPY) SEQ ID NO: 1 or Peptide-2 (VYCGPEFDES VGCM) SEQ ID NO: 2, or the peptide comprises 1, 2, or 3, amino acid substitutions, additions, or deletions relative to the amino acid sequence of Peptide-1 or Peptide-2, wherein P₁ impairs binding of the scFv to CD3 and P₁ is linked to a N-terminus of the light chain variable domain of the scFv with a linking moiety (L₁) that is a substrate for a

tumor specific protease, and P_1 is further linked to a half-life extending molecule; and a Fab that binds to a tumor cell antigen, wherein the Fab comprises a Fab light chain polypeptide and a Fab heavy chain polypeptide, wherein the Fab heavy chain polypeptide is linked to a C terminus of the heavy chain variable domain of the scFv, and wherein the Fab is linked to P_2 and L_2 , wherein P_2 comprises a peptide that impairs binding of the Fab to the tumor cell antigen; and L_2 comprises a linking moiety that connects the Fab light chain polypeptide to P_2 and is a substrate for a tumor specific protease.

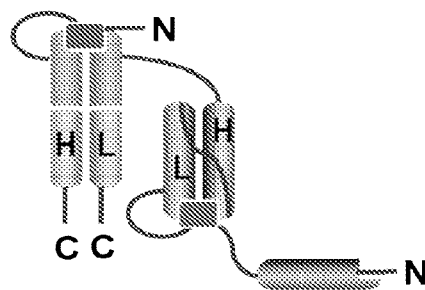
[0096] Disclosed herein, in some embodiments, are polypeptides or polypeptide complexes comprising a structural arrangement according to Configuration 2:



Configuration 2

wherein the polypeptide or polypeptide complex comprises a single chain variable fragment (scFv) comprising a light chain variable domain and a heavy chain variable domain, wherein the scFv is linked to a peptide wherein the peptide comprises an amino acid sequence according to Peptide-1 (GSQCLGPEWEVCPY) SEQ ID NO: 1 or Peptide-2 (VYCGPEFDES VGCM) SEQ ID NO: 2, or the peptide comprises 1, 2, or 3, amino acid substitutions, additions, or deletions relative to the amino acid sequence of Peptide-1 or Peptide-2, wherein the peptide impairs binding of the scFv to CD3 and the peptide is linked to the light chain variable domain of the scFv with a linking moiety that is a substrate for a tumor specific protease, and the peptide is further linked to a half-life extending molecule; and a Fab that binds to a tumor cell antigen, wherein the Fab comprises a Fab light chain polypeptide chain and a Fab heavy chain polypeptide chain, and wherein the Fab heavy chain polypeptide chain is linked to a C terminus of the heavy chain variable domain of the scFv.

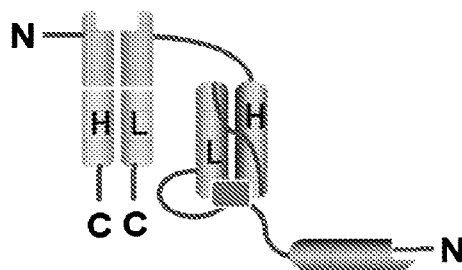
[0097] Disclosed herein, in some embodiments, are polypeptides or polypeptide complexes comprising a structural arrangement according to Configuration 3:



Configuration 3

wherein the polypeptide or polypeptide complex comprises a single chain variable fragment (scFv) comprising a light chain variable domain and a heavy chain variable domain, wherein the scFv is linked to a peptide (P₁) wherein the peptide comprises an amino acid sequence according to Peptide-1 (GSQCLGPEWEVCPY) SEQ ID NO: 1 or Peptide-2 (VYCGPEFDES VGCM) SEQ ID NO: 2, or the peptide comprises 1, 2, or 3, amino acid substitutions, additions, or deletions relative to the amino acid sequence of Peptide-1 or Peptide-2, wherein P₁ impairs binding of the scFv to CD3 and P₁ is linked to a N-terminus of the light chain variable domain of the scFv with a linking moiety that is a substrate for a tumor specific protease, and P₁ is further linked to a half-life extending molecule; and a Fab that binds to a tumor cell antigen, wherein the Fab comprises a Fab light chain polypeptide and a Fab heavy chain polypeptide, wherein the Fab light chain polypeptide is linked to a C terminus of the heavy chain variable domain of the scFv, and wherein the Fab is linked to P₂ and L₂, wherein P₂ comprises a peptide that impairs binding to the tumor cell antigen; and L₂ comprises a linking moiety that connects the Fab heavy chain polypeptide to P₂ and is a substrate for a tumor specific protease.

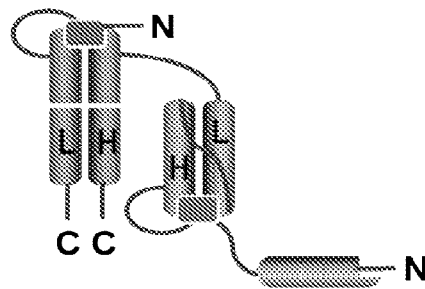
[0098] Disclosed herein, in some embodiments, are polypeptides or polypeptide complexes comprising a structural arrangement according to Configuration 4:



Configuration 4

wherein the polypeptide or polypeptide complex comprises a single chain variable fragment (scFv) comprising a light chain variable domain and a heavy chain variable domain, wherein the scFv is further linked to a peptide wherein the peptide comprises an amino acid sequence according to Peptide-1 (GSQCLGPEWEVCPY) SEQ ID NO: 1 or Peptide-2 (VYCGPEFDES VGCM) SEQ ID NO: 2, or the peptide comprises 1, 2, or 3, amino acid substitutions, additions, or deletions relative to the amino acid sequence of Peptide-1 or Peptide-2, wherein the peptide impairs binding of the scFv to CD3 and the peptide is linked to a N-terminus of the light chain variable domain of the scFv with a linking moiety that is a substrate for a tumor specific protease, and the peptide is further linked to a half-life extending molecule; and a Fab that binds to a tumor cell antigen, wherein the Fab comprises a Fab light chain polypeptide and a Fab heavy chain polypeptide, wherein the Fab light chain polypeptide is linked to a C terminus of the heavy chain variable domain of the scFv.

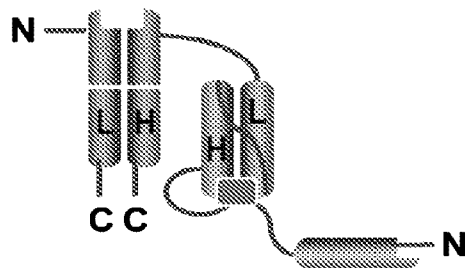
[0099] Disclosed herein, in some embodiments, are polypeptides or polypeptide complexes comprising a structural arrangement according to Configuration 5:



Configuration 5

wherein the polypeptide or polypeptide complex comprises a single chain variable fragment (scFv) comprising a light chain variable domain and a heavy chain variable domain, wherein the scFv is linked to a peptide (P₁) wherein the peptide comprises an amino acid sequence according to Peptide-1 (GSQCLGPEWEVCPY) SEQ ID NO: 1 or Peptide-2 (VYCGPEFDES VGCM) SEQ ID NO: 2, or the peptide comprises 1, 2, or 3, amino acid substitutions, additions, or deletions relative to the amino acid sequence of Peptide-1 or Peptide-2, wherein P₁ impairs binding of the scFv to CD3 and P₁ is linked to a N-terminus of the heavy chain variable domain of the scFv with a linking moiety (L₁) that is a substrate for a tumor specific protease, and P₁ is further linked to a half-life extending molecule; and a Fab that binds to a tumor cell antigen, wherein the Fab comprises a Fab light chain polypeptide and a Fab heavy chain polypeptide, wherein the Fab heavy chain polypeptide is linked to a C terminus of the light chain variable domain of the scFv, and wherein the Fab is linked to P₂ and L₂, wherein P₂ comprises a peptide that impairs binding of the Fab to the tumor cell antigen; and L₂ comprises a linking moiety that connects the Fab light chain polypeptide to P₂ and is a substrate for a tumor specific protease.

[0100] Disclosed herein, in some embodiments, are polypeptides or polypeptide complexes comprising a structural arrangement according to Configuration 6:

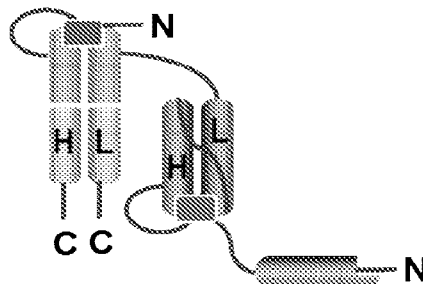


Configuration 6

wherein the polypeptide or polypeptide complex comprises a single chain variable fragment (scFv) comprising a light chain variable domain and a heavy chain variable domain, wherein the scFv is linked to a peptide wherein the peptide comprises an amino acid sequence according to Peptide-1 (GSQCLGPEWEVCPY) SEQ ID NO: 1 or Peptide-2 (VYCGPEFDES VGCM) SEQ ID NO: 2, or the peptide comprises 1, 2, or 3, amino acid substitutions, additions, or deletions relative to the amino acid sequence of Peptide-1 or Peptide-2, wherein the peptide impairs binding of the scFv to CD3 and the peptide is linked to the heavy chain variable domain of the scFv with a linking moiety that is a substrate for a tumor specific protease, and the peptide is further linked to a half-life extending molecule; and a Fab that binds to a

tumor cell antigen, wherein the Fab comprises a Fab light chain polypeptide chain and a Fab heavy chain polypeptide chain, and wherein the Fab heavy chain polypeptide chain is linked to a C terminus of the light chain variable domain of the scFv.

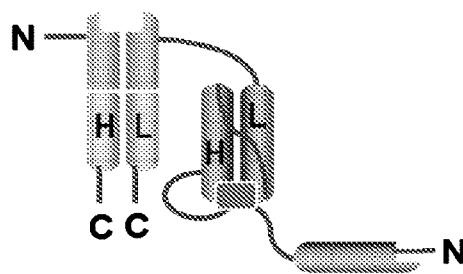
[0101] Disclosed herein, in some embodiments, are polypeptides or polypeptide complexes comprising a structural arrangement according to Configuration 7:



Configuration 7

wherein the polypeptide or polypeptide complex comprises a single chain variable fragment (scFv) comprising a light chain variable domain and a heavy chain variable domain, wherein the scFv is linked to a peptide (P₁) wherein the peptide comprises an amino acid sequence according to Peptide-1 (GSQCLGPEWEVCPY) SEQ ID NO: 1 or Peptide-2 (VYCGPEFDES VGCM) SEQ ID NO: 2, or the peptide comprises 1, 2, or 3, amino acid substitutions, additions, or deletions relative to the amino acid sequence of Peptide-1 or Peptide-2, wherein P₁ impairs binding of the scFv to CD3 and P₁ is linked to a N-terminus of the heavy chain variable domain of the scFv with a linking moiety (L₁) that is a substrate for a tumor specific protease, and P₁ is further linked to a half-life extending molecule; and a Fab that binds to a tumor cell antigen, wherein the Fab comprises a Fab light chain polypeptide and a Fab heavy chain polypeptide, wherein the Fab light chain polypeptide is linked to a C terminus of the light chain variable domain of the scFv, and wherein the Fab is linked to P₂ and L₂, wherein P₂ comprises a peptide that impairs binding of the Fab to the tumor cell antigen; and L₂ comprises a linking moiety that connects the Fab heavy chain polypeptide to P₂ and is a substrate for a tumor specific protease.

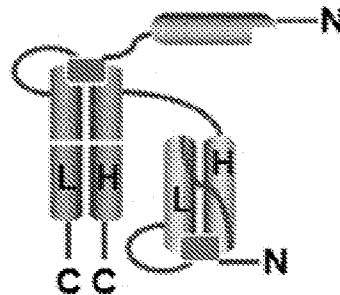
[0102] Disclosed herein, in some embodiments, are polypeptides or polypeptide complexes comprising a structural arrangement according to Configuration 8:



Configuration 8

wherein the polypeptide or polypeptide complex comprises a single chain variable fragment (scFv) comprising a light chain variable domain and a heavy chain variable domain, wherein the scFv is linked to a peptide wherein the peptide comprises an amino acid sequence according to Peptide-1 (GSQCLGPEWEVCPY) SEQ ID NO: 1 or Peptide-2 (VYCGPEFDES VGCM) SEQ ID NO: 2, or the peptide comprises 1, 2, or 3, amino acid substitutions, additions, or deletions relative to the amino acid sequence of Peptide-1 or Peptide-2, wherein the peptide impairs binding of the scFv to CD3 and the peptide is linked to a N-terminus of the heavy chain variable domain of the scFv with a linking moiety that is a substrate for a tumor specific protease, and the peptide is further linked to a half-life extending molecule; and a Fab that binds to a tumor cell antigen, wherein the Fab comprises a Fab light chain polypeptide and a Fab heavy chain polypeptide, wherein the Fab light chain polypeptide is linked to a C terminus of the light chain variable domain of the scFv.

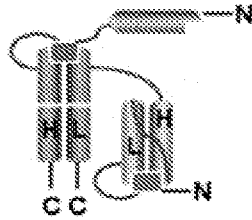
[0103] Disclosed herein, in some embodiments, are polypeptides or polypeptide complexes comprising a structural arrangement according to Configuration 9:



Configuration 9

wherein the polypeptide or polypeptide complex comprises a Fab that binds to a tumor cell antigen, the Fab comprising a Fab light chain polypeptide and a Fab heavy chain polypeptide, wherein the Fab is linked to a peptide (P_1) that impairs binding of the Fab to the tumor cell antigen and P_1 is linked to a N terminus of the Fab light chain polypeptide with a linking moiety (L_1) that is a substrate for a tumor specific protease, and the P_1 is further linked to a half-life extending molecule; and a single chain variable fragment (scFv) that binds to an effector cell antigen, the scFv comprising a light chain variable domain and a heavy chain variable domain, wherein the heavy chain variable domain of the scFv is linked to an N terminus of the Fab heavy chain polypeptide, wherein the scFv is linked to P_2 and L_2 , wherein P_2 comprises a peptide according to an amino acid sequence according to Peptide-1 (GSQCLGPEWEVCPY) SEQ ID NO: 1 or Peptide-2 (VYCGPEFDES VGCM) SEQ ID NO: 2, or P_2 comprises 1, 2, or 3, amino acid substitutions, additions, or deletions relative to the amino acid sequence of Peptide-1 or Peptide-2, wherein P_2 impairs binding of the scFv to CD3, and L_2 comprises a linking moiety that connects the light chain variable domain of the scFv to P_2 and is a substrate for a tumor specific protease.

[0104] Disclosed herein, in some embodiments, are polypeptides or polypeptide complexes comprising a structural arrangement according to Configuration 10:

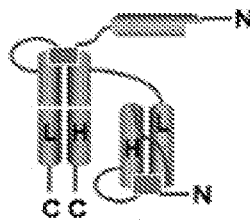


Configuration 10

wherein the polypeptide or polypeptide complex comprises a Fab that binds to a tumor cell antigen, the Fab comprising a Fab light chain polypeptide and a Fab heavy chain polypeptide, wherein the Fab is linked to a peptide (P_1) that impairs binding of the Fab to the tumor cell antigen and P_1 is linked to a N terminus of the Fab heavy chain polypeptide with a linking moiety (L_1) that is a substrate for a tumor specific protease, and P_1

is further linked to a half-life extending molecule; and a single chain variable fragment (scFv) that binds to an effector cell antigen, the scFv comprising a light chain variable domain and a heavy chain variable domain, wherein the heavy chain variable domain of the scFv is linked to an N terminus of the Fab light chain polypeptide, wherein the scFv further is linked to P_2 and L_2 , wherein P_2 comprises a peptide according to an amino acid sequence according to Peptide-1 (GSQCLGPEWEVCPY) SEQ ID NO: 1 or Peptide-2 (VYCGPEFDES VGCM) SEQ ID NO: 2, or P_2 comprises 1, 2, or 3, amino acid substitutions, additions, or deletions relative to the amino acid sequence of Peptide-1 or Peptide-2, wherein P_2 impairs binding of the scFv to CD3, and L_2 comprises a linking moiety that connects the light chain variable domain of the scFv to P_2 and is a substrate for a tumor specific protease.

[0105] Disclosed herein, in some embodiments, are polypeptides or polypeptide complexes comprising a structural arrangement according to Configuration 11:



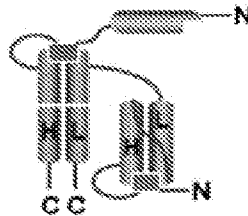
Configuration 11

wherein the polypeptide or polypeptide complex comprises a Fab that binds to a tumor cell antigen, the Fab comprising a Fab light chain polypeptide and a Fab heavy chain polypeptide, wherein the Fab is linked to a peptide (P_1) that impairs binding of the Fab to the tumor cell antigen and P_1 is linked to a N terminus of the Fab light chain polypeptide with a linking moiety (L_1) that is a substrate for a tumor specific protease, and P_1

is further linked to a half-life extending molecule; and a single chain variable fragment (scFv) that binds to an effector cell antigen, the scFv comprising a light chain variable domain and a heavy chain variable domain, wherein the light chain variable domain of the scFv is linked to an N terminus of the Fab heavy

chain polypeptide, wherein the scFv is linked to P₂ and L₂, wherein P₂ comprises a peptide according to an amino acid sequence according to Peptide-1 (GSQCLGPEWEVCPY) SEQ ID NO: 1 or Peptide-2 (VYCGPEFDES VGCM) SEQ ID NO: 2, or P₂ comprises 1, 2, or 3, amino acid substitutions, additions, or deletions relative to the amino acid sequence of Peptide-1 or Peptide-2, wherein P₂ impairs binding of the scFv to CD3, and L₂ comprises a linking moiety that connects the heavy chain variable domain of the scFv to P₂ and is a substrate for a tumor specific protease.

[0106] Disclosed herein, in some embodiments, are polypeptides or polypeptide complexes comprising a structural arrangement according to Configuration 12:



Configuration 12

wherein the polypeptide or polypeptide complex comprises a Fab that binds to a tumor cell antigen, the Fab comprising a Fab light chain polypeptide and a Fab heavy chain polypeptide, wherein the Fab is linked to a (P₁) that impairs binding of the Fab to the tumor cell antigen and P₁ is linked to a N terminus of the Fab heavy chain polypeptide with a linking moiety (L₁) that is a substrate for a tumor specific protease, and P₁ is further linked to a half-life extending molecule; and a single chain variable fragment (scFv) that binds to an effector cell antigen, the scFv comprising a light chain variable domain and a heavy chain variable domain, wherein the light chain variable domain of the scFv is linked to an N terminus of the Fab light chain polypeptide, wherein the scFv is linked to P₂ and L₂, wherein P₂ comprises a peptide according to an amino acid sequence according to Peptide-1 (GSQCLGPEWEVCPY) SEQ ID NO: 1 or Peptide-2 (VYCGPEFDES VGCM) SEQ ID NO: 2, or P₂ comprises 1, 2, or 3, amino acid substitutions, additions, or deletions relative to the amino acid sequence of Peptide-1 or Peptide-2, wherein P₂ impairs binding of the scFv to CD3, and L₂ comprises a linking moiety that connects the heavy chain variable domain of the scFv to P₂ and is a substrate for a tumor specific protease.

Polypeptides or polypeptide complexes, in some embodiments, comprise a sequence set forth in **Table 5**. In some embodiments, the sequence comprises at least or about 70%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to any one of SEQ ID NOs: 13-18.

Table 5. Exemplary amino acid sequences of isolated polypeptides or polypeptide complexes (PC) comprising Peptide-1 or Peptide-2

Construct Description	Amino Acid Sequence (N to C)	SEQ ID NO:
PC-1 – light chain	QGQSGQLSCEGWAMNREQCRAGSSGGSGGS GGSGISSGLLSGRSDNHGSSGTDILLTQSPVILS VSPGERVSFSCRAS OSIGT NIHWYQQRTNGSP RLLIK YASE SISGIPSRFSGSGSGTDFTLINSVE SEDIADYY CQONNNWPTT FGAGTKLELKRT	13

	VAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC	
PC-1 – heavy chain	EVQLVESGGGLVQPGNSLRSLCAAS <u>SGFTFSK</u> <u>FGMSWVRQAPGKGLEWVSSI</u> <u>SGSGRDTLYA</u> DSVKGRFTISRDNAKTTLYLQMNSLRPEDTAVYYC <u>TIGGSLSV</u> SSQGTLLTVSSGGGGSGGGSGGGSQCLGPEWEVCPYGGGGSGGGSGGISSGLLSGRSDNHGGGSEVQLVESGGGLVQPGGSLKLSCAAS <u>SGFTFNKYA</u> MNWVRQAPGKLEWVAR <u>IRSKYNNYATYY</u> ADSVKDRFTISRDDSNTAYLQMNNLKTEDTAVYYC <u>VRHGNFGN</u> <u>SYISYWAY</u> WGQGTLLTVSSGGGGSGGGSGSGGGSQTVVTQEPSLTVSPGGTVTLTCGSST <u>TGA</u> <u>VTSGNY</u> PNWVQQKPGQAPRGLIG <u>GTK</u> FLAPGTPARFSGSLLGGKAALTLSGVQPEDEAEYY <u>CYLWYSNRWV</u> FGGGTKLTVLGGGGSQVQLKQSGPGLVQPSQSLITCTVSG <u>FSLTNYGVH</u> WVRQSPGKGLEWLGV <u>IWSGGNT</u> DYNTPTFSRLSINKDNSKSQVFFKMNSLQSQDTAIYYC <u>CAR</u> <u>ALTYDYEFAY</u> WGQGTLLTVSAASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSC	14
PC-2 – light chain	QQQSGQLSCEGWAMNREQCRAGSSGGSGGSGSGGISSGLLSGRSDNHGSSGTDILLTQSPVILSVSPGERVSFSCRAS <u>OSIGTNI</u> HWYQQRTNGSPRLLIK <u>YASE</u> SISGIPSRFSGSGSGTDFTLSINSVESIDIADYYC <u>QONNNWPTT</u> FGAGTKLELKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC	15
PC-2 – heavy chain	EVQLVESGGGLVQPGNSLRSLCAAS <u>SGFTFSK</u> <u>FGMSWVRQAPGKGLEWVSSI</u> <u>SGSGRDTLYA</u> DSVKGRFTISRDNAKTTLYLQMNSLRPEDTAVYYC <u>TIGGSLSV</u> SSQGTLLTVSSGGGGSGGGSGGGSQCLGPEFDES VGCMGGGGSGGGSGGISSGLLSGRSDNHGGGSEVQLVESGGGLVQPGGSLKLSCAAS <u>SGFTFNKYA</u> MNWVRQAPGKLEWVAR <u>IRSKYNNYATYY</u> ADSVKDRFTISRDDSNTAYLQMNNLKTEDTAVYYC <u>VRHGNFGN</u> <u>SYISYWAY</u> WGQGTLLTVSSGGGGSGGGSGSGGGSQTVVTQEPSLTVSPGGTVTLTCGSST <u>TGA</u> <u>VTSGNY</u> PNWVQQKPGQAPRGLIG <u>GTK</u> FLAPGTPARFSGSLLGGKAALTLSGVQPEDEAEYY <u>CYLWYSNRWV</u> FGGGTKLTVLGGGGSQVQLKQSGPGLVQPSQSLITCTVSG <u>FSLTNYGVH</u> WVRQSPGKGLEWLGV <u>IWSGGNT</u> DYNTPTFSRLSINKDNSKSQVFFKMNSLQSQDTAIYYC <u>CAR</u> <u>ALTYDYEFAY</u> WGQGTLLTVSAASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSC	16
PC-3 – light chain	QILLTQSPVILSVSPGERVSFSCRAS <u>OSIGTNI</u> HWYQQRTNGSPRLLIK <u>YASE</u> SISGIPSRFSGSGS	17

	GTDFTLSINSVESEDIADYYC <u>QONNNWPTTF</u> GAGTKLELKRTVAAPSVFIFPPSDEQLKSGTA SVVCLNNFYPREAKVQWKVDNALQSGNSQ ESVTEQDSKDYSLSTLTLSKADYEKHKVY ACEVTHQGLSPVTKSFNRGEC	
PC-3 – heavy chain	EVQLVESGGGLVQPGGSLKLSCAAS <u>SGFTFNK</u> <u>YAMN</u> WVRQAPGKGLEWVAR <u>IRSKYNNYAT</u> YYADSVKDRFTISRDDSKNTAYLQMNNLKTE DTAVYYC <u>VRHGNFGNSYISYWAY</u> WGQGLT VTVSSGGGGSGGGGSGGGGSQTVVTQEPSLT VSPGGTVLTCGSS <u>TGAVTSGNYP</u> NWVQQKP GQAPRGLIG <u>GTK</u> FLAPGTPARFSGSLLGGKAA LTLGSGVQPEDEAEYYC <u>VLWYSNRWV</u> FGGGT KLTVLGGGGSQVQLKQSGPGLVQPSQSLITC TVS <u>GFSLTNYG</u> VHWVVRQSPGKGLEWLGVIW <u>SGGNT</u> DYNTPFTSRLSINKDNSKSQVFFKMNS LQSDTAIYYC <u>CARALTYDYEFAY</u> WGQGLT VTVSAASTKGPSVFPLAPSSKSTSGGTAALGC LVKDYFPEPVTVSWNSGALTSGVHTFPAVLQ SSGLYSLSSVTVPSSSLGTQTYICNVNHKPSN TKVDKKVEPKSC	18

Polynucleotides Encoding Polypeptides or Polypeptide Complexes

[0107] Disclosed herein, in some embodiments, are isolated recombinant nucleic acid molecules encoding polypeptides or polypeptide complexes as disclosed herein. In some embodiments, the polypeptides or polypeptide complexes comprise an antibody or an antibody fragment.

[0108] Disclosed herein, in some embodiments, are isolated recombinant nucleic acid molecules encoding polypeptide or polypeptide complex comprising a peptide that impairs binding of an anti-CD3 binding domain to CD3 wherein the peptide comprises an amino acid sequence according to Peptide-1 (GSQCLGPEWEVCPY) SEQ ID NO: 1 or Peptide-2 (VYCGPEFDES VGCM) SEQ ID NO: 2, or the peptide comprises 1, 2, or 3, amino acid substitutions, additions, or deletions relative to the amino acid sequence of Peptide-1 or Peptide-2. Disclosed herein, in some embodiments, are isolated recombinant nucleic acid molecules encoding polypeptide or polypeptide complex comprising a peptide that impairs binding of an anti-CD3 binding domain to CD3 wherein the peptide comprises an amino acid sequence according to Peptide-1 (GSQCLGPEWEVCPY) SEQ ID NO: 1 or Peptide-2 (VYCGPEFDES VGCM) SEQ ID NO: 2.

[0109] Disclosed herein, in some embodiments, are isolated recombinant nucleic acid molecules encoding polypeptides or polypeptide complexes comprising a peptide that impairs binding of an anti-CD3 binding domain to CD3 wherein the peptide comprises an amino acid sequence selected from SEQ ID NOs: 1-2, 19-55 or the peptide comprises 1, 2, or 3, amino acid substitutions, additions, or deletions relative to an amino acid sequence selected from SEQ ID NOs: 1-2, 19-55. In some embodiments, the peptide comprises an amino acid sequence selected from SEQ ID NOs: 1-2, and 19-55. In some embodiments, the peptide comprises the amino acid sequence of SEQ ID NO: 1. In some embodiments, the peptide comprises the amino acid sequence of SEQ ID NO: 2. In some embodiments, the peptide comprises the amino acid sequence of SEQ ID NO: 54.

[0110] Disclosed herein, in some embodiments, are isolated recombinant nucleic acid molecules encoding polypeptides or polypeptide complexes comprising an anti-CD3 binding domain that is linked to a peptide that impairs binding of the anti-CD3 binding domain to CD3 wherein the peptide comprises an amino acid sequence according to Z_1 - Z_2 -C- Z_4 -P- Z_6 - Z_7 - Z_8 - Z_9 - Z_{10} - Z_{11} - Z_{12} -C- Z_{14} and Z_1 is selected from D, Y, F, I, N, V, H, L, A, T, S, and P; Z_2 is selected from D, Y, L, F, I, N, A, V, H, T, and S; Z_4 is selected from G and W; Z_6 is selected from E, D, V, and P; Z_7 is selected from W, L, F, V, G, M, I, and Y; Z_8 is selected from E, D, P, and Q; Z_9 is selected from E, D, Y, V, F, W, P, L, and Q; Z_{10} is selected from S, D, Y, T, I, F, V, N, A, P, L, and H; Z_{11} is selected from I, Y, F, V, L, T, N, S, D, A, and H; Z_{12} is selected from F, D, Y, L, I, V, A, N, T, P, S, and H; and Z_{14} is selected from D, Y, N, F, I, P, V, A, T, H, L and S. In some embodiments, Z_1 is selected from D, Y, F, I, and N; Z_2 is selected from D, Y, L, F, I, and N; Z_4 is selected from G and W; Z_6 is selected from E and D; Z_7 is selected from W, L, F, and V; Z_8 is selected from E and D; Z_9 is selected from E, D, Y, and V; Z_{10} is selected from S, D, Y, T, and I; Z_{11} is selected from I, Y, F, V, L, and T; Z_{12} is selected from F, D, Y, L, I, V, A, and N; and Z_{14} is selected from D, Y, N, F, I, and P. In some embodiments, Z_1 is selected from D, Y, and F; Z_2 is selected from D, Y, L, and F; Z_4 is selected from G and W; Z_6 is selected from E and D; Z_7 is selected from W, L, and F; Z_8 is selected from E and D; Z_9 is selected from E and D; Z_{10} is selected from S, D, and Y; Z_{11} is selected from I, Y, and F; Z_{12} is selected from F, D, Y, and L; and Z_{14} is selected from D, Y, and N. In some embodiments, the peptide comprises an amino acid sequence of **Table 17**. In some embodiments, the peptide comprises an amino acid sequence according to any one of SEQ ID NOs: 44-55. In some embodiments, the peptide comprises the amino acid sequence of SEQ ID NO: 2. In some embodiments, the peptide comprises the amino acid sequence of SEQ ID NO: 54.

[0111] Disclosed herein, in some embodiments, are isolated recombinant nucleic acid molecules encoding polypeptides or polypeptide complexes comprising an anti-CD3 binding domain that is linked to a peptide that impairs binding of the anti-CD3 binding domain to CD3 wherein the peptide comprises an amino acid sequence according to U_1 - U_2 -C- U_4 -P- U_6 - U_7 - U_8 - U_9 - U_{10} - U_{11} - U_{12} -C- U_{14} and U_1 is selected from D, Y, F, I, N, V, H, L, A, T, S, and P; U_2 is selected from D, Y, L, F, I, N, A, V, H, T, and S; U_4 is selected from G and W; U_6 is selected from E, D, V, and P; U_7 is selected from W, L, F, V, G, M, I, and Y; U_8 is selected from E, D, P, and Q; U_9 is selected from E, D, Y, V, F, W, P, L, and Q; U_{10} is selected from S, D, Y, T, I, F, V, N, A, P, L, and H; U_{11} is selected from I, Y, F, V, L, T, N, S, D, A, and H; U_{12} is selected from F, D, Y, L, I, V, A, N, T, P, S, G, and H; and U_{14} is selected from D, Y, N, F, I, P, V, A, T, H, L, M, and S. In some embodiments, U_1 is selected from D, Y, F, I, V, and N; U_2 is selected from D, Y, L, F, I, and N; U_4 is selected from G and W; U_6 is selected from E and D; U_7 is selected from W, L, F, G, and V; U_8 is selected from E and D; U_9 is selected from E, D, Y, and V; U_{10} is selected from S, D, Y, T, and I; U_{11} is selected from I, Y, F, V, L, and T; U_{12} is selected from F, D, Y, L, I, V, A, G, and N; and U_{14} is selected from D, Y, N, F, I, M, and P. In some embodiments, U_1 is selected from D, Y, V, and F; U_2 is selected from D, Y, L, and F; U_4 is selected from G and W; U_6 is selected from E and D; U_7 is selected from W, L, G, and F; U_8 is selected from E and D; U_9 is selected from E and D; U_{10} is selected from S, D, T, and Y; U_{11} is selected from I, Y, V, L, and F; U_{12} is selected from F, D, Y, G, A, and L; and U_{14} is selected from D, Y, M, and N. In some

embodiments, the peptide comprises an amino acid sequence of **Table 17**. In some embodiments, the peptide comprises an amino acid sequence according to any one of SEQ ID NOs: 44-55. In some embodiments, the peptide comprises the amino acid sequence of SEQ ID NO: 2. In some embodiments, the peptide comprises the amino acid sequence of SEQ ID NO: 54.

[0112] In some embodiments, the peptide is connected to the anti-CD3 binding domain in a configuration according to: $A_1-L_1-P_1$ (Formula I) wherein A_1 comprises the anti-CD3 binding domain; L_1 comprises a cleavable linker that is a substrate for a tumor specific protease; P_1 comprises the peptide that impairs binding of the anti-CD3 binding domain to CD3. In some embodiments, the peptide is connected to the anti-CD3 binding domain in a configuration according to: A_1-L_1-P (Formula I) wherein A_1 is the anti-CD3 binding domain; L_1 is a cleavable linker that is a substrate for a tumor specific protease; P_1 is the peptide that impairs binding of the anti-CD3 binding domain to CD3. In some embodiments, the peptide is connected to the anti-CD3 binding domain in a configuration comprising Formula I: $A_1-L_1-P_1$ (Formula I) wherein A_1 comprises the anti-CD3 binding domain; L_1 comprises a cleavable linker that is a substrate for a tumor specific protease; P_1 comprises the peptide that impairs binding of the anti-CD3 binding domain to CD3. In some embodiments, the peptide is connected to the anti-CD3 binding domain in a configuration comprising Formula I: $A_1-L_1-P_1$ (Formula I) wherein A_1 is the anti-CD3 binding domain; L_1 is a cleavable linker that is a substrate for a tumor specific protease; P_1 is the peptide that impairs binding of the anti-CD3 binding domain to CD3. In some embodiments, A_1 is further linked to a tumor antigen binding domain (A_2). In some embodiments, the polypeptide or polypeptide complex is according to Formula Ia: $P_2-L_2-A_2-A_1-L_1-P_1$ (Formula Ia) wherein P_2 comprises a peptide that impairs binding of A_2 to a tumor antigen; and L_2 comprises a second cleavable linker that connects A_2 to P_2 and is a substrate for a tumor specific protease.

Pharmaceutical Compositions

[0113] Disclosed herein, in some embodiments, are pharmaceutical compositions comprising: (a) the polypeptides or polypeptide complexes as disclosed herein; and (b) a pharmaceutically acceptable excipient.

[0114] In some embodiments, the polypeptide or polypeptide complex further comprises a detectable label, a therapeutic agent, or a pharmacokinetic modifying moiety. In some embodiments, the detectable label comprises a fluorescent label, a radiolabel, an enzyme, a nucleic acid probe, or a contrast agent.

[0115] For administration to a subject, the polypeptide or polypeptide complex as disclosed herein, may be provided in a pharmaceutical composition together with one or more pharmaceutically acceptable carriers or excipients. The term "pharmaceutically acceptable carrier" includes, but is not limited to, any carrier that does not interfere with the effectiveness of the biological activity of the ingredients and that is not toxic to the patient to whom it is administered. Examples of suitable pharmaceutical carriers are well known in the art and include phosphate buffered saline solutions, water, emulsions, such as oil/water emulsions, various types of wetting agents, sterile solutions etc. Such carriers can be formulated by conventional methods and can be administered to the subject at a suitable dose. Preferably, the compositions are sterile. These compositions may also contain adjuvants such as preservative, emulsifying agents and dispersing agents.

Prevention of the action of microorganisms may be ensured by the inclusion of various antibacterial and antifungal agents.

[0116] The pharmaceutical composition may be in any suitable form, (depending upon the desired method of administration). It may be provided in unit dosage form, may be provided in a sealed container and may be provided as part of a kit. Such a kit may include instructions for use. It may include a plurality of said unit dosage forms.

[0117] The pharmaceutical composition may be adapted for administration by any appropriate route, including a parenteral (e.g., subcutaneous, intramuscular, or intravenous) route. Such compositions may be prepared by any method known in the art of pharmacy, for example by mixing the active ingredient with the carrier(s) or excipient(s) under sterile conditions.

[0118] Dosages of the substances of the present disclosure can vary between wide limits, depending upon the disease or disorder to be treated, the age and condition of the individual to be treated, etc. and a physician will ultimately determine appropriate dosages to be used.

[0119] In some embodiments, polypeptides described herein (e.g., antibodies and its binding fragments) are produced using any method known in the art to be useful for the synthesis of polypeptides (e.g., antibodies), in particular, by chemical synthesis or by recombinant expression, and are preferably produced by recombinant expression techniques.

[0120] In some instances, an antibody or its binding fragment thereof is expressed recombinantly, and the nucleic acid encoding the antibody or its binding fragment is assembled from chemically synthesized oligonucleotides (e.g., as described in Kutmeier et al., 1994, *BioTechniques* 17:242), which involves the synthesis of overlapping oligonucleotides containing portions of the sequence encoding the antibody, annealing and ligation of those oligonucleotides, and then amplification of the ligated oligonucleotides by PCR.

[0121] Alternatively, a nucleic acid molecule encoding an antibody is optionally generated from a suitable source (e.g., an antibody cDNA library, or cDNA library generated from any tissue or cells expressing the immunoglobulin) by PCR amplification using synthetic primers hybridizable to the 3' and 5' ends of the sequence or by cloning using an oligonucleotide probe specific for the particular gene sequence.

[0122] In some instances, an antibody or its binding is optionally generated by immunizing an animal, such as a mouse, to generate polyclonal antibodies or, more preferably, by generating monoclonal antibodies, e.g., as described by Kohler and Milstein (1975, *Nature* 256:495-497) or, as described by Kozbor et al. (1983, *Immunology Today* 4:72) or Cole et al. (1985 in *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, Inc., pp. 77-96). Alternatively, a clone encoding at least the Fab portion of the antibody is optionally obtained by screening Fab expression libraries (e.g., as described in Huse et al., 1989, *Science* 246:1275-1281) for clones of Fab fragments that bind the specific antigen or by screening antibody libraries (See, e.g., Clackson et al., 1991, *Nature* 352:624; Hane et al., 1997 *Proc. Natl. Acad. Sci. USA* 94:4937).

[0123] In some embodiments, techniques developed for the production of "chimeric antibodies" (Morrison et al., 1984, *Proc. Natl. Acad. Sci.* 81:851-855; Neuberger et al., 1984, *Nature* 312:604-608; Takeda et al., 1985, *Nature* 314:452-454) by splicing genes from a mouse antibody molecule of appropriate antigen specificity together with

genes from a human antibody molecule of appropriate biological activity are used. A chimeric antibody is a molecule in which different portions are derived from different animal species, such as those having a variable region derived from a murine monoclonal antibody and a human immunoglobulin constant region.

[0124] In some embodiments, techniques described for the production of single chain antibodies (U.S. Pat. No. 4,694,778; Bird, 1988, *Science* 242:423-42; Huston et al., 1988, *Proc. Natl. Acad. Sci. USA* 85:5879-5883; and Ward et al., 1989, *Nature* 334:544-54) are adapted to produce single chain antibodies. Single chain antibodies are formed by linking the heavy and light chain fragments of the Fv region via an amino acid bridge, resulting in a single chain polypeptide. Techniques for the assembly of functional Fv fragments in *E. coli* are also optionally used (Skerra et al., 1988, *Science* 242:1038-1041).

[0125] In some embodiments, an expression vector comprising the nucleotide sequence of an antibody or the nucleotide sequence of an antibody is transferred to a host cell by conventional techniques (e.g., electroporation, liposomal transfection, and calcium phosphate precipitation), and the transfected cells are then cultured by conventional techniques to produce the antibody. In specific embodiments, the expression of the antibody is regulated by a constitutive, an inducible or a tissue, specific promoter.

[0126] In some embodiments, a variety of host-expression vector systems is utilized to express an antibody, or its binding fragment described herein. Such host-expression systems represent vehicles by which the coding sequences of the antibody is produced and subsequently purified, but also represent cells that are, when transformed or transfected with the appropriate nucleotide coding sequences, express an antibody or its binding fragment in situ. These include, but are not limited to, microorganisms such as bacteria (e.g., *E. coli* and *B. subtilis*) transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vectors containing an antibody or its binding fragment coding sequences; yeast (e.g., *Saccharomyces Pichia*) transformed with recombinant yeast expression vectors containing an antibody or its binding fragment coding sequences; insect cell systems infected with recombinant virus expression vectors (e.g., baculovirus) containing an antibody or its binding fragment coding sequences; plant cell systems infected with recombinant virus expression vectors (e.g., cauliflower mosaic virus (CaMV) and tobacco mosaic virus (TMV)) or transformed with recombinant plasmid expression vectors (e.g., Ti plasmid) containing an antibody or its binding fragment coding sequences; or mammalian cell systems (e.g., COS, CHO, BH, 293, 293T, 3T3 cells) harboring recombinant expression constructs containing promoters derived from the genome of mammalian cells (e.g., metallothionein promoter) or from mammalian viruses (e.g. the adenovirus late promoter; the vaccinia virus 7.5K promoter).

[0127] For long-term, high-yield production of recombinant proteins, stable expression is preferred. In some instances, cell lines that stably express an antibody are optionally engineered. Rather than using expression vectors that contain viral origins of replication, host cells are transformed with DNA controlled by appropriate expression control elements (e.g., promoter, enhancer, sequences, transcription terminators, polyadenylation sites, etc.), and a selectable marker. Following the introduction of the foreign DNA, engineered cells are then allowed to grow for 1-2 days in an enriched media, and then are switched to a selective media. The selectable marker in the recombinant plasmid confers resistance to the selection and allows cells to stably integrate the plasmid into their chromosomes

and grow to form foci that in turn are cloned and expanded into cell lines. This method can advantageously be used to engineer cell lines which express the antibody or its binding fragments.

[0128] In some instances, a number of selection systems are used, including but not limited to the herpes simplex virus thymidine kinase (Wigler et al., 1977, *Cell* 11:223), hypoxanthine-guanine phosphoribosyltransferase (Szybalska & Szybalski, 1972, *Proc. Natl. Acad. Sci. USA* 48:202), and adenine phosphoribosyltransferase (Lowy et al., 1980, *Cell* 22:817) genes are employed in tk⁻, hgp⁺ or apt⁻ cells, respectively. Also, antimetabolite resistance are used as the basis of selection for the following genes: dhfr, which confers resistance to methotrexate (Wigler et al., 1980, *Proc. Natl. Acad. Sci. USA* 77:357; O'Hare et al., 1981, *Proc. Natl. Acad. Sci. USA* 78:1527); gpt, which confers resistance to mycophenolic acid (Mulligan & Berg, 1981, *Proc. Natl. Acad. Sci. USA* 78:2072); neo, which confers resistance to the aminoglycoside G-418 (*Clinical Pharmacy* 12:488-505; Wu and Wu, 1991, *Biotherapy* 3:87-95; Tolstoshev, 1993, *Ann. Rev. Pharmacol. Toxicol.* 32:573-596; Mulligan, 1993, *Science* 260:926-932; and Morgan and Anderson, 1993, *Ann. Rev. Biochem.* 62:191-217; May 1993, *TIB TECH* 11(5):155-215) and hyg⁺, which confers resistance to hygromycin (Santerre et al., 1984, *Gene* 30:147). Methods commonly known in the art of recombinant DNA technology which can be used are described in Ausubel et al. (eds., 1993, *Current Protocols in Molecular Biology*, John Wiley & Sons, NY; Kriegler, 1990, *Gene Transfer and Expression, A Laboratory Manual*, Stockton Press, NY; and in Chapters 12 and 13, Dracopoli et al. (eds), 1994, *Current Protocols in Human Genetics*, John Wiley & Sons, NY.; Colberre-Garapin et al., 1981, *J. Mol. Biol.* 150:1).

[0129] In some instances, the expression levels of an antibody are increased by vector amplification (for a review, see Bebbington and Hentschel, the use of vectors based on gene amplification for the expression of cloned genes in mammalian cells in DNA cloning, Vol. 3. (Academic Press, New York, 1987)). When a marker in the vector system expressing an antibody is amplifiable, an increase in the level of inhibitor present in culture of host cell will increase the number of copies of the marker gene. Since the amplified region is associated with the nucleotide sequence of the antibody, production of the antibody will also increase (Crouse et al., 1983, *Mol. Cell Biol.* 3:257).

[0130] In some instances, any method known in the art for purification of an antibody is used, for example, by chromatography (e.g., ion exchange, affinity, particularly by affinity for the specific antigen after Protein A, and sizing column chromatography), centrifugation, differential solubility, or by any other standard technique for the purification of proteins.

Expression Vectors

[0131] In some embodiments, vectors include any suitable vectors derived from either a eukaryotic or prokaryotic sources. In some cases, vectors are obtained from bacteria (e.g. *E. coli*), insects, yeast (e.g. *Pichia pastoris*), algae, or mammalian sources. Exemplary bacterial vectors include pACYC177, pASK75, pBAD vector series, pBADM vector series, pET vector series, pETM vector series, pGEX vector series, pHAT, pHAT2, pMal-c2, pMal-p2, pQE vector series, pRSET A, pRSET B, pRSET C, pTrcHis2 series, pZA31-Luc, pZE21-MCS-1, pFLAG ATS, pFLAG CTS, pFLAG MAC, pFLAG Shift-12c, pTAC-MAT-1, pFLAG CTC, or pTAC-MAT-2.

[0132] Exemplary insect vectors include pFastBac1, pFastBac DUAL, pFastBac ET, pFastBac HTa, pFastBac HTb, pFastBac HTc, pFastBac M30a, pFastBac M30b, pFastBac M30c, pVL1392, pVL1393, pVL1393 M10,

pVL1393 M11, pVL1393 M12, FLAG vectors such as pPolh-FLAG1 or pPolh-MAT 2, or MAT vectors such as pPolh-MAT1, or pPolh-MAT2.

[0133] In some cases, yeast vectors include Gateway® pDEST™ 14 vector, Gateway® pDEST™ 15 vector, Gateway® pDEST™ 17 vector, Gateway® pDEST™ 24 vector, Gateway® pYES-DEST52 vector, pBAD-DEST49 Gateway® destination vector, pAO815 Pichia vector, pFLD1 Pichi pastoris vector, pGAPZA,B, & C Pichia pastoris vector, pPIC3.5K Pichia vector, pPIC6 A, B, & C Pichia vector, pPIC9K Pichia vector, pTEF1/Zeo, pYES2 yeast vector, pYES2/CT yeast vector, pYES2/NT A, B, & C yeast vector, or pYES3/CT yeast vector.

[0134] Exemplary algae vectors include pChlamy-4 vector or MCS vector.

[0135] Examples of mammalian vectors include transient expression vectors or stable expression vectors. Mammalian transient expression vectors may include pRK5, p3xFLAG-CMV 8, pFLAG-Myc-CMV 19, pFLAG-Myc-CMV 23, pFLAG-CMV 2, pFLAG-CMV 6a,b,c, pFLAG-CMV 5.1, pFLAG-CMV 5a,b,c, p3xFLAG-CMV 7.1, pFLAG-CMV 20, p3xFLAG-Myc-CMV 24, pCMV-FLAG-MAT1, pCMV-FLAG-MAT2, pBICEP-CMV 3, or pBICEP-CMV 4. Mammalian stable expression vector may include pFLAG-CMV 3, p3xFLAG-CMV 9, p3xFLAG-CMV 13, pFLAG-Myc-CMV 21, p3xFLAG-Myc-CMV 25, pFLAG-CMV 4, p3xFLAG-CMV 10, p3xFLAG-CMV 14, pFLAG-Myc-CMV 22, p3xFLAG-Myc-CMV 26, pBICEP-CMV 1, or pBICEP-CMV 2.

[0136] In some instances, a cell-free system is a mixture of cytoplasmic and/or nuclear components from a cell and is used for in vitro nucleic acid synthesis. In some cases, a cell-free system utilizes either prokaryotic cell components or eukaryotic cell components. Sometimes, a nucleic acid synthesis is obtained in a cell-free system based on for example Drosophila cell, Xenopus egg, or HeLa cells. Exemplary cell-free systems include, but are not limited to, E. coli S30 Extract system, E. coli T7 S30 system, or PURExpress®.

Host Cells

[0137] In some embodiments, a host cell includes any suitable cell such as a naturally derived cell or a genetically modified cell. In some instances, a host cell is a production host cell. In some instances, a host cell is a eukaryotic cell. In other instances, a host cell is a prokaryotic cell. In some cases, a eukaryotic cell includes fungi (e.g., yeast cells), animal cell or plant cell. In some cases, a prokaryotic cell is a bacterial cell. Examples of bacterial cell include gram-positive bacteria or gram-negative bacteria. Sometimes the gram-negative bacteria is anaerobic, rod-shaped, or both.

[0138] In some instances, gram-positive bacteria include Actinobacteria, Firmicutes or Tenericutes. In some cases, gram-negative bacteria include Aquificae, Deinococcus-Thermus, Fibrobacteres–Chlorobi/Bacteroidetes (FCB group), Fusobacteria, Gemmatimonadetes, Nitrospirae, Planctomycetes–Verrucomicrobia/ Chlamydiae (PVC group), Proteobacteria, Spirochaetes or Synergistetes. Other bacteria can be Acidobacteria, Chloroflexi, Chrysiogenetes, Cyanobacteria, Deferribacteres, Dictyoglomi, Thermodesulfobacteria or Thermotogae. A bacterial cell can be Escherichia coli, Clostridium botulinum, or Coli bacilli.

[0139] Exemplary prokaryotic host cells include, but are not limited to, BL21, Mach1™, DH10B™, TOP10, DH5α, DH10Bac™, OmniMax™, MegaX™, DH12S™, INV110, TOP10F', INVαF, TOP10/P3, ccdB Survival, PIR1, PIR2, Stbl2™, Stbl3™, or Stbl4™.

[0140] In some instances, animal cells include a cell from a vertebrate or from an invertebrate. In some cases, an animal cell includes a cell from a marine invertebrate, fish, insects, amphibian, reptile, or mammal. In some cases, a fungus cell includes a yeast cell, such as brewer's yeast, baker's yeast, or wine yeast.

[0141] Fungi include ascomycetes such as yeast, mold, filamentous fungi, basidiomycetes, or zygomycetes. In some instances, yeast includes Ascomycota or Basidiomycota. In some cases, Ascomycota includes Saccharomycotina (true yeasts, e.g. *Saccharomyces cerevisiae* (baker's yeast)) or Taphrinomycotina (e.g. Schizosaccharomycetes (fission yeasts)). In some cases, Basidiomycota includes Agaricomycotina (e.g. Tremellomycetes) or Pucciniomycotina (e.g. Microbotryomycetes).

[0142] Exemplary yeast or filamentous fungi include, for example, the genus: *Saccharomyces*, *Schizosaccharomyces*, *Candida*, *Pichia*, *Hansenula*, *Kluyveromyces*, *Zygosaccharomyces*, *Yarrowia*, *Trichosporon*, *Rhodospiridi*, *Aspergillus*, *Fusarium*, or *Trichoderma*. Exemplary yeast or filamentous fungi include, for example, the species: *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Candida utilis*, *Candida boidini*, *Candida albicans*, *Candida tropicalis*, *Candida stellatoidea*, *Candida glabrata*, *Candida krusei*, *Candida parapsilosis*, *Candida guilliermondii*, *Candida viswanathii*, *Candida lusitaniae*, *Rhodotorula mucilaginosa*, *Pichia metanolica*, *Pichia angusta*, *Pichia pastoris*, *Pichia anomala*, *Hansenula polymorpha*, *Kluyveromyces lactis*, *Zygosaccharomyces rouxii*, *Yarrowia lipolytica*, *Trichosporon pullulans*, *Rhodospiridium toru*-*Aspergillus niger*, *Aspergillus nidulans*, *Aspergillus awamori*, *Aspergillus oryzae*, *Trichoderma reesei*, *Yarrowia lipolytica*, *Brettanomyces bruxellensis*, *Candida stellata*, *Schizosaccharomyces pombe*, *Torulaspora delbrueckii*, *Zygosaccharomyces bailii*, *Cryptococcus neoformans*, *Cryptococcus gattii*, or *Saccharomyces boulardii*.

[0143] Exemplary yeast host cells include, but are not limited to, *Pichia pastoris* yeast strains such as GS115, KM71H, SMD1168, SMD1168H, and X-33; and *Saccharomyces cerevisiae* yeast strain such as INVSc1.

[0144] In some instances, additional animal cells include cells obtained from a mollusk, arthropod, annelid or sponge. In some cases, an additional animal cell is a mammalian cell, e.g., from a primate, ape, equine, bovine, porcine, canine, feline or rodent. In some cases, a rodent includes mouse, rat, hamster, gerbil, hamster, chinchilla, fancy rat, or guinea pig.

[0145] Exemplary mammalian host cells include, but are not limited to, 293A cell line, 293FT cell line, 293F cells, 293 H cells, CHO DG44 cells, CHO-S cells, CHO-K1 cells, FUT8 KO CHOK1, Expi293F™ cells, Flp-In™ T-REx™ 293 cell line, Flp-In™-293 cell line, Flp-In™-3T3 cell line, Flp-In™-BHK cell line, Flp-In™-CHO cell line, Flp-In™-CV-1 cell line, Flp-In™-Jurkat cell line, FreeStyle™ 293-F cells, FreeStyle™ CHO-S cells, GripTite™ 293 MSR cell line, GS-CHO cell line, HcpaRG™ cells, T-REx™ Jurkat cell line, Per.C6 cells, T-REx™-293 cell line, T-REx™-CHO cell line, and T-REx™-HeLa cell line.

[0146] In some instances, a mammalian host cell is a stable cell line, or a cell line that has incorporated a genetic material of interest into its own genome and has the capability to express the product of the genetic material after many generations of cell division. In some cases, a mammalian host cell is a transient cell line, or a cell line that has not incorporated a genetic material of interest into its own genome and does not have the capability to express the product of the genetic material after many generations of cell division.

[0147] Exemplary insect host cells include, but are not limited to, *Drosophila* S2 cells, Sf9 cells, Sf21 cells, High Five™ cells, and expresSF+® cells.

[0148] In some instances, plant cells include a cell from algae. Exemplary insect cell lines include, but are not limited to, strains from *Chlamydomonas reinhardtii* 137c, or *Synechococcus elongatus* PPC 7942.

Articles of Manufacture

[0149] In another aspect of the invention, an article of manufacture containing materials useful for the treatment, prevention and/or diagnosis of the disorders described above is provided. The article of manufacture 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 the condition and may have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper that is pierceable by a hypodermic injection needle). At least one active agent in the composition is a bispecific antibody comprising a first antigen-binding site that specifically binds to CD3 and a second antigen-binding site that specifically binds to a tumor antigen defined herein before.

[0150] The label or package insert indicates that the composition is used for treating the condition of choice. Moreover, the article of manufacture may comprise (a) a first container with a composition contained therein, wherein the composition comprises the bispecific antibody of the invention; and (b) a second container with a composition contained therein, wherein the composition comprises a further cytotoxic or otherwise therapeutic agent. The article of manufacture in this embodiment of the invention may further comprise a package insert indicating that the compositions can be used to treat a particular condition.

[0151] Alternatively, or additionally, the article of manufacture 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.

Certain Definitions

[0152] The terminology used herein is for the purpose of describing particular cases only and is not intended to be limiting. As used herein, the singular forms “a”, “an” and “the” are intended to include the plural forms as well, unless the context clearly indicates otherwise. Furthermore, to the extent that the terms “including”, “includes”, “having”, “has”, “with”, or variants thereof are used in either the detailed description and/or the claims, such terms are intended to be inclusive in a manner similar to the term “comprising.”

[0153] The term “antibody” is used in the broadest sense and covers fully assembled antibodies, antibody fragments that can bind antigen, for example, Fab, F(ab')₂, Fv, single chain antibodies (scFv), diabodies, antibody chimeras, hybrid antibodies, bispecific antibodies, and the like.

[0154] The term “complementarity determining region” or “CDR” is a segment of the variable region of an antibody that is complementary in structure to the epitope to which the antibody binds and is more variable than the

rest of the variable region. Accordingly, a CDR is sometimes referred to as hypervariable region. A variable region comprises three CDRs. CDR peptides can be obtained by constructing genes encoding the CDR of an antibody of interest. Such genes are prepared, for example, by using the polymerase chain reaction to synthesize the variable region from RNA of antibody-producing cells. See, for example, Larrick et al., *Methods: A Companion to Methods in Enzymology* 2: 106 (1991); Courtenay-Luck, "Genetic Manipulation of Monoclonal Antibodies," in *Monoclonal Antibodies: Production, Engineering and Clinical Application*, Ritter et al. (eds.), pages 166-179 (Cambridge University Press 1995); and Ward et al., "Genetic Manipulation and Expression of Antibodies," in *Monoclonal Antibodies: Principles and Applications*, Birch et al., (eds.), pages 137-185 (Wiley-Liss, Inc. 1995).

[0155] The term "Fab" refers to a protein that contains the constant domain of the light chain and the first constant domain (CH1) of the heavy chain. Fab fragments differ from Fab' fragments by the addition of a few residues at the carboxy terminus of the heavy chain CH1 domain including one or more cysteines from the antibody hinge region. Fab'-SH is the designation herein for Fab' in which the cysteine residue(s) of the constant domains bear a free thiol group. Fab' fragments are produced by reducing the F(ab')₂ fragment's heavy chain disulfide bridge. Other chemical couplings of antibody fragments are also known.

[0156] A "single-chain variable fragment (scFv)" is a fusion protein of the variable regions of the heavy (VH) and light chains (VL) of an antibody, connected with a short linker peptide of ten to about 25 amino acids. The linker is usually rich in glycine for flexibility, as well as serine or threonine for solubility, and can either connect the N-terminus of the VH with the C-terminus of the VL, or vice versa. This protein retains the specificity of the original antibody, despite removal of the constant regions and the introduction of the linker. scFv antibodies are, e.g. described in Houston, J. S., *Methods in Enzymol.* 203 (1991) 46-96). In addition, antibody fragments comprise single chain polypeptides having the characteristics of a VH domain, namely being able to assemble together with a VL domain, or of a VL domain, namely being able to assemble together with a VH domain to a functional antigen binding site and thereby providing the antigen binding property of full length antibodies.

[0157] While preferred embodiments of the present disclosure have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the disclosure. It should be understood that various alternatives to the embodiments of the disclosure described herein may be employed in practicing the disclosure. It is intended that the following claims define the scope of the disclosure and that methods and structures within the scope of these claims and their equivalents be covered thereby.

EMBODIMENTS

[0158] Embodiment 1 comprises an isolated polypeptide or polypeptide complex comprising a peptide that impairs binding of an anti-CD3 binding domain to CD3 wherein the peptide comprises an amino acid sequence selected from SEQ ID NOs: 1-2, 19-55 or the peptide comprises 1, 2, or 3, amino acid substitutions, additions, or deletions relative to an amino acid sequence selected from SEQ ID NOs: 1-2, 19-55.

[0159] Embodiment 2 comprises an isolated polypeptide or polypeptide complex of embodiment 1, wherein the peptide comprises an amino acid sequence selected from SEQ ID NOs: 1-2, and 19-55.

[0160] Embodiment 3 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 1-2, wherein the peptide comprises the amino acid sequence of SEQ ID NO: 1.

[0161] Embodiment 4 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 1-2, wherein the peptide comprises the amino acid sequence of SEQ ID NO: 2.

[0162] Embodiment 5 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 1-2, wherein the peptide comprises the amino acid sequence of SEQ ID NO: 54.

[0163] Embodiment 6 comprises an isolated polypeptide or polypeptide complex comprising an anti-CD3 binding domain that is linked to a peptide that impairs binding of the anti-CD3 binding domain to CD3 wherein the peptide comprises an amino acid sequence according to Z_1 - Z_2 -C- Z_4 -P- Z_6 - Z_7 - Z_8 - Z_9 - Z_{10} - Z_{11} - Z_{12} -C- Z_{14} and Z_1 is selected from D, Y, F, I, N, V, H, L, A, T, S, and P; Z_2 is selected from D, Y, L, F, I, N, A, V, H, T, and S; Z_4 is selected from G and W; Z_6 is selected from E, D, V, and P; Z_7 is selected from W, L, F, V, G, M, I, and Y; Z_8 is selected from E, D, P, and Q; Z_9 is selected from E, D, Y, V, F, W, P, L, and Q; Z_{10} is selected from S, D, Y, T, I, F, V, N, A, P, L, and H; Z_{11} is selected from I, Y, F, V, L, T, N, S, D, A, and H; Z_{12} is selected from F, D, Y, L, I, V, A, N, T, P, S, and H; and Z_{14} is selected from D, Y, N, F, I, P, V, A, T, H, L and S.

[0164] Embodiment 7 comprises an isolated polypeptide or polypeptide complex of embodiment 6, wherein Z_1 is selected from D, Y, F, I, and N; Z_2 is selected from D, Y, L, F, I, and N; Z_4 is selected from G and W; Z_6 is selected from E and D; Z_7 is selected from W, L, F, and V; Z_8 is selected from E and D; Z_9 is selected from E, D, Y, and V; Z_{10} is selected from S, D, Y, T, and I; Z_{11} is selected from I, Y, F, V, L, and T; Z_{12} is selected from F, D, Y, L, I, V, A, and N; and Z_{14} is selected from D, Y, N, F, I, and P.

[0165] Embodiment 8 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 6-7, wherein Z_1 is selected from D, Y, and F; Z_2 is selected from D, Y, L, and F; Z_4 is selected from G and W; Z_6 is selected from E and D; Z_7 is selected from W, L, and F; Z_8 is selected from E and D; Z_9 is selected from E and D; Z_{10} is selected from S, D, and Y; Z_{11} is selected from I, Y, and F; Z_{12} is selected from F, D, Y, and L; and Z_{14} is selected from D, Y, and N.

[0166] Embodiment 9 comprises an isolated polypeptide or polypeptide complex comprising an anti-CD3 binding domain that is linked to a peptide that impairs binding of the anti-CD3 binding domain to CD3 wherein the peptide comprises an amino acid sequence according to U_1 - U_2 -C- U_4 -P- U_6 - U_7 - U_8 - U_9 - U_{10} - U_{11} - U_{12} -C- U_{14} and U_1 is selected from D, Y, F, I, N, V, H, L, A, T, S, and P; U_2 is selected from D, Y, L, F, I, N, A, V, H, T, and S; U_4 is selected from G and W; U_6 is selected from E, D, V, and P; U_7 is selected from W, L, F, V, G, M, I, and Y; U_8 is selected from E, D, P, and Q; U_9 is selected from E, D, Y, V, F, W, P, L, and Q; U_{10} is selected from S, D, Y, T, I, F, V, N, A, P, L, and H; U_{11} is selected from I, Y, F, V, L, T, N, S, D, A, and H; U_{12} is selected from F, D, Y, L, I, V, A, N, T, P, S, G, and H; and U_{14} is selected from D, Y, N, F, I, P, V, A, T, H, L, M, and S.

[0167] Embodiment 10 comprises an isolated polypeptide or polypeptide complex of embodiment 9, wherein U_1 is selected from D, Y, F, I, V, and N; U_2 is selected from D, Y, L, F, I, and N; U_4 is selected from G and W; U_6 is selected from E and D; U_7 is selected from W, L, F, G, and V; U_8 is selected from E and D; U_9 is selected from E,

D, Y, and V; U₁₀ is selected from S, D, Y, T, and I; U₁₁ is selected from I, Y, F, V, L, and T; U₁₂ is selected from F, D, Y, L, I, V, A, G, and N; and U₁₄ is selected from D, Y, N, F, I, M, and P.

[0168] Embodiment 11 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 9-10, wherein U₁ is selected from D, Y, V, and F; U₂ is selected from D, Y, L, and F; U₄ is selected from G and W; U₆ is selected from E and D; U₇ is selected from W, L, G, and F; U₈ is selected from E and D; U₉ is selected from E and D; U₁₀ is selected from S, D, T, and Y; U₁₁ is selected from I, Y, V, L, and F; U₁₂ is selected from F, D, Y, G, A, and L; and U₁₄ is selected from D, Y, M, and N.

[0169] Embodiment 12 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 6-11, wherein the peptide comprises an amino acid sequence of **Table 17**.

[0170] Embodiment 13 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 6-11, wherein the peptide comprises an amino acid sequence according to any one of SEQ ID NOs: 44-55.

[0171] Embodiment 14 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 6 and 9-12, wherein the peptide comprises the amino acid sequence of SEQ ID NO: 2.

[0172] Embodiment 15 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 6-7 and 9-13, wherein the peptide comprises the amino acid sequence of SEQ ID NO: 54.

[0001] Embodiment 16 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 1-15, wherein the peptide is connected to the anti-CD3 binding domain in a configuration according to Formula I: A₁-L₁-P₁ wherein A₁ comprises the anti-CD3 binding domain; L₁ comprises a cleavable linker that is a substrate for a tumor specific protease; P₁ comprises the peptide that impairs binding of the anti-CD3 binding domain to CD3.

[0173] Embodiment 17 comprises an isolated polypeptide or polypeptide complex of embodiment 16, wherein P₁ is connected N-terminal to the cleavable linker and A₁ is connected C-terminal to the cleavable linker.

[0174] Embodiment 18 comprises an isolated polypeptide or polypeptide complex of embodiment 16, wherein P₁ is connected C-terminal to the cleavable linker and A₁ is connected N-terminal to the cleavable linker.

[0175] Embodiment 19 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 16-18, wherein P₁ is bound to A₁ through ionic interactions, electrostatic interactions, hydrophobic interactions, Pi-stacking interactions, or H-bonding interactions, or a combination thereof.

[0176] Embodiment 20 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 16-19, wherein A₁ is further linked to a tumor antigen binding domain (A₂).

[0177] Embodiment 21 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 16-20, wherein the polypeptide or polypeptide complex is according to Formula Ia: P₂-L₂-A₂-A₁-L₁-P₁ wherein P₂ comprises a peptide that impairs binding of A₂ to a tumor antigen; and L₂ comprises a second cleavable linker that connects A₂ to P₂ and is a substrate for a tumor specific protease.

[0178] Embodiment 22 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 16-21, wherein A₁ comprises an antibody or antibody fragment.

[0179] Embodiment 23 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 16-22, wherein A₁ comprises an antibody or antibody fragment that is human or humanized.

- [0180] Embodiment 24 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 16-17 and 19-23, wherein L_1 is bound to N-terminus of the antibody or antibody fragment of A_1 .
- [0181] Embodiment 25 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 16 and 18-23, wherein A_2 is bound to N-terminus of the antibody or antibody fragment of A_1 .
- [0182] Embodiment 26 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 16, 18-23, and 25, wherein L_1 is bound to the C-terminus of the antibody or antibody fragment of A_1 .
- [0183] Embodiment 27 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 16-17 and 19-24, wherein A_2 is bound to the C-terminus of the antibody or antibody fragment of A_1 .
- [0184] Embodiment 28 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 22-27, wherein the antibody or antibody fragment of A_1 comprises a single chain variable fragment, a single domain antibody, or a Fab fragment.
- [0185] Embodiment 29 comprises an isolated polypeptide or polypeptide complex of embodiment 28, wherein A_1 is the single chain variable fragment (scFv).
- [0186] Embodiment 30 comprises an isolated polypeptide or polypeptide complex of embodiment 29, wherein the scFv comprises a scFv heavy chain polypeptide and a scFv light chain polypeptide.
- [0187] Embodiment 31 comprises an isolated polypeptide or polypeptide complex of embodiment 28, wherein A_1 is the single domain antibody.
- [0188] Embodiment 32 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 22-27, wherein the antibody or antibody fragment thereof comprises a single chain variable fragment (scFv), a heavy chain variable domain (VH domain), a light chain variable domain (VL domain), or a variable domain (VHH) of a camelid derived single domain antibody.
- [0189] Embodiment 33 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 16-30 and 32, wherein A_1 comprises an anti-CD3e single chain variable fragment.
- [0190] Embodiment 34 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 16-32, wherein A_1 comprises an anti-CD3e single chain variable fragment that has a KD binding of 1 μ M or less to CD3 on CD3 expressing cells.
- [0191] Embodiment 35 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 16-30 and 32-34, wherein A_1 comprises a variable light chain and variable heavy chain each of which is capable of specifically binding to human CD3.
- [0192] Embodiment 36 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 28-30 and 32-35, wherein A_1 comprises the scFv comprising a scFv heavy chain variable domain and an scFv light chain variable domain comprising complementarity determining regions (CDRs): HC-CDR1, HC-CDR2, and HC-CDR3, wherein the HC-CDR1, the HC-CDR2, and the HC-CDR3 of the scFv heavy chain variable domain comprise: HC-CDR1: SEQ ID NO: 3; HC-CDR2: SEQ ID NO: 4; HC-CDR3: SEQ ID NO: 5; and the scFv light chain variable domain comprises complementarity determining regions (CDRs): LC-CDR1, LC-CDR2, and LC-CDR3, wherein the LC-CDR1, the LC-CDR2, and the LC-CDR3 of the scFv light chain variable domain comprise: LC-CDR1: SEQ ID NO: 6; LC-CDR2: SEQ ID NO: 7; and LC-CDR3: SEQ ID NO: 8.

[0193] Embodiment 37 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 16-30 and 32-35, wherein A₁ comprises a heavy chain variable domain and a light chain variable domain comprising complementarity determining regions (CDRs): HC-CDR1, HC-CDR2, and HC-CDR3, wherein the HC-CDR1, the HC-CDR2, and the HC-CDR3 of the heavy chain variable domain comprise: HC-CDR1: SEQ ID NO: 3; HC-CDR2: SEQ ID NO: 4; HC-CDR3: SEQ ID NO: 5; and the light chain variable domain comprises complementarity determining regions (CDRs): LC-CDR1, LC-CDR2, and LC-CDR3, wherein the LC-CDR1, the LC-CDR2, and the LC-CDR3 of the light chain variable domain comprise: LC-CDR1: SEQ ID NO: 6; LC-CDR2: SEQ ID NO: 7; and LC-CDR3: SEQ ID NO: 8.

[0194] Embodiment 38 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 16-37, wherein the polypeptide or polypeptide complex of formula I binds to an effector cell when L₁ is cleaved by the tumor specific protease.

[0195] Embodiment 39 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 16-38, wherein the polypeptide or polypeptide complex of formula I binds to an effector cell when L₁ is cleaved by the tumor specific protease and A₁ binds to the effector cell.

[0196] Embodiment 40 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 38-39, wherein the effector cell is a T cell.

[0197] Embodiment 41 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 38-40, wherein A₁ binds to a polypeptide that is part of a TCR-CD3 complex on the effector cell.

[0198] Embodiment 42 comprises an isolated polypeptide or polypeptide complex of embodiment 41, wherein the polypeptide that is part of the TCR-CD3 complex is human CD3ε.

[0199] Embodiment 43 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 20-42, wherein A₂ comprises an antibody or antibody fragment.

[0200] Embodiment 44 comprises an isolated polypeptide or polypeptide complex of embodiment 43, wherein the antibody or antibody fragment thereof of A₂ comprises a single chain variable fragment, a single domain antibody, Fab', or a Fab.

[0201] Embodiment 45 comprises an isolated polypeptide or polypeptide complex of embodiment 43, wherein the antibody or antibody fragment thereof of A₂ comprises a single chain variable fragment (scFv), a heavy chain variable domain (VH domain), a light chain variable domain (VL domain), or a variable domain (VHH) of a camelid derived single domain antibody.

[0202] Embodiment 46 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 43-45, wherein the antibody or antibody fragment thereof of A₂ is humanized or human.

[0203] Embodiment 47 comprises an isolated polypeptide or polypeptide complex of embodiment 44, wherein A₂ is the Fab or Fab'.

[0002] Embodiment 48 comprises an isolated polypeptide or polypeptide complex of embodiment 47, wherein the Fab or Fab' comprises (a) a Fab light chain polypeptide and (b) a Fab heavy chain polypeptide.

[0204] Embodiment 49 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 20-48, wherein A₂ comprises an epidermal growth factor receptor (EGFR) binding domain.

- [0205] Embodiment 50 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 20-48, wherein A₂ comprises a carcinoembryonic antigen-related cell adhesion molecule CEACAM5 binding domain.
- [0206] Embodiment 51 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 16-50, wherein P₁ is further linked to a half-life extending moiety (H₁).
- [0207] Embodiment 52 comprises an isolated polypeptide or polypeptide complex of embodiment 51, wherein the half-life extending moiety is a single-domain antibody.
- [0208] Embodiment 53 comprises an isolated polypeptide or polypeptide complex of embodiment 51, wherein H₁ comprises a polymer.
- [0209] Embodiment 54 comprises an isolated polypeptide or polypeptide complex of embodiment 53, wherein the polymer is polyethylene glycol (PEG).
- [0210] Embodiment 55 comprises an isolated polypeptide or polypeptide complex of embodiment 51, wherein H₁ comprises albumin.
- [0211] Embodiment 56 comprises an isolated polypeptide or polypeptide complex of embodiment 51, wherein H₁ comprises an Fc domain.
- [0212] Embodiment 57 comprises an isolated polypeptide or polypeptide complex of embodiment 55, wherein the albumin is serum albumin.
- [0213] Embodiment 58 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 55 and 57, wherein the albumin is human serum albumin.
- [0214] Embodiment 59 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 51-58, wherein H₁ comprises a polypeptide, a ligand, or a small molecule.
- [0215] Embodiment 60 comprises an isolated polypeptide or polypeptide complex of embodiment 59, wherein the polypeptide, the ligand or the small molecule binds serum protein or a fragment thereof, a circulating immunoglobulin or a fragment thereof, or CD35/CR1.
- [0216] Embodiment 61 comprises an isolated polypeptide or polypeptide complex of embodiment 60, wherein the serum protein comprises a thyroxine-binding protein, a transthyretin, a 1-acid glycoprotein, a transferrin, transferrin receptor or a transferrin-binding portion thereof, a fibrinogen, or an albumin.
- [0003] Embodiment 62 comprises an isolated polypeptide or polypeptide complex of embodiment 60, wherein the circulating immunoglobulin molecule comprises IgG1, IgG2, IgG3, IgG4, sIgA, IgM or IgD.
- [0217] Embodiment 63 comprises an isolated polypeptide or polypeptide complex of embodiment 60 or 61, wherein the serum protein is albumin.
- [0218] Embodiment 64 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 59-63, wherein the polypeptide is an antibody.
- [0219] Embodiment 65 comprises an isolated polypeptide or polypeptide complex of embodiment 64, wherein the antibody comprises a single domain antibody, a single chain variable fragment, or a Fab.
- [0220] Embodiment 66 comprises an isolated polypeptide or polypeptide complex of embodiment 65, wherein the single domain antibody comprises a single domain antibody that binds to albumin.

- [0221] Embodiment 67 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 65-66, wherein the single domain antibody is a human or humanized antibody.
- [0222] Embodiment 68 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 65-67, wherein the single domain antibody is 645gH1gL1.
- [0223] Embodiment 69 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 65-67, wherein the single domain antibody is 645dsgH5gL4.
- [0224] Embodiment 70 comprises an isolated polypeptide or polypeptide complex of embodiment 65, wherein the single domain antibody is 23-13-A01 -sc02.
- [0225] Embodiment 71 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 65-66, wherein the single domain antibody is A10m3 or a fragment thereof.
- [0226] Embodiment 72 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 65-66, wherein the single domain antibody is DOM7r-31.
- [0227] Embodiment 73 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 65-66, wherein the single domain antibody is DOM7h-11-15.
- [0228] Embodiment 74 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 65-66, wherein the single domain antibody is Alb-1, Alb-8, or Alb-23.
- [0229] Embodiment 75 comprises an isolated polypeptide or polypeptide complex of embodiment 65, wherein the single domain antibody is 10G or 10GE.
- [0230] Embodiment 76 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 65-66, wherein the single domain antibody is SA21.
- [0231] Embodiment 77 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 1-76, wherein the polypeptide or polypeptide complex comprises a modified amino acid, a non-natural amino acid, a modified non-natural amino acid, or a combination thereof.
- [0232] Embodiment 78 comprises an isolated polypeptide or polypeptide complex of embodiment 77, wherein the modified amino acid or modified non-natural amino acid comprises a post-translational modification.
- [0233] Embodiment 79 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 51-78, wherein H₁ comprises a linking moiety (L₃) that connects H₁ to P₁.
- [0234] Embodiment 80 comprises an isolated polypeptide or polypeptide complex of embodiment 79, wherein L₃ is a peptide sequence having at least 5 to no more than 50 amino acids.
- [0235] Embodiment 81 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 79-80, wherein L₃ is a peptide sequence having at least 10 to no more than 30 amino acids.
- [0236] Embodiment 82 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 79-81, wherein L₃ is a peptide sequence having at least 10 amino acids.
- [0237] Embodiment 83 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 79-82, wherein L₃ is a peptide sequence having at least 18 amino acids.
- [0238] Embodiment 84 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 79-83, wherein L₃ is a peptide sequence having at least 26 amino acids.

[0239] Embodiment 85 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 79-84, wherein L_3 has a formula selected from the group consisting of $(G_2S)_n$, $(GS)_n$, $(GSGGS)_n$ (SEQ ID NO: 917), $(GGGS)_n$ (SEQ ID NO: 918), $(GGGGS)_n$ (SEQ ID NO: 919), and $(GSSGGS)_n$ (SEQ ID NO: 920), wherein n is an integer of at least 1.

[0240] Embodiment 86 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 79-84, wherein L_3 comprises an amino acid sequence according to $(GGGSGGGS)$ (SEQ ID NO: 921)).

[0241] Embodiment 87 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 48-86, wherein the Fab light chain polypeptide of A_2 is bound to a C-terminus of the single chain variable fragment (scFv) of A_1 .

[0242] Embodiment 88 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 48-86, wherein the Fab heavy chain polypeptide of A_2 is bound to a C-terminus of the single chain variable fragment (scFv) A_1 .

[0243] Embodiment 89 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 48-86, wherein the Fab light chain polypeptide of A_2 is bound to a N-terminus of the single chain variable fragment (scFv) of A_1 .

[0244] Embodiment 90 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 48-86, wherein the Fab heavy chain polypeptide of A_2 is bound to a N-terminus of the single chain variable fragment (scFv) A_1 .

[0245] Embodiment 91 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 48-86, 88, and 90, wherein the Fab heavy chain polypeptide of A_2 is bound to the scFv heavy chain polypeptide of A_1 .

[0246] Embodiment 92 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 48-86, 87, and 89, wherein the Fab light chain polypeptide of A_2 is bound to the scFv heavy chain polypeptide of A_1 .

[0247] Embodiment 93 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 48-86, 88, and 90, wherein the Fab heavy chain polypeptide of A_2 is bound to the scFv light chain polypeptide of A_1 .

[0248] Embodiment 94 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 48-86, 87, and 89, wherein the Fab light chain polypeptide of A_2 is bound to the scFv light chain polypeptide of A_1 .

[0249] Embodiment 95 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 48-86, 88, 90, and 91, wherein the Fab heavy chain polypeptide of A_2 is bound to the scFv heavy chain polypeptide of A_1 and L_2 is bound to the Fab light chain polypeptide of A_2 .

[0250] Embodiment 96 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 48-87, 89, and 92, wherein the Fab light chain polypeptide of A_2 is bound to the scFv heavy chain polypeptide of A_1 and L_2 is bound to the Fab heavy chain polypeptide of A_2 .

[0251] Embodiment 97 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 1-86, 88, 90, and 93, wherein the Fab heavy chain polypeptide of A_2 is bound to the scFv light chain polypeptide of A_1 and L_2 is bound to the Fab light chain polypeptide of A_2 .

[0252] Embodiment 98 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 1-87, 89, and 94, wherein the Fab light chain polypeptide of A₂ is bound to the scFv light chain polypeptide of A₁ and L₂ is bound to the Fab heavy chain polypeptide of A₂.

[0253] Embodiment 99 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 21-98, wherein P₂ impairs binding of A₂ to the tumor antigen.

[0254] Embodiment 100 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 21-99, wherein P₂ is bound to A₂ through ionic interactions, electrostatic interactions, hydrophobic interactions, Pi-stacking interactions, or H-bonding interactions, or a combination thereof.

[0255] Embodiment 101 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 21-100, wherein P₂ is bound to A₂ at or near an antigen binding site.

[0256] Embodiment 102 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 21-101, wherein P₂ has less than 70% sequence homology to the tumor antigen.

[0257] Embodiment 103 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 21-102, wherein P₂ comprises a peptide sequence of at least 10 amino acids in length.

[0258] Embodiment 104 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 21-103, wherein P₂ comprises a peptide sequence of at least 10 amino acids in length and no more than 20 amino acids in length.

[0259] Embodiment 105 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 21-104, wherein P₂ comprises a peptide sequence of at least 16 amino acids in length.

[0260] Embodiment 106 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 21-103 and 105, wherein P₂ comprises a peptide sequence of no more than 40 amino acids in length.

[0261] Embodiment 107 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 21-106, wherein P₂ comprises at least two cysteine amino acid residues.

[0262] Embodiment 108 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 21-107, wherein P₂ comprises a cyclic peptide or a linear peptide.

[0263] Embodiment 109 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 21-108, wherein P₂ comprises a cyclic peptide.

[0264] Embodiment 110 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 21-108, wherein P₂ comprises a linear peptide.

[0265] Embodiment 111 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 16-17, 19-24, 27-88, and 91-110, wherein L₁ is bound to N-terminus of A₁.

[0266] Embodiment 112 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 16, 18-23, 25-26, 28-86, 89-110, wherein L₁ is bound to C-terminus of A₁.

[0267] Embodiment 113 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 21-23, 25-26, 28-86, 89-110, and 112, wherein L₂ is bound to N-terminus of A₂.

Embodiment 114 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 21, 24, 27-88, and 91-111, wherein L₂ is bound to C-terminus of A₂.

[0268] Embodiment 115 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 16-114, wherein L_1 or L_2 is a peptide sequence having at least 5 to no more than 50 amino acids.

[0269] Embodiment 116 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 16-115, wherein L_1 or L_2 is a peptide sequence having at least 10 to no more than 30 amino acids.

[0270] Embodiment 117 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 16-116, wherein L_1 or L_2 is a peptide sequence having at least 10 amino acids.

[0271] Embodiment 118 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 16-117, wherein L_1 or L_2 is a peptide sequence having at least 18 amino acids.

[0272] Embodiment 119 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 16-118, wherein L_1 or L_2 is a peptide sequence having at least 26 amino acids.

[0273] Embodiment 120 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 16-115, wherein L_1 or L_2 has a formula comprising $(G_2S)_n$ (SEQ ID NO: 922), wherein n is an integer from 1 to 3.

[0274] Embodiment 121 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 16-120, wherein L_1 has a formula selected from the group consisting of $(G_2S)_n$, $(GS)_n$, $(GSGGS)_n$ (SEQ ID NO: 917), $(GGGS)_n$ (SEQ ID NO: 918), $(GGGGS)_n$ (SEQ ID NO: 919), and $(GSSGGS)_n$ (SEQ ID NO: 920), wherein n is an integer of at least 1.

[0275] Embodiment 122 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 16-121, wherein P_1 becomes unbound from A_1 when L_1 is cleaved by the tumor specific protease thereby exposing A_1 to CD3.

[0276] Embodiment 123 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 21-122, wherein P_2 becomes unbound from A_2 when L_2 is cleaved by the tumor specific protease thereby exposing A_2 to the tumor antigen.

[0277] Embodiment 124 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 16-123, wherein the tumor specific protease is selected from the group consisting of metalloprotease, serine protease, cysteine protease, threonine protease, and aspartic protease.

[0278] Embodiment 125 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 16-124, wherein L_1 or L_2 comprises a urokinase cleavable amino acid sequence, a matriptase cleavable amino acid sequence, a matrix metalloprotease cleavable amino acid sequence, or a legumain cleavable amino acid sequence.

[0279] Embodiment 126 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 16-119 and 122-125, wherein L_1 or L_2 comprises an amino acid sequence selected from the group consisting of GGGGSLSGRSDNHGSSGT (SEQ ID NO: 923), GGGGSSGGSGGSLSGRSDNHGSSGT (SEQ ID NO: 924), ASGRSDNH (SEQ ID NO: 925), LAGRSDNH (SEQ ID NO: 926), ISSGLASGRSDNH (SEQ ID NO: 927), ISSGLLAGRSDNH (SEQ ID NO: 928), LSGRSDNH (SEQ ID NO: 929), ISSGLLSGRSDNP (SEQ ID NO: 930), ISSGLLSGRSDNH (SEQ ID NO: 931), LSGRSDNHSPLGLAGS (SEQ ID NO: 932), SPLGLAGSLSGRSDNH (SEQ ID NO: 933), SPLGLSGRSDNH (SEQ ID NO: 934), LAGRSDNHSPLGLAGS (SEQ ID NO: 935), LSGRSDNHVPLSLKMG (SEQ ID NO: 936), and LSGRSDNHVPLSLSMG (SEQ ID NO: 937).

[0280] Embodiment 127 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 16-119 and 122-126, wherein L_1 or L_2 comprises an amino acid sequence selected from the group consisting of ASGRSDNH (SEQ ID NO: 925), LAGRSDNH (SEQ ID NO: 926), ISSGLASGRSDNH (SEQ ID NO: 927), and ISSGLLAGRSDNH (SEQ ID NO: 928).

[0281] Embodiment 128 comprises a pharmaceutical composition comprising: (i) the polypeptide or polypeptide complex of any one of embodiments 1-127; and (ii) a pharmaceutically acceptable excipient.

[0282] Embodiment 129 comprises an isolated recombinant nucleic acid molecule encoding the polypeptide or polypeptide complex of any one of embodiments 1-127.

[0283] Embodiment 130 comprises an isolated polypeptide or polypeptide complex according to Formula II: L_{1a} - P_{1a} - H_{1a} wherein: L_{1a} comprises a tumor specific protease-cleaved linking moiety that when uncleaved connects P_{1a} to an anti-CD3 binding domain that binds to CD3 and; P_{1a} comprises a peptide that impairs binding of the anti-CD3 binding domain to CD3 when L_{1a} is uncleaved wherein the peptide comprises an amino acid sequence selected from SEQ ID NOs: 1-2, and 19-55 or the peptide comprises 1, 2, or 3, amino acid substitutions, additions, or deletions relative to amino acid sequences of SEQ ID NOs: 1-2, and 19-55; and H_{1a} comprises a half-life extending molecule.

[0284] Embodiment 131 comprises an isolated polypeptide or polypeptide complex of embodiment 130, wherein the peptide comprises an amino acid sequence selected from SEQ ID NOs: 1-2, and 19-55.

[0285] Embodiment 132 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 130-131, wherein the peptide comprises the amino acid sequence of SEQ ID NO: 1.

[0286] Embodiment 133 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 130-131, wherein the peptide comprises the amino acid sequence of SEQ ID NO: 2.

[0287] Embodiment 134 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 130-131, wherein the peptide comprises the amino acid sequence of SEQ ID NO: 54.

[0288] Embodiment 135 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 130-134, wherein H_{1a} comprises a polymer.

[0289] Embodiment 136 comprises an isolated polypeptide or polypeptide complex of embodiment 135, wherein the polymer is polyethylene glycol (PEG).

[0290] Embodiment 137 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 130-134, wherein H_{1a} comprises albumin.

[0291] Embodiment 138 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 130-134, wherein H_{1a} comprises an Fc domain.

[0292] Embodiment 139 comprises an isolated polypeptide or polypeptide complex of embodiment 137, wherein the albumin is serum albumin.

[0293] Embodiment 140 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 137 and 139, wherein the albumin is human serum albumin.

[0294] Embodiment 141 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 130-140, wherein H_{1a} comprises a polypeptide, a ligand, or a small molecule.

[0295] Embodiment 142 comprises an isolated polypeptide or polypeptide complex of embodiment 141, wherein the polypeptide, the ligand or the small molecule binds a serum protein or a fragment thereof, a circulating immunoglobulin or a fragment thereof, or CD35/CR1.

[0296] Embodiment 143 comprises an isolated polypeptide or polypeptide complex of embodiment 142, wherein the serum protein comprises a thyroxine-binding protein, a transthyretin, a 1-acid glycoprotein, a transferrin, transferrin receptor or a transferrin-binding portion thereof, a fibrinogen, or an albumin.

[0297] Embodiment 144 comprises an isolated polypeptide or polypeptide complex of embodiment 142, wherein the circulating immunoglobulin molecule comprises IgG1, IgG2, IgG3, IgG4, sIgA, IgM or IgD.

[0298] Embodiment 145 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 142-143, wherein the serum protein is albumin.

[0004] Embodiment 146 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 141-145, wherein the polypeptide is an antibody.

[0299] Embodiment 147 comprises an isolated polypeptide or polypeptide complex of embodiment 146, wherein the antibody comprises a single domain antibody, a single chain variable fragment or a Fab.

[0300] Embodiment 148 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 146-147, wherein the antibody comprises a single domain antibody that binds to albumin.

[0301] Embodiment 149 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 146-148, wherein the antibody is a human or humanized antibody.

[0302] Embodiment 150 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 147-149, wherein the single domain antibody is 645gH1gL1.

[0303] Embodiment 151 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 147-149, wherein the single domain antibody is 645dsgH5gL4.

[0304] Embodiment 152 comprises an isolated polypeptide or polypeptide complex of embodiment 147, wherein the single domain antibody is 23-13-A01 -sc02.

[0305] Embodiment 153 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 147-148, wherein the single domain antibody is A10m3 or a fragment thereof.

[0306] Embodiment 154 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 147-148, wherein the single domain antibody is DOM7r-31.

[0307] Embodiment 155 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 147-148, wherein the single domain antibody is DOM7h-11-15.

[0308] Embodiment 156 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 147-148, wherein the single domain antibody is Alb-1, Alb-8, or Alb-23.

[0309] Embodiment 157 comprises an isolated polypeptide or polypeptide complex of embodiment 147, wherein the single domain antibody is 10G or 10GE.

[0310] Embodiment 158 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 147-148, wherein the single domain antibody is SA21.

[0311] Embodiment 159 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 130-158, wherein H_{1a} comprises a linking moiety (L_{3a}) that connects H_{1a} to P_{1a}.

[0312] Embodiment 160 comprises an isolated polypeptide or polypeptide complex of embodiment 159, wherein L_{3a} is a peptide sequence having at least 5 to no more than 50 amino acids.

[0313] Embodiment 161 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 159-160, wherein L_{3a} is a peptide sequence having at least 10 to no more than 30 amino acids.

[0005] Embodiment 162 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 159-161, wherein L_{3a} is a peptide sequence having at least 10 amino acids.

[0314] Embodiment 163 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 159-162, wherein L_{3a} is a peptide sequence having at least 18 amino acids.

[0315] Embodiment 164 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 159-163, wherein L_{3a} is a peptide sequence having at least 26 amino acids.

[0316] Embodiment 165 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 159-164, wherein L_{3a} has a formula selected from the group consisting of (G₂S)_n, (GS)_n, (GSGGS)_n (SEQ ID NO: 917), (GGGS)_n (SEQ ID NO: 918), (GGGGS)_n (SEQ ID NO: 919), and (GSSGGS)_n (SEQ ID NO: 920), wherein n is an integer of at least 1.

[0317] Embodiment 166 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 159-164, wherein L_{3a} comprises an amino acid sequence according to (GGGGSGGGS (SEQ ID NO: 921)).

[0318] Embodiment 167 comprises an isolated polypeptide or polypeptide complex according to **Formula III**: L_{1b}-P_{1b}-H_{1b} wherein: L_{1b} comprises a tumor specific protease-cleaved linking moiety that when uncleaved connects P_{1b} to an anti-CD3 binding domain that binds to CD3 and; P_{1b} comprises a peptide that impairs binding of the anti-CD3 binding domain to CD3 when L_{1b} is uncleaved wherein the peptide comprises the amino acid sequence according to Z₁-Z₂-C-Z₄-P-Z₆-Z₇-Z₈-Z₉-Z₁₀-Z₁₁-Z₁₂-C-Z₁₄ and Z₁ is selected from D, Y, F, I, N, V, H, L, A, T, S, and P; Z₂ is selected from D, Y, L, F, I, N, A, V, H, T, and S; Z₄ is selected from G and W; Z₆ is selected from E, D, V, and P; Z₇ is selected from W, L, F, V, G, M, I, and Y; Z₈ is selected from E, D, P, and Q; Z₉ is selected from E, D, Y, V, F, W, P, L, and Q; Z₁₀ is selected from S, D, Y, T, I, F, V, N, A, P, L, and H; Z₁₁ is selected from I, Y, F, V, L, T, N, S, D, A, and H; Z₁₂ is selected from F, D, Y, L, I, V, A, N, T, P, S, and H; and Z₁₄ is selected from D, Y, N, F, I, P, V, A, T, H, L, and S; and H_{1b} comprises a half-life extending molecule.

[0319] Embodiment 168 comprises an isolated polypeptide or polypeptide complex of embodiment 167, wherein Z₁ is selected from D, Y, F, I, and N; Z₂ is selected from D, Y, L, F, I, and N; Z₄ is selected from G and W; Z₆ is selected from E and D; Z₇ is selected from W, L, F, and V; Z₈ is selected from E and D; Z₉ is selected from E, D, Y, and V; Z₁₀ is selected from S, D, Y, T, and I; Z₁₁ is selected from I, Y, F, V, L, and T; Z₁₂ is selected from F, D, Y, L, I, V, A, and N; and Z₁₄ is selected from D, Y, N, F, I, and P.

[0320] Embodiment 169 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 167-168, wherein Z₁ is selected from D, Y, and F; Z₂ is selected from D, Y, L, and F; Z₄ is selected from G and W; Z₆ is selected from E and D; Z₇ is selected from W, L, and F; Z₈ is selected from E and D; Z₉ is selected from E and

D; Z₁₀ is selected from S, D, and Y; Z₁₁ is selected from I, Y, and F; Z₁₂ is selected from F, D, Y, and L; and Z₁₄ is selected from D, Y, and N.

[0321] Embodiment 170 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 167-169, wherein P_{1b} comprises an amino acid sequence of **Table 17**.

[0322] Embodiment 171 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 167-170, wherein P_{1b} comprises an amino acid sequences according to any one of SEQ ID NOs: 44-55.

[0323] Embodiment 172 comprises an isolated polypeptide or polypeptide complex of embodiment 167, wherein P_{1b} comprises the amino acid sequences according to SEQ ID NO: 2.

[0324] Embodiment 173 comprises an isolated polypeptide or polypeptide complex of embodiment 167-168, wherein P_{1b} comprises the amino acid sequences according to SEQ ID NO: 54.

[0325] Embodiment 174 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 167-173, wherein H_{1b} comprises a polymer.

[0326] Embodiment 175 comprises an isolated polypeptide or polypeptide complex of embodiment 174, wherein the polymer is polyethylene glycol (PEG).

[0327] Embodiment 176 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 167-173, wherein H_{1b} comprises albumin.

[0328] Embodiment 177 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 167-173, wherein H_{1b} comprises an Fc domain.

[0329] Embodiment 178 comprises an isolated polypeptide or polypeptide complex of embodiment 176, wherein the albumin is serum albumin.

[0330] Embodiment 179 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 176 and 178, wherein the albumin is human serum albumin.

[0331] Embodiment 180 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 167-179, wherein H_{1b} comprises a polypeptide, a ligand, or a small molecule.

[0332] Embodiment 181 comprises an isolated polypeptide or polypeptide complex of embodiment 180, wherein the polypeptide, the ligand or the small molecule binds a serum protein or a fragment thereof, a circulating immunoglobulin or a fragment thereof, or CD35/CR1.

[0333] Embodiment 182 comprises an isolated polypeptide or polypeptide complex of embodiment 181, wherein the serum protein comprises a thyroxine-binding protein, a transthyretin, a 1-acid glycoprotein, a transferrin, transferrin receptor or a transferrin-binding portion thereof, a fibrinogen, or an albumin.

[0334] Embodiment 183 comprises an isolated polypeptide or polypeptide complex of embodiment 181, wherein the circulating immunoglobulin molecule comprises IgG1, IgG2, IgG3, IgG4, sIgA, IgM or IgD.

[0335] Embodiment 184 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 181-182, wherein the serum protein is albumin.

[0336] Embodiment 185 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 180-184, wherein the polypeptide is an antibody.

- [0337] Embodiment 186 comprises an isolated polypeptide or polypeptide complex of embodiment 185, wherein the antibody comprises a single domain antibody, a single chain variable fragment or a Fab.
- [0338] Embodiment 187 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 185-186, wherein the antibody comprises a single domain antibody that binds to albumin.
- [0339] Embodiment 188 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 185-187, wherein the antibody is a human or humanized antibody.
- [0006] Embodiment 189 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 186-188, wherein the single domain antibody is 645gH1gL1.
- [0340] Embodiment 190 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 186-188, wherein the single domain antibody is 645dsgH5gL4.
- [0341] Embodiment 191 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 186, wherein the single domain antibody is 23-13-A01 -sc02.
- [0342] Embodiment 192 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 186-187, wherein the single domain antibody is A10m3 or a fragment thereof.
- [0343] Embodiment 193 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 186-187, wherein the single domain antibody is DOM7r-31.
- [0344] Embodiment 194 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 186-187, wherein the single domain antibody is DOM7h-11-15.
- [0345] Embodiment 195 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 186-187, wherein the single domain antibody is Alb-1, Alb-8, or Alb-23.
- [0346] Embodiment 196 comprises an isolated polypeptide or polypeptide complex of embodiment 186, wherein the single domain antibody is 10G or 10GE.
- [0347] Embodiment 197 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 186-187, wherein the single domain antibody is SA21.
- [0348] Embodiment 198 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 167-197, wherein H_{1b} comprises a linking moiety (L_{3b}) that connects H_{1b} to P_{1b}.
- [0349] Embodiment 199 comprises an isolated polypeptide or polypeptide complex of embodiment 198, wherein L_{3b} is a peptide sequence having at least 5 to no more than 50 amino acids.
- [0350] Embodiment 200 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 198-199, wherein L_{3b} is a peptide sequence having at least 10 to no more than 30 amino acids.
- [0351] Embodiment 201 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 198-200, wherein L_{3b} is a peptide sequence having at least 10 amino acids.
- [0352] Embodiment 202 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 198-201, wherein L_{3b} is a peptide sequence having at least 18 amino acids.
- [0353] Embodiment 203 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 198-202, wherein L_{3b} is a peptide sequence having at least 26 amino acids.

[0354] Embodiment 204 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 198-203, wherein L_{3b} has a formula selected from the group consisting of $(G_2S)_n$, $(GS)_n$, $(GSGGS)_n$ (SEQ ID NO: 917), $(GGGS)_n$ (SEQ ID NO: 918), $(GGGGS)_n$ (SEQ ID NO: 919), and $(GSSGGS)_n$ (SEQ ID NO: 920), wherein n is an integer of at least 1.

[0355] Embodiment 205 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 198-203, wherein L_{3b} comprises an amino acid sequence according to $(GGGGSGGGS)$ (SEQ ID NO: 921)).

[0356] Embodiment 206 comprises an isolated polypeptide or polypeptide complex according to **Formula IV**: L_{1c} - P_{1c} - H_{1c} wherein: L_{1c} comprises a tumor specific protease-cleaved linking moiety that when uncleaved connects P_{1c} to an anti-CD3 binding domain that binds to CD3 and; P_{1c} comprises a peptide that impairs binding of the anti-CD3 binding domain to CD3 when L_{1b} is uncleaved wherein the peptide comprises the amino acid sequence according to U_1 - U_2 -C- U_4 -P- U_6 - U_7 - U_8 - U_9 - U_{10} - U_{11} - U_{12} -C- U_{14} and U_1 is selected from D, Y, F, I, N, V, H, L, A, T, S, and P; U_2 is selected from D, Y, L, F, I, N, A, V, H, T, and S; U_4 is selected from G and W; U_6 is selected from E, D, V, and P; U_7 is selected from W, L, F, V, G, M, I, and Y; U_8 is selected from E, D, P, and Q; U_9 is selected from E, D, Y, V, F, W, P, L, and Q; U_{10} is selected from S, D, Y, T, I, F, V, N, A, P, L, and H; U_{11} is selected from I, Y, F, V, L, T, N, S, D, A, and H; U_{12} is selected from F, D, Y, L, I, V, A, N, T, P, S, G, and H; and U_{14} is selected from D, Y, N, F, I, P, V, A, T, H, L, M, and S; and H_{1c} comprises a half-life extending molecule.

[0357] Embodiment 207 comprises an isolated polypeptide or polypeptide complex of embodiment 206, wherein U_1 is selected from D, Y, F, I, V, and N; U_2 is selected from D, Y, L, F, I, and N; U_4 is selected from G and W; U_6 is selected from E and D; U_7 is selected from W, L, F, G, and V; U_8 is selected from E and D; U_9 is selected from E, D, Y, and V; U_{10} is selected from S, D, Y, T, and I; U_{11} is selected from I, Y, F, V, L, and T; U_{12} is selected from F, D, Y, L, I, V, A, G, and N; and U_{14} is selected from D, Y, N, F, I, M, and P.

[0358] Embodiment 208 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 206-207, wherein U_1 is selected from D, Y, V, and F; U_2 is selected from D, Y, L, and F; U_4 is selected from G and W; U_6 is selected from E and D; U_7 is selected from W, L, G, and F; U_8 is selected from E and D; U_9 is selected from E and D; U_{10} is selected from S, D, T, and Y; U_{11} is selected from I, Y, V, L, and F; U_{12} is selected from F, D, Y, G, A, and L; and U_{14} is selected from D, Y, M, and N.

[0359] Embodiment 209 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 206-208, wherein P_{1c} comprises an amino acid sequence of **Table 17**.

[0360] Embodiment 210 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 206-209, wherein P_{1c} comprises an amino acid sequences according to any one of SEQ ID NOs: 44-55.

[0361] Embodiment 211 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 206-209, wherein P_{1c} comprises the amino acid sequences according to SEQ ID NO: 2.

[0362] Embodiment 212 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 206-210, wherein P_{1c} comprises the amino acid sequences according to SEQ ID NO: 54.

[0363] Embodiment 213 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 206-212, wherein H_{1c} comprises a polymer.

- [0364] Embodiment 214 comprises an isolated polypeptide or polypeptide complex of embodiment 213, wherein the polymer is polyethylene glycol (PEG).
- [0365] Embodiment 215 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 206-212, wherein H_{1c} comprises albumin.
- [0366] Embodiment 216 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 206-212, wherein H_{1c} comprises an Fc domain.
- [0367] Embodiment 217 comprises an isolated polypeptide or polypeptide complex of embodiment 215, wherein the albumin is serum albumin.
- [0368] Embodiment 218 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 215 and 217, wherein the albumin is human serum albumin.
- [0369] Embodiment 219 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 206-218, wherein H_{1c} comprises a polypeptide, a ligand, or a small molecule.
- [0370] Embodiment 220 comprises an isolated polypeptide or polypeptide complex of embodiment 219, wherein the polypeptide, the ligand or the small molecule binds a serum protein or a fragment thereof, a circulating immunoglobulin or a fragment thereof, or CD35/CR1.
- [0371] Embodiment 221 comprises an isolated polypeptide or polypeptide complex of embodiment 220, wherein the serum protein comprises a thyroxine-binding protein, a transthyretin, a l-acid glycoprotein, a transferrin, transferrin receptor or a transferrin-binding portion thereof, a fibrinogen, or an albumin.
- [0372] Embodiment 222 comprises an isolated polypeptide or polypeptide complex of embodiment 220, wherein the circulating immunoglobulin molecule comprises IgG1, IgG2, IgG3, IgG4, slgA, IgM or IgD.
- [0373] Embodiment 223 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 220-221, wherein the serum protein is albumin.
- [0374] Embodiment 224 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 219-223, wherein the polypeptide is an antibody.
- [0375] Embodiment 225 comprises an isolated polypeptide or polypeptide complex of embodiment 224, wherein the antibody comprises a single domain antibody, a single chain variable fragment or a Fab.
- [0376] Embodiment 226 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 224-225, wherein the antibody comprises a single domain antibody that binds to albumin.
- [0377] Embodiment 227 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 224-226, wherein the antibody is a human or humanized antibody.
- [0378] Embodiment 228 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 225-227, wherein the single domain antibody is 645gH1gL1.
- [0379] Embodiment 229 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 225-227, wherein the single domain antibody is 645dsgH5gL4.
- [0380] Embodiment 230 comprises an isolated polypeptide or polypeptide complex of embodiment 225, wherein the single domain antibody is 23-13-A01 -sc02.

[0381] Embodiment 231 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 225-226, wherein the single domain antibody is A10m3 or a fragment thereof.

[0382] Embodiment 232 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 225-226, wherein the single domain antibody is DOM7r-31.

[0383] Embodiment 233 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 225-226, wherein the single domain antibody is DOM7h-11-15.

[0384] Embodiment 234 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 225-226, wherein the single domain antibody is Alb-1, Alb-8, or Alb-23.

[0385] Embodiment 235 comprises an isolated polypeptide or polypeptide complex of embodiment 225, wherein the single domain antibody is 10G or 10GE.

[0386] Embodiment 236 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 225-226, wherein the single domain antibody is SA21.

[0387] Embodiment 237 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 206-236, wherein H_{1c} comprises a linking moiety (L_{3c}) that connects H_{1c} to P_{1c} .

[0388] Embodiment 238 comprises an isolated polypeptide or polypeptide complex of embodiment 237, wherein L_{3c} is a peptide sequence having at least 5 to no more than 50 amino acids.

[0389] Embodiment 239 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 237-238, wherein L_{3c} is a peptide sequence having at least 10 to no more than 30 amino acids.

[0390] Embodiment 240 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 237-239, wherein L_{3c} is a peptide sequence having at least 10 amino acids.

[0391] Embodiment 241 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 237-240, wherein L_{3c} is a peptide sequence having at least 18 amino acids.

[0392] Embodiment 242 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 237-241, wherein L_{3c} is a peptide sequence having at least 26 amino acids.

[0393] Embodiment 243 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 237-242, wherein L_{3c} has a formula selected from the group consisting of $(G_2S)_n$, $(GS)_n$, $(GSGGS)_n$ (SEQ ID NO: 917), $(GGGS)_n$ (SEQ ID NO: 918), $(GGGGS)_n$ (SEQ ID NO: 919), and $(GSSGGS)_n$ (SEQ ID NO: 920), wherein n is an integer of at least 1.

[0394] Embodiment 244 comprises an isolated polypeptide or polypeptide complex of any one of embodiments 237-242, wherein L_{3c} comprises an amino acid sequence according to $(GGGGSGGGS)$ (SEQ ID NO: 921)).

EXAMPLES

Example 1: Structure activity relationships for Peptide-1 and Peptide 2 by Ala scanning

[0395] Sequence activity relationships were established for Peptide-1 and Peptide-2 by mutating each individual residue within the peptide to alanine and measuring binding and inhibition against SP34.185 scFv. Peptide residues whose alanine mutations significantly weakened binding and inhibition were considered key residues where mutations were not tolerated. Peptide residues whose alanine mutations

performed similarly to the non-mutated sequence were considered non-critical sites where mutations were indeed tolerated.

SP34.185 scFv binding to peptides by ELISA

[0396] Peptides were evaluated for their ability to bind SP34.185 scFv by standard enzyme linked immunosorbent assays (ELISAs). Briefly, biotinylated peptides were captured on neutravidin coated plates, quench with biocytin followed by a washing step. SP34.185 scFv was then titrated onto the peptide captured plates. Plates were then washed and bound SP34.185 scFv was detected using a secondary horse radish peroxidase antibody conjugate. After washing again, plates were developed using standard ELISA techniques and stopped using acid. The concentration of SP34.185 scFv required to achieve 50% maximal signal or EC50 was calculated using Graphpad prism. Data is shown in **Figs. 1A-1F**.

Table 6. Summary of Figure 1B

ELISA	Peptide-1	Peptide-3	Peptide-4	Peptide-5	Peptide-6	Peptide-7	Peptide-8
EC50 nM	1.013	0.9429	1.018	0.9738	1.27	47.5	346.2

Table 7. Summary of Figure 1C

ELISA	Peptide-1	Peptide-9	Peptide-10	Peptide-11	Peptide-12	Peptide-13	Peptide-14
EC50 nM	0.986	310.8	3.134	1,960	4.363	2.76	1.546

Table 8. Summary of Figure 1E

ELISA	Peptide-16	Peptide-17	Peptide-18	Peptide-19	Peptide-20	Peptide-21
EC50 nM	1.356	2.359	30.04	47.50	457.1	4.762

Table 9. Summary of Figure 1F

ELISA	Peptide-22	Peptide-23	Peptide-24	Peptide-25	Peptide-26	Peptide-27
EC50 nM	39.90	2168	1.916	1.948	2.012	1.833

Peptide inhibition of SP34.185 scFv by ELISA

[0397] Peptides were evaluated for their ability to inhibit SP34.185 scFv from binding CD3e by standard enzyme linked immunosorbent assays (ELISAs). Briefly, a fixed concentration of SP34.185 scFv was incubated with varying concentrations of peptides in solution. SP34.185scFv and peptide solutions were incubated for 1hr prior to addition to CD3 coated plates. Binding was allowed to proceed for 30 min prior to washing. After washing, bound SP34.185 scFv was measured using a secondary horse radish peroxidase antibody conjugate. After washing again, plates were developed using standard ELISA techniques and stopped using acid. The concentration of peptide required to inhibit 50% of the SP34.185 scFv CD3 binding signal (IC50) was calculated using Graphpad prism. Data is shown in **Figs. 2A-2F**.

Table 10. Summary of Figure 2B

ELISA	Peptide-1	Peptide-3	Peptide-4	Peptide-5	Peptide-6	Peptide-7	Peptide-8
IC50 uM	0.1926	0.1025	0.2318	0.1905	5.484	>100	>100

Table 11. Summary of Figure 2C

ELISA	Peptide-1	Peptide-9	Peptide-10	Peptide-11	Peptide-12	Peptide-13	Peptide-14
IC50 uM	0.1138	>100	63.18	>100	86.78	36.66	3.009

Table 12. Summary of Figure 2E

ELISA	Peptide-16	Peptide-17	Peptide-18	Peptide-19	Peptide-20	Peptide-21
IC50 uM	0.1473	3.333	>100	>100	>100	41.46

Table 13. Summary of Figure 2F

ELISA	Peptide-22	Peptide-23	Peptide-24	Peptide-25	Peptide-26	Peptide-27
IC50 uM	>100	>100	1.912	0.6992	1.456	0.1180

Table 14. Summary of Peptide Sequences

Peptide-ID	anti-CD3 Panned target	Sequence	SEQ ID NO:
Peptide-1	SP34.185	GSQCLGPEWEVCPY	1
Peptide-3	SP34.185	ASQCLGPEWEVCPY	19
Peptide-4	SP34.185	GAQCLGPEWEVCPY	20
Peptide-5	SP34.185	GSACLGPEWEVCPY	21
Peptide-6	SP34.185	GSQCAGPEWEVCPY	22
Peptide-7	SP34.185	GSQCLAPEWEVCPY	23
Peptide-8	SP34.185	GSQCLGAWEVCPY	24
Peptide-9	SP34.185	GSQCLGPAWEVCPY	25
Peptide-10	SP34.185	GSQCLGPEAEVCPY	26
Peptide-11	SP34.185	GSQCLGPEWAVCPY	27
Peptide-12	SP34.185	GSQCLGPEWEACPY	28
Peptide-13	SP34.185	GSQCLGPEWEVCAY	29
Peptide-14	SP34.185	GSQCLGPEWEVCPA	30
Peptide-15	SP34.185	GSQCLGPEWEVCPY	31
Peptide-2	SP34.185	VYCGPEFDES VGCM	2
Peptide-16	SP34.185	AYCGPEFDES VGCM	32
Peptide-17	SP34.185	VACGPEFDES VGCM	33

Peptide-18	SP34.185	VYCAPEFDES VGCM	34
Peptide-19	SP34.185	VYCGAEFDES VGCM	35
Peptide-20	SP34.185	VYCGPAFDES VGCM	36
Peptide-21	SP34.185	VYCGPEADES VGCM	37
Peptide-22	SP34.185	VYCGPEFAES VGCM	38
Peptide-23	SP34.185	VYCGPEFDAS VGCM	39
Peptide-24	SP34.185	VYCGPEFDEAVGCM	40
Peptide-25	SP34.185	VYCGPEFDESAGCM	41
Peptide-26	SP34.185	VYCGPEFDES VACM	42
Peptide-27	SP34.185	VYCGPEFDES VGCA	43

Example 2: Polypeptide binding to CD3 by ELISA

[0398] Peptide masks were incorporated into bispecific polypeptide molecules via tethering to SP34.185 scFv using a cleavable linker. Polypeptide molecules were then evaluated for their ability to bind CD3 before and after protease treatment via standard enzyme linked immunosorbent assays (ELISAs). Briefly, biotinylated CD3 was captured on neutravidin coated plates, quenched with biocytin, and washed. Polypeptide molecules were treated with protease when indicated. Polypeptide molecules were then titrated in buffer and added to the CD3 captured ELISA plate. After a brief incubation, plates were washed, and bound polypeptide molecules were detected using a standard secondary horse radish peroxidase conjugate antibody. After another wash, ELISA plates were developed and stopped in acid. The concentration of polypeptide required to achieve a 50% maximal signal (EC50) was calculated in Graphpad prism. Tethering of inhibitory peptides to SP34.185 scFv significantly hindered the ability to bind CD3 relative to the control polypeptide that lacks inhibitory peptide masking.

Table 15. Summary of Figure 3

	PC-1	PC-1+ MTSP1	PC-2	PC-2 + MTSP1	PC-3
EC50 nM	13.66	0.05722	13.28	0.06099	0.05401
Fold shift	252.9x	1.1x	245.9x	1.1x	1x

Example 3: Polypeptide mediated tumor cell killing

Peptide masks were incorporated into bispecific polypeptide molecules via tethering to SP34.185 scFv using a cleavable linker. Bispecific polypeptide molecules' functional activities were then evaluated before and after protease treatment via tumor cell line killing assays. Tumor cell killing was measured using an xCelligence real time cell analyzer (RTCA from Agilent) that relies on surface impedance to monitor cell growth kinetics. Briefly, tumor cell lines were seeded on E-plates and allowed to adhere overnight in a humidified incubator at 37C with 5% carbon dioxide atmosphere. After the overnight incubation, bispecific polypeptide molecules were titrated in cell culture medium and added to the plates. In addition, effector cells such as PBMCs or CD8+ T cells were added to the plate at the indicated effector cell to tumor cell ratio. Bispecific polypeptides were treated with protease when indicated. Plates were then placed back on the

RTCA inside the humidified incubator and impedance measurements (cell index) collected every 10minutes for multiple days. After multiple days, the area under the tumor growth curves (cell index times hours) were plotted against the logarithmic concentration of polypeptide molecule. The concentration of polypeptide molecule required to inhibit tumor cell growth by 50% (IC50) was calculated in Graphpad Prism. Masking of the polypeptide binding domains significantly inhibited functional tumor killing while treatment of masked polypeptides with protease restored functional tumor killing.

Table 16. Summary of Figure 4

	PC-1	PC-1+ MTSP1	PC-2	PC-2 + MTSP1	PC-3
IC50 pM	0.2463	144.3	0.1234	239.5	0.4171
Fold shift	1x	585.9x	1x	1940.8x	control

Example 4: Optimized phage library construction

[0399] Using the peptide sequence activity relationships (SAR) of Example 1, DNA oligo libraries were constructed where codons encoding critical residues within each peptide sequence were minimally mutated and codons encoding non-critical residues were heavily mutated. The resulting oligos were cloned into bacteriophage vectors used to display the SAR guided peptides via fusion to the pIII filament of the bacteriophage. The relevant vectors were then used to produce the phage optimization libraries via amplification in bacteria using standard techniques in the field.

Example 5: Panning of the optimized phage library

[0400] Once the phage optimization libraries were completed, phage libraries were bio-panned using SP34.185 scFv loaded beads. Multiple rounds of panning were performed where bacteriophage was allowed to bind to SP34.185 scFv loaded beads, washed, eluted, and amplified. Additional selective pressure was included during each round of panning using a fixed concentration of CD3, peptide-1, or peptide-2. After panning, phage infected bacteria were plated out and colonies picked into 96 well blocks. Clonal phage was then amplified and separated from bacterial cells via centrifugation. Phage containing supernatants were tested in binding ELISAs against SP34.185 scFv coated plates in the presence or absence of saturating concentration of CD3. Phage able to bind SP34.185 scFv were selected for sequence analysis if the binding signal was reduced in the presence of CD3.

Example 6: Panning ELISAs

[0401] Clonal phage were harvested as crude supernatants and screened via standard enzyme linked immunosorbent assays (ELISAs). Briefly, biotinylated SP34.185 scFv was captured on neutravidin coated plates. Prior to the addition of clonal phage, wells were incubated with blocking buffer and CD3 or blocking buffer alone. Without washing or aspirating, clonal phage supernatants were then added to the wells and incubated for a short time. Wells were then washed followed by detection of bound phage using a horse

radish peroxidase conjugated anti-M13 antibody. Clonal phage of interest were then sent for sequence analysis.

[0402] Phage panning results of Peptide-2 library sequences are shown in **Table 17**.

[0403] Peptide-29 to Peptide-40 were selected for sequence analysis and sequences are shown in **Table 18**. Peptide binding to SP34.185 scFv to CD3 is shown in **Figs. 5A-5B** and summarized in **Tables 19-20**, respectively. Peptide inhibition of SP34.185 scFv binding to CD3 by ELISA is shown in **Figs. 6A-6B** and summarized in **Tables 21-22**, respectively.

[0404] The core sequence motif for Peptide-2 is shown in **Fig. 7**.

Table 17. Clonal Phage Peptide Sequences from the Peptide-2 Optimization Library Panning (-) indicates same amino acid as in Peptide-2 corresponding position (e.g. Phage-1 position).

Phage ID	Amino acid position sequence														Phage binding ELISA			SEQ ID NO:
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	Background signal	SP34.185 scFv signal	SP34.185 scFv signal in presence of CD3	
Phage-1/Peptide 2	V	Y	C	G	P	E	F	D	E	S	V	G	C	M	0.06	2.79	0.09	56
Phage-2	D	D	-	W	-	D	W	E	F	D	F	A	-	A	0.08	2.75	0.09	57
Phage-3	Y	I	-	-	L	D	-	P	D	F	L	Y	-	D	0.08	2.88	0.10	58
Phage-4	F	D	-	W	-	D	W	E	-	Y	F	V	-	D	0.08	2.79	0.09	59
Phage-5	Y	I	-	W	-	D	W	E	-	Y	F	D	-	D	0.08	2.74	0.09	60
Phage-6	N	I	-	W	-	D	W	E	D	D	Y	F	-	F	0.09	2.54	0.09	61
Phage-7	N	F	-	W	-	D	W	E	Y	I	Y	P	-	I	0.07	2.77	0.09	62
Phage-8	-	D	-	W	-	D	W	E	-	D	F	L	-	I	0.08	2.54	0.08	63
Phage-9	H	A	-	W	-	D	W	E	-	Y	F	P	-	N	0.08	2.85	0.09	64
Phage-10	Y	D	-	-	-	D	V	-	-	-	Y	V	-	V	0.09	2.63	0.10	65
Phage-11	I	D	-	W	-	D	W	E	D	D	T	F	-	Y	0.09	2.73	0.08	66
Phage-12	Y	L	-	-	-	D	G	-	-	T	L	A	-	Y	0.08	2.66	0.15	67
Phage-13	-	D	-	-	-	D	G	-	-	-	I	L	-	Y	0.11	2.13	0.08	68
Phage-14	F	I	-	W	-	D	W	E	-	D	Y	F	-	A	0.07	2.44	0.09	69
Phage-15	G	D	-	W	-	D	W	E	W	D	F	Y	-	D	0.07	2.71	0.07	70
Phage-16	Y	L	-	W	-	D	W	E	Y	I	D	L	-	D	0.12	2.67	0.08	71
Phage-17	S	F	-	W	-	D	W	E	-	Y	F	D	-	D	0.10	2.60	0.07	72
Phage-18	D	D	-	W	-	D	W	E	-	Y	A	S	-	D	0.09	2.57	0.07	73
Phage-19	N	L	-	W	-	D	W	E	Y	P	F	F	-	D	0.09	2.52	0.09	74
Phage-20	F	D	-	W	-	D	W	E	-	-	F	V	-	D	0.08	2.34	0.09	75
Phage-21	D	I	-	-	-	D	G	-	-	T	I	I	-	D	0.13	2.30	0.10	76
Phage-22	D	D	-	W	-	D	W	E	Y	Y	A	V	-	D	0.09	2.28	0.09	77
Phage-23	Y	D	-	W	-	D	W	E	-	Y	S	N	-	D	0.10	2.17	0.08	78
Phage-24	I	N	-	W	-	D	W	E	D	Y	F	F	-	D	0.07	2.16	0.07	79

Phage-25	N	I	-	W	-	D	W	E	D	D	T	F	-	F	0.06	2.87	0.07	80
Phage-26	N	I	-	W	-	D	W	E	P	N	S	F	-	F	0.09	2.87	0.08	81
Phage-27	Y	D	-	-	-	-	M	-	-	-	I	D	-	F	0.09	2.39	0.08	82
Phage-28	D	F	-	W	-	D	W	E	F	P	F	I	-	H	0.11	2.73	0.12	83
Phage-29	D	F	-	-	-	-	M	-	-	-	I	T	-	I	0.07	2.36	0.08	84
Phage-30	Y	D	-	-	-	-	-	-	-	-	T	V	-	I	0.10	2.32	0.08	85
Phage-31	H	D	-	W	-	D	W	E	W	D	I	F	-	I	0.07	2.26	0.08	86
Phage-32	H	A	-	W	-	D	W	E	-	Y	N	P	-	N	0.11	2.71	0.11	87
Phage-33	D	V	-	W	-	D	W	E	W	D	F	F	-	N	0.08	2.65	0.08	88
Phage-34	N	-	-	W	-	D	W	E	Y	Y	I	P	-	N	0.10	2.57	0.08	89
Phage-35	I	I	-	W	-	D	W	E	F	I	D	Y	-	N	0.08	2.10	0.07	90
Phage-36	S	L	-	W	-	D	W	E	Y	D	I	A	-	P	0.07	2.53	0.08	91
Phage-37	D	L	-	-	-	-	L	-	-	-	I	F	-	P	0.08	2.49	0.09	92
Phage-38	T	N	-	W	-	D	W	E	W	V	L	P	-	P	0.14	2.47	0.10	93
Phage-39	I	E	-	W	-	D	W	E	P	N	Y	F	-	P	0.13	2.29	0.09	94
Phage-40	I	F	-	W	-	D	W	E	D	Y	-	D	-	P	0.07	2.28	0.07	95
Phage-41	I	D	-	W	-	D	W	E	Y	D	F	F	-	P	0.07	2.26	0.08	96
Phage-42	L	F	-	W	-	D	W	E	D	-	F	F	-	P	0.18	2.11	0.13	97
Phage-43	-	D	-	W	-	D	W	E	D	Y	A	D	-	T	0.11	2.20	0.10	98
Phage-44	-	I	-	W	-	D	W	E	Q	Y	F	P	-	V	0.11	2.34	0.09	99
Phage-45	I	E	-	W	-	D	W	E	P	I	Y	P	-	Y	0.09	2.85	0.09	100
Phage-46	I	T	-	W	-	D	W	E	V	Y	F	P	-	Y	0.07	2.55	0.08	101
Phage-47	I	D	-	W	-	D	W	E	Y	I	H	P	-	Y	0.06	2.51	0.09	102
Phage-48	I	D	-	W	-	D	W	E	Y	I	N	P	-	Y	0.12	2.50	0.12	103
Phage-49	A	D	-	W	-	D	W	E	-	A	F	P	-	Y	0.09	2.44	0.09	104
Phage-50	I	D	-	W	-	D	W	E	Y	I	Y	P	-	Y	0.09	2.31	0.07	105
Phage-51	N	I	-	W	-	D	W	E	D	D	N	F	-	F	0.09	2.08	0.09	106
Phage-52	Y	D	-	W	-	D	W	E	Y	V	D	A	-	Y	0.09	2.06	0.09	107
Phage-53	F	-	-	-	-	D	G	-	-	-	Y	V	-	D	0.09	2.03	0.11	108
Phage-54	D	I	-	W	-	D	W	E	Y	I	N	I	-	S	0.11	2.02	0.11	109
Phage-55	F	V	-	W	-	D	W	E	D	F	N	F	-	D	0.07	2.01	0.08	110
Phage-56	F	A	-	W	-	D	W	E	D	Y	-	A	-	D	0.07	2.01	0.09	111
Phage-57	D	N	-	W	-	D	W	E	Y	D	F	F	-	V	0.08	1.99	0.09	112
Phage-58	Y	D	-	W	-	D	W	E	-	Y	N	D	-	A	0.09	1.96	0.11	113
Phage-59	D	D	-	-	-	D	G	-	-	T	I	I	-	V	0.07	1.91	0.09	114
Phage-60	F	P	-	W	-	D	W	E	-	Y	A	I	-	D	0.10	1.89	0.10	115
Phage-61	P	D	-	-	-	D	G	-	-	-	L	F	-	T	0.12	1.86	0.07	116

Phage-62	D	N	-	W	-	D	W	E	Y	D	Y	F	-	V	0.07	1.83	0.07	117
Phage-63	I	F	-	W	-	D	W	E	-	F	Y	D	-	Y	0.12	1.82	0.08	118
Phage-64	A	D	-	W	-	D	W	E	-	Y	F	P	-	N	0.08	1.82	0.08	119
Phage-65	H	T	-	W	-	D	W	E	D	D	I	F	-	N	0.12	1.81	0.10	120
Phage-66	F	A	-	W	-	D	W	E	-	A	F	L	-	L	0.09	1.80	0.09	121
Phage-67	Y	D	-	-	-	-	L	-	-	-	I	A	-	D	0.08	1.77	0.08	122
Phage-68	N	S	-	W	-	D	W	E	Y	D	I	I	-	D	0.08	1.77	0.10	123
Phage-69	F	A	-	W	-	D	W	E	-	V	A	P	-	Y	0.07	1.75	0.07	124
Phage-70	L	D	-	-	-	D	G	-	-	T	L	T	-	Y	0.10	1.75	0.12	125
Phage-71	-	L	-	W	-	D	W	E	-	F	Y	D	-	P	0.07	1.74	0.09	126
Phage-72	H	A	-	W	-	V	W	E	-	Y	F	P	-	N	0.07	1.72	0.08	127
Phage-73	N	E	-	W	-	N	G	E	P	T	F	P	-	T	0.08	1.71	0.07	128
Phage-74	L	T	-	-	-	D	G	-	-	T	L	Y	-	D	0.08	1.70	0.07	129
Phage-75	Y	D	-	-	-	-	Y	-	-	-	-	P	-	I	0.13	1.67	0.09	130
Phage-76	I	E	-	W	-	D	W	E	P	N	S	F	-	D	0.09	1.66	0.08	131
Phage-77	Y	D	-	-	-	-	L	-	-	-	I	H	-	Y	0.12	1.66	0.09	132
Phage-78	I	-	-	-	-	-	-	-	-	-	T	I	-	N	0.08	1.63	0.08	133
Phage-79	I	-	-	-	-	-	V	E	-	A	Y	L	-	Y	0.09	1.62	0.10	134
Phage-80	F	D	-	-	-	D	G	-	-	T	-	Y	-	D	0.09	1.61	0.08	135
Phage-81	I	D	-	-	-	D	G	-	-	T	I	S	-	Y	0.08	1.57	0.11	136
Phage-82	N	-	-	-	-	-	-	-	-	-	S	T	-	L	0.10	1.55	0.11	137
Phage-83	Y	D	-	-	-	D	G	-	-	-	Y	F	-	D	0.08	1.53	0.08	138
Phage-84	N	F	-	W	-	D	W	E	Y	F	N	D	-	N	0.09	1.53	0.09	139
Phage-85	-	L	-	W	-	D	W	E	A	F	F	D	-	D	0.07	1.47	0.07	140
Phage-86	I	-	-	-	-	-	W	E	W	P	-	A	-	N	0.16	1.47	0.10	141
Phage-87	-	F	-	W	-	D	W	E	D	N	F	F	-	N	0.08	1.46	0.10	142
Phage-88	-	V	-	W	-	D	W	E	T	F	F	P	-	D	0.08	1.46	0.08	143
Phage-89	D	N	-	-	-	D	G	-	-	T	Y	I	-	N	0.10	1.45	0.09	144
Phage-90	D	N	-	W	-	D	W	E	Y	N	F	F	-	V	0.07	1.45	0.08	145
Phage-91	F	-	-	-	-	-	V	E	-	D	Y	L	-	I	0.10	1.43	0.10	146
Phage-92	D	N	-	W	-	D	W	E	Y	D	I	F	-	V	0.07	1.43	0.07	147
Phage-93	I	D	-	-	-	-	-	-	-	-	I	A	-	P	0.08	1.42	0.08	148
Phage-94	Y	F	-	-	-	-	V	E	-	Y	T	L	-	F	0.10	1.42	0.10	149
Phage-95	F	-	-	-	-	-	-	-	-	-	A	P	-	N	0.06	1.37	0.08	150
Phage-96	F	D	-	-	-	-	V	E	-	Y	F	Y	-	A	0.11	1.36	0.08	151
Phage-97	D	F	-	W	-	D	W	E	D	F	F	F	-	A	0.18	1.35	0.12	152
Phage-98	F	F	-	-	-	D	G	-	-	T	L	S	-	N	0.08	1.35	0.09	153

Phage-99	F	I	-	-	-	-	-	-	-	-	-	A	-	L	0.14	1.35	0.09	154
Phage-100	Y	D	-	-	-	-	-	-	-	A	I	-	-	Y	0.09	1.32	0.10	155
Phage-101	Y	I	-	W	-	D	W	E	-	Y	L	Y	-	P	0.10	1.32	0.15	156
Phage-102	F	D	-	W	-	D	W	E	-	P	T	T	-	H	0.08	1.31	0.08	157
Phage-103	Y	D	-	W	-	D	W	E	D	F	P	I	-	D	0.14	1.31	0.10	158
Phage-104	-	V	-	W	-	D	W	E	Y	I	D	D	-	S	0.08	1.30	0.07	159
Phage-105	I	N	-	W	-	D	W	E	V	I	S	F	-	D	0.12	1.30	0.08	160
Phage-106	L	S	-	W	-	D	W	E	-	V	T	P	-	L	0.10	1.29	0.10	161
Phage-107	F	A	-	W	-	D	W	E	-	V	D	I	-	Y	0.09	1.28	0.08	162
Phage-108	Y	D	-	-	-	-	M	-	-	-	I	V	-	D	0.10	1.25	0.08	163
Phage-109	Y	D	-	W	-	D	W	E	V	F	I	V	-	D	0.06	1.25	0.07	164
Phage-110	D	N	-	W	-	D	W	E	H	N	F	F	-	V	0.10	1.25	0.08	165
Phage-111	Y	D	-	-	-	D	G	-	-	-	I	Y	-	P	0.07	1.23	0.08	166
Phage-112	Y	D	-	-	-	-	-	E	F	P	Y	Y	-	F	0.12	1.23	0.12	167
Phage-113	A	D	-	-	-	-	Y	-	-	-	-	P	-	V	0.11	1.22	0.09	168
Phage-114	F	L	-	-	-	-	V	E	-	V	H	Y	-	S	0.08	1.22	0.10	169
Phage-115	T	D	-	W	-	D	W	E	Y	I	T	S	-	S	0.08	1.22	0.08	170
Phage-116	A	F	-	-	-	-	L	-	-	-	I	T	-	D	0.09	1.21	0.09	171
Phage-117	N	D	-	W	-	D	W	E	-	Y	F	S	-	Y	0.09	1.19	0.09	172
Phage-118	F	D	-	-	-	-	W	E	I	V	T	D	-	Y	0.08	1.19	0.09	173
Phage-119	N	L	-	-	-	-	M	-	-	-	I	I	-	P	0.13	1.19	0.11	174
Phage-120	D	L	-	-	-	-	M	-	-	-	I	Y	-	D	0.10	1.19	0.14	175
Phage-121	F	D	-	-	-	D	G	V	-	D	Y	I	-	D	0.09	1.18	0.09	176
Phage-122	Y	A	-	W	-	D	W	E	-	D	F	A	-	Y	0.11	1.18	0.08	177
Phage-123	H	D	-	-	-	-	M	-	-	-	I	V	-	V	0.10	1.17	0.10	178
Phage-124	-	F	-	-	-	-	-	E	F	I	F	L	-	A	0.07	1.17	0.08	179
Phage-125	Y	D	-	-	-	-	L	-	-	-	I	L	-	D	0.08	1.16	0.09	180
Phage-126	S	V	-	W	-	D	W	E	-	F	Y	S	-	D	0.11	1.16	0.10	181
Phage-127	P	-	-	-	-	D	G	-	-	T	A	I	-	T	0.13	1.16	0.10	182
Phage-128	D	D	-	-	-	-	L	E	W	Y	Y	P	-	Y	0.09	1.16	0.08	183
Phage-129	F	I	-	-	-	-	-	-	-	-	L	P	-	N	0.08	1.14	0.09	184
Phage-130	I	D	-	-	-	-	-	-	-	-	L	P	-	D	0.11	1.14	0.33	185
Phage-131	F	L	-	-	-	-	-	E	-	D	A	P	-	Y	0.08	1.13	0.08	186
Phage-132	I	F	-	-	-	D	G	-	-	T	H	I	-	H	0.10	1.13	0.07	187
Phage-133	-	F	-	W	-	D	W	E	Y	I	D	F	-	N	0.10	1.11	0.21	188
Phage-134	I	F	-	-	-	-	Y	-	-	-	L	H	-	I	0.12	1.11	0.11	189
Phage-135	H	L	-	W	-	D	W	E	W	Y	-	D	-	P	0.08	1.11	0.10	190

Phage-136	F	I	-	-	-	-	M	-	-	-	I	A	-	N	0.08	1.11	0.09	191
Phage-137	I	F	-	-	-	-	V	E	M	I	F	L	-	N	0.09	1.10	0.08	192
Phage-138	Y	D	-	-	-	-	W	E	F	P	-	D	-	I	0.11	1.09	0.11	193
Phage-139	N	L	-	-	-	-	L	-	-	-	I	T	-	F	0.10	1.09	0.08	194
Phage-140	F	-	-	-	-	-	V	E	D	F	Y	F	-	Y	0.08	1.09	0.08	195
Phage-141	D	-	-	-	-	-	-	-	-	-	L	I	-	N	0.11	1.07	0.11	196
Phage-142	D	-	-	-	-	-	-	-	-	-	L	P	-	D	0.08	1.07	0.08	197
Phage-143	A	I	-	-	-	-	L	-	-	-	I	A	-	P	0.09	1.07	0.09	198
Phage-144	-	I	-	-	-	-	V	E	D	Y	N	L	-	Y	0.08	1.07	0.09	199
Phage-145	H	T	-	W	-	D	W	E	D	Y	T	V	-	P	0.10	1.06	0.09	200
Phage-146	S	D	-	W	-	D	W	E	Y	F	Y	D	-	N	0.10	1.06	0.08	201
Phage-147	-	F	-	-	-	D	G	-	-	T	-	H	-	D	0.09	1.05	0.08	202
Phage-148	D	-	-	-	-	-	Y	-	-	-	-	H	-	I	0.09	1.05	0.08	203
Phage-149	A	D	-	-	-	D	G	-	-	-	I	I	-	H	0.07	1.05	0.08	204
Phage-150	F	-	-	-	-	-	L	-	-	-	L	T	-	V	0.10	1.05	0.08	205
Phage-151	I	L	-	-	-	-	V	E	-	D	Y	Y	-	Y	0.11	1.04	0.09	206
Phage-152	H	L	-	W	-	D	W	E	-	Y	H	S	-	D	0.09	1.04	0.09	207
Phage-153	I	F	-	W	-	D	W	E	D	Y	N	F	-	T	0.08	1.04	0.11	208
Phage-154	I	V	-	-	-	D	G	-	-	T	L	I	-	H	0.12	1.04	0.11	209
Phage-155	A	D	-	W	-	D	W	E	W	D	Y	T	-	D	0.12	1.03	0.11	210
Phage-156	I	T	-	-	-	-	-	-	-	-	T	T	-	N	0.20	1.02	0.21	211
Phage-157	Y	H	-	W	-	D	W	E	-	Y	T	S	-	D	0.20	1.02	0.09	212
Phage-158	N	-	-	-	-	-	V	E	-	Y	A	L	-	T	0.11	1.01	0.10	213
Phage-159	F	I	-	-	-	-	M	-	-	-	I	H	-	D	0.15	1.00	0.19	214
Phage-160	D	N	-	W	-	D	W	E	-	F	A	V	-	P	0.14	1.00	0.10	215
Phage-161	Y	D	-	-	-	-	L	-	-	T	-	V	-	D	0.10	1.00	0.09	216
Phage-162	Y	D	-	-	-	-	-	-	-	-	I	A	-	Y	0.08	0.99	0.08	217
Phage-163	I	D	-	W	-	D	W	E	Y	T	-	H	-	D	0.07	0.97	0.09	218
Phage-164	D	D	-	-	-	-	L	-	-	-	I	I	-	I	0.09	0.96	0.09	219
Phage-165	-	-	-	-	-	-	Y	-	-	-	S	F	-	F	0.09	0.91	0.08	220
Phage-166	F	N	-	W	-	D	W	E	D	P	Y	F	-	V	0.09	0.86	0.07	221
Phage-167	Y	D	-	-	-	-	Y	-	-	-	S	Y	-	S	0.08	0.82	0.07	222
Phage-168	-	A	-	W	-	D	W	E	Y	T	D	S	-	F	0.13	0.79	0.09	223
Phage-169	T	D	-	-	-	-	-	-	-	-	-	A	-	Y	0.10	0.77	0.09	224
Phage-170	T	D	-	W	-	D	W	E	F	Y	A	D	-	D	0.07	0.75	0.08	225
Phage-171	Y	D	-	-	-	-	L	-	-	-	-	I	-	H	0.09	0.69	0.09	226
Phage-172	S	D	-	-	-	D	G	-	-	-	I	I	-	T	0.07	0.69	0.07	227

Phage-173	Y	-	-	-	-	-	-	-	-	-	I	D	-	D	0.08	0.67	0.09	228
Phage-174	F	F	-	-	-	-	I	-	-	-	I	A	-	V	0.08	0.62	0.09	229
Phage-175	D	-	-	-	-	-	-	-	-	-	T	F	-	D	0.16	0.60	0.10	230
Phage-176	Y	D	-	-	-	-	W	E	W	P	I	D	-	V	0.10	0.59	0.10	231
Phage-177	F	-	-	-	-	-	I	E	L	F	S	F	-	Y	0.13	0.59	0.11	232
Phage-178	Y	-	-	-	-	-	V	-	-	-	I	T	-	P	0.15	0.42	0.11	233
Phage-179	I	L	-	-	-	-	-	-	-	-	I	N	-	N	0.09	0.37	0.25	234
Phage-180	-	V	-	-	-	A	M	G	Q	H	Y	L	-	D	0.08	0.09	0.08	235
Phage-181	-	V	-	-	T	K	M	G	-	H	Y	L	-	S	0.08	0.08	0.08	236
Phage-182	Y	D	-	W	-	D	W	E	Y	V	Y	A	-	Y	0.08	0.98	0.08	237
Phage-183	D	L	-	-	-	-	L	-	-	-	-	N	-	D	0.09	0.98	0.08	238
Phage-184	Y	-	-	-	-	-	-	-	-	-	T	V	-	Y	0.14	0.97	0.16	239
Phage-185	L	D	-	W	-	D	W	E	W	P	Y	S	-	N	0.08	0.96	0.09	240
Phage-186	F	I	-	W	-	D	W	E	D	D	F	F	-	Y	0.08	0.96	0.09	241
Phage-187	D	L	-	-	-	-	V	E	W	Y	F	F	-	N	0.11	0.95	0.10	242
Phage-188	Y	D	-	-	-	-	L	-	-	-	I	V	-	F	0.07	0.94	0.08	243
Phage-189	L	N	-	W	-	V	W	E	D	D	-	F	-	Y	0.09	0.92	0.09	244
Phage-190	F	N	-	W	-	D	W	E	D	P	N	F	-	V	0.09	0.91	0.09	245
Phage-191	-	I	-	W	-	D	W	E	D	D	Y	F	-	P	0.10	0.91	0.13	246
Phage-192	F	L	-	-	-	-	-	-	-	-	S	V	-	Y	0.10	0.91	0.08	247
Phage-193	Y	D	-	-	-	-	L	-	-	-	I	F	-	Y	0.10	0.91	0.09	248
Phage-194	H	L	-	-	-	D	G	-	-	-	F	T	-	F	0.11	0.90	0.10	249
Phage-195	Y	F	-	-	-	-	M	-	-	-	L	Y	-	I	0.08	0.90	0.08	250
Phage-196	Y	-	-	-	-	-	V	E	-	Y	A	N	-	Y	0.07	0.90	0.07	251
Phage-197	N	T	-	-	-	-	-	-	-	-	T	A	-	Y	0.16	0.90	0.58	252
Phage-198	I	D	-	W	-	D	W	E	-	A	F	N	-	Y	0.09	0.90	0.08	253
Phage-199	A	-	-	-	-	-	L	E	-	F	F	L	-	T	0.09	0.89	0.08	254
Phage-200	I	-	-	-	-	-	V	E	-	V	H	H	-	Y	0.08	0.89	0.08	255
Phage-201	F	F	-	-	-	-	-	-	-	-	-	A	-	D	0.10	0.89	0.11	256
Phage-202	Y	D	-	-	-	-	L	-	-	T	I	I	-	A	0.08	0.89	0.08	257
Phage-203	I	L	-	-	-	-	W	E	Y	P	L	D	-	S	0.09	0.89	0.10	258
Phage-204	F	I	-	-	-	-	-	-	-	-	T	T	-	N	0.09	0.88	0.10	259
Phage-205	F	-	-	-	-	-	L	-	-	-	-	S	-	D	0.17	0.88	0.15	260
Phage-206	H	L	-	-	-	-	L	-	-	-	-	T	-	F	0.10	0.87	0.10	261
Phage-207	L	I	-	-	-	-	V	E	D	Y	S	L	-	H	0.09	0.87	0.09	262
Phage-208	Y	F	-	-	-	-	M	-	-	-	-	Y	-	D	0.08	0.87	0.08	263
Phage-209	H	-	-	-	-	-	M	-	-	-	I	Y	-	I	0.13	0.87	0.09	264

Phage-210	F	D	-	-	-	-	L	-	-	-	I	N	-	D	0.08	0.87	0.09	265
Phage-211	Y	-	-	-	-	-	V	E	-	Y	I	Y	-	T	0.07	0.87	0.08	266
Phage-212	L	A	-	W	-	V	R	E	-	I	N	A	-	I	0.08	0.85	0.07	267
Phage-213	I	D	-	W	-	D	W	E	D	I	T	F	-	D	0.08	0.85	0.08	268
Phage-214	I	V	-	-	-	-	L	-	-	-	I	T	-	P	0.11	0.85	0.15	269
Phage-215	F	-	-	-	-	-	-	E	L	P	A	D	-	D	0.08	0.85	0.09	270
Phage-216	F	D	-	-	-	-	-	-	-	-	N	P	-	F	0.10	0.85	0.09	271
Phage-217	D	A	-	W	-	D	W	E	-	Y	S	S	-	D	0.10	0.83	0.10	272
Phage-218	D	H	-	W	-	D	W	E	P	N	Y	F	-	V	0.08	0.83	0.09	273
Phage-219	D	-	-	W	-	D	W	E	I	N	Y	I	-	F	0.09	0.83	0.10	274
Phage-220	I	-	-	W	-	D	W	E	Y	V	Y	A	-	N	0.10	0.82	0.09	275
Phage-221	D	F	-	-	-	-	V	E	-	D	Y	L	-	D	0.07	0.82	0.08	276
Phage-222	H	D	-	-	-	D	G	R	-	D	Y	D	-	A	0.11	0.82	0.09	277
Phage-223	L	A	-	W	-	D	W	E	D	D	Y	F	-	V	0.08	0.82	0.09	278
Phage-224	D	I	-	W	-	D	W	E	D	Y	L	P	-	V	0.10	0.82	0.10	279
Phage-225	I	L	-	-	-	-	I	E	V	Y	A	L	-	P	0.08	0.81	0.10	280
Phage-226	I	F	-	-	-	-	W	E	F	-	-	L	-	N	0.10	0.81	0.11	281
Phage-227	T	-	-	-	-	-	V	E	D	F	S	L	-	V	0.07	0.80	0.08	282
Phage-228	F	I	-	-	-	-	W	E	F	V	D	A	-	F	0.11	0.80	0.09	283
Phage-229	F	A	-	W	-	D	W	E	-	D	S	P	-	D	0.06	0.80	0.07	284
Phage-230	I	L	-	-	-	-	V	E	-	L	I	F	-	P	0.12	0.80	0.08	285
Phage-231	F	-	-	-	-	-	V	E	-	Y	I	Y	-	Y	0.08	0.80	0.08	286
Phage-232	D	S	-	-	-	-	L	-	-	-	I	I	-	D	0.10	0.79	0.09	287
Phage-233	F	L	-	-	-	D	G	-	-	T	S	V	-	D	0.11	0.79	0.08	288
Phage-234	F	N	-	W	-	N	G	E	P	T	Y	F	-	V	0.11	0.79	0.08	289
Phage-235	L	A	-	W	-	V	W	E	Y	P	-	T	-	I	0.09	0.78	0.09	290
Phage-236	D	-	-	-	-	-	V	E	-	D	-	Y	-	Y	0.09	0.78	0.09	291
Phage-237	I	T	-	W	-	D	W	E	-	Y	A	N	-	T	0.08	0.77	0.07	292
Phage-238	F	F	-	-	-	D	G	-	-	T	Y	S	-	I	0.15	0.77	0.13	293
Phage-239	T	D	-	W	-	D	W	E	Y	A	T	S	-	D	0.09	0.76	0.09	294
Phage-240	F	N	-	-	-	D	G	Y	-	D	Y	L	-	D	0.10	0.76	0.11	295
Phage-241	Y	D	-	W	-	D	W	E	V	D	F	H	-	P	0.11	0.76	0.08	296
Phage-242	N	I	-	W	-	D	W	E	D	D	S	F	-	F	0.08	0.76	0.08	297
Phage-243	A	T	-	-	-	-	-	-	-	-	I	-	-	S	0.13	0.75	0.09	298
Phage-244	S	-	-	-	-	-	-	-	-	-	T	F	-	D	0.10	0.74	0.09	299
Phage-245	P	I	-	-	-	-	Y	-	-	-	D	V	-	A	0.08	0.74	0.08	300
Phage-246	Y	-	-	-	-	D	G	-	-	Y	N	S	-	I	0.11	0.74	0.11	301

Phage-247	-	D	-	W	-	D	W	E	V	F	I	A	-	D	0.11	0.74	0.10	302
Phage-248	D	L	-	-	-	-	V	E	-	V	N	L	-	L	0.12	0.74	0.10	303
Phage-249	F	D	-	-	-	-	M	-	-	-	T	T	-	F	0.09	0.74	0.09	304
Phage-250	N	F	-	W	-	D	W	E	P	I	Y	F	-	T	0.13	0.74	0.14	305
Phage-251	-	D	-	-	-	D	G	-	-	-	F	F	-	L	0.08	0.73	0.08	306
Phage-252	-	D	-	-	-	D	G	-	-	T	A	F	-	I	0.10	0.73	0.09	307
Phage-253	N	I	-	-	-	-	M	-	-	-	L	V	-	I	0.10	0.73	0.09	308
Phage-254	I	I	-	-	-	-	-	-	-	-	F	F	-	F	0.08	0.73	0.09	309
Phage-255	N	F	-	-	-	-	Y	-	-	-	I	S	-	I	0.10	0.73	0.38	310
Phage-256	H	L	-	-	-	-	I	E	-	A	D	I	-	N	0.11	0.73	0.51	311
Phage-257	D	-	-	-	-	-	V	E	-	D	Y	L	-	D	0.08	0.72	0.09	312
Phage-258	D	-	-	-	-	-	L	-	-	-	I	N	-	D	0.11	0.72	0.10	313
Phage-259	F	-	-	-	-	-	L	-	-	-	L	F	-	V	0.09	0.72	0.08	314
Phage-260	P	D	-	-	-	-	W	E	F	Y	-	T	-	N	0.12	0.72	0.08	315
Phage-261	F	D	-	-	-	-	-	E	Y	I	Y	A	-	T	0.09	0.72	0.08	316
Phage-262	D	-	-	-	-	-	-	-	-	-	S	I	-	N	0.12	0.72	0.11	317
Phage-263	D	F	-	-	-	-	V	E	-	Y	I	F	-	F	0.08	0.72	0.07	318
Phage-264	P	V	-	W	-	D	W	E	Y	V	S	S	-	D	0.08	0.71	0.08	319
Phage-265	Y	I	-	-	-	-	R	-	-	-	N	L	-	L	0.09	0.71	0.09	320
Phage-266	Y	D	-	-	-	-	L	-	-	-	I	V	-	D	0.11	0.71	0.11	321
Phage-267	H	D	-	W	-	D	W	E	D	F	Y	F	-	V	0.09	0.71	0.08	322
Phage-268	H	-	-	-	-	-	Y	-	-	-	I	D	-	Y	0.12	0.71	0.10	323
Phage-269	L	F	-	-	-	-	M	P	-	D	I	F	-	N	0.08	0.71	0.08	324
Phage-270	H	D	-	-	-	-	L	E	F	H	Y	A	-	Y	0.10	0.71	0.12	325
Phage-271	D	F	-	-	-	-	L	-	-	-	I	N	-	F	0.08	0.70	0.08	326
Phage-272	Y	F	-	-	-	-	L	-	-	-	I	A	-	N	0.10	0.70	0.10	327
Phage-273	T	D	-	W	-	D	W	E	D	D	I	I	-	D	0.10	0.70	0.08	328
Phage-274	Y	D	-	-	-	-	L	-	-	-	I	Y	-	F	0.09	0.70	0.08	329
Phage-275	Y	-	-	W	-	D	W	W	-	Y	-	T	-	D	0.10	0.70	0.11	330
Phage-276	P	I	-	-	-	-	L	E	-	-	Y	L	-	N	0.13	0.69	0.52	331
Phage-277	F	D	-	-	-	-	-	-	-	-	I	V	-	Y	0.12	0.69	0.11	332
Phage-278	-	L	-	-	-	D	G	I	-	F	F	D	-	P	0.09	0.68	0.07	333
Phage-279	A	-	-	-	-	-	Y	-	-	-	L	T	-	V	0.07	0.68	0.07	334
Phage-280	D	F	-	-	-	-	L	-	-	-	I	I	-	A	0.14	0.68	0.14	335
Phage-281	Y	D	-	-	-	-	-	-	-	-	L	D	-	N	0.08	0.68	0.08	336
Phage-282	A	I	-	-	-	-	-	-	-	-	-	A	-	D	0.14	0.67	0.08	337
Phage-283	L	L	-	-	-	D	G	V	-	D	F	F	-	D	0.10	0.67	0.10	338

Phage-284	N	F	-	-	-	-	L	P	-	D	I	F	-	F	0.12	0.66	0.13	339
Phage-285	F	-	-	-	-	-	V	E	-	V	S	L	-	N	0.08	0.66	0.08	340
Phage-286	Y	D	-	-	-	D	G	Y	-	A	F	Y	-	H	0.12	0.66	0.10	341
Phage-287	N	F	-	-	-	-	I	E	F	D	Y	L	-	D	0.08	0.65	0.08	342
Phage-288	D	-	-	-	-	D	G	V	-	D	F	I	-	N	0.06	0.65	0.08	343
Phage-289	T	D	-	W	-	D	W	E	Y	I	Y	S	-	S	0.08	0.65	0.07	344
Phage-290	F	-	-	-	-	-	-	E	-	I	T	N	-	I	0.14	0.65	0.11	345
Phage-291	F	D	-	W	-	D	W	E	-	-	F	F	-	H	0.07	0.64	0.08	346
Phage-292	D	F	-	-	-	D	G	-	-	-	-	F	-	P	0.08	0.63	0.08	347
Phage-293	H	N	-	-	-	-	L	-	-	-	L	V	-	D	0.13	0.63	0.09	348
Phage-294	I	-	-	-	-	D	G	A	-	D	Y	T	-	D	0.07	0.63	0.07	349
Phage-295	F	D	-	-	-	-	-	E	F	P	-	I	-	F	0.08	0.62	0.08	350
Phage-296	Y	N	-	-	-	-	L	-	-	-	-	T	-	D	0.09	0.62	0.08	351
Phage-297	F	D	-	-	-	-	L	-	-	-	I	H	-	A	0.07	0.62	0.08	352
Phage-298	D	I	-	-	-	-	V	E	-	Y	F	L	-	F	0.15	0.61	0.10	353
Phage-299	F	D	-	-	-	-	V	-	-	-	L	T	-	F	0.10	0.61	0.09	354
Phage-300	F	D	-	-	-	-	I	E	-	F	H	L	-	F	0.08	0.61	0.08	355
Phage-301	A	-	-	-	-	-	L	-	-	-	I	I	-	D	0.12	0.61	0.10	356
Phage-302	Y	N	-	-	-	-	L	-	-	-	I	T	-	N	0.09	0.61	0.10	357
Phage-303	F	D	-	W	-	D	W	E	-	P	-	D	-	L	0.08	0.61	0.07	358
Phage-304	D	V	-	-	-	-	L	-	-	-	-	L	-	P	0.08	0.60	0.08	359
Phage-305	N	-	-	-	-	-	L	-	-	-	L	P	-	P	0.09	0.60	0.08	360
Phage-306	Y	-	-	W	-	D	W	E	Y	D	I	F	-	S	0.08	0.60	0.10	361
Phage-307	D	D	-	-	-	-	-	-	-	-	T	Y	-	N	0.08	0.60	0.09	362
Phage-308	F	-	-	-	-	-	-	E	-	V	F	H	-	Y	0.09	0.60	0.09	363
Phage-309	T	D	-	W	-	D	W	E	-	Y	F	L	-	D	0.07	0.60	0.09	364
Phage-310	-	-	-	-	-	-	W	E	-	-	Y	L	-	P	0.09	0.60	0.09	365
Phage-311	D	D	-	-	-	N	G	Y	A	T	F	I	-	Y	0.06	0.59	0.08	366
Phage-312	F	L	-	-	-	-	I	E	D	D	T	H	-	Y	0.16	0.59	0.38	367
Phage-313	F	A	-	W	-	D	W	E	-	T	I	P	-	H	0.08	0.59	0.08	368
Phage-314	-	L	-	-	-	-	-	-	-	-	Y	N	-	Y	0.10	0.58	0.08	369
Phage-315	Y	D	-	-	-	-	-	-	-	-	I	S	-	I	0.09	0.58	0.09	370
Phage-316	Y	D	-	-	-	-	-	-	-	-	-	N	-	Y	0.12	0.57	0.10	371
Phage-317	A	I	-	W	-	D	W	E	-	F	-	D	-	Y	0.10	0.57	0.09	372
Phage-318	L	T	-	W	-	V	R	E	-	I	F	A	-	D	0.07	0.57	0.08	373
Phage-319	-	L	-	-	-	-	-	-	-	-	Y	Y	-	N	0.09	0.57	0.09	374
Phage-320	N	V	-	-	-	-	Y	-	-	-	A	P	-	N	0.07	0.56	0.08	375

Phage-321	H	D	-	-	-	-	-	-	-	-	I	S	-	V	0.11	0.55	0.09	376
Phage-322	F	D	-	-	-	-	L	-	-	T	-	D	-	N	0.12	0.55	0.41	377
Phage-323	Y	F	-	-	-	-	V	E	-	H	F	Y	-	Y	0.09	0.55	0.08	378
Phage-324	D	-	-	-	-	-	L	-	-	-	I	I	-	H	0.09	0.54	0.09	379
Phage-325	D	D	-	-	-	-	V	P	-	D	I	T	-	Y	0.11	0.54	0.08	380
Phage-326	D	N	-	-	-	-	L	-	-	-	-	V	-	D	0.10	0.54	0.08	381
Phage-327	-	H	-	W	-	D	W	E	P	N	Y	V	-	D	0.10	0.54	0.08	382
Phage-328	D	-	-	-	-	-	L	-	-	-	L	F	-	L	0.09	0.53	0.09	383
Phage-329	D	D	-	-	-	-	L	-	-	-	-	V	-	A	0.08	0.53	0.11	384
Phage-330	A	A	-	-	-	-	L	-	-	-	I	V	-	D	0.13	0.53	0.09	385
Phage-331	D	F	-	-	-	-	-	E	-	I	N	N	-	F	0.14	0.52	0.48	386
Phage-332	Y	-	-	-	-	-	-	-	-	-	N	A	-	Y	0.08	0.52	0.07	387
Phage-333	-	L	-	-	-	-	-	-	-	-	N	S	-	Y	0.10	0.52	0.11	388
Phage-334	Y	D	-	-	-	-	-	-	-	-	I	D	-	D	0.09	0.52	0.09	389
Phage-335	D	S	-	-	-	-	-	E	F	Y	Y	V	-	F	0.11	0.52	0.14	390
Phage-336	Y	I	-	-	-	-	L	-	-	-	L	I	-	H	0.10	0.52	0.08	391
Phage-337	F	D	-	-	-	-	V	E	-	D	Y	F	-	Y	0.11	0.52	0.10	392
Phage-338	F	D	-	-	-	-	Y	-	-	-	L	Y	-	F	0.08	0.52	0.07	393
Phage-339	-	L	-	-	-	D	G	-	-	Y	S	F	-	H	0.10	0.51	0.10	394
Phage-340	I	P	-	-	-	-	M	-	-	-	-	V	-	N	0.12	0.51	0.09	395
Phage-341	I	I	-	-	-	D	G	Y	-	D	F	T	-	D	0.12	0.51	0.09	396
Phage-342	I	F	-	-	-	-	L	-	-	-	I	I	-	Y	0.11	0.51	0.08	397
Phage-343	-	D	-	-	-	-	-	-	-	-	Y	D	-	Y	0.18	0.51	0.18	398
Phage-344	L	S	-	-	-	-	M	-	-	-	L	Y	-	D	0.12	0.51	0.08	399
Phage-345	Y	D	-	W	-	D	W	E	Y	N	I	D	-	T	0.08	0.51	0.09	400
Phage-346	N	H	-	-	-	D	G	-	-	T	I	V	-	F	0.09	0.51	0.08	401
Phage-347	N	F	-	-	-	-	L	-	-	-	I	P	-	H	0.11	0.50	0.12	402
Phage-348	F	H	-	-	-	-	I	E	-	Y	A	L	-	D	0.08	0.50	0.09	403
Phage-349	-	-	-	-	-	-	V	E	D	Y	N	L	-	Y	0.07	0.50	0.08	404
Phage-350	-	-	-	-	-	D	G	-	-	L	A	N	-	Y	0.09	0.50	0.08	405
Phage-351	L	I	-	-	-	V	I	A	-	D	L	P	-	N	0.17	0.50	0.26	406
Phage-352	D	I	-	-	-	-	I	P	-	D	-	S	-	D	0.10	0.50	0.08	407
Phage-353	I	-	-	-	-	-	W	E	-	A	D	Y	-	D	0.11	0.50	0.45	408
Phage-354	I	D	-	W	-	D	W	E	D	D	S	I	-	Y	0.10	0.50	0.10	409
Phage-355	-	L	-	-	-	-	V	E	D	F	T	L	-	D	0.09	0.50	0.11	410
Phage-356	L	-	-	-	-	V	I	E	-	I	Y	Y	-	Y	0.09	0.49	0.09	411
Phage-357	F	F	-	-	-	-	-	E	V	H	S	D	-	N	0.14	0.49	0.38	412

Phage-358	N	D	-	-	-	-	V	E	L	V	S	D	-	N	0.10	0.49	0.08	413
Phage-359	D	L	-	-	-	-	L	-	-	-	T	V	-	D	0.09	0.49	0.08	414
Phage-360	I	P	-	-	-	-	V	E	D	Y	N	L	-	N	0.08	0.49	0.08	415
Phage-361	Y	-	-	-	-	-	L	E	W	P	-	V	-	N	0.10	0.49	0.10	416
Phage-362	Y	D	-	-	-	-	L	-	-	-	-	I	-	N	0.08	0.49	0.10	417
Phage-363	-	-	-	-	-	D	G	-	-	-	F	D	-	A	0.08	0.49	0.08	418
Phage-364	N	D	-	-	-	-	W	E	D	T	Y	F	-	L	0.08	0.49	0.10	419
Phage-365	P	-	-	-	-	-	M	E	-	L	S	N	-	S	0.13	0.48	0.16	420
Phage-366	D	D	-	-	-	-	-	E	V	I	S	D	-	Y	0.15	0.48	0.10	421
Phage-367	D	L	-	-	-	-	-	P	-	D	-	P	-	D	0.08	0.48	0.08	422
Phage-368	I	-	-	-	-	-	-	-	-	-	F	V	-	Y	0.11	0.48	0.10	423
Phage-369	A	-	-	-	-	-	Y	E	V	F	A	D	-	N	0.10	0.48	0.11	424
Phage-370	I	D	-	-	-	-	Y	-	-	-	-	D	-	L	0.09	0.48	0.09	425
Phage-371	H	I	-	W	-	D	W	E	-	F	H	D	-	N	0.07	0.48	0.08	426
Phage-372	Y	D	-	-	-	-	L	-	-	T	I	T	-	L	0.08	0.48	0.08	427
Phage-373	Y	L	-	-	-	-	L	-	-	T	I	L	-	N	0.09	0.48	0.08	428
Phage-374	F	F	-	-	-	-	-	E	-	A	F	L	-	F	0.10	0.48	0.14	429
Phage-375	I	L	-	-	-	-	L	-	-	-	F	T	-	A	0.07	0.47	0.08	430
Phage-376	F	H	-	-	-	-	V	E	L	Y	T	D	-	N	0.09	0.47	0.08	431
Phage-377	N	L	-	-	-	-	V	E	-	Y	N	F	-	Y	0.08	0.47	0.08	432
Phage-378	F	D	-	-	-	-	V	E	-	T	Y	Y	-	F	0.21	0.47	0.09	433
Phage-379	-	F	-	-	-	-	-	E	-	D	H	Y	-	Y	0.12	0.47	0.37	434
Phage-380	A	I	-	-	-	-	W	E	V	V	A	D	-	N	0.11	0.47	0.11	435
Phage-381	F	I	-	W	-	D	W	E	-	D	N	Y	-	N	0.12	0.47	0.25	436
Phage-382	I	-	-	-	-	-	-	-	-	-	F	I	-	D	0.08	0.47	0.10	437
Phage-383	N	L	-	-	-	-	V	E	D	V	Y	D	-	H	0.12	0.47	0.43	438
Phage-384	H	-	-	-	-	-	V	E	-	Y	H	N	-	N	0.09	0.47	0.09	439
Phage-385	D	I	-	-	-	-	Y	-	-	-	Y	S	-	T	0.09	0.47	0.09	440
Phage-386	D	-	-	-	-	-	L	-	-	T	L	I	-	A	0.13	0.46	0.10	441
Phage-387	I	A	-	-	-	-	M	P	-	D	I	D	-	Y	0.12	0.46	0.09	442
Phage-388	I	D	-	-	-	-	L	-	-	-	I	F	-	D	0.10	0.46	0.10	443
Phage-389	Y	F	-	-	-	D	V	E	-	D	F	A	-	D	0.11	0.46	0.11	444
Phage-390	Y	N	-	-	-	-	W	E	Y	A	I	L	-	D	0.12	0.46	0.34	445
Phage-391	I	-	-	-	-	-	V	E	D	Y	I	V	-	N	0.14	0.46	0.22	446
Phage-392	Y	D	-	-	-	-	I	-	-	-	T	P	-	A	0.07	0.46	0.07	447
Phage-393	D	T	-	W	-	D	W	E	H	I	Y	A	-	D	0.09	0.46	0.09	448
Phage-394	D	I	-	-	-	-	M	-	-	-	-	T	-	N	0.13	0.45	0.12	449

Phage-395	Y	D	-	W	-	D	W	E	R	Y	F	P	-	I	0.10	0.45	0.09	450
Phage-396	H	L	-	-	-	-	L	-	-	-	-	A	-	S	0.13	0.45	0.11	451
Phage-397	Y	D	-	-	-	D	G	-	-	T	T	I	-	A	0.09	0.45	0.09	452
Phage-398	Y	-	-	-	-	-	Y	E	D	V	L	D	-	F	0.07	0.45	0.08	453
Phage-399	D	F	-	-	-	-	M	-	-	T	I	S	-	D	0.14	0.45	0.10	454
Phage-400	I	L	-	-	-	-	L	-	-	-	L	V	-	D	0.08	0.44	0.07	455
Phage-401	L	I	-	-	-	-	W	E	V	I	T	N	-	D	0.12	0.44	0.40	456
Phage-402	Y	D	-	-	-	-	-	-	-	Y	F	-	-	P	0.09	0.44	0.09	457
Phage-403	A	L	-	-	-	-	V	E	V	Y	D	V	-	V	0.08	0.44	0.08	458
Phage-404	Y	H	-	W	-	D	W	E	D	V	N	F	-	Y	0.10	0.44	0.09	459
Phage-405	F	L	-	-	-	M	G	G	L	T	F	Y	-	Y	0.09	0.44	0.08	460
Phage-406	I	I	-	-	-	-	-	-	-	Y	-	-	-	F	0.09	0.43	0.19	461
Phage-407	F	F	-	-	-	-	M	-	-	-	-	H	-	F	0.11	0.43	0.09	462
Phage-408	A	F	-	-	-	-	-	-	-	-	L	F	-	A	0.09	0.43	0.09	463
Phage-409	N	-	-	-	-	D	G	-	-	T	N	I	-	D	0.08	0.43	0.08	464
Phage-410	Y	L	-	-	-	-	W	E	W	V	H	N	-	L	0.13	0.43	0.09	465
Phage-411	A	T	-	-	-	D	G	-	-	-	H	I	-	A	0.08	0.43	0.09	466
Phage-412	-	-	-	-	-	-	V	E	V	L	D	Y	-	D	0.07	0.42	0.08	467
Phage-413	I	H	-	-	-	-	W	E	F	Y	T	D	-	D	0.08	0.42	0.08	468
Phage-414	D	D	-	-	-	-	L	-	-	T	-	A	-	D	0.13	0.42	0.32	469
Phage-415	Y	L	-	-	-	-	-	-	-	-	I	D	-	N	0.11	0.42	0.17	470
Phage-416	L	L	-	-	-	-	V	E	D	V	F	A	-	Y	0.09	0.42	0.09	471
Phage-417	Y	D	-	-	-	-	L	-	-	-	L	T	-	D	0.08	0.42	0.08	472
Phage-418	F	D	-	-	-	-	L	-	-	T	-	N	-	Y	0.09	0.41	0.09	473
Phage-419	F	A	-	W	-	D	W	E	-	I	N	D	-	H	0.08	0.41	0.09	474
Phage-420	Y	-	-	-	-	-	Y	E	-	D	I	Y	-	N	0.09	0.41	0.09	475
Phage-421	N	V	-	-	-	-	V	E	D	Y	T	F	-	Y	0.09	0.40	0.08	476
Phage-422	A	-	-	-	-	-	L	E	-	Y	D	F	-	T	0.12	0.40	0.08	477
Phage-423	F	D	-	-	-	-	I	-	-	-	T	I	-	T	0.08	0.40	0.09	478
Phage-424	N	L	-	-	-	-	L	-	-	T	L	V	-	A	0.10	0.40	0.09	479
Phage-425	Y	S	-	W	-	D	W	E	-	Y	L	A	-	N	0.08	0.40	0.08	480
Phage-426	G	I	-	-	-	-	V	E	D	Y	N	Y	-	D	0.09	0.40	0.10	481
Phage-427	F	F	-	-	-	-	L	-	-	-	-	N	-	H	0.07	0.40	0.07	482
Phage-428	Y	-	-	-	-	-	Y	E	-	D	F	Y	-	F	0.11	0.40	0.09	483
Phage-429	L	-	-	-	-	-	Y	-	-	-	T	D	-	Y	0.11	0.40	0.10	484
Phage-430	D	-	-	-	-	-	V	E	-	D	F	L	-	Y	0.10	0.40	0.08	485
Phage-431	T	L	-	-	-	-	V	E	L	Y	I	F	-	D	0.08	0.40	0.09	486

Phage-432	F	-	-	-	-	-	-	E	Q	I	A	D	-	Y	0.11	0.40	0.09	487
Phage-433	D	D	-	-	-	-	V	E	-	Y	H	L	-	D	0.11	0.40	0.18	488
Phage-434	D	-	-	-	-	-	L	E	D	V	T	L	-	H	0.13	0.39	0.09	489
Phage-435	-	L	-	-	-	-	V	E	D	V	N	L	-	Y	0.09	0.39	0.08	490
Phage-436	D	I	-	-	-	-	L	-	-	T	I	D	-	Y	0.09	0.39	0.09	491
Phage-437	T	-	-	-	-	-	V	E	-	D	I	N	-	Y	0.08	0.39	0.08	492
Phage-438	I	V	-	W	-	D	W	E	-	Y	P	N	-	D	0.08	0.39	0.08	493
Phage-439	S	D	-	-	-	-	L	-	-	-	I	I	-	T	0.11	0.39	0.10	494
Phage-440	Y	D	-	-	-	-	L	P	-	D	Y	D	-	N	0.15	0.39	0.10	495
Phage-441	N	L	-	W	-	D	W	E	-	Y	Y	A	-	D	0.12	0.39	0.17	496
Phage-442	D	D	-	-	-	-	L	-	-	-	L	P	-	H	0.10	0.39	0.08	497
Phage-443	S	L	-	-	-	D	G	Q	-	D	Y	T	-	F	0.08	0.39	0.08	498
Phage-444	L	I	-	W	-	D	W	E	-	Y	N	F	-	T	0.13	0.39	0.11	499
Phage-445	F	H	-	-	-	D	G	-	-	T	-	P	-	I	0.08	0.39	0.08	500
Phage-446	F	D	-	-	-	-	W	E	W	I	Y	D	-	F	0.08	0.38	0.08	501
Phage-447	I	-	-	-	-	-	W	-	-	-	L	D	-	D	0.08	0.38	0.09	502
Phage-448	L	I	-	-	-	-	I	-	-	-	A	S	-	N	0.10	0.38	0.11	503
Phage-449	T	S	-	W	V	D	W	E	-	F	S	D	-	I	0.11	0.38	0.34	504
Phage-450	Y	-	-	-	-	-	V	E	-	D	Y	V	-	D	0.10	0.38	0.08	505
Phage-451	D	D	-	-	-	-	Q	E	F	I	Y	A	-	I	0.09	0.38	0.08	506
Phage-452	A	D	-	W	-	D	W	E	-	Y	A	D	-	Y	0.11	0.38	0.10	507
Phage-453	Y	-	-	-	-	-	-	-	-	-	I	H	-	I	0.08	0.38	0.07	508
Phage-454	S	E	-	W	-	D	W	E	P	F	F	D	-	N	0.08	0.37	0.09	509
Phage-455	Y	H	-	-	-	-	M	-	-	-	L	I	-	T	0.07	0.37	0.07	510
Phage-456	S	D	-	W	-	D	W	E	D	A	Y	F	-	I	0.09	0.37	0.07	511
Phage-457	-	D	-	-	-	-	V	E	-	Y	Y	H	-	D	0.08	0.37	0.08	512
Phage-458	D	N	-	-	-	-	Y	-	-	-	I	A	-	N	0.10	0.37	0.10	513
Phage-459	L	-	-	-	-	V	-	E	F	Y	D	Y	-	Y	0.08	0.37	0.10	514
Phage-460	Y	T	-	-	-	-	M	-	-	-	-	T	-	I	0.09	0.37	0.08	515
Phage-461	N	F	-	W	-	D	W	E	V	N	S	F	-	D	0.09	0.37	0.08	516
Phage-462	N	A	-	W	-	D	W	E	Y	I	D	F	-	N	0.12	0.36	0.09	517
Phage-463	Y	N	-	-	-	-	M	-	-	-	I	F	-	S	0.09	0.36	0.07	518
Phage-464	L	D	-	-	-	-	L	-	-	-	I	T	-	Y	0.08	0.36	0.09	519
Phage-465	H	-	-	-	-	-	-	-	-	-	I	N	-	D	0.11	0.36	0.08	520
Phage-466	I	I	-	-	-	-	L	P	-	D	Y	V	-	T	0.08	0.36	0.08	521
Phage-467	D	I	-	-	-	-	-	-	-	-	I	D	-	S	0.08	0.36	0.08	522
Phage-468	P	-	-	-	-	-	-	-	-	-	-	L	-	F	0.10	0.36	0.09	523

Phage-469	-	F	-	-	-	-	-	-	-	-	-	D	-	Y	0.07	0.36	0.08	524
Phage-470	Y	D	-	W	-	D	W	E	-	A	L	P	-	A	0.08	0.36	0.08	525
Phage-471	D	D	-	W	-	D	W	E	D	Y	-	F	-	F	0.10	0.36	0.10	526
Phage-472	H	F	-	-	-	-	W	E	L	F	S	D	-	Y	0.11	0.36	0.11	527
Phage-473	I	T	-	W	-	D	W	E	V	N	F	P	-	Y	0.07	0.35	0.07	528
Phage-474	P	D	-	-	-	-	L	-	-	-	I	T	-	N	0.20	0.35	0.16	529
Phage-475	N	L	-	W	-	D	W	E	A	F	F	P	-	Y	0.08	0.35	0.07	530
Phage-476	F	-	-	-	-	-	-	E	Y	I	R	D	-	Y	0.08	0.35	0.07	531
Phage-477	F	F	-	-	-	-	-	-	-	-	I	I	-	D	0.09	0.35	0.10	532
Phage-478	-	L	-	-	-	K	G	G	P	T	Y	N	-	S	0.08	0.35	0.10	533
Phage-479	L	A	-	W	-	V	W	E	-	P	G	H	-	D	0.11	0.35	0.10	534
Phage-480	D	-	-	-	-	-	V	E	D	V	N	D	-	Y	0.07	0.35	0.08	535
Phage-481	D	-	-	-	-	-	-	E	-	A	H	Y	-	N	0.08	0.35	0.07	536
Phage-482	-	L	-	-	-	-	L	-	-	T	L	T	-	I	0.08	0.35	0.07	537
Phage-483	Y	-	-	-	-	-	I	E	D	Y	N	L	-	N	0.10	0.34	0.09	538
Phage-484	Y	I	-	-	-	-	V	E	-	Y	Y	N	-	F	0.13	0.34	0.14	539
Phage-485	D	I	-	-	-	-	L	-	-	-	I	F	-	F	0.08	0.34	0.09	540
Phage-486	D	I	-	-	-	-	V	E	-	D	Y	L	-	Y	0.07	0.34	0.08	541
Phage-487	T	L	-	-	-	-	-	E	-	D	A	P	-	I	0.10	0.34	0.08	542
Phage-488	N	-	-	W	-	D	W	E	Y	I	N	S	-	V	0.14	0.34	0.09	543
Phage-489	N	D	-	-	-	-	V	E	-	Y	Y	Y	-	T	0.07	0.34	0.09	544
Phage-490	I	T	-	-	-	-	M	-	-	-	I	D	-	N	0.08	0.34	0.08	545
Phage-491	Y	-	-	-	-	-	M	A	-	D	L	I	-	D	0.10	0.34	0.29	546
Phage-492	I	D	-	-	-	-	L	-	-	-	I	V	-	T	0.11	0.33	0.09	547
Phage-493	H	T	-	W	-	D	W	E	W	D	-	Y	-	D	0.08	0.33	0.07	548
Phage-494	I	H	-	-	-	-	W	E	L	I	D	D	-	L	0.08	0.33	0.10	549
Phage-495	Y	T	-	-	-	-	L	-	-	-	I	T	-	T	0.08	0.33	0.08	550
Phage-496	F	H	-	-	-	-	V	E	-	T	-	Y	-	F	0.11	0.33	0.09	551
Phage-497	D	-	-	-	-	-	L	-	-	-	L	I	-	N	0.07	0.33	0.08	552
Phage-498	I	-	-	-	-	-	-	-	-	-	D	Y	-	I	0.08	0.33	0.10	553
Phage-499	D	L	-	-	-	-	I	E	-	D	L	V	-	T	0.09	0.33	0.09	554
Phage-500	N	I	-	-	-	-	L	Q	-	D	I	V	-	P	0.09	0.33	0.09	555
Phage-501	N	N	-	-	-	-	M	-	-	-	I	T	-	Y	0.08	0.33	0.08	556
Phage-502	H	T	-	-	-	-	L	-	-	-	I	V	-	V	0.08	0.33	0.08	557
Phage-503	Y	-	-	-	-	-	I	E	D	I	L	V	-	T	0.12	0.33	0.23	558
Phage-504	N	T	-	-	-	-	-	E	F	V	H	L	-	P	0.07	0.33	0.11	559
Phage-505	D	I	-	-	-	-	M	-	-	-	T	V	-	D	0.10	0.33	0.09	560

Phage-506	A	I	-	-	-	-	V	E	I	V	N	Y	-	Y	0.09	0.32	0.07	561
Phage-507	H	L	-	-	-	-	V	E	D	P	T	A	-	V	0.10	0.32	0.28	562
Phage-508	A	D	-	-	-	-	L	-	-	-	I	S	-	T	0.10	0.32	0.09	563
Phage-509	F	D	-	-	-	-	L	-	-	-	-	I	-	D	0.07	0.32	0.09	564
Phage-510	D	V	-	-	-	-	-	-	-	-	I	D	-	N	0.10	0.32	0.08	565
Phage-511	Y	L	-	-	-	-	V	E	-	I	S	I	-	F	0.08	0.32	0.07	566
Phage-512	S	A	-	-	-	-	-	-	-	-	L	H	-	V	0.10	0.31	0.30	567
Phage-513	H	L	-	W	-	D	W	E	-	D	S	A	-	N	0.08	0.31	0.08	568
Phage-514	H	T	-	W	-	D	W	E	Y	D	Y	D	-	F	0.10	0.31	0.08	569
Phage-515	Y	D	-	-	-	-	W	E	-	V	A	L	-	N	0.10	0.31	0.10	570
Phage-516	T	L	-	-	-	-	I	E	-	Y	I	V	-	Y	0.09	0.31	0.23	571
Phage-517	S	-	-	-	-	-	-	-	-	-	I	F	-	T	0.09	0.31	0.09	572
Phage-518	D	I	-	-	-	-	-	-	-	-	L	H	-	Y	0.08	0.31	0.08	573
Phage-519	L	F	-	-	-	-	-	E	-	A	Y	L	-	I	0.08	0.31	0.08	574
Phage-520	I	F	-	-	-	-	I	E	-	D	F	V	-	T	0.10	0.31	0.10	575
Phage-521	D	D	-	-	-	-	W	E	Y	Y	-	A	-	V	0.08	0.31	0.08	576
Phage-522	D	D	-	-	-	-	L	-	-	T	T	I	-	Y	0.10	0.31	0.09	577
Phage-523	A	S	-	-	-	-	L	-	-	-	I	A	-	D	0.10	0.31	0.09	578
Phage-524	Y	L	-	-	-	-	V	E	D	Y	D	Y	-	Y	0.08	0.31	0.09	579
Phage-525	D	L	-	-	-	-	W	E	-	T	I	F	-	A	0.12	0.30	0.09	580
Phage-526	D	F	-	-	-	D	G	E	-	F	Y	I	-	P	0.12	0.30	0.11	581
Phage-527	-	-	-	-	-	-	V	E	-	N	I	L	-	H	0.15	0.30	0.17	582
Phage-528	D	-	-	-	-	-	V	E	-	N	Y	F	-	F	0.12	0.30	0.21	583
Phage-529	N	D	-	W	-	D	W	Y	-	F	L	S	-	D	0.10	0.30	0.10	584
Phage-530	R	D	-	W	-	D	W	E	V	P	Y	F	-	D	0.08	0.30	0.09	585
Phage-531	N	L	-	-	-	-	V	E	-	A	-	Y	-	Y	0.10	0.30	0.13	586
Phage-532	F	D	-	W	-	D	G	E	L	N	Y	L	-	T	0.23	0.30	0.08	587
Phage-533	Y	-	-	-	-	-	V	E	D	V	N	L	-	I	0.18	0.30	0.10	588
Phage-534	D	N	-	-	-	-	Y	-	-	-	I	T	-	L	0.11	0.29	0.09	589
Phage-535	L	N	-	W	-	D	W	E	-	D	Y	S	-	N	0.09	0.29	0.09	590
Phage-536	P	T	-	-	-	-	V	E	-	L	L	S	-	N	0.12	0.29	0.26	591
Phage-537	D	H	-	-	-	-	V	E	L	I	F	Y	-	H	0.11	0.29	0.08	592
Phage-538	F	H	-	-	-	-	L	-	-	-	I	F	-	Y	0.08	0.29	0.08	593
Phage-539	F	D	-	-	-	-	L	E	-	T	-	V	-	P	0.16	0.29	0.16	594
Phage-540	D	F	-	-	-	-	L	-	-	-	L	P	-	A	0.08	0.29	0.08	595
Phage-541	D	S	-	-	-	-	L	-	-	-	I	Y	-	D	0.09	0.29	0.09	596
Phage-542	A	D	-	W	-	D	W	E	-	F	L	L	-	F	0.12	0.29	0.10	597

Phage-543	I	L	-	-	-	-	V	E	-	L	D	F	-	N	0.10	0.28	0.08	598
Phage-544	F	F	-	-	-	-	-	E	-	I	F	L	-	Y	0.09	0.28	0.07	599
Phage-545	Y	N	-	-	-	D	G	-	-	-	Y	D	-	H	0.07	0.28	0.07	600
Phage-546	D	N	-	-	-	-	L	-	-	T	I	T	-	F	0.11	0.28	0.11	601
Phage-547	H	N	-	-	-	D	G	-	-	A	F	I	-	N	0.09	0.28	0.23	602
Phage-548	D	-	-	-	-	-	V	E	-	D	L	V	-	P	0.10	0.28	0.23	603
Phage-549	D	-	-	-	-	-	L	-	-	-	L	N	-	F	0.08	0.28	0.07	604
Phage-550	D	-	-	-	-	-	-	Q	-	D	F	H	-	H	0.11	0.28	0.27	605
Phage-551	Y	D	-	-	-	-	W	E	F	T	D	D	-	I	0.10	0.28	0.18	606
Phage-552	D	I	-	-	-	-	Y	E	-	D	I	I	-	Y	0.14	0.28	0.19	607
Phage-553	F	D	-	-	-	-	L	-	-	T	-	P	-	P	0.11	0.28	0.08	608
Phage-554	N	-	-	-	-	-	L	-	-	T	S	V	-	D	0.09	0.28	0.26	609
Phage-555	Y	-	-	-	-	-	W	E	F	-	F	D	-	D	0.17	0.28	0.11	610
Phage-556	Y	A	-	-	-	-	L	-	-	-	-	T	-	D	0.09	0.28	0.08	611
Phage-557	F	L	-	-	-	-	V	E	Q	D	Y	F	-	V	0.08	0.28	0.10	612
Phage-558	D	N	-	-	-	-	-	-	-	-	-	R	-	D	0.09	0.27	0.26	613
Phage-559	N	D	-	-	-	D	G	I	-	T	-	D	-	Y	0.10	0.27	0.24	614
Phage-560	Y	F	-	-	-	-	V	E	D	Y	N	D	-	F	0.08	0.27	0.09	615
Phage-561	N	L	-	-	-	-	-	-	-	-	I	F	-	Y	0.10	0.27	0.07	616
Phage-562	I	D	-	W	-	D	W	E	-	Y	I	P	-	T	0.10	0.27	0.08	617
Phage-563	I	-	-	-	-	D	G	-	-	-	F	I	-	A	0.07	0.27	0.08	618
Phage-564	D	-	-	-	-	-	-	-	-	-	-	V	-	Y	0.08	0.27	0.07	619
Phage-565	-	F	-	-	-	-	W	E	D	I	T	D	-	D	0.13	0.27	0.09	620
Phage-566	L	D	-	-	-	-	V	-	-	T	F	T	-	H	0.08	0.26	0.08	621
Phage-567	D	D	-	-	-	-	Y	-	-	-	F	A	-	H	0.13	0.26	0.10	622
Phage-568	I	-	-	-	-	-	Y	Q	-	D	L	P	-	N	0.12	0.26	0.11	623
Phage-569	L	D	-	-	-	-	V	E	-	Y	N	Y	-	V	0.09	0.26	0.08	624
Phage-570	Y	V	-	-	-	-	-	-	-	-	S	A	-	N	0.14	0.26	0.08	625
Phage-571	T	P	-	-	-	-	L	E	-	A	I	-	-	Y	0.10	0.26	0.10	626
Phage-572	Y	F	-	-	-	-	-	-	-	-	A	D	-	N	0.08	0.26	0.08	627
Phage-573	D	D	-	-	-	-	-	E	-	D	I	I	-	D	0.12	0.26	0.25	628
Phage-574	L	-	-	-	-	V	V	E	-	L	N	H	-	N	0.08	0.26	0.09	629
Phage-575	-	I	-	-	-	D	G	E	-	L	I	A	-	A	0.09	0.26	0.27	630
Phage-576	F	A	-	W	-	D	W	Q	-	T	Y	V	-	N	0.09	0.25	0.08	631
Phage-577	Y	I	-	-	-	-	V	E	F	L	F	F	-	N	0.08	0.25	0.08	632
Phage-578	T	Q	-	-	-	K	G	E	P	T	Y	H	-	Y	0.12	0.25	0.12	633
Phage-579	N	-	-	-	-	-	V	E	-	Y	H	N	-	D	0.10	0.25	0.17	634

Phage-580	-	-	-	-	-	-	W	E	F	F	S	D	-	A	0.08	0.25	0.07	635
Phage-581	F	-	-	-	-	-	L	E	-	-	F	F	-	Y	0.10	0.25	0.22	636
Phage-582	D	-	-	-	-	-	I	E	-	N	F	Y	-	Y	0.13	0.25	0.09	637
Phage-583	Y	A	-	-	-	-	V	E	-	Y	-	Y	-	A	0.08	0.25	0.09	638
Phage-584	-	D	-	-	-	-	L	-	-	-	I	I	-	D	0.08	0.25	0.07	639
Phage-585	N	D	-	-	-	-	M	-	-	-	I	A	-	Y	0.07	0.25	0.07	640
Phage-586	-	-	-	-	-	-	-	-	-	Y	L	A	-	A	0.09	0.25	0.21	641
Phage-587	-	I	-	-	-	-	-	-	-	-	A	N	-	D	0.08	0.25	0.09	642
Phage-588	L	T	-	W	-	D	W	E	-	D	F	F	-	N	0.07	0.24	0.07	643
Phage-589	F	-	-	-	-	-	Q	E	-	I	N	Y	-	Y	0.10	0.24	0.23	644
Phage-590	T	-	-	-	-	-	L	E	-	F	F	L	-	Y	0.13	0.24	0.08	645
Phage-591	P	D	-	-	-	-	L	-	-	-	-	A	-	H	0.12	0.24	0.09	646
Phage-592	N	D	-	-	-	-	-	E	-	I	I	F	-	V	0.09	0.24	0.24	647
Phage-593	Y	I	-	-	-	-	-	-	-	-	F	Y	-	N	0.25	0.24	0.08	648
Phage-594	H	A	-	-	-	-	L	-	-	-	L	L	-	N	0.20	0.24	0.07	649
Phage-595	Y	-	-	-	-	-	W	E	-	A	-	L	-	A	0.09	0.24	0.21	650
Phage-596	A	F	-	-	-	-	V	E	-	Y	D	L	-	N	0.10	0.24	0.16	651
Phage-597	Y	N	-	-	-	-	-	-	-	-	-	A	-	S	0.15	0.23	0.09	652
Phage-598	Y	L	-	-	-	-	V	E	D	D	T	L	-	A	0.08	0.23	0.09	653
Phage-599	A	V	-	-	-	-	-	-	-	-	-	N	-	D	0.08	0.23	0.08	654
Phage-600	N	-	-	-	-	-	W	E	V	Y	S	L	-	P	0.13	0.23	0.08	655
Phage-601	D	F	-	-	-	-	V	E	-	D	T	Y	-	H	0.07	0.23	0.07	656
Phage-602	I	S	-	-	-	-	Y	E	W	D	Y	A	-	N	0.08	0.23	0.10	657
Phage-603	-	N	-	-	-	-	L	-	-	-	I	I	-	Y	0.08	0.23	0.08	658
Phage-604	Y	D	-	-	-	-	-	-	-	T	A	P	-	Y	0.07	0.23	0.08	659
Phage-605	Y	L	-	-	-	-	-	E	-	N	F	L	-	T	0.09	0.23	0.22	660
Phage-606	F	D	-	-	-	-	V	-	-	-	-	D	-	A	0.08	0.22	0.09	661
Phage-607	Y	D	-	-	-	-	Q	E	-	I	S	F	-	N	0.09	0.22	0.09	662
Phage-608	I	D	-	-	-	-	I	E	L	Y	D	D	-	F	0.09	0.22	0.09	663
Phage-609	T	F	-	-	-	-	L	-	-	-	-	Y	-	Y	0.08	0.22	0.07	664
Phage-610	F	F	-	-	-	-	I	-	-	-	N	A	-	V	0.09	0.22	0.07	665
Phage-611	Y	H	-	W	-	D	W	E	P	I	Y	I	-	I	0.12	0.22	0.10	666
Phage-612	A	I	-	-	-	-	Y	E	-	D	H	Y	-	Y	0.08	0.22	0.08	667
Phage-613	P	L	-	-	-	D	G	F	-	N	Y	N	-	F	0.12	0.22	0.08	668
Phage-614	F	P	-	W	-	D	W	E	W	D	N	N	-	H	0.09	0.22	0.09	669
Phage-615	-	D	-	-	-	D	G	-	-	L	A	A	-	H	0.10	0.22	0.11	670
Phage-616	-	D	-	W	-	D	W	E	-	Y	Y	S	-	D	0.08	0.22	0.07	671

Phage-617	-	-	-	-	-	-	Y	-	-	-	Y	D	-	T	0.07	0.21	0.10	672
Phage-618	N	L	-	-	-	-	W	E	N	F	A	D	-	F	0.08	0.21	0.08	673
Phage-619	Y	L	-	-	-	-	L	E	V	F	F	V	-	D	0.12	0.21	0.10	674
Phage-620	I	F	-	-	-	-	L	E	D	Y	S	I	-	F	0.09	0.21	0.08	675
Phage-621	D	-	-	-	-	-	L	E	Q	Y	D	L	-	F	0.09	0.21	0.08	676
Phage-622	L	L	-	-	-	V	N	E	D	P	L	D	-	Y	0.11	0.21	0.13	677
Phage-623	I	D	-	-	-	-	-	-	-	-	-	F	-	Y	0.08	0.21	0.08	678
Phage-624	I	I	-	-	-	-	V	E	-	I	D	I	-	S	0.08	0.21	0.08	679
Phage-625	I	A	-	W	-	D	W	E	D	Y	S	S	-	P	0.08	0.21	0.11	680
Phage-626	Y	-	-	-	-	-	V	E	D	I	N	D	-	I	0.09	0.21	0.07	681
Phage-627	N	I	-	-	-	-	M	-	-	-	I	D	-	I	0.08	0.21	0.07	682
Phage-628	F	D	-	W	-	D	W	E	-	L	-	S	-	Y	0.07	0.21	0.08	683
Phage-629	Y	F	-	-	-	-	W	E	D	H	F	F	-	D	0.09	0.21	0.19	684
Phage-630	T	-	-	-	-	-	-	E	-	D	S	Y	-	D	0.12	0.20	0.09	685
Phage-631	N	L	-	-	-	-	V	E	L	I	D	I	-	S	0.11	0.20	0.09	686
Phage-632	D	N	-	-	-	-	W	E	-	V	Y	L	-	N	0.08	0.20	0.08	687
Phage-633	F	L	-	-	-	-	-	-	-	-	D	L	-	F	0.08	0.20	0.09	688
Phage-634	H	I	-	-	-	-	Q	-	-	-	I	-	-	T	0.09	0.20	0.19	689
Phage-635	F	D	-	W	-	D	W	E	D	N	S	Y	-	D	0.10	0.20	0.09	690
Phage-636	T	A	-	-	-	-	W	E	F	D	F	N	-	D	0.08	0.20	0.07	691
Phage-637	H	H	-	W	-	D	W	E	D	Y	S	T	-	P	0.10	0.20	0.11	692
Phage-638	Y	-	-	-	-	-	-	-	-	-	-	N	-	F	0.07	0.20	0.08	693
Phage-639	L	H	-	W	-	D	W	E	-	I	D	I	-	D	0.08	0.20	0.09	694
Phage-640	D	I	-	-	-	D	G	Q	-	D	F	V	-	S	0.08	0.20	0.09	695
Phage-641	D	V	-	W	-	D	W	E	V	N	Y	F	-	D	0.09	0.20	0.07	696
Phage-642	-	N	-	-	-	-	M	-	-	-	I	D	-	A	0.12	0.20	0.10	697
Phage-643	D	N	-	-	-	-	-	-	-	A	T	V	-	N	0.11	0.19	0.19	698
Phage-644	D	L	-	-	-	-	-	E	-	V	H	N	-	N	0.08	0.19	0.08	699
Phage-645	-	N	-	-	-	-	-	-	-	-	S	Y	-	F	0.13	0.19	0.09	700
Phage-646	N	I	-	W	-	D	W	E	-	D	N	F	-	S	0.08	0.19	0.08	701
Phage-647	F	V	-	-	-	-	W	E	V	Y	D	D	-	D	0.08	0.19	0.08	702
Phage-648	A	-	-	-	-	-	L	E	V	V	H	L	-	V	0.10	0.19	0.17	703
Phage-649	P	F	-	-	-	-	M	-	-	T	I	D	-	Y	0.07	0.19	0.09	704
Phage-650	L	L	-	-	-	V	M	E	D	V	F	A	-	Y	0.08	0.19	0.08	705
Phage-651	D	L	-	-	-	-	-	-	-	-	T	N	-	Y	0.07	0.19	0.08	706
Phage-652	H	D	-	-	-	-	M	E	-	Y	Y	L	-	P	0.10	0.18	0.10	707
Phage-653	T	D	-	-	-	-	Y	-	-	-	I	I	-	P	0.08	0.18	0.09	708

Phage-654	-	L	-	W	-	D	W	E	D	Y	A	D	-	N	0.09	0.18	0.08	709
Phage-655	N	D	-	-	-	-	L	-	-	-	L	T	-	D	0.07	0.18	0.09	710
Phage-656	I	-	-	-	-	-	L	-	-	-	I	A	-	Y	0.11	0.18	0.08	711
Phage-657	N	-	-	-	-	-	V	E	-	F	N	F	-	H	0.11	0.18	0.14	712
Phage-658	D	V	-	-	-	-	I	E	-	Y	S	F	-	I	0.08	0.18	0.09	713
Phage-659	D	L	-	-	-	-	V	E	-	I	T	D	-	A	0.10	0.18	0.12	714
Phage-660	H	D	-	-	-	-	-	-	-	-	-	F	-	I	0.12	0.18	0.12	715
Phage-661	P	L	-	-	-	V	L	E	-	D	I	Y	-	Y	0.10	0.18	0.13	716
Phage-662	D	L	-	-	-	-	-	E	D	I	I	D	-	N	0.10	0.18	0.11	717
Phage-663	D	-	-	-	-	-	V	E	V	P	S	N	-	N	0.10	0.18	0.18	718
Phage-664	I	I	-	-	-	-	L	-	-	-	T	A	-	D	0.10	0.18	0.09	719
Phage-665	D	H	-	-	-	-	-	-	-	-	-	N	-	D	0.10	0.18	0.14	720
Phage-666	F	D	-	-	-	-	-	-	-	-	L	Y	-	S	0.07	0.18	0.07	721
Phage-667	F	A	-	W	-	D	W	E	-	V	Y	I	-	Y	0.08	0.18	0.08	722
Phage-668	L	-	-	-	-	-	-	-	-	-	L	D	-	S	0.08	0.18	0.09	723
Phage-669	D	L	-	-	-	-	L	E	-	A	F	L	-	A	0.09	0.18	0.08	724
Phage-670	F	A	-	-	-	-	L	-	-	T	L	T	-	L	0.10	0.18	0.08	725
Phage-671	F	D	-	-	-	-	V	E	-	I	S	N	-	D	0.17	0.18	0.10	726
Phage-672	-	H	-	-	-	-	L	E	Y	P	F	D	-	N	0.09	0.17	0.16	727
Phage-673	A	-	-	-	-	-	-	E	-	H	T	T	-	N	0.10	0.17	0.15	728
Phage-674	L	-	-	-	-	V	S	E	Q	F	T	F	-	I	0.08	0.17	0.08	729
Phage-675	D	-	-	-	-	-	L	-	-	-	Y	D	-	N	0.10	0.17	0.09	730
Phage-676	F	-	-	-	-	-	W	E	-	F	D	V	-	I	0.13	0.17	0.15	731
Phage-677	F	T	-	-	-	-	V	E	-	Y	D	H	-	I	0.08	0.17	0.09	732
Phage-678	Y	N	-	-	-	-	-	-	-	-	-	T	-	F	0.12	0.17	0.11	733
Phage-679	A	V	-	-	-	-	N	-	-	-	N	S	-	A	0.08	0.17	0.08	734
Phage-680	I	-	-	W	-	D	W	E	V	P	N	D	-	A	0.10	0.17	0.09	735
Phage-681	Y	F	-	-	-	-	-	E	-	F	F	H	-	Y	0.12	0.17	0.12	736
Phage-682	Y	V	-	-	-	D	G	-	-	-	S	F	-	D	0.12	0.17	0.12	737
Phage-683	I	S	-	-	-	-	V	E	-	F	F	Y	-	Y	0.10	0.17	0.08	738
Phage-684	L	I	-	-	-	V	-	E	-	D	-	Y	-	D	0.17	0.17	0.15	739
Phage-685	-	-	-	-	-	-	V	E	D	H	N	Y	-	A	0.14	0.17	0.16	740
Phage-686	L	D	-	-	-	-	-	E	F	V	Y	I	-	A	0.08	0.17	0.10	741
Phage-687	Y	D	-	-	-	-	-	E	-	D	L	P	-	I	0.17	0.17	0.11	742
Phage-688	D	V	-	-	-	-	V	E	-	D	Y	Y	-	D	0.10	0.17	0.14	743
Phage-689	N	D	-	W	-	D	W	E	Y	D	N	V	-	V	0.08	0.17	0.10	744
Phage-690	D	L	-	-	-	-	-	E	V	A	N	D	-	N	0.10	0.16	0.16	745

Phage-691	H	D	-	-	-	-	L	-	-	-	I	S	-	N	0.09	0.16	0.07	746
Phage-692	L	D	-	W	-	D	W	E	-	T	T	H	-	D	0.08	0.16	0.08	747
Phage-693	I	I	-	-	-	-	V	E	-	D	D	Y	-	L	0.09	0.16	0.09	748
Phage-694	Y	-	-	W	-	D	W	E	-	V	I	I	-	D	0.09	0.16	0.09	749
Phage-695	F	D	-	-	-	-	I	-	-	Y	T	N	-	N	0.12	0.16	0.09	750
Phage-696	I	T	-	-	-	-	L	-	-	T	I	N	-	D	0.08	0.16	0.11	751
Phage-697	D	S	-	-	-	-	V	E	-	D	I	Y	-	I	0.07	0.16	0.08	752
Phage-698	Y	L	-	-	-	-	-	-	-	-	G	N	-	H	0.07	0.16	0.08	753
Phage-699	D	N	-	-	-	-	L	P	-	D	Y	F	-	D	0.08	0.16	0.10	754
Phage-700	-	L	-	-	-	-	-	E	-	V	S	N	-	N	0.11	0.16	0.08	755
Phage-701	-	D	-	W	-	D	W	E	-	D	I	V	-	D	0.10	0.16	0.09	756
Phage-702	-	-	-	W	-	D	W	E	D	N	F	P	-	Y	0.07	0.16	0.07	757
Phage-703	D	-	-	-	-	-	V	E	-	H	F	N	-	H	0.08	0.16	0.08	758
Phage-704	A	D	-	-	-	-	I	E	-	D	A	Y	-	Y	0.12	0.16	0.09	759
Phage-705	I	L	-	W	-	D	W	E	D	A	T	F	-	Y	0.09	0.16	0.07	760
Phage-706	I	H	-	W	-	D	W	E	D	F	N	I	-	P	0.09	0.16	0.08	761
Phage-707	T	I	-	-	-	-	V	E	D	Y	N	D	-	I	0.07	0.16	0.07	762
Phage-708	D	D	-	-	-	-	L	-	-	-	-	A	-	I	0.08	0.16	0.08	763
Phage-709	D	D	-	W	-	D	W	E	D	H	I	F	-	F	0.13	0.16	0.08	764
Phage-710	-	N	-	-	-	-	V	E	-	I	I	F	-	D	0.12	0.15	0.12	765
Phage-711	I	F	-	W	-	D	W	E	D	D	T	V	-	I	0.08	0.15	0.09	766
Phage-712	I	I	-	-	-	-	-	E	-	I	S	D	-	L	0.12	0.15	0.15	767
Phage-713	F	D	-	-	-	-	V	E	-	Y	N	D	-	D	0.11	0.15	0.09	768
Phage-714	N	D	-	-	-	-	L	-	-	T	L	Y	-	I	0.08	0.15	0.10	769
Phage-715	A	I	-	-	-	-	L	E	-	D	I	S	-	N	0.12	0.15	0.17	770
Phage-716	H	L	-	-	-	-	-	-	-	-	T	N	-	Y	0.07	0.15	0.07	771
Phage-717	S	-	-	-	-	-	L	-	-	-	-	A	-	I	0.10	0.15	0.11	772
Phage-718	L	-	-	-	-	-	-	E	Q	L	A	D	-	T	0.08	0.15	0.08	773
Phage-719	I	D	-	-	-	-	L	-	-	-	I	A	-	N	0.07	0.15	0.08	774
Phage-720	F	D	-	-	-	D	G	Q	-	D	L	V	-	N	0.10	0.15	0.08	775
Phage-721	N	L	-	-	-	-	-	E	-	F	F	D	-	Y	0.09	0.15	0.15	776
Phage-722	S	I	-	-	-	-	L	Q	-	D	I	V	-	P	0.09	0.14	0.14	777
Phage-723	N	P	-	-	-	-	Y	-	-	-	A	H	-	D	0.08	0.14	0.08	778
Phage-724	Y	D	-	-	-	-	L	-	-	Y	Y	N	-	N	0.12	0.14	0.11	779
Phage-725	-	D	-	-	-	-	L	-	-	T	I	F	-	D	0.07	0.14	0.08	780
Phage-726	D	N	-	-	-	-	L	-	-	-	-	T	-	T	0.09	0.14	0.10	781
Phage-727	-	D	-	-	-	-	L	-	-	-	S	Y	-	D	0.10	0.14	0.13	782

Phage-728	Y	-	-	-	-	-	-	E	F	I	D	F	-	F	0.07	0.14	0.07	783
Phage-729	Y	D	-	W	-	D	W	E	V	I	T	Y	-	N	0.08	0.14	0.09	784
Phage-730	F	D	-	-	-	-	I	E	-	D	F	F	-	V	0.06	0.14	0.07	785
Phage-731	I	F	-	W	-	D	W	-	D	I	N	F	-	D	0.10	0.14	0.14	786
Phage-732	N	F	-	-	-	-	L	P	-	D	I	T	-	Y	0.37	0.14	0.09	787
Phage-733	S	L	-	-	-	-	-	E	-	Y	Y	H	-	L	0.09	0.14	0.07	788
Phage-734	A	S	-	-	-	-	L	-	-	-	L	D	-	L	0.12	0.14	0.13	789
Phage-735	S	F	-	-	-	-	R	E	W	D	L	A	-	Y	0.09	0.14	0.08	790
Phage-736	H	L	-	-	-	-	-	E	D	V	L	D	-	I	0.08	0.14	0.12	791
Phage-737	L	D	-	-	-	D	G	-	-	F	Y	Y	-	L	0.20	0.14	0.09	792
Phage-738	D	N	-	W	-	D	W	E	-	D	I	A	-	T	0.16	0.14	0.11	793
Phage-739	S	D	-	-	-	-	L	-	-	T	I	H	-	I	0.09	0.14	0.08	794
Phage-740	N	S	-	-	-	D	G	-	-	-	-	D	-	L	0.08	0.14	0.08	795
Phage-741	D	L	-	-	-	-	L	-	-	-	T	L	-	I	0.07	0.14	0.08	796
Phage-742	F	D	-	-	-	-	S	-	-	-	F	N	-	Y	0.09	0.14	0.11	797
Phage-743	D	L	-	-	-	-	-	E	-	D	D	I	-	Y	0.12	0.14	0.13	798
Phage-744	H	A	-	-	-	-	-	E	-	D	T	Y	-	F	0.10	0.14	0.14	799
Phage-745	S	D	-	-	-	-	L	-	-	-	-	A	-	I	0.11	0.14	0.08	800
Phage-746	I	V	-	-	-	-	L	P	-	D	Y	N	-	Y	0.09	0.13	0.11	801
Phage-747	D	L	-	-	-	-	-	-	-	-	F	I	-	F	0.09	0.13	0.08	802
Phage-748	Y	D	-	-	-	-	-	-	-	T	L	T	-	N	0.13	0.13	0.08	803
Phage-749	Y	D	-	-	-	-	V	E	-	I	-	N	-	D	0.11	0.13	0.12	804
Phage-750	-	F	-	-	-	-	I	E	D	D	H	V	-	I	0.07	0.13	0.07	805
Phage-751	F	I	-	-	-	-	W	E	D	D	Y	A	-	S	0.12	0.13	0.12	806
Phage-752	N	F	-	-	-	-	-	-	-	-	-	-	-	N	0.10	0.13	0.09	807
Phage-753	N	L	-	-	-	-	V	E	-	I	L	I	-	D	0.07	0.13	0.07	808
Phage-754	D	S	-	-	-	-	V	E	-	Y	D	L	-	N	0.11	0.13	0.08	809
Phage-755	T	L	-	-	-	-	-	E	-	I	T	D	-	N	0.09	0.13	0.08	810
Phage-756	T	V	-	-	-	K	M	E	M	N	S	T	-	D	0.09	0.13	0.09	811
Phage-757	-	H	-	W	-	D	W	E	D	A	-	S	-	N	0.10	0.12	0.11	812
Phage-758	D	L	-	-	-	D	G	N	-	L	D	F	-	F	0.08	0.12	0.08	813
Phage-759	D	L	-	-	-	D	G	E	-	H	Y	Y	-	D	0.09	0.12	0.07	814
Phage-760	F	N	-	-	-	-	V	E	-	I	L	L	-	T	0.11	0.12	0.12	815
Phage-761	H	-	-	-	-	-	V	E	N	I	N	D	-	I	0.09	0.12	0.07	816
Phage-762	I	D	-	-	-	-	L	-	-	-	-	I	-	D	0.09	0.12	0.08	817
Phage-763	I	F	-	-	-	-	I	E	Q	P	A	L	-	Y	0.08	0.12	0.09	818
Phage-764	Y	D	-	-	-	-	-	Q	-	D	L	V	-	P	0.10	0.12	0.08	819

Phage-765	H	A	-	W	-	D	W	E	-	P	N	Y	-	D	0.13	0.12	0.09	820
Phage-766	N	V	-	W	-	D	W	E	-	D	Y	N	-	Y	0.11	0.12	0.10	821
Phage-767	S	F	-	-	Q	-	L	G	D	N	Y	D	-	I	0.09	0.12	0.12	822
Phage-768	D	D	-	-	-	-	L	-	-	T	T	V	-	Y	0.08	0.12	0.09	823
Phage-769	N	F	-	-	-	-	W	E	V	A	T	L	-	L	0.09	0.12	0.14	824
Phage-770	D	L	-	-	-	-	V	E	-	D	T	Y	-	N	0.09	0.11	0.07	825
Phage-771	A	L	-	-	-	-	V	E	Q	V	D	L	-	T	0.08	0.11	0.08	826
Phage-772	D	D	-	-	-	-	L	-	-	-	-	N	-	N	0.08	0.11	0.08	827
Phage-773	I	F	-	-	-	-	-	E	Q	I	I	Y	-	D	0.09	0.11	0.11	828
Phage-774	S	D	-	W	-	D	W	E	-	V	Y	Y	-	S	0.09	0.11	0.10	829
Phage-775	F	F	-	-	-	D	G	-	-	V	A	I	-	D	0.09	0.11	0.07	830
Phage-776	D	D	-	W	-	D	W	E	D	D	-	Y	-	Y	0.11	0.11	0.07	831
Phage-777	Y	D	-	-	-	-	-	-	-	T	-	V	-	P	0.08	0.11	0.08	832
Phage-778	S	-	-	-	-	-	L	-	-	-	-	N	-	V	0.10	0.11	0.08	833
Phage-779	A	F	-	V	S	F	Q	Q	S	L	P	H	-	D	0.09	0.11	0.10	834
Phage-780	-	N	-	-	-	-	V	E	-	Y	F	V	-	F	0.09	0.11	0.09	835
Phage-781	T	N	-	W	-	D	W	E	-	D	F	A	-	V	0.07	0.11	0.08	836
Phage-782	D	D	-	-	-	-	-	E	-	I	I	L	-	F	0.08	0.10	0.08	837
Phage-783	N	S	-	-	-	-	L	E	D	Y	H	L	-	P	0.08	0.10	0.10	838
Phage-784	I	H	-	-	-	D	S	-	G	F	D	F	-	D	0.09	0.10	0.08	839
Phage-785	F	D	-	-	-	-	L	E	-	D	H	L	-	F	0.08	0.10	0.08	840
Phage-786	T	V	-	W	-	D	-	E	-	Y	A	D	-	D	0.10	0.10	0.08	841
Phage-787	Y	F	-	W	-	D	W	E	-	A	A	D	-	L	0.09	0.10	0.09	842
Phage-788	-	S	-	-	-	-	-	-	-	-	-	D	-	I	0.08	0.10	0.07	843
Phage-789	A	V	-	-	-	-	L	P	-	D	I	V	-	Y	0.08	0.10	0.08	844
Phage-790	-	L	-	-	-	-	V	E	-	Y	H	L	-	A	0.08	0.10	0.30	845
Phage-791	S	L	-	W	-	D	W	E	-	V	D	N	-	F	0.12	0.10	0.11	846
Phage-792	T	I	-	-	-	D	G	Q	-	D	Y	N	-	H	0.07	0.10	0.07	847
Phage-793	F	L	-	-	-	-	G	E	P	T	Y	L	-	T	0.12	0.10	0.09	848
Phage-794	D	D	-	W	L	-	Q	H	D	I	Y	V	-	A	0.10	0.10	0.08	849
Phage-795	I	F	-	-	-	-	V	E	-	V	A	F	-	F	0.07	0.10	0.08	850
Phage-796	A	L	-	-	-	-	V	E	D	D	Y	D	-	L	0.09	0.10	0.09	851
Phage-797	D	D	-	-	-	-	I	E	L	Y	L	T	-	A	0.08	0.10	0.08	852
Phage-798	T	D	-	W	-	D	W	E	D	D	S	I	-	D	0.07	0.10	0.07	853
Phage-799	S	I	-	-	-	-	L	E	-	I	F	L	-	N	0.08	0.10	0.08	854
Phage-800	N	D	-	-	-	-	L	E	-	D	I	L	-	F	0.07	0.10	0.09	855
Phage-801	D	D	-	-	-	-	L	-	-	T	Y	S	-	Y	0.09	0.09	0.08	856

Phage-802	F	V	-	-	-	-	W	E	-	I	D	L	-	I	0.10	0.09	0.09	857
Phage-803	Y	D	-	-	-	-	L	-	-	-	L	S	-	P	0.07	0.09	0.07	858
Phage-804	N	L	-	-	-	-	L	-	-	-	I	I	-	P	0.08	0.09	0.08	859
Phage-805	I	D	-	W	-	D	W	E	-	F	N	N	-	F	0.08	0.09	0.09	860
Phage-806	A	-	-	-	-	-	L	E	-	H	D	Y	-	Y	0.08	0.09	0.09	861
Phage-807	D	D	-	S	-	Q	-	-	Q	I	D	L	-	D	0.14	0.09	0.09	862
Phage-808	S	-	-	-	-	-	S	-	-	-	I	Y	-	Y	0.08	0.09	0.07	863
Phage-809	S	L	-	-	-	-	-	Q	-	D	A	P	-	N	0.07	0.09	0.07	864
Phage-810	D	A	-	-	-	-	L	-	-	T	T	H	-	D	0.09	0.09	0.08	865
Phage-811	N	D	-	-	-	-	V	E	-	V	A	D	-	F	0.07	0.09	0.08	866
Phage-812	Y	I	-	-	-	-	-	E	Q	D	Y	F	-	F	0.08	0.09	0.08	867
Phage-813	T	D	-	-	-	D	G	-	-	T	N	Y	-	F	0.11	0.09	0.08	868
Phage-814	Y	D	-	-	-	-	V	-	-	-	-	L	-	P	0.08	0.09	0.08	869
Phage-815	I	D	-	-	-	-	-	-	-	-	A	Y	-	T	0.08	0.09	0.08	870
Phage-816	F	D	-	-	-	-	-	E	-	F	F	H	-	Y	0.09	0.09	0.09	871
Phage-817	F	P	-	-	-	-	I	E	-	Y	D	Y	-	V	0.09	0.09	0.08	872
Phage-818	A	D	-	-	-	I	-	E	S	I	D	I	-	V	0.09	0.09	0.07	873
Phage-819	I	S	-	-	-	D	L	W	P	T	D	I	-	T	0.10	0.09	0.10	874
Phage-820	D	D	-	-	-	D	G	-	-	V	H	T	-	N	0.09	0.09	0.07	875
Phage-821	I	D	-	W	-	D	W	E	G	-	F	A	-	N	0.09	0.09	0.08	876
Phage-822	L	N	-	-	-	D	G	-	-	T	F	Y	-	D	0.08	0.08	0.07	877
Phage-823	I	H	-	-	-	-	L	G	A	Y	I	S	-	S	0.08	0.08	0.09	878
Phage-824	T	I	-	W	-	D	W	E	-	D	Y	F	-	Y	0.08	0.08	0.08	879
Phage-825	H	S	-	L	A	Q	-	-	Q	D	L	V	-	I	0.07	0.08	0.07	880
Phage-826	N	N	-	A	S	D	L	S	-	D	N	S	-	I	0.07	0.08	0.08	881
Phage-827	-	N	-	W	-	D	W	E	-	D	-	A	-	N	0.09	0.08	0.07	882
Phage-828	-	-	-	-	-	-	L	-	-	-	F	Y	-	F	0.08	0.08	0.07	883
Phage-829	L	F	-	-	T	V	L	-	-	F	F	D	-	D	0.07	0.08	0.07	884
Phage-830	F	D	-	-	-	-	L	-	-	-	-	S	-	A	0.08	0.08	0.07	885
Phage-831	Y	-	-	-	-	-	V	E	-	N	-	Y	-	I	0.07	0.08	0.08	886
Phage-832	D	-	-	-	-	-	L	-	D	-	T	I	-	H	0.12	0.08	0.10	887
Phage-833	-	F	-	-	-	Q	W	A	-	A	N	A	-	F	0.09	0.08	0.07	888
Phage-834	F	T	-	-	-	-	Y	-	-	-	I	T	-	P	0.09	0.08	0.09	889
Phage-835	L	N	-	-	-	V	N	-	-	-	S	V	-	I	0.07	0.08	0.08	890
Phage-836	A	I	-	W	-	D	W	E	-	F	S	D	-	H	0.07	0.08	0.07	891
Phage-837	Y	-	-	-	V	D	L	G	A	N	-	Y	-	Y	0.10	0.08	0.09	892
Phage-838	H	-	-	-	-	-	V	E	-	D	Y	H	-	D	0.07	0.08	0.07	893

Phage-839	N	D	-	-	S	L	Q	Y	D	I	P	T	-	V	0.08	0.08	0.07	894
Phage-840	Y	V	-	R	-	Q	L	-	V	Y	H	Y	-	N	0.15	0.08	0.07	895
Phage-841	H	D	-	-	-	D	G	-	-	-	I	I	-	S	0.08	0.08	0.07	896
Phage-842	F	D	-	-	-	-	L	-	-	T	I	I	-	P	0.08	0.08	0.08	897
Phage-843	-	D	-	-	S	D	R	G	-	N	A	A	-	H	0.07	0.08	0.07	898
Phage-844	N	I	-	L	A	Q	-	N	-	D	P	T	-	N	0.07	0.08	0.08	899
Phage-845	T	N	-	-	S	K	S	Q	V	-	D	H	-	I	0.10	0.08	0.09	900
Phage-846	N	H	-	H	-	Q	-	W	-	L	T	N	-	N	0.09	0.07	0.07	901
Phage-847	L	L	-	H	-	Q	G	-	L	Y	H	L	-	H	0.09	0.07	0.08	902
Phage-848	T	N	-	D	S	K	L	E	G	D	D	N	-	F	0.09	0.07	0.07	903
Phage-849	N	D	-	-	-	-	M	-	-	-	L	L	-	D	0.08	0.07	0.07	904
Phage-850	F	H	-	-	-	-	V	-	-	-	I	N	-	N	0.07	0.07	0.07	905
Phage-851	D	F	-	-	-	D	G	-	-	T	Y	V	-	S	0.07	0.07	0.08	906
Phage-852	-	S	-	-	-	Q	-	-	-	N	N	T	-	N	0.07	0.07	0.08	907
Phage-853	-	-	-	H	L	-	S	E	Q	F	D	I	-	I	0.08	0.07	0.08	908
Phage-854	D	D	-	-	-	-	W	E	F	V	F	F	-	D	0.08	0.07	0.08	909
Phage-855	Y	N	-	E	Q	Q	Q	-	-	D	P	S	-	I	0.07	0.07	0.07	910
Phage-856	N	T	-	-	T	-	Q	H	-	F	N	-	-	L	0.08	0.07	0.08	911
Phage-857	H	P	-	Q	-	G	-	E	-	V	D	Y	-	V	0.08	0.07	0.08	912
Phage-858	-	A	-	S	R	Q	L	G	-	D	A	Y	-	N	0.07	0.07	0.07	913
Phage-859	D	I	-	-	A	Q	E	V	H	V	Y	T	-	P	0.07	0.07	0.07	914
Phage-860	F	F	-	E	G	N	L	-	A	Y	L	L	-	L	0.08	0.07	0.08	915
Phage-861	Y	-	-	-	-	D	G	E	-	N	I	V	-	D	0.07	0.07	0.07	916

Table 18. Summary of Peptide Sequences

Peptide-ID	anti-CD3 Panned target	Sequence	SEQ ID NO:
Peptide-29	SP34.185	DDCWPDWEFDACA	44
Peptide-30	SP34.185	YICGLDFPDFLYCD	45
Peptide-31	SP34.185	FDCWPDWEEYFVCD	46
Peptide-32	SP34.185	YICWPDWEEYFDCD	47
Peptide-33	SP34.185	NICWPDWEDDYFCF	48
Peptide-34	SP34.185	NFCWPDWEYIYPCI	49
Peptide-35	SP34.185	VDCWPDWEEDFLCI	50
Peptide-36	SP34.185	HACWPDWEEYFPCN	51
Peptide-37	SP34.185	YDCGPDVDESYVCV	52
Peptide-38	SP34.185	IDCWPDWEDDTFCY	53
Peptide-39	SP34.185	YLCGPDGDETLACY	54
Peptide-40	SP34.185	VDCGPDGDESILCY	55

Table 19. Summary of Figure 5A

ELISA	Peptide-29	Peptide-30	Peptide-31	Peptide-32	Peptide-33	Peptide-34	Peptide-2
EC50 nM	2.163	1.564	2.035	1.713	2.174	2.752	1.972

Table 20. Summary of Figure 5B

ELISA	Peptide-35	Peptide-36	Peptide-37	Peptide-38	Peptide-39	Peptide-40	Peptide-2
EC50 nM	2.804	2.695	344.3	1.652	1.286	1.895	1.623

Table 21. Summary of Figure 6A

ELISA	Peptide-29	Peptide-30	Peptide-31	Peptide-32	Peptide-33	Peptide-34	Peptide-2
IC50 uM	7.9	3.2	8.5	6.5	17.2	11.7	0.28

Table 22. Summary of Figure 6A

ELISA	Peptide-35	Peptide-36	Peptide-37	Peptide-38	Peptide-39	Peptide-40	Peptide-2
IC50 uM	17.3	15.2	>100	2.9	0.01	0.35	0.15

CLAIMS

WHAT IS CLAIMED IS:

1. An isolated polypeptide or polypeptide complex comprising a peptide that impairs binding of an anti-CD3 binding domain to cluster of differentiation 3 (CD3) wherein the peptide comprises an amino acid sequence selected from SEQ ID NOs: 1-2 and 19-55 or the peptide comprises 1, 2, or 3, amino acid substitutions, additions, or deletions relative to an amino acid sequence selected from SEQ ID NOs: 1-2 and 19-55.
2. The isolated polypeptide or polypeptide complex of claim 1, wherein the peptide comprises an amino acid sequence selected from SEQ ID NOs: 1-2 and 19-55.
3. The isolated polypeptide or polypeptide complex of claim 2, wherein the peptide comprises the amino acid sequence of SEQ ID NO: 1.
4. The isolated polypeptide or polypeptide complex of claim 2, wherein the peptide comprises the amino acid sequence of SEQ ID NO: 2.
5. The isolated polypeptide or polypeptide complex of claim 2, wherein the peptide comprises the amino acid sequence of SEQ ID NO: 54.
6. An isolated polypeptide or polypeptide complex comprising an anti-CD3 binding domain that is linked to a peptide that impairs binding of the anti-CD3 binding domain to CD3 wherein the peptide comprises an amino acid sequence according to Z_1 - Z_2 -C- Z_4 -P- Z_6 - Z_7 - Z_8 - Z_9 - Z_{10} - Z_{11} - Z_{12} -C- Z_{14} and Z_1 is selected from D, Y, F, I, N, V, H, L, A, T, S, and P; Z_2 is selected from D, Y, L, F, I, N, A, V, H, T, and S; Z_4 is selected from G and W; Z_6 is selected from E, D, V, and P; Z_7 is selected from W, L, F, V, G, M, I, and Y; Z_8 is selected from E, D, P, and Q; Z_9 is selected from E, D, Y, V, F, W, P, L, and Q; Z_{10} is selected from S, D, Y, T, I, F, V, N, A, P, L, and H; Z_{11} is selected from I, Y, F, V, L, T, N, S, D, A, and H; Z_{12} is selected from F, D, Y, L, I, V, A, N, T, P, S, and H; and Z_{14} is selected from D, Y, N, F, I, P, V, A, T, H, L and S.
7. The isolated polypeptide or polypeptide complex of claim 6, wherein Z_1 is selected from D, Y, F, I, and N; Z_2 is selected from D, Y, L, F, I, and N; Z_4 is selected from G and W; Z_6 is selected from E and D; Z_7 is selected from W, L, F, and V; Z_8 is selected from E and D; Z_9 is selected from E, D, Y, and V; Z_{10} is selected from S, D, Y, T, and I; Z_{11} is selected from I, Y, F, V, L, and T; Z_{12} is selected from F, D, Y, L, I, V, A, and N; and Z_{14} is selected from D, Y, N, F, I, and P.
8. The isolated polypeptide or polypeptide complex of claim 7, wherein Z_1 is selected from D, Y, and F; Z_2 is selected from D, Y, L, and F; Z_4 is selected from G and W; Z_6 is selected from E and D; Z_7 is selected from W, L, and F; Z_8 is selected from E and D; Z_9 is selected from E and D; Z_{10} is selected from S, D, and Y; Z_{11} is selected from I, Y, and F; Z_{12} is selected from F, D, Y, and L; and Z_{14} is selected from D, Y, and N.
9. An isolated polypeptide or polypeptide complex comprising an anti-CD3 binding domain that is linked to a peptide that impairs binding of the anti-CD3 binding domain to CD3 wherein the peptide comprises an amino acid sequence according to U_1 - U_2 -C- U_4 -P- U_6 - U_7 - U_8 - U_9 - U_{10} - U_{11} - U_{12} -C- U_{14} and U_1 is selected from D, Y, F, I, N, V, H, L, A, T, S, and P; U_2 is selected from D, Y, L, F, I, N, A, V, H, T, and S;

U₄ is selected from G and W; U₆ is selected from E, D, V, and P; U₇ is selected from W, L, F, V, G, M, I, and Y; U₈ is selected from E, D, P, and Q; U₉ is selected from E, D, Y, V, F, W, P, L, and Q; U₁₀ is selected from S, D, Y, T, I, F, V, N, A, P, L, and H; U₁₁ is selected from I, Y, F, V, L, T, N, S, D, A, and H; U₁₂ is selected from F, D, Y, L, I, V, A, N, T, P, S, G, and H; and U₁₄ is selected from D, Y, N, F, I, P, V, A, T, H, L, M, and S.

10. The isolated polypeptide or polypeptide complex of claim 9, wherein U₁ is selected from D, Y, F, I, V, and N; U₂ is selected from D, Y, L, F, I, and N; U₄ is selected from G and W; U₆ is selected from E and D; U₇ is selected from W, L, F, G, and V; U₈ is selected from E and D; U₉ is selected from E, D, Y, and V; U₁₀ is selected from S, D, Y, T, and I; U₁₁ is selected from I, Y, F, V, L, and T; U₁₂ is selected from F, D, Y, L, I, V, A, G, and N; and U₁₄ is selected from D, Y, N, F, I, M, and P.

11. The isolated polypeptide or polypeptide complex of claim 10, wherein U₁ is selected from D, Y, V, and F; U₂ is selected from D, Y, L, and F; U₄ is selected from G and W; U₆ is selected from E and D; U₇ is selected from W, L, G, and F; U₈ is selected from E and D; U₉ is selected from E and D; U₁₀ is selected from S, D, T, and Y; U₁₁ is selected from I, Y, V, L, and F; U₁₂ is selected from F, D, Y, G, A, and L; and U₁₄ is selected from D, Y, M, and N.

12. The isolated polypeptide or polypeptide complex of claim 9, wherein the peptide comprises an amino acid sequence of **Table 17**.

13. The isolated polypeptide or polypeptide complex of claim 9, wherein the peptide comprises an amino acid sequence according to any one of SEQ ID NOs: 44-55.

14. The isolated polypeptide or polypeptide complex of claim 11, wherein the peptide comprises the amino acid sequence of SEQ ID NO: 2.

15. The isolated polypeptide or polypeptide complex of claim 11, wherein the peptide comprises the amino acid sequence of SEQ ID NO: 54.

16. The isolated polypeptide or polypeptide complex of claim 9, wherein the peptide is connected to the anti-CD3 binding domain in a configuration according to Formula I: A₁-L₁-P₁ wherein A₁ comprises the anti-CD3 binding domain; L₁ comprises a cleavable linker that is a substrate for a tumor specific protease; and P₁ comprises the peptide that impairs binding of the anti-CD3 binding domain to CD3.

17. The isolated polypeptide or polypeptide complex of claim 16, wherein P₁ is connected N-terminal to the cleavable linker and A₁ is connected C-terminal to the cleavable linker.

18. The isolated polypeptide or polypeptide complex of claim 16, wherein P₁ is connected C-terminal to the cleavable linker and A₁ is connected N-terminal to the cleavable linker.

19. The isolated polypeptide or polypeptide complex of claim 16, wherein P₁ is bound to A₁ through ionic interactions, electrostatic interactions, hydrophobic interactions, Pi-stacking interactions, or H-bonding interactions, or a combination thereof.

20. The isolated polypeptide or polypeptide complex of claim 16, wherein A₁ is further linked to a tumor antigen binding domain (A₂).

21. The isolated polypeptide or polypeptide complex of claim 16, wherein the polypeptide or polypeptide complex is according to Formula Ia: $P_2-L_2-A_2-A_1-L_1-P_1$ wherein P_2 comprises a peptide that impairs binding of A_2 to a tumor antigen; and L_2 comprises a second cleavable linker that connects A_2 to P_2 and is a substrate for a tumor specific protease.
22. The isolated polypeptide or polypeptide complex of claim 21, wherein A_1 comprises an antibody or antibody fragment.
23. The isolated polypeptide or polypeptide complex of claim 21, wherein A_1 comprises an antibody or antibody fragment that is human or humanized.
24. The isolated polypeptide or polypeptide complex of claim 22, wherein L_1 is bound to a N-terminus of the antibody or antibody fragment of A_1 .
25. The isolated polypeptide or polypeptide complex of claim 22, wherein A_2 is bound to a N-terminus of the antibody or antibody fragment of A_1 .
26. The isolated polypeptide or polypeptide complex of claim 22, wherein L_1 is bound to a C-terminus of the antibody or antibody fragment of A_1 .
27. The isolated polypeptide or polypeptide complex of claim 22, wherein A_2 is bound to a C-terminus of the antibody or antibody fragment of A_1 .
28. The isolated polypeptide or polypeptide complex of claim 22, wherein the antibody or antibody fragment of A_1 comprises a single chain variable fragment, a single domain antibody, or a Fab fragment.
29. The isolated polypeptide or polypeptide complex of claim 28, wherein A_1 is the single chain variable fragment (scFv).
30. The isolated polypeptide or polypeptide complex of claim 29, wherein the scFv comprises a scFv heavy chain polypeptide and a scFv light chain polypeptide.
31. The isolated polypeptide or polypeptide complex of claim 28, wherein A_1 is the single domain antibody.
32. The isolated polypeptide or polypeptide complex of claim 23, wherein the antibody or antibody fragment thereof comprises a single chain variable fragment (scFv), a heavy chain variable domain (VH domain), a light chain variable domain (VL domain), or a variable domain (VHH) of a camelid derived single domain antibody.
33. The isolated polypeptide or polypeptide complex of claim 32, wherein A_1 comprises an anti-CD3e single chain variable fragment.
34. The isolated polypeptide or polypeptide complex of claim 22, wherein A_1 comprises an anti-CD3e single chain variable fragment that has a KD binding of 1 μ M or less to CD3 on CD3 expressing cells.
35. The isolated polypeptide or polypeptide complex of claim 22, wherein A_1 comprises a variable light chain and variable heavy chain each of which is capable of specifically binding to human CD3.
36. The isolated polypeptide or polypeptide complex of claim 30, wherein A_1 comprises the scFv comprising a scFv heavy chain variable domain and an scFv light chain variable domain comprising

complementarity determining regions (CDRs): HC-CDR1, HC-CDR2, and HC-CDR3, wherein the HC-CDR1, the HC-CDR2, and the HC-CDR3 of the scFv heavy chain variable domain comprise: HC-CDR1: SEQ ID NO: 3; HC-CDR2: SEQ ID NO: 4; HC-CDR3: SEQ ID NO: 5; and the scFv light chain variable domain comprises complementarity determining regions (CDRs): LC-CDR1, LC-CDR2, and LC-CDR3, wherein the LC-CDR1, the LC-CDR2, and the LC-CDR3 of the scFv light chain variable domain comprise: LC-CDR1: SEQ ID NO: 6; LC-CDR2: SEQ ID NO: 7; and LC-CDR3: SEQ ID NO: 8.

37. The isolated polypeptide or polypeptide complex of claim 22, wherein A₁ comprises a heavy chain variable domain and a light chain variable domain comprising complementarity determining regions (CDRs): HC-CDR1, HC-CDR2, and HC-CDR3, wherein the HC-CDR1, the HC-CDR2, and the HC-CDR3 of the heavy chain variable domain comprise: HC-CDR1: SEQ ID NO: 3; HC-CDR2: SEQ ID NO: 4; HC-CDR3: SEQ ID NO: 5; and the light chain variable domain comprises complementarity determining regions (CDRs): LC-CDR1, LC-CDR2, and LC-CDR3, wherein the LC-CDR1, the LC-CDR2, and the LC-CDR3 of the light chain variable domain comprise: LC-CDR1: SEQ ID NO: 6; LC-CDR2: SEQ ID NO: 7; and LC-CDR3: SEQ ID NO: 8.

38. The isolated polypeptide or polypeptide complex of claim 16, wherein the polypeptide or polypeptide complex of formula I binds to an effector cell when L₁ is cleaved by the tumor specific protease.

39. The isolated polypeptide or polypeptide complex of claim 16, wherein the polypeptide or polypeptide complex of formula I binds to an effector cell when L₁ is cleaved by the tumor specific protease and A₁ binds to the effector cell.

40. The isolated polypeptide or polypeptide complex of claim 39, wherein the effector cell is a T cell.

41. The isolated polypeptide or polypeptide complex of claim 40, wherein A₁ binds to a polypeptide that is part of a TCR-CD3 complex on the effector cell.

42. The isolated polypeptide or polypeptide complex of claim 41, wherein the polypeptide that is part of the TCR-CD3 complex is human CD3ε.

43. The isolated polypeptide or polypeptide complex of claim 30, wherein A₂ comprises an antibody or antibody fragment.

44. The isolated polypeptide or polypeptide complex of claim 43, wherein the antibody or antibody fragment thereof of A₂ comprises a single chain variable fragment, a single domain antibody, Fab', or a Fab.

45. The isolated polypeptide or polypeptide complex of claim 43, wherein the antibody or antibody fragment of A₂ comprises a single chain variable fragment (scFv), a heavy chain variable domain (VH domain), a light chain variable domain (VL domain), or a variable domain (VHH) of a camelid derived single domain antibody.

46. The isolated polypeptide or polypeptide complex of claim 43, wherein the antibody or antibody fragment of A₂ is humanized or human.

47. The isolated polypeptide or polypeptide complex of claim 44, wherein A₂ is the Fab or Fab'.

48. The isolated polypeptide or polypeptide complex of claim 47, wherein the Fab or Fab' comprises (a) a Fab light chain polypeptide and (b) a Fab heavy chain polypeptide.
49. The isolated polypeptide or polypeptide complex of claim 48, wherein A₂ comprises an epidermal growth factor receptor (EGFR) binding domain.
50. The isolated polypeptide or polypeptide complex of claim 48, wherein A₂ comprises a carcinoembryonic antigen-related cell adhesion molecule CEACAM5 binding domain.
51. The isolated polypeptide or polypeptide complex of claim 48, wherein P₁ is further linked to a half-life extending moiety (H₁).
52. The isolated polypeptide or polypeptide complex of claim 51, wherein the half-life extending moiety is a single-domain antibody.
53. The isolated polypeptide or polypeptide complex of claim 51, wherein H₁ comprises a polymer.
54. The isolated polypeptide or polypeptide complex of claim 53, wherein the polymer is polyethylene glycol (PEG).
55. The isolated polypeptide or polypeptide complex of claim 51, wherein H₁ comprises albumin.
56. The isolated polypeptide or polypeptide complex of claim 51, wherein H₁ comprises an Fc domain.
57. The isolated polypeptide or polypeptide complex of claim 55, wherein the albumin is serum albumin.
58. The isolated polypeptide or polypeptide complex of claim 57, wherein the albumin is human serum albumin.
59. The isolated polypeptide or polypeptide complex of claim 51, wherein H₁ comprises a polypeptide, a ligand, or a small molecule.
60. The isolated polypeptide or polypeptide complex of claim 59, wherein the polypeptide, the ligand or the small molecule binds serum protein or a fragment thereof, a circulating immunoglobulin or a fragment thereof, or CD35/CR1.
61. The isolated polypeptide or polypeptide complex of claim 60, wherein the serum protein comprises a thyroxine-binding protein, a transthyretin, a 1-acid glycoprotein, a transferrin, transferrin receptor or a transferrin-binding portion thereof, a fibrinogen, or an albumin.
62. The isolated polypeptide or polypeptide complex of claim 60, wherein the circulating immunoglobulin comprises IgG1, IgG2, IgG3, IgG4, sIgA, IgM or IgD.
63. The isolated polypeptide or polypeptide complex of claim 60, wherein the serum protein is albumin.
64. The isolated polypeptide or polypeptide complex of claim 59, wherein the polypeptide is an antibody.

65. The isolated polypeptide or polypeptide complex of claim 64, wherein the antibody comprises a single domain antibody, a single chain variable fragment, or a Fab.

66. The isolated polypeptide or polypeptide complex of claim 65, wherein the single domain antibody comprises a single domain antibody that binds to albumin.

67. The isolated polypeptide or polypeptide complex of claim 65, wherein the single domain antibody is a human or humanized antibody.

68. The isolated polypeptide or polypeptide complex of claim 65, wherein the single domain antibody is 645gH1gL1.

69. The isolated polypeptide or polypeptide complex of claim 65, wherein the single domain antibody is 645dsgH5gL4.

70. The isolated polypeptide or polypeptide complex of claim 65, wherein the single domain antibody is 23-13-A01 -sc02.

71. The isolated polypeptide or polypeptide complex of claim 65, wherein the single domain antibody is A10m3 or a fragment thereof.

72. The isolated polypeptide or polypeptide complex of claim 65, wherein the single domain antibody is DOM7r-31.

73. The isolated polypeptide or polypeptide complex of claim 65, wherein the single domain antibody is DOM7h-11-15.

74. The isolated polypeptide or polypeptide complex of claim 65, wherein the single domain antibody is Alb-1, Alb-8, or Alb-23.

75. The isolated polypeptide or polypeptide complex of claim 65, wherein the single domain antibody is 10G or 10GE.

76. The isolated polypeptide or polypeptide complex of claim 65, wherein the single domain antibody is SA21.

77. The isolated polypeptide or polypeptide complex of claim 1, wherein the polypeptide or polypeptide complex comprises a modified amino acid, a non-natural amino acid, a modified non-natural amino acid, or a combination thereof.

78. The isolated polypeptide or polypeptide complex of claim 77, wherein the modified amino acid or modified non-natural amino acid comprises a post-translational modification.

79. The isolated polypeptide or polypeptide complex of claim 51, wherein H_1 comprises a linking moiety (L_3) that connects H_1 to P_1 .

80. The isolated polypeptide or polypeptide complex of claim 79, wherein L_3 is a peptide sequence having at least 5 to no more than 50 amino acids.

81. The isolated polypeptide or polypeptide complex of claim 79, wherein L_3 is a peptide sequence having at least 10 to no more than 30 amino acids.

82. The isolated polypeptide or polypeptide complex of claim 79, wherein L_3 is a peptide sequence having at least 10 amino acids.

83. The isolated polypeptide or polypeptide complex of claim 79, wherein L_3 is a peptide sequence having at least 18 amino acids.

84. The isolated polypeptide or polypeptide complex of claim 79, wherein L_3 is a peptide sequence having at least 26 amino acids.

85. The isolated polypeptide or polypeptide complex of claim 79, wherein L_3 has a formula selected from the group consisting of $(G_2S)_n$, $(GS)_n$, $(GSGGS)_n$ (SEQ ID NO: 917), $(GGGS)_n$ (SEQ ID NO: 918), $(GGGGS)_n$ (SEQ ID NO: 919), and $(GSSGGS)_n$ (SEQ ID NO: 920), wherein n is an integer of at least 1.

86. The isolated polypeptide or polypeptide complex of claim 79, wherein L_3 comprises an amino acid sequence according to $(GGGGSGGGS)$ (SEQ ID NO: 921)).

87. The isolated polypeptide or polypeptide complex of claim 48, wherein the Fab light chain polypeptide of A_2 is bound to a C-terminus of the single chain variable fragment (scFv) of A_1 .

88. The isolated polypeptide or polypeptide complex of claim 48, wherein the Fab heavy chain polypeptide of A_2 is bound to a C-terminus of the single chain variable fragment (scFv) A_1 .

89. The isolated polypeptide or polypeptide complex of claim 48, wherein the Fab light chain polypeptide of A_2 is bound to a N-terminus of the single chain variable fragment (scFv) of A_1 .

90. The isolated polypeptide or polypeptide complex of claim 48, wherein the Fab heavy chain polypeptide of A_2 is bound to a N-terminus of the single chain variable fragment (scFv) A_1 .

91. The isolated polypeptide or polypeptide complex of claim 48, wherein the Fab heavy chain polypeptide of A_2 is bound to the scFv heavy chain polypeptide of A_1 .

92. The isolated polypeptide or polypeptide complex of claim 48, wherein the Fab light chain polypeptide of A_2 is bound to the scFv heavy chain polypeptide of A_1 .

93. The isolated polypeptide or polypeptide complex of claim 48, wherein the Fab heavy chain polypeptide of A_2 is bound to the scFv light chain polypeptide of A_1 .

94. The isolated polypeptide or polypeptide complex of claim 48, wherein the Fab light chain polypeptide of A_2 is bound to the scFv light chain polypeptide of A_1 .

95. The isolated polypeptide or polypeptide complex of claim 48, wherein the Fab heavy chain polypeptide of A_2 is bound to the scFv heavy chain polypeptide of A_1 and L_2 is bound to the Fab light chain polypeptide of A_2 .

96. The isolated polypeptide or polypeptide complex of claim 48, wherein the Fab light chain polypeptide of A_2 is bound to the scFv heavy chain polypeptide of A_1 and L_2 is bound to the Fab heavy chain polypeptide of A_2 .

97. The isolated polypeptide or polypeptide complex of claim 48, wherein the Fab heavy chain polypeptide of A_2 is bound to the scFv light chain polypeptide of A_1 and L_2 is bound to the Fab light chain polypeptide of A_2 .

98. The isolated polypeptide or polypeptide complex of claim 48, wherein the Fab light chain polypeptide of A_2 is bound to the scFv light chain polypeptide of A_1 and L_2 is bound to the Fab heavy chain polypeptide of A_2 .

99. The isolated polypeptide or polypeptide complex of claim 21, wherein P₂ impairs binding of A₂ to the tumor antigen.
100. The isolated polypeptide or polypeptide complex of claim 21, wherein P₂ is bound to A₂ through ionic interactions, electrostatic interactions, hydrophobic interactions, Pi-stacking interactions, or H-bonding interactions, or a combination thereof.
101. The isolated polypeptide or polypeptide complex of claim 21, wherein P₂ is bound to A₂ at or near an antigen binding site.
102. The isolated polypeptide or polypeptide complex of claim 21, wherein P₂ has less than 70% sequence homology to the tumor antigen.
103. The isolated polypeptide or polypeptide complex of claim 21, wherein P₂ comprises a peptide sequence of at least 10 amino acids in length.
104. The isolated polypeptide or polypeptide complex of claim 21, wherein P₂ comprises a peptide sequence of at least 10 amino acids in length and no more than 20 amino acids in length.
105. The isolated polypeptide or polypeptide complex of claim 21, wherein P₂ comprises a peptide sequence of at least 16 amino acids in length.
106. The isolated polypeptide or polypeptide complex of claim 21, wherein P₂ comprises a peptide sequence of no more than 40 amino acids in length.
107. The isolated polypeptide or polypeptide complex of claim 21, wherein P₂ comprises at least two cysteine amino acid residues.
108. The isolated polypeptide or polypeptide complex of claim 21, wherein P₂ comprises a cyclic peptide or a linear peptide.
109. The isolated polypeptide or polypeptide complex of claim 21, wherein P₂ comprises a cyclic peptide.
110. The isolated polypeptide or polypeptide complex of claim 21, wherein P₂ comprises a linear peptide.
111. The isolated polypeptide or polypeptide complex of claim 16, wherein L₁ is bound to a N-terminus of A₁.
112. The isolated polypeptide or polypeptide complex of claim 16, wherein L₁ is bound to a C-terminus of A₁.
113. The isolated polypeptide or polypeptide complex of claim 21, wherein L₂ is bound to a N-terminus of A₂.
114. The isolated polypeptide or polypeptide complex of claim 21, wherein L₂ is bound to a C-terminus of A₂.
115. The isolated polypeptide or polypeptide complex of claim 21, wherein L₁ or L₂ is a peptide sequence having at least 5 to no more than 50 amino acids.
116. The isolated polypeptide or polypeptide complex of claim 21, wherein L₁ or L₂ is a peptide sequence having at least 10 to no more than 30 amino acids.

117. The isolated polypeptide or polypeptide complex of claim 21, wherein L_1 or L_2 is a peptide sequence having at least 10 amino acids.
118. The isolated polypeptide or polypeptide complex of claim 21, wherein L_1 or L_2 is a peptide sequence having at least 18 amino acids.
119. The isolated polypeptide or polypeptide complex of claim 21, wherein L_1 or L_2 is a peptide sequence having at least 26 amino acids.
120. The isolated polypeptide or polypeptide complex of claim 21, wherein L_1 or L_2 has a formula comprising $(G_2S)_n$ (SEQ ID NO: 922), wherein n is an integer from 1 to 3.
121. The isolated polypeptide or polypeptide complex of claim 21, wherein L_1 has a formula selected from the group consisting of $(G_2S)_n$, $(GS)_n$, $(GSGGS)_n$ (SEQ ID NO: 917), $(GGGS)_n$ (SEQ ID NO: 918), $(GGGGS)_n$ (SEQ ID NO: 919), and $(GSSGGS)_n$ (SEQ ID NO: 920), wherein n is an integer of at least 1.
122. The isolated polypeptide or polypeptide complex of claim 21, wherein P_1 becomes unbound from A_1 when L_1 is cleaved by the tumor specific protease thereby exposing A_1 to CD3.
123. The isolated polypeptide or polypeptide complex of claim 21, wherein P_2 becomes unbound from A_2 when L_2 is cleaved by the tumor specific protease thereby exposing A_2 to the tumor antigen.
124. The isolated polypeptide or polypeptide complex of claim 21, wherein the tumor specific protease is selected from the group consisting of metalloprotease, serine protease, cysteine protease, threonine protease, and aspartic protease.
125. The isolated polypeptide or polypeptide complex of claim 21, wherein L_1 or L_2 comprises a urokinase cleavable amino acid sequence, a matriptase cleavable amino acid sequence, a matrix metalloprotease cleavable amino acid sequence, or a legumain cleavable amino acid sequence.
126. The isolated polypeptide or polypeptide complex of claim 21, wherein L_1 or L_2 comprises an amino acid sequence selected from the group consisting of GGGGSLSGRSDNHGSSGT (SEQ ID NO: 923), GGGGSSGGSGGSGLSGRSDNHGSSGT (SEQ ID NO: 924), ASGRSDNH (SEQ ID NO: 925), LAGRSDNH (SEQ ID NO: 926), ISSGLASGRSDNH (SEQ ID NO: 927), ISSGLLAGRSDNH (SEQ ID NO: 928), LSGRSDNH (SEQ ID NO: 929), ISSGLLSGRSDNP (SEQ ID NO: 930), ISSGLLSGRSDNH (SEQ ID NO: 931), LSGRSDNHSPLGLAGS (SEQ ID NO: 932), SPLGLAGSLSGRSDNH (SEQ ID NO: 933), SPLGLSGRSDNH (SEQ ID NO: 934), LAGRSDNHSPLGLAGS (SEQ ID NO: 935), LSGRSDNHVPLSLKMG (SEQ ID NO: 936), and LSGRSDNHVPLSLSMG (SEQ ID NO: 937).
127. The isolated polypeptide or polypeptide complex of claim 21, wherein L_1 or L_2 comprises an amino acid sequence selected from the group consisting of ASGRSDNH (SEQ ID NO: 925), LAGRSDNH (SEQ ID NO: 926), ISSGLASGRSDNH (SEQ ID NO: 927), and ISSGLLAGRSDNH (SEQ ID NO: 928).
128. A pharmaceutical composition comprising: (i) the polypeptide or polypeptide complex of claim 1; and (ii) a pharmaceutically acceptable excipient.
129. An isolated recombinant nucleic acid molecule encoding the polypeptide or polypeptide complex of claim 1.

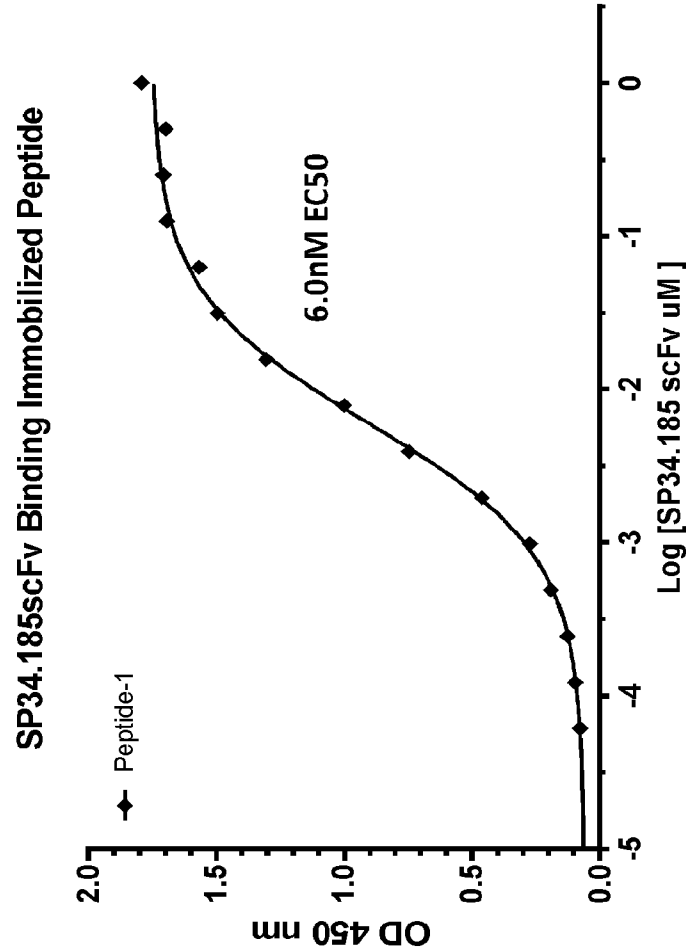


FIG. 1A

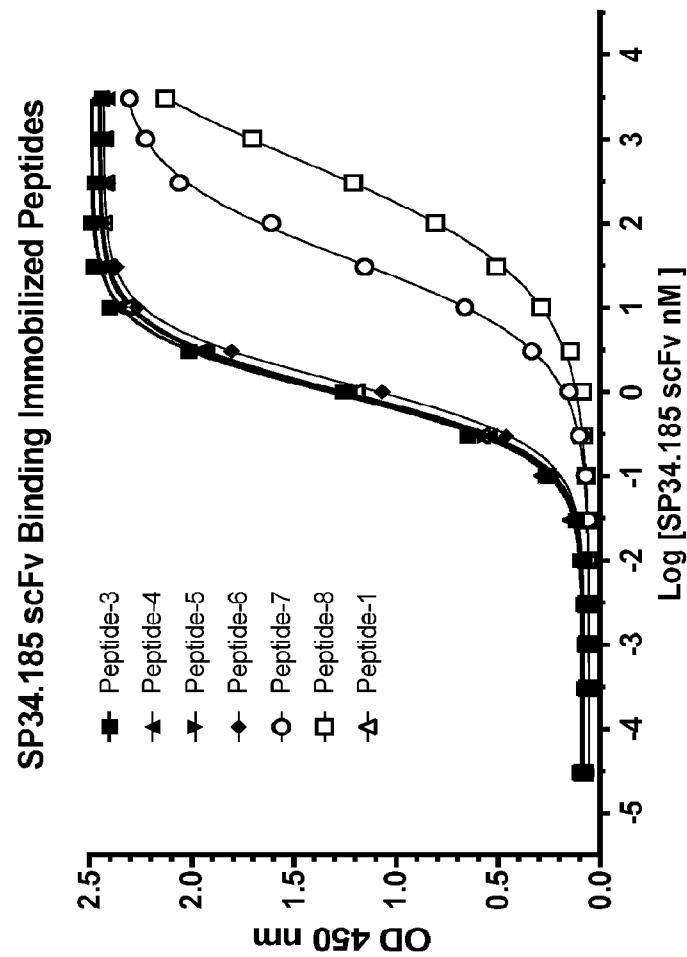


FIG. 1B

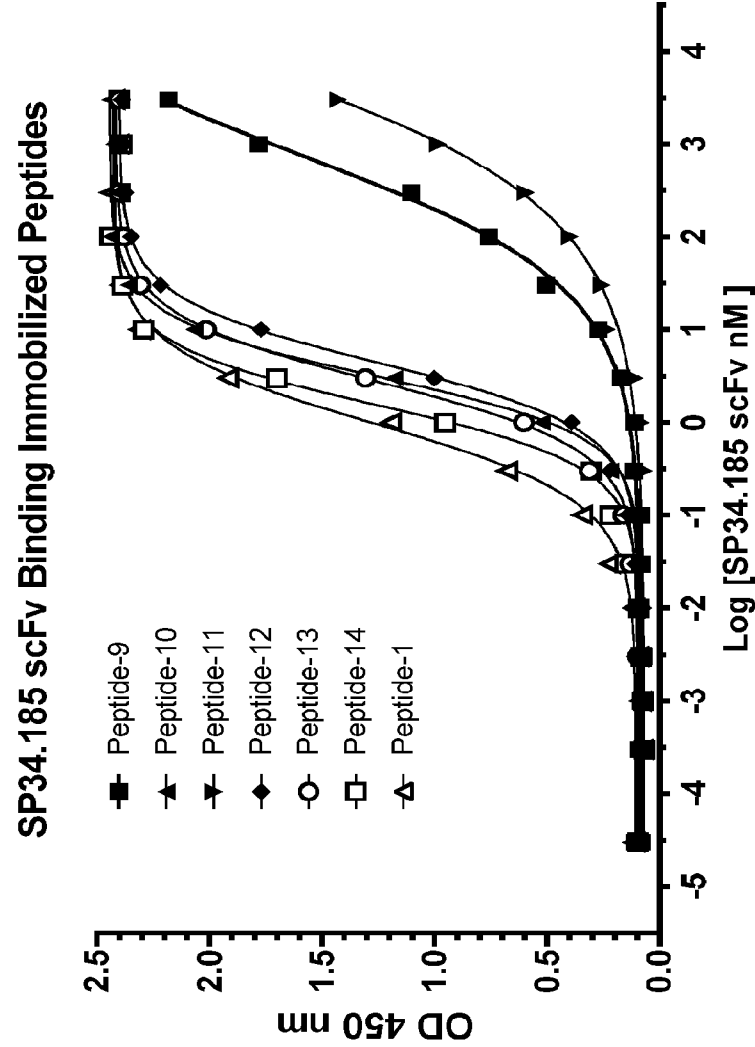


FIG. 1C

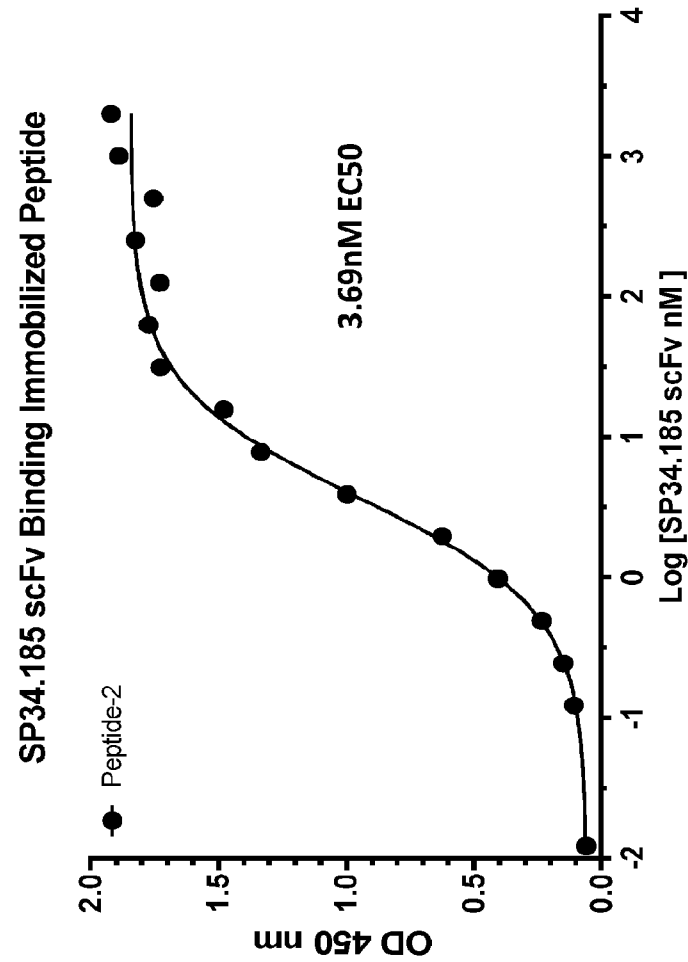


FIG. 1D

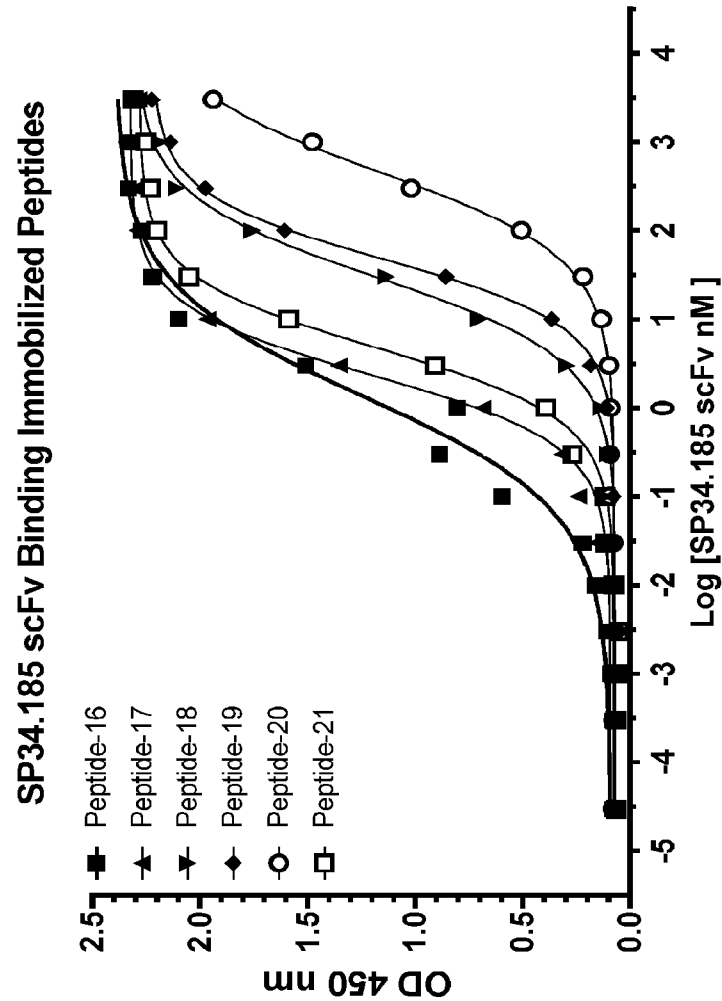


FIG. 1E

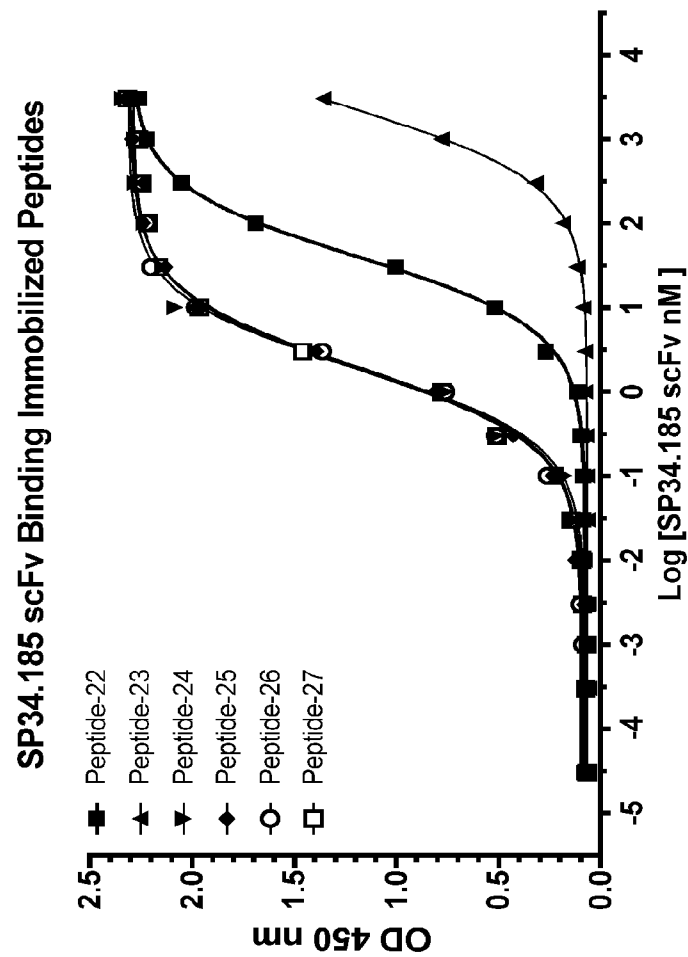


FIG. 1F

7/19

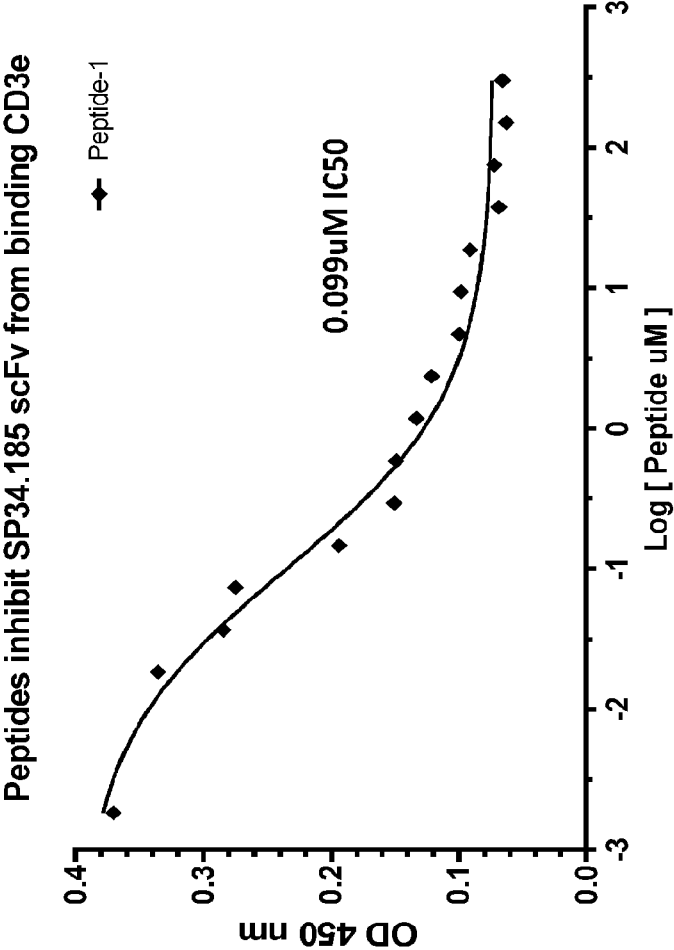


FIG. 2A

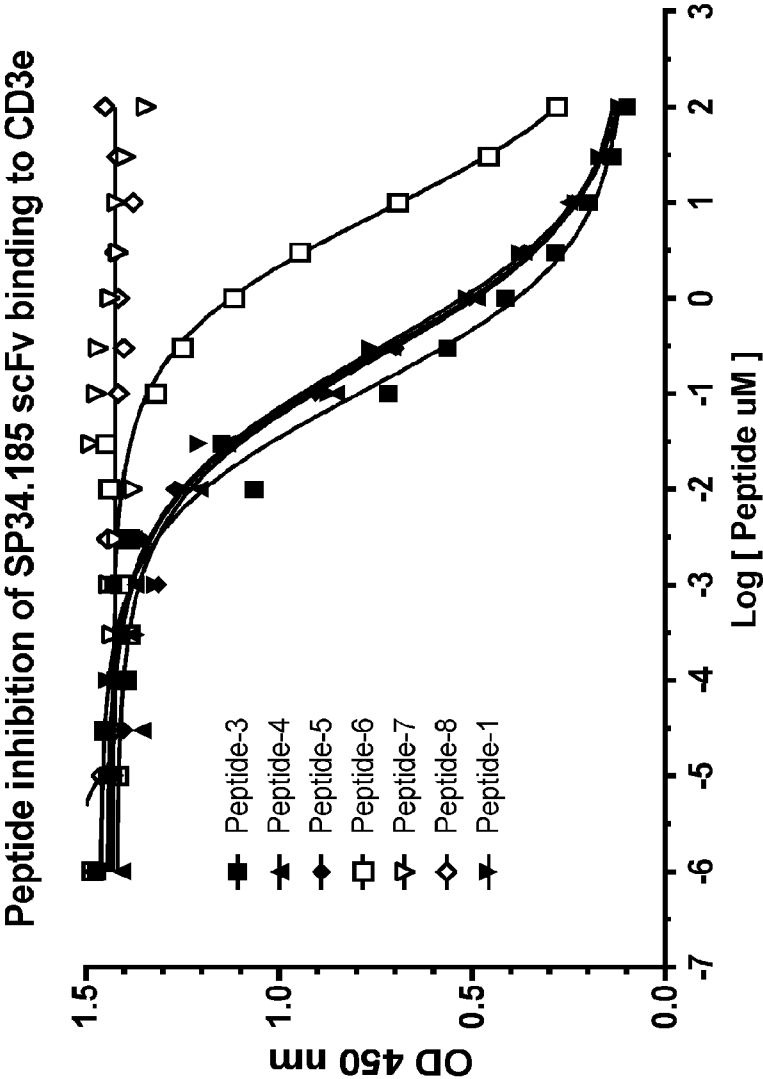


FIG. 2B

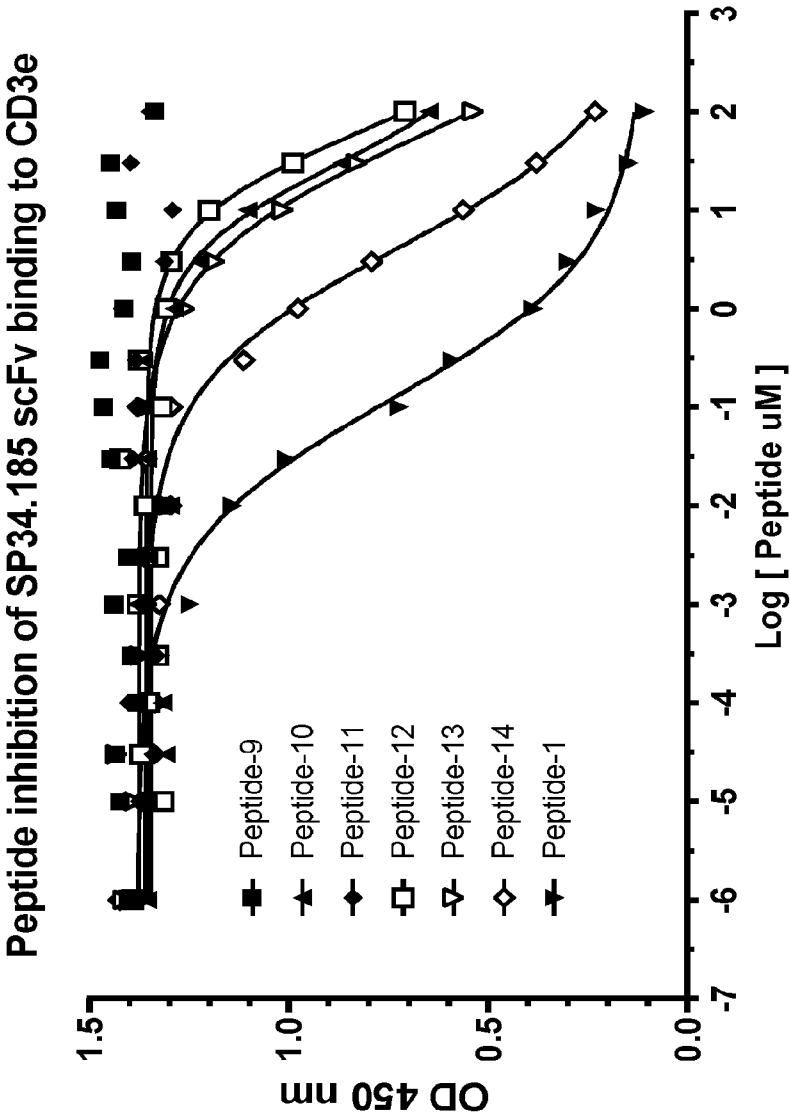


FIG. 2C

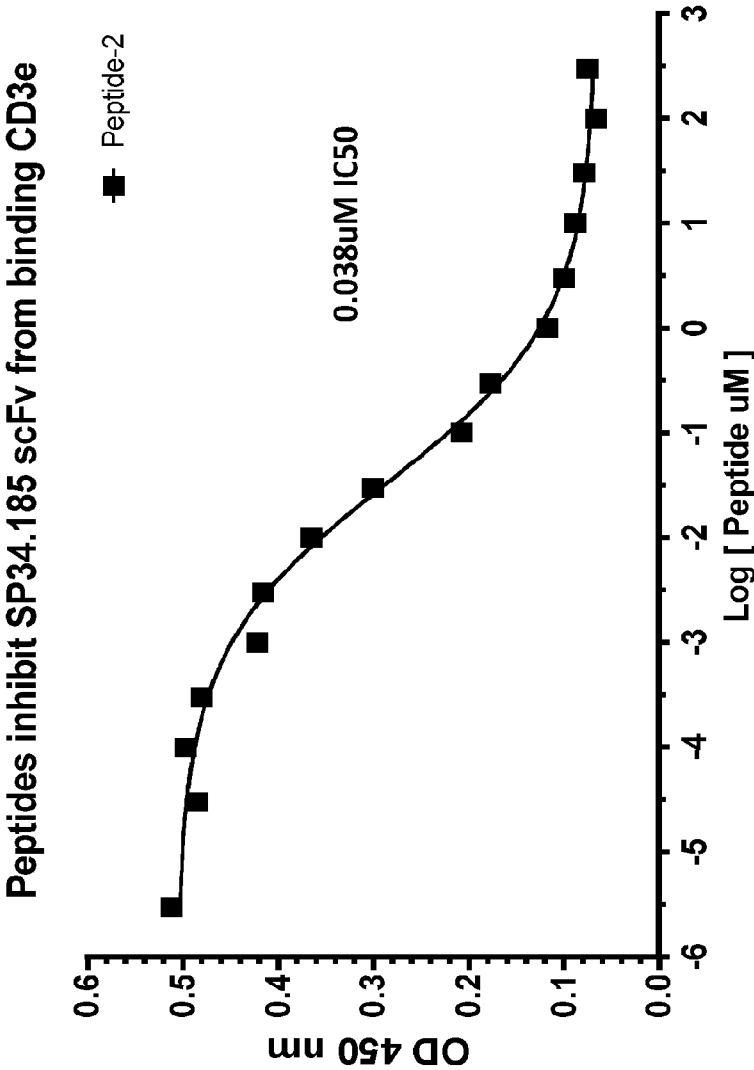


FIG. 2D

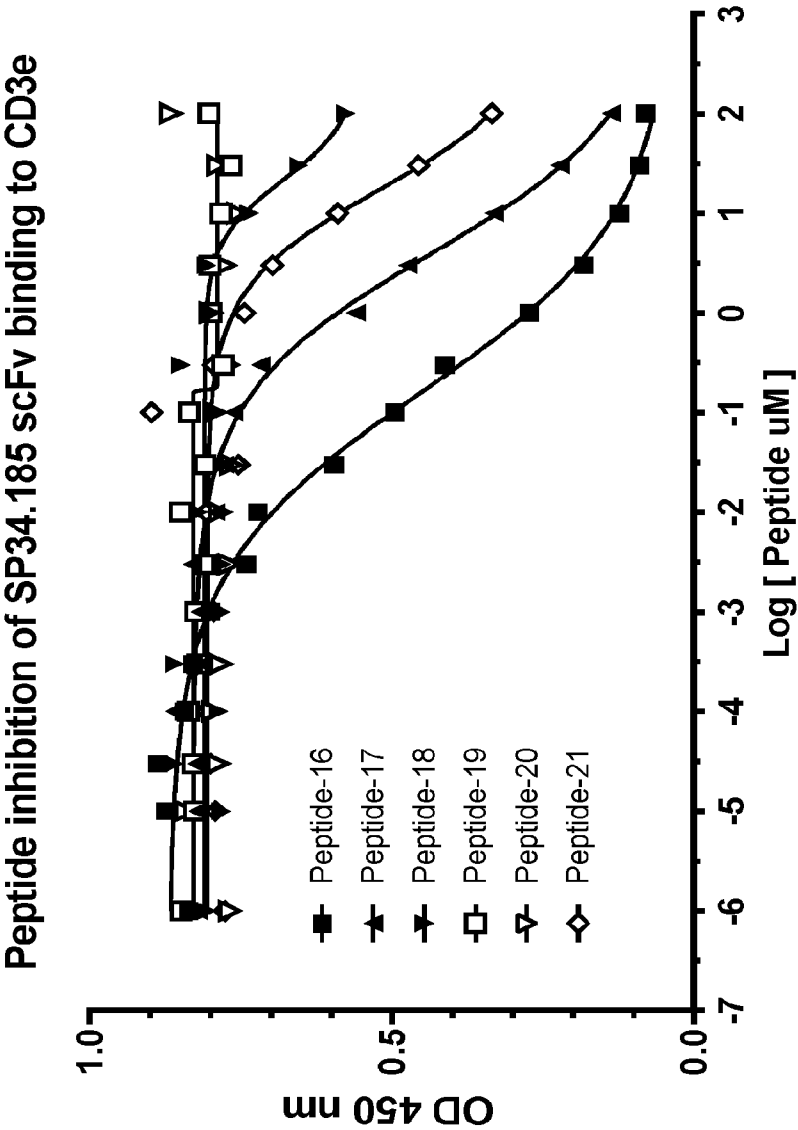


FIG. 2E

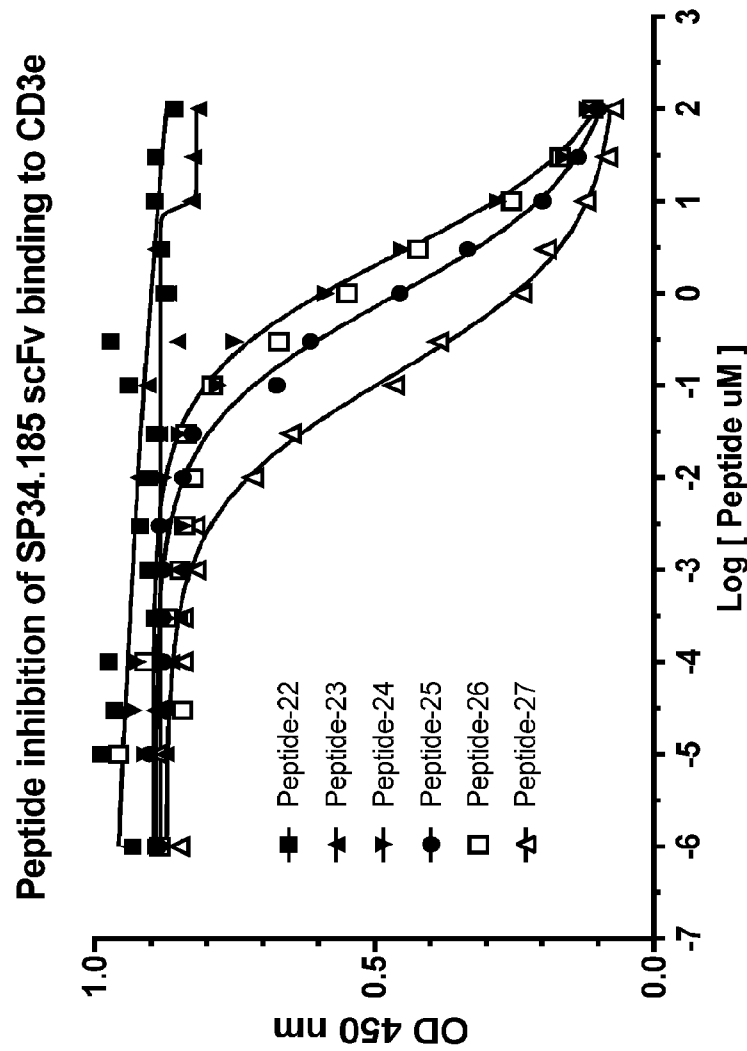


FIG. 2F

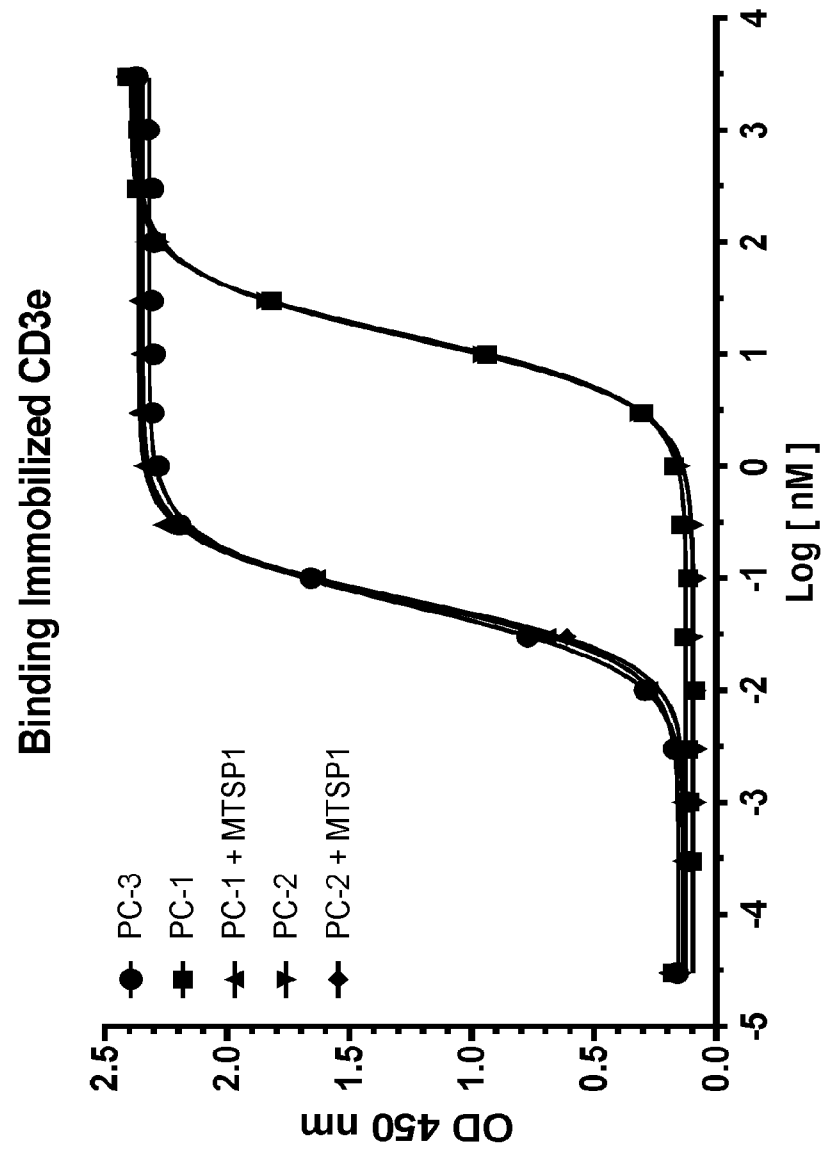


FIG. 3

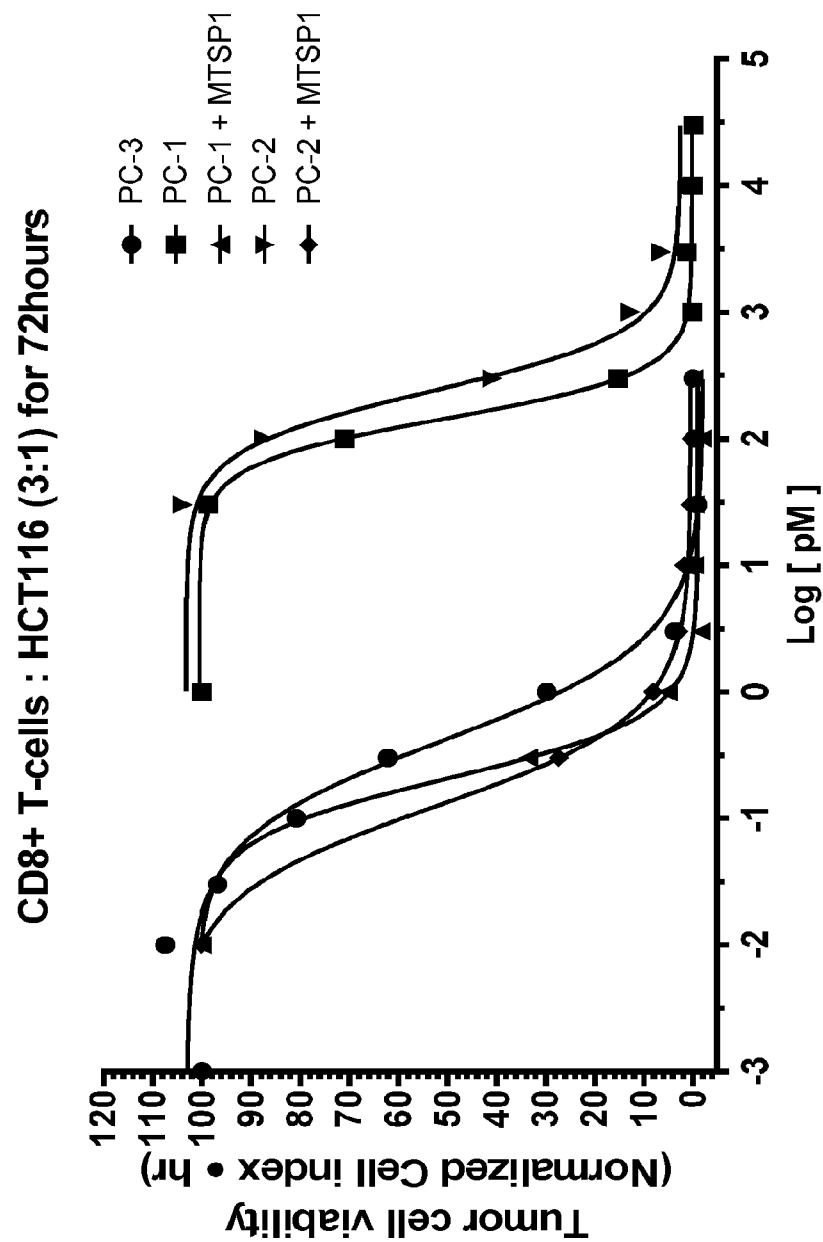


FIG. 4

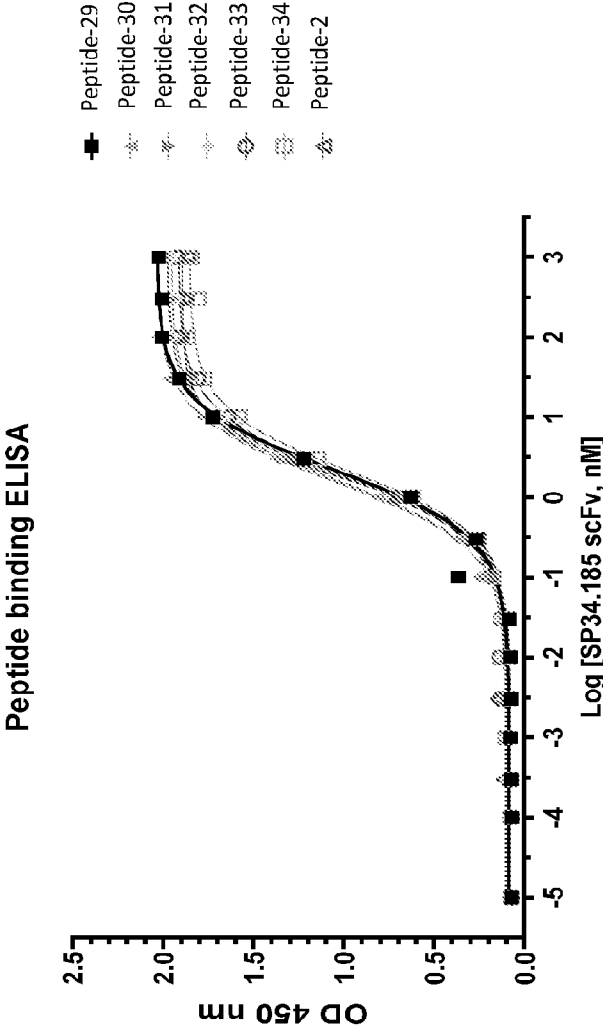


FIG. 5A

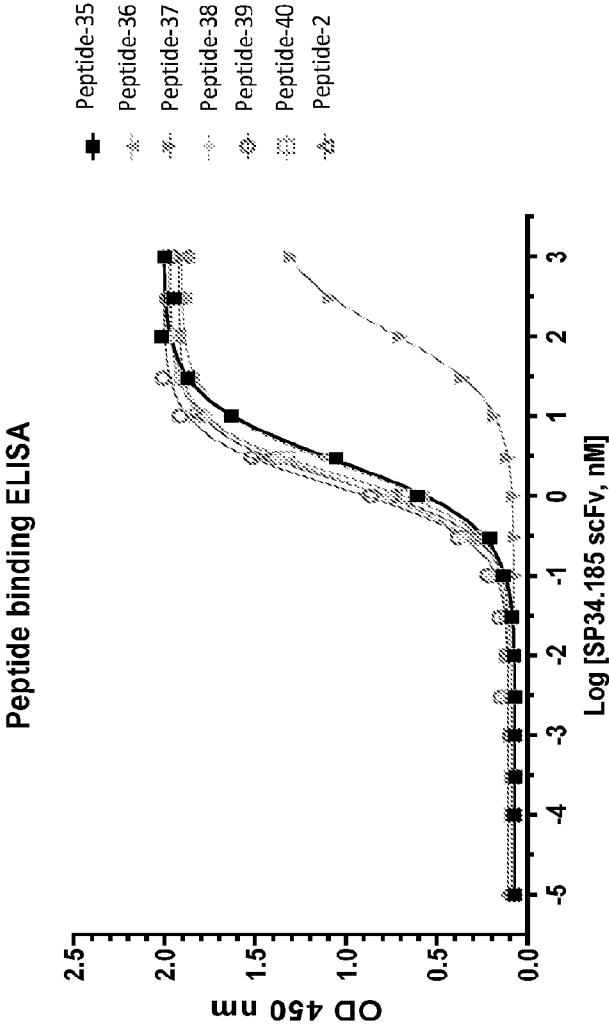


FIG. 5B

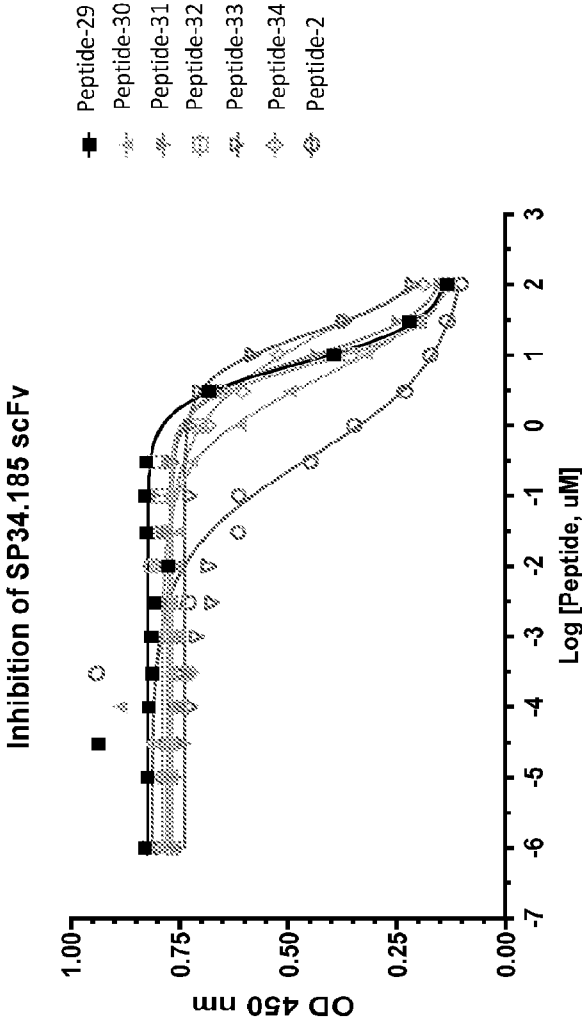


FIG. 6A

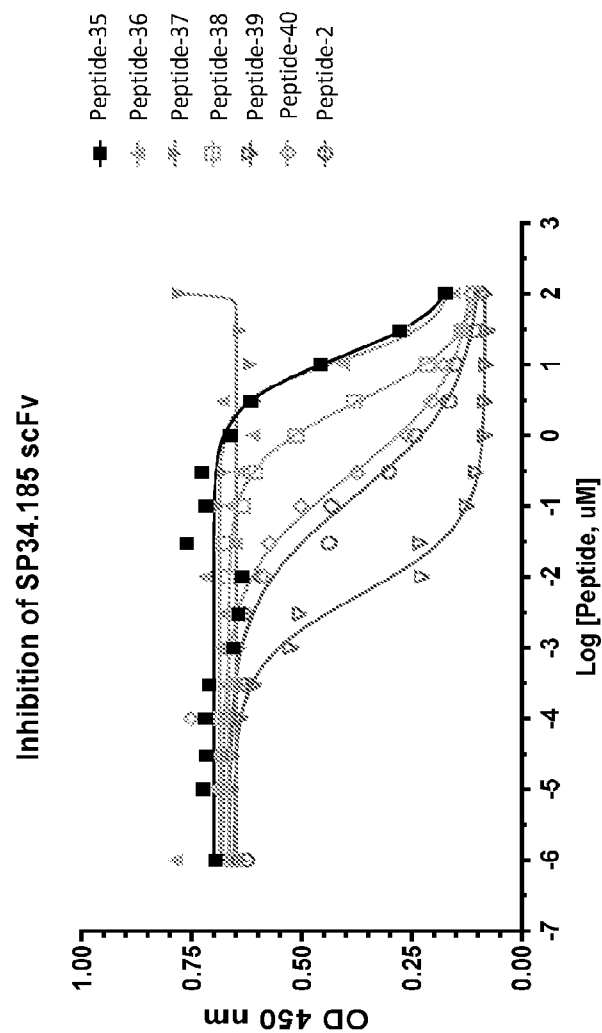


FIG. 6B

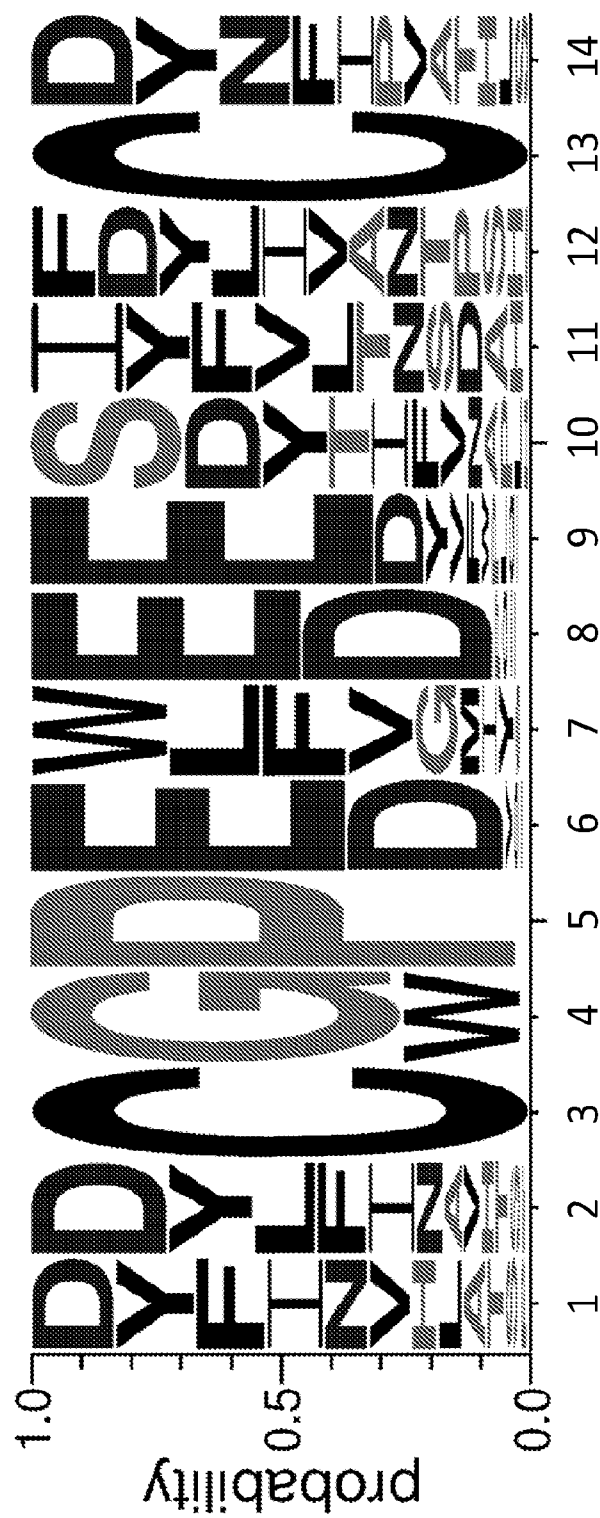


FIG. 7

Peptide binding ELISA

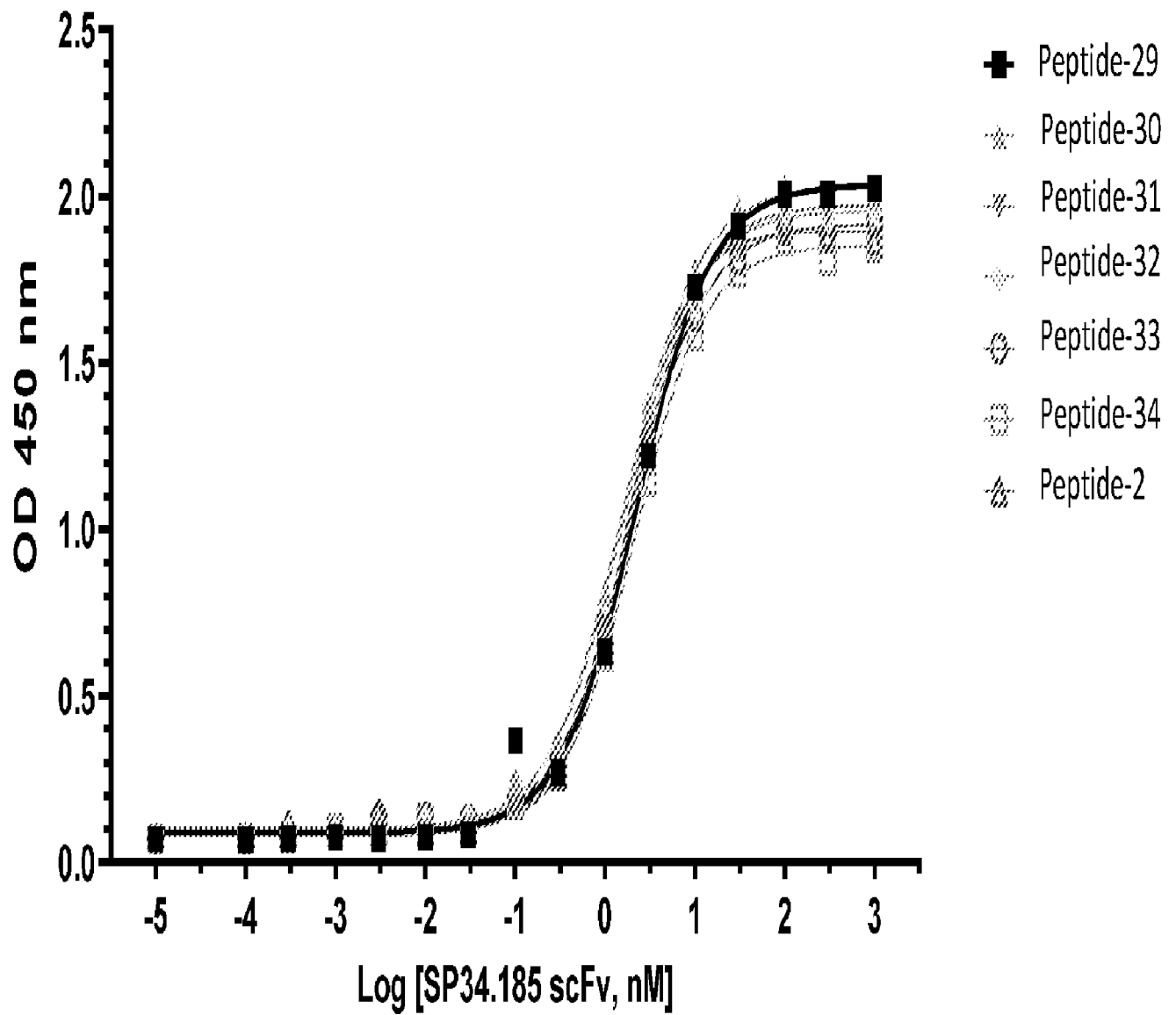


FIG. 5A