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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
22 September 2016 (22.09.2016)

WIPO | PCT

(10) International Publication Number
WO 2016/149695 A1

(51) International Patent Classification:
A61P 31/18 (2006.01) *C12P 21/08* (2006.01)
C07K 16/28 (2006.01)

(21) International Application Number:
PCT/US2016/023355

(22) International Filing Date:
21 March 2016 (21.03.2016)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

62/135,309	19 March 2015 (19.03.2015)	US
62/191,095	10 July 2015 (10.07.2015)	US
62/222,115	22 September 2015 (22.09.2015)	US
62/260,100	25 November 2015 (25.11.2015)	US
62/301,993	1 March 2016 (01.03.2016)	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,

[Continued on next page]

(54) Title: HIV-1 NEUTRALIZING ANTIBODIES AND USES THEREOF (CD4bs ANTIBODIES)

>DH542_nt_HC (SEQ ID NO: 1)

CAGGTGCAGCTGGTGCAGTCTGGGGCTCAAATGAAGAACCCTGGGGCCT
CAGTGAAGGTCTCCTGCGCGCCTTCTGGATATACCTTCACCGACTTTTACA
TACATTGGTTGCGCCAGGCCCCTGGCCAGGGGCTTCAGTGGATGGGATG
GATGAACCCTCAGACTGGTCGCACAAACACTGCACGAAACTTTCAGGGG
AGGGTCACCATGACCAGGGACACGTCCATCGGCACAGCCTACATGGAGT
TGAGAAGCCTGACATCTGACGACACGGCCATATATTACTGTACGACAGG
GGGATGGATCAGTCTTTACTATGATAGTAGTTATTACCCCAACTTTGACC
ACTGGGGTCAGGGAACCCTGCTCACCGTCTCCTCAG

>DH542_nt_LC (SEQ ID NO: 2)

ACCAGTCTGCTGACTCAGCCTGCCTCCGTGTCTGGGTCTCCTGGACAGTC
GATCACCATCTCCTGCACTGGAACCAAGTATGATGTTGGGAGTCATGACC
TTGTCTCCTGGTACCAACAGTACCCAGGCAAAGTCCCCAAATACATGATTT
ATGAAGTCAATAAACGGCCCTCAGGAGTTTCTAATCGCTTCTCTGGCTCC
AAATCTGGCAACACGGCCTCCCTGACAATCTCTGGGCTCCGGGCTGAGGA
CGAGGCTGACTATTATTGCTGTTCATTTGGAGGGAGTGCCACCGTGGTCT
GCGGCGGCGGGACCAAGGTGACCGTCCTAG

>DH542_aa_HC (SEQ ID NO: 3)

QVQLVQSGAQMKNPGASVKVSCAPSGYTFTDFYIHWLRQAPGQGLQWM
GWMNPQTGRTNTARNFQGRVTMTRDTSIGTAYMELRSLTSDDTAIYYCTT
GGWISLYYDSSYYPNFDHWGQGTLLTVSS

>DH542_aa_LC (SEQ ID NO: 4)

TSLLTQPASVSGSPGQSITISCTGTKYDVGSHDLVSWYQQYPGKVPKYMIYE
VNKRPSGVSNRFSGSKSGNTASLTISGLRAEDEADYYCCSFGGSATVVCGGG
TKVTVL

FIG. 1

(57) Abstract: The invention relates to the identification of monoclonal HIV-1 neutralizing antibodies, such as, but not limited to, antibodies that bind to the CD4 binding site (CD4bs) of HIV-1 gp120, their recombinant expression and purification and uses. In certain aspects, the invention provides a pharmaceutical composition comprising anyone of the antibodies of the invention or fragments thereof or any combination thereof. In certain aspects the invention provides methods to treat or prevent HIV-1 infection in a subject comprising administering to the subject a pharmaceutical composition comprising any one of the inventive antibodies or fragments thereof.

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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, 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))*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

HIV-1 NEUTRALIZING ANTIBODIES AND USES THEREOF (CD4bs antibodies)

[0001] This invention claims the benefit of and priority to U.S. Serial No. 62/135,309 filed March 19, 2015, U.S. Serial No. 62/260,100 filed November 25, 2015, 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, the contents of each of which are hereby incorporated by reference in their entireties.

[0002] This patent disclosure contains material that is subject to copyright protection. The copyright owner has no objection to the facsimile reproduction by anyone of the patent document or the patent disclosure as it appears in the U.S. Patent and Trademark Office patent file or records, but otherwise reserves any and all copyright rights.

[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.

GOVERNMENT SUPPORT

[0004] This invention was made with government support under Center for HIV/AIDS Vaccine Immunology-Immunogen Design grant UM1-AI100645 from the NIH, NIAID, Division of AIDS. The government has certain rights in the invention.

FIELD OF THE INVENTION

[0005] The invention relates to the identification of monoclonal HIV-1 neutralizing antibodies, such as, but not limited to, antibodies that bind to the CD4 binding site (CD4bs) of HIV-1 gp120, their recombinant expression and purification and uses.

BACKGROUND

[0006] It is well documented that essentially all HIV-1 infected individuals develop antibodies capable of binding HIV-1 envelope, but that only a small subset of these antibodies are neutralizing and capable of blocking viral entry in target cells. See e.g. Doria-Rose N. "HIV Neutralizing Antibodies: Clinical Correlates and Implications for Vaccines" The Journal of Infectious Diseases (2010) Volume 201, Issue 7, Pp. 981-983. Over the time of an infection, some individuals develop neutralizing antibodies, and with some of these neutralizing antibodies having activity against diverse primary HIV-1 isolates. A number of broad neutralizing monoclonal antibodies (mAbs) have been identified from HIV-1 infected individuals and these define specific regions on the virus

envelope, e.g. CD4 binding site, V3 loop, membrane proximal region (MPER) of gp41, that are vulnerable to neutralizing Abs.

[0007] Broadly neutralizing HIV-1 antibodies have been isolated only from natural HIV-1 infection. See e.g. Mascola and Haynes, Immunological Reviews (2013) Vol. 254: 225-244. Some examples of broadly neutralizing antibodies (bnAbs) targeting CD4 binding site or V3 loop are VRC01, CH103, CH31, CH98, 8ANC131, PGT121, PGT128. Unfortunately, so far none of these antibodies have been developed for HIV prevention or treatment. Thus, the need exists for monoclonal broadly neutralizing antibodies that can be developed and used for prevention and treatment for an infectious agent, such as HIV.

SUMMARY OF THE INVENTION

[0008] In certain aspects the invention provides an antibody or fragment thereof with the binding specificity of CD4 binding site antibody DH491 or CH493, or CH558, or CH557.

[0009] In certain aspects, the invention provides a recombinant antibody or fragment thereof comprising: a variable heavy chain (VH) amino acid sequence, or fragment thereof, selected from the group of VH amino acid sequences of an antibody CH490, CH491, CH492, CH493, CH555, CH556 and CH557 and a variable light chain (VL) amino acid sequence or fragment thereof, selected from the group of VL amino acid sequences of an antibody CH490, CH491, CH492, CH493, CH555, CH556 and CH557, wherein the recombinant antibody or fragment thereof binds to the CD4 binding site of the HIV-1 envelope. In certain aspects the antibodies bind to the CD4 binding site on the HIV-1 envelope and are neutralizing.

[0010] In certain embodiments, the antibody or fragment thereof is fully human and recombinantly produced. In certain embodiments, some of the VH and VL chains are identified from human subject who have been naturally infected with HIV-1. In certain embodiments the antibody is not naturally occurring. In certain embodiments the antibody comprises naturally occurring pair of VH and VL chains. In certain embodiments the antibody comprises naturally occurring pair of VH and VL chains wherein the Fc portion of the antibody is not the natural isotype or portion of the naturally occurring pair of VH and VL chains. In certain embodiments the antibody is computationally designed, for example based on some naturally identified VH and VL sequences. In certain embodiments the antibody is computationally designed, e.g. UCA, Intermediates in the antibody lineages. In certain embodiments the antibody comprises a non-naturally occurring pairing of VH and VL chains, wherein the VH or VL individually could be identified from a subject. In some embodiments, the antibody comprises VH chain or HCDRs of a VH chain of one clonal member, and VL or LCDRs of another clonal member, i.e., a non-naturally occurring

antibody comprising sequences derived from natural pairs. In certain embodiments the antibody comprises naturally occurring VH and VL chains modified by substituting one or more amino acids.

[0011] In certain embodiments, the antibody or fragment thereof comprises a VH chain that is 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical to the VH chain of antibody CH557, or any of the other lineage members. In certain embodiments, the antibody or fragment thereof comprises a VL chain that is 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical to the VL chain of antibody CH557, or any of the other lineage members.

[0012] In certain embodiments, the antibody or fragment thereof comprises a VH chain that is 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical to the VH chain of antibody DH542, or any of the other lineage members. In certain embodiments, the antibody or fragment thereof comprises a VL chain that is 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical to the VL chain of antibody DH542, or any of the other lineage members.

[0013] In certain embodiments, the antibody or fragment thereof comprises a VH chain that is 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical to the VH chain of antibody DH511, DH512, DH513, DH514, DH515, DH516, DH517, DH518, DH536, DH537, CH491 or CH493 and further comprises a VL chain that is 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical to the VL chain of antibody DH511, DH512, DH513, DH514, DH515, DH516, DH517, DH518, DH536, DH537, CH491 or CH493.

[0014] In certain embodiments, the antibody or fragment thereof comprises a VH which comprises the HCDR1, HCDR2, and HCDR3 of antibody CH557. In certain embodiments, the antibody or fragment thereof comprises a VL which comprises the LCDR1, LCDR2, and LCDR3 of antibody CH557.

[0015] In certain embodiments, the antibody or fragment thereof comprises a VH which comprises the HCDR3 of CH557 and further comprises a VL which comprises the LCDR3 of CH557.

[0016] In certain embodiments, the antibody or fragment thereof comprises a VH which comprises the HCDR1, HCDR2, and HCDR3 of antibody DH542. In certain embodiments, the antibody or fragment thereof comprises a VL which comprises the LCDR1, LCDR2, and LCDR3 of antibody DH542.

[0017] In certain embodiments, the antibody or fragment thereof comprises a VH which comprises the HCDR1, HCDR2, and HCDR3 of antibody CH557 and further comprises the complementary VL which comprises the LCDR1, LCDR2, LCDR3 of antibody CH557.

[0018] In certain embodiments, the antibody or fragment thereof comprises VH and VL of antibody CH557.

[0019] In certain embodiments, the antibody or fragment thereof comprises a VH which comprises the HCDR1, HCDR2, and HCDR3 of antibody DH542 and further comprises the complementary VL which comprises the LCDR1, LCDR2, LCDR3 of antibody DH542. In certain embodiments, the antibody or fragment thereof comprises VH and VL of antibody DH542.

[0020] In certain embodiments, the antibody is DH542. In certain embodiments, the antibody is CH557.

[0021] In certain embodiments, the invention provides a recombinant antibody or fragment thereof with the binding specificity of CD4 binding site antibody DH491 or CH493, or CH558, or CH557 comprising an engineered constant domain. In some embodiments, the recombinant antibody or fragment thereof is capable of neutralizing an HIV-1 Env pseudovirus with an IC50 of less than 50 μg/mL in an in vitro assay.

[0022] In certain aspects, the invention provides a pharmaceutical composition comprising anyone of the antibodies of the invention or fragments thereof or any combination thereof.

[0023] In certain aspects, the invention provides a pharmaceutical composition comprising anyone of the antibodies of the invention, or a combination thereof.

[0024] In certain embodiments, the composition comprises an antibody or a fragment thereof which is recombinantly produced in CHO cells.

[0025] In certain aspects, the invention provides a pharmaceutical composition comprising a vector comprising a nucleic acid encoding anyone of inventive antibodies or fragments. In certain embodiments, the nucleic acids are optimized for expression in human host cells. In other 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. Non-limiting examples of such vectors include adenoviral vectors (Ads), adeno associated virus based vectors (AAVs), or a combination thereof. In certain aspects, the invention provides isolated cells comprising vectors and/or nucleic acids for expression of the inventive antibodies and fragments thereof. In certain aspects, the invention provides compositions of cells comprising vectors and/or nucleic acids for expression of the inventive antibodies and fragments thereof.

[0026] In certain embodiments, the compositions further comprise an additional antibody or fragment thereof. In certain embodiments, the compositions further comprise an antibody or a fragment thereof comprising CDR1, 2, and/or 3 of the VH and VL chains, or the VH and VL chains of antibody DH540. In certain embodiments, the compositions further comprise an antibody or a fragment thereof comprising CDR1, 2, and/or 3 of the VH and VL chains, or the VH and VL chains of antibody DH512.

[0027] In certain embodiments, the compositions further comprise an antibody or a fragment thereof comprising VH and VL chain of antibody DH429 or DH270IA1.

[0028] In certain embodiments, the antibody or antigen binding fragment can include an Fc domain that has been modified compared to a native Fc domain. 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.

[0029] In certain embodiments, the invention provides antibodies or fragments comprising a CDR(s) of the VH and/or VL chains, or VH and/or VL chains of the inventive antibodies, as the HIV-1 binding arm(s) of a bispecific molecules, e.g. but not limited to DARTS, diabodies, toxin labeled HIV-1 binding molecules.

[0030] In certain aspects the invention provides methods to treat or prevent HIV-1 infection in a subject comprising administering to the subject a pharmaceutical composition comprising any one of the inventive antibodies or fragments thereof in a therapeutically effective amount. The methods of the invention contemplate combination therapeutic methods, including but not limited to administering combinations of various antibodies or fragments thereof.

[0031] In certain embodiments of the methods, the pharmaceutical compositions are administered in a therapeutically effective dose and regimen.

BRIEF DESCRIPTION OF THE DRAWINGS

[0032] 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.

[0033] FIG. 1 shows DH542 sequences (CDRs are bolded and underlined) (SEQ ID NOs: 1-4).

[0034] FIG. 2 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).

[0035] FIG. 3 shows Neutralizing Breadth and Potency of various HIV-1 BnAbs that are candidates for being combined with the inventive antibodies or other antibodies for a potent mixture of bnAbs. DH270IA1 is I1 in the DH270 lineage.

[0036] FIG. 4 shows Neutralizing Breadth and Potency of some candidate bnAbs for single or combination use.

[0037] FIG. 5 shows comparison of some of the bnAbs of the invention with other bnAbs in the same class. % breadth refers to number of tested HIV-1 strains.

[0038] FIG. 6 shows nucleic acid sequences of antibodies DH511-518, DH537 and 538 (SEQ ID NOs: 53-72).

[0039] FIG. 7 shows amino acid sequences of antibodies DH511-518, DH537 and 538 (SEQ ID NOs: 73-93).

[0040] FIGS. 8A and 8B show Alignment of VH (8A) and VL (8B) Sequences of BnAb DH511 Clonal Lineage. Bolded is the sequence of CDR1, underlined is the sequence of CDR2 and italicized is the sequence of CDR3 of the DH511 VH chain and DH511 VL chain. The CDRs of the VH and VL sequences of the other antibodies DH512, DH513, DH514, DH515, and DH516 can be readily determined based on the sequence alignment. FIG. 8A shows SEQ ID NOs: 94-99 (in order of appearance from DH511-DH516). FIG. 8B shows SEQ ID NOs: 100-105 (in order of appearance from DH511-DH516).

[0041] FIGS. 9A and 9B show Alignment of VH (Figure 9A) and VL (Figure 9B) sequences of MPER BnAbs. Bolded is the sequence of CDR1, italicized is the sequence of CDR2 and underlined is the sequence of CDR3 of VH or VL of the listed MPER antibodies. FIG. 9A shows SEQ ID NOs: 106-114 (in order of appearance from DH511-2F5). FIG. 9B shows SEQ ID NOs: 115-123 (in order of appearance from DH511-2F5).

[0042] FIG. 10 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.

[0043] FIG. 11 shows CH557 gene information.

[0044] FIG. 12 shows sequences of CH557 (SEQ ID NOs: 124-127). CDRs are bolded and underlined.

[0045] FIG. 13 shows a sequence alignment of the CD540-VRC40 antibodies, listing the heavy and light chain variable region sequences, kabat and IMGT CDR and framework regions, and kabat numbering. The heavy and light chain variable region sequences of the CH540-VRC40.01, CH540-VRC40.02, CH540-VRC40.03, CH540-VRC40.04 are shown.

[0046] FIG. 14 shows gene information for antibodies in the CH235 lineage.

[0047] 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).

[0048] 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).

[0049] 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).

[0050]

[0051] 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).

[0052] 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).

[0053] 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.

[0054] FIG. 21 shows ELISA binding of CH490, CH491, CH492 and CH493 antibodies to various antigens as listed in the figure.

[0055] 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.

[0056] 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.

[0057] 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.

[0058] FIG. 25 shows results of HEp-2 cell IF staining for CH557.

[0059] FIGS. 26A and 26B shows summary of data from CH557 microarray polyreactivity.

[0060] 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.

[0061] FIG. 27B shows the mean IC50 and percent of isolates neutralized at different IC50 values.

[0062] 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.

[0063] FIG. 28B shows the mean IC80 and percent of isolates neutralized at different IC80<50ug/ml values.

[0064] 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 V_H were complemented with full heavy chain gene regions and paired with the V_L 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 IC_{50}. See also Figures 36A-B, 40C, and 41.

[0065] 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) V_H-gene SHM of CD4bs-directed antibodies *vs* neutralization breadth. See also Figures 37A-G, and 42.

[0066] 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 V_H1-46-derived CD4bs mAbs and selected V_H1-2-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) V_H-gene mutability accounts for the majority of positional conformity of CH235 lineage. The mutability of the V_H-gene for V_H1-46 (top) and V_H1-2 (bottom) 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 V_H1-46-derived antibodies. See also Figures 38A-E, and 43A-C.

[0067] FIGS. 32A-32D show binding Kinetics of CH103 and CH235 Lineage Antibodies. Binding association (k_a) and dissociation (k_d) 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 (K_d). K_ds are shown in A and C, k_a (solid lines, plotted on the left y-axis) and k_d (dashed lines, plotted on the right y-axis) are shown in B and D. See also Figures 44A-B.

[0068] 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 IC_{50}. 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 IC_{50} 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 β20- β21 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.

[0069] 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.

[0070] 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).

[0071] FIGS. 36A-36B show CH235 Lineage: Sequences and Neutralization Fingerprint Dendrogram, Related to Figure 29. (A) Alignment of NGS sequences 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).

[0072] 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.

[0073] 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 and CH235 to the IGHV1-46 germline gene 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.

[0074] 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.

[0075] 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.

[0076] FIG. 40C shows a Table with characteristics of the V(D)J rearrangements of key CH235 lineage antibodies. Related to Figure 29.

[0077] 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.

[0078] FIG. 42 shows a Table with crystallographic Data Collection and Refinement Statistics. Related to Figures 30A-30E.

[0079] 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 V_H 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) ($P_{uniform}$), or the mutation frequency at each residue position derived from the VH1-46 antibodies ($P_{VH1\text{-}46}$). (B) The probability of a conforming VH1-46 antibody with x V_H 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) ($P_{uniform}$), or the mutation frequency at each residue position derived from the VH1-46 antibodies ($P_{VH1\text{-}46}$). (C) Pearson correlation coefficients of positional somatic mutation frequency between VH1-46, VH1-2 and three others.

[0080] 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.

[0081] FIG. 45 shows CH235 lineage autologous neutralization. Related to Figure 33A-C.

[0082] FIG. 46 shows CH235 lineage antibodies and CH235.9 mutants neutralization of CH505 TF loop D mutant viruses. Related to Figure 33A-C.

[0083] FIG. 47 shows binding of antibodies in the CH235 and CH103 lineages to CH505 autologous Env glycoproteins, Related to Figure 34A-B.

[0084] Figures 48A-48D show non-limiting examples of mutations in the VH chain of DH511 and DH512, and non-limiting examples of sequences including mutations in DH512 VH chain. Figure 48A shows positions in the VHCDR3 chain of DH511 which could be mutated. Amino acid positions refer to Kabat numbering. Most mutations are to changes to W, but F, L or possibly other substitutions can also be tried. Figure 48B shows positions in the VHCDR3 chain of DH512 which could be mutated. Amino acid positions refer to Kabat numbering for the DH512VH chain: QVQLVQSGGGLVKPGGSLTLSCSASGFFFDNSWMGWVRQAPGKGLEWVGRIRRLKDGAT GEYGAAVKDRFTISRDDSRNMLYLHMRTLKTEDSGTYYCTMDEGTPVTRFLEWGYFYYY MAVWGRGTTVIVSS (SEQ ID NO: 213). Most mutations are to changes to W, but F, L or possibly other substitutions can also be tried. For both DH511 and DH512 position V100 can be changed to I. Position L100d can be changed to F. For both DH511 and DH512 combination mutations in the DH512 or DH511 VHCDR3 could include VH_L100dF together with T100aW; VH_L100dW together with T100aW. Figure 48C shows positions outside of VHCDR3 which could be mutated. Most mutations are to changes to W, F, L or possibly other substitutions can also be tried. Figure 48D shows amino acid sequences (SEQ ID NOs: 195-212) of some of the DH512 mutants from Figure 48B.

DETAILED DESCRIPTION

[0085] 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.

[0086] 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.

[0087] 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.

[0088] 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.

[0089] 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. In non-limiting embodiments, the human monoclonal antibodies disclosed herein can be used to detect HIV-1 in a biological sample or interfere with the HIV-1 activity, for example to diagnose or treat a subject having an HIV-1 infection and/or AIDS. For example, the antibodies can be used to determine HIV-1 titer in a subject. The antibodies disclosed herein also can be used to study the biology of the human immunodeficiency virus. The antibodies of the invention can be used for therapeutic purposes for treatment or prevention of HIV-1 infection, alone or in combination with other therapeutic modalities, including ART and/or combination with other HIV-1 targeting antibodies, neutralizing antibodies and/or ADCC inducing antibodies.

[0090] In some embodiments, the antibodies of the invention are expected not to exhibit self-reactivity-- they do not bind or bind very weakly to self-antigens, such as human protein. For example, the antibodies of clone DH511 are not self-reactive although their UCA and some IAs are polyreactive. For use as preventive or therapeutic agents, what matters is whether the mature antibody will be polyreactive or not, and for example DH542 is not. DH270IA1 does not show self-reactivity, while DH491 and DH493 antibodies are polyreactive to varying degrees. Broadly neutralizing antibody CH557 displays exceptional neutralization breadth and high potency (Figure

10) and it is not autoreactive nor polyreactive as determined by lack of binding to known human antigens associated with autoimmune disorders), negativity in Hep-2 cells IF staining (Figure 25) and lack of binding to an array of 9,400 human antigens (Figure 26A and B), including UBE3A and STUB-1 proteins, known to be bound by previously described broadly neutralizing antibodies targeting the CD4bs of gp120 Env (Liu et al J Virol 2014, Bonsignori et al JCI 2014).

[0091] 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.

[0092] 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.

[0093] 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.

[0094] 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.

[0095] 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.

[0096] 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.

[0097] 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% 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 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.

[0098] In certain embodiments, the invention provides antibodies 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.

[0099] 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.

[0100] In certain embodiments the invention provides 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.

[0101] 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.

[0102] 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).

[0103] To create a single chain antibody, (scFv) the VH- and VL-encoding DNA fragments are operatively linked to another fragment encoding a flexible linker, e.g., encoding the amino acid sequence $(G1Y_4\text{-Ser})_3$, such that the VH and VL sequences can be expressed as a contiguous single-chain protein, with the VH and VL domains joined by the flexible linker (see, e.g., Bird et al., Science 242:423-426, 1988; Huston et al., Proc. Natl. Acad. Sci. USA 85:5879-5883, 1988; McCafferty et al., Nature 348:552-554, 1990). Optionally, a cleavage site can be included in a linker, such as a furin cleavage site.

[0104] In some embodiments, a single chain antibody may be monovalent, if only a single VH and VL are used, bivalent, if two VH and VL are used, or polyvalent, if more than two VH and VL are used. Bispecific or polyvalent antibodies may be generated that bind specifically to different epitopes within the envelope. Bispecific or polyvalent antibodies may be generated that bind specifically to different epitopes within the envelope, and/or to another molecule.

[0105] 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.

[0106] The antibodies described herein, or fragments thereof, 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 antibody 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. Thus, in certain embodiments, the invention provides an antibody, or antibody 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, wherein the antibody or antibody fragment was recombinantly produced in a mammalian cell-line, and wherein the antibody or antibody 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.

[0107] Furthermore, large-scale production of therapeutic-grade antibodies are much different than those for laboratory scale. There are extreme purity requirements for therapeutic-grade. Large-scale production of therapeutic-grade antibodies 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 antibody variants that are unique to the recombinant production process (*i.e.,* antibody 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 antibody 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 antibody)). In other embodiments, the antibody 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 antibody or antibody fragment (*i.e.,* protein A is a standard approach for purifying antibodies 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.)

[0108] In certain embodiments, the invention provides monoclonal antibodies. In certain embodiments the monoclonal antibodies are produced by a clone of B-lymphocytes. In certain embodiments the monoclonal antibody is a recombinant and is produced by a host cell into which the light and heavy chain genes of a single antibody have been transfected. Any suitable cell could be used for transfection and expression of the antibodies of the invention. Suitable cell lines include without limitation 293T cells or CHO cells.

[0109] Monoclonal antibodies are produced by any suitable method known to those of skill in the art. In some embodiments, monoclonal antibodies are produced by immortalizing B-cell expressing an antibody. Methods for immortalizing B-cells are known in the art, for example but

not limited to using EBV transformation, treatment with various stimulants, and/or apoptotic inhibitors (Bonsignori et al. J. Virol. 85: 9998-10009, 2011). In some embodiments, monoclonal antibodies are produced by making hybrid antibody-forming cells from a fusion of myeloma cells with immune spleen cells to make hybridomas. In some embodiments monoclonal antibodies are identified from a subject, for example but not limited as described in Example 1 (Liao HX et al. J Virol Methods. 2009 Jun;158(1-2):171-9). The amino acid and nucleic acid sequences of such identified monoclonal antibodies can be determined.

[0110] The antibodies of the invention can be of any isotype. In certain embodiments, the antibodies of the invention can be used as IgG1, IgG2, IgG3, IgG4, whole IgG1 or IgG3s, whole monomeric IgAs, dimeric IgAs, secretory IgAs, IgMs as monomeric, pentameric or other polymer forms of IgM. The class of an antibody comprising the VH and VL chains described herein can be specifically switched to a different class of antibody by methods known in the art.

[0111] In some embodiments, the nucleic acid encoding the VH and VL can encode an Fc domain (immunoadhesin). The Fc domain can be an IgA, IgM 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 one example, the immunoadhesin is an IgG1 Fc. In one example, the immunoadhesin is an IgG3 Fc.

[0112] In certain embodiments the antibodies comprise amino acid alterations, or combinations thereof, for example in the Fc region 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 region mutation, incorporated by reference in their entirety. In certain embodiments, the antibodies 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). Other antibody mutations have been reported to improve antibody half-life or function or both and can be incorporated in sequences of the antibodies. 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 region 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 antibody 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 antibody fucosylation, may affect interaction with Fc receptors (See e.g. Moldt, et al. JVI 86(11): 66189-6196, 2012). In some embodiments, the antibodies can comprise modifications, for example but not limited to glycosylation, which reduce or eliminate polyreactivity of an antibody. See e.g. Chuang, et al. Protein Science 24: 1019-1030, 2015. In some embodiments the antibodies 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 Fc.gamma.RIIA or to Fc.gamma.RIIIA; decreased binding to Fc.gamma.RIIB; or increased binding to Fc.gamma.RIIB. See e.g. US Pub 20140328836.

[0113] In certain embodiments, antibodies of the invention including but not limited to antibodies comprising a CDR(s) of VH and/or VL chains, or antibody fragments of the inventive antibodies can be used as the HIV-1 binding arm(s) of a bispecific molecule, e.g. DARTS, diabodies, toxin labeled HIV-1 binding molecules.

[0114] In accordance with the methods of the present invention, either the intact antibody or a fragment thereof can be used. Either single chain Fv, bispecific antibody for T cell engagement, or chimeric antigen receptors can be used (Chow et al, Adv. Exp. Biol. Med. 746:121-41 (2012)). That is, in non-limiting embodiments, intact antibody, a Fab fragment, a diabody, or a bispecific whole antibody can be used to inhibit HIV-1 infection in a subject (e.g., a human). A bispecific $F(ab)_2$ can also be used with one arm a targeting molecule like CD3 to deliver it to T cells and the other arm the arm of the native antibody (Chow et al, Adv. Exp. Biol. Med. 746:121-41 (2012)). Toxins that can be bound to the antibodies or antibody fragments described herein include unbound antibody, radioisotopes, biological toxins, boronated dendrimers, and immunoliposomes (Chow et al, Adv. Exp. Biol. Med. 746:121-41 (2012)). Toxins (e.g., radionucleotides or other radioactive species) can be conjugated to the antibody or antibody fragment using methods well known in the art (Chow et al, Adv. Exp. Biol. Med. 746:121-41 (2012)). 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 methods of the invention.

[0115] Antibodies of the invention and fragments thereof can be produced recombinantly using nucleic acids comprising nucleotide sequences encoding VH and VL sequences selected from those shown in the figures and examples.

[0116] In certain embodiments the invention provides intact/whole antibodies. In certain embodiments the invention provides antigen binding fragments thereof. Typically, fragments 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 can be produced by recombinant DNA techniques, or by enzymatic or chemical separation of intact immunoglobulins.

[0117] In certain embodiments the invention provides a bispecific antibody. A bispecific or bifunctional/dual targeting antibody is an artificial hybrid antibody having 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); Garber "Bispecific antibodies rise again" Nature Reviews Drug Discovery 13, 799–801 (2014), Figure 1a; 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 antibody is a whole antibody of any isotype. In other embodiments it is a bispecific fragment, for example but not limited to $F(ab)_2$ fragment. In some embodiments, the bispecific antibodies do not include Fc portion, which makes these diabodies relatively small in size and easy to penetrate tissues.

[0118] In certain embodiments, the bispecific antibodies could include Fc region. Fc bearing diabodies, for example but not limited to Fc bearing DARTs 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, incorporated by reference in their entirety. In certain embodiments, the invention encompasses diabody 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 diabody 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 diabody 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)).

[0119] 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. The hinge domain, or optionally the hinge-Fc domain, may be engineered in to a polypeptide of the invention in any position relative to other domains or portions of the polypeptide chain. In certain embodiments, a polypeptide chain of the invention comprises a hinge domain, which hinge domain is at the C-terminus of the polypeptide chain, wherein the polypeptide chain does not comprise an Fc domain. In yet other embodiments, a polypeptide chain of the invention comprises a hinge-Fc domain, which hinge-Fc domain is at the C-terminus of the polypeptide chain. In further embodiments, a polypeptide chain of the invention comprises a hinge-Fc domain, which hinge-Fc domain is at the N-terminus of the polypeptide chain.

[0120] In some embodiments, the invention encompasses multimers of 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 some embodiments, the polypeptide chains in multimers further comprise an Fc domain. Dimerization of the Fc domains leads to formation of a diabody molecule that exhibits immunoglobulin-like functionality, i.e., Fc mediated function (e.g., Fc-Fc.gamma.R interaction, complement binding, etc.).

[0121] In yet other embodiments, diabody molecules of the invention encompass tetramers of polypeptide chains, each of which polypeptide chain comprises a VH and VL domain. In certain embodiments, two polypeptide chains of the tetramer further comprise an Fc domain. The tetramer is therefore comprised of two `heavier` polypeptide chains, each comprising a VL, VH and Fc domain, and two `lighter` polypeptide chains, comprising a VL and VH domain. Interaction of a heavier and lighter chain into a bivalent monomer coupled with dimerization of the monomers via the Fc domains of the heavier chains will lead to formation of a tetravalent immunoglobulin-like molecule (exemplified in Example 6.2 and Example 6.3). In certain aspects the monomers are the same, and the tetravalent diabody molecule is monospecific or bispecific. In other aspects the monomers are different, and the tetra valent molecule is bispecific or tetraspecific.

[0122] Formation of a tetraspecific diabody molecule as described supra requires the interaction of four 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 increase 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 diabody molecule, 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).

[0123] The invention also encompasses diabody molecules comprising variant Fc or variant hinge-Fc domains (or portion 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.

[0124] The bispecific diabodies 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 preferred 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 diabody molecule binds to the effector cell determinant and also activates the effector cell. In this regard, diabody molecules of the invention 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 (e.g., ADCC assay).

[0125] Non-limiting examples of bispecific antibodies can also be (1) a dual-variable-domain antibody (DVD-Ig), where each light chain and heavy chain contains two variable domains in tandem through a short peptide linkage (Wu et al., Generation and Characterization of a Dual Variable Domain Immunoglobulin (DVD-Ig.TM.) Molecule, In: Antibody Engineering, Springer Berlin Heidelberg (2010)); (2) a Tandab, which is a fusion of two single chain diabodies resulting in a tetravalent bispecific antibody that has two binding sites for each of the target antigens; (3) a flexibody, which is a combination of scFvs with a diabody resulting in a multivalent molecule; (4) a so called "dock and lock" molecule, based on the "dimerization and docking domain" in Protein Kinase A, which, when applied to Fabs, can yield a trivalent bispecific binding protein consisting of two identical Fab fragments linked to a different Fab fragment; (5) a so-called Scorpion molecule, comprising, e.g., two scFvs fused to both termini of a human Fc-region. Examples of platforms useful for preparing bispecific antibodies include but are not limited to BiTE (Micromet), DART (MacroGenics) (e,g, US Patents 8,795,667; 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), Fcab and Mab2 (F-star), Fc-engineered IgG1 (Xencor) or DuoBody (based on Fab arm exchange, Genmab).

[0126] In certain embodiments, the bispecific antibody comprises an HIV envelope binding fragment, for example but not limited to an HIV envelope binding fragment from any of the antibodies described herein. In other embodiments, the bispecific antibody further comprises a second antigen-interaction-site/fragment. In other embodiments, the bispecific antibody further comprises at least one effector domain.

[0127] In certain embodiments the bispecific antibodies engage cells for Antibody-Dependent Cell-mediated Cytotoxicity (ADCC). In certain embodiments the bispecific antibodies engage natural killer cells, neutrophil polymorphonuclear leukocytes, monocytes and macrophages. In certain embodiments the bispecific antibodies are T-cell engagers. In certain embodiments, the bispecific antibody comprises an HIV envelope binding fragment and CD3 binding fragment. Various CD3 antibodies are known in the art. See for example US Patent 8,784,821. In certain embodiments, the bispecific antibody comprises an HIV envelope binding fragment and CD16 binding fragment.

[0128] In certain embodiments the invention provides antibodies with dual targeting specificity. In certain aspects the invention provides bi-specific 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 antibodies bind with one "arm" to a surface antigen on target cells, and with the second "arm" to an activating, invariant component of the T cell receptor (TCR) complex. The simultaneous binding of such an antibody 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 antibody to them, i.e. the immunological synapse is mimicked. Particularly desirable are bispecific antibodies that do not require lymphocyte preconditioning or co-stimulation in order to elicit efficient lysis of target cells.

[0129] Several bispecific antibody formats have been developed and their suitability for T cell mediated immunotherapy investigated. Out of these, the so-called BiTE (bispecific T cell engager) molecules have been very well characterized and already shown some promise in the clinic (reviewed in Nagorsen and Bauerle, Exp Cell Res 317, 1255-1260 (2011)). BiTEs are tandem scFv molecules wherein two scFv molecules are fused by a flexible linker. Further bispecific formats being evaluated for T cell engagement include diabodies (Holliger et al., Prot Eng 9, 299-305

(1996)) and derivatives thereof, such as tandem diabodies (Kipriyanov et al., J Mol Biol 293, 41-66 (1999)). DART (dual affinity retargeting) molecules are based on the diabody format that separates cognate variable domains of heavy and light chains of the two antigen binding specificities on two separate polypeptide chains but feature a C-terminal disulfide bridge for additional stabilization (Moore et al., Blood 117, 4542-51 (2011)). The invention also contemplates Fc-bearing DARTs. The so-called triomabs, which are whole hybrid mouse/rat IgG molecules and also currently being evaluated in clinical trials, represent a larger sized format (reviewed in Seimetz et al., Cancer Treat Rev 36, 458-467 (2010)).

[0130] The invention also contemplates bispecific molecules with enhanced pharmacokinetic properties. In some embodiments, such molecules are expected to have increased serum half-life. In some embodiments, these are Fc-bearing DARTs (see supra).

[0131] 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, CD16, or another suitable target. 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 induce T cell-mediated killing. In certain embodiments, the bispecific antibodies are whole antibodies. In other embodiments, the dual targeting antibodies consist essentially of Fab fragments. In other embodiments, the dual targeting antibodies comprise a heavy chain constant region (CH1. In certain embodiments, the bispecific antibody does not comprise Fc region. In certain embodiments, the bispecific antibodies have improved effector function. In certain embodiments, the bispecific antibodies have improved cell killing activity. Various methods and platforms for design of bispecific antibodies are known in the art. See for example US Pub. 20140206846, US Pub. 20140170149, US Pub. 20090060910, US Pub 20130295121, US Pub. 20140099318, US Pub. 20140088295 which contents are herein incorporated by reference in their entirety.

[0132] In certain embodiments the invention provides human, humanized and/or chimeric antibodies.

[0133] **Pharmaceutical compositions**

[0134] In certain aspects the invention provides a pharmaceutical composition comprising an antibody of the invention wherein the composition is used for therapeutic purposes such as but not limited to prophylaxis, treatments, prevention, and/or cure. In certain aspects the invention

provides a pharmaceutical composition comprising an antibody of the invention in combination with any other suitable antibody. In certain embodiments, the pharmaceutical compositions comprise nucleic acids which encode the antibodies of the invention. In certain embodiments, these nucleic acids can be expressed by any suitable vector for expression of antibodies. Non-limiting examples include attenuated viral hosts or vectors or bacterial vectors, recombinant vaccinia virus, adenovirus, adeno-associated virus (AAV), herpes virus, retrovirus, cytomegalovirus or other viral vectors can be used to express the antibody.

[0135] Various methods to make pharmaceutical compositions are known in the art and are contemplated by the invention. In some embodiments, the compositions include excipient suitable for a biologic molecule such as the antibodies of the invention. In some embodiments, the antibodies could be produced in specific cell lines and conditions so as to control glycosylation of the antibody. In some embodiments, the antibody framework for example, could comprise specific modification so as to increase stability of the antibody.

[0136] In certain aspects, the invention provides that the antibodies, and fragments thereof, described herein can be formulated as a composition (e.g., a pharmaceutical composition). Suitable compositions can comprise an inventive antibody (or antibody fragment) dissolved or dispersed in a pharmaceutically acceptable carrier (e.g., an aqueous medium). The compositions can be sterile and can be in an injectable form (e.g. but not limited to a form suitable for intravenous injection, intramascular injection). The antibodies (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 antibodies (and fragments thereof) can also be formulated as a composition appropriate for intranasal administration. The antibodies (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.

[0137] The antibody (and fragments thereof), 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 antibodies described herein (or fragments thereof) and 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 antibodies described herein (or fragments thereof) can be administered as post-exposure prophylaxis, e.g., IV or topically, and

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

[0138] In accordance with the invention, the antibodies (or antibody fragments) 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.

[0139] In addition, various forms of the antibodies described herein can be administered to chronically or acutely infected HIV patients and used to kill remaining virus infected cells by virtue of these antibodies binding to the surface of virus infected cells and being able to deliver a toxin to these reservoir cells.

[0140] Suitable dose ranges can depend on the antibody (or fragment) 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 antibodies in the range of 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 or 10 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). In other embodiments, the antibodies 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.

[0141] In certain aspects the invention provides use of the antibodies of the invention, including bispecific antibodies, 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 the antibodies of the invention in a pharmaceutically acceptable form. In certain embodiment, the methods include a composition which includes more than one HIV-1 targeting antibody. In certain embodiments, the HIV-1 targeting antibodies in such combination bind different epitopes on the HIV-1 envelope. In certain embodiments, such combinations of bispecific antibodies targeting more than one HIV-1 epitope provide increased killing of HIV-1 infected cells. In other embodiments, such combinations of bispecific antibodies targeting more than one HIV-1 epitope provide increased breadth in recognition of different HIV-1 subtypes.

[0142] In certain embodiments, the composition comprising the antibodies of the invention alone or in any combination can be administered via IM, subcutaneous, or IV delivery, or could be deposited at mucosal sites, such as the oral cavity to prevent maternal to child transmission, the

rectal space or the vagina as a microbicide. In certain embodiments, the antibodies can be administered locally in the rectum, vagina, or in the oral cavity, and can be formulated as a microbiocide (Hladik F et al ELIFE Elife. 2015 Feb 3;4. doi: 10.7554/eLife.04525.; Multipurpose prevention technologies for reproductive and sexual health. Stone A. Reprod Health Matters. 2014 Nov;22(44):213-7. doi: 10.1016/S0968-8080(14)44801-8). In other embodiments, antibodies can be formulated such that the therapeutic antibody or combination thereof is impregnated on a vaginal ring (Chen Y et al. Drug Des. Devel. Ther 8: 1801-15, 2014;Malcolm RK et al BJOG 121 Suppl 5: 62-9, 2014). Antibodies can be administered alone or with anti-retroviral drugs for a combination microbicide (Hladik F et al ELIFE Elife. 2015 Feb 3;4. doi: 10.7554/eLife.04525).

[0143] Alternatively they can be administered in complex with a form of HIV Env, optimally gp120, but also an Env trimer, to enhance Env immunogenicity. In certain embodiments, the antibodies can be delivered by viral vector mediated delivery of genes encoding the antibodies of the invention (See e.g. Yang et al. Viruses 2014, 6, 428-447). In certain embodiments, the antibodies can be administered in viral vector, for example but not limited to adenoassociated viral vector, for expression in muscle and plasma.

[0144] In certain embodiments, antibodies with different binding specificities are combined for use in pharmaceutical compositions and therapeutic methods. For example: CD4 binding site antibodies are combined with V3 antibodies, MPER antibodies and so forth. Figures 2, 3, and 4 show a selection of potent HIV-1 neutralizing antibodies which can be used in pharmaceutical compositions, and therapeutic methods. Non-limiting examples of selections of combinations of certain antibodies include: DH542, DH542_L4, DH542_QSA, DH429 and DH512 (or any of the DH512 variants); DH512 and CH31 (See US Publication20140205607); DH512 (or any of the DH512 variants) and DH540 (See Example 9, and this antibody will be described elsewhere); DH542, DH542_L4, DH542_QSA, DH429, DH512 and DH540; DH542, DH542_L4, DH542_QSA, DH429 and CH557; CH557 and DH512 (or any of the DH512 variants). These combinations are expected to give a greater overall potency and breadth. A polyclonal mixture of Abs is expected reduce or eliminate viral escape. It is readily understood by skilled artisans that in some embodiments a combination therapy envisions a composition which combines various antibodies. In other embodiments a combination therapy is provided wherein antibodies are administered as individual compositions, for example at different times, by different means, or at administered at different locations. In other embodiments, a combination therapy is provides wherein a therapeutic antibody or antibodies is combined with other therapeutic means, for example anti-retroviral drug cocktails, or drugs which activate latently infected HIV-1 cells.

[0145] In some embodiments, the disclosed antibodies or antigen binding fragments thereof are used to determine whether HIV-1 envelope(s) is a suitable antigen for inclusion in a vaccine composition. For example the antibodies can be used to determine whether an antigen in a vaccine composition including gp120 assumes a conformation including an epitope bound by the inventive antibodies or fragments thereof. This can be readily determined by a method which includes contacting a sample containing the vaccine, such as a gp120 antigen, with a disclosed antibody or antigen binding fragment under conditions sufficient for formation of an immune complex, and detecting the immune complex, to detect an HIV-1 antigen including an epitope of an inventive antibody in the sample. In one example, the detection of the immune complex in the sample indicates that vaccine component, such as a HIV-1 Env antigen assumes a conformation capable of binding the antibody or antigen binding fragment.

[0146] 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

[0147] 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.

[0148] 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.

[0149] Recombinant antibodies are grown and supernatants and/or purified antibodies are functionally characterized.

[0150] 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).

[0151] 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.

[0152] 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

[0153] 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.

[0154] The TZMbl assay was conducted to determine neutralization potency and breadth of the various antibodies of the invention on different HIV-1 pseudoviruses.

[0155] 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.

[0156] 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.

[0157] Figure 28B shows the mean IC80 and percent of isolates neutralized at different IC80<50ug/ml values.

Example 3: Epitope mapping of antibodies

[0158] 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

[0159] 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.

[0160] Various assays and experiments can be designed to analyze prevention, treatment and/or cure.

Example 5: Assay for self-reactivity

[0161] **Table 3** 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

[0162] 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. In our experiments the criteria for positivity is as follows: an antibody is positive for autoreactivity if reactive at 25 μg/ml.

[0163] **Table 4.** Summary of immunoflourescent (IF) staining of Hep2 cells data for antibodies DH270IA1, CH491, CH493. DH270IA1 does not show self-reactivity. CH491 and CH493 show some self-reactivity in this assay.

Antibody ID	Concentration	Score	Staining Pattern
DH270_IA1/293i	[50ug/mL}	-	
DH270_IA1/293i	[25ug/mL}	-	
CH491_4A/293i	[50ug/mL}	2+	nuclear diffuse, cytoplasmic
CH491_4A/293i	[25ug/mL}	1+	nuclear diffuse, cytoplasmic
CH493_4A/293i	[50ug/mL}	2+	cytoplasmic
CH493_4A/293i	[25ug/mL}	1+	cytoplasmic

[0164] **Table 5**. Summary of Athena data for DH270IA1, CH491, CH493. DH270IA1 does not show self-reactivity. CH491 and CH493 show some self-reactivity in this assay.

Lot	Antibody ID		SSA	SSB	Sm	RNP	Scl 70	Jo 1	dsDNA	Cent B	Histone
294HC	**DH270_IA1/293i**	50	5	6	5	2	4	5	32	10	9
		25	3	6	5	2	1	3	18	5	4
		12.5	3	6	5	3	2	6	11	3	4

		6.25	5	1	3	2	2	5	6	2	2
21RKK	**CH491_4A**	50	68	144	6	43	16	169	47	54	59
		25	44	95	4	30	9	118	39	45	44
		12.5	28	60	3	24	7	79	29	34	34
		6.25	21	42	4	18	4	56	11	26	26
23RKK	**CH493_4A**	50	155	70	37	69	36	198	0	94	166
		25	100	38	53	55	34	167	0	129	202
		12.5	54	22	42	49	26	102	0	165	200
		6.25	22	15	46	32	17	54	0	177	174

[0165] 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. We have previously reported that, in subject CH505, the CD4bs CH103 bnAb lineage is polyreactive and, similarly to CD4 mimic VRC01-class bnAbs, bound to human ubiquitin ligase E3A (UBE3A) with avidity correlated with neutralization (Liao et al Nature 2013; Liu et al J Virol 2015). Since CH557 is a potent and extremely broad CD4 mimic CD4bs bnAb, we compared the auto- and polyreactivity profiles of CH557 with those of early precursors of the CH235 antibody lineage (UCA, IA4, IA3, IA2, IA1, CH235, CH236, CH239, CH240 and CH241). In line with previous observations, reactivity against autoantigens developed among early CH235 lineage members with maturation. However, bnAb CH557 itself became exquisitely HIV-1 specific: it does not react with cardiolipin or other antigens associated with autoimmune disorders, it is negative in Hep-2 IF staining (Figure 25), or any of 9,400 human antigens, including UBE3A (Figure 26A-B). Albeit reactivity against other human antigens cannot be formally ruled out, these data demonstrate that bnAb CH557 lost the auto and polyreactivity developed by its precursors, and demonstrates that decoupling neutralization breadth of CD4 mimic CDbs bnAbs from auto- and polyreactivity is an achievable goal.

[0166] **Table 6**. Summary of Athena assay results for CH557. Results are expressed as relative luminescence units. Readings <100 are considered negative, results between 100 and 120 are considered "indeterminate" and results >120 are considered positive. CH557 is negative for all the antigens tested at all antibody concentrations ranging from 6.25 ug/ml to 50 ug/ml.

Antibody	**ug/ml**	**SSA**	**SSB**	**Sm**	**RNP**	**Scl 70**	**Jo 1**	**dsDNA**	**Cent B**	**Histone**
4 e 10	50	49	263	5	4	1	190	2	3	16

	25	33	227	2	3	1	160	2	3	10
	12.5	21	199	3	3	0	131	3	1	7
	6.25	17	178	3	3	1	113	0	2	6
synagis	50	5	6	11	10	3	5	25	9	11
	25	3	7	3	4	2	2	11	4	5
	12.5	2	5	7	3	3	6	7	2	3
	6.25	2	5	2	3	2	1	0	2	3
CH557_4A /293i	50	6	15	8	10	6	10	29	46	16
	25	4	12	6	6	4	2	18	25	8
	12.5	3	9	6	3	3	5	10	16	6
	6.25	4	5	4	4	3	5	9	11	4

[0167] **Table** 7. Summary of Athena assay results for various other antibodies of the CH235 lineage. Results are expressed as relative luminescence units. Readings <100 are considered negative, results between 100 and 120 are considered "indeterminate" and results >120 are considered positive. CH236, CH239, CH235 IA1 and IA2 are positive for multiple antigens.

Antibody ID		SSA	SSB	Sm	RNP	Scl 70	Jo 1	dsDNA	Cent B	Histone
synagis	50	13	9	6	2	1	4	3	3	0
	25	11	4	4	0	2	4	0	2	2
4E10 IgG1	50	99	207	55	28	5	227	14	14	32
	25	81	189	46	20	4	206	14	9	24
CH235_4A	50	12	7	16	11	8	7	25	11	14
	25	13	5	7	5	4	4	13	6	7
	12.5	13	5	8	6	2	3	0	5	5
	6.25	12	6	5	3	2	2	3	3	5
CH236_4A/293i	50	177	207	128	65	57	145	2	84	138
	25	224	175	165	69	62	72	42	129	193
	12.5	185	63	258	78	50	19	179	233	234
	6.25	44	15	184	40	31	7	228	208	154

CH239_4A/293i	50	289	10	250	93	kriss	15	38	173	228
	25	306	8	237	96	51	11	61	199	253
	12.5	277	6	277	85	48	8	108	216	263
	6.25	178	5	285	78	49	8	181	260	266
CH240_4A/293i	50	16	17	33	17	6	13	75	37	42
	25	16	11	22	11	6	11	50	24	27
	12.5	14	5	13	8	4	7	37	15	18
	6.25	12	5	9	6	3	5	14	8	9
CH241	50	23	10	12	8	5	8	23	21	30
	25	15	6	11	6	4	5	18	14	20
	12.5	14	8	7	5	3	6	2	8	13
	6.25	15	4	4	3	1	2	6	6	9
CH235UA/293i	50	11	3	8	5	3	3	7	5	7
	25	9	3	6	4	3	5	0	3	3
	12.5	9	4	6	3	3	5	4	3	4
	6.25	10	5	5	2	3	3	3	2	2
CH235VH_UCAtk_v2_ 4A/293i	50	14	10	13	11	5	6	22	28	17
	25	11	6	10	6	3	4	13	18	11
	12.5	10	7	9	7	4	6	9	10	7
	6.25	10	5	7	6	3	3	4	7	5
CH235VH_I1_v2_4A/ 293i	50	149	217	104	80	57	176	12	80	100
	25	150	197	110	67	52	171	19	80	99
	12.5	151	167	77	56	40	152	58	81	100
	6.25	175	117	77	46	31	129	61	87	118
CH235VH_I2_v2_4A/ 293i	50	73	69	259	101	69	55	444	256	371
	25	43	36	256	93	49	26	496	228	302
	12.5	34	31	279	85	44	20	617	225	287
	6.25	18	15	204	66	28	12	599	183	207
CH235VH_I3_v2_4A/ 293i	50	14	10	18	10	5	6	35	37	17

	25	10	9	13	7	4	8	33	27	12
	12.5	12	6	12	5	3	3	23	15	7
	6.25	12	3	7	4	2	2	15	9	5
CH235VH_I4_v2_4A/ 293i	50	12	6	12	9	4	5	15	14	13
	25	12	4	12	5	2	2	7	10	9
	12.5	11	3	6	4	2	3	11	5	6
	6.25	11	5	6	3	2	4	4	5	4
Cat-CH106	50	12	3	5	2	3	3	6	2	2
	25	8	3	1	3	2	2	3	2	3
	12.5	11	5	4	2	3	3	5	2	2
	6.25	10	6	5	1	2	4	7	1	2

[0168] **Table 8**. Summary of ELISA cardiolipin assay results for CH557 and various other antibodies. Antibodies were tested at concentrations ranging from 100ug/ml to 12.5 ug/ml. Results are expressed as optical density at wavelength of 450nm (OD450). OD450 < 0.2 are negative. Synagis is used as negative control and 4E10 is used as positive control. CH557 did not bind to cardiolipin.

Antibody	**lot**	**ug/ml**	**100**	**50**	**25**	**12.5**
4 e 10		11.94	2.0997	2.346	1.9027	1.6277
synagis		1.05	0.0424	0.0474	0.0408	0.0408
Ab901754RhK/PEI	ZRJ070	3.86	0.0382	0.0859	0.0299	0.0394
Ab901754RhKMut58_9	ZRJ93	2.8	0.6665	0.428	0.2898	0.1681
AbTr900114147Rh/293i	226JAH	7.44	0.4615	0.2596	0.1778	0.1018
DH522	64RKK	6.67	0.1206	0.079	0.0613	0.0491
DH522UCA_Rh/293i	362HC	13.19	0.6156	0.415	0.2179	0.1302
DH522_v2Rh/293i	363HC	6.93	0.3497	0.1652	0.1081	0.0828
DH522I1.2Rh/293i	372HC	13.99	0.5506	0.2738	0.183	0.1073
CH557_4A/293i	70RKK	11.94	0.0942	0.0761	0.0591	0.0475
DH542-293i	014RM	2.51	0.062	0.0503	0.0525	0.0511

[0169] **Table 9**. Summary of binding of listed antibodies to cardiolipin in ELISA. Antibodies were tested at concentrations ranging from 100ug/ml to 12.5 ug/ml. Results are expressed as optical density at wavelength of 450nm (OD450). OD450 < 0.2 are negative. Synagis is used as negative control and 4E10 is used as positive control. The majority of CH235 lineage antibodies, with the exception of CH235_IA3, IA4, CH235 and CH240 (the former has borderline binding), bound to cardiolipin.

		100	33.33333	11.11111	3.703704	1.234568	0.411523	0.137174	0.045725
	synagis	0.0391	0.0375	0.0359	0.0378				
	4E10 IgG1	2.3179	2.2324	2.1165	1.941				
105SJA	CH235_4A	0.1198	0.0555	0.0423	0.0411	0.0413	0.0377	0.0378	0.0387
121SMI	CH236_4A/293i	0.5104	0.1936	0.0849	0.0524	0.0423	0.0382	0.0425	0.0385
98JAH	CH239_4A/293i	0.5001	0.2554	0.1078	0.0612	0.0476	0.0424	0.0386	0.0405
96GEH	CH240_4A/293i	0.194	0.0893	0.0543	0.0434	0.0399	0.0408	0.0444	0.0421
108SJA	CH241	0.248	0.1061	0.0617	0.0491	0.0405	0.0437	0.0409	0.0384
132SMI	CH235UA/293i	0.2337	0.1074	0.0738	0.0504	0.0436	0.0417	0.0395	0.0391
137SMI	DH235VH_UCAtk_v2_4A/293i	0.5112	0.253	0.1353	0.0658	0.0486	0.0428	0.0415	0.0427
121JAH	DH235VH_I1_v2_4A/293i	0.5321	0.2638	0.0955	0.0637	0.0473	0.0409	0.0414	0.041

138SMI	DH235 VH_I2_v2_4A/293i	0.9691	0.4951	0.1929	0.0894	0.0603	0.0488	0.0582	0.0442
119JAH	DH235 VH_I3_v2_4A/293i	0.1317	0.0743	0.0518	0.046	0.0439	0.0412	0.0429	0.0426
	synagis	0.0405	0.0453	0.0414	0.0414				
	4E10 IgG1	2.249	2.2353	2.105	1.9205				
120JAH	DH235 VH_I4_v2_4A/293i	0.1076	0.0609	0.0475	0.0416	0.0439	0.0399	0.0366	0.0403
	Cat-CH106	0.0437	0.0443	0.0413	0.04	0.043	0.0404	0.0399	0.0407

Example 6: Antibodies from CH235 lineage

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

[0170] This example describes the design and making of non-naturally occurring CD4bs broad neutralizing HIV-1 antibodies

[0171] 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.

[0172] 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.

[0173] 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.

[0174] 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,

[0175] 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).

[0176] 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.

[0177] 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).

[0178] 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.

[0179] 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%.

[0180] In summary these antibodies were designed to include heavy chains form 454 sequencing and the heavy chains were paired with VL from observed antibodies:

[0181] CH490 and CH492 VH from 454 were paired with CH241 VL.

[0182] 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.

[0183] 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

[0184] 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

[0185] 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.

[0186] **INTRODUCTION**

[0187] 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).

[0188] 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).

[0189] 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.

[0190] 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.

[0191] **RESULTS**

[0192] **Sequencing of B cell antibody gene rearrangements in longitudinal samples.**

[0193] 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.

[0194] 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**).

[0195] **CH235 lineage HIV-1 neutralization.**

[0196] 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.

[0197] 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**).

[0198] **Crystal structures of CH235-lineage members with HIV-1 gp120.**

[0199] 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**).

[0200] 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**).

[0201] 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**).

[0202] 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).

[0203] **Negative stain EM of CH235-lineage members with trimeric HIV-1 Env.**

[0204] 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**).

[0205] 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**).

[0206] **Maturation focuses CH235 lineage recognition to a conserved site of CD4 vulnerability.**

[0207] 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).

[0208] 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.

[0209] 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.

[0210] **Conformity of sequence evolution of CH235 lineage.**

[0211] 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**).

[0212] 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.

[0213] 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.

[0214] 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.

[0215] 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).

[0216] **Interaction between CH235 and CH103 bnAb lineages.**

[0217] 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).

[0218] 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**).

[0219] 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.

[0220] 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.

[0221] 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 x 10^3 $M^{-1}s^{-1}$) which was maintained across the intermediate and mature mAbs (k_a = 11.9 - 37.3 x10^3 $M^{-1}s^{-1}$), 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 x10^{-3} s^{-1} of IA4 to 0.33 x10^{-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 x10^3 $M^{-1}s^{-1}$) 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**).

[0222] 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.

[0223] **Late CH235 lineage broadly neutralizing antibodies neutralize autologous loop D escape viruses selected by early CH235 lineage members.**

[0224] 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**).

[0225] 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.

[0226] 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**).

[0227] 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.

[0228] 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.

[0229] **CH235 and CH103 lineage antibody binding to CH505 gp120 Env.**

[0230] 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**).

[0231] 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**).

[0232] **Autoreactivity in the CH235 B cell lineage.**

[0233] 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**).

[0234] 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.

[0235] **DISCUSSION**

[0236] 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.

[0237] 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.

[0238] 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.

[0239] **EXPERIMENTAL PROCEDURES**

[0240] **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.

[0241] **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.

[0242] **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.

[0243] **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.

[0244] **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.

[0245] **Neutralization signature.** Antibody neutralization signatures were computed and compared as described in Supplemental Experimental Procedures.

[0246] **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.

[0247] **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).

[0248] **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.

[0249] **Focused maturation and conformity analysis**. Focused maturation and mAb conformity analysis are described in the Supplemental Experimental Procedures.

[0250] **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.

[0251] **Direct-Binding ELISA.** Direct-binding ELISAs were performed as described in Supplemental Experimental Procedures.

[0252] **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.

[0253] **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).

[0254] **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).

[0255] **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).

[0256] **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).

[0257] **Supplemental Experimental Procedures**

[0258] **Donor and Sample Information.**

[0259] 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).

[0260] 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.

[0261] **Flow Cytometry, Memory B Cell Cultures and mAb Isolation.**

[0262] 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).

[0263] 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.

[0264] 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).

[0265] 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.

[0266] 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).

[0267] **454 Pyrosequencing of CH235 lineage heavy chains.**

[0268] 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.

[0269] **Antibody production.**

[0270] 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).

[0271] 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.

[0272] **Direct-binding ELISA.**

[0273] 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.

[0274] 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.

[0275] 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.

[0276] 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.

[0277] Anti-cardiolipin ELISA was performed using the QUANTA Lite ACA IgG III kit (708625; INOVA Diagnostics) following manufacturer's protocol.

[0278] **Assessment of virus neutralization using a large panel and calculation of neutralization dendrograms.**

[0279] 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).

[0280] 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.

[0281] **Antibody neutralization fingerprinting analysis.**

[0282] 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.

[0283] **VH1-46 and VH1-2 antibody dendrogram calculation.**

[0284] 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.

[0285] **Production and purification of HIV-1 Env protein complexed to antigen-binding fragments.**

[0286] 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.

[0287] **X-ray crystallography.**

[0288] 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.

[0289] 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.

[0290] 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.

[0291] 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.

[0292] **Surface Plasmon Resonance Affinity and Kinetics Measurements.**

[0293] 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).

[0294] **Electron microscopy data collection and processing.**

[0295] 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).

[0296] 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).

[0297] **Epitope visualization.**

[0298] 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.

[0299] **Monoclonal antibody CH235.9 amino acid reversion.**

[0300] 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.

[0301] Presence of mutations in plasmid products was confirmed by single-colony sequencing.

[0302] **Structural bioinformatics.**

[0303] 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 $\log IC_{50}$ cutoffs = 0.60 to 1.62 μg/ml.

[0304] **Sample preparation for 5' RACE method and 454 pyrosequencing.**

[0305] 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.

[0306] **Germline V gene specific substitution profile.**

[0307] 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.

[0308] 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.

[0309] **Conformity analysis.**

[0310] 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:

[0311] $c_p(A;B) = \frac{|M_A \cap M_B|}{|M_A|}$

[0312] 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%

[0313] 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%

[0314] **Targeting precision of CD4bs-directed antibodies.**

[0315] 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.

[0316] **Antibody binding orientation calculation.**

[0317] 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.

[0318] **Supplemental References**

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[0337] **ACCESSION NUMBERS**

[0338] 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).

[0339] **Antibodies Names correlation**

[0340] 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.

[0341] **REFERENCES FOR EXAMPLE 8**

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Example 9: DH540 antibody is described elsewhere.

[0386] DH540 sequences are described in Figure 13 and the antibody is described in detail in US Ser. No. 62/170,558, filed June 3, 2015.

Example 10: DH542 antibodies

[0387] 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

[0388] >DH542_HC_nt (SEQ ID NO: 178)

CAGGTGCAGCTGGTGCAGTCTGGGGCTCAAATGAAGAACCCTGGGGCCTCAGTGAAGGTCTCCTGCGCGCCT
TCTGGATATACCTTCACCGACTTTTACATACATTGGTTGCGCCAGGCCCCTGGCCAGGGGCTTCAGTGGATG
GGATGGATGAACCCTCAGACTGGTCGCACAAACACTGCACGAAACTTTCAGGGGAGGGTCACCATGACCAGG
GACACGTCCATCGGCACAGCCTACATGGAGTTGAGAAGCCTGACATCTGACGACACGGCCATATATTACTGT
ACGACAGGGGGATGGATCAGTCTTTACTATGATAGTAGTTATTACCCCAACTTTGACCACTGGGGTCAGGGA
ACCCTGCTCACCGTCTCCTCAG

[0389] >DH542_HC_aa (SEQ ID NO: 179)

QVQLVQSGAQMKNPGASVKVSCAPSGYTFTDFYIHWLRQAPGQGLQWMGWMNPQTGRTNTARNFQGRVTMTR
DTSIGTAYMELRSLTSDDTAIYYCTTGGWISLYYDSSYYPNFDHWGQGTLLTVSS

[0390] >DH542_LC_nt_corrected (DH542_QSA) (SEQ ID NO: 180)

CAGTCTGCCCTGACTCAGCCTGCCTCCGTGTCTGGGTCTCCTGGACAGTCGATCACCATCTCCTGCACTGGA
ACCAAGTATGATGTTGGGAGTCATGACCTTGTCTCCTGGTACCAACAGTACCCAGGCAAAGTCCCCAAATAC
ATGATTTATGAAGTCAATAAACGGCCCTCAGGAGTTTCTAATCGCTTCTCTGGCTCCAAATCTGGCAACACG
GCCTCCCTGACAATCTCTGGGCTCCGGGCTGAGGACGAGGCTGACTATTATTGCTGTTCATTTGGAGGGAGT
GCCACCGTGGTCTGCGGCGGCGGGACCAAGGTGACCGTCCTAg

[0391] >DH542_LC_aa_corrected (DH542_QSA) (SEQ ID NO: 181)

QSALTQPASVSGSPGQSITISCTGTKYDVGSHDLVSWYQQYPGKVPKYMIYEVNKRPSGVSNRFSGSKSGNT
ASLTISGLRAEDEADYYCCSFGGSATVVCGGGTKVTVL

[0392] DH542-L4 is an antibody that has a VH of DH542 and VL of DH429 (Figure 2).

Example 11: MPER antibodies

[0393] DH512_K3 is a combination of VH DH512 and VL called DH511_2AVK

[0394] >DH511_2AVK Kappa Chain Nucleotide Sequence (SEQ ID NO: 182)

GACATCCAGATGACCCAGTCTCCGTCTTTCCTGTACGGCTCTGTAGGCGATAGAGTCACCATCACTTGCCGG
GCAAGTCAGAATATTAAGGACTATTTAAATTGGTATCAGCAGAGACCAGGGAGAGCCCCTAGACTCCTGATC
TATGCTGCATCCAATTTGCAAAGTGGGGTCCCGTCAAGGTTCAGTGGCAGTGGATATGGGACAGACTTTACT

CTCATCATCAGCAGTCTGCAACCTGAGGACTTTGCGACTTATTTCTGTCAAGAGAGTTATAGTTCTACGCCC
ACACACATTTTTGGCCTGGGGACCAAATTGGAGAAGAAAC

[0395] >DH511_2AVK Kappa Chain Amino Acid Sequence (SEQ ID NO: 183)

DIQMTQSPSFLYGSVGDRVTITCRASQNIKDYLNWYQQRPGRAPRLLIYAASNLQSGVPSRFSGSGYGTDFT
LIISSLQPEDFATYFCQESYSSTPTHIFGLGTKLEKKX

[0396] There are also MPER antibodies which have a mutated VH from DH512 (See Figure 48).

Other assays

[0397] Epitope mapping of antibodies: Binding and/or neutralization assays using various envelope antigens can be used to determine the epitope recognized by these antibodies.

[0398] The stability and properties of the antibodies, for example as formulated in a composition for treatment will be tested.

[0399] Animal studies (PK and PD studies) could be conducted to determine the distribution and half life of the antibodies.

[0400] Various assays and experiments can be designed to analyze prevention, treatment and/or cure.

[0401] The antibodies will be expressed in a CHO line, e.g. CHO-DG44 cell line for preparation of pharmaceutical compositions. These CHO-expressed antibodies will be analyzed in various suitable assays.

WHAT IS CLAIMED IS:

1. A recombinant antibody or fragment thereof with the binding specificity of the CD4 binding site antibody CH557.
2. The antibody or fragment thereof of claim 1 wherein the antibody or fragment thereof is fully human.
3. A recombinant antibody or fragment thereof comprising: a variable heavy chain (VH) amino acid sequence, or fragment thereof, selected from the group of VH amino acid sequences of an antibody CH490, CH491, CH492, CH493, CH555, CH556 and CH557 and
   a variable light chain (VL) amino acid sequence or fragment thereof, selected from the group of VL amino acid sequences of an antibody CH490, CH491, CH492, CH493, CH555, CH556 and CH557, wherein the recombinant antibody or fragment thereof binds to the CD4 binding site of the HIV-1 envelope.
4. The antibody or fragment thereof of claim 1 wherein the antibody or fragment thereof comprises a VH chain that is 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical to the VH chain of antibody CH557 and comprises a VL chain that is 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical to the VL chain of antibody CH557.
5. The antibody or fragment thereof of claim 1 wherein the antibody or fragment thereof comprises a VH chain which comprises the HCDR1, HCDR2, and HCDR3 of antibody CH557 and a VL chain which comprises the LCDR1, LCDR2, and LCDR3 of antibody CH557.
6. The antibody or fragment thereof of claim 1 wherein the antibody or fragment thereof comprises the VH chain and the VL chain of antibody CH557.
7. The antibody or fragment thereof of claim 1 wherein the antibody or fragment thereof comprises a modified Fc portion.
8. The antibody or fragment thereof of claim 1 wherein the antibody or fragment thereof is bispecific.
9. A pharmaceutical composition comprising anyone of the antibodies or fragments thereof of any one of claims 1-8.
10. A pharmaceutical composition comprising anyone of the antibodies of any one of claim 1-8 and another HIV-1 broad neutralizing antibody.
11. A composition comprising a vector comprising a nucleic acid encoding the antibody or fragment thereof of any one of claims 1-8.
12. The composition of claim 11, wherein the vector is suitable for gene delivery and expression.

13. A method to treat or prevent HIV-1 infection in a subject comprising administering to the subject a composition comprising an antibody or fragment thereof with the binding specificity of CH557 in a therapeutically effective amount.
14. The method of claim 13 wherein the pharmaceutical composition is administered in a therapeutically effective dose and regimen.
15. The method of claim 13 further comprising administering an additional HIV-1 broad neutralizing antibody.

>DH542_nt_HC (SEQ ID NO: 1)

CAGGTGCAGCTGGTGCAGTCTGGGGCTCAAATGAAGAACCCTGGGGCCT
CAGTGAAGGTCTCCTGCGCGCCTTCT**GGATATACCTTCACCGACTTTTAC**A
TACATTGGTTGCGCCAGGCCCCTGGCCAGGGGCTTCAGTGGATGGGATG
G**ATGAACCCTCAGACTGGTCGCACA**AACACTGCACGAAACTTTCAGGGG
AGGGTCACCATGACCAGGGACACGTCCATCGGCACAGCCTACATGGAGT
TGAGAAGCCTGACATCTGACGACACGGCCATATATTAC**TGTACGACAGG**
GGGATGGATCAGTCTTTACTATGATAGTAGTTATTACCCCAACTTTGACC
ACTGGGGTCAGGGAACCCTGCTCACCGTCTCCTCAG

>DH542_nt_LC (SEQ ID NO: 2)

ACCAGTCTGCTGACTCAGCCTGCCTCCGTGTCTGGGTCTCCTGGACAGTC
GATCACCATCTCCTGCACTGGAACC**AAGTATGATGTTGGGAGTCATGACC**
TTGTCTCCTGGTACCAACAGTACCCAGGCAAAGTCCCCAAATACATGATTT
AT**GAAGTCAAT**AAACGGCCCTCAGGAGTTTCTAATCGCTTCTCTGGCTCC
AAATCTGGCAACACGGCCTCCCTGACAATCTCTGGGCTCCGGGCTGAGGA
CGAGGCTGACTATTAT**TGCTGTTCATTTGGAGGGAGTGCCACCGTGGTCT**
GCGGCGGCGGGACCAAGGTGACCGTCCTAG

>DH542_aa_HC (SEQ ID NO: 3)

QVQLVQSGAQMKNPGASVKVSCAPS**GYTFTDFY**IHWLRQAPGQGLQWM
GW**MNPQTGRT**NTARNFQGRVTMTRDTSIGTAYMELRSLTSDDTAIYY**CTT**
GGWISLYYDSSYYPNFDHWGQGTLLTVSS

>DH542_aa_LC (SEQ ID NO: 4)

TSLLTQPASVSGSPGQSITISCTGT**KYDVGSHDL**VSWYQQYPGKVPKYMIY**E**
VNKRPSGVSNRFSGSKSGNTASLTISGLRAEDEADYY**CCSFGGSATVVC**GGG
TKVTVL

FIG. 1

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. 2

```
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. 2 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. 2 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. 2 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. 2 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. 2 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. 2 cont.

Antibody	IC50	IC80	IC50 <50ug/ml	IC80 <5ug/ml	Antibody Specificity
PGT121	0.06	0.27	63%	48%	V3-glycan
PGT128	0.07	NA	63%	NA	V3-glycan
DH270IA1	0.07	0.22	63%	61%	V3-glycan
DH429	0.06	0.22	63%	60%	V3-glycan
VRC01	0.27	0.73	87%	81%	VH1-2 CD4bs
CH31	0.10	0.42	83%	80%	VH1-2 CD4bs (VRC01-like)
CH01	3.79	NA	46%	NA	V1V2-glycan
CH01+ CH31	3.73	NA	93%	NA	V1V2-glycan + VH1-2 CD4bs
CH103	4.54	NA	55%	NA	HCDR3 binder CD4 bs
CH98	4.20	NA	63%	NA	HCDR3 binder CD4 bs
DH493	5.08	NA	83%	NA	VH1-46 CD4bs (ANC131-like)
DH540	0.10	NA	90%	NA	N276-dependent CD4bs Ab (HJ16-like)

FIG. 3

Antibody	IC50	IC80	IC50 <50ug/ml	IC80 <5ug/ml	Antibody Specificity
DH429	0.06	0.22	63%	60%	V3-glycan
DH512	0.65	5.12	100%	50%	MPER (10E8-like)
CH31	0.10	0.42	83%	80%	VH1-2 CD4bs (VRC01-like)
CH01	3.79	NA	46%	NA	V1V2-glycan
CH01+ CH31	3.73	NA	93%	NA	V1V2-glycan + VH1-2 CD4bs
DH493	5.98	NA	83%	NA	VH1-46 CD4bs (ANC131-like)
DH540	0.10	NA	90%	NA	N276-dependent CD4bs Ab (HJ16-like)

FIG. 4

BnAb	Specificity	Breadth	Median IC50(ug/ml)
DH512	gp41	100%	1.09
10E8	gp41	98%	0.39
CH557	CD4bs	100%	0.62
VRC01	CD4bs	90%	0.38
VRC07	CD4bs	93%	0.15
DH542	V3-glycan	71%	0.06
PGT121	V3-glycan	70%	0.03
PGT128	V3-glycan	72%	0.02

FIG. 5

>DH511VH (SEQ ID NO: 53)

GAGGTTCAGCTGGTGGAGTCTGGGGGAGGCTTGGTGAAGCCGGGGGGGTCTCT
TAGACTCCCCGGTGCAGCCTCTGGTTTCACTTTCACCAACACGTGGATGAGTT
GGGTCCGTCAGGCGCCAGGGAAGGGACTGGAGTGGGTCGGTCGGATTAGCCGG
AACAAAGATGGCGCGAAAACAGAGTACGCCGCACCCGTGAGAGGCAGATTCAC
CATCTCAAGAGATGACTCCAGAGACACATTGTATCTGCAGATGACCAGCCTGA
AAATAGAGGATTCAGGCCGGTATTTTTGCACCGCAGATCTTGGGGAGCCCGTG
GTGTCACGATCCATTTTTGAGTGGGGGTCTTATTATTATTATATGGACCTCTG
GGGCAAGGGGACCACGGTCACCGTCTCTTCA

>DH511VK (SEQ ID NO: 54)

GACATCCAGTTGACCCAGTCTCCATCTCCCCTGTCTGCGTCTGTGGGAGACAC
AGTCACTATCACTTGTCGGGCCAGCCAGAAGATTAGCGACTATTTGAACTGGT
ACCAACAGAAGCCGGGGAGAGCCCCCAAAATACTCATTTACGCTGCGTCCAAG
TTGGGGAGTGGCGTCCCATCAAGGTTCAGTGGCAGTGGATATGGCAGAGATTT
CACTCTCACCATCACCGGTCTGCAGCCTGAAGATTTTGCAACCTATTATTGTC
AGGAGGCTTACAGTTCTACTCCCACGTTAACTTTTGGCCAGGGGACCAGGCTG
GATCTCAAAC

>DH512VH (SEQ ID NO: 55)

CAGGTGCAGCTGGTACAGTCTGGGGGAGGTCTGGTGAAGCCGGGGGGGTCCCT
CACACTCTCCTGTTCAGCCTCTGGATTCTTTTTCGATAATTCATGGATGGGGT
GGGTCCGTCAGGCGCCAGGGAAGGGACTGGAGTGGGTTGGCCGCATTAGAAGG
CTCAAAGACGGTGCGACAGGAGAATATGGTGCAGCCGTGAAGGACAGATTCAC
CATTTCAAGAGATGACAGTAGAAATATGCTGTACCTGCACATGAGGACCCTGA
AAACCGAGGACTCAGGCACTTATTATTGTACCATGGATGAGGGGACCCCAGTA
ACACGCTTCTTAGAATGGGGCTACTTCTATTATTATATGGCCGTTTGGGGCAG
AGGGACCACGGTCATCGTCTCTTCA

FIG. 6

>DH512VK (SEQ ID NO: 56)

GACATCGTGATGACCCAGTCTCCGTCCTCCGTGTCTGCATCTGTGGGAGACAG
AGTCACCATCACTTGCCGGGCAAGTCAGAATATTAGAGACTATTTAAATTGGT
ATCAACATAAACCCGGGGGATCCCCTAGACTCCTAATTTATGCTGCGTCAACT
TTGCAAACTGGGGTCCCGTCCAGATTCAGCGGCAGTGGATCTGGGAACCTTTT
CACTCTCACCATTACCAATCTGCAACCTGAAGATTTTGCAACTTATTATTGTC
AAGAGAATTATAATACTATCCCCTCGCTCAGCTTTGGTCAGGGGACCAAGGTG
GACATCAGGC

>DH513VH (SEQ ID NO: 57)

GAGGTTCAGCTGGTGGAGTCTGGGGGAGGCTTGGTGAAGCCGGGGGGGTCTCT
TAGACTCTCCTGTGTAGCCTCTGGCTTCACTTTCAGCAACACGTGGATGAGTT
GGGTCCGTCAGGCGCCAGGGAAGGGACTGGAGTGGGTCGGTCGGATTAGCCGG
AACAAAGATGGCGCGAAAACAGAGTACGCCGCACCCGTGAGAGGCAGATTCAC
CATCTCAAGAGATGACTCCAGAGACACATTGTATCTGCAGATGAGCAGCCTGA
AAATAGAGGATTCAGGCCGGTATTTTTGCACCGCAGATCTTGGGGAGGCCGTT
GTGTCACGATTTTTTGAGTGGGGGTCCTATTATTACTACATGGACTTCTGGGG
CAAGGGGACCACGGTCACCGTCTCTTCA

>DH513VK (SEQ ID NO: 58)

GACATTCAGATGACCCAATCTCCATCTCCCCTGTCTGCGTCTGTGGGAGACAC
AGTCACTATCACTTGCCGGGCCAGCCAGAAGATTAGCGACTATTTGAACTGGT
ACCAACAGAGGCCGGGGAGAGCCCCCAAGATCCTCATTTACGCTGCGTCCAAG
TTGGCAAGCGACGTCCCATCAAGATTTAGTGGCAGTGGATATGGCAGAGATTT
CACTCTCACCATAACCGGTCTGCAGCCTGAAGATTTTGCAACCTATTATTGTC
AGGAGGCTTACAGTTCTACCCCCACGTTAACTTTTGGCCAGGGGACCAGGCTG
GATCTCAAAC

>DH514VH (SEQ ID NO: 59)

GAGGTGCAGCTGGTGGAGTCTGGGGGCGGCTTGATAAAGCCGGGACAGTCACT
CACACTATTCTGTGTGGGCTTTGGATTCAACTTCGCTAACGACTGGATGGGCT
GGGTCCGCCAGGCTCCAGGGAAGGGACTGGAATGGGTTGGGCGTATAAGGAGA
CTGAAAGATGGTGCGAAAGCTGAATATGGATCTTCCGTGAAGGGTAGATTCAC
CATCTCAAGGGATGATTCCAAAAACACCCTATACTTGCACATGAGCAGCCTCA
AGGTCGAAGACACAGCCGTCTACTATTGCACCCGAGACGAGGGGGCCCCAGTT
ACCCGGTTTCTGGAGTGGGGCTCCTATTACTACTACATGGCCGTCTGGGGCAA
AGGGACCACGGTCACCGTCTCTTCA

FIG. 6 cont.

>DH514VK (SEQ ID NO: 60)

GACATCCAGTTGACCCAGTCTCCAGCCTCTCTGTCTGCATCTGTAGGAGACAC
AGTGACTATCACTTGCCGGGCAAGTCAGAGTATAAAAGATTACATAAATTGGT
ATCAACACAAATCCGGGAGCGCCCCTAGACTCCTGATTTATGCTGCGTCAACC
TTACAAAGTGGAATCTCGTCAAGGTTCACTGGCAGTGGGTCTGGGACACAGTT
CACTCTCACCATTAACAGTCTGCAACCTGAAGATTTTGCGACTTATTATTGTC
AAGAGGCTTATAACACCAACCCCACACTCTCCTTTGGTCAGGGGACCAGGGTG
GACAAGAAGC

>DH515VH (SEQ ID NO: 61)

GAGGTTCAGCTGGTGGAGTCTGGGGGCGGCTTGGTGAAGCCGGGACAGTCACT
CACACTTTCCTGTGTGGGCTTTGGATTCAATTTCGCTAACGACTGGATGGGCT
GGGTCCGCCAGGCTCCAGGGAAGGGACTGGAATGGGTTGGTCGAATAAGGAGA
CTAAAAGACGGTGCGACAACAGAATATTCTTCATCCGTGAAGGGGAGATTCAG
TGTCTCAAGAGATGATTCAAGGAACACAGTATACTTACACATGAGTAGCCTCA
AAGTCCAGGACATTGGCGTCTATTATTGTACTCGAGACGAGGGGGCCCCGGTT
ACTCGATTTCTGGAGTGGGGCTCCTATTACTACTATATGGCCGTCTGGGGCAG
AGGGACCACGGTCACCGTCTCTTCA

>DH515VK (SEQ ID NO: 62)

GACATCCAGATGACCCAGTCTCCAACCTCTCTGTCTGCATCTGTAGGAGACAC
AGTTGCTATCACTTGCCGGGCAAGTCAGAGTGTTAAAGATTATGTGAATTGGT
ATCAACACAAATCCGGGAGCGCCCCTCGACTCCTGATTTATGCTGCCTCAGTC
TTACATACTGGAGTCTCGTCAAGGTTCACTGGCAGTGGGTCTGGGACACAGTT
CACTCTCACCATTAGCAGTCTACAACCTGAAGATTTTGCTACTTATTATTGTC
AAGAGGCTTATAACACCTATCCCACACTCTCCTTTGGTCAGGGGACCAGGGTG
GACAGGAAAC

FIG. 6 cont.

>DH516VH (SEQ ID NO: 63)

GAGGTTCAGCTGGTGGAGTCTGGGGGAGGCTTGGTGAAGCCGGGGGGGTCTCT
TAGACTCTCCTGTGTAGCCTCTGGCTTCACTTTCAGCAACACGTGGATGAGTT
GGGTCCGTCAGGCGCCAGGGAAGGGACTGGAGTGGGTCGGTCGGATTAGCCGG
AACAAAGATGGCGCGAAAACAGACTACGCCGCACCCGTGAGAGGCAGATTCAC
CATCTCCAGAGATGACTCCAGAGACACATTGTATCTGCAGATGAGCAGCCTGA
AAATAGAGGATTCAGGCCGGTATTTTTGCACCGCAGATCTTGGGGAGGCCGTG
GTGTCACGATTTTTTGAGTGGGGGTCCTATTATTACTACATGGACTTCTGGGG
CAAGGGGACCACGGTCACCGTCTCTTCA

>DH516VK (SEQ ID NO: 64)

GATATTGTGATGACCCAGTCTCCACCTCCCCTGTCTGCGTCTGTGGGAGACAC
AGTCACTATCACTTGCCGGGCCAGCCAGAAGATTAGCGACTATTTGAACTGGT
ACCAACAGAGGCCGGGGAGAGCCCCCAAAATACTCATTTACGCTGCGTCCAAG
TTGGGAAGCGACGTCCCATCAAGGTTCAGTGGCAGTGGATATGGCAGAGATTT
CACTCTCACCATCACCGGTCTGCAGCCTGAAGATTTTGCAACCTATTATTGTC
AGGAGGCTTACAGTTCTACTCCCACGTTAAGTTTTGGCCAGGGGACCAGGCTG
GATCTCAAAC

>DH517VH (SEQ ID NO: 65)

GAAAGGCAGGTGGTGGAATATGGGGGAGGCTTGGTGAAGCCGGGGGGGTCTCT
TAGACTCTCTTGTTTACCGTTTGCCTTTGGGTTCAGGGCCCCCTGGAGGAGTT
CTGTCCGTCACGCGCCTGGGGGCGGAGCGGAGTGGGTCGGTCGGATTAGCCGG
AACAAAGATGGCGCGAAAACAGAGTACGCCGCACCCGTGAGAGGCAGATTCAC
CATCTCAAGAGATGACTCCAGAGACACATTGTATCTGCAGATGACCAGCCTGA
AAATAGAGGATTCAGGCCGGTATTTTTGCACCGCAGATCTTGGGGAGCCCGTG
GTGTCACGATTTTTTGAGTGGGGGTCTTATTATTATTATATGGACCTCTGGGG
CAAGGGGACCACGGTCACCGTCTCTTCA

>DH517VL (SEQ ID NO: 66)

TCTTCTGAGCTGACTCAGGACCCCACTGTGTCTGTGGCCTTGGGCCAGACAGT
CAAGATCAGATGCCAAGGAGCCAGCCTCAGAGACTGTTATGCGACCTGGTACC
GGCAGAAGCCAGGACAGGCCCCAACACTTCTCATTTATGATATAAATAAGAGG
CCCTCAGGTATCCCAGACCGATTCTCTGCCTCCTACTCAGGGAGCACTTCTTC
CTTGACCATTATTGGGGCTCAGCCGGAAGATGAGGCTGACTATTTTTGTGCTT
CGCGGGACAGGAGTGGTGACCGTCTTGGCGTCTTCGGCGGTGGGACCAAACTG
ACCGTCCTG

FIG. 6 cont.

>DH518VH (SEQ ID NO: 67)

CAGCTGCAGGAGTCGGGTCCCAGACTGGTGAGGCCTTCGGAGACCCTGTCCCT
CACCTGCACTGTATCTGGCTCTGGTGTCTCCGTCAGTCGTGGGAGTTATTATT
GGGGCTGGATACGCCAGTCCCCAGAAAAGGGACTCGAATGGATTGGAAGTGTC
TATTCCACTACTAGTGGAAAAACCTACTACAACCCGTCCCTCAAGAGTCGAGT
CACCTTTTCGAAGGACACGTCCCAGAACGCCTTCTCCCTGACTCTGACGTCTA
TTACCGCCGCGGACACGGCCGTCTATTACTGTGCAAGACAATTTGGCTTCATG
GGGGGCTTTTTGGAGTGGTATCCGCACTATTTTGACTTCTGGGGCCCGGGAAT
CCAGGTCGTCGTGTCTTCT

>DH518VK (SEQ ID NO: 68)

GACATTGTGATGACCCAGTCTCCATCCTACCTGTCTACATCTGTCGGTGACAG
CATCACCATCACTTGCCGGGCAAGTCAGAGTATTAAAACATATGTAAATTGGT
ATCAACAAAGACCAGGGAGAGCCCCTAAACTCCTCATCTATTCTTCATCCACT
TTGCAACCTGGGGTCCCGTCAAGATTCAGCGCCAGTGGATCTGGGACAGATTT
CGTTCTCTCCATCACCAATTTGCAGTCTGAAGATTTTGCAACTTACTACTGTC
AACAGACCTACTACACCCCCTCTACTTTTGGCCAGGGGACCACACTGGACATC
AAG

>DH536VH (SEQ ID NO: 69)

CAGGTGCAGCTGGTACAGTCTGGGGCTGAGGTGAAGAAGCCTGGGTCCTCGGT
CAAGGTCTCCTGCAAGGCCTCTGGAGGCTCCTTCTACACCTATACTATCAACT
GGGTGCGACAGGCCCCTGGACAAGGGCTTGAGTGGATGGGCAGGGTCACCACT
ATGTTTGGTGTAACACTTTACGCACAGAAATTCCAGGGCAGAGTCACACTTAC
CGCGGACAAATCCACGAGCACAGCCTACATGGAACTGAGCAGTCTAAGATCTG
AGGACACGGCCGTCTATTATTGTGCGACAGATGGGCCTGACAATTTTTGGAGT
GGCTTGTCTCATGCTTTCGATCTCTGGGGCCAGGGGACAATGGTCACCGTCTC
TTCA

>DH536VL (SEQ ID NO: 70)

CAGTCTGCCCTGACTCAGCCTGCCTCCGTGGCTGGGTCTCCTGGACAGTCGAT
CACCATCTCCTGCACTGGAACCAGCAGTGACATTGGTGATTCTAAGTATGTCT
CCTGGTACCAACAGTTCCCAGGCAAAGCCCCCAAAGTCATGATTTATGAGGTC
AGTTATCGGCCCTCAGGAGTCTCTAGCCGCTTCTCTGGCTCCAAGTCTGGCAA
CACGGCCTCCCTGACCATCTCTGGACTCCAGACTGAGGACGAGGCTGATTATT
ATTGCATGGCATATACAGGCACCTTCACTGCTATTTTCGGCGGAGGGACCAAG
CTGACCGTCCTG

FIG. 6 cont.

>DH537VH (SEQ ID NO: 71)

CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAGGAAGGCTGGGTCGTCGGT
GAAGGTCTCCTGCAAGGCTTCTGGAGGCACCTTCACCAGCTATGGCTTCAGCT
GGATACGGCAGGCCCCTGGCCAAGGGCTTGAGTGGATGGGAAACGTCATCCCT
GTCTTTGGTTCAACAAACTACGCACAGAAATTTCAGGGCAGAGTCAGTATTAC
CGCGGACGAAGCCACGGGCACAGTCCACATGGACCTCACCAGCCTGACATCTG
ACGACACGGCCGTTTATTACTGTGTGAGGTCGAGTAGAGAACTGCCAACGTCA
ATGGAACGGTGGTTCGACCCCTGGGGCCAGGGAACCCAGGTCATTGTCTCCTC
G

>DH537VK (SEQ ID NO: 72)

GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTGGGAGACAG
CGTCACCATTACTTGCCGGGCAAGTCAGAGCATTAACACCTATTTAAATTGGT
ATCAGCAGAAACCAGGGAAGGCCCCTAAACTCCTGATCTATTCTGCATCCAAT
TTACACAATGGGGTCCCATCGAGGTTCAGTGGCAGTGGATCTGGGACATCTTT
CACTCTCACCATCAACAATCTACAACCTGAAGATTTTGCAACTTACTACTGTC
AACAGAGTTACAGTGCCCCTTACACTTTTGGCCAGGGGACCAAGTCAGACACC
AAA

FIG. 6 cont.

>DH511VH (SEQ ID NO: 73)

EVQLVESGGGLVKPGGSLRLPGAASGFTFTNTWMSWVRQAPGKGLEWVGRISR
NKDGAKTEYAAPVRGRFTISRDDSRDTLYLQMTSLKIEDSGRYFCTADLGEPV
VSRSIFEWGSYYYYMDLWGKGTTVTVSS

>DH511VK (SEQ ID NO: 74)

DIQLTQSPSPLSASVGDTVTITCRASQKISDYLNWYQQKPGRAPKILIYAASK
LGSGVPSRFSGSGYGRDFTLTITGLQPEDFATYYCQEAYSSTPTLTFGQGTRL
DLK

>DH512VH (SEQ ID NO: 75)

QVQLVQSGGGLVKPGGSLTLSCSASGFFFDNSWMGWVRQAPGKGLEWVGRIRR
LKDGATGEYGAAVKDRFTISRDDSRNMLYLHMRTLKTEDSGTYYCTMDEGTPV
TRFLEWGYFYYYMAVWGRGTTVIVSS

>DH512VK (SEQ ID NO: 76)

DIVMTQSPSSVSASVGDRVTITCRASQNIRDYLNWYQHKPGGSPRLLIYAAST
LQTGVPSRFSGSGSGNLFTLTITNLQPEDFATYYCQENYNTIPSLSFGQGTKV
DIR

>DH513VH (SEQ ID NO: 77)

EVQLVESGGGLVKPGGSLRLSCVASGFTFSNTWMSWVRQAPGKGLEWVGRISR
NKDGAKTEYAAPVRGRFTISRDDSRDTLYLQMSSLKIEDSGRYFCTADLGEAV
VSRFFEWGSYYYYMDFWGKGTTVTVSS

>DH513 (SEQ ID NO: 78)

VKDIQMTQSPSPLSASVGDTVTITCRASQKISDYLNWYQQRPGRAPKILIYAA
SKLASDVPSRFSGSGYGRDFTLTITGLQPEDFATYYCQEAYSSTPTLTFGQGT
RLDLK

>DH514VH (SEQ ID NO: 79)

EVQLVESGGGLIKPGQSLTLFCVGFGFNFANDWMGWVRQAPGKGLEWVGRIRR
LKDGAKAEYGSSVKGRFTISRDDSKNTLYLHMSSLKVEDTAVYYCTRDEGAPV
TRFLEWGSYYYYMAVWGKGTTVTVSS

FIG. 7

>DH514VK (SEQ ID NO: 80)

DIQLTQSPASLSASVGDTVTITCRASQSIKDYINWYQHKSGSAPRLLIYAAST
LQSGISSRFTGSGSGTQFTLTINSLQPEDFATYYCQEAYNTNPTLSFGQGTRV
DKK

>DH515VH (SEQ ID NO: 81)

EVQLVESGGGLVKPGQSLTLSCVGFGFNFANDWMGWVRQAPGKGLEWVGRIRR
LKDGATTEYSSSVKGRFSVSRDDSRNTVYLHMSSLKVQDIGVYYCTRDEGAPV
TRFLEWGSYYYYMAVWGRGTTVTVSS

>DH515VK (SEQ ID NO: 82)

DIQMTQSPTSLSASVGDTVAITCRASQSVKDYVNWYQHKSGSAPRLLIYAASV
LHTGVSSRFTGSGSGTQFTLTISSLQPEDFATYYCQEAYNTYPTLSFGQGTRV
DRK

>DH516VH (SEQ ID NO: 83)

EVQLVESGGGLVKPGGSLRLSCVASGFTFSNTWMSWVRQAPGKGLEWVGRISR
NKDGAKTDYAAPVRGRFTISRDDSRDTLYLQMSSLKIEDSGRYFCTADLGEAV
VSRFFEWGSYYYYMDFWGKGTTVTVSS

>DH516VK (SEQ ID NO: 84)

DIVMTQSPPPLSASVGDTVTITCRASQKISDYLNWYQQRPGRAPKILIYAASK
LGSDVPSRFSGSGYGRDFTLTITGLQPEDFATYYCQEAYSSTPTLSFGQGTRL
DLK

>DH517VH (SEQ ID NO: 85)

ERQVVEYGGGLVKPGGSLRLSCLPFAFGFRAPWRSSVRHAPGGGAEWVGRISR
NKDGAKTEYAAPVRGRFTISRDDSRDTLYLQMTSLKIEDSGRYFCTADLGEPV
VSRFFEWGSYYYYMDLWGKGTTVTVSS

>DH517VL (SEQ ID NO: 86)

SSELTQDPTVSVALGQTVKIRCQGASLRDCYATWYRQKPGQAPTLLIYDINKR
PSGIPDRFSASYSGSTSSLTIIGAQPEDEADYFCASRDRSGDRLGVFGGGTKL
TVL

FIG. 7 cont.

>DH518VH (SEQ ID NO: 87)

QLQESGPRLVRPSETLSLTCTVSGSGVSVSRGSYYWGWIRQSPEKGLEWIGSV
YSTTSGKTYYNPSLKSRVTFSKDTSQNAFSLTLTSITAADTAVYYCARQFGFM
GGFLEWYPHYFDFWGPGIQVVVSS

>DH536VH (SEQ ID NO: 88)

QVQLVQSGAEVKKPGSSVKVSCKASGGSFYTYTINWVRQAPGQGLEWMGRVTT
MFGVTLYAQKFQGRVTLTADKSTSTAYMELSSLRSEDTAVYYCATDGPDNFWS
GLSHAFDLWGQGTMVTVSS

>DH518VK (SEQ ID NO: 89)

DIVMTQSPSYLSTSVGDSITITCRASQSIKTYVNWYQQRPGRAPKLLIYSSST
LQPGVPSRFSASGSGTDFVLSITNLQSEDFATYYCQQTYYTPSTFGQGTTLDI
K

>DH536VH (SEQ ID NO: 90)

QVQLVQSGAEVKKPGSSVKVSCKASGGSFYTYTINWVRQAPGQGLEWMGRVTT
MFGVTLYAQKFQGRVTLTADKSTSTAYMELSSLRSEDTAVYYCATDGPDNFWS
GLSHAFDLWGQGTMVTVSS

>DH536VL (SEQ ID NO: 91)

QSALTQPASVAGSPGQSITISCTGTSSDIGDSKYVSWYQQFPGKAPKVMIYEV
SYRPSGVSSRFSGSKSGNTASLTISGLQTEDEADYYCMAYTGTFTAIFGGGTK
LTVL

>DH537VH (SEQ ID NO: 92)

QVQLVQSGAEVRKAGSSVKVSCKASGGTFTSYGFSWIRQAPGQGLEWMGNVIP
VFGSTNYAQKFQGRVSITADEATGTVHMDLTSLTSDDTAVYYCVRSSRELPTS
MERWFDPWGQGTQVIVSS

>DH537VK (SEQ ID NO: 93)

DIQMTQSPSSLSASVGDSVTITCRASQSINTYLNWYQQKPGKAPKLLIYSASN
LHNGVPSRFSGSGSGTSFTLTINNLQPEDFATYYCQQSYSAPYTFGQGTKSDT
K

FIG. 7 cont.

```
DH511VH  EVQLVESGGG LVKPGGSLRL PGAASGTTFT NTYMSWVRQA
DH512VH  Q-----Q----- ----------T- SCS-----F-D -S--G-------
DH513VH  ---------- ---------- SCV-------S ----------
DH514VH  ---------- -I---Q--T- FCVGF--N-A -D--G-------
DH515VH  ---------- -----Q--T- SCVGF--N-A -D--G-------
DH516VH  ---------- ---------- SCV-------S ---------- 40

DH511VH  PGKGLEWVGR ISRNKDGAKT EYAAPVRGRF
DH512VH  ---------- -R-L-----TG --G-A-KD--
DH513VH  ---------- ---------- ----------
DH514VH  ---------- -R-L------A --GSS-K---
DH515VH  ---------- -R-L-----T- --SSS-K---
DH516VH  ---------- ---------- D--------- 70

DH511VH  TISRDDSRDT LYLQMTSLKI EDSGRYFCTA
DH512VH  ---------- ---H-RT--T ----T-Y--M
DH513VH  ---------- -----S---- ----------
DH514VH  ---------- ---H-S---V --TAV-Y--R
DH515VH  SV-------N- V--H-S---V Q-I-V-Y--R
DH516VH  ---------- -----S---- ---------- 100

DH511VH  DLGEPVVSRS IEEWGSYYYY MDLWGKGTTV TVSS
DH512VH  -E-T--.T-. FL---YF--- -AV--R---- I---
DH513VH  ----A-----. F---------- --F------- -----
DH514VH  -E-A--.T-. FL---------- -AV------- -----
DH515VH  -E-A--.T-. FL---------- -AV--R---- ----
DH516VH  ----A-----. F---------- --F------- ----- 134
```

FIG 8A

```
DH511VK  DIQLTQSPSP LSASVGDTVT ITCRASQXIX DXLNWYQQKP
DH512VK  --VM-----S V-------R-- ---------N-R ---------H--
DH513VK  ---M------ ---------- ---------- ---------R-
DH514VK  --------AS ---------- ---------S-K --I----H-S
DH515VK  ---M-----TS ---------- ---------SVK --V----H-S
DH516VK  --VM----P- ---------- ---------- ---------R-  40

DH511VK  GRAPKILIYA ASKLGSGVPS RFSGSGYGRD
DH512VK  -GS-RL----- --T-QT----- -------S-NL
DH513VK  ---------- ---A-D---- ----------
DH514VK  -S--RL----- --T-Q--IS- --T---S-TQ
DH515VK  -S--RL----- --V-HT--S- --T---S-TQ
DH516VK  ---------- -------D--- ----------  70

DH511VK  FTLTITGLQP EDFATYYCQE AYSSTPTLTF GQGTRLDLK
DH512VK  -------N---- ---------- N-NTI-S-S- ----KV-IR
DH513VK  ---------- ---------- ---------- ---------
DH514VK  -----NS---- ---------- --NTN---S- -----V-K-
DH515VK  -----SS---- ---------- --NTY---S- -----V-R-
DH516VK  ---------- ---------- ----------S- ---------  109
```

FIG. 8B

```
DH511VH   EVQLVESGGG LVKPGGSLRL PGAASGFTFT NT..WMSWVR QAPGKGLEWV GRISRNKDGA KTEYAAPVRG
DH512VH   Q----Q---- --------T- SCS----F-D -S..--G--- ---------- ---R-L---- TG--G-A-KD
DH513VH   ---------- ---------- SCV------S --..------ ---------- ---------- ----------
DH514VH   ---------- -I---Q--T- FCVGF--N-A -D..--G--- ---------- ---R-L---- -A--GSS-K-
DH515VH   ---------- -----Q--T- SCVGF--N-A -D..--G--- ---------- ---R-L---- T---SSS-K-
DH516VH   ---------- ---------- SCV------S --..------ ---------- ---------- --D-------
10E8VH    ---------- ---------- SCS----D-D -A..--T--- -P-------- ---TGPGE-W SVD-----E-
4E10VH    Q----Q--AE VKR--S-VTV SCK---GS-S TY..AL---- ----R----M -GVIPLL..T I-N--PRFQ-
2F5VH     RIT-K---PP ----TQT-T- TCSF---SLS DFGVGVG-I- -P---A---L AI-YSDD... DKR-SPSLNT
                                                                                  70

DH511VH   RFTISRDDSR DTLYLQMTSL KIEDSGRYFC TADLGEPVVS RSIFEWGSY. ..YYYMDLWG KGTTVTVSS
DH512VH   ---------- NM---H-RT- -T----T-Y- -M-E-T--.T -.FL---YF- ------AV-- R----I---
DH513VH   ---------- -------S-- ---------- ------A--- -.F------- -------F-- ---------
DH514VH   ---------K N----H-S-- -V--TAV-Y- -R-E-A--.T -.FL------ ------AV-- ---------
DH515VH   --SV------ N-V--H-S-- -VQ-I-V-Y- -R-E-A--.T -.FL------ ------AV-- R--------
DH516VH   ---------- -------S-- ---------- ------A--- -.F------- -------F-- ---------
10E8VH    ------LN-I NF---E-NN- RM----L--- ARTGKY.... ..YDF-SG-P PGEE-FQD-- R--L-----
4E10VH    -I--TA-R-T S-A--ELN-- RP--TAV-Y- AREGTT.... ....G--WLG KPIGAFAH-- Q--L-----
2F5VH     -L--TK-T-K NQVV-V--RV SPV-TAT--- AHRR-PTT.. ..L-GVPIAR GPVNA--V-- Q-I---I--
                                                                                 139
```

FIG. 9A

```
DH511VK   DIQLTQSPSP LSASVGDTVT ITCRASQKIS .DYLNWYQQK PGRAPKILIY AASKLGSGVP
DH512VK   --VM-----S V------R-- -------N-R .-------H- --GS-RL--- ---T-QT---
DH513VK   ---M------ ---------- ---------- .--------R ---------- -----A-D--
DH514VK   --------AS ---------- -------S-K .--I----H- S-S--RL--- ---T-Q--IS
DH515VK   ---M----TS ---------A -------SVK .--V----H- S-S--RL--- ---V-HT--S
DH516VK   --VM----P- ---------- ---------- .--------R ---------- -------D--
4E10VK    E-V-----GT Q-L-P-ERA- LS-----SVG NNK-A----R --Q--RL--- G--SRP---A
2F5VK     AL-------S -------RI- -------GVT .SA-A--R-- --SP-QL--- D--S-E----
 10E8VL   SYE---.ETG V-VAL-R--- ----GDSL.R SH-AS---K- --Q--IL-F- GKNNRP----
                                                                           60

DH511VK   SRFSGSGYGR DFTLTITGLQ PEDFATYYCQ EAYSST.PTL TFGQGTRLDL K
DH512VK   -------S-N L------N-- ---------- -N-NTI--S- S-----KV-I R
DH513VK   ---------- ---------- ---------- ---------- ---------- -
DH514VK   ---T---S-T Q-----NS-- ---------- ---NTN---- S------V-K -
DH515VK   ---T---S-T Q-----SS-- ---------- ---NTY---- S------V-R -
DH516VK   ---------- ---------- ---------- ---------- S--------- -
4E10VK    D------S-T ------SR-E -----V---- QYGQ..-SLS ------KVEV -
2F5VK     -------S-T E-----ST-R ---------- QLHF..-YPH ---G---V-V R
10E8VL    D-----AS-N RAS---S-A- A--D-E---S SRDK-GSRLS V--G--K-TV L    111
```

FIG. 9B

		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. 10

		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. 10 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. 10 cont.

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. 11

>CH557_aa_HC (SEQ ID NO: 124)

QVRLAQYGGGVKRLGATMTLSCVAS**GYTFNDYY**IHWVRQAPGQGFEL
LGY**IDPANGRP**DYAGALRERLSFYRDKSMETLYMDLRSLRYDDTAMY
Y**CVRNVGTAGSLLHYDHW**GSGSPVIVSS

>CH557_aa_LC (SEQ ID NO: 125)

EIVLTQSPATLSASPGERVTLTCRAS**RSVRNN**VAWYQHKGGQSPRLL
IY**DAS**TRAAGVPARFSGSASGTEFTLAISNLESEDFTVYF**CLQYNNW**
WTFGQGTRVDIK

>CH557_nt_HC (SEQ ID NO: 126)

CAGGTCCGACTAGCCCAATATGGTGGTGGGGTGAAGAGGCTAGGGGC
CACAATGACCCTTTCCTGCGTGGCATCT**GGATACACCTTCAACGACT**
ACTACATACATTGGGTGCGGCAGGCCCCTGGACAAGGCTTTGAGTTG
TTGGGATAC**ATCGACCCCCGCTAATGGTCGCCCA**GACTACGCAGGGGC
GTTGAGGGAGAGACTCTCCTTCTACAGGGACAAGTCCATGGAGACGC
TGTACATGGACCTGAGGAGCCTAAGATATGACGACACGGCCATGTAT
TATTGT**GTTAGAAATGTGGGGACCGCTGGCAGCTTGCTGCATTATGA**
CCACTGGGGCTCGGGAAGCCCGGTCATCGTCTCCTCC

>CH557_nt_LC (SEQ ID NO: 127)

GAAATTGTGTTGACGCAGTCTCCAGCCACCCTGTCCGCGTCTCCAGG
GGAAAGAGTCACCCTAACTTGCAGGGCCAGT**CGGAGTGTCCGAAACA**
ACGTGGCCTGGTATCAGCACAAGGGTGGCCAGAGTCCCAGGCTCCTC
ATTTAT**GATGCGTCC**ACGAGGGCCGCTGGTGTCCCAGCCAGGTTCAG
CGGCAGTGCATCTGGGACAGAGTTCACTCTCGCCATCAGCAACTTGG
AGTCTGAAGATTTTACAGTCTACTTCTGT**CTGCAGTATAATAACTGG**
TGGACCTTCGGCCAAGGGACCAGGGTGGACATCAAA

FIG. 12

IMGT

Heavy

40.01	QVQLIQSGPQFKTPGASVTVSCKAS	GYIFTDYL	IHWVRLVPGKGLEWLGR	INTNAGLM
40.02	QVRLMQSGPQLKTPGASVTVSCKAS	GYIFTDYL	IHWVRLVPGKGLEWLGR	INTNAGLM
40.03	QVQLIQSGPQLKTPGASVTVSCKAS	GYVFEADYL	IHWVRLVPGKGLEWLGR	INTNAGLM
40.04	QVRLMQSGTEFKTPGASVKVSCKTS	GYIFSDYL	IHWVRLVPGKGLEWLGR	INTNAGLM
	----------FR1----------	--CDR1--	----------FR2----------	--CDR2--

continued:

YLSHKFEGRLILRRVVDWRTPSLGTVYMELRMVRSDDSAIYFC	GRVVDGFNAAGPLEF	WGQGSPVIVSS
YLSYKFEGRLILRRDVDWRTPSLGTVYMELKNLRSDDSAIYFC	GRVVDGFNAAGPLEF	WGQGSPVIVSS
YLSHKFEGRLILRRDRDWRTPSLGTLYMELRNLKSDDSAIYFC	GRVVDGFNAAGPLEF	WGQGSPVIVSS
YLSPRFEGRVILRRESSFRTPSLGTVYMELKMLKFDDSAVYFC	GRVVDGFNAAGPLEF	WGQGSLVIVSS
----------FR3----------	----------CDR3----------	----FR4----

Figure 13

IMGT

Light
40.01 QVVMTQSPATLSLSPGETAAVSCRAS QYVDRS ISWYQLKTGRAPRLLVY
40.02 QVVMTQSPVTLSVSPGETAAVSCRAS QYVDRS ISWYQLKTGRAPRLLVY
40.03 QVLMTQSPATLSVSPGETAAVSCRAS QYVDRS ISWYQVKSGRAPRLLVY
40.04 EVVMTQSPATLSVSPGEEAALSCGAS DYIDRS VSWYQLKPGRAPRLLVY
_____FR1_____ -CDR1- _____FR2_____

continued:

AAS SRSIGVPDRFSGSGSGRDFTLTIRGVQSDDFALYYC QQDYYMPVTF GQGTRLDMK
AAS SRSIGVPDRFSGSGSGRDFTLTIRGVQSDDFAVYYC QQDYYMPVTF GQGTRLDMK
AAS SRSIGVPDRFSGSGSGTDFTLTIRGVQSDDFALYYC QQDYGMPVTF GQGTRLDMK
AAS SRSIGIPDRFSGSGSGTARTLTIRGVQSDDFALYYC QQDKYMPVTF GQGTRLDMK
CDR2 _____FR3_____ ---CDR3--- ---FR4---

Figure 13 cont.

KABAT
Heavy

```
                1         2         3      4        5    6
      123456789012345678901234567890 12345 67890123456789 012A3456789012345
40.01 QVQLIQSGPQFKTPGASVTVSCKASGYIFT DYLIH WVRLVPGKGLEWLG RINTNAGLMYLSHKFEG
40.02 QVRLMQSGPQLKTPGASVTVSCKASGYIFT DYLIH WVRLVPGKGLEWLG RINTNGGLMYLSYKFEG
40.03 QVQLIQSGPQLKTPGASVTVSCKASGYVFA DYLIH WVRLVPGKGLEWLG RINTNAGLMYLSHKFEG
40.04 QVRLMQSGTEFKTPGASVKVSCKTSGYIFS DYLIH WVRLVPGKGLEWLG RINTNAGLMYLSPRFEG
      ------------FR1------------- -CDR1 -----FR2----- -------CDR2------
```

continued:

```
                                             1             1
 7                  8         9              0             1
6789012A-----BC3456789012345678901234 567890ABCDE12 34567890123
RLILRRVVDWRTPSLGTVNMELRNVRSDDSAIYFCGR VVDGFNAAGPLEF WGQGSPVIVSS
RLILRRDVDWRTPSLGTVYMELKNLRSDDSAIYFCGR VVDGFNAAGPLEF WGQGSPVIVSS
RLILRRDRDWRTPSLGTLYMELRNLKSDDSAIYFCGR VVDGFNAAGPLEF WGQGSPVIVSS
RVILRRESSFRTPSLGTVYMELRNLKFDDSAVYFCGR VVDGFNAAGPLEF WGQGSLVIVSS
----------------FR3----------------- ----CDR3---- ---FR4---
```

Figure 13 cont.

KABAT

Light

	FR1	CDR1	FR2	CDR2
	1 2	3	4	5
	12345678901234567890123	45678901234	567890123456789	0123456
40.01	QVVMTQSPATLSLSPGETAAVSC	RASQYVDRSIS	WYQLKTGRAPRLLVY	AASSRSI
40.02	QVVMTQSPVTLSVSPGETAAVSC	RASQYVDRSIS	WYQLKTGRAPRLLVY	AASSRSI
40.03	QVLMTQSPATLSVSPGETAAVSC	RASQYVDRSIS	WYQVKSGRAPRLLVY	AASSRSI
40.04	EVVMTQSPATLSVSPGEEAALSC	GASDYIDRSVS	WYQLKPGRAPRLLVY	AASSRSI

continued:

FR3	CDR3	FR4
6 7 8	9	0
78901234567890123456789012345678	901234567	8901234567
GVPDRFSGSGSGRDFTLTIRGVQSDDFALYYC	QQDYYWPVT	FGQGTRLDMK
GVPDRFSGSGSGRDFTLTIRGVQSDDFAVYYC	QQDYYWPVT	FGQGTRLDMK
GVPDRFSGSGSGTDFTLTIRGVQSDDFALYYC	QQDYGWPVT	FGQGTRLDMK
GIPDRFSGSGSGTAFTLTIRGVQSDDFALYYC	QQDKYWPVT	FGQGTRLDMK

Figure 13 cont.

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
                    |....|....|....|....|....|....|....|....|
UCA_HC              QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYYMHWVRQA
CH240_HC_           ----------T--R----------TI----------MF-V----------
CH235_HC_           ----------T---Q----------N--V----------
CH239_HC_           ----------R----------T----------MF-V----------
CH236_HC_           ----------A--R----------TI--R----------T--I----------
CH241_HC_           ----------R----------R----------SH----------
CH491_HC_           ----------A--R----------TI--R----------T--I----------
CH493_HC_           --R-L-Y-GG--R----------MI--V----------N-MD--I----------
CH555_HC_           ----------T----R----------TL--RT----------N-ID-FI----R-
CH558_HC_           ----------GG--R--STTTI--V----------S-MD--I----------
CH556_HC_           ----------GT--S--T--TL--T----------N-TD--I----R-
CH557_HC_           --R-A-Y-GG--RL--TMTL--V----------MD--I----------
```

Figure 15A

```
                    50        60        70        80        90
          ....|....|....|....|....|....|....|....|....|....|
UCA HC    PGQGLEWMGIINPSGGSTSYAQKFQGRVTMTRDTSTSTVYMELSSLRSED
CH240_HC  ------C--W-D--V-RI--G------------R-------G--------
CH235_HC  -----QL--W-D--W-R-N---N----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-RPTT-G-----------RY---A--D--------
CH491_HC  ------L--W---R--R-D-SYR-ED--S-Y----M-I---D-RN-K-A-
CH493_HC  ------L--W-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-F-D--N-R-N--GA-GD-FS-Y--K-ME-L--D-RN---D-
CH556_HC  ---RP-L--Y-D--H-RPD-EG--RD-ISLY------V---DVRG--LD-
CH557_HC  ----F-LL-Y-D-AN-RPD--GALRE-LSFY--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.........S
CH241 LC  .............................R..I...............H
CH235 LC  .............................R........R..........
CH239 LC  .........................T...R..V................S
CH558 LC  ............A.....V..T....RG.RN.V....HNV..S......I
CH557 LC  ............A.....V..T....R..RN.V....H.G..S......I
CH555 LC  ........D...A.....V..........GTKV...RHVR..P......
CH556 LC  .TT.....D...A.....V......A...G.QV..FRHIR..P.....S
```

Figure 15B

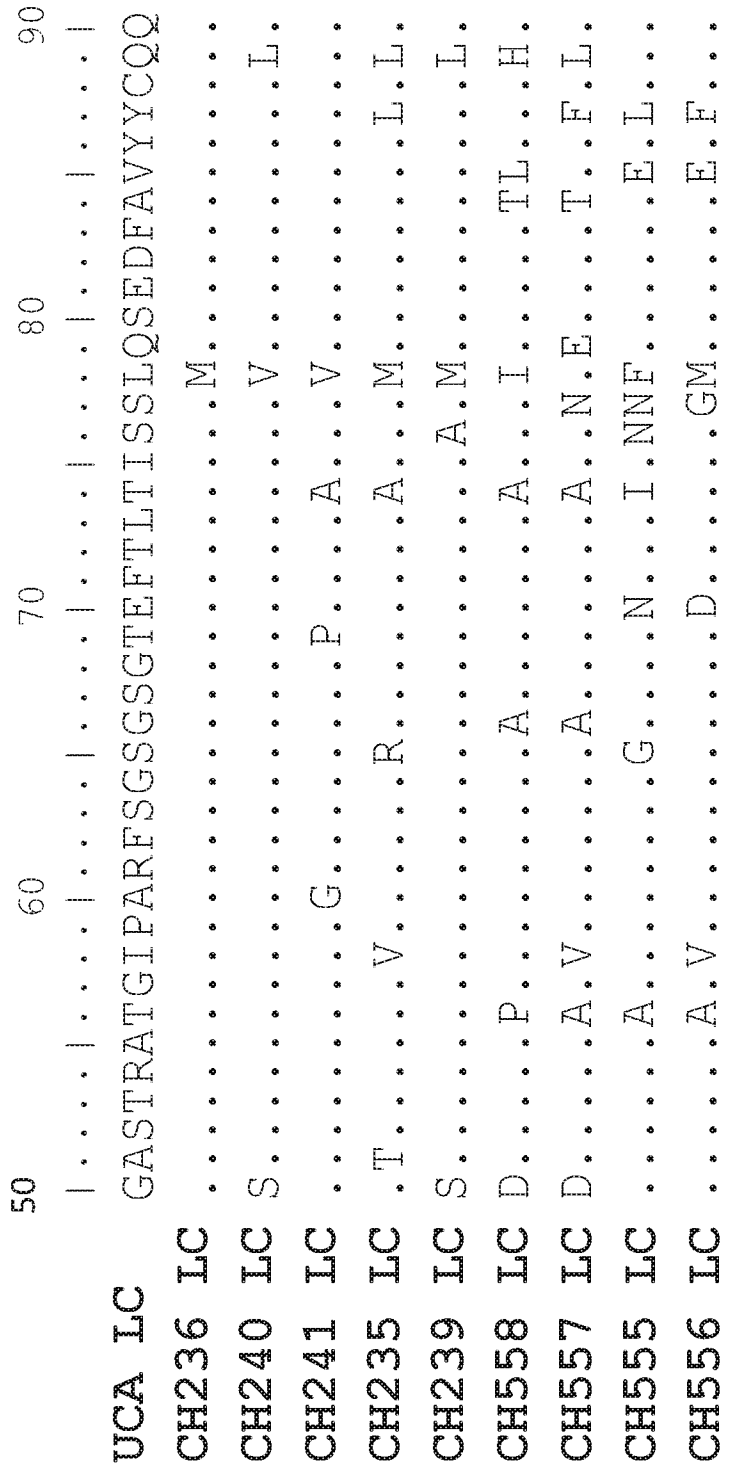


Figure 15B cont.

```
                        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 IDPANGRPDY

                ----------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)

QVQLVQSGAAVKKPGASVRVSCKASGYTFTSSHIHWVRQAPGQALEWLGMIDP
RFGRPTRPQKFQGRVTLTRDTYTTTVYMSLSSLTPEDTAVYYCARSVETSESY
LHFDYWGQGTLVTVSS

>CH235_68w100 (SEQ ID NO: 160)

QVQLVQSGAAVKRPGASVTISCRASGYTFTTYYIHWVRQAPGQGLELMGWINP
RGGRTDYSYRFEDRVSMYRDTSMSIVYMDLRNLKSADTAVYYCVRNVGTSGSL
LHYDFWGQGSLVTVSS

>CH235_115w100 (SEQ ID NO: 161)

QVQLVQSGAAVKKPGASVRVSCKASGYTFTSSHIHWVRQAPGQGPEWMGMVDP
RFGRPTYAQKFQGRVAMTRDIYTSTVYMDLRSLKSEDTAIYFCVRNAETEGSL
LHIEYWGQGTRVTVSS

>CH235_75w152 (SEQ ID NO: 162)

QVRLLQYGGGVKRPGASMTISCVASGYNFNDYYIHWVRQAPGQGLELMGWIDP
SGGRTDYAGAFGDRVSMYRDKSMNTLYMDLRSLRSGDTAMYYCVRNVGTAGSL
LHYDHWGLGVMVTVSS

>CH236_VK_aa (SEQ ID NO: 163)

EIVLTQSPATLSVSPGERVTLSCRASQSVRNNLAWYRQKRGQAPRLLIYGAST
RATGIPARFSGSGSGTEFTLTISSMQSEDFAVYYCQQYNNWWTFGQGTKVEIK

>CH241_VK_aa (SEQ ID NO: 164)

EIVLTQSPATLSVSPGERATLSCRASQSVRSNIAWYQQKPGQAPRLLIHGAST
RATGIPGRFSGSGSGPEFTLAISSVQSEDFAVYYCQQYNDWWTFGQGTKVEIK

FIG. 18

Clean Sequences for Plasmid Production

PtID	Ab ID	CH #	TX	VH	VL	Specificity
703-01-050-5	HES65W202GPOPB129w66.1.1	490	n/a	CH235_129w66	pCK001826	In CH235 lineage from 454. Paired with CH241 LC.
703-01-050-5	HES65W202GU3T768w100.1.2	491	n/a	CH235_68w100	pCK001690	In CH235 lineage from 454. Paired with CH236 LC.
703-01-050-5	HES65W201B95MO115w100.1.1	492	n/a	CH235_115w100	pCK001826	In CH235 lineage from 454. Paired with CH241 LC.
703-01-050-5	HES65W201DXWBB75w152.4.3	493	n/a	CH235_75w152	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)

CAGGTGCAGCTGGTgCAGTCTGGGGCTGCGGTGAAGAAGCCTGGGGCCTCAGTGAGGGTTTC
CTGCAAGGCATCTGGATACACCTTCACCAGTTCTCATATCCACTGGGTGCGACAGGCCCCTG
GACAAGCACTTGAGTGGTTGGGAATGATCGACCCTCGTTTTGGTAGGCCAACCCGCCCTCAG
AAGTTCCAGGGCAGAGTCACCCTGACCAGAGACACGTACACGACTACAGTGTACATGTCACT
GAGCAGCCTGACACCTGAAGACACGGCCGTTTACTACTGTGCGAGAAGCGTGGAAACGAGTG
AGAGCTATCTCCACTTTGACTACTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCAg

>CH235_68w100 (SEQ ID NO: 166)

CAGGTGCAGCTGGTgCAGTCTGGGGCTGCGGTGAAGAGGCCTGGGGCCTCAGTGACGATTTC
CTGCAGGGCATCTGGATACACCTTCACCACCTACTATATACACTGGGTGCGACAGGCCCCTG
GACAAGGACTTGAGTTGATGGGATGGATCAACCCTCGTGGCGGTCGCACAGACTACTCTTAC
AGATTTGAGGACAGAGTCAGTATGTACAGGGACACGTCCATGAGTATAGTCTACATGGACTT
GAGGAACCTGAAATCTGCGGACACGGCCGTCTACTATTGTGTGAGAAATGTGGGAACCAGTG
GGAGCTTGCTCCACTATGACTTCTGGGGCCAGGGAAGCCTGGTCACCGTCTCCTCAg

>CH235_115w100 (SEQ ID NO: 167)

CAGGTGCAGCTGGTgCAGTCTGGGGCTGCGGTGAAGAAGCCTGGGGCCTCAGTGAGGGTTTC
CTGCAAGGCATCTGGATACACCTTCACCAGTTCTCATATCCACTGGGTGCGACAGGCCCCTG
GACAAGGCCCTGAGTGGATGGGCATGGTCGACCCCCGTTTTGGTCGCCCAACCTACGCACAG
AAGTTTCAGGGCAGGGTCGCCATGACCAGGGACATTTACACGAGCACAGTCTACATGGACTT
GAGGAGCCTAAAATCTGAGGACACGGCCATCTATTTCTGTGTGAGAAATGCGGAAACGGAGG
GCAGCTTACTCCACATTGAGTACTGGGGCCAGGGAACCCGGGTCACCGTCTCCTCAg

FIG. 19

>CH235_75w152 (SEQ ID NO: 168)

CAGGTGCGACTACTACAATATGGGGGTGGAGTGAAGAGGCCTGGGGCCTCAAT
GACGATTTCCTGCGTGGCGTCTGGATACAACTTCAACGACTACTATATACACT
GGGTGCGACAGGCCCCTGGACAAGGCCTCGAATTGATGGGATGGATCGACCCT
AGTGGTGGTCGCACAGATTACGCAGGGGCGTTTGGGGACAGAGTGTCCATGTA
CAGGGACAAGTCCATGAACACACTCTACATGGACCTGAGGAGCCTGAGATCTG
GCGACACGGCCATGTATTATTGTGTTAGAAATGTGGGAACGGCTGGCAGCTTG
CTCCACTATGACCACTGGGGCCTGGGAGTTATGGTCACCGTCTCCTCAg

>CH236_VK_nt (SEQ ID NO: 169)

GAAATTGTGTTGACGCAGTCTCCAGCCACCCTGTCTGTATCTCCAGGGGAAAG
AGTCACCCTCTCCTGCAGGGCCAGTCAGAGTGTTAGAAACAACTTAGCCTGGT
ACCGGCAGAAACGTGGCCAGGCTCCCAGACTCCTCATCTATGGTGCATCCACC
AGGGCCACTGGTATCCCAGCCAGGTTCAGTGGCAGTGGGTCTGGGACAGAGTT
CACTCTTACCATCAGCAGCATGCAGTCTGAAGATTTTGCAGTTTATTACTGTC
AGCAGTATAATAACTGGTGGACGTTCGGCCAAGGGACCAAGGTGGAAATCAAA
C

>CH241_VK_nt (SEQ ID NO: 170)

GAAATTGTGTTGACGCAGTCTCCAGCCACCCTGTCTGTGTCTCCAGGGGAAAG
AGCCACCCTCTCCTGCAGGGCCAGTCAGAGTGTTAGAAGCAACATAGCCTGGT
ACCAACAAAAACCTGGCCAGGCTCCCAGGCTCCTCATCCATGGTGCATCCACC
AGGGCCACAGGTATCCCAGGCAGGTTCAGTGGCAGTGGGTCTGGGCCAGAGTT
CACTCTCGCCATCAGCAGCGTGCAGTCTGAAGATTTTGCAGTTTATTACTGTC
AGCAGTATAATGACTGGTGGACGTTCGGCCAAGGGACCAAGGTGGAAATCAAA
C

FIG. 19 cont.

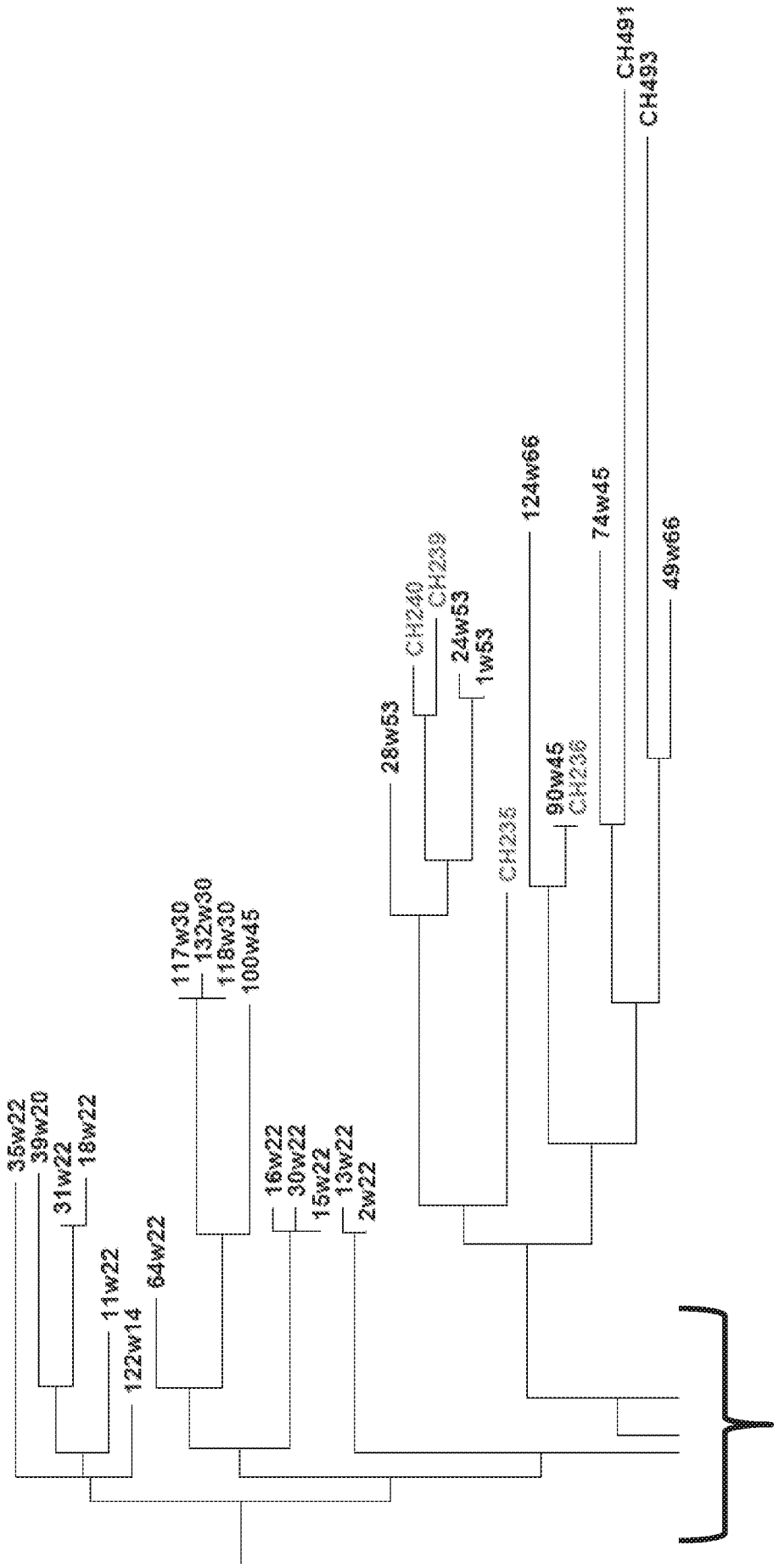

CH491
CH493
124w66
74w45
49w66
CH240
CH239
24w53
1w53
28w53
90w45
CH238
CH236
117w30
132w30
118w30
100w45
16w22
30w22
15w22
13w22
2w22
35w22
39w20
31w22
18w22
11w22
122w14
64w22
continued below

FIG. 20

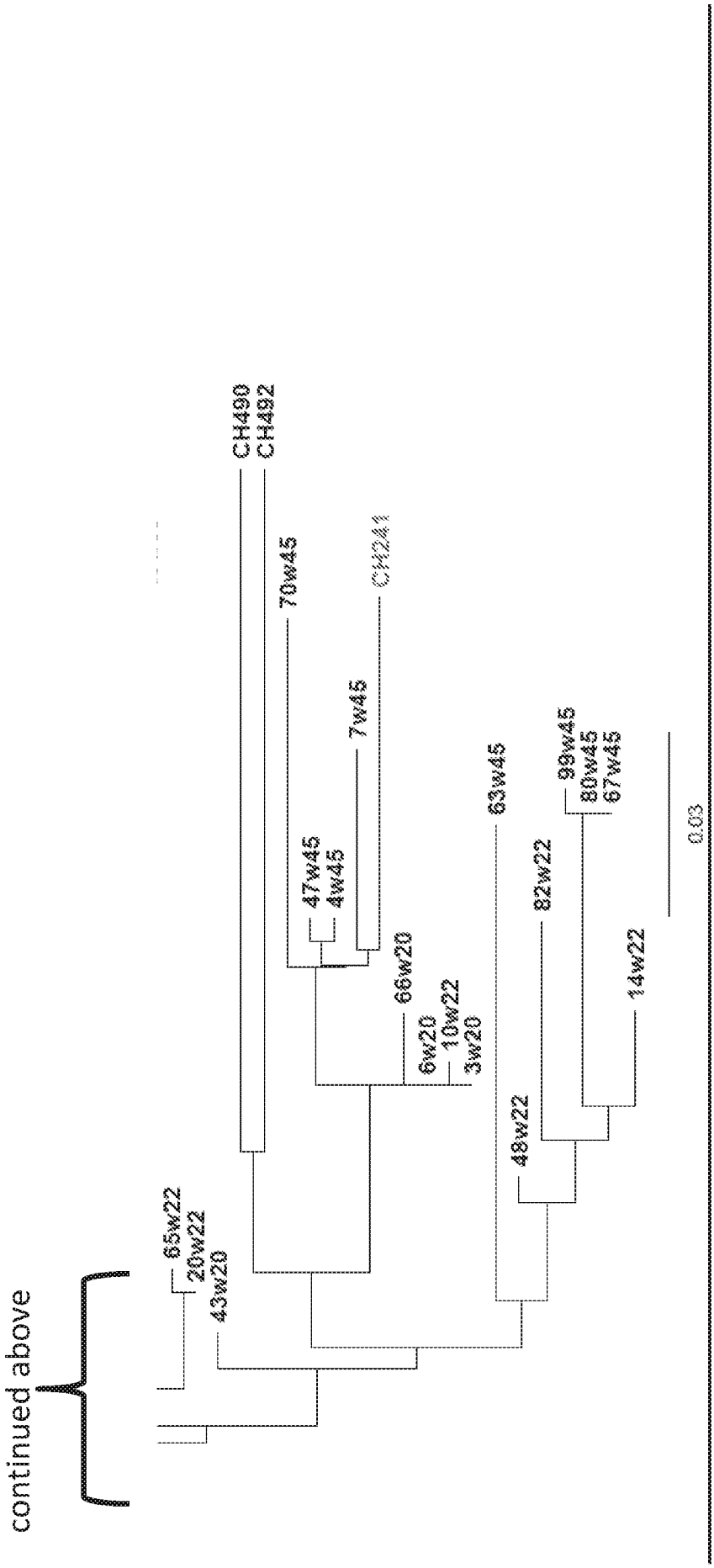

continued above
65w22
20w22
43w20
CH490
CH492
70w45
CH241
47w45
4w45
7w45
66w20
6w20
10w22
3w20
63w45
48w22
82w22
99w45
80w45
67w45
14w22
0.03

FIG. 20 cont.

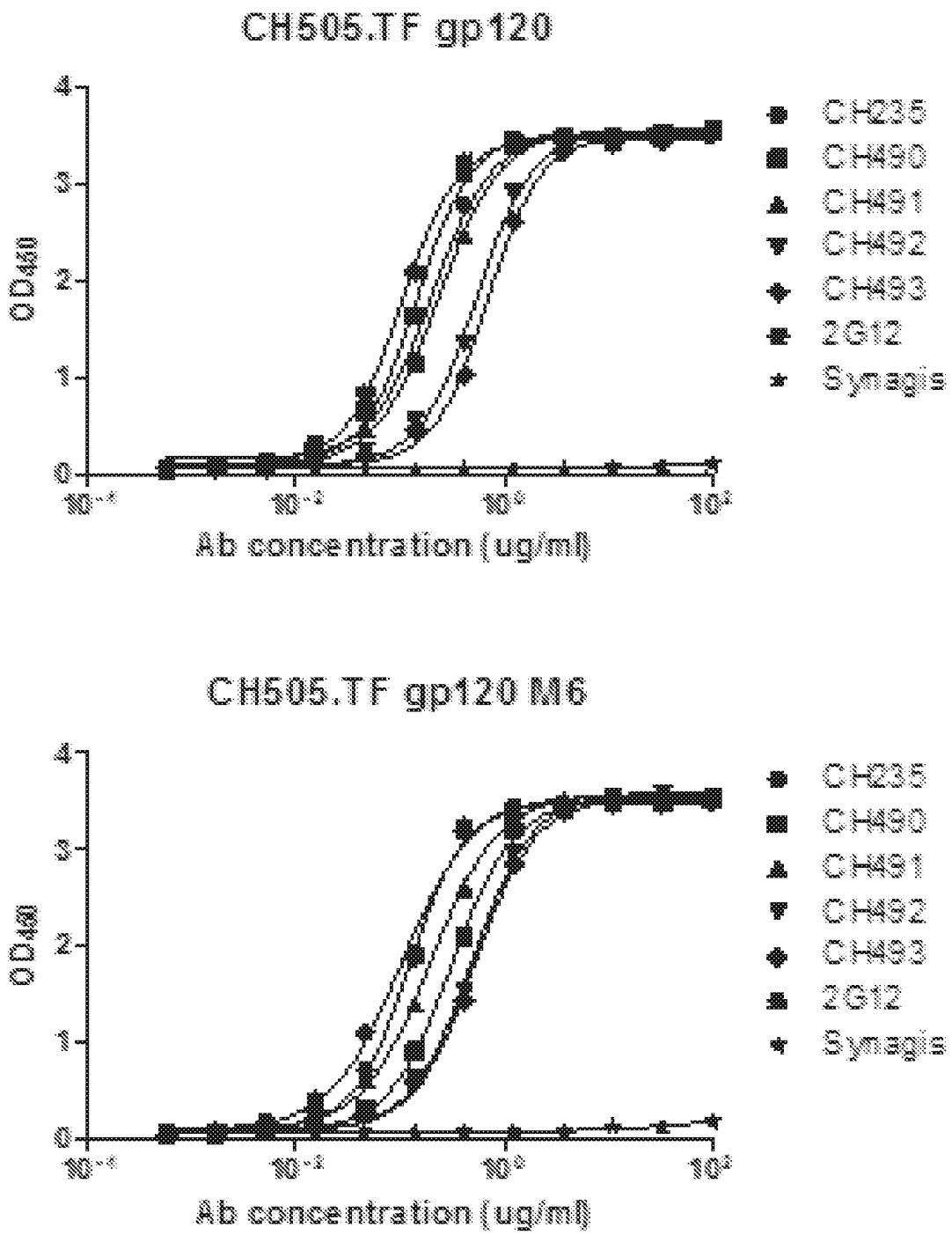

CH505.TF gp120
OD450
Ab concentration (ug/ml)
CH235
CH490
CH491
CH492
CH493
2G12
Synagis
CH505.TF gp120 M6
OD450
Ab concentration (ug/ml)
CH235
CH490
CH491
CH492
CH493
2G12
Synagis

FIG. 21

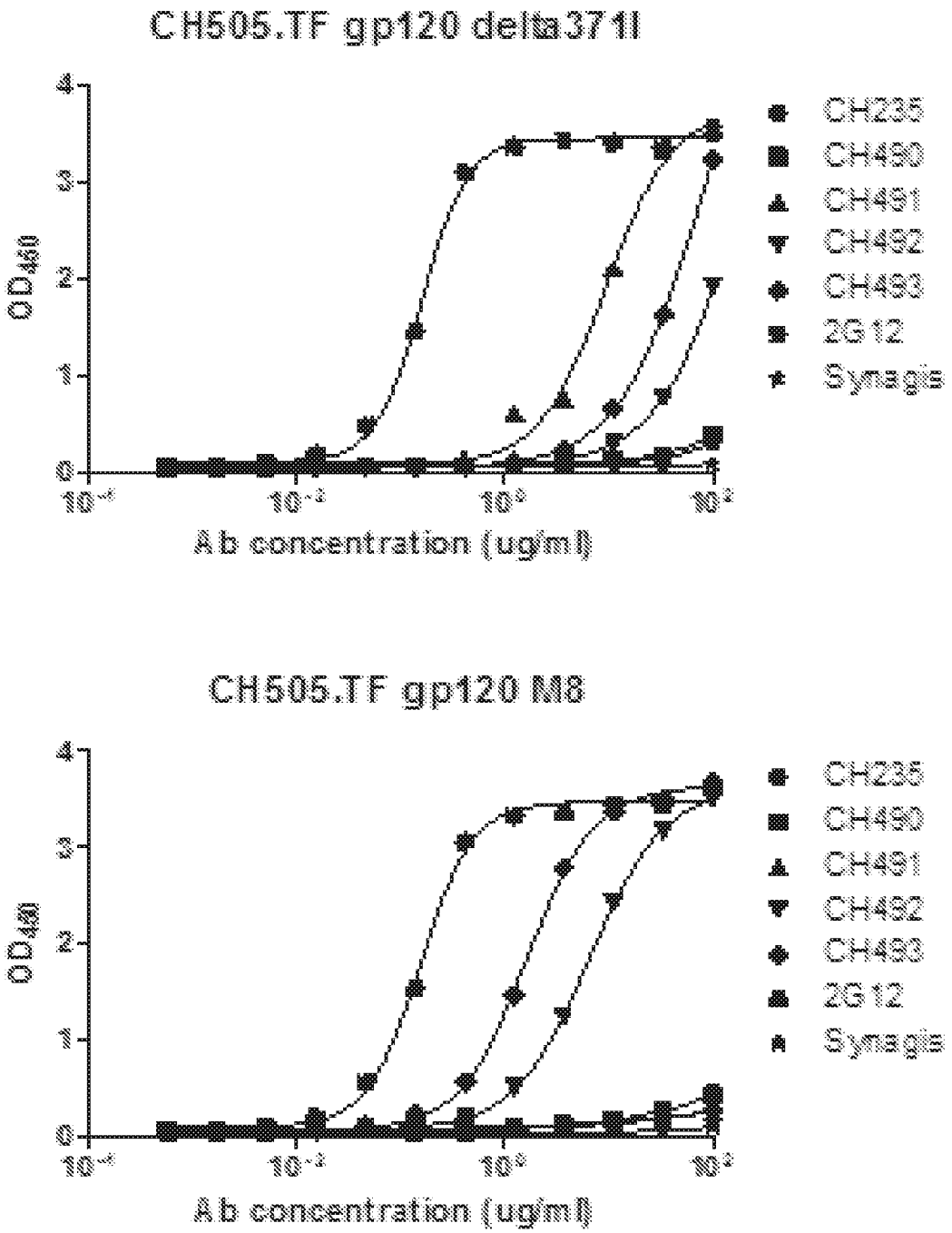

CH505.TF gp120 delta371I
OD460
Ab concentration (ug/ml)
CH235
CH490
CH491
CH492
CH493
2G12
Synagis
CH505.TF gp120 M8
OD460
Ab concentration (ug/ml)
CH235
CH490
CH491
CH492
CH493
2G12
Synagis

FIG. 21 cont.

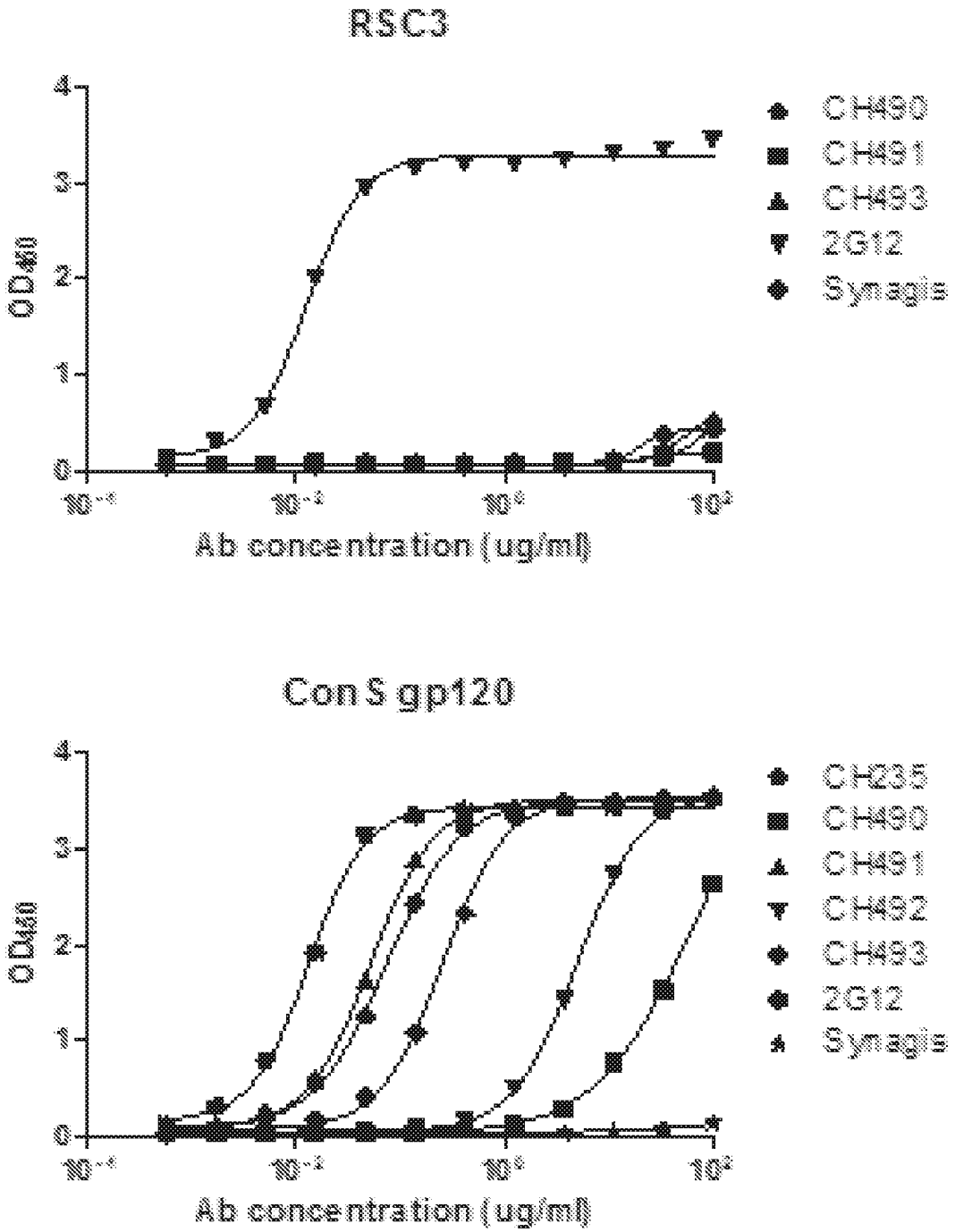

RSC3
OD450
Ab concentration (ug/ml)
CH490
CH491
CH493
2G12
Synagis
ConS gp120
OD450
Ab concentration (ug/ml)
CH235
CH490
CH491
CH492
CH493
2G12
Synagis

FIG. 21 cont.

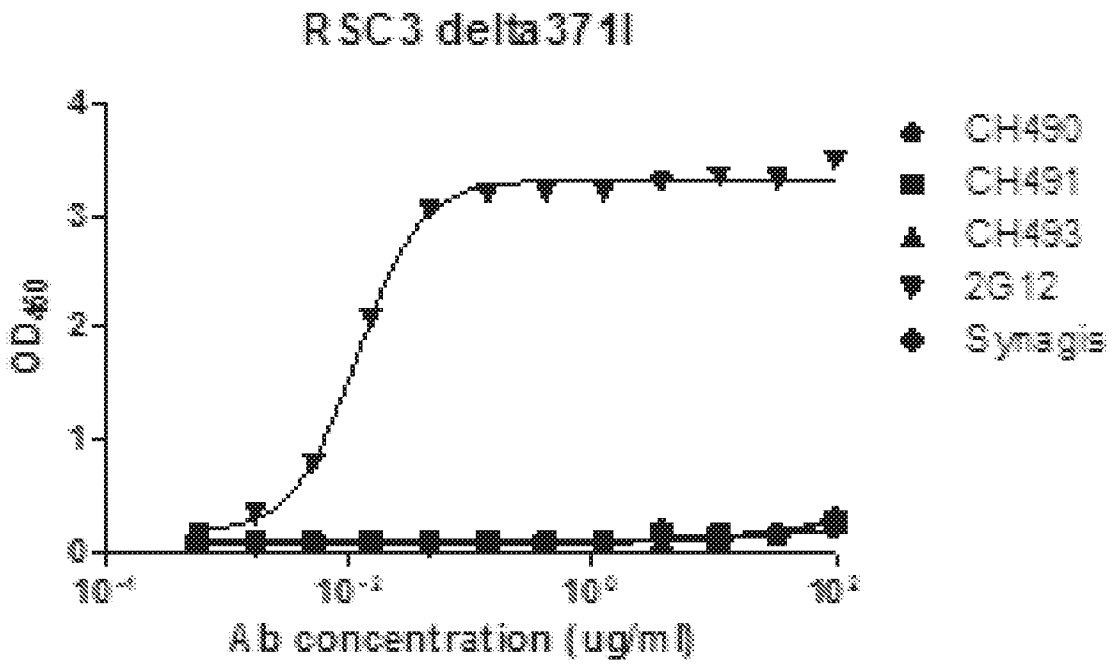

RSC3 delta371I
OD450
Ab concentration (ug/ml)
CH490
CH491
CH493
2G12
Synagis

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	**23**	>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	1.6	>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

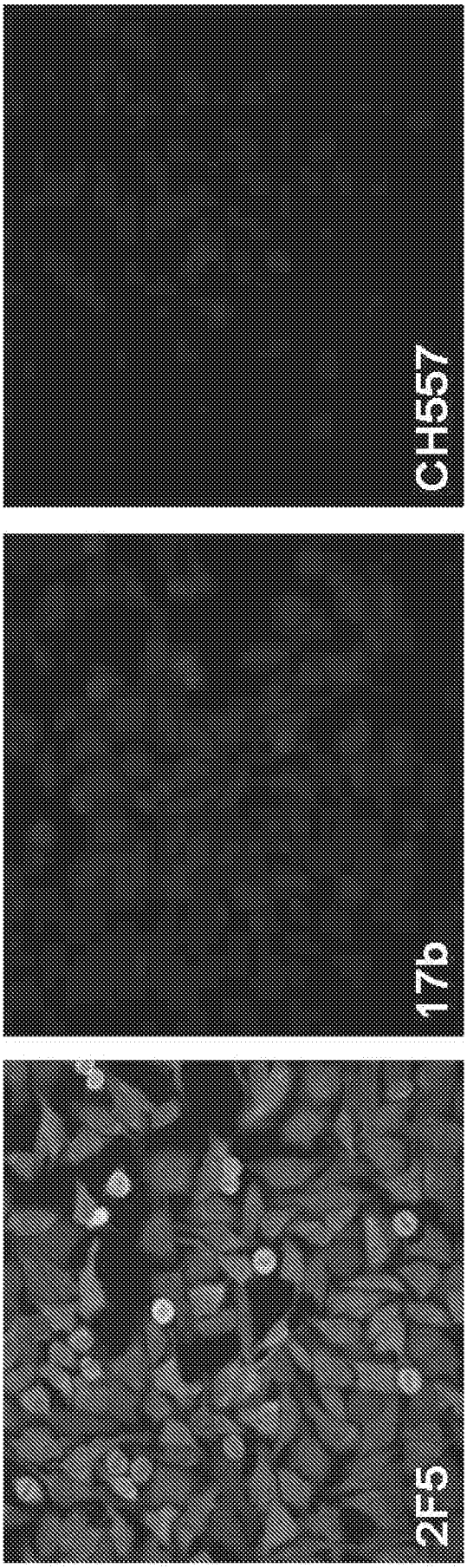


FIG. 25

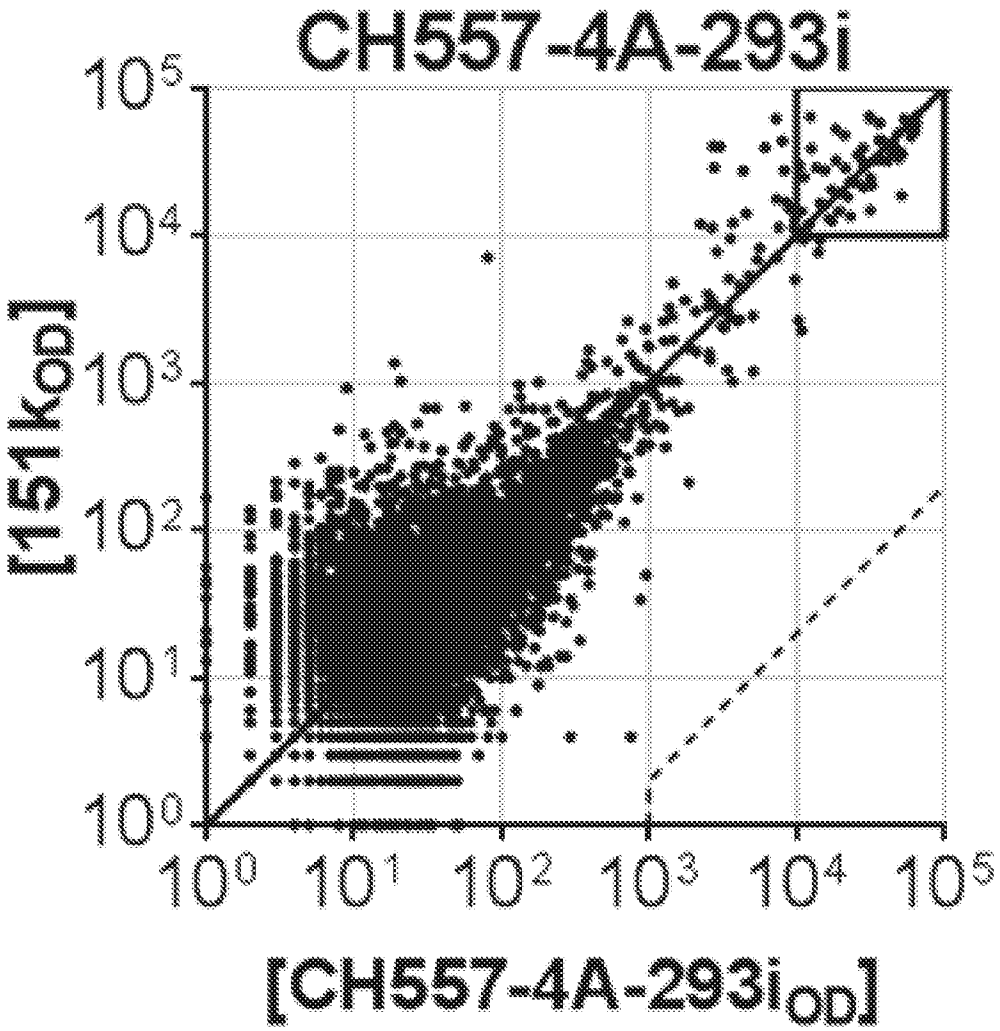

CH557-4A-293i
10^5
10^4
10^3
10^2
10^1
10^0
[151kOD]
10^0 10^1 10^2 10^3 10^4 10^5
[CH557-4A-293iOD]

FIG. 26A

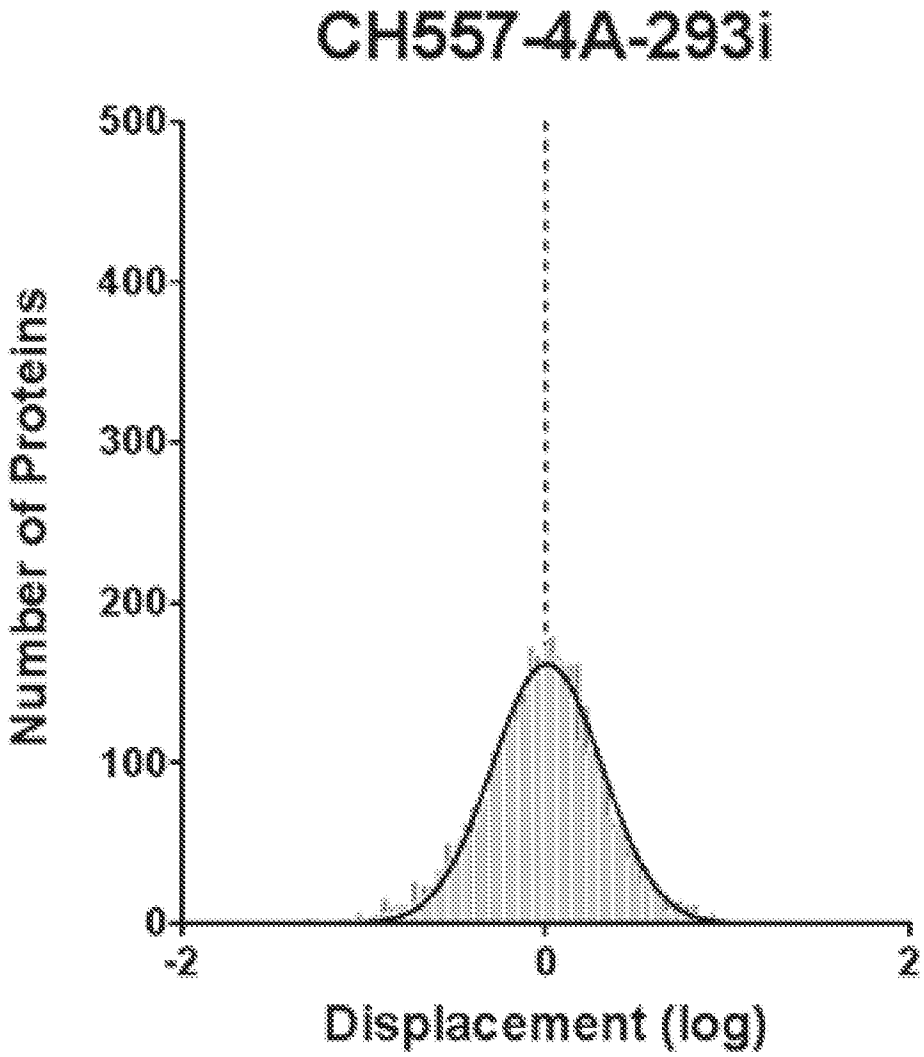

CH557-4A-293i
Number of Proteins
500
400
300
200
100
0
-2
0
2
Displacement (log)

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 CH522
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	0.013	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	23.6	>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	18.8	>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	0.039	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		10.2	>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	31.3	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	16.3	
7165.18	B	>50	>50	45.4	1.43	0.912	6.54	>50	>50	>50	>50	
45_01dG5	B	0.058	>50	0.011	0.0008	0.0063			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	0.004	0.023	0.012	0.132	0.676	>50	>50	
Bal.26	B	0.214	>50	0.069	0.0010	0.015	0.006	0.061	0.091	102	27.9	
BG1168.01	B	1.42	>50	0.738	0.080	0.077	0.179	0.562	4.17	>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	26.4	>50	>50	
CNE12	B	2.56	>50	0.695	0.129	0.200	0.085	1.42	16.4	>50	>50	
CNE14	B	0.594	>50	0.199	0.018	0.023	0.024	5.19	0.522	>50	>50	
CNE4	B	1.16	>50	0.639	0.129	0.137	0.113	19.2	45.5	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	0.0005	0.003	0.0010	0.046	0.021	>50	>50	
MN.3	B	0.142	>50	0.011	0.0009	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	76.4	1.43	0.494	0.377	0.175	>50	18.2	>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	28.6	
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	14.5	>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		17.2	>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	2.75	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	48.6	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		29.7	>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	0.0023	>50	>50	28.6	>50	>50
0921.V2.C14	C	0.344	109	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	0.060	0.106	3.67	>50	>50	>50
25711-2.4	C	0.974	>50	0.555	0.078	0.055	>50	>50	17.7	>50	>50
25925-2.22	C	0.641	>50	0.474	0.062	0.086	0.136	13.9	28.7	>50	>50
26191-2.48	C	0.583	>50	0.166	0.046	0.019	0.043	11.0	>50	>50	>50
3168.V4.C10	C	0.372	>50	0.255	0.030	0.077	0.110	9.47	16.3	>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	0.033	0.025	0.281	0.462	23.0	>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	0.094	0.070	>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	0.093	0.073	0.330	>50	>50	>50
CAP256.206.C9	C	1.32	14.5	0.971		0.081	0.494	>50		>50	>50
CAP45.G3	C	0.568	>50	7.00	0.037	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	>50
CNE53	C	0.274	1.77	0.097	0.010	0.041	0.051	2.08	6.92	>50	>50	>50
CNE58	C	1.95	>50	0.225	0.020	0.014	0.389	1.20	0.543	>50	>50	>50
DU123.06	C	4.25	>50	7.92	0.133	0.058	0.183	>50	>50	>50	>50	>50
DU151.02	C	0.287	3.94	3.49	0.076	0.041	>50	>50	>50	>50	>50	>50
DU156.12	C	0.285	9.48	0.095	0.007	0.009	0.045	11.4	>50	>50	>50	>50
DU172.17	C	0.361	1.92	>50	0.067	0.044	0.289	>50	>50	>50	>50	>50
DU422.01	C	0.944	>50	>50	2.36	0.034	>50	>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	>50	1.23
SO18.18	C	0.110	>50	0.098	0.006	0.006	0.032	1.50	1.88	>50	>50	>50
TV1.29	C	4.63	>50	>50	1.79	>50	>50	>50	>50	>50	>50	>50
TZA125.17	C	>50	>50	>50	1.41	0.746	>50	47.4	>50	>50	>50	>50
TZBD.02	C	0.219	>50	0.078	0.003	0.013	43.9	>50	5.55	>50	>50	>50
ZA012.29	C	0.971	1.72	0.384	0.046	0.033	0.063	20.7	28.0	>50	>50	>50
ZM106.9	C	0.620	>50	0.311	0.032	0.026	0.083	6.22	4.82	>50	>50	>50
ZM109.4	C	0.416	>50	0.177	0.031	0.059	0.041	17.1	21.1	>50	>50	>50
ZM135.10a	C	>50	>50	2.25	0.107	0.139			>50	>50	>50	>50
ZM176.66	C	0.183	>50	0.083	0.0010	0.005	>50	>50	0.227	>50	>50	>50
ZM197.7	C	1.40	>50	0.428	0.191	0.019	0.398	>50	35.4	>50	>50	>50
ZM214.15	C	2.22	>50	0.893	0.277	0.068	0.088	1.63	41.4	>50	>50	>50
ZM215.8	C	0.315	6.19	0.215	0.050	0.049	0.010	7.96	2.57	>50	>50	>50
ZM233.6	C	1.25	5.71	1.02	0.094	0.067	0.202	>50	7.35	>50	>50	>50
ZM249.1	C	0.273	9.99	0.057	0.017	0.015	0.039	10.0	7.23	>50	>50	>50
ZM53.12	C	0.558	>50	0.625	0.149	0.149	0.212	15.5	>50	>50	>50	>50
ZM55.28a	C	0.665	>50	0.285	0.022	0.023	0.040	0.565	4.59	>50	>50	>50
3326.V4.C3	CD	0.114	>50	0.068	0.0098	0.003	48.1	1.44	>50	>50	>50	>50
3337.V2.C6	CD	0.429	>50	0.890	0.011	0.012	0.008	0.047	0.693	>50	>50	>50
3817.v2.c59	CD	3.63	>50	>50	0.137	0.382	0.216	>50	>50	>50	>50	>50

FIG. 27A cont.

191821.E6.1	D	118	>50	0.353	0.030	0.025	0.035	1.07	31.1	>50	>50		
231965.c1	D	0.691	>50	1.84	0.014	0.077	0.028	14.1	34.0	>50	>50		
247-23	D	>50	>50	0.155	0.875	0.012	0.648	>50	4.15	>50	>50		
3016.v5.c45	D	6.59	>50	>50	0.015	0.382	0.432	10.8	>50	0.940	4.72		
57128.vrc15	D	>50	>50	1.55	0.666	0.066	0.171	43.5	>50	>50	>50		
6405.v4.c34	D	4.08	>50	4.10	0.212	0.655	0.512	5.09	>50	>50	>50		
A03349M1.vrc4a	D	0.351	>50	0.032	0.940	0.027			>50	>50	>50		
A07412M1.vrc12	D	0.466	4.61	0.596	0.020	0.147	0.089	0.510	15.9	>50	>50		
NKU3006.ec1	D				0.169		0.410	>50	>50	0.382			
UG021.16	D						0.057	5.70	>50	0.043			
UG024.2	D				0.013				33.4	>50			
P0402.c2.11	G								>50	>50			
P1981.C5.3	G	2.19	>50	0.330	0.036	0.082			>50	>50			
X1193.c1	G	0.972	>50	0.154	0.029	0.861			11.0	>50			
X1254.c3	G	1.98	>50	0.053	0.029	0.036			>50	>50			
X1632.S2.B10	G	0.484	>50	0.130	0.003	0.055			1.48	>50			
X2088.c9	G	>50	>50	>50	3.06	0.050	>50	0.630	>50	>50			
X2131.C1.B5	G	2.58	>50	0.537		0.102				>50			
SIVmac251.30.SG3	NA	>50	>50	>50	>50	>50	>50	>50	>50	>50			
SVA.MLV	NA	>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

values represent IC80 in ug/ml **IC80**

									<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		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	47.3	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	0.026	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	10.3	>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

FIG. 28A cont.

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
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	0.673	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	>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	11.0
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	49.1	>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	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
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
JRFL.JB	B	0.402	17.6	0.126	0.004	0.011	0.013	0.174	0.067	>50	>50
MN.3	B	0.437	>50	0.041	0.002	0.005	>50	>50	1.11	>50	11.3
PVO.04	B	3.41	>50	1.22	0.306	0.197	0.294	2.40	>50	>50	>50
QH0515.01	B	4.25	>50	3.45	1.98	0.847	1.14	>50	37.3	>50	>50
QH0692.42	B	5.69	>50	2.92	2.22	1.15	1.47	12.1	>50	>50	>50
REJO.67	B	2.71	>50	0.228	0.032	0.047	0.132	0.201	>50	>50	>50
RHPA.7	B	0.224	>50	0.134	0.054	0.040	0.071	12.2	32.4	>50	>50
SC422.8	B	2.27	>50	0.386	0.180	0.157	0.192	0.830	9.15	>50	>50
SF162.LS	B	2.57	>50	0.656	0.127	0.118	0.064	0.453	2.92	40.4	13.7
SS1196.01	B	3.05	>50	0.622	0.228	0.189	0.144	1.21	2.58	>50	>50
THRO.18	B	>50	>50	16.3	5.36	4.03	13.6	>50	>50	>50	>50
TRJO.58	B	1.55	>50	0.292	0.143	0.216	0.216	44.7	>50	>50	>50
TRO.11	B	1.98	>50	1.27	0.316	0.255	0.125	33.5	17.2	>50	>50
WITO.33	B	1.12	>50	0.356	0.220	0.144	0.161	33.0	31.5	>50	>50
X2278.C2.B6	B	1.16	>50	0.409		0.077			5.44	>50	>50
YU2.DG	B	0.548	>50	0.292	0.070	0.079	0.099	0.792		>50	>50
BJOX002000.03.2	BC	2.68	>50	>50		0.140				>50	>50
CH038.12	BC	>50	>50	1.18	0.165	0.208	>50	>50	7.73	>50	>50
CH070.1	BC	7.54	>50	>50	0.350	0.441	>50	12.7	>50	>50	>50
CH117.4	BC	0.898	>50	0.256	0.044	0.074	15.8	>50	14.9	>50	>50
CH119.10	BC	3.01	>50	1.97		0.127				>50	>50
CH181.12	BC	1.80	>50	1.25	0.265	0.171	0.414	>50	12.1	>50	>50
CNE15	BC	0.687	>50	0.292	0.051	0.072	>50	>50	>50	>50	>50

FIG. 28A cont.

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	18.8	>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		22.3	>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	
426c	C												
6322.V4.C1	C		5.15	>50	>50	0.136	0.112	>50	>50	>50	>50	>50	
6471.V1.C16	C		>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	
6631.V3.C10	C		15.4	>50	>50	0.614	0.222	>50	>50	>50	>50	>50	
6644.V2.C33	C		>50	>50	0.455	0.160	0.190	0.229	>50	1.51	11.5	>50	

FIG. 28A cont.

6785.V5.C14	C	>50	>50	0.734	0.229	0.247	0.679	>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
96ZM651.02	C	4.18	>50	2.29	0.366	0.165	1.77	>50	>50	>50	>50
BR025.9	C	>50	>50	3.08	0.060	0.058	>50	0.262	>50	>50	>50
CAP210.E8	C	>50	>50	>50	6.05	>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
CAP256.206.C9	C	3.36	>50	2.52		0.287	1.86	>50		>50	>50
CAP45.G3	C	1.77	>50	40.1	0.162	0.101	32.1	>50	>50	>50	>50
Ce1176.A3	C	3.55	>50	6.78		0.662				>50	>50
CE703010217.B6	C	0.948	>50	0.935		0.097				>50	>50
CNE30	C	3.64	>50	2.18	0.476	0.574	1.05	6.52	>50	>50	>50
CNE31	C	>50	>50	2.11	0.373	0.827	>50	>50	>50	>50	>50
CNE53	C	0.725	6.06	0.268	0.064	0.145	0.327	31.9	>50	>50	>50
CNE58	C	4.17	>50	0.586	0.086	0.041	1.41	10.3	1.46	>50	>50
DU123.06	C	18.5	>50	>50	0.565	0.152	1.77	>50	>50	>50	>50
DU151.02	C	0.827	15.7	>50	0.344	0.125	>50	>50	>50	>50	>50
DU156.12	C	0.829	>50	0.230	0.043	0.027	0.121	19.5	>50	>50	>50
DU172.17	C	1.12	6.87	>50	0.454	0.231	2.46	>50	>50	>50	>50
DU422.01	C	2.76	>50	>50	31.5	0.096	>50	>50	>50	>50	>50
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	19.3	>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	24.7	>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

FIG. 28A cont.

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	17.4	>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	37.5	>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	13.4	>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	16.1	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
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	42.6	>50	>50
UG021.16	D						5.09	>50	>50	2.53	
UG024.2	D				0.105		0.194	>50	>50	0.569	

FIG. 28A cont.

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	CH322
# 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

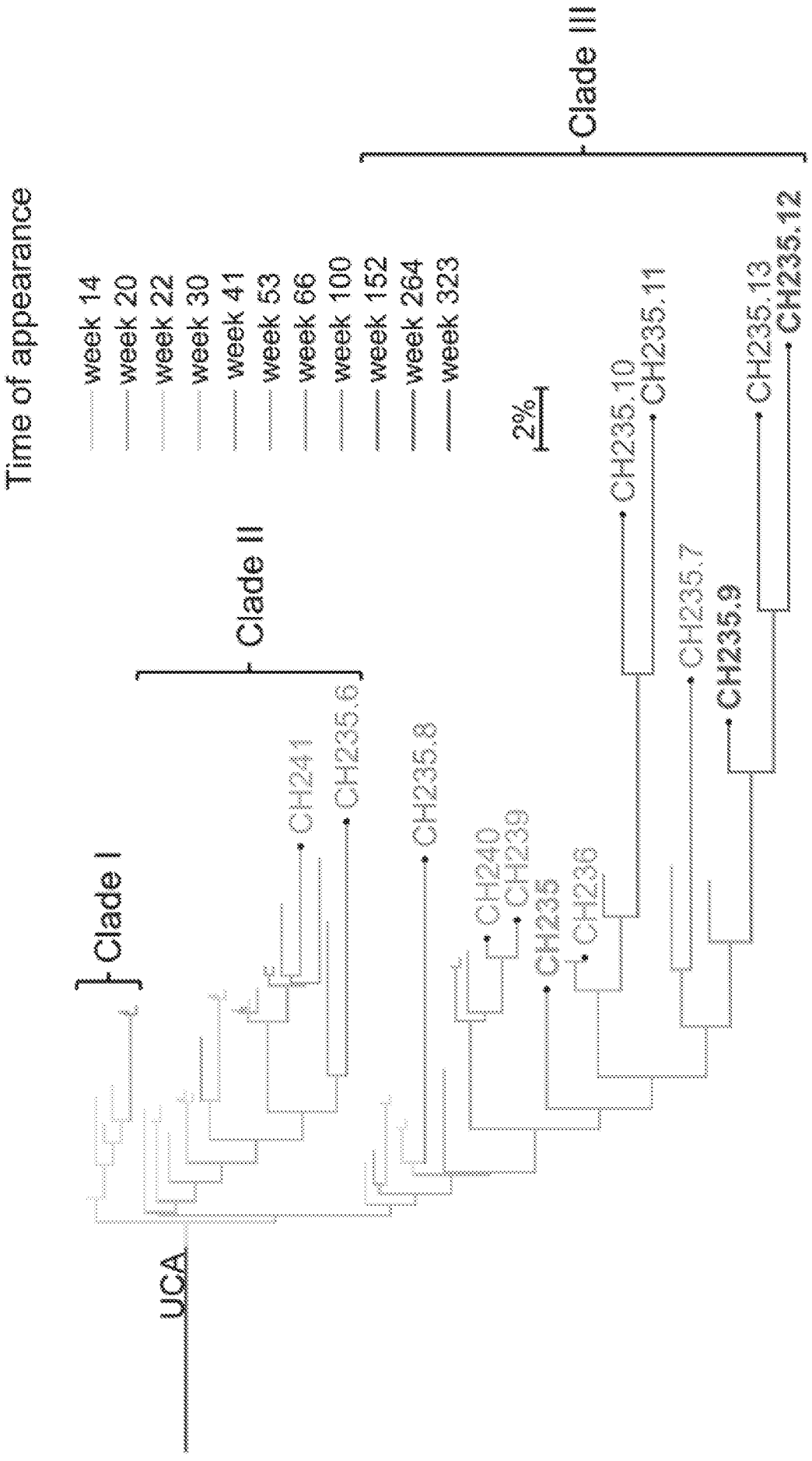
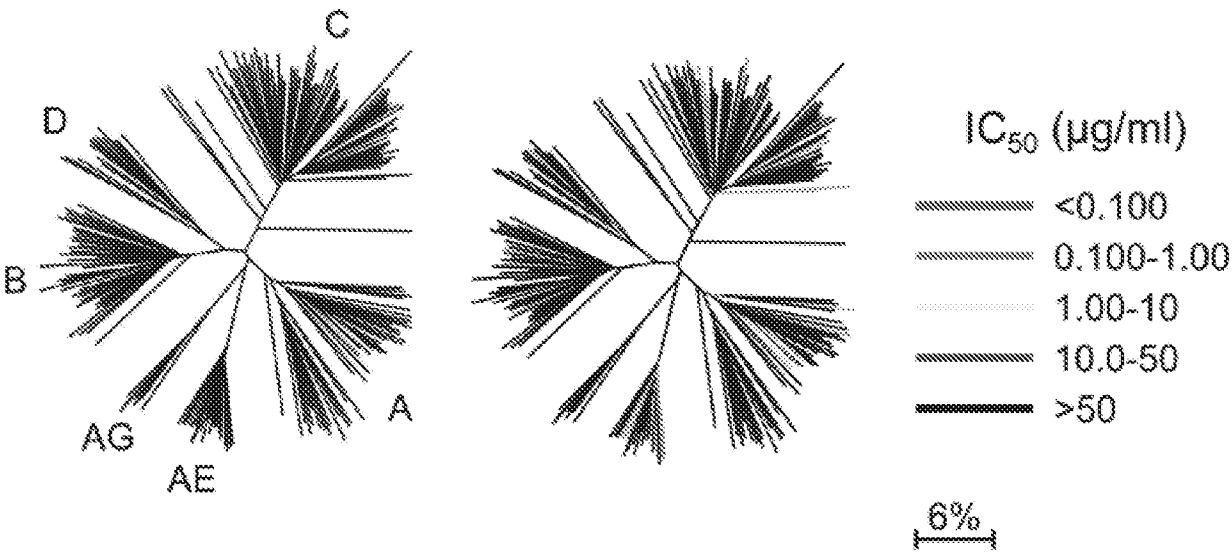

Time of appearance
week 14
week 20
week 22
week 30
week 41
week 53
week 66
week 100
week 152
week 264
week 323
2%
Clade I
Clade II
Clade III
UCA
CH241
CH235.6
CH235.8
CH240
CH239
CH235
CH236
CH235.10
CH235.11
CH235.7
CH235.9
CH235.13
CH235.12

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

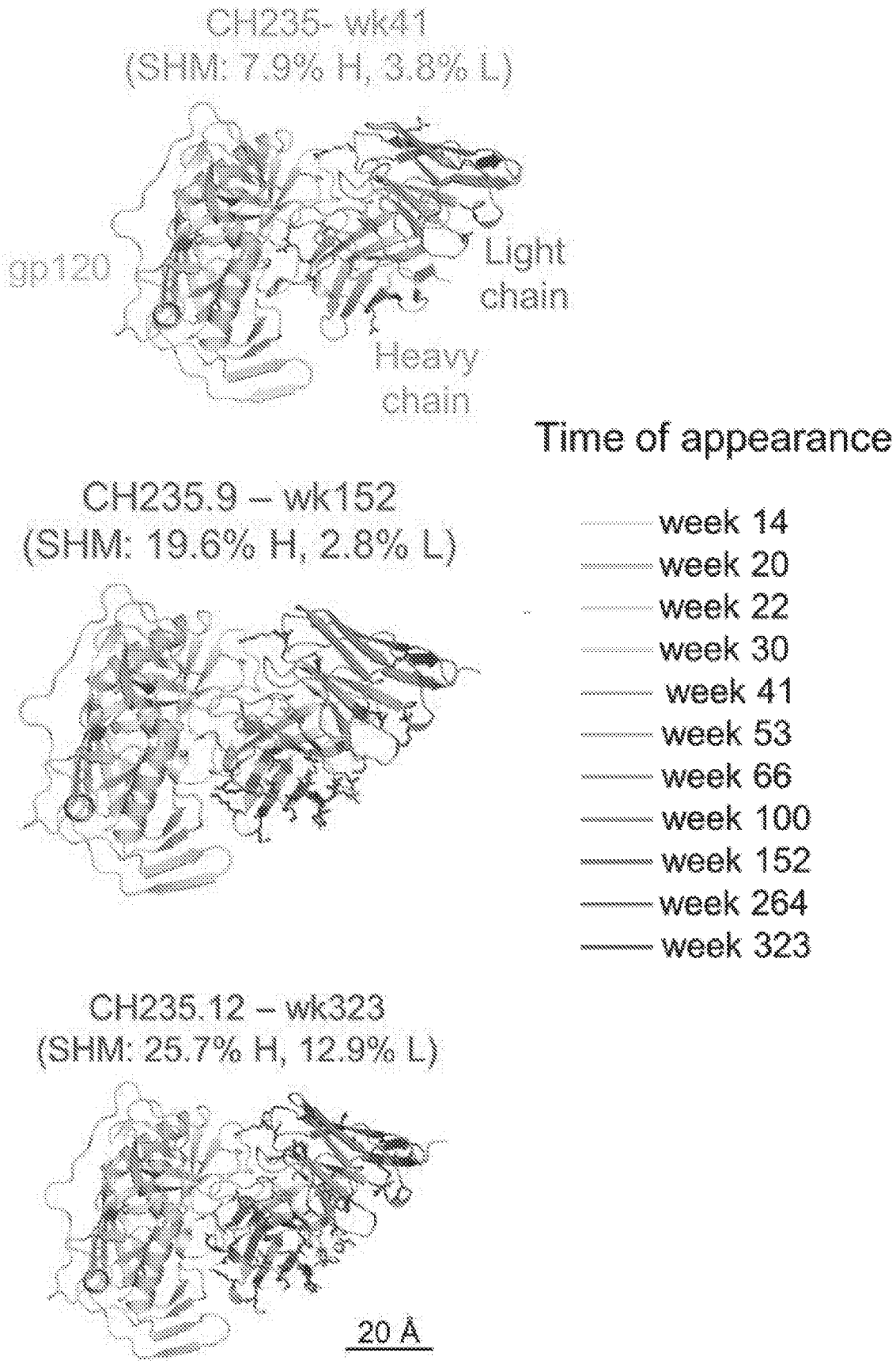

CH235- wk41
(SHM: 7.9% H, 3.8% L)
gp120
Light chain
Heavy chain
Time of appearance
CH235.9 – wk152
(SHM: 19.6% H, 2.8% L)
week 14
week 20
week 22
week 30
week 41
week 53
week 66
week 100
week 152
week 264
week 323
CH235.12 – wk323
(SHM: 25.7% H, 12.9% L)
20 Å

FIG. 30A

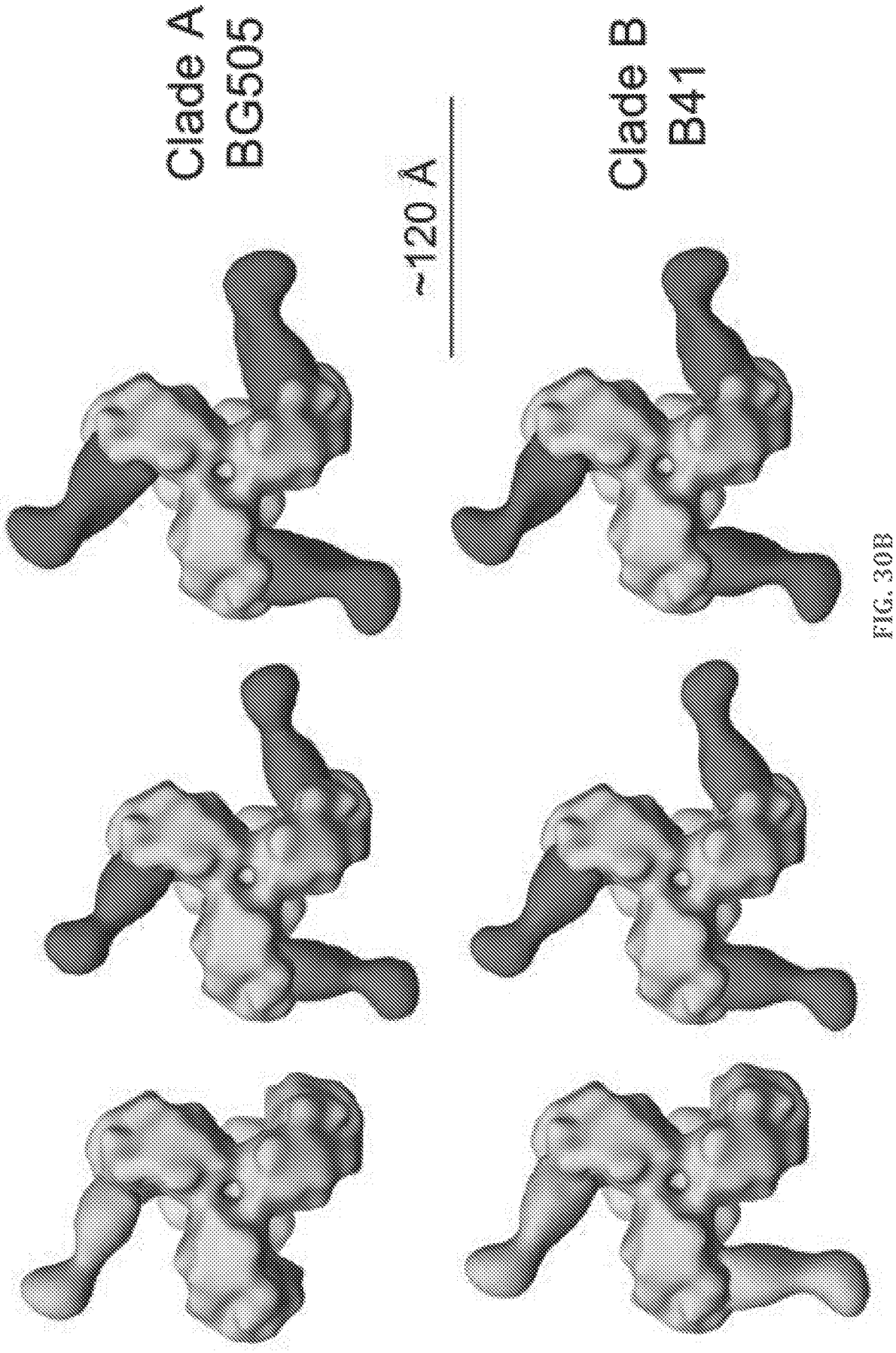


FIG. 30B

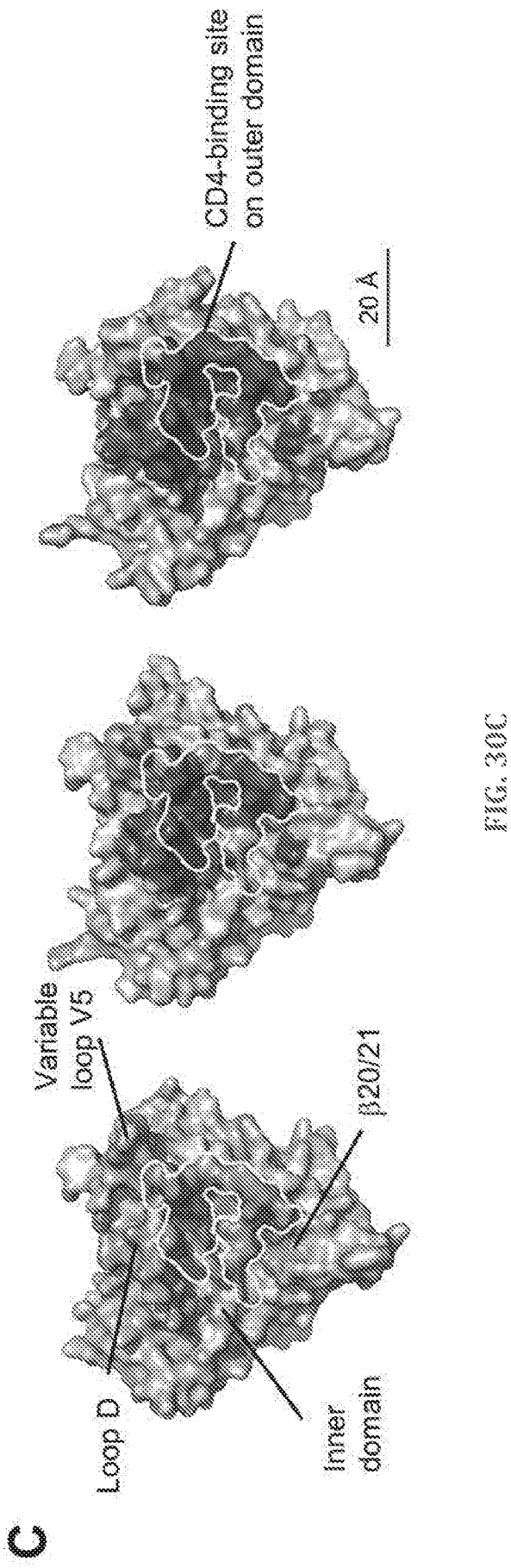

C
Loop D
Variable loop V5
Inner domain
β20/21
CD4-binding site on outer domain
20 Å

FIG. 30C

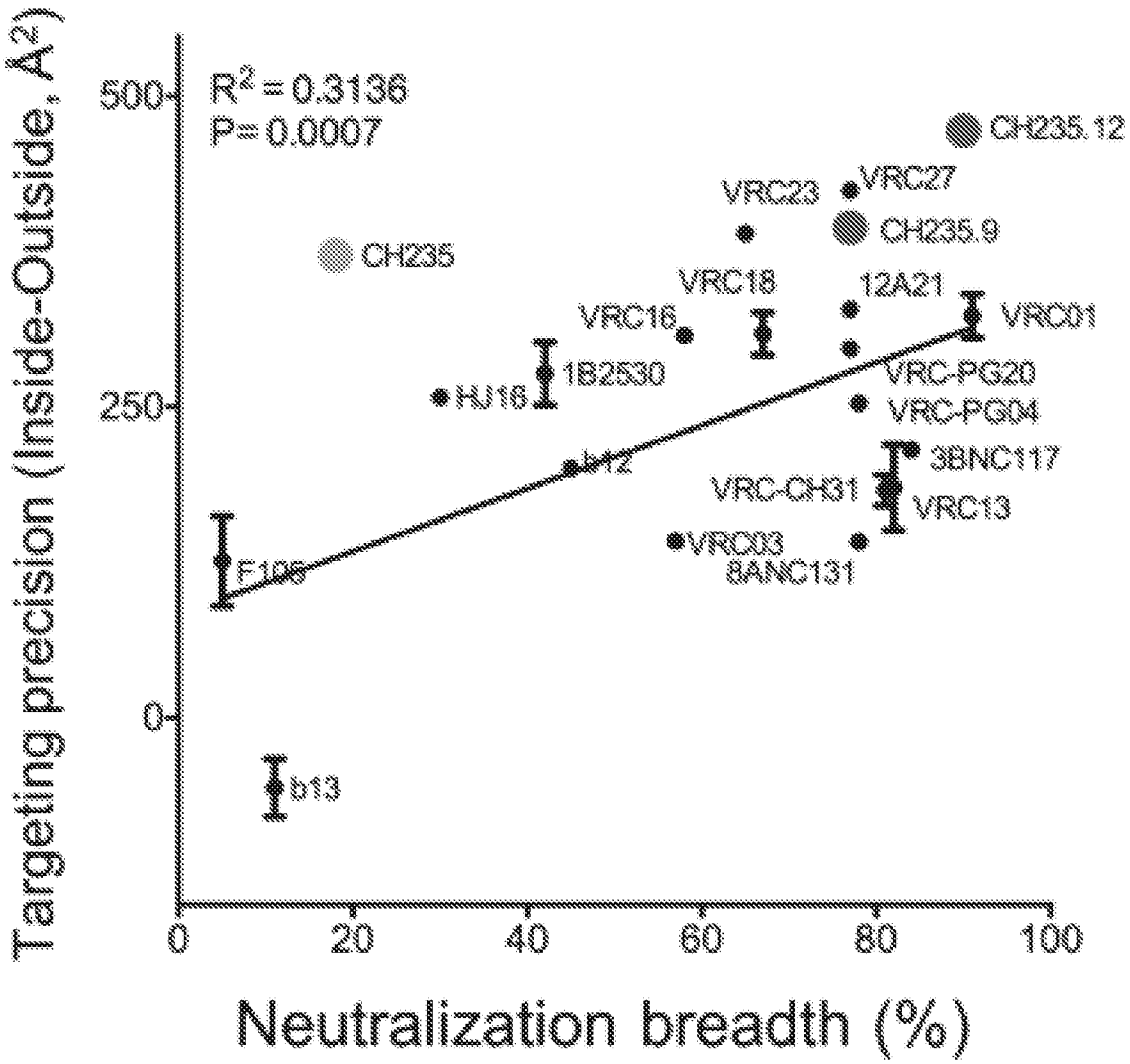

Targeting precision (Inside-Outside, Å²)
R² = 0.3136
P = 0.0007
500
250
0
CH235.12
VRC23
VRC27
CH235.9
CH235
VRC18
12A21
VRC16
VRC01
1B2530
HJ16
VRC-PG20
VRC-PG04
b12
3BNC117
VRC-CH31
VRC13
VRC03
8ANC131
F105
b13
0
20
40
60
80
100
Neutralization breadth (%)

FIG. 30D

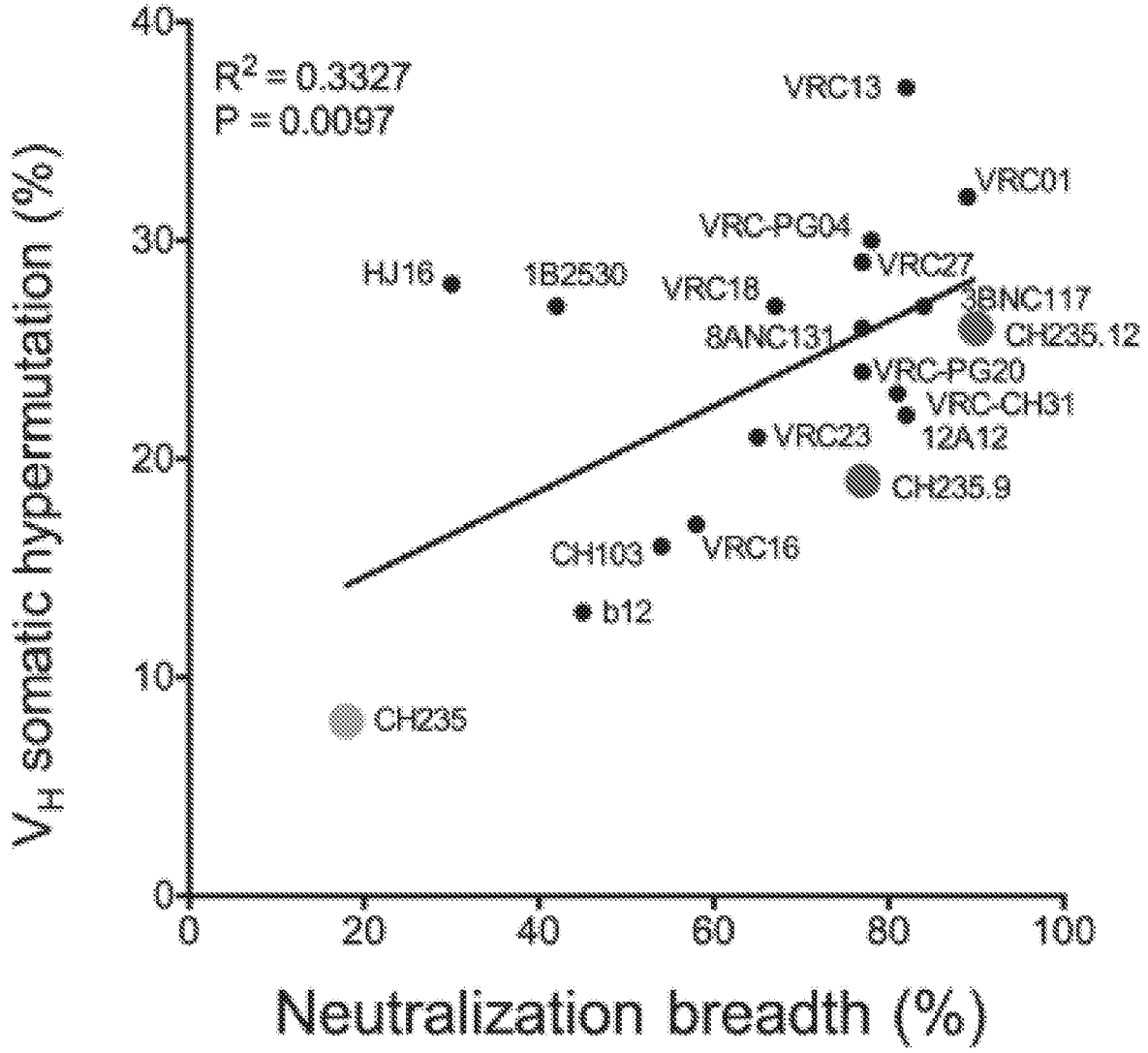

$R^2 = 0.3327$
P = 0.0097
VRC13
VRC01
VRC-PG04
VRC27
HJ16
1B2530
VRC18
3BNC117
8ANC131
CH235.12
VRC-PG20
VRC-CH31
VRC23
12A12
CH235.9
CH103
VRC16
b12
CH235
V_H somatic hypermutation (%)
Neutralization breadth (%)
0
10
20
30
40
0
20
40
60
80
100

FIG. 30E

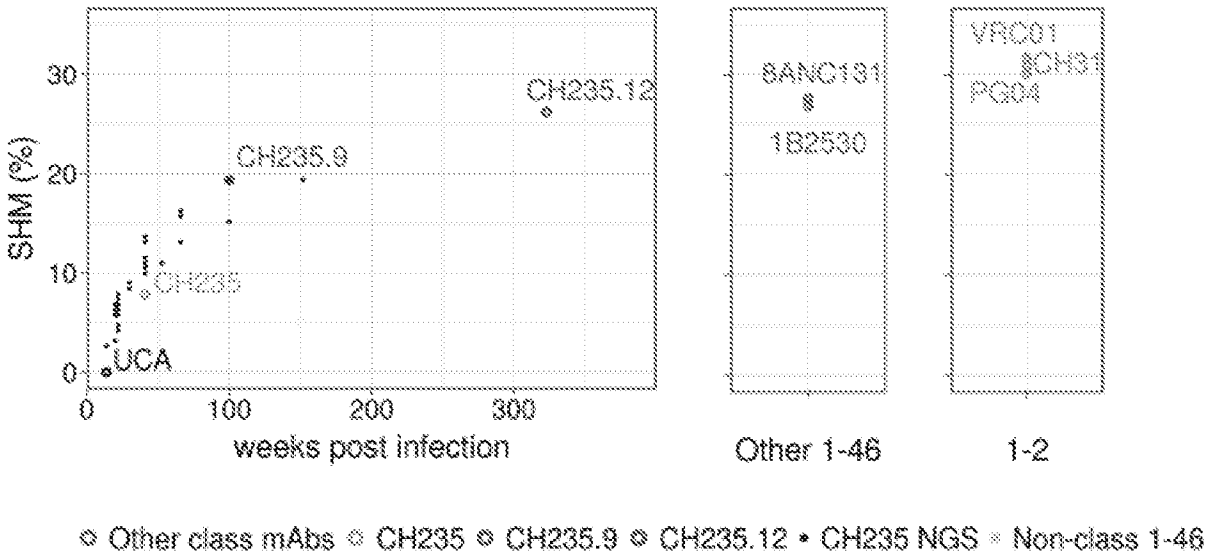


FIG. 31A

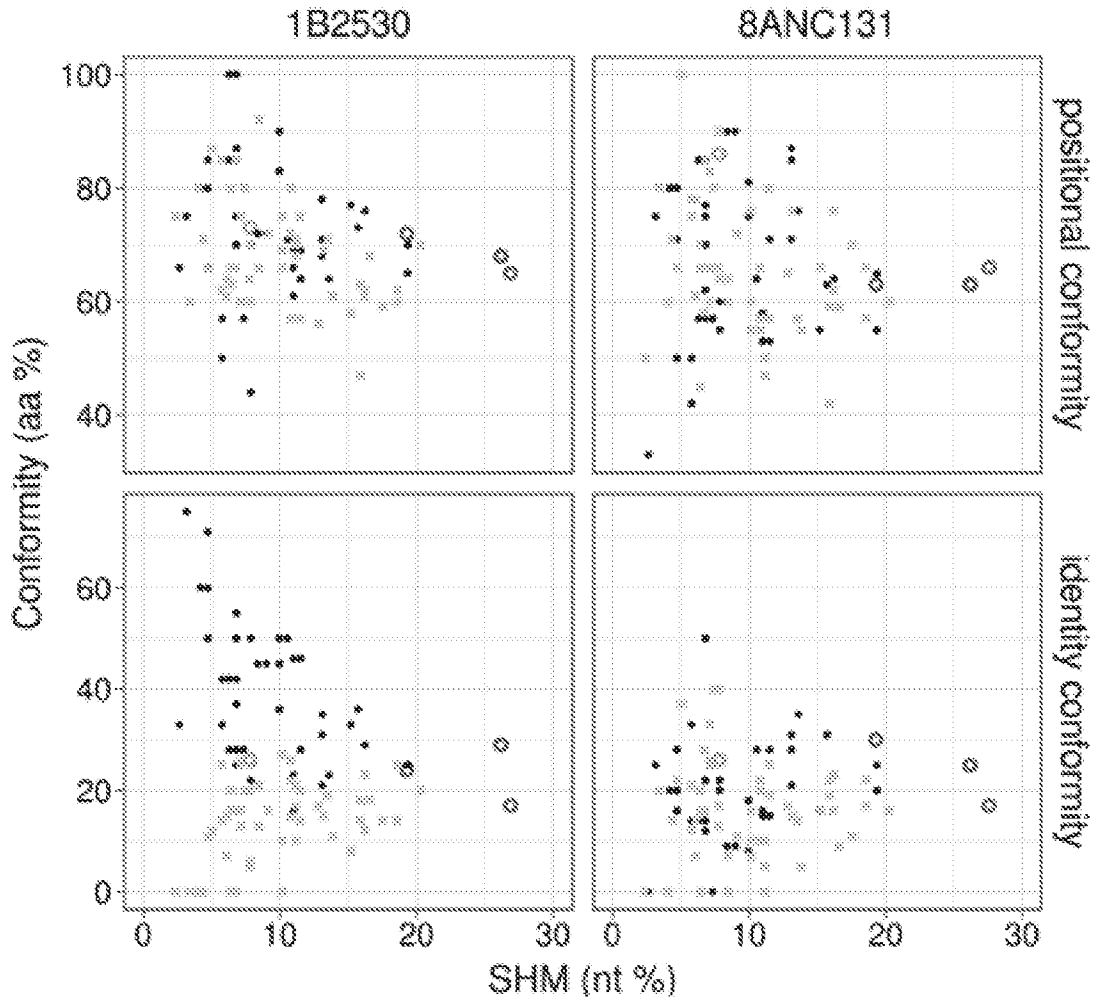


FIG. 31B

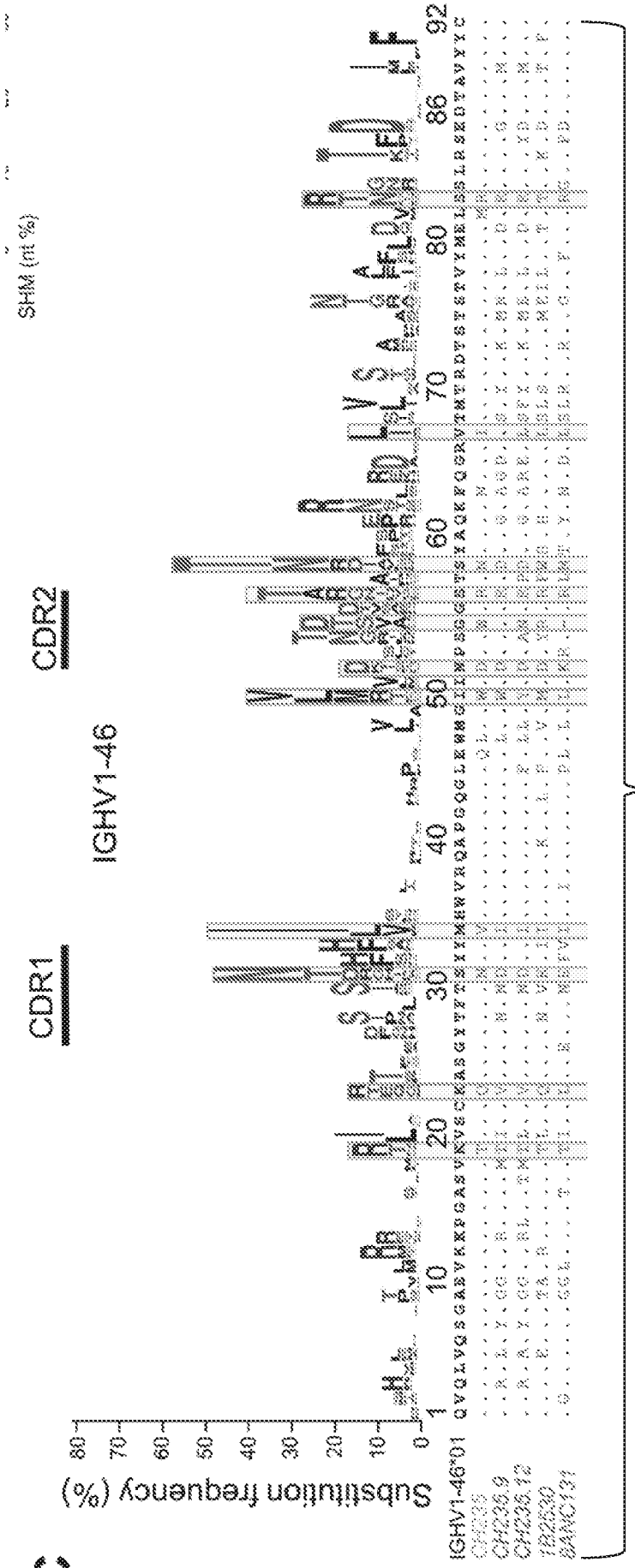

C
CDR1
CDR2
IGHV1-46
SHM (nt %)
Substitution frequency (%)
IGHV1-46*01
CH235
CH235.9
CH235.12
1B2530
BANC131
continued on next sheet

FIG. 31C

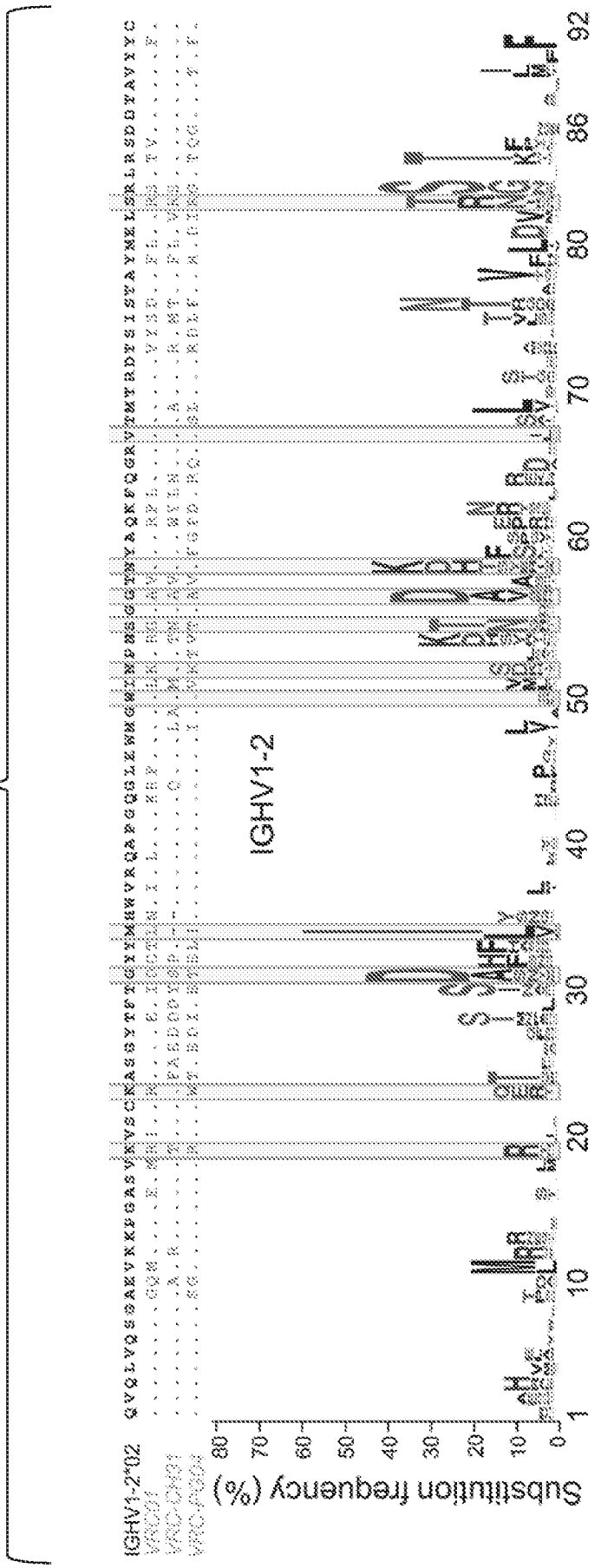
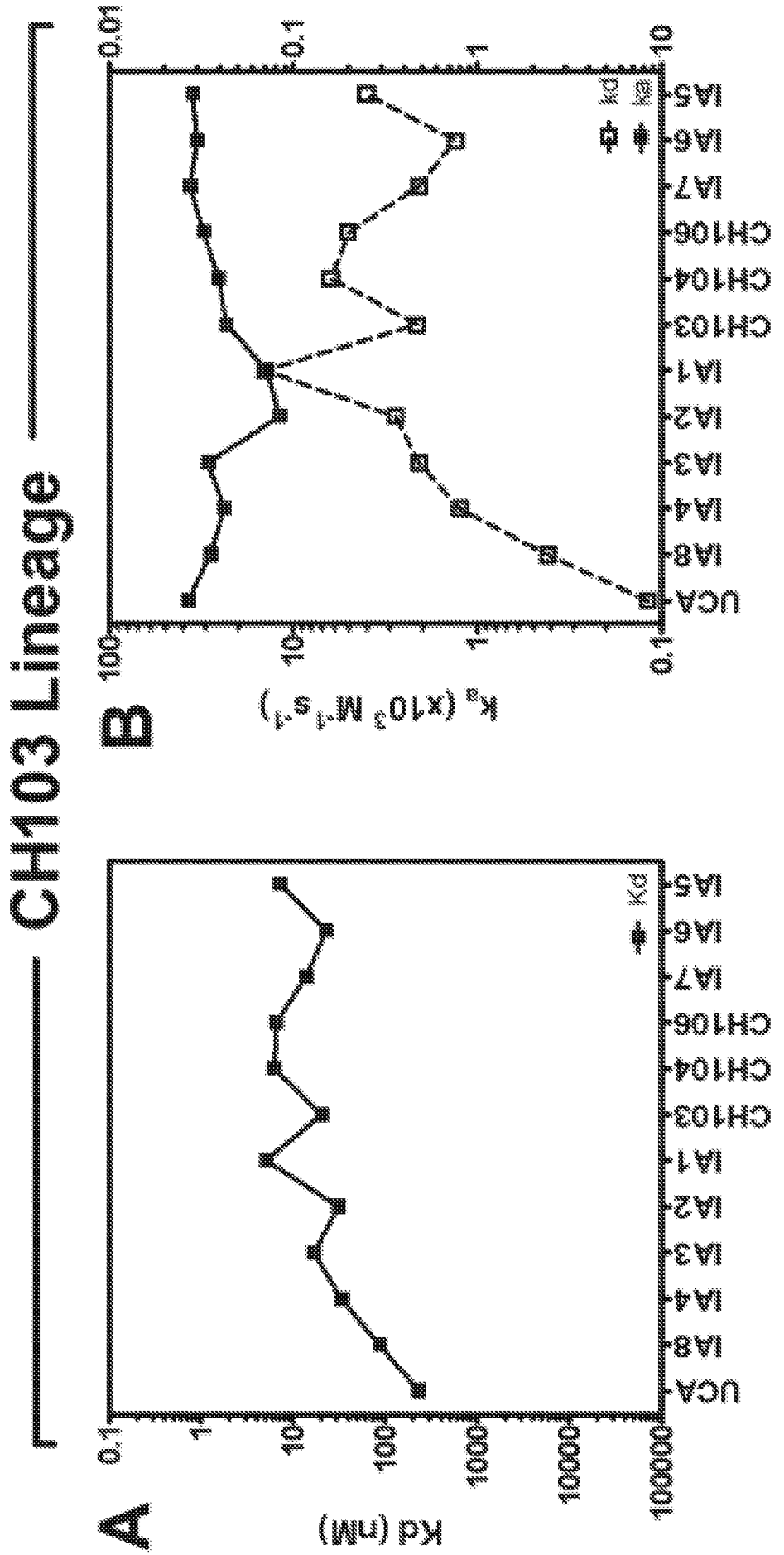
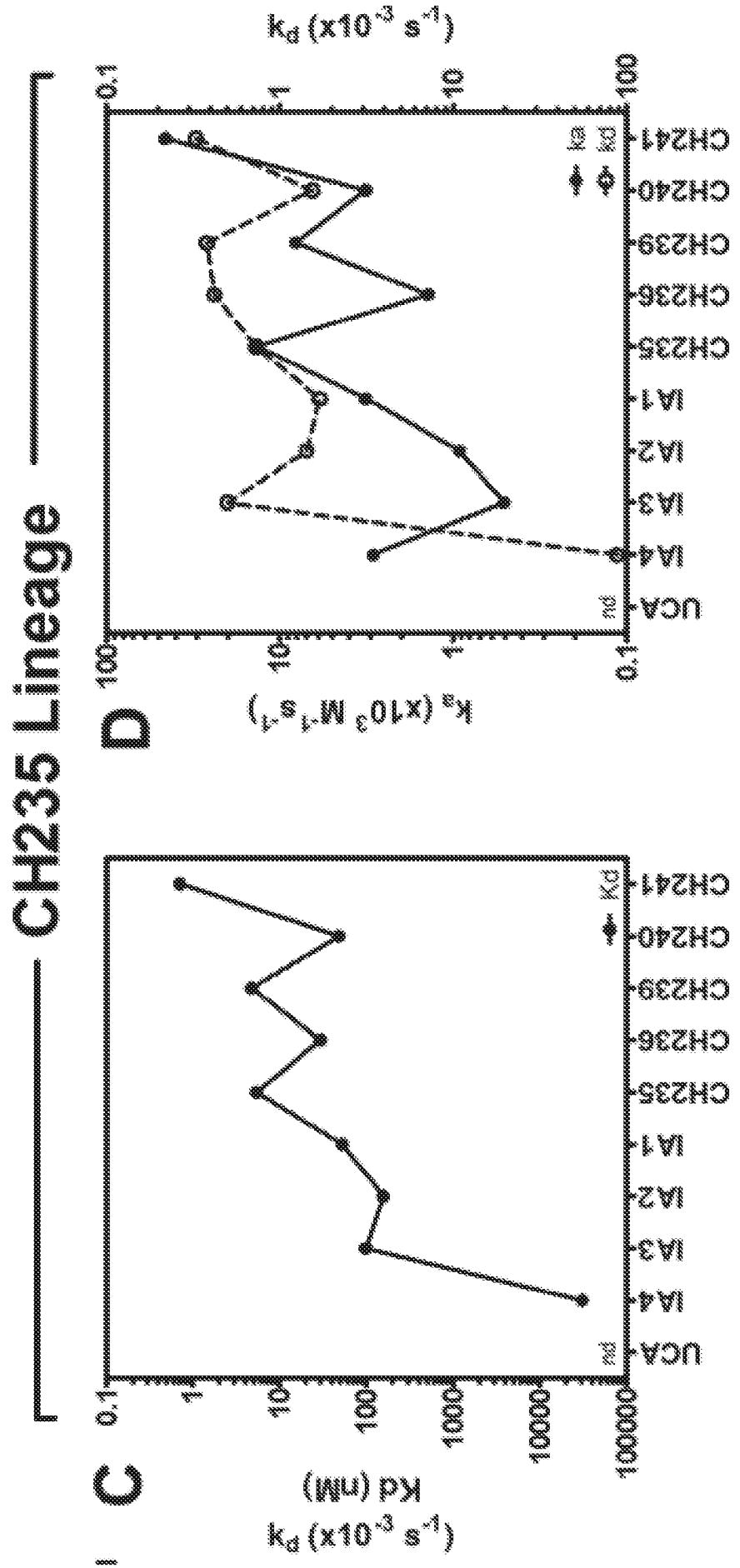

continued from above
IGHV1-2
Substitution frequency (%)
Position

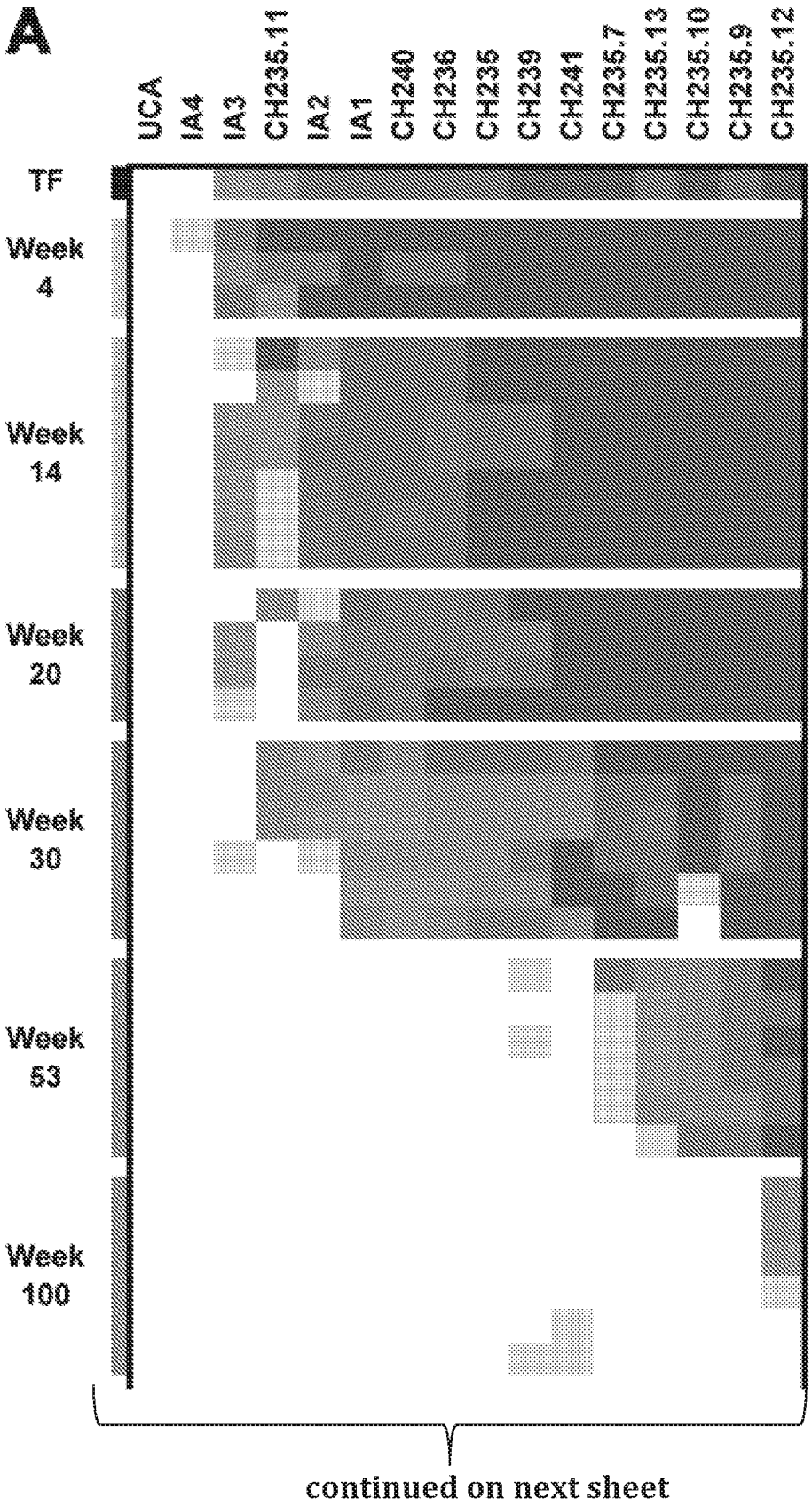
FIG. 31C cont.


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

FIGS. 32A-B


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

FIGS. 32C-D



continued on next sheet

FIG. 33A

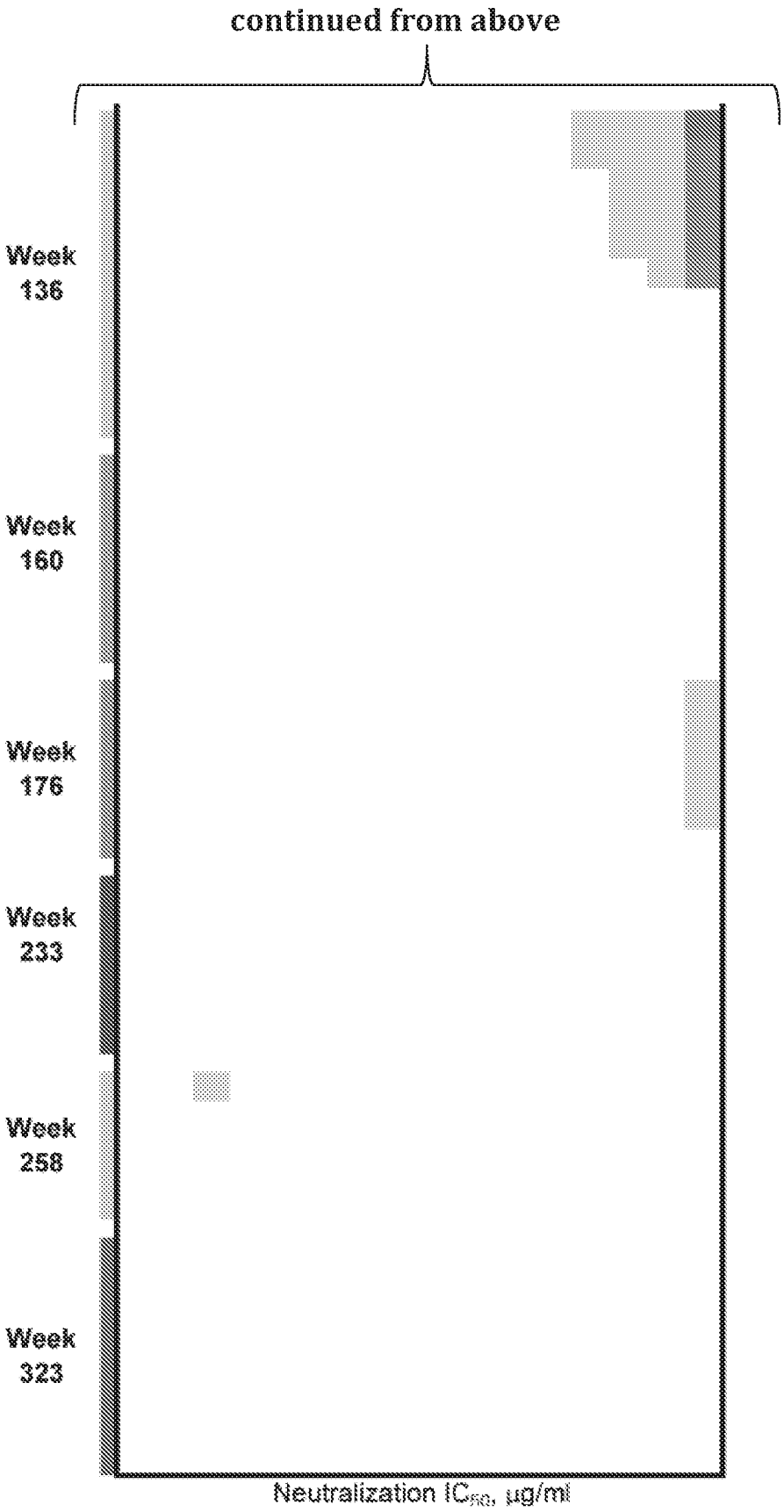
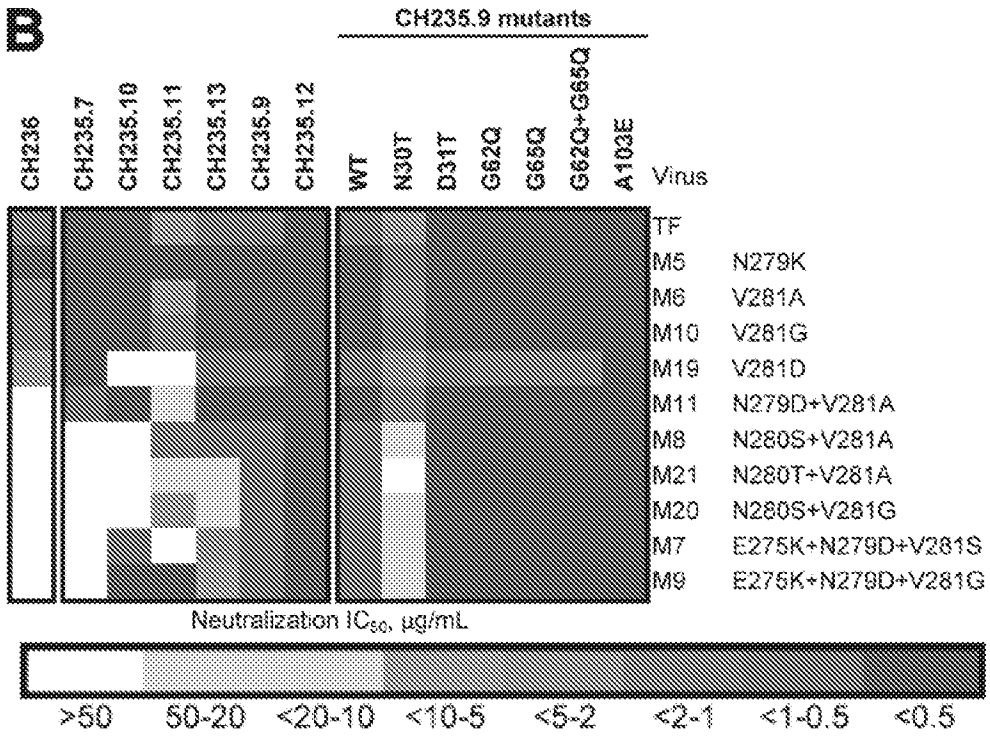
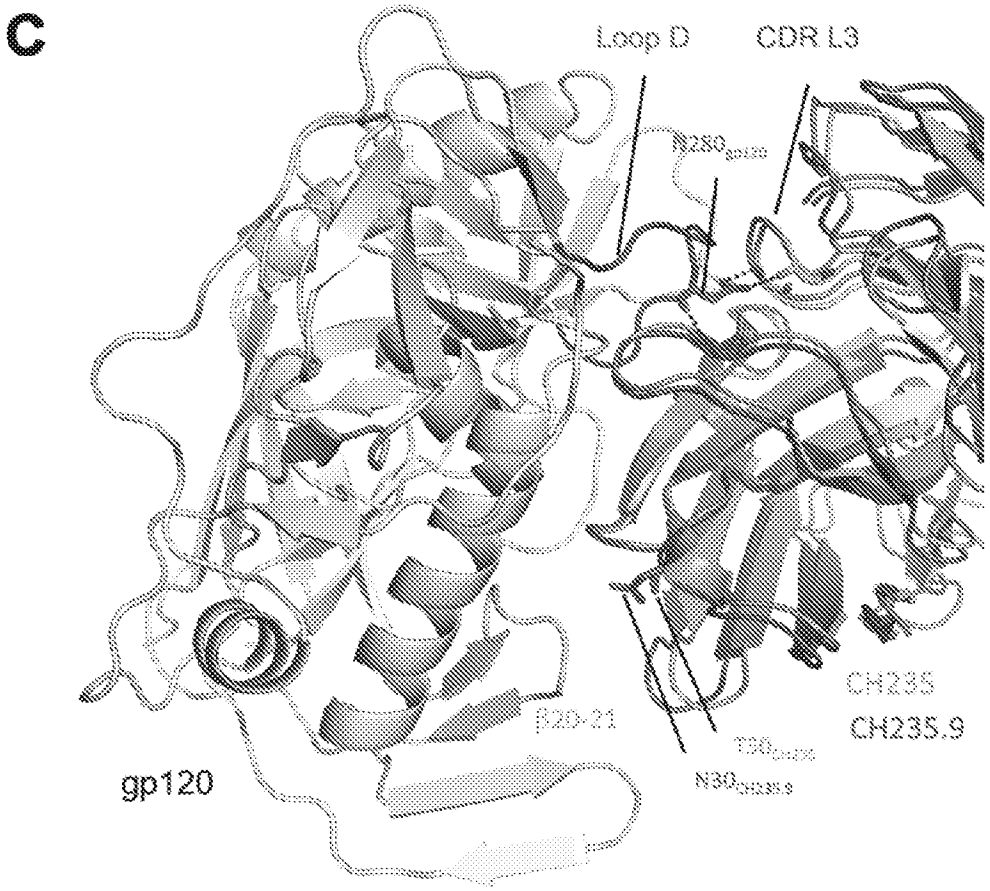

continued from above
Week 136
Week 160
Week 176
Week 233
Week 258
Week 323
Neutralization IC50, µg/ml

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 IC50, µg/mL
>50
50-20
<20-10
<10-5
<5-2
<2-1
<1-0.5
<0.5


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

FIGS. 33B-C

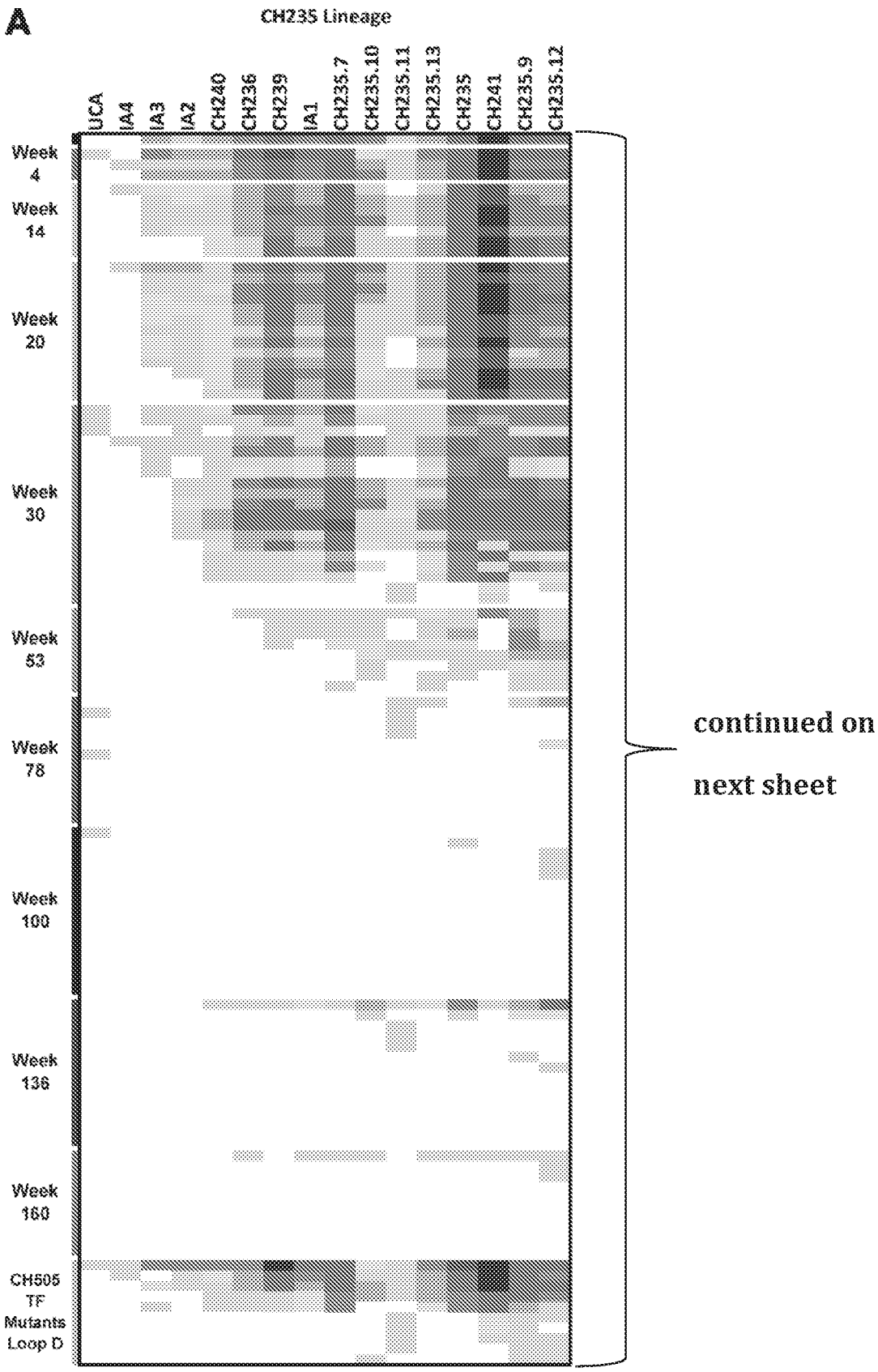

A
CH235 Lineage
UCA
IA4
IA3
IA2
CH240
CH236
CH239
IA1
CH235.7
CH235.10
CH235.11
CH235.13
CH235
CH241
CH235.9
CH235.12
Week 4
Week 14
Week 20
Week 30
Week 53
Week 78
Week 100
Week 136
Week 160
CH505 TF Mutants Loop D
continued on next sheet

FIG. 34A

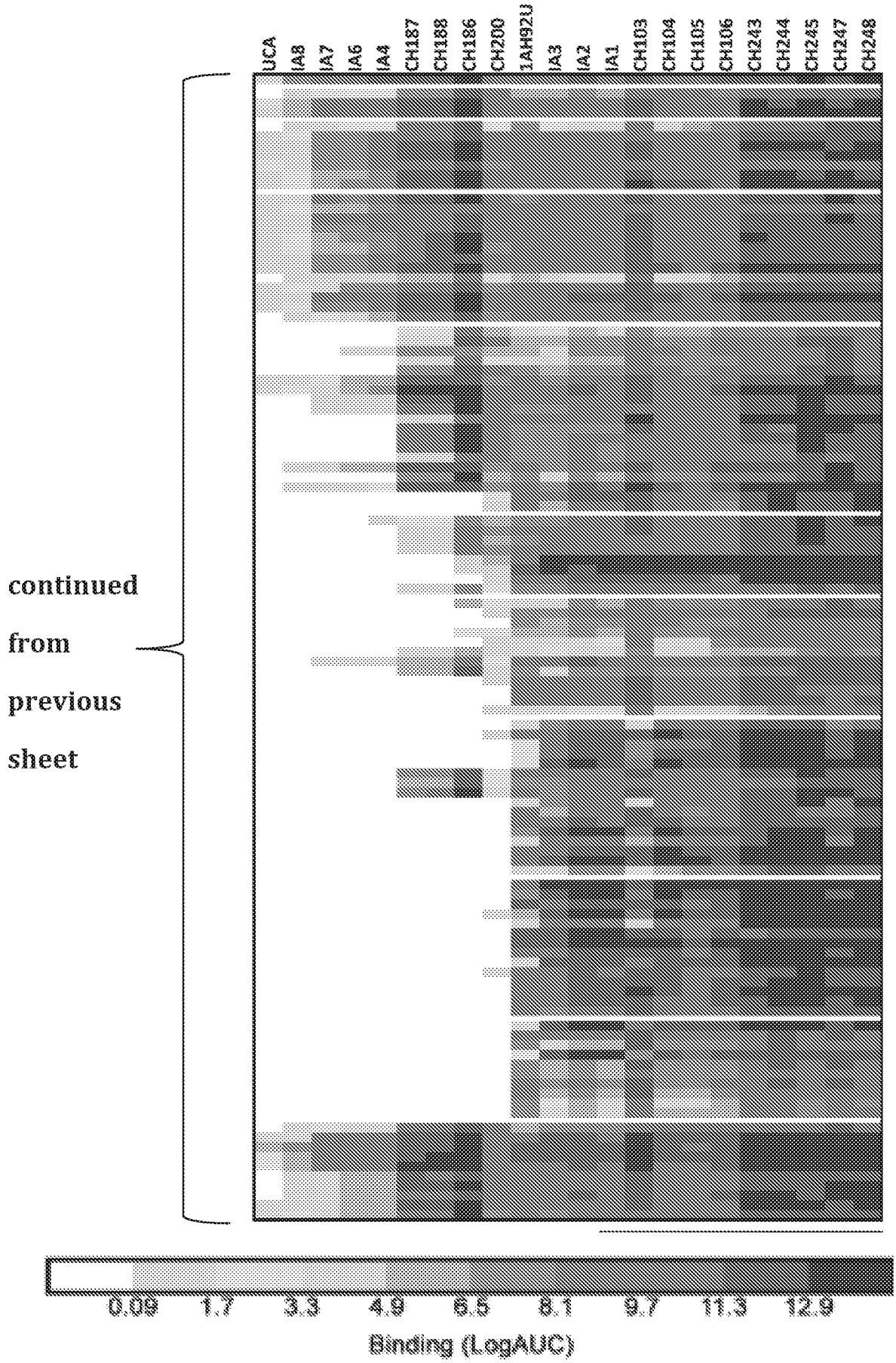

UCA
IA8
IA7
IA6
IA4
CH187
CH188
CH186
CH200
1AH92U
IA3
IA2
IA1
CH103
CH104
CH105
CH106
CH243
CH244
CH245
CH247
CH248
continued
from
previous
sheet
0.09
1.7
3.3
4.9
6.5
8.1
9.7
11.3
12.9
Binding (LogAUC)

FIG. 34A cont.

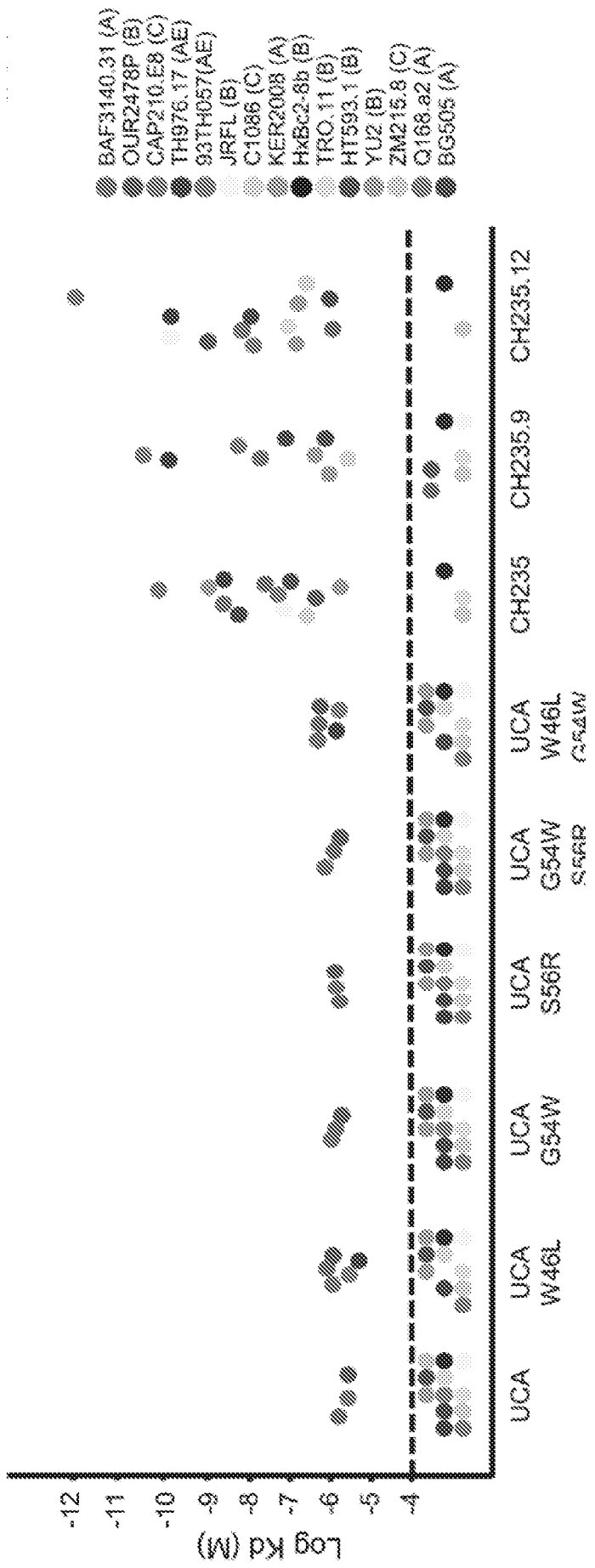

BAF3140.31 (A)
OUR2478P (B)
CAP210.E8 (C)
TH976.17 (AE)
93TH057(AE)
JRFL (B)
C1086 (C)
KER2008 (A)
HxBc2-8b (B)
TRO.11 (B)
HT593.1 (B)
YU2 (B)
ZM215.8 (C)
Q168.a2 (A)
BG505 (A)
Log Kd (M)
-12
-11
-10
-9
-8
-7
-6
-5
-4
UCA
UCA W46L
UCA G54W
UCA S56R
UCA G54W S56R
UCA W46L G54W S56R
CH235
CH235.9
CH235.12

FIG. 34B

	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	-	-	-	-	-	-	-	-	-

A

FIG. 35A

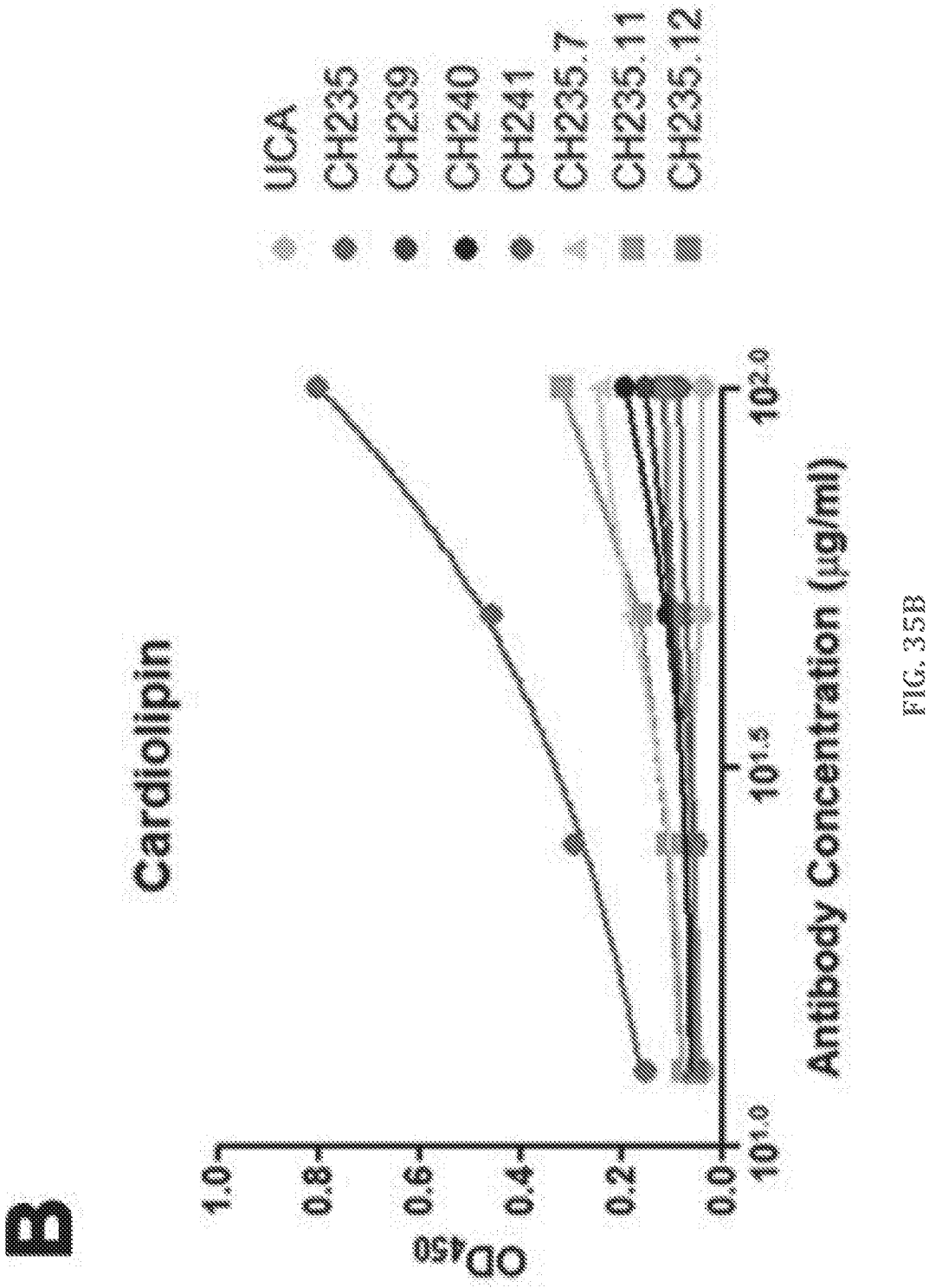

B
Cardiolipin
OD450
1.0
0.8
0.6
0.4
0.2
0.0
10^1.0
10^1.5
10^2.0
Antibody Concentration (μg/ml)
UCA
CH235
CH239
CH240
CH241
CH235.7
CH235.11
CH235.12

FIG. 35B

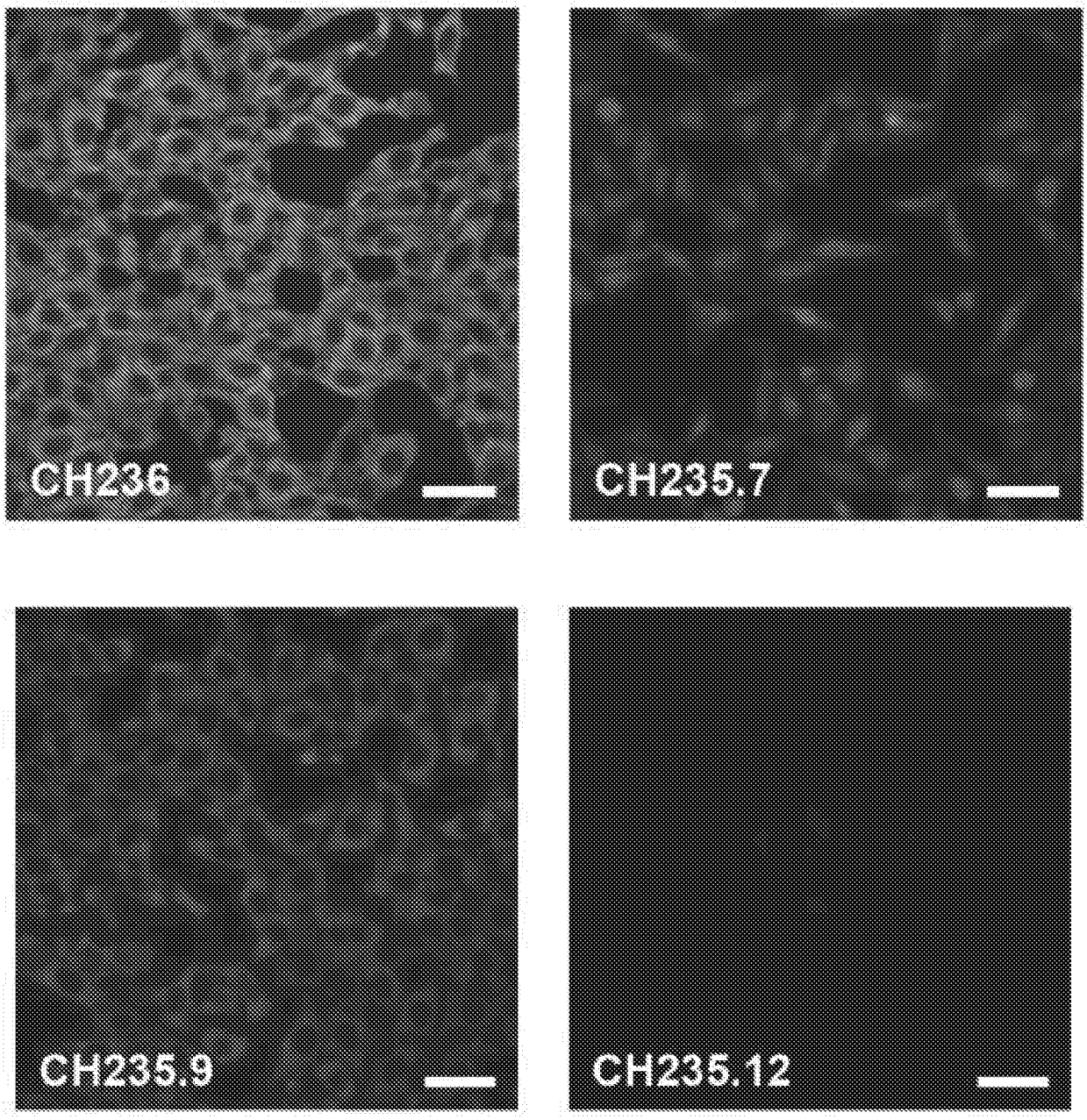


FIG. 35C

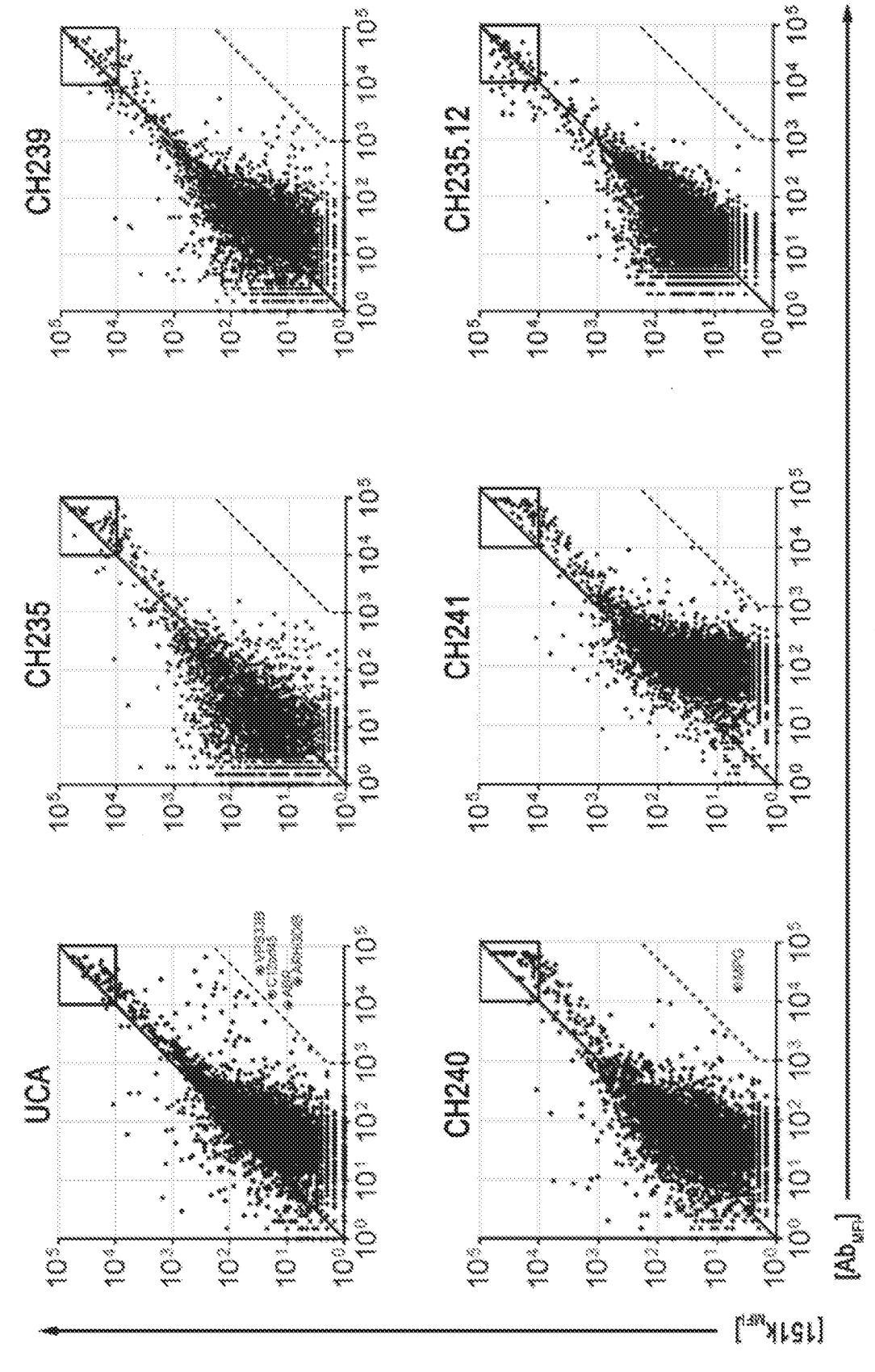


FIG. 35-D

FIG. 39A

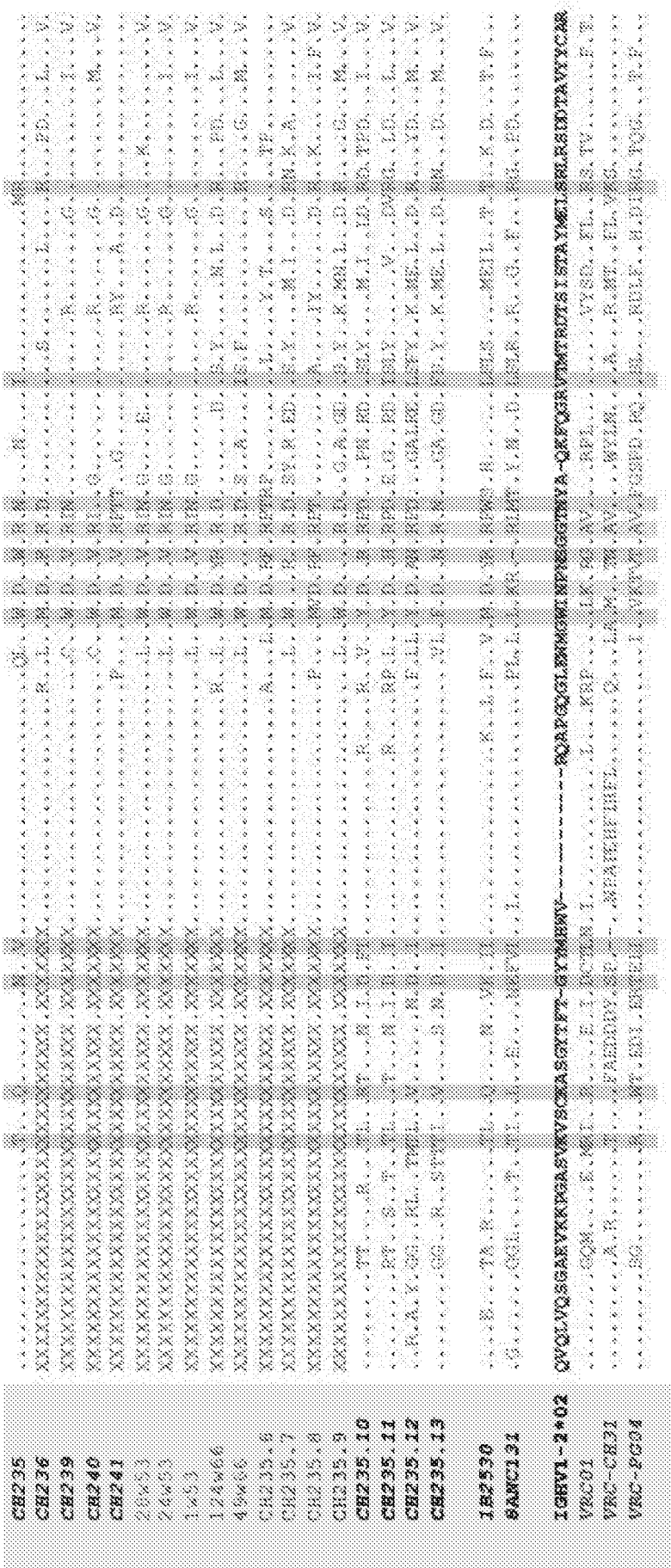

CH235
CH236
CH239
CH240
CH241
26w53
26w63
1w53
124w66
49w66
CH235.6
CH235.7
CH235.8
CH235.9
CH235.10
CH235.11
CH235.12
CH235.13
IB2530
BARC131
IGHV1-2*02
VRC01
VRC-CH31
VRC-PG04
Time of appearance
week 14
week 20
week 22
week 30
week 41
week 53
week 66
week 100
week 152
week 264
week 323

FIG. 36A cont.

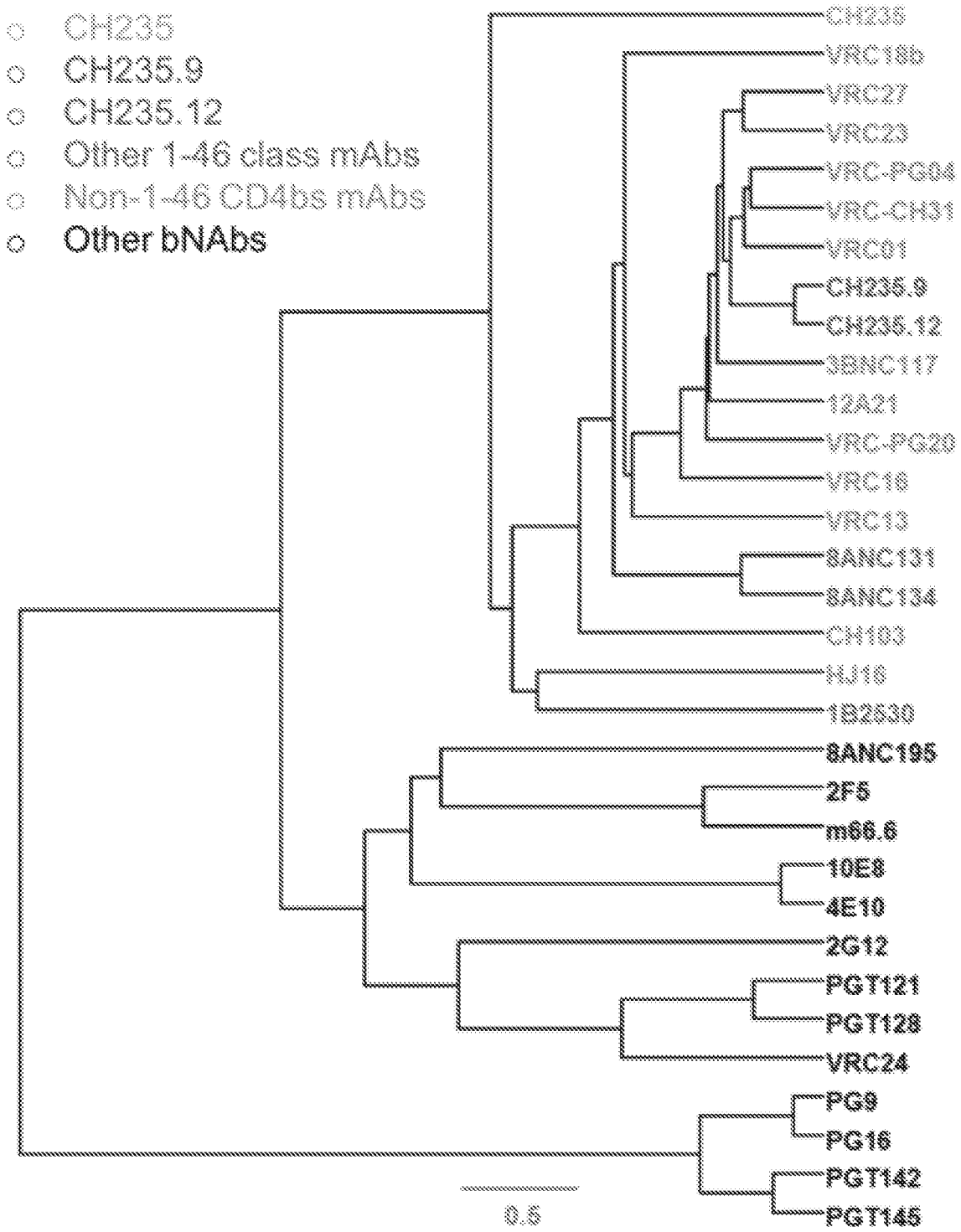

CH235
CH235.9
CH235.12
Other 1-46 class mAbs
Non-1-46 CD4bs mAbs
Other bNAbs
CH235
VRC16b
VRC27
VRC23
VRC-PG04
VRC-CH31
VRC01
CH235.9
CH235.12
3BNC117
12A21
VRC-PG20
VRC16
VRC13
8ANC131
8ANC134
CH103
HJ16
1B2530
8ANC195
2F5
m66.6
10E8
4E10
2G12
PGT121
PGT128
VRC24
PG9
PG16
PGT142
PGT145
0.5

FIG. 36B

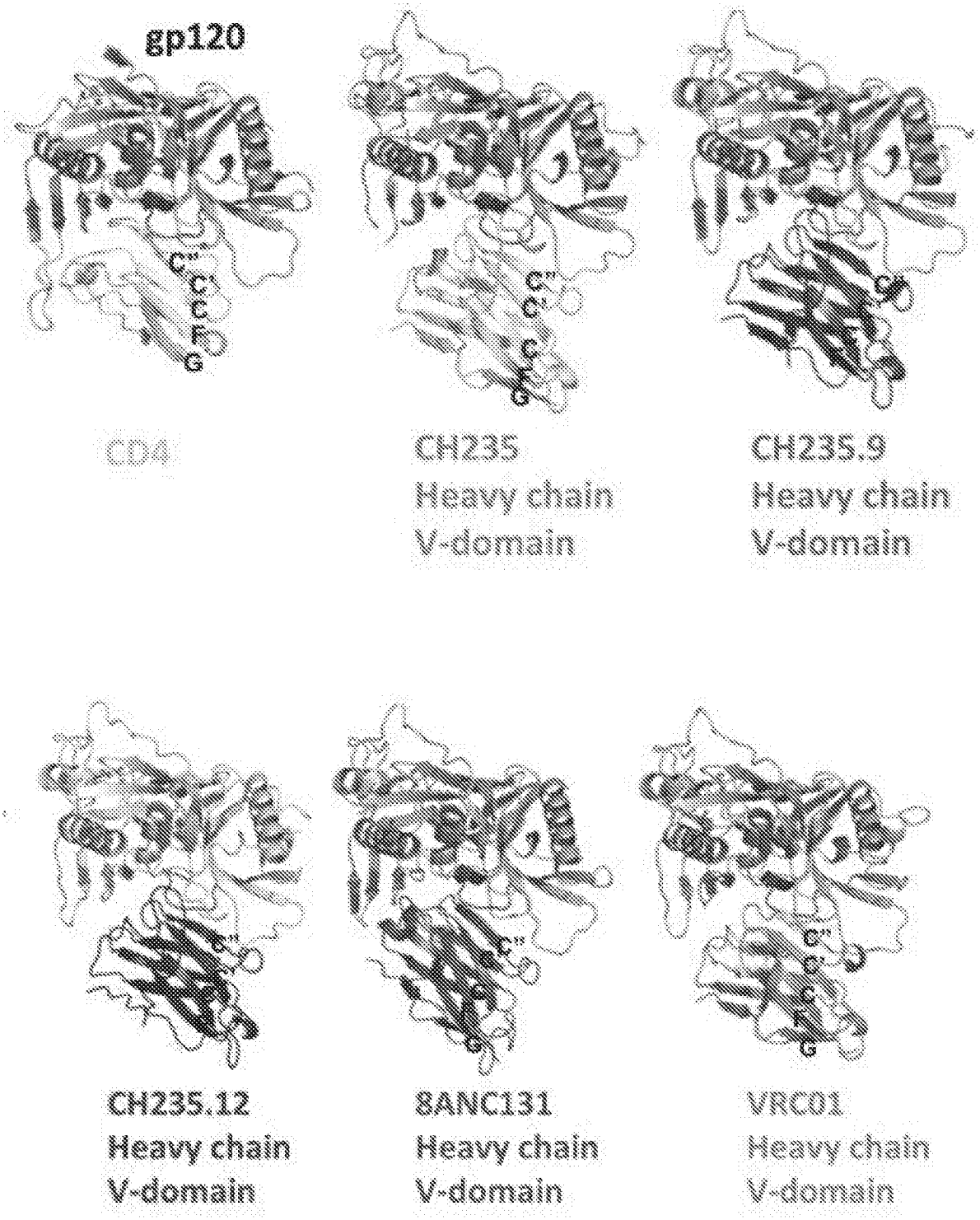

gp120
CD4
CH235
Heavy chain
V-domain
CH235.9
Heavy chain
V-domain
CH235.12
Heavy chain
V-domain
8ANC131
Heavy chain
V-domain
VRC01
Heavy chain
V-domain

FIG. 37A

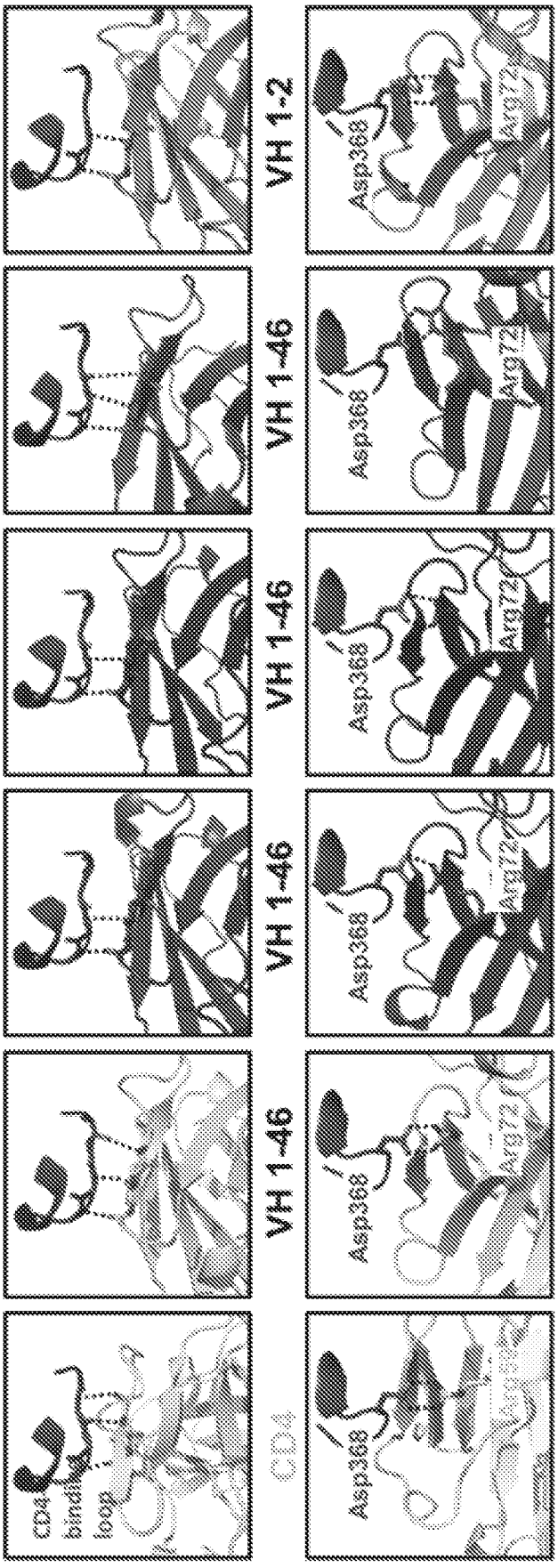
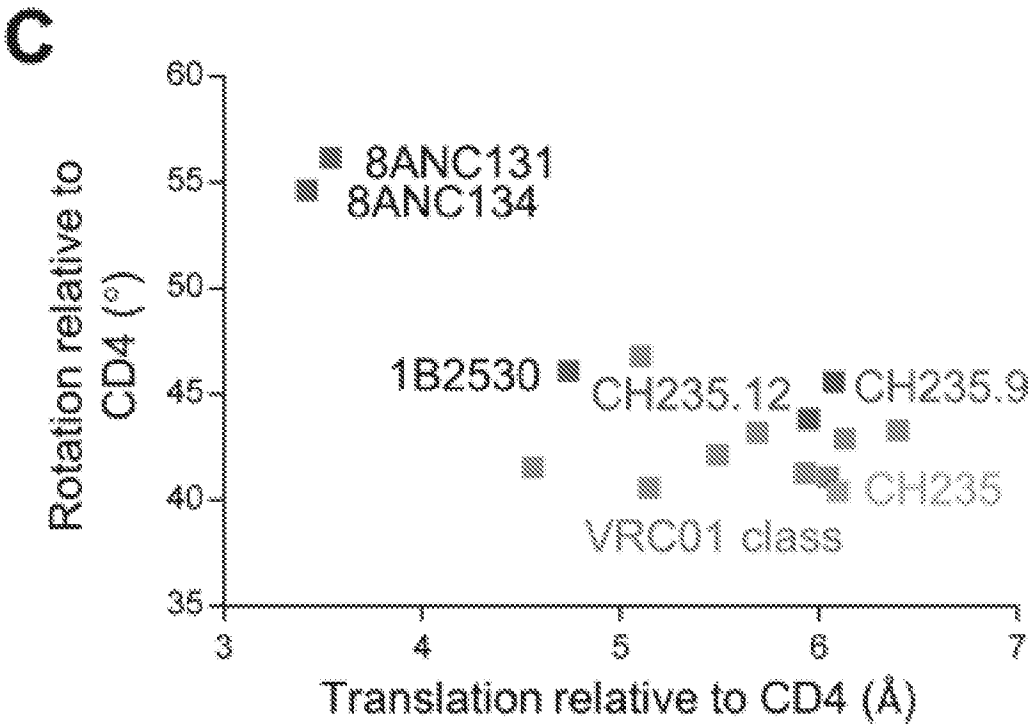
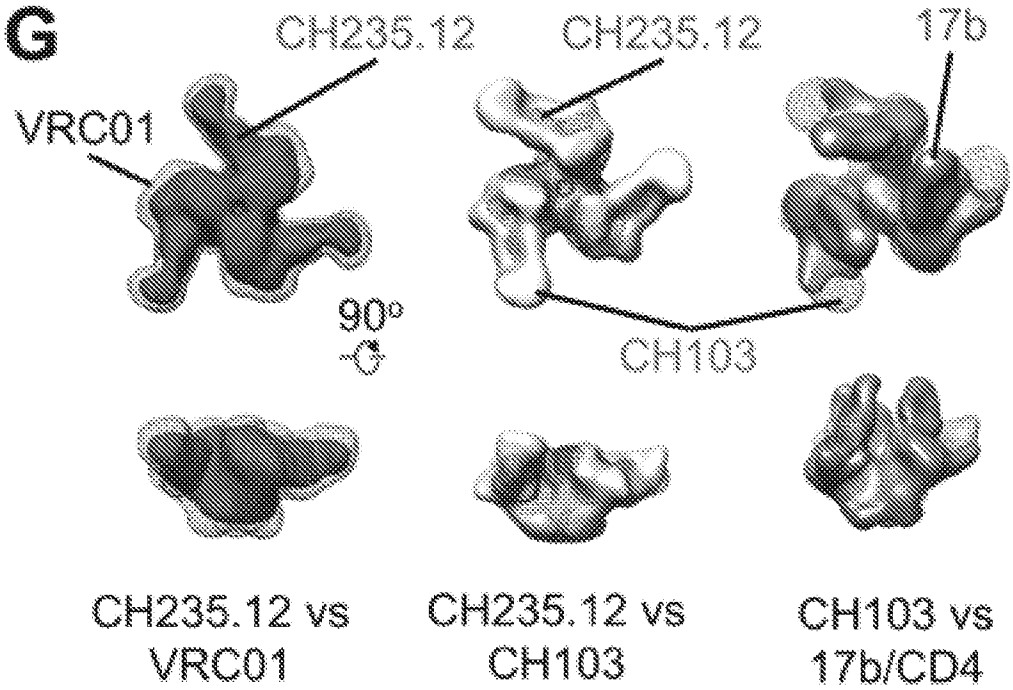

CD4 binding loop
CD4
VH 1-46
VH 1-46
VH 1-46
VH 1-46
VH 1-2
Asp368
Arg72

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

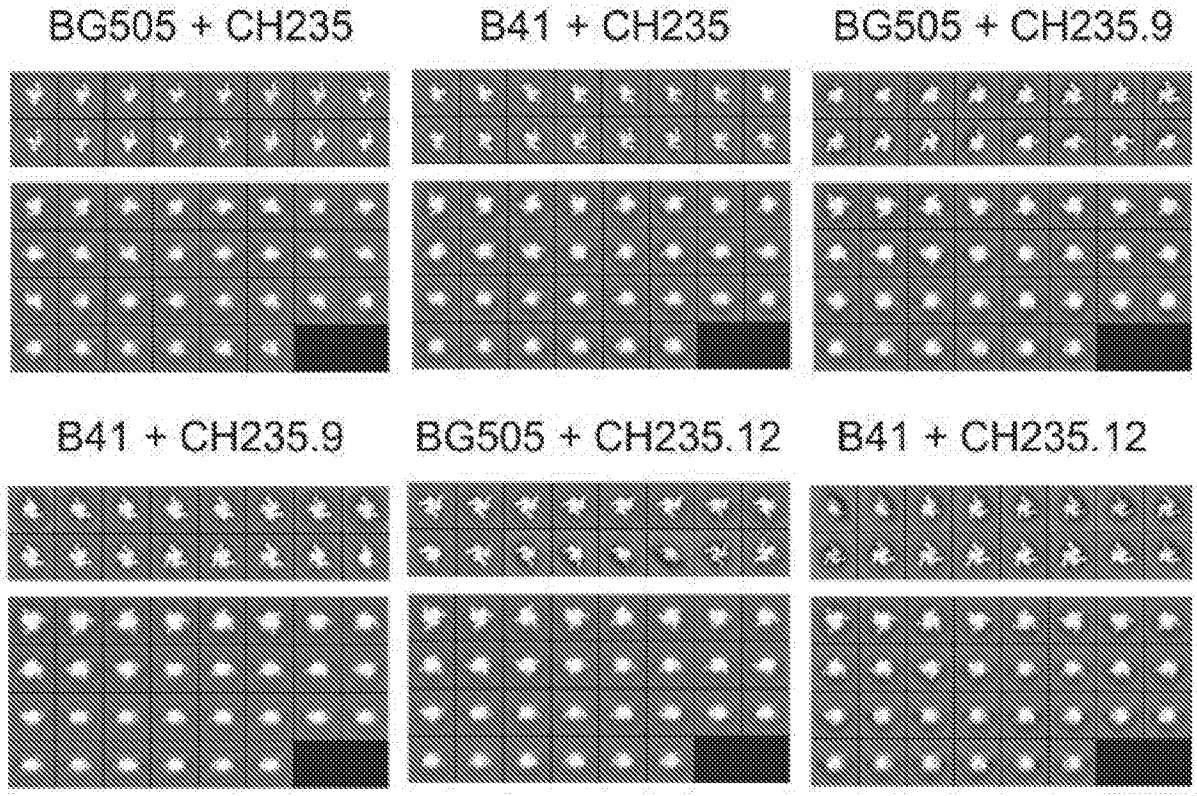

BG505 + CH235
B41 + CH235
BG505 + CH235.9
B41 + CH235.9
BG505 + CH235.12
B41 + CH235.12

FIG. 37D

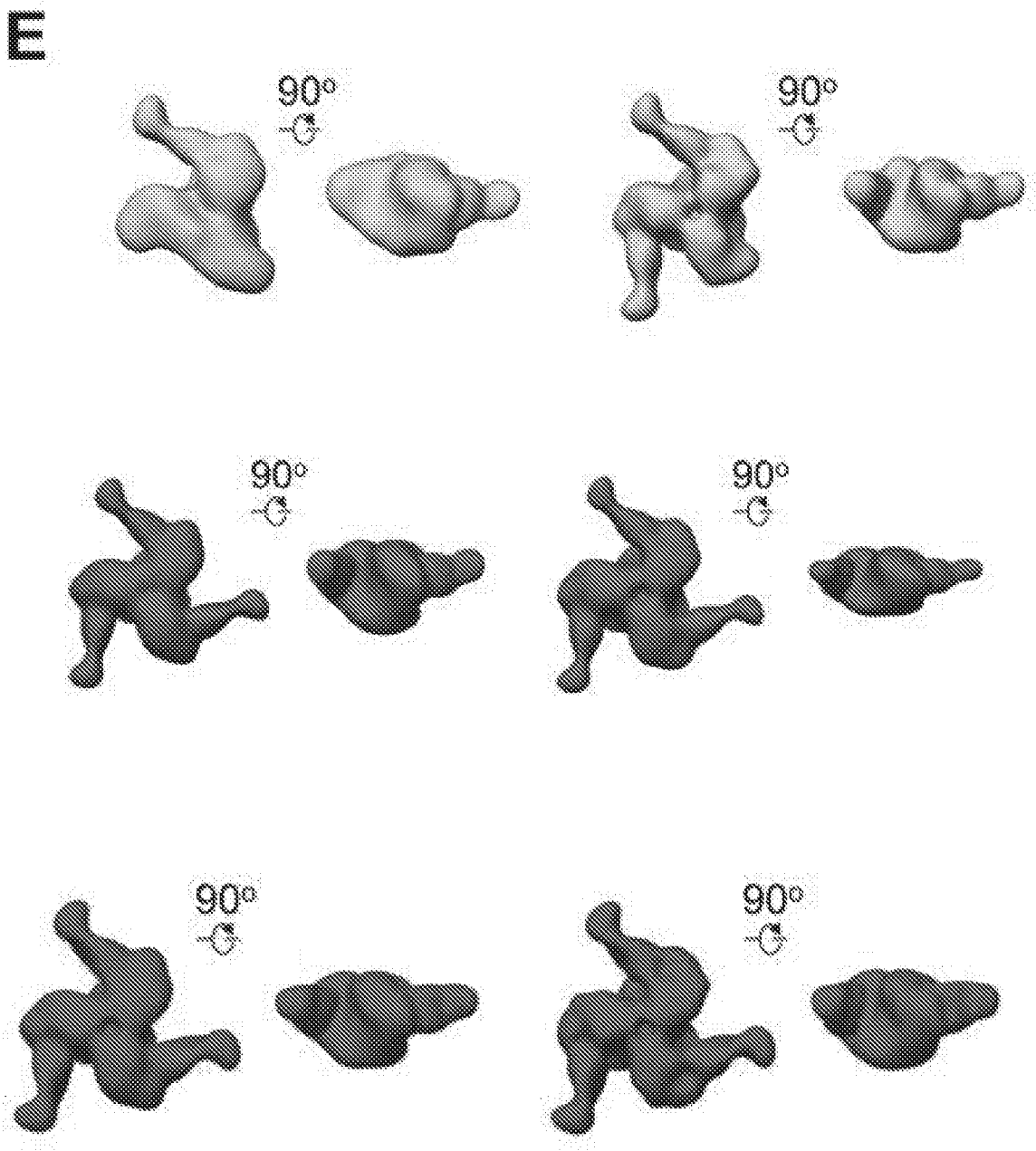


FIG. 37E

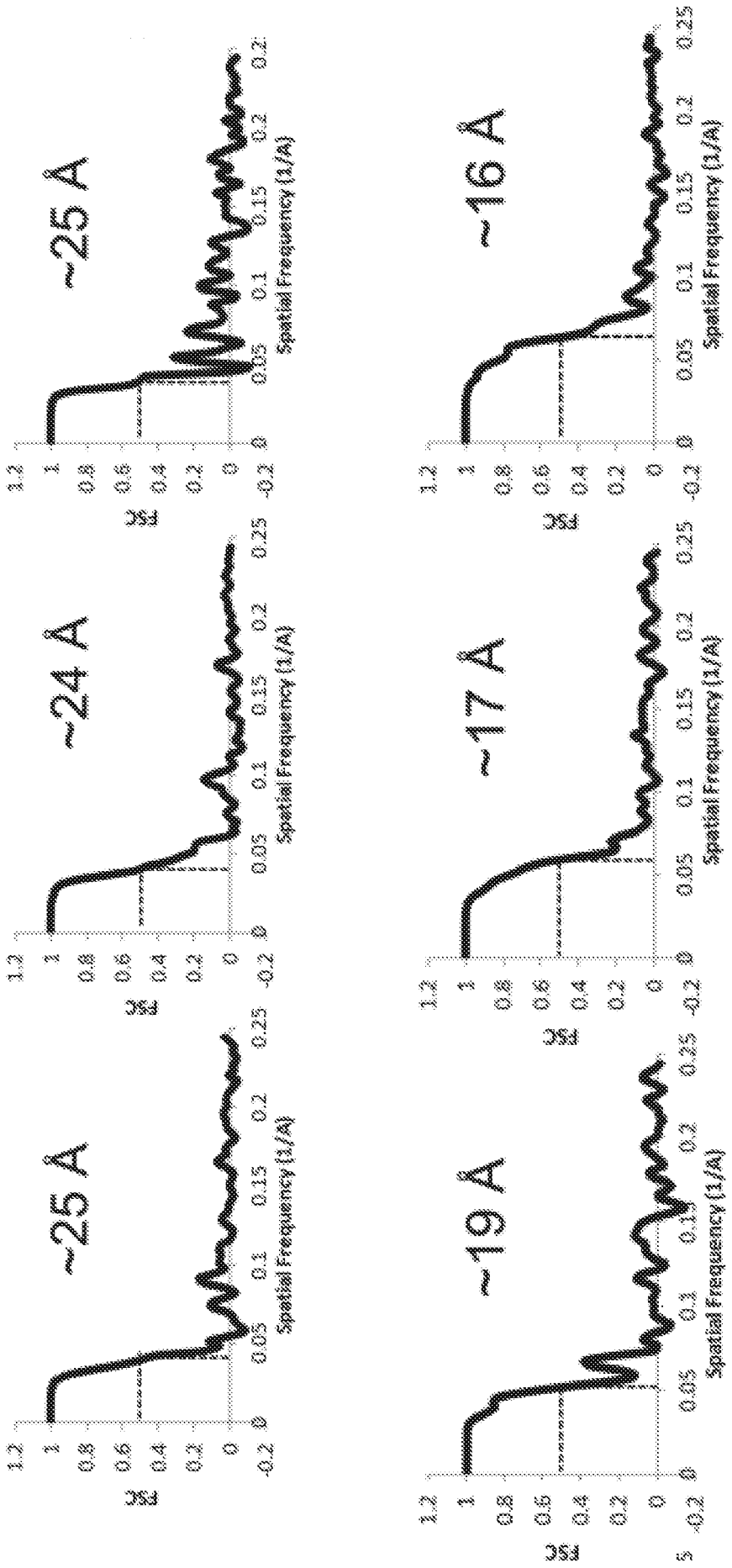


FIG. 37F

a

```
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.-.PLMT.YN..D.LSLR..R..G..F...RG..PD.........
CH235       W.D..W.P.N...N....I..............MP..............
```

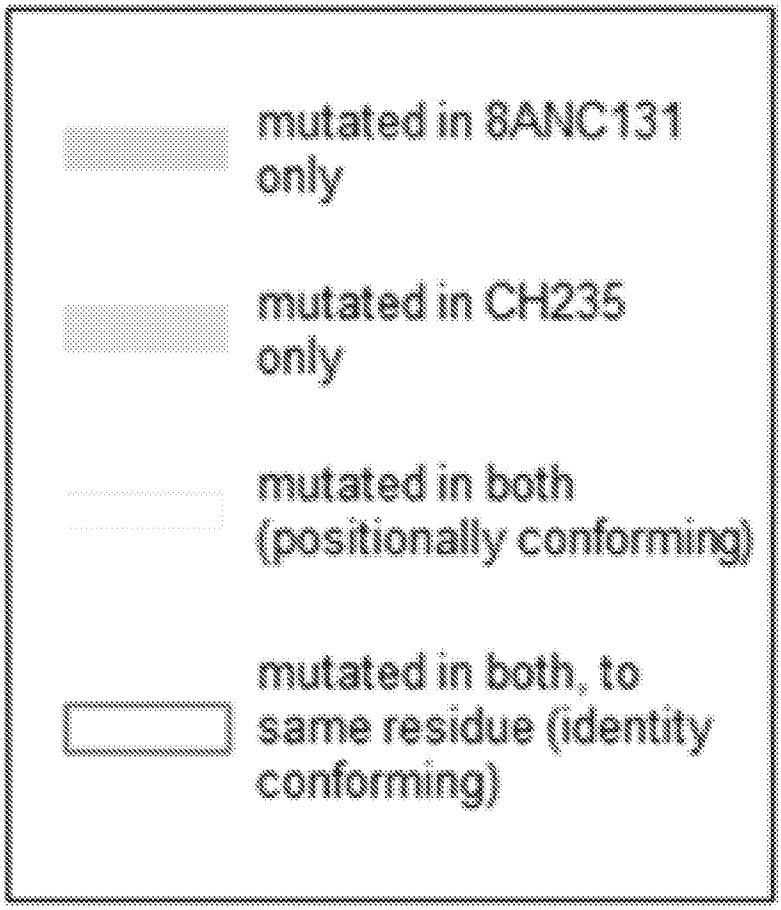


FIG. 38A

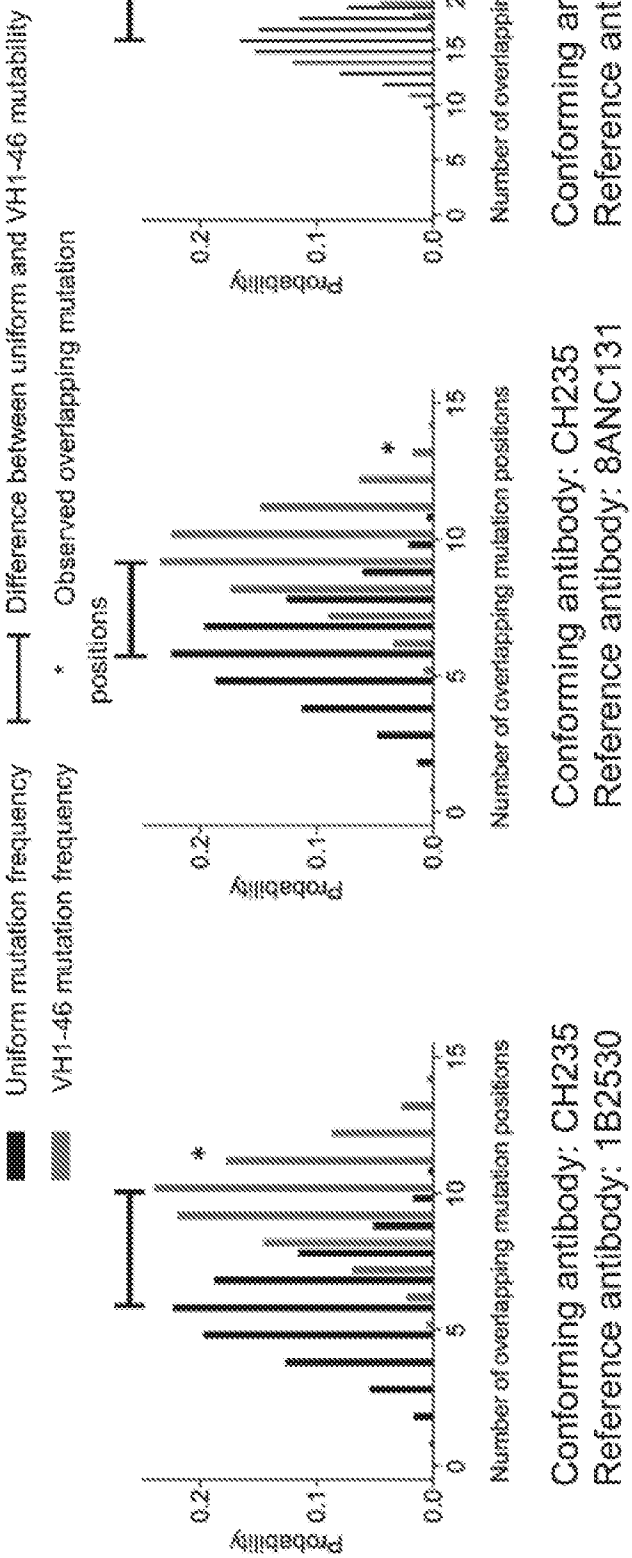

Uniform mutation frequency
VH1-46 mutation frequency
Difference between uniform and VH1-46 mutability
* Observed overlapping mutation positions
Probability
Number of overlapping mutation positions
Conforming antibody: CH235
Reference antibody: 1B2530
Conforming antibody: 1B2530
Reference antibody: 8ANC131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

FIG. 38B

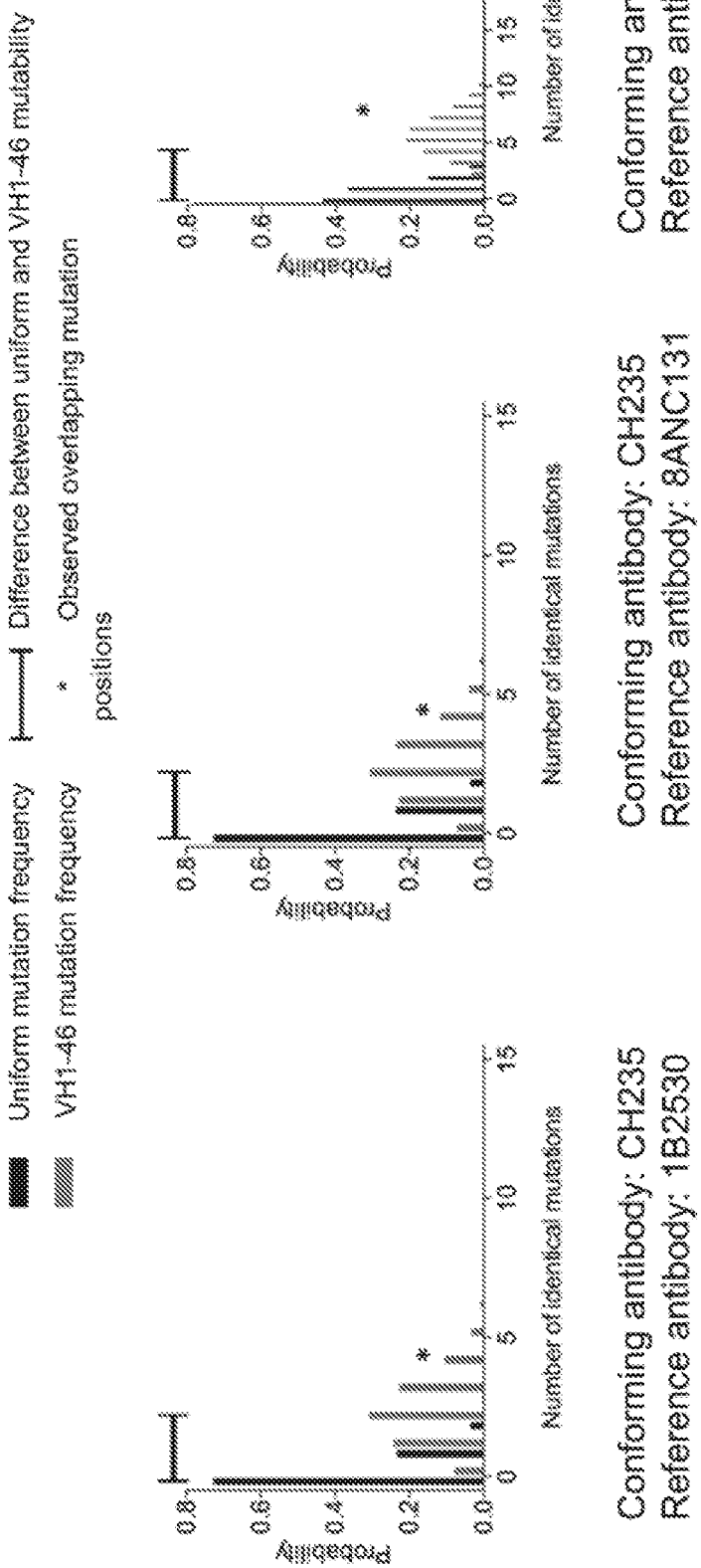
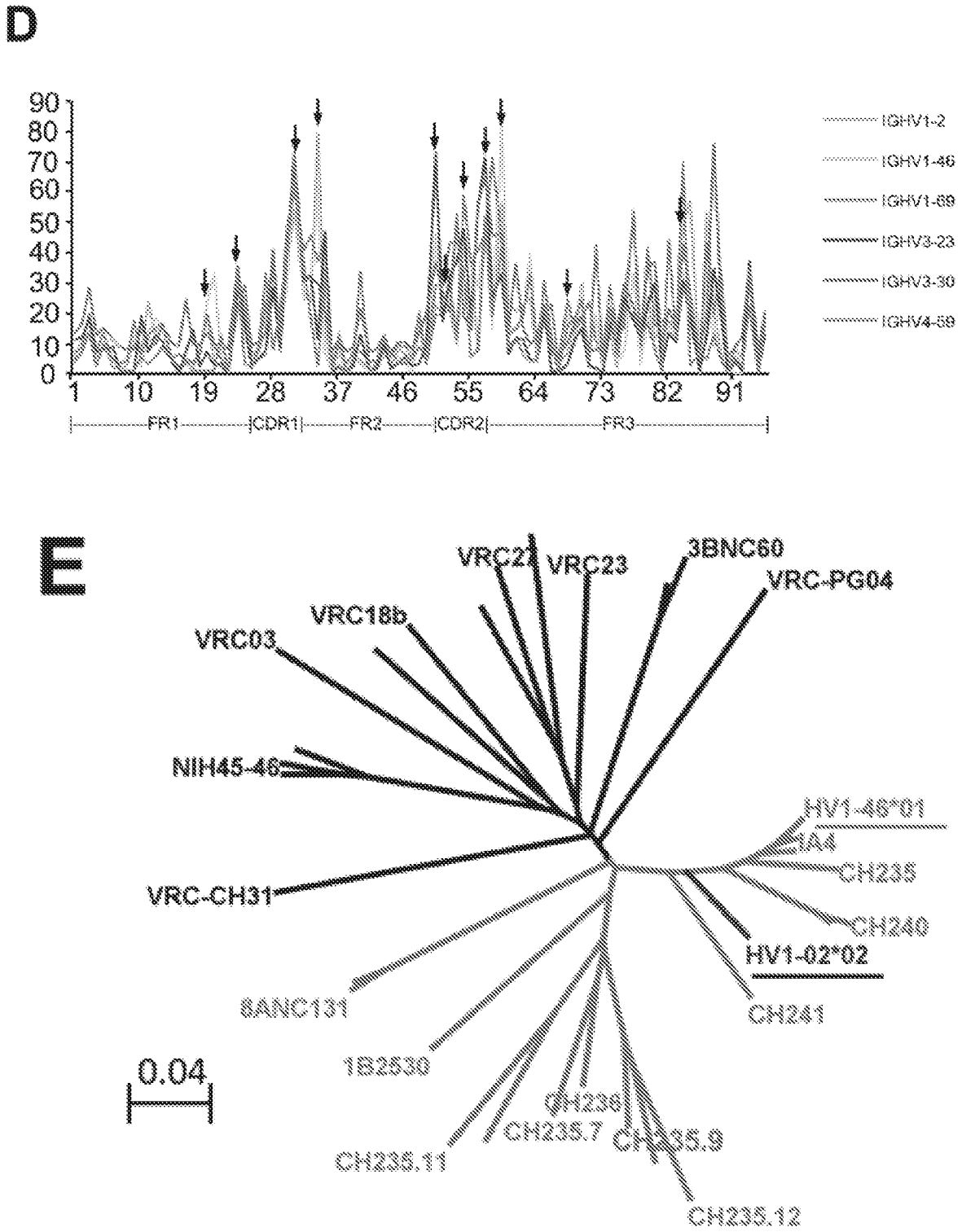

Uniform mutation frequency
VH1-46 mutation frequency
Difference between uniform and VH1-46 mutability
* Observed overlapping mutation positions
Probability
Number of identical mutations
Conforming antibody: CH235
Reference antibody: 1B2530
Conforming antibody: CH235
Reference antibody: 8ANC131
Conforming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

FIG. 38C


D
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
IGHV1-2
IGHV1-46
IGHV1-69
IGHV3-23
IGHV3-30
IGHV4-59
E
VRC23
3BNC60
VRC-PG04
VRC18b
VRC03
NIH45-46
VRC-CH31
8ANC131
1B2530
CH235.11
CH235.7
CH235.9
CH235.12
HV1-46*01
CH235
CH240
HV1-02*02
CH241
0.04

FIGS. 38D-E

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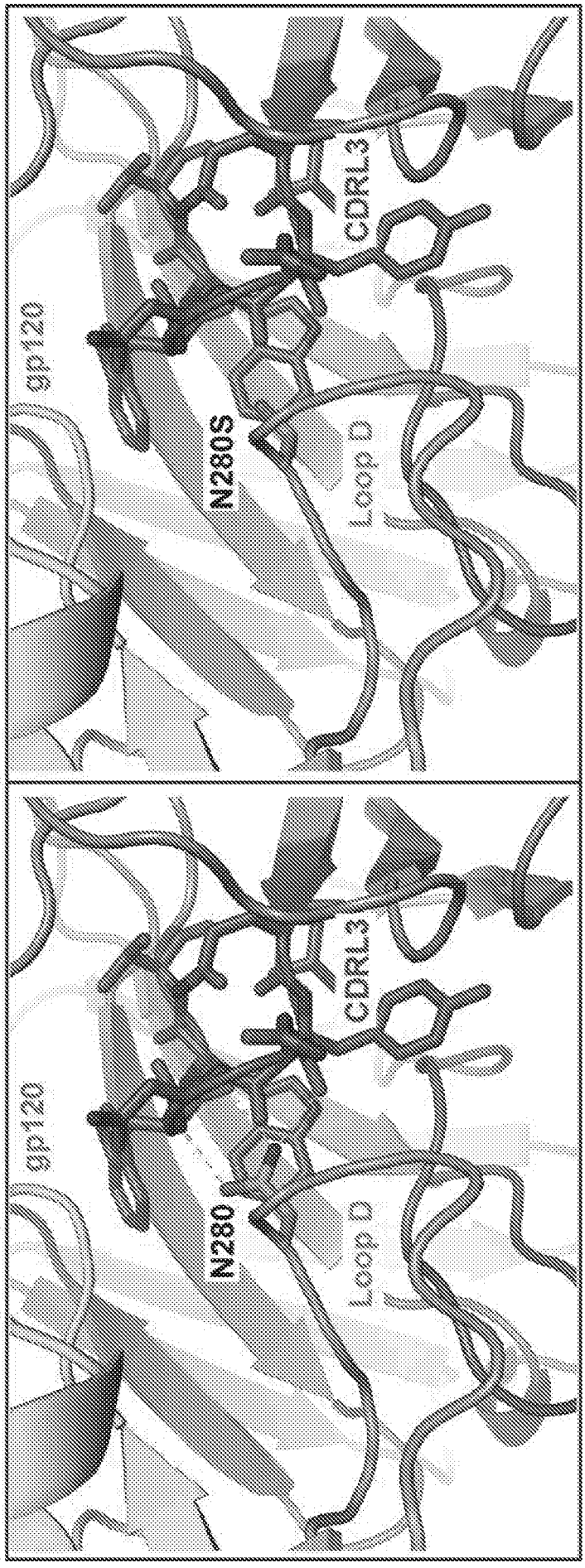

FIG. 39A

```
|----------FR1----------|--CDR1--|----------FR2----------|--CDR2--|--
                          *  *                            *******
CH236_VH      QVQLVQSGAA VKRPGASVTI SCRASGYTFT TYYIHWVRQA PGQRLELMGM IDPSRGRTDY
CH235.9_VH    QVRLLQYGGG VKRPGASMTI SCVASGYNFM DYYIHWVRQA PGQGLELMGW IDPSGGRTDY
CH235.12_VH   QVRLAQYGGG VKRLGATMTL SCVASGYTFN DYYIHWVRQA PGQGFELLGY IDPANGRPDY
CH235.13_VH   QVQLVQSGGG VKRPGSTTTI SCVASGYSFN DYYIHWVRQA PGQGLEVLGF IDPSNGRTNY
CH235.10_VH   QVQLVQSGAT VKKPRASVTL SCRTSGYNFI DYFIHWVRRA PGQRLEVMGY IDPSRGRPDY
CH235.11_VH   QVQLVQSGGT VKSPGTSVTL SCKTSGYNFI DYYIHWVRRA PGQRPELMGY IDPSHGRPDY
CH235.7_VH    QVQLVQSGAA VKRPGASVTI SCRASGYTFT TYYIHWVRQA PGQGLELMGW INPRGGRTDY

----------FR3----------|------|----CDR3----|---FR4---|
**  *   *                      *******
CH236_VH      AQKFQGRVTM SRDTSTSTLY MELRSLRPDD TALYYCVRNV GTWGSLLHYD YWGQGTLVTVSS
CH235.9_VH    AGAFGDRVSM YRDKSMNTLY MDLRSLRSGD TAMYYCVRNV GTAGSLLHYD HWGLGVMVTVSS
CH235.12_VH   AGALRERLSF YRDKSMETLY MDLRSLRYDD TAMYYCVRNV GTAGSLLHYD HWGSGSPVIVSS
CH235.13_VH   AGAFGDRFSM YRDKSMETLY MDLRNLRSDD TAMYYCVRNV GTAGSLLHYD HWGTGSKIIVSS
CH235.10_VH   APNFRDRVSL YRDTSMSIVY LDLRDLTPDD TAIYYCVRSE GTEGTVLHYD HWGPGTRVEVSP
CH235.11_VH   EGKFRDRISL YRDTSTSVVY MDVRGLRLDD TALYYCVRGG GVEVSSNHYD HWGPGTMVFVSP
CH235.7_VH    SYRFEDRVSM YRDTSMSIVY MDLRNLKSAD TAVYYCVRNV GTSGSLLHYD FWGQGSLVTVSS
```

FIG. 39B

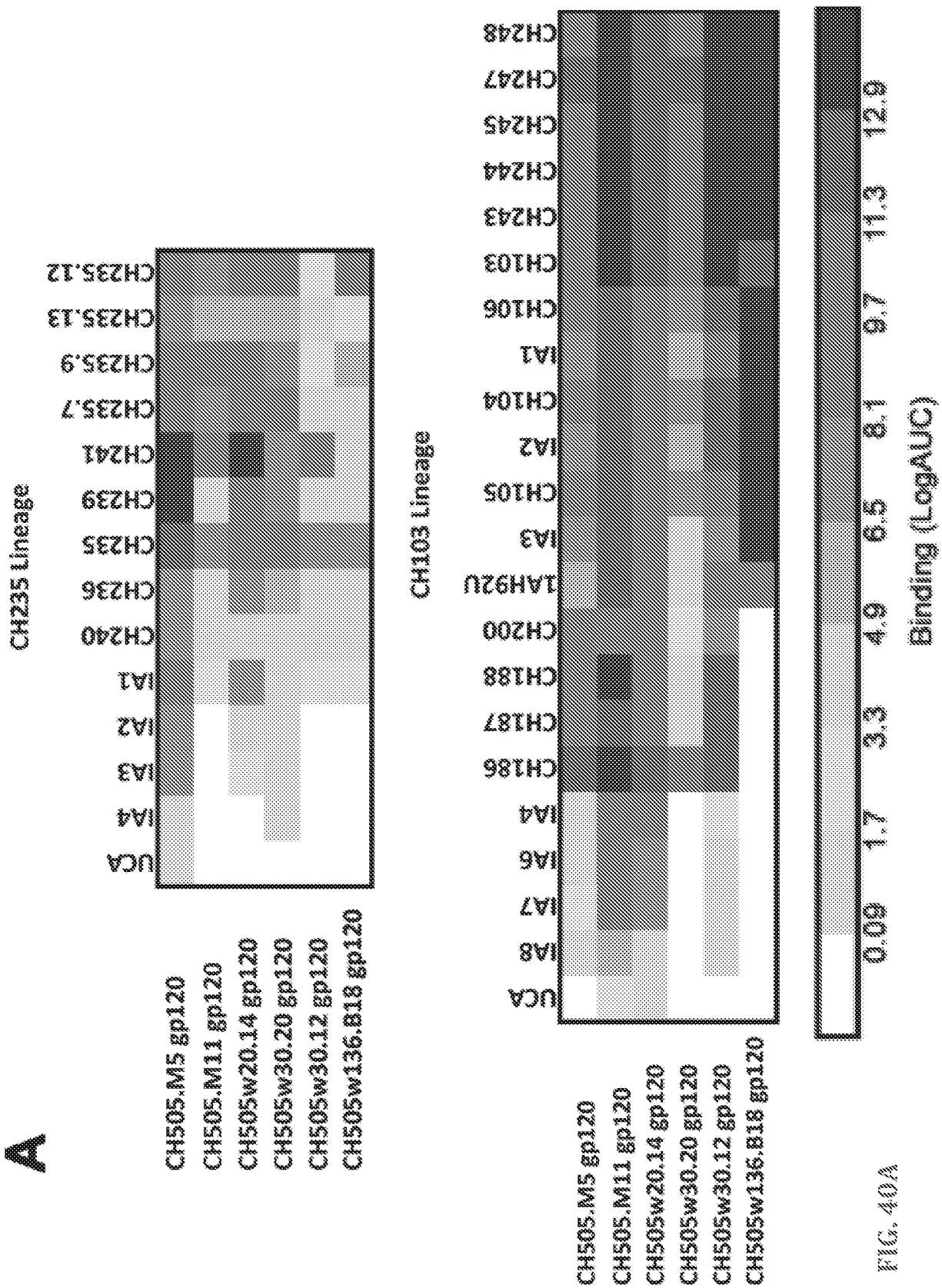
A
CH505.M5 gp120
CH505.M11 gp120
CH505w20.14 gp120
CH505w30.20 gp120
CH505w30.12 gp120
CH505w136.B18 gp120
CH235 Lineage
UCA
IA4
IA3
IA2
IA1
CH240
CH236
CH235
CH239
CH241
CH235.7
CH235.9
CH235.13
CH235.12
CH103 Lineage
UCA
IA8
IA7
IA6
IA4
CH186
CH187
CH188
CH200
IAH92U
IA3
CH105
IA2
CH104
IA1
CH106
CH103
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

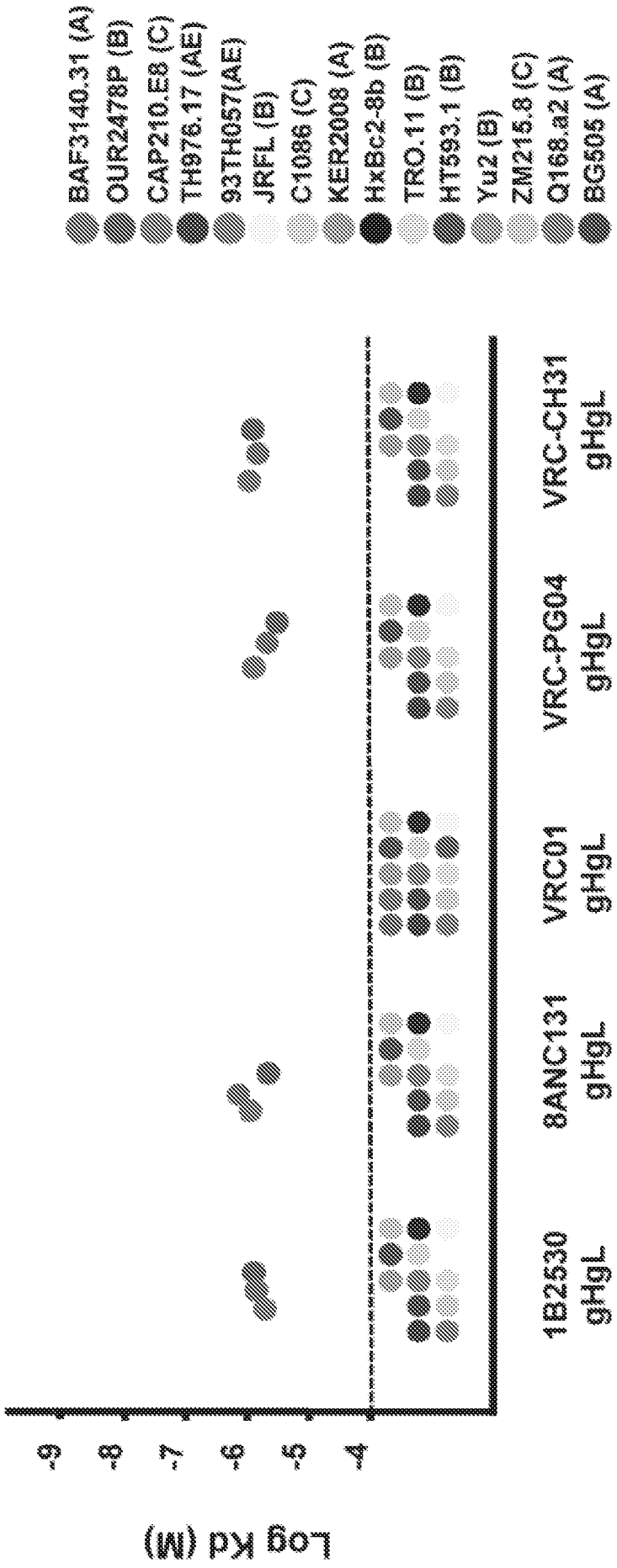
BAF3140.31 (A)
OUR2478P (B)
CAP210.E8 (C)
TH976.17 (AE)
93TH057(AE)
JRFL (B)
C1086 (C)
KER2008 (A)
HxBc2-8b (B)
TRO.11 (B)
HT593.1 (B)
Yu2 (B)
ZM215.8 (C)
Q168.a2 (A)
BG505 (A)
Log Kd (M)
-6
-8
-7
-6
-5
-4
1B2530 gHgL
VH 1-46
8ANC131 gHgL
VH 1-46
VRC01 gHgL
VH 1-2
VRC-PG04 gHgL
VH 1-2
VRC-CH31 gHgL
VH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

	Envelope ID	UCA	IA4	IA3	IA2	CH240	CH236	CH239	IA1	CH235.7	CH235.10	CH235.11	CH235.13	CH235	CH241	CH235.9	CH235.12
	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	9.73	13.27	8.67	8.39
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	10.88	14.01	8.78	9.10
	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	9.85	13.34	8.09	7.69
	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	10.26	13.88	8.44	8.23
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	9.07	12.73	5.08	6.31
	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	9.02	12.59	7.08	6.44
	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	10.62	13.47	7.86	7.63
	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	9.62	12.99	8.06	8.04
	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	8.19	11.69	6.20	6.26
	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	9.43	13.26	8.74	8.00
	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	10.58	14.40	9.70	7.95
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	9.99	13.40	8.44	7.81
	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	8.63	12.19	7.29	7.31
	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	9.86	13.58	8.70	7.97
	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	9.91	13.08	8.15	8.33
	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	10.09	13.36	8.19	7.31
	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	9.61	12.83	6.91	6.61
	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	8.59	12.34	6.56	6.41
	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	9.84	13.45	8.07	6.73
	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	7.81	11.65	4.49	5.52
	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	8.95	12.06	6.33	6.28
	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	9.75	13.59	9.79	8.66
	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	9.63	13.32	9.59	8.85
	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	8.58	12.68	7.80	7.06

CH235 Lineage

FIG. 47

		CH235 Lineage														
	Envelope ID	UCA	IA4	IA3	IA2	CH240	CH236	CH239	IA1	CH235.7	CH235.10	CH235.11	CH235.13	CH235	CH241	CH235.9	CH235.12
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	8.57	7.54	7.67	6.86
	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	7.01	5.74	7.02	7.21
	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	5.05	7.32	2.11	1.80
	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	8.33	8.03	8.09	8.59
	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	8.85	7.94	8.02	8.48
	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	6.86	9.57	4.02	2.90
	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	7.18	10.04	4.63	3.28
	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	10.05	8.90	9.33	8.06
	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	9.11	6.52	8.63	7.00
	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	10.07	9.56	8.57	8.90
	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	10.54	9.42	10.19	9.56
	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	10.40	9.09	10.52	10.28
	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	7.79	6.56	7.78	7.33
	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	8.50	4.24	8.54	8.42
	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	6.72	9.62	4.16	3.18
	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	7.57	0.13	6.75	5.54
	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	7.28	9.60	3.17	2.37
	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	0.00	3.27	0.00	1.01
	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	0.00	1.47	0.00	0.00
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	4.63	7.19	5.76	4.75
	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	3.81	0.00	6.23	4.67
	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	4.99	0.00	7.22	4.26
	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	2.18	0.00	6.53	5.79
	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	1.11	1.41	5.23	6.36
	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	0.64	0.52	3.05	3.95
	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	0.13	0.00	4.89	4.76
	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	0.00	0.00	0.25	3.44

FIG. 47 cont.

	Envelope ID	UCA	A4	A3	A2	CH240	CH236	CH239	A1	CH235.7	CH235.10	CH235.11	CH235.13	CH235	CH241	CH235.9	CH235.12
	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	0.00	0.00	4.69	6.11
	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	0.00	0.00	0.00	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	0.00	0.00	0.00	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	0.00	0.00	0.09	0.09
	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	0.00	0.00	0.00	3.01
	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	0.00	0.00	0.00	0.00
Week 78	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	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	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	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	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	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	0.00	0.00	0.00	0.00
	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	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	0.28	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	0.00	0.00	0.00	0.13
	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	0.00	0.00	0.00	0.25
	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	0.00	0.00	0.00	0.13
	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	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	0.00	0.00	0.00	0.00
Week 100	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	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	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	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	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	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	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	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	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	0.00	0.00	0.00	0.00

CH235 Lineage

FIG. 47 cont.

		CH235 Lineage													
	Envelope ID	UCA	A4	A3	A2	CH240	CH236	CH239	A1	CH235.7	CH235.10	CH235.11	CH235.13	CH235	CH241	CH235.9	CH235.12
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	7.53	0.89	5.76	8.31
	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	0.74	0.00	2.38	2.58
	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	0.00	0.00	0.00	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	0.00	0.00	0.00	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	0.00	0.00	0.00	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	0.00	0.00	0.34	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	0.00	0.00	0.00	0.11
	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	0.00	0.00	0.00	0.07
	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	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	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	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	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	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	0.00	0.00	0.00	0.00
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	0.98	0.34	0.82	3.32
	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	0.00	0.00	0.00	0.31
	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	0.00	0.00	0.00	0.16
	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	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	0.00	0.00	0.00	0.09
	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	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	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	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	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	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	CH235	CH241	CH235.9	CH235.12
	CH505.M5D8gp120293F	0.24	1.36	7.01	6.95	7.39	7.27	12.94	9.18	10.58	2.62	1.26	6.53	11.36	14.51	9.26	8.20
	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	10.63	14.40	10.17	7.63
	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	9.46	13.17	8.11	8.21
	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	7.59	9.68	8.26	7.71
CH505 TF	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	7.13	10.44	5.95	4.61
Mutants	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	0.00	2.18	4.36	1.14
Loop D	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	0.00	2.15	1.04	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	0.00	4.82	1.13	0.19
	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	0.00	0.00	1.31	0.38
	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	0.00	0.00	0.80	0.69

CH235 Lineage

FIG. 47 cont.

	Envelope ID	UCA	IA8	IA7	IA6	IA4	CH187	CH188	CH186	CH200	1AH92U	IA3	IA2	IA1	CH103	CH104
	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	11.26	12.45	10.15
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	6.17	9.21	8.13
	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	10.28	12.35	11.08
	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	10.38	12.63	10.41
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	3.38	11.22	3.86
	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	9.14	10.88	9.49
	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	9.53	11.93	10.91
	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	9.62	11.89	10.63
	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	8.70	10.26	8.25
	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	9.38	11.92	10.74
	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	9.68	13.06	11.36
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	9.89	11.76	10.54
	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	8.64	10.34	8.31
	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	10.14	11.49	10.70
	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	10.27	12.55	10.39
	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	9.88	12.38	10.52
	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	8.86	12.43	9.80
	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	9.84	11.71	10.08
	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	9.85	12.83	11.56
	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	1.83	9.59	2.18
	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	8.99	11.85	8.86
	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	11.34	12.50	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	10.85	12.46	11.11
	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	8.92	11.27	8.67

CH103 Lineage

FIG. 47 cont.

	Envelope ID	CH103 Lineage						
		CH105	CH106	CH243	CH244	CH245	CH247	CH248
	CH0505_CON D7gp120/293i	10.62	11.88	12.52	12.35	13.23	12.87	12.91
Week 4	CH505w004.10D8gp120/293F	8.33	8.06	10.08	9.96	10.25	11.11	10.76
	CH505.w4.26D8gp120 /293F	10.45	11.05	13.00	12.79	13.38	12.82	13.05
	505.s.03.D8.gp120/293F	11.18	11.28	13.40	12.96	13.86	13.08	13.34
Week 14	CH505w014.8D8gp120	6.01	7.94	10.86	10.07	10.64	12.59	11.68
	CH505w014.2D8gp120/293F	9.62	9.62	11.86	11.41	11.97	12.48	12.04
	CH505w014.32D8gp120/293F	10.70	10.54	12.94	12.92	13.42	12.65	13.05
	CH505w014.3D8gp120	10.81	10.64	12.81	12.78	12.72	13.00	13.32
	CH505.08.D11gp120/293F	8.30	9.20	10.80	10.19	10.66	11.06	10.92
	CH505w014.10D8gp120	10.81	10.37	13.24	12.98	12.86	13.12	12.85
	CH505w014.21D8gp120/293F	11.40	11.09	13.48	13.40	13.65	13.41	13.57
Week 20	CH505w020.15D8gp120	10.84	10.46	12.82	12.42	12.80	13.27	12.55
	CH505w020.13D8gp120	8.22	9.44	10.36	9.82	9.71	10.68	10.78
	CH505w020.22D8gp120/293F	10.45	10.68	12.46	12.48	12.68	13.06	12.68
	CH505w020.14D8gp120	10.42	11.23	12.61	12.32	12.11	12.08	12.48
	CH505w020.8D8gp120/293F	10.55	10.96	12.97	12.73	12.68	12.60	12.89
	CH505w020.3D8gp120	10.11	10.25	12.73	12.78	12.68	12.69	12.27
	CH505w020.30D8gp120	10.26	10.80	12.67	12.53	12.28	12.38	12.71
	CH505w020.23D8gp120	11.20	11.30	13.53	13.17	12.97	13.24	13.24
	CH505w020.11D8gp120	3.61	5.37	7.38	7.08	8.43	10.44	9.22
	CH505w020.9D8gp120	9.31	10.02	12.22	11.92	11.43	12.54	12.30
	CH505w020.4D8gp120/293F	11.14	11.49	13.28	13.39	13.66	13.48	13.30
	CH505w020.7D8gp120	10.62	11.29	12.76	12.41	12.40	12.35	12.88
	CH505w020.26D8gp120	9.01	9.56	11.61	11.45	11.37	12.22	12.01

FIG. 47cont.

		CH103 Lineage												
	Envelope ID	UCA	IA8	IA7	IA6	IA4	CH187	CH188	CH186	CH200	†AH92U	IA3	IA2	IA1	CH103	CH104
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	4.51	8.05	6.13
	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	6.69	9.08	7.83
	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	7.05	10.66	8.20
	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	5.65	9.48	7.49
	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	7.31	9.12	8.17
	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	10.10	12.50	10.34
	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	10.47	12.89	11.29
	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	9.08	12.50	10.33
	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	8.40	10.92	9.48
	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	9.92	13.05	10.91
	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	7.75	11.43	9.44
	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	8.42	11.08	9.58
	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	7.79	10.23	8.94
	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	8.04	10.06	9.67
	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	10.07	12.78	10.68
	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	5.79	9.92	6.99
	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	10.62	13.25	11.62
	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	11.78	12.25	11.30
	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	8.23	12.85	8.88
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	8.79	11.62	9.94
	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	10.00	11.64	10.61
	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	9.21	11.21	10.41
	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	9.93	11.22	9.99
	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	13.36	13.63	13.73
	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	13.56	13.49	13.82
	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	11.37	12.66	12.05
	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	8.79	10.17	9.80

FIG. 47 cont.

	Envelope ID	CH103 Lineage						
		CH105	CH106	CH243	CH244	CH245	CH247	CH248
Week 30	CH505w030.6D8gp120/293F	6.47	6.56	8.07	7.95	9.25	10.28	8.88
	CH505w030.36D8gp120	7.46	8.47	10.01	9.61	12.14	11.98	11.14
	CH505.w30.12D8gp140	7.63	8.13	10.29	9.96	9.48	11.85	9.71
	CH505w030.20D8gp120/293F	7.10	7.25	9.03	10.06	9.90	11.61	9.97
	CH505w030.27D8gp120/293F	7.88	8.17	10.10	9.49	11.87	11.61	10.96
	CH505w030.10D8gp120	9.26	11.14	12.62	12.40	12.77	13.78	12.69
	CH505w030.13D8gp120/293F	10.46	11.88	13.79	13.74	13.97	14.50	13.48
	CH505w030.25D8gp120	9.96	10.19	12.12	11.82	13.44	12.58	12.82
	CH505w030.11D8gp120	8.97	9.41	11.56	11.41	13.73	12.68	11.85
	CH505w030.18D8gp120	11.14	10.54	13.24	13.55	14.17	13.07	12.96
	CH505w030.5D8gp120	8.98	9.41	11.45	11.09	13.15	12.93	12.40
	CH505w030.23D8gp120	9.18	9.98	11.69	11.44	12.97	12.36	12.26
	CH505w030.9D8gp120	8.67	9.61	11.06	8.65	13.08	12.77	11.97
	CH505w030.15D8gp120	9.27	9.33	11.17	10.60	12.57	11.18	11.98
	CH505w030.28D8gp120	9.13	11.71	12.65	12.46	12.42	13.77	12.74
	CH505w030.17D8gp120	6.73	8.20	9.93	9.56	12.83	13.03	11.50
	CH505.w30.12D8gp120	10.68	11.76	13.89	13.68	13.80	13.89	13.22
	CH505w030.21D8gp120	10.55	10.91	12.86	12.95	12.50	11.55	13.33
	CH505w030.19D8gp120/293F	9.46	7.39	12.61	13.20	11.64	12.72	13.55
Week 53	CH505w053.16D8gp120	9.00	9.78	11.64	11.51	13.45	12.38	12.94
	CH505w053.25D8gp120	10.00	10.88	11.76	11.82	13.14	12.17	12.61
	CH505w053.3D8gp120	9.41	10.09	11.74	11.52	13.30	11.93	12.78
	CH505w053.13D8gp120/293F	9.37	10.17	11.45	11.80	12.34	11.53	12.19
	CH505w053.31D8gp120/293F	13.52	13.35	14.44	15.25	15.04	14.15	15.19
	CH505.w53.19gp D8gp120	13.75	13.24	14.43	15.75	15.37	14.20	14.82
	CH505w053.6D8gp120	11.74	10.93	13.06	14.13	13.65	13.02	13.46
	CH505w053.29D8gp120	8.51	9.70	10.73	10.38	11.51	11.14	11.51

FIG. 47 cont.

		CH103 Lineage													
	Envelope ID	UCA	IA8	IA7	IA6	IA4	CH187	CH188	CH186	CH200	1AH92U	IA3	IA2	IA1	CH103	CH104
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	5.64	10.24	7.67
	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	8.24	11.08	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	7.53	11.03	8.34
	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	6.29	8.93	6.85
	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	3.00	9.94	4.50
	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	4.41	7.69	4.22
	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	10.91	10.70	10.83
	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	6.63	11.13	8.09
	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	8.99	11.32	9.98
	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	8.94	11.38	9.49
	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	9.80	11.72	10.08
	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	2.40	8.75	3.47
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	8.86	4.78	9.20
	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	12.56	12.89	12.94
	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	12.21	7.07	11.02
	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	11.90	7.77	11.62
	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	12.87	8.66	13.49
	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	9.89	11.73	10.08
	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	8.72	11.29	9.55
	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	8.82	12.54	10.20
	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	8.56	4.70	10.41
	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	8.54	11.40	9.97
	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	11.00	12.14	11.60
	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	13.74	9.73	13.07
	CH505_w100V115A13.D7gp120/293F	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.20	8.26	9.84	8.86	6.13	10.83
	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	12.20	12.62	13.47
	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	13.50	12.52	14.02
	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	7.33	3.22	6.13

FIG. 47 cont.

		CH103 Lineage						
	Envelope ID	CH105	CH106	CH243	CH244	CH245	CH247	CH248
Week 78	CH0505.w78.env5.D11gp120/293F	6.61	7.89	10.04	11.29	11.15	12.47	10.90
	CH505w078.33D8gp120/293F	8.86	9.48	10.77	11.46	12.22	11.66	12.08
	CH505w078.1D8gp120/293F	7.48	7.29	10.55	11.24	8.36	11.02	10.38
	CH505w078.9D8gp120	6.59	8.34	8.96	9.22	11.09	10.34	10.60
	CH505w078.6D8gp120	3.80	6.72	8.99	9.77	10.16	10.78	9.68
	CH505w078.38D8gp120	3.98	5.25	6.04	6.32	7.60	9.77	7.02
	CH505w078.15D8gp120/293F	10.08	10.95	12.13	11.86	11.70	11.62	11.57
	CH505w078.10D8gp120	7.61	7.84	10.95	10.91	12.38	12.76	11.44
	CH505w078.17D8gp120	9.53	8.72	11.65	12.18	11.57	11.40	11.67
	CH505w078.7D8gp120/293F	9.41	8.71	11.51	12.05	10.76	11.12	11.07
	CH0505.w78.env4.D11gp120/293i	9.53	9.25	12.00	12.40	11.73	11.11	11.99
	CH505w078.25D8gp120	3.33	4.64	6.47	6.68	10.41	10.10	9.71
Week 100	CH505w100.C7D8gp120/293F	8.96	9.30	11.30	11.78	12.06	7.94	10.76
	CH505w100.A13D8gp120	11.40	11.99	13.90	13.85	14.42	12.88	14.01
	CH505w100.B6D8gp120	11.04	11.77	13.62	13.91	14.47	12.58	13.82
	CH505w100.B7D8gp120/293F	11.58	11.53	13.14	13.62	14.04	12.28	13.27
	CH505_w100V115b7.D7gp120/293F	12.44	12.16	14.32	14.61	15.30	13.25	14.93
	CH505w100.A10D8gp120	9.06	10.37	12.10	11.83	12.99	12.69	12.43
	505_w100.A4.D8.gp120/293F	8.51	9.43	11.03	10.97	12.50	12.61	12.19
	CH505_w100V115A10.D7gp120	9.20	10.79	12.90	12.73	13.65	13.70	12.79
	CH505w100.A12D8gp120	9.56	9.65	12.26	12.81	12.90	9.01	13.17
	CH505w100.A3D8gp120/293F	7.21	8.59	11.91	11.99	10.84	11.19	11.45
	CH505w100.A6D8gp120	10.12	11.11	12.23	12.53	12.74	11.93	12.39
	CH505w100.B4D8gp120	11.91	12.55	12.48	13.29	13.35	12.56	13.00
	CH505_w100V115A13.D7gp120/293F	10.55	10.12	12.60	13.35	13.34	9.78	13.74
	CH505_w100V115A6.D11gp120	12.24	11.93	13.62	14.17	14.54	13.15	14.09
	CH505_w100V115B4.D11gp120	13.55	12.87	13.75	14.61	14.78	13.54	14.28
	CH505w100.B2D8gp120/293F	7.51	7.80	11.29	11.41	11.60	8.61	11.13

FIG. 47 cont.

CH103 Lineage

	Envelope ID	UCA	IA8	IA7	IA6	IA4	CH187	CH188	CH186	CH200	1AH92U	IA3	IA2	IA1	CH103	CH104
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	14.36	11.79	14.25
	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	13.25	10.80	13.20
	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	12.50	12.90	12.17
	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	13.10	9.93	13.10
	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	9.48	4.33	10.59
	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	12.94	11.90	11.73
	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	13.76	13.75	13.84
	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	12.40	12.43	11.66
	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	12.47	8.72	12.70
	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	11.24	12.60	11.51
	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	12.25	12.73	11.37
	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	11.76	13.38	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	12.24	12.70	10.66
	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	9.19	11.48	8.94
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	13.55	9.03	12.85
	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	9.62	12.16	9.24
	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	5.26	12.19	7.23
	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	12.91	7.76	11.33
	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	5.84	12.15	6.83
	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	6.35	10.71	7.31
	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	7.70	11.89	7.44
	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	6.39	11.50	5.71
	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	3.78	10.95	4.51
	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	4.68	11.15	5.41

FIG. 47 cont.

	Envelope ID	CH103 Lineage							
		CH105	CH106	CH243	CH244	CH245	CH247	CH248
Week 136	CH505w136.B18D8gp120	13.69	13.35	14.26	14.83	15.42	13.40	14.27
	CH505w136.B2D8gp120/293F	12.38	12.74	13.48	13.40	13.98	12.27	13.40
	CH505_w137V201B12.D11gp120	10.96	12.53	13.17	13.91	14.34	13.53	13.41
	CH505w136.B3D8gp120/293F	12.00	12.57	13.71	14.11	14.30	13.17	13.97
	CH505w136.B5D8gp120	10.55	10.90	13.19	14.39	14.35	13.33	14.55
	CH505w136.B8D8gp120	9.86	11.71	10.95	12.28	12.32	11.97	12.29
	CH505w136.B36D8gp120	12.82	13.09	14.06	14.72	15.30	14.43	14.37
	CH505w136.B20D8gp120	10.66	12.09	13.02	13.10	13.50	12.53	12.92
	CH505_w137V209C12.D11gp120	12.67	12.21	13.97	14.45	14.78	13.49	14.68
	CH505w136.B27D8gp120	10.38	11.20	12.79	12.85	13.13	12.22	12.89
	CH505w136.B29D8gp120	10.21	12.02	12.42	12.96	13.58	12.71	12.32
	CH505w136.B4D8gp120	10.74	11.81	13.44	13.98	14.40	13.51	13.38
	CH505w136.B12D8gp120	9.67	11.90	12.75	13.29	13.34	12.90	12.94
	CH505w136.B10D8gp120	8.52	8.97	11.74	11.91	11.75	11.40	11.46
Week 160	CH505w160.T4D8gp120/293F	12.21	12.33	13.39	13.89	14.23	12.29	13.52
	CH505w160.C2D8gp120	8.61	9.29	11.24	11.88	10.53	11.47	11.09
	CH505w160.C4D8gp120	6.55	6.80	10.61	12.06	9.83	11.17	10.48
	CH505w160.C12D8gp120	11.14	12.17	12.09	12.61	12.05	11.82	12.89
	CH505w160.C14D8gp120	6.58	6.94	11.03	12.24	10.05	11.80	10.29
	CH505w160.A1D8gp120	6.66	7.60	9.67	9.63	8.96	9.31	8.62
	CH505w160.C11D8gp120	7.70	8.15	10.91	11.50	10.40	11.36	10.67
	CH505w160.D1D8gp120	6.03	7.06	9.51	10.81	8.34	10.47	8.75
	CH505w160.D5D8gp120/293F	4.58	5.64	8.57	10.05	7.32	10.29	7.84
	CH505w160.T2D8gp120	4.94	6.16	9.14	10.83	7.85	10.31	8.34

FIG. 47 cont.

	Envelope ID	UCA	IA8	IA7	IA6	IA4	CH187	CH188	CH186	CH200	1AH92U	IA3	IA2	IA1	CH103	CH104
	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	7.04	9.84	9.02
	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	11.08	13.25	11.68
	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	11.79	13.09	11.58
CH505	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	12.19	13.38	12.03
TF	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	11.30	13.29	11.26
Mutants	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	9.49	11.52	10.11
Loop D	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	10.38	12.21	10.85
	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	11.22	11.40	11.23
	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	9.85	11.60	10.84
	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	8.70	11.06	9.73

CH103 Lineage

FIG. 47 cont.

	Envelope ID	CH105	CH106	CH243	CH244	CH245	CH247	CH248
	CH505.M5D8gp120293F	8.63	8.37	10.41	10.38	10.74	11.47	10.98
	CH505.M10D8gp120	11.30	11.81	13.61	13.40	13.74	13.09	13.77
	CH505.M6D8gp120/293F	11.05	12.60	13.65	13.24	13.62	13.57	13.64
CH505 TF	CH505.M11D8gp120/293F	11.72	12.77	13.97	13.66	13.76	13.67	13.64
	CH505.M19D8gp120	10.92	12.18	13.79	13.72	14.06	13.82	13.21
Mutants	CH505.M8D8gp120	9.33	10.19	11.50	11.57	11.51	12.61	12.32
Loop D	CH505.M20D8gp120/293F	10.57	10.69	12.82	12.66	12.57	13.39	13.20
	CH505.M21D8gp120/293F	11.19	11.46	13.41	13.05	12.66	13.31	13.78
	CH505.M9D8gp120/293F	10.58	10.98	13.04	12.95	13.07	12.54	13.01
	CH505.M7D8gp120/293F	10.17	10.99	12.85	12.35	12.37	12.49	12.42

CH103 Lineage

FIG. 47 cont.

Region	R#	DH511	DH511 mutation
CDRH3	92	C	
	93	T	
	94	A	
	95	D	
	96	L	W
	97	G	W,F
	98	E	W
	99	P	W
	100	V	F,L
	100a	V	W
	100b	S	W
	100c	R	W
	100d	F	W
	100e	F	W
	100f	E	W
	100g	W	
	100h	G	W
	100i	S	W
	100j	Y	W
	100k	Y	
	100k	Y	W
	100l	Y	
	100m	M	
	101	D	
	102	L	
	103	W	
	104	G	

Figure 48A

Region	R#	DH512	DH512 mutation
CDRH3	92	C	
	93	T	
	94	M	
	95	D	
	96	E	W
	97	G	W,F
	98	T	W
	99	P	W
	100	V	F,L
	100a	T	W
	100b	R	W
	100c	F	W
	100d	L	W,F
	100e	E	W
	100f	W	
	100g	G	W
	100h	Y	W
	100i	F	W
	100j	Y	
	100k	Y	W
	100l	Y	
	100m	M	
	101	A	
	102	V	
	103	W	
	104	G	

Figure 48B

Mutations outside of CDRH3

Region	R#	DH511	DH511 mutation	Region	R#	DH512	DH512 mutation
CDRH1	26	G	W	CDRH1	26	G	W
	27	F			27	F	
	28	T	W		28	F	W
	29	F			29	F	
	30	S			30	D	
	31	N	W		31	N	W
	32	T			32	S	
	33	W			33	W	

Region	R#	DH511	DH511 mutation	Region	R#	DH512	DH512 mutation
CDRH2	51	I		CDRH2	51	I	
	52	S	W		52	R	W
	52a	R			52a	R	
	52b	N	W		52b	L	W
	52c	K	W		52c	K	W
	53	D	W		53	D	W
	54	G			54	G	
	55	A			55	A	
	56	K			56	T	
	57	T			57	G	

Region	R#	DH511	DH511 mutation	Region	R#	DH512	DH512 mutation
FR3	72	D		FR3	72	D	
	73	D	W		73	D	W
	74	S	W		74	S	W
	75	R	W		75	R	W

Figure 48C

DH512 Nucleotide Sequence (SEQ ID NO: 195)

CAGGTGCAGCTGGTACAGTCTGGGGGAGGTCTGGTGAAGCCGGGGGGGTCCCTCACACTCTCCTGTTC
AGCCTCTGGATTCTTTTTCGATAATTCATGGATGGGGTGGGTCCGTCAGGCGCCAGGGAAGGGACTGG
AGTGGGTTGGCCGCATTAGAAGGCTCAAAGACGGTGCGACAGGAGAATATGGTGCAGCCGTGAAGGAC
AGATTCACCATTTCAAGAGATGACAGTAGAAATATGCTGTACCTGCACATGAGGACCCTGAAAACCGA
GGACTCAGGCACTTATTATTGTACCATGGATGAGGGGACCCCAGTAACACGCTTCTTAGAATGGGGCT
ACTTCTATTATTATATGGCCGTTTGGGGCAGAGGGACCACGGTCATCGTCTCTTCA

DH512 Translated (Amino Acid) Sequence (SEQ ID NO: 196)

QVQLVQSGGGLVKPGGSLTLSCSASGFFFDNSWMGWVRQAPGKGLEWVGRIRRLKDGATGEYGAAVKD
RFTISRDDSRNMLYLHMRTLKTEDSGTYYCTMDEGTPVTRFLEWGYFYYYMAVWGRGTTVIVSS

Amino Acid Sequences of DH512 Heavy Chain Mutants

>DH512_E96W (SEQ ID NO: 197)

QVQLVQSGGGLVKPGGSLTLSCSASGFFFDNSWMGWVRQAPGKGLEWVGRIRRLKDGATGEYGAAVKD
RFTISRDDSRNMLYLHMRTLKTEDSGTYYCTMDWGTPVTRFLEWGYFYYYMAVWGRGTTVIVSS

>DH512_G97W (SEQ ID NO: 198)

QVQLVQSGGGLVKPGGSLTLSCSASGFFFDNSWMGWVRQAPGKGLEWVGRIRRLKDGATGEYGAAVKD
RFTISRDDSRNMLYLHMRTLKTEDSGTYYCTMDEWTPVTRFLEWGYFYYYMAVWGRGTTVIVSS

>DH512_T98W (SEQ ID NO: 199)

QVQLVQSGGGLVKPGGSLTLSCSASGFFFDNSWMGWVRQAPGKGLEWVGRIRRLKDGATGEYGAAVKD
RFTISRDDSRNMLYLHMRTLKTEDSGTYYCTMDEGWPVTRFLEWGYFYYYMAVWGRGTTVIVSS

>DH512_P99W (SEQ ID NO: 200)

QVQLVQSGGGLVKPGGSLTLSCSASGFFFDNSWMGWVRQAPGKGLEWVGRIRRLKDGATGEYGAAVKD
RFTISRDDSRNMLYLHMRTLKTEDSGTYYCTMDEGTWVTRFLEWGYFYYYMAVWGRGTTVIVSS

>DH512_V100F (SEQ ID NO: 201)

QVQLVQSGGGLVKPGGSLTLSCSASGFFFDNSWMGWVRQAPGKGLEWVGRIRRLKDGATGEYGAAVKD
RFTISRDDSRNMLYLHMRTLKTEDSGTYYCTMDEGTPFTRFLEWGYFYYYMAVWGRGTTVIVSS

>DH512_V100I (SEQ ID NO: 202)

QVQLVQSGGGLVKPGGSLTLSCSASGFFFDNSWMGWVRQAPGKGLEWVGRIRRLKDGATGEYGAAVKD
RFTISRDDSRNMLYLHMRTLKTEDSGTYYCTMDEGTPITRFLEWGYFYYYMAVWGRGTTVIVSS

>DH512_T100aW (SEQ ID NO: 203)
QVQLVQSGGGLVKPGGSLTLSCSASGFFFDNSWMGWVRQAPGKGLEWVGRIRRLKDGATGEYGAAVKD
RFTISRDDSRNMLYLHMRTLKTEDSGTYYCTMDEGTPVWRFLEWGYFYYYMAVWGRGTTVIVSS

Figure 48D

>DH512_R100bW (SEQ ID NO: 204)

QVQLVQSGGGLVKPGGSLTLSCSASGFFFDNSWMGWVRQAPGKGLEWVGRIRRLKDGATGEYGAAVKD
RFTISRDDSRNMLYLHMRTLKTEDSGTYYCTMDEGTPVTWFLEWGYFYYYMAVWGRGTTVIVSS

>DH512_F100cW (SEQ ID NO: 205)

QVQLVQSGGGLVKPGGSLTLSCSASGFFFDNSWMGWVRQAPGKGLEWVGRIRRLKDGATGEYGAAVKD
RFTISRDDSRNMLYLHMRTLKTEDSGTYYCTMDEGTPVTRWLEWGYFYYYMAVWGRGTTVIVSS

>DH512_L100dW (SEQ ID NO: 206)

QVQLVQSGGGLVKPGGSLTLSCSASGFFFDNSWMGWVRQAPGKGLEWVGRIRRLKDGATGEYGAAVKD
RFTISRDDSRNMLYLHMRTLKTEDSGTYYCTMDEGTPVTRFWEWGYFYYYMAVWGRGTTVIVSS

>DH512_L100dF (SEQ ID NO: 207)

QVQLVQSGGGLVKPGGSLTLSCSASGFFFDNSWMGWVRQAPGKGLEWVGRIRRLKDGATGEYGAAVKD
RFTISRDDSRNMLYLHMRTLKTEDSGTYYCTMDEGTPVTRFFEWGYFYYYMAVWGRGTTVIVSS

>DH512_E100eW (SEQ ID NO: 208)

QVQLVQSGGGLVKPGGSLTLSCSASGFFFDNSWMGWVRQAPGKGLEWVGRIRRLKDGATGEYGAAVKD
RFTISRDDSRNMLYLHMRTLKTEDSGTYYCTMDEGTPVTRFLWWGYFYYYMAVWGRGTTVIVSS

>DH512_G100gW (SEQ ID NO: 209)

QVQLVQSGGGLVKPGGSLTLSCSASGFFFDNSWMGWVRQAPGKGLEWVGRIRRLKDGATGEYGAAVKD
RFTISRDDSRNMLYLHMRTLKTEDSGTYYCTMDEGTPVTRFLEWWYFYYYMAVWGRGTTVIVSS

>DH512_Y100hW (SEQ ID NO: 210)

QVQLVQSGGGLVKPGGSLTLSCSASGFFFDNSWMGWVRQAPGKGLEWVGRIRRLKDGATGEYGAAVKD
RFTISRDDSRNMLYLHMRTLKTEDSGTYYCTMDEGTPVTRFLEWGWFYYYMAVWGRGTTVIVSS

>DH512_F100iW (SEQ ID NO: 211)

QVQLVQSGGGLVKPGGSLTLSCSASGFFFDNSWMGWVRQAPGKGLEWVGRIRRLKDGATGEYGAAVKD
RFTISRDDSRNMLYLHMRTLKTEDSGTYYCTMDEGTPVTRFLEWGYWYYYMAVWGRGTTVIVSS

>DH512_Y100kW (SEQ ID NO: 212)

QVQLVQSGGGLVKPGGSLTLSCSASGFFFDNSWMGWVRQAPGKGLEWVGRIRRLKDGATGEYGAAVKD
RFTISRDDSRNMLYLHMRTLKTEDSGTYYCTMDEGTPVTRFLEWGYFYWYMAVWGRGTTVIVSS

Figure 48D cont.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2016/023355

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61P 31/18; C07K 16/28; C12P 21/08 (2016.01)
CPC - A61K 2039/505; C07K 16/2812; C07K 2317/76 (2016.05)
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 - A61P 31/18; C07K 16/28; C12P 21/08
CPC - A61K 2039/505; C07K 16/2812; C07K 2317/76

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC - 424/154.1; 530/388.75 (keyword delimited)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PatBase, Google Patents, Google Scholar, ProQuest, PubMed

Search terms used: CD4 antibody CH557 HIV

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	BONSIGNORI et al. "An autoreactive antibody from an SLE/HIV-1 individual broadly neutralizes HIV-1," J Clin Invest. 10 March 2014 (10.03.2014), Vol. 124, Pgs. 1835-43. entire document	1-15
A	FERA et al. "Affinity maturation in an HIV broadly neutralizing B-cell lineage through reorientation of variable domains," Proc Natl Acad Sci USA. 30 June 2014 (30.06.2014), Vol. 111, Pgs. 10275-80. entire document	1-15
A	US 2004/0162298 A1 (HO et al) 19 August 2004 (19.08.2004) entire document	1-15
A	LIU et al. "Polyreactivity and autoreactivity among HIV-1 antibodies," J Virol. 29 October 2014 (29.10.2014), Vol. 89, Pgs. 784-98. entire document	1-15
A	WO 2001/043779 A2 (TANOX, INC.) 21 June 2001 (21.06.2001) entire document	1-15
A	US 2014/0248295 A1 (THE ROCKEFELLER UNIVERSITY) 04 September 2014 (04.09.2014) entire document	1-15

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
01 July 2016	29 JUL 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