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(54) Title: ANTIGEN BINDING CONSTRUCTS TO TARGET MOLECULES

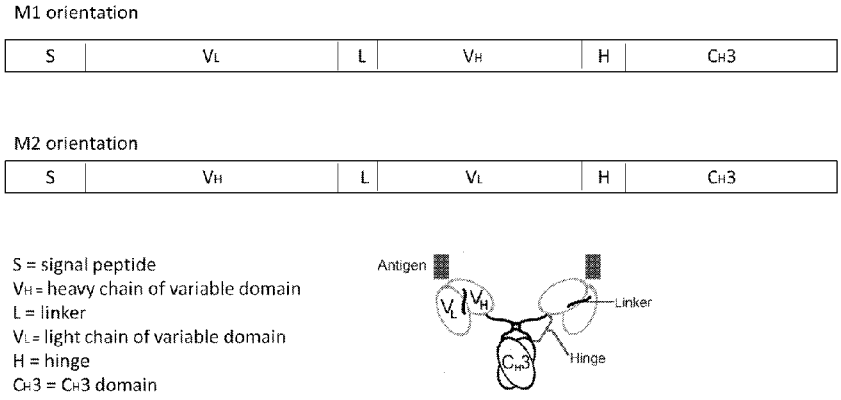


FIG. 1

(57) Abstract: Antigen binding constructs that bind to desired targets are disclosed, for example antibodies, including antibody fragments (such as scFv and minibodies) that bind to a target molecule, and have one or more of the disclosed hinges are described herein. Methods of use are described herein.

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ANTIGEN BINDING CONSTRUCTS TO TARGET MOLECULES

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Application No. 62/202,665, filed on August 7, 2015, which is hereby incorporated by reference in its entirety.

REFERENCE TO SEQUENCE LISTING

[0002] The present application is being filed along with a Sequence Listing in electronic format. The Sequence Listing is provided as a file entitled IGNAB030WOSEQUENCE.TXT, which was created and last modified on August 3, 2016, which is 270,446 bytes in size. The information in the electronic Sequence Listing is hereby incorporated by reference in its entirety.

FIELD

[0003] Embodiments described herein relate generally to hinge structures in antigen binding constructs (such as any scFv fusion proteins, such as minibodies), as well as the antigen binding constructs themselves, as well as methods for their use.

BACKGROUND

[0004] There are a wide variety of antigen binding constructs known in the art. Such constructs frequently vary by the sequences in their CDR sections, less frequently with variations in their framework regions and other sections of the antibodies (such as C_H3 and hinge regions).

SUMMARY

[0005] In some aspects, an amino acid hinge region comprising a sequence of SEQ ID NO: 1 ($X_{n1}CX_{n2}X_{n3}CX_{n4}X_{n5}C$) is provided. X_{n1} can be any amino acid that does not

naturally form a covalent crosslinking bond. X_{n2} is one of: A, R, N, D, E, Q, G, H, I, L, K, M, F, P, S, T, W, Y, or V. X_{n3} can be any amino acid. X_{n4} can be any amino acid. X_{n5} can be any amino acid.

[0006] In some aspects, X_{n1} does not form a covalent crosslinking bond with another amino acid (SEQ ID NO: 191). In some aspects, X_{n1} is not a cysteine (SEQ ID NO: 192). In some aspects, X_{n1} is one of: A, R, N, D, E, Q, G, H, I, L, K, M, F, P, S, T, W, Y, or V (SEQ ID NO: 193). In some aspects, X_{n2} is P, V, or E (SEQ ID NO: 194). In some aspects, X_{n2} is P or V (SEQ ID NO: 195). In some aspects, X_{n4} is P, V, or E (SEQ ID NO: 196). In some aspects, X_{n4} P or V (SEQ ID NO: 197). In some aspects, X_{n3} is P or E (SEQ ID NO: 198). In some aspects, X_{n5} is P or E (SEQ ID NO: 199). In some aspects, X_{n3} P or E (SEQ ID NO: 200). In some aspects, $X_{n2}X_{n3}$ is VE (SEQ ID NO: 201). In some aspects, $X_{n2}X_{n3}$ is PP (SEQ ID NO: 202). In some aspects, $X_{n4}X_{n5}$ is VE (SEQ ID NO: 203). In some aspects, $X_{n4}X_{n5}$ is PP (SEQ ID NO: 204). In some aspects, $X_{n2}X_{n3}$ is VE and $X_{n4}X_{n5}$ is PP (SEQ ID NO: 205). In some aspects, $X_{n2}X_{n3}$ is PP and $X_{n4}X_{n5}$ is PP or VE (SEQ ID NO: 206). In some aspects, $X_{n2}X_{n3}$ is VE and $X_{n4}X_{n5}$ is VE or PP (SEQ ID NO: 207). In some aspects, the hinge further comprises an extension or lower hinge sequence C-terminal to the last cysteine in $X_{n1}CX_{n2}X_{n3}CX_{n4}X_{n5}C$ (SEQ ID NO: 1). In some aspects, the extension or lower hinge sequence comprises at least one of S, G, A, P, or V. In some aspects, the extension sequence comprises at least GGGSSGGGSG (SEQ ID NO: 59). In some aspects, the linker sequence comprises at least APPVAGP (SEQ ID NO: 60). In some aspects, the hinge region of claim 1 is part of a core hinge region. In some aspects, the hinge further comprises an upper hinge region adjacent to the core hinge region. In some aspects, the hinge further comprises a lower hinge or extension region adjacent to the core hinge region. In some aspects, it further comprises an upper hinge region adjacent to the core hinge region. In some aspects, X_{n1} comprises a serine, a threonine, or an alanine (SEQ ID NO: 209). In some aspects, X_{n1} comprises a serine (SEQ ID NO: 210). In some aspects, X_{n1} comprises an alanine (SEQ ID NO: 211). In some aspects, the amino acid hinge region comprises at least one of the following sequences: SCVECPPCP (SEQ ID NO: 56) or TCPPCPPC (SEQ ID NO: 166). In some aspects, the amino acid hinge region comprises at least one of the following sequences: ERKSCVECPPCP (SEQ ID NO: 167), EPKSSDKTHT (SEQ ID NO: 46), and CPPCPPC (SEQ ID NO: 52). In some aspects, the amino acid hinge region

comprises at least one of the following sequences: ERKSCVECPPCPGGGSSGGGSG (SEQ ID NO: 34) or ERKSCVECPPCPAPPVAGP (SEQ ID NO: 33) or EPKSSDKTHTCPPCPPCGGGSSGGGSG (SEQ ID NO: 26) or EPKSSDKTHTCPPCPPCAPELLGGP (SEQ ID NO: 25).

[0007] In some aspects, an amino acid hinge region is provided. The amino acid hinge region comprises a sequence of SEQ ID NO: 2 ($X_{n1}X_{n2}X_{n3}X_{n4}X_{n5}X_{n6}CX_{n7}X_{n8}CX_{n9}X_{n10}C$). X_{n1} can be any m amino acids (where m is any number of amino acids of any type). X_{n2} can be any amino acid. X_{n3} can be any amino acid. X_{n4} can be any amino acid. X_{n5} can be any amino acid. X_{n6} can be any amino acid other than a cysteine. X_{n7} can be any amino acid. X_{n8} can be any amino acid. X_{n9} can be any amino acid. X_{n10} can be any amino acid (*see, e.g.,* SEQ ID NO: 212). In some aspects, X_{n1} is not a cysteine (*see, e.g.,* SEQ ID NO: 213). In some aspects, X_{n2} is not a cysteine (*see, e.g.,* SEQ ID NO: 214). In some aspects, X_{n2} is a D (*see, e.g.,* SEQ ID NO: 215). In some aspects, X_{n3} is a K (*see, e.g.,* SEQ ID NO: 216). In some aspects, X_{n4} is a T (*see, e.g.,* SEQ ID NO: 217). In some aspects, X_{n5} is a H (*see, e.g.,* SEQ ID NO: 218). In some aspects, X_{n6} is a T (*see, e.g.,* SEQ ID NO: 219). In some aspects, X_{n7} is a P or a V (*see, e.g.,* SEQ ID NO: 220). In some aspects, X_{n8} is a P or a E (*see, e.g.,* SEQ ID NO: 221). In some aspects, X_{n9} is a P or a V (*see, e.g.,* SEQ ID NO: 222). In some aspects, X_{n10} is a P or a E (*see, e.g.,* SEQ ID NO: 223). In some aspects, the amino acid hinge region further comprises a CXXC (*see, e.g.,* SEQ ID NO: 224) or CXXC (*see, e.g.,* SEQ ID NO: 225) motif that is positioned in front of X_{n1}. In some aspects, the amino acid hinge region further comprises a $X_{n11}X_{n12}C$ sequence immediately attached to the c-terminal cysteine in SEQ ID NO: 2, wherein X_{n11} can be any amino acid, and wherein X_{n12} can be any amino acid (*see, e.g.,* SEQ ID NO: 226). In some aspects, X_{n11} is a P or a V, and X_{n12} is a P or an E (*see, e.g.,* SEQ ID NO: 227). In some aspects, X_{n1} is a serine, X_{n2} is a D, X_{n3} is a K, X_{n4} is a T, X_{n5} is a H, X_{n6} is a T, X_{n7} is a P, X_{n8} is a P, X_{n9} is a P, and X_{n10} is a P (*see, e.g.,* SEQ ID NO: 228). In some aspects, the hinge region comprises at least one of the following sequences: CPPCPPC (SEQ ID NO: 52), CPPCVECPPC (SEQ ID NO: 53), or CPPCPPCPPC (SEQ ID NO: 54). In some aspects, the hinge region comprises at least one of the following sequences: EPKSSDKTHTCPPCPPC (SEQ ID NO: 168), EPKSSDKTHTCPPCVECPPC (SEQ ID NO: 169), or EPKSSDKTHTCPPCPPCPPC (SEQ ID NO: 170). In some aspects, the hinge region comprises at least one of the following

sequences: EPKSSDKTHTCPPCPPCGGGSSGGGSG (SEQ ID NO: 26), EPKSSDKTHTCPPCVECPPCGGGSSGGGSG (SEQ ID NO: 28), or EPKSSDKTHTCPPCPPCPPCGGGSSGGGSG (SEQ ID NO: 30).

[0008] In some aspects, an amino acid hinge region is provided. The hinge region comprises a core hinge sequence of at least one of: CVECPPCP (SEQ ID NO: 57), CPPCPPC (SEQ ID NO: 52), or CPPCPPCPPC (SEQ ID NO: 54), or CPPCVECPPC (SEQ ID NO: 53) linked to an upper hinge sequence of ELKTPLGDTTHT (SEQ ID NO: 48) or EPKSSDKTHT (SEQ ID NO: 46).

[0009] In some aspects, an amino acid hinge region for an antibody is provided, it can comprise an upper hinge region that comprises no amino acids capable of crosslinking with a corresponding amino acid; and a core hinge region connected to a C-terminus of the upper hinge region, wherein the core hinge region comprises at least three cysteines per strand. In some aspects, the amino acid hinge region further comprises a lower hinge or extension region connected C-terminal to the core hinge region, wherein the lower hinge or extension sequence is at least one of: APPVAGP (SEQ ID NO: 60), APELLGGP (SEQ ID NO: 58), and/or GGGSSGGGSG (SEQ ID NO: 59). In some aspects, the upper hinge region comprises no cysteines that crosslink within the upper hinge region. In some aspects, the upper hinge region comprises no cysteines. In some aspects, it further comprises a lower hinge or extension region. In some aspects, the lower hinge or extension region comprises at least one of: GGGSSGGGSG (SEQ ID NO: 59) or APPVAGP (SEQ ID NO: 60) or APELLGGP (SEQ ID NO: 58). In some aspects, when located within a minibody, and wherein when the minibody is administered to a human subject, clearance of the minibody from the subject occurs primarily through a liver. In some aspects, when located within a minibody, and wherein when the minibody is administered to a human subject, clearance of the minibody from the subject does not occur primarily through a kidney. In some aspects, the hinge region is within an antibody. In some aspects, the hinge region is within an antibody binding fragment. In some aspects, the hinge region is within a minibody. In some aspects, the hinge region is within a monospecific antibody. In some aspects, the hinge region comprises at least three cysteines per strand. In some aspects, the hinge region comprises at least four cysteines per strand. In some aspects, the hinge region comprises at least five cysteines per strand. In some aspects, cysteines are distributed throughout the

amino acid hinge region in a repeating CXX or CXY motif. In some aspects, the hinge region is within a bispecific antibody. In some aspects, the bispecific antibody is assembled in a 1:1 ratio. In some aspects, the bispecific antibody comprises an antibody fragment. In some aspects, the bispecific antibody is a minibody.

[0010] In some aspects, a pharmaceutical composition is provided. The pharmaceutical composition can comprise the amino acid hinge region of any of those disclosed herein. In some embodiments, this results in less than 5% aggregation of an antibody is present in the composition.

[0011] In some aspects, a pharmaceutical composition comprising the amino acid hinge region of any of those provided herein is provided. In some aspects, at least 1 microgram to 100 mg of the antibody is present.

[0012] In some aspects, a minibody comprising a core hinge region is provided. The core hinge region comprises at least three cysteines per strand forming at least three disulfide bonds within the core hinge region. In some aspects, the first residue of the core region is a serine. In some aspects, the core hinge region comprises SCVECPPCP (SEQ ID NO: 56).

[0013] In some aspects, a minibody is provided. The minibody can comprise a sequence $X_{n1}CX_{n2}X_{n3}CX_{n4}X_{n5}C$ (SEQ ID NO: 3). This sequence can be located as the core hinge region of the minibody. X_{n1} can be any amino acid or no amino acid. X_{n2} can be any amino acid. X_{n3} can be any amino acid. X_{n4} can be any amino acid. X_{n5} can be any amino acid. In some aspects, X_{n1} is any amino acid other than a cysteine (SEQ ID NO: 229). In some aspects, X_{n1} is a serine (SEQ ID NO: 230).

[0014] In some aspects, a variant minibody hinge is provided. The variant hinge can comprise a first altered amino acid position. The first altered position is an amino acid that in a native antibody hinge would be a cysteine, and has been altered in the first altered position so that it does not form a disulfide bond. The variant hinge can also comprise at least three cysteines per strand C-terminal to the first altered amino acid position. In some aspects, the hinge region consists of SEQ ID NO: 1. In some aspects, SEQ ID NO: 1 is a core hinge region, and wherein the core hinge region essentially consists of SEQ ID NO: 1. In some aspects, the core hinge region consists of SEQ ID NO: 1.

[0015] In some aspects, a minibody is provided. The minibody that binds to a target antigen, wherein the target antigen is at least one of CD3, CD8, 5T4, PSMA, or PSCA. The minibody comprising a polypeptide that comprises: a single-chain variable fragment (scFv) that binds to the target antigen, the scFv comprising a variable heavy (V_H) domain linked a variable light (V_L) domain; and a variant hinge region comprising at least three cysteines on each strand of the hinge.

[0016] In some aspects, the minibody further comprises a human IgG C_H3 sequence. In some aspects, the minibody further comprises a detectable marker selected from the group consisting of a radioactive substance, a dye, a contrast agent, a fluorescent compound, a bioluminescent compound, an enzyme, an enhancing agent, and a nanoparticle.

[0017] In some aspects, the minibody comprises: a HCDR1 of the HCDR1 as disclosed herein; a HCDR2 of the HCDR2 as disclosed herein; a HCDR3 of the HCDR3 as disclosed herein; a LCDR1 of the LCDR1 as disclosed herein; a LCDR2 of the LCDR2 as disclosed herein; and a LCDR3 of the LCDR3 as disclosed herein.

[0018] In some aspects, the variable heavy (V_H) domain and the variable light (V_L) domain are human sequences.

[0019] In some aspects, a nucleic acid encoding a minibody as disclosed herein is provided.

[0020] In some aspects, a cell line producing the minibody as disclosed herein is provided.

[0021] In some aspects, a kit comprising any of the minibodies provided herein and a detectable marker is provided.

[0022] In some aspects, a method of detecting the presence or absence of a target antigen is provided. The target antigen is at least one of CD3, CD8, 5T4, PSMA, or PSCA. The method comprises applying a minibody as disclosed herein to a sample; and detecting a binding or an absence of binding of the antigen binding construct thereof to the target antigen.

[0023] In some aspects, the minibody comprises a detectable marker selected from the group consisting of a radioactive substance, a dye, a contrast agent, a fluorescent compound, a bioluminescent compound, an enzyme, an enhancing agent, and a nanoparticle. In some aspects, applying the minibody comprises administering the minibody to a subject.

In some aspects, detecting binding or absence of binding of the minibody thereof to target antigen comprises positron emission tomography. In some aspects, the method further comprising applying a secondary antibody or fragment thereof to the sample, wherein the secondary antibody or fragment thereof binds specifically to the minibody. In some aspects, the minibody thereof is incubated with the sample for no more than 1 hour.

[0024] In some aspects, a method of targeting a therapeutic agent to a target antigen is provided. The target antigen is at least one of CD3, CD8, 5T4, PSMA, or PSCA. The method comprises administering to a subject a minibody as disclosed herein, wherein the minibody is conjugated to a therapeutic agent.

[0025] In some aspects, a method of neutralizing a B or T lymphocyte cell in a subject in need thereof is provided. The method comprising administering to the subject a minibody as disclosed herein that binds to CD8 and/or CD3. In some aspects, the subject has at least one of the disorders as noted herein.

[0026] In some aspects, an antibody and/or minibody that binds to a target antigen (such as CD8, CD3, 5T4, PSMA, PSCA) is provided. The antibody and/or minibody comprises a hinge region, wherein the hinge region comprises at least one of the following:

a) an amino acid hinge region comprising a sequence of SEQ ID NO: 1 ($X_{n1}CX_{n2}X_{n3}CX_{n4}X_{n5}C$), wherein X_{n1} can be any amino acid that does not naturally form a covalent crosslinking bond, wherein X_{n2} is one of: A, R, N, D, E, Q, G, H, I, L, K, M, F, P, S, T, W, Y, or V, wherein X_{n3} can be any amino acid, wherein X_{n4} can be any amino acid, and wherein X_{n5} can be any amino acid;

b) an amino acid hinge region comprising a sequence of SEQ ID NO: 2 (X_{n1} X_{n2} X_{n3} $X_{n4}X_{n5}$ $X_{n6}CX_{n7}X_{n8}CX_{n9}X_{n10}C$), wherein X_{n1} can be any m amino acids (where m is any number of amino acids of any type), wherein X_{n2} can be any amino acid, wherein X_{n3} can be any amino acid, wherein X_{n4} can be any amino acid, wherein X_{n5} can be any amino acid, wherein X_{n6} can be any amino acid other than a cysteine, wherein X_{n7} can be any amino acid, wherein X_{n8} can be any amino acid, wherein X_{n9} can be any amino acid, and wherein X_{n10} can be any amino acid;

c) a core hinge sequence of at least one of: CVECPPCP (SEQ ID NO: 57), CPPCPPC (SEQ ID NO: 52), or CPPCPPCPPC (SEQ ID NO: 54), or

CPPCVECPPC (SEQ ID NO: 53) linked to; an upper hinge sequence of ELKTPLGDTTHT (SEQ ID NO: 48) or EPKSSDKTHT (SEQ ID NO: 46);

d) an upper hinge region that comprises no amino acids capable of crosslinking with a corresponding amino acid; and a core hinge region connected to a C-terminus of the upper hinge region, wherein the core hinge region comprises at least three cysteines per strand;

e) an antibody and/or minibody comprising a core hinge region, wherein the core hinge region comprises at least three cysteines per strand forming at least three disulfide bonds within the core hinge region; or

f) a first altered amino acid position, wherein the first altered position is an amino acid that in a native antibody hinge would be a cysteine, and has been altered in the first altered position so that it does not form a disulfide bond; and at least three cysteines per strand C-terminal to the first altered amino acid position.

[0027] In some aspects, any minibody provided herein can instead be formatted as a full length antibody. In some embodiments, the minibody body comprises a humanized amino acid sequence.

[0028] In some aspects, a method of manufacturing the minibody provided herein comprises expressing the minibody in a cell line.

[0029] In some aspects, a method of treating a condition in a subject in need thereof is provided. The method comprises administering to the subject any one or more of the minibodies provided herein to treat any one or more of a CD3, CD8, PSCA, PSMA, and/or 5T4 disorder provided herein.

BRIEF DESCRIPTION OF THE DRAWINGS

[0030] FIG. 1 shows an illustration of an engineered minibody (Mb).

[0031] FIG. 2 shows an illustration of various embodiments of Mb hinges based on human IgG1 (γ1 EH1 top and γ1 EH2 bottom).

[0032] FIG. 3 shows an illustration of various embodiments of Mb hinges based on human IgG2 (γ2 EH1 top and γ2 EH2 bottom).

[0033] FIG. 4 shows an illustration of various embodiments of additional Mb hinges based on human IgG2 (γ2 NH1 top and γ2 NH2 bottom).

[0034] FIG. 5A shows images of non-reduced SDS-PAGE analysis of IAB2M hinge variants.

[0035] FIG. 5B shows protein sequence information for some embodiments of IAB2M γ1 EH1.

[0036] FIG. 5C shows protein sequence information for some embodiments of IAB2M γ1 EH2.

[0037] FIG. 5D shows protein sequence information for some embodiments of IAB2M γ2 EH2.

[0038] FIG. 5E shows protein sequence information for some embodiments of IAB2M- γ2 EH1.

[0039] FIG. 6A shows intact mass analysis of IAB2M γ1 EH1 variant. Upper panel shows the total ion chromatogram under reverse phase conditions. Middle panel shows the deconvoluted intact masses confirming the presence of half molecules and the lower panel shows the full size masses that were identified.

[0040] FIG. 6B shows intact mass analysis of IAB2M γ2 EH1 variant. Upper panel shows the total ion chromatogram under reverse phase conditions. Middle panel shows the deconvoluted intact masses confirming the presence of half molecules and the lower panel shows the full size molecular masses that were identified.

[0041] FIG. 6C shows intact mass analysis of IAB2M γ1 EH2 variant. Upper panel shows the total ion chromatogram under reverse phase conditions. Middle panel shows the deconvoluted intact masses confirming the presence of half molecules and the lower panel shows the full size molecular masses that were identified.

[0042] FIG. 6D shows intact mass analysis of IAB2M γ2 EH2 variant. Upper panel shows the total ion chromatogram. Lower panel shows the deconvoluted intact masses confirming the presence of the full-size mass molecules. No half molecules were detected.

[0043] FIG. 7A shows binding curves and EC50 binding values of IAB2M engineered hinge variants determined by FACS using LNCaP-AR cells.

[0044] FIG. 7B shows binding curves and EC50 binding values of IAB2M engineered hinge variants determined by FACS using C4-2 XCL cells.

[0045] FIG. 7C shows protein sequence information for some embodiments of IAB2M γ1 EH3 without a canonical signal sequence.

[0046] FIG. 7D shows protein sequence information for some embodiments of IAB2M γ1 EH3 without a canonical signal sequence.

[0047] FIG. 7E shows DNA sequence information for IAB2M γ1 EH3 without a canonical signal sequence.

[0048] FIG. 8 shows a list of disulfide-containing peptides identified within IAB2M γ1 EH1 dimer.

[0049] FIG. 9 shows an illustration of a mapping of the disulfide bonds of IAB2M γ1 EH1 indicating where mispaired cysteines are formed.

[0050] FIG. 10 shows an illustration of a mapping of the disulfide bonds of IAB2M γ2 EH1 suggesting the absence of mispaired cysteines.

[0051] FIG. 11A shows images of PET/CT scans of ^{89}Zr-Df-IAB2M γ1 EH1 in a nude mouse harboring 22Rv1 (PSMA+) tumor xenograft.

[0052] FIG. 11B shows a graph of biodistribution of ^{89}Zr-Df-IAB2M γ1 EH1 in nude mice.

[0053] FIG. 12A shows images of PET/CT scans of ^{89}Zr-Df-IAB2M γ1 EH2 in a nude mouse harboring 22Rv1 (PSMA+) tumor xenograft.

[0054] FIG. 12B shows a graph of biodistribution of ^{89}Zr-Df-IAB2M γ1 EH2 in nude mice.

[0055] FIG. 13A shows images of PET/CT scans of ^{89}Zr-Df-IAB2M γ2 EH2 in a nude mouse harboring 22Rv1 (PSMA+) tumor xenograft.

[0056] FIG. 13B shows a graph of biodistribution of ^{89}Zr-Df-IAB2M γ2 EH2 in nude mice.

[0057] FIG. 14A shows the intact mass analysis of IAB22M γ2 EH1 variant. Upper panel shows the total ion chromatogram. Middle panel shows the deconvoluted intact masses confirming the presence of half molecules and the lower panel shows the full-size masses that were identified.

[0058] FIG. 14B shows protein sequence information for some embodiments of IAB22M γ2 EH1.

[0059] FIG. 15A shows intact mass analysis of IAB22 γ2 EH2 variant. The protein was separated under reverse phase conditions. Upper panel shows the full mass range scan. The zoomed-in full-size mass region emphasizes the presence of the intact, i.e.

disulfide-bridge bonded protein (middle panel) while the zoomed-in half-molecule region showed that essentially no half-molecule is present (lower panel).

[0060] FIG. 15B shows protein sequence information for some embodiments of IAB22M γ2 EH2 variant.

[0061] FIG. 16A shows PET/CT scan images of mice harboring HPB-ALL (CD8+) tumor xenograft comparing ^{89}Zr-Df-IAB22M minibodies with human IgG1 and human IgG2 derived hinge sequences.

[0062] FIG. 16B shows protein sequence information for some embodiments of IAB22M γ1 EH1.

[0063] FIG. 16C shows protein sequence information for some embodiments of IAB22M γ2 NH1.

[0064] FIG. 16D shows protein sequence information for some embodiments of IAB22M γ2 NH2.

[0065] FIG. 17 shows PET/CT scan images comparing uptake of ^{89}Zr-Df-IAB22M γ1 EH1 in a NOD-SCID mouse with antigen-positive HPB-ALL tumor and antigen-negative Daudi tumor (right panel only).

[0066] FIG. 18 shows a graph of biodistribution of ^{89}Zr-Df-IAB22M γ1 EH1 in mice with antigen-positive HPB-ALL tumor and antigen-negative Daudi tumor. Numbers are shown on the right.

[0067] FIG. 19 is an illustration of some embodiments of hinge variants listed in Table 3 (γ1 EH1 top, γ1 EH3 middle, γ1 EH4 bottom).

[0068] FIG. 20A shows a graph summarizing the percentage of half molecules present in different hinge variants for IAB2M as determined by mass spectrometry.

[0069] FIG. 20B shows a graph summarizing the percentage of half molecules present in different hinge variants for IAB22M as determined by mass spectrometry.

[0070] FIG. 20C shows protein sequence information for some embodiments of IAB22M γ1 EH3.

[0071] FIG. 20D shows protein sequence information for some embodiments of IAB22M γ1 EH5.

[0072] FIG. 20E shows protein sequence information for some embodiments of IAB22M γ3/γ1 EH6.

[0073] FIG. 20F shows protein sequence information for some embodiments of IAB22M γ3/γ1 EH7.

[0074] FIG. 20G shows protein sequence information for some embodiments of IAB22M γ3/γ1 EH8.

[0075] FIG. 21A shows an image of non-reduced SDS-PAGE analysis (left panel) of IAB2M hinge Mb variants and percentage of half molecules quantified by densitometry (right panel).

[0076] FIG. 21B shows protein sequence information for some embodiments of IAB2M γ1 EH5.

[0077] FIG. 21C shows protein sequence information for some embodiments of IAB2M γ3/γ1 EH6.

[0078] FIG. 21D shows protein sequence information for some embodiments of IAB2M γ3/γ1 EH7.

[0079] FIG. 21E shows protein sequence information for some embodiments of IAB2M γ3/γ1 EH8.

[0080] FIG. 22A shows an image of non-reduced SDS-PAGE analysis (left panel) of IAB22M hinge Mb variants and percentage of half molecules quantified by densitometry (right panel).

[0081] FIG. 22B shows protein sequence information for some embodiments IAB22M γ1 EH2.

[0082] FIG. 23 shows the intact mass analysis of IAB22M γ1 EH1 variant. Upper panel shows the total ion chromatogram. Middle panel shows the deconvoluted intact masses confirming the presence of half molecules and the lower panel shows the full-size masses that were identified.

[0083] FIG. 24 shows intact mass analysis of IAB22M γ1 EH3 variant. The protein was separated under reverse phase conditions. Upper panel shows the full mass range scan. Zoomed-in full-size mass region emphasizes the presence of the intact protein (Middle panel). Zoomed-in half-molecule region showed that essentially no half-molecule is present (lower panel).

[0084] FIGs. 25A and 25B shows intact mass analyses of IAB22M γ2 hinge variants. FIG. 25A shows the total ion chromatogram of the IAB22M γ2 EH1 variant (FIG. 14B) under reverse phase conditions (upper panel). Middle panel shows the deconvoluted intact masses confirming the presence of half molecules and the lower panel shows the full-size masses that were identified. Note that the heterogeneity results from the terminal lysine clipping. FIG. 25B shows a deconvoluted full range scan (upper panel) of the IAB22M γ2 EH2 variant (FIG. 15B) identifies the sole species with m/z of 79053.1 Da. Zoomed-in full-size molecular weight is shown on the middle panel and the half molecule is of low abundance and just above the level of noise (bottom panel).

[0085] FIG. 26 shows binding curves and EC50 binding values of IAB2M engineered hinge variants determined by FACS using C4-2 XCL cells.

[0086] FIG. 27 shows binding curves and EC50 binding values of IAB22M engineered hinge variants determined by FACS using HPB-ALL cells.

[0087] FIG. 28A shows an image of non-reduced SDS-PAGE analysis of IAB20M hinge Mb variants (left panel) and a percentage of half molecules quantified by densitometry (right panel).

[0088] FIG. 28B shows protein sequence information for some embodiments of IAB20M γ1 EH1.

[0089] FIG. 28C shows protein sequence information for some embodiments of IAB20M γ1 EH3.

[0090] FIG. 28D shows protein sequence information for some embodiments of IAB20M γ1 EH2.

[0091] FIG. 28E shows protein sequence information for some embodiments of IAB20M γ1 EH5.

[0092] FIG. 29A shows an image of non-reduced SDS-PAGE analysis of IAB1M hinge Mb variants (left panel) and percentage of half molecules quantified by densitometry (right panel).

[0093] FIG. 29B shows protein sequence information for some embodiments of IAB1M γ1 EH1.

[0094] FIG. 29C shows protein sequence information for some embodiments of IAB1M γ2 EH2.

[0095] FIG. 29D shows protein sequence information for some embodiments of IAB1M γ1 EH3.

[0096] FIG. 30 shows PET/CT scan images comparing uptake of ^{89}Zr radiolabeled IAB22 Mbs with different hinge sequences in NOD-SCID mice bearing antigen positive HPB-ALL xenografts on the left shoulder.

[0097] FIG. 31 shows a graph of biodistribution data of ^{89}Zr radiolabeled IAB22 Mbs with different hinge sequences in NOD-SCID mice bearing antigen positive HPB-ALL xenografts on the left shoulder.

[0098] FIG. 32 shows protein sequence information for some embodiments of IAB20M γ2 EH2.

[0099] FIG. 33 shows protein sequence information for some embodiments of IAB25M-γ2 EH2.

[0100] FIG. 34A shows the DNA sequence encoding some embodiments of the IAB2M Mb.

[0101] FIG. 34B shows the DNA sequence encoding some embodiments of the IAB2M Mb.

[0102] FIG. 34C shows the DNA sequence encoding some embodiments of the IAB2M Mb.

[0103] FIG. 34D shows the DNA sequence encoding some embodiments of the IAB2M Mb.

[0104] FIG. 34E shows the DNA sequence encoding some embodiments of the IAB2M Mb.

[0105] FIG. 34F shows the DNA sequence encoding some embodiments of the IAB2M Mb.

[0106] FIG. 35A shows protein sequence information of IAB2M Mb hinge variants.

[0107] FIG. 35B shows protein sequence information of IAB2M Mb hinge variants.

[0108] FIG. 35C shows protein sequence information of IAB2M Mb hinge variants.

[0109] FIG. 36A shows protein sequence information of VL and VH domains of Mbs with different antigen-specificities.

[0110] FIG. 36B shows protein sequence information of VL and VH domains of Mbs with different antigen-specificities.

[0111] FIG. 36C shows protein sequence information of VL and VH domains of Mbs with different antigen-specificities.

[0112] FIG. 36D shows protein sequence information of VL and VH domains of Mbs with different antigen-specificities.

[0113] FIG. 36E shows protein sequence information of VL and VH domains of Mbs with different antigen-specificities.

[0114] FIG. 37 shows protein sequence information of various embodiments of linker sequences.

[0115] FIG. 38 shows protein sequence information of various embodiments of hinge regions.

[0116] FIG. 39 shows protein sequence information of various embodiments of C_H3 domains.

[0117] FIG. 40 shows an alignment of protein sequences of an embodiment each of IAB22M γ1 EH1(M1) and IAB22M γ1 EH3(M1). Sequence differences are shown in boxes.

[0118] FIG. 41 shows the DNA and translated protein sequence of an embodiment of IAB22M γ1 EH3(M1). In boxes are shown the signal, CDR, linker and hinge sequences.

[0119] FIG. 42 shows the DNA and translated protein sequence of an embodiment of IAB22M γ1 EH5(M1).

[0120] FIG. 43 shows the DNA and translated protein sequence of an embodiment of IAB22M γ1 EH7(M1).

[0121] FIG. 44 shows the DNA and translated protein sequence of an embodiment of IAB22M γ1 EH8(M1).

[0122] FIG. 45 shows the DNA and translated protein sequence of an embodiment of IAB22M γ2 EH2(M1).

[0123] FIG. 46 shows the DNA and translated protein sequence of an embodiment of IAB22M γ2 EH2(M1) with VH-K67R polymorphism.

[0124] FIG. 47 shows an alignment of protein sequences of an embodiment each of IAB2M γ1 EH1(M2) and IAB2M γ1 EH3(M2). Sequence differences are shown in boxes.

[0125] FIG. 48 shows the DNA and translated protein sequence of an embodiment of IAB2M γ1 EH3(M2). In boxes are shown the signal, CDR, linker and hinge sequences.

[0126] FIG. 49 shows the DNA and translated protein sequence of an embodiment of IAB2M γ1 EH3 (M2) (G1m1).

[0127] FIG. 50 shows the DNA and translated protein sequence of an embodiment of IAB2M γ1 EH5(M2).

[0128] FIG. 51 shows the DNA and translated protein sequence of an embodiment of IAB2M γ1 EH7(M2).

[0129] FIG. 52 shows the DNA and translated protein sequence of an embodiment of IAB2M γ1 EH8(M2).

[0130] FIG. 53 shows the DNA and translated protein sequence of an embodiment of IAB2M γ1 EH3(M1).

[0131] FIG. 54 shows an alignment of protein sequences of an embodiment each of IAB20M γ1 EH1 (M2) and IAB20M γ1 EH3 (M2) with VL-Q79E, V83E; VH-Q1E, Q6E polymorphisms. Sequence differences are shown in boxes.

[0132] FIG. 55 shows the DNA and translated protein sequence of an embodiment of IAB20M γ1 EH3(M2) with VL-Q79E, V83E; VH-Q1E, Q6E polymorphisms. In boxes are shown the signal, CDR, linker and hinge sequences.

[0133] FIG. 56 shows the DNA and translated protein sequence of an embodiment of IAB20M γ1 EH3(M2).

[0134] FIG. 57 shows the DNA and translated protein sequence of an embodiment of IAB20M γ1 EH3 (M2) with VL-A9D, T10S, S12A, P15L, L21I, S22N; VH-Q1E, Q6E polymorphisms.

[0135] FIG. 58 shows the DNA and translated protein sequence of an embodiment of IAB20M γ1 EH5 (M2) with VL-Q79E, V83E; VH-Q1E, Q6E polymorphisms.

[0136] FIG. 59 shows the DNA and translated protein sequence of an embodiment of IAB20M γ1 EH5 (M2) with VL-A9D, T10S, S12A, P15L, L21I, S22N; VH-Q1E, Q6E polymorphisms.

[0137] FIG. 60 shows an alignment of protein sequences of an embodiment each of IAB1M γ1 EH1(M1) and IAB1M γ1 EH3 (M1). Sequence differences are shown in boxes.

[0138] FIG. 61 shows the DNA and translated protein sequence of an embodiment of IAB1M γ1 EH3(M1). In boxes are shown the signal, CDR, linker and hinge sequences.

[0139] FIG. 62 shows the DNA and translated protein sequence of an embodiment of IAB1M γ2 EH2 (M1).

[0140] FIG. 63 shows the DNA and translated protein sequence of an embodiment of IAB1M γ1 EH5 (M1).

[0141] FIG. 64 shows the DNA and translated protein sequence of an embodiment of IAB1M γ1 EH7 (M1).

[0142] FIG. 65A shows the DNA and translated protein sequence of an embodiment of IAB1M γ1 EH8(M1).

[0143] FIG. 65B shows the DNA and translated protein sequence of an embodiment of IAB25M γ2 NH(M1).

[0144] FIG. 65C shows the DNA and translated protein sequence of an embodiment of IAB25M γ2 EH(M1).

[0145] FIG. 66 shows the protein sequence of an embodiment of IAB22M VH domain.

[0146] FIG. 67 shows the protein sequence of some embodiments of IAB20 VL domain.

[0147] FIG. 68 shows the protein sequence of an embodiment of IAB20 VH domain.

[0148] FIG. 69 shows the protein sequence of some embodiments of anti-CD3 VL and VH domains.

[0149] FIG. 70 shows the protein sequence of an embodiment of PSMA (Prostate-Specific Membrane Antigen) also known as glutamate carboxypeptidase 2 from *Homo sapiens*.

[0150] FIG. 71 shows the protein sequence of an embodiment of PSCA (Prostate Stem Cell Antigen) from *Homo sapiens*.

[0151] FIG. 72 shows the protein sequence of an embodiment of 5T4 oncofetal trophoblast glycoprotein from *Homo sapiens*.

[0152] FIG. 73 shows the protein sequence of an embodiment of T-cell surface glycoprotein CD8 alpha chain from *Homo sapiens*.

[0153] FIG. 74 shows the protein sequence of an embodiment of T-cell surface glycoprotein CD8 beta chain from *Homo sapiens*.

[0154] FIG. 75 shows the protein sequence of an embodiment of T-cell surface glycoprotein CD3 delta chain from *Homo sapiens*.

[0155] FIG. 76 shows the protein sequence of an embodiment of T-cell surface glycoprotein CD3 gamma chain from *Homo sapiens*.

[0156] FIG. 77 shows the protein sequence of an embodiment of T-cell surface glycoprotein CD3 epsilon chain from *Homo sapiens*.

[0157] FIG. 78 shows the protein sequence of an embodiment of T-cell surface glycoprotein CD3 zeta chain from *Homo sapiens*.

[0158] FIG. 79 shows protein sequence information for some embodiments of IAB25M- γ2 EH2.

[0159] FIG. 80 shows protein sequence information for some embodiments of IAB20M- γ2 EH2.

DETAILED DESCRIPTION

[0160] Described herein are components for antigen binding constructs, including antibodies and fragments thereof, such as minibodies, that bind to a target molecule. In some embodiments, these components are novel hinge sequences and/or sequences associated with and/or part of the hinge sequence. These hinge sequences can provide various benefits. Also

provided herein are the antigen binding constructs (such as antibodies, minibodies, etc.) that include one or more of the hinge sequences or subsequences provided herein.

[0161] In some embodiments, the antigen binding constructs can be useful for targeting therapeutic agents to cells that express the target molecule. In some embodiments, methods are provided for detecting the presence or absence of a target molecule (or "target") using antigen binding constructs (including antibodies, and constructs such as minibodies). In some embodiments, methods are provided for using the antigen binding constructs for therapeutic purposes.

[0162] In some embodiments, scFv and minibody antibodies can have superior pharmacokinetic properties for faster diagnostic imaging while maintaining the binding specificity and affinity of the parental antibody. Current technology utilizes imaging with the full length antibodies which often requires significantly longer times (~7-8 days post-injection) to produce high contrast images due to the slow serum clearance of the intact antibody. Some embodiments of the minibodies provided herein provide the opportunity for same-day or next-day imaging. Same-day or next-day imaging also provides a logistical solution to the problem facing many patients who travel great distances to receive treatment/diagnosis since the duration of travel stays or the need to return one week later would be eliminated when imaging with minibodies versus intact antibodies.

[0163] As detailed below, in some embodiments, the antigen binding constructs are for diagnostics. When labeled with an appropriate radionuclides (e.g., the positron emitter Iodine-124, Copper-64, Fluorine-18, Gallium-68 and/or Zirconium-89 for PET imaging) or fluorophore (for fluorescent imaging), the antibody fragments can be used for preclinical imaging as shown herein and for clinical imaging in patients. These antigen binding constructs can also be used as potential SPECT imaging agents by simply changing the radiolabel to single photon emitting radionuclides such as Indium-111, Iodine-123 and Lutitium-177.

[0164] In some embodiments, the antigen binding constructs can be clinical imaging agents (PET/SPECT) in humans. Accordingly, in some embodiments, antigen binding constructs can be used for targeted diagnostic detection for these disorders. In some embodiments, the antigen binding construct can be used as a therapeutic.

Definitions and various embodiments

[0165] The term "hinge" denotes at least a part of a hinge region for an antigen binding construct, such as an antibody or a minibody. A hinge region can include a combination of the upper hinge, core (or middle) hinge and lower hinge regions. In some embodiments, the hinge is defined according to any of the antibody hinge definitions. Native IgG1, IgG2, and IgG4 antibodies have hinge regions having of 12-15 amino acids. IgG3 has an extended hinge region, having 62 amino acids, including 21 prolines and 11 cysteines. The functional hinge region of naturally occurring antibodies, deduced from crystallographic studies, extends from amino acid residues 216-237 of the IgG1 H chain (EU numbering; ref. 12) and includes a small segment of the N terminus of the CH2 domain in the lower hinge, with the lower hinge being the N terminus of CH2 domain. The hinge can be divided into three regions; the "upper hinge," the "core," and the "lower hinge".

[0166] The term "artificial" or "non-natural" when modifying a hinge (or a subpart thereof) denotes that the sequence in question is not present, in the noted state, in nature. In the present context the hinges have been altered from their native state, so that their sequences are no longer those found in wild-type antibodies. As will be appreciated by those of skill in the art, minibodies do not naturally occur in nature, and thus, any construct which is a minibody construct is also not found in nature. This also applies to at least some of the constructs found in and/or incorporating the sequences of any of the hinge sequence tables provided herein (for example, Table 0.2). In some embodiments, any of the hinge subparts or full hinge sequences in Table 0.1 can be artificial hinge sequences, as long as the sequence (or resulting combination for the hinge) does not occur in nature.

[0167] The term "full hinge region" or "entire hinge region" denotes the presence of the entire upper, core, and lower hinge regions as a single construct. The upper, core, and lower regions can be positioned immediately adjacent to one another, or additional residues can be added between, or N- or C-terminal to the regions. In some embodiments, the native lower hinge can be replaced with an extension sequence. In some embodiments, one can combine a native lower hinge with the extension sequence. In some embodiments, an extension or other set of sequences can be added after the upper and/or core sequences.

[0168] The phrase "effective hinge region" denotes that an adequate amount of part of at least one of the upper, core and lower hinge regions is present to allow the hinge

region to be effective for its intended purpose. Thus, the phrase encompasses variants of hinge regions and fragments of the various hinge regions. In some embodiments, the function of the hinge region is one or more of the following: to link the scFv with the C_H3 domain, provide flexibility and spacing for the two scFvs to bind to the target properly, to link two half molecules together, to provide overall stability to the molecule, and/or to provide a site for site-specific conjugation due to its solvent exposure. In some embodiments, the hinge should be close to natural as to reduce potential immunogenicity. In some embodiments, the upper hinge provides flexibility to scFv (starts at residue 216 in native IgGs), the middle hinge provides stability, and the lower hinge mediates flexibility to C_H3 (starts at residue 231 in native IgGs).

[0169] The term "upper hinge" denotes the first part of the hinge that starts at the end of the scFv. Examples of upper hinge regions can be found in Table 0.1. The upper hinge includes the amino acids from the end of the scFv up to, but not including, the first cysteine residue in the core hinge as shown in Table 0.1. As above, the term "effective upper hinge" denotes that enough of the sequence is present to allow the section to function as an upper hinge; the term encompasses functional variants and fragments of the designated hinge section.

[0170] The term "core hinge" denotes the second part of the hinge region that is C-terminal to the upper hinge. Examples of core hinge regions can be found in Table 0.1. The core hinge contains the inter-chain disulfide bridges and a high content of prolines. As above, the term "effective core hinge" denotes that enough of the sequence is present to allow the section to function as a core hinge; the term encompasses functional variants and fragments of the designated hinge section.

[0171] The term "lower hinge" denotes the third part of the hinge region that is C-terminal to the core hinge. Examples of lower hinge regions can be found in Table 0.1. In the context of a minibody or antibody fragment, the lower hinge connects to the C_H3 domain Mb. As above, the term "effective lower hinge" denotes that enough of the sequence is present to allow the section to function as a lower hinge; the term encompasses functional variants and fragments of the designated hinge section. The term "lower hinge" as used herein can encompass various amino acid sequences including naturally occurring IgG lower hinge sequences and artificial extension sequences in place of one another or a combination

thereof provided herein. In some embodiments, the various extensions can be considered to be a lower hinge region in its entirety or a replacement.

[0172] The term "treating" or "treatment" of a condition can refer to preventing the condition, slowing the onset and/or rate of development of the condition, reducing the risk of developing the condition, preventing and/or delaying the development of symptoms associated with the condition, reducing or ending symptoms associated with the condition, generating a complete or partial regression of the condition, or some combination thereof. The term "prevent" does not require the absolute prohibition of the disorder or disease.

[0173] A "therapeutically effective amount" or a "therapeutically effective dose" is an amount that produces a desired therapeutic effect in a subject, such as preventing, treating a target condition, delaying the onset of the disorder and/or symptoms, and/or alleviating symptoms associated with the condition. This amount will vary depending upon a variety of factors, including but not limited to the characteristics of the therapeutic compound (including activity, pharmacokinetics, pharmacodynamics, and bioavailability), the physiological condition of the subject (including age, sex, disease type and stage, general physical condition, responsiveness to a given dosage, and type of medication), the nature of the pharmaceutically acceptable carrier or carriers in the formulation, and/or the route of administration. One skilled in the clinical and pharmacological arts will be able to determine a therapeutically effective amount through routine experimentation, for example by monitoring a subject's response to administration of a compound and adjusting the dosage accordingly, given the present disclosure. For additional guidance, *see* Remington: The Science and Practice of Pharmacy 21st Edition, Univ. of Sciences in Philadelphia (USIP), Lippincott Williams & Wilkins, Philadelphia, PA, 2005.

[0174] The term "antigen binding construct" includes all varieties of antibodies, including binding fragments thereof. Further included are constructs that include 1, 2, 3, 4, 5, and/or 6 CDRs. In some embodiments, tandem scFvs can be provided, which can provide two arms with bivalent binding. In some embodiments, these CDRs can be distributed between their appropriate framework regions in a traditional antibody. In some embodiments, the CDRs can be contained within a heavy and/or light chain variable region. In some embodiments, the CDRs can be within a heavy chain and/or a light chain. In some embodiments, the CDRs can be within a single peptide chain. Unless otherwise denoted

herein, the antigen binding constructs described herein bind to the noted target molecule. The term "target" or "target molecule" denotes the protein to which the antigen binding construct binds. Examples of target proteins are known in the art, and include, for example PSMA (such as FOLH1) (FIG. 70; SEQ ID NO: 131), PSCA (FIG. 71; SEQ ID NO: 132), 5T4 (such as TPBG) (FIG. 72; SEQ ID NO: 133), CD8 (such as the α-chain) (FIG. 73; SEQ ID NO: 134), CD8 (such as the β-chain) (FIG. 74; SEQ ID NO: 135), CD3 (such as the δ-chain) (FIG. 75; SEQ ID NO: 136), CD3 (such as the γ-chain) (FIG. 76; SEQ ID NO: 137), CD3 (such as the ε-chain) (FIG. 77; SEQ ID NO: 138), CD3 (such as the ζ-chain) (FIG. 78; SEQ ID NO: 139).

[0175] The term "antibody" includes, but is not limited to, genetically engineered or otherwise modified forms of immunoglobulins, such as intrabodies, chimeric antibodies, fully human antibodies, humanized antibodies, antibody fragments, scFv, and heteroconjugate antibodies (for example, bispecific antibodies, diabodies, triabodies, tetrabodies, etc.). The term "antibody" includes scFv and minibodies. Thus, each and every embodiment provided herein in regard to "antibodies" is also envisioned as scFv and/or minibody embodiments, unless explicitly denoted otherwise. The term "antibody" includes a polypeptide of the immunoglobulin family or a polypeptide comprising fragments of an immunoglobulin that is capable of noncovalently, reversibly, and in a specific manner binding a corresponding antigen. An exemplary antibody structural unit comprises a tetramer. In some embodiments, a full length antibody can be composed of two identical pairs of polypeptide chains, each pair having one "light" and one "heavy" chain (connected through a disulfide bond). The recognized immunoglobulin genes include the kappa, lambda, alpha, gamma, delta, epsilon, hinge, and mu constant region genes, as well as the myriad immunoglobulin variable region genes. For full length chains, the light chains are classified as either kappa or lambda. For full length chains, the heavy chains are classified as gamma, mu, alpha, delta, or epsilon, which in turn define the immunoglobulin classes, IgG, IgM, IgA, IgD, and IgE, respectively. The N-terminus of each chain defines a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. The terms variable light chain (V_L) and variable heavy chain (V_H) refer to these regions of light and heavy chains respectively. As used in this application, an "antibody" encompasses all variations of antibody and fragments thereof. Thus, within the scope of this

concept are full length antibodies, chimeric antibodies, humanized antibodies, single chain antibodies (scFv), Fab, Fab', and multimeric versions of these fragments (for example, $F(ab')_2$) with the same binding specificity. In some embodiments, the antibody binds specifically to a desired target.

[0176] The term "complementarity-determining domains" or "complementarity-determining regions ("CDRs") interchangeably refer to the hypervariable regions of V_L and V_H. The CDRs are the target molecule-binding site of the antibody chains that harbors specificity for such target molecule. In some embodiments, there are three CDRs (CDR1-3, numbered sequentially from the N-terminus) in each V_L and/or V_H, constituting about 15-20% of the variable domains. The CDRs are structurally complementary to the epitope of the target molecule and are thus directly responsible for the binding specificity. The remaining stretches of the V_L or V_H, the so-called framework regions (FRs), exhibit less variation in amino acid sequence (Kuby, Immunology, 4th ed., Chapter 4. W.H. Freeman & Co., New York, 2000).

[0177] The positions of the CDRs and framework regions can be determined using various well known definitions in the art, for example, Kabat (Wu, T. T. et al., "An analysis of the sequences of the variable regions of Bence Jones proteins and myeloma light chains and their implications for antibody complementarity," J. Exp. Med., Vol. 132, No. 2, pp. 211-250, 1970; Kabat, E. A. et al., "Sequences of Proteins of Immunological Interest," 5th Ed., NIH Publication No. 91–3242, Bethesda, MD, 1991, Chothia C. et al., "Canonical structures for the hypervariable regions of immunoglobulins," J. Mol. Biol., Vol. 196, No. 4, pp. 901-917, 1987; Chothia C. et al., "Conformations of immunoglobulin hypervariable regions," Nature, Vol. 342, No. 6252, pp. 877-883, 1989; Chothia C. et al., "Structural repertoire of the human VH segments," J. Mol. Biol., Vol. 227, No. 3, pp. 799-817, 1992; Al-Lazikani B. et al., "Standard conformations for the canonical structures of immunoglobulins," J. Mol. Biol., Vol. 273, No. 4, pp. 927-748, 1997), ImMunoGeneTics database (IMGT) (on the worldwide web at imgt.org/) (Giudicelli, V. et al., "IMGT/LIGM-DB, the IMGT® comprehensive database of immunoglobulin and T cell receptor nucleotide sequences," Nucleic Acids Res., Vol. 34 (Database Issue), pp. D781-D784, 2006; Lefranc, M. P. et al., "IMGT unique numbering for immunoglobulin and T cell receptor variable domains and Ig superfamily V-like domains," Dev. Comp. Immunol., Vol. 27, No. 1, pp. 55-

77, 2003; Brochet, X. et al., "IMGT/V-QUEST: the highly customized and integrated system for IG and TR standardized V-J and V-D-J sequence analysis," Nucleic Acids Res., Vol. 36 (Web Server Issue), pp. W503-508, 2008; AbM (Martin, A. C. et al., "Modeling antibody hypervariable loops: a combined algorithm,"Proc. Natl. Acad. Sci. U.S.A., Vol. 86, No. 23, pp. 9268-9272, 1989); the contact definition (MacCallum, R. M. et al., "Antibody-antigen interactions: contact analysis and binding site topography," J. Mol. Biol., Vol. 262, No. 5, pp. 732-745, 1996), and/or the automatic modeling and analysis tool (Honegger, A. et al., Accessible on the world wide web at bioc.uzh.ch/plueckthun/antibody/Numbering/).

[0178] The term "binding specificity determinant" or "BSD" interchangeably refer to the minimum contiguous or non-contiguous amino acid sequence within a complementarity determining region necessary for determining the binding specificity of an antibody. A minimum binding specificity determinant can be within one or more CDR sequences. In some embodiments, the minimum binding specificity determinants reside within (i.e., are determined solely by) a portion or the full-length of the CDR3 sequences of the heavy and light chains of the antibody. In some embodiments, CDR3 of the heavy chain variable region is sufficient for the antigen binding construct specificity.

[0179] An "antibody variable light chain" or an "antibody variable heavy chain" as used herein refers to a polypeptide comprising the V_L or V_H, respectively. The endogenous V_L is encoded by the gene segments V (variable) and J (junctional), and the endogenous V_H by V, D (diversity), and J. Each of V_L or V_H includes the CDRs as well as the framework regions. In this application, antibody variable light chains and/or antibody variable heavy chains may, from time to time, be collectively referred to as "antibody chains." These terms encompass antibody chains containing mutations that do not disrupt the basic structure of V_L or V_H, as one skilled in the art will readily recognize. In some embodiments, full length heavy and/or light chains are contemplated. In some embodiments, only the variable region of the heavy and/or light chains are contemplated as being present.

[0180] Antibodies can exist as intact immunoglobulins or as a number of fragments produced by digestion with various peptidases. Thus, for example, pepsin digests an antibody below the disulfide linkages in the hinge region to produce $F(ab)'_2$, a dimer of Fab' which itself is a light chain (V_L-C_L) joined to V_H-C_H1 by a disulfide bond. The $F(ab)'_2$ may be reduced under mild conditions to break the disulfide linkage in the hinge region,

thereby converting the $F(ab)'_2$ dimer into an Fab' monomer. The Fab' monomer is a Fab with part of the hinge region. (Paul, W. E., "Fundamental Immunology," 3d Ed., New York: Raven Press, 1993). While various antibody fragments are defined in terms of the digestion of an intact antibody, one of skill will appreciate that such fragments may be synthesized *de novo* either chemically or by using recombinant DNA methodology. Thus, the term "antibody," as used herein, also includes antibody fragments either produced by the modification of whole antibodies, or those synthesized *de novo* using recombinant DNA methodologies (for example, single chain Fv) or those identified using phage display libraries (*see*, for example, McCafferty, J. et al., "Phage antibodies: filamentous phage displaying antibody variable domains," Nature, Vol. 348, No. 66301, pp. 552-554, 1990).

[0181] For preparation of monoclonal or polyclonal antibodies, any technique known in the art can be used (*see*, for example, Kohler, G. et al., "Continuous cultures of fused cells secreting antibody of predefined specificity," Nature, Vol. 256, No. 5517, pp. 495-497, 1975; Kozbor, D. et al., "The production of monoclonal antibodies from human lymphocytes," Immunology Today, Vol. 4, No. 3, pp. 72-79, 1983; Cole, et al., "Monoclonal Antibodies and Cancer Therapy," Alan R. Liss, Inc., pp. 77-96, 1985; Wang, S., "Advances in the production of human monoclonal antibodies," Antibody Technology Journal, Vol. 1, pp. 1-4, 2011; Sharon, J. et al., "Recombinant polyclonal antibodies for cancer therapy," J. Cell Biochem., Vol. 96, No. 2, pp. 305-313, 2005;; Haurum, J. S., "Recombinant polyclonal antibodies: the next generation of antibody therapeutics?," Drug Discov. Today, Vol. 11, No. 13-14, pp. 655-660, 2006). Techniques for the production of single chain antibodies (U.S. Pat. No. 4,946,778) can be adapted to produce antibodies to polypeptides of this invention. Also, transgenic mice, or other organisms such as other mammals, may be used to express fully human monoclonal antibodies. Alternatively, phage display technology can be used to identify high affinity binders to selected antigens (*see*, for example, McCafferty et al., supra; Marks, J. D. et al., "By-passing immunization: building high affinity human antibodies by chain shuffling," Biotechnology (N. Y.), Vol. 10, No. 7, pp. 779-783, 1992).

[0182] Methods for humanizing or primatizing non-human antibodies are well known in the art. Generally, a humanized antibody has one or more amino acid residues introduced into it from a source which is non-human. These non-human amino acid residues are often referred to as import residues, which are typically taken from an import variable

domain. In some embodiments, the terms "donor" and "acceptor" sequences can be employed. Humanization can be essentially performed following the method of Winter and co-workers (*see*, for example, Jones, P. T. et al., "Replacing the complementarity-determining regions in a human antibody with those from a mouse," Nature, Vol. 321, No. 6069, pp. 522-525, 1986; Riechmann, L. et al., "Reshaping human antibodies for therapy," Nature, Vol. 332, No. 6162, pp. 323-327, 1988; Verhoeyen, M. et al., "Reshaping human antibodies: grafting an antilysozyme activity," Science, Vol. 239, No. 4847, pp. 1534-1536, 1988; Presta, L. G., "Antibody engineering,", Curr. Op. Struct. Biol., Vol. 2, No. 4, pp. 593-596, 1992), by substituting rodent CDRs or CDR sequences for the corresponding sequences of a human antibody. Accordingly, such humanized antibodies are chimeric antibodies (U.S. Pat. No. 4,816,567), wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In practice, humanized antibodies are typically human antibodies in which some complementarity determining region ("CDR") residues and possibly some framework ("FR") residues are substituted by residues from analogous sites in rodent antibodies.

[0183] A "chimeric antibody" is an antibody molecule in which (a) the constant region, or a portion thereof, is altered, replaced or exchanged so that the antigen binding site (variable region) is linked to a constant region of a different or altered class, effector function and/or species, or an entirely different molecule which confers new properties to the chimeric antibody, for example, an enzyme, toxin, hormone, growth factor, and drug; or (b) the variable region, or a portion thereof, is altered, replaced or exchanged with a variable region having a different or altered antigen specificity.

[0184] Antibodies further include one or more immunoglobulin chains that are chemically conjugated to, or expressed as, fusion proteins with other proteins. It also includes bispecific antibodies. A bispecific or bifunctional antibody is an artificial hybrid antibody having two different heavy/light chain pairs and two different binding sites.

[0185] Other antigen-binding fragments or antibody portions of the invention include, bispecific scFv antibodies where the antibody molecule recognizes two different epitopes, single binding domains (sdAb or nanobodies), and minibodies.

[0186] The term "antibody fragment" includes, but is not limited to one or more antigen binding fragments of antibodies alone or in combination with other molecules,

including, but not limited to Fab', $F(ab')_2$, Fab, Fv, rIgG (reduced IgG), scFv fragments, single domain fragments (nanobodies), peptibodies, minibodies. The term "scFv" refers to a single chain Fv ("fragment variable") antibody in which the variable domains of the heavy chain and of the light chain of a traditional two chain antibody have been joined to form one chain.

[0187] A pharmaceutically acceptable carrier may be a pharmaceutically acceptable material, composition, or vehicle that is involved in carrying or transporting a compound of interest from one tissue, organ, or portion of the body to another tissue, organ, or portion of the body. For example, the carrier may be a liquid or solid filler, diluent, excipient, solvent, or encapsulating material, or some combination thereof. Each component of the carrier is "pharmaceutically acceptable" in that it is be compatible with the other ingredients of the formulation. It also must be suitable for contact with any tissue, organ, or portion of the body that it may encounter, meaning that it must not carry a risk of toxicity, irritation, allergic response, immunogenicity, or any other complication that excessively outweighs its therapeutic benefits. The pharmaceutical compositions described herein may be administered by any suitable route of administration. A route of administration may refer to any administration pathway known in the art, including but not limited to aerosol, enteral, nasal, ophthalmic, oral, parenteral, rectal, transdermal (for example, topical cream or ointment, patch), or vaginal. "Transdermal" administration may be accomplished using a topical cream or ointment or by means of a transdermal patch. "Parenteral" refers to a route of administration that is generally associated with injection, including infraorbital, infusion, intraarterial, intracapsular, intracardiac, intradermal, intramuscular, intraperitoneal, intrapulmonary, intraspinal, intrasternal, intrathecal, intrauterine, intravenous, subarachnoid, subcapsular, subcutaneous, transmucosal, or transtracheal. In some embodiments, the antigen binding construct can be delivered intraoperatively as a local administration during an intervention or resection.

[0188] The term "target molecule dependent disorder" "or "target molecule associated disorder" includes any disorder in which the target molecule plays a role in the disorder itself. In some embodiments, this denotes over-expression of the target molecule. In some embodiments, the disorders can include any of the disorders discussed herein. In some embodiments, the disorder can be any for which there is a target molecule that can be

targeted by binding, whose binding will result in the detection and/or treatment of the disorder. Without limitation, the target molecule can be CD8, CD3, 5T4, PSMA, and/or PSCA, for example.

[0189] A minibody is an antibody format that has a smaller molecular weight than the full-length antibody while maintaining the bivalent binding property against an antigen. Because of its smaller size, absence of CH2 domain that binds Fc-gamma and FcRN receptors, absence of glycosylation, the minibody has a faster clearance from the system and potentially enhanced penetration when targeting tumor tissue. With the ability for strong targeting combined with rapid clearance, the minibody is advantageous for diagnostic imaging and delivery of radioactive payloads for which prolonged circulation times may result in adverse patient dosing or dosimetry. In some embodiments, it can also be advantageous for delivery of a cytotoxic payload due to the above-mentioned features such as tumor penetration and faster clearance. A "minibody" as described herein, encompasses a homodimer, wherein each monomer is a single-chain variable fragment (scFv) linked to a human IgG C_H3 domain by a hinge sequence. In some embodiments, a minibody is a bivalent or bispecific, covalently bound homodimer of ~80 kDa. In some embodiments, each monomer (half-molecule) is comprised of a variable heavy (V_H) domain linked to the corresponding variable light (V_L) domain by an approximate 15-18 amino acid Gly-Ser-rich linker sequence.

[0190] In some embodiments, each single-chain variable fragment (scFv) is linked to a human IgG1, IgG2, IgG3 or IgG4 C_H3 domain by a hinge sequence.

[0191] In some embodiments a lower hinge/extension sequence can be a native IgG1, 2, 3 or 4 lower hinge, an/or $(G_3)S_n$ or $(G_4)S_n$ (n can be any number of S's; in some embodiments it is 1 or 2) and/or no lower hinge and/or any combination of amino acids (doesn't have to be G's and S's). In some embodiments, the lower hinge/extension sequence can comprise GGGSSGGGSG (SEQ ID NO: 59).

[0192] In some embodiments, a linker can be any that will work. In some embodiments, a linker sequence can include a motif that is $(G_3)S_n$ or $(G_4)S_n$ (n can be any number of S's; in some embodiments it is 1 or 2). In some embodiments, the linker can comprise GSTSGGGSGGGSGGGGSS (SEQ ID NO: 62).

[0193] The phrase "specifically (or selectively) bind," when used in the context of describing the interaction between an antigen, for example, a protein, to an antibody or antibody-derived binding agent, refers to a binding reaction that is determinative of the presence of the antigen in a heterogeneous population of proteins and other biologics, for example, in a biological sample, for example, a blood, serum, plasma or tissue sample. Thus, under designated immunoassay conditions, in some embodiments, the antibodies or binding agents with a particular binding specificity bind to a particular antigen at least two times the background and do not substantially bind in a significant amount to other antigens present in the sample. Specific binding to an antibody or binding agent under such conditions may require the antibody or agent to have been selected for its specificity for a particular protein. A variety of immunoassay formats may be used to select antibodies specifically immunoreactive with a particular protein. For example, solid-phase ELISA immunoassays are routinely used to select antibodies specifically immunoreactive with a protein (*see*, for example, Harlow, E. & Lane D., "Using Antibodies, A Laboratory Manual," Cold Spring Harbor Laboratory Press, 1998, for a description of immunoassay formats and conditions that can be used to determine specific immunoreactivity). Typically a specific or selective binding reaction will produce a signal at least twice over the background signal and more typically at least than 10 to 100 times over the background.

[0194] The term "equilibrium dissociation constant (K_D, M)" refers to the dissociation rate constant (k_d, time^{-1}) divided by the association rate constant (k_a, time^{-1} M^{-1}). Equilibrium dissociation constants can be measured using any known method in the art. The antibodies of the present invention generally will have an equilibrium dissociation constant of less than about 10^{-7} or 10^{-8} M, for example, less than about 10^{-9} M or 10^{-10} M, in some embodiments, less than about 10^{-11} M, 10^{-12} M, or 10^{-13} M.

[0195] The term "isolated," when applied to a nucleic acid or protein, denotes that the nucleic acid or protein is essentially free of other cellular components with which it is associated in the natural state. In some embodiments, it can be in either a dry or aqueous solution. Purity and homogeneity can be determined using analytical chemistry techniques such as polyacrylamide gel electrophoresis or high performance liquid chromatography. A protein that is the predominant species present in a preparation is substantially purified. In particular, an isolated gene is separated from open reading frames that flank the gene and

encode a protein other than the gene of interest. The term "purified" denotes that a nucleic acid or protein gives rise to essentially one band in an electrophoretic gel. In some embodiments, this can denote that the nucleic acid or protein is at least 85% pure, more preferably at least 95% pure, and most preferably at least 99% pure of molecules that are present under *in vivo* conditions.

[0196] The term "nucleic acid" or "polynucleotide" refers to deoxyribonucleic acids (DNA) or ribonucleic acids (RNA) and polymers thereof in either single- or double-stranded form. Unless specifically limited, the term encompasses nucleic acids containing known analogues of natural nucleotides that have similar binding properties as the reference nucleic acid and are metabolized in a manner similar to naturally occurring nucleotides. Unless otherwise indicated, a particular nucleic acid sequence also implicitly encompasses conservatively modified variants thereof (for example, degenerate codon substitutions), alleles, orthologs, SNPs, and complementary sequences as well as the sequence explicitly indicated. Specifically, degenerate codon substitutions may be achieved by generating sequences in which the third position of one or more selected (or all) codons is substituted with mixed-base and/or deoxyinosine residues (Batzer, M. A. et al., "Enhanced evolutionary PCR using oligonucleotides with inosine at the 3'-terminus," Nucleic Acid Res., Vol. 19, No. 18, pp. 5081, 1991; Ohtsuka, E. et al., "An alternative approach to deoxyoligonucleotides as hybridization probes by insertion of deoxyinosine at ambiguous codon positions," J. Biol. Chem., Vol. 260, No. 5, pp. 2605-2608, 1985; Rossolini, G. M. et al., "Use of deoxyinosine-containing primers vs degenerate primers for polymerase chain reaction based on ambiguous sequence information," Mol. Cell. Probes, Vol. 8, No. 2, pp. 91-98, 1994).

[0197] The terms "polypeptide," "peptide," and "protein" are used interchangeably herein to refer to a polymer of amino acid residues. The terms apply to amino acid polymers in which one or more amino acid residue is an artificial chemical mimetic of a corresponding naturally occurring amino acid, as well as to naturally occurring amino acid polymers and non-naturally occurring amino acid polymer.

[0198] The term "amino acid" refers to naturally occurring and synthetic amino acids, as well as amino acid analogs and amino acid mimetics that function in a manner similar to the naturally occurring amino acids. Naturally occurring amino acids are those encoded by the genetic code, as well as those amino acids that are later modified, for

example, hydroxyproline, gamma-carboxyglutamate, and O-phosphoserine. Amino acid analogs refer to compounds that have the same basic chemical structure as a naturally occurring amino acid, for example, an alpha-carbon that is bound to a hydrogen, a carboxyl group, an amino group, and an R group, for example, homoserine, norleucine, methionine sulfoxide, methionine methyl sulfonium. Such analogs have modified R groups (for example, norleucine) or modified peptide backbones, but retain the same basic chemical structure as a naturally occurring amino acid. Amino acid mimetics refers to chemical compounds that have a structure that is different from the general chemical structure of an amino acid, but that functions in a manner similar to a naturally occurring amino acid.

[0199] The term "conservatively modified variants" applies to both amino acid and nucleic acid sequences. With respect to particular nucleic acid sequences, conservatively modified variants refers to those nucleic acids which encode identical or essentially identical amino acid sequences, or where the nucleic acid does not encode an amino acid sequence, to essentially identical sequences. Because of the degeneracy of the genetic code, a large number of functionally identical nucleic acids encode any given protein. For instance, the codons GCA, GCC, GCG and GCU all encode the amino acid alanine. Thus, at every position where an alanine is specified by a codon, the codon can be altered to any of the corresponding codons described without altering the encoded polypeptide. Such nucleic acid variations are "silent variations," which are one species of conservatively modified variations. Every nucleic acid sequence herein which encodes a polypeptide also describes every possible silent variation of the nucleic acid. One of skill will recognize that each codon in a nucleic acid (except AUG, which is ordinarily the only codon for methionine, and TGG, which is ordinarily the only codon for tryptophan) can be modified to yield a functionally identical molecule. Accordingly, each silent variation of a nucleic acid that encodes a polypeptide is implicit in each described sequence.

[0200] As to amino acid sequences, one of skill will recognize that individual substitutions, deletions or additions to a nucleic acid, peptide, polypeptide, or protein sequence which alters, adds or deletes a single amino acid or a small percentage of amino acids in the encoded sequence is a "conservatively modified variant" where the alteration results in the substitution of an amino acid with a chemically similar amino acid. Conservative substitution tables providing functionally similar amino acids are well known

in the art. Such conservatively modified variants are in addition to and do not exclude polymorphic variants, interspecies homologs, and alleles of the invention.

[0201] The following eight groups each contain amino acids that are conservative substitutions for one another: 1) Alanine (A), Glycine (G); 2) Aspartic acid (D), Glutamic acid (E); 3) Asparagine (N), Glutamine (Q); 4) Arginine (R), Lysine (K); 5) Isoleucine (I), Leucine (L), Methionine (M), Valine (V); 6) Phenylalanine (F), Tyrosine (Y), Tryptophan (W); 7) Serine (S), Threonine (T); and 8) Cysteine (C), Methionine (M) (*see*, for example, Creighton, T. E., "Proteins - Structures and Molecular Properties," W. H. Freeman & Co. Ltd., 1984).

[0202] The term "percentage of sequence identity" can be determined by comparing two optimally aligned sequences over a comparison window, wherein the portion of the polynucleotide sequence in the comparison window may comprise additions or deletions (i.e., gaps) as compared to the reference sequence (for example, a polypeptide of the invention), which does not comprise additions or deletions, for optimal alignment of the two sequences. The percentage is calculated by determining the number of positions at which the identical nucleic acid base or amino acid residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison and multiplying the result by 100 to yield the percentage of sequence identity.

[0203] The terms "identical" or percent "identity," in the context of two or more nucleic acids or polypeptide sequences, refer to two or more sequences or subsequences that are the same sequences. Two sequences are "substantially identical" if two sequences have a specified percentage of amino acid residues or nucleotides that are the same (for example, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% sequence identity over a specified region, or, when not specified, over the entire sequence of a reference sequence), when compared and aligned for maximum correspondence over a comparison window, or designated region as measured using one of the following sequence comparison algorithms or by manual alignment and visual inspection. Some embodiments provided herein provide polypeptides or polynucleotides that are substantially identical to the polypeptides or polynucleotides, respectively, exemplified herein (for example, any one or more of the variable regions exemplified in any one of Tables 0.1, 0.2, 1, 2, or 3 and FIGs. 5B-5E, 7C,

7D, 14B, 15B, 16B-16D, 20C-20G, 21B-21E, 22B, 28B-28E, 29B-29D, 32, 33, 35A-35C, 36A-36E, 40-69, 79, 80 and any one or more of the nucleic acid sequences exemplified in any one of FIGs. 7E, 34A-34F, 41-46, 48-53, 55-59, 61-64, 65A, 65B, and 65C). Optionally, the identity exists over a region that is at least about 15, 25 or 50 nucleotides in length, or more preferably over a region that is 100 to 500 or 1000 or more nucleotides in length, or over the full length of the reference sequence. With respect to amino acid sequences, identity or substantial identity can exist over a region that is at least 5, 10, 15 or 20 amino acids in length, optionally at least about 25, 30, 35, 40, 50, 75 or 100 amino acids in length, optionally at least about 150, 200 or 250 amino acids in length, or over the full length of the reference sequence. With respect to shorter amino acid sequences, for example, amino acid sequences of 20 or fewer amino acids, in some embodiments, substantial identity exists when one or two amino acid residues are conservatively substituted, according to the conservative substitutions defined herein.

[0204] In some embodiments, the percent identity is over the hinge regions noted herein (the hinge region and/or its subparts of upper, core, and lower hinge regions). In such situations, the percent identity of the hinge region or its subpart can be identified separately from the rest of the protein or nucleic acid sequence. Thus, two hinge regions (or upper, core, and/or lower regions) can have a specified percentage of amino acid residues or nucleotides that are the same (for example, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% sequence identity over a specified region, or, when not specified, over the entire sequence of a reference sequence), while allowing for the remainder of the protein to either stay 100% identical to the comparison protein, our while also allowing the remainder of the protein to also have variation by a specified percent identity.

[0205] For sequence comparison, typically one sequence acts as a reference sequence, to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are entered into a computer, subsequence coordinates are designated, if necessary, and sequence algorithm program parameters are designated. Default program parameters can be used, or alternative parameters can be designated. The sequence comparison algorithm then calculates the percent sequence identities for the test sequences relative to the reference sequence, based on the program parameters.

[0206] A "comparison window", as used herein, includes reference to a segment of any one of the number of contiguous positions selected from the group consisting of from 20 to 600, usually about 50 to about 200, more usually about 100 to about 150 in which a sequence may be compared to a reference sequence of the same number of contiguous positions after the two sequences are optimally aligned. Methods of alignment of sequences for comparison are well known in the art. Optimal alignment of sequences for comparison can be conducted, for example, by the local homology algorithm of Smith and Waterman (1970) Adv. Appl. Math. 2:482c, by the homology alignment algorithm of Needleman, S. B. et al., "A general method applicable to the search for similarities in the amino acid sequence of two proteins," J. Mol. Biol., Vol. 48, No. 3, pp. 443-453, 1970, by the search for similarity method of Pearson, W. R. et al., "Improved tools for biological sequence comparison," Proc. Natl. Acad. Sci. U.S.A., Vol. 85, No. 8, pp. 2444-2448, 1988, by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, Wis.), or by manual alignment and visual inspection (*see*, for example, Ausubel, F. M. et al., Current Protocols in Molecular Biology, Supplement, 1995).

[0207] Two examples of algorithms that are suitable for determining percent sequence identity and sequence similarity are the BLAST and BLAST 2.0 algorithms, which are described in Altschul, S. F. et al., "Gapped BLAST and PSI-BLAST: a new generation of protein database search programs," Nucleic Acids Res., Vol. 25, No. 17, pp. 3389-3402, 1977, and Altschul, S. F. et al., "Basic local alignment search tool," J. Mol. Biol., Vol. 215, No. 3, pp. 403-410, 1990, respectively. Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information. This algorithm involves first identifying high scoring sequence pairs (HSPs) by identifying short words of length W in the query sequence, which either match or satisfy some positive-valued threshold score T when aligned with a word of the same length in a database sequence. T is referred to as the neighborhood word score threshold (Altschul, S. F. et al., supra). These initial neighborhood word hits act as seeds for initiating searches to find longer HSPs containing them. The word hits are extended in both directions along each sequence for as far as the cumulative alignment score can be increased. Cumulative scores are calculated using, for nucleotide sequences, the parameters M (reward score for a pair of matching residues; always

>0) and N (penalty score for mismatching residues; always <0). For amino acid sequences, a scoring matrix is used to calculate the cumulative score. Extension of the word hits in each direction are halted when: the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T, and X determine the sensitivity and speed of the alignment. The BLASTN program (for nucleotide sequences) uses as defaults a wordlength (W) of 11, an expectation (E) or 10, M=5, N=-4 and a comparison of both strands. For amino acid sequences, the BLASTP program uses as defaults a wordlength of 3, and expectation (E) of 10, and the BLOSUM62 scoring matrix (*see*, Henikoff, S. et al., "Amino acid substitution matrices from protein blocks," Proc. Natl. Acad. Sci. U.S.A., Vol. 89, No. 22, pp. 10915-10919, 1992) alignments (B) of 50, expectation (E) of 10, M=5, N=-4, and a comparison of both strands.

[0208] The BLAST algorithm also performs a statistical analysis of the similarity between two sequences (*see*, for example, Karlin, S. et al., "Applications and statistics for multiple high-scoring segments in molecular sequences," Proc. Natl. Acad. Sci. U.S.A., Vol. 90, No. 12, pp. 5873-5787, 1993). One measure of similarity provided by the BLAST algorithm is the smallest sum probability (P(N)), which provides an indication of the probability by which a match between two nucleotide or amino acid sequences would occur by chance. For example, a nucleic acid is considered similar to a reference sequence if the smallest sum probability in a comparison of the test nucleic acid to the reference nucleic acid is less than about 0.2, more preferably less than about 0.01, and most preferably less than about 0.001.

[0209] An indication that two nucleic acid sequences or polypeptides are substantially identical is that the polypeptide encoded by the first nucleic acid is immunologically cross reactive with the antibodies raised against the polypeptide encoded by the second nucleic acid, as described below. Thus, in some embodiments, a polypeptide is typically substantially identical to a second polypeptide, for example, where the two peptides differ only by conservative substitutions. Another indication that two nucleic acid sequences are substantially identical is that the two molecules or their complements hybridize to each other under stringent conditions, as described below. Yet another indication that two nucleic

acid sequences are substantially identical is that the same primers can be used to amplify the sequence.

[0210] The terms "subject," "patient," and "individual" interchangeably refer to an entity that is being examined and/or treated. This can include, for example, a mammal, for example, a human or a non-human primate mammal. The mammal can also be a laboratory mammal, for example, mouse, rat, rabbit, hamster. In some embodiments, the mammal can be an agricultural mammal (for example, equine, ovine, bovine, porcine, camelid) or domestic mammal (for example, canine, feline).

[0211] The term "co-administer" refers to the administration of two active agents in the blood of an individual or in a sample to be tested. Active agents that are co-administered can be concurrently or sequentially delivered.

Antigen binding constructs

[0212] It is herein appreciated that the sequences within the hinge region can be of special relevance for various antigen binding constructs. In some embodiments, the value of the hinge region can be especially high, such as in a minibody arrangement. The sequences within the hinge can include disulfide bonds which are useful for hinge function but can have varying results when altered. As disclosed herein, and shown in the examples below, in some embodiments, the hinge region sequences can be configured to prevent and/or reduce undesirable disulfide scrambling with cysteine residues present in other regions of the protein and/or contain sufficient numbers of cysteine pairs to maintain dimer integrity *in vivo,* prevent concatamers from forming and/or allow for site specific conjugation to one of the paired cysteines.

[0213] In some embodiments, the stability of a minibody dimer *in vivo* can be attributed to natural Van-der-Waals association between the C_H3 domains, formation of disulfide bonds within the hinge regions, and/or interactions between V_H and V_L. To date most minibodies have been engineered using the human IgG1 upper and core hinge regions with an extension sequence linked to the human IgG1 C_H3 domain. In addition to the above noted variables, both orientations of a minibody (e.g., V_L-V_H (M1) and V_H-V_L (M2) scFv variants) are herein provided and characterized, as the orientation of the constructs can also alter their characteristics (as shown in the examples below).

[0214] Some previous hinges (e.g., huIgG1 hinge-extension or γ1 EH1) have included a native huIgG1 in the upper hinge (FIG. 2). This cysteine at position 245 in the context of human IgG1 as defined by Kabat and indicated by the first circle, creates problems with both protein heterogeneity and stability in the minibody format based on the disulfide mapping by LC/MS, SDS-PAGE analysis and *in vivo* biodistribution data. As shown in the examples below, in some embodiments, Cys245 can be mutated to serine, resulting in an artificial hinge or "EH2 hinge" as described herein. Thus, removal of this cysteine from a strand (as shown in the examples below), has resulted in an improved hinge region. In some embodiments, the resulting construct (which lacks the first cysteine in the hinge region) is not strong enough to maintain minibody dimer stability *in vivo* with the two remaining disulfide bridges (in regard to γ1 EH/EH2). Thus, as shown in the examples below, the introduction of a third or greater number of cysteines per strand can be made (for example, within the core region) to create an improved construct. In some embodiments, providing a 3rd (or more) cysteine in the hinge (an additional cysteine over IgG1 native hinge, per strand) increases disulfide bonding and increases protein stability. In some embodiments, the added stability afforded by increasing disulfide bonds in the hinge region allows for site specific conjugation to one or more than one of the cysteines with maintenance of intact dimer. In some embodiments, the huIgG2 minibodies of IAB2M, 20M, 1M, and IAB22M involve human IgG2 (huIgG2) hinge/extension sequence linked to the huIgG2 C_H3 domain. Thus, in some embodiments, the C_H3 domain can be a huIgG2 C_H3 domain. In some embodiments, any option for creating a covalent bond between the two strands of the peptide can be employed instead of cysteines for forming a disulfide bond.

[0215] In the present application, when a reference to an introduction of a cysteine is made (unless otherwise noted), it denotes the introduction of a cysteine per strand of the hinge (which comprises two strands). Thus, 3, 4, 5, or 6 cysteines within a strand will mean 6, 8, 10, or 12 cysteines within a hinge, and 3, 4, 5, or 6 possible disulfide bonds being present (of course, unless noted, the number of disulfide bonds can be different, as some could be used for labels and other uses).

[0216] In some embodiments, the IgG2 hinge/extension - γ2 EH1 - resulted in an increase in aggregation, where the reactive first hinge cysteine was responsible for

concatamer formation in IAB2M-γ2 EH1 format. In such embodiments, the native huIgG2 upper hinge can be employed.

[0217] The above aspects were then considered in developing a second configuration of some of the embodiments of a hinge construct. In this second arrangement, the first hinge cysteine (one that pairs with the kappa light chain in a native antibody) was mutated to serine resulting in IgG2 EH2 (γ2 EH2). This construct expressed well and had good stability *in vivo*, had all 3 disulfide bonds formed properly as shown by intact mass analysis, and had stability demonstrated with both IAB2M and IAB22M constructs (as shown in the examples below). Thus, in some embodiments an IgG2 artificial or modified hinge, in which the first cysteine has been altered is provided herein and provides for an improved minibody construct.

[0218] As demonstrated in the examples below, the results showed that it was advantageous to mutate the first hinge cysteine of the IgG1 and IgG2 hinges to prevent cysteine mis-pairing. As demonstrated in the examples below, the results showed that it was advantageous to have more than 2 cysteine residues in core hinge region per strand to maintain structural integrity of protein. As demonstrated in the examples below, the results showed that the IgG2 hinge provides one solution to increase stability *in vivo*. Thus, higher *in vivo* stability antigen binding constructs, such as minibodies, are provided herein.

[0219] In some embodiments, an IgG2 hinge can be employed. In some embodiments, an IgG2 hinge can be employed where the first hinge cysteine is mutated. In some embodiments, mutation of the terminal lysine (K) in the Mb constructs did not impact protein expression but did generate protein with more uniform charge.

[0220] As discussed in the examples, additional Mb variants were evaluated based on upper IgG3 hinge and modified IgG1 core hinge sequences where at least 3 cysteine residues were present in the core hinge region per strand. As outlined in the examples below, IAB2M, IAB22M and IAB20M constructs were evaluated to demonstrate universality of findings across various constructs having different orientations and different targets. The results indicate that the advantages described herein regarding the presently disclosed hinges would be available and applicable to any and all antigen binding constructs and minibodies in particular.

[0221] In some embodiments, the hinges can be peptide hinges and/or nucleic acid sequences that encode for the peptide hinges. All discussions regarding the peptide form of the hinges provided herein also designate corresponding structures and functions for nucleic acid sequences encoding the peptides. In some embodiments, the hinges can be incorporated into an antigen binding construct, such as an antibody, such as a minibody. Any hinge or subpart of a hinge (such as an upper hinge, core hinge, and/or lower hinge region) can be incorporated into any of the antigen binding constructs provided herein, including, but not limited to those in: Tables 0.1, 0.2, 1, 2, or 3 and FIGs. 5B-5E, 7C, 7D, 14B, 15B, 16B-16D, 20C-20G, 21B-21E, 22B, 28B-28E, 29B-29D, 32, 33, 35A-35C, 36A-36E, 40-69, 79, 80 and any one or more of the nucleic acid sequences exemplified in any one of FIGs. 7E, 34A-34F, 41-46, 48-53, 55-59, 61-64, 65A, 65B, and 65C. In some embodiments, the hinge can include any one or more of the sequences or subparts shown in Table 0.1. In some embodiments, any of the lower hinges can then be connected directly or indirectly to a C_H3 domain.

TABLE 0.1

Hinge variants (FIG. 38)

Full hinge	Upper hinge	Core hinge	Lower hinge
Human IgG1 NH1 (SEQ ID NO: 21)	EPKSCDKTHT (SEQ ID NO: 45)	CPPCP (SEQ ID NO: 51)	APELLGGP (SEQ ID NO: 58)
Human IgG1 EH1 (SEQ ID NO: 22)	EPKSCDKTHT (SEQ ID NO: 45)	CPPC (SEQ ID NO: 50)	GGGSSGGGSG (SEQ ID NO: 59)
Human IgG1 NH2 (SEQ ID NO: 23)	EPKSSDKTHT (SEQ ID NO: 46)	CPPCP (SEQ ID NO: 51)	APELLGGP (SEQ ID NO: 58)
Human IgG1 EH2 (SEQ ID NO: 24)	EPKSSDKTHT (SEQ ID NO: 46)	CPPC (SEQ ID NO: 50)	GGGSSGGGSG (SEQ ID NO: 59)
Human IgG1 NH3 (SEQ ID NO: 25)	EPKSSDKTHT (SEQ ID NO: 46)	CPPCPPC (SEQ ID NO: 52)	APELLGGP (SEQ ID NO: 58)
Human IgG1 EH3 (SEQ ID NO: 26)	EPKSSDKTHT (SEQ ID NO: 46)	CPPCPPC (SEQ ID NO: 52)	GGGSSGGGSG (SEQ ID NO: 59)
Human IgG1 NH4 (SEQ ID NO: 27)	EPKSSDKTHT (SEQ ID NO: 46)	CPPCVECPPC (SEQ ID NO: 53)	APELLGGP (SEQ ID NO: 58)
Human IgG1 EH4 (SEQ ID NO: 28)	EPKSSDKTHT (SEQ ID NO: 46)	CPPCVECPPC (SEQ ID NO: 53)	GGGSSGGGSG (SEQ ID NO: 59)
Human IgG1 NH5 (SEQ ID NO: 29)	EPKSSDKTHT (SEQ ID NO: 46)	CPPCPPCPPC (SEQ ID NO: 54)	APELLGGP (SEQ ID NO: 58)
Human IgG1 EH5 (SEQ ID NO: 30)	EPKSSDKTHT (SEQ ID NO: 46)	CPPCPPCPPC (SEQ ID NO: 54)	GGGSSGGGSG (SEQ ID NO: 59)
Human IgG2 NH1 (SEQ ID NO: 31)	ERK (SEQ ID NO: 47)	CCVECPPCP (SEQ ID NO: 55)	APPVAGP (SEQ ID NO: 60)
Human IgG2 EH1 (SEQ	ERK (SEQ ID NO: 47)	CCVECPPCP (SEQ	GGGSSGGGSG (SEQ

ID NO: 32)		ID NO: 55)	ID NO: 59)
Human IgG2 NH2 (SEQ ID NO: 33)	ERK (SEQ ID NO: 47)	SCVECPPCP (SEQ ID NO: 56)	APPVAGP (SEQ ID NO: 60)
Human IgG2 EH2 (SEQ ID NO: 34)	ERK (SEQ ID NO: 47)	SCVECPPCP (SEQ ID NO: 56)	GGGSSGGGSG (SEQ ID NO: 59)
IgG3/IgG1 EH6 (SEQ ID NO: 35)	ELKTPLGDTTHT (SEQ ID NO: 48)	CVECPPC (SEQ ID NO: 57)	GGGSSGGGSG (SEQ ID NO: 59)
IgG3/IgG1 EH7 (SEQ ID NO: 36)	ELKTPLGDTTHT (SEQ ID NO: 48)	CPPCPPC (SEQ ID NO: 52)	GGGSSGGGSG (SEQ ID NO: 59)
IgG3/IgG1 EH8 (SEQ ID NO: 37)	ELKTPLGDTTHT (SEQ ID NO: 48)	CPPCPPCPPC (SEQ ID NO: 54)	GGGSSGGGSG (SEQ ID NO: 59)
IgG4 NH (SEQ ID NO: 38)	ESKYGPP (SEQ ID NO: 49)	CPPCP (SEQ ID NO: 51)	APEFLGGP (SEQ ID NO: 61)
IgG4 EH (SEQ ID NO: 39)	ESKYGPP (SEQ ID NO: 49)	CPPCP (SEQ ID NO: 51)	GGGSSGGGSG (SEQ ID NO: 59)

[0222] In some embodiments, the hinge comprises, consists of, or consists essentially of any of the sequences in Table 0.1 above. In some embodiments, the hinge consists of each of a designated upper, core, and lower hinge region in Table 0.1. In some embodiments, any one of the above subparts (such as the upper, core and/or lower hinge) can also be provided as a variant thereof. In some embodiments, the variants have substantial identity to the sequences above. In some embodiments, the variants have one, two, or three amino acids difference from the sequences noted above. In some embodiments, the variants have one, two, or three amino acids difference from the sequences noted above and the differences are conservative differences.

[0223] In some embodiments the CPPC (SEQ ID NO: 50) motif in the core region can be altered to any other effective core motif, as long as the number and/or effectiveness of the covalent bonds presented in the table above are adequately replaced.

[0224] In some embodiments, a peptide hinge region for an antibody is provided. The hinge region can include an upper hinge region that comprises no amino acids capable of crosslinking with a corresponding amino acid. The amino acid hinge region can further include a core hinge region connected C-terminal to the upper hinge region. In some embodiments, the core hinge region comprises at least three cysteines on each side of the hinge (so at least six cysteines for the assembled structure). In some embodiments, the amino acid hinge region further comprises a lower hinge or extension region connected C-terminal to the core hinge region. In some embodiments, the lower hinge or extension

region can include at least one of: APPVAGP (SEQ ID NO: 60), APELLGGP (SEQ ID NO: 58), or GGGSSGGGSG (SEQ ID NO: 59).

[0225] In some embodiments, the upper hinge region comprises no cysteines that crosslink within the upper hinge region. In some embodiments, the upper hinge region comprises no cysteines. In some embodiments, the amino acid hinge region further comprises a lower hinge region. In some embodiments, these constructs are non-naturally occurring constructs.

[0226] In some embodiments, a variant minibody hinge region is provided. The variant minibody hinge region comprises a first altered amino acid position. The first altered amino acid position is an amino acid that in a native antibody hinge would be a cysteine, and has been altered in the first altered position so that it does not form a disulfide bond. The variant minibody hinge region further includes at least three cysteines C-terminal to the first altered amino acid position per strand. In some embodiments, 4, 5, 6, or more cysteines can be present C-terminal to the first altered amino acid position per strand. In some embodiments, these additional cysteines are fully contained within the core region of the hinge (of course, the core hinge can be modified and expanded beyond wild-type sequences such that the additional cysteines can be added). In some embodiments, the hinge region consists of SEQ ID NO: 1 ($X_{n1}CX_{n2}X_{n3}CX_{n4}X_{n5}C$). In some embodiments, SEQ ID NO: 1 is a core hinge region, and the core hinge region essentially consists of SEQ ID NO: 1. In some embodiments, the core hinge region consists of SEQ ID NO: 1. In the description noted above, the number of cysteines relates to the number of cysteines in a single hinge region of the peptide chain. It will be understood that the number of cysteines in the dimeric form of the hinge (where the peptide chains are crosslinked to one another) will be twice as many, as the noted cysteines are each part of a disulfide bind involving two cysteines.

[0227] In some embodiments, Mb constructs contain antigen binding scFvs with variable linker lengths that can be in either V_L-V_H or V_H-V_L orientation. Any disclosure provided herein regarding one orientation also contemplates the reverse orientation and both orientations. In some embodiments, any hinge sequence described in Table 0.1 can be added C-terminal to scFvs. In some embodiments, any C_H3 domain from IgG1, IgG2, IgG3, or IgG4 antibodies can be added C-terminal to scFvs and appropriate hinges. In some embodiments, to ensure appropriate disulfide bonding, the first hinge cysteine (cysteine that

usually pairs with light chain in native IgG1, IgG2, IgG3, and IgG4 antibodies) is mutated to a serine or alanine or other appropriate amino acid to prevent disulfide scrambling and/or concatemerization. In some embodiments, to reduce the presence or prevent formation of half-molecules and ensure optimal stability *in vivo*, the hinge region can contain at least 3 disulfide bonds, in some embodiments it can have more. In some embodiments, at least 3 disulfide bonds are present in the hinge located at appropriate distances from one another are included to prevent improper disulfide bonds forming and ensure proper disulfide bond formation. In some embodiments, 3 disulfide bonds in the hinge are located at appropriate distances from one another are included for protein stability *in vivo* and clearance through liver rather than renal clearance. In some embodiments, at least 3 disulfides bonds (for example, 4 or more) in the hinge are provided to afford using a cysteine as a handle, following gentle reduction of the disulfides, of drugs, metal chelators, radionuclides, radioisotopes, site specific conjugation of a chelator then attachment of a radioisotope to the chelator, or fluorescent dyes using cysteine as a site of attachment. As appreciated by one of skill in the art, the presence of each single disulfide bond indicates the presence of two cysteines, one on each peptide sequence of the hinge.

[0228] In some embodiments, Mbs are constructed so as to include any of the hinge components provided herein and retain similar (or the same) affinity to parent antibodies. In some embodiments, Mbs constructed as described above can be engineered to contain two different antigen binding domains.

[0229] In some embodiments, superior hinges for antigen binding constructs can be obtained by removing a first cysteine in the hinge region, as noted in Table 0.1 above and in the examples below. Thus, in some embodiments, a hinge region in which this cysteine (which would otherwise be present in the natural hinge) is removed or altered to another residue (for example a residue that cannot form a covalent bond) can be provided. In some embodiments, this adjustment is made in a human IgG1 context, or any other hinge region that includes the cysteine in the noted position in Table 0.1 (for example, γ2 EH1 altered in γ2 EH2 (Cys to Ser), and γ2 NH1 altered in γ2 NH2 (Cys to Ser)).

[0230] In some embodiments, an amino acid hinge region is provided that comprises a sequence of SEQ ID NO: 1 ($X_{n1}CX_{n2}X_{n3}CX_{n4}X_{n5}C$). X_{n1} can be any amino acid that does not form a covalent crosslinking bond. In some embodiments, X_{n2} is any one of:

A, R, N, D, E, Q, G, H, I, L, K, M, F, P, S, T, W, Y, or V. X_{n3} can be any amino acid. X_{n4} is any amino acid. X_{n5} is any amino acid. In some embodiments, X_{n1} comprises a serine or an alanine (SEQ ID NO: 235). In some embodiments, X_{n1} comprises a serine (SEQ ID NO: 210). In some embodiments, X_{n1} comprises an alanine (SEQ ID NO: 211). In some embodiments, X_{n1} comprises a T (SEQ ID NO: 236). In some embodiments, the first cysteine in SEQ ID NO: 1 that is altered is a cysteine in a γ2 domain. In some embodiments, the amino acid hinge region further comprises additional amino acids in front of SEQ ID NO: 1 ($X_{n1}CX_{n2}X_{n3}CX_{n4}X_{n5}C$).

[0231] In some embodiments of $X_{n1}CX_{n2}X_{n3}CX_{n4}X_{n5}C$ (SEQ ID NO: 1), X_{n1} does not form a covalent crosslinking bond with another amino acid (SEQ ID NO: 191). In some embodiments, X_{n1} is not a cysteine (SEQ ID NO: 192). In some embodiments of SEQ ID NO: 1, X_{n1} is one of: A, R, N, D, E, Q, G, H, I, L, K, M, F, P, S, T, W, Y, or V (SEQ ID NO: 193). In some embodiments of SEQ ID NO: 1, X_{n2} is P, V, or E (SEQ ID NO: 194). In some embodiments of SEQ ID NO: 1, X_{n4} is P, V, or E (SEQ ID NO: 196). In some embodiments of SEQ ID NO: 1, X_{n4} P or V (SEQ ID NO: 197). In some embodiments of SEQ ID NO: 1, X_{n2} is P or V (SEQ ID NO: 195). In some embodiments of SEQ ID NO: 1, X_{n3} is P or E (SEQ ID NO: 198). In some embodiments of SEQ ID NO: 1, X_{n5} is P or E (SEQ ID NO: 199). In some embodiments of SEQ ID NO: 1, X_{n3} is P or E and X_{n5} is P or E (SEQ ID NO: 237). In some embodiments of SEQ ID NO: 1, $X_{n2}X_{n3}$ is VE (SEQ ID NO: 201). In some embodiments of SEQ ID NO: 1, $X_{n2}X_{n3}$ is PP (SEQ ID NO: 202). In some embodiments of SEQ ID NO: 1, $X_{n4}X_{n5}$ is VE (SEQ ID NO: 203). In some embodiments of SEQ ID NO: 1, $X_{n4}X_{n5}$ is PP (SEQ ID NO: 204). In some embodiments of SEQ ID NO: 1, $X_{n2}X_{n3}$ is VE and $X_{n4}X_{n5}$ is PP (SEQ ID NO: 205). In some embodiments of SEQ ID NO: 1, $X_{n2}X_{n3}$ is VE or PP (SEQ ID NO: 238). In some embodiments of SEQ ID NO: 1, $X_{n2}X_{n3}$ is VE (SEQ ID NO: 239).

[0232] In some embodiments, any of the above noted hinge regions further comprises a lower hinge or extension sequence C-terminal to the last cysteine in $X_{n1}CX_{n2}X_{n3}CX_{n4}X_{n5}C$ (SEQ ID NO: 1). In some embodiments, any lower hinge region can be employed. In some embodiments, any of the above noted hinge regions further comprises an extension or lower hinge sequence C-terminal to the last cysteine in $X_{n1}CX_{n2}X_{n3}CX_{n4}X_{n5}C$ (SEQ ID NO: 1).

[0233] In some embodiments, the hinge region of SEQ ID NO: 1 and/or 2 is a core hinge region. In some embodiments, the hinge further comprises an upper hinge region adjacent to the core hinge region. In some embodiments, the hinge further comprises a lower hinge or extension region adjacent to the core hinge region. In some embodiments, the amino acid hinge region further comprises an upper hinge region adjacent to the core hinge region.

[0234] In some embodiments, the amino acid hinge region comprises the following sequence: SCVECPPCP (SEQ ID NO: 56). In some embodiments, the amino acid hinge region comprises the following sequence: ERKSCVECPPCP (SEQ ID NO: 167). In some embodiments, the amino acid hinge region comprises the following sequence: EPKSSDKTHTCPPCPPC (SEQ ID NO: 168). In some embodiments, the amino acid hinge region comprises at least one of the following sequences: ERKSCVECPPCPGGGSSGGGSG (SEQ ID NO: 34) or ERKSCVECPPCPAPPVAGP (SEQ ID NO: 33). In some embodiments, the amino acid hinge region comprises at least TCPPCPPC (SEQ ID NO: 166). In some embodiments, the amino acid hinge region comprises at least EPKSSDKTHTCPPCPPCGGGSSGGGSG (SEQ ID NO: 26) or EPKSSDKTHTCPPCPPCAPELLGGP (SEQ ID NO: 25).

[0235] In some embodiments, superior hinges can be provided by removing the hinge cysteine that is responsible for linking the heavy and light chains of IgG1, IgG2, IgG3 and IgG4 respectively, as shown in Table 0.1 and 3 (for example, γ1 EH1 to γ1 EH2). Furthermore, in some embodiments, using a core region with at least three cysteines (e.g., γ1 EH3, γ1 EH4, and γ1 EH5) per strand can result in a superior construct. In some embodiments, a hinge can be provided by removing a cysteine from an upper hinge region, as shown in table 0.1 and 3 (for example, γ1 EH1 to γ1 EH2).

[0236] In some embodiments, such constructs can be described as an amino acid hinge region comprising a sequence of SEQ ID NO: 2 ($X_{n1}X_{n2}X_{n3}X_{n4}X_{n5}$ $X_{n6}CX_{n7}X_{n8}CX_{n9}X_{n10}C$), wherein X_{n1} is any amino acid that does not form a covalent crosslinking bond with another amino acid, wherein X_{n2} is any amino acid, wherein X_{n3} is any amino acid, wherein X_{n4} is any amino acid, wherein X_{n5} is any amino acid, wherein X_{n6} is any amino acid, wherein X_{n7} is any amino acid, wherein X_{n8} is any amino acid, wherein X_{n9} is any amino acid, and wherein X_{n10} is any amino acid (SEQ ID NO: 240).

[0237] In some embodiments, such constructs can be described as an amino acid hinge region comprising a sequence of SEQ ID NO: 2 ($X_{n1}X_{n2}X_{n3}X_{n4}X_{n5}$ $X_{n6}CX_{n7}X_{n8}CX_{n9}X_{n10}C$), wherein X_{n1} can be any amino acid, wherein X_{n2} can be any amino acid, wherein X_{n3} can be any amino acid, wherein X_{n4} can be any amino acid, wherein X_{n5} can be any amino acid, wherein X_{n6} can be any amino acid other than a cysteine, wherein X_{n7} can be any amino acid, wherein X_{n8} can be any amino acid, wherein X_{n9} can be any amino acid, and wherein X_{n10} can be any amino acid (SEQ ID NO: 241).

[0238] In some embodiments of SEQ ID NO: 2, X_{n1} is not a cysteine (SEQ ID NO: 213). In some embodiments of SEQ ID NO: 2, X_{n1} is a serine or alanine (SEQ ID NO: 242). In some embodiments of SEQ ID NO: 2, X_{n2} is not a cysteine (SEQ ID NO: 214). In some embodiments of SEQ ID NO: 2, X_{n2} is a D (SEQ ID NO: 215). In some embodiments, X_{n3} is a K (SEQ ID NO: 216). In some embodiments of SEQ ID NO: 2, X_{n4} is a T (SEQ ID NO: 217). In some embodiments of SEQ ID NO: 2, X_{n5} is a H (SEQ ID NO: 218). In some embodiments of SEQ ID NO: 2, X_{n6} is a T (SEQ ID NO: 219). In some embodiments of SEQ ID NO: 2, X_{n7} is a P or a V (SEQ ID NO: 220). In some embodiments of SEQ ID NO: 2, X_{n8} is a P or a E (SEQ ID NO: 221). In some embodiments of SEQ ID NO: 2, X_{n9} is a P or a V (SEQ ID NO: 222). In some embodiments of SEQ ID NO: 2, X_{n10} is a P or a E (SEQ ID NO: 223). In some embodiments of SEQ ID NO: 2, X_{n2} is a D, X_{n3} is a K, X_{n4} is a T, X_{n5} is a H, X_{n6} is a T, X_{n7} is a P or a V, X_{n8} is a P or a E, and X_{n9} is a P or a V (SEQ ID NO: 243).

[0239] In some embodiments, the amino acid hinge region further comprises a $X_{n11}X_{n12}C$ sequence. In some embodiments, this can be immediately attached to the C-terminal cysteine in SEQ ID NO: 1 (SEQ ID NO: 244) or SEQ ID NO: 2 (SEQ ID NO: 245). In some embodiments of SEQ ID NO: 2, X_{n11} can be any amino acid, and X_{n12} can be any amino acid (SEQ ID NO: 245). In some embodiments of SEQ ID NO: 2, X_{n11} is a P or a V, and wherein X_{n12} is a P or an E (SEQ ID NO: 246). In some embodiments of SEQ ID NO: 2, X_{n1} is a serine, X_{n2} is a D, X_{n3} is a K, X_{n4} is a T, X_{n5} is a H, X_{n6} is a T, X_{n7} is a P, X_{n8} is a P, X_{n9} is a P, and X_{n10} is a P (SEQ ID NO: 247).

[0240] In some embodiments, any of the hinge sequences can be preceded by any number of initial amino acids. Thus, additional linkers or spacers of amino acids can be added to the molecule before the noted sequence (such as SEQ ID NO: 1 and/or NO: 2). In

some embodiments, any of the hinge sequences can be followed by any number of additional amino acids. Thus, additional linkers or spacers of amino acids can be added to the molecule after the noted sequence (such as SEQ ID NO: 1 and/or NO: 2).

[0241] In some embodiments, the hinge region comprises at least one of the following sequences: CPPCPPC (SEQ ID NO: 52), CPPCVECPPC (SEQ ID NO: 53), or CPPCPPCPPC (SEQ ID NO: 54). In some embodiments, the hinge region comprises at least one of the following sequences: ERKSCVECPPCPGGGSSGGGSG (SEQ ID NO: 34), EPKSSDKTHTCPPCPPC (SEQ ID NO: 168), EPKSSDKTHTCPPCVECPPC (SEQ ID NO: 169), or EPKSSDKTHTCPPCPPCPPC (SEQ ID NO: 170). In some embodiments, the hinge region comprises at least one of the following sequences: EPKSSDKTHTCPPCPPCGGGSSGGGSG (SEQ ID NO: 26), EPKSSDKTHTCPPCVECPPCGGGSSGGGSG (SEQ ID NO: 28), or EPKSSDKTHTCPPCPPCPPCGGGSSGGGSG (SEQ ID NO: 30).

[0242] In some embodiments, the lower hinge region/sequence can be an extension sequence (e.g., 8-25 amino acids, such as 8-20, 10-25, 10-22, 12-20, 14-16, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 amino acids). Examples of the lower hinge are shown in Table 0.1 and Table 3. In some embodiments, the lower hinge can be a native lower hinge from γ1, γ2, γ3 or γ4. In some embodiments, one can have a native hinge sequence and add one or more spacer amino acids (for example, 1, 2, 3, 4 or more amino acids, such as G or S).

[0243] In some embodiments, an amino acid hinge region comprises a core hinge sequence of at least one of: CVECPPCP (SEQ ID NO: 57), CPPCPPC (SEQ ID NO: 52), CPPCPPCPPC (SEQ ID NO: 54), or CPPCVECPPC (SEQ ID NO: 53) linked to an upper hinge sequence of ELKTPLGDTTHT (SEQ ID NO: 48) or EPKSSDKTHT (SEQ ID NO: 46).

[0244] In some embodiments, any of the embodiments described herein can be modified or supplemented by any one or more of the following options.

[0245] In some embodiments, the hinge region comprises at least three cysteines (on each chain, for a total of at least 6 cysteines between the two strands in the dimeric hinge construct). In some embodiments, the hinge region comprises at least four cysteines (on each strand). In some embodiments, the hinge region comprises at least five cysteines (on each

strand). In some embodiments, 6, 7, 8, 9, 10 or more cysteines are present (on each strand). In some embodiments, the hinge region as a whole has the above noted number of cysteines (on each strand and/or in the dimeric construct). In some embodiments, all of the noted cysteines are present in the core hinge region. In some embodiments, all of the noted cysteines are present in the core hinge region and there are no additional cysteines present in the upper hinge region and/or the lower hinge region. In some embodiments, the cysteines are distributed throughout the amino acid hinge region in a repeating "CXX" motif (such as CXXCXXCXX (SEQ ID NO: 171) or CXXCXX (SEQ ID NO: 172), or CXXCXXCXXCXX (SEQ ID NO: 173). In some embodiments, the cysteines are distributed throughout the core hinge region in a repeating CXX motif. In some embodiments, the motif repeats 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more times. In some embodiments, each X can be either a P, V, or E (SEQ ID NO: 171, SEQ ID NO: 172, SEQ ID NO: 173). In some embodiments, X is not a cysteine (SEQ ID NO: 248, SEQ ID NO: 249, SEQ ID NO: 250).

[0246] In some embodiments, the core hinge region comprises at least three cysteines per strand forming at least three disulfide bonds within the core hinge region. In some embodiments, the constructs express much better in mammalian cells and give higher titers as well as lower aggregation and more uniform product. In some embodiments, each of the cysteines present in the core hinge region can form a disulfide bond with a corresponding cysteine on its paired chain in the hinge. In some embodiments, the disulfide bond is between corresponding cysteines on two separate protein chains, each being a hinge region. In some embodiments, at least 3 disulfide bonds are formed, for example, 3, 4, 5, 6, 7, 8, 9, 10 or more. In some embodiments, at least 3 disulfide bonds are capable of being formed, for example, 3, 4, 5, 6, 7, 8, 9, 10 or more disulfide bonds can be formed at the same time within the structure.

[0247] In some embodiments, the first residue of the core hinge region is a serine. In some embodiments, the core hinge region comprises SCVECPPCP (SEQ ID NO: 56).

[0248] In some embodiments, the C_H3 domain for any construct can be from γ1, γ2, or γ3 and any naturally occurring allele thereof. As used herein "γ" is an abbreviation for gamma.

[0249] In some embodiments, the extension or lower hinge region comprises at least one of S, G, A, P, or V. In some embodiments, the extension sequence comprises at

least GGGSSGGGSG (SEQ ID NO: 59). In some embodiments, the extension or lower hinge region comprises at least APPVAGP (SEQ ID NO: 60). In some embodiments, the lower hinge region comprises at least one of: GGGSSGGGSG (SEQ ID NO: 59) or APPVAGP (SEQ ID NO: 60). In some embodiments, the lower hinge sequence can be a GS linker, extension sequence, and/or any native lower hinge region from γ1, γ2, γ3 and γ4.

Properties

[0250] In some embodiments, the hinges provided herein can allow for a lower percentage of the half-molecule to form and/or be present in any minibody or other antigen binding construct composition. For example, less can 7% of the composition can be the half molecule, including ranges such as less than 6, 5, 4, 3, 2, 1, and/or 0.5% of composition being the half-molecule.

[0251] In some embodiments when the hinge is located within a minibody, and when the minibody is administered to a human subject, clearance of the minibody from the subject may occur through liver and/or kidney. In some embodiments, clearance through the liver is at least 10% or more, for example 10, 20, 30, 40, 50, 60, 70, 80, 90, 95, 99, or 100%. In some embodiments, when located within a minibody, and when the minibody is administered to a human subject, clearance of the minibody from the subject may occur primarily through the kidneys. In some embodiments, at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 fold or more of the antibody is cleared through the kidney.

[0252] In some embodiments, less than 30% aggregation of an minibody is present in an minibody composition. In some embodiments, less than 20% aggregation of an minibody is present in an minibody composition. In some embodiments, less than 10% aggregation of an minibody is present in an minibody composition. In some embodiments, less than 5% aggregation of an minibody is present in an minibody composition. In some embodiments, less than 1% aggregation of a minibody is present in an minibody composition.

[0253] When the minibody remains as a dimer in circulation *in vivo*, then protein clearance is predicted to occur through the liver primarily. In contrast, when the half-molecule is present, then its clearance will be through the kidney. Thus, in some embodiments, the above clearance aspects are a way of characterizing the amount of

resulting half-molecule present in a subject, *in vivo*, allowing one to determine if the administered whole molecule remains as a whole molecule *in vivo*, or if it breaks down to the half molecule. In some embodiments, the minibody composition is one in which clearance through the liver is relatively high compared to clearance through the kidney, and tumor uptake of the minibody is relatively high. In some embodiments, the ratio of kidney to liver is less than 1. In some embodiments, at least 1% more uptake occurs through the liver than then kidney, for example, at least 1, 10, 20, 30, 40, 50, 60, 70 80, 90, 100, or more uptake occurs through the liver than through the kidney. In some embodiments, uptake of kidney to liver is 0.9:1, 1:10, 1:100, 1:000, 1:10,000, etc. In some embodiments, the various hinge constructs provided herein have lower clearance through the kidneys than the EH1 hinge from IgG1.

[0254] In some embodiments, mutation of the first hinge cysteine to serine in IgG1 hinge region (γ1 EH2) to prevent undesired interaction with other unpaired cysteine generated a protein with a better profile *in vitro*. However, the high clearance through the kidneys, compared to the liver, may be indicative of protein instability and dissociation into half molecules *in vivo*. In some embodiments, minibodies having an IgG2 hinge are provided wherein the first hinge cysteine is mutated to a serine (γ2 EH2 and others), have the property of being cleared through the liver as predicted for proteins of approx. 80 kDa. This indicates that the molecule remains intact *in vivo*. In some embodiments, the molecule remains intact for 48 hours or longer.

[0255] In some embodiments, the hinge construct provided herein have less than 9% half molecules present due to an unpaired first hinge cysteine. In some embodiments, an IgG2 hinge with a first cysteine to serine has greater than 99% intact dimer protein present, such as that with γ2 EH2. In some embodiments, a γ2 hinge variant results in lower kidney uptake. In some embodiments, a Mb made with an huIgG1 hinge (γ1 EH1) is rapidly cleared through the kidney resulting in low tumor targeting. In some embodiments, a Mb made with huIgG2 hinge variants, either Extended Hinge (γ2 EH2) or Natural Hinge (γ2 NH1) shows greater stability *in vivo* with high tumor targeting and lower kidney clearance.

[0256] In some embodiments, γ1 minibodies with EH2 hinge assemble properly into intact dimeric molecules but inclusion of only 2 cysteine yields protein with a high amount of half molecule. In some embodiments, γ1 minibodies with EH3 hinge assemble

properly into intact dimeric molecules and addition of 3rd cysteine per strand yield protein with very low levels of half molecule. In some embodiments, the level of half molecule is less than 15%, for example less than 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or less than 1% of half molecule is present.

[0257] In some embodiments, even though the hinge region has been varied and is different from that described in the past, the CDRs, V_H/V_Ls or full amino acid sequences of the minibodies still bind to the target molecule with the same affinity as an antibody (such as a minibody) with the previous style hinge. Thus, in some embodiments, the presence of a hinge as provided herein does not alter or negatively impact the binding affinity of the antigen binding construct, while still providing one or more of the benefits and/or structures provided herein.

[0258] In some embodiments, a minibody comprising a sequence $X_{n1}CX_{n2}X_{n3}CX_{n4}X_{n5}C$ (SEQ ID NO: 3) is provided. In some embodiments, SEQ ID NO: 3 is located as the core hinge region of the minibody. In some embodiments, X_{n1} can be any amino acid or no amino acid, X_{n2} can be any amino acid, X_{n3} can be any amino acid, X_{n4} can be any amino acid, and X_{n5} can be any amino acid. In some embodiments, X_{n1} cannot be a cysteine, X_{n2} cannot be a cysteine, X_{n3} cannot be a cysteine, X_{n4} cannot be a cysteine, and/or X_{n5} cannot be a cysteine (SEQ ID NO: 251). In some embodiments, X_{n1} is any amino acid other than a cysteine (SEQ ID NO: 229). In some embodiments, X_{n1} is a serine (SEQ ID NO: 230).

[0259] In some embodiments, a minibody comprising a sequence $X_{n1}CX_{n2}X_{n3}CX_{n4}X_{n5}CX_{n6}$ (SEQ ID NO: 4) is provided. In some embodiments, SEQ ID NO: 4 is located as the core hinge region of the minibody. In some embodiments, X_{n1} can be any amino acid or no amino acid, X_{n2} can be any amino acid, X_{n3} can be any amino acid, X_{n4} can be any amino acid, and X_{n5} can be any amino acid (SEQ ID NO: 252). In some embodiments, X_{n1} cannot be a cysteine, X_{n2} cannot be a cysteine, X_{n3} cannot be a cysteine, X_{n4} cannot be a cysteine, and/or X_{n5} cannot be a cysteine (SEQ ID NO: 253). In some embodiments, X_{n1} is any amino acid other than a cysteine (SEQ ID NO: 254). In some embodiments, X_{n1} is a serine (SEQ ID NO: 255). In some embodiments, X_{n6} can be any amino acid, no amino acid, or P (SEQ ID NO: 256).

[0260] In some embodiments, any of the amino acid hinge regions or full hinges provided herein is within an antibody. In some embodiments, any of the amino acid hinge regions or full hinges provided herein is within an antibody binding fragment. In some embodiments, any of the amino acid hinge regions or full hinges provided herein is within a minibody.

[0261] In some embodiments, any of the amino acid hinge regions or full hinges provided herein is within a monospecific antibody.

[0262] In some embodiments, any of the amino acid hinge regions or full hinges provided herein is within a bispecific antibody. In some embodiments, any of the amino acid hinge regions or full hinges provided herein is assembled in a 1:1 ratio. In some embodiments, any of the amino acid hinge regions or full hinges provided herein is part of an antibody fragment that is a bispecific construct. In some embodiments, the bispecific antibody is a minibody.

Specific Antigen Binding Constructs

[0263] Antigen binding constructs that bind to the target are described herein. An antigen binding construct is a molecule that includes one or more portions of an immunoglobulin or immunoglobulin-related molecule that specifically binds to, or is immunologically reactive with the target molecule. In some embodiments, any of the hinge embodiments provided herein can be applied to any desired antigen binding construct. In some embodiments, any of the hinge embodiments provided herein can be applied to a minibody. In some embodiments, any of the hinge embodiments provided herein can be applied to a minibody that binds to CD8, CD3, 5T4, PSCA, or PSMA. A non-limiting embodiment of the PSMA antigen is shown in FIG. 70. A non-limiting embodiment of the PSCA antigen is shown in FIG. 71. A non-limiting embodiment of the 5T4 antigen is shown in FIG. 72. Some non-limiting embodiments of the CD8 antigen are shown in FIGs. 73 and 74. Some non-limiting embodiments of the CD3 antigen are shown in FIGs. 75 – 78. In some embodiments, any of the hinge embodiments provided herein can be applied to a minibody that specifically and/or selectively binds to CD8, CD3, 5T4, PSCA, or PSMA. In some embodiments, any of the hinge embodiments provided in Tables 0.1 or 3 can be applied to a minibody that binds to CD8, CD3, 5T4, PSCA, or PSMA. In some embodiments, any of the full hinge embodiments can be applied to a minibody that binds to CD8, CD3, 5T4,

PSCA, or PSMA. In some embodiments, any of the upper hinge embodiments can be applied to a minibody that binds to CD8, CD3, 5T4, PSCA, or PSMA. In some embodiments, any of the core hinge embodiments can be applied to a minibody that binds to CD8, CD3, 5T4, PSCA, or PSMA. In some embodiments, any of the lower hinge embodiments can be applied to a minibody that binds to CD8, CD3, 5T4, PSCA, or PSMA. In some embodiments, any of the upper and core hinge embodiments can be applied to a minibody that binds to CD8, CD3, 5T4, PSCA, or PSMA. In some embodiments, any of the core and lower hinge embodiments can be applied to a minibody that binds to CD8, CD3, 5T4, PSCA, or PSMA. In some embodiments, any of the upper and lower hinge embodiments can be applied to a minibody that binds to CD8, CD3, 5T4, PSCA, or PSMA.

[0264] Additional antibody fragments, such as minibodies are provided below. The antibody fragments can be used, for example, for imaging or therapeutic purposes. Schematic representations of exemplary minibodies are illustrated in the Figures and/or outlined in Table 0.2 below (in either the M1 orientation or the M2 orientation).

TABLE 0.2

Minibodies (N-terminus to C-terminus) – M1 structural orientation

1	2	3	4	5	6	7	8	9
Name	**Signal peptide**	**Region 1**	**Linker**	**Region 2**	**Full hinge**			**Remainder**
					Upper hinge	**Core hinge**	**Lower hinge**	
IAB2M γ1 EH1	(SEQ ID NO: 69)	V_L (SEQ ID NO: 13)	18aa (SEQ ID NO: 62)	V_H (SEQ ID NO: 14)	(SEQ ID NO: 22)			IgG1 CH3 domain (SEQ ID NO: 40 or SEQ ID NO: 41)
					(SEQ ID NO: 45)	(SEQ ID NO: 50)	(SEQ ID NO: 59)	
IAB2M γ1 EH2	(SEQ ID NO: 69)	V_L (SEQ ID NO: 13)	18aa (SEQ ID NO: 62)	V_H (SEQ ID NO: 14)	(SEQ ID NO: 24)			IgG1 CH3 domain (SEQ ID NO: 40 or SEQ ID NO: 41)
					(SEQ ID NO: 46)	(SEQ ID NO: 50)	(SEQ ID NO: 59)	
IAB2M γ 2 EH1	(SEQ ID NO: 69)	V_L (SEQ ID NO: 13)	18aa (SEQ ID NO: 62)	V_H (SEQ ID NO: 14)	(SEQ ID NO: 32)			IgG2 CH3 domain (SEQ ID NO: 42)
					(SEQ ID NO: 47)	(SEQ ID NO: 55)	(SEQ ID NO: 59)	
IAB2M γ	(SEQ	V_L	18aa	V_H	(SEQ ID NO: 34)			IgG2 CH3

2 EH2	ID NO: 69)	(SEQ ID NO: 13)	(SEQ ID NO: 62)	(SEQ ID NO: 14)	(SEQ ID NO: 47)	(SEQ ID NO: 56)	(SEQ ID NO: 59)	domain (SEQ ID NO: 42)
IAB2M γ1 EH3	(SEQ ID NO: 69)	V_L (SEQ ID NO: 13)	18aa (SEQ ID NO: 62)	V_H (SEQ ID NO: 14)	(SEQ ID NO: 26)			IgG1 CH3 domain (SEQ ID NO: 40 or SEQ ID NO: 41)
					(SEQ ID NO: 46)	(SEQ ID NO: 52)	(SEQ ID NO: 59)	
IAB22M γ2 EH1	(SEQ ID NO: 69)	V_L (SEQ ID NO: 15)	18aa (SEQ ID NO: 62)	V_H (SEQ ID NO: 16)	(SEQ ID NO: 32)			IgG2 CH3 domain (SEQ ID NO: 42)
					(SEQ ID NO: 47)	(SEQ ID NO: 55)	(SEQ ID NO: 59)	
IAB22M 1 γ2 EH2	(SEQ ID NO: 69)	V_L (SEQ ID NO: 15)	18aa (SEQ ID NO: 62)	V_H (SEQ ID NO: 16)	(SEQ ID NO: 34)			IgG2 CH3 domain (SEQ ID NO: 42)
					(SEQ ID NO: 47)	(SEQ ID NO: 56)	(SEQ ID NO: 59)	
IAB22M γ1 EH1	(SEQ ID NO: 69)	V_L (SEQ ID NO: 15)	18aa (SEQ ID NO: 62)	V_H (SEQ ID NO: 16)	(SEQ ID NO: 22)			IgG1 CH3 domain (SEQ ID NO: 40 or SEQ ID NO: 41)
					(SEQ ID NO: 45)	(SEQ ID NO: 50)	(SEQ ID NO: 59)	
IAB22M γ2 NH1	(SEQ ID NO: 69)	V_L (SEQ ID NO: 15)	18aa (SEQ ID NO: 62)	V_H (SEQ ID NO: 16)	(SEQ ID NO: 31)			IgG2 CH3 domain (SEQ ID NO: 42)
					(SEQ ID NO: 47)	(SEQ ID NO: 55)	(SEQ ID NO: 60)	
IAB22M γ2 NH2	(SEQ ID NO: 69)	V_L (SEQ ID NO: 15)	18aa (SEQ ID NO: 62)	V_H (SEQ ID NO: 16)	(SEQ ID NO: 33)			IgG2 CH3 domain (SEQ ID NO: 42)
					(SEQ ID NO: 47)	(SEQ ID NO: 56)	(SEQ ID NO: 60)	
IAB22M γ1 EH3	(SEQ ID NO: 69)	V_L (SEQ ID NO: 15)	18aa (SEQ ID NO: 62)	V_H (SEQ ID NO: 16)	(SEQ ID NO: 26)			IgG1 CH3 domain (SEQ ID NO: 40 or SEQ ID NO: 41)
					(SEQ ID NO: 46)	(SEQ ID NO: 52)	(SEQ ID NO: 59)	
IAB22M γ1 EH5	(SEQ ID NO: 69)	V_L (SEQ ID NO: 15)	18aa (SEQ ID NO: 62)	V_H (SEQ ID NO: 16)	(SEQ ID NO: 30)			IgG1 CH3 domain (SEQ ID NO: 40 or SEQ ID NO: 41)
					(SEQ ID NO: 46)	(SEQ ID NO: 54)	(SEQ ID NO: 59)	
IAB22M γ3/γ1 EH6	(SEQ ID NO: 69)	V_L (SEQ ID NO: 15)	18aa (SEQ ID NO: 62)	V_H (SEQ ID NO: 16)	(SEQ ID NO: 35)			IgG1 CH3 domain (SEQ ID NO: 40 or SEQ ID NO: 41)
					(SEQ ID NO: 48)	(SEQ ID NO: 57)	(SEQ ID NO: 59)	
IAB22M γ3/γ1 EH7	(SEQ ID NO: 69)	V_L (SEQ ID NO: 15)	18aa (SEQ ID NO: 62)	V_H (SEQ ID NO: 16)	(SEQ ID NO: 36)			IgG1 CH3 domain (SEQ ID NO: 40 or SEQ ID NO: 41)
					(SEQ ID NO: 48)	(SEQ ID NO: 52)	(SEQ ID NO: 59)	
IAB22M γ3/γ1 EH8	(SEQ ID NO: 69)	V_L (SEQ ID NO: 15)	18aa (SEQ ID NO: 62)	V_H (SEQ ID NO: 16)	(SEQ ID NO: 37)			IgG1 CH3 domain (SEQ ID NO:
					(SEQ	(SEQ	(SEQ	

					ID NO: 48)	ID NO: 54)	ID NO: 59)	40 or SEQ ID NO: 41)
IAB2M γ 1 EH5	(SEQ ID NO: 69)	V_L (SEQ ID NO: 13)	18aa (SEQ ID NO: 62)	V_H (SEQ ID NO: 14)	(SEQ ID NO: 30)			IgG1 CH3 domain (SEQ ID NO: 40 or SEQ ID NO: 41)
					(SEQ ID NO: 46)	(SEQ ID NO: 54)	(SEQ ID NO: 59)	
IAB2M γ3/γ1 EH6	(SEQ ID NO: 69)	V_L (SEQ ID NO: 13)	18aa (SEQ ID NO: 62)	V_H (SEQ ID NO: 14)	(SEQ ID NO: 35)			IgG1 CH3 domain (SEQ ID NO: 40 or SEQ ID NO: 41)
					(SEQ ID NO: 48)	(SEQ ID NO: 57)	(SEQ ID NO: 59)	
IAB2M γ 3/γ1 EH7	(SEQ ID NO: 69)	V_L (SEQ ID NO: 13)	18aa (SEQ ID NO: 62)	V_H (SEQ ID NO: 14)	(SEQ ID NO: 36)			IgG1 CH3 domain (SEQ ID NO: 40 or SEQ ID NO: 41)
					(SEQ ID NO: 48)	(SEQ ID NO: 52)	(SEQ ID NO: 59)	
IAB2M-γ3/γ1 EH8	(SEQ ID NO: 69)	V_L (SEQ ID NO: 13)	18aa (SEQ ID NO: 62)	V_H (SEQ ID NO: 14)	(SEQ ID NO: 37)			IgG1 CH3 domain (SEQ ID NO: 40 or SEQ ID NO: 41)
					(SEQ ID NO: 48)	(SEQ ID NO: 54)	(SEQ ID NO: 59)	
IAB22M γ1 EH2	(SEQ ID NO: 69)	V_L (SEQ ID NO: 15)	18aa (SEQ ID NO: 62)	V_H (SEQ ID NO: 16)	(SEQ ID NO: 24)			IgG1 CH3 domain (SEQ ID NO: 40 or SEQ ID NO: 41)
					(SEQ ID NO: 46)	(SEQ ID NO: 50)	(SEQ ID NO: 59)	
IAB20M γ1 EH1	(SEQ ID NO: 69)	V_L (SEQ ID NO: 13)	18aa (SEQ ID NO: 62)	V_H (SEQ ID NO: 14)	(SEQ ID NO: 22)			IgG1 CH3 domain (SEQ ID NO: 40 or SEQ ID NO: 41)
					(SEQ ID NO: 45)	(SEQ ID NO: 50)	(SEQ ID NO: 59)	
IAB20M γ1 EH3	(SEQ ID NO: 69)	V_L (SEQ ID NO: 17)	18aa (SEQ ID NO: 62)	V_H (SEQ ID NO: 18)	(SEQ ID NO: 26)			IgG1 CH3 domain (SEQ ID NO: 40 or SEQ ID NO: 41)
					(SEQ ID NO: 46)	(SEQ ID NO: 52)	(SEQ ID NO: 59)	
IAB20M γ1 EH2	(SEQ ID NO: 69)	V_L (SEQ ID NO: 17)	18aa (SEQ ID NO: 62)	V_H (SEQ ID NO: 18)	(SEQ ID NO: 24)			IgG1 CH3 domain (SEQ ID NO: 40 or SEQ ID NO: 41)
					(SEQ ID NO: 46)	(SEQ ID NO: 50)	(SEQ ID NO: 59)	
IAB20M γ1 EH5	(SEQ ID NO: 69)	V_L (SEQ ID NO: 17)	18aa (SEQ ID NO: 62)	V_H (SEQ ID NO: 18)	(SEQ ID NO: 30)			IgG1 CH3 domain (SEQ ID NO: 40 or SEQ ID NO: 41)
					(SEQ ID NO: 46)	(SEQ ID NO: 54)	(SEQ ID NO: 59)	
IAB1M γ 1 EH1	(SEQ ID NO: 69)	V_L (SEQ ID NO: 67)	18aa (SEQ ID NO: 62)	V_H (SEQ ID NO: 68)	(SEQ ID NO: 22)			IgG1 CH3 domain (SEQ ID NO: 40 or SEQ ID NO: 41)
					(SEQ ID NO: 45)	(SEQ ID NO: 50)	(SEQ ID NO: 59)	
IAB1M γ	(SEQ	V_L	18aa	V_H	(SEQ ID NO: 24)			IgG1 CH3

1 EH2	ID NO: 69)	(SEQ ID NO: 67)	(SEQ ID NO: 62)	(SEQ ID NO: 68)	(SEQ ID NO: 46)	(SEQ ID NO: 50)	(SEQ ID NO: 59)	domain (SEQ ID NO: 40 or SEQ ID NO: 41)
IAB1M γ1 EH3	(SEQ ID NO: 69)	V_L (SEQ ID NO: 67)	18aa (SEQ ID NO: 62)	V_H (SEQ ID NO: 68)	(SEQ ID NO: 26)			IgG1 CH3 domain (SEQ ID NO: 40 or SEQ ID NO: 41)
					(SEQ ID NO: 46)	(SEQ ID NO: 52)	(SEQ ID NO: 59)	
IAB20M γ2 EH2	(SEQ ID NO: 69)	V_L (SEQ ID NO: 17)	18aa (SEQ ID NO: 62)	V_H (SEQ ID NO: 18)	(SEQ ID NO: 34)			IgG2 CH3 domain (SEQ ID NO: 42)
					(SEQ ID NO: 47)	(SEQ ID NO: 56)	(SEQ ID NO: 59)	
IAB20M γ2 EH2	(SEQ ID NO: 69)	V_L (SEQ ID NO: 17)	18aa (SEQ ID NO: 62)	V_H (SEQ ID NO: 18)	(SEQ ID NO: 34)			IgG2 CH3 T26S, L28A, M57V domain (SEQ ID NO: 181)
					(SEQ ID NO: 47)	(SEQ ID NO: 56)	(SEQ ID NO: 59)	
IAB20M γ2 EH2	(SEQ ID NO: 69)	V_L (SEQ ID NO: 17)	18aa (SEQ ID NO: 62)	V_H (SEQ ID NO: 18)	(SEQ ID NO: 34)			IgG2 CH3 T26W domain (SEQ ID NO: 182)
					(SEQ ID NO: 47)	(SEQ ID NO: 56)	(SEQ ID NO: 59)	
IAB25M γ2 EH2	(SEQ ID NO: 69)	V_L (SEQ ID NO: 19)	18aa (SEQ ID NO: 62)	V_H (SEQ ID NO: 20)	(SEQ ID NO: 34)			IgG2 CH3 domain (SEQ ID NO: 42)
					(SEQ ID NO: 47)	(SEQ ID NO: 56)	(SEQ ID NO: 59)	
IAB25M γ2 EH2	(SEQ ID NO: 69)	V_L (SEQ ID NO: 19)	18aa (SEQ ID NO: 62)	V_H (SEQ ID NO: 20)	(SEQ ID NO: 34)			IgG2 CH3 T26S, L28A, M57V domain (SEQ ID NO: 181)
					(SEQ ID NO: 47)	(SEQ ID NO: 56)	(SEQ ID NO: 59)	
IAB25M γ2 EH2	(SEQ ID NO: 69)	V_L (SEQ ID NO: 19)	18aa (SEQ ID NO: 62)	V_H (SEQ ID NO: 20)	(SEQ ID NO: 34)			IgG2 CH3 T26W domain (SEQ ID NO: 182)
					(SEQ ID NO: 47)	(SEQ ID NO: 56)	(SEQ ID NO: 59)	

TABLE 0.2 CONTINUED

Minibodies (N- to C-terminal) – M2 structural orientation

1	2	3	4	5	6	7	8	9
Name	**Signal peptide**	**Region 1**	**Linker**	**Region 2**	**Full hinge**			**Remainder**
					Upper hinge	**Core hinge**	**Lower hinge**	
IAB2M γ1 EH1	(SEQ ID NO: 69)	V_H (SEQ ID NO: 14)	18aa (SEQ ID NO: 62)	V_L (SEQ ID NO: 13)	(SEQ ID NO: 22)			IgG1 CH3 domain (SEQ ID NO: 40 or SEQ ID NO: 41)
					(SEQ ID NO: 45)	(SEQ ID NO: 50)	(SEQ ID NO: 59)	
IAB2M γ1 EH2	(SEQ ID NO: 69)	V_H (SEQ ID NO: 14)	18aa (SEQ ID NO: 62)	V_L (SEQ ID NO: 13)	(SEQ ID NO: 24)			IgG1 CH3 domain (SEQ ID NO: 40 or SEQ ID NO: 41)
					(SEQ ID NO: 46)	(SEQ ID NO: 50)	(SEQ ID NO: 59)	
IAB2M γ2 EH1	(SEQ ID NO: 69)	V_H (SEQ ID NO: 14)	18aa (SEQ ID NO: 62)	V_L (SEQ ID NO: 13)	(SEQ ID NO: 32)			IgG2 CH3 domain (SEQ ID NO: 42)
					(SEQ ID NO: 47)	(SEQ ID NO: 55)	(SEQ ID NO: 59)	
IAB2M γ2 EH2	(SEQ ID NO: 69)	V_H (SEQ ID NO: 14)	18aa (SEQ ID NO: 62)	V_L (SEQ ID NO: 13)	(SEQ ID NO: 34)			IgG2 CH3 domain (SEQ ID NO: 42)
					(SEQ ID NO: 47)	(SEQ ID NO: 56)	(SEQ ID NO: 59)	
IAB2M γ1 EH3	(SEQ ID NO: 69)	V_H (SEQ ID NO: 14)	18aa (SEQ ID NO: 62)	V_L (SEQ ID NO: 13)	(SEQ ID NO: 26)			IgG1 CH3 domain (SEQ ID NO: 40 or SEQ ID NO: 41)
					(SEQ ID NO: 46)	(SEQ ID NO: 52)	(SEQ ID NO: 59)	
IAB22M γ2 EH1	(SEQ ID NO: 69)	V_H (SEQ ID NO: 16)	18aa (SEQ ID NO: 62)	V_L (SEQ ID NO: 15)	(SEQ ID NO: 32)			IgG2 CH3 domain (SEQ ID NO: 42)
					(SEQ ID NO: 47)	(SEQ ID NO: 55)	(SEQ ID NO: 59)	
IAB22M1 γ2 EH2	(SEQ ID NO: 69)	V_H (SEQ ID NO: 16)	18aa (SEQ ID NO: 62)	V_L (SEQ ID NO: 15)	(SEQ ID NO: 34)			IgG2 CH3 domain (SEQ ID NO: 42)
					(SEQ ID NO: 47)	(SEQ ID NO: 56)	(SEQ ID NO: 59)	
IAB22M γ1 EH1	(SEQ ID NO: 69)	V_H (SEQ ID NO: 16)	18aa (SEQ ID NO: 62)	V_L (SEQ ID NO: 15)	(SEQ ID NO: 22)			IgG1 CH3 domain (SEQ ID NO: 40 or SEQ ID NO: 41)
					(SEQ ID NO: 45)	(SEQ ID NO: 50)	(SEQ ID NO: 59)	
IAB22M γ2 NH1	(SEQ ID NO: 69)	V_H (SEQ ID NO: 16)	18aa (SEQ ID NO: 62)	V_L (SEQ ID NO: 15)	(SEQ ID NO: 31)			IgG2 CH3 domain (SEQ ID NO: 42)
					(SEQ ID NO: 47)	(SEQ ID NO: 55)	(SEQ ID NO: 60)	

IAB22M γ2 NH2	(SEQ ID NO: 69)	V_H (SEQ ID NO: 16)	18aa (SEQ ID NO: 62)	V_L (SEQ ID NO: 15)	(SEQ ID NO: 33)			IgG2 CH3 domain (SEQ ID NO: 42)
					(SEQ ID NO: 47)	(SEQ ID NO: 56)	(SEQ ID NO: 60)	
IAB22M γ1 EH3	(SEQ ID NO: 69)	V_H (SEQ ID NO: 16)	18aa (SEQ ID NO: 62)	V_L (SEQ ID NO: 15)	(SEQ ID NO: 26)			IgG1 CH3 domain (SEQ ID NO: 40 or SEQ ID NO: 41)
					(SEQ ID NO: 46)	(SEQ ID NO: 52)	(SEQ ID NO: 59)	
IAB22M γ1 EH5	(SEQ ID NO: 69)	V_H (SEQ ID NO: 16)	18aa (SEQ ID NO: 62)	V_L (SEQ ID NO: 15)	(SEQ ID NO: 30)			IgG1 CH3 domain (SEQ ID NO: 40 or SEQ ID NO: 41)
					(SEQ ID NO: 46)	(SEQ ID NO: 54)	(SEQ ID NO: 59)	
IAB22M γ3/ γ1 EH6	(SEQ ID NO: 69)	V_H (SEQ ID NO: 16)	18aa (SEQ ID NO: 62)	V_L (SEQ ID NO: 15)	(SEQ ID NO: 35)			IgG1 CH3 domain (SEQ ID NO: 40 or SEQ ID NO: 41)
					(SEQ ID NO: 48)	(SEQ ID NO: 57)	(SEQ ID NO: 59)	
IAB22M γ3/ γ1 EH7	(SEQ ID NO: 69)	V_H (SEQ ID NO: 16)	18aa (SEQ ID NO: 62)	V_L (SEQ ID NO: 15)	(SEQ ID NO: 36)			IgG1 CH3 domain (SEQ ID NO: 40 or SEQ ID NO: 41)
					(SEQ ID NO: 48)	(SEQ ID NO: 52)	(SEQ ID NO: 59)	
IAB22M γ3/ γ1 EH8	(SEQ ID NO: 69)	V_H (SEQ ID NO: 16)	18aa (SEQ ID NO: 62)	V_L (SEQ ID NO: 15)	(SEQ ID NO: 37)			IgG1 CH3 domain (SEQ ID NO: 40 or SEQ ID NO: 41)
					(SEQ ID NO: 48)	(SEQ ID NO: 54)	(SEQ ID NO: 59)	
IAB2M γ1 EH5	(SEQ ID NO: 69)	V_H (SEQ ID NO: 14)	18aa (SEQ ID NO: 62)	V_L (SEQ ID NO: 13)	(SEQ ID NO: 30)			IgG1 CH3 domain (SEQ ID NO: 40 or SEQ ID NO: 41)
					(SEQ ID NO: 46)	(SEQ ID NO: 54)	(SEQ ID NO: 59)	
IAB2M γ3/γ 1 EH6	(SEQ ID NO: 69)	V_H (SEQ ID NO: 14)	18aa (SEQ ID NO: 62)	V_L (SEQ ID NO: 13)	(SEQ ID NO: 35)			IgG1 CH3 domain (SEQ ID NO: 40 or SEQ ID NO: 41)
					(SEQ ID NO: 48)	(SEQ ID NO: 57)	(SEQ ID NO: 59)	
IAB2M γ3/γ 1 EH7	(SEQ ID NO: 69)	V_H (SEQ ID NO: 14)	18aa (SEQ ID NO: 62)	V_L (SEQ ID NO: 13)	(SEQ ID NO: 36)			IgG1 CH3 domain (SEQ ID NO: 40 or SEQ ID NO: 41)
					(SEQ ID NO: 48)	(SEQ ID NO: 52)	(SEQ ID NO: 59)	
IAB2M γ3/γ 1 EH8	(SEQ ID NO: 69)	V_H (SEQ ID NO: 14)	18aa (SEQ ID NO: 62)	V_L (SEQ ID NO: 13)	(SEQ ID NO: 37)			IgG1 CH3 domain (SEQ ID NO: 40 or SEQ ID NO: 41)
					(SEQ ID NO: 48)	(SEQ ID NO: 54)	(SEQ ID NO: 59)	
IAB22M γ1 EH2	(SEQ ID NO: 69)	V_H (SEQ ID NO: 16)	18aa (SEQ ID NO: 62)	V_L (SEQ ID NO: 15)	(SEQ ID NO: 24)			IgG1 CH3 domain (SEQ ID NO: 40 or SEQ ID NO: 41)
					(SEQ ID NO: 46)	(SEQ ID NO: 50)	(SEQ ID NO: 59)	

IAB20M γ1 EH1	(SEQ ID NO: 69)	V_H (SEQ ID NO: 14)	18aa (SEQ ID NO: 62)	V_L (SEQ ID NO: 13)	(SEQ ID NO: 22)			IgG1 CH3 domain (SEQ ID NO: 40 or SEQ ID NO: 41)
					(SEQ ID NO: 45)	(SEQ ID NO: 50)	(SEQ ID NO: 59)	
IAB20M γ1 EH3	(SEQ ID NO: 69)	V_H (SEQ ID NO: 18)	18aa (SEQ ID NO: 62)	V_L (SEQ ID NO: 17)	(SEQ ID NO: 26)			IgG1 CH3 domain (SEQ ID NO: 40 or SEQ ID NO: 41)
					(SEQ ID NO: 46)	(SEQ ID NO: 52)	(SEQ ID NO: 59)	
IAB20M γ1 EH2	(SEQ ID NO: 69)	V_H (SEQ ID NO: 18)	18aa (SEQ ID NO: 62)	V_L (SEQ ID NO: 17)	(SEQ ID NO: 24)			IgG1 CH3 domain (SEQ ID NO: 40 or SEQ ID NO: 41)
					(SEQ ID NO: 46)	(SEQ ID NO: 50)	(SEQ ID NO: 59)	
IAB20M γ1 EH5	(SEQ ID NO: 69)	V_H (SEQ ID NO: 18)	18aa (SEQ ID NO: 62)	V_L (SEQ ID NO: 17)	(SEQ ID NO: 30)			IgG1 CH3 domain (SEQ ID NO: 40 or SEQ ID NO: 41)
					(SEQ ID NO: 46)	(SEQ ID NO: 54)	(SEQ ID NO: 59)	
IAB1M γ1 EH1	(SEQ ID NO: 69)	V_H (SEQ ID NO: 68)	18aa (SEQ ID NO: 62)	V_L (SEQ ID NO: 67)	(SEQ ID NO: 22)			IgG1 CH3 domain (SEQ ID NO: 40 or SEQ ID NO: 41)
					(SEQ ID NO: 45)	(SEQ ID NO: 50)	(SEQ ID NO: 59)	
IAB1M γ1 EH2	(SEQ ID NO: 69)	V_H (SEQ ID NO: 68)	18aa (SEQ ID NO: 62)	V_L (SEQ ID NO: 67)	(SEQ ID NO: 24)			IgG1 CH3 domain (SEQ ID NO: 40 or SEQ ID NO: 41)
					(SEQ ID NO: 46)	(SEQ ID NO: 50)	(SEQ ID NO: 59)	
IAB1M γ1 EH3	(SEQ ID NO: 69)	V_H (SEQ ID NO: 68)	18aa (SEQ ID NO: 62)	V_L (SEQ ID NO: 67)	(SEQ ID NO: 26)			IgG1 CH3 domain (SEQ ID NO: 40 or SEQ ID NO: 41)
					(SEQ ID NO: 46)	(SEQ ID NO: 52)	(SEQ ID NO: 59)	
IAB20M γ2 EH2	(SEQ ID NO: 69)	V_H (SEQ ID NO: 18)	18aa (SEQ ID NO: 62)	V_L (SEQ ID NO: 17)	(SEQ ID NO: 34)			IgG2 CH3 domain (SEQ ID NO: 42)
					(SEQ ID NO: 47)	(SEQ ID NO: 56)	(SEQ ID NO: 59)	
IAB20M γ2 EH2	(SEQ ID NO: 69)	V_H (SEQ ID NO: 18)	18aa (SEQ ID NO: 62)	V_L (SEQ ID NO: 17)	(SEQ ID NO: 34)			IgG2 CH3 T26S, L28A, M57V domain (SEQ ID NO: 181)
					(SEQ ID NO: 47)	(SEQ ID NO: 56)	(SEQ ID NO: 59)	
IAB20M γ2 EH2	(SEQ ID NO: 69)	V_H (SEQ ID NO: 18)	18aa (SEQ ID NO: 62)	V_L (SEQ ID NO: 17)	(SEQ ID NO: 34)			IgG2 CH3 T26W domain (SEQ ID NO: 182)
					(SEQ ID NO: 47)	(SEQ ID NO:	(SEQ ID NO:	

						56)	59)	
IAB25M γ2 EH2	(SEQ ID NO: 69)	V_H (SEQ ID NO: 20)	18aa (SEQ ID NO: 62)	V_L (SEQ ID NO: 19)	(SEQ ID NO: 34)			IgG2 CH3 domain (SEQ ID NO: 42)
					(SEQ ID NO: 47)	(SEQ ID NO: 56)	(SEQ ID NO: 59)	
IAB25M γ2 EH2	(SEQ ID NO: 69)	V_H (SEQ ID NO: 20)	18aa (SEQ ID NO: 62)	V_L (SEQ ID NO: 19)	(SEQ ID NO: 34)			IgG2 CH3 T26S, L28A, M57V domain (SEQ ID NO: 181)
					(SEQ ID NO: 47)	(SEQ ID NO: 56)	(SEQ ID NO: 59)	
IAB25M γ2 EH2	(SEQ ID NO: 69)	V_H (SEQ ID NO: 20)	18aa (SEQ ID NO: 62)	V_L (SEQ ID NO: 19)	(SEQ ID NO: 34)			IgG2 CH3 T26W domain (SEQ ID NO: 182)
					(SEQ ID NO: 47)	(SEQ ID NO: 56)	(SEQ ID NO: 59)	

[0265] Depicted in Table 0.2 are arrangements of sequences for monomers that can be used in minibodies (Table 0.2). Each row of the table represents the sequence of a monomer construct, with left-to-right representing N-terminus to C-terminus. In some embodiments, the shown sequences of each monomer construct are directly linked to each other. Thus, in some embodiments, the construct can include any of the constructs on a single row in Table 0.2. In some embodiments, the constructs can include any combination in Table 0.2. In some embodiments, for example, the first item in the first row, column 3 can be combined with the first row, column 4 to the first row column 5, to the first row column 6-8, to the first row, column 9. In some embodiments, column 3 and column 5 can be swapped with one another. In some embodiments, the first item in the first row, column 3 can be combined with the first row, column 4 to the second row column 5, to the second row column 6-8, to the second row, column 9. Thus, the tables represent all possible combinations, both within a single row and across various rows (and with columns swapped).

[0266] In some embodiments, an antigen binding construct against CD8 includes a heavy chain CDR1 (HCDR1) of the HCDR 1 in SEQ ID NO: 75; a heavy chain CDR2 (HCDR2) of the HCDR2 in SEQ ID NO: 76; a heavy chain CDR3 (HCDR3) of the HCDR3 in SEQ ID NO: 77; a light chain CDR1 (LCDR1) of the LCDR1 in SEQ ID NO: 78; a light chain CDR2 (LCDR2) of the LCDR2 in SEQ ID NO: 79; and/or a light chain CDR3 (LCDR3) of the LCDR3 in SEQ ID NO: 80. In some embodiments, an antigen binding construct against PSMA includes a heavy chain CDR1 (HCDR1) of the HCDR 1 in SEQ ID

NO: 81; a heavy chain CDR2 (HCDR2) of the HCDR2 in SEQ ID NO: 82; a heavy chain CDR3 (HCDR3) of the HCDR3 in SEQ ID NO: 83; a light chain CDR1 (LCDR1) of the LCDR1 in SEQ ID NO: 84; a light chain CDR2 (LCDR2) of the LCDR2 in SEQ ID NO: 85; and/or a light chain CDR3 (LCDR3) of the LCDR3 in SEQ ID NO: 86. In some embodiments, an antigen binding construct against 5T4 includes a heavy chain CDR1 (HCDR1) of the HCDR 1 in SEQ ID NO: 87; a heavy chain CDR2 (HCDR2) of the HCDR2 in SEQ ID NO: 88; a heavy chain CDR3 (HCDR3) of the HCDR3 in SEQ ID NO: 89; a light chain CDR1 (LCDR1) of the LCDR1 in SEQ ID NO: 90; a light chain CDR2 (LCDR2) of the LCDR2 in SEQ ID NO: 91; and/or a light chain CDR3 (LCDR3) of the LCDR3 in SEQ ID NO: 92. In some embodiments, an antigen binding construct against PSCA includes a heavy chain CDR1 (HCDR1) of the HCDR1 in SEQ ID NO: 93; a heavy chain CDR2 (HCDR2) of the HCDR2 in SEQ ID NO: 94; a heavy chain CDR3 (HCDR3) of the HCDR3 in SEQ ID NO: 95; a light chain CDR1 (LCDR1) of the LCDR1 in SEQ ID NO: 96; a light chain CDR2 (LCDR2) of the LCDR2 in SEQ ID NO: 97; and/or a light chain CDR3 (LCDR3) of the LCDR3 in SEQ ID NO: 98. In some embodiments, an antigen binding construct against CD3 includes a heavy chain CDR1 (HCDR1) of the HCDR1 in FIG. 65B or 65C; a heavy chain CDR2 (HCDR2) of the HCDR2 in FIG. 65B or 65C; a heavy chain CDR3 (HCDR3) of the HCDR3 in FIG. 65B or 65C; a light chain CDR1 (LCDR1) of the LCDR1 in FIG. 65B or 65C; a light chain CDR2 (LCDR2) of the LCDR2 in FIG. 65B or 65C; and/or a light chain CDR3 (LCDR3) of the LCDR3 in FIG. 65B or 65C. Some embodiments of CDR sequences that can be used with any one or more of the hinge sequences provided herein are shown in Table 8.

TABLE 8

CDR sequences

	IAB22M (CD8)	**IAB2M (PSMA)**	**IAB20M (5T4)**	**IAB1M (PSCA)**	**IAB25M (CD3)**
HCDR 1	GFNIKDT (SEQ ID NO: 75)	GYTFTEY (SEQ ID NO: 81)	GYSFTGY (SEQ ID NO: 87)	GFNIKDY (SEQ ID NO: 93)	GYTFTRY (SEQ ID NO: 174)
HCDR 2	DPANDN (SEQ ID NO: 76)	NINPNNGG (SEQ ID NO: 82)	NPNNGV (SEQ ID NO: 88)	DPENGD (SEQ ID NO: 94)	NPSRGY (SEQ ID NO: 175)

HCDR 3	GYGYYVFDH (SEQ ID NO: 77)	GWNFDY (SEQ ID NO: 83)	STMITNYVM DY (SEQ ID NO: 89)	GGF (SEQ ID NO: 95)	YYDDHYSLDY (SEQ ID NO: 176)
LCDR 1	RTSRSISQYLA (SEQ ID NO: 78)	KASQDVGTAVD (SEQ ID NO: 84)	KASQSVSND VA (SEQ ID NO: 90)	SASSSVRFIH (SEQ ID NO: 96)	SASSSVSYMN (SEQ ID NO: 177)
LCDR 2	SGSTLQS (SEQ ID NO: 79)	WASTRHT (SEQ ID NO: 85)	YTSSRYA (SEQ ID NO: 91)	DTSKLAS (SEQ ID NO: 97)	DTSKLAS (SEQ ID NO: 97)
LCDR 3	QQHNENPLT (SEQ ID NO: 80)	QQYNSYPLT (SEQ ID NO: 86)	QQDYNSPPT (SEQ ID NO: 92)	QQWGSSPFT (SEQ ID NO: 98)	QQWSSNPFT (SEQ ID NO: 178)

[0267] These constructs can be in any of the forms provided herein, including minibodies, scFv, etc. In some embodiments, the antigen binding construct includes 6, 5, 4, 3, 2, or 1, the above CDRs (some embodiments of the CDRs are indicated in FIG. 41, 48, 55, 61, 65B, or 65C). In some embodiments, any of the hinge sections provided herein can be combined with any of the CDRs provided herein. In some embodiments, any of the upper hinge sections provided herein can be combined with any of the CDRs provided herein. In some embodiments, any of the core hinge sections provided herein can be combined with any of the CDRs provided herein. In some embodiments, any of the lower hinge sections provided herein can be combined with any of the CDRs provided herein. In some embodiments, any of the full hinge sections provided herein can be combined with any of the CDRs provided herein. In some embodiments, any of the upper and core hinge sections provided herein can be combined with any of the CDRs provided herein. In some embodiments, any of the core and lower hinge sections provided herein can be combined with any of the CDRs provided herein. In some embodiments, any of these CDR based embodiments can be part of a minibody. In some embodiments, the antigen binding construct includes heavy chain CDR3 (HCDR3). In some embodiments, the antigen binding construct binds specifically to the target molecule. In some embodiments, the antigen binding construct competes for binding with one or more of the antibodies having the herein provided CDRs. In some embodiments, the antigen binding construct includes at least the 3 heavy chain CDRs noted herein. In some embodiments, the antigen binding construct includes heavy chain CDR3. In some embodiments, the antigen binding construct further

includes any one of the heavy chain CDR2 sequences provided herein. In some embodiments, any of these embodiments can be applied to a minibody that binds to CD8, CD3, 5T4, PSCA, or PSMA.

[0268] In some embodiments, the antigen binding construct is human or humanized. When used in the context of a minibody, the term human does not denote that the construct is identical to that found in nature in humans, but rather that the construct has sequences that are human. In some embodiments, the antigen binding construct includes at least one human framework region (for example, those sections shown before, between, and after the CDRs shown in FIG. 41, 48, 55, 61, 65B, or 65C, or a framework region with at least about 80% sequence identity, for example at least about 80%, 85%, 90%, 93%, 95%, 97%, or 99% identity to a human framework region. In some embodiments, the antigen binding construct includes 8, 7, 6, 5, 4, 3, 2, or 1 of the listed FRs. In some embodiments, any of the hinge sections provided herein can be combined with any of the FRs provided herein. In some embodiments, any of the upper hinge sections provided herein can be combined with any of the FRs provided herein. In some embodiments, any of the core hinge sections provided herein can be combined with any of the FRs provided herein. In some embodiments, any of the lower hinge sections provided herein can be combined with any of the FRs provided herein. In some embodiments, any of the full hinge sections provided herein can be combined with any of the FRs provided herein. In some embodiments, any of the upper and core hinge sections provided herein can be combined with any of the FRs provided herein. In some embodiments, any of the core and lower hinge sections provided herein can be combined with any of the FRs provided herein. In some embodiments, any of these FR based embodiments can be part of a minibody. In some embodiments, any of these embodiments can be applied to a minibody that binds to CD8, CD3, 5T4, PSCA, or PSMA.

[0269] In some embodiments, the antigen binding construct has a heavy chain variable region of the heavy chain variable region in SEQ ID NOs: 14 (PSMA), 16 or 99 (CD8), 18 or 102 (5T4), 68 (PSCA), or, 20 or 104 (or FIGs. 65B and 65C) (CD3). In some embodiments, the antigen binding construct has a heavy chain variable region that includes a sequence with at least about 80% identity, for example at least about 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NOs: 14 (PSMA), 16 or 99 (CD8), 18 or 102 (5T4), 68 (PSCA), or, 20 or 104 (or FIGs. 65B

and 65C) (CD3). In some embodiments, the antigen binding construct has a light chain variable region that includes SEQ ID NO: 13 (PSMA), 15 (CD8), 17 or 100 or 101 (5T4), 67 (PSCA), or, 19 or 103 (or FIGs. 65B and 65C) (CD3). In some embodiments, the antigen binding construct has a light chain variable region that includes a sequence with least about 80% identity, for example at least about 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO: 13 (PSMA), 15 (CD8), 17 or 100 or 101 (5T4), 67 (PSCA), or, 19 or 103 (or FIGs. 65B and 65C) (CD3). In some embodiments, the antigen binding construct is a human antigen binding construct and has a heavy chain variable region, a light chain variable region, or a heavy and light chain that is at least as identical as at least the heavy and/or light chain variable sequences noted above. In some embodiments, any one of the above embodiments is combined with any one of the hinge embodiments provided herein, including, for example, in a minibody context. In some embodiments, any of the hinge sections provided herein can be combined with any of the V_H and V_L pairings provided herein. In some embodiments, any of the upper hinge sections provided herein can be combined with any of the V_H and V_L pairings provided herein. In some embodiments, any of the core hinge sections provided herein can be combined with any of the V_H and V_L pairings provided herein. In some embodiments, any of the lower hinge sections provided herein can be combined with any of the V_H and V_L pairings provided herein. In some embodiments, any of the full hinge sections provided herein can be combined with any of the V_H and V_L pairings provided herein. In some embodiments, any of the upper and core hinge sections provided herein can be combined with any of the V_H and V_L pairings provided herein. In some embodiments, any of the core and lower hinge sections provided herein can be combined with any of the V_H and V_L pairings provided herein. In some embodiments, any of these V_H and V_L pairings based embodiments can be part of a minibody. In some embodiments, any of these embodiments can be applied to a minibody that binds to CD8, CD3, 5T4, PSCA, or PSMA.

[0270] In some embodiments, the antigen binding construct includes a detectable marker. In some embodiments, the antigen binding construct includes a therapeutic agent.

[0271] In some embodiments, the antigen binding construct is bivalent. Bivalent antigen binding construct can include at least a first antigen binding domain, for example a first scFv, and at least a second antigen binding domain, for example a second scFv. In some

embodiments, a bivalent antigen binding construct is a multimer that includes at least two monomers, for example at least 2, 3, 4, 5, 6, 7, or 8 monomers, each of which has an antigen binding domain. In some embodiments, the antigen binding construct is a minibody. In some embodiments, one or more subparts of the hinges provided herein are included in the bivalent construct. The minibody can include any of the CDR and heavy chain variable region and/or light chain variable region embodiments provided herein (for example, the CDR sequences provided in FIG. 41, 48, 55, 61, 65B, or 65C). In some embodiments, the antigen binding construct is a monovalent scFv. In some embodiments, a monovalent scFv is provided that includes the heavy chain CDR1 (HCDR1) in the HCDR1 of FIG. 41, 48, 55, 61, 65B, or 65C the heavy chain CDR2 (HCDR2) in the HCDR2 of FIG. 41, 48, 55, 61, 65B, or 65C, the HCDR3 in the HCDR3 of FIG. 41, 48, 55, 61, 65B, or 65C, the light chain CDR1 (LCDR1) in the LCDR1 of FIG. 41, 48, 55, 61, 65B, or 65C, the light chain CDR2 (LCDR2) in the LCDR2 of FIG. 41, 48, 55, 61, 65B, or 65C, and the light chain CDR3 (LCDR3) in the LCDR3 of FIG. 41, 48, 55, 61, 65B, or 65C. In some embodiments, the monovalent scFv includes the heavy chain variable region of the heavy chain variable region in FIG. 36A, 36B, 36C, 36D, 36E, 65B, or 65C. In some embodiments, the monovalent scFv includes the light chain variable region of the light chain variable region in FIG. 36A, 36B, 36C, 36D, 36E, 65B, or 65C. In some embodiments, the monovalent scFv includes the heavy chain variable region of the heavy chain variable region in FIG. 66, 68 or 69, and the light chain variable region of the light chain variable region in FIG. 67 or 69.

[0272] In some embodiments, the antigen binding construct is bispecific. Bispecific constructs can include at least a first binding domain, for example an scFv that binds specifically to a first epitope, and at least a second binding domain, for example an scFv that binds specifically to a second epitope. Thus, bispecific antigen binding constructs can bind to two or more epitopes. In some embodiments, the first epitope and the second epitope are part of the same antigen, and the bispecific antigen binding construct can thus bind to two epitopes of the same antigen. In some embodiments, the first epitope is part of a first antigen, and the second epitope is part of a second antigen, and the bispecific antigen binding construct can thus bind to two different antigens. In some embodiments, the antigen binding construct binds to two epitopes simultaneously.

[0273] In some embodiments, the antigen binding construct has a heavy chain variable region of the heavy chain variable region in SEQ ID NO: 14, 16, 18, 20, 68, 99, 102 or 104, and/or the sequences in FIGs. 65B and 65C. In some embodiments, the antigen binding construct has a heavy chain variable region that includes a sequence with at least about 80% identity, for example at least about 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO: 14, 16, 18, 20, 68, 99, 102 or 104 and/or the sequences in FIGs. 65B and 65C. In some embodiments, the antigen binding construct has a light chain variable region that includes SEQ ID NO: 13, 15, 17, 19, 67, 100, 101 or 103 and/or the sequences in FIGs. 65B and 65C. In some embodiments, the antigen binding construct has a light chain variable region that includes a sequence with least about 80% identity, for example at least about 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO: 13, 15, 17, 19, 67, 100, 101 or 103 and/or the sequences in FIGs. 65B and 65C. In some embodiments, the antigen binding construct is a human antigen binding construct and has a heavy chain variable region, a light chain variable region, or a heavy and light chain that is at least as identical as at least the heavy and/or light chain variable sequences noted above.

[0274] Some embodiments provided herein include an antigen binding construct that competes for binding to the target molecule with one or more antigen binding constructs provided herein, and includes one or more of the hinge subparts provided herein (such as in Table 0.1). In some embodiments, the competing antigen binding construct binds to the same epitope on the target molecule as the reference antigen binding construct. In some embodiments, the reference antigen binding construct binds to a first epitope of the target molecule, and the competing antigen binding construct binds to a second epitope of the target molecule, but interferes with binding of the reference antigen binding construct to the target molecule, for example by sterically blocking binding of the reference antigen binding construct, or by inducing a conformational change in the target molecule. In some embodiments, the first epitope overlaps with the second epitope.

[0275] In some embodiments, the scFv and/or minibody formats have advantageous pharmacokinetic characteristics for diagnostic imaging and certain therapeutic applications while maintaining the high binding affinity and specificity of a parental antibody. Compared to imaging with the full-length parental antibody, the pharmacokinetics

are more desirable for these fragments in that they are able to target the antigen and then rapidly clear the system for rapid high-contrast imaging. In some embodiments, the shorter circulation half-lives for the minibody allow for optimal imaging over a range of times from approximately 6-48 72 hours (depends on clearance and level of antigen "sink" and half-life of isotope that is chelated to Mb) hours post injection for the minibody. The rapid blood clearance together with better tissue penetration can allow for same day imaging, which can provide a significant advantage in the clinic with respect to patient care management.

[0276] In some embodiments, the antibody fragments can comprise one, two, or three of the variable light region CDRs and/or one, two, or three of the variable heavy region CDRs from a PSCA, 5T4, PSMA, CD3 or CD8 antibody. For example, an antibody fragment may contain one, two or three of the variable region CDRs and/or one, two, or three of the variable heavy region CDRs of murine versions of the PSCA, 5T4, PSMA, CD3 or CD8 antibody. In some embodiments, an antibody fragment comprises one or more CDR regions from the variable heavy or light regions of a humanized PSCA, 5T4, PSMA, CD3 or CD8 antibody.

[0277] In some embodiments, antigen binding constructs that bind the PSCA antigen can be antibodies, minibodies and/or fragments thereof such as scFv. Some non-limiting embodiments of antigen binding constructs against PSCA are shown in FIGs. 29B – 29D, 36E, 60 (SEQ ID NOs: 125 and 126), 61 (SEQ ID NO: 126), 62 (SEQ ID NO: 127), 63 (SEQ ID NO: 128), 64 (SEQ ID NO: 129), 65A (SEQ ID NO: 130). In some embodiments, antigen binding constructs that bind the 5T4 antigen can be antibodies, minibodies and/or fragments thereof such as scFv. Some non-limiting embodiments of antigen binding constructs against 5T4 are shown in FIGs. 28B - 28E, 32, 36C, 54 (SEQ ID NOs: 119 and 120), 55 (SEQ ID NO: 120), 56 (SEQ ID NO: 121), 57 (SEQ ID NO: 122), 58 (SEQ ID NO: 123), 59 (SEQ ID NO: 124), 67, 68. In some embodiments, antigen binding constructs that bind the PSMA antigen can be antibodies, minibodies and/or fragments thereof such as scFv. Some non-limiting embodiments of antigen binding constructs against PSMA are shown in FIGs. 5B – 5E, 7C - 7E, 21B - 21E, 34A - 34F, 35A - 35C, 36A, 47 (SEQ ID NOs: 112 and 113), 48 (SEQ ID NO: 113), 49 (SEQ ID NO: 114), 50 (SEQ ID NO: 115), 51 (SEQ ID NO: 116), 52 (SEQ ID NO: 117), 53 (SEQ ID NO: 118). In some embodiments, antigen binding constructs that bind the CD3 antigen can be antibodies, minibodies and/or fragments thereof

such as scFv. Some non-limiting embodiments of antigen binding constructs against CD3 are shown in FIGS. 33, 36D, 69, 65B, and 65C. In some embodiments, antigen binding constructs that bind the CD8 antigen can be antibodies, minibodies and/or fragments thereof such as scFv. Some non-limiting embodiments of antigen binding constructs against CD8 are shown in FIGs. 14B, 15B, 16B - 16D, 20C - 20G, 22B, 36B, 40 (SEQ ID NOs: 105 and 106), 41 (SEQ ID NO: 106), 42 (SEQ ID NO: 107), 43 (SEQ ID NO: 108), 44 (SEQ ID NO: 109), 45 (SEQ ID NO: 110), 46 (SEQ ID NO: 111), and 66.

Linker Options

[0278] In some embodiments, for individual antigen binding constructs, the heavy and light chain variable domains can associate in different ways. For this reason, the use of different linker lengths between the V_H and V_L domains allows for conformational flexibility and range-of-motion to ensure formation of the disulfide bonds.

[0279] In some embodiments, the two linker lengths can be somewhere between (and including) about 1 to 50 amino acids, for example, 2 to 15, 2 to 14, 3 to 13, 4 to 10, or 5 amino acids to 8 amino acids. In some embodiments, each linker within a pair for a minibody can be the same length. In some embodiments, each linker within the pair can be a different length. In some embodiments, any combination of linker length pairs can be used, as long as they allow and/or promote the desired combinations. In some embodiments, a modified amino acid can be used.

[0280] Table 0.2 provides minibody variants. Producing and testing the expression and binding of the variants allows for identification of a desired format for protein production for each new minibody.

[0281] In some embodiments, the linker is a GlySer linker. The GlySer linker can be a polypeptide that is rich in Gly and/or Ser residues. In some embodiments, at least about 40% of the amino acid residues of the GlySer linker are Gly, Ser, or a combination of Gly and Ser, for example at least about 40%, 50%, 60%, 70%, 80%, or 90%. In some embodiments, the GlySer linker is at least about 2 amino acids long, for example at least about 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, or 40 amino acids long. In some embodiments, the linker motif can be $(G_3)S_n$ or $(G_4)S_n$ (n can be any number of S's; and in some embodiments is 1 or 2). For example, a) GGGGS 5 aa, (SEQ ID NO: 66); b) GGGGSGGGGS 10 aa, (SEQ ID NO: 65); c) GGGGSGGGGSGGGGS 15 aa,

(SEQ ID NO: 64); d) GSTSGGGSGGGS 12 aa, (SEQ ID NO: 63); or e) GSTSGGGSGGGSGGGGSS 18 aa (SEQ ID NO: 62). In some embodiments, the linker comprises at least one threonine.

[0282] Tables 0.1 and 0.2 depict various optional sequences that can be used in some embodiments of the scFv and/or minibodies. In some embodiments, the scFv can be any of those provided herein, but with any of the sequences provided in Table 0.1. In some embodiments, the minibody can be any of those provided herein, but with any of the sequences provided in a full row in Table 0.1. In some embodiments, any of the antigen binding constructs provided herein can include any of the hinge sequence subparts provided in Table 0.1.

[0283] A "minibody" as described herein, encompasses a homodimer, wherein each monomer is a single-chain variable fragment (scFv) linked to a human C_H3 domain by a hinge sequence. In some embodiments, the hinge sequence is a human IgG1 hinge sequence. In some embodiments, the hinge sequence is any one or more of the hinge sequences provided herein, such as any of those described herein and/or in Table 0.1 (including any subpart or combination of subparts thereof).

[0284] In some embodiments, the hinge sequence is an artificial hinge sequence. In some embodiments, the hinge sequence can be a natural or artificial IgG hinge, or subpart or combination of subparts thereof, from any one or more of the four classes of IgG hinges.

[0285] In some embodiments, the minibody sequence can include CDR and/or FR, and or variable region sequences that are similar a sequence described herein (for example, as found in the Figures or Table 0.2. In some embodiments, the minibody has a sequence (CDR, CDRs, full set of 6 CDRS, heavy chain variable region, light chain variable region, heavy and light chain variable regions, etc) that is at identical to a scFv of a minibody described herein.

[0286] In some embodiments, the polypeptide of the monomer includes sequences as shown in Table 0.2. In some embodiments, the polypeptide of the monomer includes a sequence with least about 80% identity, for example at least about 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to the sequence of the V_LV_H minibody monomer shown in Table 0.2.

[0287] In some embodiments, the minibody has a variable chain region (heavy, light or heavy and light chain variable region) that is at least about 80% identical to a heavy chain sequence in SEQ ID NO: 14, 16, 18, 20, 68, 99, 102 or 104 (and/or the relevant sequences in FIG. 65B or 65C) and/or a light chain sequence in SEQ ID NO: 13, 15, 17, 19, 67, 100, 101 or 103 (and/or the sequences in FIG. 65B or 65C), for example at least about 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93, 94, 95%, 96%, 97%, 98%, or 99% identity.

[0288] The scFv can have a V_HV_L or a V_LV_H orientation. In some embodiments, the V_H and V_L are linked to each other by an amino acid linker sequence. The amino acid linker can be a linker as described herein. In some embodiments, the linker is Gly-Ser-rich and approximately 15-20 amino acids in length. In another embodiment, the linker is GlySer rich and is 18 amino acids in length. In some embodiments, the linker length varies between (and including) about 1 to 50 amino acids, for example, 2 to 30, 3 to 20, 4 to 15, or 5 amino acids to 8 amino acids. In some embodiments, the linker is GSTSGGGSGGGSGGGGSS (SEQ ID NO. 62). In some embodiments, the minibody scFv has a sequence that is at least about 80% identical to a scFv described herein, for example at least about 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93, 94, 95%, 96%, 97%, 98%, or 99% identity. The scFv can have a V_HV_L or a V_LV_H orientation.

[0289] In some embodiments, each monomer of the minibody includes the following elements, from N-terminus to C-terminus: (a) an scFv sequence that includes a V_H domain linked to a V_L domain and that binds to the target molecule, (b) a hinge domain (e.g., an upper and core hinge region combined with a lower hinge and/or extension region), and (c) a human IgG C_H3 sequence. In some embodiments, each monomer of the minibody includes a IgG1, IgG2, an IgG3, or an IgG4 C_H3 domain. In some embodiments, the minibody is encoded by a nucleic acid can be expressed by a cell, a cell line or other suitable expression system as described herein. Thus, a signal sequence can be fused to the N-terminus of the scFv to enable secretion of the minibody when expressed in the cell or cell line. In some embodiments, other signal sequences such as human serum albumin (SEQ ID NO: 70) can be used. In some embodiments, different signal sequences can improve secretion depending on context and cell line used for expression. In some embodiments, a human immunoglobulin kappa light chain signal sequence (SEQ ID NO: 69) can be

employed and/or included in a nucleic acid encoding for an amino acid sequence provided herein. In some embodiments, a single sequence can be selected from the list shown in Table 7 depending on the context and cell line used for expression.

TABLE 7

Signal peptide sequences

Signal peptide source	Signal peptide sequence
Mouse Ig kappa light chain	METDTLLLWVLLLWVPGSTG (SEQ ID NO: 69)
Human Serum Albumin (HSA)	MKWVTFISLLFLFSSAYS (SEQ ID NO: 70)
Human Azurocidin (HAZ)	MTRLTVLALLAGLLASSRA (SEQ ID NO: 71)
Prolactin	MDSKGSSQKGSRLLLLLWSNLLLCQGWS (SEQ ID NO: 72)
LCMV-GPC	MGQIVTMFEALPHIIDEVININVIIVLIIITSIKAVYNFATCGILALVSFLFLAGRSCG (SEQ ID NO: 73)
MMTV-Rem	MPNHQSGSPTGSSDLLLSGKKQRPHLALRRKRRREMRKINRKVRRMNLAPIKEKT AWQHLQALISEAEEVLKTSQTPQNSLTLFLALLSVLGPPPVTG (SEQ ID NO: 74)
Mammalia	Accessible on the world wide web at signalpeptide.de/index.php?m=listspdb_mammalia**
Drosophila	Accessible on the world wide web at signalpeptide.de/index.php?m=listspdb_drosophila**
Bacteria	Accessible on the world wide web at signalpeptide.de/index.php?m=listspdb_bacteria**
Viruses	Accessible on the world wide web at signalpeptide.de/index.php?m=listspdb_viruses**
**Signal Peptides referred to at the world wide web locations referenced above are described in the Signal Peptide Database, which can be accessed on the world wide web at signalpeptide.de and via the hyperlinks above, is hereby incorporated by reference in its entirety.	

[0290] In some embodiments, a chimeric minibody that binds to the target molecule is provided. In some embodiments, the chimeric minibody includes a monomer in the V_LV_H format, and includes the sequence of a light chain variable region that includes

SEQ ID NO: 13, 15, 17, 19, 67, 100, 101 or 103 (and/or the relevant sequences in FIG. 65B or 65C) and the sequence of a heavy chain variable region that includes SEQ ID NO: 14, 16, 18, 20, 68, 99, 102 or 104 (and/or the sequences in FIG. 65B or 65C) or a monomer as shown in Table 0.2, or a sequence having at least about 80% identity thereto, for example at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%% identity thereto. In some embodiments, the chimeric minibody includes a monomer in the V_HV_L format, and includes the sequence of a light chain variable region that includes SEQ ID NO: 13, 15, 17, 19, 67, 100, 101 or 103 (and/or the relevant sequences in FIG. 65B or 65C) and the sequence of a heavy chain variable region that includes SEQ ID NO: 14, 16, 18, 20, 68, 99, 102 or 104 (and/or the relevant sequences in FIG. 65B or 65C) or a monomer as shown in Table 0.2, or a sequence having at least about 80% identity thereto, for example at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%% identity thereto. In some embodiments, the minibody comprises one or more of the CDRs outlined in FIG. 41, 48, 55, 61, 65B, or 65C. In some embodiments, the minibody comprises one or more of the variable regions in the FIG. 36A, 36B, 36C, 36D, 36E, 66, 67, 68, 69, 65B, or 65C or Table 0.2.

[0291] In some embodiments, the minibody includes the heavy chain variable region as outlined in the FIG. 36A, 36B, 36C, 36D, 36E, 66, 68, 69, 65B, or 65C. In some embodiments, the minibody includes the light chain variable region as outlined in the FIG. 36A, 36B, 36C, 36D, 36E, 67, 69, 65B, or 65C.

[0292] In some embodiments, the minibody includes one or more of the CDRs provided in the CDRs in FIG. 41, 48, 55, 61, 65B, or 65C.

Nucleic Acids

[0293] In some embodiments, the polypeptides of the antigen binding constructs can be encoded by nucleic acids and expressed *in vivo* or *in vitro*, or these peptide can be synthesized chemically. Thus, in some embodiments, a nucleic acid encoding an antigen binding construct is provided. In some embodiments, the nucleic acid encodes one part or monomer of a minibody or other antigen binding construct. In some embodiments, the nucleic acid encodes two or more monomers, for example, at least 2 monomers. Nucleic acids encoding multiple monomers can include nucleic acid cleavage sites between at least two monomers, can encode transcription or translation start site between two or more monomers, and/or can encode proteolytic target sites between two or more monomers.

[0294] In some embodiments, an expression vector contains a nucleic acid encoding an antigen binding construct as disclosed herein. In some embodiments, the expression vector includes pcDNA3.1™/myc-His (-) Version A vector for mammalian expression (Invitrogen, Inc.) or a variant thereof. The pcDNA3.1 expression vector features a CMV promoter for mammalian expression and both mammalian (Neomycin) and bacterial (Ampicillin) selection markers. In some embodiments, the expression vector includes a plasmid. In some embodiments, the vector includes a viral vector, for example a retroviral or adenoviral vector. In embodiments, the vector includes a cosmid, YAC, or BAC.

[0295] In some embodiments, the nucleotide sequence encoding at least one of the minibody monomers comprises at least one of SEQ ID NOs: provided herein, including those disclosed herein for binding to CD3, CD8, 5T4, PSMA, and PSCA, or a sequence having at least about 80% identity, for example about 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93, 94, 95%, 96%, 97%, 98%, 99%, or greater identity thereto.

Cell Lines

[0296] In some embodiments, a cell line is provided that expresses at least one of the antigen binding constructs described herein (which can include at least a subpart of a hinge provided herein). In some embodiments, a mammalian cell line (for example, CHO-K1 cell line) is an expression system to produce the minibodies, scFv, or other antibodies as described herein. In some embodiments, the minibodies, scFv, and other antibodies or antibody fragments described herein are non-glycosylated, and a mammalian expression system is not required, as such post-translational modifications are not needed. Thus, in some embodiments, one or more of a wide variety of mammalian or non-mammalian expression systems are used to produce the antigen binding constructs disclosed herein (for example, minibodies) including, but not limited to mammalian expression systems (for example, CHO-K1 cells), bacterial expression systems (for example, *E. coli*, *B. subtilis*) yeast expression systems (for example, *Pichia*, *S. cerevisiae*) or any other known expression system. Other systems can include insect cells and/or plant cells.

Antigen Binding Construct Modifications

[0297] In some embodiments, the antigen binding construct includes at least one modification. Exemplary modifications include, but are not limited to, antigen binding

constructs that have been modified by glycosylation, acetylation, pegylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, and linkage to a cellular ligand or other protein. Any of numerous chemical modifications may be carried out by known techniques, including, but not limited to, specific chemical cleavage, acetylation, and metabolic synthesis of tunicamycin. In some embodiments, the derivative can contain one or more non-natural amino acids.

[0298] In some embodiments, the antigen binding construct is conjugated to another substance to form an anti-target conjugate. The conjugates described herein can be prepared by known methods of linking antigen binding constructs with lipids, carbohydrates, protein or other atoms and molecules. In some embodiments, the conjugate is formed by site-specific conjugation using a suitable linkage or bond. Site-specific conjugation is more likely to preserve the binding activity of an antigen binding construct. The substance may be conjugate or attached at the hinge region of a reduced antigen binding construct via thioether bond formation. In some embodiments, tyrosine conjugation can be employed. Other linkages or bonds used to form the conjugate can include, but are not limited to, a covalent bond, a non-covalent bond, a disulfide linkage, a hydrazone linkage, an ester linkage, an amido linkage, and amino linkage, an imino linkage, a thiosemicarbazone linkage, a semicarbazone linkage, an oxime linkage and a carbon-carbon linkage. In some embodiments, no cysteine or other linking aspect, need be included in the antigen binding construct.

Detectable markers

[0299] In some embodiments, a modified antigen binding construct is conjugated to a detectable marker. As used herein, a "detectable marker" includes an atom, molecule, or compound that is useful in diagnosing, detecting or visualizing a location and/or quantity of a target molecule, cell, tissue, organ and the like. Detectable markers that can be used in accordance with the embodiments herein include, but are not limited to, radioactive substances (for example, radioisotopes, radionuclides, radiolabels or radiotracers), dyes, contrast agents, fluorescent compounds or molecules, bioluminescent compounds or molecules, enzymes and enhancing agents (for example, paramagnetic ions). In addition, some nanoparticles, for example quantum dots and metal nanoparticles (described below) can

be suitable for use as a detection agent. In some embodiments, the detectable marker is IndoCyanine Green (ICG), Zirconium-89, IR800, and/or another near infrared dye.

[0300] Exemplary radioactive substances that can be used as detectable markers in accordance with the embodiments herein include, but are not limited to, ^{18}F, ^{18}F-FAC, ^{32}P, ^{33}P, ^{45}Ti, ^{47}Sc, ^{52}Fe, ^{59}Fe, ^{62}Cu, ^{64}Cu, ^{67}Cu, ^{67}Ga, ^{68}Ga, ^{75}Sc, ^{77}As, ^{86}Y, ^{90}Y, ^{89}Sr, ^{89}Zr, ^{94}Tc, ^{94}Tc, ^{99}mTc, ^{99}Mo, ^{105}Pd, ^{105}Rh, ^{111}Ag, ^{111}In, ^{123}I, ^{124}I, ^{125}I, ^{131}I, ^{142}Pr, ^{143}Pr, ^{149}Pm, ^{153}Sm, $^{154-158}Gd$, ^{161}Tb, ^{166}Dy, ^{166}Ho, ^{169}Er, ^{175}Lu, ^{177}Lu, ^{186}Re, ^{188}Re, ^{189}Re, ^{194}Ir, ^{198}Au, ^{199}Au, ^{211}At, ^{211}Pb, ^{212}Bi, ^{212}Pb, ^{213}Bi, ^{223}Ra and ^{225}Ac. Exemplary paramagnetic ions substances that can be used as detectable markers include, but are not limited to ions of transition and lanthanide metals (for example metals having atomic numbers of 6 to 9, 21-29, 42, 43, 44, or 57-71). These metals include ions of Cr, V, Mn, Fe, Co, Ni, Cu, La, Ce, Pr, Nd, Pm, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb and Lu.

[0301] When the detectable marker is a radioactive metal or paramagnetic ion, in some embodiments, the marker can be reacted with a reagent having a long tail with one or more chelating groups attached to the long tail for binding these ions. In some embodiments the reagent may carry a reactive group designed to covalently tether the antibody fragment chains. The long tail can be a polymer such as a polylysine, polysaccharide, polyethylene glycol (PEG) or other derivatized or derivatizable chain having pendant groups to which may be bound to a chelating group for binding the ions. Examples of chelating groups that may be used according to the embodiments herein include, but are not limited to, ethylenediaminetetraacetic acid (EDTA), diethylenetriaminepentaacetic acid (DTPA), DOTA, NOTA, NODAGA, NETA, deferoxamine (Df, which may also be referred to as DFO), porphyrins, polyamines, crown ethers, bis-thiosemicarbazones, polyoximes, and like groups. The same chelates, when complexed with non-radioactive metals, such as manganese, iron and gadolinium are useful for MRI, when used along with the antigen binding constructs and carriers described herein. Macrocyclic chelates such as NOTA, NODAGA, DOTA, and TETA are of use with a variety of metals and radiometals including, but not limited to, radionuclides of gallium, yttrium and copper, respectively. Other ring-type chelates such as macrocyclic polyethers, which are of interest for stably binding radionuclides, such as Radium-223 for RAIT may be used. In certain embodiments,

chelating moieties may be used to attach a PET imaging agent, such as an Aluminum-^{18}F or Zirconium-89 complex, to a targeting molecule for use in PET analysis.

[0302] Exemplary contrast agents that can be used as detectable markers in accordance with the embodiments of the disclosure include, but are not limited to, barium, diatrizoate, ethiodized oil, gallium citrate, iocarmic acid, iocetamic acid, iodamide, iodipamide, iodoxamic acid, iogulamide, iohexyl, iopamidol, iopanoic acid, ioprocemic acid, iosefamic acid, ioseric acid, iosulamide meglumine, iosemetic acid, iotasul, iotetric acid, iothalamic acid, iotroxic acid, ioxaglic acid, ioxotrizoic acid, ipodate, meglumine, metrizamide, metrizoate, propyliodone, thallous chloride, or combinations thereof.

[0303] Bioluminescent and fluorescent compounds or molecules and dyes that can be used as detectable markers in accordance with the embodiments of the disclosure include, but are not limited to, fluorescein, fluorescein isothiocyanate (FITC), OREGON GREEN™, rhodamine, Texas red, tetrarhodimine isothiocynate (TRITC), Cy3, Cy5, and the like, fluorescent markers (for example, green fluorescent protein (GFP), phycoerythrin, and the like), autoquenched fluorescent compounds that are activated by tumor-associated proteases, enzymes (for example, luciferase, horseradish peroxidase, alkaline phosphatase, and the like), nanoparticles, biotin, digoxigenin or combination thereof.

[0304] Enzymes that can be used as detectable markers in accordance with the embodiments of the disclosure include, but are not limited to, horseradish peroxidase, alkaline phosphatase, acid phoshatase, glucose oxidase, β-galactosidase, β-glucoronidase or β-lactamase. Such enzymes may be used in combination with a chromogen, a fluorogenic compound or a luminogenic compound to generate a detectable signal.

[0305] In some embodiments, the antigen binding construct is conjugated to a nanoparticle. The term "nanoparticle" refers to a microscopic particle whose size is measured in nanometers, for example, a particle with at least one dimension less than about 100 nm. Nanoparticles can be used as detectable substances because they are small enough to scatter visible light rather than absorb it. For example, gold nanoparticles possess significant visible light extinction properties and appear deep red to black in solution. As a result, compositions comprising antigen binding constructs conjugated to nanoparticles can be used for the *in vivo* imaging of T-cells in a subject. At the small end of the size range, nanoparticles are often referred to as clusters. Metal, dielectric, and semiconductor

nanoparticles have been formed, as well as hybrid structures (for example core-shell nanoparticles). Nanospheres, nanorods, and nanocups are just a few of the shapes that have been grown. Semiconductor quantum dots and nanocrystals are examples of additional types of nanoparticles. Such nanoscale particles, when conjugated to an antigen binding construct, can be used as imaging agents for the *in vivo* detection of T-cells as described herein.

[0306] In some embodiments, having a stable hinge will impart stability to the targeting agent. In some embodiments, this results in better biodistribution, higher sensitivity, and/or reduced renal clearance. Therefore, these constructs can be more applicable for higher degree of specific activity. In some embodiments, this imparts substantially improved sensitivity without high dose to the kidney, which is a radiosensitive organ. In some embodiments, these constructs are more suitable for RIT for the same reason – low renal uptake. In some embodiments, a site-specific conjugation to cysteines can now be employed because chemical reduction under mild condition will break 1 or 2 disulfide bonds to use these as a handle but keep the remaining disulfides intact. In some embodiments, this will preserve the integrity of the minibody conjugate.

Therapeutic agents and Compositions

[0307] In some embodiments, the pharmaceutical composition can also include a pharmaceutically acceptable carrier. A pharmaceutically acceptable carrier can be a pharmaceutically acceptable material, composition, or vehicle that is involved in carrying or transporting a compound of interest from one tissue, organ, or portion of the body to another tissue, organ, or portion of the body. For example, the carrier can be a liquid or solid filler, diluent, excipient, solvent, or encapsulating material, or some combination thereof. Each component of the carrier is "pharmaceutically acceptable" in that it is compatible with the other ingredients of the formulation. It is also suitable for contact with any tissue, organ, or portion of the body that it can encounter, meaning that, ideally it will not carry a significant risk of toxicity, irritation, allergic response, immunogenicity, or any other complication that excessively outweighs its therapeutic benefits.

[0308] The pharmaceutical compositions described herein can be administered by any suitable route of administration. A route of administration can refer to any administration pathway known in the art, including but not limited to aerosol, enteral, nasal, ophthalmic, oral, parenteral, rectal, transdermal (e.g., topical cream or ointment, patch), or

vaginal. "Transdermal" administration can be accomplished using a topical cream or ointment or by means of a transdermal patch. "Parenteral" refers to a route of administration that is generally associated with injection, including infraorbital, infusion, intraarterial, intracapsular, intracardiac, intradermal, intramuscular, intraperitoneal, intrapulmonary, intraspinal, intrasternal, intrathecal, intrauterine, intravenous, subarachnoid, subcapsular, subcutaneous, transmucosal, or transtracheal. In some embodiments, the antigen binding construct can be delivered intraoperatively as a local administration during an intervention or resection.

[0309] In some embodiments, an antigen binding construct is conjugated to a therapeutic agent. A "therapeutic agent" as used herein is an atom, molecule, or compound that is useful in the treatment of a disorder related to a target molecule. Examples of therapeutic agents include, but are not limited to, drugs, chemotherapeutic agents, therapeutic antibodies and antibody fragments, toxins, radioisotopes, enzymes (for example, enzymes to cleave prodrugs to a cytotoxic agent at the site of the antigen binding construct binding), nucleases, hormones, immunomodulators, antisense oligonucleotides, chelators, boron compounds, photoactive agents and dyes, and nanoparticles. Examples of disorders include those related to one or more target molecules. In some embodiments, the target molecules can be one or more of: CD8, CD3, PSMA, PSCA, and 5T4.

[0310] Chemotherapeutic agents are often cytotoxic or cytostatic in nature and may include alkylating agents, antimetabolites, anti-tumor antibiotics, topoisomerase inhibitors, mitotic inhibitors hormone therapy, targeted therapeutics and immunotherapeutics. In some embodiments the chemotherapeutic agents that may be used as detectable markers in accordance with the embodiments of the disclosure include, but are not limited to, 13-cis-Retinoic Acid, 2-Chlorodeoxyadenosine, 5-Azacitidine, 5-Fluorouracil, 6-Mercaptopurine, 6-Thioguanine, actinomycin-D, adriamycin, aldesleukin, alemtuzumab, alitretinoin, all-transretinoic acid, alpha interferon, altretamine, amethopterin, amifostine, anagrelide, anastrozole, arabinosylcytosine, arsenic trioxide, amsacrine, aminocamptothecin, aminoglutethimide, asparaginase, azacytidine, bacillus calmette-guerin (BCG), bendamustine, bevacizumab, bexarotene, bicalutamide, bortezomib, bleomycin, busulfan, calcium leucovorin, citrovorum factor, capecitabine, canertinib, carboplatin, carmustine, cetuximab, chlorambucil, cisplatin, cladribine, cortisone, cyclophosphamide, cytarabine,

darbepoetin alfa, dasatinib, daunomycin, decitabine, denileukin diftitox, dexamethasone, dexasone, dexrazoxane, dactinomycin, daunorubicin, decarbazine, docetaxel, doxorubicin, doxifluridine, eniluracil, epirubicin, epoetin alfa, erlotinib, everolimus, exemestane, estramustine, etoposide, filgrastim, fluoxymesterone, fulvestrant, flavopiridol, floxuridine, fludarabine, fluorouracil, flutamide, gefitinib, gemcitabine, gemtuzumab ozogamicin, goserelin, granulocyte - colony stimulating factor, granulocyte macrophage-colony stimulating factor, hexamethylmelamine, hydrocortisone hydroxyurea, ibritumomab, interferon alpha, interleukin – 2, interleukin-11, isotretinoin, ixabepilone, idarubicin, imatinib mesylate, ifosfamide, irinotecan, lapatinib, lenalidomide, letrozole, leucovorin, leuprolide, liposomal Ara-C, lomustine, mechlorethamine, megestrol, melphalan, mercaptopurine, mesna, methotrexate, methylprednisolone, mitomycin C, mitotane, mitoxantrone, nelarabine, nilutamide, octreotide, oprelvekin, oxaliplatin, paclitaxel, pamidronate, pemetrexed, panitumumab, PEG Interferon, pegaspargase, pegfilgrastim, PEG-L-asparaginase, pentostatin, plicamycin, prednisolone, prednisone, procarbazine, raloxifene, rituximab, romiplostim, ralitrexed, sapacitabine, sargramostim, satraplatin, sorafenib, sunitinib, semustine, streptozocin, tamoxifen, tegafur, tegafur-uracil, temsirolimus, temozolamide, teniposide, thalidomide, thioguanine, thiotepa, topotecan, toremifene, tositumomab, trastuzumab, tretinoin, trimitrexate, alrubicin, vincristine, vinblastine, vindestine, vinorelbine, vorinostat, or zoledronic acid.

[0311] Toxins that may be used in accordance with the embodiments of the disclosure include, but are not limited to, Auristatin E, Auristatin F, Dolastatin 10, Dolastatin 15, combretastatin and their analogs, maytansinoid, calicheamicin, alpha-amanitin, pyrrolobenzodiazepine dimers, epothilones, duocarmycin and their analogs, tubulysin D, basillistatins, ricin, abrin, ribonuclease (RNase), DNase I, Staphylococcal enterotoxin-A, pokeweed antiviral protein, gelonin, diphtheria toxin, Pseudomonas exotoxin, and Pseudomonas endotoxin.

[0312] In some embodiments nanoparticles are used in therapeutic applications as drug carriers that, when conjugated to an antigen binding construct, deliver chemotherapeutic agents, hormonal therapeutic agents, radiotherapeutic agents, toxins, or any other cytotoxic or anti-cancer agent known in the art to cancerous cells that overexpress the target on the cell surface.

[0313] Any of the antigen binding constructs described herein may be further conjugated with one or more additional therapeutic agents, detectable markers, nanoparticles, carriers or a combination thereof. For example, an antigen binding construct may be radiolabeled with Iodine-131 and conjugated to a lipid carrier, such that the anti-target molecule-lipid conjugate forms a micelle. The micelle can incorporate one or more therapeutic or detectable markers. Alternatively, in addition to the carrier, the antigen binding construct may be radiolabeled with Iodine-131 I (for example, at a tyrosine residue) and conjugated to a drug (for example, at the epsilon amino group of a lysine residue), and the carrier may incorporate an additional therapeutic or detectable marker.

[0314] In some embodiments, antigen binding constructs are conjugated to a therapeutic agent. While these antigen binding constructs can have a shorter circulation half-life compared to a full-length antibody, in some embodiments, these formats can exhibit improved tumor penetration based on their smaller size and be therapeutically effective when appropriately armed with a cytotoxic drug or radioisotope. In some embodiments, an antibody drug-conjugate approach can be employed. In some embodiments, a therapeutic approach includes radioimmunotherapy by attaching an appropriate radiolabel such as, Iodine-131, a beta-emitter, such as, Yttrium-90, Lutetium-177, Copper-67, Astatine-211, Lead-212/Bismuth-212, Actinium-225/Bismuth-213, , and Thorium, which can deliver cell damage and death to a target tissue. In some embodiments, treatment with these fragments armed with a cytotoxic drug or radionuclide result in less nonspecific toxicity as they will be cleared from the body more rapidly.

[0315] In some embodiments, the antigen binding construct can be connected to a therapeutic agent to a disorder associated with the expression of the target molecule.

[0316] In some embodiments, target molecule antigen binding constructs are used as a stand-alone medicament (e.g. antigen binding construct in the absence of therapeutic agent) in the treatment of a disorder associated with the expression of the target molecule.

[0317] In some embodiments, a pharmaceutical composition is provided comprising the amino acid hinge region of any of the embodiments provided herein. In some embodiments, less than 30% aggregation (or high molecular weight construct) of a minibody is present in the composition, for example, less than 20, 10, 5, or 1% aggregation.

[0318] In some embodiments, a pharmaceutical composition comprising the amino acid hinge region of any of the constructs provided herein is provided. In some embodiments, an amount of 1 micrograms to 100 mg can be used. In some embodiments, such a composition can have a reduced level of aggregation compared to constructs without the noted hinge arrangements.

[0319] In some embodiments, the disorder is one associated with 5T4, CD8, CD3, PSCA, or PSMA. However, the various hinges disclosed herein can be applied to any target molecule or antigen binding construct that includes a hinge.

Kits

[0320] In some embodiments, kits are provided. In some embodiments, the kit includes an antigen binding construct as described herein. In some embodiments, the kit includes a nucleic acid that encodes an antigen binding construct as described herein. In some embodiments, the kit includes a cell line that produces an antigen binding construct as described herein. In some embodiments, the kit includes a detectable marker as described herein. In some embodiments, the kit includes a therapeutic agent as described herein. In some embodiments, the kit includes buffers. In some embodiments, the kit includes positive controls, for example target specific cells, or fragments thereof. In some embodiments, the kit includes negative controls, for example a surface or solution that is substantially free of the target. In some embodiments, the kit includes packaging. In some embodiments, the kit includes instructions.

Methods of detecting the presence or absence of the target molecule

[0321] Antigen binding constructs can be used to detect the presence or absence of the target molecule *in vivo* and/or *in vitro*. Accordingly, some embodiments include methods of detecting the presence or absence of the target. The method can include applying an antigen binding construct to a sample. The method can include detecting a binding or an absence of binding of the antigen binding construct to the target molecule. In some embodiments, any target molecule could be detected through the options provided herein. In some embodiments, the target molecule to be detected is one or more of 5T4, CD8, CD3, PSCA, or PSMA. In some embodiments, the target molecule is detected using one or more

of the minibody arrangements provided in the figures and/or in the Tables, such as Table 0.2 (or at least employing a hinge arrangement as outlined in Table 0.1).

[0322] Methods of detecting the presence or absence of the target molecule are provided herein. It will be appreciated that the processes below can be performed in any sequence, and/or can be optionally repeated and/or eliminated, and that additional steps can optionally be added to the method. In some embodiments, an antigen binding construct as described herein can be applied to a sample. In some embodiments, an optional wash can be performed. Optionally, a secondary antigen binding construct can be applied to the sample. An optional wash can be performed. In some embodiments, a binding or absence of binding of the antigen binding construct to the target molecule can be detected.

[0323] In some embodiments, an antigen binding construct as described herein is applied to a sample *in vivo*. The antigen binding construct can be administered to a subject. In some embodiments, the subject is a human. In some embodiments, the subject is a non-human mammal, for example a rat, mouse, guinea pig, hamster, rabbit, dog, cat, cow, horse, goat, sheep, donkey, pig, monkey, or ape. In some embodiments, the antigen binding construct is infused into the subject. In some embodiments, the infusion is intravenous. In some embodiments, the infusion is intraperitoneal. In some embodiments, the antigen binding construct is applied topically or locally (as in the case of an interventional or intraoperative application) to the subject. In some embodiments, a capsule containing the antigen binding construct is applied to the subject, for example orally or intraperitoneally. In some embodiments, the antigen binding construct is selected to reduce the risk of an immunogenic response by subject. For example, for a human subject, the antigen binding construct can be humanized as described herein. In some embodiments, following *in vivo* application of the antigen binding construct, the sample, or a portion of the sample is removed from the host. In some embodiments, the antigen binding construct is applied *in vivo*, is incubated *in vivo* for a period of time as described herein, and a sample is removed for analysis *in vitro*, for example *in vitro* detection of antigen binding construct bound to the target molecule or the absence thereof as described herein.

[0324] In some embodiments, the antigen binding construct is applied to a sample *in vitro*. In some embodiments, the sample is freshly harvested from a subject, for example a biopsy. In some embodiments, the sample is incubated following harvesting from a subject.

In some embodiments, the sample is fixed. In some embodiments the sample includes a whole organ and/or tissue. In some embodiments, the sample includes one or more whole cells. In some embodiments the sample is from cell extracts, for example lysates. In some embodiments, antigen binding construct in solution is added to a solution in the sample. In some embodiments, antigen binding construct in solution is added to a sample that does not contain a solution, for example a lyophilized sample, thus reconstituting the sample. In some embodiments, lyophilized antigen binding construct is added to a sample that contains solution, thus reconstituting the antigen binding construct.

[0325] In some embodiments, the antigen binding construct is optionally incubated with the sample. The antigen binding construct can be incubated for a period of no more than about 14 days, for example no more than about 14 days, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 day, or no more than about 23 hours, for example no more than about 23 hours, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1, 0.75, 0.5, 0.25, or 0.1 hour, including ranges between any two of the listed values. In some embodiments, the incubation is within a subject to which the antigen binding construct was administered. In some embodiments, the incubation is within an incubator. In some embodiments, the incubator is maintained at a fixed temperature, for example about 21°C, room temperature, 25°C, 29°C, 34°C, 37°C, or 40°C.

[0326] In some embodiments, the antigen binding construct that is not bound to the target is optionally removed from the sample. In some embodiments, the sample is washed. Washing a sample can include removing the solution that contains unbound antigen binding construct, and adding solution that does not contain antigen binding construct, for example buffer solution. In some embodiments, an *in vitro* sample is washed, for example by aspirating, pipetting, pumping, or draining solution that contains unbound antigen binding construct, and adding solution that does not contain antigen binding construct. In some embodiments, an *in vivo* sample is washed, for example by administering to the subject solution that does not contain antigen binding construct, or by washing a site of topical antigen binding construct administration. In some embodiments, the wash is performed at least two times, for example at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, or 20 times. In some embodiments, following the wash or washes, at least about 50% of unbound antibody is

removed from the sample, for example at least about 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or greater.

[0327] In some embodiments, unbound antigen binding construct is eliminated from the sample. Following application of the antigen binding construct to the sample, antigen binding construct bound to the target reaches an equilibrium with antigen binding construct unbound to the target, so that at some time after application of the antigen binding construct, the amount of antigen binding construct bound to the target does not substantially increase. After this time, at least part of the quantity of the antigen binding construct that is unbound to the target can be eliminated. In some embodiments, unbound antigen binding construct is eliminated by metabolic or other bodily processes of the subject to whom the antibody or fragment was delivered. In some embodiments, unbound antigen binding construct is eliminated by the addition of an agent that destroys or destabilized the unbound antigen binding construct, for example a protease or a neutralizing antibody. In some embodiments, 1 day after application of the antigen binding construct, at least about 30% of the antigen binding construct that was applied has been eliminated, for example at least about 30%, 40%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 99.9%. In some embodiments, 2 days after application of the antigen binding construct, at least about 40% of the antigen binding construct that was applied has been eliminated, for example at least about 40%, 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 99.9%.

[0328] In some embodiments, the presence or absence of the target molecule is detected. The presence or absence of the target can be detected based on the presence or absence of the antigen binding construct in the sample. After removal and/or elimination of the antigen binding construct from the sample, for example by washing and/or metabolic elimination, remaining antigen binding construct in the sample can indicate the presence of the target, while an absence of the antigen binding construct in the sample can indicate the absence of the target.

[0329] In some embodiments, the antigen binding construct includes a detectable marker as described herein. Thus, the presence of the antigen binding construct can be inferred by detecting the detectable marker.

[0330] In some embodiments, a secondary antigen binding construct is used to detect the antigen binding construct. The secondary antigen binding construct can bind

specifically to the antigen binding construct. For example, the secondary antigen binding construct can include a polyclonal or monoclonal antibody, diabody, minibody, etc. against the host type of the antibody, or against the antigen binding construct itself. The secondary antigen binding construct can be conjugated to a detectable marker as described herein. The secondary antigen binding construct can be applied to the sample. In some embodiments, the secondary antigen binding construct is applied to the sample in substantially the same manner as the antigen binding construct. For example, if the antigen binding construct was infused into a subject, the secondary antigen binding construct can also be infused into the subject.

[0331] In some embodiments, binding or the absence of binding of the antigen binding construct is detected via at least one of: positron emission tomography (PET), single-photon emission computed tomography (SPECT), magnetic resonance imaging (NMR), or detection of fluorescence emissions. PET can include, but is not limited to small animal PET imaging. In some embodiments, binding of the absence of binding of the antigen binding construct is detected via two or more forms of imaging. In some embodiments, detection can be via near-infrared (NIR) and/or Cerenkov.

[0332] In some embodiments, any combination of imaging modalities is possible, including, by way of example, PET + NIR, PET + SPECT etc.

Methods of targeting a therapeutic agent to a cell

[0333] Antigen binding constructs can be used to target a therapeutic molecule, for example a cytotoxin, to a target positive cell, such as a cell expressing the target molecule. Thus, some embodiments include methods of targeting a therapeutic agent to a target positive cell. The method can include administering an antigen binding construct as described herein to a subject. The subject can be a subject in need, for example a subject in need of elimination or neutralization of at least some target positive cells. In some embodiments, the antigen binding construct includes at least on therapeutic agent as described herein. In some embodiments, the therapeutic can be directly conjugated to the antigen binding construct via a covalent bond, such as a disulfide bond. In some embodiments, the subject can benefit from the localization of a target molecule positive cell to another cell or agent.

[0334] Optionally, before and/or after administration of the antigen binding construct that includes at least one therapeutic agent, the number and/or localization of the target positive cells of the patient is determined. For example, determining the number and/or localization of target positive cells prior to administration can indicate whether the patient is likely to benefit from neutralization and/or elimination of the target positive cells. Determining the number and/or localization of the target positive cells after administration can indicate whether the target positive cells were eliminated in the patient.

[0335] In some embodiments, the antigen binding construct can be used as a therapeutic agent as stand-alone construct (for example, without a toxin conjugated thereto). While the fragments have a shorter half-life compared to the intact antibody which would be less optimal for therapy, these formats can exhibit improved tumor penetration based on their smaller size and be therapeutically effective when appropriately armed with a cytotoxic drug or radioisotope.

[0336] In some embodiments, another therapeutic approach is radioimmunotherapy via attaching an appropriate radiolabel such as the Iodine-131, a beta-emitter, such as, Yttrium-90, Lutetium-177, Copper-67, Astatine-211, Lead-212/Bismuth-212, Actinium-225/Bismuth-213, , and Thorium, which can deliver cell damage and death.

[0337] In some embodiments, the antigen binding construct is used to target cells expressing the target molecule. In some embodiments, the therapeutic agent is appropriate for one or more of a disorder associated with at least one of the following: CD8, CD3, PSMA, PSCA, or 5T4, for example. In some embodiments, the therapeutic agent is appropriate for treating one or more of the following disorders:

Specific Targets/Constructs

[0338] In some embodiments, any of the compositions, methods (for example, methods of treatment, methods of making, methods of detection, etc.), kits, agents, antigen binding construct modifications, cell lines, nucleic acids, etc. provided herein can be used for any target molecule. In some embodiments, the target molecule can be one associated with cancer immunotherapy. In some embodiments, the target molecule can be one or more of CD8, CD3, 5T4, PSCA, or PSMA, including variants thereof.

[0339] In some embodiments, one or more of the antigen binding constructs, such as the minibody, can be used for the treatment of a subject having a target molecule

associated disorder. In some embodiments, the target molecule can be any target molecule for which one has an antigen binding construct that will bind. In some embodiments, the target molecule can be at least one of the following: CD8, CD3, PSMA, PSCA, or 5T4, and thus, the disorder to be treated can be one relating to at least one of the following: CD8, CD3, PSMA, PSCA, or 5T4. In some embodiments, one or more of the antigen binding constructs, such as the minibody, can be used for the diagnostic of a subject as to whether or not they have a target molecule associated disorder.

[0340] As used herein, the term "subject" refers to any animal (e.g., a mammal), including but not limited to humans, non-human primates, rodents, dogs, pigs, and the like.

[0341] Each of the sections below outlines various embodiments for some of the various antigen binding constructs provided herein. All of these embodiments are provided as various forms of antigen binding constructs, including minibodies and scFvs. The below sections are explicitly provided for target specific embodiments; however, they are contemplated as aspects that are combinable with any of the appropriate options provided elsewhere in the present specification. Thus, the present embodiments are not exclusive of the other embodiments, but instead are options that can be combined with any of the other embodiments provided herein. For example, any of the hinge arrangements provided herein can be used in any one or more of the noted constructs and/or methods. Similarly, the various embodiments outlined below in regard to any one of the five targets of CD8, CD3, PSMA, PSCA, or 5T4 can also be swapped with the other noted targets. Thus, while the PSCA discussion is directed to PSCA, it will be understood that the embodiments can also be applied to CD8, CD3, PSMA, or 5T4 constructs. Similarly, disclosures to CD8 can also be applied to CD3, PSMA, PSCA, or 5T4, for example.

[0342] In some embodiments, chemotherapeutic agents can be used with any one or more of the CD8, CD3, PSMA, PSCA, or 5T4 constructs provided herein. Chemotherapeutic agents are often cytotoxic or cytostatic in nature and can include alkylating agents, antimetabolites, anti-tumor antibiotics, topoisomerase inhibitors, mitotic inhibitors hormone therapy, targeted therapeutics and immunotherapeutics. In some embodiments the chemotherapeutic agents that can be used as diagnostic agents in accordance with the embodiments of the disclosure include, but are not limited to,13-cis-Retinoic Acid, 2-Chlorodeoxyadenosine, 5-Azacitidine, 5-Fluorouracil, 6-Mercaptopurine, 6-

Thioguanine, actinomycin-D, adriamycin, aldesleukin, alemtuzumab, alitretinoin, all-transretinoic acid, alpha interferon, altretamine, amethopterin, amifostine, anagrelide, anastrozole,arabinosylcytosine, arsenic trioxide, amsacrine, aminocamptothecin, aminoglutethimide, asparaginase, azacytidine, bacillus calmette-guerin (BCG), bendamustine, bevacizumab, bexarotene, bicalutamide, bortezomib, bleomycin, busulfan, calcium leucovorin, citrovorum factor, capecitabine, canertinib, carboplatin, carmustine, cetuximab, chlorambucil, cisplatin, cladribine, cortisone, cyclophosphamide, cytarabine, darbepoetin alfa, dasatinib, daunomycin, decitabine, denileukin diftitox, dexamethasone, dexasone, dexrazoxane, dactinomycin, daunorubicin, decarbazine, docetaxel, doxorubicin, doxifluridine, eniluracil, epirubicin, epoetin alfa, erlotinib, everolimus, exemestane, estramustine, etoposide, filgrastim, fluoxymesterone, fulvestrant, flavopiridol, floxuridine, fludarabine, fluorouracil, flutamide, gefitinib, gemcitabine, gemtuzumab ozogamicin, goserelin, granulocyte - colony stimulating factor, granulocyte macrophage-colony stimulating factor, hexamethylmelamine, hydrocortisone hydroxyurea, ibritumomab, interferon alpha, interleukin – 2, interleukin-11, isotretinoin, ixabepilone, idarubicin, imatinib mesylate, ifosfamide, irinotecan, lapatinib, lenalidomide, letrozole, leucovorin, leuprolide, liposomal Ara-C, lomustine, mechlorethamine, megestrol, melphalan, mercaptopurine, mesna, methotrexate, methylprednisolone, mitomycin C, mitotane, mitoxantrone, nelarabine, nilutamide, octreotide, oprelvekin, oxaliplatin, paclitaxel, pamidronate, pemetrexed, panitumumab, PEG Interferon, pegaspargase, pegfilgrastim, PEG-L-asparaginase, pentostatin, plicamycin, prednisolone, prednisone, procarbazine, raloxifene, rituximab, romiplostim, ralitrexed, sapacitabine, sargramostim, satraplatin, sorafenib, sunitinib, semustine, streptozocin, tamoxifen, tegafur, tegafur-uracil, temsirolimus, temozolamide, teniposide, thalidomide, thioguanine, thiotepa, topotecan, toremifene, tositumomab, trastuzumab, tretinoin, trimitrexate, alrubicin, vincristine, vinblastine, vindestine, vinorelbine, vorinostat, or zoledronic acid.

[0343] In some embodiments, any one or more of the constructs, for example, CD8, CD3, PSMA, PSCA, or 5T4 antigen binding constructs, can be linked via one or more of the cysteine in the hinge to chelators, drug, or any of the other components described herein.

PSCA-IAB1M

[0344] Prostate stem cell antigen (PSCA) is a cell surface glycoprotein expressed in normal human prostate and bladder and is over-expressed in prostate cancers (40% of primary tumors and 60-100% of lymph node and bone marrow metastases). It is also highly expressed in transitional carcinomas of the bladder and pancreatic carcinoma. An example of PSCA protein is shown with SEQ ID NO: 132. IG8, an anti-PSCA mouse monoclonal antibody specific for PSCA demonstrated anti-tumor targeting activity *in vivo* (Gu, Z. et al., "Anti-prostate stem cell antigen monoclonal antibody 1G8 induces cell death in vitro and inhibits tumor growth in vivo via a Fc-independent mechanism," Cancer Res., Vol. 65, No. 20, pp. 9495-9500, 2005). This antibody was humanized by grafting on a human framework (Trastuzumab) and named 2B3 (Olafsen, T. et al., "Targeting, imaging, and therapy using a humanized antiprostate stem cell antigen (PSCA) antibody," Immunotherapy, Vol. 30, No. 4, pp. 396-405, 2007).

[0345] In some embodiments, embodiments involving antigen binding constructs to PSCA can provide agents that have appropriate pharmacodynamics properties to target and image tumors that express PSCA. There is a value in the field for effective agents to image cancers with sensitivity and specificity, particularly early stage tumors or ones with early metastasis not imageable by traditional means. As PSCA is highly expressed by most prostate, bladder and pancreatic tumors, it is a valuable target in the detection, diagnosis, prognosis, and treatment of these cancers. Some embodiments provided herein provide constructs with characteristics for tumor imaging and targeting. They can also be used for tumor targeting of gene therapy, radioactivity therapy, and can have therapeutic utility by themselves.

[0346] Provided herein are engineered antigen binding constructs that recognize a novel cell surface marker in prostate and other cancers with high affinity. These genetically engineered antigen binding constructs can be tailored specifically for *in vivo* use for targeting and detection. PSCA is highly expressed by most prostate, bladder and pancreatic tumors and is a promising target. Embodiments provided herein describe an innovative molecule with optimal characteristics for tumor imaging. It can also be useful for tumor targeting of gene therapy, radioactivity or can have therapeutic utility by itself.

[0347] In some embodiments, the PSCA targeted by the anti-PSCA antibody is human PSCA. In some embodiments, the immunoconjugate can be used for targeting the effector moiety to a PSCA-positive cell, particularly cells, which overexpress the PCSA protein. In some embodiments, the PSCA targeted by the anti-PSCA minibody is human PSCA.

[0348] In some embodiments, the constructs provided herein can provide agents that have appropriate pharmacodynamics properties to target and image tumors that express PSCA. In some embodiments, the minibody provides an effective agent to image cancers with sensitivity and specificity, particularly early stage tumors or ones with early metastasis not imageable by traditional means.

[0349] In some embodiments, antigen binding constructs that bind the PSCA antigen can be antibodies, minibodies and/or other fragments of antibodies such as scFv. Some non-limiting embodiments of antigen binding constructs against PSCA are shown in FIGs. 29B – 29D, 36E, 60 (SEQ ID NOs: 125 and 126), 61 (SEQ ID NO: 126), 62 (SEQ ID NO: 127), 63 (SEQ ID NO: 128), 64 (SEQ ID NO: 129), and 65A (SEQ ID NO: 130).

[0350] In some embodiments, the construct allows for imaging of cancer, in early diagnosis or diagnosis of metastatic disease. In particular, there is value in better agents for imaging prostate cancer for detection and staging. PSCA antigen binding construct imaging will be very useful for imaging bone metastases and assessing response to treatment. In some embodiments, a method of the detection of pancreatic cancer is provided. In some embodiments, a high-affinity, highly specific engineered minibody is tailored for *in vivo* targeting and detection of PSCA in prostate cancer, bladder cancer, and pancreatic cancer patients. In some embodiments, a "PSCA dependent disorder" can include a prostate tumor, a prostate cancer, a bladder tumor, a transitional carcinoma of the urinary bladder, a pancreatic tumor, a pancreatic carcinoma, any tumor associated with PSCA expression, any cancer associated with PSCA expression, or any disorder associated with PSCA expression.

[0351] In some embodiments, a high affinity PSCA antigen binding construct which can be used in the treatment and detection of cancers which overexpress PSCA is provided.

[0352] In some embodiments, the antigen binding construct can also be linked to therapeutic agents or detectable markers. In some embodiments, the therapeutic agent is a

cytotoxic agent. For instance, the agent can be ricin, ricin A-chain, doxorubicin, daunorubicin, taxol, ethidium bromide, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicine, dihydroxy anthracin dione, actinomycin D, diphteria toxin, Pseudomonas exotoxin (PE) A, PE40, abrin, arbrin A chain, modeccin A chain, alpha-sarcin, gelonin mitogellin, retstrictocin, phenomycin, enomycin, curicin, crotin, calicheamicin, sapaonaria officinalis inhibitor, maytansinoids, or glucocorticoidricin. In other embodiments, the therapeutic agent is a radioactive isotope. The radioactive isotope can be selected, for instance, from the group consisting of ^{212}Bi, ^{131}I, ^{111}In, ^{90}Y and ^{186}Re. In other embodiments the construct is linked to an anti-cancer pro-drug activating enzyme capable of converting a pro-drug to its active form.

[0353] In some embodiments, the anti-PSCA construct is labeled with a detectable marker. The marker can be for instance, a radioisotope, a fluorescent compound, a bioluminescent compound, chemiluminescent compound, a metal chelator or an enzyme. Many radionuclides can be used as imaging labels, including without limitation, ^{124}I, ^{86}Y, ^{18}F, ^{94m}Tc, and the like. One of skill in the art will know of other radionuclides particularly well suited for use in the present embodiment. In some embodiments, the method can kill the cancer cell. In some embodiments, the construct recognizes and binds the PSCA protein as shown below beginning with leucine at amino acid position 22 and ending with alanine at amino acid position 99. In additional embodiments, the method further comprises administering to a chemotherapeutic drug, radiation therapy. In some embodiments, the subject is also treated with hormone ablation therapy or hormone antagonist therapy.

[0354] In some embodiments, the treatments can be given to the patient or subject by intravenously, intraperitoneally, intramuscularly, intratumorally, or intradermally. In some embodiments, contacting comprises administering the construct directly into a prostate cancer, a bladder cancer, a pancreatic cancer or a metastasis thereof.

[0355] In some embodiments, methods of detecting a cancerous cell in a subject by contacting the cancer cell with a construct which bears a detectable marker is provided. The methods can be used in screening patients at increased risk of cancer or to monitory response to therapy or to develop a prognosis for the cancer (e.g., prostate, bladder, or pancreatic cancers. The methods are particularly advantageous in detecting metastases of the cancer. Provided herein are minibody fragments with tumor targeting/imaging aptitude. In

some embodiments, with regard to the constructs provided herein, there is a proviso that the construct comprises a VH or VL domain that is not identical to a corresponding domain or the 2B3 antibody.

[0356] In some embodiments, the antigen binding construct is conjugated to an "effector" moiety. The effector moiety can be any number of molecules, including labeling moieties such as radioactive labels or fluorescent labels, or can be a therapeutic moiety. In one aspect the antibody modulates the activity of the protein. Such effector moieties include, but are not limited to, an anti-tumor drug, a toxin, a radioactive agent, a cytokine, a second antibody or an enzyme. Further, herein provided are embodiments wherein the antigen binding construct is linked to an enzyme that converts a prodrug into a cytotoxic agent. Examples of cytotoxic agents include, but are not limited to ricin, doxorubicin, daunorubicin, taxol, ethiduim bromide, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicine, dihydroxy anthracin dione, actinomycin D, diphtheria toxin, Pseudomonas exotoxin (PE) A, PE40, abrin, and glucocorticoid and other chemotherapeutic agents, as well as radioisotopes. Suitable detectable markers include, but are not limited to, a radioisotope, a fluorescent compound, a bioluminescent compound, chemiluminescent compound, a metal chelator or an enzyme.

[0357] In some embodiments, the antigen binding protein constructs can be used to systemically to treat cancer alone or when conjugated with an effector moiety. PSCA-targeting constructs conjugated with toxic agents, such as ricin, as well as unconjugated antibodies can be useful therapeutic agents naturally targeted to PSCA bearing cancer cells. Such constructs can be useful in blocking invasiveness.

[0358] In some embodiments, the antigen-binding protein constructs can be used to treat cancer. In such a situation, the construct is joined to at least a functionally active portion of a second protein or toxic molecule having therapeutic activity. The second protein can include, but is not limited to, an enzyme, lymphokine, oncostatin or toxin. Suitable toxins include doxorubicin, daunorubicin, taxol, ethiduim bromide, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicine, dihydroxy anthracin dione, actinomycin D, diphteria toxin, Pseudomonas exotoxin (PE) A, PE40, ricin, abrin, glucocorticoid and radioisotopes.

[0359] In some embodiments, treatment will generally involve the repeated administration of the constructs and their immunoconjugates via an acceptable route of administration such as intravenous injection (N), at an effective dose. Dosages will depend upon various factors generally appreciated by those of skill in the art, including without limitation the type of cancer and the severity, grade, or stage of the cancer, the binding affinity and half-life of the agents used, the desired steady-state antibody concentration level, frequency of treatment, and the influence of chemotherapeutic agents used in combination with the treatment method of the embodiments provided herein. Typical daily doses can range from about 0.1 to 100 mg/kg. Doses in the range of 10-500 mg of the constructs or their immunoconjugates per week can be effective and well tolerated, although even higher weekly doses can be appropriate and/or well tolerated. The principal determining factor in defining the appropriate dose is the amount of a particular agent necessary to be therapeutically effective in a particular context. Repeated administrations can be required in order to achieve tumor inhibition or regression. Initial loading doses can be higher. The initial loading dose can be administered as an infusion. Periodic maintenance doses can be administered similarly, provided the initial dose is well tolerated.

[0360] In some embodiments, direct administration of the constructs is also possible and can have advantages in certain contexts. For example, for the treatment of bladder carcinoma, the agents can be injected directly into the bladder.

[0361] In some embodiments, the compositions can be administered for therapeutic or prophylactic treatments. In therapeutic applications, compositions are administered to a patient suffering from a disease (e.g., cancer) in a "therapeutically effective dose." Amounts effective for this use will depend upon the severity of the disease and the general state of the patient's health. Single or multiple administrations of the compositions can be administered depending on the dosage and frequency as required and tolerated by the patient. Other known cancer therapies can be used in combination with the methods provided herein. For example, the compositions provided herein can also be used to target or sensitize a cell to other cancer therapeutic agents such as 5FU, vinblastine, actinomycin D, cisplatin, methotrexate, and the like.

[0362] In other embodiments, the methods can be practiced together with other cancer therapies (e.g, radical prostatectomy), radiation therapy (external beam or

brachytherapy), hormone therapy (e.g., orchiectomy, LHRH-analog therapy to suppress testosterone production, anti-androgen therapy), or chemotherapy.

[0363] In some embodiments, methods of imaging cancer cells or tumors *in vivo* through administration of antibodies, such as the minibodies provided herein, are provided. In one embodiment, a method of imaging a cancer cell *in vivo* is provided, the method comprising administering a labeled anti-PSCA antibody to a mammal and imaging the antibody *in vivo*. The methods can be used to image a cancer cell in mammal, including without limitation, a mouse, rat, hamster, rabbit, pig, human, and the like.

[0364] In some embodiments, provided herein are methods for treating a subject having cancer, or inhibiting the growth of a prostate cancer cell expressing a Prostate Stem Cell Antigen (PSCA) protein comprising contacting the cancer cell (e.g., prostate, bladder, pancreatic cancer cell, with a construct as provided herein, in an amount effective to inhibit the growth of the cancer cell. In some embodiments, the methods find particular application in the diagnosis, prognosis and treatment of cancers which overexpress PSCA, for example, prostate, pancreatic and bladder cancers. In certain embodiments the methods are applied to hormone refractory or therapy resistant cancers. In certain embodiments the methods are applied to metastatic cancers.

[0365] In some embodiments, a minibody that binds to PSCA is provided. The minibody comprises a polypeptide that comprises a single-chain variable fragment (scFv) that binds to PSCA, the scFv comprising a variable heavy (V_H) domain linked a variable light (V_L) domain; and a variant hinge region comprising at least three cysteines on each strand of the hinge. In some embodiments, the minibody further comprises a human IgG C_H3 sequence. In some embodiments, the minibody further comprises a detectable marker selected from the group consisting of a radioactive substance, a dye, a contrast agent, a fluorescent compound, a bioluminescent compound, an enzyme, an enhancing agent, and a nanoparticle.

[0366] In some embodiments, the minibody provided herein comprises: a HCDR1 of the HCDR1 in SEQ ID NO: 93; a HCDR2 of the HCDR2 in SEQ ID NO: 94; a HCDR3 of the HCDR3 in SEQID NO: 95; a LCDR1 of the LCDR1 in SEQ ID NO: 96; a LCDR2 of the LCDR2 in SEQ ID NO: 97; and a LCDR3 of the LCDR3 in SEQ ID NO: 98. In some

embodiments of the minibody provided herein the variable heavy (V_H) domain and the variable light (V_L) domain are human sequences.

[0367] In some embodiments, a nucleic acid encoding an antibody of any of the embodiments described herein is provided. In some embodiments, a cell line producing any of the minibody embodiments described herein is provided. In some embodiments, a kit comprising any of the embodiments of the minibody described herein and a detectable marker are provided.

[0368] In some embodiments, a method of detecting the presence or absence of PSCA is provided. The method comprises applying any of the minibody embodiments provided herein to a sample; and detecting a binding or an absence of binding of the antigen binding construct thereof to PSCA. In some embodiments of the method, the minibody comprises a detectable marker selected from the group consisting of a radioactive substance, a dye, a contrast agent, a fluorescent compound, a bioluminescent compound, an enzyme, an enhancing agent, and a nanoparticle. In some embodiments of the method, applying the minibody comprises administering the minibody to a subject. In some embodiments of the method, detecting binding or absence of binding of the minibody thereof to a target antigen comprises positron emission tomography. In some embodiments, the method further comprises applying a secondary antibody or fragment thereof to the sample, wherein the secondary antibody or fragment thereof binds specifically to the minibody. In some embodiments of the method, the minibody thereof is incubated with the sample for no more than 1 hour.

[0369] In some embodiments, a method of targeting a therapeutic agent to PSCA is provided. The method comprises administering to a subject any of the embodiments of the minibody provided herein, wherein the minibody is conjugated to a therapeutic agent.

[0370] In some embodiments, a method of targeting PSCA in a subject in need thereof is provided. The method comprises administering to the subject a minibody of any one of the embodiments provided herein. In some embodiments of the method, the subject has at least one or more of a prostate tumor, a prostate cancer, a bladder tumor, a transitional carcinoma of the urinary bladder, a pancreatic tumor, a pancreatic carcinoma, any tumor associated with PSCA expression, any cancer associated with PSCA expression, or any disorder associated with PSCA expression.

[0371] In some embodiments, an antigen binding construct (such as a minibody or scFv) that binds to PSCA is provided, the antigen binding construct (such as a minibody or scFv) comprising a hinge region, wherein the hinge region comprises at least one of the following: a) a peptide sequence of SEQ ID NO: 1 ($X_{n1}CX_{n2}X_{n3}CX_{n4}X_{n5}C$), wherein X_{n1} can be any amino acid that does not form a covalent crosslinking bond, wherein X_{n2} is one of: A, R, N, D, E, Q, G, H, I, L, K, M, F, P, S, T, W, Y, or V, wherein X_{n3} can be any amino acid, wherein X_{n4} can be any amino acid, and wherein X_{n5} can be any amino acid; b) a peptide sequence of SEQ ID NO: 2 ($X_{n1}X_{n2}X_{n3}X_{n4}X_{n5}$ $X_{n6}CX_{n7}X_{n8}CX_{n9}X_{n10}C$), wherein X_{n1} can be any amino acid that does not form a covalent crosslinking bond with another identical amino acid, wherein X_{n2} can be any amino acid, wherein X_{n3} can be any amino acid, wherein X_{n4} can be any amino acid, wherein X_{n5} can be any amino acid, wherein X_{n6} can be any amino acid, wherein X_{n7} can be any amino acid, wherein X_{n8} can be any amino acid, wherein X_{n9} can be any amino acid, and wherein X_{n10} can be any amino acid (SEQ ID NO: 208); c) a core hinge sequence of at least one of: CVECPPCP (SEQ ID NO: 57), CPPCPPC (SEQ ID NO: 52), or CPPCPPCPPC (SEQ ID NO: 54), linked to an upper hinge sequence of ELKTPLGDTTHT (SEQ ID NO: 48); or d) an upper hinge region that comprises no amino acids capable of crosslinking with a corresponding amino acid, and a core hinge region connected to a C-terminus of the upper hinge region, wherein the core hinge region comprises at least three cysteines. In some embodiments, the antigen binding construct is a full length antibody. In some embodiments, the antibody is a minibody. In some embodiments of the antigen binding construct (such as a minibody or scFv), apart from the hinge region, the antigen binding construct (such as a minibody or scFv) comprises a humanized amino acid sequence. In some embodiments, the antigen binding construct (such as a minibody or scFv) comprises: a HCDR1 of the HCDR1 in SEQ ID NO: 93; a HCDR2 of the HCDR2 in SEQ ID NO: 94; a HCDR3 of the HCDR3 in SEQID NO: 95; a LCDR1 of the LCDR1 in SEQ ID NO: 96; a LCDR2 of the LCDR2 in SEQ ID NO: 97; and a LCDR3 of the LCDR3 in SEQ ID NO: 98.

[0372] In some embodiments, a nucleic acid encoding the hinge region of any of the antigen binding construct (such as a minibody or scFv) embodiments described herein is provided. In some embodiments of the nucleic acid, apart from the sequence encoding the hinge region, the nucleic acid comprises a human sequence. In some embodiments, a cell

line expressing the antigen binding construct (such as a minibody or scFv) encoded by the nucleic acid is provided.

[0373] In some embodiments, a method of manufacturing the antigen binding construct (such as a minibody or scFv) of any of the embodiments described herein is provided, the method comprising expressing the antibody in a cell line.

[0374] In some embodiments, a method of treating a condition in a subject in need thereof is provided, the method comprising administering to the subject the antibody of any of the embodiments provided herein.

PSMA-IAB2M

[0375] Full-length antibodies that target PSMA have been developed, some of which are in various stages of preclinical and clinical development. PSMA was originally defined by a murine antibody (mAb), 7E11, which recognized an intracellular epitope of PSMA (Olson, W. C. et al., "Clinical trials of cancer therapies targeting prostate-specific membrane antigen," Rev. Recent Clin. Trials, Vol. 2, No. 3, pp. 182-190, 2007). The 7E11 mAb was later developed into a FDA-approved SPECT imaging agent called Prostascint for the detection and imaging of prostate cancer in soft tissue. However, since 7E11 recognizes an intracellular epitope, Prostascint is a relatively poor imaging agent which is limited to detecting necrotic tumor tissue (Olson, W. C. et al., 2007). Having the pharmacokinetic properties of a full-length antibody, Prostascint also requires a long period of time between injection and imaging .. Furthermore, Prostascint is a murine antibody which elicits strong immune responses that prevent multiple dosing (Olson, W. C. et al., 2007).

[0376] Another full-length antibody that targets PSMA, J591, was discovered and subsequently deimmunized, the deimmunized version known as huJ591 (Liu, H. et al., "Monoclonal antibodies to the extracellular domain of prostate-specific membrane antigen also react with tumor vascular endothelium", Cancer Research, Vol. 57, No. 17, pp. 3629-3634, 1997; Bander, N. H. et al., "Targeting Metastatic Prostate Cancer with Radiolabeled Monoclonal Antibody J591 to the Extracellular Domain of Prostate Specific Membrane Antigen," J. Urol., Vol. 170, No. 5, pp. 1717-1721, 2003). The deimmunized huJ591 is an anti-human PSMA antibody that recognizes and binds an extracellular epitope on PSMA (Bander, N. H. et al., 2003). The huJ591 antibody is being developed as a potential radioimmunotherapy agent against prostate cancer. In Phase I trials, DOTA-conjugated

huJ591 antibody labeled with gamma emitting isotopes Indium-111 and Lutetium-177 demonstrated excellent targeting to metastatic sites, no immunogenicity, and multiple doses were well tolerated (Bander, N. H. et al., 2003, Milowsky, M. I. et al., "Phase I Trial of yttrium-90-labeled anti-prostate-specific membrane antigen monoclonal antibody J591 for androgen-independent prostate cancer," J. Clin. Oncol., Vol. 22, No. 13, pp. 2522-2531, 2004; Bander, N. H. et al., "Phase I trial of 177 Lutetium-labeled J591, a monoclonal antibody to prostate-specific membrane antigen, in patients with androgen-independent prostate cancer," J. Clin. Oncol., Vol. 23, No. 21, pp. 4591-4601, 2005; Olson, W. C. et al., 2007). Beyond prostate cancer, Phase I studies with ^{111}In-DOTA huJ591 demonstrated specific targeting of tumor neovasculature of advanced solid tumors (Milowsky, M. I. et al., "Vascular targeted therapy with anti-prostate-specific membrane antigen monoclonal antibody J591 in advanced solid tumors," J. Clin. Oncol., Vol. 25, No. 5, pp. 540-547, 2007).

[0377] In some embodiments, antigen binding constructs that bind the PSMA antigen can be antibodies, minibodies and/or other fragments of antibodies such as scFv. Some non-limiting embodiments of antigen binding constructs against PSMA are shown in FIGs. 5B – 5E, 7C - 7E, 21B - 21E, 34A - 34F, 35A - 35C, 36A, 47 (SEQ ID NOs: 112 and 113), 48 (SEQ ID NO: 113), 49 (SEQ ID NO: 114), 50 (SEQ ID NO: 115), 51 (SEQ ID NO: 116), 52 (SEQ ID NO: 117), 53 (SEQ ID NO: 118).

[0378] Prostate Specific Membrane Antigen (PSMA), a cell-surface biomarker that is associated with prostate cancer (Slovin, S. F., "Targeting novel antigens for prostate cancer treatment: focus on prostate-specific membrane antigen," Expert Opin. Ther. Targets, Vol. 9, No. 3, pp. 561-570, 2005), is a single-pass Type II transmembrane protein possessing glutamate carboxypeptidase activity, although the functional role of PSMA is not well understood (Olson, W. C. et al., 2007). Expression of PSMA is relatively limited in normal tissues outside of the prostate including the brain, small intestines, liver, proximal kidney tubules, and salivary gland (Olson, W. C. et al., 2007). An example of PSMA protein is shown with SEQ ID NO: 131.

[0379] In some embodiments, provided herein are antigen binding constructs, such as minibodies, that targets prostate specific membrane antigen (PSMA). The PSMA antigen binding construct thereof can be conjugated to a substance such as a diagnostic agent, a therapeutic agent or a nanoparticle to form an anti-PSMA conjugate. Also disclosed are

methods that include the use of the PSMA antigen binding construct or the anti-PSMA conjugate for diagnosing, visualizing, monitoring, or treating cancer or other conditions associated with overexpression of PSMA.

[0380] PSMA antigen binding constructs are provided herein according to the embodiments described herein. A PSMA antigen binding construct is a molecule that includes one or more portions of an immunoglobulin or immunoglobulin-related molecule that specifically binds to, or is immunologically reactive with a PSMA.

[0381] The PSMA antigen binding construct or anti-PSMA conjugate can be used to target a PSMA positive cell, such as cancer cells that overexpress PSMA.

[0382] In some embodiments, a method for diagnosing a cancer associated with PSMA expression in a subject is provided. Such a method includes administering an anti-PSMA minibody conjugated to a diagnostic agent to a subject having or suspected of having a cancer associated with PSMA expression; exposing the subject to an imaging method to visualize the labeled minibody *in vivo*; and determining that the subject has a cancer associated with PSMA expression when the labeled minibody localizes to a tumor site.

[0383] In some embodiments, a method for treating a cancer associated with PSMA expression in a subject is provided. Some embodiments include administering a therapeutically effective amount of a pharmaceutical composition to the subject, the composition comprising an anti-PSMA minibody. In some embodiments, the anti-PSMA minibody is conjugated to a therapeutic agent.

[0384] In some embodiments, the anti-PSMA conjugate can include a PSMA antigen binding construct (such as a minibody or scFv) conjugated to a therapeutic agent. A "therapeutic agent" as used herein is an atom, molecule, or compound that is useful in the treatment of cancer or other conditions associated with PSMA. Examples of therapeutic agents include, but are not limited to, drugs, chemotherapeutic agents, therapeutic antigen binding constructs, toxins, radioisotopes, enzymes (e.g., enzymes to cleave prodrugs to a cytotoxic agent at the site of the tumor), nucleases, hormones, immunomodulators, antisense oligonucleotides, chelators, boron compounds, photoactive agents and dyes.

[0385] Chemotherapeutic agents are often cytotoxic or cytostatic in nature and can include alkylating agents, antimetabolites, anti-tumor antibiotics, topoisomerase inhibitors, mitotic inhibitors hormone therapy, targeted therapeutics and immunotherapeutics.

In some embodiments the chemotherapeutic agents that can be used as diagnostic agents in accordance with the embodiments of the disclosure include, but are not limited to,13-cis-Retinoic Acid, 2-Chlorodeoxyadenosine, 5-Azacitidine, 5-Fluorouracil, 6-Mercaptopurine, 6-Thioguanine, actinomycin-D, adriamycin, aldesleukin, alemtuzumab, alitretinoin, all-transretinoic acid, alpha interferon, altretamine, amethopterin, amifostine, anagrelide, anastrozole,arabinosylcytosine, arsenic trioxide, amsacrine, aminocamptothecin, aminoglutethimide, asparaginase, azacytidine, bacillus calmette-guerin (BCG), bendamustine, bevacizumab, bexarotene, bicalutamide, bortezomib, bleomycin, busulfan, calcium leucovorin, citrovorum factor, capecitabine, canertinib, carboplatin, carmustine, cetuximab, chlorambucil, cisplatin, cladribine, cortisone, cyclophosphamide, cytarabine, darbepoetin alfa, dasatinib, daunomycin, decitabine, denileukin diftitox, dexamethasone, dexasone, dexrazoxane, dactinomycin, daunorubicin, decarbazine, docetaxel, doxorubicin, doxifluridine, eniluracil, epirubicin, epoetin alfa, erlotinib, everolimus, exemestane, estramustine, etoposide, filgrastim, fluoxymesterone, fulvestrant, flavopiridol, floxuridine, fludarabine, fluorouracil, flutamide, gefitinib, gemcitabine, gemtuzumab ozogamicin, goserelin, granulocyte - colony stimulating factor, granulocyte macrophage-colony stimulating factor, hexamethylmelamine, hydrocortisone hydroxyurea, ibritumomab, interferon alpha, interleukin – 2, interleukin-11, isotretinoin, ixabepilone, idarubicin, imatinib mesylate, ifosfamide, irinotecan, lapatinib, lenalidomide, letrozole, leucovorin, leuprolide, liposomal Ara-C, lomustine, mechlorethamine, megestrol, melphalan, mercaptopurine, mesna, methotrexate, methylprednisolone, mitomycin C, mitotane, mitoxantrone, nelarabine, nilutamide, octreotide, oprelvekin, oxaliplatin, paclitaxel, pamidronate, pemetrexed, panitumumab, PEG Interferon, pegaspargase, pegfilgrastim, PEG-L-asparaginase, pentostatin, plicamycin, prednisolone, prednisone, procarbazine, raloxifene, rituximab, romiplostim, ralitrexed, sapacitabine, sargramostim, satraplatin, sorafenib, sunitinib, semustine, streptozocin, tamoxifen, tegafur, tegafur-uracil, temsirolimus, temozolamide, teniposide, thalidomide, thioguanine, thiotepa, topotecan, toremifene, tositumomab, trastuzumab, tretinoin, trimitrexate, alrubicin, vincristine, vinblastine, vindestine, vinorelbine, vorinostat, or zoledronic acid.

[0386] Therapeutic antibodies and functional fragments thereof, that can be used as diagnostic agents in accordance with the embodiments of the disclosure include, but are

not limited to, alemtuzumab, bevacizumab, cetuximab, edrecolomab, gemtuzumab, ibritumomab tiuxetan, panitumumab, rituximab, tositumomab, and trastuzumab.

[0387] The PSMA antigen binding construct or anti-PSMA conjugate can be used to target a PSMA positive cell, such as cancer cells that overexpress PSMA. Therefore, methods for diagnosing, detecting, visualizing, monitoring or treating a cancer or other condition associated with PSMA expression can include administering the PSMA antigen binding construct or anti-PSMA conjugate to a subject having or suspected of having a cancer or other condition associated with PSMA expression.

[0388] In some embodiments, methods for treating cancer or other condition associated with overexpression of PSMA are provided. Such methods include administering to a subject a therapeutically effective amount of a pharmaceutical composition that includes a PSMA antigen binding construct as described herein. In one embodiment, the PSMA antigen binding construct is a minibody, derived from a J591 antibody such as those J591 minibodies described herein.

[0389] In some embodiments, the pharmaceutical composition can include a therapeutic anti-PSMA conjugate, wherein the conjugate includes a PSMA antigen binding construct conjugated to one or more therapeutic agent as described herein. In some embodiments, the PSMA antigen binding construct, derived from a J591 antibody such as those J591 minibodies described herein. For example, the J591 minibodies described herein can be used in a radioimmunotherapy approach, wherein one or more of the J591 minibodies is radiolabeled with an appropriate beta-emitting radiolabel such as Yttrium-90. The radiolabeled J591 minibody or minibodies can be used to deliver cell damage and death to local cancerous tissue that expresses PSMA. Further, the use of radiolabeled J591 minibodies would likely exhibit improved tumor penetration as compared to radiolabeled full-length parental huJ591 antibody.

[0390] The therapeutic anti-PSMA conjugate can be conjugated to or associated with one or more additional substances described herein, such as diagnostic anti-PSMA conjugates (described herein), unconjugated diagnostic agents, contrast solutions, carrier lipids or nanoparticles.

[0391] PSMA expression in prostate cancer increases with tumor aggressiveness and is the highest in high-grade tumors, metastatic lesions, and androgen-independent disease

(Olson, W. C. et al., 2007). Therefore, PSMA is a cancer biomarker that is a good candidate for targeting by an imaging agent. PSMA expression is also upregulated in the neovasculature of many non-prostatic solid tumors including lung, colon, breast, renal, liver and pancreatic carcinomas as well as sarcomas and melanoma (Olson, W. C. et al., 2007).

[0392] The cancer associated with PSMA expression in a subject can be lung cancer, colorectal cancer, breast cancer, renal cancer, liver cancer, bladder cancer, pancreatic cancer or melanoma. Thus, in some embodiments "PSMA dependent disorder" can include: lung cancer, colorectal cancer, breast cancer, renal cancer, liver cancer, bladder cancer, pancreatic cancer or melanoma.

[0393] Furthermore, "PSMA dependent disorder" can also include those cancers that are associated with PSMA expression including those having a cancer tumor tissue that overexpresses PSMA (e.g., prostate cancer) or those having solid tumor neovasculature that overexpresses PSMA (e.g., prostate cancer, lung cancer, colon (or colorectal) cancer, breast cancer, renal cancer, liver cancer, bladder cancer and pancreatic cancer as well as sarcomas and melanoma). Most solid tumor neovasculature expresses PSMA, making PSMA a neovasculature biomarker. Thus, in addition to cancer cells that express PSMA, a cancer that is associated with PSMA expression can include any cancer tissue with neovasculature including, but not limited to, carcinomas such as prostate cancer, lung cancer, colon (or colorectal) cancer, breast cancer, renal cancer, liver cancer, bladder cancer and pancreatic cancer as well as sarcomas and melanoma.

[0394] In some embodiments, a minibody that binds to PSMA is provided. The minibody comprises a polypeptide that comprises a single-chain variable fragment (scFv) that binds to PSMA, the scFv comprising a variable heavy (V_H) domain linked a variable light (V_L) domain; and a variant hinge region comprising at least three cysteines on each strand of the hinge. In some embodiments, the minibody further comprises a human IgG C_H3 sequence. In some embodiments, the minibody further comprises a detectable marker selected from the group consisting of a radioactive substance, a dye, a contrast agent, a fluorescent compound, a bioluminescent compound, an enzyme, an enhancing agent, and a nanoparticle.

[0395] In some embodiments, the minibody provided herein comprises: a HCDR1 of the HCDR1 in SEQ ID NO: 81; a HCDR2 of the HCDR2 in SEQ ID NO: 82; a HCDR3 of

the HCDR3 in SEQID NO: 83; a LCDR1 of the LCDR1 in SEQ ID NO: 84; a LCDR2 of the LCDR2 in SEQ ID NO: 85; and a LCDR3 of the LCDR3 in SEQ ID NO: 86. In some embodiments of the minibody provided herein the variable heavy (V_H) domain and the variable light (V_L) domain are human sequences.

[0396] In some embodiments, a nucleic acid encoding an antibody of any of the embodiments described herein is provided. In some embodiments, a cell line producing any of the minibody embodiments described herein is provided. In some embodiments, a kit comprising any of the embodiments of the minibody described herein and a detectable marker are provided.

[0397] In some embodiments, a method of detecting the presence or absence of PSMA is provided. The method comprising: applying any of the minibody embodiments provided herein to a sample; and detecting a binding or an absence of binding of the antigen binding construct thereof to PSMA. In some embodiments of the method, the minibody comprises a detectable marker selected from the group consisting of a radioactive substance, a dye, a contrast agent, a fluorescent compound, a bioluminescent compound, an enzyme, an enhancing agent, and a nanoparticle. In some embodiments of the method, applying the minibody comprises administering the minibody to a subject. In some embodiments of the method, detecting binding or absence of binding of the minibody thereof to target antigen comprises positron emission tomography. In some embodiments, the method further comprises applying a secondary antibody or fragment thereof to the sample, wherein the secondary antibody or fragment thereof binds specifically to the minibody. In some embodiments of the method, the minibody thereof is incubated with the sample for no more than 1 hour.

[0398] In some embodiments, a method of targeting a therapeutic agent to PSMA is provided. The method comprises administering to a subject any of the embodiments of the minibody provided herein, wherein the minibody is conjugated to a therapeutic agent.

[0399] In some embodiments, a method of targeting PSMA in a subject in need thereof is provided. The method comprises administering to the subject a minibody of any one of the embodiments provided herein. In some embodiments of the method, the subject has at least one of a tumor or a cancer or a disorder of prostate, brain, small intestines, liver,

proximal kidney tubules, salivary gland. In some embodiments, a method of targeting PSMA in a tumor neovasculature of an advanced solid tumor is provided.

[0400] In some embodiments, an antibody that binds to PSMA is provided. The antibody comprises a hinge region, wherein the hinge region comprises at least one of the following: a) a peptide sequence of SEQ ID NO: 1 ($X_{n1}CX_{n2}X_{n3}CX_{n4}X_{n5}C$), wherein X_{n1} can be any amino acid that does not form a covalent crosslinking bond, wherein X_{n2} is one of: A, R, N, D, E, Q, G, H, I, L, K, M, F, P, S, T, W, Y, or V, wherein X_{n3} can be any amino acid, wherein X_{n4} can be any amino acid, and wherein X_{n5} can be any amino acid; b) a peptide sequence of SEQ ID NO: 2 ($X_{n1}X_{n2}X_{n3}X_{n4}X_{n5}$ $X_{n6}CX_{n7}X_{n8}CX_{n9}X_{n10}C$), wherein X_{n1} can be any amino acid that does not form a covalent crosslinking bond with another identical amino acid, wherein X_{n2} can be any amino acid, wherein X_{n3} can be any amino acid, wherein X_{n4} can be any amino acid, wherein X_{n5} can be any amino acid, wherein X_{n6} can be any amino acid, wherein X_{n7} can be any amino acid, wherein X_{n8} can be any amino acid, wherein X_{n9} can be any amino acid, and wherein X_{n10} can be any amino acid (SEQ ID NO: 208); c) a core hinge sequence of at least one of: CVECPPCP (SEQ ID NO: 57), CPPCPPC (SEQ ID NO: 52), or CPPCPPCPPC (SEQ ID NO: 54), linked to an upper hinge sequence of ELKTPLGDTTHT (SEQ ID NO: 48); or d) an upper hinge region that comprises no amino acids capable of crosslinking with a corresponding amino acid, and a core hinge region connected to a C-terminus of the upper hinge region, wherein the core hinge region comprises at least three cysteines. In some embodiments, the antibody is a full length antibody. In some embodiments, the antibody is a minibody. In some embodiments of the antibody, apart from the hinge region, the antibody comprises a humanized amino acid sequence. In some embodiments, the antibody comprises: a HCDR1 of the HCDR1 in SEQ ID NO: 81; a HCDR2 of the HCDR2 in SEQ ID NO: 82; a HCDR3 of the HCDR3 in SEQID NO: 83; a LCDR1 of the LCDR1 in SEQ ID NO: 84; a LCDR2 of the LCDR2 in SEQ ID NO: 85; and a LCDR3 of the LCDR3 in SEQ ID NO: 86.

[0401] In some embodiments, a nucleic acid encoding the hinge region of any of the antibody embodiments described herein is provided. In some embodiments of the nucleic acid, apart from the sequence encoding the hinge region, the nucleic acid comprises a human sequence. In some embodiments, a cell line expressing the antibody encoded by the nucleic acid is provided.

[0402] In some embodiments, a method of manufacturing the antibody of any of the embodiments described herein is provided, the method comprising expressing the antibody in a cell line.

[0403] In some embodiments, a method of treating a condition in a subject in need thereof is provided, the method comprising administering to the subject the antibody of any of the embodiments provided herein.

CD8-IAB22M

[0404] CD8 (cluster of differentiation 8) is a transmembrane glycoprotein that is a specific marker for a subclass of T-cells including cytotoxic T-cells. Expression of CD8 is also present on some natural killer and dendritic cells as well as on a subset of T cell lymphomas. CD8 assembles as either a heterodimer of the CD8 alpha and CD8 beta subunits or a CD8 alpha homodimer. The assembled dimeric CD8 complex acts as a co-receptor together with the T-cell receptor (TCR) to recognize antigen presentation by MHC class I cells. CD8 plays a role in the development of T-cells and activation of mature T-cells. Changes in T-cell localization can reflect the progression of an immune response and can occur over time. Examples of CD8 subunits are shown with SEQ ID NOs: 134 and 135.

[0405] Described herein are antigen binding constructs, including antibodies and fragments thereof, such as minibodies that bind to a target molecule, CD8. In some embodiments, antigen binding constructs that bind the CD8 antigen can be antibodies, minibodies and/or other fragments of antibodies such as scFv. Some embodiments of antigen binding constructs against CD8 are shown in FIGs. 14E, 15D, 16B - 16D, 20C - 20G, 22B, 36B, 40 (SEQ ID NOs: 105 and 106), 41 (SEQ ID NO: 106), 42 (SEQ ID NO: 107), 43 (SEQ ID NO: 108), 44 (SEQ ID NO: 109), 45 (SEQ ID NO: 110), 46 (SEQ ID NO: 111), 66.

[0406] Some embodiments provided herein relate to a method of targeting a therapeutic agent to a CD8. The method can include administering to a subject an antigen binding construct as described herein, for example a CD8 antigen binding construct. In some embodiments, the antigen binding construct is conjugated to a therapeutic agent.

[0407] Antigen binding constructs can be useful for detecting the presence, localization, and/or quantities of the target molecule (CD8 and/or CD8+ cells, for example, certain classes of T-cells). Such antigen binding constructs can also be useful for targeting therapeutic agents to cells that express the target molecule. In some embodiments, methods

are provided for detecting the presence or absence of the target molecule (or "target") using antigen binding constructs (including antibodies, and constructs such as minibodies). In some embodiments, methods are provided for using the antigen binding constructs for therapeutic purposes.

[0408] In some embodiments, the antigen binding constructs allow for the detection of human CD8 which is a specific biomarker found on the surface of a subset of T-cells for diagnostic imaging of the immune system. Imaging of CD8 allows for the *in vivo* detection of T-cell localization. Changes in T-cell localization can reflect the progression of an immune response and can occur over time as a result various therapeutic treatments or even disease states.

[0409] In addition, CD8 plays a role in activating downstream signaling pathways that are important for the activation of cytolytic T cells that function to clear viral pathogens and provide immunity to tumors. CD8 positive T cells can recognize short peptides presented within the MHCI protein of antigen presenting cells. In some embodiments, engineered fragments directed to CD8 can potentiate signaling through the T cell receptor and enhance the ability of a subject to clear viral pathogens and respond to tumor antigens. Thus, in some embodiments, the antigen binding constructs provided herein can be agonists and can activate the CD8 target.

[0410] In some embodiments, the presence or absence of the target, CD8, is detected. The presence or absence of the target can be detected based on the presence or absence of the antigen binding construct in the sample. After removal and/or elimination of the antigen binding construct from the sample, for example by washing and/or metabolic elimination, remaining antigen binding construct in the sample can indicate the presence of the target, while an absence of the antigen binding construct in the sample can indicate the absence of the target.

[0411] Some embodiments include detection of human CD8 which is a specific biomarker found on the surface of a subset of T-cells for diagnostic imaging of the immune system. Imaging of the target molecule can allow for the *in vivo* detection of T-cell localization. Changes in T-cell localization can reflect the progression of an immune response and can occur over time as a result various therapeutic treatments or even disease states. For example, imaging T-cell localization can be useful in immunotherapy. Adoptive

immunotherapy is a form of therapy where a patient's own T-cells are manipulated *in vitro* and re-introduced into the patient. For this form of treatment, imaging of T-cells can be useful for monitoring and/or determining the status of the treatment. Thus, in some embodiments, monitoring the localization of the target molecule can be a useful for analyzing a mechanism of action, efficacy, and/or safety in the development of drugs and/or can aid in the clinical management of disease.

[0412] Some embodiments provided herein relate to a method of targeting a therapeutic agent to a CD8. The method can include administering to a subject an antigen binding construct as described herein, for example a CD8 antigen binding construct. In some embodiments, the antigen binding construct is conjugated to a therapeutic agent.

[0413] Toxins that can be used as detectable markers in accordance with the embodiments of the disclosure include, but are not limited to, ricin, abrin, ribonuclease (RNase), DNase I, Staphylococcal enterotoxin-A, pokeweed antiviral protein, gelonin, diphtheria toxin, Pseudomonas exotoxin, and Pseudomonas endotoxin.

[0414] In some embodiments nanoparticles are used in therapeutic applications as drug carriers that, when conjugated to an antigen binding construct, deliver chemotherapeutic agents, hormonal therapeutic agents, radiotherapeutic agents, toxins, or any other cytotoxic or anti-cancer agent known in the art to cancerous cells that overexpress the target on the cell surface.

[0415] Antigen binding constructs can be used to target a therapeutic molecule, for example a cytotoxin to a target positive cell, such as a cell expressing CD8. Thus, some embodiments include methods of targeting a therapeutic agent to a target positive cell. The method can include administering an antigen binding construct as described herein to a subject. The subject can be a subject in need, for example a subject in need of elimination or neutralization of at least some target positive cells. In some embodiments, the antigen binding construct includes at least one therapeutic agent as described herein. In some embodiments, the therapeutic can be directly conjugated to the antigen binding construct via a covalent bond, such as a disulfide bond. In some embodiments, the subject can benefit from the localization of a CD8 positive cell to another cell or agent.

[0416] Optionally, before and/or after administration of the antigen binding construct that includes at least one therapeutic agent, the number and/or localization of the

target positive cells of the patient is determined. For example, determining the number and/or localization of target positive cells prior to administration can indicate whether the patient is likely to benefit from neutralization and/or elimination of the target positive cells. Determining the number and/or localization of the target positive cells after administration can indicate whether the target positive cells were eliminated in the patient.

[0417] The term "CD8 dependent disorder" includes cancers for which there is an immunological component (including response to cancer immunotherapies), autoimmune disorders inflammation disorders, etc.

[0418] In some embodiments, a minibody that binds to CD8 is provided. The minibody comprises a polypeptide that comprises a single-chain variable fragment (scFv) that binds to CD8, the scFv comprising a variable heavy (V_H) domain linked a variable light (V_L) domain; and a variant hinge region comprising at least three cysteines on each strand of the hinge. In some embodiments, the minibody further comprises a human IgG C_H3 sequence. In some embodiments, the minibody further comprises a detectable marker selected from the group consisting of a radioactive substance, a dye, a contrast agent, a fluorescent compound, a bioluminescent compound, an enzyme, an enhancing agent, and a nanoparticle.

[0419] In some embodiments, the minibody provided herein comprises: a HCDR1 of the HCDR1 in SEQ ID NO: 75; a HCDR2 of the HCDR2 in SEQ ID NO: 76; a HCDR3 of the HCDR3 in SEQID NO: 77; a LCDR1 of the LCDR1 in SEQ ID NO: 78; a LCDR2 of the LCDR2 in SEQ ID NO: 79; and a LCDR3 of the LCDR3 in SEQ ID NO: 80. In some embodiments of the minibody provided herein the variable heavy (V_H) domain and the variable light (V_L) domain are human sequences.

[0420] In some embodiments, a nucleic acid encoding an antibody of any of the embodiments described herein is provided. In some embodiments, a cell line producing any of the minibody embodiments described herein is provided. In some embodiments, a kit comprising any of the embodiments of the minibody described herein and a detectable marker are provided.

[0421] In some embodiments, a method of detecting the presence or absence of CD8 is provided. The method comprising: applying any of the minibody embodiments provided herein to a sample; and detecting a binding or an absence of binding of the antigen

binding construct thereof to CD8. In some embodiments of the method, the minibody comprises a detectable marker selected from the group consisting of a radioactive substance, a dye, a contrast agent, a fluorescent compound, a bioluminescent compound, an enzyme, an enhancing agent, and a nanoparticle. In some embodiments of the method, applying the minibody comprises administering the minibody to a subject. In some embodiments of the method, detecting binding or absence of binding of the minibody thereof to CD8 comprises positron emission tomography. In some embodiments, the method further comprises applying a secondary antibody or fragment thereof to the sample, wherein the secondary antibody or fragment thereof binds specifically to the minibody. In some embodiments of the method, the minibody thereof is incubated with the sample for no more than 1 hour.

[0422] In some embodiments, a method of targeting a therapeutic agent to CD8 is provided. The method comprises administering to a subject any of the embodiments of the minibody provided herein, wherein the minibody is conjugated to a therapeutic agent.

[0423] In some embodiments, a method of targeting a T lymphocyte cell expressing CD8 in a subject in need thereof is provided. The method comprises administering to the subject a minibody of any one of the embodiments provided herein. In some embodiments, a method of neutralizing a T lymphocyte cell expressing CD8 is provided. In some embodiments, the subject has at least one of anergic CD8 T cells, dysfunctional CD8 T cells, auto-reactive CD8 T cells, over-reactive CD8 T cells, inhibitory CD8 T cells, mislocalized CD8 T cells, or CD8 T cell lymphoma. In some embodiments, the subject has CD8 T cells associated with at least one of rheumatoid arthritis, multiple sclerosis, diabetes, systemic lupus erythematosus, autoimmune, inflammatory condition, signaling defect, or co-stimulatory defect.

[0424] In some embodiments, an antibody that binds to CD8 is provided. The antibody comprises a hinge region, wherein the hinge region comprises at least one of the following: a) a peptide sequence of SEQ ID NO: 1 ($X_{n1}CX_{n2}X_{n3}CX_{n4}X_{n5}C$), wherein X_{n1} can be any amino acid that does not form a covalent crosslinking bond, wherein X_{n2} is one of: A, R, N, D, E, Q, G, H, I, L, K, M, F, P, S, T, W, Y, or V, wherein X_{n3} can be any amino acid, wherein X_{n4} can be any amino acid, and wherein X_{n5} can be any amino acid; b) a peptide sequence of SEQ ID NO: 2 ($X_{n1}X_{n2}X_{n3}X_{n4}X_{n5}$ $X_{n6}CX_{n7}X_{n8}CX_{n9}X_{n10}C$), wherein X_{n1} can be any amino acid that does not form a covalent crosslinking bond with another identical amino

acid, wherein X_{n2} can be any amino acid, wherein X_{n3} can be any amino acid, wherein X_{n4} can be any amino acid, wherein X_{n5} can be any amino acid, wherein X_{n6} can be any amino acid, wherein X_{n7} can be any amino acid, wherein X_{n8} can be any amino acid, wherein X_{n9} can be any amino acid, and wherein X_{n10} can be any amino acid (SEQ ID NO: 208); c) a core hinge sequence of at least one of: CVECPPCP (SEQ ID NO: 57), CPPCPPC (SEQ ID NO: 52), or CPPCPPCPPC (SEQ ID NO: 54), linked to an upper hinge sequence of ELKTPLGDTTHT (SEQ ID NO: 48); or d) an upper hinge region that comprises no amino acids capable of crosslinking with a corresponding amino acid, and a core hinge region connected to a C-terminus of the upper hinge region, wherein the core hinge region comprises at least three cysteines. In some embodiments, the antibody is a full length antibody. In some embodiments, the antibody is a minibody. In some embodiments of the antibody, apart from the hinge region, the antibody comprises a humanized amino acid sequence. In some embodiments, the antibody comprises: a HCDR1 of the HCDR1 in SEQ ID NO: 75; a HCDR2 of the HCDR2 in SEQ ID NO: 76; a HCDR3 of the HCDR3 in SEQID NO: 77; a LCDR1 of the LCDR1 in SEQ ID NO: 78; a LCDR2 of the LCDR2 in SEQ ID NO: 79; and a LCDR3 of the LCDR3 in SEQ ID NO: 80.

[0425] In some embodiments, a nucleic acid encoding the hinge region of any of the antibody embodiments described herein is provided. In some embodiments of the nucleic acid, apart from the sequence encoding the hinge region, the nucleic acid comprises a human sequence. In some embodiments, a cell line expressing the antibody encoded by the nucleic acid is provided.

[0426] In some embodiments, a method of manufacturing the antibody of any of the embodiments described herein is provided, the method comprising expressing the antibody in a cell line.

[0427] In some embodiments, a method of treating a condition in a subject in need thereof is provided, the method comprising administering to the subject the antibody of any of the embodiments provided herein.

5T4-IAB20M

[0428] 5T4 is an oncofetal glycoprotein which has weak expression in select adult tissues, but strong expression in various types of carcinomas including colorectal, renal, breast, ovarian, gastric, lung, and prostate cancer. 5T4 expression is found in both primary

and metastatic cancers and expression level correlates with progression of the disease making 5T4 a very promising biomarker. An example of 5T4 protein is shown with SEQ ID NO: 133.

[0429] A 5T4-specific imaging agent can provide a significant advantage in specificity over other imaging agents such as FDG which are based on detecting changes in metabolism. Furthermore, imaging agents which specifically target 5T4 can be a valuable tool during the development of therapies targeting 5T4. For example, the imaging agent can be used to monitor patients following treatment of therapy targeting 5T4. In the case of prostate cancer, a FDA approved SPECT imaging agent is on the market called Prostascint but has considerable limitations since the intact antibody is murine (immunogenicity issue with repeat administration) and the epitope is an internal epitope of the biomarker PSMA (limits accuracy). The present 5T4 antigen binding constructs can have significant advantages over Prostascint in that they can bind an extracellular epitope of 5T4, have optimized pharmacokinetics for imaging, and are humanized.

[0430] The 5T4 protein is also known as trophoblast glycoprotein or TPBG. Examples of 5T4 proteins are known in the art, and include, for example the 5T4 protein of SEQ ID NO: 133.

[0431] Therapeutic antibodies and antibody fusion proteins have been in development against the human 5T4 target including the murine antibody "H8". Also, a cancer vaccine named Trovax is currently in clinical development against the 5T4 antigen by Oxford Biomedica.

[0432] A 5T4-specific imaging agent can provide a significant advantage in specificity over other imaging agents such as FDG which are based on detecting changes in metabolism. Furthermore, imaging agents which specifically target 5T4 can be a valuable tool during the development of therapies targeting 5T4. For example, the imaging agent can be used to monitor patients following treatment of therapy targeting 5T4. In the case of prostate cancer, a FDA approved SPECT imaging agent is on the market called Prostascint but has considerable limitations since the intact antibody is murine (immunogenicity issue with repeat administration) and the epitope is an internal epitope of the biomarker PSMA (limits accuracy). The present antigen binding constructs can have significant advantages

over Prostascint in that they can bind an extracellular epitope of 5T4, have optimized pharmacokinetics for imaging, and are humanized.

[0433] In some embodiments, antigen binding constructs that bind the 5T4 antigen can be antibodies, minibodies and/or other fragments of antibodies such as scFv. Some non-limiting embodiments of antigen binding constructs against 5T4 are shown in FIGs. 28B - 28E, 32, 36C, 54 (SEQ ID NOs: 119 and 120), 55 (SEQ ID NO: 120), 56 (SEQ ID NO: 121), 57 (SEQ ID NO: 122), 58 (SEQ ID NO: 123), 59 (SEQ ID NO: 124), 67, 68.

[0434] In some embodiments, the antigen binding construct can be connected to a therapeutic to treat a form of cancer, including, but not limited to: colorectal, renal, breast, ovarian, gastric, lung, and/or prostate cancer.

[0435] In some embodiments, the 5T4 antigen binding construct can be used as a therapeutic agent as a stand-alone construct (for example, without a toxin conjugated thereto). While the fragments have a shorter half-life compared to the intact antibody which would be less optimal for therapy, these formats can exhibit improved tumor penetration based on their smaller size and be therapeutically effective when appropriately armed with a cytotoxic drug or radioisotope.

[0436] 5T4 is a rapidly internalizing antigen which can make it suitable for antibody drug-conjugate approaches. In some embodiments, another therapeutic approach is radio immunotherapy via attaching an appropriate radiolabel such as the beta-emitter Yttrium-90 which can deliver cell damage and death to local cancerous tissue.

[0437] In some embodiments, the antigen binding construct is used to target cells expressing 5T4 antigen, including, for example, colorectal, renal, breast, ovarian, gastric, lung, and/or prostate cancer cells.

[0438] The ability to image a patient's entire body for the presence of an antibody's target prior to and during treatment provides valuable information for personalized patient management. During the testing of an antibody therapy's safety and efficacy, it is useful to be able to select and test the treatment on patients who express the antibody's target as part of their disease progression.

[0439] 5T4 is overexpressed in many different carcinomas including colorectal, renal, breast, ovarian, gastric, lung, and prostate. Imaging agents such as FDG-PET have

proven quite effective for detection of many of these cancer types but current imaging practices are not sufficiently accurate for ovarian and prostate cancer. In some embodiments, any of these disorders can be diagnosed and/or treated with various 5T4 constructs provided herein.

[0440] In some embodiments, the antigen binding constructs can be clinical imaging agents (PET/SPECT) in humans. Since 5T4 is overexpressed in multiple cancer types (including colorectal, renal, breast, ovarian, gastric, lung, and prostate, these 5T4 antigen binding constructs can be used for targeted diagnostic detection for these cancers. In some embodiments, this can be used for detection of ovarian and/or prostate cancer.

[0441] The term "5T4 dependent disorder" includes any disorder in which 5T4 plays a role in the disorder itself. In some embodiments, this denotes over-expression of 5T4. Examples of the disorders include, multiple cancer types, such as colorectal, renal, breast, ovarian, gastric, lung, and prostate cancer, for example.

[0442] In some embodiments, a minibody that binds to 5T4 is provided. The minibody comprises a polypeptide that comprises a single-chain variable fragment (scFv) that binds to 5T4, the scFv comprising a variable heavy (V_H) domain linked a variable light (V_L) domain; and a variant hinge region comprising at least three cysteines on each strand of the hinge. In some embodiments, the minibody further comprises a human IgG C_H3 sequence. In some embodiments, the minibody further comprises a detectable marker selected from the group consisting of a radioactive substance, a dye, a contrast agent, a fluorescent compound, a bioluminescent compound, an enzyme, an enhancing agent, and a nanoparticle.

[0443] In some embodiments, the minibody provided herein comprises: a HCDR1 of the HCDR1 in SEQ ID NO: 87; a HCDR2 of the HCDR2 in SEQ ID NO: 88; a HCDR3 of the HCDR3 in SEQID NO: 89; a LCDR1 of the LCDR1 in SEQ ID NO: 90; a LCDR2 of the LCDR2 in SEQ ID NO: 91; and a LCDR3 of the LCDR3 in SEQ ID NO: 92. In some embodiments of the minibody provided herein the variable heavy (V_H) domain and the variable light (V_L) domain are human sequences.

[0444] In some embodiments, a nucleic acid encoding an antibody of any of the embodiments described herein is provided. In some embodiments, a cell line producing any of the minibody embodiments described herein is provided. In some embodiments, a kit

comprising any of the embodiments of the minibody described herein and a detectable marker are provided.

[0445] In some embodiments, a method of detecting the presence or absence of 5T4 is provided. The method comprising: applying any of the minibody embodiments provided herein to a sample; and detecting a binding or an absence of binding of the antigen binding construct thereof to 5T4. In some embodiments of the method, the minibody comprises a detectable marker selected from the group consisting of a radioactive substance, a dye, a contrast agent, a fluorescent compound, a bioluminescent compound, an enzyme, an enhancing agent, and a nanoparticle. In some embodiments of the method, applying the minibody comprises administering the minibody to a subject. In some embodiments of the method, detecting binding or absence of binding of the minibody to a target antigen comprises positron emission tomography. In some embodiments, the method further comprises applying a secondary antibody or fragment thereof to the sample, wherein the secondary antibody or fragment thereof binds specifically to the minibody. In some embodiments of the method, the minibody thereof is incubated with the sample for no more than 1 hour.

[0446] In some embodiments, a method of targeting a therapeutic agent to 5T4 is provided. The method comprises administering to a subject any of the embodiments of the minibody provided herein, wherein the minibody is conjugated to a therapeutic agent.

[0447] In some embodiments, a method of targeting 5T4 in a subject in need thereof is provided. The method comprises administering to the subject a minibody of any one of the embodiments provided herein. In some embodiments of the method, the subject has at least one of colorectal, renal, breast, ovarian, gastric, lung, or prostate cancer tumor or cancer. In some embodiment, the subject has at least one of a primary cancer, or metastatic cancer. In some embodiments, the subject has an early stage disorder. In some embodiments, the subject has an intermediate stage disorder. In some embodiments, the subject has a late stage disorder.

[0448] In some embodiments, an antibody that binds to 5T4 is provided. The antibody comprises a hinge region, wherein the hinge region comprises at least one of the following: a) a peptide sequence of SEQ ID NO: 1 ($X_{n1}CX_{n2}X_{n3}CX_{n4}X_{n5}C$), wherein X_{n1} can be any amino acid that does not form a covalent crosslinking bond, wherein X_{n2} is one of: A,

R, N, D, E, Q, G, H, I, L, K, M, F, P, S, T, W, Y, or V, wherein X_{n3} can be any amino acid, wherein X_{n4} can be any amino acid, and wherein X_{n5} can be any amino acid; b) a peptide sequence of SEQ ID NO: 2 ($X_{n1}X_{n2}X_{n3}X_{n4}X_{n5}$ $X_{n6}CX_{n7}X_{n8}CX_{n9}X_{n10}C$), wherein X_{n1} can be any amino acid that does not form a covalent crosslinking bond with another identical amino acid, wherein X_{n2} can be any amino acid, wherein X_{n3} can be any amino acid, wherein X_{n4} can be any amino acid, wherein X_{n5} can be any amino acid, wherein X_{n6} can be any amino acid, wherein X_{n7} can be any amino acid, wherein X_{n8} can be any amino acid, wherein X_{n9} can be any amino acid, and wherein X_{n10} can be any amino acid (SEQ ID NO: 208); c) a core hinge sequence of at least one of: CVECPPCP (SEQ ID NO: 57), CPPCPPC (SEQ ID NO: 52), or CPPCPPCPPC (SEQ ID NO: 54), linked to an upper hinge sequence of ELKTPLGDTTHT (SEQ ID NO: 48); or d) an upper hinge region that comprises no amino acids capable of crosslinking with a corresponding amino acid, and a core hinge region connected to a C-terminus of the upper hinge region, wherein the core hinge region comprises at least three cysteines. In some embodiments, the antibody is a full length antibody. In some embodiments, the antibody is a minibody. In some embodiments of the antibody, apart from the hinge region, the antibody comprises a humanized amino acid sequence.

[0449] In some embodiments, the antibody comprises: a HCDR1 of the HCDR1 in SEQ ID NO: 87; a HCDR2 of the HCDR2 in SEQ ID NO: 88; a HCDR3 of the HCDR3 in SEQID NO: 89; a LCDR1 of the LCDR1 in SEQ ID NO: 90; a LCDR2 of the LCDR2 in SEQ ID NO: 91; and a LCDR3 of the LCDR3 in SEQ ID NO: 92.

[0450] In some embodiments, a nucleic acid encoding the hinge region of any of the antibody embodiments described herein is provided. In some embodiments of the nucleic acid, apart from the sequence encoding the hinge region, the nucleic acid comprises a human sequence. In some embodiments, a cell line expressing the antibody encoded by the nucleic acid is provided.

[0451] In some embodiments, a method of manufacturing the antibody of any of the embodiments described herein is provided. The method comprises expressing the antibody in a cell line.

[0452] In some embodiments, a method of treating a condition in a subject in need thereof is provided, the method comprising administering to the subject the antibody of any of the embodiments provided herein.

CD3-IAB25M

[0453] CD3 (cluster of differentiation 3) was discovered concurrently with the monoclonal antibody OKT3. Initially, OKT3 was found to bind to all mature, peripheral T cells, and later the CD3 epsilon subunit as part of the TCR-CD3 complex was determined to be the cell surface antigen bound by OKT3. (Kung, P. et al., "Monoclonal antibodies defining distinctive human T cell surface antigens," Science, Vol. 206, No. 4416, pp. 347-349, 1979). Examples of CD3 subunits are shown with SEQ ID NOs: 136-139. OKT3 was subsequently tested as an immunosuppressant for transplant rejection with the initial trial studying acute kidney allograft rejection (Cosimi, A. B. et al., "Treatment of acute renal allograft rejection with OKT3 monoclonal antibody," Transplantation, Vol. 32, No. 6, pp. 535-539, 1981).

[0454] In some embodiments, antigen binding constructs, including antibodies and fragments thereof, such as minibodies, that bind to a target molecule, CD3, are provided. Such antigen binding constructs can be useful for detecting the presence, localization, and/or quantities of the target molecule (CD3 and/or CD3+ cells). Such antigen binding constructs can also be useful for modulating the biologic activity associated with CD3 expression on immune cells and for targeting therapeutic agents to cells that express the CD3 protein. In some embodiments, methods are provided for detecting the presence or absence of the target molecule (or "target") using antigen binding constructs (including antibodies, and constructs such as minibodies). In some embodiments, methods are provided for using the antigen binding constructs for therapeutic purposes.

[0455] Some embodiments of the CD3 minibodies disclosed herein can also be used as a therapeutic minibody, for example to modulate immune system reaction by neutralizing T cells via the CD3 epsilon domain of the TCR complex and by upregulating T regulatory cells via upregulation of FOXP3 (Saruta, M. et al., "Characterization of FOXP3+CD4+ regulatory T cells in Crohn's disease," Clin. Immunol., Vol. 125, No. 3, pp. 281-290, 2007). Such therapeutics have utility in treating not only tissue/organ allograft

transplants but also autoimmune diseases such as Rheumatoid Arthritis, Multiple Sclerosis, Type 1 Diabetes, and Lupus Erythematosus to name a few.

[0456] Initial proof-of-concept preclinical imaging has been performed with a humanized anti-CD3 antibody, Visilizumab which was not derived from OKT3 (Malviya, G. et al., "Radiolabeled humanized anti-CD3 monoclonal antibody Visilizumab for imaging human T-lymphocytes," Vol. 50, No. 10, pp. 1683-1691, 2009). Imaging of CD3+ T-cells is useful for anti-CD3 therapy since the treatment is effective if the organ of interested has been entirely infiltrated with CD3+ T-cells. A potential CD3 imaging agent would allow for the selection of the patient and also a way to monitor treatment. Imaging with a full-length antibody typically requires a longer time postinjection for optimal imaging than with the fragments provided herein.

[0457] Anti-CD3 antigen binding constructs, such as minibodies are provided in some embodiments. The antigen binding constructs can be used, for example, for imaging and for treating a variety of disorders involving the immune system.

[0458] In some embodiments, antigen binding constructs that bind the CD3 antigen can be antibodies, minibodies and/or other fragments of antibodies such as scFv. Some non-limiting embodiments of antigen binding constructs against CD3 are shown in FIGS. 33, 36D, 69.

[0459] In some embodiments, the antigen binding construct can be used as a therapeutic without linkage to another molecule such as a toxin (*see*, for example, Chatenoud, L. et al., "CD3-specific antibodies: a portal to the treatment of autoimmunity," Nat. Rev. Immunol., Vol. 7, No. 8, pp. 622-632, 2007). Such antigen binding constructs can also be useful for modulating the biologic activity associated with CD3 expression on immune cells to treat a variety of diseases including cancer, diabetes, autoimmune and inflammatory conditions. In some embodiments, the antigen binding construct alone can be used as an immunosuppressant and shows activity to inhibit CD3 signaling.

[0460] The CD3 antigen binding constructs disclosed herein can also be used as a therapeutic antigen binding construct, for example to modulate immune system reaction by neutralizing T cells via the CD3 epsilon domain of the TCR complex and by upregulating T regulatory cells via upregulation of FOXP3 (Saruta, M. et al., 2007). Such therapeutics have utility in treating not only tissue/organ allograft transplants but also autoimmune

diseases such as Rheumatoid Arthritis, Multiple Sclerosis, Type 1 Diabetes, and Lupus Erythematosus to name a few.

[0461] In some embodiments an antigen binding construct, such as a minibody, can contain one or more CDRs from the variable heavy or light regions of Teplizumab.

[0462] In some embodiments, one or more of the antigen binding constructs provided herein can be combined with other immune cell targeting agents such as antibodies directed to OX40, CD134, CD40, CD154, CD80, CD86, ICOS, CD137 and/or IL-1 receptor antagonists. In some embodiments, the minibody directed to CD3 decreases an immune response and no additional therapeutic agent need be conjugated to the antigen binding construct. Thus, in some embodiments, minibodies are provided for the treatment of autoimmune diabetes or other autoimmune conditions that involve T cells that are not conjugated to or involve a therapeutic agent.

[0463] In some embodiments, the CD3 antigen binding constructs can be used as a therapeutic antigen binding construct to modulate immune system reaction by stimulating and tolerizing T cells via the CD3 epsilon domain of the TCR complex and/or by upregulating T regulatory cells via upregulation of FOXP3 (Saruta, M. et al., 2007) Such therapeutics can be useful in treating not only tissue/organ allograft transplants but also autoimmune diseases such as Rheumatoid Arthritis, Multiple Sclerosis, Type 1 Diabetes, Lupus Erythematosus, etc.

[0464] In some embodiments, the antigen binding construct can be used as a therapeutic without linkage to another molecule such as a toxin (*see*, for example, Chatenoud, L. et al., 2007). Such antigen binding constructs can also be useful for modulating the biologic activity associated with CD3 expression on immune cells to treat a variety of diseases including cancer, diabetes, autoimmune and inflammatory conditions. In some embodiments, the antigen binding construct alone can be used as an immunosuppressant and shows activity to inhibit CD3 signaling.

[0465] Without being limited to any one theory, in some embodiments, a bispecific antigen binding construct binds to the target on the target positive cell, and binds to the first antigen (which can be different from CD3) on the first cell, and thus brings the target positive cell in proximity to the first cell. For example, a CD3+ cell can be brought into proximity of a cancer cell, and can facilitate an immune response against that cancer cell.

[0466] In some embodiments, the minibody can be conjugated to a therapeutic agent for the treatment of the CD3 dependent disorder.

[0467] The subject can have any of a number of CD3 dependent disorders, which, for example, can be Rheumatoid Arthritis, Multiple Sclerosis, Type 1 Diabetes or Lupus Erythematosus.

[0468] In some embodiments, an antigen binding construct as provided herein allows for significant decrease in undesirable side effects, including, but not limited to, pro-inflammatory cytokine release, T cell activation, and/or cell proliferation.

[0469] In some embodiments, a method of treatment is provided whereby the subject can benefit from the application of CD3 directed antigen binding constructs, but rather than a full length construct, a minibody is instead administered, so as to result in a lower stimulation of immune cell activation after binding of the antigen binding construct to CD3 *in vivo*.

[0470] In some embodiments, the amount of the minibody in a pharmaceutical composition is greater than the amount that could otherwise be administered for a full length construct (for example, full length OKT3). In some embodiments, the amount administered would induce a cytokine storm in the subject, if the amount had been administered as a full length construct. In some embodiments, the amount that can be administered without resulting in a cytokine storm or in cytokine release syndrome (CRS), is at least 10 micrograms/m2, for example, at least 10, 17, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 300, 400, 500, 600, 700, 800, 900, or at least 1000 micrograms/m^2 of the minibody format can be administered. In some embodiments, this amount will not result in cytokine release syndrome. In some embodiments, this amount will not result in Grade 1 and/or Grade 2 cytokine release syndrome. In some embodiments, this amount will not result in one or more of the following symptoms: rash, headache, nausea, vomiting, and/or chills/rigors/pyrexia. In some embodiments, these symptoms, which would normally occur on Day 1 or Day 5 or 6 of dosing if a full length antibody is used, will not be present if a minibody is employed.

[0471] In some embodiments, a method is provided for the treatment of an individual in need of a CD3 blocking therapy, and wherein the individual is also at risk of developing and/or experiencing a cytokine storm. In some embodiments, the method comprises administering to the subject at risk of experiencing a cytokine storm a minibody

construct of a full length antibody; however, the minibody format will allow for a reduction in the intensity of any immune cell activation that would otherwise occur, had a full length antibody been administered to the subject. In some embodiments, the minibody can share the same CDRs (or similar CDRs) and/or the same heavy and light chain variable regions (or similar heavy and light chain variable regions). In some embodiments, the administration of the minibody allows for lower stimulation of immune cell activation after binding to CD3 occurs. In some embodiments, the administration of the minibody allows for a reduction in the level of cytokines released after the binding to CD3 occurs. In some embodiments, the administration of the minibody allows for lower amounts of activation to occur following the binding to CD3, than if a full length construct had been employed.

[0472] In some embodiments, one can administer a CD3 therapy without having to administer a counter therapy, wherein the counter therapy would be designed to reduce or block a cytokine storm. In some embodiments, this comprises administering a minibody antigen binding construct to the subject, instead of a full length construct.

[0473] In some embodiments, a method of reducing an intensity and/or likelihood of a cytokine storm is provided, the method can include identifying a subject who is to receive an antigen binding construct that is to bind CD3, and administering to the subject an effective amount of a minibody that binds to CD3. In some embodiments, the construct is one or more of those provided herein.

[0474] As described herein, the ability to reduce and/or avoid a cytokine storm (or other aspect, such as reducing activation and/or proliferation) can be especially relevant for antigen binding constructs that bind to CD3. In some embodiments, the process (e.g., using a minibody format) to avoid such issues can be employed for other target proteins, for example, any target protein for which the binding of an antibody to the target results in a cytokine storm or activation/proliferation. In some embodiments, the method can be employed to allow one to avoid inappropriate and/or damaging activation in a subject, in which the activation occurs in response to a bivalent antigen binding protein. In some embodiments, rather than a CD3 antigen binding protein, one can employ a CD28 antigen binding protein (e.g., minibody) that binds to CD28).

[0475] In some embodiments, the use of a CD3 minibody allows for a subject to receive adequate levels of the antigen binding construct to allow for treatment of a CD3

dependent disorder. In some embodiments, the subject can receive the CD3 binding molecules without also and/or subsequently having to receive insulin injections. In some embodiments, the use of a CD3 minibody construct allows for one to dampen a CD3 driven response without destroying too many B cells in the process. In some embodiments, the activation/proliferation that is reduced includes activation and/or proliferation of a T cell and/or a Natural Killer (NK) cell.

[0476] In some embodiments, providing an antigen binding construct to a subject in need comprises providing an effective amount of an antigen binding construct to CD3, wherein said antigen binding construct binds to CD3, but does not result in a significant increase in an amount of at least one of cytokine release, activation, or proliferation.

[0477] In some embodiments, a method of administering a therapeutic agent that binds CD3 on an immune cell is provided. The method comprises providing an antigen binding construct as provided herein (e.g., any of the minibodies to CD3) to a subject in need of lowering an amount of CD3. The antigen binding construct binds to CD3; however, as shown by the data provided herein, the antigen binding construct does not result in a significant amount of an increase in at least one of cytokine release, activation, or proliferation. In some embodiments, the lowering of CD3 denotes a lowering of the amount of free CD3 protein available. In some embodiments, the lowering of CD3 denotes the lowering of cells expressing CD3 protein.

[0478] In some embodiments, the construct does not result in a cytokine storm at a level that would be unacceptably detrimental to the subject.

[0479] In some embodiments, a method of treating a subject having a CD3 dependent disorder is provided. The method comprises providing to the subject an effective amount of an antigen binding construct. The antigen binding construct is a minibody that binds to CD3. In some embodiments, the antigen binding construct is conjugated to a therapeutic agent. In some embodiments, the antigen binding construct comprises a bivalent arrangement comprising a first binding site and a second binding site, wherein the first binding site binds to CD3, and wherein the second binding site binds to CD3. The effective amount is a Molar amount that, had the antigen binding construct been administered in a different format—in particular in the full antibody format (for example OKT3), the Molar amount would induce an cytokine storm at an intensity that would make it unacceptable for

therapeutic purposes. However, this same Molar amount, when the antigen binding construct is a minibody, is still effective for the CD3 based therapy, but does not induce a cytokine storm at unacceptable levels.

[0480] In some embodiments, as the present constructs can be used for therapies, even though they are bivalent and bind to CD3, therapeutic constructs of CD3 antigen binding constructs can be employed. In some embodiments, an antigen binding construct that binds to CD3 *in vivo*, but does not result in a significant amount of at least one of cytokine release, activation, or proliferation is provided.

[0481] The term "CD3 dependent disorder" includes rheumatoid arthritis, multiple sclerosis, type 1 diabetes, lupus erythematosus, inflammatory bowel disease, diabetes, organ transplant rejection, autoimmune diseases, allergies and other disorders where T and/or Natural Killer (NK) cells play a role in the pathology.

[0482] A "cytokine storm," also known as a "cytokine cascade" or "hypercytokinemia" is a potentially fatal immune reaction that involves of a positive feedback loop between cytokines and immune cells, with highly elevated levels of various cytokines. Symptoms of a cytokine storm are high fever, swelling and redness, extreme fatigue and nausea. In some cases the immune reaction may be fatal. Typically, some level of cytokine release is acceptable and necessary for instance when fighting an infection. When one has fever chills associated with flu, such symptoms are the result of the immune system fighting the virus so such levels are acceptable and are not a "cytokine storm". However, when the activation arm is overstimulated and not balanced by an inhibitory signal, these cytokines can lead to organ and tissue damage and ultimately death. In some embodiments, the constructs provided herein can be useful for avoiding and/or minimizing cytokine release syndrome, and thus, can be applied to situations where anti-T cell full length antibodies might otherwise be used. In some embodiments, any of the methods provided herein directed to cytokine storms can also be applied to cytokine release syndrome.

[0483] In some embodiments, the subject has an inflammatory and/or autoimmune condition. In some embodiments, the condition is selected from at least one of rheumatoid arthritis, multiple sclerosis, type 1 diabetes, or lupus erythematosus.

[0484] In some embodiments, the cytokines are those that are involved in cytokine storms induced from full length OKT3 antibody administration. In some embodiments, the cytokines include at least one of IFNγ, IL-2, TNF-α, or IL-17.

[0485] In some embodiments, a minibody that binds to CD3 is provided. The minibody comprises a polypeptide that comprises a single-chain variable fragment (scFv) that binds to CD3, the scFv comprises a variable heavy (V_H) domain linked a variable light (V_L) domain; and a variant hinge region comprising at least three cysteines on each strand of the hinge. In some embodiments, the minibody further comprises a human IgG C_H3 sequence. In some embodiments, the minibody further comprises a detectable marker selected from the group consisting of a radioactive substance, a dye, a contrast agent, a fluorescent compound, a bioluminescent compound, an enzyme, an enhancing agent, and a nanoparticle.

[0486] In some embodiments, a nucleic acid encoding an antibody of any of the embodiments described herein is provided. In some embodiments, a cell line producing any of the minibody embodiments described herein is provided. In some embodiments, a kit comprising any of the embodiments of the minibody described herein and a detectable marker are provided.

[0487] In some embodiments, a method of detecting the presence or absence of CD3 is provided. The method comprises: applying any of the minibody embodiments provided herein to a sample; and detecting a binding or an absence of binding of the antigen binding construct thereof to CD3. In some embodiments of the method, the minibody comprises a detectable marker selected from the group consisting of a radioactive substance, a dye, a contrast agent, a fluorescent compound, a bioluminescent compound, an enzyme, an enhancing agent, and a nanoparticle. In some embodiments of the method, applying the minibody comprises administering the minibody to a subject. In some embodiments of the method, detecting binding or absence of binding of the minibody thereof to target antigen comprises positron emission tomography. In some embodiments, the method further comprises applying a secondary antibody or fragment thereof to the sample, wherein the secondary antibody or fragment thereof binds specifically to the minibody. In some embodiments of the method, the minibody thereof is incubated with the sample for no more than 1 hour.

[0488] In some embodiments, a method of targeting a therapeutic agent to CD3 is provided. The method comprises administering to a subject any of the embodiments of the minibody provided herein, wherein the minibody is conjugated to a therapeutic agent.

[0489] In some embodiments, a method of targeting a T lymphocyte cell expressing CD3 in a subject in need thereof is provided. The method comprises administering to the subject a minibody of any one of the embodiments provided herein. In some embodiments, a method of neutralizing a T lymphocyte cell expressing CD3 is provided. In some embodiments, the subject has at least one of anergic CD3 T cells, dysfunctional CD3 T cells, auto-reactive CD3 T cells, over-reactive CD3 T cells, inhibitory CD3 T cells, mislocalized CD3 T cells, or CD3 T cell lymphoma. In some embodiments, the subject has CD3 T cells associated with at least one of rheumatoid arthritis, multiple sclerosis, diabetes, systemic lupus erythematosus, autoimmune, inflammatory condition, signaling defect, or co-stimulatory defect.

[0490] In some embodiments, an antibody that binds to CD3 is provided. The antibody comprises a hinge region, wherein the hinge region comprises at least one of the following: a) a peptide sequence of SEQ ID NO: 1 ($X_{n1}CX_{n2}X_{n3}CX_{n4}X_{n5}C$), wherein X_{n1} can be any amino acid that does not form a covalent crosslinking bond, wherein X_{n2} is one of: A, R, N, D, E, Q, G, H, I, L, K, M, F, P, S, T, W, Y, or V, wherein X_{n3} can be any amino acid, wherein X_{n4} can be any amino acid, and wherein X_{n5} can be any amino acid; b) a peptide sequence of SEQ ID NO: 2 ($X_{n1}X_{n2}X_{n3}X_{n4}X_{n5}$ $X_{n6}CX_{n7}X_{n8}CX_{n9}X_{n10}C$), wherein X_{n1} can be any amino acid that does not form a covalent crosslinking bond with another identical amino acid, wherein X_{n2} can be any amino acid, wherein X_{n3} can be any amino acid, wherein X_{n4} can be any amino acid, wherein X_{n5} can be any amino acid, wherein X_{n6} can be any amino acid, wherein X_{n7} can be any amino acid, wherein X_{n8} can be any amino acid, wherein X_{n9} can be any amino acid, and wherein X_{n10} can be any amino acid (SEQ ID NO: 208); c) a core hinge sequence of at least one of: CVECPPCP (SEQ ID NO: 57), CPPCPPC (SEQ ID NO: 52), or CPPCPPCPPC (SEQ ID NO: 54), linked to an upper hinge sequence of ELKTPLGDTTHT (SEQ ID NO: 48); or d) an upper hinge region that comprises no amino acids capable of crosslinking with a corresponding amino acid, and a core hinge region connected to a C-terminus of the upper hinge region, wherein the core hinge region comprises at least three cysteines. In some embodiments, the antibody is a full length

antibody. In some embodiments, the antibody is a minibody. In some embodiments of the antibody, apart from the hinge region, the antibody comprises a humanized amino acid sequence.

[0491] In some embodiments, the antibody comprises: a HCDR1 of the HCDR1 in Table 8 SEQ ID NO: 174; a HCDR2 of the HCDR2 in Table 8 SEQ ID NO: 175; a HCDR3 of the HCDR3 in Table 8 SEQ ID NO: 176; a LCDR1 of the LCDR1 in Table 8 SEQ ID NO: 177; a LCDR2 of the LCDR2 in Table 8 SEQ ID NO: 97; and a LCDR3 of the LCDR3 in Table 8 SEQ ID NO: 178 for CD3.

[0492] In some embodiments, the antibody comprises: a HCDR1 of the HCDR1 in FIGs. 65B or 65C; a HCDR2 of the HCDR2 in FIGs. 65B or 65C; a HCDR3 of the HCDR3 in FIGs. 65B or 65C; a LCDR1 of the LCDR1 in FIGs. 65B or 65C; a LCDR2 of the LCDR2 in FIGs. 65B or 65C; and a LCDR3 of the LCDR3 in FIGs. 65B or 65C.

[0493] In some embodiments, antigen binding constructs that bind the CD3 antigen can be antibodies, minibodies and/or other fragments of antibodies such as scFv. Some non-limiting embodiments of antigen binding constructs against CD3 are shown in FIGs. 65B and 65C.

[0494] In some embodiments, the minibody comprises: a HCDR1 of the HCDR1 in FIGs. 65B or 65C; a HCDR2 of the HCDR2 in FIGs. 65B or 65C; a HCDR3 of the HCDR3 in FIGs. 65B or 65C; a LCDR1 of the LCDR1 in FIGs. 65B or 65C; a LCDR2 of the LCDR2 in FIGs. 65B or 65C; and a LCDR3 of the LCDR3 in FIGs. 65B or 65C. In some embodiments of the minibody provided herein the variable heavy (V_H) domain and the variable light (V_L) domain are human sequences.

[0495] In some embodiments, a nucleic acid encoding the hinge region of any of the antibody embodiments described herein is provided. In some embodiments of the nucleic acid, apart from the sequence encoding the hinge region, the nucleic acid comprises a human sequence. In some embodiments, a cell line expressing the antibody encoded by the nucleic acid is provided.

[0496] In some embodiments, a method of manufacturing the antibody of any of the embodiments described herein is provided. The method comprises expressing the antibody in a cell line.

[0497] In some embodiments, a method of treating a condition in a subject in need thereof is provided, the method comprising administering to the subject the antibody of any of the embodiments provided herein.

Additional Embodiments

[0498] In some embodiments, the scFv or minibody have superior pharmacokinetic properties for diagnostic imaging. Current technology utilizes imaging with the intact antibody which requires significantly longer time (~7 days post-injection) to produce high contrast images due to the slow serum clearance of full length antibodies. The minibody provide the opportunity for same-day or next-day imaging. Each day is vital for patients with an aggressively progressing disease, and the ability to identify the proper therapeutic approach at an earlier time-point has the potential to improve patient survival. Same-day or next-day imaging also provides a logistical solution to the problem facing many patients who travel great distances to receive treatment/diagnosis since the duration of travel stays or the need to return one week later would be eliminated when imaging with minibody versus full length antibodies. In some embodiments, exposure to lower doses of radiation allows one to image multiple times and follow disease progression over time.

[0499] Additionally, in some embodiments, the minibody component monomers contain hinge cysteine residues that form disulfide bonds. These covalently bound cysteine residues can be opened via mild chemical reduction to provide an active thiol groups for cysteine specific conjugation while maintaining the integrity of the dimeric minibody molecule. Current chemical conjugation methods include the following site specific platforms: Introducing a cysteine in F(ab)'2 region of antibody, e.g. Thio-Mabs (Roche); a cysteine in the position of 239 in the Fc (DISH-Mab, Seattle Genetics), non-natural amino-acids, such as keto-phenylalanine (Ambrx) for alkoxyamine linker formation, phenylalanineazidomethane (Sutro) for click-cycloaddition, glutamine in the Fc region for transglutaminase-dependent conjugation (Rinat-Pfizer). Hinge-cysteines in mAbs provide a chemical handle for non-site-specific thiol-conjugation chemistry. Conjugation to lysines is also non-site-specific or random and results in the highest heterogeneity. In some embodiments, one can introduce a site specific cysteine in the scFv, framework or another portion of the antigen binding construct provided herein.

[0500] The ability to image a patient's entire body for the presence of an antibody's target prior to and during treatment provides valuable information for personalized patient management. During the testing of an antibody therapy's safety and efficacy, it is useful to be able to select and test the treatment on patients who express the antibody's target as part of their disease progression.

[0501] In some embodiments, scFv or minibody diagnostic fragments matching available antibody therapies allow matching of the patient's disease state with the appropriate antibody therapy.

[0502] In some embodiments, a method of targeting a first antigen on a target molecule positive cell to is provided. The method can include applying a bispecific antigen binding construct to a sample. The bispecific antigen binding construct can include an antigen binding construct as described herein. The bispecific antibody or fragment thereof can include an antigen binding construct that binds to the first antigen, for example 1, 2, 3, 4, 5, or 6 CDR's, a scFv, or a monomer of a minibody. In some embodiments, the bispecific antibody includes 1, 2, or 3 HCDR's of an antigen binding construct as described herein, and/or 1, 2, or 3 LCDR's of an antigen binding construct as described herein. In some embodiments, the bispecific antigen binding construct includes a scFv of an antigen binding construct as described herein. In some embodiment, the bispecific antigen binding construct includes a V_H or V_L sequence as described herein. In some embodiments, the bispecific antigen binding construct includes a minibody monomer as described herein. In some embodiments, the bispecific antigen binding construct is applied to a sample *in vivo*, for example an organ or tissue of a subject. In some embodiments, the bispecific antigen binding construct is applied to an *in vitro* sample. Without being limited to any one theory, in some embodiments, the bispecific antigen binding construct binds to the target on the target positive cell, and binds to the first antigen (which can be different from the first target molecule) on the first cell, and thus brings the target positive cell in proximity to the first cell. For example, a first target molecule+ cell can be brought into proximity of a cancer cell, and can facilitate an immune response against that cancer cell. In some embodiments, a bispecific antigen binding construct comprised of a first target molecule and a second target molecule fragment can bring a cytotoxic T cell in proximity of an activated first target molecule expressing immune cell or first target molecule expressing tumor cell to result in

killing of the target cell. In some embodiments, one can target any immune cells (NK or B cell or dendritic cell) and bridge it to an antigen expressing cell as determined by the specificity of the second scFv.

[0503] Minibody constructs contain antigen binding scFvs with variable linker lengths that can be in either V_L-V_H or V_H-V_L orientation. scFvs can be linked to any of 12 hinge sequences as described on Table 0.1. scFvs and appropriate hinges can be linked with C_H3 domains from IgG1, IgG2, IgG3, or IgG4 antibodies. In some embodiments, the first hinge cysteine that usually pairs with light chain in native IgG1, IgG2 and IgG3 antibodies should be mutated to a serine or alanine or other appropriate amino acid to prevent disulfide scrambling and/or concatamerization. To reduce the presence or reduce the formation of half-molecules and enhance stability *in vivo*, the hinge region should contain at least three cysteins to form at least three disulfide bonds with the other monomer. Three cysteines per strand in the hinge located at appropriate distances from one another are useful to allow for proper disulfide bond formation (Table 3). Three disulfide bonds in the hinge within the minibody located at appropriate distances from one another are useful for protein stability *in vivo* and clearance through liver rather than renal clearance. At least three disulfides (or more) in the hinge are beneficial for site specific conjugation of drugs, chelators or fluorescent dyes. Mbs constructed as described above retain similar affinity to parent antibodies. In some embodiments, involving the IgG1 construct, the first cys can be left intact and it does not seem too deleterious.

[0504] In some embodiments, the hinge of a minibody or a bispecific minibody comprising an upper hinge, a core hinge and a lower hinge can be generated by combining any one of the upper hinge, any one of the core hinge and any one of the lower hinge sequences as shown in Table 3.

[0505] In some embodiments, the first cysteine residue in the hinge can be changed to any amino acid. In some embodiments, one cysteine residue in the upper hinge can be changed to any amino acid. In some embodiments, one cysteine residue in the core hinge can be changed to any amino acid. In some embodiments, two cysteine residues in the core hinge can be changed to any amino acid. In some embodiments, one cysteine residue in the upper hinge and one cysteine residue in the core hinge can be changed to any amino acid.

In some embodiments, one cysteine in the upper hinge and two cysteine residues in the core hinge can be changed to any amino acid.

[0506] The sequences of the constructs for the examples are discussed in the example sections below as appropriate and are provided in Table 0.2 as well.

EXAMPLE 1 - MINIBODY STRUCTURE

[0507] The minibody is a bivalent, covalently bound homodimer of ~80 kDa. Each monomer (half-molecule) is comprised of a variable heavy (V_H) domain linked to the corresponding variable light (V_L) domain by an approximate 15-18 amino acid Gly-Ser-rich linker sequence. Each single-chain variable fragment (scFv) is linked to a human IgG C_H3 domain by a hinge sequence.

[0508] The sequences encompassing the disulfide bonds of the hinge are important and were designed to prevent undesirable disulfide scrambling with cysteine residues present in other regions of the protein as well as contain sufficient numbers of cysteine pairs to maintain dimer integrity *in vivo* and also as a possible site for site-specific conjugation.

[0509] To date most minibodies have been engineered using the native human IgG1 upper and core hinge regions with an extension sequence linked to the human IgG1 C_H3 domain as shown in FIG. 1. As outlined in the following examples, scFv variants with both orientations – V_L-V_H (M1) and V_H-V_L (M2) – were often evaluated for the various target molecules.

Engineering Based On Human IgG1

[0510] In previous hinges (e.g., γ1 EH1), the first cysteine in the hinge (FIG. 2 top) created problems resulted in protein heterogeneity as demonstrated by the intact mass analysis results using LC/MS.. Despite the clear importance of cysteines in the hinge region, it was decided to mutate this cysteine to a serine, resulting in γ1 EH2 hinge (FIG. 2 bottom). However, it was determined that this hinge construct did not achieve certain desired aspects, as it appeared that the two disulfide bonds that were formed between the two remaining cysteine residues in the core hinge (FIG. 2 bottom) were not adequate to maintain dimer stability *in vivo*.

IgG2 Mbs to Overcome Problems with IgG1 Hinge Sequence

[0511] To address the newly introduced aspects noted above, the minibodies were further engineered. Engineered minibodies based on huIgG2 with an extension sequence provided a third cysteine in the core hinge (an additional cysteine over IgG1 native core hinge) to increase disulfide bonding and increase protein stability. HuIgG2 minibodies of IAB2M and IAB22M were engineered using human IgG2 (huIgG2) hinge sequence linked to the huIgG2 C_H3 domain.

[0512] In the native IgG2 upper and core hinge sequences combined with the extension sequence as the lower hinge (γ2 EH1) an increase in aggregation was observed. The reactive cysteine (FIG. 3 top; first cysteine of the hinge) was responsible for concatamer formation in IAB2M- γ2 EH1 format with huIgG2 native hinge (FIG. 3 top) as observed by SDS-PAGE analysis shown in FIG. 5A.

[0513] Thus, similar to what was done for the IgG1 hinge (γ1 EH1) the first cysteine in the hinge (that pairs with light chain) was mutated to serine resulting in IgG2 EH2 (γ2 EH2) (FIG. 3 bottom). Antigen binding constructs with this hinge (γ2 EH2) expressed well and had good stability *in vivo*. All three disulfide bonds in the core hinge formed properly as detected by mass spectrometry. Stability was demonstrated with both IAB2M and IAB22M constructs with γ2 EH2.

[0514] Further engineered huIgG2 minibodies with the first cysteine altered to a serine and native upper and lower hinge sequence (γ2 NH2) was evaluated and the proteins were found to be stable *in vivo* (FIG. 4 bottom).

EXAMPLE 2 - *IN VITRO* DATA FOR IAB2M

[0515] Table 1 shows an overview of the IAB2M variants (SEQ ID NOS are shown in FIGs. 5B – 5E). Additional embodiments of IAB2M variants are shown in FIGs. 47 - 53.

TABLE 1

Name	Cys-Ser Hinge Mut	Remove Term Lys	N- to C-terminal (SEQ ID NOs)

Name	Cys-Ser Hinge Mut	Remove Term Lys	N- to C-terminal (SEQ ID NOs)
IAB2M-γ1 EH1	No	No	VL (SEQ ID NO: 13); linker (SEQ ID NO: 62); VH (SEQ ID NO: 14); full hinge (SEQ ID NO: 22) [upper hinge (SEQ ID NO: 45), core hinge (SEQ ID NO: 50), lower hinge (SEQ ID NO: 59)]; IgG1 CH3 (SEQ ID NO: 40 or SEQ ID NO: 41)
IAB2M-γ1 EH2	Yes	Yes	VL (SEQ ID NO: 13); linker (SEQ ID NO: 62); VH (SEQ ID NO: 14); full hinge (SEQ ID NO: 24) [upper hinge (SEQ ID NO: 46), core hinge (SEQ ID NO: 50), lower hinge (SEQ ID NO: 59)]; IgG1 CH3 (SEQ ID NO: 40 or SEQ ID NO: 41)
IAB2M-γ2 EH1	Yes	Yes	VL (SEQ ID NO: 13); linker (SEQ ID NO: 62); VH (SEQ ID NO: 14); full hinge (SEQ ID NO: 32) [upper hinge (SEQ ID NO: 47), core hinge (SEQ ID NO: 55), lower hinge (SEQ ID NO: 59)]; IgG2 CH3 (SEQ ID NO: 42)
IAB2M-γ2 EH2	Yes	Yes	VL (SEQ ID NO: 13); linker (SEQ ID NO: 62); VH (SEQ ID NO: 14); full hinge (SEQ ID NO: 34) [upper hinge (SEQ ID NO: 47), core hinge (SEQ ID NO: 56), lower hinge (SEQ ID NO: 59)]; IgG2 CH3 (SEQ ID NO: 42)

[0516] SDS-PAGE analysis (FIG. 5A) of IAB2M with hinge variants γ1 EH1, γ1 EH2 and γ2 EH1 and γ2 EH2 showed that the half molecule to dimer ratios were greatly improved (half molecule can be decreased from 20-30% to less than 1%) with mutation of the first hinge cysteine in the EH2 variants. The γ2 EH2 version had proper disulfide bonding and no concatemers compared to γ2 EH1 (SEQ IDS NOS. in FIGs. 5B – 5E). Due to the presence of the additional cysteine in the core hinge (3 instead of 2), the γ2 EH2 hinge variant exhibited improved stability and had less half-molecules present compared to the γ1 EH2 hinge variant.

[0517] Confirmation of the presence of half-molecules was performed by intact mass analysis. The protein samples were separated under reverse phase conditions using TSKgel Phenyl-5PW column (2 x 75 mm, Tosoh Biosciences) at 60°C and analyzed by Agilent ESI-QTOF model 6538. FIG. 6A shows intact mass analysis of IAB2M γ1 EH1

variant. The total ion chromatograms of the separated half-molecule and full-size molecule are shown in the upper panel. The UV280 trace (not shown) was used for quantitation of the percent of half-molecules present in the minibody variant by peak integration. The deconvoluted masses were used for assignment and confirmed the identity of half molecules (Middle panel) and of the full size masses (Lower panel). FIG. 6B shows intact mass analysis of IAB2M γ2 EH1 variant. Upper panel shows the total ion chromatogram under reverse phase conditions. Middle panel shows the deconvoluted intact masses confirming the presence of half molecules and the lower panel shows the full size molecular masses that were identified. FIG. 6C shows intact mass analysis of IAB2M γ1 EH2 variant. Upper panel shows the total ion chromatogram under reverse phase conditions. Middle panel shows the deconvoluted intact masses confirming the presence of half molecules and the lower panel shows the full size molecular masses that were identified. FIG. 6D shows intact mass analysis of IAB2M γ2 EH2 variant. Upper panel shows the total ion chromatogram. Lower panel shows the deconvoluted intact masses confirming the presence of the full-size mass molecules. No half molecules were detected.

[0518] FAC analysis (FIGs. 7A and 7B) of IAB2M variants showed that changing the hinge from the previously used standard hinge, γ1 EH1, did not impact the binding affinity for target antigen expressed either on LNCap-AR cells (FIGs. 5B, 5C, 5D) or C4-2 XCL cells (FIGs. 5B, 5C, 5E, 7C).

[0519] For disulfide mapping to help understand the half-molecule formation, samples were prepared by denaturing the protein with 6 M guanidine HCl in Tris pH 7.5. To this, 4-vinylpyridine was added to a concentration of 30 mM followed by a 1 hr incubation in the dark to cap free cysteine. The solution was diluted to bring the concentration of guanidine to 1 M followed by digestion using trypsin/Lys-C. The digestion was allowed to proceed overnight at 37°C. The samples were frozen and dried down prior to separation. This was followed by LC/MS in which peptide mix was separated in 0.05% aqueous TFA in the gradient of 0.05% TFA in 90% acetonitrile using gradient on the Waters Xbridge BEH130 C18 4.6 x 150 mm column, and masses were identified using ESI-QTOT mass spectrometer 6538 (Agilent) in the positive mode and deconvoluted using the Agilent Mass Hunter software. Disulfide-containing peptides identified within IAB2M γ1 EH1 dimer is shown in FIG. 8.

[0520] Disulfide Mapping of IAB2M γ1 EH1 demonstrated the presence of properly formed e.g. expected disulfides in the hinge region and also detected disulfide scrambling. FIG. 8 shows a table of the identified disulfides while FIG. 9 shows an illustration of the results. The disulfides (FIG. 8) occurred between the expected cysteine residues (cysteines of SEQ ID NO: 183 and SEQ ID NO: 184; cysteines of SEQ ID NO: 183 and SEQ ID NO: 185; SEQ ID NO: 186) and between unexpected cysteine residues present in other domains of the protein. It was hypothesized that the unpaired cysteine in the hinge could give rise to disulfide scrambling whereby conventional disulfide formation (solid lines) was discouraged (FIG. 9). Furthermore, the V_H disulfides were not observed (broken lines) presumably due to the tryptic disulfide-bonded peptide not ionizing (and hence, not detected) in the mass spectrometer (FIG. 9).

[0521] Reformatting to huIgG2 EH1 (γ2 EH1) did not improve stability of IAB2M. FIG. 10 shows a graphical representation of the identified disulfides. Significant concatamer formation was observed for γ2 EH1 by SDS-PAGE (FIG. 5A). As in IAB2M γ1 EH1, the V_H disulfides were not observed (broken lines) presumably due to the tryptic disulfide-bonded peptide not ionizing efficiently and hence, not being detected in the mass spectrometer. IAB2M γ2 EH1 (FIG. 5D) exhibited >10% of half-molecule (39,469.5 Da by LC/MS) (FIG. 10 and FIG.6B). A dominant half molecule (peak 1) was seen in chromatograms as shown on the panel (A) and (B) in FIG. 10. FIG. 6A (upper panel) shows total ion current (TIC) chromatogram of IAB2Mγ1 EH1 where peaks 1 and 2 are half molecule and full-size molecule, respectively. FIG. 6A middle panel shows UV280 channel wth peak integration allowing determination of the percent half-molecule, 11.1%. FIG. 6A (Lower panel) shows molecular masses comprising the peak 2.

EXAMPLE 3 - *IN VIVO* DATA FOR IAB2M

[0522] PET/CT and biodistribution of ^{89}Zr-Df-IAB2M-γ1 EH1 (FIG. 5B) was performed in nude mice. MIP PET/CT images of a nude mouse bearing a PSMA positive 22Rv1 xenograft on the right shoulder after administration of ^{89}Zr-Df-IAB2M-γ1-EH1 at 24h and 48h are shown in FIG. 11A. The average radioactive uptakes in the tissues from 3 mice at 48h are shown as percentage of injected dose per gram (ID/g) (FIG. 11B). The tumor uptake was relatively low (6.1±1.2% ID/g) and the kidney uptake was high (23.3±6.2%

ID/g); almost 2-fold higher than the liver uptake (14.1±1.6% ID/g). If the minibody remains as a dimer *in vivo*, then it would be expected that protein clearance would occur through the liver. The kidney signal being greater than the liver signal suggested instability and half molecule formation *in vivo*.

[0523] PET/CT and biodistribution of ^{89}Zr-Df-IAB2M-γ1 EH2 (FIG. 5C) was performed in nude mice. MIP PET/CT images of nude mouse bearing a PSMA positive 22Rv1 xenograft on the right shoulder after administration of ^{89}Zr-Df-IAB2M-γ1 EH2 at 24h and 47h are shown in FIG. 12A. The radioactive uptakes in the tissues from 2 mice at 47h are shown as percentage of injected dose per gram (ID/g) (FIG. 12B). Again a 2- to 3-fold higher radioactive uptake was seen in the kidneys. As above, the kidney signal being greater than the liver signal indicated instability and half molecule formation *in vivo*.

[0524] PET/CT and biodistribution of ^{89}Zr-Df-IAB2M-γ2 EH2 (FIG. 5D) was performed in nude mice. MIP PET/CT images of a nude mouse bearing a PSMA positive 22Rv1 xenograft on the right shoulder after administration of ^{89}Zr-Df-IAB2M-γ2 EH2 at 24h and 47h are shown in FIG. 13A. The radioactive uptakes in the tissues from the 2 mice at 47h are shown as percentage of injected dose per gram (ID/g) (FIG. 13B). Low kidney uptake along with kidney to liver ratios <1 were obtained which indicated that the γ1 EH2 was a stable protein.

EXAMPLE 4 - SUMMARY OF IAB2M *IN VIVO* STUDIES (TABLE 2)

[0525] Minibodies made with hinge sequences derived from hinge region of IgG1 showed high clearance through the kidneys suggesting protein instability and dissociation into half molecules *in vivo* (FIG. 11).

[0526] Mutation of the first hinge cysteine to serine in the IgG1 hinge region (γ1 EH2) (FIG. 5C) to prevent undesired interaction with other unpaired cysteine generated a protein with a better profile *in vitro* (for example, SDS-PAGE data of FIG. 5). However, the high clearance through the kidneys suggested protein instability and dissociation into half molecules *in vivo* (FIG. 12).

[0527] Minibodies made with an IgG2 hinge wherein the first hinge cysteine is mutated to a serine (γ2 EH2 (FIG. 5D) and others (Table 3, presenting summary of hinge regions)) were cleared through the liver as predicted for proteins of ~80 kDa and indicated

that the molecule remained intact *in vivo* (FIGs. 13A and 13B). Results are also presented in Table 2.

TABLE 2

	Conjugation Strategy	Tumor Model	Mouse strain	Time	Tumor % ID/g	Kidney % ID/g	Liver % ID/g	N- to C-terminal (SEQ ID NOS)
IAB2M-γ1 EH1	^{89}Zr-Df-Lys	22Rv1	Nude	48h	6.1%	23.3%	14%	VL (SEQ ID NO: 13); linker (SEQ ID NO: 62); VH (SEQ ID NO: 14); full hinge (SEQ ID NO: 22) [upper hinge (SEQ ID NO: 45), core hinge (SEQ ID NO: 50), lower hinge (SEQ ID NO: 59)]; IgG1 CH3 (SEQ ID NO: 40 or SEQ ID NO: 41)
IAB2M-γ1 EH1	^{89}Zr-Df-Lys	22Rv1	Nude	48h	9.3%	26%	16.3%	VL (SEQ ID NO: 13); linker (SEQ ID NO: 62); VH (SEQ ID NO: 14); full hinge (SEQ ID NO: 22) [upper hinge (SEQ ID NO: 45), core hinge (SEQ ID NO: 50), lower hinge (SEQ ID NO: 59)]; IgG1 CH3 (SEQ ID NO: 40 or SEQ ID NO: 41)
IAB2M-γ1 EH2	^{89}Zr-Df-Lys	22Rv1	Nude	48h	9.4%	23.5%	9.9%	VL (SEQ ID NO: 13); linker (SEQ ID NO: 62); VH (SEQ ID NO: 14); full hinge (SEQ ID NO: 24) [upper hinge (SEQ ID NO: 46), core hinge (SEQ ID NO: 50), lower hinge (SEQ ID NO: 59)]; IgG1 CH3 (SEQ ID NO: 40 or SEQ ID NO: 41)
IAB2M-γ2 EH2	^{89}Zr-Df-Lys	22Rv1	Nude	48h	7.8%	3.6%	5.1%	VL (SEQ ID NO: 13); linker (SEQ ID NO: 62); VH (SEQ ID NO: 14); full hinge (SEQ ID NO:

	Conjugation Strategy	Tumor Model	Mouse strain	Time	Tumor % ID/g	Kidney % ID/g	Liver % ID/g	N- to C-terminal (SEQ ID NOS)
								34) [upper hinge (SEQ ID NO: 47), core hinge (SEQ ID NO: 56), lower hinge (SEQ ID NO: 59)]; IgG2 CH3 (SEQ ID NO: 42)

TABLE 3

	N- to C-terminal				
Hinge Name	**Full hinge**			**CH3 domain**	
	Upper hinge	**Core hinge**	**Lower Hinge**		
γ1 EH1	Full hinge (SEQ ID NO: 22)			γ1 (SEQ ID NO: 40 or SEQ ID NO: 41)	Native upper and core IgG1 hinge
	EPKSCDKTHT (SEQ ID NO: 45)	CPPC (SEQ ID NO: 50)	GGGSSGGGSG (SEQ ID NO: 59)		
γ1 EH2	Full hinge (SEQ ID NO: 24)			γ1 (SEQ ID NO: 40 or SEQ ID NO: 41)	C → S
	EPKSSDKTHT (SEQ ID NO: 46)	CPPC (SEQ ID NO: 50)	GGGSSGGGSG (SEQ ID NO: 59)		
γ1 EH3	Full hinge (SEQ ID NO: 26)			γ1 (SEQ ID NO: 40 or SEQ ID NO: 41)	C → S
	EPKSSDKTHT (SEQ ID NO: 46)	CPPCPPC (SEQ ID NO: 52)	GGGSSGGGSG (SEQ ID NO: 59)		
γ1 EH4	Full hinge (SEQ ID NO: 28)			γ1 (SEQ ID NO: 40 or SEQ ID NO: 41)	C → S
	EPKSSDKTHT (SEQ ID NO: 46)	CPPCVECPPC (SEQ ID NO: 53)	GGGSSGGGSG (SEQ ID NO: 59)		
γ1 EH5	Full hinge (SEQ ID NO: 30)			γ1 (SEQ ID NO: 40 or SEQ ID NO: 41)	C → S
	EPKSSDKTHT (SEQ ID NO: 46)	CPPCPPCPPC (SEQ ID NO: 54)	GGGSSGGGSG (SEQ ID NO: 59)		
γ2 EH1	Full hinge (SEQ ID NO: 32)			γ2 (SEQ ID NO: 42)	Native upper and core IgG2 hinge
	ERK (SEQ ID NO: 47)	CCVECPPCP (SEQ ID NO: 55)	GGGSSGGGSG (SEQ ID NO: 59)		
γ2 EH2	Full hinge (SEQ ID NO: 34)			γ2 (SEQ ID NO: 42)	C → S
	ERK (SEQ ID NO:	SCVECPPCP (SEQ ID	GGGSSGGGSG		

	47)	NO: 56)	(SEQ ID NO: 59)		
γ2 EH2	Full hinge (SEQ ID NO: 34)			γ2 (SEQ ID NO: 181)	T26S, L28A, M57V
	ERK (SEQ ID NO: 47)	SCVECPPCP (SEQ ID NO: 56)	GGGSSGGGSG (SEQ ID NO: 59)		
γ2 EH2	Full hinge (SEQ ID NO: 34)			γ2 (SEQ ID NO: 182)	T26W
	ERK (SEQ ID NO: 47)	SCVECPPCP (SEQ ID NO: 56)	GGGSSGGGSG (SEQ ID NO: 59)		
γ2 NH1	Full hinge (SEQ ID NO: 31)			γ2 (SEQ ID NO: 42)	Native IgG2 hinge
	ERK (SEQ ID NO: 47)	CCVECPPCP (SEQ ID NO: 55)	APPVAGP (SEQ ID NO: 60)		
γ2 NH2	Full hinge (SEQ ID NO: 33)			γ2 (SEQ ID NO: 42)	C → S
	ERK (SEQ ID NO: 47)	SCVECPPCP (SEQ ID NO: 56)	APPVAGP (SEQ ID NO: 60)		
γ3/γ1 EH6	Full hinge (SEQ ID NO: 35)			γ1 (SEQ ID NO: 40 or SEQ ID NO: 41)	Native γ3 upper hinge
	ELKTPLGDTTHT (SEQ ID NO: 48)	CVECPPCP (SEQ ID NO: 57)	GGGSSGGGSG (SEQ ID NO: 59)		
γ3/γ1 EH7	Full hinge (SEQ ID NO: 36)			γ1 (SEQ ID NO: 40 or SEQ ID NO: 41)	Native γ3 upper hinge
	ELKTPLGDTTHT (SEQ ID NO: 48)	CPPCPPC (SEQ ID NO: 52)	GGGSSGGGSG (SEQ ID NO: 59)		
γ3/γ1 EH8	Full hinge (SEQ ID NO: 37)			γ1 (SEQ ID NO: 40 or SEQ ID NO: 41)	Native γ3 upper hinge
	ELKTPLGDTTHT (SEQ ID NO: 48)	CPPCPPCPPC (SEQ ID NO: 54)	GGGSSGGGSG (SEQ ID NO: 59)		
γ1 NH11 NH1	EPKSCDKTHT (SEQ ID NO: 45)	CPPC (SEQ ID NO: 50)	APELLGGP (SEQ ID NO: 58)	γ1 (SEQ ID NO: 40 or SEQ ID NO: 41)	Native γ1 hinge
γ3 NH13 NH1	ELKTPLGDTTHT (SEQ ID NO: 48)	CPRCP $(EPKSCDTPPPCPRCP)_3$ (SEQ ID NO: 256)	APELLGGP (SEQ ID NO: 58)	γ3 (SEQ ID NO: 43)	Native γ3 hinge

EXAMPLE 5 - *IN VITRO* DATA FOR IAB22M IGG2 MINIBODIES

[0528] Intact mass analysis (FIG. 14A) of the IAB22M- γ2 EH1 variant (FIG. 14B) was performed by mass spectrometry. IAB22M with γ2 EH1 hinge had a potential unpaired first hinge cysteine which resulted in ~9.2% of half molecules (FIG. 20B). For

intact mass analysis of IAB22M γ2 EH1, the samples were separated by a reverse phase chromatography column (TSKgel Phenyl-5PW (Tosoh, 2 x 75 mm) at 60°C and analyzed by Agilent ESI-QTOF mass spectrometer. FIG. 14A shows the total ion chromatograms of the separated half-molecule and full-size molecule in the upper panel. The UV280 trace (not shown) was used for quantitation of the percent of half-molecules present in the minibody variant by peak integration. The deconvoluted masses were used for assignment and confirmed the identity of half molecules (middle panel) and of the full size masses (lower panel).

[0529] However, the intact mass analysis (FIG. 15A) of the IAB22M1-γ2 EH2 variant (FIG. 15B) with the first cysteine in IgG2 hinge mutated to serine showed greater than 99% intact dimeric protein with γ2 EH2 (theoretical MW = 79051.2; obtained MW 79052). For intact mass analysis of IAB22M1 γ2 EH2, a protein sample was analyzed by LC/MS consisting of the following: Waters Synapt G2 HDMS fitted with a Trizaic nanoESI source coupled to a Waters nanoAcquity UPLC. The protein was separated using the reverse phase C4 nanotile column (150µm ID x 50mm, Waters) operated at 3µl/min with 0.1% formic acid in water and 0.1% formic acid in acetonitrile as the mobile phases. FIG. 15A upper panel shows full range scan showing the single dominant molecular mass of the full-size minibody. FIG. 15A middle panel shows zoomed-in region of the dimers e.g. full size protein. FIG. 15A lower panel shows zoomed in region of the half-molecules. A half molecule is detected but its relative abundance is nearly 3 orders of magnitude lower than the full size protein.

EXAMPLE 6 - *IN VIVO* DATA FOR IAB22M IGG2 MBS

[0530] Comparison of the IAB22M Mbs with huIgG1 and huIgG2 derived hinge sequences was performed by PET/CT of ^{89}Zr-Df-IAB22M variants. MIP PET/CT images of NOD-SCID mice bearing CD8 positive HPB-ALL xenografts on the left shoulder after administration of ^{89}Zr-Df-IAB22M-γ1-EH1 (FIG. 16B), -γ2 NH1 (FIG. 16C) and -γ2 EH2 (FIG. 15B) variants are shown in FIG. 16A. The γ2 hinge variants resulted in lower kidney uptake.

[0531] IAB22M Mb made with a huIgG1 hinge (γ1 EH1) (FIG. 16B) was rapidly cleared through the kidney resulting in low tumor targeting. IAB22M Mbs made with huIgG2 hinge variants, either extended hinge (γ2 EH2) (FIG. 15B) or natural hinge (γ2 NH1)

(FIG. 16C) showed greater stability *in vivo* with high tumor targeting and lower kidney clearance (FIG. 16A).

[0532] PET/CT of HPB-ALL tumors was performed for ^{89}Zr-Df-IAB22M-γ1 EH1 (FIG. 16B). Serial coronal images of one mouse with antigen-positive HPB-ALL tumor at 4, 24 and 41hrs are shown in FIG. 17. Radioactive uptake was clearly seen in the tumor at 24 and 41hrs. Some background activity was also observed in the antigen negative Daudi tumor. High background activity was present in the abdominal region suggesting undesired clearance through the kidney.

[0533] Biodistribution analysis was performed for ^{89}Zr-Df-IAb22M-γ1 EH1 (FIG. 16B) in HPB-ALL tumor (FIG. 18). The tumor uptake in HPB-ALL was ~6-fold higher compared to Daudi (Daudi tumor does not express target antigen). The ratio of biodistribution between HPB-ALL tumor and blood was 13.5 and ratio of biodistribution between Daudi tumor and blood was 3.5. Kidney signal was higher than liver signal suggesting higher clearance through kidneys (FIG. 18) and a less stable *in vivo* construct.

Lessons learned from initial constructs used for engineering optimized minibodies

[0534] The above results showed that it was important to mutate the first hinge cysteine to prevent cysteine mis-pairing. However, more than 2 cysteine residues are beneficial in the hinge region to maintain the structural integrity of protein. Employing the IgG2 hinge provides a solution to increase stability *in vivo*. Mutation of the terminal lysine (K) in the Mb constructs did not impact protein expression but did generate protein with a more uniform charge.

[0535] Additional Mb variants were evaluated based on IgG3 and modification of IgG1 hinge sequences where the first hinge cysteine was mutated and at least 3 cysteine residues were present in the hinge region. Table 3 shows a list of hinge variants. The first native cysteine in the hinge sequence can be mutated to serine or alanine or any other amino acid. The lower hinge sequence can be an extension sequence (8-25 amino acids) or native lower hinge from γ1, γ2, γ3 or γ4. C_H3 domain for any construct can be from γ1, γ2 or γ3 or γ4 and any naturally occurring allele thereof. An illustration of some hinge variants is shown in FIG. 19. IAB2M, IAB22M, IAB20M and IAB1M constructs were evaluated to demonstrate universality of findings.

EXAMPLE 7 - *IN VITRO* DATA FOR HINGE VARIANTS OF IAB2M, IAB22M, IAB20M AND IAB1M

[0536] Intact mass analysis was performed on IAB2M (FIG. 20A) hinge variants (FIGs. 5B, 5C, 7C, 5E, 5D). Intact mass analysis was also performed on IAB22M (FIG. 20B) hinge variants (FIGs. 14B, 15B, 20C, 20D, 20E, 20F, 20G). Expressed minibodies with engineered hinges (EH) were analyzed for intact mass and the amount of the half molecule (FIGs. 20A and 20B) by LC/MS using a Waters Synapt G2 HDMS fitted with a Trizaic nanoESI source coupled to a Waters nanoAcquity UPLC Waters C4 nanotile column (150µm ID x 50mm length) operated at 3µl/min with 0.1% formic acid in water and 0.1% formic acid in acetonitrile as the buffers. For some molecules the samples were separated by reverse phase chromatography column (TSKgel Phenyl-5PW (Tosoh, 2 x 75 mm) and analyzed by Agilent ESI-QTOF mass spectrometer.

[0537] SDS-PAGE analyses of IAB2M hinge Mb variants (FIGs. 5C, 7C, 21B, 21C, 21D, 21E) and IAB22M hinge Mb variants (FIGs. 16B, 15B, 20C, 20D, 20E, 20F, 20G) were performed. The resulting data are shown in FIGs. 21A and 22A.

[0538] Mass spectrometry analysis was performed to obtained data to support the SDS-PAGE analyses data of FIGs. 21A and 22A. Two IAB22M constructs were evaluated using mass spectrometry to confirm the exact molecular weight, amount of half-molecule and post-translational modification, e.g. C-terminal lysine clipping. The protein constructs included minibodies with engineered hinges EH2, EH3 derived from IgG1 sequence (γ1 EH2 (FIG. 22B) and γ1 EH3 (FIG. 20C)). γ1 minibodies with EH2 hinge assembled properly into intact dimeric molecules but inclusion of only two cysteine yielded protein with high amount of half molecule. γ1 minibodies with EH3 hinge assembled properly into intact dimeric molecules and addition of third cysteine yielded protein with very low levels of half molecule.

[0539] Intact mass analysis by mass spectrometry of IAB22M-γ1 EH1 (FIG. 16B) is shown in FIG. 23. The obtained molecular masses indicated C-terminal clipping. Of 3 species present (MW 79318.9, 79450.1 and 79575.1) only one matched the predicted MW of 79576. The obtained molecular masses indicated C-terminal clipping. Of 3 species present (MW 79318.9, 79450.1 and 79575.1) only one matched the predicted MW of 79576. The remainder were minibody with either 1 or both C-terminal lysines being clipped. Mbs

constructed with only 2 disulfides in the hinge produced high levels of half molecule (~15%). For intact mass analysis of IAB22M γ1 EH1, the samples were separated by reverse phase chromatography column (TSKgel Phenyl-5PW (Tosoh, 2 x 75 mm) and analyzed using an Agilent ESI-QTOF mass spectrometer. FIG. 23 shows the total ion chromatograms of the separated half-molecule and full-size molecule (upper panel). The UV280 trace (not shown) was used for quantitation of the percent of half-molecules present in the minibody variant by peak integration. The deconvoluted masses were used for assignment and confirmed the identity of half molecules (middle panel) and of the full size masses (lower panel).

[0540] Intact mass analysis by mass spectrometry of IAB22M-γ1 EH3 variant (FIG. 20C) is shown in FIG. 24. Theoretical MW was 79946 matched the obtained MW of 79946. Modified IgG1 hinge constructed with three disulfide bonds in hinge yielded a minibody with greater than 99% full size protein. For intact mass analysis, a protein sample in acetate buffer was analyzed by LC/MS consisting of the following: Waters Synapt G2 HDMS fitted with a Trizaic nanoESI source coupled to a Waters nanoAcquity UPLC. The protein was separated using the reverse phase C4 nanotile column (150µm ID x 50mm, Waters) operated at 3µl/min with 0.1% formic acid in water and 0.1% formic acid in acetonitrile as the mobile phases. FIG. 24 (upper panel) shows full mass range scan. Middle panel shows zoomed in region showing intact molecular mass. Lower panel shows zoomed in region showing half-molecule.

[0541] Table 9 shows a listing summarizing the theoretical predicted molecular weight (MW) mass and obtained MW mass by intact mass analysis for five IAB2M hinge variants (top; FIGs. 5B – 5E, 7C) and seven IAB22M hinge variants (bottom; FIGs. 14B, 15B, 20C, 20D; 20E, 43, 44) and the respective content of half molecule. Actual masses of proteins matched their theoretically calculated molecular masses except where C terminal lysine residues were clipped leading to MW heterogeneity (FIGs. 6, 14A, 15A, 23 and 24).

TABLE 9

Half-molecule content obtained by intact mass analysis of IAB2M and IAB22M

(*C-terminal lysine clipping gives rise to heterogeneity)

Minibody	Construct	Predicted Mass	Obtained mass	Percent Half Molecule

IAB2M	IAB2M-γ1 EH1	79433.6	79176.6,* 79304, 79433.1	7.6
	IAB2M-γ2 EH1	79197.6	78938.7, 79069.7, 79195.9	11.1
	IAB2M-γ1 EH2	79145	79148	7.1
	IAB2M2-γ2 EH2	78909	78909.2	<0.5
	IAB2M2-γ1 EH3	79739.8	79741.8	<0.5
IAB22M	IAB22M-γ2 EH1	79576	79318.9, 79450.1, 79575.1	9.2
	IAB22M1-γ2 EH2	79051.5	79053	<0.5
	IAB22M1-γ1 EH3	79946.4	79947.5	<0.1
	IAB22M1-γ1 EH5	80541.2	80540	<0.1
	IAB22M1-γ3/γ1 EH6	80575.2	80576	<0.5
	IAB22M1-γ1 EH7	80312.8	80313.3	<0.5
	IAB22M1-γ1 EH8	80907.6	80907	<0.5

[0542] FAC analysis of was performed for IAB2M hinge variants (FIGs. 5E, 7C, 7D, 21B, 21C, 21D, 21E). All minibody hinge variants bound with similar affinity to the target antigen expressed on C4-2 XCL cells (FIG. 26).

[0543] FAC analysis was performed for IAB22M hinge variants (FIGs. 15B, 20C, 20D, 20E, 20F, and 20G). Minibodies made with same scFv and C_H3 domains but different hinges all bound to cell surface CD8 on HPB-ALL cell with similar affinity (FIG. 27).

[0544] SDS-PAGE analysis (FIG. 28A) was performed for IAB20M hinge Mb variants (FIGs. 28B – 28E) further confirming that the aspects of the hinge region noted herein can be applied across a wide range of target molecules (for example, that it works for a variety of minibodies directed to different target molecules). Additional embodiments of IAB20M variants are shown in FIGs. 54 - 59.

[0545] SDS-PAGE analysis (FIG. 29A) was performed for IAB1M hinge Mb variants (FIGs. 29B – 29D) further confirming that the aspects of the hinge region noted

herein can be applied across a wide range of target molecules (for example, that it works for a variety of minibodies directed to different target molecules). Additional embodiments of IAB1M variants are shown in FIGs. 60 – 65A, 65B, and 65C.

EXAMPLE 8 - *IN VIVO* DATA FOR HINGE VARIANTS OF IAB22M

[0546] PET/CT analysis of ^{89}Zr radiolabeled IAB22 Mbs with different hinge sequences (FIGs. 15B, 20C, 20D, 20F, 20G) was performed in NOD-SCID mice. MIP PET/CT images of NOD-SCID mice bearing CD8 positive HPB-ALL xenografts on the left shoulder after administration ^{89}Zr-labeled Df-IAB22M hinge variants at 24h are shown in FIG. 30. All new hinge variants resulted in lower kidney uptake. Good tumor targeting that ranged from 17-28% ID/g was obtained with all hinge variants. Overall, the liver signal was similar to the kidney signal.

[0547] Biodistribution analysis (FIG. 31) was performed for ^{89}Zr Radiolabeled IAB22 Mbs with the different hinge variants of FIG. 30. Similar biodistribution was observed for the IAB22M hinge variants. Excellent tumor targeting and uptake was detected compared to γ1 EH1. High clearance though liver was observed as predicted based on the data provided herein for a Mb that remained a dimer.

EXAMPLE 9

[0548] A PSCA minibody from Table 0.2 is conjugated with a relevant chelating agent via cysteine residues on the minibody and subsequently radiolabeled with an isotope of In-111 (or in the alternative, Zirconium-89 or Copper-64). Alternatively, the minibody can be radiolabeled by directly radiolabeling with iodine via tyrosine residues.

[0549] The minibody is infused intravenously into a healthy human subject. The minibody is incubated in the human subject for 10 minutes post-infusion. On the same day as the incubation, the localization of the minibody is detected via a PET scan or external scintillation system.

[0550] Localization of minibody is used to determine localization of elevated levels of PSCA in the subject.

EXAMPLE 10

[0551] A PSMA minibody from Table 0.2 is conjugated with a relevant chelating agent via cysteine residues on the minibody and subsequently radiolabeled with an isotope of In-111 (or in the alternative, Zirconium-89 or Copper-64). Alternatively, the minibody can be radiolabeled by directly radiolabeling with iodine via tyrosine residues.

[0552] The minibody is infused intravenously into a healthy human subject. The minibody is incubated in the human subject for 10 minutes post-infusion. On the same day as the incubation, the localization of the minibody is detected via a PET scan or external scintillation system.

[0553] Localization of minibody is used to determine localization of elevated levels of PSMA in the subject.

EXAMPLE 11

[0554] Upon successful imaging of PSMA positive tumors by a PSMA minibody from Table 0.2, the biodistribution of the minibody may be investigated according to embodiments of the disclosure. These biodistribution studies can investigate the localization of the minibody at the tumor site versus other selected tissues over time following injection. These studies may be used to demonstrate high tumor to background ratios. Use of a minibody would likely produce a high tumor to background ratio when imaging a tumor that overexpresses PSMA, such as in prostate cancer. Positive results from these imaging and biodistribution experiments may lead to toxicology experiments in preparation for clinical studies.

[0555] Further, the ability of a minibody to target human PSMA *in vivo* by PET imaging studies may be demonstrated through clinical trials in cancer patients. Briefly, radiolabeled minibody can be injected intravenously into cancer patients having a form of cancer that is known to overexpress PSMA. At specific time points post-injection, each patient may be serially scanned by PET. After the final scan, patients may be scanned by CT for anatomical reference. The PET and CT images for each patient may then be analyzed to evaluate tumor targeting and specificity.

EXAMPLE 12

[0556] A 5T4 minibody from Table 0.2 is conjugated with a relevant chelating agent via cysteine residues on the minibody and subsequently radiolabeled with an isotope of In-111 (or in the alternative, Zirconium-89 or Copper-64). Alternatively, the minibody can be radiolabeled by directly radiolabeling with iodine via tyrosine residues.

[0557] The minibody is infused intravenously into a healthy human subject. The minibody is incubated in the human subject for 10 minutes post-infusion. On the same day as the incubation, the localization of the minibody is detected via a PET scan or external scintillation system.

[0558] Localization of minibody is used to determine localization of elevated levels of 5T4 in the subject.

EXAMPLE 13

[0559] A 5T4 minibody of Table 0.2 is provided. The minibody is infused intravenously into a subject having colorectal, renal, breast, ovarian, gastric, lung, and/or prostate cancer in an amount adequate to bind to sufficient levels of 5T4 in the subject to provide a lessening of the symptoms of colorectal, renal, breast, ovarian, gastric, lung, and/or prostate cancer in the subject.

EXAMPLES 14

[0560] A CD8 minibody from Table 0.2 is conjugated with a relevant chelator via cysteine residues on the minibody and subsequenty radiolabeled with an isotope of In-111 (or in the alternative, Zirconium-89 or Copper-64). Alternatively, the minibody can be radiolabeled by directly radiolabeling with Iodine via tyrosine residues.

[0561] The minibody is infused intravenously into a healthy human subject. The minibody is incubated in the human subject for 10 minutes post-infusion. On the same day as the incubation, the localization of the minibody is detected via a PET scan or external scintillation system.

[0562] Localization of minibody is used to determine localization of CD8 in the subject.

EXAMPLE 15

[0563] A CD8 minibody of Table 0.2 is provided. The minibody is infused intravenously into a healthy human subject.

[0564] The minibody is incubated in the human subject for 1 hour post-infusion. A secondary antibody, a humanized minibody that binds specifically to the CD8 minibody and is conjugated to ^{33}P is provided. Within the same day as the incubation, the secondary antibody is infused into to subject. The secondary antibody is incubated for one hour. The localization of the minibody is detected via PET imaging, via a marker on the secondary antibody.

[0565] Localization of minibody is used to determine localization of CD8 in the subject.

EXAMPLE 16

[0566] A CD8 minibody of Table 0.2 is provided. The minibody is infused intravenously into a subject having a CD8 related disorder in an amount adequate to bind to sufficient levels of CD8 in the subject to provide a lessening of the symptoms of the CD8 related disorder. The minibody is conjugated to Yttrium-90.

EXAMPLE 17

[0567] A CD8 minibody of Table 0.2 is provided. The minibody is injected into a patient who has been vaccinated with an antigen to an infectious disease or with a tumor associated antigen. The CD8 directed fragments augment the immune response and enhance the cytolytic activity of CD8 expressing T cells.

EXAMPLE 18

[0568] A CD8 minibody of Table 0.2 is provided. The minibody is infused intravenously into a subject having a CD8 related disorder in an amount adequate to bind to sufficient levels of CD8 in the subject to provide a lessening of the symptoms of the CD8 related disorder. The minibody is conjugated to Lu-177. The CD8 minibody binds to a cell expressing CD8 and the Lu-177 results in the killing of the cell.

[0569] The results indicated that the constructs still bind to cellular human CD8.

EXAMPLE 19

[0570] A CD3 minibody from Table 0.2 is conjugated with a relevant chelating agent via cysteine residues on the minibody and subsequently radiolabeled with an isotope of In-111 (or in the alternative, Zirconium-89 or Copper-64). Alternatively, the minibody can be radiolabeled by directly radiolabeling with iodine via tyrosine residues.

[0571] The minibody is infused intravenously into a healthy human subject. The minibody is incubated in the human subject for 10 minutes post-infusion. On the same day as the incubation, the localization of the minibody is detected via a PET scan or external scintillation system.

[0572] Localization of minibody is used to determine localization of elevated levels of CD3 in the subject.

EXAMPLE 20

[0573] A CD3 minibody from Table 0.2 is provided. The minibody is infused intravenously into a subject having rheumatoid arthritis in an amount adequate to bind to sufficient levels of CD3 in the subject to provide a lessening of the symptoms of rheumatoid arthritis in the subject.

EXAMPLE 21

[0574] A subject at risk of developing an unacceptably intense cytokine storm from the administration of OKT3 is identified. The subject has a need for reducing the level of CD3 proteins in his system as the subject has a CD3 dependent disorder. One of the minibodies to CD3 provided herein is selected and administered to the subject at a level that is effective for adequate binding to CD3 to occur, without a cytokine storm occurring.

EXAMPLE 22

[0575] A humanized CD3 minibody of Table 0.2 is provided. The minibody is infused intravenously into a healthy human subject. The minibody is incubated in the human subject for 1 hour post-infusion. A secondary antibody, a humanized minibody that binds specifically to the CD3 minibody and is conjugated to ^{33}P is provided. Immediately after the one-hour incubation, the secondary antibody is infused into to subject. The secondary

antibody is incubated for one hour. Immediately after the one-hour incubation of the secondary antibody, the localization of the minibody is detected via PET imaging.

[0576] Localization of minibody is used to determine localization of CD3 in the subject.

EXAMPLE 23

[0577] A subject at risk of developing an unacceptably intense cytokine storm from the administration of OKT3 is identified. The subject has a need for reducing the level of CD3 proteins in his system as the subject has a CD3 dependent disorder. One of the minibody or minibodies from the herein examples is selected and administered to the subject at a level that is effective for adequate binding to CD3 to occur, without a cytokine storm occurring.

[0578] If needed, the minibody or minibodies can be conjugated to a therapeutic agent for the treatment of the CD3 dependent disorder.

[0579] The subject can have any of a number of particular CD3 dependent disorders, which, each in the alternative can be rheumatoid arthritis, multiple sclerosis, type 1 diabetes, or lupus erythematosus.

EXAMPLE 24

[0580] A PSCA minibody of Table 0.2 is provided. The minibody is infused intravenously into a subject having a PSCA related disorder in an amount adequate to bind to sufficient levels of PSCA in the subject to provide a lessening of the symptoms of the PSCA related disorder. The minibody is conjugated to Yttrium-90.

EXAMPLE 25

[0581] A PSMA minibody of Table 0.2 is provided. The minibody is infused intravenously into a subject having a PSMA related disorder in an amount adequate to bind to sufficient levels of PSMA in the subject to provide a lessening of the symptoms of the PSMA related disorder. The minibody is conjugated to Yttrium-90.

[0582] In this application, the use of the singular can include the plural unless specifically stated otherwise or unless, as will be understood by one of skill in the art in light of the present disclosure, the singular is the only functional embodiment. Thus, for example,

"a" can mean more than one, and "one embodiment" can mean that the description applies to multiple embodiments.

[0583] All references cited herein, including patents, patent applications, papers, text books, and the like, and the references cited therein, to the extent that they are not already, are hereby incorporated by reference in their entirety. In the event that one or more of the incorporated literature and similar materials differs from or contradicts this application; including but not limited to defined terms, term usage, described techniques, or the like, this application controls.

[0584] The foregoing description and examples detail certain embodiments. It will be appreciated, however, that no matter how detailed the foregoing may appear in text, the invention may be practiced in many ways and the invention should be construed in accordance with the appended claims and any equivalents thereof.

WHAT IS CLAIMED IS:

1. An amino acid hinge region comprising a sequence of SEQ ID NO: 1 ($X_{n1}CX_{n2}X_{n3}CX_{n4}X_{n5}C$), wherein X_{n1} can be any amino acid that does not naturally form a covalent crosslinking bond, wherein X_{n2} is one of: A, R, N, D, E, Q, G, H, I, L, K, M, F, P, S, T, W, Y, or V, wherein X_{n3} can be any amino acid, wherein X_{n4} can be any amino acid, and wherein X_{n5} can be any amino acid.

2. The amino acid hinge region of claim 1, wherein X_{n1} does not form a covalent crosslinking bond with another amino acid (SEQ ID NO: 191).

3. The amino acid hinge region of any one of claims 1-2, wherein X_{n1} is not a cysteine (SEQ ID NO: 192).

4. The amino acid hinge region of any one of claims 1-3, wherein X_{n1} is one of: A, R, N, D, E, Q, G, H, I, L, K, M, F, P, S, T, W, Y, or V (SEQ ID NO: 193).

5. The amino acid hinge region of any one of claims 1-4, wherein X_{n2} is P, V, or E (SEQ ID NO: 194).

6. The amino acid hinge region of any one of claims 1-5, wherein X_{n2} is P or V (SEQ ID NO: 195).

7. The amino acid hinge region of any one of claims 1-6, wherein X_{n4} is P, V, or E (SEQ ID NO: 196).

8. The amino acid hinge region of any one of claims 1-7, wherein X_{n4} is P or V (SEQ ID NO: 197).

9. The amino acid hinge region of any one of claims 1-8, wherein X_{n3} is P or E (SEQ ID NO: 198).

10. The amino acid hinge region of any one of claims 1-9, wherein X_{n5} is P or E (SEQ ID NO: 199).

11. The amino acid hinge region of claim 9, wherein X_{n3} is P or E (SEQ ID NO: 200).

12. The amino acid hinge region of any one of claims 1-11, wherein $X_{n2}X_{n3}$ is VE (SEQ ID NO: 201).

13. The amino acid hinge region of any one of claims 1-2, wherein $X_{n2}X_{n3}$ is PP (SEQ ID NO: 202).

14. The amino acid hinge region of any one of claims 1-13, wherein $X_{n4}X_{n5}$ is VE (SEQ ID NO: 203).

15. The amino acid hinge region of any one of claims 1-14, wherein $X_{n4}X_{n5}$ is PP (SEQ ID NO: 204).

16. The amino acid hinge region of any one of claims 1-12 or 15, wherein $X_{n2}X_{n3}$ is VE and $X_{n4}X_{n5}$ is PP (SEQ ID NO: 205).

17. The amino acid hinge region of any one of claims 1-11, 13, or 14, wherein $X_{n2}X_{n3}$ is PP and $X_{n4}X_{n5}$ is PP or VE (SEQ ID NO: 206).

18. The amino acid hinge region of any one of claims 1-12, 14, or 17, wherein $X_{n2}X_{n3}$ is VE and $X_{n4}X_{n5}$ is VE or PP (SEQ ID NO: 207).

19. The amino acid hinge region of any one of claims 1-18, further comprising an extension or lower hinge sequence C-terminal to the last cysteine in SEQ ID NO: 1.

20. The amino acid hinge region of claim 19, wherein the extension or lower hinge sequence comprises at least one of S, G, A, P, or V.

21. The amino acid hinge region of any one of claims 19-20, wherein the extension sequence comprises at least GGGSSGGGSG (SEQ ID NO: 59).

22. The amino acid hinge region of any one of claims 1-21, comprising a linker sequence comprising at least APPVAGP (SEQ ID NO: 60).

23. The amino acid hinge region of any one of claims 1-22, wherein the hinge region of claim 1 is part of a core hinge region.

24. The amino acid hinge region of claim 23, further comprising an upper hinge region adjacent to the core hinge region.

25. The amino acid hinge region of claim 23, further comprising a lower hinge or extension region adjacent to the core hinge region.

26. The amino acid hinge region of claim 25, further comprising an upper hinge region adjacent to the core hinge region.

27. The amino acid hinge region of any one of claims 1-26, wherein X_{n1} comprises a serine, a threonine, or an alanine (SEQ ID NO: 209).

28. The amino acid hinge region of any one of claims 1-26, wherein X_{n1} comprises a serine (SEQ ID NO: 210).

29. The amino acid hinge region of any one of claims 1-26, wherein X_{n1} comprises an alanine (SEQ ID NO: 211).

30. The amino acid hinge region of any one of claims 1-28, wherein the amino acid hinge region comprises at least one of the following sequences: SCVECPPCP (SEQ ID NO: 56) or TCPPCPPC (SEQ ID NO: 166).

31. The amino acid hinge region of any one of claims 1-30, wherein the amino acid hinge region comprises at least one of the following sequences: ERKSCVECPPCP (SEQ ID NO: 167), EPKSSDKTHT (SEQ ID NO: 46), and CPPCPPC (SEQ ID NO: 52).

32. The amino acid hinge region of any one of claims 1-31, wherein the amino acid hinge region comprises at least one of the following sequences: ERKSCVECPPCPGGGSSGGGSG (SEQ ID NO: 34) or ERKSCVECPPCPAPPVAGP (SEQ ID NO: 33) or EPKSSDKTHTCPPCPPCGGGSSGGGSG (SEQ ID NO: 26) or EPKSSDKTHTCPPCPPCAPELLGGP (SEQ ID NO: 25).

33. An amino acid hinge region comprising a sequence of SEQ ID NO: 2 (X_{n1} X_{n2} X_{n3} $X_{n4}X_{n5}$ $X_{n6}CX_{n7}X_{n8}CX_{n9}X_{n10}C$), wherein X_{n1} can be any m amino acids, wherein m is any number of amino acids of any type, wherein X_{n2} can be any amino acid, wherein X_{n3} can be any amino acid, wherein X_{n4} can be any amino acid, wherein X_{n5} can be any amino acid, wherein X_{n6} can be any amino acid other than a cysteine, wherein X_{n7} can be any amino acid, wherein X_{n8} can be any amino acid, wherein X_{n9} can be any amino acid, and wherein X_{n10} can be any amino acid.

34. The amino acid hinge region of claim 33, wherein X_{n1} is not a cysteine (SEQ ID NO: 213).

35. The amino acid hinge region of any one of claims 33-34, wherein X_{n2} is not a cysteine (SEQ ID NO: 214).

36. The amino acid hinge region of any one of claims 33-35, wherein X_{n2} is a D (SEQ ID NO: 215).

37. The amino acid hinge region of any one of claims 33-36, wherein X_{n3} is a K (SEQ ID NO: 216).

38. The amino acid hinge region of any one of claims 33-37, wherein X_{n4} is a T (SEQ ID NO: 217).

39. The amino acid hinge region of any one of claims 33-38, wherein X_{n5} is a H (SEQ ID NO: 218).

40. The amino acid hinge region of any one of claims 33-39, wherein X_{n6} is a T (SEQ ID NO: 219).

41. The amino acid hinge region of any one of claims 33-40, wherein X_{n7} is a P or a V (SEQ ID NO: 220).

42. The amino acid hinge region of any one of claims 33-41, wherein X_{n8} is a P or a E (SEQ ID NO: 221).

43. The amino acid hinge region of any one of claims 33-42, wherein X_{n9} is a P or a V (SEQ ID NO: 222).

44. The amino acid hinge region of any one of claims 33-43, wherein X_{n10} is a P or a E (SEQ ID NO: 223).

45. The amino acid hinge region of claim 45, further comprising a CXXC (SEQ ID NO: 224) or CXXC (SEQ ID NO: 225) motif that is positioned in front of X_{n1}.

46. The amino acid hinge region of claim 46, further comprising a $X_{n11}X_{n12}C$ sequence immediately attached to the C-terminal cysteine in SEQ ID NO: 1, wherein X_{n11} can be any amino acid, and wherein X_{n12} can be any amino acid (SEQ ID NO: 244).

47. The amino acid hinge region of claim 46, wherein X_{n11} is a P or a V, and wherein X_{n12} is a P or an E (SEQ ID NO: 227).

48. The amino acid hinge region of any one of claims 33-47, wherein X_{n1} is a serine, X_{n2} is a D, X_{n3} is a K, X_{n4} is a T, X_{n5} is a H, X_{n6} is a T, X_{n7} is a P, X_{n8} is a P, X_{n9} is a P, and X_{n10} is a P (SEQ ID NO: 228).

49. The amino acid hinge region of any one of claims 33-48, wherein the hinge region comprises at least one of the following sequences: CPPCPPC (SEQ ID NO: 52), CPPCVECPPC (SEQ ID NO: 53), or CPPCPPCPPC (SEQ ID NO: 54).

50. The amino acid hinge region of any one of claims 33-49, wherein the hinge region comprises at least one of the following sequences: EPKSSDKTHTCPPCPPC (SEQ ID NO: 168), EPKSSDKTHTCPPCVECPPC (SEQ ID NO: 169), or EPKSSDKTHTCPPCPPCPPC (SEQ ID NO: 170).

51. The amino acid hinge region of any one of claims 33-50, wherein the hinge region comprises at least one of the following sequences: EPKSSDKTHTCPPCPPCGGGSSGGGSG (SEQ ID NO: 26), EPKSSDKTHTCPPCVECPPCGGGSSGGGSG (SEQ ID NO: 28), or EPKSSDKTHTCPPCPPCPPCGGGSSGGGSG (SEQ ID NO: 30).

52. An amino acid hinge region comprising:

a core hinge sequence of at least one of: CVECPPCP (SEQ ID NO: 57), CPPCPPC (SEQ ID NO: 52), or CPPCPPCPPC (SEQ ID NO: 54), or CPPCVECPPC (SEQ ID NO: 53) linked to;

an upper hinge sequence of ELKTPLGDTTHT (SEQ ID NO: 48) or EPKSSDKTHT (SEQ ID NO: 46).

53. An amino acid hinge region for an antibody comprising:

an upper hinge region that comprises no amino acids capable of crosslinking with a corresponding amino acid; and

a core hinge region connected to a C-terminus of the upper hinge region, wherein the core hinge region comprises at least three cysteines per strand.

54. The amino acid hinge region of any one of claims 52-53, wherein the amino acid hinge region further comprises a lower hinge or extension region connected C-terminal to the core hinge region, wherein the lower hinge or extension sequence is at least one of:

APPVAGP (SEQ ID NO: 60), APELLGGP (SEQ ID NO: 58), and/or GGGSSGGGSG (SEQ ID NO: 59).

55. The amino acid hinge region of any one of claims 52-54, wherein the upper hinge region comprises no cysteines that crosslink within the upper hinge region.

56. The amino acid hinge region of any one of claims 52-54, wherein the upper hinge region comprises no cysteines.

57. The amino acid hinge region of any one of claims 52-54, further comprising a lower hinge or extension region.

58. The amino acid hinge region of any one of claims 52-53, wherein the lower hinge or extension region comprises at least one of: GGGSSGGGSG (SEQ ID NO: 59) or APPVAGP (SEQ ID NO: 60) or APELLGGP (SEQ ID NO: 58).

59. The amino acid hinge region of any one of claims 1-58, wherein when located within a minibody, and wherein when the minibody is administered to a human subject, clearance of the minibody from the subject occurs primarily through a liver.

60. The amino acid hinge region of any one of claims 1-58, wherein, when located within a minibody, and wherein when the minibody is administered to a human subject, clearance of the minibody from the subject does not occur primarily through a kidney.

61. The amino acid hinge region of any one of claims 1-58, wherein the hinge region is within an antibody.

62. The amino acid hinge region of any one of claims 1-58, wherein the hinge region is within an antibody binding fragment.

63. The amino acid hinge region of any one of claims 1-60, wherein the hinge region is within a minibody.

64. The amino acid hinge region of any one of claims 1-58, wherein the hinge region is within a monospecific antibody.

65. The amino acid hinge region of any one of claims 1-64, wherein the hinge region comprises at least three cysteines per strand.

66. The amino acid hinge region of any one of claims 1-64, wherein the hinge region comprises at least four cysteines per strand.

67. The amino acid hinge region of any one of claims 1-64, wherein the hinge region comprises at least five cysteines per strand.

68. The amino acid hinge region of any one of claims 61-63, wherein cysteines are distributed throughout the amino acid hinge region in a repeating CXX or CXY motif.

69. The amino acid hinge region of any one of claims 1-58, wherein the hinge region is within a bispecific antibody.

70. The amino acid hinge region of claim 69, wherein the bispecific antibody is assembled in a 1:1 ratio.

71. The amino acid hinge region of claim 69, wherein the bispecific antibody comprises an antibody fragment.

72. The amino acid hinge region of claim 71, wherein the bispecific antibody is a minibody.

73. A pharmaceutical composition comprising the amino acid hinge region of any one of claims 1-72, wherein less than 5% aggregation of an antibody is present in the composition.

74. A pharmaceutical composition comprising the amino acid hinge region of any one of claims 1-72.

75. The pharmaceutical composition of any one of claims 73-74, wherein at least 1 microgram to 100 mg of the antibody is present.

76. A minibody comprising a core hinge region, wherein the core hinge region comprises at least three cysteines per strand forming at least three disulfide bonds within the core hinge region.

77. The minibody of claim 76, wherein the first residue of the core region is a serine.

78. The minibody of any one of claims 76-77, wherein the core hinge region comprises SCVECPPCP (SEQ ID NO: 56).

79. A minibody comprising a sequence $X_{n1}CX_{n2}X_{n3}CX_{n4}X_{n5}C$ (SEQ ID NO: 3), wherein SEQ ID NO: 3 is located as the core hinge region of the minibody, and wherein X_{n1} can be any amino acid or no amino acid, X_{n2} can be any amino acid, X_{n3} can be any amino acid, X_{n4} can be any amino acid, and X_{n5} can be any amino acid.

80. The minibody of claim 79, wherein X_{n1} is any amino acid other than a cysteine (SEQ ID NO: 229).

81. The minibody of claim 79, wherein X_{n1} is a serine (SEQ ID NO: 229).

82. A variant minibody hinge comprising:

a first altered amino acid position, wherein the first altered position is an amino acid that in a native antibody hinge would be a cysteine, and has been altered in the first altered position so that it does not form a disulfide bond; and

at least three cysteines per strand C-terminal to the first altered amino acid position.

83. The amino acid hinge region of any one of claims 1-72, wherein the hinge region consists of SEQ ID NO: 1.

84. The amino acid hinge region of any one of claims 1-72, wherein SEQ ID NO: 1 is a core hinge region, and wherein the core hinge region essentially consists of SEQ ID NO: 1.

85. The amino acid hinge region of claim 84, wherein the core hinge region consists of SEQ ID NO: 1.

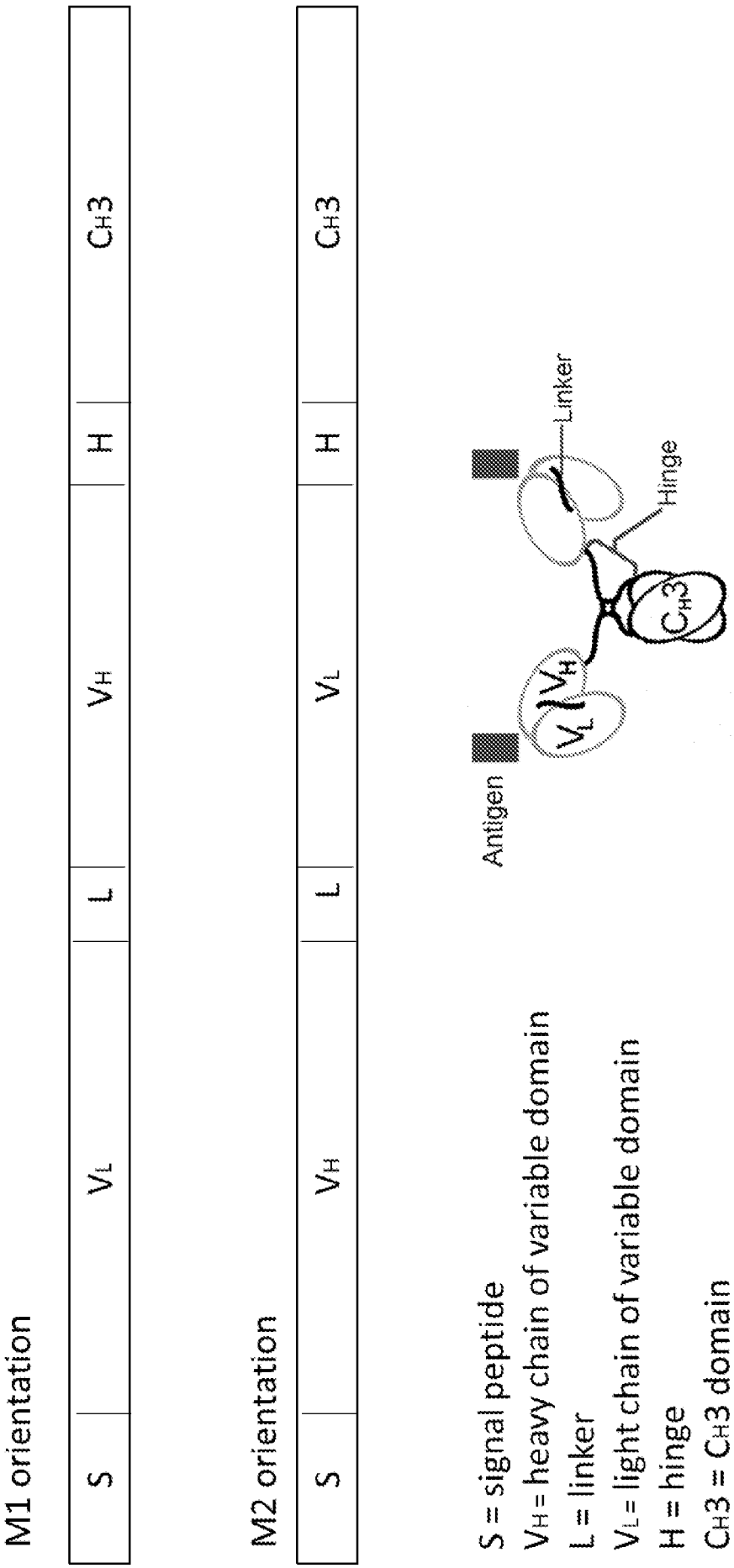

M1 orientation
S
VL
L
VH
H
CH3
M2 orientation
S
VH
L
VL
H
CH3
S = signal peptide
VH = heavy chain of variable domain
L = linker
VL = light chain of variable domain
H = hinge
CH3 = CH3 domain
Antigen
Linker
Hinge

FIG. 1

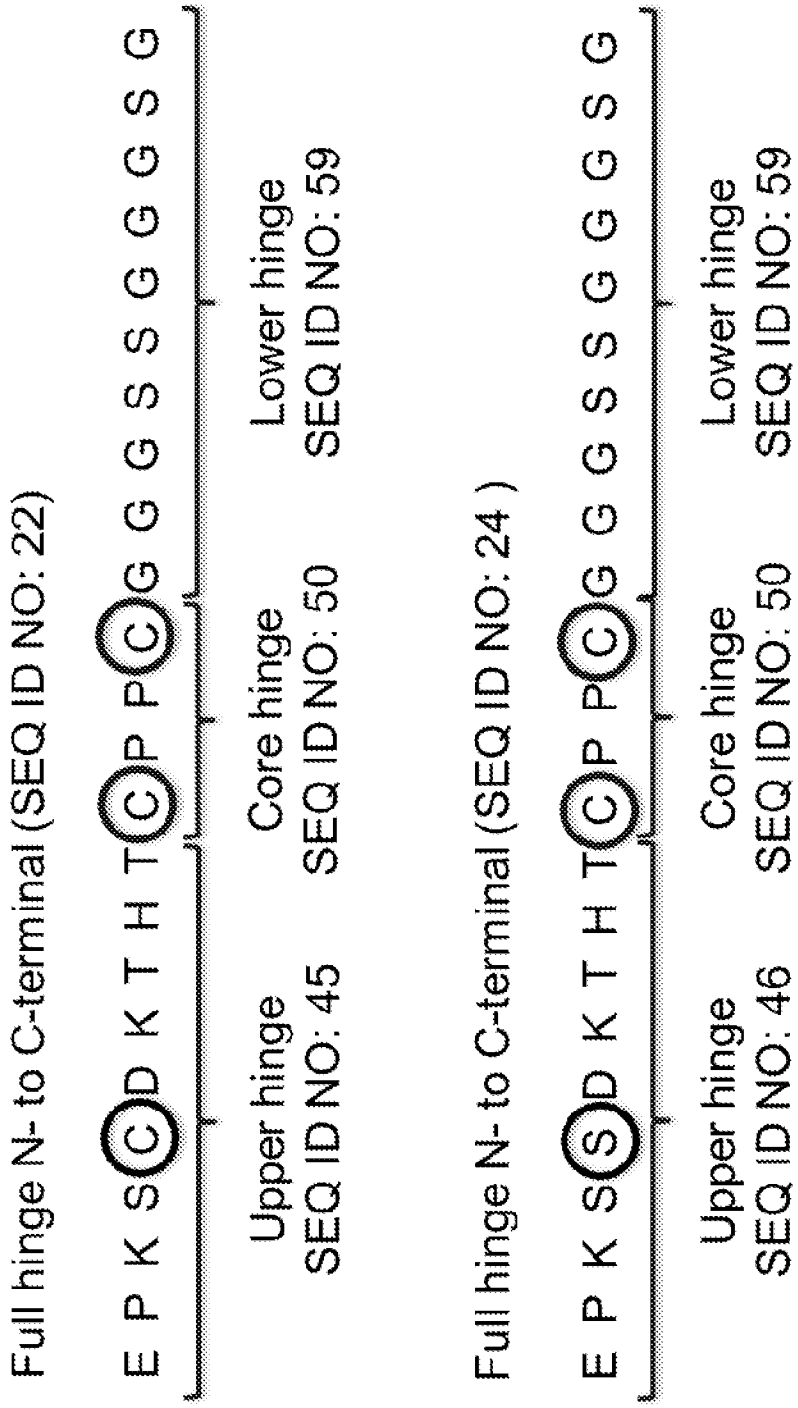

Full hinge N- to C-terminal (SEQ ID NO: 22)
E P K S C D K T H T C P P C G G G G S S G G G G S G
Upper hinge SEQ ID NO: 45
Core hinge SEQ ID NO: 50
Lower hinge SEQ ID NO: 59
Full hinge N- to C-terminal (SEQ ID NO: 24)
E P K S S D K T H T C P P C G G G G S S G G G G S G
Upper hinge SEQ ID NO: 46
Core hinge SEQ ID NO: 50
Lower hinge SEQ ID NO: 59

FIG. 2

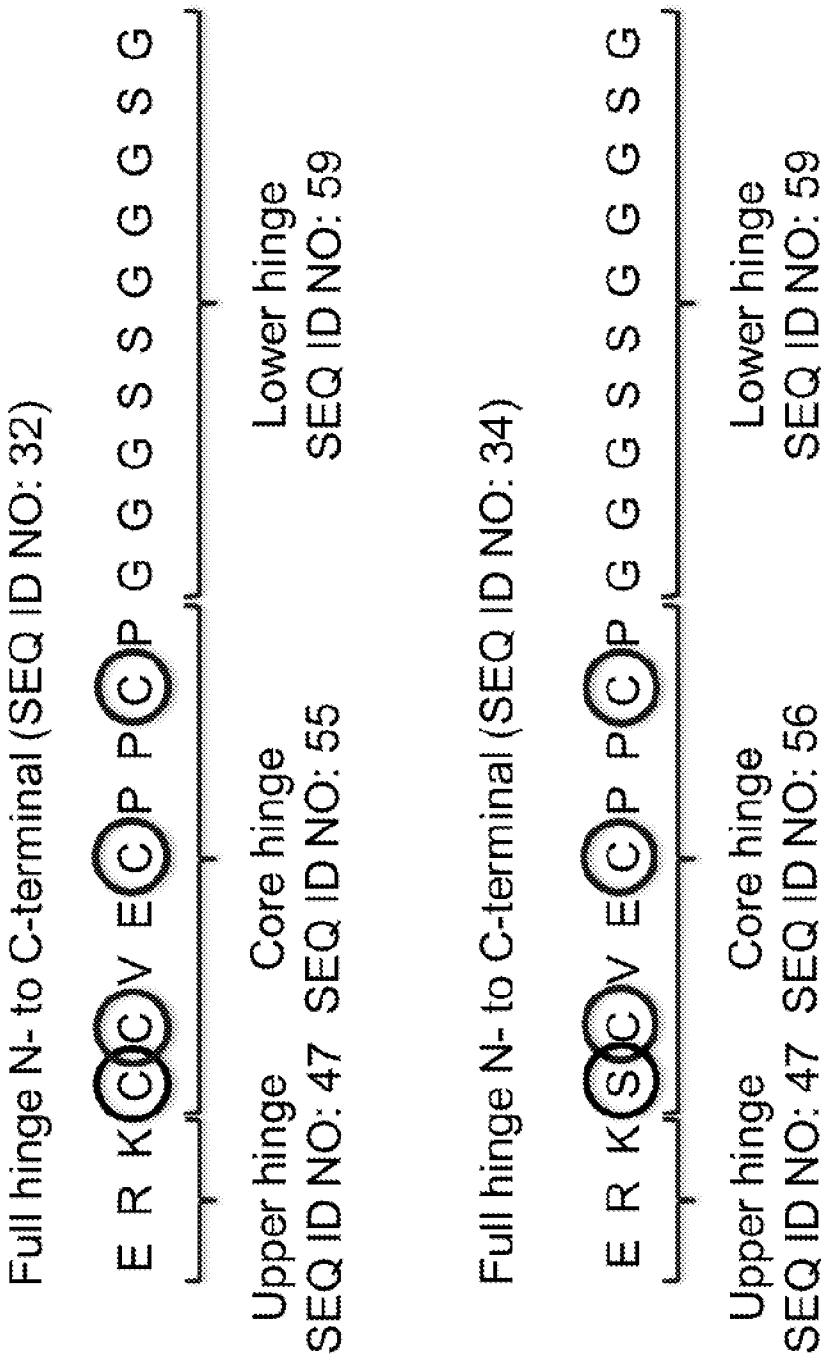

Full hinge N- to C-terminal (SEQ ID NO: 32)
E R K C C V E C P P C P G G G G S S G G G G S G
Upper hinge SEQ ID NO: 47
Core hinge SEQ ID NO: 55
Lower hinge SEQ ID NO: 59
Full hinge N- to C-terminal (SEQ ID NO: 34)
E R K S C V E C P P C P G G G G S S G G G G S G
Upper hinge SEQ ID NO: 47
Core hinge SEQ ID NO: 56
Lower hinge SEQ ID NO: 59

FIG. 3

Full hinge N- to C-terminal (SEQ ID NO: 31)
E R K C C V E C P P C P A P P V A G P
Upper hinge SEQ ID NO: 47
Core hinge SEQ ID NO: 55
Lower hinge SEQ ID NO: 60
Full hinge N- to C-terminal (SEQ ID NO: 33)
E R K S C V E C P P C P A P P V A G P
Upper hinge SEQ ID NO: 47
Core hinge SEQ ID NO: 56
Lower hinge SEQ ID NO: 60

FIG. 4

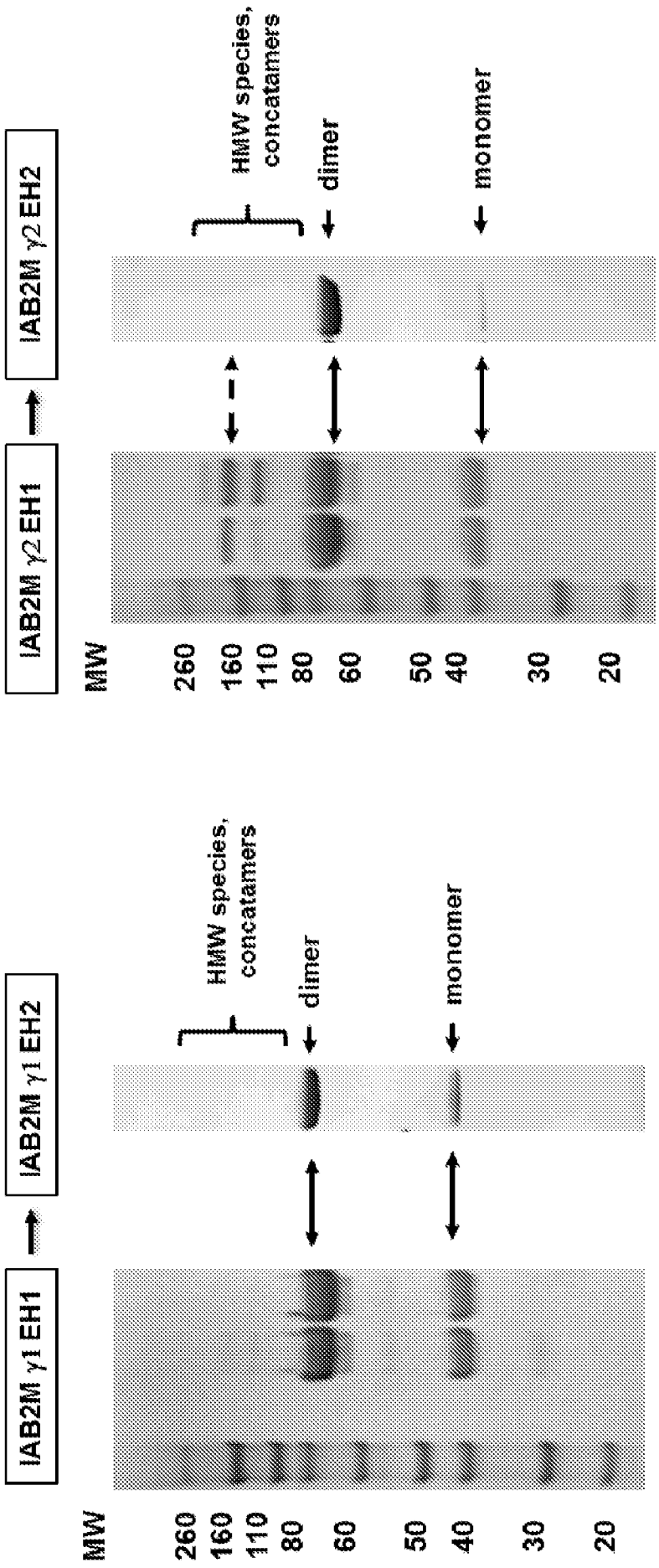

IAB2M γ1 EH1
IAB2M γ1 EH2
IAB2M γ2 EH1
IAB2M γ2 EH2
MW
260
160
110
80
60
50
40
30
20
HMW species, concatamers
dimer
monomer

FIG. 5A

IAB2M γ1 EH1 (N- to C-terminal)

Anti-PSMA variable light (VL) sequence
DIVMTQSPSSLSASVGDRVTITCKASQDVGTAVDWYQQKPGKAPKLLIYWASTRHTGVPDRFTGSGSGTDFTLT
ISSLQPEDFADYFCQQYNSYPLTFGGGTKLEIK (SEQ ID NO: 13)

Linker
GSTSGGGSGGGSGGGGSS (SEQ ID NO: 62)

Anti-PSMA variable heavy (VH) sequence
EVQLVQSGAEVKKPGASVKISCKTSGYTFTEYTIHWVKQASGKGLEWIGNINPNNGGTTYNQKFEDRATLTVDK
STSTAYMELSSLRSEDTAVYYCAAGWNFDYWGQGTTVTVSS (SEQ ID NO: 14)

Full hinge (EH1)
EPKSCDKTHTCPPCGGGSSGGGSG (SEQ ID NO: 22)

Upper hinge
EPKSCDKTHT (SEQ ID NO: 45)

Core hinge
CPPC (SEQ ID NO: 50)

Lower hinge
GGGSSGGGSG (SEQ ID NO: 59)

Human IgG1 CH3 (G1m1 allotype)
GQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDK
SRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 40)

Human IgG1 CH3 (nG1m1 allotype)
GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDK
SRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 41)

FIG. 5B

IAB2M γ1 EH2 (N- to C-terminal)

Anti-PSMA variable light (VL) sequence
DIVMTQSPSSLSASVGDRVTITCKASQDVGTAVDWYQQKPGKAPKLLIYWASTRHTGVPDRFTGSGSGTDFTL
TISSLQPEDFADYFCQQYNSYPLTFGGGTKLEIK (SEQ ID NO: 13)

Linker
GSTSGGGSGGGSGGGGSS (SEQ ID NO: 62)

Anti-PSMA variable heavy (VH) sequence
EVQLVQSGAEVKKPGASVKISCKTSGYTFTEYTIHWVKQASGKGLEWIGNINPNNGGTTYNQKFEDRATLTVD
KSTSTAYMELSSLRSEDTAVYYCAAGWNFDYWGQGTTVTVSS (SEQ ID NO: 14)

Full hinge (EH2)
EPKSSDKTHTCPPCGGGSSGGGSG (SEQ ID NO: 24)

Upper hinge
EPKSSDKTHT (SEQ ID NO: 46)

Core hinge
CPPC (SEQ ID NO: 50)

Lower hinge
GGGSSGGGSG (SEQ ID NO: 59)

Human IgG1 CH3 (G1m1 allotype)
GQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVD
KSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 40)

Human IgG1 CH3 (nG1m1 allotype)
GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVD
KSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 41)

FIG. 5C

IAB2M γ2 EH2 (N- to C-terminal)

Anti-PSMA variable light (VL) sequence
DIVMTQSPSSLSASVGDRVTITCKASQDVGTAVDWYQQKPGKAPKLLIYWASTRHTGVPDRFTGSGSGTDFT
LTISSLQPEDFADYFCQQYNSYPLTFGGGTKLEIK (SEQ ID NO: 13)

Linker
GSTSGGGSGGGSGGGGSS (SEQ ID NO: 62)

Anti-PSMA variable heavy (VH) sequence
EVQLVQSGAEVKKPGASVKISCKTSGYTFTEYTIHWVKQASGKGLEWIGNINPNNGGTTYNQKFEDRATLTVD
KSTSTAYMELSSLRSEDTAVYYCAAGWNFDYWGQGTTVTVSS (SEQ ID NO: 14)

Full hinge (EH2)
ERKSCVECPPCPGGGSSGGGGSG (SEQ ID NO: 34)

Upper hinge
ERK (SEQ ID NO: 47)

Core hinge
SCVECPPCP (SEQ ID NO: 56)

Lower hinge
GGGSSGGGSG (SEQ ID NO: 59)

Human IgG2 CH3
GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTV
DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 42)

FIG. 5D

IAB2M γ2 EH1 (N- to C-terminal)

Anti-PSMA variable light (VL) sequence
DIVMTQSPSSLSASVGDRVTITCKASQDVGTAVDWYQQKPGKAPKLLIYWASTRHTGVPDRFTGSGSGTDFTL
TISSLQPEDFADYFCQQYNSYPLTFGGGTKLEIK (SEQ ID NO: 13)

Linker
GSTSGGGSGGGSGGGGSS (SEQ ID NO: 62)

Anti-PSMA variable heavy (VH) sequence
EVQLVQSGAEVKKPGASVKISCKTSGYTFTEYTIHWVKQASGKGLEWIGNINPNNGGTTYNQKFEDRATLTVD
KSTSTAYMELSSLRSEDTAVYYCAAGWNFDYWGQGTTVTVSS (SEQ ID NO: 14)

Full hinge (EH1)
ERKCCVECPPCPGGGSSGGGSG (SEQ ID NO: 32)

Upper hinge
ERK (SEQ ID NO: 47)

Core hinge
CCVECPPCP (SEQ ID NO: 55)

Lower hinge
GGGSSGGGSG (SEQ ID NO: 59)

Human IgG2 CH3
GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTV
DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 42)

FIG. 5E

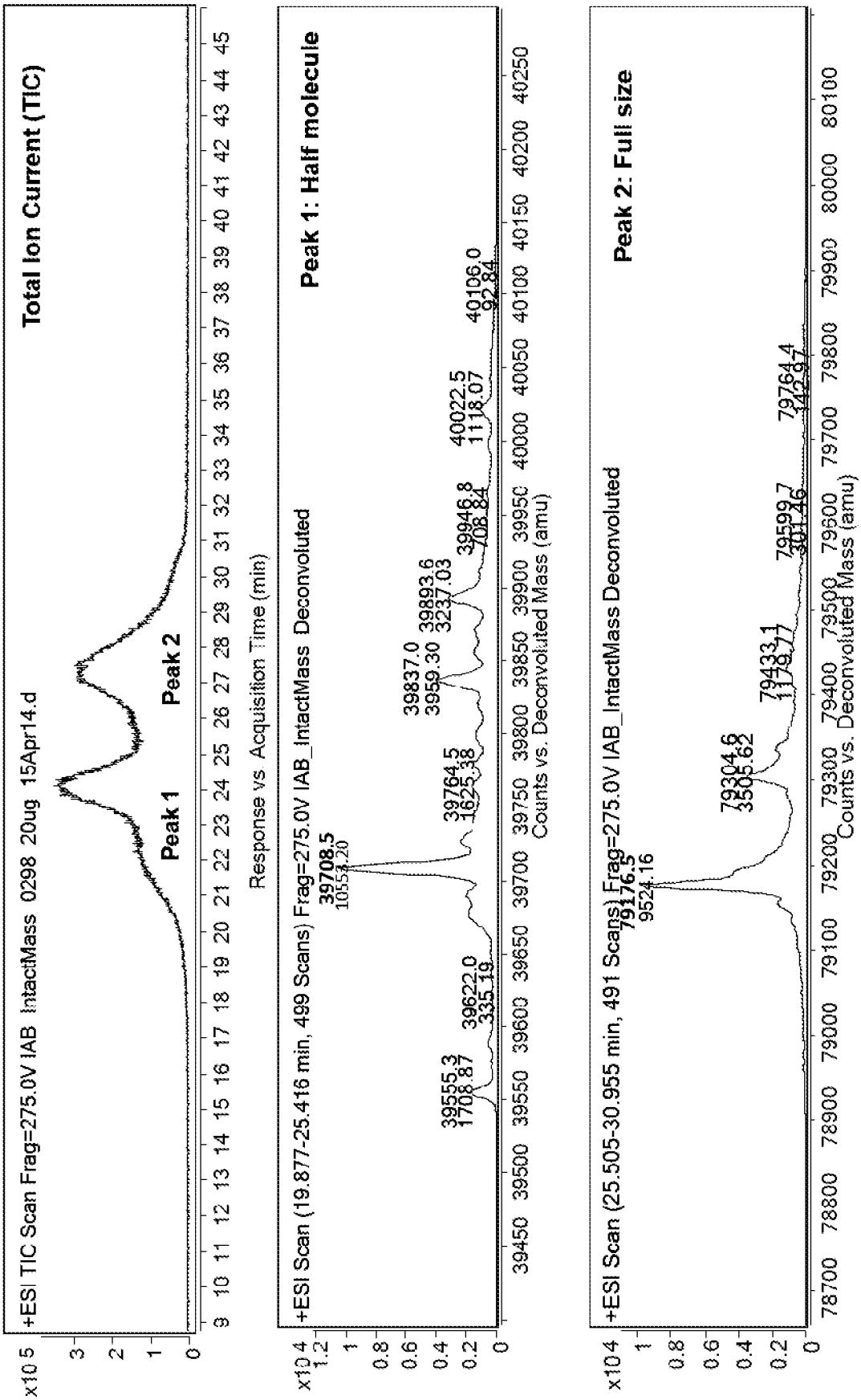

Intact mass analysis of IAB2M γ1 EH1
+ESI TIC Scan Frag=275.0V IAB IntactMass 0298 20ug 15Apr14.d
Total Ion Current (TIC)
Peak 1
Peak 2
Response vs Acquisition Time (min)
+ESI Scan (19.877-25.416 min, 499 Scans) Frag=275.0V IAB_IntactMass Deconvoluted
Peak 1: Half molecule
39708.5
39555.3
39622.0
39764.5
39837.0
39893.6
39946.8
40022.5
40106.0
Counts vs. Deconvoluted Mass (amu)
+ESI Scan (25.505-30.955 min, 491 Scans) Frag=275.0V IAB_IntactMass Deconvoluted
Peak 2: Full size
79176.5
79304.6
79433.1
79599.7
79764.4
Counts vs. Deconvoluted Mass (amu)

FIG. 6A

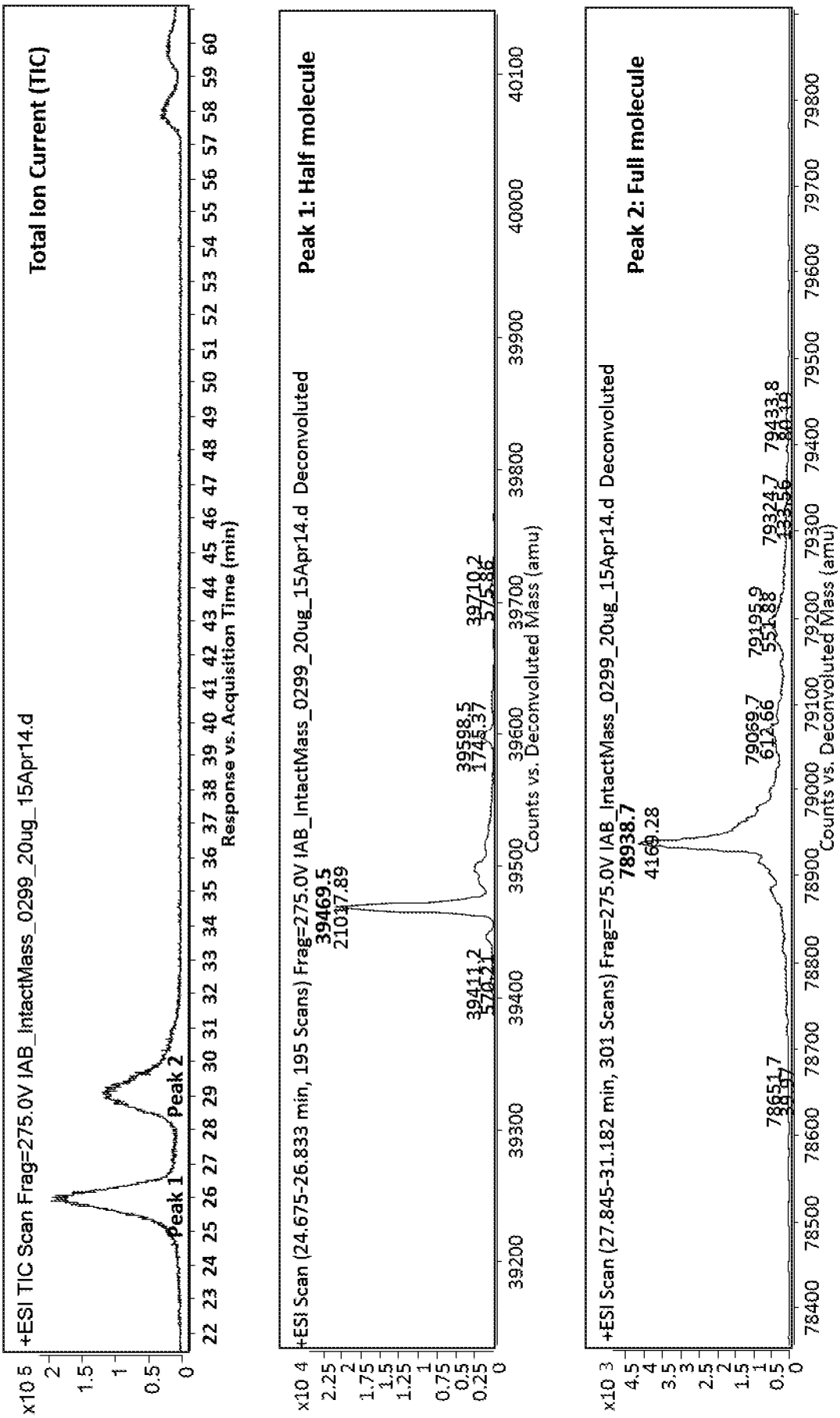

Intact mass analysis of IAB2M γ2 EH1
Total Ion Current (TIC)
+ESI TIC Scan Frag=275.0V IAB_IntactMass_0299_20ug_15Apr14.d
Peak 1
Peak 2
Response vs. Acquisition Time (min)
Peak 1: Half molecule
+ESI Scan (24.675-26.833 min, 195 Scans) Frag=275.0V IAB_IntactMass_0299_20ug_15Apr14.d Deconvoluted
39469.5
Counts vs. Deconvoluted Mass (amu)
Peak 2: Full molecule
+ESI Scan (27.845-31.182 min, 301 Scans) Frag=275.0V IAB_IntactMass_0299_20ug_15Apr14.d Deconvoluted
78938.7
Counts vs. Deconvoluted Mass (amu)

FIG. 6B

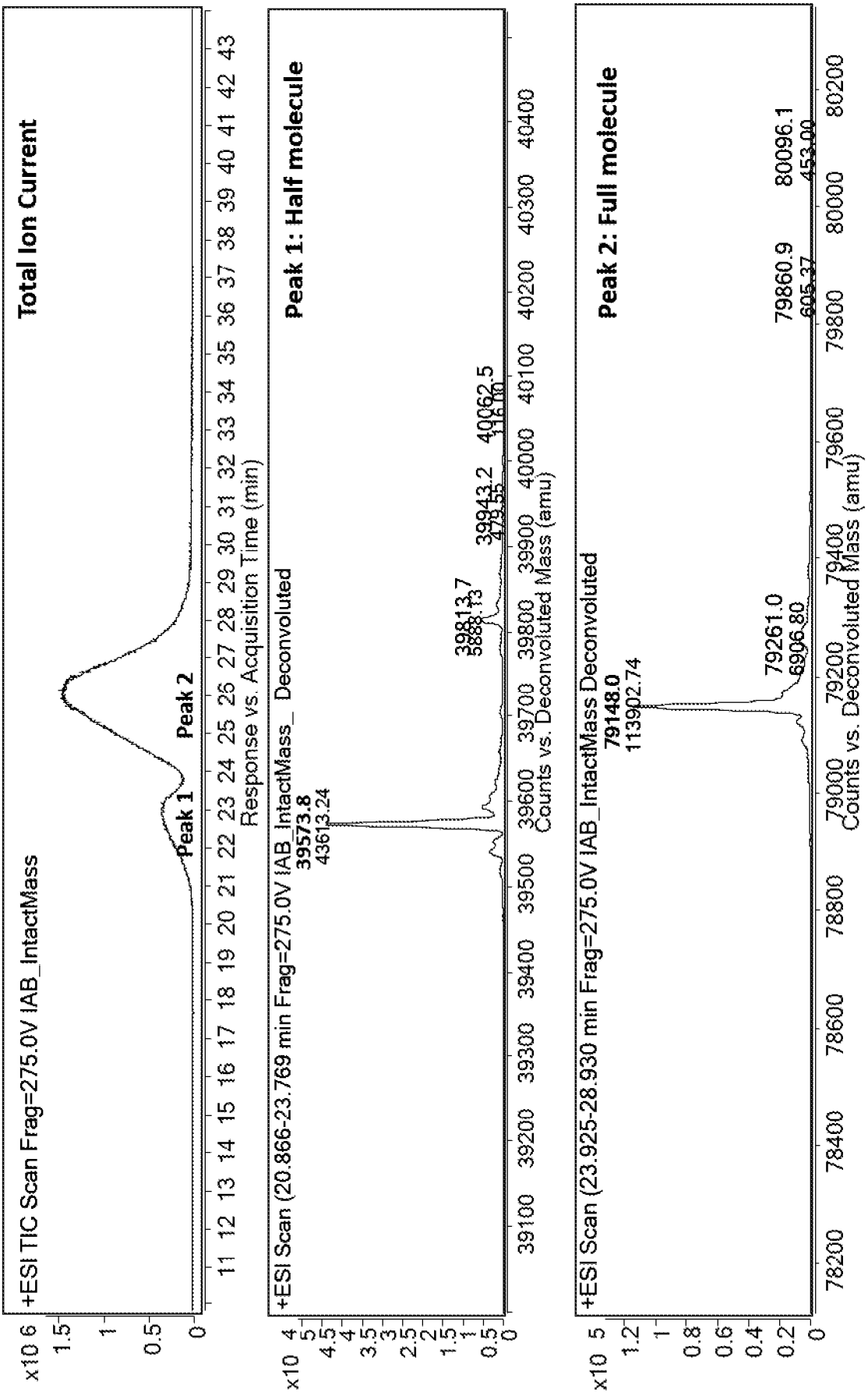

Intact mass analysis of IAB2M γ1 EH2
+ESI TIC Scan Frag=275.0V IAB_IntactMass
Total Ion Current
Peak 1
Peak 2
Response vs. Acquisition Time (min)
+ESI Scan (20.866-23.769 min Frag=275.0V IAB_IntactMass_ Deconvoluted
Peak 1: Half molecule
39573.8
43613.24
39813.7
39943.2
40062.5
Counts vs. Deconvoluted Mass (amu)
+ESI Scan (23.925-28.930 min Frag=275.0V IAB_IntactMass Deconvoluted
Peak 2: Full molecule
79148.0
113902.74
79261.0
79860.9
80096.1
Counts vs. Deconvoluted Mass (amu)

FIG. 6C

Intact mass analysis of IAB2M γ2 EH2

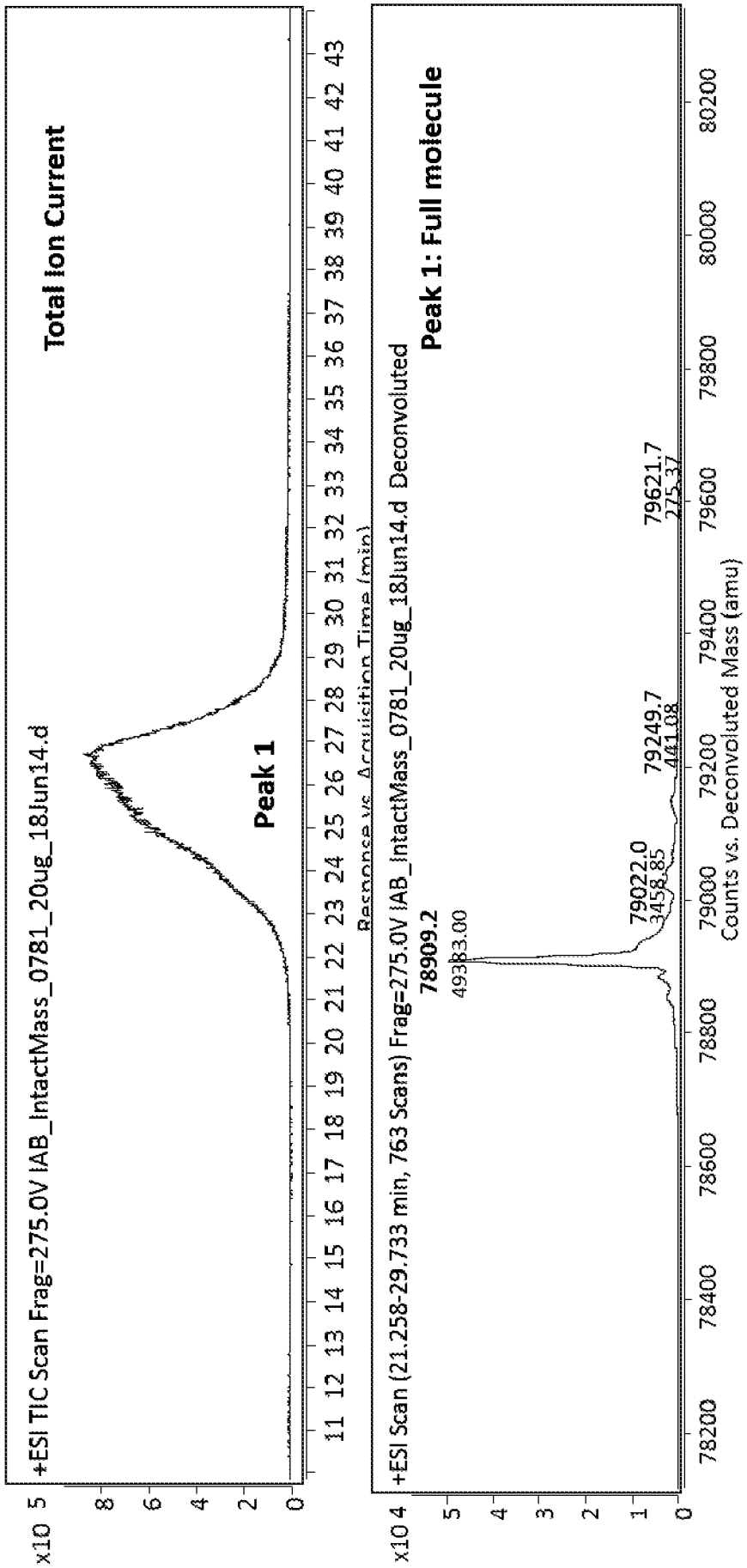
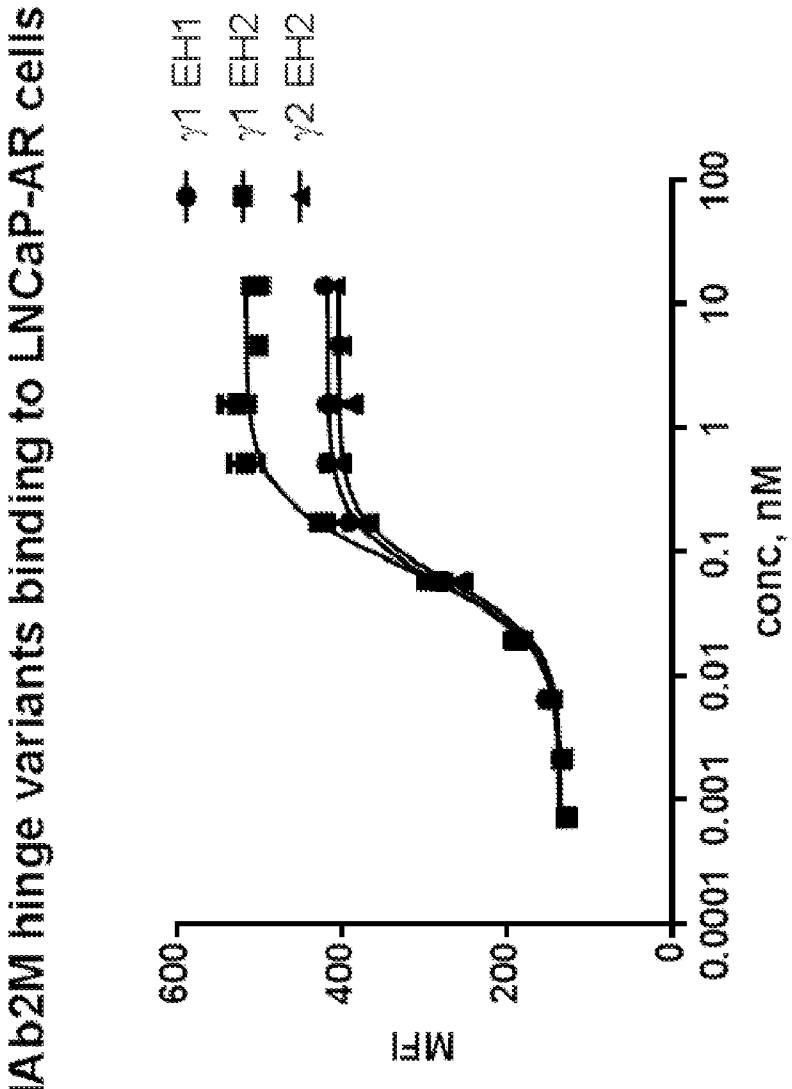
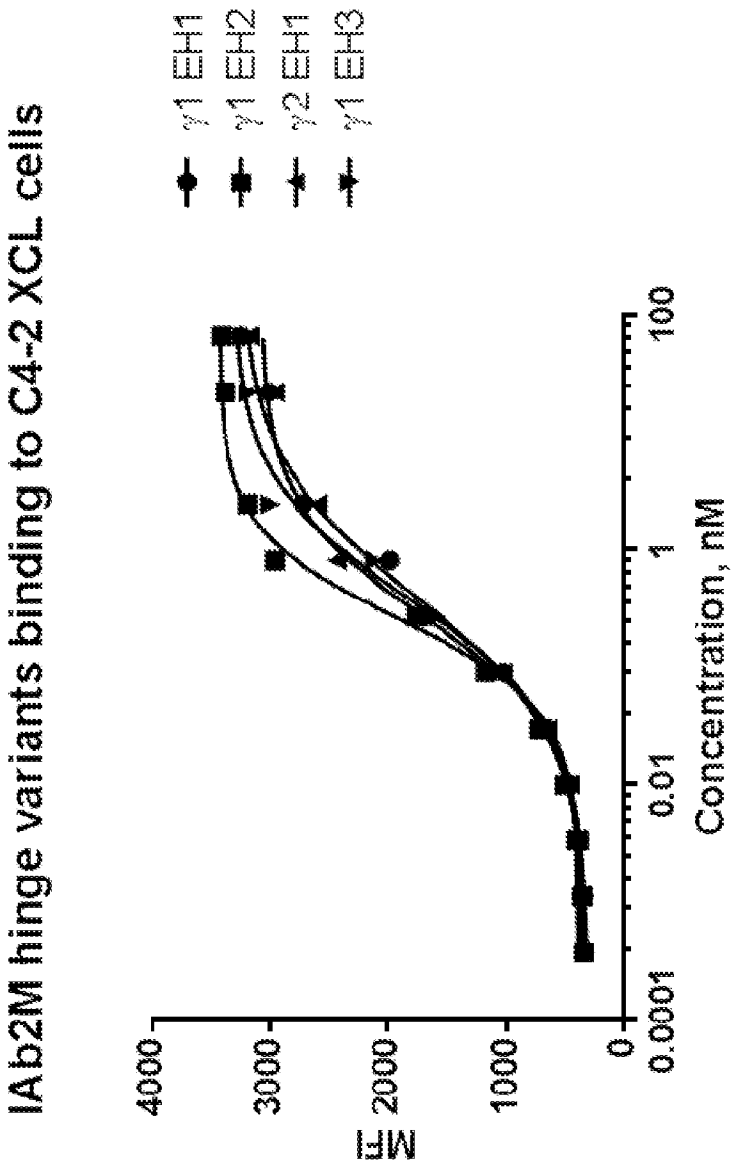


FIG. 6D



variant	γ1 EH1	γ1 EH2	γ2 EH2
EC50, nM	0.05	0.06	0.05

FIG. 7A



variant	γ1 EH1	γ1 EH2	γ2 EH1	γ1 EH3
EC50, nM	0.4	0.26	0.27	0.38

FIG. 7B

IAB2M γ1 EH3 (N- to C-terminal)

Anti-PSMA variable light (VL) sequence
DIVMTQSPSSLSASVGDRVTITCKASQDVGTAVDWYQQKPGKAPKLLIYWASTRHTGVPDRFTGSGSGTDFT
LTISSLQPEDFADYFCQQYNSYPLTFGGGTKLEIK (SEQ ID NO: 13)

Linker
GSTSGGGSGGGSGGGGSS (SEQ ID NO: 62)

Anti-PSMA variable heavy (VH) sequence
EVQLVQSGAEVKKPGASVKISCKTSGYTFTEYTIHWVKQASGKGLEWIGNINPNNGGTTYNQKFEDRATLTVD
KSTSTAYMELSSLRSEDTAVYYCAAGWNFDYWGQGTTVTVSS (SEQ ID NO: 14)

Full hinge (EH3)
EPKSSDKTHTCPPCPPCGGGSSGGGSG (SEQ ID NO: 26)

Upper hinge
EPKSSDKTHT (SEQ ID NO: 46)

Core hinge
CPPCPPC (SEQ ID NO: 52)

Lower hinge
GGGSSGGGSG (SEQ ID NO: 59)

Human IgG1 CH3 (G1m1 allotype)
GQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVD
KSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 40)

Human IgG1 CH3 (nG1m1 allotype)
GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTV
DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 41)

FIG. 7C

IAB2M γ1 EH3 (N- to C-terminal)

IAB2M γ1 EH3 (M1) (SEQ ID NO: 7)
DIVMTQSPSSLSASVGDRVTITCKASQDVGTAVDWYQQKPGKAPKLLIYWASTRHTGVPDR
FTGSGSGTDFTLTISSLQPEDFADYFCQQYNSYPLTFGGGTKLEIKGSTSGGGSGGGSGGG
GSSEVQLVQSGAEVKKPGASVKISCKTSGYTFTEYTIHWVKQASGKGLEWIGNINPNNGGTT
YNQKFEDRATLTVDKSTSTAYMELSSLRSEDTAVYYCAAGWNFDYWGQGTTVTVSSEPKSS
DKTHTCPPCPPCGGGSSGGGSGGGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIA
VEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQ
KSLSLSPG (SEQ ID NO: 7)

IAB2M γ1 EH3 (M2) (SEQ ID NO: 10)
EVQLVQSGAEVKKPGASVKISCKTSGYTFTEYTIHWVKQASGKGLEWIGNINPNNGGTTYNQ
KFEDRATLTVDKSTSTAYMELSSLRSEDTAVYYCAAGWNFDYWGQGTTVTVSSGSTSGGGS
GGGSGGGGSSDIVMTQSPSSLSASVGDRVTITCKASQDVGTAVDWYQQKPGKAPKLLIYWA
STRHTGVPDRFTGSGSGTDFTLTISSLQPEDFADYFCQQYNSYPLTFGGGTKLEIKEPKSSD
KTHTCPPCPPCGGGSSGGGSGGGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVE
WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKS
LSLSPG (SEQ ID NO: 10)

FIG. 7D

IAB2M γ1 EH3

IAB2M γ1 EH3 (M1) (SEQ ID NO: 162)

gatattgtgatgacccagagcccgagcagcctgagcgcgagcgtgggcgatcgcgtgacc
attacctgcaaagcgagccaggatgtgggcaccgcggtggattggtatcagcagaaaccg
ggcaaagcgccgaaactgctgattattgggcgagcacccgccataccggcgtgccggat
cgctttaccggcagcggcagcggcaccgatttaccctgaccattagcagcctgcagccg
gaagatttgcggattattttgccagcagtataacagctatccgctgaccttggcggc
ggcaccaaactggaaattaaaggcagcaccagcggcgggcgggcagcggcagcggc
ggcggcggcagcagcgaagtgcagctggtgcagagcggcgcggaagtgaaaaaaccgggc
gcgagcgtgaaaattagctgcaaaaccagcggctatacctttaccgaatataccattcat
tgggtgaaacaggcgagcggcaaaggcctggaatggattggcaacattaacccgaacaac
ggcggcaccacctataaccagaaatttgaagatcgcgcgaccctgaccgtggataaaagc
accagcaccgcgtatatggaactgagcagcctgcgcagcgaagataccgcggtgtattat
tgcgcggcgggctggaacttttgattattgggggccagggcaccaccgtgaccgtgagcagc
gaaccgaaaaagcagcgataaaaacccatacctgcccgccgtgcccgccgtgcggcgggc
agcagcggcggcggcagcggccagccgcgcgaaccgcaggtgtataccctgccgccgcg
agccgcgaagaaatgaccaaaaaccaggtgagcctgacctgcctggtgaaaggctttat
ccgagcgatattgcggtggaatgggaaagcaacggccagccggaaaacaactataaaacc
accccgccggtgctggatagcgatggcagctttttctgtatagcaaactgaccgtggat
aaaagccgctggcagcagggcaacgtgtttagctgcagcgtgatgcatgaagcgctgcat
aaccattatacccagaaaaagcctgagcccgggc (SEQ ID NO: 162)

FIG. 7E

IAB2M γ1 EH3

IAB2M γ1 EH3 (M2) (SEQ ID NO: 165)

gaagtgcagctggtgcagagcggcgcggaagtgaaaaaaaccgggcgcgagcgtgaaaatt
agctgcaaaaccagcggctatacctttaccgaatataccattcattgggtgaaacaggcg
agcggcaaaggcctggaatggattggcaacattaacccgaacaacggcggcaccacctat
aaccagaaatttgaagatcgcgcgaccctgaccgtggataaaagcaccagcaccgcgtat
atggaactgagcagcctgcgcagcgaagataccgcggtgtattattgcgcgggctgg
aactttgattattgggggccagggcaccaccgtgaccgtgagcagcggcagcaccagcggc
ggcggcagcggcggcggcagcggcggcggcagcagcgatattgtgatgacccagagc
ccgagcagcctgagcgcgagcgtgggcgatcgcgtgaccattacctgcaaagcgagccag
gatgtgggcaccgcggtggattggtatcagcagaaaccgggcaaagcgccgaaactgctg
atttattgggcgagcacccgccataccggcgtgccggatcgcttaccggcagcggcagc
ggcaccgatttaccctgaccattagcagcctgcagccggaagatttgcggattatttt
tgccagcagtataacagctatccgctgacctttggcgggcggcaccaaactggaaattaaa
gaaccgaaaagcagcgataaaacccataccctgcccgccgtgcccgccgtgcggcggcggc
agcagcggcggcggcggccagccgcgcgaaccgcaggtgtataccctgccgccgccg
agccgcgaagaaatgaccaaaaaccaggtgagcctgacctgcctggtgaaaggctttat
ccgagcgatattgcggtggaatgggaaagcaacggccagccggaaaaacaactataaaacc
acccgccggtgctggatagcgatggcagctttttctgtatagcaaactgaccgtggat
aaaagccgctggcagcagggcaacgtgtttagctgcagcgtgatgcatgaagcgctgcat
aaccattatacccagaaaaagcctgagcccgggc (SEQ ID NO: 165)

FIG. 7E continued

Disulfide-containing peptides identified within IAB2M γ1 EH1 dimer

Link/ Modification	Sequence Location[a]	Sequence	Theor. Mass (Da)[b]	Obs. Mass (Da)[c]	Delta ppm	RT (mins)	Abundance[d]
Not observed	A22-A96	N/A	N/A	N/A	N/A	N/A	N/A
Cysteine disulfide bond(A156-A221)*	A(152-157) + A(195-236)	VTITCK [**SEQ ID NO: 183**] + FTGSGSGTDFTLTISSLQPEDFADYFCQQYNSYPL TFGGGTK [**SEQ ID NO: 184**]	5208.3897	5208.3864	-0.63	89.9	1580663
Cysteine disulfide bond(A156-A221)*	A(152-157) + A(188-236)	VTITCK + [**SEQ ID NO: 183**] HTGVPDRFTGSGSGTDFTLTISSLQPEDFADYFCQ QYNSYPLTFGGGTK [**SEQ ID NO: 185**]	5970.7669	5970.7586	-1.40	86.1	166671
Cysteine disulfide bond(A251-B251) Cysteine disulfide bond(A254-B254)	A(248-268) + B(248-268)	THTCPPCGGGSSGGGSGGQPR [**SEQ ID NO: 186**] + THTCPPCGGGSSGGGSGGQPR [**SEQ ID NO: 186**]	3707.5067	3707.5060	-0.18	22.1	583626
Cysteine disulfide bond(A245-A291)	A(244-247) + A(285-294)	SCDK [**SEQ ID NO: 187**] + NQVSLTCLVK [**SEQ ID NO: 188**]	1552.7589	1552.7590	0.07	43.8	84406
Cysteine disulfide bond(A291-A349)*	A(285-294) + A(341-363)	NQVSLTCLVK [**SEQ ID NO: 188**] + WQQGNVFSCSVMHEALHNHYTQK [**SEQ ID NO: 189**]	3844.8236	3844.8202	-0.89	55.2	4949532
Cysteine disulfide bond(A291-A349)*	A(285-294) + A(339-363)	NQVSLTCLVK [**SEQ ID NO: 188**] + SRWQQGNVFSCSVMHEALHNHYTQK [**SEQ ID NO: 190**]	4087.9568	4087.9615	1.16	54.0	647751
Alkylation (vinyl pyridine)(A291)	A(285-294)	NQVSLTCLVK [**SEQ ID NO: 188**]	1208.6587	1208.6578	-0.81	47.2	51583
Alkylation (vinyl pyridine)(A349)	A(341-363)	WQQGNVFSCSVMHEALHNHYTQK [**SEQ ID NO: 189**]	2848.2962	2848.2955	-0.24	49.1	74838

[a] Sequence location A: heavy chain #1; B: heavy chain #2.
[b] Theoretical molecular masses (mono-isotopic) were calculated using MassHunter BioConfirm software (Agilent, Santa Clara, CA).
[c] Observed masses (mono-isotopic) are all within 3 ppm of their theoretical value.
[d] Abundances were determined using MassHunter software (Agilent, Santa Clara, CA).
RT: retention time.
* Peptides displaying alternative cleavages.

FIG. 8

Illustration of a mapping of the disulfide bonds of IAB2M γ1 EH1 (SEQ ID NO: 179)

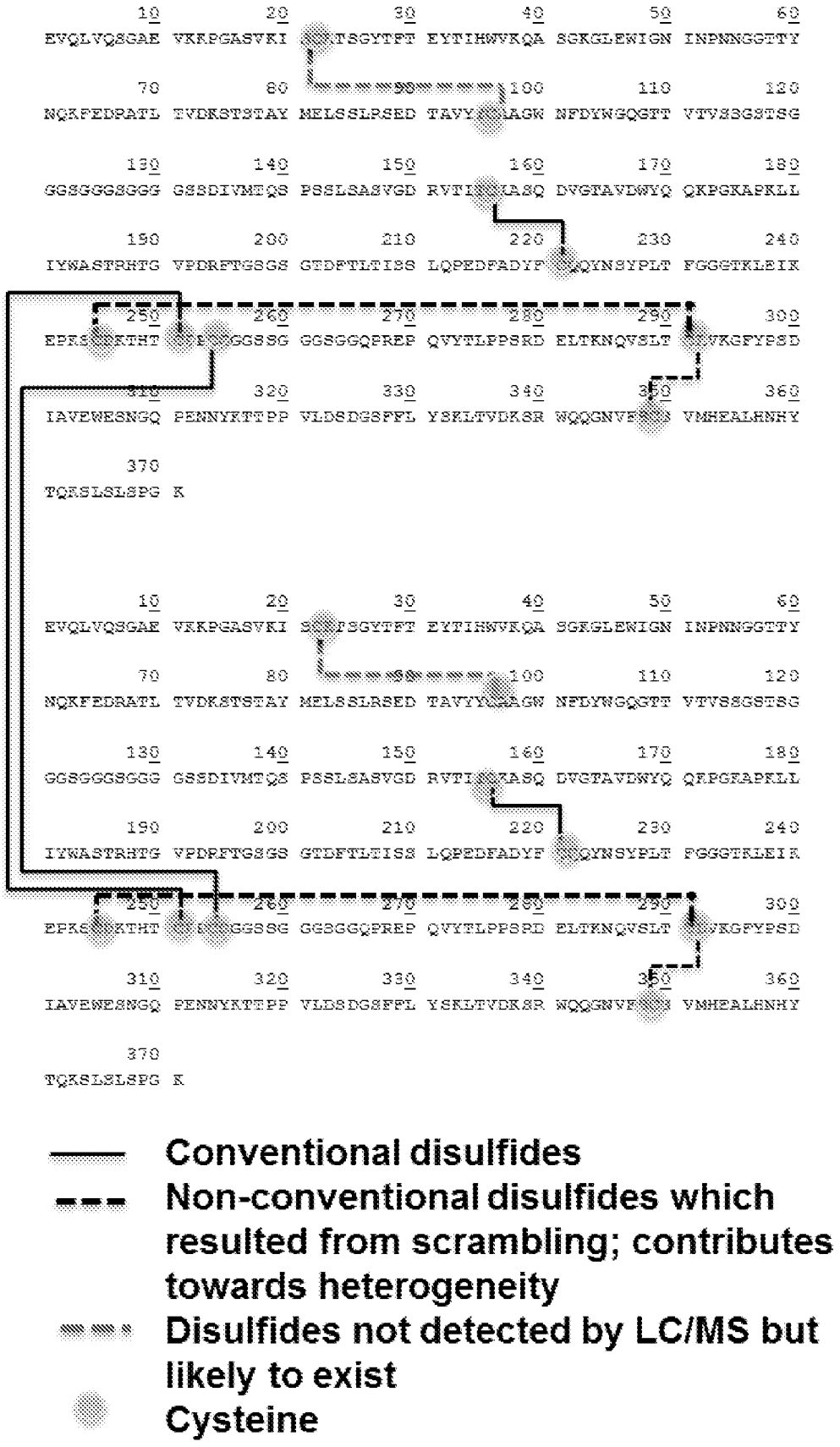


FIG. 9

Illustration of a mapping of the disulfide bonds of IAB2M γ2 EH1
(SEQ ID NO: 180)

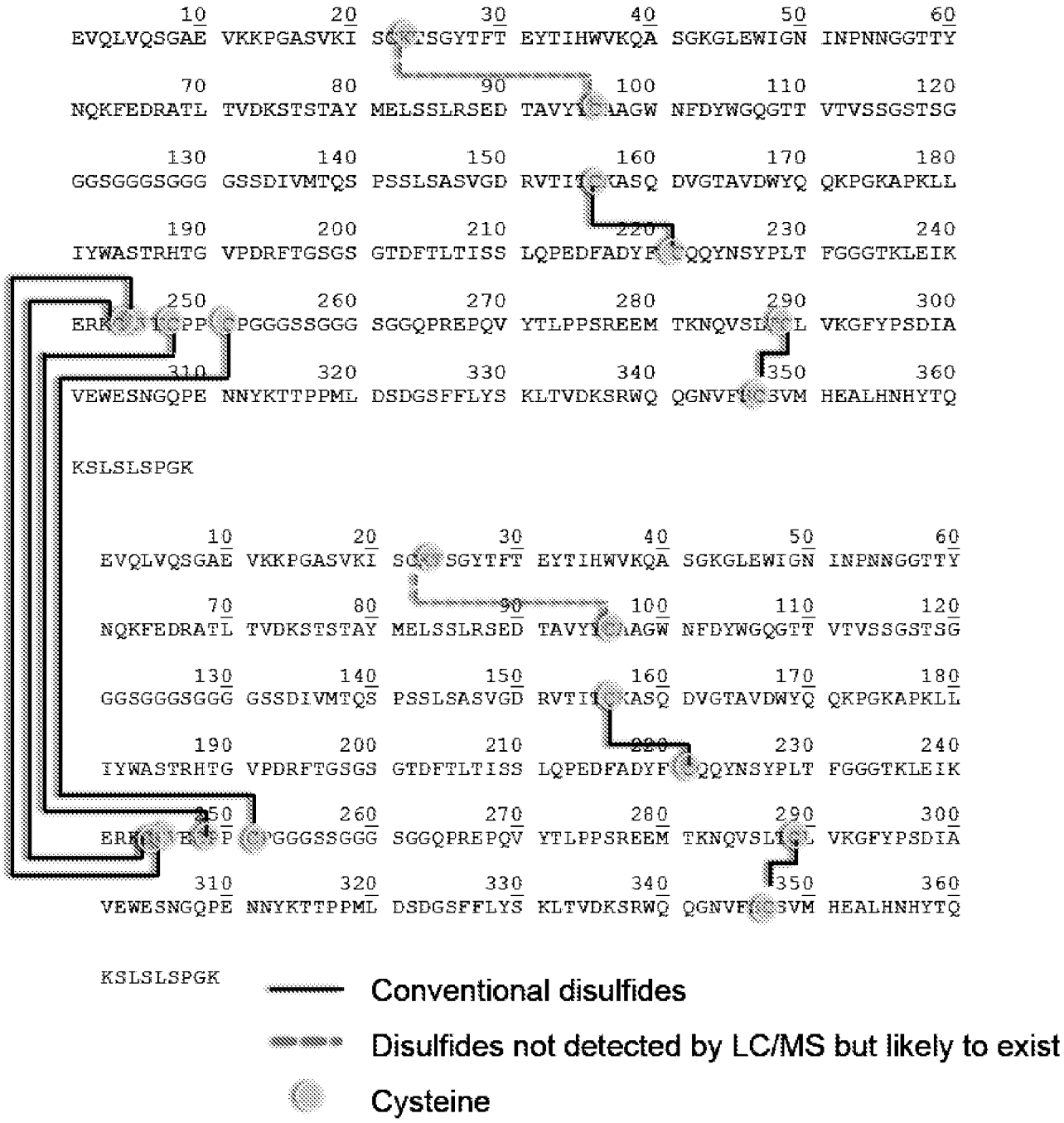


FIG. 10

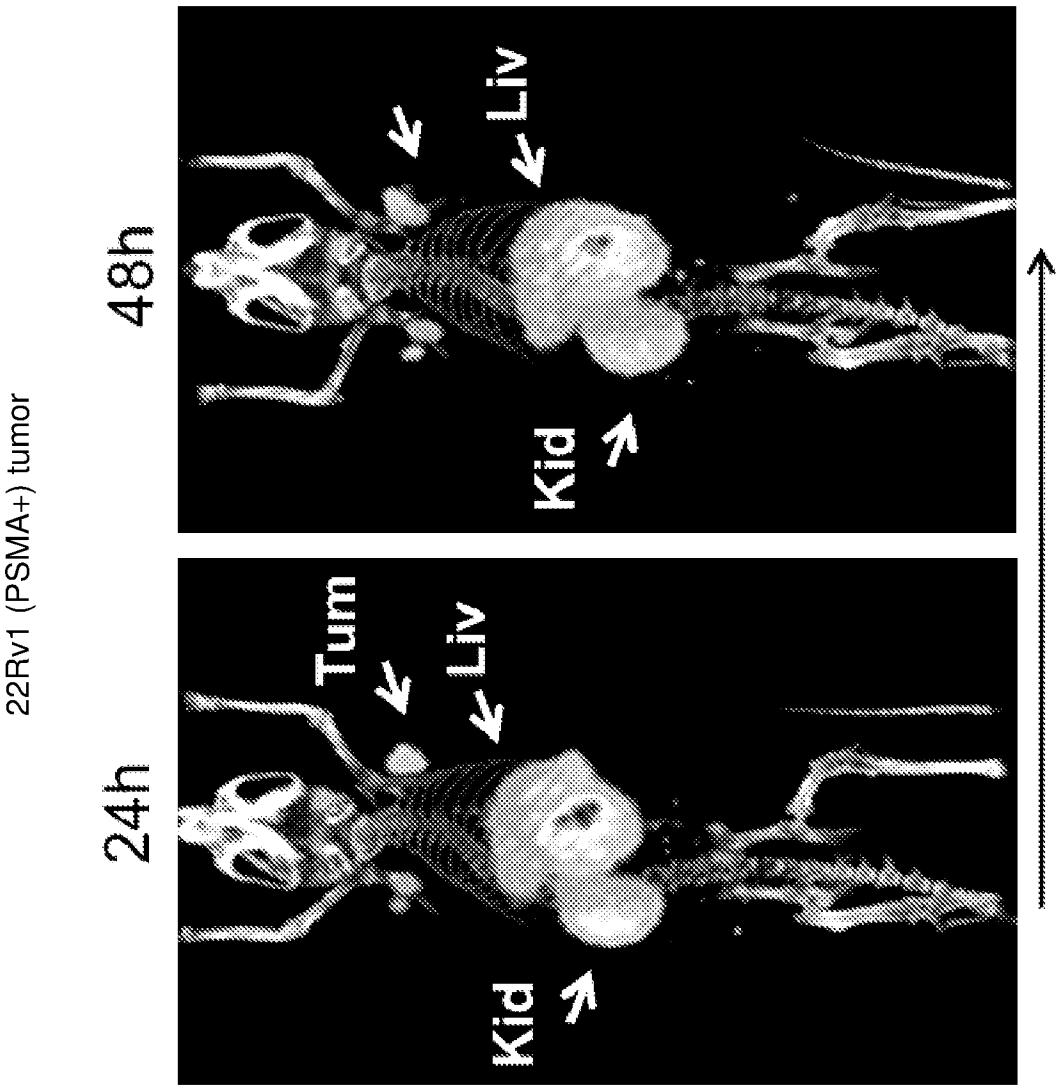


FIG. 11A

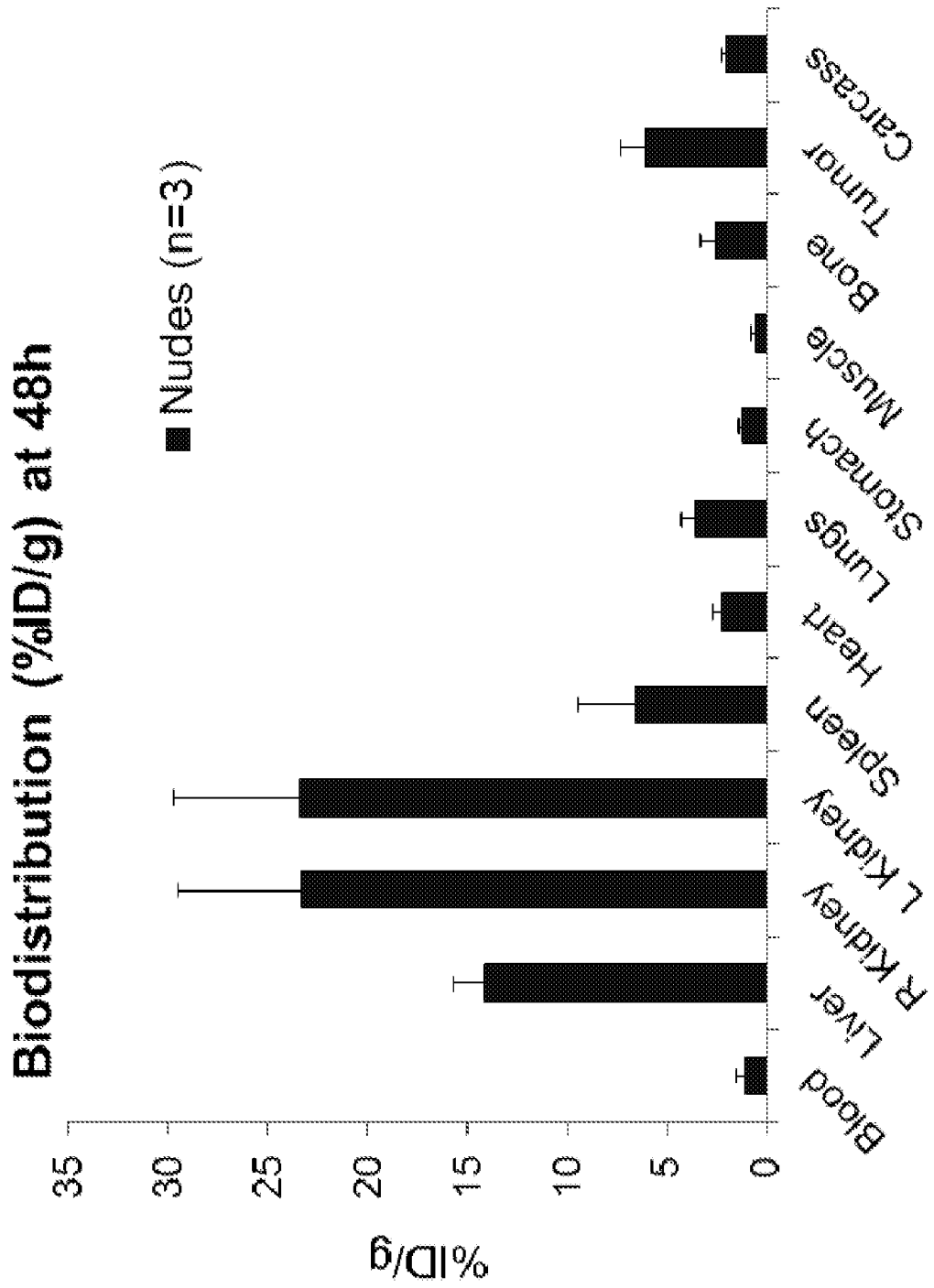

Biodistribution (%ID/g) at 48h
Nudes (n=3)
%ID/g
0
5
10
15
20
25
30
35
Blood
Liver
R Kidney
L Kidney
Spleen
Heart
Lungs
Stomach
Muscle
Bone
Tumor
Carcass

FIG. 11B

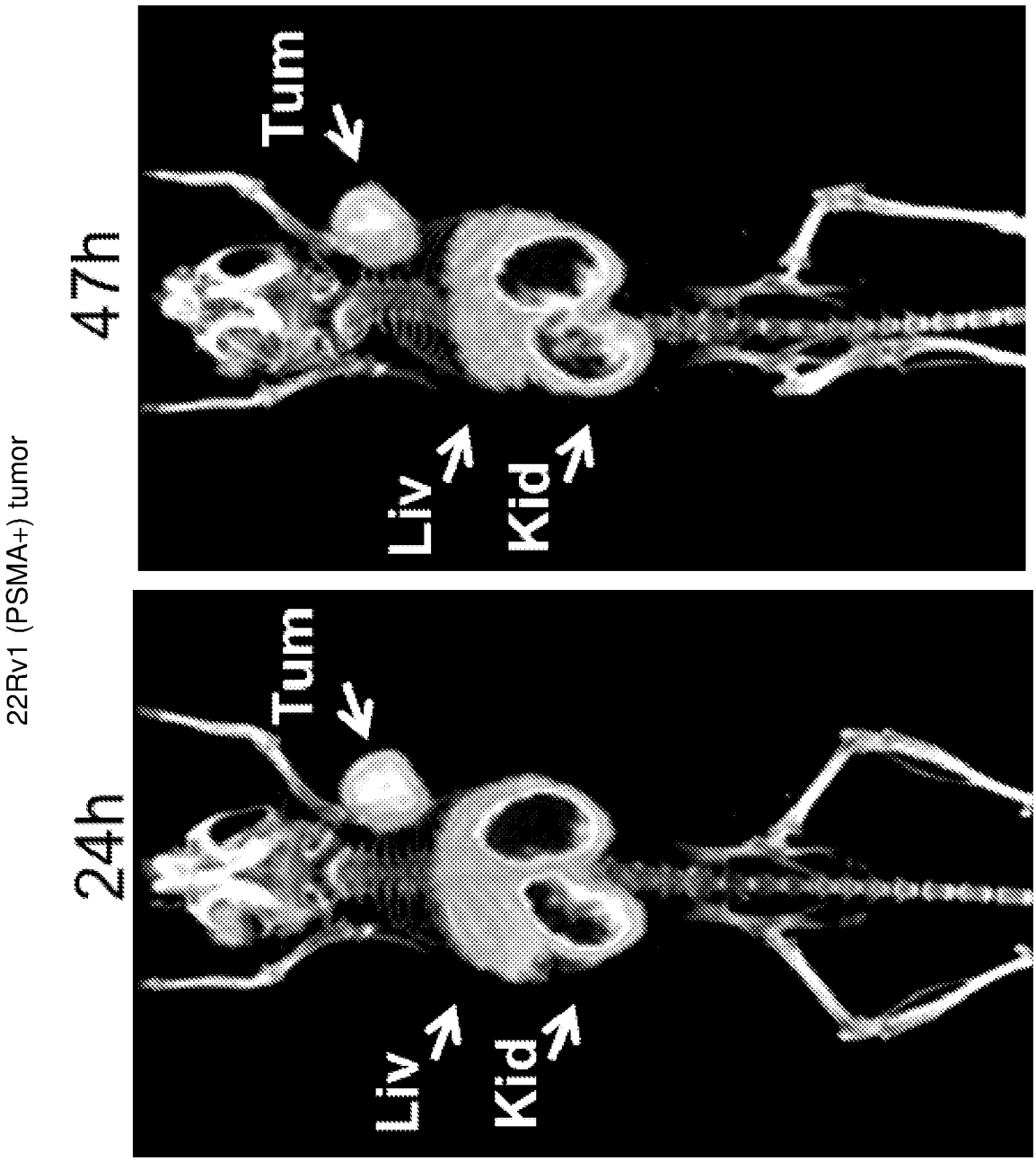


FIG. 12A

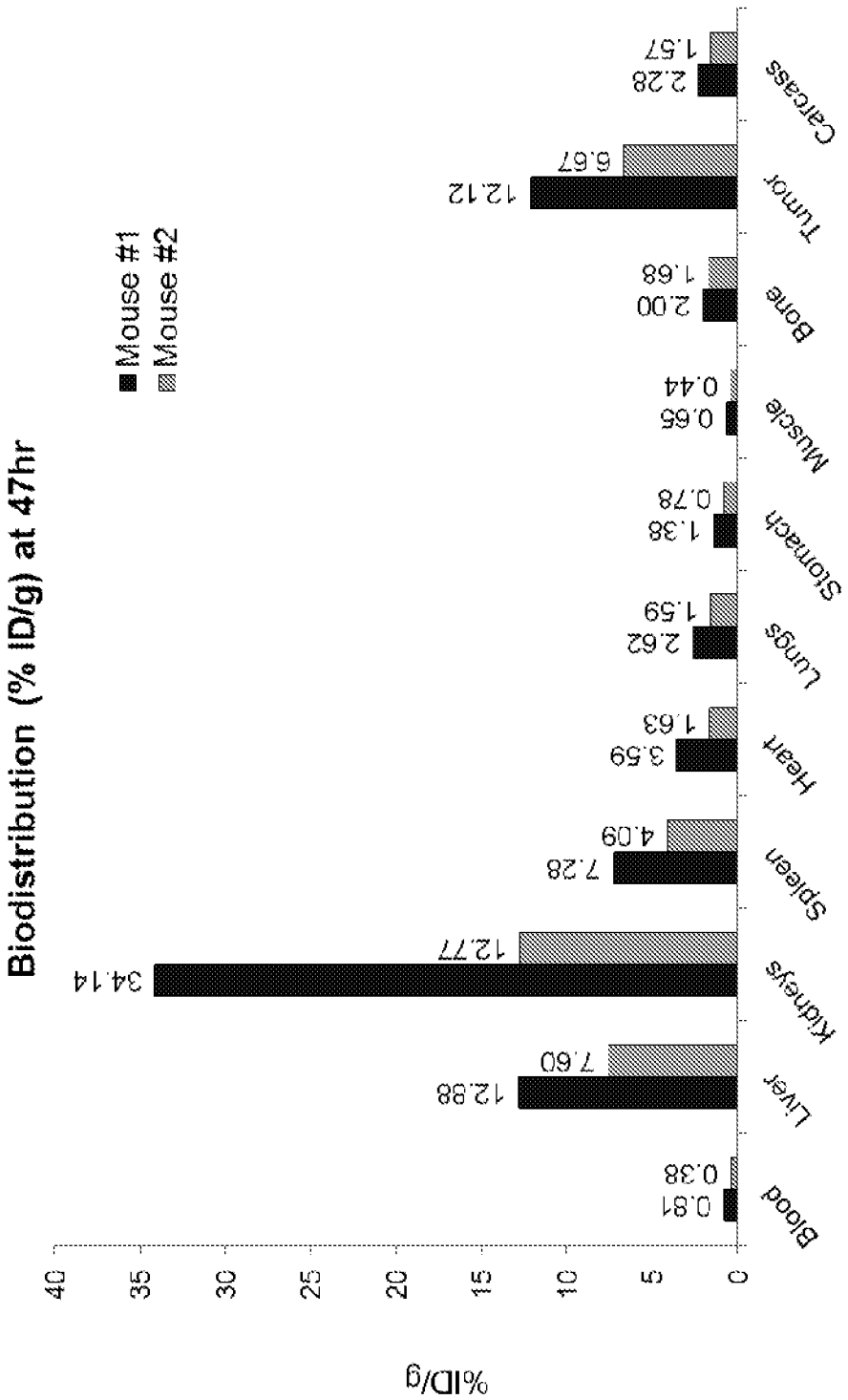

Biodistribution (% ID/g) at 47hr
Mouse #1
Mouse #2
%ID/g
0
5
10
15
20
25
30
35
40
Blood
0.81
0.38
Liver
12.88
7.60
Kidneys
34.14
12.77
Spleen
7.28
4.09
Heart
3.59
1.63
Lungs
2.62
1.59
Stomach
1.38
0.78
Muscle
0.65
0.44
Bone
2.00
1.68
Tumor
12.12
6.67
Carcass
2.28
1.57

FIG. 12B

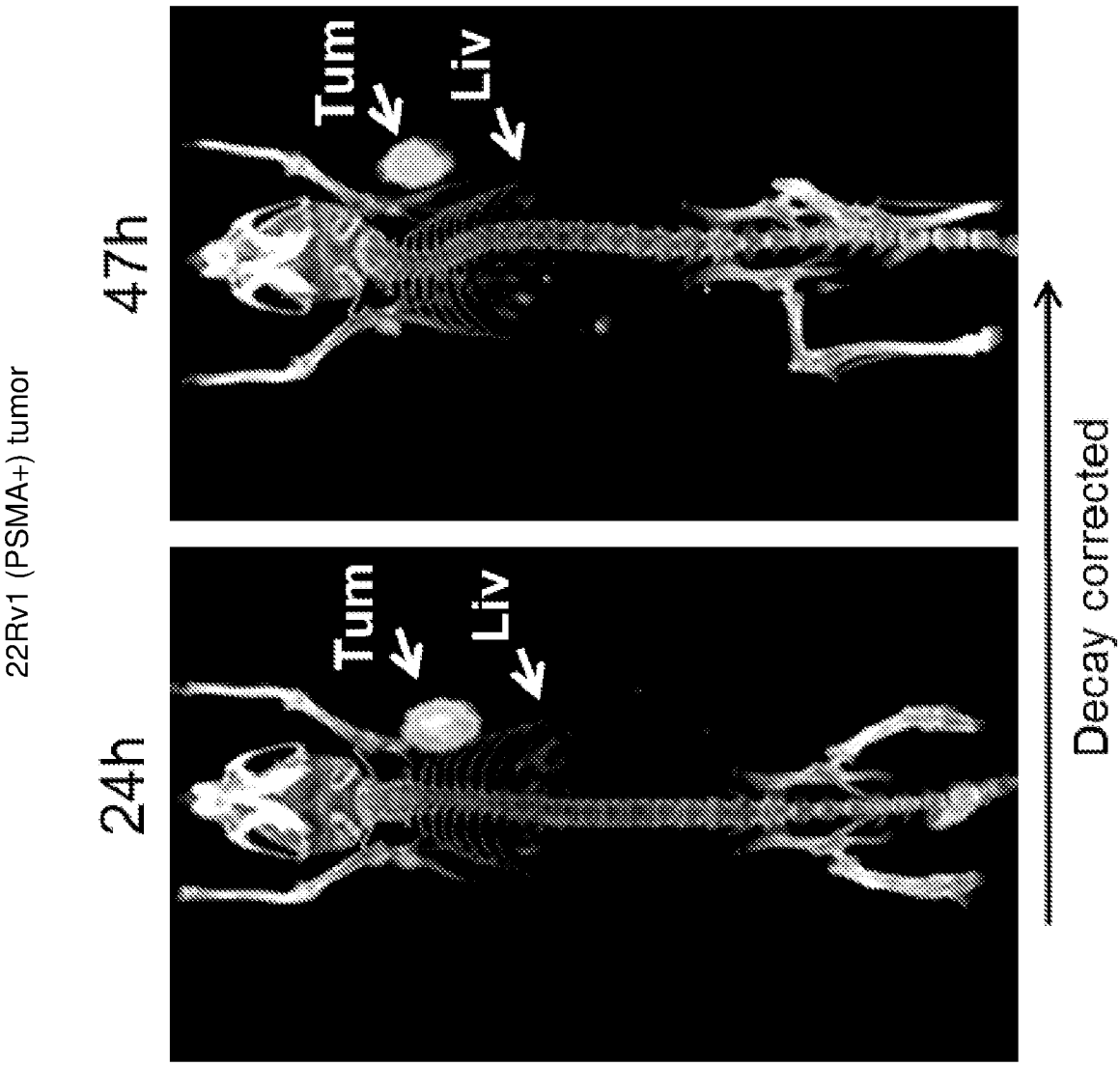


FIG. 13A

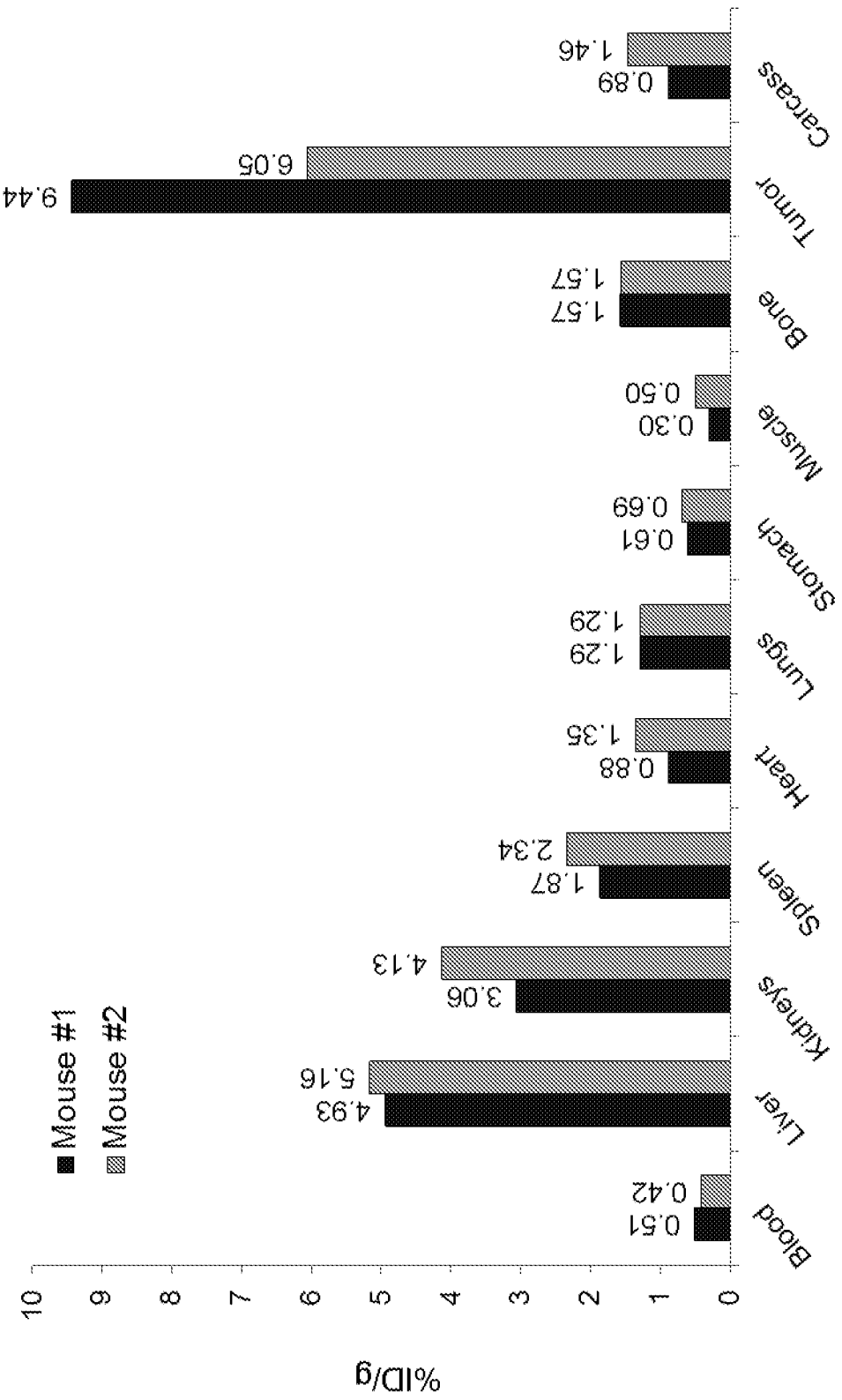

%ID/g
0
1
2
3
4
5
6
7
8
9
10
Mouse #1
Mouse #2
Blood
0.51
0.42
Liver
4.93
5.16
Kidneys
3.06
4.13
Spleen
1.87
2.34
Heart
0.88
1.35
Lungs
1.29
1.29
Stomach
0.61
0.69
Muscle
0.30
0.50
Bone
1.57
1.57
Tumor
9.44
6.05
Carcass
0.89
1.46

FIG. 13B

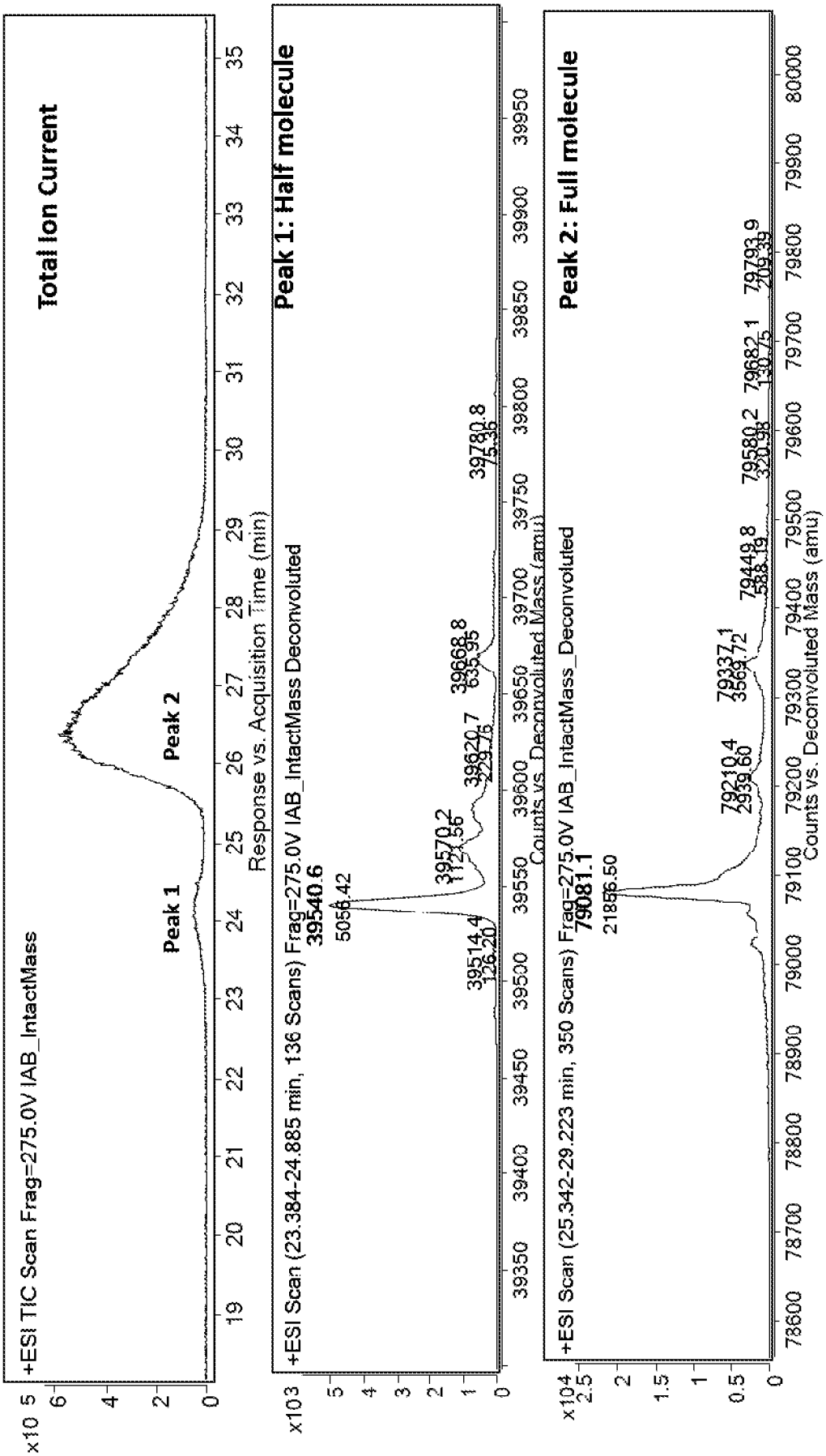

Intact mass analysis of IAB22M γ2 EH1
Totai Ion Current
+ESI TIC Scan Frag=275.0V IAB_IntactMass
Peak 1
Peak 2
Response vs. Acquisition Time (min)
Peak 1: Half molecule
+ESI Scan (23.384-24.885 min, 136 Scans) Frag=275.0V IAB_IntactMass Deconvoluted
39540.6
Counts vs. Deconvoluted Mass (amu)
Peak 2: Full molecule
+ESI Scan (25.342-29.223 min, 350 Scans) Frag=275.0V IAB_IntactMass_Deconvoluted
79081.1
Counts vs. Deconvoluted Mass (amu)

FIG. 14A

IAB22M γ2 EH1 (N- to C-terminal)

Anti-CD8 variable light (VL) sequence
DVQITQSPSSLSASVGDRVTITCRTSRSISQYLAWYQQKPGKVPKLLIYSGSTLQSGVPSRFSGSGSGTDFTLTIS
SLQPEDVATYYCQQHNENPLTFGGGTKVEIK (SEQ ID NO: 15)

Linker
GSTSGGGSGGGSGGGGSS (SEQ ID NO: 62)

Anti-CD8 variable heavy (VH) sequence
EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHFVRQAPGKGLEWIGRIDPANDNTLYASKFQGKATISADTSK
NTAYLQMNSLRAEDTAVYYCGRGYGYYVFDHWGQGTLVTVSS (SEQ ID NO: 16)

Full hinge (EH1)
ERKCCVECPPCPGGGSSGGGSG (SEQ ID NO: 32)

Upper hinge
ERK (SEQ ID NO: 47)

Core hinge
CCVECPPCP (SEQ ID NO: 55)

Lower hinge
GGGSSGGGSG (SEQ ID NO: 59)

Human IgG2 CH3
GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDK
SRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 42)

FIG. 14B

SUBSTITUTE SHEET (RULE 26)

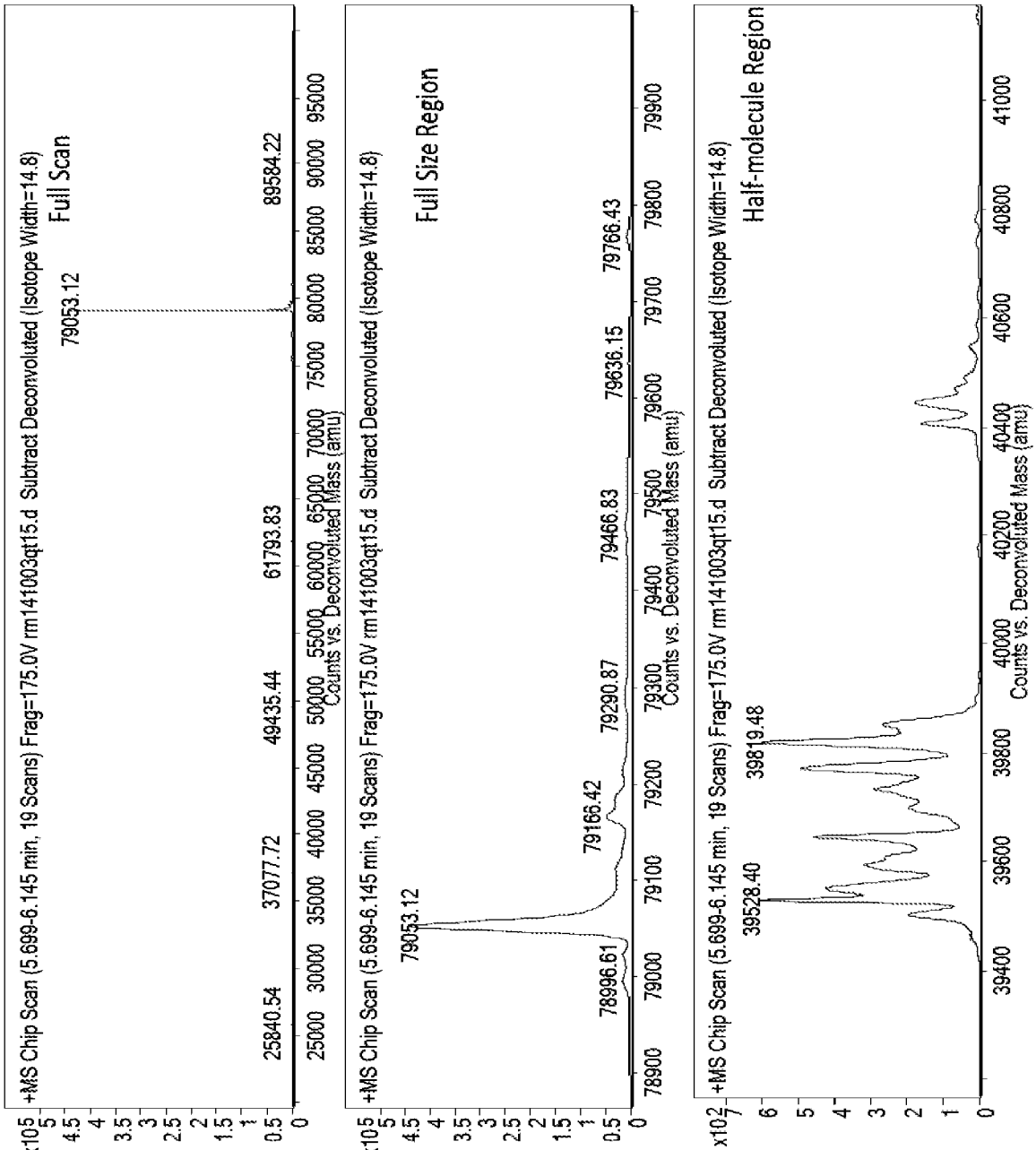

Intact mass analysis of IAB22 γ2 EH2
+MS Chip Scan (5.699-6.145 min, 19 Scans) Frag=175.0V rm141003qt15.d Subtract Deconvoluted (Isotope Width=14.8)
Full Scan
79053.12
25840.54
37077.72
49435.44
61793.83
89584.22
Counts vs. Deconvoluted Mass (amu)
+MS Chip Scan (5.699-6.145 min, 19 Scans) Frag=175.0V rm141003qt15.d Subtract Deconvoluted (Isotope Width=14.8)
Full Size Region
79053.12
78996.61
79166.42
79290.87
79466.83
79636.15
79766.43
Counts vs. Deconvoluted Mass (amu)
+MS Chip Scan (5.699-6.145 min, 19 Scans) Frag=175.0V rm141003qt15.d Subtract Deconvoluted (Isotope Width=14.8)
Half-molecule Region
39528.40
39819.48
Counts vs. Deconvoluted Mass (amu)

FIG. 15A

IAB22M γ2 EH2 (N- to C-terminal)

Anti-CD8 variable light (VL) sequence
DVQITQSPSSLSASVGDRVTITCRTSRSISQYLAWYQQKPGKVPKLLIYSGSTLQSGVPSRFSGSGSGTDFT
LTISSLQPEDVATYYCQQHNENPLTFGGGTKVEIK (SEQ ID NO: 15)

Linker
GSTSGGGSGGGSGGGGSS (SEQ ID NO: 62)

Anti-CD8 variable heavy (VH) sequence
EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHFVRQAPGKGLEWIGRIDPANDNTLYASKFQGKATISA
DTSKNTAYLQMNSLRAEDTAVYYCGRGYGYYVFDHWGQGTLVTVSS (SEQ ID NO: 16)

Full hinge (EH2)
ERKSCVECPPCPGGGSSGGGSG (SEQ ID NO: 34)

Upper hinge
ERK (SEQ ID NO: 47)

Core hinge
SCVECPPCP (SEQ ID NO: 56)

Lower hinge
GGGSSGGGSG (SEQ ID NO: 59)

Human IgG2 CH3
GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLT
VDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 42)

FIG. 15B

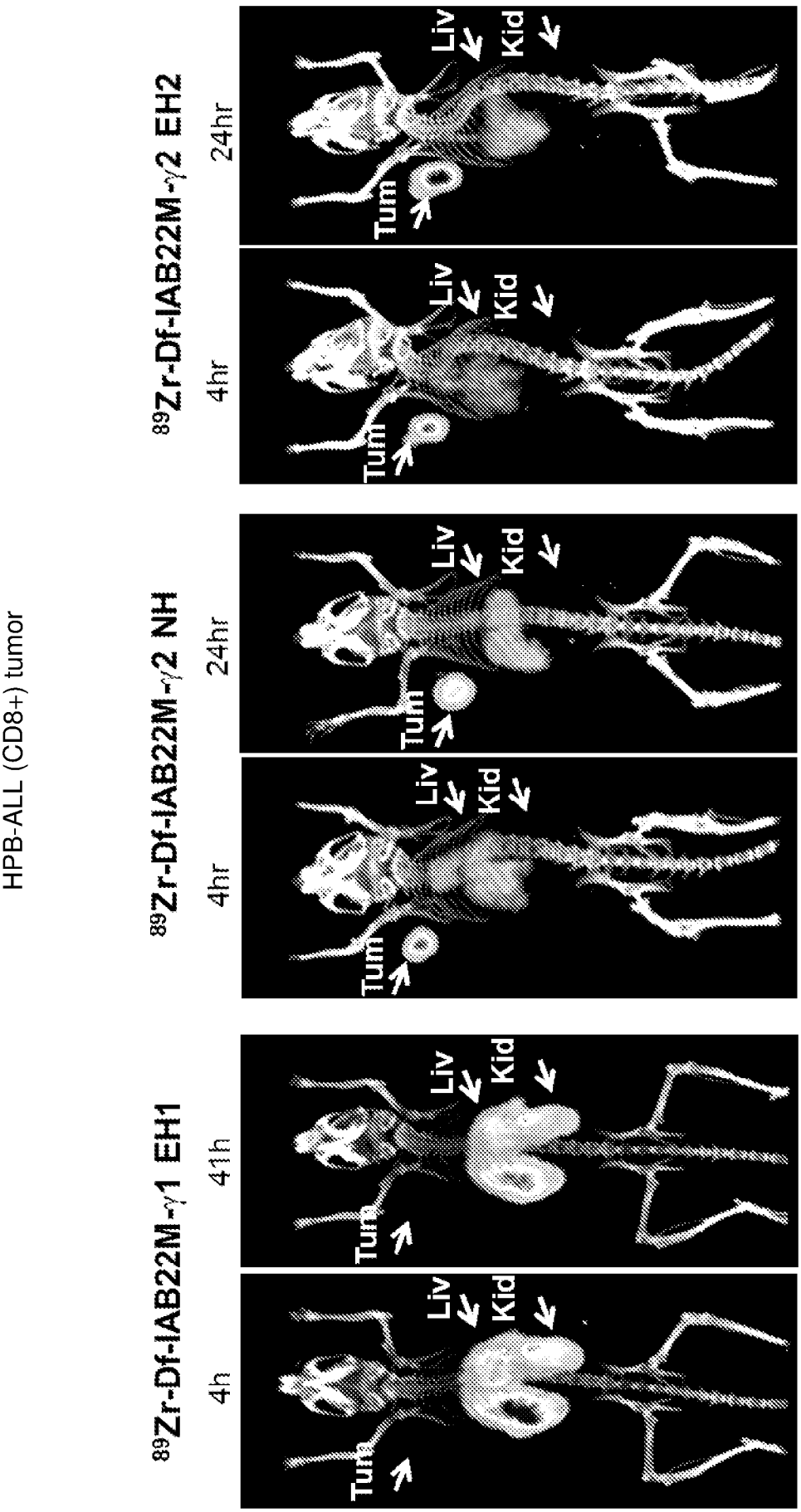

HPB-ALL (CD8+) tumor
^{89}Zr-Df-IAB22M-γ1 EH1
4h
41h
^{89}Zr-Df-IAB22M-γ2 NH
4hr
24hr
^{89}Zr-Df-IAB22M-γ2 EH2
4hr
24hr
Tum
Liv
Kid

FIG. 16A

IAB22M γ1 EH1 (N- to C-terminal)

Anti-CD8 variable light (VL) sequence
DVQITQSPSSLSASVGDRVTITCRTSRSISQYLAWYQQKPGKVPKLLIYSGSTLQSGVPSRFSGSGSGTDFTLTISSLQPE
DVATYYCQQHNENPLTFGGGTKVEIK (SEQ ID NO: 15)

Linker
GSTSGGGSGGGSGGGGSS (SEQ ID NO: 62)

Anti-CD8 variable heavy (VH) sequence
EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHFVRQAPGKGLEWIGRIDPANDNTLYASKFQGKATISADTSKNTAY
LQMNSLRAEDTAVYYCGRGYGYYVFDHWGQGTLVTVSS (SEQ ID NO: 16)

Full hinge (EH1)
EPKSCDKTHTCPPCGGGSSGGGSG (SEQ ID NO: 22)

Upper hinge
EPKSCDKTHT (SEQ ID NO: 45)

Core hinge
CPPC (SEQ ID NO: 50)

Lower hinge
GGGSSGGGSG (SEQ ID NO: 59)

Human IgG1 CH3 (G1m1 allotype)
GQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQ
QGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 40)

Human IgG1 CH3 (nG1m1 allotype)
GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQ
QGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 41)

FIG. 16B

IAB22M γ2 NH1 (N- to C-terminal)

Anti-CD8 variable light (VL) sequence
DVQITQSPSSLSASVGDRVTITCRTSRSISQYLAWYQQKPGKVPKLLIYSGSTLQSGVPSRFSGSGSGTDFTLTISSL
QPEDVATYYCQQHNENPLTFGGGTKVEIK (SEQ ID NO: 15)

Linker
GSTSGGGSGGGSGGGGSS (SEQ ID NO: 62)

Anti-CD8 variable heavy (VH) sequence
EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHFVRQAPGKGLEWIGRIDPANDNTLYASKFQGKATISADTSKN
TAYLQMNSLRAEDTAVYYCGRGYGYGYYVFDHWGQGTLVTVSS (SEQ ID NO: 16)

Full hinge (NH1)
ERKCCVECPPCPAPPVAGP (SEQ ID NO: 31)

Upper hinge
ERK (SEQ ID NO: 47)

Core hinge
CCVECPPCP (SEQ ID NO: 55)

Lower hinge
APPVAGP (SEQ ID NO: 60)

Human IgG2 CH3
GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKS
RWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 42)

FIG. 16C

IAB22M γ2 NH2 (N- to C-terminal)

Anti-CD8 variable light (VL) sequence
DVQITQSPSSLSASVGDRVTITCRTSRSISQYLAWYQQKPGKVPKLLIYSGSTLQSGVPSRFSGSGSGTDFTLTIS
SLQPEDVATYYCQQHNENPLTFGGGTKVEIK (SEQ ID NO: 15)

Linker
GSTSGGGSGGGSGGGGSS (SEQ ID NO: 62)

Anti-CD8 variable heavy (VH) sequence
EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHFVRQAPGKGLEWIGRIDPANDNTLYASKFQGKATISADTSK
NTAYLQMNSLRAEDTAVYYCGRGYGYYVFDHWGQGTLVTVSS (SEQ ID NO: 16)

Full hinge (NH2)
ERKSCVECPPCPAPPVAGP (SEQ ID NO: 33)

Upper hinge
ERK (SEQ ID NO: 47)

Core hinge
SCVECPPCP (SEQ ID NO: 56)

Lower hinge
APPVAGP (SEQ ID NO: 60)

Human IgG2 CH3
GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDK
SRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 42)

FIG. 16D

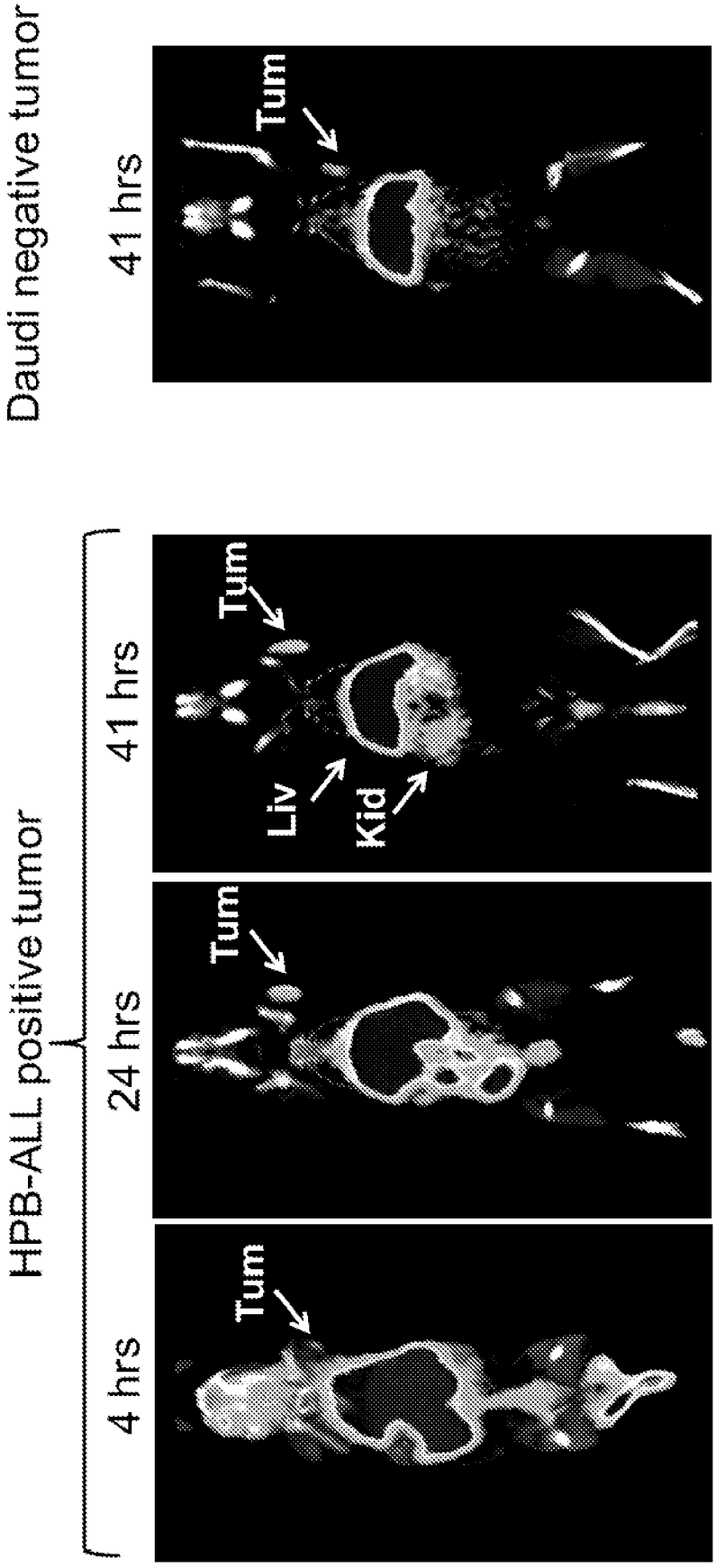
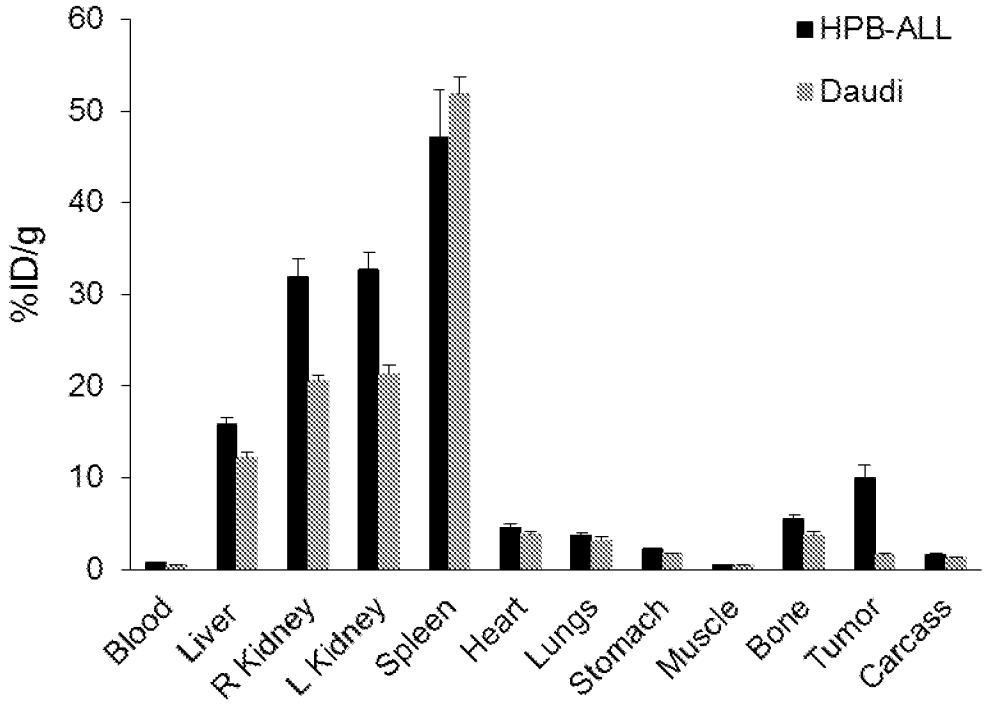

Daudi negative tumor
41 hrs
Tum
HPB-ALL positive tumor
4 hrs
24 hrs
41 hrs
Tum
Tum
Tum
Liv
Kid

FIG. 17



Biodistribution (%ID/g) at 41 hrs (n=4)

	HPB-ALL	STD	Daudi	STD
Blood	0.74	0.09	0.45	0.11
Liver	15.94	1.21	12.18	1.20
R Kidney	31.94	3.81	20.62	1.00
L Kidney	32.75	3.61	21.38	1.86
Spleen	47.19	8.88	51.91	3.15
Heart	4.56	0.76	3.89	0.57
Lungs	3.74	0.58	3.10	0.93
Stomach	2.28	0.19	1.72	0.19
Muscle	0.51	0.07	0.45	0.11
Bone	5.56	0.80	3.70	0.86
Tumor	10.04*	2.38	1.58	0.38
Carcass	1.65	0.19	1.34	0.09

*** n=3**

FIG. 18

Full hinge N- to C-terminal (SEQ ID NO: 22)
E P K S C D K T H T C P P C G G G S S G G G G S G
Upper hinge SEQ ID NO: 45
Core hinge SEQ ID NO: 50
Lower hinge SEQ ID NO: 59
Full hinge N- to C-terminal (SEQ ID NO: 26)
E P K S S D K T H T C P P C P C G G G S S G G G G S G
Upper hinge SEQ ID NO: 46
Core hinge SEQ ID NO: 52
Lower hinge SEQ ID NO: 59
Full hinge N- to C-terminal (SEQ ID NO: 28)
E P K S S D K T H T C P P C V E C P P C G G G S S G G G G S G
Upper hinge SEQ ID NO: 46
Core hinge SEQ ID NO: 53
Lower hinge SEQ ID NO: 59

FIG. 19

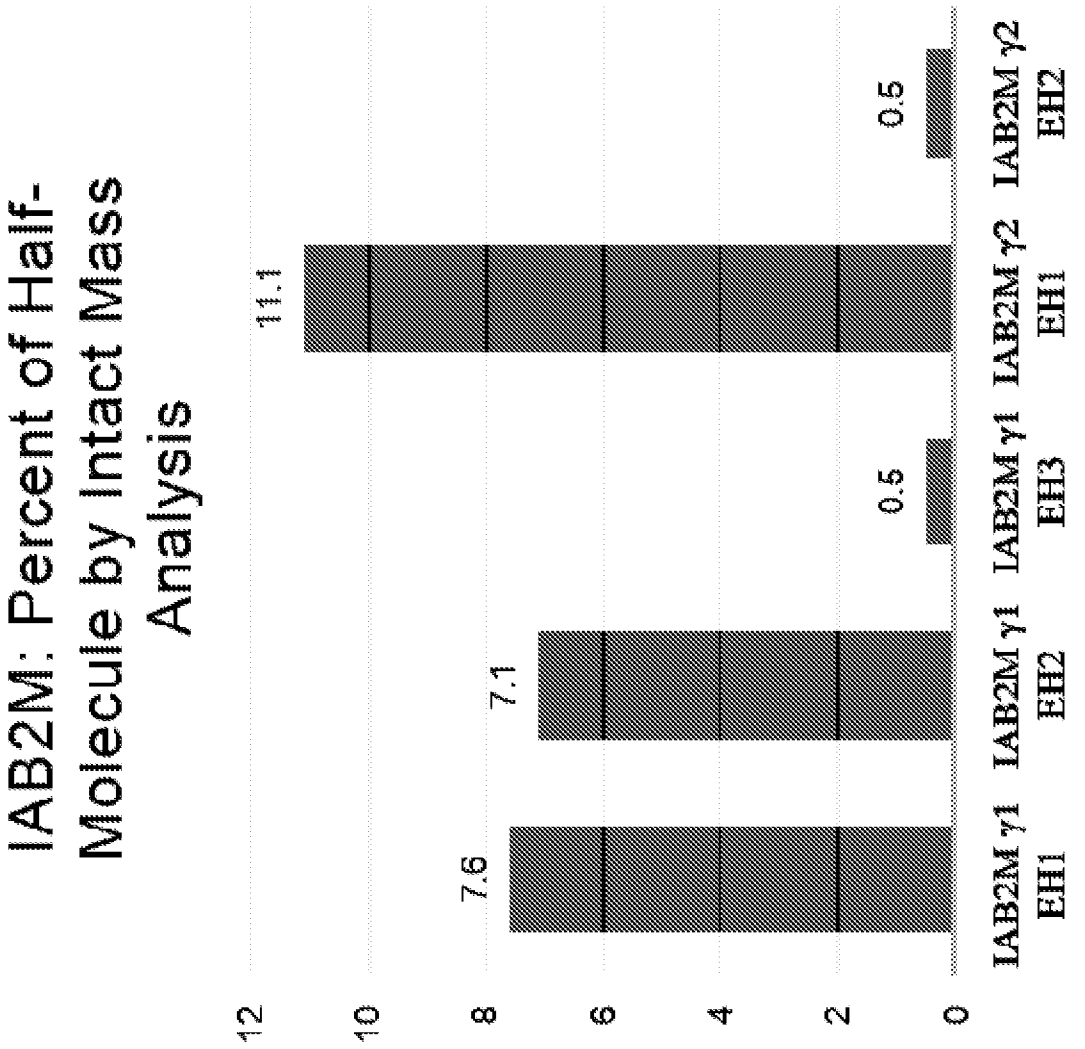

IAB2M: Percent of Half-Molecule by Intact Mass Analysis
12
10
8
6
4
2
0
7.6
7.1
0.5
11.1
0.5
IAB2M γ1 EH1
IAB2M γ1 EH2
IAB2M γ1 EH3
IAB2M γ2 EH1
IAB2M γ2 EH2

FIG. 20A

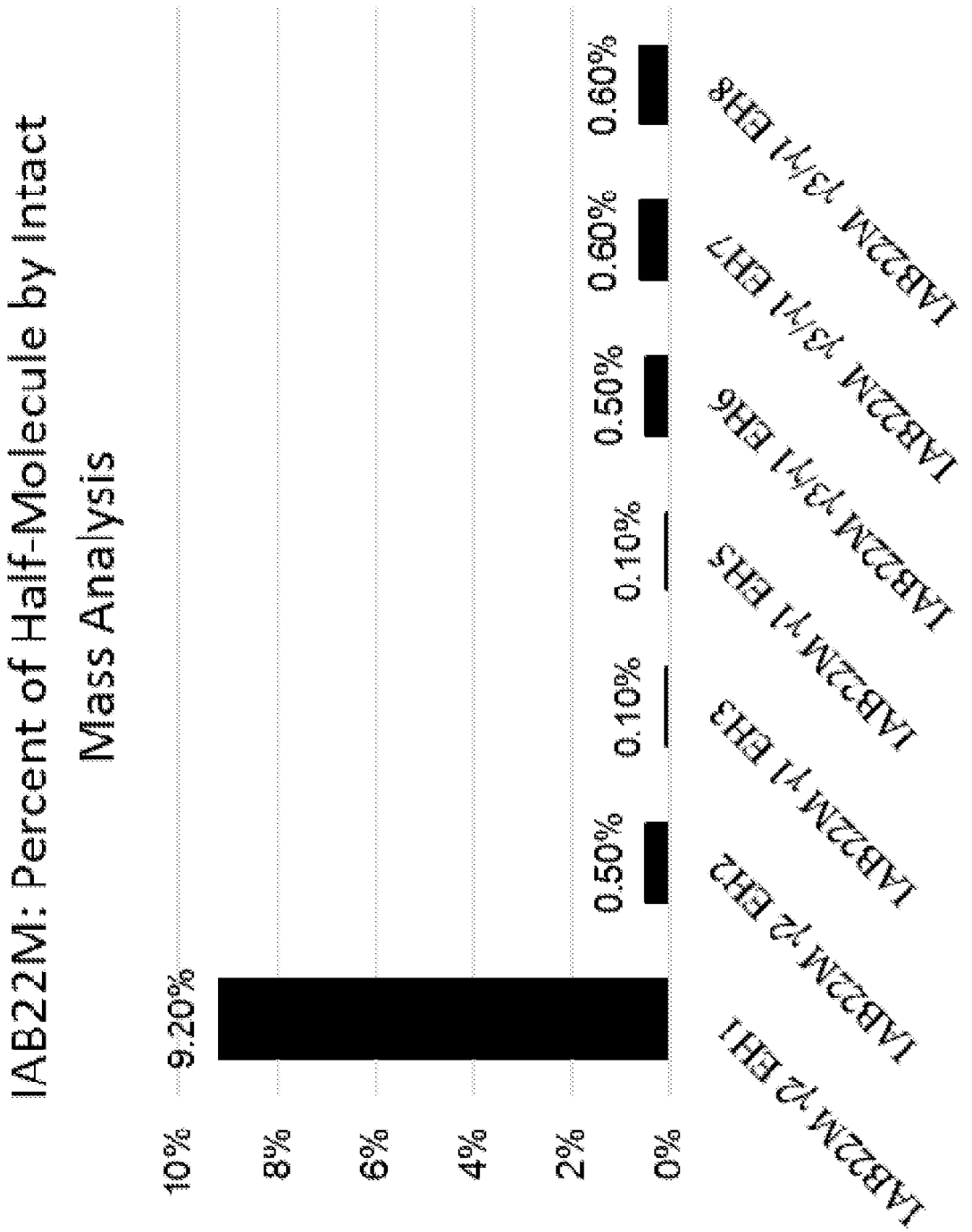

IAB22M: Percent of Half-Molecule by Intact Mass Analysis
10%
8%
6%
4%
2%
0%
9.20%
0.50%
0.10%
0.10%
0.50%
0.60%
0.60%
IAB22M *2 EH1
IAB22M *2 EH2
IAB22M *1 EH3
IAB22M *1 EH5
IAB22M *3/*1 EH6
IAB22M *3/*1 EH7
IAB22M *3/*1 EH8

FIG. 20B

IAB22M γ1 EH3 (N- to C-terminal)

Anti-CD8 variable light (VL) sequence
DVQITQSPSSLSASVGDRVTITCRTSRSISQYLAWYQQKPGKVPKLLIYSGSTLQSGVPSRFSGSGSGTDFTLTISSLQPED
VATYYCQQHNENPLTFGGGTKVEIK (SEQ ID NO: 15)

Linker
GSTSGGGSGGGSGGGGSS (SEQ ID NO: 62)

Anti-CD8 variable heavy (VH) sequence
EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHFVRQAPGKGLEWIGRIDPANDNTLYASKFQGKATISADTSKNTAYLQ
MNSLRAEDTAVYYCGRGYGYYVFDHWGQGTLVTVSS (SEQ ID NO: 16)

Full hinge (EH3)
EPKSSDKTHTCPPCPPCGGGSSGGGSG (SEQ ID NO: 26)

Upper hinge
EPKSSDKTHT (SEQ ID NO: 46)

Core hinge
CPPCPPC (SEQ ID NO: 52)

Lower hinge
GGGSSGGGSG (SEQ ID NO: 59)

Human IgG1 CH3 (G1m1 allotype)
GQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQG
NVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 40)

Human IgG1 CH3 (nG1m1 allotype)
GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQ
GNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 41)

FIG. 20C

IAB22M γ1 EH5 (N- to C-terminal)

Anti-CD8 variable light (VL) sequence
DVQITQSPSSLSASVGDRVTITCRTSRSISQYLAWYQQKPGKVPKLLIYSGSTLQSGVPSRFSGSGSGTDFTLTISSLQPEDV
ATYYCQQHNENPLTFGGGTKVEIK (SEQ ID NO: 15)

Linker
GSTSGGGSGGGSGGGGSGGGSS (SEQ ID NO: 62)

Anti-CD8 variable heavy (VH) sequence
EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHFVRQAPGKGLEWIGRIDPANDNTLYASKFQGKATISADTSKNTAYLQ
MNSLRAEDTAVYYCGRGYGYYVFDHWGQGTLVTVSS (SEQ ID NO: 16)

Full hinge (EH5)
EPKSSDKTHTCPPCPPCPPCGGGSSGGGSG (SEQ ID NO: 30)

Upper hinge
EPKSSDKTHT (SEQ ID NO: 46)

Core hinge
CPPCPPCPPC (SEQ ID NO: 54)

Lower hinge
GGGSSGGGSG (SEQ ID NO: 59)

Human IgG1 CH3 (G1m1 allotype)
GQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQG
NVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 40)

Human IgG1 CH3 (nG1m1 allotype)
GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQG
NVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 41)

FIG. 20D

IAB22M γ3/γ1 EH6 (N- to C-terminal)

Anti-CD8 variable light (VL) sequence
DVQITQSPSSLSASVGDRVTITCRTSRSISQYLAWYQQKPGKVPKLLIYSGSTLQSGVPSRFSGSGSGTDFTLTISSLQPE
DVATYYCQQHNENPLTFGGGTKVEIK (SEQ ID NO: 15)

Linker
GSTSGGGSGGGSGGGGSS (SEQ ID NO: 62)

Anti-CD8 variable heavy (VH) sequence
EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHFVRQAPGKGLEWIGRIDPANDNTLYASKFQGKATISADTSKNTAYL
QMNSLRAEDTAVYYCGRGYGYYVFDHWGQGTLVTVSS (SEQ ID NO: 16)

Full hinge (EH6)
ELKTPLGDTTHTCVECPPCGGGSSGGGSG (SEQ ID NO: 35)

Upper hinge
ELKTPLGDTTHT (SEQ ID NO: 48)

Core hinge
CVECPPC (SEQ ID NO: 57)

Lower hinge
GGGSSGGGSG (SEQ ID NO: 59)

Human IgG1 CH3 (G1m1 allotype)
GQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQ
GNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 40)

Human IgG1 CH3 (nG1m1 allotype)
GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQ
QGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 41)

FIG. 20E

IAB22M γ3/γ1 EH7 (N- to C-terminal)

Anti-CD8 variable light (VL) sequence
DVQITQSPSSLSASVGDRVTITCRTSRSISQYLAWYQQKPGKVPKLLIYSGSTLQSGVPSRFSGSGSGTDFTLTISSLQPE
DVATYYCQQHNENPLTFGGGTKVEIK (SEQ ID NO: 15)

Linker
GSTSGGGSGGGSGGGGSS (SEQ ID NO: 62)

Anti-CD8 variable heavy (VH) sequence
EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHFVRQAPGKGLEWIGRIDPANDNTLYASKFQGKATISADTSKNTAY
LQMNSLRAEDTAVYYCGRGYGYYVFDHWGQGTLVTVSS (SEQ ID NO: 16)

Full hinge (EH7)
ELKTPLGDTTHTCPPCPPCGGGSSGGGSG (SEQ ID NO: 36)

Upper hinge
ELKTPLGDTTHT (SEQ ID NO: 48)

Core hinge
CPPCPPC (SEQ ID NO: 52)

Lower hinge
GGGSSGGGSG (SEQ ID NO: 59)

Human IgG1 CH3 (G1m1 allotype)
GQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQ
QGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 40)

Human IgG1 CH3 (nG1m1 allotype)
GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQ
QGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 41)

FIG. 20F

IAB22M γ3/γ1 EH8 (N- to C-terminal)

Anti-CD8 variable light (VL) sequence
DVQITQSPSSLSASVGDRVTITCRTSRSISQYLAWYQQKPGKVPKLLIYSGSTLQSGVPSRFSGSGSGTDFTLTISSLQPED
VATYYCQQHNENPLTFGGGTKVEIK (SEQ ID NO: 15)

Linker
GSTSGGGSGGGSGGGGSS (SEQ ID NO: 62)

Anti-CD8 variable heavy (VH) sequence
EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHFVRQAPGKGLEWIGRIDPANDNTLYASKFQGKATISADTSKNTAYLQ
MNSLRAEDTAVYYCGRGYGYYVFDHWGQGTLVTVSS (SEQ ID NO: 16)

Full hinge (EH8)
ELKTPLGDTTHTCPPCPPCPPCGGGSSGGGSG (SEQ ID NO: 37)

Upper hinge
ELKTPLGDTTHT (SEQ ID NO: 48)

Core hinge
CPPCPPCPPC (SEQ ID NO: 54)

Lower hinge
GGGSSGGGSG (SEQ ID NO: 59)

Human IgG1 CH3 (G1m1 allotype)
GQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQG
NVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 40)

Human IgG1 CH3 (nG1m1 allotype)
GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQ
GNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 41)

FIG. 20G

SUBSTITUTE SHEET (RULE 26)

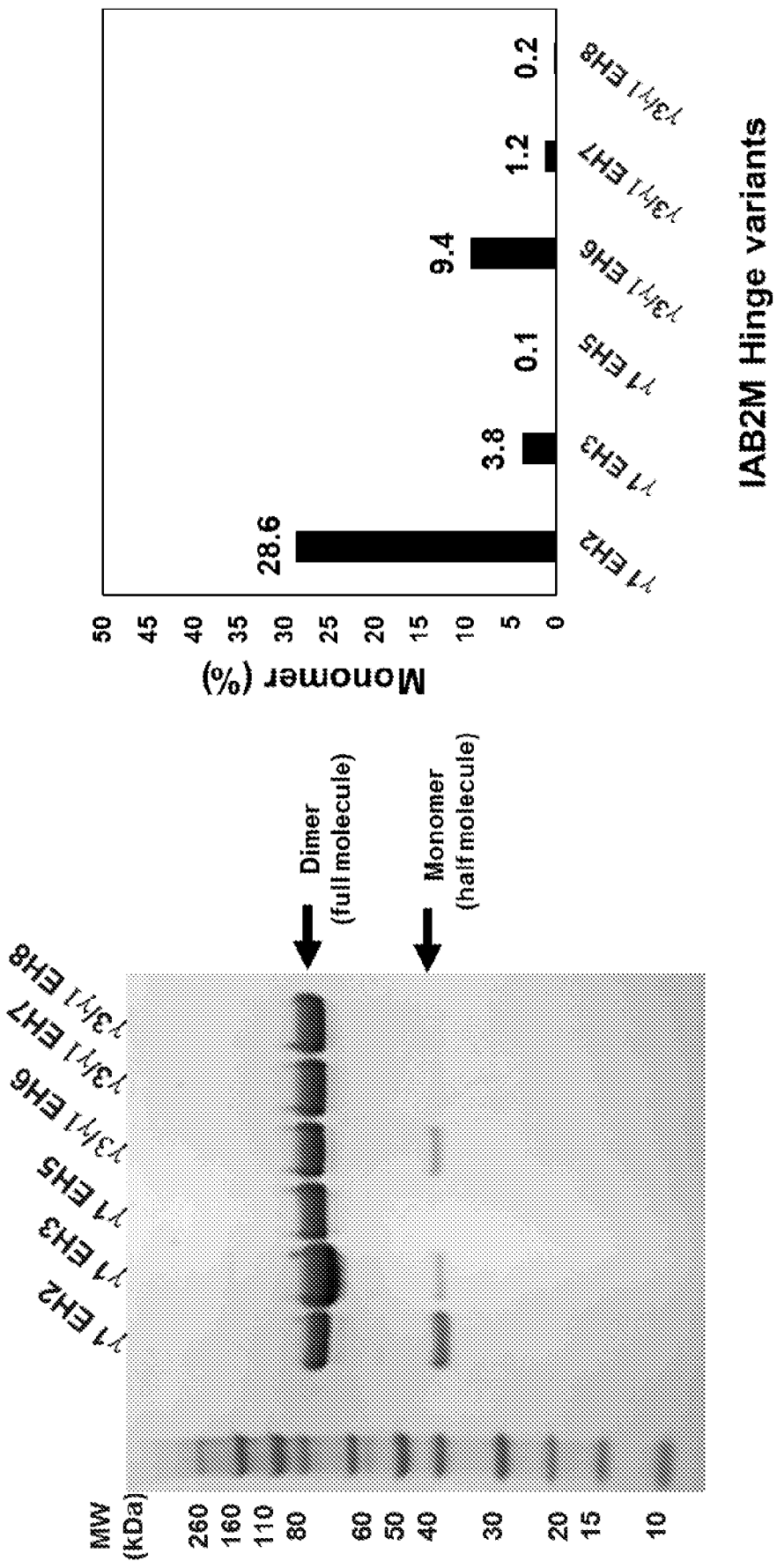

IAB2M Hinge variants
Monomer (%)
0
5
10
15
20
25
30
35
40
45
50
28.6
3.8
0.1
9.4
1.2
0.2
γ1 EH2
γ1 EH3
γ1 EH5
γ3h1 EH6
γ3h1 EH7
γ3h1 EH8
Dimer (full molecule)
Monomer (half molecule)
MW (kDa)
260
160
110
80
60
50
40
30
20
15
10

FIG. 21A

IAB2M γ1 EH5 (N- to C-terminal)

Anti-PSMA variable light (VL) sequence
DIVMTQSPSSLSASVGDRVTITCKASQDVGTAVDWYQQKPGKAPKLLIYWASTRHTGVPDRFTGSGSGTDFTLTISSLQPE
DFADYFCQQYNSYPLTFGGGTKLEIK (SEQ ID NO: 13)

Linker
GSTSGGGSGGGSGGGGSS (SEQ ID NO: 62)

Anti-PSMA variable heavy (VH) sequence
EVQLVQSGAEVKKPGASVKISCKTSGYTFTEYTIHWVKQASGKGLEWIGNINPNNGGTTYNQKFEDRATLTVDKSTSTAYM
ELSSLRSEDTAVYYCAAGWNFDYWGQGTTVTVSS (SEQ ID NO: 14)

Full hinge (EH5)
EPKSSDKTHTCPPCPPCPPCGGGSSGGGSG (SEQ ID NO: 30)

Upper hinge
EPKSSDKTHT (SEQ ID NO: 46)

Core hinge
CPPCPPCPPC (SEQ ID NO: 54)

Lower hinge
GGGSSGGGSG (SEQ ID NO: 59)

Human IgG1 CH3 (G1m1 allotype)
GQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQG
NVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 40)

Human IgG1 CH3 (nG1m1 allotype)
GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQ
GNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 41)

FIG. 21B

IAB2M γ3/γ1 EH6 (N- to C-terminal)

Anti-PSMA variable light (VL) sequence
DIVMTQSPSSLSASVGDRVTITCKASQDVGTAVDWYQQKPGKAPKLLIYWASTRHTGVPDRFTGSGSGTDFTLTISSLQPE
DFADYFCQQYNSYPLTFGGGTKLEIK (SEQ ID NO: 13)

Linker
GSTSGGGSGGGSGGGGSS (SEQ ID NO: 62)

Anti-PSMA variable heavy (VH) sequence
EVQLVQSGAEVKKPGASVKISCKTSGYTFTEYTIHWVKQASGKGLEWIGNINPNNGGTTYNQKFEDRATLTVDKSTSTAYM
ELSSLRSEDTAVYYCAAGWNFDYWGQGTTVTVSS (SEQ ID NO: 14)

Full hinge (EH6)
ELKTPLGDTTHTCVECPPCGGGSSGGGSG (SEQ ID NO: 35)

Upper hinge
ELKTPLGDTTHT (SEQ ID NO: 48)

Core hinge
CVECPPC (SEQ ID NO: 57)

Lower hinge
GGGSSGGGSG (SEQ ID NO: 59)

Human IgG1 CH3 (G1m1 allotype)
GQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQ
GNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 40)

Human IgG1 CH3 (nG1m1 allotype)
GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQ
GNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 41)

FIG. 21C

IAB2M γ3/γ1 EH7 (N- to C-terminal)

Anti-PSMA variable light (VL) sequence
DIVMTQSPSSLSASVGDRVTITCKASQDVGTAVDWYQQKPGKAPKLLIYWASTRHTGVPDRFTGSGSGTDFTLTISSLQPEDF
ADYFCQQYNSYPLTFGGGTKLEIK (SEQ ID NO: 13)

Linker
GSTSGGGSGGGSGGGGSS (SEQ ID NO: 62)

Anti-PSMA variable heavy (VH) sequence
EVQLVQSGAEVKKPGASVKISCKTSGYTFTEYTIHWVKQASGKGLEWIGNINPNNGGTTYNQKFEDRATLTVDKSTSTAYMEL
SSLRSEDTAVYYCAAGWNFDYWGQGTTVTVSS (SEQ ID NO: 14)

Full hinge (EH7)
ELKTPLGDTTHTCPPCPPCGGGSSGGGSG (SEQ ID NO: 36)

Upper hinge
ELKTPLGDTTHT (SEQ ID NO: 48)

Core hinge
CPPCPPC (SEQ ID NO: 52)

Lower hinge
GGGSSGGGSG (SEQ ID NO: 59)

Human IgG1 CH3 (G1m1 allotype)
GQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNV
FSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 40)

Human IgG1 CH3 (nG1m1 allotype)
GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNV
FSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 41)

FIG. 21D

IAB2M γ3/γ1 EH8 (N- to C-terminal)

Anti-PSMA variable light (VL) sequence
DIVMTQSPSSLSASVGDRVTITCKASQDVGTAVDWYQQKPGKAPKLLIYWASTRHTGVPDRFTGSGSGTDFTLTISSL
QPEDFADYFCQQYNSYPLTFGGGTKLEIK (SEQ ID NO: 13)

Linker
GSTSGGGSGGGSGGGGSS (SEQ ID NO: 62)

Anti-PSMA variable heavy (VH) sequence
EVQLVQSGAEVKKPGASVKISCKTSGYTFTEYTIHWVKQASGKGLEWIGNINPNNGGTTYNQKFEDRATLTVDKSTST
AYMELSSLRSEDTAVYYCAAGWNFDYWGQGTTVTVSS (SEQ ID NO: 14)

Full hinge (EH8)
ELKTPLGDTTHTCPPCPPCPPCGGGSSGGGSG (SEQ ID NO: 37)

Upper hinge
ELKTPLGDTTHT (SEQ ID NO: 48)

Core hinge
CPPCPPCPPC (SEQ ID NO: 54)

Lower hinge
GGGSSGGGSG (SEQ ID NO: 59)

Human IgG1 CH3 (G1m1 allotype)
GQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW
QQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 40)

Human IgG1 CH3 (nG1m1 allotype)
GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW
QQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 41)

FIG. 21E

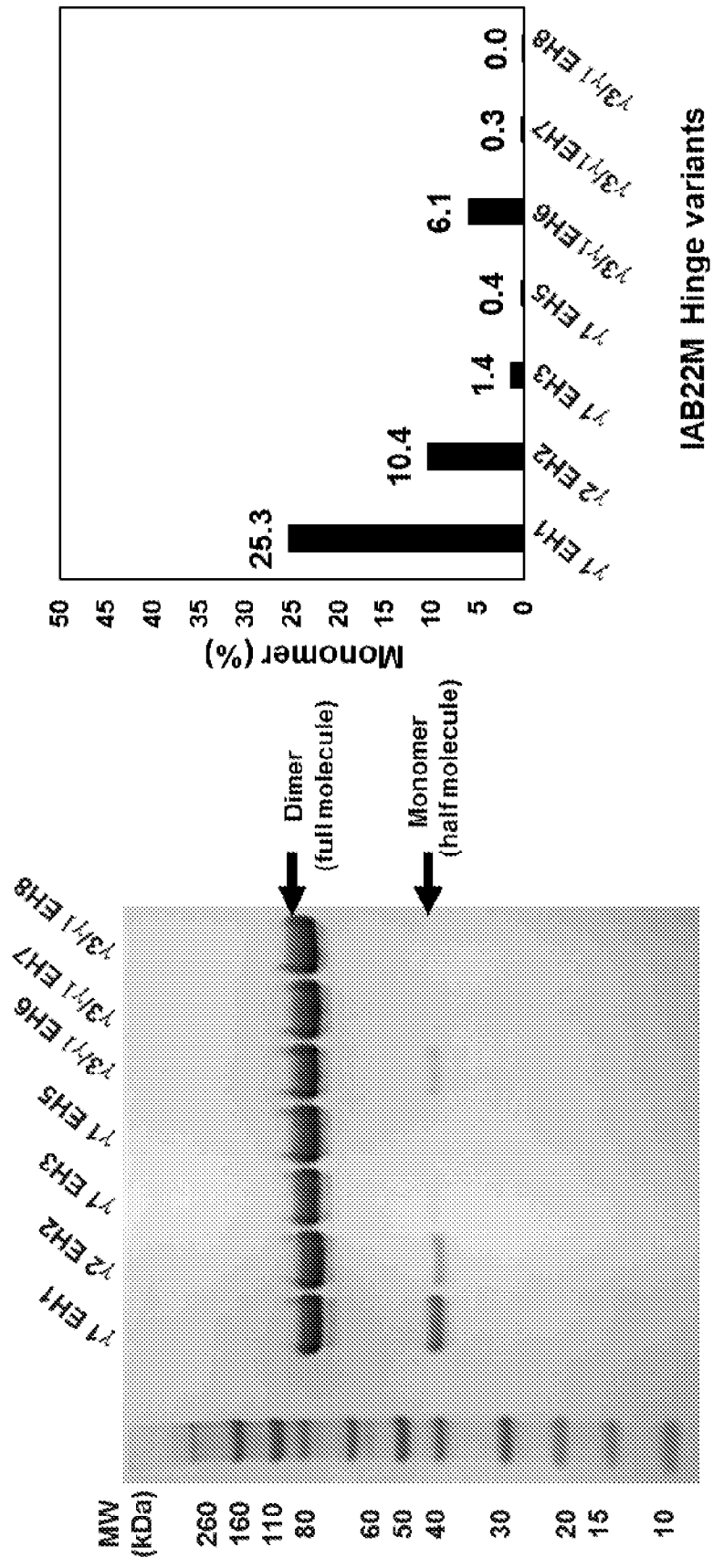

IAB22M Hinge variants
Monomer (%)
0
5
10
15
20
25
30
35
40
45
50
25.3
10.4
1.4
0.4
6.1
0.3
0.0
γ1 EH1
γ2 EH2
γ1 EH3
γ1 EH5
γ3/γ1 EH6
γ3/γ1 EH7
γ3/γ1 EH8
Dimer
(full molecule)
Monomer
(half molecule)
MW
(kDa)
260
160
110
80
60
50
40
30
20
15
10

FIG. 22A

IAB22M γ1 EH2 (N- to C-terminal)

Anti-CD8 variable light (VL) sequence
DVQITQSPSSLSASVGDRVTITCRTSRSISQYLAWYQQKPGKVPKLLIYSGSTLQSGVPSRFSGSGSGTDFTLTI
SSLQPEDVATYYCQQHNENPLTFGGGTKVEIK (SEQ ID NO: 15)

Linker
GSTSGGGSGGGSGGGGSS (SEQ ID NO: 62)

Anti-CD8 variable heavy (VH) sequence
EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHFVRQAPGKGLEWIGRIDPANDNTLYASKFQGKATISADT
SKNTAYLQMNSLRAEDTAVYYCGRGYGYYVFDHWGQGTLVTVSS (SEQ ID NO: 16)

Full length (EH2)
EPKSSDKTHTCPPCGGGSSGGGSG (SEQ ID NO: 24)

Upper hinge
EPKSSDKTHT (SEQ ID NO: 46)

Core hinge
CPPC (SEQ ID NO: 50)

Lower hinge
GGGSSGGGSG (SEQ ID NO: 59)

Human IgG1 CH3 (G1m1 allotype)
GQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVD
KSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 40)

Human IgG1 CH3 (nG1m1 allotype)
GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVD
KSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 41)

FIG. 22B

Intact mass analysis of IAB22M γ1 EH1

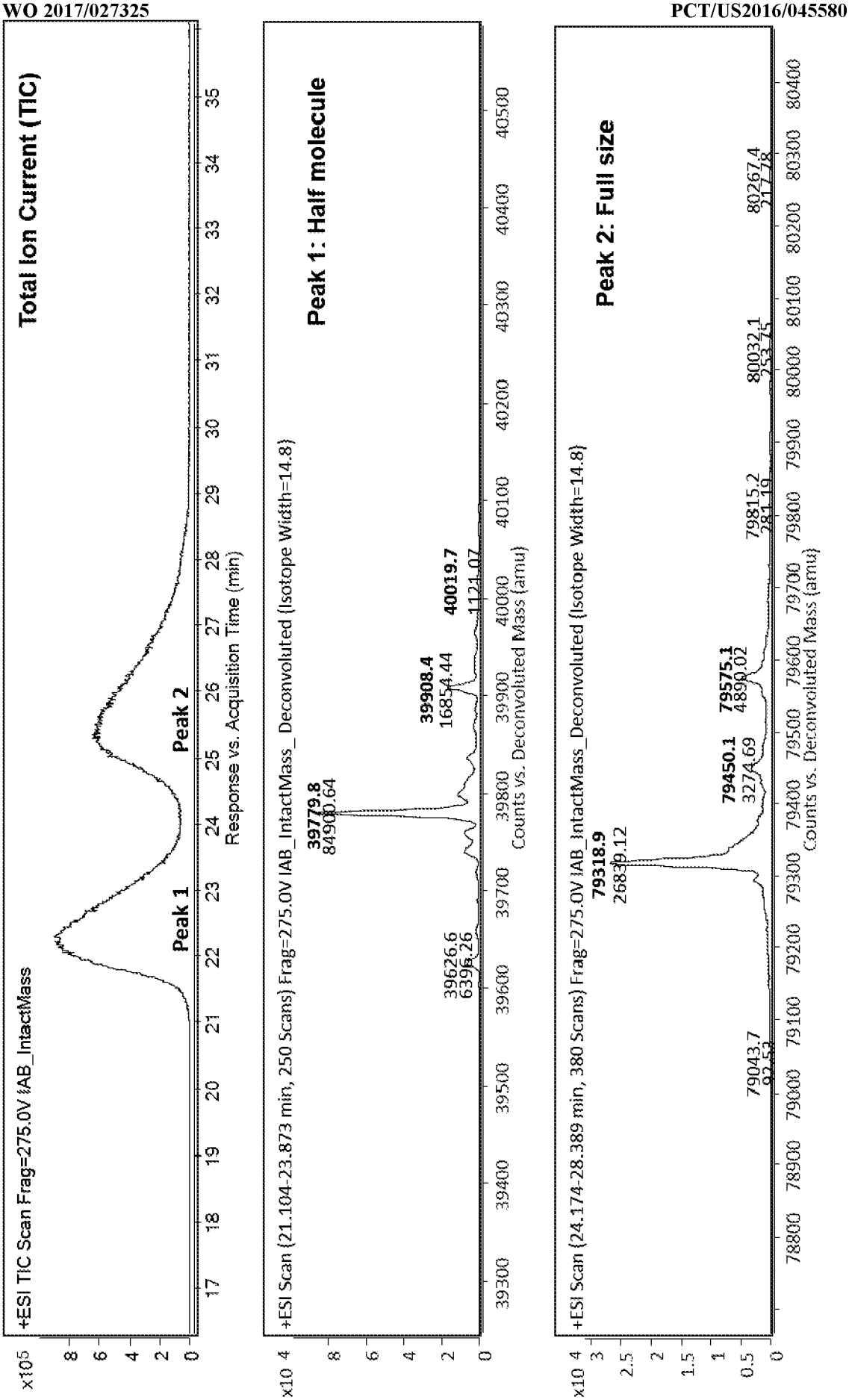


FIG. 23

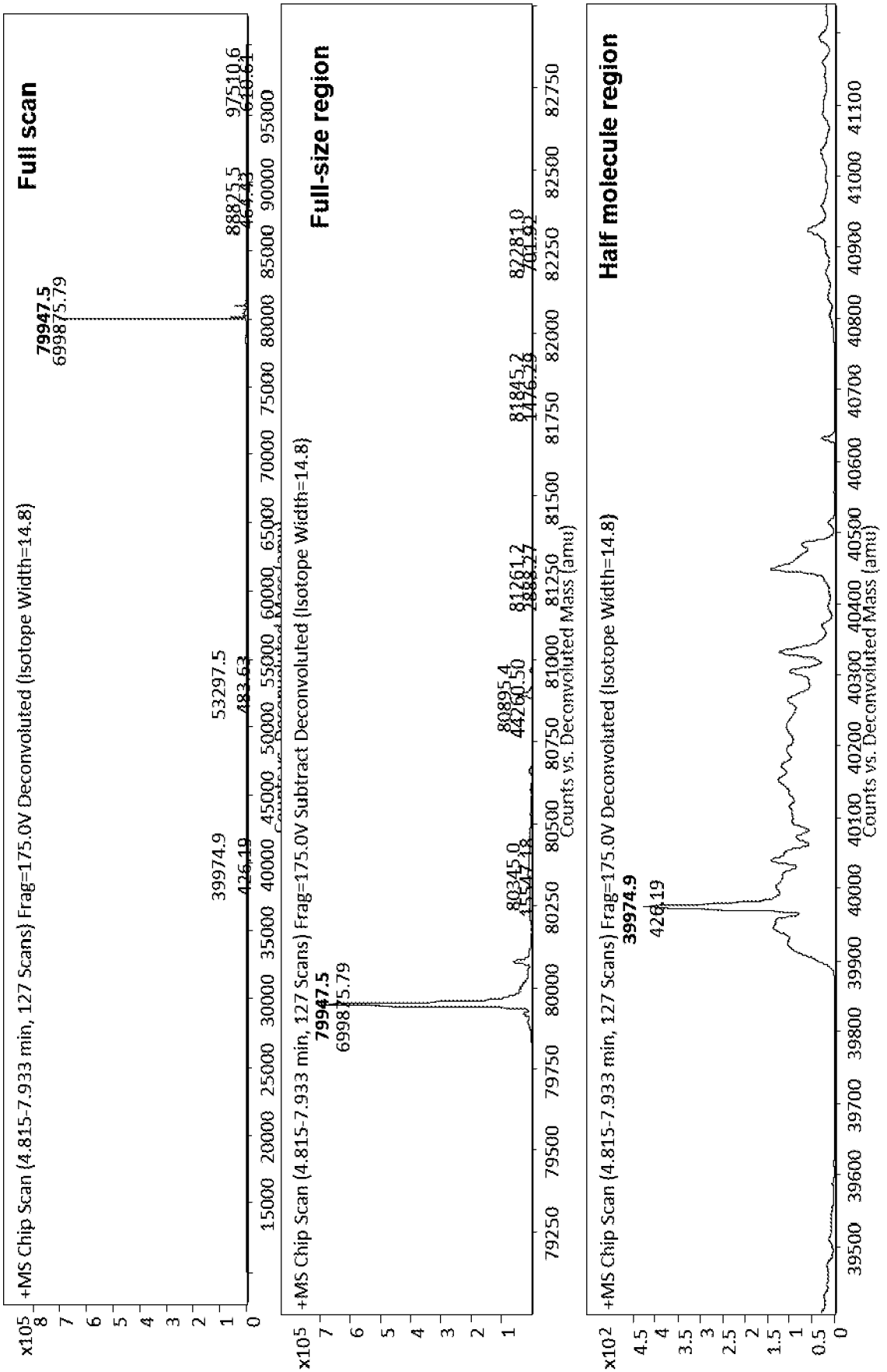

Intact mass analysis of IAB22M γ1 EH3
Full scan
+MS Chip Scan (4.815-7.933 min, 127 Scans) Frag=175.0V Deconvoluted (Isotope Width=14.8)
79947.5
699875.79
39974.9
426.19
53297.5
483.63
88825.5
97510.6
Full-size region
+MS Chip Scan (4.815-7.933 min, 127 Scans) Frag=175.0V Subtract Deconvoluted (Isotope Width=14.8)
79947.5
699875.79
80345.0
80895.4
81261.2
81845.2
82281.0
Counts vs. Deconvoluted Mass (amu)
Half molecule region
+MS Chip Scan (4.815-7.933 min, 127 Scans) Frag=175.0V Deconvoluted (Isotope Width=14.8)
39974.9
426.19
Counts vs. Deconvoluted Mass (amu)

FIG. 24

Intact mass analysis of the IAB22M γ2 EH1

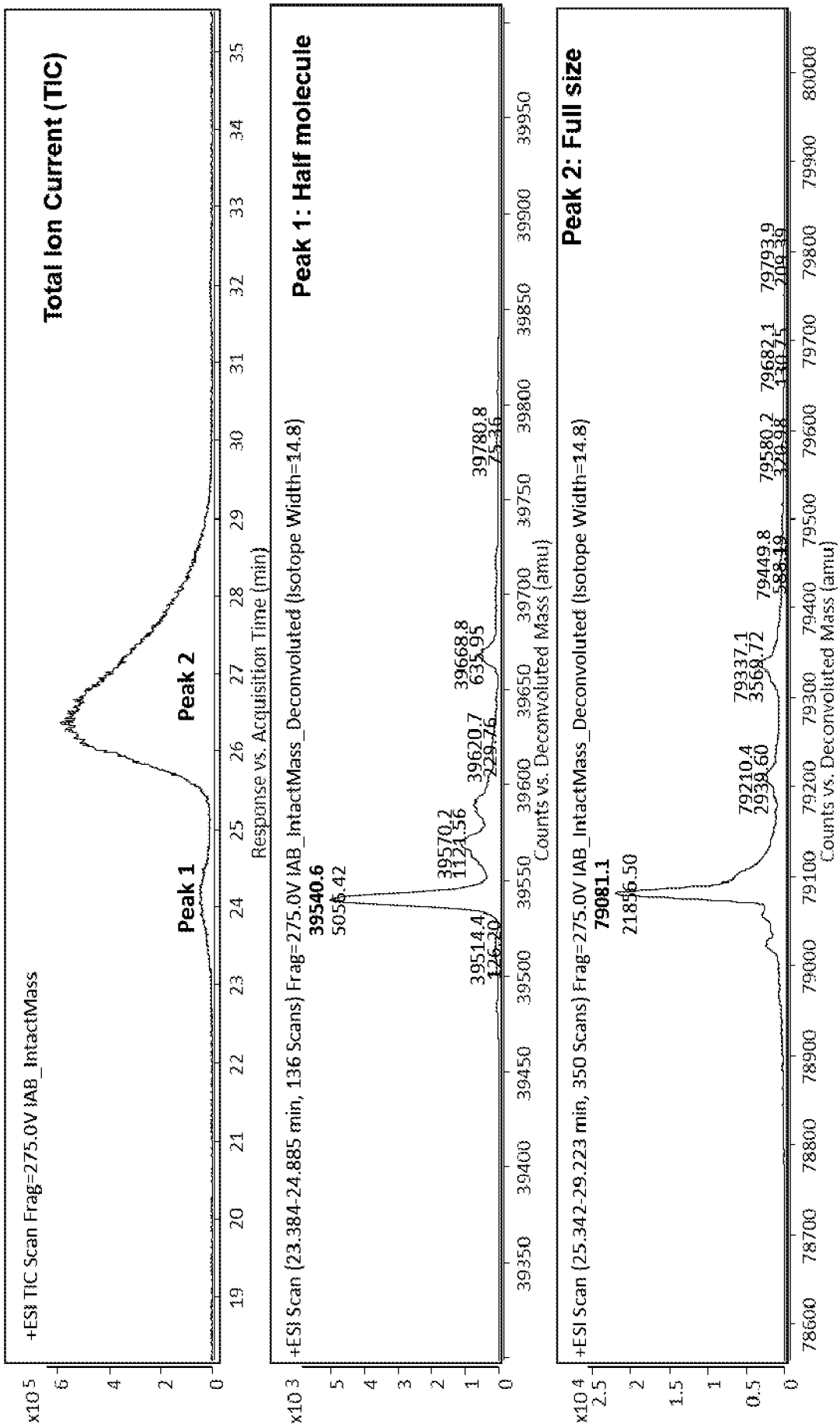


FIG. 25A

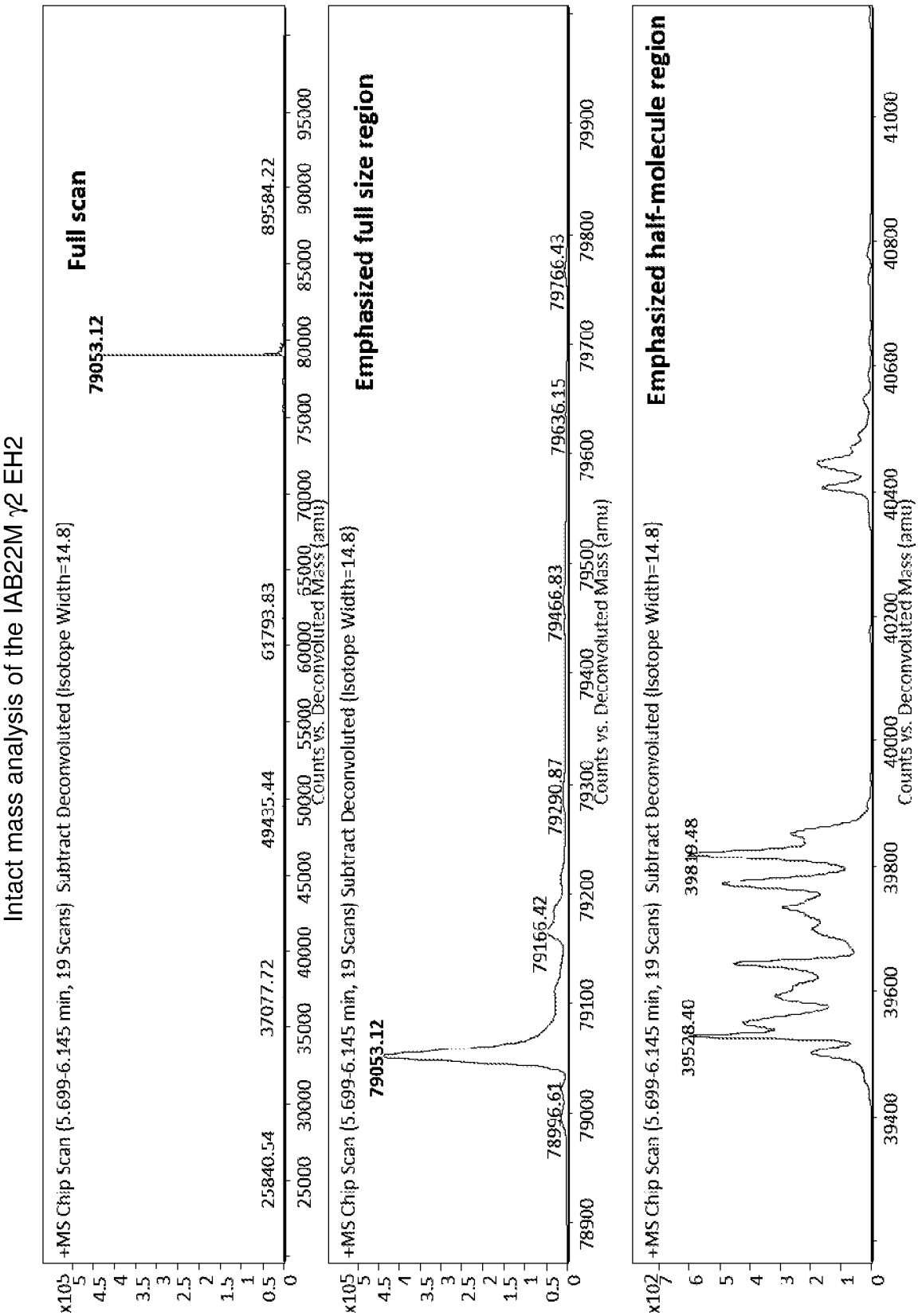
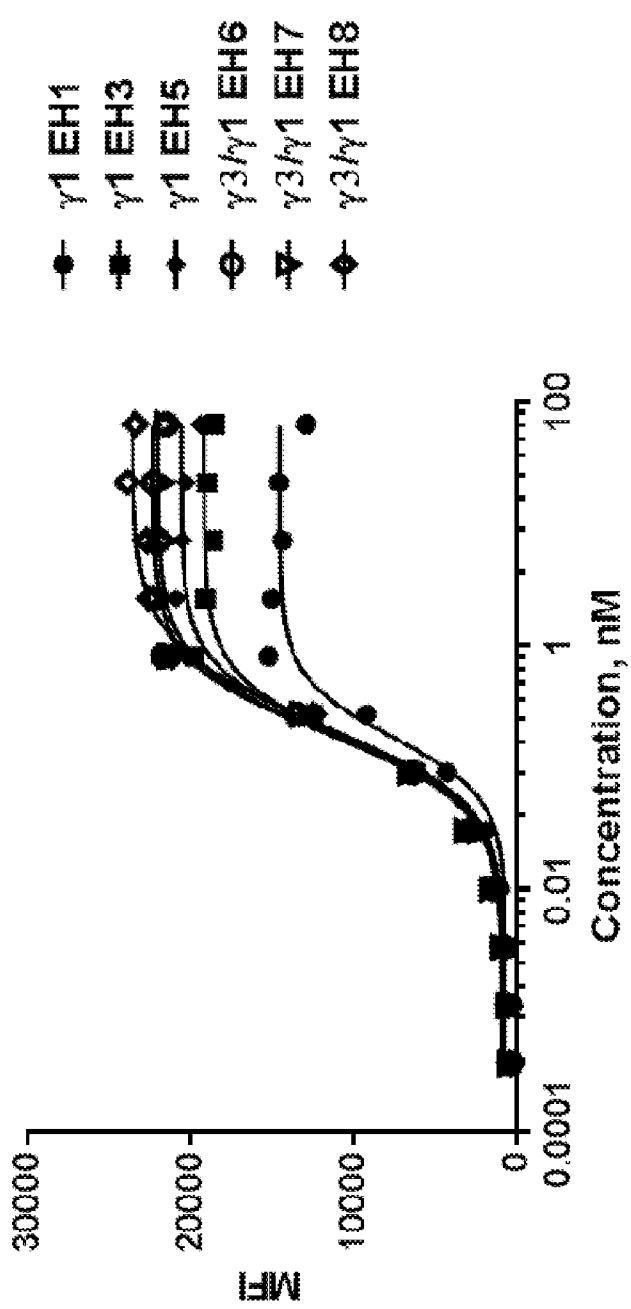
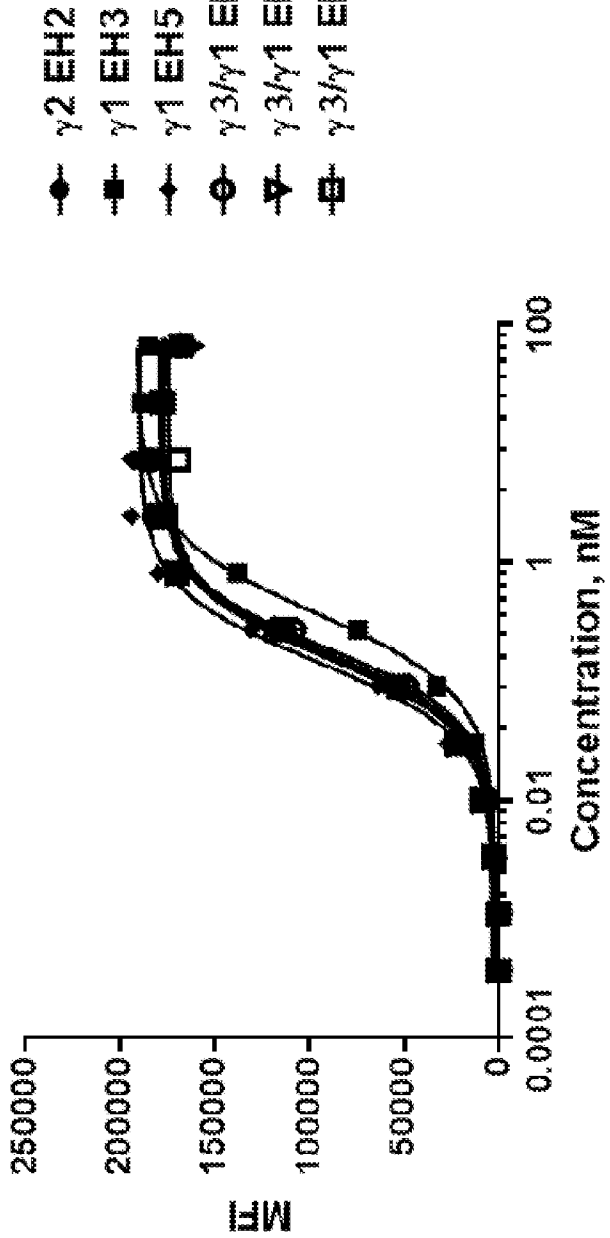

Intact mass analysis of the IAB22M γ2 EH2
+MS Chip Scan (5.699-6.145 min, 19 Scans) Subtract Deconvoluted (Isotope Width=14.8)
Full scan
79053.12
25840.54
37077.72
49435.44
61793.83
89584.22
Counts vs. Deconvoluted Mass (amu)
+MS Chip Scan (5.699-6.145 min, 19 Scans) Subtract Deconvoluted (Isotope Width=14.8)
Emphasized full size region
79053.12
78996.61
79166.42
79290.87
79466.83
79636.15
79766.43
Counts vs. Deconvoluted Mass (amu)
+MS Chip Scan (5.699-6.145 min, 19 Scans) Subtract Deconvoluted (Isotope Width=14.8)
Emphasized half-molecule region
39528.40
39810.48
Counts vs. Deconvoluted Mass (amu)

FIG. 25B



variant	γ2 EH2	γ1 EH3	γ1 EH5	γ3/γ1 EH6	γ3/γ1 EH7	γ3/γ1 EH8
EC50, nM	0.176	0.1618	0.1831	0.1876	0.1859	0.2274

FIG. 26



variant	γ2 EH2	γ1 EH3	γ1 EH5	γ3/γ1 EH6	γ3/γ1 EH7	γ3/γ1 EH8
EC50, nM	0.1561	0.3701	0.1457	0.1884	0.1701	0.1632

FIG. 27

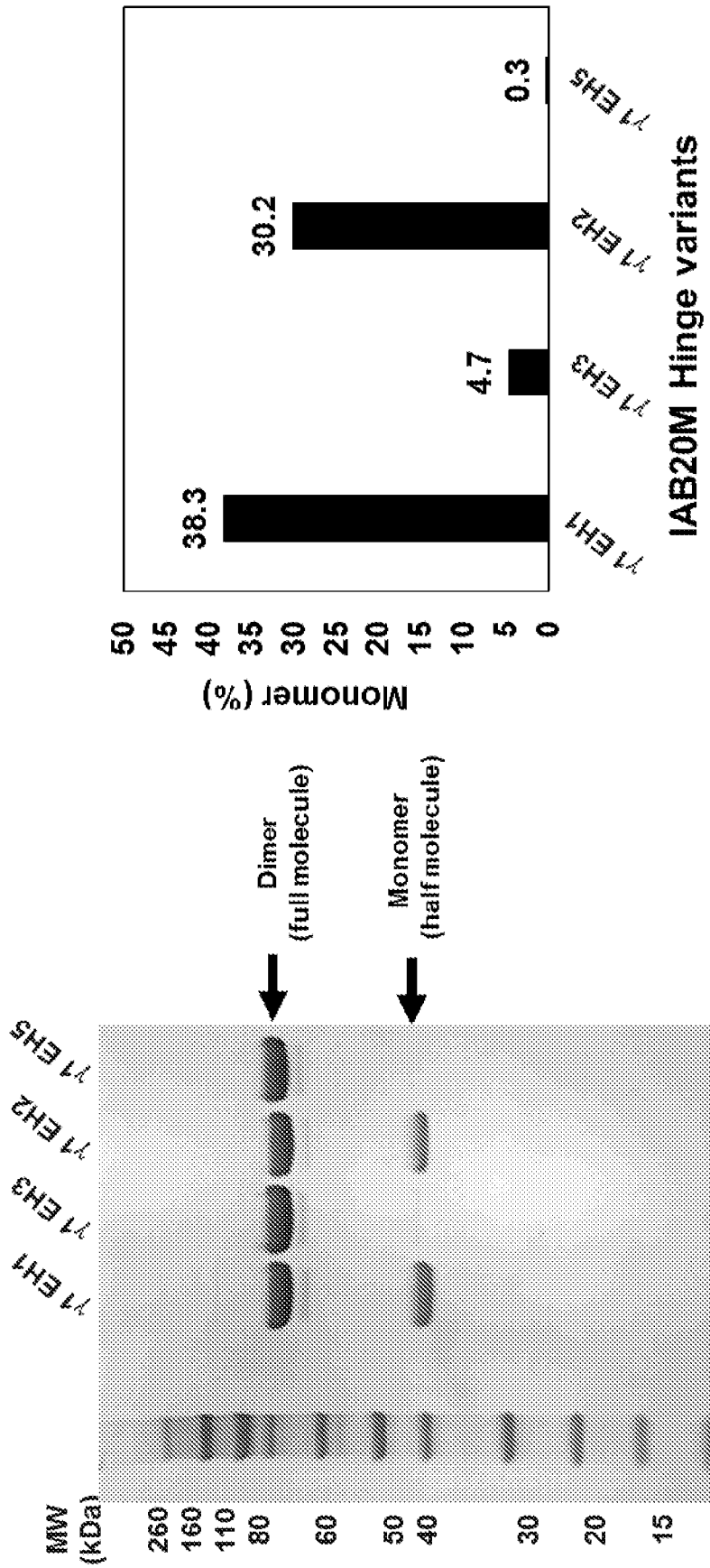

IAB20M Hinge variants
γ1 EH1
γ1 EH3
γ1 EH2
γ1 EH5
38.3
4.7
30.2
0.3
Monomer (%)
0
5
10
15
20
25
30
35
40
45
50
Dimer (full molecule)
Monomer (half molecule)
MW (kDa)
260
160
110
80
60
50
40
30
20
15
10

FIG. 28A

IAB20M γ1 EH1 (N- to C-terminal)

Anti-5T4 variable light (VL) sequence
DIVMTQSPATLSVSPGERATLSCKASQSVSNDVAWYQQKPGQAPRLLISYTSSRYAGVPDRFSGSGSGTDFTLTISSLQ
AEDVAVYYCQQDYNSPPTFGQGTKVEIK (SEQ ID NO: 17)

Linker
GSTSGGGSGGGSGGGGSS (SEQ ID NO: 62)

Anti-5T4 variable heavy (VH) sequence
QVQLVQSGAEVKKPGASVKVSCKASGYSFTGYYMHWVRQAPGQGLEWIGRINPNNGVTLYNQKFKDRVTMTVDTSIS
TAYMELSRLRSDDTAVYYCARSTMITNYVMDYWGQGTLVTVSS (SEQ ID NO: 18)

Full hinge (EH1)
EPKSCDKTHTCPPCGGGSSGGGSG (SEQ ID NO: 22)

Upper hinge
EPKSCDKTHT (SEQ ID NO: 45)

Core hinge
CPPC (SEQ ID NO: 50)

Lower hinge
GGGSSGGGSG (SEQ ID NO: 59)

Human IgG1 CH3 (G1m1 allotype)
GQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQ
QGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 40)

Human IgG1 CH3 (nG1m1 allotype)
GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQ
QGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 41)

FIG. 28B

IAB20M γ1 EH3 (N- to C-terminal)

Anti-5T4 variable light (VL) sequence
DIVMTQSPATLSVSPGERATLSCKASQSVSNDVAWYQQKPGQAPRLLISYTSSRYAGVPDRFSGSGSGTDFTLTISSLQA
EDVAVYYCQQDYNSPPTFGQGTKVEIK (SEQ ID NO: 17)

Linker
GSTSGGGSGGGSGGGGSS (SEQ ID NO: 62)

Anti-5T4 variable heavy (VH) sequence
QVQLVQSGAEVKKPGASVKVSCKASGYSFTGYYMHWVRQAPGQGLEWIGRINPNNGVTLYNQKFKDRVTMTVDTSIST
AYMELSRLRSDDTAVYYCARSTMITNYVMDYWGQGTLVTVSS (SEQ ID NO: 18)

Full hinge (EH3)
EPKSSDKTHTCPPCPPCGGGSSGGGSG (SEQ ID NO: 26)

Upper hinge
EPKSSDKTHT (SEQ ID NO: 46)

Core hinge
CPPCPPC (SEQ ID NO: 52)

Lower hinge
GGGSSGGGSG (SEQ ID NO: 59)

Human IgG1 CH3 (G1m1 allotype)
GQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQ
QGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 40)

Human IgG1 CH3 (nG1m1 allotype)
GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQ
QGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 41)

FIG. 28C

IAB20M γ1 EH2 (N- to C-terminal)

Anti-5T4 variable light (VL) sequence
DIVMTQSPATLSVSPGERATLSCKASQSVSNDVAWYQQKPGQAPRLLISYTSSRYAGVPDRFSGSGSGTDFTLTISSLQA
EDVAVYYCQQDYNSPPTFGQGTKVEIK (SEQ ID NO: 17)

Linker
GSTSGGGSGGGGSGGGGSS (SEQ ID NO: 62)

Anti-5T4 variable heavy (VH) sequence
QVQLVQSGAEVKKPGASVKVSCKASGYSFTGYYMHWVRQAPGQGLEWIGRINPNNGVTLYNQKFKDRVTMTVDTSIST
AYMELSRLRSDDTAVYYCARSTMITNYVMDYWGQGTLVTVSS (SEQ ID NO: 18)

Full hinge (EH2)
EPKSSDKTHTCPPCGGGSSGGGSG (SEQ ID NO: 24)

Upper hinge
EPKSSDKTHT (SEQ ID NO: 46)

Core hinge
CPPC (SEQ ID NO: 50)

Lower hinge
GGGSSGGGSG (SEQ ID NO: 59)

Human IgG1 CH3 (G1m1 allotype)
GQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQ
GNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 40)

Human IgG1 CH3 (nG1m1 allotype)
GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQ
QGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 41)

FIG. 28D

IAB20M γ1 EH5 (N- to C-terminal)

Anti-5T4 variable light (VL) sequence
DIVMTQSPATLSVSPGERATLSCKASQSVSNDVAWYQQKPGQAPRLLISYTSSRYAGVPDRFSGSGSGTDFTLTISSLQAE
DVAVYYCQQDYNSPPTFGQGTKVEIK (SEQ ID NO: 17)

Linker
GSTSGGGSGGGSGGGGSS (SEQ ID NO: 62)

Anti-5T4 variable heavy (VH) sequence
QVQLVQSGAEVKKPGASVKVSCKASGYSFTGYYMHWVRQAPGQGLEWIGRINPNNGVTLYNQKFKDRVTMTVDTSISTA
YMELSRLRSDDTAVYYCARSTMITNYVMDYWGQGTLVTVSS (SEQ ID NO: 18)

Full hinge (EH5)
EPKSSDKTHTCPPCPPCPPCGGGSSGGGSG (SEQ ID NO: 30)

Upper hinge
EPKSSDKTHT (SEQ ID NO: 46)

Core hinge
CPPCPPCPPC (SEQ ID NO: 54)

Lower hinge
GGGSSGGGSG (SEQ ID NO: 59)

Human IgG1 CH3 (G1m1 allotype)
GQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQ
GNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 40)

Human IgG1 CH3 (nG1m1 allotype)
GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQ
GNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 41)

FIG. 28E

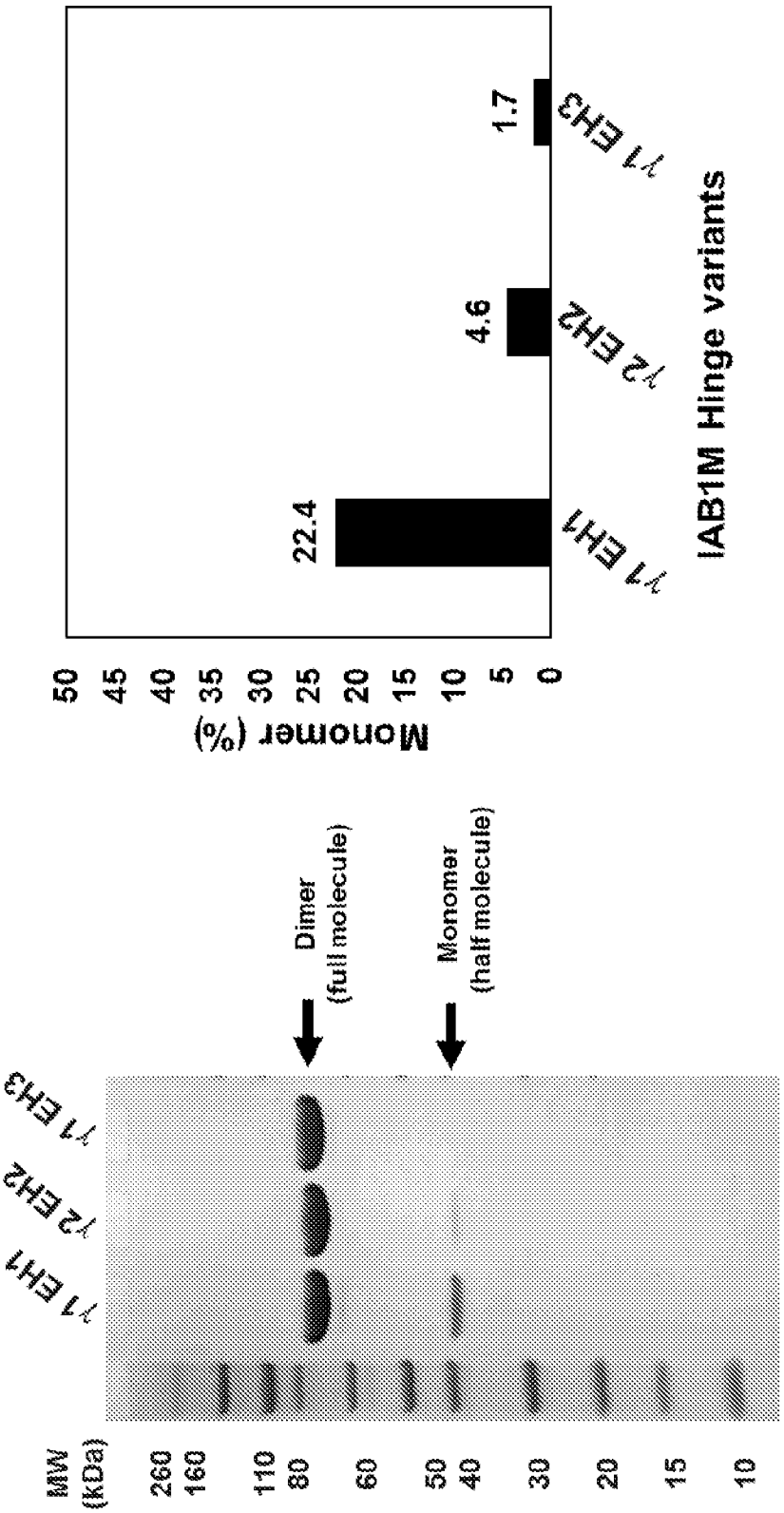

MW (kDa)
260
160
110
80
60
50
40
30
20
15
10
γ1 EH1
γ2 EH2
γ1 EH3
Dimer (full molecule)
Monomer (half molecule)
Monomer (%)
0
5
10
15
20
25
30
35
40
45
50
22.4
4.6
1.7
γ1 EH1
γ2 EH2
γ1 EH3
IAB1M Hinge variants

FIG. 29A

IAB1M γ1 EH1 (N- to C-terminal)

Anti-PSCA (A11l) variable light (VL) sequence
DIQLTQSPSTLSASVGDRVTITCSASSSVRFIHWYQQKPGKAPKRLIYDTSKLASGVPSRFSGSGSGTDFTLTISS
LQPEDFATYYCQQWGSSPFTFGQGTKVEIK (SEQ ID NO: 67)

Linker
GSTSGGGSGGGSGGGGSS (SEQ ID NO: 62)

Anti-PSCA (A11) variable heavy (VH) sequence
EVQLVEYGGGLVQPGGSLRLSCAASGFNIKDYYIHWVRQAPGKGLEWVAWIDPENGDTEFVPKFQGRATMSAD
TSKNTAYLQMNSLRAEDTAVYYCKTGGFWGQGTLVTVSS (SEQ ID NO: 68)

Full hinge (EH1)
EPKSCDKTHTCPPCGGGSSGGGGSG (SEQ ID NO: 22)

Upper hinge
EPKSCDKTHT (SEQ ID NO: 45)

Core hinge
CPPC (SEQ ID NO: 50)

Lower hinge
GGGSSGGGSG (SEQ ID NO: 59)

Human IgG1 CH3 (G1m1 allotype)
GQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDK
SRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 40)

Human IgG1 CH3 (nG1m1 allotype)
GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDK
SRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 41)

FIG. 29B

IAB1M γ2 EH2 (N- to C-terminal)

Anti-PSCA (A11) variable light (VL) sequence
DIQLTQSPSTLSASVGDRVTITCSASSSVRFIHWYQQKPGKAPKRLIYDTSKLASGVPSRFSGSGSGTDFTLTI
SSLQPEDFATYYCQQWGSSPFTFGQGTKVEIK (SEQ ID NO: 67)

Linker
GSTSGGGSGGGSGGGGSS (SEQ ID NO: 62)

Anti-PSCA (A11) variable heavy (VH) sequence
EVQLVEYGGGLVQPGGSLRLSCAASGFNIKDYYIHWVRQAPGKGLEWVAWIDPENGDTEFVPKFQGRATMS
ADTSKNTAYLQMNSLRAEDTAVYYCKTGGFWGQGTLVTVSS (SEQ ID NO: 68)

Full hinge (EH2)
ERKSCVECPPCPGGGSSGGGSG (SEQ ID NO: 34)

Upper hinge
ERK (SEQ ID NO: 47)

Core hinge
SCVECPPCP (SEQ ID NO: 56)

Lower hinge
GGGSSGGGSG (SEQ ID NO: 59)

Human IgG2 CH3
GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTV
DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 42)

FIG. 29C

IAB1M γ1 EH3 (N- to C-terminal)

Anti-PSCA (A11) variable light (VL) sequence
DIQLTQSPSTLSASVGDRVTITCSASSSVRFIHWYQQKPGKAPKRLIYDTSKLASGVPSRFSGSGSGTDFTLTIS
SLQPEDFATYYCQQWGSSPFTFGQGTKVEIK (SEQ ID NO: 67)

Linker
GSTSGGGSGGGSGGGGSS (SEQ ID NO: 62)

Anti-PSCA (A11) variable heavy (VH) sequence
EVQLVEYGGGLVQPGGSLRLSCAASGFNIKDYYIHWVRQAPGKGLEWVAWIDPENGDTEFVPKFQGRATMSA
DTSKNTAYLQMNSLRAEDTAVYYCKTGGFWGQGTLVTVSS (SEQ ID NO: 68)

Full hinge (EH3)
EPKSSDKTHTCPPCPPCGGGSSGGGGSG (SEQ ID NO: 26)

Upper hinge
EPKSSDKTHT (SEQ ID NO: 46)

Core hinge
CPPCPPC (SEQ ID NO: 52)

Lower hinge
GGGSSGGGSG (SEQ ID NO: 59)

Human IgG1 CH3 (G1m1 allotype)
GQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDK
SRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 40)

Human IgG1 CH3 (nG1m1 allotype)
GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVD
KSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 41)

FIG. 29D

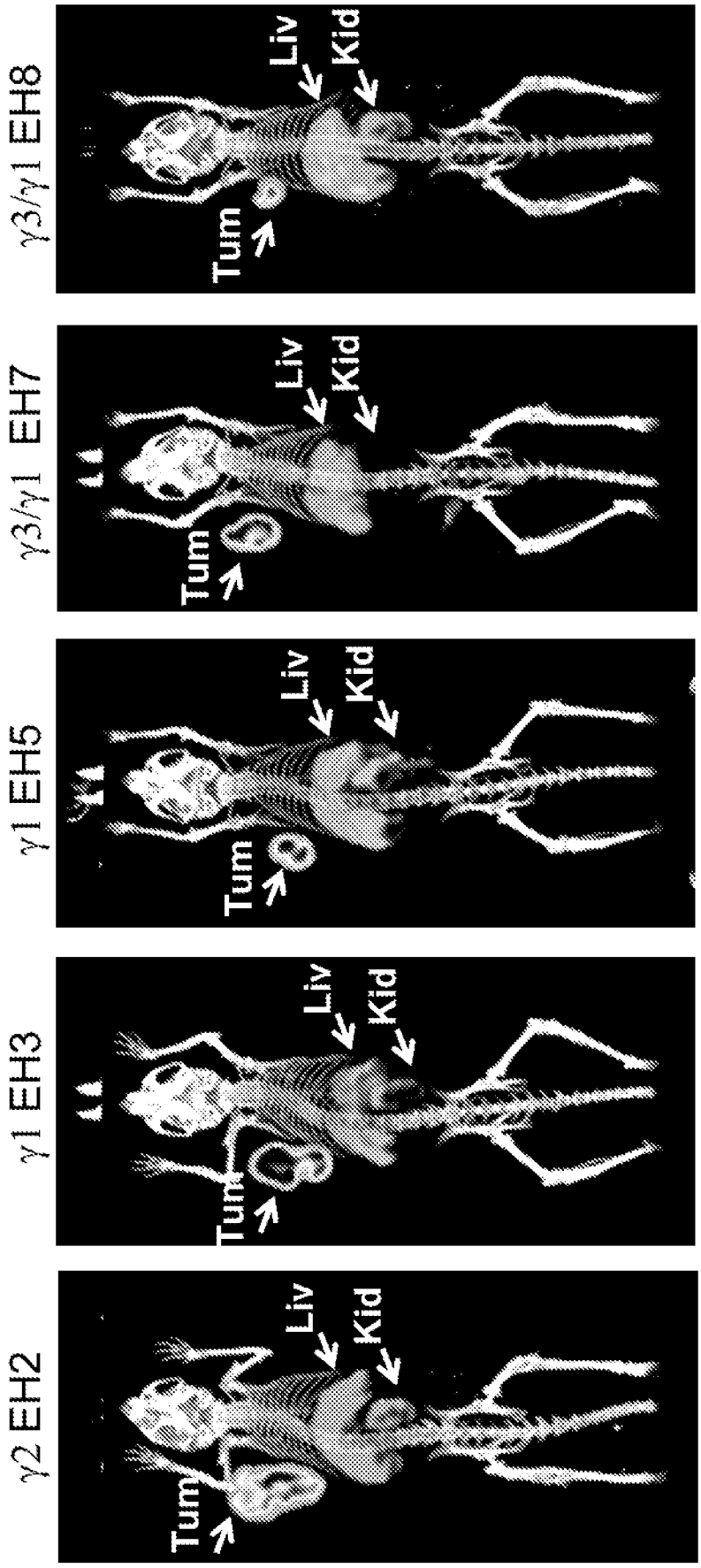

γ2 EH2
Tum
Liv
Kid
γ1 EH3
Tum
Liv
Kid
γ1 EH5
Tum
Liv
Kid
γ3/γ1 EH7
Tum
Liv
Kid
γ3/γ1 EH8
Tum
Liv
Kid

FIG. 30

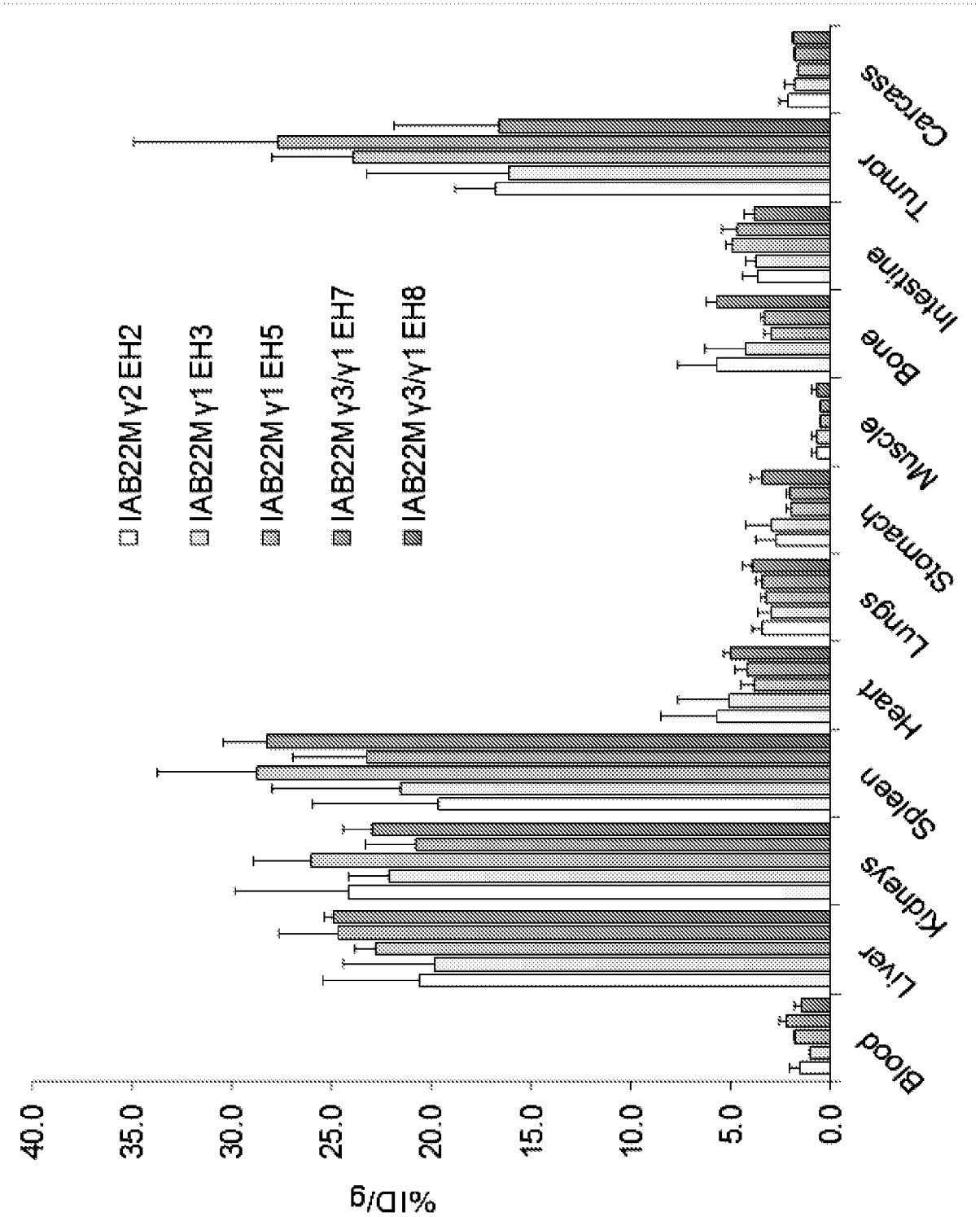

IAB22M γ2 EH2
IAB22M γ1 EH3
IAB22M γ1 EH5
IAB22M γ3/γ1 EH7
IAB22M γ3/γ1 EH8
%ID/g
40.0
35.0
30.0
25.0
20.0
15.0
10.0
5.0
0.0
Blood
Liver
Kidneys
Spleen
Heart
Lungs
Stomach
Muscle
Bone
Intestine
Tumor
Carcass

FIG. 31

IAB20M γ2 EH2 (N- to C-terminal)

Anti-5T4 variable light (VL) sequence
DIVMTQSPATLSVSPGERATLSCKASQSVSNDVAWYQQKPGQAPRLLISYTSSRYAGVPDRFSGSGSGTDFTLTIS
SLQAEDVAVYYCQQDYNSPPTFGQGTKVEIK (SEQ ID NO: 17)

Linker
GSTSGGGSGGGSGGGGSS (SEQ ID NO: 62)

Anti-5T4 variable heavy (VH) sequence
QVQLVQSGAEVKKPGASVKVSCKASGYSFTGYYMHWVRQAPGQGLEWIGRINPNNGVTLYNQKFKDRVTMTVD
TSISTAYMELSRLRSDDTAVYYCARSTMITNYVMDYWGQGTLVTVSS (SEQ ID NO: 18)

Full hinge (EH2)
ERKSCVECPPCPGGGSSGGGGSG (SEQ ID NO: 34)

Upper hinge
ERK (SEQ ID NO: 47)

Core hinge
SCVECPPCP (SEQ ID NO: 56)

Lower hinge
GGGSSGGGSG (SEQ ID NO: 59)

Human IgG2 CH3
GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKS
RWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 42)

FIG. 32

IAB25M γ2 EH2 (N- to C-terminal)

Anti-CD3 variable light (VL) sequence
EIVLTQSPATLSLSPGERATLSCSASSSVSYMNWYQQKPGQAPRLLIYDTSKLASGVPAHFRGSGSGTDFTLTI
SSLEPEDFAVYYCQQWSSNPFTFGQGTKVEIK (SEQ ID NO: 19)

Linker
GSTSGGGSGGGSGGGGSS (SEQ ID NO: 62)

Anti-CD3 variable heavy (VH) sequence
QVQLVQSGAEVKKPGASVKVSCKASGYTFTRYTMHWVRQAPGQGLEWMGYINPSRGYTNYNQKFKDRVTM
TTDTSISTAYMELSRLRSDDTAVYYCARYYDDHYSLDYWGQGTLVTVSS (SEQ ID NO: 20)

Full hinge (EH2)
ERKSCVECPPCPGGGSSGGGSG (SEQ ID NO: 34)

Upper hinge
ERK (SEQ ID NO: 47)

Core hinge
SCVECPPCP (SEQ ID NO: 56)

Lower hinge
GGGSSGGGSG (SEQ ID NO: 59)

Human IgG2 CH3
GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVD
KSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 42)

FIG. 33

M1 = VL – VH
M2 = VH - VL

IAB2M γ1 EH3 (M1) (SEQ ID NO: 162)

gatattgtgatgacccagagcccgagcctgagcgcgagcgtgggcgatcgcgtgacc
attacctgcaaagcgagccaggatgtgggcaccgcggtggattggtatcagcagaaaccg
ggcaaagcgccgaaactgctgatttatttgggcgagcaccgcgccataccggcgtgccgggat
cgctttaccggcagcgcggcaccgattttaccctgaccattagcagcctgcagccg
gaagatttgcggattatttttgccagcagtataacagctatccgctgacctttggcggc
ggcaccaaactggaaattaaagcgcagcaccagcggcgcgcgcagcgggcagcgcggc
ggcgcggcggcagcagcgaagtgcagctggtgcagagcggcgcggaagtgaaaaaaccgggc
gcgagcgtgaaaaattagctgcaaaaaccagcgcggctataccctttaccgaatataccattcat
tgggtgaaacaggcgagcgcggcaaagcgcctggaatggattggcaacatttaacccgaacaac
ggcggcaccacctataaccagaaatttgaagatcgcgcgaccctgaccgtgggataaaagc
accagcaccgcgtatatggaactgagcagcctgcgcagcgaagataccgcggtgtattat
tgcgcgggcgggctggaacttttgattattggggccaggggcaccaccgtgaccgtgagcagc
gaaccgaaaaagcagcgataaaaacccatacctgccccgcctgcccgtgcgcggcgggcgggc
agcagcggcggcggcagcggccagccgcgcgaaccgcaggtgtataccctgccgccg
agccgcgaagaaatgaccaaaaaccaggtgagcctgacctgcctggtgaaaggctttttat
ccgagcgatattgcggtggaatgggaaagcaacgggccagccgggaaaacaactataaaacc
accccgccgggtgctgggatagcgatgggcagcttttttctgtatagcaaactgaccgtgggat
aaaagccgctggcagcagggcaacgtgtttagctgcagcgtgatgcatgaagcgctgcat
aaccattatacccagaaaagcctgagccctgagcccgggc **(SEQ ID NO: 162)**

FIG. 34A

IAB2M γ1 EH5 (M1) (SEQ ID NO: 163)

gatattgtgatgacccagagccccgagcctgagcgcgagcgtgggcgatcgcgtgacc
attacctgcaaagcgagccaggatgtgggcaccgcggtgggattggtatcagcagaaaccgg
ggcaaagcgccgaaactgctgatttattgggcgagcacccgccataccggcgtgccggat
cgctttaccgggcagcgggcaccgattttaccctgaccattagcagcctgcagccgg
gaagatttttgcgggattattttttgccagcagtataacagctatccgctgacctttggcgggc
ggcaccaaactggaaaattaaagggcagcaccagcgggcgggcgggcagcgggc
ggcggcggcagcagcgaagtgcagctggtgcagagcggcgcggaagtgaaaaaaccgggc
gcgagcgtgaaaattagctgcaaaaccagcggctataccctttaccgaatataccattcat
tgggtgaaacagggcgagcgggcaaagggcctggaatgggattggcaacattaacccgaacaac
ggcggcaccacctataaccagaaatttgaagatcgcgcgaccctgaccgtggataaaagc
accagcaccgcgtatatggaactgagcagcctgcgcagcgaagataccgcgggtgtattat
tgcgcggcgcgggctggaactttgattattgggggccaggggcaccaccgtgaccgtgagcagc
gaaccgaaaagcagcgataaaacccatacctgcccgccgtgcccgccgtgc
ggcggcggcagcagcggcggcggccagccgcgcgaaccgcagggtgtataccc
ctgccgccgagccgcgaagaaatgaccaaaaaccaggtgagcctgacctgcctggtgaaa
ggctttatatccgagcgatattgcggtgggaatgggaaagcaacgggccagccgggaaaacaac
tataaaaccacccccgccggtgctggatagcgatggcagctttttctgtatagcaaactg
accgtggataaaagccgctggcagcagggcaacgtgtttagctgcagcgtgatgcatgaa
gcgctgcataaccatttataccccagaaaagccctgagccccgggc **(SEQ ID NO: 163)**

FIG. 34B

IAB2M γ1 EH8 (M1) (SEQ ID NO: 164)

gatattgtgatgacccagagccctgagcgcgagcgtgggcgatcgcgtgacc
attacctgcaaagcgagccaggatgtgggcaccgcggtgggattggtatcagcagaaaccg
ggcaaagcgccgaaactgctgatttattgggcgagcacccgccataccggcgtgccgggat
cgcttaccggcagcgcggcaccgatttttaccctgaccattagcagcctgcagccg
gaagatttttgcgggattatttttgccagcagtataacagctatccgctgacctttggcgggc
ggcaccaaactggaaaattaaaggcagcaccagcgggcgggcagcgggcaggcgggc
ggcggcggcagcagcgaagtgcagctggtgcagagcggcgcggaagtgaaaaaaccgggc
gcgagcgtgaaaattagctgcaaaaccagcggctataccttttaccgaatataccattcat
tgggtgaaacaggcgagcggcaaagggcctggaatggattggcaacattaacccgaacaac
ggcggcaccacctataaccagaaatttgaagatcgcgcgacccctgaccgtggataaaagc
accagcaccgcgtatatggaactgagcagccctgcgcagcgaagataccgcggtgtattat
tgcgcggcgggctggaacttttgattattgggggccagggcaccaccgtgaccgtgagcagc
gaactgaaaaccccgctgggcgataccacccatacctgcccgccgtgcccgtgcccg
ccgtgcggcgggcagcagcgggcgggccagccgcgcgaaccgcaggtg
tataccctgccgccgcgaagaaatgaccaaaaaccaggtgagcctgacctgcctg
gtgaaaggctttatccgagcgatattgcggtgggaatgggaaagcaacgggccagccgga
aacaactataaaaccaccccgccggtgctggatagcgatggcagctttttttctgtatagc
aaactgaccgtggataaaagccgctggcagcagggcaaccgtgtttagctgcagcgtgatg
catgaagcgctgcataaccattataccccagaaaagccctgagccctgagccccgggc **(SEQ ID NO: 164)**

FIG. 34C

IAB2M γ1 EH3 (M2) (SEQ ID NO: 165)

gaagtgcagctggtgcagagcggcgcggaagtgaaaaaaaccgggcgcgagcgtgaaaatt
agctgcaaaaaccagcggctatacctttaccgaatataccattcattgggtgaaacaggcg
agcggcaaaaggcctggaatggattggcaacatttaacccgaacaacggcggcaccacctat
aaccagaaatttgaagatcgcgcgaccctgaccgtggataaaagcaccagcaccgcgtat
atggaactgagcagcctgcgcagcgaagataccgcggtgtattatgcgcgggctggg
aacttttgattattgggggccagggcaccaccgtgaccgtgagcagcacccagcgggc
ggcggcagcggcggcggcagcggcggcggcagcagcgatattgtgatgacccagagc
ccgagcagcctgagcgcgagcgtgggcgatcgcgtgaccattacctgcaaagcgagccag
gatgtgggcaccgcggtggattggtatcagcagaaaccgggcaaagcgcccgaaactgctg
atttattgggcgagcacccgccataccgggcgtgccgggatcgcgtttaccggcagcggcagc
ggcaccgatttttaccctgaccattagcagcctgcagccggaagatttttgcggattatttt
tgccagcagtataacagctatccgctgacctttggcgggcggcaccaaaactgggaaattaaa
gaaccgaaaaagcagcgataaaacccatacctgcccgtgcccgccgtgcggcgggcgggc
agcagcggcggcggcggccagccgcgcgaaccgcaggtgtataccctgccgccgccg
agccgcgaagaaatgaccaaaaaccaggtgagcctgacctgcctggtgaaagggcttttat
ccgagcgatattgcggtgggaatgggaaagcaacgggccagccggaaaacaactataaaacc
acccccgccgggtgctggatagcgatggcagctttttctctgtatagcaaactgaccgtggat
aaaagccgctggcagcagggcaacgtgtttagctgcagcgtgatgcatgaagcgctgcat
aaccattatacccagaaaagcctgagccctgggggc **(SEQ ID NO: 165)**

FIG. 34D

IAB2M γ1 EH5 (M2) (SEQ ID NO: 5)

gaagtgcagctggtgcagagcggcgcggaagtgaaaaaaaccgggcgcgagcgtgaaaatt
agctgcaaaaaccagcggctatacctttaccgaataccattcattgggtgaaacaggcg
agcggcaaaaggcctggaatggattggcaacatttaacccgaacaacggcggcaccacctat
aaccagaaatttgaagatcgcgcgaccctgacccgtggataaaagcaccagcaccgcgtat
atggaactgagcagcctgcgcagcgaagataccgcggtgtattatgcgcgcgggctgg
aacttttgattattgggggccagggcaccaccgtgaccgtgagcagcgcaccagcaccgcgc
ggcggcagcggcggcggcagcggcggcggcagcagcgatattgtgatgacccagagc
ccgagcagcctgagcgcgagcgtgggcgatcgcgtgaccattacctgcaaagcgagccag
gatgtgggcaccgcggtggattggtatcagcagaaaccgggcaaagcgcccgaaactgctg
atttattgggcgagcacccgccataccgggcgtgccgggatcgcgtttaccggcagcggcagc
ggcaccgatttttaccctgaccattagcagcctgcagccggaagatttttgcggattatttt
tgccagcagtataacagctatccgctgacctttggcgggcgcaccaaactgggaaattaaa
gaaccgaaaagcagcgataaaacccatacctgcccgtgcccgccgtgcccgctgc
ggcggcggcagcagcggcggcggccagccgcgcgaaccgcaggtgtataccc
ctgccgccgagccgcgaagaaatgaccaaaaaccaggtgagcctgaccctgcctggtgaaa
ggctttttatccgagcgatattgcggtgggaatgggaaagcaacgggccagccgggaaaacaac
tataaaaccacccccgccggtgctgggatagcgatggcagctttttctgtatagcaaactg
accgtggataaaagcccgctgggcagggcaacgtgtttagctgcagcgtgatgcatgaa
gcgctgcataaccattataccccagaaaagcctgagccctgaggcccgggc **(SEQ ID NO: 5)**

FIG. 34E

IAB2M γ1 EH8 (M2) (SEQ ID NO: 6)

gaagtgcagctggtgcagagcggcgcggaagtgaaaaaaaccgggcgcgagcgtgaaaatt
agctgcaaaaaccagcggctataccttttaccgaatataccattcatttgggtgaaacaggcg
agcggcaaaggcctggaatggattgcaacaacattaacccgaacaacggcggcaccacctat
aaccagaaatttgaagatcgcgcgaccctgaccgtggataaaagcaccagcaccgcgtat
atggaactgagcagcctgcgcagcgaagataccgcggtgtattattgcgcgcgggctgg
aacttttgattattggggccaggggcaccaccgtgaccgtgagcagcaccaccagcggc
ggcggcagcggcggcggcggcggcggcagcagcgatattgtgatgacccagagc
ccgagcagcctgagcgcgagcgtgggcgatcgcgtgaccattacctgcaaagcgagccag
gatgtgggcaccgcgtgggattggtatcagcagaaaccgggcaaagcgccgaaactgctg
attattgggcgagcaccgccgccataccggcgtgccggatcgcttttaccggcagcggcagc
ggcaccgattttttaccctgaccattagcagcctgcagccggaagatttttgcgattattt
tgccagcagtataacagctatccgctgacctttggcggcggcaccaaactggaaattaaa
gaactgaaaaaccccgctgggcgataccacccctgcccgtgccccgtgcccg
ccgtgcggcggcggcagcagcggcggcggccagccgcgcgaaccgcaggtg
tataccctgccgccgagccgcgaagaaatgaccaaaaaccaggtgagcctgacctgcctg
gtgaaaggctttatccgagcgatattgcggtggaatgggaaagcaacgggccagccgga
aacaactataaaaccaccccgccgtgctggatagcgatggcagctttttttctgtatagc
aaactgaccgtggataaaagccgctgggcaggggcaacgtgtttagctgcagcgtgatg
catgaagcgctgcataaccattataccccagaaaagcctgagccctgagccccgggc **(SEQ ID NO: 6)**

FIG. 34F

M1 = VL – VH
M2 = VH – VL
Linkers
Hinges

IAB2M γ1 EH3 (M1) (SEQ ID NO: 7)
DIVMTQSPSSLSASVGDRVTITCKASQDVGTAVDWYQQKPGKAPKLLIYWASTRHTGVPDRFTGSGSGTDFTLTI
SSLQPEDFADYFCQQYNSYPLTFGGGTKLEIKGSTSGGGSGGGGSGGGGSEVQLVQSGAEVKKPGASVKISCKTS
GYTFTEYTIHWVKQASGKGLEWIGNINPNNGGTTYNQKFEDRATLTVDKSTSTAYMELSSLRSEDTAVYYCAAG
WNFDYWGQGTTVTVSSEPKSSDKTHTCPPCPPCGGGSSGGGGSGGQPREPQVYTLPPSREEMTKNQVSLTCLVK
GFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLS
PG **(SEQ ID NO: 7)**

IAB2M γ1 EH5 (M1) (SEQ ID NO: 8)
DIVMTQSPSSLSASVGDRVTITCKASQDVGTAVDWYQQKPGKAPKLLIYWASTRHTGVPDRFTGSGSGTDFTLTI
SSLQPEDFADYFCQQYNSYPLTFGGGTKLEIKGSTSGGGSGGGGSGGGGSEVQLVQSGAEVKKPGASVKISCKTS
GYTFTEYTIHWVKQASGKGLEWIGNINPNNGGTTYNQKFEDRATLTVDKSTSTAYMELSSLRSEDTAVYYCAAG
WNFDYWGQGTTVTVSSEPKSSDKTHTCPPCPPCPPCGGGSSGGGGSGGQPREPQVYTLPPSREEMTKNQVSLTC
LVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSL
SLSPG **(SEQ ID NO: 8)**

FIG. 35A

IAB2M γ1 EH8 (M1) (SEQ ID NO: 9)
DIVMTQSPSSLSASVGDRVTITCKASQDVGTAVDWYQQKPGKAPKLLIYWASTRHTGVPDRFTGSGSGTDFTLTI
SSLQPEDFADYFCQQYNSYPLTFGGGTKLEIKGSTSGGGSGGGGSGGGSEVQLVQSGAEVKKPGASVKISCKTS
GYTFTEYTIHWVKQASGKGLEWIGNINPNNGGTTYNQKFEDRATLTVDKSTSTAYMELSSLRSEDTAVYYCAAG
WNFDYWGQGTTVTVSSELKTPLGDTTHTCPPCPPCPGGGSGGGSGGQPREPQVYTLPPSREEMTKNQVSL
TCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQK
SLSLSPG **(SEQ ID NO: 9)**

IAB2M γ1 EH3 (M2) (SEQ ID NO: 10)
EVQLVQSGAEVKKPGASVKISCKTSGYTFTEYTIHWVKQASGKGLEWIGNINPNNGGTTYNQKFEDRATLTVDKS
TSTAYMELSSLRSEDTAVYYCAAGWNFDYWGQGTTVTVSSGSTSGGGSGGGGSGGGSSDIVMTQSPSSLSASV
GDRVTITCKASQDVGTAVDWYQQKPGKAPKLLIYWASTRHTGVPDRFTGSGSGTDFTLTISSLQPEDFADYFCQ
QYNSYPLTFGGGTKLEIKEPKSSDKTHTCPPCPPCGGGSSGGGSGQPREPQVYTLPPSREEMTKNQVSLTCLVK
GFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLS
PG **(SEQ ID NO: 10)**

FIG. 35B

IAB2M γ1 EH5 (M2) (SEQ ID NO: 11)

EVQLVQSGAEVKKPGASVKISCKTSGYTFTEYTIHWVKQASGKGLEWIGNINPNNGGTTYNQKFEDRATLTVDKS
TSTAYMELSSLRSEDTAVYYCAAGWNFDYWGQGTTVTVSSGSTSGGGSGGGSGGGSSDIVMTQSPSSLSASV
GDRVTITCKASQDVGTAVDWYQQKPGKAPKLLIYWASTRHTGVPDRFTGSGSGTDFTLTISSLQPEDFADYFCQ
QYNSYPLTFGGGTKLEIKEPKSSDKTHTCPPCPPCGGGSSGGGSGGGSGQPREPQVYTLPPSREEMTKNQVSLTC
LVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSL
SLSPG **(SEQ ID NO: 11)**

IAB2M γ1 EH8 (M2) (SEQ ID NO: 12)

EVQLVQSGAEVKKPGASVKISCKTSGYTFTEYTIHWVKQASGKGLEWIGNINPNNGGTTYNQKFEDRATLTVDKS
TSTAYMELSSLRSEDTAVYYCAAGWNFDYWGQGTTVTVSSGSTSGGGSGGGSGGGSSDIVMTQSPSSLSASV
GDRVTITCKASQDVGTAVDWYQQKPGKAPKLLIYWASTRHTGVPDRFTGSGSGTDFTLTISSLQPEDFADYFCQ
QYNSYPLTFGGGTKLEIKELKTPLGDTTHTCPPCPPCPGGGSSGGGSGQPREPQVYTLPPSREEMTKNQVSL
TCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQK
SLSLSPG **(SEQ ID NO: 12)**

FIG. 35C

VH and VL domains

IAB2M variable light (VL) and heavy (VH) sequences

VL
DIVMTQSPSSLSASVGDRVTITCKASQDVGTAVDWYQQKPGKAPKLLIYWASTRH
TGVPDRFTGSGSGTDFTLTISSLQPEDFADYFCQQYNSYPLTFGGGTKLEIK
(SEQ ID NO: 13)

VH
EVQLVQSGAEVKKPGASVKISCKTSGYTFTEYTIHWVKQASGKGLEWIGNINPNN
GGTTYNQKFEDRATLTVDKSTSTAYMELSSLRSEDTAVYYCAAGWNFDYWGQGTT
VTVSS **(SEQ ID NO: 14)**

FIG. 36A

IAB22M variable light (VL) and heavy (VH) sequences

VL
DVQITQSPSSLSASVGDRVTITCRTSRSISQYLAWYQQKPGKVPKLLIYSGSTL
QSGVPSRFSGSGSGTDFTLTISSLQPEDVATYYCQQHNENPLTFGGGTKVEIK
(SEQ ID NO: 15)

VH
EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHFVRQAPGKGLEWIGRIDPA
NDNTLYASKFQGKATISADTSKNTAYLQMNSLRAEDTAVYYCGRGYGYYVFDHW
GQGTLVTVSS **(SEQ ID NO: 16)**

FIG. 36B

IAB20M variable light (VL) and heavy (VH) sequences

VL
DIVMTQSPATLSVSPGERATLSCKASQSVSNDVAWYQQKPGQAPRLLISYTSSR
YAGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQDYNSPPTFGQGTKVEIK
(SEQ ID NO: 17)

VH
QVQLVQSGAEVKKPGASVKVSCKASGYSFTGYYMHWVRQAPGQGLEWIGRINPN
NGVTLYNQKFKDRVTMTVDTSISTAYMELSRLRSDDTAVYYCARSTMITNYVMD
YWGQGTLVTVSS **(SEQ ID NO: 18)**

FIG. 36C

Anti-CD3 variable light (VL) and heavy (VH) sequences

VL
EIVLTQSPATLSLSPGERATLSCSASSSVSYMNWYQQKPGQAPRLLIYDTSKLA
SGVPAHFRGSGSGTDFTLTISSLEPEDFAVYYCQQWSSNPFTFGQGTKVEIK
(SEQ ID NO: 19)

VH
QVQLVQSGAEVKKPGASVKVSCKASGYTFTRYTMHWVRQAPGQGLEWMGYINPS
RGYTNYNQKFKDRVTMTTDTSISTAYMELSRLRSDDTAVYYCARYYDDHYSLDY
WGQGTLVTVSS **(SEQ ID NO: 20)**

FIG. 36D

IAB1M (A11) variable light (VL) and heavy (VH) sequences

VL
DIQLTQSPSTLSASVGDRVTITCSASSSVRFIHWYQQKPGKAPKRLIYDTSKLA
SGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQWGSSPFTFGQGTKVEIK
(SEQ ID NO: 67)

VH
EVQLVESGGGLVQPGGSLRLSCAASGFNIKDYYIHWVRQAPGKGLEWVAWIDPE
NGDTEFVPKFQGRATMSADTSKNTAYLQMNSLRAEDTAVYYCKTGGFWGQGTLV
TVSS **(SEQ ID NO: 68)**

FIG. 36E

Linker sequences:

GGGGS	5 aa	(SEQ ID NO: 66)
GGGGSGGGGS	10 aa	(SEQ ID NO: 65)
GGGGSGGGGSGGGGS	15 aa	(SEQ ID NO: 64)
GSTSGGGSGGGS	12 aa	(SEQ ID NO: 63)
GSTSGGGSGGGSGGGGSS	18 aa	(SEQ ID NO: 62)

FIG. 37

Full hinge:	Upper	Core	Lower
Human IgG1 NH1 (SEQ ID NO: 21)	EPKSCDKTHT (SEQ ID NO: 45)	CPPCP (SEQ ID NO: 51)	APELLGGP (SEQ ID NO: 58)
Human IgG1 EH1 (SEQ ID NO: 22)	EPKSCDKTHT (SEQ ID NO: 45)	CPPC (SEQ ID NO: 50)	GGGSSGGGSG (SEQ ID NO: 59)
Human IgG1 NH2 (SEQ ID NO: 23)	EPKSSDKTHT (SEQ ID NO: 46)	CPPCP (SEQ ID NO: 51)	APELLGGP (SEQ ID NO: 58)
Human IgG1 EH2 (SEQ ID NO: 24)	EPKSSDKTHT (SEQ ID NO: 46)	CPPC (SEQ ID NO: 50)	GGGSSGGGSG (SEQ ID NO: 59)
Human IgG1 NH3 (SEQ ID NO: 25)	EPKSSDKTHT (SEQ ID NO: 46)	CPPCPPC (SEQ ID NO: 52)	APELLGGP (SEQ ID NO: 58)
Human IgG1 EH3 (SEQ ID NO: 26)	EPKSSDKTHT (SEQ ID NO: 46)	CPPCPPC (SEQ ID NO: 52)	GGGSSGGGSG (SEQ ID NO: 59)
Human IgG1 NH4 (SEQ ID NO: 27)	EPKSSDKTHT (SEQ ID NO: 46)	CPPCVECPPC (SEQ ID NO: 53)	APELLGGP (SEQ ID NO: 58)
Human IgG1 EH4 (SEQ ID NO: 28)	EPKSSDKTHT (SEQ ID NO: 46)	CPPCVECPPC (SEQ ID NO: 53)	GGGSSGGGSG (SEQ ID NO: 59)
Human IgG1 NH5 (SEQ ID NO: 29)	EPKSSDKTHT (SEQ ID NO: 46)	CPPCPPCPPC (SEQ ID NO: 54)	APELLGGP (SEQ ID NO: 58)
Human IgG1 EH5 (SEQ ID NO: 30)	EPKSSDKTHT (SEQ ID NO: 46)	CPPCPPCPPC (SEQ ID NO: 54)	GGGSSGGGSG (SEQ ID NO: 59)

FIG. 38

Full hinge:	Upper	Core	Lower
Human IgG2 NH1 (SEQ ID NO: 31)	ERK (SEQ ID NO: 47)	CCVECPPCP (SEQ ID NO: 55)	APPVAGP (SEQ ID NO: 60)
Human IgG2 EH1 (SEQ ID NO: 32)	ERK (SEQ ID NO: 47)	CCVECPPCP (SEQ ID NO: 55)	GGGSSGGGSG (SEQ ID NO: 59)
Human IgG2 NH2 (SEQ ID NO: 33)	ERK (SEQ ID NO: 47)	SCVECPPCP (SEQ ID NO: 56)	APPVAGP (SEQ ID NO: 60)
Human IgG2 EH2 (SEQ ID NO: 34)	ERK (SEQ ID NO: 47)	SCVECPPCP (SEQ ID NO: 56)	GGGSSGGGSG (SEQ ID NO: 59)
IgG3/IgG1 EH6 (SEQ ID NO: 35)	ELKTPLGDTTHT (SEQ ID NO: 48)	CVECPPC (SEQ ID NO: 57)	GGGSSGGGSG (SEQ ID NO: 59)
IgG3/IgG1 EH7 (SEQ ID NO: 36)	ELKTPLGDTTHT (SEQ ID NO: 48)	CPPCPPC (SEQ ID NO: 52)	GGGSSGGGSG (SEQ ID NO: 59)
IgG3/IgG1 EH8 (SEQ ID NO: 37)	ELKTPLGDTTHT (SEQ ID NO: 48)	CPPCPPCPPC (SEQ ID NO: 54)	GGGSSGGGSG (SEQ ID NO: 59)
IgG4 NH (SEQ ID NO: 38)	ESKYGPP (SEQ ID NO: 49)	CPPCP (SEQ ID NO: 51)	APEFLGGP (SEQ ID NO: 61)
IgG4 EH (SEQ ID NO: 39)	ESKYGPP (SEQ ID NO: 49)	CPPCP (SEQ ID NO: 51)	GGGSSGGGSG (SEQ ID NO: 59)

FIG. 38 continued

CH3 domains:

Human IgG1 CH3 (G1m1 allotype)
GQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVD
KSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 40)

Human IgG1 CH3 (nG1m1 allotype)
GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVD
KSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 41)

Human IgG2 CH3
GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVD
KSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 42)

Human IgG3 CH3
GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESSGQPENNYNTTPPMLDSDGSFFLYSKLTVD
KSRWQQGNIFSCSVMHEALHNRFTQKSLSLSPGK (SEQ ID NO: 43)

Human IgG4 CH3
GQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVD
KSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK (SEQ ID NO: 44)

FIG. 39

Alignment compared IAB22M γ1 EH1(M1) (SEQ ID NO: 105) vs IAB22M γ1 EH3(M1) (SEQ ID NO: 106), differences shown in box.

```
IAB22M γ1 EH1(M1) METDTLLLWVLLLWVPGSTGDVQITQSPSSLSASVGDRVTITCRTSRSISQYLAWYQQKP 60

IAB22M γ1 EH3(M1) METDTLLLWVLLLWVPGSTGDVQITQSPSSLSASVGDRVTITCRTSRSISQYLAWYQQKP 60

IAB22M γ1 EH1(M1) GKVPKLLIYSGSTLQSGVPSRFSGSGSGTDFTLTISSLQPEDVATYYCQQHNENPLTFGG 120

IAB22M γ1 EH3(M1) GKVPKLLIYSGSTLQSGVPSRFSGSGSGTDFTLTISSLQPEDVATYYCQQHNENPLTFGG 120

IAB22M γ1 EH1(M1) GTKVEIKGSTSGGGSGGGSGGGSSEVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIH 180

IAB22M γ1 EH3(M1) GTKVEIKGSTSGGGSGGGSGGGSSEVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIH 180

IAB22M γ1 EH1(M1) FVRQAPGKGLEWIGRIDPANDNTLYASKFQGKATISADTSKNTAYLQMNSLRAEDTAVYY 240

IAB22M γ1 EH3(M1) FVRQAPGKGLEWIGRIDPANDNTLYASKFQGKATISADTSKNTAYLQMNSLRAEDTAVYY 240

IAB22M γ1 EH1(M1) CGRGYGYYVFDHWGQGTLVTVSSEPKS[C DKTHTCP ---] PCGGGSSGGGSGGQPREPQVYT 297

IAB22M γ1 EH3(M1) CGRGYGYYVFDHWGQGTLVTVSSEPKS[S DKTHTCP PCP] PCGGGSSGGGSGGQPREPQVYT 300

IAB22M γ1 EH1(M1) LPPSR[DEL] TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKL 357

IAB22M γ1 EH3(M1) LPPSR[EEM] TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKL 360

IAB22M γ1 EH1(M1) TVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG[K] 394 (SEQ ID NO: 105)

IAB22M γ1 EH3(M1) TVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG[-] 396 (SEQ ID NO: 106)
```

FIG. 40

IAB22M γ1 EH3(M1), CDR regions are defined by Chothia definition (SEQ ID NO: 106)

atggagaccgataccctgctgctgtgggtgctgctgctgtgggtgcccggctccacaggc

M E T D T L L L W V L L L W V P G S T G

Signal peptide sequence

gatgtgcagatcacacagagccctagcagcctgagcgccagcgtgggagatagagtcacc

D V Q I T Q S P S S L S A S V G D R V T

VL

Atcacatgc aggaccagcagaagcattagccagtacctggcc tggtaccagcaaaagccc

I T C R T S R S I S Q Y L A W Y Q Q K P

LCDR1

Ggcaaggtgcccaagctgctgatctac agcggctccaccctgcagagc ggcgtgcccagc

G K V P K L L I Y S G S T L Q S G V P S

LCDR2

agattctccggctccggaagcggcacagactttaccctgaccatctcctccctgcagccc

R F S G S G S G T D F T L T I S S L Q P

gaggatgtcgccacctactactgc cagcagcacaacgaaaaccccctgaca tttggcggc

E D V A T Y Y C Q Q H N E N P L T F G G

LCDR3

ggcaccaaggtggagatcaag ggcagcaccagcggtggaggaagtggaggtggaagtgga

G T K V E I K G S T S G G G S G G G S G

Linker

Ggaggcggaagcagc gaggtgcagctggtggagagtggtggaggactggtgcagcccgga

G G G S S E V Q L V E S G G G L V Q P G

Linker **VH**

Ggcagcctgagactgagctgtgctgcctcc ggattcaatatcaaggacacc tacatccac

G S L R L S C A A S G F N I K D T Y I H

HCDR1

ttcgtgagacaggcccccggcaagggactggagtggattggaaggatc gaccccgccaac

F V R Q A P G K G L E W I G R I D P A N

HCDR2

gacaac accctgtacgccagcaaattccagggcaaggccacaatcagcgccgacaccagc

D N T L Y A S K F Q G K A T I S A D T S

HCDR2

aagaacaccgcctatctgcagatgaactccctgagagccgaggacacagccgtgtactac

K N T A Y L Q M N S L R A E D T A V Y Y

FIG. 41

tgcggcagg ggctacggctattacgtgttcgaccac tggggccagggcaccctggtgaca

C G R G Y G Y Y V F D H W G Q G T L V T

HCDR3

gtgagcagc gaacccaagagctccgacaagacccacacc tgtccccttgccctccttgt

V S S E P K S S D K T H T C P P C P P C

Upper hinge Core hinge

ggcggaggaagctccggaggcggaagcggag gacagcctagggagccccaggtgtatacc

G G G S S G G G S G G Q P R E P Q V Y T

Lower hinge CH3

ctccccccctccagggaagagatgaccaagaaccaggtgagcctgacctgcctcgtgaag

L P P S R E E M T K N Q V S L T C L V K

ggcttttatccctccgatatcgccgtggagtgggagagcaacggccagcctgagaacaat

G F Y P S D I A V E W E S N G Q P E N N

tacaagaccacccccctgtgctggactccgatggcagcttcttcctgtattccaagctg

Y K T T P P V L D S D G S F F L Y S K L

accgtcgacaagtccaggtggcaacagggcaacgtcttcagctgcagcgtgatgcacgag

T V D K S R W Q Q G N V F S C S V M H E

gccctgcacaatcactacacccagaagtccctctccctgagccccggctga **(SEQ ID NO: 140)**

A L H N H Y T Q K S L S L S P G Stop **(SEQ ID NO: 106)**

FIG. 41
continued

IAB22M γ1 EH5(M1) (SEQ ID NO: 107)

atggagacagacaccctcctcctgtgggtgctgctgctgtgggtgcccggatccaccgga

M E T D T L L L W V L L L W V P G S T G

gacgtgcagatcacacagagccccagctccctgtccgctagcgtgggcgacagagtgacc

D V Q I T Q S P S S L S A S V G D R V T

atcacctgcaggaccagcaggagcatctcccagtacctcgcttggtaccagcagaagcct

I T C R T S R S I S Q Y L A W Y Q Q K P

ggcaaggtgcccaagctgctgatttacagcggatccaccctgcagagcggcgtgcctagc

G K V P K L L I Y S G S T L Q S G V P S

aggtttagcggcagcggatccggaacagacttcaccctgaccatcagcagcctgcagcct

R F S G S G S G T D F T L T I S S L Q P

gaagatgtggccacctactactgtcagcagcacaacgaaaaccccctcaccttcggcggc

E D V A T Y Y C Q Q H N E N P L T F G G

ggcacaaaggtggaaatcaagggcagcacctccggaggaggcagcggcggaggcagcgga

G T K V E I K G S T S G G G S G G G S G

ggcggcggctccagcgaagtgcagctggtcgagagcggaggcggactggtgcaacccgga

G G G S S E V Q L V E S G G G L V Q P G

ggaagcctgaggctgagctgtgccgccagcggcttcaacatcaaggacacatacattcac

G S L R L S C A A S G F N I K D T Y I H

tttgtgaggcaggctcctggaaagggcctggagtggatcggcagaatcgaccccgctaac

F V R Q A P G K G L E W I G R I D P A N

gacaacaccctgtacgccagcaagttccagggcaaggccaccatctccgccgacacaagc

D N T L Y A S K F Q G K A T I S A D T S

aagaataccgcctacctgcagatgaactccctgagggccgaggataccgccgtgtactac

K N T A Y L Q M N S L R A E D T A V Y Y

tgcggcaggggctatggctactacgtgtttgaccactggggccagggcacactggtgaca

C G R G Y G Y Y V F D H W G Q G T L V T

gtgagctccgagcccaagagctccgacaagacacacacctgccctccttgccccccttgt

V S S E P K S S D K T H T C P P C P P C

cctccctgtggaggaggaagcagcggaggaggaagcggcggacagcccagagagcctcaa

P P C G G G S S G G G S G G Q P R E P Q

gtgtataccctgccccccctccagggaagagatgaccaagaaccaggtgagcctgacatgc

V Y T L P P S R E E M T K N Q V S L T C

ctggtcaaaggcttctaccccagcgatattgctgtggagtgggagagcaacggccagccc

L V K G F Y P S D I A V E W E S N G Q P

gagaacaactacaagaccacacccccgtcctggatagcgatggcagcttcttcctgtac

FIG. 42

E N N Y K T T P P V L D S D G S F F L Y

agcaagctgaccgtggacaagtccaggtggcagcagggcaacgtcttctcctgcagcgtg

S K L T V D K S R W Q Q G N V F S C S V

atgcacgaggctctgcataaccactacacacagaagtccctcagcctgagccctggatga **(SEQ ID NO: 141)**

M H E A L H N H Y T Q K S L S L S P G Stop **(SEQ ID NO: 107)**

FIG. 42
continued

IAB22M γ1 EH7(M1) (SEQ ID NO: 108)

```
atggagacagacaccctcctcctgtgggtgctgctgctgtgggtgcccggatccaccgga
 M  E  T  D  T  L  L  L  W  V  L  L  L  W  V  P  G  S  T  G
gacgtgcagatcacacagagccccagctccctgtccgctagcgtgggcgacagagtgacc
 D  V  Q  I  T  Q  S  P  S  S  L  S  A  S  V  G  D  R  V  T
atcacctgcaggaccagcaggagcatctcccagtacctcgcttggtaccagcagaagcct
 I  T  C  R  T  S  R  S  I  S  Q  Y  L  A  W  Y  Q  Q  K  P
ggcaaggtgcccaagctgctgatttacagcggatccaccctgcagagcggcgtgcctagc
 G  K  V  P  K  L  L  I  Y  S  G  S  T  L  Q  S  G  V  P  S
aggtttagcggcagcggatccggaacagacttcaccctgaccatcagcagcctgcagcct
 R  F  S  G  S  G  S  G  T  D  F  T  L  T  I  S  S  L  Q  P
gaagatgtggccacctactactgtcagcagcacaacgaaaaccccctcaccttcggcggc
 E  D  V  A  T  Y  Y  C  Q  Q  H  N  E  N  P  L  T  F  G  G
ggcacaaaggtggaaatcaagggcagcacctccggaggaggcagcggcggaggcagcgga
 G  T  K  V  E  I  K  G  S  T  S  G  G  G  S  G  G  G  S  G
ggcggcggctccagcgaagtgcagctggtcgagagcggaggcggactggtgcaacccgga
 G  G  G  S  S  E  V  Q  L  V  E  S  G  G  G  L  V  Q  P  G
ggaagcctgaggctgagctgtgccgccagcggcttcaacatcaaggacacatacattcac
 G  S  L  R  L  S  C  A  A  S  G  F  N  I  K  D  T  Y  I  H
tttgtgaggcaggctcctggaaagggcctggagtggatcggcagaatcgaccccgctaac
 F  V  R  Q  A  P  G  K  G  L  E  W  I  G  R  I  D  P  A  N
gacaacaccctgtacgccagcaagttccagggcaaggccaccatctccgccgacacaagc
 D  N  T  L  Y  A  S  K  F  Q  G  K  A  T  I  S  A  D  T  S
aagaataccgcctacctgcagatgaactccctgagggccgaggataccgccgtgtactac
 K  N  T  A  Y  L  Q  M  N  S  L  R  A  E  D  T  A  V  Y  Y
tgcggcaggggctatggctactacgtgtttgaccactggggccagggcacactggtgaca
 C  G  R  G  Y  G  Y  Y  V  F  D  H  W  G  Q  G  T  L  V  T
gtgagctccgagctgaagacacctctgggcgacacaacacacacctgccccccttgtcct
 V  S  S  E  L  K  T  P  L  G  D  T  T  H  T  C  P  P  C  P
ccctgtggaggaggaagcagcggaggaggaagcggcggacagcccagagagcctcaagtg
 P  C  G  G  G  S  S  G  G  G  S  G  G  Q  P  R  E  P  Q  V
tataccctgccccccctccagggaagagatgaccaagaaccaggtgagcctgacatgcctg
 Y  T  L  P  P  S  R  E  E  M  T  K  N  Q  V  S  L  T  C  L
gtcaaaggcttctaccccagcgatattgctgtggagtgggagagcaacggccagcccgag
 V  K  G  F  Y  P  S  D  I  A  V  E  W  E  S  N  G  Q  P  E
aacaactacaagaccacacccccgtcctggatagcgatggcagcttcttcctgtacagc
 N  N  Y  K  T  T  P  P  V  L  D  S  D  G  S  F  F  L  Y  S
```

FIG. 43

aagctgaccgtggacaagtccaggtggcagcagggcaacgtcttctcctgcagcgtgatg

K L T V D K S R W Q Q G N V F S C S V M

cacgaggctctgcataaccactacacacagaagtccctcagcctgagccctggatga **(SEQ ID NO: 142)**

H E A L H N H Y T Q K S L S L S P G Stop **(SEQ ID NO: 108)**

FIG. 43
continued

IAB22M γ1 EH8(M1) (SEQ ID NO: 109)

```
atggagacagacaccctcctcctgtgggtgctgctgctgtgggtgcccggatccaccgga
 M  E  T  D  T  L  L  L  W  V  L  L  L  W  V  P  G  S  T  G
gacgtgcagatcacacagagccccagctccctgtccgctagcgtgggcgacagagtgacc
 D  V  Q  I  T  Q  S  P  S  S  L  S  A  S  V  G  D  R  V  T
atcacctgcaggaccagcaggagcatctcccagtacctcgcttggtaccagcagaagcct
 I  T  C  R  T  S  R  S  I  S  Q  Y  L  A  W  Y  Q  Q  K  P
ggcaaggtgcccaagctgctgatttacagcggatccaccctgcagagcggcgtgcctagc
 G  K  V  P  K  L  L  I  Y  S  G  S  T  L  Q  S  G  V  P  S
aggtttagcggcagcggatccggaacagacttcaccctgaccatcagcagcctgcagcct
 R  F  S  G  S  G  S  G  T  D  F  T  L  T  I  S  S  L  Q  P
gaagatgtggccacctactactgtcagcagcacaacgaaaacccccctcaccttcggcggc
 E  D  V  A  T  Y  Y  C  Q  Q  H  N  E  N  P  L  T  F  G  G
ggcacaaaggtggaaatcaagggcagcacctccggaggaggcagcggcggaggcagcgga
 G  T  K  V  E  I  K  G  S  T  S  G  G  G  S  G  G  G  S  G
ggcggcggctccagcgaagtgcagctggtcgagagcggaggcggactggtgcaacccgga
 G  G  G  S  S  E  V  Q  L  V  E  S  G  G  G  L  V  Q  P  G
ggaagcctgaggctgagctgtgccgccagcggcttcaacatcaaggacacatacattcac
 G  S  L  R  L  S  C  A  A  S  G  F  N  I  K  D  T  Y  I  H
tttgtgaggcaggctcctggaaagggcctggagtggatcggcagaatcgaccccgctaac
 F  V  R  Q  A  P  G  K  G  L  E  W  I  G  R  I  D  P  A  N
gacaacaccctgtacgccagcaagttccagggcaaggccaccatctccgccgacacaagc
 D  N  T  L  Y  A  S  K  F  Q  G  K  A  T  I  S  A  D  T  S
aagaataccgcctacctgcagatgaactccctgagggccgaggataccgccgtgtactac
 K  N  T  A  Y  L  Q  M  N  S  L  R  A  E  D  T  A  V  Y  Y
tgcggcaggggctatggctactacgtgtttgaccactggggccagggcacactggtgaca
 C  G  R  G  Y  G  Y  Y  V  F  D  H  W  G  Q  G  T  L  V  T
gtgagctccgagctgaagacacctctgggcgacacaacacacacctgccctccttgcccc
 V  S  S  E  L  K  T  P  L  G  D  T  T  H  T  C  P  P  C  P
ccttgtcctccctgtggaggaggaagcagcggaggaggaagcggcggacagcccagagag
 P  C  P  P  C  G  G  G  S  S  G  G  G  S  G  G  Q  P  R  E
cctcaagtgtataccctgccccccctccagggaagagatgaccaagaaccaggtgagcctg
 P  Q  V  Y  T  L  P  P  S  R  E  E  M  T  K  N  Q  V  S  L
acatgcctggtcaaaggcttctaccccagcgatattgctgtggagtgggagagcaacggc
 T  C  L  V  K  G  F  Y  P  S  D  I  A  V  E  W  E  S  N  G
cagcccgagaacaactacaagaccacacccccgtcctggatagcgatggcagcttcttc
 Q  P  E  N  N  Y  K  T  T  P  P  V  L  D  S  D  G  S  F  F
```

FIG. 44

ctgtacagcaagctgaccgtggacaagtccaggtggcagcagggcaacgtcttctcctgc

L Y S K L T V D K S R W Q Q G N V F S C

agcgtgatgcacgaggctctgcataaccactacacacagaagtccctcagcctgagccct

S V M H E A L H N H Y T Q K S L S L S P

ggatga **(SEQ ID NO: 143)**

G Stop **(SEQ ID NO: 109)**

FIG. 44
continued

IAB22M γ2 EH2(M1) (SEQ ID NO: 110)

```
atggaaaccgacacactgctgctgtgggtgctgctgctgtgggtccctggctccaccgga
 M  E  T  D  T  L  L  L  W  V  L  L  L  W  V  P  G  S  T  G
gacgtccagatcacacagagccccagcagcctgtccgccagcgtgggagacagggtgacc
 D  V  Q  I  T  Q  S  P  S  S  L  S  A  S  V  G  D  R  V  T
atcacctgcaggaccagcagatccatctcccagtatctggcctggtaccaacagaagccc
 I  T  C  R  T  S  R  S  I  S  Q  Y  L  A  W  Y  Q  Q  K  P
ggcaaggtccccaaactgctgatctacagcggctccaccctgcagtccggcgtgcctagc
 G  K  V  P  K  L  L  I  Y  S  G  S  T  L  Q  S  G  V  P  S
aggttctccggcagcggatccggcaccgacttcaccctgaccatcagctccctgcagcct
 R  F  S  G  S  G  S  G  T  D  F  T  L  T  I  S  S  L  Q  P
gaggacgtggctacctactactgccaacagcacaacgagaaccccctgacctttggaggc
 E  D  V  A  T  Y  Y  C  Q  Q  H  N  E  N  P  L  T  F  G  G
ggcaccaaggtggaaatcaagggcagcaccagcggcggaggaagcggaggaggatccgga
 G  T  K  V  E  I  K  G  S  T  S  G  G  G  S  G  G  G  S  G
ggaggcggaagctccgaggtgcagctggtggaaagcggcggcggactggtgcagcctgga
 G  G  G  S  S  E  V  Q  L  V  E  S  G  G  G  L  V  Q  P  G
ggaagcctcagactgagctgtgccgccagcggattcaacatcaaagacacctacattcat
 G  S  L  R  L  S  C  A  A  S  G  F  N  I  K  D  T  Y  I  H
ttcgtgagacaggcccccggcaagggcctcgaatggatcggaaggatcgaccccgctaac
 F  V  R  Q  A  P  G  K  G  L  E  W  I  G  R  I  D  P  A  N
gacaataccctgtacgcctccaagttccagggaaaggccaccatctccgccgatacctcc
 D  N  T  L  Y  A  S  K  F  Q  G  K  A  T  I  S  A  D  T  S
aagaacaccgcctacctccagatgaactccctgagggccgaagataccgccgtctactac
 K  N  T  A  Y  L  Q  M  N  S  L  R  A  E  D  T  A  V  Y  Y
tgtggcaggggctacggctactatgtgttcgatcactggggccaaggaaccctggtgacc
 C  G  R  G  Y  G  Y  Y  V  F  D  H  W  G  Q  G  T  L  V  T
gtgagcagcgaaaggaagagctgcgtggagtgtcctccttgtcccggcggcggctccagc
 V  S  S  E  R  K  S  C  V  E  C  P  P  C  P  G  G  G  S  S
ggcggaggctccggcggccagcctagagaacctcaggtgtacaccctcccccccctccaga
 G  G  G  S  G  G  Q  P  R  E  P  Q  V  Y  T  L  P  P  S  R
gaggagatgaccaagaaccaggtgtccctgacctgcctggtgaaaggcttctatcccagc
 E  E  M  T  K  N  Q  V  S  L  T  C  L  V  K  G  F  Y  P  S
gacatcgccgtggaatgggagtccaacggccagcccgagaacaactacaagaccacccct
 D  I  A  V  E  W  E  S  N  G  Q  P  E  N  N  Y  K  T  T  P
cccatgctggattccgacggcagctttttcctgtacagcaagctcaccgtggacaagagc
 P  M  L  D  S  D  G  S  F  F  L  Y  S  K  L  T  V  D  K  S
```

FIG. 45

agatggcagcagggcaacgtgttcagctgcagcgtgatgcacgaggctctgcataaccac

R W Q Q G N V F S C S V M H E A L H N H

tacacccagaagagcctgtccctgtcccccggatga **(SEQ ID NO: 144)**

Y T Q K S L S L S P G Stop **(SEQ ID NO: 110)**

FIG. 45
continued

IAB22M γ2 EH2(M1)(VH-K67R) (SEQ ID NO: 111)

```
atggagaccgacaccctcctcctgtgggtgctgctgctgtgggtgcctggaagcaccggc
 M  E  T  D  T  L  L  L  W  V  L  L  L  W  V  P  G  S  T  G
gatgtgcagatcacccagagccctagcagcctgtccgcttccgtgggcgacagggtgacc
 D  V  Q  I  T  Q  S  P  S  S  L  S  A  S  V  G  D  R  V  T
atcacctgtaggacctccaggagcatctcccagtacctggcctggtaccagcagaagccc
 I  T  C  R  T  S  R  S  I  S  Q  Y  L  A  W  Y  Q  Q  K  P
ggcaaggtgcccaaactgctcatctactccggcagcacactccagagcggcgtccctagc
 G  K  V  P  K  L  L  I  Y  S  G  S  T  L  Q  S  G  V  P  S
agattcagcggaagcggcagcggcaccgacttcaccctgaccatcagctccctgcagccc
 R  F  S  G  S  G  S  G  T  D  F  T  L  T  I  S  S  L  Q  P
gaggacgtggccacctattactgtcagcagcacaacgaaaaccccctgaccttcggcggc
 E  D  V  A  T  Y  Y  C  Q  Q  H  N  E  N  P  L  T  F  G  G
ggcacaaaagtggagatcaagggcagcaccagcggaggcggatccggcggcggcagcggc
 G  T  K  V  E  I  K  G  S  T  S  G  G  G  S  G  G  G  S  G
ggcggaggatccagcgaagtgcagctggtcgaaagcggaggcggactggtgcagcctgga
 G  G  G  S  S  E  V  Q  L  V  E  S  G  G  G  L  V  Q  P  G
ggaagcctgagactcagctgcgccgcctccggattcaacatcaaggacacctacatccac
 G  S  L  R  L  S  C  A  A  S  G  F  N  I  K  D  T  Y  I  H
ttcgtgaggcaggctcccggcaaaggcctcgagtggattggaaggattgaccccgccaac
 F  V  R  Q  A  P  G  K  G  L  E  W  I  G  R  I  D  P  A  N
gacaacaccctgtacgccagcaagttccaaggaagggccaccatctccgccgacaccagc
 D  N  T  L  Y  A  S  K  F  Q  G  R  A  T  I  S  A  D  T  S
aagaataccgcctacctgcagatgaactccctgagggctgaggacaccgccgtgtactac
 K  N  T  A  Y  L  Q  M  N  S  L  R  A  E  D  T  A  V  Y  Y
tgcggcagaggctacggctactacgtgttcgaccactggggacagggcacactggtgaca
 C  G  R  G  Y  G  Y  Y  V  F  D  H  W  G  Q  G  T  L  V  T
gtgagcagcgagaggaaaagctgcgtggagtgccccccctgccctggcggcggcagctcc
 V  S  S  E  R  K  S  C  V  E  C  P  P  C  P  G  G  G  S  S
ggcggaggaagcggaggacaacccagggagccccaggtgtacacactccccctagcagg
 G  G  G  S  G  G  Q  P  R  E  P  Q  V  Y  T  L  P  P  S  R
gaagagatgaccaagaaccaggtgtccctgacctgcctcgtgaagggattctaccccagc
 E  E  M  T  K  N  Q  V  S  L  T  C  L  V  K  G  F  Y  P  S
gacattgccgtcgagtgggagagcaacggccagcctgagaacaactacaagacaaccccc
 D  I  A  V  E  W  E  S  N  G  Q  P  E  N  N  Y  K  T  T  P
cctatgctcgatagcgatggctccttcttcctgtactccaagctcaccgtcgacaagagc
 P  M  L  D  S  D  G  S  F  F  L  Y  S  K  L  T  V  D  K  S
```

FIG. 46

aggtggcagcagggcaacgtcttctcctgtagcgtgatgcacgaggctctgcacaaccac

R W Q Q G N V F S C S V M H E A L H N H

tacacccagaagagcctgagcctgagccccggctga **(SEQ ID NO: 145)**

Y T Q K S L S L S P G Stop **(SEQ ID NO: 111)**

FIG. 46
continued

Alignment compared IAB2M γ1 EH1(M2) (SEQ ID NO: 112) vs IAB2M γ1 EH3(M2) (SEQ ID NO: 113), differences shown in box.

```
IAB2M γ1 EH1(M2)  METDTLLLWVLLLWVPGSTGEVQLVQSGAEVKKPGASVKISCKTSGYTFTEYTIHWVKQA 60

IAB2M γ1 EH3(M2)  METDTLLLWVLLLWVPGSTGEVQLVQSGAEVKKPGASVKISCKTSGYTFTEYTIHWVKQA 60

IAB2M γ1 EH1(M2)  SGKGLEWIGNINPNNGGTTYNQKFEDRATLTVDKSTSTAYMELSSLRSEDTAVYYCAAGW 120

IAB2M γ1 EH3(M2)  SGKGLEWIGNINPNNGGTTYNQKFEDRATLTVDKSTSTAYMELSSLRSEDTAVYYCAAGW 120

IAB2M γ1 EH1(M2)  NFDYWGQGTTVTVSSGSTSGGSGGGSGGGGSSDIVMTQSPSSLSASVGDRVTITCKASQ 180

IAB2M γ1 EH3(M2)  NFDYWGQGTTVTVSSGSTSGGSGGGSGGGGSSDIVMTQSPSSLSASVGDRVTITCKASQ 180

IAB2M γ1 EH1(M2)  DVGTAVDWYQQKPGKAPKLLIYWASTRHTGVPDRFTGSGSGTDFTLTISSLQPEDFADYF 240

IAB2M γ1 EH3(M2)  DVGTAVDWYQQKPGKAPKLLIYWASTRHTGVPDRFTGSGSGTDFTLTISSLQPEDFADYF 240

IAB2M γ1 EH1(M2)  CQQYNSYPLTFGGGTKLEIKEPKS[C]DKTHTCP[---]PCGGGSSGGGSGGGQPREPQVYTLPP 297

IAB2M γ1 EH3(M2)  CQQYNSYPLTFGGGTKLEIKEPKS[S]DKTHTCP[PCP]PCGGGSSGGGSGGGQPREPQVYTLPP 300

IAB2M γ1 EH1(M2)  SR[DEL]TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVD 357

IAB2M γ1 EH3(M2)  SR[EEM]TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVD 360

IAB2M γ1 EH1(M2)  KSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG[K] 391 (SEQ ID NO: 112)

IAB2M γ1 EH3(M2)  KSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG[_] 393 (SEQ ID NO: 113)
```

FIG. 47

IAB2M γ1 EH3(M2), CDR regions are defined by Chothia definition (SEQ ID NO: 113)

atggaaaccgacaccctgctgctgtgggtgctgctgctgtgggtgccaggatctacaggc

M E T D T L L L W V L L L W V P G S T G

Signal peptide sequence

gaggtgcagctggtgcagtctggcgccgaagtgaagaaacctggcgcctccgtgaagatc

E V Q L V Q S G A E V K K P G A S V K I

VH

tcctgcaagacctcc ggctacaccttcaccgagtac accatccactgggtcaagcaggcc

S C K T S G Y T F T E Y T I H W V K Q A

HCDR1

tccggcaagggcctggaatggatcggc aacatcaaccccaacaacggcggc accacctac

S G K G L E W I G N I N P N N G G T T Y

HCDR2

aaccagaagttcgaggaccgggccaccctgaccgtggacaagtctacctccaccgcctac

N Q K F E D R A T L T V D K S T S T A Y

atggaactgtcctccctgcggagcgaggacaccgccgtgtactattgtgccgct ggctgg

M E L S S L R S E D T A V Y Y C A A G W

HCDR3

aacttcgactac tggggccagggcaccaccgtgacagtgtcctct ggctctacctccggc

N F D Y W G Q G T T V T V S S G S T S G

HCDR3 **Linker**

ggaggaagtggcggaggatcaggcggaggcggctcctctg atatcgtgatgacccagtcc

G G S G G G S G G G G S S D I V M T Q S

Linker **VL**

ccctccagcctgtctgcttccgtgggcgacagagtgaccatcacatgc aaggcctcccag

P S S L S A S V G D R V T I T C K A S Q

LCDR1

gacgtgggcaccgctgtggac tggtatcagcagaagcctggcaaggcccccaagctgctg

D V G T A V D W Y Q Q K P G K A P K L L

LCDR1

atctac tgggcctctaccagacacacc ggcgtgcccgatagattcaccggctctggatcc

I Y W A S T R H T G V P D R F T G S G S

LCDR2

ggcaccgactttaccctgaccatcagctccctgcagcccgaggacttcgccgactacttc

G T D F T L T I S S L Q P E D F A D Y F

tgc cagcagtacaactcctacccccctgacc tttggcggaggcaccaagctggaaatcaaa

C Q Q Y N S Y P L T F G G G T K L E I K

FIG. 48

LCDR3

gagcccaagtcctccgacaagacccacacc tgtccccttgccctccatgt ggtggcgga

E P K S S D K T H T C P P C P P C G G G

Upper hinge **Core hinge** **Lower hinge**

agttctgggggaggttctggt ggccagcctcgggaacctcaggtgtacacactgccccct

S S G G G S G G Q P R E P Q V Y T L P P

Lower hinge **CH3**

agccgggaagagatgaccaagaaccaggtgtccctgacctgcctcgtgaagggcttctac

S R E E M T K N Q V S L T C L V K G F Y

ccctccgatatcgccgtggaatgggagtccaacggccagcccgagaacaactacaagacc

P S D I A V E W E S N G Q P E N N Y K T

acccccccctgtgctggactccgacggctcattcttcctgtactccaagctgacagtggat

T P P V L D S D G S F F L Y S K L T V D

aagtcccggtggcagcagggcaacgtgttctcctgctccgtgatgcacgaggccctgcac

K S R W Q Q G N V F S C S V M H E A L H

aaccactatacccagaagtccctgtccctgagccccggctga **(SEQ ID NO: 146)**

N H Y T Q K S L S L S P G Stop **(SEQ ID NO: 113)**

FIG. 48
continued

IAB2M γ1 EH3(M2) (G1m1 allotype) (SEQ ID NO: 114)

```
atggaaaccgataccctgctgctgtgggtgctgctgctgtgggtgccgggcagcaccggc
 M  E  T  D  T  L  L  L  W  V  L  L  L  W  V  P  G  S  T  G
gaagtgcagctggtgcagagcggcgcggaagtgaaaaaaccgggcgcgagcgtgaaaatt
 E  V  Q  L  V  Q  S  G  A  E  V  K  K  P  G  A  S  V  K  I
agctgcaaaaccagcggctatacctttaccgaatataccattcattgggtgaaacaggcg
 S  C  K  T  S  G  Y  T  F  T  E  Y  T  I  H  W  V  K  Q  A
agcggcaaaggcctggaatggattggcaacattaacccgaacaacggcggcaccacctat
 S  G  K  G  L  E  W  I  G  N  I  N  P  N  N  G  G  T  T  Y
aaccagaaatttgaagatcgcgcgaccctgaccgtggataaaagcaccagcaccgcgtat
 N  Q  K  F  E  D  R  A  T  L  T  V  D  K  S  T  S  T  A  Y
atggaactgagcagcctgcgcagcgaagataccgcggtgtattattgcgcggcgggctgg
 M  E  L  S  S  L  R  S  E  D  T  A  V  Y  Y  C  A  A  G  W
aactttgattattggggccagggcaccaccgtgaccgtgagcagcggcagcaccagcggc
 N  F  D  Y  W  G  Q  G  T  T  V  T  V  S  S  G  S  T  S  G
ggcggcagcggcggcggcagcggcggcggcggcagcagcgatattgtgatgacccagagc
 G  G  S  G  G  G  S  G  G  G  G  S  S  D  I  V  M  T  Q  S
ccgagcagcctgagcgcgagcgtgggcgatcgcgtgaccattacctgcaaagcgagccag
 P  S  S  L  S  A  S  V  G  D  R  V  T  I  T  C  K  A  S  Q
gatgtgggcaccgcggtggattggtatcagcagaaaccgggcaaagcgccgaaactgctg
 D  V  G  T  A  V  D  W  Y  Q  Q  K  P  G  K  A  P  K  L  L
atttattgggcgagcacccgccataccggcgtgccggatcgctttaccggcagcggcagc
 I  Y  W  A  S  T  R  H  T  G  V  P  D  R  F  T  G  S  G  S
ggcaccgattttaccctgaccattagcagcctgcagccggaagattttgcggattatttt
 G  T  D  F  T  L  T  I  S  S  L  Q  P  E  D  F  A  D  Y  F
tgccagcagtataacagctatccgctgacctttggcggcggcaccaaactggaaattaaa
 C  Q  Q  Y  N  S  Y  P  L  T  F  G  G  G  T  K  L  E  I  K
gaaccgaaaagcagcgataaaacccatacctgcccgccgtgcccgccgtgcggcggcggc
 E  P  K  S  S  D  K  T  H  T  C  P  P  C  P  P  C  G  G  G
agcagcggcggcggcagcggcggccagccgcgcgaaccgcaggtgtataccctgccgccg
 S  S  G  G  G  S  G  G  Q  P  R  E  P  Q  V  Y  T  L  P  P
agccgcgatgaactgaccaaaaaccaggtgagcctgacctgcctggtgaaaggcttttat
 S  R  D  E  L  T  K  N  Q  V  S  L  T  C  L  V  K  G  F  Y
ccgagcgatattgcggtggaatgggaaagcaacggccagccggaaaacaactataaaacc
 P  S  D  I  A  V  E  W  E  S  N  G  Q  P  E  N  N  Y  K  T
accccgccggtgctggatagcgatggcagcttttttctgtatagcaaactgaccgtggat
 T  P  P  V  L  D  S  D  G  S  F  F  L  Y  S  K  L  T  V  D
```

FIG. 49

aaaagccgctggcagcagggcaacgtgtttagctgcagcgtgatgcatgaagcgctgcat

K S R W Q Q G N V F S C S V M H E A L H

aaccattatacccagaaaagcctgagcctgagcccgggctga **(SEQ ID NO: 147)**

N H Y T Q K S L S L S P G Stop **(SEQ ID NO: 114)**

FIG. 49
continued

IAB2M γ1 EH5(M2) (SEQ ID NO: 115)

```
atggaaaccgataccctgctgctgtgggtgctgctgctgtgggtgccgggcagcaccggc
 M  E  T  D  T  L  L  L  W  V  L  L  L  W  V  P  G  S  T  G
gaagtgcagctggtgcagagcggcgcggaagtgaaaaaaccgggcgcgagcgtgaaaatt
 E  V  Q  L  V  Q  S  G  A  E  V  K  K  P  G  A  S  V  K  I
agctgcaaaaccagcggctatacctttaccgaatataccattcattgggtgaaacaggcg
 S  C  K  T  S  G  Y  T  F  T  E  Y  T  I  H  W  V  K  Q  A
agcggcaaaggcctggaatggattggcaacattaacccgaacaacggcggcaccacctat
 S  G  K  G  L  E  W  I  G  N  I  N  P  N  N  G  G  T  T  Y
aaccagaaatttgaagatcgcgcgaccctgaccgtggataaaagcaccagcaccgcgtat
 N  Q  K  F  E  D  R  A  T  L  T  V  D  K  S  T  S  T  A  Y
atggaactgagcagcctgcgcagcgaagataccgcggtgtattattgcgcggcgggctgg
 M  E  L  S  S  L  R  S  E  D  T  A  V  Y  Y  C  A  A  G  W
aactttgattattggggccagggcaccaccgtgaccgtgagcagcggcagcaccagcggc
 N  F  D  Y  W  G  Q  G  T  T  V  T  V  S  S  G  S  T  S  G
ggcggcagcggcggcggcagcggcggcggcggcagcagcgatattgtgatgacccagagc
 G  G  S  G  G  G  S  G  G  G  G  S  S  D  I  V  M  T  Q  S
ccgagcagcctgagcgcgagcgtgggcgatcgcgtgaccattacctgcaaagcgagccag
 P  S  S  L  S  A  S  V  G  D  R  V  T  I  T  C  K  A  S  Q
gatgtgggcaccgcggtggattggtatcagcagaaaccgggcaaagcgccgaaactgctg
 D  V  G  T  A  V  D  W  Y  Q  Q  K  P  G  K  A  P  K  L  L
atttattgggcgagcacccgccataccggcgtgccggatcgctttaccggcagcggcagc
 I  Y  W  A  S  T  R  H  T  G  V  P  D  R  F  T  G  S  G  S
ggcaccgatttttaccctgaccattagcagcctgcagccggaagattttgcggattatttt
 G  T  D  F  T  L  T  I  S  S  L  Q  P  E  D  F  A  D  Y  F
tgccagcagtataacagctatccgctgacctttggcggcggcaccaaactggaaattaaa
 C  Q  Q  Y  N  S  Y  P  L  T  F  G  G  G  T  K  L  E  I  K
gaaccgaaaagcagcgataaaacccatacctgcccgccgtgcccgccgtgcccgccgtgc
 E  P  K  S  S  D  K  T  H  T  C  P  P  C  P  P  C  P  P  C
ggcggcggcagcagcggcggcggcagcggcggccagccgcgcgaaccgcaggtgtatacc
 G  G  G  S  S  G  G  G  S  G  G  Q  P  R  E  P  Q  V  Y  T
ctgccgccgagccgcgaagaaatgaccaaaaaccaggtgagcctgacctgcctggtgaaa
 L  P  P  S  R  E  E  M  T  K  N  Q  V  S  L  T  C  L  V  K
ggcttttatccgagcgatattgcggtggaatgggaaagcaacggccagccggaaaacaac
 G  F  Y  P  S  D  I  A  V  E  W  E  S  N  G  Q  P  E  N  N
tataaaaccaccccgccggtgctggatagcgatggcagctttttctgtatagcaaactg
 Y  K  T  T  P  P  V  L  D  S  D  G  S  F  F  L  Y  S  K  L
```

FIG. 50

accgtggataaaagccgctggcagcagggcaacgtgtttagctgcagcgtgatgcatgaa

T V D K S R W Q Q G N V F S C S V M H E

gcgctgcataaccattatacccagaaaagcctgagcctgagcccgggctga **(SEQ ID NO: 148)**

A L H N H Y T Q K S L S L S P G Stop **(SEQ ID NO: 115)**

FIG. 50
continued

IAB2M γ1 EH7(M2) (SEQ ID NO: 116)

```
atggagacagacaccctcctcctgtgggtgctgctgctgtgggtccccggaagcaccgga
 M  E  T  D  T  L  L  L  W  V  L  L  L  W  V  P  G  S  T  G
gaagtgcagctggtgcaatccggagccgaggtgaagaaacccggcgccagcgtgaagatt
 E  V  Q  L  V  Q  S  G  A  E  V  K  K  P  G  A  S  V  K  I
agctgcaaaaccagcggctacaccttcaccgagtacaccatccactgggtcaagcaggcc
 S  C  K  T  S  G  Y  T  F  T  E  Y  T  I  H  W  V  K  Q  A
agcggcaagggcctggagtggatcggcaacattaaccccaataacggcggcaccacctac
 S  G  K  G  L  E  W  I  G  N  I  N  P  N  N  G  G  T  T  Y
aatcagaaattcgaggacagggccaccctgaccgtggataagtccacctccaccgcctac
 N  Q  K  F  E  D  R  A  T  L  T  V  D  K  S  T  S  T  A  Y
atggagctgtcctccctgagaagcgaggatacagccgtctactactgcgctgccggatgg
 M  E  L  S  S  L  R  S  E  D  T  A  V  Y  Y  C  A  A  G  W
aatttcgactactggggccagggcacaaccgtgaccgtgagcagcggatccacctccggc
 N  F  D  Y  W  G  Q  G  T  T  V  T  V  S  S  G  S  T  S  G
ggcggcagcggaggcggcagcggcggaggaggcagcagcgacatcgtgatgacacagtcc
 G  G  S  G  G  G  S  G  G  G  G  S  S  D  I  V  M  T  Q  S
cccagcagcctgtccgctagcgtgggcgacagagtgaccatcacctgcaaggcctcccag
 P  S  S  L  S  A  S  V  G  D  R  V  T  I  T  C  K  A  S  Q
gacgtgggaaccgctgtggactggtaccagcagaagcccggcaaggcccccaagctgctg
 D  V  G  T  A  V  D  W  Y  Q  Q  K  P  G  K  A  P  K  L  L
atctactgggccagcaccagacacaccggcgtgcctgacagattcaccggctccggaagc
 I  Y  W  A  S  T  R  H  T  G  V  P  D  R  F  T  G  S  G  S
ggcaccgacttcaccctgaccatcagctccctccagcccgaggacttcgccgactacttc
 G  T  D  F  T  L  T  I  S  S  L  Q  P  E  D  F  A  D  Y  F
tgccagcagtataatagctacccccctgaccttcggcggcggcacaaagctcgaaatcaag
 C  Q  Q  Y  N  S  Y  P  L  T  F  G  G  G  T  K  L  E  I  K
gagctgaagaccccctctgggagacaccacccacacctgcccccccttgccctccctgtgga
 E  L  K  T  P  L  G  D  T  T  H  T  C  P  P  C  P  P  C  G
ggcggaagcagcggaggaggaagcggaggacagcccagggaaccccaggtgtacacactg
 G  G  S  S  G  G  G  S  G  G  Q  P  R  E  P  Q  V  Y  T  L
ccccccctccagagaggagatgacaaagaaccaggtgtccctcacctgcctggtgaaaggc
 P  P  S  R  E  E  M  T  K  N  Q  V  S  L  T  C  L  V  K  G
ttctatcccagcgacatcgccgtggagtgggagtccaacggccaacccgaaaacaactac
 F  Y  P  S  D  I  A  V  E  W  E  S  N  G  Q  P  E  N  N  Y
aagaccacccctcccgtgctggattccgacggctccttttttcctgtacagcaagctgacc
 K  T  T  P  P  V  L  D  S  D  G  S  F  F  L  Y  S  K  L  T
```

FIG. 51

gtggacaagtccaggtggcagcagggcaatgtctttagctgcagcgtcatgcacgaggcc

V D K S R W Q Q G N V F S C S V M H E A

ctgcataaccactatacccagaagtccctgtccctcagccctggatga **(SEQ ID NO: 149)**

L H N H Y T Q K S L S L S P G Stop **(SEQ ID NO: 116)**

FIG. 51
continued

IAB2M γ1 EH8(M2) (SEQ ID NO: 117)

```
atggaaaccgataccctgctgctgtgggtgctgctgctgtgggtgccgggcagcaccggc
 M  E  T  D  T  L  L  L  W  V  L  L  L  W  V  P  G  S  T  G
gaagtgcagctggtgcagagcggcgcggaagtgaaaaaaccgggcgcgagcgtgaaaatt
 E  V  Q  L  V  Q  S  G  A  E  V  K  K  P  G  A  S  V  K  I
agctgcaaaaccagcggctatacctttaccgaatataccattcattgggtgaaacaggcg
 S  C  K  T  S  G  Y  T  F  T  E  Y  T  I  H  W  V  K  Q  A
agcggcaaaggcctggaatggattggcaacattaacccgaacaacggcggcaccacctat
 S  G  K  G  L  E  W  I  G  N  I  N  P  N  N  G  G  T  T  Y
aaccagaaatttgaagatcgcgcgaccctgaccgtggataaaagcaccagcaccgcgtat
 N  Q  K  F  E  D  R  A  T  L  T  V  D  K  S  T  S  T  A  Y
atggaactgagcagcctgcgcagcgaagataccgcggtgtattattgcgcggcgggctgg
 M  E  L  S  S  L  R  S  E  D  T  A  V  Y  Y  C  A  A  G  W
aactttgattattggggccagggcaccaccgtgaccgtgagcagcggcagcaccagcggc
 N  F  D  Y  W  G  Q  G  T  T  V  T  V  S  S  G  S  T  S  G
ggcggcagcggcggcggcagcggcggcggcggcagcagcgatattgtgatgacccagagc
 G  G  S  G  G  G  S  G  G  G  G  S  S  D  I  V  M  T  Q  S
ccgagcagcctgagcgcgagcgtgggcgatcgcgtgaccattacctgcaaagcgagccag
 P  S  S  L  S  A  S  V  G  D  R  V  T  I  T  C  K  A  S  Q
gatgtgggcaccgcggtggattggtatcagcagaaaccgggcaaagcgccgaaactgctg
 D  V  G  T  A  V  D  W  Y  Q  Q  K  P  G  K  A  P  K  L  L
atttattgggcgagcacccgccataccggcgtgccggatcgctttaccggcagcggcagc
 I  Y  W  A  S  T  R  H  T  G  V  P  D  R  F  T  G  S  G  S
ggcaccgatttttaccctgaccattagcagcctgcagccggaagattttgcggattatttt
 G  T  D  F  T  L  T  I  S  S  L  Q  P  E  D  F  A  D  Y  F
tgccagcagtataacagctatccgctgacctttggcggcggcaccaaactggaaattaaa
 C  Q  Q  Y  N  S  Y  P  L  T  F  G  G  G  T  K  L  E  I  K
gaactgaaaaccccgctgggcgataccacccatacctgcccgccgtgcccgccgtgcccg
 E  L  K  T  P  L  G  D  T  T  H  T  C  P  P  C  P  P  C  P
ccgtgcggcggcggcagcagcggcggcggcagcggcggccagccgcgcgaaccgcaggtg
 P  C  G  G  G  S  S  G  G  G  S  G  G  Q  P  R  E  P  Q  V
tataccctgccgccgagccgcgaagaaatgaccaaaaaccaggtgagcctgacctgcctg
 Y  T  L  P  P  S  R  E  E  M  T  K  N  Q  V  S  L  T  C  L
gtgaaaggcttttatccgagcgatattgcggtggaatgggaaagcaacggccagccggaa
 V  K  G  F  Y  P  S  D  I  A  V  E  W  E  S  N  G  Q  P  E
aacaactataaaaccaccccgccggtgctggatagcgatggcagcttttttctgtatagc
 N  N  Y  K  T  T  P  P  V  L  D  S  D  G  S  F  F  L  Y  S
```

FIG. 52

aaactgaccgtggataaaagccgctggcagcagggcaacgtgtttagctgcagcgtgatg

K L T V D K S R W Q Q G N V F S C S V M

catgaagcgctgcataaccattatacccagaaaagcctgagcctgagcccgggctga **(SEQ ID NO: 150)**

H E A L H N H Y T Q K S L S L S P G Stop **(SEQ ID NO: 117)**

FIG. 52
continued

IAB2M γ1 EH3(M1) (SEQ ID NO: 118)

```
atggaaaccgataccctgctgctgtgggtgctgctgctgtgggtgccgggcagcaccggc
 M  E  T  D  T  L  L  L  W  V  L  L  L  W  V  P  G  S  T  G
gatattgtgatgacccagagcccgagcagcctgagcgcgagcgtgggcgatcgcgtgacc
 D  I  V  M  T  Q  S  P  S  S  L  S  A  S  V  G  D  R  V  T
attacctgcaaagcgagccaggatgtgggcaccgcggtggattggtatcagcagaaaccg
 I  T  C  K  A  S  Q  D  V  G  T  A  V  D  W  Y  Q  Q  K  P
ggcaaagcgccgaaactgctgatttattgggcgagcacccgccataccggcgtgccggat
 G  K  A  P  K  L  L  I  Y  W  A  S  T  R  H  T  G  V  P  D
cgctttaccggcagcggcagcggcaccgattttaccctgaccattagcagcctgcagccg
 R  F  T  G  S  G  S  G  T  D  F  T  L  T  I  S  S  L  Q  P
gaagattttgcggattatttttgccagcagtataacagctatccgctgacctttggcggc
 E  D  F  A  D  Y  F  C  Q  Q  Y  N  S  Y  P  L  T  F  G  G
ggcaccaaactggaaattaaaggcagcaccagcggcggcggcagcggcggcggcagcggc
 G  T  K  L  E  I  K  G  S  T  S  G  G  G  S  G  G  G  S  G
ggcggcggcagcagcgaagtgcagctggtgcagagcggcgcggaagtgaaaaaaccgggc
 G  G  G  S  S  E  V  Q  L  V  Q  S  G  A  E  V  K  K  P  G
gcgagcgtgaaaattagctgcaaaaccagcggctatacctttaccgaatataccattcat
 A  S  V  K  I  S  C  K  T  S  G  Y  T  F  T  E  Y  T  I  H
tgggtgaaacaggcgagcggcaaaggcctggaatggattggcaacattaacccgaacaac
 W  V  K  Q  A  S  G  K  G  L  E  W  I  G  N  I  N  P  N  N
ggcggcaccacctataaccagaaatttgaagatcgcgcgaccctgaccgtggataaaagc
 G  G  T  T  Y  N  Q  K  F  E  D  R  A  T  L  T  V  D  K  S
accagcaccgcgtatatggaactgagcagcctgcgcagcgaagataccgcggtgtattat
 T  S  T  A  Y  M  E  L  S  S  L  R  S  E  D  T  A  V  Y  Y
tgcgcggcgggctggaactttgattattggggccagggcaccaccgtgaccgtgagcagc
 C  A  A  G  W  N  F  D  Y  W  G  Q  G  T  T  V  T  V  S  S
gaaccgaaaagcagcgataaaacccatacctgcccgccgtgcccgccgtgcggcggcggc
 E  P  K  S  S  D  K  T  H  T  C  P  P  C  P  P  C  G  G  G
agcagcggcggcggcagcggcggccagccgcgcgaaccgcaggtgtataccctgccgccg
 S  S  G  G  G  S  G  G  Q  P  R  E  P  Q  V  Y  T  L  P  P
agccgcgaagaaatgaccaaaaaccaggtgagcctgacctgcctggtgaaaggcttttat
 S  R  E  E  M  T  K  N  Q  V  S  L  T  C  L  V  K  G  F  Y
ccgagcgatattgcggtggaatgggaaagcaacggccagccggaaaacaactataaaacc
 P  S  D  I  A  V  E  W  E  S  N  G  Q  P  E  N  N  Y  K  T
accccgccggtgctggatagcgatggcagcttttttctgtatagcaaactgaccgtggat
 T  P  P  V  L  D  S  D  G  S  F  F  L  Y  S  K  L  T  V  D
```

FIG. 53

aaaagccgctggcagcagggcaacgtgtttagctgcagcgtgatgcatgaagcgctgcat

K S R W Q Q G N V F S C S V M H E A L H

aaccattatacccagaaaagcctgagcctgagcccgggctga **(SEQ ID NO: 151)**

N H Y T Q K S L S L S P G Stop **(SEQ ID NO: 118)**

FIG. 53
continued

Alignment compared IAB20M γ1 EH1(M2) (SEQ ID NO: 119) vs IAB20M γ1 EH3(M2)(VL-Q79E, V83E; VH-Q1E, Q6E) (SEQ ID NO: 120), differences shown in box.

```
IAB20M γ1 EH1(M2) METDTLLLWVLLLWVPGSTG [Q] VQLV [Q] SGAEVKKPGASVKVSCKASGYSFTGYYMHWVRQA 60
IAB20M γ1 EH3(M2) METDTLLLWVLLLWVPGSTG [E] VQLV [E] SGAEVKKPGASVKVSCKASGYSFTGYYMHWVRQA 60

IAB20M γ1 EH1(M2) PGQGLEWIGRINPNNGVTLYNQKFKDRVTMTVDTSISTAYMELSRLRSDDTAVYYCARST 120

IAB20M γ1 EH3(M2) PGQGLEWIGRINPNNGVTLYNQKFKDRVTMTVDTSISTAYMELSRLRSDDTAVYYCARST 120

IAB20M γ1 EH1(M2) MITNYVMDYWGQGTLVTVSSGSTSGGGSGGGGSGGGGSSDIVMTQSPATLSVSPGERATLS 180

IAB20M γ1 EH3(M2) MITNYVMDYWGQGTLVTVSSGSTSGGGSGGGGSGGGGSSDIVMTQSPATLSVSPGERATLS 180

IAB20M γ1 EH1(M2) CKASQSVSNDVAWYQQKPGQAPRLLISYTSSRYAGVPDRFSGSGSGTDFTLTISSL [Q] AED 240

IAB20M γ1 EH3(M2) CKASQSVSNDVAWYQQKPGQAPRLLISYTSSRYAGVPDRFSGSGSGTDFTLTISSL [E] AED 240

IAB20M γ1 EH1(M2) [V] AVYYCQQDYNSPPTFGQGTKVEIKEPKS [C] DKTHTCP [---] PCGGGSGGGSGGQPREPQV 297
IAB20M γ1 EH3(M2) [E] AVYYCQQDYNSPPTFGQGTKVEIKEPKS [S] DKTHTCP [PCP] PCGGGSGGGSGGQPREPQV 300

IAB20M γ1 EH1(M2) YTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYS 357

IAB20M γ1 EH3(M2) YTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYS 360

IAB20M γ1 EH1(M2) KLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG [K] 396 (SEQ ID NO: 119)

IAB20M γ1 EH3(M2) KLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG [-] 398 (SEQ ID NO: 120)
```

FIG. 54

IAB20M γ1 EH3(M2)(VL-Q79E, V83E; VH-Q1E, Q6E), CDR regions are defined by Chothia definition (SEQ ID NO: 120)

```
atggaaaccgataccctgctgctgtgggtgctgctgctgtgggtgcctggcagcacagga
 M  E  T  D  T  L  L  L  W  V  L  L  L  W  V  P  G  S  T  G
```

Signal peptide sequence

```
gaggtgcagctggtggagagcggcgctgaagtgaagaagcccggcgccagcgtgaaggtg
 E  V  Q  L  V  E  S  G  A  E  V  K  K  P  G  A  S  V  K  V
```

VH

```
agctgcaaggccagc ggctacagcttcacaggctac tacatgcactgggtgagacaagcc
 S  C  K  A  S  G  Y  S  F  T  G  Y  Y  M  H  W  V  R  Q  A
```

HCDR1

```
cctggccagggcctcgagtggattggaaggatc aaccccaacaacggagtg acactgtac
 P  G  Q  G  L  E  W  I  G  R  I  N  P  N  N  G  V  T  L  Y
```

HCDR2

```
aaccagaaattcaaggacagggtcaccatgaccgtggacaccagcatcagcaccgcctat
 N  Q  K  F  K  D  R  V  T  M  T  V  D  T  S  I  S  T  A  Y
atggagctgtccaggctgaggagcgacgacaccgctgtgtactactgcgccagg agcacc
 M  E  L  S  R  L  R  S  D  D  T  A  V  Y  Y  C  A  R  S  T
```

HCDR3

```
atgatcaccaactacgtgatggattat tggggccagggcacactggtgacagtgagcagc
 M  I  T  N  Y  V  M  D  Y  W  G  Q  G  T  L  V  T  V  S  S
```

HCDR3

```
ggcagcaccagcggaggaggcagcggaggaggaagcggcggaggcggctcctcc gatatc
 G  S  T  S  G  G  G  S  G  G  G  S  G  G  G  G  S  S  D  I
```

Linker **VL**

```
gtcatgacccagagccccgctaccctcagcgtgtcccctggcgagagagccaccctgagc
 V  M  T  Q  S  P  A  T  L  S  V  S  P  G  E  R  A  T  L  S
tgc aaggccagccagtccgtgtccaacgacgtggcc tggtatcaacagaagcccggccag
 C  K  A  S  Q  S  V  S  N  D  V  A  W  Y  Q  Q  K  P  G  Q
```

LCDR1

```
gctcctaggctgctgatctcc tacaccagcagcaggtacgcc ggcgtgcctgataggttc
 A  P  R  L  L  I  S  Y  T  S  S  R  Y  A  G  V  P  D  R  F
```

LCDR2

```
agcggctccggcagcggcacagacttcaccctgacaatcagcagcctggaggccgaggat
 S  G  S  G  S  G  T  D  F  T  L  T  I  S  S  L  E  A  E  D
```

FIG. 55

gaggccgtgtactactgt cagcaggactacaactcccccccacc ttcggccagggcaca

E A V Y Y C Q Q D Y N S P P T F G Q G T

LCDR3

aaggtc gagatcaaggagcccaagtcctccgacaagacccacacc tgtcccccctgccct

K V E I K E P K S S D K T H T C P P C P

Upper hinge Core hinge

ccttgt ggaggcggaagcagcggaggcgggagcgga ggacagcccagagaaccccaggtg

P C G G G S S G G G S G G Q P R E P Q V

Core Lower hinge CH3

tataccctgcccccctccagggaggagatgaccaaaaaccaggtgagcctgacatgcctg

Y T L P P S R E E M T K N Q V S L T C L

gtgaagggcttctacccctccgacatcgccgtggagtgggaaagcaacggccagcccgag

V K G F Y P S D I A V E W E S N G Q P E

aacaactacaagaccacacccccgtgctggatagcgacggctccttctttctgtacagc

N N Y K T T P P V L D S D G S F F L Y S

aagctgaccgtggataaaagcaggtggcagcaaggcaacgtgttctcctgctccgtcatg

K L T V D K S R W Q Q G N V F S C S V M

cacgaggctctgcataaccactacacccaaaaatccctgagcctgagccccggctga **(SEQ ID NO: 152)**

H E A L H N H Y T Q K S L S L S P G Stop **(SEQ ID NO: 120)**

FIG. 55
continued

IAB20M γ1 EH3(M2) (SEQ ID NO: 121)

```
atggaaaccgataccctgctgctgtgggtgctgctgctgtgggtgccgggcagcaccggc
 M  E  T  D  T  L  L  L  W  V  L  L  L  W  V  P  G  S  T  G
caggtgcagctggtgcagagcggcgcggaagtgaaaaaaccgggcgcgagcgtgaaagtg
 Q  V  Q  L  V  Q  S  G  A  E  V  K  K  P  G  A  S  V  K  V
agctgcaaagcgagcggctatagctttaccggctattatatgcattgggtgcgccaggcg
 S  C  K  A  S  G  Y  S  F  T  G  Y  Y  M  H  W  V  R  Q  A
ccgggccagggcctggaatggattggccgcattaacccgaacaacggcgtgaccctgtat
 P  G  Q  G  L  E  W  I  G  R  I  N  P  N  N  G  V  T  L  Y
aaccagaaatttaaagatcgcgtgaccatgaccgtggataccagcattagcaccgcgtat
 N  Q  K  F  K  D  R  V  T  M  T  V  D  T  S  I  S  T  A  Y
atggaactgagccgcctgcgcagcgatgataccgcggtgtattattgcgcgcgcagcacc
 M  E  L  S  R  L  R  S  D  D  T  A  V  Y  Y  C  A  R  S  T
atgattaccaactatgtgatggattattggggccagggcaccctggtgaccgtgagcagc
 M  I  T  N  Y  V  M  D  Y  W  G  Q  G  T  L  V  T  V  S  S
ggcagcaccagcggcggcggcagcggcggcggcagcggcggcggcggcagcagcgatatt
 G  S  T  S  G  G  G  S  G  G  G  S  G  G  G  G  S  S  D  I
gtgatgacccagagcccggcgaccctgagcgtgagcccgggcgaacgcgcgaccctgagc
 V  M  T  Q  S  P  A  T  L  S  V  S  P  G  E  R  A  T  L  S
tgcaaagcgagccagagcgtgagcaacgatgtggcgtggtatcagcagaaaccgggccag
 C  K  A  S  Q  S  V  S  N  D  V  A  W  Y  Q  Q  K  P  G  Q
gcgccgcgcctgctgattagctataccagcagccgctatgcgggcgtgccggatcgctttt
 A  P  R  L  L  I  S  Y  T  S  S  R  Y  A  G  V  P  D  R  F
agcggcagcggcagcggcaccgattttaccctgaccattagcagcctgcaggcggaagat
 S  G  S  G  S  G  T  D  F  T  L  T  I  S  S  L  Q  A  E  D
gtggcggtgtattattgccagcaggattataacagcccgccgacctttggccagggcacc
 V  A  V  Y  Y  C  Q  Q  D  Y  N  S  P  P  T  F  G  Q  G  T
aaagtggaaattaaagaaccgaaaagcagcgataaaacccatacctgcccgccgtgcccg
 K  V  E  I  K  E  P  K  S  S  D  K  T  H  T  C  P  P  C  P
ccgtgcggcggcggcagcagcggcggcggcagcggcggccagccgcgcgaaccgcaggtg
 P  C  G  G  G  S  S  G  G  G  S  G  G  Q  P  R  E  P  Q  V
tataccctgccgccgagccgcgaagaaatgaccaaaaaccaggtgagcctgacctgcctg
 Y  T  L  P  P  S  R  E  E  M  T  K  N  Q  V  S  L  T  C  L
gtgaaaggcttttatccgagcgatattgcggtggaatgggaaagcaacggccagccggaa
 V  K  G  F  Y  P  S  D  I  A  V  E  W  E  S  N  G  Q  P  E
aacaactataaaaccaccccgccggtgctggatagcgatggcagcttttttctgtatagc
 N  N  Y  K  T  T  P  P  V  L  D  S  D  G  S  F  F  L  Y  S
```

FIG. 56

aaactgaccgtggataaaagccgctggcagcagggcaacgtgtttagctgcagcgtgatg

K L T V D K S R W Q Q G N V F S C S V M

catgaagcgctgcataaccattatacccagaaaagcctgagcctgagcccgggctga **(SEQ ID NO: 153)**

H E A L H N H Y T Q K S L S L S P G Stop **(SEQ ID NO: 121)**

FIG. 56
continued

IAB20M γ1 EH3(M2)(VL-A9D, T10S, S12A, P15L, L21I, S22N; VH-Q1E, Q6E) (SEQ ID NO: 122)

```
atggaaaccgataccctgctgctgtgggtgctgctgctgtgggtgcctggcagcacagga
 M  E  T  D  T  L  L  L  W  V  L  L  L  W  V  P  G  S  T  G
gaggtgcagctggtggagagcggcgctgaagtgaagaagcccggcgccagcgtgaaggtg
 E  V  Q  L  V  E  S  G  A  E  V  K  K  P  G  A  S  V  K  V
agctgcaaggccagcggctacagcttcacaggctactacatgcactgggtgagacaagcc
 S  C  K  A  S  G  Y  S  F  T  G  Y  Y  M  H  W  V  R  Q  A
cctggccagggcctcgagtggattggaaggatcaaccccaacaacggagtgacactgtac
 P  G  Q  G  L  E  W  I  G  R  I  N  P  N  N  G  V  T  L  Y
aaccagaaattcaaggacagggtcaccatgaccgtggacaccagcatcagcaccgcctat
 N  Q  K  F  K  D  R  V  T  M  T  V  D  T  S  I  S  T  A  Y
atggagctgtccaggctgaggagcgacgacaccgctgtgtactactgcgccaggagcacc
 M  E  L  S  R  L  R  S  D  D  T  A  V  Y  Y  C  A  R  S  T
atgatcaccaactacgtgatggattattggggccagggcacactggtgacagtgagcagc
 M  I  T  N  Y  V  M  D  Y  W  G  Q  G  T  L  V  T  V  S  S
ggcagcaccagcggaggaggcagcggaggaggaagcggcggaggcggctcctccgatatc
 G  S  T  S  G  G  G  S  G  G  G  S  G  G  G  G  S  S  D  I
gtcatgacccagagccccgatagcctggccgtgagcctgggcgagagagccaccatcaat
 V  M  T  Q  S  P  D  S  L  A  V  S  L  G  E  R  A  T  I  N
tgcaaggccagccagtccgtgtccaacgacgtggcctggtatcaacagaagcccggccag
 C  K  A  S  Q  S  V  S  N  D  V  A  W  Y  Q  Q  K  P  G  Q
gctcctaggctgctgatctcctacaccagcagcaggtacgccggcgtgcctgataggttc
 A  P  R  L  L  I  S  Y  T  S  S  R  Y  A  G  V  P  D  R  F
agcggctccggcagcggcacagacttcaccctgacaatcagcagcctgcaagccgaagat
 S  G  S  G  S  G  T  D  F  T  L  T  I  S  S  L  Q  A  E  D
gtggccgtgtactactgtcagcaggactacaactccccccccaccttcggccagggcaca
 V  A  V  Y  Y  C  Q  Q  D  Y  N  S  P  P  T  F  G  Q  G  T
aaggtcgagatcaaggagcccaagtcctccgacaagacccacacctgtccccctgccct
 K  V  E  I  K  E  P  K  S  S  D  K  T  H  T  C  P  P  C  P
ccttgtggaggcggaagcagcggaggcgggagcggaggacagcccagagaaccccaggtg
 P  C  G  G  G  S  S  G  G  G  S  G  G  Q  P  R  E  P  Q  V
tataccctgccccctccagggaggagatgaccaaaaaccaggtgagcctgacatgcctg
 Y  T  L  P  P  S  R  E  E  M  T  K  N  Q  V  S  L  T  C  L
gtgaagggcttctacccctccgacatcgccgtggagtgggaaagcaacggccagcccgag
 V  K  G  F  Y  P  S  D  I  A  V  E  W  E  S  N  G  Q  P  E
aacaactacaagaccacacccccgtgctggatagcgacggctccttctttctgtacagc
 N  N  Y  K  T  T  P  P  V  L  D  S  D  G  S  F  F  L  Y  S
```

FIG. 57

aagctgaccgtggataaaagcaggtggcagcaaggcaacgtgttctcctgctccgtcatg

K L T V D K S R W Q Q G N V F S C S V M

cacgaggctctgcataaccactacacccaaaaatccctgagcctgagccccggctga **(SEQ ID NO: 154)**

H E A L H N H Y T Q K S L S L S P G Stop **(SEQ ID NO: 122)**

FIG. 57
continued

IAB20M γ1 EH5(M2)(VL-Q79E, V83E; VH-Q1E, Q6E) (SEQ ID NO: 123)

```
atggagacagacaccctcctcctgtgggtcctgctcctgtgggtgcccggatccacaggc
 M  E  T  D  T  L  L  L  W  V  L  L  L  W  V  P  G  S  T  G
gaggtccagctggtggaatccggagccgaggtgaagaaacccggcgcctccgtgaaagtc
 E  V  Q  L  V  E  S  G  A  E  V  K  K  P  G  A  S  V  K  V
tcctgcaaggcctccggctactccttcaccggctactacatgcactgggtgaggcaggcc
 S  C  K  A  S  G  Y  S  F  T  G  Y  Y  M  H  W  V  R  Q  A
cctggacagggactggaatggatcggcaggattaaccccaacaacggagtgaccctgtac
 P  G  Q  G  L  E  W  I  G  R  I  N  P  N  N  G  V  T  L  Y
aaccagaagttcaaggacagggtcaccatgaccgtggacaccagcatttccaccgcctac
 N  Q  K  F  K  D  R  V  T  M  T  V  D  T  S  I  S  T  A  Y
atggagctgtccaggctgagaagcgacgacaccgctgtgtactactgcgccaggagcacc
 M  E  L  S  R  L  R  S  D  D  T  A  V  Y  Y  C  A  R  S  T
atgatcaccaactacgtgatggattattggggccagggcaccctcgtgacagtgagcagc
 M  I  T  N  Y  V  M  D  Y  W  G  Q  G  T  L  V  T  V  S  S
ggatccacaagcggaggcggaagcggaggcggcagcggcggcggcggcagcagcgatatc
 G  S  T  S  G  G  G  S  G  G  G  S  G  G  G  G  S  S  D  I
gtgatgacccagagccccgccaccctgagcgtgagccccggcgaaagggccacactgagc
 V  M  T  Q  S  P  A  T  L  S  V  S  P  G  E  R  A  T  L  S
tgcaaggccagccagtccgtctccaacgatgtggcctggtaccagcagaagcccggacag
 C  K  A  S  Q  S  V  S  N  D  V  A  W  Y  Q  Q  K  P  G  Q
gcccctaggctcctgatctcctacaccagctccaggtacgccggcgtccctgacagattc
 A  P  R  L  L  I  S  Y  T  S  S  R  Y  A  G  V  P  D  R  F
tccggcagcggcagcggcaccgatttcaccctcaccatcagcagcctggaagccgaagat
 S  G  S  G  S  G  T  D  F  T  L  T  I  S  S  L  E  A  E  D
gaagctgtgtattactgccagcaggactacaacagccccccccacatttggccagggaacc
 E  A  V  Y  Y  C  Q  Q  D  Y  N  S  P  P  T  F  G  Q  G  T
aaggtggagatcaaggagcccaagagcagcgacaagacccatacctgccctccttgccct
 K  V  E  I  K  E  P  K  S  S  D  K  T  H  T  C  P  P  C  P
ccttgtcctccctgtggaggcggaagctccggaggaggatccggaggccagcctagagag
 P  C  P  P  C  G  G  G  S  S  G  G  G  S  G  G  Q  P  R  E
ccccaggtgtacaccctgccccctagcagggaggagatgaccaagaaccaggtgagcctg
 P  Q  V  Y  T  L  P  P  S  R  E  E  M  T  K  N  Q  V  S  L
acatgtctggtcaagggcttttaccccagcgacatcgccgtggagtgggagagcaacggc
 T  C  L  V  K  G  F  Y  P  S  D  I  A  V  E  W  E  S  N  G
cagcctgagaacaactacaagaccacccctcctgtgctggactccgacggcagcttttc
 Q  P  E  N  N  Y  K  T  T  P  P  V  L  D  S  D  G  S  F  F
```

FIG. 58

ctgtacagcaagctgaccgtggataagagcaggtggcagcagggcaacgtcttcagctgc

L Y S K L T V D K S R W Q Q G N V F S C

agcgtgatgcacgaggctctgcataaccactacacccagaagagcctgagcctgagccct

S V M H E A L H N H Y T Q K S L S L S P

ggatga **(SEQ ID NO: 155)**

G Stop **(SEQ ID NO: 123)**

FIG. 58
continued

IAB20M γ1 EH5(M2)(VL-A9D, T10S, S12A, P15L, L21I, S22N; VH-Q1E, Q6E) (SEQ ID NO: 124)

atggagacagacaccctcctcctgtgggtcctgctcctgtgggtgcccggatccacaggc

M E T D T L L L W V L L L W V P G S T G

gaggtccagctggtggaatccggagccgaggtgaagaaacccggcgcctccgtgaaagtc

E V Q L V E S G A E V K K P G A S V K V

tcctgcaaggcctccggctactccttcaccggctactacatgcactgggtgaggcaggcc

S C K A S G Y S F T G Y Y M H W V R Q A

cctggacagggactggaatggatcggcaggattaaccccaacaacggagtgaccctgtac

P G Q G L E W I G R I N P N N G V T L Y

aaccagaagttcaaggacagggtcaccatgaccgtggacaccagcatttccaccgcctac

N Q K F K D R V T M T V D T S I S T A Y

atggagctgtccaggctgagaagcgacgacaccgctgtgtactactgcgccaggagcacc

M E L S R L R S D D T A V Y Y C A R S T

atgatcaccaactacgtgatggattattggggccagggcaccctcgtgacagtgagcagc

M I T N Y V M D Y W G Q G T L V T V S S

ggatccacaagcggaggcggaagcggaggcggcagcggcggcggcggcagcagcgatatc

G S T S G G G S G G G S G G G G S S D I

gtgatgacccagagccccgattccctcgccgtgagcctgggcgaaagggccacaatcaac

V M T Q S P D S L A V S L G E R A T I N

tgcaaggccagccagtccgtctccaacgatgtggcctggtaccagcagaagcccggacag

C K A S Q S V S N D V A W Y Q Q K P G Q

gcccctaggctcctgatctcctacaccagctccaggtacgccggcgtccctgacagattc

A P R L L I S Y T S S R Y A G V P D R F

tccggcagcggcagcggcaccgatttcaccctcaccatcagcagcctgcaggccgaggac

S G S G S G T D F T L T I S S L Q A E D

gtggctgtgtattactgccagcaggactacaacagccccccccacatttggccagggaacc

V A V Y Y C Q Q D Y N S P P T F G Q G T

aaggtggagatcaaggagcccaagagcagcgacaagacccatacctgccctccttgccct

K V E I K E P K S S D K T H T C P P C P

ccttgtcctccctgtggaggcggaagctccggaggaggatccggaggccagcctagagag

P C P P C G G G S S G G G S G G Q P R E

ccccaggtgtacaccctgcccccctagcagggaggagatgaccaagaaccaggtgagcctg

P Q V Y T L P P S R E E M T K N Q V S L

acatgtctggtcaagggcttttaccccagcgacatcgccgtggagtgggagagcaacggc

T C L V K G F Y P S D I A V E W E S N G

cagcctgagaacaactacaagaccacccctcctgtgctggactccgacggcagctttttc

Q P E N N Y K T T P P V L D S D G S F F

FIG. 59

ctgtacagcaagctgaccgtggataagagcaggtggcagcagggcaacgtcttcagctgc

L Y S K L T V D K S R W Q Q G N V F S C

agcgtgatgcacgaggctctgcataaccactacacccagaagagcctgagcctgagccct

S V M H E A L H N H Y T Q K S L S L S P

ggatga **(SEQ ID NO: 156)**

G Stop **(SEQ ID NO: 124)**

FIG. 59
continued

Alignment compared IAB1M γ1 EH1(M1) (SEQ ID NO: 125) vs IAB1M γ1 EH3(M1) (SEQ ID NO: 126), differences shown in box.

```
IAB1M γ1 EH1(M1) METDTLLLWVLLLWVPGSTGDIQLTQSPSTLSASVGDRVTITCSASSVRFIHWYQQKPG 60

IAB1M γ1 EH3(M1) METDTLLLWVLLLWVPGSTGDIQLTQSPSTLSASVGDRVTITCSASSVRFIHWYQQKPG 60

IAB1M γ1 EH1(M1) KAPKRLIYDTSKLASGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQWGSSPFTFGQG 120

IAB1M γ1 EH3(M1) KAPKRLIYDTSKLASGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQWGSSPFTFGQG 120

IAB1M γ1 EH1(M1) TKVEIKGSTSGGGSGGGSGGGSSEVQLVEYGGGLVQPGGSLRLSCAASGFNIKDYYIHW 180

IAB1M γ1 EH3(M1) TKVEIKGSTSGGGSGGGSGGGSSEVQLVEYGGGLVQPGGSLRLSCAASGFNIKDYYIHW 180

IAB1M γ1 EH1(M1) VRQAPGKGLEWVAWIDPENGDTEFVPKFQGRATMSADTSKNTAYLQMNSLRAEDTAVYYC 240

IAB1M γ1 EH3(M1) VRQAPGKGLEWVAWIDPENGDTEFVPKFQGRATMSADTSKNTAYLQMNSLRAEDTAVYYC 240

IAB1M γ1 EH1(M1) KTGGFWGQGTLVTVSSEPKS[C]DKTHTCP[---]PCGGGSSGGGSGGQPREPQVYTLPPSR[DE]297

IAB1M γ1 EH3(M1) KTGGFWGQGTLVTVSSEPKS[S]DKTHTCP[PCP]PCGGGSSGGGSGGQPREPQVYTLPPSR[EE]300

IAB1M γ1 EH1(M1) [L]TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW 357

IAB1M γ1 EH3(M1) [M]TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW 360

IAB1M γ1 EH1(M1) QQGNVFSCSVMHEALHNHYTQKSLSLSPG[K]387 (SEQ ID NO: 125)

IAB1M γ1 EH3(M1) QQGNVFSCSVMHEALHNHYTQKSLSLSPG[-]389 (SEQ ID NO: 126)
```

FIG. 60

IAB1M γ1 EH3(M1) (SEQ ID NO: 126), CDR regions are defined by Chothia definition

atggagaccgataccctgctgctgtgggtgctgctgctgtgggtgcctggcagcaccggc

M E T D T L L L W V L L L W V P G S T G

Signal peptide sequence

gacattcagctgacccagtccccttccacactgagcgcctccgtgggcgacagggtcaca

D I Q L T Q S P S T L S A S V G D R V T

VL

attacatgt tccgcctccagcagcgtcaggtttatccac tggtatcagcagaagcccggc

I T C S A S S S V R F I H W Y Q Q K P G

LCDR1

aaggcccccaagagactgatctac gacacaagcaagctggcctcc ggagtgcccagcaga

K A P K R L I Y D T S K L A S G V P S R

LCDR2

ttcagcggcagcggatccggcaccgatttcacactgaccatcagcagcctgcagcccgaa

F S G S G S G T D F T L T I S S L Q P E

gacttcgccacctactactgc cagcagtggggctcctccccttcacc ttcggacagggc

D F A T Y Y C Q Q W G S S P F T F G Q G

LCDR3

accaaggtggagatcaag ggctccacaagcggcggcggaagcggcggcggcagcggcggc

T K V E I K G S T S G G G S G G G S G G

Linker

ggcggctccagc gaagtgcagctggtggagtacggcggcggactggtccagcctggagga

G G S S E V Q L V E Y G G G L V Q P G G

Linker **VH**

agcctgaggctgagctgcgccgcctcc ggctttaacatcaaagattac tacattcactgg

S L R L S C A A S G F N I K D Y Y I H W

HCDR1

gtcagacaggcccctggcaaaggcctggagtgggtggcctggatc gatcccgagaacgga

V R Q A P G K G L E W V A W I D P E N G

HCDR2

gac accgagttcgtgcccaagttccagggaagagccaccatgagcgccgacaccagcaag

D T E F V P K F Q G R A T M S A D T S K

HCDR2

aacaccgcctacctgcagatgaattccctcagggccgaggataccgctgtgtactactgc

N T A Y L Q M N S L R A E D T A V Y Y C

aagacc ggcggattt tggggccagggaaccctggtgaccgtcagctcc gagcctaagagc

K T G G F W G Q G T L V T V S S E P K S

FIG. 61

HCDR3 Upper hinge

agcgataagacccacacc tgccctccttgccccccttgt ggcggaggatcctccggagga

S D K T H T C P P C P P C G G G S S G G

Upper hinge Core hinge Lower hinge

ggatccgga ggacagcccagggagcctcaggtgtacaccctccctcccagcagggaggag

G S G G Q P R E P Q V Y T L P P S R E E

Lower hinge CH3

atgaccaagaaccaggtgtccctgacctgcctcgtcaagggcttctaccccagcgacatc

M T K N Q V S L T C L V K G F Y P S D I

gctgtggagtgggagagcaacggccagcccgaaaacaactacaagaccacccctcccgtg

A V E W E S N G Q P E N N Y K T T P P V

ctggacagcgacggcagcttcttcctctactccaagctgacagtggacaagagcaggtgg

L D S D G S F F L Y S K L T V D K S R W

cagcagggcaacgtgttcagctgctccgtgatgcacgaggccctgcacaatcactatacc

Q Q G N V F S C S V M H E A L H N H Y T

cagaagtccctgtccctctccccggctag **(SEQ ID NO: 157)**

Q K S L S L S P G Stop **(SEQ ID NO: 126)**

FIG. 61
continued

IAB1M γ2 EH2(M1) (SEQ ID NO: 127)

atggagaccgataccctgctgctgtgggtgctgctgctgtgggtgcctggcagcaccggc

M E T D T L L L W V L L L W V P G S T G

gacattcagctgacccagtccccttccacactgagcgcctccgtgggcgacagggtcaca

D I Q L T Q S P S T L S A S V G D R V T

attacatgttccgcctccagcagcgtcaggtttatccactggtatcagcagaagcccggc

I T C S A S S S V R F I H W Y Q Q K P G

aaggcccccaagagactgatctacgacacaagcaagctggcctccggagtgcccagcaga

K A P K R L I Y D T S K L A S G V P S R

ttcagcggcagcggatccggcaccgatttcacactgaccatcagcagcctgcagcccgaa

F S G S G S G T D F T L T I S S L Q P E

gacttcgccacctactactgccagcagtggggctcctccccccttcaccttcggacagggc

D F A T Y Y C Q Q W G S S P F T F G Q G

accaaggtggagatcaagggctccacaagcggcggcggaagcggcggcggcagcggcggc

T K V E I K G S T S G G G S G G G S G G

ggcggctccagcgaagtgcagctggtggagtacggcggcggactggtccagcctggagga

G G S S E V Q L V E Y G G G L V Q P G G

agcctgaggctgagctgcgccgcctccggctttaacatcaaagattactacattcactgg

S L R L S C A A S G F N I K D Y Y I H W

gtcagacaggcccctggcaaaggcctggagtgggtggcctggatcgatcccgagaacgga

V R Q A P G K G L E W V A W I D P E N G

gacaccgagttcgtgcccaagttccagggaagagccaccatgagcgccgacaccagcaag

D T E F V P K F Q G R A T M S A D T S K

aacaccgcctacctgcagatgaattccctcagggccgaggataccgctgtgtactactgc

N T A Y L Q M N S L R A E D T A V Y Y C

aagaccggcggattttggggccagggaaccctggtgaccgtcagctccgagagaaagagc

K T G G F W G Q G T L V T V S S E R K S

tgcgtggagtgtcctccttgtcccggcggaggatcctccggaggaggatccggaggacag

C V E C P P C P G G G S S G G G S G G Q

cccagggagcctcaggtgtacaccctccctcccagcagggaggagatgaccaagaaccag

P R E P Q V Y T L P P S R E E M T K N Q

gtgtccctgacctgcctcgtcaagggcttctaccccagcgacatcgctgtggagtgggag

V S L T C L V K G F Y P S D I A V E W E

agcaacggccagcccgaaaacaactacaagaccacccctcccatgctggacagcgacggc

S N G Q P E N N Y K T T P P M L D S D G

agcttcttcctctactccaagctgacagtggacaagagcaggtggcagcagggcaacgtg

FIG. 62

S F F L Y S K L T V D K S R W Q Q G N V

ttcagctgctccgtgatgcacgaggccctgcacaatcactatacccagaagtccctgtcc

F S C S V M H E A L H N H Y T Q K S L S

ctctcccccggctag **(SEQ ID NO: 158)**

L S P G Stop **(SEQ ID NO: 127)**

FIG. 62
continued

IAB1M γ1 EH5(M1) (SEQ ID NO: 128)

```
atggaaaccgataccctgctgctgtgggtgctgctgctgtgggtgccgggcagcaccggc
 M  E  T  D  T  L  L  L  W  V  L  L  L  W  V  P  G  S  T  G
gatattcagctgacccagagcccgagcaccctgagcgcgagcgtgggcgatcgcgtgacc
 D  I  Q  L  T  Q  S  P  S  T  L  S  A  S  V  G  D  R  V  T
attacctgcagcgcgagcagcagcgtgcgctttattcattggtatcagcagaaaccgggc
 I  T  C  S  A  S  S  S  V  R  F  I  H  W  Y  Q  Q  K  P  G
aaagcgccgaaacgcctgatttatgataccagcaaactggcgagcggcgtgccgagccgc
 K  A  P  K  R  L  I  Y  D  T  S  K  L  A  S  G  V  P  S  R
tttagcggcagcggcagcggcaccgattttaccctgaccattagcagcctgcagccggaa
 F  S  G  S  G  S  G  T  D  F  T  L  T  I  S  S  L  Q  P  E
gattttgcgacctattattgccagcagtggggcagcagcccgtttacctttggccagggc
 D  F  A  T  Y  Y  C  Q  Q  W  G  S  S  P  F  T  F  G  Q  G
accaaagtggaaattaaaggcagcaccagcggcggcggcagcggcggcggcagcggcggc
 T  K  V  E  I  K  G  S  T  S  G  G  G  S  G  G  G  S  G  G
ggcggcagcagcgaagtgcagctggtggaatatggcggcggcctggtgcagccgggcggc
 G  G  S  S  E  V  Q  L  V  E  Y  G  G  G  L  V  Q  P  G  G
agcctgcgcctgagctgcgcggcgagcggctttaacattaaagattattatattcattgg
 S  L  R  L  S  C  A  A  S  G  F  N  I  K  D  Y  Y  I  H  W
gtgcgccaggcgccgggcaaaggcctggaatgggtggcgtggattgatccggaaaacggc
 V  R  Q  A  P  G  K  G  L  E  W  V  A  W  I  D  P  E  N  G
gataccgaatttgtgccgaaatttcagggccgcgcgaccatgagcgcggataccagcaaa
 D  T  E  F  V  P  K  F  Q  G  R  A  T  M  S  A  D  T  S  K
aacaccgcgtatctgcagatgaacagcctgcgcgcggaagataccgcggtgtattattgc
 N  T  A  Y  L  Q  M  N  S  L  R  A  E  D  T  A  V  Y  Y  C
aaaaccggcggcttttggggccagggcaccctggtgaccgtgagcagcgaaccgaaaagc
 K  T  G  G  F  W  G  Q  G  T  L  V  T  V  S  S  E  P  K  S
agcgataaaacccatacctgcccgccgtgcccgccgtgcccgccgtgcggcggcggcagc
 S  D  K  T  H  T  C  P  P  C  P  P  C  P  P  C  G  G  G  S
agcggcggcggcagcggcggccagccgcgcgaaccgcaggtgtataccctgccgccgagc
 S  G  G  G  S  G  G  Q  P  R  E  P  Q  V  Y  T  L  P  P  S
cgcgaagaaatgaccaaaaaccaggtgagcctgacctgcctggtgaaaggcttttatccg
 R  E  E  M  T  K  N  Q  V  S  L  T  C  L  V  K  G  F  Y  P
agcgatattgcggtggaatgggaaagcaacggccagccggaaaacaactataaaaccacc
 S  D  I  A  V  E  W  E  S  N  G  Q  P  E  N  N  Y  K  T  T
ccgccggtgctggatagcgatggcagcttttttctgtatagcaaactgaccgtggataaa
 P  P  V  L  D  S  D  G  S  F  F  L  Y  S  K  L  T  V  D  K
```

FIG. 63

agccgctggcagcagggcaacgtgtttagctgcagcgtgatgcatgaagcgctgcataac

S R W Q Q G N V F S C S V M H E A L H N

cattatacccagaaaagcctgagcctgagcccgggctga **(SEQ ID NO: 159)**

H Y T Q K S L S L S P G Stop **(SEQ ID NO: 128)**

FIG. 63
continued

IAB1M γ1 EH7(M1) (SEQ ID NO: 129)

```
atggaaaccgataccctgctgctgtgggtgctgctgctgtgggtgccgggcagcaccggc
 M  E  T  D  T  L  L  L  W  V  L  L  L  W  V  P  G  S  T  G
gatattcagctgacccagagcccgagcaccctgagcgcgagcgtgggcgatcgcgtgacc
 D  I  Q  L  T  Q  S  P  S  T  L  S  A  S  V  G  D  R  V  T
attacctgcagcgcgagcagcagcgtgcgctttattcattggtatcagcagaaaccgggc
 I  T  C  S  A  S  S  S  V  R  F  I  H  W  Y  Q  Q  K  P  G
aaagcgccgaaacgcctgatttatgataccagcaaactggcgagcggcgtgccgagccgc
 K  A  P  K  R  L  I  Y  D  T  S  K  L  A  S  G  V  P  S  R
tttagcggcagcggcagcggcaccgattttaccctgaccattagcagcctgcagccggaa
 F  S  G  S  G  S  G  T  D  F  T  L  T  I  S  S  L  Q  P  E
gattttgcgacctattattgccagcagtggggcagcagcccgtttacctttggccagggc
 D  F  A  T  Y  Y  C  Q  Q  W  G  S  S  P  F  T  F  G  Q  G
accaaagtggaaattaaaggcagcaccagcggcggcggcagcggcggcggcagcggcggc
 T  K  V  E  I  K  G  S  T  S  G  G  G  S  G  G  G  S  G  G
ggcggcagcagcgaagtgcagctggtggaatatggcggcggcctggtgcagccgggcggc
 G  G  S  S  E  V  Q  L  V  E  Y  G  G  G  L  V  Q  P  G  G
agcctgcgcctgagctgcgcggcgagcggctttaacattaaagattattatattcattgg
 S  L  R  L  S  C  A  A  S  G  F  N  I  K  D  Y  Y  I  H  W
gtgcgccaggcgccgggcaaaggcctggaatgggtggcgtggattgatccggaaaacggc
 V  R  Q  A  P  G  K  G  L  E  W  V  A  W  I  D  P  E  N  G
gataccgaatttgtgccgaaatttcagggccgcgcgaccatgagcgcggataccagcaaa
 D  T  E  F  V  P  K  F  Q  G  R  A  T  M  S  A  D  T  S  K
aacaccgcgtatctgcagatgaacagcctgcgcgcggaagataccgcggtgtattattgc
 N  T  A  Y  L  Q  M  N  S  L  R  A  E  D  T  A  V  Y  Y  C
aaaaccggcggcttttggggccagggcaccctggtgaccgtgagcagcgaactgaaaacc
 K  T  G  G  F  W  G  Q  G  T  L  V  T  V  S  S  E  L  K  T
ccgctgggcgataccacccatacctgcccgccgtgcccgccgtgcggcggcggcagcagc
 P  L  G  D  T  T  H  T  C  P  P  C  P  P  C  G  G  G  S  S
ggcggcggcagcggcggccagccgcgcgaaccgcaggtgtataccctgccgccgagccgc
 G  G  G  S  G  G  Q  P  R  E  P  Q  V  Y  T  L  P  P  S  R
gaagaaatgaccaaaaaccaggtgagcctgacctgcctggtgaaaggcttttatccgagc
 E  E  M  T  K  N  Q  V  S  L  T  C  L  V  K  G  F  Y  P  S
gatattgcggtggaatgggaaagcaacggccagccggaaaacaactataaaaccaccccg
 D  I  A  V  E  W  E  S  N  G  Q  P  E  N  N  Y  K  T  T  P
ccggtgctggatagcgatggcagcttttttctgtatagcaaactgaccgtggataaaagc
 P  V  L  D  S  D  G  S  F  F  L  Y  S  K  L  T  V  D  K  S
```

FIG. 64

cgctggcagcagggcaacgtgtttagctgcagcgtgatgcatgaagcgctgcataaccat

R W Q Q G N V F S C S V M H E A L H N H

tatacccagaaaagcctgagcctgagcccgggctga **(SEQ ID NO: 160)**

Y T Q K S L S L S P G Stop **(SEQ ID NO: 129)**

FIG. 64
continued

IAB1M γ1 EH8(M1) (SEQ ID NO: 130)

```
atggaaaccgataccctgctgctgtgggtgctgctgctgtgggtgccgggcagcaccggc
 M  E  T  D  T  L  L  L  W  V  L  L  L  W  V  P  G  S  T  G
gatattcagctgacccagagcccgagcaccctgagcgcgagcgtgggcgatcgcgtgacc
 D  I  Q  L  T  Q  S  P  S  T  L  S  A  S  V  G  D  R  V  T
attacctgcagcgcgagcagcagcgtgcgctttattcattggtatcagcagaaaccgggc
 I  T  C  S  A  S  S  S  V  R  F  I  H  W  Y  Q  Q  K  P  G
aaagcgccgaaacgcctgatttatgataccagcaaactggcgagcggcgtgccgagccgc
 K  A  P  K  R  L  I  Y  D  T  S  K  L  A  S  G  V  P  S  R
tttagcggcagcggcagcggcaccgattttaccctgaccattagcagcctgcagccggaa
 F  S  G  S  G  S  G  T  D  F  T  L  T  I  S  S  L  Q  P  E
gattttgcgacctattattgccagcagtggggcagcagcccgtttacctttggccagggc
 D  F  A  T  Y  Y  C  Q  Q  W  G  S  S  P  F  T  F  G  Q  G
accaaagtggaaattaaaggcagcaccagcggcggcggcagcggcggcggcagcggcggc
 T  K  V  E  I  K  G  S  T  S  G  G  G  S  G  G  G  S  G  G
ggcggcagcagcgaagtgcagctggtggaatatggcggcggcctggtgcagccgggcggc
 G  G  S  S  E  V  Q  L  V  E  Y  G  G  G  L  V  Q  P  G  G
agcctgcgcctgagctgcgcggcgagcggctttaacattaaagattattatattcattgg
 S  L  R  L  S  C  A  A  S  G  F  N  I  K  D  Y  Y  I  H  W
gtgcgccaggcgccgggcaaaggcctggaatgggtggcgtggattgatccggaaaacggc
 V  R  Q  A  P  G  K  G  L  E  W  V  A  W  I  D  P  E  N  G
gataccgaatttgtgccgaaatttcagggccgcgcgaccatgagcgcggataccagcaaa
 D  T  E  F  V  P  K  F  Q  G  R  A  T  M  S  A  D  T  S  K
aacaccgcgtatctgcagatgaacagcctgcgcgcggaagataccgcggtgtattattgc
 N  T  A  Y  L  Q  M  N  S  L  R  A  E  D  T  A  V  Y  Y  C
aaaaccggcggcttttggggccagggcaccctggtgaccgtgagcagcgaactgaaaacc
 K  T  G  G  F  W  G  Q  G  T  L  V  T  V  S  S  E  L  K  T
ccgctgggcgataccacccatacctgcccgccgtgcccgccgtgcccgccgtgcggcggc
 P  L  G  D  T  T  H  T  C  P  P  C  P  P  C  P  P  C  G  G
ggcagcagcggcggcggcagcggcggccagccgcgcgaaccgcaggtgtataccctgccg
 G  S  S  G  G  G  S  G  G  Q  P  R  E  P  Q  V  Y  T  L  P
ccgagccgcgaagaaatgaccaaaaaccaggtgagcctgacctgcctggtgaaaggcttt
 P  S  R  E  E  M  T  K  N  Q  V  S  L  T  C  L  V  K  G  F
tatccgagcgatattgcggtggaatgggaaagcaacggccagccggaaaacaactataaa
 Y  P  S  D  I  A  V  E  W  E  S  N  G  Q  P  E  N  N  Y  K
accaccccgccggtgctggatagcgatggcagcttttttctgtatagcaaactgaccgtg
 T  T  P  P  V  L  D  S  D  G  S  F  F  L  Y  S  K  L  T  V
```

FIG. 65A

gataaaagccgctggcagcagggcaacgtgtttagctgcagcgtgatgcatgaagcgctg

D K S R W Q Q G N V F S C S V M H E A L

cataaccattatacccagaaaagcctgagcctgagcccgggctga **(SEQ ID NO: 161)**

H N H Y T Q K S L S L S P G Stop **(SEQ ID NO: 130)**

FIG. 65A
continued

IAB25M γ2 NH (M1)

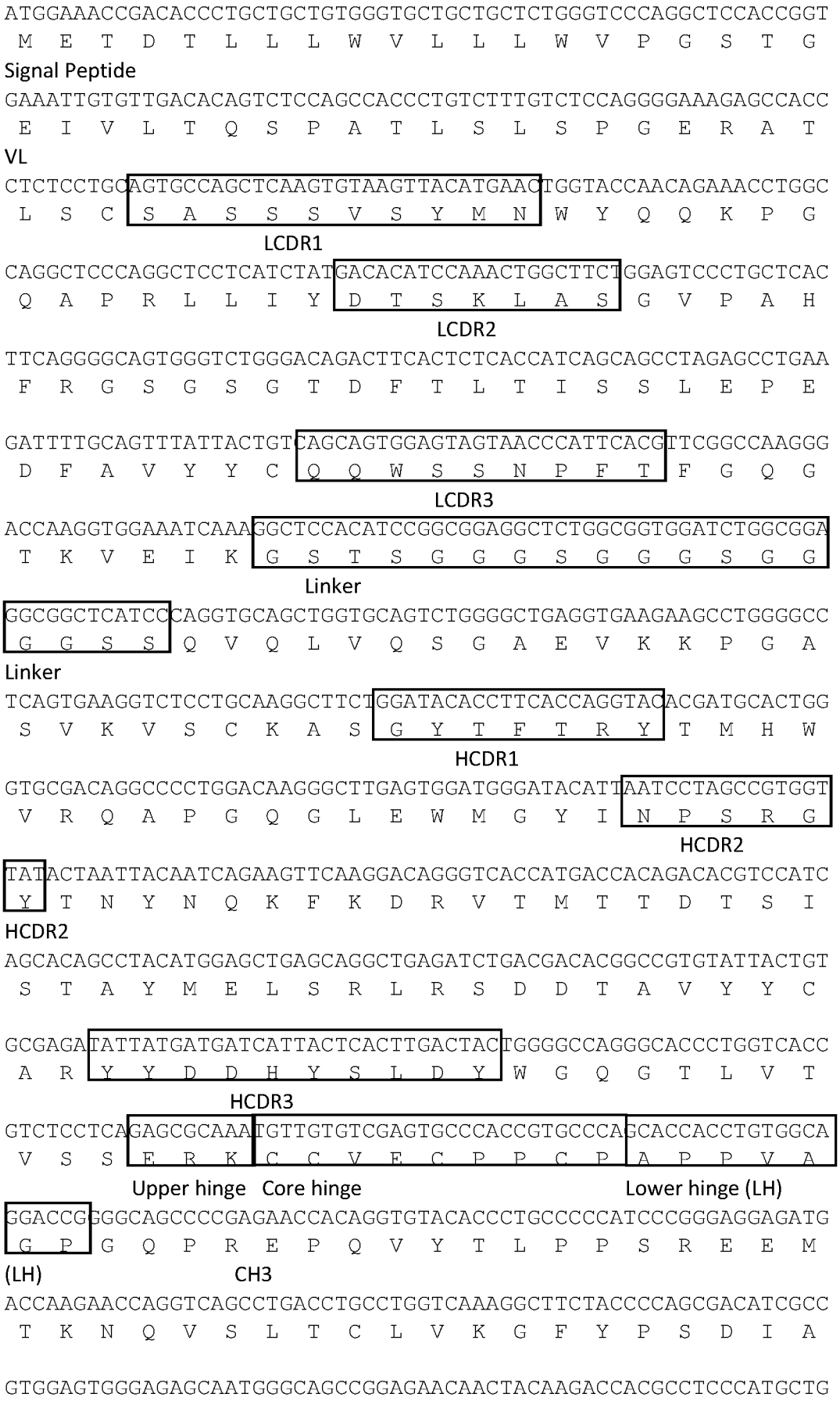


FIG. 65B

```
 V  E  W  E  S  N  G  Q  P  E  N  N  Y  K  T  T  P  P  M  L

GACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAG
 D  S  D  G  S  F  F  L  Y  S  K  L  T  V  D  K  S  R  W  Q

CAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAG
 Q  G  N  V  F  S  C  S  V  M  H  E  A  L  H  N  H  Y  T  Q

AAGAGCCTCTCCCTGTCTCCGGGTAAA (SEQ ID NO: 231)
 K  S  L  S  L  S  P  G  K  (SEQ ID NO: 232)
```

FIG. 65B
continued

IAB25M γ2 EH (M1)

```
ATGGAGACCGACACCCTCCTCCTGTGGGTCCTGCTGCTGTGGGTGCCTGGAAGCACCGGC
 M  E  T  D  T  L  L  L  W  V  L  L  L  W  V  P  G  S  T  G
```

Signal Peptide Sequence

```
GAGATTGTGCTGACCCAGTCCCCTGCCACCCTGAGCCTGTCCCCTGGAGAAAGAGCCACA
 E  I  V  L  T  Q  S  P  A  T  L  S  L  S  P  G  E  R  A  T
```

VL

```
CTGAGCTGTTCCGCCTCCTCCAGCGTGAGCTACATGAACTGGTACCAGCAGAAGCCCGGA
 L  S  C  S  A  S  S  S  V  S  Y  M  N  W  Y  Q  Q  K  P  G
```

LCDR1

```
CAGGCTCCCAGGCTGCTCATCTACGACACAAGCAAGCTGGCTAGCGGCGTGCCCGCTCAT
 Q  A  P  R  L  L  I  Y  D  T  S  K  L  A  S  G  V  P  A  H
```

LCDR2

```
TTCAGAGGCAGCGGAAGCGGCACAGATTTTACCCTGACCATTTCCTCCCTGGAGCCTGAG
 F  R  G  S  G  S  G  T  D  F  T  L  T  I  S  S  L  E  P  E

GACTTCGCCGTGTATTACTGCCAGCAGTGGAGCTCCAACCCCTTCACATTCGGCCAGGGC
 D  F  A  V  Y  Y  C  Q  Q  W  S  S  N  P  F  T  F  G  Q  G
```

LCDR3

```
ACCAAGGTGGAAATCAAGGGATCCACAAGCGGAGGCGGCAGCGGCGGCGGCAGCGGAGGC
 T  K  V  E  I  K  G  S  T  S  G  G  G  S  G  G  G  S  G  G
```

Linker

```
GGAGGCAGCAGCCAGGTGCAACTGGTGCAGAGCGGAGCCGAGGTGAAGAAGCCCGGAGCC
 G  G  S  S  Q  V  Q  L  V  Q  S  G  A  E  V  K  K  P  G  A
```

Linker VH

```
AGCGTGAAGGTGTCCTGCAAAGCCTCCGGATACACCTTCACCAGGTACACAATGCACTGG
 S  V  K  V  S  C  K  A  S  G  Y  T  F  T  R  Y  T  M  H  W
```

HCDR1

```
GTGAGGCAGGCTCCCGGCCAGGGCCTGGAGTGGATGGGATACATCAACCCCAGCAGGGGC
 V  R  Q  A  P  G  Q  G  L  E  W  M  G  Y  I  N  P  S  R  G
```

HCDR2

```
TACACCAACTATAACCAGAAGTTCAAGGACAGGGTGACCATGACCACCGACACCAGCATT
 Y  T  N  Y  N  Q  K  F  K  D  R  V  T  M  T  T  D  T  S  I
```

HCDR2

```
TCCACCGCTTATATGGAGCTCAGCAGACTGAGGTCCGACGACACCGCCGTGTACTACTGC
 S  T  A  Y  M  E  L  S  R  L  R  S  D  D  T  A  V  Y  Y  C

GCCAGGTATTACGACGACCACTACAGCCTGGACTACTGGGGCCAGGGAACACTCGTGACC
 A  R  Y  Y  D  D  H  Y  S  L  D  Y  W  G  Q  G  T  L  V  T
```

HCDR3

```
GTGTCCAGCGAGAGGAAGTGCTGCGTGGAATGTCCCCCTTGTCCTGGAGGCGGAAGCTCC
 V  S  S  E  R  K  C  C  V  E  C  P  P  C  P  G  G  G  S  S
```

Upper hinge Core hinge Lower hinge

```
GGAGGAGGATCCGGCGGACAGCCCAGGGAACCTCAGGTGTACACCCTGCCCCCCAGCAGG
 G  G  G  S  G  G  Q  P  R  E  P  Q  V  Y  T  L  P  P  S  R
```

Lower hinge CH3

```
GAGGAGATGACAAAGAACCAGGTGAGCCTGACCTGTCTGGTGAAGGGCTTCTACCCCAGC
 E  E  M  T  K  N  Q  V  S  L  T  C  L  V  K  G  F  Y  P  S
```

FIG. 65C

GACATCGCCGTGGAGTGGGAGAGCAACGGCCAGCCTGAGAACAACTACAAGACAACCCCC
D I A V E W E S N G Q P E N N Y K T T P

CCTATGCTGGATAGCGACGGCAGCTTCTTCCTGTACTCCAAGCTCACCGTGGATAAGAGC
P M L D S D G S F F L Y S K L T V D K S

AGGTGGCAGCAGGGAAACGTCTTTAGCTGTTCCGTGATGCACGAGGCCCTGCACAACCAC
R W Q Q G N V F S C S V M H E A L H N H

TACACCCAGAAGAGCCTCTCCCTGTCCCCCGGCAAG **(SEQ ID NO: 233)**
Y T Q K S L S L S P G K **(SEQ ID NO: 234)**

FIG. 65C
continued

IAB22M variable heavy (VH-K67R) sequence

VH

EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHFVRQAPGKGLEWIGRIDP
ANDNTLYASKFQGRATISADTSKNTAYLQMNSLRAEDTAVYYCGRGYGYYVFD
HWGQGTLVTVSS **(SEQ ID NO: 99)**

FIG. 66

IAB20M variable light (VL-Q79E, V83E)
VL
DIVMTQSPATLSVSPGERATLSCKASQSVSNDVAWYQQKPGQAPRLLISYTSSRY
AGVPDRFSGSGSGTDFTLTISSLEAEDEAVYYCQQDYNSPPTFGQGTKVEIK
(SEQ ID NO: 100)

IAB20M variable light (VL-A9D, T10S, S12A, P15L, L21I, S22N)
VL
DIVMTQSPDSLAVSLGERATINCKASQSVSNDVAWYQQKPGQAPRLLISYTSSRY
AGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQDYNSPPTFGQGTKVEIK
(SEQ ID NO: 101)

FIG. 67

IAB20M variable heavy (VH-Q1E, Q6E)
VH
EVQLVESGAEVKKPGASVKVSCKASGYSFTGYYMHWVRQAPGQGLEWIGRINP
NNGVTLYNQKFKDRVTMTVDTSISTAYMELSRLRSDDTAVYYCARSTMITNYV
MDYWGQGTLVTVSS **(SEQ ID NO: 102)**

FIG. 68

Anti-CD3 variable light (VL, R62S) sequence
EIVLTQSPATLSLSPGERATLSCSASSSVSYMNWYQQKPGQAPRLLIYDTSKLASGVP
AHFSGSGSGTDFTLTISSLEPEDFAVYYCQQWSSNPFTFGQGTKVEIK **(SEQ ID NO: 103)**

Anti-CD3 variable heavy (VH, Q1E, Q6E) sequence
EVQLVESGAEVKKPGASVKVSCKASGYTFTRYTMHWVRQAPGQGLEWMGYINPSRGYT
NYNQKFKDRVTMTTDTSISTAYMELSRLRSDDTAVYYCARYYDDHYSLDYWGQGTLVT
VSS **(SEQ ID NO: 104)**

FIG. 69

PSMA (Prostate-Specific Membrane Antigen), Glutamate carboxypeptidase 2, Homo sapiens
UniProtKB/SwissProt: Q04609 (SEQ ID NO: 131)

MWNLLHETDSAVATARRPRWLCAGALVLAGGFFLLGFLFGWFIKSSNEATNITPKHNMKAFLDELKAENI

KKFLYNFTQIPHLAGTEQNFQLAKQIQSQWKEFGLDSVELAHYDVLLSYPNKTHPNYISIINEDGNEIFN

TSLFEPPPPGYENVSDIVPPFSAFSPQGMPEGDLVYVNYARTEDFFKLERDMKINCSGKIVIARYGKVFR

GNKVKNAQLAGAKGVILYSDPADYFAPGVKSYPDGWNLPGGGVQRGNILNLNGAGDPLTPGYPANEYAYR

RGIAEAVGLPSIPVHPIGYYDAQKLLEKMGGSAPPDSSWRGSLKVPYNVGPGFTGNFSTQKVKMHIHSTN

EVTRIYNVIGTLRGAVEPDRYVILGGHRDSWVFGGIDPQSGAAVVHEIVRSFGTLKKEGWRPRRTILFAS

WDAEEFGLLGSTEWAEENSRLLQERGVAYINADSSIEGNYTLRVDCTPLMYSLVHNLTKELKSPDEGFEG

KSLYESWTKKSPSPEFSGMPRISKLGSGNDFEVFFQRLGIASGRARYTKNWETNKFSGYPLYHSVYETYE

LVEKFYDPMFKYHLTVAQVRGGMVFELANSIVLPFDCRDYAVVLRKYADKIYSISMKHPQEMKTYSVSFD

SLFSAVKNFTEIASKFSERLQDFDKSNPIVLRMMNDQLMFLERAFIDPLGLPDRPFYRHVIYAPSSHNKY

AGESFPGIYDALFDIESKVDPSKAWGEVKRQIYVAAFTVQAAAETLSEVA **(SEQ ID NO: 131)**

FIG. 70

PSCA (Prostate Stem Cell Antigen), Homo sapiens
UniProtKB/SwissProt: O43653 (SEQ ID NO: 132)

MKAVLLALLMAGLALQPGTALLCYSCKAQVSNEDCLQVENCTQLGEQCWTARIRAVGLLTVISKGCSLNC

VDDSQDYYVGKKNITCCDTDLCNASGAHALQPAAAILALLPALGLLLWGPGQL **(SEQ ID NO: 132)**

FIG. 71

5T4 (gi|435655|e5T4 oncofetal trophoblast glycoprotein, Homo sapiens UniProtKB/SwissProt: Q13641 (SEQ ID NO: 133)

MPGGCSRGPAAGDGRLRLARLALVLLGWVSSSSPTSSASSFSSSAPFLASAVSAQPPLPDQCPALCECSE

AARTVKCVNRNLTEVPTDLPAYVRNLFLTGNQLAVLPAGAFARRPPLAELAALNLSGSRLDEVRAGAFEH

LPSLRQLDLSHNPLADLSPFAFSGSNASVSAPSPLVELILNHIVPPEDERQNRSFEGMVVAALLAGRALQ

GLRRLELASNHFLYLPRDVLAQLPSLRHLDLSNNSLVSLTYVSFRNLTHLESLHLEDNALKVLHNGTLAE

LQGLPHIRVFLDNNPWVCDCHMADMVTWLKETEVVQGKDRLTCAYPEKMRNRVLLELNSADLDCDPILPP

SLQTSYVFLGIVLALIGAIFLLVLYLNRKGIKKWMHNIRDACRDHMEGYHYRYEINADPRLTNLSSNSDV

(SEQ ID NO: 133)

FIG. 72

T-cell surface glycoprotein CD8 alpha chain, Homo sapiens
UniProtKB/SwissProt: P01732 (SEQ ID NO: 134)

MALPVTALLLPLALLLHAARPSQFRVSPLDRTWNLGETVELKCQVLLSNPTSGCSWLFQPRGAAASPTFL
LYLSQNKPKAAEGLDTQRFSGKRLGDTFVLTLSDFRRENEGYYFCSALSNSIMYFSHFVPVFLPAKPTTT
PAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCNHRN
RRRVCKCPRPVVKSGDKPSLSARYV **(SEQ ID NO: 134)**

FIG. 73

T-cell surface glycoprotein CD8 beta chain, Homo sapiens
UniProtKB/SwissProt: P10966 (SEQ ID NO: 135)

MRPRLWLLLAAQLTVLHGNSVLQQTPAYIKVQTNKMVMLSCEAKISLSNMRIYWLRQRQAPSSDSHHEFLALWDSAK

GTIHGEEVEQEKIAVFRDASRFILNLTSVKPEDSGIYFCMIVGSPELTFGKGTQLSVVDFLPTTAQPTKKSTLKKRV

CRLPRPETQKGPLCSPVTLGLLVAGVLVLLVSLGVAMHLCCRRRRARLRFMKQFYK **(SEQ ID NO: 135)**

FIG. 74

T-cell surface glycoprotein CD3 delta chain, Homo sapiens
UniProtKB/SwissProt: P04234 (SEQ ID NO: 136)

MEHSTFLSGLVLATLLSQVSPFKIPIEELEDRVFVNCNTSITWVEGTVGTLLSDITRLDLGKRILDPRGI

YRCNGTDIYKDKESTVQVHYRMCQSCVELDPATVAGIIVTDVIATLLLALGVFCFAGHETGRLSGAADTQ

ALLRNDQVYQPLRDRDDAQYSHLGGNWARNK **(SEQ ID NO: 136)**

FIG. 75

T-cell surface glycoprotein CD3 gamma chain, Homo sapiens
UniProtKB/SwissProt: P09693 (SEQ ID NO: 137)

MEQGKGLAVLILAIILLQGTLAQSIKGNHLVKVYDYQEDGSVLLTCDAEAKNITWFKDGKMIGFLTEDKK

KWNLGSNAKDPRGMYQCKGSQNKSKPLQVYYRMCQNCIELNAATISGFLFAEIVSIFVLAVGVYFIAGQD

GVRQSRASDKQTLLPNDQLYQPLKDREDDQYSHLQGNQLRRN **(SEQ ID NO: 137)**

FIG. 76

T-cell surface glycoprotein CD3 epsilon chain
UniProtKB/SwissProt: P07766 (SEQ ID NO: 138)

MQSGTHWRVL GLCLLSVGVW GQDGNEEMGG ITQTPYKVSI SGTTVILTCP
QYPGSEILWQ HNDKNIGGDE DDKNIGSDED HLSLKEFSEL EQSGYYVCYP
RGSKPEDANF YLYLRARVCE NCMEMDVMSV ATIVIVDICI TGGLLLLVYY
WSKNRKAKAK PVTRGAGAGG RQRGQNKERP PPVPNPDYEP IRKGQRDLYS
GLNQRRI **(SEQ ID NO: 138)**

FIG. 77

T-cell surface glycoprotein CD3 zeta chain, Homo sapiens
UniProtKB/SwissProt: P20963 (SEQ ID NO: 139)

MKWKALFTAA ILQAQLPITE AQSFGLLDPK LCYLLDGILF IYGVILTALF

LRVKFSRSAD APAYQQGQNQ LYNELNLGRR EEYDVLDKRR GRDPEMGGKP

QRRKNPQEGL YNELQKDKMA EAYSEIGMKG ERRRGKGHDG LYQGLSTATK

DTYDALHMQA LPPR **(SEQ ID NO: 139)**

FIG. 78

IAB25M γ2 EH2 (N- to C-terminal)

IAB25 variable light (VL) sequence
EIVLTQSPATLSLSPGERATLSCSASSSVSYMNWYQQKPGQAPRLLIYDTSKLASGVPAHFRGSGSGTDFTLTISSLEPE
DFAVYYCQQWSSNPFTFGQGTKVEIK (SEQ ID NO: 19)

Linker
GSTSGGGSGGGSGGGGSS (SEQ ID NO: 62)

IAB25 variable heavy (VH) sequence
QVQLVQSGAEVKKPGASVKVSCKASGYTFTRYTMHWVRQAPGQGLEWMGYINPSRGYTNYNQKFKDRVTMTTDTSIS
TAYMELSRLRSDDTAVYYCARYYDDHYSLDYWGQGTLVTVSS (SEQ ID NO: 20)

Full hinge (EH2)
ERKSCVECPPCPGGGSSGGGSG (SEQ ID NO: 34)

Upper hinge
ERK (SEQ ID NO: 47)

Core hinge
SCVECPPCP (SEQ ID NO: 56)

Lower hinge
GGGSSGGGSG (SEQ ID NO: 59)

Human IgG2 CH3
GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQ
QGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 42)

Human IgG2 CH3 T26S, L28A, M57V
GQPREPQVYTLPPSREEMTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRW
QQGNVFSCSVMHEALHNHYTQKSLSLSPG (SEQ ID NO: 181)

Human IgG2 CH3 T26W
GQPREPQVYTLPPSREEMTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW
QQGNVFSCSVMHEALHNHYTQKSLSLSPG (SEQ ID NO: 182)

FIG. 79

IAB20M γ2 EH2 (N- to C-terminal)

Anti-5T4 variable light (VL) sequence
DIVMTQSPATLSVSPGERATLSCKASQSVSNDVAWYQQKPGQAPRLLISYTSSRYAGVPDRFSGSGSGTDFTLTISSLQA
EDVAVYYCQQDYNSPPTFGQGTKVEIK (SEQ ID NO: 17)

Linker
GSTSGGGSGGGSGGGGSS (SEQ ID NO: 62)

Anti-5T4 variable heavy (VH) sequence
QVQLVQSGAEVKKPGASVKVSCKASGYSFTGYYMHWVRQAPGQGLEWIGRINPNNGVTLYNQKFKDRVTMTVDTSIST
AYMELSRLRSDDTAVYYCARSTMITNYVMDYWGQGTLVTVSS (SEQ ID NO: 18)

Full hinge (EH2)
ERKSCVECPPCPGGGSSGGGGSG (SEQ ID NO: 34)

Upper hinge
ERK (SEQ ID NO: 47)

Core hinge
SCVECPPCP (SEQ ID NO: 56)

Lower hinge
GGGSSGGGSG (SEQ ID NO: 59)

Human IgG2 CH3
GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQ
QGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 42)

Human IgG2 CH3 T26S, L28A, M57V
GQPREPQVYTLPPSREEMTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQ
QGNVFSCSVMHEALHNHYTQKSLSLSPG (SEQ ID NO: 181)

Human IgG2 CH3 T26W
GQPREPQVYTLPPSREEMTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQ
QGNVFSCSVMHEALHNHYTQKSLSLSPG (SEQ ID NO: 182)

FIG. 80

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 16/45580

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 39/395, C07K 16/00 (2016.01)
CPC - A61K 39/39591, C07K 16/00, C07K 16/24, C07K 2317/40, C07K 2317/52, C07K 2317/522
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8) - A61K 39/395, C07K 16/00 (2016.01)
CPC - A61K 39/39591, C07K 16/00, C07K 16/24, C07K 2317/40, C07K 2317/52, C07K 2317/522

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
CPC - C07K 2317/53, C07K 2317/94
USPC- 530/387.1, 424/133.1, 435/320.1, 435/328

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PubWEST(PGPB,USPT,USOC,EPAB,JPAB); PatBase, Google/Scholar: IMAGINAB, INC; GUDAS, Jean; OLAFSEN, Tove; SATPAYEV, Daulet; TORGOV, Michael, minibody, hinge, upper, middle, core, IAB22M [gamma]3/[gamma]1 EH6
GenCore 6.4.1; SEQ ID NO: 35, 48, 57, 191, wherein Xaa1 is Ala

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2013/0323236 A1 (Humphreys, et al.) 05 December 2013 (05.12.2013) para [0137], SEQ ID NO 284, amino acids 4-11	1-3, 13
X	US 2014/0286951 A1 (OncoMed Pharmaceuticals, Inc.) 25 September 2014 (25.09.2014) para [0403], Table 2, SEQ ID NO: 46, amino acids 1-18, 100% identity to SEQ ID NO:214; amino acids 20-26, 100% identity to SEQ ID NO: 60	33-35, 53, (54,58)/53
A	US 2014/0302035 A1 (ADIMAB, LLC, et al.) 09 October 2014 (09.10.2014) Abstract, Fig 2D, SEQ ID NO:7, wilde type; para [0433], Table 2, SEQ ID NO: 8, 100% identity to SEQ ID NO:48; para [0504]	52, (54,58)/52

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
15 December 2016	05 JAN 2017

Name and mailing address of the ISA/US	Authorized officer:
Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 16/45580

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 4-12, 14-32, 36-51, 55-57, 59-75, 83-85
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I+: claims 1-3, 13, 33-35, 52-54, 58 directed to a hinge region polypeptide. The hinge region polypeptide will be searched to the extent that a core hinge sequence encompasses SEQ ID NO:57 and upper hinge sequence encompasses SEQ ID NO:48. It is believed that claims 1-3, 13, 33-35, 52-54, 58 encompass this first named invention, and thus these claims will be searched without fee to the extent that they encompass core hinge sequence SEQ ID NO:57 and upper hinge sequence SEQ ID NO:48. Additional specific combination(s) of a specific core hinge sequence and upper hinge sequence will be searched upon the payment of additional fees. Applicants must specify the claims that encompass any additionally elected combination(s) of a specific core hinge sequence and upper hinge sequence.

********** See Supplemental Sheet to continue **********

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-3, 13, 33-35, 52-54, 58, restricted to SEQ ID NO:57 and SEQ ID NO:48

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 16/45580

In Continuation of Box III. Observations where unity of invention is lacking:

Applicants must further indicate, if applicable, the claims which encompass the first named invention, if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched. An exemplary election would be a hinge region polypeptide comprising a core hinge sequence of SEQ ID NO:52 and upper hinge sequence encompasses SEQ ID NO:46, i.e., claims 1-3, 13, 33-35, 52-54, 58.

Group II: claims 76-82, directed to a minibody.

The inventions listed as Groups I+ and II do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Special Technical Features
The special technical feature of some inventions of Group I+ is a specific combination of a specific core hinge sequence and upper hinge sequence recited therein.

The inventions of Group I+ do not include the shared or common technical feature of a minibody, as required by Group II.

Common Technical Features
The inventions of Groups I+ and II share the technical feature of a hinge domain. Some inventions of Groups I+ and II share the technical feature of a hinge region comprising a core hinge sequence comprising a CPPCP motif and an upper hinge sequence. However, this shared technical feature does not represent a contribution over prior art as being anticipated by US 2014/0086903 A1 to TAN, et al. (27 March 2014) (hereinafter "Tan").
Tan discloses a hinge domain comprising the CPPCP motif (para [0182], TABLE 2, "Exemplary Hinge Regions for CD37-Specific Binding Proteins", SEQ ID NO: 90 (EPKSCDKTHTCPPCP) and SEQ ID NO:92 (EPKSSDKTHTCPPCP).).
Tan further discloses a hinge region comprising a core hinge sequence and an upper hinge sequence (para [0069], "An exemplary wild type immunoglobulin hinge region is human IgG1 hinge region as set forth in SEQ ID NO:90, in which from its amino terminus to its carboxyl terminus, the first ten amino acids (EPKSCDKTHT, SEQ ID NO:263) form the upper hinge region, the next four amino acids (CPPC, SEQ ID NO:264) form the core hinge region, and the last amino acid (i.e., proline) is the first amino acid in the lower hinge region"). As said technical features were known in the art at the time of the invention, this cannot be considered special technical feature that would otherwise unify the inventions.

Groups I+ and II therefore lack unity under PCT Rule 13 because they do not share a same or corresponding special technical feature.