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(54) **Title:** BROADLY NEUTRALIZING ANTI-HIV ANTIBODIES AND EPITOPE THEREFOR

(57) **Abstract:** The present invention relates to broadly neutralizing anti-HIV-1 antibodies and isolated antigens. Also disclosed are related methods and compositions.



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BROADLY NEUTRALIZING ANTI-HIV ANTIBODIES AND EPITOPE THEREFOR

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority to U.S. Provisional Application No. 61/934,359
5 filed January 31, 2014, the contents of which are incorporated herein by reference.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

The invention disclosed herein was made, at least in part, with Government support under Grant Nos. HIVRAD P01 AI100148 and 1UM1 AI100663-01 from the
10 National Institutes of Health. Accordingly, the U.S. Government has certain rights in this invention.

BACKGROUND OF THE INVENTION

The only target of neutralizing anti-HIV-1 antibodies is the envelope (Env) spike, a heterotrimer of gp120 and gp41 subunits. Single cell-based antibody cloning techniques
15 have recently uncovered a large number of antibodies that can potentially neutralize highly diverse HIV-1 variants by targeting Env (Klein et al., *Science* 341, 1199 (2013)). When transferred passively, broadly neutralizing antibodies (bNAbs) can prevent infection by HIV-1 or SHIV in humanized mice and macaques, respectively. Moreover, combinations of bNAbs can also suppress established HIV-1 and SHIV infections (Klein et al., *Nature*
20 492, 118 (2012); Barouch et al., *Nature* 503, 224 (2013); Shingai et al., *Nature* 503, 277 (2013)).

Most of the bNAbs characterized to date target one of four major sites of vulnerability on HIV-1 Env: on gp120, the CD4 binding site, the V2 loop, and the base of the V3 loop, and on gp41, the membrane proximal region (Klein et al., *Science* 341, 1199
25 (2013); Burton et al., *Science* 337, 183 (2012); Mascola et al., *Immunological Reviews* 254, 225 (2013)). 8ANC195 is among a small group of bNAbs that does not appear to target any of these sites. Although only two of the B cells originally isolated from the 8ANC195 donor, an HIV-1 elite controller, belonged to the 8ANC195 clone, the antibodies produced by this clone complemented the neutralizing activity of antibodies

produced by a more expanded B cell clone that targeted the CD4 binding site (Scheid et al., *Science* 333, 1633 (2011)).

8ANC195 is classified as a bNAb because it neutralized 66% of viruses in a diverse viral panel (Scheid et al., *Science* 333, 1633 (2011)). Like other anti-HIV-1 bNAbs, 8ANC195 is highly somatically mutated, including insertions and deletions in the complementarity determining regions (CDRs) and framework regions (FWRs) of its heavy chain (HC) and light chain (LC). Although initial efforts to map the 8ANC195 epitope were unsuccessful (*Ibid.*) computational analyses of neutralization data predicted that intact potential *N*-linked glycosylation sites (PNGSs) at positions 234_{gp120} and 276_{gp120} were essential for its activity. These predictions were confirmed by evaluating the neutralization potency of 8ANC195 against mutant HIV-1 strains *in vitro* and *in vivo* (West, Jr. et al., *Proceedings of the National Academy of Sciences of the United States of America* 110, 10598 (2013); Chuang et al., *Journal of Virology* 87, 10047 (2013)). However, the precise 8ANC195 epitope on HIV-1 Env has heretofore remained elusive.

SUMMARY OF INVENTION

This invention relates, in part, to the isolation of broadly-neutralizing antibodies (bNAbs) directed at an epitope on the HIV-1 envelope spike that spans the gp120 and gp41 subunits.

In one embodiment, the antibody comprises a heavy chain having one of the following amino acid sequences:

QIHLVQSGTEVKKPGSSVTVSCKAYGVNTFGLYAVNWVRQAPGQSLEYIG
QIWRWKSSASHHFRGRVLISAVDLTGSSPPISSLEIKNLTSDDTAVYFCTTTSTYDR
WSGLHHDGVMAFSSWGQGTLSVSAASTKG (g52; SEQ ID NO:1);

QIHLVQSGTEVKKPGSSVTVSCKAYGVNTFGLYAVNWVRQAPGQSLEYIG
QIWRWKSSASHHFRGRVIISAVDLTGSSPPISSLEIKNLTSDDTAVYFCTTTSTSDY
WSGLHHDGVMAFSSWGQGTLSVSAASTKG (g23; SEQ ID NO:2);

QIHLVQSGTG VKKPGSSVTVSCKAYGVNTFGLYAVNWVRQAPQGLEYI
GQIWRWKSSASHHFRGRVLISAVDLTGSSPPISSLEIKNLTSDDTAVYFCTTTSTYD
KWSGLYHDGVMAFSSWGQGTLSVSAASTKG (g8; SEQ ID NO:3);

QIHLVQSGTEVKKPGSSVAVSCKAYGVNTFGLYAVNWVRQAPGQSLEYIG
QIWRWKSSASHDFRGRVIISAVDLTGSSPPISSLEIKNLTSDDTAVYFCTATSTPDY
WSGLHHDGVMAFSSWGQGLTISVSAASTKG (g20; SEQ ID NO:4);

QIHLVQSGTEVKKPGSSVTVSCKAYGVNTFGLYAVNWVRQAPGQGLEYIG
5 QIWRWKSSASHHFRGRVLISAVDLTGSSPPISSLEIKNVTSDDTAVYFCTTTSTYDE
WSDLHHDGVMAFSSWGQGLTISVSAASTKG (g59; SEQ ID NO:5);

QIHLVQSGTEVKKPGSSVTVSCKAYGVNTFGLYAVNWVRQAPGQSLEYIG
QIWRWKSSASHHFRGRVLISAVDLTGSSPPISSLEIKNLTSDDTAVYFCTTTSTYDK
WSGLHHDGVMAFSSRGQGLTISVSAASTKG (g62; SEQ ID NO:6);

10 QIHLVQSGTEVKKPGSSVTVSCKAYGVNTFGLYAVNWVRQAPGQSLEYIG
QIWRWKSSASHHFRGRVLISAVDLTGSPPISSLEIKNLTSDDTAVYFCTTTSTYDK
WSGLHHDGVMAFSSWGQGLTISVSAASTKG (g22; SEQ ID NO:7);

QIHLVQSGTEVKKPGSSVTVSCKAYGVNTFGLYAVNWVRQAPGQSLEYIG
QIWRWKSSASHHFRGRVIISAVDLTGSSPPISSLEIKNLTSDDTAVYFCTTASTYDK
15 WSG LHHDGVMAFSSWGQGLTISVSAASTKG (g15; SEQ ID NO:8);

QIHLVQSGTEVKKPGSSVTVSCKAYGVNTFGLYAVNWVRQAPGQSLEYIG
QIWRWKSSASHHFRGRVIISAVDLTGSSPPISSLEIKNLTSDDTAVYFCTTTSTSDR
WSGLHHDGVMAFSSWGQGLTISVSAASTKG (g4; SEQ ID NO:9);

QIHLVQSGTEVKKPGSSVTVSCKAYGVNTFGLYAVNWVRQAPGQGLEYIG
20 QIWRWKSSASHHFRGRVLISAVDLTGSSPPISSLEIKNVTSDDTAVYFCTTTSTYDK
WSGLHHDGVMAFSSWGQGLTISVSAASTKG (g46; SEQ ID NO:10);

QIHLVQSGTEVKKPGSSVTVSCKAYEVNTFGLYAVNWVRQAPGQSLEYIG
QIWRWKSSASHHFRGRVLISAVDLTGSSPPISSLEIKNVTSDDTAVYFCTTTSTHDK
WSGLHHDGVMAFSSWGQGLTISVSAASTKG (g44; SEQ ID NO:11);

25 QIHLVQSGTEVKKPGSSVTVSCKAYGVNTFGLYAVNWVRQAPGQGLEYIG
QIWRWKSSASHHFRGRVLISAIDLTGSSPPISSLEIKNVTSDDTAVYFCTTMSTYDK
WSGLHHDGVMAFSSWGQGLTISVSAASTKG (g50; SEQ ID NO:12);

QIHLVQSGTEVKKPGSSVTVSCKAYGVNTFGLYAVSWVRQAPGQRLEYIG
QIRRWKSSASHHFRGRVTLSAVDPTGSSPPISSLEIRDLTTDDTAVYFCTTTSTSDY
WSGLHNERGTAFSSWGQGTLSVSAASTKG (g3; SEQ ID NO:13);

QIHLVQSGTEVKKPGSSVTVSCKAYGVNTFGLYAVNWVRQAPGQSLEYIG
5 QIWRWKSSASHHFRGRVLISAVDLTGSSPPISSLEIKNLTSDDTAVYFCTTTSTYDQ
WSGLHHDGVMAFSSWGQGTLSVSAASTKG (3040HC; SEQ ID NO:14);

QIHLVQSGTEVKKPGSSVTVSCKAYGVNTFGLYAVNWVRQAPGQSLEYIG
QIWRWKSSASHHFRGRVIISAVDLTGSSPPISSLEIKNLTSDDTAVYFCTTTSTSDY
WSGLHHDGVMAFSSWGQGTLSVSAASTKG (3430HC; SEQ ID NO:15);

10 QIHLVQSGTEVKKPGSSVTVSCKAYGVNTFGLYAVNWVRQAPGQSLEYIG
QIWRWKSSASHHFRGRVLISAVDLTGSSPPISSLEIKNLTSDDTAVYFCTTTSTYDR
WSGLHHDGVMAFSSWGQGTLSVSAASTKG (3484HC; SEQ ID NO:16);

QIHLVQSGTEVRKPGSSVTVSCKAYGVNTFGLYAVNWVRQAPGQSLEYIG
QIWRWKSSASHHFRGRVLISAVDLTGSSPPISSLEIKNLTSDDTAVYFCTTTSTYDK
15 WSG LHHDGVMAFSSWGQGTLSVSAASTKG (3044HC; SEQ ID NO:17); and

QIHLVQSGTEVKKPGSSVTVSCKAYGVNTFGLYAVNWVRQAPGQSLEYIG
QIWRWKSSASHHFRGRVLISAVDLTGSSPPISSLEIKNLTSDDTAVYFCTTTSTYDR
WSGLHHDGVMAFSSWGQGTLSVSAASTKG (3630HC; SEQ ID NO:18).

20 In one embodiment, the antibody comprises a light chain having one of the
following amino acid sequences:

DIQMTQSPSTLSASIGDTRVSCRASQSITGNWLAWYHQRPGKAPRLLIYRG
SRLGGVPSRFRGSAAGTDFTLTIANLQAEDFGTFYCQQYDTPGTFGQGTKVEV
KRTVAAPSVF (k3; SEQ ID NO:19);

DIQMTQSPSTLSASTGDTVRISCRASQSITGNWVAWYQQRPGKAPRLLIYR
25 GAALLGGVPSRFRGSAAGTDFTLTIGNLQAEDFGTFYCQQYDTPGTFGQGTKVE
VKRTVAAPSVF (k5; SEQ ID NO:20);

DIQMTQSPSTLSASIGDTRISCRASQSITGGWLAWYHQRPGKAPRLLIYRG
SRLLLGGVPSKFSGSAAGTDFTLTIANLQAEDFGTFYCQQYDTPGTFGQGKVEV
KRTVAAPSVF (k59; SEQ ID NO:21);

DIQMTQSPSTLSASIGDTRISCRASQSITGGWLAWYHQRPGKAPRLLIYRG
5 SRLVGGVPSRFSGSAAGTDFTLTIGNLQAEDFGTFYCQQYDTPGTFGQGKVEV
KRTVAAPSVF (k62; SEQ ID NO:22);

DIQMTQSPSTLSASVGDTRISCRASQSITGGWLAWYHQRPGKAPRLLIYR
GSRLLLGGVPSRFSGSAAGADFTLTIANLQAEDFGTFYCQQYDTPGTFGQGKVE
VKRTVAAPSVF (k18; SEQ ID NO:23);

10 DIQMTQSPSTLSASIGDTRMISCRASQSITGGWLAWYHQRPGKAPRLLIYR
GSKLLGGVPSRFSGSAAGTGFTLTIGNLQAEDFGTFYCQQYDTPGTFGQGKVE
VKRTVAAPSVF (k53; SEQ ID NO:24);

DIQMTQSPSTLSASIGDTRISCRASQSITGNWVAWYHQRPGKAPRLLIYR
GAALLGGVPSRFSGSAAGTDFTLTIGNLQAEDFGTFYCQQYDTPGTFGQGKVE
15 VKRTVAAPSVF (k61; SEQ ID NO:25);

DIQMTQSPSTLSASVGGTTRISCRASQSITGGWLAWYHQRPGKAPRLLIYR
GSRLLLGGVPSRFSGSAAGTDFTLTIANLQAEDFGTFYCQQYDTPGTFGQGKVE
VKRTVAAPSVF (k11; SEQ ID NO:26);

DIQMTQSPSTLSASVGDTRISCRASQSITGGWLAWYHQRPGKAPRLLIYR
20 GSRLLLGGVPSRFSGSAAGTGFTLTIANLQAEDFGTFYCQQYDTPGTFGQGKVE
VKRTVAAPSVF (k19; SEQ ID NO:27);

DIQMTQSPSTLSASIGDTRISCRASQSITGGWVAWYHQRPGKAPRLLIYR
GSRLLLGGVPSRFSGSAAGTDFTLTIGNLQAEDFGTFYCQQYDTPGTFGQGKVE
VKRTVAAPSVF (k81; SEQ ID NO:28)

25 DIQMTQSPSTLSASIGDTRISCRASQSITGNWVAWYQQRPGKAPRLLIYR
GAALLGGVPSRFSGSAAGTDFTLTIGNLQAEDFGTFYCQQYDTPGTFGQGKVE
VKRTVAAPSVF (3040LC; SEQ ID NO:29);

DIQMTQSPSTLSASVGD TVRISCRASQSITGGW LAWYHQRPGKAPRL LIYR
 GSRL LGGVPSRFRGSAAGTDFTLTIANLQAEDFGTFYCQQYDTYPGTFGQG GTKVE
 VKRTVAAPSVF (3430LC; SEQ ID NO:30);

DIQMTQSPSTLSASIGD TVRISCRASQSITGNWVAWYQQRPGKAPRL LIYR
 5 GAALLGGVPSRFRGSAAGTDFTLTIGNLQAEDFGTFYCQQYDTYPGTFGQG GTKVE
 VKRTVAAPSVF (3484LC; SEQ ID NO:31);

DIQMTQSPSTLSASIGD TVRISCRASQSITGNWVAWYQQRPGKAPRL LIYR
 GAALLGGVPSRFRGSAAGTDFTLTIGNLQTEDFGTFYCQQYDTYPGTFGQG GTKVE
 VKRTVAAPSVF (3044LC; SEQ ID NO:32); and

10 DIQMTQSPSTLSASIGD TVRISCRASQSITGGW LAWYHQRPGKAPRL LIYRG
 SRL LGGVPSRFRGSAAGTDFTLTIANLQAEDFGTFYCQQYDTYPGTFGQG GTKVEV
 KRTVAAPSVF (3630LC; SEQ ID NO:33).

Accordingly, one aspect of this invention features an isolated polypeptide comprising the sequence of any one of SEQ ID NOs: 1-33. The invention also provides an
 15 isolated anti-HIV antibody comprising one or both of a heavy chain comprising the sequence of any one of SEQ ID NOs: 1-18 and a light chain comprising the sequence any one of SEQ ID NOs: 19-33.

The above-mentioned antibody can be a human antibody, a chimeric antibody, or a humanized antibody. It can be an IgG1, IgG2, IgG3, or IgG4. The antibodies of the
 20 invention recognize the epitope on the HIV-1 envelope spike recognized by 8ANC195 and are broadly neutralizing.

In another aspect, the invention provides an isolated nucleic acid encoding the isolated polypeptide or anti-HIV-1 antibody described above. Also provided are a vector comprising the nucleic acid and a cultured cell comprising the nucleic acid.

25 In another aspect, the invention provides a composition comprising at least one of the above-described isolated polypeptide or anti-HIV-1 antibody or a fragment thereof. In one embodiment, the composition comprises a pharmaceutically acceptable carrier.

In another aspect, the invention provides a method of preventing or treating an HIV-1 infection or an HIV-related disease. The method includes steps of identifying a

patient in need of such prevention or treatment, and administering to the patient a first therapeutic agent comprising a therapeutically effective amount of at least one of the above-described isolated polypeptide or anti-HIV-1 antibody. The method can further comprise administering a second therapeutic agent, such as an antiviral agent.

5 In another embodiment, the present invention provides an isolated antigen comprising an epitope-scaffold that mimics the HIV-1 envelope spike epitope of broadly neutralizing antibody 8ANC195. In one aspect, the epitope-scaffold comprises a discontinuous epitope and a scaffold. In another aspect, the epitope is derived from HIV-1 gp120 and gp41, and at least part of the scaffold is not derived from gp120 or gp41. In
10 another aspect, the discontinuous epitope comprises amino acids corresponding to amino acid numbers 44-47, 90-94, 97, 234, 236-238, 240, 274-278, 352-354, 357, 456, 463, 466, 487, and 625-641 of gp140 from HIV strain 93TH057 numbered using standard numbering for HIV strain HXBC2. The amino acids corresponding to amino acid numbers 234 and 276 may be glycosylated.

15 In another aspect, the invention provides an isolated nucleic acid encoding the isolated antigen described above. Also provided are a vector comprising the nucleic acid and a cultured cell comprising the nucleic acid.

 In another aspect, the invention provides a composition comprising the isolated antigen. In one embodiment, the composition further comprises a pharmaceutically
20 acceptable carrier. In one embodiment, the composition further comprises an adjuvant.

 In another aspect, the present invention provides a method for generating an immune response in a subject in need thereof, comprising administering to said subject a composition comprising the above-described isolated antigen in an amount effective to generate an immune response.

25 In another aspect, the invention provides a method of preventing or treating an HIV-1 infection or an HIV-related disease. The method includes steps of identifying a patient in need of such prevention or treatment, and administering to the patient a first therapeutic agent comprising a therapeutically effective amount of the above-described antigen. The method can further comprise administering a second therapeutic agent, such
30 as an antiviral agent.

 In another aspect, the present invention provides a method for detecting or isolating an HIV-1 binding antibody in a subject comprising obtaining a biological sample

from the subject, contacting the sample with the above-described antigen, and conducting an assay to detect or isolate an HIV-1 binding antibody.

The details of one or more embodiments of the invention are set forth in the description below. Other features, objects, and advantages of the invention will be
 5 apparent from the description and from the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

Figs. 1A and B illustrates alignments of (A) VH and (B) VL sequences of mature 8ANC195 and its putative germ-line progenitor (GL). 8ANC195 HC has the sequence of SEQ ID NO:34. 8ANC195 LC has the sequence of SEQ ID NO:35. GL HC has the
 10 sequence of SEQ ID NO:36. GL LC has the sequence of SEQ ID NO:37. Residues forming the CDR loops are labeled (CDR1), (CDR2) and (CDR3). 8ANC195 is one of the most heavily mutated bNAbs isolated to date, with 49 of 103 amino acid mutations in the HC and 25 of 90 in the LC. The HC was too highly somatically mutated to accurately assign D and J gene segments, but the LC showed sufficient homology to assign its J
 15 segment as IGKJ5*01.

Figs. 2A-C illustrates conformations of 8ANC195 CDRH1 and CDRH3 loops. (A) The hook-like conformation of CDRH1 is stabilized by burial of the hydrophobic Phe30HC side chain and hydrogen bonds within CDRH1 and with CDRH3 and FWR1 and FWR3 residues (Ala24HC and Asp73HC, respectively). (B) The complexed CDRH3
 20 conformation consists of a protruding loop (residues 95HC-100HC) and a small β -sheet subdomain (residues 100dHC-100kHC) stabilized by multiple hydrogen bonds within CDRH3 as well as with CDRH1 and CDRL3. A hydrogen bond between Tyr92LC and Gly100cHC stabilized the bifurcation of CDRH3 into its two subdomains. CDRH1 and CDRH3 loop backbone atoms are shown as sticks and side chains of residues important
 25 for stabilizing the loop conformations are shown as sticks (involved in direct contacts) or lines (backbone involved in contacts) (other side chains Tyr98HC, Lys100HC and Trp100aHC, are shown for clarity). (C) Comparison of CDRH3 loops in 8ANC195 and other anti-HIV-1 bNAbs. CDRH3 residues corresponding to 8ANC195HC residues 90-105 of NIH45-46 (PDB 3U7Y), PG16 (PDB 4DQO), PGT121 (PDB 4FQC) and PGT128
 30 (PDB 3TYG) are shown as C α traces.

Figs. 3A-E illustrates CD4 interactions with 8ANC195. (A) Superimposition of sCD4 D1D2/gp120 structures (ribbon diagrams) from complexes with 8ANC195, 17b (PDB 1GCI) and 21c PDB 3LQA). (B) Competition ELISA of 8ANC195 IgG binding to 93TH057 gp120 in the presence of increasing concentrations of potential competitors (sCD4, diamonds; J3 VHH, triangles; 3BNC60 Fab, squares; NIH45-46 Fab, circles). No competition was observed with small, single-Ig domain CD4-binding site ligands (sCD4, J3 VHH), but larger Fab fragments of CD4 binding site antibodies (3BNC60, NIH45-46) competed for binding. (C) In vitro assay comparing neutralization of YU2 by sCD4 (squares), 8ANC195 IgG (triangles), and an equimolar mixture of 8ANC195 and sCD4 (circles). (D) Packing of 8ANC195/sCD4/gp120 crystals. Several symmetry mates are shown as surface representations (8ANC195 HC; 8ANC195 LC; 93TH057 gp120; sCD4 D1D2). Areas where two complexes form crystal contacts are indicated. (E) *In vitro* assay comparing neutralization of YU2 by 8ANC195 IgG (squares), 3BNC60 IgG (triangles), and an 8ANC195 IgG mutant that lacks the FWR3 insertion (Ser77a-Pro77b-Pro77c-Ile77d) that results in the protruding “FWR3_{HC} thumb” (circles).

Figs. 4A-I illustrates surface area buried at interface of 8ANC195 Fab and gp120. Left panels, surface area buried on 8ANC195 Fab by (A) gp120 protein residues, (C) Asn276gp120 glycan or (E) Asn234gp120 glycan; right panels, surface area buried by 8ANC195 Fab on (B) gp120 protein residues, (D) Asn234gp120 glycan or (F) Asn276gp120 glycan. Atoms buried at these interfaces are shown as surface representations overlaid onto ribbon diagrams of 8ANC195 Fab and gp120 or stick representations of glycans. 8ANC195 Fab: HC; LC; CDRH1; CDRH2; CDRH3; gp120: inner domain; outer domain; loop D; loop V5; Asn234gp120 glycan: Asn276gp120 glycan. 2Fo-Fc annealed omit electron density maps (grey mesh, $\sigma=1$) used to build (E) Asn234_{gp120} glycan and (H) Asn276_{gp120} glycan. (I) Modeled fucose residue α 1-6-linked to the first *N*-acetylglucosamine residue of the Asn276_{gp120} glycan shows that the core fucose of a complex-type *N*-glycan could be accommodated by the 8ANC195. Glycan residues are shown as sticks, and gp120, 8ANC195 HC and CD4 are shown as surface representations.

Figs. 5A and B illustrates a comparison of glycan-dependent bNAbs. 8ANC195 is “bracketed” by two glycans (Asn234_{gp120} glycan; Asn276_{gp120} glycan) in the 8ANC195 Fab/gp120/sCD4 complex structure (left panels). For comparison, crystal structures of

PG16 (middle panels, PDB 4DQO) bound to a V1/V2 loop scaffold and PGT128 (right panels, PDB 3TYG) bound to a V3 loop scaffold are shown with (A) the antibody HCs aligned to the 8ANC195 HC or (B) an alternative view showing their interactions with bracketing glycans (for PG16: Asn160_{gp120} glycan/Asn172_{gp120} glycan; for PGT128:

5 Asn301_{gp120} glycan/Asn332_{gp120} glycan). The proteins are shown as ribbon diagrams and the glycans as stick representations.

Figs. 6A-C illustrates green EM refinement statistics. (A) Electron micrograph at 52,000x magnification and -0.8 μm defocus. (B) Reference-free 2D class averages of the SOSIP trimer in complex with 8ANC195 Fab showing various orientations. (C) Fourier
10 Shell Correlation (FSC) graph resulting from refinement. The resolution was determined as 18.7 Å at an FSC cut-off of 0.5.

Figs. 7A and B illustrates negative stain EM reconstruction of BG505 SOSIP.664 in complex with 8ANC195 Fab fit two ways. (A) When the gp120-8ANC195 Fab structure was fit into the EM density, the gp120 from the complex structure was displaced
15 slightly outwards in comparison to the gp120 in the SOSIP trimer structure. The HC and LC of the Fab are shown. The Asn234_{gp120} and Asn276_{gp120} glycans are shown as spheres. (B) Close up of the Fab-Env interface. The position of Asn637_{gp120} can be deduced from the position of the C-terminus of HR2, which corresponds to residue Gly664_{gp41}. This residue is in close proximity to the LC and the glycan at this position could interact with
20 the 8ANC195 Fab.

Figs. 8A-D illustrates EM reconstruction of 8ANC195 Fab/BG505 SOSIP.664 showing gp41 contacts. Top view of EM density with the X-ray structures of BG505 SOSIP.664 (PDB ID 4NCO; gp120, grey; gp41) and 8ANC195 Fab (HC; LC with a map contour level of 0.0176 (A) and 0.030 (B). Areas of contact between 8ANC195 and gp41
25 are marked with circles, those between 8ANC195 and gp120 with black circles. (C,D) Close-up of 8ANC195 LC and HR2 region in EM complex structure (HR2 coordinates in PDB 4NCO with presumptive sidechains for strain YU2 added to the polyalanine coordinates). (C) Fab is shown as a surface representation with highlights (CDRL1; CDRL2; CDRH3, and gp41 HR2 is shown as a ribbon diagram. The position of
30 Asn637_{gp41} was deduced from the position of the C-terminus of the SOSIP.664 trimer (Gly664_{gp41}). (D) 8ANC195 HC and LC residues (sticks) positioned to contact HR2,

with side chains of surface-exposed residues that vary between newly isolated 8ANC195 (γ/κ) variants shown as sticks.

Figs 9A-C relate to Single Cell Variants of 8ANC195. (A) Strategy of large scale single cell sorting. (B) IgH and IgL chain genes from isolated single cell variants of 8ANC195. Identical members are grouped together. The HC CDR3 of 8ANC195 has the sequence of SEQ ID NO:38. The HC CDR3 of 8ANC142 has the sequence of SEQ ID NO:39. The HC CDR3 of 8ANC3430 has the sequence of SEQ ID NO:40. The HC CDR3 of 8ANC3484 has the sequence of SEQ ID NO:41. The HC CDR3 of 8ANC3044 has the sequence of SEQ ID NO:42. The HC CDR3 of 8ANC3630 has the sequence of SEQ ID NO:43. The LC CDR3 of 8ANC195 has the sequence of SEQ ID NO:44. The LC CDR3 of 8ANC142 has the sequence of SEQ ID NO:45. The LC CDR3 of 8ANC3430 has the sequence of SEQ ID NO:46. The LC CDR3 of 8ANC3484 has the sequence of SEQ ID NO:47. The LC CDR3 of 8ANC3044 has the sequence of SEQ ID NO:48. The LC CDR3 of 8ANC3630 has the sequence of SEQ ID NO:49. (C) IC₅₀ neutralization titers of distinct single cell versions of the 8ANC195 clone compared to 8ANC195 against a 15 virus Tier 2 panel.

Figs 10A and B depict the alignment of amino acid sequences of all distinct single cell versions of the 8ANC195 clone. HC (A) and LC (B) sequences were aligned with the respective germline genes. Mutations introduced by somatic hypermutation are indicated.

Figs. 11A-C are directed to Bulk Sorted Variants of 8ANC195. (A) Strategy of bulk memory B cell sorting without antigen. (B) PCR strategy for the amplification of 8ANC195 HC and LC clone members. Shown are the priming sites aligned with the original nucleotide sequence of 8ANC195 at the respective sites. Mismatches with the respective germline genes are indicated. Primers 1 and 2 for the 8ANC195 HC FWR1 have SEQ ID Nos: 50 and 51, respectively. Primers 1 and 2 for the 8ANC195 LC FWR1 have SEQ ID Nos: 52 and 53, respectively. The primer for the 8ANC195 HC J-gene has SEQ ID NO:54. Primers 1 and 2 for the 8ANC195 LC J-gene have SEQ ID Nos: 55 and 56, respectively. (C) Phylogenetic tree of 128 isolated HC and 100 LC sequences. Representative members chosen for alignment are indicated.

Figs. 12A and B depict alignment of amino acid sequences of selected bulk sorted versions of the 8ANC195 clone. HC (A) and LC (B) sequences were aligned with the

respective germline genes as well as the original 8ANC195 sequence. All mutations introduced by somatic hypermutation are indicated.

Figs. 13A and B show that $\kappa 52_{\text{HC}} \kappa 5_{\text{LC}}$ is more potent than 8ANC195. IC_{50} values of $\kappa 52_{\text{HC}} \kappa 5_{\text{LC}}$ and 8ANC195 against Tier 2 15 virus panel shown as dot plot (A) and Table (B). NT, not tested.

Figs. 14A-C show that somatic mutations in the 8ANC195 LC CDRs and FWRs could affect contacts with gp41. (A) Surface representation of 8ANC195 Fab (HC; LC; somatically mutated, surface-exposed LC residues; residue 64_{LC}). (B) Surface representation of 8ANC195 Fab and BG505 gp41 HR2 with a modeled Man6 sugar attached to Asn637gp41. (C) Surface representation of 8ANC195 Fab (CDRL1; CDRL2; CDRH3; residue 64_{LC}) and BG505 gp41 HR2 with a modeled Man6 sugar attached to Asn637gp41.

Fig. 15 illustrates locations of bNAbs epitopes on HIV-1 Env Trimer. EM density map of Env trimer including MPER region showing approximate epitope locations for antibodies targeting the 8ANC195 epitope, CD4 binding site, V3 loop/Asn332 glycan (332 glycan shown as spheres), V1/V2 loop/Asn160 glycan (160 glycan shown as spheres), and MPER.

Figs. 16A and C illustrate crystal structures of 8ANC195 Fab and 8ANC195/gp120/sCD4 complex. (A) Superimposition of unbound and bound (HC and LC) structures of 8ANC195 Fab shown as ribbon diagrams. CDR loops are highlighted (CDRH1/CDRL1; CDRH2/CDRL2; CDRH3; CDRL3) and a “thumb”-like loop formed by an insertion in FWR3 is indicated. Disordered loops are shown as dashed lines. (B) Space-filling model (inset) and ribbon diagram of ternary complex of 8ANC195 (HC and LC), sCD4, and 93TH057 gp120 core (inner domain; outer domain; bridging sheet; loop D; loop V5; CD4 binding loop). Ordered glycans attached to Asn234_{gp120} and Asn276_{gp120} are shown as sticks. Fab CDR loops are indicated as in (A). sCD4 was omitted from the right panel for clarity. (C) Approximate locations of bNAbs epitopes on a surface representation of the gp120 core. The epitopes of V3 and V1/V2 antibodies include regions of loops (dotted lines) not present in the gp120 core structure. CD4 binding site and 8ANC195 epitopes are outlined by black (CD4 binding site) and (8ANC195) dots. Glycans included in the 8ANC195 epitope are indicated. Subdomains of gp120 are indicated as in (B).

Figs. 17A-E show contacts made by 8ANC195 HC with gp120 protein residues and glycans. Labels for gp120 protein and glycan residues are italicized. Hydrogen bonds are shown as dashed lines. (A) FWR3_{HC} loop contacts with loop D, loop V5, and outer domain loop. (B) 8ANC195 HC CDRH1 and CDRH3 contacts with gp120 inner domain. (C) Buried surface area between the Asn234_{gp120} glycan (transparent surface with glycan residues shown as sticks) and 8ANC195 (HC FWR residues and CDRH2 are indicated). Antibody atoms buried by glycan interactions are shown as surfaces. (D) Buried surface area between the Asn276_{gp120} glycan (transparent surface with glycan residues shown as sticks) and 8ANC195 (HC FWR residues and CDRH1 are indicated). Antibody atoms buried by glycan interactions are shown as surfaces. (E) Top: Contacts made by 8ANC195 HC FWR residues and CDRH2 with Asn234_{gp120} glycan. Glycan and protein residues involved in hydrogen bonds are shown as sticks. Bottom: schematic of ordered high mannose glycans on Asn234_{gp120} and Asn276_{gp120} (bottom).

Figs. 18A and B show the EM structure of 8ANC195/Env trimer complex and model of 8ANC195 LC interactions with gp41 HR2. (A) EM reconstruction of 8ANC195 Fab/BG505 SOSIP.664. Side (left) and top (right) views of EM density with the X-ray structures of BG505 SOSIP.664 (PDB ID 4NCO; gp120, gp41) and 8ANC195 Fab fit in two ways: (i) fitting 8ANC195 Fab independently of gp140 coordinates to the EM density (best fit/independently placed), and (ii) by aligning the gp120 of the gp120/8ANC195 complex structure onto the gp120 of PDB 4NCO fit to the EM density. (B) Close-up of 8ANC195 LC/HR2 region of EM complex structure (Fab placement is best fit/independently placed as in (A)). Left: Fab is shown as a surface representation with highlights (CDRL1; CDRL2; CDRH1; CDRH3), and gp140 is shown as a ribbon diagram (gp120; gp41). The position of Asn637_{gp41} was deduced from the position of the C-terminus of the SOSIP.664 trimer (Gly664_{gp41}). Right: 8ANC195 HC and LC residues (sticks) positioned to contact HR2, which is shown as a surface representation calculated from HR2 coordinates in PDB 4NCO with presumptive sidechains added to the polyalanine coordinates.

Figs 19A-C show effects of LC sequence changes on 8ANC195 neutralization potency. (A) Sequences of LC CDRs in constructs used with 8ANC195 HC to make chimeric IgGs (left) and location of CDRs on 8ANC195 structure (right). Sequences derived from the mature antibody are shown and those derived from the germline

precursor are shown on a grey background. The mutations introduced into CDRL3 in glCDRL3Ala are shown on a white background. (B) Effects of changes in 8ANC195 LC on binding to 93TH057 and YU2 gp120s and neutralization of viral strains, expressed as fold changes over results for 8ANC195 IgG. KD and IC₅₀ values for these experiments are shown in table S3. (C) Heat map showing the expression and neutralization of randomly paired HCs and LCs from the bulk sort on a Tier 2 15-virus panel. Average IC₅₀ values (arithmetic means) between 0.1 and 2 µg/ml; between 2.1 and 10 µg/ml, between 10.1 and 14.9 µg/ml, and above 15 µg/ml are indicated with varying degrees of shaded squares. Empty squares represent insufficient antibody expression.

Fig. 20 depicts the alignment of gp140 sequences from HIV strains HXBC2 and 93TH057 using standard HXBC2 numbering of amino acid residues. Amino acid residues contacted by 8ANC195 in the complex crystal structure with 93TH057 gp120 core are indicated on the 93TH057 sequence. Glycans contacted by 8ANC195 in the complex crystal structure with 93TH057 gp120 core are shown as the asparagine residues to which they are attached, highlighted in cyan on the 93TH057 sequence. The region of gp41 contacted by 8ANC195 based on the EM complex structure is indicated. Select glycans are shown as diagrams on the asparagine residues to which they are attached.

DETAILED DESCRIPTION OF THE INVENTION

The present invention is based, at least in part, on the identification of the epitope recognized by 8ANC195, a broadly neutralizing antibody to the HIV-1 envelope glycoprotein. The present invention, in one embodiment, provides an isolated antigen comprising the epitope, compositions comprising the antigen, and methods of using the antigen. In other embodiments, the present invention provides isolated anti-HIV-1 antibodies that recognize the epitope on the HIV-1 envelope spike recognized by 8ANC195, compositions comprising the antibodies, and methods of using the antibodies.

In one embodiment, the present invention is directed to an isolated anti-HIV antibody comprising one or both of a heavy chain comprising the sequence of any one of SEQ ID NOs: 1-18 and a light chain comprising the sequence any one of of SEQ ID NOs: 19-33. In one preferred embodiment, the heavy chain comprises the sequence of SEQ ID NO:1 and the light chain comprises the sequence of SEQ ID NO:20. In another

embodiment, the present invention provides an isolated polypeptide comprising the sequence of any one of SEQ ID NOs: 1-33.

The above-mentioned antibody can be a human antibody, a chimeric antibody, or a humanized antibody. It can be an IgG1, IgG2, IgG3, or IgG4. The antibodies of the invention recognize the epitope on the HIV-1 envelope spike recognized by 8ANC195 and are broadly neutralizing. 8ANC195 is known in the art and disclosed, for example, by Scheid et al., *Science*, 333, 1633 (2011). The heavy chain of 8ANC195 has the sequence of SEQ ID NO:34 and the light chain of 8ANC195 has the sequence of SEQ ID NO:35.

The term "antibody" (Ab) as used herein includes monoclonal antibodies, polyclonal antibodies, multispecific antibodies (for example, bispecific antibodies and polyreactive antibodies), and antibody fragments. Thus, the term "antibody" as used in any context within this specification is meant to include, but not be limited to, any specific binding member, immunoglobulin class and/or isotype (e.g., IgG1, IgG2, IgG3, IgG4, IgM, IgA, IgD, IgE and IgM); and biologically relevant fragment or specific binding member thereof, including but not limited to Fab, F(ab')₂, Fv, and scFv (single chain or related entity). It is understood in the art that an antibody is a glycoprotein comprising at least two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds, or an antigen binding portion thereof. A heavy chain is comprised of a heavy chain variable region (VH) and a heavy chain constant region (CH1, CH2 and CH3). A light chain is comprised of a light chain variable region (VL) and a light chain constant region (CL). The variable regions of both the heavy and light chains comprise framework regions (FWR) and complementarity determining regions (CDR). The four FWR regions are relatively conserved while CDR regions (CDR1, CDR2 and CDR3) represent hypervariable regions and are arranged from NH₂ terminus to the COOH terminus as follows: FWR1, CDR1, FWR2, CDR2, FWR3, CDR3, and FWR4. The variable regions of the heavy and light chains contain a binding domain that interacts with an antigen while, depending of the isotype, the constant region(s) may mediate the binding of the immunoglobulin to host tissues or factors.

Also included in the definition of "antibody" as used herein are chimeric antibodies, humanized antibodies, and recombinant antibodies, human antibodies generated from a transgenic non-human animal, as well as antibodies selected from libraries using enrichment technologies available to the artisan.

The term "variable" refers to the fact that certain segments of the variable (V) domains differ extensively in sequence among antibodies. The V domain mediates antigen binding and defines specificity of a particular antibody for its particular antigen. However, the variability is not evenly distributed across the 110-amino acid span of the variable regions. Instead, the V regions consist of relatively invariant stretches called framework regions (FRs) of 15-30 amino acids separated by shorter regions of extreme variability called "hypervariable regions" that are each 9-12 amino acids long. The variable regions of native heavy and light chains each comprise four FRs, largely adopting a beta sheet configuration, connected by three hypervariable regions, which form loops connecting, and in some cases forming part of, the beta sheet structure. The hypervariable regions in each chain are held together in close proximity by the FRs and, with the hypervariable regions from the other chain, contribute to the formation of the antigen-binding site of antibodies (see, for example, Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991)).

The term "hypervariable region" as used herein refers to the amino acid residues of an antibody that are responsible for antigen binding. The hypervariable region generally comprises amino acid residues from a "complementarity determining region" ("CDR").

The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. The term "polyclonal antibody" refers to preparations that include different antibodies directed against different determinants ("epitopes").

The monoclonal antibodies herein include "chimeric" antibodies in which a portion of the heavy and/or light chain is identical with, or homologous to, corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with, or homologous to, corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity (see, for example, U.S. Pat. No. 4,816,567; and Morrison et al., Proc. Natl. Acad. Sci. USA, 81:6851-6855 (1984)).

Chimeric antibodies include antibodies having one or more human antigen binding sequences (for example, CDRs) and containing one or more sequences derived from a non-human antibody, for example, an FR or C region sequence. In addition, chimeric antibodies included herein are those comprising a human variable region antigen binding
5 sequence of one antibody class or subclass and another sequence, for example, FR or C region sequence, derived from another antibody class or subclass.

A "humanized antibody" generally is considered to be a human antibody that has one or more amino acid residues introduced into it from a source that is non-human. These non-human amino acid residues often are referred to as "import" residues, which
10 typically are taken from an "import" variable region. Humanization may be performed following the method of Winter and co-workers (see, for example, Jones et al., *Nature* 321:522-525 (1986); Reichmann et al., *Nature* 332:323-327 (1988); Verhoeyen et al., *Science* 239:1534-1536 (1988)), by substituting import hypervariable region sequences for the corresponding sequences of a human antibody. Accordingly, such "humanized"
15 antibodies are chimeric antibodies (see, for example, U.S. Pat. No. 4,816,567), where substantially less than an intact human variable region has been substituted by the corresponding sequence from a non-human species.

An "antibody fragment" comprises a portion of an intact antibody, such as the antigen binding or variable region of the intact antibody. Examples of antibody fragments
20 include, but are not limited to, Fab, Fab', F(ab')₂, and Fv fragments; diabodies; linear antibodies (see, for example, U.S. Pat. No. 5,641,870; Zapata et al., *Protein Eng.* 8(10): 1057-1062 [1995]); single-chain antibody molecules; and multispecific antibodies formed from antibody fragments.

"Fv" is the minimum antibody fragment that contains a complete antigen-
25 recognition and antigen-binding site. This fragment contains a dimer of one heavy- and one light-chain variable region domain in tight, non-covalent association. From the folding of these two domains emanate six hypervariable loops (three loops each from the H and L chain) that contribute the amino acid residues for antigen binding and confer antigen binding specificity to the antibody. However, even a single variable region (or half of an
30 Fv comprising only three CDRs specific for an antigen) has the ability to recognize and bind antigen, although at a lower affinity than the entire binding site.

"Single-chain Fv" ("sFv" or "scFv") are antibody fragments that comprise the VH and VL antibody domains connected into a single polypeptide chain. The sFv polypeptide can further comprise a polypeptide linker between the VH and VL domains that enables the sFv to form the desired structure for antigen binding. For a review of sFv, see, for example, Pluckthun in *The Pharmacology of Monoclonal Antibodies*, vol. 113, Rosenberg and Moore eds., Springer-Verlag, New York, pp. 269-315 (1994); Borrebaeck 1995, *infra*.

The term "diabodies" refers to small antibody fragments prepared by constructing sFv fragments with short linkers (about 5-10 residues) between the VH and VL domains such that inter-chain but not intra-chain pairing of the V domains is achieved, resulting in a bivalent fragment, i.e., fragment having two antigen-binding sites. Bispecific diabodies are heterodimers of two "crossover" sFv fragments in which the VH and VL domains of the two antibodies are present on different polypeptide chains. Diabodies are described more fully in, for example, EP 404,097; WO 93/11161; and Hollinger et al., *Proc. Natl. Acad. Sci. USA*, 90:6444-6448 (1993).

Domain antibodies (dAbs), which can be produced in fully human form, are the smallest known antigen-binding fragments of antibodies, ranging from about 11 kDa to about 15 kDa. DAbs are the robust variable regions of the heavy and light chains of immunoglobulins (VH and VL, respectively). They are highly expressed in microbial cell culture, show favorable biophysical properties including, for example, but not limited to, solubility and temperature stability, and are well suited to selection and affinity maturation by in vitro selection systems such as, for example, phage display. DAbs are bioactive as monomers and, owing to their small size and inherent stability, can be formatted into larger molecules to create drugs with prolonged serum half-lives or other pharmacological activities. Examples of this technology have been described in, for example, WO9425591 for antibodies derived from Camelidae heavy chain Ig, as well in US20030130496 describing the isolation of single domain fully human antibodies from phage libraries.

Fv and sFv are the only species with intact combining sites that are devoid of constant regions. Thus, they are suitable for reduced nonspecific binding during in vivo use. sFv fusion proteins can be constructed to yield fusion of an effector protein at either the amino or the carboxy terminus of an sFv. See, for example, *Antibody Engineering*, ed. Borrebaeck, *supra*. The antibody fragment also can be a "linear antibody", for example, as

described in U.S. Pat. No. 5,641,870 for example. Such linear antibody fragments can be monospecific or bispecific.

In certain embodiments, antibodies of the described invention are bispecific or multi-specific. Bispecific antibodies are antibodies that have binding specificities for at least two different epitopes. Exemplary bispecific antibodies can bind to two different epitopes of a single antigen. Other such antibodies can combine a first antigen binding site with a binding site for a second antigen. Alternatively, an anti-HIV arm can be combined with an arm that binds to a triggering molecule on a leukocyte, such as a T-cell receptor molecule (for example, CD3), or Fc receptors for IgG (Fc gamma R), such as Fc gamma RI (CD64), Fc gamma RII (CD32) and Fc gamma RIII (CD16), so as to focus and localize cellular defense mechanisms to the infected cell. Bispecific antibodies also can be used to localize cytotoxic agents to infected cells. Bispecific antibodies can be prepared as full length antibodies or antibody fragments (for example, F(ab')₂ bispecific antibodies). For example, WO 96/16673 describes a bispecific anti-ErbB2/anti-Fc gamma RIII antibody and U.S. Pat. No. 5,837,234 discloses a bispecific anti-ErbB2/anti-Fc gamma RI antibody. For example, a bispecific anti-ErbB2/Fc alpha antibody is reported in WO98/02463; U.S. Pat. No. 5,821,337 teaches a bispecific anti-ErbB2/anti-CD3 antibody. See also, for example, Mouquet et al., Polyreactivity Increases The Apparent Affinity Of Anti-HIV Antibodies By Heterologation. NATURE. 467, 591-5 (2010).

Methods for making bispecific antibodies are known in the art. Traditional production of full length bispecific antibodies is based on the co-expression of two immunoglobulin heavy chain-light chain pairs, where the two chains have different specificities (see, for example, Millstein et al., Nature, 305:537-539 (1983)). Similar procedures are disclosed in, for example, WO 93/08829, Traunecker et al., EMBO J., 10:3655-3659 (1991) and see also; Mouquet et al., Polyreactivity Increases The Apparent Affinity Of Anti-HIV Antibodies By Heterologation. NATURE. 467, 591-5 (2010).

Alternatively, antibody variable regions with the desired binding specificities (antibody-antigen combining sites) are fused to immunoglobulin constant domain sequences. The fusion is with an Ig heavy chain constant domain, comprising at least part of the hinge, CH₂, and CH₃ regions. According to some embodiments, the first heavy-chain constant region (CH₁) containing the site necessary for light chain bonding, is present in at least one of the fusions. DNAs encoding the immunoglobulin heavy chain

fusions and, if desired, the immunoglobulin light chain, are inserted into separate expression vectors, and are co-transfected into a suitable host cell. This provides for greater flexibility in adjusting the mutual proportions of the three polypeptide fragments in embodiments when unequal ratios of the three polypeptide chains used in the construction provide the optimum yield of the desired bispecific antibody. It is, however, possible to insert the coding sequences for two or all three polypeptide chains into a single expression vector when the expression of at least two polypeptide chains in equal ratios results in high yields or when the ratios have no significant affect on the yield of the desired chain combination.

Techniques for generating bispecific antibodies from antibody fragments also have been described in the literature. For example, bispecific antibodies can be prepared using chemical linkage. For example, Brennan et al., Science, 229: 81 (1985) describe a procedure wherein intact antibodies are proteolytically cleaved to generate F(ab')₂ fragments. These fragments are reduced in the presence of the dithiol complexing agent, sodium arsenite, to stabilize vicinal dithiols and prevent intermolecular disulfide formation. The Fab' fragments generated then are converted to thionitrobenzoate (TNB) derivatives. One of the Fab'-TNB derivatives then is reconverted to the Fab'-thiol by reduction with mercaptoethylamine and is mixed with an equimolar amount of the other Fab'-TNB derivative to form the bispecific antibody. The bispecific antibodies produced can be used as agents for the selective immobilization of enzymes.

Other modifications of the antibody are contemplated herein. For example, the antibody can be linked to one of a variety of nonproteinaceous polymers, for example, polyethylene glycol, polypropylene glycol, polyoxyalkylenes, or copolymers of polyethylene glycol and polypropylene glycol. The antibody also can be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization (for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacrylate)microcapsules, respectively), in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules), or in macroemulsions. Such techniques are disclosed in, for example, Remington's Pharmaceutical Sciences, 16th edition, Oslo, A., Ed., (1980).

Typically, the antibodies of the described invention are produced recombinantly, using vectors and methods available in the art. Human antibodies also can be generated

by in vitro activated B cells (see, for example, U.S. Pat. Nos. 5,567,610 and 5,229,275). General methods in molecular genetics and genetic engineering useful in the present invention are described in the current editions of *Molecular Cloning: A Laboratory Manual* (Sambrook, et al., 1989, Cold Spring Harbor Laboratory Press), *Gene Expression Technology* (Methods in Enzymology, Vol. 185, edited by D. Goeddel, 1991. Academic Press, San Diego, CA), "Guide to Protein Purification" in *Methods in Enzymology* (M.P. Deutscher, ed., (1990) Academic Press, Inc.); *PCR Protocols: A Guide to Methods and Applications* (Innis, et al. 1990. Academic Press, San Diego, CA), *Culture of Animal Cells: A Manual of Basic Technique*, 2nd Ed. (R.I. Freshney. 1987. Liss, Inc. New York, NY), and *Gene Transfer and Expression Protocols*, pp. 109-128, ed. E.J. Murray, The Humana Press Inc., Clifton, N.J.). Reagents, cloning vectors, and kits for genetic manipulation are available from commercial vendors such as BioRad, Stratagene, Invitrogen, ClonTech and Sigma-Aldrich Co.

Human antibodies also can be produced in transgenic animals (for example, mice) that are capable of producing a full repertoire of human antibodies in the absence of endogenous immunoglobulin production. For example, it has been described that the homozygous deletion of the antibody heavy-chain joining region (JH) gene in chimeric and germ-line mutant mice results in complete inhibition of endogenous antibody production. Transfer of the human germ-line immunoglobulin gene array into such germ-line mutant mice results in the production of human antibodies upon antigen challenge. See, for example, Jakobovits et al., *Proc. Natl. Acad. Sci. USA*, 90:2551 (1993); Jakobovits et al., *Nature*, 362:255-258 (1993); Bruggemann et al., *Year in Immuno.*, 7:33 (1993); U.S. Pat. Nos. 5,545,806, 5,569,825, 5,591,669 (all of GenPharm); U.S. Pat. No. 5,545,807; and WO 97/17852. Such animals can be genetically engineered to produce human antibodies comprising a polypeptide of the described invention.

Various techniques have been developed for the production of antibody fragments. Traditionally, these fragments were derived via proteolytic digestion of intact antibodies (see, for example, Morimoto et al., *Journal of Biochemical and Biophysical Methods* 24:107-117 (1992); and Brennan et al., *Science*, 229:81 (1985)). However, these fragments can now be produced directly by recombinant host cells. Fab, Fv and ScFv antibody fragments can all be expressed in and secreted from *E. coli*, thus allowing the facile production of large amounts of these fragments. Fab'-SH fragments can be directly

recovered from *E. coli* and chemically coupled to form F(ab')₂ fragments (see, for example, Carter et al., *Bio/Technology* 10:163-167 (1992)). According to another approach, F(ab')₂ fragments can be isolated directly from recombinant host cell culture. Fab and F(ab')₂ fragment with increased in vivo half-life comprising a salvage receptor binding epitope residues are described in U.S. Pat. No. 5,869,046. Other techniques for the production of antibody fragments will be apparent to the skilled practitioner.

Other techniques that are known in the art for the selection of antibody fragments from libraries using enrichment technologies, including but not limited to phage display, ribosome display (Hanes and Pluckthun, 1997, *Proc. Nat. Acad. Sci.* 94: 4937-4942), bacterial display (Georgiou, et al., 1997, *Nature Biotechnology* 15: 29-34) and/or yeast display (Kieck, et al., 1997, *Protein Engineering* 10: 1303-1310) may be utilized as alternatives to previously discussed technologies to select single chain antibodies. Single-chain antibodies are selected from a library of single chain antibodies produced directly utilizing filamentous phage technology. Phage display technology is known in the art (e.g., see technology from Cambridge Antibody Technology (CAT)) as disclosed in U.S. Patent Nos. 5,565,332; 5,733,743; 5,871,907; 5,872,215; 5,885,793; 5,962,255; 6,140,471; 6,225,447; 6,291,650; 6,492,160; 6,521,404; 6,544,731; 6,555,313; 6,582,915; 6,593,081, as well as other U.S. family members, or applications which rely on priority filing GB 9206318, filed 24 May 1992; see also Vaughn, et al. 1996, *Nature Biotechnology* 14: 309-314). Single chain antibodies may also be designed and constructed using available recombinant DNA technology, such as a DNA amplification method (e.g., PCR), or possibly by using a respective hybridoma cDNA as a template.

Variant antibodies also are included within the scope of the invention. Thus, variants of the sequences recited in the application also are included within the scope of the invention. Further variants of the antibody sequences having improved affinity can be obtained using methods known in the art and are included within the scope of the invention. For example, amino acid substitutions can be used to obtain antibodies with further improved affinity. Alternatively, codon optimization of the nucleotide sequence can be used to improve the efficiency of translation in expression systems for the production of the antibody.

The present invention provides for antibodies, either alone or in combination with other antibodies, such as, but not limited to, VRC01, anti-V3 loop, CD4bs, and CD4i

antibodies as well as PG9/PG16-like antibodies, that have broad neutralizing activity in serum.

The present invention also relates to isolated polypeptides comprising the amino acid sequences of the light chains and heavy chains of the antibodies of the invention. In
5 one embodiment, the isolated polypeptide comprises the sequence of any one of SEQ ID NOs: 1-33.

The term "polypeptide" is used in its conventional meaning, i.e., as a sequence of amino acids. The polypeptides are not limited to a specific length of the product. Peptides, oligopeptides, and proteins are included within the definition of polypeptide, and
10 such terms can be used interchangeably herein unless specifically indicated otherwise. This term also includes post-expression modifications of the polypeptide, for example, glycosylations, acetylations, phosphorylations and the like, as well as other modifications known in the art, both naturally occurring and non-naturally occurring. A polypeptide can be an entire protein, or a subsequence thereof.

15 A polypeptide "variant," as the term is used herein, is a polypeptide that typically differs from a polypeptide specifically disclosed herein in one or more substitutions, deletions, additions and/or insertions. Such variants can be naturally occurring or can be synthetically generated, for example, by modifying one or more of the above polypeptide sequences of the invention and evaluating one or more biological activities of the
20 polypeptide as described herein and/or using any of a number of techniques well known in the art.

For example, certain amino acids can be substituted for other amino acids in a protein structure without appreciable loss of its ability to bind other polypeptides (for example, antigens) or cells. Since it is the binding capacity and nature of a protein that
25 defines that protein's biological functional activity, certain amino acid sequence substitutions can be made in a protein sequence, and, accordingly, its underlying DNA coding sequence, whereby a protein with like properties is obtained. It is thus contemplated that various changes can be made in the peptide sequences of the disclosed compositions, or corresponding DNA sequences that encode said peptides without
30 appreciable loss of their biological utility or activity.

In many instances, a polypeptide variant will contain one or more conservative substitutions. A "conservative substitution" is one in which an amino acid is substituted

for another amino acid that has similar properties, such that one skilled in the art of peptide chemistry would expect the secondary structure and hydrophobic nature of the polypeptide to be substantially unchanged.

Amino acid substitutions generally are based on the relative similarity of the amino acid side-chain substituents, for example, their hydrophobicity, hydrophilicity, charge, size, and the like. Exemplary substitutions that take various of the foregoing characteristics into consideration are well known to those of skill in the art and include: arginine and lysine; glutamate and aspartate; serine and threonine; glutamine and asparagine; and valine, leucine and isoleucine.

In another embodiment, the present invention provides an isolated antigen comprising an epitope-scaffold that mimics the HIV-1 envelope spike epitope of broadly neutralizing antibody 8ANC195. On one embodiment, the epitope-scaffold comprises a discontinuous epitope and a scaffold, wherein the epitope is derived from HIV-1 gp120 and gp41, and wherein at least part of the scaffold is not derived from gp120 or gp41. In one embodiment, the discontinuous epitope comprises amino acids corresponding to amino acid numbers 44-47, 90-94, 97, 234, 236-238, 240, 274-278, 352-354, 357, 456, 463, 466, 487, and 625-641 of gp140 from HIV strain 93TH057 numbered using standard numbering for HIV strain HXBc2 as depicted in Fig. 20 and disclosed by Korber et al. (1998, Numbering positions in HIV relative to HXBc2, p. III-102-IV-103. *In* B. Korber, C. L. Kuiken, B. Foley, B. Hahn, F. McCutchan, J. W. Mellors, and J. Sodroski (ed.), Human retroviruses and AIDS. Los Alamos National Laboratories, Los Alamos, N.Mex.). In another embodiment, the amino acids corresponding to amino acid numbers 234 and 276 are glycosylated.

Methods of making epitope-scaffolds are known in the art and disclosed, for example, by Correia et al. *Journal of Molecular Biology* 405, 284 (2011), Correia et al. *Structure* 18, 1116 (2010), Ofek et al. *Proc Natl Acad Sci USA* 107, 17780 (2010), McLellan et al. *J Mol Biol* 409, 853 (2011), Azoitei et al. *Science* 334, 373 (2011) and in US2010/0068217. Briefly, information obtained from the crystallographic analysis disclosed herein is used to design epitope-scaffolds that mimic the 8ANC195 epitope on the HIV-1 envelope spike. First, computational methods are utilized to identify non-HIV scaffold proteins capable of supporting the discontinuous epitope identified herein. Epitope-scaffolds are then designed and produced, and their immunological properties are

characterized. For example, in the method of Azoitei et al., the Protein Data Bank (www.pdb.org) is searched for suitable scaffolds for the discontinuous epitope, for example by using an algorithm such as Multigraft Match. An algorithm such as Multigraft Design disclosed by Azoitei et al. is used for scaffold design in which regions of the scaffold are deleted and new segments are built to connect the epitope to the scaffold. Candidate epitope-scaffolds may be expressed in a host cell and purified, and tested for binding to 8ANC195 or another antibody that binds to the epitope recognized by 8ANC195.

The invention also includes isolated nucleic acid sequences encoding part or all of the light and heavy chains of the described inventive antibodies, and fragments thereof. Due to redundancy of the genetic code, variants of these sequences will exist that encode the same amino acid sequences. In one embodiment, the present invention provides an isolated nucleic acid encoding a polypeptide having the sequence of any one of SEQ ID Nos:1-33. In another embodiment, the isolated nucleic acid encodes an antibody comprising a heavy chain comprising the sequence of any one of SEQ ID Nos: 1-18 and a light chain comprising the sequence of any one of SEQ ID Nos:19-33.

The invention also includes isolated nucleic acid sequences that encode the antigen comprising the epitope-scaffold of the invention.

The terms "nucleic acid" and "polynucleotide" are used interchangeably herein to refer to single-stranded or double-stranded RNA, DNA, or mixed polymers. Polynucleotides can include genomic sequences, extra-genomic and plasmid sequences, and smaller engineered gene segments that express, or can be adapted to express polypeptides.

An "isolated nucleic acid" is a nucleic acid that is substantially separated from other genome DNA sequences as well as proteins or complexes such as ribosomes and polymerases, which naturally accompany a native sequence. The term encompasses a nucleic acid sequence that has been removed from its naturally occurring environment, and includes recombinant or cloned DNA isolates and chemically synthesized analogues or analogues biologically synthesized by heterologous systems. A substantially pure nucleic acid includes isolated forms of the nucleic acid. Accordingly, this refers to the nucleic acid as originally isolated and does not exclude genes or sequences later added to the isolated nucleic acid by the hand of man.

A polynucleotide "variant," as the term is used herein, is a polynucleotide that typically differs from a polynucleotide specifically disclosed herein in one or more substitutions, deletions, additions and/or insertions. Such variants can be naturally occurring or can be synthetically generated, for example, by modifying one or more of the polynucleotide sequences of the invention and evaluating one or more biological activities of the encoded polypeptide as described herein and/or using any of a number of techniques well known in the art.

Modifications can be made in the structure of the polynucleotides of the described invention and still obtain a functional molecule that encodes a variant or derivative polypeptide with desirable characteristics. When it is desired to alter the amino acid sequence of a polypeptide to create an equivalent, or even an improved, variant or portion of a polypeptide of the invention, one skilled in the art typically will change one or more of the codons of the encoding DNA sequence.

Typically, polynucleotide variants contain one or more substitutions, additions, deletions and/or insertions, such that the immunogenic binding properties of the polypeptide encoded by the variant polynucleotide is not substantially diminished relative to a polypeptide encoded by a polynucleotide sequence specifically set forth herein.

In some embodiments, the polypeptide encoded by the polynucleotide variant or fragment has the same binding specificity (i.e., specifically or preferentially binds to the same epitope or HIV strain) as the polypeptide encoded by the native polynucleotide. In some embodiments, the described polynucleotides, polynucleotide variants, fragments and hybridizing sequences, encode polypeptides that have a level of binding activity of at least about 50%, at least about 70%, and at least about 90% of that for a polypeptide sequence specifically set forth herein.

The polynucleotides of the described invention, or fragments thereof, regardless of the length of the coding sequence itself, can be combined with other DNA sequences, such as promoters, polyadenylation signals, additional restriction enzyme sites, multiple cloning sites, other coding segments, and the like, such that their overall length can vary considerably. A nucleic acid fragment of almost any length is employed. For example, illustrative polynucleotide segments with total lengths of about 10000, about 5000, about 3000, about 2000, about 1000, about 500, about 200, about 100, about 50 base pairs in

length, and the like, (including all intermediate lengths) are included in many implementations of this invention.

Further included within the scope of the invention are vectors such as expression vectors, comprising a nucleic acid sequence according to the invention. Cells transformed
5 with such vectors also are included within the scope of the invention.

The present invention also provides vectors and host cells comprising a nucleic acid of the invention, as well as recombinant techniques for the production of a polypeptide of the invention. Vectors of the invention include those capable of replication in any type of cell or organism, including, for example, plasmids, phage, cosmids, and
10 mini chromosomes. In some embodiments, vectors comprising a polynucleotide of the described invention are vectors suitable for propagation or replication of the polynucleotide, or vectors suitable for expressing a polypeptide of the described invention. Such vectors are known in the art and commercially available.

"Vector" includes shuttle and expression vectors. Typically, the plasmid construct
15 also will include an origin of replication (for example, the ColE1 origin of replication) and a selectable marker (for example, ampicillin or tetracycline resistance), for replication and selection, respectively, of the plasmids in bacteria. An "expression vector" refers to a vector that contains the necessary control sequences or regulatory elements for expression of the antibodies including antibody fragment of the invention, in bacterial or eukaryotic
20 cells.

As used herein, the term "cell" can be any cell, including, but not limited to, that of a eukaryotic, multicellular species (for example, as opposed to a unicellular yeast cell), such as, but not limited to, a mammalian cell or a human cell. A cell can be present as a single entity, or can be part of a larger collection of cells. Such a "larger collection of
25 cells" can comprise, for example, a cell culture (either mixed or pure), a tissue (for example, endothelial, epithelial, mucosa or other tissue), an organ (for example, lung, liver, muscle and other organs), an organ system (for example, circulatory system, respiratory system, gastrointestinal system, urinary system, nervous system, integumentary system or other organ system), or an organism (e.g., a bird, mammal, or the like).

Polynucleotides of the invention may be synthesized, whole or in parts that then are combined, and inserted into a vector using routine molecular and cell biology techniques, including, for example, subcloning the polynucleotide into a linearized vector using
30

appropriate restriction sites and restriction enzymes. Polynucleotides of the described invention are amplified by polymerase chain reaction using oligonucleotide primers complementary to each strand of the polynucleotide. These primers also include restriction enzyme cleavage sites to facilitate subcloning into a vector. The replicable
5 vector components generally include, but are not limited to, one or more of the following: a signal sequence, an origin of replication, and one or more marker or selectable genes.

In order to express a polypeptide of the invention, the nucleotide sequences encoding the polypeptide, or functional equivalents, may be inserted into an appropriate expression vector, i.e., a vector that contains the necessary elements for the transcription
10 and translation of the inserted coding sequence. Methods well known to those skilled in the art may be used to construct expression vectors containing sequences encoding a polypeptide of interest and appropriate transcriptional and translational control elements. These methods include in vitro recombinant DNA techniques, synthetic techniques, and in vivo genetic recombination. Such techniques are described, for example, in Sambrook, J.,
15 et al. (1989) Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Press, Plainview, N.Y., and Ausubel, F. M. et al. (1989) Current Protocols in Molecular Biology, John Wiley & Sons, New York. N.Y.

According to another embodiment, the present invention provides methods for the preparation and administration of an HIV antibody composition that is suitable for
20 administration to a human or non-human primate patient having HIV infection, or at risk of HIV infection, in an amount and according to a schedule sufficient to induce a protective immune response against HIV, or reduction of the HIV virus, in a human.

According to another embodiment, the present invention provides methods for the preparation and administration of an HIV antigen composition that is suitable for
25 administration to a human or non-human primate patient having HIV infection, or at risk of HIV infection, in an amount and according to a schedule sufficient to induce a protective immune response against HIV, or reduction of the HIV virus, in a human.

According to another embodiment, the present invention provides a composition comprising at least one antibody or polypeptide of the invention and a pharmaceutically
30 acceptable carrier. The composition may include a plurality of the antibodies having the characteristics described herein in any combination and can further include antibodies neutralizing to HIV as are known in the art. According to another embodiment, the

present invention provides a composition comprising at least one antigen of the invention and a pharmaceutically acceptable carrier, can further include antibodies neutralizing to HIV as are known in the art, and can further include an adjuvant.

It is to be understood that compositions can be a single or a combination of
5 antibodies disclosed herein, which can be the same or different, in order to prophylactically or therapeutically treat the progression of various subtypes of HIV infection after vaccination. Such combinations can be selected according to the desired immunity. When an antibody or antigen is administered to an animal or a human, it can be combined with one or more pharmaceutically acceptable carriers, excipients or adjuvants
10 as are known to one of ordinary skilled in the art. The composition can further include broadly neutralizing antibodies known in the art, including but not limited to, VRC01, b12, anti-V3 loop, CD4bs, and CD4i antibodies as well as PG9/PG16-like antibodies.

Further, with respect to determining the effective level in a patient for treatment of HIV, in particular, suitable animal models are available and have been widely
15 implemented for evaluating the in vivo efficacy against HIV of various gene therapy protocols (Sarver et al. (1993b), supra). These models include mice, monkeys and cats. Even though these animals are not naturally susceptible to HIV disease, chimeric mice models (for example, SCID, bg/nu/xid, NOD/SCID, SCID-hu, immunocompetent SCID-hu, bone marrow-ablated BALB/c) reconstituted with human peripheral blood
20 mononuclear cells (PBMCs), lymph nodes, fetal liver/thymus or other tissues can be infected with lentiviral vector or HIV, and employed as models for HIV pathogenesis. Similarly, the simian immune deficiency virus (SIV)/monkey model can be employed, as can the feline immune deficiency virus (FIV)/cat model. The pharmaceutical composition can contain other pharmaceuticals, in conjunction with a vector according to the invention,
25 when used to therapeutically treat AIDS. These other pharmaceuticals can be used in their traditional fashion (i.e., as agents to treat HIV infection).

According to another embodiment, the present invention provides an antibody-based pharmaceutical composition comprising an effective amount of an isolated antibody of the invention, or an affinity matured version, which provides a prophylactic or
30 therapeutic treatment choice to reduce infection of the HIV virus. According to another embodiment, the present invention provides an antigen-based pharmaceutical composition comprising an effective amount of an isolated antigen of the invention, which provides a

prophylactic or therapeutic treatment choice to reduce infection of the HIV virus. The pharmaceutical compositions of the present invention may be formulated by any number of strategies known in the art (e.g., see McGoff and Scher, 2000, Solution Formulation of Proteins/Peptides: In McNally, E.J., ed. Protein Formulation and Delivery. New York, NY: Marcel Dekker; pp. 139-158; Akers and Defilippis, 2000, Peptides and Proteins as Parenteral Solutions. In: Pharmaceutical Formulation Development of Peptides and Proteins. Philadelphia, PA: Talyor and Francis; pp. 145-177; Akers, et al., 2002, Pharm. Biotechnol. 14:47-127). A pharmaceutically acceptable composition suitable for patient administration will contain an effective amount of the antibody in a formulation which both retains biological activity while also promoting maximal stability during storage within an acceptable temperature range. The pharmaceutical compositions can also include, depending on the formulation desired, pharmaceutically acceptable diluents, pharmaceutically acceptable carriers and/or pharmaceutically acceptable excipients, or any such vehicle commonly used to formulate pharmaceutical compositions for animal or human administration. The diluent is selected so as not to affect the biological activity of the combination. Examples of such diluents are distilled water, physiological phosphate-buffered saline, Ringer's solutions, dextrose solution, and Hank's solution. The amount of an excipient that is useful in the pharmaceutical composition or formulation of this invention is an amount that serves to uniformly distribute the antibody throughout the composition so that it can be uniformly dispersed when it is to be delivered to a subject in need thereof. It may serve to dilute the antibody or antigen to a concentration which provides the desired beneficial palliative or curative results while at the same time minimizing any adverse side effects that might occur from too high a concentration. It may also have a preservative effect. Thus, for an active ingredient having a high physiological activity, more of the excipient will be employed. On the other hand, for any active ingredient(s) that exhibit a lower physiological activity, a lesser quantity of the excipient will be employed. Compositions comprising an antigen of the invention may further comprise one or more adjuvants.

The above described antibodies and antibody compositions, comprising at least one or a combination of the antibodies described herein, can be administered for the prophylactic and therapeutic treatment of HIV viral infection.

The above described antigens and antigen compositions, comprising at least one or a combination of the antigens described herein, can be administered for the prophylactic and therapeutic treatment of HIV viral infection.

5 The present invention also provides kits useful in performing diagnostic and prognostic assays using the antibodies, polypeptides and nucleic acids of the present invention. Kits of the present invention include a suitable container comprising an HIV antibody, an antigen, a polypeptide or a nucleic acid of the invention in either labeled or unlabeled form. In addition, when the antibody, antigen, polypeptide or nucleic acid is supplied in a labeled form suitable for an indirect binding assay, the kit further includes
10 reagents for performing the appropriate indirect assay. For example, the kit may include one or more suitable containers including enzyme substrates or derivatizing agents, depending on the nature of the label. Control samples and/or instructions may also be included. The present invention also provides kits for detecting the presence of the HIV antibodies or the nucleotide sequence of the HIV antibody of the present invention in a
15 biological sample by PCR or mass spectrometry.

"Label" as used herein refers to a detectable compound or composition that is conjugated directly or indirectly to the antibody so as to generate a "labeled" antibody. A label can also be conjugated to a polypeptide and/or a nucleic acid sequence disclosed herein. The label can be detectable by itself (for example, radioisotope labels or
20 fluorescent labels) or, in the case of an enzymatic label, can catalyze chemical alteration of a substrate compound or composition that is detectable. Antibodies and polypeptides of the described invention also can be modified to include an epitope tag or label, for example, for use in purification or diagnostic applications. Suitable detection means include the use of labels such as, but not limited to, radionucleotides, enzymes,
25 coenzymes, fluorescers, chemiluminescers, chromogens, enzyme substrates or co-factors, enzyme inhibitors, prosthetic group complexes, free radicals, particles, dyes, and the like.

Methods for reducing an increase in HIV virus titer, virus replication, virus proliferation or an amount of an HIV viral protein in a subject are further provided. According to another aspect, a method includes administering to the subject an amount of
30 an HIV antibody of the invention effective to reduce an increase in HIV titer, virus replication or an amount of an HIV protein of one or more HIV strains or isolates in the subject. According to another aspect, a method includes administering to the subject an

amount of an HIV antigen of the invention effective to reduce an increase in HIV titer, virus replication or an amount of an HIV protein of one or more HIV strains or isolates in the subject.

According to another embodiment, the present invention provides a method of
5 reducing viral replication or spread of HIV infection to additional host cells or tissues comprising contacting a mammalian cell with the antibody, or a portion thereof, which binds to the 8ANC195 antigenic epitope on gp120. According to another embodiment, the present invention provides a method of reducing viral replication or spread of HIV infection to additional host cells or tissues comprising contacting a mammalian cell with
10 the antigen that mimics the 8ANC195 antigenic epitope on gp120.

According to another embodiment, the present invention provides a method for treating a mammal infected with a virus infection, such as, for example, HIV, comprising administering to said mammal a pharmaceutical composition comprising the HIV antibodies disclosed herein. According to one embodiment, the method for treating a
15 mammal infected with HIV comprises administering to said mammal a pharmaceutical composition that comprises an antibody of the present invention, or a fragment thereof. The compositions of the invention can include more than one antibody having the characteristics disclosed (for example, a plurality or pool of antibodies). It also can include other HIV neutralizing antibodies as are known in the art, for example, but not
20 limited to, VRC01, PG9 and b12.

Passive immunization has proven to be an effective and safe strategy for the prevention and treatment of viral diseases. (See, for example, Keller et al., Clin. Microbiol. Rev. 13:602-14 (2000); Casadevall, Nat. Biotechnol. 20:114 (2002); Shibata et al., Nat. Med. 5:204-10 (1999); and Igarashi et al., Nat. Med. 5:211-16 (1999). Passive
25 immunization using human monoclonal antibodies provides an immediate treatment strategy for emergency prophylaxis and treatment of HIV.

According to another embodiment, the present invention provides a method of inducing an HIV antigen-specific immune response in a mammal infected with HIV or at risk of infection with HIV comprising administering to the mammal a pharmaceutical
30 composition comprising the antigen of the invention.

According to another embodiment, the present invention provides a method of inducing an HIV antigen-specific immune response in a mammal infected with HIV or at

risk of infection with HIV comprising administering to the mammal a pharmaceutical composition comprising a nucleic acid encoding the antigen of the invention.

Subjects at risk for HIV-related diseases or disorders include patients who have come into contact with an infected person or who have been exposed to HIV in some other way. Administration of a prophylactic agent can occur prior to the manifestation of symptoms characteristic of HIV-related disease or disorder, such that a disease or disorder is prevented or, alternatively, delayed in its progression.

For in vivo treatment of human and non-human patients, the patient is administered or provided a pharmaceutical formulation including an HIV antibody or antigen of the invention. When used for in vivo therapy, the antibodies and antigens of the invention are administered to the patient in therapeutically effective amounts (i.e., amounts that eliminate or reduce the patient's viral burden). The antibodies or antigens are administered to a human patient, in accord with known methods, such as intravenous administration, for example, as a bolus or by continuous infusion over a period of time, by intramuscular, intraperitoneal, intracerebrospinal, subcutaneous, intra-articular, intrasynovial, intrathecal, oral, topical, or inhalation routes. The antibodies can be administered parenterally, when possible, at the target cell site, or intravenously. In some embodiments, antibody is administered by intravenous or subcutaneous administration. Therapeutic compositions of the invention may be administered to a patient or subject systemically, parenterally, or locally. The above parameters for assessing successful treatment and improvement in the disease are readily measurable by routine procedures familiar to a physician.

For parenteral administration, the antibodies or antigens may be formulated in a unit dosage injectable form (solution, suspension, emulsion) in association with a pharmaceutically acceptable, parenteral vehicle. Examples of such vehicles include, but are not limited, water, saline, Ringer's solution, dextrose solution, and 5% human serum albumin. Nonaqueous vehicles include, but are not limited to, fixed oils and ethyl oleate. Liposomes can be used as carriers. The vehicle may contain minor amounts of additives such as substances that enhance isotonicity and chemical stability, such as, for example, buffers and preservatives. The antibodies can be formulated in such vehicles at concentrations of about 1 mg/ml to 10 mg/ml.

The dose and dosage regimen depends upon a variety of factors readily determined by a physician, such as the nature of the infection, for example, its therapeutic index, the

patient, and the patient's history. Generally, a therapeutically effective amount of an antibody or antigen is administered to a patient. In some embodiments, the amount of antibody or antigen administered is in the range of about 0.1 mg/kg to about 50 mg/kg of patient body weight. Depending on the type and severity of the infection, about 0.1 mg/kg to about 50 mg/kg body weight (for example, about 0.1-15 mg/kg/dose) of antibody is an initial candidate dosage for administration to the patient, whether, for example, by one or more separate administrations, or by continuous infusion. The progress of this therapy is readily monitored by conventional methods and assays and based on criteria known to the physician or other persons of skill in the art. The above parameters for assessing successful treatment and improvement in the disease are readily measurable by routine procedures familiar to a physician.

A dosage regimen for administration of an antigen to a patient may be a suitable immunization regimen, including for example at least three separate inoculations. The second inoculation may be administered more than at least two weeks after the first inoculation. The third inoculation may be administered at least several months after the second administration.

Other therapeutic regimens may be combined with the administration of the HIV antibody or antigen of the present invention. The combined administration includes co-administration, using separate formulations or a single pharmaceutical formulation, and consecutive administration in either order, wherein preferably there is a time period while both (or all) active agents simultaneously exert their biological activities. Such combined therapy can result in a synergistic therapeutic effect. The above parameters for assessing successful treatment and improvement in the disease are readily measurable by routine procedures familiar to a physician.

The terms "treating" or "treatment" or "alleviation" are used interchangeably and refer to both therapeutic treatment and prophylactic or preventative measures; wherein the object is to prevent or slow down (lessen) the targeted pathologic condition or disorder. Those in need of treatment include those already with the disorder as well as those prone to have the disorder or those in whom the disorder is to be prevented. A subject or mammal is successfully "treated" for an infection if, after receiving a therapeutic amount of an antibody or antigen according to the methods of the present invention, the patient shows observable and/or measurable reduction in or absence of one or more of the

following: reduction in the number of infected cells or absence of the infected cells; reduction in the percent of total cells that are infected; and/or relief to some extent, one or more of the symptoms associated with the specific infection; reduced morbidity and mortality, and improvement in quality of life issues. The above parameters for assessing
5 successful treatment and improvement in the disease are readily measurable by routine procedures familiar to a physician.

The term "therapeutically effective amount" refers to an amount of an antibody or a drug effective to treat a disease or disorder in a subject or mammal.

Administration "in combination with" one or more further therapeutic agents
10 includes simultaneous (concurrent) and consecutive administration in any order.

"Carriers" as used herein include pharmaceutically acceptable carriers, excipients, or stabilizers that are nontoxic to the cell or mammal being exposed thereto at the dosages and concentrations employed. Often the physiologically acceptable carrier is an aqueous pH buffered solution. Examples of physiologically acceptable carriers include, but not
15 limited to, buffers such as phosphate, citrate, and other organic acids; antioxidants including, but not limited to, ascorbic acid; low molecular weight (less than about 10 residues) polypeptide; proteins, such as, but not limited to, serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as, but not limited to, polyvinylpyrrolidone; amino acids such as, but not limited to, glycine, glutamine, asparagine, arginine or lysine;
20 monosaccharides, disaccharides, and other carbohydrates including, but not limited to, glucose, mannose, or dextrans; chelating agents such as, but not limited to, EDTA; sugar alcohols such as, but not limited to, mannitol or sorbitol; salt-forming counterions such as, but not limited to, sodium; and/or nonionic surfactants such as, but not limited to, TWEEN.; polyethylene glycol (PEG), and PLURONICS.

25 According to another embodiment, the present invention provides diagnostic methods. Diagnostic methods generally involve contacting a biological sample obtained from a patient, such as, for example, blood, serum, saliva, urine, sputum, a cell swab sample, or a tissue biopsy, with an HIV antibody and determining whether the antibody preferentially binds to the sample as compared to a control sample or predetermined cut-
30 off value, thereby indicating the presence of the HIV virus.

According to another embodiment, the present invention provides methods to detect the presence of the HIV antibodies of the present invention in a biological sample

from a patient. Detection methods generally involve obtaining a biological sample from a patient, such as, for example, blood, serum, saliva, urine, sputum, a cell swab sample, or a tissue biopsy and isolating HIV antibodies or fragments thereof, or the nucleic acids that encode an HIV antibody, and assaying for the presence of an HIV antibody in the biological sample. Also, the present invention provides methods to detect the nucleotide sequence of an HIV antibody in a cell. The nucleotide sequence of an HIV antibody may also be detected using the primers disclosed herein. The presence of the HIV antibody in a biological sample from a patient may be determined utilizing known recombinant techniques and/or the use of a mass spectrometer.

According to another embodiment, the present invention provides methods for detecting or isolating an HIV-1 binding antibody in a subject comprising obtaining a biological sample from the subject, contacting said sample with the antigen of the invention, and conducting an assay to detect or isolate an HIV-1 binding antibody.

The term "assessing" includes any form of measurement, and includes determining if an element is present or not. The terms "determining", "measuring", "evaluating", "assessing" and "assaying" are used interchangeably and include quantitative and qualitative determinations. Assessing may be relative or absolute. "Assessing the presence of" includes determining the amount of something present, and/or determining whether it is present or absent. As used herein, the terms "determining," "measuring," and "assessing," and "assaying" are used interchangeably and include both quantitative and qualitative determinations.

Where a value of ranges is provided, it is understood that each intervening value, to the tenth of the unit of the lower limit unless the context clearly dictates otherwise, between the upper and lower limit of that range and any other stated or intervening value in that stated range is encompassed within the invention. The upper and lower limits of these smaller ranges which may independently be included in the smaller ranges is also encompassed within the invention, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either both of those included limits are also included in the invention.

EXAMPLE 1

This example describes materials and methods used in EXAMPLES 2-5 below.

Protein expression and purification

The antibodies used in this study were produced and purified as in previously-described studies (Diskin et al., *Science* 334, 1289 (2011).) Briefly, 8ANC195, 3BNC60 and chimeric antibody (mature HC / various LC; κ/κ combinations of newly isolated 8ANC195 variants) IgGs were expressed by transiently transfecting HEK293-6E cells with vectors containing the appropriate heavy and light chain genes. Secreted IgGs were purified from cell supernatants using protein A affinity chromatography (GE Healthcare). For neutralization assays, IgGs were diluted to 1 mg/mL stocks in 20 mM Tris pH 8.0, 150 mM sodium chloride (TBS buffer). 8ANC195 Fab was expressed with a 6x-His tag on the C-terminus of C_H1 as described for IgGs and purified using Ni²⁺-NTA affinity chromatography (GE Healthcare) and Superdex 200 16/60 size exclusion chromatography (GE Healthcare).

A truncated gp120 from the HIV-1 strain 93TH057 containing mutations Asn88Gln_{gp120}, Asn289Gln_{gp120}, Asn334Gln_{gp120}, Asn392Gln_{gp120}, Asn448Gln_{gp120} was produced by transiently transfecting HEK293-S (GnTI^{-/-}) cells adapted for growth in suspension by the Caltech Protein Expression Center with a pTT5 vector encoding His-tagged gp120. Secreted gp120 was captured on Ni²⁺-NTA resin (GE Healthcare) and further purified using Superdex 200 16/60 size exclusion chromatography (GE Healthcare).

Soluble CD4 domains 1 and 2 (sCD4) and sCD4_{K75T} were produced as described previously (Diskin et al. *Nat Struct Mol Biol* 17, 608 (2010)). Briefly, the pACgp67b vector encoding 6x-His-tagged sCD4 or sCD4_{K75T} (residues 1–186 of mature CD4) was used to make infectious baculovirus particles using BaculoGold (BD Biosynthesis). Protein was expressed in Hi5 cells, captured on a Ni²⁺-NTA column (GE Healthcare) and further purified using Superdex 200 16/60 size exclusion chromatography (GE Healthcare). To remove an N-linked glycan introduced by mutation in sCD4_{K75T}, the protein was treated with Endoglycosidase H (New England Biolabs) for 16 hours at 25° C and then purified by Superdex 200 16/60 size exclusion chromatography (GE Healthcare).

For complex crystallization trials, purified 8ANC195 Fab, 93TH057 gp120 and EndoH-treated sCD4_{K75T} were incubated at a 1:1:1 molar ratio for 2 hours at 25 °C. The complex was purified by Superdex 200 10/300 size exclusion chromatography (GE Healthcare) and the peak corresponding to 8ANC195 Fab/gp120/sCD4_{K75T} complex

concentrated to 16 mg/mL in TBS buffer. For crystallization of 8ANC195 Fab alone, the protein was concentrated to 20 mg/mL in TBS buffer.

Purified BG505 SOSIP trimers (Julien et al., *PLoS pathogens* 9, e1003342 (2013); Lyumkis et al., *Science* 342, 1484 (2013); Sanders et al., *PLoS pathogens* 9, e1003618 (2013)) for EM studies were the gift of Dr. John P. Moore (Weill Cornell Medical College).

Crystallization

Crystallization conditions were screened using vapor diffusion in sitting drops set using a Mosquito® crystallization robot (TTP labs) in a final volume of 200 nL per drop (1:1 protein to reservoir ratio) utilizing commercially available crystallization screens (Hampton Research, Microlytic). Initial crystallization hits for 8ANC195 Fab and for 8ANC195 Fab/93TH057 gp120/sCD4_{K75T} complex were identified using the MCSG-1 (Microlytic) and PEGRx (Hampton) screens and then manually optimized. Crystals of 8ANC195 Fab (space group P4₁2₁2, $a = 66.5 \text{ \AA}$, $b = 66.5 \text{ \AA}$, $c = 219.0 \text{ \AA}$; one molecule per asymmetric unit) were obtained upon mixing a protein solution at 11 mg/mL with 0.1M Hepes pH 7, 20% PEG 6,000, 10 mM zinc chloride at 20°C. Crystals were briefly soaked in mother liquor solution supplemented with 20% ethylene glycol before flash cooling in liquid nitrogen. Crystals of the 8ANC195 Fab/93TH057 gp120/sCD4_{K75T} complex (space group P2₁2₁2₁, $a = 66.5 \text{ \AA}$, $b = 132.5 \text{ \AA}$, $c = 142.8 \text{ \AA}$; one molecule per asymmetric unit) were obtained upon mixing a protein solution at 16 mg/mL with 14% polyethylene glycol 3,350, 0.1 M HEPES pH 7.3, 2% benzamidinium HCl at 20°C. Crystals were briefly soaked in mother liquor solution supplemented with 30% ethylene glycol before flash cooling in liquid nitrogen.

Crystallographic data collection, structure solution and refinement

X-ray diffraction data for 8ANC195 Fab crystals were collected at the Argonne National Laboratory Advanced Photon Source (APS) beamline 23-ID-D using a MAR 300 CCD detector. X-ray diffraction data for 8ANC195 Fab/93TH057 gp120/sCD4_{K75T} complex crystals were collected at the Stanford Synchrotron Radiation Lightsource (SSRL) beamline 12-2 using a Pilatus 6M pixel detector (Dectris). The data were indexed, integrated and scaled using XDS (Kabsch, *Acta Crystallogr D Biol Crystallogr* 66, 133 (2010)).

The 8ANC195 Fab structure was solved by molecular replacement using Phenix (Adams et al., *Acta Crystallogr D Biol Crystallogr* 66, 213 (2010)) and the V_HV_L and C_H1C_L domains of NIH45-46 Fab (PDB code 3U7W) lacking all CDR loops as two separate search models. The model was then refined to 2.13 Å resolution using an iterative approach involving refinement and verification of model accuracy with simulated annealing composite omit maps using the Phenix crystallography package, and manually fitting models into electron density maps using Coot (Emsley et al., *Acta Crystallogr D Biol Crystallogr* 60, 2126 (2004)). The final model (R_{work} = 21.4%; R_{free} = 25.7%) includes 3,279 protein atoms and 127 water molecules as shown in Table 1.

	8ANC195 Fab/gp120/sCD4 complex	8ANC195 Fab
Data collection		
Resolution range (Å)	39.22 - 3.0 (3.22 - 3.0)	29.73 - 2.1 (2.21 - 2.1)
Space group	P 2 ₁ 2 ₁ 2 ₁	P 4 ₁ 2 ₁ 2
Cell dimensions		
a, b, c (Å)	66.53, 132.49, 142.77	66.48, 66.48, 219.03
α, β, γ (°)	90, 90, 90	90, 90, 90
Total reflections	229212 (12539)	239217 (24708)
Unique reflections	36730 (3064)	28097 (2766)
Multiplicity	6.2 (6.3)	8.4 (8.9)
Completeness (%)	97.65 (99.80)	98.92 (91.00)
Mean I/σ(I)	7.86 (2.1)	11.90 (3.16)
Wilson B-factor	61.95	32.47
R _{merge}	0.1747 (0.765)	0.1225 (0.5802)
CC1/2	0.996 (0.854)	0.996 (0.876)
CC*	0.999 (0.960)	0.999 (0.966)
Refinement		
R _{work} /R _{free}	0.2655/0.3149	0.2431/0.2772
Number of atoms	7272	3311
Protein	6861	3311
Ligands	391	0
Water	0	0
Protein residues	939	437
RMS (bonds)	0.023	0.008
RMS (angles)	1.33	1.17
Clashscore	22.78	12.43
Average B-factor	81.5	36.5
Protein	81	36.5
Ligands	89	-
Water	-	-

Statistics for the highest-resolution shell are shown in parentheses.

Table 1

96.75%, 2.78% and 0.0% of the residues were in the favored, allowed and disallowed regions, respectively, of the Ramachandran plot. Disordered residues that were not included in the model include residues 146-153, 233-238 and the 6x-His tag of the 8ANC195 heavy chain, and residues 214-215 of the light chain.

5 The 8ANC195 Fab/93TH057 gp120/sCD4_{K75T} complex structure was solved by molecular replacement using Phaser (Adams et al., *Acta Crystallogr D Biol Crystallogr* 66, 213 (2010)) and the V_HV_L and C_H1C_L domains of 8ANC195 (lacking all CDR loops), 93TH057 gp120 (taken from PDB code 3U7Y), and sCD4 (taken from PDB code 3LQA) as separate search models. The complex structure was refined to 3.0 Å resolution as described for the Fab structure. In addition to considering $1/\sigma_1$ and completeness of the highest resolution shell (2.1% and 99.9%, respectively), CC_{1/2} statistic (Karplus et al., *Science* 336, 1030 (2012)) (correlation coefficient between two random halves of the data set where CC_{1/2} > 10%) was used to determine the high-resolution cutoff for the data. Phenix was used to compute CC_{1/2} (85.4% for the highest resolution shell and 99.8% for the entire data set), supporting our high-resolution cutoff determination.

10 The final model (R_{work} = 23.4%; R_{free} = 28.6%) includes x protein atoms and y atoms of carbohydrates (Table S1). 96.2%, 3.8% and 0.0% of the residues were in the favored, allowed and disallowed regions, respectively, of the Ramachandran plot. Disordered residues that were not included in the model include residues 146-153, 206-208, 233-238 and the 6x-His tag of the 8ANC195 heavy chain, residues 213-215 of the light chain, residues 125-197 (V1/V2 substitution), 302-324 (V3 substitution), residues 397-409 (a total of 6 residues from V4), residues 492-494 and the 6x-His tag of 93TH057 gp120 and residues 106-111, 154-155, 177-186 of sCD4_{K75T}.

25 Buried surface areas were calculated using PDBePISA (Krissinel et al., *Journal of molecular biology* 372, 774 (2007)) and a 1.4 Å probe. Superimposition calculations were done and molecular representations were generated using PyMol (Schrödinger (The PyMOL Molecular Graphics System, 2011). Pairwise C α alignments were performed using PDBeFold (Krissinel et al., *Acta Crystallogr D Biol Crystallogr* 60, 2256 (2004)).

ELISAs

30 High-binding 96-well ELISA plates (Costar) were coated overnight with 5 µg/well of purified gp120 in 100 mM sodium carbonate pH 9.6. After washing with TBS

containing 0.05% Tween 20, the plates were blocked for 2 h with 1% BSA, 0.05% Tween-TBS (blocking buffer) and then incubated for 2 h with 8ANC195 IgG (1 $\mu\text{g/mL}$) mixed with 1:2 serially diluted solutions of potential antibody competitors (sCD4, J3 VHH, 3BNC60 Fab, NIH45-46 Fab) in blocking buffer (competitor concentration range from 5 to 320 $\mu\text{g/mL}$). After washing with TBS containing 0.05% Tween 20, the plates were incubated with HRP-conjugated goat anti-human IgG antibodies (Jackson ImmunoResearch) (at 0.8 $\mu\text{g/mL}$ in blocking buffer) for 1 hour. The ELISAs were developed by addition of HRP chromogenic substrate (TMB solution, BioLegend) and the color development stopped by addition of 10% sulfuric acid. Experiments were performed in duplicate.

Surface plasmon resonance

Experiments were performed using a Biacore T100 (Biacore) using a standard single-cycle kinetics method. YU-2 and 93TH057 gp120 proteins were primary amine-coupled on CM5 chips (Biacore) at a coupling density of 1,000 RUs and one flow cell was mock coupled using HBS-EP+ buffer. 8ANC195 and chimeric IgGs were injected over flow cells at increasing concentrations (62.5 to 1,000 nM), at flow rates of 20 $\mu\text{L/min}$ with 5 consecutive cycles of 2 min association/1 min dissociation and a final 10 min dissociation phase. Flow cells were regenerated with 3 pulses of 10 mM glycine pH 2.5. Apparent binding constants (K_D (M)) were calculated from single-cycle kinetic analyses after subtraction of backgrounds using a 1:1 binding model without a bulk reflective index (RI) correction (Biacore T100 Evaluation software). Binding constants for bivalent IgGs are referred to as “apparent” affinities to emphasize that the K_D values include potential avidity effects.

Neutralization assays

A TZM-bl/pseudovirus neutralization assay was used to evaluate the neutralization potencies of the antibodies as described (Montefiori, *Current protocols in immunology* edited by John E. Coligan et al., Chapter 12, Unit 12 11 (2005)). Pseudoviruses were generated by cotransfection of HEK 293T cells with an Env expression plasmid and a replication-defective backbone plasmid. Neutralization was determined by measuring the reduction in luciferase reporter gene expression in the presence of antibody following a single round of pseudovirus infection in TZM-bl cells. Nonlinear regression analysis was

used to calculate the concentrations at which half-maximal inhibition was observed (IC₅₀ values).

Negative-stain EM

The BG505 SOSIP.664/8ANC195 Fab complex and grids were prepared as described previously (Kong et al., *Nat Struct Mol Biol* 20, 796 (2013)). The data were collected on an FEI Tecnai T12 electron microscope coupled with a Tietz TemCam-F416 4k x 4k CMOS camera using the LEGINON interface. Images were collected in 10° increments from 0° to -40° using a defocus range of 0.6 - 0.9 μm at a magnification of 52,000x, resulting in a pixel size of 2.05 Å at the specimen plane. Particles were selected using DogPicker (Voss et al., *Journal of structural biology* 166, 205 (2009)) within the Appion software package (Lander et al., *Journal of structural biology* 166, 95 (2009)), and sorted from reference-free 2D class averages using the SPARX package (Penczek et al., *Ultramicroscopy* 40, 33 (1992)). An initial model was generated by common lines from class averages using the EMAN2 package (Tang et al., *Journal of structural biology* 157, 38 (2007)) and was refined using 11,637 unbinned particles. The refinement was carried out using the SPARX package (Penczek et al., *Ultramicroscopy* 53, 251 (1994)) with C3 symmetry applied. The resulting resolution at a 0.5 Fourier Shell Correlation (FSC) cut-off was 18.7 Å (Figs. 6A,B).

Human Samples

Human samples were collected after signed informed consent in accordance with Institutional Review Board (IRB)-reviewed protocols by all participating institutions. Patient 8 was selected from a cohort of elite controllers that were followed at the Ragon Institute in Boston.

Isolation of 8ANC195 variants

Single Cell clonal variants of 8ANC195 were isolated by 2CC core-specific single cell sorting, followed by reverse transcription and immunoglobulin gene amplification as described previously (Scheid et al., *Science* 333, 1633 (2011)). Immunoglobulin genes were cloned into heavy and light chain expression vectors and co-transfected for IgG production as described previously (Tiller et al., *Journal of immunological methods* 329, 112 (2008)).

IgG+ CD19+ memory B cells were bulk sorted on a FACS AriaIII cell sorter. Bulk mRNA was extracted using TRIzol (Invitrogen) and reverse transcribed as previously described (Scheid et al., *Science* 333, 1633 (2011)). 8ANC195-related heavy and light chain genes were PCR amplified using the following clone-specific primers:

- 5 For heavy chain amplification: 5' GGTGTACATTCTCAGATACACCTCGTACAA 3' (SEQ ID NO:50) and
5' CAGGTGTCCAGTCTCAGATACA 3' (SEQ ID NO:51) as forward primers and
5' GCGGAGACGGAGATGAGGGTT 3' (SEQ ID NO:57) as a reverse primer. For light chain amplification:
- 10 5' GACATCCAGATGACCCAGTCTCCTTCCACCCTGTCTGCATCTATAGGT 3' (SEQ ID NO:52) and
5' GACATCCAGATGACCCAGTCTCCTTCCACCCTGTCTGCATCT 3' (SEQ ID NO:53) as forward and
5' GTTTCACCTCAACTTTAGTCCCTT 3' (SEQ ID NO:58) as well as
15 5' GTTTCACCTCAACTTTAGTCCCTTGGCCGAAGGTC 3' (SEQ ID NO:59) as reverse primers.

Amplification products were gel purified and cloned into TOPO TA sequencing vectors (Invitrogen) and expression vectors as described previously (T. Tiller et al., *Journal of immunological methods* 329, 112 (2008)).

20 **Phylogenetic tree and Alignment assembly.**

Phylogenetic trees were assembled using Geneious Neighbor-Joining Tree Software. Sequence Alignments were performed using DNA Star Clustal W alignment software.

Computational analysis.

- 25 The program AntibodyDatabase (West, Jr. et al., *Proceedings of the National Academy of Sciences of the United States of America* 110, 10598 (2013)) was used to analyze 8ANC195 neutralization panel data from Scheid et al., *Science* 333, 1633 (2011) and Chuang et al., *Journal of virology* 87, 10047 (2013). This method attempts to model the variation in neutralization potency across strains based on a sum of terms (“rules”)
30 corresponding to specific residues or potential N-linked glycosylation site (PNGS) positions. With the free residual option deselected, the analysis finds a rule corresponding

to ~3-fold better 8ANC195 neutralization for strains with Glu632_{gp41}. This correlation appears to hold across clades based on neutralization data for strains having the most favorable glycosylation pattern (PNGS at 234_{gp120} and 276_{gp120}, and not at 230_{gp120}) (22). For all clades, the residue at 632_{gp41} versus geometric mean IC₅₀s for 8ANC195 on strains
 5 with the most favorable glycosylation pattern was as follows: Glu, 0.43 µg/mL (n=53) versus Asp, 1.31 µg/mL (n=51). For separate clades, the correlations were Clade A: Glu, 0.47 µg/mL (n=3); Asp, 1.30 µg/mL (n=24); Clade B: Glu, 0.18 µg/mL (n=15); Asp, 0.72 µg/mL (n=6); Clade C: Glu, 0.32 µg/mL (n=2); Asp, 1.31 µg/mL (n=20).

Statistical analysis of neutralization potencies of 8ANC195 variants

10 IC₅₀ values derived from neutralization assays with 8ANC195 and its κ 52_{HC}/ κ 5_{LC} variant against 11 sensitive virus strains (IC₅₀ < 50) were analyzed by G-test for the relationship between the amino acid identity at position 636_{gp41} and the antibody IC₅₀. For each antibody the partition between “high” and “low” IC₅₀s was chosen such that approximately half of the strains had high IC₅₀s (0.8 µg/mL for 8ANC195, 0.1 µg/mL for
 15 κ 52_{HC}/ κ 5_{LC}).

EXAMPLE 2

Determination of the crystal structure of the Fab fragment of 8ANC195 alone and complexed with HIV-1 gp120 core and CD4 domains 1-2 (sCD4)

20 To determine the epitope recognized by 8ANC195 and investigate its neutralization mechanism, crystal structures were solved of the Fab fragment of 8ANC195 alone and complexed with an HIV-1 clade A/E 93TH057 gp120 core and CD4 domains 1-2 (sCD4) at 1.9 Å and 2.9 Å resolution, respectively (Fig. 16A,B; Table 1). Five PNGSs on the core gp120 were removed by mutation (Asn88Gln_{gp120}, Asn289Gln_{gp120},
 25 Asn334Gln_{gp120}, Asn392Gln_{gp120}, Asn448Gln_{gp120}) to reduce glycan heterogeneity.

Comparison of 8ANC195 Fab in its free versus gp120-bound states revealed high structural similarity (RMSD = 0.7 Å for 236 C α atoms of V_H-V_L) except for a 3.5 Å displacement of the loop connecting strands D and E in HC FWR3 (Fig. 16A). The CDRH1 and CDRH3 loops were folded into hook-like tertiary structures in free and
 30 gp120-bound Fabs; therefore the conformations were not induced upon binding to gp120 (Fig. 16A and Figs. 2A,B). The CDRH3 architecture differed from CDRH3s in other

antibodies including anti-HIV-1 antibodies with long CDR loops (Fig. 2C). The CDRH1 loop conformation was stabilized by a hydrogen bond network among backbone atoms of CDRH1, burial of Phe30_{HC}, and hydrogen bonds with Asp73_{HC} and Thr104_{HC} (Fig. 2A). CDRH3 had a complex tertiary structure in which residues 102_{HC}-110_{HC} formed a loop protruding ~10 Å from the antibody surface, and residues 111_{HC}-118_{HC} formed a β-sheet subdomain that was stabilized by hydrophobic stacking between His113_{HC} and Trp33_{LC} and a hydrogen bond between Met117_{HC} and Gln90_{LC} (Fig. 2B). The side chain of Tyr92_{LC} hydrogen bonded with the Gly110_{HC} carbonyl oxygen, stabilizing a kink in the loop that formed the transition between these secondary structure elements (Fig. 2B).

The complex structure showed independent binding of sCD4 and 8ANC195 Fab to distinct sites on gp120 (Fig. 16B). sCD4 interacted with the gp120 core as in other sCD4-gp120 structures (Kwong et al., *Nature* 393, 648 (1998)) (Fig. 3A), thus its binding was not altered by the presence of the adjacent antibody, consistent with binding and neutralization experiments showing no effects of CD4 addition on 8ANC195 activity (Fig. 3B,C). sCD4 did, however, contribute to crystal packing (Fig. 3D), rationalizing why diffraction-quality crystals failed to grow in its absence. In the ternary complex structure, 8ANC195 bound to a gp120 region adjacent to the CD4 binding site, contacting mainly the gp120 inner domain, loops D and V5, and a small patch of the gp120 outer domain (His352_{gp120}-Asn354_{gp120}) (Fig. 16B,C).

8ANC195 Fab bound gp120 core exclusively with its HC, using residues in FWRs and its three CDR loops to form an extensive interface (3,671 Å² total buried surface area; 1287 Å² HC-gp120 protein contacts; 2,384 Å² HC-gp120 glycan contacts) (Fig. 16B, 2, Fig. 4; Table 2).

Buried Surface Area (BSA) at Interfaces				Hydrogen Bonds at Interfaces				
gp120	BSA (Å ²)	SANC195 HC	BSA (Å ²)	gp120		SANC195 HC		Distances (Å)
VAL 44	19.4	ASN 28	28.6	THR 278	Oγ1	THR 75	O	2.43
TRP 46	17.4	THR 29	32.9	ARG 456	NH2	GLY 76	O	3.37
LYS 48	35.1	GLY 31	26.3	ASN 354	Nδ2	SER 77	Oγ	2.88
ASP 47	39.7	LEU 32	56.9	THR 278	Oγ1	SER 78	O	3.34
THR 90	39.1	ARG 54	64.5	ASN 92	Nδ2	THR 104	O	2.88
GLU 91	5.7	TRP 55	4.2	ASN 92	Nδ2	TYR 105	O	3.08
ASN 92	67.3	LYS 56	4.4	HIS 352	O	THR 75	Oγ1	3.14
PHE 93	9.0	LEU 74	65.7	ASN 354	Oδ1	THR 75	Oγ1	3.06
ASN 94	38.0	THR 75	44.3	ASP 47	Oδ2	TYR 105	OH	3.09
LYS 97	7.2	GLY 76	81.0	LYS 487	NC	TYR 105	OH	3.49
THR 236	27.7	SER 77	33.9					
GLY 237	21.1	SER 78	5.5					
PRO 238	51.8	PRO 79	3.9					
LYS 240	4.1	THR 104	11.7					
SER 274	0.2	TYR 105	100.2					
GLU 275	7.0	ASP 106	16.8					
ASN 276	15.9	LYS 107	24.9					
LEU 277	37.8	TRP 108	70.3					
THR 278	89.6							
HIS 352	17.0							
PHE 353	10.0							
ASN 354	48.1							
LYS 357	3.5							
ARG 456	13.8							
THR 463	0.2							
GLU 466	0.1							
LYS 487	8.0							
Total gp120	633.4	Total SANC195 HC	683.9					

gp120	BSA (Å ²)	SANC195 HC	BSA (Å ²)
gly276 NAG ¹	121.2	TYR 25	12.8
		GLY 26	36.7
		VAL 27	6.5
		ASN 28	16.3
		LEU 74	21.2
		PRO 79	4.2
gly276 NAG ²	109.5	GLN 1	18.8
		HIS 3	9.0
		TYR 25	40.8
		GLY 26	13.8
gly276 BMA ³	65.5	HIS 3	35.3
		VAL 5	6.6
		TYR 25	19.4
gly276 MAN ⁴	88.5	GLN 1	14.9
		ILE 2	1.8
		HIS 3	40.0
gly276 MAN ⁵	45.2	VAL 5	18.4
		TYR 25	18.7
Total gly276	399.8	Total SANC195 HC	331.8

Table 2

Buried Surface Area (BSA) at interfaces			
gp120	BSA (Å ²)	8ANC195 HC	BSA (Å ²)
gly234 NAG ¹	108.4	ASN 28	1.5
		THR 29	9.8
		TRP 55	24.9
		ASP 73	29.7
		LEU 74	16.8
gly234 NAG ²	128.4	ARG 54	1.9
		TRP 55	53.4
		ALA 71	2.8
		VAL 72	11.6
		ASP 73	13.1
gly234 BMA ³	100.6	ILE 52	9.5
		TRP 55	24.5
		LYS 56	0.2
		SER 57	3.4
		ILE 69	0.8
		SER 70	16.5
		ALA 71	11.4
		VAL 72	10.5
gly234 MAN ⁴	55.2	SER 70	8.8
		ALA 71	5.2
		VAL 72	31.0
gly234 MAN ⁵	129.8	SER 70	11.9
		ALA 71	1.5
		VAL 72	18.1
		ILE 81	18.5
gly234 MAN ⁶	118.4	SER 83	18.6
		THR 19	18.7
		LEU 68	24.3
		SER 70	8.9
		SER 83	7.8
gly234 MAN ⁷	84.2	GLU 65	25.7
		ILE 52	3.3
		TRP 55	13.4
		LYS 56	1.8
		SER 57	28.2
		LEU 68	16.2
		ILE 69	3.8
gly234 MAN ⁹	68.3	SER 70	2.1
		SER 57	30.2
		SER 58	0.2
		VAL 67	2.5
		LEU 68	24.4
gly234 MAN ¹⁰	198.7	ILE 69	1.2
		SER 57	26.9
		SER 58	11.0
		ALA 59	21.8
		ARG 64	14.3
		GLY 65	14.2
		VAL 67	13.3
		LEU 68	10.9
ILE 69	3.5		
Total gly234	992.0	Total 8ANC195 HC	881.2

Hydrogen Bonds at interfaces				
gp120		8ANC195 HC		Distance (Å)
gly234 NAG ¹	O4	TRP 55	Nε1	2.99
gly234 NAG ¹	O3	ASP 73	Oα2	3.30
gly234 NAG ²	O6	ASP 73	N	3.13
gly234 MAN ⁵	O6	VAL 72	N	2.78
gly234 MAN ⁶	O6	ILE 81	O	2.84
gly234 MAN ⁶	O3	GLU 85	Oα2	3.17
gly234 MAN ⁶	O4	GLU 85	Oα2	2.34
gly234 MAN ¹⁰	O3	ALA 59	N	3.50
gly234 MAN ¹⁰	O2	ALA 59	N	3.03
gly234 MAN ¹⁰	O6	VAL 67	O	3.17
gly234 MAN ¹⁰	O6	GLY 65	N	2.58
gly234 MAN ¹⁰	O2	SER 58	O	3.28
gly234 MAN ¹⁰	O2	SER 58	N	3.19
gly234 MAN ¹⁰	O3	SER 57	O	2.79

Table 2 (continued)

A loop in FWR3_{HC}, consisting of somatically-mutated residues and extended by a
 5 four-residue insertion, reached like a thumb into the pocket formed by loops D, V5 and
 outer domain residues 352_{gp120}-358_{gp120} (Fig. 16A,B and 17A, Fig. 4B). CDRH1 and
 CDRH3 contacted the gp120 inner domain (Fig. 16B, Fig. 4B), contributing to a 1287 Å²
 interface between the 8ANC195 HC and gp120 protein residues. The CDRH1 and CDRH3
 loop conformations, conserved in the free Fab (Fig. 16A, Fig. 2A,B), were necessary for

binding gp120 since extending these loops would result in clashes with gp120. The resulting antibody combining site was exquisitely suited to contacting portions of the inner domain of gp120 not targeted by other bNAbs (Fig. 16C).

The 8ANC195 Fab also made extensive interfaces with glycans attached to

5 Asn234_{gp120} (buried surface area = 1,653 Å²) and Asn276_{gp120} (buried surface area = 731 Å²), rationalizing its dependence on these PNGSs for neutralization (West, Jr. et al., *Proceedings of the National Academy of Sciences of the United States of America* 110, 10598 (2013); Chuang et al., *Journal of virology* 87, 10047 (2013)). Together with CDRH2, somatically-mutated FWR residues in strands B, C", D and E contributed to an
 10 extensive interface with the Asn234_{gp120}-associated *N*-glycan (usually high mannose in native HIV-1 Envs (Go et al., *Journal of virology* 85, 8270 (2011) that involved 10 sugar moieties, including specific interactions with terminal mannose residues (Fig. 17C,E, Fig. 4C,D). A two-residue deletion at the CDRH2–FWR3_{HC} boundary compared to the
 15 inner domain residue Asn234_{gp120} and its neighbors. The Asn276_{gp120} glycan (a complex-type *N*-glycan in native HIV-1 Envs (Go et al., *Journal of virology* 85, 8270 (2011); Binley et al., *Journal of virology* 84, 5637 (2010)), but high mannose in the crystallized gp120) was wedged between 8ANC195 and sCD4, where it contacted FWR residues in strands A and B and the N-terminal portion of CDRH1, forming an interface involving
 20 only the core pentasaccharide common to both high mannose and complex-type *N*-glycans (Fig. 17D, Fig. 4E,F).

The 8ANC195 HC was bracketed by the Asn234_{gp120} and Asn276_{gp120} glycans in a manner analogous to interactions of HIV-1 antibodies that penetrate the Env glycan shield, such as PG16 (interactions with Asn156_{gp120}/Asn173_{gp120} and Asn160_{gp120} glycans)

25 (Pancera et al., *Nature structural & molecular biology* 20, 804 (2013), PGT128 (with Asn301_{gp120} and Asn332_{gp120} glycans) (Pejchal et al., *Science* 334, 1097 (2011)) and PGT121 (with Asn137_{gp120} and Asn332_{gp120} glycans) (Mouquet et al., *Proceedings of the National Academy of Sciences of the United States of America* 109, E3268 (2012); Julien et al., *Science* 342, 1477 (2013) Julien et al., *PLoS pathogens* 9, e1003342 (2013)) (Fig.
 30 5). However, in contrast to these antibodies, 8ANC195 contacts with gp120 were made exclusively by its HC; indeed, 33% of 8ANC195 V_H domain residues not buried at the LC interface contacted gp120. In summary, the 8ANC195-gp120 structure demonstrated that

8ANC195 recognizes a novel epitope involving the Asn234_{gp120} and Asn276_{gp120} glycans, the gp120 inner domain, loop D and loop V5, which would be adjacent to gp41 in Env trimer (Julien et al., *Science* 342, 1477 (2013); Lyumkis et al., *Science* 342, 1484 (2013)).

5 EXAMPLE 3

Negative stain single particle electron microscopy (EM) to determine the structure of 8ANC195 Fab bound to a soluble HIV-1 SOSIP trimer

To investigate portions of the 8ANC195 epitope beyond the gp120 core, including potential contacts with gp41, negative stain single particle EM was used to determine the structure of 8ANC195 Fab bound to a soluble HIV-1 SOSIP trimer derived from strain BG505 (Fig. 6) (Julien et al., *Science* 342, 1477 (2013); Lyumkis et al., *Science* 342, 1484 (2013); Sanders et al., *PLoS pathogens* 9, e1003618 (2013)). Independent docking of the BG505 Env trimer structure (PDB 4NCO) (Julien et al., *Science* 342, 1477 (2013)) and 8ANC195 Fab resulted in a model wherein the Fab contacted both gp120 and gp41 within a single protomer (Fig. 18A, Fig. 7). The EM model placed the CDRL1, CDRL2, and portions of FWR3_{LC} and CDRH3 in close proximity to the HR2 helix of gp41 (Fig. 18B). Although gp41 residues were not definitively identified in the trimer crystal structure (Julien et al., *Science* 342, 1477 (2013)), based on the assignment of the HR2 C-terminus as Gly664_{gp41} (Lyumkis et al., *Science* 342, 1484 (2013), the kink in the HR2 helix was assigned as Asn637_{gp41} (Fig. 18B, Fig. 8), the asparagine of a highly conserved PNGS. The EM model predicted that the Asn637_{gp41}-linked glycan and adjacent amino acid residues on HR2 interacted with 8ANC195 CDRH3, CDRL1 and CDRL2.

Docking of the gp120-8ANC195 portion of the ternary crystal structure onto the SOSIP trimer structure resulted in a slightly different angular placement of the Fab in the EM density than when the 8ANC195 Fab was fit independently (Fig. 18A, Fig. 7). The Fab, especially the LC, was pushed further away from gp41 by comparison to the placement suggested by the complex crystal structure. The LC position in the EM model was more likely to be accurate since it left space for bulky side chains at positions 625_{gp41}-640_{gp41} that were modeled as alanines in the trimer crystal structure (Julien et al., *Science* 342, 1477 (2013); Lyumkis et al., *Science* 342, 1484 (2013)). The slightly different placements could be due to crystal packing effects, spatial restraints imposed by the gp41 glycans that were not present in the 8ANC195-gp120 complex, removal of the PNGS at

Asn88_{gp120} in the gp120 core, which may have allowed for a closer association of 8ANC195 and gp120 in the crystal structure, and/or a small conformational change in the gp120 region of the trimer to accommodate the Fab orientation trapped by crystallization.

5 EXAMPLE 4

Neutralization and binding assays

The EM reconstruction highlighted a potential role for 8ANC195 LC contacts to gp41. To assess LC contacts with trimeric Env, chimeras consisting of the 8ANC195 HC paired with different Lcs were tested in neutralization and binding assays. The chimeras
10 included a full germline LC, a mature LC with individual CDR loops reverted to their germline sequences or CDRL3 partially mutated to alanines, or the LC from the CD4 binding site antibody 3BNC117 (Fig. 19A). As expected from the crystal structure in which all gp120 contacts were made by the 8ANC195 HC, the chimeras bound normally to gp120 core and to a full-length 93TH057 gp120 (Fig. 19B, table 3), thus changes in the
15 LC did not disrupt the HC portion of the antibody combining site.

Antibody	K _D (nM)		IC ₅₀ (μg/mL)							
	93TH057 gp120	YU2 gp120	YU2	Tro11	SF162	6535_3	SC4226618	PVO4	REJO4541	RHPA4259
8ANC195 IgG	33.1	82.0	0.4	0.31	0.30	0.43	0.69	0.52	0.249	0.17
8ANC195 mHC/giLC	38.0	56.1	6.76	>100	12.9	8.9	8.1	11.5	11.2	14.6
8ANC195 giCDRL1	38.0	105.4	66	>100	65	24	97	>100	62.4	0.96
8ANC195 giCDRL2	40.5	119.5	6.28	16.9	44	13.3	69	97	62.9	0.52
8ANC195 giCDRL3	44.4	107.1	0.79	0.75	0.49	1.06	1.29	3.1	1.045	0.27
8ANC195 CDRL3Ala	ND	ND	39	ND	>100	>100	>100	>100	>100	0.81
8ANC195HC/3BNC117LC	ND	ND	4.77	5.33	5.6	>100	22	ND	10.9	4.52
3BNC80	ND	ND	0.027	0.04	0.05	0.33	0.07	0.06	0.063	0.02

Table 3

In contrast to gp120 binding, neutralization potencies assayed against native Env
20 spike trimers were decreased by changes in the 8ANC195 LC. For example, reverting CDRL1 and CDRL2 sequences to germline precursor sequences (changing 3 of 7 and 3 of

3 residues, respectively) almost completely abrogated neutralization of YU2, an 8ANC195-sensitive strain. Changes to CDRL3 led to a moderate reduction in neutralization potency, as did substituting the 3BNC117 LC for the cognate LC (Fig. 19B, table 3). A chimeric IgG with one of the most conservatively-substituted LCs (Thr–Gly–Asn, mature CDRL1 containing a one-residue insertion, reverted to Ser–Ser, germline CDRL1) displayed unchanged binding to gp120, yet showed reductions in neutralization potency of up to 250-fold. Similarly, conservative changes in CDRL2 (Arg–Gly–Ala, the mature CDRL2, reverted to the germline Lys–Ala–Ser sequence) caused large reductions in neutralization potencies but had little effect on gp120 binding. Overall the data showed differential sensitivities of the binding and neutralization assays to changes in the 8ANC195 LC that were distant from the gp120 surface, which supported the EM results suggesting that LC, and CDRL1 and CDRL2 in particular, contacted gp41.

EXAMPLE 5

Isolation of Antibodies

To further investigate Env recognition by 8ANC195, additional members of this antibody clone were isolated from the original donor by single cell sorting using gp120 stabilized in the CD4-bound conformation (2CC core) as bait (Fig. 9). From 1536 single 2CC core-binding B cells, 10 (0.7%) were clonally related to 8ANC195, and of these, only four differed slightly from the two previously-described members (1 to 3 and 1 to 7 residue differences in the HCs and LCs, respectively) (Fig. 10). Consistent with the limited sequence diversity, these antibodies exhibited similar potencies to 8ANC195 in neutralization assays against a panel of 15 Tier 2 viruses (Fig. 9C and Table 4).

Virus	8ANC3040	8ANC3484	8ANC3630	8ANC3044	8ANC3430	8ANC195
REJO4541.57	0.198	0.117	2.652	0.278	0.198	0.08
PVO.4	0.284	0.077	0.102	0.260	0.206	0.52
YU2.DG	0.617	0.461	0.468	0.747	0.545	0.79
3415.v1.c1	3.059	0.589	27.977	7.557	>23	2.404
3365.v2.c20	>25	>30	>30	>30	>23	>30
ZM53M.PB12	14.910	11.581	>30	15.164	>23	9.626
ZM109F.PB4	NT	>30	>30	>30	>23	>30
3016.v5.c45	0.427	0.131	0.138	0.242	0.271	0.195
231985.c1	1.174	0.294	0.375	1.332	1.190	0.514
X1254_c3	2.909	2.192	2.377	4.538	4.284	1.524
251-18	0.571	0.391	0.730	0.858	6.170	0.284
R1166.c1	2.370	1.027	1.453	2.381	3.642	0.986
H086.8	NT	0.394	0.300	3.830	>23	0.095
Du172.17	NT	4.011	>30	>30	>23	10.797
250-4	NT	>30	>30	>30	>23	>50
MuLV	>30	>30	>30	>30	>23	>23

Table 4

Reasoning that the 2CC core bait might fail to capture some 8ANC195 family members, clone-specific primers were used to amplify 8ANC195 variants from purified populations of CD19+ IgG+ memory B cells (Fig. 11). 128 HC and 100 LC sequences were obtained that were clonally related to 8ANC195 and displayed greater sequence diversity than antibodies obtained using antigen-specific selection (Figs. 10, 12). Of the 13 HC and 6 LC genes exhibiting greatest diversity, all combinations were co-transfected in order to evaluate their neutralizing activity against a 15-member Tier 2 virus panel. 3 of 39 (7.7%) new antibodies were at least as broad and potent as 8ANC195 (Fig. 19C and Table 5).

	y3	y4	y8	y15	y20	y22	y23	y44	y46	y52	y59
Virus	y3c3	y4c3	y8c3	y15c3	y20c3	y22c3				y52c3	y59c3
RE_JC4541.07	0.280	>15	>15	>15	>15	>15				>15	8.543
PvC1.4	0.179	>15	>15	5.316	>15	>15				1.918	>15
Y112.DG	0.420	>15	5.418	1.088	>15	>15				5.751	8.978
34>15.v1.c1	>15	>15	>15	>15	>15	>15				>15	>15
3385.v2.c29	>15	>15	>15	>15	>15	>15				>15	>15
ZM63M.PB12	>15	>15	>15	>15	>15	>15				>15	>15
ZM100F.PB4	>15	>15	>15	>15	>15	>15				>15	>15
3015.v5.c45	0.347	>15	2.383	1.268	>15	>15				3.850	>15
Z31905.c1	0.727	>15	3.477	1.611	>15	>15				6.584	>15
X1254.c3	1.822	18.047	4.689	3.487	14.782	>15				>15	3.392
253.18	>15	>15	>15	>15	>15	>15				14.557	>15
R1106.c1	2.208	>15	7.596	3.843	>15	>15				23.408	24.226
1880.8	3.307	>15	>15	>15	>15	>15				>15	>15
1kn172.17	>15	>15	>15	>15	>15	>15				>15	>15
258.4	>15	>15	>15	>15	>15	>15				>15	>15
NAcV	>30	NT	>15	>30	>21	NT				>30	>30

Virus	y3c5
RE_JC4541.07	0.897
PvC1.4	0.081
Y112.DG	0.209
34>15.v1.c1	>15
3385.v2.c29	>15
ZM63M.PB12	>15
ZM100F.PB4	>15
3015.v5.c45	0.314
Z31905.c1	0.897
X1254.c3	1.717
253.18	0.721
R1106.c1	2.074
1880.8	0.434
1kn172.17	3.726
258.4	>15
NAcV	>15

	y22c5	y23c5
	0.795	0.981
	0.352	0.943
	0.569	0.135
	>15	1.479
	>15	>15
	>15	5.876
	>15	>15
	0.103	0.091
	0.880	0.291
	1.821	0.919
	1.698	0.176
	2.388	1.075
	>15	0.474
	>15	1.814
	>15	>15
	>30	>30

	y46c3	y52c3	y59c3
	0.198	0.935	4.865
	0.329	0.818	>15
	0.359	0.885	7.278
	>15	0.128	>15
	>15	>15	>15
	12.482	3.134	24.057
	>15	>15	>15
	5.111	0.817	>15
	0.525	0.894	>15
	1.782	0.884	5.416
	0.669	0.848	6.859
	0.922	0.319	28.291
	1.768	0.178	>15
	>15	NT	>15
	>15	>15	>15
	NT	NT	>30

Virus	y3c11
RE_JC4541.07	0.891
PvC1.4	0.074
Y112.DG	0.275
34>15.v1.c1	>15
3385.v2.c29	>15
ZM63M.PB12	>15
ZM100F.PB4	>15
3015.v5.c45	0.143
Z31905.c1	0.455
X1254.c3	2.308
253.18	>15
R1106.c1	1.792
1880.8	0.773
1kn172.17	>15
258.4	>15
NAcV	>30

	y8c11	y15c11	y20c11	y22c11	y23c11	y44c11	y46c11	y52c11	y59c11
	>15	>15	>15	>15	0.140	>15	>15	3.473	1.572
	8.153	6.764	>15	>15	0.188	>15	2.521	0.193	>15
	9.349	4.122	>15	>15	0.348	>15	2.101	0.298	6.911
	>15	>15	>15	>15	>15	>15	>15	>15	>15
	>15	>15	>15	>15	>15	>15	>15	>15	>15
	>15	>15	>15	>15	>15	>15	>15	>15	>15
	>15	>15	>15	>15	>15	>15	>15	>15	>15
	2.483	1.325	>15	>15	0.159	>15	1.823	0.161	>15
	2.495	5.103	>15	>15	0.543	>15	2.953	0.298	>15
	4.144	13.687	7.583	>15	1.696	>15	5.662	2.419	4.865
	>15	>15	>15	>15	2.496	>15	>15	0.547	>15
	3.883	9.813	26.237	>15	1.611	>15	3.390	1.159	12.580
	>15	>15	>15	>15	>15	>15	>15	1.638	>15
	>15	>15	>15	>15	11.288	>15	>15	>15	>15
	>15	>15	>15	>15	>15	>15	>15	>15	>15
	NT	>30	>30	>30	>30	>15	>30	>30	>30

Table 5

	Y3	Y4	Y8	Y15	Y20	Y22	Y23	Y44	Y46	Y52	Y59
Virus	Y3K18	Y4K18		Y15K18	Y20K18	Y22K18	Y23K18		Y46K18	Y52K18	Y59K18
RE-NC4541.87	0.048	>15		>15	>15	>15	0.061		>15	0.132	1.227
PvO.4	0.008	>15		1.088	>15	>15	0.047		0.100	0.001	>15
Y12.1G	0.078	>15		1.168	>15	8.229	0.117		2.360	0.288	7.787
34x15.v1.c1	>15	>15		>15	>15	>15	>15		>15	>15	>15
3365.v2.c20	>15	>15		>15	>15	>15	>15		>15	>15	>15
ZM1381.P812	>15	>15		>15	>15	>15	>15		>15	>15	>15
ZM1381.P814	>15	>15		>15	>15	>15	>15		>15	>15	>15
3018.v5.c45	0.057	>15		0.064	>15	13.380	0.047		2.993	0.129	>15
231905.c3	0.163	>15		1.481	>15	26.888	0.128		4.167	0.264	>15
X1254.c3	0.618	16.224		2.849	5.221	1.563	0.548		4.282	1.567	4.066
251.18	>15	>15		>15	>15	>15	1.670		>15	0.118	25.839
D1306.c3	0.579	>15		1.988	29.036	7.311	0.708		4.098	1.351	11.701
H880.8	0.289	>15		>15	>15	>15	>15		>15	0.484	>15
De172.17	11.818	>15		>15	>15	>15	2.953		>15	>15	>15
250.4	>15	>15		>15	>15	>15	>15		>15	>15	>15
888.V	>15	>15		NT	>15	>15	>15		>15	>15	>15

Virus	Y20K19
RE-NC4541.87	>15
PvO.4	>15
Y12.1G	>15
34x15.v1.c1	>15
3365.v2.c20	>15
ZM1381.P812	>15
ZM1381.P814	>15
3018.v5.c45	>15
231905.c3	>15
X1254.c3	2.981
251.18	>15
D1306.c3	>15
H880.8	>15
De172.17	>15
250.4	>15
888.V	>15

Virus	Y15K01	Y46K01	Y52K01	Y59K01
RE-NC4541.87	2.029	>15	0.378	0.115
PvO.4	1.236	>15	0.244	0.078
Y12.1G	3.446	>15	0.918	0.406
34x15.v1.c1	>15	>15	>15	6.681
3365.v2.c20	>15	>15	>15	>15
ZM1381.P812	>15	>15	27.433	8.435
ZM1381.P814	>15	>15	>15	>15
3018.v5.c45	1.314	>15	0.383	0.182
231905.c3	5.231	>15	1.576	0.618
X1254.c3	16.884	>15	4.488	2.668
251.18	4.656	>15	1.842	0.273
D1306.c3	0.548	>15	2.578	1.836
H880.8	>15	>15	0.884	0.242
De172.17	>15	>15	36.883	7.275
250.4	>15	>15	>15	>15
888.V	>15	>15	>15	>15

Table 5 (continued)

Of these, γ 52_{HC5_{LC}} was 5-fold more potent than 8ANC195 (neutralized 12 of 15 viruses with a mean IC₅₀ of 0.45 μ g/ml as compared to 2.3 μ g/ml for 8ANC195) (Fig. 13), a potency and breadth against this virus panel that was comparable to those of other bNAbs, such as VRC01 (neutralized 12 of 15 viruses with a 0.56 μ g/ml mean IC₅₀) and 10-1074 (neutralized 6 of 15 viruses with a mean of 0.09 μ g/ml), that target non-

overlapping sites (Wu et al., *Science* 329, 856 (2010); Mouquet et al., *Proceedings of the National Academy of Sciences of the United States of America* 109, E3268 (2012)).

The LC was critical to the activity of more potent $\kappa 52_{\text{HCK}5_{\text{LC}}}$ variant, as demonstrated by diminished neutralization potencies when $\kappa 5_{\text{LC}}$ was swapped for either $\kappa 3_{\text{LC}}$ or $\kappa 11_{\text{LC}}$ (Fig. 19C). The weaker neutralization could be explained by differences between $\kappa 5_{\text{LC}}$ and $\kappa 3_{\text{LC}}$ at solvent-exposed residues in CDRL2 (53_{LC} and 54_{LC}) and FWRL3 (64_{LC}), and a nearby buried residue (34_{LC}) that may affect the structural integrity of CDRL1. Modeling of YU2 gp41 residues into the Env trimer structure (Julien et al., *Science* 342, 1477 (2013)) suggested that 8ANC195 positions 53_{LC} and 54_{LC} were adjacent to the Asn637_{gp41} PNGS (Figs. 8, 14). The improved neutralizing activity of $\kappa 5_{\text{LC}}$ compared with the other newly-isolated LCs was associated with small side chains at positions 34_{LC} (Val), 53_{LC} (Ala) and 54_{LC} (Ala), whereas $\kappa 3_{\text{LC}}$ or $\kappa 11_{\text{LC}}$, which were less broadly neutralizing when paired with identical HCs, included bulkier and/or charged side chains that would clash with the nearby gp41 glycan. $\kappa 5_{\text{LC}}$ was the only LC containing an S64R_{LC} substitution and this single change compared to the 8ANC195 LC may account for the 5-fold improved potency of $\kappa 52_{\text{HCK}5_{\text{LC}}}$. Residues in the immediate vicinity of Asn637_{gp41} might also modify neutralization; all six viral strains that were potentially neutralized by the $\kappa 52_{\text{HCK}5_{\text{LC}}}$ variant had Asp636_{gp41} or Asn636_{gp41} whereas the remaining eight strains had Ser636_{gp41} ($p < 0.001$ by G-test). The same association between Asp636_{gp41}/Asn636_{gp41} and neutralization potency was also statistically significant for 8ANC195 ($p < 0.01$ by G-test), consistent an interaction between the N-terminal portion of gp41 HR2 (residues ~625 to 640) and 8ANC195 LC (Fig. 8). Also consistent with changes in the gp41 HR2 region affecting 8ANC195 neutralization, a computational analysis of neutralization panel data using the Antibody Database program (West, Jr. et al., *Proceedings of the National Academy of Sciences of the United States of America* 110, 10598 (2013)) suggested that Glu632_{gp41} was associated with stronger neutralization.

In conclusion, 8ANC195 defines a novel site of HIV-1 Env vulnerability to neutralizing antibodies that spans gp120 and gp41 (Fig. 15). Rather than penetrating the glycan shield using only a single CDR loop, a strategy employed by antibodies such as PG9 and PGT128 (Pejchal et al., *Science* 334, 1097 (2011); McLellan et al., *Nature* 480, 336 (2011)) 8ANC195 inserted its entire HC variable region into a gap in the shield to form a large interface, of which >50% involved contacts to gp120 glycans (Fig. 17).

Although it was not possible to obtain large numbers of 8ANC195 variants by standard single cell cloning techniques (Scheid et al., *J Immunol Methods* 343, 65 (2009)), randomly combining HCs and LCs obtained from memory B cells without antigen-specific sorting demonstrated that the target of this antibody supported neutralization activity
5 comparable to that against the most vulnerable sites on Env. Potent variants of 8ANC195 are particularly since the epitope does not overlap with the targets of CD4 binding site, V2 loop, V3 loop or MPER antibodies.

The foregoing examples and description of the preferred embodiments should be taken as illustrating, rather than as limiting the present invention as defined by the claims.
10 As will be readily appreciated, numerous variations and combinations of the features set forth above can be utilized without departing from the present invention as set forth in the claims. Such variations are not regarded as a departure from the scope of the invention, and all such variations are intended to be included within the scope of the following claims. All references cited herein are incorporated herein by reference in their entireties.

CLAIMS

WHAT IS CLAIMED IS:

1. An isolated anti-HIV-1 antibody comprising one or both of a heavy chain comprising the sequence of any one of SEQ ID NOs: 1-18 and a light chain comprising the sequence
5 of any one of SEQ ID NOs: 19-33.
2. The isolated antibody of Claim 1 wherein the heavy chain comprises the sequence of SEQ ID NO:1 and the light chain comprises the sequence of SEQ ID NO:20.
3. The isolated antibody of Claim 1 wherein the antibody is a human antibody.
4. The isolated antibody of Claim 1 wherein the antibody is a chimeric antibody.
- 10 5. An isolated polypeptide comprising the sequence of any one of SEQ ID NOs: 1-33.
6. An isolated nucleic acid encoding the antibody of Claim 1 or the polypeptide of Claim 5.
7. A vector comprising the isolated nucleic acid of Claim 6.
8. A cultured cell comprising the vector of Claim 7.
- 15 9. A composition comprising the antibody of Claim 1 or a fragment thereof that binds to the 8ANC195 epitope of the HIV-1 envelope spike.
10. A method of preventing or treating an HIV-1 infection in a subject in need thereof comprising administering to said subject the composition of Claim 9 in an amount effective to prevent or treat said HIV-1 infection.
- 20 11. An isolated antigen comprising an epitope-scaffold that mimics the HIV-1 envelope spike epitope of broadly neutralizing antibody 8ANC195.
12. The antigen of Claim 11 wherein the epitope-scaffold comprises a discontinuous epitope and a scaffold, wherein the epitope is derived from HIV-1 gp120 and gp41, and wherein at least part of the scaffold is not derived from gp120 or gp41.
- 25 13. The antigen of Claim 12 wherein the discontinuous epitope comprises amino acids corresponding to amino acid numbers 44-47, 90-94, 97, 234, 236-238, 240, 274-278, 352-

354, 357, 456, 463, 466, 487, and 625-641 of gp140 from HIV strain 93TH057 numbered using standard numbering for HIV strain HXBC2.

14. The antigen of Claim 13 wherein the amino acids corresponding to amino acid numbers 234 and 276 are glycosylated.

5 15. An isolated nucleic acid encoding the antigen of Claim 11.

16. A vector comprising the isolated nucleic acid of Claim 15.

17. A cultured cell comprising the vector of Claim 16.

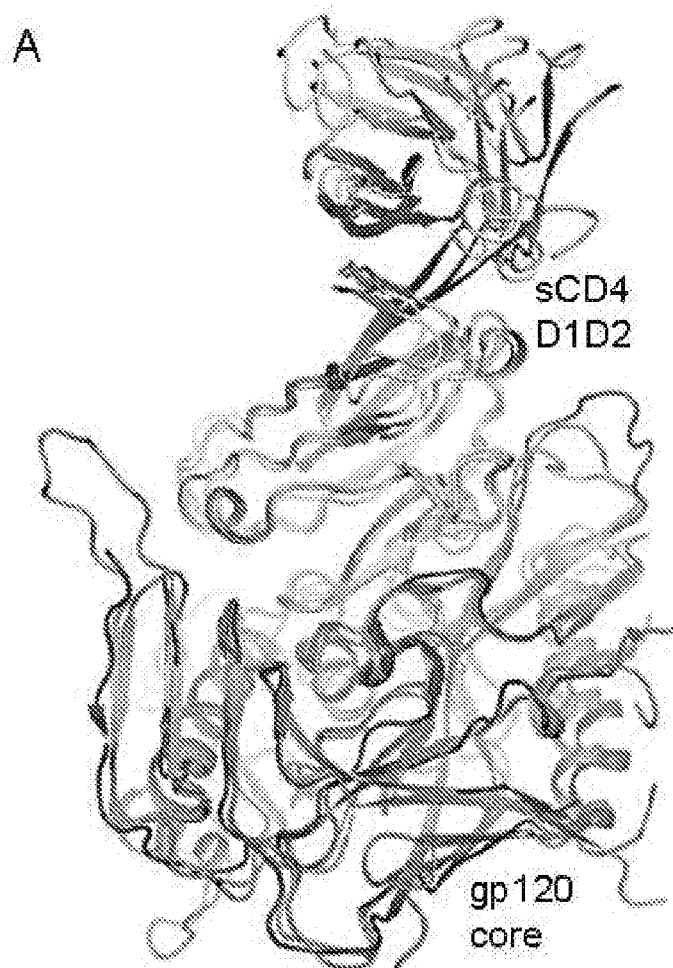
18. A composition comprising the antigen of Claim 11.

10 19. A method for inducing an HIV antigen-specific immune response in a subject in need thereof, comprising administering to said subject the composition of Claim 18 in an amount effective to generate an immune response.

20. A method of preventing or treating an HIV-1 infection in a subject in need thereof comprising administering to said subject the composition of Claim 19 in an amount effective to generate an immune response.

15 21. A method for detecting or isolating an HIV-1 binding antibody in a subject comprising obtaining a biological sample from said subject, contacting said sample with the antigen of Claim 11, and conducting an assay to detect or isolate an HIV-1 binding antibody.





From complex with 8ANC195 Fab

From complex with 17b Fab (PDB 1GCI)

From complex with 21c Fab (PDB 3LQA)

FIG. 3A

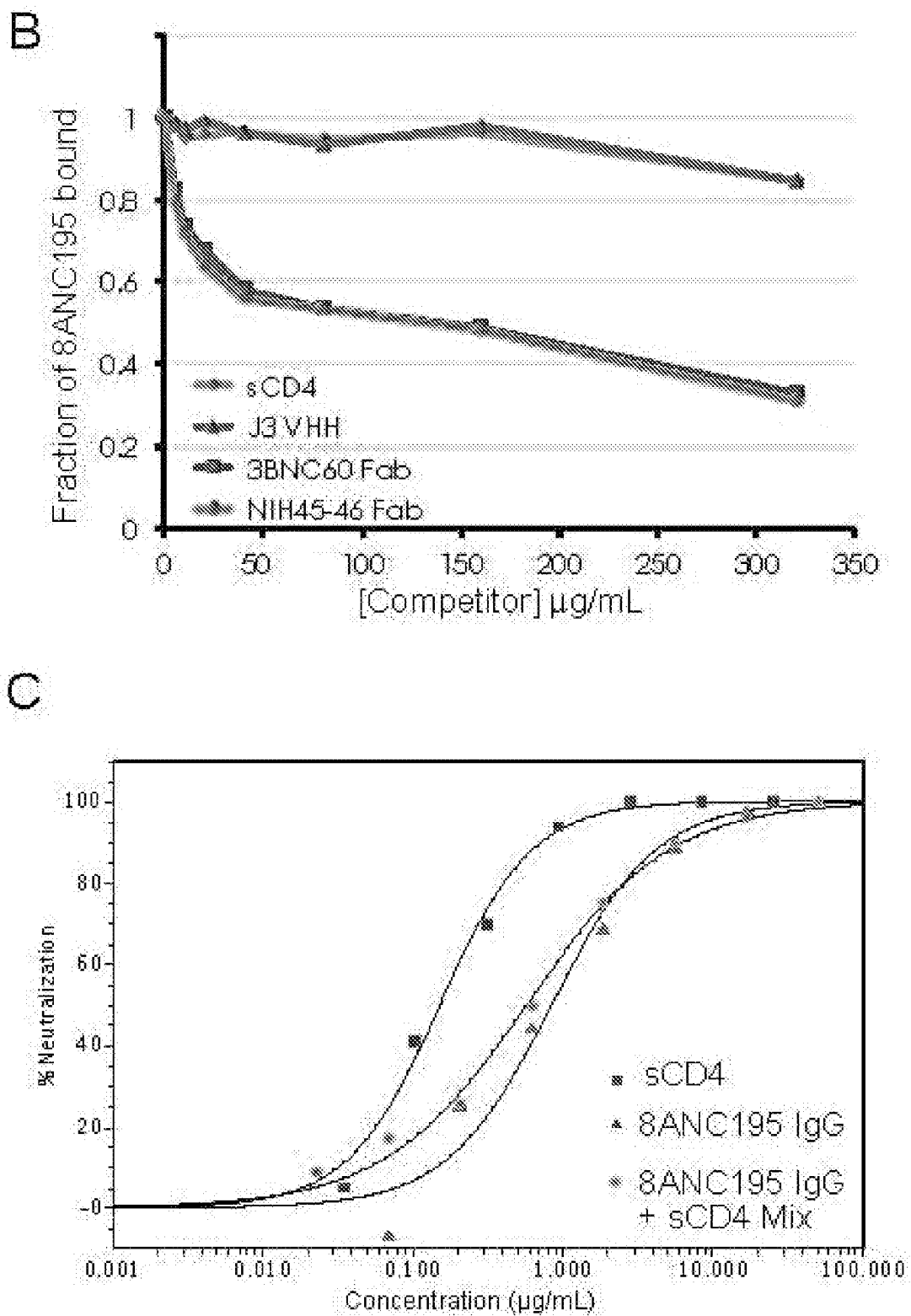


FIG. 3B-C

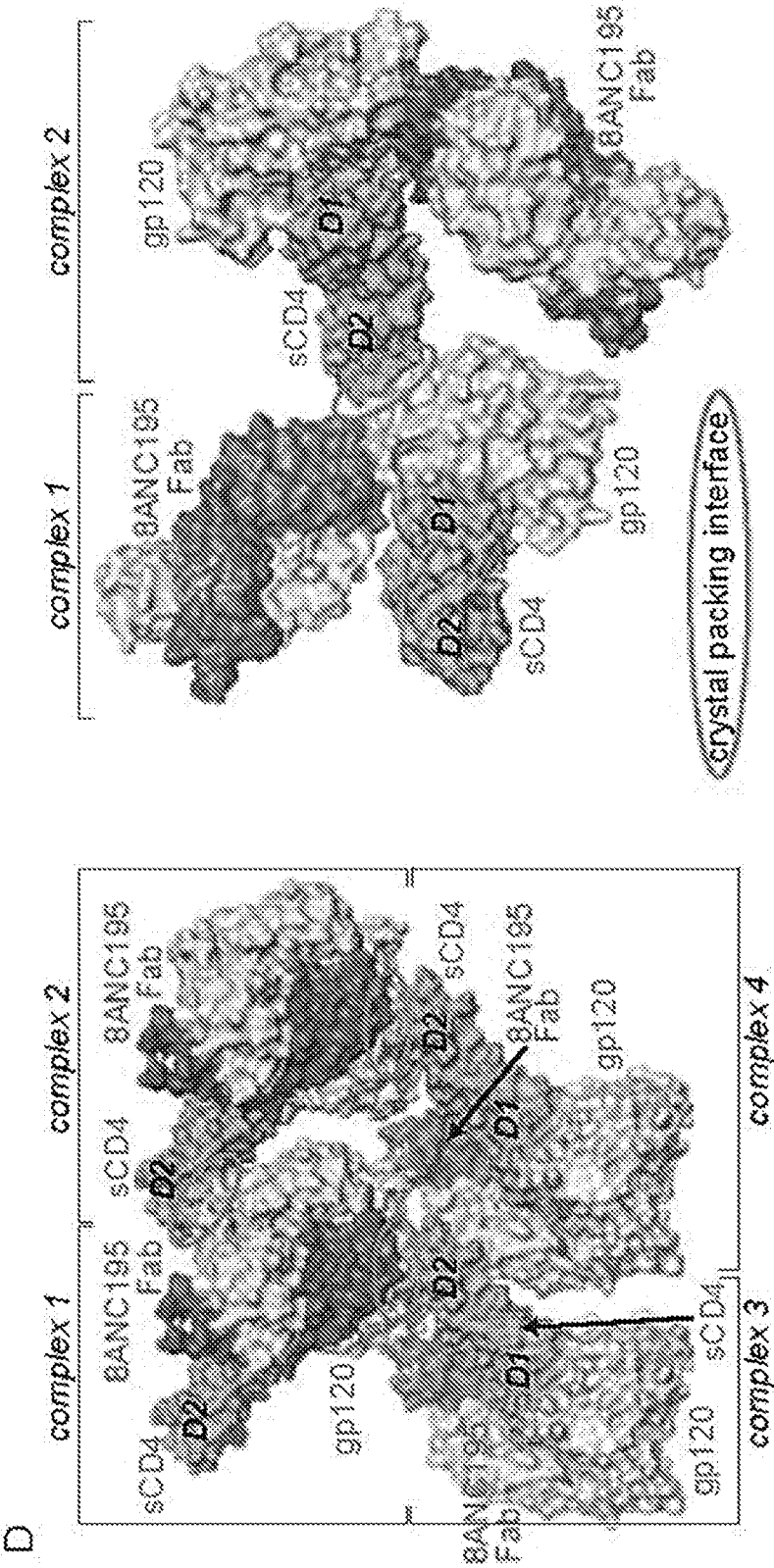


FIG. 3D

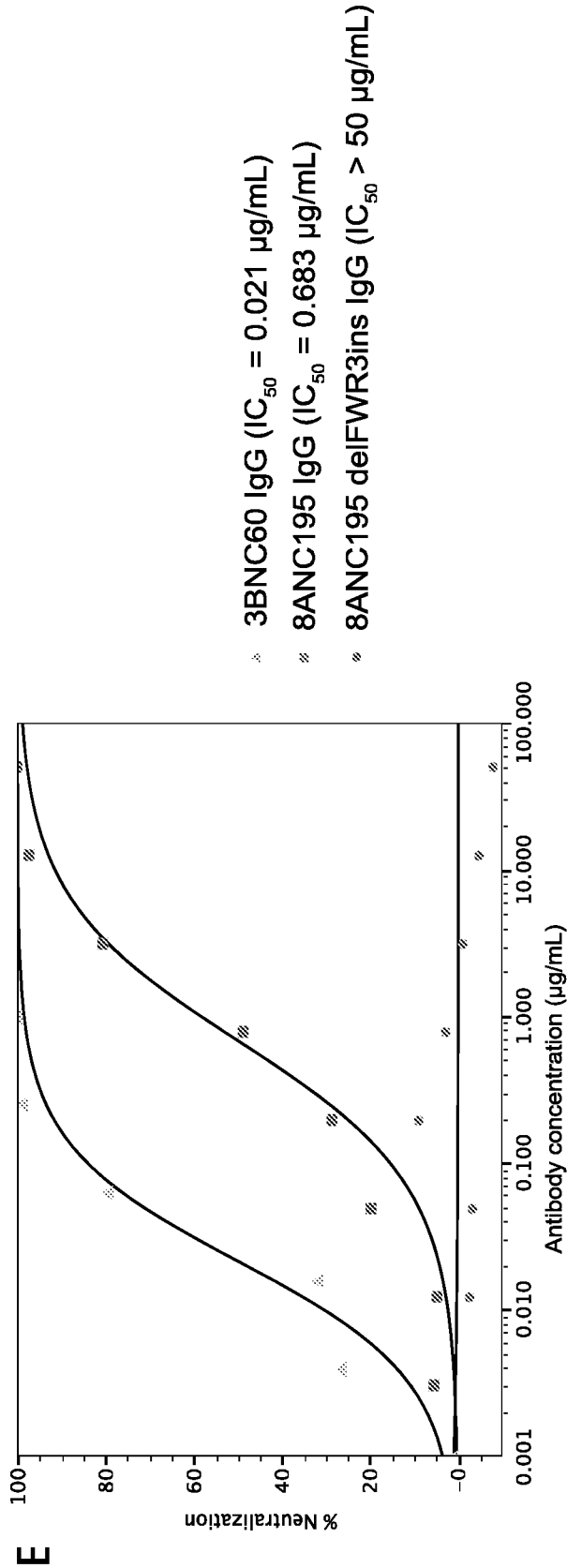
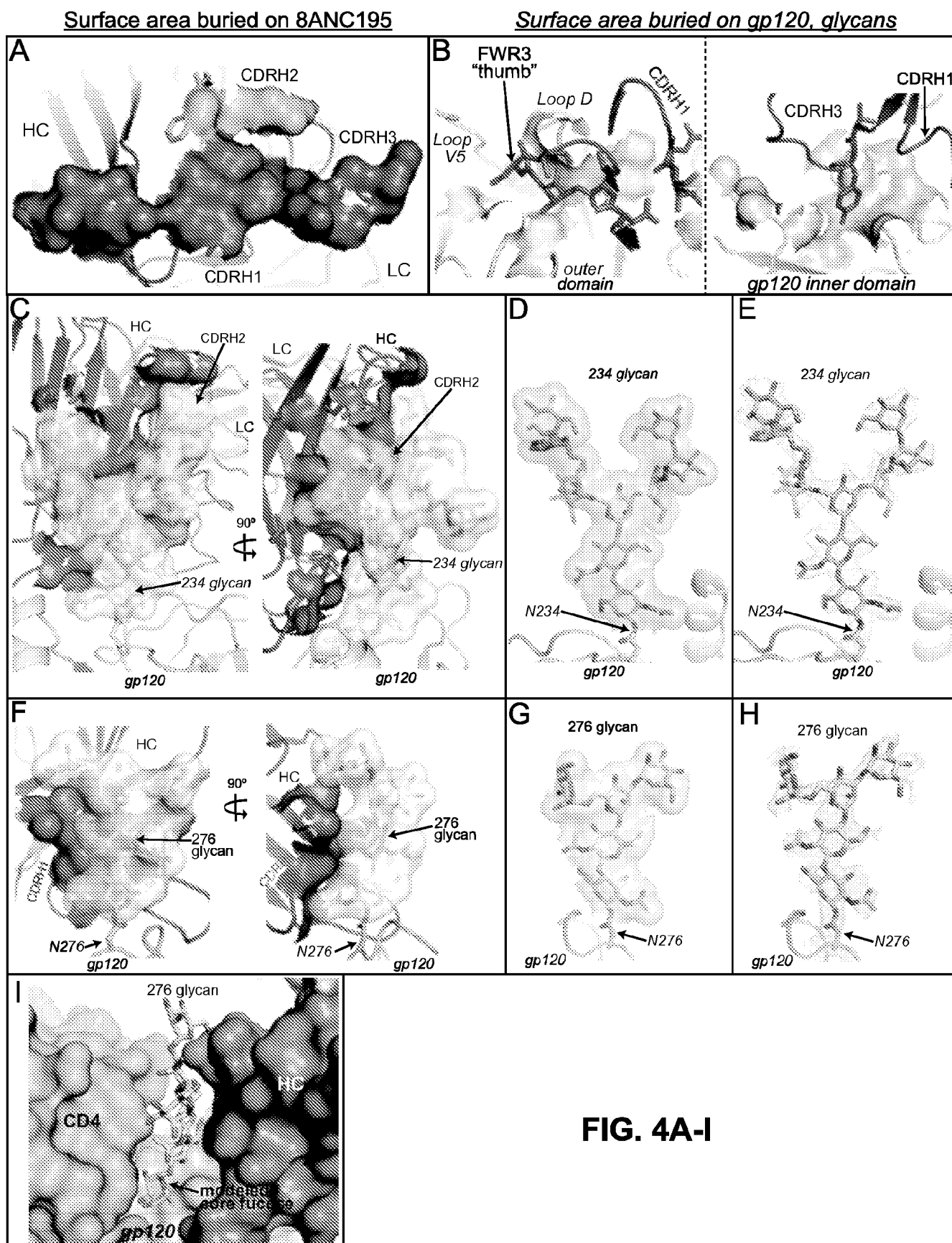
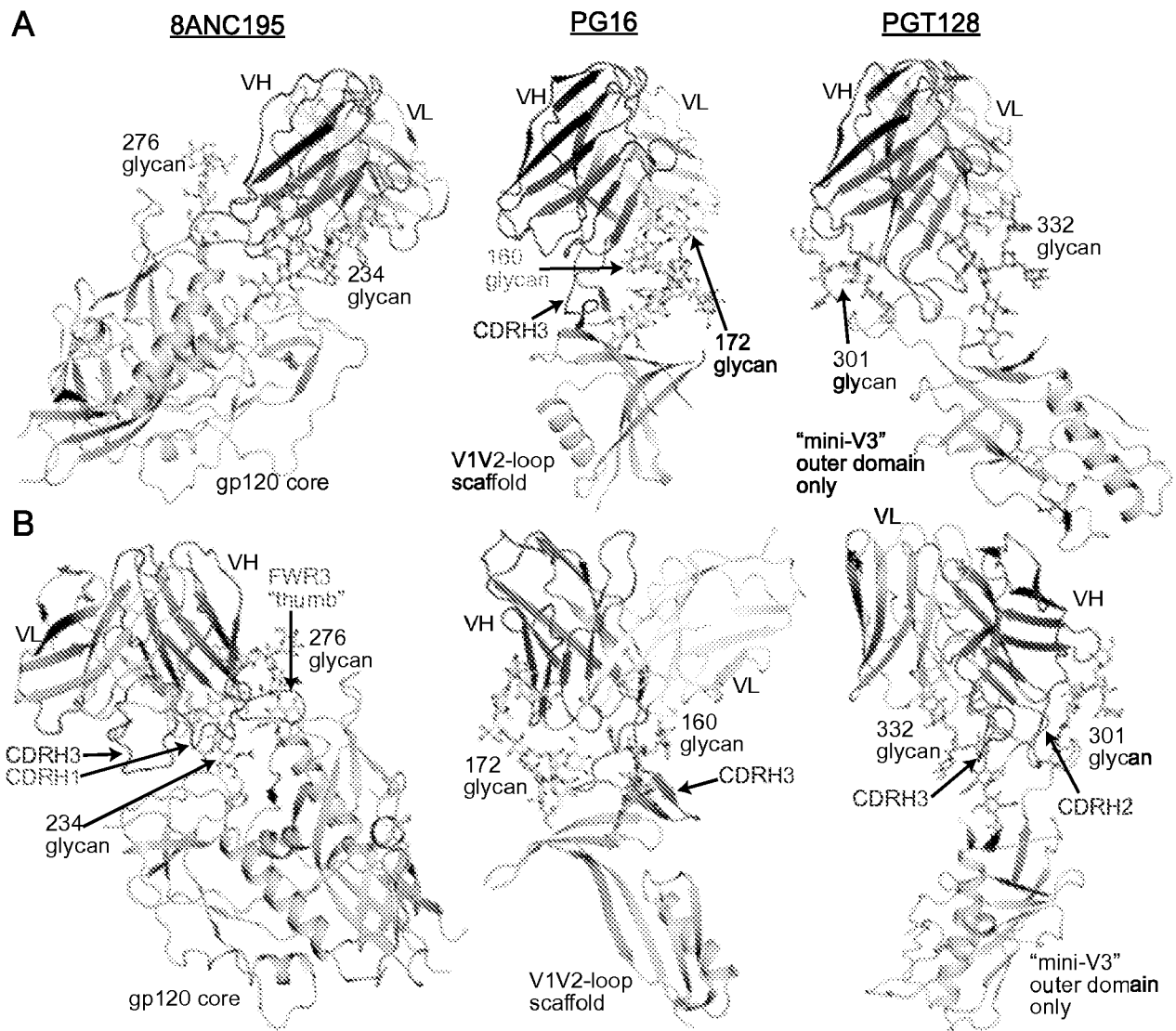


FIG. 3E



**FIG. 5A-B**

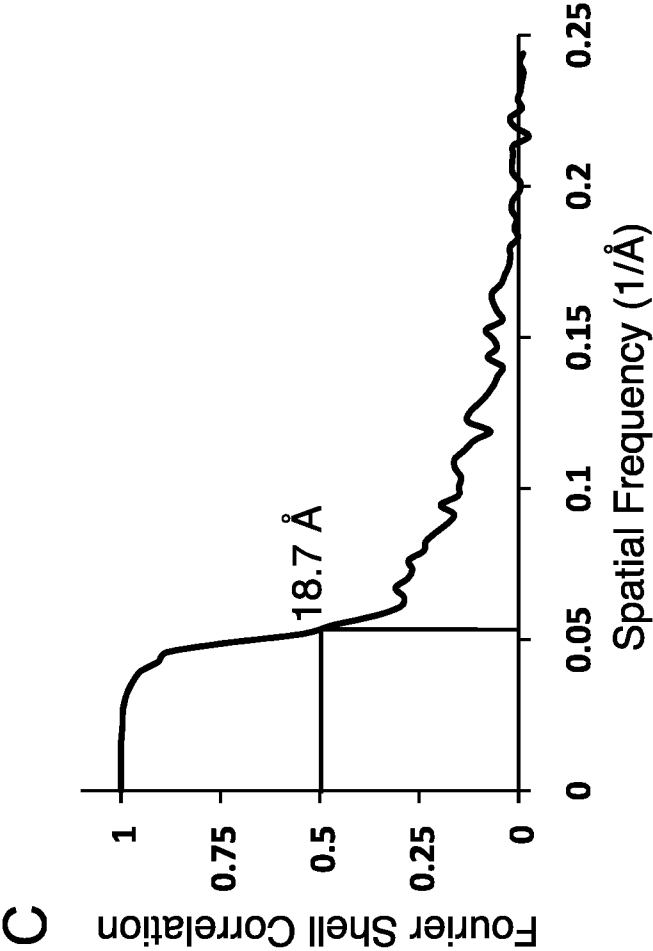
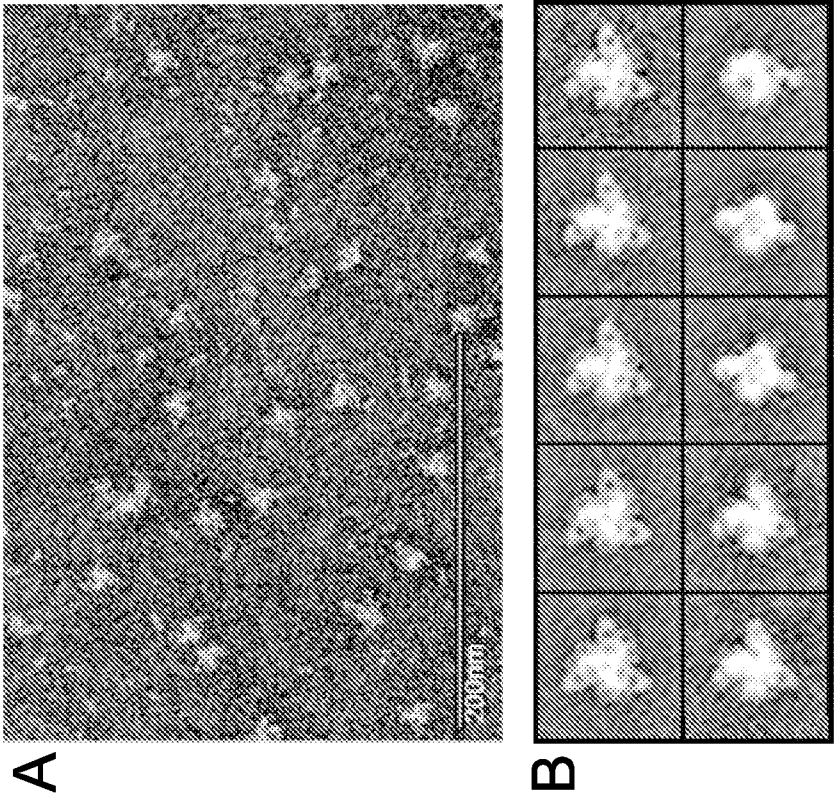
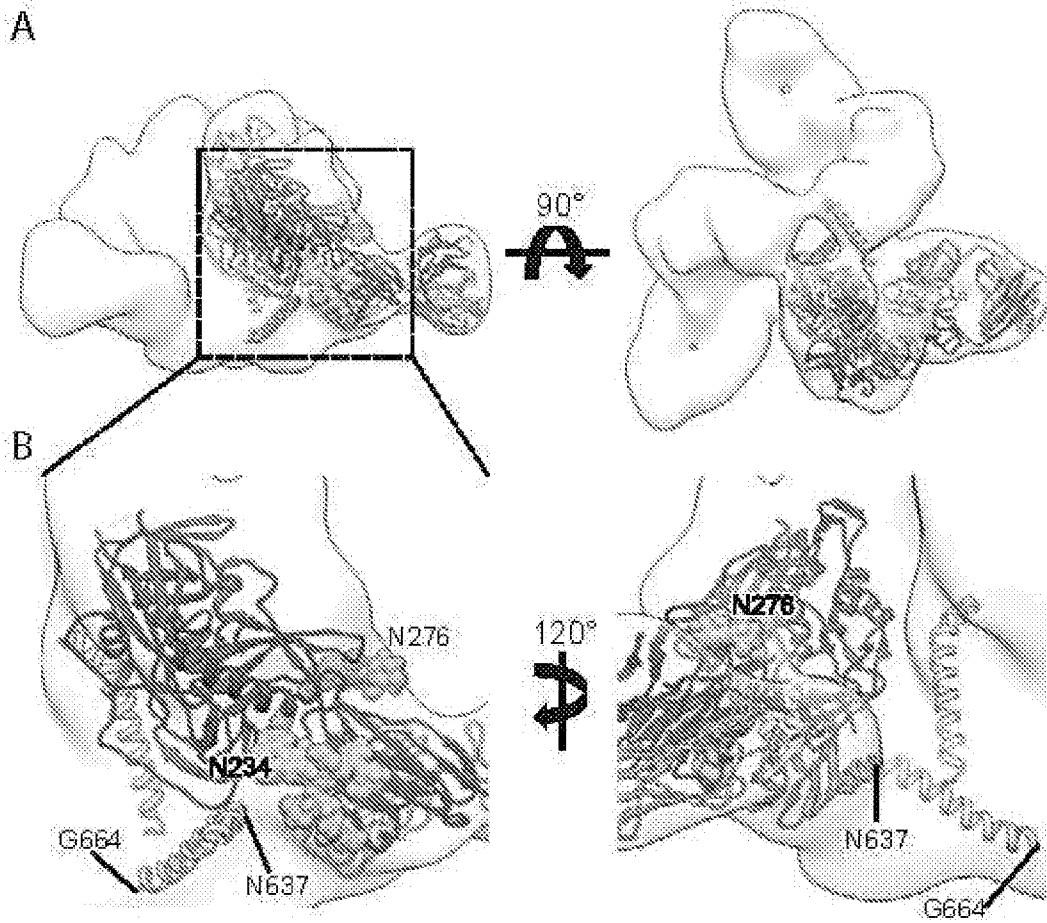


FIG. 6A-C

**FIG. 7A-B**

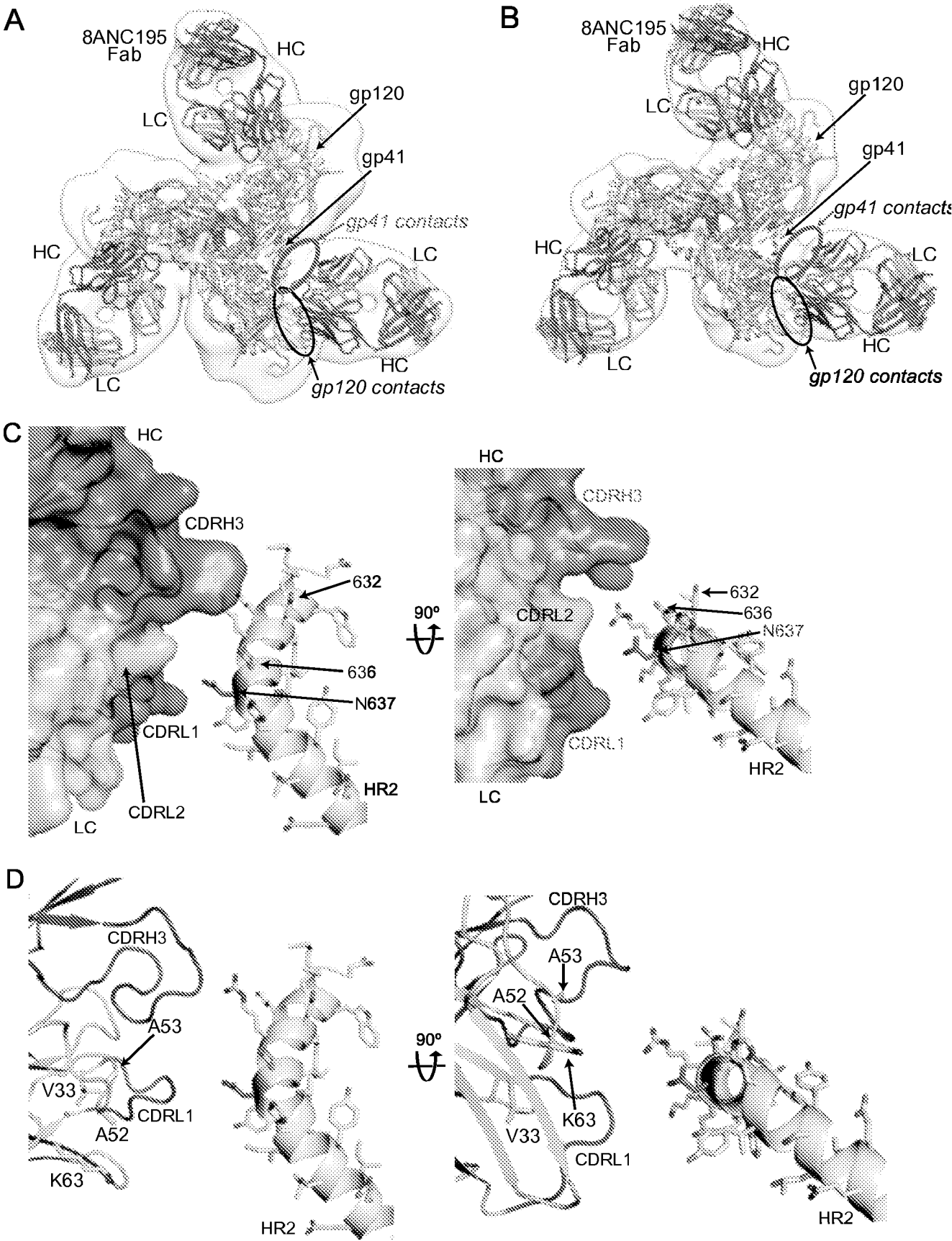
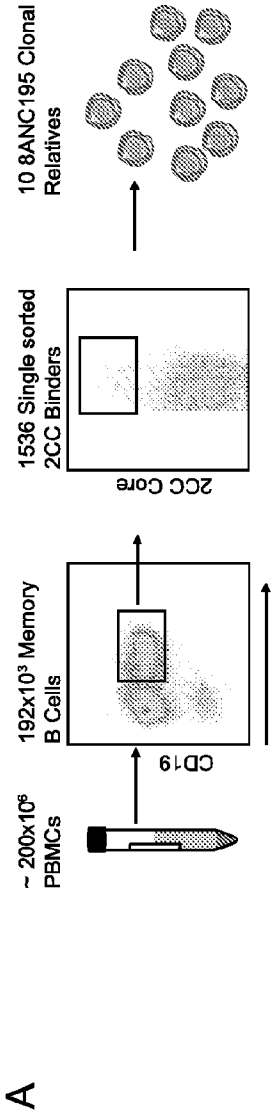


FIG. 8A-D



B

Heavy Chains

Ab name	VH	D	JH	(C)	CDR3 (aa)	(+)	Length	Mutations HC
8ANC165	1-69	3-3	ND	2	TSTVDKWSGLHHDGVMAFSS	3	20	73
8ANC200	1-69	3-3	ND	2	TSTVDKWSGLHHDGVMAFSS	3	20	71
8ANC3393	1-69	3-3	ND	2	TSTVDKWSGLHHDGVMAFSS	3	20	73
8ANC3465	1-69	3-3	ND	2	TSTVDKWSGLHHDGVMAFSS	3	20	73
8ANC3675	1-69	3-3	ND	2	TSTVDKWSGLHHDGVMAFSS	3	20	73
8ANC147	1-69	3-3	ND	2	TSTVDQWSGLHHDGVMAFSS	2	20	72
8ANC3040	1-69	3-3	ND	2	TSTVDQWSGLHHDGVMAFSS	2	20	72
8ANC3264	1-69	3-3	ND	2	TSTVDQWSGLHHDGVMAFSS	2	20	72
8ANC3430	1-69	3-3	ND	2	TSTSDYWSGLHHDGVMAFSS	2	20	71
8ANC3464	1-69	3-3	ND	2	TSTVDKWSGLHHDGVMAFSS	3	20	75
8ANC3044	1-69	3-3	ND	2	TSTVDKWSGLHHDGVMAFSS	3	20	74
8ANC3630	1-69	3-3	ND	2	TSTVDKWSGLHHDGVMAFSS	3	20	82

Light Chains

Ab name	VL	Jk/Jl	(C)	CDR3 (aa)	(+)	Length	Mutations LC	NEUT
8ANC146	1-15	1/5	1	QGYDTYPGT	0	9	41	+
8ANC200	1-15	1/5	1	QGYDTYPGT	0	9	41	+
8ANC3115	1-15	1/5	1	QGYDTYPGT	0	9	41	+
8ANC3369	1-15	1/5	1	QGYDTYPGT	0	9	41	+
8ANC3675	1-15	1/5	1	QGYDTYPGT	0	9	41	+
8ANC147	1-15	1/5	1	QGYDTYPGT	0	9	43	+
8ANC3040	1-15	1/5	1	QGYDTYPGT	0	9	43	+
8ANC3264	1-15	1/5	1	QGYDTYPGT	0	9	43	+
8ANC3430	1-15	1/5	1	QGYDTYPGT	0	9	39	+
8ANC3464	1-15	1/5	1	QGYDTYPGT	0	9	42	+
8ANC3044	1-15	1/5	1	QGYDTYPGT	0	9	40	+
8ANC3630	1-15	1/5	1	QGYDTYPGT	0	9	40	+

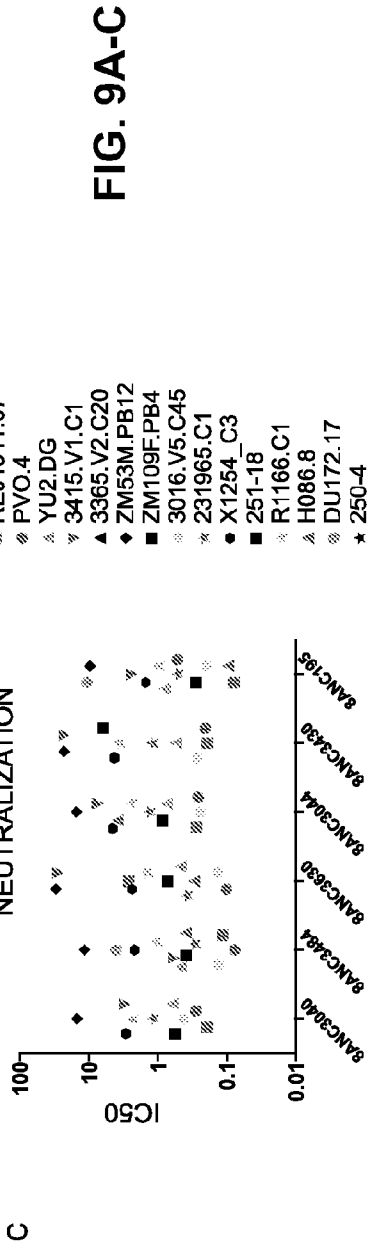
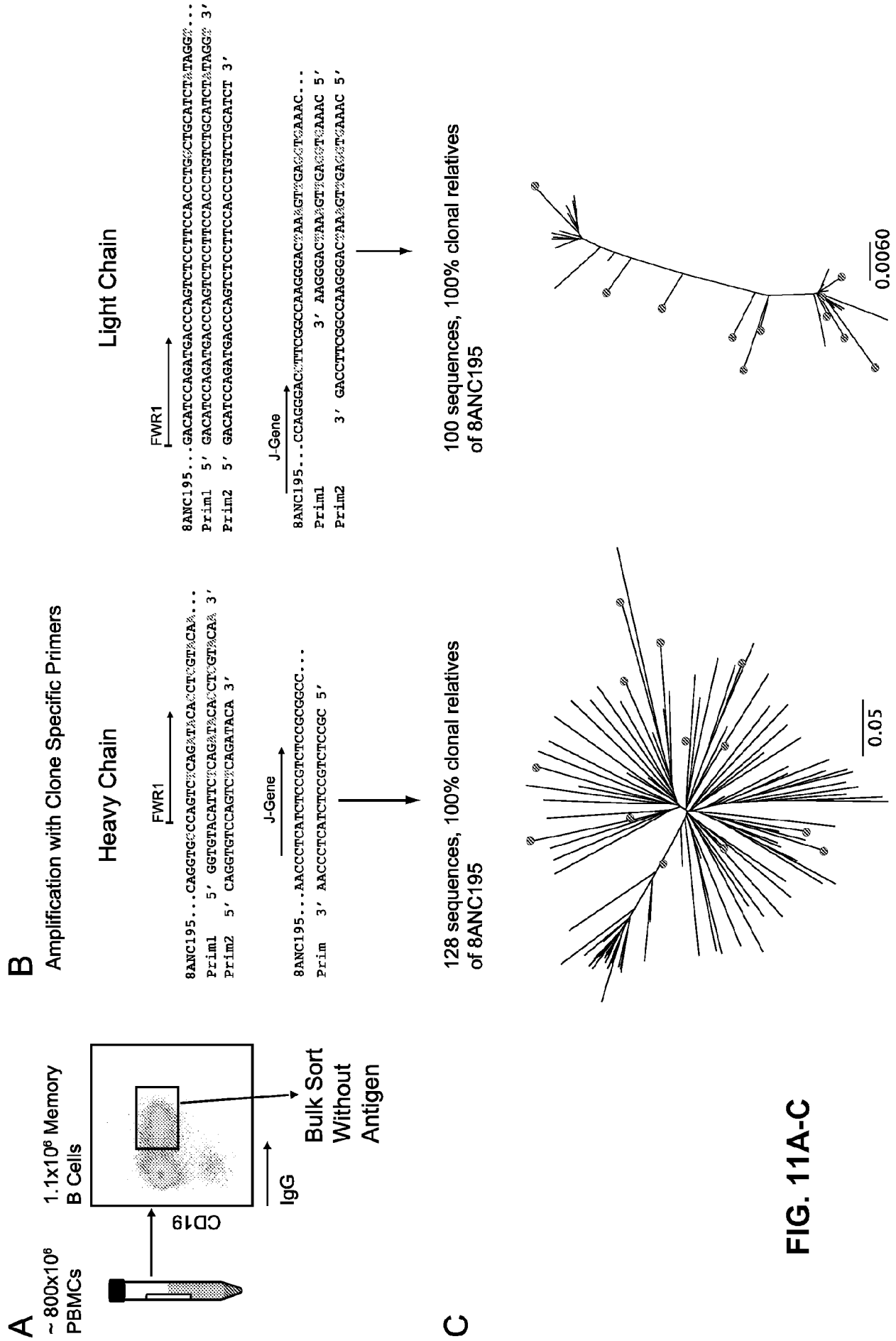
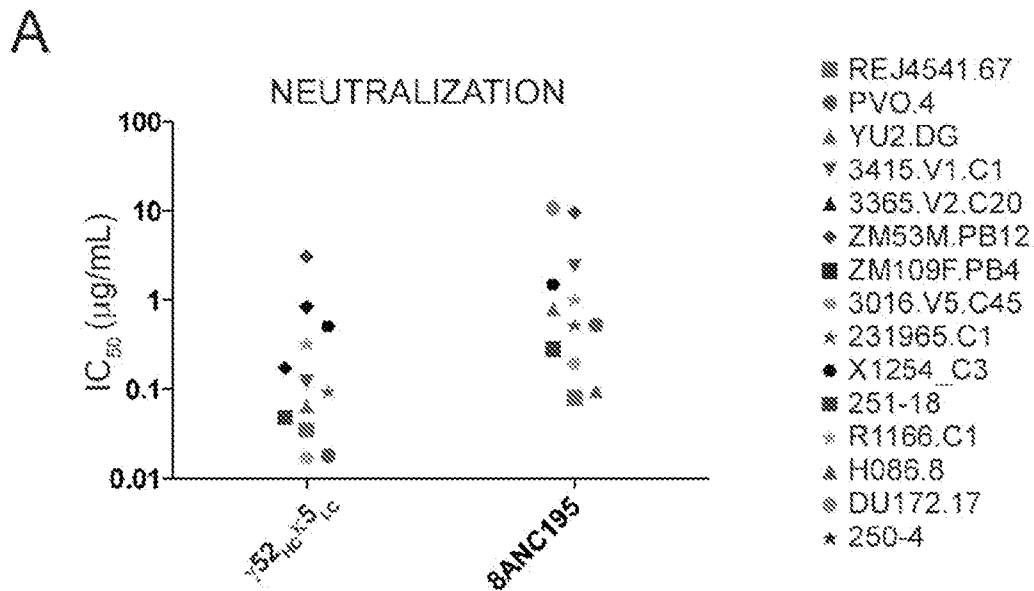


FIG. 9A-C

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D	HC	FWR1										CDR1										FWR2										CDR2										FWR3										CDR3																																										
		10	20	30	40	50	60	70	80	90	100	110	120	10	20	30	40	50	60	70	80	90	100	110	120	10	20	30	40	50	60	70	80	90	100	110	120																																																									
VH1-69GL	8ANC2080	Q	V	L	V	Q	S	G	A	E	V	K	P	G	S	S	V	K	S	C	A	S	G	T	F	S	S	A	I	S	W	R	O	A	P	G	O	G	L	E	M	M	G	L	I	P	E	G	T	A	N	A	Y	A	K	F	O	G	R	V	T	L	A	E	S	T	S	T	A	M	E	L	S	S	L	R	S	E	D	T	A	V	I	C	A	R								
	8ANC2080	Q	L	V	Q	S	G	A	E	V	K	P	G	S	S	V	K	S	C	A	S	G	T	F	S	S	A	I	S	W	R	O	A	P	G	O	G	L	E	M	M	G	L	I	P	E	G	T	A	N	A	Y	A	K	F	O	G	R	V	T	L	A	E	S	T	S	T	A	M	E	L	S	S	L	R	S	E	D	T	A	V	I	C	A	R									
	8ANC3035	Q	L	V	Q	S	G	A	E	V	K	P	G	S	S	V	K	S	C	A	S	G	T	F	S	S	A	I	S	W	R	O	A	P	G	O	G	L	E	M	M	G	L	I	P	E	G	T	A	N	A	Y	A	K	F	O	G	R	V	T	L	A	E	S	T	S	T	A	M	E	L	S	S	L	R	S	E	D	T	A	V	I	C	A	R									
	8ANC3369	Q	L	V	Q	S	G	A	E	V	K	P	G	S	S	V	K	S	C	A	S	G	T	F	S	S	A	I	S	W	R	O	A	P	G	O	G	L	E	M	M	G	L	I	P	E	G	T	A	N	A	Y	A	K	F	O	G	R	V	T	L	A	E	S	T	S	T	A	M	E	L	S	S	L	R	S	E	D	T	A	V	I	C	A	R									
	8ANC3625	Q	L	V	Q	S	G	A	E	V	K	P	G	S	S	V	K	S	C	A	S	G	T	F	S	S	A	I	S	W	R	O	A	P	G	O	G	L	E	M	M	G	L	I	P	E	G	T	A	N	A	Y	A	K	F	O	G	R	V	T	L	A	E	S	T	S	T	A	M	E	L	S	S	L	R	S	E	D	T	A	V	I	C	A	R									
	8ANC3040	Q	L	V	Q	S	G	A	E	V	K	P	G	S	S	V	K	S	C	A	S	G	T	F	S	S	A	I	S	W	R	O	A	P	G	O	G	L	E	M	M	G	L	I	P	E	G	T	A	N	A	Y	A	K	F	O	G	R	V	T	L	A	E	S	T	S	T	A	M	E	L	S	S	L	R	S	E	D	T	A	V	I	C	A	R									
	8ANC3288	Q	L	V	Q	S	G	A	E	V	K	P	G	S	S	V	K	S	C	A	S	G	T	F	S	S	A	I	S	W	R	O	A	P	G	O	G	L	E	M	M	G	L	I	P	E	G	T	A	N	A	Y	A	K	F	O	G	R	V	T	L	A	E	S	T	S	T	A	M	E	L	S	S	L	R	S	E	D	T	A	V	I	C	A	R									
	8ANC3630	Q	L	V	Q	S	G	A	E	V	K	P	G	S	S	V	K	S	C	A	S	G	T	F	S	S	A	I	S	W	R	O	A	P	G	O	G	L	E	M	M	G	L	I	P	E	G	T	A	N	A	Y	A	K	F	O	G	R	V	T	L	A	E	S	T	S	T	A	M	E	L	S	S	L	R	S	E	D	T	A	V	I	C	A	R									
	8ANC3430	Q	L	V	Q	S	G	A	E	V	K	P	G	S	S	V	K	S	C	A	S	G	T	F	S	S	A	I	S	W	R	O	A	P	G	O	G	L	E	M	M	G	L	I	P	E	G	T	A	N	A	Y	A	K	F	O	G	R	V	T	L	A	E	S	T	S	T	A	M	E	L	S	S	L	R	S	E	D	T	A	V	I	C	A	R									
	8ANC3484	Q	L	V	Q	S	G	A	E	V	K	P	G	S	S	V	K	S	C	A	S	G	T	F	S	S	A	I	S	W	R	O	A	P	G	O	G	L	E	M	M	G	L	I	P	E	G	T	A	N	A	Y	A	K	F	O	G	R	V	T	L	A	E	S	T	S	T	A	M	E	L	S	S	L	R	S	E	D	T	A	V	I	C	A	R									
8ANC3044	Q	L	V	Q	S	G	A	E	V	K	P	G	S	S	V	K	S	C	A	S	G	T	F	S	S	A	I	S	W	R	O	A	P	G	O	G	L	E	M	M	G	L	I	P	E	G	T	A	N	A	Y	A	K	F	O	G	R	V	T	L	A	E	S	T	S	T	A	M	E	L	S	S	L	R	S	E	D	T	A	V	I	C	A	R										
8ANC3509	Q	L	V	Q	S	G	A	E	V	K	P	G	S	S	V	K	S	C	A	S	G	T	F	S	S	A	I	S	W	R	O	A	P	G	O	G	L	E	M	M	G	L	I	P	E	G	T	A	N	A	Y	A	K	F	O	G	R	V	T	L	A	E	S	T	S	T	A	M	E	L	S	S	L	R	S	E	D	T	A	V	I	C	A	R										
E	LC	DI	Q	M	T	Q	S	P	S	T	L	S	A	S	V	G	D	R	V	I	T	T	C	R	A	S	Q	S	I	S	S	N	L	A	W	Y	Q	O	R	P	G	K	A	P	K	I	L	L	I	Y	D	A	S	S	L	E	S	G	V	P	S	R	F	S	G	S	G	T	E	F	T	L	T	I	S	S	L	O	P	D	D	F	A	T	Y	C	Q	O	O	N	S	Y	S	
		8ANC2080	DI	Q	M	T	Q	S	P	S	T	L	S	A	S	V	G	D	R	V	I	T	T	C	R	A	S	Q	S	I	S	S	N	L	A	W	Y	Q	O	R	P	G	K	A	P	K	I	L	L	I	Y	D	A	S	S	L	E	S	G	V	P	S	R	F	S	G	S	G	T	E	F	T	L	T	I	S	S	L	O	P	D	D	F	A	T	Y	C	Q	O	O	N	S	Y	S
		8ANC3035	DI	Q	M	T	Q	S	P	S	T	L	S	A	S	V	G	D	R	V	I	T	T	C	R	A	S	Q	S	I	S	S	N	L	A	W	Y	Q	O	R	P	G	K	A	P	K	I	L	L	I	Y	D	A	S	S	L	E	S	G	V	P	S	R	F	S	G	S	G	T	E	F	T	L	T	I	S	S	L	O	P	D	D	F	A	T	Y	C	Q	O	O	N	S	Y	S
		8ANC3369	DI	Q	M	T	Q	S	P	S	T	L	S	A	S	V	G	D	R	V	I	T	T	C	R	A	S	Q	S	I	S	S	N	L	A	W	Y	Q	O	R	P	G	K	A	P	K	I	L	L	I	Y	D	A	S	S	L	E	S	G	V	P	S	R	F	S	G	S	G	T	E	F	T	L	T	I	S	S	L	O	P	D	D	F	A	T	Y	C	Q	O	O	N	S	Y	S
		8ANC3625	DI	Q	M	T	Q	S	P	S	T	L	S	A	S	V	G	D	R	V	I	T	T	C	R	A	S	Q	S	I	S	S	N	L	A	W	Y	Q	O	R	P	G	K	A	P	K	I	L	L	I	Y	D	A	S	S	L	E	S	G	V	P	S	R	F	S	G	S	G	T	E	F	T	L	T	I	S	S	L	O	P	D	D	F	A	T	Y	C	Q	O	O	N	S	Y	S
		8ANC3040	DI	Q	M	T	Q	S	P	S	T	L	S	A	S	V	G	D	R	V	I	T	T	C	R	A	S	Q	S	I	S	S	N	L	A	W	Y	Q	O	R	P	G	K	A	P	K	I	L	L	I	Y	D	A	S	S	L	E	S	G	V	P	S	R	F	S	G	S	G	T	E	F	T	L	T	I	S	S	L	O	P	D	D	F	A	T	Y	C	Q	O	O	N	S	Y	S
		8ANC3288	DI	Q	M	T	Q	S	P	S	T	L	S	A	S	V	G	D	R	V	I	T	T	C	R	A	S	Q	S	I	S	S	N	L	A	W	Y	Q	O	R	P	G	K	A	P	K	I	L	L	I	Y	D	A	S	S	L	E	S	G	V	P	S	R	F	S	G	S	G	T	E	F	T	L	T	I	S	S	L	O	P	D	D	F	A	T	Y	C	Q	O	O	N	S	Y	S
		8ANC3630	DI	Q	M	T	Q	S	P	S	T	L	S	A	S	V	G	D	R	V	I	T	T	C	R	A	S	Q	S	I	S	S	N	L	A	W	Y	Q	O	R	P	G	K	A	P	K	I	L	L	I	Y	D	A	S	S	L	E	S	G	V	P	S	R	F	S	G	S	G	T	E	F	T	L	T	I	S	S	L	O	P	D	D	F	A	T	Y	C	Q	O	O	N	S	Y	S
		8ANC3430	DI	Q	M	T	Q	S	P	S	T	L	S	A	S	V	G	D	R	V	I	T	T	C	R	A	S	Q	S	I	S	S	N	L	A	W	Y	Q	O	R	P	G	K	A	P	K	I	L	L	I	Y	D	A	S	S	L	E	S	G	V	P	S	R	F	S	G	S	G	T	E	F	T	L	T	I	S	S	L	O	P	D	D	F	A	T	Y	C	Q	O	O	N	S	Y	S
		8ANC3484	DI	Q	M	T	Q	S	P	S	T	L	S	A	S	V	G	D	R	V	I	T	T	C	R	A	S	Q	S	I	S	S	N	L	A	W	Y	Q	O	R	P	G	K	A	P	K	I	L	L	I	Y	D	A	S	S	L	E	S	G	V	P	S	R	F	S	G	S	G	T	E	F	T	L	T	I	S	S	L	O	P	D	D	F	A	T	Y	C	Q	O	O	N	S	Y	S
8ANC3044	DI	Q	M	T	Q	S	P	S	T	L	S	A	S	V	G	D	R	V	I	T	T	C	R	A	S	Q	S	I	S	S	N	L	A	W	Y	Q	O	R	P	G	K	A	P	K	I	L	L	I	Y	D	A	S	S	L	E	S	G	V	P	S	R	F	S	G	S	G	T	E	F	T	L	T	I	S	S	L	O	P	D	D	F	A	T	Y	C	Q	O	O	N	S	Y	S		
8ANC3509	DI	Q	M	T	Q	S	P	S	T	L	S	A	S	V	G	D	R	V	I	T	T	C	R	A	S	Q	S	I	S	S	N	L	A	W	Y	Q	O	R	P	G	K	A	P	K	I	L	L	I	Y	D	A	S	S	L	E	S	G	V	P	S	R	F	S	G	S	G	T	E	F	T	L	T	I	S	S	L	O	P	D	D	F	A	T	Y	C	Q	O	O	N	S	Y	S		

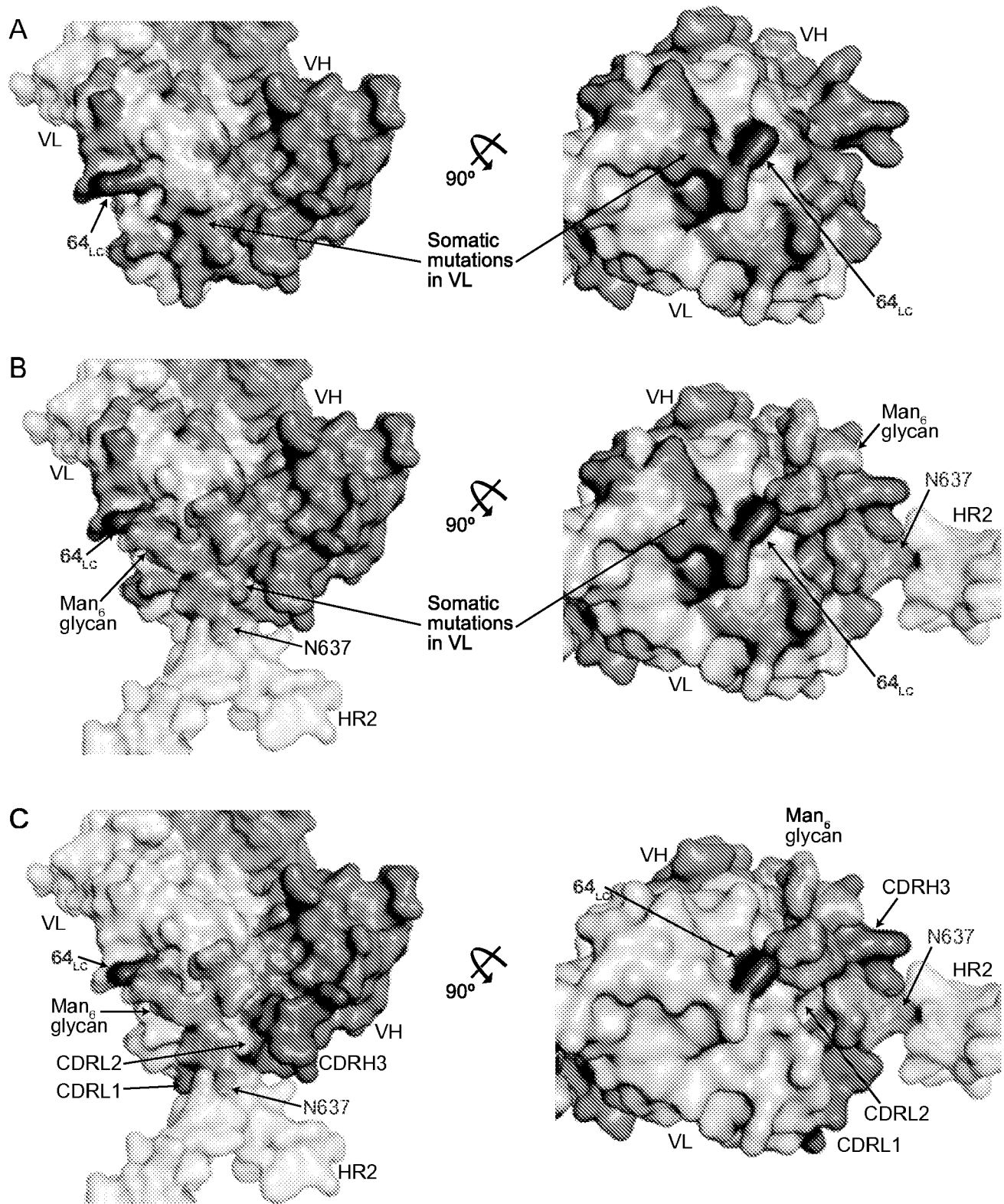


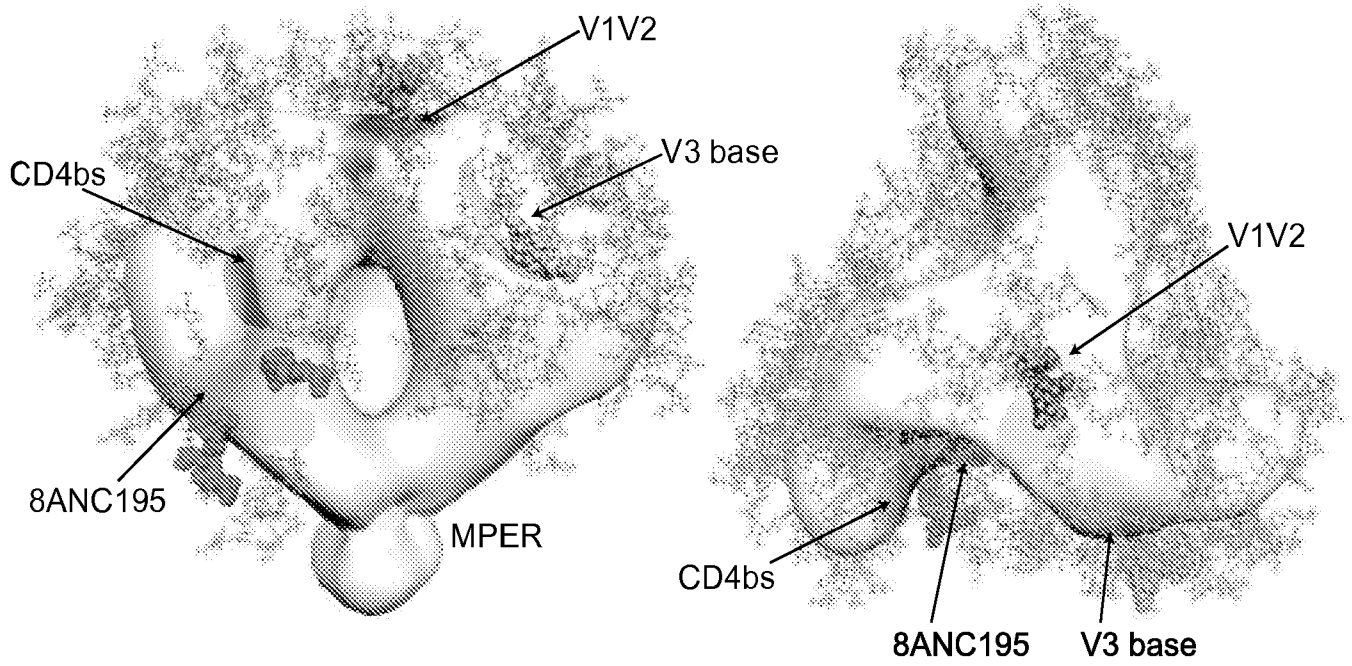


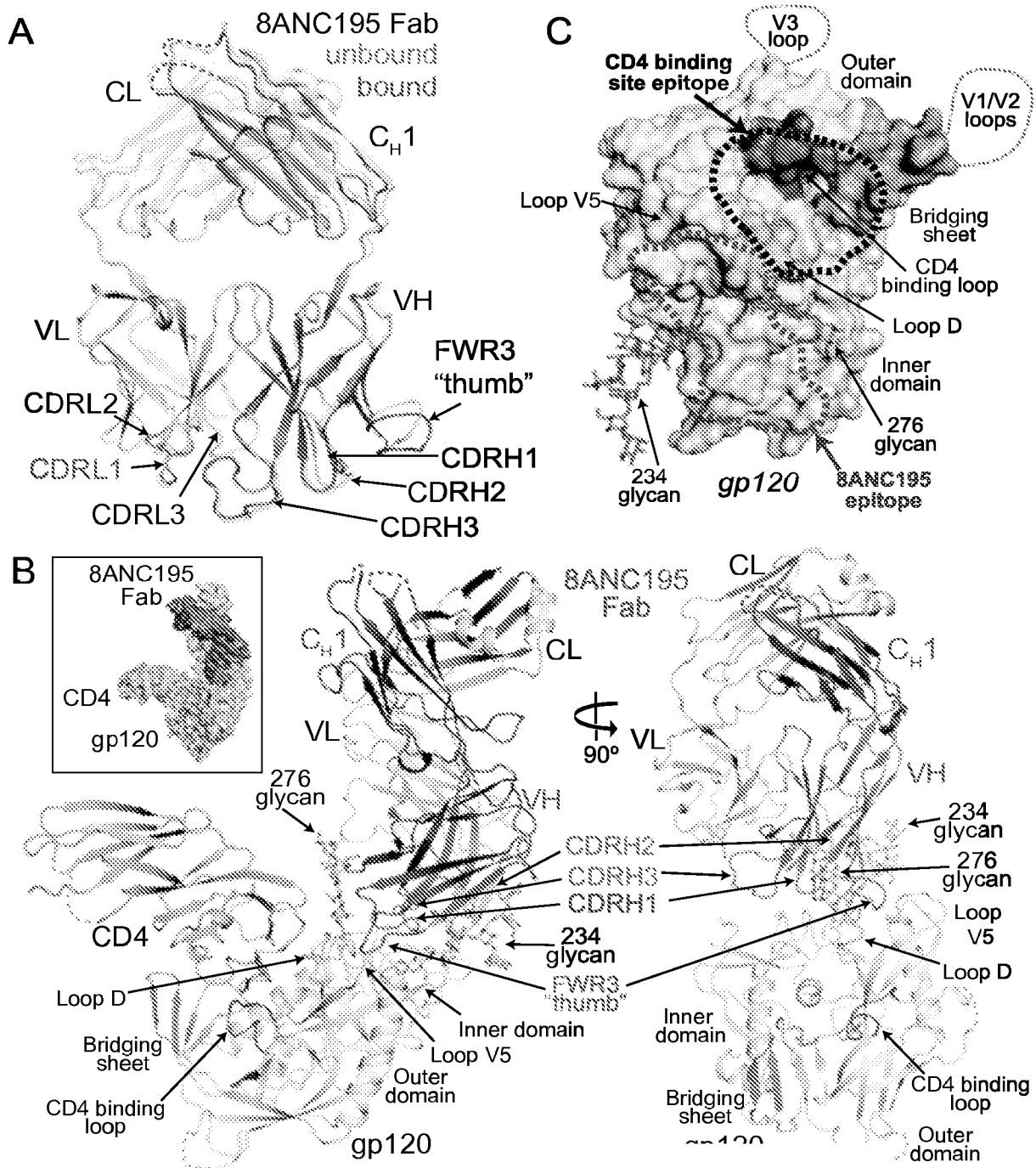
B

IC ₅₀	Virus	γ52 _{HC} K5 _{LG}	8ANC195
	REJO4541.67	0.035	0.08
	PVO.4	0.018	0.52
	YU2.DG	0.065	0.79
	3415.v1.c1	0.120	2.404
	3365.v2.c20	>50	>30
	ZM53M.PB12	3.134	9.626
	ZM109F.PB4	>50	>30
	3016.v5.c45	0.017	0.195
	231965.c1	0.094	0.514
	X1254_c3	0.504	1.524
	251-18	0.048	0.284
	R1166.c1	0.319	0.986
	H086.8	0.175	0.095
	Du172.17	0.841	10.797
	250-4	>50	>50

FIG. 13A-B

**FIG. 14A-C**

**FIG. 15**

**FIG. 16A-C**

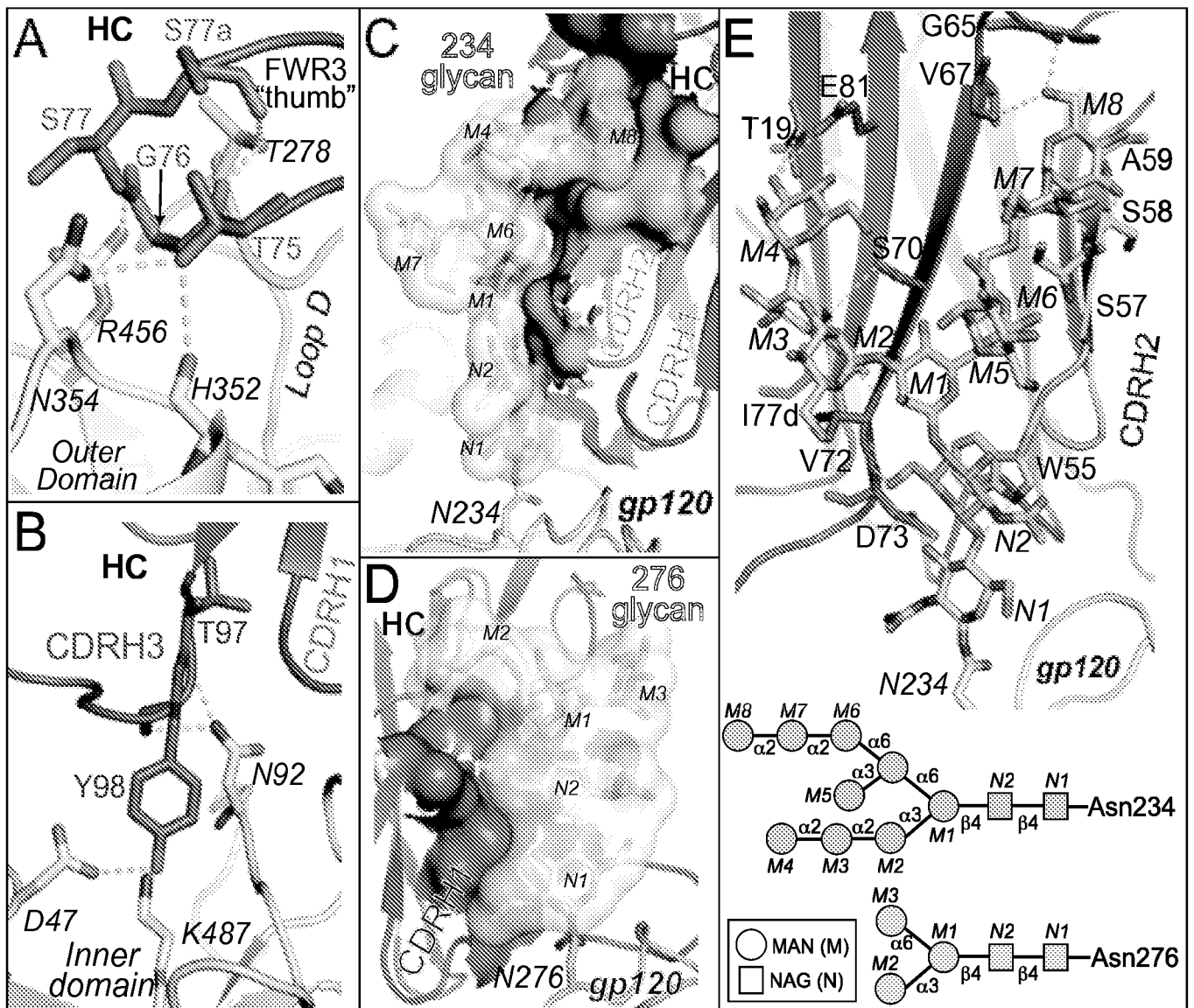
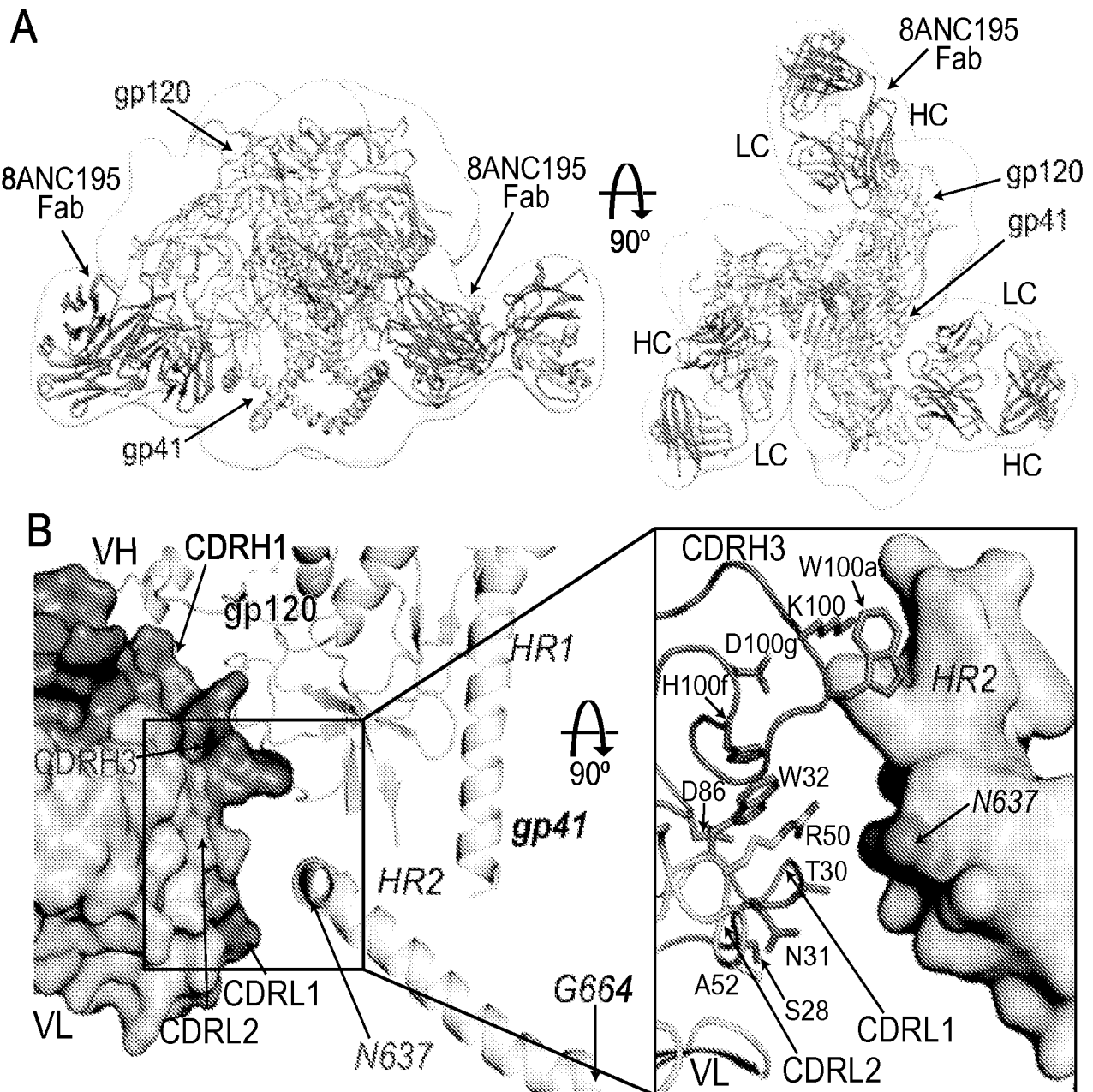


FIG. 17A-E

**FIG. 18A-B**

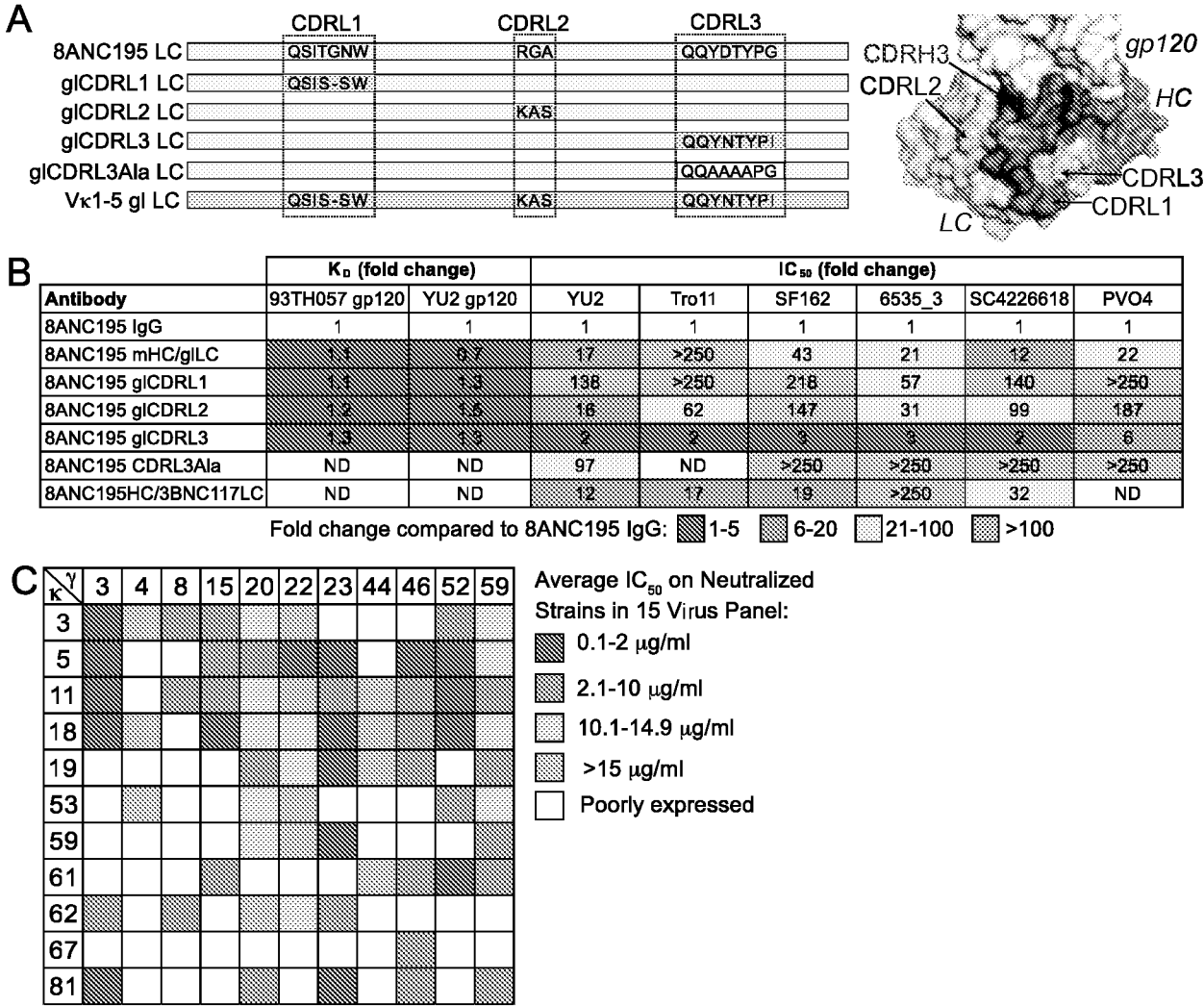


FIG. 19A-C

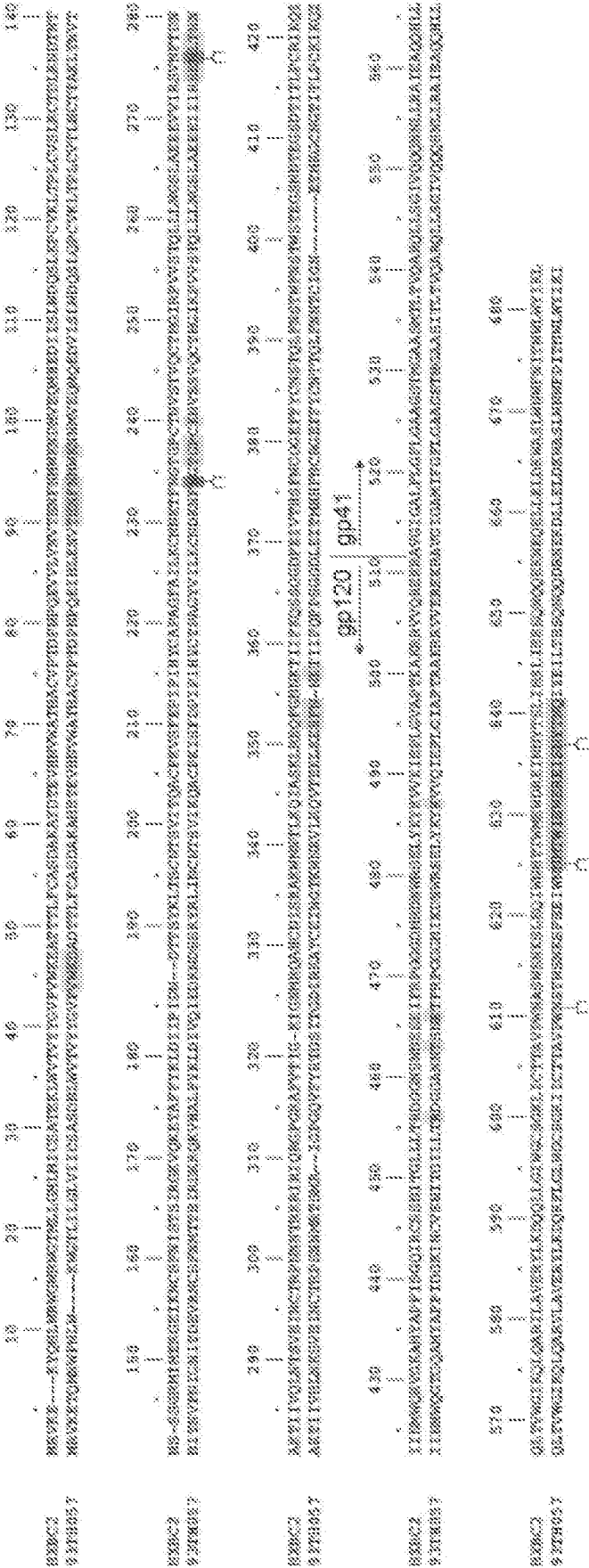


FIG. 20

Legend:

- Glycan
- Amino acid contacts
- Glycan contacts
- Potential contacts based on EM structure