



(51) International Patent Classification:

A61K 38/00 (2006.01) C07K 16/10 (2006.01)
A61K 39/395 (2006.01) C07K 16/46 (2006.01)
A61K 39/42 (2006.01) C07K 19/00 (2006.01)

(21) International Application Number:

PCT/US2017/051877

(22) International Filing Date:

15 September 2017 (15.09.2017)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

62/395,788 16 September 2016 (16.09.2016) US

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(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK,

(54) Title: BISPECIFIC MOLECULES COMPRISING AN HIV-1 ENVELOPE TARGETING ARM

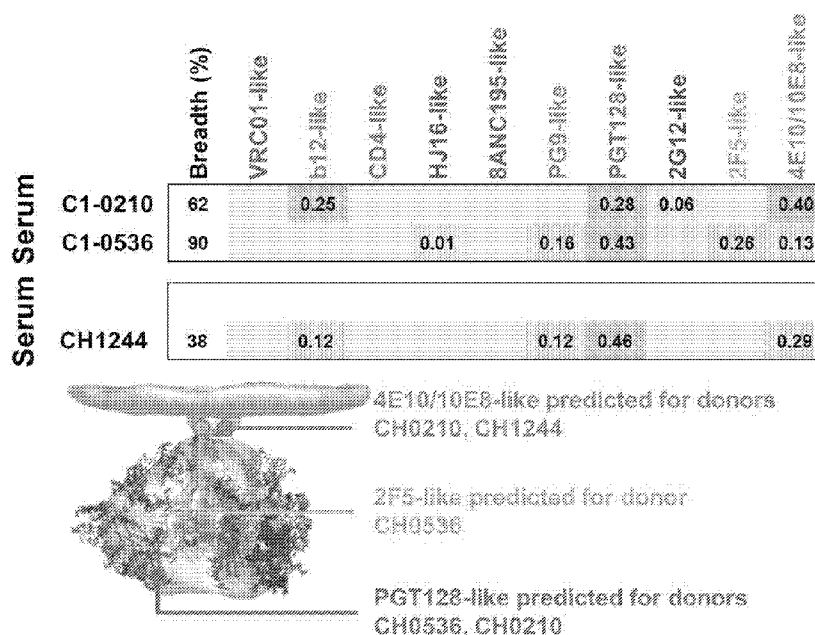


Figure 1

(57) Abstract: The invention is directed to multispecific molecules comprising an HIV-1 envelope targeting arm and an arm targeting an effector cell, compositions comprising these molecules and methods of use.



EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV,
MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM,
TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW,
KM, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*

Bispecific Molecules Comprising an HIV-1 Envelope Targeting Arm

[0001] This invention claims the benefit of and priority to U.S. Serial No. 62/395,788, filed September 16, 2016, the contents of which is hereby incorporated by reference in its entirety.

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[0003] All patents, patent applications and publications cited herein are hereby incorporated by reference in their entirety. The disclosure of these publications in their entireties are hereby incorporated by reference into this application in order to more fully describe the state of the art as known to those skilled therein as of the date of the invention described herein.

FIELD OF THE INVENTION

[0004] The invention is directed to multispecific molecules, such as but not limited to bispecific and trispecific molecules (*e.g.* bispecific antibodies, bispecific diabodies, and trivalent binding molecules) comprising an HIV-1 binding domain and an effector cell binding domain, and their uses.

BACKGROUND

[0005] Highly Active Antiretroviral Therapy (HAART) has been effective in reducing the viral burden and ameliorating the effects of HIV-1 infection in infected individuals. However, despite this therapy the virus persists in the individual due to latent reservoir of HIV-1 infected cells which evade this treatment. Thus, there is a need for therapeutic agents for treatment of HIV-1 infected individuals, as well as agents that target virus infected cells and have the potential to reduce the latent reservoir of HIV-1 infected cells.

GOVERNMENT SUPPORT

[0006] This invention was made with government support under Grant No. UM1AI126619 awarded by National Institute of Allergy and Infectious Diseases. The Government has certain rights to the invention.

SUMMARY OF THE INVENTION

[0007] In some aspects the invention is directed to multispecific molecules, such as but not limited to bispecific and trispecific molecules (*e.g.*, bispecific antibodies, bispecific diabodies, trivalent binding molecules, *etc.*) which comprise epitope-binding fragments of antibodies

(*e.g.*, VL and VH Domains) that enable them to coordinately bind immunospecifically to at least one target on HIV-1 envelope (*e.g.* but not limited to a membrane-proximal extracellular region of HIV-1 gp41 epitope) and at least one epitope of a second molecule that is not HIV-1 *Env*, for example but not limited to an effector cell which expresses CD2, CD3, CD8, CD16, T-Cell Receptor (TCR), NKG2D, *etc.* epitope. In some embodiments, the MPER binding specificity of the molecules of the invention is different from the specificity of the MPER antibody 10E8.

[0008] Selection of the VL and VH Domains of the polypeptide domains of the multispecific molecules of the invention is coordinated so that the polypeptides chains that make up such multispecific molecules assemble to form at least one functional epitope-binding site that is specific for at least one epitope of HIV-1 *Env* and at least one functional epitope-binding site that is specific for at least one epitope of a molecule that is not HIV-1 *Env*. In some embodiments, the multispecific molecules of the invention comprise an Fc Domain (Fc bearing multispecific molecules of the invention).

[0009] In certain aspects, the invention provides multispecific molecules comprise an epitope-binding site that specifically binds the membrane-proximal extracellular region (MPER) of HIV-1 gp41. In non-limiting embodiments the multispecific molecules comprise the MPER binding portions from an antibody from Figure 13, Figure 55, Figure 56, Figure 57 or Figures 30-33 (antibodies with mutations in the DH512 or DH511 VH chain). In non-limiting embodiments, combination mutations in the DH512 or DH511 VHCDR3 could include VH_L100dF together with T100aW (Figures 31 and 32); VH_L100dW together with T100aW (Figures 31 and 32). In some embodiments, the MPER binding portions of the multispecific molecule do not comprise portions for the antibody 10E8.

[0010] Non-limiting examples include multispecific molecules comprising VH or VL chains from DH511, DH512, DH512_K3, DH512-L100dF, DH513, DH514, DH515, DH516, DH517, DH518, DH536, DH537, lineage members, chimeric and mutant antibodies with non-limiting embodiments described in Examples 10, 11 or 12.

[0011] In non-limiting embodiments, the multispecific molecules comprise 1, 2 or all 3 of the **CDR_{HS}** of a VH Domain with the specificity of the gp41 MPER binding antibody DH511, DH512, DH512_K3, DH512-L100dF, DH513, DH514, DH515, DH516, DH517, DH518, DH536, DH537, lineage members, chimeric and mutant antibodies with non-limiting embodiments described in Example 11, and/or 1, 2 or all 3 of the **CDR_{LS}** of a VL Domain of

the gp41 MPER binding antibody DH511, DH512, DH512_K3, DH512-L100dF, DH513, DH514, DH515, DH516, DH517, DH518, DH536, DH537, lineage members, chimeric and mutant antibodies with non-limiting embodiments described in Examples 10, 11 or 12.

[0012] In non-limiting embodiments, the multispecific molecules comprise the VH Domain with the specificity of the gp41 MPER binding antibody DH511, DH512, DH512_K3, DH512-L100dF, DH513, DH514, DH515, DH516, DH517, DH518, DH536, DH537, lineage members, chimeric and mutant antibodies with non-limiting embodiments described in Example 11, and/or the VL Domain, of the gp41 MPER binding antibody DH511, DH512, DH512_K3, DH512-L100dF, DH513, DH514, DH515, DH516, DH517, DH518, DH536, DH537, lineage members, chimeric and mutant antibodies with non-limiting embodiments described in Examples 10, 11 or 12.

[0013] In certain embodiments an antibody (or a molecule comprising the CDRs, or the variable domains of such antibody) binds specifically to a particular target, peptide, or polysaccharide (such as an antigen present on the surface of a pathogen, for example gp120, gp41, or CD3), even where the specific epitope may not be known, and do not bind in a significant amount to other proteins or polysaccharides present in the sample or subject. Specific binding can be determined by methods known in the art. Various competitive binding assays are known in the art. With reference to an antibody antigen complex, in certain embodiments specific binding of the antigen and antibody has a K_D of less than about 10^6 Molar, such as less than about 10^6 Molar, 10^7 Molar, 10^8 Molar, 10^9 , or even less than about 10^{10} Molar.

[0014] In some aspects, the present invention is directed to bispecific molecules, *e.g.* covalently linked polypeptide chains to form bispecific antibodies, covalently linked diabodies and/or trivalent binding molecules and their use in the treatment of HIV-1. In certain aspects, the bispecific molecules of the present invention can bind to two different targets or epitopes on two different cells wherein the first epitope is expressed on a different cell type than the second epitope, such that the bispecific molecules can bring the two cells together. In certain aspects, the bispecific molecules of the present invention can bind to two different cells, wherein the bispecific molecules comprises an arm with the binding specificity for an HIV-1 envelope, for example as provided by the binding specificity of the gp41 MPER binding antibody DH511.2, a variant of DH511.2 called DH511.2_K3 and/or other antibodies from the DH511 lineage, and/or provided by any of the antibodies DH511, DH513, DH514,

DH515, DH516, DH517, DH518, DH536, or DH537, which arm binds to the HIV-1 envelope expressed on a first cell, *e.g.* HIV-1 infected cell, and a second arm with the binding specificity for an epitope expressed on a different cell type than the first cell, such that the bispecific molecules can bring the two cells together. In certain embodiment, the second cell is in effector cell which expresses CD2, CD3, CD8, CD16, T-Cell Receptor (TCR), NKG2D, *etc.* epitope.

[0015] In certain aspects, the invention provides a bispecific molecule comprising a first polypeptide chain and a second polypeptide chain, covalently bonded to one another, wherein:

(I) the first polypeptide chain comprises in the N- to C-terminal direction:

- (i)** a domain (A) comprising a binding region of the light chain variable domain of a first immunoglobulin (VL1) having the binding specificity of a gp41 MPER binding HIV-1 antibody (1);
- (ii)** a domain (B) comprising a binding region of a heavy chain variable domain of a second immunoglobulin (VH2) specific for an epitope (2); and
- (iii)** a domain (C) comprising a heterodimer promoting domain;

(II) the second polypeptide chain comprises in the N- to C-terminal direction:

- (i)** a domain (D) comprising a binding region of a light chain variable domain of the second immunoglobulin (VL2) specific for the epitope (2);
- (ii)** a domain (E) comprising a binding region of a heavy chain variable domain of the first immunoglobulin (VH1) having the binding specificity of a gp41 MPER binding HIV-1 antibody (1); and
- (iii)** a domain (F) comprising a heterodimer promoting domain; and wherein:

the domains (A) and (B) are linked so that they do not associate with one another to form an epitope binding site;

the domains (D) and (E) are linked so that they do not associate with one another to form an epitope binding site; and

the domains (A) and (E) associate to form a binding site that binds the HIV-1 envelope like a gp41 MPER binding HIV-1 antibody (1); and

the domains (B) and (D) associate to form a binding site that binds the epitope (2).

[0016] In certain aspects the invention provides such bispecific molecules wherein:

- (i)** domains (A) and (B) are separated by a peptide linker 1;
- (ii)** domains (C) and (B) are separated by a peptide linker 2;

- (iii) domains (D) and (E) are separated by a peptide linker 1; and
- (iv) domains (F) and (E) are separated by a peptide linker 2.

[0017] In certain aspects the invention provides bispecific molecules comprising a first polypeptide chain and a second polypeptide chain, covalently bonded to one another, wherein:

(I) the first polypeptide chain comprises in the N- to C-terminal direction:

- (i) a domain (A) comprising a binding region of the light chain variable domain of a first immunoglobulin (VL1) having the binding specificity of the gp41 MPER binding antibody DH511.2, a variant of DH511.2 called DH511.2_K3, or any antibody of the DH511 lineage (1);
- (ii) a domain (B) comprising a binding region of a heavy chain variable domain of a second immunoglobulin (VH2) specific for an epitope (2), wherein domains (A) and (B) are separated from one another by a peptide linker 1; and
- (iii) a domain (C) comprising a heterodimer promoting domain, including but not limited to a K coil or E coil; wherein domain (C) and domain (B) are separated by a peptide linker 2;

(II) the second polypeptide chain comprises in the N- to C-terminal direction:

- (i) a domain (D) comprising a binding region of a light chain variable domain of the second immunoglobulin (VL2) specific for the epitope (2);
- (ii) a domain (E) comprising a binding region of a heavy chain variable domain of the first immunoglobulin (VH1) having the binding specificity of the gp41 MPER binding antibody DH511.2, a variant of DH511.2 called DH511.2_K3, or any antibody of the DH511 lineage (1), wherein domains (D) and (E) are separated from one another by a peptide linker 1; and
- (iii) a domain (F) comprising a heterodimer promoting domain, including but not limited to a K coil or E coil; wherein domain (F) and domain (E) are separated by a peptide linker 2; and wherein:

the domains (A) and (B) do not associate with one another to form an epitope binding site;

the domains (D) and (E) do not associate with one another to form an epitope binding site;

and

the domains (A) and (E) associate to form a binding site that binds the HIV-1 envelope like the gp41 MPER binding antibody DH511.2, a variant of DH511.2 called DH511.2_K3, or any

antibody of the DH511 lineage (1); and the domains (B) and (D) associate to form a binding site that binds the epitope (2).

[0018] In certain aspects the invention provides such bispecific molecules, wherein the first or second polypeptide chain further comprises an Fc Domain. The invention also provides such bispecific molecules wherein the first or second polypeptide chain further comprises an Fc Domain and the bispecific molecule further comprises a third polypeptide chain.

[0019] In certain aspects, the invention provides bispecific molecules comprising a first polypeptide chain and a second polypeptide chain, covalently bonded to one another, wherein:

(I) the first polypeptide chain comprises in the N- to C-terminal direction:

(i) a domain (A) comprising SEQ ID NO: 553, 570, 574, 578, 582, 586, 590, 594, 598, 602, 606, or 625;

(ii) a domain (B) comprising SEQ ID NO: 500 or 504; and

(iii) a domain (C) comprising SEQ ID NO: 520, 521, 518, or 519;

(II) the second polypeptide chain comprises in the N- to C-terminal direction:

(i) a domain (D) comprising SEQ ID NO: 502, or 506;

(ii) a domain (E) comprising SEQ ID NO: 551, 568, 572, 576, 580, 584, 588, 592, 596, 600, 604, 608-624, or 626 (Table 4); and

(iii) a domain (F) comprising SEQ ID NO: 518, 519, 520, or 521; and wherein:

the domains (A) and (B) are linked so that they do not associate with one another to form an epitope binding site;

the domains (D) and (E) are linked so that they do not associate with one another to form an epitope binding site; and

the domains (A) and (E) associate to form a binding site that binds the HIV-1 envelope like a gp41 MPER binding antibody (1); and

the domains (B) and (D) associate to form a binding site that binds an epitope (2).

[0020] In certain aspects the invention provides such bispecific molecules wherein:

(i) domains (A) and (B) are separated by SEQ ID NO: 508;

(ii) domains (C) and (B) are separated by SEQ ID NO: 509 or 510;

(iii) domains (D) and (E) are separated by SEQ ID NO: 508; and

(iv) domains (F) and (E) are separated by SEQ ID NO: 509 or 510.

[0021] In certain aspects the invention provides bispecific molecules comprising a first polypeptide chain and a second polypeptide chain, covalently bonded to one another, wherein:

(I) the first polypeptide chain comprises in the N- to C-terminal direction:

(i) a domain (A) comprising SEQ ID NO: 553, 570, 574, 578, 582, 586, 590, 594, 598, 602, 606, or 625;

(ii) a domain (B) comprising SEQ ID NO: 500 or 504, wherein domains (A) and (B) are separated from one another by SEQ ID NO: 508; and

(iii) a domain (C) comprising SEQ ID NO: 520, 521, 518, or 519; wherein domain (C) and domain (B) are separated by SEQ ID NO: 509 or 510;

(II) the second polypeptide chain comprises in the N- to C-terminal direction:

(i) a domain (D) comprising SEQ ID NO: 502, or 506;

(ii) a domain (E) comprising SEQ ID NO: 551, 568, 572, 576, 580, 584, 588, 592, 596, 600, 604 608-624, or 626 (Table 4), wherein domains (D) and (E) are separated from one another by SEQ ID NO: 508; and

(iii) a domain (F) comprising SEQ ID NO: 518, 519, 520, or 521; wherein domain (F) and domain (E) are separated by SEQ ID NO: 509 or 510; and wherein:

the domains (A) and (B) do not associate with one another to form an epitope binding site;

the domains (D) and (E) do not associate with one another to form an epitope binding site;

and

wherein if the domain (A) comprises SEQ ID NO: 553 and domain (E) comprises SEQ ID NO: 551 they associate to form a binding site that binds the HIV-1 envelope like antibody DH511.2_K3;

wherein if the domain (A) comprises SEQ ID NO: 570 and domain (E) comprises SEQ ID NO: 568 they associate to form a binding site that binds the HIV-1 envelope like antibody DH511;

wherein if the domain (A) comprises SEQ ID NO: 574 and domain (E) comprises SEQ ID NO: 572 they associate to form a binding site that binds the HIV-1 envelope like antibody DH512 (also referred to as DH511.2);

wherein if the domain (A) comprises SEQ ID NO: 578 and domain (E) comprises SEQ ID NO: 576 they associate to form a binding site that binds the HIV-1 envelope like antibody DH513;

wherein if the domain (A) comprises SEQ ID NO: 582 and domain (E) comprises SEQ ID NO: 580 they associate to form a binding site that binds the HIV-1 envelope like antibody DH514;

wherein if the domain (A) comprises SEQ ID NO: 586 and domain (E) comprises SEQ ID NO: 584 they associate to form a binding site that binds the HIV-1 envelope like antibody DH515;

wherein if the domain (A) comprises SEQ ID NO: 590 and domain (E) comprises SEQ ID NO: 588 they associate to form a binding site that binds the HIV-1 envelope like antibody DH516;

wherein if the domain (A) comprises SEQ ID NO: 594 and domain (E) comprises SEQ ID NO: 592 they associate to form a binding site that binds the HIV-1 envelope like antibody DH517;

wherein if the domain (A) comprises SEQ ID NO: 598 and domain (E) comprises SEQ ID NO: 596 they associate to form a binding site that binds the HIV-1 envelope like antibody DH518;

wherein if the domain (A) comprises SEQ ID NO: 602 and domain (E) comprises SEQ ID NO: 600 they associate to form a binding site that binds the HIV-1 envelope like antibody DH536; or

wherein if the domain (A) comprises SEQ ID NO: 606 and domain (E) comprises SEQ ID NO: 604 they associate to form a binding site that binds the HIV-1 envelope like antibody DH537; or

wherein if the domain (A) comprises SEQ ID NO: 625 and domain (E) comprises SEQ ID NO: 624 or 626 they associate to form a binding site that binds the HIV-1 envelope like antibody DH511_5a or DH511_5b; and

wherein if the domain (B) comprises SEQ ID NO: 500 and domain (D) comprises SEQ ID NO: 502 they associate to form a binding site that binds CD3; or

wherein if the domain (B) comprises SEQ ID NO: 504 and domain (D) comprises SEQ ID NO: 506 they associate to form a binding site that binds CD16.

[0022] In certain aspects the invention provides a bispecific molecule comprising a first polypeptide chain, a second polypeptide chain, and a third polypeptide chain, wherein some of the polypeptides are covalently bonded (See Figure 103), and wherein:

(I) the first polypeptide chain comprises in the N- to C-terminal direction:

- (i) a domain (A) comprising a binding region of the light chain variable domain of a first immunoglobulin (VL1) having the binding specificity of a gp41 MPER binding antibody (1);
 - (ii) a domain (B) comprising a binding region of a heavy chain variable domain of a second immunoglobulin (VH2) specific for an epitope (2);
 - (iii) a domain (C) comprising a heterodimer promoting domain; and
 - (iv) a CH2-CH3 domain;
- (II) the second polypeptide chain comprises in the N- to C-terminal direction:
- (i) a domain (D) comprising a binding region of a light chain variable domain of the second immunoglobulin (VL2) specific for the epitope (2);
 - (ii) a domain (E) comprising a binding region of a heavy chain variable domain of the first immunoglobulin (VH1) having the binding specificity of a gp41 MPER binding antibody (1); and
 - (iii) a domain (F) comprising a heterodimer promoting domain;
- (III) the third polypeptide chain comprises in the N- to C-terminal direction:
- (i) a CH2-CH3 domain, and wherein:
- the domains (A) and (B) are linked so that they do not associate with one another to form an epitope binding site;
- the domains (D) and (E) are linked so that they do not associate with one another to form an epitope binding site;
- the domains (A) and (E) associate to form a binding site that binds the HIV-1 envelope like a gp41 MPER binding antibody (1);
- the domains (B) and (D) associate to form a binding site that binds the epitope (2); and
- the CH2-CH3 domains of the first and third polypeptide form an Fc Domain.

[0023] In certain aspects the invention provides such bispecific molecules wherein:

- (i) the third polypeptide chain further comprises a peptide linker 3 N-terminal to the CH2-CH3 domain;
- (ii) domains (A) and (B) are separated by a peptide linker 1;
- (iii) domains (C) and (B) are separated by a peptide linker 2;
- (iv) the CH2-CH3 domain and domain (C) are separated by a peptide linker 3 or a spacer linker 3;
- (v) domains (D) and (E) are separated by a peptide linker 1; and

(vi) domains (F) and (E) are separated by a peptide linker 2.

[0024] In certain aspects the invention provides bispecific molecules comprising a first polypeptide chain, a second polypeptide chain, and a third polypeptide chain, wherein some of the polypeptides are covalently bonded (See Figure 103), and wherein:

(I) the first polypeptide chain comprises in the N- to C-terminal direction:

- (i) a domain (A) comprising a binding region of the light chain variable domain of a first immunoglobulin (VL1) having the binding specificity of the gp41 MPER binding antibody DH511.2, a variant of DH511.2 called DH511.2_K3, or any antibody of the DH511 lineage (1);
- (ii) a domain (B) comprising a binding region of a heavy chain variable domain of a second immunoglobulin (VH2) specific for an epitope (2), wherein domains (A) and (B) are separated from one another by a peptide linker 1;
- (iii) a domain (C) comprising a heterodimer promoting domain, including but not limited to a K coil or E coil; wherein domain (C) and domain (B) are separated by a peptide linker 2;
- (iv) a CH2-CH3 domain, wherein the CH2-CH3 domain and domain (C) are separated by a peptide linker 3 or a spacer-linker 3;

(II) the second polypeptide chain comprises in the N- to C-terminal direction:

- (i) a domain (D) comprising a binding region of a light chain variable domain of the second immunoglobulin (VL2) specific for the epitope (2);
- (ii) a domain (E) comprising a binding region of a heavy chain variable domain of the first immunoglobulin (VH1) having the binding specificity of the gp41 MPER binding antibody DH511.2, a variant of DH511.2 called DH511.2_K3, or any antibody of the DH511 lineage (1), wherein domains (D) and (E) are separated from one another by a peptide linker 1; and
- (iii) a domain (F) comprising a heterodimer promoting domain, including but not limited to a K coil or E coil; wherein domain (F) and domain (E) are separated by a peptide linker 2;

(III) the third polypeptide chain comprises in the N- to C-terminal direction:

- (i) a peptide linker 3,
- (ii) a CH2-CH3 domain, and wherein:

the domains (A) and (B) do not associate with one another to form an epitope binding site;
 the domains (D) and (E) do not associate with one another to form an epitope binding site;
 the domains (A) and (E) associate to form a binding site that binds the HIV-1 envelope like the gp41 MPER binding antibody DH511.2, a variant of DH511.2 called DH511.2_K3, or any antibody of the DH511 lineage (1);
 the domains (B) and (D) associate to form a binding site that binds the epitope (2); and
 the CH2-CH3 domains of the first and third polypeptide form an Fc Domain.

[0025] In certain aspects said first and second polypeptide chains are covalently bonded to one another; and said first and third polypeptide chains are covalently bonded to one another.

[0026] In certain aspects the invention provides a bispecific molecule comprising a first polypeptide chain, a second polypeptide chain, and a third polypeptide chain, wherein the first and second polypeptide chains are covalently bonded and the first and third polypeptide chains are covalently bonded, and wherein:

(I) the first polypeptide chain comprises in the N- to C-terminal direction:

- (i)** a domain (A) comprising SEQ ID NO: 553, 570, 574, 578, 582, 586, 590, 594, 598, 602, 606, or 625;
- (ii)** a domain (B) comprising SEQ ID NO: 500 or 504;
- (iii)** a domain (C) comprising SEQ ID NO: 520, 521, 518, or 519; and
- (iv)** a CH2-CH3 domain comprising SEQ ID NO: 531, 532, 533, or 534;

(II) the second polypeptide chain comprises in the N- to C-terminal direction:

- (i)** a domain (D) comprising SEQ ID NO: 502, or 506;
- (ii)** a domain (E) comprising SEQ ID NO: 551, 568, 572, 576, 580, 584, 588, 592, 596, 600, 604, 608-624, or 626 (Table 4); and
- (iii)** a domain (F) comprising SEQ ID NO: 518, 519, 520, or 521;

(III) the third polypeptide chain comprises in the N- to C-terminal direction:

- (i)** a CH2-CH3 domain comprising SEQ ID NO: 531, 532, 533, or 534; and wherein:

the domains (A) and (B) are linked so that they do not associate with one another to form an epitope binding site;

the domains (D) and (E) are linked so that they do not associate with one another to form an epitope binding site;

the domains (A) and (E) associate to form a binding site that binds the HIV-1 envelope like a gp41 MPER binding antibody (1);

the domains (B) and (D) associate to form a binding site that binds an epitope (2); and

the CH2-CH3 domains of the first and third polypeptide form an Fc Domain.

[0027] In certain aspects the invention provides such bispecific molecules wherein:

- (i) the third polypeptide chain further comprises SEQ ID NO: 523 N-terminal to the CH2-CH3 domain;
- (ii) domains (A) and (B) are separated by SEQ ID NO: 508;
- (iii) domains (C) and (B) are separated by SEQ ID NO: 509 or 510;
- (iv) the CH2-CH3 domain and domain (C) are separated by SEQ ID NO: 523 or 522;
- (v) domains (D) and (E) are separated by SEQ ID NO: 508; and
- (vi) domains (F) and (E) are separated by SEQ ID NO: 509 or 510.

[0028] In certain aspects the invention provides bispecific molecules comprising a first polypeptide chain, a second polypeptide chain, and a third polypeptide chain, wherein the first and second polypeptide chains are covalently bonded and the first and third polypeptide chains are covalently bonded, and wherein:

(I) the first polypeptide chain comprises in the N- to C-terminal direction:

- (i)** a domain (A) comprising SEQ ID NO: 553, 570, 574, 578, 582, 586, 590, 594, 598, 602, 606, or 625;
- (ii)** a domain (B) comprising SEQ ID NO: 500 or 504, wherein domains (A) and (B) are separated by SEQ ID NO: 508;
- (iii)** a domain (C) comprising SEQ ID NO: 513, 520, 511, or 518; wherein domain (C) and domain (B) are separated by SEQ ID NO: 509 or 510;
- (iv)** a CH2-CH3 domain comprising SEQ ID NO: 531, 532, 533, or 534, wherein the CH2-CH3 domain and domain (C) are separated by SEQ ID NO: 523 or 522;

(II) the second polypeptide chain comprises in the N- to C-terminal direction:

- (i)** a domain (D) comprising SEQ ID NO: 502, or 506;
- (ii)** a domain (E) comprising SEQ ID NO: 551, 568, 572, 576, 580, 584, 588, 592, 596, 600, 604, 608-624, or 626 (Table 4), wherein domains (D) and (E) are separated from one another by SEQ ID NO: 508; and

(iii) a domain (F) comprising SEQ ID NO: 513, 520, 511, or 518; wherein domain (F) and domain (E) are separated by SEQ ID NO: 509 or 510;

(III) the third polypeptide chain comprises in the N- to C-terminal direction:

(i) SEQ ID NO: 523; and

(ii) a CH2-CH3 domain comprising SEQ ID NO: 531, 532, 533, or 534; and wherein: the domains (A) and (B) do not associate with one another to form an epitope binding site; the domains (D) and (E) do not associate with one another to form an epitope binding site; and

wherein if the domain (A) comprises SEQ ID NO: 553 and domain (E) comprises SEQ ID NO: 551 they associate to form a binding site that binds the HIV-1 envelope like antibody DH511.2_K3;

wherein if the domain (A) comprises SEQ ID NO: 570 and domain (E) comprises SEQ ID NO: 568 they associate to form a binding site that binds the HIV-1 envelope like antibody DH511;

wherein if the domain (A) comprises SEQ ID NO: 574 and domain (E) comprises SEQ ID NO: 572 they associate to form a binding site that binds the HIV-1 envelope like antibody DH512 (also referred to as DH511.2);

wherein if the domain (A) comprises SEQ ID NO: 578 and domain (E) comprises SEQ ID NO: 576 they associate to form a binding site that binds the HIV-1 envelope like antibody DH513;

wherein if the domain (A) comprises SEQ ID NO: 582 and domain (E) comprises SEQ ID NO: 580 they associate to form a binding site that binds the HIV-1 envelope like antibody DH514;

wherein if the domain (A) comprises SEQ ID NO: 586 and domain (E) comprises SEQ ID NO: 584 they associate to form a binding site that binds the HIV-1 envelope like antibody DH515;

wherein if the domain (A) comprises SEQ ID NO: 590 and domain (E) comprises SEQ ID NO: 588 they associate to form a binding site that binds the HIV-1 envelope like antibody DH516;

wherein if the domain (A) comprises SEQ ID NO: 594 and domain (E) comprises SEQ ID NO: 592 they associate to form a binding site that binds the HIV-1 envelope like antibody DH517;

wherein if the domain (A) comprises SEQ ID NO: 598 and domain (E) comprises SEQ ID NO: 596 they associate to form a binding site that binds the HIV-1 envelope like antibody DH518;

wherein if the domain (A) comprises SEQ ID NO: 602 and domain (E) comprises SEQ ID NO: 600 they associate to form a binding site that binds the HIV-1 envelope like antibody DH536;

wherein if the domain (A) comprises SEQ ID NO: 606 and domain (E) comprises SEQ ID NO: 604 they associate to form a binding site that binds the HIV-1 envelope like antibody DH537; or

wherein if the domain (A) comprises SEQ ID NO: 625 and domain (E) comprises SEQ ID NO: 624 or 626 they associate to form a binding site that binds the HIV-1 envelope like antibody DH511_5a or DH511_5b; and

wherein if the domain (B) comprises SEQ ID NO: 500 and domain (D) comprises SEQ ID NO: 502 they associate to form a binding site that binds CD3; or

wherein if the domain (B) comprises SEQ ID NO: 504 and domain (D) comprises SEQ ID NO: 506 they associate to form a binding site that binds CD16; and the CH2-CH3 domains of the first and third polypeptide form an Fc Domain.

[0029] In certain aspects the invention provides bispecific molecules comprising a first polypeptide chain, a second polypeptide chain, and a third polypeptide chain, wherein some of the polypeptides are covalently bonded (See Figure 103), and wherein:

(I) the first polypeptide chain comprises in the N- to C-terminal direction:

(i) a CH2-CH3 domain;

(ii) a domain (A) comprising a binding region of the light chain variable domain of a first immunoglobulin (VL1) having the binding specificity of a gp41 MPERbinding antibody (1);

(iii) a domain (B) comprising a binding region of a heavy chain variable domain of a second immunoglobulin (VH2) specific for an epitope (2); and

(iv) a domain (C) comprising a heterodimer promoting domain;

(II) the second polypeptide chain comprises in the N- to C-terminal direction:

(i) a domain (D) comprising a binding region of a light chain variable domain of the second immunoglobulin (VL2) specific for the epitope (2);

- (ii) a domain (E) comprising a binding region of a heavy chain variable domain of the first immunoglobulin (VH1) having the binding specificity of a gp41 MPERbinding antibody (1); and
 - (iii) a domain (F) comprising a heterodimer promoting domain;
- (III) the third polypeptide chain comprises in the N- to C-terminal direction:
- (i) a CH2-CH3 domain, and wherein:
- the domains (A) and (B) are linked so that they do not associate with one another to form an epitope binding site;
- the domains (D) and (E) are linked so that they do not associate with one another to form an epitope binding site;
- the domains (A) and (E) associate to form a binding site that binds the HIV-1 envelope like a gp41 MPER binding antibody (1); the domains (B) and (D) associate to form a binding site that binds the epitope (2); and
- the CH2-CH3 domains of the first and third polypeptide form an Fc Domain.

[0030] In certain aspects the invention provides such bispecific molecules wherein:

- (i) the CH2-CH3 domain and domain (A) are separated by a peptide linker 4;
- (ii) domains (A) and (B) are separated by a peptide linker 1;
- (iii) domains (C) and (B) are separated by a peptide linker 2;
- (iv) domains (D) and (E) are separated by a peptide linker 1;
- (v) domains (F) and (E) are separated by a peptide linker 2;
- (vi) the first polypeptide chain further comprises a peptide linker 3 N-terminal to the CH2-CH3 domain; and
- (vii) the third polypeptide chain further comprises a peptide linker 3 N-terminal to the CH2-CH3 domain.

[0031] In other aspects the invention provides bispecific molecules comprising a first polypeptide chain, a second polypeptide chain, and a third polypeptide chain, wherein some of the polypeptides are covalently bonded (See Figure 103), and wherein:

- (I) the first polypeptide chain comprises in the N- to C-terminal direction:
 - (i) a peptide linker 3 followed by a CH2-CH3 domain;
 - (ii) a domain (A) comprising a binding region of the light chain variable domain of a first immunoglobulin (VL1) having the binding specificity of the gp41 MPER binding

antibody DH511.2, a variant of DH511.2 called DH511.2_K3, or any antibody of the DH511 lineage (1), wherein the CH2-CH3 domain and domain (A) are separated by a peptide linker 4;

(iii) a domain (B) comprising a binding region of a heavy chain variable domain of a second immunoglobulin (VH2) specific for an epitope (2), wherein domains (A) and (B) are separated by a peptide linker 1;

(iv) a domain (C) comprising a heterodimer promoting domain, including but not limited to a K coil or E coil; wherein domain (C) and domain (B) are separated by a peptide linker 2;

(II) the second polypeptide chain comprises in the N- to C-terminal direction:

(i) a domain (D) comprising a binding region of a light chain variable domain of the second immunoglobulin (VL2) specific for the epitope (2);

(ii) a domain (E) comprising a binding region of a heavy chain variable domain of the first immunoglobulin (VH1) having the binding specificity of the gp41 MPER binding antibody DH511.2, a variant of DH511.2 called DH511.2_K3, or any antibody of the DH511 lineage (1), wherein domains (D) and (E) are separated by a peptide linker 1; and

(iii) a domain (F) comprising a heterodimer promoting domain, including but not limited to a K coil or E coil; wherein domain (F) and domain (E) are separated by a peptide linker 2;

(III) the third polypeptide chain comprises in the N- to C-terminal direction:

(i) a peptide linker 3,

(ii) a CH2-CH3 domain, and wherein:

the domains (A) and (B) do not associate with one another to form an epitope binding site;

the domains (D) and (E) do not associate with one another to form an epitope binding site;

the domains (A) and (E) associate to form a binding site that binds the HIV-1 envelope like the gp41 MPER binding antibody DH511.2, a variant of DH511.2 called DH511.2_K3, or any antibody of the DH511 lineage (1);

the domains (B) and (D) associate to form a binding site that binds the epitope (2); and

the CH2-CH3 domains of the first and third polypeptide form an Fc Domain.

[0032] In certain aspects said first and second polypeptide chains are covalently bonded to one another; and said first and third polypeptide chains are covalently bonded to one another.

[0033] In certain aspects the invention provides bispecific molecules comprising a first polypeptide chain, a second polypeptide chain, and a third polypeptide chain, wherein the first and second polypeptide chains are covalently bonded and the first and third polypeptide chains are covalently bonded (See Figure 103), and wherein:

(I) the first polypeptide chain comprises in the N- to C-terminal direction:

- (i) a CH2-CH3 domain comprising SEQ ID NO: 531, 532, 533, or 534;
- (ii) a domain (A) comprising SEQ ID NO: 553, 570, 574, 578, 582, 586, 590, 594, 598, 602, 606, or 625;
- (iii) a domain (B) comprising SEQ ID NO: 500 or 504; and
- (iv) a domain (C) comprising SEQ ID NO: 520, 521, 518, or 519;

(II) the second polypeptide chain comprises in the N- to C-terminal direction:

- (i) a domain (D) comprising SEQ ID NO: 502, or 506;
- (ii) a domain (E) comprising SEQ ID NO: 551, 568, 572, 576, 580, 584, 588, 592, 596, 600, 604, 608-624, or 626 (Table 4); and
- (iii) a domain (F) comprising SEQ ID NO: 518, 519, 520, or 521;

(III) the third polypeptide chain comprises in the N- to C-terminal direction:

- (i) a CH2-CH3 domain comprising SEQ ID NO: 531, 532, 533, or 534, and wherein:
 - the domains (A) and (B) are linked so that they do not associate with one another to form an epitope binding site;
 - the domains (D) and (E) are linked so that they do not associate with one another to form an epitope binding site;
 - the domains (A) and (E) associate to form a binding site that binds the HIV-1 envelope like a gp41 MPER binding antibody (1); the domains (B) and (D) associate to form a binding site that binds an epitope (2); and
 - the CH2-CH3 domains of the first and third polypeptide form an Fc Domain.

[0034] In certain aspects the invention provides such bispecific molecules wherein:

- (i) the CH2-CH3 domain and domain (A) are separated by SEQ ID NO: 524 or 525;
- (ii) domains (A) and (B) are separated by SEQ ID NO: 508;
- (iii) domains (C) and (B) are separated by SEQ ID NO: 509 or 510;

- (iv) domains (D) and (E) are separated by SEQ ID NO: 508;
- (v) domains (F) and (E) are separated by SEQ ID NO: 509 or 510;
- (vi) the first polypeptide chain further comprises SEQ ID NO: 523 N-terminal to the CH2-CH3 domain; and
- (vii) the third polypeptide chain further comprises SEQ ID NO: 523 N-terminal to the CH2-CH3 domain.

[0035] In other aspects the invention provides bispecific molecules comprising a first polypeptide chain, a second polypeptide chain, and a third polypeptide chain, wherein the first and second polypeptide chains are covalently bonded and the first and third polypeptide chains are covalently bonded (See Figure 103), and wherein:

- (I)** the first polypeptide chain comprises in the N- to C-terminal direction:
 - (i) SEQ ID NO: 523 followed by a CH2-CH3 domain comprising SEQ ID NO: 531, 532, 533, or 534;
 - (ii) a domain (A) comprising SEQ ID NO: 553, 570, 574, 578, 582, 586, 590, 594, 598, 602, 606, or 625, wherein the CH2-CH3 domain and domain (A) are separated by SEQ ID NO: 524 or 525;
 - (iii) a domain (B) comprising SEQ ID NO: 500 or 504, wherein domains (A) and (B) are separated by SEQ ID NO: 508;
 - (iv) a domain (C) comprising SEQ ID NO: 520, 521, 518, or 519; wherein domain (C) and domain (B) are separated by SEQ ID NO: 509 or 510;
- (II)** the second polypeptide chain comprises in the N- to C-terminal direction:
 - (i) a domain (D) comprising SEQ ID NO: 502, or 506;
 - (ii) a domain (E) comprising SEQ ID NO: 551, 568, 572, 576, 580, 584, 588, 592, 596, 600, 604, 608-624, or 626 (Table 4), wherein domains (D) and (E) are separated by SEQ ID NO: 508; and
 - (iii) a domain (F) comprising SEQ ID NO: 518, 519, 520, or 521; wherein domain (F) and domain (E) are separated by SEQ ID NO: 509 or 510;
- (III)** the third polypeptide chain comprises in the N- to C-terminal direction:
 - (i) SEQ ID NO: 523,
 - (ii) a CH2-CH3 domain comprising SEQ ID NO: 531, 532, 533, or 534, and wherein:
 - the domains (A) and (B) do not associate with one another to form an epitope binding site;
 - the domains (D) and (E) do not associate with one another to form an epitope binding site;

wherein if the domain (A) comprises SEQ ID NO: 553 and domain (E) comprises SEQ ID NO: 551 they associate to form a binding site that binds the HIV-1 envelope like antibody DH511.2_K3;

wherein if the domain (A) comprises SEQ ID NO: 570 and domain (E) comprises SEQ ID NO: 568 they associate to form a binding site that binds the HIV-1 envelope like antibody DH511;

wherein if the domain (A) comprises SEQ ID NO: 574 and domain (E) comprises SEQ ID NO: 572 they associate to form a binding site that binds the HIV-1 envelope like antibody DH512 (also referred to as DH511.2);

wherein if the domain (A) comprises SEQ ID NO: 578 and domain (E) comprises SEQ ID NO: 576 they associate to form a binding site that binds the HIV-1 envelope like antibody DH513;

wherein if the domain (A) comprises SEQ ID NO: 582 and domain (E) comprises SEQ ID NO: 580 they associate to form a binding site that binds the HIV-1 envelope like antibody DH514;

wherein if the domain (A) comprises SEQ ID NO: 586 and domain (E) comprises SEQ ID NO: 584 they associate to form a binding site that binds the HIV-1 envelope like antibody DH515;

wherein if the domain (A) comprises SEQ ID NO: 590 and domain (E) comprises SEQ ID NO: 588 they associate to form a binding site that binds the HIV-1 envelope like antibody DH516;

wherein if the domain (A) comprises SEQ ID NO: 594 and domain (E) comprises SEQ ID NO: 592 they associate to form a binding site that binds the HIV-1 envelope like antibody DH517;

wherein if the domain (A) comprises SEQ ID NO: 598 and domain (E) comprises SEQ ID NO: 596 they associate to form a binding site that binds the HIV-1 envelope like antibody DH518;

wherein if the domain (A) comprises SEQ ID NO: 602 and domain (E) comprises SEQ ID NO: 600 they associate to form a binding site that binds the HIV-1 envelope like antibody DH536; or

wherein if the domain (A) comprises SEQ ID NO: 606 and domain (E) comprises SEQ ID NO: 604 they associate to form a binding site that binds the HIV-1 envelope like antibody DH537; or

wherein if the domain (A) comprises SEQ ID NO: 625 and domain (E) comprises SEQ ID NO: 624 or 626 they associate to form a binding site that binds the HIV-1 envelope like antibody DH511_5a or DH511_5b; and

wherein if the domain (B) comprises SEQ ID NO: 500 and domain (D) comprises SEQ ID NO: 502 they associate to form a binding site that binds CD3; or

wherein if the domain (B) comprises SEQ ID NO: 504 and domain (D) comprises SEQ ID NO: 506 they associate to form a binding site that binds CD16; and the CH2-CH3 domains of the first and third polypeptide form an Fc Domain.

[0036] In certain aspects the invention provides a bispecific molecule comprising a first polypeptide chain, a second polypeptide chain, a third polypeptide chain, and a fourth polypeptide chain, wherein some of the polypeptides are covalently bonded, and wherein:

(I) the first and the third polypeptide chains each comprise in the N- to C-terminal direction:

- (i) a domain (A) comprising a binding region of the light chain variable domain of a first immunoglobulin (VL1) having the binding specificity of a gp41 MPER binding antibody (1);
- (ii) a domain (B) comprising a binding region of a heavy chain variable domain of a second immunoglobulin (VH2) specific for an epitope (2);
- (iii) a domain (C) comprising a heterodimer promoting domain; and
- (iv) a CH2-CH3 domain;

(II) the second and fourth polypeptide chains each comprise in the N- to C-terminal direction:

- (i) a domain (D) comprising a binding region of a light chain variable domain of the second immunoglobulin (VL2) specific for the epitope (2);
- (ii) a domain (E) comprising a binding region of a heavy chain variable domain of the first immunoglobulin (VH1) having the binding specificity of a gp41 MPER binding antibody (1);
- (iii) a domain (F) comprising a heterodimer promoting domain; and wherein

the domains (A) and (B) are linked so that they do not associate with one another to form an epitope binding site;

the domains (D) and (E) are linked so that they do not associate with one another to form an epitope binding site;

the domains (A) and (E) associate to form a binding site that binds the HIV-1 envelope like a gp41 MPER binding antibody (1);

the domains (B) and (D) associate to form a binding site that binds the epitope (2); and

the CH2-CH3 domains of the first and third polypeptide form an Fc Domain.

[0037] In certain aspects the invention provides such bispecific molecules wherein:

- (i) domains (A) and (B) are separated by a peptide linker 1;
- (ii) domains (C) and (B) are separated by a peptide linker 2;
- (iii) the CH2-CH3 domain and domain (C) are separated by a peptide linker 3 or a spacer linker 3;
- (iv) domains (D) and (E) are separated by a peptide linker 1; and
- (v) domains (F) and (E) are separated by a peptide linker 2.

[0038] In certain aspects the invention provides a bispecific molecule comprising a first polypeptide chain, a second polypeptide chain, a third polypeptide chain, and a fourth polypeptide chain, wherein some of the polypeptides are covalently bonded, and wherein:

(I) the first and the third polypeptide chains each comprise in the N- to C-terminal direction:

- (i) a domain (A) comprising a binding region of the light chain variable domain of a first immunoglobulin (VL1) having the binding specificity of the gp41 MPER binding antibody DH511.2, a variant of DH511.2 called DH511.2_K3, or any antibody of the DH511 lineage (1);
- (ii) a domain (B) comprising a binding region of a heavy chain variable domain of a second immunoglobulin (VH2) specific for an epitope (2), wherein domains (A) and (B) are separated by a peptide linker 1;
- (iii) a domain (C) comprising a heterodimer promoting domain, wherein domains (C) and (B) are separated by a peptide linker 2; and
- (iv) a CH2-CH3 domain, wherein the CH2-CH3 domain and domain (C) are separated by a peptide linker 3 or a spacer linker 3;

(II) the second and fourth polypeptide chains each comprise in the N- to C-terminal direction:

- (i) a domain (D) comprising a binding region of a light chain variable domain of the second immunoglobulin (VL2) specific for the epitope (2);

- (ii) a domain (E) comprising a binding region of a heavy chain variable domain of the first immunoglobulin (VH1) having the binding specificity of the gp41 MPER binding antibody DH511.2, a variant of DH511.2 called DH511.2_K3, or any antibody of the DH511 lineage (1), wherein domains (D) and (E) are separated by a peptide linker 1;
- (iii) a domain (F) comprising a heterodimer promoting domain, wherein domains (F) and (E) are separated by a peptide linker 2; and wherein

the domains (A) and (B) do not associate with one another to form an epitope binding site;
 the domains (D) and (E) do not associate with one another to form an epitope binding site;
 the domains (A) and (E) associate to form a binding site that binds the HIV-1 envelope like the gp41 MPER binding antibody DH511.2, a variant of DH511.2 called DH511.2_K3, or any antibody of the DH511 lineage (1);

the domains (B) and (D) associate to form a binding site that binds the epitope (2); and
 the CH2-CH3 domains of the first and third polypeptide form an Fc Domain.

[0039] In certain aspects said first and second polypeptide chains are covalently bonded to one another; said third and fourth polypeptide chains are covalently bonded to one another; and said first and third chains are covalently bonded to one another.

[0040] In certain aspects the invention provides a bispecific molecule comprising a first polypeptide chain, a second polypeptide chain, a third polypeptide chain, and a fourth polypeptide chain, wherein the first and second polypeptide chains are covalently bonded, the third and fourth polypeptide chains are covalently bonded, and the first and third chains are covalently bonded, and wherein:

(I) the first and the third polypeptide chains each comprise in the N- to C-terminal direction:

- (i) a domain (A) comprising SEQ ID NO: 553, 570, 574, 578, 582, 586, 590, 594, 598, 602, 606, or 625;
- (ii) a domain (B) comprising SEQ ID NO: 500 or 504;
- (iii) a domain (C) comprising SEQ ID NO: 520, 521, 518, or 519; and
- (iv) a CH2-CH3 domain comprising SEQ ID NO: 527, 528, or 529;

(II) the second and fourth polypeptide chains each comprise in the N- to C-terminal direction:

- (i) a domain (D) comprising SEQ ID NO: 502, or 506;
- (ii) a domain (E) comprising SEQ ID NO: 551, 568, 572, 576, 580, 584, 588, 592, 596, 600, 604, 608-624, or 626 (Table 4);

(iii) a domain (F) comprising SEQ ID NO: 518, 519, 520, or 521; and wherein
 the domains (A) and (B) are linked so that they do not associate with one another to form an epitope binding site;
 the domains (D) and (E) are linked so that they do not associate with one another to form an epitope binding site;
 the domains (A) and (E) associate to form a binding site that binds the HIV-1 envelope like a gp41 MPER binding antibody (1);
 the domains (B) and (D) associate to form a binding site that binds an epitope (2); and
 the CH2-CH3 domains of the first and third polypeptide form an Fc Domain.

[0041] In certain aspects the invention provides such bispecific molecules wherein:

- (i) domains (A) and (B) are separated by SEQ ID NO: 508;
- (ii) domains (C) and (B) are separated by SEQ ID NO: 509 or 510;
- (iii) the CH2-CH3 domain and domain (C) are separated by SEQ ID NO: 523 or 522;
- (iv) domains (D) and (E) are separated by SEQ ID NO: 508; and
- (v) domains (F) and (E) are separated by SEQ ID NO: 509 or 510.

[0042] In certain aspects the invention provides a bispecific molecule comprising a first polypeptide chain, a second polypeptide chain, a third polypeptide chain, and a fourth polypeptide chain, wherein the first and second polypeptide chains are covalently bonded, the third and fourth polypeptide chains are covalently bonded, and the first and third chains are covalently bonded, and wherein:

(I) the first and the third polypeptide chains each comprise in the N- to C-terminal direction:

- (i)** a domain (A) comprising SEQ ID NO: 553, 570, 574, 578, 582, 586, 590, 594, 598, 602, 606, or 625;
- (ii)** a domain (B) comprising SEQ ID NO: 500 or 504, wherein domains (A) and (B) are separated by SEQ ID NO: 508;
- (iii)** a domain (C) comprising SEQ ID NO: 520, 521, 518, or 519, wherein domains (C) and (B) are separated by SEQ ID NO: 509 or 510; and
- (iv)** a CH2-CH3 domain comprising SEQ ID NO: 527, 528, or 529, wherein the CH2-CH3 domain and domain (C) are separated by SEQ ID NO: 523 or 522;

(II) the second and fourth polypeptide chains each comprise in the N- to C-terminal direction:

- (i) a domain (D) comprising SEQ ID NO: 502, or 506;
- (ii) a domain (E) comprising SEQ ID NO: 551, 568, 572, 576, 580, 584, 588, 592, 596, 600, 604, 608-624, or 626 (Table 4), wherein domains (D) and (E) are separated by SEQ ID NO: 508;
- (iii) a domain (F) comprising SEQ ID NO: 518, 519, 520, or 521, wherein domains (F) and (E) are separated by SEQ ID NO: 509 or 510; and wherein

the domains (A) and (B) do not associate with one another to form an epitope binding site;
the domains (D) and (E) do not associate with one another to form an epitope binding site;

wherein if the domain (A) comprises SEQ ID NO: 553 and domain (E) comprises SEQ ID NO: 551 they associate to form a binding site that binds the HIV-1 envelope like antibody DH511.2_K3;

wherein if the domain (A) comprises SEQ ID NO: 570 and domain (E) comprises SEQ ID NO: 568 they associate to form a binding site that binds the HIV-1 envelope like antibody DH511;

wherein if the domain (A) comprises SEQ ID NO: 574 and domain (E) comprises SEQ ID NO: 572 they associate to form a binding site that binds the HIV-1 envelope like antibody DH512 (also referred as DH511.2);

wherein if the domain (A) comprises SEQ ID NO: 578 and domain (E) comprises SEQ ID NO: 576 they associate to form a binding site that binds the HIV-1 envelope like antibody DH513;

wherein if the domain (A) comprises SEQ ID NO: 582 and domain (E) comprises SEQ ID NO: 580 they associate to form a binding site that binds the HIV-1 envelope like antibody DH514;

wherein if the domain (A) comprises SEQ ID NO: 586 and domain (E) comprises SEQ ID NO: 584 they associate to form a binding site that binds the HIV-1 envelope like antibody DH515;

wherein if the domain (A) comprises SEQ ID NO: 590 and domain (E) comprises SEQ ID NO: 588 they associate to form a binding site that binds the HIV-1 envelope like antibody DH516;

wherein if the domain (A) comprises SEQ ID NO: 594 and domain (E) comprises SEQ ID NO: 592 they associate to form a binding site that binds the HIV-1 envelope like antibody DH517;

wherein if the domain (A) comprises SEQ ID NO: 598 and domain (E) comprises SEQ ID NO: 596 they associate to form a binding site that binds the HIV-1 envelope like antibody DH518;

wherein if the domain (A) comprises SEQ ID NO: 602 and domain (E) comprises SEQ ID NO: 600 they associate to form a binding site that binds the HIV-1 envelope like antibody DH536; or

wherein if the domain (A) comprises SEQ ID NO: 606 and domain (E) comprises SEQ ID NO: 604 they associate to form a binding site that binds the HIV-1 envelope like antibody DH537; or

wherein if the domain (A) comprises SEQ ID NO: 625 and domain (E) comprises SEQ ID NO: 624 or 626 they associate to form a binding site that binds the HIV-1 envelope like antibody DH511_5a or DH511_5b; and

wherein if the domain (B) comprises SEQ ID NO: 500 and domain (D) comprises SEQ ID NO: 502 they associate to form a binding site that binds CD3; or

wherein if the domain (B) comprises SEQ ID NO: 504 and domain (D) comprises SEQ ID NO: 506 they associate to form a binding site that binds CD16; and the CH2-CH3 domains of the first and third polypeptide form an Fc Domain.

[0043] In certain aspects the invention provides trivalent binding molecules comprising a first, second, third and fourth polypeptide chain, wherein some of the polypeptides are covalently bonded and wherein:

- (I) the first polypeptide chain comprises in the N-terminus to C-terminus direction:
 - (i) a domain (A) comprising a binding region of a light chain variable domain of a first immunoglobulin (VL1) specific for an epitope (1);
 - (ii) a domain (B) comprising a binding region of a heavy chain variable domain of a second immunoglobulin (VH2) specific for an epitope (2);
 - (iii) a domain (C) comprising a heterodimer promoting domain; and
 - (iv) a CH2-CH3 Domain;
- (II) the second polypeptide chain comprises, in the N-terminus to C-terminus direction:
 - (i) a domain (D) comprising a binding region of a light chain variable domain of the first immunoglobulin (VL1) specific for the epitope (2);

- (ii) a domain (E) comprising a binding region of a heavy chain variable domain of the second immunoglobulin (VH1) specific for the epitope (1); and
- (iii) a domain (F) comprising a heterodimer promoting domain;
- (III) the third polypeptide chain that comprises, in the N-terminus to C-terminus direction:
 - (i) a domain (G) comprising a binding region of a heavy chain variable domain of a third immunoglobulin (VH3) specific for an epitope (3); and
 - (ii) a CH1-Hinge Domain, and a CH2-CH3 Domain; and
- (IV) the fourth polypeptide chain that comprises, in the N-terminus to C-terminus direction:
 - (i) a domain (H) comprising a binding region of a light chain variable domain of the third immunoglobulin (VL3) specific for the epitope (3); and
 - (ii) CL Kappa Domain or a CL Lambda Domain; and wherein

the domains (A) and (B) are linked so that they do not associate with one another to form an epitope binding site;

the domains (D) and (E) are linked so that they do not associate with one another to form an epitope binding site;

the domains (A) and (E) associate to form a binding site that binds the epitope (1);

the domains (B) and (D) associate to form a binding site that binds the epitope (2);

the domains (G) and (H) associate to form a binding site that bind the epitope (3);

at least one of epitope (1), epitope (2), and epitope (3) is an epitope bound by a gp41 MPER binding antibody, and at least one of epitope (1), epitope (2), and epitope (3) is an epitope of, for example, but not limited to, CD3, CD8, or CD16, or an epitope on any suitable effector cell;

the CH2-CH3 domains of the first and third polypeptide form an Fc Domain;

said first and second polypeptide chains are covalently bonded to one another;

said first and third polypeptide chains are covalently bonded to one another; and

said third and fourth polypeptide chains are covalently bonded to one another.

[0044] In certain aspects the invention provides such trivalent molecules wherein:

- (i) domains (A) and (B) are separated by a peptide linker 1;
- (ii) domains (C) and (B) are separated by a peptide linker 2 or a peptide linker 2-C;
- (iii) the CH2-CH3 domain and domain (C) are separated by a peptide linker 3 or a spacer linker 3;

- (iv) domains (D) and (E) are separated by a peptide linker 1; and
- (v) domains (F) and (E) are separated by a peptide linker 2 or a peptide linker 2-C.

[0045] In certain aspects the invention provides trivalent binding molecules comprising a first, second, third and fourth polypeptide chain, wherein some of the polypeptides are covalently bonded and wherein:

- (I)** the first polypeptide chain comprises in the N-terminus to C-terminus direction:
 - (i)** a domain (A) comprising a binding region of a light chain variable domain of a first immunoglobulin (VL1) specific for an epitope (1);
 - (ii)** a domain (B) comprising a binding region of a heavy chain variable domain of a second immunoglobulin (VH2) specific for an epitope (2), wherein domains (A) and (B) are separated by a Peptide Linker 1;
 - (iii)** a domain (C) comprising:
 - (a)** a heterodimer promoting domain; wherein domain (C) and domain (B) are separated by a Peptide Linker 2-C; or
 - (b)** a heterodimer promoting domain; wherein domain (C) and domain (B) are separated by a Peptide Linker 2; and
 - (iv)** a CH2-CH3 Domain, wherein the CH2-CH3 domain and domain (C) are separated by a Peptide Linker 3 or a Spacer-Linker 3;
- (II)** the second polypeptide chain comprises, in the N-terminus to C-terminus direction:
 - (i)** a domain (D) comprising a binding region of a light chain variable domain of the first immunoglobulin (VL1) specific for the epitope (2);
 - (ii)** a domain (E) comprising a binding region of a heavy chain variable domain of the second immunoglobulin (VH1) specific for the epitope (1), wherein domains (D) and (E) are separated by a Peptide Linker 1;
 - (iii)** a domain (F) comprising
 - (a)** a heterodimer promoting domain; wherein domain (F) and domain (E) are separated by a Peptide Linker 2-C; or
 - (b)** a heterodimer promoting domain; wherein domain (F) and domain (E) are separated by a Peptide Linker 2;
- (III)** the third polypeptide chain that comprises, in the N-terminus to C-terminus direction:

- (i) a domain (G) comprising a binding region of a heavy chain variable domain of a third immunoglobulin (VH3) specific for an epitope (3); and
 - (ii) a CH1-Hinge Domain, and a CH2-CH3 Domain; and
 - (IV) the fourth polypeptide chain that comprises, in the N-terminus to C-terminus direction:
 - (i) a domain (H) comprising a binding region of a light chain variable domain of the third immunoglobulin (VL3) specific for the epitope (3); and
 - (ii) CL Kappa Domain or a CL Lambda Domain; and wherein
- the domains (A) and (B) do not associate with one another to form an epitope binding site;
 the domains (D) and (E) do not associate with one another to form an epitope binding site;
 the domains (A) and (E) associate to form a binding site that binds the epitope (1);
 the domains (B) and (D) associate to form a binding site that binds the epitope (2);
 the domains (G) and (H) associate to form a binding site that bind the epitope (3);
 at least one of epitope (1), epitope (2), and epitope (3) is an epitope bound by the gp41 MPER binding antibody DH511.2, a variant of DH511.2 called DH511.2_K3, or any antibody of the DH511 lineage, and at least one of epitope (1), epitope (2), and epitope (3) is an epitope of for example, but not limited to, CD3, CD8, or CD16, or an epitope on any suitable effector cell;
 the CH2-CH3 domains of the first and third polypeptide form an Fc Domain;
 said first and second polypeptide chains are covalently bonded to one another;
 said first and third polypeptide chains are covalently bonded to one another; and
 said third and fourth polypeptide chains are covalently bonded to one another.

[0046] In certain aspects the invention provides trivalent binding molecules comprising a first, second, third and fourth polypeptide chain, wherein some of the polypeptides are covalently bonded and wherein:

- (I) the first polypeptide chain comprises in the N-terminus to C-terminus direction:
 - (i) a domain (A) comprising SEQ ID NO: 553, 570, 574, 578, 582, 586, 590, 594, 598, 602, 606, or 625;
 - (ii) a domain (B) comprising SEQ ID NO:500;
 - (iii) a domain (C) comprising SEQ ID NO: 520, 521, 518, or 519; and
 - (iv) a CH2-CH3 Domain comprising SEQ ID NO: 531, 532, 533, or 534;
- (II) the second polypeptide chain comprises, in the N-terminus to C-terminus direction:
 - (i) a domain (D) comprising SEQ ID NO: 502;

- (ii) a domain (E) comprising SEQ ID NO: 551, 568, 572, 576, 580, 584, 588, 592, 596, 600, 604, 608-624, or 626 (Table 4); and
- (iii) a domain (F) comprising SEQ ID NO: 518, 519, 520, or 521;
- (III) the third polypeptide chain that comprises, in the N-terminus to C-terminus direction:
 - (i) a domain (G) comprising SEQ ID NO: 551, 568, 572, 576, 580, 584, 588, 592, 596, 600, 604, 608-624, or 626 (Table 4), 543, or 547; and
 - (ii) a CH1-Hinge Domain comprising SEQ ID NO: 515, and a CH2-CH3 Domain comprising SEQ ID NO: 531, 532, 533, or 534; and
- (IV) the fourth polypeptide chain that comprises, in the N-terminus to C-terminus direction:
 - (i) a domain (H) comprising SEQ ID NO: 553, 570, 574, 578, 582, 586, 590, 594, 598, 602, 606, 545, or 549; and
 - (ii) CL Kappa Domain comprising SEQ ID NO: 516 or a CL Lambda Domain comprising SEQ ID NO: 517; and wherein

the domains (A) and (B) are linked so that they do not associate with one another to form an epitope binding site;

the domains (D) and (E) are linked so that they do not associate with one another to form an epitope binding site;

the domains (A) and (E) associate to form a binding site that binds an epitope (1);

the domains (B) and (D) associate to form a binding site that binds an epitope (2);

the domains (G) and (H) associate to form a binding site that bind an epitope (3);

wherein epitope (1) is an epitope bound by a gp41 MPER binding antibody, epitope (2) is an epitope of CD3, and epitope (3) is an epitope bound by a gp41 MPER binding antibody or is an epitope of CD8;

the CH2-CH3 domains of the first and third polypeptide form an Fc Domain;

said first and second polypeptide chains are covalently bonded to one another;

said first and third polypeptide chains are covalently bonded to one another; and

said third and fourth polypeptide chains are covalently bonded to one another.

[0047] In certain aspects the invention provides such trivalent molecules wherein:

- (i) domains (A) and (B) are separated by SEQ ID NO: 508;
- (ii) domains (C) and (B) are separated by SEQ ID NO: 509 or 510;
- (iii) the CH2-CH3 domain and domain (C) are separated by SEQ ID NO: 522, or 523;

- (iv) domains (D) and (E) are separated by SEQ ID NO: 508; and
- (v) domains (F) and (E) are separated by SEQ ID NO: 509 or 510.

[0048] In certain aspects the invention provides trivalent binding molecules comprising a first, second, third and fourth polypeptide chain, wherein some of the polypeptides are covalently bonded and wherein:

- (I)** the first polypeptide chain comprises in the N-terminus to C-terminus direction:
 - (i)** a domain (A) comprising SEQ ID NO: 553, 570, 574, 578, 582, 586, 590, 594, 598, 602, 606, or 625;
 - (ii)** a domain (B) comprising SEQ ID NO: 500, wherein domains (A) and (B) are separated by SEQ ID NO: 508;
 - (iii)** a domain (C) comprising SEQ ID NO: 520, 521, 518, or 519, wherein domains (C) and (B) are separated by SEQ ID NO: 509 or 510;
 - (iv)** a CH2-CH3 Domain comprising SEQ ID NO: 531, 532, 533, or 534, wherein the CH2-CH3 domain and domain (C) are separated by SEQ ID NO: 522, or 523;
- (II)** the second polypeptide chain comprises, in the N-terminus to C-terminus direction:
 - (i)** a domain (D) SEQ ID NO: 502;
 - (ii)** a domain (E) comprising SEQ ID NO: 551, 568, 572, 576, 580, 584, 588, 592, 596, 600, 604, 608-624, or 626 (Table 4), wherein domains (D) and (E) are separated from one another by SEQ ID NO: 508;
 - (iii)** a domain (F) comprising SEQ ID NO: 518, 519, 520, or 521, wherein domain (F) and domain (E) are separated by SEQ ID NO: 509 or 510;
- (III)** the third polypeptide chain that comprises, in the N-terminus to C-terminus direction:
 - (i)** a domain (G) comprising SEQ ID NO: 551, 568, 572, 576, 580, 584, 588, 592, 596, 600, 604, 608-624, or 626 (Table 4), 543, or 547; and
 - (ii)** a CH1-Hinge Domain SEQ ID NO: 515, and a CH2-CH3 Domain comprising SEQ ID NO: 531, 532, 533, or 534; and
- (IV)** the fourth polypeptide chain that comprises, in the N-terminus to C-terminus direction:
 - (i)** a domain (H) comprising SEQ ID NO: 553, 570, 574, 578, 582, 586, 590, 594, 598, 602, 606, 625, 545, or 549; and
 - (ii)** CL Kappa Domain comprising SEQ ID NO: 516 or a CL Lambda Domain comprising SEQ ID NO: 517; and wherein

the domains (A) and (B) do not associate with one another to form an epitope binding site;
the domains (D) and (E) do not associate with one another to form an epitope binding site;
the domains (A) and (E) associate to form a binding site that binds an epitope (1);
the domains (B) and (D) associate to form a binding site that binds an epitope (2);
the domains (G) and (H) associate to form a binding site that bind an epitope (3);

wherein if the domain (A) comprises SEQ ID NO: 553 and domain (E) comprises SEQ ID NO: 551 they associate to form a binding site that binds the HIV-1 envelope like antibody DH511.2_K3;

wherein if the domain (A) comprises SEQ ID NO: 570 and domain (E) comprises SEQ ID NO: 568 they associate to form a binding site that binds the HIV-1 envelope like antibody DH511;

wherein if the domain (A) comprises SEQ ID NO: 574 and domain (E) comprises SEQ ID NO: 572 they associate to form a binding site that binds the HIV-1 envelope like antibody DH512 (also referred as DH511.2);

wherein if the domain (A) comprises SEQ ID NO: 578 and domain (E) comprises SEQ ID NO: 576 they associate to form a binding site that binds the HIV-1 envelope like antibody DH513;

wherein if the domain (A) comprises SEQ ID NO: 582 and domain (E) comprises SEQ ID NO: 580 they associate to form a binding site that binds the HIV-1 envelope like antibody DH514;

wherein if the domain (A) comprises SEQ ID NO: 586 and domain (E) comprises SEQ ID NO: 584 they associate to form a binding site that binds the HIV-1 envelope like antibody DH515;

wherein if the domain (A) comprises SEQ ID NO: 590 and domain (E) comprises SEQ ID NO: 588 they associate to form a binding site that binds the HIV-1 envelope like antibody DH516;

wherein if the domain (A) comprises SEQ ID NO: 594 and domain (E) comprises SEQ ID NO: 592 they associate to form a binding site that binds the HIV-1 envelope like antibody DH517;

wherein if the domain (A) comprises SEQ ID NO: 598 and domain (E) comprises SEQ ID NO: 596 they associate to form a binding site that binds the HIV-1 envelope like antibody DH518;

wherein if the domain (A) comprises SEQ ID NO: 602 and domain (E) comprises SEQ ID NO: 600 they associate to form a binding site that binds the HIV-1 envelope like antibody DH536; or

wherein if the domain (A) comprises SEQ ID NO: 606 and domain (E) comprises SEQ ID NO: 604 they associate to form a binding site that binds the HIV-1 envelope like antibody DH537; or

wherein if the domain (A) comprises SEQ ID NO: 625 and domain (E) comprises SEQ ID NO: 624 or 626 they associate to form a binding site that binds the HIV-1 envelope like antibody DH511_5a or DH511_5b; and

wherein if the domain (B) comprises SEQ ID NO: 500 and domain (D) comprises SEQ ID NO: 502 they associate to form a binding site that binds CD3; and

wherein if the domain (H) comprises SEQ ID NO: 553 and domain (G) comprises SEQ ID NO: 551 they associate to form a binding site that binds the HIV-1 envelope like antibody DH511.2_K3;

wherein if the domain (H) comprises SEQ ID NO: 570 and domain (G) comprises SEQ ID NO: 568 they associate to form a binding site that binds the HIV-1 envelope like antibody DH511;

wherein if the domain (H) comprises SEQ ID NO: 574 and domain (G) comprises SEQ ID NO: 572 they associate to form a binding site that binds the HIV-1 envelope like antibody DH512 (also referred as DH511.2);

wherein if the domain (H) comprises SEQ ID NO: 578 and domain (G) comprises SEQ ID NO: 576 they associate to form a binding site that binds the HIV-1 envelope like antibody DH513;

wherein if the domain (H) comprises SEQ ID NO: 582 and domain (G) comprises SEQ ID NO: 580 they associate to form a binding site that binds the HIV-1 envelope like antibody DH514;

wherein if the domain (H) comprises SEQ ID NO: 586 and domain (G) comprises SEQ ID NO: 584 they associate to form a binding site that binds the HIV-1 envelope like antibody DH515;

wherein if the domain (H) comprises SEQ ID NO: 590 and domain (G) comprises SEQ ID NO: 588 they associate to form a binding site that binds the HIV-1 envelope like antibody DH516;

wherein if the domain (H) comprises SEQ ID NO: 594 and domain (G) comprises SEQ ID NO: 592 they associate to form a binding site that binds the HIV-1 envelope like antibody DH517;

wherein if the domain (H) comprises SEQ ID NO: 598 and domain (G) comprises SEQ ID NO: 596 they associate to form a binding site that binds the HIV-1 envelope like antibody DH518;

wherein if the domain (H) comprises SEQ ID NO: 602 and domain (G) comprises SEQ ID NO: 600 they associate to form a binding site that binds the HIV-1 envelope like antibody DH536; or

wherein if the domain (H) comprises SEQ ID NO: 606 and domain (G) comprises SEQ ID NO: 604 they associate to form a binding site that binds the HIV-1 envelope like antibody DH537; or

wherein if the domain (A) comprises SEQ ID NO: 625 and domain (E) comprises SEQ ID NO: 624 or 626 they associate to form a binding site that binds the HIV-1 envelope like antibody DH511_5a or DH511_5b; or

wherein if the domain (H) comprises SEQ ID NO: 545 and domain (G) comprises SEQ ID NO: 543 they associate to form a binding site that binds CD8; or

wherein if the domain (H) comprises SEQ ID NO: 549 and domain (G) comprises SEQ ID NO: 547 they associate to form a binding site that binds CD8; and wherein

epitope (1) is an epitope bound by the antibody DH511, DH511.2_K3, DH512 (also referred to as DH511.2), DH513, DH514, DH515, DH516, DH517, DH518, DH536, or DH537, epitope (2) is an epitope of CD3, and epitope (3) is an epitope bound by the antibody DH511, DH511.2_K3, DH512 (also referred to as DH511.2), DH513, DH514, DH515, DH516, DH517, DH518, DH536, or DH537 or is an epitope of CD8; and wherein the CH2-CH3 domains of the first and third polypeptide form an Fc Domain; said first and second polypeptide chains are covalently bonded to one another; said first and third polypeptide chains are covalently bonded to one another; and said third and fourth polypeptide chains are covalently bonded to one another.

[0049] In certain aspects the invention also provides trivalent binding molecules comprising a first, second, and third polypeptide chain, wherein some of the polypeptides are covalently bonded and wherein:

(I) the first polypeptide chain comprises in the N-terminus to C-terminus direction:

- (i) a domain (A) comprising a binding region of a light chain variable domain of a first immunoglobulin (VL1) specific for an epitope (1);
 - (ii) a domain (B) comprising a binding region of a heavy chain variable domain of a second immunoglobulin (VH2) specific for an epitope (2);
 - (iii) a domain (C) comprising a heterodimer promoting domain; and
 - (iv) a CH2-CH3 Domain;
- (II) the second polypeptide chain comprises, in the N-terminus to C-terminus direction:
- (i) a domain (D) comprising a binding region of a light chain variable domain of the first immunoglobulin (VL1) specific for the epitope (2);
 - (ii) a domain (E) comprising a binding region of a heavy chain variable domain of the second immunoglobulin (VH1) specific for the epitope (1);
 - (iii) a domain (F) comprising a heterodimer promoting domain; and
- (III) the third polypeptide chain that comprises, in the N-terminus to C-terminus direction:
- (i) a domain (H) comprising a binding region of a light chain variable domain of a third immunoglobulin (VL3) specific for an epitope (3)
 - (ii) a domain (G) comprising a binding region of a heavy chain variable domain of the third immunoglobulin (VH3) specific for the epitope (3);
 - (iii) a CH2-CH3 Domain; and wherein
- the domains (A) and (B) are linked so that they do not associate with one another to form an epitope binding site;
- the domains (D) and (E) are linked so that they do not associate with one another to form an epitope binding site;
- the domains (A) and (E) associate to form a binding site that binds the epitope (1);
- the domains (B) and (D) associate to form a binding site that binds the epitope (2);
- the domains (G) and (H) associate to form a binding site that bind the epitope (3);
- at least one of epitope (1), epitope (2), and epitope (3) is an epitope bound by a gp41 MPER antibody, and at least one of epitope (1), epitope (2), and epitope (3) is and epitope of for example, but not limited to, CD3, CD8, or CD16, or any other suitable epitope on an effector cell;
- the CH2-CH3 domains of the first and third polypeptide form an Fc Domain;
- said first and second polypeptide chains are covalently bonded to one another;
- said first and third polypeptide chains are covalently bonded to one another; and

said third and fourth polypeptide chains are covalently bonded to one another.

[0050] In certain aspects the invention provides such trivalent molecules wherein:

- (i) domains (A) and (B) are separated by a peptide linker 1;
- (ii) domains (C) and (B) are separated by a peptide linker 2 or a peptide linker 2-C;
- (iii) the CH2-CH3 domain and domain (C) are separated by a peptide linker 3 or a spacer linker 3;
- (iv) domains (D) and (E) are separated by a peptide linker 1;
- (v) domains (F) and (E) are separated by a peptide linker 2 or a peptide linker 2-C;
- (vi) domains (H) and (G) are separated by a peptide linker 5; and
- (vii) the CH2-CH3 domain and domain (G) are separated by a peptide linker 3.

[0051] In certain aspects the invention also provides trivalent binding molecules comprising a first, second, and third polypeptide chain, wherein some of the polypeptides are covalently bonded and wherein:

- (I)** the first polypeptide chain comprises in the N-terminus to C-terminus direction:
 - (i) a domain (A) comprising a binding region of a light chain variable domain of a first immunoglobulin (VL1) specific for an epitope (1);
 - (ii) a domain (B) comprising a binding region of a heavy chain variable domain of a second immunoglobulin (VH2) specific for an epitope (2), wherein domains (A) and (B) are separated by a Peptide Linker 1;
 - (iii) a domain (C) comprising:
 - (a) a heterodimer promoting domain; wherein domain (C) and domain (B) are separated by a Peptide Linker 2-C; or
 - (b) a heterodimer promoting domain; wherein domain (C) and domain (B) are separated by a Peptide Linker 2; and
 - (iv) a CH2-CH3 Domain, wherein the CH2-CH3 domain and domain (C) are separated by a Peptide Linker 3 or a Spacer-Linker 3;
- (II)** the second polypeptide chain comprises, in the N-terminus to C-terminus direction:
 - (i) a domain (D) comprising a binding region of a light chain variable domain of the first immunoglobulin (VL1) specific for the epitope (2);

- (ii) a domain (E) comprising a binding region of a heavy chain variable domain of the second immunoglobulin (VH1) specific for the epitope (1), wherein domains (D) and (E) are separated by a Peptide Linker 1;
- (iii) a domain (F) comprising:
 - (a) a heterodimer promoting domain; wherein domain (F) and domain (E) are separated by a Peptide Linker 2-C; or
 - (b) a heterodimer promoting domain; wherein domain (F) and domain (E) are separated by a Peptide Linker 2;
- (III) the third polypeptide chain that comprises, in the N-terminus to C-terminus direction:
 - (i) a domain (H) comprising a binding region of a light chain variable domain of a third immunoglobulin (VL3) specific for an epitope (3)
 - (ii) a domain (G) comprising a binding region of a heavy chain variable domain of the third immunoglobulin (VH3) specific for the epitope (3), wherein domains (H) and (G) are separated by a Peptide Linker 5;
 - (iii) a Peptide Linker 3; and
 - (iv) a CH2-CH3 Domain; and wherein

the domains (A) and (B) do not associate with one another to form an epitope binding site;

the domains (D) and (E) do not associate with one another to form an epitope binding site;

the domains (A) and (E) associate to form a binding site that binds the epitope (1);

the domains (B) and (D) associate to form a binding site that binds the epitope (2);

the domains (G) and (H) associate to form a binding site that bind the epitope (3);

at least one of epitope (1), epitope (2), and epitope (3) is an epitope bound by the gp41 MPER binding antibody DH511.2, a variant of DH511.2 called DH511.2_K3, or any antibody of the DH511 lineage, and at least one of epitope (1), epitope (2), and epitope (3) is and epitope of for example, but not limited to, CD3, CD8 or CD16, or an epitope on any suitable effector cell;

the CH2-CH3 domains of the first and third polypeptide form an Fc Domain;

said first and second polypeptide chains are covalently bonded to one another; and

said first and third polypeptide chains are covalently bonded to one another.

[0052] In certain aspects the invention also provides trivalent binding molecules comprising a first, second, and third polypeptide chain, wherein some of the polypeptides are covalently bonded and wherein:

- (I) the first polypeptide chain comprises in the N-terminus to C-terminus direction:
 - (i) a domain (A) comprising SEQ ID NO: 553, 570, 574, 578, 582, 586, 590, 594, 598, 602, 606, or 625;
 - (ii) a domain (B) comprising SEQ ID NO: 500;
 - (iii) a domain (C) comprising SEQ ID NO: 520, 521, 518, or 519; and
 - (iv) a CH2-CH3 Domain comprising SEQ ID NO: 531, 532, 533, or 534;
- (II) the second polypeptide chain comprises, in the N-terminus to C-terminus direction:
 - (i) a domain (D) comprising SEQ ID NO: 502;
 - (ii) a domain (E) comprising SEQ ID NO: 551, 568, 572, 576, 580, 584, 588, 592, 596, 600, 604, 608-624, or 626 (Table 4);
 - (iii) a domain (F) comprising SEQ ID NO: 518, 519, 520, or 521; and
- (III) the third polypeptide chain that comprises, in the N-terminus to C-terminus direction:
 - (i) a domain (H) comprising SEQ ID NO: 545 or 549;
 - (ii) a domain (G) comprising SEQ ID NO: 543 or 547;
 - (iii) a CH2-CH3 Domain comprising SEQ ID NO: 531, 532, 533, or 534; and

wherein

the domains (A) and (B) are linked so that they do not associate with one another to form an epitope binding site;

the domains (D) and (E) are linked so that they do not associate with one another to form an epitope binding site;

the domains (A) and (E) associate to form a binding site that binds an epitope bound by a gp41 MPER antibody;

the domains (B) and (D) associate to form a binding site that binds CD3;

the domains (G) and (H) associate to form a binding site that binds CD8; wherein

the CH2-CH3 domains of the first and third polypeptide form an Fc Domain;

said first and second polypeptide chains are covalently bonded to one another;

said first and third polypeptide chains are covalently bonded to one another; and

said third and fourth polypeptide chains are covalently bonded to one another.

[0053] In certain aspects the invention provides such trivalent molecules wherein:

- (i) domains (A) and (B) are separated by SEQ ID NO: 508;
- (ii) domains (C) and (B) are separated by SEQ ID NO: 509 or 510;

- (iii) the CH2-CH3 domain and domain (C) are separated by SEQ ID NO: 522, or 523;
- (iv) domains (D) and (E) are separated by SEQ ID NO: 508;
- (v) domains (F) and (E) are separated by SEQ ID NO: 509 or 510;
- (vi) domains (H) and (G) are separated by SEQ ID NO: 526; and
- (vii) the CH2-CH3 domain and domain (G) are separated by SEQ ID NO: 523 or a CH1-Hinge Domain comprising SEQ ID NO: 515.

[0054] In certain aspects the invention also provides trivalent binding molecules comprising a first, second, and third polypeptide chain, wherein some of the polypeptides are covalently bonded and wherein:

- (I)** the first polypeptide chain comprises in the N-terminus to C-terminus direction:
 - (i)** a domain (A) comprising SEQ ID NO: 553, 570, 574, 578, 582, 586, 590, 594, 598, 602, 606, or 625;
 - (ii)** a domain (B) comprising SEQ ID NO: 500, wherein domains (A) and (B) are separated by SEQ ID NO: 508;
 - (iii)** a domain (C) comprising SEQ ID NO: 520, 521, 518, and 519, wherein domains (C) and (B) are separated by SEQ ID NO: 509 or 510; and
 - (iv)** a CH2-CH3 Domain comprising SEQ ID NO: 531, 532, 533, or 534, wherein the CH2-CH3 domain and domain (C) are separated by SEQ ID NO: 522, or 523;
- (II)** the second polypeptide chain comprises, in the N-terminus to C-terminus direction:
 - (i)** a domain (D) comprising SEQ ID NO: 502;
 - (ii)** a domain (E) comprising SEQ ID NO: 551, 568, 572, 576, 580, 584, 588, 592, 596, 600, 604, 608-624, or 626 (Table 4), wherein domains (D) and (E) are separated by SEQ ID NO: 508;
 - (iii)** a domain (F) comprising SEQ ID NO: 518, 519, 520, or 521, wherein domains (F) and (E) are separated by SEQ ID NO: 509 or 510;
- (III)** the third polypeptide chain that comprises, in the N-terminus to C-terminus direction:
 - (i)** a domain (H) comprising SEQ ID NO: 545 or 549;
 - (ii)** a domain (G) comprising SEQ ID NO: 543 or 547, wherein domains (H) and (G) are separated by SEQ ID NO: 526;
 - (iii)** SEQ ID NO: 523, or a CH1-Hinge Domain comprising SEQ ID NO: 515; and
 - (iv)** a CH2-CH3 Domain comprising SEQ ID NO: 531, 532, 533, or 534; and

wherein

the domains (A) and (B) do not associate with one another to form an epitope binding site;
the domains (D) and (E) do not associate with one another to form an epitope binding site;
the domains (A) and (E) associate to form a binding site that binds the epitope (1);
the domains (B) and (D) associate to form a binding site that binds CD3;
the domains (G) and (H) associate to form a binding site that binds CD8; and

wherein if the domain (A) comprises SEQ ID NO: 553 and domain (E) comprises SEQ ID NO: 551 they associate to form a binding site that binds the HIV-1 envelope like antibody DH511.2_K3;

wherein if the domain (A) comprises SEQ ID NO: 570 and domain (E) comprises SEQ ID NO: 568 they associate to form a binding site that binds the HIV-1 envelope like antibody DH511;

wherein if the domain (A) comprises SEQ ID NO: 574 and domain (E) comprises SEQ ID NO: 572 they associate to form a binding site that binds the HIV-1 envelope like antibody DH512 (also referred as DH511.2);

wherein if the domain (A) comprises SEQ ID NO: 578 and domain (E) comprises SEQ ID NO: 576 they associate to form a binding site that binds the HIV-1 envelope like antibody DH513;

wherein if the domain (A) comprises SEQ ID NO: 582 and domain (E) comprises SEQ ID NO: 580 they associate to form a binding site that binds the HIV-1 envelope like antibody DH514;

wherein if the domain (A) comprises SEQ ID NO: 586 and domain (E) comprises SEQ ID NO: 584 they associate to form a binding site that binds the HIV-1 envelope like antibody DH515;

wherein if the domain (A) comprises SEQ ID NO: 590 and domain (E) comprises SEQ ID NO: 588 they associate to form a binding site that binds the HIV-1 envelope like antibody DH516;

wherein if the domain (A) comprises SEQ ID NO: 594 and domain (E) comprises SEQ ID NO: 592 they associate to form a binding site that binds the HIV-1 envelope like antibody DH517;

wherein if the domain (A) comprises SEQ ID NO: 598 and domain (E) comprises SEQ ID NO: 596 they associate to form a binding site that binds the HIV-1 envelope like antibody DH518;

wherein if the domain (A) comprises SEQ ID NO: 602 and domain (E) comprises SEQ ID NO: 600 they associate to form a binding site that binds the HIV-1 envelope like antibody DH536; or

wherein if the domain (A) comprises SEQ ID NO: 606 and domain (E) comprises SEQ ID NO: 604 they associate to form a binding site that binds the HIV-1 envelope like antibody DH537; or

wherein if the domain (A) comprises SEQ ID NO: 625 and domain (E) comprises SEQ ID NO: 624 or 626 they associate to form a binding site that binds the HIV-1 envelope like antibody DH511_5a or DH511_5b; and

wherein if the domain (H) comprises SEQ ID NO: 545 and domain (G) comprises SEQ ID NO: 543 they associate to form a binding site that binds CD8; or

wherein if the domain (H) comprises SEQ ID NO: 549 and domain (G) comprises SEQ ID NO: 547 they associate to form a binding site that binds CD8;

wherein the CH2-CH3 domains of the first and third polypeptide form an Fc Domain;

said first and second polypeptide chains are covalently bonded to one another; and

said first and third polypeptide chains are covalently bonded to one another.

[0055] In certain aspects the invention provides such trivalent binding molecules wherein one of epitope (1), epitope (2), and epitope (3) is an epitope of HIV-1 Envelope, one of epitope (1), epitope (2), and epitope (3) is an epitope of CD3, and one of epitope (1), epitope (2), and epitope (3) is an epitope of CD8. In particular aspects of such trivalent binding molecules:

(a) epitope (1) is an epitope of HIV-1 Envelope gp41 MPER, epitope (2) is an epitope of CD3, and epitope (3) is an epitope of CD8;

(b) epitope (1) is an epitope of HIV-1 Envelope gp41 MPER, epitope (2) is an epitope of CD8, and epitope (3) is an epitope of CD3;

(c) epitope (1) is an epitope of CD3, epitope (2) is an epitope of HIV-1 envelope gp41 MPER, and epitope (3) is an epitope of CD8;

(d) epitope (1) is an epitope of CD3, epitope (2) is an epitope of CD8, and epitope (3) is an epitope of HIV-1 Envelope gp41 MPER;

(e) epitope (1) is an epitope of CD8, epitope (2) is an epitope of CD3, and epitope (3) is an epitope of HIV-1 Envelope gp41 MPER; or

(f) epitope (1) is an epitope of CD8, epitope (2) is an epitope of HIV-1 Envelope gp41 MPER, and epitope (3) is an epitope of CD3.

[0056] In certain aspects the invention provides such trivalent binding molecules wherein two of epitope (1), epitope (2), and epitope (3) are epitopes of HIV-1 Envelope, and one of epitope (1), epitope (2), and epitope (3) is an epitope of CD3, where said epitopes of HIV-1 Envelope may be the same epitope or different epitopes. In particular aspects of such trivalent binding molecules:

- (a) epitope (1) is an epitope of HIV-1 Envelope gp41 MPER, epitope (2) is an epitope of HIV-1 Envelope gp41 MPER, and epitope (3) is an epitope of CD3;
- (b) epitope (1) is an epitope of HIV-1 Envelope gp41 MPER, epitope (2) is an epitope of CD3, and epitope (3) is an epitope of HIV-1 Envelope gp41 MPER; or
- (c) epitope (1) is an epitope of CD3, epitope (2) is an epitope of HIV-1 Envelope gp41 MPER, and epitope (3) is an epitope of HIV-1 Envelope gp41 MPER.

[0057] In certain aspects, domain (H) comprises a binding region of a light chain variable domain of an anti-CD8 antibody, an anti-CD3 antibody, an anti-CD16 antibody, an HIV-1 envelope gp41 MPER antibody. In certain aspects, domain (G) comprises a binding region of a heavy chain variable domain of an anti-CD8 antibody, an anti-CD3 antibody, an anti-CD16 antibody, an HIV-1 envelope gp41 MPER antibody.

[0058] In certain aspects, domain (B) comprises the heavy chain variable domain of an anti-CD3 antibody, an anti-CD8 antibody, or an anti-CD16 antibody. In certain embodiment, domain (D) comprises the light chain variable domain of an anti-CD3 antibody, an anti-CD8 antibody, or an anti-CD16 antibody.

[0059] In certain aspects, the CH2-CH3 domain of the first polypeptide chain of any of the multispecific molecules of the invention is the of the “knob” design and the CH2-CH3 domain of the third polypeptide chain of any of the multivalent molecules of the invention is of the “hole” design.

[0060] In certain aspects, the CH2-CH3 domain of the third polypeptide chain of any of the multispecific molecules of the invention is the of the “knob” design and the CH2-CH3 domain of the first polypeptide chain of any of the multivalent molecules of the invention is of the “hole” design.

[0061] In certain aspects, the CH2-CH3 domain of the first polypeptide chain is the of the “knob” design (SEQ ID NOs: 531 or 532) and the CH2-CH3 domain of the third polypeptide chain is of the “hole” design (SEQ ID NOs: 533 or 534). In certain aspects, the CH2-CH3 domain of the first polypeptide comprises SEQ ID NO: 531 and the CH2-CH3 domain of the

third polypeptide chain comprises SEQ ID NO: 533. In certain aspects, the CH2-CH3 domain of the third polypeptide chain is the of the “knob” design (SEQ ID NOs: 531 or 532) and the CH2-CH3 domain of the first polypeptide chain is of the “hole” design (SEQ ID NOs: 533 or 534). In certain aspects, the CH2-CH3 domain of the third polypeptide comprises SEQ ID NO: 531 and the CH2-CH3 domain of the first polypeptide chain comprises SEQ ID NO: 533.

[0062] In certain aspects, the epitope (2) is a CD3 epitope, CD8 epitope or a CD16 epitope. In certain embodiments, the bispecific or trivalent molecule binds HIV-1 envelope with the specificity of the gp41 MPER binding antibody DH511.2, a variant of DH511.2 called DH511.2_K3, or any antibody of the DH511 lineage and also binds CD3. In certain embodiments, the bispecific or trivalent molecule binds HIV-1 envelope with the specificity of the gp41 MPER binding antibody DH511.2, a variant of DH511.2 called DH511.2_K3, or any antibody of the DH511 lineage and also binds CD3, CD8, or CD16.

[0063] In certain aspects, domain (A) comprises the CDR1, CDR2, and CDR3 of the light chain variable domain of any one of the immunoglobulin DH511, DH512, DH511.2_K3, DH513, DH514, DH515, DH516, DH517, DH518, DH536, or DH537, or any of the other antibodies described herein. In certain aspects, the domain (E) comprises the CDR1, CDR2, and CDR3 of the heavy chain variable domain of any one of the immunoglobulin DH511, DH512, DH511.2_K3, DH513, DH514, DH515, DH516, DH517, DH518, DH536, or DH537, or any of the other antibodies described herein. In certain aspects, domain (A) comprises the light chain variable domain of any one of the immunoglobulin DH511, DH512, DH511.2_K3, DH513, DH514, DH515, DH516, DH517, DH518, DH536, or DH537. In certain aspects, domain (E) comprises the heavy chain variable domain of any one of the immunoglobulin DH511, DH512, DH511.2_K3, DH513, DH514, DH515, DH516, DH517, DH518, DH536, or DH537.

[0064] In certain aspects, domain (A) comprises the CDR1, CDR2, and CDR3 of the light chain variable domain of immunoglobulin DH511.2 or DH511.2_K3. In certain aspects, the domain (E) comprises the CDR1, CDR2, and CDR3 of the heavy chain variable domain of immunoglobulin DH511.2 or DH511.2_K3. In certain aspects, domain (A) comprises the light chain variable domain of immunoglobulin DH511.2 or DH511.2_K3. In certain aspects, domain (E) comprises the heavy chain variable domain of immunoglobulin DH511.2 or DH511.2_K3.

[0065] In certain aspects, the first polypeptide comprises SEQ ID NO: 555. In certain aspects, the second polypeptide comprises SEQ ID NO: 557. In certain aspects, the third polypeptide comprises SEQ ID NO: 559.

[0066] In certain aspects, the bispecific molecule comprises the first polypeptide of SEQ ID NO: 555, the second polypeptide of SEQ ID NO: 557, and the third polypeptide of SEQ ID NO: 559.

[0067] In certain aspects, the bispecific molecule consists essentially of the first polypeptide of SEQ ID NO: 555, the second polypeptide of SEQ ID NO: 557, and the third polypeptide of SEQ ID NO: 559. In certain aspects, the bispecific molecule consists of the first polypeptide of SEQ ID NO: 555, the second polypeptide of SEQ ID NO: 557, and the third polypeptide of SEQ ID NO: 559.

[0068] In certain aspects, a four chain trivalent binding molecule is a trispecific molecule and comprises the first polypeptide of SEQ ID NO: 555, the second polypeptide of SEQ ID NO: 557, the third polypeptide of SEQ ID NO: 561, the fourth polypeptide of SEQ ID NO: 562 (See Figure 104A).

[0069] In certain aspects, a three chain trivalent binding molecule is a trispecific molecule and comprises the first polypeptide of SEQ ID NO: 555, the second polypeptide of SEQ ID NO: 557, the third polypeptide of SEQ ID NO: 563 (See Figure 104D).

[0070] In certain aspects, the invention provides a composition comprising any one of the multispecific molecules or any combination thereof. In certain aspects, the composition comprises a composition comprising a bispecific molecule comprising a first arm with the binding specificity of a HIV-1 envelope gp41 MPER binding site antibody and a second arm targeting CD3, CD8, or CD16. In certain aspects, the bispecific molecule comprises an Fc portion or any other modification which extends its serum half-life. In certain aspects, the composition further comprises a second bispecific molecule or trivalent binding molecule comprising a first arm with an HIV-1 envelope binding specificity different from the HIV-1 binding specificity of the first multispecific molecule, and a second arm targeting CD3, CD8, or CD16, wherein the first and second multispecific molecules are different in either the HIV-1 binding specificity and/or the specificity of the second arm.

[0071] In certain aspects, the invention provides a method to treat or prevent HIV-1 infection in a subject in need thereof comprising administering to the subject a composition comprising any one of the multispecific molecules of the invention or a combination of any one of the multispecific molecules in a therapeutically effective amount. In certain embodiments, the methods further comprise administering a latency activating agent. In some embodiments, the latency activating agent is vorinostat, romidepsin, panobinostat, disulfiram, JQ1, bryostatins, PMA, inonomecin, or any combination thereof.

[0072] In certain aspects, the invention provides nucleic acids comprising nucleotides encoding the multispecific molecules of the invention. In certain aspects, the invention provides a vector comprising nucleic acids comprising nucleotides encoding the multispecific molecules of the invention. Provided are also compositions comprising a vector comprising a nucleic acid encoding the multispecific molecules. In certain aspects the invention provides a cell line comprising vectors or nucleic acids encoding the multispecific molecules of the invention, wherein the vectors encode polypeptide chains for expression of the multispecific molecules of the invention, *e.g.* but not limited to, polypeptide chain 1 and polypeptide chain 2, or polypeptide chain 1, polypeptide chain 2 and polypeptide chain 3. In certain embodiments, the vector is suitable for gene delivery and expression. In certain embodiment, the vector is an adenoviral vector, an adeno associated virus based vector, or a combination thereof.

[0073] In certain embodiments, the multispecific molecule binds to the HIV-1 envelope like the HIV-1 antibody from which it is derived. In certain embodiments, the multispecific molecule binds to the DH511-HIV-1 envelope epitope, *i.e.* the multispecific molecule binds to the HIV-1 envelope like the DH511 antibody, and also binds CD3, CD8, or CD16.

[0074] In certain embodiments a multispecific molecule of the invention comprises, consists essentially of or consists of sequences as described herein, (*e.g.*, Table 4).

[0075] In certain aspects the invention provides compositions comprising any of the multispecific molecules described herein, or a combination thereof. In certain embodiments, these compositions are formulated as pharmaceutical composition for therapeutic use.

[0076] In certain aspects the invention is directed to nucleic acids which encode the multispecific molecule of the invention. In certain embodiments, these nucleic acids are comprised in a vector, and are operably linked to a promoter. In certain aspects the invention provides cell lines, or isolated cells, which comprise nucleic acids for the expression of the multispecific molecule of the invention.

[0077] In certain aspects, the invention provides compositions comprising the multispecific molecule of the invention or nucleic acids encoding the same for use in methods of treating or preventing HIV-1 infection. In some embodiments, these methods further comprise administering a Latency Activating Reagent. Non-limiting examples of these include HDAC inhibitors, e.g., vorinostat, romidepsin, panobinostat, disulfiram, JQ1, bryostatin, PMA, inonomecin, or any combination thereof. In some embodiments, this combination therapy targets the pool of latently infected HIV-1 cells.

[0078] In certain aspects, the invention provides methods of treating or preventing an HIV-1 infection in a subject, the method comprising administering to the subject a composition comprising any one of the multispecific molecules the invention, or a combination thereof in a therapeutically sufficient amount. In certain embodiments, the methods further comprise administering a latency activating agent.

BRIEF DESCRIPTION OF THE DRAWINGS

[0079] To conform to the requirements for PCT patent applications, many of the figures presented herein are black and white representations of images originally created in color.

[0080] Figure 1 shows Neutralization-based Epitope Prediction (NEP) Analysis.

Neutralization-based epitope prediction analysis. The predicted relevant prevalence of antibody clusters [(10 epitopes targeting sites of vulnerability (CD4 binding site, V1/V2, MPER, glycan V3)] is shown as a heat map, with dark color intensity (higher fractional number) corresponding to a stronger neutralization signal. Plasma neutralization breadth is shown, and numbers in each row add up to 1.00. NEP algorithm reference: [Georgiev IS *et al Science* 340: 751-756].

[0081] Figure 2 shows MPR.03 Hook sequence (SEQ ID NOs: 1-2). MPR.03 is a biotinylated peptide containing lysines at both ends for solubility (KKKNEQELLELDKWASLWNWFDITNWLWYIRKKK-biotin) (SEQ ID NO: 463) used to

pull out gp41 antibodies from blood memory B cell sorts. See Morris L. et al. (2011) PLoS ONE 6(9): e23532.

[0082] Figure 3 shows a representative CH0210 mper03 sort (sort #1).

[0083] Figure 4 shows V(D)J Rearrangement of MPER Antibodies Isolated from Four HIV-1 Infected Individuals. * indicates that these mAbs neutralized the tier 1 isolate MN in TZM-bl cells. Mutation refers to VH nucleotide sequence somatic mutation percentages in the variable heavy (VH) immunoglobulin (Ig) genes.

[0084] Figure 5 shows Neutralization Titers of MPER Antibodies Isolated from Four HIV-1 Infected Individuals using a small panel of HIV-1 isolates in the TZMbl pseudovirus inhibition assay.

[0085] Figure 6 shows the MPER BnAb DH511 VH Phylogram of the B Cell Clonal Lineage Derived from Subject 0210. Antibodies in clone DH511 include the following: DH511, DH512, DH513, DH514, DH515, DH516 and DH520.

[0086] Figure 7 shows summary results of neutralization of gp41 antibodies against a panel of 30 HIV-1 tier 2 isolates in the TZMbl pseudovirus neutralization assay. Data show that antibodies in the DH511 B cell clonal lineage (DH511-DH516) all neutralize 100% of 30 HIV-1 isolates tested in the TZMbl Env pseudovirus neutralization assay.

[0087] Figure 8 shows Neutralizing Breadth and Potency of DH512, DH517 and DH518 HIV-1 BnAbs compared to 10E8, VRC01 and a mixture of CH01 and CH31 bnAbs. DH512 neutralizes 100% of HIV strains and is as at least as potent as 10E8.

[0088] Figure 9 shows Neutralizing Breadth and Potency of various HIV-1 BnAbs that are candidates for being combined with DH512 or other antibodies in Figure 4 for a potent mixture of bnAbs. DH270IA1 is I1 in the DH270 lineage (See Figure 26, and US Ser. No. 62/056,568 filed September 28, 2014)

[0089] Figure 10 shows Neutralizing Breadth and Potency of some candidate bnAbs for single or combination use.

[0090] Figure 11 shows summary of Clone DH511 binding to the indicated peptides (SEQ ID NOs: 3-14) in ELISA. Clone DH511 antibodies bind at the C-terminus of the MPER. "+" indicates that antibodies in the Clone DH511 bind to the peptide. The summary shows that DH511 clone antibodies do not bind the peptides when D674 is mutated to S674. The twelve sequences of the peptides (without the three lysines at the N- and C- end) are shown in SEQ ID NOs: 703 to 714. The twelve sequences of the peptides (with the three lysines at the N-

and C- end) are shown in SEQ ID NOs: 3 to 14. Thus, antibody DH511 requires an aspartic acid at amino acid position 674 for binding.

[0091] Figure 12 shows nucleic acid sequences of antibodies DH511-518, DH536 and 537 (SEQ ID Nos: 15 to 34).

[0092] Figure 13 shows amino acid sequences of antibodies DH511-518, DH536 and 537. (SEQ ID Nos: 35 to 55)

[0093] Figures 14A-B show Alignment of VH (Fig. 14A; (SEQ ID Nos: 56-61)) and VL (Fig. 14B (SEQ ID Nos: 62-67)) Sequences of BnAb DH511 Clonal Lineage. Bolded is the sequence of CDR1, underlined is the sequence of CDR2 and italicized is the sequence of CDR3 of the DH511 VH chain and DH511 VL chain. The CDRs of the VH and VL sequences of the other antibodies DH512, DH513, DH514, DH515, and DH516 can be readily determined based on the sequence alignment.

[0094] Figures 15A-B show Alignment of VH (Fig. 15A (SEQ ID Nos: 68-76)) and VL (Fig. 15B (SEQ ID Nos: 77-85)) sequences of MPER BnAbs. Bolded is the sequence of CDR1, italicized is the sequence of CDR2 and underlined is the sequence of CDR3 of VH or VL of the listed MPER antibodies.

[0095] Figure 16 shows sequences of MPER alanine mutants (SEQ ID NOs: 86-112) screened in ELISA. All antibodies in the DH51 clone showed weak binding to this peptide set. DH517 (Ab510053) strongly bound to MPER656 peptide and showed decreased binding to several residues (A4, A6-A13, A16-A18, A20, A23, A24, A26) using the ala substituted peptides in table.

[0096] Figure 17 shows Binding of DH517 (Ab510053) to alanine substituted MPER-26 peptides. The binding studies do not conclusively map the DH517epitope.

[0097] Figure 18 shows MPER656 variants (SEQ ID NOs: 113-124) screened in ELISA. Residues shown in light blue (underlined) indicate positions that differ from MPER656-biotin.

[0098] Figure 19 shows Binding of DH511 (Ab510056) to MPER656 variants

[0099] Figure 20 shows Binding of DH512 (Ab510049) to MPER656 variants

[0100] Figure 21 shows Binding of DH513 (Ab570022) to MPER656 variants

[0101] Figure 22 shows Binding of DH514 (Ab570029) to MPER656 variants

[0102] Figure 23 shows Binding of DH515 (Ab510052) to MPER656 variants

[0103] Figure 24 shows Binding of DH516 (Ab510048) to MPER656 variants

[0104] Figure 25 shows Binding of DH518 (Ab570010) to MPER656 variants.

[0105] Figure 26 shows the amino acids sequences of VH (SEQ ID NOs: 137-148) and VL (SEQ ID NOs: 161-172) chains of antibodies of the DH270 lineage, and nucleic acid sequences (SEQ ID NOs: 125-136 (VH); SEQ ID NOs: 149-160 (VL)) encoding these amino acids. CDRs are highlighted and underlined in the UCA.

[0106] Figure 27A shows amino acid (SEQ ID Nos: 173 and 174) and nucleic acid sequences (SEQ ID Nos: 175 and 176) of CD4bs antibody CH557. Figure 27B shows amino acid sequences of VH chains of antibodies from CH235 lineage (SEQ ID NOs: 177-188). Figure 27C shows amino acid sequences of VL chains of antibodies from CH235 lineage (SEQ ID NOs: 189-198).

[0107] Figure 28A shows neutralization Breadth and Potency of Plasma and Memory B cell (MBC)-derived MPER bnAbs. Figure 28B shows neutralization Breadth and Potency of chimeric MPER bnAbs (n=30 cross-clade HIV-1 isolates)

[0108] Figure 29A and B show neutralization data from TZM-bl assay (Titer in TZM.bl cells (ug/ml) for DH512_K3 and other chimeric antibodies compared to DH512 and 10E8. The data in the first column is historic data when DH512 was run in this panel previously. DH512 was run at the same time as DH512_K3 but is listed as Ab510049 in this assay; therefore, data from columns DH512_K3 and AA&AB DH512/Ab510049 should be compared.

[0109] Figure 30 shows positions in the VHCDR3 chain of DH511 (SEQ ID NO.: 471) which could be mutated. Amino acid positions refer to Kabat numbering. Most mutations are to changes to W, but F, L or possibly other substitutions can be tried.

[0110] Figure 31 shows positions in the VHCDR3 chain of DH512 (SEQ ID NO.: 472) which could be mutated. Amino acid positions refer to Kabat numbering for the DH512VH chain: QVQLVQSGGGLVKPGGSLTLSCSASGFFFDNSWMGWVRQAPGKGLEWVGRIRRLK DGATGEYGA AVKDRFTISRDDSRNMLYLHMRTLKTEDSGTYYCTMDEGTPVTRFLE WGYFYYYMAVWGRGTTIVIVSS (SEQ ID NO.: 469). Most mutations are to changes to W, but F, L or possibly other substitutions can also be tried. Position V100 can be changed to I. Position L100d can be changed to F.

[0111] Figure 32 shows positions outside of VHCDR3 which could be mutated (SEQ ID NOS: 473-478, reselectively, in order of appearance). Most mutations are to changes to W, but F, L or possibly other substitutions can also be tried.

[0112] Figure 33 shows amino acid sequences (SEQ ID NOs: 199-216) of some of the DH512 mutants from Figure 31.

[0113] Figure 34 shows neutralization data for a set of 16 mutations from Figure 31. In this figure DH512 is referred to as DH512 (Ab510049_4A): its heavy chain is H510049_4 and its light chain is K510032

[0114] Figure 35 shows summary of anti-cardiolipin activity of various antibodies as measured by QUANTA Lite® ACA IgG III kit. Data plotted are representative of 2 independent experiments. mAb were run in duplicate in the second assay. Mean error and standard deviation are shown. Data were consistent between assays. Dotted line indicates positivity cut-off of 0.18. mAbs with OD values above 0.18 are bolded in the figure legend (DH514, DH518-315 HC, DH511-I6-4a through DH511_I1_4A; 4E10).

[0115] Figure 36 shows a summary of self-reactivity data of MPER antibodies.

[0116] Figure 37 shows summary results of neutralization data of DH512 and 10E8 against a panel of HIV-1 isolates in the TZMbl pseudovirus neutralization assay. Values represent IC₅₀ in µg/ml. Figure 37 also shows the mean IC₅₀ and percent of isolates neutralized at different IC₅₀ values.

[0117] Figure 38 shows summary results of neutralization data of DH512 and 10E8 against a panel of HIV-1 isolates in the TZMbl pseudovirus neutralization assay. Values represent IC₈₀ in µg/ml. Figure 38 also shows the mean IC₈₀ and percent of isolates neutralized at different IC₈₀ values.

[0118] Figure 39 shows Experimental Overview of Paired VH-VL Sequencing and antibody identification (Example 10). V gene repertoire sequencing. Identification of individual monoclonal antibodies requires the generation of a sample-specific database of IgG VH sequences constructed by next-generation sequencing of mature B cells isolated from the PBMCs of the donor. Reads are processed bioinformatically to obtain a database of unique VH sequences, which then are clustered into clonotypes according to their CDR3 sequences. The obtained database is used to interpret the MS spectra. F(ab)₂ purification and proteomic analysis. F(ab)₂ fragments are prepared from total serum IgG and subjected to antigen-affinity chromatography (monomeric gp120). Proteins in the elution and flow-through are denatured and reduced, alkylated, trypsin-digested and analyzed by high resolution LC-MS/MS. Spectra are interpreted with the sample-specific VH database and peptides uniquely associated with a single CDR3 are used to identify full-length VH sequences.

[0119] Figure 40 shows MPER BnAb DH511 Clonal Lineage Derived from African Individual CH0210 (the heavy chain for DH511_1A is not included).

[0120] Figure 41 shows Neutralization Activity (IC₅₀) of MPER Antibodies Identified by Paired VH:VL Sequencing Technology (Example 10). Summary data of two independent assays.

[0121] Figure 42 shows Neutralization Activity (IC₈₀) of MPER Antibodies Identified by Paired VH:VL Sequencing Technology (Example 10). Summary data of two independent assays.

[0122] Figure 43 shows Nucleotide Alignment of MPER Antibody Heavy Chain Sequences (SEQ ID NOs: 217-229).

[0123] Figure 44 shows Amino Acid Alignment of MPER Antibody Heavy Chain Sequences (SEQ ID NOs: 230-242).

[0124] Figure 45 shows Nucleotide Alignment of MPER Antibody Light Chain Sequences (SEQ ID NOs: 243-252).

[0125] Figure 46 shows Amino Acid Alignment of MPER Antibody Light Chain Sequences (SEQ ID NOs: 253-262).

[0126] Figure 47 shows Immunogenetic Characteristics of MPER Antibodies – Original Pairings.

[0127] Figures 48 shows epitope mapping of antibodies of Example 10. Binding to various MPER peptides in an ELISA assay was used to map the epitopes of these MPER antibodies.

[0128] Figures 49 show epitope mapping of antibodies of Example 10. Binding to various MPER peptides in an ELISA assay was used to map the epitopes of these MPER antibodies.

[0129] Figures 50 show epitope mapping of antibodies of Example 10. Binding to various MPER peptides in an ELISA assay was used to map the epitopes of these MPER antibodies.

[0130] Figures 51 show epitope mapping of antibodies of Example 10. Binding to various MPER peptides in an ELISA assay was used to map the epitopes of these MPER antibodies.

[0131] Figures 52 show epitope mapping of antibodies of Example 10. Binding to various MPER peptides in an ELISA assay was used to map the epitopes of these MPER antibodies.

[0132] Figure 53 shows Poly/Autoreactivity analysis of DH511_5a. Antibody DH511_5a appears to be autoreactive with one protein (NUDC).

[0133] Figure 54 shows Poly/Autoreactivity analysis of DH511_5b. Antibody DH511_5b appears to be polyreactive.

[0134] Figure 55 shows Antibody Pairings – Heavy and Light Chain Chimeric Antibodies from Example 11.

[0135] Figure 56A shows neutralization activity of Heavy and Light Chain Chimeric Antibodies chimeric pairings 1-32 (from Figure 55). Figure 56B shows Neutralization Activity on New Pairings in rows 33-67 (from Figure 55). Figure 56C shows Neutralization Activity on New Pairings in rows 68-91 (from Figure 55). Figure 56D shows that 8 chimeric antibodies were selected for large scale expression and neutralization activity analysis.

[0136] Figure 57 shows nucleic acid and amino acid sequences of VH and VL sequences of antibodies from Example 10 (SEQ ID NOs: 263-300).

[0137] Figure 58 shows sequences of DH511_5a and 5b as Fabs (SEQ ID NOs: 301-304).

[0138] Figures 59A-F show isolation of MPER-directed broadly neutralizing antibodies. (a) Fluorescently-labeled MPR.03 peptide tetramers were used to stain peripheral blood mononuclear cells from donor CH0210. A representative flow cytometric plot is shown. Square represents frequency of MPR.03 double positive memory B cells that were single-cell sorted for Ig gene amplification and expression. Colored dots within the square show individual cells that yielded MPER-specific monoclonal antibodies DH511.1-DH511.6 as revealed by index sorting. Memory B cells were gated as live CD16-CD14-CD3-CD235-CD19+IgD-CD38hi. (b) Phylogenetic tree of VHDHJH sequences of the DH511 clonal lineage. Ancestral reconstruction of the evolutionary pathway from the inferred unmutated common ancestor (UCA) to the mature mAbs including 6 maturational intermediates (circles, I1-I6) is indicated. (c) Neutralization activity of probe-identified MPER antibodies against a panel of 199 cross-clade HIV-1 isolates. Median and geometric mean neutralization potency against viruses neutralized with a median IC₅₀/IC₈₀<50 µg/ml is indicated. Percentage of 199 viruses neutralized by mAbs DH511.1-DH511.6, 10E8, and VRC01 at IC₅₀<50 µg/ml, IC₅₀<1 µg/ml, and IC₅₀<0.1 µg/ml. (d) Neutralization potency and breadth of DH511.2 compared to 10E8 and VRC01 against a 199 HIV-1 Env pseudovirus panel displayed as potency-breadth curves. Percentage of isolates neutralized at IC₅₀ (top panel) and IC₈₀ (bottom panel) values is plotted against mAb concentration. (e) Percent maximum neutralization of each isolate by DH511.2 is shown. (f) Identification of MPER-directed broadly neutralizing plasma antibodies by proteomics. Phylogenetic tree of heavy chain sequences identified in the plasma (black) and in the memory B cell compartment (grey, see

Figure 59b). The bar on the right shows the relative abundance of the three identified clonotypes in serum (IV: 95%, II: 4%, III: 1%).

[0139] Figures 60A-E shows structural analysis of the DH511 lineage. (a) Ribbon model of crystal structures of DH511.1 and DH511.2 Fabs in complex with gp41 MPER peptides 656-683 and 662-683, respectively, oriented based on Ca-atom superposition of distal MPER residues 671-683. (b) Close-up view of antibody-peptide contacts. gp41 residues that interact with antibody VH3-15 region residues, HCDR3 residues, or both, are shown in cyan, red, and brown, respectively. (c) Ribbon model of crystal structures of Fabs of plasma-derived variants DH511.11P and DH511.12P are shown in complex with gp41 MPER peptide 662-683 [511.11P is placeholder here]. Residues shown in surface representation differ in sequence from DH511.1 or DH511.2. Of the residues that are unique to DH511.11P and DH511.12P, those at the interface with gp41 are colored red and are predominantly located within their HCDR3 loops. (d) Close-up view of DH511.11P and DH511.12P antibody-peptide contacts, with gp41 contacting residues colored as in b. (e) Sequence alignment of DH511 lineage antibodies (SEQ ID NOs: 305-310), antibody 10E8, and their shared VH3-15 germ line gene precursor. Residues that contact gp41 are labeled with closed circles, and somatically-mutated residues shaded red, orange blue, and green, for 10E8, DH511.1, DH511.2, and DH511.11P and DH511.12P, respectively.

[0140] Figures 61A-E shows comparison with other MPER-specific antibodies. (a) Crystal structures of DH511.1 and DH511.2 Fab in complex with gp41 MPER peptides 656-683 and 662-683, respectively, oriented based on Ca-atom superposition of distal MPER residues 671-683. (b) Crystal structures of antibodies 10E8 and 4E10 in complex with MPER peptide epitopes, oriented as in (a). (c) Surface representations of antibodies DH511.1, DH511.2, and 10E8, colored as in (a) and (b) and rotated by 60°. gp41 contact footprints within the HCDR3 loops are colored red and those within the variable heavy chain VH3-15 regions are colored green. VH3-15 contacting residues positions that are shared by antibodies DH511.1 and DH511.2 and antibody 10E8 are colored cyan. (d) Angles of approach to distal gp41 MPER by antibodies DH511.1, DH511.2, 10E8, and 4E10. Shown is a superposition of the structures of antibody-bound gp41 MPER, with lines representing the longitudinal and latitudinal axes of antibody variable regions colored as in (a) and (b). The longitudinal axis is drawn to the Ca atom of gp41 residue 672 from the center of the latitudinal axis, defined as the point midway between heavy and light chain intra-chain disulfide bonds (spheres).

[0141] Figures 62A-C show standard experimental mapping and neutralization-based epitope prediction analysis to delineate the specificities that mediate plasma neutralization breadth.

(a) Plasma from donor CH0210 showed potent MPER-directed neutralizing activity against the HIV-2/HIV-1 MPER chimeric pseudovirus C1C. Neutralization titer is reported as median inhibitory dilution (ID50). (b) Neutralization activity adsorbed with MPER peptide. Anti-MPER antibodies were depleted from plasma using MPER peptide-coated magnetic beads. The depleted fraction was tested for neutralization activity against the indicated heterologous viruses. Neutralization was considerably diminished by removal of anti-MPER from both plasmas, indicating that MPER antibodies were largely responsible for neutralization breadth. ND, not determined. (c) Neutralization-based epitope prediction (NEP) analysis. The predicted relative prevalence of antibody clusters [(10 epitopes targeting sites of vulnerability (CD4 binding site, V1/V2, MPER, glycan V3)] is shown as a heat map, with dark color intensity (higher fractional number) corresponding to a stronger neutralization signal. Plasma neutralization breadth is shown, and numbers in each row add up to 1.00. Shown below are the locations on the Env trimer of the epitopes identified by NEP for this donor and confirmed to be targeted by standard experimental mapping methods.

[0142] Figures 63A-B show frequency and identity of CDR3 peptides from MPER affinity chromatography. (a) Representative histogram of antibody clonotype frequencies identified proteomically in the F(ab)² elution and flow through fractions following MPER affinity purification. Clonotypes were defined as genes with the same V- and J- gene usage and >85% sequence identity in the HCDR3. Frequencies of the identified clonotypes were based on the average peak areas of the detected CDR peptides. (b) Identified clonotypes and gene usage (SEQ ID NOs: 311-320).

[0143] Figure 64 shows Phylogenetic tree of VHDHJH sequences of memory B cell and plasma-derived DH511 clonal lineage members.

[0144] Figure 65A and 65B show Epitope mapping by alanine scanning mutagenesis of C-terminal MPER residues. Values listed are mean measurements from two independent experiments. Epitope residues were defined as residues where log AUC relative to wild-type (WT) for alanine mutations was reduced by 50%.

[0145] Figure 66A-C show Surface-plasmon resonance analysis of binding of the DH511 clonal lineage to MPR.03 peptide. Figure 66C shows Association (ka) and dissociation (kd) rate constants and binding affinities (Kd) for each Fab.

[0146] Figure 67A-C show Surface-plasmon resonance analysis of binding of the DH511 clonal lineage to MPER liposomes (SEQ ID NOs: 321-325).

[0147] Figure 68A-C show poly/autoreactivity analysis of MPER bNAbs. Reactivity of DH511 clonal lineage members with self-antigens as measured by indirect immunofluorescence Hep-2 cell staining (b) and a multiplex bead array anti-nuclear antibody (ANA) assay (a) panel consisting of several autoantigens: SSA, SSB, Smith antigen (Sm), ribonucleoprotein (RNP), Scl-70, Jo-1, double-stranded DNA (dsDNA), Cent B, Histone, and anti-cardiolipin. None of the antibodies were identified as reactive with Hep-2 cells.

DH511.1 UCA reacted with ribonucleoprotein, and DH511 I6 reacted with dsDNA. (c) Protein microarrays were used to assess binding to >9400 human proteins. Autoantigens identified: PPP1R1C (protein phosphatase 1, regulatory (inhibitor) subunit 1C) [DH511.1]; FYN (FYN oncogene related to SRC, FGR, YES, transcription variant 1 [DH511.1, DH511.3, DH511.6, DH511_I3, DH511_I4]; NECAP endocytosis associated 1 (NECAP1) [DH511.1, DH511.6]; SIAHBP1 (fuse-binding protein-interacting repressor, transcription variant 1, mRNA) [DH511.1]; STUB 1 (STIP1 homology and U-box containing protein 1) [DH511.2, DH511.6] STIP1 (stress-induced phosphoprotein 1) [DH511_I1, DH511_I2]; OR1F1 (olfactory receptor, family 1, subfamily F, member 1) [DH511]; C6orf145 (Px-domain containing protein) [DH511.1]; FLJ36032 [DH511_UCA]; TTC1(tetratricopeptide repeat domain 1) [DH511_I1], nuclear distribution gene C homolog (A. nidulans) (NUDC) [DH511.11P, DH511.12P], Scm-like with four MBT domains protein 1 [DH511.12P].

[0148] Figure 69 shows ELISA binding of DH511 lineage members to U1 snRNP components. The DH511_UCA bound specifically to U1-snRNPA while no binding was observed to the other components. Results shown represent one experiment.

[0149] Figure 70 shows potential mechanistic differences in binding of 4E10 versus DH511.2/10E8 to MPER liposomes. 4E10 bound to MPER656.1 in a biphasic association/dissociation mode and the binding could be fit to a 2-step conformational change model. DH512 appears to have a different mechanistic mode and its binding could be fit to a 1:1 Langmuir model.

[0150] Figure 71A-C show DH511.2 recognizes a transiently exposed intermediate state of gp41, and the lifetime of DH511.2 epitope exposure is the same as that of 10E8 and 4E10. Time course of neutralization of tier 2 HIV-1 isolate B.BG1168 was measured by addition of

mAbs to TZM-bl cells pre-incubated with virus. Half-life values were similar among the three antibodies.

[0151] Figure 72 shows Sequence Comparison of DH511, DH512, and 10E8 HCDR3 Loops (SEQ ID NOs: 326-328). The figure shows that while HCDR3 loops of DH511 and 10E8 lineages are both encoded by D3-3 precursor, substantial differences are observed in their final matured lengths and sequences. One conserved sequence motif between DH511/DH512 and 10E8 HCDR3s appears to be a hydrophobic residue doublet at the center of the loop (boxed).

[0152] Figure 73A-D shows Structural Comparison of DH511 (A), DH512 (B), and 10E8 (C)HCDR3 Loops. Conserved DH511/DH512 and 10E8 hydrophobic residue doublets at apex of HCDR3 loops are spatially co-localized (D), relative to MPER. Comparison is based on Ca superposition of MPER residues 671-683.

[0153] Figures 74A-B shows Comparison of DH511, DH512, and 10E8 HCDR3 Loops. (a) Sequence alignment of HCDR3 loops of DH511, DH512, and 10E8 (SEQ ID NOs: 329-331). (b) Structural comparison of HCDR3 loops based on alignment of distal MPER gp41 residues (that CDRH3 orientation differs from Figure 73). The HCDR3 loops of bNabs that target the gp41 MPER have been shown to be critical for their capacity to neutralize the HIV-1 virus, largely through interactions with the viral membrane. Mutations that reduce hydrophobicity of the HCDR3 loops ablate virus neutralization, while mutations that augment hydrophobicity in turn augment neutralization potency. Given that the DH511 lineage shares a common D3-3 gene with 10E8, we sought to compare the sequences and structures of their respective HCDR3 loops to assess whether common characteristics could be discerned. While sequence alignment of their matured amino acid sequences were quite different, as were their lengths, a conserved hydrophobic residue doublet at the centers of both loops was observed. These two residues have previously been shown to be critical for 10E8 epitope binding and neutralization. Remarkably, despite the overall differences in sequence and length of the DH511/12 and 10E8 HCDR3 loops, when they were compared structurally based on an alignment of MPER distal residues, the conserved hydrophobic residue doublets at their tips ended up spatially co-localized relative to MPER. Studies are underway to assess the importance of these two residues in the DH511 context, and the structures are being utilized to introduce additional mutations that are aimed at improving the neutralization potency of DH511-lineage antibodies as immunotherapeutics. 1Huang, J. et al. Broad and potent

neutralization of HIV-1 by a gp41-specific human antibody. *Nature* 491, 406-412, doi:10.1038/nature11544 (2012).

[0154] Figure 75 shows sequence characteristics of MPER antibodies isolated from memory B cells (SEQ ID NOs: 332-359). Figure 75 corresponds to Supplementary Table 1 as referenced in Example 12.

[0155] Figure 76 shows neutralization activity of MPER mAbs against a cross-clade 30 isolate HIV-1 Env-pseudovirus panel (IC50 values). Figure 76 corresponds to Supplementary Table 2a as referenced in Example 12.

[0156] Figure 77 shows neutralization activity of MPER mAbs against a cross-clade 30 isolate HIV-1 Env-pseudovirus panel (IC80 values). Figure 77 corresponds to Supplementary Table 2b as referenced in Example 12.

[0157] Figure 78 shows neutralization activity of DH511.2 against a cross-clade 199 isolate HIV-1 Env-pseudovirus panel. Figure 78 corresponds to Supplementary Table 3 as referenced in Example 12.

[0158] Figure 79 shows neutralization activity of DH511.2 against a panel of 200 clade C HIV-1 primary isolates. Figure 79 corresponds to Supplementary Table 4 as referenced in Example 12.

[0159] Figure 80 shows neutralization activity of 16 DH511.2 heavy chain mutant antibodies. Figure 80 corresponds to Supplementary Table 27 as referenced in Example 12.

[0160] Figure 81 shows sequence characteristics and pairing of plasma-derived heavy and light chains identified by mass spectrometry and paired VH-VL next-generation sequencing (SEQ ID NOs: 360-367 and 479-489, respectively, in order of appearance). Figure 81 corresponds to Supplementary Table 6 as referenced in Example 12.

[0161] Figure 82 shows neutralization activity of 16 plasma mAbs against a 4 indicator HIV-1 Env pseudovirus panel. Figure 82 corresponds to Supplementary Table 7 as referenced in Example 12.

[0162] Figure 83 shows neutralization activity of plasma mAbs DH511.11P and DH511.12P against a cross-clade 203 isolate HIV-1 Env-pseudovirus panel. Figure 83 corresponds to Supplementary Table 8 as referenced in Example 12.

[0163] Figure 84 shows sequences of alanine substituted MPR.03 peptides (SEQ ID NOs: 368-381). Figure 84 corresponds to Supplementary Table 9 as referenced in Example 12.

[0164] Figure 85 shows sequences of COT6.15 MPER mutant viruses (SEQ ID NOs: 382-403). Figure 85 corresponds to Supplementary Table 10 as referenced in Example 12.

[0165] Figure 86 shows neutralization Activity Against a series of MPER alanine mutant pseudoviruses in the COT6.15 Env background. Figure 86 corresponds to Supplementary Table 11 as referenced in Example 12.

[0166] Figure 87 shows crystallization peptides (SEQ ID NOs: 404-406). Figure 87 corresponds to Supplementary Table 12 as referenced in Example 12.

[0167] Figure 88 shows crystallographic data collection and refinement statistics. Figure 88 corresponds to Supplementary Table 13 as referenced in Example 12.

[0168] Figure 89 shows antibody contact interfaces by CDR loop. Figure 89 corresponds to Supplementary Table 14 as referenced in Example 12.

[0169] Figure 90 shows bonded and non-bonded contacts DH511.1-MPER. (Non-Kabat numbering). Figure 90 corresponds to Supplementary Table 15 as referenced in Example 12.

[0170] Figure 91 shows bonded and non-bonded contacts DH511.2-MPER. (Non-Kabat numbering). Figure 91 corresponds to Supplementary Table 16 as referenced in Example 12.

[0171] Figure 92 shows bonded and non-bonded contacts DH511.11P-MPER. Figure 92 corresponds to Supplementary Table 17 as referenced in Example 12.

[0172] Figure 93 shows bonded and non-bonded contacts DH511.12P-MPER. (Non-Kabat numbering). Figure 93 corresponds to Supplementary Table 18 as referenced in Example 12.

[0173] Figure 94 shows neutralization of the DH511 clonal lineage against a panel of 12 global HIV-1 reference strains. Figure 94 corresponds to Supplementary Table 19 as referenced in Example 12.

[0174] Figures 95A-C show primers and PCR conditions for paired VH:VL NGS. Figure 95A shows overlap extension oligonucleotides for framework region 1 (5' - 3') (SEQ ID NOs: 407-427). Figure 95B shows overlap extension oligonucleotides for leader peptide (5' - 3') (SEQ ID NOs: 428-441). Figure 95C shows nested constant region oligonucleotides (5' - 3') (SEQ ID NOs: 442-446). Figure 95A corresponds to Supplementary Table 28 as referenced in Example 12. Figure 95B corresponds to Supplementary Table 29 as referenced in Example 12. Figure 95C corresponds to Supplementary Table 30 as referenced in Example 12.

[0175] Figure 96 shows DH511 clonal lineage membrane insertion scores and HCDR3 analysis (SEQ ID NOs: 447-455). The membrane insertion scores can be recalculated to

exclude the C in the CDR3. HCDR3s score for the .P antibodies will be calculated. Figure 96 corresponds to Supplementary Table 21 as referenced in Example 12.

[0176] Figure 97 shows cardiolipin reactivity of the DH511 clonal lineage. Figure 97 corresponds to Supplementary Table 22 as referenced in Example 12.

[0177] Figure 98 shows neutralization activity of 91 chimeric MPER mAbs against the tier 2 HIV-1 isolate B.BG1168. Figure 98 corresponds to Supplementary Table 23 as referenced in Example 12.

[0178] Figure 99 shows neutralization activity of chimeric mAb DH511.2_K3 against a cross-clade 30 isolate Env-pseudovirus panel. Figure 99 corresponds to Supplementary Table 24 as referenced in Example 12.

[0179] Figures 100A-C show primers and PCR conditions for paired VH:VL NGS. Figure 100A shows PCR conditions for isotype specific amplification. Figure 100B shows oligonucleotides for isotype specific amplification (5' - 3') (SEQ ID NOs: 456-462). Figure 100C shows PCR conditions for MiSeq Barcoding. Figure 100A corresponds to Supplementary Table 30 as referenced in Example 12. Figure 100B corresponds to Supplementary Table 31 as referenced in Example 12. Figure 100C corresponds to Supplementary Table 32 as referenced in Example 12.

[0180] **Figure 101** provides a schematic of a representative covalently bonded diabody having two epitope-binding sites composed of two polypeptide chains, each having an E-coil or K-coil Heterodimer-Promoting Domain (alternative Heterodimer-Promoting Domains are provided below). A cysteine residue may be present in a linker and/or in the Heterodimer-Promoting Domain as shown in **Figure 102B**. VL and VH Domains that recognize the same epitope are shown using the same shading or fill pattern.

[0181] **Figures 102A-102C** provide schematics showing representative covalently bonded tetravalent diabodies having four epitope-binding sites composed of two heterodimer pairs of polypeptide chains (*i.e.*, four polypeptide chains in all). One polypeptide of each heterodimer pair possesses a CH2 and CH3 Domain, such that the associated chains form all or part of an Fc Domain. VL and VH Domains that recognize the same epitope are shown using the same shading or fill pattern. The two pairs of polypeptide chains may be the same. In such embodiments wherein the two pairs of polypeptide chains are the same and the VL and VH Domains recognize different epitopes (as shown in **Figures 102A-102B**), the resulting molecule possesses four epitope-binding sites and is bispecific and bivalent with respect to

each bound epitope. In such embodiments wherein the VL and VH Domains recognize the same epitope (*e.g.*, the same VL Domain CDRs and the same VH Domain CDRs are used on all chains) the resulting molecule possesses four epitope-binding sites and is monospecific and tetravalent with respect to a single epitope. Alternatively, the two pairs of polypeptides may be different. In such embodiments wherein the two pairs of polypeptide chains are different and the VL and VH Domains of each pair of polypeptides recognize different epitopes (as shown by the different shading and patterns in **Figure 102C**), the resulting molecule possesses four epitope-binding sites and is tetraspecific and monovalent with respect to each bound epitope. **Figure 102A** shows an Fc Domain-containing diabody which contains a peptide Heterodimer-Promoting Domain comprising a cysteine residue. **Figure 102B** shows an Fc Domain-containing diabody, which contains E-coil and K-coil Heterodimer-Promoting Domains comprising a cysteine residue and a linker (with an optional cysteine residue). **Figure 102C**, shows an Fc-Region-Containing diabody, which contains antibody CH1 and CL domains which could serve as Heterodimer Promoting Domains.

[0182] **Figures 103A and 103B** provide schematics of a representative covalently bonded Fc bearing diabody molecule having two epitope-binding sites composed of three polypeptide chains. Two of the polypeptide chains comprise a CH2 and CH3 Domain, such that the associated chains form all or part of an Fc Domain. The polypeptide chains comprising the VL and VH Domain further comprise a Heterodimer-Promoting Domain. VL and VH Domains that recognize the same epitope are shown using the same shading or fill pattern.

[0183] **Figures 104A-104F** provide schematics of representative Fc Domain-containing trivalent binding molecules having three epitope-binding sites. **Figures 104A and 104B**, respectively, illustrate schematically the domains of trivalent binding molecules comprising two diabody-type binding domains and a Fab-type binding domain having different domain orientations in which the diabody-type binding domains are N-terminal or C-terminal to an Fc Domain. The molecules in **Figures 104A and 104B** comprise four chains. **Figures 104C and 104D**, respectively, illustrate schematically the domains of trivalent binding molecules comprising two diabody-type binding domains N-terminal to an Fc Domain, and a Fab-type binding domain in which the light chain and heavy chain are linked via a polypeptide spacer, or an scFv-type binding domain. The trivalent binding molecules in **Figures 104E and 104F**, respectively, illustrate schematically the domains of trivalent binding molecules comprising

two diabody-type binding domains C-terminal to an Fc Domain, and a Fab-type binding domain in which the light chain and heavy chain are linked via a polypeptide spacer, or an scFv-type binding domain. The trivalent binding molecules in **Figures 104C-104F** comprise three chains. VL and VH Domains that recognize the same epitope are shown using the same shading or fill pattern.

[0184] **Figures 105A-105B** provide schematics of a representative HIV $\times$ CD3 bispecific monovalent diabody comprising three polypeptide chains. **Figure 105A** shows the domains of each of the three polypeptide chains, dashed lines represent disulfide bonds which form between the chains, and the arrows indicate the interactions of the Variable Domains. **Figure 105B** provides a schematic of the assembled chains. Such diabodies contain an anti-HIV-1 binding arm (*e.g.*, DH511, DH511.2_K3, DH512, DH513, DH514, DH515, DH516, DH517, DH518, DH536, or DH537) combined with an anti-CD3 binding arm (*e.g.*, hXR32). They are composed of two polypeptide chains: one with the VL of an anti-CD3 antibody linked to the VH of an anti-HIV-1 antibody; the second with the VL of an anti-HIV-1 antibody linked to the VH of an anti-CD3 antibody. The first and the second polypeptide chains are linked by interchain disulfide bond and paired via oppositely charged E-coil/K-coil Heterodimer-Promoting Domains. The amino acid and nucleotide sequences of Chain 1, 2 and 3 which form the bispecific monovalent diabody designated DH511.2_K3 $\times$ CD3 Fc are provided in Table 4 (SEQ ID NOs: 555-560). Control molecules have one of the arms replaced by a non-HIV-1 envelope binding arm derived, for example, from an anti-FITC antibody (4420) or from an anti-RSV antibody (palivizumab).

DETAILED DESCRIPTION

[0185] Highly active anti-retroviral therapy (HAART) alone or in combination with latency reversing agents fails to reduce the pool of latently infected cells. This is due to limited ability of the CD8 $^{+}$ T cells to eliminate HIV-1 latently infected cells. Dual Affinity Re-Targeting proteins (DARTs) are multispecific, antibody-based diabody molecules that can bind at least two distinct antigens simultaneously. HIV-1 diabodies contain an HIV-1 binding arm combined with an effector cell binding arm (*e.g.*, but not limited to CD3 effector cells) are designed to redirect effector cells (*e.g.* but not limited to cytotoxic CD3 $^{+}$ T cells) to engage and kill HIV-1-infected cells. Additionally, multispecific molecules such as trivalent binding

molecules containing one or more HIV-1 binding arms combined with one or more effector cell binding arms, may also be designed to redirected effector cells to engage and kill HIV-1-infected cells.

[0186] The provision of multispecific/non-mono-specific molecules provides a significant advantage over typical mono-specific antibodies: the capacity to co-ligate and co-localize cells that express different epitopes. Bivalent diabodies have wide-ranging applications including therapy and immunodiagnosis. Bi-valency allows for great flexibility in the design and engineering of the diabody in various applications, providing enhanced avidity to multimeric antigens, the cross-linking of differing antigens, and directed targeting to specific cell types relying on the presence of both target antigens. Due to their increased valency, low dissociation rates and rapid clearance from the circulation (for diabodies of small size, at or below ~50 kDa), diabody molecules known in the art have also shown particular use in the field of tumor imaging (Fitzgerald *et al.* (1997) "*Improved Tumour Targeting By Disulphide Stabilized Diabodies Expressed In Pichia pastoris*," Protein Eng. 10:1221). Of particular importance is the co-ligating of differing cells, for example, the cross-linking of cytotoxic T cells to tumor cells (Staerz *et al.* (1985) "*Hybrid Antibodies Can Target Sites For Attack By T Cells*," Nature 314:628-631, and Holliger *et al.* (1996) "*Specific Killing Of Lymphoma Cells By Cytotoxic T-Cells Mediated By A Bispecific Diabody*," Protein Eng. 9:299-305).

[0187] Diabody epitope binding domains may also be directed to a surface determinant of a B cell, such as CD19, CD20, CD22, CD30, CD37, CD40, and CD74 (Moore, P.A. *et al.* (2011) "*Application Of Dual Affinity Retargeting Molecules To Achieve Optimal Redirected T-Cell Killing Of B-Cell Lymphoma*," Blood 117(17):4542-4551; Cheson, B.D. *et al.* (2008) "*Monoclonal Antibody Therapy For B-Cell Non-Hodgkin's Lymphoma*," N. Engl. J. Med. 359(6):613-626; Castillo, J. *et al.* (2008) "*Newer monoclonal antibodies for hematological malignancies*," Exp. Hematol. 36(7):755-768. In many studies, diabody binding to effector cell determinants, *e.g.*, Fc γ receptors (Fc γ R), was also found to activate the effector cell (Holliger *et al.* (1996) "*Specific Killing Of Lymphoma Cells By Cytotoxic T-Cells Mediated By A Bispecific Diabody*," Protein Eng. 9:299-305; Holliger *et al.* (1999) "*Carcinoembryonic Antigen (CEA)-Specific T-Cell Activation In Colon Carcinoma Induced By Anti-CD3 x Anti-CEA Bispecific Diabodies And B7 x Anti-CEA Bispecific Fusion Proteins*," Cancer Res. 59:2909-2916; WO 2006/113665; WO 2008/157379; WO 2010/080538; WO 2012/018687; WO 2012/162068). Normally, effector cell activation is triggered by the binding of an

antigen bound antibody to an effector cell via Fc-FcγR interaction; thus, in this regard, diabody molecules may exhibit Ig-like functionality independent of whether they comprise an Fc Domain (*e.g.*, as assayed in any effector function assay known in the art or exemplified herein (*e.g.*, ADCC assay)). By cross-linking tumor and effector cells, the diabody not only brings the effector cell within the proximity of the tumor cells but leads to effective tumor killing (see *e.g.*, Cao *et al.* (2003) “*Bispecific Antibody Conjugates In Therapeutics*,” *Adv. Drug. Deliv. Rev.* 55:171-197).

[0188] The formation of such non-mono-specific diabodies requires the successful assembly of two or more distinct and different polypeptides (*i.e.*, such formation requires that the diabodies be formed through the heterodimerization of different polypeptide chain species). This fact is in contrast to mono-specific diabodies, which are formed through the homodimerization of identical polypeptide chains. Because at least two dissimilar polypeptides (*i.e.*, two polypeptide species) must be provided in order to form a non-mono-specific diabody, and because homodimerization of such polypeptides leads to inactive molecules (Takemura, S. *et al.* (2000) “*Construction Of A Diabody (Small Recombinant Bispecific Antibody) Using A Refolding System*,” *Protein Eng.* 13(8):583-588), the production of such polypeptides must be accomplished in such a way as to prevent covalent bonding between polypeptides of the same species (*i.e.*, so as to prevent homodimerization) (Takemura, S. *et al.* (2000) “*Construction Of A Diabody (Small Recombinant Bispecific Antibody) Using A Refolding System*,” *Protein Eng.* 13(8):583-588). The art has therefore taught the non-covalent association of such polypeptides (see, *e.g.*, Olafsen *et al.* (2004) “*Covalent Disulfide-Linked Anti-CEA Diabody Allows Site-Specific Conjugation And Radiolabeling For Tumor Targeting Applications*,” *Prot. Engr. Des. Sel.* 17:21-27; Asano *et al.* (2004) “*A Diabody For Cancer Immunotherapy And Its Functional Enhancement By Fusion Of Human Fc Domain*,” Abstract 3P-683, *J. Biochem.* 76(8):992; Takemura, S. *et al.* (2000) “*Construction Of A Diabody (Small Recombinant Bispecific Antibody) Using A Refolding System*,” *Protein Eng.* 13(8):583-588; Lu, D. *et al.* (2005) “*A Fully Human Recombinant IgG-Like Bispecific Antibody To Both The Epidermal Growth Factor Receptor And The Insulin-Like Growth Factor Receptor For Enhanced Antitumor Activity*,” *J. Biol. Chem.* 280(20):19665-19672).

[0189] The art has recognized that bispecific diabodies composed of non-covalently associated polypeptides are unstable and readily dissociate into non-functional monomers

(see, e.g., Lu, D. *et al.* (2005) “A Fully Human Recombinant IgG-Like Bispecific Antibody To Both The Epidermal Growth Factor Receptor And The Insulin-Like Growth Factor Receptor For Enhanced Antitumor Activity,” J. Biol. Chem. 280(20):19665-19672).

[0190] In the face of this challenge, the invention provides stable, covalently bonded heterodimeric multispecific diabodies, termed DARTs™ (see, e.g., United States Patent Publications No. 2014-0099318; 2013-0295121; 2010-0174053 and 2009-0060910; European Patent Publication No. EP 2714079; EP 2601216; EP 2376109; EP 2158221 and PCT Publications No. WO 2015/026894; WO2015/026892; WO 2015/021089; WO 2014/159940; WO 2012/162068; WO 2012/018687; WO 2010/080538; Moore, P.A. *et al.* (2011) “Application Of Dual Affinity Retargeting Molecules To Achieve Optimal Redirected T-Cell Killing Of B-Cell Lymphoma,” Blood 117(17):4542-4551; Veri, M.C. *et al.* (2010) “Therapeutic Control Of B Cell Activation Via Recruitment Of Fcγ Receptor IIb (CD32B) Inhibitory Function With A Novel Bispecific Antibody Scaffold,” Arthritis Rheum. 62(7):1933-1943; Johnson, S. *et al.* (2010) “Effector Cell Recruitment With Novel Fv-Based Dual-Affinity Re-Targeting Protein Leads To Potent Tumor Cytolysis And *in vivo* B-Cell Depletion,” J. Mol. Biol. 399(3):436-449), the contents of which publications are herein incorporated by reference in their entirety). Such multispecific molecules comprise two or more covalently complexed polypeptides and involve engineering one or more cysteine residues into each of the employed polypeptide species. For example, the addition of a cysteine residue to the C-terminus of such constructs has been shown to allow disulfide bonding between the polypeptide chains, stabilizing the resulting heterodimer without interfering with the binding characteristics of the bivalent molecule.

[0191] The invention provides multispecific, antibody-based molecules that can bind at least two distinct antigens simultaneously, wherein at least one of the antigens is comprised in an HIV-1 envelope. In certain aspects, the present invention is directed to HIV-1 multispecific molecules that are capable of simultaneous binding to an epitope of HIV-1 envelope and an epitope of an antigen on a number of effector cells, e.g. but not limited to an effector cell which expresses CD2, CD3, CD8, CD16, T-Cell Receptor (TCR), NKG2D, *etc.* epitope) and to the uses of such molecules in the treatment of HIV-1 infection.

[0192] In certain embodiments the invention provides molecules with dual targeting specificity (including but not limited to bispecific antibodies, bispecific diabodies, and trivalent binding molecules). In certain aspects the invention provides bispecific molecules

that are capable of localizing an immune effector cell to an HIV-1 envelope expressing cell, so as facilitate the killing of the HIV-1 envelope expressing cell. In this regard, bispecific molecules bind with one "arm" to an epitope of a surface antigen on target cells, and with the second "arm" to an epitope of an activating, invariant component of the T cell receptor (TCR) complex. The simultaneous binding of such a bispecific molecule to both of its targets will force a temporary interaction between target cell and T cell, causing activation of any cytotoxic T cell and subsequent lysis of the target cell. Hence, the immune response is re-directed to the target cells and is independent of peptide antigen presentation by the target cell or the specificity of the T cell as would be relevant for normal MHC-restricted activation of CTLs. In this context it is crucial that CTLs are only activated when a target cell is presenting the bispecific molecule to them, *i.e.* the immunological synapse is mimicked. Particularly desirable are bispecific molecules that do not require lymphocyte preconditioning or co-stimulation in order to elicit efficient lysis of target cells. In certain embodiments, such molecule may further comprise a third binding "arm" and be trivalent. In some embodiments, the third arm binds to an epitope of a surface antigen on target cells, which may be the same epitope or a different epitope as bound by the first arm. In some embodiments, the third arm binds to an epitope of an activating, invariant component of the TCR complex, which may be the same epitope or a different epitope as bound by the second arm. In alternative embodiments, the third arm binds to a different epitope to that bound by the first arm or second arm, such as an epitope of a surface antigen on target cells or an epitope expressed on the surface of an effector cell (*e.g.*, but not limited to an epitope of CD2, CD3, CD8, CD16, T-Cell Receptor (TCR), NKG2D, *etc.*).

[0193] In certain embodiments, such bispecific molecules comprise one portion which targets HIV-1 envelope and a second portion which binds a second target. In certain embodiments, the first portion comprises VH and VL sequences, or CDRs from the antibodies described herein. In certain embodiments, the second target could be, for example but not limited to an effector cell. In certain embodiments the second portion is a T-cell engager. In certain embodiments, the second portion comprises a sequence/paratope which targets CD3. In certain embodiments, the second portion is an antigen-binding region derived from a CD3 antibody, optionally a known CD3 antibody. In certain embodiments, the anti-CD3 antibody induces T cell-mediated killing. In certain embodiments, the bispecific molecules comprise whole antibodies. In other embodiments, the dual targeting bispecific molecules consist

essentially of Fab fragments. In other embodiments, the dual targeting bispecific molecules comprise a heavy chain constant region (CH1). In certain embodiments, the bispecific molecule does not comprise Fc Domain. In certain embodiments, the bispecific molecules have improved effector function. In certain embodiments, the bispecific molecules have improved cell killing activity. Various methods and platforms for design of bispecific molecules (including but not limited to bispecific antibodies, bispecific diabodies, *etc.*) are known in the art. See for example US Pub. 20140206846, US Pub. 20140170149, 20100174053, US Pub. 20090060910, US Pub 20130295121, US Pub. 20140099318, US Pub. 20140088295 which contents are herein incorporated by reference in their entirety.

[0194] A bispecific or bifunctional molecule is an artificial hybrid antibody that can comprise two different heavy/light chain pairs and two different binding sites (see, *e.g.*, Romain Rouet & Daniel Christ “Bispecific antibodies with native chain structure” *Nature Biotechnology* 32, 136–137 (2014); Byrne *et al.* “A tale of two specificities: bispecific antibodies for therapeutic and diagnostic applications” *Trends in Biotechnology*, Volume 31, Issue 11, November 2013, Pages 621–632 Songvilai and Lachmann, *Clin. Exp. Immunol.*, 79:315-321 (1990); Kostelny *et al.*, *J. Immunol.* 148:1547-53 (1992) (and references therein)). In certain embodiments the bispecific molecule is a whole antibody of any isotype. In other embodiments the bispecific molecule is a bispecific fragment, for example but not limited to F(ab)₂ fragment. In some embodiments, the bispecific molecules do not include Fc portion, which makes these bispecific molecules relatively small in size and easy to penetrate tissues.

[0195] In some embodiments, the invention encompasses polypeptide chains, each of which polypeptide chains comprise a VH and VL domain, comprising CDRs as described herein. In certain embodiments, the VL and VH domains comprising each polypeptide chain have the same specificity, and the multimer molecule is bivalent and monospecific. In other embodiments, the VL and VH domains comprising each polypeptide chain have differing specificity and the multimer is bivalent and bispecific. In still other embodiments, the VH and VL domains of each polypeptide chain have differing specificity and the multimer is trivalent and bispecific or trivalent and trispecific.

[0196] The multispecific molecules of the invention can simultaneously bind two separate and distinct epitopes. In certain embodiments the epitopes are from the same antigen. In other embodiments, the epitopes are from different antigens. In non-limiting embodiments at least one epitope binding site is specific for a determinant expressed on an immune effector cell

(*e.g.* CD3, CD16, CD32, CD64, *etc.*) which are expressed on T lymphocytes, natural killer (NK) cells or other mononuclear cells. In one embodiment, the multispecific molecule binds to the effector cell determinant and also activates the effector cell. In this regard, multispecific molecules of the invention (*e.g.*, bispecific antibodies, bispecific diabodies, trivalent binding molecules, *etc.*) may exhibit Ig-like functionality independent of whether they further comprise an Fc domain (*e.g.*, as assayed in any effector function assay known in the art or exemplified herein).

[0197] In certain embodiments, the multispecific molecule comprises an HIV-1 envelope binding fragment, for example but not limited to an HIV-1 envelope binding fragment from any of the antibodies described herein. In other embodiments, the multispecific molecule further comprises a second antigen-interaction-site/fragment. In other embodiments, the multispecific molecule further comprises at least one effector cell targeting arm.

[0198] In certain embodiments the multispecific molecules engage cells for Antibody-Dependent Cell-mediated Cytotoxicity (ADCC). In certain embodiments the multispecific molecules engage natural killer cells, neutrophil polymorphonuclear leukocytes, monocytes and macrophages. In certain embodiments the multispecific molecules are T-cell engagers. In certain embodiments, the bispecific molecule comprises an HIV-1 envelope binding fragment and CD3 binding fragment. Various CD3 antibodies are provided herein (see, *e.g.*, Table 4) and others are known in the art. See for example US Patent 8,784,821, and United States Patent Publications No. 2014-0099318 providing various disclosure on various CD3 antibodies, which disclosure is incorporated by reference in its entirety. In certain embodiments, the bispecific molecule comprises an HIV-1 envelope binding fragment and CD16 binding fragment. Various CD16 antibodies are provided herein (see *e.g.*, Table 4) and others are known in the art. See for example WO 03/101485, which disclosure is incorporated by reference in its entirety.

[0199] In certain embodiments, the invention provides molecules or fragments comprising a CDR(s) of the VH and/or VL chains, or VH and/or VL chains of any suitable HIV-1 antibody, as the HIV-1 binding arm(s) of multispecific molecules, *e.g.*, but not limited to bispecific antibodies, bispecific diabodies, trivalent binding molecules, *etc.*, or toxin labeled HIV-1 binding molecules. Exemplary HIV-1 antibodies are provided in Table 4.

[0200] The invention also includes variants of the antibodies (and fragments) disclosed herein, including variants that retain the ability to bind to recombinant Env protein, the ability

to bind to the surface of virus-infected cells and/or ADCC-mediating properties of the antibodies specifically disclosed, and methods of using same to, for example, reduce HIV-1 infection risk. Combinations of the antibodies, or fragments thereof, disclosed herein can also be used in the generation of the multispecific molecules of the invention.

[0201] Homologs and variants of a VL or a VH of an antibody that specifically binds a polypeptide are typically characterized by possession of at least about 75%, for example at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity counted over the full length alignment with the amino acid sequence of interest. Proteins with even greater similarity to the reference sequences will show increasing percentage identities when assessed by this method, such as at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99% sequence identity. When less than the entire sequence is being compared for sequence identity, homologs and variants will typically possess at least 80% sequence identity over short windows of 10-20 amino acids, and may possess sequence identities of at least 85% or at least 90% or 95% depending on their similarity to the reference sequence. Methods for determining sequence identity over such short windows are available at the NCBI website on the internet. One of skill in the art will appreciate that these sequence identity ranges are provided for guidance only; it is entirely possible that strongly significant homologs could be obtained that fall outside of the ranges provided.

[0202] In certain embodiments, the invention provides multispecific molecules comprising the VL and VH domains of antibodies which are 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%, 85%, 84%, 83%, 82%, 81%, 80% identical to the VH and VL amino acid sequences of the antibodies described herein and still maintain their epitope binding breadth and/or potency. In certain embodiments, the invention provides multispecific molecules comprising the CDR 1, 2, and/or 3 of the VH and CDR1, 2, and/or 3 of the VL which are 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%, 85%, 84%, 83%, 82%, 81%, 80% identical to the CDR1, 2, and/or 3 of VH and CDR1, 2, and/or 3 VL amino acid sequences of the antibodies described herein and still maintain their epitope binding breadth and/or potency.

[0203] In certain embodiments, the invention provides multispecific molecules comprising polypeptide chains which are 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%, 85%, 84%, 83%, 82%, 81%, 80% identical to SEQ ID NOs:551.

[0204] In certain embodiments, the invention provides multispecific molecules comprising polypeptide chains which are 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%, 85%, 84%, 83%, 82%, 81%, 80% identical to SEQ ID NOs:553.

[0205] In some aspects the invention provides recombinant, multispecific molecules, polyclonal or monoclonal antibodies, variants, fusion proteins comprising an antibody portion with an antigen recognition site of the required specificity, humanized antibodies, and chimeric antibodies, and any other modified configuration of the immunoglobulin molecule that comprises an antigen recognition site of the required specificity. Throughout this application, the numbering of amino acid residues of the light and heavy chains of antibodies is according to the EU index as in Kabat *et al.* (1992) SEQUENCES OF PROTEINS OF IMMUNOLOGICAL INTEREST, National Institutes of Health Publication No. 91-3242. Amino acids from the Variable Domains of the mature heavy and light chains of immunoglobulins are designated by the position of an amino acid in the chain. Kabat described numerous amino acid sequences for antibodies, identified an amino acid consensus sequence for each subgroup, and assigned a residue number to each amino acid. The Kabat numbering scheme is extendible to antibodies not included in his compendium by aligning the antibody in question with one of the consensus sequences in Kabat by reference to conserved amino acids. This method for assigning residue numbers has become standard in the field and readily identifies amino acids at equivalent positions in different antibodies, including chimeric or humanized variants.

[0206] In some embodiments, antigen-binding fragment of an antibody is a portion of an antibody that possesses at least one antigen recognition site. Fragments include for example but not limited to, Fab, Fab', F(ab')₂ Fv, and single chain (scFv).

[0207] In certain embodiments the invention provides recombinant molecules. In certain embodiments, recombinant molecules encompasses not only intact monoclonal antibodies and full-length monoclonal antibodies, but also fragments thereof (such as Fab, Fab', F(ab')₂ Fv), single chain (scFv), mutants thereof, fusion proteins and multispecific molecules comprising an antibody portion, humanized monoclonal antibodies, chimeric monoclonal antibodies, and any other modified configuration of the immunoglobulin molecule that comprises an antigen recognition site of the required specificity and the ability to bind to an antigen. Recombinant molecules are not limited as regards to the source of the molecule or the manner in which it is made (*e.g.*, by hybridoma, phage selection, recombinant expression, transgenic animals, *etc.*).

[0208] Methods of making recombinant molecules are known in the art. In certain embodiments, the molecules are produced recombinantly by any means known in the art. In one embodiment, the polynucleotide sequence encoding such a recombinant molecule is cloned into a vector for expression or propagation. The sequence encoding an antibody of interest may be maintained in a vector in a host cell and the host cell can then be expanded and frozen for future use. The polynucleotide sequence of such antibodies may be used for genetic manipulation to generate the multispecific molecules of the invention (*e.g.*, bispecific antibodies, bispecific diabodies, trivalent binding molecules, *etc.*) as well as a chimeric antibody, a humanized antibody, or a caninized antibody, to improve the affinity, or other characteristics of the antibody. The general principle in humanizing an antibody involves retaining the basic sequence of the antigen-binding portion of the antibody, while swapping the non-human remainder of the antibody with human antibody sequences. There are four general steps to humanize a monoclonal antibody. These are: (1) determining the nucleotide and predicted amino acid sequence of the starting antibody light and heavy variable Domains (2) designing the humanized antibody or caninized antibody, *i.e.*, deciding which antibody framework region to use during the humanizing or canonizing process (3) the actual humanizing or caninizing methodologies/techniques and (4) the transfection and expression of the humanized antibody. See, for example, U.S. Patents Nos. 4,816,567; 5,807,715; 5,866,692; and 6,331,415.

[0209] The antibodies described herein, or fragments thereof, or molecules comprising such fragments, may be recombinantly produced in prokaryotic or eukaryotic expression systems. These systems are well described in the art. In general, protein therapeutics are produced from mammalian cells. The most widely used host mammalian cells are Chinese hamster ovary (CHO) cells and mouse myeloma cells, including NS0 and Sp2/0 cells. Two derivatives of the CHO cell line, CHO-K1 and CHO pro-3, gave rise to the two most commonly used cell lines in large scale production, DUKX-X11 and DG44. (*See, e.g.*, Kim, J., *et al.*, "CHO cells in biotechnology for production of recombinant proteins: current state and further potential," *Appl. Microbiol. Biotechnol.*, 2012, 93:917-30, which is hereby incorporated-by-reference.) Other mammalian cell lines for recombinant protein expression include, but are not limited to, COS, HeLa, HEK293T, U2OS, A549, HT1080, CAD, P19, NIH 3T3, L929, N2a, HEK 293, MCF-7, Y79, SO-Rb50, HepG2, J558L, and BHK. If the aim is large-scale production, the most currently used cells for this application are CHO cells.

Guidelines to cell engineering for mAbs production were also reported. (Costa *et al.*, “Guidelines to cell engineering for monoclonal antibody production,” *Eur J Pharm Biopharm*, 2010, 74:127–38, which is hereby incorporated-by-reference.) Using heterologous promoters, enhancers and amplifiable genetic markers, the yields of antibody and antibody fragments can be increased. Similar methods are utilized for the expression of the multispecific molecules of the invention. Thus, in certain embodiments, the invention provides an antibody, or antibody fragment, or molecule comprising such fragment, that is recombinantly produced from a mammalian cell-line, including a CHO cell-line. In certain embodiments, the invention provides a composition comprising an antibody, or antibody fragment, or molecule comprising such fragment, wherein the antibody, or antibody fragment, or molecule comprising such fragment was recombinantly produced in a mammalian cell-line, and wherein the antibody, or antibody fragment, or molecule comprising such fragment is present in the composition at a concentration of at least 1, 10, 100, 1000 micrograms/mL, or at a concentration of at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 50, or 100 milligrams/mL.

[0210] Furthermore, large-scale production of therapeutic-grade molecules are much different than those for laboratory scale. There are extreme purity requirements for therapeutic-grade. Large-scale production of therapeutic-grade molecules requires multiples steps, including product recovery for cell-culture harvest (removal of cells and cell debris), one or more chromatography steps for antibody purification, and formulation (often by tangential filtration). Because mammalian cell culture and purification steps can introduce variants that are unique to the recombinant production process (*i.e.*, protein aggregates, N- and C- terminal variants, acidic variants, basic variants, different glycosylation profiles), there are recognized approaches in the art for analyzing and controlling these variants. (See, Fahrner, *et al.*, Industrial purification of pharmaceutical antibodies: Development, operation, and validation of chromatography processes, *Biotech. Gen. Eng. Rev.*, 2001, 18:301-327, which is hereby incorporated-by-reference.) In certain embodiments of the invention, the composition comprises less than 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 35, 50, or 100 nanograms of host cell protein (*i.e.*, proteins from the cell-line used to recombinantly produce the molecule). In other embodiments, the composition comprises less than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, or 25 ng of protein A per milligram of molecule (*i.e.*, protein A is a standard approach for purifying antibodies and other Fc bearing molecules from recombinant cell culture, but steps should be done to limit the amount of protein A in the composition, as it may be immunogenic). (See,

e.g., U.S. Patent No. 7,458,704, Reduced protein A leaching during protein A affinity chromatography; which is hereby incorporated-by-reference.)

[0211] Multispecific molecules

[0212] Various non-limiting multispecific molecule designs are provided in Figures 101-105.

[0213] As provided herein, the invention contemplates designs of multispecific molecules, which include, but are not limited to bispecific antibodies, bispecific diabodies, Fc Domain bearing diabodies, trivalent binding molecules, Fc Domain bearing trivalent binding molecules *etc.* The multispecific molecules provided herein comprise various domains, including, but not limited to peptide linkers, Heterodimer Promoting Domains, VL and VH domains, and Fc Domains. Specific non-limiting embodiments of exemplary multispecific molecules are provided herein. Alternative combinations of the various domains described herein can be employed in the multispecific molecules of the invention.

[0214] As provided herein, the invention contemplates designs of multispecific molecules with various peptide linkers (also referred to herein as “intervening peptide linkers”) separating the domains comprised in the polypeptide chains. Any of a variety of peptide linkers can be used to separate the domains in the polypeptide chains of the multispecific molecules of the invention. Typically, such peptide linkers will comprise 1-20, 1-19, 1-18, 1-17, 1-16, 1-15, 1-14, 1-13, 1-12, 1-11, 1-10, 1-9, 1-8, 1-7, 1-6, 1-5, 1-4, 1-3, 1-2, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 amino acid residues. Such polypeptide linkers can include a series of glycine residues (Gly) and/or Serine (Ser) residues and may optionally comprise cysteine residue(s). Specific non-limiting embodiments of exemplary polypeptide linkers (*e.g.*, Peptide Linker 1, Peptide Linker 2, Spacer Linker 3, *etc.*) are provided herein. Alternative peptide linkers are well-known in the art and can be employed in the multispecific molecules of the invention. Other linkers can be readily determined. Some additional examples of linkers are disclosed in US Pub 20100174053, incorporated by reference in its entirety.

[0215] Typically, the VH and VL domains of a polypeptide chain of the multispecific molecules are linked so that they do not associate with each other. In certain embodiments the length of the peptide linker, which separates such VL and VH domains of a polypeptide chain is selected to substantially or completely prevent such VL and VH domains from binding to one another. Thus the VL and VH domains of a polypeptide chain are substantially or completely incapable of binding to one another. In certain embodiments this

is due to the peptide linker which separates the VH and VL domains. As provided herein, the invention also contemplates designs of multispecific molecules wherein the domains comprising the polypeptide chains (*e.g.*, Heterodimer Promoting Domains, VL and VH domains, and Fc Domains *etc.*) are directly linked (*i.e.* no peptide linker is used between the domain). In such multispecific molecules the domains can be linked by a peptide bond.

[0216] One embodiment of the present invention relates to multispecific molecules, which are bispecific, that are capable of binding to a “**first epitope**” and a “**second epitope**,” such epitopes not being identical to one another. Such bispecific molecules comprise “VL1” / “VH1” domains that are capable of binding to the first epitope, and “VL2” / “VH2” domains that are capable of binding to the second epitope. The notation “VL1” and “VH1” denote respectively, the Variable Light Chain Domain and Variable Heavy Chain Domain of such bispecific molecules that bind the “first” epitope. Similarly, the notation “VL2” and “VH2” denote respectively, the Light Chain Variable Domain and Heavy Chain Variable Domain of such bispecific molecules that bind the “second” epitope. It is irrelevant whether a particular epitope is designated as the first vs. the second epitope; such notation having relevance only with respect to the presence and orientation of domains of the polypeptide chains of such multispecific molecules of the present invention. In one embodiment, one of such epitopes is an epitope of HIV-1 *Env* (for example but not limited to a gp41 MPER epitope), and the other is an epitope of a molecule that is not HIV-1 *Env*. In particular embodiments, one of such epitopes is an epitope of HIV-1 *Env* and the other is an epitope of a molecule (*e.g.*, CD2, CD3, CD8, CD16, T-Cell Receptor (TCR), NKG2D, *etc.*) present on the surface of an effector cell, for example but not limited to a T lymphocyte, a natural killer (NK) cell or other mononuclear cell (see, *e.g.*, **Figures 101 and 103A-103B**). In certain embodiments, a bispecific molecule comprises more than two epitope-binding sites (see, *e.g.*, **Figures 102A-102C**). Such bispecific molecules will bind at least one epitope of HIV-1 *Env* and at least one epitope of a molecule that is not HIV-1 *Env*.

[0217] One embodiment of the present invention also relates to trivalent binding molecules that are capable of binding to a “**first epitope**,” a “**second epitope**,” and a “**third epitope**,” wherein at least one of such epitopes is not identical to another. Such trivalent binding molecules comprise VL1 / VH1 domains that are capable of binding to the first epitope, VL2 / VH2 domains that are capable of binding to the second epitope, and further comprise “VL3” / “VH3” domains that are capable of binding to the third epitope, wherein the notation “VL3” /

and “VH3” denote respectively, the Variable Light Chain Domain and Variable Heavy Chain Domain of such trivalent binding molecules that bind the “third” epitope. The capacity to bind a third epitope provides additional and/or enhanced functionality. In one embodiment, one (or two) of such epitopes is an epitope of HIV-1 *Env* (particularly a gp41 MPER epitope), and two (or one) of such epitopes is an epitope of a molecule that is not HIV-1 *Env*. In particular embodiments, one (or two) of such epitopes is an epitope of HIV-1 *Env* and two (or one) of such epitopes is an epitope of a molecule (*e.g.*, CD2, CD3, CD8, CD16, T-Cell Receptor (TCR), NKG2D, *etc.*) present on the surface of an effector cell, such as a T lymphocyte, a natural killer (NK) cell or other mononuclear cell (see, *e.g.*, **Figures 104A-104F**). Such trivalent binding molecules will bind at least one epitope of HIV-1 *Env* and at least one epitope of a molecule that is not HIV-1 *Env*, and may bind two epitopes of HIV-1 *Env* and one epitope of a molecule that is not HIV-1 *Env* or may bind one epitope of HIV-1 *Env* and two epitopes that are not epitopes of HIV-1 *Env*.

[0218] In some embodiments, such molecules comprise two polypeptide chains, wherein each of the two polypeptide chains comprises three Domains (**Figure 101**). The first polypeptide chain comprises: (i) a Domain that comprises a binding region of a light chain variable Domain of a first immunoglobulin (VL1), (ii) a second Domain that comprises a binding region of a heavy chain variable Domain of a second immunoglobulin (VH2), and (iii) a third Domain that serves to promote heterodimerization with the second polypeptide chain and to covalently bond the first polypeptide to the second polypeptide chain of the molecule. The second polypeptide chain contains a complementary first Domain (a VL2 Domain), a complementary second Domain (a VH1 Domain) and a third Domain that complexes with the third Domain of the first polypeptide chain in order to promote heterodimerization and covalent bonding with the first polypeptide chain. Such molecules are stable, potent and have the ability to simultaneously bind two or more antigens. They are able to promote redirected T cell (CD3) or NK (CD16) cell mediated killing of cells expressing target antigens.

[0219] In certain embodiments, the HIV-1 multispecific molecules of the present invention are composed of two polypeptide chains which associate with one another to form one binding site specific for an epitope of HIV-1, and one binding site specific for an epitope of CD2, CD3, CD8, CD16, T-Cell Receptor (TCR), NKG2D, *etc.*, so as to be capable of simultaneously binding to HIV-1 and, for example, to CD3. Thus, such diabodies bind to a “first epitope,” which may be either an epitope of CD3 or HIV-1, and a “second epitope,”

which is an epitope of HIV-1 when the first epitope is an epitope of CD3, and is an epitope of CD3 when the first epitope is from HIV-1. Alternatively, such diabodies bind to a “first epitope,” which may be either an epitope of CD16 or HIV-1, and a “second epitope,” which is an epitope of HIV-1 when the first epitope is of CD16, and is an epitope of CD16 when the first epitope is an epitope of HIV-1.

[0220] In certain embodiments, the first of such two polypeptide chains will contain, in the N-terminal to C-terminal direction, an N-terminus, the Antigen-Binding Domain of a Light Chain Variable Domain (VL) of an antibody that binds to a “first” epitope of a “first” antigen (*e.g.*, either CD3 or HIV-1 envelope), the Antigen-Binding Domain of a Heavy Chain Variable Domain (VH) of an antibody that binds to a “second” epitope of a “second” antigen (HIV-1, if the first antigen was CD3; CD3, if the first antigen was HIV-1), a Heterodimerization-Promoting Domain, and a C-terminus. An intervening peptide linker (Peptide Linker 1) separates the Antigen-Binding Domain of the Light Chain Variable Domain from the Antigen-Binding Domain of the Heavy Chain Variable Domain. In certain embodiments the Antigen-Binding Domain of the Heavy Chain Variable Domain is linked to the Heterodimerization-Promoting Domain by an intervening peptide linker (Peptide Linker 2). In certain embodiments the first of the two polypeptide chains will thus contain, in the N-terminal to C-terminal direction: VL_{First Antigen} – Peptide Linker 1 – VH_{Second Antigen} – Peptide Linker 2 – Heterodimerization-Promoting Domain.

[0221] The terms VL/VH for first and second antigens, VL_{first antigen}/VH_{first antigen} VL_{second antigen}/VH_{second antigen}, and VL1/VH1 and VL2/VH2 are used interchangeably throughout the application.

[0222] In certain embodiments, the second of such two polypeptide chains will contain, in the N-terminal to C-terminal direction, an N-terminus, the Antigen-Binding Domain of a Light Chain Variable Domain (VL) of an antibody that binds to the second epitope of the second antigen, the Antigen-Binding Domain of a Heavy Chain Variable Domain (VH) of an antibody that binds to the first epitope of the first antigen, a Heterodimerization-Promoting Domain and a C-terminus. An intervening peptide linker (Peptide Linker 1) separates the Antigen-Binding Domain of the Light Chain Variable Domain from the Antigen-Binding Domain of the Heavy Chain Variable Domain. In certain embodiments, the Antigen-Binding Domain of the Heavy Chain Variable Domain is linked to the Heterodimerization-Promoting Domain by an intervening peptide linker (Peptide Linker 2). In certain embodiments the

second of the two polypeptide chains will thus contain, in the N-terminal to C-terminal direction: VL_{Second Antigen} – Peptide Linker 1 – VH_{First Antigen} – Peptide Linker 2 – Heterodimerization-Promoting Domain.

[0223] The Antigen-Binding Domain of the Light Chain Variable Domain of the first polypeptide chain interacts with the Antigen-Binding Domain of the Heavy Chain Variable Domain of the second polypeptide chain in order to form a functional antigen-binding site that is specific for the first antigen (*e.g.*, either HIV-1 envelope or CD3). Likewise, the Antigen-Binding Domain of the Light Chain Variable Domain of the second polypeptide chain interacts with the Antigen-Binding Domain of the Heavy Chain Variable Domain of the first polypeptide chain in order to form a second functional antigen-binding site that is specific for the second antigen (*e.g.*, either CD3 or HIV-1 envelope, depending upon the identity of the first antigen). Thus, the selection of the Antigen-Binding Domain of the Light Chain Variable Domain and the Antigen-Binding Domain of the Heavy Chain Variable Domain of the first and second polypeptide chains are coordinated, such that the two polypeptide chains collectively comprise Antigen-Binding Domains of light and Heavy Chain Variable Domains capable of binding to the intended targets, in certain embodiments *e.g.* HIV-1 envelope and CD3, or CD16.

[0224] In certain embodiments the length of Peptide Linker 1, which separates such VL and VH domains of a polypeptide chain is selected to substantially or completely prevent such VL and VH domains from binding to one another. Thus the VL and VH domains of the first polypeptide chain are substantially or completely incapable of binding to one another. Likewise, the VL and VH domains of the second polypeptide chain are substantially or completely incapable of binding to one another. In certain embodiments this is due to the peptide linker which separates the VH and VL domains. In certain embodiments, the peptide linker is 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, but no more than 20 amino acids. In some embodiments, the peptide linker is less than 12 amino acids in length. In certain embodiments an intervening spacer peptide (Peptide Linker 1) has the sequence (SEQ ID NO:508): GGGSGGGG.

[0225] Peptide Linker 2 separates the VH Domain of a polypeptide chain from the Heterodimer-Promoting Domain of that polypeptide chain. Any of a variety of linkers can be used for the purpose of Peptide Linker 2. The length and composition of Peptide Linker 2 may be selected based on the choice of heterodimer-promoting domains. Typically, the

second intervening peptide linker (Peptide Linker 2) will comprise 1-20 amino acid residues. In certain embodiments, where the heterodimer-promoting domains do not comprise a cysteine residue a cysteine-containing second intervening peptide linker (Peptide Linker 2) is utilized. Optionally, both a cysteine-containing Peptide Linker 2 (Peptide Linker 2-C) and a cysteine-containing Heterodimer-Promoting Domain are used. In certain embodiments a sequence for such Peptide Linker 2 has the amino acid sequence: GGCGGG (SEQ ID NO:509), which has a cysteine residue that may be used to covalently bond the first and second polypeptide chains to one another via a disulfide bond. In certain embodiments, a sequence for Peptide Linker 2-C has the amino acid sequence: ASTKG (SEQ ID NO: 510). Peptide Linker 2 and Peptide Linker 2-C could be used interchangeably.

[0226] The formation of heterodimers of the first and second polypeptide chains can be driven by the inclusion of specific sequences referred to as Heterodimer-Promoting Domains (HPDs). Such domains include without limitation GVEPKSC (SEQ ID NO:511) or VEPKSC (SEQ ID NO:512) on one polypeptide chain and GFNRGEC (SEQ ID NO:513) or FNRGEC (SEQ ID NO:514) on the other polypeptide chain (See US2007/0004909 herein incorporated by reference in its entirety). Various HPD sequences are contemplated by the invention and disclosed in the specification. Table 4 discloses non-limiting embodiments of various HPDs. In some embodiments the HPD include E/K-coils (SEQ ID NOs: 518, 520) or cysteine engineered E/K-coils (SEQ ID NOs: 519, 521). In some embodiments HPD includes combinations of SEQ ID NOs: 511, 512, 513, and 514 (*e.g.*, SEQ ID NOs: 511 and 513; SEQ ID NOs: 512 and 513; SEQ ID NOs: 511 and 514; SEQ ID NOs: 512 and 514). In some embodiments HPDs include any suitable sequences with a Cysteine residue to permit disulfide bond. In some embodiments HPDs includes suitable CH1 and CL domains (See for example CH domain (SEQ ID NO: 515) and kappa and lambda light chain constant domains (SEQ ID NOs: 516 and 517).

[0227] In certain embodiments, the Heterodimer-Promoting Domains of the present invention are formed from one, two, three or four tandemly repeated coil domains of opposing charge that comprise a sequence of at least six, at least seven or at least eight charged amino acid residues (Apostolovic, B. *et al.* (2008) “*pH-Sensitivity of the E3/K3 Heterodimeric Coiled Coil*,” *Biomacromolecules* 9:3173–3180; Arndt, K.M. *et al.* (2001) “*Helix-stabilized Fv (hsFv) Antibody Fragments: Substituting the Constant Domains of a Fab Fragment for a Heterodimeric Coiled-coil Domain*,” *J. Molec. Biol.* 312:221-228; Arndt, K.M. *et al.* (2002)

“Comparison of In Vivo Selection and Rational Design of Heterodimeric Coiled Coils,” Structure 10:1235-1248; Boucher, C. *et al.* (2010) *“Protein Detection By Western Blot Via Coiled-Coil Interactions,”* Analytical Biochemistry 399:138-140; Cachia, P.J. *et al.* (2004) *“Synthetic Peptide Vaccine Development: Measurement Of Polyclonal Antibody Affinity And Cross-Reactivity Using A New Peptide Capture And Release System For Surface Plasmon Resonance Spectroscopy,”* J. Mol. Recognit. 17:540-557; De Crescenzo, G.D. *et al.* (2003) *“Real-Time Monitoring of the Interactions of Two-Stranded de novo Designed Coiled-Coils: Effect of Chain Length on the Kinetic and Thermodynamic Constants of Binding,”* Biochemistry 42:1754-1763; Fernandez-Rodriguez, J. *et al.* (2012) *“Induced Heterodimerization And Purification Of Two Target Proteins By A Synthetic Coiled-Coil Tag,”* Protein Science 21:511-519; Ghosh, T.S. *et al.* (2009) *“End-To-End And End-To-Middle Interhelical Interactions: New Classes Of Interacting Helix Pairs In Protein Structures,”* Acta Crystallographica D65:1032-1041; Grigoryan, G. *et al.* (2008) *“Structural Specificity In Coiled-Coil Interactions,”* Curr. Opin. Struc. Biol. 18:477-483; Litowski, J.R. *et al.* (2002) *“Designing Heterodimeric Two-Stranded α -Helical Coiled-Coils: The Effects Of Hydrophobicity And α -Helical Propensity On Protein Folding, Stability, And Specificity,”* J. Biol. Chem. 277:37272-37279; Steinkruger, J.D. *et al.* (2012) *“The d'-d-d' Vertical Triad is Less Discriminating Than the α' - α - α' Vertical Triad in the Antiparallel Coiled-coil Dimer Motif,”* J. Amer. Chem. Soc. 134(5):2626-2633; Straussman, R. *et al.* (2007) *“Kinking the Coiled Coil – Negatively Charged Residues at the Coiled-coil Interface,”* J. Molec. Biol. 366:1232-1242; Tripet, B. *et al.* (2002) *“Kinetic Analysis of the Interactions between Troponin C and the C-terminal Troponin I Regulatory Region and Validation of a New Peptide Delivery/Capture System used for Surface Plasmon Resonance,”* J. Molec. Biol. 323:345-362; Woolfson, D.N. (2005) *“The Design Of Coiled-Coil Structures And Assemblies,”* Adv. Prot. Chem. 70:79-112; Zeng, Y. *et al.* (2008) *“A Ligand-Pseudoreceptor System Based On de novo Designed Peptides For The Generation Of Adenoviral Vectors With Altered Tropism,”* J. Gene Med. 10:355-367).

[0228] Such repeated coil domains may be exact repeats or may have substitutions. For example, the Heterodimer-Promoting Domain of the first polypeptide chain may comprise a sequence of eight negatively charged amino acid residues and the Heterodimerization-Promoting Domain of the second polypeptide chain may comprise a sequence of eight negatively charged amino acid residues. It is immaterial which coil is provided to the first or

second polypeptide chains, provided that a coil of opposite charge is used for the other polypeptide chain.

[0229] In certain embodiments a multispecific molecule of the present invention has a first polypeptide chain having a negatively charged coil. The positively charged amino acid may be lysine, arginine, histidine, *etc.* and/or the negatively charged amino acid may be glutamic acid, aspartic acid, *etc.* In certain embodiments the positively charged amino acid is lysine and/or the negatively charged amino acid is glutamic acid. It is possible for only a single Heterodimer-Promoting Domain to be employed (since such domain will inhibit homodimerization and thereby promote heterodimerization). In certain embodiments both the first and second polypeptide chains of the multispecific molecules of the present invention contain Heterodimer-Promoting Domains.

[0230] In certain embodiments, one of the Heterodimer-Promoting Domains will comprise four tandem “E-coil” helical domains (SEQ ID NO:518 (EVAALEK-EVAALEK-EVAALEK-EVAALEK)), whose glutamate residues will form a negative charge at pH 7, while the other of the Heterodimer-Promoting Domains will comprise four tandem “K-coil” domains (SEQ ID NO:520 (KVAALEK-KVAALEK-KVAALEK-KVAALEK)), whose lysine residues will form a positive charge at pH 7. The presence of such charged domains promotes association between the first and second polypeptides, and thus fosters heterodimerization. In other embodiments, one of the four tandem “E-coil” helical domains of SEQ ID NO: 518 has been modified to contain a cysteine residue: EVAACEK-EVAALEK-EVAALEK-EVAALEK (SEQ ID NO: 519). In some embodiments, a Heterodimer-Promoting Domain in which one of the four tandem “K-coil” helical domains of SEQ ID NO: 520 has been modified to contain a cysteine residue: KVAACEK-KVAALEK-KVAALEK-KVAALEK (SEQ ID NO: 521). Such cysteine modified Heterodimer-Promoting Domains may be used to covalently bond the first and second polypeptide chains to one another via a disulfide bond.

[0231] In some embodiments, the number of K coil and E coil domains can vary and a skilled artisan can readily determine whether a different number of K-coil or E-coil domain lead to heterodimerization.

[0232] In certain embodiments, the multispecific molecules of the invention, for example but not limited to bispecific monovalent diabodies and trivalent binding molecules, are engineered so that their first and second polypeptide chains covalently bond to one another

via one or more cysteine residues positioned along their length. Such cysteine residues may be introduced into the intervening peptide linker that separates the VL and VH domains of the polypeptides. Alternatively, as provided above Peptide Linker 2 and/or the HPDs may contain such cysteine residues.

[0233] The invention also includes variants of the multispecific molecules, or fragments thereof disclosed herein, including variants that retain the ability to bind to recombinant Env protein, the ability to bind to the surface of virus-infected cells and/or ADCC-mediating properties of the antibodies specifically disclosed, and methods of using same to, for example, reduce HIV-1 infection risk. Combinations of the multispecific molecules, antibodies, or fragments thereof, disclosed herein can also be used in the methods of the invention.

[0234] Formation of multispecific molecule as described herein requires the interaction of differing polypeptide chains. Such interactions are difficult to achieve with efficiency within a single cell recombinant production system, due to the many variants of potential chain mispairings. One solution to decrease the probability of mispairings, is to engineer "knobs-into-holes" type mutations into the desired polypeptide chain pairs. Such mutations favor heterodimerization over homodimerization. For example, with respect to Fc-Fc-interactions, an amino acid substitution (preferably a substitution with an amino acid comprising a bulky side group forming a 'knob', *e.g.*, tryptophan) can be introduced into the CH2 or CH3 domain such that steric interference will prevent interaction with a similarly mutated domain and will obligate the mutated domain to pair with a domain into which a complementary, or accommodating mutation has been engineered, *i.e.*, 'the hole' (*e.g.*, a substitution with glycine). Such sets of mutations can be engineered into any pair of polypeptides comprising the multispecific molecule, and further, engineered into any portion of the polypeptides chains that comprise a multispecific molecule. Methods of protein engineering to favor heterodimerization over homodimerization are well known in the art, in particular with respect to the engineering of immunoglobulin-like molecules, and are encompassed herein (see *e.g.*, Ridgway *et al.* (1996) "'Knobs-Into-Holes' Engineering Of Antibody CH3 Domains For Heavy Chain Heterodimerization," Protein Engr. 9:617-621, Atwell *et al.* (1997) "Stable Heterodimers From Remodeling The Domain Interface Of A Homodimer Using A Phage Display Library," J. Mol. Biol. 270: 26-35, and Xie *et al.* (2005) "A New Format Of Bispecific Antibody: Highly Efficient Heterodimerization, Expression And Tumor Cell

Lysis," J. Immunol. Methods 296:95-101; each of which is hereby incorporated herein by reference in its entirety).

[0235] In some embodiments the invention provides multispecific molecules comprising variant Fc domain (or portions thereof), which variant Fc domain comprises at least one amino acid modification (*e.g.* substitution, insertion deletion) relative to a comparable wild-type Fc domain or hinge-Fc domain (or portion thereof). Molecules comprising variant Fc domains or hinge-Fc domains (or portion thereof) (*e.g.*, antibodies) normally have altered phenotypes relative to molecules comprising wild-type Fc domains or hinge-Fc domains or portions thereof. The variant phenotype may be expressed as altered serum half-life, altered stability, altered susceptibility to cellular enzymes or altered effector function as assayed in an NK dependent or macrophage dependent assay. Fc domain variants identified as altering effector function are known in the art. For example International Application WO04/063351, U.S. Patent Application Publications 2005/0037000 and 2005/0064514.

[0236] In some embodiments the invention provides multispecific molecules comprising a hinge domain. The hinge domain be derived from any immunoglobulin isotype or allotype including IgA, IgD, IgG, IgE and IgM. In preferred embodiments, the hinge domain is derived from IgG, wherein the IgG isotype is IgG1, IgG2, IgG3 or IgG4, or an allotype thereof. The hinge domain may be engineered into a polypeptide chain comprising the multispecific molecule together with an Fc domain such that the multispecific molecule comprises a hinge-Fc domain. In certain embodiments, the hinge and Fc domain are independently selected from any immunoglobulin isotype known in the art or exemplified herein. In other embodiments the hinge and Fc domain are separated by at least one other domain of the polypeptide chain, *e.g.*, the VL domain.

[0237] In another aspect, the invention provides multispecific molecules which include Fc domain(s)-- Fc bearing multispecific molecules. While some of the disclosure regarding Fc domain(s) refers to specific designs, a skilled artisan appreciates that the Fc disclosure is pertinent to any Fc bearing design of multispecific molecules, including but not limited to the designs described in Figures 101-105.

[0238] Fc bearing multispecific molecules, for example but not limited to Fc bearing diabodies are heavier, and could bind neonatal Fc receptor, increasing their circulating half-life. See Garber "Bispecific antibodies rise again" Nature Reviews Drug Discovery 13, 799–801 (2014), Figure 1a; See US Pub 20130295121, US Pub 20140099318 incorporated by

reference in their entirety. In certain embodiments, the invention encompasses multispecific molecules comprising an Fc domain or portion thereof (*e.g.* a CH2 domain, or CH3 domain). The Fc domain or portion thereof may be derived from any immunoglobulin isotype or allotype including, but not limited to, IgA, IgD, IgG, IgE and IgM. In some embodiments, the Fc domain (or portion thereof) is derived from IgG. In some embodiments, the IgG isotype is IgG1, IgG2, IgG3 or IgG4 or an allotype thereof. In some embodiments, the multispecific molecule comprises an Fc domain, which Fc domain comprises a CH2 domain and CH3 domain independently selected from any immunoglobulin isotype (*i.e.* an Fc domain comprising the CH2 domain derived from IgG and the CH3 domain derived from IgE, or the CH2 domain derived from IgG1 and the CH3 domain derived from IgG2, *etc.*). In some embodiments, the Fc domain may be engineered into a polypeptide chain comprising the multispecific molecule of the invention in any position relative to other domains or portions of the polypeptide chain (*e.g.*, the Fc domain, or portion thereof, may be C-terminal to both the VL and VH domains of the polypeptide of the chain; may be N-terminal to both the VL and VH domains; or may be N-terminal to one domain and C-terminal to another (*i.e.*, between two domains of the polypeptide chain)).

[0239] Other modifications of the multispecific molecules are contemplated to increase the half-life of the molecules. In some embodiments, these modifications include addition of a polypeptide portion of a serum binding protein. See US20100174053 A1, incorporated by reference.

[0240] In some embodiments, the Fc variants of the multispecific molecules of the invention are expected to have increased serum half-life compared to the non-Fc variants. Skilled artisan can readily carry out various assays, including pharmacokinetic studies, to determine the half-life of these molecules.

[0241] In some embodiments, the polypeptide chains in multispecific molecules further comprise an Fc domain. Dimerization of the Fc domains leads to formation of a multispecific molecule that exhibits immunoglobulin-like functionality, *i.e.*, Fc mediated function (*e.g.*, Fc- γ interaction, complement binding, *etc.*).

[0242] As provided in **Figures 102-103**, one or both of the polypeptide chains of bispecific diabodies may additionally comprise the sequence of a CH2-CH3 Domain, such that complexing between the two diabody polypeptides forms an Fc Domain that may be capable of binding to the Fc receptor of cells (such as B lymphocytes, dendritic cells, natural killer

cells, macrophages, neutrophils, eosinophils, basophils and mast cells). Similarly, the first and third polypeptide chains of trivalent binding molecules can comprise the sequence of a CH2-CH3 Domain, such that complexing between these two polypeptide chains forms an Fc Domain. As provided in more detail below, the CH2 and/or CH3 Domains of such polypeptide chains need not be identical in sequence, and advantageously are modified to foster complexing between the two polypeptide chains. Many variations of such molecules have been described (see, *e.g.*, United States Patent Publications No. 2014-0099318; 2013-0295121; 2010-0174053 and 2009-0060910; European Patent Publication No. EP 2714079; EP 2601216; EP 2376109; EP 2158221 and PCT Publications No. WO 2015/026894; WO 2015/026892; WO 2015/021089; WO 2014/159940; WO 2012/162068; WO 2012/018687; WO 2010/080538, the content of each of these publications is herein incorporated by reference in its entirety).

[0243] In some embodiments, Fc -bearing bispecific diabodies may comprise two pairs of polypeptide chains (or four different chains, as provided below). The first and third polypeptide chains of such a bispecific molecule (*e.g.*, diabodies) contain three domains: (i) a VL1-containing Domain, (ii) a VH2-containing Domain, (iii) Heterodimer-Promoting Domain and (iv) a Domain containing a CH2-CH3 sequence. The second and fourth polypeptide chains contain: (i) a VL2-containing Domain, (ii) a VH1-containing Domain and (iii) a Heterodimer-Promoting Domain, where the Heterodimer-Promoting Domains promote the dimerization of the first/third chains with the second/fourth chains. The VL and/or VH Domains of the third and fourth polypeptide chains, and VL and/or VH Domains of the first and second polypeptide chains may be the same or different so as to permit tetravalent binding that is either monospecific, bispecific or tetraspecific. Such molecules are tetravalent and have enhanced potency. The general structure of the polypeptide chains of a representative four-chain Fc Domain-containing multispecific molecules of invention is provided in **Table 1**:

Table 1		
Bispecific	2 nd Chain	NH ₂ -VL2-VH1-Heterodimer-Promoting Domain-COOH
	1 st Chain	NH ₂ -VL1-VH2-Heterodimer-Promoting Domain-CH ₂ -CH ₃ -COOH
	1 st Chain	NH ₂ -VL1-VH2-Heterodimer-Promoting Domain-CH ₂ -CH ₃ -COOH
	2 nd Chain	NH ₂ -VL2-VH1-Heterodimer-Promoting Domain-COOH
Tetraspecific	2 nd Chain	NH ₂ -VL2-VH1-Heterodimer-Promoting Domain-COOH
	1 st Chain	NH ₂ -VL1-VH2-Heterodimer-Promoting Domain-CH ₂ -CH ₃ -COOH
	3 rd Chain	NH ₂ -VL3-VH4-Heterodimer-Promoting Domain-CH ₂ -CH ₃ -COOH
	4 th Chain	NH ₂ -VL4-VH3-Heterodimer-Promoting Domain-COOH

[0244] HIV-1 bispecific bivalent Fc bearing diabodies can be composed of two pairs of polypeptide chains (*i.e.*, two first polypeptide chain and two second polypeptide chains) which associate with one another to form two binding sites specific for an epitope of HIV-1 and two binding sites specific for an epitope, for example but not limited to CD3 (see, **Figures 102A-102C**), so as to be capable of simultaneously binding to HIV-1 and to CD3. Thus such molecules binding to a “first” epitope, on a “first” antigen which may be either CD3 or HIV-1, and a “second” epitope on a “second” antigen, which is HIV-1 when the first epitope is CD3, and is CD3 when the first epitope is HIV-1.

[0245] As shown in **Figures 102A-102C**, the first polypeptide chain comprises (in the N-terminal to C-terminal direction): an N-terminus, the Antigen-Binding Domain of a Light Chain Variable Domain (VL1) of an antibody that binds to a “first” epitope of a “first” antigen (either an effector cell epitope such as but not limited to CD3, or HIV-1), the Antigen-Binding Domain of a Heavy Chain Variable Domain (VH2) of an antibody that binds to a “second” epitope of a “second” antigen (HIV-1, if the first antigen as CD3; CD3, if the first antigen was HIV-1), a Heterodimer-Promoting Domain which may comprise a cysteine

residue, the CH2-CH3 domains of an Fc Domain (“Fc Domain”) and a C-terminus. The second polypeptide contains (in the N-terminal to C-terminal direction): an N-terminus, the Antigen-Binding Domain the Light Chain Variable Domain (**VL2**) of an antibody that binds to the second epitope of the second antigen (VL2), the Antigen-Binding Domain of the Heavy Chain Variable Domain (**VH1**) of an antibody that binds to the first epitope of the first antigen, a Heterodimer-Promoting Domain that promotes heterodimerization with the first polypeptide chain, and a C-terminus. Here, two first polypeptides complex with each other to form an Fc Domain. An intervening peptide linker (**Peptide Linker 1**) separates the Antigen-Binding Domain of the Light Chain Variable Domain from the Antigen-Binding Domain of the Heavy Chain Variable Domain. In non-limiting embodiments the Antigen-Binding Domain of the Heavy Chain Variable Domain is linked to the Heterodimer-Promoting Domain by an intervening peptide linker (**Peptide Linker 2**). In other non-limiting embodiments, the Heterodimer-Promoting Domain is linked to the Fc Domain by an intervening peptide linker (**Peptide Linker 3**) or by an intervening spacer-linker peptide (**Spacer-Linker 3**). In certain embodiments, the first and second polypeptide chains form a disulfide bond between cysteine residues, which may be present in Peptide Linker 2 (*e.g.*, but not limited to **Peptide Linker 2-C**) and/or in the Heterodimer-Promoting Domains (*e.g.* but not limited to **E-coil-C/K-coil-C**). **Figures 102A-102C** provide schematics of three variations of such diabodies utilizing different Heterodimer-Promoting Domains. In non-limiting embodiments, the first polypeptide chains will contain, in the N-terminal to C-terminal direction: VL1 - Peptide Linker 1 – VH2 – Peptide Linker 2 – Heterodimer-Promoting Domain – Spacer-Linker 3 – Fc Domain, and the second polypeptide chains will contain, in the N-terminal to C-terminal direction: VL2 - Peptide Linker 1 – VH1 – Peptide Linker 2 – Heterodimer-Promoting Domain.

[0246] In some embodiments, Fc bearing diabodies may comprise three polypeptide chains. The first polypeptide of such a molecule contains three Domains: (i) a VL1-containing Domain, (ii) a VH2-containing Domain, (iii) a Domain that promotes heterodimerization and covalent bonding with the diabody’s first polypeptide chain and (iv) a Domain containing a CH2-CH3 sequence. The second polypeptide of such diabodies contains: (i) a VL2-containing Domain, (ii) a VH1-containing Domain and (iii) a Domain that promotes heterodimerization and covalent bonding with the diabody’s first polypeptide chain. The third polypeptide of such diabodies comprises a CH2-CH3 sequence. Thus, the first and second

polypeptide chains of such diabodies associate together to form a VL1/VH1 binding site that is capable of binding to the epitope, as well as a VL2/VH2 binding site that is capable of binding to the second epitope. The first and second polypeptides are bonded to one another through a disulfide bond involving cysteine residues in their respective third Domains. Notably, the first and third polypeptide chains complex with one another to form an Fc Domain that is stabilized via a disulfide bond. Such diabodies have enhanced potency. Such Fc bearing diabodies may have either of two orientations (**Table 2**):

Table 2		
First Orientation	3 rd Chain	NH ₂ -CH ₂ -CH ₃ -COOH
	1 st Chain	NH ₂ -VL1-VH2-Heterodimer Promoting Domain-CH ₂ -CH ₃ -COOH
	2 nd Chain	NH ₂ -VL2-VH1-Heterodimer Promoting Domain-COOH
Second Orientation	3 rd Chain	NH ₂ -CH ₂ -CH ₃ -COOH
	1 st Chain	NH ₂ -CH ₂ -CH ₃ - VL1-VH2-Heterodimer Promoting Domain-COOH
	2 nd Chain	NH ₂ -VL2-VH1-Heterodimer Promoting Domain-COOH

[0247] HIV-1 bispecific monovalent Fc bearing diabodies can be composed of three polypeptide chains which associate with one another to form one binding site specific for an epitope of HIV-1 and one binding site specific for another epitope, for example but not limited to an epitope of CD3 (see, **Figure 103A-103B**), so as to be capable of simultaneously binding to HIV-1 and to CD3. Thus, such molecules bind to a “first” antigen, which may be either CD3 or HIV-1, and a “second” antigen, which is HIV-1 when the first epitope is CD3, and is CD3 when the first epitope is HIV-1.

[0248] As shown in **Figure 103A**, the first of such three polypeptide chains will contain, in the N-terminal to C-terminal direction, an N-terminus, the Antigen-Binding Domain of a Light Chain Variable Domain (VL) of an antibody that binds to a “first” epitope of a “first” antigen (for example but not limited to either CD3 or HIV-1), the Antigen-Binding Domain of a Heavy Chain Variable Domain (VH) of an antibody that binds to a “second” epitope of a “second” antigen (for example but not limited to HIV-1, if the first antigen was CD3; CD3, if the first antigen was HIV-1), a Heterodimerization-Promoting Domain, and a C-terminus. An intervening peptide linker (**Peptide Linker 1**) separates the Antigen-Binding Domain of the

Light Chain Variable Domain from the Antigen-Binding Domain of the Heavy Chain Variable Domain. In non-limiting embodiments, the Antigen-Binding Domain of the Heavy Chain Variable Domain is linked to the Heterodimerization-Promoting Domain by an intervening peptide linker (**Peptide Linker 2**). In a non-limiting example of an HIV-1 bispecific monovalent Fc bearing diabody, the C-terminus of the Heterodimerization-Promoting Domain is linked to the CH2-CH3 domains of an Fc Domain ("Fc Domain") by an intervening peptide linker (**Peptide Linker 3**) or by an intervening spacer-linker peptide (**Spacer-Linker 3**). In non-limiting embodiments, the first of the three polypeptide chains will thus contain, in the N-terminal to C-terminal direction: VL_{First Antigen} – Peptide Linker 1 – VH_{Second Antigen} – Peptide Linker 2 – Heterodimerization-Promoting Domain – Spacer-Linker 3 – Fc Domain.

[0249] Alternatively, as shown in **Figure 103B**, the first of such three polypeptide chains will contain, in the N-terminal to C-terminal direction, an N-terminus, **Peptide Linker 3**, the CH2-CH3 domains of an Fc Domain ("Fc Domain"), an intervening spacer peptide (**Peptide Linker 4**), having, for example the amino acid sequence: APSSS (**SEQ ID NO:524**) or the amino acid sequence APSSSPME (**SEQ ID NO:525**), the Antigen-Binding Domain of a Light Chain Variable Domain (VL) of an antibody that binds to the first epitope of the first antigen (for example but not limited to CD3 or HIV-1), the Antigen-Binding Domain of a Heavy Chain Variable Domain (VH) of an antibody that binds to the second epitope of the second antigen (for example but not limited to HIV-1, if the first antigen was CD3; CD3, if the first antigen was HIV-1), a Heterodimerization-Promoting Domain, and a C-terminus. An intervening peptide linker (**Peptide Linker 1**) separates the Antigen-Binding Domain of the Light Chain Variable Domain from the Antigen-Binding Domain of the Heavy Chain Variable Domain. In non-limiting embodiments, the Antigen-Binding Domain of the Heavy Chain Variable Domain is linked to the Heterodimerization-Promoting Domain by an intervening peptide linker peptide (**Peptide Linker 2**). In non-limiting embodiments, the first of the three polypeptide chains will thus contain, in the N-terminal to C-terminal direction: **Peptide Linker 3** – Fc Domain – **Peptide Linker 4** – VL_{First Antigen} – Peptide Linker 1 – VH_{Second Antigen} – Peptide Linker 2 – Heterodimerization-Promoting Domain.

[0250] In non-limiting embodiments, the second of such three polypeptide chains will contain, in the N-terminal to C-terminal direction, an N-terminus, the Antigen-Binding Domain of a Light Chain Variable Domain (VL) of an antibody that binds to the second

epitope of the second antigen, the Antigen-Binding Domain of a Heavy Chain Variable Domain (VH) of an antibody that binds to the first epitope of the first antigen, a Heterodimerization-Promoting Domain and a C-terminus. An intervening peptide linker (**Peptide Linker 1**) separates the Antigen-Binding Domain of the Light Chain Variable Domain from the Antigen-Binding Domain of the Heavy Chain Variable Domain. In non-limiting embodiments, the Antigen-Binding Domain of the Heavy Chain Variable Domain is linked to the Heterodimerization-Promoting Domain by an intervening peptide linker (**Peptide Linker 2**). In non-limiting embodiments, the second of the three polypeptide chains will thus contain, in the N-terminal to C-terminal direction: VL_{Second Antigen} – Peptide Linker 1 – VH_{First Antigen} – Peptide Linker 2 – Heterodimerization-Promoting Domain.

[0251] In non-limiting embodiments, the third of such three polypeptide chains will contain a peptide linker (**Peptide Linker 3**) and the CH2-CH3 domains of an Fc Domain (“Fc Domain”).

[0252] For the various Fc bearing multispecific molecules described herein, the Antigen-Binding Domain of the Light Chain Variable Domain of the first polypeptide chain interacts with the Antigen-Binding Domain of the Heavy Chain Variable Domain of the second polypeptide chain in order to form a functional antigen-binding site that is specific for the first antigen (*e.g.*, either HIV-1 or CD3). Likewise, the Antigen-Binding Domain of the Light Chain Variable Domain of the second polypeptide chain interacts with the Antigen-Binding Domain of the Heavy Chain Variable Domain of the first polypeptide chain in order to form a second functional antigen-binding site that is specific for the second antigen (*e.g.*, either CD3 or HIV-1, depending upon the identity of the first antigen). Thus, the selection of the Antigen-Binding Domain of the Light Chain Variable Domain and the Antigen-Binding Domain of the Heavy Chain Variable Domain of the first and second polypeptide chains are coordinated, such that the two polypeptide chains collectively comprise Antigen-Binding Domains of light and Heavy Chain Variable Domains capable of binding to the first and second antigens (*e.g.*, HIV-1 and CD3).

[0253] The Fc Domain of the Fc bearing multispecific molecules, including but not limited to bispecific and trispecific molecules (*e.g.* bispecific antibodies, bispecific diabodies, and trivalent binding molecules), of the present invention may be either a complete Fc Domain (*e.g.*, a complete IgG Fc Domain) or only a fragment of a complete Fc Domain. In some embodiments the Fc Domain of the molecules of the present invention may possess the ability

to bind to one or more Fc receptors (*e.g.*, FcγR(s)). In non-limiting embodiments the Fc Domain will cause reduced binding to FcγRIA (CD64), FcγRIIA (CD32A), FcγRIIB (CD32B), FcγRIIA (CD16a) or FcγRIIB (CD16b) (relative to the binding exhibited by a wild-type Fc Domain) or will substantially eliminate the ability of such Fc Domain to bind to such receptor(s). The Fc bearing multispecific molecules of the present invention may include some or all of the CH2 Domain and/or some or all of the CH3 Domain of a complete Fc Domain, or may comprise a variant CH2 and/or a variant CH3 sequence (that may include, for example, one or more insertions and/or one or more deletions with respect to the CH2 or CH3 domains of a complete Fc Domain). The Fc Domain of the Fc bearing multispecific molecules of the present invention may comprise non-Fc polypeptide portions, or may comprise portions of non-naturally complete Fc Domains, or may comprise non-naturally occurring orientations of CH2 and/or CH3 domains (such as, for example, two CH2 domains or two CH3 domains, or in the N-terminal to C-terminal direction, a CH3 Domain linked to a CH2 Domain, *etc.*).

[0254] Polymorphisms have been observed at a number of different positions within antibody constant regions (*e.g.*, Fc positions, including but not limited to positions 270, 272, 312, 315, 356, and 358 as numbered by the EU index as set forth in Kabat), and thus slight differences between the presented sequence and sequences in the prior art can exist. Polymorphic forms of human immunoglobulins have been well-characterized. At present, 18 Gm allotypes are known: G1m (1, 2, 3, 17) or G1m (a, x, f, z), G2m (23) or G2m (n), G3m (5, 6, 10, 11, 13, 14, 15, 16, 21, 24, 26, 27, 28) or G3m (b1, c3, b3, b0, b3, b4, s, t, g1, c5, u, v, g5) (Lefranc, *et. al.*, “*The Human IgG Subclasses: Molecular Analysis Of Structure, Function And Regulation.*” Pergamon, Oxford, pp. 43-78 (1990); Lefranc, G. *et. al.*, 1979, Hum. Genet.: 50, 199-211). It is specifically contemplated that the molecules of the present invention may be incorporate any allotype, isoallotype, or haplotype of any immunoglobulin gene, and are not limited to the allotype, isoallotype or haplotype of the sequences provided herein. Furthermore, in some expression systems the C-terminal amino acid residue (bolded above) of the CH3 Domain may be post-translationally removed. Accordingly, the C-terminal residue of the CH3 Domain is an optional amino acid residue. Specifically encompassed by the instant invention are molecules lacking the C-terminal residue of the CH3 Domain. Also specifically encompassed by the instant invention are such constructs comprising the C-terminal lysine residue of the CH3 Domain.

[0255] In non-limiting embodiments the first and third polypeptide chains of the Fc bearing multispecific molecules of the present invention each comprise CH2-CH3 domains that complex together to form an immunoglobulin (IgG) Fc Domain. The amino acid sequence of the CH2-CH3 domain of human IgG1 is (SEQ ID NO: 527):

```

APELLGGPSV FLFPPKPKDT LMISRTPEVT CVVVDVSHED PEVKFNWYVD
GVEVHNAKTK PREEQYNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKALPA
PIEKTISKAK GQPREPQVYT LPSSREEMTK NQVSLTCLVK GFYPSDIAVE
WESNGQPENN YKTTTPVLDS DGSFFLYSKL TVDKSRWQQG NVFSCSVME
ALHNHYTQKS LSLSPGK

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[0256] Thus the CH2 and/or CH3 Domains of the first and third polypeptide chains may both be composed of SEQ ID NO: 527, or a variant thereof (*e.g.*, SEQ ID NO: 528, 529, 530).

[0257] In non-limiting embodiments the CH2-CH3 domains of the first and third polypeptide chains of the Fc bearing multispecific molecules of the present invention exhibit decreased (or substantially no) binding to FcγRIA (CD64), FcγRIIA (CD32A), FcγRIIB (CD32B), FcγRIIIA (CD16a) or FcγRIIIB (CD16b) (relative to the binding exhibited by the wild-type Fc Domain). Fc variants and mutant forms capable of mediating such altered binding are well known in the art and include amino acid substitutions at positions 234 and 235, a substitution at position 265 or a substitution at position 297 (see, for example, US Patent No. 5,624,821, herein incorporated by reference). In non-limiting embodiments the CH2-CH3 Domain of the first and/or third polypeptide chains of the Fc bearing multispecific molecules of the present invention include a substitution at position 234 with alanine and 235 with alanine.

[0258] The CH2 and/or CH3 Domains of the first and third polypeptide chains need not be identical in sequence, and in some embodiment are modified to foster complexing between the two polypeptide chains. For example, an amino acid substitution (for example a substitution with an amino acid comprising a bulky side group forming a ‘knob’, *e.g.*, tryptophan) can be introduced into the CH2 or CH3 Domain such that steric interference will prevent interaction with a similarly mutated domain and will obligate the mutated domain to pair with a domain into which a complementary, or accommodating mutation has been engineered, *i.e.*, ‘the hole’ (*e.g.*, a substitution with glycine). Such sets of mutations can be engineered into any pair of polypeptides comprising Fc bearing multispecific molecules of the invention, and further, engineered into any portion of the polypeptides chains of the pair. Methods of protein engineering to favor heterodimerization over homodimerization are well

known in the art, in particular with respect to the engineering of immunoglobulin-like molecules, and are encompassed herein (see *e.g.*, Ridgway *et al.* (1996) “*Knobs-Into-Holes’ Engineering Of Antibody CH3 Domains For Heavy Chain Heterodimerization*,” *Protein Engr.* 9:617-621, Atwell *et al.* (1997) “*Stable Heterodimers From Remodeling The Domain Interface Of A Homodimer Using A Phage Display Library*,” *J. Mol. Biol.* 270: 26-35, and Xie *et al.* (2005) “*A New Format Of Bispecific Antibody: Highly Efficient Heterodimerization, Expression And Tumor Cell Lysis*,” *J. Immunol. Methods* 296:95-101; each of which is hereby incorporated herein by reference in its entirety). In non-limiting embodiments the ‘knob’ is engineered into the CH2-CH3 Domains of the first polypeptide chain and the ‘hole’ is engineered into the CH2-CH3 Domains of the third polypeptide chain. Thus, the ‘knob’ will help in preventing the first polypeptide chain from homodimerizing via its CH2 and/or CH3 Domains. In non-limiting embodiments, as the third polypeptide chain contains the ‘hole’ substitution it will heterodimerize with the first polypeptide chain as well as homodimerize with itself. In non-limiting embodiments a knob is created by modifying a native IgG Fc Domain to contain the modification T366W. In non-limiting embodiments a hole is created by modifying a native IgG Fc Domain to contain the modification T366S, L368A and Y407V. To aid in purifying the third polypeptide chain homodimer from the final bispecific monovalent Fc bearing diabody comprising the first, second and third polypeptide chains, the protein A binding site of the CH2 and CH3 Domains of the third polypeptide chain is mutated by amino acid substitution at position 435 (H435R). Thus, the third polypeptide chain homodimer will not bind to protein A, whereas the bispecific monovalent Fc bearing diabody will retain its ability to bind protein A via the protein A binding site on the first polypeptide chain.

[0259] In non-limiting embodiments a sequence for the CH2 and CH3 Domains of the first polypeptide chain of the Fc bearing multispecific molecules of the present invention will have the “**knob-bearing**” sequence (SEQ ID NO: 531):

```

APEAAGGPSV FLFPPKPKDT LMISRTPEVT CVVVDVSHED PEVKFNWYVD
GVEVHNAKTK PREEQYNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKALPA
PIEKTISKAK GQPREPQVYT LPPSREEMTK NQVSLWCLVK GFYPSDIAVE
WESNGQPENN YKTTTPVLDS DGSFFLYSKL TVDKSRWQQG NVFSCSVMHE
ALHNHYTQKS LSLSPGK

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[0260] In non-limiting embodiments a sequence for the CH2 and CH3 Domains of the third polypeptide chain of the Fc bearing multispecific molecules of the present invention will have the “hole-bearing” sequence (SEQ ID NO: 533):

```
APEAAAGGPSV FLFPPKPKDT LMISRTPEVT CVVVDVSHED PEVKFNWYVD
GVEVHNAKTK PREEQYNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKALPA
PIEKTISKAK GQPREPQVYT LPPSREEMTK NQVSLSCAVK GFYPSDIAVE
WESNGQPENN YKTTTPVLDS DGSFFLVSKL TVDKSRWQQG NVFSCSVME
ALHNRYYTQKS LSLSPGK
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[0261] As will be noted, the CH2-CH3 Domains of SEQ ID NO: 531 and SEQ ID NO: 533 include a substitution at position 234 with alanine and 235 with alanine, and thus form an Fc Domain exhibit decreased (or substantially no) binding to FcγRIA (CD64), FcγRIIA (CD32A), FcγRIIB (CD32B), FcγRIIIA (CD16a) or FcγRIIIB (CD16b) (relative to the binding exhibited by the wild-type Fc Domain (SEQ ID NO: 527)).

[0262] In non-limiting embodiments, the first polypeptide chain will have a “knob-bearing” CH2-CH3 sequence, such as that of SEQ ID NO: 531 or 532. However, as will be recognized, a “hole-bearing” CH2-CH3 Domain (*e.g.*, SEQ ID NO: 533 or 534) could be employed in the first polypeptide chain, in which case, a “knob-bearing” CH2-CH3 Domain (*e.g.*, SEQ ID NO: 531 or 532) would be employed in the third polypeptide chain.

[0263] In non-limiting embodiments, the Fc domain can be modified by amino acid substitution to increase binding to the neonatal Fc receptor and therefore the half-life of the antibody when administered to a subject. The Fc domain can be an IgA, IgM, IgD, IgE or IgG Fc domain. The Fc domain can be an optimized Fc domain, as described in U.S. Published Patent Application No. 20100093979, incorporated herein by reference. In certain embodiments the Fc bearing multispecific molecules comprise amino acid alterations, or combinations thereof, for example in the Fc domain(s) outside of epitope binding, which alterations can improve their properties. Various Fc modifications are known in the art. Amino acid numbering is according to the EU Index in Kabat. In some embodiments, the invention contemplates antibodies comprising mutations that affect neonatal Fc receptor (FcRn) binding, antibody half-life, and localization and persistence of antibodies at mucosal sites. See *e.g.* Ko SY *et al.*, Nature 514: 642-45, 2014, at Figure 1a and citations therein; Kuo, T. and Averson, V., mAbs 3(5): 422-430, 2011, at Table 1, US Pub 20110081347 (an aspartic acid at Kabat residue 288 and/or a lysine at Kabat residue 435), US Pub 20150152183 for various Fc Domain mutation, incorporated by reference in their entirety.

[0264] In certain embodiments, the Fc bearing multispecific molecules comprise AAAA substitution in and around the Fc Domain of the Fc bearing multispecific molecule that has been reported to enhance ADCC via NK cells (AAA mutations) containing the Fc Domain aa of S298A as well as E333A and K334A (Shields RI *et al.* JBC, 276: 6591-6604, 2001) and the 4th A (N434A) is to enhance FcR neonatal mediated transport of the IgG to mucosal sites (Shields RI *et al.* *ibid*).

[0265] Other mutations have been reported to improve antibody half-life or function or both and can be incorporated into the Fc Domain of the Fc bearing multispecific molecules. These include the DLE set of mutations (Romain G, *et al.* Blood 124: 3241, 2014), the LS mutations M428L/N434S, alone or in a combination with other Fc Domain mutations, (Ko SY *et al.* Nature 514: 642-45, 2014, at Figure 1a and citations therein; Zlevsky *et al.*, Nature Biotechnology, 28(2): 157-159, 2010; US Pub 20150152183); the YTE Fc mutations (Robbie G *et al.*, Antimicrobial Agents and Chemotherapy 12: 6147-53, 2013) as well as other engineered mutations to the Fc Domain such as QL mutations, IHH mutations (Ko SY *et al.* Nature 514: 642-45, 2014, at Figure 1a and relevant citations; See also Rudicell R *et al.*, J. Virol 88: 12669-82, 201). In some embodiments, modifications, such as but not limited to fucosylation, which may affect interaction with Fc receptors (See *e.g.* Moldt, *et al.* JVI 86(11): 66189-6196, 2012). In some embodiments, the Fc bearing multispecific molecules can comprise modifications, for example but not limited to glycosylation, which reduce or eliminate polyreactivity of such a molecule. See *e.g.* Chuang, *et al.* Protein Science 24: 1019-1030, 2015. In some embodiments the Fc bearing multispecific molecules can comprise modifications in the Fc domain such that the Fc domain exhibits, as compared to an unmodified Fc domain enhanced antibody dependent cell mediated cytotoxicity (ADCC); increased binding to FcγRIIA or to FcγRIIIA; decreased binding to FcγRIIB; or increased binding to FcγRIIB. See *e.g.* US Pub 20140328836.

[0266] In another aspect, the invention provides trivalent structures incorporating two diabody-type binding domains and one non-diabody-type domain and an Fc Domain (see, *e.g.*, **Figures 104A-104F** and PCT Publication Nos. WO 2015/184207 and WO 2015/184203). Such trivalent binding molecules may be utilized to generate monospecific, bispecific or trispecific molecules. The ability to bind three different epitopes provides enhanced capabilities.

[0267] A further embodiment of the present invention relates to trivalent binding molecules comprising an Fc Domain. The Fc Domain bearing trivalent binding molecules can simultaneously bind a first epitope, a second epitope, and a third epitope, wherein at least one of such epitopes is not identical to another. Such trivalent binding molecules comprise three epitope-binding sites, two of which are Diabody-Type Binding Domains, which provide binding Site A and binding Site B, and one of which is a Fab-Type Binding Domain, or an scFv-Type Binding Domain, which provides binding Site C (see, *e.g.*, **Figures 104A-104F**, and PCT Publication Nos. WO 2015/184207 and WO 2015/184203). Such trivalent binding molecules thus comprise “VL1” / “VH1” domains that are capable of binding to the first epitope and “VL2” / “VH2” domains that are capable of binding to the second epitope and “VL3” and “VH3” domains that are capable of binding to the “third” epitope of such trivalent binding molecule. A “Diabody-Type Binding Domain” is the type of epitope-binding site present in a diabody, and especially, a DART® diabody, as described above. Each of a “Fab-Type Binding Domain” and an “scFv-Type Binding Domain” are epitope-binding sites that are formed by the interaction of the VL Domain of an immunoglobulin light chain and a complementing VH Domain of an immunoglobulin heavy chain. Fab-Type Binding Domains differ from Diabody-Type Binding Domains in that the two polypeptide chains that form a Fab-Type Binding Domain comprise only a single epitope-binding site, whereas the two polypeptide chains that form a Diabody-Type Binding Domain comprise at least two epitope-binding sites. Similarly, scFv-Type Binding Domains also differ from Diabody-Type Binding Domains in that they comprise only a single epitope-binding site. Thus, as used herein Fab-Type, and scFv-Type Binding Domains are distinct from Diabody-Type Binding Domains.

[0268] Typically, the trivalent binding molecules of the present invention will comprise four different polypeptide chains (see **Figures 104A-104B**), however, the molecules may comprise fewer or greater numbers of polypeptide chains, for example by fusing such polypeptide chains to one another (*e.g.*, via a peptide bond) or by dividing such polypeptide chains to form additional polypeptide chains, or by associating fewer or additional polypeptide chains via disulfide bonds. **Figures 104C-104F** illustrate this aspect of the present invention by schematically depicting such molecules having three polypeptide chains. As provided in **Figures 104A-104F**, the trivalent binding molecules of the present invention may have alternative orientations in which the Diabody-Type Binding Domains are N-terminal (**Figures 104A, 104C and 104D**) or C-terminal (**Figures 104B, 104E and 104F**) to an Fc Domain.

[0269] In certain embodiments, the first polypeptide chain of such trivalent binding molecules of the present invention contains: (i) a VL1-containing Domain, (ii) a VH2-containing Domain, (iii) a Heterodimer-Promoting Domain, and (iv) a Domain containing a CH2-CH3 sequence. The VL1 and VL2 Domains are located N-terminal or C-terminal to the CH2-CH3-containing domain as presented in **Table 3** (also see, **Figures 104A and 104B**). The second polypeptide chain of such embodiments contains: (i) a VL2-containing Domain, (ii) a VH1-containing Domain, and (iii) a Heterodimer-Promoting Domain. The third polypeptide chain of such embodiments contains: (i) a VH3-containing Domain, (ii) a CH1-containing Domain and (iii) a Domain containing a CH2-CH3 sequence. The third polypeptide chain may be the heavy chain of an antibody that contains a VH3 and a heavy chain constant region, or a polypeptide that contains such domains. The fourth polypeptide of such embodiments contains: (i) a VL3-containing Domain and (ii) a CL-containing Domain. The fourth polypeptide chains may be a light chain of an antibody that contains a VL3 complementary to the VH3 of the third polypeptide chain, or a polypeptide that contains such domains. The third or fourth polypeptide chains may be isolated from naturally occurring antibodies. Alternatively, they may be constructed recombinantly, synthetically or by other means.

[0270] The Light Chain Variable Domain of the first and second polypeptide chains are separated from the Heavy Chain Variable Domains of such polypeptide chains by an intervening spacer peptide having a length that is too short to permit their VL1/VH2 (or their VL2/VH1) domains to associate together to form epitope-binding site capable of binding to either the first or second epitope. A preferred intervening peptide linker (**Peptide Linker 1**) for this purpose has the sequence (**SEQ ID NO:508**): GGGSGGGG. Other Domains of the trivalent binding molecules may be separated by one or more intervening peptide linkers (Peptide Linkers), optionally comprising a cysteine residue. In particular, as provided above, such Peptide Linkers will typically be incorporated between Variable Domains (*i.e.*, VH or VL) and peptide Heterodimer-Promoting Domains (*e.g.*, an E-coil or K-coil) and between such peptide Heterodimer-Promoting Domains (*e.g.*, an E-coil or K-coil) and CH2-CH3 Domains. Exemplary peptide linkers (*e.g.*, **Peptide Linker 2**, **Peptide Linker 2-C**, **Peptide Linker 3**, **Spacer Linker 3**, *etc.*) useful for the generation of trivalent binding molecules are provided above. Such linkers are also provided in PCT Publication Nos. WO 2015/184207 and WO 2015/184203. Thus, the first and second polypeptide chains of such trivalent binding molecules associate together to form a VL1/VH1 binding site capable of binding a first

epitope, as well as a VL2/VH2 binding site that is capable of binding to a second epitope. The third and fourth polypeptide chains of such trivalent binding molecules associate together to form a VL3/VH3 binding site that is capable of binding to a third epitope.

[0271] As described above, the trivalent binding molecules of the present invention may comprise three polypeptides. Trivalent binding molecules comprising three polypeptide chains may be obtained by linking the domains of the fourth polypeptide N-terminal to the VH3-containing Domain of the third polypeptide (*e.g.*, using an intervening spacer peptide (**Peptide Linker 5**)). Alternatively, a third polypeptide chain of a trivalent binding molecule of the invention containing the following domains is utilized: (i) a VL3-containing Domain, (ii) a VH3-containing Domain, and (iii) a Domain containing a CH2-CH3 sequence, wherein the VL3 and VH3 are spaced apart from one another by an intervening spacer peptide that is sufficiently long (at least 9 or more amino acid residues) so as to allow the association of these domains to form an epitope-binding site. One preferred intervening spacer peptide for this purpose has the sequence: GGGGSGGGGSGGGGS (**SEQ ID NO: 526**).

[0272] It will be understood that the VL1/VH1, VL2/VH2, and VL3/VH3 Domains of such trivalent binding molecules may be different so as to permit binding that is monospecific, bispecific, or trispecific. In particular, the VL and VH Domains may be selected such that a trivalent binding molecule comprises two binding sites for a first epitope and one binding sites for a second epitope, or one binding site for a first epitope and two binding sites for a second epitope, or one binding site for a first epitope, one binding site for a second epitope and one binding site for a third epitope.

[0273] In one embodiment, these domains are selected so as to bind an epitope of HIV-1 *Env*, an epitope of second molecule, and an epitope of a third molecule, wherein the second molecule and the third molecule (*e.g.*, CD2, CD3, CD8, CD16, T-Cell Receptor (TCR), NKG2D, *etc.*) are present on the surface of an effector cell, such as a T lymphocyte, a natural killer (NK) cell or other mononuclear cell.

[0274] The general structure of the polypeptide chains of representative trivalent binding molecules of invention is provided in **Figures 104A-104F** and in **Table 3**:

Table 3		
Four Chain	2 nd Chain	NH ₂ -VL2-VH1-HPD-COOH

Table 3		
1 st Orientation	1 st Chain	NH ₂ -VL1-VH2-HPD-CH2-CH3-COOH
	3 rd Chain	NH ₂ -VH3-CH1-CH2-CH3-COOH
	2 nd Chain	NH ₂ -VL3-CL-COOH
Four Chain 2 nd Orientation	2 nd Chain	NH ₂ -VL2-VH1-HPD-COOH
	1 st Chain	NH ₂ -CH2-CH3-VL1-VH2-HPD-COOH
	3 rd Chain	NH ₂ -VH3-CH1-CH2-CH3-COOH
	2 nd Chain	NH ₂ -VL3-CL-COOH
Three Chain 1 st Orientation	2 nd Chain	NH ₂ -VL2-VH1-HPD-COOH
	1 st Chain	NH ₂ -VL1-VH2-HPD-CH2-CH3-COOH
	3 rd Chain	NH ₂ -VL3-VH3-HPD-CH2-CH3-COOH
Three Chain 2 nd Orientation	2 nd Chain	NH ₂ -VL2-VH1-HPD-COOH
	1 st Chain	NH ₂ -CH2-CH3-VL1-VH2-HPD-COOH
	3 rd Chain	NH ₂ -VL3-VH3-HPD-CH2-CH3-COOH

HPD = Heterodimer-Promoting Domain

[0275] One embodiment of the present invention relates to trivalent binding molecules that comprise two epitope-binding sites for HIV-1 *Env* and one epitope-binding site for a second molecule. The two epitope-binding sites for HIV-1 *Env* may bind the same epitope or different epitopes. Another embodiment of the present invention relates to trivalent binding molecules that comprise, one epitope-binding site for HIV-1 *Env* and two epitope-binding sites for a second molecule. The two epitope-binding sites for the second molecule may bind the same epitope or different epitopes of the second molecule. A further embodiment of the present invention relates to trispecific trivalent binding molecules that comprise, one epitope-binding site for HIV-1 *Env*, one epitope-binding site for a second molecule, and one epitope-binding site for a third molecule. In certain embodiments, the second molecule is a molecule (*e.g.*, CD2, CD3, CD8, CD16, T-Cell Receptor (TCR), NKG2D, *etc.*) present on the surface of an effector cell, such as a T lymphocyte, a natural killer (NK) cell or other mononuclear cell. In certain embodiments, the second molecule is CD3 and the third molecule is CD8. As provided above, such trivalent binding molecules may comprise three, four, five, or more polypeptide chains.

[0276] In one embodiment, these domains are selected so as to bind two epitopes of HIV-1 *Env*, which may be the same epitopes or different epitopes, and an epitope of second molecule, wherein the second molecule is a molecule (*e.g.*, CD2, CD3, CD8, CD16, T-Cell Receptor (TCR), NKG2D, *etc.*) present on the surface of an effector cell, such as a T lymphocyte, a natural killer (NK) cell or other mononuclear cell. In a specific embodiment, the two epitopes of HIV-1 *Env* are the same, and the second molecule is CD3. In an alternative embodiment, the two epitopes of HIV-1 *Env* are different, and the second molecule is CD3.

[0277] The molecules, and fragments thereof, described above can be formulated as a composition (*e.g.*, a pharmaceutical composition). Suitable compositions can comprise the molecules (or fragments thereof) in a pharmaceutically acceptable carrier *e.g.*, dissolved or dispersed in an aqueous medium, or lyophilized. The compositions can be sterile and can be in an injectable form (*e.g.* but not limited to a form suitable for intravenous injection, or intramuscular injection). The molecules (and fragments thereof) can also be formulated as a composition appropriate for topical administration to the skin or mucosa. Such compositions can take the form of liquids, ointments, creams, gels and pastes. The molecules (and fragments thereof) can also be formulated as a composition appropriate for intranasal administration. The molecules (and fragments thereof) can be formulated so as to be administered as a post-coital douche or with a condom. Standard formulation techniques can be used in preparing suitable compositions.

[0278] In certain embodiments the invention provides multispecific molecules such as but not limited to bispecific and trispecific molecules (*e.g.*, bispecific antibodies, bispecific diabodies, trivalent binding molecules, *etc.*) comprising the binding domains from human, humanized and/or chimeric antibodies. Methods to construct such antibodies are well known in the art.

[0279] In certain aspects the invention provides use of the multispecific molecules of the invention such as but not limited to bispecific and trispecific molecules (*e.g.*, bispecific antibodies, bispecific diabodies, trivalent binding molecules, *etc.*), in methods of treating and preventing HIV-1 infection in an individual, comprising administering to the individual a therapeutically effective amount of a composition comprising a multispecific molecule of the invention in a pharmaceutically acceptable form. In certain embodiment, the methods include a composition which includes more than one HIV-1 targeting multispecific molecule. In certain embodiments, the HIV-1 targeting multispecific molecule in such combination bind

different epitopes on the HIV-1 envelope. In certain embodiments, such combinations of multispecific molecule targeting more than one HIV-1 epitope provide increased killing of HIV-1 infected cells. In other embodiments, such combinations of multispecific molecule targeting more than one HIV-1 epitope provide increased breadth in recognition of different HIV-1 subtypes.

[0280] The various multispecific molecule described herein have utility, for example, in settings including but not limited to the following:

i) in the setting of anticipated known exposure to HIV-1 infection, the multispecific molecule described herein can be administered prophylactically (*e.g.*, IV, topically or intranasally) as a microbiocide,

ii) in the setting of known or suspected exposure, such as occurs in the setting of rape victims, or commercial sex workers, or in any homosexual or heterosexual transmission without condom protection, the multispecific molecule described herein can be administered as post-exposure prophylaxis, *e.g.*, IV or topically, and

iii) in the setting of Acute HIV-1 infection (AHI), the multispecific molecule described herein can be administered as a treatment for AHI to control the initial viral load, or for the elimination of virus-infected CD4 T cells.

[0281] In accordance with the invention, the multispecific molecules described herein can be administered prior to contact of the subject or the subject's immune system/cells with HIV-1 or within about 48 hours of such contact. Administration within this time frame can maximize inhibition of infection of vulnerable cells of the subject with HIV-1.

[0282] In addition, various forms of the multispecific molecules described herein can be administered to chronically or acutely infected HIV-1 patients and used to kill remaining virus infected cells by virtue of these multispecific molecule binding to the surface of virus infected cells and being able to mediate redirected cell killing of such infected cells.

[0283] In certain embodiments, the multispecific molecules of the invention can be administered in combination with latency activating agents, so as to activate latent reservoir of HIV-1-infected cells. The expectation is that by activating latent proviral HIV-1 DNA in resting cells, once inactive cells will start producing new virus and they will be recognized and eliminated by the immune system. Non-limiting examples of latency activating agents are HDAC inhibitors, *e.g.*, vorinostat, romidepsin, panobinostat, disulfiram, JQ1, bryostatin,

PMA, inonomeycin, or any combination thereof. See Bullen *et al.* Nature Medicine 20, 425–429 (2014).

[0284] In certain embodiments the multispecific molecules of the invention can be administered in combination with anti-retroviral agents.

[0285] Suitable dose ranges can depend on the multispecific molecule and on the nature of the formulation and route of administration. Optimum doses can be determined by one skilled in the art without undue experimentation. For example, doses of antibodies in the range of 1-50 mg/kg of unlabeled or labeled antibody (with toxins or radioactive moieties) can be used. If antibody fragments, with or without toxins are used or antibodies are used that can be targeted to specific CD4 infected T cells, then less antibody can be used (*e.g.*, from 5 mg/kg to 0.01 mg/kg). If multispecific molecules are used, doses in the range of 0.01 µg/kg to about 30 mg/kg or more of the subject's body weight can be used. Suitable dose ranges can depend on the antibody (or fragment, or multispecific molecule) and on the nature of the formulation and route of administration. Optimum doses can be determined by one skilled in the art without undue experimentation. For example but not limited, doses of multispecific molecules in the range of 0.01-100 µg/kg, 0.1-50 mg/kg, 1-50 mg/kg, 1-10 mg/kg, 1, 1.25, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 mg/kg of unlabeled or labeled multispecific molecule (with toxins or radioactive moieties) can be used. If antibody fragments, with or without toxins are used or antibodies are used that can be targeted to specific CD4 infected T cells, then less antibody can be used (*e.g.*, from 5 mg/kg to 0.01 mg/kg). In other embodiments, the molecules of the invention can be administered at a suitable fixed dose, regardless of body size or weight. See Bai *et al.* Clinical Pharmacokinetics February 2012, Volume 51, Issue 2, pp 119-135.

[0286] Multispecific molecules of the invention can be produced recombinantly using nucleic acids comprising nucleotide sequences encoding VH and VL sequences selected from those shown in the figures and examples, or those known in the art.

[0287] In certain embodiments the invention provides multispecific binding molecules comprising antigen binding fragments. Typically, multispecific binding molecules compete with the intact antibody from which they were derived for specific binding to the target including separate heavy chains, light chains Fab, Fab', F(ab').sub.2, F(ab)c, diabodies, Dabs,

nanobodies, and Fv. Fragments that can be produced by recombinant DNA techniques, or by enzymatic or chemical separation of intact immunoglobulins.

[0288] Nucleic acid sequences encoding polypeptides for the production of multispecific molecules with specificities as described herein can be used to produce plasmids for stable expression of such multispecific molecules. Methods for recombinant expression and purification are known in the art. In certain embodiments of Fc bearing multispecific molecules, the plasmids also comprise any of the changes to the Fc portion described herein.

[0289] In certain embodiments, the nucleic acids are optimized for recombinant expression in a suitable host cell. In certain embodiments, the vector is suitable for gene delivery and expression. There are numerous expression systems available for expression of proteins including *E. coli*, other bacterial hosts, yeast, and various higher eukaryotic cells such as the COS, CHO, HeLa and myeloma cell lines.

[0290] Any suitable cell line can be used for expression of the polypeptides of the invention, including but not limited to CHO cells, 293T cells. In some aspects, the invention provides nucleic acids encoding these antibodies, expression cassettes and vectors including these nucleic acids, and isolated cells that express the nucleic acids which encode the multispecific molecules of the invention are also provided. The polypeptides of the invention can be purified by any suitable method for purification of polypeptides and/or antibodies.

[0291] Table 4. Summary listing of various sequences listed throughout the specification; starting at SEQ ID NO: 500

SEQ ID NO:	Brief Desc.	Sequence
500	hCD3 VH (hXR32VH D65G) (amino acid sequence)	EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPGK GLEWVGRIRSKYNNYATYYADSVKGRFTISRDDSKNSLYLQMN SLKTEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLLTVSS

501	hCD3 VH (hXR32VH D65G) (nucleotide sequence)	gaggtgcagctggtggagtctggggaggcttggtccagcctg gagggccctgagactctcctgtgcagcctctggattcacctt cagcacatacgtatgaattgggtccgccaggctccaggggaag gggctggagtgggttggaaggatcaggtccaagtacaacaatt atgcaacctactatgccgactctgtgaagggtagattcaccat ctcaagagatgattcaaagaactcactgtatctgcaaatgaac agcctgaaaaccgaggacacggccgtgtattactgtgtgagac acggtaacttcggcaattcttacgtgtcttgggttgcttattg gggacaggggacactggtgactgtgtcttcc
502	hCD3 VL (hXR32VL) (amino acid sequence)	QAVVTQEPSLTVSPGGTVTLTCRSSTGAVTTSNYANWVQKPG QAPRGLIGGTNKRAPWTPARFSGSLLGGKAALTITGAQAEDEA DYICALWYSNLWVFGGGTKLTVLG
503	hCD3 VL (hXR32VL) (nucleotide sequence)	caggctgtggtgactcaggagccttcactgaccgtgtccccag gcggaactgtgaccctgacatgcagatccagcacaggcgcagt gaccacatctaactacgccaattgggtgcagcagaagccagga caggcaccaaggggctgatcgggggtacaaacaaaagggtc cctggaccctgcacgggttttctggaagtctgctgggcggaaa ggccgctctgactattaccggggcacaggccgaggacgaagcc gattactattgtgctctgtggtatagcaatctgtgggtgttcg ggggtggcacaaaactgactgtgctgggc
504	hCD16 VH (h3G8VH5) (amino acid sequence)	QVTLRSEGPALVKPTQTLTLTCTFSGFSLSTSGMGVGVIRQPP GKALEWLAHIWDDDKRYNPALKSRLTISKDTSKNQVLTMTN MDPVDTATYYCAQINPAWFAYWGQGLVTVSS
505	hCD16 VH (h3G8VH5) (nucleotide sequence)	caggttaccctgagagagtctggccctgcgctggtgaagcca cacagaccctcacactgacttgtagcttctctgggttttact gagcacttctggtatgggtgtaggctggattcgtcagcctccc gggaaggctctagagtggctggcacacatttggtgggatgatg acaagcgctataatccagccctgaagagccgactgacaatctc caaggatacctccaaaaaccaggtagtcctcacaatgaccaac atggaccctgtggatactgccacatactactgtgctcaaataa accccgctgggttgcttactggggccaagggactctggtcac tgtgagctccgg

506	hCD16 VL (h3G8VL1) (amino acid sequence)	DIVMTQSPDSLAVSLGERATINCKASQSVDFDGDSEFMNWIYQQK PGQPPKLLIYTTNLESGVPDRFSGSGSGTDFTLTITSSLAED VAVYYCQQSNEDPYTFGGQGTKLEIK
507	hCD16 VL (h3G8VL1) (nucleotide sequence)	atgggtgacaatgacatccactttgcctttctctccacaggtg tccactccgacatcgtgatgaccaatctccagactctttggc tgtgtctctaggggagagggccaccatcaactgcaaggccagc caaagtgttgattttgatgggtgatagttttatgaactggtacc aacagaaaccaggacagccacccaaactcctcatctatactac atccaatctagaatctgggggtcccagacaggttttagtggcagt gggtctgggacagacttcaccctcaccatcagcagcctgcagg ctgaggatgtggcagttttattactgtcagcaaagtaataaga tccgtacacgttcggacaggggaccaagcttgagatcaaa
508	Peptide Linker 1	GGGSGGGG
509	Peptide Linker 2-C	GGCGGG
510	Peptide Linker 2	ASTKG
511	HPD 1	GVEPKSC
512	HPD 2	VEPKSC
513	HPD 3	GFNRGEC
514	HPD 4	FNRGEC
515	IgG CH1-hinge Domain	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTWNS GALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVN HKPSNTKVDKRVEPKSCDKTHTCPPCP
516	Ig CL Kappa Domain	RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKV DNALQSGNSQESVTEQDSKSTYSLSTLTLSKADYEKHKVYA CEVTHQGLSPVTKSFNRGEC
517	Ig CL Lambda Domain	QPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKA DSSPVKAGVETTPSKQSNKYAASSYLSLTPEQWKSHRYSYSCQ VTHEGSTVEKTVAPTECS
518	HPD 5: E-coil	EVAALEKEVAALEKEVAALEKEVAALEK

519	HPD 6: E-coil-C	EVAACEKEVAALEKEVAALEKEVAALEK
520	HPD 7: K-coil	KVAALKEKVAALKEKVAALKEKVAALKE
521	HPD 8: K-coil-C	KVAACKEKVAALKEKVAALKEKVAALKE
522	Spacer Linker 3	GGGDKTHTCPPCP
523	Peptide Linker 3	DKTHTCPPCP
524	Peptide Linker 4	APSSS
525	Peptide Linker 4.1	APSSSPME
526	Peptide Linker 5	GGGGSGGGSGGGGS
527	IgG1 Fc WT	<p>APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEV KFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMT KNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDG SFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSP <u>G</u><u>X</u></p> <p>wherein, X is a lysine (K) or is absent</p>
528	IgG1 Fc (AA)	<p>APE<u>AA</u>GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEV KFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMT KNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDG SFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSP <u>G</u><u>X</u></p> <p>wherein, X is a lysine (K) or is absent</p>
529	IgG1 Fc YTE	<p>APELLGGPSVFLFPPKPKDTL<u>Y</u><u>I</u><u>T</u><u>R</u><u>E</u>PEVTCVVVDVSHEDPEV KFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMT KNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDG SFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSP <u>G</u><u>X</u></p> <p>wherein, X is a lysine (K) or is absent</p>

530	IgG1 Fc (AA/YTE)	<p>APEA<u>AA</u>GGPSVFLFPPKPKDTL<u>YI</u><u>TR</u><u>E</u>PEVTCVVVDVSHEDPEV KFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMT KNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDG SFFFLYSKLTVDKSRWQQGNVFSVMSHEALHNHYTQKSLSLSP G<u>X</u></p> <p>wherein, X is a lysine (K) or is absent</p>
531	IgG1 Fc Knob (AA)	<p>APEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEV KFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMT KNQVSL<u>W</u>CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDG SFFFLYSKLTVDKSRWQQGNVFSVMSHEALHNHYTQKSLSLSP G<u>X</u></p> <p>wherein, X is a lysine (K) or is absent</p>
532	IgG1 Fc Knob (AA/YTE)	<p>APEAAGGPSVFLFPPKPKDTL<u>YI</u><u>TR</u><u>E</u>PEVTCVVVDVSHEDPEV KFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMT KNQVSL<u>W</u>CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDG SFFFLYSKLTVDKSRWQQGNVFSVMSHEALHNHYTQKSLSLSP G<u>X</u></p> <p>wherein, X is a lysine (K) or is absent</p>
533	IgG1 Fc Hole (AA/R)	<p>APEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEV KFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMT KNQVSL<u>SCA</u>VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDG SFFFL<u>V</u>SKLTVDKSRWQQGNVFSVMSHEALHN<u>R</u>YTQKSLSLSP G<u>X</u></p> <p>wherein, X is a lysine (K) or is absent</p>
534	IgG1 Fc Hole (AA/R/YTE)	<p>APEAAGGPSVFLFPPKPKDTL<u>YI</u><u>TR</u><u>E</u>PEVTCVVVDVSHEDPEV KFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMT KNQVSL<u>SCA</u>VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDG SFFFL<u>V</u>SKLTVDKSRWQQGNVFSVMSHEALHN<u>R</u>YTQKSLSLSP G<u>X</u></p> <p>wherein, X is a lysine (K) or is absent</p>
535	Pali VH (amino acid sequence)	<p>QVTLRESGPALVKPTQTLTLTCTFSGFSLSTSGMSVGWIRQPP GKALEWLADIWDDKDYNP SLKSR LTISKDTSKNQVVLKVTN MDPADTATYYCARSMTNWFVDVWGAGTTTVTVSS</p>

536	Pali VH (nucleotide sequence)	caggttacacttagagaatctggctcctgctcttgtgaagccta ctcagactcttactcttacctgcaccttctctggtttcagcct ttctacttctggatgtctgtgggttggttagacaacctcct ggtaaggctcttgaatggcttgctgatatttgggtgggatgata agaaggattacaacccaagcctgaagtctaggctgaccatttc taaggataccagcaagaatcagggtggtgcttaagggtgaccaat atggaccctgctgatactgctacttactactgcgctagatcca tgatcaccaactggctactttgatgtttggggtgctggtactac tgtgaccgtttcttcc
537	Pali VL (amino acid sequence)	DIQMTQSPSTLSASVGDRVTITCRASQSVGYMHYQQKPGKAP KLLIYDTSKLASGVPSRFSGSGSGTEFTLTISSLQPDFATYY CFQGSQGYPTFGGGTKLEIK
538	Pali VL (nucleotide sequence)	gatattcagatgactcagtctccttctaccctgtctgcttctg ttggtgatagggttaccattacatgcagggttctcaatctgt gggttacatgcactggtaccaacaaaagcctggtaaggctcct aagctgcttatctacgatacctctaagcttgcttctggtgtgc cttctagggttttctggttctggatctggtactgagttcacct taccatttctagccttcagcctgatgatttcgctacctactat tgctttcagggtagcggttaccctttcacttttgggtggtggta ctaagcttgagattaag
539	Anti-FITC (4420) VH (amino acid sequence)	EVKLDDETGGG LVQFGRPMKL SCVASGFTEF DYWMNWVRQS PEKGLEWVAQ IRNKPYNYET YYSDSVKGRF TISRDDSKSS VYLQMNNLRV EDMGIYYCTG SYYGMDYWQ GTSVTVSS
540	Anti-FITC (4420) VH (nucleotide sequence)	gagggtgaagctggatgagactggaggaggcttggtgcaacctg ggaggcccatgaaactctcctgtgttgctctggttaccattt tagtgactactggatgaactgggtccgccagtctccagagaaa ggactggagtgggttagcacaattagaaacaaaccttataatt atgaaacatattattcagattctgtgaaaggcagattcaccat ctcaagagatgattccaaaagtagtgtctacctgcaaatgaac aacttaagagttgaagacatgggtatctattactgtacgggtt cttactatggtatggactactgggtcaaggaaacctcagtcac cgtctcatcc
541	Anti-FITC (4420) VL (amino acid sequence)	DVVMTQTPFS LPVSLGDQAS ISCRSSQSLV HSNNTYLRW YLQKPGQSPK VLIYKVSNRFS GVPDRFSGS GSGTDFTLKI SRVEAEDLGV YFCSQSTHVP WTFGGGTKLE IK

542	Anti-FITC (4420) VL (nucleotide sequence)	gatgtcgtgatgacccaaactccattctccctgcctgtcagtc ttggagatcaagcctccatctcttgcagatctagtcagagcct tgtacacagtaatggaaacacctatttacgttgggtacctgcag aagccaggccagtcctcaaaggctcctgatctacaaagtttcca accgattttctgggggtcccagacagggttcagtggcagtggatc agggacagatttcacactcaagatcagcagagtggaggctgag gatctgggagttttatttctgctctcaaagtacacatgttccgt ggacgttcggtggaggcaccaagctggaaatcaaa
543	OKT8 VH (CD8 VH) (amino acid sequence)	QVQLLESQPELLKPGASVKMSCKASGYTFTDYNMHWVKQSHGK SLEWIGYIYPYTGGTGYNQKFKNKATLTVDSSSSSTAYMELRSL TSEDSAVYYCARNFRYTYWYFDVWGQGTTVTVSS
544	OKT8 VH (CD8 VH) (nucleotide sequence)	cagggtgcagctggttgagctctggacctgagctggtgaaacctg gggcctcagtgaagatgtcctgcaaggcttctggatacacatt cactgactacaacatgcactgggtgaagcagagccatggaaag agccttgagtggattggatatatttatccttacactgggtggca ctggctacaaccagaagttcaagaacaaggccacattgactgt agacagttcctccagcacagcctacatggagctccgcagcctg acatctgaggactctgcagtctattactgtgcaaggaacttta ggtacacctactggtacttcgatgtctggggccaaggcaccac ggtcaccgtctcctca
545	OKT8 VL (CD8 VL) (amino acid sequence)	DIVMTQSPASLAVSLGQRATISCRASESVDSYDNSLMHWYQQK PGQPPKVLIIYLASNLESGVPARFSGSGSRTDFTLTIDPVEADD AATYYCQNNEDPYTFGGGTKLEIK
546	OKT8 VL (CD8 VL) (nucleotide sequence)	gacatcgtgatgaccagtcctccagcttctttggctgtgtctc tagggcagagggccaccatatacctgcagagccagtgaagtgt tgatagttatgacaatagtcttatgcactgggtaccagcagaaa ccaggacagccacccaaagtcctcatctatcttgcacccaacc tagaatctgggggtccctgcccagggttcagtggcagtgggtctag gacagacttcaccctcaccattgatcctgtggaggctgatgat gctgcaacctattactgtcagcaaaaataatgaggatccgtaca cgttcggggggggggaccaagctggagatcaaacgt

547	TRX2 VH (CD8 VH) (amino acid sequence)	QVQLVESGGGVVQPGRSRLRLSCAASGFTFSDFGMNWRQAPGK GLEWVAL IYYDGSNKFYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAV YYCAKPHYDGYHYHFFDSWGQGT LVTVSS
548	TRX2 VH (CD8 VH) (nucleotide sequence)	caggttcaattggtggagtctggaggaggcgttgtagcagcctg gaaggtccctgagactctcatgtgcagcttctggattcacttt cagtgacttttggcatgaactgggttcgacaggctcccgggaag gggctggaatgggtggcactgatttactatgatggtagtaaca agttctatgcagactctgtgaagggtcgattcaccatctccag ggacaattctaagaacaccctatacctgcaaataaacagcctg agagctgaggacacagccgtgtattactgtgcaaaacccact atgatggttattatcacttctttgattcctggggccaaggga actagtcacagtctcctca
549	TRX2 VL (CD8 VL) (amino acid sequence)	DIQMTQSPSSLSASVGDRVTITCKGSQDINNYLAWYQQKPGKA PKLLIYNTDILHTGVPSRFSGSGSGTDFTFTISSLQPEDATY YCYQYNNGYTFGQGTKVEIK
550	TRX2 VL (CD8 VL) (nucleotide sequence)	gacatccagatgacccagagcccaagcagcctgagcgccagcg tggttgacagagtgaccatcacctgtaaaggaagtcaggatat taacaattacttagcctggtaccagcagaagccaggtaaggct ccaaagctgctgatctacaatacagacatttttgacacgggtg tgccaagcagattcagcggttagcggttagcggtaccgacttcac cttcaccatcagcagcctccagccagaggacatcgccacctac tactgctatcagtataacaacgggtacacgttcggccaaggga ccaaggtggaaatcaaa
551	DH511.2_K3 VH (amino acid sequence)	See Figure 13 (DH512VH) QVQLVQSGGGLVKPGGSLTLSCSASGFFDNSWMGWVRQAPGK GLEWVGRIRRLKDGATGEYGA AVKDRFTISRDDSRNMLYLHMR TLKTEDSGTYICTMDEGTPVTRFLEWGYFY YMAVWGRGTTVI VSS
552	DH511.2_K3 VH (exemplary nucleotide sequence)	See Figure 12 (DH512VH) for one embodiment (SEQ ID NO:17)

553	DH511.2_K3 VL (amino acid sequence)	See Figure 57 DH511-2AVK DIQMTQSPSFLYGSVGDRVITITCRASQNIKDYLNWYQQRPGRA PRLLIYAASNLSQGVPSRFSGSGYGTDFTLIISSSLQPEDFATY FCQESYSSTPTHIFGLGTKLEKK
554	DH511.2_K3 VL (exemplary nucleotide sequence)	See Figure 57 for one embodiment (SEQ ID NO: 275)
555	Exemplary DH511.2_K3 xCD3 Fc CHAIN 1 (amino acid sequence)	DIQMTQSPSFLYGSVGDRVITITCRASQNIKDYLNWYQQRPGRA PRLLIYAASNLSQGVPSRFSGSGYGTDFTLIISSSLQPEDFATY FCQESYSSTPTHIFGLGTKLEKKGGGSGGGGEVQLVESGGGLV QPGGSLRLSCAASGFTFSTYAMNWVRQAPGKGLEWVGRIRSKY NNYATYYADSVKGRFTISRDDSKNSLYLQMNSLKTEDTAVYYC VRHGNFGNSYVSWFAYWGQGLVTVSSASTKGEVAACEKEVAA LEKEVAALEKEVAALEKGGGDKTHTCPPCPAPEAAGGPSVFLF PPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHN AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP APIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLWCLVKGF YPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKS RWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

556	<p>Exemplary DH511.2_K3 xCD3 Fc</p> <p>CHAIN 1 (nucleotide sequence)</p>	<p>gacatccagatgaccagtgccccctccttctgtacgggtccg tgggcgaccgcgtgaccatcacctgccgcgcctcccagaacat caaggactacctgaactggtagcagcagcgcgcgcgcgcgc ccccgcctgctgatctacgcgcgcctccaacctgcagtcggcg tgccctcccgcttctccggctccggctacggcaccgacttcac cctgatcatctcctccctgcagcccgaggacttcgccacctac ttctgccaggagtcctactcctccacccccaccacatcttcg gcctgggacccaagctggagaagaagggtggaggatccggcgg cggaggcgagggtgcagctgggtggagtctgggggaggcttggtc cagcctggagggtccctgagactctcctgtgcagcctctggat tcaccttcagcacatacgtatgaattgggtccgccaggctcc aggggaaggggctggagtgggttggaaggatcaggtccaagtac aacaattatgcaacctactatgccgactctgtgaagggtagat tcaccatctcaagagatgattcaaagaactcactgtatctgca aatgaacagcctgaaaaccgaggacacggcgcgtgtattactgt gtgagacacggtaacttcggcaattcttacgtgtcttggtttg cttattggggacaggggacactgggtgactgtgtcttcgcctc caccaagggcgaagtggccgatgtgagaaagagggttgctgct ttggagaaggaggtcgctgcacttgaaaaggaggtcgagccc tgagagaaggcggcggggacaaaactcacacatgccaccgtg cccagcacctgaagccgcggggggaccgtcagtccttcctcttc ccccaaaacccaaggacacctcatgatctcccgacccttg aggtcacatgcgtgggtgggtggacgtgagccacgaagacctga gggtcaagttcaactggtacgtggacggcgtggagggtgcataat gccaaagacaaagccgcgggaggagcagtacaacagcacgtacc gtgtgggtcagcgtcctcacctgcctgcaccaggactggctgaa tggcaaggagtacaagtgaagggtctccaacaaagccctcca gcccccatcgagaaaaccatctccaaagccaaagggcagcccc gagaaccacaggtgtacacctgcccccatcccgggaggagat gaccaagaaccaggtcagcctgtggtgcctgggtcaaaggcttc tatcccagcgacatcgccgtggagtgggagagcaatgggcagc cggagaacaactacaagaccacgcctcccgtgctggactccga cggctccttcttctctacagcaagctcacctggacaagagc aggtggcagcaggggaacgtcttctcatgctccgtgatgcatg aggctctgcacaaccactacacgcagaagagcctctcctgtc tccgggtaaa</p>
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557	Exemplary DH511.2_K3 xCD3 Fc CHAIN 2 (amino acid sequence)	QAVVTQEPSTLVSPGGTVTLTCRSSTGAVTTSNYANWVQKPG QAPRGLIGGTNKRAPWTPARFSGSLLGGKAALTITGAQAEDEA DYICALWYSNLWVFGGKTCLTVLGGGSGGGGQVQLVQSGGGL VKPGGSLTLSCSASGFFFDNSWMGWVRQAPGKLEWVGRIIRRL KDGATGEYGAAVKDRFTISRDDSRNMLYLHMRTLKTEDSGTYY CTMDEGTPVTRFLEWGYFYYYMAVWGRGTTVIVSSASTKGKVA ACKEKVAALKEKVAALKEKVAALKE
558	Exemplary DH511.2_K3 xCD3 Fc CHAIN 2 (nucleotide sequence)	caggctgtggtgactcaggagccttcactgaccgtgtccccag gcggaactgtgaccctgacatgcagatccagcacaggcgagcgt gaccacatctaactacgccaattgggtgcagcagaagccagga caggcaccaaggggcctgatcggggtacaaacaaaagggtc cctggaccctgcacggttttctggaagtctgctgggcgaaa ggccgctctgactattaccggggcacaggccgaggacgaagcc gattactattgtgctctgtggtatagcaatctgtgggtgttcg ggggtggcacaaaactgactgtgctgggaggggtggatccgg cggaggtggacaggtgcagctggtgcagtccggcgggcgctg gtgaagcccggcggtccctgaccctgtcctgctccgcctccg gcttcttcttcgacaactcctggatgggtggtgcccaggc cccggcaagggcctggagtgggtgggcccgcacccgcctg aaggacggcgccaccggcgagtacggcgccgcctgaaggacc gcttcaccatctcccgcgacgactcccgcaacatgctgtacct gcacatgcgaccctgaagaccgaggactccggcacctactac tgcaccatggacgagggcaccctcgtagcccgcttcctggagt ggggtacttctactactacatggccgtgtggggccgcggcac caccgtgatcgtgtcctcccagggtccgactagcccaatatggt ggtgggtgaagaggctaggggccacaatgacccttctctgcg tggcatctggatacaccttcaacgactactacatacattgggt gcggcaggccccctggacaaggctttgagttgttgggatacatc gaccccgctaattggtcgcccagactacgcagggcggttgagg agagactctccttctacagggacaagtccatggagacgctgta catggacctgaggagcctaagatatgacgacacggccatgtat tattgtgttagaaatgtggggaccgctggcagcttgctgcatt atgaccactggggctcggaagcccgtcatcgtctcctccgc ctccaccaagggcaaagtggccgcagtaaggagaaagttgct gctttgaaagagaaggtcgccgcacttaaggaaaaggtcgag ccctgaaagag

559	Exemplary CHAIN 3 (amino acid sequence) for a DH511.2_K3 xCD3 Fc design	DKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVV VDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVL TVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVY TLPPSREEMTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYK TTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSQVMHEALHNR YTQKSLSLSPGK
560	Exemplary CHAIN 3 (nucleotide sequence) for DH511.2_K3 xCD3 Fc design	gacaaaactcacacatgccaccgtgccagcacctgaagccg cggggggaccgtcagtccttccctcttcccccaaaacccaagga caccctcatgatctcccgaccctgaggtcacatgctgtggtg gtggacgtgagccacgaagaccctgaggtcaagttcaactggt acgtggacggcggtggaggtgcataatgccaagacaaagccgcg ggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctc accgtcctgcaccaggactggctgaatggcaaggagtacaagt gcaaggtctccaacaaagccctcccagccccatcgagaaaac catctcaaagccaaagggcagccccgagaaccacaggtgtac accctgcccccatcccgggaggagatgaccaagaaccaggtca gcctgagttgcgcagtc aaaggcttctatcccagcgacatcgc cgtggagtgggagagcaatgggcagccggagaacaactacaag accacgcctcccgtgctggactccgacggctccttcttccctcg tcagcaagctcaccgtggacaagagcaggtggcagcaggggaa cgtcttctcatgctccgtgatgcatgaggctctgcacaaccgc tacacgcagaagagcctctccctgtctccgggtaaa
561	Chain 3 of exemplary four chain trivalent (HIVxCD3xCD 8) binding molecule (amino acid sequence)	EVQLQQSGAELVKPGASVKLSCTASGFNIKDTYIHFVRQRPEQ GLEWIGRIDPANDNTLYASKFQGKATITADTSSNTAYMHLCSL TSGDTAVYYCGRGYGYVFDHWGQGTTTLTVSSASTKGPSVFPL APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFP AVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKR VEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPE VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTY RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE PQVYTLPPSREEMTKNQVSLSCAVKGFYPSDIAVEWESNGQP PENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSQVMH EALHNRYTQKSLSLSPGK

562	Chain 4 of exemplary four chain trivalent (HIV _x CD3 _x CD8) binding molecule (amino acid sequence)	DVQINQSPSFLAASPGETITINCRTSRISISQYLAWYQEKPGKT NKLLIYSGSTLQSGIPSRFSGSGSGTDFTLTISGLEPEDFAMY YCQQHNNENPLTFGAGTKLELRRTVAAPSVFIFPPSDEQLKSGT ASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDST YSLSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
563	Chain 3 of exemplary three chain trivalent (HIV _x CD3 _x CD8) binding molecule (amino acid sequence)	DVQINQSPSFLAASPGETITINCRTSRISISQYLAWYQEKPGKT NKLLIYSGSTLQSGIPSRFSGSGSGTDFTLTISGLEPEDFAMY YCQQHNNENPLTFGAGTKLELRGGGSGGGGSGGGGSEVQLQQS GAELVKPGASVKLSCTASGFNIKDTYIHFVRQRPEQGLEWIGR IDPANDNTLYASKFQGKATITADTSSNTAYMHLCSLTSGDTAV YYCGRGYGYVFDHWGQGTTTLTVSSASTKGPSVFPLAPSSKST SGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG LYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCD KTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVV DVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLT VLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVY TPPSREEMTKNQVSLTSCAVKGFPYSDIAVEWESNGQPENNYKT TPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSQSVMEALHNRY TQKSLSLSPGK
564	A32 VH (amino acid sequence)	QVQLQESGPGLVKPSQTLTSLCTVSGGSSSSGAHYWSWIRQYP GKGLEWIGYIHYSGNTYYNPSLKSRLTISQHTSENQFSLKLNS VTVADTAVYYCARGTRLRLRNAFDIWGQGTTLTVSS
565	A32 VH (nucleotide sequence)	caggtgcagctgcaggagtcgccccggactggtcaaaccct ctcagactctgtctctgtcatgtaccgtgtcaggcggctcttc cagctccggggcacactactggagctggatcaggcagtatccc ggcaaggggctggagtgatcggtacattcattatagcggca acacatactataatccttctctgaagagtcggatcactatttc acagcacaccagcgaaaaccagttcagcctgaagctgaacagc gtgaccgtcgccgacacagccgtgtactattgcgccccgggca ccagactgagaactctgagaaacgcatttgacatctggggaca ggggacactggtgacagtgcctcc
566	A32 VL (amino acid sequence)	QSALTQPPSASGSPGQSVTISCTGTSSDVGGYNYVSWYQHHPG KAPKLIISEVNNRPSGVPDRFSGSKSGNTASLTVSGLQAEDEA EYYCSSYTDIHNFFVFGGKLTVL

567	A32 VL (nucleotide sequence)	cagagcgcactgactcagcccccttccgcctccgggtctcctg gacagagcgtgacaatctcatgcactgggacttcaagcgatgt gggcgggtacaactatgtgagttggtaccagcaccatcccggg aaggcacctaaactgatcattagcgaagtgaacaatcgaccaa gcggcgtccccgaccggttcagcggcagcaagtctggcaatac cgccagtctgacagtctcaggcctgcaggccgaggatgaagct gagtactattgctcatcacactgacatccataacttcgtct tcggcggcggaactaaactgaccgtgctg
568	DH511 VH (amino acid sequence)	See Figure 13 (SEQ ID NO: 35)
569	DH511 VH (nucleotide sequence)	See Figure 12 (SEQ ID NO:15)
570	DH511 VL (amino acid sequence)	See Figure 13 (SEQ ID NO: 36)
571	DH511 VL (nucleotide sequence)	See Figure 12 (SEQ ID NO: 16)
572	DH512 VH (amino acid sequence)	See Figure 13 (SEQ ID NO: 37; same as SEQ ID NO: 551)
573	DH512 VH (nucleotide sequence)	See Figure 12 (SEQ ID NO: 17)
574	DH512 VL (amino acid sequence)	See Figure 13 (SEQ ID NO: 38)
575	DH512 VL (nucleotide sequence)	See Figure 12 (SEQ ID NO: 18)
576	DH513 VH (amino acid sequence)	See Figure 13 (SEQ ID NO: 39)

577	DH513 VH (nucleotide sequence)	See Figure 12 (SEQ ID NO: 19)
578	DH513 VL (amino acid sequence)	See Figure 13 (SEQ ID NO: 40)
579	DH513 VL (nucleotide sequence)	See Figure 12 (SEQ ID NO: 20)
580	DH514 VH (amino acid sequence)	See Figure 13 (SEQ ID NO: 41)
581	DH514 VH (nucleotide sequence)	See Figure 12 (SEQ ID NO: 21)
582	DH514 VL (amino acid sequence)	See Figure 13 (SEQ ID NO: 42)
583	DH514 VL (nucleotide sequence)	See Figure 12 (SEQ ID NO: 22)
584	DH515 VH (amino acid sequence)	See Figure 13 (SEQ ID NO: 43)
585	DH515 VH (nucleotide sequence)	See Figure 12 (SEQ ID NO: 23)
586	DH515 VL (amino acid sequence)	See Figure 13 (SEQ ID NO: 44)
587	DH515 VL (nucleotide sequence)	See Figure 12 (SEQ ID NO: 24)

588	DH516 VH (amino acid sequence)	See Figure 13 (SEQ ID NO: 45)
589	DH516 VH (nucleotide sequence)	See Figure 12 (SEQ ID NO: 25)
590	DH516 VL (amino acid sequence)	See Figure 13 (SEQ ID NO: 46)
591	DH516 VL (nucleotide sequence)	See Figure 12 (SEQ ID NO: 26)
592	DH517 VH (amino acid sequence)	See Figure 13 (SEQ ID NO: 47)
593	DH517 VH (nucleotide sequence)	See Figure 12 (SEQ ID NO: 27)
594	DH517 VL (amino acid sequence)	See Figure 13 (SEQ ID NO: 48)
595	DH517 VL (nucleotide sequence)	See Figure 12 (SEQ ID NO: 28)
596	DH518 VH (amino acid sequence)	See Figure 13 (SEQ ID NO: 49)
597	DH518 VH (nucleotide sequence)	See Figure 12 (SEQ ID NO: 29)
598	DH518 VL (amino acid sequence)	See Figure 13 (SEQ ID NO: 51)

599	DH518 VL (nucleotide sequence)	See Figure 12 (SEQ ID NO: 30)
600	DH536 VH (amino acid sequence)	See Figure 13 (SEQ ID NO: 52)
601	DH536 VH (nucleotide sequence)	See Figure 12 (SEQ ID NO: 31)
602	DH536 VL (amino acid sequence)	See Figure 13 (SEQ ID NO: 53)
603	DH536 VL (nucleotide sequence)	See Figure 12 (SEQ ID NO: 32)
604	DH537 VH (amino acid sequence)	See Figure 13 (SEQ ID NO: 54)
605	DH537 VH (nucleotide sequence)	See Figure 12 (SEQ ID NO: 33)
606	DH537 VL (amino acid sequence)	See Figure 13 (SEQ ID NO: 55)
607	DH537 VL (nucleotide sequence)	See Figure 12 (SEQ ID NO: 34)
608	DH512_E96W	See Figure 33 for DH512 VH mutants (SEQ ID NO: 201)
609	DH512_G97W	See Figure 33 for DH512 VH mutants (SEQ ID NO: 202)
610	DH512_T98W	See Figure 33 for DH512 VH mutants (SEQ ID NO: 203)
611	DH512_P99W	See Figure 33 for DH512 VH mutants (SEQ ID NO: 204)
612	DH512_V100F	See Figure 33 for DH512 VH mutants (SEQ ID NO: 205)

613	DH512_V100I	See Figure 33 for DH512 VH mutants (SEQ ID NO: 206)
614	DH512_T100a W	See Figure 33 for DH512 VH mutants (SEQ ID NO: 207)
615	DH512_R100b W	See Figure 33 for DH512 VH mutants (SEQ ID NO: 208)
616	DH512_F100c W	See Figure 33 for DH512 VH mutants (SEQ ID NO: 209)
617	DH512_L100d W	See Figure 33 for DH512 VH mutants (SEQ ID NO: 210)
618	DH512_L100d F	See Figure 33 for DH512 VH mutants (SEQ ID NO: 211)
619	DH512_E100e W	See Figure 33 for DH512 VH mutants (SEQ ID NO: 212)
620	DH512_G100g W	See Figure 33 for DH512 VH mutants (SEQ ID NO: 213)
621	DH512_Y100h W	See Figure 33 for DH512 VH mutants (SEQ ID NO: 214)
622	DH512_F100i W	See Figure 33 for DH512 VH mutants (SEQ ID NO: 215)
623	DH512_Y100k W	See Figure 33 for DH512 VH mutants (SEQ ID NO: 216)
624	DH511_5AVH- 4A	<p>Amino acid sequence of VH of antibody DH511_5a(DH511.11P), See Ex. 10,</p> <p>See Figure 57 for DH511_5AVH_4A (SEQ ID NO: 291)</p> <p>QVQLVQSGGGLVKPGGSLTLSCVTSGFTFSNTWMSWVRQTPGK GLEWVARISRVDGPIIDYAAPVKGRFIISRDDSRNTLFLHMN NLKTEDTAVYYCTADEGAPILRFFEWGYNYNYMDVWGKGTTVI VSS</p>

625	DH511_5AVK	<p>Amino acid sequence of VL of antibodies DH511_5a(DH511.11P) AND DH511_5b (DH511.12P), See Ex. 10,</p> <p>See Figure 57 for DH511_5AVK (SEQ ID NO: 300)</p> <p>DIRLTQSPSSLSASVGDRITITCRASQSIKDYLNWYKHRPGE APKLLIYSASKLRSGVSSRFSGSGYGSFAFTLTISLQPEDFA TYYCQESYSSVPMYIFGQGTKVDLK</p>
626	DH511_5BVH_4A	<p>Amino acid sequence of VH of antibody DH511_5b(DH511.12P), See Ex. 10,</p> <p>See Figure 57 for DH511_5BVH_4A (SEQ ID NO: 292)</p> <p>QVQLVQSGGGLVKPGGSLTPSCVTSGFTFSNTWMSWVRQTPG KGLEWVARISRVGDGPIIDYAAPVKGRFIIISRRDSRNTLFLH MNNLKTEDTAVYYCTADEGAPILRFFEWGYNYNYMDVWGKGT TVIVSS</p>
627	Chain 3 of exemplary four chain trivalent (HIVxCD3xHIV) binding molecule (amino acid sequence)	<p>QVQLVQSGGGLVKPGGSLTLSCSASGFFFDNSWMGWVRQAPGK GLEWVGRIRRLKDGATGEYGA AVKDRFTISRDDSRNMLYLHMR TLKTEDSGTYYCTMDEGTPVTRFLEWGYFY YMAVWGRGTTVI VSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYIC NVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPEAAGGPSVFLF PPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP APIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLSCAVKGF YPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKS RWQQGNVFSCSVMHEALHNRYTQKSLSLSPGK</p>
628	Chain 4 of exemplary four chain trivalent (HIVxCD3xHIV) binding molecule (amino acid sequence)	<p>DIQMTQSPSFYLGSGDRVTITCRASQNIKDYLNWYQQRPGRA PRLLIYAASNLSGVPSRFSGSGYGTDFTLIISLQPEDFATY FCQESYSSTPTHIFGLGTKLEKKRTVAAPSVFIFPPSDEQLKS GTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKD STYSLSSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGE C</p>

629	Chain 1 of exemplary four chain trivalent (HIVxHIVxCD 3) binding molecule (amino acid sequence)	DIQMTQSPSFYLGSGVDRVTITCRASQNIKDYLNWYQQRPGRA PRLLIYAASNLSQGVPSRFSGSGYGTDFTLIISSSLQPEDFATY FCQESYSSTPTTHIFGLGTKLEKKGGSGGGGQVQLVQSGGGLV KPGGSLTLSCSASGFFFDNSWMGWVRQAPGKGLEWVGRIRRLK DGATGEYGA AVKDRFTISRDDSRNMLYLHMRTLKTEDSGTYYC TMDEGTPVTRFLEWGYFY YMAVWGRGTTVIVSSASTKGEVAA CEKEVAALKEKEVAALKEKEVAALKEGGGDKTHTCPPCPAPEAAG GPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCK VSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSL WCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYS KLTVDKSRWQQGNV FSCSVMHEALHNHYTQKSLSLSPGK
630	Chain 2 of exemplary four chain trivalent (HIVxHIVxCD 3) binding molecule (amino acid sequence)	DIQMTQSPSFYLGSGVDRVTITCRASQNIKDYLNWYQQRPGRA PRLLIYAASNLSQGVPSRFSGSGYGTDFTLIISSSLQPEDFATY FCQESYSSTPTTHIFGLGTKLEKKGGSGGGGQVQLVQSGGGLV KPGGSLTLSCSASGFFFDNSWMGWVRQAPGKGLEWVGRIRRLK DGATGEYGA AVKDRFTISRDDSRNMLYLHMRTLKTEDSGTYYC TMDEGTPVTRFLEWGYFY YMAVWGRGTTVIVSSASTKGVAA CKEKVAALKEKVAALKEKVAALKE
631	Chain 3 of exemplary four chain trivalent (HIVxHIVxCD 3) binding molecule (amino acid sequence)	EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPGK GLEWVGRIRSKYNNYATYYADSVKGRFTISRDDSKNSLYLQMN SLKTEDTAVYYCVRHGNFGNSYVSWFAYWGQGLTVTVSSASTK GPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALT SGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPS NTKVDKRVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDT LMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPRE EQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKT I SKAKGQPREPQVYTLPPSREEMTKNQVSLSCAVKGFYPSDIAV EWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNV FSCSVMHEALHNRYTQKSLSLSPGK
632	Chain 4 of exemplary four chain trivalent (HIVxHIVxCD 3) binding molecule (amino acid sequence)	QAVVTQEPSTLVSPGGTVTLTCRSSTGAVTTSNYANWVQQKPG QAPRGLIGGTNKRAPWTPARFSGSLLGGKAALTITGAQAEDEA DYICALWYSNLWVFGGKTCLTVLGRVAAPSVEFI FPPSDEQLK SGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSK DSTYSLSSLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRG EC

633	Exemplary DH511.11P (and DH511.12P) xCD3 Fc CHAIN 1 (amino acid sequence)	DIRLTQSPSSLSASVGDRTITTCRASQSIKDYLNWYKHRPGEA PKLLIYSASKLRSGVSSRFSGSGYGSFAFTLTISSLQPEDFATY YCQESYSSVPMYIFGQGTKVDLKGGGSGGGGEVQLVESGGGLV QPGGSLRLSCAASGFTFSTYAMNWVRQAPGKGLEWVGRIRSKY NNYATYYADSVKGRFTISRDDSKNSLYLQMNSLKTEDTAVYYC VRHGNFGNSYVSWFAYWGQGTLLTVSSASTKGEVAACEKEVAA LEKEVAALEKEVAALEKGGGDKTHTCPPCPAPEAAGGPSVFLF PPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP APIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLWCLVKGF YPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKS RWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
634	Exemplary DH511.11P xCD3 Fc CHAIN 2 (amino acid sequence)	QAVVTQEPSTLVSPGGTVTLTCRSSTGAVTTSNYANWVQQKPG QAPRGLIGGTNKRAPWTPARFSGSLLGGKAALTITGAQAEDEA DYICALWYSNLWVFGGGTKLTVLGGGSGGGGQVQLVQSGGGL VKPGGSLTLSCVTSGETFSNTWMSWVRQTPGKGLEWVARISRV GDGPIIDYAAPVKGRFIIISRDDSRNTLFLHMNNLKTEDTAVYY CTADEGAPILRFFFEWGYNYMDVWGKGTIVIVSSASTKGKVA ACKEKVAALKEKVAALKEKVAALKE
635	Exemplary DH511.12P xCD3 Fc CHAIN 2 (amino acid sequence)	QAVVTQEPSTLVSPGGTVTLTCRSSTGAVTTSNYANWVQQKPG QAPRGLIGGTNKRAPWTPARFSGSLLGGKAALTITGAQAEDEA DYICALWYSNLWVFGGGTKLTVLGGGSGGGGQVQLVQSGGGL VKPGGSLTPSCVTSGETFSNTWMSWVRQTPGKGLEWVARISRV GDGPIIDYAAPVKGRFIIISRDDSRNTLFLHMNNLKTEDTAVYY CTADEGAPILRFFFEWGYNYMDVWGKGTIVIVSSASTKGKVA ACKEKVAALKEKVAALKEKVAALKE

[0292] Various antigen binding domains capable of binding to an epitope of HIV-1, CD3, CD16 and CD8 are contemplated by the invention and are disclosed in the specification. Table 4 discloses non-limiting embodiments of such antigen binding domains which may be incorporated into the multispecific molecules of the invention. Additional, alternative antigen binding domains from other antibodies having specificity for the desired antigens may be utilized. Many such antibodies are known in the art, for example additional anti-CD3 antibodies are described in WO2012/162067 and WO 2014/110601 the contents of each of which are hereby incorporated by reference; additional anti-CD16 antibodies are described in WO 03/101485 the contents of which is hereby incorporated by reference; and additional anti-

CD8 antibodies are described in WO 2014/164553 the contents of which is hereby incorporated by reference.

[0293] Various Heterodimer-Promoting Domain (HPD) sequences are contemplated by the invention and disclosed in the specification. Table 4 discloses non-limiting embodiments of various HPDs. In some embodiments the HPD include E/K-coils (SEQ ID NOs: 518, 510) or cysteine engineered E/K-coils (SEQ ID NOs: 519, 521). In some embodiments HPD includes combinations of SEQ ID NOs: 511, 512, 513, and 514 sequences (*e.g.*, SEQ ID NOs: 511 and 513; SEQ ID NOs: 512 and 513; SEQ ID NOs: 511 and 514; SEQ ID NOs: 512 and 514); kappa and lambda light chain constant domains (SEQ ID NOs: 516 and 517). In some embodiments HPD include any suitable sequences with a Cysteine residue to permit disulfide bond. In some embodiments HPD includes suitable CH1 and CL domains.

[0294] A skilled artisan readily appreciates that the disclosure throughout the application of various design elements such as but not limited to HPDs and sequences, linkers, CH2-CH3 domain and Fc domain and their variants is applicable and could be used in any of the designs of the multispecific molecules of the invention that comprise these design elements.

[0295] *HIV-1 Antibodies*

[0296] Broadly neutralizing and potent HIV-1 envelope glycoprotein (Env) antibodies are now being developed for both prevention of HIV-1 (Rudicell RS *et al.* J. Virol 88: 12669-82, 2014) and for treatment of HIV-1 infected individuals (Barouch DH, *et al.* Nature 503: 224-8, 2013; Shingai M *et al.* Nature 503: 277-80, 2013). Thus, human recombinant antibodies either alone or in combinations have great prophylactic and therapeutic potential for the prevention and treatment of HIV-1 infection. Moreover, antibodies that bind with high affinity to Env may be useful in eliminating the latent pool of HIV-1 –infected CD4 T cells and curing HIV-1 infection, when either used to sensitize HIV-1 expressing target cells with bispecific bnAbs for NK or CD8 T cell killing or when bnAbs are conjugated with toxins or radionucleotides.

[0297] In certain aspects the invention provides fully human antibodies and fragments that specifically bind to and potently neutralize various isolates of HIV-1. In some embodiments, the antibodies bind to HIV-1 env gp41 membrane-proximal extracellular region (MPER). In some embodiments, the antibodies of the invention are combined in compositions with antibodies to other HIV-1 env binding sites.

[0298] In certain aspects the invention provides pharmaceutical compositions including these human antibodies and a pharmaceutically acceptable carrier. In certain aspects the invention provides antibodies for passive immunization against HIV/AIDS. Nucleic acids encoding these antibodies, expression cassettes and vectors including these nucleic acids, and isolated cells that express the nucleic acids which encode the antibodies of the invention are also provided.

[0299] In some embodiments, the invention provides antibodies which are clonal variants. In some embodiments, clonal variants are sequences that differ by one or more nucleotides or amino acids, and have a V region with shared mutations compared to the germline, identical VHDJH or VJH gene usage, identical or similar HCDR3 length, and the same VL and JL usage. The germline sequence (unmutated common ancestor “UCA”) is intended to be the sequence coding for the antibody/immunoglobulin (or of any fragment thereof) deprived of mutations, for example somatic mutations. Antibodies in a clone that are designated as UCA and/or I (for “Intermediate”) are typically not identified from a biological sample, but are derived computationally based on VH and/or VL sequences isolated from subjects infected with HIV-1.

[0300] Compositions including the human antibodies of the invention, including gp41 MPER binding antibodies, can be used for any purpose including but not limited to research, diagnostic and therapeutic purposes.

[0301] The neutralization breadth of the inventive antibodies is demonstrated by the diversity of viruses which are neutralized in the TZMbl Env pseudovirus inhibition assay. In certain embodiments, the neutralization breadth and/or binding of the antibodies of the invention can be maintained in the presence of tolerate changes to the epitope. Comparing the sequences of the neutralized viruses, versus viruses that are not neutralized, a skilled artisan can readily determine the % virus changes, including changes in the epitope, which can be tolerated while neutralization and/or binding is maintained.

[0302] Comparing the sequences of the antibodies and their neutralization properties, a skilled artisan can readily determine sequence identity, compare sequence length and determine the % sequence identity and/or changes, including % sequence identity and/or changes in the VH and VL sequences, including % sequence identity and/or changes in the CDRs, as well as the specific positions and types of substitutions which can be tolerated while neutralization potency and breadth is maintained.

[0303] Various algorithms for sequence alignment are known in the art. The similarity between amino acid sequences is expressed in terms of the similarity between the sequences, otherwise referred to as sequence identity. Sequence identity is frequently measured in terms of percentage identity (or similarity or homology); the higher the percentage, the more similar the two sequences are. Homologs or variants of a polypeptide will possess a relatively high degree of sequence identity when aligned using standard methods.

[0304] Methods of alignment of sequences for comparison are well known in the art. Various programs and alignment algorithms are described in: Smith and Waterman, *Adv. Appl. Math.* 2:482, 1981; Needleman and Wunsch, *J. Mol. Biol.* 48:443, 1970; Pearson and Lipman, *Proc. Natl. Acad. Sci. U.S.A.* 85:2444, 1988; Higgins and Sharp, *Gene* 73:237, 1988; Higgins and Sharp, *CABIOS* 5:151, 1989; Corpet *et al.*, *Nucleic Acids Research* 16:10881, 1988; and Pearson and Lipman, *Proc. Natl. Acad. Sci. U.S.A.* 85:2444, 1988. Altschul *et al.*, *Nature Genet.* 6:119, 1994, presents a detailed consideration of sequence alignment methods and homology calculations.

[0305] The NCBI Basic Local Alignment Search Tool (BLAST) (Altschul *et al.*, *J. Mol. Biol.* 215:403, 1990) is available from several sources, including the National Center for Biotechnology Information (NCBI, Bethesda, Md.) and on the internet, for use in connection with the sequence analysis programs blastp, blastn, blastx, tblastn and tblastx. A description of how to determine sequence identity using this program is available on the NCBI website on the internet.

[0306] Homologs and variants of a VL or a VH of an antibody that specifically binds a polypeptide are typically characterized by possession of at least about 75%, for example at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity counted over the full length alignment with the amino acid sequence of interest. Proteins with even greater similarity to the reference sequences will show increasing percentage identities when assessed by this method, such as at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99% sequence identity. When less than the entire sequence is being compared for sequence identity, homologs and variants will typically possess at least 80% sequence identity over short windows of 10-20 amino acids, and may possess sequence identities of at least 85% or at least 90% or 95% depending on their similarity to the reference sequence. Methods for determining sequence identity over such short windows are available at the NCBI website on the internet. One of skill in the art will

appreciate that these sequence identity ranges are provided for guidance only; it is entirely possible that strongly significant homologs could be obtained that fall outside of the ranges provided.

[0307] As used herein, reference to antibodies, without explicit mention of antibody fragments and antibody-fragment comprising molecules, may encompass antibody fragments and antibody-fragment comprising molecules.

[0308] In certain embodiments, the invention provides antibodies and antibody-fragment comprising molecules, including multispecific molecules, such as, but not limited to bispecific and trispecific molecules (*e.g.*, bispecific antibodies, bispecific diabodies, trivalent binding molecules, *etc.*) which are 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%, 85%, 84%, 83%, 82%, 81%, 80% identical to the VH and VL amino acid sequences of the antibodies described herein and still maintain the neutralization breadth, binding and/or potency. In certain embodiments, the invention provides antibodies and antibody-fragment comprising molecules, including multispecific molecules, such as, but not limited to bispecific and trispecific molecules (*e.g.*, bispecific antibodies, bispecific diabodies, trivalent binding molecules, *etc.*) which are 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%, 85%, 84%, 83%, 82%, 81%, 80% identical to the CDR1, 2, and/or 3 of VH and CDR1, 2, and/or 3 VL amino acid sequences of the antibodies described herein and still maintain the neutralization breadth, binding and/or potency.

[0309] In certain embodiments, the invention provides antibodies and antibody-fragment comprising molecules, including multispecific molecules, such as, but not limited to bispecific and trispecific molecules (*e.g.*, bispecific antibodies, bispecific diabodies, trivalent binding molecules, *etc.*) which can tolerate a larger percent variation in the sequences outside of the VH and/VL sequences of the antibodies. In certain embodiments, the invention provides antibodies which are 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%, 85%, 84%, 83%, 82%, 81%, 80%, 79%, 78%, 77%, 76%, 75%, 74%, 73%, 72%, 71%, 70%, 69%, 68%, 67%, 66%, 65% identical, wherein the identity is outside of the VH or VL regions, or the CDRs of the VH or VL chains of the antibodies described herein.

[0310] Antibodies of the invention are expected to have the same binding specificity, for example as intact immunoglobulins and antigen binding variants or fragments *e.g.* as a number of well characterized fragments produced by digestion with various peptidases. For instance, and without limitation, Fabs, Fvs, scFvs are fragments which are expected to have

the same binding specificities as intact antibodies. Binding specificity can be determined by any suitable assay in the art, for example but not limited competition binding assays, epitope mapping, *etc.* Assays to determine glycan dependence and glycan specificity binding are also known in the art. A scFv protein is a fusion protein in which a light chain variable region of an immunoglobulin and a heavy chain variable region of an immunoglobulin are bound by a linker, while in dsFvs, the chains have been mutated to introduce a disulfide bond to stabilize the association of the chains. Provided are also genetically engineered forms such as chimeric antibodies and heteroconjugate antibodies such as bispecific antibodies. See also, Pierce Catalog and Handbook, 1994-1995 (Pierce Chemical Co., Rockford, Ill.); Kuby, Immunology, 3rd Ed., W.H. Freeman & Co., New York, 1997.

[0311] In certain embodiments the invention provides antibody fragments and molecules comprising antibody fragments, which have the binding specificity and/or properties of the inventive antibodies. Non-limiting examples include: (1) Fab, the fragment which contains a monovalent antigen-binding fragment of an antibody molecule produced by digestion of whole antibody with the enzyme papain to yield an intact light chain and a portion of one heavy chain; (2) Fab', the fragment of an antibody molecule obtained by treating whole antibody with pepsin, followed by reduction, to yield an intact light chain and a portion of the heavy chain; two Fab' fragments are obtained per antibody molecule; (3) (Fab').sub.2, the fragment of the antibody obtained by treating whole antibody with the enzyme pepsin without subsequent reduction; (4) F(ab').sub.2, a dimer of two Fab' fragments held together by two disulfide bonds; (5) Fv, a genetically engineered fragment containing the variable region of the light chain and the variable region of the heavy chain expressed as two chains; and (6) single chain antibody ("SCA"), a genetically engineered molecule containing the variable region of the light chain, the variable region of the heavy chain, linked by a suitable polypeptide linker as a genetically fused single chain molecule. In certain embodiments, the antibody fragments can be produced recombinantly.

[0312] In certain embodiments, VH refers to the variable region of an immunoglobulin heavy chain, including but not limited to that of an antibody fragment, such as Fv, scFv, dsFv or Fab. In certain embodiments, VL refers to the variable region of an immunoglobulin light chain, including but not limited to that of an Fv, scFv, dsFv or Fab.

[0313] Any of the nucleic acids encoding any of the antibodies, or fragment thereof can be expressed in a recombinantly engineered cell such as bacteria, plant, yeast, insect and

mammalian cells. The nucleic acid sequences include any sequence necessary for expression, including but not limited to a promoter, a leader sequence. These antibodies can be expressed as individual VH and/or VL chain, or can be expressed as a fusion protein. In certain embodiments, the antibodies can be expressed by viral vector mediated delivery of genes encoding the antibodies of the invention (See *e.g.* Yang *et al.* Viruses 2014, 6, 428-447).

[0314] The present invention also encompasses molecules comprising a hinge domain. The hinge domain be derived from any immunoglobulin isotype or allotype including IgA, IgD, IgG, IgE and IgM. In preferred embodiments, the hinge domain is derived from IgG, wherein the IgG isotype is IgG1, IgG2, IgG3 or IgG4, or an allotype thereof. The hinge domain may be engineered into a polypeptide chain comprising the diabody molecule together with an Fc domain such that the diabody molecule comprises a hinge-Fc domain. In certain embodiments, the hinge and Fc domain are independently selected from any immunoglobulin isotype known in the art or exemplified herein. In other embodiments the hinge and Fc domain are separated by at least one other domain of the polypeptide chain, *e.g.*, the VL domain.

[0315] Various antibodies names are used throughout the application. Antibodies names correlation is as follows: Memory B cell antibodies: DH511=DH511.1; DH512=DH511.2; DH513=DH511.3; DH514=DH511.4; DH515=DH511.5; DH516=DH511.6;

[0316] Plasma antibodies: DH511_1a=DH511.7P; DH511_2a=DH511.8P; DH511_3a=DH511.9P; DH511_4a=DH511.10P; DH511_5a=DH511.11P; DH511_5b=DH511.12P.

[0317] Chimeric antibodies which combine a heavy and light chain from different antibodies are typically indicated by the designation of the heavy and light chain of each parent antibody.

[0318] Mutations in the VH chain are referenced with respect to Kabat numbering of the indicated VH chain.

[0319] It is readily understood that the nucleic acid sequences disclosed in the application are non-limiting embodiments of representative nucleotide sequences encoding the respective amino acid sequences.

[0320] The contents of the various publications cited throughout the specification are incorporated by reference in their entirety.

[0321] The following examples are provided to illustrate particular features of certain embodiments, but the scope of the claims should not be limited to those features exemplified.

EXAMPLES

[0322] The following examples are provided to illustrate particular features of certain embodiments, but the scope of the claims should not be limited to those features exemplified.

Example 1: MPER antibodies

[0323] Figure 1 shows the three HIV infected individual plasma that was evaluated for HIV neutralizing activity and the specificities profiled by the Georgiev algorithm (Georgiev IS et al Science 340: 751-6, 2013). From this analysis we found three subjects (CH0210, CH0536, CH1244) with gp41 bnAb activity (**Figure 1**).

[0324] Methods to identify and isolate MPER reactive antibodies were carried out as described in Liao HX et al. J. Virol. Methods 158: 171-9, 2009. MPER specific hooks were designed to identify to antibodies which bind to HIV-1 gp41 MPER region. Using one such hook, the MPR.03-biotin hook tetramerized (**Figure 2**), with fluorophor labeled streptavidin in two colors (**Figure 3**), we sorted by flow cytometry into single wells, the diagonally (that reacted with both colors hooks) reactive memory B cells (**Figure 3**). B cells from 10 million PBMC were sorted and PCR was carried out according to the protocol in Liao HX et al. J. Virol. Methods 158: 171-9, 2009. PCR amplifications were carried out to amplify rearranged VH and VL fragment pairs from the diagonally sorted memory B cells (Liao et al JVM). Overlapping PCR was used to construct full length Ig heavy and Ig light linear genes comprising the rearranged VH and VL fragment pairs. RT-PCR and PCR reactions was carried out essentially as described in Liao HX et al. J. Virol. Methods 158: 171-9, 2009, see for example Figure 1, Section 3.3. Sequence analysis of the VH and VL genes was carried out to determine the VH and VL gene usage, CDR lengths, the % mutation of HCDR3 and LCDR3. Based on this sequence analysis, one to two pairs of linear VH and VL genes were selected and made in linear cassettes (essentially as described in Liao HX et al. J. Virol. Methods 158: 171-9, 2009, see for example Figure 1, Section 3.3) to produce recombinant monoclonal antibodies by transient transfection in 293T cells.

[0325] Pairs of VH and VL genes as selected above can also be used to produce plasmids for stable expression of recombinant antibodies.

[0326] In certain embodiments, the plasmids or linear constructs for recombinant antibody expression also comprise AAAA substitution in and around the Fc region of the antibody that has been reported to enhance ADCC via NK cells (AAA mutations) containing the Fc region

aa of S298A as well as E333A and K334A (Shields RI et al JBC, 276: 6591-6604, 2001) and the 4th A (N434A) is to enhance FcR neonatal mediated transport of the IgG to mucosal sites (Shields RI et al. *ibid*).

[0327] The antibodies of the invention were selected based on a combination of criteria including sequence analyses, and functional analyses including but not limited as neutralization breadth, and potency.

[0328] In certain embodiments, the antibodies of the invention comprise naturally rearranged VH and VL fragment pairs, wherein the rest of the Ig gene is not naturally occurring with the isolated rearranged VH and VL fragments. In certain embodiments, the antibodies of the invention are recombinantly produced by synth

[0329] **Figure 4 and Example 12** shows a summary of some of the characteristics of the recombinant MPER antibodies of the invention. DH511-DH517 are antibodies with VH and VL chains from individual CH0210. DH518 is an antibody with VH and VL chains from individual CH0536. DH536 is an antibody with VH and VL chains from individual CH1244. CH537 is an antibody with VH and VL chains from individual CH0585. DH 511-DH516 antibodies are all members of the same B cell clonal lineage (Figure 6). **Figure 5** shows the neutralizing capacity of these antibodies with all but DH536 and DH537 able to neutralize difficult to neutralized (tier 2) HIV strains B.BG1168, C.CH505, and C.DU172). **Figure 6** shows the phylogram of the DH511 clonal lineage.

Example 2: TZMbl neutralization assay

[0330] TZMbl neutralization assay is a standard way to evaluate antibody breadth and potency. See Montefiori, D. *Methods Mol Biol.* 2009;485:395-405; HIV-1 Env-pseudoviruses infection of TZM-bl cells. Exemplary pseudovirus neutralization assays and panels of HIV-1 pseudovirus are described for example, in Li et al., *J Virol* 79, 10108-10125, 2005; Seaman et al, *J. Virol.*, 84:1439-1452, 2010; Sarzotti-Kelsoe et al., *J. Immunol. Methods*, 409:131-46, 2014; and WO2011/038290, each of which is incorporated by reference herein. Various HIV-1 isolates, both Tier 1 and Tier 2 viruses can be included in this assay.

[0331] The TZMbl assay was conducted to determine neutralization potency and breadth of the various antibodies of the invention on different HIV-1 pseudoviruses.

[0332] **Figure 7** shows the results of neutralization of 8 of the gp41 antibodies against a panel of 30 HIV tier 2 isolates in the TZMbl pseudovirus neutralization assay. The DH511 clonal lineage members all neutralized 100% (30/30) isolates while DH517 neutralized 50% and DH518 neutralized 83%. This in contrast to 10E8 gp41 antibody that only neutralized 29/30 isolates. **Figure 8** shows the mean IC₅₀, IC₈₀ and percent of isolates neutralized at an IC₅₀ <50ug/ml and at an IC₈₀ of <5ug/ml (confirm). Thus, mAb DH512 is equally as potent and slightly more broad in neutralization breadth than the mAb 10E8. **Figure 9** shows other mAbs and their breadth and potency. Various figures, including without limitation, Figures 37, 38, 28, 56 and 34, and Figures from Example 12 show neutralization data of various antibodies against various panels of pseudoviruses.

Example 3: Epitope mapping of MPER antibodies

[0333] Binding of antibodies to various MPER peptides in an ELISA assay was used to map the epitopes of the MPER antibodies.

[0334] **Figure 11** shows that Antibody epitopes maps to the C-terminus of gp41 to a similar region where 10E8 binds (Huang J et al. Nature 491 406, 2012; See US Pub 20140348785). Figures 11, 15-25 show binding of antibodies to MPER peptide variants. These mapping studies show that the antibodies of the invention are 10E8 like Abs. In non-limiting embodiments, DH512 shows the broadest and most potent neutralization among the antibodies tested.

[0335] **Figure 12** shows an alanine substituted gp41 peptide set used to map DH517 mab and **Figure 13** shows a summary of ala mutants to which the antibody is sensitive for binding to gp41. **Figures 14 and 15** show the VH and VL sequences of the DH511-DH516 antibodies.

[0336] **Figures 12-13** show the nucleotide and amino acid sequences of all the certain antibodies of the invention.

[0337] Figures 16-25 show that DH517 displayed a unique mapping pattern in that it depends on DKW at the N terminus and several residues at the C terminus important for 10E8 binding and neutralization. Clone DH511 mAbs bound strongly to the majority of the MPER656 variants, showing decreased binding to MPER656.2 and MPER656.2dYIK683R-biotin. These data indicate that the asparagine at position 674 is critical for binding, thus providing evidence that these mAbs bind at the C-terminus.

[0338] All the antibodies used in the above Examples had the AAAA substitution in and around the Fc region of the antibody that has been reported to enhance ADCC via NK cells (AAA mutations) containing the Fc region aa of S298A as well as E333A and K334A (Shields RI et al JBC, 276: 6591-6604, 2001) and the 4th A (N434A) is to enhance FcR neonatal mediated transport of the IgG to mucosal sites (Shields RI et al. ibid).

[0339] Epitope mapping studies are also described in Example 12.

Example 4: Binding assays and Kd determination

[0340] Kd measurements of antibody binding to HIV-1 envelope, e.g. gp41 or any other suitable peptide for the MPER antibodies, will be determined by Surface Plasmon Resonance measurements, for example using Biacore, or any other suitable technology which permits detection of interaction between two molecules in a quantitative way.

Example 5: Various assays

[0341] Various assays for self-reactivity of human antibodies are known in the art. AtheNA Multi-Lyte ANA Plus Test System is one such assay. ELISA cardiolipin assay is another assay to measure autoreactivity.

[0342] The stability and properties of the antibodies, for example as formulated in a composition for treatment will be tested.

[0343] Animal studies (PK and PD studies) could be conducted to determine the distribution and half-life of the antibodies.

[0344] Various assays and experiments can be designed to analyze prevention, treatment and/or cure.

Example 6: Antibodies from CH235 lineage

[0345] CH557 is one example of a CD4bs broad neutralizing HIV-1 antibody, from a series of clonal antibodies (Figure 28) which can be used in combination with the antibodies of the invention.

Example 7: V3 glycan Antibodies from DH270 lineage

[0346] Antibodies from DH270 lineage are shown in Figure 26. I1 (DH270IA1), I2, I4, I3 and UCA in Figure 26 are not isolated from human subjects but are derived computationally

based on VH and VL sequences of other observed antibodies from the clone: DH471, DH429, DH473, DH391 and DH270. The VH and VL sequences of DH471, DH429, DH473, DH391 and DH270 are derived from a human subject infected with HIV-1.

[0347] The VH and VL sequences of DH471, DH429, DH473, DH391 and DH270 are derived essentially as described in Example 1, except that cell were sorted with a different hook.

[0348] Neutralization data for antibodies I1 (DH270IA1) and DH429 is summarized in Figure 9, and Figure 10.

[0349] DH542, DH542-QSA, DH542_K3 are non-limiting examples of V3 antibodies, which can be used in combination with the antibodies of the invention. The nucleotide and amino acid sequences of the VH and VL of DH542 QSA are shown below. DH542 QSA antibody has the VH of DH542 and the VL called DH542-QSA

[0350] >DH542_HC_nt (SEQ ID NO: 465)

CAGGTGCAGCTGGTGCAGTCTGGGGCTCAAATGAAGAACCCTGGGGCCTCAGTGAAGGTCTC
CTGCGCGCCTTCTGGATATACCTTCACCGACTTTTACATACATTGGTTGCGCCAGGCCCTG
GCCAGGGGCTTCAGTGGATGGGATGGATGAACCCTCAGACTGGTCGCACAAACACTGCACGA
AACTTTCAGGGGAGGGTCACCATGACCAGGGACACGTCCATCGGCACAGCCTACATGGAGTT
GAGAAGCCTGACATCTGACGACACGGCCATATATTACTGTACGACAGGGGGATGGATCAGTC
TTTACTATGATAGTAGTTATTACCCCAACTTTGACCACTGGGGTCAGGGAACCCTGCTCACC
GTCTCCTCAG

[0351] >DH542_HC_aa (SEQ ID NO: 466)

QVQLVQSGAQMKNP GASVKVSCAPSGYTFTDFYIHWLRQAPGQGLQWMGWMNPQTGRNTAR
NFGQGRVTMTRDTSIGTAYMELRSLTSDDTAIYYCTTGGWISLYYDSSYYPNFDHWGQGTLT
VSS

[0352] >DH542_LC_nt_corrected (DH542_QSA) (SEQ ID NO: 467)

CAGTCTGCCCTGACTCAGCCTGCCTCCGTGTCTGGGTCTCCTGGACAGTCGATCACCATCTC
CTGCACTGGAACCAAGTATGATGTTGGGAGTCATGACCTTGTCTCCTGGTACCAACAGTACC
CAGGCAAAGTCCCCAAATACATGATTTATGAAGTCAATAAACGGCCCTCAGGAGTTTCTAAT
CGCTTCTCTGGCTCCAAATCTGGCAACACGGCCTCCCTGACAATCTCTGGGCTCCGGGCTGA
GGACGAGGCTGACTATTATTGCTGTTTCATTTGGAGGGAGTGCCACCGTGGTCTGCGGCGGCG
GGACCAAGGTGACCGTCCTAg

[0353] >DH542_LC_aa_corrected (DH542_QSA) (SEQ ID NO: 468)

QSALTQPASVSGSPGQSITISCTGTKYDVGSHDLVSWYQQYPGKVPKYMIYEVNKRPSGVSN
RFSGSKSGNTASLTISGLRAEDEADYCCSFGGSATVVCGGGTKVTVL

[0354] DH542-L4 is an antibody that has a VH of DH542 and VL of DH429 (Figure 26)

Example 8: DH540 antibody is described elsewhere.

[0355] DH540 antibody is described in detail in US Ser. No. 62/170,558, filed June 3, 2015.

Example 9: TZMbl neutralization assay

[0356] TZMbl neutralization assay was conducted to determine neutralization potency and breadth of different HIV-1 viral species by DH512 and mAb 10E8. **Figures 37 and 38** show the results of neutralization against a panel of HIV isolates in the TZMbl pseudovirus neutralization assay. **Figures 37 and 38** also show the mean IC₅₀, IC₈₀ and percent of isolates neutralized at different IC₅₀ or IC₈₀ values.

Example 10: Isolation of additional antibodies from the DH511 lineage

[0357] High throughput native VH:VL sequencing from single B cells

[0358] Additional antibodies were isolated from the individual CH0210 by high-throughput sequencing of the paired human immunoglobulin heavy and light chain repertoire. See Figure 39. For detailed methods, see DeKosky *et al.* Nature Biotechnology, 31, 166-169 (2013), and DeKosky *et al.* Nature Medicine, 21, 86-91 (2015). Briefly B cells were isolated from PBMCs via negative depletion. The heavy and light chain transcripts were co-localized on RNA binding beads, and then physically tied together using overlap extension RT-PCR. The paired VH:VL amplicons were then used to generate 3 libraries for sequencing: a heavy chain database, a light chain database, and a paired database. The necessity for three databases stems from the fact that MiSeq currently limits the forward and reverse reads to ~300 bp each (approximate read lengths are shown below as arrows). As the heavy and light chains are both longer than 300 nucleotides, the full length heavy and full length light chains were sequenced separately and the paired database was used as a key to stitch the heavy and light chains together by matching unique CDR3 sequences.

[0359] F(ab)₂ fragments were prepared from total serum IgG and subjected to antigen-affinity chromatography using the MPER peptide. Proteins in the elution and flow-through were denatured and reduced, alkylated, trypsin-digested and analyzed by high resolution LC-

MS/MS. Spectra were interpreted with the heavy chain database obtained from next-generation sequencing, and peptides uniquely associated with a single CDR (“informative peptides”) were used to identify full-length VH sequences. Clonotypes are defined as VH sequences having the same germline V and J and at least 85% aa identity in the CDRH3. To identify the MPER-binding antibodies, the focus was on the clonotypes that contain the identified CDR3 peptides and were highly enriched in the elution. This identified three clonotypes: 137, 335 and 195. All three clonotypes use the same VDJ combination (VH3-15, DH3-3, and JH6), which was also utilized by the DH511 series MPER lineages.

[0360] Based on VH sequences it was apparent that the antibodies pulled out by the paired VH:VL sequencing technology were members of the DH511 clonal lineage. Therefore, all of the antibodies are named starting with DH511. The numbers after the underscore correspond to the cluster names that were designated by the VH:VL sequencing. Antibodies were clustered by 96% nucleotide identity in the CDR3.

[0361] The above analysis identified additional MPER antibodies listed below:

PTID	Ab ID	H ID	K/L ID
704-01-021-0	DH511_1a_4A	DH511_1AVH_4A	DH511_1AVK
704-01-021-0	DH511_1b_4A	DH511_1BVH_4A	DH511_1AVK
704-01-021-0	DH511_2a_4A	DH511_2AVH_4A	DH511_2AVK
704-01-021-0	DH511_2b_4A	DH511_2BVH_4A	DH511_2AVK
704-01-021-0	DH511_2c_4A	DH511_2CVH_4A	DH511_2AVK
704-01-021-0	DH511_3a_4A	DH511_3AVH_4A	DH511_3AVK
704-01-021-0	DH511_3b_4A	DH511_3AVH_4A	DH511_3BVK
704-01-021-0	DH511_3c_4A	DH511_3AVH_4A	DH511_3CVK
704-01-021-0	DH511_4a_4A	DH511_4AVH_4A	DH511_4A4CVK
704-01-021-0	DH511_4a_4bK_4A	DH511_4AVH_4A	DH511_4BVK

704-01-021-0	DH511_4b_4aK_4A	DH511_4BVH_4A	DH511_4A4CVK
704-01-021-0	DH511_4b_4A	DH511_4BVH_4A	DH511_4BVK
704-01-021-0	DH511_4c_4A	DH511_4CVH_4A	DH511_4A4CVK
704-01-021-0	DH511_4c_4bK_4A	DH511_4CVH_4A	DH511_4BVK
704-01-021-0	DH511_5a_4A	DH511_5AVH_4A	DH511_5AVK
704-01-021-0	DH511_5b_4A	DH511_5BVH_4A	DH511_5AVK

[0362] VH and VL genes were selected and made in linear cassettes (essentially as described in Liao HX et al. J. Virol. Methods 158: 171-9, 2009, see for example Figure 1, Section 3.3) to produce recombinant monoclonal antibodies by transient transfection in 293T cells. See also Example 1 for variations in the backbone.

Example 11: Heavy and Light Chain Chimeric Antibodies; Antibodies with changes in the amino acids of the VH chain

[0363] This example describes chimeric antibodies comprising non-natural VH and VL chain pairs. Naturally occurring VH or VL chain are combined in non-natural pairs as described in Figure 55, chimeras 1-91.

[0364] Chimeras 1-91 were recombinantly expressed and their neutralization profile was determined in the TZMBI assay (Figure 56). Based on neutralization data for chimeras 68-91 as shown in Figure 56, three antibodies DH512_K2_4A (VH: H510049_4A (DH512) and VL: DH511_1AVK), DH512_K3_4A (VH: H510049_4A (DH512) and VL: DH511_2AVK) and DH512_K4_4A (VH: H510049_4A (DH512) and VL: DH511_5AVK) antibodies were produced large scale and will be tested for neutralization against a larger panel of viruses (see panels for DU512). DH512_K3_4A is also referred to as DH512_K3 and DH511.2_K3.

[0365] The invention contemplates antibodies which comprise amino acid changes, or combination of such changes, in the VH chains of antibodies form the DH511 lineage. Non-limiting examples of antibodies with mutations are provided in Figures 30-33, or any combination thereof. Most mutations are to changes to W, but can also try F, L or possibly other substitutions, e.g. without limitation I, V, A. Additional mutations include without

limitation the following: T100aF; T100aL; T100aI; T100aV; T100aA; L100dW, or any combination thereof.

[0366] In some embodiment, such double mutants: T100aW-L100dF; T100aW-L100dW; T100aF-L100dF; T100aL-L100dF; T100aL-L100dW.

[0367] Neutralization data for a subset of these antibodies is provided in Figure 34. The data show that some of the mutations abrogate neutralization while others enhance potency. One candidate, DH512_L100dF_4A, is more potent than 10E8 and has similar potency to DH512_K3.

[0368] In some embodiments, L100d could be changed to Trp.

[0369] Data in Figure 34 and 80 show that single mutant L100dF, and single mutant T100aW have improved neutralization. These single mutants will be tested against a panel of additional viruses (see panel for DH512, DH512_K3).

[0370] Contemplated are also combination mutations, for example but not limited combination T100aW with L100dF, combination L100dW with T100aW.

[0371] Mutated VH chain as contemplated above could be combined with VH chain from DH512, or with VL chain from DH512_K3 (DH511_2AVK).

Example 12: Shared Memory and Plasma Repertoires of HIV-1 Neutralizing Antibodies

[0372] Shared Memory and Plasma Repertoires of HIV-1 Neutralizing Antibodies

[0373] Understanding the relationship of the memory B cell and plasma immunoglobulin repertoires of HIV-1-infected individuals who develop broadly neutralizing antibodies (bnAbs) is important, since plasma antibody responses are required to achieve maximum protection from infectious agents. Using HIV-1 envelope gp41 membrane-proximal external region (MPER)-specific memory B cell sorting and next-generation sequencing, coupled with mass spectrometry analysis of plasma antibodies, we probed the memory B cell and plasma antibody repertoires of an HIV-1-infected donor with a plasma bnAb signature that mapped to Env gp41 distal MPER. We found potent IgG bnAbs from the same B cell clonal lineage in memory B cells and plasma that neutralized 99% of HIV-1 isolates. Structural analysis demonstrated clonal lineage antibodies from memory B cells and plasma both recognized the envelope gp41 epitope identically in an alpha helical conformation. Thus, a major source of potentially protective plasma HIV-1 bnAbs is the memory B cell pool.

[0374] Introduction

[0375] Inducing broadly reactive neutralizing antibodies (bnAbs) is critical for developing a protective HIV-1 vaccine. Some of the broadest bnAbs isolated are to the envelope gp41 membrane proximal external region (MPER), with two of these, 10E8 and 4E10, the most broad (1, 2). Monoclonal antibody (mAb) 4E10, while extremely broad in neutralization breadth, is not potent, and is highly polyreactive with many non-HIV-1 proteins and autoreactive with the human protein splicing factor 3b subunit 3 (SF3B3) (3) as well as with lipids (4). In contrast, mAb 10E8 is not as polyreactive as 4E10, and is both more broad and potent (1), although it does have a degree of lipid reactivity (5) and is autoreactive with the host protein family of sequence similarity 84 member A (FAM84A) (6).

[0376] To date, all HIV-1 broadly neutralizing antibodies have been isolated from memory B cells, either with clonal memory B cell cultures or using fluorophore-labeled Env and flow cytometry cell sorting. However, most correlates of protection for infectious agents with successful vaccines are the levels of plasma neutralizing antibodies. Moreover, the correlate of decreased transmission risk in the only HIV-1 vaccine trial to demonstrate a degree of efficacy was plasma antibodies to the second variable loop (V2) region (7).

[0377] In HIV-1 infection, 60% of HIV-1-specific antibodies derive from abnormal B cell subsets, that are either activated or exhausted and express Fc receptor-like-4 (FcRL4) (8, 9). However, many of the antibodies reflected in HIV-1 memory B cells are not expressed in plasma (8). Similarly, many of the memory B cell specificities of antibodies in other settings are also not represented in plasma (10-12). Thus, it is not known if envelope-reactive memory B cells with bnAb B cell receptors are a major source of plasma broad neutralizing activity.

[0378] Here we have isolated memory B cell and plasma broad and potent envelope gp41 bnAbs from an African donor and demonstrated broad and potent plasma gp41 bnAbs to be in the same B cell clonal lineage as those isolated from memory B cells. Chimeric antibodies consisting of memory bnAb V_H and plasma bnAb V_L as well as engineering memory bnAb heavy chain complementary determining regions yield antibodies with greater potency than naturally paired antibodies. Thus, the class-switched memory B cell pool contributes to plasma bnAbs.

[0379] Results**[0380] Isolation of Memory B Cell gp41 Neutralizing Antibodies**

[0381] Neutralization-based epitope prediction analysis revealed that plasma from HIV-1 clade C-infected individual CH0210 contained C-terminal MPER bnAb activity (13) (**Figure 62**). Six clonally-related mAbs, designated DH511.1-DH511.6, were isolated by antigen-specific single memory B cell sorting using MPER peptide fluorophore-labeled probes (14) (**Fig. 59a, 59b, and Supplementary Table 1**). The DH511 B cell clonal lineage was distinguished by HCDR3 loops of 24 amino acids in DH511.1, DH511.3, and DH511.6, while DH511.2, DH511.4, and DH511.5 antibodies had a one amino acid deletion in the HCDR3, resulting in a length of 23 amino acids (**Supplementary Table 1**). V_H and V_L somatic mutation rates were 15-22% and 14-18%, respectively. The DH511 clonal lineage was derived from the same heavy-chain germ line gene as previously isolated gp41 neutralizing antibody 10E8 (V_H 3-15), but utilized a different V_L germ line gene (DH511: V_L 1-39, 10E8: V_L 3-19) (1) (**Supplementary Table 1**). Antibody DH517, derived from a second clonal lineage arising from the same donor, was similarly isolated. DH517 utilized V_H 4-34 and V_L 3-19 germ line genes, was 22.8% and 14.3% mutated, respectively, and had a long HCDR3 comprised of 24 amino acids.

[0382] DH511.1-DH511.6 and DH517 mAbs were assessed for neutralization breadth and potency against a panel of 30 cross-clade HIV-1 isolates. All six DH511 clonal members neutralized 30 of 30 isolates tested with median 50% inhibitory concentrations (IC_{50}) ranging from 0.7 to 4.2 μ g/ml (**Supplementary Table 2a**). DH517 had less breadth than DH511 clone antibodies, neutralizing 15 of 30 isolates with a median IC_{50} of 5.7 μ g/ml (**Supplementary Table 2a**). The most potent DH511 clone bnAb (DH511.2) in a large cross-clade panel of 199 geographically and genetically diverse HIV-1 Env pseudoviruses, neutralized 197/199 (99%) viruses but was less potent than 10E8 (195/200, 98%) (median IC_{50} , DH511.2 = 1.1 μ g/ml and 10E8 = 0.4 μ g/ml) (**Fig. 59c, 59d, and Supplementary Table 3**). Neutralization curves revealed that DH511.2 achieved >99% maximal neutralization for 93% of the isolates (**Fig. 59e**), and showed similar potency and breadth of neutralization against a second panel of 200 clade C primary HIV-1 isolates (**Supplementary Table 4**).

[0383] Isolation of Plasma gp41 Neutralizing Antibodies

[0384] We next analyzed the MPER-specific plasma antibody repertoire from donor CH0210 using an independent proteomics-based approach for the identification and semi-quantitative determination of antigen-specific antibodies in human serum (15, 16). MPER-specific antibodies were isolated from a 2 ml plasma sample by affinity chromatography, processed

for proteomics (10) and subjected to liquid chromatography high-resolution tandem mass spectrometry (LC-MS/MS) analysis. For peptide identification, a donor-specific V_H database comprising 98,413 unique high quality sequences was derived from a natively paired V_H:V_L repertoire from 845,000 peripheral single B cells from total PBMCs (isolated using MACS negative selection: CD2⁺CD14⁻CD16⁻CD43⁻CD235a⁺) (17-19). These V_H sequences were then clustered into 4,428 clonotypes, using a cut-off of $\geq 85\%$ amino acid identity in the HCDR3 region.

[0385] Using stringent data filtering protocols (10), high confidence peptide-spectrum matches (PSMs) from HCDR3 peptides were identified and their respective LC peak intensities were used for relative quantification. As we have shown previously, an estimated >80% of all HCDR3 peptides within a sample are typically identified in this manner (detection limit approximately 0.4 ng/ml), and peak intensities correlate well with absolute peptide concentrations (10, 15). Plasma Ig clonotypes were defined as V_H sequences having the same germline V and J and 85% aa identity in the HCDR3.

[0386] We found that the MPER-specific plasma antibody repertoire consisted of 10 clonotypes, three of which used the same VDJ combination (V_H3-15, D_H3-3, J_H6) as the DH511 clonal lineage (**Figure 63**). Clonotype IV comprised 95% of the total intensity of HCDR3 peptides detected in the MPER-specific antibody repertoire (*i.e.* in antibodies eluted following affinity chromatography with immobilized MPR.03 peptide); we noted that detection of HCDR1 and HCDR2 peptides unique to Clonotype IV provided further unambiguous support for the prevalence of these antibodies in the CH0210 plasma (**Fig. 59f**). Clonotype II, which included antibodies DH511.2, DH511.4 and DH511.5 isolated by single-cell sorting, and Clonotype III were detected at 4% and 1% relative abundance, respectively (**Fig. 59f**). All three HCDR3 clonotypes utilized the same VDJ genes (V_H3-15, D_H 3-3 and J_H6), displayed similar HCDR3 lengths of 23-24 amino acids and V_H gene mutation rates of 15-20% (**Supplementary Table 6**). Whereas 11 V_H DH511 clonal lineage members were found by mass spectrometry (**Supplementary Table 6, Figure 64**), the phylogram was collapsed to represent the most prevalent members (**Fig. 59f**). It is noteworthy that Clonotype I (**Fig. 59g**), that includes DH511.1, DH511.3 and DH511.6, was isolated by memory B cell sorting but was not detected in the plasma; we validated that recombinant DH511.1, DH511.3 and DH511.6 antibodies were readily detectable by mass spectrometry, indicating that their absence from the CH0210 plasma was not a technical artifact.

[0387] Using the proteomically identified HCDR3 sequences, we searched the native V_H:V_L sequence database comprising ~200,000 heavy-light chain pairs from single B cells to determine the respective full-length light-chain sequence belonging to each clonotype (**Supplementary Table 6**). For clonotypes in which multiple V_H:V_L somatic variants were detected, only the two most frequent variants, as quantified by the number of sequencing reads, were selected for expression and characterization (**Supplementary Table 6**). The light-chains belonging to these three clonotypes all shared the same V- and J- gene identity (IGKV1-39, IGKJ2) as the light-chains of the DH511 clonal lineage isolated by memory B cell single-cell sorting. Six plasma mAbs belonging to the DH511 clonal lineage (designated DH511.7P-DH511.12P), showed potent tier 2 neutralizing activity against a panel of four HIV-1 isolates (**Supplementary Table 7**), with mAbs DH511.11P and DH511.12P demonstrating the most potent neutralizing activity. DH511.11P and DH511.12P were selected for further characterization of their neutralization breadth and potency against a panel of 203 cross-clade isolates and had slightly more breadth (99.5% of isolates tested) and greater potency than memory B cell-derived DH511.2 but were less potent than 10E8 (median IC₅₀: 0.7 µg/ml for DH511.11P and DH511.12P versus 0.4 µg/ml for 10E8) (**Supplementary Table 8**).

[0388] Structural Analysis of DH511 Lineage Antibodies

[0389] We used a panel of alanine substituted MPER peptides that span gp41 residues 671-683 (**Supplementary Table 9**) to define the epitopes of DH511.1-DH511.12P by enzyme linked immunosorbent assay (ELISA). Similar to the epitopes of 4E10 and 10E8 (1), DH511.1-DH511.12P binding was sensitive to alanine mutations at Asn671_{gp41} and Trp672_{gp41}, but unlike 4E10 and 10E8, was also sensitive to Asp674Ala_{gp41}, and to a lesser extent Leu679Ala_{gp41} mutations (**Figure 63**). Assessment of the neutralization activity of DH511.1-DH511.12P (not DH511.7-DH511.10) mAbs against clade C COT6.15 Env pseudoviruses bearing alanine substitutions across the MPER (20, 21) (**Supplementary Table 10**) demonstrated sensitivity of neutralization to Env mutations of Phe673Ala_{gp41}, Asp674Ala_{gp41}, and Asp674Ser_{gp41}, with the most prominent resistance observed against the Trp672Ala_{gp41} mutant virus (**Supplementary Table 11**). These data demonstrated that the epitope recognized by DH511 lineage antibodies was similar to but distinct with those of gp41 bnAbs 4E10 and 10E8, requiring the aspartic acid at position 674 for binding and neutralization.

[0390] Crystal structures of the antigen-binding fragments (Fab) of the DH511.1 antibody in complex with a peptide spanning the full gp41 MPER (residues 656-683) and of the DH511.2 antibody in complex with gp41 peptides spanning residues 662-683 and 670-683 were determined to 2.7 Å, 2.6 Å and 2.2 Å resolution, respectively (**Fig. 60, Figure 62 and Supplementary Tables 12 and 13**). Both DH511.1 and DH511.2 recognized an alpha-helical conformation of the distal portion of the gp41 MPER (residues 671-683) (**Fig. 60a**), similar to the conformation recognized by neutralizing antibodies 10E8 and 4E10 (**Fig. 61b**). C α RMSDs for this region of gp41 across all four antibody-bound structures did not exceed 0.46 Å. Ordered electron density for the bound peptides was also observed upstream of the distal gp41 MPER helix. In the case of DH511.1, an additional α -helix was present between residues 656-661, followed by an extended conformation between residues 662-670 (**Fig. 60a**). DH511.2-bound MPER also adopted an extended conformation between residues 662-670, upstream of the distal helix, with the highest degree of overall structural homology to DH511.1-bound MPER occurring between gp41 residues 668-683 (C α RMSD = 0.39 Å) (**Fig. 60a**). Interactions between DH511.1 and DH511.2 and gp41 MPER were mediated exclusively by their heavy chains, with V_H3-15-encoded regions accounting for 45-50% of the antibody contact interface with gp41, and HCDR3 loops accounting for 50% or more of the remaining interface (**Fig. 60b and 61c, Supplementary Table 14**). A total of 751.1 and 681.4 Å² interactive surface area was buried on DH511.1 and DH511.2, respectively, and 797.2 and 780.1 Å² on gp41 MPER in the two respective structures (**Supplementary Table 14**). The larger interface observed for the DH511.1 complex was due to the longer gp41 MPER peptide of that complex and the additional interface observed between its N-terminus and the antibody. It is likely that this additional interface is due to crystal lattice constraints, since alanine scan mutagenesis of N-terminal gp41 MPER residues did not result in reduction of antibody binding (**Figure 65**). Contacts between DH511.1 and DH511.2 and gp41 MPER were highly conserved in both structures (**Fig. 60c and Supplementary Tables 15-16**). V_H3-15-encoded residues of both DH511.1 and DH511.2 mediated interactions with gp41 residues L669, W670, N671, W672, F673, and D674, while their HCDR3 loop residues contacted gp41 residues W672, T676, L678, W679 and R683, as well as I675 in the case of DH511.2 (**Fig. 60c and Supplementary Tables 15-16**). The interactions observed in the structures were consistent with alanine scan analyses that revealed reduced antibody binding upon mutation of gp41 residues 671-674 and 679 (**Figure 65**). Interactions between DH511.1 and

DH511.2 and main-chain atoms of gp41, which would be difficult to detect in alanine scan analyses, were also observed, including between antibody residue N31 and the carbonyl oxygen of gp41 W670 (**Fig. 60c and Supplementary Tables 15-16**).

[0391] To compare atomic-level recognition of gp41 MPER by plasma-derived versus memory B-cell-derived antibodies, structural studies of the plasma-derived DH511-lineage antibodies DH511.11P and DH511.12P were undertaken in complex with gp41 MPER peptides. Crystal structures of DH511.11P and DH511.12P Fabs were determined in complex with a peptide spanning gp41 MPER residues 662-683, to 2.47 and 1.88 Å, respectively (**Fig. 60c and Supplementary Tables 12 and 13**). The structures revealed that both plasma derived variants recognized a conformation of the MPER similar to that recognized by DH511.1 and DH511.2, adopting an α -helix between residues 671-683 and an extended conformation upstream, between residues 662-670. The highest degree of structural homology occurred between residues 668-683. As in the case of DH511.1 and DH511.2, interactions between DH511.11P and DH511.12P and gp41 were mediated exclusively by their heavy chains (**Fig. 60d and Supplementary Table 14**). The plasma-derived variants recognized the very same gp41 residues as those recognized in common by DH511.1 and DH511.2, although the respective antibody residues that mediated these contacts with gp41 differed in some cases (**Fig. 60b, 60d, 60e and Supplementary Tables 15-18**). While contacts between HCDR1 loop residues of the DH511.11P and DH511.12P and gp41 were largely conserved relative to those of DH511.1 and DH511.2, gp41 contacts mediated by their HCDR2 loops diverged relative to those of DH511.1 and DH511.2 (**Fig. 60**). The substitution of DH511.1 and DH511.2 HCDR2 residue K52c with a glycine in DH511.11P and DH511.12P, led to the loss of a salt bridge mediated by K52c and gp41 residue D674 – one that was replaced by an additional salt bridge mediated by conserved residue R52a (**Figures 60b, 60d, 60e and Supplementary Tables 15-18**). Examination of additional gp41-contacting residues that were unique to the plasma-derived variants revealed that unique residues of their HCDR3 loops, which differed from the DH511.1 and DH511.2 HCDR3 loops at ~7 residue positions, mediated many of these contacts (**Fig. 60c and Supplementary Tables 17-18**). Despite their overall sequence divergence from DH511.1 and DH511.2, ~26-28% in heavy chain variable regions, the structures of the DH511.11P and DH511.12P were highly homologous to those of DH511.1 and DH511.2. In sum, the plasma-derived variants examined here recognized a similar conformation of the gp41 MPER as that recognized by

memory B-cell derived variants, contacted a similar set of gp41 residues, and did so through modified antibody contacts that did not significantly alter the backbone conformations of their paratopes or common epitope.

[0392] We next compared the structures of DH511 lineage antibodies to those of other antibodies that target the distal gp41 MPER (**Fig. 61a and 61b**). Since the DH511 lineage shares a common V_H3-15 heavy chain precursor as the 10E8 lineage, we were especially interested in determining if a structural basis for usage of this precursor to target the MPER could be discerned. As a first step, we compared the directions of approach of DH511 lineage antibodies to the distal MPER helix, relative to those of 10E8 and 4E10. All four antibodies were oriented by superimposing residues 671-683 of their respective epitopes, and their directions of approach were defined by a line drawn from the C α atom of epitope residue 672 to a point midway between the variable region intra-chain heavy and light chain disulfide bonds, which represented the longitudinal axis of the antibody variable regions. Pairwise comparison of the directions of approach of DH511.1 versus those of DH511.2, 10E8 and 4E10 yielded differences of 4.7°, 13.4° and 25.2°, respectively, suggesting the DH511 lineage most closely resembled 10E8 in its approach to the epitope (**Fig. 61d**). While the longitudinal axes of the DH511.1 and DH511.2 variable regions and that of 10E8 were highly similar, the orientations of their heavy and light chains relative to this longitudinal axis differed more substantially – by ~54° (**Fig. 61d**). This difference resulted in a rotational shift of the gp41 footprint on 10E8 relative to the footprint on DH511 lineage antibodies (**Fig. 61c**). Thus, while DH511 lineage antibodies share an identical heavy chain V_H3-15 precursor as antibody 10E8, and approached gp41 MPER from similar angles, the orientations of their heavy and light chains relative to the epitope differed more substantially.

[0393] To determine if a common structural basis for V_H3-15 precursor usage could nonetheless be discerned between the two lineages, we compared V_H3-15-encoded gp41-contacting residues in DH511.1, DH511.2 and 10E8. Of the total number of residue interactions that exist between the V_H3-15 regions of three respective antibodies and gp41 (8 for DH511.1, 10 for DH511.2, and 10 for 10E8), five common residue positions were involved interactions with gp41 in all three antibodies: 28, 31, and 33 within the HCDR1 and 52c and 53 within the HCDR2 (**Fig. 61c and 60e**). Heavy chain residues 31 and 33 are asparagine and tryptophan in all three antibodies and are un-mutated from the germ-line precursor. Residue 53 is aspartate in DH511.1 and DH511.2, as it is in the germ-line

precursor, and a chemically similar glutamate in 10E8. Residue positions 28 and 52c are somatically mutated from germ-line in all three antibodies, to disparate amino acids (**Fig. 61e**). While all five residues maintain contact with gp41 in both the DH511.1 and 10E8 lineages, the rotational shift in the orientations of the heavy and light chains between the two lineages results in distinct modes of gp41 recognition (**Fig. 60b and 61e**). Yet, the five common V_H3-15 encoded gp41-contacting residues in both lineages end up interacting with many of the same gp41 MPER residues, including L669, W670, N671, W672, and F673 (**Fig. 60b, 60e, and 61e**). V_H3-15 germ line encoded residue W33, shown in previous studies to be required for 10E8 recognition of gp41 (1), interacts with gp41 residues W672 and F673 in both the DH511.1 and 10E8 lineages, although from a distinct spatial position in each case (**Fig. 60b and 61e**). Thus, despite a relative shift in heavy and light chain orientations, a common subset of DH511.1 and 10E8 lineage V_H3-15 residues interact with the same subset of distal MPER residues. It remains to be determined if the observed differences in the heavy and light chain orientations of two lineages, relative to gp41 MPER, were determined at inception of naïve antibody recognition or if they were added during antibody development and maturation.

[0394] Origin and Development of the DH511 Clonal Lineage

[0395] A maximum likelihood phylogenetic tree was constructed from the VDJ sequences recovered from memory B cell sorting and was used to infer the unmutated common ancestor (UCA) of clone DH511 and six maturational intermediate antibodies (**Fig. 59b**). A global panel of 12 HIV-1 isolates was used to assess the development of neutralization breadth in the DH511 clonal lineage. None of the isolates were neutralized by the UCA or intermediate (I) 6 antibody that was most closely related to the DH511 UCA. Antibody I2 and later members of the lineage acquired the ability to neutralize 12/12 isolates (**Supplementary Table 19**). DH511 clone acquisition of breadth was associated with the accumulation of somatic mutations, but neutralization potency did not directly correlate with percent V_H mutation frequency. Analysis of a panel of MPER peptides and MPER peptide liposomes did not reveal constructs that bound to the UCA. Binding to the MPER peptides was acquired at the I5 stage of maturation (**Figures 66 and 67**).

[0396] Polyreactivity/autoreactivity of the DH511 Clonal Lineage

[0397] The DH511 inferred UCA and intermediates I1-I3 and I6 reacted with several autoantigens as measured by ELISA (**Figures 68-69**) and were found to exhibit polyreactivity

in a protein microarray against 9,400 human proteins (3) (**Figure 68**). The mature members of the lineage were not polyreactive by ELISA, although some members demonstrated polyreactivity by microarray analysis (DH511.1, DH511.5, DH511.6, and DH511.12P). All DH511 lineage members lacked reactivity by indirect immunofluorescence human epithelial (HEp-2) cell staining assay. Regarding higher affinity autoreactivity with single proteins, mature bnAb DH511.2 reacted with the E3 ubiquitin ligase STIP1 Homology and U-Box Containing Protein 1 (STUB1) while both DH511.11P and DH511.12P reacted with nuclear distribution gene C homolog (*A. nidulans*) (NUDC); DH511.12 also reacted with Scm-like with four MBT domains protein 1 (SFMBT1) (**Figure 68**).

[0398] To characterize the lipid reactivity of the DH511 clonal lineage, we first determined propensity for lipid membrane binding/insertion of DH511.1-DH511.6 based on HCDR3 hydrophobicity. Three or more Phe or Trp amino acid residues were contained within the HCDR3 sequences of each DH511 clonal lineage member, and several members were found to have at least one Pro, with the exception of DH511.3 and DH511.6. A membrane insertion score was calculated based on the Wimley-White hydrophobicity scale, which measures the propensity of amino acids to sit at the interface of the head and tail group in a lipid bilayer. Notably, membrane insertion scores were similar between the most potent neutralizer DH511.2 and 4E10/10E8 but differed from 2F5 (**Supplementary Table 21**).

[0399] To further delineate the interaction of DH511 clonal members with the lipid bilayer interface, we determined cardiolipin reactivity and kinetics of binding to MPER peptide versus MPER peptide-liposome conjugates. The UCA and members of the memory B cell clonal lineage did not bind cardiolipin in ELISA (**Supplementary Table 22**). The binding of gp41 bnAbs 2F5 and 4E10 to gp41-lipid complex has been proposed as a sequential two-step process, in which encountering the lipid membrane takes place first, presumably to aid in docking of the antibody with the transiently exposed gp41 intermediate neutralizing epitope during the virion-host cell fusion process (4, 22, 23). Surface plasmon resonance (SPR) analysis of DH511 lineage fragments of antigen binding (Fabs) demonstrated that DH511.1-DH511.6 and intermediates I1-I5 bound the MPER peptide (NEQELLELDKWASLWNWFDITNWLWYIR (SEQ ID NO:2)) with nanomolar affinity (K_d range: 11.1-99.9 nM), while the inferred UCA and intermediate 6 (most closely related to the UCA) did not bind (**Figure 66**). Binding kinetics studies as show in **Figure 71**), support

the hypothesis that like 2F5 and 4E10, DH511 lineage antibodies bind in a two-step conformational change model.

[0400] To determine the impact of timing of the gp41 intermediate epitope exposure on HIV-1 neutralization (24), we compared the window of time in which bnAbs DH511.2, 10E8, and 4E10 could neutralize the tier 2 HIV-1 strain B.BG1168 after virus addition to TZM-bl cells. The lifetime of neutralization for DH511.2 ($t_{1/2}$: 26.8 ± 2.3 min) was the same as that for bnAbs 10E8 ($t_{1/2}$: 25.6 ± 2.5 min) and 4E10 ($t_{1/2}$: 28.2 ± 3.5 min), similar to the published half-life of fusion inhibition by the gp41 intermediate mimic T20 (20.2 ± 0.5 min) (24). These results suggest that DH511.2 recognizes a transiently exposed intermediate state of gp41 (25).

[0401] Engineering DH511 Clonal Lineage Members for Enhanced Potency

[0402] To identify more potent variants of the DH511 clonal lineage, we generated 91 chimeric mAbs by swapping the heavy and light-chains of DH511.2 with those of DH511 lineage members derived from the plasma. Of the 91 chimeric antibodies, one variant, DH511.2_K3 (comprised of the DH511.2 heavy-chain reconstituted with the plasma light-chain of DH511.8P), showed greater potency than 10E8 (**Supplementary Table 24**). DH511.2_K3 neutralization data are shown in Figure 28 and 58.

[0403] Sixteen HCDR3 mutations of DH511.2 were made (**Figures 30-33**) to determine effect on DH511.2 potency. Figure 34 shows neutralization data for sixteen of these antibodies. Additional mutations will be made, including combinations of mutations, from the mutations listed in Figures 30-31.

[0404] Discussion

[0405] We have used a combination of memory B cell sorting (26, 27) and plasma antigen-specific antibody characterization by HCDR3 mass spectrometry sequencing to simultaneously characterize class-switched memory B cell antibodies and plasma antibodies (15, 28-30). The memory B cell repertoire contains multiple specificities of antibodies reflective of an individual's immune history (30) whereas primary contributors to plasma antibodies are both long lived plasma cells as well as shorter lived plasma cells derived from terminally differentiated memory B cells in response to current antigens (16). However, evidence exists that for non-HIV-1 antigens such as influenza (11) and West Nile virus (12), not all of the memory B cell repertoire is found in plasma. Here we demonstrate that class-switched memory B cells and plasma shared the same clonal lineage members of highly broad and potent HIV-1 gp41 neutralizing antibodies.

[0406] In the case of HIV-1 antibody responses, the relationship of the memory B cell and plasma antibody pools is complicated by the damage that HIV-1 inflicts on the B cell lineage with disruption of the germinal center in the earliest stages of infection (31), and the accumulation of FcRL4+ memory B cells in chronic infection (8). Interestingly, HIV-1-specific B cell responses are enriched in the FcRL4+ memory B cell compartment and exhibit many features of premature exhaustion (8). Regarding antibodies that target the Env bnAb epitope at the CD4 binding site, it has been shown that ~60% of this response is contained within the exhausted FcRL4+ memory B cell compartment, thus preventing their progression to plasma cells and production of secreted antibody (8, 9). In contrast, Scheid and colleagues studied antigen-specific memory B cell repertoires in HIV-1 infected individuals and found broad diversity of neutralizing antibodies (32). Moreover, analysis has demonstrated bnAb activity in plasma can predict isolation of bnAb variable heavy (V_H) and variable light (V_L) from memory B cells from the same individual (13, 33-38). Moreover, only a limited number of bnAb specificities are generally present in HIV-1-infected plasma (38, 39), and when bnAbs are isolated from memory B cells in clonal memory B cell cultures, the bnAbs are the minority of the Env specificities isolated (26, 37, 40). Thus, in spite of early damage to B cell follicles and accumulation of memory B cells with an exhaustion phenotype, HIV-1 infected individuals can make productive, albeit subdominant, bnAb responses that progress to plasma cell differentiation and secretion into blood plasma.

[0407] A critical question is whether memory B cells in HIV-1 infected individuals are differentiating into the long-lived plasma cell pool that resides in bone marrow and is responsible for long-lived plasma antibody responses (41). We have previously studied the effect of anti-retroviral treatment in HIV-1 infection on the half-lives of Env gp120 and gp41 as well as Gag antibody responses, and demonstrated whereas Env antibody half-life was short for gp120 (81 weeks) and gp41 (33 weeks), antibody half-life was longer for Gag (648 weeks). In contrast, in the same individuals, the half-life of influenza antibodies did not decay over the time studied (42). These data demonstrate that in chronic HIV-1-infection, the cells making plasma gp41 antibodies are not long-lived plasma cells.

[0408] Thus, by directly measuring the gp41 broad neutralizing repertoire in memory B cells and plasma, we have directly demonstrated the survival from immune damage of memory B cells to produce plasma broadly neutralizing antibodies. Finally, we show that blood plasma is a rich source for isolation of potent bnAbs for recombinant antibody production and for

constructing chimeric memory B cell/plasma antibodies for enhancing antibody potency and breadth.

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[0452] Supplementary Materials and Methods

[0453] Donor Information

[0454] Plasma and peripheral blood mononuclear cells were collected from South African donor CH0210, chronically infected with a clade C virus for an unknown period at the time of enrollment in the Center for HIV/AIDS Vaccine Immunology (CHAVI) 001 chronic HIV-1 infection cohort (previously described in (33)). Informed consent was obtained under clinical protocols approved by the Institutional Review Board of the Duke University Health System and clinical site in South Africa. The DH511 bnAb lineage was isolated from PBMC and plasma collected at 8 weeks post-study enrollment, where the viral load was 5,180 copies/ml and CD4 T cell count was unknown, at which time donor CH0210 had not initiated anti-retroviral therapy (ART).

[0455] Epitope Mapping and Neutralization-based Epitope Prediction Analysis

[0456] Donor CH0210 plasma was screened for neutralization breadth utilizing standard experimental mapping and computational methods for epitope prediction (13, 43). Anti-MPER bnAb activity was detected using two different assays: plasma neutralization of the HIV-2/HIV-1 MPER chimeric pseudovirus C1C and plasma adsorption with MPER peptide coated magnetic beads, followed by testing of adsorbed plasmas for reduction of neutralization activity as described previously (44). An algorithm for Neutralization-based Epitope Prediction (NEP) (13, 43) was used to delineate the specificities mediating breadth against a panel of 21 diverse HIV-1 strains. The resulting linear coefficients on a scale of (0 to 1) from the computational procedure was used to predict the relative prevalence of each of the reference antibody specificities in donor CH0210 plasma.

[0457] Antigen-specific Single Memory B Cell Sorting and Antibody Expression

[0458] As previously described (14), fluorescently-labeled MPER peptide tetramer probes were generated using biotinylated MPR.03 peptide (KKKNEQELLELDKWASLWNWFDITNWLWYIRKKK-biotin SEQ ID NO:463) (CPC Scientific Inc., San Jose, CA) conjugated to fluorophore-labeled streptavidins, yielding a tetramer with four MPER epitopes for surface Ig cross-linking. Eleven and a half million PBMC from donor CH0210 were stained with MPR.03-Alexa647 and MPR.03-Brilliant Violet 421 peptide tetramers and a cocktail of antibodies to identify MPER-specific memory B cells: surface IgM (FITC), surface IgD (phycoerythrin [PE]), CD3 (PE-Cy5), CD16 (Brilliant Violet 570), CD235a (PE-Cy5), and CD19 (allophycocyanin [APC]-Cy7) (BD Biosciences, San Jose, CA); CD14 (Brilliant Violet 605) (Invitrogen, Carlsbad, CA); CD27 (PE-Cy7), CD38 (APC-Alexa Fluor 700) (Beckman Coulter, Brea, CA), and CD10 (ECD) (Beckman Coulter, Brea, CA). Aqua blue vital dye (Invitrogen, Carlsbad, CA) was used to stain dead cells. Using a four laser FACS Aria cell sorter and FACSDiva software (BD Biosciences, San Jose, CA), MPR.03 double positive CD16-CD14-CD3-CD235-CD19+IgD-CD38hi memory B cells were single cell sorted into individual wells of a 96-well plate containing reverse transcription (RT) reaction buffer (5 μ L of 5' first-strand cDNA buffer, 0.5 μ L of RNaseOUT [Invitrogen, Carlsbad, CA], 1.25 μ L of dithiothreitol, 0.0625 μ L Igepal CA-630 [Sigma, St. Louis, MO], 13.25 μ L of distilled H₂O [dH₂O; Invitrogen, Carlsbad, CA]). Data were further analyzed using FlowJo software (TreeStar, Ashland, OR). Plates were stored at -80°C until PCR could be performed.

[0459] PCR Amplification and Expression of Ig Genes

[0460] Immunoglobulin genes were amplified from RNA of isolated cells by reverse transcription- polymerase chain reaction (RT-PCR). For RT, 10 mM dNTPs (New England Biolabs, Ipswich, MA), 3 μ L random hexamers at 150 ng/ml (GeneLink, Hawthorne, NY), and 1 μ L SuperScript ® III (Invitrogen, Carlsbad, CA) were added to each well and subjected to thermocycling under the following conditions: 42°C for 10 minutes, 25°C for 10 minutes, 50°C for 60 minutes and 94°C for 5 minutes. IgH, Ig κ , and Ig λ variable region genes were separately amplified from the cDNA by nested PCR, using AmpliTaq Gold ® 360 Mastermix (Invitrogen, Carlsbad, CA), heavy-chain (45) and light-chain gene-specific primers as previously described (46). PCR amplicons were purified and sequenced, and V_HDJ_H and V_LJ_L genes, mutation frequencies, and CDR3 lengths were determined using the Clonanalyst

software (47). Clonal relatedness and inference of the unmutated common ancestor (UCA) and intermediate antibodies were determined by computational methods as described in (26, 40, 48). Maximum likelihood phylogenetic trees were constructed from V(D)J sequences using the Phylogeny Inference Package (PHYLP) (version 3.69; (49)). Transient small-scale expression of antibodies was achieved by overlapping PCR assembly of variable heavy and light-chain gene pairs into IgH, Igκ, and Igλ linear expression cassettes for production of full length IgG1 mAbs by transfection into 293T cells as described previously (46). Supernatants were screened for HIV-1 Env binding by ELISA and neutralization activity in TZM-bl cells. For large scale antibody production, antibody variable heavy-chain and light-chain genes were de novo synthesized (GenScript, Township, NJ), cloned into pcDNA3.1 expression vectors containing the constant regions of IgG1 (46), and co-transfected at equal ratios in Expi 293i cells using ExpiFectamine 293 transfection reagents (Thermo Fischer Scientific, Waltham, MA) according to the manufacturer's instructions. Culture supernatants were harvested and concentrated after 4-5 days incubation at 37°C and 8% CO₂, followed by affinity purification by protein A column (Pierce, Thermo Fisher Scientific, Waltham, MA). Antibody purity was evaluated by SDS-Page and Coomassie Blue staining for heavy and light-chains of the appropriate size.

[0461] ELISA Assays

[0462] Binding of transiently transfected supernatants and mAbs to HIV-1 Env proteins and peptides was detected by enzyme-linked immunosorbent assay (ELISA). High-binding 384-well plates (Corning, Oneonta, NY) were coated overnight at 4°C or for 2 hours at room temperature with 2 µg/ml HIV-1 protein or streptavidin (for detection of binding to biotinylated peptides) in 0.1 M sodium bicarbonate (Sigma Aldrich, St. Louis, MO). Plates were blocked for 1 hour at room temperature with assay diluent comprised of phosphate buffered saline (PBS), 4% (weight/volume) whey protein (BiPro USA, Prairie, MN), 15% normal goat serum (Invitrogen, Carlsbad, CA), 0.5% Tween 20, and 0.05% sodium azide (Sigma Aldrich, St. Louis, MO), followed by a 1 hour incubation with antibody at a starting concentration of 100 µg/ml, serially diluted 3-fold. Horseradish peroxidase-conjugated goat anti-human IgG Fc antibody (Jackson ImmunoResearch Laboratories, West Grove, PA) was added to each well and incubated for 1 hour, after which plates were washed with PBS/0.1% Tween 20 and developed with SureBlue Reserve TMB One Component Microwell Peroxidase Substrate for 15 minutes (KPL, Gaithersburg, MD). Development was stopped

with 0.1 M HCl, and plates were read at 450 nm. Experiments were performed in duplicate, and results were reported as logarithm area under the curve (Log AUC). For epitope mapping, purified mAbs were screened as listed above against a panel of MPR.03 alanine scanned peptides. Epitope positions were defined by MPR.03 alanine scan mutations that reduced the Log AUC by >50% compared to the wild-type peptide.

[0463] Neutralization Assays

[0464] Neutralization assays were performed using HIV-1 Env pseudoviruses to infect TZM-bl cells as previously described (50, 51). A five-parameter hill slope equation was used to fit neutralization curves by non-linear regression and for determination of maximum percent inhibition (MPI) values. Titers were calculated as 50% or 80% inhibitory concentrations (IC₅₀ and IC₈₀) and reported as the concentration of antibody causing a 50% or 80% reduction in relative luminescence units compared to virus control wells. Mapping of the MPER residues critical for neutralization was performed using a panel of alanine scanned COT6.15 Env pseudoviruses as described previously (20, 21).

[0465] Poly/autoreactivity Analysis

[0466] Antibody binding to a panel of nine autoantigens, including Sjogren's syndrome antigen (SSA), SSB, Smith antigen (Sm), ribonucleoprotein (RNP), scleroderma 70 (Scl-70), Jo-1, double-stranded DNA (dsDNA), centromere B (Cent B), and histone, was quantified by ELISA. Anti-cardiolipin reactivity was measured using the QUANTA Lite ACA IgG III ELISA kit (Nova Diagnostics, San Diego, CA) per the manufacturer's instructions as previously described (52). Antibodies were assayed for reactivity to the human epithelial cell line (HEp-2) by indirect immunofluorescence staining using the IFA ANA/Hep-2 Test System (Zeus Scientific, Somerville, NJ) per the manufacturer's protocol. Antibodies were diluted to 50 µg/ml and 25 µg/ml and scored negative or positive (1+ to 4+) at each dilution. Antibodies were also screened for binding to a panel of >9,400 human proteins using a Protoarray microarray (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions and as described in (6). Briefly, the array was blocked and incubated on ice with 2 µg/ml HIV-1 antibody or the isotype control antibody, human myeloma protein, 151K (Southern Biotech, Birmingham, AL) for 90 minutes. Antibody binding was detected with 1 µg/ml anti-human IgG-Alexa-647 secondary antibody (Invitrogen). Arrays were scanned using a GenePix 4000B scanner (Molecular Devices, Sunnyvale, CA) at a wavelength of 635 nm, 10 µm resolution, using 100% power and 650 gain. The fluorescence intensity of antibody

binding was measured with the GenePix Pro 5.0 program (Molecular Devices, Sunnyvale, CA).

[0467] Surface Plasmon Resonance Affinity and Kinetics Measurements

[0468] Surface plasmon resonance analysis was performed on a Biacore 3000 instrument (GE Healthcare, Little Chalfont, UK) at 25°C and data analyzed using the BIAevaluation 4.1 software (Biacore) as described previously (Alam et al. JI 2007). To determine the affinity, association and dissociation rate constants of the DH511 clonal lineage to MPER, biotinylated MPR.03 peptide was coated on streptavidin sensors at a density of 58 response units (RUs). DH511 lineage Fabs were injected over flow cells at increasing concentrations at a flow and minute dissociation steps. Curves were blank surface and CH58 Fab analyte subtracted. Peptide-liposome conjugates were generated with MPER656.1-GTH1 peptides using an extrusion method (4) and analyzed for binding in a two-step encounter docking model as described previously (4).

[0469] Time Course of DH511.2 Neutralization

[0470] The time course of DH511.2 neutralization was determined using a post-attachment HIV-1 pseudotyped virus neutralization assay described previously (53). Inhibitory concentrations of DH511.2, 10E8, and 4E10 mAb were added to TZM-bl cells incubated with B.BG1168 virus at different time intervals after infection. Infectivity was measured in relative light units (RLUs).

[0471] High-throughput Paired VH:VL Sequencing of Immunoglobulin Transcripts

[0472] Material & reagents. Protein G Plus agarose, NeutrAvidin agarose, immobilized pepsin resin and Hypersep SpinTip C18 columns (C18-SpinTips) were acquired from Pierce (Thermo Fisher Scientific, Rockford, IL). TRIS hydrochloride (Tris-HCl), ammonium bicarbonate (NH_4HCO_3), 2,2,2-trifluoroethanol (TFE), dithiothreitol (DTT), and iodoacetamide (IAM) were obtained from Sigma-Aldrich (St. Louis, MO). LC-MS grade water, acetonitrile (ACN), and formic acid were purchased from EMD (Billerica, MA).

[0473] Isolation of memory B cells. Frozen PBMCs (10 million cells in 1 mL) were thawed at 37°C, resuspended in 50 mL of RPMI 1640 (Lonza) supplemented with 10% Fetal Bovine Serum, 1x non-essential amino acids, 1x sodium pyruvate, 1x glutamine, 1x penicillin/streptomycin, and 20 U/mL DNase I, and recovered via centrifugation (300 g for 10 min at 20°C). The cells were then resuspended in 4 mL of RPMI and allowed to recover at 37°C for 30 min. The cells were diluted with 10 mL of cold MACS buffer (PBS

supplemented with 0.5% BSA and 2 mM EDTA), collected by centrifugation (300 g for 10 min at 4°C), and depleted of non-B cells using the Human Memory B Cell Isolation Kit with an LD column (Miltenyi Biotec) as per the manufacturer's instructions. This yielded 400,000 – 500,000 B cells per vial.

[0474] Amplification of the paired VH:VL repertoire. The paired VH and VL sequences were then determined using a custom designed axisymmetric flow focusing device (19) that is comprised of three concentric tubes. Total B cells were suspended in 6 mL of cold PBS and passed through the innermost tube at a rate of 0.5 mL/min. Oligo d(T)₂₅ magnetic beads (1 µm diameter at a concentration of 45 µL beads/mL solution; NEB) were washed, subjected to focused ultrasonication (Covaris) to dissociate any aggregates, resuspended in 6 mL of lysis buffer (100 mM Tris-HCl pH 7.5, 500 mM LiCl, 10 mM EDTA, 1% Lithium dodecyl sulfate (LiDS), 5 mM DTT), and passed through the middle tube at a rate of 0.5 mL/min. The outer tubing contained an oil phase (mineral oil containing 4.5% Span-80, 0.4% Tween-80, and 0.05% Triton X-100; Sigma-Aldrich) flowing at 3 mL/min. The cells, beads, and lysis buffer were emulsified as they passed through a custom designed 120 µm diameter orifice, and were subsequently collected in 2 mL microcentrifuge tubes. Each tube was inverted several times, incubated at 20°C for 3 minutes, and then placed on ice. Following the collection phase, emulsions were pooled into 50 mL conicals, and centrifuged (4,000 g for 5 min at 4°C). The mineral oil (upper phase) was decanted, and the emulsions (bottom phase) were broken with water-saturated cold diethyl ether (Fischer). Magnetic beads were recovered following a second centrifugation step (4,000 g for 5 min at 4°C) and resuspended in 1 mL of cold Buffer 1 (100 mM Tris pH 7.5, 500 mM LiCl, 10 mM EDTA, 1% LiDS, 5 mM DTT). The beads were then serially pelleted using a magnetic rack, and washed with the following buffers: 1 mL lysis buffer, 1 mL Buffer 1, and 0.5 mL Buffer 2 (20 mM Tris pH 7.5, 50 mM KCl, 3 mM MgCl). The beads were split into two aliquots, and each was then pelleted one final time and resuspended in an RT-PCR mixture (19) containing VH and VL Framework Region 1 (FR1) linkage primers or VH and VL leader peptide (LP) linkage primers (**Supplementary Tables 28 and 29**). The RT-PCR mixtures were then added dropwise to 9 mL of chilled oil phase in an IKA dispersing tube (DT-20, VWR) and emulsified using an emulsion dispersing apparatus (Ultra-Turrax® Tube Drive; IKA) for 5 min. The emulsions were aliquoted into 96-well PCR plates (100 µL/well), and subjected to RT-PCR under the following conditions: 30 min at 55°C followed by 2 min at 94°C; 4 cycles of 94°C for 30 s, 50°C for 30 s, 72°C for 2

min; 4 cycles of 94°C for 30 s, 55°C for 30 s, 72°C for 2 min; 32 cycles of 94°C for 30 s, 60°C for 30 s, 72°C for 2 min; 72°C for 7 min; held at 4°C.

[0475] Following RT-PCR, the emulsions were collected in 2 mL microcentrifuge tubes and centrifuged (16000 g for 10 min at 20°C). The mineral oil (upper phase) was decanted, and water-saturated ether was used to break the emulsions. The aqueous phase (containing the DNA) was extracted three times by sequentially adding ether, centrifuging the samples (16000 g for 30 s at 20°C), and removing the upper ether phase. Trace amounts of ether were removed using a SpeedVac for 30 min at 20°C. The DNA amplicons were purified using a silica spin column (Zymo-Spin™ I, Zymo Research) according to the manufacturer's instructions, and eluted in 40 µL H₂O. The two samples were then amplified through a nested PCR (see **Supplementary Table 30** for primers) using Platinum Taq (Life Technologies) under the following conditions: (FR1 primer derived sample) 2 min at 94°C, 32 cycles of 94°C for 30 s, 62°C for 30 s, 72°C for 20 s; 72°C for 7 min; held at 4°C; (LP primer derived sample) 2 min at 94°C, 27 cycles of 94°C for 30 s, 62°C for 30 s, 72°C for 20 s; 72°C for 7 min; held at 4°C. The amplicons, approximately 850 bp in length, were gel purified from 1% agarose using a gel extraction kit (Zymo Research) according to the manufacturer's instructions, and eluted in 20 µL H₂O.

[0476] To determine the full length VH and VL reads for antibody expression studies, the paired amplicon was subjected to an additional PCR using NEBNext high fidelity polymerase (NEB) to specifically amplify the full VH chain and the full VL chain separately in addition to the paired chains (Note: the paired reads sequence the entire J- and D-regions, and the fragment of the V regions spanning FR2 to CDR3). Each sample was split into 5 reactions and subjected to the following PCR conditions: 30 s at 98°C, X cycles of 98°C for 10 s, 62°C for 30 s, 72°C for Y s; 72°C for 7 min; held at 4°C (See **Supplementary Table 31** for the PCR conditions and **Supplementary Table 32** for the primer sequences). Finally, these sequences were amplified one final time with TSBC compatible barcoding primers following the protocol shown in **Supplementary Table 33**, gel purified from 1% agarose using a gel purification kit according to manufacturer's instructions, and submitted for paired-end Illumina NGS.

[0477] Bioinformatic analysis of NGS data. Raw 2x300 MiSeq reads were quality filtered (minimum Phred score of 20 over half of the nucleotide sequence) and submitted to MiXCR (54) for CDR3 identification and gene annotation. Productive VH and VL reads were paired

by Illumina MiSeq ID using a custom python script. Full length VH and VL reads were stitched together using FLAsH (55) and then quality filtered. Full length VH and VL constructs were designed by matching the paired CDRH3:CDRL3 nucleotide sequences to the respective CDR3 in the full length VH and VL libraries.

[0478] Sample preparation & LC-MS/MS analysis. Serum IgG from donor 0210 was purified by Protein G Plus agarose affinity chromatography, and F(ab')₂ fragments were generated by digestion with immobilized pepsin. Antigen-specific F(ab')₂ was isolated by affinity chromatography with the biotinylated MPER peptide coupled to NeutrAvidin agarose and eluted in 100 mM glycine pH 2.7. The collected fractions were neutralized and the protein containing fractions were pooled and prepared for LC-MS/MS as described previously (10). Briefly, protein samples were concentrated and resuspended in 50% (v/v) TFE, 50 mM NH₄HCO₃ and 2.5 mM DTT and incubated at 55°C for 45 min. The reduced samples were then alkylated with IAM in the dark, at room temperature for 30 min. The reaction was quenched by addition of DTT and the samples were diluted to 5% TFE and digested with trypsin (trypsin/protein ration of 1:75 at 37°C for 5 h). The digestion was stopped by addition of formic acid to 1% (v/v). The samples were then concentrated by SpeedVac, resuspended in 5% ACN, 0.1% formic acid and the peptides were washed on C18-SpinTips according to the manufacturer's protocol. Subsequently, the peptides were separated by reverse phase chromatography (Dionex UltiMate 3000 RSLCnano system with Dionex Acclaim PepMapRSLC C18 column, Thermo Scientific) and analyzed on-line by nano-ESI tandem MS on an Orbitrap Velos Pro (Thermo Scientific). MS1 scans were collected in the orbitrap at 60,000 resolution and ions with >+1 charge were fragmented by CID with up to 20 MS2 spectra collected per MS1.

[0479] Computational interpretation of peptide mass spectra. Full length VH and VL sequencing data (see above) was submitted to the IMGT/HighV-Quest Tool (56) for annotation and unique full length VH sequences were clustered into clonotypes according to their CDRH3 sequences with a cut-off of 85% identity as described previously (29). The sample-specific target protein sequence database was constructed from the full-length VH and VL sequences mentioned above (≥2 reads), Ensembl human protein-coding sequences and common contaminants (maxquant.org). The spectra were then searched against this database using the SEQUEST (Proteome Discoverer 1.4, Thermo Scientific) with previously described settings (15). The resulting PSMs were filtered with Percolator (Proteome Discoverer 1.4) to

control false discovery rates (FDR) to <1% and the average mass deviation (AMD) was calculated for all high-confidence PSMs and peptides with an AMD of <1.5 ppm were kept for the final dataset. Informative peptides, as defined previously (15), were grouped by their CDRH1, 2 or 3 association and for each group the abundances of the corresponding clonotypes were determined by the sum of the extracted-ion chromatograms of the respective precursor ions.

[0480] Crystallization, Structure Determination, and Structural Analysis.

[0481] Purified DH511.1 and DH511.2 fragments of antigen binding (Fabs) were set up in crystallization trials in complex with a panel of gp41 MPER peptides. For each complex, 576 initial conditions from commercially available screens (Hampton Research, Rigaku) were set up as vapor diffusion sitting drops robotically (TTP Labtech). Crystals of DH511 Fab in complex with gp41 MPER peptide 656-683 were obtained in a condition composed of 30% PEG 1500, while those of DH511.2 Fab in complex with peptides MPR.03.DN4 and MPR.03.DN14, were obtained in 30% PEG 1500, 10% Isopropanol, 0.1 M CaCl₂, 0.1 M Imidazole pH 6.5 and in 20% PEG 8000, 10% PEG 400, 0.5 M NaCl, 0.1 M C₂H₃NaO₂ pH 5.5, respectively. Crystal hits were hand optimized and X-ray diffraction data extended to 2.8, 2.65, and 2.2 Å, respectively. Data was processed with HKL-2000 (57) and structures were solved by molecular replacement using the DH514 Fab unliganded structure as a search model in Phaser (58). The structures were refined to R_{crystal}/R_{free} of 21.28/25.57, 25.61/28.99, and 19.03/22.63%, respectively, using Phenix (59) combined with iterative model building in Coot (60). Interactive surfaces were determined using Pisa (61) and structural alignments using LSQKAB (62). All graphical images were prepared with Pymol (PyMOL Molecular Graphics System). X-ray diffraction data was collected at SER CAT ID-22 or BM-22 beamlines of the Advanced Photon Source (Argonne, IL), under General User Proposal 44127 (G.O.).

[0482] Supplemental References

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Example 13: Exemplary HIVxCD3 Bispecific Diabodies with Fc Domains

[0545] Provided herein are stable, structurally compact, bispecific diabodies that have inter-chain disulfide bonds that may be engineered with an Fc Domain to extend serum half-life. The antigen binding arms of such bispecific diabodies are advantageously selected to co-engage immune effector cells (*e.g.*, T cells, NK cells, *etc.*) with antigen-expressing target cells (*e.g.*, HIV-1 infected cells) and activate and redirect the cytolytic activity of immune effector cell against the antigen expressing target cells. Provided herein are multispecific molecules which comprise fully human antigen binding portions of HIV-1 antibodies, and variants thereof, that specifically bind to and potently neutralize various isolates of HIV-1. The HIV-1 antibodies provided herein (*e.g.*, DH511, DH512, DH511.2_K3, DH513, DH514, DH515, DH516, DH517, DH518, DH536, or DH537) specifically bind the membrane-proximal extracellular region (MPER) of gp41. Such antibodies are utilized in the generation of HIVxCD3 bispecific diabodies (and/or trivalent binding molecule as provided herein) having binding specificity for the MPER of gp41. Exemplary, HIVxCD3 bispecific diabodies, having three polypeptide chains, an HIV-1 binding arm derived from an anti-gp41 MPER antibody described herein, a CD3 effector cell binding arm derived from a humanized anti-CD3 ϵ mAb (hXRCD3), and CH2-CH3 IgG1 Fc Domains may have the general structure shown in **Figures 105A-105B**.

[0546] Three exemplary HIVxCD3 bispecific diabodies with an Fc Domain designated “DH511.2_K3xCD3 Fc,” “DH511.11PxCD3 Fc,” and “DH511.12P Fc” were designed and were expressed and tested. These molecules comprise three polypeptide chains and include

an HIV-1 binding arm derived from the DH511.2_K3, DH511.11P, or DH511.12P antibodies described herein, a CD3 effector cell binding arm derived from a humanized anti-CD3 ϵ mAb (hXRCD3), and CH2-CH3 IgG1 Fc Domains. The amino acid sequences and a representative polynucleotide encoding each polypeptide chain are presented in **Table 4**. Corresponding negative control bispecific diabodies with an irrelevant binding arm [*e.g.*, α RSV derived from palivizumab, or α fluorescein derived from 4-4-20] instead of the DH511.2_K3 HIV-1 arm, or having an HIV-1 arm [*e.g.*, α HIV derived from DH511.2_K3] with an irrelevant binding arm [α RSV or α fluorescein] instead of the CD3 arm may also be designed. In addition, a comparator bispecific diabody having an HIV-1 binding arm derived from an alternative anti-HIV-1 env antibody [*e.g.*, the A32 antibody (Protein Data Bank (PDB) ID Code 3TNM)] instead of DH511.2_K3 may be designed. The HIVxCD3 Fc bispecific diabodies are capable of simultaneously binding to HIV-1 and CD3. The control bispecific diabodies are capable of simultaneously binding to RSV (or any other non-HIV antigen) and CD3, or of simultaneously binding to HIV-1 and an irrelevant antigen. Each of the exemplary bispecific diabodies is a heterotrimer of polypeptide Chains 1, 2 and 3 have the general structure provided in **Figure 103A** (also see, *e.g.*, **Figures 105A-105B**). Chains 1 and 2 comprise the VH and VL Domains of DH511.2_K3, XRCD3, 4-4-20, palivizumab, or an alternative anti-HIV env antibody, as detailed above, while Chain 3 is common to other diabody molecules with this configuration.

[0547] Methods for forming bispecific diabodies, including such diabodies comprising an Fc Domain, are provided in WO 2006/113665, WO 2008/157379, WO 2010/080538, WO 2012/018687, WO 2012/162068, WO 2012/162067, WO 2014/159940, WO 2015/021089, WO 2015/026892 and WO 2015/026894.

[0548] **Table 5** provides the SEQ ID NOs for each of the polypeptide chains present in DH511.2_K3xCD3 Fc molecule. **Table 4** provides SEQ ID NOs for the VL and VH Domains of DH511.2_K3 (as provided herein), A32 (additional HIV-1 *Env* antibodies are known in the art, see, *e.g.*, WO 2016/054101), hXRCD3 (additional anti-CD3 antibodies are known in the art, see, *e.g.*, WO 2012/162067), 4-4-20, and palivizumab (additional anti-RSV antibodies are known in the art, see, *e.g.*, US 6,818,216), the SEQ ID NOs for the VL and VH Domains of the exemplary anti-CD16 mAb h3G8, anti-CD8 mAb OKT8 and anti-CD8 mAb

TRX2 are also provided. In addition, the SEQ ID NOs for representative nucleic acid encoding sequences are provided.

Table 5		
Bispecific Molecule	Polypeptide Chain Amino Acid Sequences	Nucleic Acid Encoding Sequences
HIVxCD3 Bispecific Diabody with Fc Domain (“DH511.2_K3xCD3 Fc”) (Variable domain from DH511.2_K3, binds to HIV-1 gp41 MPER)	SEQ ID NO: 555 SEQ ID NO: 557 SEQ ID NO: 559	SEQ ID NO: 556 SEQ ID NO: 558 SEQ ID NO: 560
HIVxCD3 Bispecific Diabody with Fc Domain (“DH511.11PxCD3 Fc”) (Variable domain from DH511.11P, binds to HIV-1 gp41 MPER)	SEQ ID NO: 633 SEQ ID NO: 634 SEQ ID NO: 559	
HIVxCD3 Bispecific Diabody with Fc Domain (“DH511.12PxCD3 Fc”) (Variable domain from DH511.12P, binds to HIV-1 gp41 MPER)	SEQ ID NO: 633 SEQ ID NO: 635 SEQ ID NO: 559	

[0549] Chain 1 of the **DH511.2_K3xCD3 Fc** exemplary molecule comprises: an N-terminus, the VL Domain of DH511.2_K3 (SEQ ID NO: 553), a Peptide Linker 1 (SEQ ID NO: 508), the VH Domain of hXR32 (SEQ ID NO: 500), a Peptide Linker 2 (SEQ ID NO: 510), a cysteine engineered E-coil, a Spacer Linker 3 (SEQ ID NO: 522), a knob bearing CH2-CH3 (SEQ ID NO: 531), and a C-terminus. Chain 1 of **DH511.11PxCD3 Fc** is the same as for **DH511.12PxCD3 Fc** and differs from that of **DH511.2_K3xCD3 Fc** only in it comprises VL Domain DH511_5AVK (SEQ ID NO: 625).

[0550] Chain 2 of the **DH511.2_K3xCD3 Fc** exemplary molecule comprises: an N-terminus, the VL Domain of hXR32 (SEQ ID NO: 503), a Peptide Linker 1 (SEQ ID NO: 508), the VH Domain of DH511.2_K3 (SEQ ID NO: 551), a Peptide Linker 2 (SEQ ID NO: 510), a cysteine engineered K-coil, a Spacer Linker 3 (SEQ ID NO: 521), and a C-terminus. Chain 2 of **DH511.11PxCD3 Fc** differs from that of **DH511.2_K3xCD3 Fc** only in that it comprises VH Domain DH511_5AVH-4A (SEQ ID NO: 624). Chain 2 of **DH511.12PxCD3 Fc** differs from that of **DH511.2_K3xCD3 Fc** only in that it comprises VH Domain DH511_5BVH-4A (SEQ ID NO: 626).

[0551] Chain 3 of the **DH511.2_K3xCD3 Fc** exemplary molecule comprises: an N-terminus, a Peptide Linker 3 (SEQ ID NO: 523), a hole bearing CH2-CH3 (SEQ ID NO: 533), and a C-terminus. Such bispecific diabodies, may be generated using the methods as provided above. This Chain 3 is common to each of **DH511.2_K3xCD3 Fc**, **DH511.11PxCD3 Fc**, and **DH511.12P Fc**.

[0552] For additional sequences related to this example see Table 4.

[0553] The exemplary bispecific molecules were expressed and tested in an MPER peptide binding ELISA essentially as described above to confirm HIV antigen binding activity. The exemplary bispecific binding molecules were also tested in sCD3 binding ELISA to confirm CD3 binding activity. Briefly, soluble CD3 (1 µg/mL) was coated onto a solid support, and then incubated with the exemplary bispecific molecules (at a starting concentration of 2 nM, followed by 1:4 serial dilutions). At the conclusion of the assay, binding was blocked with phosphate buffered saline (PBS) containing bovine serum albumin (BSA). Binding was detected by ELISA using anti-human IgG-that had been conjugated to horseradish peroxidase (HRP). All the molecules were found to bound to both the MPER peptide and sCD3.

[0554] **DH511.11PxCD3 Fc** and **DH511.2_K3xCD3 Fc** were also tested in a cytotoxic T lymphocyte (CTL) assay. Briefly, Jurkat 522F/Y GF HIV-1 Env-expressing target cells were treated with serial dilutions of the exemplary bispecific molecules, together with Pan T cells (isolated from healthy human PBMCs) at an E/T ratio of 10:1, and incubated at 37°C for 48 hours. Cytotoxicity was measured by luminescence. Specific lysis is calculated from luminiscence counts (RLU): cytotoxicity (%) = $100 \times (1 - [\text{RLU of Sample} / \text{RLU of Control}])$, where control equals the average RLU of target cells incubated with effector cells in the

absence of a test article. Minimal or no activity was observed for these bispecific molecules in these assays under these conditions using Pan T cells from two different donors.

[0555] The cytotoxic activity of these and other molecules of the invention will be tested in a killing assay using HIV-1 infected cells expressing HIV-1 envelope on their surface.

[0556] Without being bound by theory, other bispecific formats, for example but not limited to trivalent molecules having two HIV-1 binding arms, are expected to have improved properties, including but not limited to improved activity in the CTL assay. Non-limiting embodiments of such trivalent molecules are described in Example 14B.

Example 14A: Additional multispecific molecules

[0557] Sequences from any one of the MPER antibodies contemplated by this disclosure, including but not limited to DH511, DH512, DH512_K3, DH513, DH514, DH515, DH516, DH517, DH518, DH536, DH537, lineage members, chimeric and mutant antibodies with non-limiting embodiments shown in Example 11 could be used to construct any one of the multispecific molecule formats described herein. The effector arm could target be any one of the non-limiting examples of CD2, CD3, CD8, CD16, T-Cell Receptor (TCR), NKG2D, *etc.* epitopes. Non-limiting examples are provided in the Table below. The information in the specification can be readily used for alternative designs of the listed molecules, and for design of other bispecific molecules, for example molecules based on DH511, DH512, DH512_K3, DH513, DH514, DH515, DH516, DH517, DH518, DH536, DH537, lineage members chimeric and mutant antibodies with non-limiting embodiments shown in Example 11 lineage members using CDRs, or VH and VL chains from these HIV-1 antibodies.

[0558] **Table 6** shows a summary of some additional non-limiting embodiments of bispecific diabody (with and without Fc Domains) that may be generated comprising alternative HIV-1 and/or alternative effector cell binding specificities. The information in specification can be readily used for alternative design of the listed bispecific diabodies, and for design of other bispecific diabodies, for example bispecific diabodies comprising a CD16 binding specificity instead of CD3 and/or incorporating the HIV-1 binding specificity of alternative DH511 lineage members, DH512, DH512_K3, DH513, DH514, DH515, DH516,

DH517, DH518, DH536, DH537 using CDRs, or VH and VL chains from these antibodies as provided herein.

Table 6					
Bivalent HIVxCD3 (or HIV-1 x CD16)					
Heterodimer of Chains 1 and 2 (see, <i>e.g.</i> , Figure 101)					
Polypeptide Chain 1		Polypeptide Chain 2		Polypeptide Chain 3	none
NH ₂ -VL(HIV-1)	SEQ ID NOs: 553 570 574 578 582 586 590 594 598 602 606 625	NH ₂ -VL(CD3) or NH ₂ -VL(CD16)	SEQ ID NO: 502 or SEQ ID NO: 506		
Peptide Linker 1	SEQ ID NO: 508	Peptide Linker 1	SEQ ID NO: 508		
VH(CD3) or VH(CD16)	SEQ ID NO: 500 or SEQ ID NO: 504	VH(HIV-1)	SEQ ID NOs: 551 568 572 576 580 584 588 592 596 600 604 608-624 625		

Peptide Linker 2	SEQ ID NO: 509 or 510	Peptide Linker 2	SEQ ID NO: 509 or 510		
K-coil	SEQ ID NO: 520 or 521	E-coil	SEQ ID NO: 518 or 519		
or	or	or	or		
E-coil	SEQ ID NO: 518 or 519	K-coil	SEQ ID NO: 520 or 521		
Bivalent HIVxCD3 Fc (or HIV x CD16 Fc) version 1 Heterotrimer of Chains 1, 2 and 3 (see, e.g., Figure 103A)					
Polypeptide Chain 1	(e.g., SEQ ID NO: 555)	Polypeptide Chain 2	(e.g., SEQ ID NO: 557)	Polypeptide Chain 3	(e.g., SEQ ID NO: 559)
NH ₂ - VL(HIV-1)	SEQ ID NOs: 553 570 574 578 582 586 590 594 598 602 606 625	NH ₂ - VL(CD3) or NH ₂ - VL(CD16)	SEQ ID NO: 502 or SEQ ID NO: 506		
Peptide Linker 1	SEQ ID NO: 508	Peptide Linker 1	SEQ ID NO: 508		

VH(CD3) or VH(CD16)	SEQ ID NO: 500 or SEQ ID NO: 504	VH(HIV-1)	SEQ ID NOs: 551 568 572 576 580 584 588 592 596 600 604 608-624 626		
Peptide Linker 2	SEQ ID NO: 509 or 510	Peptide Linker 2	SEQ ID NO: 509 or 510		
K-coil or E-coil	SEQ ID NO: 520 or 521 or SEQ ID NO: 518 or 519	E-coil or K-coil	SEQ ID NO: 518 or 519 or SEQ ID NO: 520 or 521		
Peptide Linker 3 or Spacer Linker 3	SEQ ID NO: 523 or 522			NH ₂ -Peptide Linker 3	SEQ ID NO: 523
CH2-CH3 (knob bearing) or CH2-CH3 (hole bearing)	SEQ ID NO: 531 or 532 or SEQ ID NO: 533 or 534			CH2-CH3 (hole bearing) or CH2-CH3 (knob bearing)	SEQ ID NO: 533 or 534 or SEQ ID NO: 531 or 532
Bivalent HIVxCD3 Fc (or HIV x CD16 Fc) version 2 Heterotrimer of Chains 1, 2 and 3 (see, e.g., Figure 103B)					

Polypeptide Chain 1		Polypeptide Chain 2		Polypeptide Chain 3	
NH ₂ -Peptide Linker 3	SEQ ID NO: 523			NH ₂ - Peptide Linker 3	SEQ ID NO: 523
CH ₂ -CH ₃ (knob bearing) or CH ₂ -CH ₃ (hole bearing)	SEQ ID NO: 531 or 532 or SEQ ID NO: 533 or 534			CH ₂ -CH ₃ (hole bearing) or CH ₂ -CH ₃ (knob bearing)	SEQ ID NO: 533 or 534 or SEQ ID NO: 531 or 532
Peptide Linker 4 or 4.1	SEQ ID NO: 524 or 525				
VL(HIV-1)	SEQ ID NOs: 553 570 574 578 582 586 590 594 598 602 606 625	NH ₂ -VL(CD3) or NH ₂ -VL(CD16)	SEQ ID NO: 502 or SEQ ID NO: 506		
Peptide Linker 1	SEQ ID NO: 508	Peptide Linker 1	SEQ ID NO: 508		

VH(CD3) or VH(CD16)	SEQ ID NO: 500 or SEQ ID NO: 504	VH(HIV-1)	SEQ ID NOs: 551 568 572 576 580 584 588 592 596 600 604 608-624 626		
Peptide Linker 2	SEQ ID NO: 509 or 510	Peptide Linker 2	SEQ ID NO: 509 or 510		
K-coil or E-coil	SEQ ID NO: 520 or 521 or SEQ ID NO: 518 or 519	E-coil or K-coil	SEQ ID NO: 518 or 519 or SEQ ID NO: 520 or 521		
Tetravalent HIVxCD3 Fc (or HIV x CD16 Fc)					
Heterotetramer of Two Chain 1 and Two Chain 2 (see, e.g., Figure 102B)					
Polypeptide Chain 1 (x2)		Polypeptide Chain 2 (x2)		Polypeptide Chain 3	None

NH ₂ - VL(HIV-1)	SEQ ID NOs: 553 570 574 578 582 586 590 594 598 602 606 625	NH ₂ - VL(CD3) or NH ₂ - VL(CD16)	SEQ ID NO: 502 or SEQ ID NO: 506		
Peptide Linker 1	SEQ ID NO: 508	Peptide Linker 1	SEQ ID NO: 508		
VH(CD3) or VH(CD16)	SEQ ID NO: 500 or SEQ ID NO: 504	VH(HIV-1)	SEQ ID NOs: 551 568 572 576 580 584 588 592 596 600 604 608-624 626		
Peptide Linker 2	SEQ ID NO: 509 or 510	Peptide Linker 2	SEQ ID NO: 509 or 510		
K-coil or E-coil	SEQ ID NO: 520 or 521 or SEQ ID NO: 518 or 519	E-coil or K-coil	SEQ ID NO: 518 or 519 or SEQ ID NO: 520 or 521		

Peptide Linker 3 or Spacer Linker 3	SEQ ID NO: 523 or 522				
CH2-CH3	SEQ ID NO: 527, 528 or 529				

[0559] Wherein SEQ ID NOs: 570, 574, 578, 582, 586, 590, 594, 598, 602, and 606 correspond to the VL chain of antibody DH511, DH512, DH513, DH514, DH515, DH516, DH517, DH518, DH536, and DH537, respectively and as described in Table 4. Wherein SEQ ID NOs: 553, and 625 correspond to the VL chain of antibody DH511.2_K3, and DH511_5a/5b, respectively and as described in Table 4.

[0560] Wherein SEQ ID NOs: 568, 572, 576, 580, 584, 588, 592, 596, 600, 604, 608-623 correspond to the VH chain of antibody DH511, DH512, DH513, DH514, DH515, DH516, DH517, DH518, DH536, DH537, chimeras and mutants as described in Example 11, respectively and as described in Table 4. Wherein SEQ ID NOs: 551, 624, and 626 correspond to the VH chain of antibody DH511.2_K3, DH511_5a, and DH511_5b, respectively and as described in Table 4.

Example 14B: Exemplary Trivalent and Control Molecules with Fc Domains

[0561] As provided herein trivalent binding molecules having three Antigen-Binding Domains provide additional functionality as they can co-engage multiple epitopes present on the surface of an effector cell, such as a T lymphocyte (*e.g.*, CD3 and CD8), or they can bind multiple epitopes of HIV-1 (*e.g.*, epitopes of different HIV-1 isolates or different epitopes of HIV-1 *Env*, or the same epitope on multiple HIV-1 proteins). The antigen binding arms of such trivalent binding molecules are advantageously selected to co-engage immune effector cells (*e.g.*, T cells, NK cells, *etc.*) with antigen-expressing target cells (*e.g.*, HIV-1 infected cells) and activate and redirect the cytolytic activity of immune effector cell against the antigen expressing target cells.

[0562] **Table 7** shows a summary of some non-limiting embodiments of trivalent binding molecules (having 3 or 4 polypeptide chain) that may be generated. The information in specification can be readily used for alternative design of the listed trivalent binding molecules, and for design of other trivalent binding molecules, for example trivalent binding molecules comprising alternative CD8 binding specificities and/or incorporating the HIV-1 binding specificity of additional and/or alternative DH511 lineage members, DH512, DH512_K3, DH513, DH514, DH515, DH516, DH517, DH518, DH536, or DH537 using CDRs, or VH and VL chains from these antibodies.

Table 7					
HIVxCD3xCD8		HIVxCD3xCD8		HIVxCD3xHIV	
Heterotetramer of Chains 1-4 (see, e.g., Figure 104A)		Heterotrimer of Chains 1-3 (see, e.g., Figure 104D)		Heterotetramer of Chains 1-4 (see, e.g., Figure 104A)	
Polypeptide Chain 1	See e.g., SEQ ID NO: 555	Polypeptide Chain 1	See e.g., SEQ ID NO: 555	Polypeptide Chain 1	See e.g., SEQ ID NO: 555
NH ₂ -VL(HIV-1)	SEQ ID NOs: 553 570 574 578 582 586 590 594 598 602 606 625	NH ₂ -VL(HIV-1)	SEQ ID NOs: 553 570 574 578 582 586 590 594 598 602 606 625	NH ₂ -VL(HIV-1)	SEQ ID NOs: 553 570 574 578 582 586 590 594 598 602 606 625
Peptide Linker 1	SEQ ID NO: 508	Peptide Linker 1	SEQ ID NO: 508	Peptide Linker 1	SEQ ID NO: 508
VH(CD3)	SEQ ID NO: 500	VH(CD3)	SEQ ID NO: 500	VH(CD3)	SEQ ID NO: 500
Peptide Linker 2	SEQ ID NO: 509 or 510	Peptide Linker 2	SEQ ID NO: 509 or 510	Peptide Linker 2	SEQ ID NO: 509 or 510

K-coil	SEQ ID NO: 520 or 521	K-coil	SEQ ID NO: 520 or 521	K-coil	SEQ ID NO: 520 or 521
or	or	or	or	or	or
E-coil	SEQ ID NO: 518 or 519	E-coil	SEQ ID NO: 518 or 519	E-coil	SEQ ID NO: 518 or 519
Peptide Linker 3 or Spacer Linker 3	SEQ ID NO: 523 or 522	Peptide Linker 3 or Spacer Linker 3	SEQ ID NO: 523 or 522	Peptide Linker 3 or Spacer Linker 3	SEQ ID NO: 523 or 522
CH2-CH3 (knob bearing) or CH2-CH3 (hole bearing)	SEQ ID NO: 531 or 532 or SEQ ID NO: 533 or 534	CH2-CH3 (knob bearing) or CH2-CH3 (hole bearing)	SEQ ID NO: 531 or 532 or SEQ ID NO:533 or 534	CH2-CH3 (knob bearing) or CH2-CH3 (hole bearing)	SEQ ID NO: 531 or 532 or SEQ ID NO: 533 or 534
Polypeptide Chain 2	See <i>e.g.</i> , SEQ ID NO: 556	Polypeptide Chain 2	See <i>e.g.</i> , SEQ ID NO: 556	Polypeptide Chain 2	See <i>e.g.</i> , SEQ ID NO: 556
NH ₂ - VL(CD3)	SEQ ID NO: 502	NH ₂ - VL(CD3)	SEQ ID NO: 502	NH ₂ - VL(CD3)	SEQ ID NO: 502
Peptide Linker 1	SEQ ID NO: 508	Peptide Linker 1	SEQ ID NO: 508	Peptide Linker 1	SEQ ID NO: 508
VH(HIV-1)	SEQ ID NOs: 551 568 572 576 580 584 588 592 596 600 604 608-624 626	VH(HIV-1)	SEQ ID NOs: 551 568 572 576 580 584 588 592 596 600 604 608-624 626	VH(HIV-1)	SEQ ID NOs: 551 568 572 576 580 584 588 592 596 600 604 608-624 626

Peptide Linker 2	SEQ ID NO: 509 or 510	Peptide Linker 2	SEQ ID NO: 509 or 510	Peptide Linker 2	SEQ ID NO: 509 or 510
E-coil	SEQ ID NO: 518 or 519	E-coil	SEQ ID NO: 518 or 519	E-coil	SEQ ID NO: 518 or 519
or	or	or	or	or	or
K-coil	SEQ ID NO: 520 or 521	K-coil	SEQ ID NO: 520 or 521	K-coil	SEQ ID NO: 520 or 521
Polypeptide Chain 3	See <i>e.g.</i> , SEQ ID NO: 561	Polypeptide Chain 3	See <i>e.g.</i> , SEQ ID NO: 563	Polypeptide Chain 3	See <i>e.g.</i> , SEQ ID NO: 627
		NH ₂ - VL(CD8)	SEQ ID NO: 545 or 549		
		Peptide Linker 5	SEQ ID NO: 526		
NH ₂ - VH(CD8)	SEQ ID NO: 543 or 547	VH(CD8)	SEQ ID NO: 543 or 547	NH ₂ - VH(HIV-1)	SEQ ID NOs: 551 568 572 576 580 584 588 592 596 600 604 608-624 626
CH1-hinge	SEQ ID NO: 515	CH1-hinge	SEQ ID NO: 515	CH1-hinge	SEQ ID NO: 515
CH2-CH3 (knob bearing)	SEQ ID NO: 531 or 532	CH2-CH3 (hole bearing)	SEQ ID NO: 533 or 534	CH2-CH3 (knob bearing)	SEQ ID NO: 531 or 532
or	or	or	or	or	or
CH2-CH3 (hole bearing)	SEQ ID NO: 533 or 534	CH2-CH3 (knob bearing)	SEQ ID NO: 531 or 532	CH2-CH3 (hole bearing)	SEQ ID NO: 533 or 534

Polypeptide Chain 4	See <i>e.g.</i> , SEQ ID NO: 562	Polypeptide Chain 4	none	Polypeptide Chain 4	See <i>e.g.</i> , SEQ ID NO: 628
NH ₂ -VL(CD8)	SEQ ID NO: 545 or 549			NH ₂ -VL(HIV-1)	SEQ ID NOs: 553 570 574 578 582 586 590 594 598 602 606 625
CL kappa or CL lambda	SEQ ID NO: 516 or 517			CL kappa or CL lambda	SEQ ID NO: 516 or 517
HIVxHIVxCD3		HIVxCD3xHIV			
Heterotetramer of Chains 1-4 (see, <i>e.g.</i>, Figure 104A)		Heterotrimer of Chains 1-3 (see, <i>e.g.</i>, Figure 104D)			
Polypeptide Chain 1	See <i>e.g.</i> , SEQ ID NO: 629	Polypeptide Chain 1			
NH ₂ -VL(HIV-1)	SEQ ID NOs: 553 570 574 578 582 586 590 594 598 602 606 625	NH ₂ -VL(HIV-1)	SEQ ID NOs: 553 570 574 578 582 586 590 594 598 602 606 625		
Peptide Linker 1	SEQ ID NO: 508	Peptide Linker 1	SEQ ID NO: 508		

VH(HIV-1)	SEQ ID NOs: 551 568 572 576 580 584 588 592 596 600 604 608-624 626	VH(CD3)	SEQ ID NO: 500
Peptide Linker 2	SEQ ID NO: 509 or 510	Peptide Linker 2	SEQ ID NO: 509 or 510
K-coil or E-coil	SEQ ID NO: 520 or 521 or SEQ ID NO: 518 or 519	K-coil or E-coil	SEQ ID NO: 520 or 521 or SEQ ID NO: 518 or 519
Peptide Linker 3 or Spacer Linker 3	SEQ ID NO: 523 or 522	Peptide Linker 3 or Spacer Linker 3	SEQ ID NO: 523 or 522
CH2-CH3 (knob bearing) or CH2-CH3 (hole bearing)	SEQ ID NO: 531 or 532 or SEQ ID NO: 533 or 534	CH2-CH3 (knob bearing) or CH2-CH3 (hole bearing)	SEQ ID NO: 531 or 532 or SEQ ID NO:533 or 534
Polypeptide Chain 2	See <i>e.g.</i> , SEQ ID NO: 630	Polypeptide Chain 2	
NH ₂ - VL(HIV-1)	SEQ ID NO: 553	NH ₂ - VL(CD3)	SEQ ID NO: 502

	570 574 578 582 586 590 594 598 602 606 625		
Peptide Linker 1	SEQ ID NO: 508	Peptide Linker 1	SEQ ID NO: 508
VH(HIV-1)	SEQ ID NOs: 551 568 572 576 580 584 588 592 596 600 604 608-624 626	VH(HIV-1)	SEQ ID NOs: 551 568 572 576 580 584 588 592 596 600 604 608-624 626
Peptide Linker 2	SEQ ID NO: 509 or 510	Peptide Linker 2	SEQ ID NO: 509 or 510
E-coil or K-coil	SEQ ID NO: 518 or 519 or SEQ ID NO: 520 or 521	E-coil or K-coil	SEQ ID NO: 518 or 519 or SEQ ID NO: 520 or 521
Polypeptide Chain 3	See <i>e.g.</i> , SEQ ID NO: 631	Polypeptide Chain 3	
		NH ₂ -	SEQ ID NO: 553

		VL(HIV-1)	570 574 578 582 586 590 594 598 602 606 625
		Peptide Linker 5	SEQ ID NO: 526
NH ₂ - VH(CD3)	SEQ ID NO: 500	VH(HIV-1)	SEQ ID NO: 551 568 572 576 580 584 588 592 596 600 604 608-624 626
CH1-hinge	SEQ ID NO: 515	CH1-hinge	SEQ ID NO: 515
CH2-CH3 (knob bearing) or CH2-CH3 (hole bearing)	SEQ ID NO: 531 or 532 or SEQ ID NO: 533 or 534	CH2-CH3 (hole bearing) or CH2-CH3 (knob bearing)	SEQ ID NO: 533 or 534 or SEQ ID NO: 531 or 532
Polypeptide	See <i>e.g.</i> , SEQ	Polypeptide	none

Chain 4	ID NO: 632	Chain 4	
NH ₂ - VL(CD3)	SEQ ID NO: 502		
CL kappa or CL lambda	SEQ ID NO: 516 or 517		

[0563] Wherein SEQ ID NOs:570, 574, 578, 582, 586, 590, 594, 598, 602, and 606 correspond to the VL chain of antibody DH511, DH512, DH513, DH514, DH515, DH516, DH517, DH518, DH536, and DH537, respectively and as described in Table 4. Wherein SEQ ID NOs: 553, and 625 correspond to the VL chain of antibody DH511.2_K3, and DH511_5a/5b, respectively and as described in Table 4.

[0564] Wherein SEQ ID NOs:568, 572, 576, 580, 584, 588, 592, 596, 600, 604, 608-623 correspond to the VH chain of antibody DH511, DH512, DH513, DH514, DH515, DH516, DH517, DH518, DH536, DH537, chimeric and mutant variants as described in Example 11, respectively and as described in Table 4. Wherein SEQ ID NOs: 551, 624, and 626 correspond to the VH chain of antibody DH511.2_K3, DH511_5a, and DH511_5b, respectively and as described in Table 4.

[0565] **Table 4** provides the amino acid sequences of one such exemplary trivalent binding molecule comprising antigen binding sites for HIV-1, CD3 and CD8 (HIVxCD3xCD8), and having four polypeptide chains which may be generated are provided in SEQ ID NOs: 555, 557, 561, and 562. Chain 1 of this exemplary molecule comprises: an N-terminus, the VL Domain of DH511.2_K3 (SEQ ID NO: 553), a Peptide Linker 1 (SEQ ID NO: 508), the VH Domain of hXR32 (SEQ ID NO: 500), a Peptide Linker 2 (SEQ ID NO: 510), a cysteine engineered E-coil, a Spacer Linker 3 (SEQ ID NO: 522), a knob bearing CH2-CH3 (SEQ ID NO: 531), and a C-terminus. Chain 2 of this exemplary molecule comprises: an N-terminus, the VL Domain of hXR32 (SEQ ID NO: 503), a Peptide Linker 1 (SEQ ID NO: 508), the VH Domain of DH511.2_K3 (SEQ ID NO: 551), a Peptide Linker 2 (SEQ ID NO: 510), a cysteine engineered K-coil, a Spacer Linker 3 (SEQ ID NO: 521), and a C-terminus. Chain 3 of this exemplary molecule comprises: an N-terminus, the VH Domain of OKT8 (SEQ ID NO: 543), a CH1-Hinge Domain (SEQ ID NO: 515), a hole bearing CH2-CH3 (SEQ ID NO:

533), and a C-terminus. Chain 4 of this exemplary molecule comprises: an N-terminus, the VL Domain of OKT8 (SEQ ID NO: 545), a CL Kappa Domain (SEQ ID NO: 516), and a C-terminus. It will be noted that polypeptide chains 1 and 2 of such an exemplary trivalent binding molecule are the same as those present in the bispecific Fc bearing diabody DH511.2_K3xCD3 Fc described above.

[0566] **Table 4** provides the amino acid sequences of another such exemplary trivalent binding molecule comprising antigen binding sites for HIV-1, CD3 and CD8 (HIVxCD3xCD8), and having three polypeptide chain which may be generated are provided in SEQ ID NOs: 555, 557, and 563. Chain 1 of this exemplary molecule comprises: an N-terminus, the VL Domain of DH511.2_K3 (SEQ ID NO: 553), a Peptide Linker 1 (SEQ ID NO: 508), the VH Domain of hXR32 (SEQ ID NO: 500), a Peptide Linker 2 (SEQ ID NO: 510), a cysteine engineered E-coil, a Spacer Linker 3 (SEQ ID NO: 522), a knob bearing CH2-CH3 (SEQ ID NO: 531), and a C-terminus. Chain 2 of this exemplary molecule comprises: an N-terminus, the VL Domain of hXR32 (SEQ ID NO: 503), a Peptide Linker 1 (SEQ ID NO: 508), the VH Domain of DH511.2_K3 (SEQ ID NO: 551), a Peptide Linker 2 (SEQ ID NO: 510), a cysteine engineered K-coil, a Spacer Linker 3 (SEQ ID NO: 521), and a C-terminus. Chain 3 of this exemplary molecule comprises: an N-terminus, the VL Domain of OKT8 (SEQ ID NO: 545), a Peptide Linker 5 (SEQ ID NO: 526), the VH Domain of OKT8 (SEQ ID NO: 543), a CH1-Hinge Domain (SEQ ID NO: 515), a hole bearing CH2-CH3 (SEQ ID NO: 533), and a C-terminus. It will be noted that polypeptide chains 1 and 2 of such an exemplary trivalent binding molecule are the same as those present in the bispecific Fc bearing diabody DH511.2_K3xCD3 Fc described above.

[0567] Exemplary trivalent binding molecules having three or four polypeptide chains, comprising two HIV-1 *Env* binding sites and one CD3 binding site are readily generated, for example by replace the VL and VH Domains of the anti-CD antibody in the above described molecules with the VL and VH Domains of an anti-HIV-1 *Env* antibody provided herein (*e.g.*, DH511.2_K3) or known in the art. As provided above the HIV-1 *Env* binding arms may comprise the VL and VH Domains of the same or different anti-HIV-1 *Env* antibodies.

[0568] **Table 4** provides the amino acid sequences of one exemplary trivalent binding molecules comprising two HIV-1 *Env* binding arms having the same VH and VL Domains,

and one CD3 binding arm (HIVxCD3xHIV), and having four polypeptide chains which may be generated are provided in SEQ ID NOs: 555, 557, 627, and 628. Chain 1 of this exemplary trispecific molecule comprises: an N-terminus, the VL Domain of DH511.2_K3 (SEQ ID NO: 553), a Peptide Linker 1 (SEQ ID NO: 508), the VH Domain of hXR32 (SEQ ID NO: 500), a Peptide Linker 2 (SEQ ID NO: 510), a cysteine engineered E-coil, a Spacer Linker 3 (SEQ ID NO: 522), a knob bearing CH2-CH3 (SEQ ID NO: 531), and a C-terminus. Chain 2 of this exemplary molecule comprises: an N-terminus, the VL Domain of hXR32 (SEQ ID NO: 503), a Peptide Linker 1 (SEQ ID NO: 508), the VH Domain of DH511.2_K3 (SEQ ID NO: 551), a Peptide Linker 2 (SEQ ID NO: 510), a cysteine engineered K-coil, a Spacer Linker 3 (SEQ ID NO: 521), and a C-terminus. Chain 3 of this exemplary molecule comprises: an N-terminus, the VH Domain of DH511.2_K3 (SEQ ID NO: 551), a CH1-Hinge Domain (SEQ ID NO: 515), a hole bearing CH2-CH3 (SEQ ID NO: 533), and a C-terminus. Chain 4 of this exemplary molecule comprises: an N-terminus, the VL Domain of DH511.2_K3 (SEQ ID NO: 553), a CL Kappa Domain (SEQ ID NO: 516), and a C-terminus. It will be noted that polypeptide chains 1 and 2 of such an exemplary trivalent binding molecule are the same as those present in the bispecific Fc bearing diabody DH511.2_K3xCD3 Fc described above.

[0569] Table 4 provides the amino acid sequences of another exemplary trivalent binding molecules comprising two HIV-1 *Env* binding arms having the same VH and VL Domains, and one CD3 binding arm (HIVxHIVxCD3), and having four polypeptide chains which may be generated are provided in SEQ ID NOs: 629, 630, 631 and 632. Chain 1 of this exemplary molecule comprises: an N-terminus, the VL Domain of DH511.2_K3 (SEQ ID NO: 553), a Peptide Linker 1 (SEQ ID NO: 508), the VH Domain of DH511.2_K3 (SEQ ID NO: 551), a Peptide Linker 2 (SEQ ID NO: 510), a cysteine engineered E-coil, a Spacer Linker 3 (SEQ ID NO: 522), a knob bearing CH2-CH3 (SEQ ID NO: 531), and a C-terminus. Chain 2 of this exemplary molecule comprises: an N-terminus, the VL Domain of DH511.2_K3 (SEQ ID NO: 553), a Peptide Linker 1 (SEQ ID NO: 508), the VH Domain of DH511.2_K3 (SEQ ID NO: 551), a Peptide Linker 2 (SEQ ID NO: 510), a cysteine engineered K-coil, a Spacer Linker 3 (SEQ ID NO: 521), and a C-terminus. Chain 3 of this exemplary molecule comprises: an N-terminus, the VH Domain of hXR32 (SEQ ID NO: 500), a CH1-Hinge Domain (SEQ ID NO: 515), a hole bearing CH2-CH3 (SEQ ID NO: 533), and a C-terminus. Chain 4 of this exemplary molecule comprises: an N-terminus, the VL Domain of hXR32 (SEQ ID NO: 503), a CL Kappa Domain (SEQ ID NO: 516), and a C-terminus.

[0570] As described above, alternative trivalent binding molecules may be generated by replacing the VL and VH Domains of DH511.2_K3 with the VL and VH Domains of any of the anti-HIV-1 antibodies provided herein. In particular, the VL Domain of DH511.2_K3 (SEQ ID NO: 553) may be replaced with the VL Domain DH511_5AVK (SEQ ID NO: 625), and the VH Domain of DH511.2_K3 (SEQ ID NO: 551) may be replaced with the VH Domain DH511_5AVH-4A (SEQ ID NO: 624) or the VH Domain DH511_5BVH-4A (SEQ ID NO: 626).

[0571] The ability of such exemplary trivalent binding molecules to bind to HIV-1 *Env*, CD3, and/or CD8 may be evaluated using the methods describe above, or well known in the art (see, *e.g.*, WO2016/054101). The activity of such trivalent binding molecules to mediate redirected cell killing of target cells expressing HIV-1 *Env* may be examined using the cytotoxic T lymphocyte (CTL) assay described above, or similar assays known in the art (see, *e.g.*, Sung, JAM *et al.* Dual-Affinity Re-Targeting proteins direct T cell-mediated cytotoxicity of latently HIV-1-infected cells. *J Clin Invest.* 2015;125(11):4077-4090; and WO2016/054101).

Example 15: Combinations of multispecific antibodies

[0572] Multispecific antibodies with different HIV-1 specificity, *e.g.* but not limited to CD4 binding site and V3 glycan binding, could also be tested in combination.

[0573] Various combinations of HIV-1 multispecific antibodies to mediate redirected cell killing of target cells expressing HIV-1 *Env* may be examined using the cytotoxic T lymphocyte (CTL) assay described above, or similar assays known in the art (see, *e.g.*, Sung, JAM *et al.* Dual-Affinity Re-Targeting proteins direct T cell-mediated cytotoxicity of latently HIV-1-infected cells. *J Clin Invest.* 2015;125(11):4077-4090; and WO2016/054101). Combinations of multispecific molecules, either with different HIV-1 specificity and/or different effector cell specificity, will be tested whether they provide enhanced benefits.

Example 16: Prophetic examples

[0574] All multispecific molecules described herein could be tested in any other suitable assay. For non-limiting examples of further studies and characterization See Sung *et al.* *J Clin Invest.* 2015;125(11):4077-4090; Sloan DD, Lam C-YK, Irrinki A, Liu L, Tsai A, Pace CS, *et al.* (2015) Targeting HIV Reservoir in Infected CD4 T Cells by Dual-Affinity Re-Targeting Molecules (DARTs) that Bind HIV Envelope and Recruit Cytotoxic T Cells. *PLoS Pathog* 11(11): e1005233. doi:10.1371/journal.ppat.1005233

[0575] Non-limiting examples of these assays include: determination of binding properties of the multispecific molecules to HIV-1 envelope expressing cells and/or HIV-1 infected cells; determining whether the multispecific molecules of the invention induce redirected T-cell killing of cell lines expressing various Envelopes and concomitant T-cell activation; determining whether multispecific molecules bind to the surface of HIV-1- infected CD4⁺T cells and redirect CD8⁺ T-cells to kill HIV-1 infected CD4⁺ cells using lymphocytes from HIV-1 seronegative donors; determining whether multispecific molecules redirect CD8⁺ T-cells to clear HIV-1 (*e.g.* JR-CSF, or any other suitable HIV-1 type)-superinfected CD4⁺ cells using lymphocytes from patients on suppressive ART; determining whether multispecific molecules redirect T cells from HIV-1-infected individuals on suppressive ART to clear virus from resting CD4⁺ T cells following induction of latent virus expression.

[0576] Methods and Reagents

[0577] *Generation of Infectious Molecular Clones (IMCs).* HIV-1 IMCs for any subtype B BaL, subtype AE CM235 and subtype C 1086.C were generated with the backbone derived from NHL4-3 isolate as previously described See Edmonds TG *et al.* Replication competent molecular clones of HIV-1 expressing Renilla luciferase facilitate the analysis of antibody inhibition in PBMC. *Virology.* 2010;408(1):1–13; Adachi A *et al.* Production of acquired immunodeficiency syndrome-associated retrovirus in human and nonhuman cells transfected with an infectious molecular clone. *J Virol.* 1986;59(2):284–291). All IMCs expressed the *Renilla* luciferase reporter gene and preserved all nine viral open reading frames. The *Renilla* luciferase reporter gene was expressed under the control of the HIV-1 Tat gene. Upon HIV-1 infection of CD4⁺ T cells, expression of Tat during HIV-1 replication will induce luciferase expression, which allows quantitation of infected cells by measuring relative luminescence units (RLU).

[0578] *Cell Lines.* Jurkat-522 F/Y GF cells, which constitutively express a fusion protein of Copepod Green Fluorescent Protein (copGFP) and Firefly Luciferase (System Biosciences),

were generated at MacroGenics from Jurkat-522 F/Y cells by transduction and clone selection. HEK293-D371 cells, which have doxycycline-inducible expression of HIV-1 CM244 (subtype AE) gp140 or CH505 T/F envelope, were obtained from Dr. John Kappes (University of Alabama at Birmingham).

[0579] *Flow Cytometric Analysis of diabody or mAb Binding to Cells.* Diabodies at a desired concentration, *e.g.* 4 µg/mL are incubated with 10^5 cells in 200 µL FACS buffer containing 10% human AB serum for 30 minutes at room temperature. After washing, cells are resuspended in 100 µL of 1 µg/mL biotin-conjugated mouse anti-EK antibody (recognizes the E/K heterodimerization region of diabody proteins and/or trivalent binding molecules), are mixed with 1:500 diluted streptavidin-PE and incubated in the dark for 45 minutes at 2-8°C. Cells are washed, resuspended with FACS buffer, and analyzed with a BD Calibur flow cytometer and FlowJo software (TreeStar, Ashland OR). Binding to IMC-infected CD4⁺ T cells from normal human donors is conducted as previously described (54) for the A32 and 7B2 mAbs, and with biotin-conjugated mouse anti-EK antibody and 1:500 diluted streptavidin-PE for the HIVx4420 control diabodies.

[0580] The ability of DH511.2_K3xCD3 Fc (or any of the bispecific diabodies and/or trivalent binding molecules) to mediate redirected cell killing of *HIV-1 IMC-Infected CD4⁺* Cells may be examined using assays well known in the art (see, *e.g.*, Sung, JAM *et al.* Dual-Affinity Re-Targeting proteins direct T cell-mediated cytotoxicity of latently HIV-infected cells. *J Clin Invest.* 2015;125(11):4077-4090; and WO2016/054101). Briefly, resting PBMC from normal healthy HIV-1 seronegative donors are activated (*e.g.*, with anti-human CD3 and anti-human CD28 antibodies). A CD4⁺ enriched cell population is obtained by depletion of CD8⁺ T cells (*e.g.*, using commercially available magnetic beads) and is spinoculated in the presence of the luciferase-expressing IMC-representing an HIV-1 subtype (*e.g.*, AE (CM235); B (BaL); or C (1086.C)) and cultured for ~72 hours. The CD4⁺-infected target cells are then incubated with resting CD8⁺ effector cells (which may be isolated by negative selection from autologous PBMC, using a commercial CD8⁺ T cell isolation kit) at different E/T ratios (*e.g.*, 33:1, 11:1, 3:1, and 0:1) in the absence or presence of bispecific diabodies for 6–48 hours at concentrations ranging from 0.0001–1,000 ng/ml. Uninfected and infected target cells alone are included as additional controls. Each condition is tested in duplicate. After incubation, the percentage of specific lysis (%SL) of target cells is determined, for example by adding ViviRen Live Cell Substrate (Promega) and measuring the RLU on a luminometer. The %SL

of target cells may be determined as described previously (Pollara J *et al.* HIV-1 Vaccine-Induced C1 and V2 Env-Specific Antibodies Synergize for Increased Antiviral Activities. *J Virol.* 2014;88(14):7715–7726).

[0581] *Redirected T-Cell Cytotoxicity Assay Against HIV-1 Env-expressing Cell Lines and Assessment of T-Cell Activation.* Pan T cells are isolated from healthy human PBMCs with the Dynabeads® Untouched™ Human T Cells Kit (Invitrogen). HIV-1 *Env* expressing cell lines ($1-4 \times 10^5$ cells/mL) are treated with serial dilutions of diabodies (or trivalent binding molecules), together with human T cells at an effector:target (E:T) ratio = 10:1, or otherwise at varying E:T ratios as indicated, and incubated at 37°C, 5% CO₂ overnight. Cytotoxicity is measured by lactate dehydrogenase (LDH) release (CytoTox 96® Non-Radioactive Cytotoxicity Assay, Promega) as described previously (32). With the Jurkat-522 F/Y GF cell line, cytotoxicity is also measured by luminescence using Luciferase-Glo substrate (Promega). Specific lysis is calculated from luminiscence counts (RLU): cytotoxicity (%) = $100 \times (1 - (\text{RLU of Sample} \div \text{RLU of Control}))$, where Control = average RLU of target cells incubated with effector cells in the absence of DART. Data are fit to a sigmoidal dose-response function to obtain 50% effective concentration (EC₅₀) and percent maximum specific lysis values. T-cell activation is measured by FACS analysis after cells in the assay plate are labeled with CD8-FITC, CD4-APC, and CD25-PE antibodies (BD Biosciences), followed by cell collection by FACS Calibur flow cytometer equipped with acquisition software CellQuest Pro Version 5.2.1 (BD Biosciences). Data analysis is performed using FlowJo software (Treestar, Inc).

[0582] *Redirected T-Cell Cytotoxicity Assay Against HIV-1 IMC-Infected CD4⁺ Cells.* Cryopreserved resting PBMC from normal healthy HIV-1 seronegative donors are activated for 72 hours with anti-human CD3 (clone OKT3; eBioscience) and anti-human CD28 (clone CD28.2; BD Pharmingen). Subsequently, a CD4⁺ enriched cell population (purity >92.3%; average±standard deviation 95.73±2.6%) is obtained by depletion of CD8⁺ T cells using magnetic beads (Miltenyi Biosciences), spinoculated in presence of the luciferase-expressing IMC representing HIV-1 subtype AE (CM235), B (BaL) or C (1086.C) and cultured for 72 hours. CD4⁺ infected target cells are incubated with resting CD8⁺ effector cells (isolated by negative selection from autologous PBMC, CD8⁺ T cell Isolation Kit, Miltenyi Biosciences) at 33:1, 11:1, 3:1, and 0:1 E:T ratios in the absence or presence of diabodies (and/or trivalent binding molecules) for 6-48 hours at concentration ranging from 1,000 to 0.0001 ng/mL.

Uninfected and infected target cells alone are included as additional controls. Each condition is tested in duplicate. After incubation, ViviRen™ Live Cell Substrate (Promega) is added and RLU measured on a luminometer; percentage specific lysis (%SL) of target cells is determined as described previously. See Pollara J *et al.* HIV-1 Vaccine-Induced C1 and V2 Env-Specific Antibodies Synergize for Increased Antiviral Activities. *J Virol.* 2014;88(14):7715–7726.

[0583] *T-Cell Degranulation (CD107) Assay.* As described for the cytotoxicity assay with HIV-1 IMC-infected cells as targets, activated CD4⁺ cells infected with HIV-1 BaL IMC are plated with resting CD8⁺ effector cells at a 33:1 E:T ratio in the absence or presence of 1ng/mL diabodies and incubated for 6 hours. For the CD4 T cell degranulation, activated CD4⁺ T cells are either infected with JR-CSF and labeled with the viability (NFL1) and target specific (TFL4) markers utilized in an ADCC assay or added to targets as effectors at a 10:1 ratio prior to addition of diabodies. Each condition is tested in duplicate. CD107 PE-Cy5 (clone H4A3; eBioscience) is titrated and added during the last six hours of the incubation along with Monensin solution (BD GolgiStop). A panel of antibodies consisting of LIVE/DEAD Aqua stain, anti-CD3 APC-H7 (clone SK7; BD Pharmingen), anti-CD4 BV605 (clone OKT4; Biolegend), anti-CD8 BV650 (clone RPA-T8; Biolegend) are used to detect CD107⁺ CD8⁺ T cells. After washing and fixation, samples are acquired on a custom made LSRII (BD Bioscience, San Jose, CA) within the next 24 hours. A minimum of 300,000 total viable events is acquired for each test. The analysis of the data is performed using the Flow-Jo software (Treestar, Ashland, OR).

[0584] *T-Cell Viability and Activation Assays.* CD8⁺ T cells and CD8 depleted PBMCs obtained from HIV infected ART suppressed patients are plated at 5×10⁴ cells per well in 96 well plates with 100ng/mL of the indicated DART. Cells are cultured in 0.2mL of cIMDM media supplemented with 10% FBS, 1% Penicillin/Streptomycin and 5U/mL IL-2 for 7 days, and then are stained with the following antibodies: HLA-DR-PerCP (clone L243), CD25-PE (clone M-A251), CD8-FITC (clone HIT8a), CD8-PE (clone HIT8a), CD4-FITC (clone RPA-T4), and Annexin V-PE and 7-AAD (all BD biosciences, San Jose, CA).

[0585] *Redirected T-Cell Viral Clearance Assay.* CD8⁺ T-cells are isolated from PBMCs by positive selection (EasySep human CD8⁺ Selection Kit, Stem Cell). CD8-depleted PBMCs are first activated with 2μg/mL of PHA (Remel, Lenexa, KS) and 60U/mL of IL-2, and then infected by spinoculation at 1200xg for 90 minutes with either JR-CSF or autologous

reservoir virus (AR) at an MOI of 0.01). AR virus is obtained from pooled supernatants of replicate wells from outgrowth assays of resting CD4⁺ T-cells for each patient performed. Fifty-thousand (5×10^4) targets/well are co-cultured with CD8⁺ T cells in triplicate at the indicated E:T ratio in the absence or presence of 100 ng/mL of diabody in 0.2m of cIMDM media supplemented with 10% FBS, 1% Penicillin/Streptomycin and 5 U/mL IL-2. For experiments performed in the presence of antiretrovirals (ARVs), 24 hours after spinoculation cells are washed and 1 μ M of raltegravir and 4 μ M of abacavir are added, and then diabodies and CD8⁺ T-cells are added to cultures. Supernatant is assayed on day 7 by p24 ELISA (ABL, Rockville, MD). Results are calculated as the log (p24 of infected target cells only control divided by p24 of the test condition).

[0586] *Latency Clearance Assay (LCA)*. The reduction of virus recovery from CD4⁺ infected cells is assessed by a standard quantitative viral outgrowth assay using the resting CD4⁺ T cells of aviremic, ART-treated patients, following the addition of antiviral effector cells and/or molecules, as previously described. See Sung JA *et al.* Expanded Cytotoxic T-Cell Lymphocytes Target the Latent Hiv Reservoir. *J Infect Dis.* 2015;:1–15. . In this case the LCA is used to model the ability of diabodies to clear virus emerging from the latent reservoir under clinically and pharmacologically relevant conditions. Resting CD4⁺ T-cells are isolated from a leukapheresis product as previously described (72) and exposed to PHA (4 μ g/mL) and IL-2 (60U/mL) for 24 hours or vorinostat (VOR) (335nM, 6 hours) (Merck Research Laboratories), and plated at 0.5 to 1 x 10⁶ cells/well in 12 to 36 replicate wells depending on the size of the reservoir. The VOR is then washed off and CD8s added at an E:T of 1:10 as well as 100 ng/mL of the indicated DART. Cells are co-cultured for 24 hours (unless specified otherwise) following which the diabody (and/or trivalent binding molecule) proteins are washed off and allogeneic CD8-depleted PBMCs from an HIV negative donor are added to amplify residual virus. Supernatant is assayed for the presence of p24 antigen on day 15 for each well. Results are calculated as % viral recovery [(# of positive wells/total number plated)x100], normalized to a control in which no CD8⁺ T cells are added.

[0587] Additional animal studies will be conducted to evaluate toxicity, safety, PK and PD profiles, and efficacy in preventing or treating HIV infection of the multispecific molecules of the invention.

[0588] All documents and other information sources cited herein are hereby incorporated in their entirety by reference.

CLAIMS

1. A bispecific molecule comprising a first polypeptide chain and a second polypeptide chain, covalently bonded to one another, wherein:
 - (I) the first polypeptide chain comprises in the N- to C-terminal direction:
 - (i) a domain (A) comprising a binding region of the light chain variable domain of a first immunoglobulin (VL1) having the binding specificity of a gp41 MPER binding HIV-1 antibody (1);
 - (ii) a domain (B) comprising a binding region of a heavy chain variable domain of a second immunoglobulin (VH2) specific for an epitope (2); and
 - (iii) a domain (C) comprising a heterodimer promoting domain;
 - (II) the second polypeptide chain comprises in the N- to C-terminal direction:
 - (i) a domain (D) comprising a binding region of a light chain variable domain of the second immunoglobulin (VL2) specific for the epitope (2);
 - (ii) a domain (E) comprising a binding region of a heavy chain variable domain of the first immunoglobulin (VH1) having the binding specificity of a gp41 MPER binding HIV-1 antibody (1); and
 - (iii) a domain (F) comprising a heterodimer promoting domain; and wherein:

the domains (A) and (B) are linked so that they do not associate with one another to form an epitope binding site;

the domains (D) and (E) are linked so that they do not associate with one another to form an epitope binding site; and

the domains (A) and (E) associate to form a binding site that binds the HIV-1 envelope like a gp41 MPER binding HIV-1 antibody (1); and the domains (B) and (D) associate to form a binding site that binds the epitope (2).
2. The bispecific molecule of claim 1, wherein:
 - (i) domains (A) and (B) are separated by a peptide linker 1;
 - (ii) domains (C) and (B) are separated by a peptide linker 2;
 - (iii) domains (D) and (E) are separated by a peptide linker 1; and
 - (iv) domains (F) and (E) are separated by a peptide linker 2.

3. A bispecific molecule comprising a first polypeptide chain, a second polypeptide chain, and a third polypeptide chain, wherein the first and second polypeptide chains are covalently bonded and the second and third polypeptide chains are covalently bonded, and wherein:

(I) the first polypeptide chain comprises in the N- to C-terminal direction:

(i) a domain (A) comprising a binding region of the light chain variable domain of a first immunoglobulin (VL1) having the binding specificity of a gp41 MPER binding HIV-1 antibody (1);

(ii) a domain (B) comprising a binding region of a heavy chain variable domain of a second immunoglobulin (VH2) specific for an epitope (2);

(iii) a domain (C) comprising a heterodimer promoting domain; and

(iv) a CH2-CH3 domain;

(II) the second polypeptide chain comprises in the N- to C-terminal direction:

(i) a domain (D) comprising a binding region of a light chain variable domain of the second immunoglobulin (VL2) specific for the epitope (2);

(ii) a domain (E) comprising a binding region of a heavy chain variable domain of the first immunoglobulin (VH1) having the binding specificity of a gp41 MPER binding HIV-1 antibody (1); and

(iii) a domain (F) comprising a heterodimer promoting domain;

(III) the third polypeptide chain comprises in the N- to C-terminal direction:

(i) a CH2-CH3 domain, and wherein:

the domains (A) and (B) are linked so that they do not associate with one another to form an epitope binding site;

the domains (D) and (E) are linked so that they do not associate with one another to form an epitope binding site;

the domains (A) and (E) associate to form a binding site that binds the HIV-1 envelope like a gp41 MPER binding HIV-1 antibody (1);

the domains (B) and (D) associate to form a binding site that binds the epitope (2); and

the CH2-CH3 domains of the first and third polypeptide form an Fc Domain.

4. The bispecific molecule of claim 3, wherein:

- (i) the third polypeptide chain further comprises a peptide linker 3 N-terminal to the CH2-CH3 domain;
 - (ii) domains (A) and (B) are separated by a peptide linker 1;
 - (iii) domains (C) and (B) are separated by a peptide linker 2;
 - (iv) the CH2-CH3 domain and domain (C) are separated by a peptide linker 3 or a spacer linker 3;
 - (v) domains (D) and (E) are separated by a peptide linker 1; and
 - (vi) domains (F) and (E) are separated by a peptide linker 2.
5. A bispecific molecule comprising a first polypeptide chain, a second polypeptide chain and a third polypeptide chain, wherein the first and second polypeptide chains are covalently bonded and the second and third polypeptide chains are covalently bonded, and wherein:
- (I) the first polypeptide chain comprises in the N- to C-terminal direction:
- (i) a CH2-CH3 domain;
 - (ii) a domain (A) comprising a binding region of the light chain variable domain of a first immunoglobulin (VL1) having the binding specificity of a gp41 MPER binding HIV-1 antibody (1);
 - (iii) a domain (B) comprising a binding region of a heavy chain variable domain of a second immunoglobulin (VH2) specific for an epitope (2); and
 - (iv) a domain (C) comprising a heterodimer promoting domain;
- (II) the second polypeptide chain comprises in the N- to C-terminal direction:
- (i) a domain (D) comprising a binding region of a light chain variable domain of the second immunoglobulin (VL2) specific for the epitope (2);
 - (ii) a domain (E) comprising a binding region of a heavy chain variable domain of the first immunoglobulin (VH1) having the binding specificity of a gp41 MPER binding HIV-1 antibody (1); and
 - (iii) a domain (F) comprising a heterodimer promoting domain;
- (III) the third polypeptide chain comprises in the N- to C-terminal direction:
- (i) a CH2-CH3 domain, and wherein:
- the domains (A) and (B) are linked so that they do not associate with one another to form an epitope binding site;

the domains (D) and (E) are linked so that they do not associate with one another to form an epitope binding site;

the domains (A) and (E) associate to form a binding site that binds the HIV-1 envelope like a gp41 MPER binding HIV-1 antibody (1);

the domains (B) and (D) associate to form a binding site that binds the epitope (2); and

the CH2-CH3 domains of the first and third polypeptide form an Fc Domain.

6. The bispecific molecule of claim 5, wherein:

- (i) the CH2-CH3 domain and domain (A) are separated by a peptide linker 4;
- (ii) domains (A) and (B) are separated by a peptide linker 1;
- (iii) domains (C) and (B) are separated by a peptide linker 2;
- (iv) domains (D) and (E) are separated by a peptide linker 1;
- (v) domains (F) and (E) are separated by a peptide linker 2;
- (vi) the first polypeptide chain further comprises a peptide linker 3 N-terminal to the CH2-CH3 domain; and
- (vii) the third polypeptide chain further comprises a peptide linker 3 N-terminal to the CH2-CH3 domain.

7. A bispecific molecule comprising a first polypeptide chain, a second polypeptide chain, a third polypeptide chain, and a fourth polypeptide chain, wherein the first and second polypeptide chains are covalently bonded and the first and third polypeptide chains are covalently bonded, and wherein:

(I) the first and the third polypeptide chains each comprise in the N- to C-terminal direction:

(i) a domain (A) comprising a binding region of the light chain variable domain of a first immunoglobulin (VL1) having the binding specificity of a gp41 MPER binding HIV-1 antibody (1);

(ii) a domain (B) comprising a binding region of a heavy chain variable domain of a second immunoglobulin (VH2) specific for an epitope (2);

(iii) a domain (C) comprising a heterodimer promoting domain; and

(iv) a CH2-CH3 domain;

(II) the second and fourth polypeptide chains each comprise in the N- to C-terminal direction:

(i) a domain (D) comprising a binding region of a light chain variable domain of the second immunoglobulin (VL2) specific for the epitope (2);

(ii) a domain (E) comprising a binding region of a heavy chain variable domain of the first immunoglobulin (VH1) having the binding specificity of a gp41 MPER binding HIV-1 antibody (1);

(iii) a domain (F) comprising a heterodimer promoting domain; and wherein the domains (A) and (B) are linked so that they do not associate with one another to form an epitope binding site;

the domains (D) and (E) are linked so that they do not associate with one another to form an epitope binding site;

the domains (A) and (E) associate to form a binding site that binds the HIV-1 envelope like a gp41 MPER binding HIV-1 antibody (1);

the domains (B) and (D) associate to form a binding site that binds the epitope (2); and

the CH2-CH3 domains of the first and third polypeptide form an Fc Domain.

8. The bispecific molecule of claim 7, wherein:

- (i) domains (A) and (B) are separated by a peptide linker 1;
- (ii) domains (C) and (B) are separated by a peptide linker 2;
- (iii) the CH2-CH3 domain and domain (C) are separated by a peptide linker 3 or a spacer linker 3;
- (iv) domains (D) and (E) are separated by a peptide linker 1; and
- (v) domains (F) and (E) are separated by a peptide linker 2.

9. A trivalent binding molecule comprising a first, second, third and fourth polypeptide chain wherein:

- (I)** the first polypeptide chain comprises in the N-terminus to C-terminus direction:
 - (i) a domain (A) comprising a binding region of a light chain variable domain of a first immunoglobulin (VL1) specific for an epitope (1);
 - (ii) a domain (B) comprising a binding region of a heavy chain variable domain of a second immunoglobulin (VH2) specific for an epitope (2);
 - (iii) a domain (C) comprising a heterodimer promoting domain; and
 - (iv) a CH2-CH3 Domain;
- (II)** the second polypeptide chain comprises, in the N-terminus to C-terminus direction:

- (i) a domain (D) comprising a binding region of a light chain variable domain of the first immunoglobulin (VL1) specific for the epitope (2);
- (ii) a domain (E) comprising a binding region of a heavy chain variable domain of the second immunoglobulin (VH1) specific for the epitope (1);
- (iii) a domain (F) comprising a heterodimer promoting domain; and
- (III) the third polypeptide chain that comprises, in the N-terminus to C-terminus direction:
 - (i) a domain (G) comprising a binding region of a heavy chain variable domain of a third immunoglobulin (VH3) specific for an epitope (3); and
 - (ii) a CH1-Hinge Domain, and a CH2-CH3 Domain; and
- (IV) the fourth polypeptide chain that comprises, in the N-terminus to C-terminus direction:
 - (i) a domain (H) comprising a binding region of a light chain variable domain of the third immunoglobulin (VL3) specific for the epitope (3); and
 - (ii) CL Kappa Domain or a CL Lambda Domain; and wherein

the domains (A) and (B) are linked so that they do not associate with one another to form an epitope binding site;

the domains (D) and (E) are linked so that they do not associate with one another to form an epitope binding site;

the domains (A) and (E) associate to form a binding site that binds the epitope (1);

the domains (B) and (D) associate to form a binding site that binds the epitope (2);

the domains (G) and (H) associate to form a binding site that bind the epitope (3);

at least one of epitope (1), epitope (2), and epitope (3) is an epitope bound by an gp41 MPER binding HIV-1 antibody, and at least one of epitope (1), epitope (2), and epitope (3) is an epitope of CD3, CD8, or CD16;

the CH2-CH3 domains of the first and third polypeptide form an Fc Domain;

said first and second polypeptide chains are covalently bonded to one another;

said first and third polypeptide chains are covalently bonded to one another; and

said third and fourth polypeptide chains are covalently bonded to one another.

10. The trivalent binding molecule of claim 9, wherein:

- (i) domains (A) and (B) are separated by a peptide linker 1;
- (ii) domains (C) and (B) are separated by a peptide linker 2 or a peptide linker 2-C;
- (iii) the CH2-CH3 domain and domain (C) are separated by a peptide linker 3 or a spacer linker 3;

- (iv) domains (D) and (E) are separated by a peptide linker 1; and
 - (v) domains (F) and (E) are separated by a peptide linker 2 or a peptide linker 2-C.
11. A trivalent binding molecule comprising a first, second, and third polypeptide chain wherein:
- (I) the first polypeptide chain comprises in the N-terminus to C-terminus direction:
 - (i) a domain (A) comprising a binding region of a light chain variable domain of a first immunoglobulin (VL1) specific for an epitope (1);
 - (ii) a domain (B) comprising a binding region of a heavy chain variable domain of a second immunoglobulin (VH2) specific for an epitope (2);
 - (iii) a domain (C) comprising a heterodimer promoting domain; and
 - (iv) a CH2-CH3 Domain;
 - (II) the second polypeptide chain comprises, in the N-terminus to C-terminus direction:
 - (i) a domain (D) comprising a binding region of a light chain variable domain of the first immunoglobulin (VL1) specific for the epitope (2);
 - (ii) a domain (E) comprising a binding region of a heavy chain variable domain of the second immunoglobulin (VH1) specific for the epitope (1);
 - (iii) a domain (F) comprising a heterodimer promoting domain; and
 - (III) the third polypeptide chain that comprises, in the N-terminus to C-terminus direction:
 - (i) a domain (H) comprising a binding region of a light chain variable domain of a third immunoglobulin (VL3) specific for an epitope (3)
 - (ii) a domain (G) comprising a binding region of a heavy chain variable domain of the third immunoglobulin (VH3) specific for the epitope (3);
 - (iii) a CH2-CH3 Domain; and wherein
- the domains (A) and (B) are linked so that they do not associate with one another to form an epitope binding site;
- the domains (D) and (E) are linked so that they do not associate with one another to form an epitope binding site;
- the domains (A) and (E) associate to form a binding site that binds the epitope (1);
- the domains (B) and (D) associate to form a binding site that binds the epitope (2);
- the domains (G) and (H) associate to form a binding site that bind the epitope (3);

at least one of epitope (1), epitope (2), and epitope (3) is an epitope bound by a gp41 MPER binding HIV-1 antibody, and at least one of epitope (1), epitope (2), and epitope (3) is an epitope of CD3, CD8, or CD16;

the CH2-CH3 domains of the first and third polypeptide form an Fc Domain;

said first and second polypeptide chains are covalently bonded to one another;

said first and third polypeptide chains are covalently bonded to one another; and

said third and fourth polypeptide chains are covalently bonded to one another.

12. The trivalent binding molecule of claim 11, wherein:

- (i) domains (A) and (B) are separated by a peptide linker 1;
- (ii) domains (C) and (B) are separated by a peptide linker 2 or a peptide linker 2-C;
- (iii) the CH2-CH3 domain and domain (C) are separated by a peptide linker 3 or a spacer linker 3;
- (iv) domains (D) and (E) are separated by a peptide linker 1;
- (v) domains (F) and (E) are separated by a peptide linker 2 or a peptide linker 2-C;
- (vi) domains (H) and (G) are separated by a peptide linker 5; and
- (vii) the CH2-CH3 domain and domain (G) are separated by a peptide linker 3.

13. The trivalent binding molecule of claims 9-12, wherein one of epitope (1), epitope (2), or epitope (3) is an epitope of CD8.

14. The molecules of claims 3-8 and 9-12, wherein the CH2-CH3 domain of the first polypeptide chain is the of the “knob” design (SEQ ID NOs: 531 or 532) and the CH2-CH3 domain of the third polypeptide chain is of the “hole” design (SEQ ID NOs: 533 or 534).

15. The molecules of claim 14, wherein the CH2-CH3 domain of the first polypeptide comprises SEQ ID NO: 531 and the CH2-CH3 domain of the third polypeptide chain comprises SEQ ID NO: 533.

16. The molecules of claims 3-8 and 9-12, wherein the CH2-CH3 domain of the third polypeptide chain is the of the “knob” design (SEQ ID NOs: 531 or 532) and the CH2-CH3 domain of the first polypeptide chain is of the “hole” design (SEQ ID NOs: 533 or 534).

17. The molecules of claim 16, wherein the CH2-CH3 domain of the third polypeptide comprises SEQ ID NO: 531 and the CH2-CH3 domain of the first polypeptide chain comprises SEQ ID NO: 533.
18. The molecules of claims 1-12 wherein the epitope (2) is a CD3 epitope, CD8 epitope, or a CD16 epitope.
19. The molecules of claims 1-12, wherein the gp41 MPER binding HIV-1 antibody is DH511, DH511.2_K3, DH512, DH513, DH514, DH515, DH516, DH517, DH518, DH536, or DH537 or any of the antibodies of the DH511 lineage.
20. The molecules of claims 1-12, wherein the molecule binds HIV-1 envelope with the specificity of the gp 41 MPER binding antibody DH512, DH511.2_K3 or any antibody of the DH511 lineage and binds CD3, CD8, or CD16.
21. The molecules of claims 1-12, wherein domain (A) comprises the CDR1, CDR2, and CDR3 of the light chain variable domain of immunoglobulin DH512, DH511.2_K3 or any immunoglobulin of the DH511 lineage.
22. The molecules of claims 1-12, wherein domain (E) comprises the CDR1, CDR2, and CDR3 of the heavy chain variable domain of immunoglobulin DH512, or any immunoglobulin of the DH511 lineage.
23. The molecules of claims 1-12, wherein domain (A) comprises the light chain variable domain of immunoglobulin DH512, DH511.2_K3 or any immunoglobulin of the DH511 lineage.
24. The molecules of claims 1-12, wherein domain (E) comprises the heavy chain variable domain of immunoglobulin DH512, or any immunoglobulin of the DH511 lineage.
25. The molecules of claims 1-12, wherein domain (B) comprises the heavy chain variable domain of an anti-CD3 antibody.
26. The molecules of claims 1-12, wherein the domain (D) comprises the light chain variable domain of an anti-CD3 antibody.

27. The trivalent binding molecule of claims 9-12, wherein domain (G) comprises heavy chain variable domain of an anti-CD8 antibody.
28. The trivalent binding molecule of claims 9-12, wherein domain (H) comprises light chain variable domain of an anti-CD8 antibody.
29. The bispecific molecule of claim 3, wherein the first polypeptide comprises SEQ ID NO: 555, the second polypeptide comprises SEQ ID NO: 557, and the third polypeptide comprises SEQ ID NO: 559.
30. The trivalent binding molecule of claim 9, wherein
- a. the first polypeptide comprises SEQ ID NO: 555, the second polypeptide comprises SEQ ID NO: 557, the third polypeptide comprises SEQ ID NO: 561, and the fourth polypeptide comprises SEQ ID NO: 562; or
 - b. the first polypeptide comprises SEQ ID NO: 555, the second polypeptide comprises SEQ ID NO: 557, the third polypeptide comprises SEQ ID NO: 627, and the fourth polypeptide comprises SEQ ID NO: 628; or
 - c. comprises SEQ ID NO: 629, the second polypeptide comprises SEQ ID NO: 630, the third polypeptide comprises SEQ ID NO: 631, and the fourth polypeptide comprises SEQ ID NO: 632.
31. The trivalent binding molecule of claim 11, wherein the first polypeptide comprises SEQ ID NO: 555, the second polypeptide comprises SEQ ID NO: 557, and the third polypeptide comprises SEQ ID NO: 563.
32. A composition comprising any one of the molecules of claims 1-31 or any combination thereof and a carrier.
33. A composition comprising a bispecific molecule or trivalent molecule which comprises at least one arm with the binding specificity of HIV-1 antibody DH512, DH511.2_K3 or any antibody of the DH511 lineage, and a second arm targeting CD3, CD8 or CD16.
34. The composition of claim 33, further comprising a second bispecific molecule or trivalent molecule comprising a first arm with the binding specificity of an HIV-1 antibody

different from the binding specificity of the first bispecific molecule or trivalent molecule and a second arm targeting CD3, CD8 or CD16, wherein the first and second molecules are different.

35. A method to treat or prevent HIV-1 infection in a subject in need thereof comprising administering to the subject a composition comprising any one of the molecules of claim 1-31 or a combination of any one of these molecules in a therapeutically effective amount.
36. The method of claim 35, further comprising administering a latency activating agent.
37. The method of claim 36, wherein the latency activating agent is vorinostat, romidepsin, panobinostat, disulfiram, JQ1, bryostatin, PMA, inonomecin, or any combination thereof.
38. A vector comprising nucleic acids comprising nucleotides encoding the molecules of any one of claim 1-31.
39. A composition comprising a vector comprising a nucleic acid encoding the molecules of any one of claim 1-31.

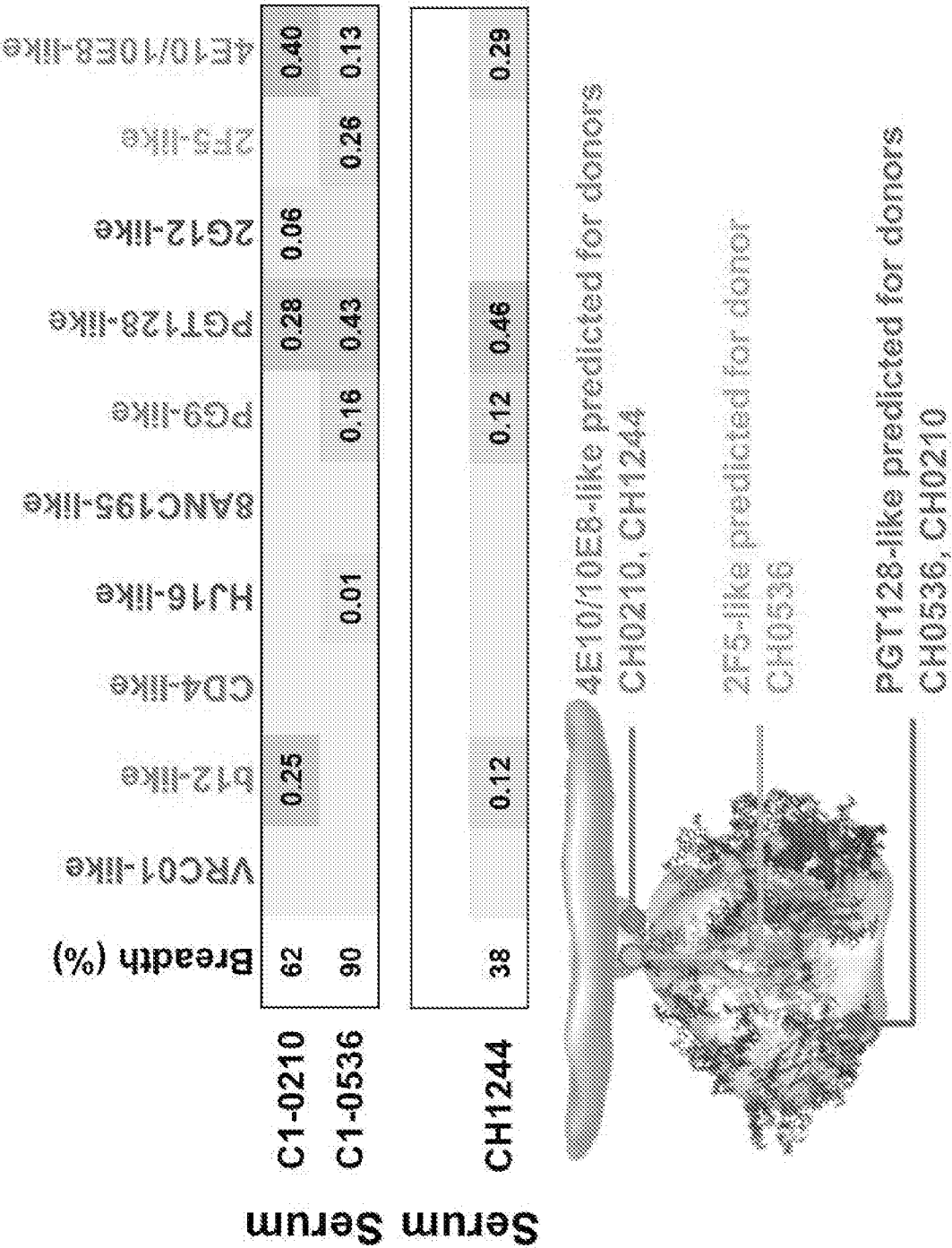


Figure 1

MPR.03-biotin: KKK-NEQELLELDKWASLWNWFDITNWLWYIR

MPR.03: NEQELLELDKWASLWNWFDITNWLWYIR

Figure 2

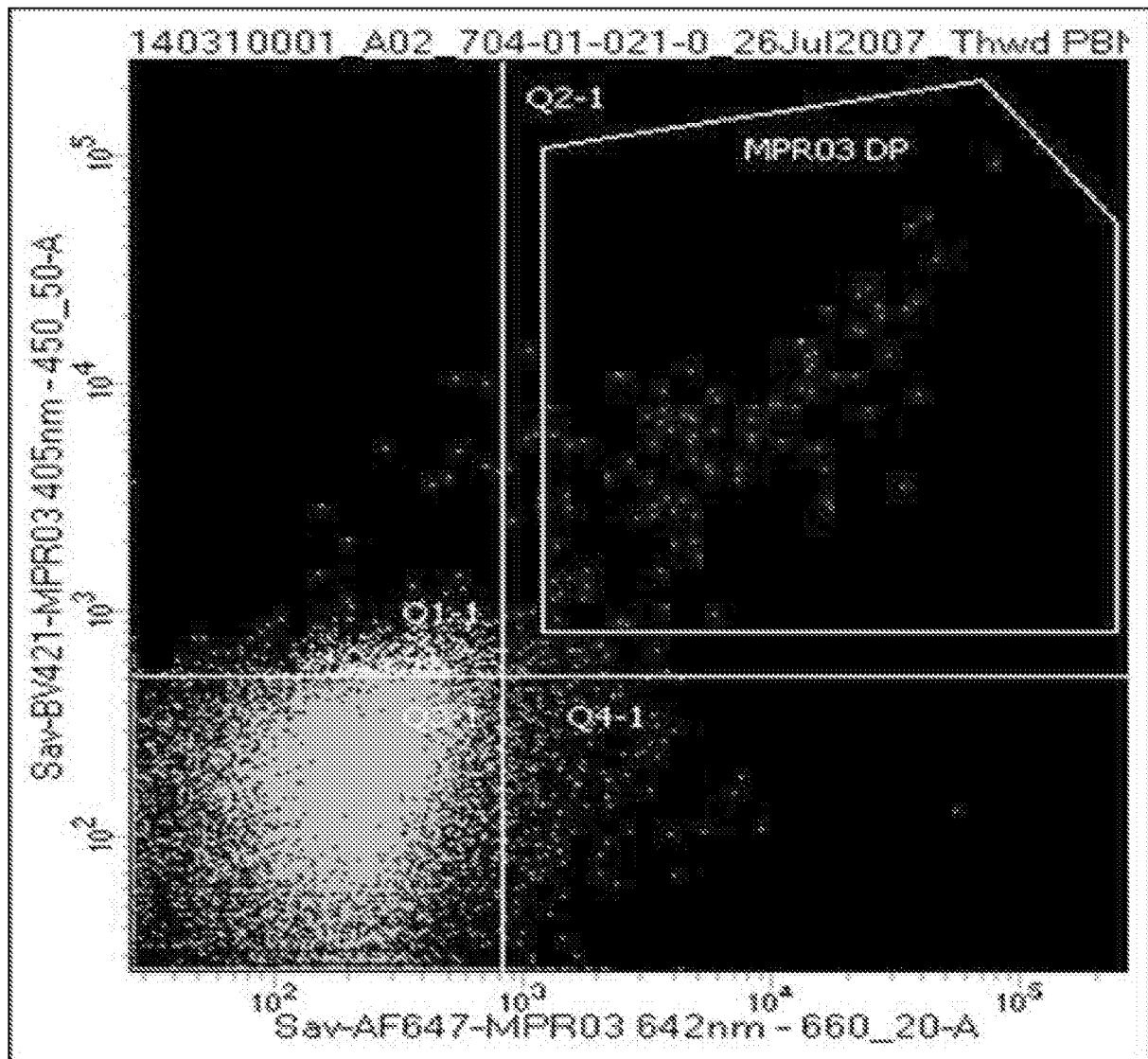


Figure 3

PTID	AntibodyID	VHID	VH	DH	JH	Mutation	HCDR3	VK/LID	VK/VL	JK/L	Mutation	LCDR3
704-01-021-0	DH511	H510056	3~15	3~3	6	15.7%	24	K510037	VK1~39	2	17.2%	11
704-01-021-0	DH512	H510049	3~15	3~3	6	20.1%	23	K510032	VK1~39	2	14.7%	11
704-01-021-0	DH513	H570022	3~15	3~3	6	15.3%	24	K570010	VK1~39	2	16.3%	11
704-01-021-0	DH514	H570029	3~15	3~3	6	20.5%	23	K570016	VK1~39	2	14.6%	11
704-01-021-0	DH515	H510052	3~15	3~3	6	21.8%	23	K510035	VK1~39	2	18.3%	11
704-01-021-0	DH516	H510048	3~15	3~3	6	16.0%	24	K510031	VK1~39	2	19.0%	11
704-01-021-0	DH517	H510053	4~34	2~2	6	22.8%	24	L510018	VL3-19	2	14.3%	12
707-01-053-6	DH518	H570010	4~39	3~3	4	16.2%	20	K570004	VK1~39	2	15.4%	9
704-01-124-4	DH536*	H510127	1~69	3~3	2	8.3%	18	L510050	VL2~14	2	5.6%	10
707-01-058-5	DH537*	H008587	1~69	1~1	5	9.0%	17	K006517	VK1~39	2	6.9%	9

Figure 4

PT ID	Antibody ID	Old Antibody ID	Netralization Titer (TCID50), ug/ml				
			BG1168	CH505	DU172	MN	MuLV
704-01-021-0	DH511	Ab510056	<0.023	18.573	1.238	<0.023	>50
704-01-021-0	DH512	Ab510049	0.22	8.537	3.43	<0.023	>50
704-01-021-0	DH513	Ab570022	0.651	24.19	3.212	<0.023	>50
704-01-021-0	DH514	Ab570029	0.809	28.229	3.815	<0.023	>50
704-01-021-0	DH515	Ab510052	0.712	40.39	6.122	0.041	>50
704-01-021-0	DH516	Ab510048	0.608	45	6.538	<0.023	>50
704-01-021-0	DH517	Ab510053	2.518	>50	16.53	0.791	>50
707-01-053-6	DH518	Ab570010	<0.023	>50	5.174	<0.023	>50
703-01-124-4	DH536	Ab510127	>50	>50	>50	9.606	>50
707-01-058-5	DH537	Ab008587	>50	>50	>50	0.246	>50
			<0.1	>0.1-1.0	>1.0-10	>10-25	>25-50

Figure 5

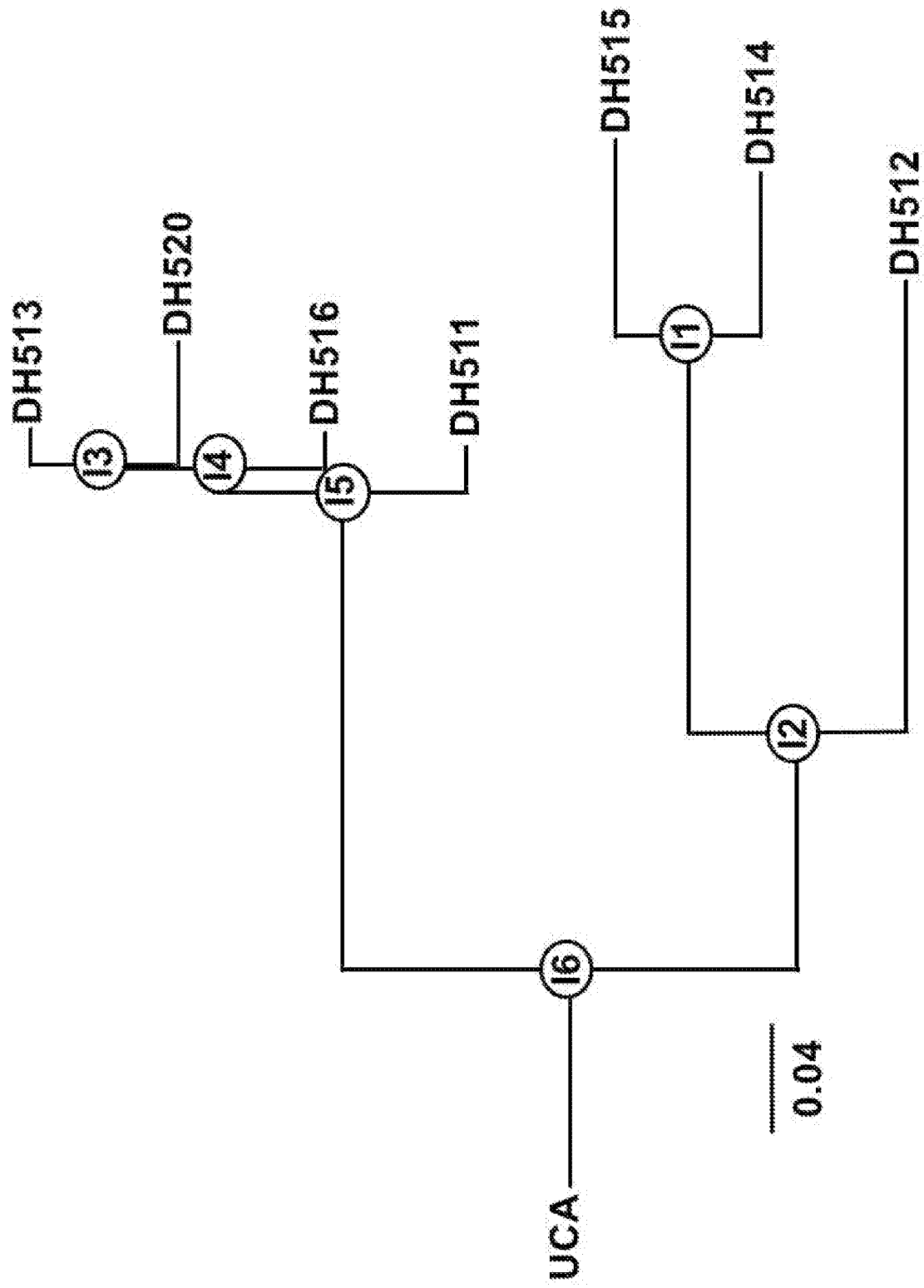
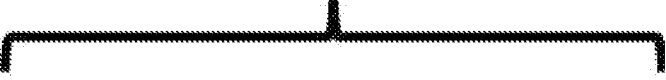


Figure 6

DH511 clonal lineage



clade	virus	DH511	DH512	DH513	DH514	DH515	DH516
		CHAVI	CHAVI	CHAVI	CHAVI	CHAVI	CHAVI
A	KER2018.11	8.150	4.630	18.500	13.400	15.801	18.700
A	Q23.17	4.460	2.520	9.500	9.000	9.550	13.400
A	Q769.h5	0.229	0.214	0.175	1.400	0.227	0.228
A	RW020.2	2.770	1.900	8.920	6.180	4.730	7.170
AC	6540.v4.c1	4.410	1.460	10.300	7.830	14.700	12.500
AD	Q168.a2	5.070	1.040	8.940	10.100	11.000	7.860
AE	C1080.c3	0.029	0.017	0.040	0.239	0.091	0.032
AE	CNE59	0.009	0.003	0.028	0.064	0.026	0.015
AE	TH966.8	0.017	0.013	0.033	0.156	0.081	0.038
AG	DJ263.8	0.027	0.066	0.140	0.732	0.218	0.318
B	6101.10	0.074	0.040	0.175	0.310	0.282	0.205
B	Bal.01	0.517	0.063	0.918	2.470	1.110	0.239
B	BG1168.01	0.283	0.219	0.705	1.320	1.070	0.609
B	CAAN.A2	4.520	3.150	9.300	11.300	9.890	16.800
B	JRC5FJB	1.590	0.674	3.980	5.090	2.790	2.500
B	JRFLJB	1.560	0.619	2.640	5.020	3.200	1.880
B	PVO.04	4.040	1.290	9.260	11.000	7.080	8.270
B	THRO.18	0.112	0.146	0.275	0.688	0.639	0.851
B	TRJO.58	6.250	2.800	8.960	6.520	7.500	8.530
B	TRO.11	0.442	0.235	1.120	0.921	0.932	0.941
B	YU2.DG	2.750	1.900	6.860	5.330	4.080	3.670
C	CNE58	2.680	0.682	5.830	4.600	3.740	8.160
C	DU156.12	0.134	0.039	0.206	0.167	0.396	0.327
C	DU172.17	0.457	2.710	6.920	4.630	2.240	9.070
C	DU422.01	0.909	0.565	2.830	1.840	2.100	3.570
C	ZA012.29	2.180	0.690	3.670	3.750	3.210	0.880
C	ZM106.9	10.100	3.350	20.800	14.500	18.800	16.500
C	ZM55.28a	10.901	5.950	16.600	10.000	24.900	15.900
D	57128.vrc.15	0.156	0.051	0.333	0.898	1.180	0.274
G	X1632.52.B10	0.314	0.159	0.409	0.323	0.705	0.671

Figure 7

DH517
clonal
lineage



clade	virus	DH517	DH518	VRC01	VRC01	CH103	10E8
		CHAVI	CHAVI	Mascola	Mascola	Mascola	Mascola
A	KER2018.11	>50	10.100	0.701	0.558	40.000	22.000
A	Q23.17	>50	5.920	0.064	0.063	10.400	0.783
A	Q769.h5	>50	0.998	0.024	0.077		2.670
A	RW020.2	>50	6.820	0.109	0.297	23.600	1.360
AC	6540.v4.c1	>50	12.400	>50	>50	1.770	3.010
AD	Q168.a2	>50	8.980	0.054	0.098	6.760	1.930
AE	C1080.c3	0.406	0.152	0.195	2.230	>50	0.099
AE	CNE59	0.558	0.019	0.344	0.555	>50	0.901
AE	TH966.8	0.044	0.044	0.036	0.320	>50	0.014
AG	DJ263.8	0.070	>50	0.064	0.030	0.784	0.015
B	6101.10	3.540	0.127	0.045	0.039	1.800	0.002
B	Bal.01	47.900	1.790	0.012	0.081	0.676	0.638
B	BG1168.01	5.540	1.160	0.218	0.908	21.700	0.405
B	CAAN.A2	>50	16.700	1.590	0.996	>50	1.140
B	JRCSEJB	>50	5.110	0.097	0.251	0.906	0.523
B	JRFLJB	28.300	3.120	0.007	0.020	0.021	0.155
B	PVO.04	>50	>50	0.254	0.348	>50	2.810
B	THRO.18	10.200	13.700	1.490	4.440	>50	0.100
B	TRJO.58	>50	8.690	0.074	0.121	>50	0.980
B	TRO.11	7.820	0.510	0.409	0.521	5.340	0.013
B	YU2.DG	>50	5.130	0.034	0.100	1.800	2.230
C	CNE58	>50	>50	0.171	0.189	0.543	0.501
C	DU156.12	5.700	0.543	0.055	0.066	>50	0.064
C	DU172.17	13.400	5.310	>50	>50	>50	0.006
C	DU422.01	13.100	6.630	>50	>50	>50	0.189
C	ZA012.29	>50	8.740	0.542	0.322	28.300	3.090
C	ZM106.9	>50	11.000	0.173	0.299	4.820	>25
C	ZM55.28a	>50	>50	0.313	0.289	4.590	2.850
D	57128.vrc.15	0.945	>50	>50	>50	>50	0.611
G	X1632.S2.B10	18.900	0.727	0.029	0.140	1.480	0.579

Figure 7cont.

<u>Antibody</u>	<u>IC50</u>	<u>IC80</u>	<u>IC50 <50ug/ml</u>	<u>IC80 <5ug/ml</u>
10E8	0.58	2.46	97%	65%
DH512	0.65	5.12	100%	50%
DH517	5.70	20.30	50%	3%
DH518	5.13	14.00	83%	20%
VRC01	0.27	0.73	87%	81%
CH01+				
CH31	3.73	NA	93%	NA

Figure 8

Antibody	IC50	IC80	IC50 <50ug/ml	IC80 <5ug/ml	Antibody Specificity
PGT121	0.06	0.27	63%	48%	V3-glycan
PGT128	0.07	NA	63%	NA	V3-glycan
DH2701A1	0.07	0.22	63%	61%	V3-glycan
DH429	0.06	0.22	63%	60%	V3-glycan
VRC01	0.27	0.73	87%	81%	VH1-2 CD4bs
CH31	0.10	0.42	83%	80%	VH1-2 CD4bs (VRC01-like)
CH01	3.79	NA	46%	NA	V1V2-glycan
CH01+					
CH31	3.73	NA	93%	NA	V1V2-glycan + VH1-2 CD4bs
CH103	4.54	NA	55%	NA	HCDR3 binder CD4 bs
CH98	4.20	NA	63%	NA	HCDR3 binder CD4 bs
DH493	5.98	NA	83%	NA	VH1-46 CD4bs (ANC131-like)
DH540	0.10	NA	90%	NA	N276-dependent CD4bs Ab (HJ16-like)

Figure 9

<u>Antibody</u>	<u>IC50</u>	<u>IC80</u>	<u>IC50 <50ug/ml</u>	<u>IC80 <5ug/ml</u>	<u>Antibody Specificity</u>
DH429	0.06	0.22	63%	60%	V3-glycan
DH512	0.65	5.12	100%	50%	MPER (10E8-like)
CH31	0.10	0.42	83%	80%	VH1-2 CD4bs (VRC01-like)
CH01	3.79	NA	46%	NA	V1V2-glycan
CH01+					
CH31	3.73	NA	93%	NA	V1V2-glycan + VH1-2 CD4bs
DH493	5.98	NA	83%	NA	VH1-46 CD4bs (ANC131-like)
DH540	0.10	NA	90%	NA	N276-dependent CD4bs Ab (HJ16-like)

Figure 10

MPER656-biotin	KKKNEQELLLELDKWASLNNWFDITNWLWYIR---	biotin	+++	511
MPER656.1-biotin	KKKNEQELLALDQWASLNNWFDISNWLWYIK---	KKK-biotin	+	+
MPER656.2-biotin	KKKNEQELLALDQWASLNNWFDITNWLWYIK---	KKK-biotin	-	-
MPER656.3-biotin	KKKNEQELLALDQWASLNNWFDITNWLWYIR---	KKK-biotin	+	+
CAP206_OmoB5_MPER656-biotin	KKKNEQELLALDQWASLNNWFDITNWLWYIK---	KKK-biotin	+	+
MPER656.3dYIK-biotin	KKKNEQELLALDQWASLNNWFDITNWLWYIR---	KKK-biotin	+	+
MPER656.3YIKd664_6A-biotin	KKKNEQELLALAKANNLWNSWFDITNWLWYIR---	KKK-biotin	+	+
MPER656.3dYIK664_6A-biotin	KKKNEQELLALAKANNLWNSWFDITNWLWYIR---	KKK-biotin	+	+
MPER656.2dYIK683R-biotin	KKKNEQELLALDQWASLNNWFDITNWLWYIR---	KKK-biotin	-	-
MPER656.3YIKd664A-biotin	KKKNEQELLALAKANNLWNSWFDITNWLWYIR---	KKK-biotin	+	+
CAP206_OmoB5_MPER656d683R-biotin	KKKNEQELLALDQWASLNNWFDITNWLWYIR---	KKK-biotin	+	+
MPER.03-biotin	KKKNEQELLLELDKWASLNNWFDITNWLWYIR---	KKK-biotin	+	+

Residues shown in light blue indicate positions that differ from MPER656-biotin.

Figure 11

>DH511VH

GAGGTTCAGCTGGTGGAGTCTGGGGGAGGCTTGGTGAAGCCGGGGGGGTCTCTTAGA
CTCCCCGGTGCAGCCTCTGGTTTCACTTTCACCAACACGTGGATGAGTTGGGTCCGT
CAGGCGCCAGGGAAGGGACTGGAGTGGGTCTGGTTCGGATTAGCCGGAACAAAGATGGC
GCGAAAACAGAGTACGCCGCACCCGTGAGAGGCAGATTACCATCTCAAGAGATGAC
TCCAGAGACACATTGTATCTGCAGATGACCAGCCTGAAAATAGAGGATTCAGGCCGG
TATTTTTCACCGCAGATCTTGGGGAGCCCGTGGTGTACGATCCATTTTGTAGTGG
GGGTCTTATTATTATTATATGGACCTCTGGGGCAAGGGGACCACGGTCACCGTCTCT
TCA

>DH511VK

GACATCCAGTTGACCCAGTCTCCATCTCCCCTGTCTGCGTCTGTGGGAGACACAGTC
ACTATCACTTGTCTGGGCCAGCCAGAAGATTAGCGACTATTTGAACTGGTACCAACAG
AAGCCGGGGAGAGCCCCCAAATACTCATTTACGCTGCGTCCAAGTTGGGGAGTGGC
GTCCCATCAAGGTTCAAGTGGCAGTGGATATGGCAGAGATTTCACTCTCACCATCACC
GGTCTGCAGCCTGAAGATTTTGCAACCTATTATTGTCAAGAGGCTTACAGTTCTACT
CCCACGTAACTTTTGGCCAGGGGACCAGGCTGGATCTCAAAC

>DH512VH

CAGGTGCAGCTGGTACAGTCTGGGGGAGGTCTGGTGAAGCCGGGGGGGTCCCTCACA
CTCTCCTGTTTCAGCCTCTGGATTCTTTTTTCGATAATTCATGGATGGGGTGGGTCCGT
CAGGCGCCAGGGAAGGGACTGGAGTGGGTGGCCGCATTAGAAGGCTCAAAGACGGT
GCGACAGGAGAATATGGTGCAGCCGTGAAGGACAGATTACCATTTCAAGAGATGAC
AGTAGAAATATGCTGTACCTGCACATGAGGACCTGAAAACCGAGGACTCAGGCACT
TATTATTGTACCATGGATGAGGGGACCCAGTAACACGCTTCTTAGAATGGGGCTAC
TTCTATTATTATATGGCCGTTTGGGGCAGAGGGACCACGGTCATCGTCTCTTCA

>DH512VK

GACATCGTGATGACCCAGTCTCCGTCCTCCGTGTCTGCATCTGTGGGAGACAGAGTC
ACCATCACTTGCCGGGCAAGTCAGAATATTAGAGACTATTTAAATTGGTATCAACAT
AAACCCGGGGGATCCCCTAGACTCCTAATTTATGCTGCGTCAACTTTGCAAACCTGGG
GTCCCGTCCAGATTCAGCGGCAGTGGATCTGGGAACCTTTTCACTCTCACCATTACC
AATCTGCAACCTGAAGATTTTGCAACTTATTATTGTCAAGAGAATTATAATACTATC
CCCTCGCTCAGCTTTGGTCAGGGGACCAAGGTGGACATCAGGC

Figure 12

>DH513VH

GAGGTTCAGCTGGTGGAGTCTGGGGGAGGCTTGGTGAAGCCGGGGGGGTCTCTTAGA
CTCTCCTGTGTAGCCTCTGGCTTCACTTTCAGCAACACGTGGATGAGTTGGGTCCGT
CAGGCGCCAGGGAAGGGACTGGAGTGGGTCTGGTTCGGATTAGCCGGAACAAAGATGGC
GCGAAAACAGAGTACGCCGCACCCGTGAGAGGCAGATTCACCATCTCAAGAGATGAC
TCCAGAGACACATTGTATCTGCAGATGAGCAGCCTGAAAATAGAGGATTACAGGCCGG
TATTTTTGCACCGCAGATCTTGGGGAGGCCGTTGTGTCACGATTTTTTTGAGTGGGGG
TCCTATTATTACTACATGGACTTCTGGGGCAAGGGGACCACGGTCACCGTCTCTTCA

>DH513VK

GACATTCAGATGACCCAATCTCCATCTCCCCTGTCTGCGTCTGTGGGAGACACAGTC
ACTATCACTTGCCGGGGCCAGCCAGAAGATTAGCGACTATTTGAACTGGTACCAACAG
AGGCCGGGGAGAGCCCCCAAGATCCTCATTTACGCTGCGTCCAAGTTGGCAAGCGAC
GTCCCATCAAGATTTAGTGGCAGTGGATATGGCAGAGATTTCACTCTCACCATAACC
GGTCTGCAGCCTGAAGATTTTGCAACCTATTATTGTCAGGAGGCTTACAGTTCTACC
CCCACGTAACTTTTGGCCAGGGGACCAGGCTGGATCTCAAAC

>DH514VH

GAGGTGCAGCTGGTGGAGTCTGGGGGCGGCTTGATAAAGCCGGGACAGTCACTCACA
CTATTCTGTGTGGGCTTTGGATTCAACTTCGCTAACGACTGGATGGGCTGGGTCCGC
CAGGCTCCAGGGAAGGGACTGGAATGGGTTGGGCGTATAAGGAGACTGAAAGATGGT
GCGAAAGCTGAATATGGATCTTCCGTGAAGGGTAGATTCACCATCTCAAGGGATGAT
TCCAAAAACACCCCTATACTTGCACATGAGCAGCCTCAAGGTCGAAGACACAGCCGTC
TACTATTGCACCCGAGACGAGGGGGCCCCAGTTACCCGGTTTCTGGAGTGGGGCTCC
TATTACTACTACATGGCCGTCTGGGGCAAAGGGACCACGGTCACCGTCTCTTCA

>DH514VK

GACATCCAGTTGACCCAGTCTCCAGCCTCTCTGTCTGCATCTGTAGGAGACACAGTG
ACTATCACTTGCCGGGGCAAGTCAGAGTATAAAAGATTACATAAATTGGTATCAACAC
AAATCCGGGAGCGCCCCCTAGACTCCTGATTTATGCTGCGTCAACCTTACAAAGTGGA
ATCTCGTCAAGGTTCACTGGCAGTGGGTCTGGGACACAGTTCACTCTCACCATTAAAC
AGTCTGCAACCTGAAGATTTTGCGACTTATTATTGTCAAGAGGCTTATAACACCAAC
CCCACACTCTCCTTTGGTCAGGGGACCAGGGTGGACAAGAAGC

Figure 12 cont.

>DH515VH

GAGGTTTCAGCTGGTGGAGTCTGGGGGCGGCTTGGTGAAGCCGGGACAGTCACTCACA
CTTTCCTGTGTGGGCTTTGGATTCAATTTTCGCTAACGACTGGATGGGCTGGGTCCGC
CAGGCTCCAGGGAAGGGACTGGAATGGGTTGGTTCGAATAAGGAGACTAAAAGACGGT
GCGACAACAGAATATTCTTCATCCGTGAAGGGGAGATTCAGTGTCTCAAGAGATGAT
TCAAGGAACACAGTATACTTACACATGAGTAGCCTCAAAGTCCAGGACATTGGCGTC
TATTATTGTACTCGAGACGAGGGGGCCCCGGTTACTCGATTTCTGGAGTGGGGCTCC
TATTACTACTATATGGCCGTCTGGGGCAGAGGGACCACGGTCACCGTCTCTTCA

>DH515VK

GACATCCAGATGACCCAGTCTCCAACCTCTCTGTCTGCATCTGTAGGAGACACAGTT
GCTATCACTTGCCGGGCAAGTCAGAGTGTTAAAGATTATGTGAATTGGTATCAACAC
AAATCCGGGAGCGCCCCCTCGACTCCTGATTTATGCTGCCTCAGTCTTACATACTGGA
GTCTCGTCAAGGTTCACTGGCAGTGGGTCTGGGACACAGTTCACTCTCACCATTAGC
AGTCTACAACCTGAAGATTTTGCTACTTATTATTGTCAAGAGGCTTATAACACCTAT
CCCACACTCTCCTTTGGTCAGGGGACCAGGGTGGACAGGAAAC

>DH516VH

GAGGTTTCAGCTGGTGGAGTCTGGGGGAGGCTTGGTGAAGCCGGGGGGGTCTCTTAGA
CTCTCCTGTGTAGCCTCTGGCTTCACTTTCAGCAACACGTGGATGAGTTGGGTCCGT
CAGGCGCCAGGGAAGGGACTGGAGTGGGTCTGGTTCGGATTAGCCGGAACAAAGATGGC
GCGAAAACAGACTACGCCGCACCCGTGAGAGGCAGATTCACCATCTCCAGAGATGAC
TCCAGAGACACATTGTATCTGCAGATGAGCAGCCTGAAAATAGAGGATTCAGGCCGG
TATTTTTTGCACCGCAGATCTTGGGGAGGCCGTGGTGTACGATTTTTTTGAGTGGGGG
TCCTATTATTACTACATGGACTTCTGGGGCAAGGGGACCACGGTCACCGTCTCTTCA

>DH516VK

GATATTGTGATGACCCAGTCTCCACCTCCCCTGTCTGCGTCTGTGGGAGACACAGTC
ACTATCACTTGCCGGGCCAGCCAGAAGATTAGCGACTATTTGAACTGGTACCAACAG
AGGCCGGGGAGAGCCCCCAAATACTCATTTACGCTGCGTCCAAGTTGGGAAGCGAC
GTCCCATCAAGGTTCACTGGCAGTGGATATGGCAGAGATTTCACTCTCACCATCACC
GGTCTGCAGCCTGAAGATTTTGCAACCTATTATTGTCAGGAGGCTTACAGTTCTACT
CCCACGTTAAGTTTTGGCCAGGGGACCAGGCTGGATCTCAAAC

Figure 12 cont.

>DH517VH

GAAAGGCAGGTGGTGGGAATATGGGGGAGGCTTGGTGAAGCCGGGGGGGTCTCTTAGA
CTCTCTTGTTTACCGTTTGCCTTTGGGTTCAGGGCCCCCTGGAGGAGTTCTGTCCGT
CACGCGCCTGGGGGCGGAGCGGAGTGGGTTCGGTCGGATTAGCCGGAACAAAGATGGC
GCGAAAACAGAGTACGCCGCACCCGTGAGAGGCAGATTCACCATCTCAAGAGATGAC
TCCAGAGACACATTGTATCTGCAGATGACCAGCCTGAAAATAGAGGATTCAGGCCGG
TATTTTTGCACCGCAGATCTTGGGGAGCCCGTGGTGTACGATTTTTTGAGTGGGGG
TCTTATTATTATTATATGGACCTCTGGGGCAAGGGGACCACGGTCACCGTCTCTTCA

>DH517VL

TCTTCTGAGCTGACTCAGGACCCCACTGTGTCTGTGGCCTTGGGCCAGACAGTCAAG
ATCAGATGCCAAGGAGCCAGCCTCAGAGACTGTTATGCGACCTGGTACCGGCAGAAG
CCAGGACAGGCCCCCAACACTTCTCATTTATGATATAAATAAGAGGCCCTCAGGTATC
CCAGACCGATTCTCTGCCTCCTACTCAGGGAGCACTTCTTCCTTGACCATTATTGGG
GCTCAGCCGGAAGATGAGGCTGACTATTTTTGTGCTTCGCGGGACAGGAGTGGTGAC
CGTCTTGGCGTCTTCGGCGGTGGGACCAAACCTGACCGTCCTG

>DH518VH

CAGCTGCAGGAGTTCGGGTCCCAGACTGGTGAGGCCTTCGGAGACCCTGTCCCTCACC
TGCACTGTATCTGGCTCTGGTGTCTCCGTCAGTCGTGGGAGTTATTATTGGGGCTGG
ATACGCCAGTCCCCAGAAAAGGGACTCGAATGGATTGGAAGTGTCTATTCCACTACT
AGTGGAACCACTACTACAACCCGTCCCTCAAGAGTCGAGTCACCTTTTCGAAGGAC
ACGTCCCAGAACGCCTTCTCCCTGACTCTGACGTCTATTACCGCCGCGGACACGGCC
GTCTATTACTGTGCAAGACAATTTGGCTTCATGGGGGGCTTTTTGGAGTGGTATCCG
CACTATTTTGACTTCTGGGGCCCCGGGAATCCAGGTCGTCGTGTCTTCT

>DH518VK

GACATTGTGATGACCCAGTCTCCATCCTACCTGTCTACATCTGTCGGTGACAGCATC
ACCATCACTTGCCGGGCAAGTCAGAGTATTAAACATATGTAAATTGGTATCAACAA
AGACCAGGGAGAGCCCCTAAACTCCTCATCTATTCTTCATCCACTTTGCAACCTGGG
GTCCCGTCAAGATTCAGCGCCAGTGGATCTGGGACAGATTTTCGTTCTCTCCATCACC
AATTTGCAGTCTGAAGATTTTGCAACTTACTACTGTCAACAGACCTACTACACCCCC
TCTACTTTTGGCCAGGGGACCACACTGGACATCAAG

Figure 12 cont.

>DH536VH

CAGGTGCAGCTGGTACAGTCTGGGGCTGAGGTGAAGAAGCCTGGGTCCTCGGTCAAG
GTCTCCTGCAAGGCCTCTGGAGGCTCCTTCTACACCTATACTATCAACTGGGTGCGA
CAGGCCCCCTGGACAAGGGCTTGAGTGGATGGGCAGGGTCACCACTATGTTTGGTGTA
ACACTTTACGCACAGAAATTCCAGGGCAGAGTCACACTTACCGCGGACAAATCCACG
AGCACAGCCTACATGGAAGTGAAGCAGTCTAAGATCTGAGGACACGGCCGTCTATTAT
TGTGCGACAGATGGGCCTGACAATTTTTGGAGTGGCTTGTCTCATGCTTTCGATCTC
TGGGGCCAGGGGACAATGGTCACCGTCTCTTCA

>DH536VL

CAGTCTGCCCTGACTCAGCCTGCCTCCGTGGCTGGGTCTCCTGGACAGTCGATCACC
ATCTCCTGCACTGGAACCAGCAGTGACATTGGTGATTCTAAGTATGTCTCCTGGTAC
CAACAGTTCCCAGGCAAAGCCCCCAAAGTCATGATTTATGAGGTCAGTTATCGGCCC
TCAGGAGTCTCTAGCCGCTTCTCTGGCTCCAAGTCTGGCAACACGGCCTCCCTGACC
ATCTCTGGACTCCAGACTGAGGACGAGGCTGATTATTATTGCATGGCATATACAGGC
ACCTTCACTGCTATTTTCGGCGGAGGGACCAAGCTGACCGTCCTG

>DH537VH

CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAGGAAGGCTGGGTCGTCTCGGTGAAG
GTCTCCTGCAAGGCTTCTGGAGGCACCTTCACCAGCTATGGCTTCAGCTGGATACGG
CAGGCCCCCTGGCCAAGGGCTTGAGTGGATGGGAAACGTCATCCCTGTCTTTGGTTCA
ACAACTACGCACAGAAATTTTCAGGGCAGAGTCAGTATTACCGCGGACGAAGCCACG
GGCACAGTCCACATGGACCTCACCAGCCTGACATCTGACGACACGGCCGTTTATTAC
TGTGTGAGGTTCGAGTAGAGAACTGCCAACGTCAATGGAACGGTGGTTCGACCCCTGG
GGCCAGGGAACCCAGGTCATTGTCTCCTCG

>DH537VK

GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTGGGAGACAGCGTC
ACCATTACTTGCCGGGCAAGTCAGAGCATTAACACCTATTTAAATTGGTATCAGCAG
AAACCAGGGAAGGCCCCCTAAACTCCTGATCTATTCTGCATCCAATTTACACAATGGG
GTCCCATCGAGGTTTCAGTGGCAGTGGATCTGGGACATCTTTCCTCTCACCATCAAC
AATCTACAACCTGAAGATTTTGCAACTTACTACTGTCAACAGAGTTACAGTGCCCCCT
TACACTTTTGGCCAGGGGACCAAGTCAGACACCAAA

Figure 12 cont.

>DH511VH

EVQLVESGGGLVKPGGSLRLPGAASGFTFTNTWMSWVRQAPGKGLEWVGRISRNDKG
AKTEYAAPVRGRFTISRDDSRDTLYLQMTSLKIEDSGRYFCTADLGEPVVSRSEFEW
GSYYYYMDLWGKGTTVTVSS

>DH511VK

DIQLTQSPSPLSASVGDTVTITCRASQKISDYLNWYQQKPGRAPKILIYAASKLGSG
VPSRFSGSGYGRDFTLTITGLQPEDFATYYCQEAYSSTPTLTFTGQGTRLDLK

>DH512VH

QVQLVQSGGGLVKPGGSLTLSCSASGFFFDNSWMGWVRQAPGKGLEWVGRIRRLKDG
ATGEYGAAVKDRFTISRDDSRNMLYLHMRCLKTEDSGTYCYCTMDEGTPVTRFLEWGY
FYYYMAVWGRGTTVIVSS

>DH512VK

DIVMTQSPSSVSASVGDRVTITCRASQNIRDYLNWYQHKPGGSPRLLIYAASLTQTG
VPSRFSGSGSGNLTITITNLQPEDFATYYCQENYNTIPSLSFGQGTKVDIR

>DH513VH

EVQLVESGGGLVKPGGSLRLSCVASGFTFSNTWMSWVRQAPGKGLEWVGRISRNDKG
AKTEYAAPVRGRFTISRDDSRDTLYLQMSSLKIEDSGRYFCTADLGEAVVSRFFEWG
SYYYMDFWKGKGTTVTVSS

>DH513

VKDIQMTQSPSPLSASVGDTVTITCRASQKISDYLNWYQQRPGRAPKILIYAASKLA
SDVPSRFSGSGYGRDFTLTITGLQPEDFATYYCQEAYSSTPTLTFTGQGTRLDLK

>DH514VH

EVQLVESGGGLIKPGQSLTLFCVGFGENFANDWMGWVRQAPGKGLEWVGRIRRLKDG
AKAEYGSSVKGRFTISRDDSKNTLYLHMSSLKVEDTAVYYCTRDEGAPVTRFLEWGS
YYYYMAVWKGKGTTVTVSS

>DH514VK

DIQLTQSPASLSASVGDTVTITCRASQSIKDYINWYQHKSGSAPRLLIYAASLTQSG
ISSRFTGSGSGTQFTLTINSLQPEDFATYYCQEAYNTNPTLSFGQGTTRVDKK

Figure 13

>DH515VH

EVQLVESGGGLVKPGQSLTSLSCVGFGENFANDWMGWVRQAPGKGLEWVGRIRRLKDG
ATTEYSSSVKGRFSVSRDDSRNTVYLHMSSLKVQDIGVYYCTRDEGAPVTRFLEWGS
YYYYMAVWGRGTTVTVSS

>DH515VK

DIQMTQSPTSLASVGDVVAITCRASQSVKDYVNWYQHKSGSAPRLLIYAASVLHTG
VSSRFTGSGSGTQFTLTISLQPEDFATYYCQEAYNTYPTLSFGQGTRVDRK

>DH516VH

EVQLVESGGGLVKPGGSLRLSCVASGFTFSNTWMSWVRQAPGKGLEWVGRISRNDKG
AKTDYAAPVRGRFTISRDDSRDTLYLQMSLKIEDSGRYFCTADLGEAVVSRFFEWG
SYYYMDFWKGKTTVTVSS

>DH516VK

DIVMTQSPFPLSASVGDVTITCRASQKISDYLWYQQRPGRAPKILIYAASKLGSD
VPSRFSGSGYGRDFTLTITGLQPEDFATYYCQEAYSSTPTLSFGQGTRLDLK

>DH517VH

ERQVVEYGGGLVKPGGSLRLSCLPFAFGFRAPWRSSVRHAPGGGAEWVGRISRNDKG
AKTEYAAPVRGRFTISRDDSRDTLYLQMTSLKIEDSGRYFCTADLGEPPVSRFFEWG
SYYYMDLWKGKTTVTVSS

>DH517VL

SSELTQDPTVSVALGQTVKIRCQGASLRDCYATWYRQKPGQAPTLLIYDINKRPSGI
PDRFSASYSGSTSSLTIIGAQPEDEADYFCASRDRSGDRLGVFGGGTKLTVL

>DH518VH

QLQESGPRLVRPSETLSLTCTVSGSGVSVSRGSYYWGWIRQSPEKGLEWIGSVYSTT
SGKTYYNPSLKSRTVFSKDTSQNAFSLTLTSITAADTAVYYCARQFGFMGGFLEWYP
HYFDFWGPGLQVVVSS

>DH536

VHQVQLVQSGAEVKKPGSSVKVSCKASGGSFYTYTINWVRQAPGQGLEWMGRVTTMF
GVTLYAQKFQGRVTLTADKSTSTAYMELSSLRSEDVAVYYCATDGPDNFWSGLSHAF
DLWGQGTMTVTVSS

>DH518VK

DIVMTQSPSYLSTSVGDSITITCRASQSIKTYVNWYQQRPGRAPKLLIYSSSTLQPG
VPSRFSASGSGTDFVLSITNLQSEDFATYYCQQTYTPSTFGQGTTLDIK

Figure 13 cont.

>DH536VH

QVQLVQSGAEVKKPGSSVKVSCKASGGSEFYTYTINWVRQAPGQGLEWMGRVTTMFGV
TLYAQKFQGRVTLTADKSTSTAYMELSSLRSEDVAVYYCATDGPDNFWSGLSHAFDL
WGQGTMTVTVSS

>DH536VL

QSALTQPASVAGSPGQSITISCTGTSSDIGDSKYVSWYQQFPGKAPKVMIEVSYRP
SGVSSRFSGSKSGNTASLTISGLQTEDEADYYCMAYTGTFTATFGGGTKLTVL

>DH537VH

QVQLVQSGAEVRKAGSSVKVSCKASGGTFTSYGFSWIRQAPGQGLEWMGNVIPVFGS
TNYAQKFQGRVSITADEATGTVHMDLTSLTSDDTAVYYCVRSSRELPTSMERWFDPW
GQGTQVIVSS

>DH537VK

DIQMTQSPSSLSASVGDSVTITCRASQSINTYLNWYQQKPGKAPKLLIYSASNLHNG
VPSRFSGSGSGTSFTLTINNLPEDFATYYCQQSYSAPTYTFGQGTKSDTK

Figure 13 cont.

DH511VH	EVQLVESGGG	LVKPGGSLRL	PGAASGFTFT	NTWMSWVRQA
DH512VH	Q-----Q-----	-----T-	SCS-----F-D	-S--G-----
DH513VH	-----	-----	SCV-----S	-----
DH514VH	-----	-I---Q--T-	FCVGF--N-A	-D--G-----
DH515VH	-----	-----Q--T-	SCVGF--N-A	-D--G-----
DH516VH	-----	-----	SCV-----S	-----

DH511VH	PGKGLEWVGR	ISRNKDGAKT	EYAAPVRGRF	
DH512VH	-----	-R-L----TG	--G-A-KD--	
DH513VH	-----	-----	-----	
DH514VH	-----	-R-L----A	--GSS-K---	
DH515VH	-----	-R-L----T-	--SSS-K---	
DH516VH	-----	-----	D-----	70

DH511VH	TISRDDSRDT	LYLQMTSLKI	EDSGRYFCTA	<u>DLGEFVVSRS</u>
DH512VH	-----NM	---H-RT--T	---T-Y--M	-E-T--.T-.
DH513VH	-----	-----S---	-----	---A-----.
DH514VH	-----KN-	---H-S---V	--TAV-Y--R	-E-A--.T-.
DH515VH	SV-----N-	V--H-S---V	Q-I-V-Y--R	-E-A--.T-.
DH516VH	-----	-----S---	-----	---A-----.

DH511VH	<u>IFEWGSYYYY</u>	MDLWGKGTTV	TVSS	
DH512VH	FL---YF---	-AV--R----	I---	
DH513VH	F-----	--F-----	----	
DH514VH	FL-----	-AV-----	----	
DH515VH	FL-----	-AV--R----	----	
DH516VH	F-----	--F-----	----	134

Figure 14A

DH511VK	DIQLTQSPSP	LSASVGDTVT	ITCRASQKIS	DYLNWYQQKP
DH512VK	--VM-----S	V-----R--	-----N-R	-----H--
DH513VK	---M-----	-----	-----	-----R-
DH514VK	-----AS	-----	-----S-K	--I---H-S
DH515VK	---M---TS	-----A	-----SVK	--V---H-S
DH516VK	--VM---P-	-----	-----	-----R-

DH511VK	GRAPKILIYA	ASKLGSGVPS	RFSGSGYGRD
DH512VK	-GS-RL----	--T-QT----	-----S-NL
DH513VK	-----	---A-D---	-----
DH514VK	-S--RL----	--T-Q--IS-	--T---S-TQ
DH515VK	-S--RL----	--V-HT--S-	--T---S-TQ
DH516VK	-----	-----D--	----- 70

DH511VK	FTLTITGLQP	EDFATYYCQE	AYSSPTTTF	GQGTRLDLK
DH512VK	-----N---	-----	N-NTI-S-S-	---KV-IR
DH513VK	-----	-----	-----	-----
DH514VK	-----NS---	-----	--NTN---S-	-----V-K-
DH515VK	-----SS---	-----	--NTY---S-	-----V-R-
DH516VK	-----	-----	-----S-	----- 109

Figure 14B

DH511VH EVQLVESGGG LVKPGGSLRL PGAASGFTFT NT..WMSWVR
 DH512VH Q-----Q----- -----T- SCS-----F-D -S..--G---
 DH513VH ----- ----- SCV-----S --..-----
 DH514VH ----- -I---Q--T- FCVGF--N-A -D..--G---
 DH515VH ----- -----Q--T- SCVGF--N-A -D..--G---
 DH516VH ----- ----- SCV-----S --..-----
 10E8VH ----- ----- SCS-----D-D -A..--T---
 4E10VH Q----Q--AE VKR--S-VTV SCK---GS-S TY..AL----
 2F5VH RIT-K---PP -----TQT-T- TCSF---SLS DFGVGVG-I-

DH511VH QAPGKGLEWV GRISRNKDGA KTEYAAPVRG
 DH512VH ----- ---R-L----- TG--G-A-KD
 DH513VH ----- -----
 DH514VH ----- ---R-L----- -A--GSS-K-
 DH515VH ----- ---R-L----- T---SSS-K-
 DH516VH ----- ----- --D-----
 10E8VH -P----- ---TGPGE-W SVD-----E-
 4E10VH ----R----M -GVIPLL..T I-N--PRFQ-
 2F5VH -P---A---L AI-YSDD... DKR-SPSLNT 70

Figure 15A

DH511VH	RFTISRDDSR	DTLYLQMTSL	KIEDSGRYFC	<u>TADLGEPVVS</u>
DH512VH	-----	NM---H-RT-	-T-----T-Y-	-M-E-T--.T
DH513VH	-----	-----S--	-----	-----A---
DH514VH	-----K	N-----H-S--	-V--TAV-Y-	-R-E-A--.T
DH515VH	--SV-----	N-V--H-S--	-VQ-I-V-Y-	-R-E-A--.T
DH516VH	-----	-----S--	-----	-----A---
10E8VH	-----LN-I	NF---E-NN-	RM----L---	ARTGKY....
4E10VH	-I--TA-R-T	S-A--ELN--	RP--TAV-Y-	AREGTT....
2F5VH	-L--TK-T-K	NQVV-V--RV	SPV-TAT----	AHRR-PTT..
DH511VH	<u>RSIFEWGSY.</u>	..YYMDLWG	KGTTVTVSS	
DH512VH	-.FL---YF-	-----AV--	R-----I---	
DH513VH	-.F-----	-----F--	-----	
DH514VH	-.FL-----	-----AV--	-----	
DH515VH	-.FL-----	-----AV--	R-----	
DH516VH	-.F-----	-----F--	-----	
10E8VH	..YDF-SG-P	PGEE-FQD--	R--L-----	
4E10VH	G--WLG	KPIGAFAH--	Q--L-----	
2F5VH	..L-GVPIAR	GPVNA--V--	Q-I---I--	

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Figure 15A cont.

DH511VK	DIQLTQSPSP	LSASVGDTVT	ITCRASQKIS
DH512VK	--VM-----S	V-----R--	-----N-R
DH513VK	---M-----	-----	-----
DH514VK	-----AS	-----	-----S-K
DH515VK	---M----TS	-----A	-----SVK
DH516VK	--VM----P-	-----	-----
4E10VK	E-V-----GT	Q-L-P-ERA-	LS-----SVG
2F5VK	AL-----S	-----RI-	-----GVT
10E8VL	SYE---.ETG	V-VAL-R---	-----GDSL.R
DH511VK	.DYLNWYQQK	PGRAPKILY	AASKLGSQVP
DH512VK	.-----H-	--GS-RL---	---T-QT---
DH513VK	.-----R	-----	-----A-D--
DH514VK	.--I----H-	S-S--RL---	---T-Q--IS
DH515VK	.--V----H-	S-S--RL---	---V-HT--S
DH516VK	.-----R	-----	-----D--
4E10VK	NNK-A----R	--Q--RL---	G--SRP---A
2F5VK	.SA-A--R--	--SP-QL---	D--S-E----
10E8VL	SH-AS---K-	--Q--IL-F-	GKNNRP----- 60

Figure 15B

DH511VK	SRFSGSGYGR	DFTLTITGLQ	PEDFATYYCQ
DH512VK	-----S-N	L-----N--	-----
DH513VK	-----	-----	-----
DH514VK	---T---S-T	Q-----NS--	-----
DH515VK	---T---S-T	Q-----SS--	-----
DH516VK	-----	-----	-----
4E10VK	D-----S-T	-----SR-E	-----V----
2F5VK	-----S-T	E-----ST-R	-----
10E8VL	D-----AS-N	RAS---S-A-	A--D-E---S

DH511VK	<u>EAYSST.PTL</u>	TFGQGTRLDL	K
DH512VK	-N-NTI--S-	S-----KV-I	R
DH513VK	-----	-----	-
DH514VK	---NTN----	S-----V-K	-
DH515VK	---NTY----	S-----V-R	-
DH516VK	-----	S-----	-
4E10VK	QYGQ..-SLS	-----KVEV	-
2F5VK	QLHF..-YPH	---G---V-V	R
10E8VL	SRDK-GSRLS	V--G--K-TV	L 111

Figure 15B cont.

	AA position	
MPER-26		Biotin-GGG-QELLELDKWASLWNWFNITNWLWYIK
MPER-26 A1	658	Biotin-GGG-AELLELDKWASLWNWFNITNWLWYIK
MPER-26 A2	659	Biotin-GGG-QALLELDKWASLWNWFNITNWLWYIK
MPER-26 A3	660	Biotin-GGG-QEAELLELDKWASLWNWFNITNWLWYIK
MPER-26 A4	661	Biotin-GGG-QELAELLELDKWASLWNWFNITNWLWYIK
MPER-26 A5	662	Biotin-GGG-QELLALLELDKWASLWNWFNITNWLWYIK
MPER-26 A6	663	Biotin-GGG-QELLEADKWASLWNWFNITNWLWYIK
MPER-26 A7	664	Biotin-GGG-QELLELAKWASLWNWFNITNWLWYIK
MPER-26 A8	665	Biotin-GGG-QELLELDAWASLWNWFNITNWLWYIK
MPER-26 A9	666	Biotin-GGG-QELLELDKAASLWNWFNITNWLWYIK
MPER-26 N10	667	Biotin-GGG-QELLELDKWNSLWNWFNITNWLWYIK
MPER-26 A11	668	Biotin-GGG-QELLELDKWAALWNWFNITNWLWYIK
MPER-26 A12	669	Biotin-GGG-QELLELDKWASAWNWFNITNWLWYIK
MPER-26 A13	670	Biotin-GGG-QELLELDKWASLANWFNITNWLWYIK
MPER-26 A14	671	Biotin-GGG-QELLELDKWASLWAWFNITNWLWYIK
MPER-26 A15	672	Biotin-GGG-QELLELDKWASLWNAFNITNWLWYIK
MPER-26 A16	673	Biotin-GGG-QELLELDKWASLWNWANITNWLWYIK
MPER-26 A17	674	Biotin-GGG-QELLELDKWASLWNWFAITNWLWYIK
MPER-26 A18	675	Biotin-GGG-QELLELDKWASLWNWFNATNWLWYIK
MPER-26 A19	676	Biotin-GGG-QELLELDKWASLWNWFNIANWLWYIK
MPER-26 A20	677	Biotin-GGG-QELLELDKWASLWNWFNITAWLWYIK
MPER-26 A21	678	Biotin-GGG-QELLELDKWASLWNWFNITNALWYIK
MPER-26 A22	679	Biotin-GGG-QELLELDKWASLWNWFNITNWAWEYIK
MPER-26 A23	680	Biotin-GGG-QELLELDKWASLWNWFNITNWLWAYIK
MPER-26 A24	681	Biotin-GGG-QELLELDKWASLWNWFNITNWLWAIK
MPER-26 A25	682	Biotin-GGG-QELLELDKWASLWNWFNITNWLWYAK
MPER-26 A26	683	Biotin-GGG-QELLELDKWASLWNWFNITNWLWYIA

Figure 16

	AA position	
MPER-26		Biotin-GGG-QELLELDKWASLWNWFNITNWLWYIK
MPER-26 A1	658	Biotin-GGG-AELLELDKWASLWNWFNITNWLWYIK
MPER-26 A2	659	Biotin-GGG-QALLELDKWASLWNWFNITNWLWYIK
MPER-26 A3	660	Biotin-GGG-QEAELELDKWASLWNWFNITNWLWYIK
MPER-26 A4	661	Biotin-GGG-QELAELDKWASLWNWFNITNWLWYIK
MPER-26 A5	662	Biotin-GGG-QELLAELDKWASLWNWFNITNWLWYIK
MPER-26 A6	663	Biotin-GGG-QELLEADKWASLWNWFNITNWLWYIK
MPER-26 A7	664	Biotin-GGG-QELLELAKWASLWNWFNITNWLWYIK
MPER-26 A8	665	Biotin-GGG-QELLELDAWASLWNWFNITNWLWYIK
MPER-26 A9	666	Biotin-GGG-QELLELDKAASLWNWFNITNWLWYIK
MPER-26 N10	667	Biotin-GGG-QELLELDKWNSLWNWFNITNWLWYIK
MPER-26 A11	668	Biotin-GGG-QELLELDKWAALWNWFNITNWLWYIK
MPER-26 A12	669	Biotin-GGG-QELLELDKWASAWNWFNITNWLWYIK
MPER-26 A13	670	Biotin-GGG-QELLELDKWASLANWFNITNWLWYIK
MPER-26 A14	671	Biotin-GGG-QELLELDKWASLWAWFNITNWLWYIK
MPER-26 A15	672	Biotin-GGG-QELLELDKWASLWNAFNITNWLWYIK
MPER-26 A16	673	Biotin-GGG-QELLELDKWASLWNWAFNITNWLWYIK
MPER-26 A17	674	Biotin-GGG-QELLELDKWASLWNWFAITNWLWYIK
MPER-26 A18	675	Biotin-GGG-QELLELDKWASLWNWFNAITNWLWYIK
MPER-26 A19	676	Biotin-GGG-QELLELDKWASLWNWFNIAFWLWYIK
MPER-26 A20	677	Biotin-GGG-QELLELDKWASLWNWFNITAFWLWYIK
MPER-26 A21	678	Biotin-GGG-QELLELDKWASLWNWFNITNAFWYIK
MPER-26 A22	679	Biotin-GGG-QELLELDKWASLWNWFNITNWFAYIK
MPER-26 A23	680	Biotin-GGG-QELLELDKWASLWNWFNITNWLAFYIK
MPER-26 A24	681	Biotin-GGG-QELLELDKWASLWNWFNITNWLWAFIK
MPER-26 A25	682	Biotin-GGG-QELLELDKWASLWNWFNITNWLWYAFK
MPER-26 A26	683	Biotin-GGG-QELLELDKWASLWNWFNITNWLWYIA

Figure 16 cont.

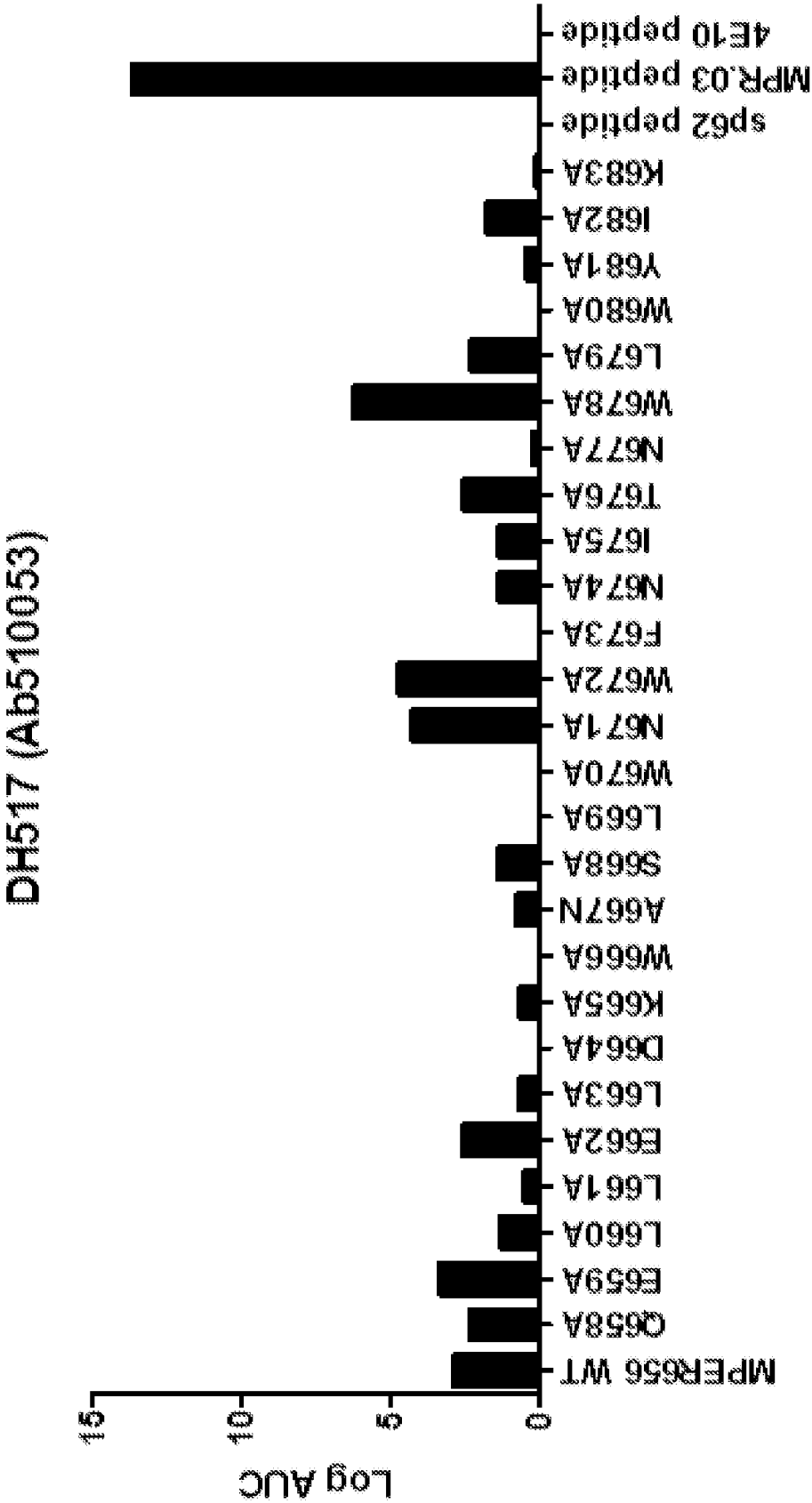


Figure 17

MPER656-biotin	KKKNEQELLELDKQASLWNWFDITNWLWYIK--biotin
MPER656.1-biotin	KKKNEQDLLALDKQASLWNWFDISNWLWYIK---KKK-biotin
MPER656.2-biotin	KKKNEKDLLALDSSWKNLWNWFSITKWLWYIK---KKK-biotin
MPER656.3-biotin	KKKNEQELLELDKQASLWNWFDITNWLWYIK---KKK-biotin
CAP206_0moB5_MPER656-biotin	KKKNEKDLLALDSSWKNLWNWFDITKWLWYIK---KKK-biotin
MPER656.3dYIK-biotin	KKKNEQELLELDKQASLWNWFDITNWLWYIK---KKK-biotin
MPER656.3YIKd664_6A-biotin	KKKNEQELLELDKQASLWNWFDITNWLWYIK---KKK-biotin
MPER656.3dYIK664_6A-biotin	KKKNEQELLELDKQASLWNWFDITNWLWYIK---KKK-biotin
MPER656.2dYIK683R-biotin	KKKNEKDLLALDSSWKNLWNWFSITKWLWYIK---KKK-biotin
MPER656.3YIKd664A-biotin	KKKNEQELLELDKQASLWNWFDITNWLWYIK---KKK-biotin
CAP206_0moB5_MPER656d683R-biotin	KKKNEKDLLALDSSWKNLWNWFDITKWLWYIK---KKK-biotin
MPR.03-biotin	KKKNEQELLELDKQASLWNWFDITNWLWYIK---KKK-biotin

Figure 18

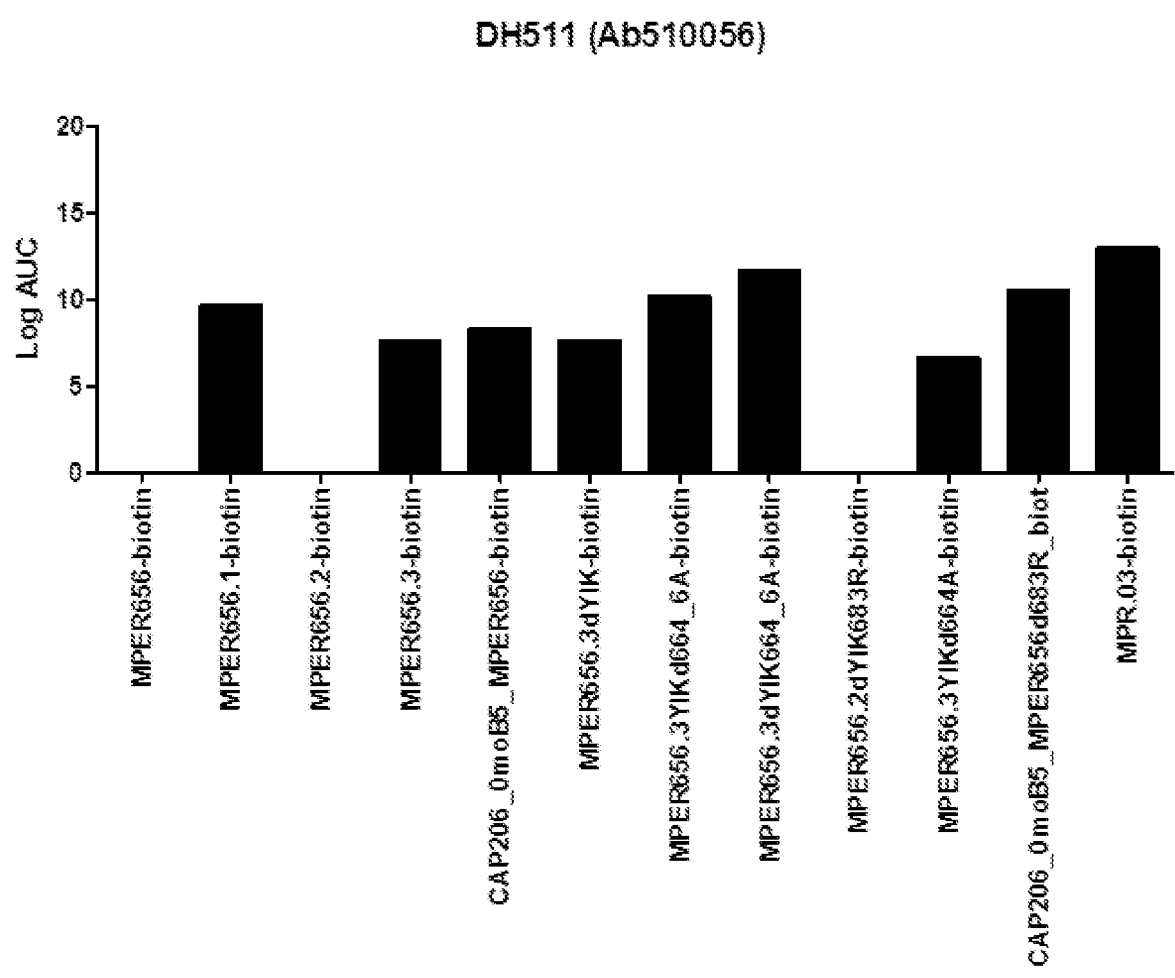


Figure 19

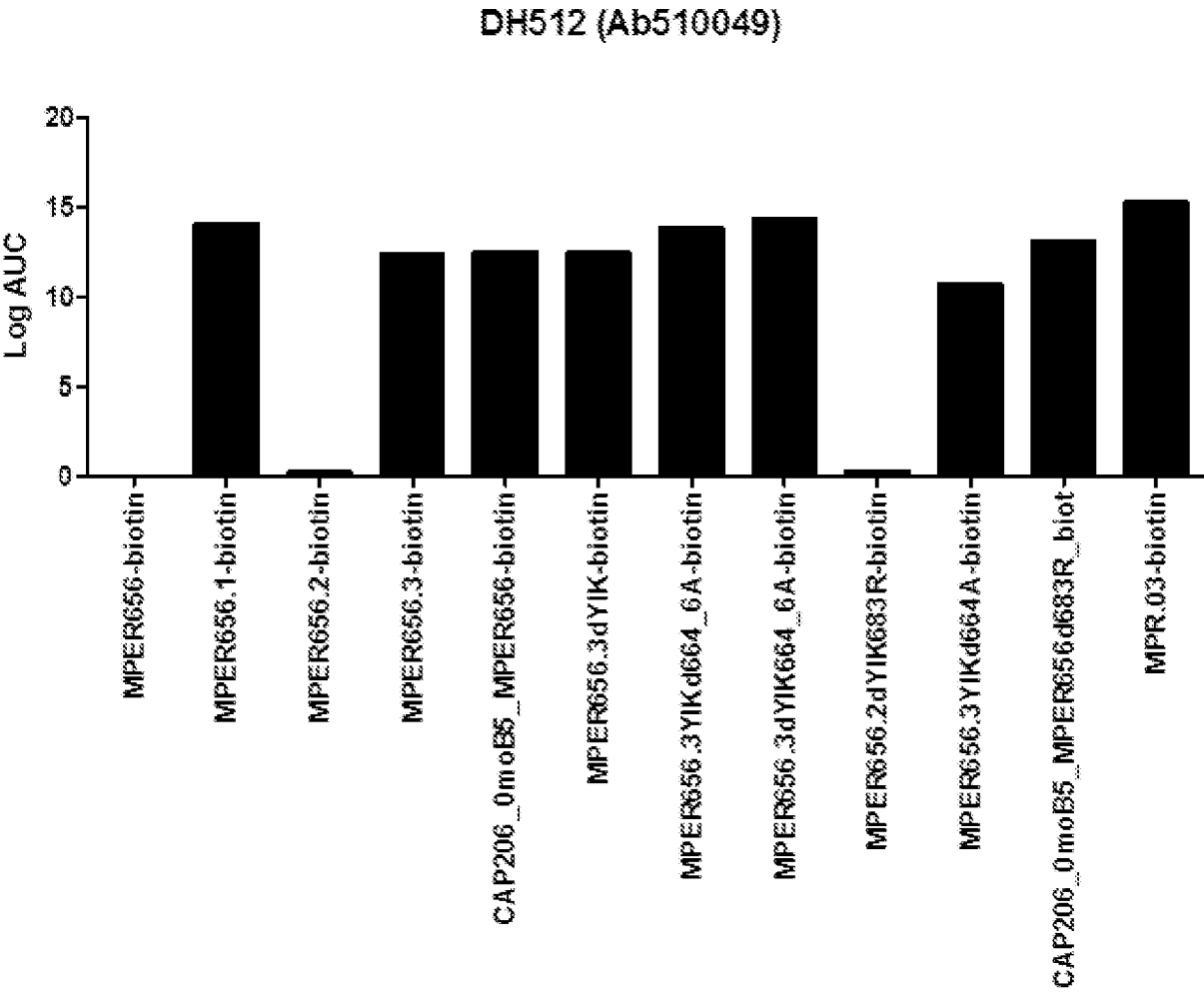


Figure 20

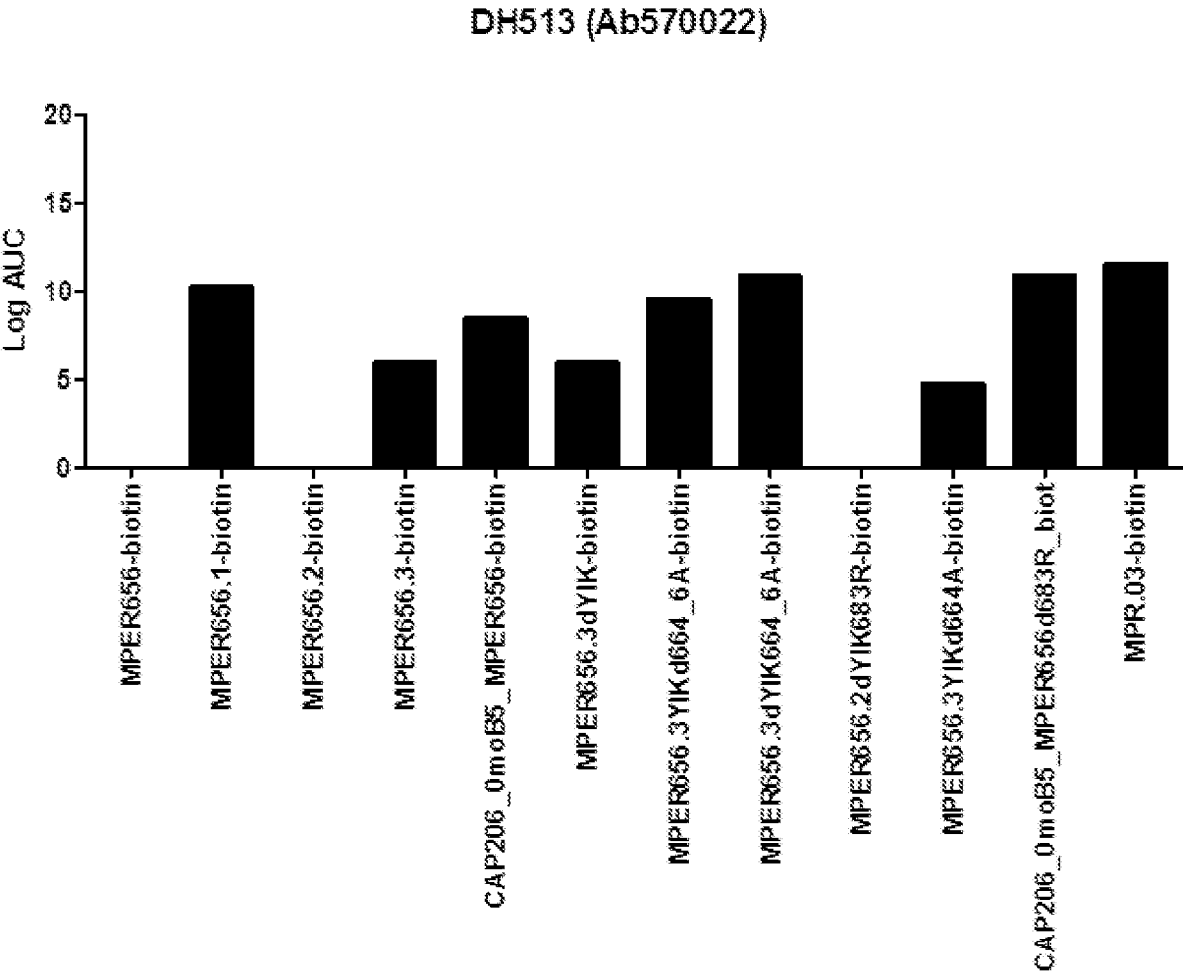


Figure 21

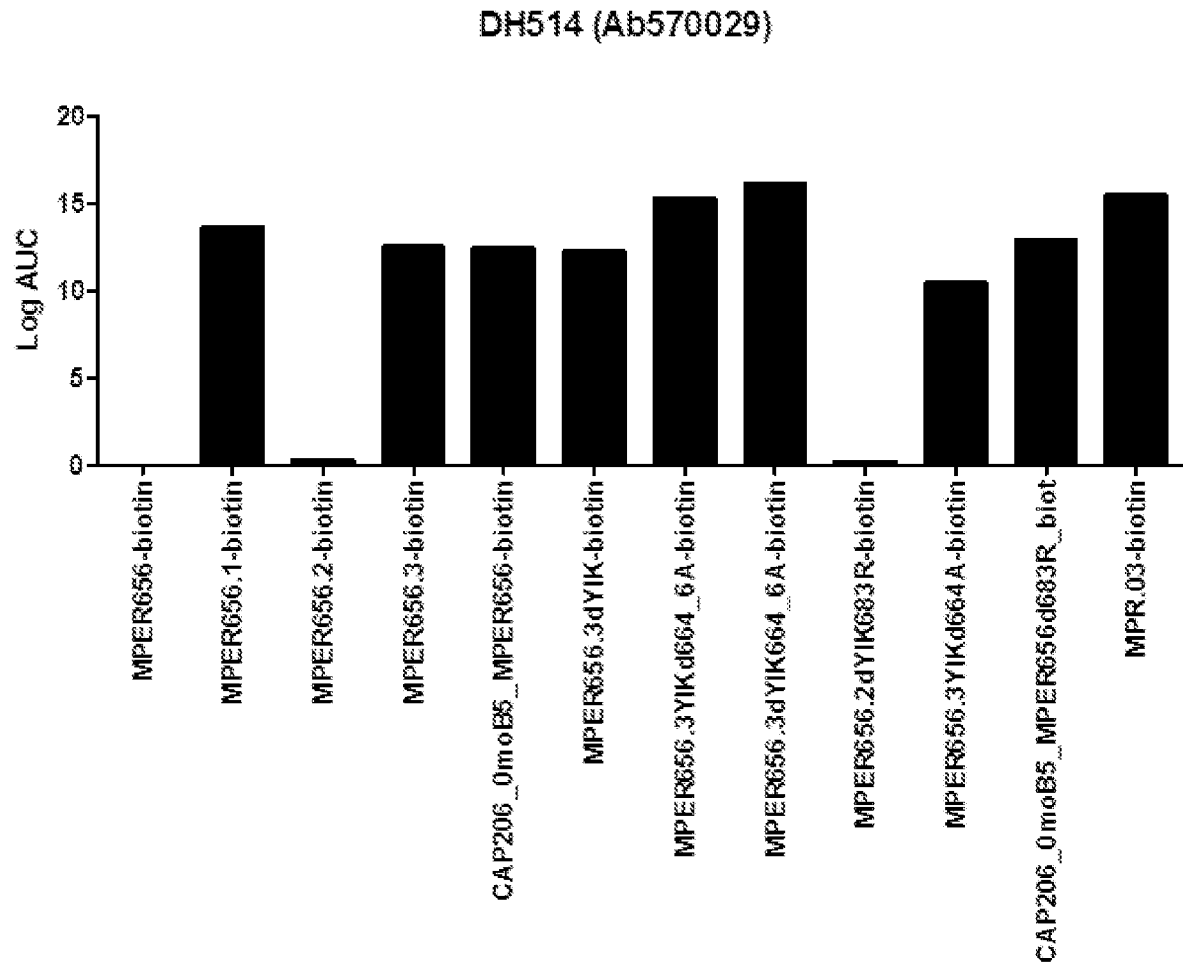


Figure 22

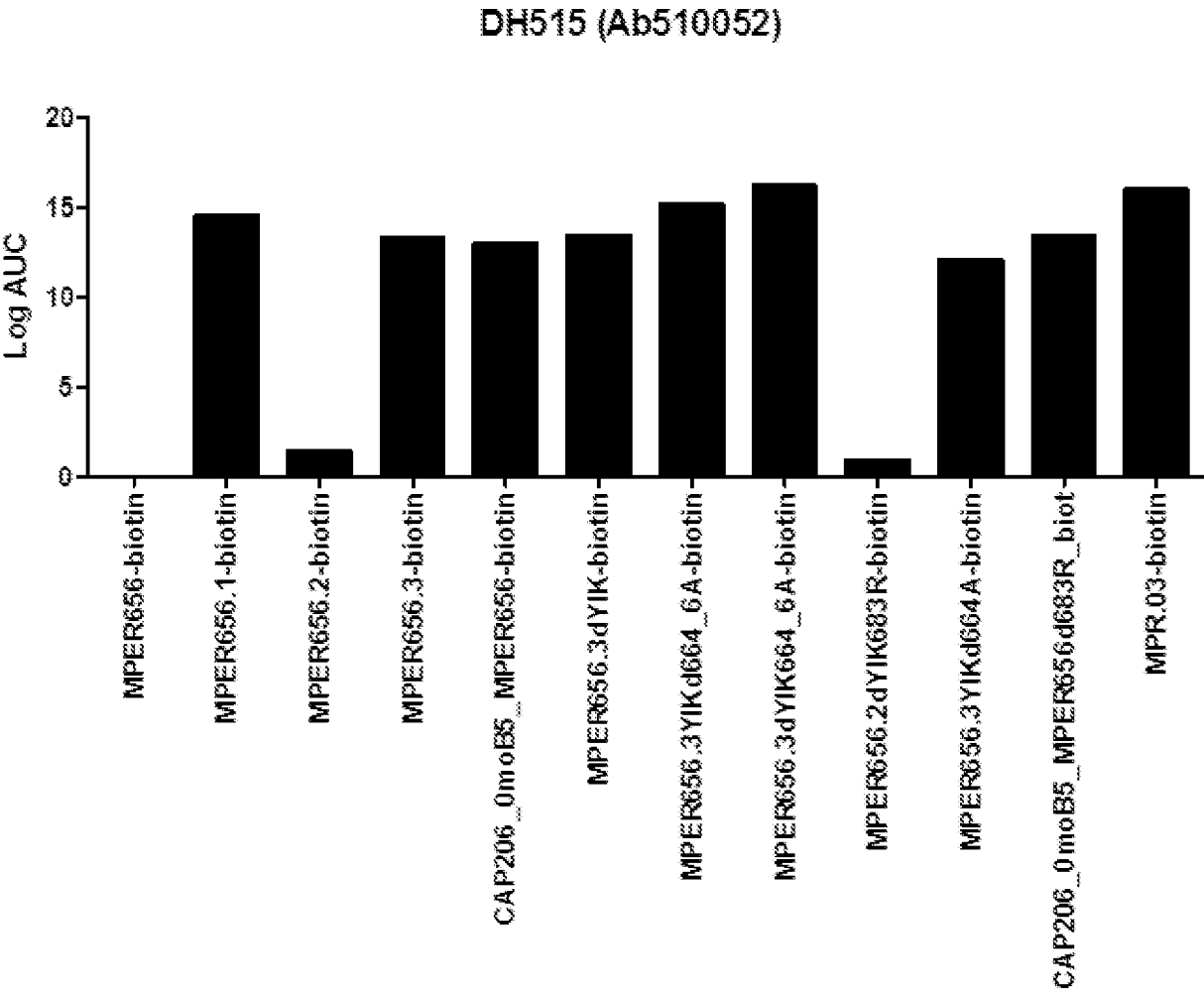


Figure 23

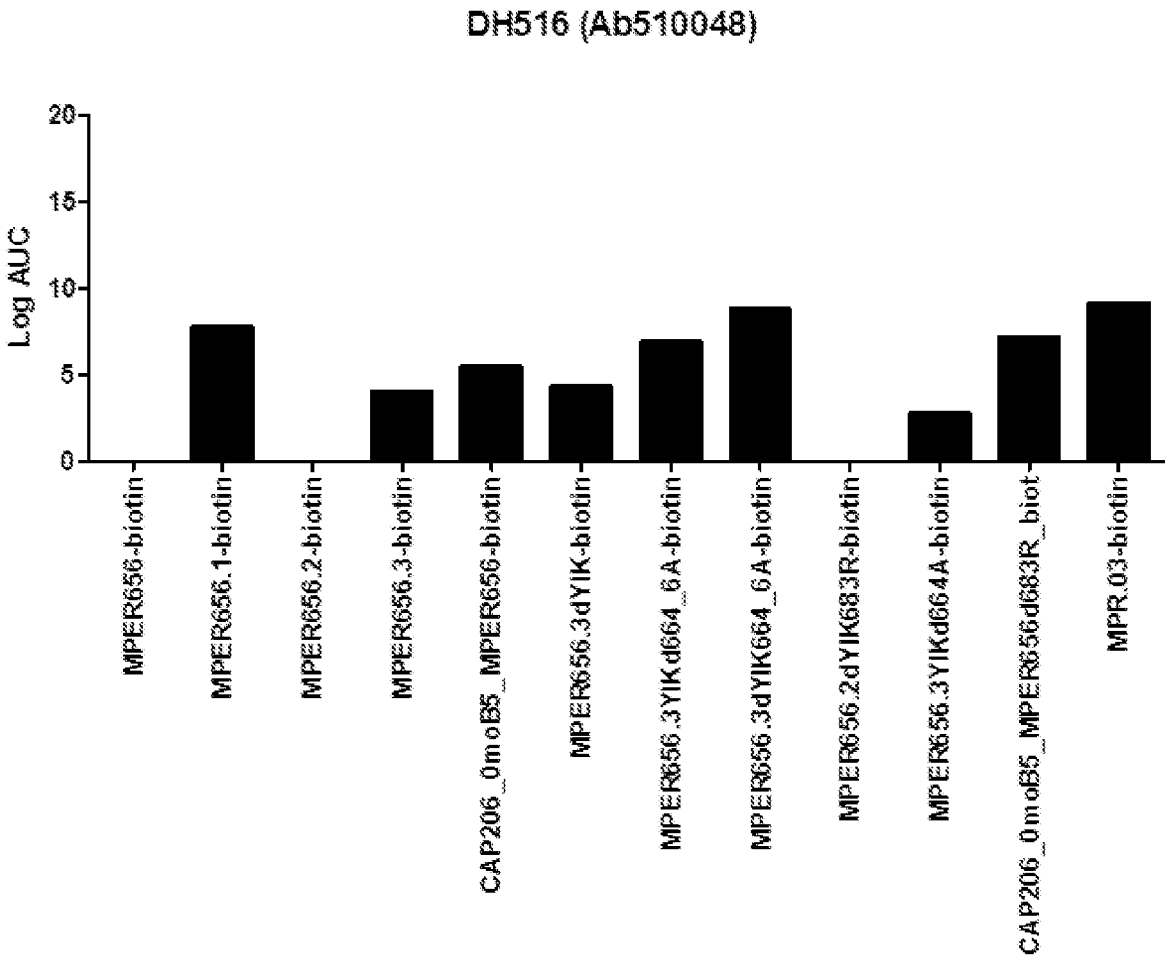


Figure 24

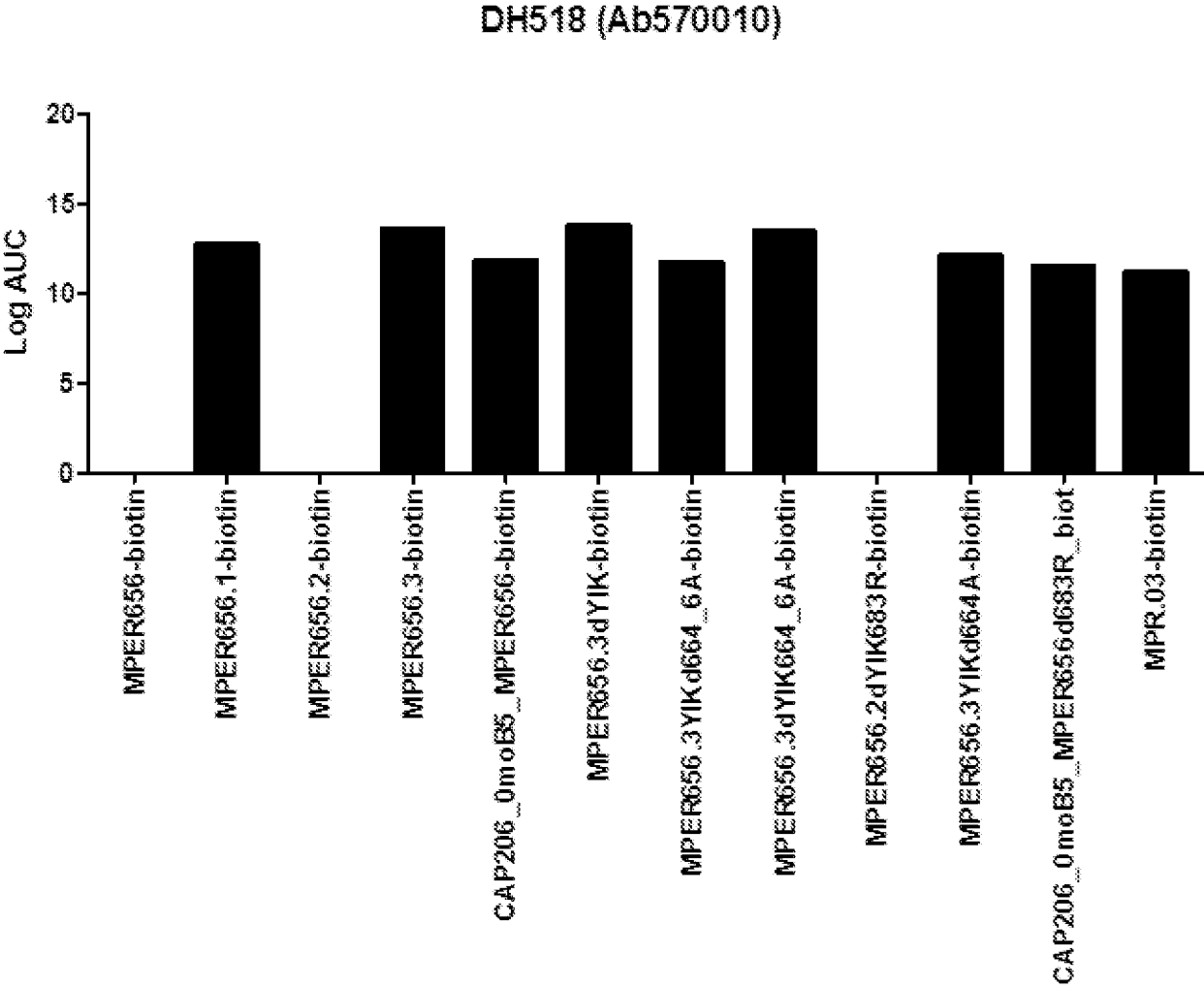


Figure 25

DH270 lineage - Heavy chain nucleotide sequences

					
	10	20	30	40	50
UCA	CAGGTGCAGC	TGGTGCAGTC	TGGGGCTGAG	GTGAAGAAGC	CTGGGGCCTC
I5	CAGGTGCAGC	TGGTGCAGTC	TGGGGCTGAG	RTGAAGAAGC	CTGGGGCCTC
I1	CAGGTGCAGC	TGGTGCAGTC	TGGGGCTGAG	DTGAAGAAGC	CTGGGGCCTC
DH473H	GAGGTTCAGC	TGGTGGAGTC	TGGGCCTGAG	TTGAAGGAGC	CTGGGGCCTC
DH391H	CAGGTGCAGC	TGGTGCAGTC	TGGGGCTGAA	CTGAAGAAGC	CTGGGGCCTC
I4	CAGGTGCAGC	TGGTGCAGTC	TGGGGCTGAG	ATGAAGAAGC	CTGGGGCCTC
I3	CAGGTGCAGC	TGGTGCAGTC	TGGGGCTGAA	ATGAAGAACC	CTGGGGCCTC
DH542H	CAGGTGCAGC	TGGTGCAGTC	TGGGGCTCAA	ATGAAGAACC	CTGGGGCCTC
I2	CAGGTGCAGC	TGGTGCAGTC	TGGGGCTGAA	ATGAAGAACC	CTGGGGCCTC
DH471H	CAGGTGCAGC	TGGTGCAGTC	TGGGGCTGAA	GTGAAGAACC	CTGGGGCCTC
DH429H	GAGGTGCAGC	TGGTGCAGTC	TGGGGCTGAA	ATGAAGAACC	CTGGGGCCTC
DH270H	CAGGTGCAGC	TGGTGCAGTC	TGGGGCTGAG	ATGAAGAAGC	CTGGGGCCTC
	CDR1				
					
	60	70	80	90	100
UCA	AGTGAAGGTC	TCCTGCAAGG	CTTCTGGATA	CACCTTCACC	GGCTACTATA
I5	AGTGAAGGTC	TCCTGCAAGG	CTTCTGGATA	CACCTTCACC	GACTACTATA
I1	AGTGAAGGTC	TCCTGCAAGG	CTTCTGGATA	CACCTTCACC	GACTACTATA
DH473H	AGTGAAGGTC	TCCTGCAAGG	CTTCTGGATA	CACCTTCACC	GACTACTACA
DH391H	AGTGAAGGTC	TCCTGCAAGG	CTTCTGGATA	CACCTTCACC	GACTACTATG
I4	AGTGAAGGTC	TCCTGCAAGG	CTTCTGGATA	CACCTTCACC	GACTACTATA
I3	AGTGAAGGTC	TCCTGCGCGS	CTTCTGGATA	TACCTTCACC	GACTTCTACA
DH542H	AGTGAAGGTC	TCCTGCGCGC	CTTCTGGATA	TACCTTCACC	GACTTTTACA
I2	AGTGAAGGTC	TCCTGCGCGS	CTTCTGGATA	TACCTTCACC	GACTTCTACA
DH471H	AGTGAAGGTC	TCCTGCGCGC	CTTCTGGATA	TACCTTCACT	GACTTCTACA
DH429H	AGTGAAGGTC	TCCTGCGCGG	CTTCTGGATA	TGGTTTCACC	GACTTCTACA
DH270H	AGTGAGGGTC	TCCTGCAAGG	CTTCTGGATA	CACCTTCACC	GACTACTATA
					
	110	120	130	140	150
UCA	TGCACTGGGT	GCGACAGGCC	CCTGGACAAG	GGCTTGAGTG	GATGGGATGG
I5	TCACTGGGT	GCGACAGGCC	CCTGGACAAG	GGCTTGAGTG	GATGGGATGG
I1	TCACTGGGT	GCGACAGGCC	CCTGGACAAG	GGCTTGAGTG	GATGGCATGG
DH473H	TCACTGGGT	GCGACAGGCC	CCTGGACAAG	GTCTTGAGTG	GATGGCATGG
DH391H	TCACTGGCT	GCGACAGGCC	CCTGGACAGG	GGCTTGAGTG	GGTGGCTTGG
I4	TCACTGGGT	GCGACAGGCC	CCTGGACAAG	GGCTTGAGTG	GATGGGATGG
I3	TCACTGGGT	GCGACAGGCC	CCTGGACAAG	GGCTTSAGTG	GATGGGATGG
DH542H	TCACTGGGT	GCGACAGGCC	CCTGGACAAG	GGCTTSAGTG	GATGGGATGG
I2	TCACTGGGT	GCGACTGGCC	CCTGGACAAG	GGCTTSAGTG	GATGGGATGG
DH471H	TCACTGGGT	GCGACTGGCC	CCTGGACAAG	GGCTTGAGTG	GCTGGGGTGG
DH429H	TCACTGGGT	GCGACTGGCC	CCTGGACACG	GGCTCCAGTG	GATGGGATGG
DH270H	TCACTGGGT	GCGACAGGCC	CCTGGACAAG	GGCTTGAGTG	GATGGGATGG

Figure 26

CDR2					
					
	160	170	180	190	200
UCA	ATCAACCCTA	ACAGTGGTGG	CACAAACTAT	GCACAGAAGT	TTCAGGGCAG
I5	ATCAACCCTA	ACASTGGTCG	CACAAACTMT	GCACAGAAGT	TTCAGGGCAG
I1	ATCAACCCTA	CCASTGGTCG	CACAACTMT	GCACGGAAGT	TTCAGGGCAG
DH473H	ATCAACCCTA	CCACTGGTCG	CTCTAGCTTT	GCCCGGGGGT	TTCAGGGCAG
DH391H	ATCAACCCTA	CCAGTGGTCG	CACAATCTCT	CCACGGAAGT	TTCAGGGCAG
I4	ATCAACCCTA	ACACTGGTCG	CACAAACTMT	GCACAGAAGT	TTCAGGGCAG
I3	ATGAACCCTA	AGACTGGTCG	CACAAACAMT	GCACAAAAC	TTCAGGGCAG
DH542H	ATGAACCCTC	AGACTGGTCG	CACAAACACT	GCACGAAACT	TTCAGGGGAG
I2	ATGAACCCTA	AGACTGGTCG	CACAAATAAT	GCACAAAAC	TTCAGGGCAG
DH471H	ATGAACCCTA	AGACTGGTCG	CACAAATCAA	GGACAAAAC	TTCAGGGCAG
DH429H	ATGAACCCTA	AGACTGGTCG	CACAAATAAT	GCACAAGATT	TTCAGGGCAG
DH270H	ATCAACCCTA	GCACTGGTCG	CACAAACTCT	CCACAGAAGT	TTCAGGGCAG
					
	210	220	230	240	250
UCA	GGTCACCATG	ACCAGGGACA	CGTCCATCAG	CACAGCCTAC	ATGGAGCTGA
I5	GGTCACCATG	ACCAGGGACA	CGTCCATCAG	CACAGCCTAC	ATGGAGCTGA
I1	GGTCACCATG	ACCAGGGACA	CGTCCATCAG	CACRGCCTAC	ATGGAAGTGA
DH473H	GGTCACCATG	ACCAGGGAAA	CGTCCGTCAG	CACGGCCTAT	ATGGAAGTGA
DH391H	GGTCACGATG	ACTACGGACA	CGTCCATGAA	TGTTGCCTAC	ATGGAAGTGA
I4	GGTCACCATG	ACCAGGGACA	CGTCCATCAG	CACAGCCTAC	ATGGAGCTGA
I3	GGTCACCATG	ACCAGGGACA	CGTCCATCGG	CACAGCCTAC	ATGGAGYTGA
DH542H	GGTCACCATG	ACCAGGGACA	CGTCCATCGG	CACAGCCTAC	ATGGAGTTGA
I2	GGTCACCATG	ACCAGGGACA	CGTCCATCGG	CACAGCCTAC	ATGGAGYTGA
DH471H	GGTCACCATG	ACCAGGGACA	CGTCCATCGG	CACAGCCTAC	ATGGAGTTGA
DH429H	GGTCACCCTG	ACCAGGGACA	CGTCCATCGG	CACAGCCTAC	ATGGAGCTGA
DH270H	GGTCACCATG	ACCAGGGACA	CGTCCATCAG	CACAGCCTAC	ATGGACCTGA
CDR3					
					
	260	270	280	290	300
UCA	GCAGGCTGAG	ATCTGACGAC	ACGGCCGTGT	ATTACTGTGC	GAGAGGGGGR
I5	GCAGVCTGAG	ATCTGACGAC	ACGGCCGTGT	ATTACTGTGC	GAGAGGGGGR
I1	GAAGMCTGAG	ATCTGACGAC	ACGGCCGTCT	ATTACTGTGC	GAGAGGGGGA
DH473H	GAAGACTGAG	ATCTGACGAC	ACGGCCGTCT	ATTACTGTGC	GAAAGCGGGA
DH391H	GAGGCTTGAG	ATCTGACGAC	ACGGCCGTCT	ATTCTGTGC	GAGAGGGGGA
I4	GCAGVCTGAC	ATCTGACGAC	ACGGCCGTGT	ATTACTGTGC	GACAGGGGGR
I3	GVAGCCTGAC	ATCTGACGAC	ACGGCCGTVT	ATTACTGTGC	GACAGGGGGR
DH542H	GAAGCCTGAC	ATCTGACGAC	ACGGCCATAT	ATTACTGTAC	GACAGGGGGA
I2	GGAGCCTGAC	ATCTGACGAC	ACGGCCGTCT	ATTACTGTGT	GACAGGGGGR
DH471H	GGAGCCTCAC	ATCTGACGAC	ACGGCCGTCT	ATTACTGTGT	GACAGGGGCC
DH429H	GGAGGCTGAC	ATCTGACGAC	ACGGCCGTCT	ATTACTGTGT	GACAGGGGGG
DH270H	ACAGACTGAC	GTCTGACGAC	ACGGCCATGT	ATTACTGTAC	GACCGGGGGG

Figure 26 cont.

					
	310	320	330	340	350
UCA	TGGATCRGTC	TTTACTATGA	TAGTAGTGGT	TACCCTAACT	TTGACTACTG
I5	TGGATCRGTC	TTTACTATGA	TAGTAGTGGT	TACCCTAACT	TTGACTACTG
I1	TGGATCRGTC	TTTACGTTGA	TTATAGTGGT	TACCCTAACT	TTGACTCCTG
DH473H	TACATCGCCC	TTTACGTTGA	CTATAGTGGT	TACCCTAACT	TTAATTCCTG
DH391H	TGGATCAGTC	TCTACGTTGA	TTACAGTTAT	TACCCTAACT	TTGACTCGTG
I4	TGGATCRGTC	TTTACTATGA	TAGTAGTGGT	TACCCTAACT	TTGACTACTG
I3	TGGATCAGTC	TTTACTATGA	TAGTAGTTAT	TACCCTAACT	TTGACCACTG
DH542H	TGGATCAGTC	TTTACTATGA	TAGTAGTTAT	TACCCCAACT	TTGACCACTG
I2	TGGATCAGTC	HTTATTATGA	TAGTAGTTAT	TACCCTAACT	TTGACCACTG
DH471H	TGGATCAGTG	ATTATTATGA	TAGTAGTTAT	TATCCTAACT	TTGACCACTG
DH429H	TGGATCAGTC	CTTATTATGA	TAGTAGTTAT	TACCCTAATT	TTGACCACTG
DH270H	TGGATCGGTC	TTTACTCTGA	TACTAGTGGT	TACCCTAACT	TTGACTACTG
					
	360	370	380		
UCA	GGGCCAGGGA	ACCCTGGTCA	CCGTCTCCTC	AG	
I5	GGGCCAGGGA	ACCCTGGTCA	CCGTCTCCTC	AG	
I1	GGGCCAGGGA	ACCCTGGTCA	CCGTCTCCTC	AG	
DH473H	GGGCCAGGGA	ACCCTGGTCA	CCGTCTCCTC	AG	
DH391H	GGGCCAGGGA	ACCCTGGTCT	CCGTCTCTTC	AG	
I4	GGGCCAGGGA	ACCCTGGTCA	CCGTCTCCTC	AG	
I3	GGGTCAGGGA	ACCCTGGTCA	CCGTCTCCTC	AG	
DH542H	GGGTCAGGGA	ACCCTGCTCA	CCGTCTCCTC	AG	
I2	GGGTCAGGGA	ACCCTGGTCA	CCGTCTCCTC	AG	
DH471H	GGGTCAGGGA	ACCCTGGTCA	CCGTCTCCTC	AG	
DH429H	GGGTCAGGGA	ACCCTGATCA	CCGTCTCCTC	AG	
DH270H	GGGCCAGGGA	ACCCTGGTCA	CCGTCTCCTC	AG	

Figure 26 cont.

DH270 lineage - Heavy chain amino acid sequences

	CDR1									
										
	10	20	30	40	50					
UCA	QVQLVQSGAE	VKKPGASVKV	SCKASGYTFT	GYMHWVRQA	PGQGLEWMGW					
I5	QVQLVQSGAE	XKKPGASVKV	SCKASGYTFT	DYYIHWVRQA	PGQGLEWMGW					
I1	QVQLVQSGAE	XKKPGASVKV	SCKASGYTFT	DYYIHWVRQA	PGQGLEWMAW					
DH473H	EVQLVESGPE	LKEPGASVKV	SCKASGYTFT	DYYIHWVRQA	PGQGLEWMAW					
DH391H	QVQLVQSGAE	LKKPGASVKV	SCKASGYTLS	DYYVHWLRQA	PGQGLEWVAW					
I4	QVQLVQSGAE	MKKPGASVKV	SCKASGYTFT	DYYIHWVRQA	PGQGLEWMGW					
I3	QVQLVQSGAE	MKNPGASVKV	SCAXSGYTFT	DFYIHWVRQA	PGQGLXWMGW					
DH542H	QVQLVQSGAQ	MKNPGASVKV	SCAPSGYTFT	DFYIHWLRQA	PGQGLQWMGW					
I2	QVQLVQSGAE	MKNPGASVKV	SCAXSGYTFT	DFYIHWVRLA	PGQGLXWMGW					
DH471H	QVQLVQSGAE	VKNPGASVKV	SCAPSGYTFT	DFYIHWVRLA	PGQGLEWLGW					
DH429H	EVQLVQSGAE	MKNPGASVKV	SCAASGYGFT	DFYIHWVRLA	PGHGLQWMGW					
DH270H	QVQLVQSGAE	MKKPGASVRV	SCKASGYTFT	DYYIHWVRQA	PGQGP EWMGW					
	CDR2									
										
	60	70	80	90	100					
UCA	INPNSGGTNY	AQKFQGRVTM	TRDTSISTAY	MELSRRLSDD	TAVYYCARGX					
I5	INPNXGRTNX	AQKFQGRVTM	TRDTSISTAY	MELSLRLSDD	TAVYYCARGX					
I1	INPTXGRTXX	ARKFQGRVTM	TRDTSISXAY	MELRXRLSDD	TAVYYCARGG					
DH473H	INPTTGRTSS	ARGFQGRVTM	TRETSVSTAY	MELRRLSDD	TAVYYCAKAG					
DH391H	INPTSGRTIS	PRKFQGRVTM	TTDTSMNVA	MELRGLSDD	TAVYFCARGG					
I4	INPNTGRTNX	AQKFQGRVTM	TRDTSISTAY	MELSLRLSDD	TAVYYCATGX					
I3	MNPKTGRTNX	AQNFQGRVTM	TRDTSIGTAY	MEXXSLTSDD	TAXYYCATGX					
DH542H	MNPQTGRTNT	ARNFQGRVTM	TRDTSIGTAY	MELRSLTSDD	TAIYYCTTGG					
I2	MNPKTGRTNN	AQNFQGRVTM	TRDTSIGTAY	MEXRSLTSDD	TAVYYCVTGX					
DH471H	MNPKTGRTNQ	GQNFQGRVTM	TRDTSIGTAY	MELRSLTSDD	TAVYYCVTGA					
DH429H	MNPKTGRTNN	AQDFQGRVTL	TRDTSIGTAY	MELRRLTSDD	TAVYYCVTGG					
DH270H	INPSTGRTNS	PQKFQGRVTM	TRDTSISTAY	MDLNRLTSDD	TAMYYCTTGG					
										
	110	120								
UCA	WIXLYYDSSG	YPNFDYWGQG	TLVTVSS							
I5	WIXLYYDSSG	YPNFDYWGQG	TLVTVSS							
I1	WIXLYVDYSG	YPNFDYWGQG	TLVTVSS							
DH473H	YIALYVDYSG	YPNFNSWGQG	TLVTVSS							
DH391H	WISLYVDYSY	YPNFDYWGQG	TLVSVSS							
I4	WIXLYYDSSG	YPNFDYWGQG	TLVTVSS							
I3	WISLYYDSSY	YPNFDHWGQG	TLVTVSS							
DH542H	WISLYYDSSY	YPNFDHWGQG	TLLTVSS							
I2	WISXYDSSY	YPNFDHWGQG	TLVTVSS							
DH471H	WISDYDSSY	YPNFDHWGQG	TLVTVSS							
DH429H	WISPYDSSY	YPNFDHWGQG	TLITVSS							
DH270H	WIGLYSDTSG	YPNFDYWGQG	TLVTVSS							

Figure 26 cont.

										
			10		20		30		40	50
UCA	CAGTCTGCCC	TGACTCAGCC	TGCCTCCGTG	TCTGGGTCTC	CTGGACAGTC					
I5	CAGTCTGCCC	TGACTCAGCC	TGCCTCCGTG	TCTGGGTCTC	CTGGACAGTC					
I1	CAGTCTGCCC	TGACTCAGCC	TGCCTCCGTG	TCTGGGTCTC	CTGGACAGTC					
DH473H	CAGTCTGCCC	TGACTCAGCC	TGCCTCCGTG	TCTGGGTCTC	CTGGCCAGTC					
DH391H	CAGCCTGTGC	TGACTCAGCC	TGCCTCCGTG	TCTGGGTCTC	CTGGACAGTC					
I4	CAGTCTGCCC	TGACTCAGCC	TGCCTCCGTG	TCTGGGTCTC	CTGGACAGTC					
I3	CAGTCTGYSC	TGACTCAGCC	TGCCTCCGTG	TCTGGGTCTC	CTGGACAGTC					
DH542H	ACCAGTCTGC	TGACTCAGCC	TGCCTCCGTG	TCTGGGTCTC	CTGGACAGTC					
I2	CAGTCTGYSC	TGACTCAGCC	TGCCTCCGTG	TCTGGGTCTC	CTGGACAGTC					
DH471H	CTGCCCTGTGC	TGACTCAGCC	TGCCTCCGTG	TCTGGGTCTC	CTGGGCAGTC					
DH429H	CAGTCTGCCC	TGACTCAGCC	TGCCTCCGTG	TCTGGGTCTC	CTGGACAGTC					
DH270H	CAGTCTGCCC	TGACTCAGCC	TGCCTCCGTG	TCTGGGTCTC	CTGGACAGTC					

		60	70	80	90	100
UCA	GATCACCATC	TCCTGCACTG	GAACCAGCAG	TGATGTTGGG	AGTTATAACC	
I5	GATCACCATC	TCCTGCACTG	GAACCAGCWR	TGATGTTGGG	AGTTATAACC	
I1	GATCACCATC	TCCTGCACTG	GAACCAGCWR	TGATGTTGGG	AGTTATAACC	
DH473H	GATCACCATC	TCCTGCACTG	GAACCAGCTA	TGATGTTGGG	AGTTATAATC	
DH391H	GATCACCATC	TCCTGCACTG	GAAGCAGCAG	TGATGTTGGG	AGTTATAACC	
I4	GATCACCATC	TCCTGCACTG	GAACCAGTTA	TGATGTTGGG	AGTTATAACC	
I3	GATCACCATC	TCCTGCACTG	GAACCAGTTA	TGATGTTGGG	AGTTATGACC	
DH542H	GATCACCATC	TCCTGCACTG	GAACCAAGTA	TGATGTTGGG	AGTCATGACC	
I2	GATCACCATC	TCCTGCACTG	GAACCAGTTA	TGATGTTGGG	AAGTTTGACC	
DH471H	GATCACCATC	TCCTGCACTG	GGACCATTTA	TGATGTTGGG	AAGTTTGACC	
DH429H	GATCACCATC	TCCTGCACTG	GAACCAGTTA	TGATGTTGCG	AAGTTTGACC	
DH270H	GATCACCATC	TCCTGCACTG	GAACCAATTA	TGATGTTGGG	AGTTATAACC	

Figure 26 cont.

	CDR2				
					
	160	170	180	190	200
UCA	TATGAGGTCA	GTAAGCGGCC	CTCAGGGGTT	TCTAATCGCT	TCTCTGGCTC
I5	TATGAGGTCA	RTAAGCGGCC	CTCAGGGGTT	TCTAATCGCT	TCTCTGGCTC
I1	TATGAGGTCA	RTAAGTGGCC	CTCAGGGGTT	TCTAATCGCT	TCTCTGGCTC
DH473H	TATGAGGTCA	GTCAGTGGCC	CTCAGGGGTT	TCTAAGCGCT	TCTCTGGCTC
DH391H	TATGAGGTCA	ATAAGTGGGC	CTCAGGGGTT	TCTGATCGCT	TCGCTGGCTC
I4	TATGAGGTCA	ATAAGCGGCC	CTCAGGGGTT	TCTAATCGCT	TCTCTGGCTC
I3	TATGAAGTCA	ATAAGCGGCC	CTCAGGAGTT	TCTAATCGCT	TCTCTGGCTC
DH542H	TATGAAGTCA	ATAAACGGCC	CTCAGGAGTT	TCTAATCGCT	TCTCTGGCTC
I2	TATGAAGTCA	ATAAGTGGCC	CTCAGGAGTT	TCTCATCGCT	TCTCTGGCTC
DH471H	TATGAAGTCA	AAAAGTGGCC	CTCAGGAGTT	TCTCATCGCT	TCTCTGGCTC
DH429H	TATGAAGTCA	ATAAGTGGCC	CTCAGGAGTT	TCTCATCGCT	TCTCTGGTTC
DH270H	TATGAGGTCA	ATAAGCGGCC	CTCAGGGGTT	TCTAATCGCT	TCTCTGGCTC
					
	210	220	230	240	250
UCA	CAAGTCTGGC	AACACGGCCT	CCCTGACAAT	CTCTGGGCTC	CAGGCTGAGG
I5	CAAGTCTGGC	AACACGGCCT	CCCTGACAAT	CTCTGGGCTC	CAGGCTGAGG
I1	CAAGTCTGGC	AACACGGCCT	CCCTGACAAT	CTCTGGGCTC	CAGGCTGAGG
DH473H	CAAGTCTGGC	AACACGGCCT	CCCTGACAAT	CTCTGGGCTC	CAGGCTGAGG
DH391H	CAAGTCTGGC	AACACGGCCT	CCCTGACAAT	CTCTAGACTC	CAGGCTGAGG
I4	CAAGTCTGGC	AACACGGCCT	CCCTGACAAT	CTCTGGGCTC	CAGGCTGAGG
I3	CAAATCTGGC	AACACGGCCT	CCCTGACAAT	CTCTGGGCTC	CAGGCTGAGG
DH542H	CAAATCTGGC	AACACGGCCT	CCCTGACAAT	CTCTGGGCTC	CGGGCTGAGG
I2	CAAATCTGGC	AACACGGCCT	CCCTGACAAT	CTCTGGGCTC	CAGGCTGAGG
DH471H	CAAATCTGGC	AACACGGCCT	CCCTGACAAT	CTCTGGGCTC	CAGGTTGAGG
DH429H	CAAATCTGGC	AACACGGCCT	CCCTGACAAT	CTCTGGGCTC	CAGGCTGAGG
DH270H	CAAGTCTGGC	AACACGGCCT	CCCTGACAAT	CTCTGGGCTC	CAGGCTGAGG
	CDR3				
					
	260	270	280	290	300
UCA	ACGAGGCTGA	TTATTACTGC	TGCTCATATG	CAGGTAGTAG	CACTGTAWTA
I5	ACGAGGCTGA	TTATTACTGY	TGCTCATATG	CAGGTAGTAG	CACTGTAWTA
I1	ACGAGGCTVA	TTATTACTGT	TGCTCATATG	CAGGTAGTAG	CACTGTAATA
DH473H	ACGAGGCTCA	TTATTACTGT	TGCTCATATG	CAGGCAGTAG	CACTGTAATA
DH391H	ACGAGGCTAA	TTACTTTTGT	TCCTCATCTA	CAAATAGTGC	CACTGTCATA
I4	ACGAGGCTGA	TTATTACTGY	TGCTCATATG	CAGGTAGTAG	CACTGTADTW
I3	ACGAGGCTGA	CTATTATTGC	TGCTCATTTG	GAGGTAGTGC	CACTGTRGTC
DH542H	ACGAGGCTGA	CTATTATTGC	TGTTTCATTTG	GAGGGAGTGC	CACCGTGGTC
I2	ACGAGGCTGA	CTATTATTGC	TGCTCATTCG	GAGGTAGTGC	CACTGTRGTC
DH471H	ACGAGGCTGA	CTATTATTGC	TGCTCATTCG	GAGGTAGTGC	CGCTGTGGTC
DH429H	ACGAGGCTGA	CTATTATTGC	TGCTCATTCG	GAGGTAGTGC	CACTGTAGTC
DH270H	ACGAGGCCAC	TTATTACTGT	TGTTTCATATG	CAGGTAGTAG	CATTATATTT

Figure 26 cont.

				
	310	320	330	
UCA	TTCGGCGGAG	GGACCAAGCT	GACCGTCCTA	G
I5	TTCGGCGGAG	GGACCAAGCT	GACCGTCCTA	G
I1	TTCGGCGGAG	GGACCAAGCT	GACCGTCCTA	G
DH473H	TTCGGCGGAG	GGACCTCGCT	GACCGTCCTA	G
DH391H	TTCGGCGGAG	GGACCAAGCT	GACCGTCCTA	G
I4	TTCGGCGGAG	GGACCAAGCT	GACCGTCCTA	G
I3	TGCGGCGGAG	GGACCAAGGT	GACCGTCCTA	G
DH542H	TGCGGCGGCG	GGACCAAGGT	GACCGTCCTA	G
I2	TGCGGCGGAG	GGACCAAGGT	GACCGTCCTA	G
DH471H	TGCGGCGGAG	GGACCAAGGT	GACCGTCCTA	G
DH429H	TGCGGCGGAG	GGACCAAGGT	GACCGTCCTA	G
DH270H	TTCGGCGGTG	GGACCAAGCT	GACCGTCATA	G

Figure 26 cont.

DH270 lineage - Light chain amino acid sequences

	CDR1									
										
	10	20	30	40	50					
UCA	QSALTQPASV	SGSPGQSITI	SCTGTSSDVG	SYNLVSWYQQ	HPGKAPKLMI					
I5	QSALTQPASV	SGSPGQSITI	SCTGTSXDVG	SYNLVSWYQQ	HPGKAPKLMI					
I1	QSALTQPASV	SGSPGQSITI	SCTGTSXDVG	SYNLVSWYQQ	HPGKAPKLMI					
DH473H	QSALTQPASV	SGSPGQSITI	SCTGTSYDVG	SYNLVSWYQQ	HPGKAPKLII					
DH391H	QPVLTQPASV	SGSPGQSITI	SCTGSSSDVG	SYNLVSWYQQ	HPGKAPKLMI					
I4	QSALTQPASV	SGSPGQSITI	SCTGTSYDVG	SYNLVSWYQQ	HPGKAPKYMI					
I3	QSXLTQPASV	SGSPGQSITI	SCTGTSYDVG	SYDLVSWYQQ	HPGKAPKYMI					
DH542H	TSLLTQPASV	SGSPGQSITI	SCTGTTYDVG	SHDLVSWYQQ	YPGKVPKYMI					
I2	QSXLTQPASV	SGSPGQSITI	SCTGTSYDVG	KFDLVSWYQQ	HPGKAPKYMI					
DH471H	LPVLTQPASV	SGSPGQSITI	SCTGTIYDVG	KFDLVSWYQH	HPGKAPKYLI					
DH429H	QSALTQPASV	SGSPGQSITI	SCTGTSYDVA	KFDLVSWFQQ	HPGKAPKYMI					
DH270H	QSALTQPASV	SGSPGQSITI	SCTGTNYDVG	SYNLVSWYQQ	HPGKVPKYII					
	CDR2					CDR3				
										
	60	70	80	90	100					
UCA	YEVSKRPSGV	SNRFGSKSG	NTASLTISGL	QAEDEADYYC	CSYAGSSTVX					
I5	YEVXKRPSGV	SNRFGSKSG	NTASLTISGL	QAEDEADYYX	CSYAGSSTVX					
I1	YEVXKWPSGV	SNRFGSKSG	NTASLTISGL	QAEDEAXYYC	CSYAGSSTVI					
DH473H	YEVSQWPSGV	SKRFGSKSG	NTASLTISGL	QAEDEAHYYC	CSYAGSSTVI					
DH391H	YEVNKWASGV	SDRFAGSKSG	NTASLTISRL	QAEDEANYFC	SSSTNSATVI					
I4	YEVNKRPSGV	SNRFGSKSG	NTASLTISGL	QAEDEADYYX	CSYAGSSTVX					
I3	YEVNKRPSGV	SNRFGSKSG	NTASLTISGL	QAEDEADYYC	CSFGGSATXV					
DH542H	YEVNKRPSGV	SNRFGSKSG	NTASLTISGL	RAEDEADYYC	CSFGGSATVV					
I2	YEVNKWPSGV	SHRFGSKSG	NTASLTISGL	QAEDEADYYC	CSFGGSATXV					
DH471H	YEVKKWPSGV	SHRFGSKSG	NTASLTISGL	QVEDEADYYC	CSFGGSAAVV					
DH429H	YEVNKWPSGV	SHRFGSKSG	NTASLTISGL	QAEDEADYYC	CSFGGSATVV					
DH270H	YEVNKRPSGV	SNRFGSKSG	NTASLTISGL	QAEDEATYYC	CSYAGSSIIF					

Figure 26 cont.

>CH557_aa_HC

QVRLAQYGGGVKRLGATMTLSCVAS**GYTFNDYY**IHWVRQAPGQGFEL
LGY**IDPANGRP**DYAGALRERLSFYRDKSMETLYMDLRSLRYDDTAMY
Y**CVRVNVTAGSLLHYDHW**GSISPVIIVSS

>CH557_aa_LC

EIVLTQSPATLSASPGERVTLTCRAS**RSVRNN**VAWYQHKGGQSPRLL
IY**DAS**TRAAGVPARFSGSASGTEFTLAISNLESEDFTVYF**CLQYNNW**
WTFGQGTRVDIK

>CH557_nt_HC

CAGGTCCGACTAGCCCAATATGGTGGTGGGGTGAAGAGGCTAGGGGC
CACAATGACCCTTTCCTGCGTGGCATCT**GGATACACCTTCAACGACT**
ACTACATACATTGGGTGCGGCAGGCCCCCTGGACAAGGCTTTGAGTTG
TTGGGATAC**ATCGACCCCGCTAATGGTCGCCC**AGACTACGCAGGGGC
GTTGAGGGAGAGACTCTCCTTCTACAGGGACAAGTCCATGGAGACGC
TGTACATGGACCTGAGGAGCCTAAGATATGACGACACGGCCATGTAT
TATTGT**GTTAGAAATGTGGGGACCGCTGGCAGCTTGCTGCATTATGA**
CCACTTGGGGCTCGGGAAGCCCGGTCATCGTCTCCTCC

>CH557_nt_LC

GAAATTGTGTTGACGCAGTCTCCAGCCACCCTGTCCGCGTCTCCAGG
GGAAAGAGTCACCCTAACTTGCAGGGCCAGT**CGGAGTGTCGAAACA**
ACGTTGGCCTGGTATCAGCACAAGGGTGGCCAGAGTCCCAGGCTCCTC
ATTTAT**GATGCGTCC**ACGAGGGCCGCTGGTGTCCCAGCCAGGTTTCAG
CGGCAGTGCATCTGGGACAGAGTTCACTCTCGCCATCAGCAACTTGG
AGTCTGAAGATTTTACAGTCTACTTCTGT**CTGCAGTATAATAACTGG**
TGGACCTTCGGCCAAGGGACCAGGGTGGACATCAAA

Figure 27A

	10	20	30	40	50	60	70	80	90
UUA HC	QVQLVQSGAEVKKPGASVKVSCHASGIFTTSTYNNHVRQAPGQGLENGIINPSGGSTSYAQKFGQKVTMTADISTSTVMESSLRSED								
CH240 HC	--T--R--TI--V--NNF--V--C--N--D--V--RI--G--								
CH235 HC	--T--Q--N--V--QL--N--D--N--R--N--N--I--								
CH239 HC	--R--T--NNF--V--C--N--D--V--RIN--								
CH236 HC	--A--R--TI--R--I--I--R--L--M--D--R--R--D--S--								
CH241 HC	--A--R--R--SHI--P--N--D--V--RPTT--G--								
CH491 HC	--A--R--TI--R--I--I--L--N--R--R--D--SYR--ED--S--Y--N--I--D--RN--K--A--								
CH493 HC	--R--L--Y--GG--R--MTI--V--N--ND--I--L--N--D--R--D--GA--GD--S--Y--K--NN--L--D--R--G--								
CH553 HC	--T--R--TL--RT--N--ID--FI--R--V--Y--D--R--R--PD--PN--ND--SLY--N--I--LD--RD--TFD--								
CH558 HC	--GG--R--STTTI--V--S--ND--I--VL--F--D--N--R--N--GA--GD--FS--Y--K--NE--L--D--RN--D--								
CH556 HC	--GI--S--I--IL--I--N--ID--I--R--RP--L--Y--D--H--RPD--EG--RD--ISLY--V--DVRG--LD--								
CH557 HC	--R--A--Y--GG--RL--TNIL--V--ND--I--F--LL--Y--D--AN--RPD--GALRE--LSFY--K--NE--L--D--R--YD--								

Figure 27B

	IC ₅₀ (µg/ml)				IC ₈₀ (µg/ml)			
	Plasma Ab	Plasma Ab	MBC Ab		Plasma Ab	Plasma Ab	MBC Ab	
	DH511_5a	DH511_5b	DH512	10E8	DH511_5a	DH511_5b	DH512	10E8
Number of Viruses	203	203	208	208	203	203	208	208
Total Viruses Neutralized								
IC50/IC80 <50ug/ml	202	202	206	203	201	201	203	203
IC50/IC80 <10ug/ml	196	198	198	202	169	172	147	193
IC50/IC80 <1.0ug/ml	122	123	103	152	37	37	33	61
IC50/IC80 <0.1ug/ml	19	18	20	42	5	6	7	10
IC50/IC80 <0.01ug/ml	4	3	7	10	0	0	3	5
Percent of Viruses Neutralized								
IC50/IC80 <50ug/ml	100	100	99	98	99	99	98	98
IC50/IC80 <10ug/ml	97	98	95	97	83	85	71	93
IC50/IC80 <1.0ug/ml	60	61	50	73	18	18	16	29
IC50/IC80 <0.1ug/ml	9	9	10	20	2	3	3	5
IC50/IC80 <0.01ug/ml	2	1	3	5	0	0	1	2
Median IC50/IC80	0.764	0.698	1.04	0.392	3.49	3.33	5.07	1.69
Geometric Mean	0.684	0.634	0.792	0.299	3.02	2.80	3.73	1.34

Figure 28A

Titer in TZM-bl cells (µg/ml)																
	Chimeric Ab		Chimeric Ab		Chimeric Ab		Chimeric Ab		Serum Ab		Serum Ab		Serum Ab		MBC Ab	
	DH512_K2	DH512_K3	DH512_K4	DH511_2b_K2	DH511_5a	DH511_5b	DH511_2b	DH511_5a	DH511_5b	DH511_2b	DH511_5b	DH511_2b	DH511_5b	DH511_2b	DH512	
	IC80	IC80	IC80	IC80	IC80	IC80	IC80	IC80	IC80	IC80	IC80	IC80	IC80	IC80	IC80	IC80
MEDIAN TITERS	1.327	5.538	0.387	1.462	0.951	4.838	1.583	7.867	1.330	4.892	0.712	4.066	1.384	6.765	0.903	4.963
			</													

Figure 28B

Virus ID	Clade	DH512*		DH512_K2	
		IC50	IC80	IC50	IC80
QH0692.42	B	3.170	15.785	3.385	12.499
SC422661.8	B	0.532	4.018	0.910	3.158
AC10.0.29	B	0.163	1.136	0.348	1.619
THRO4156.18	B	0.716	4.418	1.784	7.415
WITO4160.33	B	0.136	1.850	0.693	2.633
Du156.12	C	0.058	0.290	0.078	0.343
Du422.1	C	1.024	6.146	0.809	2.686
ZM53M.PB12	C	4.292	2.534	6.747	21.514
ZM109F.PB4	C	0.335	5.344	2.387	9.154
HIV-0013095-2.11	C	0.026	0.284	<0.023	0.617
CNE19	BC	0.266	3.853	1.403	6.256
CNE30	BC	3.043	19.041	5.780	28.975
MS208.A1	A	1.770	6.902	3.220	12.725
Q23.17	A	2.113	11.185	1.968	10.871
Q769.d22	A	0.463	7.680	1.358	10.127
0330.v4.c3	A	1.045	10.469	2.370	10.334
191955_A11	A (T/F)	0.655	3.299	0.981	2.691
928-28	CRF02_AG	0.112	0.597	0.260	1.400
T250-4	CRF02_AG	0.377	3.496	2.177	9.125
211-9	CRF02_AG	0.459	3.591	1.020	3.622
C1080.c03	CRF01_AE	0.103	1.305	0.250	1.364
R1166.c01	CRF01_AE	1.153	5.944	2.001	5.853
C4118.c09	CRF01_AE	1.252	18.623	2.753	17.337
X2131_C1_B5	G	0.174	1.096	0.202	0.972
P1981_C5_3	G	0.049	0.331	0.062	0.269
X1632_S2_B10	G	0.472	6.059	0.760	4.101
3016.v5.c45	D	0.749	3.788	1.437	5.222
A07412M1.vrc12	D	0.695	5.920	1.296	6.226
3817.v2.c59	CD	3.570	13.097	3.473	12.836
0815.v3.c3	ACD	0.158	3.334	1.160	4.420
MuLV	Neg. Control	>50	>50	>50	>50
MEDIAN TTTERS		0.592	3.938	1.327	5.538

Figure 29A

	Titer in TZM.t						
	DH512_K3			DH512_K4		DH511_5A*	
Virus ID	IC50	IC80		IC50	IC80	IC50	IC80
QH0692.42	0.982	3.587		2.775	9.974	1.614	6.942
SC422661.8	0.269	0.999		0.492	2.221	1.327	4.239
AC10.0.29	0.115	0.476		0.207	0.987	0.279	1.934
THRO4156.18	0.584	2.617		1.031	6.105	0.277	2.051
WITO4160.33	0.149	0.750		0.292	1.481	0.294	1.546
Du156.12	<0.023	0.108		0.034	0.229	0.161	0.629
Du422.1	0.320	1.172		0.638	2.357	0.942	3.605
ZM53M.PB12	2.900	11.121		4.429	13.276	10.718	36.286
ZM109F.PB4	0.729	2.690		1.966	10.797	0.692	4.090
HIV-0013095-2.11	<0.023	0.138		<0.023	0.483	0.077	0.606
CNE19	0.259	1.337		0.462	4.989	1.374	8.836
CNE30	1.499	7.869		3.947	27.253	2.630	15.432
MS208.A1	0.683	3.312		2.169	11.758	1.854	6.789
Q23.17	0.731	3.080		1.828	8.227	3.346	14.463
Q769.d22	0.254	1.587		0.596	5.661	3.124	12.578
0330.v4.c3	0.926	3.654		2.245	14.007	3.168	13.860
191955_A11	0.388	1.155		0.956	2.555	1.333	3.570
928-28	0.033	0.513		0.145	1.201	0.133	0.992
T250-4	0.866	3.326		2.339	9.437	4.869	21.225
211-9	0.386	1.139		1.040	3.680	0.857	4.307
C1080.c03	0.081	0.516		0.173	0.988	0.212	1.292
R1166.c01	0.711	2.354		1.925	6.129	1.121	4.781
C4118.c09	0.508	4.200		1.173	9.343	2.605	17.135
X2131_C1_B5	0.068	0.442		0.126	0.750	0.335	1.432
P1981_C5_3	<0.023	0.109		0.029	0.190	0.138	0.571
X1632_S2_B10	0.265	1.141		0.612	2.522	1.351	5.682
3016.v5.c45	0.531	1.952		1.383	6.571	1.435	7.036
A07412M1.vrc12	0.484	1.764		0.946	4.688	0.782	5.002
3817.v2.c59	1.214	4.276		3.210	11.531	5.564	16.415
0815.v3.c3	0.271	1.215		0.568	3.551	1.657	7.439
MuLV	>50	>50		>50	>50	>50	>50
MEDIAN TTTERS	0.387	1.462		0.851	4.839	1.338	4.892

Figure 29A cont.

DH511_5B*		DH511_2b		Virus ID
IC50	IC80	IC50	IC80	
1.213	5.184	1.444	8.256	QH0692.42
0.741	3.436	0.618	2.900	SC422661.8
0.180	1.199	0.388	1.833	AC10.0.29
0.238	1.659	0.259	1.699	THRO4156.18
0.143	0.865	0.546	2.245	WITO4160.33
0.071	0.380	0.153	0.572	Du156.12
0.919	3.651	1.284	4.527	Du422.1
6.495	21.700	9.580	33.864	ZM53M.PB12
0.522	3.262	0.922	6.499	ZM109F.PB4
0.048	0.494	<0.023	0.633	HIV-0013095-2.11
0.636	5.581	2.313	11.343	CNE19
1.856	11.326	4.025	16.713	CNE30
1.461	5.333	0.982	3.647	MS208.A1
2.197	13.298	2.057	13.126	Q23.17
0.653	9.811	2.991	16.017	Q769.d22
1.991	13.002	2.104	13.337	0330.v4.c3
0.979	2.852	0.964	4.655	191955_A11
0.099	0.783	0.330	1.494	928-28
2.594	13.554	3.319	14.860	T250-4
1.058	4.045	1.906	7.030	211-9
0.136	0.952	0.290	1.816	C1080.c03
1.040	4.517	2.984	12.222	R1166.c01
0.805	12.062	2.178	14.025	C4118.c09
0.170	0.873	0.382	1.499	X2131_C1_B5
0.108	0.335	0.121	0.423	P1981_C5_3
0.682	4.086	1.842	9.172	X1632_S2_B10
0.954	4.988	3.013	11.044	3016.v5.c45
0.524	3.057	1.194	6.046	A07412M1.vrc12
3.721	14.355	8.320	27.254	3817.v2.c59
0.570	4.595	2.107	8.562	0815.v3.c3
>50	>50	>50	>50	MuLV
0.712	4.066	1.364	6.766	MEDIAN TITERS

Figure 29B

DH511_2b_K2		Ab510049		10E8 (Haynes)		Virus ID
IC50	IC80	IC50	IC80	IC50	IC80	
2.111	13.660	1.630	8.754	0.566	2.318	QH0692.42
0.638	4.555	0.444	2.013	0.302	1.233	SC422661.8
0.436	2.943	0.153	0.823	0.172	0.882	AC10.0.29
0.287	1.748	0.194	1.984	0.120	0.566	THRO4156.18
0.371	1.900	0.261	1.275	0.159	0.858	WITO4160.33
0.184	0.791	0.064	0.336	0.028	0.130	Du156.12
2.184	7.641	0.758	2.880	0.220	0.825	Du422.1
12.531	47.720	6.127	25.371	2.721	13.377	ZM53M.PB12
1.529	7.978	1.144	5.702	0.252	1.363	ZM109F.PB4
0.091	0.862	<0.023	0.603	<0.023	0.127	HIV-0013095-2.11
2.188	14.043	1.583	7.944	0.582	3.268	CNE19
3.817	24.186	4.827	27.474	0.616	4.877	CNE30
0.888	4.384	1.480	5.405	0.442	2.001	MS208.A1
3.327	22.256	2.013	11.866	0.660	3.669	Q23.17
1.095	21.724	1.118	9.570	1.113	4.767	Q769.d22
3.598	21.324	1.784	9.736	1.405	6.461	0330.v4.c3
1.555	5.766	0.956	3.362	0.447	1.313	191955_A11
0.553	2.272	0.195	1.188	0.119	0.665	928-28
5.106	25.688	2.599	14.555	1.007	4.995	T250-4
2.490	9.001	1.066	3.778	0.635	2.174	211-9
0.388	2.146	0.154	1.176	0.029	0.317	C1080.c03
3.836	17.464	1.354	5.447	0.384	2.914	R1166.c01
2.555	32.545	0.801	12.004	0.650	4.682	C4118.c09
0.440	1.646	0.229	1.144	0.080	0.373	X2131_C1_B5
0.150	0.537	0.065	0.306	<0.023	0.106	P1981_C5_3
1.571	7.755	0.741	4.779	0.494	1.932	X1632_S2_B10
3.891	14.199	1.811	8.529	0.593	2.099	3016.v5.c45
1.407	7.393	0.649	5.147	0.221	1.152	A07412M1.vrc12
11.619	41.810	6.004	17.824	0.906	3.819	3817.v2.c59
1.989	10.166	0.850	4.019	0.238	1.091	0815.v3.c3
>50	>50	>50	>50	>50	>50	MuLV
MEDIAN TITERS						
1.563	7.867	0.903	4.963	0.413	1.848	

Figure 29B cont.

Region	R#	DH511	DH511 mutation
CDRH3	92	C	
	93	T	
	94	A	
	95	D	
	96	L	W
	97	G	W,F
	98	E	W
	99	P	W
	100	V	F,L
	100a	V	W
	100b	S	W
	100c	R	W
	100d	F	W
	100e	F	W
	100f	E	W
	100g	W	
	100h	G	W
	100i	S	W
	100j	Y	W
	100k	Y	
	100k	Y	W
	100l	Y	
	100m	M	
	101	D	
	102	L	
	103	W	
	104	G	

Figure 30

Region	R#	DH512	DH512 mutation
CDRH3	92	C	
	93	T	
	94	M	
	95	D	
	96	E	W
	97	G	W,F
	98	T	W
	99	P	W
	100	V	F,L
	100a	T	W
	100b	R	W
	100c	F	W
	100d	L	W,F
	100e	E	W
	100f	W	
	100g	G	W
	100h	Y	W
	100i	F	W
	100j	Y	
	100k	Y	W
	100l	Y	
	100m	M	
	101	A	
	102	V	
	103	W	
	104	G	

Figure 31.

Mutations outside of CDRH3

Region R# DH511 DH511 mutation Region R# DH512 DH512 mutation

CDRH1 26	G	W	CDRH1 26	G	W
27	F		27	F	
28	T	W	28	F	W
29	F		29	F	
30	S		30	D	
31	N	W	31	N	W
32	T		32	S	
33	W		33	W	

Figure 32

Region R# DH511 DH511 mutation Region R# DH512 DH512 mutation

CDRH2	51	I		CDRH2	51	I
	52	S	W		52	R
	52a	R			52a	R
	52b	N	W		52b	L
	52c	K	W		52c	K
	53	D	W		53	D
	54	G			54	G
	55	A			55	A
	56	K			56	T
	57	T			57	G

Region R# DH511 DH511 mutation Region R# DH512 DH512 mutation

FR3	72	D		FR3	72	D
	73	D	W		73	D
	74	S	W		74	S
	75	R	W		75	R

Figure 32 cont.

DH512 Nucleotide Sequence

CAGGTGCAGCTGGTACAGTCTGGGGGAGGTCTGGTGAAGCCGGGGGGGTCCCTCACACTCTCCTGTTC
 AGCCTCTGGATTCTTTTTTCGATAATTCATGGATGGGGTGGGTCCGTGAGGCGCCAGGGAAGGGACTGG
 AGTGGGTTGGCCGCATTAGAAAGGCTCAAAGACGGTGCACAGGAGAATATGGTGCAGCCGTGAAGGAC
 AGATTCACCATTTCAAGAGATGACAGTAGAAATATGCTGTACCTGCACATGAGGACCCCTGAAAACCGA
 GGACTCAGGCACTTATTATTGTACCATGGATGAGGGGACCCAGTAACACGCTTCTTAGAATGGGGCT
 ACTTCTATTATTATATGGCCGTTTGGGGCAGAGGGACCACGGTCATCGTCTCTTCA

DH512 Translated (Amino Acid) Sequence

QVQLVQSGGGLVKPGGSLTLSCSASGFFFDNSWMGWVRQAPGKGLEWVGRIRRLKDGATGEYGAAVKD
 RFTISRDDSRNMLYLHMRTLKTEDSGTYCYCTMDEGTPVTRFLEWGYFYYYMAVWGRGTTIVSS

Amino Acid Sequences of DH512 Heavy Chain Mutants

>DH512_E96W

QVQLVQSGGGLVKPGGSLTLSCSASGFFFDNSWMGWVRQAPGKGLEWVGRIRRLKDGATGEYGAAVKD
 RFTISRDDSRNMLYLHMRTLKTEDSGTYCYCTMD~~EG~~TPVTRFLEWGYFYYYMAVWGRGTTIVSS

>DH512_G97W

QVQLVQSGGGLVKPGGSLTLSCSASGFFFDNSWMGWVRQAPGKGLEWVGRIRRLKDGATGEYGAAVKD
 RFTISRDDSRNMLYLHMRTLKTEDSGTYCYCTMDE~~ET~~TPVTRFLEWGYFYYYMAVWGRGTTIVSS

>DH512_T98W

QVQLVQSGGGLVKPGGSLTLSCSASGFFFDNSWMGWVRQAPGKGLEWVGRIRRLKDGATGEYGAAVKD
 RFTISRDDSRNMLYLHMRTLKTEDSGTYCYCTMDEG~~W~~TPVTRFLEWGYFYYYMAVWGRGTTIVSS

>DH512_P99W

QVQLVQSGGGLVKPGGSLTLSCSASGFFFDNSWMGWVRQAPGKGLEWVGRIRRLKDGATGEYGAAVKD
 RFTISRDDSRNMLYLHMRTLKTEDSGTYCYCTMDEGT~~W~~TPVTRFLEWGYFYYYMAVWGRGTTIVSS

>DH512_V100F

QVQLVQSGGGLVKPGGSLTLSCSASGFFFDNSWMGWVRQAPGKGLEWVGRIRRLKDGATGEYGAAVKD
 RFTISRDDSRNMLYLHMRTLKTEDSGTYCYCTMDEGTP~~F~~TRFLEWGYFYYYMAVWGRGTTIVSS

>DH512_V100I

QVQLVQSGGGLVKPGGSLTLSCSASGFFFDNSWMGWVRQAPGKGLEWVGRIRRLKDGATGEYGAAVKD
 RFTISRDDSRNMLYLHMRTLKTEDSGTYCYCTMDEGTP~~I~~TRFLEWGYFYYYMAVWGRGTTIVSS

>DH512_T100aW

QVQLVQSGGGLVKPGGSLTLSCSASGFFFDNSWMGWVRQAPGKGLEWVGRIRRLKDGATGEYGAAVKD
 RFTISRDDSRNMLYLHMRTLKTEDSGTYCYCTMDEGTPV~~W~~TRFLEWGYFYYYMAVWGRGTTIVSS

Figure 33

>DH512_R100bW

QVQLVQSGGGLVKPGGSLTLSCSASGFFFDNSWMGWVRQAPGKGLEWVGRIRRLKDGATGEY
GAAVKDRFTISRDDSRNMLYLHMRTLKTEDSGTYYCTMDEGTPVTWFLEWGYFYYYMAVWGR
GTTVIVSS

>DH512_F100cW

QVQLVQSGGGLVKPGGSLTLSCSASGFFFDNSWMGWVRQAPGKGLEWVGRIRRLKDGATGEYGAAVKD
RFTISRDDSRNMLYLHMRTLKTEDSGTYYCTMDEGTPVTRWLEWGYFYYYMAVWGRGTTVIVSS

>DH512_L100dW

QVQLVQSGGGLVKPGGSLTLSCSASGFFFDNSWMGWVRQAPGKGLEWVGRIRRLKDGATGEYGAAVKD
RFTISRDDSRNMLYLHMRTLKTEDSGTYYCTMDEGTPVTRWEWGYFYYYMAVWGRGTTVIVSS

>DH512_L100dF

QVQLVQSGGGLVKPGGSLTLSCSASGFFFDNSWMGWVRQAPGKGLEWVGRIRRLKDGATGEYGAAVKD
RFTISRDDSRNMLYLHMRTLKTEDSGTYYCTMDEGTPVTRWEWGYFYYYMAVWGRGTTVIVSS

>DH512_E100eW

QVQLVQSGGGLVKPGGSLTLSCSASGFFFDNSWMGWVRQAPGKGLEWVGRIRRLKDGATGEYGAAVKD
RFTISRDDSRNMLYLHMRTLKTEDSGTYYCTMDEGTPVTRWWGYFYYYMAVWGRGTTVIVSS

>DH512_G100gW

QVQLVQSGGGLVKPGGSLTLSCSASGFFFDNSWMGWVRQAPGKGLEWVGRIRRLKDGATGEYGAAVKD
RFTISRDDSRNMLYLHMRTLKTEDSGTYYCTMDEGTPVTRWLEWGYFYYYMAVWGRGTTVIVSS

>DH512_Y100hW

QVQLVQSGGGLVKPGGSLTLSCSASGFFFDNSWMGWVRQAPGKGLEWVGRIRRLKDGATGEYGAAVKD
RFTISRDDSRNMLYLHMRTLKTEDSGTYYCTMDEGTPVTRWLEWGYFYYYMAVWGRGTTVIVSS

>DH512_F100iW

QVQLVQSGGGLVKPGGSLTLSCSASGFFFDNSWMGWVRQAPGKGLEWVGRIRRLKDGATGEYGAAVKD
RFTISRDDSRNMLYLHMRTLKTEDSGTYYCTMDEGTPVTRWLEWGYFYYYMAVWGRGTTVIVSS

>DH512_Y100kW

QVQLVQSGGGLVKPGGSLTLSCSASGFFFDNSWMGWVRQAPGKGLEWVGRIRRLKDGATGEYGAAVKD
RFTISRDDSRNMLYLHMRTLKTEDSGTYYCTMDEGTPVTRWLEWGYFYYYMAVWGRGTTVIVSS

Figure 33 cont.

Antibody ID	Heavy ID	Light ID	Lot Number	IC50 (µg/ml)	IC80 (µg/ml)	IC50 (µg/ml)	IC80 (µg/ml)	CH505.T F	IC50 (µg/ml)	IC80 (µg/ml)	DU172	IC50 (µg/ml)	IC80 (µg/ml)	MN	IC50 (µg/ml)	IC80 (µg/ml)	SVA
10E8				0.262	1.189	4.764	13.642	13.642	0.124	0.684	0.684	<0.023	<0.023	<0.023	<0.023	<0.023	>50
DH512 (Ab510049_4A)	H510049_4A	K510032	314HC	0.156	0.913	8.974	28.095	28.095	3.411	13.048	13.048	<0.023	<0.023	<0.023	<0.023	<0.023	>50
DH512_93i	H510049_4A	DH511_2 AVK	132RKR	0.044	0.376	2.813	13.222	13.222	3.673	11.587	11.587	<0.023	<0.023	<0.023	<0.023	<0.023	>50
DH512_E96W_4A/293i	H510049_4A_E96W	K510032	76TCB	1.918	5.716	30.990	>50.32	>50.32	4.775	19.394	19.394	0.051	0.276	0.051	0.276	>50	>50
DH512_T98W_4A/293i	H510049_4A_T98W	K510032	77TCB	5.799	17.307	>50	>50	>50	14.417	>50	>50	0.434	1.513	0.434	1.513	>50	>50
DH512_P99W_4A/293i	H510049_4A_P99W	K510032	78TCB	2.458	12.903	>50	>50	>50	>50	>50	>50	0.232	0.729	0.232	0.729	>50	>50
DH512_L100dW_4A/293i	H510049_4A_L100dW	K510032	79TCB	0.398	1.299	6.823	23.229	23.229	0.606	3.308	3.308	<0.023	0.100	<0.023	0.100	>50	>50
DH512_E100eW_4A/293i	H510049_4A_E100eW	K510032	80TCB	1.411	6.738	>50	>50	>50	>50	>50	>50	0.254	0.877	0.254	0.877	>50	>50
DH512_Y100hW_4A/293i	H510049_4A_Y100hW	K510032	81TCB	1.568	5.143	45.094	>50	>50	18.482	>50	>50	0.071	0.387	0.071	0.387	>50	>50
DH512_F100iW_4A/293i	H510049_4A_F100iW	K510032	135LDW	1.642	7.094	39.299	>50	>50	>50	>50	>50	0.054	0.327	0.054	0.327	>50	>50
DH512_Y100kW_4A/293i	H510049_4A_Y100kW	K510032	136LDW	>50	>50	>50	>50	>50	>50	>50	>50	9.341	32.530	9.341	32.530	>50	>50
DH512_V100F_4A/293i	H510049_4A_V100F	K510032	137LDW	>50	>50	>50	>50	>50	>50	>50	>50	20.119	>50	20.119	>50	>50	>50
DH512_T100aW_4A/293i	H510049_4A_T100aW	K510032	138LDW	0.099	0.696	6.240	20.059	20.059	0.134	0.950	0.950	<0.023	<0.023	<0.023	<0.023	>50	>50

Figure 34

DH512_R100bW_4A/2 93i	H510049_4A_R100b W	K510032	139LDW	5.448	15.619	>50	>50	>50	>50	0.428	1.190	>50	>50
DH512_F100cW_4A/2 93i	H510049_4A_F100c W	K510032	140LDW	1.326	6.575	30.590	>50	>50	>50	0.050	0.316	>50	>50
DH512_G100gW_4A/2 93i	H510049_4A_G100 gW	K510032	141LDW	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
DH512_V100i_4A/293i	H510049_4A_V100i	K510032	142LDW	2.734	10.558	>50	>50	>50	>50	0.304	1.107	>50	>50
DH512_L100f_4A/293i	H510049_4A_L100d F	K510032	143LDW	0.048	0.349	2.733	10.178	0.229	1.673	<0.023	<0.023	>50	>50
DH512_G97W_4A/293i	H510049_4A_G97W	K510032	144LDW	>50	>50	>50	>50	>50	>50	33.197	>50	>50	>50
Ab82				>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
CH01+CH31				1.441	5.337	<0.011	0.059	1.515	10.121	0.575	3.315	>25	>25

Figure 34 cont.

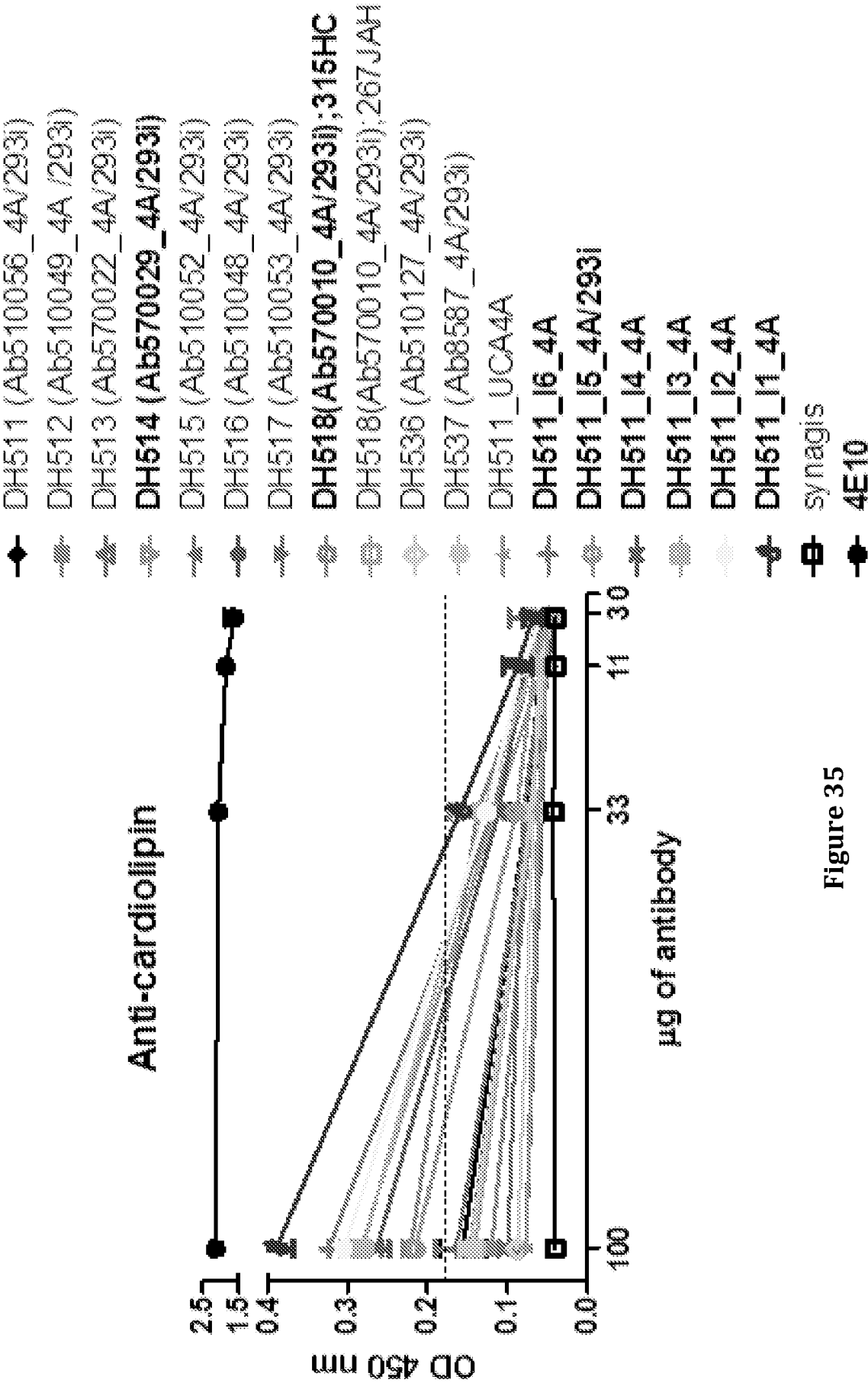


Figure 35

Ab ID	Old Ab ID	CL	SSA	SSB	Sm	RVP	Scl-70	Jo-1	Cent B	Hist	dsDNA	Hep-2
DH511	Ab510056	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
DH512	Ab510049	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
DH513	Ab570022	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
DH514	Ab570029	(+/-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
DH515	Ab510052	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(+/-)	(-)	(+/-)	(-)
DH516	Ab510048	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
DH517	Ab510053	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(+/-)	(-)
DH518	Ab570010	(+/-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
DH536	Ab510127	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
DH537	Ab008587	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
DH511_UCA		(-)	(-)	(-)	(-)	(+/-)	(-)	(-)	(-)	(-)	(-)	(-)
DH511_I6		(+/-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(+/-)	(-)
DH511_I5		(+/-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
DH511_I4		(+/-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
DH511_I3		(+/-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
DH511_I2		(+/-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
DH511_I1		(+/-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)

Figure 36

High Throughput Antibody Screen - Panel P

Assay - Luc/TZM-bl

values represent IC50 in ug/ml

IC50

Panel O Panel M

<0.100
0.100-1.00
1.00-10.0
>10.0

Virus ID	Clade	DH512	10E8
0260.v5.c36	A	17.0	9.87
0330.v4.c3	A	1.42	1.12
0439.v5.c1	A	2.36	1.23
3365.v2.c20	A	2.29	1.60
3415.v1.c1	A	12.6	4.69
3718.v3.c11	A	3.80	0.838
398-F1 F6 20	A	2.49	0.704
BB201.B42	A	2.82	0.613
BB539.2B13	A		
BG505.W6M.C2	A	1.33	0.689
BI369.9A	A	1.61	0.356
BS208.B1	A	0.803	0.319
KER2008.12	A	0.723	>50
KER2018.11	A	4.31	1.89
KNH1209.18	A	0.410	0.406
MB201.A1	A	0.844	0.411
MB539.2B7	A	>50	>50
MI369.A5	A	0.510	0.671
MS208.A1	A	0.927	0.187
Q23.17	A	1.18	0.461
Q259.17	A	12.4	4.76
Q769.d22	A	2.44	1.91
Q769.h5	A	5.73	2.89
Q842.d12	A	5.73	2.82
QH209.14M.A2	A	2.20	1.30
RW020.2	A	3.46	0.902
UG037.8	A	0.273	0.048
246-F3.C10.2	AC	0.802	0.210
3301.V1.C24	AC	12.5	2.97
3589.V1.C4	AC	3.26	5.77
6540.v4.c1	AC	2.84	2.24
6545.V4.C1	AC	7.79	2.54
0815.V3.C3	ACD	2.04	0.491
6095.V1.C10	ACD	0.0003	0.0005
3468.V1.C12	AD	2.00	0.381
Q168.a2	AD	1.45	0.463
Q461.e2	AD	2.45	2.29
620345.c1	AE	1.77	0.989
BJOX009000.02.4	AE	0.580	0.251
BJOX010000.06.2	AE	0.178	0.060
BJOX025000.01.1	AE	0.751	0.228
BJOX028000.10.3	AE	0.853	0.167
C1080.c3	AE	0.521	0.108

Figure 37

C2101.c1	AE	4.64	1.20
C3347.c11	AE	0.046	0.019
C4118.09	AE	1.66	0.421
CM244.ec1	AE		0.365
CNE3	AE	2.81	1.37
CNE5	AE	1.54	1.17
CNE55	AE	0.287	0.038
CNE56	AE	0.212	0.060
CNE59	AE	0.0003	0.001
CNE8	AE	0.045	0.140
M02138	AE		
R1166.c1	AE	0.742	0.488
R2184.c4	AE	1.12	0.576
R3265.c6	AE	6.31	1.58
TH023.6	AE		
TH966.8	AE	0.081	0.039
TH976.17	AE	0.762	0.392
235-47	AG	0.361	0.244
242-14	AG	0.069	0.568
263-8	AG	0.407	0.229
269-12	AG	0.420	0.124
271-11	AG	1.23	0.891
928-28	AG	0.215	0.079
DJ263.8	AG	0.190	0.009
T250-4	AG	3.54	1.07
T251-18	AG	7.52	0.666
T253-11	AG	3.51	1.21
T255-34	AG	1.40	0.228
T257-31	AG	3.42	0.336
T266-60	AG	0.953	>50
T278-50	AG	1.89	0.357
T280-5	AG	4.21	
T33-7	AG	2.34	0.818
3988.25	B	0.284	0.070
5768.04	B	5.70	1.63
6101.10	B	0.012	0.001
6535.3	B	1.39	0.190
7165.18	B	0.805	0.659
45 01dG5	B	0.753	0.106
89.6.DG	B	0.676	0.318
AC10.29	B	0.115	0.102
ADA.DG	B	0.042	0.055
Bal.01	B	0.620	0.421
Bal.26	B	2.57	0.518
BG1168.01	B	0.528	0.396
BL01.DG	B	1.08	0.362
BR07.DG	B	0.106	0.118
BX08.16	B	0.565	0.213
CAAN.A2	B	5.29	1.45
CNE10	B	0.121	0.014
CNE12	B	0.509	0.301

Figure 37 cont.

CNE14	B	0.950	0.151
CNE4	B	0.279	0.059
CNE57	B	0.120	0.059
HO86.8	B	2.55	0.326
HT593.1	B	0.140	0.049
HXB2.DG	B	0.0003	0.003
JRCSEFJB	B	2.04	0.429
JRFLJB	B	0.862	0.174
MN.3	B	0.0003	0.0003
PVO.04	B	2.32	1.60
QH0515.01	B	4.97	2.25
QH0692.42	B	2.03	0.531
REJO.67	B	0.544	0.302
RHPA.7	B	5.45	1.01
SC422.8	B	1.63	0.343
SF162.LS	B	1.88	0.245
SS1196.01	B	0.857	0.244
THRO.18	B	0.711	0.092
TRJO.58	B	3.86	1.13
TRO.11	B	0.228	0.028
WITO.33	B	0.314	0.031
X2278.C2.B6	B	3.79	0.442
YU2.DG	B	8.00	1.17
BJOX002000.03.2	BC	1.17	0.384
CH038.12	BC	1.57	0.271
CH070.1	BC	12.1	6.65
CH117.4	BC	0.458	0.270
CH119.10	BC	0.916	0.591
CH181.12	BC	0.959	0.754
CNE15	BC	1.27	0.844
CNE19	BC	0.383	0.251
CNE20	BC	0.370	0.131
CNE21	BC	1.09	0.979
CNE40	BC	0.0003	0.001
CNE7	BC	0.119	0.130
286.36	C	3.22	1.19
288.38	C	2.19	0.435
0013095-2.11	C	0.014	0.009
001428-2.42	C	3.33	1.71
0077.V1.C16	C	5.56	1.86
00836-2.5	C	1.89	0.666
0921.V2.C14	C	5.33	0.908
16055-2.3	C	2.16	1.10
16845-2.22	C	0.016	0.030
16936-2.21	C	0.312	0.264
25710-2.43	C	0.046	0.064
25711-2.4	C	6.14	0.516
25925-2.22	C	0.504	0.402
26191-2.48	C	1.82	1.83
3168.V4.C10	C	2.52	2.83
3637.V5.C3	C	2.83	2.12

Figure 37 cont.

3873.V1.C24	C	10.0	5.51
426c	C		
6322.V4.C1	C	2.90	0.923
6471.V1.C16	C	11.0	4.98
6631.V3.C10	C	7.08	0.934
6644.V2.C33	C	0.229	0.013
6785.V5.C14	C	1.58	0.701
6838.V1.C35	C	0.813	0.292
96ZM651.02	C	0.035	0.033
BR025.9	C	1.10	0.307
CAP210.E8	C	0.911	0.474
CAP244.D3	C	1.13	0.369
CAP256.206.C9	C	0.779	0.713
CAP45.G3	C	2.84	0.722
Ce1176.A3	C	0.998	0.252
CE703010217.B6	C	0.159	0.996
CNE30	C	3.30	0.456
CNE31	C	5.23	1.32
CNE53	C	0.208	0.213
CNE58	C	0.453	0.229
DU123.06	C	0.250	0.132
DU151.02	C	0.928	0.461
DU156.12	C	0.019	0.023
DU172.17	C	7.22	0.957
DU422.01	C	0.821	0.224
MW965.26	C	0.0003	0.001
SO18.18	C	7.52	1.60
TV1.29	C	0.802	0.248
TZA125.17	C	0.750	0.217
TZBD.02	C	2.44	1.41
ZA012.29	C	3.66	1.47
ZM106.9	C	5.68	>50
ZM109.4	C	0.715	0.161
ZM135.10a	C	0.246	0.033
ZM176.66	C	1.10	0.267
ZM197.7	C	0.232	0.995
ZM214.15	C	5.39	2.22
ZM215.8	C	0.107	0.044
ZM233.6	C	0.549	0.270
ZM249.1	C	1.58	0.830
ZM53.12	C	6.11	2.62
ZM55.28a	C	7.88	2.34
3326.V4.C3	CD	3.62	1.40
3337.V2.C6	CD	1.50	1.09
3817.v2.c59	CD	2.12	0.229
191821.E6.1	D		
231965.c1	D	19.6	11.0
247-23	D	0.618	0.344
3016.v5.c45	D	0.607	0.710
57128.vrc15	D	0.352	0.212
6405.v4.c34	D	1.93	0.461

Figure 37 cont.

A03349M1.vrc4a	D	0.402	0.270
A07412M1.vrc12	D		0.140
NKU3006.ec1	D	0.989	0.673
UG021.16	D		
UG024.2	D		
P0402.c2.11	G	0.094	0.057
P1981.C5.3	G	0.020	0.024
X1193.c1	G	0.746	0.341
X1254.c3	G	2.77	3.67
X1632.S2.B10	G	0.925	0.387
X2088.c9	G	>50	>50
X2131.C1.B5	G	0.118	0.039
SIVmac251.30.SG3	NA	>50	>50
SVA.MLV	NA	>50	>50

	DH512	10E8
# Viruses	199	200
Total VS Neutralized		
IC50 <50ug/ml	197	195
IC50 <10ug/ml	189	194
IC50 <1.0ug/ml	97	145
IC50 <0.1ug/ml	19	38
IC50 <0.01ug/ml	6	9
% VS Neutralized		
IC50 <50ug/ml	99	98
IC50 <10ug/ml	95	97
IC50 <1.0ug/ml	49	73
IC50 <0.1ug/ml	10	19
IC50 <0.01ug/ml	3	5
Median IC50	1.09	0.392
Geometric Mean	0.802	0.315

Note: Median and Geometric Mean titers are calculated only for samples with IC50 <50ug/ml

Values less than the lowest concentration assayed were assigned a value 2-fold less for calculation purposes.
Indicated in italics

Figure 37 cont.

High Throughput Antibody Screen - Panel P

Assay - Luc/TZM-bl

values represent IC80 in ug/ml

IC80

<0.100
0.100-1.00
1.00-10.0
>10.0

Virus ID	Clade	Panel O	Panel M
		DH512	10E8
0260.v5.c36	A	>50	21.7
0330.v4.c3	A	7.37	3.64
0439.v5.c1	A	9.57	3.95
3365.v2.c20	A	7.89	4.56
3415.v1.c1	A	22.1	11.5
3718.v3.c11	A	13.5	4.42
398-F1 F6 20	A	18.0	6.17
BB201.B42	A	9.87	1.96
BB539.2B13	A		
BG505.W6M.C2	A	5.60	2.14
BI369.9A	A	4.93	1.29
B5208.B1	A	3.35	3.27
KER2008.12	A	4.29	>50
KER2018.11	A	17.0	7.16
KNH1209.18	A	2.25	2.39
MB201.A1	A	4.22	1.36
MB539.2B7	A	>50	>50
MI369.A5	A	3.41	1.77
M5208.A1	A	3.56	1.14
Q23.17	A	5.85	1.60
Q259.17	A	30.7	12.0
Q769.d22	A	8.64	4.47
Q769.h5	A	16.7	7.44
Q842.d12	A	12.8	7.58
QH209.14M.A2	A	10.0	4.09
RW020.2	A	10.0	2.92
UG037.8	A	1.33	0.353
246-F3.C10.2	AC	5.10	1.49
3301.V1.C24	AC	33.8	9.50
3589.V1.C4	AC	10.2	11.7
6540.v4.c1	AC	13.4	7.01
6545.V4.C1	AC	15.4	7.50
0815.V3.C3	ACD	7.05	1.81
6095.V1.C10	ACD	0.001	0.004
3468.V1.C12	AD	7.45	2.04
Q168.a2	AD	5.18	2.88
Q461.e2	AD	8.68	4.68
620345.c1	AE	9.05	3.73
BJOX009000.02.4	AE	5.03	1.47
BJOX010000.06.2	AE	1.23	0.476
BJOX025000.01.1	AE	4.77	1.54
BJOX028000.10.3	AE	4.59	0.876
C1080.c3	AE	2.50	0.613
C2101.c1	AE	15.6	4.12

Figure 38

C3347.c11	AE	0.274	0.089
C4118.09	AE	11.6	2.30
CM244.ec1	AE		1.46
CNE3	AE	9.17	4.01
CNE5	AE	7.34	2.52
CNE55	AE	2.00	0.605
CNE56	AE	1.18	0.314
CNE59	AE	0.023	0.010
CNE8	AE	0.483	1.42
M02138	AE		
R1166.c1	AE	3.70	2.02
R2184.c4	AE	5.40	2.20
R3265.c6	AE	21.9	9.28
TH023.6	AE		
TH966.8	AE	0.611	0.291
TH976.17	AE	4.08	1.75
235-47	AG	1.61	0.786
242-14	AG	0.750	3.17
263-8	AG	2.63	0.991
269-12	AG	2.10	0.475
271-11	AG	7.60	4.34
928-28	AG	1.28	0.365
DJ263.8	AG	0.903	0.100
T250-4	AG	13.1	3.45
T251-18	AG	20.5	2.55
T253-11	AG	12.0	4.05
T255-34	AG	6.11	1.14
T257-31	AG	10.5	1.58
T266-60	AG	4.78	>50
T278-50	AG	8.95	2.10
T280-5	AG	17.0	
T33-7	AG	10.2	2.83
3988.25	B	0.883	0.293
5768.04	B	16.0	5.26
6101.10	B	0.137	0.003
6535.3	B	7.62	1.28
7165.18	B	3.55	2.71
45 01dG5	B	4.87	0.703
89.6.DG	B	2.23	1.48
AC10.29	B	0.604	0.512
ADA.DG	B	0.482	0.358
Bal.01	B	4.09	1.91
Bal.26	B	11.0	2.39
BG1168.01	B	2.38	1.48
BL01.DG	B	6.87	1.57
BR07.DG	B	1.03	0.445
BX08.16	B	3.02	1.30
CAAN.A2	B	16.3	5.70
CNE10	B	0.932	0.169
CNE12	B	2.46	1.09
CNE14	B	4.24	0.649

Figure 38 cont.

CNE4	B	2.45	0.437
CNE57	B	1.28	0.317
HO86.8	B	10.7	1.52
HT593.1	B	0.780	0.285
HXB2.DG	B	0.012	0.015
JRC5F.JB	B	9.61	1.89
JRFL.JB	B	5.40	0.768
MN.3	B	0.004	0.001
PVO.04	B	15.9	6.43
QH0515.01	B	15.8	5.54
QH0692.42	B	11.1	2.35
REJO.67	B	3.31	1.18
RHPA.7	B	25.9	5.10
SC422.8	B	4.60	1.15
SF162.LS	B	7.75	1.06
SS1196.01	B	4.72	1.25
THRO.18	B	2.39	0.587
TRJO.58	B	17.7	4.18
TRO.11	B	1.22	0.286
WITO.33	B	1.60	0.305
X2278.C2.B6	B	14.1	2.24
YU2.DG	B	50	5.46
BJOX002000.03.2	BC	5.64	1.56
CH038.12	BC	6.10	1.41
CH070.1	BC	44.6	13.5
CH117.4	BC	2.29	0.859
CH119.10	BC	5.48	2.36
CH181.12	BC	3.99	2.79
CNE15	BC	6.77	2.97
CNE19	BC	3.05	1.11
CNE20	BC	2.43	0.732
CNE21	BC	8.40	3.25
CNE40	BC	0.030	0.009
CNE7	BC	0.672	0.603
286.36	C	14.0	5.00
288.38	C	8.90	3.08
0013095-2.11	C	0.229	0.077
001428-2.42	C	16.3	6.28
0077.V1.C16	C	36.0	7.11
00836-2.5	C	5.40	1.77
0921.V2.C14	C	18.4	3.03
16055-2.3	C	9.40	3.31
16845-2.22	C	0.238	0.172
16936-2.21	C	1.99	1.31
25710-2.43	C	0.408	0.304
25711-2.4	C	12.6	1.69
25925-2.22	C	2.95	1.53
26191-2.48	C	8.77	4.90
3168.V4.C10	C	19.3	8.18
3637.V5.C3	C	11.1	6.68
3873.V1.C24	C	13.8	15.7

Figure 38 cont.

426c	C		
6322.V4.C1	C	9.72	3.68
6471.V1.C16	C	41.9	14.9
6631.V3.C10	C	15.5	3.36
6644.V2.C33	C	1.14	0.124
6785.V5.C14	C	6.70	2.42
6838.V1.C35	C	3.67	1.01
96ZM651.02	C	0.358	0.177
BR025.9	C	5.33	1.11
CAP210.E8	C	3.37	2.01
CAP244.D3	C	4.56	1.48
CAP256.206.C9	C	3.92	2.97
CAP45.G3	C	9.41	3.41
Ce1176.A3	C	4.13	1.15
CE703010217.B6	C	0.944	0.679
CNE30	C	16.3	2.29
CNE31	C	11.5	3.57
CNE53	C	1.41	1.01
CNE58	C	2.98	1.09
DU123.06	C	0.948	0.423
DU151.02	C	3.60	1.71
DU156.12	C	0.158	0.120
DU172.17	C	19.9	0.238
DU422.01	C	3.95	0.812
MW965.26	C	0.002	0.007
SO18.18	C	20.7	4.48
TV1.29	C	2.66	0.719
TZA125.17	C	4.05	1.19
TZBD.02	C	7.78	4.31
ZA012.29	C	11.3	4.12
ZM106.9	C	21.8	>50
ZM109.4	C	4.64	1.07
ZM135.10a	C	2.20	0.408
ZM176.66	C	5.65	1.73
ZM197.7	C	1.16	0.369
ZM214.15	C	15.5	5.98
ZM215.8	C	0.591	0.230
ZM233.6	C	2.33	0.737
ZM249.1	C	5.78	2.27
ZM53.12	C	18.5	6.72
ZM55.28a	C	19.8	6.78
3326.V4.C3	CD	13.3	4.29
3337.V2.C6	CD	5.60	4.87
3817.v2.c59	CD	6.33	1.43
191821.E6.1	D		
231965.c1	D	>50	20.4
247-23	D	3.24	1.29
3016.v5.c45	D	3.42	2.17
57128.vrc15	D	2.59	1.50
6405.v4.c34	D	6.76	1.80
A03349M1.vrc4a	D	1.32	0.663

Figure 38 cont.

A07412M1.vrc12	D		0.873
NKU3006.ec1	D	4.86	2.46
UG021.16	D		
UG024.2	D		
P0402.c2.11	G	0.815	0.460
P1981.C5.3	G	0.211	0.124
X1193.c1	G	3.28	1.15
X1254.c3	G	14.5	15.7
X1632.S2.B10	G	3.93	1.76
X2088.c9	G	>50	>50
X2131.C1.B5	G	0.611	0.175
SIVmac251.30.SG3	NA	>50	>50
SVA.MLV	NA	>50	>50

	DH512	10E8
# Viruses	199	200
Total VS Neutralized		
IC80 <50ug/ml	194	195
IC80 <10ug/ml	140	186
IC80 <1.0ug/ml	29	57
IC80 <0.1ug/ml	6	9
IC80 <0.01ug/ml	3	5
% VS Neutralized		
IC80 <50ug/ml	97	98
IC80 <10ug/ml	70	93
IC80 <1.0ug/ml	15	29
IC80 <0.1ug/ml	3	5
IC80 <0.01ug/ml	2	3
Median IC80	5.10	1.71
Geometric Mean	3.82	1.37

Note: Median and Geometric Mean titers are calculated only for samples with IC80
Values less than the lowest concentration assayed were assigned a value 2-fold less for calculation purposes. Indicated in italics

Figure 38 cont.

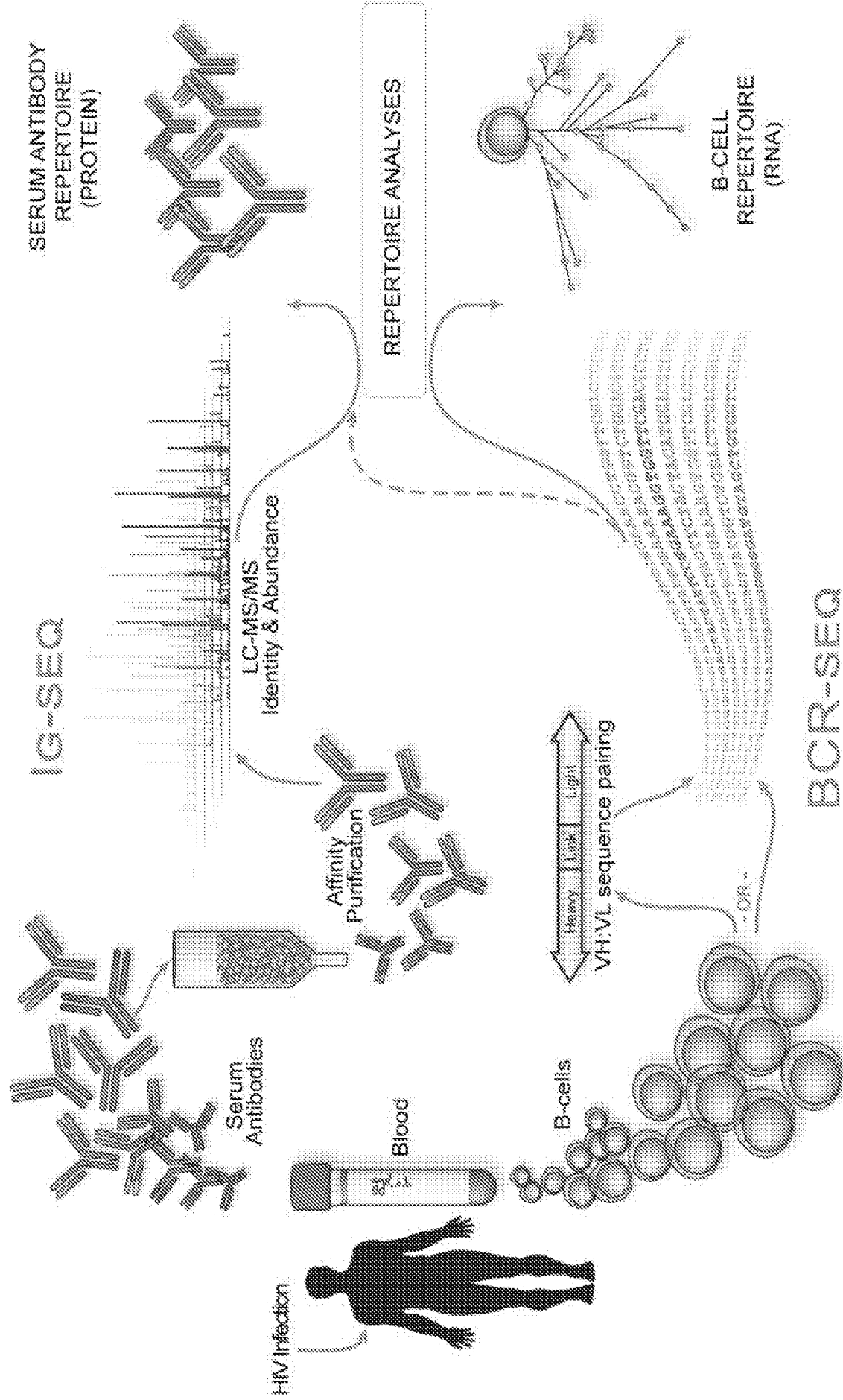


Figure 39

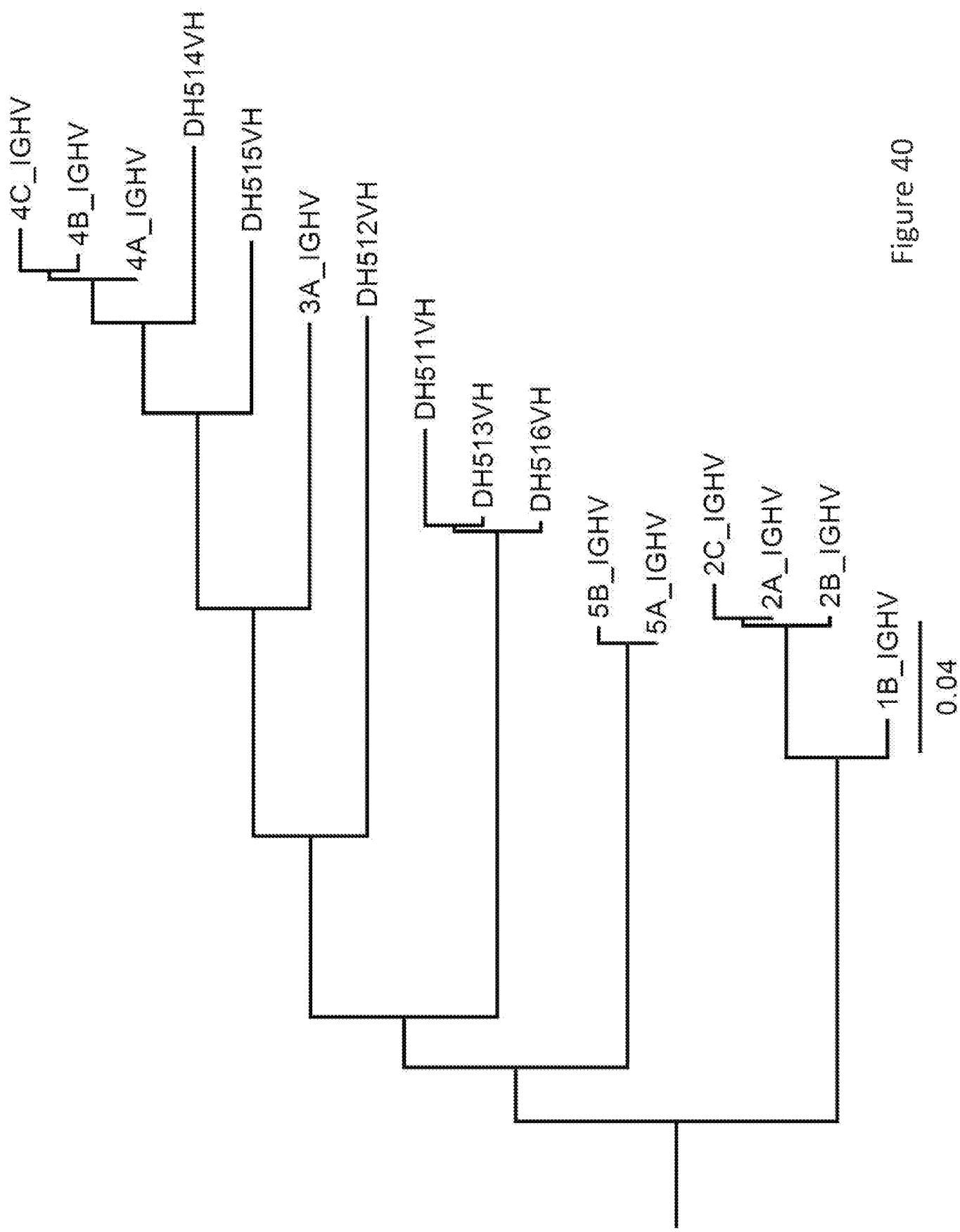


Figure 40

Figure 41

PTID	Ab Name	Heavy ID	Light ID	IC50<50 µg/ml							
				BG1168 (20151002)	BG1168 (20151022)	CH505.TF (20151002)	CH505.TF (20151022)	DU172 (20151002)	DU172 (20151022)	MN (20151002)	MN (20151022)
704-01-021-0	DH511_1a_4A/293i	DH511_1AVH_4A	DH511_1AVK	1.937	0.771	32.077	30.361	8.389	12.841	<0.023	<0.023
704-01-021-0	DH511_1b_4A/293i	DH511_1BVH_4A	DH511_1AVK	1.558	0.793	20.570	25.607	6.526	8.889	<0.023	<0.023
704-01-021-0	DH511_2a_4A/293i	DH511_2AVH_4A	DH511_2AVK	0.896	0.813	17.790	16.462	0.224	0.185	<0.023	<0.023
704-01-021-0	DH511_2b_4A/293i	DH511_2BVH_4A	DH511_2AVK	0.767	0.549	16.668	11.334	0.136	0.147	<0.023	<0.023
704-01-021-0	DH511_2c_4A/293i	DH511_2CVH_4A	DH511_2AVK	10.607	NT	>50	NT	3.056	NT	0.610	NT
704-01-021-0	DH511_3a_4A/293i	DH511_3AVH_4A	DH511_3AVK	1.000	0.751	20.446	21.124	1.762	1.342	<0.023	<0.023
704-01-021-0	DH511_3b_4A/293i	DH511_3AVH_4A	DH511_3BVK	1.922	NT	43.788	NT	2.837	NT	0.027	NT
704-01-021-0	DH511_3c_4A/293i	DH511_3AVH_4A	DH511_3CVK	1.199	0.458	22.389	18.698	1.498	1.193	<0.023	<0.023
704-01-021-0	DH511_4a_4A/293i	DH511_4AVH_4A	DH511_4A4CVK	12.294	7.089	>50	>50	17.317	>50	0.468	0.449
704-01-021-0	DH511_4a_4bK_4A/293i	DH511_4AVH_4A	DH511_4BVK	5.089	5.231	>50	>50	1.650	16.167	0.278	0.352
704-01-021-0	DH511_4b_4bK_4A/293i	DH511_4BVH_4A	DH511_4A4CVK	30.034	NT	>50	NT	>50	NT	1.055	NT
704-01-021-0	DH511_4b_4A/293i	DH511_4BVH_4A	DH511_4BVK	26.071	NT	>50	NT	>50	NT	0.740	NT
704-01-021-0	DH511_4c_4A/293i	DH511_4CVH_4A	DH511_4A4CVK	31.722	>50	>50	>50	>50	>50	2.063	1.328
704-01-021-0	DH511_4c_4bK_4A/293i	DH511_4CVH_4A	DH511_4BVK	33.591	>50	>50	>50	>50	>50	1.267	0.898
704-01-021-0	DH511_5a_4A/293i	pDH511_5AVH_4A	pDH511_5AVK	0.239	0.214	7.000	7.697	0.053	0.067	<0.023	<0.023
704-01-021-0	DH511_5b_4A/293i	pDH511_5BVH_4A	pDH511_5AVK	0.163	0.109	9.385	5.967	<0.023	0.061	<0.023	<0.023
704-01-021-0	DH512 (Ab510049_4A/293i)	H510049_4A	K510032	NT	0.174	NT	7.580	NT	2.905	NT	<0.023
10E8				0.188	0.089	3.314	3.178	<0.023	<0.023	<0.023	<0.023
2F5				0.907	0.841	NT	NT	NT	NT	<0.023	<0.023
4E10				NT	NT	NT	NT	0.199	0.116	NT	NT
CH31				NT	NT	<0.023	<0.023	NT	NT	NT	NT

Key

<0.2 µg/ml

0.2-2 µg/ml

2-20 µg/ml

>20 µg/ml

NT, not tested

Figure 42

PTID	Ab Name	Heavy ID	Light ID	IC50<50 µg/ml									
				BG1168 (20151002)	BG1168 (20151021)	CH505.TF (20151002)	CH505.TF (20151022)	DU172 (20151002)	DU172 (20151022)	MN (20151002)	MN (20151022)	SVA (20151002)	SVA (20151022)
704-01-021-0	DH511_1a_4A/293i	DH511_1AVH_4A	DH511_1AVK	6.969	2.806	>50	>50	37.248	>50	0.051	0.041	>50	>50
704-01-021-0	DH511_1b_4A/293i	DH511_1BVH_4A	DH511_1AVK	6.238	3.754	>50	>50	27.138	>50	0.063	0.021	>50	>50
704-01-021-0	DH511_2a_4A/293i	DH511_2AVH_4A	DH511_2AVK	3.319	3.218	46.876	46.887	1.427	1.307	0.037	<0.023	>50	>50
704-01-021-0	DH511_2b_4A/293i	DH511_2BVH_4A	DH511_2AVK	2.637	2.405	43.664	43.281	1.122	0.809	0.066	<0.023	>50	>50
704-01-021-0	DH511_2c_4A/293i	DH511_2CVH_4A	DH511_2AVK	42.914	NT	>50	NT	22.124	NT	1.648	NT	>50	>50
704-01-021-0	DH511_3a_4A/293i	DH511_3AVH_4A	DH511_3AVK	4.958	2.390	>50	>50	10.523	5.580	0.044	<0.023	>50	>50
704-01-021-0	DH511_3b_4A/293i	DH511_3AVH_4A	DH511_3BVK	6.904	NT	>50	NT	17.279	NT	0.130	NT	>50	>50
704-01-021-0	DH511_3c_4A/293i	DH511_3AVH_4A	DH511_3CVK	5.549	1.885	>50	>50	11.008	5.053	0.077	<0.023	>50	>50
704-01-021-0	DH511_4a_4A/293i	DH511_4AVH_4A	DH511_4A4CVK	39.577	>50	>50	>50	>50	>50	1.323	0.498	>50	>50
704-01-021-0	DH511_4a_4bK_4A/293i	DH511_4AVH_4A	DH511_4BVK	24.986	39.218	>50	>50	>50	>50	0.649	0.536	>50	>50
704-01-021-0	DH511_4b_4A/293i	DH511_4BVH_4A	DH511_4A4CVK	>50	NT	>50	NT	>50	NT	4.022	NT	>50	>50
704-01-021-0	DH511_4b_4A/293i	DH511_4BVH_4A	DH511_4BVK	>50	NT	>50	NT	>50	NT	2.042	NT	>50	>50
704-01-021-0	DH511_4c_4A/293i	DH511_4CVH_4A	DH511_4A4CVK	>50	>50	>50	>50	>50	>50	6.885	4.577	>50	>50
704-01-021-0	DH511_4c_4bK_4A/293i	DH511_4CVH_4A	DH511_4BVK	>50	>50	>50	>50	>50	>50	3.769	2.456	>50	>50
704-01-021-0	DH511_5a_4A/293i	pDH511_5AVH_4A	pDH511_5AVK	1.141	1.064	24.104	23.995	0.477	0.505	<0.023	<0.023	>50	>50
704-01-021-0	DH511_5b_4A/293i	pDH511_5BVH_4A	pDH511_5AVK	0.944	0.448	20.798	18.442	0.443	0.440	<0.023	<0.023	>50	>50
704-01-021-0	DH512 (Ab510049_4A/293i)	H510049_4A	K510032	NT	0.813	NT	21.759	NT	9.813	NT	<0.023	NT	>50
10E8				0.773	0.433	9.597	7.213	0.196	0.193	<0.023	<0.023	>50	>50
2F5				4.035	4.239	NT	NT	NT	NT	<0.023	<0.023	NT	NT
4E10				NT	NT	NT	NT	2.301	1.449	NT	NT	>50	>50
CH31				NT	NT	NT	0.029	0.030	NT	NT	NT	NT	NT

Key

<0.2 µg/ml

0.2-2 µg/ml

2-20 µg/ml

>20 µg/ml

NT, not tested

Figure 43

	10	20	30	40	50	60	70	80	90	100	
DH512_VH	...	...	...	...	...	...	...	...	...	...	
DH511_1AVH	...	...	...	...	...	...	...	...	...	...	
DH511_1BVH	...	...	...	...	...	...	...	...	...	...	
DH511_2AVH	...	...	...	...	...	...	...	...	...	...	
DH511_2BVH	...	...	...	...	...	...	...	...	...	...	
DH511_2CVH	...	...	...	...	...	...	...	...	...	...	
DH511_3AVH	...	...	...	...	...	...	...	...	...	...	
DH511_4AVH	...	...	...	...	...	...	...	...	...	...	
DH511_4BVH	...	...	...	...	...	...	...	...	...	...	
DH511_4CVH	...	...	...	...	...	...	...	...	...	...	
DH511_5AVH	...	...	...	...	...	...	...	...	...	...	
DH511_5BVH	...	...	...	...	...	...	...	...	...	...	
10E8_VH	...	...	...	...	...	...	...	...	...	...	
	110	120	130	140	150	160	170	180	190	200	
	...	...	...	...	...	...	...	...	...	...	
DH512_VH	...	...	...	...	...	...	...	...	...	...	
DH511_1AVH	...	...	...	...	...	...	...	...	...	...	
DH511_1BVH	...	...	...	...	...	...	...	...	...	...	
DH511_2AVH	...	...	...	...	...	...	...	...	...	...	
DH511_2BVH	...	...	...	...	...	...	...	...	...	...	
DH511_2CVH	...	...	...	...	...	...	...	...	...	...	
DH511_3AVH	...	...	...	...	...	...	...	...	...	...	
DH511_4AVH	...	...	...	...	...	...	...	...	...	...	
DH511_4BVH	...	...	...	...	...	...	...	...	...	...	
DH511_4CVH	...	...	...	...	...	...	...	...	...	...	
DH511_5AVH	...	...	...	...	...	...	...	...	...	...	
DH511_5BVH	...	...	...	...	...	...	...	...	...	...	
10E8_VH	...	...	...	...	...	...	...	...	...	...	

Figure 43 cont.

	210	220	230	240	250	260	270	280	290	300	
DH512_VH	GGACAGATTCACCACTTCACAGATGACAGATGAGAAATATGCTGTGACCTGACATGAGGACCCCTGAATAACCGAGGACTCAGGGACACTTAAATATTGTAACCATG										CDR3
DH511_1AVH	A.G.....C.....C.....TTCA.....C.C.....T.G.A.....TGG.....G.T.....C.A.....CGGTG.....GCT										
DH511_1BVH	A.G.....C.....C.....TTCA.....C.C.....T.G.A.....TGG.....G.T.....C.A.....CAGTG.....GCT										
DH511_2AVH	A.G.....C.....G.C.....TTCA.....C.CA.....T.G.G.....TGG.....T.....A.T.C.GTG.....GCT										
DH511_2BVH	A.G.....C.....G.C.....TTCA.....C.CA.....T.G.G.....TGG.....T.....A.T.C.GTG.....GCT										
DH511_2CVH	A.G.....A.C.....G.C.....TTCA.....C.CA.....T.G.G.....TGG.....T.....A.T.C.GTG.....GCT										
DH511_3AVH	.GA.....C.....G.....TCA.AG.C.CAG.....T.A.....AT.A.....A.T.A.....TGTAA.....T.GA										
DH511_4AVH	.GT.....C.....C.....TTCA.....C.C.A.TT.....AT.G.....C.GGT.....A.A.....C.GTC.....TCCA										
DH511_4BVH	.GT.....C.....C.....TTCA.....C.C.A.TT.....AT.G.....C.GGT.....A.A.....C.GTC.....TCCA										
DH511_4CVH	.GT.....C.....C.....TTCA.....C.C.A.TT.....AT.G.....C.GGT.....A.A.....C.GTC.....TCCA										
DH511_5AVH	A.G.....TA.C.....TCA.....C.CA.....C.CT.T.T.....AC.A.....A.A.....C.GTG.....GCT										
DH511_5BVH	A.G.....TA.C.....TCA.....C.CA.....C.CT.T.T.....AC.A.....A.A.....C.GTG.....GCT										
10E8_VH	A.G.....T.....C.G.....CTCA.TTCA.T.....T.CT.A.TT.....G.G.....AC.AAT.A.G.TG.A.A.....CT.....C.TC.....G.....C.										
	310	320	330	340	350	360	370	380	390	400	
DH512_VH	GAUGAGGGGACCCAGTAACACGC.....TTCTTAGAATGGGGCTACCTCT.....ATTATTAAATGGCCGTTTGGGGAGAGGGACACGGTCAATCGCTCTCT										
DH511_1AVH	...CG....G.....CTT.....T.....T.GG..G.....ATG.....C.C.C.....AGT.C.....T.....C.....C										
DH511_1BVH	...CG....G.....CTT.....T.....T.GG..G.....ATG.....C.C.C.....AGT.C.....T.....C.....C										
DH511_2AVH	...CG....G.....CTT.....T.....T.GG..G.....T.TG.....C.C.C.....AGT.C.....A.....T.....C.....C										
DH511_2BVH	...CG....G.....CTT.....T.....T.GG..G.....T.TG.....C.C.C.....AGT.C.....A.....T.....C.....C										
DH511_2CVH	...CG....G.....CTT.....T.....T.GG..G.....T.TG.....C.C.C.....AGT.C.....A.....T.....C.....C										
DH511_3AVH	...C.....G.....ACGG..TC.G.G.....C.....C.....C.....A.....C.....C										
DH511_4AVH	...C.....G.....T.C.A.A.....TC.G.G.....C.AT.....C.C.C.....C.....C										
DH511_4BVH	...C.....G.....T.C.A.A.....TC.G.G.....C.AT.....C.C.C.....C.....C										
DH511_4CVH	...C.....G.....T.C.A.A.....C.TC.G.G.....C.AT.....C.C.C.....C.....C										
DH511_5AVH	...G.....A.TTT.A.A.....T.T.G.....T.T.A.A.....C.C.C.....A.A.....AG.....C										
DH511_5BVH	...G.....A.TTT.A.A.....T.T.G.....T.T.A.A.....C.C.C.....A.A.....AG.....C										
10E8_VH	C.C.G.AA.....A.TT.T.A.....T.G.G.....CTA.C.GC.GGGAG.AG.A.CT.CCAAC.....C.G..C...CT.....C.....C										

Figure 43 cont.

DH512_VH	...
DH511_1AVH	2CA
DH511_1BVH	...
DH511_2AVH	...
DH511_2BVH	...
DH511_2CVH	...
DH511_3AVH	C..
DH511_4AVH	...
DH511_4BVH	...
DH511_4CVH	...
DH511_5AVH	..G
DH511_5BVH	..G
10E8_VH	...

[illegible]

Figure 45 cont.

	210	220	230	240	250	260	270	280	290	300
										
DH512_VK	TGGGAAACCTTTTCACCTCTCACCATTACCAATCTGGCAACCTGAGAGATTTGGCACTTATTATTTGT									
DH511_1AVK	CAGAC.....T...C.G.G.....G...C.....G...C.C.....G...T.C.CG...A.A.A..C.									
DH511_2AVK	CAGAC...T...T...C.G.G.....G...C.....G...TC.....G...T...CG...A.A.A..TT									
DH511_3AVK	AA.....A..G.....A..G.....A.....G...CC.G...CG.T...A.A...C.									
DH511_3BVK	AA.....A..G.....A..G.....A.....G...CC.G...CG.T...A.A...C.									
DH511_3CVK	AA.....A..G.....A..G.....A.....G...CC.G...CG.T...A.A...C.									
DH511_4A4CVK	CA.AG.....A..G.....A..G.....A.....G...GC.....C.C.A...A.A...TC.									
DH511_4BVK	CA.AG.....A..G.....A..G.....A.....G...GC.....C.C.A...A.A...TC.									
DH511_5AVK	TCGGCC.....A..G.....C.G.G.....G.....G...C.GC.GCG.T...AT.TA...TT									
10E8_VL	A..A...AGAGCTT.CT.G.....CT.TGGGGCT...GG.G....CGAC..GGAA.....AGTTCG.G.CA.G.G.GGC.G...GTCT.TCGGTT.									
										
	310	320	330							
										
DH512_VK	TTTGGTCAAGGGGACCAAGGTGGACATTCAGG-									
DH511_1AVK	C.T.....AT.....G...G..A.AC									
DH511_2AVK	C.T.....AT.....G..AG.AAC									
DH511_3AVK	C.....G.....GG.A.C									
DH511_3BVK	C.....G.....GG.A.C									
DH511_3CVK	C.....G.....GG.A.C									
DH511_4A4CVK	C.....G.....GG.AAC									
DH511_4BVK	C.....G.....GG.AAC									
DH511_5AVK	C.C.....C...AAC									
10E8_VL	..C..CGG.....AC..AC.G...CTCA									

CDR3

Figure 47

PTID	Ab Name	Heavy ID	Light ID	VH	DH	JH	HCDR3 Length	VH Mut (%)	VK	JK	K CDR3 Length	VK Mut (%)
CH0210	DH511_1a_4A	DH511_1AVH_4A	DH511_1AVK	3-15*02	3-3*01	6*03	23	12.29	1-39*01	2*01	11	11.74
CH0210	DH511_1b_4A	DH511_1BVH_4A	DH511_1AVK	3-15*02	3-3*01	6*03	23	11.60	1-39*01	2*01	11	11.74
CH0210	DH511_2a_4A	DH511_2AVH_4A	DH511_2AVK	3-15*02	3-3*01	6*03	23	13.99	1-39*01	2*01	11	11.36
CH0210	DH511_2b_4A	DH511_2BVH_4A	DH511_2AVK	3-15*02	3-3*01	6*03	23	14.68	1-39*01	2*01	11	11.36
CH0210	DH511_2c_4A	DH511_2CVH_4A	DH511_2AVK	3-15*02	3-3*01	6*03	23	14.97	1-39*01	2*01	11	11.36
CH0210	DH511_3a_4A	DH511_3AVH_4A	DH511_3AVK	3-15*01	3-3*01	6*03	24	20.41	1-39*01	2*01	11	13.64
CH0210	DH511_3b_4A	DH511_3AVH_4A	DH511_3BVK	3-15*01	3-3*01	6*03	24	20.41	1-39*01	2*01	11	14.02
CH0210	DH511_3c_4A	DH511_3AVH_4A	DH511_3CVK	3-15*01	3-3*01	6*03	24	20.41	1-39*01	2*01	11	13.64
CH0210	DH511_4a_4A	DH511_4AVH_4A	DH511_4A4CVK	3-15*01	3-3*01	6*03	23	19.05	1-39*01	2*01	11	13.64
CH0210	DH511_4a_4bK_4A	DH511_4AVH_4A	DH511_4BVK	3-15*01	3-3*01	6*03	23	19.05	1-39*01	2*01	11	14.39
CH0210	DH511_4b_4aK_4A	DH511_4BVH_4A	DH511_4A4CVK	3-15*01	3-3*01	6*03	23	19.80	1-39*01	2*01	11	13.64
CH0210	DH511_4b_4A	DH511_4BVH_4A	DH511_4BVK	3-15*01	3-3*01	6*03	23	19.80	1-39*01	2*01	11	14.39
CH0210	DH511_4c_4A	DH511_4CVH_4A	DH511_4A4CVK	3-15*01	3-3*01	6*03	23	20.48	1-39*01	2*01	11	13.64
CH0210	DH511_4c_4bK_4A	DH511_4CVH_4A	DH511_4BVK	3-15*01	3-3*01	6*03	23	20.48	1-39*01	2*01	11	14.39
CH0210	DH511_5a_4A	DH511_5AVH_4A	DH511_5AVK	3-15*01	3-3*01	6*03	23	10.56	1-39*01	2*01	11	15.53
CH0210	DH511_5b_4A	DH511_5BVH_4A	DH511_5AVK	3-15*01	3-3*01	6*03	23	11.22	1-39*01	2*01	11	15.53

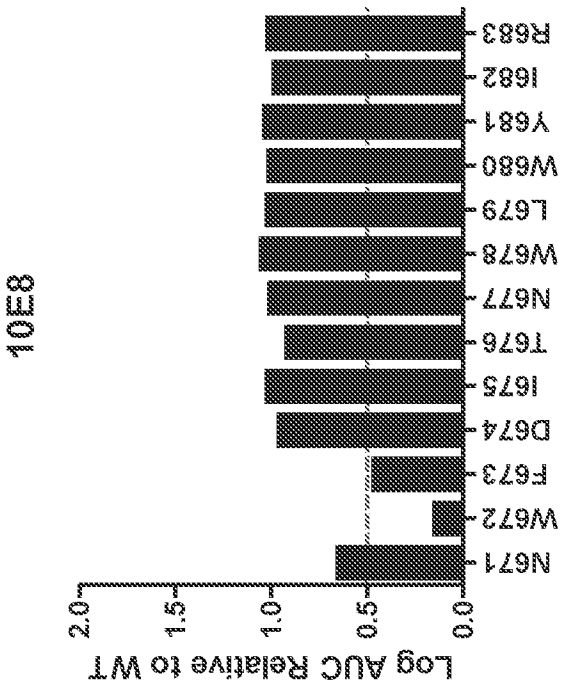
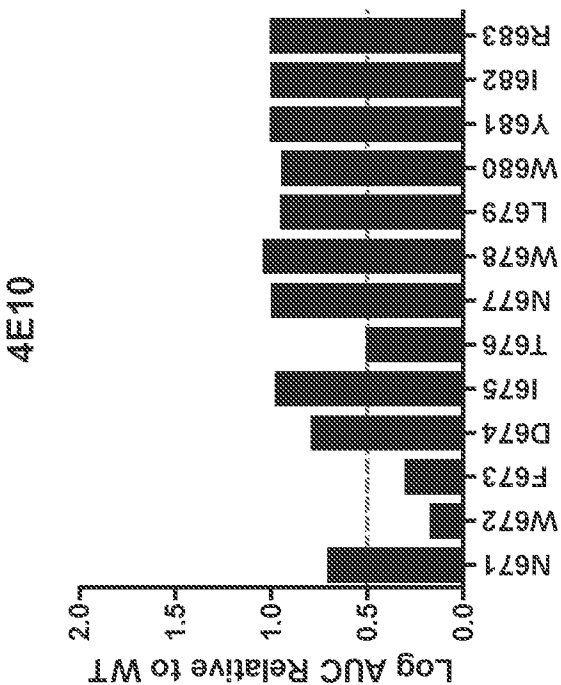
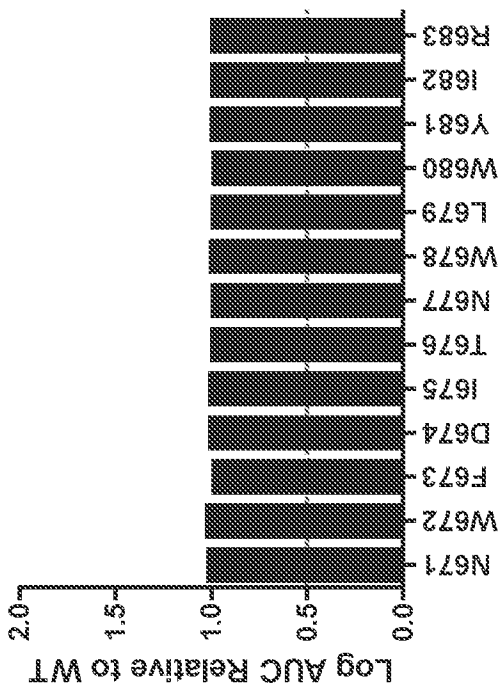


Figure 48

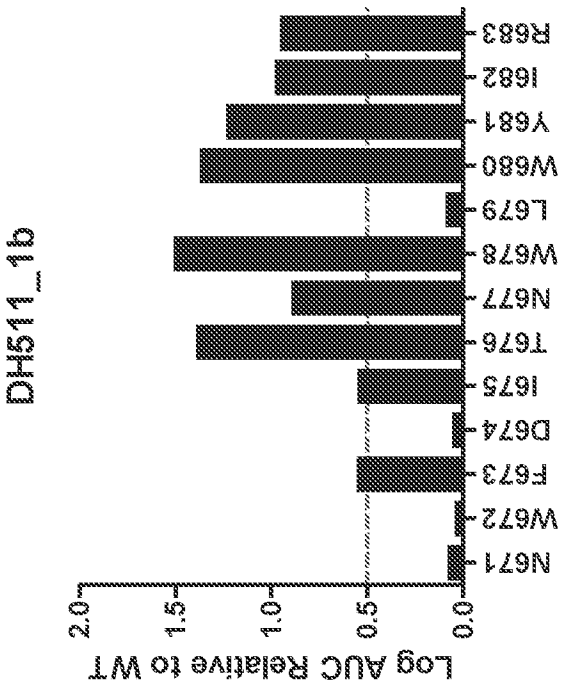
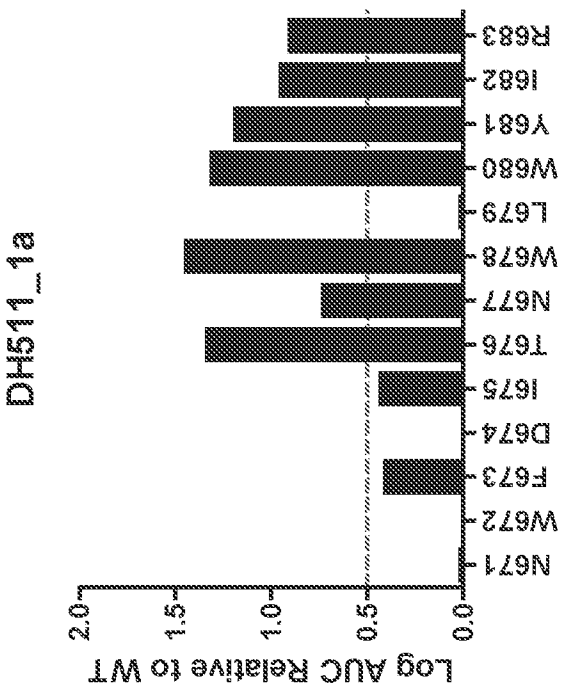
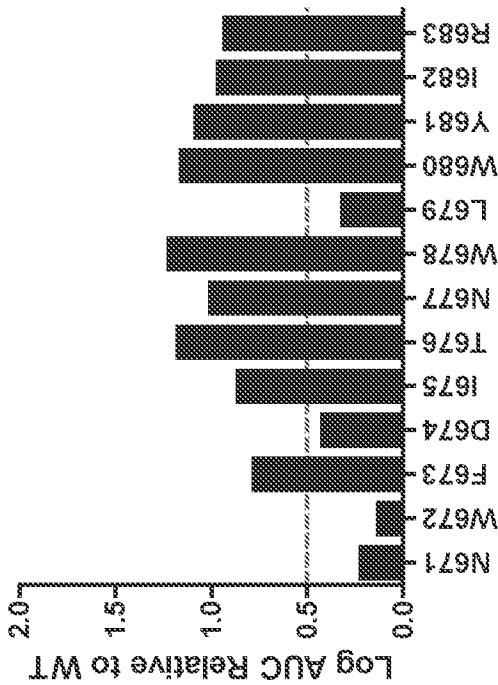
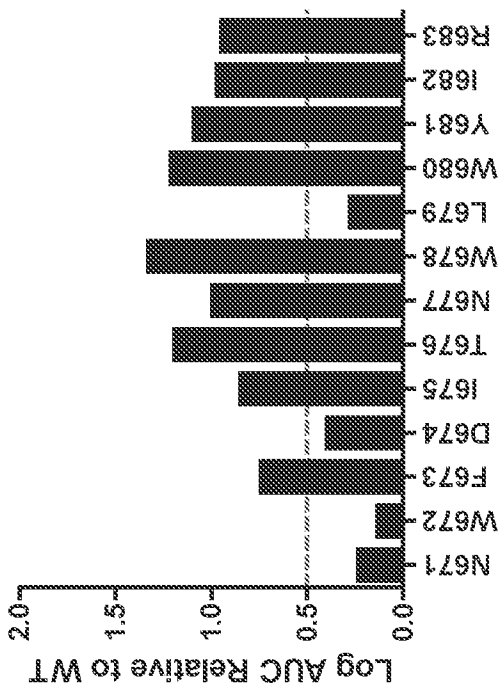


Figure 49

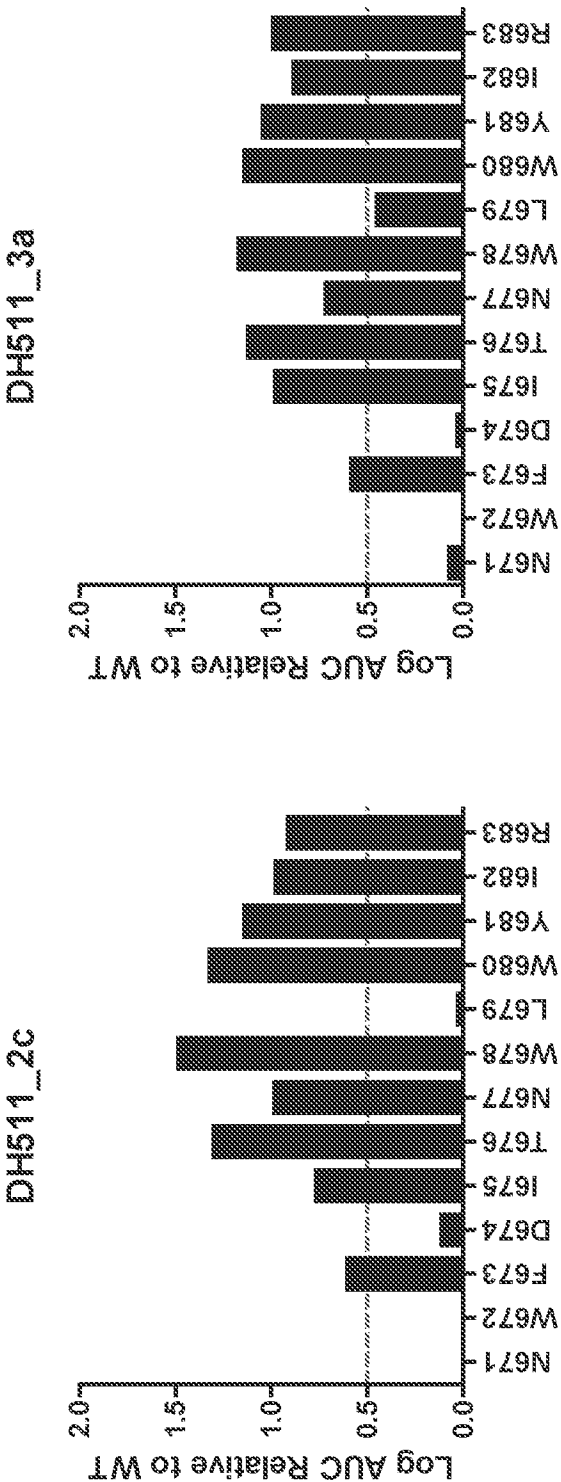
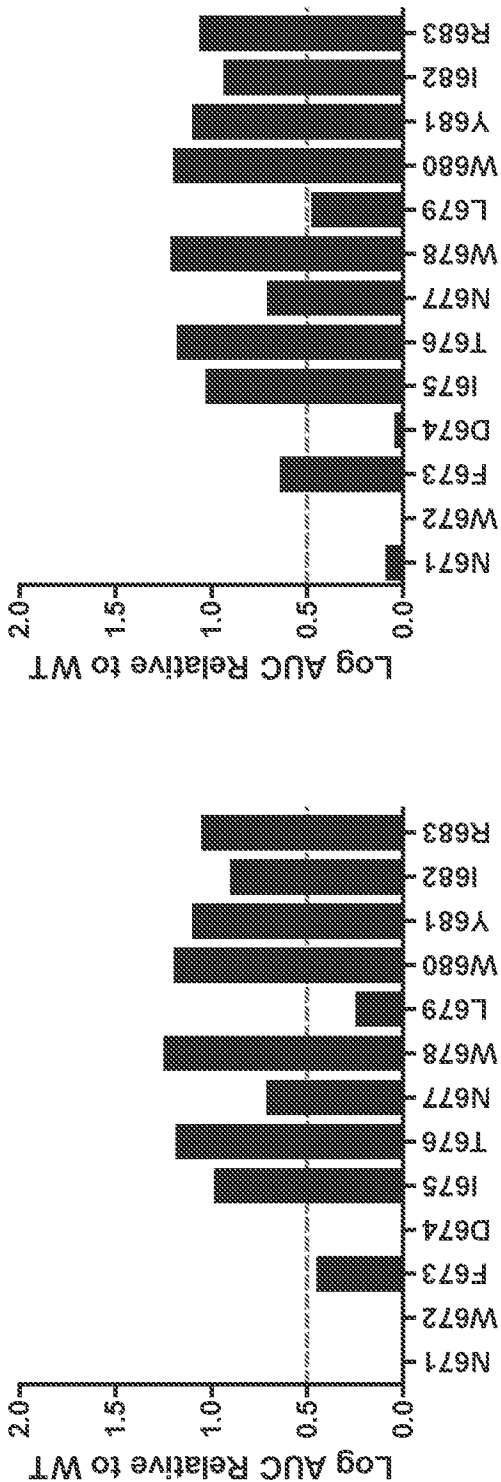
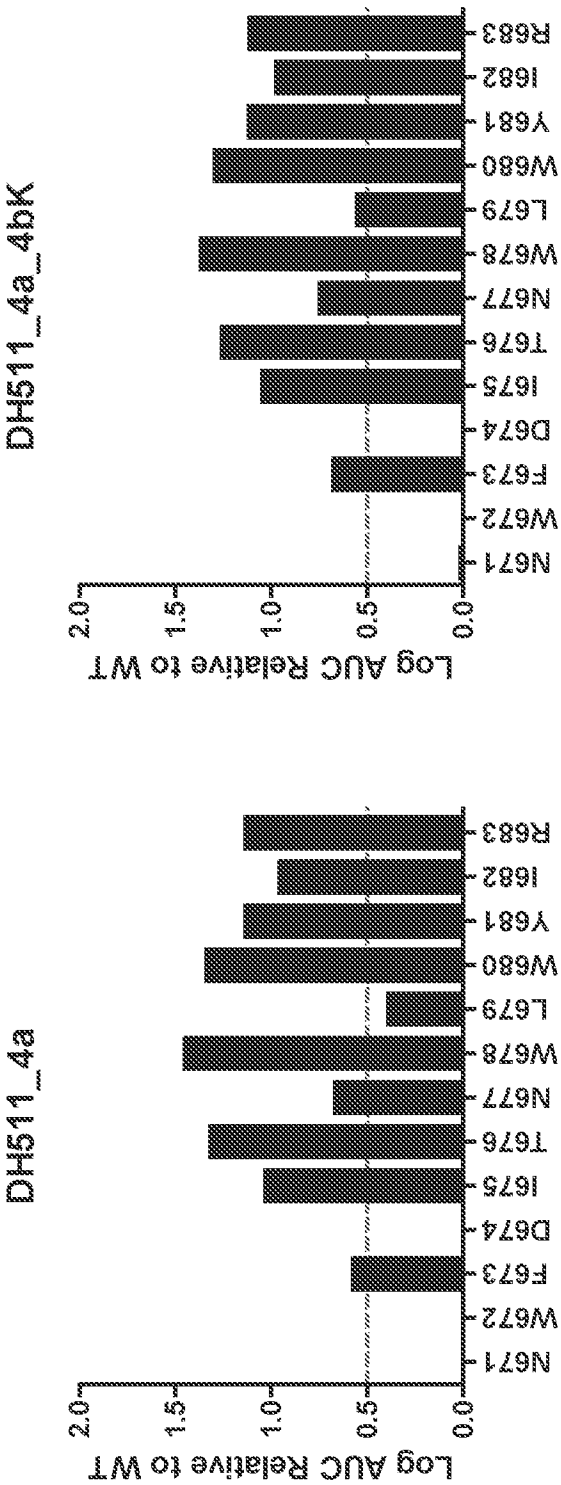
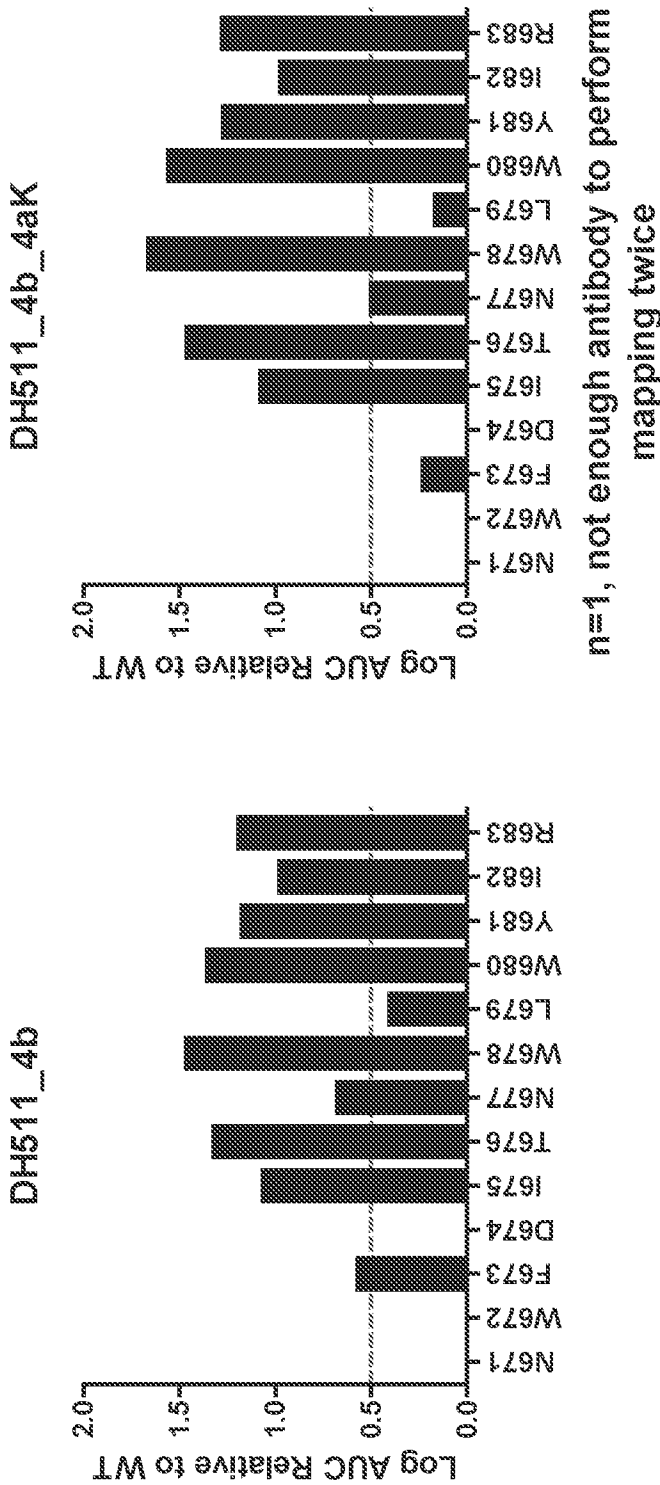


Figure 50





n=1, not enough antibody to perform mapping twice

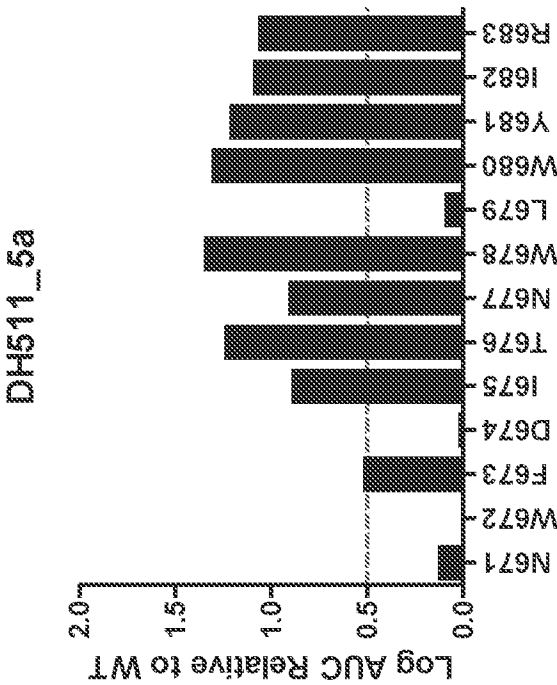
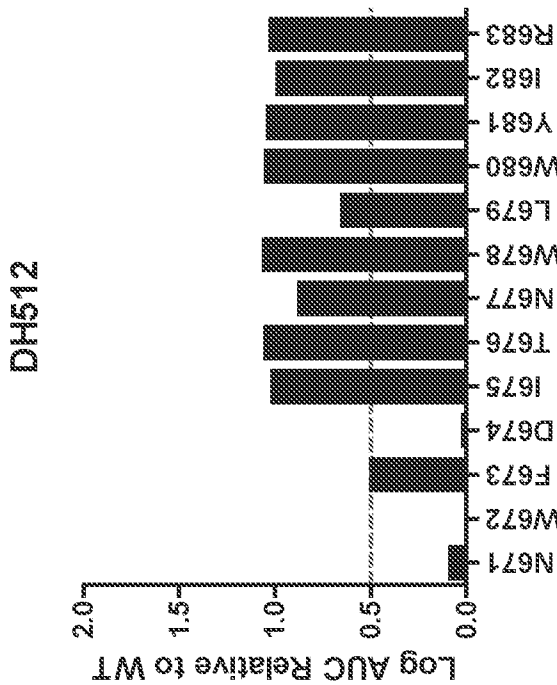
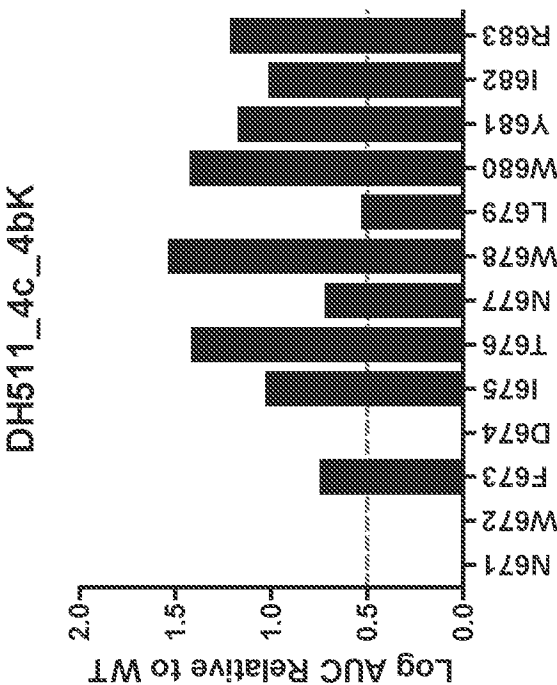
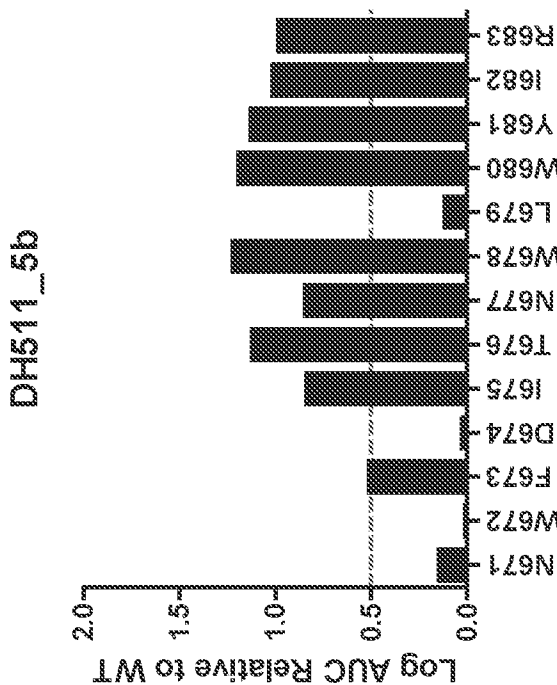


Figure 52

PI=0.02826

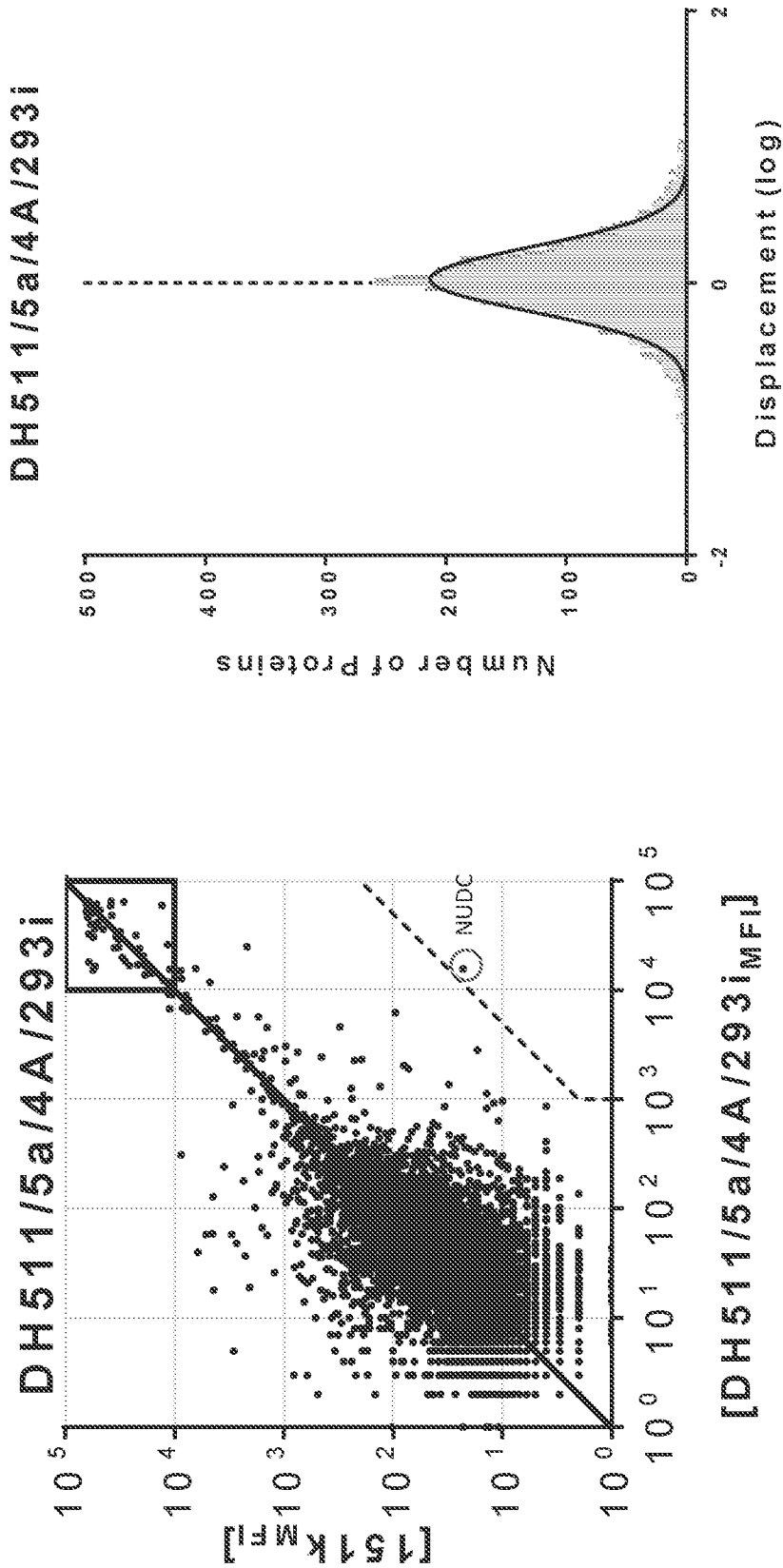


Figure 53

PI=0.2738

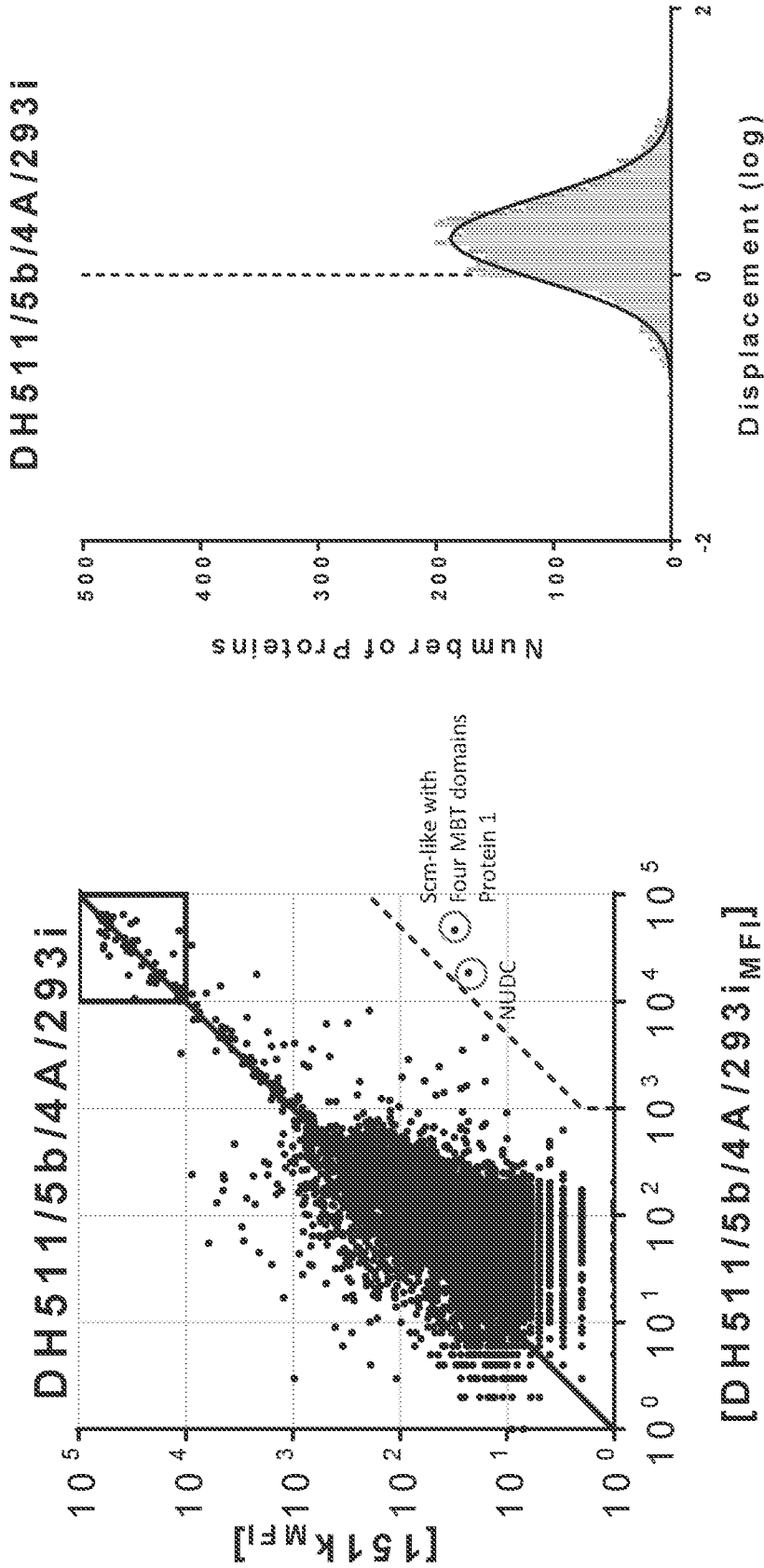


Figure 54

Figure 55

Heavy ID		Light ID	Heavy ID		Light ID	Heavy ID		Light ID
1	DH511_1AVH_4A	DH511_2AVK	33	DH511_2CVH_4A	DH511_1AVK	61	DH511_4CVH_4A	DH511_1AVK
2	DH511_1AVH_4A	DH511_3AVK	34	DH511_2CVH_4A	DH511_3AVK	62	DH511_4CVH_4A	DH511_2AVK
3	DH511_1AVH_4A	DH511_3BVK	35	DH511_2CVH_4A	DH511_3BVK	63	DH511_4CVH_4A	DH511_3AVK
4	DH511_1AVH_4A	DH511_3CVK	36	DH511_2CVH_4A	DH511_3CVK	64	DH511_4CVH_4A	DH511_3BVK
5	DH511_1AVH_4A	DH511_4A4CVK	37	DH511_2CVH_4A	DH511_4A4CVK	65	DH511_4CVH_4A	DH511_3CVK
6	DH511_1AVH_4A	DH511_4BVK	38	DH511_2CVH_4A	DH511_4BVK	66	DH511_4CVH_4A	DH511_5AVK
7	DH511_1AVH_4A	DH511_5AVK	39	DH511_2CVH_4A	DH511_5AVK	67	DH511_4CVH_4A	K510032 (DH512)
8	DH511_1AVH_4A	K510032 (DH512)	40	DH511_2CVH_4A	K510032 (DH512)	68	DH511_5AVH_4A	DH511_1AVK
9	DH511_1BVH_4A	DH511_2AVK	41	DH511_3AVH_4A	DH511_1AVK	69	DH511_5AVH_4A	DH511_2AVK
10	DH511_1BVH_4A	DH511_3AVK	42	DH511_3AVH_4A	DH511_2AVK	70	DH511_5AVH_4A	DH511_3AVK
11	DH511_1BVH_4A	DH511_3BVK	43	DH511_3AVH_4A	DH511_4A4CVK	71	DH511_5AVH_4A	DH511_3BVK
12	DH511_1BVH_4A	DH511_3CVK	44	DH511_3AVH_4A	DH511_4BVK	72	DH511_5AVH_4A	DH511_3CVK
13	DH511_1BVH_4A	DH511_4A4CVK	45	DH511_3AVH_4A	DH511_5AVK	73	DH511_5AVH_4A	DH511_4A4CVK
14	DH511_1BVH_4A	DH511_4BVK	46	DH511_3AVH_4A	K510032 (DH512)	74	DH511_5AVH_4A	DH511_4BVK
15	DH511_1BVH_4A	DH511_5AVK	47	DH511_4AVH_4A	DH511_1AVK	75	DH511_5AVH_4A	K510032 (DH512)
16	DH511_1BVH_4A	K510032 (DH512)	48	DH511_4AVH_4A	DH511_2AVK	76	DH511_5BVH_4A	DH511_1AVK
17	DH511_2AVH_4A	DH511_1AVK	49	DH511_4AVH_4A	DH511_3AVK	77	DH511_5BVH_4A	DH511_2AVK
18	DH511_2AVH_4A	DH511_3AVK	50	DH511_4AVH_4A	DH511_3BVK	78	DH511_5BVH_4A	DH511_3AVK
19	DH511_2AVH_4A	DH511_3BVK	51	DH511_4AVH_4A	DH511_3CVK	79	DH511_5BVH_4A	DH511_3BVK
20	DH511_2AVH_4A	DH511_3CVK	52	DH511_4AVH_4A	DH511_5AVK	80	DH511_5BVH_4A	DH511_3CVK
21	DH511_2AVH_4A	DH511_4A4CVK	53	DH511_4AVH_4A	K510032 (DH512)	81	DH511_5BVH_4A	DH511_4A4CVK
22	DH511_2AVH_4A	DH511_4BVK	54	DH511_4BVH_4A	DH511_1AVK	82	DH511_5BVH_4A	DH511_4BVK
23	DH511_2AVH_4A	DH511_5AVK	55	DH511_4BVH_4A	DH511_2AVK	83	DH511_5BVH_4A	K510032 (DH512)
24	DH511_2AVH_4A	K510032 (DH512)	56	DH511_4BVH_4A	DH511_3AVK	84	H510049_4A (DH512)	DH511_1AVK
25	DH511_2BVH_4A	DH511_1AVK	57	DH511_4BVH_4A	DH511_3BVK	85	H510049_4A (DH512)	DH511_2AVK
26	DH511_2BVH_4A	DH511_3AVK	58	DH511_4BVH_4A	DH511_3CVK	86	H510049_4A (DH512)	DH511_3AVK
27	DH511_2BVH_4A	DH511_3BVK	59	DH511_4BVH_4A	DH511_5AVK	87	H510049_4A (DH512)	DH511_3BVK
28	DH511_2BVH_4A	DH511_3CVK	60	DH511_4BVH_4A	K510032 (DH512)	88	H510049_4A (DH512)	DH511_3CVK
29	DH511_2BVH_4A	DH511_4A4CVK				89	H510049_4A (DH512)	DH511_4A4CVK
30	DH511_2BVH_4A	DH511_4BVK				90	H510049_4A (DH512)	DH511_4BVK
31	DH511_2BVH_4A	DH511_5AVK				91	H510049_4A (DH512)	DH511_5AVK
32	DH511_2BVH_4A	K510032 (DH512)						

PTID	Heavy ID	Light ID	IgG Quant ($\mu\text{g/ml}$)	1:6 Initial Dilution ($\mu\text{g/ml}$)
704-01-021-0	DH511_1AVH_4A	DH511_2AVK	53	10.50
704-01-021-0	DH511_1AVH_4A	DH511_3AVK	57.2	11.20
704-01-021-0	DH511_1AVH_4A	DH511_3BVK	52.35	10.39
704-01-021-0	DH511_1AVH_4A	DH511_3CVK	109.25	18.21
704-01-021-0	DH511_1AVH_4A	DH511_4A4CVK	24.5	4.09
704-01-021-0	DH511_1AVH_4A	DH511_4BVK	57	11.17
704-01-021-0	DH511_1AVH_4A	DH511_5AVK	38.75	6.46
704-01-021-0	DH511_1AVH_4A	K510032 (DH512)	59.35	11.39
704-01-021-0	DH511_1BVH_4A	DH511_2AVK	40.55	6.91
704-01-021-0	DH511_1BVH_4A	DH511_3AVK	51.9	10.32
704-01-021-0	DH511_1BVH_4A	DH511_3BVK	36	6.00
704-01-021-0	DH511_1BVH_4A	DH511_3CVK	51.45	8.58
704-01-021-0	DH511_1BVH_4A	DH511_4A4CVK	44.5	7.42
704-01-021-0	DH511_1BVH_4A	DH511_4BVK	38.75	6.46
704-01-021-0	DH511_1BVH_4A	DH511_5AVK	37.5	6.25
704-01-021-0	DH511_1BVH_4A	K510032 (DH512)	72.7	12.12
704-01-021-0	DH511_2AVH_4A	DH511_1AVK	55.3	11.05
704-01-021-0	DH511_2AVH_4A	DH511_3AVK	59.55	9.98
704-01-021-0	DH511_2AVH_4A	DH511_3BVK	54.1	10.69
704-01-021-0	DH511_2AVH_4A	DH511_3CVK	92.4	15.40
704-01-021-0	DH511_2AVH_4A	DH511_4A4CVK	29.5	4.75
704-01-021-0	DH511_2AVH_4A	DH511_4BVK	24.1	4.02
704-01-021-0	DH511_2AVH_4A	DH511_5AVK	23.2	3.87
704-01-021-0	DH511_2AVH_4A	K510032 (DH512)	75.75	12.79
704-01-021-0	DH511_2BVH_4A	DH511_1AVK	57.45	11.24
704-01-021-0	DH511_2BVH_4A	DH511_3AVK	152.55	25.34
704-01-021-0	DH511_2BVH_4A	DH511_3BVK	103.5	17.25
704-01-021-0	DH511_2BVH_4A	DH511_3CVK	227.2	37.87
704-01-021-0	DH511_2BVH_4A	DH511_4A4CVK	45.9	7.65
704-01-021-0	DH511_2BVH_4A	DH511_4BVK	40.55	6.78
704-01-021-0	DH511_2BVH_4A	DH511_5AVK	92.55	15.44
704-01-021-0	DH511_2BVH_4A	K510032 (DH512)	118.75	19.79
704-01-021-0	DH511_1AVH_4A	DH511_1AVK	59.3	11.55
704-01-021-0	DH511_1BVH_4A	DH511_1AVK	55	10.83
704-01-021-0	DH511_2AVH_4A	DH511_2AVK	58.5	11.42
704-01-021-0	DH511_2BVH_4A	DH511_2AVK	55.4	14.23
704-01-021-0	H510049_4A (DH512)	K510032 (DH512)	170	28.33
Positive Control	HV13221	HV13501	55	9.17
Mock Transfection			20	3.33
10E8				

Figure 56A

	IC50 (µg/ml)	IC80 (µg/ml)	IC50 (µg/ml)	IC80 (µg/ml)
PTID	B G1168	B G1168	SVA	SVA
704-01-021-0	1.272	6.006	>10.5	>10.5
704-01-021-0	9.314	>11.2	>11.2	>11.2
704-01-021-0	>10.39	>10.39	>10.39	>10.39
704-01-021-0	8.174	>18.21	>18.21	>18.21
704-01-021-0	>4.08	>4.08	>4.08	>4.08
704-01-021-0	>11.17	>11.17	>11.17	>11.17
704-01-021-0	>6.46	>6.46	>6.46	>6.46
704-01-021-0	2.818	>11.39	>11.39	>11.39
704-01-021-0	1.3	>6.81	>6.81	>6.81
704-01-021-0	>10.32	>10.32	>10.32	>10.32
704-01-021-0	>6	>6	>6	>6
704-01-021-0	7.843	>8.58	>8.58	>8.58
704-01-021-0	>7.42	>7.42	>7.42	>7.42
704-01-021-0	>6.46	>6.46	>6.46	>6.46
704-01-021-0	>6.25	>6.25	>6.25	>6.25
704-01-021-0	3.458	>12.12	>12.12	>12.12
704-01-021-0	1.047	5.776	>11.05	>11.05
704-01-021-0	7.925	>9.98	>9.98	>9.98
704-01-021-0	>10.65	>10.68	>10.68	>10.68
704-01-021-0	6.174	>15.4	>15.4	>15.4
704-01-021-0	>4.75	>4.75	>4.75	>4.75
704-01-021-0	>4.02	>4.02	>4.02	>4.02
704-01-021-0	>3.87	>3.87	>3.87	>3.87
704-01-021-0	2.091	>12.79	>12.79	>12.79
704-01-021-0	0.513	2.523	>11.24	>11.24
704-01-021-0	8.241	>25.34	>25.34	>25.34
704-01-021-0	7.890	>17.25	>17.25	>17.25
704-01-021-0	6.789	>37.87	>37.87	>37.87
704-01-021-0	>7.65	>7.65	>7.65	>7.65
704-01-021-0	>6.78	>6.78	>6.78	>6.78
704-01-021-0	5.087	>15.44	>15.44	>15.44
704-01-021-0	2.002	9.339	>19.79	>19.79
704-01-021-0	2.236	>11.55	>11.55	>11.55
704-01-021-0	3.239	>10.83	>10.83	>10.83
704-01-021-0	1.023	6.96	>11.42	>11.42
704-01-021-0	0.244	1.503	>4.23	>4.23
704-01-021-0	0.522	2.750	>28.33	>28.33
Positive Control	1.581	>9.17	>9.17	>9.17
Mock Transfection	>3.33	>3.33	>3.33	>3.33
10E8	0.152	0.841	>50	>50

Figure 56A cont.

PTID	Heavy ID	Light ID	IgG Quant ($\mu\text{g/ml}$)	1:6 Initial Dilution ($\mu\text{g/ml}$)
704-01-021-0	DH511_2C\H_4A	DH511_1AVK	2.511	0.419
704-01-021-0	DH511_2C\H_4A	DH511_3AVK	12.818	2.137
704-01-021-0	DH511_2C\H_4A	DH511_3BVK	0.397	0.066
704-01-021-0	DH511_2C\H_4A	DH511_3CVK	12.785	2.131
704-01-021-0	DH511_2C\H_4A	DH511_4A4C\K	0.314	0.052
704-01-021-0	DH511_2C\H_4A	DH511_4BVK	0.493	0.082
704-01-021-0	DH511_2C\H_4A	DH511_5AVK	5.005	0.834
704-01-021-0	DH511_2C\H_4A	K510032 (DH512)	1.826	0.304
704-01-021-0	DH511_3AVH_4A	DH511_1AVK	113.8	18.987
704-01-021-0	DH511_3AVH_4A	DH511_2AVK	80.144	13.357
704-01-021-0	DH511_3AVH_4A	DH511_4A4C\K	0.425	0.071
704-01-021-0	DH511_3AVH_4A	DH511_4BVK	1.324	0.221
704-01-021-0	DH511_3AVH_4A	DH511_5AVK	35.459	5.910
704-01-021-0	DH511_3AVH_4A	K510032 (DH512)	123.19	20.532
704-01-021-0	DH511_4AVH_4A	DH511_1AVK	88.918	14.820
704-01-021-0	DH511_4AVH_4A	DH511_2AVK	100.574	16.782
704-01-021-0	DH511_4AVH_4A	DH511_3AVK	173.528	28.921
704-01-021-0	DH511_4AVH_4A	DH511_3BVK	154.783	25.797
704-01-021-0	DH511_4AVH_4A	DH511_3CVK	274.885	45.811
704-01-021-0	DH511_4AVH_4A	DH511_5AVK	85.743	10.957
704-01-021-0	DH511_4AVH_4A	K510032 (DH512)	142.347	23.725
704-01-021-0	DH511_4B\H_4A	DH511_1AVK	72.442	12.074
704-01-021-0	DH511_4B\H_4A	DH511_2AVK	152.552	25.425
704-01-021-0	DH511_4B\H_4A	DH511_3AVK	154.118	25.688
704-01-021-0	DH511_4B\H_4A	DH511_3BVK	12.751	2.125
704-01-021-0	DH511_4B\H_4A	DH511_3CVK	185.783	30.984
704-01-021-0	DH511_4B\H_4A	DH511_5AVK	24.781	4.132
704-01-021-0	DH511_4B\H_4A	K510032 (DH512)	139.712	23.285
704-01-021-0	DH511_4C\H_4A	DH511_1AVK	123.445	20.574
704-01-021-0	DH511_4C\H_4A	DH511_2AVK	169.294	28.216
704-01-021-0	DH511_4C\H_4A	DH511_3AVK	253.605	42.268
704-01-021-0	DH511_4C\H_4A	DH511_3BVK	451.084	75.181
704-01-021-0	DH511_4C\H_4A	DH511_3CVK	444.052	74.009
704-01-021-0	DH511_4C\H_4A	DH511_5AVK	93.456	15.576
704-01-021-0	DH511_4C\H_4A	K510032 (DH512)	181.158	30.193
704-01-021-0	DH511_2C\H_4A	DH511_2AVK	2.124	0.354
704-01-021-0	DH511_3AVH_4A	DH511_3AVK	78.025	13.004
704-01-021-0	DH511_3AVH_4A	DH511_3BVK	48.033	8.006
704-01-021-0	DH511_3AVH_4A	DH511_3CVK	182.475	30.413
704-01-021-0	DH511_4AVH_4A	DH511_4A4C\K	3.868	0.681
704-01-021-0	DH511_4AVH_4A	DH511_4BVK	11.367	1.895
704-01-021-0	DH511_4B\H_4A	DH511_4A4C\K	0.818	0.103
704-01-021-0	DH511_4B\H_4A	DH511_4BVK	2.234	0.372
704-01-021-0	DH511_4C\H_4A	DH511_4A4C\K	4.813	0.802
704-01-021-0	DH511_4C\H_4A	DH511_4BVK	12.499	2.083
704-01-021-0	H510049_4A (DH512)	K510032 (DH512)	119.527	19.521
Positive Control	HV13221		87.848	14.841
Mock Transfection			0.003	0.001
10E 8				
2F5				

Figure 56B

	IC 50 (µg/ml)	IC 80 (µg/ml)	IC 50 (µg/ml)	IC 80 (µg/ml)
PTID	BG1168	BG1168	SVA	SVA
704-01-021-0	>0.419	>0.419	>0.419	>0.419
704-01-021-0	>2.137	>2.137	>2.137	>2.137
704-01-021-0	>0.066	>0.066	>0.066	>0.066
704-01-021-0	>2.131	>2.131	>2.131	>2.131
704-01-021-0	>0.052	>0.052	>0.052	>0.052
704-01-021-0	>0.082	>0.082	>0.082	>0.082
704-01-021-0	>0.834	>0.834	>0.834	>0.834
704-01-021-0	>0.304	>0.304	>0.304	>0.304
704-01-021-0	3.238	14.836	>18.967	>18.967
704-01-021-0	0.774	3.790	>13.357	>13.357
704-01-021-0	>0.071	>0.071	>0.071	>0.071
704-01-021-0	>0.221	>0.221	>0.221	>0.221
704-01-021-0	2.433	>5.91	>5.91	>5.91
704-01-021-0	6.858	>20.532	>20.532	>20.532
704-01-021-0	0.958	4.132	>14.82	>14.82
704-01-021-0	1.396	5.769	>16.762	>16.762
704-01-021-0	1.731	7.303	>28.921	>28.921
704-01-021-0	1.428	5.567	>25.797	>25.797
704-01-021-0	1.305	4.523	>45.811	>45.811
704-01-021-0	0.71	4.336	>10.957	>10.957
704-01-021-0	1.731	7.428	>23.725	>23.725
704-01-021-0	0.913	5.042	>12.074	>12.074
704-01-021-0	0.48	1.495	>25.425	>25.425
704-01-021-0	0.817	3.173	>25.686	>25.686
704-01-021-0	0.673	>2.125	>2.125	>2.125
704-01-021-0	1.135	4.096	>30.964	>30.964
704-01-021-0	1.213	>4.132	>4.132	>4.132
704-01-021-0	1.312	6.339	>23.285	>23.285
704-01-021-0	10.673	>20.574	>20.574	>20.574
704-01-021-0	3.408	>28.216	>28.216	>28.216
704-01-021-0	8.719	>42.268	>42.268	>42.268
704-01-021-0	9.393	50.853	>75.181	>75.181
704-01-021-0	5.180	29.485	>74.009	>74.009
704-01-021-0	11.992	>15.576	>15.576	>15.576
704-01-021-0	10.671	>30.193	>30.193	>30.193
704-01-021-0	>0.354	>0.354	>0.354	>0.354
704-01-021-0	1.277	3.565	>13.004	>13.004
704-01-021-0	1.928	5.549	>8.006	>8.006
704-01-021-0	0.899	3.789	>30.413	>30.413
704-01-021-0	>0.661	>0.661	>0.661	>0.661
704-01-021-0	>1.895	>1.895	>1.895	>1.895
704-01-021-0	>0.103	>0.103	>0.103	>0.103
704-01-021-0	>0.372	>0.372	>0.372	>0.372
704-01-021-0	>0.802	>0.802	>0.802	>0.802
704-01-021-0	>2.083	>2.083	>2.083	>2.083
704-01-021-0	0.725	2.441	>19.921	>19.921
Positive Control	3.686	>14.641	>14.641	>14.641
Mock Transfection	>0.001	>0.001	>0.001	>0.001
10E 8	0.262	0.779	>50	>50
2F5	0.888	4.793	>50	>50

Figure 56B cont.

PTID	Heavy ID	Light ID	IgG Quant ($\mu\text{g/ml}$)	1:6 Initial Dilution ($\mu\text{g/ml}$)
704-01-021-0	DH511_5AVH_4A	DH511_1AVK	199.465	33.24
704-01-021-0	DH511_5AVH_4A	DH511_2AVK	218.318	38.39
704-01-021-0	DH511_5AVH_4A	DH511_3AVK	427.911	71.32
704-01-021-0	DH511_5AVH_4A	DH511_3BVK	333.05	55.51
704-01-021-0	DH511_5AVH_4A	DH511_3CVK	134.35	22.39
704-01-021-0	DH511_5AVH_4A	DH511_4A4CVK	17.539	2.92
704-01-021-0	DH511_5AVH_4A	DH511_4BVK	80.406	13.40
704-01-021-0	DH511_5AVH_4A	K510032 (DH512)	155.052	25.84
704-01-021-0	DH511_5AVH_4A	DH511_5AVK	178.536	29.76
704-01-021-0	DH511_5BVH_4A	DH511_1AVK	18.997	3.17
704-01-021-0	DH511_5BVH_4A	DH511_2AVK	11.844	1.97
704-01-021-0	DH511_5BVH_4A	DH511_3AVK	189.142	28.19
704-01-021-0	DH511_5BVH_4A	DH511_3BVK	2.571	0.43
704-01-021-0	DH511_5BVH_4A	DH511_3CVK	111.504	18.58
704-01-021-0	DH511_5BVH_4A	DH511_4A4CVK	2.187	0.36
704-01-021-0	DH511_5BVH_4A	DH511_4BVK	15.98	2.66
704-01-021-0	DH511_5BVH_4A	K510032 (DH512)	50.982	8.50
704-01-021-0	DH511_5BVH_4A	DH511_5AVK	204.937	34.16
704-01-021-0	H510049_4A (DH512)	DH511_1AVK	130.55	21.76
704-01-021-0	H510049_4A (DH512)	DH511_2AVK	145.575	24.26
704-01-021-0	H510049_4A (DH512)	DH511_3AVK	597.214	99.54
704-01-021-0	H510049_4A (DH512)	DH511_3BVK	1072.598	178.77
704-01-021-0	H510049_4A (DH512)	DH511_3CVK	646.48	107.75
704-01-021-0	H510049_4A (DH512)	DH511_4A4CVK	3.676	0.61
704-01-021-0	H510049_4A (DH512)	DH511_4BVK	14.861	2.48
704-01-021-0	H510049_4A (DH512)	DH511_5AVK	156.182	26.03
704-01-021-0	H510049_4A (DH512)	K510032 (DH512)	188.551	31.43
Positive Control	HV13221	HV13501	100.04	18.67
Mock Transfection			0.026	0.0043
Ab ID				
DH512 (from 20151022)	H510049_4A (DH512)	K510032 (DH512)	50	NA
10E8 (from 20151022)			50	NA

Figure S6C

	IC 50<50 µg/ml	IC 80<50 µg/ml	IC 50<50 µg/ml	IC 80<50 µg/ml
PTID	BG1168	BG1168	SVA	SVA
704-01-021-0	31.35	>33.24	>33.24	>33.24
704-01-021-0	8.322	>36.39	>36.39	>36.39
704-01-021-0	>71.32	>71.32	>71.32	>71.32
704-01-021-0	>55.51	>55.51	>55.51	>55.51
704-01-021-0	>22.39	>22.39	>22.39	>22.39
704-01-021-0	>2.92	>2.92	>2.92	>2.92
704-01-021-0	>13.4	>13.4	>13.4	>13.4
704-01-021-0	11.195	>25.84	>25.84	>25.84
704-01-021-0	0.963	4.494	>29.76	>29.76
704-01-021-0	>3.17	>3.17	>3.17	>3.17
704-01-021-0	>1.97	>1.97	>1.97	>1.97
704-01-021-0	>28.19	>28.19	>28.19	>28.19
704-01-021-0	>0.43	>0.43	>0.43	>0.43
704-01-021-0	>18.58	>18.58	>18.58	>18.58
704-01-021-0	>0.36	>0.36	>0.36	>0.36
704-01-021-0	>2.66	>2.66	>2.66	>2.66
704-01-021-0	>8.5	>8.5	>8.5	>8.5
704-01-021-0	1.145	5.634	>34.16	>34.16
704-01-021-0	0.699	3.225	>21.76	>21.76
704-01-021-0	0.258	0.886	>24.26	>24.26
704-01-021-0	1.846	9.286	>99.54	>99.54
704-01-021-0	3.414	13.355	>178.77	>178.77
704-01-021-0	2.115	7.925	>107.75	>107.75
704-01-021-0	>0.61	>0.61	>0.61	>0.61
704-01-021-0	>2.48	>2.48	>2.48	>2.48
704-01-021-0	0.355	1.243	>26.03	>26.03
704-01-021-0	1.602	6.039	>31.43	>31.43
Positive Control	3.674	14.218	>16.67	>16.67
Mock Transfection	>0	>0	>0	>0
Ab ID				
DH512 (from 20151022)	0.174	0.813	>50	>50
10E 8 (from 20151022)	0.099	0.433	>50	>50

Figure 56C cont.

Description	Antibody ID	Heavy ID	Light ID	IgG Quant (μ g/ml)
MBC-derived	DH512	DH512_VH	DH512_VK	188.551
Chimeric mAb	DH512_K2	DH512_VH	DH511_1AVK	130.55
Chimeric mAb	DH512_K3	DH512_VH	DH511_2AVK	145.575
Chimeric mAb	DH512_K4	DH512_VH	DH511_5AVK	156.132
Chimeric mAb	DH511_2b_K2	DH511_2BVH	DH511_1AVK	67.45
Chimeric mAb	DH511_3a_K2	DH511_3AVH	DH511_2AVK	80.144
Chimeric mAb	DH511_4a_K2	DH511_4AVH	DH511_5AVK	65.743
Chimeric mAb	DH511_4b_K2	DH511_4BVH	DH511_2AVK	152.552
Chimeric mAb	DH511_4b_K3	DH511_4BVH	DH511_3BVK	12.751

MBC, memory B cell

Figure 56D

Key (IC50/IC80 in µg/ml)

		<0.1	>0.1-1.0	>1.0-10.0	>10.0
Antibody ID	1:6 Initial Dilution (µg/ml)	IC50 (µg/ml)	IC80 (µg/ml)	IC50 (µg/ml)	IC80 (µg/ml)
		BG1168	BG1168	SVA	SVA
DH512	31.43	1.602	6.039	>31.43	>31.43
DH512_K2	21.76	0.699	3.225	>21.76	>21.76
DH512_K3	24.26	0.258	0.886	>24.26	>24.26
DH512_K4	26.03	0.355	1.243	>26.03	>26.03
DH511_2b_K2	11.24	0.513	2.523	>11.24	>11.24
DH511_3a_K2	13.36	0.774	3.790	>13.357	>13.357
DH511_4a_K2	10.96	0.710	4.336	>10.957	>10.957
DH511_4b_K2	25.43	0.480	1.495	>25.425	>25.425
DH511_4b_K3	2.13	0.673	>2.125	>2.125	>2.125

||

Figure 56D cont.

Note that for the AbDH511 series of antibodies, multiple heavy chains will be paired with different light chains and vice-versa. Eleven unique heavy chain and eight unique light chains will be ordered for this antibody series.

Raw Sequences in fasta format

Heavy Chain Sequences

```
>DH511_1AVH_4A
CAGGTCCAGCTGGTACAGTCTGGGGGAGCCCTGGTAAAGCCCGGGGGGGCCCTTAGACTCTCCTGTGA
AGCCTCTGGATTCACTTTTCAGCGATACGTGGATGAGCTGGGTCCGCCATCTTCCCGGGAAGGGACTGG
AGTGGATTGGCCGCATTAGAAGGACCACTGATGGTGGGACAACAGAATACGCTTCACCCGTGAAAGGC
AGATTCACCATCTCAAGAGACGATTCAAGAAACACGCTGTATCTGGAAATGAGTGGCCTGAGAATCGA
CGACACAGCGGTGTATTATTGTACCGCTGATCGGGGGGGCCCCAGTCTTACGTTTTTGGGAGTGGGGCT
ACTATGACTACTACATGGAGTTCTGGGGCAGAGGGACCTCGGTCACCGTCTCCTCA
```

```
>DH511_1BVH_4A

CAGGTGCAGCTGGTGGAATCTGGGGGAGCCCTGGTAAAGCCCGGGGGGGCCCTTAGACTCTCCTGTGA
AGCCTCTGGATTCACTTTTCAGCGATACGTGGATGAGCTGGGTCCGCCATCTTCCCGGGAAGGGACTGG
AGTGGATTGGCCGCATTAGAAGGACCACTGATGGTGGGACAACAGAATACGCTTCACCCGTGAAAGGC
AGATTCACCATCTCAAGAGACGATTCAAGAAACACGCTGTATCTGGAAATGAGTGGCCTGAGAATCGA
CGACACAGCAGTGTATTATTGTACCGCTGATCGGGGGGGCCCCAGTCTTACGTTTTTGGGAGTGGGGCT
ACTATGACTACTACATGGAGTTCTGGGGCAGAGGGACCTCGGTCACCGTCTCCTCA
```

```
>DH511_2AVH_4A
CAGGTGCAGCTGGTGGAGTCTGGGGGAGCCCTGGTAAAGCCCGGGGGGGCCCTTAGACTCTCCTGTGA
AGCCTCCGGATTCCCTTTTCAGCGCTACCTGGATGAGCTGGGTCCGCCATCTCCCCGGAAGGGACTGG
AGTGGATTGGCCGCATTAGAGCGACCACTAATGGTGGGACAACAGAATACGCTTCACCCGTGAAAGGC
AGATTCACCATCTCAAGGGACGATTCAAGAAACACACTGTATCTGGAGATGAGTGGCCTGAAAATCGA
GGACACTGCCGTGTATTATTGTACCGCTGATCGGGGGGGCCCCAGTCTTACGTTTTTGGGAGTGGGGCT
ATTTTGACTACTACATGGAGTTCTGGGGCAAAGGGACCTCGGTCACCGTCTCCTCA
```

```
>DH511_2BVH_4A
CAGGTGCAGCTGGTGCAATCTGGGGGAGCCCTGGTAAAGCCCGGGGGGGCCCTTAGACTCTCCTGTGA
AGCCTCCGGATTCCCTTTTCAGCGCTACCTGGATGAGCTGGGTCCGCCATCTCCCCGGAAGGGACTGG
AGTGGATTGGCCGCATTAGAGCGACCACTAATGGTGGGACAACAGAATACGCTTCACCCGTGAAAGGC
AGATTCACCATCTCAAGGGACGATTCAAGAAACACACTGTATCTGGAGATGAGTGGCCTGAAAATCGA
GGACACTGCCGTGTATTATTGTACCGCTGATCGGGGGGGCCCCAGTCTTACGTTTTTGGGAGTGGGGCT
ATTTTGACTACTACATGGAGTTCTGGGGCAAAGGGACCTCGGTCACCGTCTCCTCA
```

Figure 57

>DH511_2CVH_4A

GAAGTGCAGCTGGTGGAGTCTGGGGGAGCCCTGGTAAAGCCCGGGGGGGCCCTTAGACCCCTCCTGTGA
AGCCTCCGATTCCCTTTTCAGCGCTACCTGGATGAGCTGGGTCCGCCATCTCCCCGAAAGGGACTGG
AGTGGATTGGCCGCATTAGAGCGACCACTAATGGTGGGACAACAGAATACGCTTCACCCGTGAAAGGC
AGATTCACAATCTCAAGGGACGATTCAAGAAACACACTGTATCTGGAGATGAGTGGCCTGAAAATCGA
GGACACTGCCGTGTATTATTGTACCGCTGATCGGGGGGGCCCCAGTCTTACGTTTTTGGGAGTGGGGCT
ATTTTGACTACTACATGGAGTTCTGGGGCAAAGGGACCTCGGTCACCGTCTCCTCA

>DH511_3AVH_4A

GAGGTGCAGTTGGTGGAGTCTGGGGGCGGCTTGGTGAAGGCGGGAGAGAGAGTCCACAGTCTCCTGTGA
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AATGGGTTGGTCGTATAAGAAGAGTAAAGACGGTGCAGACGACAGAATATGGTGTACCCGTGAAGGGA
AGATTCACCATCTCAAGGGATGACTCAAAGAACACAGTGTATCTACACATGAATAACCTGAAAATCGA
AGACACAGGTGTATATTATTGTACTAGAGATGAGGGGGGGCCCCAGTTACACGACGGTTTTCTGGAGTGGG
GCTACTTCTACTATTACATGGCCGTCTGGGGCAGAGGGACAACGGTCACCGTCTCTCCA

>DH511_4AVH_4A

GAGGTGCAGCTGGTGCAGTCTGGGGGCGGCTTGGTAAAGCCGGGACAGTCAGTCACACTTTCCTGTGT
GGGCTTTGGATTCAATTTTCGCTAATGACTGGATGGGCTGGGTCCGCCAGGCTCCAGGGAAGGGACTGG
AATGGGTTGGTCGTATAAGGAGACTAAAAGATGGTGCAGAAAGCGGAATATGGATCATCCGTGAAGGGT
AGATTCACCATCTCAAGAGATGATTCAAGAAACACGCTATATTTGCACATGAATAGCCTCAAGGTCTGA
AGACACAGCCGTCTATTATTGTACTCGAGACGAGGGGGGGCCCCAGTTACCCGATTTCTGGAGTGGGGCT
CCTATTACTACTACATGGCCGTCTGGGGCAGAGGGACCACGGTCATCGTCTCTTCA

>DH511_4BVH_4A

CAGGTGCAGCTGGTGCAATCTGGGGGCGGCTTGGTAAAGCCGGGACAGTCAGTCACACCTTCCTGTGT
GGGCTTTGGATTCAATTTTCGCTAATGACTGGATGGGCTGGGTCCGCCAGGCTCCAGGGAAGGGACTGG
AATGGGTTGGTCGTATAAGGAGACTAAAAGATGGTGCAGAAAGCGGAATATGGATCATCCGTGAAGGGT
AGATTCACCATCTCAAGAGATGATTCAAGAAACACGCTATATTTGCACATGAATAGCCTCAAGGTCTGA
AGACACAGCCGTCTATTATTGTACTCGAGACGAGGGGGGGCCCCAGTTACCCGATTTCTGGAGTGGGGCT
CCTATTACTACTACATGGCCGTCTGGGGCAGAGGGACCACGGTCATCGTCTCTTCA

>DH511_4CVH_4A

CAGGTCCAGCTTGTACAGTCTGGGGGCGGCTTGGTAGAGCCGGGACAGTCAGTCACACTTTCCTGTGT
GGGCTTTGGATTCAATTTTCGCTAATGACTGGATGGGCTGGGTCCGCCAGGCTCCAGGGAAGGGACTGG
AATGGGTTGGTCGTATAAGGAGACTAAAAGATGGTGCAGAAAGCGGAATATGGATCATCCGTGAAGGGT
AGATTCACCATCTCAAGAGATGATTCAAGAAACACGCTATATTTGCACATGAATAGCCTCAAGGTCTGA
AGACACAGCCGTCTATTATTGTACTCGAGACGAGGGGGGGCCCCAGTTACCCGACTTCTGGAGTGGGGCT
CCTATTACTACTACATGGCCGTCTGGGGCAGAGGGACCACGGTCATCGTCTCTTCA

>DH511_5AVH_4A

CAGGTCCAGCTGGTACAGTCTGGGGGAGGCTTGGTAAAGCCTGGGGGGTCCCTTACACTCTCCTGTGT
CACCTCTGGATTTACTTTTCAGCAACACGTGGATGAGTTGGGTCCGCCAGACTCCAGGGAAGGGACTGG
AGTGGGTTGCCCGTATTAGTAGGGTCGGGGATGGCCCAATAATAGACTACGCTGCTCCCGTGAAAGGC
AGATTCATAATCTCAAGAGATGACTCAAGAAACACACTCTTTCTTCACATGAACAACCTGAAAACCGA
GGACACAGCCGTGTATTATTGTACCGCTGATGAGGGGGGGCCCCAATTTTAAGATTTTTTGAGTGGGGTT
ATTACAATACTACTACATGGACGTCTGGGGCAAAGGGGACCACGGTCATCGTCTCTCTCG

FIGURE 57 Cont

>DH511_5BVH_4A

CAGGTCCAGCTTGTACAGTCTGGGGGAGGCTTGGTAAAGCCTGGGGGGTCCCTTACACCCCTCCTGTGT
CACCTCTGGATTTACTTTTCAGCAACACGTGGATGAGTTGGGTCCGCCAGACTCCAGGGAAGGGACTGG
AGTGGGTTGCCCCGATTAGTAGGGTCGGGGATGGCCCAATAATAGACTACGCTGCTCCCGTGAAAGGC
AGATTCATAATCTCAAGAGATGACTCAAGAAACACACTCTTCTTCACATGAACAACCTGAAAACCGA
GGACACAGCCGTGTATTATTGTACCGCTGATGAGGGGGCCCCAATTTTAAGATTTTTTTGAGTGGGGTT
ATTACAATACTACTACATGGACGTCTGGGGCAAGGGGACCACGGTCATCGTCTCCTCG

Kappa Chain Sequences

>DH511_1AVK

GACATCCAGATCACGCAGTCTCCGTCTTTCTGTACGGCTCTGTAGGCGATAGGGTCACCATCACTTG
CCGGGCAAGTCAGAATATTAAGGACTATTTAAATTGGTATCAGCAGAGACCAGGGAGAGCCCCCTAGAC
TCCTGGTCTATGCTGCATCCAATTTGCAAAGTGGGGTCCCGTCAAGGTTCAGTGGCAGTGGATATGGG
ACAGACTTCACTCTCATCATCAGCAGTCTGCAACCTGAGGACTTTGCGACTTACTACTGTCAAGAGAG
TTATAGTTCCACGCCACACACACCTTTGGCCTGGGGACCAAATTGGAGATGAAA

>DH511_2AVK

GACATCCAGATGACCCAGTCTCCGTCTTTCTGTACGGCTCTGTAGGCGATAGAGTCACCATCACTTG
CCGGGCAAGTCAGAATATTAAGGACTATTTAAATTGGTATCAGCAGAGACCAGGGAGAGCCCCCTAGAC
TCCTGATCTATGCTGCATCCAATTTGCAAAGTGGGGTCCCGTCAAGGTTCAGTGGCAGTGGATATGGG
ACAGACTTTACTCTCATCATCAGCAGTCTGCAACCTGAGGACTTTGCGACTTATTTCTGTCAAGAGAG
TTATAGTTCTACGCCACACACATTTTTGGCCTGGGGACCAAATTGGAGAAGAAA

>DH511_3AVK

GACATCCAGATGACCCAGTTTCCATCCTCCCTGTCTGCATCTGTGGGAGACAGAGTCACCATGACTTG
CCGGGCAAGTCAGAGCATTAAAGGACTATTTAAATTGGTATCAACACACCCCCGGGGAAGGCCCTCGAC
TCCTGATTTATGGTGCGACGACTTTACAGAGAGGGGTCCCATCAAGATTCAGTGGCAGTGGGTCTGGG
AACCAATTCACTCTCACCATTAACAGTCTGCAACCAGAAGATTTTGCAACTTATTATTGTCAAGAGAG
TTACCAGACCGTTCCACACTCACCTTTGGTCCGGGGACCAGGGTGGACAGGAAG

>DH511_3BVK

GACATCCAGATGACTCAGCCTCCATCCTCCCTGTCTGCATCTGTGGGAGACAGAGTCACCATGACTTG
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TCCTGATTTATGGTGCGACGACTTTACAGAGAGGGGTCCCATCAAGATTCAGTGGCAGTGGGTCTGGG
AACCAATTCACTCTCACCATTAACAGTCTGCAACCAGAAGATTTTGCAACTTATTATTGTCAAGAGAG
TTACCAGACCGTTCCACACTCACCTTTGGTCCGGGGACCAGGGTGGACAGGAAG

>DH511_3CVK

GACATCCGGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTGGGAGACAGAGTCACCATGACTTG
CCGGGCAAGTCAGAGCATTAAAGGACTATTTAAATTGGTATCAACACACCCCCGGGGAAGGCCCTCGAC
TCCTGATTTATGGTGCGACGACTTTACAGAGAGGGGTCCCATCAAGATTCAGTGGCAGTGGGTCTGGG
AACCAATTCACTCTCACCATTAACAGTCTGCAACCAGAAGATTTTGCAACTTATTATTGTCAAGAGAG
TTACCAGACCGTTCCACACTCACCTTTGGTCCGGGGACCAGGGTGGACAGGAAG

FIGURE 57 Cont

>DH511_4A4CVK

GACATCCAGGTGACCCAGTCTCCAACCTCTCTGTCTGCATCTGTAGGAGACACAGTCACTATCACTTG
CCGGGCAAGTCAGAGCATTAAAAATTATGTAAATTGGTATCAACACAAATCCGGGAGCGCCCCCTAGAC
TCCTGATTTATGCTGCGTCAGCCTTACATAGTGGGATCCCGTCAAGGTTCACTGGCAGTGGGTCTGGG
ACACAGTTCACTCTCACCATTAACAGTCTGCAACCTGAAGATTTTGCAACTTATTATTGTCAAGAGGC
TTATAACACCAACCCACACTCTCCTTTGGTCAGGGGACCAGGGTGGACAGGAAA

>DH511_4BVK

GACATCCAGATGACACAGTTTCCAACCTCTCTGTCTGCATCTGTAGGAGACACCGTCACTATCACTTG
CCGGGCAAGTCAGAGCATTAAAAATTATGTAAATTGGTATCAACACAAATCCGGGAGCGCCCCCTAGAC
TCCTGATTTATGCTGCGTCAGCCTTACATAGTGGGATCCCGTCAAGGTTCACTGGCAGTGGGTCTGGG
ACACAGTTCACTCTCACCATTAACAGTCTGCAACCTGAAGATTTTGCAACTTATTATTGTCAAGAGGC
TTATAACACCAACCCACACTCTCCTTTGGTCAGGGGACCAGGGTGGACAGGAAA

>DH511_5AVK

GACATCCGGTTGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTTGGGGACAGAATCACCATCACTTG
TCGGGCCAGCCAAAGCATTAAAGACTATTTAAATTGGTATAAACACCGGCCAGGGGAAGCCCCCAAAC
TCCTCATTTATTCTGCATCCAAGTTGAGAAGTGGGGTCTCATCAAGGTTCACTGGCAGTGGATATGGG
TCGGCCTTCACACTGACCATCAGCAGTCTGCAGCCTGAAGATTTTGCGACTTATTATTGTCAAGAGAG
TTACAGCAGCGTTCCCATGTACATTTTCGGCCAGGGGACCAAGGTGGACCTCAA

Translation of sequences- V(D)J only- in fasta format

Heavy Chain Sequences

>DH511_1AVH_4A

QVQLVQSGGALVKPGGALRLSCEASGFTFSDTWMSWVRHLPKGLEWIGRIRRTTDGGTTEYASPVK
GRFTISRDDSRNTLYLEMSGLRIDDTAVYYCTADRGAPVLRFEWGYDYDYMEFWGRGTSVTVSS

>DH511_1BVH_4A

QVQLVESGGALVKPGGALRLSCEASGFTFSDTWMSWVRHLPKGLEWIGRIRRTTDGGTTEYASPVK
GRFTISRDDSRNTLYLEMSGLRIDDTAVYYCTADRGAPVLRFEWGYDYDYMEFWGRGTSVTVSS

>DH511_2AVH_4A

QVQLVESGGALVKPGGALRLSCEASGFPFSATWMSWVRHLPKGLEWIGRIRATTNGGTTEYASPVK
GRFTISRDDSRNTLYLEMSGLKIEDTAVYYCTADRGAPVLRFEWGYFDYDYMEFWGKGTSVTVSS

FIGURE 57 Cont

>DH511_2BHV_4A

QVQLVQSGGALVKPGGALRLSCEASGFPFSATWMSWVRHLPKGKLEWIGRIRATTNGGTTEYASPVK
GRFTISRDDSRNTLYLEMSGLKIEDTAVYYCTADRGAPVLRFEWGYFDYYMEFWGKGTSTVTVSS

>DH511_2CVH_4A

EVQLVESGGALVKPGGALRPSCEASGFPFSATWMSWVRHLPKGKLEWIGRIRATTNGGTTEYASPVK
GRFTISRDDSRNTLYLEMSGLKIEDTAVYYCTADRGAPVLRFEWGYFDYYMEFWGKGTSTVTVSS

>DH511_3AVH_4A

EVQLVESGGGLVKAGERVTVSCEGYGFRFDDDMGWVRQAPGRGLEWVGRIRRVKDGAATTEYGVFVK
GRFTISRDDSKNTVYLHMNNLKIEDTGYYCTRDEGAPVTRRFLEWGYFYYYMAVWGRGTTVTVPSP

>DH511_4AVH_4A

EVQLVQSGGGLVKPGQSVTLSCVGFGFNFANDWMGWVRQAPGKLEWVGRIRRLKDGAKEYGSSVK
GRFTISRDDSRNTLYLHMNSLKVEDTAVYYCTRDEGAPVTRFLEWGSYYYYMAVWGRGTTVIVSS

>DH511_4BVH_4A

QVQLVQSGGGLVKPGQSVTPSCVGFGFNFANDWMGWVRQAPGKLEWVGRIRRLKDGAKEYGSSVK
GRFTISRDDSRNTLYLHMNSLKVEDTAVYYCTRDEGAPVTRFLEWGSYYYYMAVWGRGTTVIVSS

>DH511_4CVH_4A

QVQLVQSGGGLVEPGQSVTLSCVGFGFNFANDWMGWVRQAPGKLEWVGRIRRLKDGAKEYGSSVK
GRFTISRDDSRNTLYLHMNSLKVEDTAVYYCTRDEGAPVTRLLEWGSYYYYMAVWGRGTTVIVSS

>DH511_5AVH_4A

QVQLVQSGGGLVKPGGSLTLSCVTSGFTFSNTWMSWVRQTPGKLEWVARISRVGDGPIIDYAAPVK
GRFIISRDDSRNTLFLHMNNLKTEDTAVYYCTADEGAPILRFFEWGYNNYMDVWGKGTTVIVSS

>DH511_5BVH_4A

QVQLVQSGGGLVKPGGSLTPSCVTSGFTFSNTWMSWVRQTPGKLEWVARISRVGDGPIIDYAAPVK
GRFIISRDDSRNTLFLHMNNLKTEDTAVYYCTADEGAPILRFFEWGYNNYMDVWGKGTTVIVSS

FIGURE 57 Cont

Kappa Chain Sequences

>DH511_1AVK

DIQITQSPSFLYGSVGDRVTITCRASQNIKDYLNWYQQRPGRAPRLLVYAASNLSQSGVPSRFSGSGY
GTDFTLIISLQPEDFATYYCQESYSSTPTHFTFGLGTKLEMK

>DH511_2AVK

DIQMTQSPSFLYGSVGDRVTITCRASQNIKDYLNWYQQRPGRAPRLLIYAASNLSQSGVPSRFSGSGY
GTDFTLIISLQPEDFATYFCQESYSSTPTHIFGLGTKLEKK

>DH511_3AVK

DIQMTQFPSSLSASVGDRVTMTCRASQSIKDYLNWYQHTPGKAPRLLIYGATTLQRGVPSRFSGSGS
GNQFTLTINSLQPEDFATYYCQESYQTVPTLTFGPGTRVDRK

>DH511_3BVK

DIQMTQPPSSLSASVGDRVTMTCRASQSIKDYLNWYQHTPGKAPRLLIYGATTLQRGVPSRFSGSGS
GNQFTLTINSLQPEDFATYYCQESYQTVPTLTFGPGTRVDRK

>DH511_3CVK

DIRMTQSPSSLSASVGDRVTMTCRASQSIKDYLNWYQHTPGKAPRLLIYGATTLQRGVPSRFSGSGS
GNQFTLTINSLQPEDFATYYCQESYQTVPTLTFGPGTRVDRK

>DH511_4A4CVK

DIQVTQSPTSLSASVGDTVITITCRASQSIKNYVNWYQHKSGSAPRLLIYAASALHSGIPSRFTGSGS
GTQFTLTINSLQPEDFATYYCQEAYNTNPTLSFGQGTRVDRK

>DH511_4BVK

DIQMTQFPTSLSASVGDTVITITCRASQSIKNYVNWYQHKSGSAPRLLIYAASALHSGIPSRFTGSGS
GTQFTLTINSLQPEDFATYYCQEAYNTNPTLSFGQGTRVDRK

>DH511_5AVK

DIRLTQSPSSLSASVGDRITITCRASQSIKDYLNWYKHRPGEAPKLLIYSASKLRSGVSSRFSGSGYGSFTL
TISSLQPEDFATYYCQESYSSVPMYIFGQGTKVDLK**FIGURE 57 Cont**

Target gene: pDH511_5AVH_4A was made previously as construct #15ACIZXC under Order#:29691872 SQ

>pDH511_5AVH_4A

GCTAGCACCATGGAGACAGACACACTCCTGCTATGGGTAAGTGTGCTCTGGGTTCCAGGTTCCACTGG
TGACCAGGTCCAGCTGGTACAGTCTGGGGGAGGCTTGGTAAAGCCTGGGGGGTCCCTTACACTCTCCT
GTGTCACCTCTGGATTTACTTTTTCAGCAACACGTGGATGAGTTGGGTCCGCCAGACTCCAGGGAAGGGA
CTGGAGTGGGTTGCCCCGTATTAGTAGGGTCGGGGATGGCCCAATAATAGACTACGCTGCTCCCGTGAA
AGGCAGATTTCATAATCTCAAGAGATGACTCAAGAAACACACTCTTTCTTCACATGAACAACCTGAAAA
CCGAGGACACAGCCGTGTATTATTGTACCGCTGATGAGGGGGCCCCAATTTTAAGATTTTTTGAGTGG
GGTTATTACAACACTACTACATGGACGTCTGGGGCAAGGGGACCACGGTCATCGTCTCCTCGGCGTCGAC

Task 1: Subclone the pDH511_5AVH_4A into **HV1301089FabH** at NheI-SalI sites (remove the old insert), The resulting product should be called as **pDH511_5AFabH** that has the gene insert sequence between NheI-XbaI sites in pcDNA3.1(+)/hygromycin backbone as shown below.

>pDH511_5AFabH

GCTAGCACCATGGAGACAGACACACTCCTGCTATGGGTAAGTGTGCTCTGGGTTCCAGGTTCCACTGG
TGACCAGGTCCAGCTGGTACAGTCTGGGGGAGGCTTGGTAAAGCCTGGGGGGTCCCTTACACTCTCCT
GTGTCACCTCTGGATTTACTTTTTCAGCAACACGTGGATGAGTTGGGTCCGCCAGACTCCAGGGAAGGGA
CTGGAGTGGGTTGCCCCGTATTAGTAGGGTCGGGGATGGCCCAATAATAGACTACGCTGCTCCCGTGAA
AGGCAGATTTCATAATCTCAAGAGATGACTCAAGAAACACACTCTTTCTTCACATGAACAACCTGAAAA
CCGAGGACACAGCCGTGTATTATTGTACCGCTGATGAGGGGGCCCCAATTTTAAGATTTTTTGAGTGG
GGTTATTACAACACTACTACATGGACGTCTGGGGCAAGGGGACCACGGTCATCGTCTCCTCGGCGTCGAC
CAAGGGGCCCATGGGTCCTTCCCCCTGGCACCCCTCTTCCAAAGAGCACCTCTGGGGGGCACAGCGGCCCTGG
GCTGGCCTGGTCAAGGACTACTTCCCGGAACCGGTGACGGTGTGTGGAACTCAGGCGCCCTGACCCAGC
GGGCTSCACACCTTCCCGGTGTCTCTACAGTCTCTCAGGACTCTACTCCCTCAGCAGCGTGGTGGACCGT
GCCCCCAGCAGCTTGGGTTACCCAGACCTACATCTGCAACGTGAATCACAAAGCCAGCAACACCAAGG
TGGACAAGAGAGTTGAGCCCAAAATCTTGTGACAAATGATCTAGA

Target gene: pDH511_5BVH_4A was made previously as construct# **15ACIZYC** under the Order ID: **29691872 SQ**

>pDH511_5BVH_4A

GCTAGCACCATGGAGACAGACACACTCCTGCTATGGGTAAGTGTGCTCTGGGTTCCAGGTTCCACTGG
TGACCAGGTCCAGCTTGTACAGTCTGGGGGAGGCTTGGTAAAGCCTGGGGGGTCCCTTACACCCTCCT
GTGTCACCTCTGGATTTACTTTTTCAGCAACACGTGGATGAGTTGGGTCCGCCAGACTCCAGGGAAGGGA
CTGGAGTGGGTTGCCCCGTATTAGTAGGGTCGGGGATGGCCCAATAATAGACTACGCTGCTCCCGTGAA
AGGCAGATTTCATAATCTCAAGAGATGACTCAAGAAACACACTCTTTCTTCACATGAACAACCTGAAAA
CCGAGGACACAGCCGTGTATTATTGTACCGCTGATGAGGGGGCCCCAATTTTAAGATTTTTTGAGTGG
GGTTATTACAACACTACTACATGGACGTCTGGGGCAAGGGGACCACGGTCATCGTCTCCTCGGCGTCGAC

Figure 58

Task 2: Subclone the pDH511_5BVH_4A into **HV1301089FabH** in HV200221 backbone at NheI-SalI sites (remove the old insert), The resulting product should be called as **pDH511_5BFabH** that has the gene insert sequence between NheI-XbaI sites in pcDNA3.1(+)/hygromycin backbone as shown below.

>pDH511_5BFabH

```
GCTAGCACCATGGAGACAGACACACTCCTGCTATGGGTACTGCTGCTCTGGGFTCCAGGFTCCACTGG
TGAOCAGGTCCAGCTTGTACAGTCTGGGGGAGGCTTGGTAAAGCCTGGGGGGTCCCTTACACCCTCCT
GTGTCACCTCTGGATTTACTTTTACAGCAACACGTGGATGAGTTGGGTCCGCCAGACTCCAGGGAAGGGA
CTGGAGTGGGTTGCCCGTATTAGTAGGGTCGGGGATGGCCCAATAATAGACTACGCTGCTCCCGTGAA
AGGCAGATTTCATAATCTCAAGAGATGACTCAAGAAACACACTCTTTCTTCACATGAACAACCTGAAAA
CCGAGGACACAGCCGTGTATTATTGTACCGCTGATGAGGGGGCCCCAATTTTAAGATTTTTTGAGTGG
GGTTATTACAACACTACTACATGGACGTCTGGGGGCAAGGGGACCACGGTCATCGTCTCCTCGTCGTGAC
CAAGGGGCCATCGGTCTTCCCCCTTGGCACCCCTCCTCCAAGAGCACCTCTGGGGGGCACAGCGGCCCTGG
GCTGCCTTGGTCAAGGACTACTTCTCCGAACTGGTGAAGGTGTCTGTTGAACTCAGGCGCCCTGACCAGC
GGCGTGACACACCTTCCCGGCTGTCTACAGTCTCAGGACTCTACTCCCTCAGCAGCGTGGTGGACCGT
GCCCTCCAGCAGCTTGGGTACCCAGACCTACATCTGCAACGTGAATCACAAGCCCAGCAACACCAAGG
TGGACAAGAGAGTTGAGCCCCAAATCTTGTGACAAATGATCTAGA
```

Figure 58 cont.

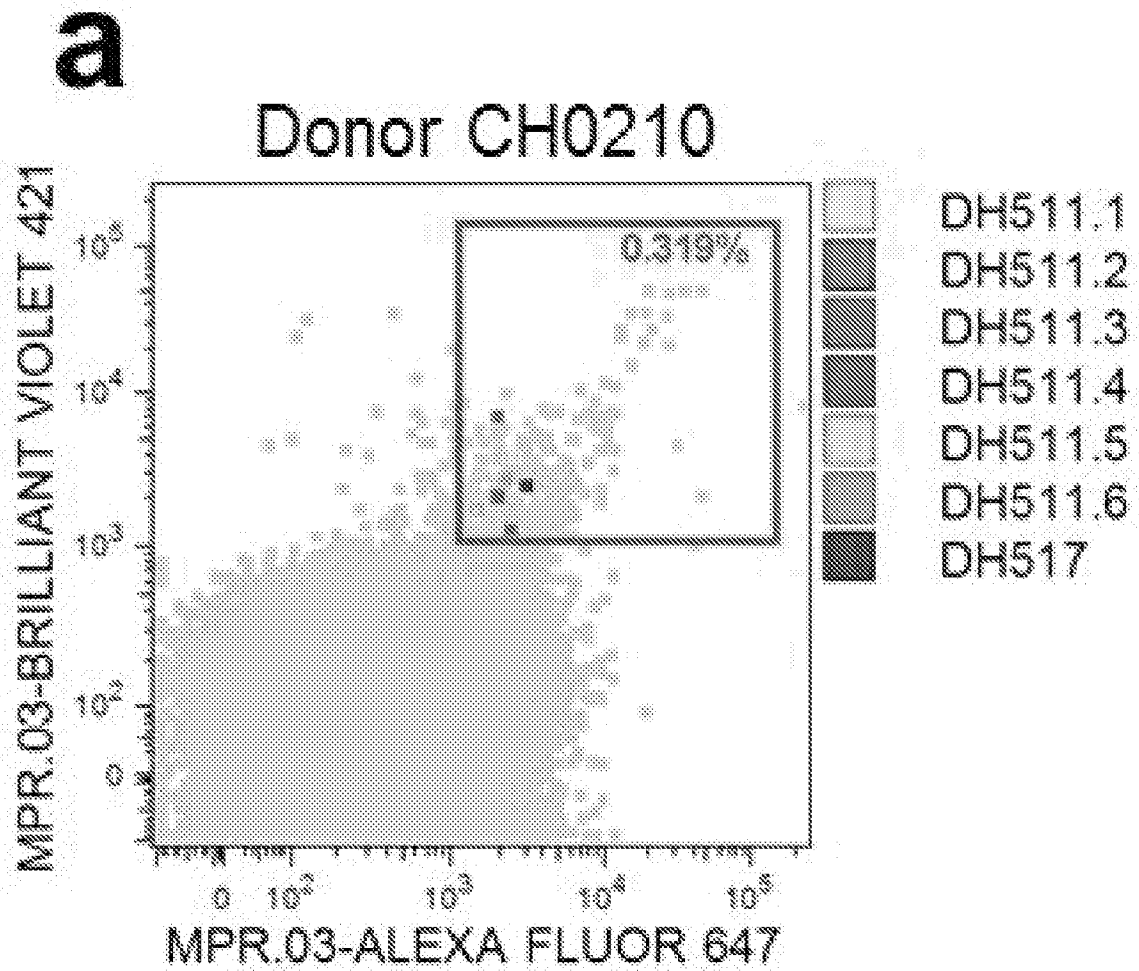
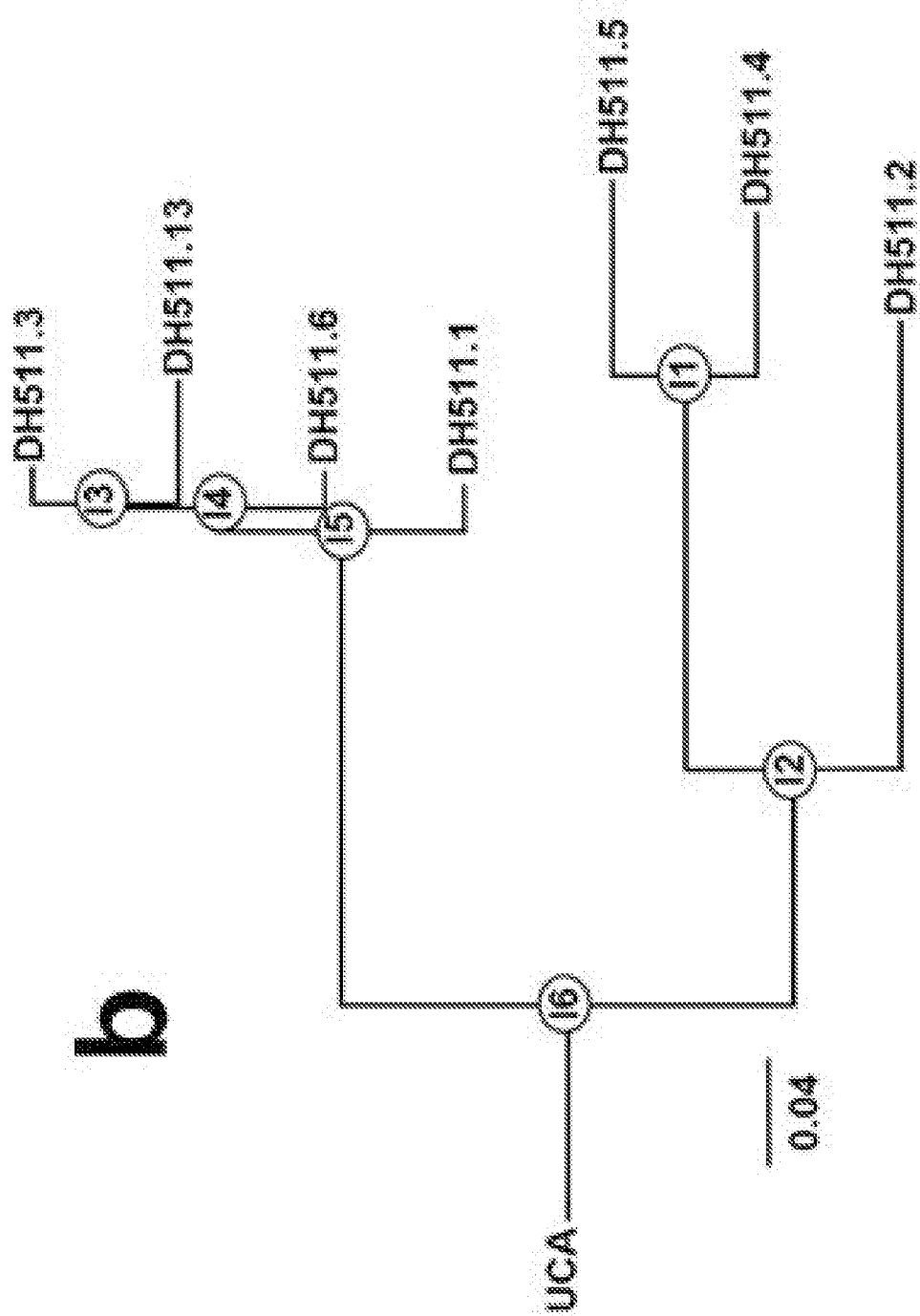


Figure 59A



595 2164

C

Antibody ID	Median IC ₅₀	Geom. IC ₅₀ Mean
DH511.2	1.09	0.802
10E8	0.392	0.315
VR001	0.261	0.287

Antibody ID	Median IC ₅₀	Geom. IC ₅₀ Mean
DH511.2	5.1	3.82
10E8	1.71	1.37
VR001	0.839	0.843

Key	
	< 0.2 µg/ml
	0.2-2 µg/ml
	2-20 µg/ml

Percent of Viruses Neutralized		
IC ₅₀ < 50 µg/ml	IC ₅₀ < 1 µg/ml	IC ₅₀ < 0.1 µg/ml
99	49	10
98	73	19
90	73	26

IC ₅₀ < 50 µg/ml	IC ₅₀ < 1 µg/ml	IC ₅₀ < 0.1 µg/ml
97	15	3
98	29	5
87	49	6

Key	
	91-100%
	61-90%
	31-60%
	1-30%

Figure 59C

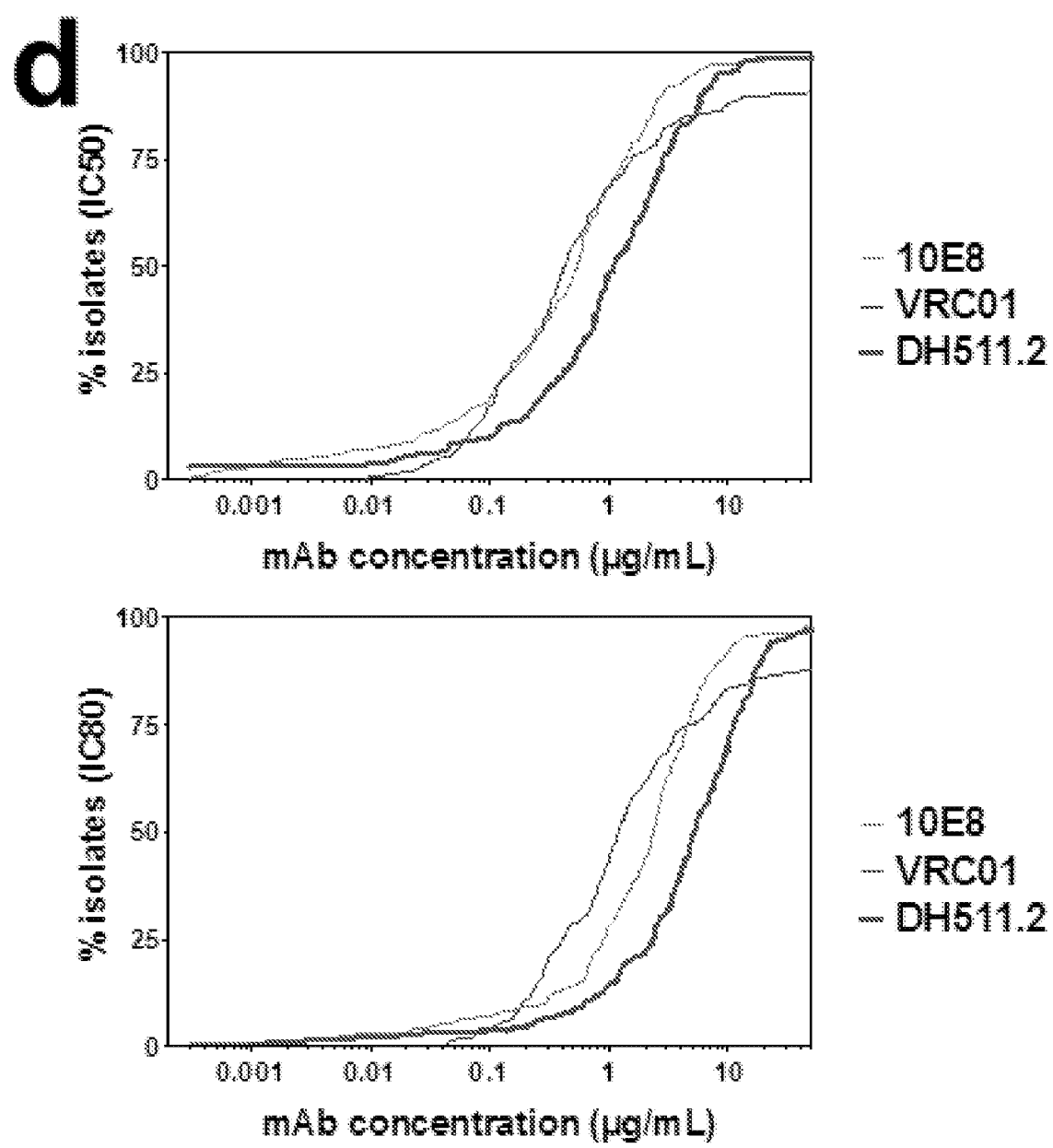


Figure 59D

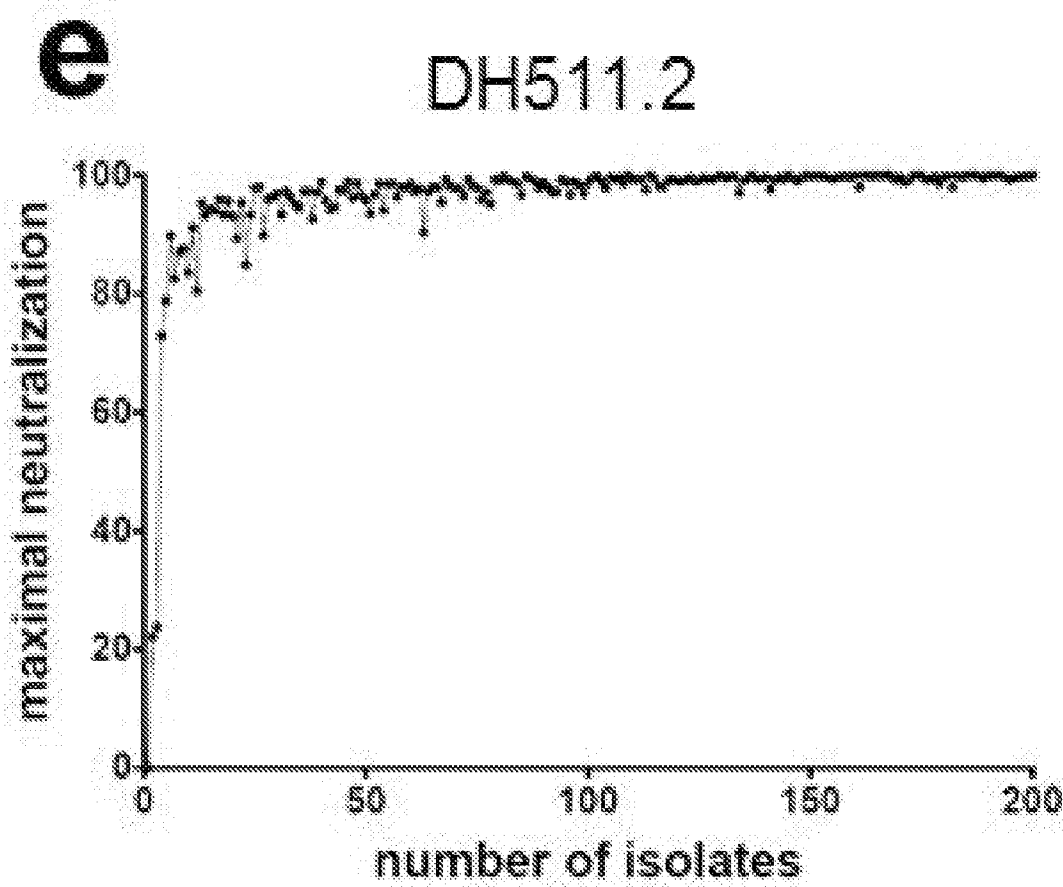


Figure 59E

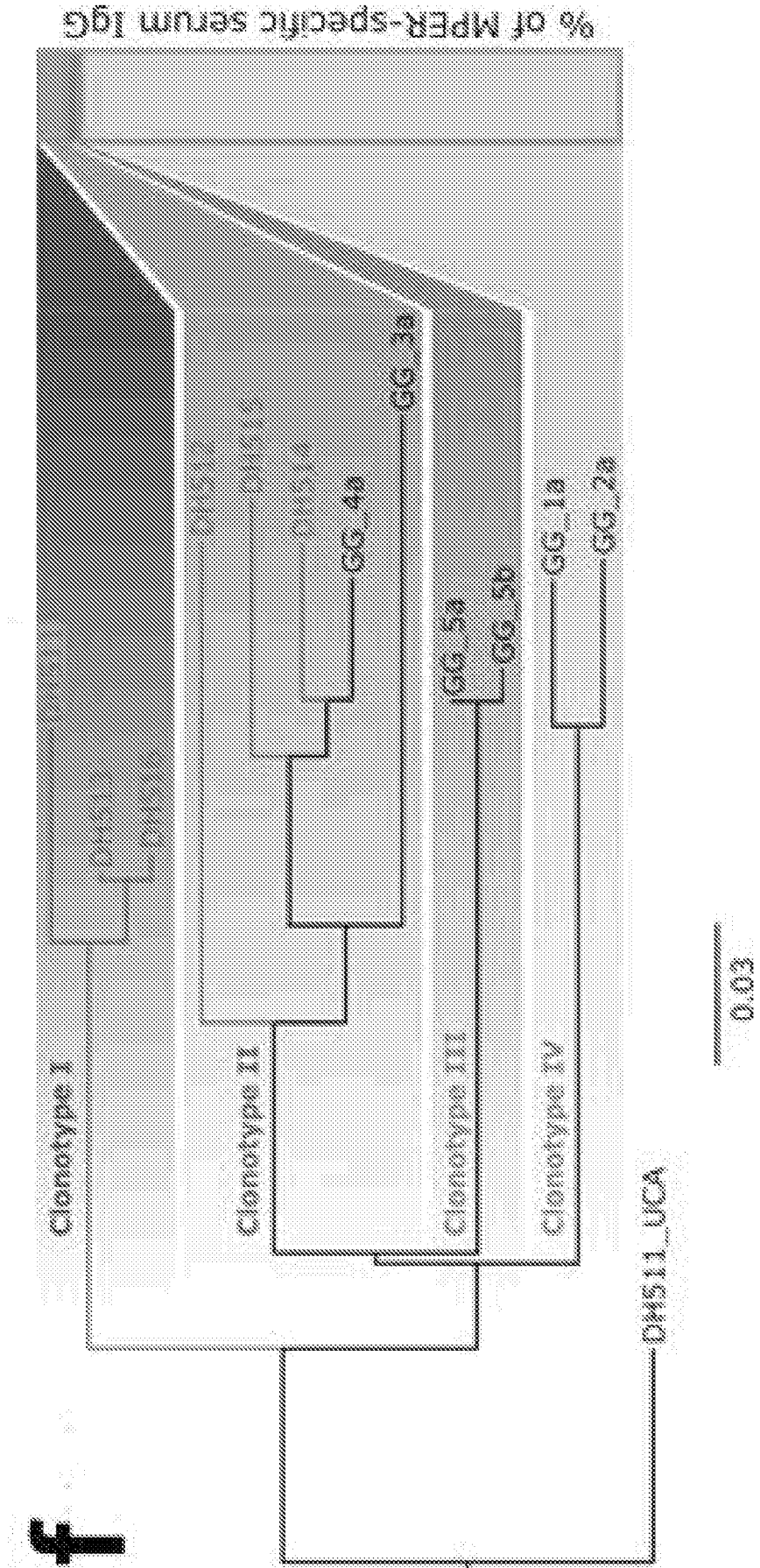


Figure 59f

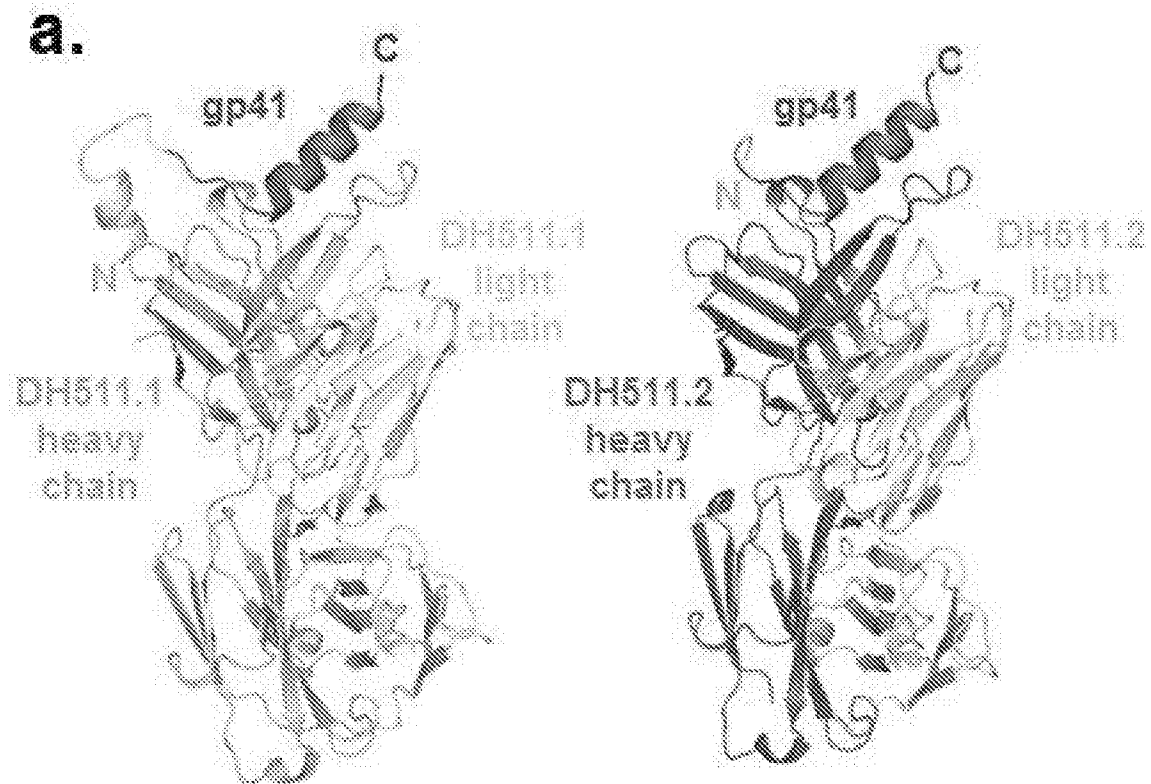


Figure 60A

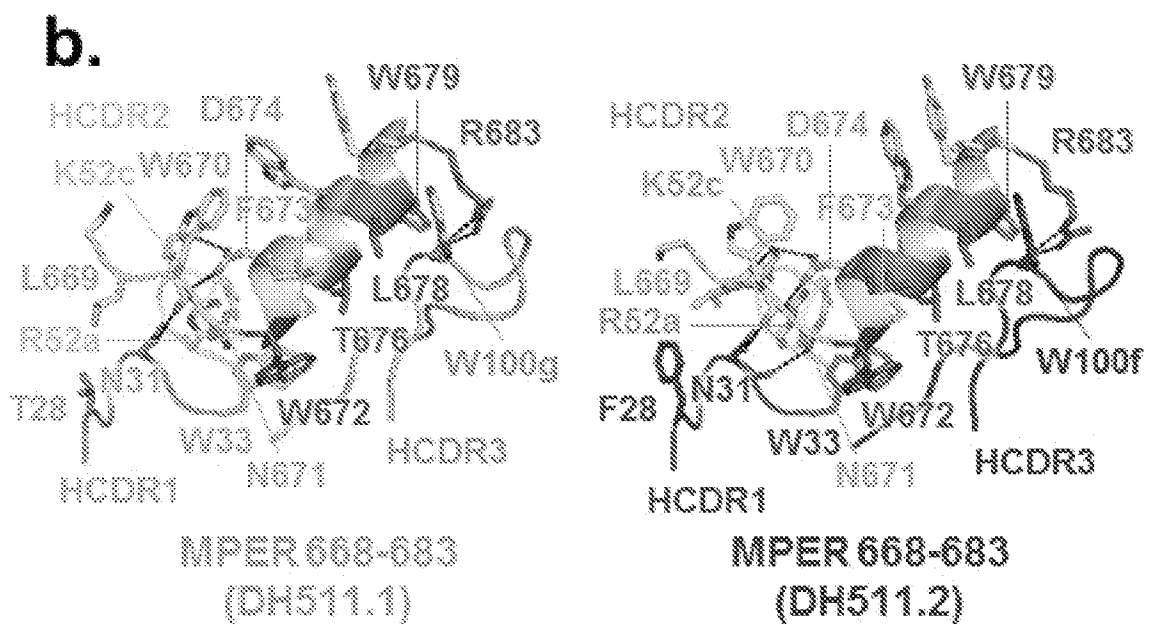


Figure 60B

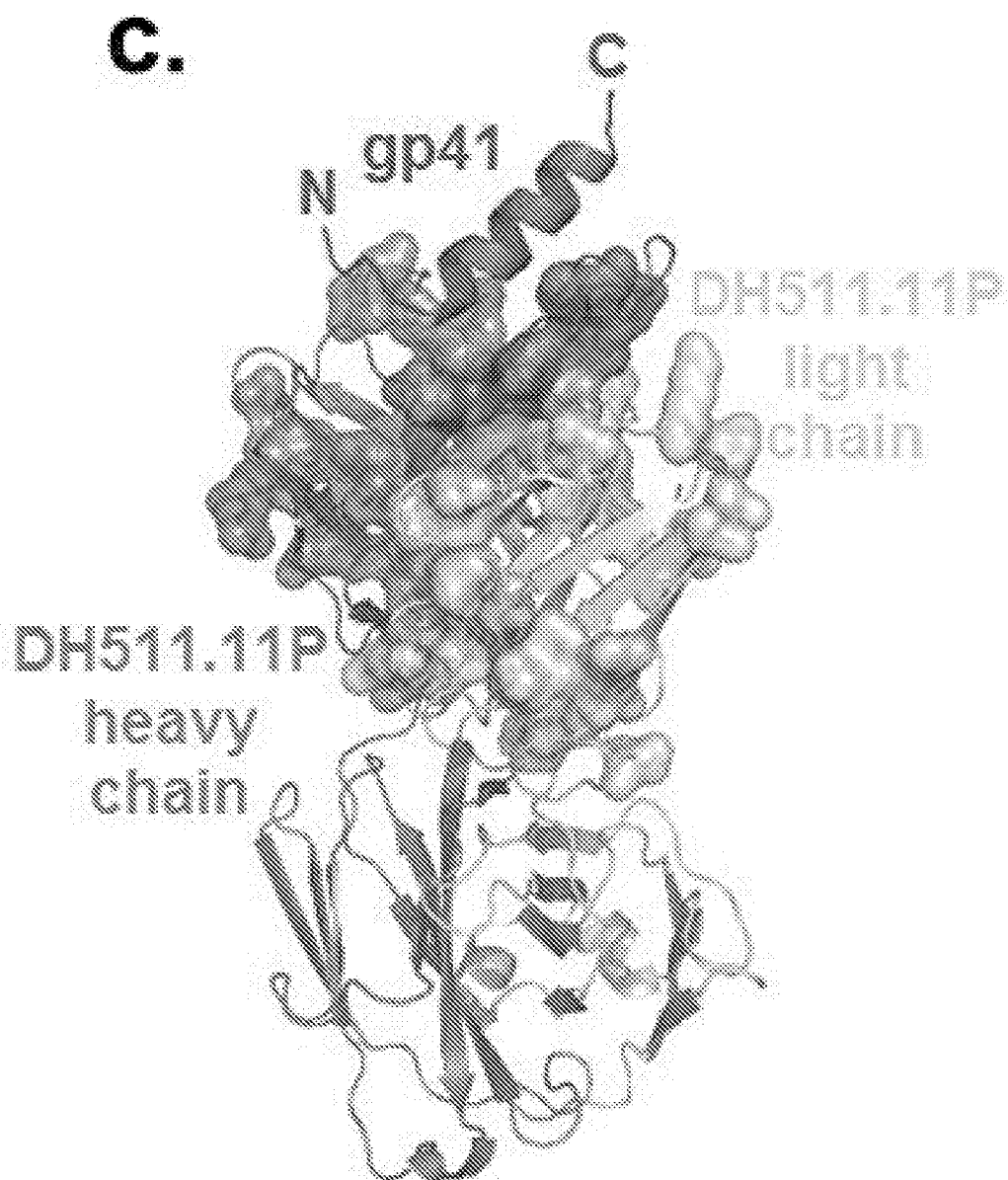


Figure 60C

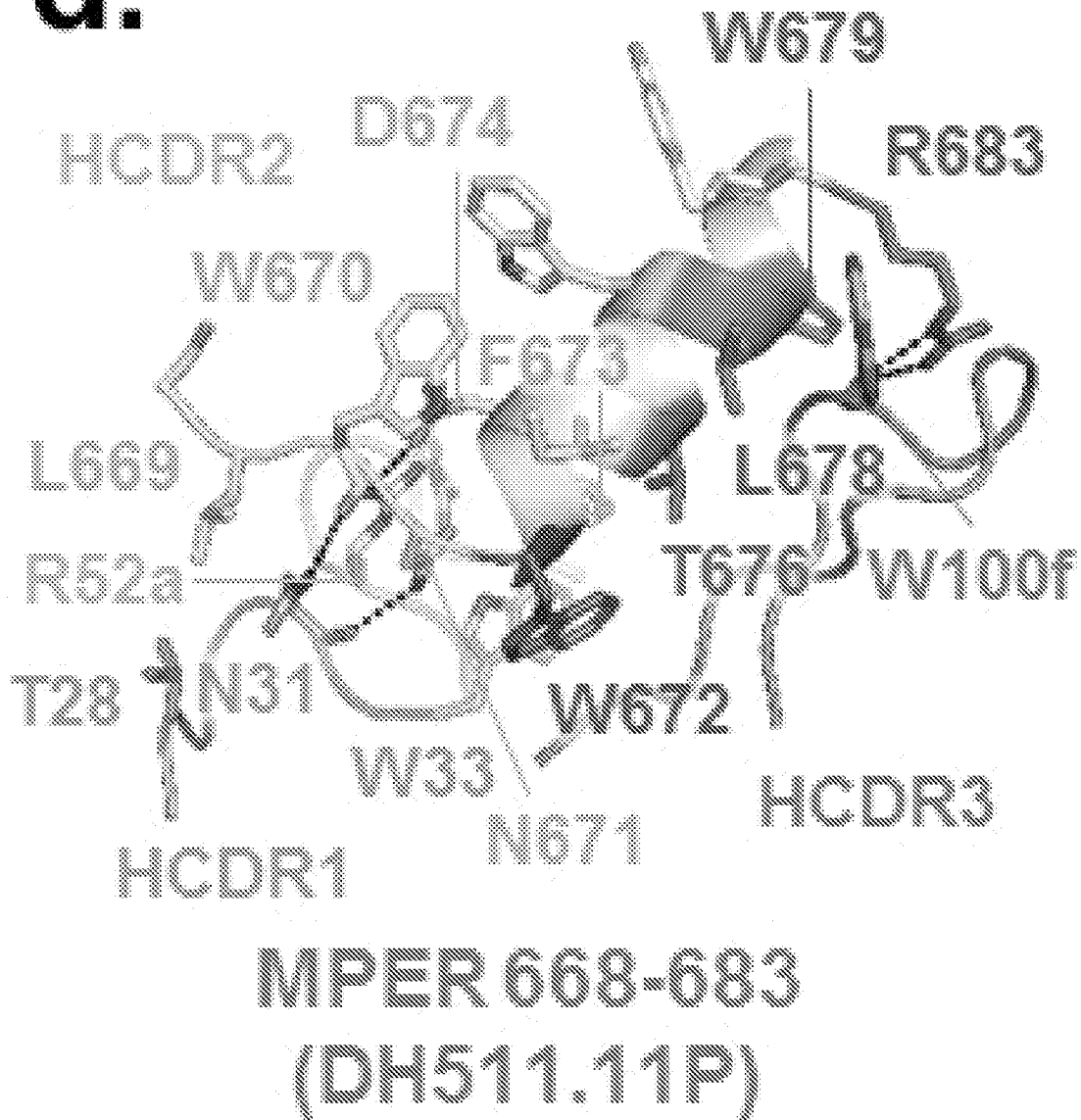
d.

Figure 60D

1	10	20	30	40	50
	FR1				
	CDR1				
	FR2				
IGHV3-15	EVQLVESGGGLV	KPGGSLRLSCAAS	GTFSNAW	MSWVRQAPGKGLEWVGR	
			● ● ●		●
10E8 VH	EVQLVESGGGLV	KPGGSLRLSCAAS	GFDFDNAM	MTWVRQPPGKGLEWVGR	
			● ● ● ●		
DH511.1 VH	EVQLVESGGGLV	KPGGSLRLSCAAS	GTFSNTW	MSWVRQAPGKGLEWVGR	
			● ● ● ●		
DH511.2 VH	QVQLVQSGGGLV	KPGGSLTLSCAAS	GFDFDNEM	MGWVRQAPGKGLEWVGR	
			● ● ● ●		
DH511.11P VH	QVQLVQSGGGLV	KPGGSLTLSCVTS	GTFSNTW	MSWVRQTPGKGLEWVAR	
			● ● ● ●		
DH511.12P VH	QVQLVQSGGGLV	KPGGSLTPSCVTS	GTFSNTW	MSWVRQTPGKGLEWVAR	

Figure 60E

IGHV3-15	52ABC	60	70	8082ABC	90
	CDR2	FR3			
	IKSKTDGGTT	DYAAPVKGRFTISRDDSKNTLYLQMN	SLKTEDTAVYYCTT		
	● ● ● ● ●				
10E8 VH	ITGEGEGWSV	DYAAPVGRFTISRRLNSINFLYLEMNNLRMEDSGLYFCAR			
	● ● ●	●			
DH511.1 VH	ISRNKDGAKT	EYAAPVGRFTISRDDSRD	TLYLQMTSLKIEDSGRYFCTA		
	● ● ● ● ●	●			
DH511.2 VH	IRRLKDGATG	EYGAAPVGRFTISRDDSRNNMLYLHMRTLKTEDSGTYVCTM			
	● ● ● ● ●				
DH511.11P VH	ISRVGDGPIT	DYAAPVKGRFTISRDDSRNTLF	LHMNNLKTEDTAVYYCTA		
	● ● ● ● ●				
DH511.12P VH	ISRVGDGPIT	DYAAPVKGRFTISRDDSRNTLF	LHMNNLKTEDTAVYYCTA		

Figure 60E cont.

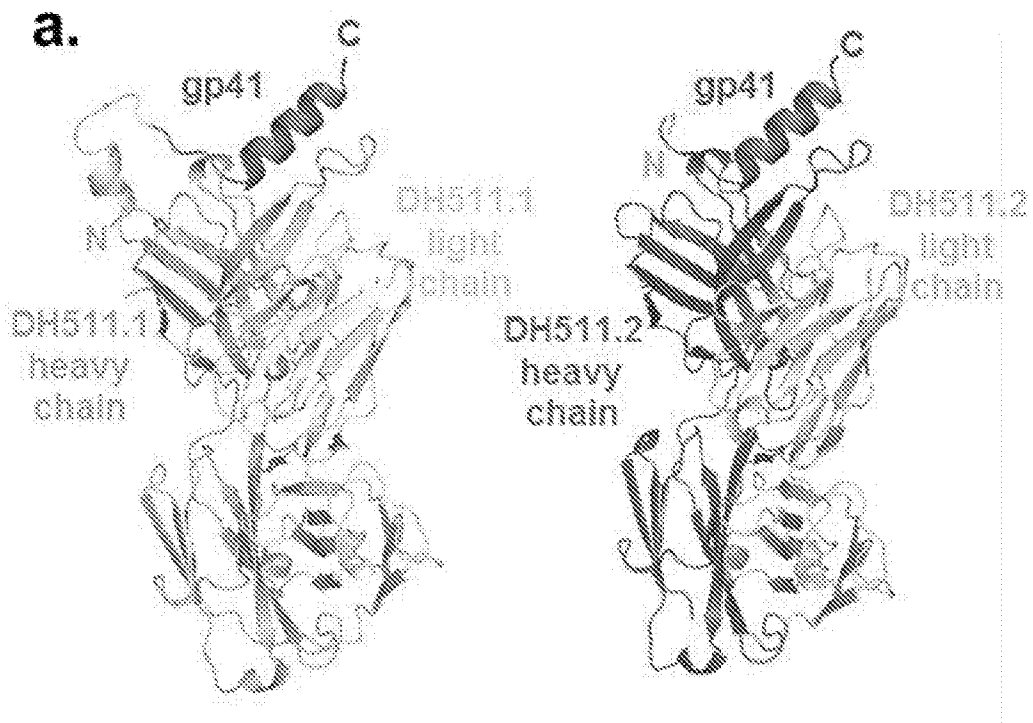


Figure 61A

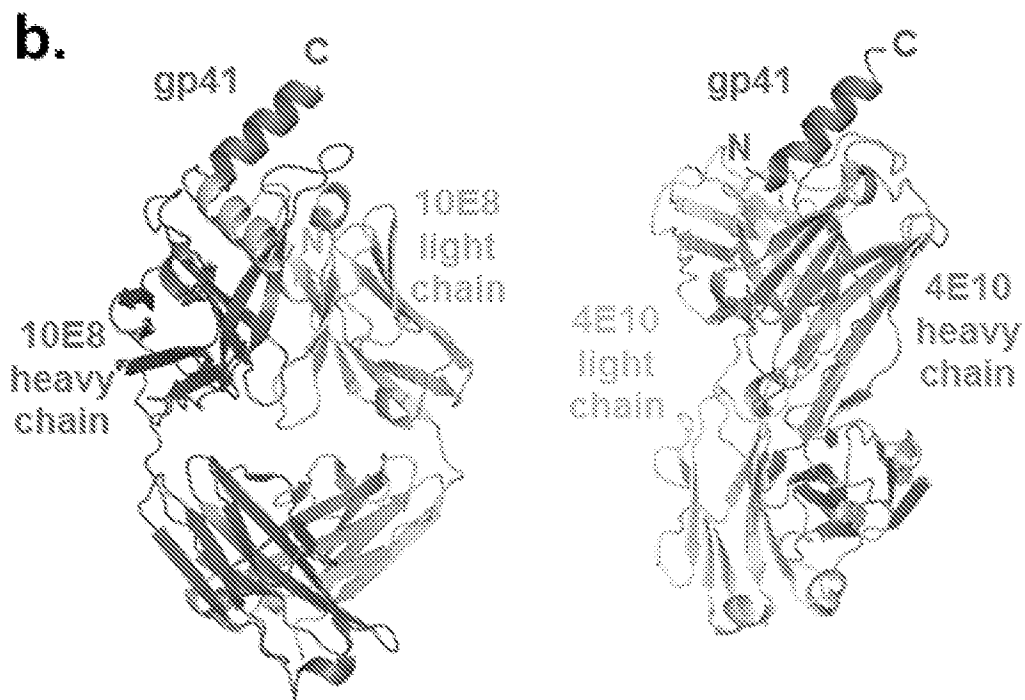


Figure 61B

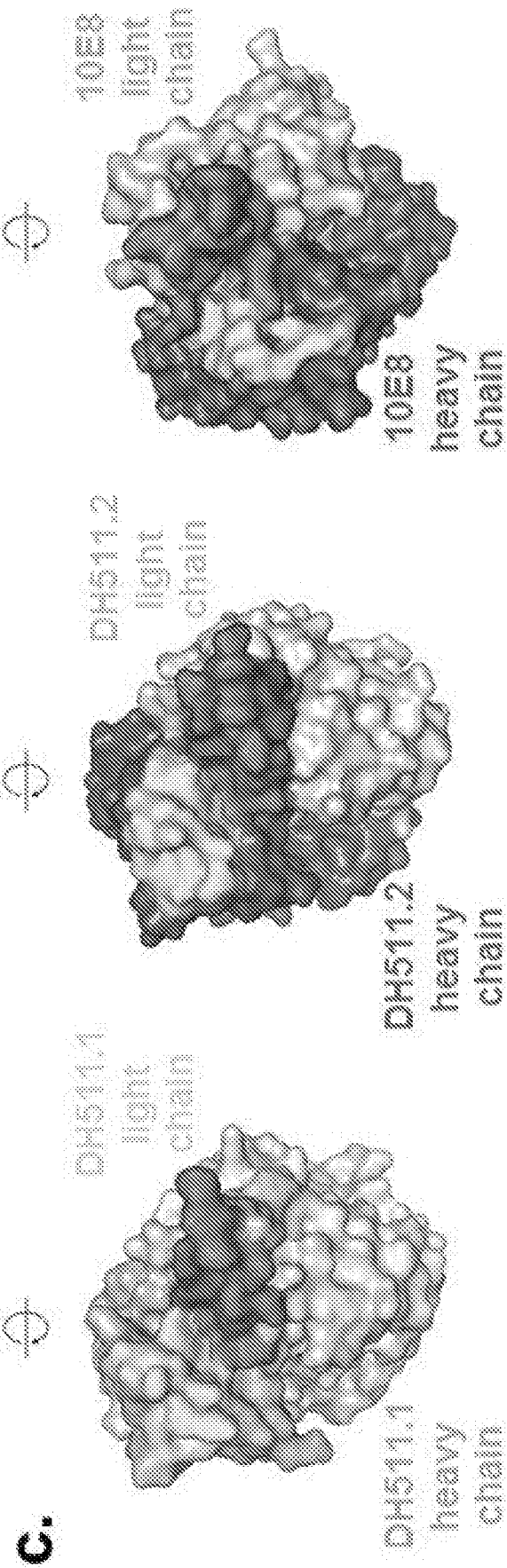


Figure 61C

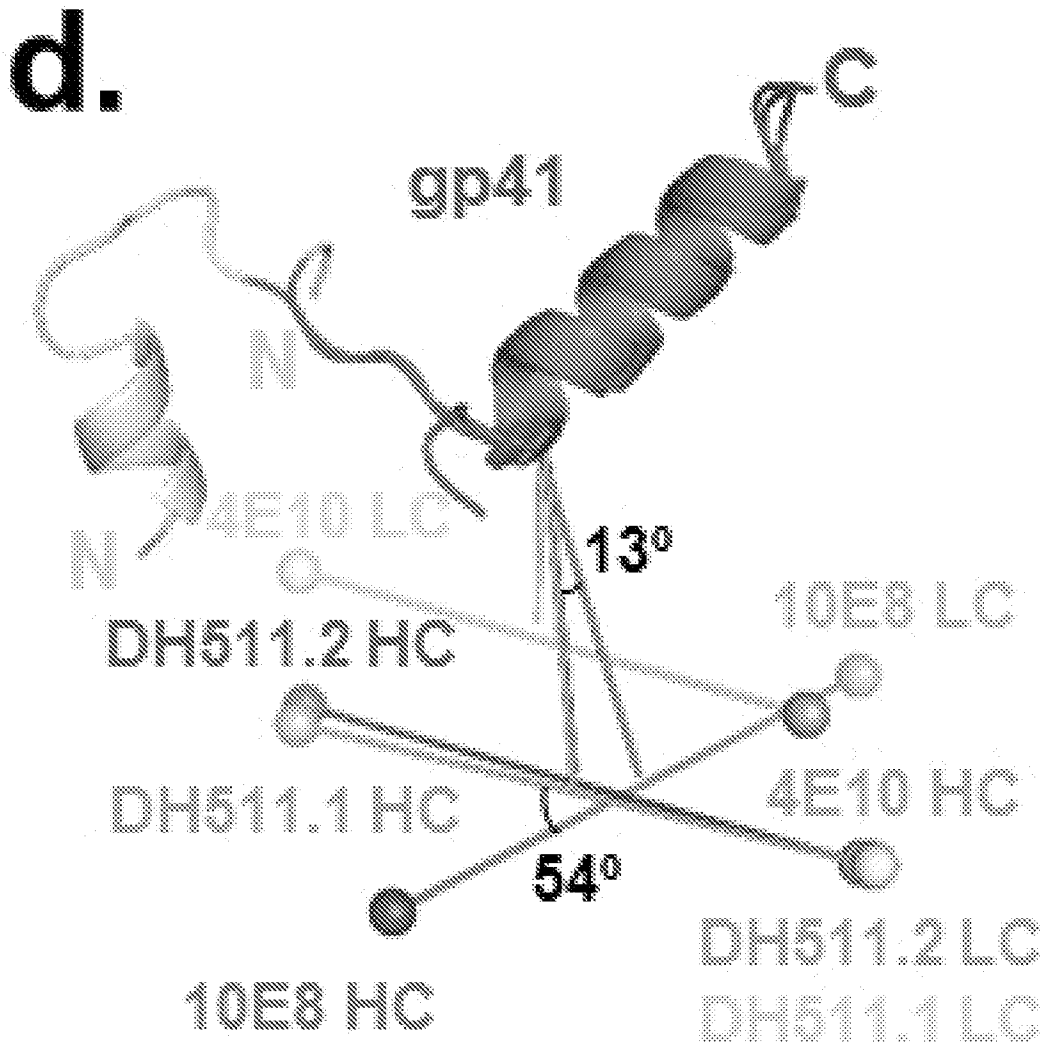


Figure 61D

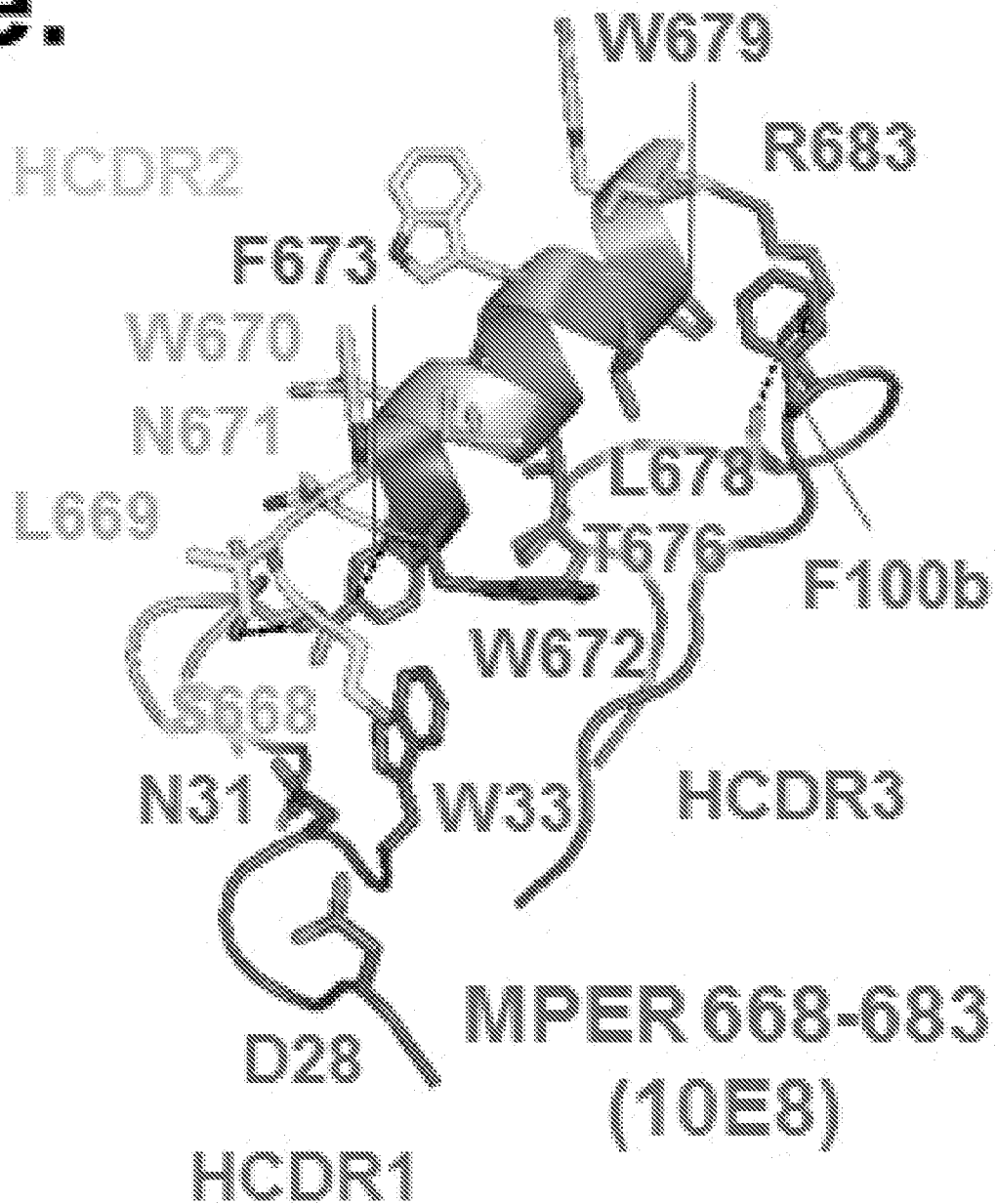
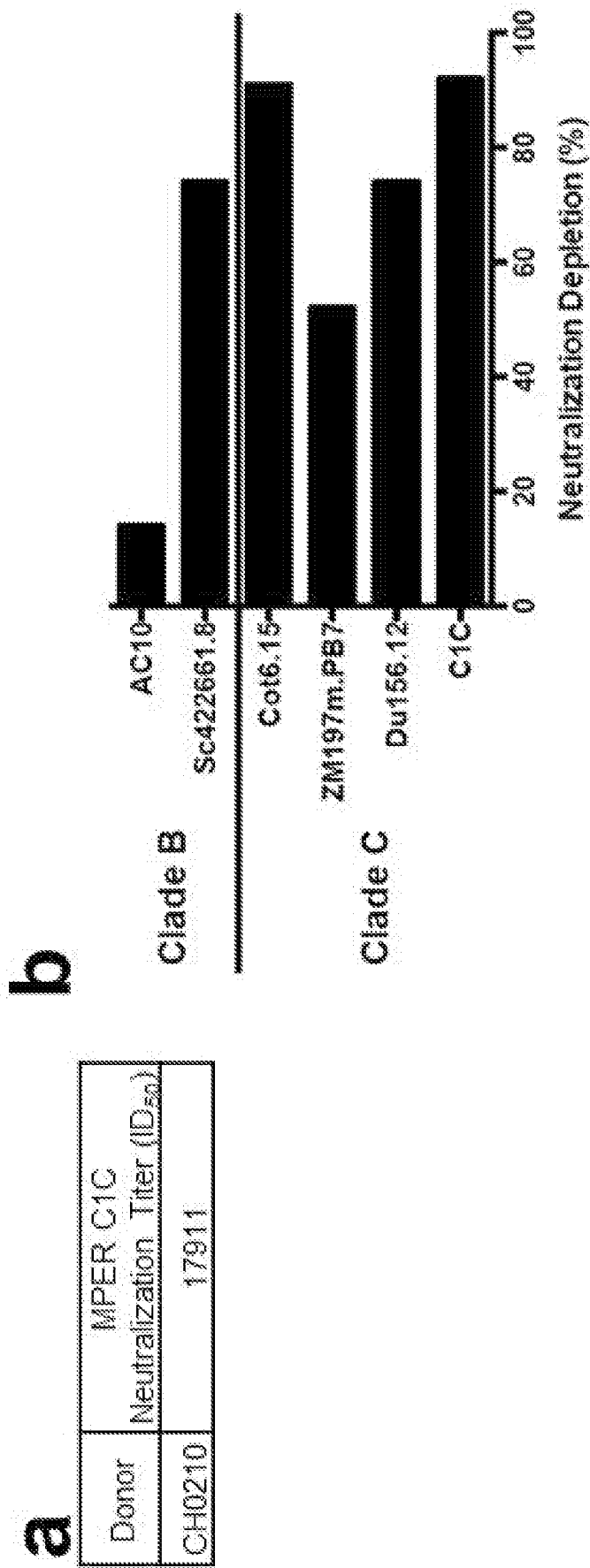
e.

Figure 61E



Figures 62A-B

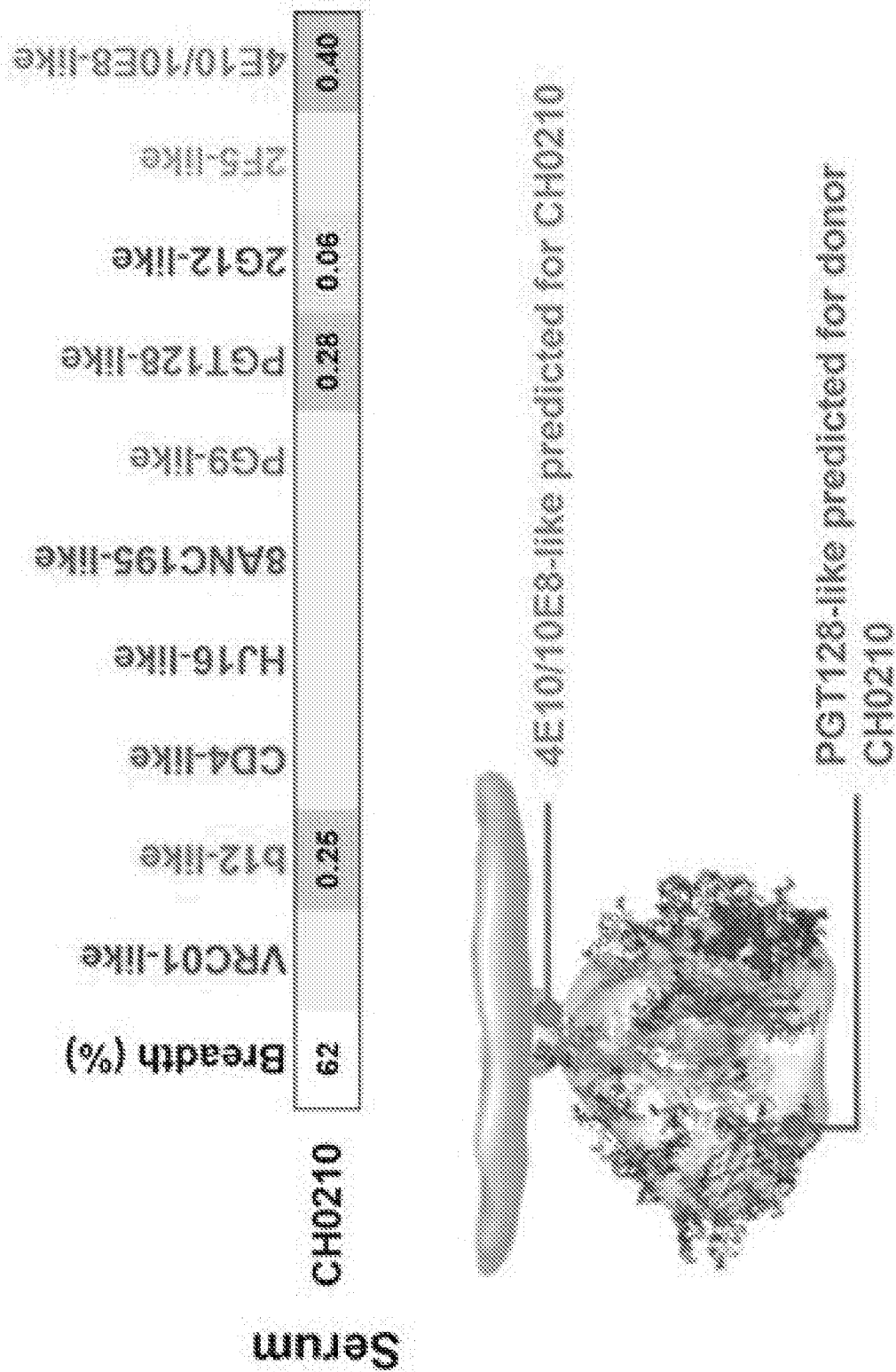


Figure 62C

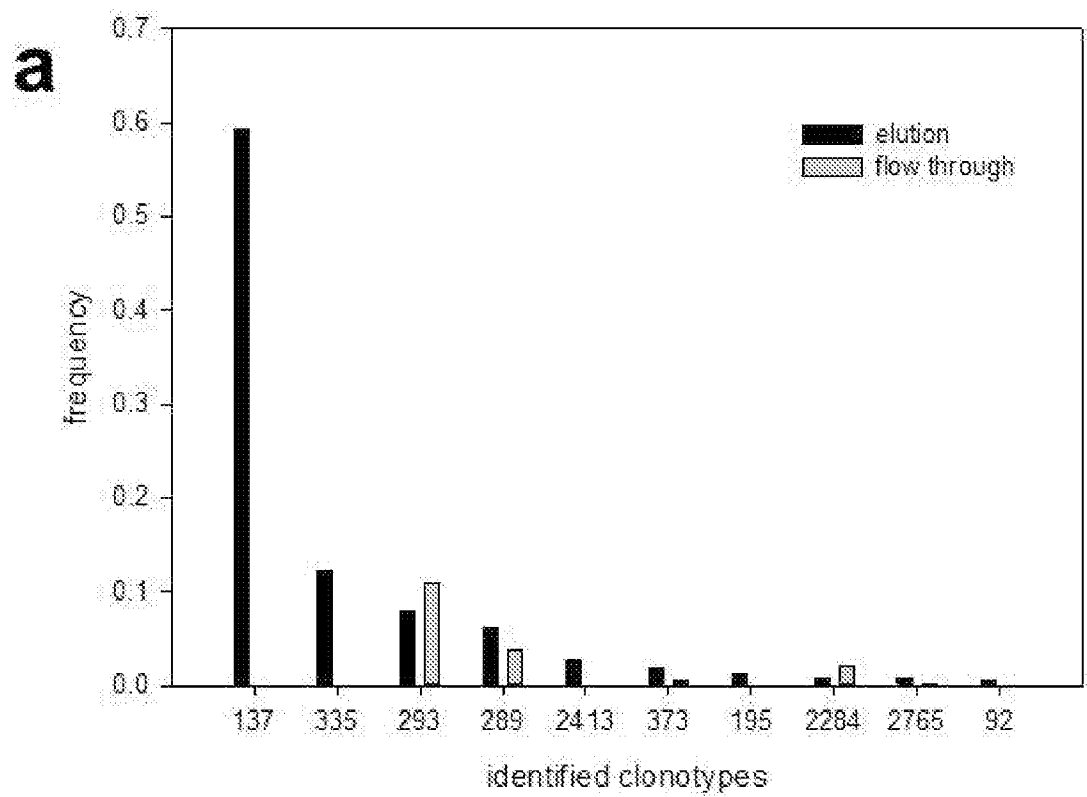
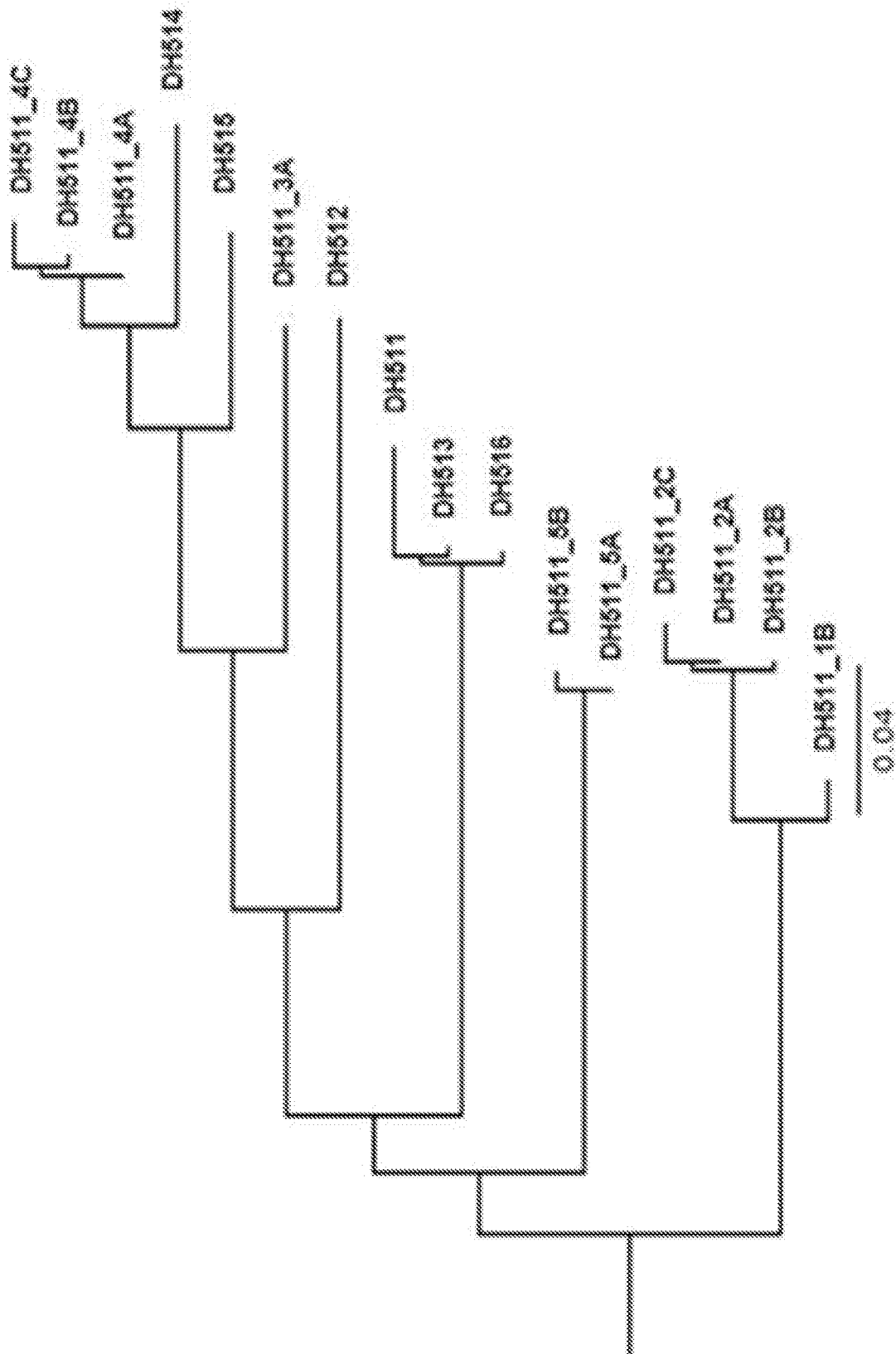


Figure 63A

b

Clonotype	V gene	J gene	Mutation rate ² [%]	CDR3 sequence	Comment
137	3-15	6	15	TADRGAPVLRFEWGWGYFDYYMEF	no complaints
335	3-15	6	15	TADEGAPILRFFEWGYNYNMDV	no complaints
293	3-9	4	11	VKGATPSITIFGRVAFDH	high abundance in FT
289	1-69	2	9	ARVERPGSDWYFDL	CDR1 peptide only
2413	4-16	4	4	ARTAVAGTNYFDY	low confidence
373	3-9	3	6	TKDIVWRYSLSEGAFDL	low confidence
195	3-15	6	21	TRDEGAPVTRFLEWGSYYYYMAV	no complaints
2284	3-7	3	7	ARDGPATFRLLLEYVFMSSFDM	rel. high abundance in FT
2765	3-74	4	12	ARGGSGIFRFPNY	low confidence
92	3-9	3	10	AKDTMGHCSSAFCFAFDF	no CDR3 peptide

Figure 63B



6301

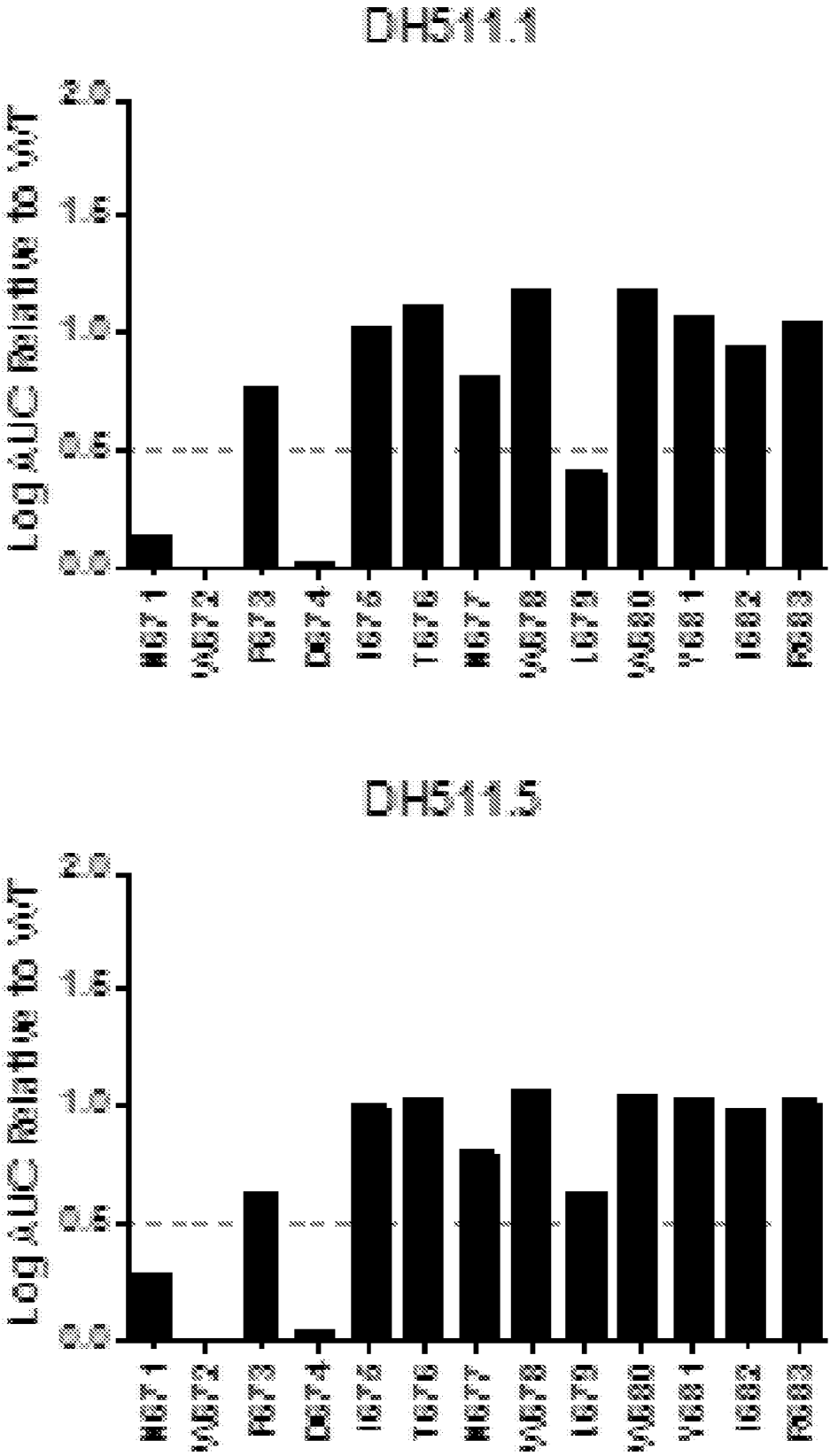


Figure 65A

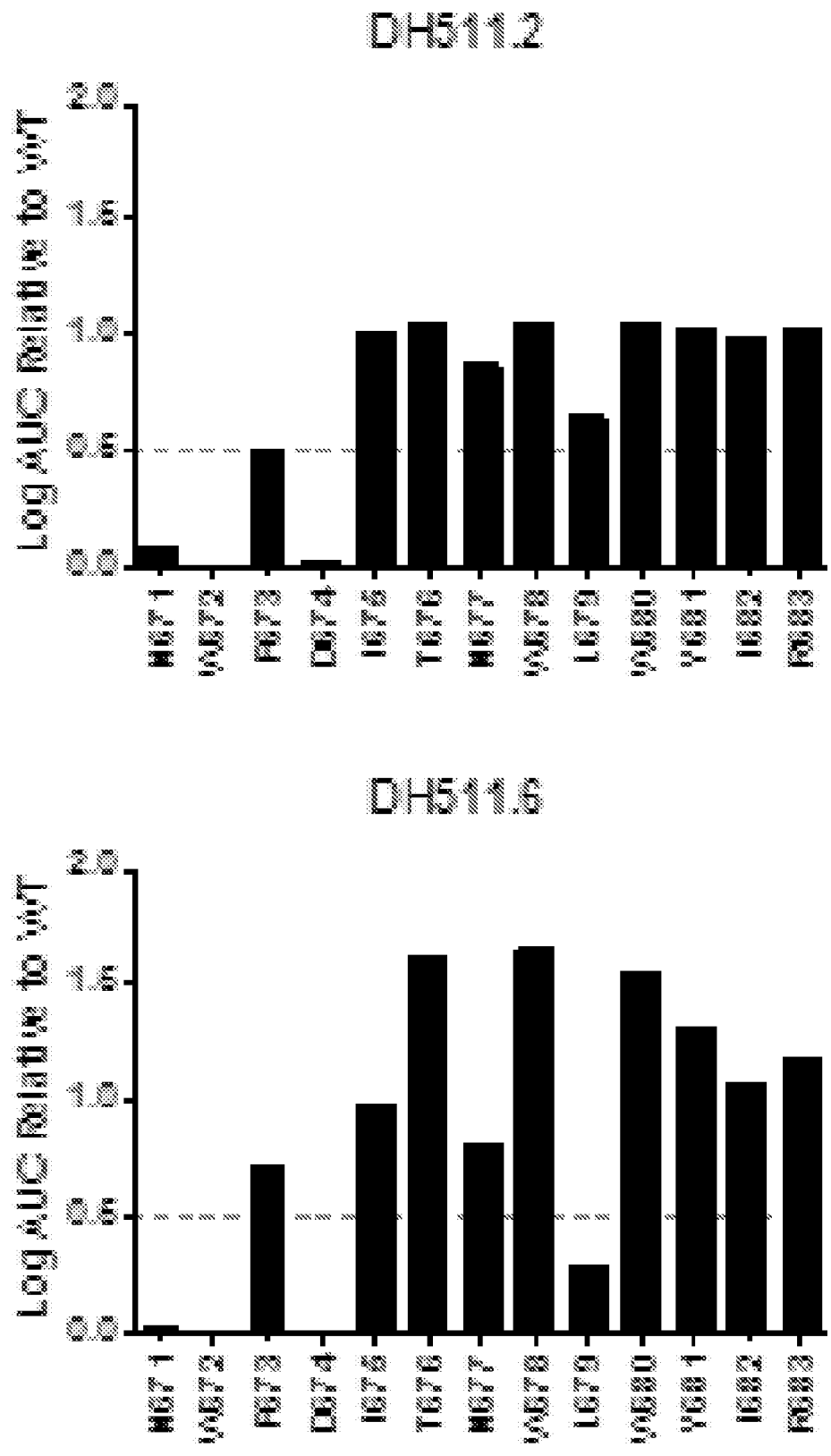


Figure 65A cont.

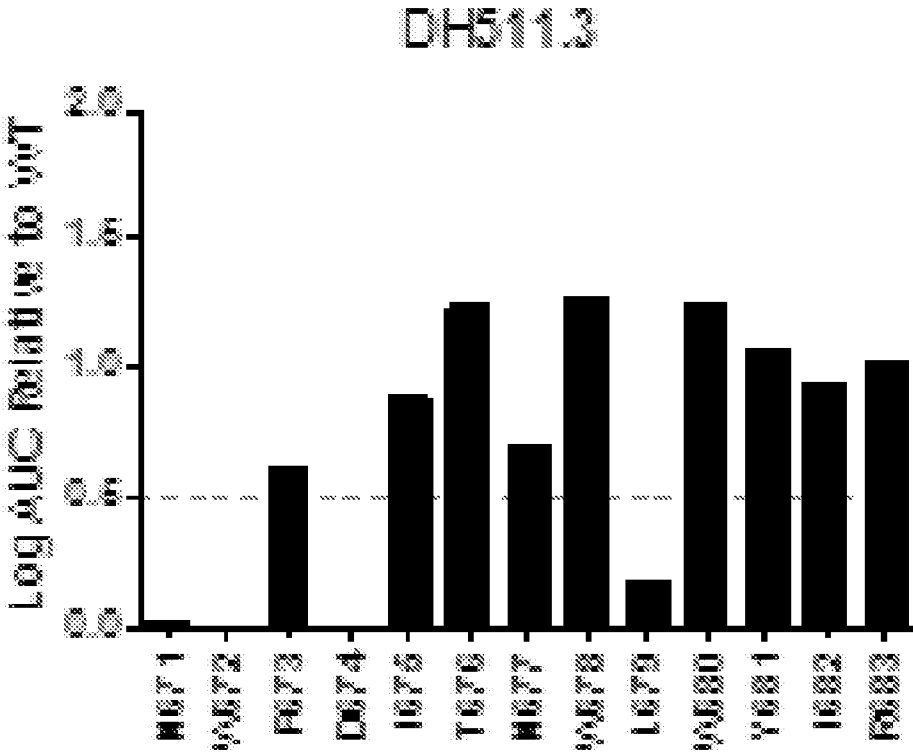
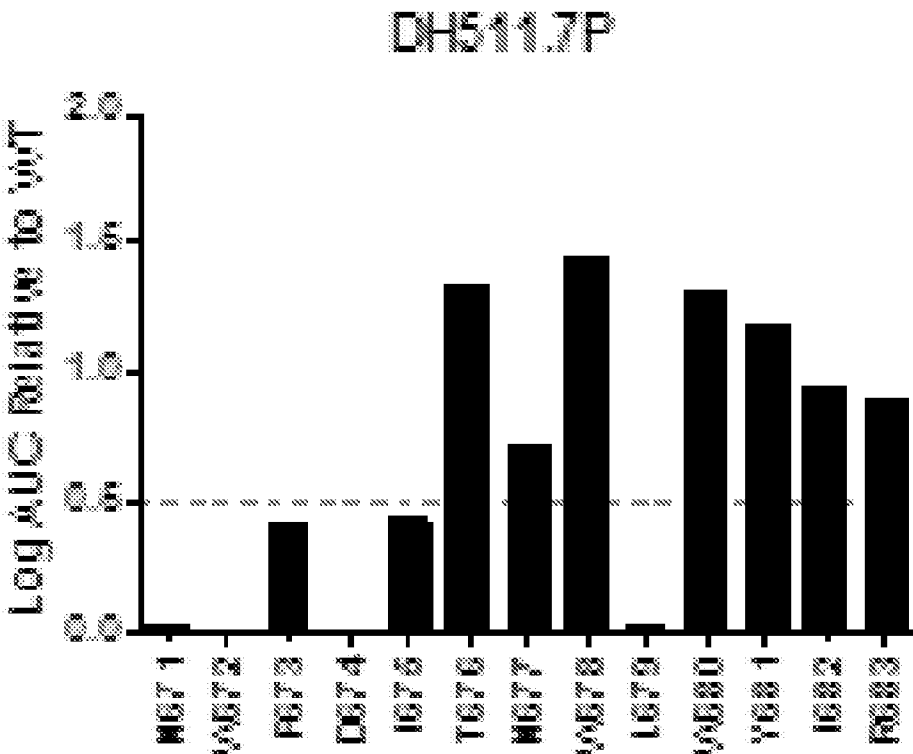


Figure 65A cont.

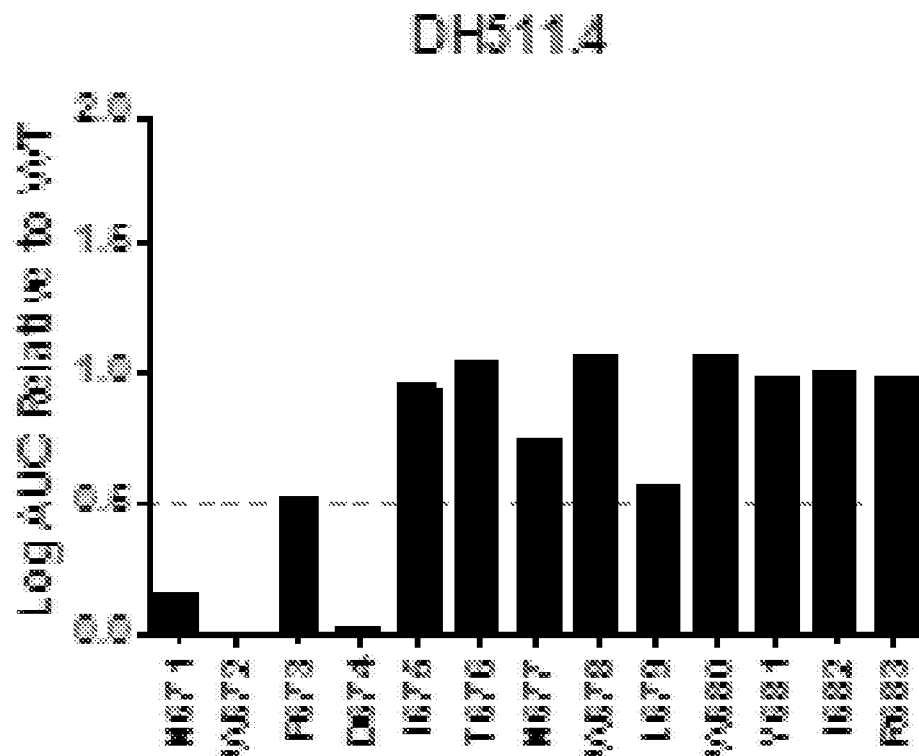
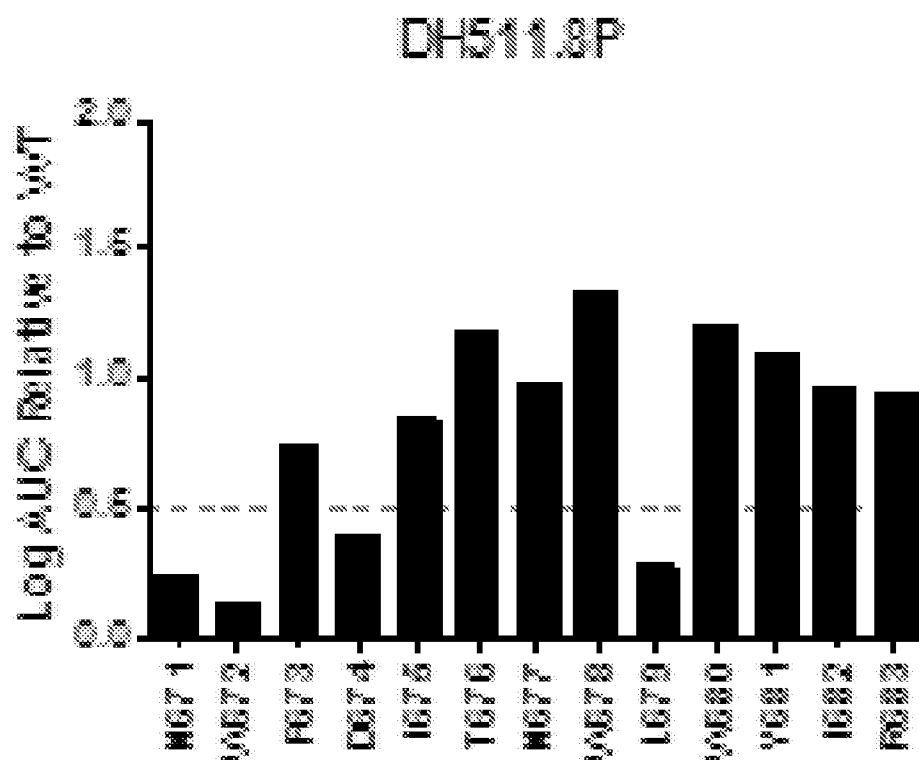


Figure 65A cont.

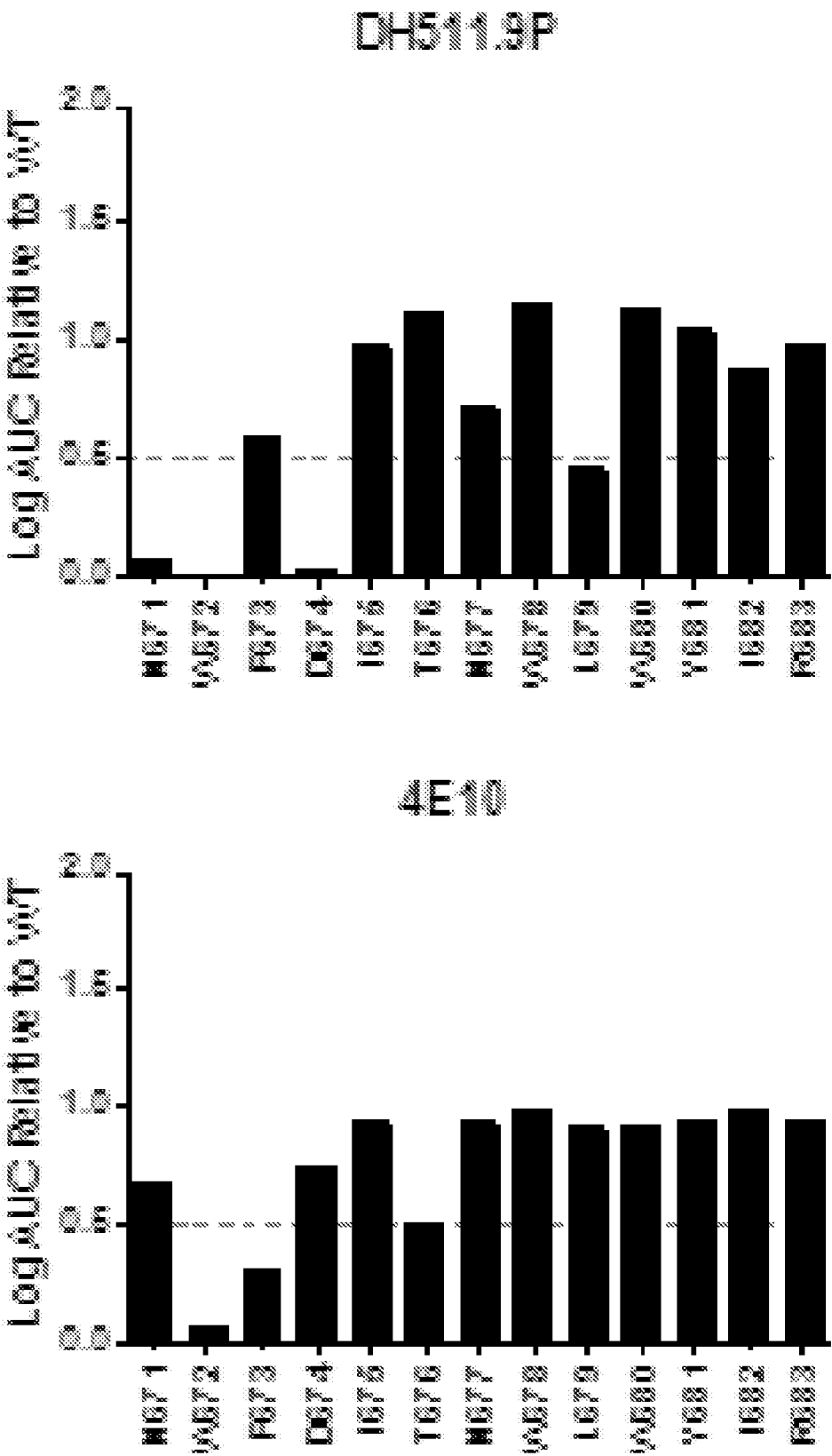


Figure 65B

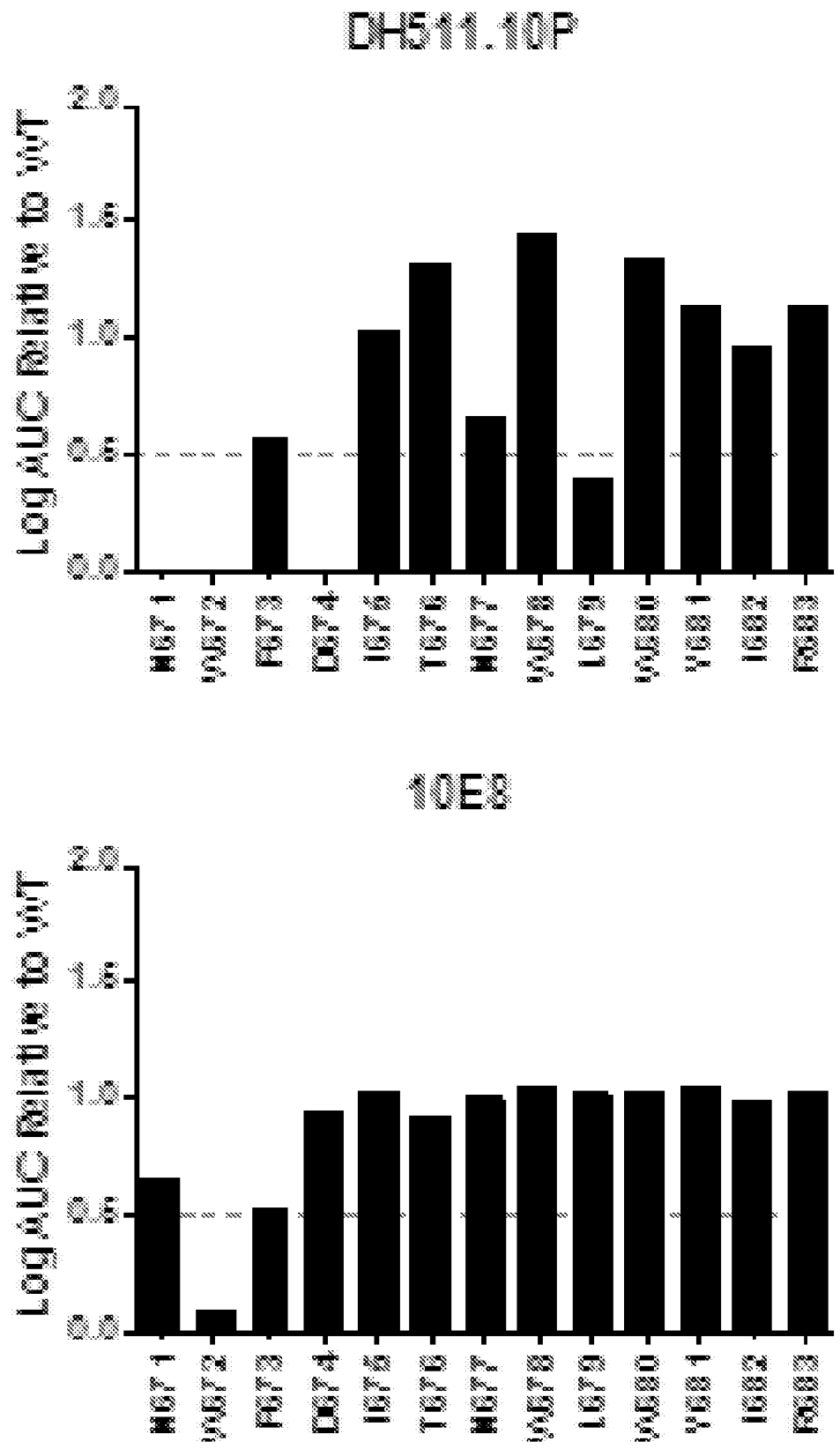


Figure 65B cont.

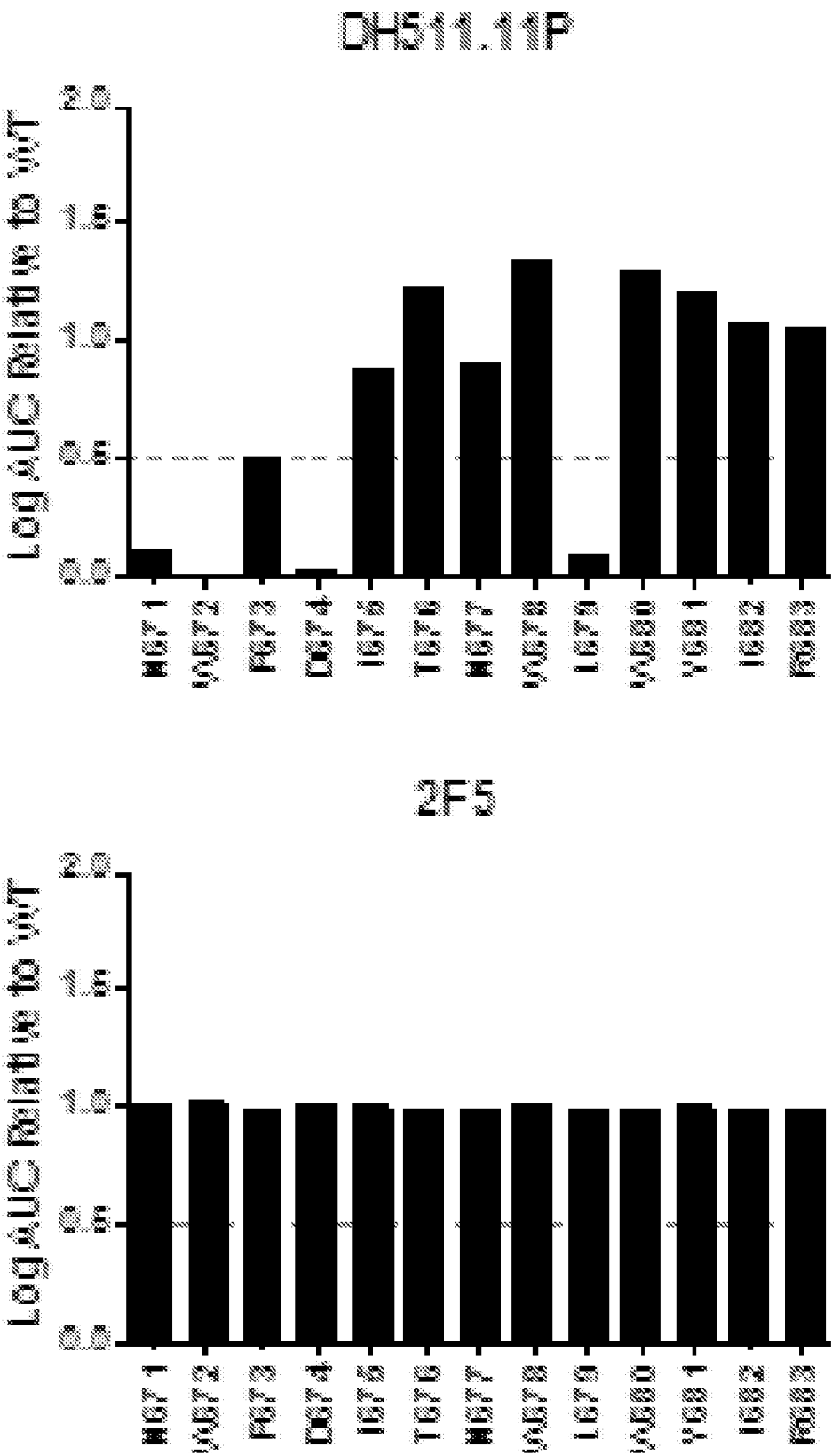


Figure 65B cont.

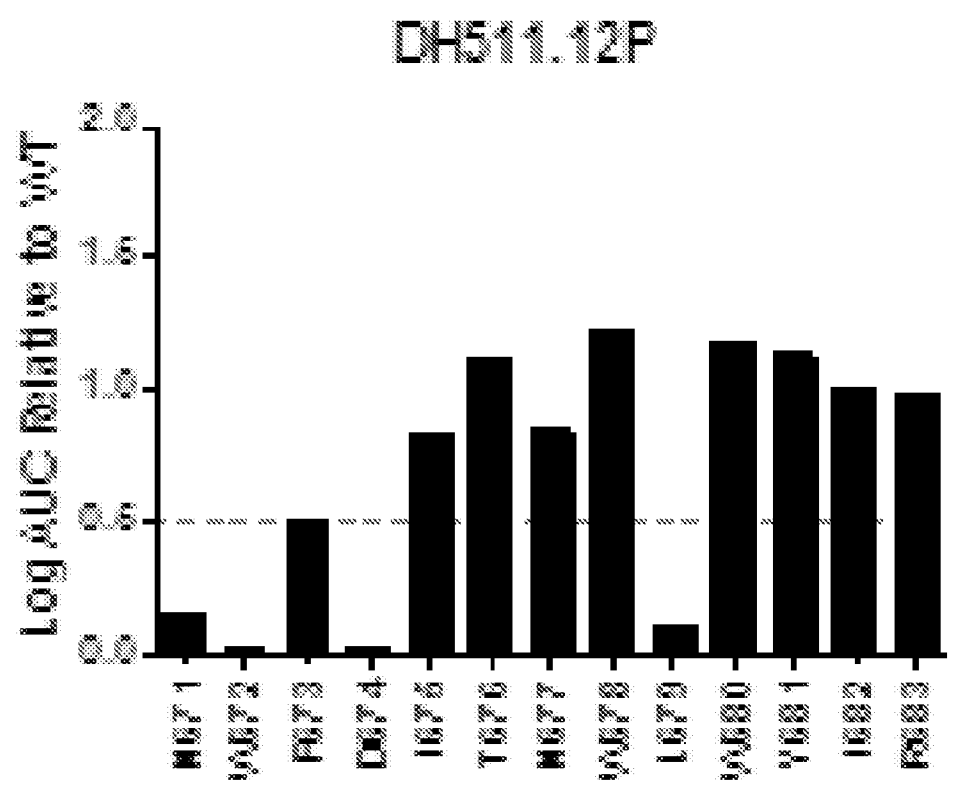


Figure 65B cont.

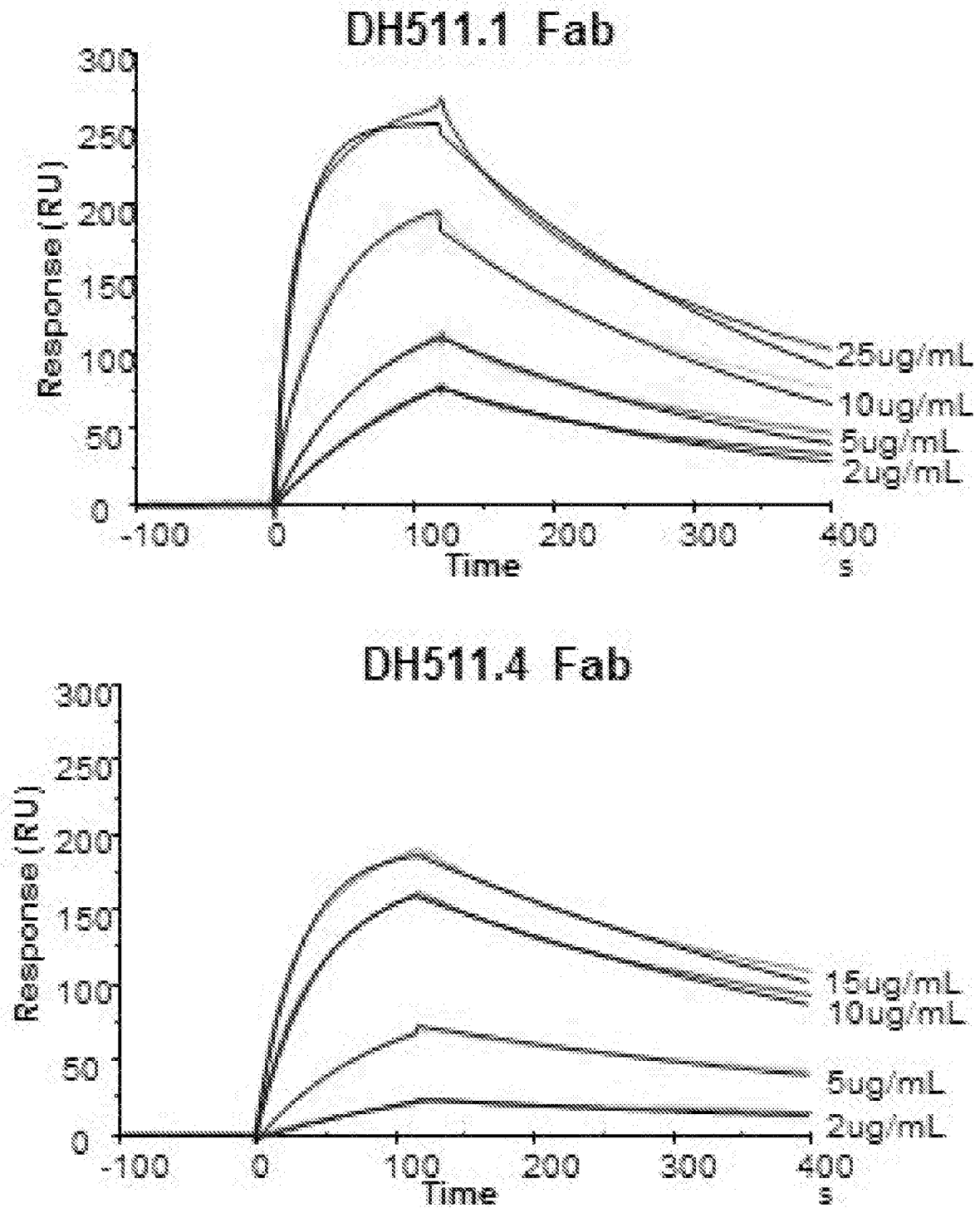


Figure 66A

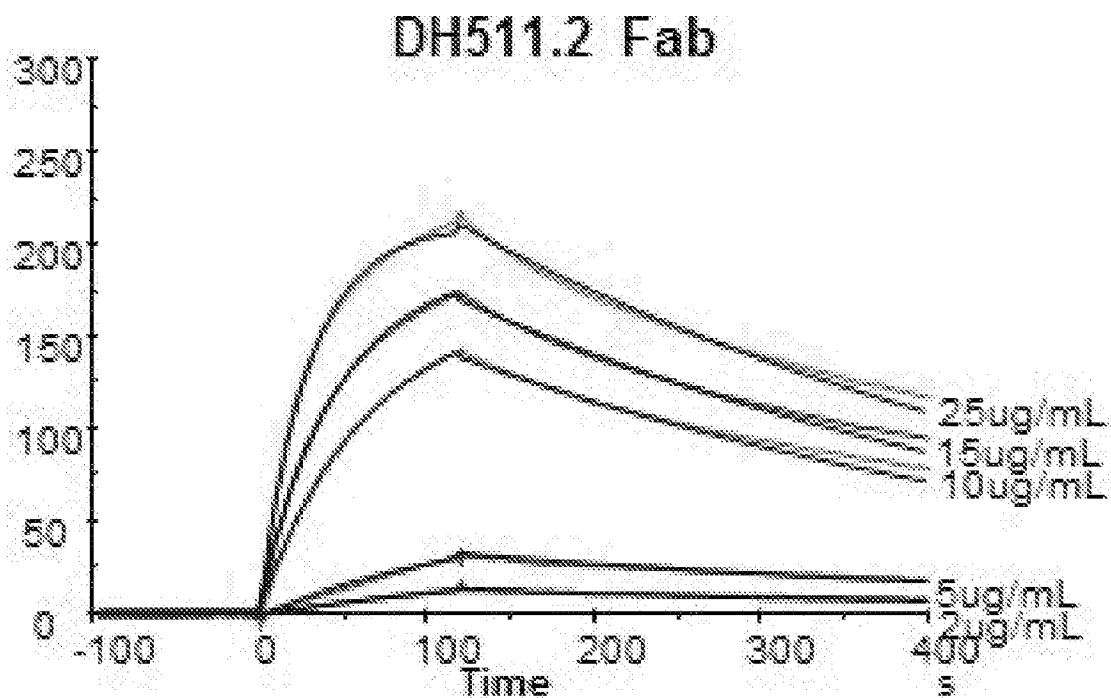
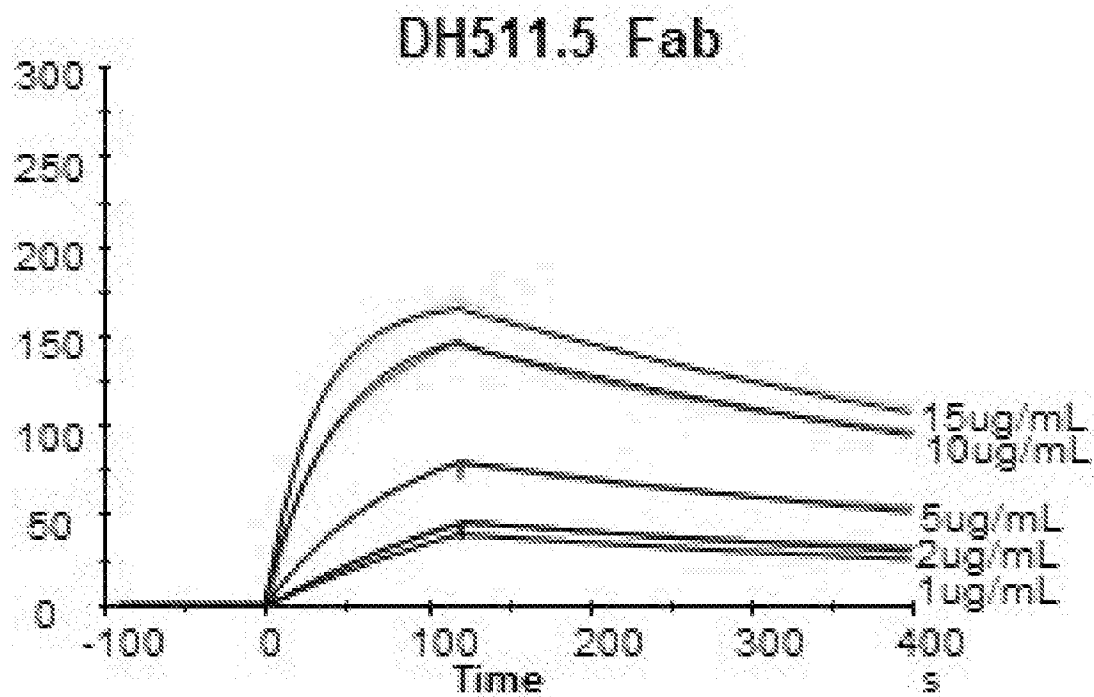


Figure 66A cont.

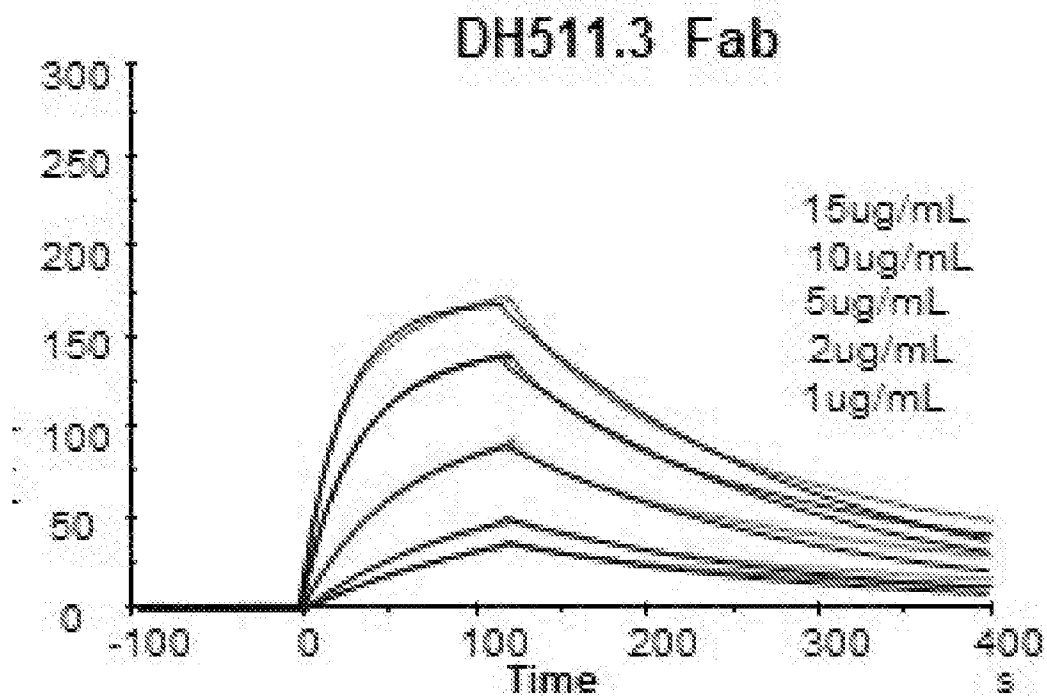
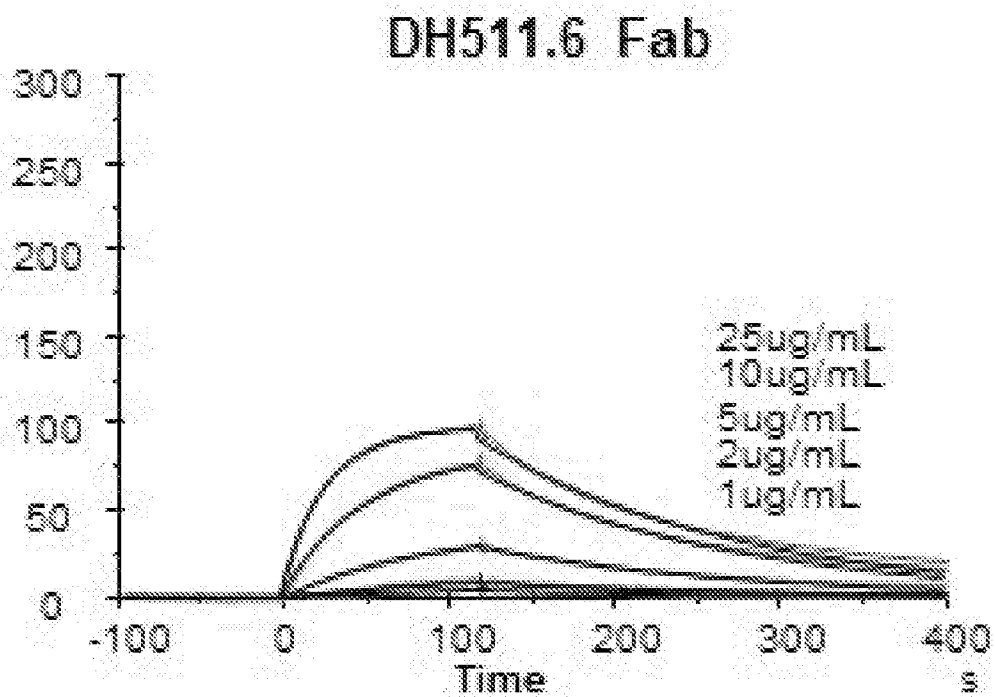


Figure 66A cont.

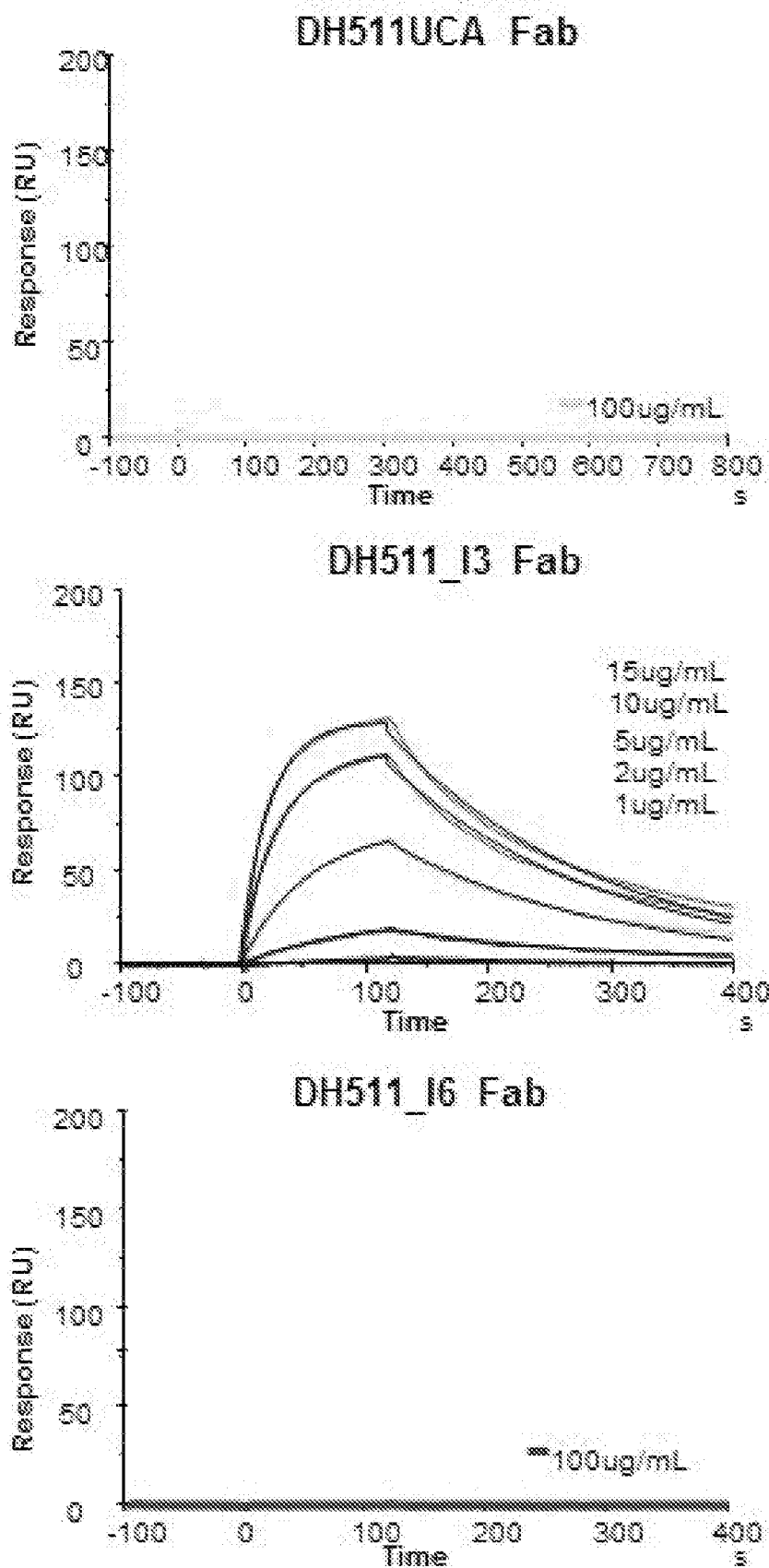


Figure 66B

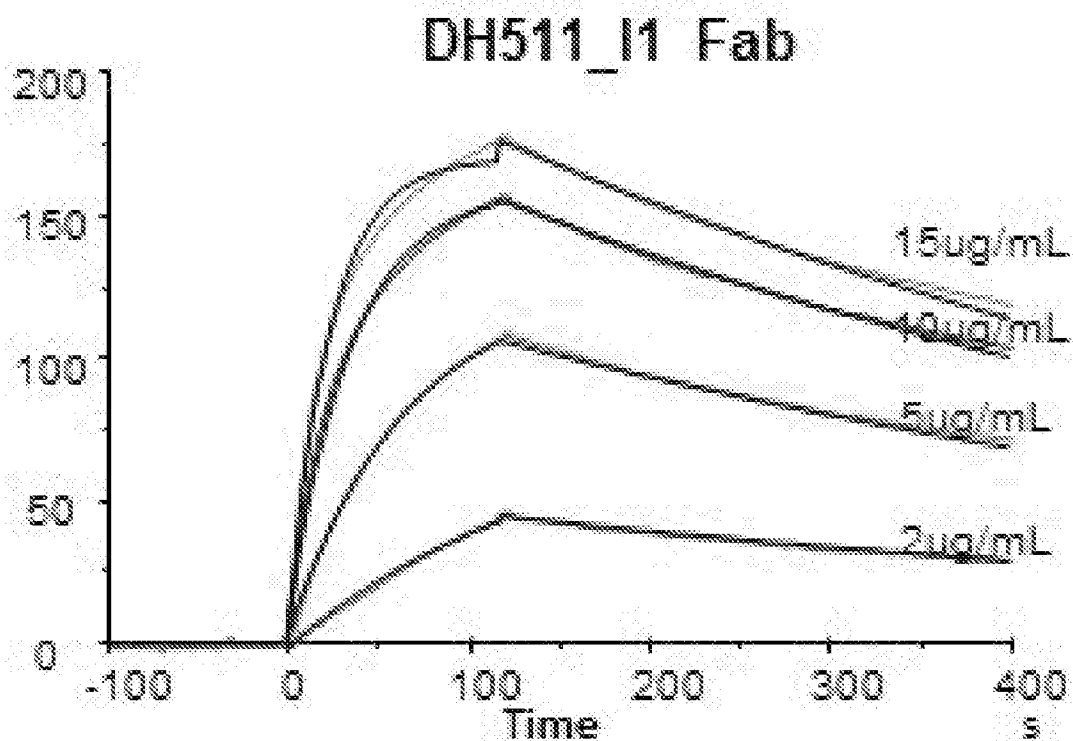
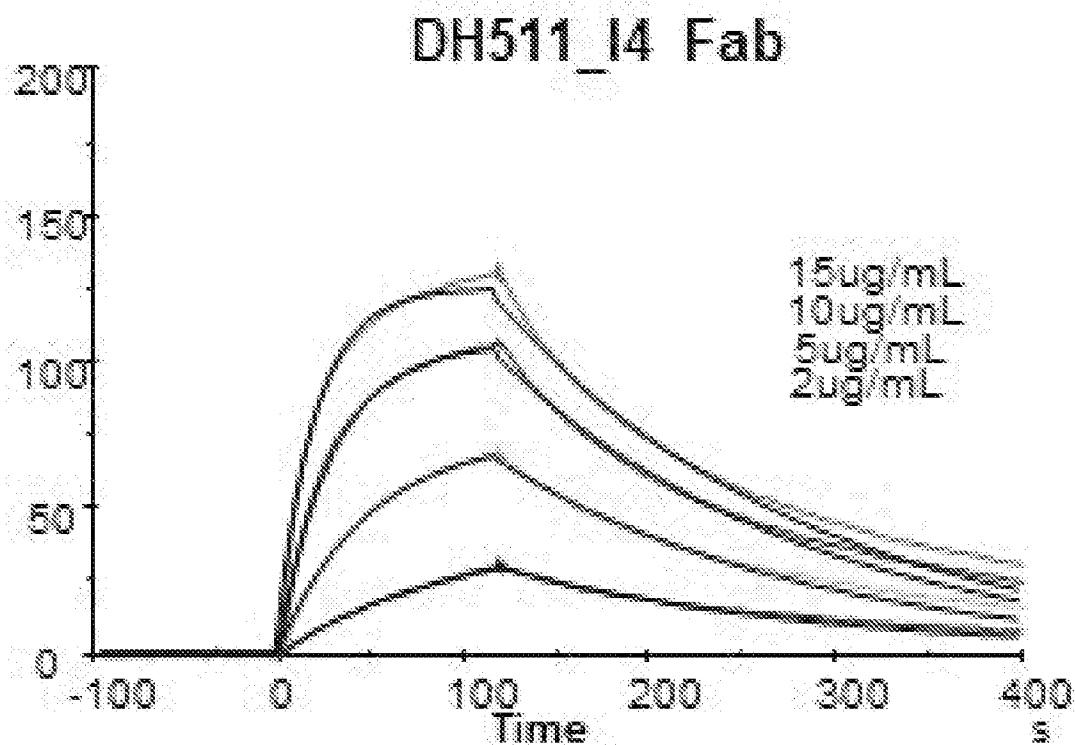


Figure 66B cont.

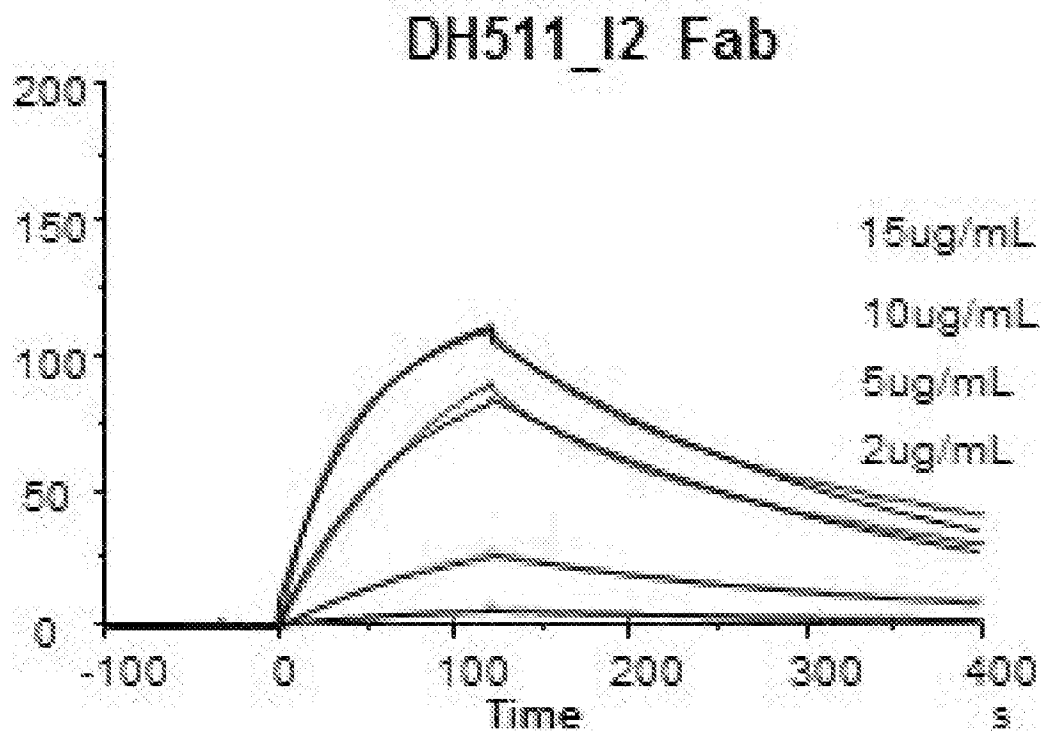
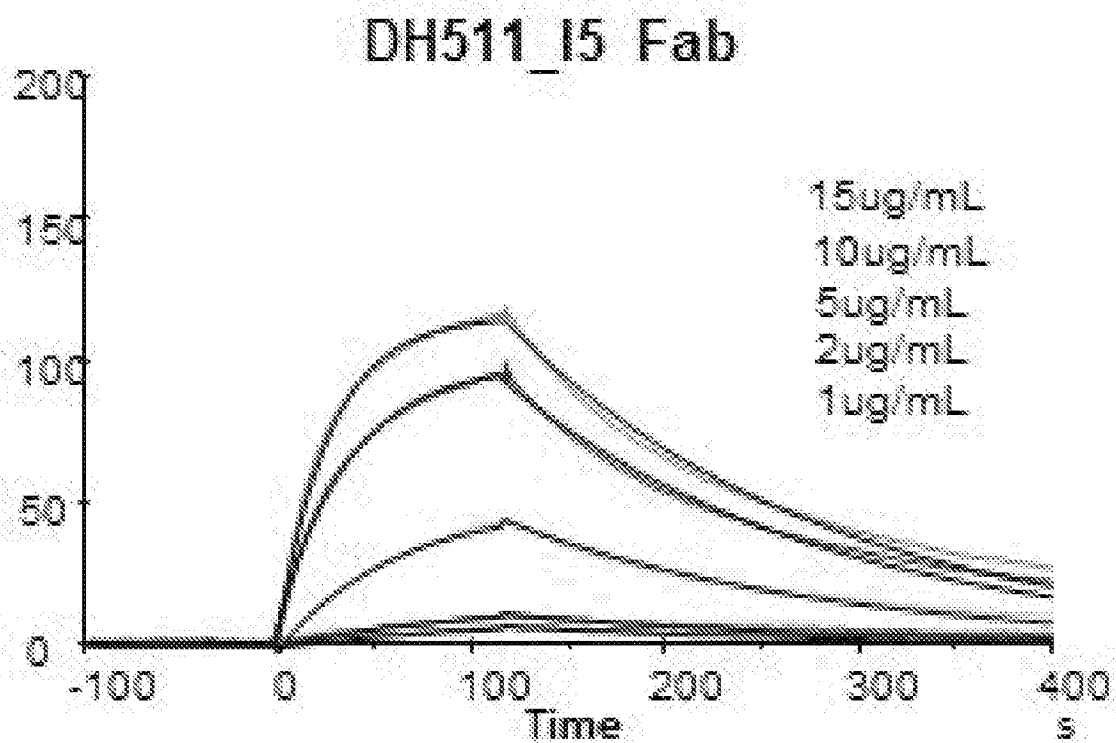


Figure 66B cont.

Fab ID	$k_a (M s^{-1}) \times 10^4$	$k_d (s^{-1}) \times 10^{-4}$	$K_d (nM)$
DH511.1 Fab	10.4	35.8	34.4
DH511.2 Fab	8.35	23.8	37.2
DH511.3 Fab	11.9	52.8	44.4
DH511.4 Fab	10.2	21.1	20.8
DH511.5 Fab	10.5	15.2	14.5
DH511.6 Fab	8.4	84	99.9
DH511_UCA Fab		No binding	
DH511_11 Fab	13.9	15.5	11.1
DH511_12 Fab	5.3	39.8	75.1
DH511_13 Fab	12.8	57.4	45.8
DH511_14 Fab	14.3	59.8	41.8
DH511_15 Fab	10.5	81	58.1
DH511_16 Fab		No binding	

Figure 66C

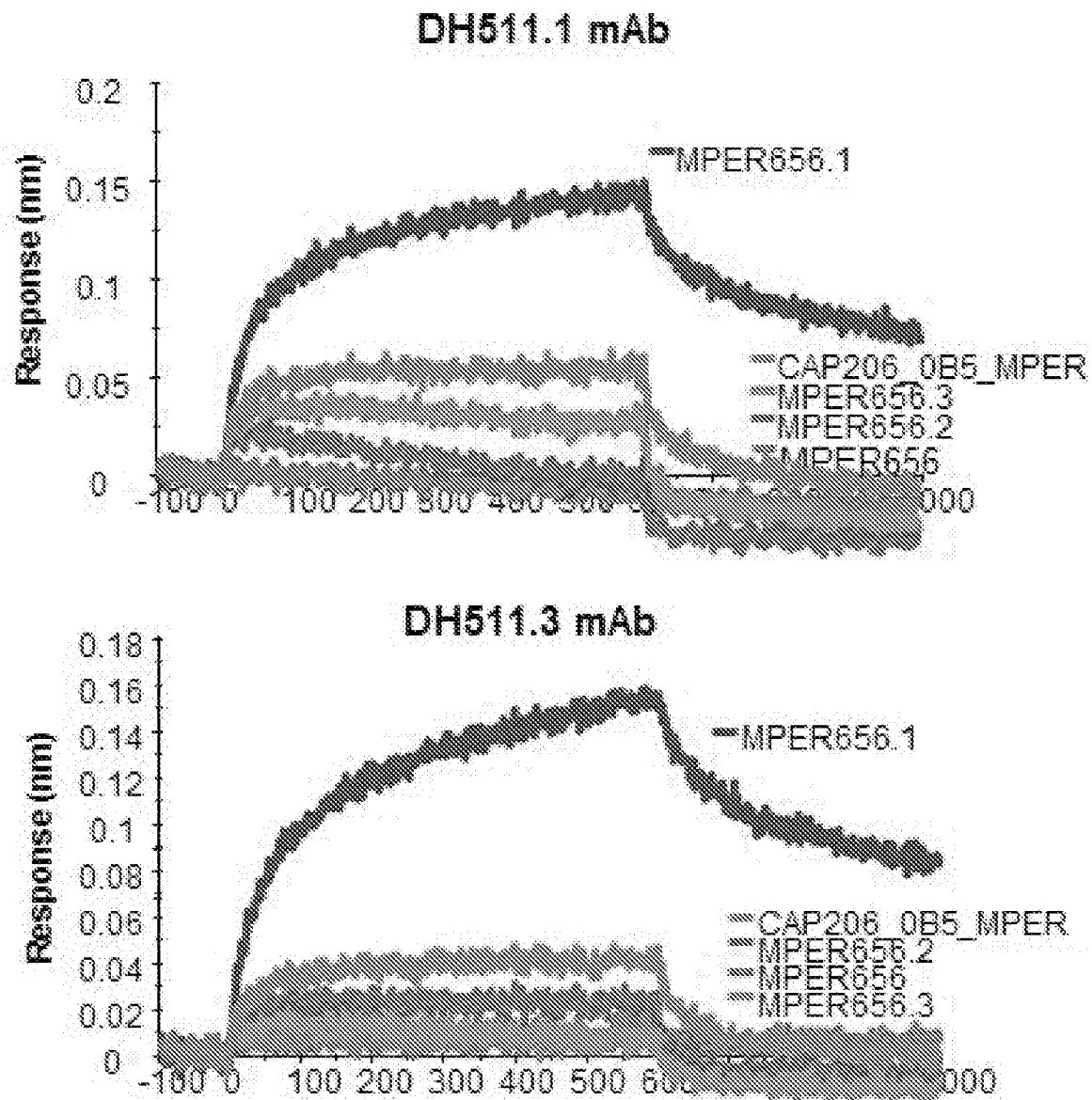


Figure 67A

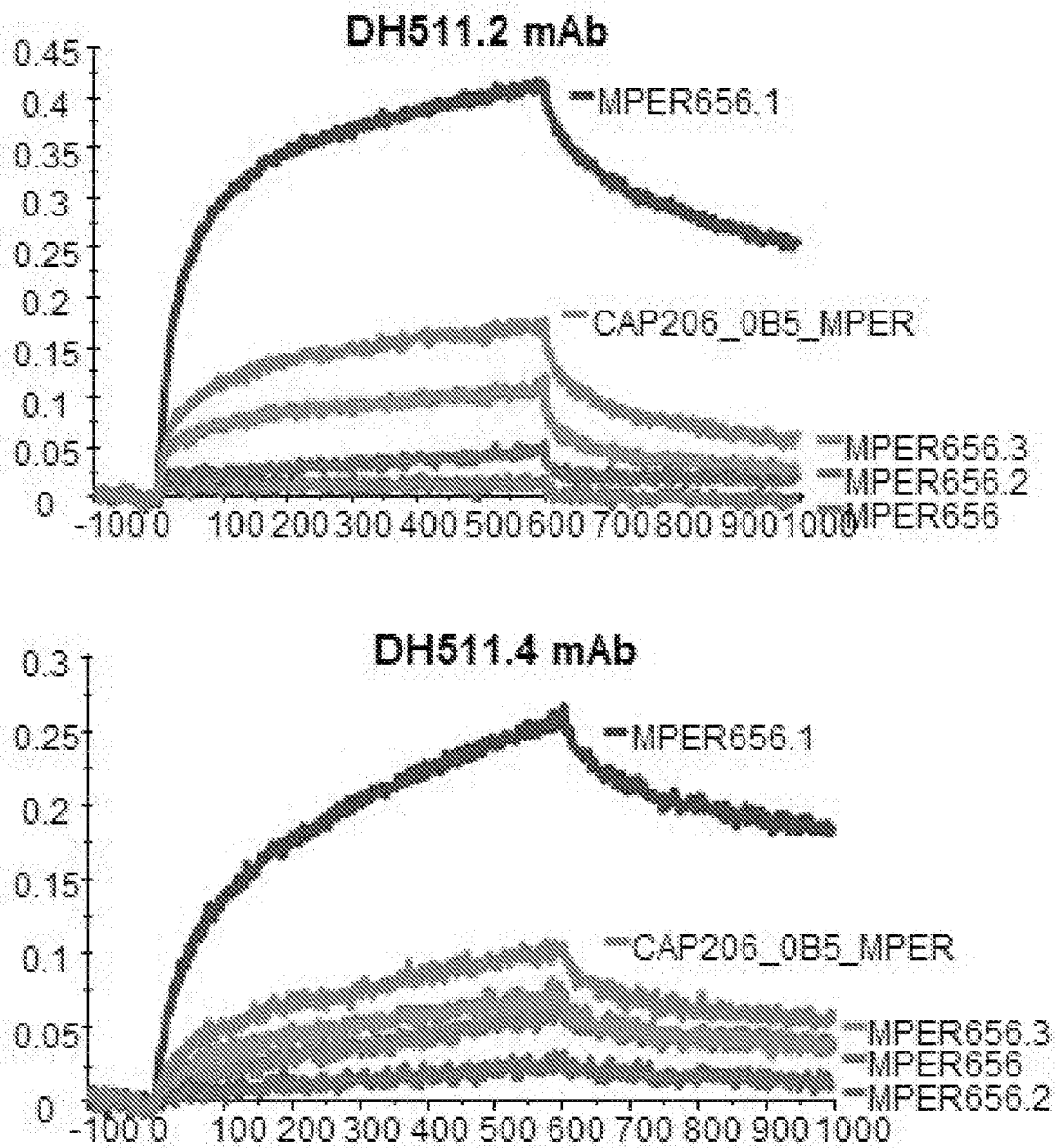


Figure 67A cont.

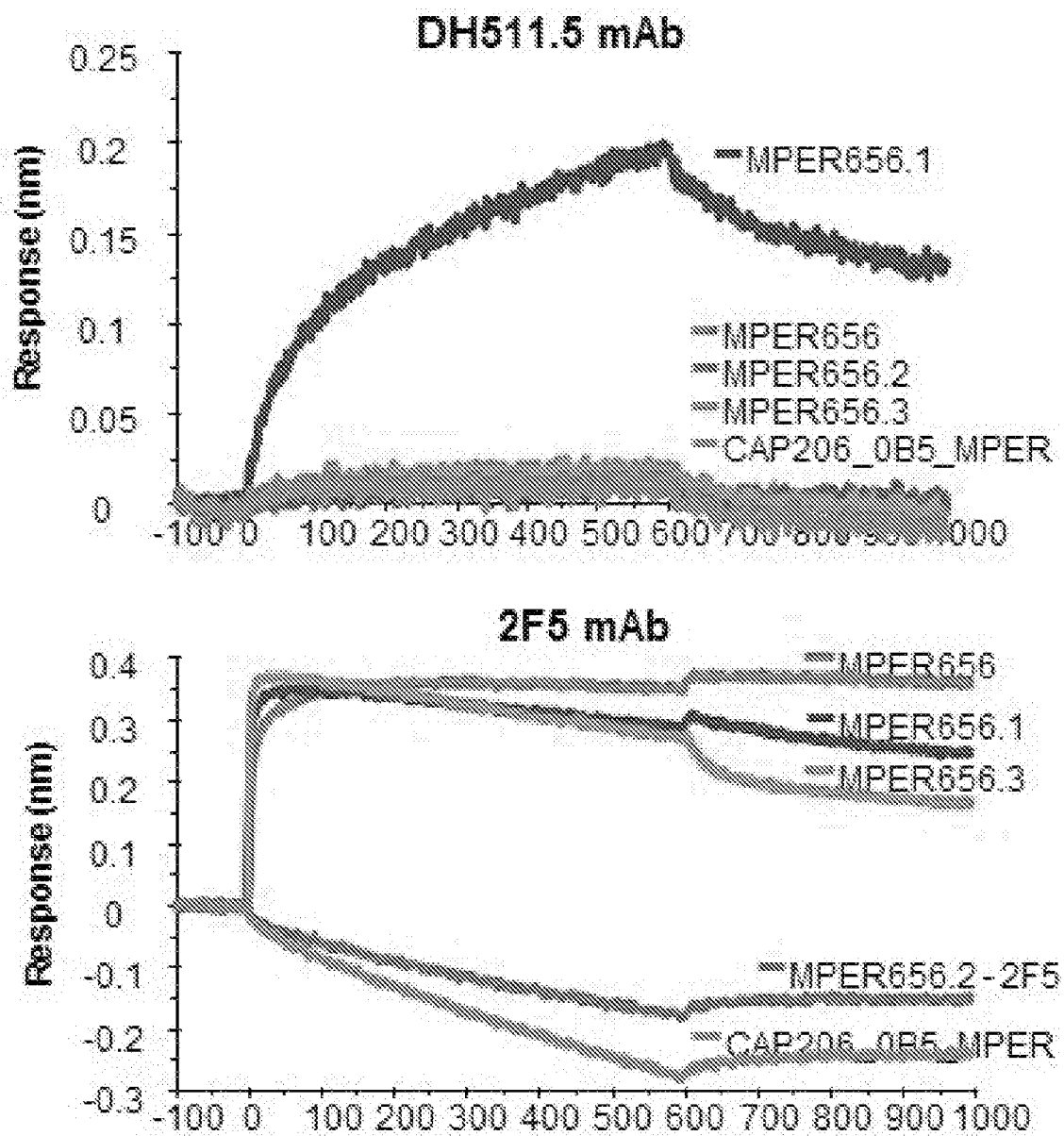


Figure 67B

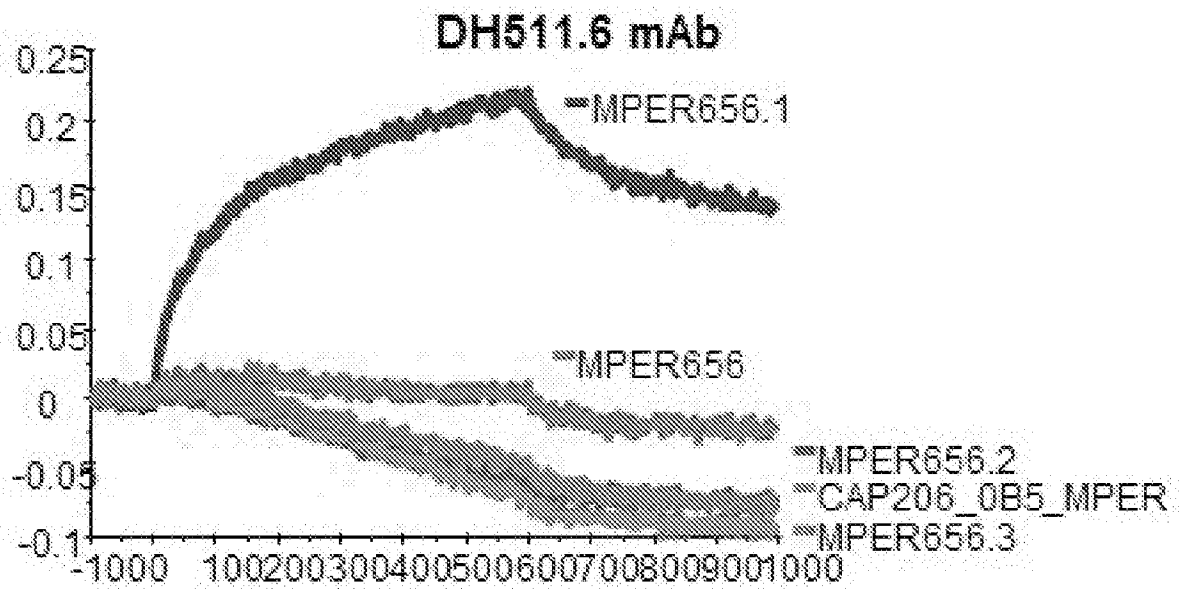


Figure 67B cont.

Peptide name	Sequence
MPER656-biotin	NEQEELLELDKWKASLNWNFETNNWLWYIK-GTHL
MPER656.1-biotin	NEQDILLALDQKWKASLNWNFDSNWLWYIK-GTHL
MPER656.2-biotin	NEKDLLALDQWKNNLNWNFSTKWLWYIK-GTHL
MPER656.3-biotin	NEQEELLELDKWKNNLNWNFETNNWLWYIR-GTHL
CAP206 OncoB5 MPER656-biotin	NEKDLLALDQWKNNLNWNFETKWLWYIK-GTHL

Figure 67C

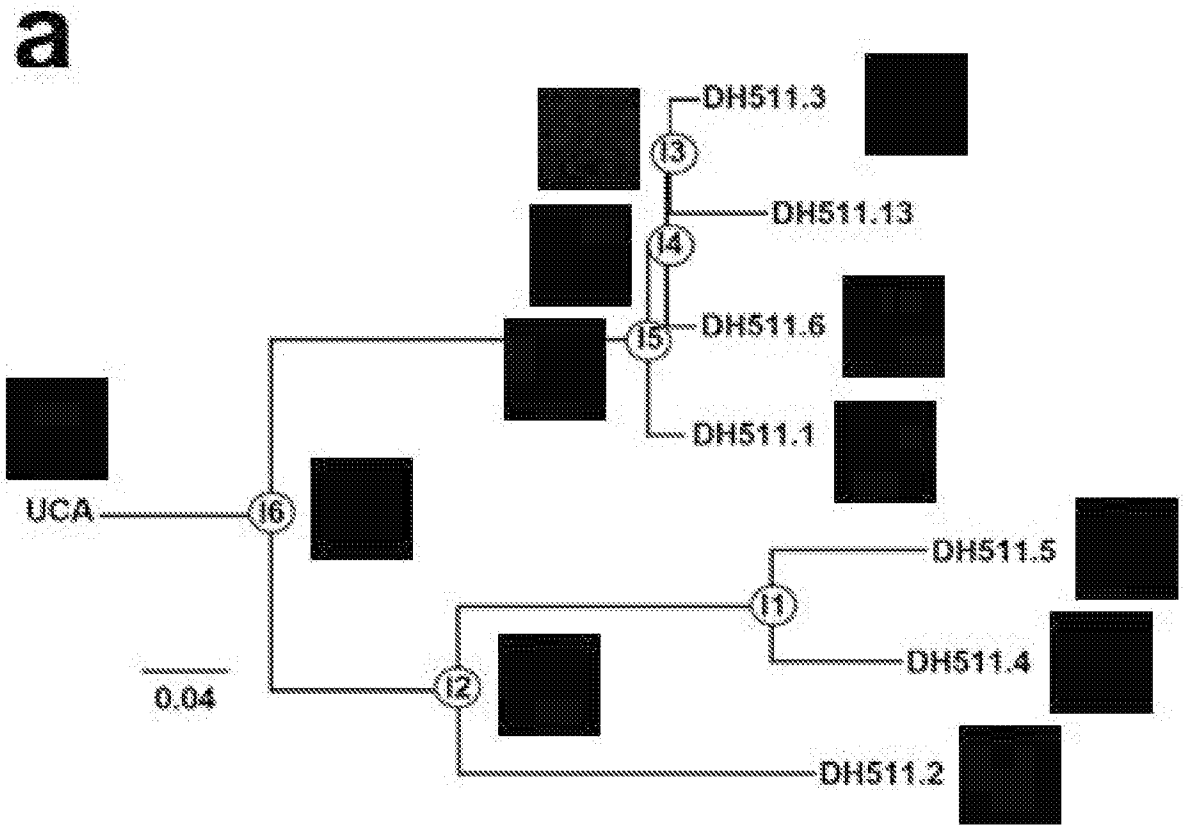


Figure 68A

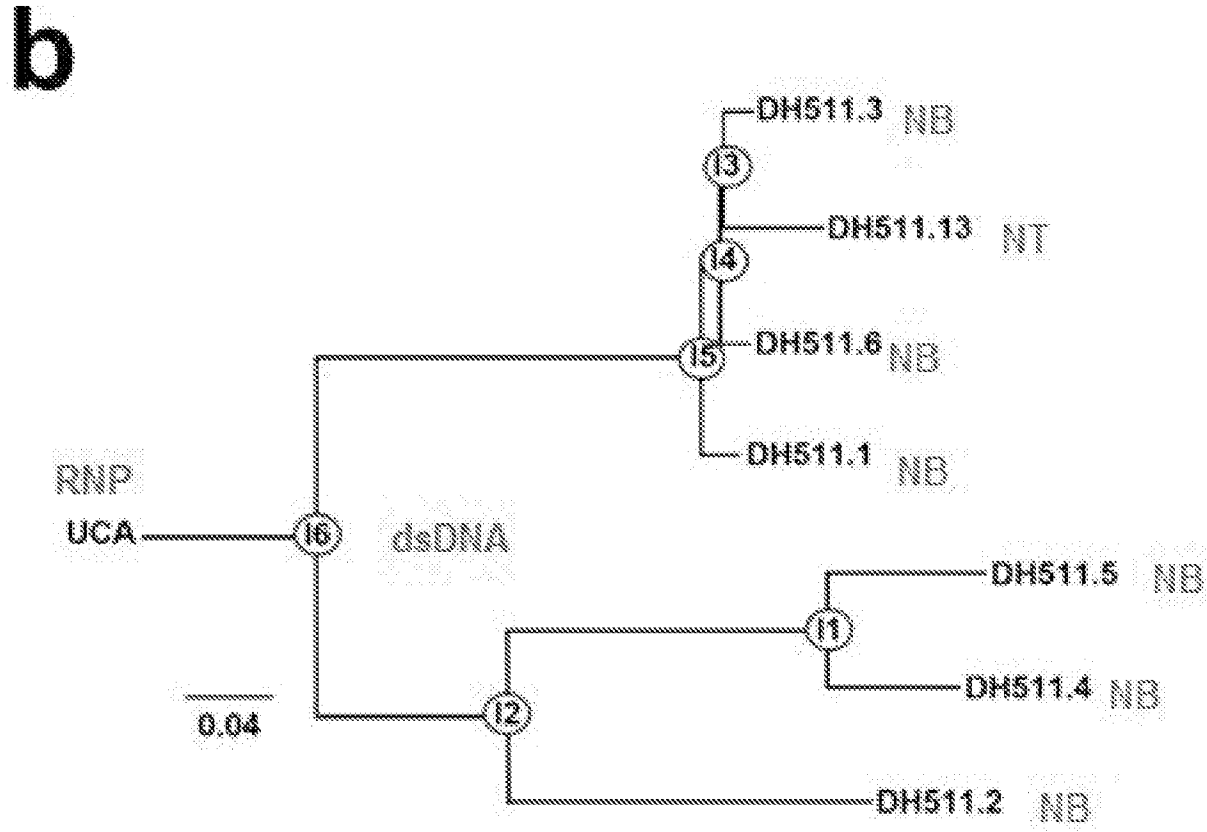


Figure 68B

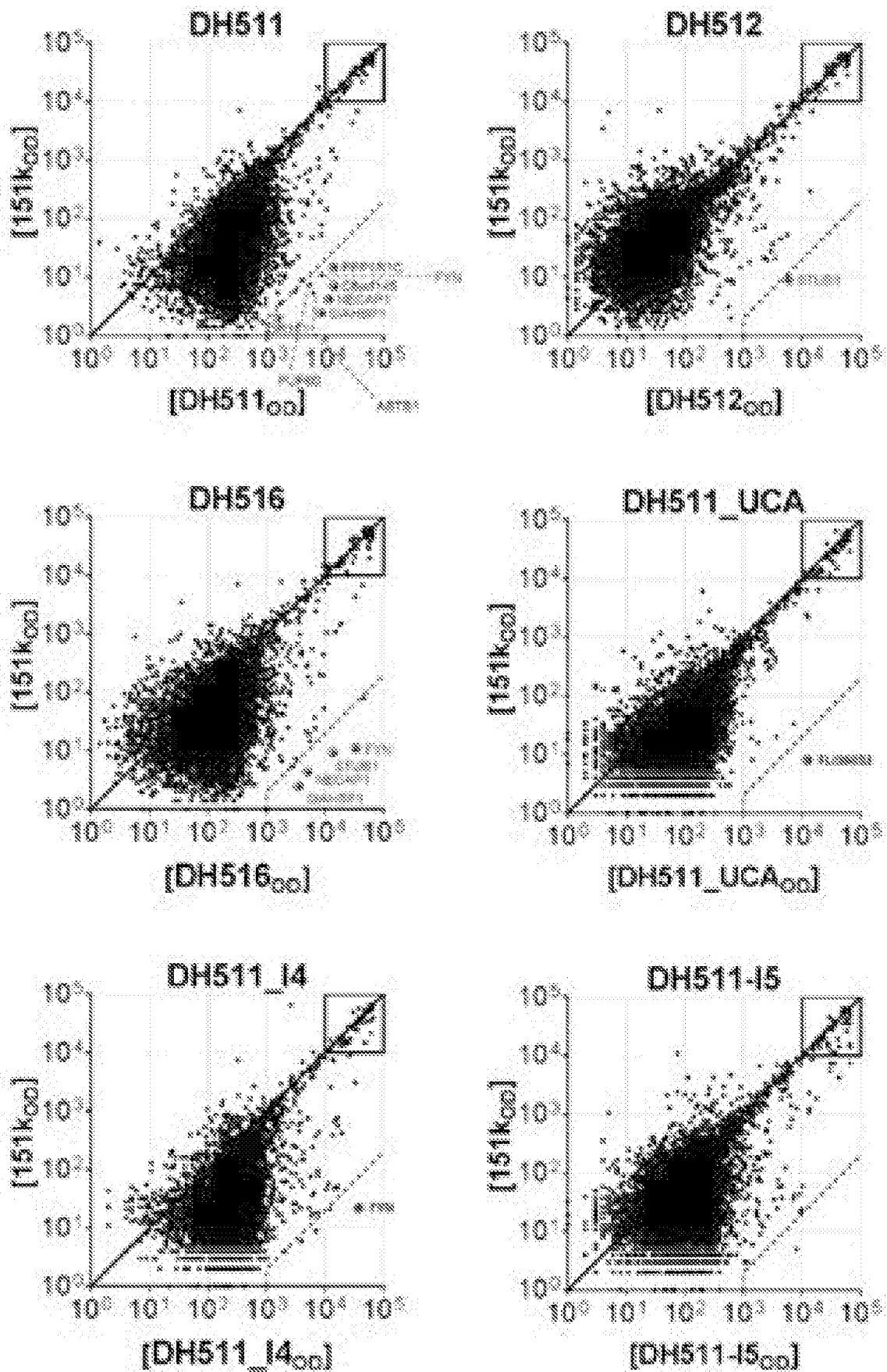


Figure 68C

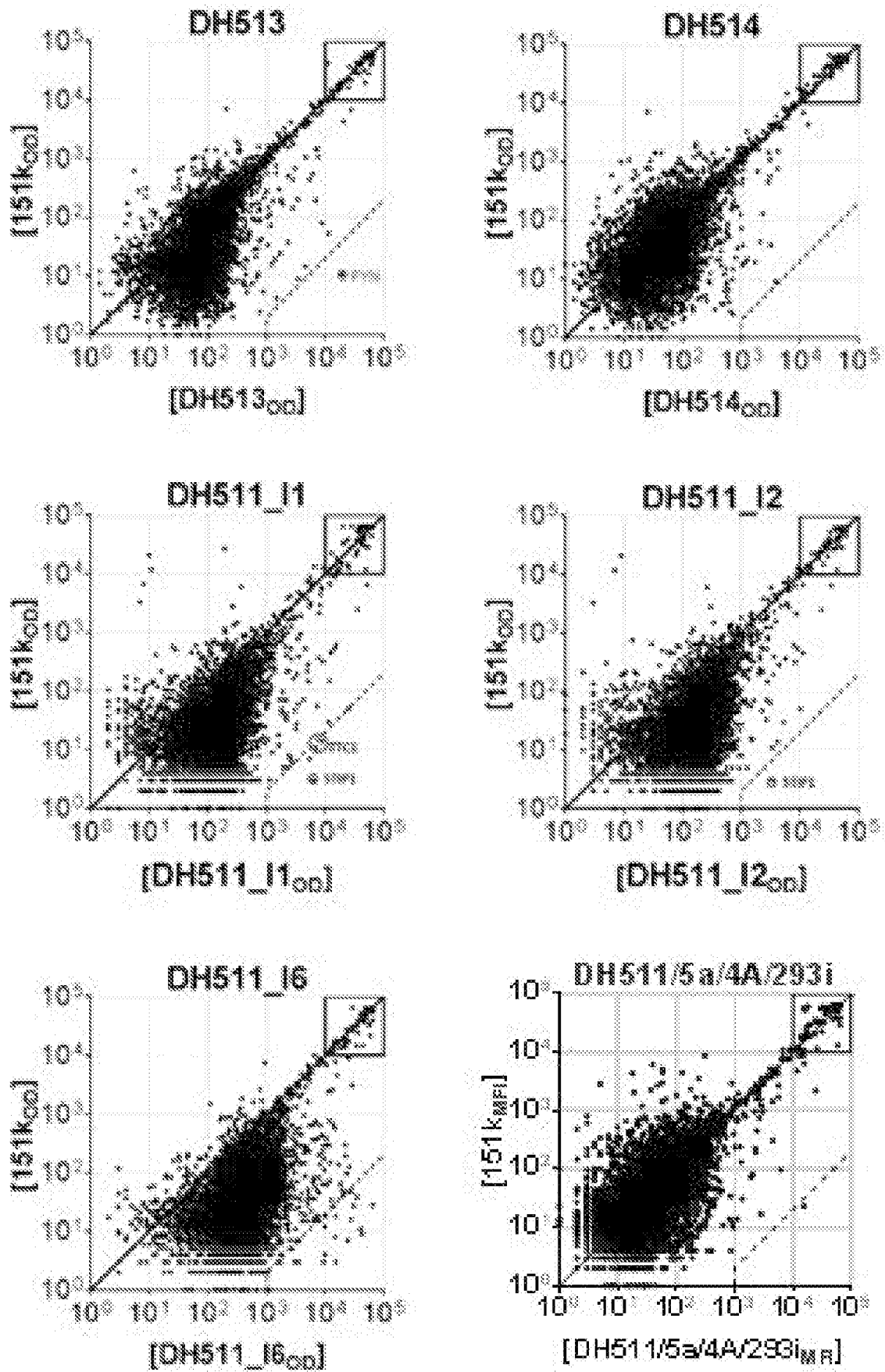


Figure 68C cont.

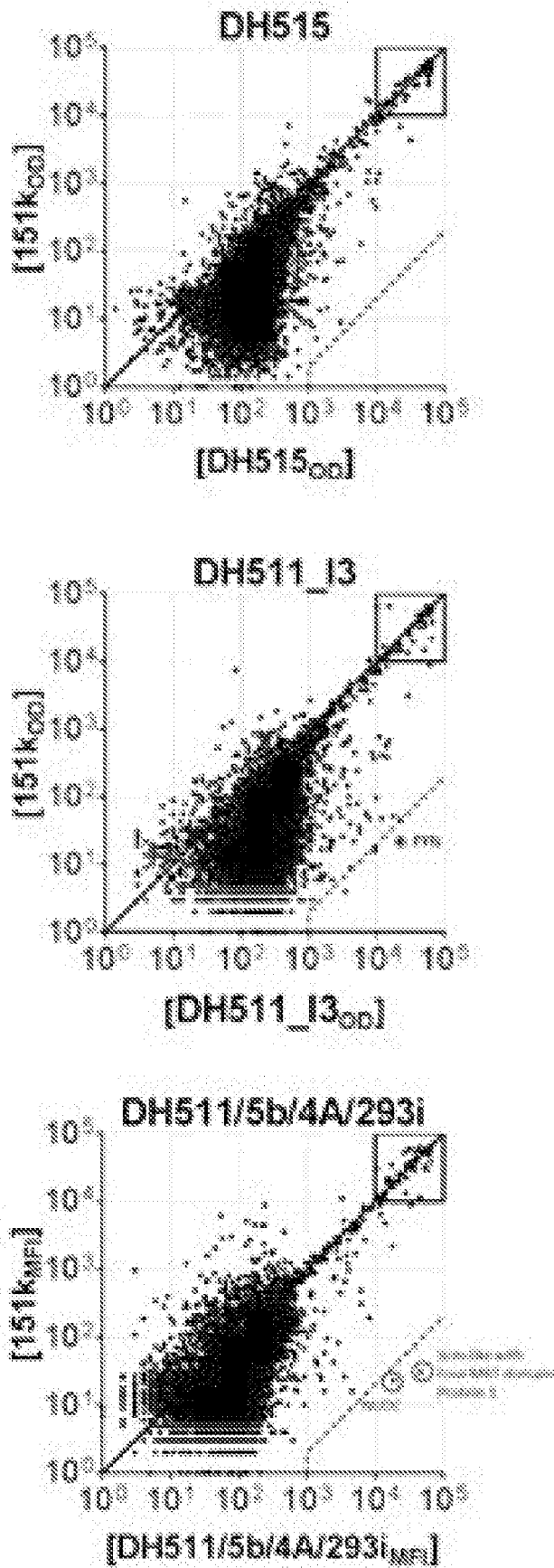


Figure 68C cont.

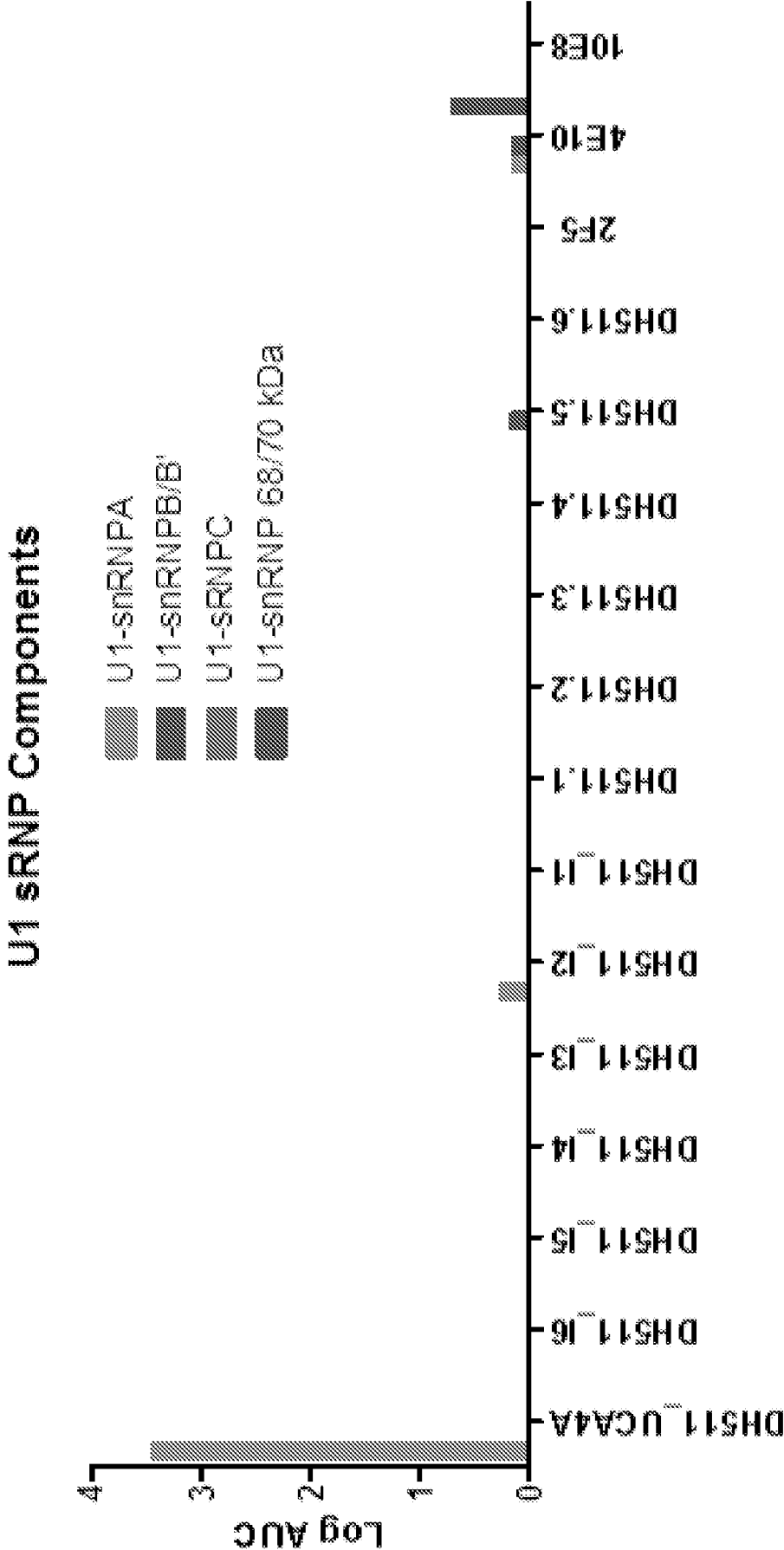


Figure 69

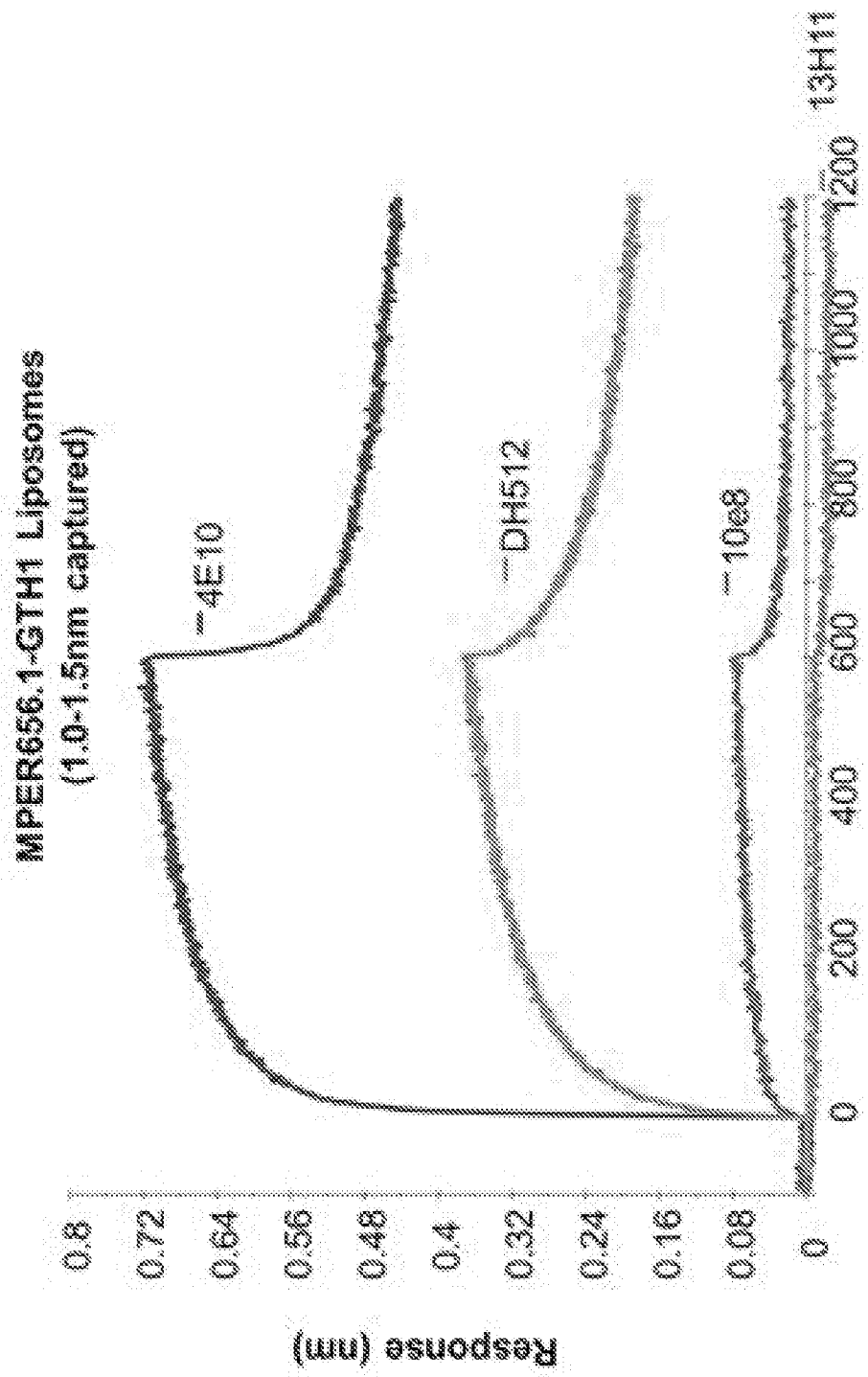


Figure 70

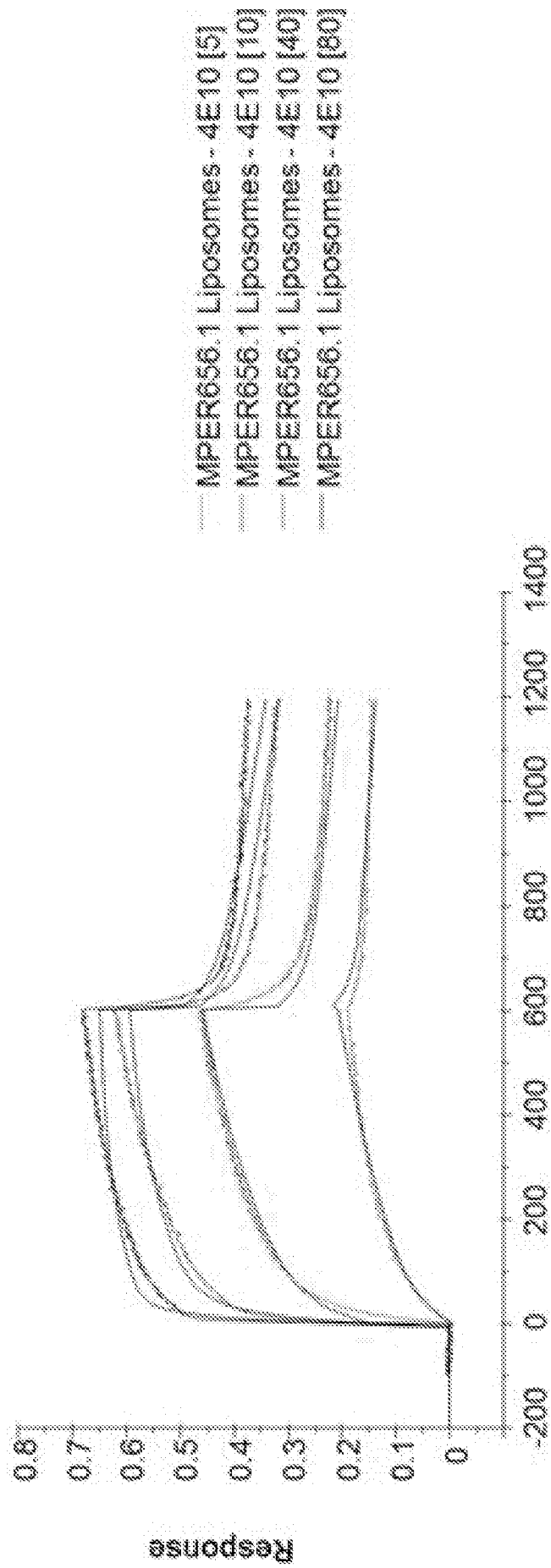


Figure 70 cont.

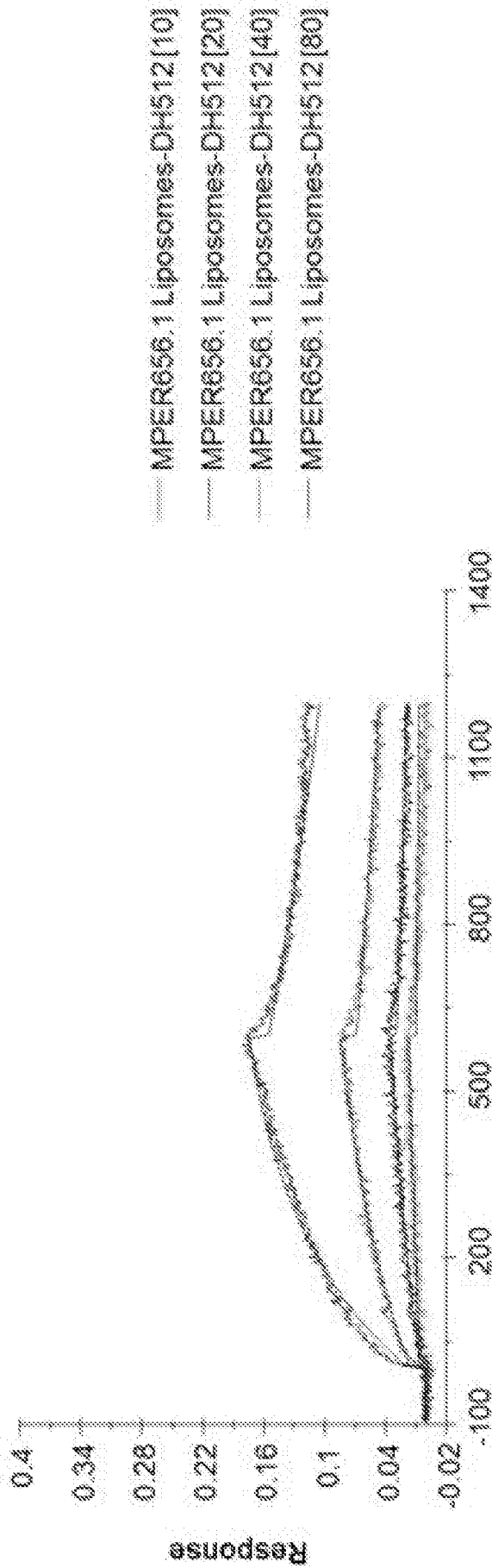


Figure 70 cont.

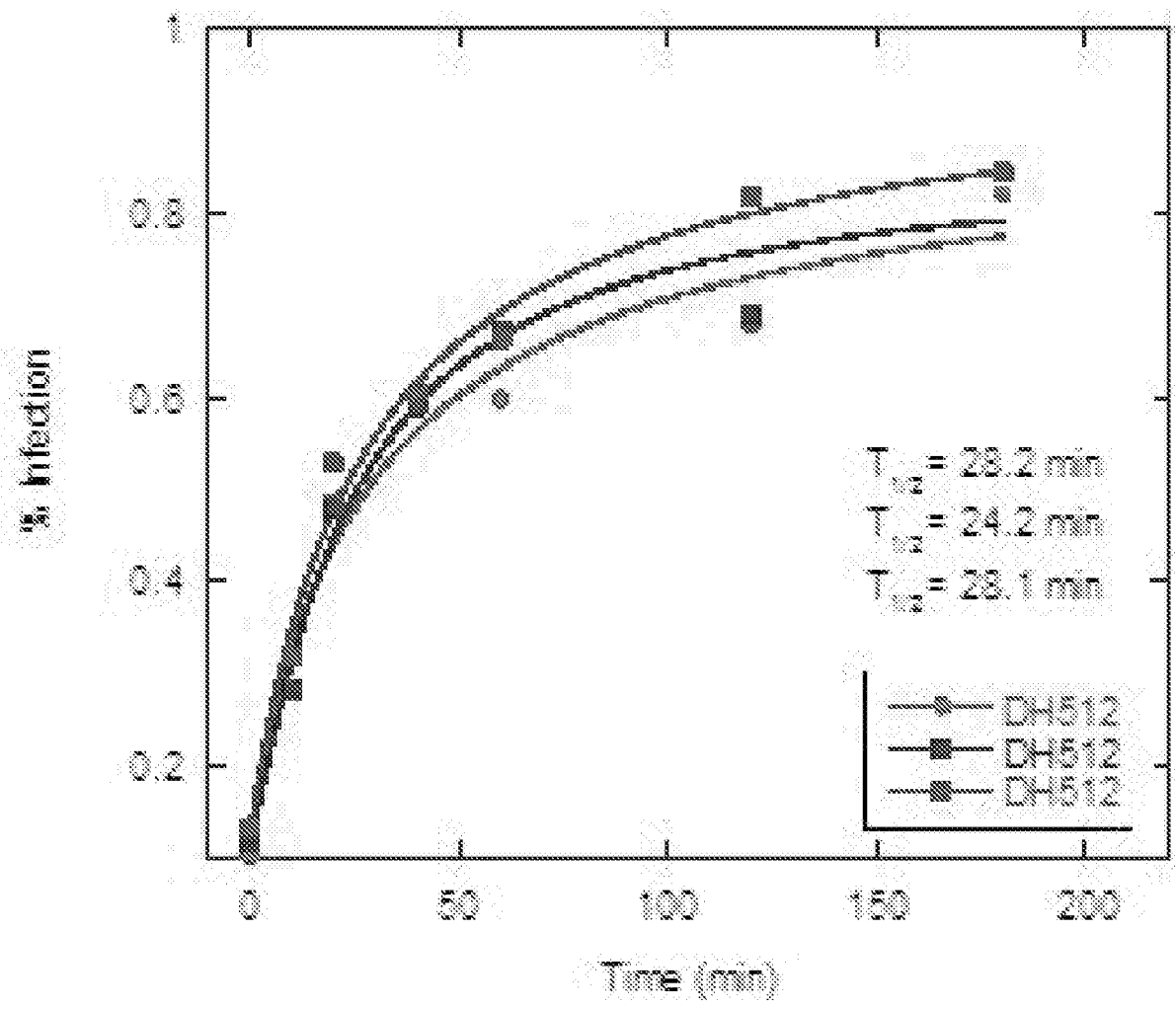


Figure 71A

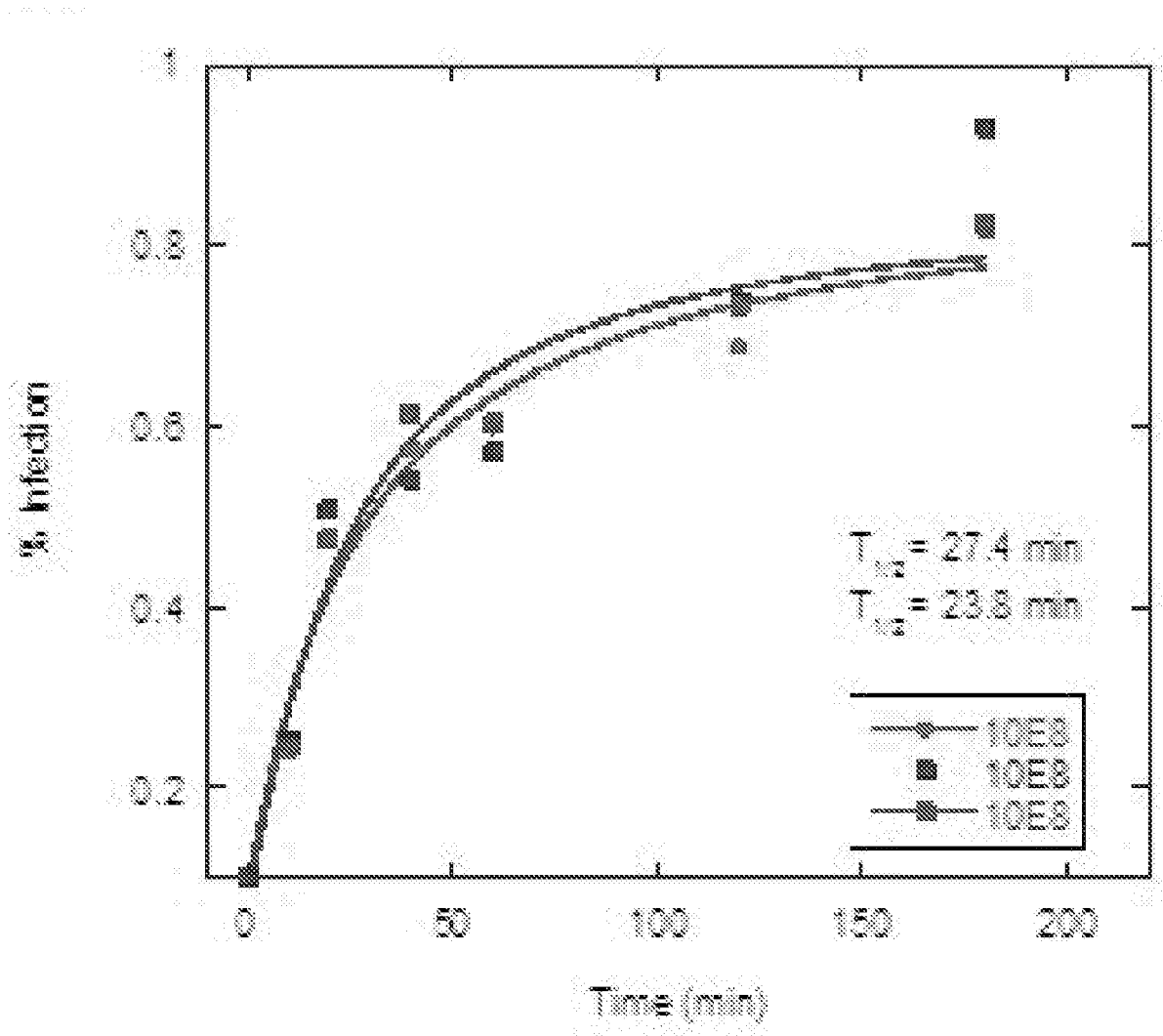


Figure 71B

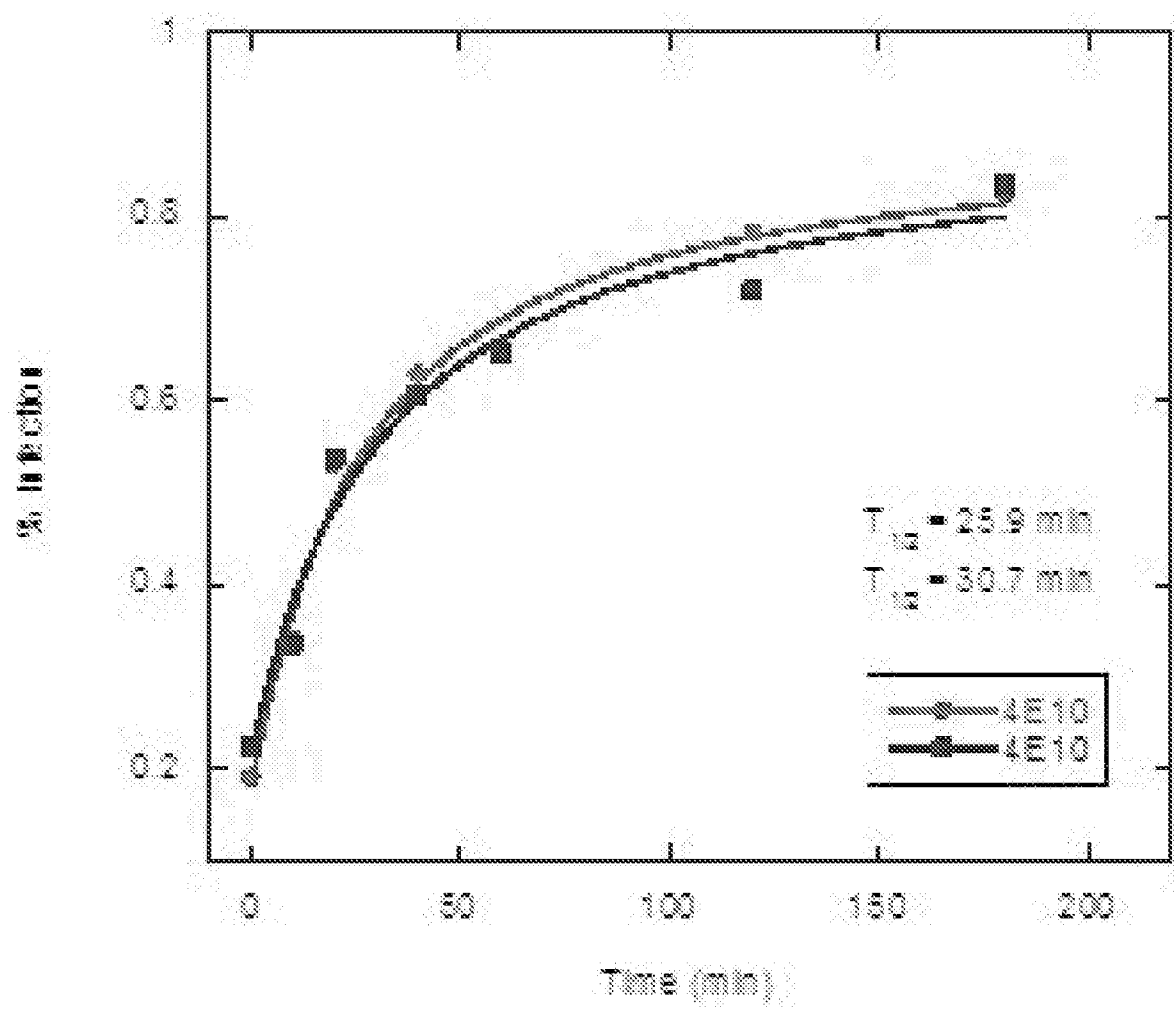


Figure 71C

HCDR3 Sequence Alignment

	92	104
10E8	CARTGK---YYDFWSGYPPEEYFQDWG	
DH511	CTADLGEPVVSRRFFEWGSYYYIMDLWG	
DH512	CTMDEGTPV-TRFLEWGYFYYYMAVWG	

Figure 72

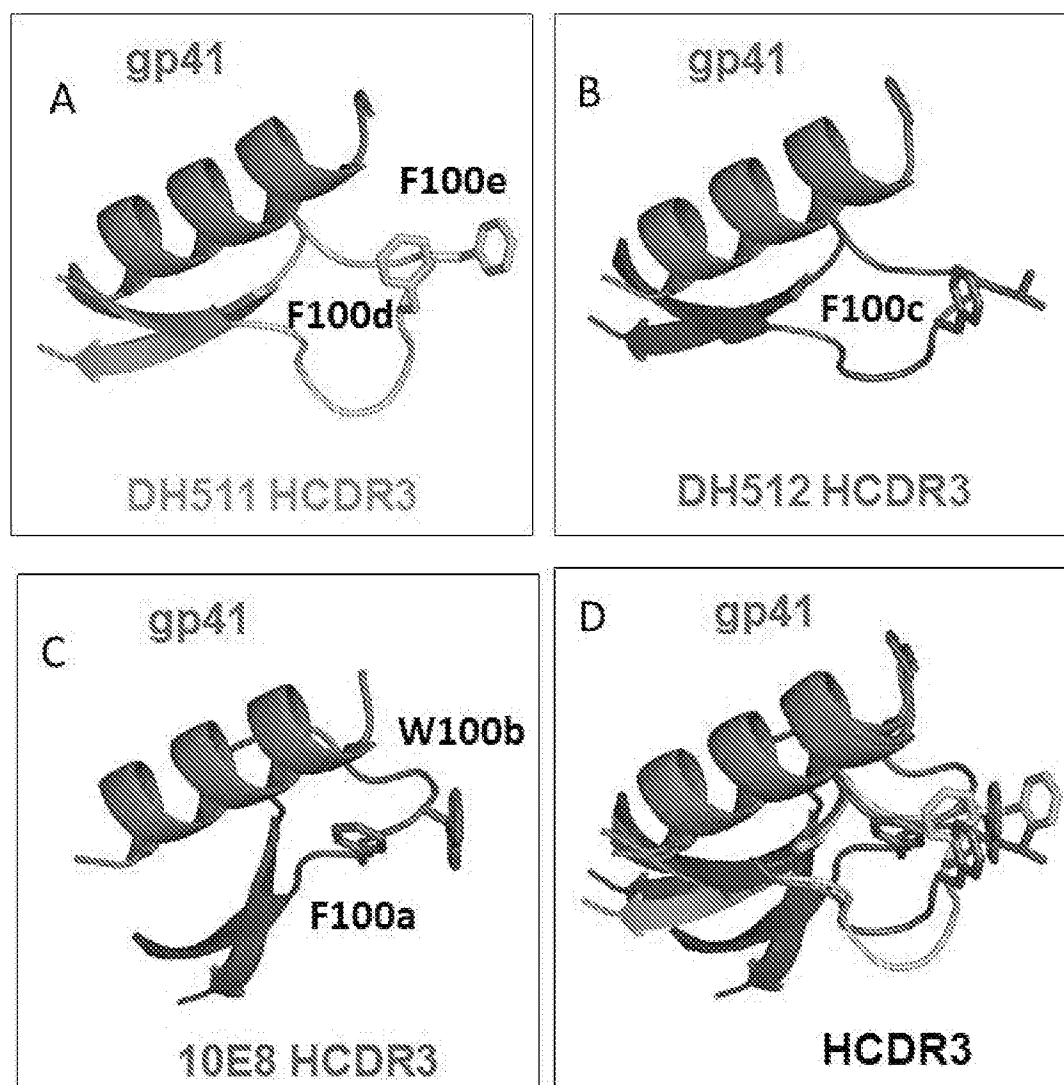


Figure 73A-D

a. HCDR3 Sequence Alignment

	92	104
10E8	CARTGK---YYDFTW	SGYPPPEEYFQDWG
DH511	CTADLGEPVVSRFF	EWGSYYYYMDLWG
DH512	CTMDEGTPV--TRF	EWGYFYYMAVWG

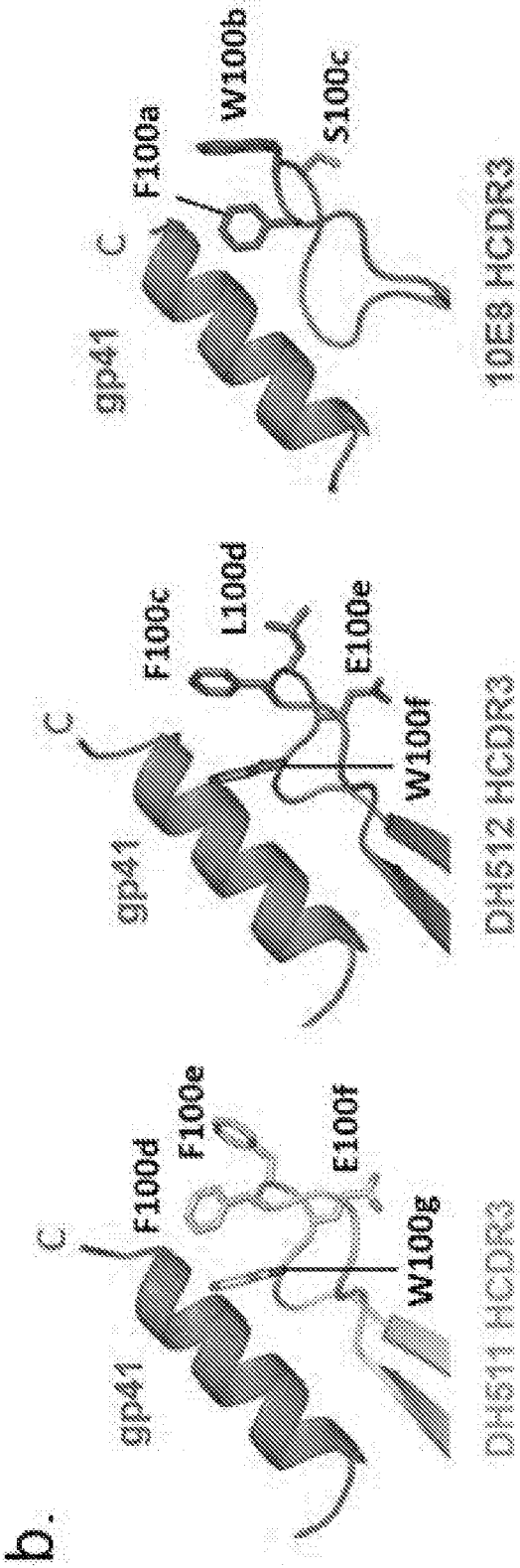


Figure 74A and 74B

**Heavy Chain
Sequences**
Mature Monoclonal
Antibodies

Donor	Antibody ID	IGHV	IGHD	IGHJ	CDR3 length (Amino Acids) ^a	CDR3 Sequence (Amino Acids)	Somatic Mutations (Nucleotides) ^b	Mutation Frequency (Nucleotides) ^b	Mutation Frequency (Amino Acids) ^b
CH0210	DH511.1	3-15*01	3-3*01	6*04	24	TADLGEPVVSRFFEWGSYYYYMDL	46	15.7%	17.7%
CH0210	DH511.2	3-15*01	4-4*01	6*03	23	TMDEGTPVTRFLEWGYFYYYMAV	52	17.6%	27.5%
CH0210	DH511.3	3-15*01	3-3*01	6*03	24	TADLGEAVVSRFFEWGSYYYYMDF	47	16.0%	18.7%
CH0210	DH511.4	3-15*01	3-3*01	6*03	23	TRDEGAPVTRFLEWGSYYYYMAV	63	21.4%	26.5%
CH0210	DH511.5	3-15*01	3-3*01	6*03	23	TRDEGAPVTRFLEWGSYYYYMAV	64	21.8%	29.5%
CH0210	DH511.6	3-15*01	3-3*01	6*03	24	TADLGEAVVSRFFEWGSYYYYMDF	47	16.0%	17.7%
CH0210	DH517	4-34*01	3-16*01	6*01	24	ARCTGVVVGGSWTVPFGMAYILDV	54	18.1%	

Figure 75

Inferred UCA and Intermediate Antibodies

Donor	Antibody ID	IGHV	IGHD	IGH J	CDR3 length (Amino Acids) ^a	CDR3 Sequence (Amino Acids)	Somatic Mutations (Nucleotides) _b	Mutation Frequency (Nucleotides) _b	Mutation Frequency (Amino Acids) _b
CH0210	DH511_UCA	3-15*01	3-3*01	6*03	24	TTDLEGAPVLRFLFWGYYYYMDV	0	0.0%	0.0%
CH0210	DH511_I6	3-15*01	3-3*01	6*03	24	TTDLEGAPVSRFLFWGYYYYMDV	12	4.1%	6.9%
CH0210	DH511_I5	3-15*01	3-3*01	6*03	24	TADLGEAVVSRFFFWGSGYYYYMDF	45	15.3%	16.7%
CH0210	DH511_I4	3-15*01	3-3*01	6*03	24	TADLGEAVVSRFFFWGSGYYYYMDF	46	15.6%	17.7%
CH0210	DH511_I3	3-15*01	3-3*01	6*03	24	TADLGEAVVSRFFFWGSGYYYYMDF	46	15.6%	17.7%
CH0210	DH511_I2	3-15*01	3-3*01	6*03	23	TTDEGAPVTRFLFWGYYYYMAV	18	6.1%	13.8%
CH0210	DH511_I1	3-15*01	3-3*01	6*03	23	TRDEGAPVTRFLFWGSGYYYYMAV	50	17.0%	23.6%

Figure 75 cont.

Light Chain Sequences
Mature Monoclonal Antibodies

Donor	Antibody ID	IGKV	IGKJ	CDR3 length (Amino Acids) ^a	CDR3 Sequence (Amino Acids)	Somatic Mutations (Nucleotides) ^b	Mutation Frequency (Nucleotides) ^b	Mutation Frequency (Amino Acids) ^b
CH0210	DH511.1	1-39*01	2*01	11	QEAYSSPTLT	43	16.3%	17.6%
CH0210	DH511.2	1-39*01	2*03	11	QENYNTIPSLS	37	14.0%	20.7%
CH0210	DH511.3	1-39*01	2*01	11	QEAYSSPTLT	45	17.1%	19.6%
CH0210	DH511.4	1-39*01	2*01	11	QEAYNTNPTLS	42	15.9%	21.7%
CH0210	DH511.5	1-39*01	2*01	11	QEAYNTYPTLS	45	17.1%	22.7%
CH0210	DH511.6	1-39*01	2*03	11	QEAYSSPTLS	45	17.6%	21.7%
CH0210	DH517	3-19*01	2*01	12	ASDRSGDRLGV	35	13.4%	

Figure 75 cont.

Inferred UCA and intermediate antibodies

Donor	Antibody ID	IGKV	IGKJ	CDR3 length (Amino Acids) ^a	CDR3 Sequence (Amino Acids)	Somatic Mutations (Nucleotides) ^b	Mutation Frequency (Nucleotides) ^b	Mutation Frequency (Amino Acids) ^b
CH0210	DH511_UCA	1-39*01	2-1*01	11	QQSYSTPPCT	0	0.0%	1.1%
CH0210	DH511_I6	1-39*01	2-1*01	11	QETYSTTPTFT	10	3.8%	6.2%
CH0210	DH511_I5	1-39*01	2-1*01	11	QEAYSSSTPILT	41	15.5%	17.6%
CH0210	DH511_I4	1-39*01	2-1*01	11	QEAYSSSTPILT	44	16.7%	19.6%
CH0210	DH511_I3	1-39*01	2-1*01	11	QEAYSSSTPILT	44	16.7%	19.6%
CH0210	DH511_I2	1-39*01	2-1*01	11	QETYNTPILT	19	7.2%	12.4%
CH0210	DH511_I1	1-39*01	2-1*01	11	QEAYNTNPILS	37	14.0%	18.6%

^aCDR3 length listed according to the IMGT definition.

^bSomatic mutations were determined over the entire variable region and are reported as the number/frequency of nucleotides or amino acids that differed from the putative germ line V-gene sequences.

Figure 75 cont.

Key (IC₅₀ in µg/ml)

<0.100	0.100-1.00	1.00-10.0	>10.0
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Virus	Clade	IC ₅₀ (µg/ml)								
		DH511.1	DH511.2	DH511.3	DH511.4	DH511.5	DH511.6	DH517	VRC01	10E8
KER2018.11	A	8.15	4.63	18.5	15.4	15.8	18.7	>50	0.701	22.0
Q23.17		4.46	2.52	9.50	9.00	9.55	13.4	>50	0.064	0.783
Q769.h5		0.029	0.014	0.075	1.40	0.037	0.008	>50	0.005	2.67
RW020.2		2.77	1.30	8.92	6.18	4.73	7.17	>50	0.109	1.36
6540.v4.c1	AC	4.41	1.46	10.3	7.83	14.7	12.5	>50	>50	3.01
Q168.a2	AD	5.07	1.04	8.94	10.1	11.0	7.86	>50	0.055	1.93
C1080.c3	AE	0.029	0.017	0.040	0.239	0.091	0.032	0.406	0.195	0.099
CNE59		0.009	0.003	0.020	0.064	0.036	0.015	0.558	0.344	0.001
TH966.8		0.017	0.011	0.015	0.156	0.031	0.010	0.094	0.026	0.024
DJ263.8	AG	0.027	0.066	0.140	0.732	0.218	0.318	0.070	0.088	0.015
6101.10	B	0.074	0.045	0.175	0.310	0.282	0.205	3.54	0.045	0.002
Bal.01		0.517	0.063	0.918	2.47	1.11	0.239	47.9	0.012	0.638
BG1168.01		0.283	0.219	0.705	1.32	1.07	0.609	5.54	0.218	0.405
CAAN.A2		4.52	3.15	9.30	11.3	9.89	14.8	>50	1.59	1.14
JRC5F.JB		1.59	0.674	3.98	5.09	2.79	2.50	>50	0.097	0.523
JRFL.JB		1.56	0.619	2.64	5.02	3.20	1.88	28.3	0.007	0.155
PVO.04		4.04	1.29	9.26	11.0	7.08	8.27	>50	0.254	2.81
THRO.18		0.112	0.146	0.275	0.688	0.639	0.851	10.2	1.49	0.100
TRJO.58		6.25	2.80	8.96	6.52	7.50	8.53	>50	0.078	0.980
TRO.11		0.442	0.235	1.12	0.921	0.932	0.941	7.82	0.409	0.023
YU2.DG		2.75	1.30	6.36	5.33	4.08	3.67	>50	0.034	2.23
CNE58	C	2.68	0.682	5.83	4.60	3.74	8.16	>50	0.171	0.501
DU156.12		0.134	0.036	0.206	0.167	0.396	0.327	5.70	0.053	0.004
DU172.17		0.457	2.71	6.92	4.63	2.24	9.07	15.4	>50	0.026
DU422.01		0.909	0.565	2.83	1.84	2.10	3.57	18.1	>50	0.189
ZA012.29		2.18	0.690	3.67	3.75	3.21	0.880	>50	0.542	3.09
ZM106.9		10.1	3.35	20.8	14.5	16.8	16.3	>50	0.173	>25
ZM55.28a		10.9	5.95	16.6	10.0	24.9	32.9	>50	0.313	2.85
57128.vrc.15	D	0.156	0.061	0.333	0.898	1.18	0.274	0.945	>50	0.611
X1632.S2.B10	G	0.314	0.159	0.409	0.323	0.705	0.671	18.8	0.023	0.579
AC10.29	B	ND	ND	ND	ND	ND	ND	ND	0.138	0.171
AC10.29A332(S334A)		ND	ND	ND	ND	ND	ND	ND	0.562	ND
6644.V2.C33	C	ND	ND	ND	ND	ND	ND	ND	0.202	0.007
		DH511.1	DH511.2	DH511.3	DH511.4	DH511.5	DH511.6	DH517	VRC01	10E8
# Viruses		30	30	30	30	30	30	30	30	30
Total Viruses Neutralized										
IC ₅₀ <50µg/ml		30	30	30	30	30	30	15	26	29
IC ₅₀ <1µg/ml		15	18	12	10	10	14	5	24	19
IC ₅₀ <0.1µg/ml		6	9	4	1	4	4	2	13	8
Percent of Viruses Neutralized										
IC ₅₀ <50µg/ml		100	100	100	100	100	100	50	87	97
IC ₅₀ <1µg/ml		50	60	40	33	33	47	17	80	63
IC ₅₀ <0.1µg/ml		20	30	13	3	13	13	7	43	27
Median IC ₅₀		1.23	0.647	3.25	4.18	2.52	2.19	5.70	0.103	0.579
Geometric Mean		0.669	0.336	1.50	2.17	1.64	1.34	3.39	0.107	0.290

Figure 76

Key (IC₅₀ in µg/ml)

<0.100	0.100-1.00	1.00-10.0	>10.0
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Virus	Clade	IC ₅₀ (µg/ml)								
		DH511.1	DH511.2	DH511.3	DH511.4	DH511.5	DH511.6	DH517	VRC01	10E8
KER2018.11	A	30.1	16.4	48.0	39.5	>50	>50	>50	2.19	6.38
Q23.17		17.0	11.6	37.0	27.7	29.7	43.6	>50	0.265	3.02
Q769.h5		3.01	0.697	8.02	13.5	23.8	49.5	>50	0.046	6.62
RW020.2		16.1	7.75	34.2	18.3	26.7	43.6	>50	0.589	4.10
6540.v4.c1	AC	32.7	24.5	>50	>50	>50	>50	>50	>50	7.86
Q168.a2	AD	34.6	12.7	>50	>50	>50	>50	>50	0.351	5.23
C1080.c3	AE	0.543	0.333	1.06	1.97	1.34	0.776	13.6	2.91	0.683
CNE59		0.066	0.033	0.181	0.262	0.247	0.125	3.06	2.48	0.038
TH966.8		0.196	0.178	0.475	1.08	1.24	0.360	>50	0.423	0.315
DJ263.8	AG	0.297	0.697	2.16	4.93	5.01	5.91	10.5	0.434	0.176
6101.10	B	0.620	0.574	1.32	2.04	1.98	1.85	25.4	0.215	0.021
Bal.01		8.37	3.23	19.3	19.3	23.2	49.7	>50	0.097	2.49
BG1168.01		2.40	1.94	5.46	6.79	7.86	6.83	28.7	1.64	1.52
CAAN.A2		27.3	16.2	49.4	>50	>50	>50	>50	5.62	5.97
JRCSE.JB		13.7	5.99	28.1	24.7	19.1	30.0	>50	0.660	2.38
JRFL.JB		10.1	6.91	22.9	21.9	17.4	22.9	>50	0.050	0.824
PVO.04		35.2	13.0	>50	>50	>50	>50	>50	1.31	8.54
THRO.18		2.23	1.86	3.54	4.32	8.87	10.1	>50	27.0	0.715
TRJO.58		21.5	11.1	20.6	28.5	33.9	45.2	>50	0.293	4.33
TRO.11		2.17	1.27	4.68	3.92	3.69	4.28	20.3	1.67	0.398
YU2.DG		28.8	16.2	>50	>50	>50	>50	>50	0.179	9.46
CNE58		11.3	4.25	29.5	22.1	21.2	46.9	>50	0.556	2.52
DU156.12		0.828	0.309	1.37	0.928	1.66	1.75	31.3	0.248	0.051
DU172.17		4.09	11.6	19.3	15.6	17.8	>50	>50	>50	0.300
DU422.01	C	5.67	2.93	10.6	7.58	8.28	13.0	>50	>50	1.10
ZA012.29		13.3	6.70	28.9	18.5	28.2	>50	>50	2.66	6.43
ZM106.9		43.1	24.0	>50	>50	>50	>50	>50	0.979	>25
ZM55.28a		28.8	12.0	>50	>50	>50	>50	>50	1.37	11.8
57128.vrc.15	D	7.32	2.23	9.06	8.04	9.76	8.71	>50	>50	2.46
X1632.S2.B10	G	3.54	1.82	6.60	4.68	7.00	9.37	>50	0.344	2.35
AC10.29	B	ND	ND	ND	ND	ND	ND	ND	4.40	0.947
AC10.29Δ332(S334A)		ND	ND	ND	ND	ND	ND	ND	5.75	ND
6644.V2.C33	C	ND	ND	ND	ND	ND	ND	ND	1.31	0.116
		DH511.1	DH511.2	DH511.3	DH511.4	DH511.5	DH511.6	DH517	VRC01	10E8
# Viruses		30	30	30	30	30	30	30	30	30
Total Viruses Neutralized										
IC50 <50ug/ml		30	30	24	23	22	20	7	26	29
IC50 <1ug/ml		6	7	2	2	1	3	0	16	10
IC50 <0.1ug/ml		1	1	0	0	0	0	0	3	3
Percent of Viruses Neutralized										
IC50 <50ug/ml		100	100	80	77	73	67	23	87	97
IC50 <1ug/ml		20	23	7	7	3	10	0	53	33
IC50 <0.1ug/ml		3	3	0	0	0	0	0	10	10
Median IC50		9.24	5.12	9.83	8.04	9.32	9.74	20.3	0.608	2.460
Geometric Mean		5.64	3.24	7.73	7.36	7.86	8.09	15.4	0.670	1.444

Figure 77

Key (IC₅₀/IC₈₀ in µg/ml)

<0.100	0.100-1.00	1.00-10.0	>10.0
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Virus	Clade	IC ₅₀ (µg/ml)			IC ₈₀ (µg/ml)		
		DH511.2	10E8	VRC01	DH511.2	10E8	VRC01
0260.v5.c36	A	17.0	9.87	0.502	>50	21.7	1.24
0330.v4.c3	A	1.42	1.12	0.056	7.37	3.64	0.184
0439.v5.c1	A	2.36	1.23	0.113	9.57	3.95	0.369
3365.v2.c20	A	2.29	1.60	0.054	7.89	4.56	0.129
3415.v1.c1	A	12.6	4.69	0.091	22.1	11.5	0.238
3718.v3.c11	A	3.80	0.838	0.572	13.5	4.42	>50
398-F1_F6_20	A	2.49	0.704	0.134	18.0	6.17	0.493
BB201.B42	A	2.82	0.613	0.338	9.87	1.96	0.926
BG505.W6M.C2	A	1.33	0.689	0.049	5.60	2.14	0.164
B369.9A	A	1.61	0.356	0.220	4.93	1.29	0.769
BS208.B1	A	0.803	0.319	0.030	3.35	3.27	0.080
KER2008.12	A	0.723	>50	0.485	4.29	>50	1.58
KER2018.11	A	4.31	1.89	0.674	17.0	7.16	1.73
KNH1209.18	A	0.410	0.406	0.068	2.25	2.39	0.213
MB201.A1	A	0.844	0.411	0.170	4.22	1.36	0.496
MB539.2B7	A	>50	>50	0.326	>50	>50	0.890
MI369.A5	A	0.510	0.671	0.228	3.41	1.77	0.742
MS208.A1	A	0.927	0.187	0.195	3.56	1.14	0.661
Q23.17	A	1.18	0.461	0.058	5.85	1.60	0.166
Q259.17	A	12.4	4.76	0.060	30.7	12.0	0.182
Q769.d22	A	2.44	1.91	0.033	8.64	4.47	0.102
Q769.h5	A	5.73	2.89	0.068	16.7	7.44	0.149
Q842.d12	A	5.73	2.62	0.025	12.8	7.58	0.082
QH209.14MA2	A	2.20	1.30	0.033	10.0	4.09	0.093
RW020.2	A	3.46	0.902	0.366	10.0	2.92	0.947
UG037.8	A	0.273	0.046	0.087	1.33	0.353	0.282
246-F3.C10.2	AC	0.802	0.210	0.215	5.10	1.49	0.760
3301.V1.C24	AC	12.5	2.97	0.097	33.8	9.50	0.234
3589.V1.C4	AC	3.26	5.77	0.079	10.2	11.7	0.222
6540.v4.c1	AC	2.84	2.24	>50	13.4	7.01	>50
6545.V4.C1	AC	7.79	2.54	>50	15.4	7.50	>50
0815.V3.C3	ACD	2.04	0.491	0.023	7.05	1.81	0.072
6095.V1.C10	ACD	0.0003	0.0005	0.674	0.0010	0.004	2.42
3468.V1.C12	AD	2.00	0.381	0.040	7.45	2.04	0.135
Q168.a2	AD	1.45	0.463	0.084	5.18	2.88	0.193
Q461.e2	AD	2.45	2.29	0.398	8.68	4.68	1.14
620345.c1	AE	1.77	0.989	>50	9.05	3.73	>50
BJOX009000.02.4	AE	0.580	0.251	2.27	5.03	1.47	5.95
BJOX010000.06.2	AE	0.178	0.060	8.13	1.23	0.478	23.3
BJOX025000.01.1	AE	0.751	0.228	7.50	4.77	1.54	16.6
BJOX028000.10.3	AE	0.853	0.167	0.181	4.59	0.876	0.811
C1080.c3	AE	0.521	0.108	3.26	2.50	0.613	10.8
C2101.c1	AE	4.64	1.20	0.243	15.6	4.12	0.678
C3347.c11	AE	0.046	0.019	0.119	0.274	0.083	0.333
C4118.09	AE	1.66	0.421	0.149	11.6	2.30	0.633
CM244.ec1	AE		0.365			1.46	
CNE3	AE	2.81	1.37	1.37	9.17	4.01	8.70
CNE5	AE	1.54	1.17	0.233	7.34	2.52	0.890
CNE55	AE	0.287	0.038	0.264	2.00	0.605	0.902
CNE56	AE	0.212	0.060	0.295	1.18	0.314	1.25

Figure 78

Key (IC₅₀/IC₉₀ in µg/ml)

<0.100	0.100-1.00	1.00-10.0	>10.0
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Virus	Clade	IC ₅₀ (µg/ml)			IC ₉₀ (µg/ml)		
		DH511.2	10E8	VRC01	DH511.2	10E8	VRC01
CNE59	AE	0.0003	0.0010	0.498	0.023	0.010	1.71
CNE8	AE	0.045	0.140	0.414	0.483	1.42	1.27
R1166.c1	AE	0.742	0.488	1.93	3.70	2.02	6.61
R2184.c4	AE	1.12	0.576	0.998	5.40	2.20	0.311
R3265.c6	AE	6.31	1.58	0.443	21.9	9.28	1.61
TH966.8	AE	0.061	0.039	0.482	0.611	0.291	1.39
TH976.17	AE	0.762	0.392	0.360	4.08	1.75	1.11
235-47	AG	0.361	0.244	0.042	1.61	0.786	0.122
242-14	AG	0.069	0.568	>50	0.750	3.17	>50
263-8	AG	0.407	0.229	0.108	2.63	0.991	0.415
269-12	AG	0.420	0.124	0.211	2.10	0.475	0.537
271-11	AG	1.23	0.891	0.042	7.60	4.34	0.147
928-28	AG	0.215	0.079	0.353	1.28	0.365	1.08
DJ263.8	AG	0.190	0.009	0.057	0.903	0.100	0.305
T250-4	AG	3.54	1.07	>50	13.1	3.45	>50
T251-18	AG	7.52	0.666	5.67	20.5	2.55	12.7
T253-11	AG	3.51	1.21	0.403	12.0	4.05	0.994
T255-34	AG	1.40	0.228	0.773	6.11	1.14	2.54
T257-31	AG	3.42	0.336	2.23	10.5	1.58	6.43
T266-60	AG	0.953	>50	3.39	4.78	>50	11.9
T278-50	AG	1.89	0.357	>50	8.95	2.10	>50
T280-5	AG	4.21		0.029	17.0		0.080
T33-7	AG	2.34	0.818	0.016	10.2	2.83	0.057
3988.25	B	0.284	0.070	0.475	0.863	0.293	1.25
5768.04	B	5.70	1.63	0.323	16.0	5.26	0.824
6101.10	B	0.012	0.0010	0.095	0.137	0.005	0.153
6535.3	B	1.39	0.190	1.78	7.62	1.28	4.60
7165.18	B	0.805	0.659	16.1	3.55	2.71	>50
45_01dG5	B	0.753	0.106	0.013	4.87	0.703	0.030
89.6.DG	B	0.676	0.318	0.975	2.23	1.48	3.29
AC10.29	B	0.115	0.102	0.973	0.604	0.512	2.98
ADA.DG	B	0.042	0.056	0.322	0.482	0.358	1.29
Bal.01	B	0.620	0.421	0.067	4.09	1.91	0.259
Bal.26	B	2.57	0.518	0.061	11.0	2.39	0.203
BG1168.01	B	0.528	0.396	0.977	2.38	1.48	3.45
BL01.DG	B	1.08	0.362	>50	6.87	1.57	>50
BR07.DG	B	0.106	0.118	1.36	1.03	0.445	5.14
BX08.16	B	0.565	0.213	0.429	3.02	1.30	1.03
CAANA2	B	5.29	1.45	1.29	16.3	5.70	3.48
CNE10	B	0.121	0.014	0.782	0.932	0.169	1.99
CNE12	B	0.509	0.301	0.563	2.46	1.09	1.93
CNE14	B	0.950	0.151	0.218	4.24	0.649	0.742
CNE4	B	0.279	0.059	0.800	2.45	0.437	2.43
CNE57	B	0.120	0.059	0.507	1.28	0.317	1.43
HO86.8	B	2.55	0.326	>50	10.7	1.52	>50
HT593.1	B	0.140	0.049	0.647	0.780	0.285	2.04
HXB2.DG	B	0.0003	0.003	0.039	0.012	0.015	0.116
JRC5F.JB	B	2.04	0.429	0.254	9.61	1.89	0.840
JRFL.JB	B	0.862	0.174	0.024	5.40	0.768	0.068
MN.3	B	0.0003	0.0003	0.016	0.004	0.0010	0.037

Figure 78 cont.

Key (IC₅₀/IC₈₀ in µg/ml)

<0.100	0.100-1.00	1.00-10.0	>10.0
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Virus	Clade	IC ₅₀ (µg/ml)			IC ₈₀ (µg/ml)		
		DH511.2	10E8	VRC01	DH511.2	10E8	VRC01
PVO.04	B	2.32	1.60	0.395	15.9	6.43	1.03
QH0515.01	B	4.97	2.25	1.17	15.8	5.54	3.58
QH0692.42	B	2.03	0.531	1.77	11.1	2.35	4.92
REJO.67	B	0.544	0.302	0.078	3.31	1.18	0.203
RHPA.7	B	5.45	1.01	0.040	25.9	5.10	0.132
SC422.8	B	1.63	0.343	0.197	4.60	1.15	0.501
SF162.LS	B	1.88	0.245	0.367	7.75	1.06	0.875
SS1196.01	B	0.857	0.244	0.408	4.72	1.25	1.08
THRO.18	B	0.711	0.992	6.24	2.39	0.587	23.2
TRJO.58	B	3.86	1.13	0.111	17.7	4.18	0.292
TRO.11	B	0.228	0.928	0.541	1.22	0.286	1.54
WITO.33	B	0.314	0.931	0.125	1.60	0.305	0.372
X2278.C2.B1	B	3.79	0.442	0.116	14.1	2.24	0.320
YU2.DG	B	8.00	1.17	0.087	50	5.46	0.238
BJOX002000.03.2	BC	1.17	0.384	>50	5.64	1.56	>50
CH038.12	BC	1.57	0.271	0.378	6.10	1.41	0.939
CH070.1	BC	12.1	6.65	8.57	44.6	13.5	>50
CH117.4	BC	0.458	0.270	0.062	2.29	0.859	0.194
CH119.10	BC	0.916	0.591	0.577	5.48	2.36	1.84
CH181.12	BC	0.959	0.754	0.407	3.99	2.79	1.21
CNE15	BC	1.27	0.844	0.078	6.77	2.97	0.282
CNE19	BC	0.383	0.251	0.141	3.05	1.11	0.577
CNE20	BC	0.370	0.131	7.20	2.43	0.732	>50
CNE21	BC	1.09	0.979	0.232	8.40	3.25	0.882
CNE40	BC	0.0003	0.0010	0.301	0.030	0.009	3.54
CNE7	BC	0.119	0.130	0.183	0.672	0.603	0.837
286.36	C	3.22	1.19	0.325	14.8	5.00	0.748
288.38	C	2.19	0.435	1.31	8.90	3.08	4.05
0013095-2.11	C	0.014	0.009	0.111	0.229	0.077	0.316
001428-2.42	C	3.33	1.71	0.010	16.3	6.28	0.038
0077.V1.C16	C	5.56	1.86	0.655	36.0	7.11	2.37
00836-2.5	C	1.89	0.666	0.126	5.40	1.77	0.639
0921.V2.C14	C	5.33	0.908	0.220	18.4	3.03	0.727
16055-2.3	C	2.16	1.10	0.094	9.40	3.31	0.244
16845-2.22	C	0.015	0.020	4.69	0.238	0.172	17.7
16936-2.21	C	0.312	0.264	0.179	1.99	1.31	0.474
25710-2.43	C	0.048	0.064	0.577	0.408	0.304	1.83
25711-2.4	C	6.14	0.516	0.496	12.6	1.69	1.59
25925-2.22	C	0.504	0.402	0.469	2.95	1.53	1.32
25191-2.48	C	1.82	1.83	0.139	8.77	4.90	0.490
3168.V4.C10	C	2.52	2.83	0.139	19.3	8.18	0.394
3637.V5.C3	C	2.83	2.12	3.84	11.1	6.68	8.30
3873.V1.C24	C	10.0	5.51	0.767	13.8	15.7	2.64
6322.V4.C1	C	2.90	0.923	>50	9.72	3.68	>50
6471.V1.C16	C	11.0	4.98	>50	41.9	14.9	>50
6631.V3.C10	C	7.08	0.934	>50	15.5	3.36	>50
6644.V2.C33	C	0.229	0.913	0.182	1.14	0.124	0.731
6785.V5.C14	C	1.58	0.701	0.281	6.70	2.42	0.900
6838.V1.C35	C	0.813	0.292	0.181	3.67	1.01	0.694
96ZM651.02	C	0.035	0.933	0.979	0.358	0.177	2.27

Figure 78. cont

Key (IC₅₀/IC₈₀ in µg/ml)

<0.100	0.100-1.00	1.00-10.0	>10.0
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Virus	Clade	IC ₅₀ (µg/ml)			IC ₈₀ (µg/ml)		
		DH511.2	10E8	VRC01	DH511.2	10E8	VRC01
BR025.9	C	1.10	0.307	0.222	5.33	1.11	1.61
CAP210.E8	C	0.911	0.474	>50	3.37	2.01	>50
CAP244.D3	C	1.13	0.369	1.21	4.56	1.48	3.37
CAP256.206.C9	C	0.779	0.713	0.915	3.92	2.97	2.10
CAP45.G3	C	2.84	0.722	7.52	9.41	3.41	45.3
Ce1176.A3	C	0.998	0.252	2.64	4.13	1.15	7.56
CE703010217.B6	C	0.159	0.096	0.295	0.944	0.679	0.758
CNE30	C	3.30	0.456	0.808	16.3	2.29	2.10
CNE31	C	5.23	1.32	0.947	11.5	3.57	2.02
CNE53	C	0.208	0.213	0.062	1.41	1.01	0.221
CNE58	C	0.453	0.229	0.132	2.98	1.09	0.436
DU123.06	C	0.250	0.132	9.08	0.948	0.423	25.5
DU151.02	C	0.928	0.461	17.3	3.60	1.71	>50
DU156.12	C	0.019	0.023	0.081	0.158	0.120	0.230
DU172.17	C	7.22	0.087	>50	19.9	0.238	>50
DU422.01	C	0.821	0.224	>50	3.95	0.812	>50
MMV965.26	C	0.0003	0.0010	0.023	0.002	0.007	0.065
SO18.18	C	7.52	1.60	0.067	20.7	4.48	0.162
TV1.29	C	0.802	0.248	>50	2.66	0.719	>50
TZA125.17	C	0.750	0.217	>50	4.05	1.19	>50
TZBD.02	C	2.44	1.41	0.068	7.78	4.31	0.198
ZA012.29	C	3.66	1.47	0.296	11.3	4.12	0.755
ZM106.9	C	5.68	>50	0.208	21.8	>50	0.627
ZM109.4	C	0.715	0.161	0.144	4.64	1.07	0.410
ZM135.10a	C	0.246	0.033	1.38	2.20	0.408	4.26
ZM176.66	C	1.10	0.267	0.063	5.65	1.73	0.266
ZM197.7	C	0.232	0.085	0.729	1.16	0.369	2.00
ZM214.15	C	5.39	2.22	1.37	19.5	5.98	4.95
ZM215.8	C	0.107	0.044	0.344	0.591	0.230	1.32
ZM233.6	C	0.549	0.270	2.27	2.33	0.737	7.04
ZM249.1	C	1.58	0.830	0.077	5.78	2.27	0.283
ZM53.12	C	6.11	2.62	1.17	18.5	6.72	3.59
ZM55.28a	C	7.88	2.34	0.334	19.8	6.78	1.01
3326.V4.C3	CD	3.62	1.40	0.088	13.3	4.29	17.8
3337.V2.C6	CD	1.50	1.09	0.086	5.60	4.87	0.203
3817.v2.c59	CD	2.12	0.229	>50	6.33	1.43	>50
231965.c1	D	19.6	11.0	0.448	>50	20.4	1.12
247-23	D	0.618	0.344	1.53	3.24	1.29	9.17
3016.v5.c45	D	0.607	0.710	0.121	3.42	2.17	0.272
57128.vrc15	D	0.352	0.212	>50	2.59	1.50	>50
6405.v4.c34	D	1.93	0.461	1.82	6.76	1.80	3.05
A03349M1.vrc4a	D	0.402	0.270	3.48	1.32	0.663	11.7
A07412M1.vrc12	D		0.140			0.873	
NKU3006.ec1	D	0.989	0.673	0.441	4.86	2.46	1.23
P0402.c2.11	G	0.094	0.097	0.095	0.815	0.460	0.288
P1981.C5.3	G	0.020	0.024	0.187	0.211	0.124	0.683
X1193.c1	G	0.746	0.341	0.092	3.28	1.15	0.388
X1254.c3	G	2.77	3.67	0.041	14.5	15.7	0.128
X1632.S2.B10	G	0.925	0.387	0.096	3.93	1.76	0.632
X2088.c9	G	>50	>50	>50	>50	>50	>50

Figure 78. cont

Key (IC₅₀/IC₈₀ in µg/ml)

<0.100	0.100-1.00	1.00-10.0	>10.0
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Virus	Clade	IC ₅₀ (µg/ml)				IC ₈₀ (µg/ml)		
		DH511.2	10E8	VRC01		DH511.2	10E8	VRC01
X2131.C1.B5	G	0.118	0.039	0.466		0.611	0.175	1.64
SIVmac251.30.SG3	Control	>50	>50	>50		>50	>50	>50
SVA.MLV	Control	>50	>50	>50		>50	>50	>50
		DH511.2	10E8	VRC01		DH511.2	10E8	VRC01
Number of Viruses		199	200	199		199	200	199
Total Viruses Neutralized								
IC50/80 <50ug/ml		197	195	179		194	195	174
IC50/80 <10ug/ml		189	194	177		140	186	163
IC50/80 <1.0ug/ml		97	145	146		29	57	98
IC50/80 <0.1ug/ml		19	38	51		6	9	11
IC50/80 <0.01ug/ml		6	9	0		3	5	0
Percent of Viruses Neutralized								
IC50/80 <50ug/ml		99	98	90		97	98	87
IC50/80 <10ug/ml		95	97	89		70	93	82
IC50/80 <1.0ug/ml		49	73	73		15	29	49
IC50/80 <0.1ug/ml		10	19	26		3	5	6
IC50/80 <0.01ug/ml		3	5	0		2	3	0
Median IC50/80		1.09	0.392	0.281		5.10	1.71	0.839
Geometric Mean		0.802	0.315	0.287		3.82	1.37	0.843

Figure 78. cont

Virus ID	DH511.2			Virus ID	DH511.2		
	IC ₅₀	IC ₈₀	MPI*		IC ₅₀	IC ₈₀	MPI*
Du156.12	0.092	0.475	100	21261106_C12_H2	3.53	17.7	98
Du172.17	1.65	8.54	98	21369737_G11_F2	6.80	33.3	90
Du422.1	0.749	4.80	100	21399975_E2_B3	3.29	16.7	97
ZM197M.PB7	0.053	0.601	99	21492713_B11_E3	2.18	10.6	98
ZM214M.PL15	0.385	4.95	99	21502011_F12_E2	0.587	4.07	99
ZM233M.PB6	0.590	4.08	100	21561324_D3_B5	7.84	24.8	93
ZM53M.PB12	5.64	22.6	96	19314479_A2_5	0.943	5.35	99
ZM109F.PB4	0.682	6.08	98	98-F4_H5-13	12.7	41.1	85
ZM135M.PL10a	0.690	3.36	100	234-F1-16-57	1.33	7.19	99
CAP45.2.00.G3	0.268	6.84	98	541-F1_A7_2	20.8	>50	75
Du151.2	1.27	6.60	99	569-F1_37_10	9.21	36.6	86
CAP244.2.00.D3	1.07	3.08	100	304_F2_1_11	7.21	31.9	91
ZM215F.PB8	0.065	0.400	100	346_F4_D2_12	0.147	1.08	99
ZM55F.PB28a	3.27	16.4	98	556_F2_3_25	1.93	10.4	97
ZM106F.PB9	6.18	36.9	92	CAP69.1.12_TA7.1	0.799	6.48	99
6644.v2.c33	0.231	0.965	100	CAP136.1.16_E6_1	0.243	5.92	97
0077.v1.c16	1.91	18.9	93	CAP174.1.06_F3_1B	0.085	1.31	99
3728.v2.c6	2.17	11.2	97	CAP220.2.00_A8_5B	2.10	11.2	98
6838.v1.c35	1.12	5.00	100	CAP224.1.18_C7_3	0.962	3.09	99
6785.v5.c14	2.89	13.3	98	CAP225.1.06_A2_18	2.31	10.9	99
0041.v3.c18	1.56	7.99	99	CAP269.2.00_F11_1	1.24	7.87	99
3168.v4.c10	1.64	14.3	97	CAP37.1.18_D2_19	11.2	40.4	87
3873.v1.c24	4.14	21.3	94	CAP40.2.01_A3_5	0.189	2.48	100
6322.v4.c1	2.96	16.6	98	CAP129.1.15_B2_13	1.60	13.9	96
0921.v2.c14	2.66	10.5	98	CAP237.1.22_B2_2_39	0.546	4.11	99
3637.v5.c3	3.31	18.8	94	CAP258.2.00_X_23	0.512	3.93	99
6471.v1.c16	8.00	47.3	91	CAP260.2.00_TA1_1B	3.38	19.5	95
0984.v2.c2	14.4	42.8	84	CAP266.2.00_E9_h6	3.78	14.3	95
6040.v4.c15	1.50	12.7	95	TRP290.2.00_23_6	0.650	4.83	100
6631.v3.c10	2.43	12.4	98	TRP292.2.00_12_4	0.430	2.30	100
933.v4.c4	3.27	9.32	100	TRP307.2.00_24_1	0.741	5.87	98
6980.v0.c31	5.10	17.5	98	TRP310.2.00_20_2	0.070	0.544	99
3426.v5.c17	0.087	0.459	100	TRP343.2.00_21_2	2.20	16.8	86
377.v4.c09	6.33	23.6	94	TRP347.2.00_B1_1	0.818	5.91	99
Ce1086_B2	0.176	2.29	99	TRP363.2.00_10_3	0.208	2.30	100
Ce0393_C3	1.22	7.00	100	722_G4_16	0.762	2.54	100
Ce1176_A3	0.868	5.10	99	So431_C1_1	0.157	2.34	98
Ce2010_F5	>50	>50	35	CT885_H3_2	2.69	13.2	98
Ce0682_E4	0.574	4.85	99	So186_H6_5	1.09	5.21	99
Ce1172_H1	0.141	1.71	100	So405_T24_5	0.054	0.272	100
Ce2060_G9	2.67	14.7	97	So225_H11_12	>50	>50	29
Ce2103_E8	13.3	46.7	69	So706_T10b_3	23.6	>50	69
Ce0985_H7(Rev-)	17.7	43.1	85	CT072_56_7	0.353	2.30	100
Ce703010131_1E2	2.95	18.0	94	CT966_E1-7	0.831	3.80	100
Ce703010054_2A2	2.05	8.71	100	CT977_69_12	0.372	2.64	100
Ce703010217_B6	0.172	1.27	100	CT140_140_B6	1.50	6.59	100
Ce703010228_1C4	1.53	7.19	99	CT349_39_16	0.142	1.37	100
Ce704010083_B8	0.538	3.13	100	CT431_G6_6	0.410	1.84	100
Ce704809221_1B3	0.104	0.640	100	CT823_B6_1	0.939	4.55	100
Ce0626_E6	0.708	3.23	99	Ko426_T78_10	1.30	5.79	100

*MPI: Maximum Percent Inhibition

Figure 79

Virus ID	DH511.2			Virus ID	DH511.2		
	IC ₅₀	IC ₈₀	MPI*		IC ₅₀	IC ₈₀	MPI*
Ce0665_F2	0.026	0.117	100	Ko459_T68_4	1.20	6.53	99
Ce0089_G2	2.32	10.2	100	Ko870_C2_10	0.837	4.31	100
Ce2052_G10	0.813	4.86	100	Ko224_T87_2_4	0.697	10.6	95
Ce704010069_C6	1.30	6.44	98	Ko756_38_Tb12	0.314	2.50	100
Ce703010010_C4	1.46	7.23	100	CA327_D2_2	0.677	3.57	100
Ce704810053_2B7	1.50	7.02	100	CA392_H2_6	0.692	2.36	100
CeCAP210_TA5	1.28	5.75	100	CA457_H1_1	1.18	5.36	100
CeCAP200_B8a	3.40	15.7	98	ME067_A10-15	2.85	13.2	98
CeCAP221_B14	5.07	16.9	98	CA146_H3_3	0.070	0.504	100
CeCAP188_1_D1_14(Rev-)	2.34	11.2	98	CA240_A5.5	3.96	20.3	97
CeCAP177_1A3	1.16	5.78	100	1811_B3.23	2.59	9.22	99
246F_C1G	2.66	12.8	99	2865_A11.12	4.99	16.9	97
ZM249M.PL1	1.03	8.38	100	3603_C11.13	1.17	5.18	99
ZM247v1(Rev-)	1.83	10.1	98	3312_D6.2	5.44	49.7	85
7060101641A7(Rev-)	0.245	2.95	100	CAP301.2.00_C3.20	1.36	10.3	99
7030102001E5(Rev-)	6.53	37.4	87	CAP340.2.00_B3.16	0.303	4.32	99
1394C9G1(Rev-)	1.08	8.48	98	CAP382.2.00_D7.19	4.07	22.9	96
235080_3G7env2(Rev-)	4.16	18.0	98	CAP317.2.00_D4.10	0.788	12.5	95
BF1266.431a	3.73	24.4	91	CAP341.2.00.C10.18	0.145	0.675	98
704010042	0.548	2.40	100	CAP304.2.00_F6.6	6.77	30.7	91
705010185	5.05	19.0	96	CAP306.2.00_F9.1	2.68	14.8	96
705010198	4.39	21.8	97	CAP308.2.00_E11.35	>50	>50	40
705010067	1.73	10.8	99	CAP323.2.00_B6.45	2.04	10.0	98
2833264	1.82	13.8	99	CAP330.2.00_F2.41	1.52	6.65	100
1245045	9.09	34.6	93	CAP326.2.00_D9.2	16.4	43.8	90
19157834_V1	0.511	4.06	100	20417927.07	2.79	17.6	93
2969249	1.61	9.57	97	20355851.20	39.1	>50	57
3514597	8.15	28.0	93	20358510.01	9.71	36.7	94
20258279_V2	7.89	27.9	91	1107356.07	1.03	7.01	100
20721190	5.06	17.9	96	1143465.14	0.133	0.494	100
20803520	6.19	27.1	93	1170887.08	6.50	20.5	94
20915593	0.210	1.33	100	B005582-7_G7.8	>50	>50	38
20927783	7.92	28.4	96	B005018-8_F6.3	0.269	2.15	100
20965238	11.1	44.2	91	CT810_G4-7	0.721	6.37	100
21197826_V1	11.1	36.5	90	CT184_D3.15	1.86	10.4	99
21283649	15.3	45.5	83	SO607_B6.9	13.8	45.4	84
2759058_F10_B6	1.75	8.49	98	Me178_G6.16	1.48	7.98	100
2768732_C5_16	13.4	50.0	87	CAP291.2.00_H2.15	1.08	4.46	100
2869751_A4_D3	6.06	18.4	96	CAP327.2.00_C6.37	2.88	12.3	98
2891391_A2_E1	6.05	17.2	98	CAP378.2.00_D2.5	3.06	22.0	94
3545883_G1_E1	1.47	9.71	99	SO032_A2.8-1	0.085	0.909	100
18814602_H8_F3	0.234	2.56	100	CT565_C7.48	2.87	13.0	99
19252094_A5_G2	5.32	34.6	92	Ko243_H6.3	4.25	19.2	96
19707346_E8_C6	1.47	8.58	100	CAP331.2.00_D7.39	4.29	34.1	95
19715820_A10_H2	0.389	2.76	99	CAP332.2.00_C10.1	2.06	10.4	99
20104663_E11_D2	0.960	4.37	99	CAP206.1.B5.Y681D	0.895	4.28	99
20198102_E9_G1	1.33	9.90	99	CH0505.w4.3	0.020	0.131	100
20286961_C1_H8	0.442	2.68	100	6022.v7.c24	2.45	10.6	99
20883229_C9_H6	0.549	4.24	100	6146.v7.c23	14.6	44.4	84
21203310_G7_C3	1.09	8.49	98	CH0694.env	12.0	47.0	84

Figure 79 cont.

Early/Acute Clade C Virus Panel (n=200)							
		Highest Concentration Tested (ug/ml)	% Viruses Neutralized		Median Titers (All Viruses)		Median Titers (Sensitive Viruses Only)
	Epitope Target		IC50 Cut-off	IC80 Cut-off	IC50	IC80	
bnAb							
VRC01	CD4bs	50	84.0	77.5	0.748	2.970	IC50 0.553 IC80 1.750
10E8	MPER	25	97.5	96.5	0.570	3.458	0.559 3.399
DH511.2	MPER	50	98.0	96.5	1.547	9.443	1.510 8.580

Figure 79 cont.

		Key (IC ₅₀ /IC ₅₀ in $\mu\text{g/ml}$)							
		<0.100		0.100-1.00		1.00-10.0		>10.0	
PTID	Antibody ID	Heavy ID	Light ID	IC ₅₀ ($\mu\text{g/ml}$)	IC ₅₀ ($\mu\text{g/ml}$)	CH50/5.TF	IC ₅₀ ($\mu\text{g/ml}$)	IC ₅₀ ($\mu\text{g/ml}$)	IC ₅₀ ($\mu\text{g/ml}$)
	10E8			0.262	0.124	4.76	<0.023	5.9A	>50
704-01-021-0	DH511.2 (A6510349)	DH511.2 VH_4A	DH511.2 VK	0.156	3.41	8.97	<0.023	>50	>50
704-01-021-0	DH511.2 K3	DH511.2 VH_4A	DH511.2 VK	0.044	3.67	2.81	<0.023	>50	>50
704-01-021-0	DH511.2 E98W	DH511.2 VH_4A E98W	DH511.2 VK	1.92	4.78	31.0	0.051	>50	>50
704-01-021-0	DH511.2 T58W	DH511.2 VH_4A T58W	DH511.2 VK	5.80	14.4	>50	0.434	>50	>50
704-01-021-0	DH511.2 F99W	DH511.2 VH_4A F99W	DH511.2 VK	2.46	>50	>50	0.232	>50	>50
704-01-021-0	DH511.2 L100dW	DH511.2 VH_4A L100dW	DH511.2 VK	0.396	0.606	6.82	<0.023	>50	>50
704-01-021-0	DH511.2 E100dW	DH511.2 VH_4A E100dW	DH511.2 VK	1.41	>50	>50	0.254	>50	>50
704-01-021-0	DH511.2 Y100dW	DH511.2 VH_4A Y100dW	DH511.2 VK	1.57	18.5	45.1	0.071	>50	>50
704-01-021-0	DH511.2 F100dW	DH511.2 VH_4A F100dW	DH511.2 VK	1.64	>50	39.3	0.054	>50	>50
704-01-021-0	DH511.2 Y100dW	DH511.2 VH_4A Y100dW	DH511.2 VK	>50	>50	>50	9.34	>50	>50
704-01-021-0	DH511.2 V100dF	DH511.2 VH_4A V100dF	DH511.2 VK	>50	>50	>50	20.1	>50	>50
704-01-021-0	DH511.2 T100dW	DH511.2 VH_4A T100dW	DH511.2 VK	0.099	0.134	6.24	<0.023	>50	>50
704-01-021-0	DH511.2 R100dW	DH511.2 VH_4A R100dW	DH511.2 VK	5.45	>50	>50	0.428	>50	>50
704-01-021-0	DH511.2 F100dW	DH511.2 VH_4A F100dW	DH511.2 VK	1.33	>50	30.6	0.050	>50	>50
704-01-021-0	DH511.2 G100dW	DH511.2 VH_4A G100dW	DH511.2 VK	>50	>50	>50	>50	>50	>50
704-01-021-0	DH511.2 V100I	DH511.2 VH_4A V100I	DH511.2 VK	2.73	>50	>50	0.304	>50	>50
704-01-021-0	DH511.2 L100dF	DH511.2 VH_4A L100dF	DH511.2 VK	0.046	0.229	2.73	<0.023	>50	>50
704-01-021-0	DH511.2 G97W	DH511.2 VH_4A G97W	DH511.2 VK	>50	>50	>50	30.2	>50	>50
Control	A632			>50	>50	>50	>50	>50	>50
Control	CH-01-CH-31			1.44	1.52	<0.011	0.575	>25	>25

Figure 80

PTID	Antibody ID	Heavy ID	Light ID	IC50 (µg/ml) BG1158	IC50 (µg/ml) CH505.TF	IC50 (µg/ml) DU172	IC50 (µg/ml) MN	IC50 (µg/ml) S/A
	1OE3			1.19	13.6	0.684	<0.023	>50
704-01-021-0	DH511.2 (A6510049)	DH511.2 VH 4A	DH511.2 VK	0.913	28.1	130	0.082	>50
704-01-021-0	DH511.2 K3	DH511.2 VH 4A	DH511.3 VK	0.376	13.2	116	<0.023	>50
704-01-021-0	DH511.2 E56W	DH511.2 VH 4A E56W	DH511.2 VK	5.72	>50.32	184	0.276	>50
704-01-021-0	DH511.2 T98W	DH511.2 VH 4A T98W	DH511.2 VK	27.3	>50	>50	1.51	>50
704-01-021-0	DH511.2 F55W	DH511.2 VH 4A F55W	DH511.2 VK	12.9	>50	>50	0.729	>50
704-01-021-0	DH511.2 L100aW	DH511.2 VH 4A L100aW	DH511.2 VK	1.30	23.2	3.31	0.100	>50
704-01-021-0	DH511.2 E100bW	DH511.2 VH 4A E100bW	DH511.2 VK	6.74	>50	>50	0.877	>50
704-01-021-0	DH511.2 Y100bW	DH511.2 VH 4A Y100bW	DH511.2 VK	5.14	>50	>50	0.387	>50
704-01-021-0	DH511.2 F100W	DH511.2 VH 4A F100W	DH511.2 VK	7.09	>50	>50	0.327	>50
704-01-021-0	DH511.2 Y100aW	DH511.2 VH 4A Y100aW	DH511.2 VK	>50	>50	>50	32.5	>50
704-01-021-0	DH511.2 V100F	DH511.2 VH 4A V100F	DH511.2 VK	>50	>50	>50	>50	>50
704-01-021-0	DH511.2 T100aW	DH511.2 VH 4A T100aW	DH511.2 VK	0.696	20.1	0.960	<0.023	>50
704-01-021-0	DH511.2 R100bW	DH511.2 VH 4A R100bW	DH511.2 VK	15.6	>50	>50	1.19	>50
704-01-021-0	DH511.2 F100aW	DH511.2 VH 4A F100aW	DH511.2 VK	6.58	>50	>50	0.316	>50
704-01-021-0	DH511.2 G100aW	DH511.2 VH 4A G100aW	DH511.2 VK	>50	>50	>50	>50	>50
704-01-021-0	DH511.2 V100I	DH511.2 VH 4A V100I	DH511.2 VK	10.6	>50	>50	1.11	>50
704-01-021-0	DH511.2 L100dF	DH511.2 VH 4A L100dF	DH511.2 VK	0.249	10.2	1.67	<0.023	>50
704-01-021-0	DH511.2 G87W	DH511.2 VH 4A G87W	DH511.2 VK	>50	>50	>50	>50	>50
Control	A682			>50	>50	>50	>50	>50
Control	CH01-CH31			5.24	0.059	10.1	3.32	>25

Figure 80 cont.

Heavy Chain Sequences

Plasma monoclonal antibodies

Donor	HeAav ChAin ID	IGHV	IGHD	IGHJ	CDR3 length (Amino Acids) ^a	CDR3 Sequence (Amino Acids)	Somatic Mutations (Nucleotides) _b	Mutation Frequency (Nucleotides) _b	Mutation Frequency (Amino Acids) ^b cy
CH0210	DH511.7 AP VH	3-15*02	3-3*01	6*03	23	TADRGAPVLRFEWGYDYDYMEF	36	12.3%	in progress
CH0210	DH511.7 BP VH	3-15*02	3-3*01	6*03	23	TADRGAPVLRFEWGYDYDYMEF	34	11.6%	in progress
CH0210	DH511.8 AP VH	3-15*02	3-3*01	6*03	23	TADRGAPVLRFEWGYFDYDYMEF	41	14.0%	in progress
CH0210	DH511.8 BP VH	3-15*02	3-3*01	6*03	23	TADRGAPVLRFEWGYFDYDYMEF	43	14.7%	in progress
CH0210	DH511.8 CP VH	3-15*02	3-3*01	6*03	23	TADRGAPVLRFEWGYFDYDYMEF	44	15.0%	in progress
CH0210	DH511.9 AP VH	3-15*01	3-3*01	6*03	24	TRDEGAPVTRRFLWGYFYYYMAV	60	20.4%	in progress
CH0210	DH511.1 OAP VH	3-15*01	3-3*01	6*03	23	TRDEGAPVTRFLWGSYYYMAV	56	19.1%	in progress
CH0210	DH511.1 OBP VH	3-15*01	3-3*01	6*03	23	TRDEGAPVTRFLWGSYYYMAV	58	19.8%	in progress
CH0210	DH511.1 OCP VH	3-15*01	3-3*01	6*03	23	TRDEGAPVTRLLEWGSYYYMAV	60	20.5%	in progress
CH0210	DH511.1 1AP VH	3-15*01	3-3*01	6*03	23	TADEGAPILRFFWGYNYNMDV	32	10.6%	in progress
CH0210	DH511.1 2AP VH	3-15*01	3-3*01	6*03	23	TADEGAPILRFFWGYNYNMDV	34	11.2%	in progress

Figure 81

Light Chain Sequences
Plasma monoclonal antibodies

Donor	Light Chain ID	IGKV	IGKJ	CDR3 length (Amino Acids) ^a	CDR3 Sequence (Amino Acids)	Somatic Mutations (Nucleotides) ^b	Mutation Frequency (Nucleotides) ^b	Mutation Frequency (Amino Acids) ^b
CH0210	DH511.7PA VK	1-39*01	2*01	11	QESYSSPTHT	31	11.7%	in progress
CH0210	DH511.8PA VK	1-39*01	2*01	11	QESYSSPTTHI	30	11.4%	in progress
CH0210	DH511.9A VK	1-39*01	2*01	11	QESYQTVPTLT	36	13.6%	in progress
CH0210	DH511.9BP VK	1-39*01	2*01	11	QESYQTVPTLT	37	14.0%	in progress
CH0210	DH511.9CP VK	1-39*01	2*01	11	QESYQTVPTLT	36	13.6%	in progress
CH0210	DH511.10P VK	1-39*01	2*01	11	QEAYNTNPTLS	36	13.6%	in progress
CH0210	DH511.10BP VK	1-39*01	2*01	11	QEAYNTNPTLS	38	14.4%	in progress
CH0210	DH511.11A P VK	1-39*01	2*01	11	QESYSSVPMYI	41	15.5%	in progress

^aCDR3 length listed according to the IMGT definition.

^bSomatic mutations were determined over the entire variable region and are reported as the number/frequency of nucleotides or amino acids that differed from the putative germ line V-gene sequence.

Figure 81 cont.

Pairing of Plasma-derived Heavy and Light Chains for Expression of 16 Recombinant Monoclonal Antibodies

	Ab Name	Heavy ID	Light ID
1	DH511.7P	DH511.7AP VH	DH511.7AP VK
2	DH511.7bP	DH511.7BP VH	DH511.7AP VK
3	DH511.8P	DH511.8AP VH	DH511.8AP VK
4	DH511.8bP	DH511.8BP VH	DH511.8AP VK
5	DH511.8cP	DH511.8CP VH	DH511.8AP VK
6	DH511.9P	DH511.9AP VH	DH511.9AP VK
7	DH511.9bP	DH511.9AP VH	DH511.9BP VK
8	DH511.9cP	DH511.9AP VH	DH511.9CP VK
9	DH511.10P	DH511.10AP VH	DH511.10AP VK
10	DH511.10a10bVKP	DH511.10AP VH	DH511.10BP VK
11	DH511.10b10aVKP	DH511.10BP VH	DH511.10AP VK
12	DH511.10bP	DH511.10BP VH	DH511.10BP VK
13	DH511.10cP	DH511.10CP VH	DH511.10AP VK
14	DH511.10c10bVKP	DH511.10CP VH	DH511.10BP VK
15	DH511.11P	DH511.11AP VH	DH511.11AP VK
16	DH511.12P	DH511.12P VH	DH511.11AP VK

Figure 81 cont.

Key (IC ₅₀ /IC ₂₀ in µg/ml)												
		<0.100		0.100-1.00		1.00-10.0		>10.0				
Ab Name	IC ₅₀ (µg/ml)											
	BG1168 (Assay 1)	BG1168 (Assay 2)	CH505.TF (Assay 1)	CH505.TF (Assay 2)	DU172 (Assay 1)	DU172 (Assay 2)	IMN (Assay 1)	IMN (Assay 2)	SVA (Assay 1)	SVA (Assay 2)	SVA	SVA
10E8	0.188	0.099	3.31	3.18	<0.023	<0.023	<0.023	<0.023	<0.023	<0.023	<0.023	<0.023
DH511.2	NT	0.174	NT	7.58	NT	2.91	NT	<0.023	NT	<0.023	NT	<0.023
DH511.7P	1.94	0.771	32.1	30.4	8.39	12.8	<0.023	<0.023	<0.023	<0.023	<0.023	<0.023
DH511.7bP	1.66	0.793	20.6	25.6	6.53	8.89	<0.023	<0.023	<0.023	<0.023	<0.023	<0.023
DH511.8P	0.898	0.813	17.8	16.5	0.224	0.185	<0.023	<0.023	<0.023	<0.023	<0.023	<0.023
DH511.8bP	0.767	0.549	16.7	11.3	0.130	0.147	<0.023	<0.023	<0.023	<0.023	<0.023	<0.023
DH511.8cP	10.6	NT	>50	NT	3.06	NT	0.610	NT	<0.023	<0.023	<0.023	<0.023
DH511.9P	1.00	0.751	20.4	21.1	1.76	1.34	<0.023	<0.023	<0.023	<0.023	<0.023	<0.023
DH511.9bP	1.92	NT	43.8	NT	2.84	NT	0.027	NT	<0.023	<0.023	<0.023	<0.023
DH511.9cP	1.20	0.458	22.4	18.7	1.50	1.19	<0.023	<0.023	<0.023	<0.023	<0.023	<0.023
DH511.10P	12.3	7.09	>50	>50	17.3	>50	0.468	0.149	<0.023	<0.023	<0.023	<0.023
DH511.10a10bAP	5.09	5.23	>50	>50	1.65	16.2	0.278	0.152	<0.023	<0.023	<0.023	<0.023
DH511.10b10aAP	30.0	NT	>50	NT	>50	NT	1.07	NT	<0.023	<0.023	<0.023	<0.023
DH511.10bP	26.1	NT	>50	NT	>50	NT	0.740	NT	<0.023	<0.023	<0.023	<0.023
DH511.10cP	31.7	>50	>50	>50	>50	>50	2.06	1.33	<0.023	<0.023	<0.023	<0.023
DH511.10c10bAP	33.6	>50	>50	>50	>50	>50	1.27	0.898	<0.023	<0.023	<0.023	<0.023
DH511.11P	0.239	0.214	7.00	7.70	0.055	0.067	<0.023	<0.023	<0.023	<0.023	<0.023	<0.023
DH511.12P	0.163	0.108	9.39	5.97	<0.023	0.061	<0.023	<0.023	<0.023	<0.023	<0.023	<0.023
2F5	0.907	0.841	NT	NT	NT	NT	<0.023	<0.023	<0.023	<0.023	<0.023	<0.023
4E10	NT	NT	NT	NT	0.192	0.116	NT	NT	<0.023	<0.023	<0.023	<0.023
CH31	NT	NT	<0.023	<0.023	NT	NT	NT	NT	<0.023	<0.023	<0.023	<0.023

Figure 28

Ab Name	IC ₅₀ (μg/ml)									
	BG1168 (Assay 1)	BG1168 (Assay 2)	CH505.TF (Assay 1)	CH505.TF (Assay 2)	DU172 (Assay 1)	DU172 (Assay 2)	MMN (Assay 1)	MMN (Assay 2)	SVA (Assay 1)	SVA (Assay 2)
10E8	0.773	0.433	9.60	7.24	0.198	0.103	<0.023	<0.023	>50	>50
DH511.2	NT	0.813	NT	21.8	NT	9.81	NT	<0.023	NT	>50
DH511.7P	6.97	2.81	>50	>50	37.2	>50	0.051	0.042	>50	>50
DH511.7bP	6.24	3.75	>50	>50	27.1	>50	0.068	0.028	>50	>50
DH511.8P	3.32	3.22	48.6	48.8	1.43	1.31	0.037	<0.023	>50	>50
DH511.8bP	2.64	2.41	42.7	43.3	1.12	0.809	0.056	<0.023	>50	>50
DH511.8cP	42.9	NT	>50	NT	22.1	NT	1.65	NT	>50	>50
DH511.9P	4.96	2.39	>50	>50	10.5	5.58	0.044	<0.023	>50	>50
DH511.9bP	6.90	NT	>50	NT	17.3	NT	0.130	NT	>50	>50
DH511.9cP	5.55	1.89	>50	>50	11.0	5.05	0.077	<0.023	>50	>50
DH511.10P	39.6	>50	>50	>50	>50	>50	1.52	0.498	>50	>50
DH511.10a10c10cP	25.0	29.2	>50	>50	>50	>50	0.649	0.526	>50	>50
DH511.10b10c10cP	>50	NT	>50	NT	>50	NT	4.02	NT	>50	>50
DH511.10cP	>50	NT	>50	NT	>50	NT	2.04	NT	>50	>50
DH511.10dP	>50	>50	>50	>50	>50	>50	6.87	4.58	>50	>50
DH511.10e10c10cP	>50	>50	>50	>50	>50	>50	3.77	2.46	>50	>50
DH511.11P	1.14	1.06	24.1	24.0	0.477	0.605	<0.023	<0.023	>50	>50
DH511.12P	0.944	0.448	26.8	18.4	0.443	0.440	<0.023	<0.023	>50	>50
2F5	4.04	4.24	NT	NT	NT	NT	<0.023	<0.023	NT	NT
4E10	NT	NT	NT	NT	2.30	1.45	NT	NT	>50	>50
CH31	NT	NT	NT	0.029	0.030	NT	NT	NT	NT	NT

Results shown are derived from two independent experiments. NT, not tested.

Figure 82 cont.

		Key {IC ₅₀ /IC ₅₀ in µg/ml}			
		<0.100	0.100-1.00	1.00-10.0	>10.0

Virus	Clade	IC ₅₀ (µg/ml)			
		DH511.11P	DH511.12P	DH511.2	10E8
0260.v5.c36	A	10.9	12.0	17.0	987
0330.v4.c3	A	0.798	0.785	1.42	1.12
0439.v5.c1	A	2.02	1.74	2.36	1.23
3035.v2.c20	A	1.23	1.43	2.29	1.60
3415.v1.c1	A	10.7	9.89	12.6	4.69
3713.v3.c11	A	3.48	3.34	3.80	0.838
398-F1_F8_20	A	2.84	2.26	2.49	0.704
B8201B42	A	1.97	2.61	2.82	0.613
B8539.2B13	A	2.16	2.08	6.73	0.591
B8505.WEMC2	A	0.915	0.907	1.33	0.889
B369.9A	A	0.850	1.10	1.61	0.356
B5203.B1	A	0.425	0.398	0.803	0.319
KER2008.12	A	0.544	0.548	0.723	>50
KER2018.11	A	3.73	3.73	4.31	1.89
K14H1208.18	A	0.368	0.313	0.410	0.406
MB201.A1	A	0.679	0.657	0.844	0.411
MB539.2B7	A	10.2	13.8	>50	>50
M369.A6	A	0.562	0.347	0.510	0.571
MB208.A1	A	0.700	0.486	0.927	0.187
Q23.17	A	1.50	1.59	1.18	0.461
Q269.17	A	7.94	7.31	12.4	4.76
Q769.d22	A	1.65	1.60	2.44	1.91
Q769.h5	A	3.39	4.09	5.73	2.89

		IC ₅₀ (µg/ml)			
		DH511.11P	DH511.12P	DH511.2	10E8
23.4			306	>50	21.7
4.75			5.17	7.37	3.64
6.99			5.83	9.57	3.95
6.52			5.78	7.89	4.56
17.5			19.2	22.1	11.5
13.7			10.7	13.5	4.42
16.9			10.7	18.0	6.17
8.09			8.37	9.87	1.96
6.32			7.16	43.0	13.0
4.93			4.03	5.60	2.14
3.37			3.62	4.93	1.29
2.03			1.76	3.35	3.27
2.76			2.30	4.29	>50
13.4			14.1	17.0	7.16
1.87			1.53	2.25	2.39
3.31			3.02	4.22	1.36
>50			>50	>50	>50
2.55			2.50	3.41	1.77
2.41			2.36	3.56	1.14
6.34			6.98	5.85	1.60
24.5			21.7	30.7	12.0
6.21			6.05	8.64	4.47
13.7			14.0	16.7	7.44

Figure 83

Q842.d12	A	3.50	4.56	5.73	2.82	14.7	13.5	12.8	7.58
QH208.14M.A2	A	1.22	1.03	2.20	1.30	5.63	5.10	10.0	4.03
RW020.2	A	3.41	3.19	3.46	0.902	8.77	8.34	10.0	2.92
UG037.8	A	0.210	0.250	0.273	0.046	0.931	0.844	1.33	0.353
248-F3C10.2	AC	0.777	0.589	0.802	0.210	3.33	3.37	5.10	1.49
3301.V1.C24	AC	11.4	6.79	12.5	2.97	30.0	22.3	33.8	9.50
3589.V1.C4	AC	0.431	0.194	3.26	5.77	3.19	2.18	10.2	11.7
6540.v4.c1	AC	5.84	6.24	2.84	2.24	24.0	23.7	13.4	7.01
6545.V4.C1	AC	6.97	6.71	7.79	2.54	14.7	19.5	15.4	7.50
0815.V3.C3	ACD	1.31	0.944	2.04	0.491	5.04	4.72	7.05	1.81
6085.V1.C10	ACD	0.005	0.004	0.0006	0.0005	0.022	0.016	0.0010	0.004
3488.V1.C12	AD	0.560	0.480	2.00	0.381	2.98	2.45	7.45	2.04
Q168.a2	AD	1.53	1.42	1.45	0.463	6.39	5.63	5.18	2.88
Q481.e2	AD	1.90	1.65	2.45	2.29	6.48	5.68	8.68	4.68
620345.c1	AE	1.82	1.38	1.77	0.989	7.31	6.10	9.05	3.73
BUO\009000.02.4	AE	0.743	0.726	0.580	0.251	4.00	3.33	5.03	1.47
BUO\010000.06.2	AE	0.247	0.231	0.178	0.060	1.58	1.40	1.23	0.476
BUO\025000.01.1	AE	0.431	0.479	0.751	0.228	3.05	3.03	4.77	1.54
BUO\028000.10.3	AE	0.321	0.322	0.853	0.167	2.41	2.26	4.59	0.876
C1080.c3	AE	0.360	0.310	0.521	0.108	1.54	1.59	2.50	0.613
C2101.c1	AE	1.37	1.30	4.64	1.20	7.26	6.64	15.6	4.12
C3347.c11	AE	0.066	0.096	0.046	0.016	0.377	0.376	0.274	0.089
C4118.03	AE	0.857	0.851	1.66	0.421	6.07	5.90	11.6	2.30
CME44.ec1	AE	0.477	0.413	0.863	0.365	3.33	3.00	6.02	1.46
CNE3	AE	2.63	2.84	2.81	1.37	8.80	9.98	9.17	4.01
CNE5	AE	1.85	1.40	1.54	1.17	8.25	6.84	7.34	2.52
CME55	AE	0.363	0.310	0.287	0.036	1.99	1.45	2.00	0.605
CNE58	AE	0.175	0.170	0.212	0.060	0.944	0.715	1.18	0.314

Figure 83 cont.

CNE59	AE	0.013	0.017	0.0006	0.0010	0.052	0.058	0.023	0.010
CNE8	AE	0.310	0.322	0.045	0.140	1.63	1.71	0.483	1.42
M02138	AE	0.045	0.051	0.125	0.014	0.162	0.172	0.659	0.126
R1155 c1	AE	0.764	0.681	0.742	0.488	2.87	2.70	3.70	2.02
R2184 c4	AE	0.881	0.708	1.12	0.576	3.99	3.25	5.40	2.20
R3285 c5	AE	3.92	3.10	6.31	1.58	15.7	14.8	2.19	9.28
TH0236	AE			0.0010	0.0003			0.022	0.034
TH8658	AE	0.132	0.172	0.081	0.038	0.590	0.502	0.611	0.291
TH976 17	AE	0.437	0.480	0.762	0.392	2.84	2.46	4.08	1.75

Figure 83 cont.

		Key {IC ₅₀ /IC ₂₀ in µg/ml}			
		<0-100	0-100-100	1-100-10.0	>100

Virus	Clade	IC ₅₀ (µg/ml)			
		DH511.11P	DH511.12P	DH511.2	10E8
235-47	A/G	1.38	1.28	1.61	0.786
242-14	A/G	0.643	0.618	0.750	3.17
263-8	A/G	1.98	1.85	2.63	0.991
268-12	A/G	0.804	0.810	2.10	0.475
271-11	A/G	6.43	5.33	7.60	4.34
328-28	A/G	1.06	1.20	1.28	0.365
DJ253-8	A/G	0.348	0.344	0.903	0.100
T250-4	A/G	7.96	8.05	13.1	3.45
T251-18	A/G	12.2	10.1	20.5	2.55
T253-11	A/G	9.32	7.78	12.0	4.05
T255-24	A/G	2.22	1.83	6.11	1.14
T257-31	A/G	5.08	5.46	10.5	1.58
T266-60	A/G	3.58	3.91	4.78	>50
T278-60	A/G	5.95	5.84	8.95	2.10
T280-5	A/G	40.4	29.0	17.0	4.77
T33-7	A/G	8.61	8.51	10.2	2.83
3883-25	B	0.961	0.756	0.883	0.293
5768-04	B	16.5	15.2	16.0	5.26
6101-10	B	0.106	0.076	0.137	0.005
6535-3	B	4.81	3.67	7.62	1.28
7165-18	B	5.63	5.45	3.55	2.71
45_01d35	B	4.64	4.21	4.87	0.703

Virus	Clade	IC ₂₀ (µg/ml)			
		DH511.11P	DH511.12P	DH511.2	10E8
235-47	A/G	0.332	0.313	0.361	0.244
242-14	A/G	0.107	0.132	0.099	0.568
263-8	A/G	0.279	0.263	0.407	0.229
268-12	A/G	0.226	0.208	0.420	0.124
271-11	A/G	0.672	0.601	1.23	0.891
328-28	A/G	0.243	0.210	0.215	0.079
DJ253-8	A/G	0.052	0.046	0.190	0.009
T250-4	A/G	1.83	1.82	3.54	1.07
T251-18	A/G	5.12	3.57	7.52	0.666
T253-11	A/G	2.00	1.47	3.51	1.21
T255-24	A/G	0.284	0.274	1.40	0.228
T257-31	A/G	1.33	1.74	3.42	0.336
T266-60	A/G	0.803	1.10	0.953	>50
T278-60	A/G	0.987	1.11	1.89	0.357
T280-5	A/G	14.9	13.6	4.21	0.715
T33-7	A/G	2.38	2.25	2.34	0.818
3883-25	B	0.261	0.220	0.284	0.070
5768-04	B	4.52	4.32	5.70	1.63
6101-10	B	0.035	0.018	0.012	0.0010
6535-3	B	0.755	0.603	1.39	0.190
7165-18	B	1.18	1.01	0.805	0.659
45_01d35	B	0.803	0.678	0.753	0.106

Figure 83 cont.

88.6.DG	E	0.983	0.970	0.676	0.318	3.43	3.24	2.23	1.48
AC10.29	E	0.323	0.294	0.115	0.102	1.29	1.30	0.604	0.512
ADADG	E	0.160	0.151	0.042	0.055	0.757	0.649	0.482	0.358
Bal01	E	0.853	0.740	0.620	0.421	4.63	3.92	4.03	1.91
Bal26	E	0.786	0.903	2.57	0.518	4.45	4.41	11.0	2.38
BG1188.01	E	0.424	0.544	0.528	0.396	1.44	1.63	2.38	1.48
BL01.DG	E			1.08	0.362			6.87	1.57
BR07.DG	E	0.287	0.237	0.106	0.118	1.18	1.08	1.03	0.445
BX08.18	E			0.565	0.213			3.02	1.30
CAAN.A2	E	2.75	2.18	5.29	1.45	13.8	11.1	16.3	5.70
CNE10	E	0.047	0.040	0.121	0.014	0.415	0.401	0.932	0.169
CNE12	E	0.395	0.363	0.509	0.301	1.63	1.73	2.46	1.09
CNE14	E	0.725	0.687	0.950	0.151	3.37	3.82	4.24	0.649
CNE4	E	0.152	0.155	0.279	0.059	1.14	1.05	2.45	0.437
CNE57	E	0.233	0.361	0.120	0.059	1.14	1.67	1.28	0.317
HO88.8	E	2.19	2.46	2.55	0.326	8.38	7.94	10.7	1.52
HT593.1	E	0.125	0.116	0.140	0.049	0.678	0.643	0.780	0.285
HXB2.DG	E	0.007	0.008	0.0006	0.003	0.041	0.060	0.012	0.015
JRC5F.JB	E	1.99	1.75	2.04	0.429	8.51	7.59	9.61	1.88
JRFLJB	E	0.948	0.791	0.862	0.174	3.95	3.48	5.40	0.768
MM3	E	0.005	0.007	0.0006	0.0003	0.019	0.025	0.004	0.0010
PVO.04	E	2.10	1.72	2.32	1.60	8.94	7.91	15.9	6.43
QH0515.01	E	4.18	3.55	4.97	2.25	13.6	13.4	15.8	5.54
QH0882.42	E	1.47	1.20	2.03	0.531	5.71	5.30	11.1	2.35
REJO.67	E	0.699	0.699	0.544	0.302	3.11	3.29	3.31	1.18
RHPA.7	E	3.69	3.03	5.45	1.01	15.8	13.3	25.9	5.10
SC422.8	E	1.01	1.00	1.53	0.343	3.79	3.87	4.60	1.15

Figure 83 cont.

SF162LS	B	0.620	0.843	1.88	0.245	4.08	3.98	7.75	1.06
SS119601	B	0.174	0.232	0.857	0.244	1.21	1.18	4.72	1.25
THRO.18	B	0.326	0.291	0.711	0.062	1.52	1.26	2.39	0.587
TRJD.58	B	5.73	4.78	3.86	1.13	22.3	19.8	17.7	4.18
TRO.11	B	0.240	0.214	0.228	0.028	0.984	0.848	1.22	0.286
WITO.33	B	0.186	0.240	0.314	0.031	0.876	0.873	1.60	0.305
XZ278.C2B6	B	1.26	1.36	3.79	0.442	6.93	6.16	14.1	2.24
YL2.DG	B	4.54	5.06	8.00	1.17	37.7	32.1	50	5.46
BUO\002000.03.2	BC	0.763	0.651	1.17	0.384	4.28	3.99	5.64	1.56
CH038.12	BC	0.979	0.827	1.57	0.271	4.21	3.57	6.10	1.41
CH070.1	BC	8.22	5.55	12.1	6.65	34.8	36.8	44.6	13.5

Figure 83 cont.

		Key {IC ₅₀ /IC ₅₀ in µg/ml}			
		<0.100	0.100-1.00	1.00-10.0	>10.0

Virus	Clade	IC ₅₀ (µg/ml)			
		DH511.11P	DH511.12P	DH511.2	10E8
CH117.4	EC	1.58	1.57	2.29	0.859
CH119.10	EC	3.49	3.35	5.48	2.36
CH131.12	EC	3.97	3.46	3.99	2.79
CNE15	EC	4.38	3.42	6.77	2.97
CNE19	EC	2.27	2.33	3.05	1.11
CNE20	EC	2.08	1.81	2.43	0.732
CNE21	EC	5.24	5.26	8.40	3.25
CNE40	EC	0.043	0.044	0.030	0.009
CNE7	EC	0.911	0.814	0.672	0.603
298.36	C	3.50	2.77	14.0	5.00
298.38	C	3.72	3.90	8.90	3.08
0013095-2.11	C	0.184	0.156	0.229	0.077
001428-2.42	C	16.2	17.2	16.3	6.28
0077_V1.C16	C	10.8	11.3	36.0	7.11
00838-2.5	C	4.14	3.69	5.40	1.77
0921V2.C14	C	7.42	4.47	18.4	3.03
15055-2.3	C	3.13	2.75	9.40	3.31
18845-2.22	C	0.121	0.139	0.238	0.172
18938-2.21	C	0.803	0.872	1.99	1.31
25710-2.43	C	0.303	0.374	0.408	0.304
25711-2.4	C	4.22	3.25	12.6	1.69
26825-2.22	C	2.83	1.82	2.95	1.53

Figure 83 cont.

26191-2.48	C	1.88	1.34	1.82	1.83
3188 V4 C10	C	2.16	1.14	2.52	2.83
3637 V5 C3	C	2.26	1.58	2.83	2.12
3873 V1 C24	C	7.38	6.39	10.0	5.51
428c	C	0.821	0.497	2.22	0.445
6322 V4 C1	C	2.40	1.75	2.90	0.923
6471 V1 C16	C	8.01	5.50	11.0	4.98
6631 V3 C10	C	2.96	2.62	7.08	0.934
6644 V2 C33	C	0.502	0.498	0.229	0.013
6785 V5 C14	C	1.74	1.96	1.58	0.701
6838 V1 C35	C	0.562	0.634	0.813	0.292
962A E51.02	C	0.094	0.094	0.035	0.033
BR025.9	C	1.11	0.865	1.10	0.307
CAF210.E8	C	0.818	0.619	0.911	0.474
CAF244.D3	C	0.538	0.601	1.13	0.369
CAF258.203.C9	C	0.585	0.545	0.779	0.713
CAP45.G3	C	1.18	1.23	2.84	0.722
Ce1175.A3	C	0.458	0.489	0.998	0.252
CE703010217.E6	C	0.121	0.107	0.159	0.056
CNE30	C	1.42	1.03	3.30	0.456
CNE31	C	2.00	1.64	5.23	1.32
CNE53	C	0.240	0.214	0.208	0.213
CNE58	C	0.202	0.112	0.453	0.229
DU123.06	C	0.145	0.128	0.250	0.132
DU151.02	C	0.591	0.480	0.928	0.461
DU156.12	C	0.037	0.039	0.019	0.023
DU172.17	C	0.161	0.143	7.22	0.057
986	8.46	8.77	4.90		
123	8.42	19.3	8.18		
921	8.78	11.1	6.68		
241	19.6	13.8	15.7		
298	2.12	7.87	1.60		
111	9.07	9.72	3.68		
232	29.2	41.9	14.9		
113	9.65	15.5	3.36		
173	1.65	1.14	0.124		
648	6.03	6.70	2.42		
230	2.50	3.67	1.01		
0.396	0.345	0.358	0.177		
385	3.39	5.33	1.11		
246	2.35	3.37	2.01		
218	2.23	4.56	1.48		
293	2.93	3.92	2.97		
504	4.61	9.41	3.41		
195	1.79	4.13	1.15		
0.811	0.653	0.944	0.679		
595	4.32	16.3	2.29		
587	5.68	11.5	3.57		
118	1.20	1.41	1.01		
107	0.776	2.98	1.09		
0.665	0.604	0.948	0.423		
267	2.32	3.60	1.71		
0.177	0.165	0.158	0.120		
0.789	0.721	19.9	0.238		

Figure 83 cont.

DU422.01	C	0.593	0.575	0.821	0.224	2.53	2.05	3.95	0.812
MW965.26	C			0.0006	0.0010			0.002	0.007
SO18.18	C	1.43	1.54	7.52	1.60	7.80	6.53	20.7	4.48
TV1.29	C	0.618	0.487	0.802	0.248	1.75	1.40	2.56	0.719
TZA125.17	C	0.466	0.407	0.750	0.217	1.93	2.12	4.05	1.19
TZBD.02	C	2.40	2.17	2.44	1.41	8.18	7.56	7.78	4.31
ZA012.29	C	2.29	1.73	3.66	1.47	7.59	7.01	11.3	4.12
ZM105.9	C	5.40	4.17	5.68	>50	13.8	17.6	21.8	>50
ZM108.4	C	0.296	0.223	0.715	0.161	1.99	1.70	4.64	1.07
ZM135.10a	C	0.156	0.106	0.246	0.033	1.05	0.913	2.20	0.408
ZM178.66	C	0.563	0.443	1.10	0.267	3.21	2.80	5.65	1.73

Figure 83 cont.

		Key {IC ₅₀ /IC ₅₀ in µg/ml}			
		<0.100	0.100-1.00	1.00-10.0	>10.0

Virus	Clade	IC ₅₀ (µg/ml)			
		DH511.11P	DH511.12P	DH511.2	10E8
Zh187.7	C	0.058	0.108	0.232	0.055
Zh14.15	C	2.52	1.84	5.39	2.22
Zh15.8	C	0.077	0.066	0.107	0.044
Zh23.6	C	0.253	0.182	0.549	0.270
Zh249.1	C	1.78	1.66	1.58	0.830
Zh153.12	C	8.95	8.14	6.11	2.62
Zh55.28a	C	6.32	4.10	7.88	2.34
3326/V4.C3	CD	1.89	1.64	3.52	1.40
3337/V2.C6	CD	1.06	0.763	1.50	1.09
3817/V2.c59	CD	2.08	1.84	2.12	0.229
191821.E3.1	D	2.48	2.47	6.82	1.91
231935.c1	D	13.6	12.6	19.6	11.0
247.23	D	0.652	0.500	0.618	0.344
3018/V5.c45	D	0.826	0.749	0.607	0.710
57128.vrc15	D	0.417	0.496	0.352	0.212
6405.V4.c34	D	0.981	0.870	1.93	0.461
A03349/v1.vrc4s	D	0.508	0.532	0.402	0.270
A07412/v1.vrc12	D	0.425	0.375	0.851	0.140
NK13006.ec1	D	0.625	0.713	0.989	0.673
UG021.16	D			0.100	0.046
UG024.2	D	0.109	0.106	0.154	0.053
F0402.c2.11	G	0.151	0.149	0.094	0.057
P1981.C5.3	G	0.071	0.073	0.026	0.024

		IC ₅₀ (µg/ml)			
		DH511.11P	DH511.12P	DH511.2	10E8
		0.523	0.520	1.16	0.369
		9.67	6.40	15.5	5.98
		0.343	0.246	0.591	0.230
		0.983	1.08	2.33	0.737
		4.94	5.11	5.78	2.27
		22.2	20.3	18.5	6.72
		15.8	17.0	19.8	6.78
		8.13	6.80	13.3	4.29
		6.34	4.46	5.60	4.87
		8.07	6.31	6.33	1.43
		7.70	6.83	18.3	5.89
		33.1	31.8	>50	20.4
		2.35	2.08	3.24	1.29
		3.12	2.90	3.42	2.17
		1.96	2.14	2.59	1.50
		4.91	4.20	6.76	1.80
		1.33	1.39	1.32	0.663
		2.45	2.13	4.55	0.873
		2.72	2.69	4.86	2.46
				0.774	0.362
		0.451	0.523	0.745	0.241
		0.953	1.04	0.815	0.460
		0.280	0.221	0.211	0.124

Figure 83 cont.

X1193.c1	G	0.324	0.360	0.746	0.341
X1254.c3	G	1.34	1.06	2.77	3.67
X1832.S2B10	G	0.728	0.675	0.925	0.387
X2088.c9	G	>50	>50	>50	>50
X2131.C1.B5	G	0.079	0.089	0.118	0.039
SV/mec25130.S.G3	NA	10.3	29.9	>50	>50
SV/ALV	NA	>50	>50	>50	>50

1.45	1.54	3.28	1.15
6.12	6.13	14.5	15.7
3.47	3.22	3.93	1.76
>50	>50	>50	>50
0.382	0.386	0.611	0.175
>50	>50	>50	>50
>50	>50	>50	>50

	DH511.11P	DH511.12P	DH511.2	10E8
Number of Viruses	203	203	208	208
Total Viruses Neutralized				
IC50/IC80 <50ug/ml	202	202	206	203
IC50/IC80 <10ug/ml	196	198	198	202
IC50/IC80 <1.0ug/ml	122	123	103	152
IC50/IC80 <0.1ug/ml	19	18	20	42
IC50/IC80 <0.01ug/ml	4	3	7	10
Percent of Viruses Neutralized				
IC50/IC80 <50ug/ml	100	100	99	98
IC50/IC80 <10ug/ml	97	98	95	97
IC50/IC80 <1.0ug/ml	60	61	50	73
IC50/IC80 <0.1ug/ml	9	9	10	20
IC50/IC80 <0.01ug/ml	2	1	3	5
Median IC50/IC80	0.764	0.698	1.04	0.392
Geometric Mean	0.684	0.634	0.792	0.298

DH511.11P	DH511.12P	DH511.2	10E8
203	203	208	208
201	201	203	203
169	172	147	193
37	37	33	61
5	6	7	10
0	0	3	5
99	99	98	98
83	85	71	93
18	18	16	29
2	3	3	5
0	0	1	2
3.49	3.33	5.07	1.69
3.02	2.80	3.73	1.34

Figure 83 cont.

Peptide	Sequence
MPR.03-biotin	KKK-NEQELLELDKWASLNNWFDTNWLWYIR-KKK-biotin
MPR.03 N671A-biotin	KKK-NEQELLELDKWASLNNWFDTNWLWYIR-KKK-biotin
MPR.03 W672A-biotin	KKK-NEQELLELDKWASLNNWFDTNWLWYIR-KKK-biotin
MPR.03 F673A-biotin	KKK-NEQELLELDKWASLNNWFDTNWLWYIR-KKK-biotin
MPR.03 D674A-biotin	KKK-NEQELLELDKWASLNNWFDTNWLWYIR-KKK-biotin
MPR.03 I675A-biotin	KKK-NEQELLELDKWASLNNWFDTNWLWYIR-KKK-biotin
MPR.03 T676A-biotin	KKK-NEQELLELDKWASLNNWFDTNWLWYIR-KKK-biotin
MPR.03 N677A-biotin	KKK-NEQELLELDKWASLNNWFDTNWLWYIR-KKK-biotin
MPR.03 W678A-biotin	KKK-NEQELLELDKWASLNNWFDTNWLWYIR-KKK-biotin
MPR.03 L679A-biotin	KKK-NEQELLELDKWASLNNWFDTNWLWYIR-KKK-biotin
MPR.03 W680A-biotin	KKK-NEQELLELDKWASLNNWFDTNWLWYIR-KKK-biotin
MPR.03 Y681A-biotin	KKK-NEQELLELDKWASLNNWFDTNWLWYIR-KKK-biotin
MPR.03 I682A-biotin	KKK-NEQELLELDKWASLNNWFDTNWLWYIR-KKK-biotin
MPR.03 R683A-biotin	KKK-NEQELLELDKWASLNNWFDTNWLWYIR-KKK-biotin

Figure 84

Virus	Sequence
COT6.15 WT	QELLALDSWKNLWSWFDITKWLWYIKIFI
A662G	QELLGLDSWKNLWSWFDITKWLWYIKIFI
L663A	QELLAADSWKNLWSWFDITKWLWYIKIFI
D664A	QELLALASWKNLWSWFDITKWLWYIKIFI
S665A	QELLALDAWKNLWSWFDITKWLWYIKIFI
W666A	QELLALDSAKNLWSWFDITKWLWYIKIFI
K667A	QELLALDSWANLWSWFDITKWLWYIKIFI
N668A	QELLALDSWKALWSWFDITKWLWYIKIFI
L669A	QELLALDSWKNAWSWFDITKWLWYIKIFI
W670A	QELLALDSWKNLASWFDITKWLWYIKIFI
S671A	QELLALDSWKNLWAWFDITKWLWYIKIFI
W672A	QELLALDSWKNLWSAFDITKWLWYIKIFI
F673A	QELLALDSWKNLWSWADITKWLWYIKIFI
D674A	QELLALDSWKNLWSWFAITKWLWYIKIFI
D674S	QELLALDSWKNLWSWFSITKWLWYIKIFI
D674N	QELLALDSWKNLWSWFNITKWLWYIKIFI
I675A	QELLALDSWKNLWSWFDATKWLWYIKIFI
T676A	QELLALDSWKNLWSWFEDIAKWLWYIKIFI
K677A	QELLALDSWKNLWSWFDITAWLWYIKIFI
W678A	QELLALDSWKNLWSWFDITKALWYIKIFI
L679A	QELLALDSWKNLWSWFDITKWAWYIKIFI
W680A	QELLALDSWKNLWSWFDITKWLAYIKIFI

Figure 85

IC ₅₀ (µg/ml) in TZM-bl cells ¹										
Antibody	COT6	L663A	D664A	S665A	W666A	K667A	N668A	L669A	W670A	S671A
	WT	A662G								
DH511.1	0.21	<0.023	<0.023	0.36	0.08	<0.023	0.62	<0.023	0.17	<0.023
DH511.2	0.10	<0.023	<0.023	0.12	0.03	<0.023	0.24	<0.023	<0.023	<0.023
DH511.3	0.30	<0.023	<0.023	0.44	0.18	<0.023	0.95	0.04	0.34	0.06
DH511.4	0.32	<0.023	<0.023	0.58	0.22	<0.023	0.84	<0.023	0.35	0.09
DH511.5	0.46	0.05	0.03	0.81	0.25	<0.023	1.2	0.08	0.38	0.10
DH511.6	0.40	0.04	<0.023	0.44	0.23	<0.023	1.3	0.07	0.45	0.10
DH511.11P	0.08	<0.023	<0.023	0.12	0.05	<0.023	0.25	<0.023	0.06	<0.023
DH511.12P	0.09	<0.023	<0.023	0.12	0.05	<0.023	0.26	<0.023	0.05	<0.023
2F5	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
10E8	0.03	<0.023	<0.023	0.04	<0.023	<0.023	0.10	<0.023	<0.023	<0.023
4E10	0.54	0.04	<0.023	0.89	0.30	<0.023	2.2	0.06	0.57	0.19
CH01-31	0.12	0.14	0.10	0.13	0.13	0.04	0.10	0.06	0.09	0.11

¹Values are the antibody concentration (µg/ml) at which relative luminescence units (RLUs) were reduced 50% compared to virus control wells (no test sample).

Note: Values in bold are considered positive for neutralizing antibody activity when compared against background.

Values highlighted in yellow indicate resistance to neutralization.

Figure 86

IC ₅₀ (µg/ml) in TZM-bl cells ¹											
Antibody	D664A	F673A	D674A	D674S	I675A	T676A			L679A	W680A	D674N
			D674A				K677A	W678A			
DH511.1	>50*	1.0 *	2.5*	1.9*	<0.023	0.09	<0.023	<0.023	0.35	<0.023	0.21
DH511.2	0.19	0.31	0.71*	0.55*	<0.023	<0.023	<0.023	<0.023	<0.023	<0.023	0.07
DH511.3	>50*	1.6*	4.2*	3.4*	<0.023	0.15	0.05	0.03	0.97	<0.023	0.44
DH511.4	15*	1.4*	5.5*	3.6*	0.03	0.14	0.06	0.06	0.14	0.05	0.71
DH511.5	31*	1.6*	8.2*	2.0*	0.06	0.24	0.14	0.10	0.19	0.10	0.72
DH511.6	>50*	1.9*	7.1*	5.1*	0.03	0.15	0.09	0.08	2.6	0.05	1.0
DH511.11P	1.5*	0.27	0.56*	0.27	<0.023	<0.023	<0.023	<0.023	0.03	<0.023	0.05
DH511.12P	0.87*	0.24	0.53*	0.23	<0.023	0.02	<0.023	<0.023	0.03	<0.023	0.04
2F5	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
10E8	10*	0.72*	0.30*	0.18*	<0.023	<0.023	<0.023	<0.023	<0.023	<0.023	0.03
4E10	>50*	>50*	5.5*	4.1*	0.09	>50*	0.16	0.07	0.53	17*	0.60
CH01-31	0.07	0.07	0.09	0.10	0.09	0.07	0.07	0.06	0.10	0.08	0.11

¹Values are the antibody concentration (µg/ml) at which relative luminescence units (RLUs) were reduced 50% compared to virus control wells (no test sample).

Note: Values in bold are considered positive for neutralizing antibody activity when compared against background.

Values marked with asterix (*) indicate resistance to neutralization.

Figure 86 cont.

Antibody	Peptide	gp41 Residue Range	Peptide Sequence
DH511.1	MPER ₆₅₆₋₆₈₃	656-683	RRR ⁶⁵⁶ NEQELLEDKWASLWNWFDITNWLWYIR ⁶⁸³ RRR
DH511.2	MPR.03.DN 4	662-683	KKK ⁶⁶² ELDKWASLWNWFDITNWLWYIR ⁶⁸³ KKK
DH511.2	MPR.03.DN 14	670-683	KKK ⁶⁷⁰ WNWFDITNWLWYIR ⁶⁸³ KKK

Figure 87

		DH511.1 Fab +	DH511.2 Fab +	DH511.2 Fab +	DH511.4 Fab
		MPER ₆₅₆₋₆₈₃	MPER ₆₆₂₋₆₈₃	MPER ₆₇₀₋₆₈₃	
PDB Accession					
Code					
Data Collection					
Space group	I222				P1
Cell constants					
a, b, c (Å)	64.1, 134.0, 179.6	109.5, 75.8, 122.8	110.1, 75.6, 123.4	42.5, 48.2, 57.1	
a, b, g (°)	90.0, 90.0, 90.0	90.0, 94.5, 90.0	90.0, 94.1, 90.0	86.1, 82.3, 82.3	
Wavelength (Å)	1.0000	1.0000	1.0000	1.0000	
Resolution (Å)	50 - 2.70	50 - 2.65	50 - 2.17	50 - 1.50	
	(2.80 - 2.70)	(2.74 - 2.65)	(2.23 - 2.17)	(1.53 - 1.50)	
R_{merge}	0.133 (0.809)	0.085 (0.467)	0.077 (0.483)	0.055 (0.519)	
I / σ	12.1 (1.66)	6.54 (1.70)	15.6 (1.65)	16.1 (3.7)	
Completeness (%)	99.7 (98.4)	96.9 (97.9)	93.5 (52.5)	93.3 (93.1)	
Multiplicity	6.3 (4.6)	1.7 (1.7)	5.1 (2.6)	2.5 (2.3)	
Refinement					
Resolution (Å)	37.3 - 2.74	40.8 - 2.64	42.5 - 2.17	27.3 - 1.50	
	(2.83 - 2.74)	(2.73 - 2.64)	(2.24 - 2.17)	(1.53 - 1.50)	
Unique reflections	20466 (1711)	28726 (2832)	51031 (3495)	64453 (3551)	
$R_{\text{work}} / R_{\text{free}}$ (%)	21.28/25.57	25.61/28.99	19.03/22.63	18.80/21.11	
No. atoms	3750	7332	7403	3655	
Protein	3723	7124	7135	3464	
Ligand/ion	-	2	1	0	

Figure 88

Water	27	206	267	191
B-factors ($\text{\AA}^2$)	80.5	58.5	55.8	28.2
Protein	80.6	58.9	56.0	28.0
Ligand/ion	-	61.6	29.6	-
Water	57.7	45.8	51.0	31.3
R.m.s. deviations				
Bond lengths ($\text{\AA}$)	0.003	0.002	0.006	0.006
Bond angles ($^\circ$)	0.68	0.62	0.88	1.121
Ramachandran				
Most favored regions (%)	94.0	97.0	98.0	98.5
Additional allowed regions (%)	5.37	3.0	2	1.6
Disallowed regions (%)	0.63	0	0	0

Numbers in parentheses represent highest resolution shell.

Figure 88 cont.

Antibody contact interfaces by HCDR loop (Å²)

	DH511.1 + MPER ₆₅₆₋₆₈₃	DH511.2 + MPER ₆₆₂₋₆₈₃	DH511.12P + MPER ₆₆₂₋₆₈₃
HCDR1	207.53	138.02	130.8
HCDR2	122.54	148.85	106.55
FWR3	58.57	3.25	0
HCDR3	362.48	391.28	403.71
Total	751.12	681.4	641.06

Figure 89

Hydrogen bonds

<----- A T O M 1 ----->					<----- A T O M 2 ----->							
Atom Atom Res Res Chain					Atom Atom Res Res Chain							
no. name no. Chain					no. name no. Chain							
Distance												
1.	3574	O	TRP	670	A	<----->	214	ND2	ASN	31	H	3.14
2.	3625	OD2	ASP	674	A	<----->	397	NH2	ARG	53	H	3.32
3.	3624	OD1	ASP	674	A	<----->	414	NZ	LYS	55	H	2.97
4.	3712	NE	ARG	683	A	<----->	873	O	TRP	113	H	3.15
5.	3715	NH2	ARG	683	A	<----->	873	O	TRP	113	H	3.09

Salt bridges

<----- A T O M 1 ----->					<----- A T O M 2 ----->							
Atom Atom Res Res Chain					Atom Atom Res Res Chain							
no. name no. Chain					no. name no. Chain							
Distance												
1.	3625	OD2	ASP	674	A	<--->	397	NH2	ARG	53	H	3.32
2.	3624	OD1	ASP	674	A	<--->	414	NZ	LYS	55	H	2.97

Figure 90

ATOM 1 ---> <--- ATOM 2 --->

Figure 90 cont.

18.	3589	CB	ASN	671	A	<--->	213	OD1	ASN	31	H	3.64
19.	3573	C	TRP	670	A	<--->	214	ND2	ASN	31	H	3.60
20.	3574	O	TRP	670	A	<--->	214	ND2	ASN	31	H	3.14
21.	3589	CB	ASN	671	A	<--->	214	ND2	ASN	31	H	3.63
22.	3604	CZ2	TRP	672	A	<--->	229	CD2	TRP	33	H	3.66
23.	3604	CZ2	TRP	672	A	<--->	232	CE3	TRP	33	H	3.83
24.	3612	CG	PHE	673	A	<--->	233	CZ2	TRP	33	H	3.75
25.	3613	CD1	PHE	673	A	<--->	233	CZ2	TRP	33	H	3.69
26.	3615	CE1	PHE	673	A	<--->	233	CZ2	TRP	33	H	3.84
27.	3606	CH2	TRP	672	A	<--->	234	CZ3	TRP	33	H	3.87
28.	3615	CE1	PHE	673	A	<--->	235	CH2	TRP	33	H	3.83
29.	3590	CG	ASN	671	A	<--->	393	CD	ARG	53	H	3.88
30.	3592	ND2	ASN	671	A	<--->	393	CD	ARG	53	H	3.35
31.	3590	CG	ASN	671	A	<--->	394	NE	ARG	53	H	3.83
32.	3591	OD1	ASN	671	A	<--->	394	NE	ARG	53	H	3.78
33.	3592	ND2	ASN	671	A	<--->	394	NE	ARG	53	H	3.80
34.	3590	CG	ASN	671	A	<--->	395	CZ	ARG	53	H	3.54
35.	3591	OD1	ASN	671	A	<--->	395	CZ	ARG	53	H	3.08
36.	3592	ND2	ASN	671	A	<--->	395	CZ	ARG	53	H	3.87
37.	3625	OD2	ASP	674	A	<--->	395	CZ	ARG	53	H	3.74
38.	3590	CG	ASN	671	A	<--->	396	NH1	ARG	53	H	3.20
39.	3591	OD1	ASN	671	A	<--->	396	NH1	ARG	53	H	2.46
40.	3592	ND2	ASN	671	A	<--->	396	NH1	ARG	53	H	3.50
41.	3623	CG	ASP	674	A	<--->	396	NH1	ARG	53	H	3.90
42.	3624	OD1	ASP	674	A	<--->	396	NH1	ARG	53	H	3.86
43.	3625	OD2	ASP	674	A	<--->	396	NH1	ARG	53	H	3.39

Figure 90 cont.

44.	3566	O	LEU	669	A	<--->	397	NH2	ARG	53	H	3.62
45.	3591	OD1	ASN	671	A	<--->	397	NH2	ARG	53	H	3.67
46.	3625	OD2	ASP	674	A	<--->	397	NH2	ARG	53	H	3.32
47.	3566	O	LEU	669	A	<--->	413	CE	LYS	55	H	3.70
48.	3623	CG	ASP	674	A	<--->	414	NZ	LYS	55	H	3.86
49.	3624	OD1	ASP	674	A	<--->	414	NZ	LYS	55	H	2.97
50.	3614	CD2	PHE	673	A	<--->	420	CG	ASP	56	H	3.76
51.	3614	CD2	PHE	673	A	<--->	421	OD1	ASP	56	H	3.84
52.	3614	CD2	PHE	673	A	<--->	422	OD2	ASP	56	H	3.58
53.	3598	CG	TRP	672	A	<--->	788	N	GLY	103	H	3.62
54.	3599	CD1	TRP	672	A	<--->	788	N	GLY	103	H	3.54
55.	3600	CD2	TRP	672	A	<--->	788	N	GLY	103	H	3.55
56.	3601	NE1	TRP	672	A	<--->	788	N	GLY	103	H	3.43
57.	3602	CE2	TRP	672	A	<--->	788	N	GLY	103	H	3.43
58.	3598	CG	TRP	672	A	<--->	789	CA	GLY	103	H	3.75
59.	3600	CD2	TRP	672	A	<--->	789	CA	GLY	103	H	3.41
60.	3602	CE2	TRP	672	A	<--->	789	CA	GLY	103	H	3.69
61.	3603	CE3	TRP	672	A	<--->	789	CA	GLY	103	H	3.62
62.	3598	CG	TRP	672	A	<--->	790	C	GLY	103	H	3.86
63.	3597	CB	TRP	672	A	<--->	791	O	GLY	103	H	3.12
64.	3598	CG	TRP	672	A	<--->	791	O	GLY	103	H	3.34
65.	3600	CD2	TRP	672	A	<--->	791	O	GLY	103	H	3.83
66.	3640	CG2	THR	676	A	<--->	802	CA	PRO	105	H	3.79
67.	3640	CG2	THR	676	A	<--->	805	CB	PRO	105	H	3.66
68.	3640	CG2	THR	676	A	<--->	806	CG	PRO	105	H	3.77
69.	3711	CD	ARG	683	A	<--->	839	O	PHE	110	H	3.13

Figure 90 cont.

70.	3712	NE	ARG	683	A	<--->	839	O	PHE	110	H	3.42
71.	3710	CG	ARG	683	A	<--->	847	CE1	PHE	110	H	3.70
72.	3711	CD	ARG	683	A	<--->	848	CE2	PHE	110	H	3.73
73.	3710	CG	ARG	683	A	<--->	849	CZ	PHE	110	H	3.51
74.	3711	CD	ARG	683	A	<--->	849	CZ	PHE	110	H	3.69
75.	3712	NE	ARG	683	A	<--->	853	O	PHE	111	H	3.89
76.	3713	CZ	ARG	683	A	<--->	853	O	PHE	111	H	3.83
77.	3715	NH2	ARG	683	A	<--->	853	O	PHE	111	H	3.71
78.	3712	NE	ARG	683	A	<--->	870	N	TRP	113	H	3.60
79.	3677	CD1	TRP	680	A	<--->	873	O	TRP	113	H	3.43
80.	3712	NE	ARG	683	A	<--->	873	O	TRP	113	H	3.15
81.	3713	CZ	ARG	683	A	<--->	873	O	TRP	113	H	3.50
82.	3715	NH2	ARG	683	A	<--->	873	O	TRP	113	H	3.09
83.	3665	C	LEU	679	A	<--->	874	CB	TRP	113	H	3.86
84.	3666	O	LEU	679	A	<--->	874	CB	TRP	113	H	3.36
85.	3667	CB	LEU	679	A	<--->	874	CB	TRP	113	H	3.85
86.	3710	CG	ARG	683	A	<--->	874	CB	TRP	113	H	3.81
87.	3711	CD	ARG	683	A	<--->	874	CB	TRP	113	H	3.84
88.	3712	NE	ARG	683	A	<--->	874	CB	TRP	113	H	3.65
89.	3667	CB	LEU	679	A	<--->	875	CG	TRP	113	H	3.84
90.	3667	CB	LEU	679	A	<--->	877	CD2	TRP	113	H	3.79
91.	3666	O	LEU	679	A	<--->	880	CE3	TRP	113	H	3.31
92.	3667	CB	LEU	679	A	<--->	880	CE3	TRP	113	H	3.80
93.	3670	CD2	LEU	679	A	<--->	880	CE3	TRP	113	H	3.87
94.	3670	CD2	LEU	679	A	<--->	882	CZ3	TRP	113	H	3.83
95.	3637	O	THR	676	A	<--->	884	N	GLY	114	H	3.89

Figure 90 cont.

96.	3637	O	THR	676	A	<-->	885	CA	GLY	114	H	3.42
97.	3675	CB	TRP	680	A	<-->	885	CA	GLY	114	H	3.64
98.	3677	CD1	TRP	680	A	<-->	885	CA	GLY	114	H	3.87
99.	3638	CB	THR	676	A	<-->	887	O	GLY	114	H	3.55
100.	3605	CZ3	TRP	672	A	<-->	899	CG	TYR	116	H	3.67
101.	3605	CZ3	TRP	672	A	<-->	900	CD1	TYR	116	H	3.69
102.	3605	CZ3	TRP	672	A	<-->	901	CD2	TYR	116	H	3.82
103.	3605	CZ3	TRP	672	A	<-->	902	CE1	TYR	116	H	3.88

Figure 90 cont.

Hydrogen bonds

<----- A T O M				1	<----- A T O M				2	<----- A T O M				2
Atom		Atom		Res	Chain		Atom		Res	Chain		Atom		Res
no.		name		no.	no.		name		no.	Chain		Distance		Distance
1.	209	O	ASN	31	H	<-->	6804	ND2	ASN	671	P	3.17		
2.	213	ND2	ASN	31	H	<-->	6786	O	TRP	670	P	2.86		
3.	397	NH1	ARG	53	H	<-->	6837	OD2	ASP	674	P	2.93		
4.	415	NZ	LYS	55	H	<-->	6750	O	TRP	666	P	3.29		
5.	415	NZ	LYS	55	H	<-->	6778	O	LEU	669	P	3.02		
6.	864	O	TRP	112	H	<-->	6924	NE	ARG	683	P	2.94		
7.	864	O	TRP	112	H	<-->	6927	NH2	ARG	683	P	2.97		

Salt bridges

<----- A T O M		1	<----- A T O M		2	<----- A T O M		2
Atom		no.	Atom		no.	Atom		no.
Res		name	Res		name	Res		name
Chain			Chain			Chain		
Distance			Distance			Distance		
1.	397	NH1 ARG 53	<-->	6837	OD2 ASP 674	P	2.93	
2.	415	NZ LYS 55	<-->	6836	OD1 ASP 674	P	3.30	

Figure 91

Non-bonded contacts

<----- A T O M 1 ----->				<----- A T O M 2 ----->					
	Atom	Atom	Res	Chain	Atom	Atom	Res	Chain	Distance
	no.	name	no.		no.	name	no.		
1.	185	CE2 PHE	28	H <-->	6782	CD2 LEU	669	P	3.35
2.	186	CZ PHE	28	H <-->	6782	CD2 LEU	669	P	3.46
3.	209	O ASN	31	H <-->	6804	ND2 ASN	671	P	3.17
4.	211	CG ASN	31	H <-->	6786	O TRP	670	P	3.77
5.	212	OD1 ASN	31	H <-->	6777	C LEU	669	P	3.87
6.	212	OD1 ASN	31	H <-->	6779	CB LEU	669	P	3.51
7.	212	OD1 ASN	31	H <-->	6783	N TRP	670	P	3.68
8.	212	OD1 ASN	31	H <-->	6785	C TRP	670	P	3.86
9.	212	OD1 ASN	31	H <-->	6786	O TRP	670	P	3.86
10.	212	OD1 ASN	31	H <-->	6801	CB ASN	671	P	3.78
11.	212	OD1 ASN	31	H <-->	6804	ND2 ASN	671	P	3.90
12.	213	ND2 ASN	31	H <-->	6785	C TRP	670	P	3.57
13.	213	ND2 ASN	31	H <-->	6786	O TRP	670	P	2.86
14.	213	ND2 ASN	31	H <-->	6801	CB ASN	671	P	3.76
15.	225	CG TRP	33	H <-->	6816	CZ2 TRP	672	P	3.76
16.	227	CD2 TRP	33	H <-->	6816	CZ2 TRP	672	P	3.50
17.	227	CD2 TRP	33	H <-->	6818	CH2 TRP	672	P	3.71
18.	229	CE2 TRP	33	H <-->	6816	CZ2 TRP	672	P	3.72
19.	229	CE2 TRP	33	H <-->	6818	CH2 TRP	672	P	3.74

20.	230	CE3	TRP	33	H	<--->	6816	CZ2	TRP	672	P	3.83
21.	230	CE3	TRP	33	H	<--->	6818	CH2	TRP	672	P	3.69
22.	231	CZ2	TRP	33	H	<--->	6818	CH2	TRP	672	P	3.79
23.	231	CZ2	TRP	33	H	<--->	6824	CG	PHE	673	P	3.51
24.	231	CZ2	TRP	33	H	<--->	6825	CD1	PHE	673	P	3.45
25.	231	CZ2	TRP	33	H	<--->	6826	CD2	PHE	673	P	3.67
26.	231	CZ2	TRP	33	H	<--->	6827	CE1	PHE	673	P	3.58
27.	231	CZ2	TRP	33	H	<--->	6828	CE2	PHE	673	P	3.79
28.	231	CZ2	TRP	33	H	<--->	6829	CZ	PHE	673	P	3.75
29.	232	CZ3	TRP	33	H	<--->	6818	CH2	TRP	672	P	3.70
30.	233	CH2	TRP	33	H	<--->	6818	CH2	TRP	672	P	3.75
31.	233	CH2	TRP	33	H	<--->	6825	CD1	PHE	673	P	3.82
32.	233	CH2	TRP	33	H	<--->	6827	CE1	PHE	673	P	3.68
33.	386	NH1	ARG	52	H	<--->	6829	CZ	PHE	673	P	3.70
34.	394	CD	ARG	53	H	<--->	6804	ND2	ASN	671	P	3.32
35.	395	NE	ARG	53	H	<--->	6804	ND2	ASN	671	P	3.36
36.	396	CZ	ARG	53	H	<--->	6802	CG	ASN	671	P	3.48
37.	396	CZ	ARG	53	H	<--->	6803	OD1	ASN	671	P	3.23
38.	396	CZ	ARG	53	H	<--->	6804	ND2	ASN	671	P	3.41
39.	396	CZ	ARG	53	H	<--->	6837	OD2	ASP	674	P	3.36
40.	397	NH1	ARG	53	H	<--->	6777	C	LEU	669	P	3.88
41.	397	NH1	ARG	53	H	<--->	6778	O	LEU	669	P	3.72

Figure 91 cont.

42.	397	NH1	ARG	53	H	<--->	6779	CB	LEU	669	P	3.86
43.	397	NH1	ARG	53	H	<--->	6797	N	ASN	671	P	3.76
44.	397	NH1	ARG	53	H	<--->	6801	CB	ASN	671	P	3.90
45.	397	NH1	ARG	53	H	<--->	6802	CG	ASN	671	P	3.16
46.	397	NH1	ARG	53	H	<--->	6803	OD1	ASN	671	P	3.13
47.	397	NH1	ARG	53	H	<--->	6804	ND2	ASN	671	P	3.35
48.	397	NH1	ARG	53	H	<--->	6837	OD2	ASP	674	P	2.93
49.	398	NH2	ARG	53	H	<--->	6803	OD1	ASN	671	P	3.37
50.	398	NH2	ARG	53	H	<--->	6835	CG	ASP	674	P	3.28
51.	398	NH2	ARG	53	H	<--->	6836	OD1	ASP	674	P	3.05
52.	398	NH2	ARG	53	H	<--->	6837	OD2	ASP	674	P	2.97
53.	403	CB	LEU	54	H	<--->	6761	CZ2	TRP	666	P	3.41
54.	403	CB	LEU	54	H	<--->	6763	CH2	TRP	666	P	3.83
55.	408	CA	LYS	55	H	<--->	6760	CE3	TRP	666	P	3.72
56.	411	CB	LYS	55	H	<--->	6754	CB	TRP	666	P	3.78
57.	411	CB	LYS	55	H	<--->	6755	CG	TRP	666	P	3.75
58.	411	CB	LYS	55	H	<--->	6757	CD2	TRP	666	P	3.73
59.	411	CB	LYS	55	H	<--->	6760	CE3	TRP	666	P	3.78
60.	413	CD	LYS	55	H	<--->	6750	O	TRP	666	P	3.85
61.	413	CD	LYS	55	H	<--->	6779	CB	LEU	669	P	3.74
62.	414	CE	LYS	55	H	<--->	6750	O	TRP	666	P	3.79
63.	415	NZ	LYS	55	H	<--->	6750	O	TRP	666	P	3.29
64.	415	NZ	LYS	55	H	<--->	6778	O	LEU	669	P	3.02
65.	415	NZ	LYS	55	H	<--->	6836	OD1	ASP	674	P	3.30
66.	421	CG	ASP	56	H	<--->	6826	CD2	PHE	673	P	3.53

Figure 91 cont.

67.	421	CG	ASP	56	H	<--->	6828	CE2	PHE	673	P	3.67
68.	422	OD1	ASP	56	H	<--->	6826	CD2	PHE	673	P	3.36
69.	422	OD1	ASP	56	H	<--->	6828	CE2	PHE	673	P	3.57
70.	423	OD2	ASP	56	H	<--->	6826	CD2	PHE	673	P	3.60
71.	782	CA	GLU	102	H	<--->	6813	NE1	TRP	672	P	3.79
72.	783	C	GLU	102	H	<--->	6813	NE1	TRP	672	P	3.84
73.	790	N	GLY	103	H	<--->	6810	CG	TRP	672	P	3.53
74.	790	N	GLY	103	H	<--->	6811	CD1	TRP	672	P	3.32
75.	790	N	GLY	103	H	<--->	6812	CD2	TRP	672	P	3.55
76.	790	N	GLY	103	H	<--->	6813	NE1	TRP	672	P	3.20
77.	790	N	GLY	103	H	<--->	6814	CE2	TRP	672	P	3.35
78.	791	CA	GLY	103	H	<--->	6810	CG	TRP	672	P	3.64
79.	791	CA	GLY	103	H	<--->	6812	CD2	TRP	672	P	3.45
80.	791	CA	GLY	103	H	<--->	6814	CE2	TRP	672	P	3.70
81.	791	CA	GLY	103	H	<--->	6815	CE3	TRP	672	P	3.83
82.	792	C	GLY	103	H	<--->	6809	CB	TRP	672	P	3.76
83.	792	C	GLY	103	H	<--->	6810	CG	TRP	672	P	3.71
84.	793	O	GLY	103	H	<--->	6809	CB	TRP	672	P	3.27
85.	793	O	GLY	103	H	<--->	6810	CG	TRP	672	P	3.44
86.	793	O	GLY	103	H	<--->	6811	CD1	TRP	672	P	3.81
87.	802	CA	PRO	105	H	<--->	6852	CG2	THR	676	P	3.79
88.	805	CB	PRO	105	H	<--->	6845	CG2	ILE	675	P	3.73
89.	805	CB	PRO	105	H	<--->	6852	CG2	THR	676	P	3.72

Figure 91 cont.

90.	806	CG	PRO	105	H	<-->	6845	CG2	ILE	675	P	3.75
91.	836	O	PHE	109	H	<-->	6923	CD	ARG	683	P	3.38
92.	847	O	LEU	110	H	<-->	6925	CZ	ARG	683	P	3.77
93.	847	O	LEU	110	H	<-->	6926	NH1	ARG	683	P	3.69
94.	861	N	TRP	112	H	<-->	6924	NE	ARG	683	P	3.81
95.	863	C	TRP	112	H	<-->	6924	NE	ARG	683	P	3.86
96.	864	O	TRP	112	H	<-->	6884	CA	TRP	680	P	3.69
97.	864	O	TRP	112	H	<-->	6924	NE	ARG	683	P	2.94
98.	864	O	TRP	112	H	<-->	6925	CZ	ARG	683	P	3.38
99.	864	O	TRP	112	H	<-->	6927	NH2	ARG	683	P	2.97
100.	865	CB	TRP	112	H	<-->	6878	O	LEU	679	P	3.78
101.	865	CB	TRP	112	H	<-->	6879	CB	LEU	679	P	3.68
102.	865	CB	TRP	112	H	<-->	6924	NE	ARG	683	P	3.85
103.	866	CG	TRP	112	H	<-->	6879	CB	LEU	679	P	3.78
104.	868	CD2	TRP	112	H	<-->	6879	CB	LEU	679	P	3.82
105.	871	CE3	TRP	112	H	<-->	6878	O	LEU	679	P	3.63
106.	871	CE3	TRP	112	H	<-->	6879	CB	LEU	679	P	3.83
107.	875	N	GLY	113	H	<-->	6849	O	THR	676	P	3.70
108.	876	CA	GLY	113	H	<-->	6849	O	THR	676	P	3.15
109.	876	CA	GLY	113	H	<-->	6887	CB	TRP	680	P	3.52
110.	878	O	GLY	113	H	<-->	6850	CB	THR	676	P	3.74
111.	882	O	TYR	114	H	<-->	6852	CG2	THR	676	P	3.58
112.	895	CB	PHE	115	H	<-->	6852	CG2	THR	676	P	3.80
113.	896	CG	PHE	115	H	<-->	6817	CZ3	TRP	672	P	3.50
114.	897	CD1	PHE	115	H	<-->	6817	CZ3	TRP	672	P	3.48
115.	898	CD2	PHE	115	H	<-->	6817	CZ3	TRP	672	P	3.72
116.	899	CE1	PHE	115	H	<-->	6817	CZ3	TRP	672	P	3.70
117.	899	CE1	PHE	115	H	<-->	6818	CH2	TRP	672	P	3.87

Figure 91 cont.

Hydrogen bonds

		<----- A T O M 1 ----->		<----- A T O M 2 ----->	
		Atom	Atom Res	Atom	Atom Res
		no.	name no.	no.	name no.
		Chain	Chain	Chain	Chain
Distance					
1.	3574	OD1 ASP	674 A	<---->	399 NH1 ARG 53 H 2.43
2.	3664	NH1 ARG	683 A	<---->	844 O PHE 110 H 2.83

Salt bridges

		<----- A T O M 1 ----->		<----- A T O M 2 ----->	
		Atom	Atom Res	Atom	Atom Res
		no.	name no.	no.	name no.
		Chain	Chain	Chain	Chain
Distance					
1.	3574	OD1 ASP	674 A	<---->	399 NH1 ARG 53 H 2.43

Figure 92

Non-bonded contacts

<----- A T O M 1 ----->				<----- A T O M 2 ----->						
Atom no.	Atom name	Res no.	Chain	Atom no.	Atom name	Res no.	Chain	Distance		
1.	3519	CD1 LEU	669	A	<--->	189	CG2 THR	28	H	3.66
2.	3519	CD1 LEU	669	A	<--->	206	OG SER	30	H	3.23
3.	3540	CG ASN	671	A	<--->	210	O ASN	31	H	3.59
4.	3541	OD1 ASN	671	A	<--->	210	O ASN	31	H	3.79
5.	3542	ND2 ASN	671	A	<--->	210	O ASN	31	H	3.57
6.	3517	CB LEU	669	A	<--->	211	CB ASN	31	H	3.76
7.	3519	CD1 LEU	669	A	<--->	211	CB ASN	31	H	3.57
8.	3541	OD1 ASN	671	A	<--->	211	CB ASN	31	H	3.71
9.	3519	CD1 LEU	669	A	<--->	212	CG ASN	31	H	3.73
10.	3517	CB LEU	669	A	<--->	214	ND2 ASN	31	H	3.55
11.	3518	CG LEU	669	A	<--->	214	ND2 ASN	31	H	3.75
12.	3519	CD1 LEU	669	A	<--->	214	ND2 ASN	31	H	3.75
13.	3520	CD2 LEU	669	A	<--->	214	ND2 ASN	31	H	3.44
14.	3554	CZ2 TRP	672	A	<--->	227	CG TRP	33	H	3.79
15.	3554	CZ2 TRP	672	A	<--->	229	CD2 TRP	33	H	3.67
16.	3554	CZ2 TRP	672	A	<--->	231	CE2 TRP	33	H	3.78
17.	3562	CG PHE	673	A	<--->	233	CZ2 TRP	33	H	3.51
18.	3563	CD1 PHE	673	A	<--->	233	CZ2 TRP	33	H	3.57
19.	3564	CD2 PHE	673	A	<--->	233	CZ2 TRP	33	H	3.66

Figure 92 cont.

20.	3565	CE1	PHE	673	A	<--->	233	CZ2	TRP	33	H	3.81
21.	3566	CE2	PHE	673	A	<--->	233	CZ2	TRP	33	H	3.89
22.	3565	CE1	PHE	673	A	<--->	235	CH2	TRP	33	H	3.86
23.	3541	OD1	ASN	671	A	<--->	396	CD	ARG	53	H	3.61
24.	3542	ND2	ASN	671	A	<--->	396	CD	ARG	53	H	3.53
25.	3541	OD1	ASN	671	A	<--->	397	NE	ARG	53	H	3.51
26.	3541	OD1	ASN	671	A	<--->	398	CZ	ARG	53	H	3.23
27.	3574	OD1	ASP	674	A	<--->	398	CZ	ARG	53	H	3.45
28.	3540	CG	ASN	671	A	<--->	399	NH1	ARG	53	H	3.49
29.	3541	OD1	ASN	671	A	<--->	399	NH1	ARG	53	H	2.96
30.	3542	ND2	ASN	671	A	<--->	399	NH1	ARG	53	H	3.40
31.	3573	CG	ASP	674	A	<--->	399	NH1	ARG	53	H	3.48
32.	3574	OD1	ASP	674	A	<--->	399	NH1	ARG	53	H	2.43
33.	3575	OD2	ASP	674	A	<--->	399	NH1	ARG	53	H	3.82
34.	3574	OD1	ASP	674	A	<--->	400	NH2	ARG	53	H	3.70
35.	3575	OD2	ASP	674	A	<--->	400	NH2	ARG	53	H	3.77
36.	3564	CD2	PHE	673	A	<--->	417	CG	ASP	56	H	3.82
37.	3564	CD2	PHE	673	A	<--->	418	OD1	ASP	56	H	3.76
38.	3567	CZ	PHE	673	A	<--->	438	CD1	ILE	59	H	3.64
39.	3549	CD1	TRP	672	A	<--->	785	OE1	GLU	102	H	3.63
40.	3551	NE1	TRP	672	A	<--->	785	OE1	GLU	102	H	3.90
41.	3548	CG	TRP	672	A	<--->	787	N	GLY	103	H	3.66
42.	3549	CD1	TRP	672	A	<--->	787	N	GLY	103	H	3.60
43.	3550	CD2	TRP	672	A	<--->	787	N	GLY	103	H	3.48

Figure 92 cont.

44.	3551	NE1	TRP	672	A	<--->	787	N	GLY	103	H	3.41
45.	3552	CE2	TRP	672	A	<--->	787	N	GLY	103	H	3.32
46.	3554	CZ2	TRP	672	A	<--->	787	N	GLY	103	H	3.86
47.	3550	CD2	TRP	672	A	<--->	788	CA	GLY	103	H	3.47
48.	3552	CE2	TRP	672	A	<--->	788	CA	GLY	103	H	3.70
49.	3553	CE3	TRP	672	A	<--->	788	CA	GLY	103	H	3.60
50.	3547	CB	TRP	672	A	<--->	790	O	GLY	103	H	3.24
51.	3548	CG	TRP	672	A	<--->	790	O	GLY	103	H	3.40
52.	3550	CD2	TRP	672	A	<--->	790	O	GLY	103	H	3.72
53.	3660	CG	ARG	683	A	<--->	830	O	PHE	109	H	3.60
54.	3661	CD	ARG	683	A	<--->	830	O	PHE	109	H	3.21
55.	3658	O	ARG	683	A	<--->	839	CE2	PHE	109	H	3.86
56.	3658	O	ARG	683	A	<--->	840	CZ	PHE	109	H	3.21
57.	3664	NH1	ARG	683	A	<--->	843	C	PHE	110	H	3.46
58.	3664	NH1	ARG	683	A	<--->	844	O	PHE	110	H	2.83
59.	3664	NH1	ARG	683	A	<--->	861	N	TRP	112	H	3.88
60.	3622	CA	TRP	680	A	<--->	864	O	TRP	112	H	3.62
61.	3625	CB	TRP	680	A	<--->	864	O	TRP	112	H	3.90
62.	3661	CD	ARG	683	A	<--->	864	O	TRP	112	H	3.40
63.	3662	NE	ARG	683	A	<--->	864	O	TRP	112	H	3.50
64.	3663	CZ	ARG	683	A	<--->	864	O	TRP	112	H	3.56
65.	3664	NH1	ARG	683	A	<--->	864	O	TRP	112	H	3.48
66.	3615	C	LEU	679	A	<--->	865	CB	TRP	112	H	3.82
67.	3616	O	LEU	679	A	<--->	865	CB	TRP	112	H	3.27
68.	3617	CB	LEU	679	A	<--->	865	CB	TRP	112	H	3.85

Figure 92 cont.

69.	3661	CD	ARG	683	A	<--->	865	CB	TRP	112	H	3.87
70.	3616	O	LEU	679	A	<---->	871	CE3	TRP	112	H	3.74
71.	3587	O	THR	676	A	<--->	875	N	GLY	113	H	3.80
72.	3587	O	THR	676	A	<--->	876	CA	GLY	113	H	3.24
73.	3625	CB	TRP	680	A	<--->	876	CA	GLY	113	H	3.54
74.	3588	CB	THR	676	A	<---->	878	O	GLY	113	H	3.81
75.	3664	NH1	ARG	683	A	<--->	885	CD2	TYR	114	H	3.89
76.	3664	NH1	ARG	683	A	<--->	887	CE2	TYR	114	H	3.38
77.	3590	CG2	THR	676	A	<--->	895	CB	TYR	115	H	3.83
78.	3555	CZ3	TRP	672	A	<---->	896	CG	TYR	115	H	3.61
79.	3555	CZ3	TRP	672	A	<--->	897	CD1	TYR	115	H	3.62
80.	3555	CZ3	TRP	672	A	<--->	898	CD2	TYR	115	H	3.82
81.	3555	CZ3	TRP	672	A	<--->	899	CE1	TYR	115	H	3.85

Figure 92 cont.

Hydrogen bonds

		<----- A T O M 1 ----->		<----- A T O M 2 ----->			
		Atom	Atom Res	Atom	Atom Res	Chain	Distance
	no. name	no.	no.	no.	no.		
1.	3537 ND2 ASN	671	A	210 O	ASN 31	H	2.84
2.	3519 O TRP	670	A	214 ND2 ASN	31	H	3.17
3.	3570 OD2 ASP	674	A	399 NH1 ARG	53	H	2.83
4.	3657 NE ARG	683	A	864 O	TRP 112	H	3.05
5.	3660 NH2 ARG	683	A	864 O	TRP 112	H	3.15

Salt bridges

		<----- A T O M 1 ----->		<----- A T O M 2 ----->			
		Atom	Atom Res	Atom	Atom Res	Chain	Distance
	no. name	no.	no.	no.	no.		
1.	3570 OD2 ASP	674	A	399 NH1 ARG	53	H	2.63

Figure 93

Non-bonded contacts

		<----- A T O M 1 ----->		<----- A T O M 2 ----->			
		Atom	Atom Res	Atom	Atom Res	Chain	Distance
		no.	name	no.	name	no.	
1.	3514	CD1	LEU	669	A	<--->	
2.	3537	ND2	ASN	671	A	<--->	3.35
3.	3534	CB	ASN	671	A	<--->	3.81
4.	3535	CG	ASN	671	A	<--->	3.84
5.	3537	ND2	ASN	671	A	<--->	3.79
6.	3512	CB	LEU	669	A	<--->	2.84
7.	3514	CD1	LEU	669	A	<--->	3.64
8.	3514	CD1	LEU	669	A	<--->	3.71
9.	3514	CD1	LEU	669	A	<--->	3.36
10.	3514	CD1	LEU	669	A	<--->	3.45
11.	3515	CD2	LEU	669	A	<--->	3.76
12.	3518	C	TRP	670	A	<--->	3.75
13.	3519	O	TRP	670	A	<--->	3.81
14.	3534	CB	ASN	671	A	<--->	3.17
15.	3549	CZ2	TRP	672	A	<--->	3.40
16.	3549	CZ2	TRP	672	A	<--->	3.78
17.	3549	CZ2	TRP	672	A	<--->	3.85
18.	3549	CZ2	TRP	672	A	<--->	3.67
19.	3549	CZ2	TRP	672	A	<--->	3.84
20.	3557	CG	PHE	673	A	<--->	3.71
							3.62

Figure 93 cont.

21.	3558	CD1	PHE	673	A	<--->	233	CZ2	TRP	33	H	3.62
22.	3559	CD2	PHE	673	A	<--->	233	CZ2	TRP	33	H	3.73
23.	3560	CE1	PHE	673	A	<--->	233	CZ2	TRP	33	H	3.75
24.	3561	CE2	PHE	673	A	<--->	233	CZ2	TRP	33	H	3.88
25.	3562	CZ	PHE	673	A	<--->	233	CZ2	TRP	33	H	3.88
26.	3537	ND2	ASN	671	A	<--->	396	CD	ARG	53	H	3.48
27.	3535	CG	ASN	671	A	<--->	397	NE	ARG	53	H	3.90
28.	3536	OD1	ASN	671	A	<--->	397	NE	ARG	53	H	3.88
29.	3537	ND2	ASN	671	A	<--->	397	NE	ARG	53	H	3.52
30.	3535	CG	ASN	671	A	<--->	398	CZ	ARG	53	H	3.51
31.	3536	OD1	ASN	671	A	<--->	398	CZ	ARG	53	H	3.27
32.	3537	ND2	ASN	671	A	<--->	398	CZ	ARG	53	H	3.62
33.	3570	OD2	ASP	674	A	<--->	398	CZ	ARG	53	H	3.24
34.	3511	O	LEU	669	A	<--->	399	NH1	ARG	53	H	3.54
35.	3530	N	ASN	671	A	<--->	399	NH1	ARG	53	H	3.56
36.	3535	CG	ASN	671	A	<--->	399	NH1	ARG	53	H	3.37
37.	3536	OD1	ASN	671	A	<--->	399	NH1	ARG	53	H	3.37
38.	3537	ND2	ASN	671	A	<--->	399	NH1	ARG	53	H	3.70
39.	3570	OD2	ASP	674	A	<--->	399	NH1	ARG	53	H	2.83
40.	3536	OD1	ASN	671	A	<--->	400	NH2	ARG	53	H	3.34
41.	3568	CG	ASP	674	A	<--->	400	NH2	ARG	53	H	3.31

Figure 93 cont.

42.	3569	OD1	ASP	674	A	<-->	400	NH2	ARG	53	H	3.29
43.	3570	OD2	ASP	674	A	<-->	400	NH2	ARG	53	H	2.85
44.	3559	CD2	PHE	673	A	<-->	417	CG	ASP	56	H	3.89
45.	3559	CD2	PHE	673	A	<-->	419	OD2	ASP	56	H	3.87
46.	3561	CE2	PHE	673	A	<-->	438	CD1	ILE	59	H	3.85
47.	3562	CZ	PHE	673	A	<-->	438	CD1	ILE	59	H	3.54
48.	3546	NE1	TRP	672	A	<-->	779	CA	GLU	102	H	3.85
49.	3544	CD1	TRP	672	A	<-->	785	OE1	GLU	102	H	3.62
50.	3543	CG	TRP	672	A	<-->	787	N	GLY	103	H	3.54
51.	3544	CD1	TRP	672	A	<-->	787	N	GLY	103	H	3.53
52.	3545	CD2	TRP	672	A	<-->	787	N	GLY	103	H	3.36
53.	3546	NE1	TRP	672	A	<-->	787	N	GLY	103	H	3.36
54.	3547	CE2	TRP	672	A	<-->	787	N	GLY	103	H	3.27
55.	3549	CZ2	TRP	672	A	<-->	787	N	GLY	103	H	3.83
56.	3545	CD2	TRP	672	A	<-->	788	CA	GLY	103	H	3.48
57.	3547	CE2	TRP	672	A	<-->	788	CA	GLY	103	H	3.75
58.	3548	CE3	TRP	672	A	<-->	788	CA	GLY	103	H	3.58
59.	3542	CB	TRP	672	A	<-->	790	O	GLY	103	H	3.31
60.	3543	CG	TRP	672	A	<-->	790	O	GLY	103	H	3.49
61.	3545	CD2	TRP	672	A	<-->	790	O	GLY	103	H	3.81
62.	3585	CG2	THR	676	A	<-->	800	CB	PRO	105	H	3.72
63.	3585	CG2	THR	676	A	<-->	801	CG	PRO	105	H	3.75
64.	3656	CD	ARG	683	A	<-->	830	O	PHE	109	H	2.96
65.	3657	NE	ARG	683	A	<-->	830	O	PHE	109	H	3.59
66.	3655	CG	ARG	683	A	<-->	840	CZ	PHE	109	H	3.65

Figure 93 cont.

67.	3685	CD	LYS	686	A	<--->	840	CZ	PHE	109	H	3.81
68.	3656	CD	ARG	683	A	<--->	842	CA	PHE	110	H	3.83
69.	3656	CD	ARG	683	A	<--->	844	O	PHE	110	H	3.85
70.	3657	NE	ARG	683	A	<--->	844	O	PHE	110	H	3.46
71.	3658	CZ	ARG	683	A	<--->	844	O	PHE	110	H	3.21
72.	3659	NH1	ARG	683	A	<--->	844	O	PHE	110	H	3.29
73.	3660	NH2	ARG	683	A	<--->	844	O	PHE	110	H	3.68
74.	3657	NE	ARG	683	A	<--->	861	N	TRP	112	H	3.78
75.	3617	CA	TRP	680	A	<--->	864	O	TRP	112	H	3.66
76.	3622	CD1	TRP	680	A	<--->	864	O	TRP	112	H	3.84
77.	3657	NE	ARG	683	A	<--->	864	O	TRP	112	H	3.05
78.	3658	CZ	ARG	683	A	<--->	864	O	TRP	112	H	3.54
79.	3660	NH2	ARG	683	A	<--->	864	O	TRP	112	H	3.15
80.	3611	O	LEU	679	A	<--->	865	CB	TRP	112	H	3.72
81.	3611	O	LEU	679	A	<--->	871	CE3	TRP	112	H	3.65
82.	3582	O	THR	676	A	<--->	875	N	GLY	113	H	3.86
83.	3616	N	TRP	680	A	<--->	875	N	GLY	113	H	3.86
84.	3582	O	THR	676	A	<--->	876	CA	GLY	113	H	3.30
85.	3616	N	TRP	680	A	<--->	876	CA	GLY	113	H	3.86
86.	3617	CA	TRP	680	A	<--->	876	CA	GLY	113	H	3.88
87.	3620	CB	TRP	680	A	<--->	876	CA	GLY	113	H	3.46
88.	3583	CB	THR	676	A	<--->	878	O	GLY	113	H	3.80
89.	3660	NH2	ARG	683	A	<--->	888	CE1	TYR	114	H	3.63
90.	3585	CG2	THR	676	A	<--->	895	CB	TYR	115	H	3.88
91.	3550	CZ3	TRP	672	A	<--->	896	CG	TYR	115	H	3.53
92.	3550	CZ3	TRP	672	A	<--->	897	CD1	TYR	115	H	3.49
93.	3551	CH2	TRP	672	A	<--->	897	CD1	TYR	115	H	3.87
94.	3550	CZ3	TRP	672	A	<--->	898	CD2	TYR	115	H	3.75
95.	3550	CZ3	TRP	672	A	<--->	899	CE1	TYR	115	H	3.68
96.	3551	CH2	TRP	672	A	<--->	899	CE1	TYR	115	H	3.65
97.	3550	CZ3	TRP	672	A	<--->	901	CZ	TYR	115	H	3.87
98.	3549	CZ2	TRP	672	A	<--->	919	CE1	TYR	117	H	3.89

Figure 93 cont.

IC50 (µg/ml) in TZM-bl cells ¹							
Ab Name	398-F1-F6_20	CNE8	X2278_C2_B6	246-F3_C10_2	TRO.11	CNE55	CH119.10
DH511_UCA4A	>50.0	>50.0	>50.0	>50.0	>50.0	>50.0	>50.0
DH511_I6_4A	>50.0	>50.0	>50.0	>50.0	>50.0	>50.0	>50.0
DH511_I5_4A	23.682	4.959	11.236	1.782	0.289	4.176	2.413
DH511_I4_4A	22.127	4.758	11.862	2.791	0.298	4.409	3.209
DH511_I3_4A	24.865	4.821	12.594	2.744	0.292	2.822	2.916
DH511_I2_4A	43.109	6.616	19.674	3.300	0.485	5.542	3.224
DH511_I1_4A	26.974	6.222	12.837	2.935	0.435	4.819	1.789
DH511.1	2.9	0.03	1.0	1.1	0.25	0.63	0.85
DH511.2	1.2	<0.011	0.36	0.39	0.13	0.32	0.44
DH511.3	4.7	0.05	1.4	1.6	0.36	0.92	1.5
DH511.4	8.8	0.07	2.5	1.5	0.39	2.1	0.74
DH511.5	9.4	0.12	4.0	1.2	0.63	3.4	1.2
DH511.6	12	0.07	4.7	1.7	0.46	1.3	2.1
CH01-31	0.162	0.253	0.124	0.155	0.333	0.211	1.003
10E8*	0.73	0.03	0.29	0.5	0.02	0.14	0.5

¹Values are the antibody concentration (µg/ml) at which relative luminescence units (RLUs) were reduced 50% compared to virus control wells (no test sample).

Note: Values in bold are considered positive for neutralizing antibody activity when compared against background.

*Reference data.

Figure 94

Ab Name	IC50 (µg/ml) in TZM-bl cells ¹				
	BJOX002000.03.2	25710- 2.43	X1632_S2_B10	Ce703010217_B6	Ce1176_A3
DH511_UCA4A	>50.0	>50.0	>50.0	>50.0	>50.0
DH511_I6_4A	>50.0	>50.0	>50.0	>50.0	>50.0
DH511_I5_4A	6.513	1.132	4.426	0.952	4.601
DH511_I4_4A	7.364	1.498	5.668	0.851	4.979
DH511_I3_4A	6.235	1.283	5.528	0.941	4.024
DH511_I2_4A	13.004	2.120	5.523	4.963	9.617
DH511_I1_4A	10.444	1.548	5.963	1.213	4.102
DH511.1	1.2	0.16	0.85	0.20	1.3
DH511.2	0.52	0.10	0.42	0.16	0.59
DH511.3	1.5	0.43	1.3	0.55	2.2
DH511.4	1.7	0.46	1.0	0.67	2.7
DH511.5	2.8	0.39	1.6	0.58	1.5
DH511.6	2.2	0.56	1.6	0.89	3.2
CH01-31	7.819	0.244	0.110	0.108	0.134
10E8*	0.55	0.05	0.4	0.22	0.36

¹Values are the antibody concentration (µg/ml) at which relative luminescence units (RLUs) were reduced 50% compared to virus control wells (no test sample).

Note: Values in bold are considered positive for neutralizing antibody activity when compared against background.

*Reference data.

Figure 94 cont.

A: Overlap extension oligonucleotides for framework region 1 (5' - 3')

VH1	TA TTCCCA TC GCG GCG CCA GGTCC AGCTKG TRCAG TCTGG
VH157	TA TTCCCA TC GCG GCG CCA GGTCC AGCTGGTGSAR TCTGG
VH2	TA TTCCCA TC GCG GCG CCA GRTCA CC TTGAAGGAG TCTG
VH3	TA TTCCCA TC GCG GCG CCA GGTGC AGCTGKTGAGMNCY
VH4	TA TTCCCA TC GCG GCG CCA GGTCC AGCTGCAGGAGTCSG
VH4-DF63	TA TTCCCA TC GCG GCG CCA GGTCC AGCTACAGCAG TGGG
VH6	TA TTCCCA TC GCG GCG CCA GGTCA AGCTGCAGCAG TCA
VH3N	TA TTCCCA TC GCG GCG CCA CACAGG TTCCCA GTTA
VK1	GC GCG CCG CA TGGGA ATAGC TAGCCGACA TCCRGD TGACCCAG TCTCC
VK2	GC GCG CCG CA TGGGA ATAGC TAGCCGA TA TTGTMTGACBCAGNCTCC
VK3	GC GCG CCG CA TGGGA ATAGC TAGCCGAAA TTGTRV TBACRCAG TCTCC
VK5	GC GCG CCG CA TGGGA ATAGC TAGCCGA AACGACATCACBCAGTCTC
VL1	GC GCG CCG CA TGGGA ATAGC TAGCCCA GTCTGTSB TBACGCAGCCGCC
VL1459	GC GCG CCG CA TGGGA ATAGC TAGCCCA GCTTG TCTGAC TCARYC
VL15910	GC GCG CCG CA TGGGA ATAGC TAGCCCA GCGW GKGTGAC TCAGCCMCC
VL2	GC GCG CCG CA TGGGA ATAGC TAGCCCA GTCTGNYCTGAYTCAGCCT
VL3	GC GCG CCG CA TGGGA ATAGC TAGCCTCTCTATG WGTBACW CAGCCAA
VL-DPL16	GC GCG CCG CA TGGGA ATAGC TAGCCTCTCTTGA BCTGASTCAGGASCC
VL3-39	GC GCG CCG CA TGGGA ATAGC TAGCCTCTCTATGA BCTGAYRCAGCYACC
VL6	GC GCG CCG CA TGGGA ATAGC TAGCCAA TTTTATBCTBAC TCAGCCCC
VL78	GC GCG CCG CA TGGGA ATAGC TAGCCCA GDC TGTGG TGACYCAGGAGCC

Figure 95A

B: Overlap extension oligonucleotides for leader peptide (5' - 3')

VH1	TA TTCCCA TC GCGGCGCACAGGTGCCAC TC CCAAGGTGCAG
VH3	TA TTCCCA TC GCGGCGCAAGGTGCCAG TG TGA RGTCCAG
VH4/6	TA TTCCCA TC GCGGCGCCCCAGATGGG TCC TGTCC CAGGTGCAG
VH5	TA TTCCCA TC GCGGCGGCCAAGGAGTCTGTCCGAGGTGCAG
VL1	GC GCGCGCGA TGGGAATATCTGCTCGAGTTCGG TCA GGTCC TGGGCCCCAGTCTGTGCTG
VL2	GC GCGCGCGA TGGGAATATCTGCTCGAGTTCGG TCA GGTCC TGGGCCCCAGTCTGTGCCCCTG
VL3	GC GCGCGCGA TGGGAATATCTGCTCGAGTTCGG TCA YWCTGCA CAGGCTCTGTGACCTCCTAT
VL4/5	GC GCGCGCGA TGGGAATATCTGCTCGAGTTCGG TCA GGTCTCTCTCSCAGCTGTGCTG
VL6	GC GCGCGCGA TGGGAATATCTGCTCGAGTTCGG TCA GTTCTGGGCCAA TTTATGCTG
VL7	GC GCGCGCGA TGGGAATATCTGCTCGAGTTCGG TCA GGTCCAA TCTCAGGCTGTGGTG
VL8	GC GCGCGCGA TGGGAATATCTGCTCGAGTTCGG TCA GAGTGGATCTCAGACTGTGGTG
VK1/2	GC GCGCGCGA TGGGAATATCTGCTCGAGTTCGG TCA ATGAGGS TCCYGGTCA GCTGCTGG
VK3	GC GCGCGCGA TGGGAATATCTGCTCGAGTTCGG TCA CTCTTCC TCC TGC TACTCTGGCTCCGAG
VK4	GC GCGCGCGA TGGGAATATCTGCTCGAGTTCGG TCA ATTTCTCTGTTGCTCTGGATCTCTG

C: Nested constant region oligonucleotides (5' - 3')

IgG nested	NNNNATGGGCCCTGSGATGGGCCCTTGGTGGARGC
IgM nested	NNNNATGGGCCCTGGGTGGGGCGCGATCCAC TCC
IgA nested	NNNNATGGGCCCTGCTTGGGGCTGGTGGGGGATG
IgK nested	NNNNGTGGGCCCGCAGATGGTGCAGCCACAGTTC
IgL nested	NNNNGTGGGCCCGCAGAGGYGGGACACAGAGTGAC

Figures 95B-C

Antibody ID	CDR3	IMGT Length	Membrane Insertion Score ^a
DH511.1	CTADLGEPVVSRFFEWSYYMDLW	24	1.36
DH511.2	CTMDEGTP-VTRFLEWGYFYMAVW	23	2.11
DH511.3	CTADLGEAVVSRFFEWSYYMDFW	24	1.64
DH511.4	CTRDEGAP-VTRFLEWGSYYMAVW	23	0.85
DH511.5	CTRDEGAP-VTRFLEWGSYYMAVW	23	0.85
DH511.6	CTADLGEAVVSRFFEWSYYMDFW	24	1.64
DH511.7P			
DH511.8P			
DH511.9P			
DH511.10P			
DH511.11P			
DH511.12P			
2F5	CAHRRGPTTLFGVPIARGPVMNDVW	24	-0.25
4E10	CAREGTTGWGWLKPIGAFAHW	20	2.96
10E8	CARTGKYDFWSGYPPGEEYFQDW	22	1.5

a: Membrane insertion score is calculated as sum of Wimley-White Interfacial Hydrophobicity Scale values over the middle 50% of the HCDR3 amino acids. Positive scores are favorable for membrane insertion.

Phe or Trp residues are highlighted in red.

Pro are highlighted in blue.

Figure 96

Ab ID	OD 450 nm (33 µg/ml)
DH511.1	0.075
DH511.2	0.069
DH511.3	0.080
DH511.4	0.116
DH511.5	0.073
DH511.6	0.072
DH511.7P	
DH511.8P	
DH511.9P	
DH511.10P	
DH511.11P	
DH511.12P	
DH511_UCA	0.061
DH511_I6	0.118
DH511_I5	0.089
DH511_I4	0.111
DH511_I3	0.129
DH511_I2	0.126
DH511_I1	0.160
Synagis	0.043
4E10	2.068

Data shown
are an
average of
two
independent
experiments.
OD values
above 0.15
were
considered
positive.

Figure 97

		Key (IC50 IC80 in $\mu\text{g/ml}$)			
		<0.100	0.100-1.00	1.00-10.0	>10.0
Antibody Number	Heavy-chain ID	Light-chain ID	IgG Quant ($\mu\text{g/ml}$)	1:8 Initial Dilution ($\mu\text{g/ml}$)	IC50 ($\mu\text{g/ml}$) BG1153 IC80 ($\mu\text{g/ml}$) BG1153 SVA IC50 ($\mu\text{g/ml}$) SVA
1	DH511.7A1P_4A	DH511.5A1P	83	1050	1.272 >10.5
2	DH511.7A1P_4A	DH511.5A1P	67.2	1120	9.314 >11.2
3	DH511.7A1P_4A	DH511.5B1P	82.95	1039	>10.39 >10.39
4	DH511.7A1P_4A	DH511.5C1P	105.25	1821	8.174 >18.21
5	DH511.7A1P_4A	DH511.10A1P	24.5	408	>4.08 >4.08
6	DH511.7A1P_4A	DH511.10B1P	67	1117	>11.17 >11.17
7	DH511.7A1P_4A	DH511.11A1P	3875	848	>8.48 >8.48
8	DH511.7A1P_4A	DH511.2A1P	65.35	1139	2.818 >11.39
9	DH511.7B1P_4A	DH511.5A1P	40.55	631	1.3 >6.31
10	DH511.7B1P_4A	DH511.5A1P	81.9	1032	>10.32 >10.32
11	DH511.7B1P_4A	DH511.5B1P	36	800	>6 >6
12	DH511.7B1P_4A	DH511.5C1P	51.45	358	7.843 >8.58
13	DH511.7B1P_4A	DH511.10A1P	44.5	742	>7.42 >7.42
14	DH511.7B1P_4A	DH511.10B1P	3875	848	>8.48 >8.48
15	DH511.7B1P_4A	DH511.11A1P	37.5	825	>8.25 >8.25
16	DH511.7B1P_4A	DH511.2A1P	72.7	1212	3.458 >12.12
17	DH511.8A1P_4A	DH511.7A1P	68.3	1105	1.047 >11.05
18	DH511.8A1P_4A	DH511.5A1P	59.85	998	7.925 >9.98
19	DH511.8A1P_4A	DH511.5B1P	64.1	1068	>10.68 >10.68
20	DH511.8A1P_4A	DH511.5C1P	82.4	1540	6.174 >15.4
21	DH511.8A1P_4A	DH511.10A1P	28.5	475	>4.75 >4.75
22	DH511.8A1P_4A	DH511.10B1P	24.1	402	>4.02 >4.02
23	DH511.8A1P_4A	DH511.11A1P	23.2	357	>3.57 >3.57
24	DH511.8A1P_4A	DH511.2A1P	78.75	1279	2.091 >12.79
25	DH511.8B1P_4A	DH511.7A1P	67.45	1124	0.513 >11.24
26	DH511.8B1P_4A	DH511.5A1P	152.05	2534	8.241 >25.34
27	DH511.8B1P_4A	DH511.5B1P	103.5	1725	7.890 >17.25
28	DH511.8B1P_4A	DH511.5C1P	227.2	3787	6.789 >37.87

Figure 98

29	DH511.8EVP_4A	DH511.10ANP	45.9	7.65	>7.65	>7.65	>7.65	>7.65
30	DH511.8EVP_4A	DH511.10EVP	40.55	6.78	>6.78	>6.78	>6.78	>6.78
31	DH511.8EVP_4A	DH511.11ANP	92.65	15.44	5.087	>15.44	>15.44	>15.44
32	DH511.8EVP_4A	DH511.2ANP	113.75	19.79	2.002	9.339	>19.79	>19.79
33	DH511.8EVP_4A	DH511.7ANP	2.511	0.419	>0.419	>0.419	>0.419	>0.419
34	DH511.8EVP_4A	DH511.9ANP	12.919	2.137	>2.137	>2.137	>2.137	>2.137
35	DH511.8EVP_4A	DH511.9EVP	0.997	0.066	>0.066	>0.066	>0.066	>0.066
36	DH511.8EVP_4A	DH511.9EVP	12.785	2.131	>2.131	>2.131	>2.131	>2.131
37	DH511.8EVP_4A	DH511.10ANP	0.314	0.052	>0.052	>0.052	>0.052	>0.052
38	DH511.8EVP_4A	DH511.10EVP	0.493	0.082	>0.082	>0.082	>0.082	>0.082
39	DH511.8EVP_4A	DH511.11ANP	5.005	0.834	>0.834	>0.834	>0.834	>0.834
40	DH511.8EVP_4A	DH511.2ANP	1.826	0.304	>0.304	>0.304	>0.304	>0.304
41	DH511.9ANP_4A	DH511.7ANP	113.8	18.967	3.238	14.835	>18.967	>18.967
42	DH511.9ANP_4A	DH511.8ANP	80.144	13.357	0.774	3.780	>13.357	>13.357
43	DH511.9ANP_4A	DH511.10ANP	0.425	0.071	>0.071	>0.071	>0.071	>0.071
44	DH511.9ANP_4A	DH511.10EVP	1.324	0.221	>0.221	>0.221	>0.221	>0.221
45	DH511.9ANP_4A	DH511.11ANP	35.459	5.910	2.433	>5.91	>5.91	>5.91
46	DH511.9ANP_4A	DH511.2ANP	123.19	20.532	6.958	>20.532	>20.532	>20.532
47	DH511.10ANP_4A	DH511.7ANP	88.915	14.920	0.958	4.132	>14.92	>14.92
48	DH511.10ANP_4A	DH511.8ANP	100.574	16.782	1.396	5.759	>16.782	>16.782
49	DH511.10ANP_4A	DH511.9ANP	173.525	26.921	1.731	7.303	>26.921	>26.921
50	DH511.10ANP_4A	DH511.9EVP	164.783	25.797	1.428	5.567	>25.797	>25.797
51	DH511.10ANP_4A	DH511.9EVP	274.865	45.511	1.305	4.523	>45.511	>45.511
52	DH511.10ANP_4A	DH511.11ANP	66.743	10.937	0.71	4.336	>10.937	>10.937
53	DH511.10ANP_4A	DH511.2ANP	142.347	23.725	1.731	7.428	>23.725	>23.725
54	DH511.10EVP_4A	DH511.7ANP	72.442	12.074	0.913	5.042	>12.074	>12.074
55	DH511.10EVP_4A	DH511.8ANP	152.552	25.425	0.48	1.495	>25.425	>25.425
56	DH511.10EVP_4A	DH511.9ANP	154.118	25.036	0.817	3.173	>25.036	>25.036
57	DH511.10EVP_4A	DH511.9EVP	12.751	2.125	0.673	>2.125	>2.125	>2.125
58	DH511.10EVP_4A	DH511.9EVP	185.783	30.994	1.135	4.096	>30.994	>30.994
59	DH511.10EVP_4A	DH511.11ANP	24.738	4.132	1.213	>4.132	>4.132	>4.132
60	DH511.10EVP_4A	DH511.2ANP	139.712	23.285	1.312	6.339	>23.285	>23.285

Antibody highlighted in red showed comparable potency to Dh512.

Figure 98 cont.

Antibody Number	Heavy-chain ID	Light-chain ID	IgG Quant ($\mu\text{g/ml}$)	1:6 Initial Dilution ($\mu\text{g/ml}$)	Key (IC50/IC80 in $\mu\text{g/ml}$)			
					IC50 ($\mu\text{g/ml}$)	IC80 ($\mu\text{g/ml}$)	BG116.8	SV4
61	DH511.10C4P_4A	DH511.7A4NP	123.445	20.574	10.673	>20.574	>20.574	>20.574
62	DH511.10C4P_4A	DH511.8A4NP	189.294	28.215	3.408	>28.215	>28.215	>28.215
63	DH511.10C4P_4A	DH511.9A4NP	253.835	42.293	8.719	>42.293	>42.293	>42.293
64	DH511.10C4P_4A	DH511.9B4NP	451.094	75.181	9.393	50.853	>75.181	>75.181
65	DH511.10C4P_4A	DH511.9C4NP	444.052	74.009	5.180	29.485	>74.009	>74.009
66	DH511.10C4P_4A	DH511.11A4NP	93.455	15.575	11.992	>15.575	>15.575	>15.575
67	DH511.10C4P_4A	DH511.21NP	181.155	30.193	10.671	>30.193	>30.193	>30.193
68	DH511.11A4P_4A	DH511.7A4NP	199.455	33.24	31.35	>33.24	>33.24	>33.24
69	DH511.11A4P_4A	DH511.8A4NP	218.318	36.39	8.322	>36.39	>36.39	>36.39
70	DH511.11A4P_4A	DH511.9A4NP	427.911	71.32	>71.32	>71.32	>71.32	>71.32
71	DH511.11A4P_4A	DH511.9B4NP	333.355	55.51	>55.51	>55.51	>55.51	>55.51
72	DH511.11A4P_4A	DH511.9C4NP	134.35	22.39	>22.39	>22.39	>22.39	>22.39
73	DH511.11A4P_4A	DH511.10A4NP	17.539	2.92	>2.92	>2.92	>2.92	>2.92
74	DH511.11A4P_4A	DH511.10B4NP	80.405	13.40	>13.4	>13.4	>13.4	>13.4
75	DH511.11A4P_4A	DH511.21NP	155.052	25.94	11.195	>25.94	>25.94	>25.94
76	DH511.12A4P_4A	DH511.7A4NP	16.997	3.17	>3.17	>3.17	>3.17	>3.17
77	DH511.12A4P_4A	DH511.8A4NP	11.944	1.57	>1.97	>1.97	>1.97	>1.97
78	DH511.12A4P_4A	DH511.9A4NP	189.142	28.19	>28.19	>28.19	>28.19	>28.19
79	DH511.12A4P_4A	DH511.9B4NP	2.571	0.43	>0.43	>0.43	>0.43	>0.43
80	DH511.12A4P_4A	DH511.9C4NP	111.524	15.55	>15.55	>15.55	>15.55	>15.55
81	DH511.12A4P_4A	DH511.10A4NP	2.197	0.36	>0.36	>0.36	>0.36	>0.36
82	DH511.12A4P_4A	DH511.10B4NP	15.93	2.65	>2.65	>2.65	>2.65	>2.65
83	DH511.12A4P_4A	DH511.21NP	50.982	8.50	>8.5	>8.5	>8.5	>8.5
84	DH511.21NP	DH511.7A4NP	130.55	21.76	0.699	3.225	>21.76	>21.76
85	DH511.21NP	DH511.8A4NP	145.575	24.25	0.258	0.895	>24.25	>24.25
86	DH511.21NP	DH511.9A4NP	597.214	99.54	1.846	9.285	>99.54	>99.54

Figure 98 cont.

67	DH511.2/H	DH511.9E/WP	1072.596	178.77	3.414	13.355	>178.77	>178.77
88	DH511.2/H	DH511.9C/WP	845.43	107.75	2.115	7.925	>107.75	>107.75
89	DH511.2/H	DH511.10A/WP	3.678	0.61	>0.61	>0.61	>0.61	>0.61
90	DH511.2/H	DH511.10E/WP	14.331	2.48	>2.48	>2.48	>2.48	>2.48
91	DH511.2/H	DH511.11A/WP	156.132	26.03	0.355	1.243	>26.03	>26.03
DH511.2	DH511.2/H	DH511.2/WP	188.551	31.43	1.602	6.039	>31.43	>31.43
	DH511.2/H	DH511.2/WP	119.527	19.921	0.725	2.441	>19.921	>19.921
	DH511.2/H	DH511.2/WP	170	23.33	0.522	2.750	>23.33	>23.33
10E3 mAb					0.162	0.841	>50	>50
					0.262	0.779	>50	>50
2F5 mAb			100.04	16.57	3.674	14.218	>16.57	>16.57
			37.943	14.841	3.686	>14.841	>14.841	>14.841
			55	5.17	1.581	>5.17	>5.17	>5.17
Mock Transfection			0.028	0.0043	>0	>0	>0	>0
			0.003	0.001	>0.001	>0.001	>0.001	>0.001
			20	3.33	>3.33	>3.33	>3.33	>3.33

Antibody highlighted in red showed comparable potency to DH512.

Figure 98 cont.

Virus ID	Clade	Titer in TZM.bl cells (ug/ml)					
		DH511.2_K3*		DH511.2		10E8	
		IC ₅₀	IC ₈₀	IC ₅₀	IC ₈₀	IC ₅₀	IC ₈₀
QH0692.42	B	0.982	3.59	1.63	8.75	0.566	2.32
SC422661.8	B	0.269	0.999	0.444	2.01	0.302	1.23
AC10.0.29	B	0.115	0.476	0.153	0.823	0.172	0.882
THRO4156.18	B	0.584	2.62	0.194	1.98	0.120	0.566
WTO4160.33	B	0.149	0.750	0.261	1.28	0.159	0.858
Du156.12	C	<0.023	0.108	0.064	0.336	0.028	0.130
Du422.1	C	0.320	1.17	0.758	2.88	0.220	0.825
ZM53M.PB12	C	2.90	11.1	6.13	25.4	2.72	13.4
ZM109F.PB4	C	0.729	2.69	1.14	5.70	0.252	1.36
HIV-0013095-2.11	C	<0.023	0.138	<0.023	0.603	<0.023	0.127
CNE19	BC	0.259	1.34	1.58	7.94	0.582	3.27
CNE30	BC	1.50	7.87	4.83	27.5	0.616	4.88
MS208.A1	A	0.683	3.31	1.48	5.41	0.442	2.00
Q23.17	A	0.731	3.08	2.01	11.9	0.660	3.67
Q769.d22	A	0.254	1.59	1.12	9.57	1.11	4.77
0330.v4.c3	A	0.926	3.65	1.78	9.74	1.41	6.46
191955_A11	A (T/F)	0.388	1.16	0.956	3.36	0.447	1.31
928-28	CRF02_AG	0.033	0.513	0.195	1.19	0.119	0.665
T250-4	CRF02_AG	0.866	3.33	2.60	14.6	1.01	5.00
211-9	CRF02_AG	0.386	1.14	1.07	3.78	0.635	2.17
C1080.c03	CRF01_AE	0.081	0.516	0.154	1.18	0.028	0.317
R1166.c01	CRF01_AE	0.711	2.35	1.35	5.45	0.384	2.91
C4118.c09	CRF01_AE	0.508	4.20	0.801	12.0	0.650	4.68
X2131_C1_B5	G	0.068	0.442	0.229	1.14	0.080	0.373
P1981_C5_3	G	<0.023	0.109	0.065	0.306	<0.023	0.106
X1632_S2_B10	G	0.265	1.14	0.741	4.78	0.494	1.93
3016.v5.c45	D	0.531	1.95	1.81	8.53	0.593	2.10
A07412M1.vrc12	D	0.484	1.76	0.649	5.15	0.221	1.15
3817.v2.c59	CD	1.21	4.28	6.00	17.8	0.906	3.82
0815.v3.c3	ACD	0.271	1.22	0.850	4.02	0.238	1.09
MuLV	Neg. Control	>50	>50	>50	>50	>50	>50
MEDIAN TITERS		0.387	1.462	0.963	4.963	0.413	1.648

*DH511.2_K3 is comprised of the DH511.2 heavy-chain reconstituted with the light chain DH511.8AVK.

Figure 99

A: PCR conditions for isotype specific amplification

Amplicon	Primers	Expected Length	Extension Y (s)	Cycles X (#)
Paired IgG, IgA, IgK, IgL	IgG, IgA, IgK, IgL	1100	33	8
Paired IgM, IgK, IgL	IgM, IgK, IgL	1100	33	5
VH only IgG, IgA	IgG, IgA, VH_linker	600	20	8
VH only IgM	IgM, VH_linker	600	20	5
VL only	IgK, IgL, VL_linker	550	20	5

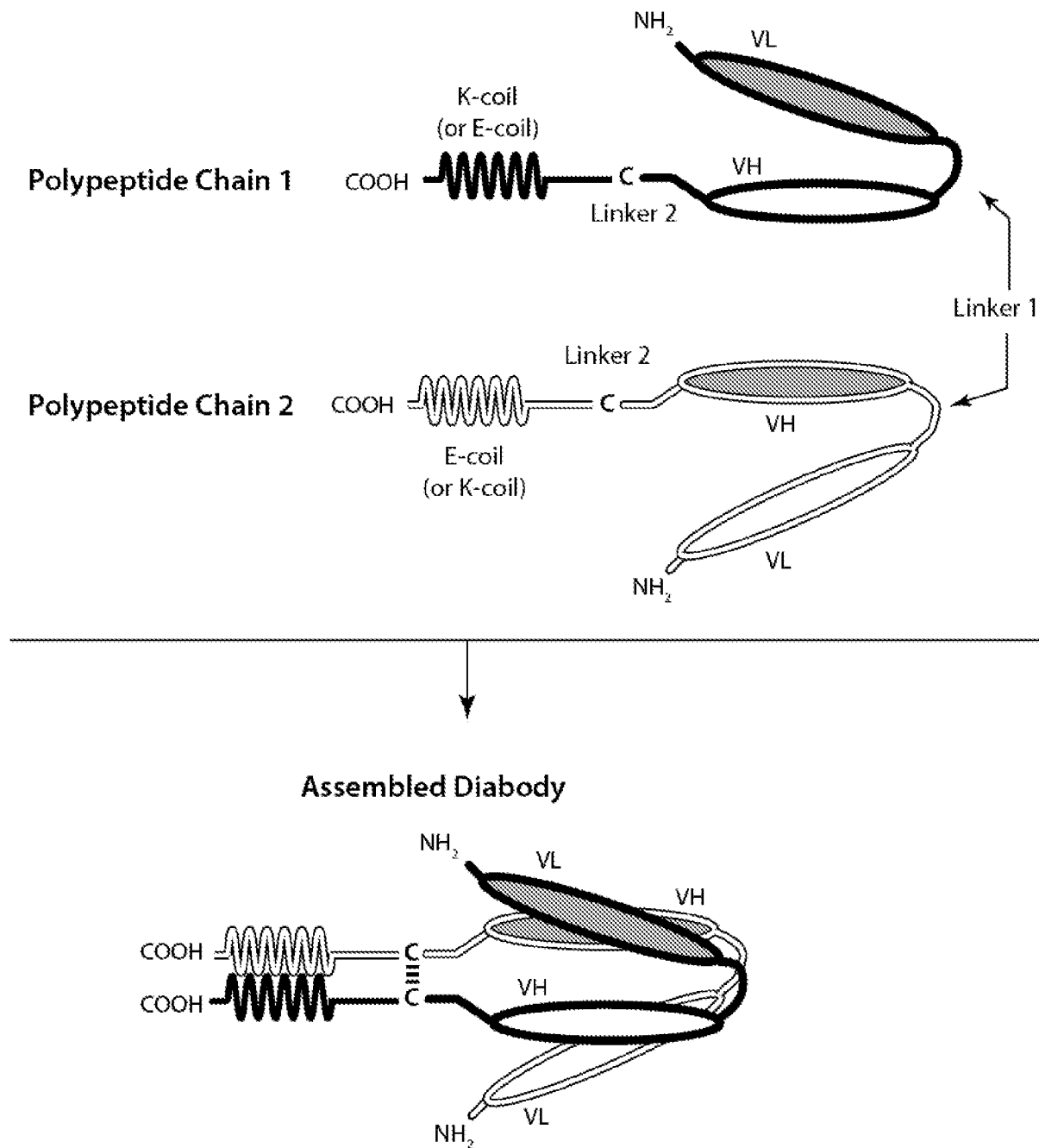
B: Oligonucleotides for isotype specific amplification (5' - 3')

IgG	GTC TCGTGGGCTCGGAGATGTGTATAAGAGACAGCAGNNNATGGGCCCCGTCGATGGGGCCC TTGGTGGARGC
IgM	GTC TCGTGGGCTCGGAGATGTGTATAAGAGACAGCAGNNNATGGGCCCCGTCGATGGGGCCCAGATGCACTCC
IgA	GTC TCGTGGGCTCGGAGATGTGTATAAGAGACAGCAGNNNATGGGCCCCGTCGATGGGGCCCCTTGGGGCTGGTGGGGATG
IgK	TGG TGGGCA GGGTCAGATGTGTATAAGAGACAGNNNNGTGGGGCCCCGCAAGATGGTGCAGCCACAGTTTC
IgL	TGG TGGGCA GGGTCAGATGTGTATAAGAGACAGNNNNGTGGGGCCCCGCAAGATGGTGCAGAGGTGAC
VL_linker	GTC TCGTGGGCTCGGAGATGTGTATAAGAGACAGNNNNGGCGCCGCGATGGGAAAT
VH_linker	TGG TGGGCA GGGTCAGATGTGTATAAGAGACAGNNNNGGCTAGCTATTCCCATGGGG

C: PCR conditions for MiSeq Barcoding

Amplicon	Expected Length	Extension Y (s)	Cycles X (#)
Paired IgG, IgA, IgK, IgL	1100	33	8
Paired IgM, IgK, IgL	1100	33	8
VH only IgG, IgA	600	20	8
VH only IgM	600	20	8
VL only	550	20	8

Figures 100A-C

**Figure 101**

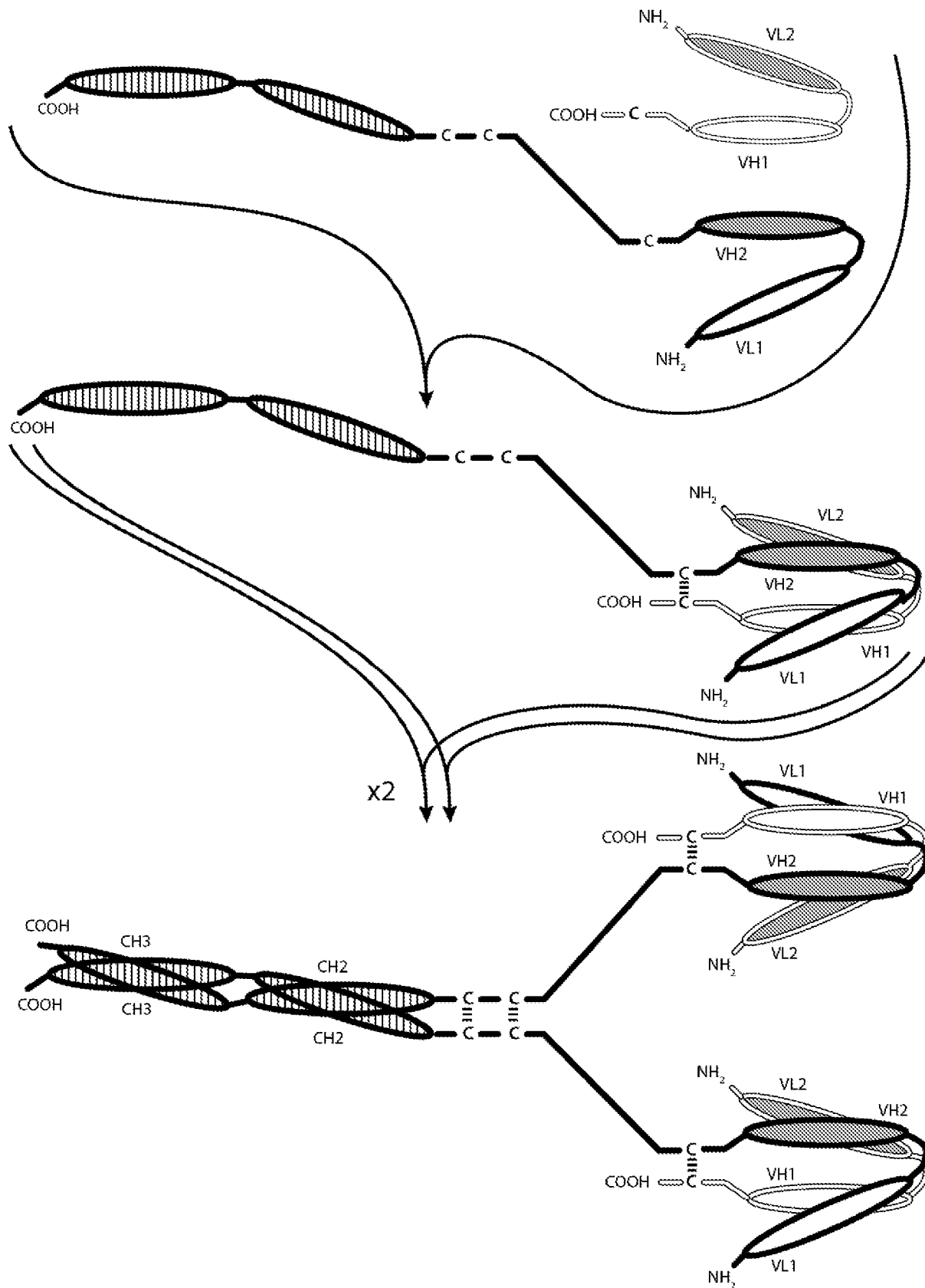
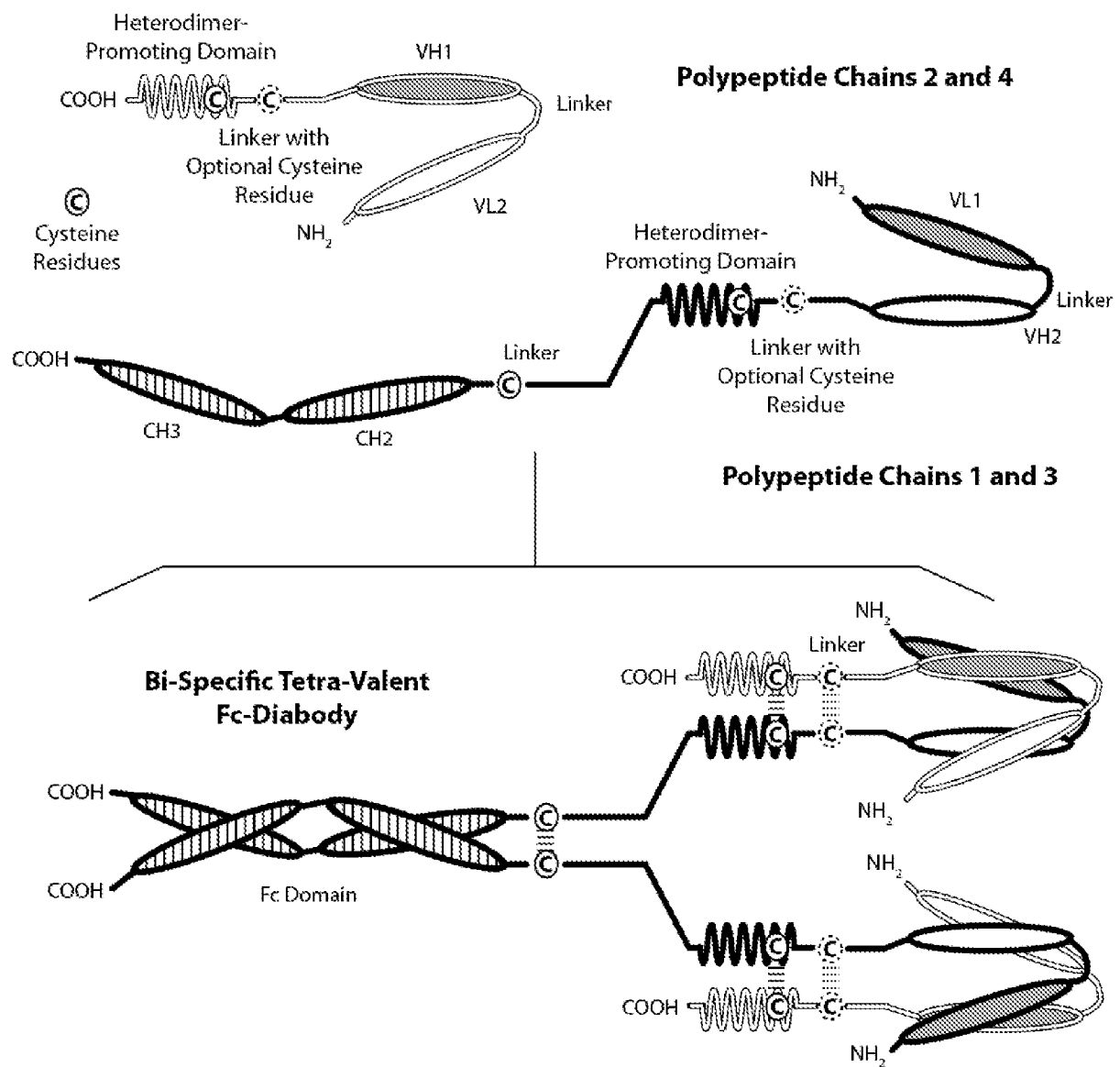
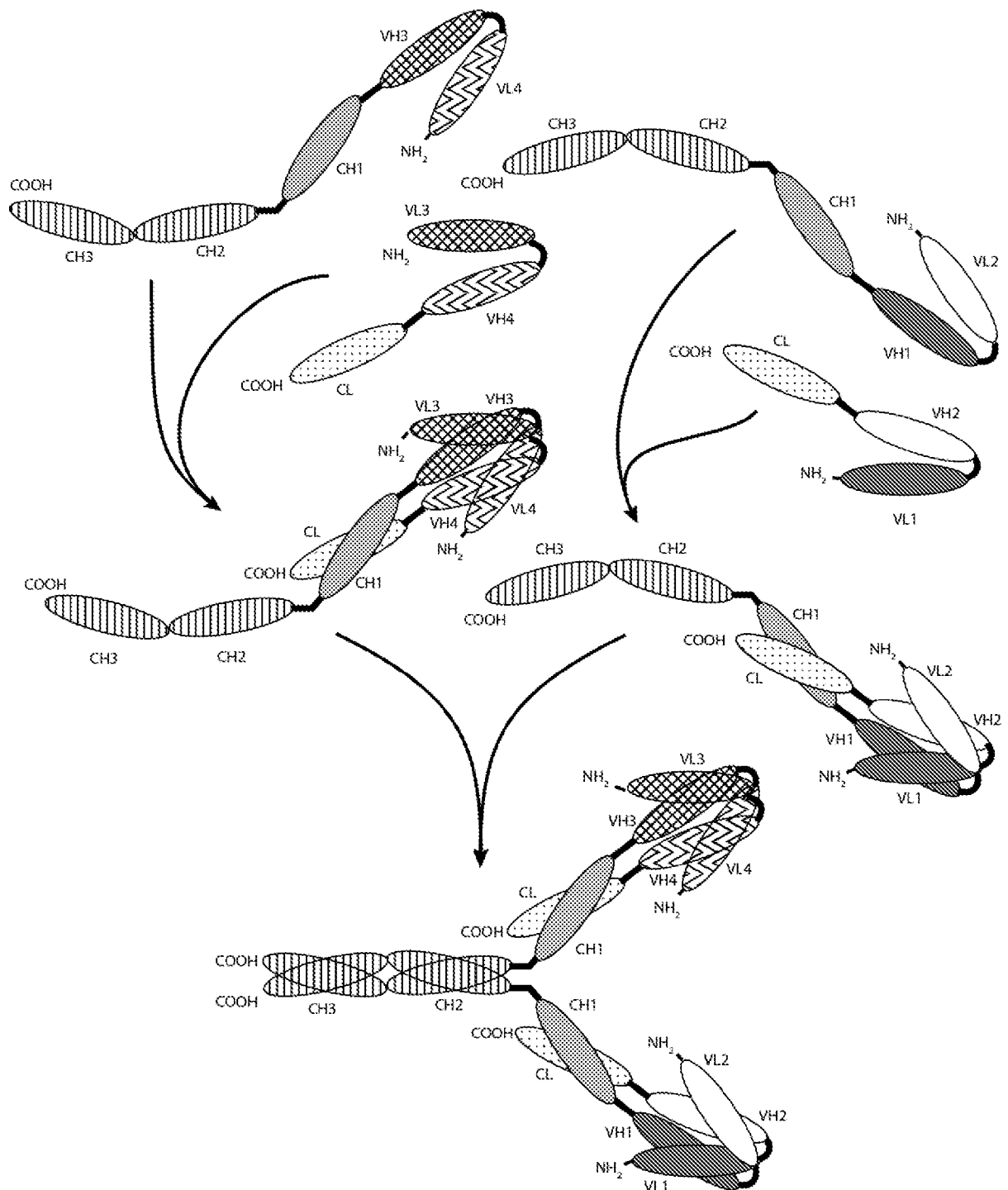
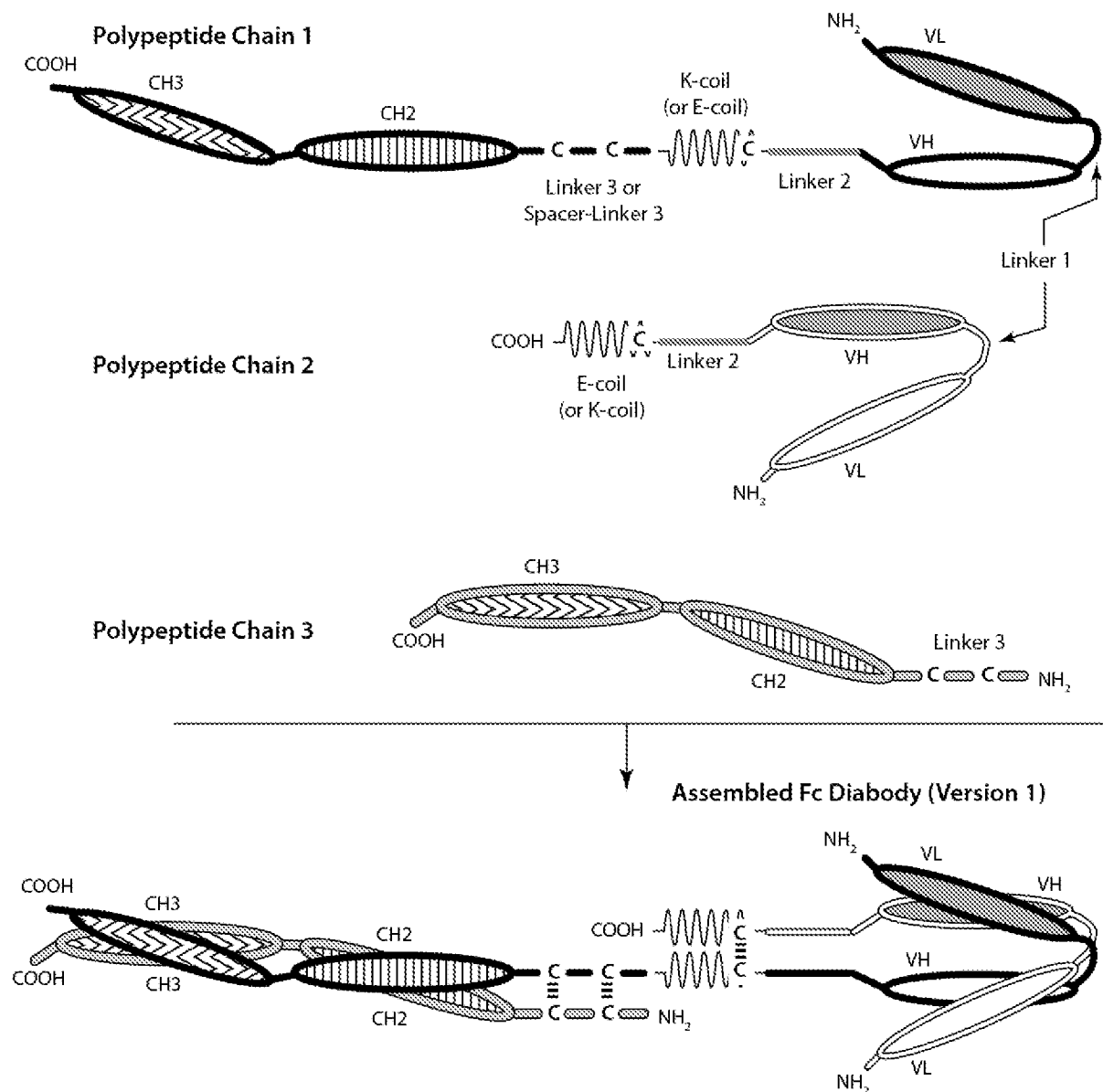
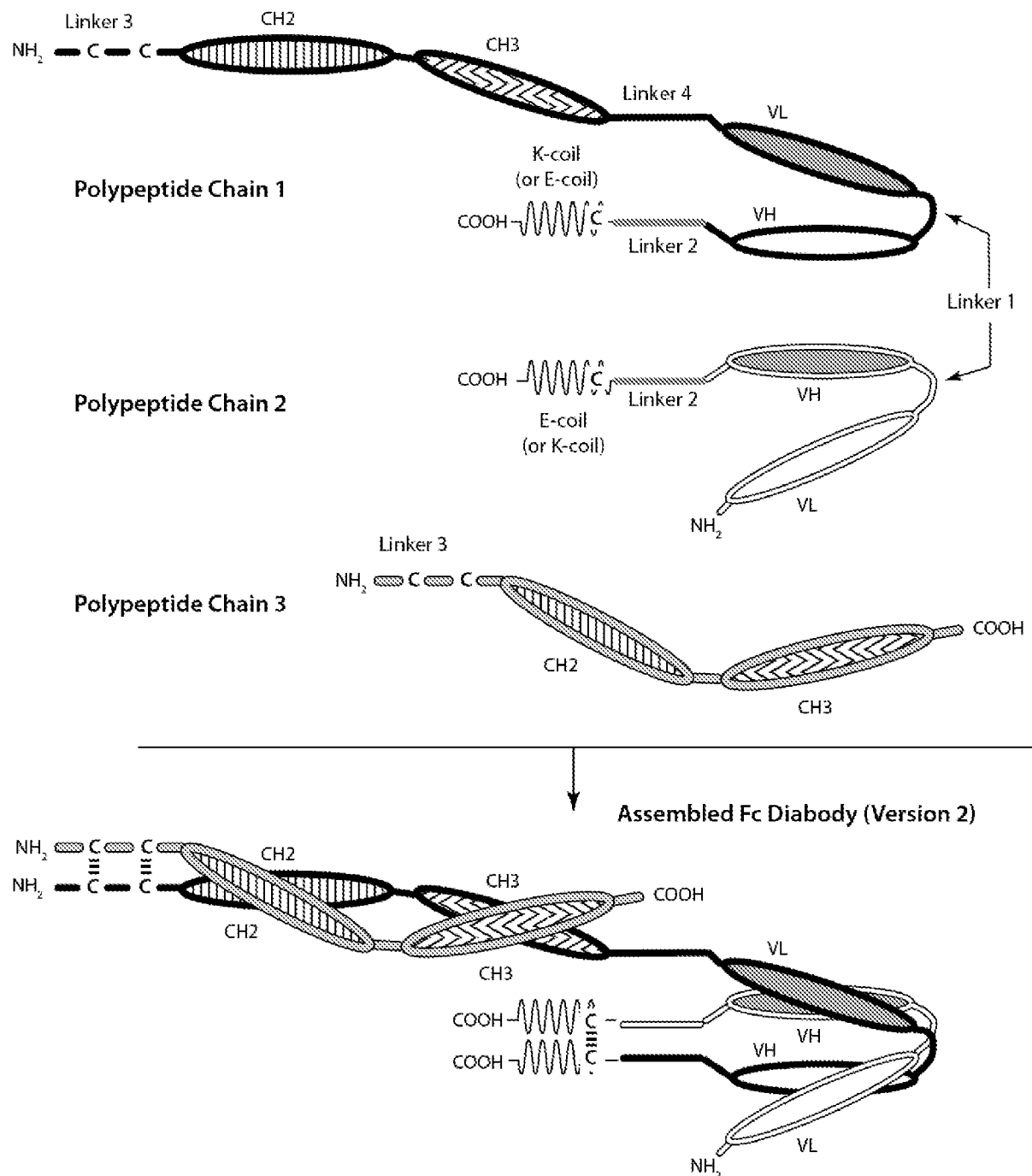


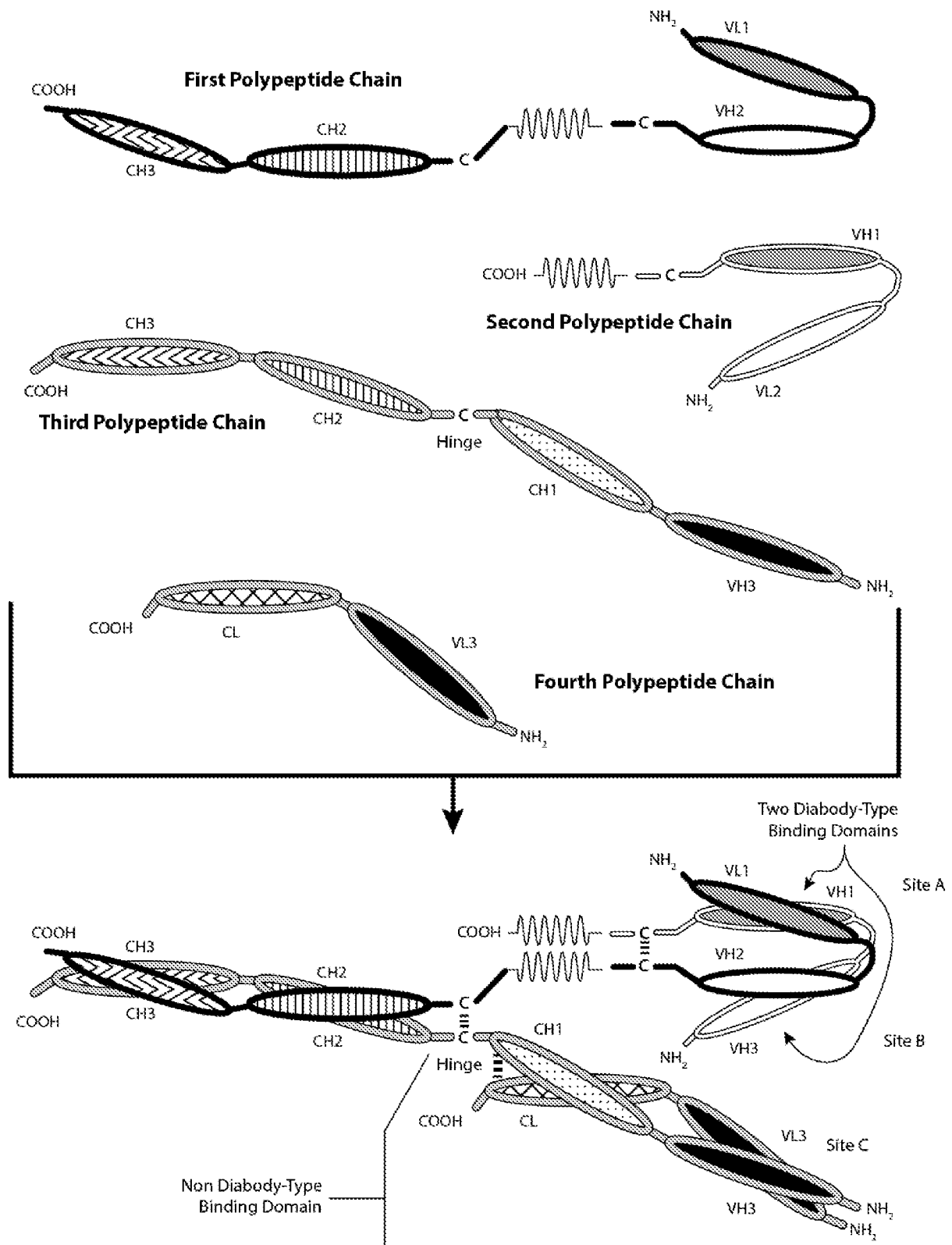
Figure 102A

**Figure 102B**

**Figure 102C**

**Figure 103A**

**Figure 103B**

**Figure 104A**

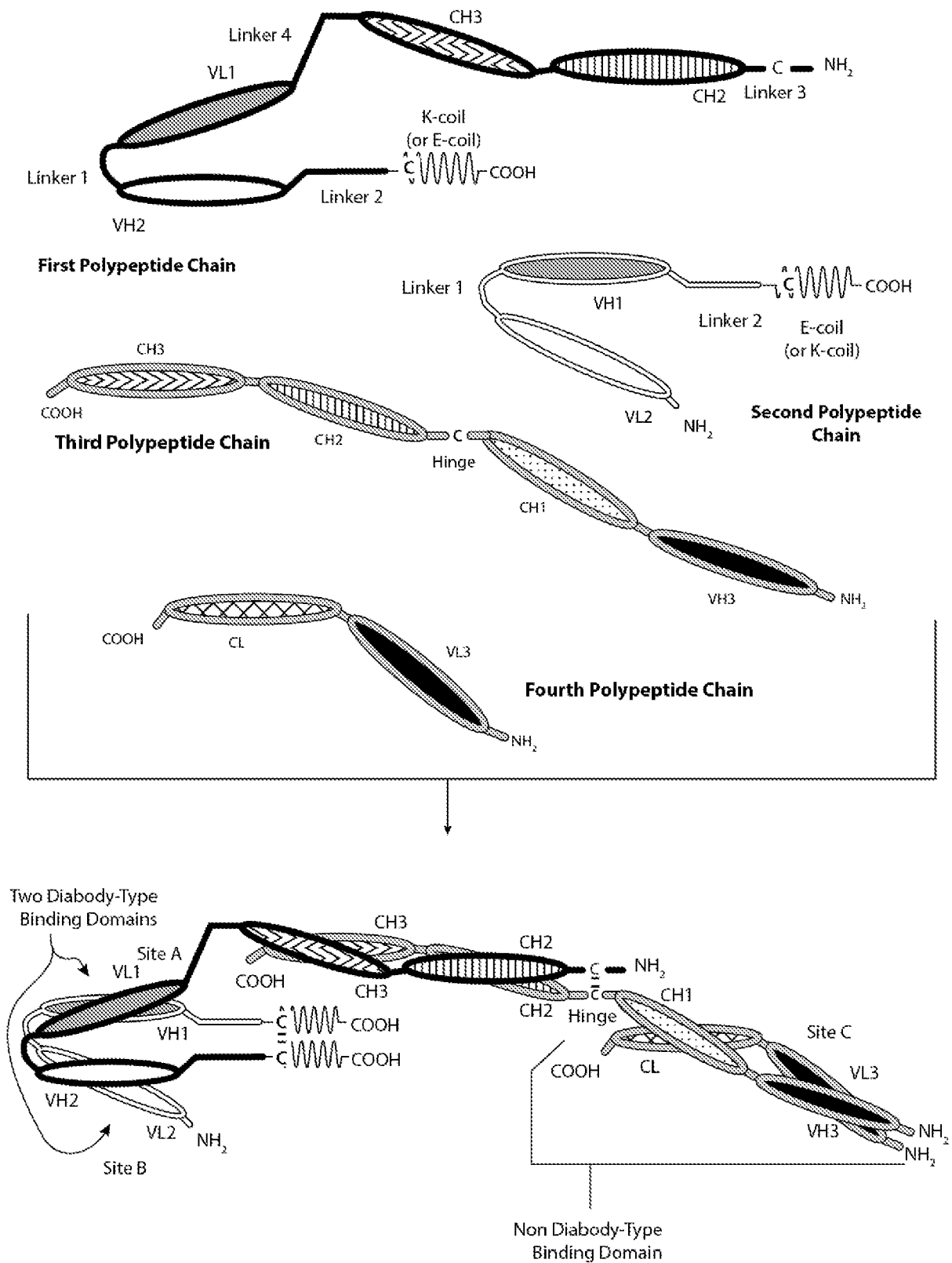


Figure 104B

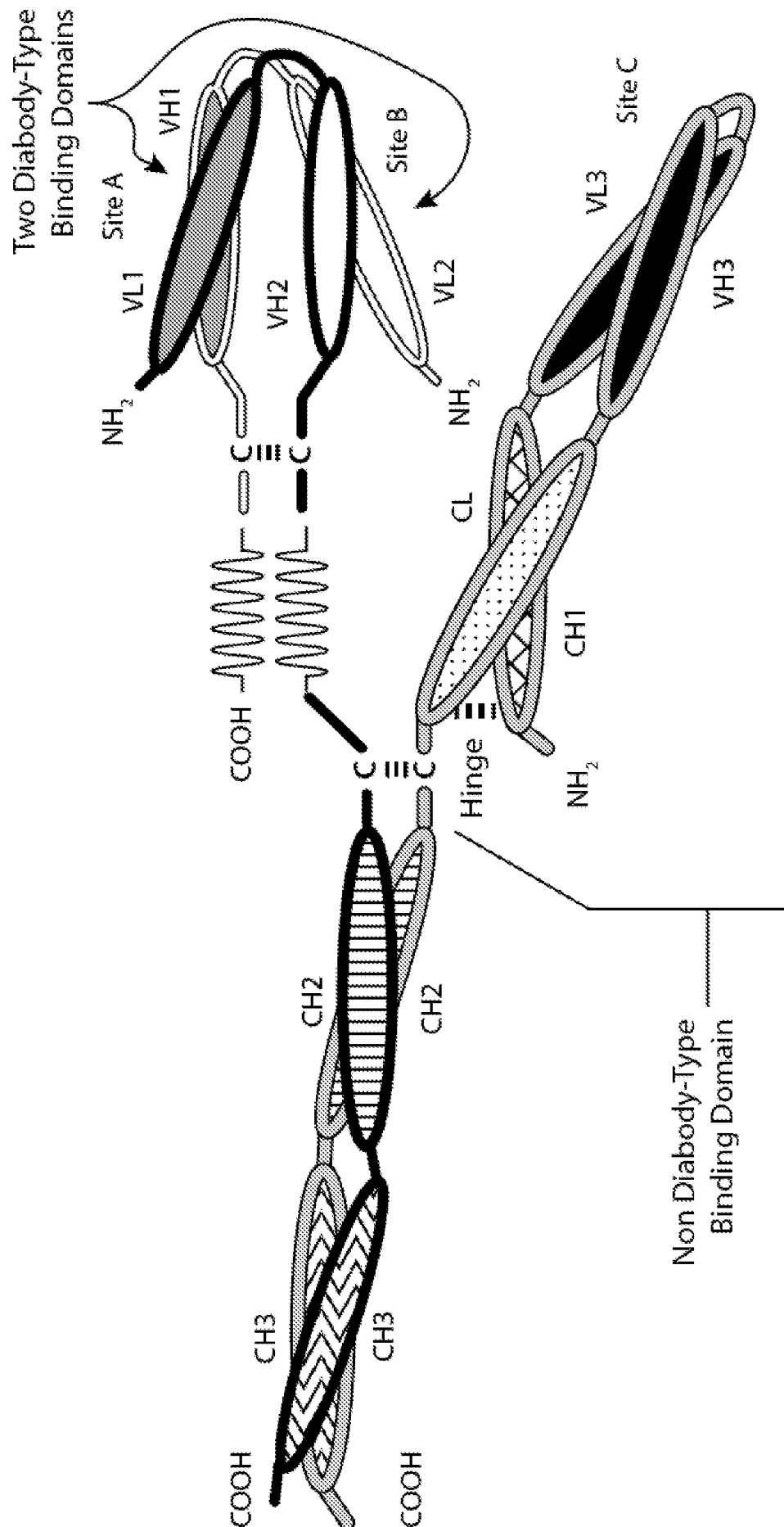


Figure 104C

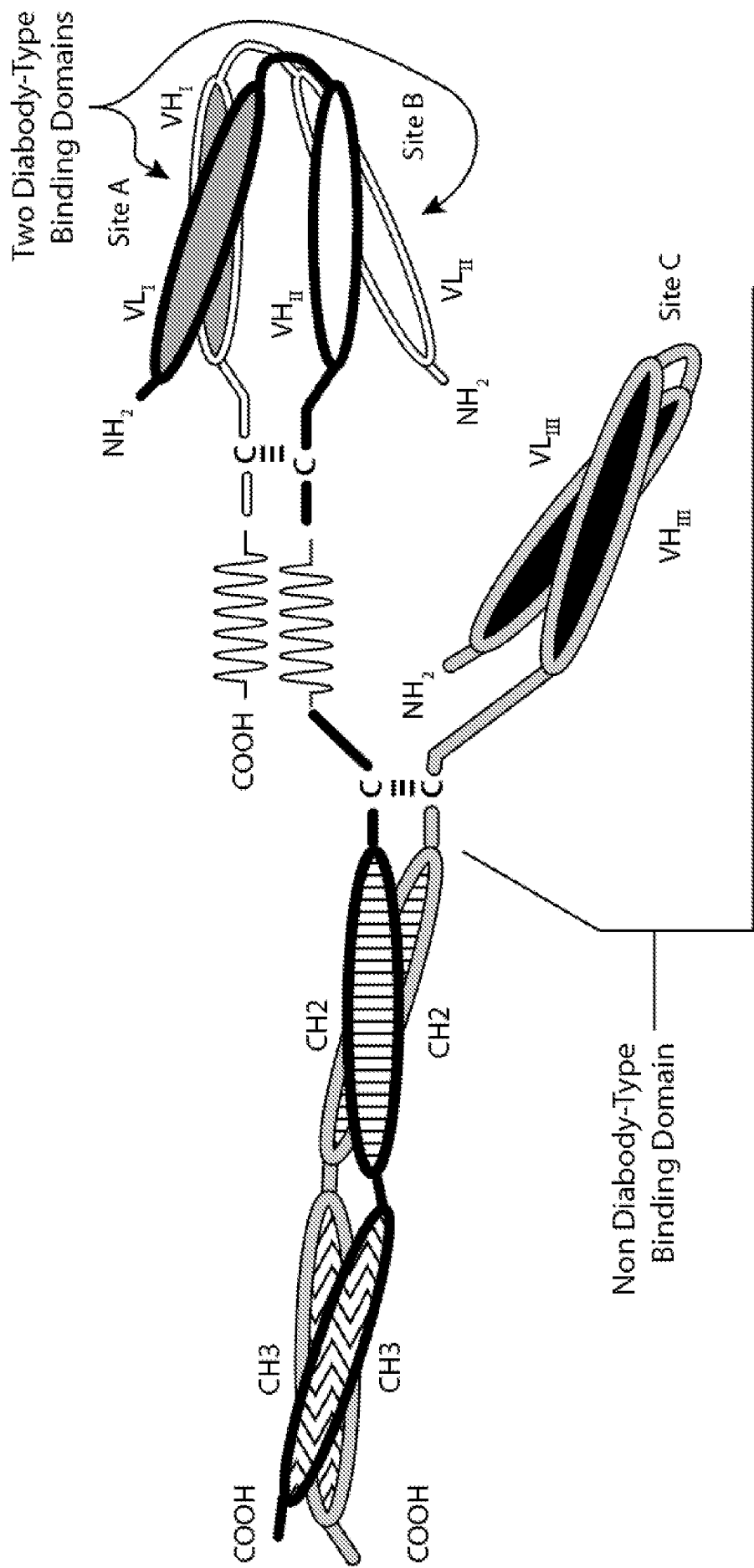


Figure 104D

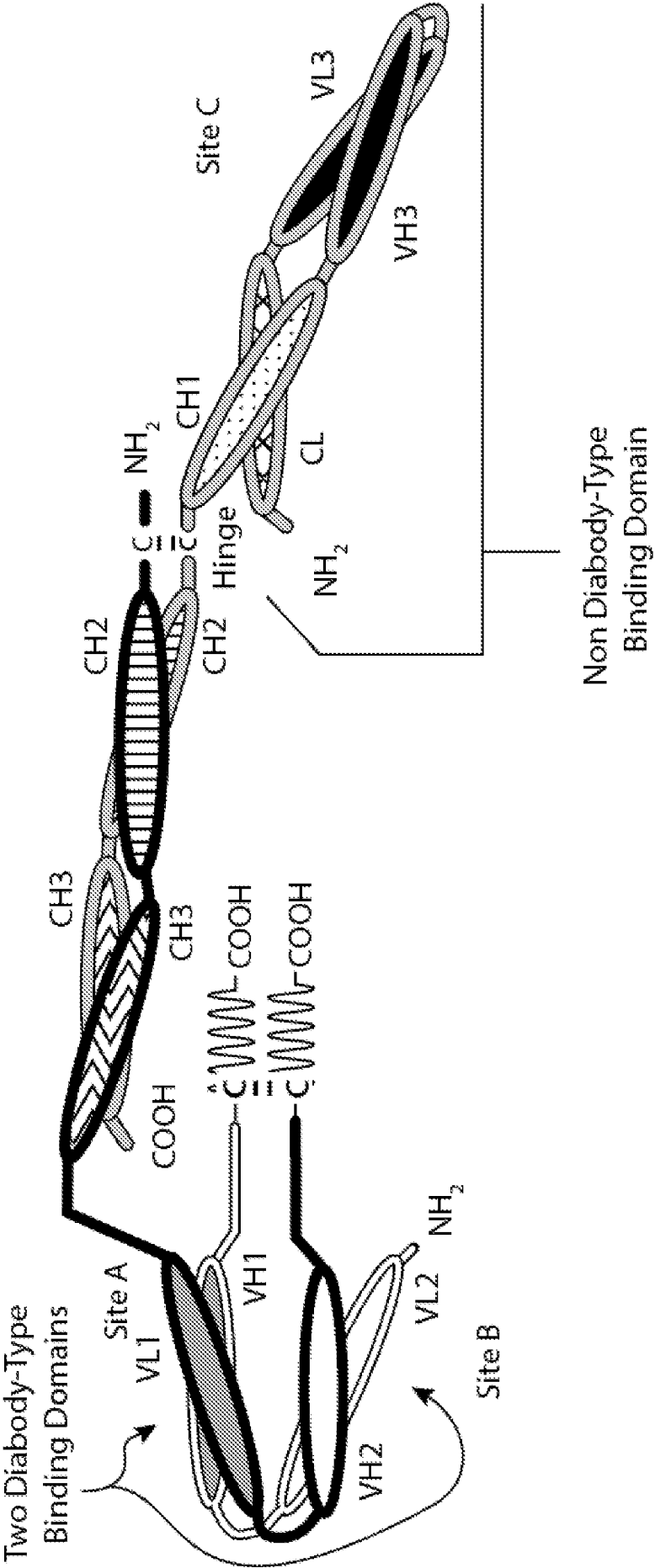


Figure 104E

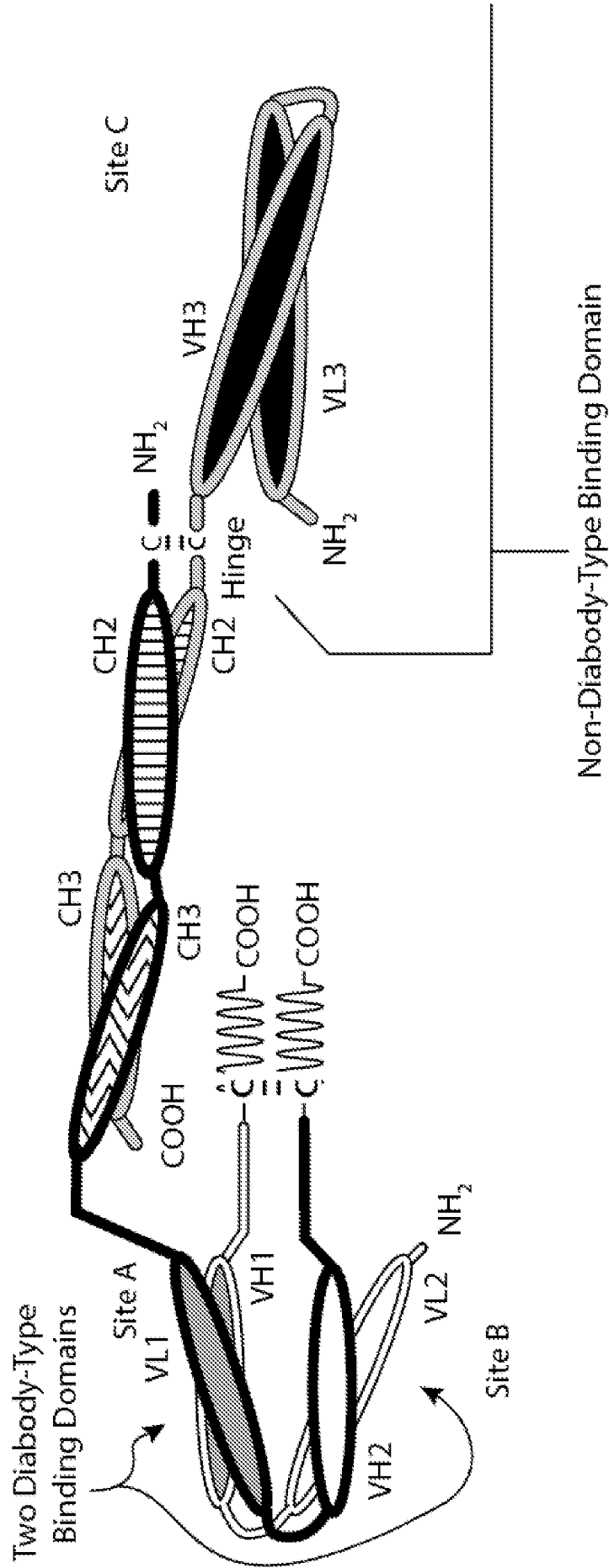
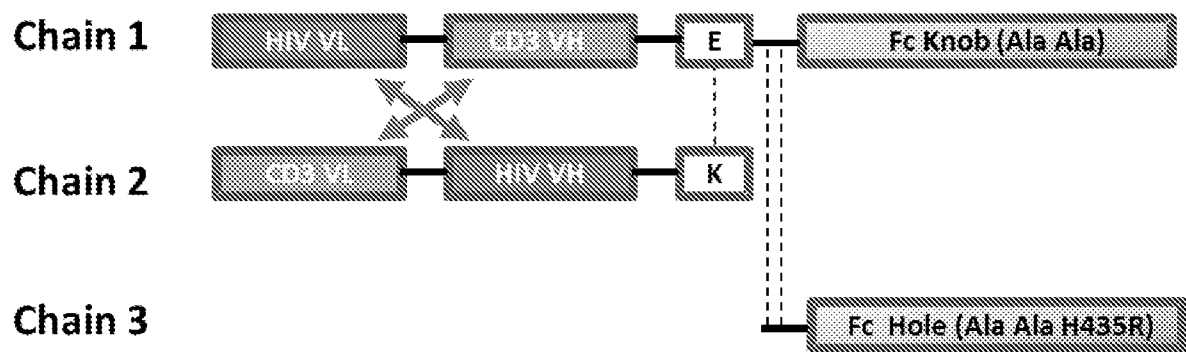
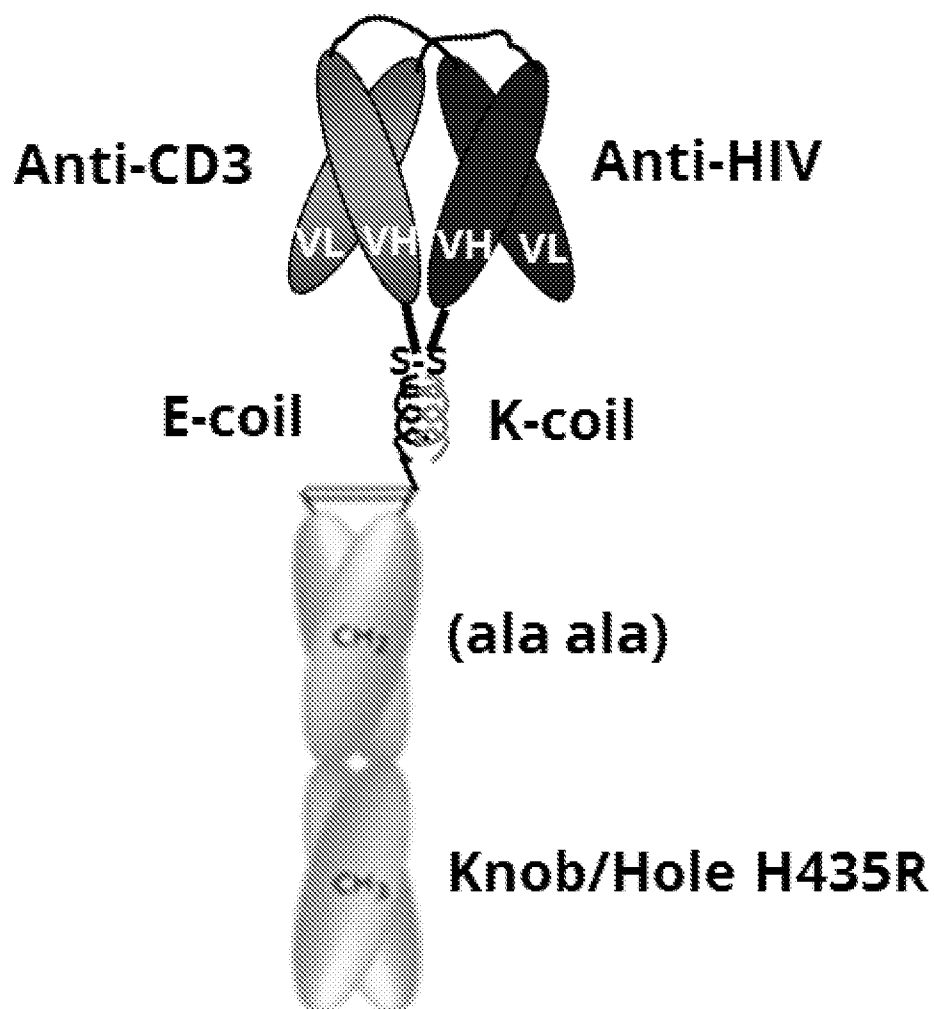


Figure 104F

**Figure 105A****Figure 105B**

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2017/051877

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61K 38/00; A61K 39/395; A61K 39/42; C07K 16/10; C07K 16/46; C07K 19/00 (2017.01)
 CPC - A61K 2039/505; C07K 16/1063; C07K 2317/31; C07K 2317/62; C07K 2319/00 (2017.08)

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)

See Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

USPC - 424/133.1; 424/134.1; 424/136.1; 424/188.1; 530/387.3 (keyword delimited)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 2015/0152167 A1 (AARON DIAMOND AIDS RESEARCH CENTER) 04 June 2015 (04.06.2015) entire document	1-12, 29-31, 33, 34
A	WO 2016/054101 A1 (DUKE UNIVERSITY et al) 07 April 2016 (07.04.2016) entire document	1-12, 29-31, 33, 34
A	US 2012/0269821 A1 (HAYNES et al) 25 October 2012 (25.10.2012) entire document	1-12, 29-31, 33, 34
A	WO 2015/184207 A1 (MACROGENICS, INC) 03 December 2015 (03.12.2015) entire document	1-12, 29-31, 33, 34
A	US 2016/0222105 A1 (MACROGENICS, INC) 04 August 2016 (04.08.2016) entire document	1-12, 29-31, 33, 34
A	WO 2013/070776 A1 (THE UNITED STATES OF AMERICA, AS REPRESENTED BY THE SECRETARY, DEPARTMENT OF HEALTH & HUMAN SERVICES) 16 May 2013 (16.05.2013) entire document	1-12, 29-31, 33, 34

☐ Further documents are listed in the continuation of Box C.☐ See patent family annex.

* 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

27 November 2017

Date of mailing of the international search report

22 DEC 2017

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Authorized officer

Blaine R. Copenheaver

PCT Helpdesk: 571-272-4300

PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2017/051877

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.: 13-28, 32, 35-39
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:

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.:

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.