(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
19 January 2017 (19.01.2017)

WIPO | PCT

(10) International Publication Number
WO 2017/011414 A1

(51) International Patent Classification:
A61K 39/395 (2006.01) *C07K 16/18* (2006.01)
A61P 35/00 (2006.01) *C07K 16/30* (2006.01)
C07K 16/00 (2006.01) *C12P 21/08* (2006.01)

(21) International Application Number:
PCT/US2016/041809

(22) International Filing Date:
11 July 2016 (11.07.2016)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
62/191,095 10 July 2015 (10.07.2015) US
62/222,115 22 September 2015 (22.09.2015) US
62/301,993 1 March 2016 (01.03.2016) US
PCT/US16/23355 21 March 2016 (21.03.2016) 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, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, 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, 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,

[Continued on next page]

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

(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.

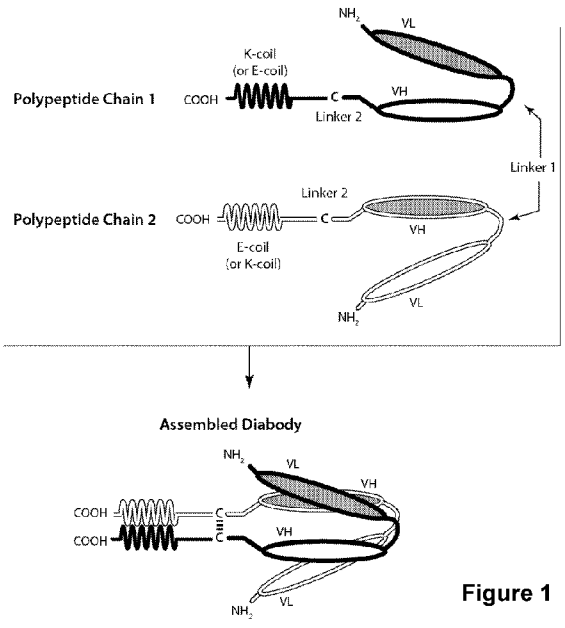

Figure 1

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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. 62/191,095 filed July 10, 2015, U.S. Serial No. 62/222,115 filed September 22, 2015, and U.S. Serial No. 62/301,993 filed March 1, 2016, and International Application No. PCT/US16/23355 filed March 21, 2016 the entire contents of each of which are hereby incorporated by reference in their entireties.

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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.

SUMMARY OF THE INVENTION

[0006] 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 V3 glycan or a CD4 binding site 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.

[0007] 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).

[0008] 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 V3 glycan binding antibody DH542 (also referred to as DH270.6), a variant of DH542 called DH542_QSA, DH542_L4, and/or other antibodies from the DH542 lineage (DH542-like antibodies, *e.g,* DH270.UCA, DH270.IA4, DH270.IA3, DH270.IA2, DH270.IA1, DH270 (also referred to as DH270.1), DH473 (also referred to as DH270.2), DH391 (also referred to as DH270.3), DH429 (also referred to as DH270.4); DH471 (also referred to as DH270.5)), and/or 1, 2 or all 3 of the **CDR_{LS}** of a VL Domain of the V3 glycan binding antibody DH542 (also referred to as DH270.6), a variant of DH542 called DH542_QSA, DH542_L4 (the VL of DH542_L4 comprises the VL from DH429 (also referred to as DH270.4)), and/or other antibodies from the DH542 lineage (DH542-like antibodies, *e.g.,* DH270.UCA, DH270.IA4, DH270.IA3, DH270.IA2, DH270.IA1, DH270 (also referred to as DH270.1), DH473 (also referred to as DH270.2), DH391 (also referred to as DH270.3), DH429 (also referred to as DH270.4); DH471 (also referred to as DH270.5)).

[0009] In non-limiting embodiments, the multispecific molecules comprise the VH Domain with the specificity of the V3 glycan binding antibody DH542, a variant of DH542 called DH542_QSA, DH542_L4, and/or other antibodies from the DH542 lineage (DH542-like antibodies , *e.g.,* DH270.UCA, DH270.IA4, DH270.IA3, DH270.IA2, DH270.IA1, DH270 (also referred to as DH270.1), DH473 (also referred to as DH270.2), DH391 (also referred to as DH270.3); DH429 (also referred to as DH270.4); DH471 (also referred to as DH270.5)), and/or the VL Domain, of the V3 glycan binding antibody DH542, a variant of DH542 called DH542_QSA, DH542_L4 (the VL of DH542_L4 comprises the VL from DH429 (also

referred to as DH270.4)), and/or other antibodies from the DH542 lineage (DH542-like antibodies, *e.g.,* DH270.UCA, DH270.IA4, DH270.IA3, DH270.IA2, DH270.IA1, DH270 (also referred to as DH270.1), DH473 (also referred to as DH270.2), DH391 (also referred to as DH270.3), DH429 (also referred to as DH270.4); DH471 (also referred to as DH270.5)).

[0010] 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 CD4 binding site antibody CH557 (also referred to as CH235.12) or any of the antibodies of the CH235 lineage (CH557-like antibodies, *e.g.,* CH556 (also referred to as CH235.11), CH555 (also referred to as CH235.10), CH493 (also referred to as CH235.9), CH492 (also referred to as CH235.8), CH491 (also referred to as CH235.7), or CH490 (also referred to as CH235.6)), and/or 1, 2 or all 3 of the **CDR_{LS}** of a VL Domain with the specificity of the CD4 binding site antibody CH557 (also referred to as CH235.12) or any of the antibodies of the CH235 lineage (CH557-like antibodies, *e.g.,* CH556 (also referred to as CH235.11), CH555 (also referred to as CH235.10), CH493 (also referred to as CH235.9), CH492 (also referred to as CH235.8), CH491 (also referred to as CH235.7), or CH490 (also referred to as CH235.6)).

[0011] In non-limiting embodiments, the multispecific molecules comprise the VH Domain with the specificity of the CD4 binding site antibody CH557 (also referred to as CH235.12) or any of the antibodies of the CH235 lineage (CH557-like antibodies, *e.g.,* CH556 (also referred to as CH235.11), CH555 (also referred to as CH235.10), CH493 (also referred to as CH235.9), CH492 (also referred to as CH235.8), CH491 (also referred to as CH235.7), or CH490 (also referred to as CH235.6)) and/or the VL Domain with the binding specificity of the CD4 binding site antibody CH557 (also referred to as CH235.12) or any of the antibodies of the CH235 lineage (CH557-like antibodies, *e.g.,* CH556 (also referred to as CH235.11), CH555 (also referred to as CH235.10), CH493 (also referred to as CH235.9), CH492 (also referred to as CH235.8), CH491 (also referred to as CH235.7), or CH490 (also referred to as CH235.6)).

[0012] 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 gpl20, 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 KD 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.

[0013] 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 V3 glycan binding antibody DH542, a variant of DH542 called DH542_QSA, DH542_L4 and/or other antibodies from the DH542 lineage, and/or provided by the binding specificity of the CD4 binding site antibody CH557 (also referred to as CH235.12) or any of the antibodies of the CH235 lineage, 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.

[0014] 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 CD4 binding site HIV-1 antibody or a V3 glycan 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); 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 CD4 binding site HIV-1 antibody or a V3 glycan binding 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 CD4 binding site HIV-1 antibody or like a V3 glycan binding antibody (1); and

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

[0015] 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.

[0016] 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 V3 glycan binding antibody DH542, a variant of DH542 called DH542_QSA, DH542_L4 and/or other antibodies from the DH542 lineage, and/or the binding specificity of the CD4 binding site antibody CH557 (also referred to as CH235.12) or any of the antibodies of the CH235 HIV-1 antibody 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 V3 glycan binding antibody DH542, a variant of DH542 called DH542_QSA, DH542_L4 and/or other antibodies from the DH542 lineage, and/or the binding specificity of the CD4 binding site antibody CH557 (also referred to as CH235.12) or any of the antibodies of the CH235 HIV-1 antibody 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 V3 glycan binding antibody DH542, a variant of DH542 called DH542_QSA, DH542_L4 and/or other antibodies from the DH542 lineage, and/or like the CD4 binding site antibody CH557 (also referred to as CH235.12) or any of the antibodies of the CH235 HIV-1 antibody lineage (1); and the domains (B) and (D) associate to form a binding site that binds the epitope (2).

[0017] 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.

[0018] 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, 565, 567, 570, 574, 578, 582, 586, 590, or 594;

(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, 564, 566, 568, 572, 576, 580, 584, 588, 592; 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 CD4 binding site HIV-1 antibody or like a V3 glycan binding antibody (1); and

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

[0019] 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.

[0020] 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, 565, 567, 570, 574, 578, 582, 586, 590, or 594;

(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, 564, 566, 568, 572, 576, 580, 584, 588, 592, 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 DH557;

wherein if the domain (A) comprises SEQ ID NO: 565 and domain (E) comprises SEQ ID NO: 564 they associate to form a binding site that binds the HIV-1 envelope like antibody DH556;

wherein if the domain (A) comprises SEQ ID NO: 567 and domain (E) comprises SEQ ID NO: 566 they associate to form a binding site that binds the HIV-1 envelope like antibody DH555;

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 DH493;

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 DH492;

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 DH491;

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 DH490;

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 DH542;

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 DH542_QSA; or

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 DH542_L4; 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.

[0021] 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, 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 CD4 binding site HIV-1 antibody or a V3 glycan 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 CD4 binding site HIV-1 antibody or a V3 glycan 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 CD4 binding site HIV-1 antibody or like a V3 glycan 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.

[0022] 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.

[0023] 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 3), 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 V3 glycan binding antibody DH542, a variant of DH542 called DH542_QSA, DH542_L4 and/or other antibodies from the DH542 lineage, and/or the binding specificity of the CD4 binding site antibody CH557 (also referred to as CH235.12) or any of the antibodies of the CH235 lineage 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), 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 V3 glycan binding antibody DH542, a variant of DH542 called DH542_QSA, DH542_L4 and/or other antibodies from the DH542 lineage, and/or the binding specificity of the CD4 binding site antibody CH557 (also referred to as CH235.12) or any of the antibodies of the CH235 lineage HIV-1 antibody (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 V3 glycan binding antibody DH542, a variant of DH542 called DH542_QSA, DH542_L4 and/or other antibodies from the DH542 lineage, and/or like the CD4 binding site antibody CH557 (also referred to as CH235.12) or any of the antibodies of the CH235 lineage 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.

[0024] In certain aspects said first and second polypeptide chains are covalently bonded to one another; and said second and third polypeptide chains are covalently bonded to one another.

[0025] 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 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 SEQ ID NO: 553, 565, 567, 570, 574, 578, 582, 586, 590, or 594;

(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, 564, 566, 568, 572, 576, 580, 584, 588, 592; 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 CD4 binding site HIV-1 antibody or like a V3 glycan 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.

[0026] 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.

[0027] 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 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 SEQ ID NO: 553, 565, 567, 570, 574, 578, 582, 586, 590, or 594;

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

(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;

(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, 564, 566, 568, 572, 576, 580, 584, 588, 592, 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;

(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 DH557;

wherein if the domain (A) comprises SEQ ID NO: 565 and domain (E) comprises SEQ ID NO: 564 they associate to form a binding site that binds the HIV-1 envelope like antibody DH556;

wherein if the domain (A) comprises SEQ ID NO: 567 and domain (E) comprises SEQ ID NO: 566 they associate to form a binding site that binds the HIV-1 envelope like antibody DH555;

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 DH493;

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 DH492;

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 DH491;

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 DH490;

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 DH542;

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 DH542_QSA; or

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 DH542_L4; 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.

[0028] 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 3), 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 CD4 binding site HIV-1 antibody or a V3 glycan binding 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 CD4 binding site HIV-1 antibody or a V3 glycan 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 CD4 binding site HIV-1 antibody or like a V3 glycan 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.

[0029] 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.

[0030] 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 3), 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 V3 glycan binding antibody DH542, a variant of DH542 called DH542_QSA, DH542_L4 and/or other antibodies from the DH542 lineage, and/or the binding specificity of the CD4 binding site antibody CH557 (also referred to as CH235.12) or any of the antibodies of the CH235 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 V3 glycan binding antibody DH542, a variant of DH542 called DH542_QSA, DH542-L4 and/or other antibodies from the DH542 lineage, and/or the binding specificity of the CD4 binding site antibody CH557 (also referred to as CH235.12) or any of the antibodies of the CH235 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 V3 glycan binding antibody DH542, a variant of DH542 called DH542_QSA, DH542_L4 and/or other antibodies from the DH542 lineage, and/or like the CD4 binding site antibody CH557 (also referred to as CH235.12) or any of the antibodies of the CH235 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.

[0031] 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.

[0032] 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 3), 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, 565, 567, 570, 574, 578, 582, 586, 590, or 594;

(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, 564, 566, 568, 572, 576, 580, 584, 588, 592; 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 CD4 binding site HIV-1 antibody or like a V3 glycan 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.

[0033] 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.

[0034] 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 3), 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, 565, 567, 570, 574, 578, 582, 586, 590, or 594, wherein the CH2-CH3 domain and domain (A) are separated 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, 564, 566, 568, 572, 576, 580, 584, 588, 592, 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 DH557;

wherein if the domain (A) comprises SEQ ID NO: 565 and domain (E) comprises SEQ ID NO: 564 they associate to form a binding site that binds the HIV-1 envelope like antibody DH556;

wherein if the domain (A) comprises SEQ ID NO: 567 and domain (E) comprises SEQ ID NO: 566 they associate to form a binding site that binds the HIV-1 envelope like antibody DH555;

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 DH493;

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 DH492;

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 DH491;

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 DH490;

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 DH542;

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 DH542_QSA; or

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 DH542_L4; 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.

[0035] 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 CD4 binding site HIV-1 antibody or a V3 glycan 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 CD4 binding site HIV-1 antibody or a V3 glycan 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 CD4 binding site HIV-1 antibody or like a V3 glycan 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.

[0036] 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.

[0037] 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 V3 glycan binding antibody DH542, a variant of DH542 called DH542_QSA, DH542_L4 and/or other antibodies from the DH542 lineage, and/or the binding specificity of the CD4 binding site antibody CH557 (also referred to as CH235.12) or any of the antibodies of the CH235 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 V3 glycan binding antibody DH542, a variant of DH542 called DH542_QSA, DH542_L4 and/or other antibodies from the DH542 lineage, and/or the binding specificity of the CD4 binding site antibody CH557 (also referred to as CH235.12) or any of the antibodies of the CH235 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 V3 glycan binding antibody DH542, a variant of DH542 called DH542_QSA, DH542_L4 and/or other antibodies from the DH542 lineage, and/or like the CD4 binding site antibody CH557 (also referred to as CH235.12) or any of the antibodies of the CH235 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.

[0038] 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.

[0039] 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, 565, 567, 570, 574, 578, 582, 586, 590, or 594;

(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, 564, 566, 568, 572, 576, 580, 584, 588, 592;

(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 CD4 binding site HIV-1 antibody or like a V3 glycan 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.

[0040] 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.

[0041] 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, 565, 567, 570, 574, 578, 582, 586, 590, or 594;

(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, 564, 566, 568, 572, 576, 580, 584, 588, 592, 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 DH557;

wherein if the domain (A) comprises SEQ ID NO: 565 and domain (E) comprises SEQ ID NO: 564 they associate to form a binding site that binds the HIV-1 envelope like antibody DH556;

wherein if the domain (A) comprises SEQ ID NO: 567 and domain (E) comprises SEQ ID NO: 566 they associate to form a binding site that binds the HIV-1 envelope like antibody DH555;

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 DH493;

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 DH492;

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 DH491;

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 DH490;

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 DH542;

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 DH542_QSA; or

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 DH542_L4; 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.

[0042] 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 CD4 binding site HIV-1 antibody or a V3 glycan 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.

[0043] 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.

[0044] 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 Peptide Linker 2-C; or

(b) a heterodimer promoting domain; wherein domain (F) and domain (E) are separated by a peptide 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 V3 glycan binding antibody DH542, a variant of DH542 called DH542_QSA, DH542_L4 and/or other antibodies from the DH542 lineage, and/or the CD4 binding site antibody CH557 (also referred to as CH235.12) or any of the antibodies of the CH235 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.

[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 SEQ ID NO: 553, 565, 567, 570, 574, 578, 582, 586, 590, or 594;

(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, 564, 566, 568, 572, 576, 580, 584, 588, 592; 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, 564, 566, 568, 572, 576, 580, 584, 588, 592, 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, 565, 567, 570, 574, 578, 582, 586, 590, 594, 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 CD4 binding site HIV-1 antibody or a V3 glycan binding antibody, epitope (2) is an epitope of CD3, and epitope (3) is an epitiope bound by a CD4 binding site HIV-1 antibody or a V3 glycan 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.

[0046] 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.

[0047] 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, 565, 567, 570, 574, 578, 582, 586, 590, or 594;

(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, 564, 566, 568, 572, 576, 580, 584, 588, 592, 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, 564, 566, 568, 572, 576, 580, 584, 588, 592, 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, 565, 567, 570, 574, 578, 582, 586, 590, 594, 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 DH557;

wherein if the domain (A) comprises SEQ ID NO: 565 and domain (E) comprises SEQ ID NO: 564 they associate to form a binding site that binds the HIV-1 envelope like antibody DH556;

wherein if the domain (A) comprises SEQ ID NO: 567 and domain (E) comprises SEQ ID NO: 566 they associate to form a binding site that binds the HIV-1 envelope like antibody DH555;

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 DH493;

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 DH492;

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 DH491;

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 DH490;

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 DH542;

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 DH542_QSA; or

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 DH542_L4; 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 (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 DH557;

wherein if the domain (H) comprises SEQ ID NO: 565 and domain (G) comprises SEQ ID NO: 564 they associate to form a binding site that binds the HIV-1 envelope like antibody DH556;

wherein if the domain (H) comprises SEQ ID NO: 567 and domain (G) comprises SEQ ID NO: 566 they associate to form a binding site that binds the HIV-1 envelope like antibody DH555;

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 DH493;

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 DH492;

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 DH491;

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 DH490;

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 DH542;

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 DH542_QSA; or

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 DH542_L4;

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 CH557, CH556, CH555, CH493, CH492, CH491, CH490, DH542, DH542_QSA, or DH542_L4, epitope (2) is an epitope of

CD3, and epitope (3) is is an epitope bound by the antibody CH557, CH556, CH555, CH493, CH492, CH491, CH490, DH542, DH542_QSA, or DH542_L4 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.

[0048] 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 CD4 binding site HIV-1 antibody or a V3 glycan 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.

[0049] 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.

[0050] 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 Peptide Linker 2-C; or

(b) a heterodimer promoting domain; wherein domain (F) and domain (E) are separated by a peptide 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 V3 glycan binding antibody DH542, a variant of DH542 called DH542_QSA, DH542_L4 and/or other antibodies from the DH542 lineage, and/or the CD4 binding site antibody CH557 (also

referred to as CH235.12) or any of the antibodies of the CH235 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.

[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 SEQ ID NO: 553, 565, 567, 570, 574, 578, 582, 586, 590, or 594;
- **(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, 564, 566, 568, 572, 576, 580, 584, 588, or 592;
- **(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 CD4 binding site HIV-1 antibody or a V3 glycan 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.

[0052] 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.

[0053] 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, 565, 567, 570, 574, 578, 582, 586, 590, or 594;

(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, 564, 566, 568, 572, 576, 580, 584, 588, or 592, wherein domains (D) and (E) are separated by SEQ ID NO: 508;

(iii) a domain (F) comprising 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 DH557;

wherein if the domain (A) comprises SEQ ID NO: 565 and domain (E) comprises SEQ ID NO: 564 they associate to form a binding site that binds the HIV-1 envelope like antibody DH556;

wherein if the domain (A) comprises SEQ ID NO: 567 and domain (E) comprises SEQ ID NO: 566 they associate to form a binding site that binds the HIV-1 envelope like antibody DH555;

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 DH493;

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 DH492;

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 DH491;

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 DH490;

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 DH542;

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 DH542_QSA; or

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 DH542_L4; 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.

[0054] 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 V3 glycan or an epitope of HIV-1 Envelope CD4 binding site, 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 V3 glycan or an epitope of HIV-1 Envelope CD4 binding site, 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 V3 glycan or an epitope of HIV-1 Envelope CD4 binding site, 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 V3 glycan or an epitope of HIV-1 Envelope CD4 binding site;

(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 V3 glycan or an epitope of HIV-1 Envelope CD4 binding site; or

(f) epitope (1) is an epitope of CD8, epitope (2) is an epitope of HIV-1 Envelope V3 glycan or an epitope of HIV-1 Envelope CD4 binding site, and epitope (3) is an epitope of CD3.

[0055] In certain aspects the invention provides such trivalent binding molecules wherein two of epitope (1), epitope (2), and epitope (3) are an 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 V3 glycan or an epitope of HIV-1 Envelope CD4 binding site, epitope (2) is an epitope of HIV-1 Envelope V3 glycan or an epitope of HIV-1 Envelope CD4 binding site, and epitope (3) is an epitope of CD3;

(b) epitope (1) is an epitope of HIV-1 Envelope V3 glycan or an epitope of HIV-1 Envelope CD4 binding site, epitope (2) is an epitope of CD3, and epitope (3) is an epitope of HIV-1 Envelope V3 glycan or an epitope of HIV-1 Envelope CD4 binding site; or

(c) epitope (1) is an epitope of CD3, epitope (2) is an epitope of HIV-1 Envelope V3 glycan or an epitope of HIV-1 Envelope CD4 binding site, and epitope (3) is an epitope of HIV-1 Envelope V3 glycan or an epitope of HIV-1 Envelope CD4 binding site.

[0056] 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 CD4 binding site antibody, or a HIV-1 envelope V3 glycan antibody. In certain aspects, domain (G) comprises a binding region of a heavy chain variable domain of an anti-CB8 antibody, an anti-CD3 antibody, an anti-CD16 antibody, an HIV-1 envelope CD4 binding site antibody, or a HIV-1 envelope V3 glycan antibody.

[0057] 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.

[0058] 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.

[0059] 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.

[0060] 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.

[0061] 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 V3 glycan binding antibody DH542, a variant of DH542 called DH542_QSA, DH542_L4 and/or other antibodies from the DH542 lineage, and/or the binding specificity of CD4 binding site antibody CH557 (also referred to as CH235.12) or any of the antibodies from the CH235 HIV-1 antibody lineage and also binds CD3. In certain embodiments, the bispecific or trivalent molecule binds HIV-1 envelope with the specificity of the V3 glycan binding antibody DH542, a variant of DH542 called DH542_QSA, DH542_L4 and/or other antibodies from the DH542 lineage, and/or the binding specificity of CD4 binding site antibody CH557 (also referred to as CH235.12) or any of the antibodies of the CH235 HIV-1 antibody lineage and also binds CD3, CD8, or CD16.

[0062] In certain aspects, domain (A) comprises the CDR1, CDR2, and CDR3 of the light chain variable domain of immunoglobulin CH557, CH556, CH555, CH493, CH492, CH491, or CH490. In certain aspects, the domain (E) comprises the CDR1, CDR2, and CDR3 of the heavy chain variable domain of immunoglobulin CH557, CH556, CH555, CH493, CH492, CH491, or CH490. In certain aspects, domain (A) comprises the light chain variable domain of immunoglobulin CH557, CH556, CH555, CH493, CH492, CH491, or CH490. In certain aspects, domain (E) comprises the heavy chain variable domain of immunoglobulin CH557, CH556, CH555, CH493, CH492, CH491, or CH490.

[0063] In certain aspects, domain (A) comprises the CDR1, CDR2, and CDR3 of the light chain variable domain of immunoglobulin DH542, DH542_QSA, DH542_L4, DH429. In certain aspects, the domain (E) comprises the CDR1, CDR2, and CDR3 of the heavy chain variable domain of immunoglobulin DH542, DH542_QSA, DH542_L4, DH429. In certain aspects, domain (A) comprises the light chain variable domain of immunoglobulin DH542, DH542_QSA, DH542_L4, DH429. In certain aspects, domain (E) comprises the heavy chain variable domain of immunoglobulin DH542, DH542_QSA, DH542_L4, DH429.

[0064] 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.

[0065] 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.

[0066] 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.

[0067] 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 4A).

[0068] 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 4D).

[0069] In certain aspects, the first polypeptide comprises SEQ ID NO: 596. In certain embodiments, the second polypeptide comprises SEQ ID NO: 597. In certain embodiments, the third polypeptide comprises SEQ ID NO: 559. An exemplary DH542 bispecific Fc bearing diabody is shown in Figure 3A.

[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 CD4 binding site antibody or HIV-1 V3 glycan 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 multipecific 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, bryostatin, PMA, inonomycin, 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 provide 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 CH557-HIV-1 envelope epitope, i.e. the multispecific molecule binds to the HIV-1 envelope like the CH557 antibody, and also binds CD3, CD8, or CD16.

[0074] In certain embodiments, the multispecific molecule binds to the DH542-HIV-1 envelope epitope, i.e. the multispecific molecule binds to the HIV-1 envelope like the DH542 antibody, and also binds CD3, CD8, or CD16.

[0075] In certain embodiments a multispecific molecule of the invention comprises, consists essentially of or consists of sequences as described herein, (*e.g.*, Table 4).

[0076] 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.

[0077] 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.

[0078] 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, inonomycin, or any combination thereof. In some embodiments, this combination therapy targets the pool of latently infected HIV-1 cells.

[0079] 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

[0080] 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.

[0081] Figure 1 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 2B**. VL and VH Domains that recognize the same epitope are shown using the same shading or fill pattern.

[0082] Figures 2A-2C 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 2A-2B**), 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 2C**), the resulting molecule possesses four epitope-binding sites and is tetraspecific and monovalent with respect to each bound epitope. **Figure 2A** shows an Fc Domain-containing diabody which contains a peptide Heterodimer-Promoting Domain comprising a cysteine residue. **Figure 2B** 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 2C**, shows an Fc-Region-Containing diabody, which contains antibody CH1 and CL domains which could serve as Heterodimer Promoting Domains.

[0083] Figures 3A and 3B 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.

[0084] Figures 4A-4F provide schematics of representative Fc Domain-containing trivalent binding molecules having three epitope-binding sites. **Figures 4A** and **4B**, 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 4A** and **4B** comprise four chains. **Figures 4C** and **4D**, 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 4E** and **4F**, 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 4C-4F** comprise three chains. VL and VH Domains that recognize the same epitope are shown using the same shading or fill pattern.

[0085] Figures 5A-5E provide schematics of a representative HIVxCD3 bispecific monovalent diabody comprising three polypeptide chains. **Figure 5A** 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 5B** provides a schematic of the assembled chains. Such diabodies contain an anti-HIV-1 binding arm (*e.g.*, DH491 or CH493, DH542, CH557, CH558, or any of the CH235 lineage antibodies) 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 CH557xCD3 Fc are provided in Table 4 (SEQ ID NOs: 555, 556, 557, 558, 559, 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).

[0086] Figures 6A-6C show antigen binding by ELISA. Binding of bispecific monovalent diabodies (CH557xCD3 Fc, the comparator A32xCD3 Fc, and the controls RSVxCD3 Fc and A32xRSV Fc) to human CD3 protein (**Figure 6A**), to M.CONS gpl40 protein (**Figure 6B**) or simultaneously binding to both human CD3 and M.CONS gp140 proteins (**Figure 6C**).

[0087] Figures 7A-7B show cell surface binding by FACS. Binding of the bispecific monovalent diabodies (CH557xCD3 Fc, the comparator A32xCD3 Fc, and the controls RSVxCD3 Fc and A32xRSV Fc) to HEK293-D375 cells expressing HIV-1 *Env*, (**Figure 7A**)

or to primary human T cells expressing CD3 (**Figure 7B**), Data are reported as mean fluorescence intensity (MFI).

[0088] Figures 8A-8B show redirected T-cell killing of HIV-1 *Env*+ target cells. The results from two different donors are shown (**Figures 8A and 8B**). Both HIVxCD3 Fc bispecific monovalent diabodies mediate concentration dependent killing of HIV-1 *Env*+ HEK293-D375 cells in the presence of human PBMC effector cells at an E:T ratio of 30:1 for 28 hours with cytolysis measured by LDH release assay. The control molecule (RSVxCD3 Fc) was inactive. The average EC50 values were 13.8 ng/mL and 12.3 ng/mL for CH557xCD3 Fc and A32xCD3 Fc, respectively.

[0089] Figures 9A-9E provide the amino acid sequences of exemplary (CH557HIVxCD3xCD8 trivalent binding molecules. **Figures 9A-9D** represent the amino acid sequences of polypeptide chains 1-4, respectively, of an exemplary HIVxCD3xCD8 trivalent binding molecule comprising four polypeptide chain (SEQ ID NOs: 555, 557, 561, 561). **Figures 9A, 9B and 9E** represent the amino acid sequences of polypeptide chains 1, 2 and 3, respectively, of an exemplary HIVxCD3xCD8 trivalent binding molecule comprising three polypeptide chains (SEQ ID NOs: 555, 557, 563).

[0090] FIG. 10A shows DH542 sequences (CDRs are bolded and underlined) (SEQ ID NOs: 1-4).

[0091] FIG. 10B shows the amino acids sequences of VH and VL chains of antibodies of the DH270 lineage, and nucleic acid sequences encoding these amino acids. CDRs are highlighted in each antibody. DH270IA1 as listed in Figure 3 is the same antibody referred to as I1 in the sequence of Figure 2. The figure shows SEQ ID NOs: 5-16 (Heavy chain nucleotide sequences in order of appearance from UCA-DH270H), SEQ ID NOs: 17-28 (Heavy chain amino acid sequences in order of appearance from UCA-DH270H), SEQ ID NOs: 29-40 (Light chain nucleotide sequences in order of appearance from UCA-DH270H), SEQ ID NOs: 41-52 (Light chain amino acid sequences in order of appearance from UCA-DH270H).

[0092] FIG. 11 shows neutralization by antibodies CH555, CH556, CH557, CH558, CH560, CH561, CH562, DH210 and DH211 identified from subject CH505 measured in TZM-bl cells. Pseudoviruses were produced by transfection in 293T cells. Values are the antibody concentration (μg/ml) at which relative luminescence units (RLUs) were reduced 50%

compared to virus control wells (no test sample). Values in bold are considered positive for neutralizing antibody activity. CH557 IC50 neutralization summary: mean IC50 = 3.66 μg/ml; geometric mean IC50 = 0.66 μg/ml; median = 0.62 μg/ml.

[0093] FIG. 12 shows sequences of CH557 (SEQ ID NOs: 124-127). CDRs are bolded and underlined.

[0094] FIG. 13 shows CH557 gene information. (SEQ ID NO: 215).

[0095]

[0096] FIG. 14 shows gene information for antibodies in the CH235 lineage.

[0097] FIGS. 15A and 15B show amino acid alignment of CH235 lineage antibody heavy chain (Figure 15A) and light chain (Figure 15B). Antibodies are listed in ascending order of somatic mutations and compared to the inferred unmutated common ancestor previously published (Gao, Bonsignori, Liao et al. Cell 2014). FIG. 15A shows SEQ ID NOs: 128-139 (in order of appearance from UCA-CH557). FIG. 15B shows SEQ ID NOs: 140-149 (in order of appearance from UCA-CH556).

[0098] FIG. 16 shows alignment of CH557 heavy chain amino acid sequence compared to CH235 lineage antibodies with increasing levels of somatic mutations and neutralization breadth. Contact sites with gp120 identified from CH235/gp120 co-crystal structure are indicated with asterisks. Amino acid mutations within the contact sites are bolded and underlined. Figure 16 shows SEQ ID NOs: 150-154 (in order of appearance from UCA-CH557).

[0099] FIG. 17 shows alignment of CH557 light chain amino acid sequence compared to CH235 lineage antibodies with increasing levels of somatic mutations and neutralization breadth. Figure 17 shows SEQ ID NOs: 155-158 (in order of appearance from UCA-CH557).

[0100]

[0101] FIG. 18 shows amino acid sequences of VH and VL chains of antibodies CH490, CH491, CH492 and CH493 (CH235_129w66 = CH490; CH235_68w100 = CH491; CH235_115w100 = CH492; CH235_75w152 = CH493) (SEQ ID NOs: 159-164).

[0102] FIG. 19 shows nucleic acid sequences of VH and VL chains of antibodies CH490, CH491, CH492 and CH493 (CH235_129w66 = CH490; CH235_68w100 = CH491; CH235_115w100 = CH492; CH235_75w152 = CH493) (SEQ ID NOs: 165-170).

[0103] FIG. 20 shows phylogenetic tree of the heavy chains of antibodies CH490, CH491, CH492 and CH493 (see table in Figure 19). See also Example 6: CH240, CH239, CH235,

CH236, CH241 VH chains were identified from cultured memory B cells. The rest of the VH chains were retrieved with deep sequencing.

[0104] FIG. 21 shows ELISA binding of CH490, CH491, CH492 and CH493 antibodies to various antigens as listed in the figure.

[0105] FIG. 22 shows a summary of neutralization data of CH490, CH491, CH492 and CH493 antibodies for various HIV-1 strains in TZMbl assay. Intermediate antibodies are described in Gao, Bonsignori, Liao et al. Cell 158, 481–491, July 31, 2014.

[0106] FIG. 23 shows a summary of neutralization data for antibodies CH235, CH490, CH491, and CH493. The viruses are CH505 TF (autologous virus) in which either point or multiple single mutations were introduced in the loop D region. The loop D mutations are described in Gao et al Cell 158, 481–491, July 31, 2014 (incorporated by reference). These mutations reflect mutations in the loop D region that naturally occurred in the autologous virus of this subject over time. They were artificially introduced into the CH505 TF to study their effect in absence of other mutations in other parts of the autologous virus that also occurred during virus evolution. These mutations were induced by CH235 lineage antibodies identified early during the course of infection. These data show that the more mutated antibody CH493 which came from a later time point and that is broadly neutralizing also acquired the ability of recognizing virus escape mutants that escaped earlier antibodies from the same lineage, before they acquired substantial breadth.

[0107] FIG. 24 shows a summary of neutralization (TZMbl assay) data of CH505 D loop mutants by various antibodies. The summary shows that CH493 neutralizes all Loop D CH505 mutants.

[0108] FIG. 25 shows results of HEp-2 cell IF staining for CH557.

[0109] FIGS. 26A and 26B shows summary of data from CH557 microarray polyreactivity.

[0110] FIG. 27A shows summary results of neutralization data of CH557, CH235, VRC01, VRC07-523-LS, N6, 3BNC117, 8ANC131, CH103, F105, and DH522 against a panel of HIV-1 isolates in the Luc/TZM-bl neutralization assay. Values represent IC50 in μg/ml.

[0111] FIG. 27B shows the mean IC50 and percent of isolates neutralized at different IC50 values.

[0112] FIG. 28A shows summary results of neutralization data of CH557, CH235, VRC01, VRC07-523-LS, N6, 3BNC117, 8ANC131, CH103, F105, and DH522 against a panel of HIV-1 isolates in the Luc/TZM-bl neutralization assay. Values represent IC80 in μg/ml.

[0113] FIG. 28B shows the mean IC80 and percent of isolates neutralized at different IC80<50ug/ml values.

[0114] FIGS. 29A-29B show CH235 Lineage, with Time of Appearance and Neutralization by Select Members. (A) Phylogram of CH235 lineage. Phylogenetic tree is colored by first time (wk post-infection) from which sequences were obtained. Key members of the CH235 lineage are labeled. CH235.6, CH235.7, CH235.8 and CH235.9 VH were complemented with full heavy chain gene regions and paired with the VL from the closest natural antibody. (B) Neutralization dendrograms display single mAb neutralization of a genetically diverse panel of 199 HIV-1 isolates. Coloration is by IC50. See also Figures 36A-B, 40C, and 41.

[0115] FIGS. 30A-30E show structures of CH235-Lineage Members in Complex with HIV-1 Env. (A) Co-crystal structures of the antigen-binding fragments (Fabs) of CH235-lineage members with core gp120. Structures are shown in ribbon diagram, with gp120 in gray and residues altered by SHM in stick representation colored by time-of-appearance. (B) Negative stain EM of Fabs of CH235-lineage members and trimeric HIV-1 Env from BG505 (top row) and B41 (bottom row). Structures in surface representation, with Env portions colored gray and Fabs by time-of-appearance. (C) Epitope displayed on the gp120 surface and colored by antibody time-of-appearance, with the vulnerable portion of the CD4bs highlighted in yellow and select regions labeled. (D) Targeting precision of CD4bs-directed antibodies vs neutralization breadth. (E) VH-gene SHM of CD4bs-directed antibodies vs neutralization breadth. See also Figures 37A-G, and 42.

[0116] FIGS. 31A-C show sequence Evolution of CH235 Lineage: SHM, Timing, and Conformity of CH235-Lineage Development from UCA to Antibody with 90% Breadth. (A) Heavy chain SHM over time for the CH235 lineage (left panel). SHM levels of other VH1-46-derived CD4bs mAbs and selected VH1-2-derived VRC01-class mAbs are shown (middle and right panels, respectively); the time since infection is unknown for these mAbs. (B) Maturation conformity vs overall heavy chain SHM. Positional conformity (top row) is defined as the number of aa positions differing from the germline sequence in both the conforming and reference sequences, divided by the total number of aa changes in the conforming antibody. Identity conformity (bottom row) is defined as the number of such positions which are additionally mutated to the same residue, divided by the total number of mutations in the conforming antibody. Conformity to 1B2530 (left) and to 8ANC131 (right) is shown for both position and identity. (C) VH-gene mutability accounts for the majority of positional conformity of CH235 lineage. The mutability of the VH-gene for VH1-46 (top;

SEQ ID NOS 224-229, respectively, in order of appearance) and VH1-2 (bottom; SEQ ID NOS 230-233, respectively, in order of appearance) is shown. Sequence logos are shown at each position; the height of each logo corresponds to the percent of mutated reads. Green bars are shown for SHM in antibody CH235, which are altered in over a quarter of VH1-46-derived antibodies. See also Figures 38A-E, and 43A-C.

[0117] FIGS. 32A-32D show binding Kinetics of CH103 and CH235 Lineage Antibodies. Binding association (ka) and dissociation (kd) rates of the CH103 (A-B, squares) and CH235 (C-D, circles) lineage mAbs to CH505.TF gp120 Env were measured with SPR and used to calculate the dissociation rate constants (Kd). Kds are shown in A and C, ka (solid lines, plotted on the left y-axis) and kd (dashed lines, plotted on the right y-axis) are shown in B and D. See also Figures 44A-B.

[0118] FIGS. 33A-33C show CH235 Lineage Antibodies Neutralization of Autologous Virus and CH505.TF Loop D Mutants. (A) Heatmap analysis of neutralization of 76 pseudoviruses (row) by 16 CH235 lineage mAbs (column). Coloration is by IC50. This analysis extends previous observations on early CH235 lineage antibodies (Gao et al., 2014) by including late mAbs CH235.7, CH235.8, CH235.10, CH235.11, CH235.12 and CH235.13 and by adding pseudoviruses isolated from wk 136 to 323 post-transmission. (B) CH505 TF and loop D mutants M5, M6, M10, M19, M11, M7, M8, M9, M20 and M21 neutralization by CH236 mAb, late mAbs CH235.7, CH235.9 CH235.10, CH235.11, CH235.12, CH235.13 (left panel) and CH235.9 mAb mutants (right panel). Neutralization is expressed as IC50 μg/ml. CH505 TF sequence mutations are shown on the right. (C) The CDR H1 N30 (sticks, dark red) in CH235.9, which interacts with the □20- □21 loop in the bridging sheet of gp120 (cyan), is over 19Å away from the N280S mutation site in loop D (orange). See also Figures 39A-B, 45, and 46.

[0119] FIGS. 34A-34B show binding of CH235 and CH103 Lineage mAbs to Autologous CH505. (A) and CH235 UCA Binding to Heterologous HIV-1 Env Glycoproteins (B). (A) Heatmap analysis of UCA, intermediate (IA) and mature CH235 and CH103 lineage mAbs binding to 113 CH505 autologous Env isolated from time of infection (TF) to 160 wks post-infection and to the CH505.TF mutants (Gao et al., Cell 2014). Mabs were tested in ELISA at concentrations ranging from 100 μg/ml to 0.6 ng/ml . Binding is expressed as a LogAUC. (B) Affinity of CH235 UCA, CH235 wild-type and select SHM variants to a panel of 15 heterologous gp120 Envs. See also Figures 40A-40B and 43.

[0120] FIGS. 35A-35D show CH235 Antibody Lineage Auto- and Polyreactivity. (A) CH235 lineage antibody binding to ANA measured in ELISA. LogAUC was calculated from duplicate samples. Results representative of duplicate experiments. (B) Binding to cardiolipin was determined using Quanta Lite ACA IgG III ELISA Assay. (C) Hep2 cell IF staining. Size bars = 50 μm. (D) Measurement of polyreactivity against 9,400 human antigens using ProtoArray 5 microchip: CH235 lineage mAbs binding (x-axis) was compared to non-polyreactive control mAb 151K (y-axis). Polyreactivity is defined as 1 log stronger binding than 151k mAb to more than 90% of the test proteins. High affinity binding was measured as a >2 log increase in binding (dotted line) (Liu et al., 2015).

[0121] FIGS. 36A-36B show CH235 Lineage: Sequences and Neutralization Fingerprint Dendrogram, Related to Figure 29. (A) Alignment of NGS sequences (SEQ ID NOS 234 and 234-294, respectively, in order of appearance) and antibodies identified from 17 time points from 6 to 323 weeks post-transmission and comparison of mutation patterns to other IGHV1-46 (1B2530 and 8ANC131) and IGHV1-2 (VRC01, VRC-CH31 and VRC-PG04) derived broadly neutralizing antibodies. Antibodies identified from single B cells are shown in bold. The positions mutated in CH235 were color coded based on the time points at which these mutations were firstly observed in the NGS reads. Mutated positions not seen in the NGS data are colored based on the time of isolation of CH235 (41 weeks). IGHV1-46*01 is used as reference for IGHV1-46 derived antibodies and IGHV1-2*02 is used as reference for the three VRC01-class antibodies. (B) The neutralization fingerprints for three antibodies from the CH235 lineage were compared to the fingerprints for other VH1-46 class antibodies and non-VH1-46 class CD4-binding-site antibodies; coloring same as in Figure 31A-C. Antibodies targeting other sites of vulnerability on HIV-1 Env are shown as control (black).

[0122] FIGS. 37A-37G show CH235 Lineage Versus Other CD4-Binding Site Antibodies and Negative-stain EM Reconstructions of gp140 SOSIP Trimers with CH235-lineage Fabs, Related to Figure 30A-E. (A) CD4-mimicry by CH235. Recognition of gp120 by the N-terminal domain of the CD4 receptor (far left) is compared to VH genes from CH235 and prototypic antibodies VRC01 (from VH1-2) and 8ANC131 (from VH1-46). (B) Conserved molecular interactions between antibody CH235, receptor CD4 and antibody VRC01. Top row shows intermolecular antiparallel strand interactions and bottom row Asp368 electrostatic interaction. (C) Binding orientation of VH-gene derived antibodies relative to CD4. (D) Negative-stain EM 3D models with BG505 SOSIP.664. (left) Top and side views of CH235.12 in complex with BG505 SOSIP (purple) aligned to the EM volume of VRC01 in

complex with BG505 (gold mesh; EMD-6252). (middle) Top and side views of the CH235.12-BG505 complex (purple mesh) aligned to the EM volume of CH103 in complex with BG505 SOSIP (gray; EMD-6250). (right) Top and side views of the CH103-BG505 complex (gray mesh) aligned to the EM reconstruction of BG505 SOSIP in complex with soluble CD4 and 17b Fab (blue; EMDB ID 5723). (E) Negative-stain EM of gp140 SOSIP trimers with CH235-lineage Fabs. (F) Top and side views of 3D reconstructions of each complex. (G) Fourier shell correlation curves for each dataset with a resolution estimate using an FSC cutoff of 0.5.

[0123] FIGS. 38A-38E show Sequence Similarity Between VH1-2 and VH1-46 Broadly Neutralizing Antibodies and Mutability of Germline Genes, Related to Figure 31A-C. (A) Amino acid alignment of 8ANC131 (SEQ ID NO: 290) and CH235 (SEQ ID NO: 271) to the IGHV1-46 germline gene (SEQ ID NO: 234) showing the definition of conformity. (B) Probability distribution of the number of sharing mutation positions for each pair of antibodies. (C) Probability distribution of the number of identical mutations for each pair of antibodies. (D) SHM frequency is shown versus VH-gene position for VH1-46, VH1-2 and three others. Sequences were aligned to VH1-46 and positions not aligned to VH1-46 (indels) were removed. (E) Dendrogram showing sequence segregation of VH1-2 and VH1-46 derived broadly neutralizing antibodies, despite similarity of VH1-2 and VH1-46 germline genes shown with underline.

[0124] FIGS. 39A-39B show Generation of CH235.9 Mutants to Evaluate the Effect of Mutations in the V-heavy Chain on the Ability of CH235.9 to Neutralize loop D Mutant CH505 Autologous Viruses, Related to Figure 33A-C. (A) The interaction between CH235 CDR L3 (purple) and N280 in the HIV-1 gp120 Env loop D (orange) from the crystal structure of the CH235-gp120 complex (left panel). Asparagine in position 280 in gp120 forms three hydrogen bonds (yellow dotted lines) with residues in the CDR L3 (left panel). Structural modeling predicted these hydrogen bonds to be disrupted in the N280S (right panel) and N280T (not shown) mutations which occur in autologous CH505 escape mutants. (B) Alignment of CH235.7 and CH235.9 through CH235.13 VH amino acid sequences to CH236 VH (SEQ ID NOs: 171-177 (in order of appearance from CH236-CH235.7)). CH235.9 aa mutations expressed as recombinant IgG and tested for neutralization of CH505 TF loop D mutants are shown in red. Asterisks indicate points of contact with gp120 derived from the CH235 crystal structure in complex with gp120 Env.

[0125] FIGS. 40A-40B show CH505 gp120 Env Quasi-species Selected as Optimized Immunogens to Induce Both CH235 and CH103-like bnAbs, Related to Figure 34A-B. (A) Heatmap of the binding data of selected CH235 and CH103 lineage members to the CH505 Env glycoproteins selected to be used as immunogens. Individual Env clone names and weeks of isolation are shown on the left. (B) Affinity of gHgL of 1B2530, 8ANC131, VRC01, VRC-PG04 and VRC-CH31 to a panel of 15 heterologous gp120 envelope glycoproteins.

[0126] FIG. 40C shows a Table with characteristics of the V(D)J rearrangements of key CH235 lineage antibodies. Related to Figure 29.

[0127] FIG. 41 shows a Table with a summary of the Breadth and Potency of Antibody Neutralization Against 199 HIV-1 Env-Pseudoviruses. Related to Figure 29.

[0128] FIG. 42 shows a Table with crystallographic Data Collection and Refinement Statistics. Related to Figures 30A-30E.

[0129] FIGS. 43A-43C show Sequence Similarity Between VH1-2 and VH1-46 Broadly Neutralizing Antibodies and Mutability of Germline Genes. Related to Figures 31A-31C. (A) The probability of a conforming VH1-46 antibody with x VH mutations, having c common mutation positions with a reference antibody were estimated based on 100,000 simulated events, with the likelihood of each residue being mutated based on uniform distribution (position) (Puniform), or the mutation frequency at each residue position derived from the VH1-46 antibodies (PVH1-46). (B) The probability of a conforming VH1-46 antibody with x VH mutations, having i identical mutations with a reference antibody were estimated based on 100,000 simulated events, with the likelihood of each residue being mutated based on uniform distribution (position and mutation type) (Puniform), or the mutation frequency at each residue position derived from the VH1-46 antibodies (PVH1-46). (C) Pearson correlation coefficients of positional somatic mutation frequency between VH1-46, VH1-2 and three others.

[0130] FIGS. 44A-44B show CH235 Lineage and CH106 Monoclonal Antibodies Cross-Blocking. Related to Figure 32. (A) CH235 lineage antibodies blocking of sCD4 and CH106 binding to CH505 TF gp120 and B.63521 gp120 Envs. Results expressed as IC50 ug/ml. nb= no blocking. (B) Monoclonal antibody CH106 blocking of CH235 lineage antibodies to CH505 TF gp120. Results expressed as IC50 ug/ml. nb= no blocking.

[0131] FIG. 45 shows CH235 lineage autologous neutralization. Related to Figure 33A-C.

[0132] FIG. 46 shows CH235 lineage antibodies and CH235.9 mutants neutralization of CH505 TF loop D mutant viruses. Related to Figure 33A-C.

[0133] FIG. 47 shows binding of antibodies in the CH235 and CH103 lineages to CH505 autologous Env glycoproteins, Related to Figure 34A-B.

[0134] Figure 48 shows levels of GFP expression detected with each Doxycycline concentration in each staining condition of the examined bispecific molecules (Example 10C).

[0135] Figure 49 shows levels of GFP expression detected with each Doxycycline concentration in each staining condition of the control molecules (Example 10C).

[0136] Figure 50 shows analysis of A32x4420 and CH557xhXR32 staining to the 491 control and 598 cell lines (Example 10C).

[0137] Figure 51 shows analysis of control antibodies staining to the 491 control and 598 cell lines (Example 10C).

DETAILED DESCRIPTION

[0138] 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.

[0139] 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).

[0140] 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.*, Fcγ receptors (FcγR), 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).

[0141] 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).

[0142] 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).

[0143] 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 Fcgamma 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.

[0144] 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.

[0145] 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.*).

[0146] 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-CD 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.

[0147] 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 Songsivilai 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.

[0148] 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.

[0149] 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).

[0150] 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.

[0151] 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.

[0152] 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.

[0153] 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.

[0154] 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.

[0155] 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.

[0156] 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: 555, 557, 559, 561, 562, 563, 596, or 597.

[0157] 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: 500-597.

[0158] 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.

[0159] In some embodiments, antigen-binding fragment of an antibody is a portion of an antibody that possesses an at least one antigen recognition site. Fragments include for example but not limited to, Fab, Fab', $F(ab')_2$ Fv), and single chain (scFv).

[0160] 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.*).

[0161] 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.

[0162] 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.

[0163] 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.)

[0164] Multispecific molecules

[0165] Various non-limiting multispecific molecule designs are provides in Figures 1-9.

[0166] 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 bind 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.

[0167] 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.

[0168] 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.

[0169] 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 V3 glycan and/or a CD4 binding site 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 1** and **3A-3B)**. In certain embodiments, a bispecific molecule comprises more than two epitope-binding sites (see, *e.g.*, **Figures 2A-2C**). 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*.

[0170] 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 aV3 glycan or a CD4 binding site 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 4A-4F)**. 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*.

[0171] In some embodiments, such molecules comprise two polypeptide chains, wherein each of the two polypeptide chains comprises three Domains (**Figure 1**). 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.

[0172] 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.

[0173] 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: $VL_{\text{First Antigen}}$ – Peptide Linker 1 – $VH_{\text{Second Antigen}}$ – Peptide Linker 2 – Heterodimerization-Promoting Domain.

[0174] The terms VL/VH for first and second antigens, $VL_{\text{first antigen}}/VH_{\text{first antigen}}$ $VL_{\text{second antigen}}/VH_{\text{second antigen}}$, and VL1/VH1 and VL2/VH2 are used interchangeably throughout the application.

[0175] 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: $VL_{\text{Second Antigen}}$ – Peptide Linker 1 – $VH_{\text{First Antigen}}$ – Peptide Linker 2 – Heterodimerization-Promoting Domain.

[0176] 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.

[0177] 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.

[0178] 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.

[0179] 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).

[0180] 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-Rodriquez, 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 a'--a--a' 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).

[0181] 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.

[0182] 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.

[0183] 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 (KVAALKE-KVAALKE-KVAALKE-KVAALKE)), 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: **E**VAA**C**E**K**-**E**VAAL**E**K-**E**VAAL**E**K-**E**VAAL**E**K (**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: **K**VAA**C**K**K**E-**K**VAAL**K**E-**K**VAAL**K**E-**K**VAAL**K**E (**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.

[0184] 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.

[0185] 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.

[0186] 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.

[0187] 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).

[0188] 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.

[0189] 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.

[0190] 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 1-9.

[0191] 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)).

[0192] 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.

[0193] 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.

[0194] 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-Fc.gamma.R interaction, complement binding, etc.).

[0195] As provided in **Figures 2-3**, 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 in herein incorporated by reference in its entirety).

[0196] 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	2nd Chain	NH_2-VL2-VH1-Heterodimer-Promoting Domain-COOH
	1st Chain	NH_2-VL1-VH2-Heterodimer-Promoting Domain-CH2-CH3-COOH
	1st Chain	NH_2-VL1-VH2-Heterodimer-Promoting Domain-CH2-CH3-COOH
	2nd Chain	NH_2-VL2-VH1-Heterodimer-Promoting Domain-COOH
Tetraspecific	2nd Chain	NH_2-VL2-VH1-Heterodimer-Promoting Domain-COOH
	1st Chain	NH_2-VL1-VH2-Heterodimer-Promoting Domain-CH2-CH3-COOH
	3rd Chain	NH_2-VL3-VH4-Heterodimer-Promoting Domain-CH2-CH3-COOH
	4th Chain	NH_2-VL4-VH3-Heterodimer-Promoting Domain-COOH

[0197] 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 2A-2C**), 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.

[0198] As shown in **Figures 2A-2C**, 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 2A-2C** 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.

[0199] 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	3rd Chain	NH_2-CH2-CH3-COOH
	1st Chain	NH_2-VL1-VH2-Heterodimer Promoting Domain-CH2-CH3-COOH
	2nd Chain	NH_2-VL2-VH1-Heterodimer Promoting Domain-COOH
Second Orientation	3rd Chain	NH_2-CH2-CH3-COOH
	1st Chain	NH_2-CH2-CH3- VL1-VH2-Heterodimer Promoting Domain-COOH
	2nd Chain	NH_2-VL2-VH1-Heterodimer Promoting Domain-COOH

[0200] 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 3A-3B**), 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.

[0201] As shown in **Figure 3A**, 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: $VL_{\text{First Antigen}}$ – Peptide Linker 1 – $VH_{\text{Second Antigen}}$ – Peptide Linker 2 – Heterodimerization-Promoting Domain – Spacer-Linker 3 – Fc Domain.

[0202] Alternatively, as shown in **Figure 3B**, 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** – $VL_{\text{First Antigen}}$ – Peptide Linker 1 – $VH_{\text{Second Antigen}}$ – Peptide Linker 2 – Heterodimerization-Promoting Domain.

[0203] 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: $VL_{\text{Second Antigen}}$ – Peptide Linker 1 – $VH_{\text{First Antigen}}$ – Peptide Linker 2 – Heterodimerization-Promoting Domain.

[0204] 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").

[0205] 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).

[0206] 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), FcγRIIIA (CD16a) or FcγRIIIB (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.).

[0207] 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.

[0208] 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
LPPSREEMTK NQVSLTCLVK GFYPSDIAVE WESNGQPENN YKTTPPVLDS DGSFFLYSKL
TVDKSRWQQG NVFSCSVMHE ALHNHYTQKS LSLSPGK

[0209] 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**).

[0210] 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.

[0211] 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.

[0212] 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**):

APE**AA**GGPSV FLFPPKPKDT LMISRTPEVT CVVVDVSHED PEVKFNWYVD GVEVHNAKTK
PREEQYNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKALPA PIEKTISKAK GQPREPQVYT
LPPSREEMTK NQVSL**W**C**L**VK GFYPSDIAVE WESNGQPENN YKTTPPVLDS DGSFFLYSKL
TVDKSRWQQG NVFSCSVMHE ALHN**H**YTQKS LSLSPGK

[0213] 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**):

APE**AA**GGPSV FLFPPKPKDT LMISRTPEVT CVVVDVSHED PEVKFNWYVD GVEVHNAKTK
PREEQYNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKALPA PIEKTISKAK GQPREPQVYT
LPPSREEMTK NQVSL**S**C**A**VK GFYPSDIAVE WESNGQPENN YKTTPPVLDS DGSFFL**V**SKL
TVDKSRWQQG NVFSCSVMHE ALHN**R**YTQKS LSLSPGK

[0214] 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**).

[0215] 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.

[0216] 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 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.

[0217] 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).

[0218] 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.

[0219] 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 4A-4F** 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.

[0220] 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 4A-4F**, 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.

[0221] Typically, the trivalent binding molecules of the present invention will comprise four different polypeptide chains (see **Figures 4A-4B**), 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 4C-4F** illustrate this aspect of the present invention by schematically depicting such molecules having three polypeptide chains. As provided in **Figures 4A-4F**, the trivalent binding molecules of the present invention may have alternative orientations in which the Diabody-Type Binding Domains are N-terminal (**Figures 4A**, **4C** and **4D**) or C-terminal (**Figures 4B**, **4E** and **4F**) to an Fc Domain.

[0222] 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 4A and 4B**). 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.

[0223] 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.

[0224] 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**).

[0225] 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.

[0226] 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.

[0227] The general structure of the polypeptide chains of representative trivalent binding molecules of invention is provided in **Figures 4A-4F** and in **Table 3**:

Table 3

Four Chain 1st Orientation	2nd Chain	NH_2-VL2-VH1-HPD-COOH
	1st Chain	NH_2-VL1-VH2-HPD-CH2-CH3-COOH
	3rd Chain	NH_2-VH3-CH1-CH2-CH3-COOH
	2nd Chain	NH_2-VL3-CL-COOH
Four Chain 2nd Orientation	2nd Chain	NH_2-VL2-VH1-HPD-COOH
	1st Chain	NH_2-CH2-CH3-VL1-VH2-HPD-COOH
	3rd Chain	NH_2-VH3-CH1-CH2-CH3-COOH
	2nd Chain	NH_2-VL3-CL-COOH
Three Chain 1st Orientation	2nd Chain	NH_2-VL2-VH1-HPD-COOH
	1st Chain	NH_2-VL1-VH2-HPD-CH2-CH3-COOH
	3rd Chain	NH_2-VL3-VH3-HPD-CH2-CH3-COOH
Three Chain 2nd Orientation	2nd Chain	NH_2-VL2-VH1-HPD-COOH
	1st Chain	NH_2-CH2-CH3-VL1-VH2-HPD-COOH
	3rd Chain	NH_2-VL3-VH3-HPD-CH2-CH3-COOH

HPD = Heterodimer-Promoting Domain

[0228] 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.

[0229] 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.

[0230] 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.

[0231] 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.

[0232] 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.

[0233] 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.

[0234] 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.

[0235] 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.

[0236] 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, inonomycin, or any combination thereof. See Bullen et al. Nature Medicine 20, 425–429 (2014).

[0237] In certain embodiments the multispecific molecules of the invention can be administered in combination with anti-retroviral agents.

[0238] 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.

[0239] 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.

[0240] 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.

[0241] 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.

[0242] 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.

[0243] 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.

[0244] 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) (amino acid sequence)	EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPGKGLEWV GRIRSKYNNYATYYADSVKGRFTISRDDSKNSLYLQMNSLKTEDTAVY YCVRHGNFGNSYVSWFAYWGQGTLVTVSS

501	hCD3 VH (hXR32VH) (nucleotide sequence)	gaggtgcagctggtggagtctgggggaggcttggtccagcctggaggg tccctgagactctcctgtgcagcctctggattcaccttcagcacatac gctatgaattgggtccgccaggctccagggaaggggctggagtgggtt ggaaggatcaggtccaagtacaacaattatgcaacctactatgccgac tctgtgaagggtagattcaccatctcaagagatgattcaaagaactca ctgtatctgcaaatgaacagcctgaaaaccgaggacacggccgtgtat tactgtgtgagacacggtaacttcggcaattcttacgtgtcttggttt gcttattggggacaggggacactggtgactgtgtcttcc
502	hCD3 VL (hXR32VL) (amino acid sequence)	QAVVTQEPSLTVSPGGTVTLTCRSSTGAVTTSNYANWVQQKPGQAPRG LIGGTNKRAPWTPARFSGSLLGGKAALTITGAQAEDEADYYCALWYSN LWVFGGGTKLTVL
503	hCD3 VL (hXR32VL) (nucleotide sequence)	caggctgtggtgactcaggagccttcactgaccgtgtccccaggcgga actgtgaccctgacatgcagatccagcacaggcgcagtgaccacatct aactacgccaattgggtgcagcagaagccaggacaggcaccaaggggc ctgatcgggggtacaaacaaaagggctccctggacccctgcacggttt tctggaagtctgctgggcggaaaggccgctctgactattaccggggca caggccgaggacgaagccgattactattgtgctctgtggtatagcaat ctgtgggtgttcgggggtggcacaaaactgactgtgctg
504	hCD16 VH (h3G8VH5) (amino acid sequence)	QVTLRESGPALVKPTQTLTLTCTFSGFSLSTSGMGVGWIRQPPGKALE WLAHIWWDDDKRYNPALKSRLTISKDTSKNQVVLTMTNMDPVDTATYY CAQINPAWFAYWGQGTLVTVSS
505	hCD16 VH (h3G8VH5) (nucleotide sequence)	caggttaccctgagagagtctggccctgcgctggtgaagcccacacag accctcacactgacttgtaccttctctgggttttcactgagcacttct ggtatgggtgtaggctggattcgtcagcctcccgggaaggctctagag tggctggcacacatttggtgggatgatgacaagcgctataatccagcc ctgaagagccgactgacaatctccaaggatacctccaaaaaccaggta gtcctcacaatgaccaacatggaccctgtggatactgccacatactac tgtgctcaaataaaccccgcctggtttgcttactggggccaagggact ctggtcactgtgagctccgg
506	hCD16 VL (h3G8VL1) (amino acid sequence)	DIVMTQSPDSLAVSLGERATINCKASQSVDFDGDSFMNWYQQKPGQPP KLLIYTTSNLESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQSN EDPYTFGQGTKLEIK

507	hCD16 VL (h3G8VL1) (nucleotide sequence)	atgggtgacaatgacatccactttgcctttctctccacaggtgtccac tccgacatcgtgatgacccaatctccagactctttggctgtgtctcta ggggagagggccaccatcaactgcaaggccagccaaagtgttgatttt gatggtgatagttttatgaactggtaccaacagaaaccaggacagcca cccaaactcctcatctatactacatccaatctagaatctggggtccca gacaggtttagtggcagtgggtctgggacagacttcaccctcaccatc agcagcctgcaggctgaggatgtggcagtttattactgtcagcaaagt aatgaagatccgtacacgttcggacaggggaccaagcttgagatcaaa
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	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTS GVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDK RVEPKSCDKTHTCPPCP
516	Ig CL Kappa Domain	RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQ SGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSS PVTKSFNRGEC
517	Ig CL Lambda Domain	QPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPV KAGVETTPSKQSNNKYAASSYLSLTPEQWKSHRSYSCQVTHEGSTVEK TVAPTECS
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	GGGGSGGGGSGGGGS
527	IgG1 Fc WT	APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWY VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK ALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYP SDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNV FSCSVMHEALHNHYTQKSLSLSPG**X** wherein, X is a lysine (K) or is absent
528	IgG1 Fc (AA)	APE**AA**GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWY VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK ALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYP SDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNV FSCSVMHEALHNHYTQKSLSLSPG**X** wherein, X is a lysine (K) or is absent
529	IgG1 Fc YTE	APELLGGPSVFLFPPKPKDTL**Y**I**T**R**E**PEVTCVVVDVSHEDPEVKFNWY VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK ALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYP SDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNV FSCSVMHEALHNHYTQKSLSLSPG**X** wherein, X is a lysine (K) or is absent
530	IgG1 Fc (AA/YTE)	APE**AA**GGPSVFLFPPKPKDTL**Y**I**T**R**E**PEVTCVVVDVSHEDPEVKFNWY VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK ALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYP SDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNV FSCSVMHEALHNHYTQKSLSLSPG**X** wherein, X is a lysine (K) or is absent

531	IgG1 Fc Knob (AA)	APEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWY VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK ALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSL**W**CLVKGFYP SDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNV FSCSVMHEALHNHYTQKSLSLSPG**X** wherein, X is a lysine (K) or is absent
532	IgG1 Fc Knob (AA/YTE)	APEAAGGPSVFLFPPKPKDTL**Y**I**T**R**E**PEVTCVVVDVSHEDPEVKFNWY VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK ALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSL**W**CLVKGFYP SDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNV FSCSVMHEALHNHYTQKSLSLSPG**X** wherein, X is a lysine (K) or is absent
533	IgG1 Fc Hole (AA/R)	APEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWY VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK ALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSL**S**C**A**VKGFYP SDIAVEWESNGQPENNYKTTPPVLDSDGSFFL**V**SKLTVDKSRWQQGNV FSCSVMHEALHN**R**YTQKSLSLSPG**X** wherein, X is a lysine (K) or is absent
534	IgG1 Fc Hole (AA/R/YTE)	APEAAGGPSVFLFPPKPKDTL**Y**I**T**R**E**PEVTCVVVDVSHEDPEVKFNWY VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK ALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSL**S**C**A**VKGFYP SDIAVEWESNGQPENNYKTTPPVLDSDGSFFL**V**SKLTVDKSRWQQGNV FSCSVMHEALHN**R**YTQKSLSLSPG**X** wherein, X is a lysine (K) or is absent
535	Pali VH (amino acid sequence)	QVTLRESGPALVKPTQTLTLTCTFSGFSLSTSGMSVGWIRQPPGKALE WLADIWWDDKKDYNPSLKSRLTISKDTSKNQVVLKVTNMDPADTATYY CARSMITNWYFDVWGAGTTVTVSS
536	Pali VH (nucleotide sequence)	caggttacacttagagaatctggtcctgctcttgtgaagcctactcag actcttactcttacctgcaccttctctggtttcagcctttctacttct ggtatgtctgtgggttggattagacaacctcctggtaaggctcttgaa tggcttgctgatatttggtgggatgataagaaggattacaacccaagc ctgaagtctaggctgaccatttctaaggataccagcaagaatcaggtg gtgcttaaggtgaccaatatggaccctgctgatactgctacttactac tgcgctagatccatgatcaccaactggtactttgatgtttggggtgct ggtactactgtgaccgtttcttcc

537	Pali VL (amino acid sequence)	DIQMTQSPSTLSASVGDRVTITCRASQSVGYMHWYQQKPGKAPKLLIY DTSKLASGVPSRFSGSGSGTEFTLTISSLQPDDFATYYCFQGSGYPFT FGGGTKLEIK
538	Pali VL (nucleotide sequence)	gatattcagatgactcagtctccttctaccctgtctgcttctgttggt gatagggttaccattacatgcagggcttctcaatctgtgggttacatg cactggtaccaacaaaagcctggtaaggctcctaagctgcttatctac gatacctctaagcttgcttctggtgtgccttctaggttttctggttct ggatctggtactgagttcacccttaccatttctagccttcagcctgat gatttcgctacctactattgctttcagggtagcggttacccttcact tttggtggtggtactaagcttgagattaag
539	A32 VH (amino acid sequence)	QVQLQESGPGLVKPSQTLSLSCTVSGGSSSSGAHYWSWIRQYPGKGLE WIGYIHYSGNTYYNPSLKSRITISQHTSENQFSLKLNSVTVADTAVYY CARGTRLRTLRNAFDIWGQGTLVTVSS
540	A32 VH (nucleotide sequence)	caggtgcagctgcaggagtccggccccggactggtcaaaccctctcag actctgtctctgtcatgtaccgtgtcaggcggctcttccagctccggg gcacactactggagctggatcaggcagtatcccggcaaggggctggag tggatcggatacattcattatagcggcaacacatactataatccttct ctgaagagtcggatcactatttcacagcacaccagcgaaaaccagttc agcctgaagctgaacagcgtgaccgtcgccgacacagccgtgtactat tgcgcccggggcaccagactgagaactctgagaaacgcatttgacatc tggggacaggggacactggtgacagtgagctcc
541	A32 VL (amino acid sequence)	QSALTQPPSASGSPGQSVTISCTGTSSDVGGYNYVSWYQHHPGKAPKL IISEVNNRPSGVPDRFSGSKSGNTASLTVSGLQAEDEAEYYCSSYTDI HNFVFGGGTKLTVL
542	A32 VL (nucleotide sequence)	cagagcgcactgactcagccccttccgcctccgggtctcctggacag agcgtgacaatctcatgcactgggacttcaagcgatgtgggcgggtac aactatgtgagttggtaccagcaccatcccgggaaggcacctaaactg atcattagcgaagtgaacaatcgaccaagcggcgtccccgaccggttc agcggcagcaagtctggcaataccgccagtctgacagtctcaggcctg caggccgaggatgaagctgagtactattgctcatcatacactgacatc cataacttcgtcttcggcggcggaactaaactgaccgtgctg
543	OKT8 VH (CD8 VH) (amino acid sequence)	QVQLLESGPELLKPGASVKMSCKASGYTFTDYNMHWVKQSHGKSLEWI GYIYPYTGGTGYNQKFKNKATLTVDSSSSTAYMELRSLTSEDSAVYYC ARNFRYTYWYFDVWGQGTTVTVSS

544	OKT8 VH (CD8 VH) (nucleotide sequence)	caggtgcagctgttggagtctggacctgagctgttgaaacctggggcc tcagtgaagatgtcctgcaaggcttctggatacacattcactgactac aacatgcactgggtgaagcagagccatggaaagagccttgagtggatt ggatatatttatccttacactggtggcactggctacaaccagaagttc aagaacaaggccacattgactgtagacagttcctccagcacagcctac atggagctccgcagcctgacatctgaggactctgcagtctattactgt gcaaggaactttaggtacacctactggtacttcgatgtctggggccaa ggcaccacggtcaccgtctcctca
545	OKT8 VL (CD8 VL) (amino acid sequence)	DIVMTQSPASLAVSLGQRATISCRASESVDSYDNSLMHWYQQKPGQPP KVLIYLASNLESGVPARFSGSGSRTDFTLTIDPVEADDAATYYCQQNN EDPYTFGGGTKLEIK
546	OKT8 VL (CD8 VL) (nucleotide sequence)	gacatcgtgatgacccagtctccagcttctttggctgtgtctctaggg cagagggccaccatatcctgcagagccagtgaaagtgttgatagttat gacaatagtcttatgcactggtaccagcagaaaccaggacagccaccc aaagtcctcatctatcttgcatccaacctagaatctggggtccctgcc aggttcagtggcagtgggtctaggacagacttcaccctcaccattgat cctgtggaggctgatgatgctgcaacctattactgtcagcaaaataat gaggatccgtacacgttcgggggggggaccaagctggagatcaaacgt
547	TRX2 VH (CD8 VH) (amino acid sequence)	QVQLVESGGGVVQPGRSLRLSCAASGFTFSDFGMNWVRQAPGKGLEWV AL IYYDGSNKFYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAK PHYDGYYHFFDSWGQGTLVTVSS
548	TRX2 VH (CD8 VH) (nucleotide sequence)	caggttcaattggtggagtctggaggaggcgttgtacagcctggaagg tccctgagactctcatgtgcagcttctggattcactttcagtgacttt ggcatgaactgggttcgacaggctcccgggaaggggctggaatgggtg gcactgatttactatgatggtagtaacaagttctatgcagactctgtg aagggtcgattcaccatctccagggacaattctaagaacaccctatac ctgcaaatgaacagcctgagagctgaggacacagccgtgtattactgt gcaaaaccccactatgatggttattatcacttctttgattcctggggc caagggacactagtcacagtctcctca
549	TRX2 VL (CD8 VL) (amino acid sequence)	DIQMTQSPSSLSASVGDRVTITCKGSQDINNYLAWYQQKPGKAPKLLI YNTDILHTGVPSRFSGSGSGTDFTFTISSLQPEDIATYYCYQYNNGYT FGQGTKVEIK

550	TRX2 VL (CD8 VL) (nucleotide sequence)	gacatccagatgacccagagcccaagcagcctgagcgccagcgtgggt gacagagtgaccatcacctgtaaaggaagtcaggatattaacaattac ttagcctggtaccagcagaagccaggtaaggctccaaagctgctgatc tacaatacagacattttgcacacgggtgtgccaagcagattcagcggt agcggtagcggtaccgacttcaccttcaccatcagcagcctccagcca gaggacatcgccacctactactgctatcagtataacaacgggtacacg ttcggccaagggaccaaggtggaaatcaaa
551	CH557 VH (amino acid sequence)	See Figure 12 (SEQ ID NO: 124)
552	CH557 VH (nucleotide sequence)	See Figure 12 (SEQ ID NO: 126)
553	CH557 VL (amino acid sequence)	See Figure 12 (SEQ ID NO: 125)
554	CH557 VL (nucleotide sequence)	See Figure 12 (SEQ ID NO: 127)
555	CH557xCD3 Fc CHAIN 1 (amino acid sequence)	EIVLTQSPATLSASPGERVTLTCRASRSVRNNVAWYQHKGGQSPRLLI YDASTRAAGVPARFSGSASGTEFTLAISNLESEDFTVYFCLQYNNWWT FGQGTRVDIKGGGSGGGGEVQLVESGGGLVQPGGSLRLSCAASGFTFS TYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRFTISRDDSK NSLYLQMNSLKTEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSA STKGEVAACEKEVAALEKEVAALEKEVAALEKGGGDKTHTCPPCPAPE AAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDG VEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP APIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLWCLVKGFYPSDI AVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSC SVMHEALHNHYTQKSLSLSPGK

556	CH557xCD3 Fc CHAIN 1 (nucleotide sequence)	gaaattgtgttgacgcagtctccagccaccctgtccgcgtctccaggg gaaagagtcaccctaacttgcagggccagtcggagtgtccgaaacaac gtggcctggtatcagcacaagggtggccagagtcccaggctcctcatt tatgatgcgtccacgagggccgctggtgtcccagccaggttcagcggc agtgcatctgggacagagttcactctcgccatcagcaacttggagtct gaagattttacagtctacttctgtctgcagtataataactggtggacc ttcggccaagggaccagggtggacatcaaaggtggaggatccggcggc ggaggcgaggtgcagctggtggagtctgggggaggcttggtccagcct ggagggtccctgagactctcctgtgcagcctctggattcaccttcagc acatacgctatgaattgggtccgccaggctccagggaaggggctggag tgggttggaaggatcaggtccaagtacaacaattatgcaacctactat gccgactctgtgaagggtagattcaccatctcaagagatgattcaaag aactcactgtatctgcaaatgaacagcctgaaaaccgaggacacggcc gtgtattactgtgtgagacacggtaacttcggcaattcttacgtgtct tggtttgcttattggggacaggggacactggtgactgtgtcttccgcc tccaccaagggcgaagtggccgcatgtgagaaagaggttgctgctttg gagaaggaggtcgctgcacttgaaaaggaggtcgcagccctggagaaa ggcggcggggacaaaactcacacatgcccaccgtgcccagcacctgaa gccgcgggggggaccgtcagtcttcctcttcccccaaaacccaaggac accctcatgatctcccggacccctgaggtcacatgcgtggtggtggac gtgagccacgaagaccctgaggtcaagttcaactggtacgtggacggc gtggaggtgcataatgccaagacaaagccgcgggaggagcagtacaac agcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactgg ctgaatggcaaggagtacaagtgcaaggtctccaacaaagccctccca gcccccatcgagaaaaccatctccaaagccaaagggcagccccgagaa ccacaggtgtacaccctgcccccatcccgggaggagatgaccaagaac caggtcagcctgtggtgcctggtcaaaggcttctatcccagcgacatc gccgtggagtgggagagcaatgggcagccggagaacaactacaagacc acgcctcccgtgctggactccgacggctccttcttcctctacagcaag ctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgc tccgtgatgcatgaggctctgcacaaccactacacgcagaagagcctc tccctgtctccgggtaaa
557	CH557xCD3 Fc CHAIN 2 (amino acid sequence)	QAVVTQEPSLTVSPGGTVTLTCRSSTGAVTTSNYANWVQQKPGQAPRG LIGGTNKRAPWTPARFSGSLLGGKAALTITGAQAEDEADYYCALWYSN LWVFGGGTKLTVLGGGGSGGGGQVRLAQYGGGVKRLGATMTLSCVASG YTFNDYYIHWVRQAPGQGFELLGYIDPANGRPDYAGALRERLSFYRDK SMETLYMDLRSLRYDDTAMYYCVRNVGTAGSLLHYDHWGSGSPVIVSS ASTKGKVAACKEKVAALKEKVAALKEKVAALKE

558	CH557xCD3 Fc CHAIN 2 (nucleotide sequence)	caggctgtggtgactcaggagccttcactgaccgtgtccccaggcgga actgtgaccctgacatgcagatccagcacaggcgcagtgaccacatct aactacgccaattgggtgcagcagaagccaggacaggcaccaaggggc ctgatcgggggtacaaacaaaagggctccctggacccctgcacggttt tctggaagtctgctgggcggaaaggccgctctgactattaccggggca caggccgaggacgaagccgattactattgtgctctgtggtatagcaat ctgtgggtgttcgggggtggcacaaaactgactgtgctgggaggggt ggatccggcggaggtggacaggtccgactagcccaatatggtggtggg gtgaagaggctaggggccacaatgacccttcctgcgtggcatctgga tacaccttcaacgactactacatacattgggtgcggcaggcccctgga caaggctttgagttgttgggatacatcgaccccgctaatggtcgccca gactacgcaggggcgttgagggagagactctccttctacagggacaag tccatggagacgctgtacatggacctgaggagcctaagatatgacgac acggccatgtattattgtgttagaaatgtggggaccgctggcagcttg ctgcattatgaccactggggctcgggaagcccggtcatcgtctcctcc gcctccaccaagggcaaagtggccgcatgtaaggagaaagttgctgct ttgaaagagaaggtcgccgcacttaaggaaaaggtcgcagccctgaaa gag
559	CH557xCD3 Fc or DH542xCD3 Fc CHAIN 3 (amino acid sequence)	DKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSH EDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVS LSCAVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTV DKSRWQQGNVFSCSVMHEALHNRYTQKSLSLSPGK
560	CH557xCD3 Fc CHAIN 3 (nucleotide sequence)	Gacaaaactcacacatgcccaccgtgcccagcacctgaagccgcgggg ggaccgtcagtcttcctcttcccccaaaacccaaggacaccctcatg atctcccggaccctgaggtcacatgcgtggtggtggacgtgagccac gaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtg cataatgccaagacaaagccgcgggaggagcagtacaacagcacgtac cgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggc aaggagtacaagtgcaaggtctccaacaaagccctcccagccccatc gagaaaaccatctccaaagccaaagggcagccccgagaaccacaggtg tacaccctgcccccatcccgggaggagatgaccaagaaccaggtcagc ctgagttgcgcagtcaaaggcttctatcccagcgacatcgccgtggag tgggagagcaatgggcagccggagaacaactacaagaccacgcctccc gtgctggactccgacggctccttcttcctcgtcagcaagctcaccgtg gacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatg catgaggctctgcacaaccgctacacgcagaagagcctctccctgtct ccgggtaaa

561	Chain 3 of exemplary four chain trivalent binding molecule (amino acid sequence)	EVQLQQSGAELVKPGASVKLSCTASGFNIKDTYIHFVRQRPEQGLEWI GRIDPANDNTLYASKFQGKATITADTSSNTAYMHLCSLTSGDTAVYYC GRGYGYYVFDHWGQGTTLTVSSASTKGPSVFPLAPSSKSTSGGTAALG CLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSS SLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPEAAGGPS VFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNA KTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKT ISKAKGQPREPQVYTLPPSREEMTKNQVSLSCAVKGFYPSDIAVEWES NGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEA LHNRYTQKSLSLSPG
562	Chain 4 of exemplary four chain trivalent binding molecule (amino acid sequence)	DVQINQSPSFLAASPGETITINCRTSRSISQYLAWYQEKPGKTNKLLI YSGSTLQSGIPSRFSGSGSGTDFTLTISGLEPEDFAMYYCQQHNENPL TFGAGTKLELRRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREA KVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVY ACEVTHQGLSSPVTKSFNRGEC
563	Chain 3 of exemplary three chain trivalent binding molecule (amino acid sequence)	DVQINQSPSFLAASPGETITINCRTSRSISQYLAWYQEKPGKTNKLLI YSGSTLQSGIPSRFSGSGSGTDFTLTISGLEPEDFAMYYCQQHNENPL TFGAGTKLELRGGGGSGGGGSGGGGSEVQLQQSGAELVKPGASVKLSC TASGFNIKDTYIHFVRQRPEQGLEWIGRIDPANDNTLYASKFQGKATI TADTSSNTAYMHLCSLTSGDTAVYYCGRGYGYYVFDHWGQGTTLTVSS ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTS GVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDK RVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTC VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVL HQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREE MTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFF LVSKLTVDKSRWQQGNVFSCSVMHEALHNRYTQKSLSLSPG
564	CH556 VH (amino acid sequence)	See Fig. 15A (SEQ ID NO 138)
565	CH556VL (amino acid sequence)	See Fig. 158 (SEQ ID NO 149)

566	CH555VH (amino acid sequence)	See Fig. 15A (SEQ ID NO 136)
567	CH555VL (amino acid sequence)	See Fig. 15B (SEQ ID NO 148)
568	CH493VH (amino acid sequence)	See Fig. 18 (SEQ ID NO 162)
569	CH493VH (nucleotide sequence)	See Fig. 19 (SEQ ID NO 168)
570	CH493VL* (amino acid sequence)	See Fig. 18 (SEQ ID NO 163) *CH493VL is VL from CH236
571	CH493VL* (nucleotide sequence)	See Fig. 19 (SEQ ID NO 169) *CH493VL is VL from CH236
572	CH492VH (amino acid sequence)	See Fig. 18 (SEQ ID NO 161)
573	CH492VH (nucleotide sequence)	See Fig. 19 (SEQ ID NO 167)

574	CH492VL* (amino acid sequence)	See Fig. 18 (SEQ ID NO 164) *CH492VL is VL from CH241
575	CH492VL* (nucleotide sequence)	See Fig. 19 (SEQ ID NO 170) *CH492VL is VL from CH241
576	CH491VH (amino acid sequence)	See Fig. 18 (SEQ ID NO 160)
577	CH491VH (nucleotide sequence)	See Fig. 19 (SEQ ID NO 166)
578	CH491VL* (amino acid sequence)	See Fig. 18 (SEQ ID NO 163) *CH491VL is VL from CH236
579	CH491VL* (nucleotide sequence)	See Fig. 19 (SEQ ID NO 169) *CH491VL is VL from CH236
580	CH490VH (amino acid sequence)	See Fig. 18 (SEQ ID NO 159)
581	CH490VH (nucleotide sequence)	See Fig. 19 (SEQ ID NO 165)

582	CH490VL* (amino acid sequence)	See Fig. 18 (SEQ ID NO 164) *CH490VL VL from CH241
583	CH490VL* (nucleotide sequence)	See Fig. 19 (SEQ ID NO 170) *CH490VL is VL from CH241
584	DH542VH (amino acid sequence)	See Fig. 1 (SEQ ID NO 3)
585	DH542VH (nucleotide sequence)	See Fig. 1 (SEQ ID NO 1)
586	DH542VL (amino acid sequence)	See Fig. 1 (SEQ ID N0 4)
587	DH542VL (nucleotide sequence)	See Fig. 1 (SEQ ID NO 2)
588	DH542_QSAVH (amino acid sequence)	See Ex.10 (SEQ ID NO 179)

589	DH542_QSAVH (nucleotide sequence)	See Ex.10 (SEQ ID NO 178)
590	DH542_QSAVL (amino acid sequence)	See Ex.10 (SEQ ID NO 181)
591	DH542_QSAVL (nucleotide sequence)	See Ex.10 (SEQ ID NO 180)
592	DH542_L4VH* (amino acid sequence)	See Fig. 1 (SEQ ID NO 3) *DH542_L4VH is DH542VH
593	DH542_L4VH* (nucleotide sequence)	See Fig. 1 (SEQ ID NO 1) *DH542_L4VH is DH542VH
594	DH542_L4VL* (amino acid sequence)	See Fig. 2 (SEQ ID NO 51) *DH542_L4VL is VL of DH429
595	DH542_L4VL* (nucleotide sequence)	See Fig. 2 (SEQ ID NO 39) *DH542_L4VL is VL of DH429

596	DH542xCD3 Fc CHAIN 1	TSLLTQPASVSGSPGQSITISCTGTKYDVGSHDLVSWYQQYPGKVPKY MIYEVNKRPSGVSNRFSGSKSGNTASLTISGLRAEDEADYYCCSFGGS ATVVCGGGTKVTVLGGGSGGGGEVQLVESGGGLVQPGGSLRLSCAASG FTFSTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRFTISR DDSKNSLYLQMNSLKTEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVT VSSASTKGEVAACEKEVAALEKEVAALEKEVAALEKGGGDKTHTCPPC PAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNW YVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSN KALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLWCLVKGFY PSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSPGK
597	DH542xCD3 Fc CHAIN 2	QAVVTQEPSLTVSPGGTVTLTCRSSTGAVTTSNYANWVQQKPGQAPRG LIGGTNKRAPWTPARFSGSLLGGKAALTITGAQAEDEADYYCALWYSN LWVFGGGTKLTVLGGGGSGGGGQVQLVQSGAQMKNPGASVKVSCAPSG YTFTDFYIHWLRQAPGQGLQWMGWMNPQTGRTNTARNFQGRVTMTRDT SIGTAYMELRSLTSDDTAIYYCTTGGWISLYYDSSYYPNFDHWGQGTL LTVSSASTKGKVAACKEKVAALKEKVAALKEKVAALKE

[0245] 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.

[0246] 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 Cystein residue to permit disulfide bond. In some embodiments HPD includes suitable CH1 and CL domains.

[0247] 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.

[0248] ***HIV-1 Antibodies***

[0249] 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.

[0250] 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 V3 glycan. In some embodiments, the antibodies of the invention bind to HIV-1 gp120 Env CD4 binding site.

[0251] 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.

[0252] 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 identified from subjects infected with HIV-1.

[0253] Compositions including the human antibodies of the invention, including V3 glycan and CD4 binding site antibodies, can be used for any purpose including but not limited to research, diagnostic and therapeutic purposes.

[0254] 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.

[0255] 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.

[0256] 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.

[0257] 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.

[0258] 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.

[0259] 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.

[0260] 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.

[0261] 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, biding 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, biding and/or potency.

[0262] 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.

[0263] 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. 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, 3.sup.rd Ed., W.H. Freeman & Co., New York, 1997.

[0264] 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 produces recombinantly.

[0265] 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.

[0266] 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).

[0267] 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.

[0268] 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.

[0269] The contents of the various publications cited throughout the specification are incorporated by reference in their entirety.

[0270] 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

Example 1: Isolating antibodies from natural HIV-1 infected individuals

[0271] Methods to identify and isolate antigen specific reactive antibodies were carried out essentially as described in Liao HX et al. J. Virol. Methods 158: 171-9, 2009. Specific hooks are designed to identify antibodies which bind to specific HIV-1 envelope targets/antigens. Using such hooks, with fluorophore labeled streptavidin in two colors, cells are sorted by flow cytometry, into single wells, and the diagonally (that reacted with both colors hooks) reactive memory B cells are picked. B cells enriched from PBMC are sorted, and plated at limiting dilution (as single cell per well). Optionally, these cultures are grown and supernatants are functionally characterized.

[0272] PCR on these cells is carried out according to the protocol in Liao HX et al. J. Virol. Methods 158: 171-9, 2009. PCR amplifications are carried out to amplify rearranged VH and VL fragment pairs from the diagonally sorted memory B cells (Liao et al JVM). Overlapping PCR is 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 is 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 are 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, e.g. in 293T cells.

[0273] Recombinant antibodies are grown and supernatants and/or purified antibodies are functionally characterized.

[0274] Pairs of VH and VL genes as selected above can also be used to produce plasmids for stable expression of recombinant antibodies. 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).

[0275] 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.

[0276] 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 identified rearranged VH and VL fragments. In certain embodiments, the antibodies of the invention are recombinantly produced.

Example 2: TZM-bl cells pseudo-viruses neutralization assay

[0277] 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.

[0278] The TZMbl assay was conducted to determine neutralization potency and breadth of the various antibodies of the invention on different HIV-1 pseudoviruses.

[0279] Figure 27A shows summary results of neutralization data of CH557, CH235, VRC01, VRC07-523-LS, N6, 3BNC117, 8ANC131, CH103, F105, and DH522 against a panel of HIV-1 isolates in the Luc/TZM-bl neutralization assay. Values represent IC50 in μg/ml.

[0280] Figure 27B shows the mean IC50 and percent of isolates neutralized at different IC50 values. Figure 28A shows summary results of neutralization data of CH557, CH235, VRC01,

VRC07-523-LS, N6, 3BNC117, 8ANC131, CH103, F105, and DH522 against a panel of HIV-1 isolates in the Luc/TZM-bl neutralization assay. Values represent IC80 in μg/ml.

[0281] Figure 28B shows the mean IC80 and percent of isolates neutralized at different IC80<50ug/ml values.

Example 3: Epitope mapping of antibodies

[0282] Binding and/or neutralization assays using various envelop antigens can be used to determine the envelop epitope recognized by these antibodies.

Example 4: Kd determination

[0283] Kd measurements of antibody binding to HIV-1 envelope, e.g. gp120 or any other suitable peptide, 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.

[0284] Various assays and experiments can be designed to analyze prevention, treatment and/or cure.

Example 5: Assay for self-reactivity

[0285] **Table 9** below summarizes some of the known types of disease associated antibodies.

Autoantibody	Disease Association (s)
SSA	SLE, Sjogrens Syndrome (SS)
SSB	Sjogrens Syndrome
Sm (Smith antigen)	SLE
RNP (ribonucleoprotein)	Mixed connective tissue disease (MCTD)
Scl-70	Scleroderma
Jo-1	Myositis
Centromere B	Scleroderma CREST variant (calcinosis), Raynaud's, esophogeal dysmotility, sclerodactyly and talangiectasia
Histones	Drug induced SLE

[0286] Various assays for self-reactivity of human antibodies are known in the art. AtheNA Multi-Lyte ANA Plus Test System is one such assay. This is luminex-based assay, which is also used to screen patient sera.

Example 6: Antibodies from CH235 lineage

DH493 (also referred as CH493) and DH491 (also referred as CH491)

[0287] This example describes the design and making of non-naturally occurring CD4bs broad neutralizing HIV-1 antibodies

[0288] Monoclonal antibody CH493 was designed as follows: the heavy chain VDJ rearrangement was derived from genomic DNA deep sequencing performed on memory B cells isolated from PBMCs of the 703-01-050-5 subject (CHAVI001 protocol) obtained 152 weeks post-infection. Other V-heavy chain VDJ rearrangement sequences were retrieved with this technology from multiple time points. Figure 20 shows the heavy chain phylogenetic tree including all the sequences retrieved with deep sequencing (in black: all except for CH240, CH239, CH235, CH236, CH241). V-light chains were not identified.

[0289] Figure 20 shows the heavy chain phylogenetic tree including all the sequences retrieved from RNA of cultured memory B cells isolated from PBMCs obtained 41 weeks post-infection (in red: CH240, CH239, CH235, CH236, CH241). V-light chains of these antibodies from cultures were also identified.

[0290] Four recombinant non-naturally occurring antibodies were produced using V-heavy VDJ rearrangements identified from deep sequencing and they are called CH490, CH491, CH492 and CH493. These V-heavy sequences were chosen because they are the most mutated.

[0291] The V-heavy chains were paired with the V-light chains of the antibodies identified from memory B cell cultures that are closest in the phylogenetic tree shown above. Therefore, CH491 and CH493 heavy chains were paired with CH236 light chain, while CH490 and CH492 heavy chains were paired with the CH241 light chain,

[0292] CH235 antibodies from week 41 are characterized by their inability to bind and neutralize CH505.TF envelope mutants with specific point mutations in the D loop (Gao, Bonsignori, Liao et al. Cell 158, 481–491, July 31, 2014, see also U.S. Provisional Application No. 62/027,427 filed July 22, 2014, and U.S. Provisional Application No. 61/972,531 filed March 31, 2014).

[0293] Figure 21 shows that CH493 restores almost completely the ability to bind the mutants not recognized by the early members of the lineage, indicating that this antibody is not constrained by the amino acid makeup of the D loop as tightly as the naturally occurring CH235 early lineage antibodies. CH493 still retained differential binding to the CH505.TF gp120 delta 371I mutant. Binding dependence to I371 is a hallmark of neutralizing CD4bs antibodies.

[0294] Most notably, CH493 neutralized 20 of 24 tier-2 HIV-1 viruses (83%) in a multiclade virus panel optimized to represent diversity among globally circulating viruses. Naturally occurring antibodies in the same lineage neutralized only 25% of the viruses in the same panel and other engineered antibodies neutralized max 46% (Figure 22).

[0295] CH235, CH236, CH239, CH240 and CH241 are all Abs with natural pairs VH and VL from week 41 of infection of CH505 individual. CH241 is the most mutated at ~11 %. CH490, CH491, CH492 and CH493 are antibodies which comprise VH chains identified by deep sequencing and are near 20% mutated. These VH chains were paired with VL chains from the closest natural pair antibodies. So in the natural tree, CH241 is the most mutated from week 41 and it hit 38% of isolates.

[0296] For Abs CH491 and CH493, the VH chains were complemented with the VL of CH236. For antibodies CH490 and CH492 that were complemented with the VL of CH241. CH491 neutralized 46% of isolates and CH493 neutralized 83%.

[0297] In summary these antibodies were designed to include heavy chains form 454 sequencing and the heavy chains were paired with VL from observed antibodies:

[0298] CH490 and CH492 VH from 454 were paired with CH241 VL.

[0299] CH491 and CH493 VH from 454 were paired with CH236 VL. Pairing was done with the observed VL that was closer to the 454 sequence in the phylogenetic tree.

[0300] VH chains source: CH490 is from week 66; CH491 and CH492 are from week 100; CH493 is from week 152. The heavy chains were selected because were the most mutated ones.

Example 7: Antibodies from CH235 lineage

Antibodies CH555, CH556, CH557and CH558

[0301] CH505 transmitted/founder (CH505.TF) gp120 Env-specific memory B cells were isolated from 20 million PBMCs using fluorescent-activated single cell sorting (FACS) collected from chronically HIV-1 infected African CHAVI subject 703-01-050-5 264 and 323

weeks post-infection (10 million PBMCs/timepoint). Viable memory B cells were defined as AquaVital Dye neg, CD16neg, CD14neg, CD3neg, CD19pos, IgDneg cells. Sorted cells were cultured overnight in RPMI + 10%FCS supplemented with 2.5 ug/mL ODN2006, 2.5 ug/mL CHK2-inhibitor, 50ng/mL rHu IL-21 and 1:1 EBV-containing supernatants in a 96-well plates well containing 5000 CD40L-expressing MS40L feeder cells. Cells were then plated at limiting dilution in 96-well plates containing feeder cells, 2.5 ug/mL ODN2006, 2.5 ug/mL CHK2-inhibitor and 50ng/mL rHu IL-21, and cultured for 14 days, with medium refresh at days 3, 7 and 10. Culture supernatants were collected at day 14 and tested for neutralization of CH505.TF virus and binding to CH505.TF gp120, CH505.TF gp120 delta371I, RSC3 protein, RSC3 protein delta371I and CH505.TF mutant envelopes M6, M8 and M20 (described in Gao, Bonsignori, Liao et al. Cell 2014. Jul 31;158(3):481-91). From selected positive cultures, recombinant monoclonal antibodies were produced as previously described (Bonsignori et al. J Virol. 2011. Oct;85(19):9998-10009). Monoclonal antibodies CH557 was identified from a culture that neutralized 91% infectivity of CH505.TF virus, differentially bound to CH505.TF gp120 Env and CH505.TF gp120 delta371I Env, bound to M6, weakly bound to M8 but did not bind to M20 CH505.TF mutant gp120s. CH557 was identified from memory B cells collected 323 weeks post-infection. CH557 is a member of the previously described CH235 clonal lineage (Gao, Bonsignori, Liao et al. Cell 2014. Jul 31;158(3):481-91). From the same experiment we also identified monoclonal antibodies CH555, CH556 and CH558 - all members of the CH235 clonal lineage. All monoclonal antibodies but CH555, which was identified from memory B cells collected 236 weeks post-infection, are from week 323 post-infection.

Example 8: Maturation Pathway from Germline to Broad HIV-1 Neutralizer of a CD4-Mimic Antibody

[0302] See Bonsignori et al. Cell 165, 1–15, April 7, 2016, published on-line March 3, 2016, the contents of which are hereby incorporated by reference in its entirety. Antibodies with ontogenies from V_H1-2 or V_H1-46-germline genes dominate the broadly neutralizing response against the CD4-binding site (CD4bs) on HIV-1. Here we define with longitudinal sampling from time-of-infection the development of a V_H1-46-derived antibody lineage that matured to neutralize 90% of HIV-1 isolates. Structures of lineage antibodies CH235 (week 41 from time-of-infection, 18% breadth), CH235.9 (week 152, 77%) and CH235.12 (week 323, 90%) demonstrated the maturing epitope to focus on the conformationally invariant portion of the

CD4bs. Similarities between CH235 lineage and five unrelated CD4bs lineages in epitope focusing, length-of-time to develop breadth, and extraordinary levels of somatic hypermutation suggested commonalities in maturation among all CD4bs antibodies. Fortunately, the required CH235-lineage hypermutation appeared substantially guided by the intrinsic mutability of the V_H1-46 gene, which closely resembled V_H1-2. The CH235-lineage findings were integrated with a second broadly neutralizing lineage and HIV-1 co-evolution to suggest a vaccination strategy for inducing both lineages.

[0303] INTRODUCTION

[0304] Understanding the pathways and mechanisms of broadly neutralizing antibody (bnAb) induction is a critical goal of HIV-1 vaccine development (Bonsignori et al., 2012; Haynes, 2015; Haynes and Bradley, 2015; Haynes et al., 2012; Mascola and Haynes, 2013). In chronic HIV-1 infections, breadth of plasma neutralization follows a uniform distribution and broad neutralization arises in ~50% of individuals after 5 years or more of infection (Hraber et al., 2014). The delayed appearance of bnAbs suggests roadblocks to their development, and one vaccine approach is to decipher these roadblocks and devise strategies to overcome them. It is possible that - because of the high diversity of antibodies resulting from recombination and somatic hypermutation (SHM) - different bnAb lineages may have different developmental pathways and roadblocks. However, for the CD4-binding site (CD4bs), a population-level analysis on 14 donors indicated only two general types of CD4bs bnAbs: V_H-gene restricted and CDR H3-dominated (Zhou et al., 2015).

[0305] The V_H-gene restricted classes arise from two highly similar V_H -genes: V_H1-2 and V_H1-46 (Scheid et al., 2011; Wu et al., 2011). V_H1-2*02 and V_H1-46*01 share 93.4% (269/288) nucleotide sequence identity. Both classes give rise to antibodies that recognize the CD4bs via V_H structural mimicry of the immunoglobulin-like N-terminal domain of CD4 (Zhou et al., 2010; Zhou et al., 2015). For the V_H1-2 gene-derived antibodies, analysis of their ontogeny suggests two roadblocks based on: (i) a requirement for high levels of SHM (Klein et al., 2013; Scheid et al., 2009; Scheid et al., 2011; Wu et al., 2010), and (ii) weak binding of the inferred unmutated common ancestor (UCA) to gp120 (Jardine et al., 2013; McGuire et al., 2013; Scheid et al., 2011; Wu et al., 2011; Zhou et al., 2010; Zhou et al., 2015), although a definitive analysis from time-of-infection had not yet provided detail. In addition, several of the CD4bs bnAbs are autoreactive with ubiquitinase enzymes (Bonsignori et al., 2014; Liao et al., 2013; Liu et al., 2015).

[0306] Structure-based design of UCA-interacting immunogens has recently demonstrated a means to overcome this second roadblock, with priming of V_H1-2 bnAb lineages in knock-in mice (Dosenovic et al., 2015; Jardine et al., 2015). However, the maturation of primed V_H1-2 CD4bs B cell lineages to broad neutralization as well as the mechanism for the development of breadth remain unresolved.

[0307] For the V_H1-46-derived antibodies, far less is known. Two chronically HIV-infected individuals, RU1 and RU8, have developed V_H1-46-derived bnAbs, 1B2530 and 8ANC131 (Scheid et al., 2011). An African individual (donor CH505) was recently described who, over time, developed a CD4bs bnAb lineage (the CH103 lineage) that recognized the CD4 supersite through a CDR H3-dominated mode of interaction (Liao et al., 2013). Analysis of the co-evolution between virus and CH103 lineage demonstrated a second B cell lineage (the CH235 lineage) that cooperated by selection of escape mutants from the CH235 lineage that drove the CH103 bnAb lineage (Gao et al., 2014). Described herein is the finding that the CH235 lineage itself progressed to bnAb over 5 years of affinity maturation. Described herein are sequences of the CH235 lineage that were identified through longitudinal samples of 17 time points spanning weeks (wks) 6-323 post infection, assessment of the neutralization breadth of sequential lineage members on a panel of ~200 diverse isolates, and determination of Env-complexed crystal and EM structures for lineage members. The conformity (i.e. the level of shared mutation positions and identical sequence mutations) of CH235 lineage development is analyzed relative to other V_H gene-specific bnAb lineages in other donors, as well as the co-evolution of virus and CH235 lineage. Despite an early near-optimal binding orientation, the CH235 lineage required over 20% SHM to reach 90% neutralization breadth. The results described herein provide insight into the difficulties in focusing recognition to the conserved site of HIV-1 vulnerability, and suggest that CD4bs-directed antibodies, whether V_H-gene restricted or CDR H3-dominated, face similar obstacles in development. For V_H1-46- and V_H1-2-derived CD4-mimic antibodies, the unique genetic mutability inherent in each of these two V_H-germline genes helps to direct maturation, potentially providing an explanation for the prevalence of effective CD4bs antibodies derived from these two germline genes.

[0308] RESULTS

[0309] Sequencing of B cell antibody gene rearrangements in longitudinal samples.

[0310] To understand the maturation of the cooperating CH235 lineage in donor CH505, we sought to identify sequences of lineage members at 17 time points, spanning wks 6 to 323 from time of infection. First it was asked when we could detect members of the CH235 lineage. Next-generation sequencing (NGS) of antibody heavy chain gene rearrangements amplified from genomic DNA template of blood mononuclear cells from wk 6 to 152 (15 time points) identified a total of 479,028 unique, non-duplicated V-heavy sequences. The first V-heavy sequences belonging to the CH235 B cell lineage were found at wk 14, and additional CH235 lineage members were found at all subsequent time points. Only unique sequences in the CH235 lineage were further investigated and they were assigned to the earliest time-point (time-of-appearance) in which they were identified. Four V-heavy sequences were paired with the closest V_L from identified antibodies and produced as recombinant monoclonal antibodies (mAbs) (CH235.6 through CH235.9). From cultured memory B cells collected 41 wks post-transmission we had previously identified five members of the CH235 lineage (CH235, CH236, CH239, CH240 and CH241) (Gao et al., 2014) and we have now identified four additional members with natural V_H and V_L pairing from cultured memory B cells collected at wks 264 and 323 post-transmission: CH235.10 through CH235.13 (**Figure 29A, Figure 36A and Figure 40C**). CH235 lineage antibodies represented 0.018% of the total memory B cell repertoire and 0.5% of the CH505 TF gp120-specific memory B cell population.

[0311] The CH235 lineage could be separated into three clades (clade I, II and III). Clade I showed a number of early lineage members, but no additional clade I sequences were observed after wk 30; clade II showed further development and included members CH241 (wk 41) and CH235.6 (wk 66), but no additional sequences were observed after wk 66; clade III developed through wk 323 and included antibodies CH235 (wk 41), CH235.9 (wk 152), and CH235.12 (wk 323) (**Figure 29A**).

[0312] CH235 lineage HIV-1 neutralization.

[0313] To characterize the development of neutralization breadth in the CH235 lineage, antibodies in clade III were assessed for their ability to neutralize diverse HIV-1 isolates in a 199-isolate panel (**Figure 29B and Figure 41**). No isolates were neutralized by the unmutated common ancestor (UCA), whereas 18% of the viruses were neutralized by CH235 at wk 41. By wk 152, CH235.9 neutralized 77% of viruses, although with a relatively weak potency of

3 μg/ml. By wk 323, however, CH235.12 was able to neutralize 90% of viruses, and the neutralization 50% inhibitory concentration (IC_{50}) potency increased by 5-fold to 0.6 μg/ml.

[0314] Next the heterologous neutralization pattern of these antibodies were analyzed to understand their development of broad neutralization (**Figure 36B**) (Georgiev et al., 2013). CH235 lineage members and previously identified HIV-1 bnAbs were clustered based on heterologous neutralization activity. CH235 neutralization activity was more similar to CD4bs bnAbs than to bnAbs with other epitope specificities. While the CH235 neutralization profile was the most divergent from other CD4bs bnAbs, CH235.9 and CH235.12 were much more similar to other CD4bs bnAbs and each other. Interestingly, despite V_H1-46 usage, the CH235.9 and CH235.12 neutralizing profile was more similar to that of V_H1-2-derived antibodies, such as VRC01, than V_H1-46-derived antibodies, such as 8ANC131 (**Figure 36B**).

[0315] Crystal structures of CH235-lineage members with HIV-1 gp120.

[0316] To provide structural insight into the recognition and maturation of the CH235 lineage, the antigen-binding fragments (Fabs) of antibodies CH235 (wk 41 from time of infection, 18% breadth), CH235.9 (wk 152, 77%) and CH235.12 (wk 323, 90%), were prepared and co-crystallized, solved and refined these in complex with the gp120 core of HIV-1 isolate strain (93TH057) (**Figure 30, Figure 42**). We mapped the location of residues altered during SHM and observed changes throughout the variable domain (**Figure 30A**).

[0317] Comparison of the orientation of the V_H of CH235 in Env binding with that of CD4, VRC01 and 8ANC131 (Scheid et al., 2011) showed that the CH235 V_H domain mimicked CD4 in Env binding and was highly similar to the V_H orientation and structure of the VRC01 and 8ANC131 V_H chains: in particular, the V_H1-46 of CH235 preserved key contacts mediated by the CDR H2 loop for the CD4 binding loop and for the gp120 D368 (**Figure 37A,B**).

[0318] Analysis of the angle of recognition for the CH235 lineage indicated little change during maturation, with CH235, CH235.9 and CH235.12 all clustering within the larger VRC01-class of antibodies. Interestingly, other V_H1-46 antibodies clustered differently, with antibody 1B2530 from HIV-1-positive donor RU1 at a highly similar angle and 1.5 Å translated, and antibodies 8ANC131 and 8ANC134 from HIV-1-positive donor RU8 occupying a cluster about 55 degrees and 3.5 Å translated related to the CD4 (**Figure 37C**).

[0319] These results suggest that the gp120-antibody orientation was determined early in bnAb lineage ontogeny, with further maturation maintaining the same general orientation.

Overall, the structures of CH235 lineage members with HIV-1 gp120 Env revealed CD4 mimicry. While the V_H gene usage classifies the CH235 lineage within the V_H1-46-derived 8ANC131 bnAb class, it is both functionally and structurally closer to the VRC01 class (Zhou et al., 2015).

[0320] Negative stain EM of CH235-lineage members with trimeric HIV-1 Env.

[0321] To visualize the recognition of the CH235 lineage in the context of the HIV-1 Env trimer, negative stain EM was used to determine 3D-reconstructions of Fabs CH235, CH235.9 and CH235.12 bound to trimeric BG505 and B41 HIV-1 Env glycoproteins (**Figure 30B**)(Pugach et al., 2015; Sanders et al., 2013). Notably, the stoichiometry increased with antibody maturation, with CH235 (8% V_H mutation) binding with a stoichiometry of 1:1 (BG505; **Figure 30B, top, Figure 37D-F**) or 2:1 (B41; **Figure 30B, bottom, Figure 37D-F**) Fabs per trimer and CH235.9 and CH235.12 (19% and 25% V_H mutation, respectively) binding with a 3:1 Fab to trimer ratio (**Figure 30B**). Next the orientation and stoichiometry of CH235.12 Fab was compared with that of CH103, a CDR H3-dependent CD4bs bnAb identified from the same subject (Liao et al., 2013). EM analysis of either CH235.12 or CH103 Fab in complex with BG505 SOSIP.664 revealed structural differences between the CDR H3-dominated CH103 class bnAb and the 8ANC131-class CH235.12 bnAb and, in accordance with crystallographic results, the angle of approach of CH235 was similar to that of VRC01 and other CD4 mimicking bnAbs (**Figure 37G**).

[0322] Despite the CD4 mimicry by CH235, the trimer remained in a closed conformation when the CH235 lineage members were bound. However, the EM-derived model of CH103 in complex with BG505 revealed that CH103 either bound to or induced a more open version of the trimer. This conformation represents an intermediate state between the closed, compact trimer in complex with CH235 or VRC01, and the CD4-induced open model in complex with soluble CD4 or 17b Fab (**Figure 37G**). Similar to more mature CH235 lineage bnAb Fabs, bnAb CH103 bound to BG505 with a stoichiometry of 3 Fabs per trimer. (**Figure 37G**).

[0323] Maturation focuses CH235 lineage recognition to a conserved site of CD4 vulnerability.

[0324] To gain insight into the structural consequences of maturation, the epitope of CH235 lineage members was mapped relative to the conformationally invariant CD4 supersite of vulnerability (Zhou et al., 2015). When the CH235 footprint was mapped on gp120, we observed portions of the CH235-binding surface on gp120 to be outside of the CD4 supersite

of vulnerability (**Figure 30C,** left). This surface was reduced in CH235.9 and CH235.12 structures, especially on variable loop V5. Recognition by the CH235.12 antibody concentrated almost entirely on the CD4 supersite of vulnerability, with little interactions with the inner domain or variable loop V5; there was, however, a large remaining interaction with the conserved loop D region (**Figure 30C**, middle and right).

[0325] To quantify targeting precision, the buried surface between antibodies and gp120 co-crystal complexes was computed, for the region overlapping the CD4 supersite of vulnerability minus the region outside the vulnerable site. Overall targeting precision correlated with neutralization breadth (P=0.0007) (**Figure 30D**). The CH235-lineage antibodies all showed good targeting precision. The correlation of SHM versus neutralization breadth was also analyzed (P=0.0097) (**Figure 30E**): While the CH235 lineage generally trended towards lower SHM relative to neutralization breadth, all CD4bs bnAbs appeared to require a high degree of SHM, independent of whether the antibody derived from a specific V_H-gene or used a CDR H3-dominated mode of recognition.

[0326] Overall, the results suggest that maturation requires a high degree of SHM to focus recognition onto the CD4 supersite of vulnerability and that this high degree of SHM is a general requirement of all CD4bs bnAb lineages, even those that begin with highly favorable orientations such as CH235.

[0327] Conformity of sequence evolution of CH235 lineage.

[0328] The mutation levels of CH235-lineage antibodies identified 41 wks post infection from memory B cell cultures was markedly lower (range 7-11%;) than that of all previously reported V_H1-46 and V_H1-2 CD4bs bnAbs (>25%) (Scheid et al., 2011; Sui et al., 2010; Wu et al., 2010; Zhou et al., 2015) (**Figure 40C**). The mutation levels of CH235-lineage antibodies identified up to 264 wks post infection increased to ~20%, but were still lower than those of most other bnAbs until 323 wks post infection (CH235.12 : 26% mutations) (**Figure 31A**).

[0329] To quantify the conformity of CH235-lineage antibodies to the two V_H1-46-derived bnAbs (1B2530 from donor RU1 and 8ANC131 from donor RU8) (Scheid et al., 2011; Zhou et al., 2015), we analyzed the similarity of shared mutation positions (positional conformity) and shared identical mutations (identity conformity) of the V_H genes (**Figure 31B, Figure 38A**). As a comparison, the positional conformity and identity conformity of non-HIV-1 targeting antibodies identified from 3 HIV-1 negative donors relative to template antibodies

1B2530 and 8ANC131 were also calculated. Positional conformity in SHM was spread over a large range (50-90%), and there did not seem to be much discrimination between V_H1-46 in antibodies that effectively neutralized HIV-1 and those that did not (**Figure 31B**, top panels). Identity conformity in SHM was also spread over a large range (0-75%) (**Figure 31B**, bottom panels), and while little discrimination was observed between V_H1-46 in antibodies that effectively neutralized HIV-1 and those that did not for antibody 8ANC131, there was discrimination among CD4bs antibodies when 1B2530 was used as a reference (**Figure 31B**, bottom left panel). The differences in CH235-lineage identify conformity to 1B2530 or to 8ANC131 may reflect the greater similarity of the recognition orientation of CH235-lineage members with1B2530 (**Figure 37C**) and suggested that slight differences in recognition orientation can substantially alter factors associated with identity conformity.

[0330] Overall, these results indicated SHM in response to HIV-1 infection to proceed in a manner that depended less on functional selection and more on intrinsic properties of the V_H1-46-gene, especially related to the position of residues that undergo SHM. To investigate further the contribution of the V_H1-46 gene, we analyzed SHM observed in V_H1-46 gene transcripts from three uninfected individuals (**Figure 31C**, top); notably, all 11 positions mutated in CH235, 1B2530 and 8ANC131 were also mutated among non-HIV-1 neutralizing antibodies with high frequency (≥20%). Moreover, the residue substitutions in CH235 were frequently found in the top three most commonly observed substitutions for that position in the V_H1-46 gene. To quantify the impact of gene mutability, we compared the difference in probability distributions of positional and identity conformity for sequences simulated with and without taking into account the intrinsic V_H1-46 gene mutability. The simulations showed that both positional and identity conformity shifted to a higher level of similarity when considering gene mutability (**Figure 38B and 38C**). Notably, a substantial shift in probability was observed for the positional conformity of CH235 (**Figure 38B, Figure 43A**). Similar shifts in identity conformity were also observed for CH235 (**Figure 38C, Figure 43B**). Thus, the intrinsic susceptibility at specific sites of the V_H1-46 germline gene to mutation as well as to the frequency of specific mutations that existed at each of these sites appeared to be a dominant factor in the SHM alteration of the CH235 lineage. These results are in line with our previous finding that selection and mutability synergized during affinity maturation of an influenza HA-reactive clone from a non-HIV-1 infected person to hemagglutinin (HA) (Kepler et al., 2014): hence, the dominant role of intrinsic susceptibility at specific sites may be a more general biological phenomenon in dictating the course of SHM.

[0331] Because V_H1-2 is genetically the most closely related germline gene to V_H1-46, we also examined the mutability of the V_H1-2 gene (**Figure 31C,** bottom). Consistent with V_H1-46 antibodies, the mutated positions among V_H1-2 derived bnAbs also showed high frequency of mutation among non-HIV-1 targeting V_H1-2 antibodies, suggesting that gene mutability contributes to V_H1-2 derived HIV-1 antibody evolution. Notably, the average mutability of the V_H1-2 gene at positions where the CH235 antibody showed SHM was generally high: 9 of 15 positions mutated in CH235 antibody were also mutated in more than 15% of V_H1-2-derived NGS reads. In 10 of these 15 positions, the mature V_H1-2-derived bnAbs (VRC01, VRC-CH31 and VRC-PG04) also showed changes. When we analyzed mutability of other V_H genes used by CD4bs bnAbs (V_H1-69, V_H3-23, V_H3-30, and V_H4-59) (Zhou et al., 2015) (**Figure 38D**), we observed gene mutability patterns different from that of V_H1-46 while, in contrast, the mutability patterns of V_H1-2 and V_H1-46 were more similar (**Figure 43C**). Despite the similarity between V_H1-2 and V_H1-46, we did observe that antibody sequences from CD4bs bnAbs of each gene segregated phylogenetically (**Figure 31E**), indicating differences in maturation pathway between bnAbs evolving from these two germline genes.

[0332] These data suggested that for both V_H1-2 and V_H1-46 germline genes-derived bnAbs, somatic mutations that lead to neutralization breadth appeared to be primarily determined by the intrinsic mutability of V_H1-46 and V_H1-2 germline genes. The differences in the intrinsic mutabilities of these V_H genes may contribute to the high occurrence of CD4bs bnAbs that originate from either V_H1-2 or V_H1-46 (Zhou et al., 2015).

[0333] Interaction between CH235 and CH103 bnAb lineages.

[0334] While gene mutability plays a role in determining the position where SHM occurs, binding between antibody and HIV-1 Env likely also plays a role in selecting or fixing a mutation. A hallmark of cooperating B cell lineages is that they interact at the same site as the bnAb lineage that is being driven (Gao et al., 2014).

[0335] To determine a mechanism whereby the initial interaction of the early CH235 and CH103 lineage members bind to the same or similar epitope and result in CH235 selection of escape mutants that stimulated the CH103 bnAb lineage (Gao et al., 2014), cross-competition between early CH235 lineage antibodies and the CH103 lineage antibody CH106 in ELISA was evaluated, as an example of early CH103 lineage development, and measured their association rate constant with surface plasmon resonance (SPR). Since both the CH235 and CH103 lineages bound to the loop D gp120 region, we asked if the early CH235 lineage

antibodies could block the binding of the CH103 lineage mature antibody CH106, or block the binding of soluble (s)CD4 to CH505 TF gp120 Env. CH241 was the only antibody in the CH235 lineage that strongly blocked CH106 bnAb and sCD4 binding to CH505 gp120 (IC_{50} = 2.6 and 1.5 μg/ml, respectively) (**Figure 44A**).

[0336] To confirm early dominance of the binding of CH103 lineage compared to the CH235 lineage to CH505 TF Env, the blocking assay was reversed and asked if bnAb CH106 could block the binding of biotinylated CH235, CH236, CH239, CH240 or CH241. CH106 strongly blocked the binding of all the CH235 mature antibodies with IC50s ranging from 2.3 μg/ml (for CH240) to 14.3 μg/ml (for CH241) (**Figure 44B**). These data suggested that the earliest maturation intermediates of the CH235 lineage antibodies could not outcompete CH106 bnAb for binding to CH505 TF gp120 Env.

[0337] Affinity maturation in germinal centers is subjected to kinetic selection and involves improvement in dissociation rate constant (K_d) that is often driven by an improvement in the kinetic association rate (k_a), which is a key variable in conferring a binding advantage for the cognate epitope to an antibody over other competing antibodies (Foote and Milstein, 1991; Kepler et al., 2014). The k_a and dissociation kinetic rate (k_d) of the CH505 TF gp120 Env binding by CH235 and CH103was measured with SPR to identify differences that might explain the relative inability of the CH235 lineage to block the binding of the CH103 lineage bnAbs to autologous CH505 TF Env and found that the two lineages followed two distinct trajectories and modalities to increase their overall affinity.

[0338] The UCA of the CH103 lineage bound to CH505 TF Env with a K_d of 227 nM which increased one order of magnitude throughout affinity maturation (**Figure 32A**). The CH103 UCA displayed a fast association rate ($k_a = 37 \times 10^3\ M^{-1}s^{-1}$) which was maintained across the intermediate and mature mAbs ($k_a = 11.9 - 37.3 \times 10^3\ M^{-1}s^{-1}$), suggesting that maintaining the fast association rate was important for survival and maturation of the CH103 lineage (**Figure 32B**). In contrast, the CH235 lineage mAb K_d increased four orders of magnitude during affinity maturation (from 30.6 mM of IA4 - the earliest intermediate mAb in the CH235 lineage for which kinetic rates could be measured - to 0.7 nM of CH241) (**Figure 32C**). Such increase was predominantly facilitated by slower dissociation rates (k_d) observed in later intermediates and mature mAbs, which decreased from $88.1 \times 10^{-3}\ s^{-1}$ of IA4 to $0.33 \times 10^{-3}\ s^{-1}$ of CH241 (**Figure 32D**). Conversely, CH235 lineage mAbs bound to CH505 TF gp120 Env with k_a that started off an order of magnitude slower than CH103 UCA and its earlier intermediates (IA4 $k_a = 2.9 \times 10^3\ M^{-1}s^{-1}$) and only modestly improved - primarily between IA1

and CH235 mAbs - with the majority of the early CH235 mAbs having slower k_a than CH103 mAbs (**Figure 32D**).

[0339] Thus, the relative inability of wk 41 CH235 lineage antibodies to block early mature CH103 lineage mAbs could be explained by the observed differences in their association rates, and these data provide an explanation of how the CH235 antibody lineage exerted its cooperating function in driving autologous virus toward better neutralization by the CH103 antibody lineage without impeding concurrent development of the CH103 antibody lineage itself.

[0340] Late CH235 lineage broadly neutralizing antibodies neutralize autologous loop D escape viruses selected by early CH235 lineage members.

[0341] It has previously been demonstrated that the CH235 lineage selected escape viruses with mutations in the loop D region of gp120 Env that rendered loop D mutant viruses more sensitive to the CH103 bnAb lineage and that autologous virus escaped from early CH235 lineage antibodies by wk 30 after infection (Gao et al., 2014). Described herein is the isolation of autologous viruses through wk 323 and the determination of the neutralization capacity of the late CH235 lineage bnAbs. Viruses partially sensitive to the later members of the CH235 lineage (particularly bnAbs CH235.9 and CH235.12) were found as late as wk 176 (**Figure 33A, Figure 45**). These viruses still contained the loop D mutations that were selected by virus escape from early antibody members of the CH235 lineage (Gao et al., 2014). Hence, the ability of the late CH235 lineage bnAbs to neutralize the panel of CH505 TF loop D mutants was tested (Gao et al., 2014). Remarkably, CH235.9, CH235.12 and CH235.13 bnAbs acquired the ability to neutralize all loop D mutants that were resistant to the early members of the CH235 lineage (**Figure 33B and Figure 46**). In particular, CH235.9, CH235.12 and CH235.13 neutralized CH505 TF gp120 M8, M20 and M21 (not neutralized by early lineage member CH236), which differed from CH505 TF gp120 M6 and M10 (neutralized by CH236) by a single mutation at position 280 (N280S for M8 and M20, and N280T for M21) (**Figure 33B**).

[0342] In the gp120-complexed structure, the side chains of N280 forms three hydrogen bonds with two residues in the CDR L3 and these hydrogen bonds are predicted to be disrupted by the N280S and N280T mutations (**Figure 39A**). Since the CH235.9 antibody had the V_L of CH236, the direct implication was that mutations in the heavy chain were responsible for the ability of CH235.9 to neutralize loop D mutant viruses. Interestingly,

CH235.7, which did not neutralize autologous viruses beyond wk 53, also had the V_L of CH236 but, in contrast to CH235.9, failed to neutralize CH236-resistant loop D mutants M7, M8, M9, M20 and M21.

[0343] Therefore, we reverted the 5 amino acids (aa) in CH235.9 V_H at gp120 contact positions that were different from those present in CH236 V_H but not shared with CH235.7 V_H: N30T and D31T in CDR H1, G62Q and G65Q in FR H3 and A103E in CDR H3 (**Figure 39B**). Five of the six CH235.9 mutants retained the ability to potently neutralize all the CH505 TF loop D mutant viruses. In contrast, the N30T mutation in CDR H1 reverted CH235.9 to the CH236 phenotype (CH236 has a threonine in position 30): M21 neutralization was abrogated, M20, M7 and M9 were near completely abrogated (CH235.9 N30T IC_{50} >44 μg/ml) and M8 IC_{50} increased 37-fold (CH235.9 IC_{50} = 0.66μg/ml *vs* CH235.9 N30T IC_{50} = 24.31 μg/ml) (**Figure 46**).

[0344] Thus, acquisition of extraordinary breadth in the CH235 bnAb lineage was associated with accumulation of somatic mutations in CDR H1 that enabled late CH235 lineage antibodies to neutralize autologous loop D mutant viruses that were escape mutants from early CH235 antibodies. CH235.9 bnAb residue N30 contacts R429 in the β20-β21 loop of the C4 region of gp120 Env, which is on the opposite face of the CD4bs from loop D (**Figure 33C**). In addition, CH505 TF has a glutamic acid in position 429 that is in close enough proximity to N30 to form a hydrogen bond.

[0345] These findings indicate a mechanism for acquiring the ability to neutralize loop D mutants via a compensatory mutation in the CH235 V_HDJ_H which strengthens the binding to the gp120 C4 region by introducing hydrogen bonds that correct the loss of neutralization due to disruption of the hydrogen bonds between loop D and the CH235 mAb light chain.

[0346] CH235 and CH103 lineage antibody binding to CH505 gp120 Env.

[0347] The CH235 lineage antibodies were tested for binding to 113 recombinant CH505 gp120 Env isolated from time of transmission to wk 160 post-transmission, including CH505 TF loop D mutant Envs (**Figure 34A** and **Figure 47**). Of note, CH235.9 and CH235.12 bound to 4/22 and 8/22 Envs isolated from wk 136 and 160 post-transmission, respectively, including Envs from viruses that were also neutralized. Env binding to the initial members of the CH103 lineage has previously been reported (Hraber et al., 2015), and described herein is the same Env binding analysis of the CH103 lineage with 10 additional matured bnAb members of the CH103 lineage (**Figure 34A** and **Figure 47**). We have used these data to

select CH505 gp120 Env quasi-species that bound to mature and precursor bnAbs of both lineages, defining a series of CH505 Env immunogens now optimized and predicted to induce both bnAb lineages (**Figure 40A**).

[0348] It has previously been reported that CH235 UCA weakly reacted with CH505 TF gp120 at ~10 μM as determined by SPR (Gao et al., 2014). Here we show stronger binding of the CH235 UCA to 8/113 autologous CH505 gp120 Envs measured in ELISA (**Figure 34A** and **Figure 47**). Moreover, in a panel of 15 heterologous Envs from multiple clades, CH235 UCA bound to 3/15 Envs and the introduction of only 3 mutations (W47L, G54W and S56R), which were selected based on the increase in surface area of interaction (G54W and S56R) or the reduction in clash score (W47L), increased this recognition (to 5/15 Envs), of which the dominant effect appeared to be reduction in clash (**Figure 34B** and **Figure 40B**).

[0349] Autoreactivity in the CH235 B cell lineage.

[0350] Development of auto- and polyreactivity during antibody maturation toward neutralization breadth is a critical aspect that may limit the ability of generating bnAbs during natural infection and upon vaccination (Bonsignori et al., 2014; Haynes et al., 2005; Haynes et al., 2012; Haynes and Verkoczy, 2014; Liu et al., 2015; Verkoczy et al., 2013; Verkoczy et al., 2010; Verkoczy et al., 2011). It has previously been reported that in HIV-1-infected individual CH505, the CD4bs CH103 bnAb lineage was polyreactive and, similar to VRC01-class bnAbs, bound to human ubiquitin ligase E3A (UBE3A) with avidity correlated with neutralization (Liao et al., 2013; Liu et al., 2015). In addition, most of the mutations introduced in VRC07 - a somatic variant of VRC01 - that enhanced neutralizing activity also resulted in increased autoreactivity (Rudicell et al., 2014). Since CH235.12 is a potent and extremely broad CD4-mimic CD4bs bnAb, we compared the auto- and polyreactivity profile of CH235.12 with other members of the CH235 lineage. Most CH235 lineage antibodies displayed reactivity against DNA and sporadic reactivity with Scl70 (CH235.7) (**Figure 35A**). CH241 bound to cardiolipin (**Figure 35B**). In Hep-2 IF staining CH236, CH235.7 and CH235.9 were all cytoplasmic positive (**Figure 35C**). Conversely, CH235.12, despite being highly mutated and broadly neutralizing, did not display autoreactivity in any of these assays (**Figure 35A-C**) Of particular note, CH235 lineage antibodies, including CH235.12, did not react with UBE3A (**Figure 35D**).

[0351] These data identify CH235.12 as an antibody that has developed neutralization breadth without being itself auto- and polyreactive, while less mutated precursor antibodies (CH235 is

in the same clade of CH235.12) did develop autoreactivity. Therefore, *in vivo* decoupling of neutralization breadth of CD4 mimic CD4bs bnAbs from auto- and polyreactivity can occur, even for bnAb lineages that have developed autoreactivity during the course of their maturation and, therefore, inducing such bnAbs from such lineages through vaccination, though difficult, is an achievable goal.

[0352] DISCUSSION

[0353] Here we have traced the ontogeny of the CH235 V_H1-46 8ANC131 class of CD4bs bnAbs from acute infection to chronic infection and defined both the structural and functional pathways of bnAb lineage induction. That the CH235 bnAb lineage that selected virus escape mutants that drove the CH103 CD4bs CDR H3-dependent bnAb lineage is itself an 8ANC131-class bnAb lineage and co-evolved with the CH103 bnAb is a remarkable demonstration of a bnAb-to virus-to bnAb interaction in the same HIV-1 infected individual. In addition, the similarity of V_H1-46 8ANC131-like and V_H1-2 VRC01 family CD4 supersite bnAbs demonstrates dramatic convergence of antibody structures to recognize the CD4 supersite. The CH235 lineage required over 20% SHM in heavy chain variable domain to achieve 90% breadth. Fortunately, a substantial portion of the V_H-gene SHM was guided by the intrinsic mutability of the V_H1-46 germline gene. Moreover, the CH235 lineage Ab that became broadly neutralizing acquired the ability to neutralize loop D mutants selected by early Ab lineage members (Gao et al., 2014) with a mechanism involving a compensatory mutation (T30N) in CDR H1, which allowed the formation of H-bonds with the HIV-1 gp120 C4 region, thus correcting the original loss of binding.

[0354] The driving forces of the CH235 lineage were the natural transmitted/founder and M5 Envs. In addition, despite near-complete autologous virus escape from CH235 lineage antibodies by wk 100, viruses arose later during the course of infection, which were sensitive to the more mature CH235 bnAb members and likely contributed to antigen drive. It is interesting to note that many of these late viruses were less sensitive to CH103 CDR H3 binder bnAbs prompting the hypothesis that the CH103 lineage may have the capacity for cooperation with the CH235 lineage after 5-6 years of co-development. Finally, the CH235.12 antibody that evolved late in CH235 development is an extraordinary broad and potent non-autoreactive antibody and is a candidate for preventive and therapeutic uses.

[0355] In summary, the acquisition of neutralization breadth in the CH235 VRC01-like V_H1-46 CD4 mimic bnAb occurred with the sequence of transmitted/founder and early mutant-

initiated antigen drive, selection of Env loop D mutants that cooperated with the CH103 bnAb lineage to drive it to bnAb breadth, followed by acquisition of the ability of the CH235 lineage itself to neutralize autologous loop D mutants coincident with potent neutralization of a broad array of heterologous HIV-1 isolates. Mapping these events points to a strategy for the simultaneous induction of both CDR H3 and VRC01-class CD4bs bnAbs, whereby sequential immunizations with transmitted founder Env followed by loop D mutant Envs comprise a rational immunization strategy.

[0356] EXPERIMENTAL PROCEDURES

[0357] Donor and sample information. Donor and sample information was previously reported (Liao et al., 2013) and is summarized in Supplemental Experimental Procedures. Memory B cell cultures were performed on PBMCs collected at 264 and 323 wks post-transmission. All work related to human subjects was in compliance with Institutional Review Board protocols approved by the Duke University Health System Institutional Review Board.

[0358] Preparation of libraries for 454 DNA pyrosequencing. 454 DNA pyrosequencing was performed on genomic DNA template isolated with Qiagen kits from PBMCs collected at 6, 7, 8, 9, 14, 20, 22, 30, 41, 53, 66, 92, 100, 144 and 152 wks post-transmission as described in (Boyd et al., 2009) and in Supplemental Experimental Procedures. Only unique V-heavy rearrangements were included in the analysis to generate the phylogeny; in the case of duplicated sequences, the earliest occurrence was included in the analysis.

[0359] Phylogenetic analysis. For clonal phylogenetics, the UCA was inferred using Cloanalyst (Kepler, 2013), which simultaneously estimates the UCA and the phylogenetic tree relating the observed sequences to each other and to the UCA. Internally, Cloanalyst uses dnaml from the PHYLIP suite of phylogenetic software (Felsenstein, 2005). The CH235 antibody lineage clonogram was displayed using the ete2 Python package.

[0360] Isolation of CH235 Lineage Antibodies from Donor CH505. Fluorescence-activated cell sorting of antigen-specific IgG^+ B cells from PBMC and the amplification and cloning of immunoglobulin genes were performed as described in (Bonsignori et al., 2011). CH505.TF gp120 Env-positive memory B cells were cultured as described in Supplemental Experimental Procedures.

[0361] Neutralization assays. Neutralization of donor CH235 mAbs were measured using single-round-of-infection HIV-1 Env pseudoviruses and TZM-bl target cells as described in Supplemental Experimental Procedures.

[0362] Neutralization signature. Antibody neutralization signatures were computed and compared as described in Supplemental Experimental Procedures.

[0363] Monoclonal Antibody and Antigen-Binding Fragment (Fab) Production. Ig genes of mAbs were amplified from RNA and expression plasmids for heavy and kappa chains were constructed. Expression and purification of recombinant IgG mAbs and preparation of Fab fragments are described in Supplemental Experimental Procedures.

[0364] Crystallization, X-Ray Data Collection, Structure Determination, and Refinement of Donor CH235 Antibodies in Complex with HIV-1 gp120. Purification, crystallization of antibody-gp120 complexes, data collection, structure solution, refinement and analysis are described in Supplemental Experimental Procedures. Diffraction data were integrated and scaled with the HKL2000 suite (Otwinowski and Minor, 1997).

[0365] Electron microscopy data collection and processing. BG505 SOSIP.664 and B41 SOSIP.664 gp140 trimers and donor CH235-derived Fab complex negative-stain electron microscopy images, analysis and visualization are described in the Supplemental Experimental Procedures.

[0366] Focused maturation and conformity analysis. Focused maturation and mAb conformity analysis are described in the Supplemental Experimental Procedures.

[0367] Surface Plasmon Resonance Affinity and Kinetics Measurements. MAb binding to autologous CH505 gp140 was measured using a BIAcore 3000 or BIAcore T200 instrument (GE Healthcare) as described in (Alam et al., 2007; Alam et al., 2009; Liao et al., 2013) and in Supplemental Experimental Procedures.

[0368] Direct-Binding ELISA. Direct-binding ELISAs were performed as described in Supplemental Experimental Procedures.

[0369] MAb CH235.9 Amino Acid Reversion. Site-directed mutagenesis of the CH235.9 mAb genes was performed using the Quikchange lightning multi-site-directed mutagenesis kit (Agilent) following manufacturer's protocol. Primers are listed in Supplemental Experimental Procedures.

[0370] Structural Modeling. Loop D mutations were structurally modeled using PyMOL with sidechains placed in the most frequently observed rotamer that did not result in steric clashing with neighboring residues. Hydrogen bonds were calculated using HBPLUS software (McDonald and Thornton, 1994).

[0371] Recombinant HIV-1 Proteins. HIV-1 genes of autologous CH505 Env were determined from samples collected from 4 to 323 wks post-infection by single genome amplification (Keele et al., 2008) and produced as described in (Liao et al., 2013).

[0372] Protein Array. MAbs were screened for binding on protein microarrays (ProtoArray) (PAH0525101; Invitrogen) pre-coated with 9,400 human proteins in duplicate and screened following manufacturer's instructions and as described in (Liu et al., 2015; Yang et al., 2013).

[0373] HEp-2 cell staining. Indirect immunofluorescence binding of mAbs or plasma to HEp-2 cells (Zeuss Scientific) was performed as previously described (Bonsignori et al., 2014; Haynes et al., 2005).

[0374] Supplemental Experimental Procedures

[0375] Donor and Sample Information.

[0376] The CH505 donor, from which the CH103 and the CH235 antibody lineages were identified, is an African male enrolled in the CHAVI001 acute HIV-1 infection cohort (Tomaras et al., 2008) and followed for over 6 years. During this time viral load ranged from 14,460 to 847,279 copies/ml (median = 173,667 copies/ml), and CD4 counts ranged from 69 to 431 cells/mm^3 (median = 294 cells/ mm^3).

[0377] The time of infection was estimated by analyzing the sequence diversity in the first available sample using the Poisson Fitter tool (Giorgi et al., 2010) as described in (Liao et al., Nature 2013). Results were consistent with a single founder virus establishing the infection and with the earliest isolated virus sequences being taken 4 weeks post-transmission.

[0378] Flow Cytometry, Memory B Cell Cultures and mAb Isolation.

[0379] The HIV-1 CH505.TF gp120 envelope glycoprotein was produced and used in flow cytometry on PBMC collected from donor CH505 at week 264 and 323 post-transmission using a two-color technique as described (Gray et al., 2011).

[0380] CH505.TF gp120 Env-positive memory B cells were cultured as described (Bonsignori et al., 2011) with the following modifications: non-irradiated MS40L cells were used as feeder cells at a concentration of 3,000 cells/well and were added to wells in which memory B cells were sorted in bulk; 50 ng/ml of recombinant human (rHu) IL-21 (200-21; Peprotech, Rocky Hill, NJ) were added to the complete medium; memory B cells were distributed by limiting dilution at a calculated concentration of 2 cells/well; culture medium was refreshed every 5 days.

[0381] Cell culture supernatants were screened for neutralization of autologous CH505.TF virus using the tzm-bl neutralization assay (Bonsignori et al., 2011; Montefiori, 2005) and for binding to CH505.TF gp120 Env, CH505.TF Δ371I gp120 Env mutant, HIV-1 Env resurface core protein 3 (RSC3) and RSC3 Δ371I (Wu et al., 2010).

[0382] MAbs CH235.10 through CH235.13 were identified from cultures that displayed differential binding of CH505.TF and CH505 TF Δ371I gp120 Env, did not bind to RSC3 (Gao et al., 2014) and neutralized 13 to 99% CH505.TF infectivity.

[0383] CH235 lineage antibody frequency over total memory B cells was calculated by dividing the number of CH235 lineage antibodies identified at week 41 (n = 5; Gao et al., 2014) for the number of memory B cells analyzed (n = 27,950). CH235 lineage antibody frequency over CH505.TF gp120 Env-specific memory B cells was calculated by dividing the number of CH235 lineage antibodies identified at weeks 264 and 323 (n = 4) for the number of CH505.Env gp120-specific memory B cells analyzed (n = 794).

[0384] 454 Pyrosequencing of CH235 lineage heavy chains.

[0385] Antibody heavy chain gene rearrangements were PCR amplified from 6 independent 100ng genomic DNA aliquots to generate 6 barcoded libraries per sample. Multiplexed primers complementary to the IGHV FR1 or FR2 framework regions, and an IGHJ-primer were modified from the BIOMED-2 consortium primers (Boyd et al., 2009; van Dongen et al., 2003). 10-nucleotide 'barcode' sequences in the primer sets encoded sample identity and replicate library identity. AmpliTaq Gold (Roche) enzyme was used for PCR following the manufacturer's instructions, with a thermocycler program: 94°C 5 min; 35 cycles of (94°C 30 sec, 60°C 45 sec, 72°C 90 sec); and final extension at 72°C for 10 min. Following quantitation, PCR products from each replicate library were pooled in equimolar amounts, then the pooled library was run on a 1.5% agarose gel and gel extracted (Qiagen). High-throughput sequencing was performed on the 454 (Roche) platform using Titanium chemistry.

[0386] Antibody production.

[0387] Immunoglobulin genes of mAbs CH235.10 through CH235.13 were amplified from RNA from isolated cells, expression cassettes made, and mAbs expressed as described (Gao et al. 2014). The V_H genes of mAbs CH235.6 through CH235.9 were retrieved from sequences obtained through genomic DNA 454 sequencing, which were restored to full length and complemented with the V_L of the phylogenetically closest identified antibody in the CH235 lineage (i.e. CH241 for CH235.6 and CH235.8, and CH236 for CH235.7 and CH235.9). We have previously described the isolation of mAbs CH235, CH236, CH239,

CH240 and CH241and the inference of unmutated common ancestor (UCA) and intermediate antibodies IA1 through IA4 (Gao et al., 2014; Kepler, 2013).

[0388] Heavy chain plasmids were co-transfected with appropriate light chain plasmids at an equal ratio in Expi 293 cells using either 293Fectin or ExpiFectamine 293 transfection reagents (Thermo Fisher Scientific) according to the manufacturer's protocols. Cultures were supplemented with AbBooster antibody expression enhancer media (ABI Scientific) at 10% of the final culture volume 24 h after transfection. . Cultures were then incubated at 33°C for 5 more days, and supernatants were harvested and passed over a protein A affinity column. Following PBS wash and low pH elution, the pH of eluate was neutralized with 1MTris pH 8.5 and samples were dialyzed against PBS. Antibodies were then aliquoted and stored at -80°C prior to use. Alternatively, for ExpiFectamine transfections we used the enhancer provided with the kit, transfected cultures were incubated at 37°C 8% CO_2 for 2-6 days, harvested, concentrated and incubated overnight with Protein A beads at 4°C on a rotating shaker before loading the bead mixture in columns for purification; following PBS/NaCl wash, eluate was neutralized with trizma hydrochloride and antibody concentration was determined by Nanodrop. Purified antibodies were tested in SDS-Page Coomassie and western blots, and stored at 4°C.

[0389] Direct-binding ELISA.

[0390] Direct-binding ELISAs were performed as described previously (Bonsignori et al., 2011) with the following modifications: plates were blocked for 1 h at room temperature (RT) or overnight at 4°C (both procedures were previously validated); primary purified antibodies were tested at a starting concentrations of 100µg/ml, serially three-fold diluted and incubated for 1 h at RT; HRP-conjugated human IgG antibody was added at optimized concentration of 1:30,000 in assay diluent for 1 hour and developed using TMB substrate; plates were read at 450 nm in a SpectraMax 384 PLUS reader (Molecular Devices, Sunnyvale, CA); results are reported as logarithm area under the curve (LogAUC) unless otherwise noted.

[0391] For cell culture supernatant screening of RSC3 and RSC3 Δ371I HIV-1 Env core proteins reactivity, plates were coated with streptavidin (2µg/ml); blocked plates were stored at -20°C until used; 10 µl/well of biotinylated avi-tagged RSC3 and RSC3 Δ371I were added at 2 µg/ml for 30 minutes at RT and culture supernatants were added at 1:3 dilution in assay diluent; plates were developed for 10 min using SureBlue Reserve TMB (53-00-03; KPL, Gaithersburg, MD) equilibrated at RT.

[0392] Competition ELISAs were performed using 10µl of primary purified monoclonal antibody, starting at 100µg/ml and diluted in a two-fold concentration, incubated for 1 h at RT; for CD4 binding site blocking assays, 10 µl of a saturating concentration soluble CD4 (Progenics Pharm Inc.) was added following antibody incubation step. Ten µl of biotinylated target Mab was added at the EC50 determined by a direct binding of biotinylated-Mab for one hour at RT. After background subtractions, percent inhibition was calculated as follows: 100-(sera triplicate mean/no inhibition control mean)*100.

[0393] Autoimmune purified antigens histones (whole), Jo-1, RNP/Sm, Scl-70, Sm, SSA (Ro), SSB (all from ImmunoVision) and centromere B (Prospec) were coated at optimal concentrations determined by lot-specific checkerboard with positive controls. All plasma antibody positive controls were purchased from ImmunoVision; lot-specific optimal ranges for standard curves were determined. All antibodies were tested using the same lots for each antigen and positive controls with the protocol described above. For DNA ELISA, plates were coated with 2µg/ml poly-lysine (Sigma-Aldrich) for 2 h at RT, washed 3X with PBS and blocked with PBS/2%BSA/0.05% Tween-20 for 2h at RT. After 3X wash, DNA (LS002195, Worthington) in saline sodium citrate buffer was added for 1 h, washed and antibodies were incubated for 1 h. Secondary antibody was diluted in PBS/0.05% Tween-20. Plates were developed for 30 min. Human recombinant monoclonal antibody Ab008391 (courteously provided by David Easterhoff, Duke Human Vaccine Institute) was used as positive control. For all autoantigen ELISAs, palivizumab was used as negative control. For each antibody, LogAUC was calculated and data are presented semi-quantitatively: no binding = $\log AUC_{Ab} \leq$ 2X negative control $\log AUC_{neg\ ctrl}$; to quantify antibody binding we divided ($\log AUC_{pos\ ctrl}$ - 2X $\log AUC_{neg\ ctrl}$) in tertiles and expressed test antibody binding as weak (+), intermediate (+ +) or strong (+ + +) if $\log AUC_{Ab}$ was in the first, second or higher tertile, respectively.

[0394] Anti-cardiolipin ELISA was performed using the QUANTA Lite ACA IgG III kit (708625; INOVA Diagnostics) following manufacturer's protocol.

[0395] Assessment of virus neutralization using a large panel and calculation of neutralization dendrograms.

[0396] Neutralizing antibody assays in TZM-bl cells were performed as described previously (Montefiori, 2005). Neutralization breadth of CH235 UCA, CH235, CH235.9 and CH235.12 neutralization breadth was assessed using the 384-well plate declination of the assay using an updated panel of 199 geographically and genetically diverse Env-pseudoviruses representing the major circulating genetic subtypes and recombinant forms as described (Seaman et al.,

2010; Wu et al., 2010). The data were calculated as a reduction in luminescence units compared with control wells, and reported as IC_{50} in μg/ml (Montefiori, 2005).

[0397] Dendrograms were calculated using the neighbor-joining method, showing the protein sequence distance from the HIV-1 Env gp160 sequences of 190 HIV-1 primary isolates. The clades of HIV-1, including circulating recombinant forms (CRFs) are indicated.

[0398] Antibody neutralization fingerprinting analysis.

[0399] Neutralization fingerprints were computed and compared for CH235, CH235.9 and CH235.12 from the CH235 lineage, other CD4-binding-site antibodies, and antibodies targeting other sites of vulnerability on HIV-1 Env. The fingerprints were computed over a common panel of 165 HIV-1 strains with neutralization data for all antibodies, and a hierarchical clustering procedure was applied for building the tree, as described in (Georgiev et al., 2013). Briefly, for each antibody, the neutralization data for the common set of 165 HIV-1 strains formed that antibody's neutralization fingerprint. The Spearman correlation coefficients for all pairs of antibody neutralization fingerprints were then computed, transforming the antibody-virus neutralization matrix into an antibody-antibody correlation matrix. This correlation matrix was then input into a hierarchical clustering procedure as a way to visualize the similarities between the neutralization fingerprints for the different antibodies. The distances in the resulting tree are thus a function of the differences between fingerprints.

[0400] VH1-46 and VH1-2 antibody dendrogram calculation.

[0401] Phylogenic trees for multiple antibodies derived from VH1-46 and VH1-2 heavy chain variable genes were calculated using the neighbor-joining method. The sequences are aligned by Clustal Omega, calculated using ClustalW2. Dendrograms were drawn in Figtree.

[0402] Production and purification of HIV-1 Env protein complexed to antigen-binding fragments.

[0403] HIV-1 gp120 protein from clade AE 93TH057 and antibodies of CH235, CH235.9 and CH235.12 were produced and purified as described previously (Zhou et al., 2010). Fab fragments of antibodies were prepared by digesting purified IgG with Lys-C at 37°C for 2-4 h. The digestion reaction was quenched by the addition of cOmplete protase inhibitors (Roche). The digested antibodies were passed over Protein A agarose to remove the Fc fragment. The Fab was further purified over a Superdex 200 gel filtration column and concentrated aliquots were stored at −80 °C.

[0404] X-ray crystallography.

[0405] The gp120-antibody complexes were formed by mixing deglycosylated gp120 with the antibody Fab in a 1:1.5 molar ratio. The complexes were purified by size exclusion chromatography (Hiload 26/60 Superdex S200 prep grade; GE Healthcare) with buffer containing 0.35 M NaCl, 2.5 mM Tris (pH 7.0), and 0.02% NaN_3. Fractions with gp120-antibody complexes were concentrated to ~10 mg/ml and used for crystallization experiments. All gp120-Fab complexes were screened against 576 crystallization conditions using a Cartesian Honeybee crystallization robot. Initial crystals were grown by the vapor diffusion method in sitting drops at 20 °C by mixing 0.2 µl of protein complex with 0.2 µl of reservoir solution. Crystals were manually reproduced in hanging drops by mixing 0.50 µl protein complex solution with 0.5 µl reservoir solution.

[0406] The 93TH057 $core_e$ gp120-CH235 complex was crystallized with a reservoir solution of 25% (w/v) of PEG2000, 0.2 M of Li_2SO_4, 0.1 M of Tris-HCl pH 8.5 and 5% (v/v) of isopropanol and was flash frozen in liquid nitrogen in mother liquor supplemented with 15% of 2R,3R-butanediol as a cryoprotectant. The 93TH057 $core_e$ gp120-CH235.9 complex was crystallized with a reservoir solution of 9% (w/v) of PEG8000, 19% (w/v) of PEG400, 0.1 M HEPES pH 7.5 and was flash frozen in mother liquor supplemented with an additional 15% PEG 400 as a cryoprotectant. The 93TH057 $core_e$ gp120-CH235.12 complex was crystallized with a reservoir solution of 10% PEG 8000, 20% PEG 400 and 100 mM HEPES, pH7.5 and was flash frozen in mother liquor supplemented with an additional 15 - 20% PEG 400 as a cryoprotectant.

[0407] Data for all crystals were collected at a wavelength of 1.00Å at SER-CAT beamlines ID-22 and BM-22 (Advanced Photon Source, Argonne National Laboratory). All diffraction data were processed with the HKL2000 suite, structures were solved by molecular replacement using PHASER, and iterative model building and refinement were performed in COOT and PHENIX, respectively. For 93TH057$core_e$ complexes with CH235.9 and CH235.12, molecular replacement solutions were obtained using EAF31403.1-CH235 complex as a search model.

[0408] Throughout the refinement processes, a cross validation (R_{free}) test set consisting of 5% of the data was used and hydrogen atoms were included in the refinement model. Structure validations were performed periodically during the model building/refinement process with MolProbity. The 93TH057 $core_e$-CH235 structure was refined to a final R_{free} value of 22.9% with 96% residues in the favored region of the Ramachandran plot, and 0.1%

outliers. The 93TH057 $core_e$-CH235.9 structure was refined to a final R_{free} value of 22% with 97.1% residues in the favored region of the Ramachandran plot, and 0% outliers. The 93TH057 $core_e$-CH235.12 structure was refined to a final R_{free} value of 23% with 97.0% residues in the favored region of the Ramachandran plot, and 0.1% outliers. All figures containing representations of the protein crystal structures were made with PyMOL. Gp120 and antibody interactions were analyzed with the PISA server.

[0409] Surface Plasmon Resonance Affinity and Kinetics Measurements.

[0410] For kinetic measurement, each antibody was captured on an anti-human IgFc immobilized sensor surface (200-500RU) and gp120 proteins at varying concentrations were injected to monitor association and dissociation phases. Buffer reference and non-specific binding to a control antibody (palivizumab) captured surface were used to derive specific binding signals. Kinetic rate constants and dissociation constant (Kd) were derived from global curve fitting analysis using a Langmuir 1:1 interaction model using the BIAevaluation 4.1 software (GE Healthcare).

[0411] Electron microscopy data collection and processing.

[0412] BG505 SOSIP.664 and B41 SOSIP.664 gp140 trimers were expressed in HEK293F cells and purified by 2G12-affinity and gel filtration chromatography as described elsewhere (Pugach et al., 2015; Sanders et al., 2013).Trimers were incubated with a 10 molar excess of Fab (CH235, CH235.9, or CH235.12) overnight at room temperature and the complexes were diluted to ~0.03 mg/mL prior to application onto a carbon-coated 400 Cu mesh grid that had been glow discharged at 20 mA for 30 s. The grids were stained with 2% (w/v) uranyl formate for 60 seconds. Samples were imaged using a FEI Tecnai T12 electron microscope operating at 120 keV, with an electron dose of ~ 25 $e^-/Å^2$ and a magnification of 52,000x that resulted in a pixel size of 2.05 Å at the specimen plane. Images were acquired with Leginon (Suloway et al., 2005) using a Tietz TemCam-F416 CMOS camera with a nominal defocus range of 1000-1500 nm. Automated particle picking, stack creation, and initial 2D classification was performed in the Appion software suite (Lander et al., 2009). Noise and junk particles were discarded and the remaining stack was subjected to 3D classification using Relion (Scheres, 2012) with an EM volume created from the x-ray structure of ligand-free BG505 SOSIP.664 (PDB: 4zmj) low pass filtered to 60 Å as the reference model. While both CH235.9 and CH235.12 bound to either B41 or BG505 at predominantly full stoichiometry (3 Fabs per trimer), CH235 bound to either trimer at sub-stoichiometric ratios (1 Fab per BG505 trimer and 2 Fabs per B41 trimer). The 3D classes representing the predominant stoichiometry for

each complex were used as the initial models (low pass filtered to 40 Å) for further refinement using Relion, with C3 symmetry imposed for complexes with CH235.9 or CH235.12. The total number of particles used in refinement and final resolution of the map using a Fourier shell correlation of 0.5 are as follows: BG505 in complex with CH235 – 3,467 particles (~25 Å); B41 in complex with CH235 – 4,248 particles (~24 Å); BG505 in complex with CH235.9 – 2,567 particles (25 Å); B41 in complex with CH235.9 – 8,061 particles (19 Å); BG505 in complex with CH235.12 – 15,565 particles (17 Å); B41 in complex with CH235.12 – 17,023 particles (16 Å).

[0413] To create figures of each Fab in complex with a representative trimer, the 3D reconstructions for each complex were fit into an EM volume created from the x-ray structure of unliganded BG505 SOSIP.664 (PDB: 4ZMJ) low pass filtered to 30 Å in UCSF Chimera (Pettersen et al., 2004) and using the "segment map" option to isolate the density of the Fab components alone. Two-dimensional back projections of the final 3D models were generated using EMAN (Tang et al., 2007).

[0414] Epitope visualization.

[0415] The HIV-1 gp120 epitopes targeted by donor CH235 antibodies were visualized using PyMOL (Schrodinger, 2010). In this graphic program, we used 5.5-Å distance for selection of epitope atom sets which were virtually identical to those defined by protein interface analysis program PISA.

[0416] Monoclonal antibody CH235.9 amino acid reversion.

[0417] Primers were designed with the online Agilent Quikchange primer designer tool (www.thermofisher.com) and were as follows (SEQ ID NOs: 184-191, in order of appearance):

CH235.9$_{N30T}$: CGTGGCGTCTGGATACAACTTCACCGACTACTATATAC;

CH235.9$_{D31T}$: CGTCTGGATACAACTTCAACACCTACTATATACACTGGGTGC;

CH235.9$_{G62Q}$: GGTCGCACAGATTACGCACAGGCGTTTGGGGA;

CH235.9$_{G65Q}$: GATTACGCAGGGGCGTTTCAGGACAGAGTGTCCA;

CH235.9$_{A103E}$: GTTAGAAATGTGGGAACGGAGGGCAGCTTGCTCCACTATG;

CH235.9$_{G62Q/G65Q}$: GGTCGCACAGATTACGCACAGGCGTTTCAGGACAGAGTGTCCA;

CH235.9$_{S54R}$: GGATCGACCCTAGGGGTGGTCGCACAG;

CH235.9$_{A61S}$: GTGGTCGCACAGATTACTCAGGGGCGTTTG.

[0418] Presence of mutations in plasmid products was confirmed by single-colony sequencing.

[0419] Structural bioinformatics.

[0420] Average buried surface area (BSA) on gp120 was calculated for residues with BSA > 1Å^2 for the gp120-antibody complexes, and the corresponding antibody neutralization potencies were averaged for each of those residues based on data from neutralization assays. Spearman correlation between BSA on gp120 and antibody potencies was calculated for BSA cutoffs = 0 to 85 Å^2 and potency logIC_{50} cutoffs = 0.60 to 1.62 μg/ml.

[0421] Sample preparation for 5' RACE method and 454 pyrosequencing.

[0422] Human PBMCs (6×10^7) were obtained from three HIV-1 and hepatitis C negative individuals (LP32647, LP08248 and LP23810). A 5'RACE approach was developed to amplify immunoglobulin genes based on previously described methods (Venturi et al., 2011). Briefly, the PBMCs were pelleted at 1200 rpm for 8 min. mRNA was then extracted and eluted in 50 μl elution buffer using μMACS mRNA isolation kit (Miltenyi Biotec) according to manufacturer's instructions. To synthesize cDNA, 10 μl mRNA was mixed with 1 μl 5'CDS Oligo dT primers (12 μM) and incubated at 70 °C for 1 min and then -20 °C for 1 min. Then 1 μl SMARTER Oligo Primer (12 μM) (Clontech), 4 μl 5X RT buffer, 1 μl DTT 20 (20mM), 1 μl dNTP (10mM), 1 μl RNAse out and 1 μl SuperScript II reverse transcriptase (Invitrogen) were added to the reaction. After 2 hours incubation at 42 °C, the cDNA products were purified using Nucleospin II kit (Macherey-Nagel) and eluted in 50 μl water. 454 pyrosequencing was performed as described previously (Wu et al., 2011). The first PCR amplification was performed with a common 5' primer II A (Clontech) and an Ig gene specific 3' primer (5'GGGGAAGACCGATGGGCCCTTGGTGG3') (SEQ ID NO: 192) using KAPA HIFI qPCR kit (Kapa Biosystems). The PCR products were purified with 2% Size Select Clonewell E-gel (Invitrogen) and Agencourt AMPure XP beads (Beckman Coulter). The second PCR amplification was performed with primers with 454 sequencing adapters (454-RACE-F: 5'CCATCTCATCCCTGCGTGTCTCCGACTCAGAAGCAGTGGTATCAACGCAGAGT3' (SEQ ID NO: 193); 454-IgG-R: 5'CCTATCCCCTGTGTGCCTTGGCAGTCTCAGGGGGAAGACCGATGGGCCCTTGGTGG3' (SEQ ID NO: 194)). The PCR products were again purified with 2% Size Select Clonewell E-gel and Agencourt AMPure XP beads.

[0423] Germline V gene specific substitution profile.

[0424] The raw reads from three healthy donors shorter than 300 nucleotides or longer than 600 nucleotides in length were not analyzed. Germline V gene was then assigned to each read using an in-house bioinformatics pipeline (Wu et al., 2015). We removed reads containing stop codons. Functional reads were then clustered using Usearch at 97% sequence identity, and one unique sequence was selected from each cluster to derive a curated dataset. To further reduce reads containing sequencing errors in the curated dataset, unique sequences having only one read in the clustering step were excluded. Finally, the curated dataset of the three donors were pooled for substitution frequency analyses.

[0425] Reads from the curated dataset that were assigned to germline V genes of interest were extracted, and were aligned using MUSCLE (Edgar, 2004). The amino acid substitution frequency or mutability of a V gene position was calculated by counting how many reads contain amino acids that are different from the germline V gene, and normalized by the total number of reads. We further calculated the frequency of the 19 types of amino acid substitutions at a position, which was used to generate positional substitution logo. The similarity of positional substitution frequency profiles between V genes of interest was measured by Pearson correlation coefficient.

[0426] Conformity analysis.

[0427] The positional conformity of a conforming antibody sequence A to a reference sequence B is defined as the number of mutated positions shared by both sequences divided by the total number of mutations in the conforming sequence. Thus:

[0428] $c_p(A;B) = \frac{|M_A \cap M_B|}{|M_A|}$

[0429] where M_i represents the set of amino acid positions in sequence i which are mutated from the germline V residue. Insertions and missing data are ignored, but deletions relative to the germline V are counted as mutations. For 8ANC131 and CH235 (Figure 38A):

$M_{8ANC131}$ = {2, 9, 10, 11, 16, 19, 20, 23, 26, 30, 31, 32, 33, 34, 37, 45, 46, 48, 50, 52, 53, 55, 57, 58, 59, 60, 62, 63, 66, 68, 69, 70, 71, 74, 77, 80, 84, 85, 88, 89}

M_{CH235} = {19, 23, 31, 34, 46, 47, 50, 52, 55, 57, 59, 63, 68, 83, 84}

$M_{8ANC131} \cap M_{CH235}$ = {19, 23, 31, 34, 46, 50, 52, 55, 57, 59, 63, 68, 84}

c_p(8ANC131, CH235) = 13 / 15 = 86.7%

[0430] Identity conformity was defined the number of positionally conforming sites in conforming antibody *A* which were also mutated to the same residue as in the reference antibody *B*. Thus:

$$c_i(A;B) = \frac{\sum_{x\in\{M_A\cap M_B\}} \delta_{A_x B_x}}{|M_A|}$$

where δ is the Kronecker delta function and A_x is the identity of the residue at position *x* of sequence *A*. For 8ANC131 and CH235 (Figure 38B): c_i(8ANC131, CH235) = 4 / 15 = 26.7%

[0431] Targeting precision of CD4bs-directed antibodies.

[0432] The targeting precision of the CD4bs-directed antibodies was defined as the buried surface area inside of the CD4 binding site minus the buried surface area outside of the CD4 binding site. The buried surface area of each antigen residue was determined by NACCESS. The buried surface area from the following residue numbers were considered inside of the CD4 binding site: 257, 279, 280, 281, 282, 283, 365, 366, 367, 368, 370, 371, 455, 456, 457, 458, 459, 460, 469, 472, 473, 474, 475, 476, and 477 (Zhou et al., 2007). The buried surface areas from the rest of the residues were considered outside of the CD4 binding site. Somatic hypermutation was defined using nucleotide sequences and *P* values were calculated based on linear regression.

[0433] Antibody binding orientation calculation.

[0434] To calculate the relative rotation angles and translation to gp120-bound CD4 for gp120-bound CD4-binding site antibodies, all antibody-gp120 complexes to be analyzed were first superposed over the outer domain of gp120 (residue ranges: 252-392, 412-422, 437-476) with gp120 in its CD4 complex (PDB ID: 2NXY). The calculations of rotation angles and translation were then carried out with the gp120-aligned structures. For comparison of position of heavy chain variable domain relative to gp120-bound CD4, the frame work regions (residues 46-52, 56-59, 66-71 and 76-82) were superimposed to regions of CD4 domain 1 (residues 34-40, 43-46, 54-59, 65-71). The superposition procedures were performed with the Superpose Molecules module in CCP4 (Collaborative Computational Project, 1994). The Chi angle and distance between centroids in the Superpose output was taken as the rotation angle and translation distance between CD4 and a CD4-binding site antibody.

[0435] Supplemental References

[0436] Collaborative Computational Project (1994). The CCP4 suite: programs for protein crystallography. Acta Crystallogr., Sect D: Biol. Crystallogr. *50*, 760-763.

[0437] Edgar, R.C. (2004). MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res. *32*, 1792-1797.

[0438] Giorgi, E.E., Funkhouser, B., Athreya, G., Perelson, A.S., Korber, B.T., Bhattacharya, T. (2010). Estimating time since infection in early homogeneous HIV-1 samples using a poisson model. BMC Bioinformatics *11,* 532.

[0439] Gray, E.S., Moody, M.A., Wibmer, C.K., Chen, X., Marshall, D., Amos, J., Moore, P.L., Foulger, A., Yu, J.S., Lambson, B., *et al.* (2011). Isolation of a monoclonal antibody that targets the alpha-2 helix of gp120 and represents the initial autologous neutralizing-antibody response in an HIV-1 subtype C-infected individual. J. Virol. *85*, 7719-7729.

[0440] Lander, G.C., Stagg, S.M., Voss, N.R., Cheng, A., Fellmann, D., Pulokas, J., Yoshioka, C., Irving, C., Mulder, A., Lau, P.W., *et al.* (2009). Appion: an integrated, database-driven pipeline to facilitate EM image processing. J. Struct. Biol. *166*, 95-102.

[0441] Montefiori, D.C. (2005). Evaluating neutralizing antibodies against HIV, SIV, and SHIV in luciferase reporter gene assays. Current protocols in immunology / edited by John E Coligan [et al] *Chapter 12*, Unit 12 11.

[0442] Pettersen, E.F., Goddard, T.D., Huang, C.C., Couch, G.S., Greenblatt, D.M., Meng, E.C., and Ferrin, T.E. (2004). UCSF Chimera--a visualization system for exploratory research and analysis. J. Comput. Chem. *25*, 1605-1612.

[0443] Scheres, S.H. (2012). A Bayesian view on cryo-EM structure determination. J. Mol. Biol. *415*, 406-418.

[0444] Seaman, M.S., Janes, H., Hawkins, N., Grandpre, L.E., Devoy, C., Giri, A., Coffey, R.T., Harris, L., Wood, B., Daniels, M.G., *et al.* (2010). Tiered categorization of a diverse panel of HIV-1 Env pseudoviruses for assessment of neutralizing antibodies. J. Virol. *84*, 1439-1452.

[0445] Schrodinger, LLC (2010). The PyMOL Molecular Graphics System, Version 1.3r1.

[0446] Suloway, C., Pulokas, J., Fellmann, D., Cheng, A., Guerra, F., Quispe, J., Stagg, S., Potter, C.S., and Carragher, B. (2005). Automated molecular microscopy: the new Leginon system. J. Struct. Biol. *151*, 41-60.

[0447] Tang, G., Peng, L., Baldwin, P.R., Mann, D.S., Jiang, W., Rees, I., and Ludtke, S.J. (2007). EMAN2: an extensible image processing suite for electron microscopy. J. Struct. Biol. *157*, 38-46.

[0448] Tomaras, G,D,, Yates, N.L., Liu, P., Qin, L., Fouda, G.G., Chavez, L.L., Decamp, A.C., Parks, R.J., Ashley, V.C., Lucas, J.T., *et al.* (2008). Initial B-cell responses to

transmitted human immunodeficiency virus type 1: virion-binding immunoglobulin M (IgM) and IgG antibodies followed by plasma anti-gp41 antibodies with ineffective control of initial viremia. J. Virol. *82,* 12449-12463

[0449] van Dongen, J.J., Langerak, A.W., Bruggemann, M., Evans, P.A., Hummel, M., Lavender, F.L., Delabesse, E., Davi, F., Schuuring, E., Garcia-Sanz, R., et al. (2003). Design and standardization of PCR primers and protocols for detection of clonal immunoglobulin and T-cell receptor gene recombinations in suspect lymphoproliferations: report of the BIOMED-2 Concerted Action BMH4-CT98-3936. Leukemia 17, 2257-2317.

[0450] Venturi, V., Quigley, M.F., Greenaway, H.Y., Ng, P.C., Ende, Z.S., McIntosh, T., Asher, T.E., Almeida, J.R., Levy, S., Price, D.A., *et al.* (2011). A mechanism for TCR sharing between T cell subsets and individuals revealed by pyrosequencing. J Immunol. *186*, 4285-4294.

[0451] Wu, X., Yang, Z.Y., Li, Y., Hogerkorp, C.M., Schief, W.R., Seaman, M.S., Zhou, T., Schmidt, S.D., Wu, L., Xu, L., *et al.* (2010). Rational design of envelope identifies broadly neutralizing human monoclonal antibodies to HIV-1. Science *329*, 856-861.

[0452] Wu, X., Zhang, Z., Schramm, C.A., Joyce, M.G., Kwon, Y.D., Zhou, T., Sheng, Z., Zhang, B., O'Dell, S., McKee, K., *et al.* (2015). Maturation and Diversity of the VRC01-Antibody Lineage over 15 Years of Chronic HIV-1 Infection. Cell *161*, 470-485.

[0453] Zhou, T., Xu, L., Dey, B., Hessell, A.J., Van Ryk, D., Xiang, S.H., Yang, X., Zhang, M.Y., Zwick, M.B., Arthos, J., *et al.* (2007). Structural definition of a conserved neutralization epitope on HIV-1 gp120. Nature *445*, 732-737.

[0454] ACCESSION NUMBERS

[0455] Coordinates and structure factors for CH235, CH235.9 and CH235.12 in complex with HIV-1 gp120 have been deposited with the Protein Data Bank (PDB ID 5F9W, 5F9O and 5F96). Next-generation sequencing data have been deposited with the NCBI Sequence Reads Archive (SRP067168). Antibody heavy and light chains have been deposited with GenBank (KU570032-KU570053).

[0456] Antibodies Names correlation

[0457] Various antibodies names are used throughout the application. Antibodies names correlation is as follows: CH490=CH235.6; CH491=CH235.7; CH492=CH235.8; CH493=CH235.9; CH555=CH235.10; CH556=CH235.11; CH557=CH235.12.

[0458] REFERENCES FOR EXAMPLE 8

[0459] Alam, S.M., McAdams, M., Boren, D., Rak, M., Scearce, R.M., Gao, F., Camacho, Z.T., Gewirth, D., Kelsoe, G., Chen, P., *et al.* (2007). The role of antibody polyspecificity and lipid reactivity in binding of broadly neutralizing anti-HIV-1 envelope human monoclonal antibodies 2F5 and 4E10 to glycoprotein 41 membrane proximal envelope epitopes. J. Immunol. *178*, 4424-4435.

[0460] Alam, S.M., Morelli, M., Dennison, S.M., Liao, H.X., Zhang, R., Xia, S.M., Rits-Volloch, S., Sun, L., Harrison, S.C., Haynes, B.F., *et al.* (2009). Role of HIV membrane in neutralization by two broadly neutralizing antibodies. Proc. Natl. Acad. Sci. U. S. A. *106*, 20234-20239.

[0461] Bonsignori, M., Alam, S.M., Liao, H.X., Verkoczy, L., Tomaras, G.D., Haynes, B.F., and Moody, M.A. (2012). HIV-1 antibodies from infection and vaccination: insights for guiding vaccine design. Trends Microbiol. *20*, 532-539.

[0462] Bonsignori, M., Hwang, K.K., Chen, X., Tsao, C.Y., Morris, L., Gray, E., Marshall, D.J., Crump, J.A., Kapiga, S.H., Sam, N.E., *et al.* (2011). Analysis of a clonal lineage of HIV-1 envelope V2/V3 conformational epitope-specific broadly neutralizing antibodies and their inferred unmutated common ancestors. J. Virol. *85*, 9998-10009.

[0463] Bonsignori, M., Wiehe, K., Grimm, S.K., Lynch, R., Yang, G., Kozink, D.M., Perrin, F., Cooper, A.J., Hwang, K.K., Chen, X., *et al.* (2014). An autoreactive antibody from an SLE/HIV-1 individual broadly neutralizes HIV-1. J. Clin. Invest. *124*, 1835-1843.

[0464] Boyd, S.D., Marshall, E.L., Merker, J.D., Maniar, J.M., Zhang, L.N., Sahaf, B., Jones, C.D., Simen, B.B., Hanczaruk, B., Nguyen, K.D., *et al.* (2009). Measurement and Clinical Monitoring of Human Lymphocyte Clonality by Massively Parallel V-D-J Pyrosequencing. Sci. Transl. Med. *1*.

[0465] Dosenovic, P., von Boehmer, L., Escolano, A., Jardine, J., Freund, N.T., Gitlin, A.D., McGuire, A.T., Kulp, D.W., Oliveira, T., Scharf, L., *et al.* (2015). Immunization for HIV-1 Broadly Neutralizing Antibodies in Human Ig Knockin Mice. Cell *161*, 1505-1515.

[0466] Felsenstein, J. (2005). PHYLIP (Phylogeny Inference Package), 3.6a3 edn (Seattle, WA: distributed by the author: Department of Genome Sciences, University of Washington).

[0467] Foote, J., and Milstein, C. (1991). Kinetic maturation of an immune response. Nature *352*, 530-532.

[0468] Gao, F., Bonsignori, M., Liao, H.X., Kumar, A., Xia, S.M., Lu, X., Cai, F., Hwang, K.K., Song, H., Zhou, T., *et al.* (2014). Cooperation of B cell lineages in induction of HIV-1-broadly neutralizing antibodies. Cell *158*, 481-491.

[0469] Georgiev, I.S., Doria-Rose, N.A., Zhou, T.Q., Do Kwon, Y., Staupe, R.P., Moquin, S., Chuang, G.Y., Louder, M.K., Schmidt, S.D., Altae-Tran, H.R., *et al.* (2013). Delineating Antibody Recognition in Polyclonal Sera from Patterns of HIV-1 Isolate Neutralization. Science *340*, 751-756.

[0470] Haynes, B.F. (2015). New approaches to HIV vaccine development. Curr. Opin. Immunol. *35*, 39-47.

[0471] Haynes, B.F., and Bradley, T. (2015). Broadly Neutralizing Antibodies and the Development of Vaccines. JAMA *313*, 2419-2420.

[0472] Haynes, B.F., Fleming, J., St Clair, E.W., Katinger, H., Stiegler, G., Kunert, R., Robinson, J., Scearce, R.M., Plonk, K., Staats, H.F., *et al.* (2005). Cardiolipin polyspecific autoreactivity in two broadly neutralizing HIV-1 antibodies. Science *308*, 1906-1908.

[0473] Haynes, B.F., Kelsoe, G., Harrison, S.C., and Kepler, T.B. (2012). B-cell-lineage immunogen design in vaccine development with HIV-1 as a case study. Nat. Biotechnol. *30*, 423-433.

[0474] Haynes, B.F., and Verkoczy, L. (2014). AIDS/HIV. Host controls of HIV neutralizing antibodies. Science *344*, 588-589.

[0475] Hraber, P., Korber, B., Wagh, K., Giorgi, E.E., Bhattacharya, T., Gnanakaran, S., Lapedes, A.S., Learn, G.H., Kreider, E.F., Li, Y., *et al.* (2015). Longitudinal Antigenic Sequences and Sites from Intra-Host Evolution (LASSIE) Identifies Immune-Selected HIV Variants. Viruses *7*, 5443-5475.

[0476] Hraber, P., Seaman, M.S., Bailer, R.T., Mascola, J.R., Montefiori, D.C., and Korber, B.T. (2014). Prevalence of broadly neutralizing antibody responses during chronic HIV-1 infection. AIDS *28*, 163-169.

[0477] Jardine, J., Julien, J.P., Menis, S., Ota, T., Kalyuzhniy, O., McGuire, A., Sok, D., Huang, P.S., MacPherson, S., Jones, M., *et al.* (2013). Rational HIV immunogen design to target specific germline B cell receptors. Science *340*, 711-716.

[0478] Jardine, J.G., Ota, T., Sok, D., Pauthner, M., Kulp, D.W., Kalyuzhniy, O., Skog, P.D., Thinnes, T.C., Bhullar, D., Briney, B., *et al.* (2015). HIV-1 VACCINES. Priming a broadly neutralizing antibody response to HIV-1 using a germline-targeting immunogen. Science *349*, 156-161.

[0479] Keele, B.F., Giorgi, E.E., Salazar-Gonzalez, J.F., Decker, J.M., Pham, K.T., Salazar, M.G., Sun, C., Grayson, T., Wang, S., Li, H., *et al.* (2008). Identification and characterization

of transmitted and early founder virus envelopes in primary HIV-1 infection. Proc. Natl. Acad. Sci. U. S. A. *105*, 7552-7557.

[0480] Kepler, T.B. (2013). Reconstructing a B-cell clonal lineage. I. Statistical inference of unobserved ancestors. F1000Res *2*, 103.

[0481] Kepler, T.B., Munshaw, S., Wiehe, K., Zhang, R., Yu, J.S., Woods, C.W., Denny, T.N., Tomaras, G.D., Alam, S.M., Moody, M.A., *et al.* (2014). Reconstructing a B-Cell Clonal Lineage. II. Mutation, Selection, and Affinity Maturation. Front. Immunol. *5*, 170.

[0482] Klein, F., Diskin, R., Scheid, J.F., Gaebler, C., Mouquet, H., Georgiev, I.S., Pancera, M., Zhou, T., Incesu, R.B., Fu, B.Z., *et al.* (2013). Somatic mutations of the immunoglobulin framework are generally required for broad and potent HIV-1 neutralization. Cell *153*, 126-138.

[0483] Liao, H.X., Lynch, R., Zhou, T., Gao, F., Alam, S.M., Boyd, S.D., Fire, A.Z., Roskin, K.M., Schramm, C.A., Zhang, Z., *et al.* (2013). Co-evolution of a broadly neutralizing HIV-1 antibody and founder virus. Nature *496*, 469-476.

[0484] Liu, M., Yang, G., Wiehe, K., Nicely, N.I., Vandergrift, N.A., Rountree, W., Bonsignori, M., Alam, S.M., Gao, J., Haynes, B.F., *et al.* (2015). Polyreactivity and autoreactivity among HIV-1 antibodies. J. Virol. *89*, 784-798.

[0485] Mascola, J.R., and Haynes, B.F. (2013). HIV-1 neutralizing antibodies: understanding nature's pathways. Immunol. Rev. *254*, 225-244.

[0486] McDonald, I.K., and Thornton, J.M. (1994). Satisfying hydrogen bonding potential in proteins. J. Mol. Biol. *238*, 777-793.

[0487] McGuire, A.T., Hoot, S., Dreyer, A.M., Lippy, A., Stuart, A., Cohen, K.W., Jardine, J., Menis, S., Scheid, J.F., West, A.P., *et al.* (2013). Engineering HIV envelope protein to activate germline B cell receptors of broadly neutralizing anti-CD4 binding site antibodies. J. Exp. Med. *210*, 655-663.

[0488] Otwinowski, Z., and Minor, W. (1997). Processing of X-ray diffraction data collected in oscillation mode. Method Enzymol. *276*, 307-326.

[0489] Pugach, P., Ozorowski, G., Cupo, A., Ringe, R., Yasmeen, A., de Val, N., Derking, R., Kim, H.J., Korzun, J., Golabek, M., *et al.* (2015). A native-like SOSIP.664 trimer based on an HIV-1 subtype B env gene. J. Virol. *89*, 3380-3395.

[0490] Rudicell, R.S., Do Kwon, Y., Ko, S.Y., Pegu, A., Louder, M.K., Georgiev, I.S., Wu, X.L., Zhu, J., Boyington, J.C., Chen, X.J., *et al.* (2014). Enhanced Potency of a Broadly

Neutralizing HIV-1 Antibody In Vitro Improves Protection against Lentiviral Infection In Vivo. J. Virol. *88*, 12669-12682.

[0491] Sanders, R.W., Derking, R., Cupo, A., Julien, J.P., Yasmeen, A., de Val, N., Kim, H.J., Blattner, C., de la Pena, A.T., Korzun, J., *et al.* (2013). A next-generation cleaved, soluble HIV-1 Env trimer, BG505 SOSIP.664 gp140, expresses multiple epitopes for broadly neutralizing but not non-neutralizing antibodies. PLoS Pathog. *9*, e1003618.

[0492] Scheid, J.F., Mouquet, H., Feldhahn, N., Seaman, M.S., Velinzon, K., Pietzsch, J., Ott, R.G., Anthony, R.M., Zebroski, H., Hurley, A., *et al.* (2009). Broad diversity of neutralizing antibodies isolated from memory B cells in HIV-infected individuals. Nature *458*, 636-640.

[0493] Scheid, J.F., Mouquet, H., Ueberheide, B., Diskin, R., Klein, F., Oliveira, T.Y., Pietzsch, J., Fenyo, D., Abadir, A., Velinzon, K., *et al.* (2011). Sequence and structural convergence of broad and potent HIV antibodies that mimic CD4 binding. Science *333*, 1633-1637.

[0494] Sui, Z.W., Chen, Q.J., Wu, R., Zhang, H.B., Zheng, M., Wang, H.Z., and Chen, Z. (2010). Cross-protection against influenza virus infection by intranasal administration of M2-based vaccine with chitosan as an adjuvant. Arch. Virol. *155*, 535-544.

[0495] Verkoczy, L., Chen, Y., Zhang, J., Bouton-Verville, H., Newman, A., Lockwood, B., Scearce, R.M., Montefiori, D.C., Dennison, S.M., Xia, S.M., *et al.* (2013). Induction of HIV-1 broad neutralizing antibodies in 2F5 knock-in mice: selection against membrane proximal external region-associated autoreactivity limits T-dependent responses. J. Immunol. *191*, 2538-2550.

[0496] Verkoczy, L., Diaz, M., Holl, T.M., Ouyang, Y.B., Bouton-Verville, H., Alam, S.M., Liao, H.X., Kelsoe, G., and Haynes, B.F. (2010). Autoreactivity in an HIV-1 broadly reactive neutralizing antibody variable region heavy chain induces immunologic tolerance. Proc. Natl. Acad. Sci. U. S. A. *107*, 181-186.

[0497] Verkoczy, L., Kelsoe, G., Moody, M.A., and Haynes, B.F. (2011). Role of immune mechanisms in induction of HIV-1 broadly neutralizing antibodies. Curr. Opin. Immunol. *23*, 383-390.

[0498] Wu, X., Yang, Z.Y., Li, Y., Hogerkorp, C.M., Schief, W.R., Seaman, M.S., Zhou, T., Schmidt, S.D., Wu, L., Xu, L., *et al.* (2010). Rational design of envelope identifies broadly neutralizing human monoclonal antibodies to HIV-1. Science *329*, 856-861.

[0499] Wu, X., Zhou, T., Zhu, J., Zhang, B., Georgiev, I., Wang, C., Chen, X., Longo, N.S., Louder, M., McKee, K., *et al.* (2011). Focused evolution of HIV-1 neutralizing antibodies revealed by structures and deep sequencing. Science *333*, 1593-1602.

[0500] Yang, G., Holl, T.M., Liu, Y., Li, Y., Lu, X., Nicely, N.I., Kepler, T.B., Alam, S.M., Liao, H.X., Cain, D.W., *et al.* (2013). Identification of autoantigens recognized by the 2F5 and 4E10 broadly neutralizing HIV-1 antibodies. J. Exp. Med. *210*, 241-256.

[0501] Zhou, T., Georgiev, I., Wu, X., Yang, Z.Y., Dai, K., Finzi, A., Kwon, Y.D., Scheid, J.F., Shi, W., Xu, L., *et al.* (2010). Structural basis for broad and potent neutralization of HIV-1 by antibody VRC01. Science *329*, 811-817.

[0502] Zhou, T., Lynch, R.M., Chen, L., Acharya, P., Wu, X., Doria-Rose, N.A., Joyce, M.G., Lingwood, D., Soto, C., Bailer, R.T., *et al.* (2015). Structural Repertoire of HIV-1-Neutralizing Antibodies Targeting the CD4 Supersite in 14 Donors. Cell *161*, 1280-1292.

[0503]

[0504]

Example 10: DH542 antibodies

[0505] 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

[0506] >DH542_HC_nt (SEQ ID NO: 178)

CAGGTGCAGCTGGTGCAGTCTGGGGCTCAAATGAAGAACCCTGGGGCCTCAGTGAAGGTCTCCTGCGC
GCCTTCTGGATATACCTTCACCGACTTTTACATACATTGGTTGCGCCAGGCCCCTGGCCAGGGGCTTC
AGTGGATGGGATGGATGAACCCTCAGACTGGTCGCACAAACACTGCACGAAACTTTCAGGGGAGGGTC
ACCATGACCAGGGACACGTCCATCGGCACAGCCTACATGGAGTTGAGAAGCCTGACATCTGACGACAC
GGCCATATATTACTGTACGACAGGGGGATGGATCAGTCTTTACTATGATAGTAGTTATTACCCCAACT
TTGACCACTGGGGTCAGGGAACCCTGCTCACCGTCTCCTCAG

[0507] >DH542_HC_aa (SEQ ID NO: 179)

QVQLVQSGAQMKNPGASVKVSCAPSGYTFTDFYIHWLRQAPGQGLQWMGWMNPQTGRTNTARNFQGRV
TMTRDTSIGTAYMELRSLTSDDTAIYYCTTGGWISLYYDSSYYPNFDHWGQGTLLTVSS

[0508] >DH542_LC_nt_corrected (DH542_QSA) (SEQ ID NO: 180)

CAGTCTGCCCTGACTCAGCCTGCCTCCGTGTCTGGGTCTCCTGGACAGTCGATCACCATCTCCTGCAC
TGGAACCAAGTATGATGTTGGGAGTCATGACCTTGTCTCCTGGTACCAACAGTACCCAGGCAAAGTCC
CCAAATACATGATTTATGAAGTCAATAAACGGCCCTCAGGAGTTTCTAATCGCTTCTCTGGCTCCAAA
TCTGGCAACACGGCCTCCCTGACAATCTCTGGGCTCCGGGCTGAGGACGAGGCTGACTATTATTGCTG
TTCATTTGGAGGGAGTGCCACCGTGGTCTGCGGCGGCGGGACCAAGGTGACCGTCCTAg

[0509] >DH542_LC_aa_corrected (DH542_QSA) (SEQ ID NO: 181)

QSALTQPASVSGSPGQSITISCTGTKYDVGSHDLVSWYQQYPGKVPKYMIYEVNKRPSGVSNRFSGSK
SGNTASLTISGLRAEDEADYYCCSFGGSATVVCGGGTKVTVL

[0510] DH542-L4 is an antibody that has a VH of DH542 and VL of DH429 (Figure 2).

Example 10: Exemplary HIVxCD3 Bispecific Diabodies with Fc Domains

[0511] 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.

[0512] An HIVxCD3 bispecific diabody with an Fc Domain designated "**CH557xCD3 Fc**" was designed and expressed. This molecule comprises three polypeptide chains and includes an HIV-1 binding arm derived from the CH557 antibody described herein, a CD3 effector cell binding arm derived from a humanized anti-CD3ε mAb (hXRCD3), and CH2-CH3 IgG1 Fc Domains. The general structure of the polypeptide chains and a schematic of the assembled chains are shown in **Figures 5A-5B**. The amino acid sequences and a representative polynucleotide encoding each polypeptide chain are presented in **Figures 5C-5D**. A corresponding negative control bispecific diabody with an irrelevant binding arm [αRSV derived from palivizumab] instead of the CH557 HIV-1 arm (designated "**RSVxCD3 Fc,**") was also generated. In addition, a comparator bispecific diabody (designated "**A32xCD3 Fc**") having an HIV-1 binding arm derived from the A32 antibody (Protein Data Bank (PBD) ID Code 3TNM) instead of CH557, and additional negative control bispecific diabodies having the A32 HIV-1 arm with an irrelevant binding arm [αRSV or αfluorescein] instead of the CD3 arm (designated "**A32xRSV Fc,**" and "**A32x4420**," respectively) were generated. The HIVxCD3 Fc bispecific diabodies are capable of simultaneously binding to HIV-1 and CD3. The control RSVxCD3 Fc bispecific diabody is capable of simultaneously binding to RSV and CD3 and the control A32xRSV Fc bispecific diabody is capable of simultaneously binding to HIV-1 and RSV. Each of the generated bispecific diabodies is a heterotrimer of polypeptide Chains 1, 2 and 3 have the general structure provided in **Figure 3A** (also see, *e.g.*,

Figures 5A-5B). Chains 1 and 2 comprise the VH and VL Domains of CH557, A32, XRCD3, or palivizumab, as detailed above, while Chain 3 is common to each diabody molecule.

[0513] 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.

[0514] Table 5 provides the SEQ ID NOs for each of the polypeptide chains present in CH557xCD3 Fc molecule. **Table 4** provides SEQ ID NOs for the VL and VH Domains of CH557 (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), 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	**SEQ ID NO: 555**	**SEQ ID NO: 556**
("CH557xCD3 Fc")	**SEQ ID NO: 557**	**SEQ ID NO: 558**
(Variable domain from CH557, binds to HIV-1 gp120)	**SEQ ID NO: 559**	**SEQ ID NO: 560**

[0515] For additional sequences related to this example see Table 4.

Example 10A: Binding Properties of HIVxCD3 Bispecific Diabodies with Fc Domains

[0516] The binding of CH557xCD3 Fc to recombinant HIV-1 *Env* and human CD3 was examined by ELISA assay. Briefly, microtiter plates were coated with recombinant proteins (human CD3ε/δ heterodimer, or M.CONS gp140 (Liao HX, *et al.* A group M consensus envelope glycoprotein induces antibodies that neutralize subsets of subtype B and C HIV-1

primary viruses. *Virology*. 2006;353(2):268–282)) in buffer and blocked. Serial dilutions of the bispecific diabodies (CH557xCD3 Fc, the comparator A32xCD3 Fc, or the controls RSVxCD3 Fc and A32xRSV Fc) were applied followed by sequential addition of biotinylated anti-EK coil antibody and streptavidin-HRP. For bispecific binding assays, the plate was coated with M.CONS gp140, and diabody application was followed by sequential addition of biotinylated CD3ε/δ and streptavidin-HRP. HRP activity was detected with a chemiluminescent substrate. For bispecific binding assays, the plate was coated with M.CONS gp140, and diabody application was followed by sequential addition of biotinylated CD3ε/δ and streptavidin-HRP. CH557xCD3 Fc and A32xCD3 Fc both exhibited binding to recombinant human CD3 and HIV-1 *Env*, individually and simultaneously, while the control molecules, RSVxCD3 Fc and A32xRSV Fc, only exhibited binding to human CD3 or HIV-1 *Env*, respectively, as shown by ELISA (**Figures 6A-6C**).

[0517] Cell surface binding of CH557xCD3 Fc to cells expressing HIV-1 *Env* (HEK293-D371 cells, expressing HIV-1 CM244 (subtype AE) gp140) and human CD3 (human primary T cells) was examined by flow cytometric analysis. Briefly, Serial dilutions of the bispecific diabodies: CH557xCD3 Fc; the comparator A32xCD3 Fc; or the control RSVxCD3 Fc, were incubated with HEK293-D371 cells or human primary T cells (Pan T cells) in FACS buffer containing a blocking agent (*e.g.*, human albumin serum). After washing, cells were resuspended in buffer containing biotin-conjugated mouse anti-EK antibody (recognizes the E/K heterodimerization region of diabody proteins), mixed with streptavidin-PE and incubated in the dark. Cells were washed, resuspended with FACS buffer, and analyzed by flow cytometry. CH557xCD3 Fc and A32xCD3 Fc exhibited binding to both HEK293-D375 and Pan T cells, while the control, RSVxCD3 Fc, only exhibited binding to Pan T cells (**Figures 7A-7B**). CH557xCD3 Fc exhibited stronger binding to HEK293-D375 cells in these studies (**Figure 7A**).

Example 10B: Cell Killing Activity of HIVxCD3 Bispecific Diabodies with Fc Domains

[0518] The ability of CH557xCD3 Fc to mediate redirected cell killing of target cells expressing HIV-1 *Env* was examined using a cytotoxic T lymphocyte (CTL) assay. Briefly, the HIV-1 *Env* expressing cell line HEK293-D371 was treated with serial dilutions of the bispecific monovalent diabodies: CH557xCD3 Fc; the comparator A32xCD3 Fc; or the control RSVxCD3 Fc, together with effector cells (human PBMCs) from two different donors

at an E/T ratio of 30:1 for 24 hours. The percentage cytotoxicity (*i.e.*, cell killing) was determined by measuring the release of lactate dehydrogenase (LDH) into the media by damaged cells as described previously (Moore PA et al. Application of dual affinity retargeting molecules to achieve optimal redirected T-cell killing of B-cell lymphoma. *Blood.* 2011;117(17):4542–4551). As measured by LDH release assays, CH557xCD3 Fc and A32xCD3 Fc both mediated redirected human immune cells derived from healthy donors to kill the HEK293-D371 cells in a concentration dependent manner at an E:T ratio of 30:1. The average EC50 values were 13.8 ng/mL and 12.3 ng/mL for CH557xCD3 Fc and A32xCD3 Fc, respectively. In contrast, no diabody-mediated redirected T-cell killing occurred with the RSVxCD3 control diabody in which the HIV-1 arm was replaced by an irrelevant one (**Figures 8A-8B**).

Example 10C: Binding Properties of HIVxCD3 Bispecific Diabodies with Fc Domains to autologous HIV-1 CH557 envelope

Methods.

[0519] The 491 (control) and 598 (CH505 Transmitted/Founder Envelope- and GFP-transfected cell lines were used to detect the ability of the diabodies of interest to bind specifically to the HIV-1 envelope of the CH505 transmitted/founder isolate. The cell lines were incubated for 24 hours with Doxycycline at concentrations of 0.5, 0.0125, and 0.0031, and 0ug/ml to achieve different levels of expression of the HIV-1 CH505 Envelope as determined in preliminary experiments. The GFP expression was used to determine the level of expression as of the transduced proteins. The A32x4420 and CH557xhXR32 diabodies were tested for staining at the concentration of 10 and 1 μg/ml. Palivizumabx4420 (PAlix4420) and PalivizumabxhXR32 (PalixhXR32) diabodies based on the anti-RSV mAb were used as control for each of the two anti-HIV-1 Env diabodies, respectively, using the same concentrations. In addition the A32 (anti-C1/C2 Env mAb; positive control), Synagis (anti-RSV mAb, negative control), and CH65 (anti-Flu HA mAb; negative control) were used as controls for the specificity of the staining.

[0520] After 24hr. incubation with the different doses of Doxycycline, each cells culture was plated with and without Palix4420, PalixhXR32, A32 x 4420, and CH557 x hXR32 DARTS at 1ug/ml and 10ug/ml. In addition, the same cell culture were plated with and without Synagis, A32, and CH65 human mAbs at 1ug/ml and 10ug/ml.

[0521] All the different cell cultures were incubated with diabodies and human mAbs for 2hrs at 37 degrees Celsius.

[0522] After 2hr incubation cells were washed 2x in PBS and stained with a LIVE/DEAD cell marker (Aqua LIVE/DEAD) for 20 minutes at RT.

[0523] After viability staining, cells were washed 2x in Wash Buffer and appropriate secondary mixes (anti-EK + IgG2b PE for DARTS or IgG PE for human mAbs) were added to wells for 30min at 4 degrees Celsius. The anti-EK and anti-EK+IgG2 conditions were used to stain the cells as additional control conditions.

[0524] Cells were washed 2x in Wash Buffer and fixed in 1% Formalin Solution until acquiring on BD LSR Fortessa.

[0525] The analysis of data was performed by gating on the viable (Aqua Live/Dead negative) and GFP positive cells for the 598 cell line and on viable cells for the 491 control cell line. Each frequency reported in the figure refers to the percentage of diabodies or mAb positive cells referred to the Live+/GFP+ cells.

[0526] Results: The levels of GFP expression detected with each Doxycycline concentration in each staining condition are reported in Figure 48 and 49 for the diabodies and control mAbs, respectively.

[0527] As expected, we observed three different levels of GFP expression ranging from >80% of cells to less than 20%. A minimal constitutive expression of GFP was also observed in absence of Doxycycline.

[0528] The analysis of the staining with the A32x4420 and CH557xhXR32 to the 491 control cell line did not reveal any non specific staining (Figure 50, top panels). In contrast, the 598 cell lines were stained proportionally to the level of GFP observed due to the stimulation with Doxycycline, ranging from 82% to 11.7% with the CH557xhXR32 DART, and CH557xhXR32 diabody binding was always better than the A32x4420 diabody binding at each concentration (Figure 50 bottom panels). A minimum background of 10% staining with the CH557xhXR32 was observed when the 598 cell line was used without incubation with Doxycycline. This is due to a minimum constitutive expression of the transduced genes as indicated in Figure 48 and 49 for GFP expression. The staining with the A32 mAb indicated the expression of HIV-1 Env on the membrane of the cells according to a similar profile and the specificity of the staining of the 598 cell line (Figure 51).

[0529] The analysis of the staining with diabody and mAb controls represented by the anti-EK, anti-EK+IgG2, Palix4420, PalixhXR32, Synagis, and CH65 mAbs combination did not reveal non-specific staining of the 598 cell line.

Example 11A: Additional multispecific molecules

[0530] Any one of the antibodies from the CH235 lineage or DH270 lineage could be constructed in 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 CH235 lineage members or DH270 lineage members using CDRs, or VH and VL chains from these HIV-1 antibodies.

[0531] **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 **CH235 lineage** antibodies or DH270 lineage antibodies using CDRs, or VH and VL chains from these antibodies.

Table 6					
Bivalent HIVxCD3 (or HIV-1 x CD16) **Heterodimer of Chains 1 and 2 (see, *e.g.*, Figure 1)**					
Polypeptide Chain 1		**Polypeptide Chain 2**		**Polypeptide Chain 3**	**none**
NH_2-VL(HIV-1)	SEQ ID NOs: 553 565 567 570 574 578 582 586 590 594	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: 551 564 566 568 572 576 580 584 588 592		
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		

Bivalent HIVxCD3 Fc (or HIV x CD16 Fc) version 1 **Heterotrimer of Chains 1, 2 and 3 (see, *e.g.*, Figure 3A)**					
Polypeptide Chain 1	(*e.g.*, SEQ ID NO: V)	**Polypeptide Chain 2**	(*e.g.*, SEQ ID NO: W)	**Polypeptide Chain 3**	(*e.g.*, SEQ ID NO: X)
NH_2-VL(HIV-1)	SEQ ID NOs: 553 565 567 570 574 578 582 586 590 594	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: 551 564 566 568 572 576 580 584 588 592		
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: 519 or 518	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 3B)**					
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
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
Peptide Linker 4 or 4.1	SEQ ID NO: 524 or 525				

VL(HIV-1)	SEQ ID NOs: 553 565 567 570 574 578 582 586 590 594	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: 551 564 566 568 572 576 580 584 588 592		
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 2B)					
Polypeptide Chain 1 (x2)		**Polypeptide Chain 2 (x2)**		**Polypeptide Chain 3**	**None**
NH_2-VL(HIV-1)	SEQ ID NOs: 553 565 567 570 574 578 582 586 590 594	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: 551 564 566 568 572 576 580 584 588 592		
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				

[0532] Wherein SEQ ID NOs: 553, 565, 567, 570, 574, 578, and 582 correspond to the VL chain of antibody CH557, CH556, CH555, CH493, CH492, CH491, and CH490, respectively and as described in Table 4. Wherein SEQ ID NOs: 586, 590, and 594 correspond to the VL chain of antibody DH542, DH542_QSA, and D542_L4, respectively and as described in Table 4.

[0533] Wherein SEQ ID NOs: 551, 564, 566, 568, 572, 576, and 580 correspond to the VH chain of antibody CH557, CH556, CH555, CH493, CH492, CH491, and CH490, respectively and as described in Table 4. Wherein SEQ ID NOs: 584, 588, and 592 correspond to the VH chain of antibody DH542, DH542_QSA, and D542_L4, respectively and as described in Table 4.

Example 11B: Exemplary Trivalent and Control Molecules with Fc Domains

[0534] 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*). 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.

[0535] **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 **CH235 lineage** antibodies or DH270 lineage antibodies using CDRs, or VH and VL chains from these antibodies.

Table 7

HIVxCD3xCD8 Heterotetramer of Chains 1-4 (see, *e.g.*, Figure 4A)		**HIVxCD3xCD8 Heterotrimer of Chains 1-3 (see, *e.g.*, Figure 4D)**		**HIVxCD3xHIV Heterotetramer of Chains 1-4 (see, *e.g.*, Figure 4A)**	
Polypeptide Chain 1	See *e.g.*, SEQ ID NO: 555	**Polypeptide Chain 1**	See *e.g.*, SEQ ID NO: 555	**Polypeptide Chain 1**	
NH_2-VL(HIV-1)	SEQ ID NOs: 553 565 567 570 574 578 582 586 590 594	NH_2-VL(HIV-1)	SEQ ID NOs: 553 565 567 570 574 578 582 586 590 594	NH_2-VL(HIV-1)	SEQ ID NOs: 553 565 567 570 574 578 582 586 590 594
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 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	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	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 *e.g.*, SEQ ID NO: 556	**Polypeptide Chain 2**	See *e.g.*, SEQ ID NO: 556	**Polypeptide Chain 2**	
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: 551 564 566 568 572 576 580 584 588 592	VH(HIV-1)	SEQ ID NOs: 551 564 566 568 572 576 580 584 588 592	VH(HIV-1)	SEQ ID NOs: 551 564 566 568 572 576 580 584 588 592
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 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	E-coil or K-coil	SEQ ID NO: 518 or 519 or SEQ ID NO: 520 or 521
Polypeptide Chain 3	See *e.g.*, SEQ ID NO: 561	**Polypeptide Chain 3**	See *e.g.*, SEQ ID NO: 563	**Polypeptide Chain 3**	
		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: 551 564 566 568 572 576 580 584 588 592
CH1-hinge	SEQ ID NO: 515	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	CH2-CH3 (knob bearing) or CH2-CH3 (hole bearing)	SEQ ID NO: 531 or 532 or SEQ ID NO: 533 or 534
Polypeptide Chain 4	See *e.g.*, SEQ ID NO: 562	**Polypeptide Chain 4**	**none**	**Polypeptide Chain 4**	
NH_2-VL(CD8)	SEQ ID NO: 545 or 549			NH_2-VL(HIV-1)	SEQ ID NOs: 553 565 567 570 574 578 582 586 590 594
CL kappa or CL lambda	SEQ ID NO: 516 or 517			CL kappa or CL lambda	SEQ ID NO: 516 or 517

[0536] Wherein SEQ ID NOs: 553, 565, 567, 570, 574, 578, and 582 correspond to the VL chain of antibody CH557, CH556, CH555, CH493, CH492, CH491, and CH490, respectively and

as described in Table 4. Wherein SEQ ID NOs: 586, 590, and 594 correspond to the VL chain of antibody DH542, DH542_QSA, and D542_L4, respectively and as described in Table 4.

[0537] Wherein SEQ ID NOs: 551, 564, 566, 568, 572, 576, and 580 correspond to the VH chain of antibody CH557, CH556, CH555, CH493, CH492, CH491, and CH490, respectively and as described in Table 4. Wherein SEQ ID NOs: 584, 588, and 592 correspond to the VH chain of antibody DH542, DH542_QSA, and D542_L4, respectively and as described in Table 4.

[0538] **Table 4** provides the amino acid sequences of one such exemplary trivalent binding molecule having four polypeptide chain 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 CH557 (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 CH557 (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 CH557xCD3 Fc described above.

[0539] **Table 4** provides the amino acid sequences of another such exemplary trivalent binding molecule 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 CH557 (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 CH557 (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 CH557xCD3 Fc described above.

[0540] 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 (*e.g.*, CH557).

[0541] 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 cytolysis of latently HIV-1-infected cells. *J Clin Invest.* 2015;125(11):4077-4090; and WO2016/054101).

Example 12: Combinations of multispecific anitbodies

[0542] Multispecific antibodies with different HIV-1 specificity, e.g CD4 binding site and V3 glycan binding, could also be tested in combination.

[0543] 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 cytolysis of latently HIV-1-infected cells. *J Clin Invest.* 2015;125(11):4077-4090; and WO2016/054101). Ccombinations of multispecific molecules, either with different HIV-1 specificity and/or different effector cell specificity, will be tested whether they provide enhanced benefits.

Example 13: Exemplary V3 glycan Specific HIVxCD3 Bispecific Diabody

[0544] As provided herein, the binding specificity of the anti-HIV-1 antibody DH542 differs from the other anti-HIV-1 antibodies provide herein, in that it is specific for the V3 glycan of HIV-1 *Env*, According, the VH and VL Domains of DH542 are utilized in the generation of HIVxCD3 bispecific diabodies (and/or trivalent binding molecule as provided herein) having binding specificity for the V3 glycan of HIV-1 *Env*. Exemplary, HIVxCD3 bispecific diabodies, having three polypeptide chains, an HIV-1 binding arm derived from the DH542 antibody described herein, a CD3 effector cell binding arm derived from a humanized anti-CD3ε mAb (hXRCD3), and CH2-CH3 IgG1 Fc Domains may have the general structure shown in **Figures 5A-5B**. **Table 8** provides the SEQ ID NOs for each of the polypeptide chains present in one non-limiting embodiment of such a molecule, designated herein as "**DH542xCD3 Fc**". It will be appreciated based on the instant disclosure that the third polypeptide chain of DH542xCD3 Fc may be identical to the HIVxCD3 bivalent diabodies having an Fc Domain provide above.

Table 8

Bispecific Molecule	Polypeptide Chain Amino Acid Sequences
HIVxCD3 Bispecific Diabody with Fc Domain **("DH542xCD3 Fc")** (Variable domain from DH542, binds to HIV-1 V3 glycan)	**SEQ ID NO: 596** **SEQ ID NO: 597** **SEQ ID NO: 559**

[0545] Chain 1 of this exemplary molecule comprises: an N-terminus, the VL Domain of DH542 (SEQ ID NO: 586), 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 DH542 (SEQ ID NO: 584), 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, 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.

[0546] The ability of such exemplary HIV-1 *Env* V3 glycan binding bispecific diabodies to bind to HIV-1 *Env* V3 glycan and CD3 may be evaluated using the methods describe above, or well known in the art (see, *e.g.*, WO2016/054101). The activity of such exemplary bispecific diabodies 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 cytolysis of latently HIV-1-infected cells. *J Clin Invest.* 2015;125(11):4077-4090; and WO2016/054101).

Example 14: Prophetic examples

[0547] 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

[0548] 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.

[0549] Methods and Reagents

[0550] *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).

[0551] *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).

[0552] *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.

[0553] The ability of CH557xCD3 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 cytolysis 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).

[0554] *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 x10^5 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% CO_2 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 x (1-(RLU of Sample ÷ 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 (EC_{50}) 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).

[0555] *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. SeePollara 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.

[0556] *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 hour. 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 titered 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).

[0557] *T-Cell Viability and Activation Assays.* $CD8^+$ T cells and CD8 depleted PBMCs obtained from HIV infected ART suppressed patients are plated at $5x10^4$ 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).

[0558] *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 ($5x10^4$) 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 1μM of raltegravir and 4μM 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)**.**

[0559] *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 (4μg/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 10^6 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.

[0560] 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.

[0561] All documents and other information sources cited herein are hereby incorporated in their entirety by reference.

What is claimed is:

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 CD4 binding site 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 CD4 binding site HIV-1 antibody 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 CD4 binding site 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 CD4 binding site 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 CD4 binding site 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 CD4 binding site 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 CD4 binding site 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 CD4 binding site 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 CD4 binding site 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 CD4 binding site 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 CD4 binding site 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 CD4 binding site 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 CD4 binding site HIV-1antibody, 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 CD4 binding site HIV-1 antibody, and at least one of epitope (1), epitope (2), and epitope (3) is and 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 CD4 binding site HIV-1 antibody is CH557 (also referred to as CH235.12) or any of the antibodies of the CH235 lineage.

20. The molecules of claims 1-12, wherein the molecule binds HIV-1 envelope with the specificity of CH557 antibody 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 CH557, CH556, CH555, CH493, CH492, CH491, or CH490.

22. The molecules of claims 1-12, wherein domain (E) comprises the CDR1, CDR2, and CDR3 of the heavy chain variable domain of immunoglobulin CH557, CH556, CH555, CH493, CH492, CH491, or CH490.

23. The molecules of claims 1-12, wherein domain (A) comprises the light chain variable domain of immunoglobulin CH557, CH556, CH555, CH493, CH492, CH491, or CH490.

24. The molecules of claims 1-12, wherein domain (E) comprises the heavy chain variable domain of immunoglobulin CH557, CH556, CH555, CH493, CH492, CH491, or CH490.

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 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.

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 1-31or 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 CH557, 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 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 secondmolecules 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, inonomycin, 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.

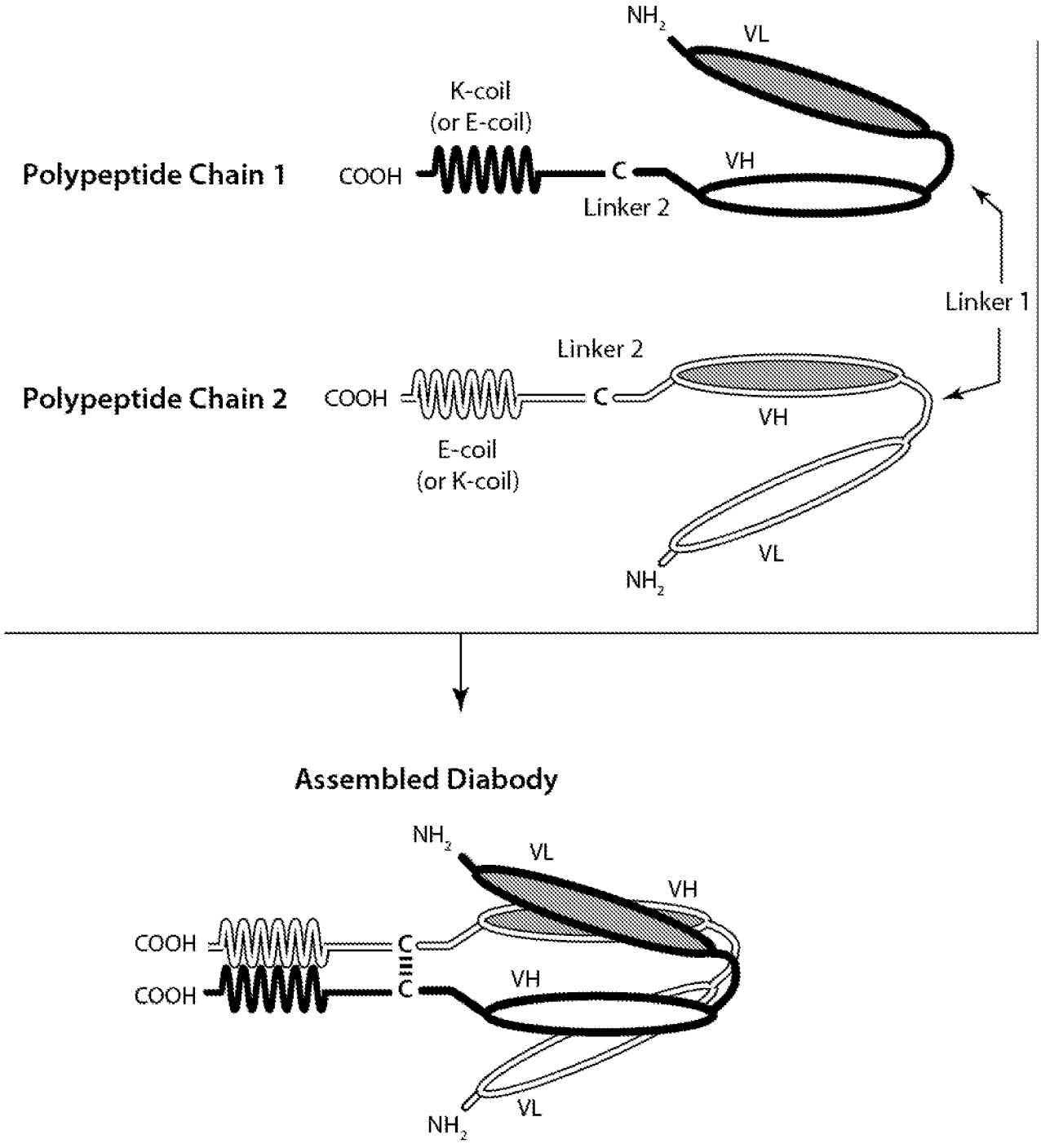
NH2
VL
K-coil
(or E-coil)
Polypeptide Chain 1
COOH
C
VH
Linker 2
Linker 1
Linker 2
Polypeptide Chain 2
COOH
C
VH
E-coil
(or K-coil)
VL
NH2
Assembled Diabody
NH2
VL
VH
COOH
C
COOH
C
VH
VL
NH2

Figure 1

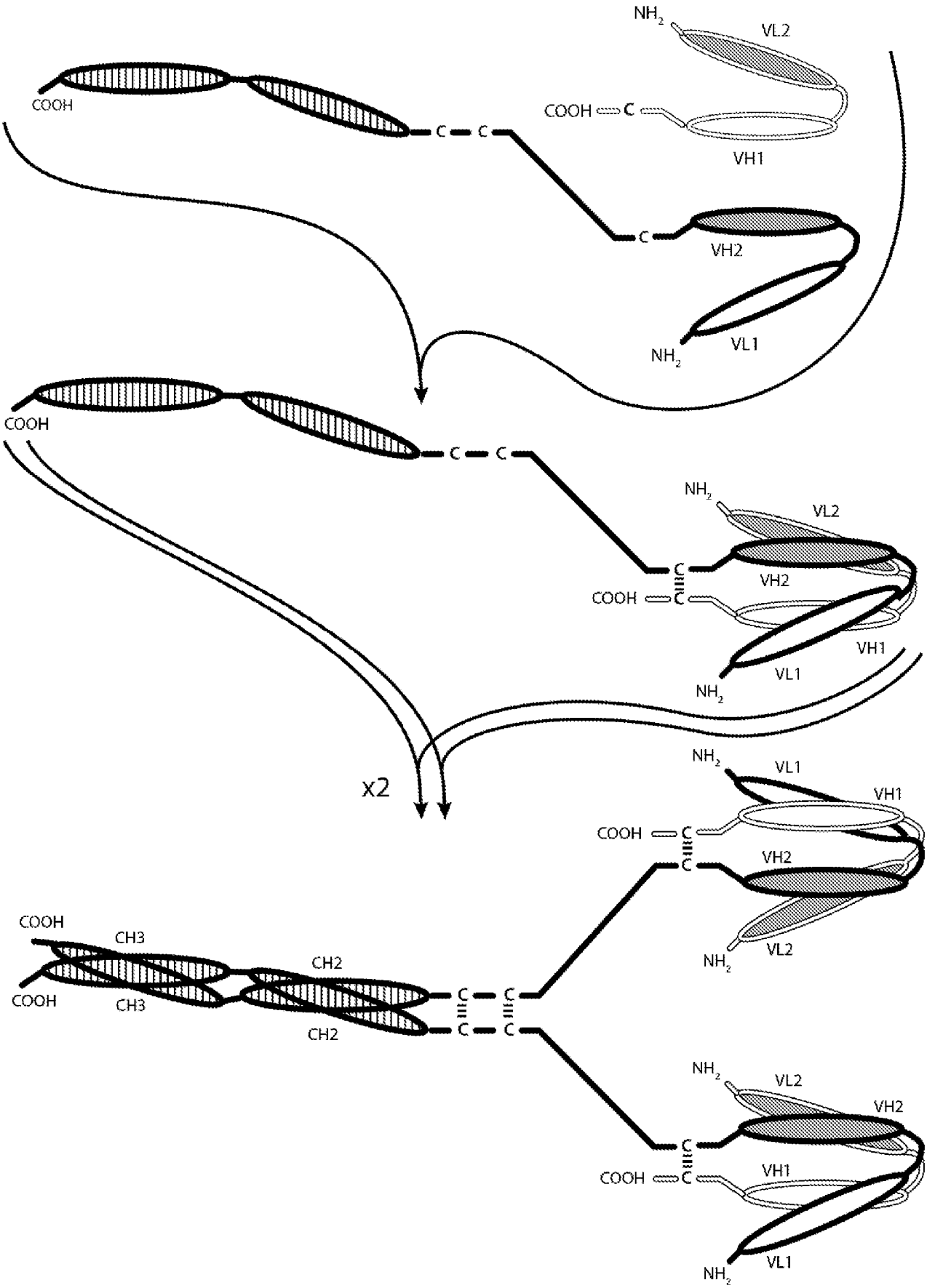
NH2
VL2
COOH
C
C
COOH
C
VH1
C
VH2
NH2
VL1
COOH
C
C
NH2
VL2
C
VH2
COOH
C
VH1
NH2
VL1
x2
NH2
VL1
VH1
COOH
C
C
VH2
NH2
VL2
COOH
CH3
CH2
COOH
CH3
CH2
C
C
C
C
NH2
VL2
VH2
C
C
COOH
VH1
NH2
VL1

Figure 2A

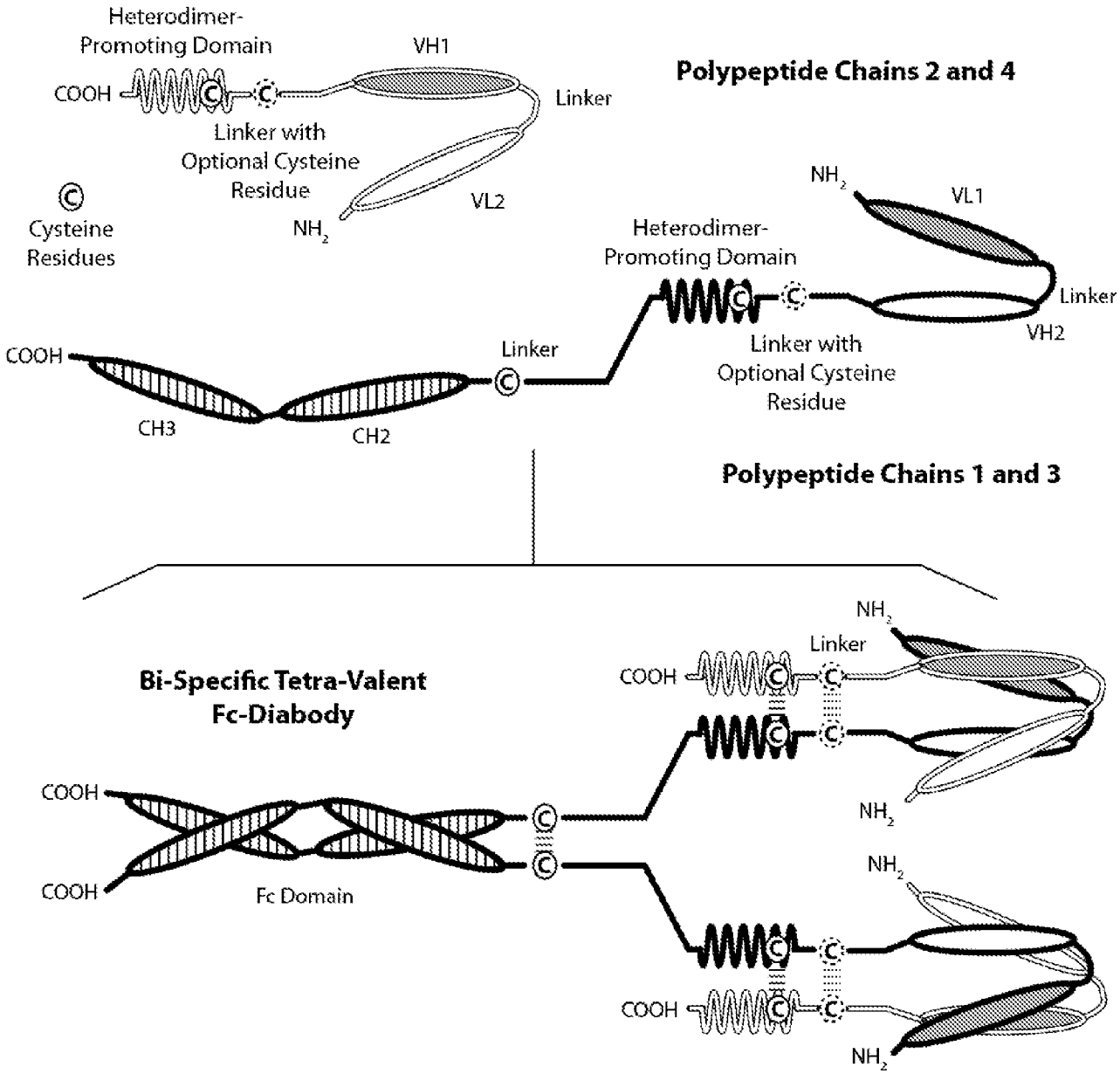
Heterodimer-
Promoting Domain
VH1
COOH
Linker
Linker with
Optional Cysteine
Residue
VL2
NH2
Cysteine
Residues
Polypeptide Chains 2 and 4
NH2
VL1
Heterodimer-
Promoting Domain
Linker
VH2
Linker with
Optional Cysteine
Residue
COOH
Linker
CH3
CH2
Polypeptide Chains 1 and 3
Bi-Specific Tetra-Valent
Fc-Diabody
NH2
Linker
COOH
COOH
COOH
Fc Domain
NH2
NH2
COOH
NH2

Figure 2B

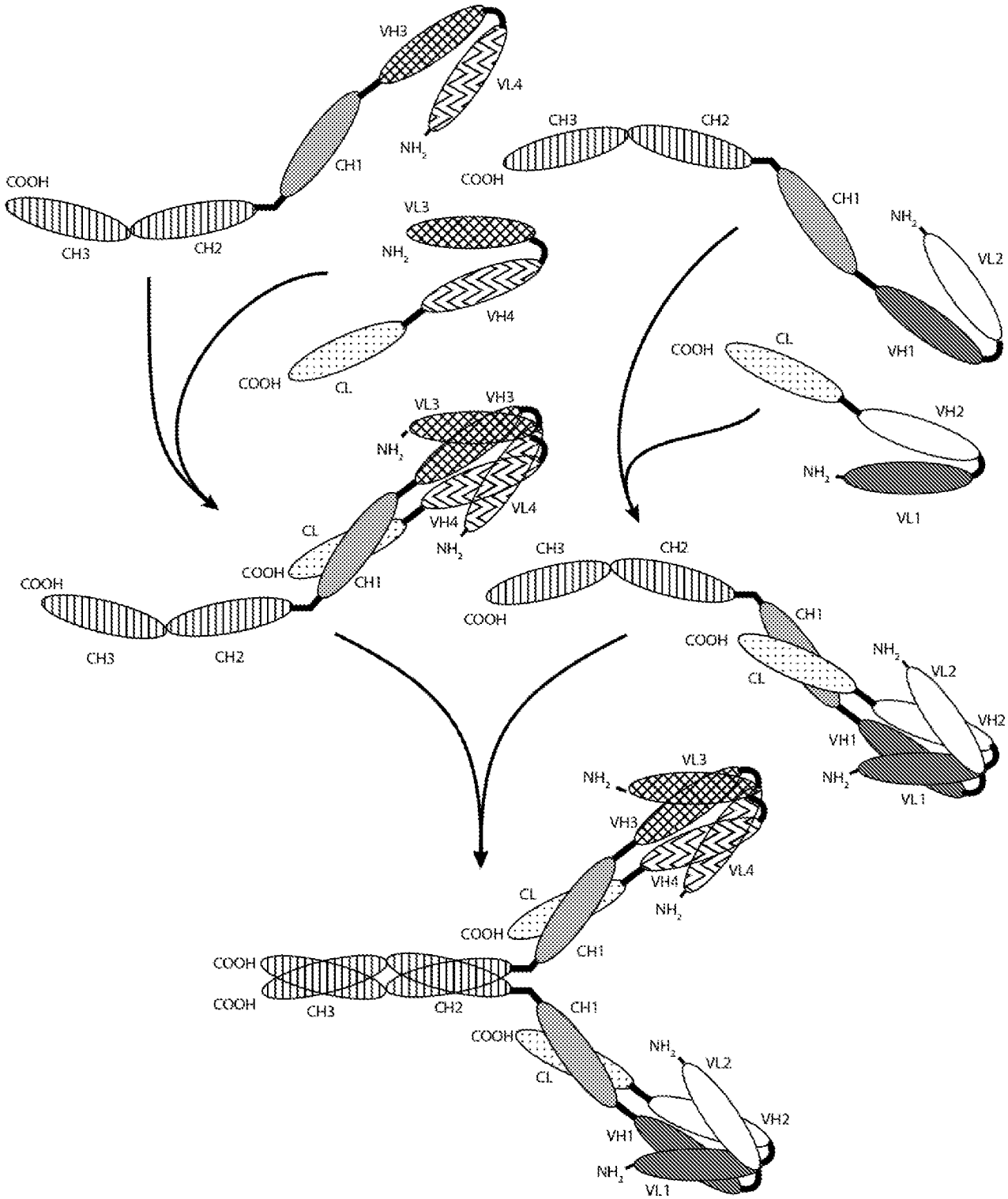

Figure 2C

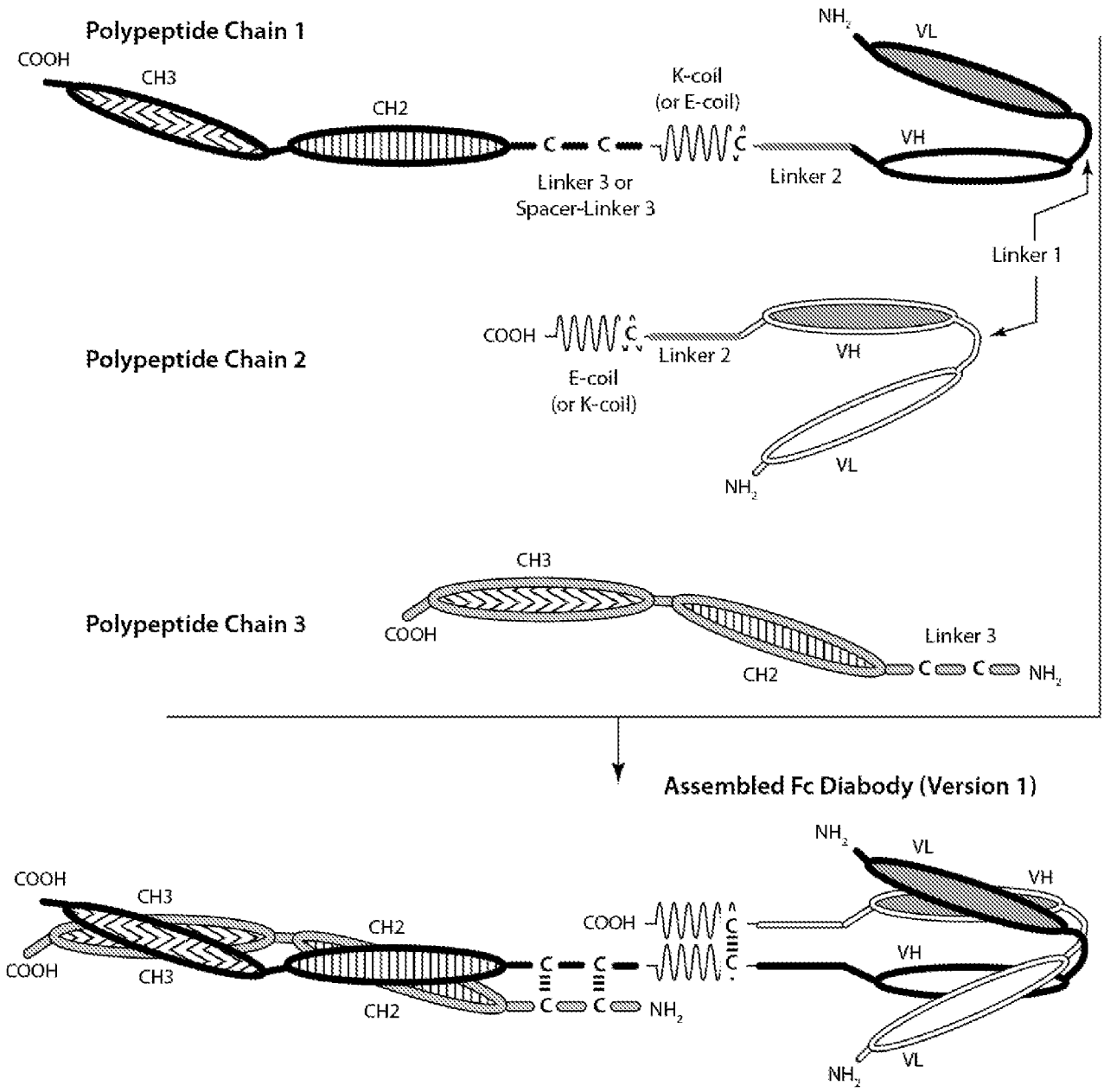
Polypeptide Chain 1
COOH
CH3
CH2
K-coil
(or E-coil)
Linker 3 or
Spacer-Linker 3
Linker 2
NH2
VL
VH
Linker 1
Polypeptide Chain 2
COOH
E-coil
(or K-coil)
Linker 2
VH
VL
NH2
Polypeptide Chain 3
CH3
COOH
CH2
Linker 3
NH2
Assembled Fc Diabody (Version 1)
COOH
CH3
CH2
COOH
CH3
CH2
NH2
VL
VH
COOH
VH
NH2
VL

Figure 3A

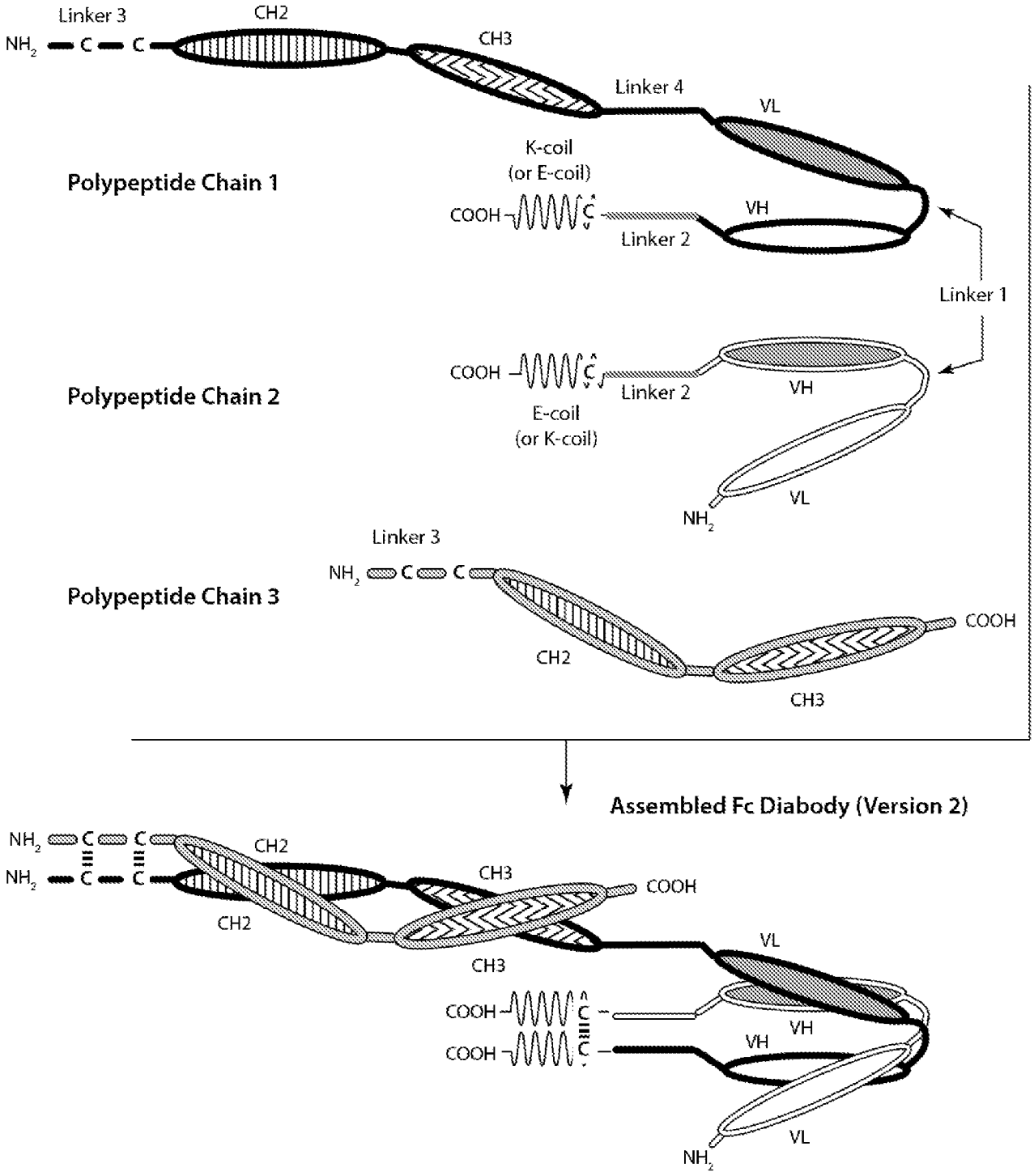
Linker 3
CH2
CH3
Linker 4
VL
K-coil
(or E-coil)
Polypeptide Chain 1
VH
Linker 2
Linker 1
Polypeptide Chain 2
E-coil
(or K-coil)
Linker 3
Polypeptide Chain 3
COOH
Assembled Fc Diabody (Version 2)

Figure 3B

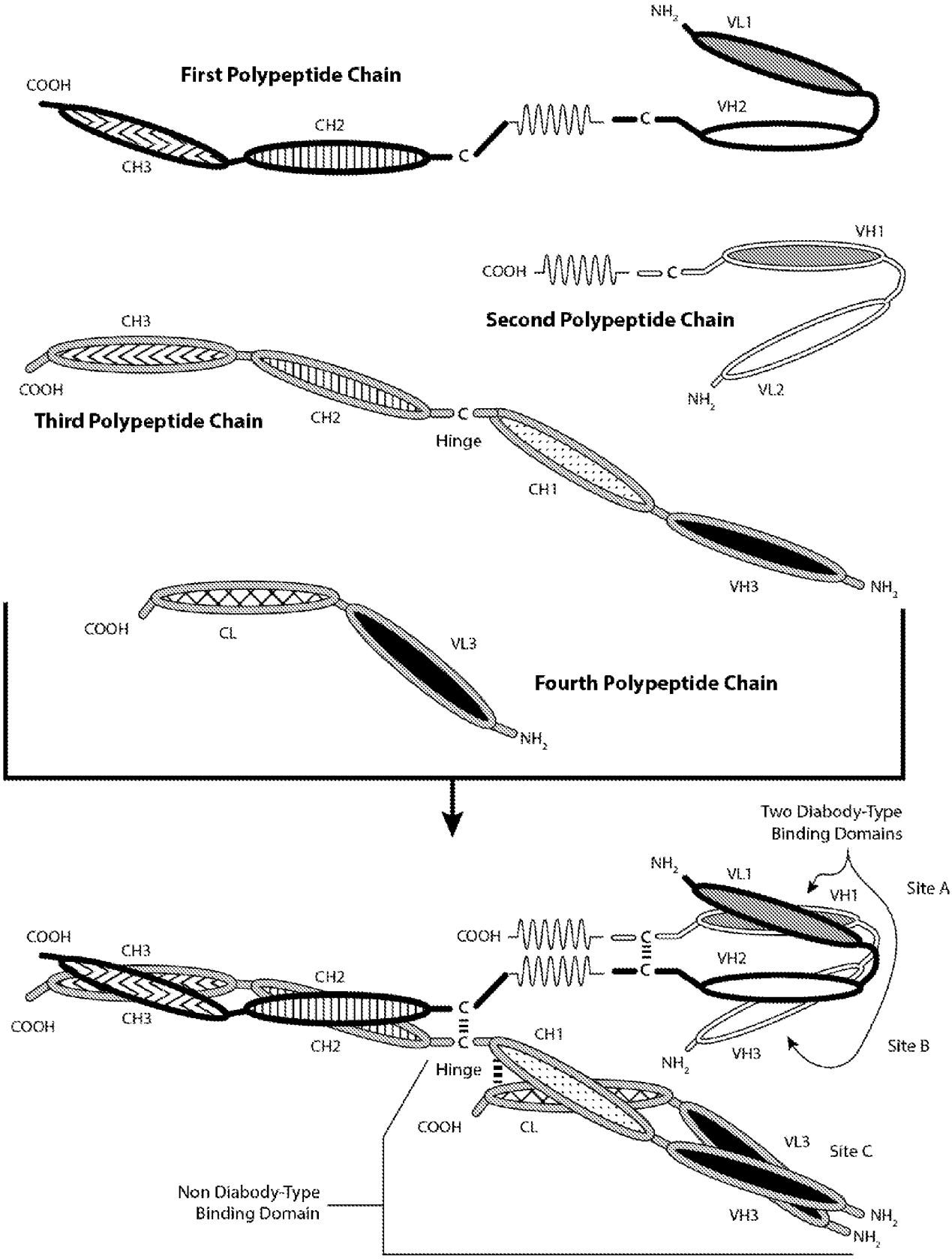
First Polypeptide Chain
COOH
CH2
CH3
C
NH2
VL1
VH2
Second Polypeptide Chain
VH1
COOH
C
NH2
VL2
Third Polypeptide Chain
CH3
COOH
CH2
C
Hinge
CH1
VH3
NH2
Fourth Polypeptide Chain
COOH
CL
VL3
NH2
Two Diabody-Type Binding Domains
Site A
Site B
Site C
Non Diabody-Type Binding Domain

Figure 4A

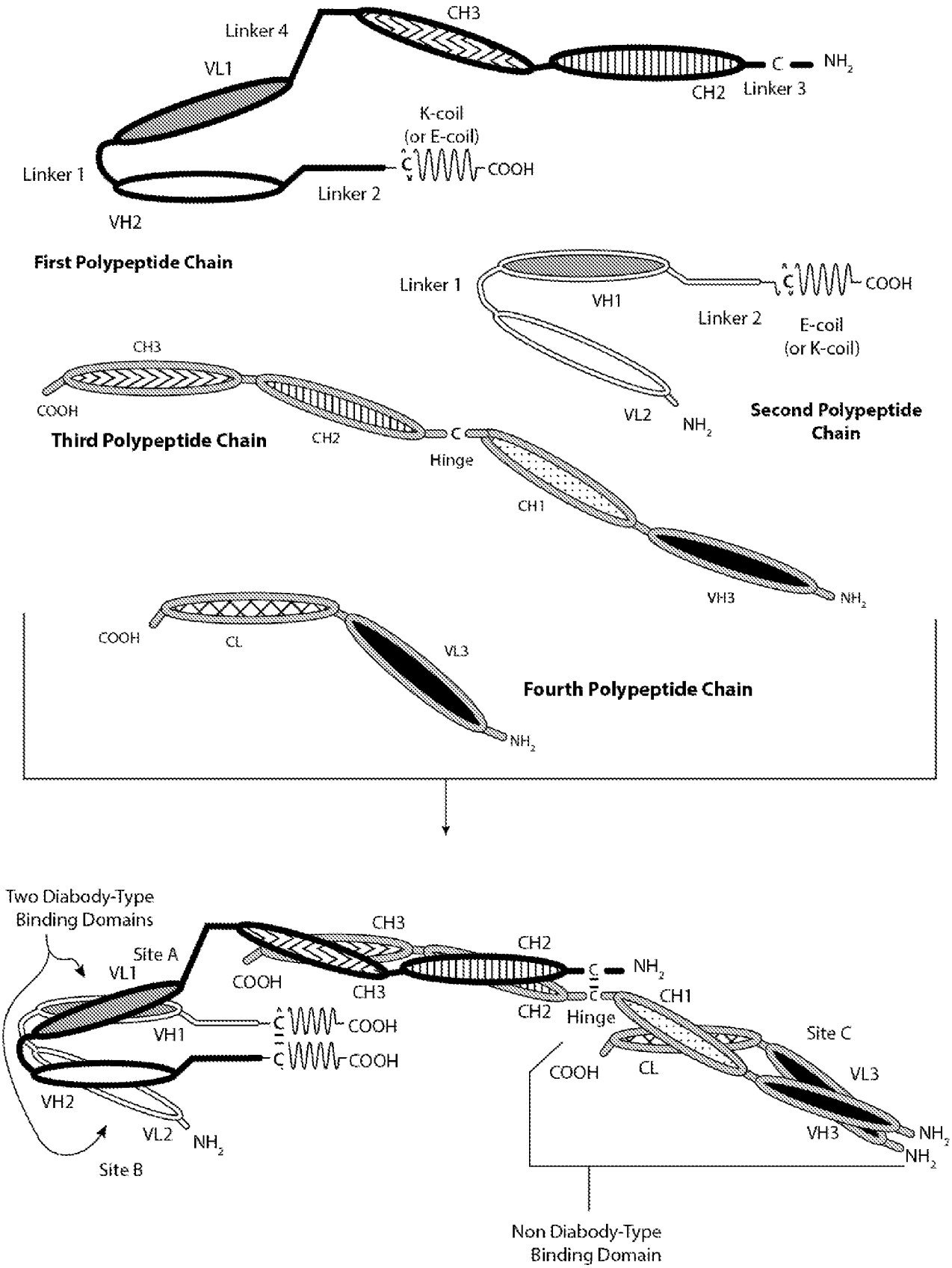
CH3
Linker 4
VL1
C
NH2
CH2
Linker 3
K-coil
(or E-coil)
COOH
Linker 1
Linker 2
VH2
First Polypeptide Chain
Linker 1
VH1
COOH
Linker 2
E-coil
(or K-coil)
CH3
COOH
VL2
NH2
Second Polypeptide Chain
Third Polypeptide Chain
CH2
C
Hinge
CH1
VH3
NH2
COOH
CL
VL3
Fourth Polypeptide Chain
NH2
Two Diabody-Type
Binding Domains
Site A
VL1
CH3
CH2
COOH
CH3
C
NH2
CH2
CH1
Hinge
VH1
COOH
COOH
Site C
COOH
CL
VL3
VH2
VH3
NH2
VL2
NH2
NH2
Site B
Non Diabody-Type
Binding Domain

Figure 4B

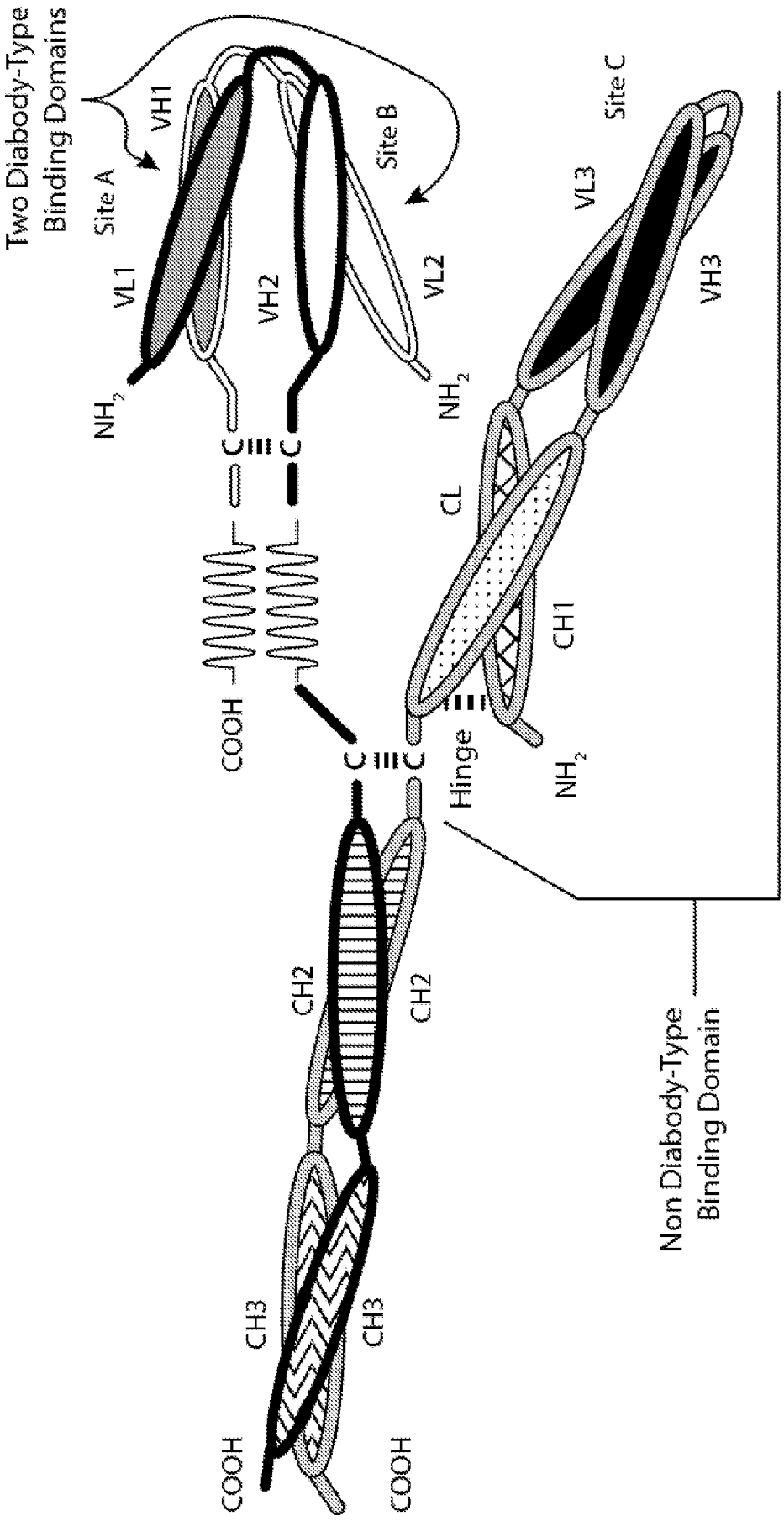
Two Diabody-Type Binding Domains
Site A
VH1
VL1
VH2
Site B
VL2
NH2
NH2
C
C
COOH
CL
VL3
Site C
VH3
CH1
NH2
Hinge
C
C
CH2
CH2
CH3
CH3
COOH
COOH
Non Diabody-Type Binding Domain

Figure 4C

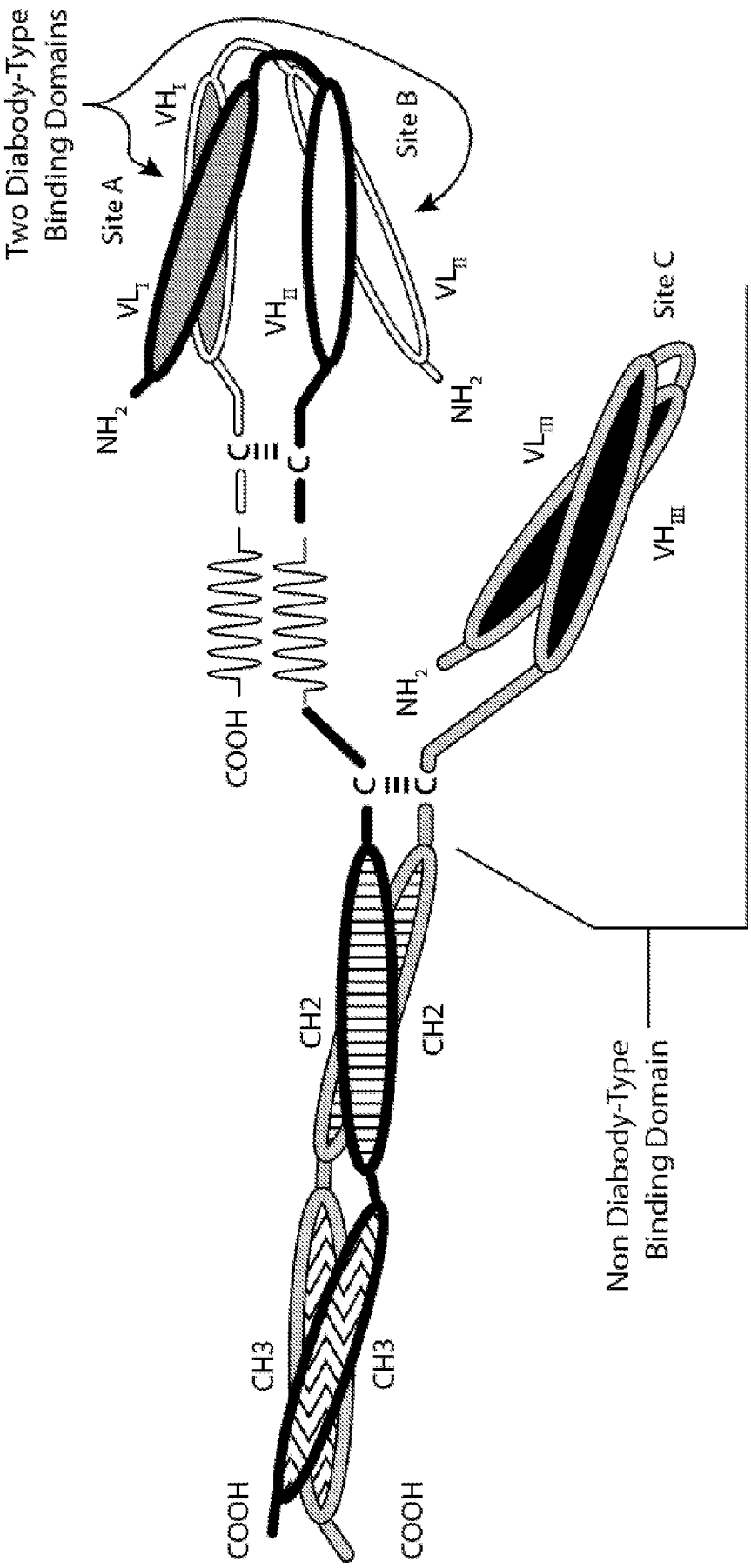
Two Diabody-Type Binding Domains
Site A
Site B
Site C
VH_I
VL_I
VH_{II}
VL_{II}
VL_{III}
VH_{III}
NH_2
NH_2
NH_2
COOH
COOH
COOH
CH2
CH2
CH3
CH3
Non Diabody-Type Binding Domain

Figure 4D

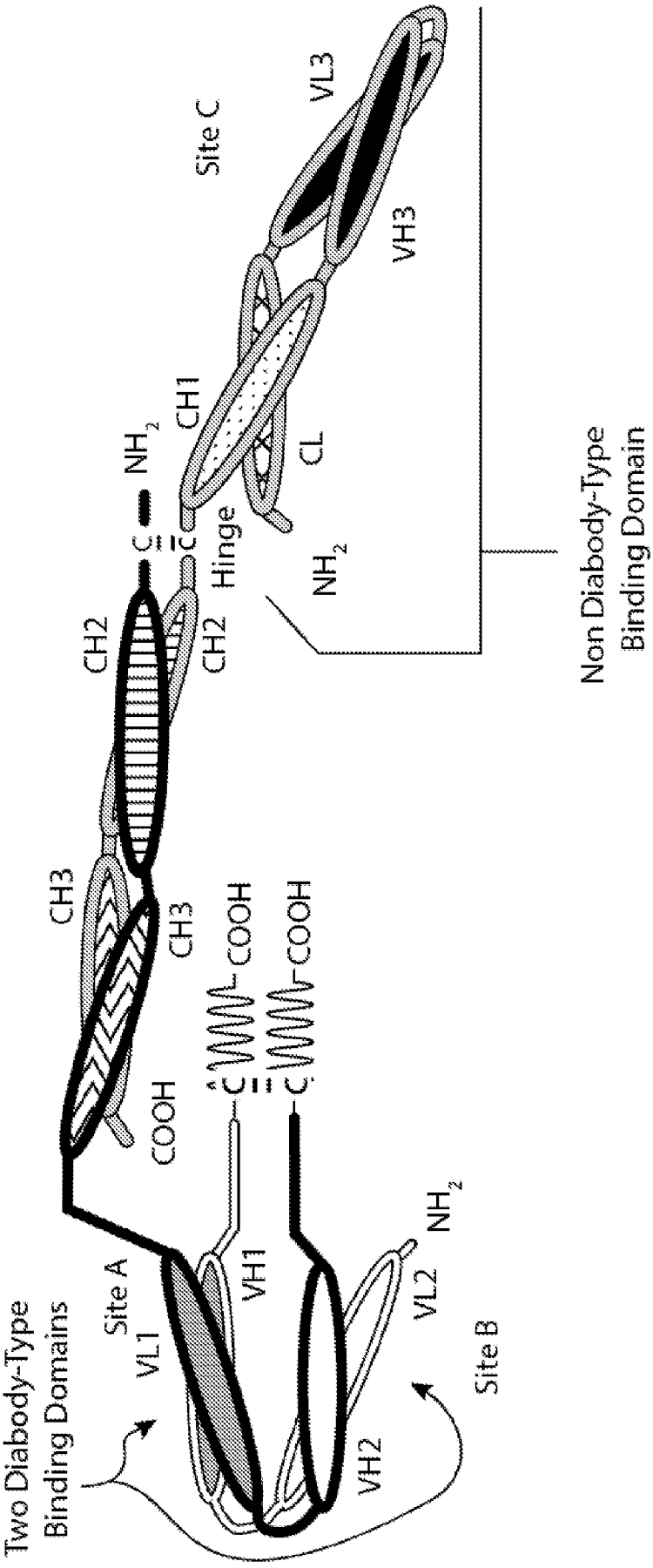
Two Diabody-Type Binding Domains
Site A
VL1
VH1
VH2
VL2
Site B
NH2
COOH
COOH
COOH
CH3
CH3
CH2
CH2
Hinge
NH2
CH1
CL
Site C
VL3
VH3
Non Diabody-Type Binding Domain

Figure 4E

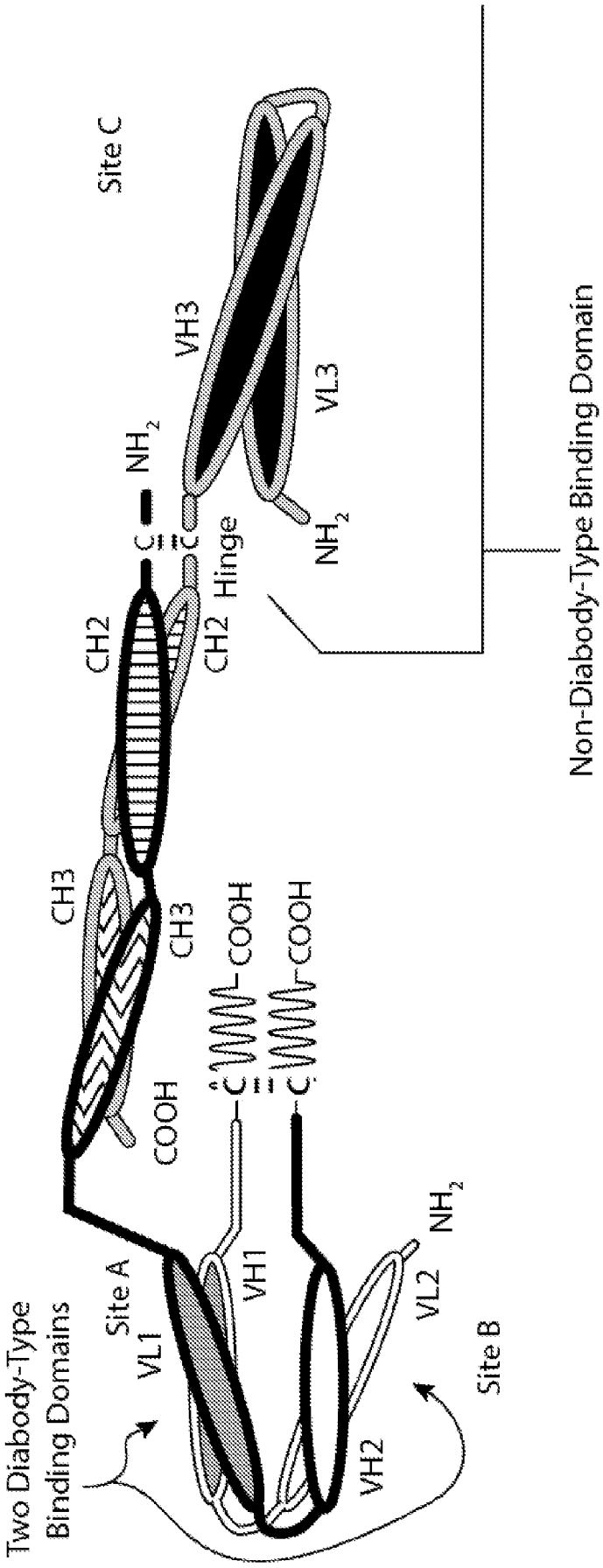
Two Diabody-Type Binding Domains
Site A
VL1
VH1
VL2
NH_2
Site B
VH2
COOH
COOH
CH3
CH3
CH2
CH2
Hinge
NH_2
VH3
VL3
Site C
Non-Diabody-Type Binding Domain

Figure 4F

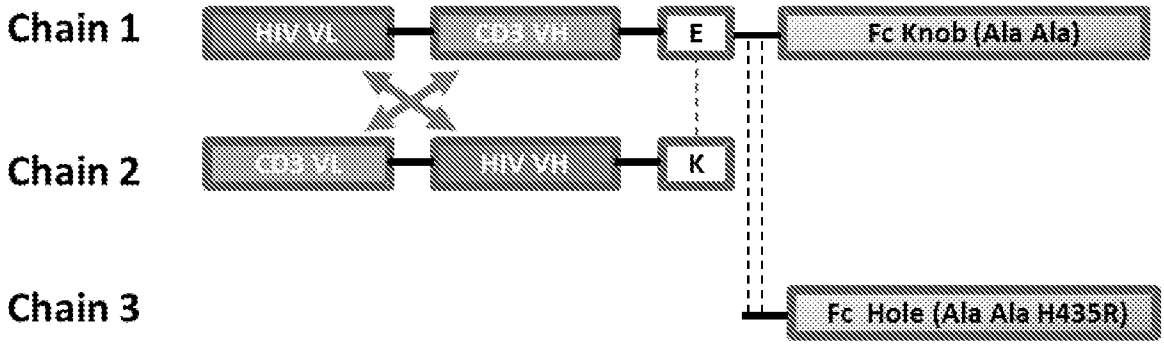

Figure 5A

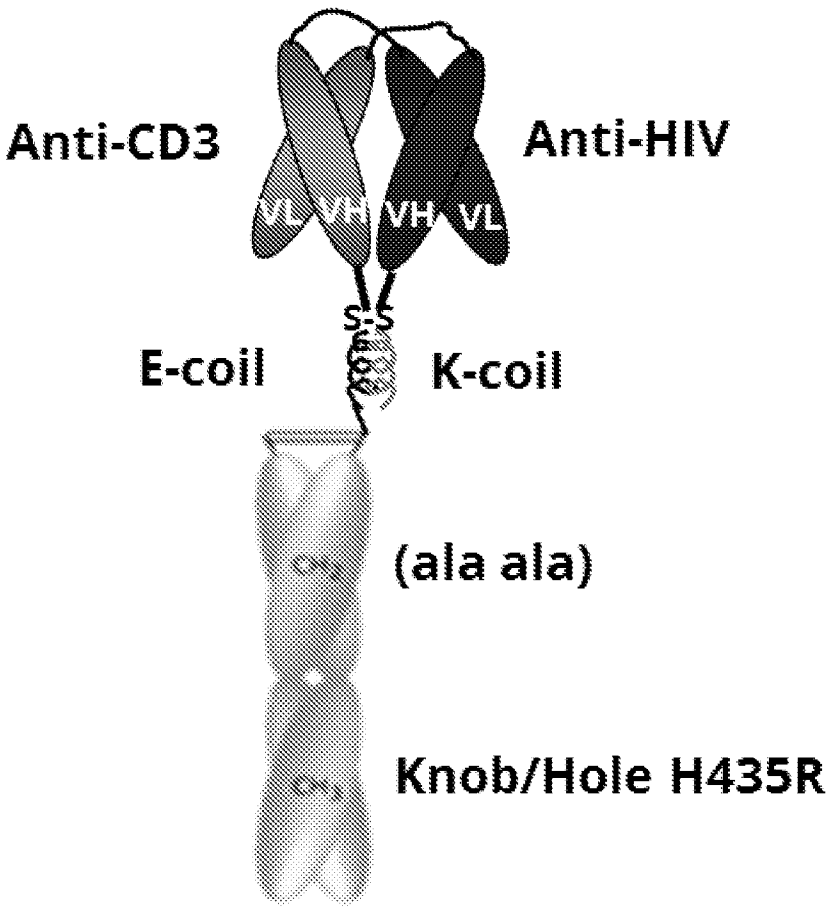

Figure 5B

>CH557xCD3 Fc Chain 1

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TRVDIKGGGSGGGGEVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNW
VRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRFTISRDDSKNSLYLQMN
SLKTEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSASTKGEVAACE
KEVAALEKEVAALEKEVAALEKGGGDKTHTCPPCPAPEAAGGPSVFLFPP
KPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ
YNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE
PQVYTLPPSREEMTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTP
PVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSP
GK (SEQ ID NO: 555)

gaaattgtgttgacgcagtctccagccaccctgtccgcgtctccagggga
aagagtcaccctaacttgcagggccagtcggagtgtccgaaacaacgtgg
cctggtatcagcacaagggtggccagagtcccaggctcctcatttatgat
gcgtccacgagggccgctggtgtcccagccaggttcagcggcagtgcatc
tgggacagagttcactctcgccatcagcaacttggagtctgaagatttta
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accagggtggacatcaaaggtggaggatccggcggcggaggcgaggtgca
gctggtggagtctgggggaggcttggtccagcctggagggtccctgagac
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cttcggcaattcttacgtgtcttggtttgcttattggggacaggggacac
tggtgactgtgtcttccgcctccaccaagggcgaagtggccgcatgtgag
aa

Figure 5C

agaggttgctgctttggagaaggaggtcgctgcacttgaaaaggaggtcg
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ccagcacctgaagccgcgggggggaccgtcagtcttcctcttcccccaaa
acccaaggacaccctcatgatctcccggacccctgaggtcacatgcgtgg
tggtggacgtgagccacgaagaccctgaggtcaagttcaactggtacgtg
gacggcgtggaggtgcataatgccaagacaaagccgcgggaggagcagta
caacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggact
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gagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcc
cgtgctggactccgacggctccttcttcctctacagcaagctcaccgtgg
acaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcat
gaggctctgcacaaccactacacgcagaagagcctctccctgtctccggg
taaa (SEQ ID NO: 556)

Figure 5C cont.

>CH557xCD3 Fc Chain 2

QAVVTQEPSLTVSPGGTVTLTCRSSTGAVTTSNYANWVQQKPGQAPRGLI
GGTNKRAPWTPARFSGSLLGGKAALTITGAQAEDEADYYCALWYSNLWVF
GGGTKLTVLGGGGSGGGGQVRLAQYGGGVKRLGATMTLSCVASGYTFNDY
YIHWVRQAPGQGFELLGYIDPANGRPDYAGALRERLSFYRDKSMETLYMD
LRSLRYDDTAMYYCVRNVGTAGSLLHYDHWGSGSPVIVSSASTKGKVAAC
KEKVAALKEKVAALKEKVAALKE (SEQ ID NO: 557)

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tgtgaccctgacatgcagatccagcacaggcgcagtgaccacatctaact
acgccaattgggtgcagcagaagccaggacaggcaccaaggggcctgatc
gggggtacaaacaaaagggctccctggacccctgcacggttttctggaag
tctgctgggcggaaaggccgctctgactattaccggggcacaggccgagg
acgaagccgattactattgtgctctgtggtatagcaatctgtgggtgttc
gggggtggcacaaaactgactgtgctgggaggggtggatccggcggagg
tggacaggtccgactagcccaatatggtggtggggtgaagaggctagggg
ccacaatgacccttcctgcgtggcatctggatacaccttcaacgactac
tacatacattgggtgcggcaggcccctggacaaggctttgagttgttggg
atacatcgaccccgctaatggtcgcccagactacgcaggggcgttgaggg
agagactctccttctacagggacaagtccatggagacgctgtacatggac
ctgaggagcctaagatatgacgacacggccatgtattattgtgttagaaa
tgtggggaccgctggcagcttgctgcattatgaccactggggctcgggaa
gcccggtcatcgtctcctccgcctccaccaagggcaaagtggccgcatgt
aaggagaaagttgctgctttgaaagagaaggtcgccgcacttaaggaaaa
ggtcgcagccctgaaagag (SEQ ID NO: 558)

Figure 5D

>CH557xCD3 Fc Chain 3

DKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED
PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYK
CKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLSCAVK
GFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQG
NVFSCSVMHEALHNRYTQKSLSLSPGK (SEQ ID NO: 559)

gacaaaactcacacatgcccaccgtgcccagcacctgaagccgcgggggg
accgtcagtcttcctcttcccccaaaacccaaggacaccctcatgatct
cccggacccctgaggtcacatgcgtggtggtggacgtgagccacgaagac
cctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgc
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gcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaag
tgcaaggtctccaacaaagccctcccagccccatcgagaaaaccatctc
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cccgggaggagatgaccaagaaccaggtcagcctgagttgcgcagtcaaa
ggcttctatcccagcgacatcgccgtggagtgggagagcaatgggcagcc
ggagaacaactacaagaccacgcctcccgtgctggactccgacggctcct
tcttcctcgtcagcaagctcaccgtggacaagagcaggtggcagcagggg
aacgtcttctcatgctccgtgatgcatgaggctctgcacaaccgctacac
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Figure 5E

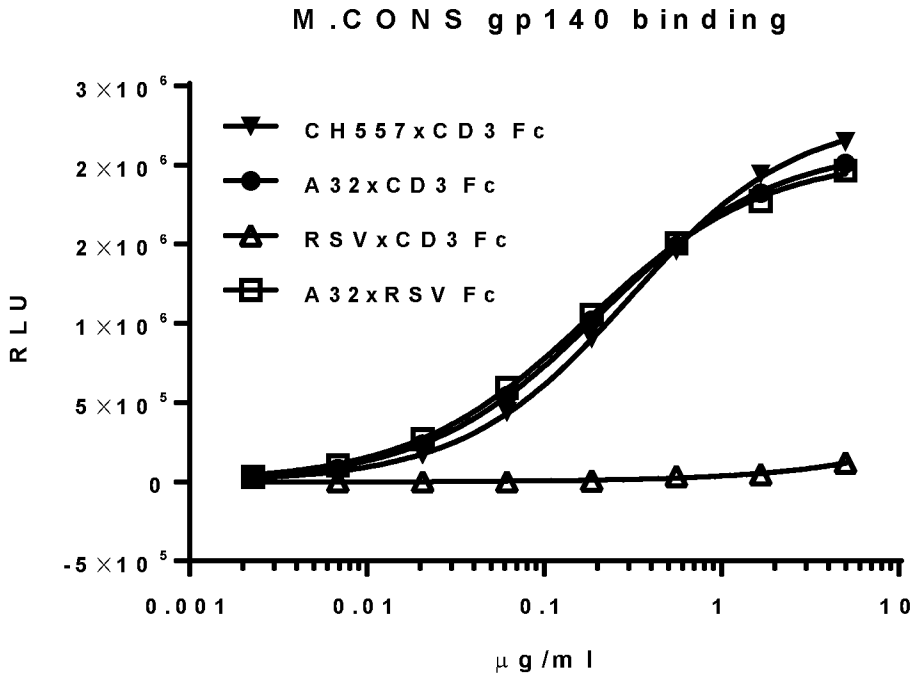


Figure 6A

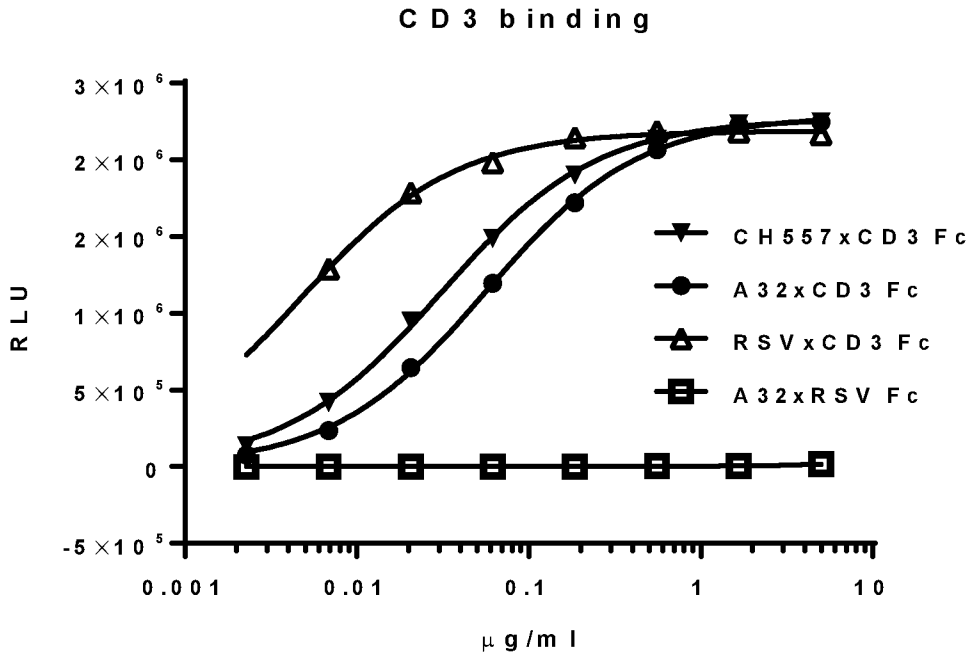


Figure 6B

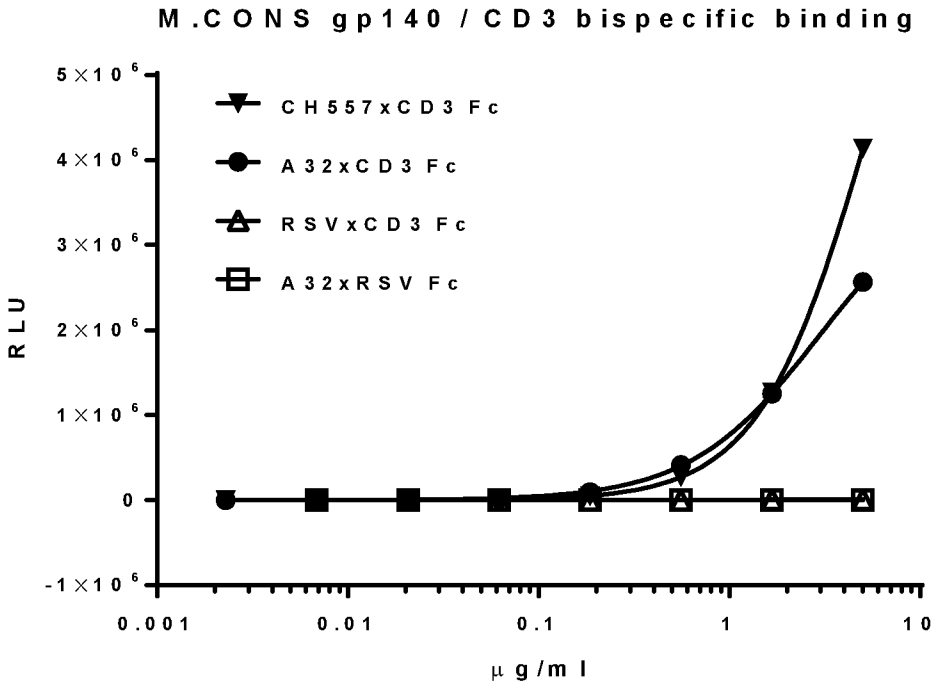

M.CONS gp140 / CD3 bispecific binding
CH557xCD3 Fc
A32xCD3 Fc
RSVxCD3 Fc
A32xRSV Fc
RLU
5×10^6
4×10^6
3×10^6
2×10^6
1×10^6
0
-1×10^6
0.001
0.01
0.1
1
10
μg/ml

Figure 6C

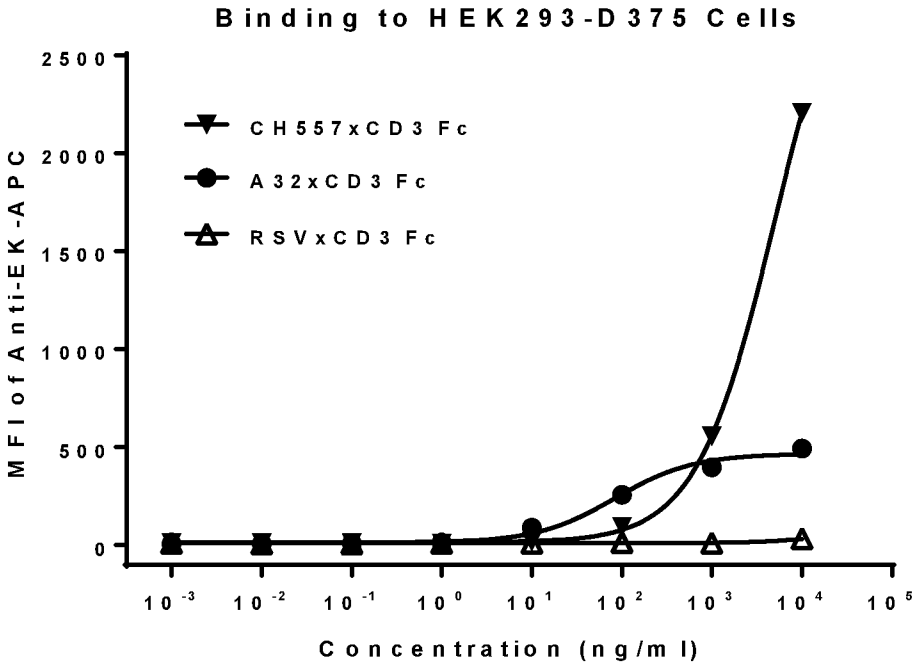


Figure 7A

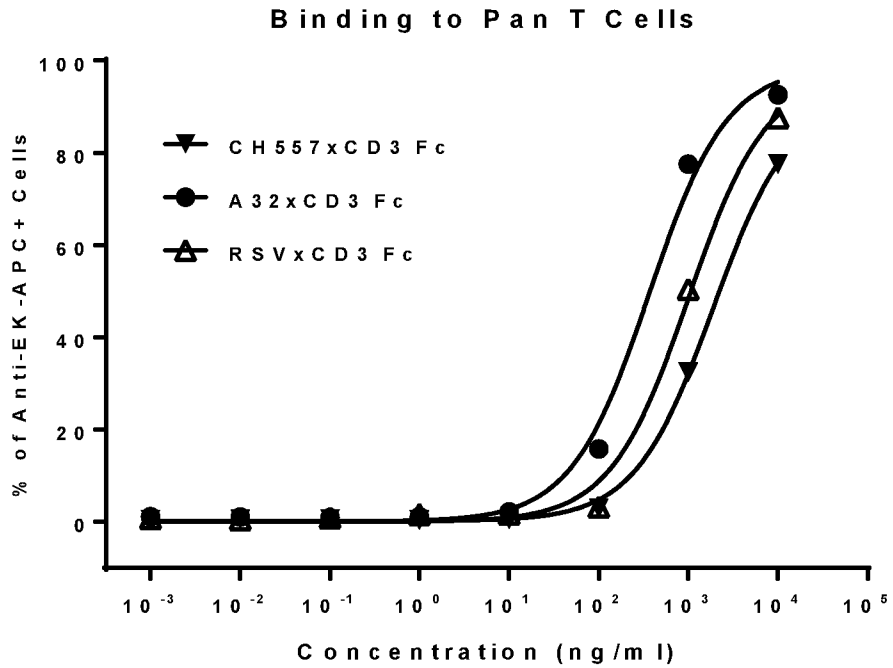
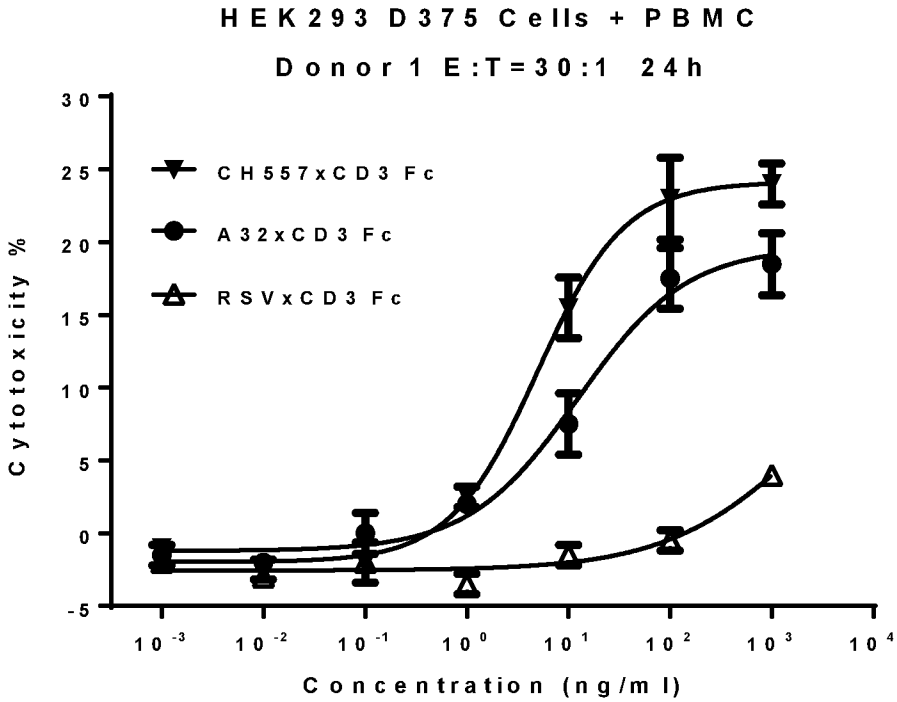
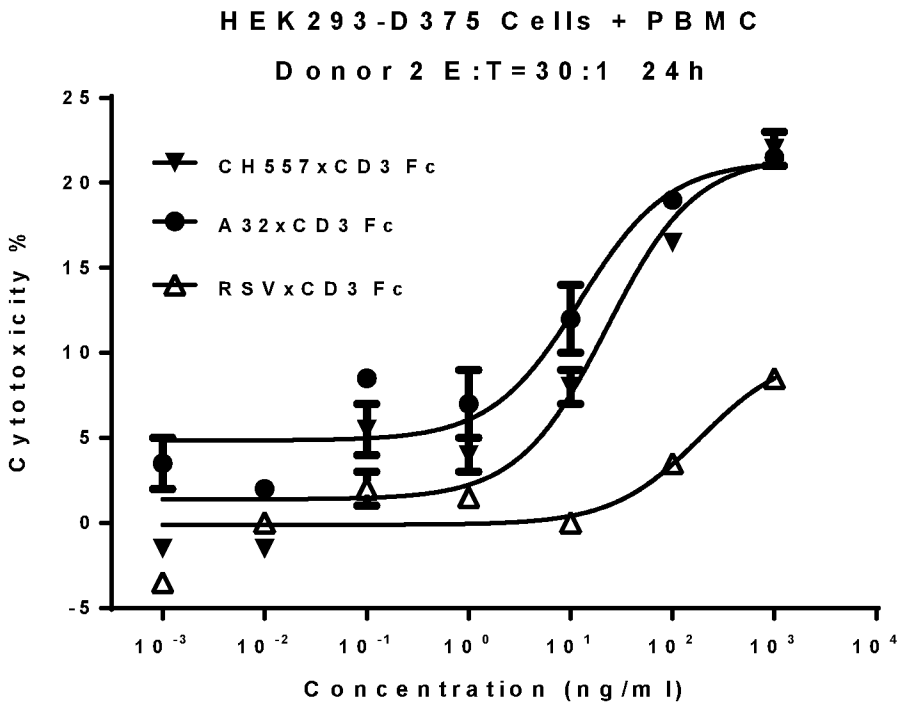


Figure 7B



	CH557xCD3 Fc	A32xCD3 Fc
EC50	4.940	12.30

Figure 8A



	CH557xCD3 Fc	A32xCD3 Fc
EC50	22.72	12.36

Figure 8B

>HIVxCD3xCD8 Trivalent Molecule Chain 1 (for trivalent molecules having three or four polypeptide chains)

EIVLTQSPATLSASPGERVTLTCRASRSVRNNVAWYQHKGGQSPRLLIYD
ASTRAAGVPARFSGSASGTEFTLAISNLESEDFTVYFCLQYNNWWTFGQG
TRVDIKGGGSGGGGEVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNW
VRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRFTISRDDSKNSLYLQMN
SLKTEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSASTKGEVAACE
KEVAALEKEVAALEKEVAALEKGGGDKTHTCPPCPAPEAAGGPSVFLFPP
KPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ
YNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE
PQVYTLPPSREEMTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTP
PVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSP
GK (SEQ ID NO: 555)

Figure 9A

> HIVxCD3xCD8 Trivalent Molecule Chain 2 (for trivalent molecules having three or four polypeptide chains)

QAVVTQEPSLTVSPGGTVTLTCRSSTGAVTTSNYANWVQQKPGQAPRGLI
GGTNKRAPWTPARFSGSLLGGKAALTITGAQAEDEADYYCALWYSNLWVF
GGGTKLTVLGGGGSGGGGQVRLAQYGGGVKRLGATMTLSCVASGYTFNDY
YIHWVRQAPGQGFELLGYIDPANGRPDYAGALRERLSFYRDKSMETLYMD
LRSLRYDDTAMYYCVRNVGTAGSLLHYDHWGSGSPVIVSSASTKGKVAAC
KEKVAALKEKVAALKEKVAALKE (SEQ ID NO: 557)

Figure 9B

> HIVxCD3xCD8 Trivalent Molecule Chain 3 (for trivalent molecules having four polypeptide chains)

EVQLQQSGAELVKPGASVKLSCTASGFNIKDTYIHFVRQRPEQGLEWIGR
IDPANDNTLYASKFQGKATITADTSSNTAYMHLCSLTSGDTAVYYCGRGY
GYYVFDHWGQGTTLTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDY
FPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYI
CNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKD
TLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST
YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVY
TLPPSREEMTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPPVLD
SDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNRYTQKSLSLSPG
(SEQ ID NO: 561)

Figure 9C

> HIVxCD3xCD8 Trivalent Molecule Chain 4 (for trivalent molecules having four polypeptide chains)

DVQINQSPSFLAASPGETITINCRTSRSISQYLAWYQEKPGKTNKLLIYS
GSTLQSGIPSRFSGSGSGTDFTLTISGLEPEDFAMYYCQQHNENPLTFGA
GTKLELRRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKV
DNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQG
LSSPVTKSFNRGEC (SEQ ID NO: 562)

Figure 9D

> HIVxCD3xCD8 Trivalent Molecule Chain 3 (for trivalent molecules having three polypeptide chains)

DVQINQSPSFLAASPGETITINCRTSRSISQYLAWYQEKPGKTNKLLIYS
GSTLQSGIPSRFSGSGSGTDFTLTISGLEPEDFAMYYCQQHNENPLTFGA

GTKLELRGGGGSGGGGSGGGGSEVQLQQSGAELVKPGASVKLSCTASGFN
IKDTYIHFVRQRPEQGLEWIGRIDPANDNTLYASKFQGKATITADTSSNT
AYMHLCSLTSGDTAVYYCGRGYGYYVFDHWGQGTTLTVSSASTKGPSVFP
LAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS
GLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCP
PCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNW
YVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKA
LPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLSCAVKGFYPSDI
AVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSV
MHEALHNRYTQKSLSLSPG (SEQ ID NO: 563)

Figure 9E

>DH542_nt_HC (SEQ ID NO: 1)

CAGGTGCAGCTGGTGCAGTCTGGGGCTCAAATGAAGAACCCTGGGGCCTCAG
TGAAGGTCTCCTGCGCGCCTTCT**GGATATACCTTCACCGACTTTTAC**ATACATT
GGTTGCGCCAGGCCCCTGGCCAGGGGCTTCAGTGGATGGGATGG**ATGAACCC**
TCAGACTGGTCGCACAAACACTGCACGAAACTTTCAGGGGAGGGTCACCATG
ACCAGGGACACGTCCATCGGCACAGCCTACATGGAGTTGAGAAGCCTGACATC
TGACGACACGGCCATATATTAC**TGTACGACAGGGGGATGGATCAGTCTTTACT**
ATGATAGTAGTTATTACCCCAACTTTGACCACTGGGGTCAGGGAACCCTGCTC
ACCGTCTCCTCAG

>DH542_nt_LC (SEQ ID NO: 2)

ACCAGTCTGCTGACTCAGCCTGCCTCCGTGTCTGGGTCTCCTGGACAGTCGATC
ACCATCTCCTGCACTGGAACC**AAGTATGATGTTGGGAGTCATGACCTT**GTCTC
CTGGTACCAACAGTACCCAGGCAAAGTCCCCAAATACATGATTTAT**GAAGTCA**
ATAAACGGCCCTCAGGAGTTTCTAATCGCTTCTCTGGCTCCAAATCTGGCAACA
CGGCCTCCCTGACAATCTCTGGGCTCCGGGCTGAGGACGAGGCTGACTATTAT
TGCTGTTCATTTGGAGGGAGTGCCACCGTGGTCTGCGGCGGCGGGACCAAGG
TGACCGTCCTAG

>DH542_aa_HC (SEQ ID NO: 3)

QVQLVQSGAQMKNPGASVKVSCAPS**GYTFTDFY**IHWLRQAPGQGLQWMGW
MNPQTGRTNTARNFQGRVTMTRDTSIGTAYMELRSLTSDDTAIYY**CTTGGWISL**
YYDSSYYPNFDHWGQGTLLTVSS

>DH542_aa_LC (SEQ ID NO: 4)

TSLLTQPASVSGSPGQSITISCTGT**KYDVGSHDL**VSWYQQYPGKVPKYMIY**EVN**KR
PSGVSNRFSGSKSGNTASLTISGLRAEDEADYY**CCSFGGSATVVC**GGGTKVTVL

FIG. 10A

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          AGTGAAAGTC TCCTGCAAGG CTTCTGGATA CACCTTCACC GACTACTACA
DH391H          AGTGAAGGTC TCCTGCAAGG CTTCTGGATA CACCCTCAGC GACTACTATG
I4              AGTGAAGGTC TCCTGCAAGG CTTCTGGATA CACCTTCACC GACTACTATA
I3              AGTGAAGGTC TCCTGCGCGS CTTCTGGATA TACCTTCACC GACTTCTACA
DH542H          AGTGAAGGTC TCCTGCGCGC CTTCTGGATA TACCTTCACC GACTTTTACA
I2              AGTGAAAGTC TCCTGCGCGS CTTCTGGATA TACCTTCACC GACTTCTACA
DH471H          AGTGAAAGTC TCCTGCGCGC CTTCTGGATA TACCTTCACT GACTTCTACA
DH429H          AGTGAAAGTC TCCTGCGCGG CTTCTGGATA TGGTTTCACC GACTTCTACA
DH270H          AGTGAGGGTC TCCTGCAAGG CTTCTGGATA CACCTTCACC GACTACTATA


                ....|....| ....|....| ....|....| ....|....| ....|....|
                     110        120        130        140        150
UCA             TGCACTGGGT GCGACAGGCC CCTGGACAAG GGCTTGAGTG GATGGGATGG
I5              TACACTGGGT GCGACAGGCC CCTGGACAAG GGCTTGAGTG GATGGGATGG
I1              TACACTGGGT GCGACAGGCC CCTGGACAAG GGCTTGAGTG GATGGCATGG
DH473H          TACACTGGGT GCGACAGGCC CCTGGACAAG GTCTTGAGTG GATGGCATGG
DH391H          TACACTGGCT GCGACAGGCC CCTGGACAGG GGCTTGAGTG GGTGGCTTGG
I4              TACACTGGGT GCGACAGGCC CCTGGACAAG GGCTTGAGTG GATGGGATGG
I3              TACACTGGGT GCGACAGGCC CCTGGACAAG GGCTTSAGTG GATGGGATGG
DH542H          TACATTGGTT GCGCCAGGCC CCTGGCCAGG GGCTTCAGTG GATGGGATGG
I2              TACACTGGGT GCGACTGGCC CCTGGACAAG GGCTTSAGTG GATGGGATGG
DH471H          TACACTGGGT GCGACTGGCC CCTGGACAAG GGCTTGAGTG GCTGGGGTGG
DH429H          TACACTGGGT GCGACTGGCC CCTGGACACG GGCTCCAGTG GATGGGATGG
DH270H          TACACTGGGT GCGACAGGCC CCTGGACAAG GGCCTGAGTG GATGGGATGG

                                      CDR2
                ....|....| ....|....| ....|....| ....|....| ....|....|
                     160        170        180        190        200
UCA             ATCAACCCTA ACAGTGGTGG CACAAACTAT GCACAGAAGT TTCAGGGCAG
I5              ATCAACCCTA ACASTGGTCG CACAAACTMT GCACAGAAGT TTCAGGGCAG
I1              ATCAACCCTA CCASTGGTCG CACAARCTMT GCACGGAAGT TTCAGGGCAG
DH473H          ATCAACCCTA CCACTGGTCG CTCTAGCTTT GCCCGGGGGT TTCAGGGCAG
```

FIG. 10B

```
DH391H      ATCAACCCTA CCAGTGGTCG CACAATCTCT CCACGGAAGT TTCAGGGCAG
I4          ATCAACCCTA ACACTGGTCG CACAAACTMT GCACAGAAGT TTCAGGGCAG
I3          ATGAACCCTA AGACTGGTCG CACAAACAMT GCACAAAACT TTCAGGGCAG
DH542H      ATGAACCCTC AGACTGGTCG CACAAACACT GCACGAAACT TTCAGGGGAG
I2          ATGAACCCTA AGACTGGTCG CACAAATAAT GCACAAAACT TTCAGGGCAG
DH471H      ATGAACCCTA AGACTGGTCG CACAAATCAA GGACAAAACT 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 ATGGAACTGA
DH473H      GGTCACCATG ACCAGGGAAA CGTCCGTCAG CACGGCCTAT ATGGAACTGA
DH391H      GGTCACGATG ACTACGGACA CGTCCATGAA TGTTGCCTAC ATGGAACTGA
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 ATTTCTGTGC 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

                    ....|....|  ....|....|  ....|....|  ....|....|  ....|....|
                          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
```

FIG. 10B cont.

```
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
```

FIG. 10B cont.

DH270 lineage - Heavy chain amino acid sequences

```
                                        CDR1
          ....|....| ....|....| ....|....| ....|....| ....|....|
                10         20         30         40         50
UCA        QVQLVQSGAE VKKPGASVKV SCKASGYTFT GYYMHWVRQA 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 PGQGPEWMGW

           CDR2                                                 CDR3
          ....|....| ....|....| ....|....| ....|....| ....|....|
                60         70         80         90        100
UCA        INPNSGGTNY AQKFQGRVTM TRDTSISTAY MELSRLRSDD TAVYYCARGX
I5         INPNXGRTNX AQKFQGRVTM TRDTSISTAY MELSXLRSDD TAVYYCARGX
I1         INPTXGRTXX ARKFQGRVTM TRDTSISXAY MELRXLRSDD TAVYYCARGG
DH473H     INPTTGRSSF ARGFQGRVTM TRETSVSTAY MELRRLRSDD TAVYYCAKAG
DH391H     INPTSGRTIS PRKFQGRVTM TTDTSMNVAY MELRGLRSDD TAVYFCARGG
I4         INPNTGRTNX AQKFQGRVTM TRDTSISTAY MELSXLTSDD 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 YPNFDSWGQG TLVTVSS
DH473H              YIALYVDYSG YPNFNSWGQG TLVTVSS
DH391H              WISLYVDYSY YPNFDSWGQG TLVSVSS
I4                  WIXLYYDSSG YPNFDYWGQG TLVTVSS
I3                  WISLYYDSSY YPNFDHWGQG TLVTVSS
DH542H              WISLYYDSSY YPNFDHWGQG TLLTVSS
I2                  WISXYYDSSY YPNFDHWGQG TLVTVSS
DH471H              WISDYYDSSY YPNFDHWGQG TLVTVSS
DH429H              WISPYYDSSY YPNFDHWGQG TLITVSS
DH270H              WIGLYSDTSG YPNFDYWGQG TLVTVSS
```

FIG. 10B cont.

DH270 lineage - Light chain nucleotide sequences

```
                 ....|....| ....|....| ....|....| ....|....| ....|....|
                       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           CTGCCTGTGC TGACTCAGCC TGCCTCCGTG TCTGGGTCTC CTGGGCAGTC
DH429H           CAGTCTGCCC TGACTCAGCC TGCCTCCGTG TCTGGGTCTC CTGGACAGTC
DH270H           CAGTCTGCCC TGACTCAGCC TGCCTCCGTG TCTGGGTCTC CTGGACAGTC

                                        CDR1
                 ....|....| ....|....| ....|....| ....|....| ....|....|
                       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

                 ....|....| ....|....| ....|....| ....|....| ....|....|
                      110        120        130        140        150
UCA              TTGTCTCCTG GTACCAACAG CACCCAGGCA AAGCCCCCAA ACTCATGATT
I5               TTGTCTCCTG GTACCAACAG CACCCAGGCA AAGCCCCCAA ACTCATGATT
I1               TTGTCTCCTG GTACCAACAG CACCCAGGCA AAGCCCCCAA ACTCATGATT
DH473H           TTGTCTCCTG GTACCAACAG CACCCAGGCA AAGCCCCCAA ACTCATTATT
DH391H           TTGTGTCCTG GTACCAGCAG CACCCAGGCA AAGCCCCCAA ACTGATGATT
I4               TTGTCTCCTG GTACCAACAG CACCCAGGCA AAGCCCCCAA ATACATGATT
I3               TTGTCTCCTG GTACCAACAG CACCCAGGCA AAGCCCCCAA ATACATGATT
DH542H           TTGTCTCCTG GTACCAACAG TACCCAGGCA AAGTCCCCAA ATACATGATT
I2               TTGTCTCCTG GTACCAACAG CACCCAGGCA AAGCCCCCAA ATACATGATT
DH471H           TTGTCTCCTG GTACCAGCAC CACCCAGGCA AAGCCCCCAA ATATTTGATT
DH429H           TTGTCTCCTG GTTCCAACAG CACCCAGGCA AAGCCCCCAA ATACATGATT
DH270H           TTGTCTCCTG GTATCAACAG CACCCAGGCA AAGTCCCCAA ATACATAATT

                                        CDR2
                 ....|....| ....|....| ....|....| ....|....| ....|....|
                      160        170        180        190        200
UCA              TATGAGGTCA GTAAGCGGCC CTCAGGGGTT TCTAATCGCT TCTCTGGCTC
I5               TATGAGGTCA RTAAGCGGCC CTCAGGGGTT TCTAATCGCT TCTCTGGCTC
I1               TATGAGGTCA RTAAGTGGCC CTCAGGGGTT TCTAATCGCT TCTCTGGCTC
```

FIG. 10B cont.

```
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 TGTTCATTTG GAGGGAGTGC CACCGTGGTC
I2          ACGAGGCTGA CTATTATTGC TGCTCATTCG GAGGTAGTGC CACTGTRGTC
DH471H      ACGAGGCTGA CTATTATTGC TGCTCATTCG GAGGTAGTGC CGCTGTGGTC
DH429H      ACGAGGCTGA CTATTATTGC TGCTCATTCG GAGGTAGTGC CACTGTAGTC
DH270H      ACGAGGCCAC TTATTACTGT TGTTCATATG CAGGTAGTAG CATTATATTT

                        ....|....| ....|....| ....|....| .
                              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
```

FIG. 10B cont.

```
DH429H      TGCGGCGGAG GGACCAAGGT GACCGTCCTA G
DH270H      TTCGGCGGTG GGACCAAGCT GACCGTCATA G
```

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 SCTGTKYDVG 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 SNRFSGSKSG NTASLTISGL QAEDEADYYC CSYAGSSTVX
I5        YEVXKRPSGV SNRFSGSKSG NTASLTISGL QAEDEADYYX CSYAGSSTVX
I1        YEVXKWPSGV SNRFSGSKSG NTASLTISGL QAEDEAXYYC CSYAGSSTVI
DH473H    YEVSQWPSGV SKRFSGSKSG NTASLTISGL QAEDEAHYYC CSYAGSSTVI
DH391H    YEVNKWASGV SDRFAGSKSG NTASLTISRL QAEDEANYFC SSSTNSATVI
I4        YEVNKRPSGV SNRFSGSKSG NTASLTISGL QAEDEADYYX CSYAGSSTVX
I3        YEVNKRPSGV SNRFSGSKSG NTASLTISGL QAEDEADYYC CSFGGSATXV
DH542H    YEVNKRPSGV SNRFSGSKSG NTASLTISGL RAEDEADYYC CSFGGSATVV
I2        YEVNKWPSGV SHRFSGSKSG NTASLTISGL QAEDEADYYC CSFGGSATXV
DH471H    YEVKKWPSGV SHRFSGSKSG NTASLTISGL QVEDEADYYC CSFGGSAAVV
DH429H    YEVNKWPSGV SHRFSGSKSG NTASLTISGL QAEDEADYYC CSFGGSATVV
DH270H    YEVNKRPSGV SNRFSGSKSG NTASLTISGL QAEDEATYYC CSYAGSSIIF
```

FIG. 10B cont.

		IC50 (ug/ml) in TZM-bl Cells[2]		
		CH555_4A/293i	CH556_4A/293i	CH557_4A/293i
		CH0505	CH0505	CH0505
		Lot#68RKK	Lot#69RKK	Lot#70RKK
Virus Name	Virus Lot	Rec'd&Aliq 11/MAY/15	Rec'd&Aliq 11/MAY/15	Rec'd&Aliq 11/MAY/15
SVA-MLV	5545	>50	>50	>50
Q23.17	2435	>50	>50	**0.09**
DJ263.8	2220	>50	>50	**0.18**
C1080.c03	3757	>50	>50	**1.6**
6540.v4.c1	2746	>50	>50	**39**
Q168.a2	1715	>50	>50	**0.08**
6101.10	737	>50	>50	**0.77**
BG1168.1	530	>50	>50	**2.0**
DU172.17	4168	>50	>50	**0.25**
DU156.12	4166	**0.42**	>50	**0.30**
DU422.1	3803	>50	>50	**0.89**
57128.vrc15	1940	>50	>50	**4.9**
X1632-S2-B10	2900	>50	>50	**0.20**
Q769.d22	4405	>50	>50	**0.08**
ZM106F.PB9	824	>50	>50	**0.5**
CNE58	6509	>50	>50	**1.7**
92RW020.2	1573	**1.3**	>50	**0.28**
CAAN5342.A2	995	>50	>50	**1.7**
JR-FL	730	>50	>50	**0.13**
PVO.4	3801	>50	>50	**1.5**
THRO4156.18	967	>50	>50	**30**
TRJO4551.58	4159	>50	>50	**0.58**
TRO.11	772	>50	>50	**0.66**
YU2	4098	>50	>50	**0.08**
ZM55F.PB28a	819	>50	>50	**1.0**

FIG. 11

		IC50 (ug/ml) in TZM-bl Cells[2]			
		CH558_4A/293i	CH560_4A	CH561_4A	CH562_4A
		CH0505	CH0505	CH0505	CH0505
		Lot#71RKK	Lot#218SMI	Lot#219SMI	Lot#220SMI
Virus Name	Virus Lot	Rec'd&Aliq 11/MAY/15	Rec'd&Aliq 11/MAY/15	Rec'd&Aliq 11/MAY/15	Rec'd&Aliq 11/MAY/15
SVA-MLV	5545	>50	>50	>50	>50
Q23.17	2435	**0.52**	>50	>50	>50
DJ263.8	2220	**33**	**2.6**	**3.8**	**2.6**
C1080.c03	3757	**25**	>50	>50	>50
6540.v4.c1	2746	>50	**1.2**	**0.51**	**0.62**
Q168.a2	1715	**0.68**	**5.1**	**42**	**37**
6101.10	737	**8.2**	**6.6**	**10.0**	**10.5**
BG1168.1	530	>50	**7.3**	**17**	**17**
DU172.17	4168	**1.1**	**14**	>50	>50
DU156.12	4166	**1.8**	>50	>50	>50
DU422.1	3803	**6.0**	>50	>50	>50
57128.vrc15	1940	>50	>50	>50	>50
X1632-S2-B10	2900	**0.93**	**2.0**	**2.1**	**1.8**
Q769.d22	4405	**1.2**	**3.2**	**3.7**	**1.5**
ZM106F.PB9	824	**2.7**	**26**	**35**	**26**
CNE58	6509	>50	**8.0**	**30**	**18**
92RW020.2	1573	**1.2**	**26**	**50**	**29**
CAAN5342.A2	995	**25**	**49**	>50	**49**
JR-FL	730	**25**	**0.19**	**0.27**	**0.26**
PVO.4	3801	**7.4**	>50	>50	>50
THRO4156.18	967	>50	**4.9**	**5.8**	**5.8**
TRJO4551.58	4159	>50	>50	>50	>50
TRO.11	772	**9.4**	>50	>50	>50
YU2	4098	**0.21**	**2.2**	**3.7**	**2.9**
ZM55F.PB28a	819	**6.4**	**10.5**	**14**	**9.2**

FIG. 11 cont.

		IC50 (ug/ml) in TZM-bl Cells[1]		
		DH210_4A/293i	DH211_4A/293i	CH01-31
		CH0505	CH0505	
		Lot#155HC	Lot#217SMI	
Virus Name	Virus Lot	Rec'd&Aliq 11/MAY/15	Rec'd&Aliq 11/MAY/15	prep. 26/MAR/15
SVA-MLV	5545	>50	>50	>25
Q23.17	2435	>50	>50	**<0.011**
DJ263.8	2220	>50	>50	**0.84**
C1080.c03	3757	>50	>50	**0.05**
6540.v4.c1	2746	>50	>50	**0.58**
Q168.a2	1715	>50	>50	**0.08**
6101.10	737	>50	>50	**0.57**
BG1168.1	530	>50	>50	**2.1**
DU172.17	4168	>50	>50	**0.73**
DU156.12	4166	>50	>50	**0.30**
DU422.1	3803	>50	>50	>25
57128.vrc15	1940	>50	>50	>25
X1632-S2-B10	2900	>50	>50	**0.07**
Q769.d22	4405	>50	>50	**0.07**
ZM106F.PB9	824	>50	>50	**9.2**
CNE58	6509	>50	>50	**0.11**
92RW020.2	1573	>50	>50	**0.05**
CAAN5342.A2	995	>50	>50	>25
JR-FL	730	>50	>50	**0.03**
PVO.4	3801	>50	>50	**0.86**
THRO4156.18	967	>50	>50	**3.1**
TRJO4551.58	4159	>50	>50	**0.42**
TRO.11	772	>50	>50	**0.25**
YU2	4098	>50	>50	**0.10**
ZM55F.PB28a	819	>50	>50	**3.1**

FIG. 11 cont.

>CH557_aa_HC (SEQ ID NO: 124)

QVRLAQYGGGVKRLGATMTLSCVAS**GYTFNDYY**IHWVRQAPGQGFELLGY
IDPANGRPDYAGALRERLSFYRDKSMETLYMDLRSLRYDDTAMYY**CVRNV**
GTAGSLLHYDHWGSGSPVIVSS

>CH557_aa_LC (SEQ ID NO: 125)

EIVLTQSPATLSASPGERVTLTCRAS**RSVRNN**VAWYQHKGGQSPRLLIY**D**
ASTRAAGVPARFSGSASGTEFTLAISNLESEDFTVYF**CLQYNNWWTF**GQG
TRVDIK

>CH557_nt_HC (SEQ ID NO: 126)

CAGGTCCGACTAGCCCAATATGGTGGTGGGGTGAAGAGGCTAGGGGCCAC
AATGACCCTTTCCTGCGTGGCATCT**GGATACACCTTCAACGACTACTAC**A
TACATTGGGTGCGGCAGGCCCCTGGACAAGGCTTTGAGTTGTTGGGATAC
ATCGACCCCGCTAATGGTCGCCCAGACTACGCAGGGGCGTTGAGGGAGAG
ACTCTCCTTCTACAGGGACAAGTCCATGGAGACGCTGTACATGGACCTGA
GGAGCCTAAGATATGACGACACGGCCATGTATTATTGT**GTTAGAAATGTG**
GGGACCGCTGGCAGCTTGCTGCATTATGACCACTGGGGCTCGGGAAGCCC
GGTCATCGTCTCCTCC

>CH557_nt_LC (SEQ ID NO: 127)

GAAATTGTGTTGACGCAGTCTCCAGCCACCCTGTCCGCGTCTCCAGGGGA
AAGAGTCACCCTAACTTGCAGGGCCAGT**CGGAGTGTCCGAAACAAC**GTGG
CCTGGTATCAGCACAAGGGTGGCCAGAGTCCCAGGCTCCTCATTTAT**GAT**
GCGTCCACGAGGGCCGCTGGTGTCCCAGCCAGGTTCAGCGGCAGTGCATC
TGGGACAGAGTTCACTCTCGCCATCAGCAACTTGGAGTCTGAAGATTTTA
CAGTCTACTTCTGT**CTGCAGTATAATAACTGGTGGACC**TTCGGCCAAGGG
ACCAGGGTGGACATCAAA

FIG. 12

mAb	VH	D	JH	Mut. Frq.	HCDR3 length (AA)	HCDR3	VL	JL	Mut. Frq.	LCDR3 length (AA)
DH557	1-46	2-21	4	25.7	15	CVRNVGTAGSLLHYDHW	K3-15	1	14.3	8

FIG. 13

Antibody ID	VH	D	JH	Mutation frequency	CDRH3 length	VK	JK	Mutation frequency	CDRL3 length	Week of isolation
UCA	1-46*01	3-10*01	4*02	0.0%	15	3-15*01	1*01	0.0%	8	-
CH235	1-46*01	3-10*01	4*02	7.9%	15	3-15*01	1*01	3.5%	8	41
CH236	1-46*01	3-10*01	4*02	8.2%	15	3-15*01	1*01	2.2%	8	41
CH239	1-46*01	3-10*01	4*02	7.9%	15	3-15*01	1*01	4.7%	8	41
CH240	1-46*01	3-10*01	4*02	7.1%	15	3-15*01	1*01	3.1%	8	41
CH241	1-46*01	3-10*01	4*02	11.4%	15	3-15*01	1*01	3.1%	8	41
CH491	1-46*01	3-10*01	4*02	14.7%	15	3-15*01	1*01	2.2%	8	100*
CH493	1-46*01	3-10*01	4*02	19.3%	15	3-15*01	1*01	2.2%	8	152*
CH555	1-46*01	3-10*01	4*02	21.5%	15	3-15*01	1*01	16.0%	8	264
CH556	1-46*01	3-10*01	4*02	25.1%	15	3-15*01	1*01	17.0%	8	323
CH557	1-46*01	3-10*01	4*02	25.6%	15	3-15*01	1*01	12.3%	8	323
CH558	1-46*01	3-10*01	4*02	23.4%	15	3-15*01	1*01	11.0%	8	323

* Paired with CH236 V-light chain

FIG. 14

Amino acid alignment of CH235 lineage antibody heavy chains

```
                    10        20        30        40
                    |....|....|....|....|....|....|....|
                    QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYYMHWVRQA
UCA_HC              ----------T--R----------TI----------MF-V----------
CH240_HC            ----------T--R----------TI----------MF-V----------
CH235_HC            ----------T--R----------T-----Q----------N--V----------
CH239_HC            ----------R----------T----------MF-V----------
CH236_HC            ----------A--R----------TI--R----------T--I----------
CH241_HC            ----------A--R----------TI--R----------T--I----------
CH491_HC            ----------A--R----------TI--R----------T--I----------
CH493_HC            --R-L-Y-GG--R----MTI--V----M-MD--I----------
CH555_HC            ----------I----R----TL--RT----M-ID-FI----R-
CH558_HC            ----------GG--R--STTTI--V----S-MD--I----------
CH556_HC            ----------GT--S--T--TL----T----M-ID--I----R-
CH557_HC            --R-A-Y-GG--RL--TMTL--V----MD--I----------
```

Figure 15A

```
                1          50        60        70        80        90
                .....|.....|.....|.....|.....|.....|.....|.....|.....|
UCA_HC    PSQGLEWMGIIMPSGGSTSYAQKFQGRVTMTRDTSTSTVYMELSSLRSED
CH240_HC  ------C--W-D--V-RI--G-------------R-------G---------
CH235_HC  ------QL--W-D--W-R-N---M---I-------------------MR----
CH239_HC  ------C--W-D--V-RIN-------------R-------G---------
CH236_HC  --R--L--M-D--R-R-D-------------S---L---R--PD-
CH241_HC  ---P---M-D--V-RFTT-G-------------RY---A--D---------
CH491_HC  ---L--N--R--R-D-SYR-ED--S-Y---M-T---D-RN-K-A-
CH493_HC  ---L--M-D---R-D--GA-GD--S-Y--K-MN--L--D-R---G-
CH555_HC  --R--V--Y-D--R-RPD---PN-RD--SLY-----M-I--LD-RD-TPD-
CH558_HC  ---VL-E-D--M-R-N---GA-GD-FS-Y--K-ME--L--D-RM---D-
CH556_HC  --RE-L--Y-D--H-RPD-EG--RD-TSLY-----V---DVRG---LD-
CH557_HC  ---F-LL-Y-D-AM-RPD---GALRE-LSEY---K-ME--L--D-R---YD-
```

Figure 15A cont.

```
                 100       110       120
             ....|....|....|....|....|....|..
UCA HC       TAVYYCARDVGTEGSLLHFDYWGQGTLVTVSS
CH240_HC     --M---V-------------H-------I--A
CH235_HC     --------N-A-------Y------------A
CH239_HC     --I---V-------------H-------I--A
CH236_HC     --L---V-N---------Y-------------
CH241_HC     --------S-E-T----Y---------I----
CH491_HC     ------V-N---S-----Y-F----S------
CH493_HC     --M---V-N---A-----Y-H--L-VM-----
CH555_HC     --I---V-SE----TV--Y-H--P--R----P
CH558_HC     --M---V-N---A-----Y-H--T-SKII---
CH556_HC     --L---V-GG-V-V-SN-Y-H--P--M-F--P
CH557_HC     --M---V-N---A-----Y-H--S-SP-I---
```

Figure 15A cont.

Amino acid alignment of CH235 lineage antibody light chains

```
                    10        20        30        40
                    |.....|.....|.....|.....|.....|.....|.....|.....|
UCA LC              EIVLTQSPATLSVSPGERATLSCRASQSVSSNLAWYQQKPGQAPRLLIY(
CH236 LC            ..........V..........RN.....R..R..................
CH240 LC            ..........T...R..........R..........................
CH241 LC            ..........R..I..........................H.
CH235 LC            ..........R..........R..........................
CH239 LC            ..........T...R..V..........................
CH558 LC            ..........A.....V..T....RG.RN.V....HNV..S.....
CH557 LC            ..........A.....V..T....R..RN.V....H.G..S.....
CH555 LC            ..........D...A.....V..........GTKV...RHVR..P.....
CH556 LC            .TT.....D...A.....V..........A...G.QV..FRHIR..P.....S
```

Figure 15B

```
                50        60        70        80        90
                |.....|.....|.....|.....|.....|.....|.....|
UCA LC          GASTRATGIPARFSGSGSGTEFTLTISSLQSEDFAVYYCQQ
CH236 LC        ..........................M...................
CH240 LC        S.........................V...................L.
CH241 LC        ..........G.....P....A...V...................
CH235 LC        .T........V.....R.....A...M...............L.L.
CH239 LC        S.....................A.M...............L.
CH558 LC        D.....P.....A.....A...I.....TL...H.
CH557 LC        D.....A.V.....A.....A..N.E....T..F.L.
CH555 LC        .....A.....G.....N....I.NNF.....E.L...
CH556 LC        .....A.V.....D.....GM.....E.F...
```

Figure 15B cont.

```
                    100
          ....|.....|.....|.
UCA LC    YNNWWTFGQGTKVEIK
CH236 LC  ................
CH240 LC  ................
CH241 LC  ..D.............
CH235 LC  ................
CH239 LC  .DD.............
CH558 LC  ..............R.D.N
CH557 LC  ..............R.D..
CH555 LC  .KS...........DN.
CH556 LC  .HM...........R.DKN
```

FIG. 15B cont.

Alignment of CH557 heavy chain amino acid sequence compared to CH235 lineage antibodies with increasing levels of somatic mutations and neutralization breadth. Contact sites with gp120 identified from CH235/gp120 co-crystal structure are indicated with asterisks. Amino acid mutations within the contact sites are bolded and underlined.

```
                  |----------FR1----------|--CDR1--|--------FR2--------|--CDR2--|--
                             * *                    ********
UCA_HC            QVQLVQSGAE VKKPGASVKV SCKASGYTFT SYYMHWVRQA PGQGLEWMGI INPSGGSTSY
CH236_HC          QVQLVQSGAA VKRPGASVTI SCRASGYTFT TYYIHWVRQA PGQRLELMGM IDPSRGRTDY
CH235_HC          QVQLVQSGAE VKKPGASVTV SCQASGYTFT NYYVHWVRQA PGQGLQLMGW IDPSWGRTNY
CH493_HC          QVRLLQYGGG VKRPGASMTI SCVASGYNFN DYYIHWVRQA PGQGLELMGW IDPSGGRTDY
CH557_HC          QVRLAQYGGG VKRLGATMTL SCVASGYTFN DYYIHWVRQA PGQGFELLGY IDPANGRPDV

                  ----------FR3----------|------CDR3------|---FR4----|
                    **  *   *                  ********
UCA_HC            AQKFQGRVTM TRDTSTSTVY MELSSLRSED TAVYYCARDV GTEGSLLHFD YWGQGTLVTVSS
CH236_HC          AQKFQGRVTM SRDTSTSTLY MELRSLRPDD TALYYCVRNV GTEGSLLHYD YWGQGTLVTVSS
CH235_HC          AQNFQGRITM TRDTSTSTVY MEMRSLRSED TAVYYCARNV ATEGSLLHYD YWGQGTLVTVSA
CH493_HC          AGAFGDRVSM YRDKSMNTLY MDLRSLRSGD TAMYYCVRNV GTAGSLLHYD HWGLGVMVTVSS
CH557_HC          AGALRERLSF YRDKSMETLY MDLRSLRYDD TAMYYCVRNV GTAGSLLHYD HWGSGSPVIVSS
```

FIG. 16

Alignment of CH557 light chain amino acid sequence compared to CH235 lineage antibodies with increasing levels of somatic mutations and neutralization breadth.

```
                |----------FR1-------------|-CDR1--|-------FR2----------|CDR2|-------
UCA_LC          EIVLTQSPAT LSVSPGERAT LSCRASQSVS SNLAWYQQKP GQAPRLLIYG ASTRATGIPA
CH236_LC        EIVLTQSPAT LSVSPGERVT LSCRASQSVR NNLAWYRQKR GQAPRLLIYG ASTRATGIPA
CH235_LC        EIVLTQSPAT LSVSPGERAT LSCRASQSVR SNLAWYQQRP GQAPRLLIYG TSTRATGVPA
CH557_LC        EIVLTQSPAT LSASPGERVT LTCRASRSVR NNVAWYQHKG GQSPRLLIYD ASTRAAGVPA

                ----------FR3-------------------|--CDR3--|---FR4----|
UCA_LC          RFSGSGSGTE FTLTISSLQS EDFAVYYCQQ YNNWWTFGQG TKVEIK
CH236_LC        RFSGSGSGTE FTLTISSMQS EDFAVYYCQQ YNNWWTFGQG TKVEIK
CH235_LC        RFSGRGSGTE FTLAISSMQS EDFAVYLCLQ YNNWWTFGQG TKVEIK
CH557_LC        RFSGSASGTE FTLAISNLES EDFTVYFCLQ YNNWWTFGQG TRVDIK
```

FIG. 17

In yellow/highlighted CDR1, 2 and 3

>CH235_129w66 (SEQ ID NO: 159)

QVQLVQSGAAVKKPGASVRVSCKASGYTFTSSHIHWVRQAPGQALEWLGMIDPRFGR
PTRPQKFQGRVTLTRDTYTTTVYMSLSSLTPEDTAVYYCARSVETSESYLHFDYWGQ
GTLVTVSS

>CH235_68w100 (SEQ ID NO: 160)

QVQLVQSGAAVKRPGASVTISCRASGYTFTTYYIHWVRQAPGQGLELMGWINPRGGR
TDYSYRFEDRVSMYRDTSMSIVYMDLRNLKSADTAVYYCVRNVGTSGSLLHYDFWGQ
GSLVTVSS

>CH235_115w100 (SEQ ID NO: 161)

QVQLVQSGAAVKKPGASVRVSCKASGYTFTSSHIHWVRQAPGQGPEWMGMVDPRFGR
PTYAQKFQGRVAMTRDIYTSTVYMDLRSLKSEDTAIYFCVRNAETEGSLLHIEYWGQ
GTRVTVSS

>CH235_75w152 (SEQ ID NO: 162)

QVRLLQYGGGVKRPGASMTISCVASGYNFNDYYIHWVRQAPGQGLELMGWIDPSGGR
TDYAGAFGDRVSMYRDKSMNTLYMDLRSLRSGDTAMYYCVRNVGTAGSLLHYDHWGL
GVMVTVSS

>CH236_VK_aa (SEQ ID NO: 163)

EIVLTQSPATLSVSPGERVTLSCRASQSVRNNLAWYRQKRGQAPRLLIYGASTRATG
IPARFSGSGSGTEFTLTISSMQSEDFAVYYCQQYNNWWTFGQGTKVEIK

>CH241_VK_aa (SEQ ID NO: 164)

EIVLTQSPATLSVSPGERATLSCRASQSVRSNIAWYQQKPGQAPRLLIHGASTRATG
IPGRFSGSGSGPEFTLAISSVQSEDFAVYYCQQYNDWWTFGQGTKVEIK

FIG. 18

Clean Sequences for Plasmid Production

PtID	Ab ID	CH #	TX	VH	VL	Specificity
703-01-050-5	HES65W202GPOPB 129w66.1.1	490	n/a	CH235_129 w66	pCK001826	In CH235 lineage from 454. Paired with CH241 LC.
703-01-050-5	HES65W202GU3T7 68w100.1.2	491	n/a	CH235_68w 100	pCK001690	In CH235 lineage from 454. Paired with CH236 LC.
703-01-050-5	HES65W201B95MO 115w100.1.1	492	n/a	CH235_115 w100	pCK001826	In CH235 lineage from 454. Paired with CH241 LC.
703-01-050-5	HES65W201DXWBB 75w152.4.3	493	n/a	CH235_75w 152	pCK001690	In CH235 lineage from 454. Paired with CH236 LC.

NOTE: VLs have already been produced (are those of CH241 and CH236).

Heavy chains

>CH235_129w66 (SEQ ID NO: 165)

CAGGTGCAGCTGGTgCAGTCTGGGGCTGCGGTGAAGAAGCCTGGGGCCTCAGTGAGGGTTTCCTGC
AAGGCATCTGGATACACCTTCACCAGTTCTCATATCCACTGGGTGCGACAGGCCCCTGGACAAGCA
CTTGAGTGGTTGGGAATGATCGACCCTCGTTTTGGTAGGCCAACCCGCCCTCAGAAGTTCCAGGGC
AGAGTCACCCTGACCAGAGACACGTACACGACTACAGTGTACATGTCACTGAGCAGCCTGACACCT
GAAGACACGGCCGTTTACTACTGTGCGAGAAGCGTGGAAACGAGTGAGAGCTATCTCCACTTTGAC
TACTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCAg

>CH235_68w100 (SEQ ID NO: 166)

CAGGTGCAGCTGGTgCAGTCTGGGGCTGCGGTGAAGAGGCCTGGGGCCTCAGTGACGATTTCCTGC
AGGGCATCTGGATACACCTTCACCACCTACTATATACACTGGGTGCGACAGGCCCCTGGACAAGGA
CTTGAGTTGATGGGATGGATCAACCCTCGTGGCGGTCGCACAGACTACTCTTACAGATTTGAGGAC
AGAGTCAGTATGTACAGGGACACGTCCATGAGTATAGTCTACATGGACTTGAGGAACCTGAAATCT
GCGGACACGGCCGTCTACTATTGTGTGAGAAATGTGGGAACCAGTGGGAGCTTGCTCCACTATGAC
TTCTGGGGCCAGGGAAGCCTGGTCACCGTCTCCTCAg

>CH235_115w100 (SEQ ID NO: 167)

CAGGTGCAGCTGGTgCAGTCTGGGGCTGCGGTGAAGAAGCCTGGGGCCTCAGTGAGGGTTTCCTGC
AAGGCATCTGGATACACCTTCACCAGTTCTCATATCCACTGGGTGCGACAGGCCCCTGGACAAGGC
CCTGAGTGGATGGGCATGGTCGACCCCCGTTTTGGTCGCCCAACCTACGCACAGAAGTTTCAGGGC
AGGGTCGCCATGACCAGGGACATTTACACGAGCACAGTCTACATGGACTTGAGGAGCCTAAAATCT
GAGGACACGGCCATCTATTTCTGTGTGAGAAATGCGGAAACGGAGGGCAGCTTACTCCACATTGAG
TACTGGGGCCAGGGAACCCGGGTCACCGTCTCCTCAg

FIG. 19

>CH235_75w152 (SEQ ID NO: 168)

CAGGTGCGACTACTACAATATGGGGGTGGAGTGAAGAGGCCTGGGGCCTCAATGACG
ATTTCCTGCGTGGCGTCTGGATACAACTTCAACGACTACTATATACACTGGGTGCGA
CAGGCCCCTGGACAAGGCCTCGAATTGATGGGATGGATCGACCCTAGTGGTGGTCGC
ACAGATTACGCAGGGGCGTTTGGGGACAGAGTGTCCATGTACAGGGACAAGTCCATG
AACACACTCTACATGGACCTGAGGAGCCTGAGATCTGGCGACACGGCCATGTATTAT
TGTGTTAGAAATGTGGGAACGGCTGGCAGCTTGCTCCACTATGACCACTGGGGCCTG
GGAGTTATGGTCACCGTCTCCTCAG

>CH236_VK_nt (SEQ ID NO: 169)

GAAATTGTGTTGACGCAGTCTCCAGCCACCCTGTCTGTATCTCCAGGGGAAAGAGTC
ACCCTCTCCTGCAGGGCCAGTCAGAGTGTTAGAAACAACTTAGCCTGGTACCGGCAG
AAACGTGGCCAGGCTCCCAGACTCCTCATCTATGGTGCATCCACCAGGGCCACTGGT
ATCCCAGCCAGGTTCAGTGGCAGTGGGTCTGGGACAGAGTTCACTCTTACCATCAGC
AGCATGCAGTCTGAAGATTTTGCAGTTTATTACTGTCAGCAGTATAATAACTGGTGG
ACGTTCGGCCAAGGGACCAAGGTGGAAATCAAAC

>CH241_VK_nt (SEQ ID NO: 170)

GAAATTGTGTTGACGCAGTCTCCAGCCACCCTGTCTGTGTCTCCAGGGGAAAGAGCC
ACCCTCTCCTGCAGGGCCAGTCAGAGTGTTAGAAGCAACATAGCCTGGTACCAACAA
AAACCTGGCCAGGCTCCCAGGCTCCTCATCCATGGTGCATCCACCAGGGCCACAGGT
ATCCCAGGCAGGTTCAGTGGCAGTGGGTCTGGGCCAGAGTTCACTCTCGCCATCAGC
AGCGTGCAGTCTGAAGATTTTGCAGTTTATTACTGTCAGCAGTATAATGACTGGTGG
ACGTTCGGCCAAGGGACCAAGGTGGAAATCAAAC

FIG. 19 cont.

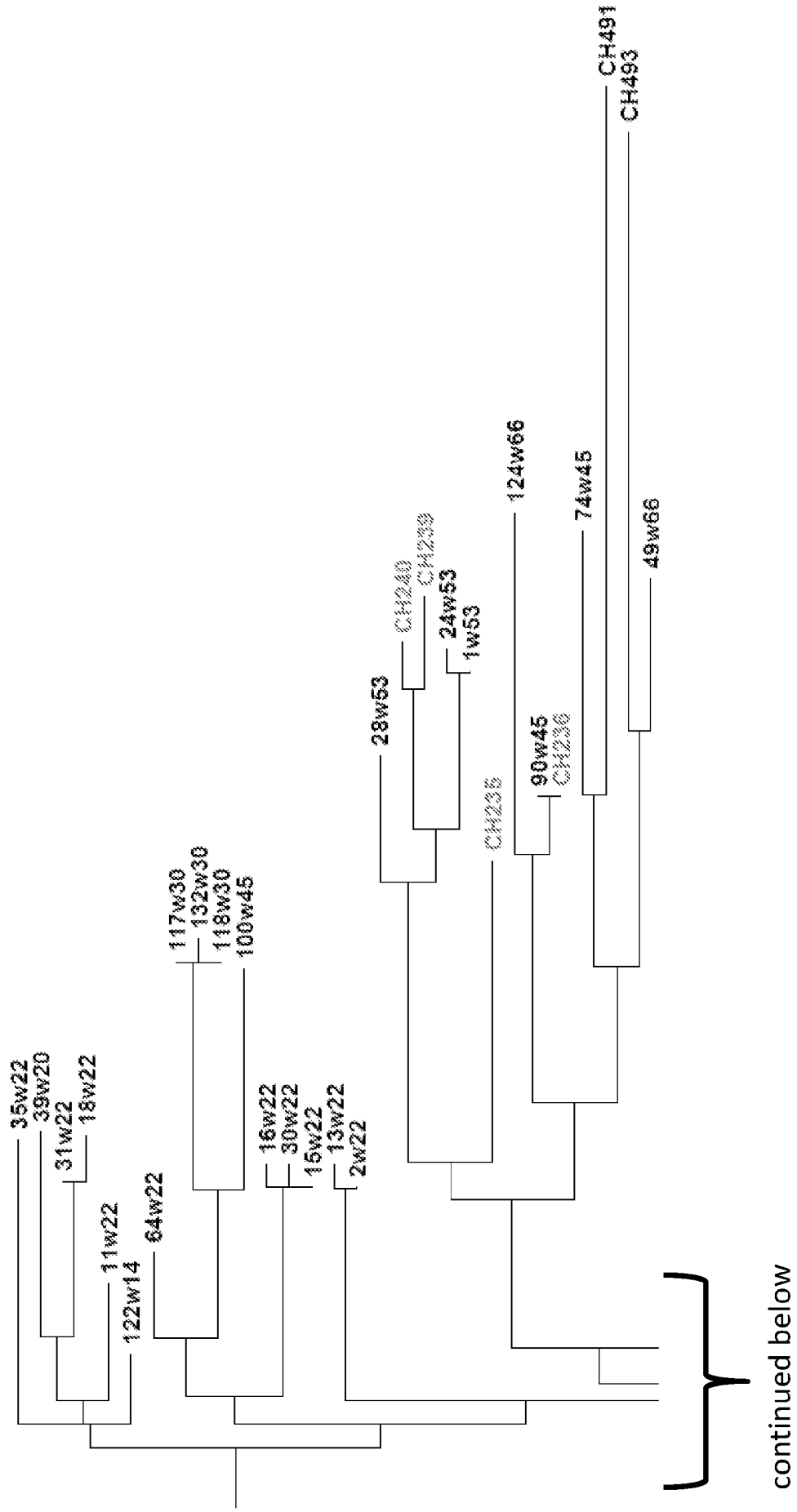

CH491
CH493
124w66
74w45
49w66
CH240
CH239
24w53
1w53
28w53
90w45
CH238
CH238
117w30
132w30
118w30
100w45
16w22
30w22
15w22
13w22
2w22
35w22
39w20
31w22
18w22
11w22
64w22
122w14
continued below

FIG. 20

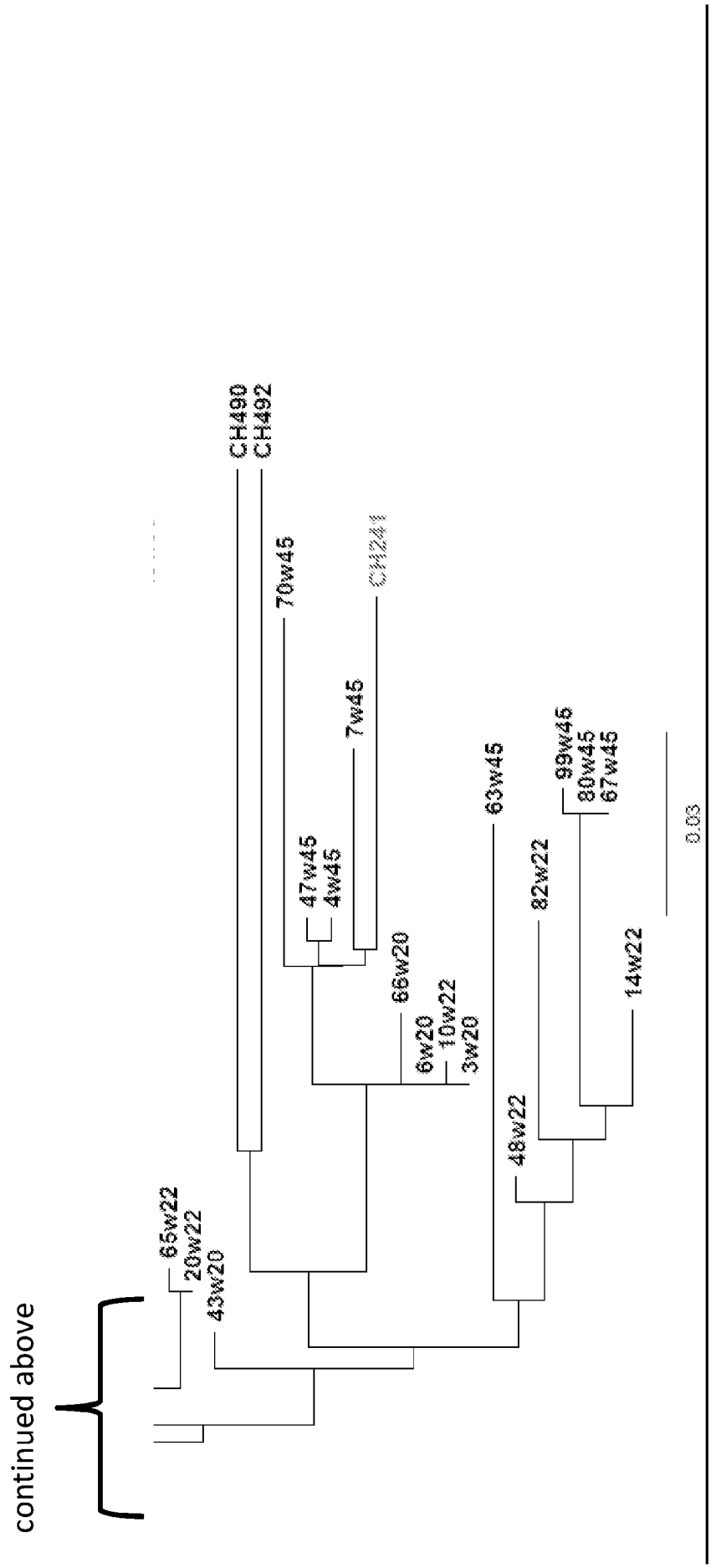

continued above
65w22
20w22
43w20
CH490
CH492
70w45
CH241
7w45
47w45
4w45
66w20
6w20
10w22
3w20
63w45
48w22
82w22
99w45
80w45
67w45
14w22
0.03

FIG. 20 cont.

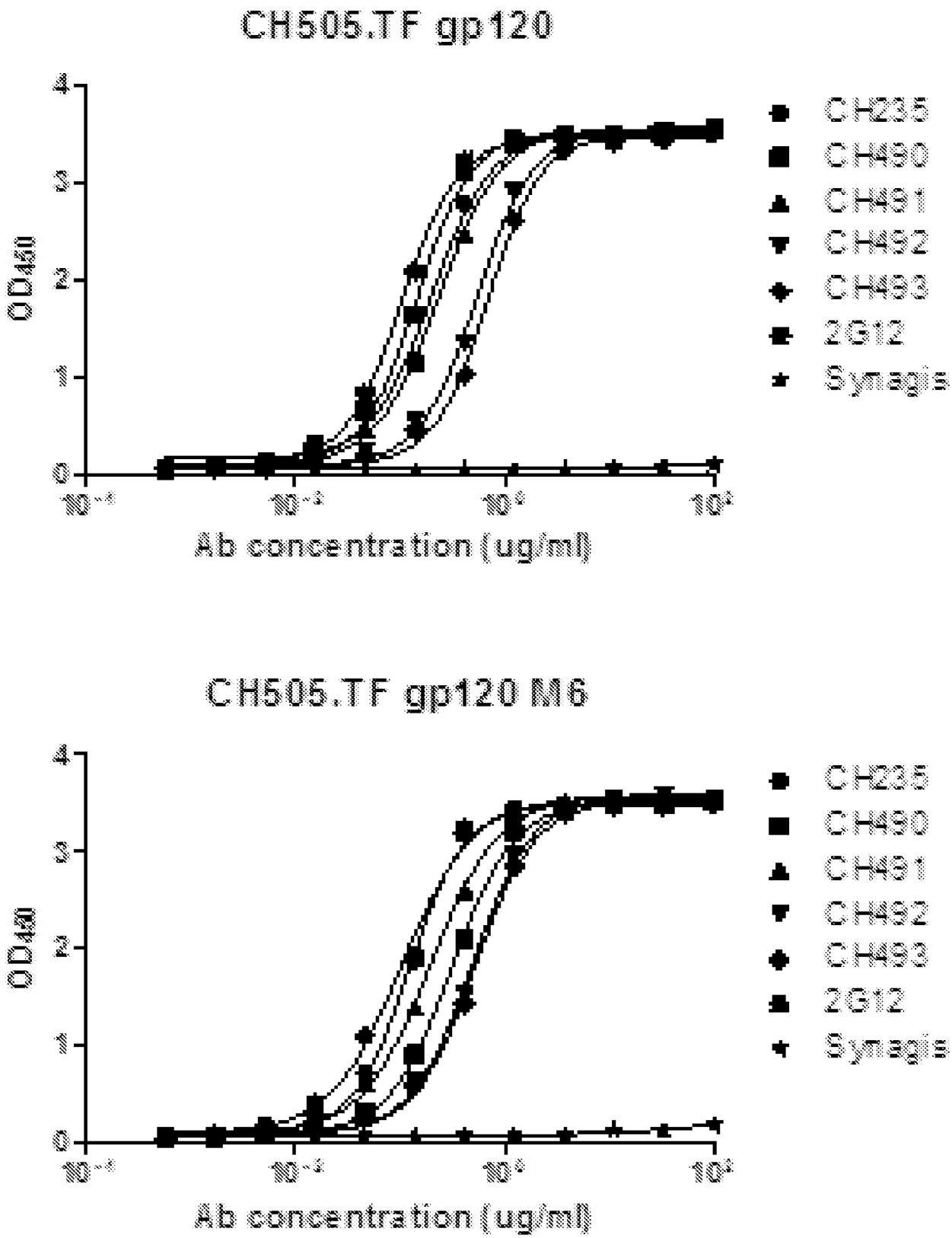
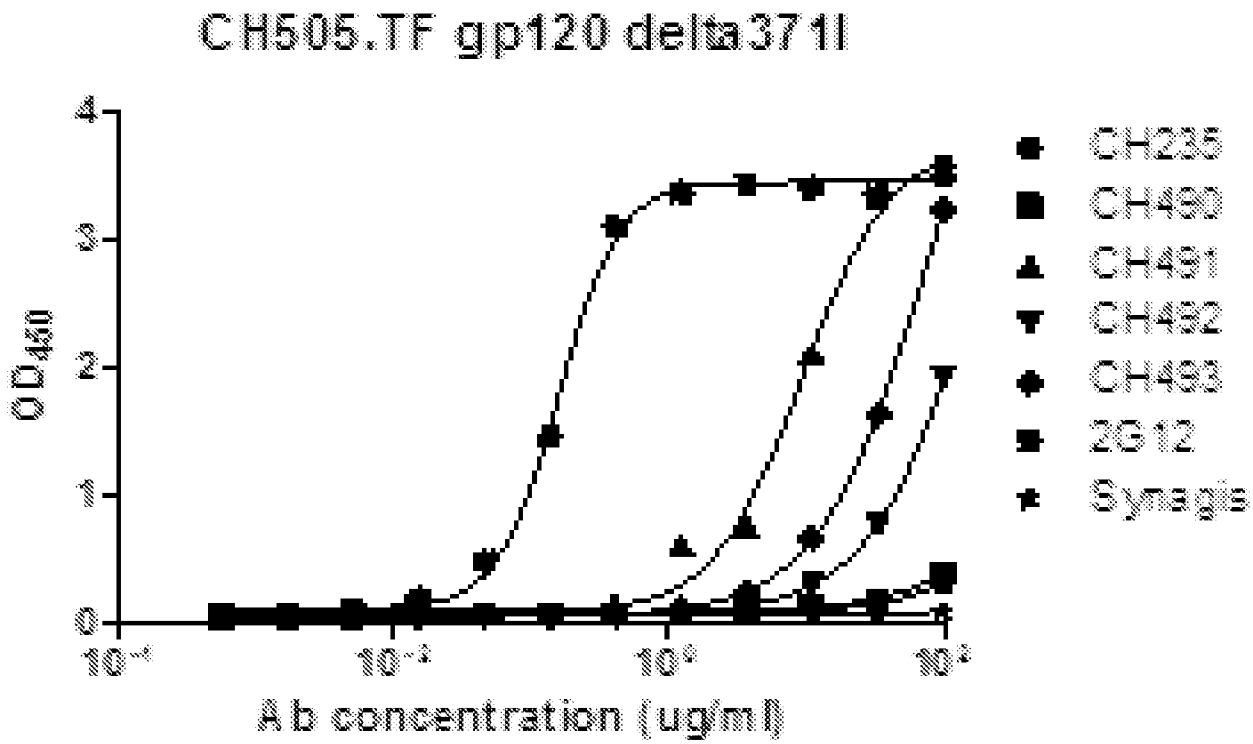

CH505.TF gp120
OD450
Ab concentration (ug/ml)
CH235
CH490
CH491
CH492
CH493
2G12
Synagis
CH505.TF gp120 M6
OD450
Ab concentration (ug/ml)
CH235
CH490
CH491
CH492
CH493
2G12
Synagis

FIG. 21


CH505.TF gp120 delta371I
OD450
Ab concentration (ug/ml)
CH235
CH490
CH491
CH492
CH493
2G12
Synagis

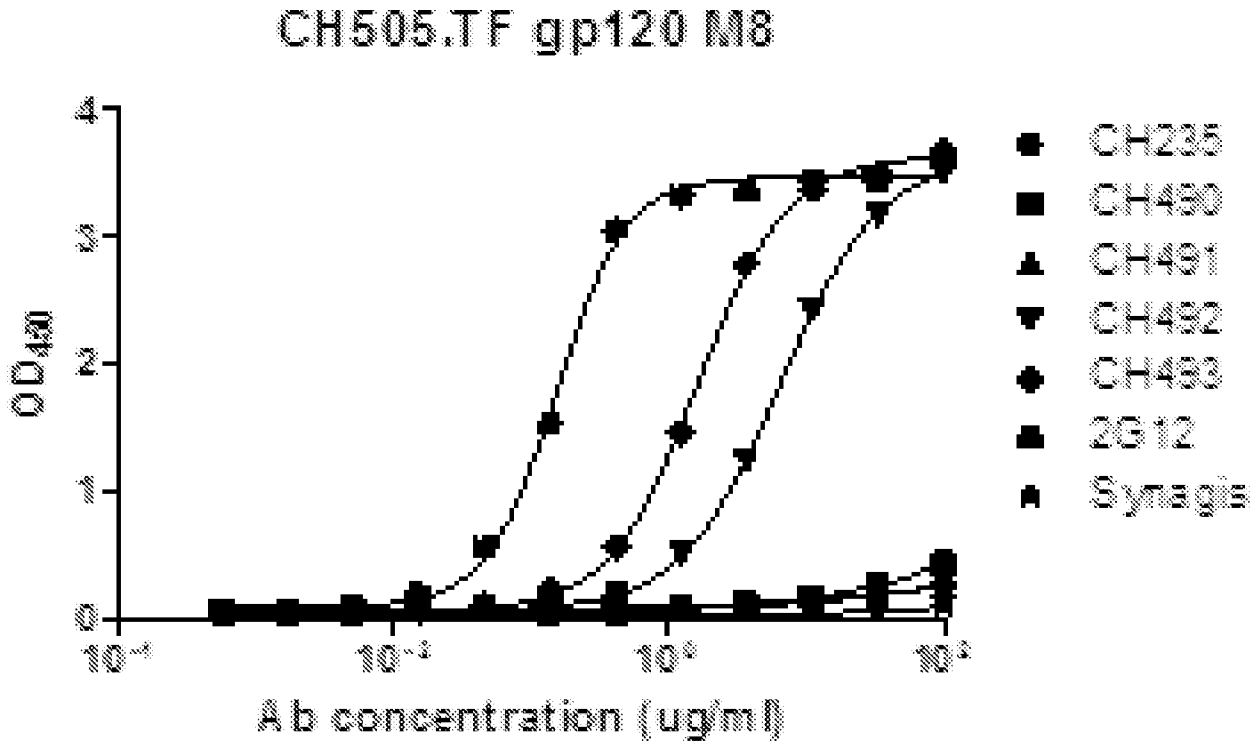

CH505.TF gp120 M8
OD450
Ab concentration (ug/ml)
CH235
CH490
CH491
CH492
CH493
2G12
Synagis

FIG. 21 cont.

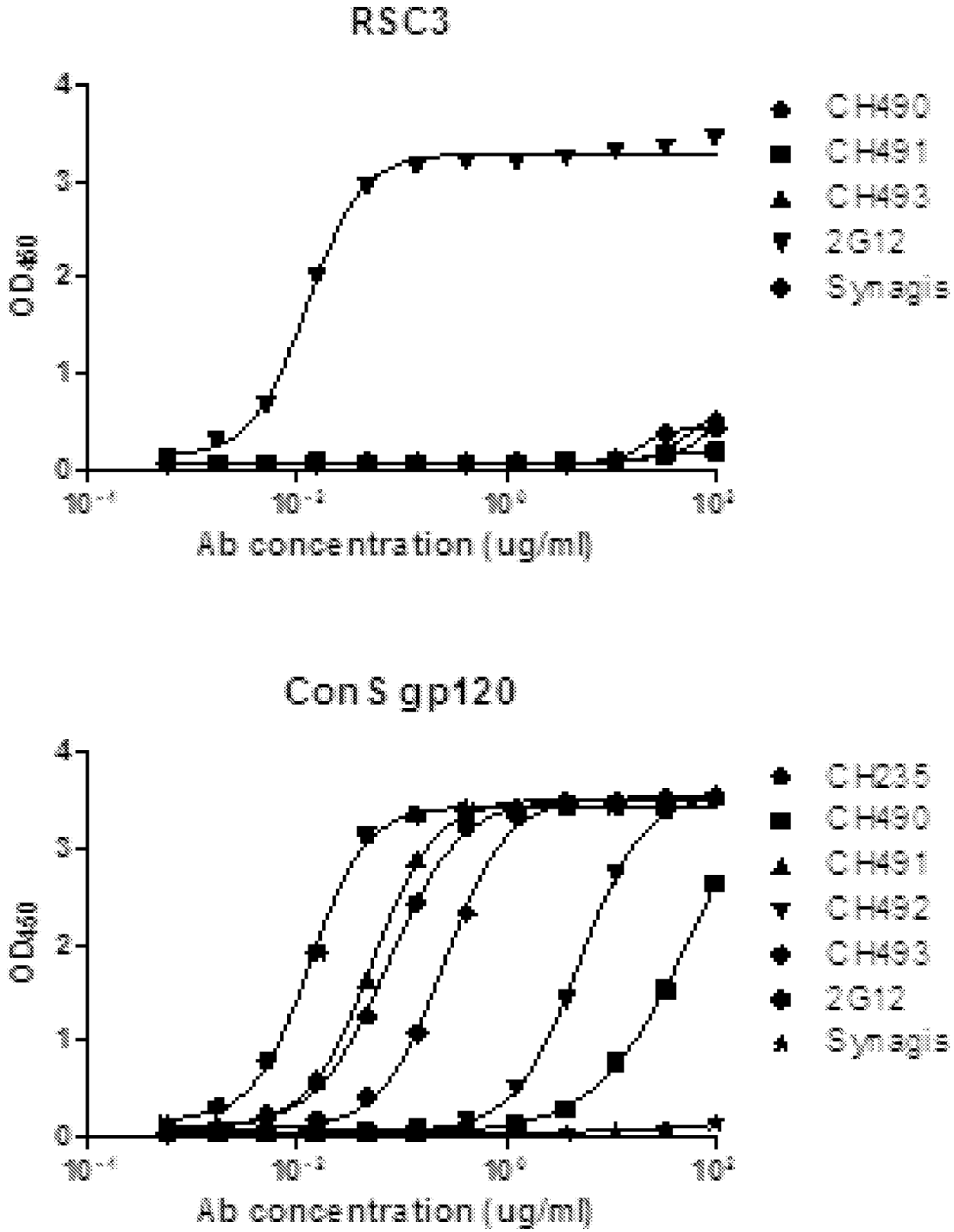

RSC3
OD450
Ab concentration (ug/ml)
CH490
CH491
CH493
2G12
Synagis
ConS gp120
OD450
Ab concentration (ug/ml)
CH235
CH490
CH491
CH492
CH493
2G12
Synagis

FIG. 21 cont.

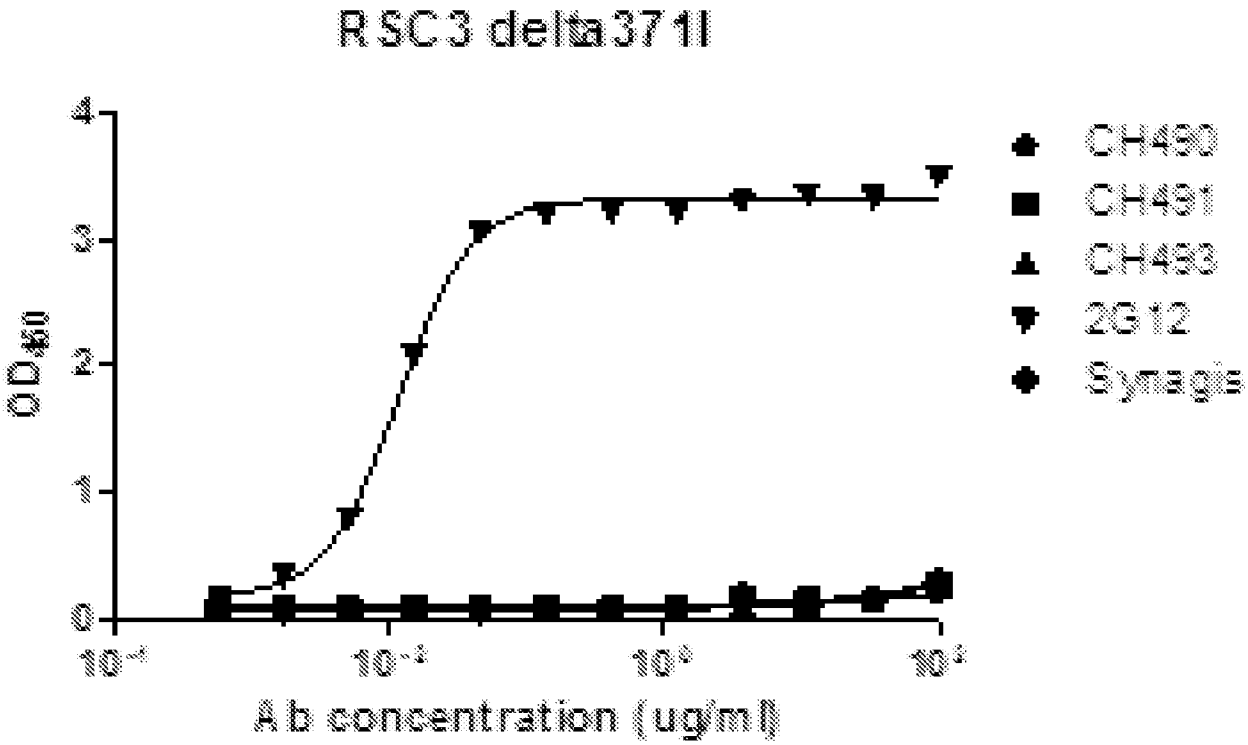

RSC3 delta371I
CH490
CH491
CH493
2G12
Synagis
OD450
Ab concentration (ug/ml)

FIG. 21 cont.

			IC50 (ug/ml) in TZM-bl Cells[1]					
			CH235UCA_LL	DH235UCAtk_V2_4A/293i	DH235VH_I1_V2_4A/293i	DH235VH_I2_V2_4A/293i	DH235VH_I3_v2_4A/293i	DH235_I4_V2_4A/293i
Virus Name	Clade	Virus Lot	217SJA	5RKK	218SJA	256JAH	257JAH	4RKK
Q23.17	A	2435	>50	>50	**21**	>50	>50	>50
DJ263.8	AG	2220	>50	>50	>50	>50	>50	>50
C1080.c03	AE	3756	>50	>50	>50	>50	>50	>50
6540.v4.c1	AC	2746	>50	>50	>50	>50	>50	>50
Q168.a2	AD	1715	>50	>50	>50	>50	>50	>50
6101.1	B	737	>50	>50	>50	>50	>50	>50
BG1168.1	B	530	>50	>50	>50	>50	>50	>50
DU172.17	C	4168	>50	>50	>50	>50	>50	>50
DU156.12	C	4166	>50	>50	>50	>50	>50	>50
DU422.1	C	3803	>50	>50	>50	>50	>50	>50
57128.vrc15	D	1940	>50	>50	>50	>50	>50	>50
X1632-S2-B10	G	2900	>50	>50	>50	>50	>50	>50
Q679.d22	A	3278	>50	>50	>50	>50	>50	>50
ZM106F.PB9	C	824	>50	>50	>50	>50	>50	>50
CNE58	C	6509	>50	>50	>50	>50	>50	>50
92RW020.2	A	1573	>50	>50	**44**	>50	>50	>50
CAAN5342.A2	B	995	>50	>50	>50	>50	>50	>50
JR-FL	B	730	>50	>50	>50	>50	>50	>50
PVO.4	B	3801	>50	>50	>50	>50	>50	>50
THRO4156.18	B	967	>50	>50	>50	>50	>50	>50
TRJO4551.58	B	4159	>50	>50	>50	>50	>50	>50
TRO.11	B	772	>50	>50	>50	>50	>50	>50
YU2	B	4098	>50	>50	>50	>50	>50	>50
ZM55F.PB28a	C	819	>50	>50	>50	>50	>50	>50
percent positive			0	0	**8**	0	0	0

[1]Values 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

nt = not tested

FIG. 22

			IC50 (ug/ml) in TZM-bl Cells[1]			
			DH235_4A	CH236_4A/293i	CH239_4A/293i	CH240_4A/293i
Virus Name	Clade	Virus Lot	223SJA	215SJA	216SJA	254JAH
Q23.17	A	2435	1.7	3.8	6.7	>50
DJ263.8	AG	2220	>50	>50	>50	>50
C1080.c03	AE	3756	>50	>50	>50	>50
6540.v4.c1	AC	2746	>50	>50	>50	>50
Q168.a2	AD	1715	>50	>50	>50	>50
6101.1	B	737	>50	>50	>50	>50
BG1168.1	B	530	>50	>50	>50	>50
DU172.17	C	4168	1.8	5.6	11	>50
DU156.12	C	4166	13	>50	>50	>50
DU422.1	C	3803	>50	>50	>50	>50
57128.vrc15	D	1940	>50	>50	>50	>50
X1632-S2-B10	G	2900	>50	>50	>50	>50
Q679.d22	A	3278	>50	>50	>50	>50
ZM106F.PB9	C	824	>50	>50	>50	>50
CNE58	C	6509	>50	>50	>50	>50
92RW020.2	A	1573	1.5	26	6.3	>50
CAAN5342.A2	B	995	>50	>50	>50	>50
JR-FL	B	730	1.6	>50	2.8	>50
PVO.4	B	3801	>50	>50	>50	>50
THRO4156.18	B	967	>50	>50	>50	>50
TRJO4551.58	B	4159	>50	>50	>50	>50
TRO.11	B	772	13	>50	30	>50
YU2	B	4098	>50	>50	16	>50
ZM55F.PB28a	C	819	>50	>50	>50	>50
percent positive			25	13	25	0

FIG. 22 cont.

			CH241_4A	CH490_4A	CH491_4A	CH492_4A/293i	CH493_4A	CH01-31
Virus Name	Clade	Virus Lot	296HC	311HC	226SJA	22RKK	29RKK	
Q23.17	A	2435	19	>50	1.1	>50	0.98	0.02
DJ263.8	AG	2220	>50	>50	>50	>50	1.5	0.25
C1080.c03	AE	3756	>50	>50	>50	>50	50	0.14
6540.v4.c1	AC	2746	>50	>50	>50	>50	>50	0.46
Q168.a2	AD	1715	>50	>50	>50	>50	1.2	0.10
6101.1	B	737	11	>50	>50	>50	8.1	0.57
BG1168.1	B	530	>50	>50	>50	>50	8.4	1.7
DU172.17	C	4168	3.2	>50	1.1	>50	1.5	0.59
DU156.12	C	4166	>50	>50	4.5	>50	1.6	0.36
DU422.1	C	3803	>50	>50	8.1	>50	5.9	>25
57128.vrc15	D	1940	>50	>50	>50	>50	>50	>25
X1632-S2-B10	G	2900	>50	>50	0.52	>50	1.1	0.09
Q679.d22	A	3278	>50	>50	>50	>50	1.1	0.08
ZM106F.PB9	C	824	48	>50	5.0	>50	2.6	12
CNE58	C	6509	>50	>50	>50	>50	>50	0.09
92RW020.2	A	1573	3.3	>50	1.7	>50	0.74	0.03
CAAN5342.A2	B	995	>50	>50	>50	>50	9.8	>25
JR-FL	B	730	2.9	>50	12	>50	5.4	0.03
PVO.4	B	3801	23	>50	8.4	nt	8.1	0.92
THRO4156.18	B	967	>50	>50	>50	nt	>50	2.8
TRJO4551.58	B	4159	>50	>50	>50	nt	2.1	0.31
TRO.11	B	772	>50	>50	>50	nt	4.5	0.26
YU2	B	4098	1.0	>50	0.20	nt	0.32	0.07
ZM55F.PB28a	C	819	29	>50	15	nt	5.0	2.9
percent positive			38	0	46	0	83	88

FIG. 22 cont.

Ab	M5	M6	M10	M19	M11	M7	M8	M9	M20	M21
CH235	0.30	0.81	0.71	2.16	26.7	>50	>50	>50	>50	>50
CH490	0.12	>50	>50	>50	>50	>50	>50	>50	>50	>50
CH491	0.16	0.34	0.18	0.40	0.50	>50*	>50	>50	>50	>50
CH493	0.19	0.40	0.24	0.63	0.39	0.77	0.66	0.85	0.94	0.74

FIG. 23

CH0505 Loop D Mutants

	ID	CH01-31	CH235	CH490	CH491	CH493
	Lot	Catalent	223SJA	311HC	226SJA	29RKK
S.No.	Sample ID	IC50 (ug/ml) in TZM-bl cells				
1	CH0505.TF.M5	3.97	0.30	0.12	0.16	0.19
2	CH0505.TF.M6	0.04	0.81	>50	0.34	0.40
3	CH0505.TF.M7	0.05	>50	>50	>50*	0.77
4	CH0505.TF.M8	0.07	>50	>50	>50	0.66
5	CH0505.TF.M9	0.71	>50	>50	>50	0.85
6	CH0505.TF.M10	0.20	0.71	>50	0.18	0.24
7	CH0505.TF.M11	0.79	26.68	>50	0.50	0.39
8	CH0505.TF.M19	2.96	2.16	>50	0.40	0.63
9	CH0505.TF.M20	0.56	>50	>50	>50	0.94
10	CH0505.TF.M21	0.06	>50	>50	>50	0.74

*48% neutralization at 50 ug/ml

FIG. 24

FIG. 25

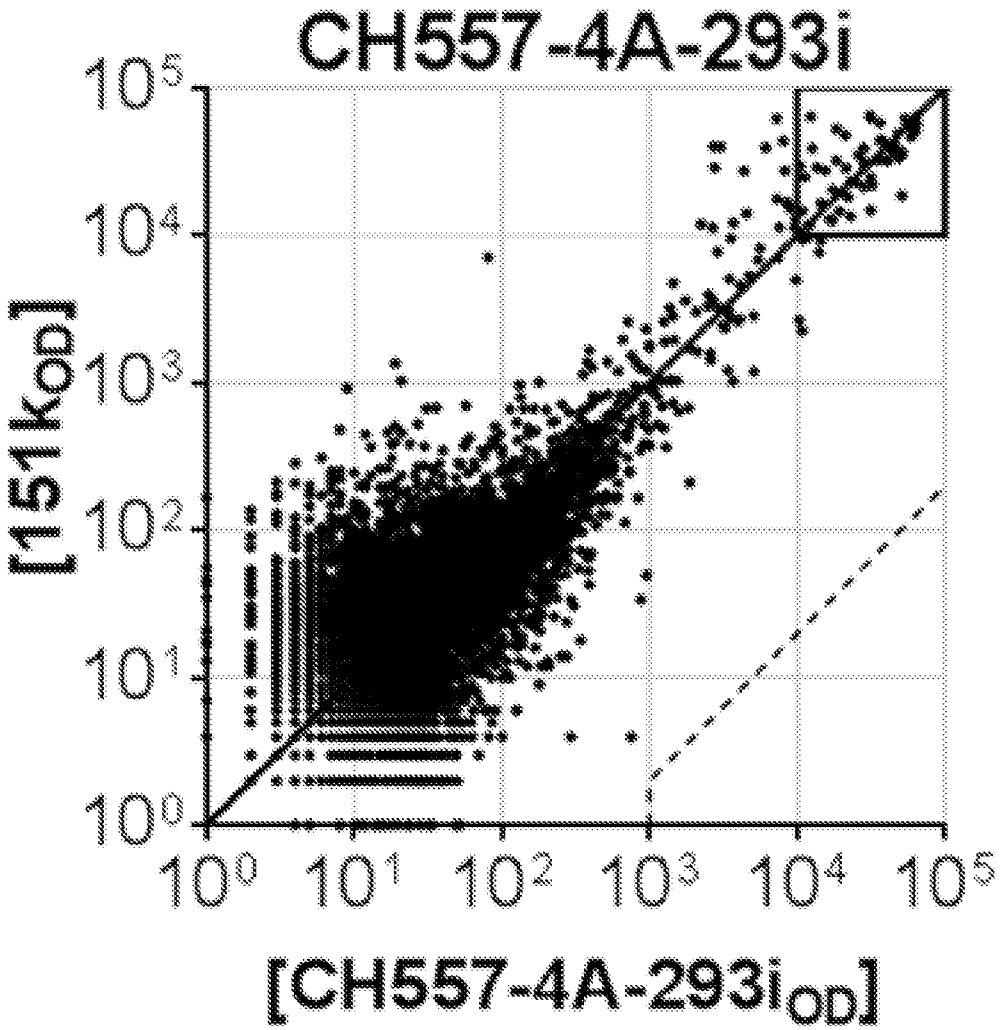

CH557-4A-293i
10^5
10^4
10^3
10^2
10^1
10^0
[151kOD]
10^0 10^1 10^2 10^3 10^4 10^5
[CH557-4A-293iOD]

FIG. 26A

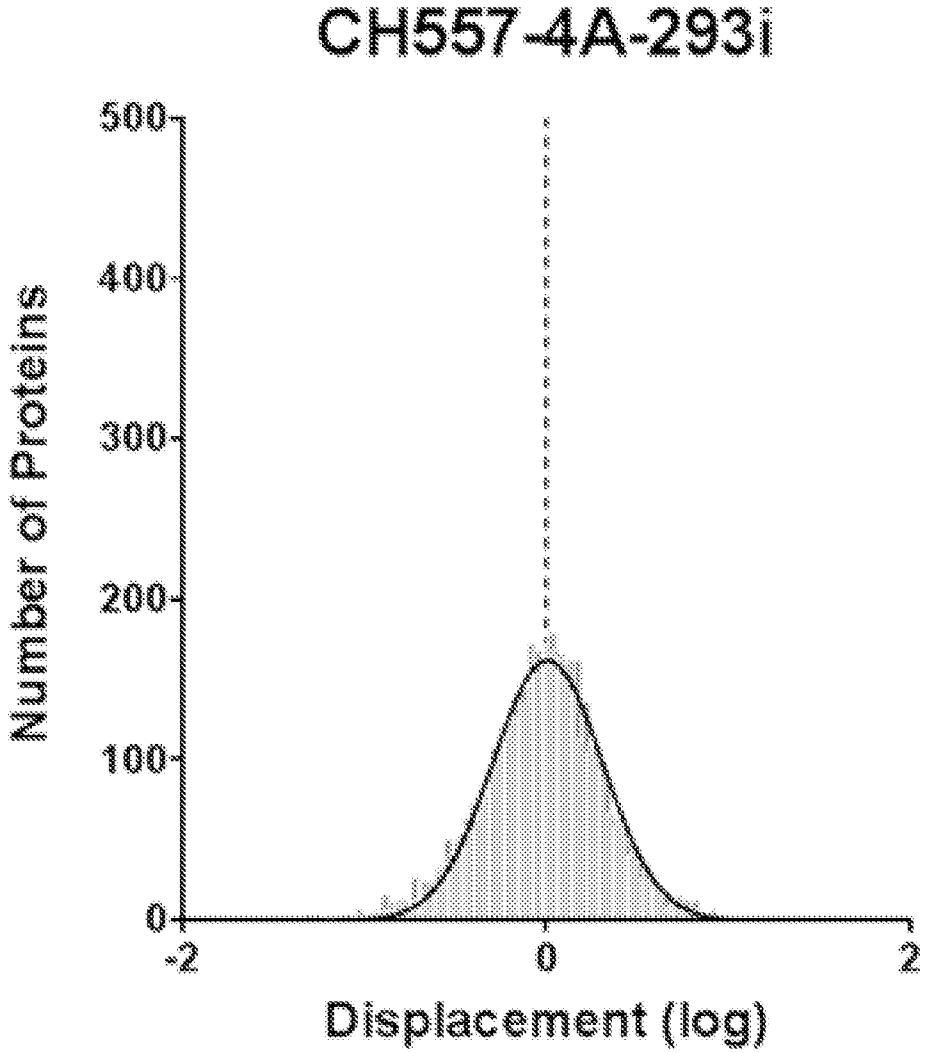

CH557-4A-293i
Number of Proteins
500
400
300
200
100
0
-2
0
2
Displacement (log)

FIG. 26B

High Throughput Antibody Screen - Panel P

Assay - Luc/TZM-bl
values represent IC50 in ug/ml

<0.100
0.100-1.00
1.00-10.0
>10.0

Virus ID	Clade	Panel P CH557	Panel P CH235	Panel P VRC01	Panel H VRC07-523-LS	Panel P N6	Panel D 3BNC117	Panel D 8ANC131	Panel F CH103	Panel A F105	Panel P DH522
0260.v5.c36	A	1.02	>50	0.468	0.175	0.048	0.200	1.18	>50	>50	>50
0330.v4.c3	A	0.313	>50	0.047	0.007	0.008	0.013	0.084	7.84	>50	>50
0439.v5.c1	A	0.374	>50	0.129	0.045	0.024	0.215	0.262	8.37	>50	>50
3365.v2.c20	A	0.068	>50	0.030	0.004	0.010	0.011	4.30	4.00	>50	>50
3415.v1.c1	A	0.450	>50	0.084	0.015	0.019	0.094	0.430	2.46	>50	>50
3718.v3.c11	A	0.360	12.3	0.165	0.009	0.011	>50	1.31	29.6	>50	>50
398-F1_F6_20	A	1.76	>50	0.181	0.025	0.019	0.071	15.9	>50	>50	>50
BB201.B42	A	0.573	>50	0.316	0.111	0.106	3.35	7.32	9.19	>50	>50
BB539.2B13	A				0.004		0.033	0.195	22.2	>50	
BG505.W6M.C2	A	0.111	>50	0.053		0.022	0.024	0.298		>50	>50
BI369.9A	A	0.290	>50	0.224	0.046	0.063	0.020	0.798	>50	>50	>50
BS208.B1	A	0.263	>50	0.022	0.0010	0.009	0.002	0.076	>50	>50	>50
KER2008.12	A	>50	>50	0.591	0.192	0.296	0.248	>50	>50	>50	>50
KER2018.11	A	2.52	>50	0.555	0.174	0.064	0.417	3.28	40.0	>50	>50
KNH1209.18	A	0.251	>50	0.099	0.034	0.029	0.040	1.99	>50	>50	>50
MB201.A1	A	0.333	>50	0.212	0.093	0.045	0.464	27.4	17.7	>50	>50
MB539.2B7	A	1.71	>50	0.500	0.102	0.067	0.087	3.10	3.70	>50	>50
MI369.A5	A	0.416	>50	0.269	0.110	0.070	0.033	0.850	>50	>50	>50
MS208.A1	A	0.463	>50	0.178	0.029	0.049	0.019	0.931	>50	>50	>50
Q23.17	A	0.132	1.35	0.052	0.022	0.018	0.017	0.274	10.4	>50	>50
Q259.17	A	0.100	>50	0.075	0.006	0.129	0.017	0.756	10.6	>50	>50

FIG. 27A

Q769.d22	A	0.110	>50	0.035	0.008	0.015	0.007	0.032	0.998	>50	>50	
Q769.h5	A	0.139	>50	0.062	0.012	0.028	0.006	0.034		>50	>50	
Q842.d12	A	0.091	8.15	0.038	0.007	0.013	0.002	0.014	1.46	>50	>50	
QH209.14M.A2	A	0.374	>50	0.060	0.006	0.012	0.008	2.29	1.16	>50	>50	
RW020.2	A	0.301	1.20	0.203	0.064	0.037	0.020	0.378	2.16	>50	>50	
UG037.8	A	0.188	>50	0.089	0.012	0.014	0.020	0.187	1.76	>50	>50	
246-F3.C10.2	AC									>50		
3301.V1.C24	AC	0.473	20.9	0.097	0.005	0.007	0.046	0.067	5.54	>50	>50	
3589.V1.C4	AC	0.309	>50	0.047	0.011	0.010	0.061	8.12	19.6	>50	>50	
6540.v4.c1	AC	>50	>50	>50	>50	0.027	>50	>50	1.77	>50	>50	
6545.V4.C1	AC	>50	>50	>50	>50	42.0	>50	42.5	>50	>50	>50	
0815.V3.C3	ACD	0.056	>50	0.015	0.0010	0.003	0.018	0.135	34.5	>50	>50	
6095.V1.C10	ACD	1.33	>50	0.506	0.210	0.132	0.096	1.53	>50	4.69	22.8	
3468.V1.C12	AD	0.070	2.47	0.050	0.006	0.010	0.073	6.79	>50	>50	>50	
Q168.a2	AD	0.261	>50	0.098	0.030	0.027	0.050	0.299	6.76	>50	>50	
Q461.e2	AD	0.818	>50	0.497	0.091	0.099	0.069	1.26	>50	>50	>50	
620345.c1	AE	1.94	>50	>50	>50	1.26	>50	>50	>50	>50	>50	
BJOX009000.02.4	AE	5.50	>50	1.54	0.311	0.187			>50	>50	>50	
BJOX010000.06.2	AE	10.6	>50	6.79	1.26	0.518			>50	>50	>50	
BJOX025000.01.1	AE	0.271	40.6	8.46	0.128	0.021			>50	>50	>50	
BJOX028000.10.3	AE	0.168	>50	0.256	0.0010	0.005			>50	>50	>50	
C1080.c3	AE	2.69	>50	2.10	0.148	0.178	0.096	>50	>50	>50	>50	
C2101.c1	AE	0.261	12.6	0.179	0.052	0.044	0.064	14.2	>50	>50	>50	
C3347.c11	AE	0.117	>50	0.095	0.024	0.010	0.029	5.30	>50	>50	>50	
C4118.09	AE	0.084	3.30	0.248	0.012	0.047	0.019	9.97	>50	>50	>50	
CM244.ec1	AE	0.160	1.19	0.089	0.005	0.011			>50	>50	>50	
CNE3	AE	2.45	>50	1.63	0.122	0.022	0.125	2.75	>50	>50	>50	
CNE5	AE	1.03	17.6	0.323	0.059	0.074	0.386	>50	>50	>50	>50	

FIG. 27A cont.

CNE55	AE	0.400	>50	0.359	0.046	0.069	0.147	16.8	>50	>50	>50
CNE56	AE	1.10	42.9	0.343	0.086	0.074	0.075	11.3	>50	>50	>50
CNE59	AE	0.943	13.6	0.623	0.057	0.100	0.043	11.3	>50	>50	>50
CNE8	AE	1.10	>50	0.510	0.118	0.096			>50	>50	>50
M02138	AE				0.231		0.154	22.4	>50	>50	
R1166.c1	AE	0.758	>50	3.00	0.434	0.453	0.230	>50	>50	>50	>50
R2184.c4	AE	0.563	5.82	0.133	0.013	0.029	0.035	9.00	>50	>50	>50
R3265.c6	AE	0.172	>50	0.710	0.111	0.079	0.020	>50	>50	>50	>50
TH023.6	AE				0.005				>50	>50	
TH966.8	AE	0.304	0.732	0.284	0.043	0.034	0.056	15.6	>50	>50	>50
TH976.17	AE	0.286	0.975	0.332	0.046	0.058	0.025	11.0	>50	>50	>50
235-47	AG	0.293	>50	0.061	0.003	0.009	0.022	4.54	0.628	>50	>50
242-14	AG	2.83	>50	>50	0.136	0.212	>50	>50	>50	>50	>50
263-8	AG	0.447	>50	0.168	0.023	0.018	0.047	0.209	>50	>50	>50
269-12	AG	>50	>50	0.293	0.046	0.025	0.151	2.92	10.1	>50	>50
271-11	AG	0.090	>50	0.054	0.007	0.011	0.007	2.18	8.52	>50	>50
928-28	AG	0.542	>50	0.476	0.086	0.067	0.155	2.26	>50	>50	>50
DJ263.8	AG	0.276	>50	0.066	0.003	0.059	0.025	0.032	0.784	>50	7.18
T250-4	AG	>50	>50	>50	>50	0.007	>50	>50	>50	>50	>50
T251-18	AG	4.02	>50	4.42	0.215	0.383	0.203	1.79	34.4	>50	>50
T253-11	AG	1.65	>50	0.501	0.064	0.109	0.116	10.9	>50	>50	>50
T255-34	AG	0.608	>50	0.725	0.032	0.056	0.051	9.76	>50	>50	>50
T257-31	AG	2.66	>50	2.47	0.266	0.173	0.181	31.4	>50	>50	>50
T266-60	AG	103	>50	2.37	0.317	0.193	0.032	1.35	>50	>50	>50
T278-50	AG	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
T280-5	AG	0.109	>50	0.059	0.003	0.021	0.019	0.042	0.986	>50	>50
T33-7	AG	0.039	>50	0.019	0.0010	0.007	0.007	0.959	8.44	>50	>50
3988.25	B	0.917	>50	0.369	0.059	0.084	>50	>50	9.84	>50	>50

FIG. 27A cont.

5768.04	B	0.715	>50	0.354	0.065	0.053	0.201	31.5	7.08	>50	>50
6101.10	B	0.467	>50	0.023	0.005	0.003	0.022	0.058	1.80	>50	>50
6535.3	B	4.85	>50	2.10	0.114	0.063	0.262	0.362	4.67	3.08	16.5
7165.18	B	>50	>50	45.0	1.43	0.912	6.54	>50	>50	>50	>50
45_01dG5	B	0.058	>50	0.011	0.0008	0.0003			0.796	>50	>50
89.6.DG	B	2.23	>50	1.30	0.092	0.195	0.109	0.810	1.40	>50	>50
AC10.29	B	2.13	>50	1.41	0.624	0.238	6.05	>50	>50	>50	>50
ADA.DG	B	0.907	>50	0.563	0.114	0.120	0.086	0.393	1.49	2.02	1.69
Bal.01	B	0.237	>50	0.124	0.034	0.023	0.011	0.132	0.676	>50	>50
BaL.26	B	0.214	>50	0.060	0.0010	0.015	0.006	0.081	0.091	10.9	27.9
BG1168.01	B	1.42	>50	0.738	0.090	0.077	0.179	0.562	21.7	>50	>50
BL01.DG	B	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
BR07.DG	B	1.51	>50	1.81	0.191	0.335	0.135	1.77	9.67	>50	>50
BX08.16	B	2.35	>50	0.389	0.040	0.047	0.102	2.26	1.15	9.73	6.44
CAAN.A2	B	2.23	>50	0.963	0.140	0.136	0.673	1.72	>50	>50	>50
CNE10	B	5.26	>50	0.689	0.042	0.104	0.050	0.941	26.9	>50	>50
CNE12	B	2.56	>50	0.695	0.129	0.200	0.085	1.42	26.4	>50	>50
CNE14	B	0.594	>50	0.199	0.019	0.028	0.024	5.19	0.522	>50	>50
CNE4	B	1.16	>50	0.639	0.129	0.137	0.113	19.2	43.5	13.6	>50
CNE57	B	1.25	>50	0.496	0.172	0.076	0.066	14.5	>50	>50	>50
HO86.8	B	0.174	>50	>50	>50	2.00	>50	>50	>50	>50	>50
HT593.1	B	0.984	>50	0.606	0.080	0.156	0.229	0.817	40.0	>50	>50
HXB2.DG	B	0.173	18.1	0.063	0.009	0.008	0.037	0.026	0.124	0.167	0.241
JRCSF.JB	B	0.596	>50	0.436	0.037	0.135	0.028	0.245	0.906	>50	>50
JRFL.JB	B	0.127	1.82	0.051	0.0009	0.003	0.0010	0.046	0.021	>50	>50
MN.3	B	0.142	>50	0.011	0.0003	0.002	>50	0.693	0.187	>50	1.81
PVO.04	B	1.47	>50	0.552	0.079	0.073	0.074	0.477	>50	>50	>50
QH0515.01	B	1.40	26.4	1.43	0.494	0.377	0.175	>50	10.2	>50	>50

FIG. 27A cont.

QH0692.42	B	2.25	>50	1.37	0.502	0.484	0.275	2.63	25.2	>50	>50
REJO.67	B	1.09	>50	0.113	0.003	0.022	0.039	0.052	2.15	>50	>50
RHPA.7	B	0.091	16.6	0.051	0.006	0.012	0.019	5.10	8.53	>50	>50
SC422.8	B	0.798	>50	0.127	0.023	0.058	0.049	0.158	2.44	>50	>50
SF162.LS	B	0.534	>50	0.228	0.019	0.040	0.019	0.103	0.843	2.92	2.83
SS1196.01	B	0.827	>50	0.246	0.047	0.069	0.038	0.271	1.34	6.24	29.6
THRO.18	B	>50	>50	4.63	0.965	0.890	2.80	19.7	>50	>50	>50
TRJO.58	B	0.524	>50	0.116	0.038	0.094	0.062	3.45	>50	>50	>50
TRO.11	B	0.714	14.8	0.502	0.093	0.099	0.033	9.97	5.34	>50	>50
WITO.33	B	0.418	>50	0.140	0.047	0.056	0.030	12.7	7.35	>50	>50
X2278.C2.B6	B	0.425	>50	0.133		0.030				>50	>50
YU2.DG	B	0.235	>50	0.113	0.020	0.030	0.029	0.221	1.80	>50	>50
BJOX002000.03.2	BC	0.739	>50	>50		0.032				>50	>50
CH038.12	BC	173	>50	0.519	0.052	0.094	>50	>50	3.54	>50	>50
CH070.1	BC	2.39	>50	9.99	0.088	0.119	7.89	3.81	>50	>50	>50
CH117.4	BC	0.340	>50	0.095	0.007	0.027	0.663	>50	4.09	>50	>50
CH119.10	BC	1.24	>50	0.577		0.045				>50	>50
CH181.12	BC	0.612	>50	0.481	0.061	0.065	0.124	9.50	5.00	>50	>50
CNE15	BC	0.249	15.2	0.100	0.007	0.025	>50	25.9	18.7	>50	>50
CNE19	BC	0.134	27.5	0.169		0.016			10.6	>50	>50
CNE20	BC	0.254	>50	9.25	0.005	0.009			1.80	>50	>50
CNE21	BC	0.527	>50	0.357	0.041	0.031			>50	>50	>50
CNE40	BC	0.207	>50	0.370	0.034	0.018	0.116	49.6	0.505	0.242	0.221
CNE7	BC	1.36	>50	0.286	0.039	0.024	>50	26.4	27.3	>50	>50
286.36	C	0.699	>50	0.322	0.070	0.027	0.067	0.713	8.36	>50	>50
288.38	C	1.62	>50	1.49	0.263	0.169	0.063	0.683	8.45	>50	>50
0013095-2.11	C	24.7	>50	0.088	0.009	0.028	0.208	37.1	5.71	>50	>50
001428-2.42	C	0.087	>50	0.008	0.0003	0.0008	0.010	0.073	1.51	>50	>50

Fig. 27A cont.

0077_V1.C16	C	6.84	>50	1.28	0.045	0.010	>50	>50	>50	>50	>50
00836-2.5	C	1.09	>50	0.119	0.0007	0.0003	>50	>50	28.6	>50	>50
0921.V2.C14	C	0.344	10.9	0.182	0.014	0.016	0.243	16.0	>50	>50	>50
16055-2.3	C	0.159	>50	0.063	0.003	0.004	3.24	0.386	>50	>50	>50
16845-2.22	C	7.47	>50	3.60	0.383	0.257	29.6	>50	>50	>50	>50
16936-2.21	C	0.500	>50	0.110	0.0010	0.012	0.059	0.094	5.57	>50	>50
25710-2.43	C	0.382	>50	0.594	0.111	0.080	0.100	3.67	>50	>50	>50
25711-2.4	C	0.974	>50	0.555	0.078	0.055	>50	>50	17.1	>50	>50
25925-2.22	C	0.641	>50	0.474	0.062	0.086	0.136	13.9	26.7	>50	>50
26191-2.48	C	0.583	>50	0.166	0.046	0.039	0.043	11.0	>50	>50	>50
3168.V4.C10	C	0.372	>50	0.255	0.030	0.077	0.110	9.47	10.3	>50	>50
3637.V5.C3	C	1.22	>50	1.45	0.255	0.130	>50	39.7	>50	>50	>50
3873.V1.C24	C	>50	>50	0.791	0.181	0.105	6.97	0.460	>50	>50	>50
426c	C										
6322.V4.C1	C	0.944	>50	>50	0.023	0.018	>50	>50	>50	>50	>50
6471.V1.C16	C	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
6631.V3.C10	C	5.83	>50	>50	0.148	0.090	>50	>50	>50	>50	>50
6644.V2.C33	C	>50	>50	0.243	0.028	0.065	0.033	19.2	0.377	2.30	4.49
6785.V5.C14	C	>50	>50	0.286	0.068	0.102	0.195	10.5	8.95	>50	>50
6838.V1.C35	C	1.08	>50	0.210	0.038	0.025	0.281	0.462	29.0	>50	>50
96ZM651.02	C	1.18	>50	0.570	0.071	0.037	0.443	>50	8.99	>50	>50
BR025.9	C	>50	>50	0.593	0.004	0.020	>50	0.022	>50	>50	>50
CAP210.E8	C	>50	>50	>50	1.91	21.4	8.16	>50	>50	>50	>50
CAP244.D3	C	1.52	>50	1.33	0.184	0.098	0.073	0.330	>50	>50	>50
CAP256.206.C9	C	1.32	14.6	0.971		0.081	0.494	>50		>50	>50
CAP45.G3	C	0.568	>50	7.00	0.032	0.034	0.589	>50	>50	>50	>50
Ce1176.A3	C	1.24	>50	2.60		0.252				>50	>50
CE703010217.B6	C	0.319	>50	0.366		0.029				>50	>50
CNE30	C	1.21	>50	0.525	0.150	0.169	0.291	1.22	>50	>50	>50

FIG. 27A cont.

CNE31	C	2.78	>50	0.786	0.131	0.330	9.95	33.9	>50	>50	>50	
CNE53	C	0.274	1.77	0.087	0.010	0.041	0.051	2.08	6.92	>50	>50	
CNE58	C	1.95	>50	0.225	0.020	0.014	0.389	1.20	0.543	>50	>50	
DU123.06	C	4.25	>50	7.92	0.133	0.058	0.183	>50	>50	>50	>50	
DU151.02	C	0.287	3.94	14.3	0.076	0.041	>50	>50	>50	>50	>50	
DU156.12	C	0.285	9.48	0.086	0.007	0.009	0.035	11.4	>50	>50	>50	
DU172.17	C	0.361	1.92	>50	0.067	0.044	0.289	>50	>50	>50	>50	
DU422.01	C	0.944	>50	>50	2.36	0.034	>50	>50	>50	>50	>50	
MW965.26	C	0.573	6.10	0.029	0.003	0.003	0.005	4.93	0.014	>50	1.23	
SO18.18	C	0.110	>50	0.058	0.006	0.006	0.032	1.50	1.88	>50	>50	
TV1.29	C	4.63	>50	>50	1.79	>50	>50	>50	>50	>50	>50	
TZA125.17	C	>50	>50	>50	1.41	0.746	>50	47.4	>50	>50	>50	
TZBD.02	C	0.219	>50	0.078	0.003	0.013	45.9	>50	5.55	>50	>50	
ZA012.29	C	0.971	13.2	0.384	0.046	0.033	0.063	20.7	28.0	>50	>50	
ZM106.9	C	0.620	>50	0.311	0.032	0.016	0.082	6.22	4.82	>50	>50	
ZM109.4	C	0.416	>50	0.177	0.031	0.059	0.041	17.1	21.2	>50	>50	
ZM135.10a	C	>50	>50	2.25	0.107	0.139			>50	>50	>50	
ZM176.66	C	0.183	>50	0.083	0.0010	0.005	>50	>50	0.227	>50	>50	
ZM197.7	C	1.40	>50	0.428	0.191	0.019	0.398	>50	35.4	>50	>50	
ZM214.15	C	2.22	>50	0.893	0.277	0.088	0.088	1.63	41.4	>50	>50	
ZM215.8	C	0.315	6.19	0.215	0.050	0.049	0.010	7.96	2.57	>50	>50	
ZM233.6	C	1.25	5.71	1.02	0.084	0.067	0.202	>50	7.35	>50	>50	
ZM249.1	C	0.273	9.99	0.057	0.017	0.015	0.039	10.0	7.23	>50	>50	
ZM53.12	C	0.558	>50	0.625	0.149	0.149	0.212	15.8	>50	>50	>50	
ZM55.28a	C	0.665	>50	0.285	0.022	0.023	0.040	0.565	4.59	>50	>50	
3326.V4.C3	CD	0.114	>50	0.068	0.0008	0.003	48.1	1.44	>50	>50	>50	
3337.V2.C6	CD	0.429	>50	0.090	0.011	0.012	0.008	0.037	0.693	>50	>50	
3817.v2.c59	CD	3.63	>50	>50	0.137	0.382	0.216	>50	>50	>50	>50	

FIG. 27A cont.

191821.E6.1	D	13.9	>50	0.353	0.030							
231965.c1	D	0.691	>50	1.84	0.014	0.025	0.035	1.07	31.1	>50	>50	
247-23	D	>50	>50	0.155	0.875	0.077	0.028	14.1	34.0	>50	>50	
3016.v5.c45	D	6.59	>50	>50	0.015	0.012	0.648	>50	4.15	>50	>50	
57128.vrc15	D	>50	>50	1.55	0.666	0.382	0.432	30.8	>50	0.940	4.72	
6405.v4.c34	D	4.08	>50	4.10	0.212	0.066	0.171	43.6	>50	>50	>50	
A03349M1.vrc4a	D	0.351	>50	0.062	0.940	0.655	0.512	5.09	>50	>50	>50	
A07412M1.vrc12	D	0.466	>50	0.596	0.020	0.027			>50	>50	>50	
NKU3006.ec1	D		4.61		0.169	0.147	0.089	0.510	15.9	>50	>50	
UG021.16	D						0.410	>50	>50	0.382		
UG024.2	D				0.019		0.057	5.70	>50	0.043		
P0402.c2.11	G								33.4	>50		
P1981.C5.3	G	2.19	>50	0.330	0.036	0.082			>50	>50	>50	
X1193.c1	G	0.972	>50	0.154	0.029	0.061			11.0	>50	>50	
X1254.c3	G	1.98	>50	0.059	0.029	0.036			>50	>50	>50	
X1632.S2.B10	G	0.484	>50	0.130	0.003	0.055			1.48	>50	>50	
X2088.c9	G	>50	>50	>50	30.6	0.050	>50	0.630	>50	>50	>50	
X2131.C1.B5	G	2.58	>50	0.537		0.102				>50	>50	
SIVmac251.30.SG3	NA	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	
SVA.MLV	NA	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	

FIG. 27A cont.

	CH557	CH235	VRC01	VRC07-523-LS	N6	3BNC117	8ANC131	CH103	F105	DH522
# Viruses	199	199	199	195	199	181	181	196	206	199
Total VS Neutralized										
IC50 <50ug/ml	179	35	179	187	195	153	140	104	14	14
IC50 <10ug/ml	173	19	177	186	193	150	102	69	12	10
IC50 <1.0ug/ml	115	2	146	180	191	141	55	19	5	2
IC50 <0.1ug/ml	10	0	47	130	146	89	17	3	1	0
IC50 <0.01ug/ml	0	0	1	45	22	11	0	0	0	0
% VS Neutralized										
IC50 <50ug/ml	90	18	90	96	98	85	77	53	7	7
IC50 <10ug/ml	87	10	89	95	97	83	56	35	6	5
IC50 <1.0ug/ml	58	1	73	92	96	78	30	10	2	1
IC50 <0.1ug/ml	5	0	24	67	73	49	9	2	0	0
IC50 <0.01ug/ml	0	0	1	23	11	6	0	0	0	0
Median IC50	0.583	9.48	0.293	0.045	0.047	0.073	1.89	6.84	2.61	4.61
Geometric Mean	0.658	6.84	0.300	0.037	0.045	0.092	1.77	4.54	1.65	3.87

FIG. 27B

High Throughput Antibody Screen - Panel P Assay - Luc/TZM-bl 1.00-10.0 <0.100

values represent IC80 in ug/ml IC80 >10.0 0.100-1.00

Virus ID	Clade	Panel P CH557	Panel P CH235	Panel P VRC01	Panel H VRC07-523-LS	Panel P N6	Panel D 3BNC117	Panel D 8ANC131	Panel F CH103	Panel A F105	Panel P DH522
0260.v5.c36	A	2.65	>50	1.54	0.568	0.148	0.733	4.66	>50	>50	>50
0330.v4.c3	A	0.759	>50	0.167	0.060	0.034	0.070	0.453	31.3	>50	>50
0439.v5.c1	A	1.29	>50	0.436	0.352	0.088	0.776	0.940	>50	>50	>50
3365.v2.c20	A	0.227	>50	0.116	0.021	0.029	0.044	15.7	40.0	>50	>50
3415.v1.c1	A	1.28	>50	0.243	0.109	0.064	0.276	1.74	4.73	>50	>50
3718.v3.c11	A	0.948	>50	2.58	0.066	0.036	>50	11.1	>50	>50	>50
398-F1_F6_20	A	4.42	>50	0.612	0.181	0.083	0.297	>50	>50	>50	>50
BB201.B42	A	1.67	>50	0.733	0.464	0.292	25.8	>50	40.5	>50	>50
BB539.2B13	A				0.039		0.197	0.915	>50	>50	
BG505.W6M.C2	A	0.314	>50	0.135		0.068	0.078	1.79		>50	>50
BI369.9A	A	0.811	>50	0.615	0.274	0.217	0.142	5.39	>50	>50	>50
BS208.B1	A	0.598	>50	0.068	0.012	0.030	0.010	0.745	>50	>50	>50
KER2008.12	A	>50	>50	1.48	0.737	0.729	1.36	>50	>50	>50	>50
KER2018.11	A	5.84	>50	1.49	0.936	0.310	1.51	14.0	>50	>50	>50
KNH1209.18	A	0.656	>50	0.305	0.179	0.117	0.159	10.5	>50	>50	>50
MB201.A1	A	0.883	>50	0.532	0.311	0.157	4.97	>50	>50	>50	>50
MB539.2B7	A	3.62	>50	1.08	0.301	0.157	0.382	27.3	14.2	>50	>50
MI369.A5	A	1.15	>50	0.826	0.352	0.219	0.210	7.25	>50	>50	>50
MS208.A1	A	1.31	>50	0.663	0.235	0.136	0.118	12.2	>50	>50	>50

FIG. 28A

Q23.17	A		0.318	5.28	0.156	0.109	0.063	0.062	0.896	12.9	>50	>50	
Q259.17	A		0.308	>50	0.234	0.044	1.56	0.067	3.72	33.9	>50	>50	
Q769.d22	A		0.293	>50	0.087	0.055	0.044	0.045	0.213	2.67	>50	>50	
Q769.h5	A		0.426	>50	0.158	0.073	0.064	0.039	0.249		>50	>50	
Q842.d12	A		0.239	18.5	0.111	0.038	0.028	0.012	0.085	5.10	>50	>50	
QH209.14M.A2	A		1.09	>50	0.148	0.051	0.024	0.043	22.0	5.75	>50	>50	
RW020.2	A		0.774	4.59	0.715	0.321	0.110	0.106	1.60	47.4	>50	>50	
UG037.8	A		0.601	>50	0.227	0.084	0.071	0.108	0.935	11.9	>50	>50	
246-F3.C10.2	AC										>50		
3301.V1.C24	AC		1.33	>50	0.269	0.026	0.021	0.109	0.245	13.2	>50	>50	
3589.V1.C4	AC		2.01	>50	0.181	0.072	0.036	0.182	16.2	>50	>50	>50	
6540.v4.c1	AC		>50	>50	>50	>50	1.19	>50	>50	7.74	>50	>50	
6545.V4.C1	AC		>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	
0815.V3.C3	ACD		0.133	>50	0.067	0.005	0.016	0.058	0.654	>50	>50	>50	
6095.V1.C10	ACD		4.09	>50	1.76	0.937	0.592	0.808	7.94	>50	>50	>50	
3468.V1.C12	AD		0.200	14.4	0.159	0.044	0.027	0.705	45.8	>50	>50	>50	
Q168.a2	AD		0.619	>50	0.240	0.112	0.082	0.172	1.20	26.4	>50	>50	
Q461.e2	AD		2.54	>50	0.968	0.361	0.200	0.330	7.46	>50	>50	>50	
620345.c1	AE		8.44	>50	>50	>50	17.1	>50	>50	>50	>50	>50	
BJOX009000.02.4	AE		21.6	>50	5.02	1.41	0.764			>50	>50	>50	
BJOX010000.06.2	AE		>50	>50	19.4	3.40	1.50			>50	>50	>50	
BJOX025000.01.1	AE		0.885	>50	>50	0.427	0.070			>50	>50	>50	
BJOX028000.10.3	AE		0.507	>50	1.06	0.012	0.013			>50	>50	>50	
C1080.c3	AE		7.74	>50	9.07	0.794	0.792	0.665	>50	>50	>50	>50	
C2101.c1	AE		0.792	>50	0.562	0.269	0.151	0.222	>50	>50	>50	>50	
C3347.c11	AE		0.332	>50	0.289	0.110	0.028	0.106	26.4	>50	>50	>50	

FIG. 28A cont.

C4118.09	AE	0.234	11.2	0.452	0.097	0.161	0.106	>50	>50	>50	>50	
CM244.ec1	AE	0.503	6.31	0.325	0.045	0.035			>50	>50	>50	
CNE3	AE	25.3	>50	8.35	0.626	0.067	0.430	23.7	>50	>50	>50	
CNE5	AE	2.78	>50	1.01	0.267	0.281	1.34	>50	>50	>50	>50	
CNE55	AE	1.25	>50	0.973	0.218	0.225	0.530	>50	>50	>50	>50	
CNE56	AE	3.25	>50	1.10	0.381	0.326	0.355	38.1	>50	>50	>50	
CNE59	AE	3.03	>50	2.32	0.353	0.422	0.573	>50	>50	>50	>50	
CNE8	AE	2.64	>50	1.38	0.411	0.233			>50	>50	>50	
M02138	AE				0.752		0.568	>50	>50	>50		
R1166.c1	AE	3.52	>50	7.96	1.96	1.24	0.805	>50	>50	>50	>50	
R2184.c4	AE	1.63	16.0	0.353	0.106	0.080	0.178	45.0	>50	>50	>50	
R3265.c6	AE	0.631	>50	1.86	0.384	0.183	0.500	>50	>50	>50	>50	
TH023.6	AE				0.071				>50	>50		
TH966.8	AE	0.889	2.43	0.814	0.208	0.108	0.363	>50	>50	>50	>50	
TH976.17	AE	0.752	2.83	0.913	0.177	0.168	0.197	42.7	>50	>50	>50	
235-47	AG	0.762	>50	0.202	0.020	0.029	0.124	25.6	10.1	>50	>50	
242-14	AG	10.5	>50	>50	0.661	1.38	>50	>50	>50	>50	>50	
263-8	AG	1.65	>50	0.485	0.112	0.090	0.188	1.03	>50	>50	>50	
269-12	AG	>50	>50	0.864	0.212	0.085	0.446	17.3	>50	>50	>50	
271-11	AG	0.201	>50	0.244	0.044	0.038	0.026	9.42	36.0	>50	>50	
928-28	AG	1.78	>50	1.20	0.282	0.200	0.619	15.4	>50	>50	>50	
DJ263.8	AG	0.962	>50	0.510	0.027	1.29	0.133	0.183	2.83	>50	>50	
T250-4	AG	>50	>50	>50	>50	0.026	>50	>50	>50	>50	>50	
T251-18	AG	13.3	>50	9.86	0.898	1.14	0.858	7.32	>50	>50	>50	
T253-11	AG	5.25	>50	1.09	0.227	0.308	0.328	42.1	>50	>50	>50	
T255-34	AG	2.04	>50	2.49	0.242	0.223	0.296	>50	>50	>50	>50	
T257-31	AG	8.40	>50	6.61	1.32	0.527	0.694	>50	>50	>50	>50	

FIG. 28A cont.

T266-60	AG	16.2	>50	6.76	1.48	0.490	0.119	7.12	>50	>50	>50
T278-50	AG	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
T280-5	AG	0.263	>50	0.190	0.025	0.050	0.060	0.143	11.6	>50	>50
T33-7	AG	0.107	>50	0.060	0.008	0.020	0.021	3.07	>50	>50	>50
3988.25	B	1.90	>50	1.04	0.284	0.285	>50	>50	46.1	>50	>50
5768.04	B	1.89	>50	0.895	0.284	0.145	1.38	>50	24.9	>50	>50
6101.10	B	1.40	>50	0.073	0.029	0.011	0.104	0.290	6.08	>50	>50
6535.3	B	16.5	>50	5.81	0.390	0.234	1.72	2.00	12.3	>50	>50
7165.18	B	>50	>50	>50	5.14	3.25	35.7	>50	>50	>50	>50
45_01dG5	B	0.154	>50	0.032	0.002	0.003			2.63	>50	>50
89.6.DG	B	8.35	>50	2.12	0.420	0.516	0.501	6.82	4.03	>50	>50
AC10.29	B	6.27	>50	3.34	1.63	0.731	>50	>50	>50	>50	>50
ADA.DG	B	3.71	>50	1.55	0.405	0.299	0.341	1.96	4.87	>50	110
Bal.01	B	0.534	>50	0.434	0.037	0.048	0.051	0.692	2.97	>50	>50
BaL.26	B	0.454	>50	0.200	0.006	0.033	0.028	0.308	0.262	>50	>50
BG1168.01	B	3.37	>50	3.50	0.246	0.192	0.676	1.98	>50	>50	>50
BL01.DG	B	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
BR07.DG	B	4.47	>50	4.82	0.927	0.879	0.688	12.7	>50	>50	>50
BX08.16	B	>50	>50	0.990	0.229	0.125	0.281	13.4	2.56	28.1	>50
CAAN.A2	B	6.21	>50	2.85	0.519	0.369	2.57	6.96	>50	>50	>50
CNE10	B	16.2	>50	2.15	0.294	0.251	0.232	3.23	>50	>50	>50
CNE12	B	7.71	>50	1.83	0.473	0.480	0.253	5.41	>50	>50	>50
CNE14	B	1.86	>50	0.736	0.097	0.075	0.096	19.4	1.30	>50	>50
CNE4	B	3.80	>50	1.93	0.586	0.392	0.481	>50	>50	>50	>50
CNE57	B	3.76	>50	1.30	0.406	0.223	0.202	44.0	>50	>50	>50

FIG. 28A cont.

HO86.8	B	0.599	>50	>50	>50	37.6	>50	>50	>50	>50	>50	>50
HT593.1	B	2.94	>50	1.87	0.365	0.427	0.922	3.22	>50	>50	>50	>50
HXB2.DG	B	0.438	>50	0.164	0.038	0.023	0.197	0.155	0.393	0.624	0.880	
JRCSF.JB	B	1.77	>50	1.02	0.194	0.346	0.119	1.34	12.5	>50	>50	>50
JRFL.JB	B	0.402	17.6	0.126	0.004	0.011	0.013	0.174	0.067	>50	>50	>50
MN.3	B	0.437	>50	0.041	0.002	0.005	>50	>50	1.11	>50	13.3	
PVO.04	B	3.41	>50	1.22	0.306	0.197	0.294	2.40	>50	>50	>50	>50
QH0515.01	B	4.25	>50	3.45	1.98	0.847	1.14	>50	37.3	>50	>50	>50
QH0692.42	B	5.69	>50	2.92	2.22	1.15	1.47	12.2	>50	>50	>50	>50
REJO.67	B	2.71	>50	0.228	0.032	0.047	0.132	0.201	>50	>50	>50	>50
RHPA.7	B	0.224	>50	0.134	0.054	0.040	0.071	14.2	32.4	>50	>50	>50
SC422.8	B	2.27	>50	0.386	0.180	0.157	0.192	0.830	9.15	>50	>50	>50
SF162.LS	B	2.57	>50	0.656	0.127	0.118	0.084	0.453	2.92	20.4	13.7	
SS1196.01	B	3.05	>50	0.622	0.228	0.189	0.144	1.21	2.58	>50	>50	>50
THRO.18	B	>50	>50	16.3	5.36	4.03	13.6	>50	>50	>50	>50	>50
TRJO.58	B	1.55	>50	0.292	0.143	0.216	0.216	44.7	>50	>50	>50	>50
TRO.11	B	1.98	>50	1.27	0.316	0.255	0.125	33.5	17.2	>50	>50	>50
WITO.33	B	1.12	>50	0.356	0.220	0.144	0.161	33.8	31.5	>50	>50	>50
X2278.C2.B6	B	1.16	>50	0.409		0.077			5.44	>50	>50	>50
YU2.DG	B	0.548	>50	0.292	0.070	0.079	0.099	0.792		>50	>50	>50
BJOX002000.03.2	BC	2.68	>50	>50		0.140				>50	>50	>50
CH038.12	BC	>50	>50	1.18	0.165	0.208	>50	>50	7.73	>50	>50	>50
CH070.1	BC	7.54	>50	>50	0.350	0.441	>50	12.7	>50	>50	>50	>50
CH117.4	BC	0.898	>50	0.256	0.044	0.074	15.8	>50	14.9	>50	>50	>50
CH119.10	BC	3.01	>50	1.97		0.127				>50	>50	>50

FIG. 28A cont.

CH181.12	BC	1.80	>50	1.25	0.265	0.171	0.414	>50	32.1	>50	>50
CNE15	BC	0.687	>50	0.292	0.051	0.072	>50	>50	>50	>50	>50
CNE19	BC	0.356	>50	0.641		0.058			>50	>50	>50
CNE20	BC	0.670	>50	>50	0.041	0.033			18.8	>50	>50
CNE21	BC	1.47	>50	1.24	0.158	0.117			>50	>50	>50
CNE40	BC	0.723	>50	3.61	0.180	0.062	0.495	>50	3.14	1.78	0.991
CNE7	BC	8.67	>50	1.01	0.202	0.088	>50	>50	>50	>50	>50
286.36	C	1.90	>50	0.756	0.191	0.090	0.401	3.39	16.8	>50	>50
288.38	C	5.81	>50	5.36	0.822	0.560	0.346	4.06	19.1	>50	>50
0013095-2.11	C	>50	>50	0.318	0.052	0.083	2.23	>50	25.0	>50	>50
001428-2.42	C	0.213	>50	0.035	0.002	0.005	0.060	0.191	4.64	>50	>50
0077_V1.C16	C	30.8	>50	4.12	0.300	0.042	>50	>50	>50	>50	>50
00836-2.5	C	>50	>50	0.708	0.002	0.004	>50	>50	>50	>50	>50
0921.V2.C14	C	1.08	>50	0.673	0.168	0.076	0.669	>50	>50	>50	>50
16055-2.3	C	0.471	>50	0.176	0.023	0.020	27.1	1.66	>50	>50	>50
16845-2.22	C	19.3	>50	21.1	2.73	1.03	>50	>50	>50	>50	>50
16936-2.21	C	1.36	>50	0.373	0.100	0.046	0.402	0.480	34.1	>50	>50
25710-2.43	C	1.07	>50	1.87	0.581	0.213	0.469	47.7	>50	>50	>50
25711-2.4	C	3.43	>50	1.69	0.279	0.185	>50	>50	46.0	>50	>50
25925-2.22	C	2.16	>50	1.27	0.277	0.264	0.549	>50	>50	>50	>50
26191-2.48	C	1.57	>50	0.558	0.236	0.116	0.183	39.2	>50	>50	>50
3168.V4.C10	C	1.23	>50	0.362	0.145	0.161	0.267	36.5	>50	>50	>50
3637.V5.C3	C	22.8	>50	5.93	1.06	0.395	>50	>50	>50	>50	>50
3873.V1.C24	C	>50	>50	2.82	0.672	0.335	>50	4.46	>50	>50	>50

FIG. 28A cont.

426c	C											
6322.V4.C1	C	5.15	>50	>50	0.136	0.112	>50	>50	>50	>50	>50	>50
6471.V1.C16	C	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
6631.V3.C10	C	154	>50	>50	0.614	0.222	>50	>50	>50	>50	>50	>50
6644.V2.C33	C	>50	>50	0.455	0.160	0.190	0.229	>50	>50	1.51	11.5	>50
6785.V5.C14	C	>50	>50	0.734	0.229	0.247	0.679	>50	>50	27.6	>50	>50
6838.V1.C35	C	3.41	>50	0.588	0.144	0.075	0.746	1.94	>50	>50	>50	>50
96ZM651.02	C	4.18	>50	2.29	0.366	0.165	1.77	>50	>50	>50	>50	>50
BR025.9	C	>50	>50	3.08	0.060	0.068	>50	0.262	>50	>50	>50	>50
CAP210.E8	C	>50	>50	>50	6.05	>50	>50	>50	>50	>50	>50	>50
CAP244.D3	C	4.13	>50	2.78	0.714	0.317	0.309	2.28	>50	>50	>50	>50
CAP256.206.C9	C	3.36	>50	2.52		0.287	1.86	>50		>50	>50	>50
CAP45.G3	C	1.77	>50	40.1	0.162	0.101	32.1	>50	>50	>50	>50	>50
Ce1176.A3	C	3.55	>50	6.78		0.662				>50	>50	>50
CE703010217.B6	C	0.948	>50	0.935		0.097				>50	>50	>50
CNE30	C	3.64	>50	2.18	0.476	0.574	1.05	6.52	>50	>50	>50	>50
CNE31	C	>50	>50	2.11	0.373	0.827	>50	>50	>50	>50	>50	>50
CNE53	C	0.725	6.06	0.268	0.064	0.145	0.327	30.9	>50	>50	>50	>50
CNE58	C	4.17	>50	0.586	0.086	0.041	1.41	10.9	1.46	>50	>50	>50
DU123.06	C	18.5	>50	>50	0.565	0.152	1.77	>50	>50	>50	>50	>50
DU151.02	C	0.827	15.7	>50	0.344	0.125	>50	>50	>50	>50	>50	>50
DU156.12	C	0.829	>50	0.230	0.043	0.027	0.121	19.9	>50	>50	>50	>50
DU172.17	C	1.12	6.87	>50	0.454	0.231	2.46	>50	>50	>50	>50	>50
DU422.01	C	2.76	>50	>50	31.6	0.096	>50	>50	>50	>50	>50	>50

FIG. 28A cont.

MW965.26	C	1.56	>50	0.081	0.026	0.008	0.023	13.4	0.091	>50	12.1
SO18.18	C	0.250	>50	0.087	0.041	0.022	0.176	7.71	7.48	>50	>50
TV1.29	C	>50	>50	>50	19.8	>50	>50	>50	>50	>50	>50
TZA125.17	C	>50	>50	>50	10.2	2.58	>50	>50	>50	>50	>50
TZBD.02	C	0.938	>50	0.217	0.019	0.034	>50	>50	23.7	>50	>50
ZA012.29	C	2.99	>50	0.823	0.166	0.067	0.319	>50	>50	>50	>50
ZM106.9	C	1.43	>50	0.641	0.122	0.065	0.325	16.1	8.11	>50	>50
ZM109.4	C	1.21	>50	0.508	0.202	0.160	0.242	>50	>50	>50	>50
ZM135.10a	C	>50	>50	8.58	0.458	0.460	0.280	>50	>50	>50	>50
ZM176.66	C	0.590	>50	0.407	0.007	0.014	>50	>50	1.25	>50	>50
ZM197.7	C	4.79	>50	1.81	0.722	0.190	1.29	>50	>50	>50	>50
ZM214.15	C	6.50	>50	2.50	0.971	0.321	0.744	17.4	>50	>50	>50
ZM215.8	C	0.764	>50	0.748	0.245	0.167	0.070	27.4	>50	>50	>50
ZM233.6	C	3.58	36.9	4.44	0.302	0.229	0.912	>50	>50	>50	>50
ZM249.1	C	0.678	43.0	0.181	0.126	0.052	0.207	>50	37.6	>50	>50
ZM53.12	C	1.85	>50	2.40	0.626	0.449	1.06	>50	>50	>50	>50
ZM55.28a	C	2.35	>50	0.519	0.089	0.059	0.179	3.92	14.3	>50	>50
3326.V4.C3	CD	0.307	>50	1.89	0.003	0.006	>50	13.3	>50	>50	>50
3337.V2.C6	CD	1.57	>50	0.225	0.057	0.033	0.040	0.170	3.76	>50	>50
3817.v2.c59	CD	9.90	>50	>50	1.06	1.39	0.752	>50	>50	>50	>50
191821.E6.1	D				0.266						
231965.c1	D	>50	>50	1.25	0.111	0.073	0.172	4.69	>50	>50	>50
247-23	D	2.03	>50	16.3	7.86	0.439	0.124	43.1	>50	>50	>50
3016.v5.c45	D	>50	>50	0.344	0.106	0.032	3.35	>50	19.5	>50	>50

FIG. 28A cont.

57128.vrc15	D	>50	>50	>50	3.12	1.70	1.84	>50	>50	>50	>50
6405.v4.c34	D	>50	>50	3.83	0.676	0.185	0.578	>50	>50	>50	>50
A03349M1.vrc4a	D	10.8	>50	9.98	3.29	2.09	2.34	13.7	>50	>50	>50
A07412M1.vrc12	D	0.945	>50	0.303	0.118	0.109			>50	>50	>50
NKU3006.ec1	D	1.33	13.7	1.42	0.903	0.574	0.323	2.40	43.6	>50	>50
UG021.16	D						5.09	>50	>50	2.53	
UG024.2	D				0.105		0.194	>50	>50	0.569	
P0402.c2.11	G								>50	>50	
P1981.C5.3	G	6.07	>50	0.807	0.216	0.176			>50	>50	>50
X1193.c1	G	2.48	>50	0.415	0.148	0.166			>50	>50	>50
X1254.c3	G	8.25	>50	0.173	0.121	0.086			>50	>50	>50
X1632.S2.B10	G	1.59	>50	0.732	0.036	0.141			6.01	>50	>50
X2088.c9	G	>50	>50	>50	>50	0.152	>50	1.93	>50	>50	>50
X2131.C1.B5	G	10.7	>50	1.75		0.422				>50	>50
SIVmac251.30.SG3	NA	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
SVA.MLV	NA	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50

FIG. 28A cont.

	CH557	CH235	VRC01	VRC07-523-LS	N6	3BNC117	8ANC131	CH103	F105	DH522
# Viruses	199	199	199	195	199	182	182	196	206	199
Total VS Neutralized										
IC80 <50ug/ml	170	16	173	186	193	146	105	67	7	6
IC80 <10ug/ml	156	7	168	183	191	140	64	32	4	2
IC80 <1.0ug/ml	59	0	97	166	176	118	26	4	2	2
IC80 <0.1ug/ml	0	0	10	54	77	25	1	2	0	0
IC80 <0.01ug/ml	0	0	0	10	6	0	0	0	0	0
% VS Neutralized										
IC80 <50ug/ml	85	8	87	95	97	80	58	34	3	3
IC80 <10ug/ml	78	4	84	94	96	77	35	16	2	1
IC80 <1.0ug/ml	30	0	49	85	88	65	14	2	1	1
IC80 <0.1ug/ml	0	0	5	28	39	14	1	1	0	0
IC80 <0.01ug/ml	0	0	0	5	3	0	0	0	0	0
Median IC80	1.66	12.5	0.807	0.219	0.151	0.303	5.39	11.6	2.53	11.6
Geometric Mean	1.73	10.1	0.815	0.188	0.142	0.335	3.99	8.00	3.76	5.26

FIG. 28B

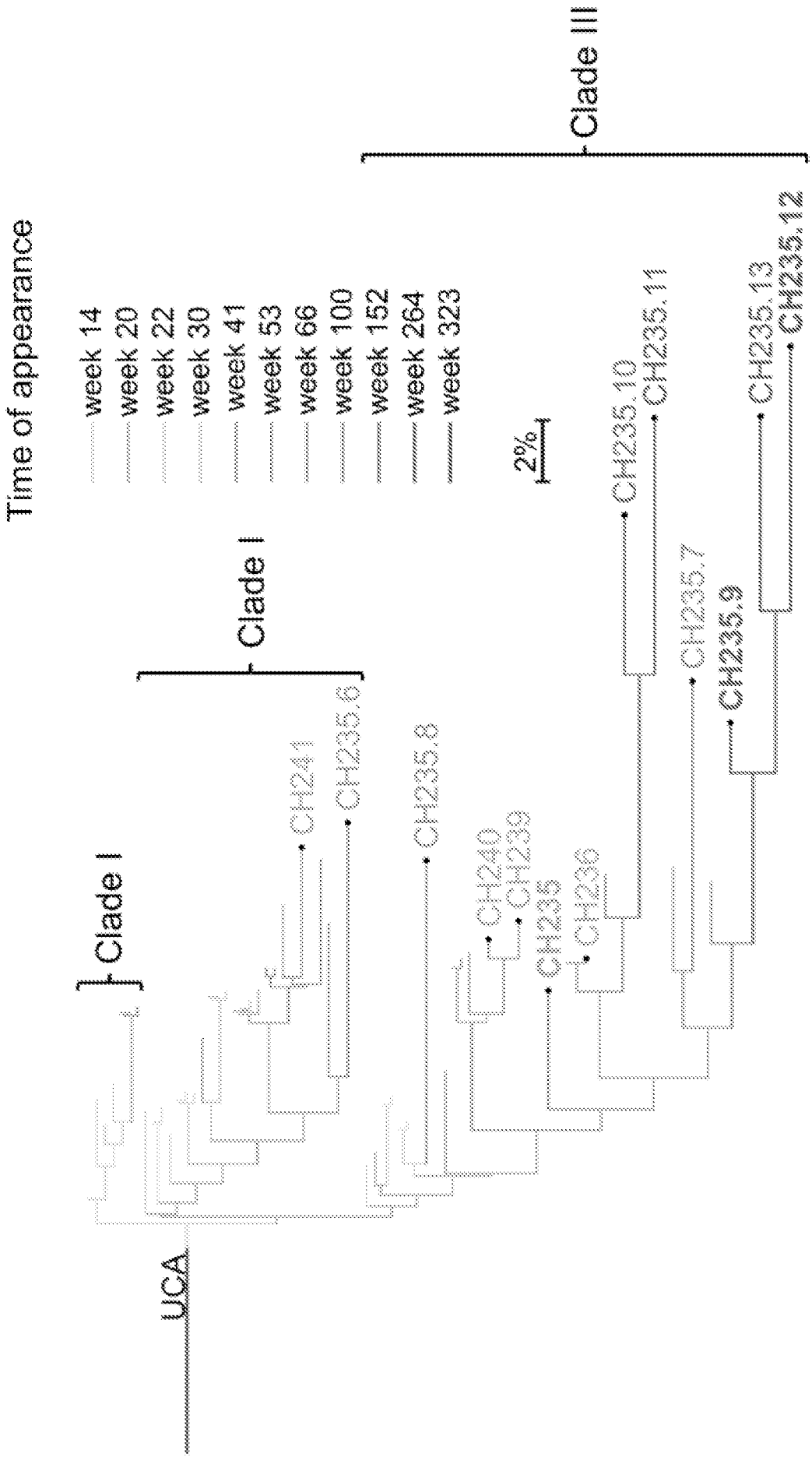
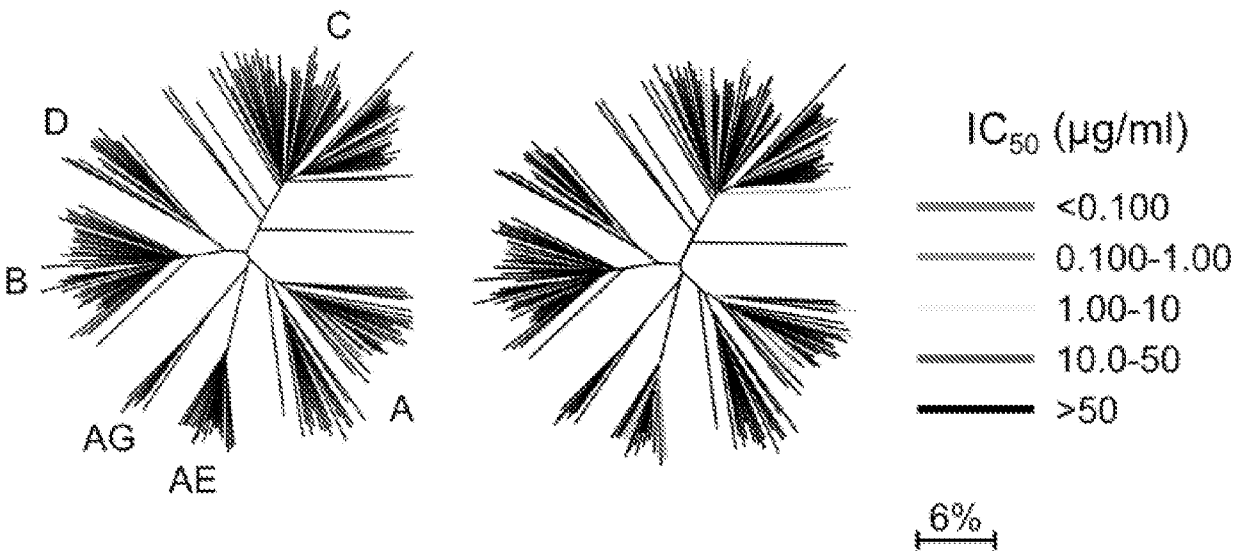

Time of appearance
week 14
week 20
week 22
week 30
week 41
week 53
week 66
week 100
week 152
week 264
week 323
2%
Clade I
Clade I
Clade III
UCA
CH241
CH235.6
CH235.8
CH240
CH239
CH235
CH236
CH235.10
CH235.11
CH235.7
CH235.9
CH235.13
CH235.12

FIG. 29A



UCA – wk14
(0% breadth;
>50 µg/ml)

CH235- wk41
(18% breadth;
9.5 µg/ml)

CH235.9 – wk152
(77% breadth;
3.0 µg/ml)

235.12– wk323
(90% breadth,
0.6 µg/ml)

FIG. 29B

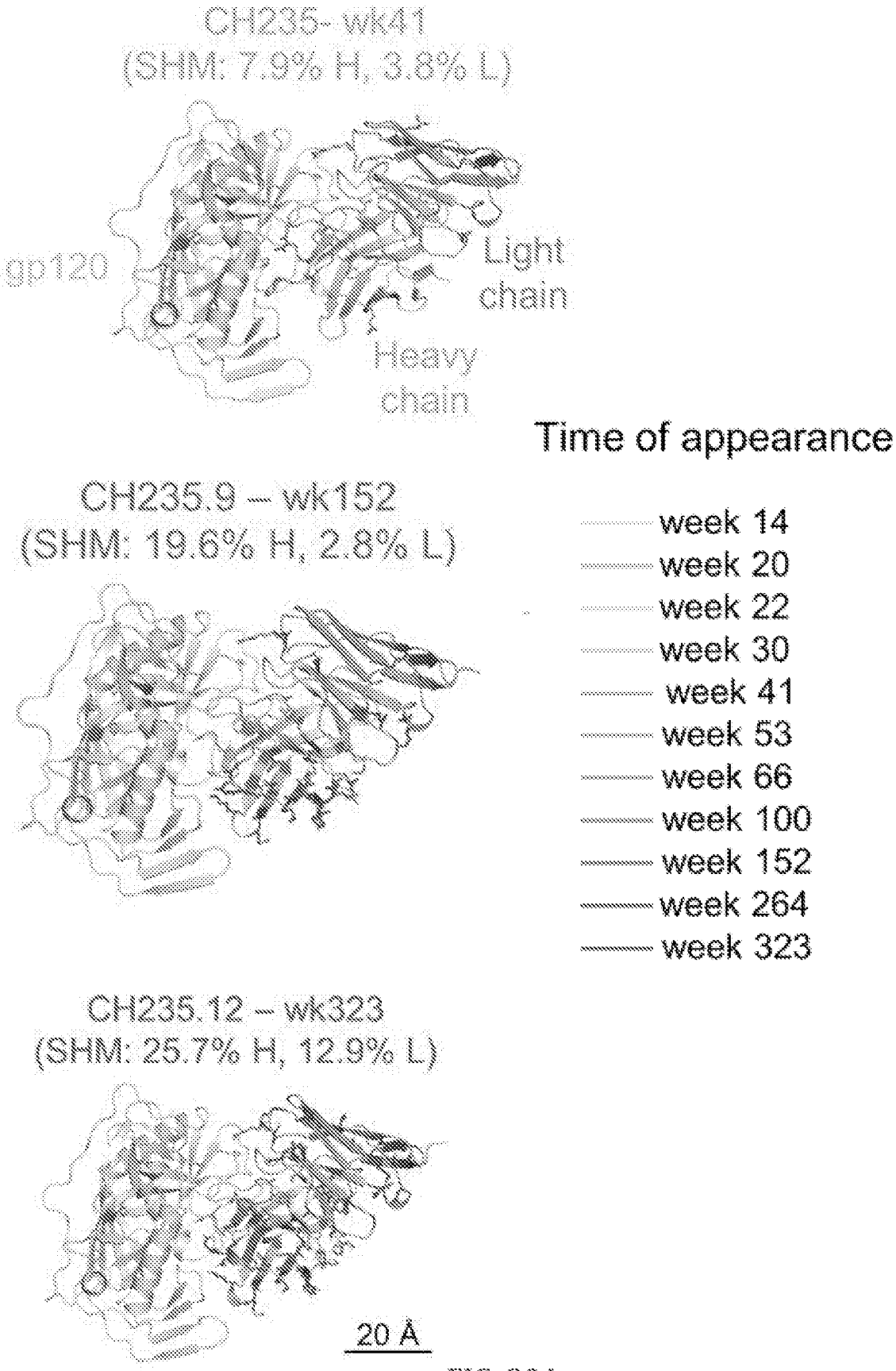

CH235- wk41
(SHM: 7.9% H, 3.8% L)
gp120
Light chain
Heavy chain
Time of appearance
CH235.9 – wk152
(SHM: 19.6% H, 2.8% L)
week 14
week 20
week 22
week 30
week 41
week 53
week 66
week 100
week 152
week 264
week 323
CH235.12 – wk323
(SHM: 25.7% H, 12.9% L)
20 Å

FIG. 30A

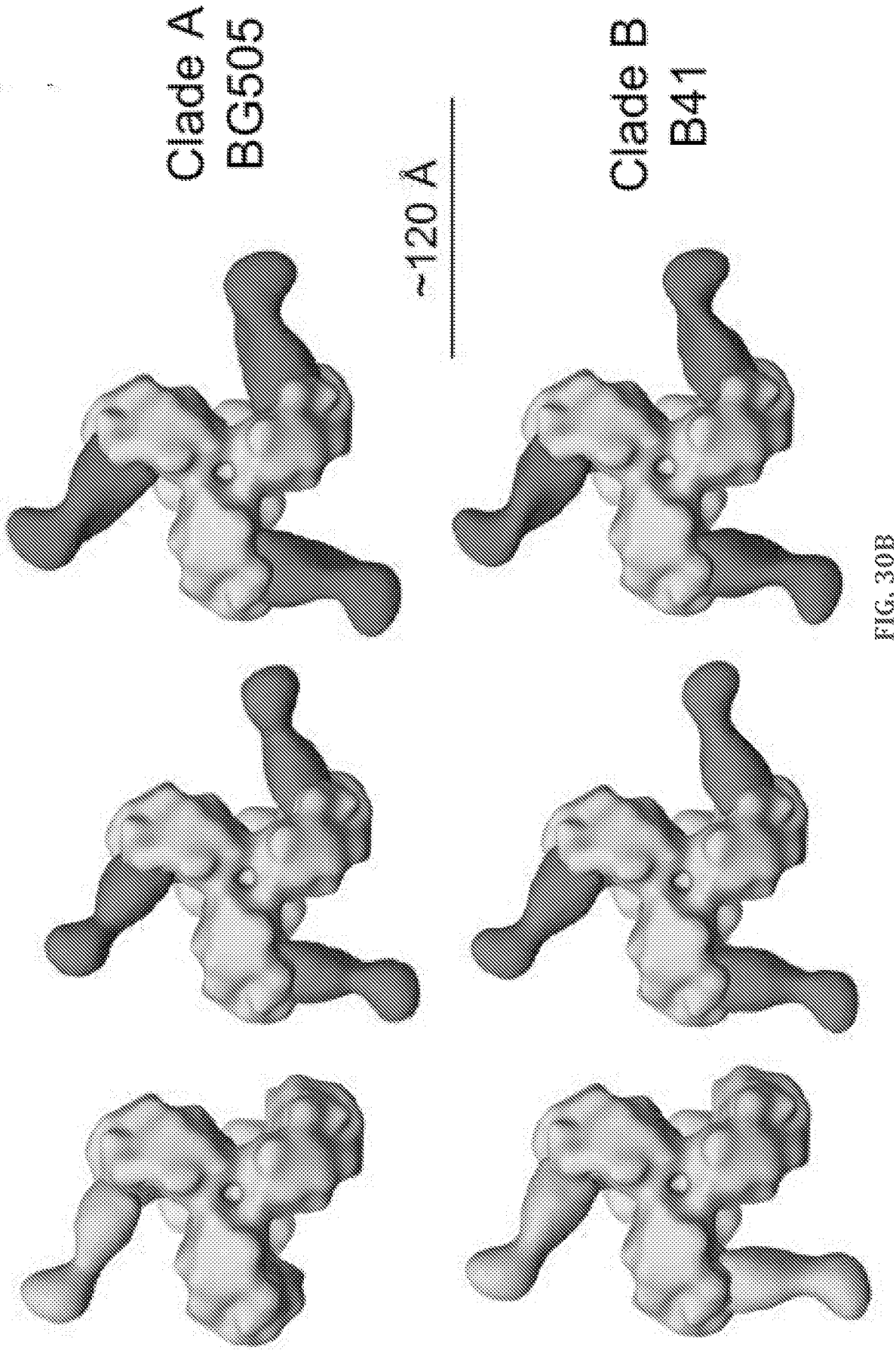


FIG. 30B

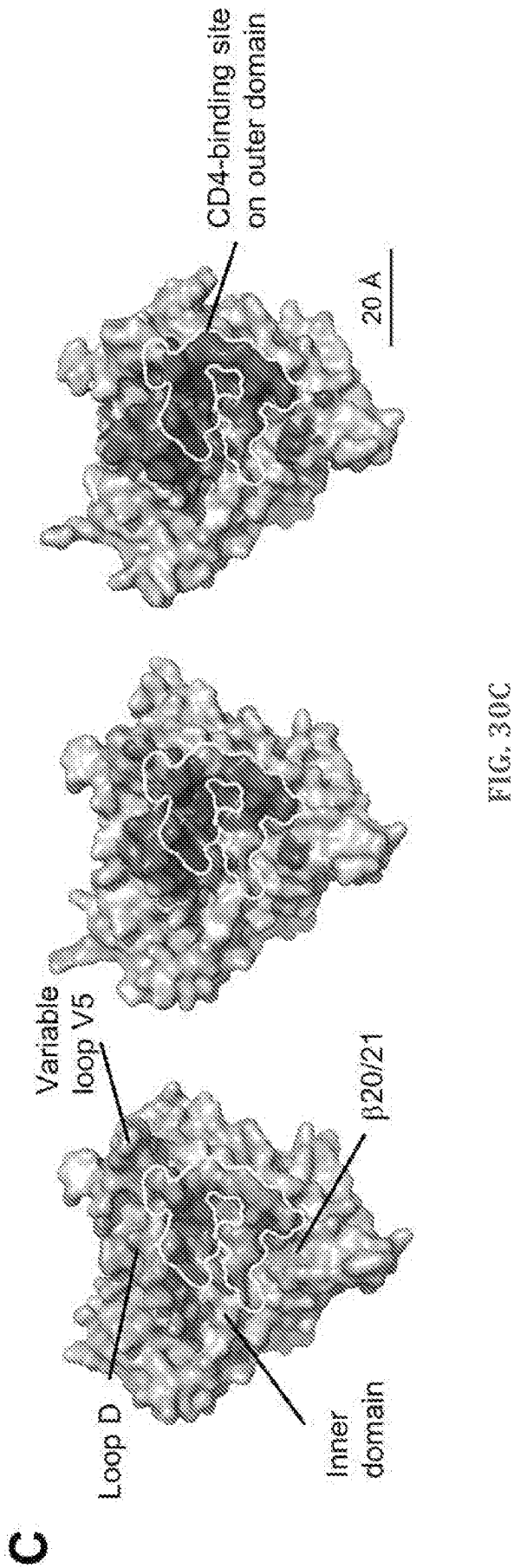

C
Loop D
Variable loop V5
Inner domain
β20/21
CD4-binding site on outer domain
20 Å

FIG. 30C

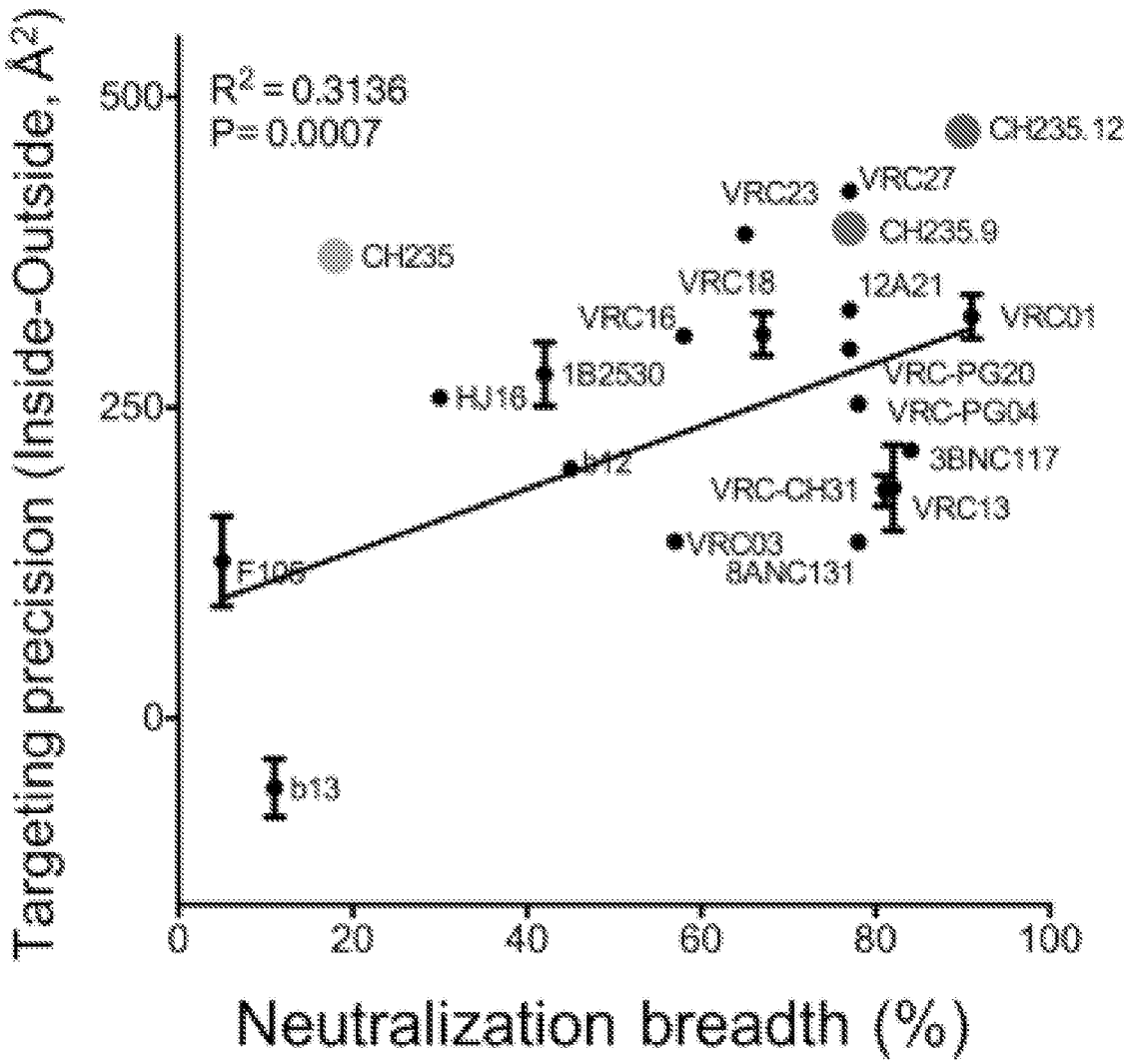

Targeting precision (Inside-Outside, Å²)
R² = 0.3136
P = 0.0007
500
250
0
0
20
40
60
80
100
Neutralization breadth (%)
CH235.12
VRC23
VRC27
CH235.9
CH235
VRC18
12A21
VRC16
VRC01
1B2530
HJ16
VRC-PG20
VRC-PG04
b12
3BNC117
VRC-CH31
VRC13
VRC03
8ANC131
F105
b13

FIG. 30D

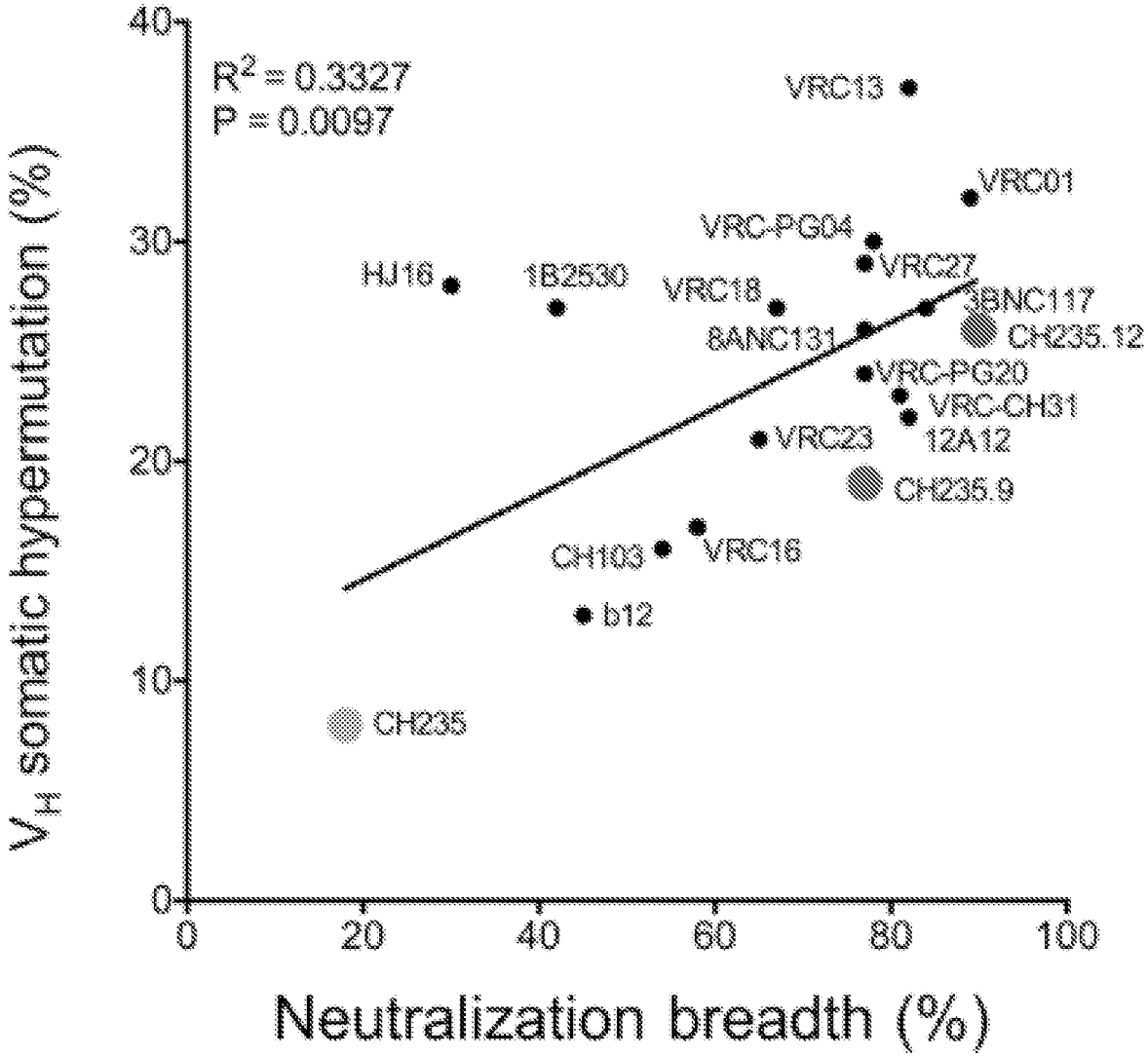

$R^2 = 0.3327$
P = 0.0097
VRC13
VRC01
VRC-PG04
VRC27
HJ16
1B2530
VRC18
3BNC117
8ANC131
CH235.12
VRC-PG20
VRC-CH31
12A12
VRC23
CH235.9
CH103
VRC16
b12
CH235
V_H somatic hypermutation (%)
0
10
20
30
40
0
20
40
60
80
100
Neutralization breadth (%)

FIG. 30E

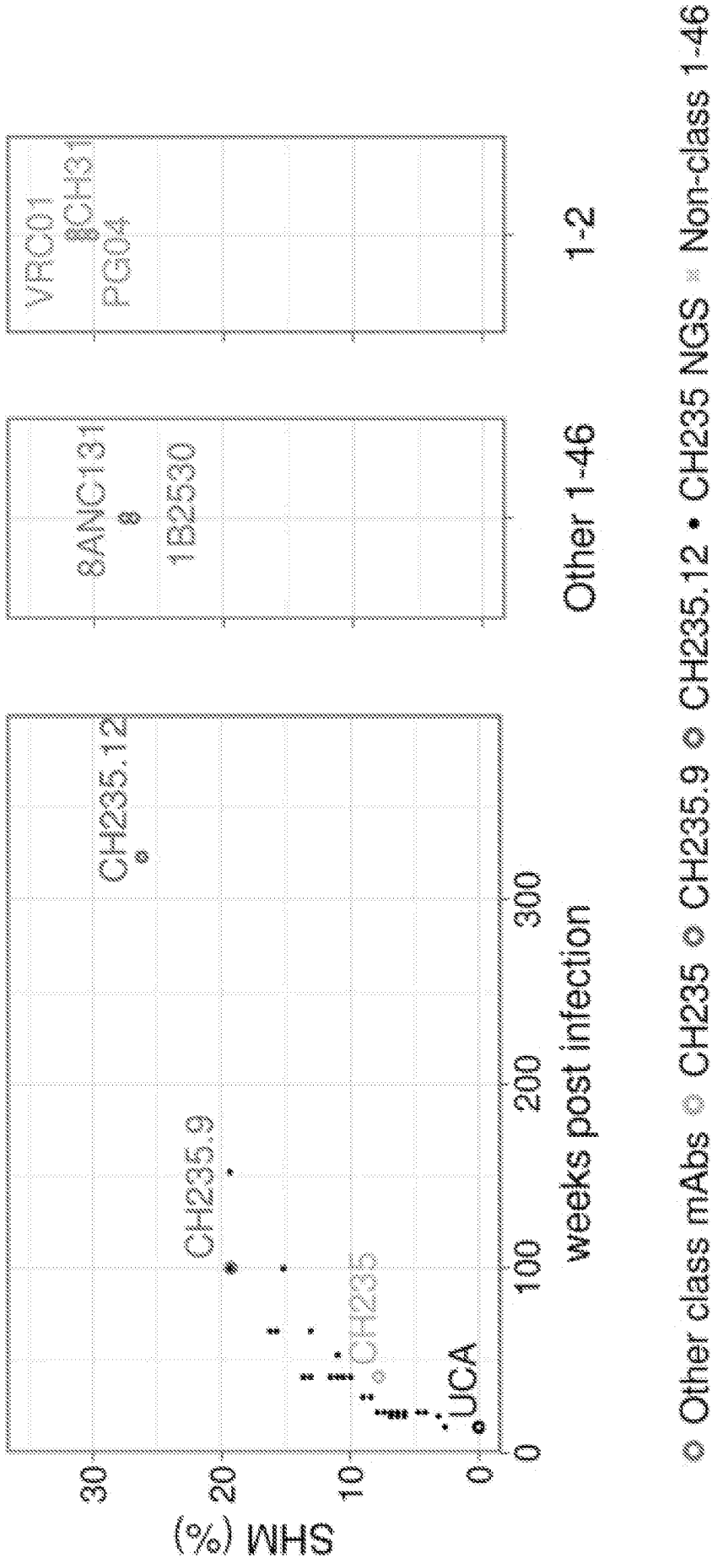

(%) WHS
30
20
10
0
0
100
200
300
weeks post infection
UCA
CH235
CH235.9
CH235.12
8ANC131
1B2530
Other 1-46
VRC01
PG04
CH31
1-2
Other class mAbs CH235 CH235.9 CH235.12 CH235 NGS Non-class 1-46

FIG. 31A

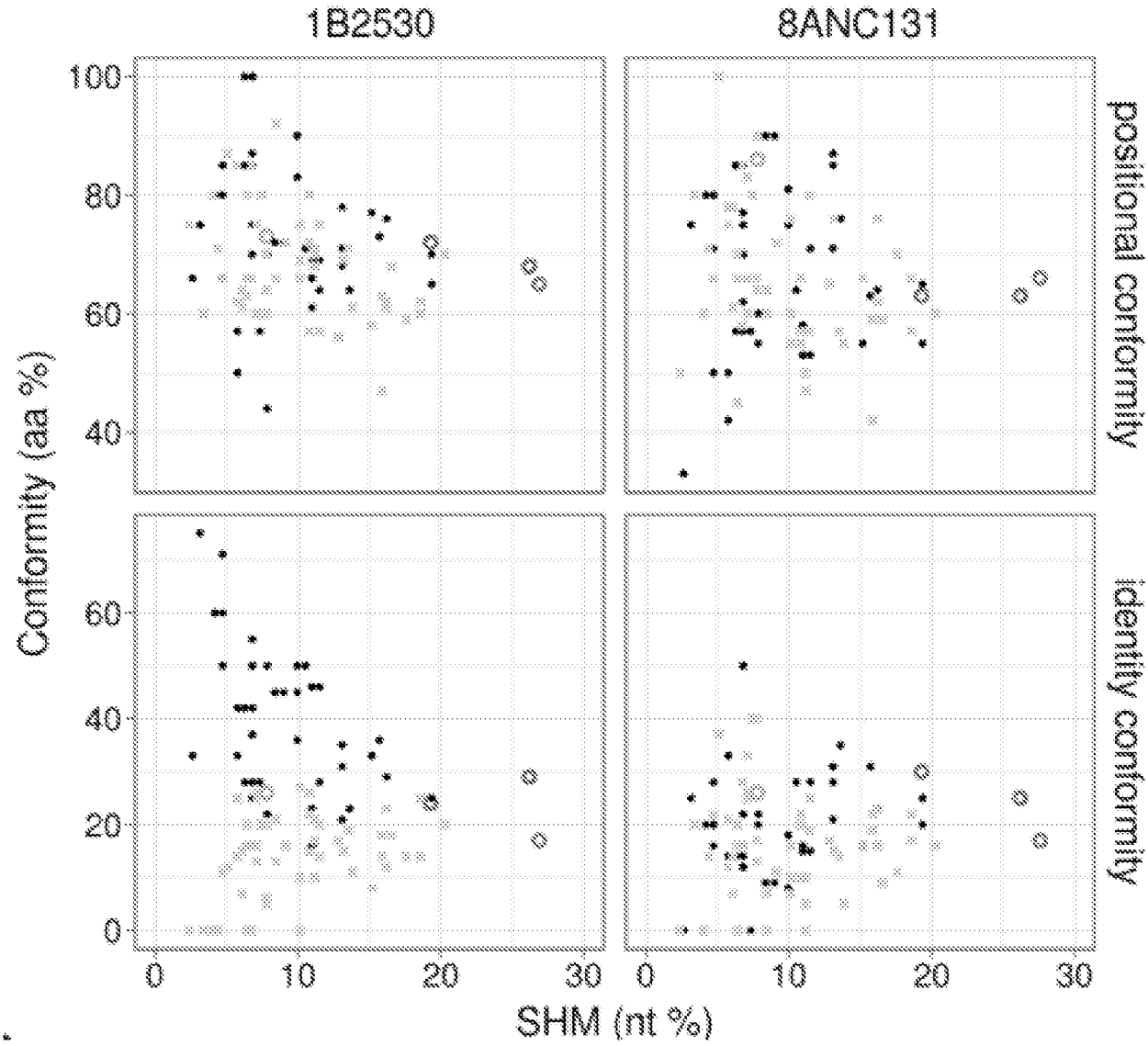
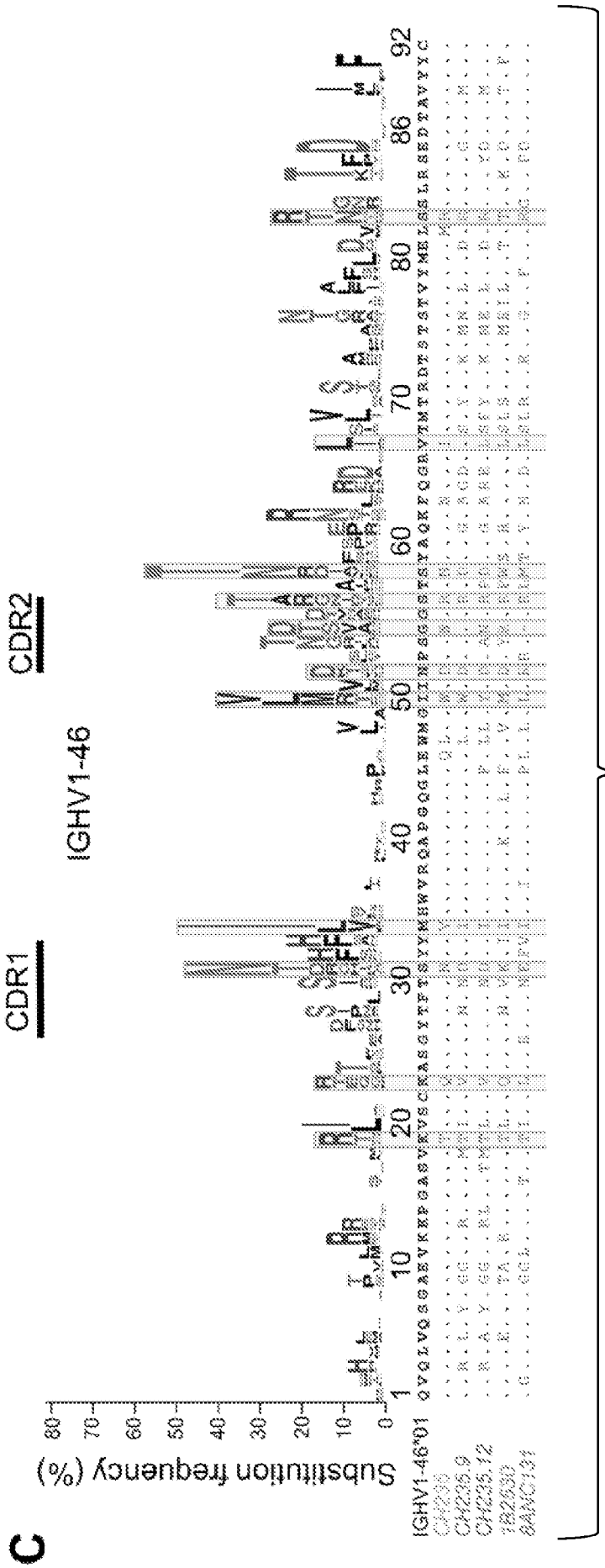

1B2530
8ANC131
positional conformity
identity conformity
Conformity (aa %)
SHM (nt %)
0
20
40
60
80
100
0
10
20
30

FIG. 31-B



continued on next sheet

FIG. 31C

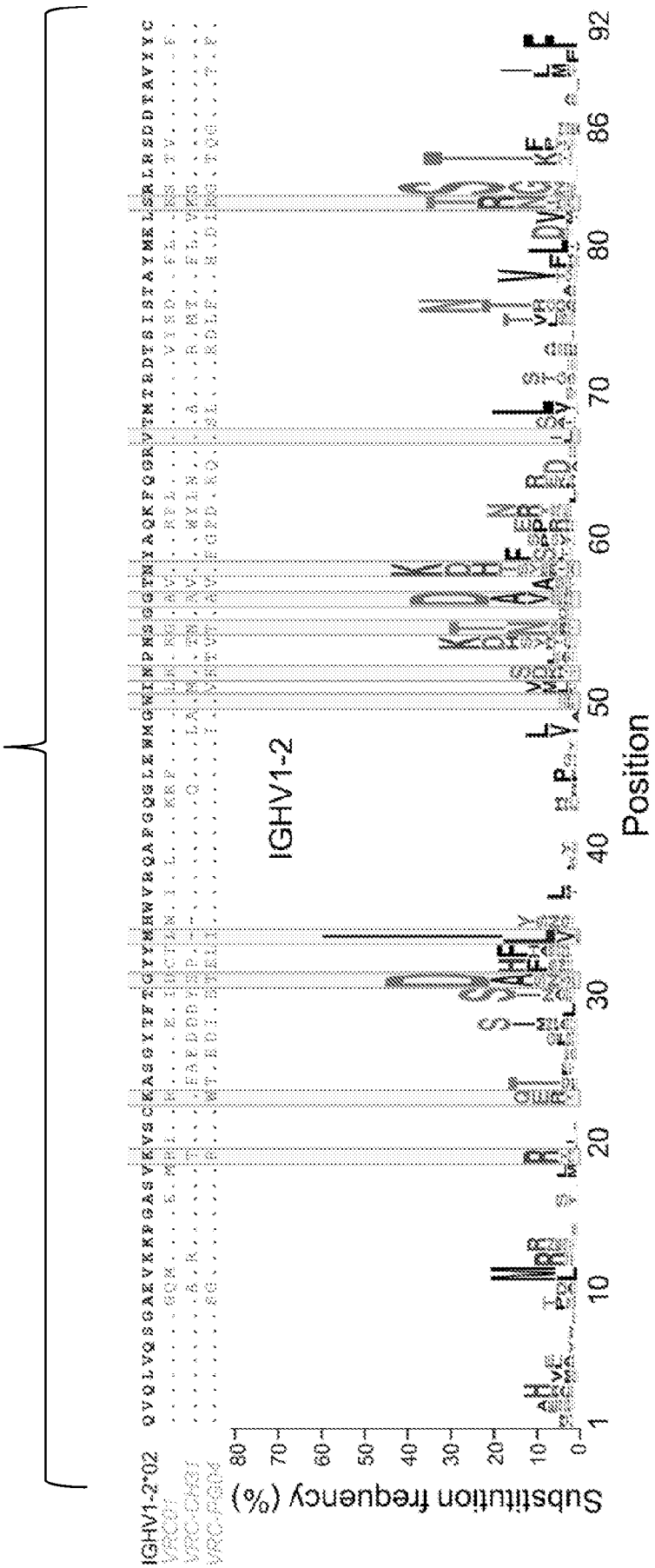
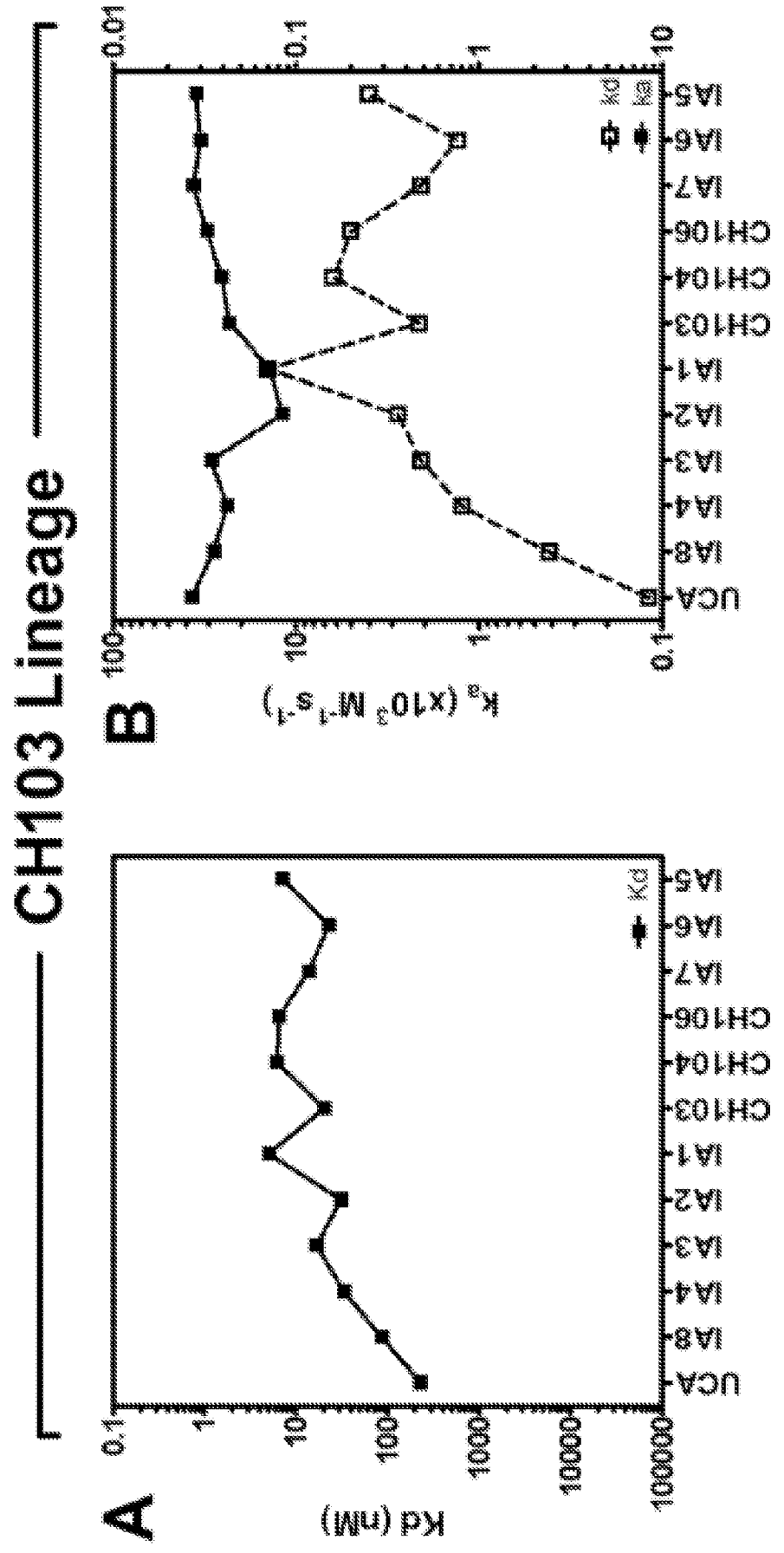
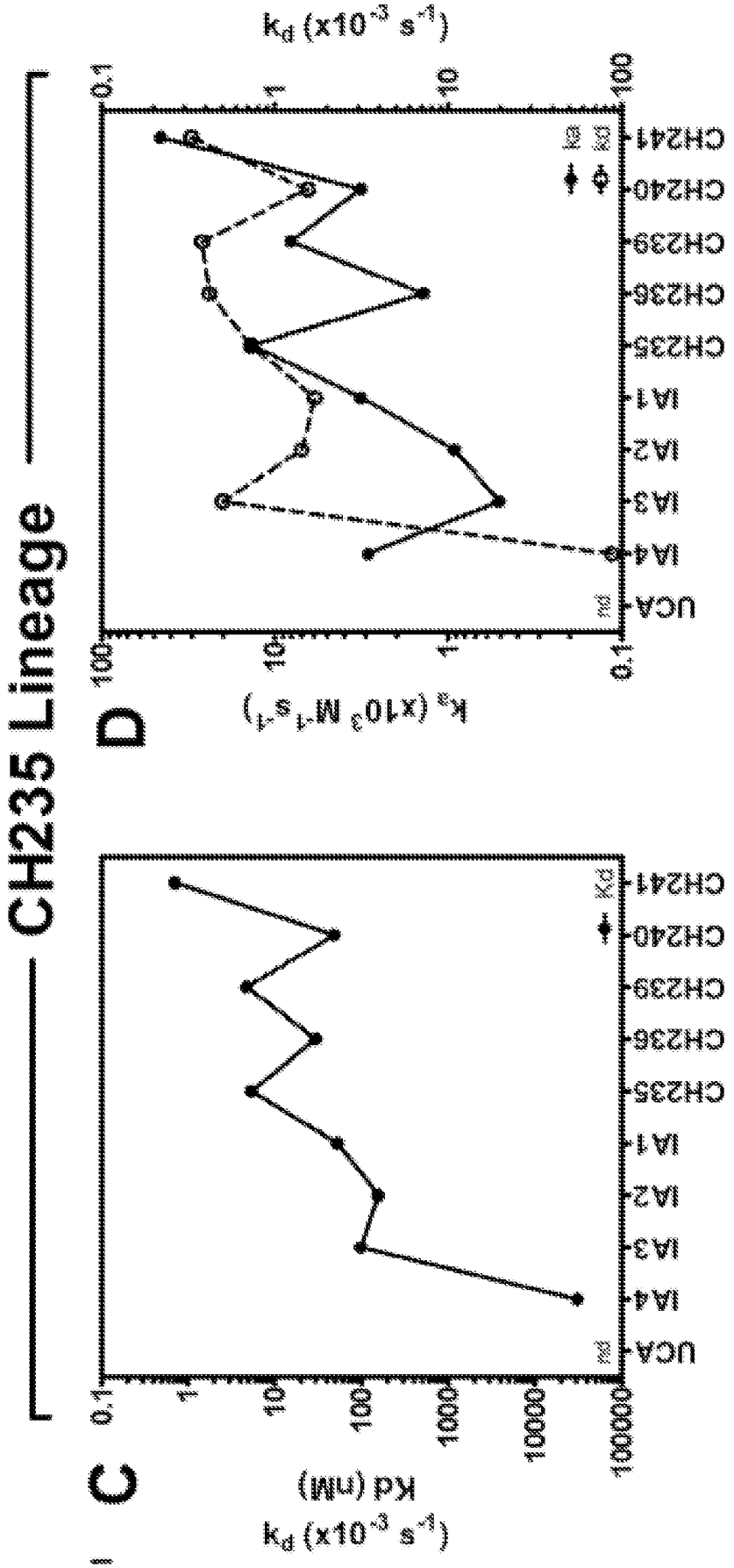

continued from above
IGHV1-2*02
IGHV1-2
Substitution frequency (%)
Position

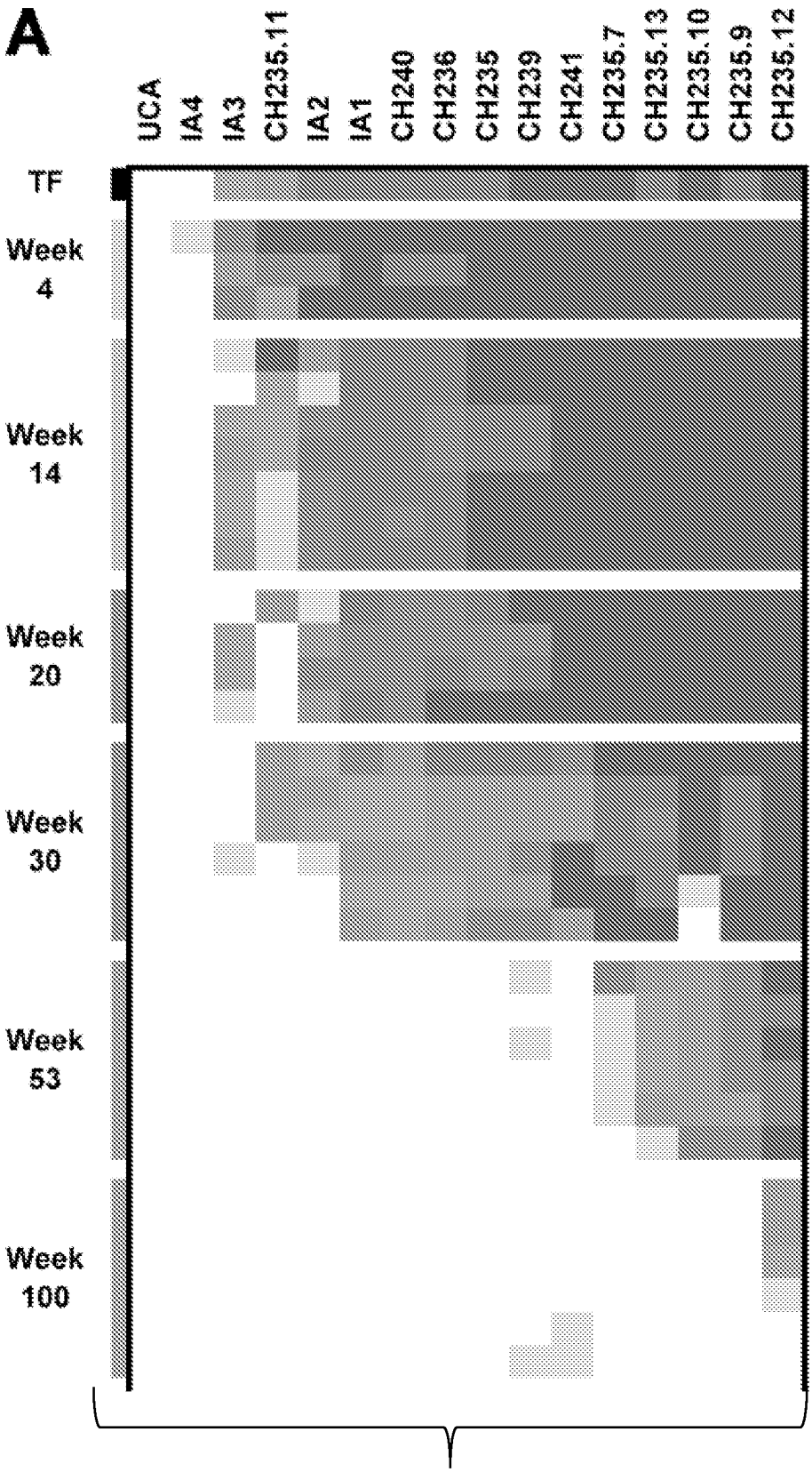
FIG. 31C cont.


CH103 Lineage
A
Kd (nM)
0.1
1
10
100
1000
10000
100000
Kd
UCA
IA8
IA4
IA3
IA2
IA1
CH103
CH104
CH106
IA7
IA6
IA5
B
k_a ($x10^3$ $M^{-1}s^{-1}$)
100
10
1
0.1
0.01
kd
ka
UCA
IA8
IA4
IA3
IA2
IA1
CH103
CH104
CH106
IA7
IA6
IA5

FIGS. 32A-B


CH235 Lineage
C
Kd (nM)
k_d ($x10^{-3}$ s^{-1})
D
k_a ($x10^3$ $M^{-1}s^{-1}$)
k_d ($x10^{-3}$ s^{-1})
UCA
IA4
IA3
IA2
IA1
CH235
CH236
CH239
CH240
CH241

FIGS. 32C-D



continued on next sheet

FIG. 33A

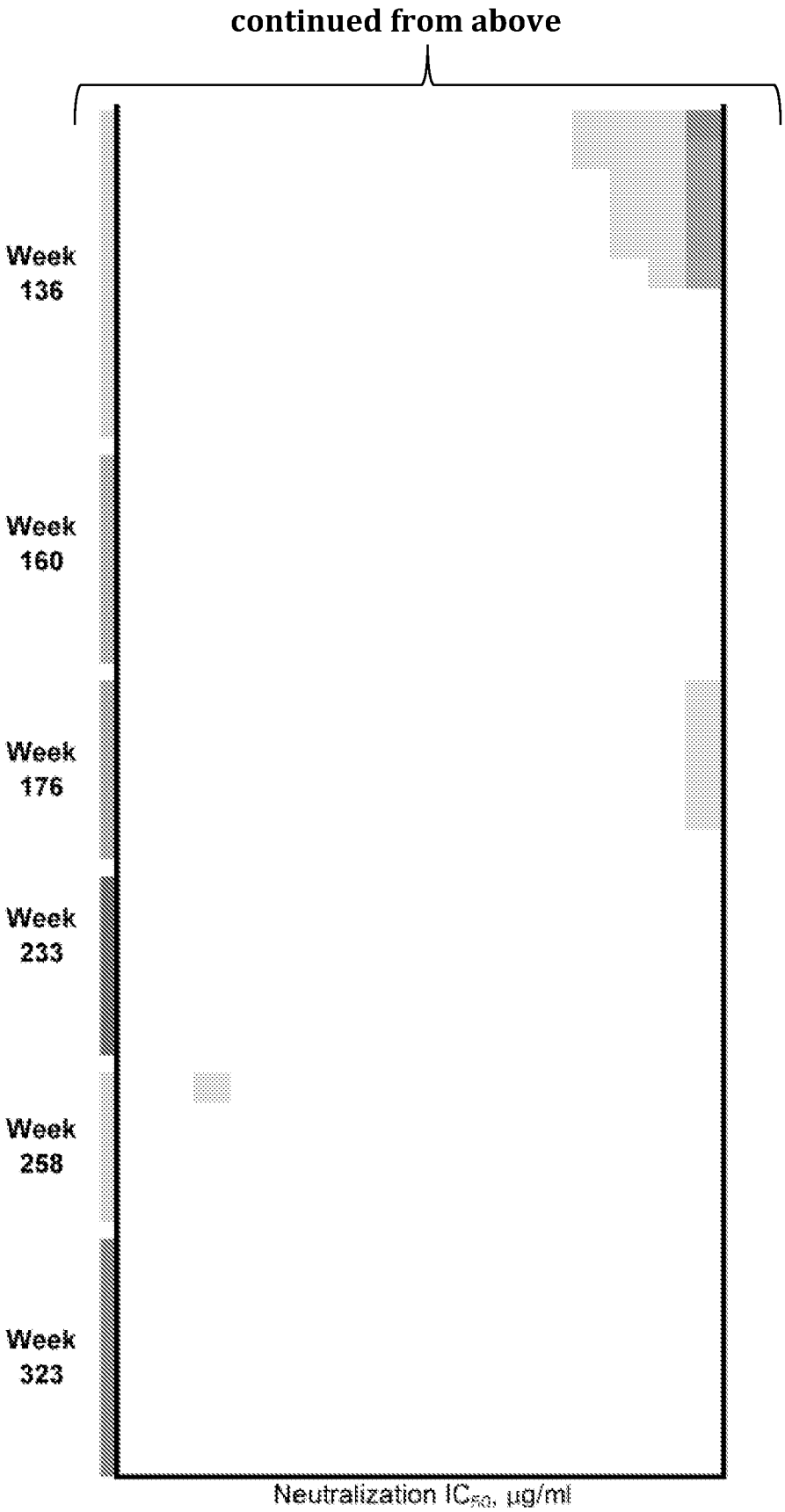

continued from above
Week 136
Week 160
Week 176
Week 233
Week 258
Week 323
Neutralization IC50, µg/ml

FIG. 33A cont.

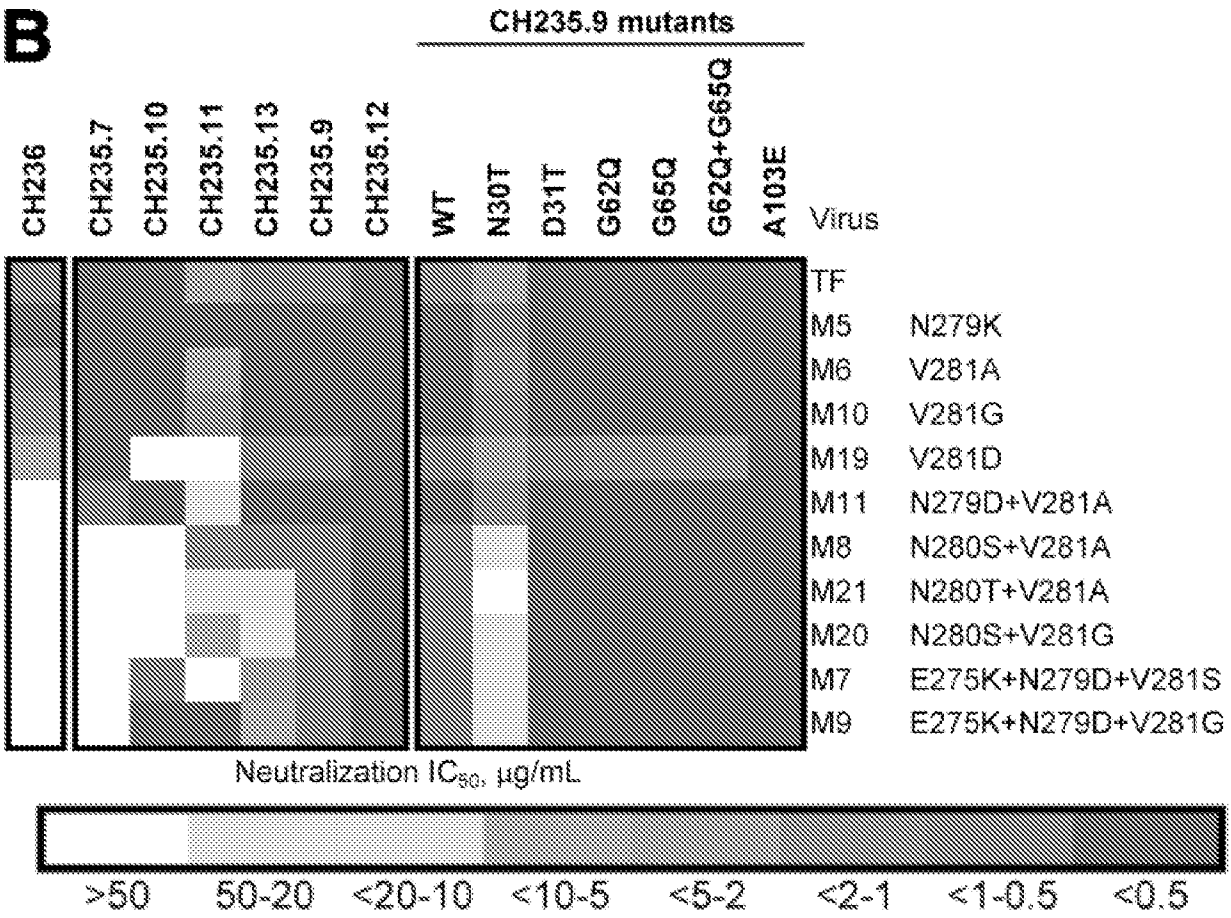

B
CH235.9 mutants
CH236
CH235.7
CH235.10
CH235.11
CH235.13
CH235.9
CH235.12
WT
N30T
D31T
G62Q
G65Q
G62Q+G65Q
A103E
Virus
TF
M5 N279K
M6 V281A
M10 V281G
M19 V281D
M11 N279D+V281A
M8 N280S+V281A
M21 N280T+V281A
M20 N280S+V281G
M7 E275K+N279D+V281S
M9 E275K+N279D+V281G
Neutralization IC80, µg/mL
>50
50-20
<20-10
<10-5
<5-2
<2-1
<1-0.5
<0.5

FIG. 33B

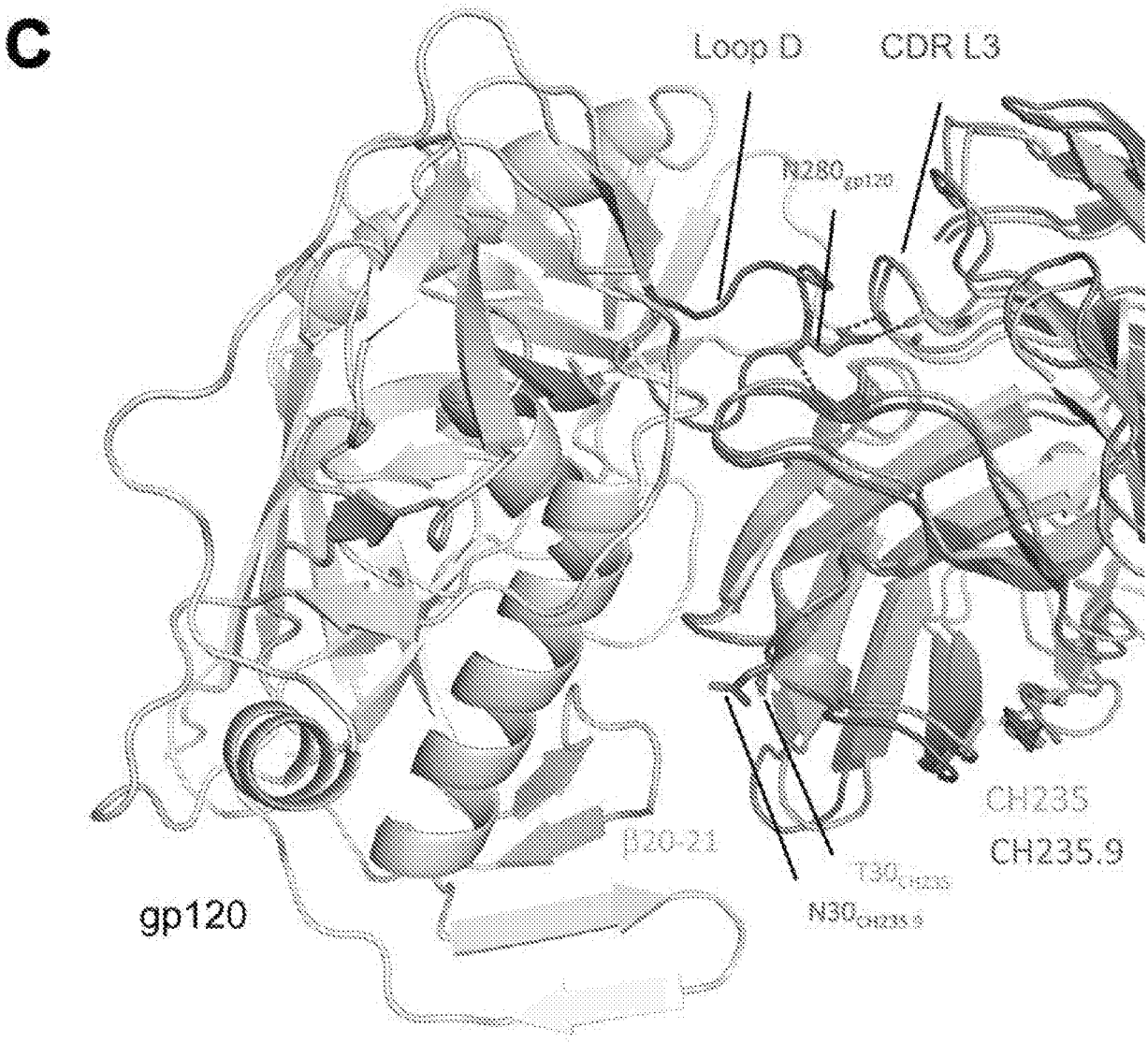

C
Loop D
CDR L3
N280gp120
CH235
CH235.9
β20-21
T30CH235
N30CH235.9
gp120

FIG. 33C

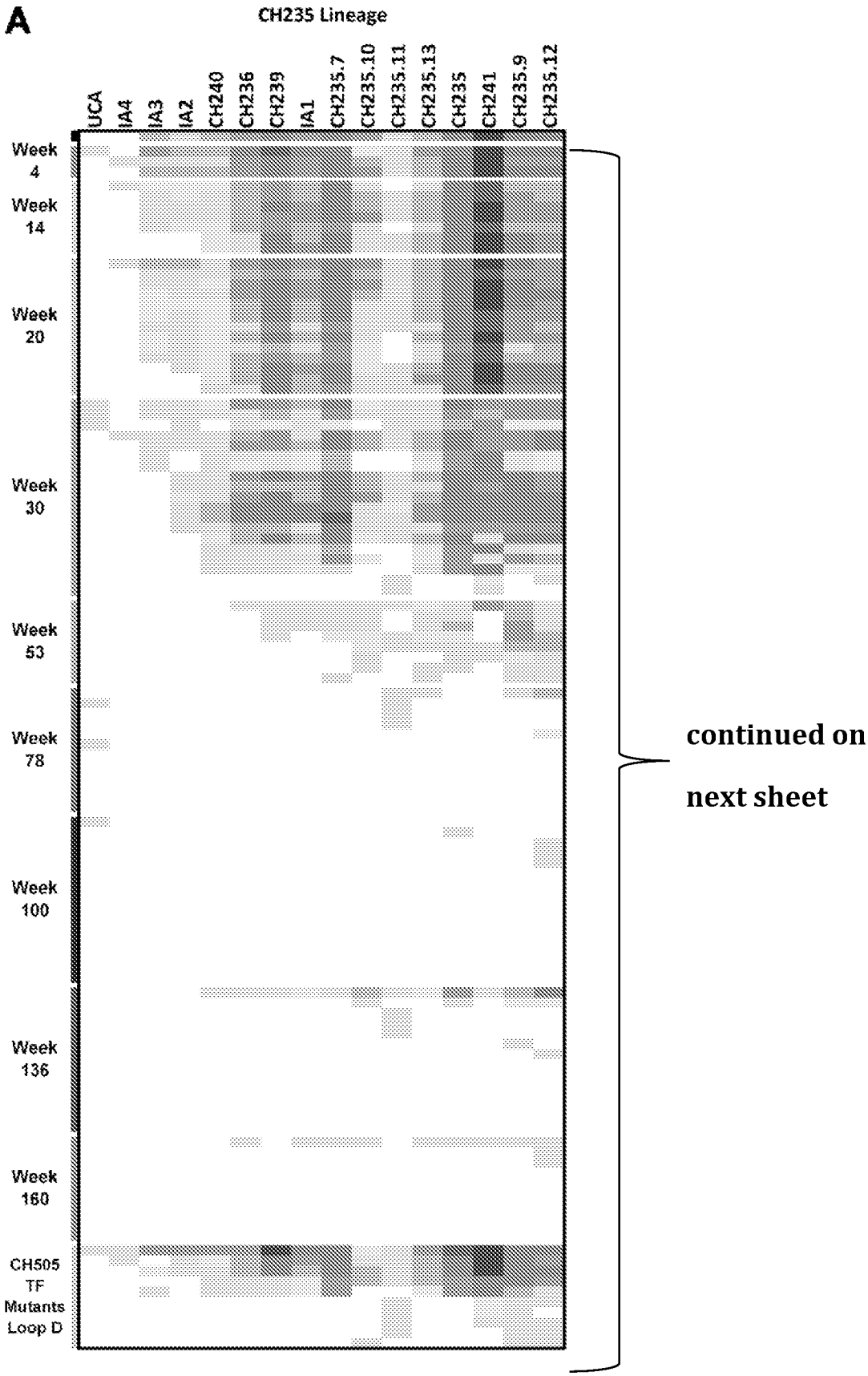

A
CH235 Lineage
UCA
IA4
IA3
IA2
CH240
CH236
CH239
IA1
CH235.7
CH235.10
CH235.11
CH235.13
CH235
CH241
CH235.9
CH235.12
Week 4
Week 14
Week 20
Week 30
Week 53
Week 78
Week 100
Week 136
Week 160
CH505 TF Mutants Loop D
continued on next sheet

FIG. 34A

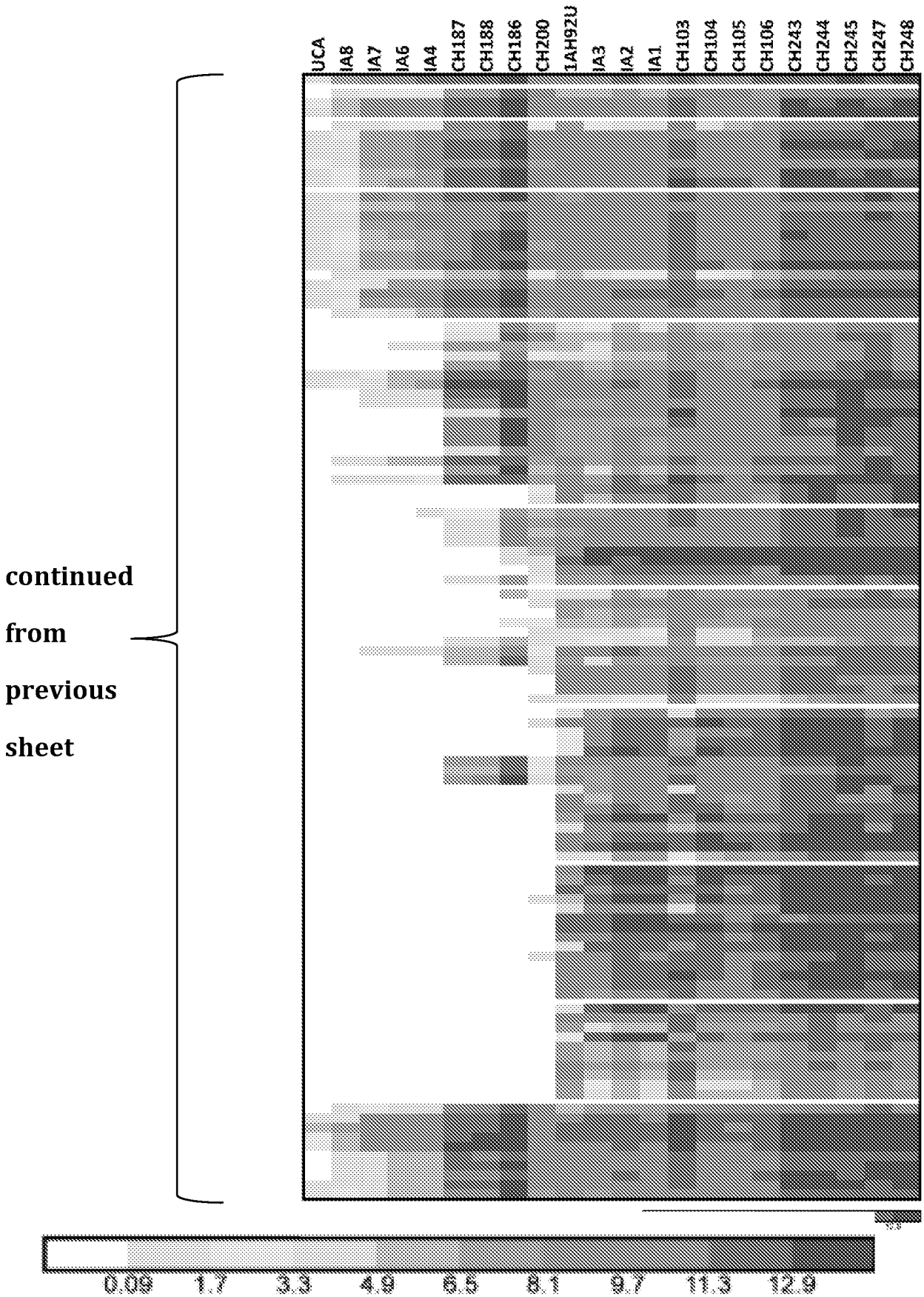

UCA
IA8
IA7
IA6
IA4
CH187
CH188
CH186
CH200
1AH92U
IA3
IA2
IA1
CH103
CH104
CH105
CH106
CH243
CH244
CH245
CH247
CH248
continued from previous sheet
0.09
1.7
3.3
4.9
6.5
8.1
9.7
11.3
12.9
Binding (LogAUC)

FIG. 34A cont.

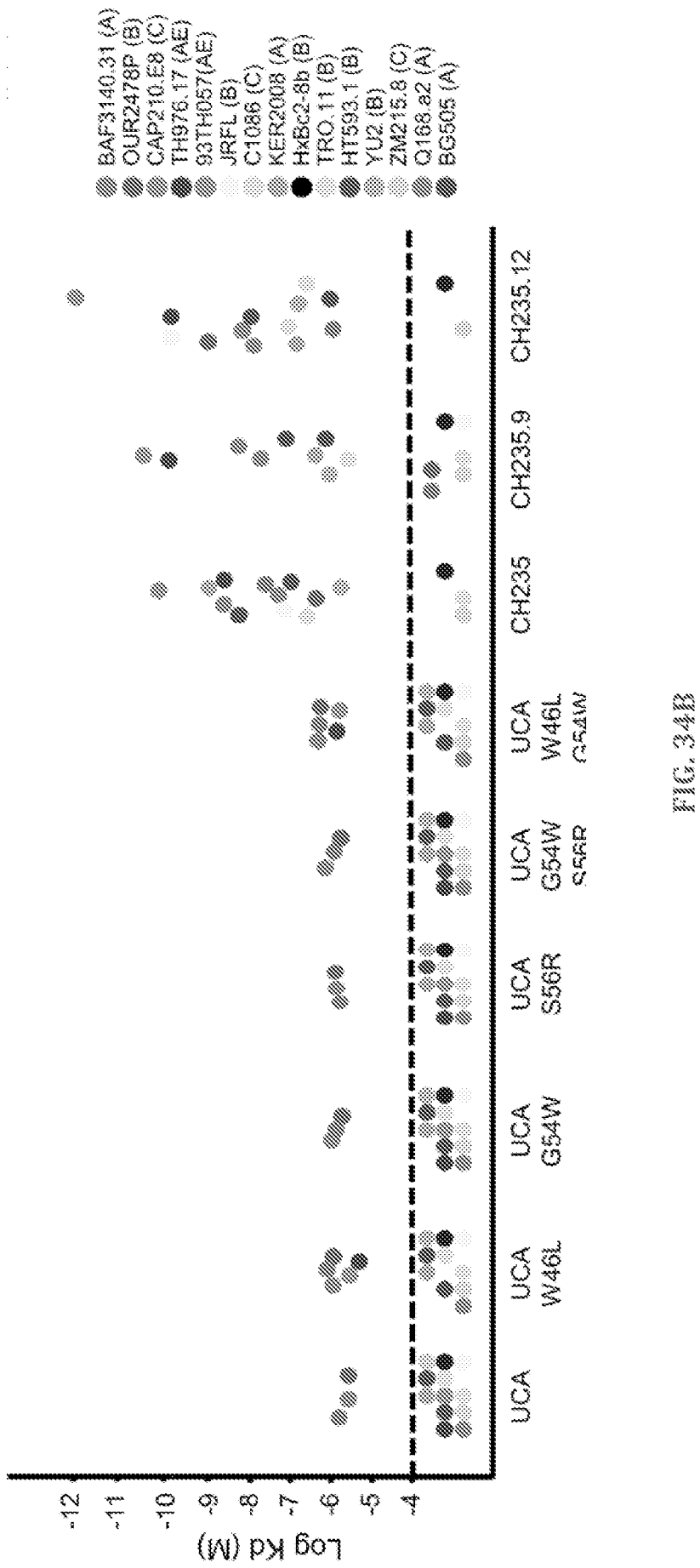

BAF3140.31 (A)
OUR2478P (B)
CAP210.E8 (C)
TH976.17 (AE)
93TH057(AE)
JRFL (B)
C1086 (C)
KER2008 (A)
HxBc2-8b (B)
TRO.11 (B)
HT593.1 (B)
YU2 (B)
ZM215.8 (C)
Q168.a2 (A)
BG505 (A)
Log Kd (M)
-12
-11
-10
-9
-8
-7
-6
-5
-4
UCA
UCA W46L
UCA G54W
UCA S56R
UCA G54W S56R
UCA W46L G54W
CH235
CH235.9
CH235.12

FIG. 34B

A

	SSA	SSB	Sm	RNP	Scl70	Jo1	DNA	Cent. B	Histone
UCA	-	-	-	-	-	-	-	-	-
IA4	-	-	-	-	-	-	-	-	-
IA1	-	-	-	-	-	-	-	-	-
CH240	-	-	-	-	-	-	-	-	-
CH239	-	-	-	-	-	-	-	-	-
IA3	-	-	-	-	-	-	+	-	-
IA2	-	-	-	-	-	-	+	-	-
CH236	-	-	-	-	-	-	+	-	-
CH235	-	-	-	-	-	-	-	-	-
CH241	-	-	-	-	-	-	+	-	-
CH235.7	-	-	-	-	+	-	+++	-	-
CH235.9	-	-	-	-	-	-	+	-	-
CH235.10	-	-	-	-	-	-	+	-	-
CH235.13	-	-	-	-	-	-	-	-	-
CH235.11	-	-	-	-	-	-	-	-	-
CH235.12	-	-	-	-	-	-	-	-	-

FIG. 35A

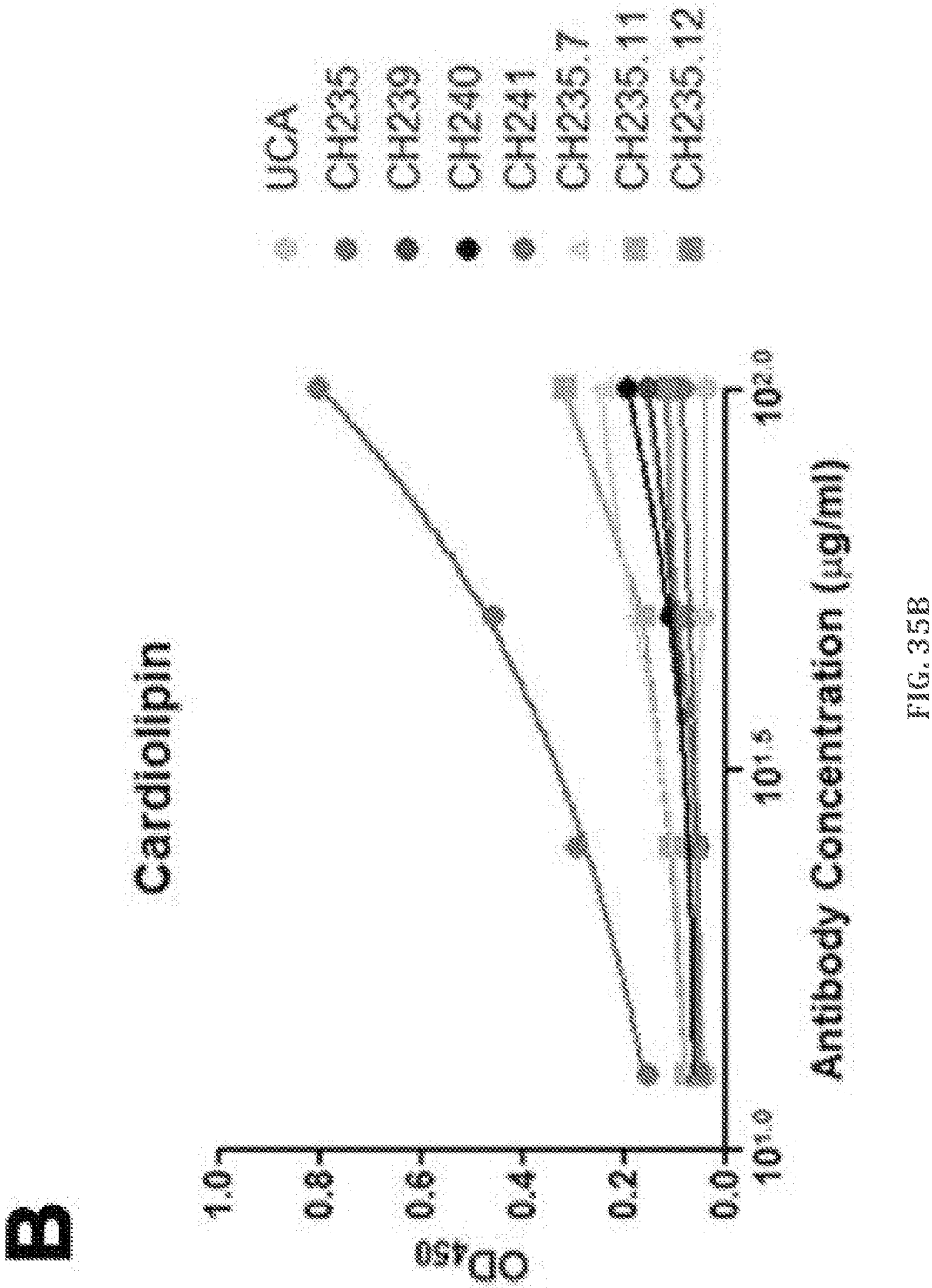

B
Cardiolipin
OD450
1.0
0.8
0.6
0.4
0.2
0.0
10^1.0
10^1.5
10^2.0
Antibody Concentration (μg/ml)
UCA
CH235
CH239
CH240
CH241
CH235.7
CH235.11
CH235.12

FIG. 35B

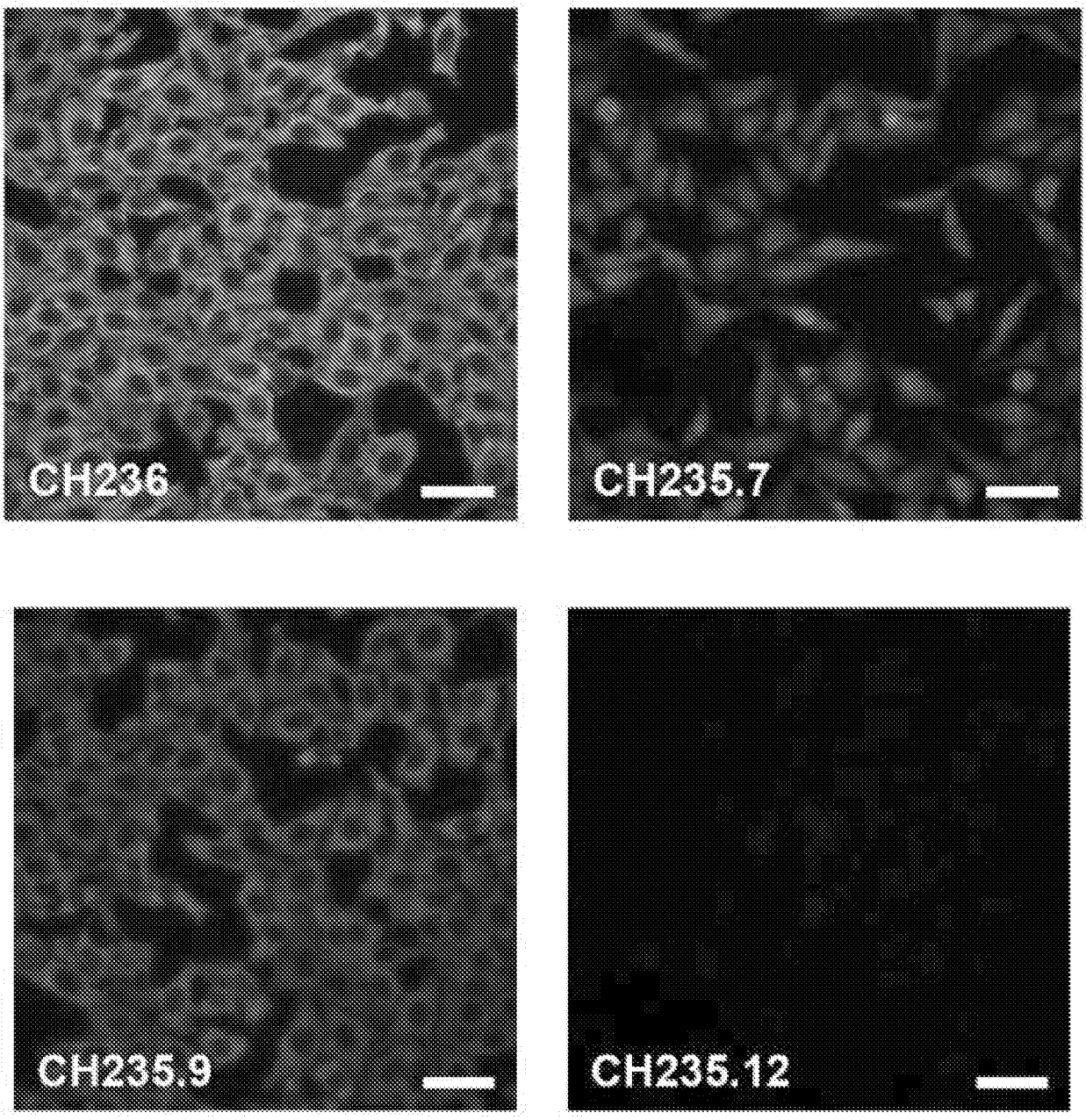


FIG. 35C

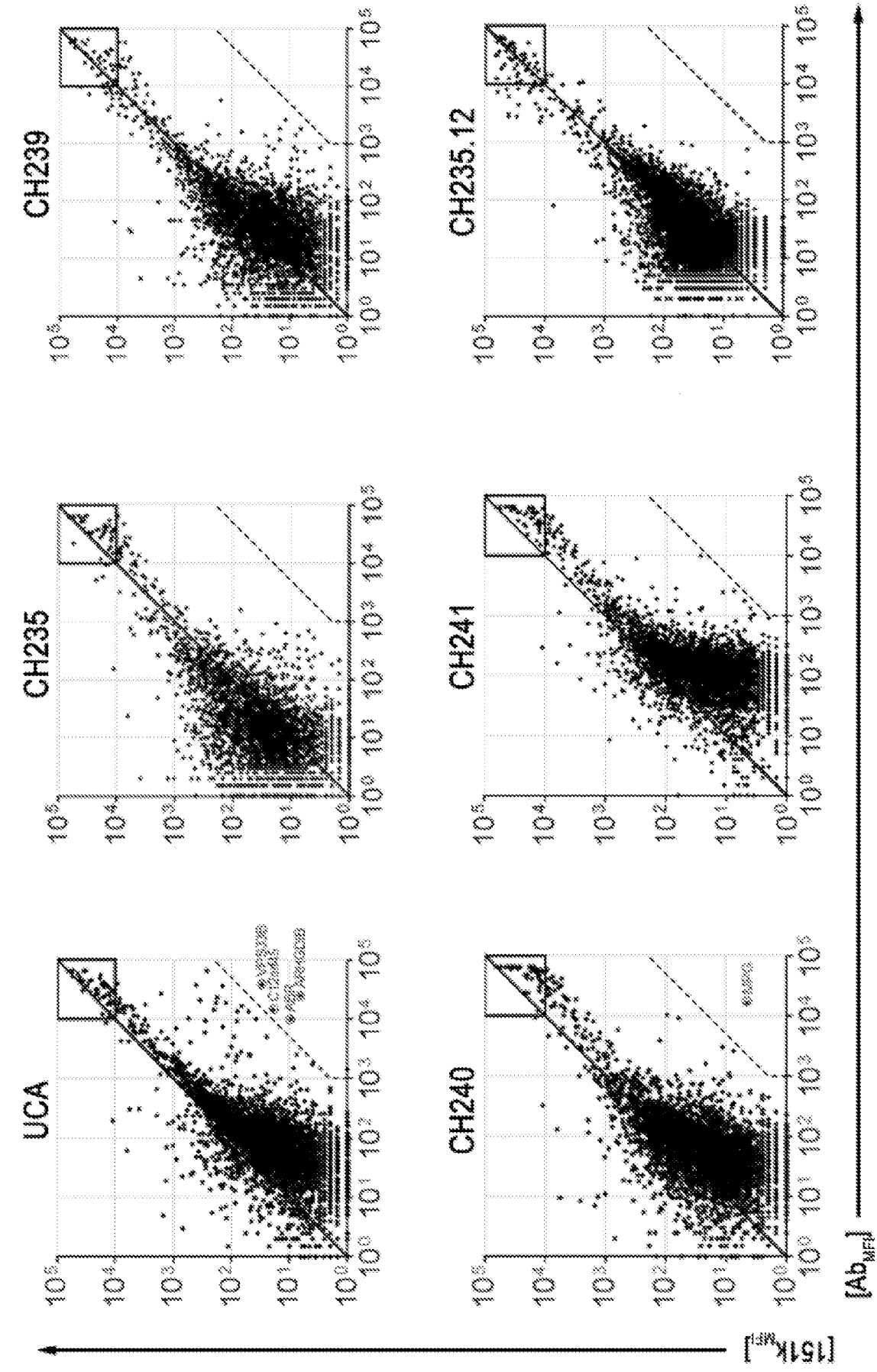


FIG. 35D

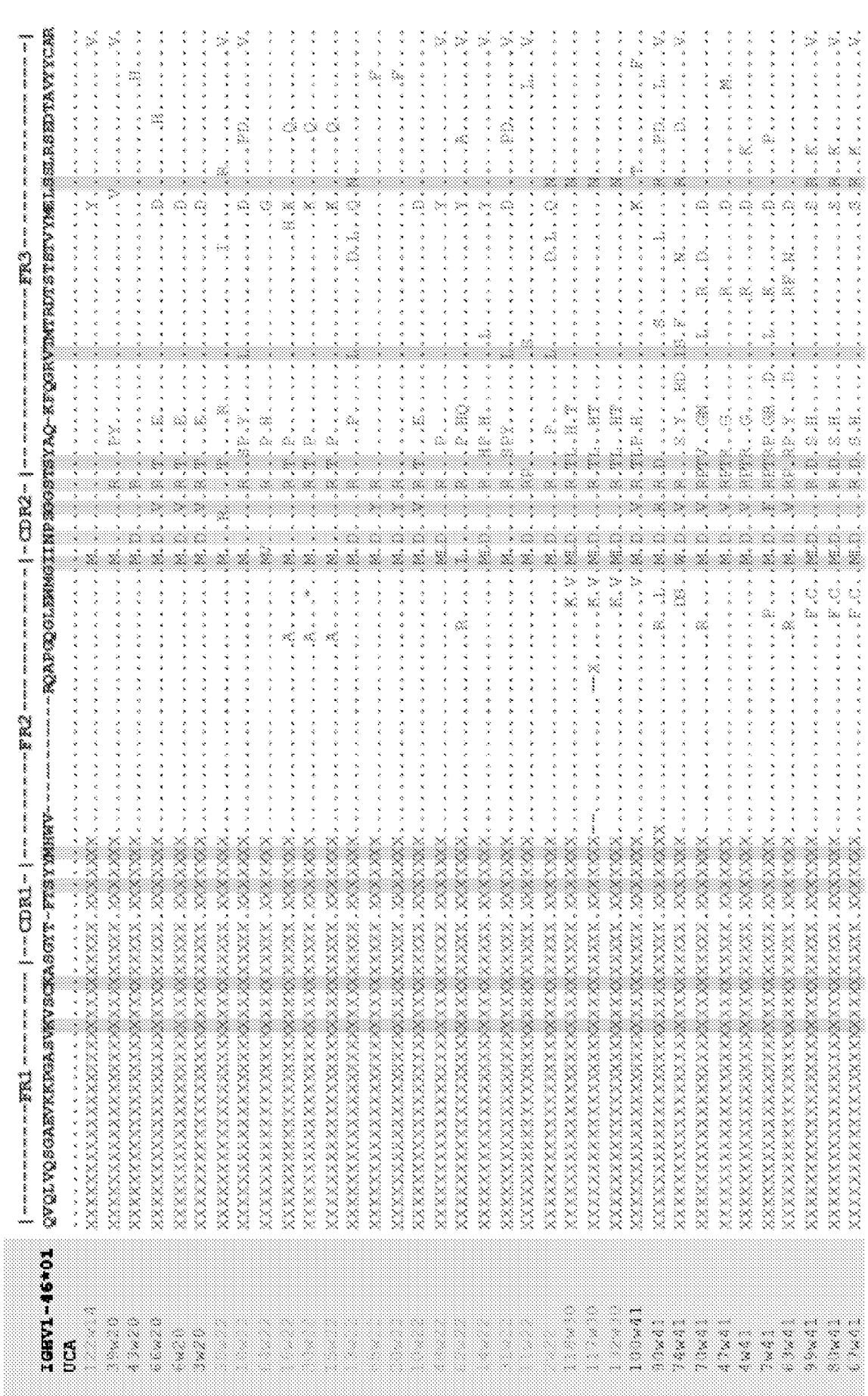


FIG. 36A

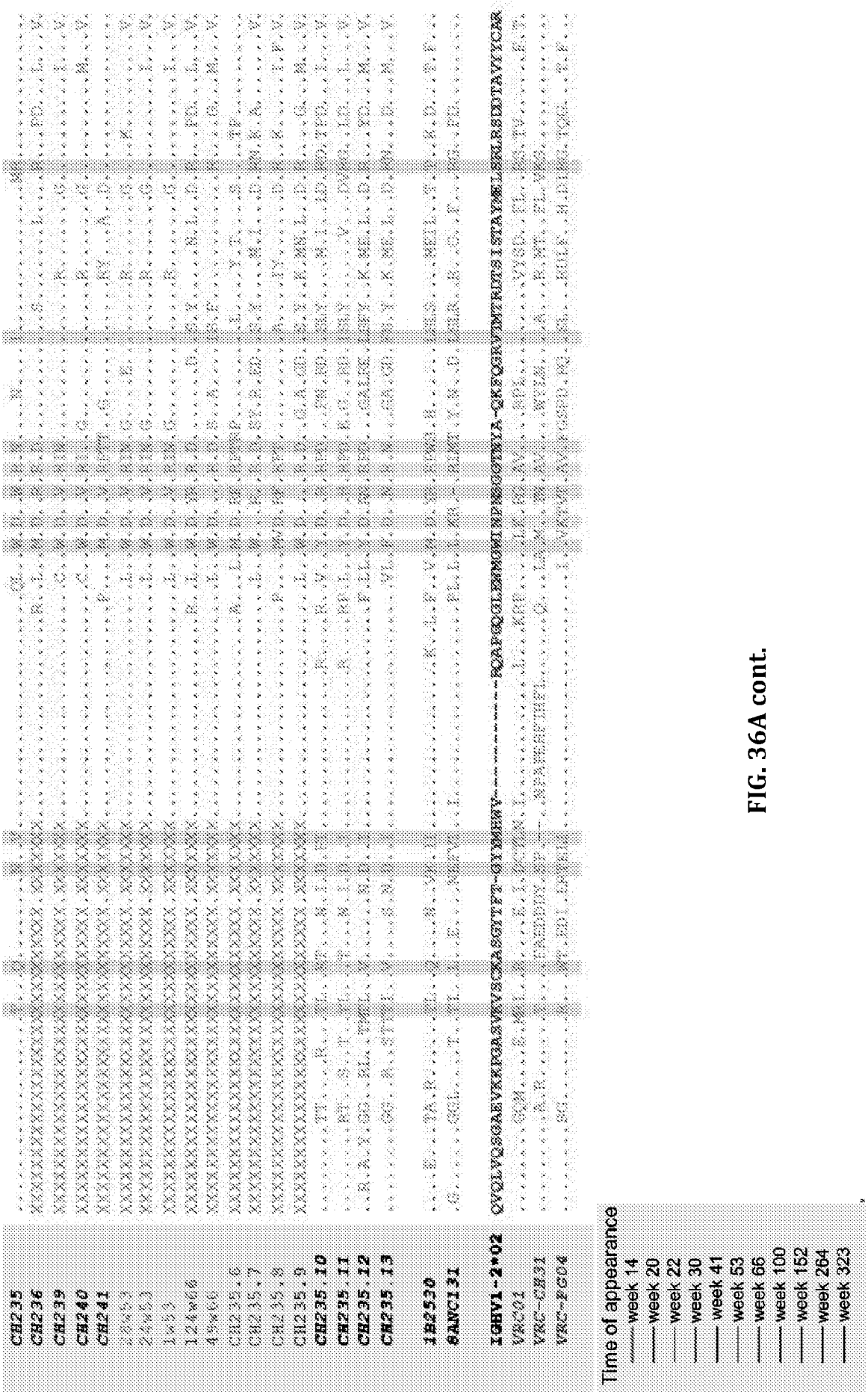

CH235
CH236
CH239
CH240
CH241
CH235.6
CH235.7
CH235.8
CH235.9
CH235.10
CH235.11
CH235.12
CH235.13
IH2530
6AYC131
IGHV1-2*02
VRC01
VRC-CH31
VRC-PG04
Time of appearance
week 14
week 20
week 22
week 30
week 41
week 53
week 66
week 100
week 152
week 264
week 323

FIG. 36A cont.

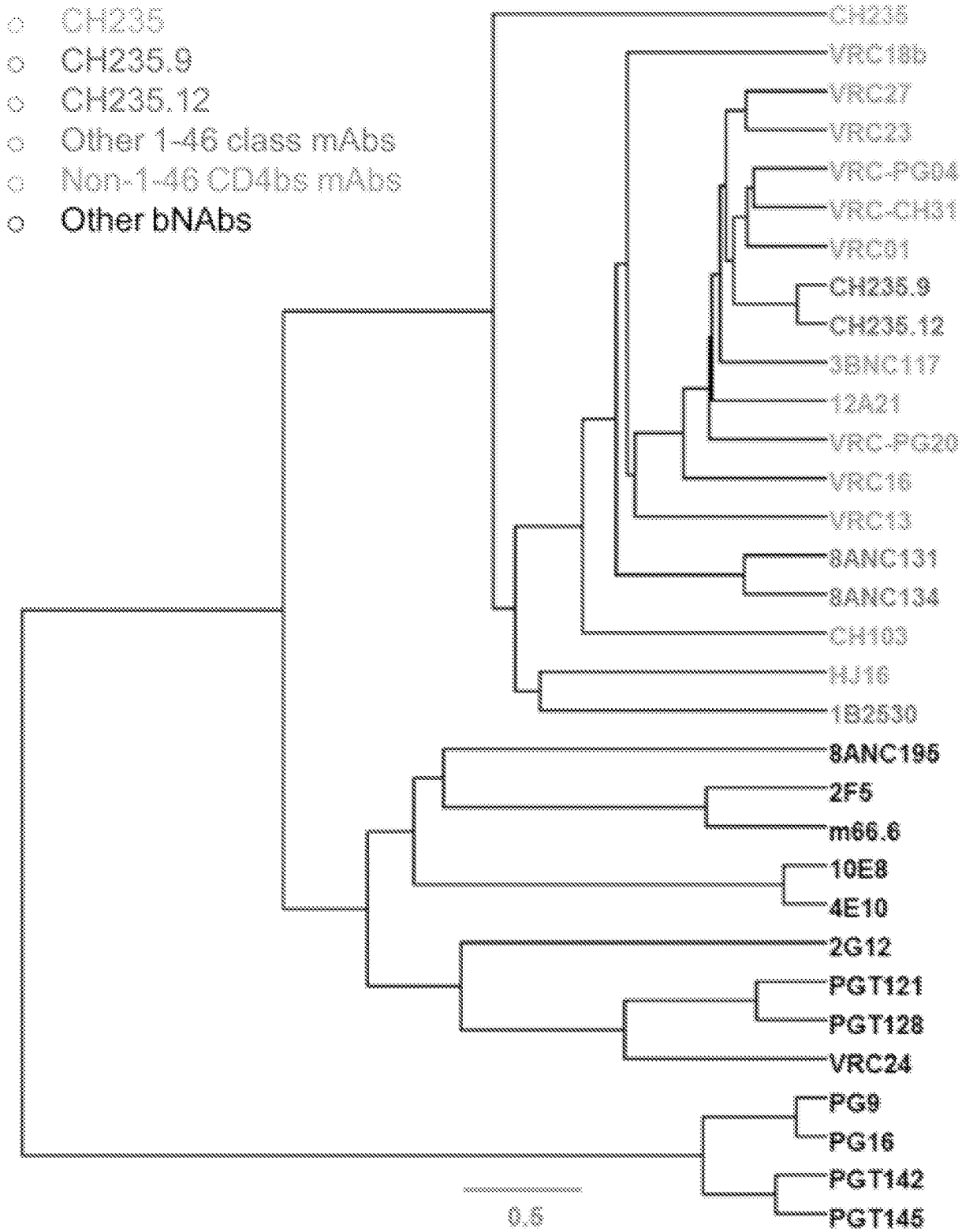

CH235
CH235.9
CH235.12
Other 1-46 class mAbs
Non-1-46 CD4bs mAbs
Other bNAbs
CH235
VRC18b
VRC27
VRC23
VRC-PG04
VRC-CH31
VRC01
CH235.9
CH235.12
3BNC117
12A21
VRC-PG20
VRC16
VRC13
8ANC131
8ANC134
CH103
HJ16
1B2530
8ANC195
2F5
m66.6
10E8
4E10
2G12
PGT121
PGT128
VRC24
PG9
PG16
PGT142
PGT145
0.5

FIG. 36B

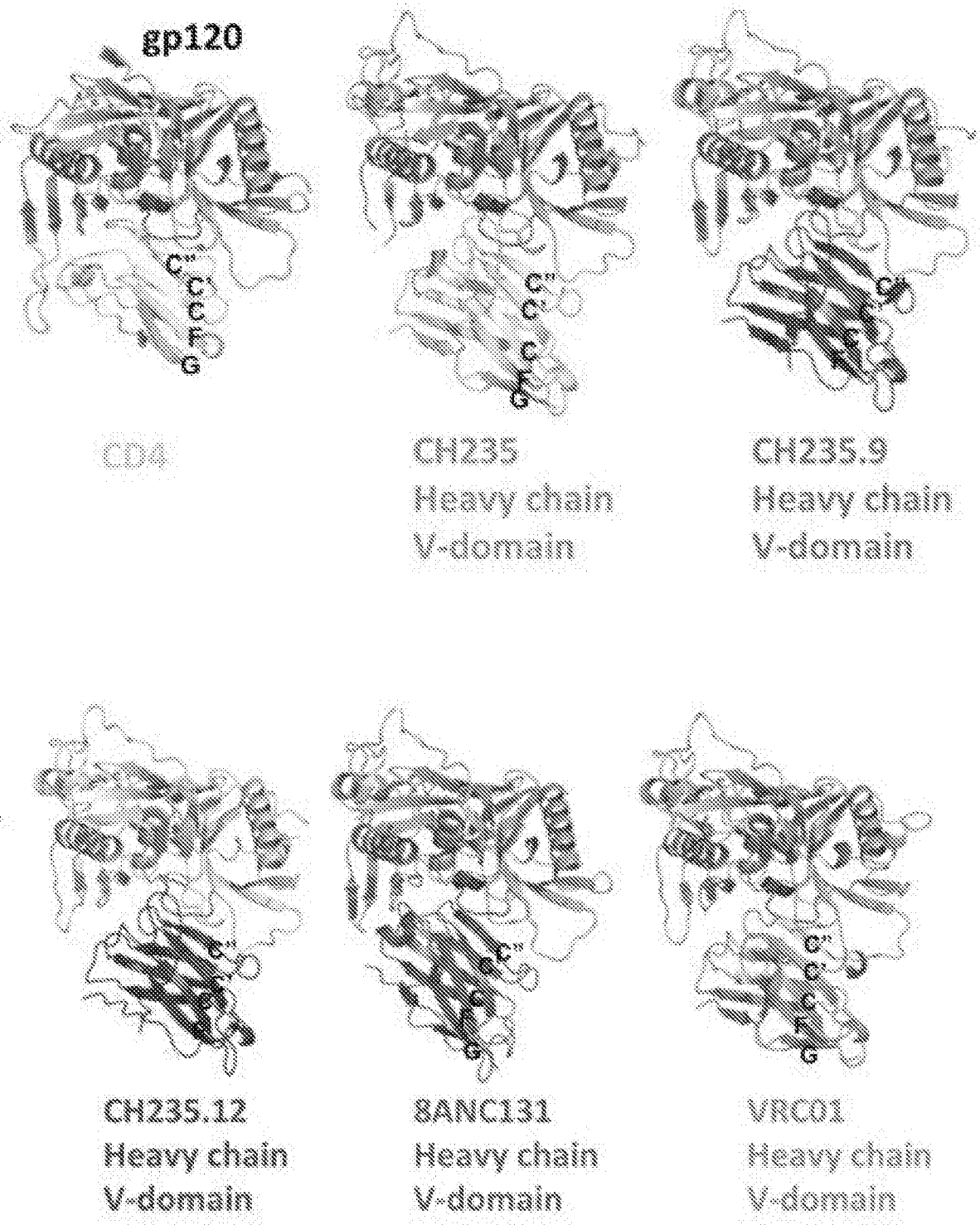

gp120
CD4
CH235
Heavy chain
V-domain
CH235.9
Heavy chain
V-domain
CH235.12
Heavy chain
V-domain
8ANC131
Heavy chain
V-domain
VRC01
Heavy chain
V-domain

FIG. 37A

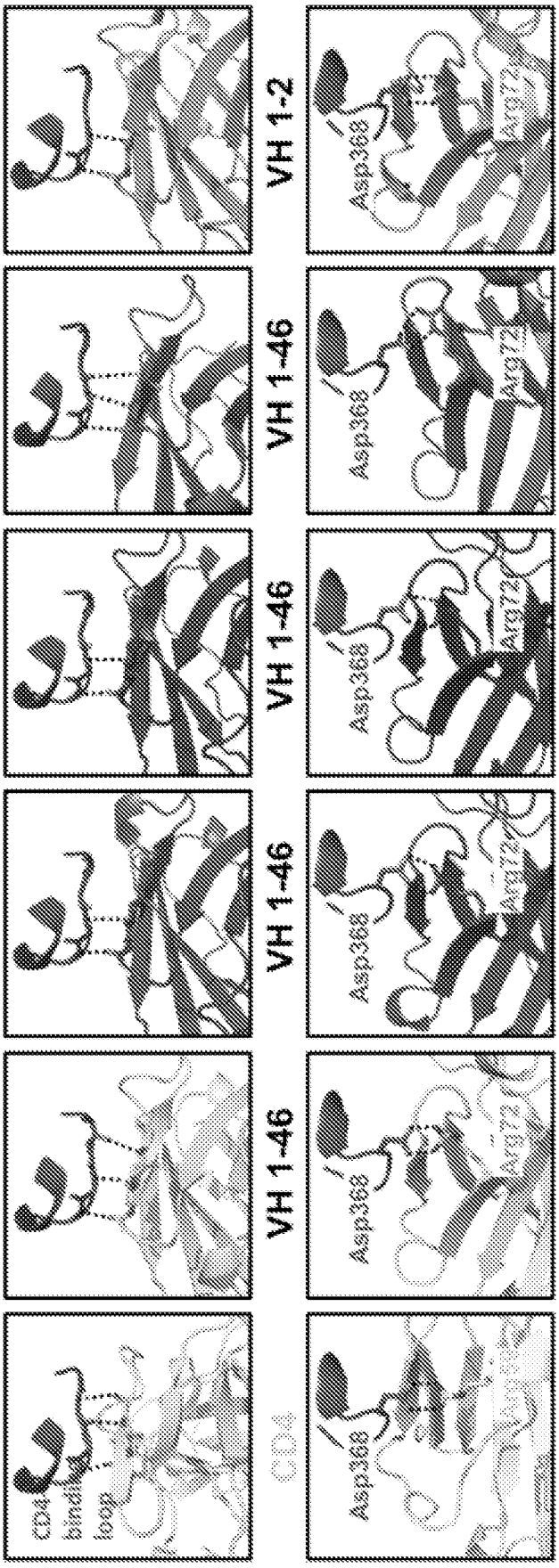
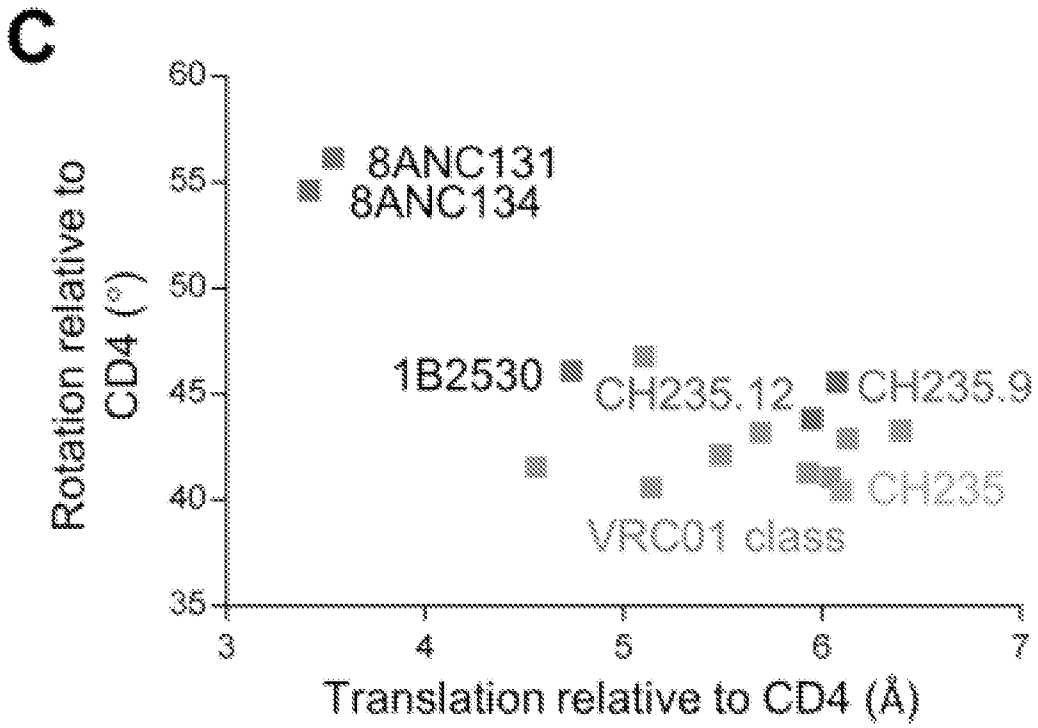
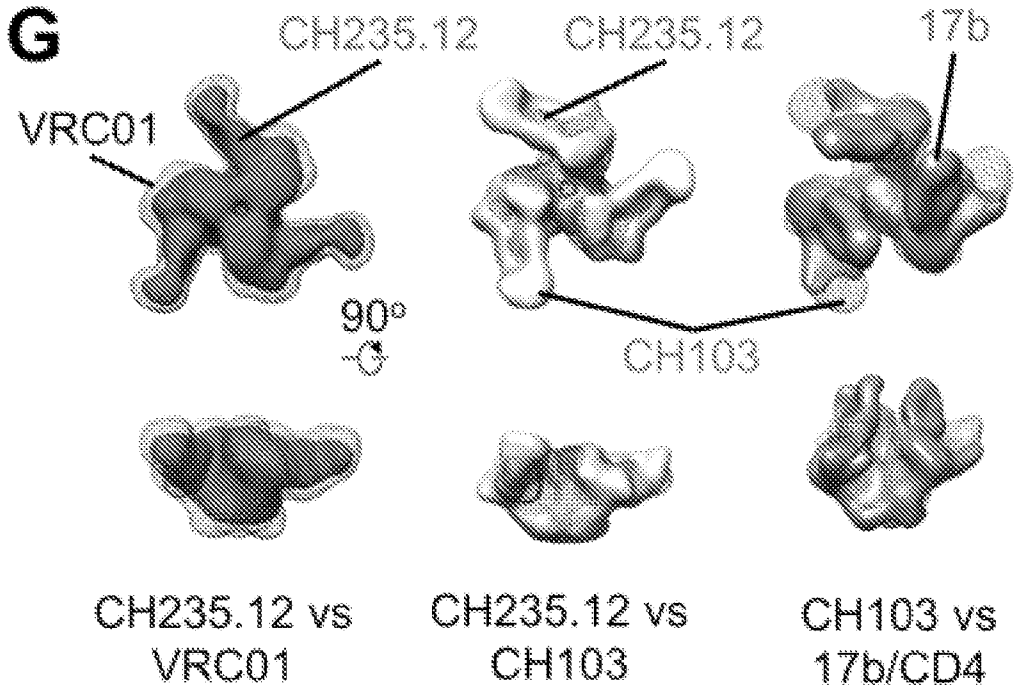

CD4 binding loop
CD4
VH 1-46
VH 1-46
VH 1-46
VH 1-46
VH 1-2
Asp368
Arg72

FIG. 37B


C
Rotation relative to CD4 (°)
60
55
50
45
40
35
3
4
5
6
7
Translation relative to CD4 (Å)
8ANC131
8ANC134
1B2530
CH235.12
CH235.9
CH235
VRC01 class


G
CH235.12
CH235.12
17b
VRC01
90°
CH103
CH235.12 vs VRC01
CH235.12 vs CH103
CH103 vs 17b/CD4

FIGS. 37C, G

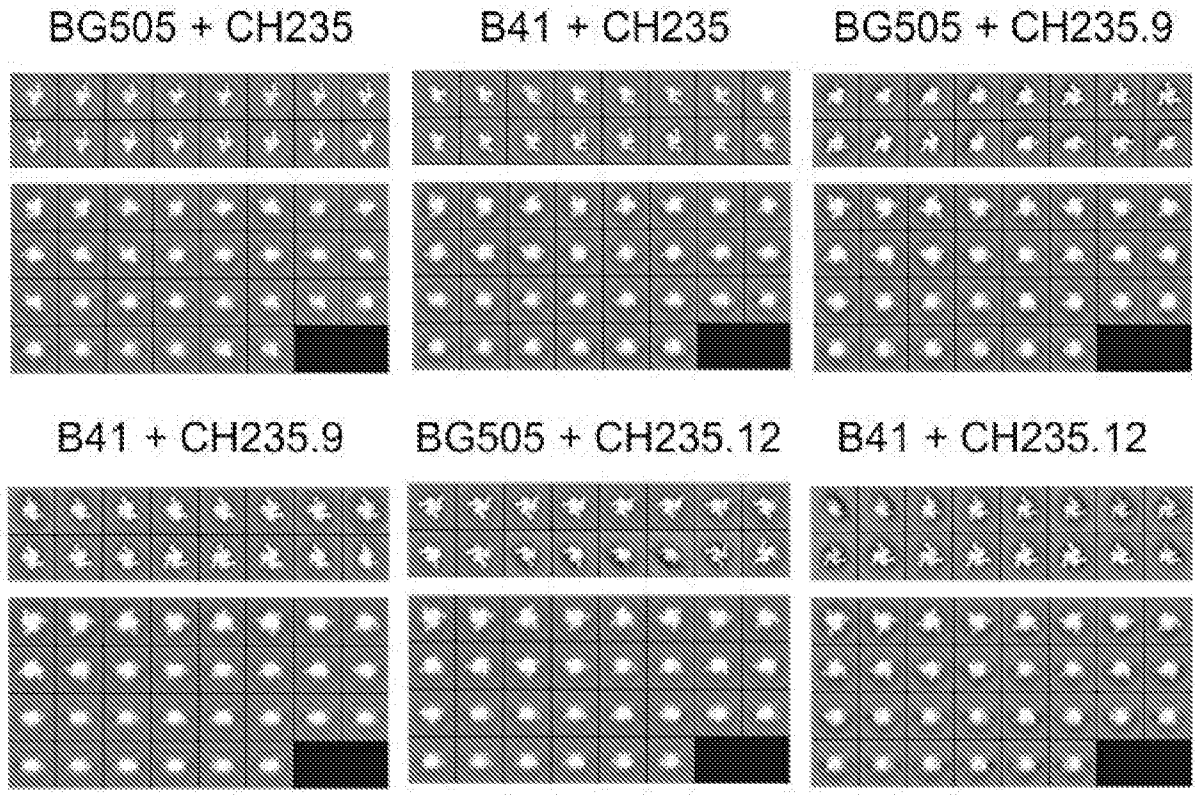

BG505 + CH235
B41 + CH235
BG505 + CH235.9
B41 + CH235.9
BG505 + CH235.12
B41 + CH235.12

FIG. 37D

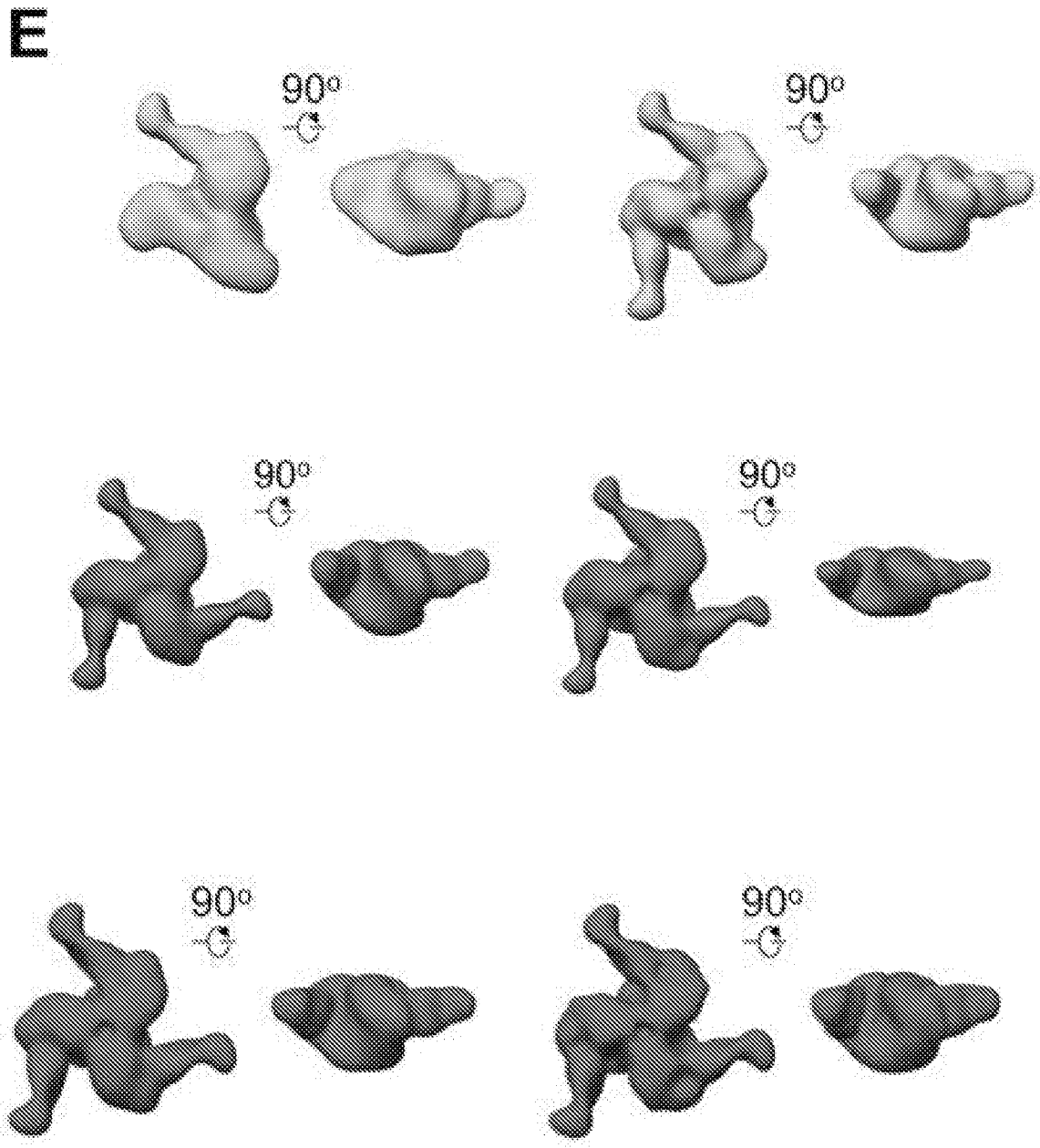


FIG. 37E

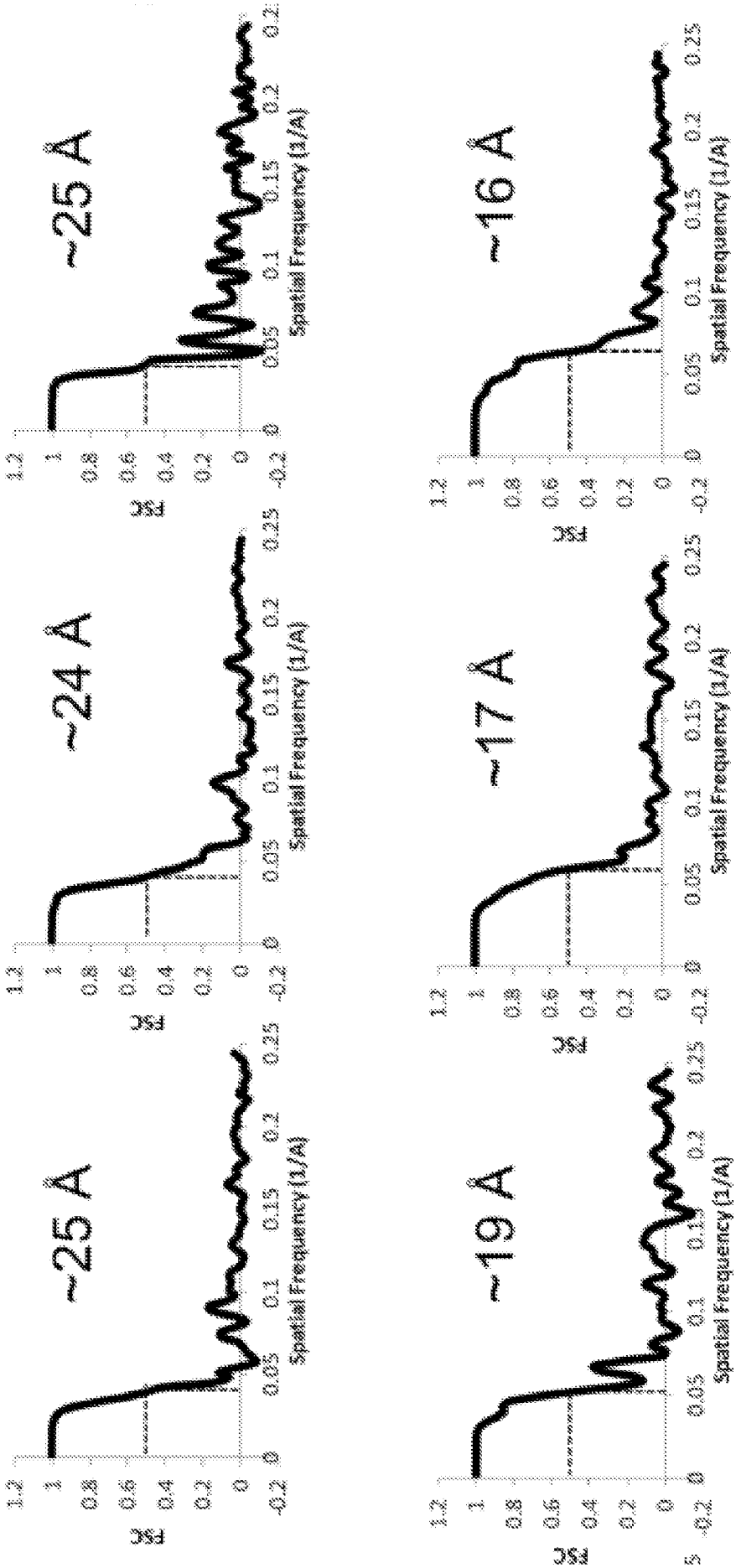


FIG. 37F

```
VH1-46*01   QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYYMHWVRQAPGQGLEWMG
8ANC131     .G......GGL....T..II..L..E...NEFVI..I.......PL.L.
CH235       ..................I...Q......N..V..........QL..

VH1-46*01   IINPSGGSTSYAQKFQGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCAR
8ANC131     L.KR.-.RLMT.YN..D.LSLR..R..G..F...NG..PD.........
CH235       W.D..W.S.N...N....I..............NS..............
```

a

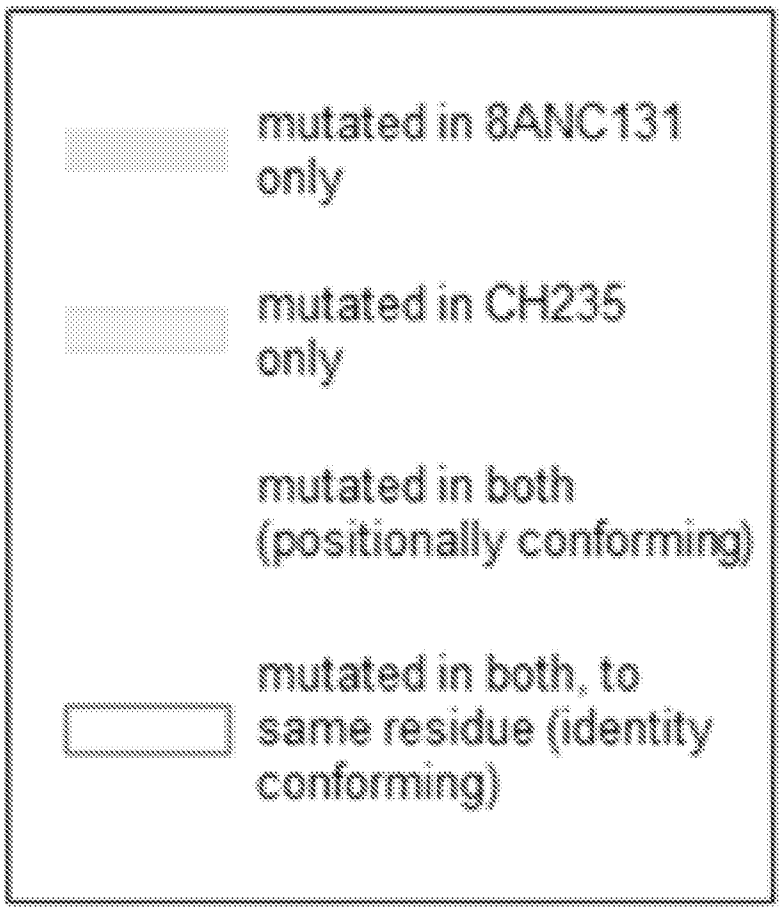


FIG. 38A

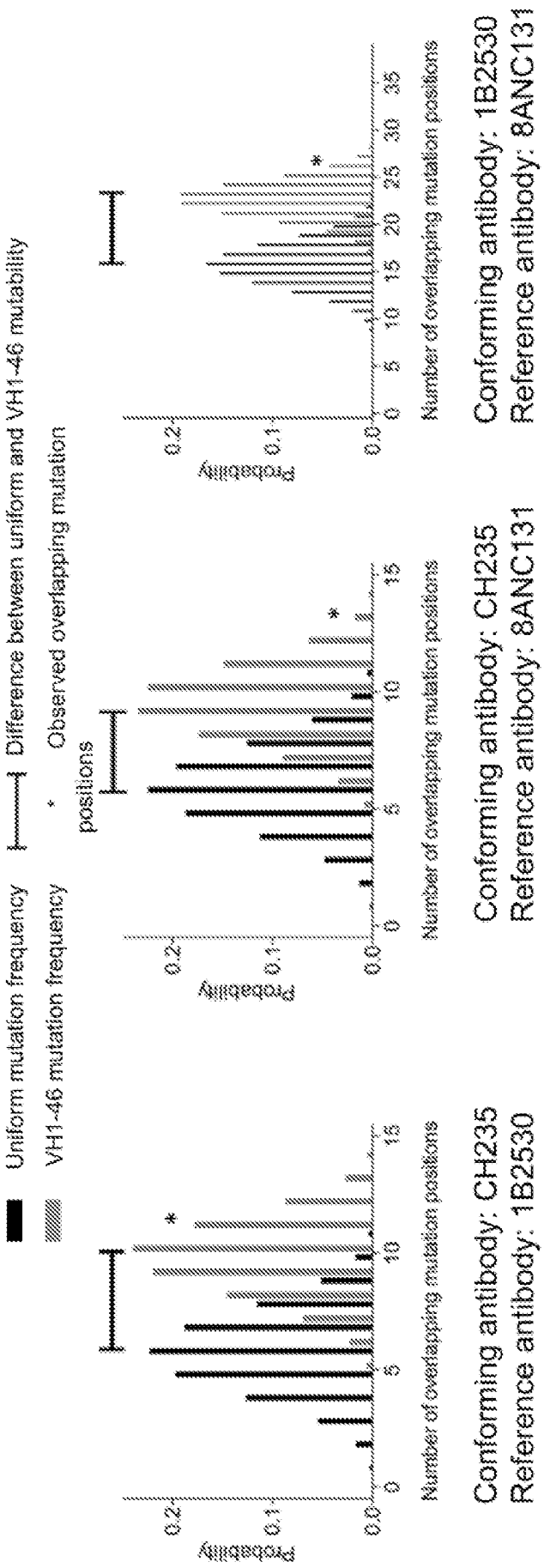

Uniform mutation frequency
VH1-46 mutation frequency
Difference between uniform and VH1-46 mutability
* Observed overlapping mutation positions
Probability
Number of overlapping mutation positions
Conforming antibody: CH235
Reference antibody: 1B2530
Conforming antibody: 1B2530
Reference antibody: 8ANC131
Conforming antibody: CH235
Reference antibody: 8ANC131
Conforming antibody: 1B2530
Reference antibody: 8ANC131

FIG. 38B

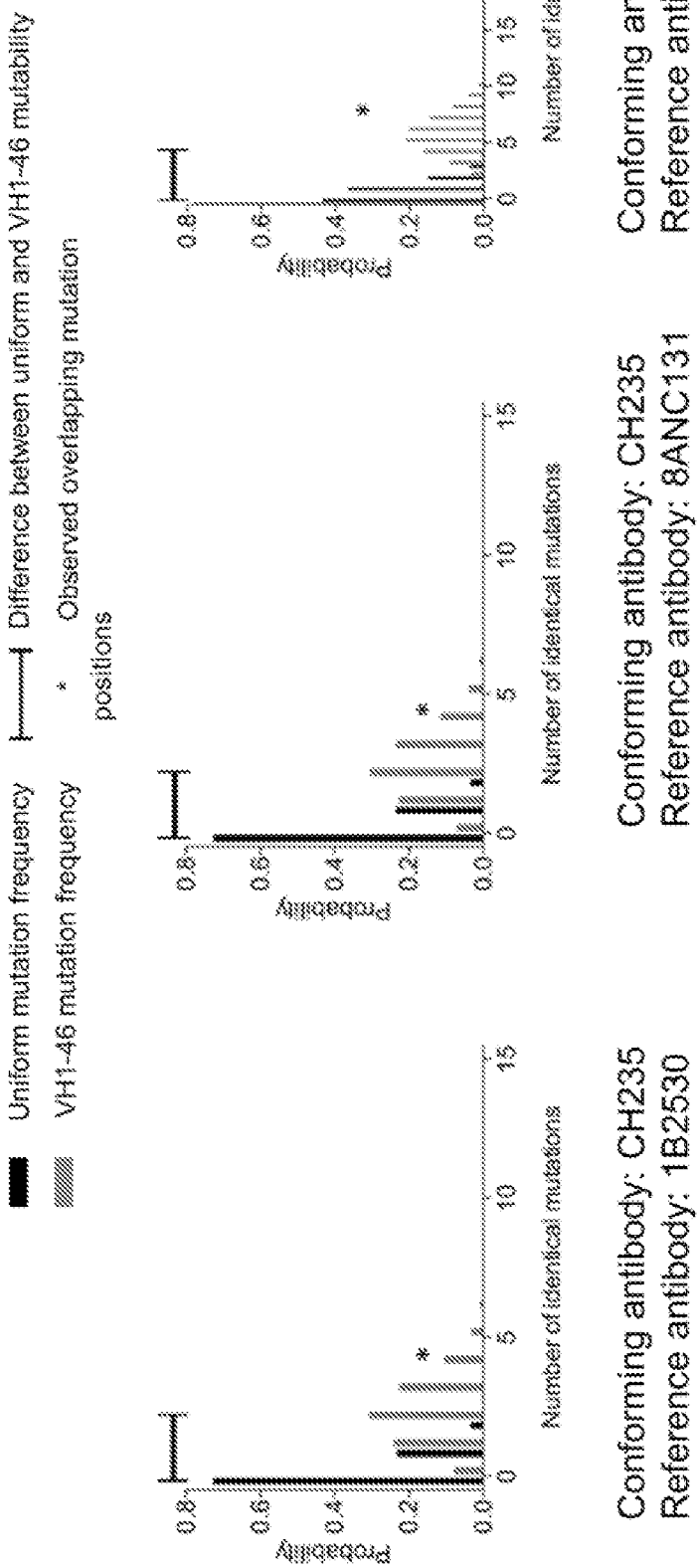

Uniform mutation frequency
VH1-46 mutation frequency
Difference between uniform and VH1-46 mutability
* Observed overlapping mutation positions
Probability
Number of identical mutations
Conforming antibody: CH235
Reference antibody: 1B2530
Conforming antibody: CH235
Reference antibody: 8ANC131
Conforming antibody: 1B2530
Reference antibody: 8ANC131

FIG. 38C

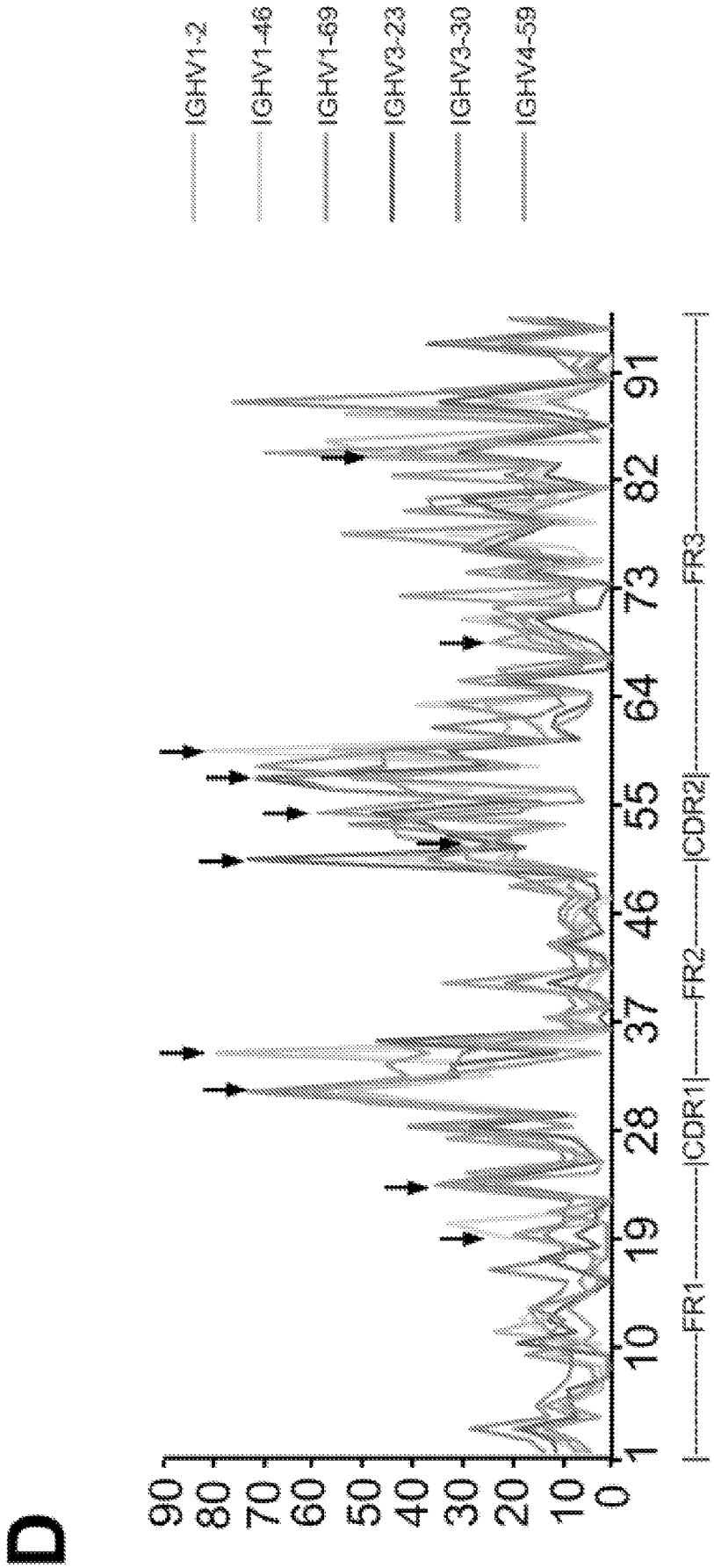

D
IGHV1-2
IGHV1-46
IGHV1-69
IGHV3-23
IGHV3-30
IGHV4-59
90
80
70
60
50
40
30
20
10
0
1
10
19
28
37
46
55
64
73
82
91
FR1
CDR1
FR2
CDR2
FR3

FIG. 37D

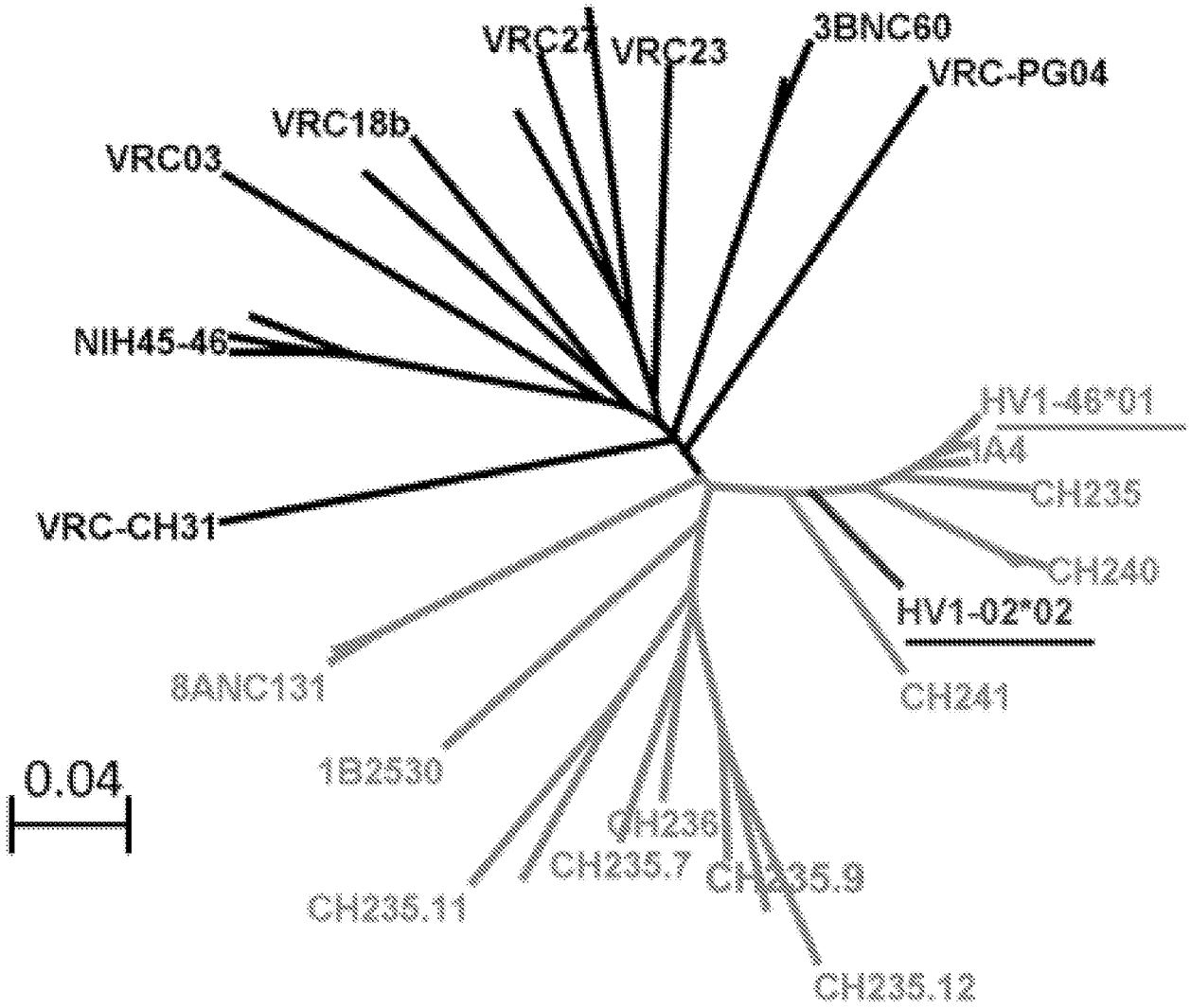

VRC23
3BNC60
VRC-PG04
VRC18b
VRC03
NIH45-46
HV1-46*01
IA4
CH235
CH240
HV1-02*02
VRC-CH31
CH241
8ANC131
1B2530
0.04
CH235.11
CH235.12

FIG. 38E

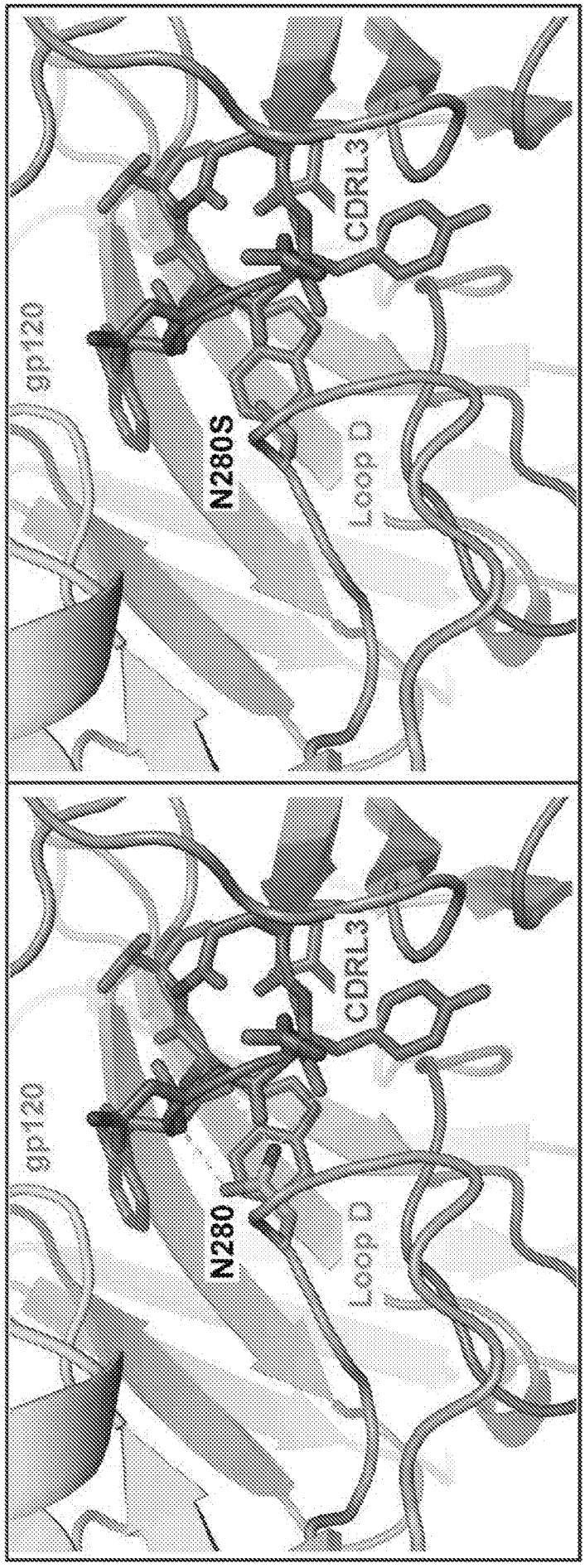


FIG. 39A

```
|----------FR1----------|--CDR1--|-------FR2-------|--CDR2--|--
                        *  *                      *********
CH2236_VH      QVQLVQSGAA VKRPGASVTI SCRASGYTFT TYYIHWVRQA PGQRLELMGM IDPSRGRTDY
CH2235.9_VH    QVRLLQYGGG VKRPGASMTI SCVASGYNFM DYYIHWVRQA PGQGLELMGW IDPSGGRTDY
CH2235.12_VH   QVRLAQYGGG VKRLGATMTL SCVASGYTFN DYYIHWVRQA PGQGFELLGY IDPANGRPDY
CH2235.13_VH   QVQLVQSGGG VKRPGSTTTI SCVASGYSFN DYYIHWVRQA PGQGLEVLGF IDPSNGRTNY
CH2235.10_VH   QVQLVQSGAT VKKPRASVTL SCRTSGYNFI DYFIHWVRRA PGQRLEVMGY IDPSRGRPDY
CH2235.11_VH   QVQLVQSGGT VKSPGTSVTL SCKTSGYNFI DYYIHWVRRA PGQRPELMGY IDPSHGRPDY
CH2235.7_VH    QVQLVQSGAA VKRPGASVTI SCRASGYTFT TYYIHWVRQA PGQGLELMGW INPRGGRTDY

|----------FR3----------|-----|-----CDR3-----|---FR4---|
**   *      *             *********
CH2236_VH      AQKFQGRVTM SRDTSTSTLY MELRSLRPDD TALYYCVRNV GTWGSLLHYD YWGQGTLVTVSS
CH2235.9_VH    AGAFGDRVSM YRDKSMNTLY MDLRSLRSGD TAMYYCVRNV GTAGSLLHYD HWGLGVMVTVSS
CH2235.12_VH   AGALRERLSF YRDKSMETLY MDLRSLRYDD TAMYYCVRNV GTAGSLLHYD HWGSGSPVIVSS
CH2235.13_VH   AGAFGDRFSM YRDKSMETLY MDLRNLRSDD TAMYYCVRNV GTAGSLLHYD HWGTGSKIIVSS
CH2235.10_VH   APNFRDRVSL YRDTSMSTVY LDLRDLTPDD TAIYYCVRSE GTEGTVLHYD HWGPGTRVTVSP
CH2235.11_VH   EGKFRDRISL YRDTSTSVVY MDVRGLRLDD TALYYCVRGG GVEVSSNHYD HWGPGTMVFVSP
CH2235.7_VH    SYRFEDRVSM YRDTSMSIVY MDLRNLKSAD TAVYYCVRNV GTSGSLLHYD FWGQGSLVTVSS
```

FIG. 39B

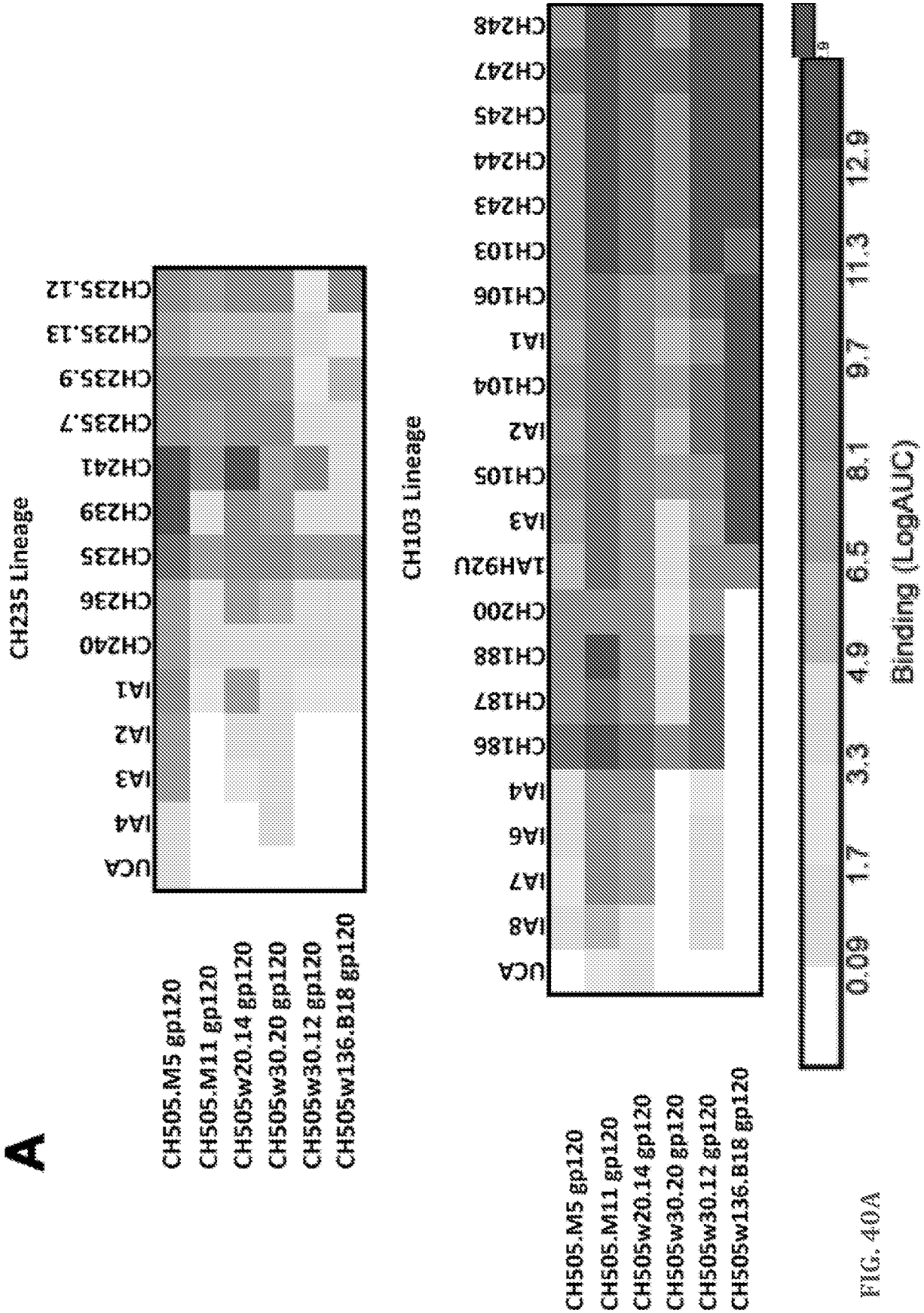

A
CH505.M5 gp120
CH505.M11 gp120
CH505w20.14 gp120
CH505w30.20 gp120
CH505w30.12 gp120
CH505w136.B18 gp120
CH235 Lineage
UCA
IA4
IA3
IA2
IA1
CH240
CH236
CH235
CH239
CH241
CH235.7
CH235.9
CH235.13
CH235.12
CH103 Lineage
UCA
IA8
IA7
IA6
IA4
CH186
CH187
CH188
CH200
IAH92U
IA3
CH105
IA2
CH104
IA1
CH106
CH103
CH243
CH244
CH245
CH247
CH248
0.09
1.7
3.3
4.9
6.5
8.1
9.7
11.3
12.9
Binding (LogAUC)

FIG. 40A

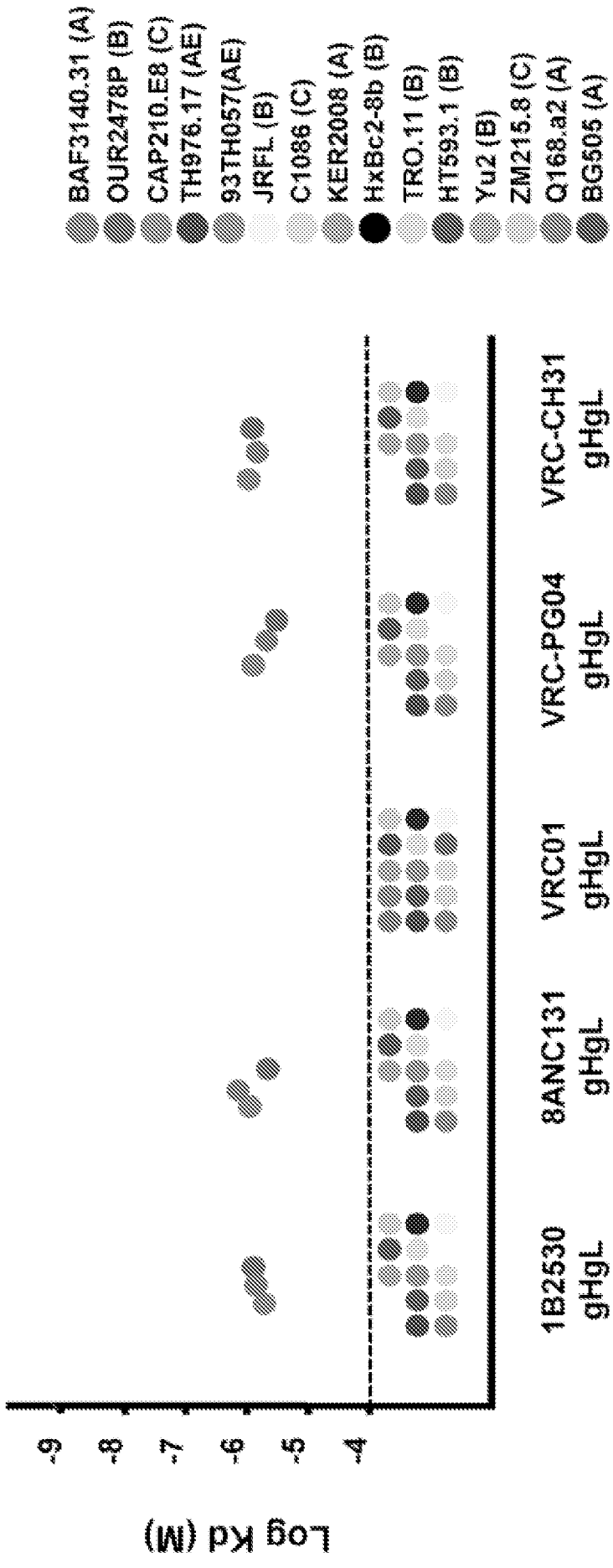

BAF3140.31 (A)
OUR2478P (B)
CAP210.E8 (C)
TH976.17 (AE)
93TH057(AE)
JRFL (B)
C1086 (C)
KER2008 (A)
HxBc2-8b (B)
TRO.11 (B)
HT593.1 (B)
Yu2 (B)
ZM215.8 (C)
Q168.a2 (A)
BG505 (A)
Log Kd (M)
-9
-8
-7
-6
-5
-4
1B2530 gHgL
VH 1-46
8ANC131 gHgL
VH 1-46
VRC01 gHgL
VH 1-2
VRC-PG04 gHgL
VH 1-2
VRC-CH31 gHgL
VH 1-2

FIG. 40B

Antibody ID	VH	D	JH	Mutation frequency	CDRH3 length
UCA	1-46*01	3-10*01	4*02	0.0%	15
CH235	1-46*01	3-10*01	4*02	7.9%	15
CH236	1-46*01	3-10*01	4*02	8.2%	15
CH239	1-46*01	3-10*01	4*02	7.9%	15
CH240	1-46*01	3-10*01	4*02	7.4%	15
CH241	1-46*01	3-10*01	4*02	11.5%	15
CH235.6	1-46*01	3-10*01	4*02	12.6%	15
CH235.7	1-46*01	3-10*01	4*02	14.8%	15
CH235.8	1-46*01	3-10*01	4*02	12.0%	15
CH235.9	1-46*01	3-10*01	4*02	19.6%	15
CH235.10	1-46*01	3-10*01	4*02	21.6%	15
CH235.11	1-46*01	3-10*01	4*02	25.1%	15
CH235.12	1-46*01	3-10*01	4*02	25.7%	15
CH235.13	1-46*01	3-10*01	4*02	23.5%	15

FIG. 40C

Antibody ID	VK	JK	Mutation frequency	CDRL3 length	Week of isolation
UCA	3-15*01	1*01	0.0%	8	-
CH235	3-15*01	1*01	3.8%	8	41
CH236	3-15*01	1*01	2.8%	8	41
CH239	3-15*01	1*01	4.7%	8	41
CH240	3-15*01	1*01	3.1%	8	41
CH241	3-15*01	1*01	3.5%	8	41
CH235.6	3-15*01	1*01	3.5%	8	66^
CH235.7	3-15*01	1*01	2.8%	8	100#
CH235.8	3-15*01	1*01	3.5%	8	100^
CH235.9	3-15*01	1*01	2.8%	8	152#
CH235.10	3-15*01	1*01	16.7%	8	264
CH235.11	3-15*01	1*01	17.6%	8	323
CH235.12	3-15*01	1*01	12.9%	8	323
CH235.13	3-15*01	1*01	11.6%	8	323

^ Paired with CH241 V-light chain and complemented with CH241 V-heavy.

Paired with CH236 V-light chain and complemented with CH236 V-heavy.

Mutation frequency is calculated on nucleotide sequences of the whole V(D)J rearrangement compared to UCA.

CDR H3 and CDR L3 lengths are expressed in amino acids.

FIG. 40C cont.

Virus ID	Clade	CH235	CH235.9	CH235.12	VRC01
0260.v5.c36	A	>50	10.5	1.02	0.468
0330.v4.c3	A	>50	1.88	0.313	0.047
0439.v5.c1	A	>50	3.49	0.374	0.129
3365.v2.c20	A	>50	1.29	0.068	0.030
3415.v1.c1	A	>50	3.20	0.450	0.084
3718.v3.c11	A	12.3	1.80	0.360	0.165
398-F1_F6_20	A	>50	5.48	1.76	0.181
BB201.B42	A	>50	7.20	0.573	0.316
BG505.W6M.C2	A	>50	0.823	0.111	0.053
BI369.9A	A	>50	1.95	0.290	0.224
BS208.B1	A	>50	1.77	0.263	0.022
KER2008.12	A	>50	>50	>50	0.591
KER2018.11	A	>50	9.89	2.52	0.555
KNH1209.18	A	>50	1.21	0.251	0.099
MB201.A1	A	>50	12.9	0.333	0.212
MB539.2B7	A	>50	11.7	1.71	0.500
MI369.A5	A	>50	2.64	0.416	0.269
MS208.A1	A	>50	2.77	0.463	0.178
Q23.17	A	1.35	0.405	0.132	0.052
Q259.17	A	>50	7.46	0.100	0.075
Q769.d22	A	>50	0.981	0.110	0.035
Q769.h5	A	>50	2.55	0.139	0.062
Q842.d12	A	8.15	0.378	0.091	0.038
QH209.14M.A2	A	>50	5.76	0.374	0.060
RW020.2	A	1.20	1.05	0.301	0.203
UG037.8	A	>50	1.10	0.188	0.089
246-F3.C10.2	AC		1.33		
3301.V1.C24	AC	20.9	1.91	0.473	0.097
3589.V1.C4	AC	>50	>50	0.309	0.047
6540.v4.c1	AC	>50	>50	>50	>50
6545.V4.C1	AC	>50	>50	>50	>50

FIG. 41

0815.V3.C3	ACD	>50	0.549	0.056	0.015
6095.V1.C10	ACD	>50	3.29	1.33	0.506
3468.V1.C12	AD	2.47	0.659	0.070	0.050
Q168.a2	AD	>50	1.10	0.261	0.098
Q461.e2	AD	>50	6.95	0.818	0.497
620345.c1	AE	>50	8.61	1.94	>50
BJOX009000.02.4	AE	>50	>50	5.50	1.54
BJOX010000.06.2	AE	>50	>50	10.6	6.79
BJOX025000.01.1	AE	40.6	0.586	0.271	8.46
BJOX028000.10.3	AE	>50	0.886	0.168	0.256
C1080.c3	AE	>50	13.3	2.69	2.10
C2101.c1	AE	12.6	3.37	0.261	0.179
C3347.c11	AE	>50	0.482	0.117	0.095
C4118.09	AE	3.30	1.04	0.084	0.248
CM244.ec1	AE	1.19		0.160	0.089
CNE3	AE	>50	>50	2.45	1.63
CNE5	AE	17.6	2.94	1.03	0.323
CNE55	AE	>50	1.90	0.400	0.359
CNE56	AE	42.9	2.96	1.10	0.343
CNE59	AE	13.6	3.79	0.943	0.623
CNE8	AE	>50	3.22	1.10	0.510
R1166.c1	AE	>50	34.4	0.758	3.00
R2184.c4	AE	5.82	6.83	0.563	0.133
R3265.c6	AE	>50	35.0	0.172	0.710
TH966.8	AE	0.732	1.70	0.304	0.284
TH976.17	AE	0.975	0.935	0.286	0.332
235-47	AG	>50	2.25	0.293	0.061
242-14	AG	>50	>50	2.83	>50
263-8	AG	>50	2.93	0.447	0.168

FIG. 41 cont.

Virus ID	Clade	CH235	CH235.9	CH235.12	VRC01
269-12	AG	>50	>50	>50	0.293
271-11	AG	>50	0.652	0.090	0.054
928-28	AG	>50	3.55	0.542	0.476
DJ263.8	AG	>50	2.90	0.276	0.066
T250-4	AG	>50	>50	>50	>50
T251-18	AG	>50	>50	4.02	4.42
T253-11	AG	>50	>50	1.65	0.501
T255-34	AG	>50	7.83	0.608	0.725
T257-31	AG	>50	13.3	2.66	2.47
T266-60	AG	>50	>50	10.3	2.37
T278-50	AG	>50	>50	>50	>50
T280-5	AG	>50	0.308	0.109	0.059
T33-7	AG	>50	0.469	0.039	0.019
3988.25	B	>50	3.49	0.917	0.369
5768.04	B	>50	3.75	0.715	0.354
6101.10	B	>50	3.14	0.467	0.023
6535.3	B	>50	>50	4.85	2.10
7165.18	B	>50	>50	>50	45.0
45_01dG5	B	>50	0.507	0.058	0.011
89.6.DG	B	>50	27.7	2.23	1.30
AC10.29	B	>50	9.55	2.13	1.41
ADA.DG	B	>50	2.88	0.907	0.563
Bal.01	B	>50	0.326	0.237	0.124
BaL.26	B	>50	1.10	0.214	0.060
BG1168.01	B	>50	4.06	1.42	0.738
BL01.DG	B	>50	>50	>50	>50
BR07.DG	B	>50	4.66	1.51	1.81
BX08.16	B	>50	>50	2.35	0.389
CAAN.A2	B	>50	7.47	2.23	0.963

FIG. 41 cont.

CNE10	B	>50	23.2	5.26	0.689
CNE12	B	>50	8.19	2.56	0.695
CNE14	B	>50	12.5	0.594	0.199
CNE4	B	>50	5.97	1.16	0.639
CNE57	B	>50	>50	1.25	0.496
HO86.8	B	>50	1.35	0.174	>50
HT593.1	B	>50	2.23	0.984	0.606
HXB2.DG	B	18.1	0.243	0.173	0.063
JRCSF.JB	B	>50	1.65	0.596	0.436
JRFL.JB	B	1.82	2.13	0.127	0.051
MN.3	B	>50	1.27	0.142	0.011
PVO.04	B	>50	3.53	1.47	0.552
QH0515.01	B	26.4	7.95	1.40	1.43
QH0692.42	B	>50	10.8	2.25	1.37
REJO.67	B	>50	>50	1.09	0.113
RHPA.7	B	16.6	0.300	0.091	0.051
SC422.8	B	>50	3.73	0.798	0.127
SF162.LS	B	>50	2.21	0.534	0.228
SS1196.01	B	>50	>50	0.827	0.246
THRO.18	B	>50	>50	>50	4.63
TRJO.58	B	>50	1.76	0.524	0.116
TRO.11	B	14.8	4.68	0.714	0.502
WITO.33	B	>50	3.65	0.418	0.140
X2278.C2.B6	B	>50	5.96	0.425	0.133
YU2.DG	B	>50	0.761	0.235	0.113
BJOX002000.03.2	BC	>50	2.74	0.739	>50
CH038.12	BC	>50	>50	17.3	0.519
CH070.1	BC	>50	>50	2.39	9.99
CH117.4	BC	>50	1.03	0.340	0.095
CH119.10	BC	>50	3.68	1.24	0.577
CH181.12	BC	>50	3.44	0.612	0.481

FIG. 41 cont.

Virus ID	Clade	CH235	CH235.9	CH235.12	VRC01
CNE15	BC	15.2	1.16	0.249	0.100
CNE19	BC	27.5	0.488	0.134	0.169
CNE20	BC	>50	1.09	0.254	9.25
CNE21	BC	>50	1.81	0.527	0.357
CNE40	BC	>50	0.477	0.207	0.370
CNE7	BC	>50	>50	1.36	0.286
286.36	C	>50	3.00	0.699	0.322
288.38	C	>50	3.62	1.62	1.49
0013095-2.11	C	>50	>50	29.7	0.088
001428-2.42	C	>50	0.417	0.087	0.008
0077_V1.C16	C	>50	41.7	6.84	1.28
00836-2.5	C	>50	>50	1.09	0.119
0921.V2.C14	C	10.9	1.76	0.344	0.182
16055-2.3	C	>50	0.768	0.159	0.063
16845-2.22	C	>50	28.0	7.47	3.60
16936-2.21	C	>50	1.85	0.500	0.110
25710-2.43	C	>50	0.983	0.382	0.594
25711-2.4	C	>50	4.57	0.974	0.555
25925-2.22	C	>50	2.51	0.641	0.474
26191-2.48	C	>50	1.65	0.583	0.166
3168.V4.C10	C	>50	6.56	0.372	0.255
3637.V5.C3	C	>50	10.5	12.2	1.45
3873.V1.C24	C	>50	>50	>50	0.791
6322.V4.C1	C	>50	4.74	0.944	>50
6471.V1.C16	C	>50	>50	>50	>50
6631.V3.C10	C	>50	>50	5.83	>50
6644.V2.C33	C	>50	>50	>50	0.243
6785.V5.C14	C	>50	>50	>50	0.286
6838.V1.C35	C	>50	4.54	1.08	0.210
96ZM651.02	C	>50	4.37	1.18	0.570

FIG. 41 cont.

BR025.9	C	>50	>50	>50	0.593
CAP210.E8	C	>50	>50	>50	>50
CAP244.D3	C	>50	13.2	1.52	1.33
CAP256.206.C9	C	14.6	3.73	1.32	0.971
CAP45.G3	C	>50	4.00	0.568	7.00
Ce1176.A3	C	>50	7.71	1.24	2.60
CE703010217.B6	C	>50	1.70	0.319	0.366
CNE30	C	>50	4.31	1.21	0.525
CNE31	C	>50	>50	2.78	0.786
CNE53	C	1.77	0.781	0.274	0.087
CNE58	C	>50	>50	1.95	0.225
DU123.06	C	>50	17.5	4.25	7.92
DU151.02	C	3.94	1.33	0.287	14.8
DU156.12	C	9.48	1.65	0.285	0.086
DU172.17	C	1.92	1.74	0.361	>50
DU422.01	C	>50	2.85	0.944	>50
MW965.26	C	6.10	3.03	0.573	0.029
SO18.18	C	>50	1.24	0.110	0.058
TV1.29	C	>50	11.0	4.63	>50
TZA125.17	C	>50	>50	>50	>50
TZBD.02	C	>50	38.9	0.219	0.078
ZA012.29	C	13.2	11.5	0.971	0.384
ZM106.9	C	>50	2.09	0.620	0.311
ZM109.4	C	>50	2.50	0.416	0.177
ZM135.10a	C	>50	>50	>50	2.25
ZM176.66	C	>50	1.21	0.183	0.083
ZM197.7	C	>50	10.5	1.40	0.428
ZM214.15	C	>50	10.1	2.22	0.893
ZM215.8	C	6.19	1.71	0.315	0.215
ZM233.6	C	5.71	5.02	1.25	1.02

IC50 (μg/ml)
<0.100
0.100-1.00
1.00-10.0
>10.0
>50

FIG. 41 cont.

Virus ID	Clade	CH235	CH235.9	CH235.12	VRC01
ZM249.1	C	9.99	0.598	0.273	0.057
ZM53.12	C	>50	4.44	0.558	0.625
ZM55.28a	C	>50	4.20	0.665	0.285
3326.V4.C3	CD	>50	1.54	0.114	0.068
3337.V2.C6	CD	>50	10.8	0.429	0.090
3817.v2.c59	CD	>50	14.6	3.63	>50
231965.c1	D	>50	>50	13.9	0.353
247-23	D	>50	3.32	0.691	1.84
3016.v5.c45	D	>50	>50	>50	0.155
57128.vrc15	D	>50	>50	6.59	>50
6405.v4.c34	D	>50	>50	>50	1.55
A03349M1.vrc4a	D	>50	7.54	4.08	4.10
A07412M1.vrc12	D	>50		0.351	0.082
NKU3006.ec1	D	4.61	1.29	0.466	0.596
P0402.c2.11	G		3.65		
P1981.C5.3	G	>50	>50	2.19	0.330
X1193.c1	G	>50	4.08	0.972	0.154
X1254.c3	G	>50	>50	1.98	0.059
X1632.S2.B10	G	>50	1.18	0.484	0.130
X2088.c9	G	>50	>50	>50	>50
X2131.C1.B5	G	>50	10.3	2.58	0.537
SIVmac251.30.SG3	NA	>50	>50	>50	>50
SVA.MLV	NA	>50	>50	>50	>50

	CH235	CH235.9	CH235.12	VRC01
# Viruses	202	202	202	202
Total VS Neutralized				
IC50 <50 µg/ml	35	153	179	179
IC50 <10 µg/ml	19	130	173	177
IC50 <1.0 µg/ml	2	25	115	146
IC50 <0.1 µg/ml	0	0	10	47
IC50 <0.01 µg/ml	0	0	0	1
% VS Neutralized				
IC50 <50 µg/ml	17	76	89	89

FIG. 41 cont.

Complex (antibody-gp120)	CH235-93TH057	CH235.9-93TH057	CH235.12-93TH057
PDB ID	5F9W	5F9O	5F96
Data collection			
Space group	$P3_2$	$P\ 2_1\ 2_1\ 2_1$	$P\ 2_1$
Cell dimensions			
a, *b*, *c* (Å)	123.4, 123.4, 127.3	63.5, 67.8, 225.6	53.7, 69.9, 127.3
α, *β*, *γ* (°)	90.0, 90.0, 120.0	90.0, 90.0, 90.0	90.0, 94.6, 90.0
Resolution (Å)	40.94-2.89 (2.99-2.89)*	50.0-1.86 (2.00-1.93; 1.93-1.86)	2.25 (2.29-2.25)
R_{sym} or R_{merge}	0.22 (0.68)	14.1 (41.8; 48.4)	12.5 (41.4)
$I/\sigma I$	8.9 (1.9)	7.06 (1.79; 1.25)	15.5 (2.1)
Completeness (%)	100 (100)	89.7 (73.4; 46.8)	97.6 (86.7)
Redundancy	7.6 (6.7)	3.4 (1.6; 1.2)	3.0 (2.1)
Refinement			
Resolution (Å)	40.9 – 2.89	35.8-1.86	34.5-2.25
No. reflections	48360	73935	43920
R_{work}/ R_{free}	17.5/22.9	20.4/22.0	18.3/23.0
No. atoms			
Protein	11932	6137	5976
Ligand/ion	393	53	213
Water	74	461	196
B-factors (Å^2)			
Protein	92.5	43.5	63.4
Ligand/ion	120.9	87.0	103.4
Water	77.5	47.2	59.3
R.m.s deviations			
Bond lengths (Å)	0.004	0.008	0.006
Bond angles (°)	0.78	1.27	0.92
Ramachandran statistics			
Favored (%)	96.0	97.1	97.0
Outliers (%)	0.1	0.0	0.1

*Values in parenthesis denote highest resolution shell.

FIG. 42

Conforming antibody	# mutations (*x*)	Reference antibody	# sharing mutation positions (*c*)	Probability of seeing *c* based on: Uniform distribution ($P_{uniform}$)	Probability of seeing *c* based on: VH1-46 mutation frequencies (P_{VH1-46})	$PVH1\text{-}46/P_{uniform}$
CH235	15	1B2530	11	0.00432	0.17751	41.1
CH235	15	8ANC131	13	0.00010	0.01744	174.4
1B2530	39	8ANC131	26	0.00001	0.04274	4274.0

FIG. 43A

Conforming antibody	# mutations (*x*)	Reference antibody	# identical mutations (*i*)	Probability of seeing *i* based on: Uniform distribution ($P_{uniform}$)	Probability of seeing *i* based on: VH1-46 mutation frequencies (P_{VH1-46})	$PVH1\text{-}46/P_{uniform}$
CH235	15	1B2530	4	0.00022	0.10433	474.2
CH235	15	8ANC131	4	0.00019	0.11546	607.7
1B2530	39	8ANC131	7	0.00001	0.14622	14622.0

FIG. 43B

	IGHV1-2	IGHV1-46	IGHV1-69	IGHV3-23	IGHV3-30
IGHV1-46	0.84				
IGHV1-69	0.74	0.74			
IGHV3-23	0.54	0.63	0.68		
IGHV3-30	0.53	0.57	0.62	0.83	
IGHV4-59	0.47	0.53	0.55	0.67	0.57

FIG. 43C

	UCA	IA4	IA3	IA2	IA1	CH240	CH236	CH235	CH239	CH241
sCD4	nb	nb	nb	nb	>100	nb	nb	26.3	92.6	2.6
CH106	nb	nb	nb	nb	82.4	68.9	82.2	16.5	45.4	1.5

FIG. 44A

	CH235	CH236	CH239	CH240	CH241	CH106
CH106	4.3	6.8	4.6	2.3	14.3	2.5

FIG. 44B

		Neutralization IC50, µg/ml			
Virus ID	**Week**	**UCA**	**IA4**	**IA3**	**CH235.11**
T/F		>50	>50	**5.22**	**2.19**
CH505.w4.10	4	>50	**20.51**	**1.02**	**0.12**
CH505.w4.26	4	>50	>50	**3.67**	**1.59**
CH505.w4.3	4	>50	>50	**1.65**	**9.58**
CH505.w8.e12	14	>50	>50	**34.99**	**0.06**
CH505.w8.e21	14	>50	>50	>50	**5.52**
CH505.w8.e29	14	>50	>50	**5.34**	**6.19**
CH505.w8.e34	14	>50	>50	**4.84**	**7.07**
CH505.w8.e6	14	>50	>50	**5.73**	**17.42**
CH505.w8.e3	14	>50	>50	**6.28**	**27.80**
CH505.w8.e4	14	>50	>50	**3.60**	**35.26**
CH505.w12.e4	20	>50	>50	>50	**2.66**
CH505.w12.e19	20	>50	>50	**8.99**	>50
CH505.w12.e25	20	>50	>50	**8.54**	>50
CH505.w12.e27	20	>50	>50	**10.28**	>50
CH505.w24.e5	30	>50	>50	>50	**2.08**
CH505.w24.e34	30	>50	>50	>50	**2.18**
CH505.w24.e37	30	>50	>50	>50	**3.56**
CH505.w24.e24	30	>50	>50	**29.77**	>50
CH505.w24.e28	30	>50	>50	>50	>50
CH505.w24.e13	30	>50	>50	>50	>50
CH505.w48.e6	53	>50	>50	>50	>50
CH505.w48.e22	53	>50	>50	>50	>50
CH505.w48.e13	53	>50	>50	>50	>50
CH505.w48.e28	53	>50	>50	>50	>50
CH505.w48.e11	53	>50	>50	>50	>50
CH505.w48.e31	53	>50	>50	>50	>50
CH505.w96.A5	100	>50	>50	>50	>50
CH505.w96.B8	100	>50	>50	>50	>50
CH505.w96.B6	100	>50	>50	>50	>50
CH505.w96.A10	100	>50	>50	>50	>50
CH505.w96.A3	100	>50	>50	>50	>50
CH505.w96.B4	100	>50	>50	>50	>50
CH0505.C.w136.e.B23	136	>50	>50	>50	>50
CH0505.C.w136.e.B18	136	>50	>50	>50	>50
CH0505.C.w136.e.B24	136	>50	>50	>50	>50

FIG. 45

Neutralization IC50, µg/ml

Virus ID	IA2	IA1	CH240	CH236	CH235
T/F	**0.97**	**0.91**	**0.94**	**0.61**	**0.63**
CH505.w4.10	**0.24**	**0.20**	**0.15**	**0.10**	**0.10**
CH505.w4.26	**1.36**	**0.32**	**0.67**	**0.78**	**0.46**
CH505.w4.3	**0.35**	**0.30**	**0.25**	**0.40**	**0.11**
CH505.w8.e12	**2.22**	**0.85**	**0.93**	**0.53**	**0.42**
CH505.w8.e21	**38.08**	**0.79**	**0.58**	**0.96**	**0.38**
CH505.w8.e29	**1.02**	**0.86**	**0.88**	**1.05**	**0.75**
CH505.w8.e34	**0.94**	**0.84**	**0.89**	**1.09**	**0.52**
CH505.w8.e6	**1.64**	**0.92**	**0.73**	**0.63**	**0.31**
CH505.w8.e3	**1.40**	**0.79**	**1.05**	**0.75**	**0.49**
CH505.w8.e4	**1.00**	**0.72**	**0.60**	**0.88**	**0.27**
CH505.w12.e4	**47.61**	**0.94**	**1.00**	**1.81**	**0.65**
CH505.w12.e19	**2.12**	**1.99**	**1.63**	**1.24**	**1.16**
CH505.w12.e25	**1.69**	**1.85**	**1.41**	**0.85**	**1.08**
CH505.w12.e27	**2.06**	**0.98**	**1.13**	**0.46**	**0.39**
CH505.w24.e5	**7.72**	**1.83**	**2.08**	**0.58**	**0.80**
CH505.w24.e34	**4.98**	**5.18**	**5.61**	**2.27**	**3.23**
CH505.w24.e37	**6.45**	**6.92**	**7.57**	**2.60**	**4.25**
CH505.w24.e24	**27.95**	**4.62**	**4.12**	**3.69**	**2.06**
CH505.w24.e28	>50	**5.28**	**5.28**	**9.14**	**2.16**
CH505.w24.e13	>50	**4.73**	**5.11**	**4.39**	**1.15**
CH505.w48.e6	>50	>50	>50	>50	>50
CH505.w48.e22	>50	>50	>50	>50	>50
CH505.w48.e13	>50	>50	>50	>50	>50
CH505.w48.e28	>50	>50	>50	>50	>50
CH505.w48.e11	>50	>50	>50	>50	>50
CH505.w48.e31	>50	>50	>50	>50	>50
CH505.w96.A5	>50	>50	>50	>50	>50
CH505.w96.B8	>50	>50	>50	>50	>50
CH505.w96.B6	>50	>50	>50	>50	>50
CH505.w96.A10	>50	>50	>50	>50	>50
CH505.w96.A3	>50	>50	>50	>50	>50
CH505.w96.B4	>50	>50	>50	>50	>50
CH0505.C.w136.e.B23	>50	>50	>50	>50	>50
CH0505.C.w136.e.B18	>50	>50	>50	>50	>50
CH0505.C.w136.e.B24	>50	>50	>50	>50	>50

FIG. 45 cont.

	Neutralization IC50, µg/ml				
Virus ID	**CH239**	**CH241**	**CH235.7**	**CH235.13**	**CH235.10**
T/F	**0.48**	**0.14**	**0.39**	**0.91**	**0.22**
CH505.w4.10	**<0.02**	**<0.02**	**0.07**	**0.17**	**0.10**
CH505.w4.26	**0.30**	**0.05**	**0.09**	**0.16**	**0.04**
CH505.w4.3	**0.10**	**<0.02**	**0.06**	**0.15**	**0.11**
CH505.w8.e12	**0.41**	**0.09**	**0.07**	**0.18**	**0.02**
CH505.w8.e21	**0.14**	**0.04**	**0.10**	**0.17**	**0.24**
CH505.w8.e29	**0.61**	**0.17**	**0.17**	**0.35**	**0.15**
CH505.w8.e34	**0.78**	**0.12**	**0.14**	**0.31**	**0.12**
CH505.w8.e6	**0.43**	**0.17**	**0.21**	**0.34**	**0.21**
CH505.w8.e3	**0.40**	**0.09**	**0.19**	**0.27**	**0.24**
CH505.w8.e4	**0.40**	**0.08**	**0.20**	**0.34**	**0.13**
CH505.w12.e4	**0.18**	**0.06**	**0.08**	**0.19**	**0.18**
CH505.w12.e19	**1.11**	**0.27**	**0.29**	**0.47**	**0.24**
CH505.w12.e25	**0.75**	**0.18**	**0.29**	**0.37**	**0.23**
CH505.w12.e27	**0.27**	**0.10**	**0.16**	**0.30**	**0.12**
CH505.w24.e5	**0.67**	**1.17**	**0.35**	**0.38**	**0.44**
CH505.w24.e34	**3.02**	**3.68**	**0.74**	**0.60**	**0.38**
CH505.w24.e37	**2.79**	**3.99**	**0.94**	**1.19**	**0.43**
CH505.w24.e24	**1.54**	**0.49**	**0.64**	**0.72**	**0.43**
CH505.w24.e28	**2.07**	**0.40**	**0.11**	**0.53**	**45.05**
CH505.w24.e13	**1.49**	**2.96**	**0.16**	**0.39**	>50
CH505.w48.e6	**19.95**	>50	**1.76**	**2.66**	**3.69**
CH505.w48.e22	>50	>50	**12.55**	**5.43**	**3.06**
CH505.w48.e13	**33.78**	>50	**27.04**	**5.03**	**2.42**
CH505.w48.e28	>50	>50	**25.29**	**5.08**	**2.58**
CH505.w48.e11	>50	>50	**34.23**	**3.26**	**3.10**
CH505.w48.e31	>50	>50	>50	**25.92**	**1.53**
CH505.w96.A5	>50	>50	>50	>50	>50
CH505.w96.B8	>50	>50	>50	>50	>50
CH505.w96.B6	>50	>50	>50	>50	>50
CH505.w96.A10	>50	>50	>50	>50	>50
CH505.w96.A3	>50	**45.37**	>50	>50	>50
CH505.w96.B4	**48.62**	**41.17**	>50	>50	>50
CH0505.C.w136.e.B23	>50	>50	>50	**29.73**	**11.41**
CH0505.C.w136.e.B18	>50	>50	>50	**32.27**	**27.81**
CH0505.C.w136.e.B24	>50	>50	>50	>50	**43.03**

FIG. 45 cont.

	Neutralization IC50, µg/ml	
Virus ID	**CH235.9**	**CH235.12**
T/F	**0.51**	**0.22**
CH505.w4.10	**0.08**	**<0.02**
CH505.w4.26	**0.16**	**0.03**
CH505.w4.3	**0.11**	**<0.02**
CH505.w8.e12	**0.08**	**<0.02**
CH505.w8.e21	**0.14**	**<0.02**
CH505.w8.e29	**0.21**	**0.06**
CH505.w8.e34	**0.16**	**0.05**
CH505.w8.e6	**0.30**	**0.09**
CH505.w8.e3	**0.25**	**0.11**
CH505.w8.e4	**0.27**	**0.06**
CH505.w12.e4	**0.08**	**<0.02**
CH505.w12.e19	**0.27**	**0.05**
CH505.w12.e25	**0.38**	**0.07**
CH505.w12.e27	**0.15**	**0.05**
CH505.w24.e5	**0.26**	**0.07**
CH505.w24.e34	**0.62**	**0.12**
CH505.w24.e37	**0.70**	**0.19**
CH505.w24.e24	**0.67**	**0.17**
CH505.w24.e28	**0.37**	**0.17**
CH505.w24.e13	**0.46**	**0.10**
CH505.w48.e6	**1.25**	**0.46**
CH505.w48.e22	**1.30**	**0.58**
CH505.w48.e13	**1.39**	**0.48**
CH505.w48.e28	**1.23**	**0.66**
CH505.w48.e11	**2.48**	**0.75**
CH505.w48.e31	**1.44**	**0.11**
CH505.w96.A5	>50	**3.68**
CH505.w96.B8	>50	**3.02**
CH505.w96.B6	>50	**8.35**
CH505.w96.A10	>50	**19.82**
CH505.w96.A3	>50	>50
CH505.w96.B4	>50	>50
CH0505.C.w136.e.B23	**10.23**	**1.75**
CH0505.C.w136.e.B18	**12.41**	**3.33**
CH0505.C.w136.e.B24	**13.58**	**4.09**

FIG. 45 cont.

		Neutralization IC50, µg/ml			
Virus ID	**Week**	**UCA**	**IA4**	**IA3**	**CH235.11**
CH0505.C.w136.e.B33	136	>50	>50	>50	>50
CH0505.C.w136.e.B38	136	>50	>50	>50	>50
CH0505.C.w136.e.B2	136	>50	>50	>50	>50
CH0505.C.w136.e.B3	136	>50	>50	>50	>50
CH0505.C.w136.e.B4	136	>50	>50	>50	>50
CH0505.C.w136.e.B5	136	>50	>50	>50	>50
CH0505.C.w136.e.B12	136	>50	>50	>50	>50
CH0505.C.w136.e.B27	136	>50	>50	>50	>50
CH0505.C.w160.C2	160	>50	>50	>50	>50
CH0505.C.w24.C9	160	>50	>50	>50	>50
CH0505.C.w24.C10	160	>50	>50	>50	>50
CH0505.C.w24.C11	160	>50	>50	>50	>50
CH0505.C.w24.C12	160	>50	>50	>50	>50
CH0505.C.w24.D2	160	>50	>50	>50	>50
CH0505.C.w24.T3	160	>50	>50	>50	>50
CH0505.w176.e11	176	>50	>50	>50	>50
CH0505.w176.e12	176	>50	>50	>50	>50
CH0505.w176.e13	176	>50	>50	>50	>50
CH0505.w176.e1	176	>50	>50	>50	>50
CH0505.w176.e2	176	>50	>50	>50	>50
CH0505.w176.e10	176	>50	>50	>50	>50
CH0505.w233.e1	233	>50	>50	>50	>50
CH0505.w233.e3	233	>50	>50	>50	>50
CH0505.w233.e4	233	>50	>50	>50	>50
CH0505.w233.e7	233	>50	>50	>50	>50
CH0505.w233.e17	233	>50	>50	>50	>50
CH0505.w233.e18	233	>50	>50	>50	>50
CH0505.w258.e4	258	>50	>50	**34.10**	>50
CH0505.w258.e1	258	>50	>50	>50	>50
CH0505.w258.e7	258	>50	>50	>50	>50
CH0505.w258.e6	258	>50	>50	>50	>50
CH0505.w258.e5	258	>50	>50	>50	>50
CH0505.w323.e1	323	>50	>50	>50	>50
CH0505.w323.e11	323	>50	>50	>50	>50
CH0505.w323.e13	323	>50	>50	>50	>50
CH0505.w323.e14	323	>50	>50	>50	>50
CH0505.w323.e15	323	>50	>50	>50	>50
CH0505.w323.e16	323	>50	>50	>50	>50
CH0505.w323.e17	323	>50	>50	>50	>50
CH0505.w323.e18	323	>50	>50	>50	>50

FIG. 45 cont.

	Neutralization IC50, µg/ml				
	IA2	IA1	CH240	CH236	CH235
CH0505.C.w136.e.B33	>50	>50	>50	>50	>50
CH0505.C.w136.e.B38	>50	>50	>50	>50	>50
CH0505.C.w136.e.B2	>50	>50	>50	>50	>50
CH0505.C.w136.e.B3	>50	>50	>50	>50	>50
CH0505.C.w136.e.B4	>50	>50	>50	>50	>50
CH0505.C.w136.e.B5	>50	>50	>50	>50	>50
CH0505.C.w136.e.B12	>50	>50	>50	>50	>50
CH0505.C.w136.e.B27	>50	>50	>50	>50	>50
CH0505.C.w160.C2	>50	>50	>50	>50	>50
CH0505.C.w24.C9	>50	>50	>50	>50	>50
CH0505.C.w24.C10	>50	>50	>50	>50	>50
CH0505.C.w24.C11	>50	>50	>50	>50	>50
CH0505.C.w24.C12	>50	>50	>50	>50	>50
CH0505.C.w24.D2	>50	>50	>50	>50	>50
CH0505.C.w24.T3	>50	>50	>50	>50	>50
CH0505.w176.e11	>50	>50	>50	>50	>50
CH0505.w176.e12	>50	>50	>50	>50	>50
CH0505.w176.e13	>50	>50	>50	>50	>50
CH0505.w176.e1	>50	>50	>50	>50	>50
CH0505.w176.e2	>50	>50	>50	>50	>50
CH0505.w176.e10	>50	>50	>50	>50	>50
CH0505.w233.e1	>50	>50	>50	>50	>50
CH0505.w233.e3	>50	>50	>50	>50	>50
CH0505.w233.e4	>50	>50	>50	>50	>50
CH0505.w233.e7	>50	>50	>50	>50	>50
CH0505.w233.e17	>50	>50	>50	>50	>50
CH0505.w233.e18	>50	>50	>50	>50	>50
CH0505.w258.e4	>50	>50	>50	>50	>50
CH0505.w258.e1	>50	>50	>50	>50	>50
CH0505.w258.e7	>50	>50	>50	>50	>50
CH0505.w258.e6	>50	>50	>50	>50	>50
CH0505.w258.e5	>50	>50	>50	>50	>50
CH0505.w323.e1	>50	>50	>50	>50	>50
CH0505.w323.e11	>50	>50	>50	>50	>50
CH0505.w323.e13	>50	>50	>50	>50	>50
CH0505.w323.e14	>50	>50	>50	>50	>50
CH0505.w323.e15	>50	>50	>50	>50	>50
CH0505.w323.e16	>50	>50	>50	>50	>50
CH0505.w323.e17	>50	>50	>50	>50	>50
CH0505.w323.e18	>50	>50	>50	>50	>50

FIG. 45 cont.

	Neutralization IC50, µg/ml				
	CH239	**CH241**	**CH235.7**	**CH235.13**	**CH235.10**
CH0505.C.w136.e.B33	>50	>50	>50	>50	**32.79**
CH0505.C.w136.e.B38	>50	>50	>50	>50	**32.68**
CH0505.C.w136.e.B2	>50	>50	>50	>50	>50
CH0505.C.w136.e.B3	>50	>50	>50	>50	>50
CH0505.C.w136.e.B4	>50	>50	>50	>50	>50
CH0505.C.w136.e.B5	>50	>50	>50	>50	>50
CH0505.C.w136.e.B12	>50	>50	>50	>50	>50
CH0505.C.w136.e.B27	>50	>50	>50	>50	>50
CH0505.C.w160.C2	>50	>50	>50	>50	>50
CH0505.C.w24.C9	>50	>50	>50	>50	>50
CH0505.C.w24.C10	>50	>50	>50	>50	>50
CH0505.C.w24.C11	>50	>50	>50	>50	>50
CH0505.C.w24.C12	>50	>50	>50	>50	>50
CH0505.C.w24.D2	>50	>50	>50	>50	>50
CH0505.C.w24.T3	>50	>50	>50	>50	>50
CH0505.w176.e11	>50	>50	>50	>50	>50
CH0505.w176.e12	>50	>50	>50	>50	>50
CH0505.w176.e13	>50	>50	>50	>50	>50
CH0505.w176.e1	>50	>50	>50	>50	>50
CH0505.w176.e2	>50	>50	>50	>50	>50
CH0505.w176.e10	>50	>50	>50	>50	>50
CH0505.w233.e1	>50	>50	>50	>50	>50
CH0505.w233.e3	>50	>50	>50	>50	>50
CH0505.w233.e4	>50	>50	>50	>50	>50
CH0505.w233.e7	>50	>50	>50	>50	>50
CH0505.w233.e17	>50	>50	>50	>50	>50
CH0505.w233.e18	>50	>50	>50	>50	>50
CH0505.w258.e4	>50	>50	>50	>50	>50
CH0505.w258.e1	>50	>50	>50	>50	>50
CH0505.w258.e7	>50	>50	>50	>50	>50
CH0505.w258.e6	>50	>50	>50	>50	>50
CH0505.w258.e5	>50	>50	>50	>50	>50
CH0505.w323.e1	>50	>50	>50	>50	>50
CH0505.w323.e11	>50	>50	>50	>50	>50
CH0505.w323.e13	>50	>50	>50	>50	>50
CH0505.w323.e14	>50	>50	>50	>50	>50
CH0505.w323.e15	>50	>50	>50	>50	>50
CH0505.w323.e16	>50	>50	>50	>50	>50
CH0505.w323.e17	>50	>50	>50	>50	>50
CH0505.w323.e18	>50	>50	>50	>50	>50

FIG. 45 cont.

	Neutralization IC50, µg/ml	
	CH235.9	**CH235.12**
CH0505.C.w136.e.B33	**16.05**	**3.62**
CH0505.C.w136.e.B38	**16.17**	**3.88**
CH0505.C.w136.e.B2	**28.62**	**5.95**
CH0505.C.w136.e.B3	>50	>50
CH0505.C.w136.e.B4	>50	>50
CH0505.C.w136.e.B5	>50	>50
CH0505.C.w136.e.B12	>50	>50
CH0505.C.w136.e.B27	>50	>50
CH0505.C.w160.C2	>50	>50
CH0505.C.w24.C9	>50	>50
CH0505.C.w24.C10	>50	>50
CH0505.C.w24.C11	>50	>50
CH0505.C.w24.C12	>50	>50
CH0505.C.w24.D2	>50	>50
CH0505.C.w24.T3	>50	>50
CH0505.w176.e11	>50	**15.43**
CH0505.w176.e12	>50	**26.20**
CH0505.w176.e13	>50	**30.25**
CH0505.w176.e1	>50	**35.54**
CH0505.w176.e2	>50	**26.11**
CH0505.w176.e10	>50	>50
CH0505.w233.e1	>50	>50
CH0505.w233.e3	>50	>50
CH0505.w233.e4	>50	>50
CH0505.w233.e7	>50	>50
CH0505.w233.e17	>50	>50
CH0505.w233.e18	>50	>50
CH0505.w258.e4	>50	>50
CH0505.w258.e1	>50	>50
CH0505.w258.e7	>50	>50
CH0505.w258.e6	>50	>50
CH0505.w258.e5	>50	>50
CH0505.w323.e1	>50	>50
CH0505.w323.e11	>50	>50
CH0505.w323.e13	>50	>50
CH0505.w323.e14	>50	>50
CH0505.w323.e15	>50	>50
CH0505.w323.e16	>50	>50
CH0505.w323.e17	>50	>50
CH0505.w323.e18	>50	>50

FIG. 45 cont.

Neutralization IC50, µg/ml

Virus ID	Virus Mutations	CH236	CH235.7	CH235.10	CH235.11	CH235.13	CH235.12
CH505.TF	-	**0.61**	**0.39**	**0.22**	**2.19**	**0.91**	**0.22**
CH505.TF.M5	N279K	**0.26**	**0.16**	**0.17**	**0.31**	**0.18**	**0.02**
CH505.TF.M6	V281A	**0.80**	**0.34**	**0.11**	**2.30**	**0.30**	**0.08**
CH505.TF.M10	V281G	**1.75**	**0.18**	**0.19**	**1.52**	**0.14**	**0.02**
CH505.TF.M19	V281D	**7.53**	**0.40**	>50	>50	**0.57**	**0.17**
CH505.TF.M11	N279D+V281A	>50	**0.50**	**0.21**	**15.35**	**0.25**	**0.08**
CH505.TF.M8	N280S+V281A	>50	>50	>50	**1.91**	**0.86**	**0.05**
CH505.TF.M21	N280T+V281A	>50	>50	>50	**10.16**	**16.72**	**0.13**
CH505.TF.M20	N280S+V281G	>50	>50	>50	**7.86**	**11.58**	**0.07**
CH505.TF.M7	E275K+N279D+V281S	>50	>50	**0.61**	>50	**2.12**	**0.13**
CH505.TF.M9	E275K+N279D+V281G	>50	>50	**0.24**	**0.46**	**5.02**	**0.11**

FIG. 46

Neutralization IC50, μg/ml

Virus ID	Virus Mutations	CH235.9	CH235.9 N30T	CH235.9 D31T	CH235.9 G62Q	CH235.9 G65Q	G62Q+ G65Q	CH235.9 A103E
CH505.TF	-	0.51	4.26	0.43	0.30	0.26	0.15	0.43
CH505.TF.M5	N279K	0.19	0.90	0.11	0.12	0.14	0.12	0.03
CH505.TF.M6	V281A	0.40	1.06	0.12	0.02	0.08	0.04	0.16
CH505.TF.M10	V281G	0.24	0.83	0.07	0.02	0.09	0.08	0.07
CH505.TF.M19	V281D	0.63	4.04	0.89	1.36	1.58	1.54	0.43
CH505.TF.M11	N279D+V281A	0.39	1.25	0.12	0.02	0.14	0.13	0.24
CH505.TF.M8	N280S+V281A	0.66	24.31	0.15	0.14	0.12	0.03	0.13
CH505.TF.M21	N280T+V281A	0.74	>50	0.23	0.15	0.25	0.27	0.19
CH505.TF.M20	N280S+V281G	0.94	44.29	0.08	0.11	0.26	0.26	0.10
CH505.TF.M7	E275K+N279D+V281S	0.77	44.21	0.38	0.37	0.49	0.38	0.19
CH505.TF.M9	E275K+N279D+V281G	0.85	42.75	0.25	0.25	0.24	0.14	0.14

FIG. 46 cont.

CH235 Lineage

	Envelope ID	CA	A4	A3	A2	CH240	CH236	CH239	A1	CH235.7	CH235.10	CH235.11	CH235.13
	CH0505_CON D7gp120/293i	0.00	0.00	5.09	4.77	5.26	6.89	8.74	6.89	9.64	7.23	0.26	6.23
Week 4	CH505w004.10D8gp120/293F	0.26	0.00	6.55	6.25	6.47	8.10	12.11	8.53	10.31	2.64	1.17	7.63
	CH505.w4.26D8gp120 /293F	0.00	0.35	4.65	4.41	4.66	6.71	8.39	6.77	9.78	6.40	0.24	5.67
	505.s.03.D8.gp120/293F	0.00	0.00	5.13	5.03	5.33	6.90	8.82	7.39	10.68	7.20	0.36	6.32
Week 14	CH505w014.8D8gp120	0.00	0.16	3.13	2.68	3.19	5.93	6.98	5.49	8.17	4.48	0.00	4.62
	CH505w014.2D8gp120/293F	0.00	0.00	3.49	3.01	3.53	5.87	7.27	5.82	8.66	5.49	0.12	5.52
	CH505w014.32D8gp120/293F	0.00	0.00	4.72	4.51	4.58	6.47	8.39	6.63	9.68	6.33	0.26	5.57
	CH505w014.3D8gp120	0.00	0.00	4.25	4.12	4.57	6.02	7.83	6.56	9.61	6.63	0.31	6.24
	CH505.08.D11gp120/293F	0.00	0.00	3.45	3.01	3.24	5.00	7.20	5.52	7.81	4.07	0.00	4.43
	CH505w014.10D8gp120	0.00	0.00	0.00	0.00	1.92	4.24	9.83	5.57	8.95	0.85	0.26	5.89
	CH505w014.21D8gp120/293F	0.00	0.00	0.00	0.00	3.00	4.89	11.21	6.59	9.95	1.29	0.26	5.81

FIG. 47

		CH235 Lineage											
	Envelope ID	UCA	IA4	IA3	IA2	CH240	CH236	CH239	IA1	CH235.7	CH235.10	CH235.11	CH235.13
Week 20	CH505w020.15D8gp120	0.00	0.14	4.94	5.35	4.80	6.82	8.52	6.84	9.90	6.79	0.38	6.28
	CH505w020.13D8gp120	0.08	0.00	3.87	3.56	3.49	5.68	7.15	5.85	8.93	6.30	0.30	5.54
	CH505w020.22D8gp120/293F	0.00	0.00	4.68	4.24	2.92	6.70	8.31	6.55	10.37	6.77	0.33	6.01
	CH505w020.14D8gp120	0.00	0.00	2.72	1.22	3.83	6.72	9.01	6.52	9.35	5.63	0.11	6.14
	CH505w020.8D8gp120/293F	0.00	0.00	2.16	2.33	3.17	6.27	9.38	6.04	9.54	4.73	0.22	5.19
	CH505w020.3D8gp120	0.00	0.00	2.00	0.76	2.32	5.80	8.18	5.32	8.34	3.80	0.10	4.93
	CH505w020.30D8gp120	0.00	0.00	1.40	0.52	1.85	4.50	7.51	4.72	7.63	4.07	0.09	4.36
	CH505w020.23D8gp120	0.00	0.00	2.57	1.09	2.85	4.95	8.93	5.89	8.65	4.12	0.09	5.03
	CH505w020.11D8gp120	0.00	0.00	2.21	1.59	2.11	4.89	6.14	4.40	7.04	2.77	0.00	3.98
	CH505w020.9D8gp120	0.00	0.00	1.67	0.52	2.20	5.60	7.81	5.02	7.88	2.86	0.07	3.99
	CH505w020.4D8gp120/293F	0.00	0.00	0.00	1.69	2.64	5.67	10.28	7.07	9.92	1.82	0.39	6.33
	CH505w020.7D8gp120	0.00	0.00	0.00	0.00	3.06	5.38	10.27	6.45	9.47	1.36	0.27	6.63
	CH505w020.26D8gp120	0.00	0.00	0.00	0.00	1.64	4.14	9.23	5.37	7.96	0.56	0.11	4.53

FIG. 47 cont.

CH235 Lineage

	Envelope ID	UCA	M4	M3	M2	CH240	CH236	CH239	M1	CH235.7	CH235.10	CH235.11	CH235.13
Week 30	CH505w030.6D8gp120/293F	0.44	0.00	0.09	1.44	2.06	6.61	8.00	5.31	8.45	2.28	0.19	4.49
	CH505w030.36D8gp120	0.56	0.00	1.03	0.55	1.21	4.82	5.51	3.43	7.60	3.34	0.28	4.53
	CH505.w30.12D8gp140	0.32	0.00	0.00	0.10	0.00	0.54	2.04	1.02	2.26	0.08	0.14	0.97
	CH505w030.20D8gp120/293F	0.00	0.24	1.16	0.53	1.53	6.23	8.02	4.82	8.82	5.62	0.69	5.41
	CH505w030.27D8gp120/293F	0.08	0.00	0.46	4.14	3.68	6.94	7.84	6.28	9.09	5.63	1.33	5.99
	CH505w030.10D8gp120	0.00	0.00	0.10	0.00	0.32	1.63	3.84	2.21	5.42	0.00	0.00	1.50
	CH505w030.13D8gp120/293F	0.00	0.00	0.11	0.00	0.34	1.78	4.03	2.67	6.05	0.00	0.00	1.82
	CH505w030.25D8gp120	0.00	0.00	0.00	3.57	4.42	7.94	9.74	7.24	10.22	5.05	1.64	5.33
	CH505w030.11D8gp120	0.00	0.00	0.00	3.40	3.32	4.98	5.83	5.86	9.15	5.91	0.10	4.61
	CH505w030.18D8gp120	0.00	0.00	0.00	2.19	3.63	7.70	9.91	6.43	9.21	6.70	0.61	5.69
	CH505w030.5D8gp120	0.00	0.00	0.00	1.57	5.73	8.82	11.24	8.48	11.14	3.50	1.90	6.73
	CH505w030.23D8gp120	0.00	0.00	0.00	0.45	5.57	8.43	11.22	8.37	11.52	4.26	4.28	7.91
	CH505w030.9D8gp120	0.00	0.00	0.00	0.97	1.37	5.55	6.37	3.86	8.73	3.77	0.59	4.34
	CH505w030.15D8gp120	0.00	0.00	0.00	0.00	2.37	5.26	8.71	5.96	8.68	2.15	1.40	5.95
	CH505w030.28D8gp120	0.00	0.00	0.00	0.00	0.31	1.51	3.57	2.38	5.01	0.00	0.00	1.84
	CH505w030.17D8gp120	0.00	0.00	0.00	0.00	0.75	3.89	3.34	3.85	7.10	1.92	0.09	2.64
	CH505.w30.12D8gp120	0.00	0.00	0.00	0.00	0.35	0.95	3.37	2.40	3.89	0.00	0.00	1.05
	CH505w030.21D8gp120	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.12	0.00
	CH505w030.19D8gp120/293F	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.11	0.00

FIG. 47 cont.

CH235 Lineage

	Envelope ID	UCA	A4	A3	A2	CH240	CH236	CH239	A1	CH235.7	CH235.10	CH235.11	CH235.13
Week 53	CH505w053.16D8gp120	0.00	0.00	0.00	0.00	0.00	0.34	3.70	0.47	3.51	1.62	0.12	1.99
	CH505w053.25D8gp120	0.00	0.00	0.00	0.00	0.00	0.00	0.36	0.25	3.33	1.44	0.00	0.74
	CH505w053.3D8gp120	0.00	0.00	0.00	0.00	0.00	0.00	0.89	0.21	3.41	1.63	0.08	1.23
	CH505w053.13D8gp120/293F	0.00	0.00	0.00	0.00	0.00	0.00	0.15	0.09	2.14	2.07	0.11	2.30
	CH505w053.31D8gp120/293F	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.25	0.11	0.35
	CH505.w53.19gp D8gp120	0.00	0.07	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.08	0.00	0.08
	CH505w053.6D8gp120	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.54	0.00	0.09
	CH505w053.29D8gp120	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	4.84	0.00	0.00	1.04
Week 78	CH0505.w78.env5.D11gp120/293F	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.07	0.09	0.23	0.46
	CH505w078.33D8gp120/293F	0.18	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.71	0.00
	CH505w078.1D8gp120/293F	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.10	0.00
	CH505w078.9D8gp120	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.13	0.00
	CH505w078.6D8gp120	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	CH505w078.38D8gp120	0.11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	CH505w078.15D8gp120/293F	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	CH505w078.10D8gp120	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	CH505w078.17D8gp120	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	CH505w078.7D8gp120/293F	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	CH0505.w78.env4.D11gp120/293i	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	CH505w078.25D8gp120	0.08	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

FIG. 47 cont.

CH235 Lineage

	Envelope ID	UCA	A4	A3	A2	CH240	CH236	CH239	A1	CH235.7	CH235.10	CH235.11	CH235.13
Week 100	CH505w100.C7D8gp120/293F	0.73	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	CH505w100.A13D8gp120	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	CH505w100.B6D8gp120	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	CH505w100.B7D8gp120/293F	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	CH505_w100V115b7.D7gp120/293F	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	CH505w100.A10D8gp120	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	505_w100.A4.D8.gp120/293F	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	CH505_w100V115A10.D7gp120	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	CH505w100.A12D8gp120	0.08	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	CH505w100.A3D8gp120/293F	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	CH505w100.A6D8gp120	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	CH505w100.B4D8gp120	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	CH505_w100V115A13.D7gp120/293F	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	CH505_w100V115A6.D11gp120	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	CH505_w100V115B4.D11gp120	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	CH505w100.B2D8gp120/293F	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

FIG. 47 cont.

		CH235 Lineage											
	Envelope ID	UCA	A4	A3	A2	CH240	CH236	CH239	A1	CH235.7	CH235.10	CH235.11	CH235.13
Week 136	CH505w136.B18D8gp120	0.00	0.00	0.00	0.00	0.25	3.51	0.74	2.36	3.53	5.54	0.11	1.76
	CH505w136.B2D8gp120/293F	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.07	0.64	0.00	0.00
	CH505_w137V201B12.D11gp120	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.28	0.00
	CH505w136.B3D8gp120/293F	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.72	0.00
	CH505w136.B5D8gp120	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.52	0.00
	CH505w136.B8D8gp120	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	CH505w136.B36D8gp120	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	CH505w136.B20D8gp120	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	CH505_w137V209C12.D11gp120	0.08	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	CH505w136.B27D8gp120	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	CH505w136.B29D8gp120	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	CH505w136.B4D8gp120	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	CH505w136.B12D8gp120	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	CH505w136.B10D8gp120	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

FIG. 47 cont.

	Envelope ID	UCA	IA4	IA3	IA2	CH240	CH236	CH239	IA1	CH235.7	CH235.10	CH235.11	CH235.13
Week 160	CH505w160.T4D8gp120/293F	0.00	0.00	0.00	0.00	0.00	0.10	0.00	0.10	0.13	0.32	0.00	0.46
	CH505w160.C2D8gp120	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	CH505w160.C4D8gp120	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	CH505w160.C12D8gp120	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	CH505w160.C14D8gp120	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	CH505w160.A1D8gp120	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	CH505w160.C11D8gp120	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	CH505w160.D1D8gp120	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	CH505w160.D5D8gp120/293F	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	CH505w160.T2D8gp120	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CH505 TF Mutants Loop D	CH505.M5D8gp120/293F	0.24	1.36	7.01	6.95	7.39	7.27	12.94	9.18	10.58	2.62	1.26	6.53
	CH505.M10D8gp120	0.00	0.12	0.08	0.43	3.13	6.07	11.17	7.08	10.55	1.06	1.06	6.38
	CH505.M6D8gp120/293F	0.00	0.00	2.96	1.22	3.52	6.31	8.97	6.10	9.21	5.67	0.09	6.07
	CH505.M11D8gp120/293F	0.00	0.00	0.00	0.00	0.51	1.43	1.35	2.85	6.75	5.05	0.11	5.35
	CH505.M19D8gp120	0.00	0.00	0.11	0.00	0.65	1.54	4.34	2.78	6.61	0.00	0.09	2.89
	CH505.M8D8gp120	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.29	0.00
	CH505.M20D8gp120/293F	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.28	0.00
	CH505.M21D8gp120/293F	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.10	0.00
	CH505.M9D8gp120/293F	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.07	0.23	0.00
	CH505.M7D8gp120/293F	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.09	0.00	0.00

CH235 Lineage

FIG. 47 cont.

	Envelope ID	CH235	CH241	CH235.9	CH235.12
	CH0505_CON D7gp120/293i	9.73	13.27	8.67	8.39
Week 4	CH505w004.10D8gp120/293F	10.88	14.01	8.78	9.10
	CH505.w4.26D8gp120 /293F	9.85	13.34	8.09	7.69
	505.s.03.D8.gp120/293F	10.26	13.88	8.44	8.23
Week 14	CH505w014.8D8gp120	9.07	12.73	5.08	6.31
	CH505w014.2D8gp120/293F	9.02	12.59	7.08	6.44
	CH505w014.32D8gp120/293F	10.62	13.47	7.86	7.63
	CH505w014.3D8gp120	9.62	12.99	8.06	8.04
	CH505.08.D11gp120/293F	8.19	11.69	6.20	6.26
	CH505w014.10D8gp120	9.43	13.26	8.74	8.00
	CH505w014.21D8gp120/293F	10.58	14.40	9.70	7.95

CH235 Lineage

FIG. 47 cont.

		CH235 Lineage			
	Envelope ID	CH235	CH241	CH235.9	CH235.12
Week 20	CH505w020.15D8gp120	9.99	13.40	8.44	7.81
	CH505w020.13D8gp120	8.63	12.19	7.29	7.31
	CH505w020.22D8gp120/293F	9.86	13.58	8.70	7.97
	CH505w020.14D8gp120	9.91	13.08	8.15	8.33
	CH505w020.8D8gp120/293F	10.09	13.36	8.19	7.31
	CH505w020.3D8gp120	9.61	12.83	6.91	6.61
	CH505w020.30D8gp120	8.59	12.34	6.56	6.41
	CH505w020.23D8gp120	9.84	13.45	8.07	6.73
	CH505w020.11D8gp120	7.81	11.65	4.49	5.52
	CH505w020.9D8gp120	8.95	12.06	6.33	6.28
	CH505w020.4D8gp120/293F	9.75	13.59	9.79	8.66
	CH505w020.7D8gp120	9.63	13.32	9.59	8.85
	CH505w020.26D8gp120	8.58	12.68	7.80	7.06

FIG. 47 cont.

		CH235 Lineage				
	Envelope ID	CH235	CH241	CH235.9	CH235.12	
Week 30	CH505w030.6D8gp120/293F	8.57	7.54	7.67	6.86	
	CH505w030.36D8gp120	7.01	5.74	7.02	7.21	
	CH505.w30.12D8gp140	5.05	7.32	2.11	1.80	
	CH505w030.20D8gp120/293F	8.33	8.03	8.09	8.59	
	CH505w030.27D8gp120/293F	8.85	7.94	8.02	8.48	
	CH505w030.10D8gp120	6.86	9.57	4.02	2.90	
	CH505w030.13D8gp120/293F	7.18	10.04	4.63	3.28	
	CH505w030.25D8gp120	10.05	8.90	9.33	8.06	
	CH505w030.11D8gp120	9.11	6.52	8.63	7.00	
	CH505w030.18D8gp120	10.07	9.56	8.57	8.90	
	CH505w030.5D8gp120	10.54	9.42	10.19	9.56	
	CH505w030.23D8gp120	10.40	9.09	10.52	10.28	
	CH505w030.9D8gp120	7.79	6.56	7.78	7.33	
	CH505w030.15D8gp120	8.50	4.24	8.54	8.42	
	CH505w030.28D8gp120	6.72	9.62	4.16	3.18	
	CH505w030.17D8gp120	7.57	0.13	6.75	5.54	
	CH505.w30.12D8gp120	7.28	9.60	3.17	2.37	
	CH505w030.21D8gp120	0.00	3.27	0.00	1.01	
	CH505w030.19D8gp120/293F	0.00	1.47	0.00	0.00	

FIG. 47 cont.

	Envelope ID	CH235 Lineage			
		CH235	CH241	CH235.9	CH235.12
Week 53	CH505w053.16D8gp120	4.63	7.19	5.76	4.75
	CH505w053.25D8gp120	3.81	0.00	6.23	4.67
	CH505w053.3D8gp120	4.99	0.00	7.22	4.26
	CH505w053.13D8gp120/293F	2.18	0.00	6.53	5.79
	CH505w053.31D8gp120/293F	1.11	1.41	5.23	6.36
	CH505.w53.19gp D8gp120	0.64	0.52	3.05	3.95
	CH505w053.6D8gp120	0.13	0.00	4.89	4.76
	CH505w053.29D8gp120	0.00	0.00	0.25	3.44
Week 78	CH0505.w78.env5.D11gp120/293F	0.00	0.00	4.69	6.11
	CH505w078.33D8gp120/293F	0.00	0.00	0.00	0.00
	CH505w078.1D8gp120/293F	0.00	0.00	0.00	0.00
	CH505w078.9D8gp120	0.00	0.00	0.09	0.09
	CH505w078.6D8gp120	0.00	0.00	0.00	3.01
	CH505w078.38D8gp120	0.00	0.00	0.00	0.00
	CH505w078.15D8gp120/293F	0.00	0.00	0.00	0.00
	CH505w078.10D8gp120	0.00	0.00	0.00	0.00
	CH505w078.17D8gp120	0.00	0.00	0.00	0.00
	CH505w078.7D8gp120/293F	0.00	0.00	0.00	0.00
	CH0505.w78.env4.D11gp120/293i	0.00	0.00	0.00	0.00
	CH505w078.25D8gp120	0.00	0.00	0.00	0.00

FIG. 47 cont.

	CH235 Lineage				
	Envelope ID	CH235	CH241	CH235.9	CH235.12
Week 100	CH505w100.C7D8gp120/293F	0.00	0.00	0.00	0.00
	CH505w100.A13D8gp120	0.28	0.00	0.00	0.00
	CH505w100.B6D8gp120	0.00	0.00	0.00	0.13
	CH505w100.B7D8gp120/293F	0.00	0.00	0.00	0.25
	CH505_w100V115b7.D7gp120/293F	0.00	0.00	0.00	0.13
	CH505w100.A10D8gp120	0.00	0.00	0.00	0.00
	505_w100.A4.D8.gp120/293F	0.00	0.00	0.00	0.00
	CH505_w100V115A10.D7gp120	0.00	0.00	0.00	0.00
	CH505w100.A12D8gp120	0.00	0.00	0.00	0.00
	CH505w100.A3D8gp120/293F	0.00	0.00	0.00	0.00
	CH505w100.A6D8gp120	0.00	0.00	0.00	0.00
	CH505w100.B4D8gp120	0.00	0.00	0.00	0.00
	CH505_w100V115A13.D7gp120/293F	0.00	0.00	0.00	0.00
	CH505_w100V115A6.D11gp120	0.00	0.00	0.00	0.00
	CH505_w100V115B4.D11gp120	0.00	0.00	0.00	0.00
	CH505w100.B2D8gp120/293F	0.00	0.00	0.00	0.00

FIG. 47 cont.

		CH235 Lineage			
	Envelope ID	CH235	CH241	CH235.9	CH235.12
Week 136	CH505w136.B18D8gp120	7.53	0.89	5.76	8.31
	CH505w136.B2D8gp120/293F	0.74	0.00	2.38	2.58
	CH505_w137V201B12.D11gp120	0.00	0.00	0.00	0.00
	CH505w136.B3D8gp120/293F	0.00	0.00	0.00	0.00
	CH505w136.B5D8gp120	0.00	0.00	0.00	0.00
	CH505w136.B8D8gp120	0.00	0.00	0.34	0.00
	CH505w136.B36D8gp120	0.00	0.00	0.00	0.11
	CH505w136.B20D8gp120	0.00	0.00	0.00	0.07
	CH505_w137V209C12.D11gp120	0.00	0.00	0.00	0.00
	CH505w136.B27D8gp120	0.00	0.00	0.00	0.00
	CH505w136.B29D8gp120	0.00	0.00	0.00	0.00
	CH505w136.B4D8gp120	0.00	0.00	0.00	0.00
	CH505w136.B12D8gp120	0.00	0.00	0.00	0.00
	CH505w136.B10D8gp120	0.00	0.00	0.00	0.00

FIG. 47 cont.

	Envelope ID	CH235 Lineage			
		CH235	CH241	CH235.9	CH235.12
Week 160	CH505w160.T4D8gp120/293F	0.98	0.34	0.82	3.32
	CH505w160.C2D8gp120	0.00	0.00	0.00	0.31
	CH505w160.C4D8gp120	0.00	0.00	0.00	0.16
	CH505w160.C12D8gp120	0.00	0.00	0.00	0.00
	CH505w160.C14D8gp120	0.00	0.00	0.00	0.09
	CH505w160.A1D8gp120	0.00	0.00	0.00	0.00
	CH505w160.C11D8gp120	0.00	0.00	0.00	0.00
	CH505w160.D1D8gp120	0.00	0.00	0.00	0.00
	CH505w160.D5D8gp120/293F	0.00	0.00	0.00	0.00
	CH505w160.T2D8gp120	0.00	0.00	0.00	0.00
CH505 TF Mutants Loop D	CH505.M5D8gp120293F	11.36	14.51	9.26	8.20
	CH505.M10D8gp120	10.63	14.40	10.17	7.63
	CH505.M6D8gp120/293F	9.46	13.17	8.11	8.21
	CH505.M11D8gp120/293F	7.59	9.68	8.26	7.71
	CH505.M19D8gp120	7.13	10.44	5.95	4.61
	CH505.M8D8gp120	0.00	2.18	4.36	1.14
	CH505.M20D8gp120/293F	0.00	2.15	1.04	0.00
	CH505.M21D8gp120/293F	0.00	4.82	1.13	0.19
	CH505.M9D8gp120/293F	0.00	0.00	1.31	0.38
	CH505.M7D8gp120/293F	0.00	0.00	0.80	0.69

FIG. 47 cont.

	Envelope ID	UCA	A8	A7	A6	A4	CH187	CH188	CH186	CH200	1AH92U	A3	A2
	CH0505_CON D7gp120/293i	4.30	7.21	10.96	10.86	11.33	11.79	11.60	13.00	9.42	11.19	11.27	11.97
Week 4	CH505w004.10D8gp120/293F	0.00	0.45	2.26	2.89	2.84	7.92	7.63	11.59	8.50	6.03	6.17	6.85
	CH505.w4.26D8gp120 /293F	3.20	5.48	9.05	9.04	9.53	11.86	11.88	13.47	9.51	10.20	10.05	10.63
	505.s.03.D8.gp120/293F	3.53	5.45	9.21	9.07	10.14	12.58	12.35	14.05	10.14	9.42	9.97	10.83
Week 14	CH505w014.8D8gp120	0.00	0.95	0.62	2.43	2.62	8.89	9.28	12.29	2.46	7.26	1.73	3.60
	CH505w014.2D8gp120/293F	0.83	3.17	7.18	6.90	8.47	10.69	10.53	12.43	9.01	8.98	9.31	9.69
	CH505w014.32D8gp120/293F	1.61	4.25	7.88	7.82	8.46	11.99	11.89	13.50	10.00	9.14	9.58	10.21
	CH505w014.3D8gp120	1.01	4.38	7.65	7.62	9.20	11.86	11.76	13.43	9.97	8.93	9.82	10.57
	CH505.08.D11gp120/293F	2.25	4.52	7.84	8.02	8.51	9.47	9.14	11.49	7.30	8.24	8.56	9.38
	CH505w014.10D8gp120	0.31	2.22	5.14	6.33	7.07	12.04	12.09	13.78	9.44	9.44	9.32	9.92
	CH505w014.21D8gp120/293F	0.39	2.82	6.25	7.78	8.05	12.48	12.40	14.16	10.14	9.83	9.28	10.21

CH103 Lineage

FIG. 47 cont.

CH103 Lineage

	Envelope ID	CH505	A8	A7	A6	A4	CH187	CH188	CH186	CH200	1AH92U	A3	A2
Week 20	CH505w020.15D8gp120	1.57	4.21	8.19	7.78	9.06	11.85	11.52	13.26	10.07	7.20	9.49	10.45
	CH505w020.13D8gp120	0.83	2.41	6.37	6.05	7.28	9.25	8.96	11.14	8.27	8.22	8.45	9.02
	CH505w020.22D8gp120/293F	1.24	3.97	8.28	8.37	8.80	11.00	11.21	12.46	10.18	9.42	10.21	10.57
	CH505w020.14D8gp120	0.33	3.40	7.23	7.94	8.64	11.17	10.85	12.83	8.68	10.41	10.50	10.98
	CH505w020.8D8gp120/293F	0.44	2.56	5.90	6.69	7.37	11.22	11.36	13.00	9.04	10.08	9.86	10.47
	CH505w020.3D8gp120	0.77	2.57	5.88	5.98	7.05	11.21	11.44	13.36	7.12	10.18	8.37	9.28
	CH505w020.30D8gp120	0.79	4.18	7.31	7.89	8.45	11.21	11.44	12.68	8.24	10.31	9.66	10.46
	CH505w020.23D8gp120	0.94	3.57	7.17	7.89	8.58	12.39	12.43	14.02	9.59	9.89	10.14	10.65
	CH505w020.11D8gp120	0.00	0.90	0.10	0.78	0.75	5.81	4.97	10.29	2.09	5.49	0.97	2.58
	CH505w020.9D8gp120	0.38	2.84	4.89	6.79	7.41	11.01	10.90	12.66	6.89	9.74	8.68	9.65
	CH505w020.4D8gp120/293F	0.93	3.63	8.18	8.66	9.41	12.13	12.26	13.64	10.34	10.78	11.18	11.61
	CH505w020.7D8gp120	0.57	2.74	7.63	8.09	8.75	11.51	11.60	12.94	9.76	10.56	10.83	11.27
	CH505w020.26D8gp120	0.00	0.72	2.73	3.97	5.10	10.40	10.34	12.41	7.61	8.21	8.18	8.99

FIG. 47 cont.

CH103 Lineage

	Envelope ID	UCA	A8	A7	A6	A4	CH187	CH188	CH186	CH200	1AH92U	A3	A2
Week 30	CH505w030.6D8gp120/293F	0.00	0.00	0.00	0.00	0.00	1.71	1.85	10.03	6.28	4.58	3.16	5.38
	CH505w030.36D8gp120	0.00	0.00	0.00	0.00	0.00	4.72	4.80	12.14	9.03	6.39	5.80	7.27
	CH505.w30.12D8gp140	0.00	0.00	0.00	0.21	0.29	7.20	7.32	7.62	5.72	6.22	3.88	7.28
	CH505w030.20D8gp120/293F	0.00	0.00	0.00	0.00	0.00	1.16	1.19	8.59	2.84	4.89	4.62	6.26
	CH505w030.27D8gp120/293F	0.00	0.00	0.00	0.00	0.00	7.13	7.07	12.75	8.96	6.52	5.98	7.90
	CH505w030.10D8gp120	0.18	1.26	3.24	5.06	5.77	12.02	12.32	12.48	7.56	9.03	7.79	10.98
	CH505w030.13D8gp120/293F	0.25	1.96	4.72	6.48	7.39	13.37	13.60	13.30	9.19	10.03	8.65	11.44
	CH505w030.25D8gp120	0.00	0.00	0.36	0.49	0.36	11.46	11.68	14.73	9.99	8.75	8.78	9.93
	CH505w030.11D8gp120	0.00	0.00	0.73	0.48	0.49	10.21	10.14	13.59	10.92	7.02	6.82	9.12
	CH505w030.18D8gp120	0.00	0.00	0.00	0.00	0.00	4.27	4.50	10.44	8.76	8.59	8.13	9.42
	CH505w030.5D8gp120	0.00	0.00	0.00	0.00	0.00	10.12	10.26	14.05	8.91	7.40	6.70	8.09
	CH505w030.23D8gp120	0.00	0.00	0.00	0.00	0.00	10.43	10.47	13.87	9.53	7.75	7.83	8.86
	CH505w030.9D8gp120	0.00	0.00	0.00	0.00	0.00	8.32	8.51	13.64	9.74	7.41	7.11	8.59
	CH505w030.15D8gp120	0.00	0.00	0.00	0.00	0.00	3.92	3.78	10.84	8.12	6.91	7.84	8.37
	CH505w030.28D8gp120	0.00	1.59	3.52	6.34	6.47	12.33	12.50	12.61	7.51	9.64	7.73	11.07
	CH505w030.17D8gp120	0.00	0.00	0.00	0.00	0.00	6.69	7.21	13.59	6.10	6.57	4.11	7.00
	CH505.w30.12D8gp120	0.00	0.36	0.98	1.22	1.91	11.70	11.64	12.02	7.29	9.35	7.58	11.64
	CH505w030.21D8gp120	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.17	9.50	11.00	11.46
	CH505w030.19D8gp120/293F	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.26	9.46	6.38	8.24

FIG. 47 cont.

CH103 Lineage

	Envelope ID	UCA	A8	A7	A6	A4	CH187	CH188	CH186	CH200	1AH92U	A3	A2
Week 53	CH505w053.16D8gp120	0.00	0.00	0.00	0.00	0.22	2.00	2.10	7.55	5.12	8.03	7.76	9.26
	CH505w053.25D8gp120	0.00	0.00	0.00	0.00	0.00	0.85	1.17	8.55	7.97	8.50	8.67	10.23
	CH505w053.3D8gp120	0.00	0.00	0.00	0.00	0.00	1.04	1.49	9.17	6.13	7.92	8.31	9.45
	CH505w053.13D8gp120/293F	0.00	0.00	0.00	0.00	0.00	0.79	1.05	8.34	7.14	8.28	9.20	10.47
	CH505w053.31D8gp120/293F	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.13	5.80	11.26	13.01	13.34
	CH505.w53.19gp D8gp120	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.42	3.36	11.78	13.22	12.61
	CH505w053.6D8gp120	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.27	9.90	11.17	11.95
	CH505w053.29D8gp120	0.00	0.00	0.00	0.00	0.00	0.46	0.82	7.64	2.42	7.56	7.72	9.16
Week 78	CH0505.w78.env5.D11gp120/293F	0.00	0.00	0.00	0.00	0.00	0.00	0.00	4.95	2.91	4.33	3.39	6.57
	CH505w078.33D8gp120/293F	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.56	8.07	7.10	8.97
	CH505w078.1D8gp120/293F	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	7.70	6.06	7.73
	CH505w078.9D8gp120	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.86	2.27	6.11	5.78	7.18
	CH505w078.6D8gp120	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.13	3.43	1.04	4.57
	CH505w078.38D8gp120	0.00	0.00	0.00	0.00	0.00	1.07	1.22	6.19	0.57	3.76	1.94	4.70
	CH505w078.15D8gp120/293F	0.00	0.00	0.68	0.99	1.32	5.54	5.65	9.55	1.86	9.25	10.66	11.49
	CH505w078.10D8gp120	0.00	0.00	0.00	0.00	0.00	5.29	5.80	12.35	1.45	6.53	4.40	6.56
	CH505w078.17D8gp120	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.09	8.31	8.95	9.91
	CH505w078.7D8gp120/293F	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	8.36	8.26	9.19
	CH0505.w78.env4.D11gp120/293i	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	8.98	9.29	10.21
	CH505w078.25D8gp120	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.16	3.59	1.37	3.19

FIG. 47 cont.

CH103 Lineage

	Envelope ID	UCA	A8	A7	A6	A4	CH187	CH188	CH186	CH200	TAH92U	A3	A2
Week 100	CH505w100.C7D8gp120/293F	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.10	6.85	9.14
	CH505w100.A13D8gp120	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.10	10.66	10.95	12.47
	CH505w100.B6D8gp120	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.84	9.81	12.11
	CH505w100.B7D8gp120/293F	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.43	10.56	12.37
	CH505_w100V115b7.D7gp120/293F	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.62	11.34	12.92
	CH505w100.A10D8gp120	0.00	0.00	0.00	0.00	0.00	7.23	7.93	12.41	3.02	8.52	8.67	10.45
	505_w100.A4.D8.gp120/293F	0.00	0.00	0.00	0.00	0.00	5.35	5.76	11.31	2.85	7.32	7.39	8.84
	CH505_w100V115A10.D7gp120	0.00	0.00	0.00	0.00	0.00	8.00	8.66	13.44	4.09	8.31	7.16	9.58
	CH505w100.A12D8gp120	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.12	7.40	9.42
	CH505w100.A3D8gp120/293F	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	8.21	6.37	9.34
	CH505w100.A6D8gp120	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	9.78	9.48	11.38
	CH505w100.B4D8gp120	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	7.83	12.89	13.42
	CH505_w100V115A13.D7gp120/293I	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.20	8.26	9.84
	CH505_w100V115A6.D11gp120	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	10.38	11.20	12.60
	CH505_w100V115B4.D11gp120	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	8.18	12.68	13.42
	CH505w100.B2D8gp120/293F	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.10	4.11	7.49

FIG. 47 cont.

CH103 Lineage

	Envelope ID	UCA	A8	A7	A6	A4	CH187	CH188	CH186	CH200	1AH92U	A3	A2
Week 136	CH505w136.B18D8gp120	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	8.70	13.82	14.27
	CH505w136.B2D8gp120/293F	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	7.65	12.60	13.07
	CH505_w137V201B12.D11gp120	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	11.40	11.13	12.81
	CH505w136.B3D8gp120/293F	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.22	5.00	12.05	13.06
	CH505w136.B5D8gp120	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.13	8.59	9.94
	CH505w136.B8D8gp120	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	11.49	11.80	13.23
	CH505w136.B36D8gp120	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	12.04	12.76	13.63
	CH505w136.B20D8gp120	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	11.08	11.21	12.70
	CH505_w137V209C12.D11gp120	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.81	11.62	12.61
	CH505w136.B27D8gp120	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.10	10.57	9.89	11.91
	CH505w136.B29D8gp120	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	11.03	10.90	12.52
	CH505w136.B4D8gp120	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	10.87	10.56	12.17
	CH505w136.B12D8gp120	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	11.18	10.85	12.40
	CH505w136.B10D8gp120	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	8.89	7.55	9.69

FIG. 47 cont.

CH103 Lineage

	Envelope ID	UCA	A8	A7	A6	A4	CH187	CH188	CH186	CH200	1AH92U	A3	A2
Week 160	CH505w160.T4D8gp120/293F	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	4.84	12.55	13.44
	CH505w160.C2D8gp120	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	9.64	9.07	10.09
	CH505w160.C4D8gp120	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	7.65	3.88	6.15
	CH505w160.C12D8gp120	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.05	12.02	12.90
	CH505w160.C14D8gp120	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	7.86	5.43	7.05
	CH505w160.A1D8gp120	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	7.68	5.85	7.58
	CH505w160.C11D8gp120	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	8.93	6.73	8.53
	CH505w160.D1D8gp120	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	8.20	5.15	7.34
	CH505w160.D5D8gp120/293F	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	7.32	3.03	4.99
	CH505w160.T2D8gp120	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	7.56	3.82	5.64
CH505 TF Mutants Loop D	CH505.M5D8gp120293F	0.00	0.57	2.33	3.30	3.76	9.44	9.01	12.22	8.90	6.12	6.88	7.79
	CH505.M10D8gp120	1.35	4.68	8.82	9.18	9.78	12.67	12.64	13.93	10.46	11.08	11.37	11.75
	CH505.M6D8gp120/293F	5.23	7.05	10.46	10.70	10.89	12.67	12.60	13.82	9.64	11.89	11.87	12.07
	CH505.M11D8gp120/293F	2.62	6.22	10.13	10.04	10.46	12.61	13.02	13.63	10.52	11.82	11.92	12.67
	CH505.M19D8gp120	1.74	5.03	8.41	8.38	9.53	13.55	13.78	13.53	10.31	9.99	9.79	11.71
	CH505.M8D8gp120	0.00	0.79	3.65	4.92	5.40	11.41	11.30	12.29	9.12	9.00	8.85	9.97
	CH505.M20D8gp120/293F	0.00	0.68	3.59	5.21	5.58	11.86	12.04	13.27	10.75	9.66	9.92	10.78
	CH505.M21D8gp120/293F	0.00	0.66	3.45	5.12	5.51	10.82	11.04	11.47	10.24	10.07	10.94	11.59
	CH505.M9D8gp120/293F	0.12	1.33	4.88	5.73	6.33	11.65	11.70	13.46	8.96	9.77	9.52	10.35
	CH505.M7D8gp120/293F	0.44	2.23	4.38	5.34	6.39	11.26	11.15	12.94	7.36	9.06	7.73	9.31

FIG. 47 cont.

CH103 Lineage

	Envelope ID	IA1	CH103	CH104	CH105	CH106	CH243	CH244	CH245	CH247	CH248
	CH0505_CON D7gp120/293i	11.26	12.45	10.15	10.62	11.88	12.52	12.35	13.23	12.87	12.91
Week 4	CH505w004.10D8gp120/293F	6.17	9.21	8.13	8.33	8.06	10.08	9.96	10.25	11.11	10.76
	CH505.w4.26D8gp120 /293F	10.28	12.35	11.08	10.45	11.05	13.00	12.79	13.38	12.82	13.05
	505.s.03.D8.gp120/293F	10.38	12.63	10.41	11.18	11.28	13.40	12.96	13.86	13.08	13.34
Week 14	CH505w014.8D8gp120	3.38	11.22	3.86	6.01	7.94	10.86	10.07	10.64	12.59	11.68
	CH505w014.2D8gp120/293F	9.14	10.88	9.49	9.62	9.62	11.86	11.41	11.97	12.48	12.04
	CH505w014.32D8gp120/293F	9.53	11.93	10.91	10.70	10.54	12.94	12.92	13.42	12.65	13.05
	CH505w014.3D8gp120	9.62	11.89	10.63	10.81	10.64	12.81	12.78	12.72	13.00	13.32
	CH505.08.D11gp120/293F	8.70	10.26	8.25	8.30	9.20	10.80	10.19	10.66	11.06	10.92
	CH505w014.10D8gp120	9.38	11.92	10.74	10.81	10.37	13.24	12.98	12.86	13.12	12.85
	CH505w014.21D8gp120/293F	9.68	13.06	11.36	11.40	11.09	13.48	13.40	13.65	13.41	13.57

FIG. 47 cont.

		CH103 Lineage									
	Envelope ID	VA	CH103	CH104	CH105	CH106	CH243	CH244	CH245	CH247	CH248
Week 20	CH505w020.15D8gp120	9.89	11.76	10.54	10.84	10.46	12.82	12.42	12.80	13.27	12.55
	CH505w020.13D8gp120	8.64	10.34	8.31	8.22	9.44	10.36	9.82	9.71	10.68	10.78
	CH505w020.22D8gp120/293F	10.14	11.49	10.70	10.45	10.68	12.46	12.48	12.68	13.06	12.68
	CH505w020.14D8gp120	10.27	12.55	10.39	10.42	11.23	12.61	12.32	12.11	12.08	12.48
	CH505w020.8D8gp120/293F	9.88	12.38	10.52	10.55	10.96	12.97	12.73	12.68	12.60	12.89
	CH505w020.3D8gp120	8.86	12.43	9.80	10.11	10.25	12.73	12.78	12.68	12.69	12.27
	CH505w020.30D8gp120	9.84	11.71	10.08	10.26	10.80	12.67	12.53	12.28	12.38	12.71
	CH505w020.23D8gp120	9.85	12.83	11.56	11.20	11.30	13.53	13.17	12.97	13.24	13.24
	CH505w020.11D8gp120	1.83	9.59	2.18	3.61	5.37	7.38	7.08	8.43	10.44	9.22
	CH505w020.9D8gp120	8.99	11.85	8.86	9.31	10.02	12.22	11.92	11.43	12.54	12.30
	CH505w020.4D8gp120/293F	11.34	12.50	11.61	11.14	11.49	13.28	13.39	13.66	13.48	13.30
	CH505w020.7D8gp120	10.85	12.46	11.11	10.62	11.29	12.76	12.41	12.40	12.35	12.88
	CH505w020.26D8gp120	8.92	11.27	8.67	9.01	9.56	11.61	11.45	11.37	12.22	12.01

FIG. 47 cont.

	Envelope ID	CH103 Lineage										
		M1	CH103	CH104	CH105	CH106	CH243	CH244	CH245	CH247	CH248
Week 30	CH505w030.6D8gp120/293F	4.51	8.05	6.13	6.47	6.56	8.07	7.95	9.25	10.28	8.88
	CH505w030.36D8gp120	6.69	9.08	7.83	7.46	8.47	10.01	9.61	12.14	11.98	11.14
	CH505.w30.12D8gp140	7.05	10.66	8.20	7.63	8.13	10.29	9.96	9.48	11.85	9.71
	CH505w030.20D8gp120/293F	5.65	9.48	7.49	7.10	7.25	9.03	10.06	9.90	11.61	9.97
	CH505w030.27D8gp120/293F	7.31	9.12	8.17	7.88	8.17	10.10	9.49	11.87	11.61	10.96
	CH505w030.10D8gp120	10.10	12.50	10.34	9.26	11.14	12.62	12.40	12.77	13.78	12.69
	CH505w030.13D8gp120/293F	10.47	12.89	11.29	10.46	11.88	13.79	13.74	13.97	14.50	13.48
	CH505w030.25D8gp120	9.08	12.50	10.33	9.96	10.19	12.12	11.82	13.44	12.58	12.82
	CH505w030.11D8gp120	8.40	10.92	9.48	8.97	9.41	11.56	11.41	13.73	12.68	11.85
	CH505w030.18D8gp120	9.92	13.05	10.91	11.14	10.54	13.24	13.55	14.17	13.07	12.96
	CH505w030.5D8gp120	7.75	11.43	9.44	8.98	9.41	11.45	11.09	13.15	12.93	12.40
	CH505w030.23D8gp120	8.42	11.08	9.58	9.18	9.98	11.69	11.44	12.97	12.36	12.26
	CH505w030.9D8gp120	7.79	10.23	8.94	8.67	9.61	11.06	8.65	13.08	12.77	11.97
	CH505w030.15D8gp120	8.04	10.06	9.67	9.27	9.33	11.17	10.60	12.57	11.18	11.98
	CH505w030.28D8gp120	10.07	12.78	10.68	9.13	11.71	12.65	12.46	12.42	13.77	12.74
	CH505w030.17D8gp120	5.79	9.92	6.99	6.73	8.20	9.93	9.56	12.83	13.03	11.50
	CH505.w30.12D8gp120	10.62	13.25	11.62	10.68	11.76	13.89	13.68	13.80	13.89	13.22
	CH505w030.21D8gp120	11.78	12.25	11.30	10.55	10.91	12.86	12.95	12.50	11.55	13.33
	CH505w030.19D8gp120/293F	8.23	12.85	8.88	9.46	7.39	12.61	13.20	11.64	12.72	13.55

FIG. 47 cont.

CH103 Lineage

	Envelope ID	IA1	CH103	CH104	CH105	CH106	CH243	CH244	CH245	CH247	CH248
Week 53	CH505w053.16D8gp120	8.79	11.62	9.94	9.00	9.78	11.64	11.51	13.45	12.38	12.94
	CH505w053.25D8gp120	10.00	11.64	10.61	10.00	10.88	11.76	11.82	13.14	12.17	12.61
	CH505w053.3D8gp120	9.21	11.21	10.41	9.41	10.09	11.74	11.52	13.30	11.93	12.78
	CH505w053.13D8gp120/293F	9.93	11.22	9.99	9.37	10.17	11.45	11.80	12.34	11.53	12.19
	CH505w053.31D8gp120/293F	13.36	13.63	13.73	13.52	13.35	14.44	15.25	15.04	14.15	15.19
	CH505.w53.19gp D8gp120	13.56	13.49	13.82	13.75	13.24	14.43	15.75	15.37	14.20	14.82
	CH505w053.6D8gp120	11.37	12.66	12.05	11.74	10.93	13.06	14.13	13.65	13.02	13.46
	CH505w053.29D8gp120	8.79	10.17	9.80	8.51	9.70	10.73	10.38	11.51	11.14	11.51
Week 78	CH0505.w78.env5.D11gp120/293F	5.64	10.24	7.67	6.61	7.89	10.04	11.29	11.15	12.47	10.90
	CH505w078.33D8gp120/293F	8.24	11.08	8.97	8.86	9.48	10.77	11.46	12.22	11.66	12.08
	CH505w078.1D8gp120/293F	7.53	11.03	8.34	7.48	7.29	10.55	11.24	8.36	11.02	10.38
	CH505w078.9D8gp120	6.29	8.93	6.85	6.59	8.34	8.96	9.22	11.09	10.34	10.60
	CH505w078.6D8gp120	3.00	9.94	4.50	3.80	6.72	8.99	9.77	10.16	10.78	9.68
	CH505w078.38D8gp120	4.41	7.69	4.22	3.98	5.25	6.04	6.32	7.60	9.77	7.02
	CH505w078.15D8gp120/293F	10.91	10.70	10.83	10.08	10.95	12.13	11.86	11.70	11.62	11.57
	CH505w078.10D8gp120	6.63	11.13	8.09	7.61	7.84	10.95	10.91	12.38	12.76	11.44
	CH505w078.17D8gp120	8.99	11.32	9.98	9.53	8.72	11.65	12.18	11.57	11.40	11.67
	CH505w078.7D8gp120/293F	8.94	11.38	9.49	9.41	8.71	11.51	12.05	10.78	11.12	11.07
	CH0505.w78.env4.D11gp120/293i	9.80	11.72	10.08	9.53	9.25	12.00	12.40	11.73	11.11	11.99
	CH505w078.25D8gp120	2.40	8.75	3.47	3.33	4.64	6.47	6.68	10.41	10.10	9.71

FIG. 47 cont.

CH103 Lineage

	Envelope ID	1A	CH103	CH104	CH105	CH106	CH243	CH244	CH245	CH247	CH248
Week 100	CH505w100.C7D8gp120/293F	8.86	4.78	9.20	8.96	9.30	11.30	11.78	12.06	7.94	10.76
	CH505w100.A13D8gp120	12.56	12.89	12.94	11.40	11.99	13.90	13.85	14.42	12.88	14.01
	CH505w100.B6D8gp120	12.21	7.07	11.02	11.04	11.77	13.62	13.91	14.47	12.58	13.82
	CH505w100.B7D8gp120/293F	11.90	7.77	11.62	11.58	11.53	13.14	13.62	14.04	12.28	13.27
	CH505_w100V115b7.D7gp120/293F	12.87	8.66	13.49	12.44	12.16	14.32	14.61	15.30	13.25	14.93
	CH505w100.A10D8gp120	9.89	11.73	10.08	9.06	10.37	12.10	11.83	12.99	12.69	12.43
	505_w100.A4.D8.gp120/293F	8.72	11.29	9.55	8.51	9.43	11.03	10.97	12.50	12.61	12.19
	CH505_w100V115A10.D7gp120	8.82	12.54	10.20	9.20	10.79	12.90	12.73	13.65	13.70	12.79
	CH505w100.A12D8gp120	8.56	4.70	10.41	9.56	9.65	12.26	12.81	12.90	9.01	13.17
	CH505w100.A3D8gp120/293F	8.54	11.40	9.97	7.21	8.59	11.91	11.99	10.84	11.19	11.45
	CH505w100.A6D8gp120	11.00	12.14	11.60	10.12	11.11	12.23	12.53	12.74	11.93	12.39
	CH505w100.B4D8gp120	13.74	9.73	13.07	11.91	12.55	12.48	13.29	13.35	12.56	13.00
	CH505_w100V115A13.D7gp120/293F	8.86	6.13	10.83	10.55	10.12	12.60	13.35	13.34	9.78	13.74
	CH505_w100V115A6.D11gp120	12.20	12.62	13.47	12.24	11.93	13.62	14.17	14.54	13.15	14.09
	CH505_w100V115B4.D11gp120	13.50	12.52	14.02	13.55	12.87	13.75	14.61	14.78	13.54	14.28
	CH505w100.B2D8gp120/293F	7.33	3.22	6.13	7.51	7.80	11.29	11.41	11.60	8.61	11.13

FIG. 47 cont.

		CH103 Lineage									
	Envelope ID	1A1	CH103	CH104	CH105	CH106	CH243	CH244	CH245	CH247	CH248
Week 136	CH505w136.B18D8gp120	14.36	11.79	14.25	13.69	13.35	14.26	14.83	15.42	13.40	14.27
	CH505w136.B2D8gp120/293F	13.25	10.80	13.20	12.38	12.74	13.48	13.40	13.98	12.27	13.40
	CH505_w137V/201B12.D11gp120	12.50	12.90	12.17	10.96	12.53	13.17	13.91	14.34	13.53	13.41
	CH505w136.B3D8gp120/293F	13.10	9.93	13.10	12.00	12.57	13.71	14.11	14.30	13.17	13.97
	CH505w136.B5D8gp120	9.48	4.33	10.59	10.55	10.90	13.19	14.39	14.35	13.33	14.55
	CH505w136.B8D8gp120	12.94	11.90	11.73	9.86	11.71	10.95	12.28	12.32	11.97	12.29
	CH505w136.B36D8gp120	13.76	13.75	13.84	12.82	13.09	14.06	14.72	15.30	14.43	14.37
	CH505w136.B20D8gp120	12.40	12.43	11.66	10.66	12.09	13.02	13.10	13.50	12.53	12.92
	CH505_w137V/209C12.D11gp120	12.47	8.72	12.70	12.67	12.21	13.97	14.45	14.78	13.49	14.68
	CH505w136.B27D8gp120	11.24	12.60	11.51	10.38	11.20	12.79	12.85	13.13	12.22	12.89
	CH505w136.B29D8gp120	12.25	12.73	11.37	10.21	12.02	12.42	12.96	13.58	12.71	12.32
	CH505w136.B4D8gp120	11.76	13.38	12.17	10.74	11.81	13.44	13.98	14.40	13.51	13.38
	CH505w136.B12D8gp120	12.24	12.70	10.66	9.67	11.90	12.75	13.29	13.34	12.90	12.94
	CH505w136.B10D8gp120	9.19	11.48	8.94	8.52	8.97	11.74	11.91	11.75	11.40	11.46

FIG. 47 cont.

	Envelope ID	IA1	CH103	CH104	CH105	CH106	CH243	CH244	CH245	CH247	CH248
CH103 Lineage											
Week 160	CH505w160.T4D8gp120/293F	13.55	9.03	12.85	12.21	12.33	13.39	13.89	14.23	12.29	13.52
	CH505w160.C2D8gp120	9.62	12.16	9.24	8.61	9.29	11.24	11.88	10.53	11.47	11.09
	CH505w160.C4D8gp120	5.26	12.19	7.23	6.55	6.80	10.61	12.06	9.83	11.17	10.48
	CH505w160.C12D8gp120	12.91	7.76	11.33	11.14	12.17	12.09	12.61	12.05	11.82	12.89
	CH505w160.C14D8gp120	5.84	12.15	6.83	6.58	6.94	11.03	12.24	10.05	11.80	10.29
	CH505w160.A1D8gp120	6.35	10.71	7.31	6.66	7.60	9.67	9.63	8.96	9.31	8.62
	CH505w160.C11D8gp120	7.70	11.89	7.44	7.70	8.15	10.91	11.50	10.40	11.36	10.67
	CH505w160.D1D8gp120	6.39	11.50	5.71	6.03	7.06	9.51	10.81	8.34	10.47	8.75
	CH505w160.D5D8gp120/293F	3.78	10.95	4.51	4.58	5.64	8.57	10.05	7.32	10.29	7.84
	CH505w160.T2D8gp120	4.68	11.15	5.41	4.94	6.16	9.14	10.83	7.85	10.31	8.34
CH505 TF Mutants Loop D	CH505.M5D8gp120/293F	7.04	9.84	9.02	8.63	8.37	10.41	10.38	10.74	11.47	10.98
	CH505.M10D8gp120	11.08	13.25	11.68	11.30	11.81	13.61	13.40	13.74	13.09	13.77
	CH505.M6D8gp120/293F	11.79	13.09	11.58	11.05	12.60	13.65	13.24	13.62	13.57	13.64
	CH505.M11D8gp120/293F	12.19	13.38	12.03	11.72	12.77	13.97	13.66	13.76	13.67	13.64
	CH505.M19D8gp120	11.30	13.29	11.26	10.92	12.18	13.79	13.72	14.06	13.82	13.21
	CH505.M8D8gp120	9.49	11.52	10.11	9.33	10.19	11.50	11.57	11.51	12.61	12.32
	CH505.M20D8gp120/293F	10.38	12.21	10.85	10.57	10.69	12.82	12.66	12.57	13.39	13.20
	CH505.M21D8gp120/293F	11.22	11.40	11.23	11.19	11.46	13.41	13.05	12.66	13.31	13.78
	CH505.M9D8gp120/293F	9.85	11.60	10.84	10.58	10.98	13.04	12.95	13.07	12.54	13.01
	CH505.M7D8gp120/293F	8.70	11.06	9.73	10.17	10.99	12.85	12.35	12.37	12.49	12.42

FIG. 47 cont.

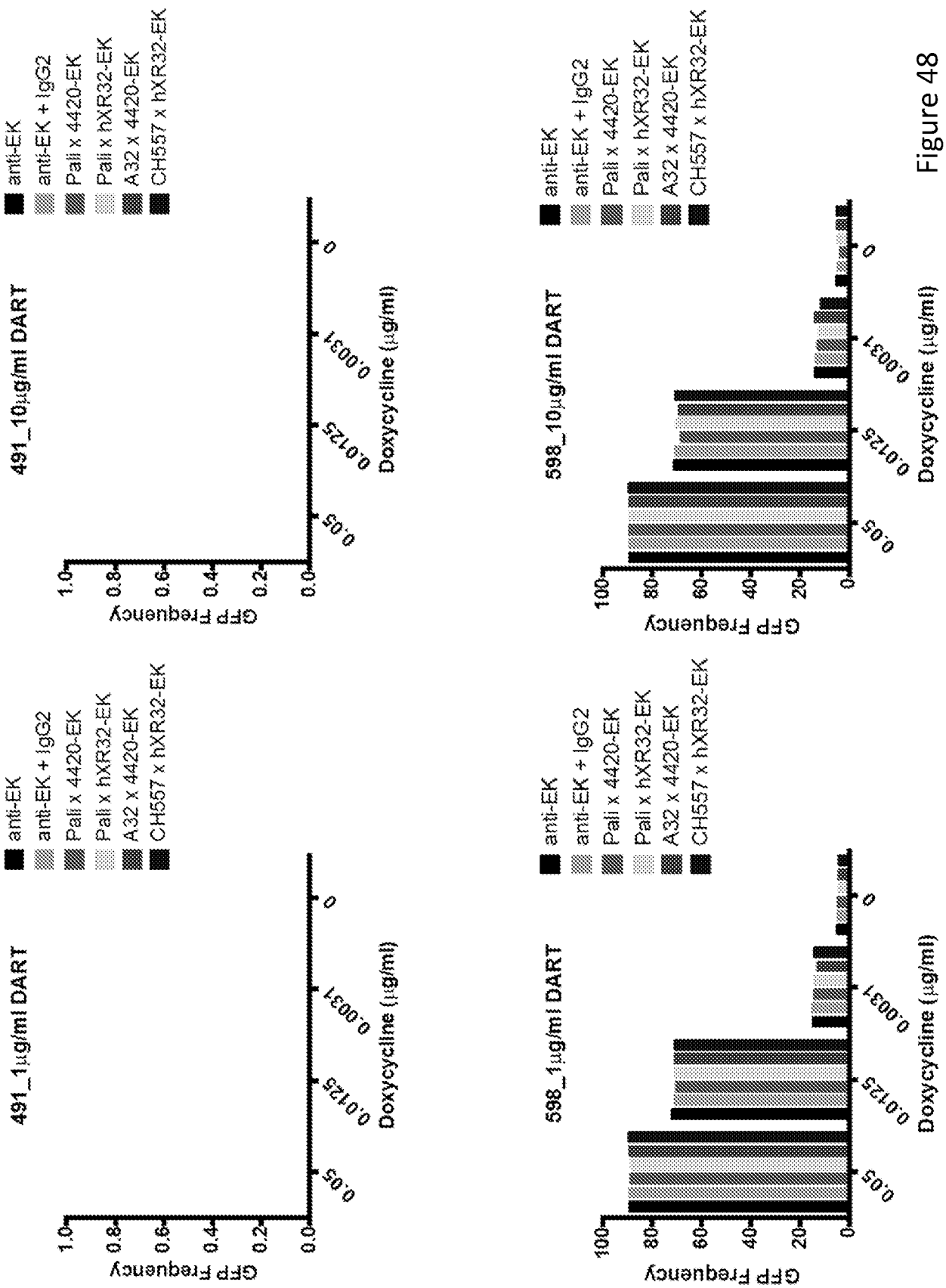

491_1µg/ml DART
491_10µg/ml DART
598_1µg/ml DART
598_10µg/ml DART
anti-EK
anti-EK + IgG2
Pali x 4420-EK
Pali x hXR32-EK
A32 x 4420-EK
CH557 x hXR32-EK
GFP Frequency
Doxycycline (µg/ml)
0
0.0031
0.0125
0.05

Figure 48

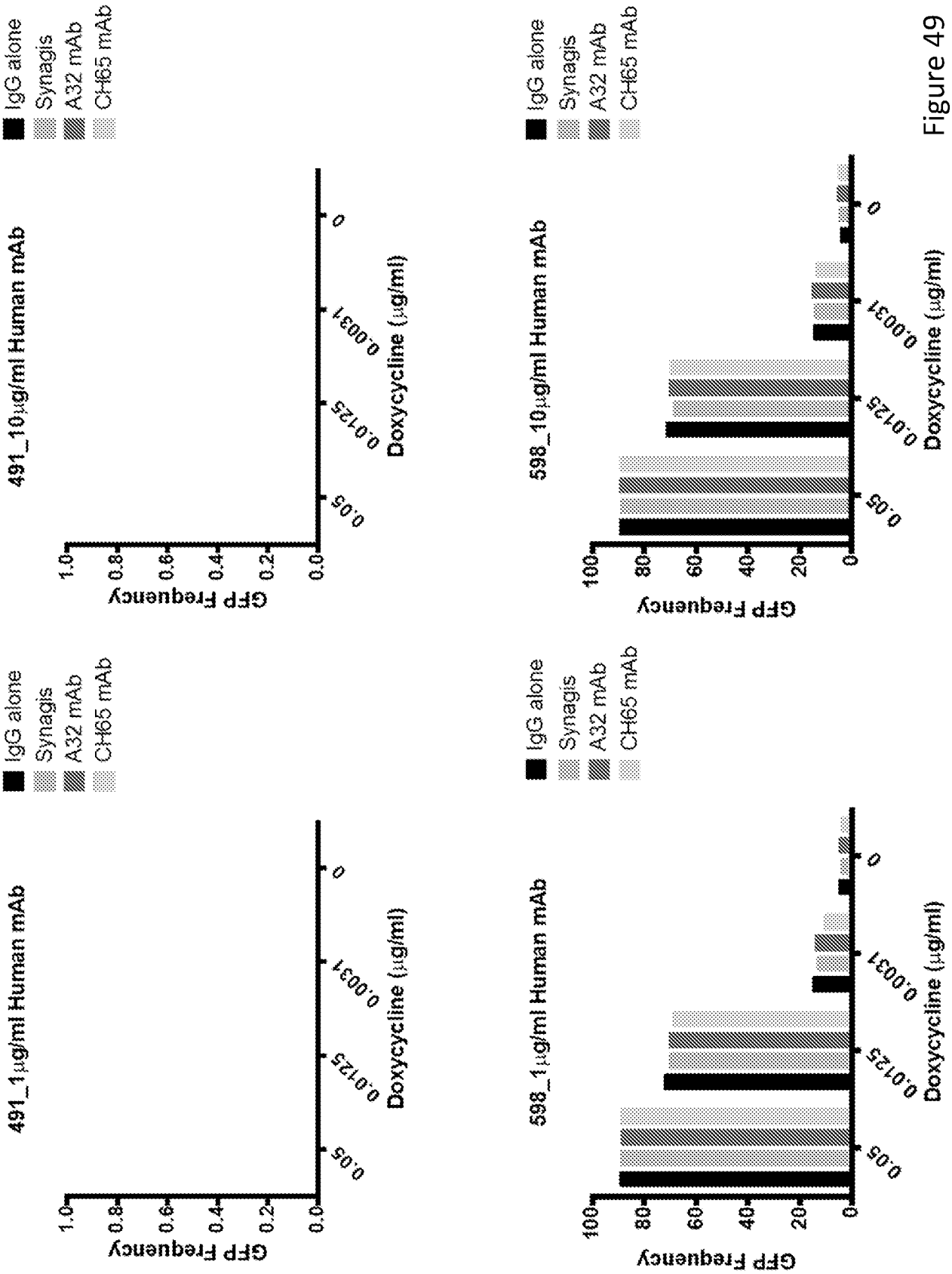

IgG alone
Synagis
A32 mAb
CH65 mAb
491_10μg/ml Human mAb
598_10μg/ml Human mAb
491_1μg/ml Human mAb
598_1μg/ml Human mAb
GFP Frequency
Doxycycline (μg/ml)
0
0.0031
0.0125
0.05
0.0
0.2
0.4
0.6
0.8
1.0
0
20
40
60
80
100

Figure 49

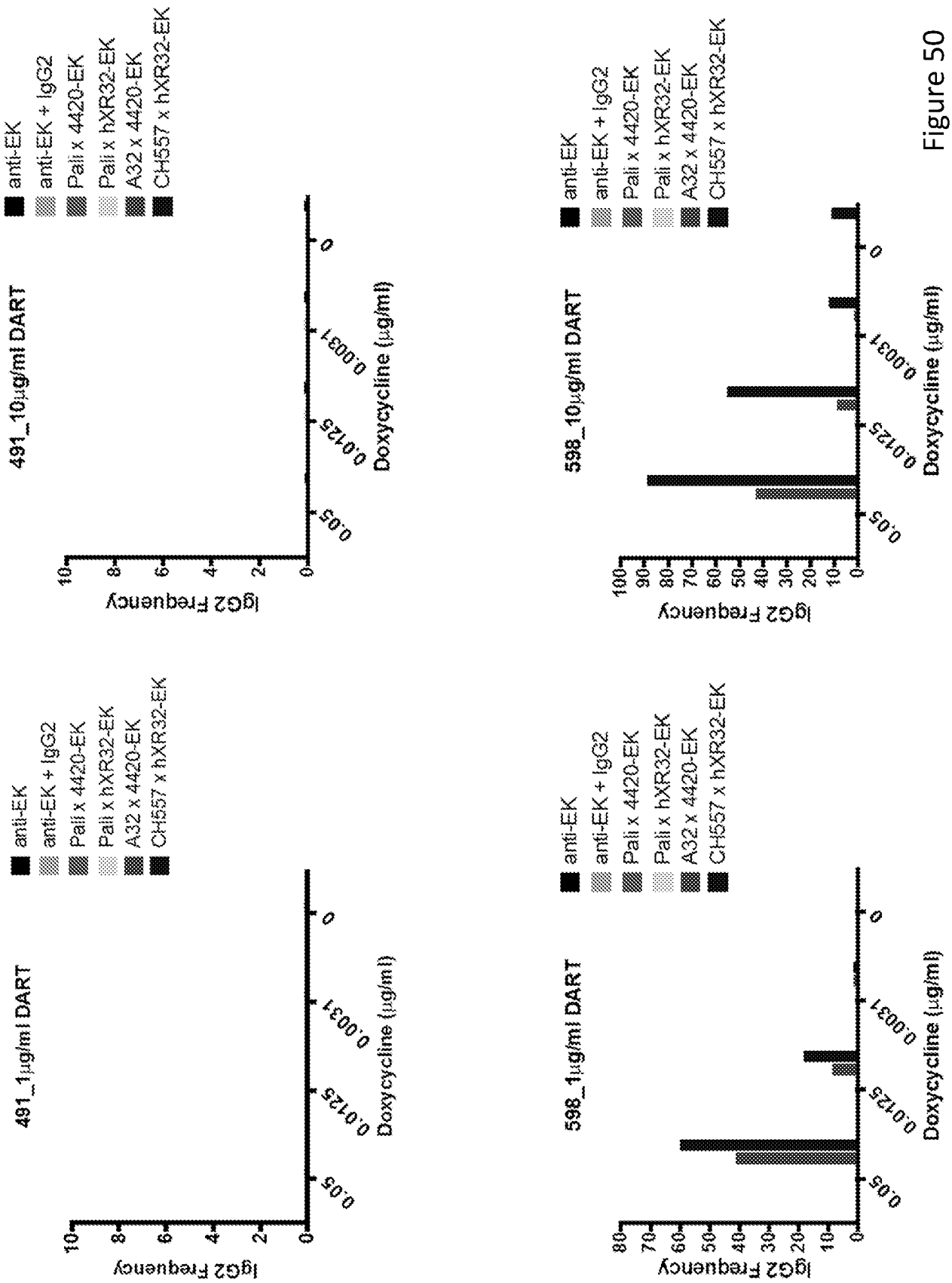

491_1µg/ml DART
491_10µg/ml DART
598_1µg/ml DART
598_10µg/ml DART
anti-EK
anti-EK + IgG2
Pali x 4420-EK
Pali x hXR32-EK
A32 x 4420-EK
CH557 x hXR32-EK
IgG2 Frequency
Doxycycline (µg/ml)
0
0.0031
0.0125
0.05

Figure 50

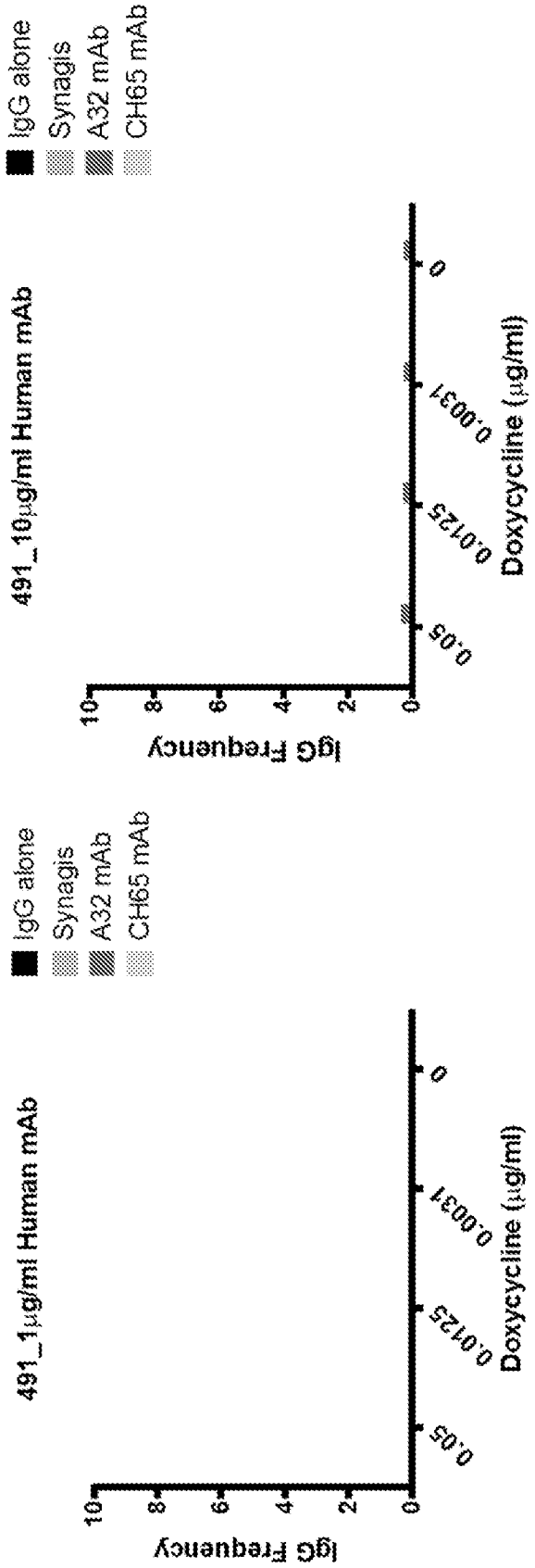

491_1μg/ml Human mAb
IgG alone
Synagis
A32 mAb
CH65 mAb
IgG Frequency
0
2
4
6
8
10
0
0.0031
0.0125
0.05
Doxycycline (μg/ml)
491_10μg/ml Human mAb
IgG alone
Synagis
A32 mAb
CH65 mAb
IgG Frequency
0
10
20
30
40
50
60
70
80
0
0.0031
0.0125
0.05
Doxycycline (μg/ml)
598_1μg/ml Human mAb
IgG alone
Synagis
A32 mAb
CH65 mAb
IgG Frequency
0
10
20
30
40
50
60
70
80
0
0.0031
0.0125
0.05
Doxycycline (μg/ml)
598_10μg/ml Human mAb
IgG alone
Synagis
A32 mAb
CH65 mAb
IgG Frequency
0
10
20
30
40
50
60
70
80
0
0.0031
0.0125
0.05
Doxycycline (μg/ml)

Figure 51

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2016/041809

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61K 39/395; A61P 35/00; C07K 16/00; C07K 16/18; C07K 16/30; C12P 21/08 (2016.01)

CPC - A61K 39/395; A61K 2039/505; C07K 16/28; C07K 16/2809; C07K 16/2815; C07K 2317/31 (2016.08)

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)

IPC - A61K 39/395; A61P 35/00; C07K 16/00; C07K 16/18; C07K 16/30; C12P 21/08

CPC - A61K 39/395; A61K 2039/505; C07K 16/28; C07K 16/2809; C07K 16/2815; C07K 2317/31; C07K 2317/60; C07K 2318/00; C07K 2319/70

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

USPC - 424/136.1; 530/387.3 (keyword delimited)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Orbit, Google Patents, Google Scholar

Search terms used: hiv cd4 gp120 antibody hiv-1 antibody (bispecific OR trivalent OR tetravalent) (CD3 OR CD8 OR CD16) (ch557 OR ch235.12) HAYNES

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X -- Y	US 2014/0294823 A1 (XENCOR, INC.) 02 October 2014 (02.10.2014) entire document	1, 3, 5, 7, 9, 11, 33, 34 ---------- 2, 4, 6, 8, 10, 12
Y	WO 2015/021089 A1 (MACROGENICS, INC.) 12 February 2015 (12.02.2015) entire document	2, 4, 6, 8, 10, 12
A	US 2014/0302037 A1 (AMGEN INC.) 09 October 2014 (09.10.2014) entire document	1-12, 29-31, 33, 34
A	WO 2015/048610 A1 (DUKE UNIVERSITY et al) 02 April 2015 (02.04.2015) entire document	1-12, 29-31, 33, 34
A	EULER et al. "Exploring the Potential of Monoclonal Antibody Therapeutics for HIV-1 Eradication," AIDS Research and Human Retroviruses, 01 January 2015 (01.01.2015), Vol. 31, Pgs. 13-24. entire document	1-12, 29-31, 33, 34
A	WO 2014/145907 A1 (XENCOR, INC. et al) 18 September 2014 (18.09.2014) entire document	1-12, 29-31, 33, 34
A	WO 2015/184203 A1 (MACROGENICS, INC.) 03 December 2015 (03.12.2015) 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	Date of mailing of the international search report
10 October 2016	28 OCT 2016
Name and mailing address of the ISA/ Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, VA 22313-1450 Facsimile No. 571-273-8300	Authorized officer Blaine R. Copenheaver PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2016/041809

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. ☐ *forming part of the international application as filed:*

      ☐ in the form of an Annex C/ST.25 text file.

      ☐ on paper or in the form of an image file.

   b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. ☒ furnished subsequent to the international filing date for the purposes of international search only:

      ☒ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

      ☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

2. ☒ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

SEQ ID NOs: 555, 557, 559, 561, 562, and 563 were searched.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2016/041809

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.