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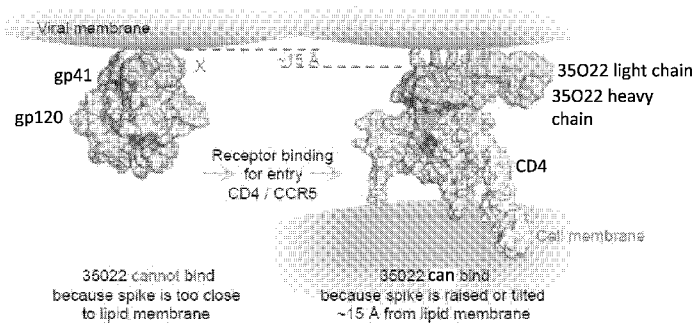
[Continued on next page]

(54) Title: NEUTRALIZING ANTIBODIES TO HIV-1 ENV AND THEIR USE

FIG. 4E



(57) Abstract: Neutralizing antibodies that specifically bind to HIV-1 Envelope protein and antigen binding fragments of these antibodies are disclosed. Nucleic acids encoding these antibodies, vectors and host cells are also provided. Methods for detecting HIV using these antibodies are disclosed. In addition, the use of these antibodies, antigen binding fragment, nucleic acids and vectors to prevent and/or treat an HIV infection is disclosed.

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NEUTRALIZING ANTIBODIES TO HIV-1 ENV AND THEIR USE

RELATED APPLICATIONS

This application claims priority to U.S. Provisional Application No. 61/923,546, filed January 3, 2014, and U.S. Provisional Application No. 62/032,886, filed August 4, 2014, the disclosure of each of which is incorporated by reference herein in its entirety.

FIELD OF THE DISCLOSURE

This relates to monoclonal antibodies and antigen binding fragments that specifically bind to HIV-1 Envelope protein and their use, for example, in methods of treating a subject with HIV-1 infection.

BACKGROUND

Human Immunodeficiency Virus (HIV) infection, and the resulting Acquired Immunodeficiency Syndrome (AIDS), remain threats to global public health, despite extensive efforts to develop anti-HIV therapeutic agents. Some HIV-infected individuals eventually develop broadly neutralizing antibodies (bNAbs), which neutralize a large panel of HIV viruses.

An enveloped virus, HIV-1 hides from humoral recognition behind a wide array of protective mechanisms. The major envelope protein of HIV-1 is a glycoprotein of approximately 160 kD (gp160). During infection proteases of the host cell cleave gp160 into gp120 and gp41. gp41 is an integral membrane protein, while gp120 protrudes from the mature virus. Together gp120 and gp41 make up the HIV envelope spike, which is a target for neutralizing antibodies. Although certain HIV-1 neutralizing antibodies that bind to the HIV-1 Envelope have been identified, there is a need to develop additional neutralizing antibodies for HIV-1 with varying HIV-1 Env recognition profiles.

SUMMARY

Isolated monoclonal antibodies and antigen binding fragments that specifically bind to an epitope on HIV-1 Env are provided herein. Also disclosed are compositions including the antibodies and antigen binding fragments, nucleic acids encoding the antibodies and antigen binding fragments, expression vectors comprising the nucleic acids, and isolated host cells that express the nucleic acids. The antibodies and antigen binding fragments can neutralize HIV-1 infection.

Surprisingly, the antibodies and antigen binding fragments specifically bind to a novel epitope on HIV-1 Env that comprises residues of both gp120 and gp41. In some embodiments, the antibody or antigen binding fragment specifically binds to an epitope comprising two or more amino acids of the C2 region of gp120 and at least one amino acid of gp41. In additional embodiments, the antibody or antigen binding fragment specifically binds to an epitope on HIV-1 Env that comprises or consists of HIV-1 Envelope protein residues 87-92 and 617-633, wherein residue 88 is glycosylated with an N-linked

glycan. In more embodiments, the antibody or antigen binding fragment specifically binds to an epitope on HIV-1 Env that comprises or consists of HIV-1 Env residues 87-92, 227-243, and 617-633, wherein residue 88 is glycosylated with an N-linked glycan. In additional embodiments, the antibody or antigen binding fragment specifically binds to an epitope on HIV-1 Env that comprises two or more of residues N88, K227, N230, N241, S243, and N625.

In some embodiments, the antibody or antigen binding fragment comprises a heavy chain variable region comprising a HCDR1, a HCDR2, and a HCDR3 of the heavy chain variable region set forth as SEQ ID NO: 1 (35O22 VH) and/or a light chain variable region comprising a LCDR1, a LCDR2, and a LCDR3 of the light chain variable region set forth as SEQ ID NO: 2 (35O22 VL).

The antibodies, antigen binding fragments, nucleic acid molecules, vectors, and compositions disclosed herein can be used for a variety of purposes, such as for detecting an HIV-1 infection or diagnosing AIDS in a subject. In further embodiments, a method is disclosed for treating or preventing an HIV infection in a subject. The methods include administering a therapeutically effective amount of one or more of the antibodies, antigen binding fragments, nucleic acid molecules, vectors, or compositions disclosed herein, to the subject, for example to a subject at risk of or having HIV infection.

The foregoing and other features and advantages of this disclosure will become more apparent from the following detailed description of several embodiments which proceeds with reference to the accompanying figures.

BRIEF DESCRIPTION OF THE FIGURES

FIGs. 1A and 1B illustrate 35O22 sequence and neutralization. (A) Inferred germline genes encoding the variable regions 35O22. Germline alleles were determined using the IMGT database (imgt.org). (B) Neutralizing activity of antibodies against a 181-isolate Env-pseudovirus panel. Dendrograms indicate the gp160 protein distance of HIV-1 primary isolate Env glycoproteins. Data below the dendrogram show the number of tested viruses, the percentage of viruses neutralized and the geometric mean or median IC_{50} for viruses neutralized with an $IC_{50} < 50$ μg ml^{-1}.

FIGs. 2A-2F illustrate the binding specificity of 35O22. (A) Neutralization of HIV_{JRCSF} pseudovirus or variants containing the indicated mutations. (B) Binding to BG505 SOSIP.664 trimer produced in cells treated with kifunensine or deficient in glycan processing (293S). Concentration is in μg ml^{-1}.(C) Binding to BG505 trimers with the indicated mutations. BG505 SOSIP.SEKS lacks the furin cleavage site. WT.664 lacks stabilizing mutations and the antigen primarily represents gp41. (D) SPR analysis of binding to immobilized BG505 SOSIP.664 trimers. (E) Binding of 35O22 (250 nM) to BG505 SOSIP.664 trimers, gp120-$gp41_{ECTO}$ protomers, or monomeric gp120. (F) Binding to BG505 SOSIP.664, BG505SOSIP SEKS, or the WT.SEKS lacking the SOSIP mutations.

FIGs. 3A-3B illustrate the structure of the 35O22 Fab and EM reconstruction of the Fab in complex with HIV-1 Env. (A) Cartoon representation of unbound 35O22 Fab (left) and with 90° rotation, looking down on combining site (right). (B) EM reconstruction of BG505 SOSIP.664 in complex with 35O22 Fab, with fitted crystal structures. gp120 and gp41 are shown with 35O22. The

approximate location of the viral membrane is indicated. Glycans N241 and N230 are not part of the BG505 sequence, but have been modelled for reference.

FIG. 4A-4E illustrates 35O22 binding to and neutralization of HIV-1 in the context of a lipid membrane. (A) Staining of cell surface expressed HIV_{JRFL} Env. (B) ELISA assay of antibody binding to WT or SOS HIV_{JRFL} VLPs in the presence or absence of sCD4. The CD4-inducible 39F antibody or gp41-specific 7B2 are used as controls. (C) Access to the HIV_{JRFL} Env trimer on pseudovirions based upon washing the antibody-pseudovirion mixture prior to infecting cells. (D) Kinetic assay of HIV_{JRFL} neutralization. See Methods for a description of individual formats. (E) Schematic of conformational change resulting in raising of the trimer spike required to permit access of 35O22 to its epitope.

FIGS. 5A-5C illustrate an analysis of 35O22 autoreactivity. (A) Reactivity of 35O22 with HEP-2 epithelial cells. 2F5 was used as a positive control and 17b as a negative control. Antibody concentration was 25 μg/ml. All pictures are shown at 400x magnification. (B) SPR analysis of 35O22 binding to anionic phospholipids. 35O22 was injected over PC-CLP liposomes or PC-PS liposomes immobilized on the BIAcore L1 sensor chip. 4E10 and 2F5 were used as positive controls and 13H1 as a negative control. (C) Reactivity of 35O22 with autoantigens detected in Luminex assay. 4E10 was used as a positive control. Synagis, an anti-RSV monoclonal antibody, was used as a negative control. SSA, Sjogren's syndrome antigen A; SSB, Sjogren syndrome antigen B; Sm, Smith antigen; RNP, ribonucleoprotein; Scl 70, scleroderma 70; Jo1, antigen; CentrB, centromere B. A positive response is >120 units.

FIGS. 6A-6C illustrate the neutralization similarities between 35O22 and other HIV-1 bNAbs. (A) Correlation (Spearman) between the neutralization potencies of 35O22 and the indicated antibody against 172 pseudoviruses. Representatives from all four major sites of vulnerability are shown. Resistant strains corresponding to values of >50 μg/ml are plotted as 50. (B) Neutralization-based clustering of bNabs over a set of 172 diverse HIV-1 strains. A putative epitope-specific clustering cutoff is shown as a dashed line. The respective target site of vulnerability that is targeted by each type of antibody is indicated (CD4bs, glycan-V3, V1V2, MPER, and other. 35O22 clusters separately from all other antibodies, indicating a novel mechanism of neutralization. (C) 35O22 competition with other bNAbs on HIV_{JRFL} VLPs with the trimer stabilizing SOS mutations in an ELISA assay. Biotin-bNAbs were titrated into the ELISA at increasing concentrations in the presence of excess (10 μg ml^{-1}) cold competitor neutralizing antibodies. Values in the table indicates percentage binding of biotin-nAbs in the presence of cold-competitor. ND = not done

FIGS. 7A and **7B** illustrate that 35O22 binds to N-linked glycans. (A) Neutralization by 35O22 plateaus below 80% against several pseudoviruses. (B) Neutralization activity of mAbs against JRCSF pseudoviruses generated in the presence of glycosidase-inhibitors, such as kifunensine (25 μM), NB-DNJ (500 μM) or swainsonine (20 μM). Error bars denote one standard error of the mean (s.e.m.).

FIGS. 8A-8C illustrate the neutralization of 35O22 against pseudovirus mutants known to knock out activity against known glycan-specific antibodies. (A) Neutralization of 35O22 against JRCSF or KER2018.11 with or without the N160K mutation. PG9 and PG16 were used as positive controls. (B)

Neutralization of 35O22 against N332A mutants of JRCSF. PGT121 was used as a positive control. (C) Neutralization of 35O22 against N234S, T236K and N276D mutants of 3337.V2.C6. 8ANC195 was used as a positive control. Error bars denote one standard error of the mean (s.e.m.).

FIGS. 9A-9C illustrate the binding specificity of 35O22. ELISA binding of indicated mAbs to HIV_{YU2} gp140 foldon trimer, gp120, and gp41 monomers (A). ELISA binding of gp120 (B) and gp140 (C) monomers from different HIV-1 subtypes.

FIGS. 10A and **10B** illustrate 35O22 Fab features. (A) 35O22 is seen looking down on the combining site from the viewpoint of antigen. Insets show structural details of the framework 3 insertion, disulfides in CDR L1 and CDR L3 with electron density $2F_0$-F_c contoured at 1σ. Location of the viral membrane. (B) Superposition of BaL gp160 negative stain (light grey surface) with the negative stain reconstruction of soluble BG505 SOSIP in complex with 35O22 (darker grey surface) gives an estimation of the viral membrane location relative to 35O22 antibody as shown in FIG. 3B.

FIG. 11 illustrates the binding site of 35O22 on the HIV Env trimer relative to those of PGT151 and 8ANC195 antibodies, as well as the CD4 binding site, the MPER, the V1V2 domain, and the N332 glycan site.

FIGs. 12A-12E illustrate a new site of HIV-1 vulnerability at the interface of gp120 and gp41 and prevalence of targeting this site. (A) Dominant sites of vulnerability to neutralizing antibody elicited by natural infection, shown in the context of an EM tomogram from the BAL viral spike. The viral membrane is positioned at the top of the spike. It is unclear if 35O22 and MPER antibodies bind to this form of the viral spike, and approximate locations for these are shown in dotted outlines. (B) Viral spike from the soluble BG505 SOSIP context, shown in the same orientation as (12A), with gp120 surface colored by conservation from 0-100%, from 4265 HIV-1 strains (white to darker grey for protomers 1, 2 and 3, with scale shown), with glycans shown in dark grey when present in more than 90% of strains, in light grey when present in 30-90% of strains and not shown otherwise. (C) 35O22-identified site of HIV-1 vulnerability, comprises both conserved amino acids and a cluster of glycans, including N88 from gp120 and N625 from gp41. N230 and N241 are not present in BG505 strain. The 35O22 epitope is shown in dotted line. (D) Neutralization fingerprints for 35O22 and for antibodies encompassing ten different epitope specificities representing the other four known major sites of Env vulnerability were used to interrogate the serum specificities of 34 HIV-infected patients. Values (with proportional color intensities) predict the fraction of serum neutralization that can be attributed to each antibody specificity. Possible 35O22-like signals were predicted for 13 of the sera (values >0.2), while strong signals were observed in 3 of the sera (values >0.3). A panel of 21 HIV-1 strains was used in the neutralization analysis and for computing serum breadth. (E) Sites of HIV-1 vulnerability to neutralizing antibody outlined by a white line. Prevalence in 34-donor cohort indicated along with critical glycans.

FIGs. 13A-13D illustrate the autologous virus Env sequence and the impact of variants on 35O22 neutralization. (A-C) A total of 12 single-genome amplicons from plasma of patient N152 were sequenced. Donor Env sequences together with the reference sequences of JRCSF and LAI are aligned. The Env sequences shown include those from the JRCSFM38429 (SEQ ID NO: 40), LAI_K02013 (SEQ

ID NO: 41), N152_061511_1 (SEQ ID NO: 42), N152_061511_8 (SEQ ID NO: 43), N152_061511_15 (SEQ ID NO: 44), N152_061511_2 (SEQ ID NO: 45), N152_061511_4 (SEQ ID NO: 46), N152_061511_17 (SEQ ID NO: 47), N152_061511_14 (SEQ ID NO: 48), N152_061511_9 (SEQ ID NO: 49), N152_061511_11 (SEQ ID NO: 50), N152_061511_3 (SEQ ID NO: 51), N152_061511_6 (SEQ ID NO: 52), and N152_061511_7 (SEQ ID NO: 53) strains of HIV-1. Amino acids critical for 35O22 neutralization of JRCSF and LAI are labeled with an asterisk. Differences between autologous and JRCSF sequences are labeled in dark grey. (D) 35O22 neutralization of JRCSF pseudovirus or variants containing the autologous virus mutations from patient N152. Error bars denote one standard error of the mean.

FIG. 14 shows the neutralizing activity of sera or mAb against HIV_{JRCSF} pseudovirus with mutation in the 35O22 epitope. *Fold change=ID_{50} of HIV_{JRCSF} WT / ID_{50} of HIV_{JRCSF} mutant. Values with fold changes >5 are highlighted in yellow. †Fold change=IC_{50} of HIV_{JRCSF} mutant / IC_{50} of HIV_{JRCSF} WT. Values with fold changes >5 are highlighted in yellow.

FIGs. 15A-15B are a set of tables showing data concerning 35O22 neutralizing breadth and potency. (A) Neutralization by 35O22 and its variants against an 8-isolate Env pseudovirus mini-panel. (B) Neutralization profile of patient N152 serum and monoclonal antibodies.

FIGs. 16A-16D show a set of sequence alignments illustrating the amino acid sequences of the variable regions of 35O22 and its variants, including the Kabat and IMGT heavy and light chain variable region CDR sequences (SEQ ID NOs: 1-16). The dot symbol denotes a residue deletion.

FIGs. 17A-17D are a set of tables showing antibody neutralization data for the 35O22 antibody and other HIV-1 neutralizing antibodies against 181 HIV-1 Env-pseudoviruses.

FIGs. 18A-18B are a set of tables showing data concerning 35O22 neutralization of pseudotyped HIV_{JRCSF} alanine mutants.

FIG. 19 is a table showing data concerning 35O22 neutralization of pseudotyped HIV_{LAI} alanine mutants.

FIG. 20 is a table showing data collection and refinement statistics for the crystal structure of the 35O22 Fab

FIG. 21 is a table showing 35O22 neutralizing activity with reversion of an 8 amino acid FR3 insertion to germline sequence.

FIG. 22 is a table showing the conservation of N-linked glycosylation sequons in HIV-1 Env.

FIGs. 23A-23E are a set of tables providing details of the HIV-1 Env binding interface for the 35O22 heavy and light chains.

SEQUENCES

The nucleic and amino acid sequences are shown using standard letter abbreviations for nucleotide bases, and three letter code for amino acids, as defined in 37 C.F.R. 1.822. Only one strand of each nucleic acid sequence is shown, but the complementary strand is understood as included by any reference to the displayed strand. The Sequence Listing is submitted as an ASCII text file in the form of

the file named "Sequence.txt" (~140 kb), which was created on December 31, 2014 which is incorporated by reference herein. In the accompanying sequence listing:

SEQ ID NO: 1 is the amino acid sequence of the V_H of the 35O22 mAb.
QGQLVQSGAELKKPGASVKISCKTSGYRFNFYHINWIRQTAGRGPEWMGWISPYSGDKNLAPAFQDRVIM
TTDTEVPVTSFTSTGAAYMEIRNLKFDDTGTYFCAKGLLRDGSSTWLPYLWGQGTLLTVSS

SEQ ID NO: 2 is the amino acid sequence of the V_L of the 35O22 mAb.
QSVLTQSASVSGSLGQSVTISCTGPNSVCCSHKSISWYQWPPGRAPTLIIYEDNERAPGISPRFSGYKSY
WSAYLTISDLRPEDETTYYCCSYTHNSGCVFGTGTKVSVL

SEQ ID NO: 3 is the amino acid sequence of the V_H of the 10J4 mAb.
QGQLVQSGGELKKPGASVKISCKTSGYRFSFYHINWIRQLVGRGPEWMGWISPYNGGTNLAPELRGRLVL
TTEREVVDTMTLSTGTAHMELRNLRSDDTGIYFCAKGLLRDGSSTWLPHLWGQGTLLTVSS

SEQ ID NO: 4 is the amino acid sequence of the V_L of the 10J4 mAb.
QSALTQPASVSGSLGQSVTISCTGPSSVCCSHKSISWYQWPPGRAPTLLIFEDSERSWGISDRFSGYKSY
WSASLTISNLRPEDETTYYCCSYTHNSGCVFGTGTNVSVL

SEQ ID NO: 5 is the amino acid sequence of the V_H of the 10M6 mAb.
QGQLVQSGGELKRPGASVKISCKTSGYRFSSYHINWIRQVIGRGPEWMGWISPYSGGTNLAPEFRGRLVL
TTEREVVDTMTLSTGTAHMELRNLKSDDTGIYFCAKGLLRDGSSTWLPHLWGQGTLLTVSS

SEQ ID NO: 6 is the amino acid sequence of the V_L of the 10M6 mAb.
QSALTQPASVSGSLGQSVTISCTGPSSVCCSHKSISWYQWPPGRAPTLLIFEDSERSWGISDRFSGYKSY
WSASLTISNLRPEDETTYFCCSYTHNSGCVFGTGTKVSVL

SEQ ID NO: 7 is the amino acid sequence of the V_H of the 13I10 mAb.
QGQLVQSGGELKKPGASVKISCKTSGYRFSFYHINWIRQVVGRGPEWMGWISPYNGGTNLAPEFRGRLVL
TTEREVVDTMTMSTGTAHMELRNLKSDDTGLYFCAKGLLRDGPSTWLPHLWGQGTLLTVSS

SEQ ID NO: 8 is the amino acid sequence of the V_L of the 13I10 mAb.
QSALTQPASVSGSLGQSVTISCTGPSSVCCSHKSISWYQWPPGRAPTLLIFEDSERSWGISDRFSGYKSY
WSASLTISNLRPEDETTYYCCSYTHNSGCVFGTGTKVSVL

SEQ ID NO: 9 is the amino acid sequence of the V_H of the 2N5mAb.
QGQLVQSGAELKKPGASVKISCKTSGYKFSFFHINWIRQTAGRGPEWLGWISPYSGDKNYAPAFQDRVIM
TTDKEVPVTSFTSTGTAYLEIRSLKPDDTGIYFCARGLLRDGSSTWLPYLWGQGTLLTVSS

SEQ ID NO: 10 is the amino acid sequence of the V_L of the 2N5mAb.
QPVLTQPASVSGSLGQSVTISCTGPSSVCCSHKSISWYRWPPGRAPTLIIYEDNKRFSEISPRFSGYKSY
WSAYLTISDLRPEDETTYYCCSYTHNSGCVFATGTKVSVL

SEQ ID NO: 11 is the amino acid sequence of the V_H of the 4O20mAb.
QGQLVQSGAELKKPGGSVKISCKTSGYRFNFYHINWIRQTAGRGPEWMGWISPYSGDKNLAPAFQDRVIM
TTDKEVPVTAFTSTGTAYMEIRNLKFDDTGIYFCAKGLLRDGSSTWLPYLWGQGTLLTVSS

SEQ ID NO: 12 is the amino acid sequence of the V_L of the 4O20mAb.
QSALTQPASVSGSLGQSVTISCTGPNSVCCSHKSISWYQWPPGRAPTLIIYEDNEKAPGISHRFSGYKSY
WSAYLTISDLRPEDETTYYCCSYTHNSGCVFGTGTKVSVL

SEQ ID NO: 13 is the amino acid sequence of the V_H of the 7B9mAb.
QGQLVQSGAELKKPGDSVKISCKTSGYRFNFYHINWIRQTAGRGPEWMGWISPYSGDKNLAPAFQDRVIM
TTDNEVPVTAFTSTGTAYMEIRNLRFDDTGIYFCAKGLLRDGSSTWLPYLWGQGTLLTVSS

SEQ ID NO: 14 is the amino acid sequence of the V_L of the 7B9mAb.

QSALTQPASVSGSLGQSVTISCTGPNSACCSHKSISWHQWPPGRAPTLIIYEDNEKAPGISHRFSGYKSY
WSAYLTISDLRPEDETTYYCCSYTHNSGCVFGTGTKVSVL

SEQ ID NO: 15 is the amino acid sequence of the V_H of the 7K3mAb.

QGQLVQSGGELKKPGASVKISCKTSGYRFSFYHINWIRQVSGRGPEWMGWISPYSGDTNLAPDFRGRVVL
TTDREVVDTMTMSTGTAHMELRNLKSDDTGLYFCAKGLLRDGSSTWLPHLWGQGTLLTVSS

SEQ ID NO: 16 is the amino acid sequence of the V_L of the 7K3mAb.

QSALTQPASVSGSLGQSVTISCTGPNSVCCSHKSISWYQWPPGRAPTLLIFEDSERSWGISDRFSGYKSY
WSASLTISNLRPEDETTYYCCSYTHNSGCVFGTGTKVSVL

SEQ ID NOs: 17-22 are consensus CDR sequences.

SEQ ID NOs: 23-32 are amino acid sequences of CAR domains.

SEQ ID NOs: 33-37 are consensus CDR sequences.

SEQ ID NOs: 38-53 are HIV-1 Env amino acid sequences.

DETAILED DESCRIPTION

I. Summary of Terms

Unless otherwise noted, technical terms are used according to conventional usage. Definitions of common terms in molecular biology may be found in Benjamin Lewin, *Genes X*, published by Jones & Bartlett Publishers, 2009; and Meyers *et al.* (eds.), *The Encyclopedia of Cell Biology and Molecular Medicine*, published by Wiley-VCH in 16 volumes, 2008; and other similar references.

As used herein, the singular forms "a," "an," and "the," refer to both the singular as well as plural, unless the context clearly indicates otherwise. For example, the term "an antigen" includes single or plural antigens and can be considered equivalent to the phrase "at least one antigen." As used herein, the term "comprises" means "includes." Thus, "comprising an antigen" means "including an antigen" without excluding other elements. It is further to be understood that any and all base sizes or amino acid sizes, and all molecular weight or molecular mass values, given for nucleic acids or polypeptides are approximate, and are provided for descriptive purposes, unless otherwise indicated. Although many methods and materials similar or equivalent to those described herein can be used, particular suitable methods and materials are described below. In case of conflict, the present specification, including explanations of terms, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting. To facilitate review of the various embodiments, the following explanations of terms are provided:

Administration: The introduction of a composition into a subject by a chosen route. Administration can be local or systemic. For example, if the chosen route is intravenous, the composition is administered by introducing the composition into a vein of the subject. Exemplary routes of administration include, but are not limited to, oral, injection (such as subcutaneous, intramuscular, intradermal, intraperitoneal, and intravenous), sublingual, rectal, transdermal (for example, topical), intranasal, vaginal, and inhalation routes. In some examples a disclosed antibody specific for an HIV Env protein or polypeptide, or a nucleic acid encoding the antibody, is administered to a subject.

Agent: Any substance or any combination of substances that is useful for achieving an end or result; for example, a substance or combination of substances useful for inhibiting HIV infection in a subject. Agents include proteins, antibodies, nucleic acid molecules, compounds, small molecules, organic compounds, inorganic compounds, or other molecules of interest. An agent can include a therapeutic agent (such as an anti-retroviral agent), a diagnostic agent or a pharmaceutical agent. In some embodiments, the agent is a polypeptide agent (such as a HIV-neutralizing antibody), or an anti-viral agent. The skilled artisan will understand that particular agents may be useful to achieve more than one result.

Amino acid substitution: The replacement of one amino acid in peptide with a different amino acid.

Antibody: An immunoglobulin, antigen-binding fragment, or derivative thereof, that specifically binds and recognizes an analyte (antigen) such as HIV-1 Env or an antigenic fragment of HIV-1 Env. The term "antibody" is used herein in the broadest sense and encompasses various antibody structures, including but not limited to monoclonal antibodies, polyclonal antibodies, multispecific antibodies (e.g., bispecific antibodies), and antibody fragments, so long as they exhibit the desired antigen-binding activity.

Non-limiting examples of antibodies include, for example, intact immunoglobulins and variants and fragments thereof known in the art that retain binding affinity for the antigen. Examples of antibody fragments include but are not limited to Fv, Fab, Fab', Fab'-SH, $F(ab')_2$; diabodies; linear antibodies; single-chain antibody molecules (e.g. scFv); and multispecific antibodies formed from antibody fragments. Antibody fragments include antigen binding fragments either produced by the modification of whole antibodies or those synthesized *de novo* using recombinant DNA methodologies (see, e.g, Kontermann and Dubel (Ed), Antibody Engineering, Vols. 1-2, 2nd Ed., Springer Press, 2010).

A single-chain antibody (scFv) is a genetically engineered molecule containing the V_H and V_L domains of one or more antibody(ies) linked by a suitable polypeptide linker as a genetically fused single chain molecule (see, for example, Bird *et al.*, *Science*, 242:423-426, 1988; Huston *et al.*, *Proc. Natl. Acad. Sci.,* 85:5879-5883, 1988; Ahmad *et al.*, *Clin. Dev. Immunol.*, 2012, doi:10.1155/2012/980250; Marbry, *IDrugs*, 13:543-549, 2010). The intramolecular orientation of the V_H-domain and the V_L-domain in a scFv, is typically not decisive for scFvs. Thus, scFvs with both possible arrangements (V_H-domain-linker domain-V_L-domain; V_L-domain-linker domain-V_H-domain) may be used.

In a dsFv the heavy and light chain variable chains have been mutated to introduce a disulfide bond to stabilize the association of the chains. Diabodies also are included, which are bivalent, bispecific antibodies in which V_H and V_L domains are expressed on a single polypeptide chain, but using a linker that is too short to allow for pairing between the two domains on the same chain, thereby forcing the domains to pair with complementary domains of another chain and creating two antigen binding sites (see, for example, Holliger *et al.*, *Proc. Natl. Acad. Sci.,* 90:6444-6448, 1993; Poljak *et al.*, *Structure,* 2:1121-1123, 1994).

Antibodies also include genetically engineered forms such as chimeric antibodies (such as humanized murine antibodies) and heteroconjugate antibodies (such as bispecific antibodies). See also, *Pierce Catalog and Handbook*, 1994-1995 (Pierce Chemical Co., Rockford, IL); Kuby, J., *Immunology*, 3rd Ed., W.H. Freeman & Co., New York, 1997.

An "antibody that binds to the same epitope" as a reference antibody refers to an antibody that blocks binding of the reference antibody to its antigen in a competition assay by 50% or more, and conversely, the reference antibody blocks binding of the antibody to its antigen in a competition assay by 50% or more. Antibody competition assays are known, and an exemplary competition assay is provided herein.

An antibody may have one or more binding sites. If there is more than one binding site, the binding sites may be identical to one another or may be different. For instance, a naturally-occurring immunoglobulin has two identical binding sites, a single-chain antibody or Fab fragment has one binding site, while a bispecific or bifunctional antibody has two different binding sites.

Typically, a naturally occurring immunoglobulin has heavy (H) chains and light (L) chains interconnected by disulfide bonds. Immunoglobulin genes include the kappa, lambda, alpha, gamma, delta, epsilon and mu constant region genes, as well as the myriad immunoglobulin variable domain genes. There are two types of light chain, lambda (λ) and kappa (κ). There are five main heavy chain classes (or isotypes) which determine the functional activity of an antibody molecule: IgM, IgD, IgG, IgA and IgE.

Each heavy and light chain contains a constant region (or constant domain) and a variable region (or variable domain; see, e.g., Kindt et al. Kuby Immunology, 6th ed., W.H. Freeman and Co., page 91 (2007).) In several embodiments, the heavy and the light chain variable regions combine to specifically bind the antigen. In additional embodiments, only the heavy chain variable region is required. For example, naturally occurring camelid antibodies consisting of a heavy chain only are functional and stable in the absence of light chain (see, *e.g.*, Hamers-Casterman *et al.*, *Nature*, 363:446-448, 1993; Sheriff *et al.*, *Nat. Struct. Biol.*, 3:733-736, 1996). References to "V_H" or "VH" refer to the variable region of an antibody heavy chain, including that of an antigen binding fragment, such as Fv, scFv, dsFv or Fab. References to "V_L" or "VL" refer to the variable domain of an antibody light chain, including that of an Fv, scFv, dsFv or Fab.

Heavy and Light chain variable regions contain a "framework" region interrupted by three hypervariable regions, also called "complementarity-determining regions" or "CDRs" (see, e.g., Kabat *et al.*, *Sequences of Proteins of Immunological Interest*, U.S. Department of Health and Human Services, 1991). The sequences of the framework regions of different light or heavy chains are relatively conserved within a species. The framework region of an antibody, that is the combined framework regions of the constituent light and heavy chains, serves to position and align the CDRs in three-dimensional space.

The CDRs are primarily responsible for binding to an epitope of an antigen. The amino acid sequence boundaries of a given CDR can be readily determined using any of a number of well-known

schemes, including those described by Kabat et al. ("Sequences of Proteins of Immunological Interest," 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD, 1991; "Kabat" numbering scheme), Al-Lazikani et al., (JMB 273,927-948, 1997; "Chothia" numbering scheme), and Lefranc et al. ("IMGT unique numbering for immunoglobulin and T cell receptor variable domains and Ig superfamily V-like domains," Dev. Comp. Immunol., 27:55-77, 2003; "IMGT" numbering scheme). The CDRs of each chain are typically referred to as CDR1, CDR2, and CDR3 (from the N-terminus to C-terminus), and are also typically identified by the chain in which the particular CDR is located. Thus, a V_H CDR3 is the CDR3 from the variable domain of the heavy chain of the antibody in which it is found, whereas a V_L CDR1 is the CDR1 from the variable domain of the light chain of the antibody in which it is found. Light chain CDRs are sometimes referred to as LCDR1, LCDR2, and LCDR3. Heavy chain CDRs are sometimes referred to as LCDR1, LCDR2, and LCDR3.

A "monoclonal antibody" is an antibody obtained from a population of substantially homogeneous antibodies, that is, the individual antibodies comprising the population are identical and/or bind the same epitope, except for possible variant antibodies, for example, containing naturally occurring mutations or arising during production of a monoclonal antibody preparation, such variants generally being present in minor amounts. In contrast to polyclonal antibody preparations, which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody of a monoclonal antibody preparation is directed against a single determinant on an antigen. Thus, the modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies may be made by a variety of techniques, including but not limited to the hybridoma method, recombinant DNA methods, phage-display methods, and methods utilizing transgenic animals containing all or part of the human immunoglobulin loci, such methods and other exemplary methods for making monoclonal antibodies being described herein. In some examples monoclonal antibodies are isolated from a subject. Monoclonal antibodies can have conservative amino acid substitutions which have substantially no effect on antigen binding or other immunoglobulin functions. (See, for example, Harlow & Lane, *Antibodies, A Laboratory Manual*, 2nd ed. Cold Spring Harbor Publications, New York (2013).)

A "humanized" antibody or antigen binding fragment includes a human framework region and one or more CDRs from a non-human (such as a mouse, rat, or synthetic) antibody or antigen binding fragment. The non-human antibody or antigen binding fragment providing the CDRs is termed a "donor," and the human antibody or antigen binding fragment providing the framework is termed an "acceptor." In one embodiment, all the CDRs are from the donor immunoglobulin in a humanized immunoglobulin. Constant regions need not be present, but if they are, they can be substantially identical to human immunoglobulin constant regions, such as at least about 85-90%, such as about 95% or more identical. Hence, all parts of a humanized antibody or antigen binding fragment, except possibly the CDRs, are substantially identical to corresponding parts of natural human antibody sequences.

A "chimeric antibody" is an antibody which includes sequences derived from two different antibodies, which typically are of different species. In some examples, a chimeric antibody includes one or more CDRs and/or framework regions from one human antibody and CDRs and/or framework regions from another human antibody.

Anti-retroviral agent: An agent that specifically inhibits a retrovirus from replicating or infecting cells. Non-limiting examples of antiretroviral drugs include entry inhibitors (*e.g.*, enfuvirtide), CCR5 receptor antagonists (*e.g.*, aplaviroc, vicriviroc, maraviroc), reverse transcriptase inhibitors (*e.g.*, lamivudine, zidovudine, abacavir, tenofovir, emtricitabine, efavirenz), protease inhibitors (*e.g.*, lopivar, ritonavir, raltegravir, darunavir, atazanavir), maturation inhibitors (*e.g.*, alpha interferon, bevirimat and vivecon).

Anti-retroviral therapy (ART): A therapeutic treatment for HIV infection involving administration of at least one anti-retroviral agents (*e.g.*, one, two, three or four anti-retroviral agents) to an HIV infected individual during a course of treatment. Non-limiting examples of antiretroviral agents include entry inhibitors (*e.g.*, enfuvirtide), CCR5 receptor antagonists (*e.g.*, aplaviroc, vicriviroc, maraviroc), reverse transcriptase inhibitors (*e.g.*, lamivudine, zidovudine, abacavir, tenofovir, emtricitabine, efavirenz), protease inhibitors (*e.g.*, lopivar, ritonavir, raltegravir, darunavir, atazanavir), maturation inhibitors (*e.g.*, alpha interferon, bevirimat and vivecon). One example of an ART regimen includes treatment with a combination of tenofovir, emtricitabine and efavirenz. In some examples, ART includes Highly Active Anti-Retroviral Therapy (HAART). One example of a HAART regimen includes treatment with a combination of tenofovir, emtricitabine and efavirenz.

Biological sample: A sample obtained from a subject. Biological samples include all clinical samples useful for detection of disease or infection (for example, HIV-1) in subjects, including, but not limited to, cells, tissues, and bodily fluids, such as blood, derivatives and fractions of blood (such as serum), cerebrospinal fluid; as well as biopsied or surgically removed tissue, for example tissues that are unfixed, frozen, or fixed in formalin or paraffin. In a particular example, a biological sample is obtained from a subject having or suspected of having an HIV-1 infection.

Bispecific antibody: A recombinant molecule composed of two different antigen binding domains that consequently binds to two different antigenic epitopes. Bispecific antibodies include chemically or genetically linked molecules of two antigen-binding domains. The antigen binding domains can be linked using a linker. The antigen binding domains can be monoclonal antibodies, antigen-binding fragments (*e.g.*, Fab, scFv), or combinations thereof. A bispecific antibody can include one or more constant domains, but does not necessarily include a constant domain. An example of a bispecific antibody is a bispecific single chain antibody including a scFv that specifically binds to HIV-1 Env joined (via a peptide linker) to a scFv that specifically binds CD3. Another example is a bispecific antibody including a Fab that specifically binds to HIV-1 Env joined to a scFv that specifically binds to CD3.

CD3 (Cluster of differentiation 3 T-cell Co-receptor): A specific protein complex including at least four polypeptide chains, which are non-covalently associated with the T-cell receptors on the

surface of T-cells. The four polypeptide chains include two CD3-epsilon chains, a CD3-delta chain and a CD3-gamma chain. CD3 is present on both helper T cells and cytotoxic T cells.

CD4: Cluster of differentiation factor 4 polypeptide; a T-cell surface protein that mediates interaction with the MHC class II molecule. CD4 also serves as the primary receptor site for HIV on T-cells during HIV-I infection. CD4 is known to bind to gp120 from HIV. The known sequence of the CD4 precursor has a hydrophobic signal peptide, an extracellular region of approximately 370 amino acids, a highly hydrophobic stretch with significant identity to the membrane-spanning domain of the class II MHC beta chain, and a highly charged intracellular sequence of 40 resides (Maddon, *Cell* 42:93, 1985).

The term "CD4" includes polypeptide molecules that are derived from CD4 include fragments of CD4, generated either by chemical (for example enzymatic) digestion or genetic engineering means. Such a fragment may be one or more entire CD4 protein domains. The extracellular domain of CD4 consists of four contiguous immunoglobulin-like regions (D1, D2, D3, and D4, see Sakihama *et al.*, *Proc. Natl. Acad. Sci.* 92:6444, 1995; U.S. Patent No. 6,117,655), and amino acids 1 to 183 have been shown to be involved in gp120 binding. For instance, a binding molecule or binding domain derived from CD4 would comprise a sufficient portion of the CD4 protein to mediate specific and functional interaction between the binding fragment and a native or viral binding site of CD4. One such binding fragment includes both the D1 and D2 extracellular domains of CD4 (D1D2 is also a fragment of soluble CD4 or sCD4 which is comprised of D1 D2 D3 and D4), although smaller fragments may also provide specific and functional CD4-like binding. The gp120-binding site has been mapped to D1 of CD4.

CD4 polypeptides also include "CD4-derived molecules" which encompasses analogs (non-protein organic molecules), derivatives (chemically functionalized protein molecules obtained starting with the disclosed protein sequences) or mimetics (three-dimensionally similar chemicals) of the native CD4 structure, as well as proteins sequence variants or genetic alleles that maintain the ability to functionally bind to a target molecule.

Chimeric Antigen Receptor (CAR): An engineered T cell receptor having an extracellular antibody-derived targeting domain (such as an scFv) joined to one or more intracellular signaling domains of a T cell receptor. A "**chimeric antigen receptor T cell**" is a T cell expressing a CAR, and has antigen specificity determined by the antibody-derived targeting domain of the CAR. Methods of making CARs (e.g., for treatment of cancer) are available (see, e.g., Park *et al.*, *Trends Biotechnol.*, 29:550-557, 2011; Grupp et al., N Engl J Med., 368:1509-1518, 2013; Han et al., J. Hematol Oncol., 6:47, 2013; PCT Pubs. WO2012/079000, WO2013/059593; and U.S. Pub. 2012/0213783, each of which is incorporated by reference herein in its entirety.)

Conditions sufficient to form an immune complex: Conditions which allow an antibody or antigen binding fragment thereof to bind to its cognate epitope to a detectably greater degree than, and/or to the substantial exclusion of, binding to substantially all other epitopes. Conditions sufficient to form an immune complex are dependent upon the format of the binding reaction and typically are those utilized in immunoassay protocols or those conditions encountered *in vivo*. See Harlow & Lane, *Antibodies, A Laboratory Manual*, 2nd ed. Cold Spring Harbor Publications, New York (2013) for a

description of immunoassay formats and conditions. The conditions employed in the methods are "physiological conditions" which include reference to conditions (e.g., temperature, osmolarity, pH) that are typical inside a living mammal or a mammalian cell. While it is recognized that some organs are subject to extreme conditions, the intra-organismal and intracellular environment normally lies around pH 7 (e.g., from pH 6.0 to pH 8.0, more typically pH 6.5 to 7.5), contains water as the predominant solvent, and exists at a temperature above 0°C and below 50°C. Osmolarity is within the range that is supportive of cell viability and proliferation.

Conjugate: A complex of two molecules linked together, for example, linked together by a covalent bond. In one embodiment, an antibody is linked to an effector molecule; for example, an antibody that specifically binds to HIV-1 Env covalently linked to an effector molecule. The linkage can be by chemical or recombinant means. In one embodiment, the linkage is chemical, wherein a reaction between the antibody moiety and the effector molecule has produced a covalent bond formed between the two molecules to form one molecule. A peptide linker (short peptide sequence) can optionally be included between the antibody and the effector molecule. Because conjugates can be prepared from two molecules with separate functionalities, such as an antibody and an effector molecule, they are also sometimes referred to as "chimeric molecules."

Contacting: Placement in direct physical association; includes both in solid and liquid form, which can take place either *in vivo* or *in vitro*. Contacting includes contact between one molecule and another molecule, for example the amino acid on the surface of one polypeptide, such as an antigen, that contacts another polypeptide, such as an antibody. Contacting can also include contacting a cell for example by placing an antibody in direct physical association with a cell.

Control: A reference standard. In some embodiments, the control is a negative control, such as sample obtained from a healthy patient not infected with HIV. In other embodiments, the control is a positive control, such as a tissue sample obtained from a patient diagnosed with HIV infection. In still other embodiments, the control is a historical control or standard reference value or range of values (such as a previously tested control sample, such as a group of HIV patients with known prognosis or outcome, or group of samples that represent baseline or normal values).

A difference between a test sample and a control can be an increase or conversely a decrease. The difference can be a qualitative difference or a quantitative difference, for example a statistically significant difference. In some examples, a difference is an increase or decrease, relative to a control, of at least about 5%, such as at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 100%, at least about 150%, at least about 200%, at least about 250%, at least about 300%, at least about 350%, at least about 400%, or at least about 500%.

Conservative variants: "Conservative" amino acid substitutions are those substitutions that do not substantially alter the biological function of a protein, such as substitutions that do not substantially decrease the binding affinity of an antibody for an antigen (for example, the binding affinity of an antibody for HIV-1 Env). For example, a human antibody that specifically binds HIV-1 Env can include

at most about 1, at most about 2, at most about 5, at most about 10, or at most about 15 conservative substitutions and specifically bind the HIV-1 Env polypeptide. The term conservative variation also includes the use of a substituted amino acid in place of an unsubstituted parent amino acid, provided that antibody retains binding affinity for HIV-1 Env.

Conservative amino acid substitution tables providing functionally similar amino acids are well known to one of ordinary skill in the art. The following six groups are examples of amino acids that are considered to be conservative substitutions for one another:

1) Alanine (A), Serine (S), Threonine (T);

2) Aspartic acid (D), Glutamic acid (E);

3) Asparagine (N), Glutamine (Q);

4) Arginine (R), Lysine (K);

5) Isoleucine (I), Leucine (L), Methionine (M), Valine (V); and

6) Phenylalanine (F), Tyrosine (Y), Tryptophan (W).

Non-conservative substitutions are those that reduce an activity or function of the HIV-1 Env-specific antibody, such as the ability to specifically bind to HIV-1 Env. For instance, if an amino acid residue is essential for a function of the protein, even an otherwise conservative substitution may disrupt that activity. Thus, a conservative substitution does not alter the basic function of a protein of interest.

Degenerate variant: In the context of the present disclosure, a "degenerate variant" refers to a polynucleotide encoding a protein (for example, an antibody that specifically binds HIV-1 Env) that includes a sequence that is degenerate as a result of the genetic code. There are twenty natural amino acids, most of which are specified by more than one codon. Therefore, all degenerate nucleotide sequences are included as long as the amino acid sequence of the antibody that binds HIV-1 Env encoded by the nucleotide sequence is unchanged.

Detectable marker: A detectable molecule (also known as a label) that is conjugated directly or indirectly to a second molecule, such as an antibody, to facilitate detection of the second molecule. For example, the detectable marker can be capable of detection by ELISA, spectrophotometry, flow cytometry, microscopy or diagnostic imaging techniques (such as CT scans, MRIs, ultrasound, fiberoptic examination, and laparoscopic examination). Specific, non-limiting examples of detectable markers include fluorophores, chemiluminescent agents, enzymatic linkages, radioactive isotopes and heavy metals or compounds (for example super paramagnetic iron oxide nanocrystals for detection by MRI). In one example, a "labeled antibody" refers to incorporation of another molecule in the antibody. For example, the label is a detectable marker, such as the incorporation of a radiolabeled amino acid or attachment to a polypeptide of biotinyl moieties that can be detected by marked avidin (for example, streptavidin containing a fluorescent marker or enzymatic activity that can be detected by optical or colorimetric methods). Various methods of labeling polypeptides and glycoproteins are known in the art and may be used. Examples of labels for polypeptides include, but are not limited to, the following: radioisotopes or radionuclides (such as ^{35}S or ^{131}I), fluorescent labels (such as fluorescein isothiocyanate (FITC), rhodamine, lanthanide phosphors), enzymatic labels (such as horseradish peroxidase, beta-

galactosidase, luciferase, alkaline phosphatase), chemiluminescent markers, biotinyl groups, predetermined polypeptide epitopes recognized by a secondary reporter (such as a leucine zipper pair sequences, binding sites for secondary antibodies, metal binding domains, epitope tags), or magnetic agents, such as gadolinium chelates. In some embodiments, labels are attached by spacer arms of various lengths to reduce potential steric hindrance. Methods for using detectable markers and guidance in the choice of detectable markers appropriate for various purposes are discussed for example in Sambrook *et al.* (Molecular Cloning: A Laboratory Manual, 4th ed, Cold Spring Harbor, New York, 2012) and Ausubel *et al.* (In Current Protocols in Molecular Biology, John Wiley & Sons, New York, through supplement 104, 2013).

Detecting: To identify the existence, presence, or fact of something. General methods of detecting are known to the skilled artisan and may be supplemented with the protocols and reagents disclosed herein. For example, included herein are methods of detecting a cell that expresses HIV-1 Env in a subject.

Effector molecule: The portion of a chimeric molecule that is intended to have a desired effect on a cell to which the chimeric molecule is targeted. Effector molecule is also known as an effector moiety (EM), therapeutic agent, or diagnostic agent, or similar terms.

Epitope: An antigenic determinant. These are particular chemical groups or peptide sequences on a molecule that are antigenic, and can be specifically bound by an antibody that targets the epitope. An epitope on an antigen can, in some embodiments, elicit a specific immune response, for example, an epitope is the region of an antigen to which B and/or T cells respond. An antibody can specifically bind to a particular epitope, such as an epitope on HIV-1 Env.

Expressed: Translation of a nucleic acid into a protein. Proteins may be expressed and remain intracellular, become a component of the cell surface membrane, or be secreted into the extracellular matrix or medium.

Expression Control Sequences: Nucleic acid sequences that regulate the expression of a heterologous nucleic acid sequence to which it is operatively linked. Expression control sequences are operatively linked to a nucleic acid sequence when the expression control sequences control and regulate the transcription and, as appropriate, translation of the nucleic acid sequence. Thus expression control sequences can include appropriate promoters, enhancers, transcription terminators, a start codon (i.e., ATG) in front of a protein-encoding gene, splicing signal for introns, maintenance of the correct reading frame of that gene to permit proper translation of mRNA, and stop codons. The term "control sequences" is intended to include, at a minimum, components whose presence can influence expression, and can also include additional components whose presence is advantageous, for example, leader sequences and fusion partner sequences. Expression control sequences can include a promoter.

A promoter is a minimal sequence sufficient to direct transcription. Also included are those promoter elements which are sufficient to render promoter-dependent gene expression controllable for cell-type specific, tissue-specific, or inducible by external signals or agents; such elements may be located in the 5' or 3' regions of the gene. Both constitutive and inducible promoters are included (see for

example, Bitter *et al.*, *Methods in Enzymology* 153:516-544, 1987). For example, when cloning in bacterial systems, inducible promoters such as pL of bacteriophage lambda, plac, ptrp, ptac (ptrp-lac hybrid promoter) and the like may be used. In one embodiment, when cloning in mammalian cell systems, promoters derived from the genome of mammalian cells (such as metallothionein promoter) or from mammalian viruses (such as the retrovirus long terminal repeat; the adenovirus late promoter; the vaccinia virus 7.5K promoter) can be used. Promoters produced by recombinant DNA or synthetic techniques may also be used to provide for transcription of the nucleic acid sequences. A polynucleotide can be inserted into an expression vector that contains a promoter sequence which facilitates the efficient transcription of the inserted genetic sequence of the host. The expression vector typically contains an origin of replication, a promoter, as well as specific nucleic acid sequences that allow phenotypic selection of the transformed cells.

Expression vector: A vector comprising a recombinant polynucleotide comprising expression control sequences operatively linked to a nucleotide sequence to be expressed. An expression vector comprises sufficient cis- acting elements for expression; other elements for expression can be supplied by the host cell or in an in vitro expression system. Expression vectors include all those known in the art, such as cosmids, plasmids (e.g., naked or contained in liposomes) and viruses (e.g., lentiviruses, retroviruses, adenoviruses, and adeno-associated viruses) that incorporate the recombinant polynucleotide.

Framework Region: Amino acid sequences interposed between CDRs in a heavy or light variable region of an antibody. Includes variable light and variable heavy framework regions. The framework regions serve to hold the CDRs in an appropriate orientation.

Fc polypeptide: The polypeptide including the constant region of an antibody excluding the first constant region immunoglobulin domain. Fc region generally refers to the last two constant region immunoglobulin domains of IgA, IgD, and IgG, and the last three constant region immunoglobulin domains of IgE and IgM. An Fc region may also include part or all of the flexible hinge N-terminal to these domains. For IgA and IgM, an Fc region may or may not include the tailpiece, and may or may not be bound by the J chain. For IgG, the Fc region includes immunoglobulin domains Cgamma2 and Cgamma3 (Cγ2 and Cγ3) and the lower part of the hinge between Cgamma1 (Cγ1) and Cγ2. Although the boundaries of the Fc region may vary, the human IgG heavy chain Fc region is usually defined to include residues C226 or P230 to its carboxyl-terminus, wherein the numbering is according to the EU index as in Kabat. For IgA, the Fc region includes immunoglobulin domains Calpha2 and Calpha3 (Cα2 and Cα3) and the lower part of the hinge between Calpha1 (Cα1) and Cα2.

Glycoprotein (gp): A protein that contains oligosaccharide chains (glycans) covalently attached to polypeptide side-chains. The carbohydrate is attached to the protein in a cotranslational or posttranslational modification. This process is known as glycosylation. In proteins that have segments extending extracellularly, the extracellular segments are often glycosylated. Glycoproteins are often important integral membrane proteins, where they play a role in cell-cell interactions. In some examples a glycoprotein is an HIV glycoprotein, such as HIV Env.

Glycosylation site: An amino acid sequence on the surface of a polypeptide, such as a protein, which accommodates the attachment of a glycan. An N-linked glycosylation site is triplet sequence of NXS/T in which N is asparagine, X is any residues except proline, S/T means serine or threonine. A glycan is a polysaccharide or oligosaccharide. Glycan may also be used to refer to the carbohydrate portion of a glycoconjugate, such as a glycoprotein, glycolipid, or a proteoglycan.

Human Immunodeficiency Virus (HIV): A retrovirus that causes immunosuppression in humans (HIV disease), and leads to a disease complex known as the acquired immunodeficiency syndrome (AIDS). "HIV disease" refers to a well-recognized constellation of signs and symptoms (including the development of opportunistic infections) in persons who are infected by an HIV virus, as determined by antibody or western blot studies. Laboratory findings associated with this disease include a progressive decline in T cells. HIV includes HIV type 1 (HIV-1) and HIV type 2 (HIV-2). Related viruses that are used as animal models include simian immunodeficiency virus (SIV), and feline immunodeficiency virus (FIV). Treatment of HIV-1 with HAART has been effective in reducing the viral burden and ameliorating the effects of HIV-1 infection in infected individuals.

HIV-1 broadly neutralizing antibody: An antibody that reduces the infectious titer of HIV-1 by binding to and inhibiting the function of related HIV-1 antigens, such as antigens that share at least 85%, 90%, 95%, 96%, 97%, 98% or 99% sequence identity with antigenic surface of the antigen. In some embodiments, broadly neutralizing antibodies to HIV are distinct from other antibodies to HIV in that they neutralize a high percentage (such as at least 50% or at least 80%) of the many types of HIV in circulation. Non-limiting examples of HIV-1 broadly neutralizing antibodies include 2G12, PGT122, VRC01, and 35O22.

HIV-1 Envelope protein (Env): The HIV envelope protein is initially synthesized as a precursor protein of 845-870 amino acids in size, designated gp160. Individual gp160 polypeptides form a homotrimer and undergo glycosylation within the Golgi apparatus as well as processing to remove the signal peptide, and cleavage by a cellular protease between approximately positions 511/512 to generate separate gp120 and gp41 polypeptide chains, which remain associated as gp120/gp41 protomers within the homotrimer. The ectodomain (that is, the extracellular portion) of the HIV-1 Env trimer undergoes several structural rearrangements from a prefusion mature (cleaved) closed conformation that evades antibody recognition, through intermediate conformations that bind to receptors CD4 and co-receptor (either CCR5 or CXCR4), to a postfusion conformation.

The numbering used in the disclosed HIV-1 Env proteins and fragments thereof is relative to the HXB2 numbering scheme as set forth in *Numbering Positions in HIV Relative to HXB2CG* Bette Korber *et al.*, Human Retroviruses and AIDS 1998: A Compilation and Analysis of Nucleic Acid and Amino Acid Sequences. Korber *et al.*, Eds. Theoretical Biology and Biophysics Group, Los Alamos National Laboratory, Los Alamos, NM, which is incorporated by reference herein in its entirety.

In one example, an HIV-1 Env protein is from the BG505 strain of HIV, which is a Clade A HIV-1 virus isolated from a six-week old HIV-1 infected infant. The amino acid sequence of BG505

Env protein is known (see, e.g., GenBank accession no. ABA61516, incorporated by reference herein as present in the database on June 20, 2014).

HIV-1 gp120: A polypeptide that is part of the HIV-1 Env protein. Mature gp120 includes approximated HIV-1 Env residues 31-511, contains most of the external, surface-exposed, domains of the HIV-1 Env trimer, and it is gp120 which binds both to cellular CD4 receptors and to cellular chemokine receptors (such as CCR5). A mature gp120 polypeptide is an extracellular polypeptide that interacts with the gp41 ectodomain to form an HIV-1 Env protomer that trimerizes to form the HIV-1 Env trimer.

The gp120 is heavily N-glycosylated giving rise to an apparent molecular weight of 120 kD. The polypeptide is comprised of five conserved regions (C1-C5) and five regions of high variability (V1-V5). Exemplary sequences of wild-type gp160 polypeptides (including a gp120 region) are shown on GENBANK®, for example Accession Nos. AAB05604 and AAD12142, which are incorporated herein by reference in their entirety as available on December 20, 2013.

The C2 region of gp120 locates between the V2 and V3 regions and includes gp120 residues 196-295 (HXB2 numbering). The C2 region contains several N-linked glycosylation (PNLG) sites, the glycosylation of which can regulate the susceptibility of HIV-1 viruses to neutralizing antibodies. For example, glycosylation of N197 in the C2 region of can determine the b12 resistance of CRF01_AE viruses (Utachee *et al.*, J. Virol. 2010;84:4311-4320). Also, glycosylation of N234 and N276 can affect HIV-1 neutralization by mAb 8ANC195, a potent broadly neutralizing antibody, because mutations removing these glycosylation sites greatly reduced the neutralization potency of 8ANC195 (West *et al.*, Proc Natl Acad Sci U S A. 2013; 110(26):10598-603). The residues that make up the C2 region of gp120 in HIV-1 Env of *HXB2* are set forth below CNTSVITQACPKVSFEPIPIHYCAPAGFAILKCNNKTFNGTGPCTNVSTVQCTHGIRPVVSTQLLLNGSL AEEEVVIRSVNFTDNAKTIIVQLNTSVEIN (SEQ ID NO: 38; from GENBANK® Accession No. K03455, incorporated by reference herein as present in GENBANK on January 3, 2014).

The gp120 core has a unique molecular structure, which comprises two domains: an "inner" domain (which faces gp41) and an "outer" domain (which is mostly exposed on the surface of the oligomeric envelope glycoprotein complex). The two gp120 domains are separated by a "bridging sheet" that is not part of either of these domains.

The core gp120 comprises 25 β-strands, 5 α-helices and 10 defined loop segments. The polypeptide chain of gp120 is folded into two major domains, plus certain excursions that emanate from this body. The inner domain (inner with respect to the N and C termini) features a two-helix, two-strand bundle with a small five-stranded β -sandwich at its termini-proximal end and a projection at the distal end from which the V1/V2 stem emanates. The outer domain is a stacked double barrel that lies alongside the inner domain so that the outer barrel and inner bundle axes are approximately parallel. The bridging sheet (β3, β2, β21, β20) packs primarily over the inner domain, although some surface residues of the outer domain, such as Phenylalanine 382, reach in to form part of its hydrophobic core.

HIV-1 gp140: A recombinant HIV Env polypeptide including gp120 and the gp41 ectodomain domain, but not the gp41 transmembrane or cytosolic domains. HIV-1 gp140 polypeptides can trimerize to form a soluble HIV-1 Env ectodomain trimer.

HIV-1 gp41: A polypeptide that is part of the HIV-1 Env protein. Mature gp41 includes approximately HIV-1 Env residues 512-860, and includes cytosolic-, transmembrane-, and ecto-domains. The gp41 ectodomain (including approximately HIV-1 Env residues 512-644) can interact with gp120 to form an HIV-1 Env protomer that trimerizes to form the HIV-1 Env trimer. The amino acid sequence of an example of gp41 is set forth in GENBANK® Accession No. CAD20975 (as available on December 20, 2013) which is incorporated by reference herein. It is understood that the sequence of gp41 can vary from that given in GENBANK® Accession No. CAD20975.

HXB2 numbering system: A reference numbering system for HIV protein and nucleic acid sequences, using HIV-1 HXB2 strain sequences as a reference for all other HIV strain sequences. The person of ordinary skill in the art is familiar with the HXB2 numbering system, and this system is set forth in "Numbering Positions in HIV Relative to HXB2CG," Bette Korber *et al.*, Human Retroviruses and AIDS 1998: A Compilation and Analysis of Nucleic Acid and Amino Acid Sequences. Korber B, Kuiken CL, Foley B, Hahn B, McCutchan F, Mellors JW, and Sodroski J, Eds. Theoretical Biology and Biophysics Group, Los Alamos National Laboratory, Los Alamos, NM, which is incorporated by reference herein in its entirety. HXB2 is also known as: HXBc2, for HXB clone 2; HXB2R, in the Los Alamos HIV database, with the R for revised, as it was slightly revised relative to the original HXB2 sequence; and HXB2CG in GENBANK™, for HXB2 complete genome. The numbering used in gp120 polypeptides disclosed herein is relative to the HXB2 numbering scheme. For reference, the amino acid sequence of HIV-1 Env of *HXB2* is set forth below:

MRVKEKYQHLWRWGWRWGTMLLGMLMICSATEKLWVTVYYGVPVWKEATTTLFCASDAKAYDTEVHNVWA
THACVPTDPNPQEVVLVNVTENFNMWKNDMVEQMHEDIISLWDQSLKPCVKLTPLCVSLKCTDLKNDTNT
NSSSGRMIMEKGEIKNCSFNISTSIRGKVQKEYAFFYKLDIIPIDNDTTSYKLTSCNTSVITQACPKVSF
EPIPIHYCAPAGFAILKCNNKTFNGTGPCTNVSTVQCTHGIRPVVSTQLLLNGSLAEEEVVIRSVNFTDN
AKTIIVQLNTSVEINCTRPNNNTRKRIRIQRGPGRAFVTIGKIGNMRQAHCNISRAKWNNTLKQIASKLR
EQFGNNKTIIFKQSSGGDPEIVTHSFNCGGEFFYCNSTQLFNSTWFNSTWSTEGSNNTEGSDTITLPCRI
KQIINMWQKVGKAMYAPPISGQIRCSSNITGLLLTRDGGNSNNESEIFRPGGGDMRDNWRSELYKYKVVK
IEPLGVAPTKAKRRVVQREKRAVGIGALFLGFLGAAGSTMGAASMTLTVQARQLLSGIVQQQNNLLRAIE
AQQHLLQLTVWGIKQLQARILAVERYLKDQQLLGIWGCSGKLICTTAVPWNASWSNKSLEQIWNHTTWME
WDREINNYTSLIHSLIEESQNQQEKNEQELLELDKWASLWNWFNITNWLWYIKLFIMIVGGLVGLRIVFA
VLSIVNRVRQGYSPLSFQTHLPTPRGPDRPEGIEEEGGERDRDRSIRLVNGSLALIWDDLRSLCLFSYHR
LRDLLLIVTRIVELLGRRGWEALKYWWNLLQYWSQELKNSAVSLLNATAIAVAEGTDRVIEVVQGACRAI
RHIPRRIRQGLERILL (SEQ ID NO: 39; GENBANK® Accession No. K03455, incorporated by reference herein as present in the database on December 20, 2013).

IgA: A polypeptide belonging to the class of antibodies that are substantially encoded by a recognized immunoglobulin alpha gene. In humans, this class or isotype includes IgA_1 and IgA_2. IgA antibodies can exist as monomers, polymers (referred to as pIgA) of predominantly dimeric form, and secretory IgA. The constant chain of wild-type IgA contains an 18-amino-acid extension at its C-terminus called the tail piece (tp). Polymeric IgA is secreted by plasma cells with a 15-kDa peptide called the J chain linking two monomers of IgA through the conserved cysteine residue in the tail piece.

IgG: A polypeptide belonging to the class or isotype of antibodies that are substantially encoded by a recognized immunoglobulin gamma gene. In humans, this class includes IgG_1, IgG_2, IgG_3, and IgG_4. In mice, this class includes IgG_1, IgG_{2a}, IgG_{2b}, and IgG_3.

Immune complex: The binding of antibody or antigen binding fragment (such as a scFv) to a soluble antigen forms an immune complex. The formation of an immune complex can be detected through conventional methods known to the skilled artisan, for instance immunohistochemistry, immunoprecipitation, flow cytometry, immunofluorescence microscopy, ELISA, immunoblotting (for example, Western blot), magnetic resonance imaging, CT scans, X-ray and affinity chromatography. Immunological binding properties of selected antibodies may be quantified using methods well known in the art.

Isolated: A biological component (such as a nucleic acid, peptide, protein or protein complex, for example an antibody) that has been substantially separated, produced apart from, or purified away from other biological components in the cell of the organism in which the component naturally occurs, that is, other chromosomal and extra-chromosomal DNA and RNA, and proteins. Thus, isolated nucleic acids, peptides and proteins include nucleic acids and proteins purified by standard purification methods. The term also embraces nucleic acids, peptides and proteins prepared by recombinant expression in a host cell, as well as, chemically synthesized nucleic acids. A isolated nucleic acid, peptide or protein, for example an antibody, can be at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% pure.

K_D: The dissociation constant for a given interaction, such as a polypeptide ligand interaction or an antibody antigen interaction. For example, for the bimolecular interaction of an antibody or antigen binding fragment (such as 35O22 or an antigen binding fragment thereof) and an antigen (such as HIV-1 Env protein) it is the concentration of the individual components of the bimolecular interaction divided by the concentration of the complex.

Linker: A bi-functional molecule that can be used to link two molecules into one contiguous molecule, for example, to link an effector molecule to an antibody. In some embodiments, the provided conjugates include a linker between the effector molecule or detectable marker and an antibody. In some embodiments, the linker is selectively cleavable, for example, cleavable under intracellular conditions, such that cleavage of the linker releases the effector molecule or detectable marker from the antibody in the intracellular environment. Selectively cleavable refers to cleaving in response to a preselected condition or stimulus. In yet other embodiments, the linker is not cleavable and the effector molecule or detectable marker can be released, for example, by antibody degradation. In some cases, a linker is a peptide within an antigen binding fragment (such as an Fv fragment) which serves to indirectly bond the variable heavy chain to the variable light chain.

The terms "conjugating," "joining," "bonding," or "linking" can refer to making two molecules into one contiguous molecule; for example, linking two polypeptides into one contiguous polypeptide, or covalently attaching an effector molecule or detectable marker radionuclide or other molecule to a polypeptide, such as an scFv. In the specific context, the terms include reference to joining a ligand, such

as an antibody moiety, to an effector molecule. The linkage can be either by chemical or recombinant means. "Chemical means" refers to a reaction between the antibody moiety and the effector molecule such that there is a covalent bond formed between the two molecules to form one molecule.

Neutralizing antibody: An antibody which reduces the infectious titer of an infectious agent by binding to a specific antigen on the infectious agent. In some examples the infectious agent is a virus. In some examples, an antibody that is specific for HIV-1 Env neutralizes the infectious titer of HIV. A "broadly neutralizing antibody" is an antibody that binds to and inhibits the function of related antigens, such as antigens that share at least 85%, 90%, 95%, 96%, 97%, 98% or 99% identity antigenic surface of antigen. With regard to an antigen from a pathogen, such as a virus, the antibody can bind to and inhibit the function of an antigen from more than one class and/or subclass of the pathogen. For example, with regard to a human immunodeficiency virus, the antibody can bind to and inhibit the function of an antigen, such as HIV-1 Env from more than one clade. In one embodiment, broadly neutralizing antibodies to HIV are distinct from other antibodies to HIV in that they neutralize a high percentage of the many types of HIV in circulation.

Nucleic acid: A polymer composed of nucleotide units (ribonucleotides, deoxyribonucleotides, related naturally occurring structural variants, and synthetic non-naturally occurring analogs thereof) linked via phosphodiester bonds, related naturally occurring structural variants, and synthetic non-naturally occurring analogs thereof. Thus, the term includes nucleotide polymers in which the nucleotides and the linkages between them include non-naturally occurring synthetic analogs, such as, for example and without limitation, phosphorothioates, phosphoramidates, methyl phosphonates, chiral-methyl phosphonates, 2-O-methyl ribonucleotides, peptide-nucleic acids (PNAs), and the like. Such polynucleotides can be synthesized, for example, using an automated DNA synthesizer. The term "oligonucleotide" typically refers to short polynucleotides, generally no greater than about 50 nucleotides. It will be understood that when a nucleotide sequence is represented by a DNA sequence (i.e., A, T, G, C), this also includes an RNA sequence (i.e., A, U, G, C) in which "U" replaces "T. "

Conventional notation is used herein to describe nucleotide sequences: the left-hand end of a single-stranded nucleotide sequence is the 5'-end; the left-hand direction of a double-stranded nucleotide sequence is referred to as the 5'-direction. The direction of 5' to 3' addition of nucleotides to nascent RNA transcripts is referred to as the transcription direction. The DNA strand having the same sequence as an mRNA is referred to as the "coding strand;" sequences on the DNA strand having the same sequence as an mRNA transcribed from that DNA and which are located 5' to the 5'-end of the RNA transcript are referred to as "upstream sequences;" sequences on the DNA strand having the same sequence as the RNA and which are 3' to the 3' end of the coding RNA transcript are referred to as "downstream sequences."

"cDNA" refers to a DNA that is complementary or identical to an mRNA, in either single stranded or double stranded form.

"Encoding" refers to the inherent property of specific sequences of nucleotides in a polynucleotide, such as a gene, a cDNA, or an mRNA, to serve as templates for synthesis of other

polymers and macromolecules in biological processes having either a defined sequence of nucleotides (i.e., rRNA, tRNA and mRNA) or a defined sequence of amino acids and the biological properties resulting therefrom. Thus, a gene encodes a protein if transcription and translation of mRNA produced by that gene produces the protein in a cell or other biological system. Both the coding strand, the nucleotide sequence of which is identical to the mRNA sequence and is usually provided in sequence listings, and non-coding strand, used as the template for transcription, of a gene or cDNA can be referred to as encoding the protein or other product of that gene or cDNA. Unless otherwise specified, a "nucleotide sequence encoding an amino acid sequence" includes all nucleotide sequences that are degenerate versions of each other and that encode the same amino acid sequence. Nucleotide sequences that encode proteins and RNA may include introns.

A polynucleotide or nucleic acid sequence refers to a polymeric form of nucleotide at least 10 bases in length. A recombinant polynucleotide includes a polynucleotide that is not immediately contiguous with both of the coding sequences with which it is immediately contiguous (one on the 5' end and one on the 3' end) in the naturally occurring genome of the organism from which it is derived. The term therefore includes, for example, a recombinant DNA which is incorporated into a vector; into an autonomously replicating plasmid or virus; or into the genomic DNA of a prokaryote or eukaryote, or which exists as a separate molecule (e.g., a cDNA) independent of other sequences. The nucleotides can be ribonucleotides, deoxyribonucleotides, or modified forms of either nucleotide. The term includes single- and double- stranded forms of DNA.

Operably linked: A first nucleic acid sequence is operably linked with a second nucleic acid sequence when the first nucleic acid sequence is placed in a functional relationship with the second nucleic acid sequence. For instance, a promoter, such as the CMV promoter, is operably linked to a coding sequence if the promoter affects the transcription or expression of the coding sequence. Generally, operably linked DNA sequences are contiguous and, where necessary to join two protein-coding regions, in the same reading frame.

Pharmaceutically acceptable carriers: The pharmaceutically acceptable carriers of use are conventional. *Remington's Pharmaceutical Sciences*, by E. W. Martin, Mack Publishing Co., Easton, PA, 19th Edition, 1995, describes compositions and formulations suitable for pharmaceutical delivery of the disclosed antibodies.

In general, the nature of the carrier will depend on the particular mode of administration being employed. For instance, parenteral formulations usually comprise injectable fluids that include pharmaceutically and physiologically acceptable fluids such as water, physiological saline, balanced salt solutions, aqueous dextrose, glycerol or the like as a vehicle. For solid compositions (*e.g.*, powder, pill, tablet, or capsule forms), conventional non-toxic solid carriers can include, for example, pharmaceutical grades of mannitol, lactose, starch, or magnesium stearate. In addition to biologically neutral carriers, pharmaceutical compositions to be administered can contain minor amounts of non-toxic auxiliary substances, such as wetting or emulsifying agents, preservatives, and pH buffering agents and the like, for example sodium acetate or sorbitan monolaurate. In particular embodiments, suitable for

administration to a subject the carrier may be sterile, and/or suspended in a unit dosage form containing one or more measured doses of the composition suitable to induce the desired anti-HIV immune response. It may also be accompanied by medications for its use for treatment purposes. The unit dosage form may be, for example, in a sealed vial that contains sterile contents or a syringe for injection into a subject.

Polypeptide: Any chain of amino acids, regardless of length or post-translational modification (e.g., glycosylation or phosphorylation). In one embodiment, the polypeptide is a gp120 polypeptide. In one embodiment, the polypeptide is a disclosed antibody or a fragment thereof. A "residue" refers to an amino acid or amino acid mimetic incorporated in a polypeptide by an amide bond or amide bond mimetic. A polypeptide has an amino terminal (N-terminal) end and a carboxy terminal (C-terminal) end. A protein can include multiple polypeptide chains; for example, HIV-1 Env protein includes a gp120 polypeptide chain and a gp41 polypeptide chain.

Polypeptide modifications: polypeptides can be modified by a variety of chemical techniques to produce derivatives having essentially the same activity and conformation as the unmodified peptides, and optionally having other desirable properties. For example, carboxylic acid groups of the protein, whether carboxyl-terminal or side chain, may be provided in the form of a salt of a pharmaceutically-acceptable cation or esterified to form a C_1-C_{16} ester, or converted to an amide of formula NR_1R_2 wherein R_1 and R_2 are each independently H or C_1-C_{16} alkyl, or combined to form a heterocyclic ring, such as a 5- or 6- membered ring. Amino groups of the peptide, whether amino-terminal or side chain, may be in the form of a pharmaceutically-acceptable acid addition salt, such as the HCl, HBr, acetic, benzoic, toluene sulfonic, maleic, tartaric and other organic salts, or may be modified to C_1-C_{16} alkyl or dialkyl amino or further converted to an amide.

Hydroxyl groups of the peptide side chains can be converted to C_1-C_{16} alkoxy or to a C_1-C_{16} ester using well-recognized techniques. Phenyl and phenolic rings of the peptide side chains can be substituted with one or more halogen atoms, such as F, Cl, Br or I, or with C_1-C_{16} alkyl, C_1-C_{16} alkoxy, carboxylic acids and esters thereof, or amides of such carboxylic acids. Methylene groups of the peptide side chains can be extended to homologous C_2-C_4 alkylenes. Thiols can be protected with any one of a number of well-recognized protecting groups, such as acetamide groups.

Purified: The term purified does not require absolute purity; rather, it is intended as a relative term. Thus, for example, a purified peptide preparation is one in which the peptide or protein (such as an antibody) is more enriched than the peptide or protein is in its natural environment within a cell. In one embodiment, a preparation is purified such that the protein or peptide represents at least 50% of the total peptide or protein content of the preparation, such as at least 80%, at least 90%, at least 95% or greater of the total peptide or protein content.

Recombinant: A recombinant nucleic acid is one that has a sequence that is not naturally occurring or has a sequence that is made by an artificial combination of two otherwise separated segments of sequence. This artificial combination can be accomplished by chemical synthesis or, more commonly, by the artificial manipulation of isolated segments of nucleic acids, for example, by genetic

engineering techniques. A recombinant protein is a protein encoded by a heterologous (for example, recombinant) nucleic acid that has been introduced into a host cell, such as a bacterial or eukaryotic cell. The nucleic acid can be introduced, for example, on an expression vector having signals capable of expressing the protein encoded by the introduced nucleic acid or the nucleic acid can be integrated into the host cell chromosome.

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

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

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

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

Terms used to describe sequence relationships between two or more nucleotide sequences or amino acid sequences include “reference sequence,” “selected from,” “comparison window,” “identical,”

"percentage of sequence identity," "substantially identical," "complementary," and "substantially complementary."

For sequence comparison of nucleic acid sequences, typically one sequence acts as a reference sequence, to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are entered into a computer, subsequence coordinates are designated, if necessary, and sequence algorithm program parameters are designated. Default program parameters are used. Methods of alignment of sequences for comparison are well known in the art. Optimal alignment of sequences for comparison can be conducted, e.g., by the local homology algorithm of Smith & Waterman, *Adv. Appl. Math.* 2:482, 1981, by the homology alignment algorithm of Needleman & Wunsch, *J. Mol. Biol.* 48:443, 1970, by the search for similarity method of Pearson & Lipman, *Proc. Nat'l. Acad. Sci. USA* 85:2444, 1988, by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, WI), or by manual alignment and visual inspection (see, e.g., Sambrook *et al.* (Molecular Cloning: A Laboratory Manual, 4th ed, Cold Spring Harbor, New York, 2012) and Ausubel *et al.* (In Current Protocols in Molecular Biology, John Wiley & Sons, New York, through supplement 104, 2013). One example of a useful algorithm is PILEUP. PILEUP uses a simplification of the progressive alignment method of Feng & Doolittle, *J. Mol. Evol.* 35:351-360, 1987. The method used is similar to the method described by Higgins & Sharp, *CABIOS* 5:151-153, 1989. Using PILEUP, a reference sequence is compared to other test sequences to determine the percent sequence identity relationship using the following parameters: default gap weight (3.00), default gap length weight (0.10), and weighted end gaps. PILEUP can be obtained from the GCG sequence analysis software package, e.g., version 7.0 (Devereaux et al., *Nuc. Acids Res.* 12:387-395, 1984.

Another example of algorithms that are suitable for determining percent sequence identity and sequence similarity are the BLAST and the BLAST 2.0 algorithm, which are described in Altschul et al., *J. Mol. Biol.* 215:403-410, 1990 and Altschul et al., *Nucleic Acids Res.* 25:3389-3402, 1977. Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information (ncbi.nlm.nih.gov). The BLASTN program (for nucleotide sequences) uses as defaults a word length (W) of 11, alignments (B) of 50, expectation (E) of 10, M=5, N=-4, and a comparison of both strands. The BLASTP program (for amino acid sequences) uses as defaults a word length (W) of 3, and expectation (E) of 10, and the BLOSUM62 scoring matrix (see Henikoff & Henikoff, *Proc. Natl. Acad. Sci. USA* 89:10915, 1989). An oligonucleotide is a linear polynucleotide sequence of up to about 100 nucleotide bases in length.

Specifically bind: When referring to an antibody or antigen binding fragment, refers to a binding reaction which determines the presence of a target protein, peptide, or polysaccharide in the presence of a heterogeneous population of proteins and other biologics. Thus, under designated conditions, an antibody binds preferentially to a particular target protein, peptide or polysaccharide (such as an antigen present on the surface of a pathogen, for example HIV-1 Env) and does not bind in a significant amount to other proteins or polysaccharides present in the sample or subject. Specific binding can be determined by

methods known in the art. With reference to an antibody antigen complex, specific binding of the antigen and antibody has a K_D of less than about 10^{-7} M, such as less than about 10^{-8} M, 10^{-9}, or even less than about 10^{-10} M.

The antibodies disclosed herein specifically bind to a defined target (or multiple targets, in the case of a bispecific antibody). Thus, an antibody that specifically binds to an epitope on HIV-1 Env is an antibody that binds substantially to HIV-1 Env, including cells or tissue expressing HIV-1 Env, substrate to which the HIV-1 Env is attached, or HIV-1 Env in a biological specimen. It is, of course, recognized that a certain degree of non-specific interaction may occur between an antibody or conjugate including an antibody (such as an antibody that specifically binds HIV-1 Env or conjugate including such antibody) and a non-target (such as a cell that does not express HIV-1 Env). Typically, specific binding results in a much stronger association between the antibody and protein or cells bearing the antigen than between the antibody and protein or cells lacking the antigen. Specific binding typically results in greater than 2-fold, such as greater than 5-fold, greater than 10-fold, or greater than 100-fold increase in amount of bound antibody (per unit time) to a protein including the epitope or cell or tissue expressing the target epitope as compared to a protein or cell or tissue lacking this epitope. Specific binding to a protein under such conditions requires an antibody that is selected for its specificity for a particular protein. A variety of immunoassay formats are appropriate for selecting antibodies or other ligands specifically immunoreactive with a particular protein. For example, solid-phase ELISA immunoassays are routinely used to select monoclonal antibodies specifically immunoreactive with a protein. See Harlow & Lane, *Antibodies, A Laboratory Manual*, 2nd ed., Cold Spring Harbor Publications, New York (2013), for a description of immunoassay formats and conditions that can be used to determine specific immunoreactivity.

Subject: Living multi-cellular vertebrate organisms, a category that includes human and non-human mammals. In an example, a subject is a human. In a particular example, the subject is a newborn infant. In an additional example, a subject is selected that is in need of inhibiting of an HIV-1 infection. For example, the subject is either uninfected and at risk of HIV-1 infection or is infected in need of treatment.

T Cell: A white blood cell critical to the immune response. T cells include, but are not limited to, $CD4^+$ T cells and $CD8^+$ T cells. A $CD4^+$ T lymphocyte is an immune cell that expresses CD4 on its surface. These cells, also known as helper T cells, help orchestrate the immune response, including antibody responses as well as killer T cell responses. Th1 and Th2 cells are functional subsets of helper T cells. Th1 cells secrete a set of cytokines, including interferon-gamma, and whose principal function is to stimulate phagocyte-mediated defense against infections, especially related to intracellular microbes. Th2 cells secrete a set of cytokines, including interleukin (IL)-4 and IL-5, and whose principal functions are to stimulate IgE and eosinophil/mast cell-mediated immune reactions and to downregulate Th1 responses.

Therapeutically effective amount: The amount of an agent (such as a HIV-1 Env specific antibody or antigen binding fragment, conjugate thereof, or nucleic acid molecule encoding such

molecules) that alone, or together with one or more additional agents, is sufficient to achieve a desired effect in a subject being treated. For instance, this can be the amount necessary to inhibit HIV-1 replication or treat HIV-1 infection. In several embodiments, a therapeutically effective amount is the amount necessary to reduce a sign or symptom of HIV-1 infection, and/or to decrease viral titer in a subject. When administered to a subject, a dosage will generally be used that will achieve target tissue concentrations that has been shown to achieve a desired *in vitro* effect. Ideally, a therapeutically effective amount provides a therapeutic effect without causing a substantial cytotoxic effect in the subject.

Several preparations disclosed herein are administered in therapeutically effective amounts. A therapeutically effective amount of an antibody or antigen binding fragment that specifically binds HIV-1 Env that is administered to a subject will vary depending upon a number of factors associated with that subject, for example the overall health of the subject. A therapeutically effective amount can be determined by varying the dosage and measuring the resulting therapeutic response, such as, for example, a decrease in viral titer. Therapeutically effective amounts also can be determined through various *in vitro*, *in vivo* or *in situ* immunoassays. The disclosed agents can be administered in a single dose, or in several doses, as needed to obtain the desired response. However, the therapeutically effective amount of can be dependent on the source applied, the subject being treated, the severity and type of the condition being treated, and the manner of administration.

Treating or preventing a disease: Inhibiting the full development of a disease or condition, for example, in a subject who is at risk of or has a disease such as an HIV-1 infection. "Treatment" refers to a therapeutic intervention that ameliorates a sign or symptom of a disease or pathological condition after it has begun to develop. The term "ameliorating," with reference to a disease or pathological condition, refers to any observable beneficial effect of the treatment. The beneficial effect can be evidenced, for example, by a delayed onset of clinical symptoms of the disease in a susceptible subject, a reduction in severity of some or all clinical symptoms of the disease, a slower progression of the disease, a reduction in the viral load, an improvement in the overall health or well-being of the subject, or by other parameters well known in the art that are specific to the particular disease. A "prophylactic" treatment is a treatment administered to a subject who does not exhibit signs of a disease or exhibits only early signs for the purpose of decreasing the risk of developing pathology.

The term "reduces" is a relative term, such that an agent reduces a disease or condition if the disease or condition is quantitatively diminished following administration of the agent, or if it is diminished following administration of the agent, as compared to a reference agent. Similarly, the term "prevents" does not necessarily mean that an agent completely eliminates the disease or condition, so long as at least one characteristic of the disease or condition is eliminated. Thus, an antibody that reduces or prevents an infection, can, but does not necessarily completely, eliminate such an infection, so long as the infection is measurably diminished, for example, by at least about 50%, such as by at least about 70%, or about 80%, or even by about 90% the infection in the absence of the agent, or in comparison to a reference agent.

Toxin: An effector molecule that induces cytotoxicity when it contacts a cell. Specific, non-limiting examples of toxins include, but are not limited to, abrin, ricin, auristatins (such as monomethyl auristatin E (MMAE; see for example, Francisco et al., Blood, 102: 1458-1465, 2003)) and monomethyl auristatin F (MMAF; see, for example, Doronina *et al.*, BioConjugate Chem., 17: 114-124, 2006), maytansinoids (such as DM1; see, for example, Phillips et al., Cancer Res., 68:9280-9290, 2008), *Pseudomonas* exotoxin (PE, such as PE35, PE37, PE38, and PE40), diphtheria toxin (DT), botulinum toxin, saporin, restrictocin or gelonin, or modified toxins thereof, or other toxic agents that directly or indirectly inhibit cell growth or kill cells. For example, PE and DT are highly toxic compounds that typically bring about death through liver toxicity. PE and DT, however, can be modified into a form for use as an immunotoxin by removing the native targeting component of the toxin (such as the domain Ia of PE and the B chain of DT) and replacing it with a different targeting moiety, such as an antibody.

Transformed: A transformed cell is a cell into which a nucleic acid molecule has been introduced by molecular biology techniques. As used herein, the term transformation encompasses all techniques by which a nucleic acid molecule might be introduced into such a cell, including transfection with viral vectors, transformation with plasmid vectors, and introduction of DNA by electroporation, lipofection, and particle gun acceleration.

Under conditions sufficient for: A phrase that is used to describe any environment that permits a desired activity. In one example the desired activity is formation of an immune complex. In particular examples the desired activity is treatment of HIV-1 infection.

Vector: A nucleic acid molecule as introduced into a host cell, thereby producing a transformed host cell. Recombinant DNA vectors are vectors having recombinant DNA. A vector can include nucleic acid sequences that permit it to replicate in a host cell, such as an origin of replication. A vector can also include one or more selectable marker genes and other genetic elements known in the art. Viral vectors are recombinant nucleic acid vectors having at least some nucleic acid sequences derived from one or more viruses. A replication deficient viral vector is a vector that requires complementation of one or more regions of the viral genome required for replication due to a deficiency in at least one replication-essential gene function. For example, such that the viral vector does not replicate in typical host cells, especially those in a human patient that could be infected by the viral vector in the course of a therapeutic method.

II. Description of Several Embodiments

Isolated monoclonal antibodies and antigen binding fragments thereof that specifically bind an epitope on HIV-1 Env protein are provided. The antibodies and antigen binding fragments can be fully human. In several embodiments, the antibodies and antigen binding fragments can be used to neutralize HIV-1 infection. Also disclosed herein are compositions including the antibodies and antigen binding fragments and a pharmaceutically acceptable carrier. Nucleic acids encoding the antibodies or antigen binding fragments, expression vectors including these nucleic acids, and isolated host cells that express the nucleic acids are also provided.

The antibodies, antigen binding fragments, nucleic acid molecules, host cells, and compositions can be used for research, diagnostic and therapeutic purposes. For example, the monoclonal antibodies and antigen binding fragments can be used to diagnose or treat a subject with an HIV-1 infection and/or AIDS. For example, the antibodies can be used to determine HIV-1 titer in a subject. The antibodies disclosed herein also can be used to study the biology of the human immunodeficiency virus.

A. Neutralizing Monoclonal Antibodies and Antigen Binding Fragments

Isolated monoclonal antibodies and antigen binding fragments that specifically bind an epitope on HIV-1 Env protein are provided. The antibodies and antigen binding fragments can neutralize HIV-1. The disclosed antibodies and antigen binding fragments specifically bind HIV-1 Env at a previously uncharacterized epitope, which is designated herein as the 35O22 epitope, based on the first antibody identified that binds to this epitope: 35O22. Epitope mapping and structural studies allowed for detailed analysis of the binding of 35O22 to HIV-1 Env, and the elucidation of the 35O22 epitope. As discussed in the Examples, the 35O22 antibody specifically binds to an epitope on HIV-1 Env that includes residues of the C2 region of gp120, and well as residues of gp41. Several of these residues are glycosylated, and the 35O22 antibody binds to the glycan moiety. Accordingly, in several embodiments, antibodies and antigen binding fragments that specifically bind to the 35O22 epitope of HIV-1 Env and which neutralize HIV-1 infection are provided.

In some embodiments, the antibody or antigen binding fragment specifically binds to an epitope of HIV-1 Env including or consisting of resides of the C2 region of gp120 and residues of gp41. In several embodiments, the C2 region of gp120 includes gp120 residues 196-295. In further embodiments, the antibody or antigen binding fragment specifically binds to an epitope including or consisting of two or more (such as 2, 3, 4, 5, 6, 7, 8, 9, or 10) amino acids of the C2 region of gp120 and at least one (such as 1, 2, 3, 4, or 5) amino acid of gp41. In some embodiments, the antibody or antigen binding fragment specifically binds to an epitope including or consisting of two or more (such as 2, 3, 4, 5, or 6) of gp120 residues N88, K227, N230, N241, and S243, and gp41 residue N625. The gp120 residue numbering used herein is according to the HXB2 numbering system.

In some embodiments, any of the gp120 and/or gp41 asparagine residues of the 35O22 epitope can be glycosylated (e.g., N-linked glycosylation), and the antibody or antigen binding fragment can specifically bind to an epitope including the glycan moiety linked to the gp120 and/or gp41 asparagine residue. In some embodiments, the antibody or antigen binding fragment can specifically bind to an epitope including a glycan moiety one or more of HIV-1 Env residues N88, N230, and/or N241. In some embodiments, the antibody or antigen binding fragment can specifically bind to an epitope that does not include a glycan moiety linked to HIV-1 Env residue N625.

As disclosed herein, in the context of the BG505 strain of HIV-1, the 35O22 antibody makes at least minimal contacts with the following HIV-1 Env residues: 87-92, 238, 240, 527-529, 532, 617-618, 620-621, 624-627, 629-630, and 633. The majority of the 35O22 contacts are with an N-linked glycan moiety attached to an asparagine residue at position 88. Accordingly, in some embodiments, the

antibody or antigen binding fragment specifically binds to an epitope of HIV-1 Env including or consisting of these HIV-1 Env residues, wherein residue 88 is glycosylated with an N-linked glycan.

In additional embodiments, the antibody or antigen binding fragment specifically binds to an epitope of HIV-1 Env including or consisting of HIV-1 Env residues 87-92 and 617-633, wherein residue 88 is glycosylated with an N-linked glycan. In additional embodiments, the antibody or antigen binding fragment specifically binds to an epitope of HIV-1 Env including or consisting of HIV-1 Env residues 87-92, 227-243, and 617-633, wherein residue 88 is glycosylated with an N-linked glycan.

In some embodiments, the antibodies and antigen binding fragments include a variable heavy (V_H) and a variable light (V_L) chain and specifically bind to HIV-1 Env and neutralize HIV-1 infection. In several embodiments, the antibodies and antigen binding fragments include a heavy chain comprising a heavy chain complementarity determining region (HCDR)1, a HCDR2 and an HCDR3, and a light chain comprising a light chain complementarity determining region (LCDR) 1, a LCDR2, and a LCDR3 and specifically bind to HIV-1 Env and neutralize HIV-1 infection. In several embodiments, the antibody or antigen binding fragment includes heavy and light chain variable regions including the HCDR1, HCDR2, and HCDR3, and LCDR1, LCDR2, and LCDR3, respectively, of one of the 35O22, 10J4, 10M6, 13I10, 2N5, 4O20, 7B9, and 7K3 antibodies, and specifically bind to HIV-1 Env and neutralize HIV-1 infection.

The discussion of monoclonal antibodies below refers to isolated monoclonal antibodies that include heavy and light chain variable domains including a CDR1, CDR2 and CDR3 with reference to the IMGT or Kabat numbering scheme (unless the context indicates otherwise). The person of ordinary skill in the art will understand that various CDR numbering schemes (such as the Kabat, Chothia or IMGT numbering schemes) can be used to determine CDR positions. The amino acid sequence and the CDR positions of the heavy and light chain of the 35O22, 10J4, 10M6, 13I10, 2N5, 4O20, 7B9, and 7K3 monoclonal antibodies according to the IMGT and Kabat numbering schemes are shown in Table 2 (IMGT) and Table 3 (Kabat).

Table 2. IMGT CDR sequences of HIV-1 Env specific antibodies

35O22					
	SEQ ID NO: 1	A.A. Sequence		SEQ ID NO: 2	A.A. Sequence
HCDR1	26-33	GYRFNFYH	LCDR1	26-34	NSVCCSHKS
HCDR2	51-58	ISPYSGDK	LCDR2	52-54	EDN
HCDR3	105-120	AKGLLRDGSSTWLPYL	LCDR3	91-100	CSYTHNSGCV
10J4					
	SEQ ID NO: 3	A.A. Sequence		SEQ ID NO: 4	A.A. Sequence
HCDR1	26-33	GYRFSFYH	LCDR1	26-34	SSVCCSHKS
HCDR2	51-58	ISPYNGGT	LCDR2	52-54	EDS
HCDR3	105-120	AKGLLRDGSSTWLPHL	LCDR3	91-100	CSYTHNSGCV
10M6					
	SEQ ID NO: 5	A.A. Sequence		SEQ ID NO: 6	A.A. Sequence
HCDR1	26-33	GYRFSSYH	LCDR1	26-34	SSVCCSHKS
HCDR2	51-58	ISPYSGGT	LCDR2	52-54	EDS
HCDR3	105-120	AKGLLRDGSSTWLPH	LCDR3	91-100	CSYTHNSGCV

		L			
13I10					
	SEQ ID NO: 7	A.A. Sequence		SEQ ID NO: 8	A.A. Sequence
HCDR1	26-33	GYRFSFYH	LCDR1	26-34	SSVCCSHKS
HCDR2	51-58	ISPYNGGT	LCDR2	52-54	EDS
HCDR3	105-120	AKGLLRDGPSTWLPH L	LCDR3	91-100	CSYTHNSGCV
2N5					
	SEQ ID NO: 9	A.A. Sequence		SEQ ID NO: 10	A.A. Sequence
HCDR1	26-33	GYKFSFFH	LCDR1	26-34	SSVCCSHKS
HCDR2	51-58	ISPYSGDK	LCDR2	52-54	EDN
HCDR3	105-120	ARGLLRDGSSTWLPY L	LCDR3	91-100	CSYTHNSGCV
4O20					
	SEQ ID NO: 11	A.A. Sequence		SEQ ID NO: 12	A.A. Sequence
HCDR1	26-33	GYRFNFYH	LCDR1	26-34	NSVCCSHKS
HCDR2	51-58	ISPYSGDK	LCDR2	52-54	EDN
HCDR3	105-120	AKGLLRDGSSTWLPY L	LCDR3	91-100	CSYTHNSGCV
7B9					
	SEQ ID NO: 13	A.A. Sequence		SEQ ID NO: 14	A.A. Sequence
HCDR1	26-33	GYRFNFYH	LCDR1	26-34	NSACCSHKS
HCDR2	51-58	ISPYSGDK	LCDR2	52-54	EDN
HCDR3	105-120	AKGLLRDGSSTWLPY L	LCDR3	91-100	CSYTHNSGCV
7K3					
	SEQ ID NO: 15	A.A. Sequence		SEQ ID NO: 16	A.A. Sequence
HCDR1	26-33	GYRFSFYH	LCDR1	26-34	NSVCCSHKS
HCDR2	51-58	ISPYSGDT	LCDR2	52-54	EDS
HCDR3	105-120	AKGLLRDGSSTWLPH L	LCDR3	91-100	CSYTHNSGCV

In some embodiments, the antibody includes IMGT CDRs, such as those listed in Table 2. For example, in some embodiments, the antibody includes a heavy chain variable region including a LCDR1, LCDR2, and/or LCDR3 including amino acids 26-33, 51-58, and/or 105-120, respectively, of one of SEQ ID NOs: 1, 3, 5, 7, 11, 13, or 15. In some embodiments, the antibody includes a light chain variable region including a LCDR1, LCDR2, and/or LCDR3 including amino acids 26-34, 52-54, and/or 91-100, respectively, of one of SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, or 16

In some embodiments, the antibody includes a heavy chain variable region including a HCDR1, a HCDR2, and a HCDR3 including amino acids 26-33, 51-58, and 105-120 of SEQ ID NO: 1, respectively, and a light chain variable region including a LCDR1, a LCDR2, and a LCDR3 including amino acids 26-34, 52-54, and 91-100 of SEQ ID NO: 2, respectively. In further embodiments, the antibody includes a heavy chain variable region including a HCDR1, a HCDR2, and a HCDR3 including amino acids 26-33, 51-58, and 105-120 of SEQ ID NO: 3, respectively, and a light chain variable region including a LCDR1, a LCDR2, and a LCDR3 including amino acids 26-34, 52-54, and 91-100 of SEQ ID NO: 4, respectively. In additional embodiments, the antibody includes a heavy chain variable region including a HCDR1, a HCDR2, and a HCDR3 including amino acids 26-33, 51-58, and 105-120 of SEQ ID NO: 5, respectively, and a light chain variable region including a LCDR1, a LCDR2, and a LCDR3

including amino acids 26-34, 52-54, and 91-100 of SEQ ID NO: 6, respectively. In more embodiments, the antibody includes a heavy chain variable region including a HCDR1, a HCDR2, and a HCDR3 including amino acids 26-33, 51-58, and 105-120 of SEQ ID NO: 7, respectively, and a light chain variable region including a LCDR1, a LCDR2, and a LCDR3 including amino acids 26-34, 52-54, and 91-100 of SEQ ID NO: 8, respectively. In some embodiments, the antibody includes a heavy chain variable region including a HCDR1, a HCDR2, and a HCDR3 including amino acids 26-33, 51-58, and 105-120 of SEQ ID NO: 9, respectively, and a light chain variable region including a LCDR1, a LCDR2, and a LCDR3 including amino acids 26-34, 52-54, and 91-100 of SEQ ID NO: 10, respectively. In further embodiments, the antibody includes a heavy chain variable region including a HCDR1, a HCDR2, and a HCDR3 including amino acids 26-33, 51-58, and 105-120 of SEQ ID NO: 11, respectively, and a light chain variable region including a LCDR1, a LCDR2, and a LCDR3 including amino acids 26-34, 52-54, and 91-100 of SEQ ID NO: 12, respectively. In additional embodiments, the antibody includes a heavy chain variable region including a HCDR1, a HCDR2, and a HCDR3 including amino acids 26-33, 51-58, and 105-120 of SEQ ID NO: 13, respectively, and a light chain variable region including a LCDR1, a LCDR2, and a LCDR3 including amino acids 26-34, 52-54, and 91-100 of SEQ ID NO: 14, respectively. In more embodiments, the antibody includes a heavy chain variable region including a HCDR1, a HCDR2, and a HCDR3 including amino acids 26-33, 51-58, and 105-120 of SEQ ID NO: 15, respectively, and a light chain variable region including a LCDR1, a LCDR2, and a LCDR3 including amino acids 26-34, 52-54, and 91-100 of SEQ ID NO: 16, respectively.

The 35O22, 10J4, 10M6, 13I10, 2N5, 4O20, 7B9, and 7K3 antibodies are clonal variants of each other, and include similar heavy and light chain CDRs that derive from the same heavy and light chain germline genes. According, the IMGT CDR sequences of these antibodies can be used to generate consensus CDR sequences for a genus of antibodies that specifically bind to HIV-1 Env and neutralize HIV-1 infection. In some embodiments, the antibody includes a heavy chain variable region including a HCDR1, a HCDR2, and/or a HCDR3 including the amino acid sequence of SEQ ID NO: 33 ($GYRFX_1X_2X_3H$, wherein X_1 is S or N, X_2 is F or S, and X_3 is Y or F), SEQ ID NO: 34 ($ISPYX_1GX_2X_3$, wherein X_1 is N or S, X_2 is G or D, and X_3 is T or K), and SEQ ID NO: 35 ($AKGLLRDGX_1STWLPX_2L$, wherein X_1 is S or P, and X_2 is H or Y), respectively. In some embodiments, the antibody includes a light chain variable region including a LCDR1, a LCDR2, and/or a LCDR3 including the amino acid sequence of SEQ ID NO: 36 ($X_1S\ X_2CCSHKS$, wherein X_1 is S or N and X_2 is V or A), SEQ ID NO: 54 (EDX_1, wherein X_1 is S or N), and SEQ ID NO: 37 (CSYTHNSGCV), respectively. In some embodiments, the antibody includes a heavy chain variable region including a HCDR1, a HCDR2, and/or a HCDR3 including the amino acid sequences of SEQ ID NO: 33, SEQ ID NO: 34, and SEQ ID NO: 35, respectively, and further includes a light chain variable region including a LCDR1, a LCDR2, and/or a LCDR3 including the amino acid sequences set forth as SEQ ID NO: 36, SEQ ID NO: 54, and SEQ ID NO: 37, respectively.

In some embodiments, the antibody includes a heavy chain variable region including a HCDR1, a HCDR2, and a HCDR3 including amino acid sequences at least 90% (such as at least 95%, 96%, 97%,

98%, or 99%) identical to amino acids 26-33, 51-58, and/or 105-120, respectively, of one of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, or 15. In some embodiments, the antibody includes a light chain variable region including a LCDR1, a LCDR2, and a LCDR3 including amino acid sequences at least 90% (such as at least 95%, 96%, 97%, 98%, or 99%) identical to amino acids amino acids 26-34, 52-54, and 91-100, respectively, of one of SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, or 16.

In additional embodiments, the antibody includes a heavy chain variable region including a HCDR1, a HCDR2, and a HCDR3 including amino acid sequences at least 90% (such as at least 95%, 96%, 97%, 98%, or 99%) identical to amino acids 26-33, 51-58, and 105-120, respectively, of SEQ ID NO: 1, and a light chain variable region including a LCDR1, a LCDR2, and a LCDR3 including amino acid sequences at least 90% (such as at least 95%, 96%, 97%, 98%, or 99%) identical to amino acids amino acids 26-34, 52-54, and 91-100, respectively, of SEQ ID NO: 2. In additional embodiments, the antibody includes (a) a heavy chain variable region including a HCDR1, a HCDR2, and a HCDR3 and (b) a light chain variable region including a LCDR1, a LCDR2, and a LCDR3, each including CDR amino acid sequences at least 90% (such as at least 95%, 96%, 97%, 98%, or 99%) identical to (a) amino acids 26-33, 51-58, and 105-120, respectively, and (b) amino acids 26-34, 52-54, and 91-100, respectively, of one of SEQ ID NOs: 1 and 2, SEQ ID NOs: 3 and 4, SEQ ID NO: 5 and 6, SEQ ID NOs: 7 and 8, SEQ ID NOs: 9 and 10, SEQ ID NOs: 11 and 12, SEQ ID NOs: 13 and 14, or SEQ ID NOs: 15 and 16.

Table 3. Kabat CDR sequences of HIV-1 Env specific antibodies

35O22					
	SEQ ID NO: 1	A.A. Sequence		SEQ ID NO: 2	A.A. Sequence
HCDR1	31-35	FYHIN	LCDR1	23-36	TGPNSVCCSHKSIS
HCDR2	50-66	WISPYSGDKNLAPAFQD	LCDR2	52-58	EDNERAP
HCDR3	107-120	GLLRDGSSTWLPYL	LCDR3	91-100	CSYTHNSGCV
10J4					
	SEQ ID NO: 3	A.A. Sequence		SEQ ID NO: 4	A.A. Sequence
HCDR1	31-35	FYHIN	LCDR1	23-36	TGPSSVCCSHKSIS
HCDR2	50-66	WISPYNGGTNLAPELRG	LCDR2	52-58	EDSERSW
HCDR3	107-120	GLLRDGSSTWLPHL	LCDR3	91-100	CSYTHNSGCV
10M6					
	SEQ ID NO: 5	A.A. Sequence		SEQ ID NO: 6	A.A. Sequence
HCDR1	31-35	SYHIN	LCDR1	23-36	TGPSSVCCSHKSIS
HCDR2	50-66	WISPYSGGTNLAPEFRG	LCDR2	52-58	EDSERSW
HCDR3	107-120	GLLRDGSSTWLPHL	LCDR3	91-100	CSYTHNSGCV
13I10					
	SEQ ID NO: 7	A.A. Sequence		SEQ ID NO: 8	A.A. Sequence
HCDR1	31-35	FYHIN	LCDR1	23-36	TGPSSVCCSHKSIS
HCDR2	50-66	WISPYNGGTNLAPEFRG	LCDR2	52-58	EDSERSW
HCDR3	107-120	GLLRDGPSTWLPHL	LCDR3	91-100	CSYTHNSGCV
2N5					
	SEQ ID NO: 9	A.A. Sequence		SEQ ID NO: 10	A.A. Sequence
HCDR1	31-35	FFHIN	LCDR1	23-36	TGPSSVCCSHKSIS
HCDR2	50-66	WISPYSGDKNYAPAFQD	LCDR2	52-58	EDNKRFS
HCDR3	107-120	GLLRDGSSTWLPYL	LCDR3	91-100	CSYTHNSGCV
4O20					
	SEQ ID NO: 11	A.A. Sequence		SEQ ID NO: 12	A.A. Sequence

HCDR1	31-35	FYHIN	LCDR1	23-36	TGPNSVCCSHKSIS
HCDR2	50-66	WISPYSGDKNLAPAFQD	LCDR2	52-58	EDNEKAP
HCDR3	107-120	GLLRDGSSTWLPYL	LCDR3	91-100	CSYTHNSGCV
7B9					
	SEQ ID NO: 13	A.A. Sequence		SEQ ID NO: 14	A.A. Sequence
HCDR1	31-35	FYHIN	LCDR1	23-36	TGPNSACCSHKSIS
HCDR2	50-66	WISPYSGDKNLAPAFQD	LCDR2	52-58	EDNEKAP
HCDR3	107-120	GLLRDGSSTWLPYL	LCDR3	91-100	CSYTHNSGCV
7K3					
	SEQ ID NO: 15	A.A. Sequence		SEQ ID NO: 16	A.A. Sequence
HCDR1	31-35	FYHIN	LCDR1	23-36	TGPNSVCCSHKSIS
HCDR2	50-66	WISPYSGDTNLAPDFRG	LCDR2	52-58	EDSERSW
HCDR3	107-120	GLLRDGSSTWLPHL	LCDR3	91-100	CSYTHNSGCV

In some embodiments, the antibody includes Kabat CDRs, such as those listed in Table 3. For example, in some embodiments, the antibody includes a heavy chain variable region including a HCDR1, HCDR2, and/or HCDR3 including amino acids 31-35, 50-66, and/or 107-120, respectively, of one of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, or 15. In some embodiments, the antibody includes a light chain variable region including a LCDR1, LCDR2, and/or LCDR3 including amino acids 23-36, 50-66, and/or 91-100, respectively, of one of SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, or 16

In some embodiments, the antibody includes a heavy chain variable region including a HCDR1, a HCDR2, and a HCDR3 including amino acids 31-35, 50-66, and 107-120 of SEQ ID NO: 1, respectively, and a light chain variable region including a LCDR1, a LCDR2, and a LCDR3 including amino acids 31-35, 50-66, and 107-120 of SEQ ID NO: 2, respectively. In further embodiments, the antibody includes a heavy chain variable region including a HCDR1, a HCDR2, and a HCDR3 including amino acids 31-35, 50-66, and 107-120 of SEQ ID NO: 3, respectively, and a light chain variable region including a LCDR1, a LCDR2, and a LCDR3 including amino acids 31-35, 50-66, and 107-120 of SEQ ID NO: 4, respectively. In additional embodiments, the antibody includes a heavy chain variable region including a HCDR1, a HCDR2, and a HCDR3 including amino acids 31-35, 50-66, and 107-120 of SEQ ID NO: 5, respectively, and a light chain variable region including a LCDR1, a LCDR2, and a LCDR3 including amino acids 31-35, 50-66, and 107-120 of SEQ ID NO: 6, respectively. In more embodiments, the antibody includes a heavy chain variable region including a HCDR1, a HCDR2, and a HCDR3 including amino acids 31-35, 50-66, and 107-120 of SEQ ID NO: 7, respectively, and a light chain variable region including a LCDR1, a LCDR2, and a LCDR3 including amino acids 31-35, 50-66, and 107-120 of SEQ ID NO: 8, respectively. In some embodiments, the antibody includes a heavy chain variable region including a HCDR1, a HCDR2, and a HCDR3 including amino acids 31-35, 50-66, and 107-120 of SEQ ID NO: 9, respectively, and a light chain variable region including a LCDR1, a LCDR2, and a LCDR3 including amino acids 31-35, 50-66, and 107-120 of SEQ ID NO: 10, respectively. In further embodiments, the antibody includes a heavy chain variable region including a HCDR1, a HCDR2, and a HCDR3 including amino acids 31-35, 50-66, and 107-120 of SEQ ID NO: 11, respectively, and a light chain variable region including a LCDR1, a LCDR2, and a LCDR3 including amino acids 31-35, 50-66, and 107-120 of SEQ ID NO: 12, respectively. In additional embodiments, the

antibody includes a heavy chain variable region including a HCDR1, a HCDR2, and a HCDR3 including amino acids 31-35, 50-66, and 107-120 of SEQ ID NO: 13, respectively, and a light chain variable region including a LCDR1, a LCDR2, and a LCDR3 including amino acids 31-35, 50-66, and 107-120 of SEQ ID NO: 14, respectively. In more embodiments, the antibody includes a heavy chain variable region including a HCDR1, a HCDR2, and a HCDR3 including amino acids 31-35, 50-66, and 107-120 of SEQ ID NO: 15, respectively, and a light chain variable region including a LCDR1, a LCDR2, and a LCDR3 including amino acids 31-35, 50-66, and 107-120 of SEQ ID NO: 16, respectively.

The 35O22, 10J4, 10M6, 13I10, 2N5, 4O20, 7B9, and 7K3 antibodies are clonal variants of each other, and include similar heavy and light chain CDRs that derive from the same heavy and light chain germline genes. According, the Kabat CDR sequences of these antibodies can be used to generate consensus CDR sequences for a genus of antibodies that specifically bind to HIV-1 Env and neutralize HIV-1 infection. In some embodiments, the antibody includes a heavy chain variable region including a HCDR1, a HCDR2, and/or a HCDR3 including the amino acid sequence of SEQ ID NO: 17 (X_1X_2HIN, wherein X_1 is F or S and X_2 is Y or F), SEQ ID NO: 18 (WISPY $X_1GX_2X_3NX_4APX_5X_6X_7X_8$, wherein X_1 is N or S, X_2 is G or D, X_3 is T or K, X_4 is L or Y, X_5 is E, D or A, X_6 is L or F, X_7 is R or Q, and X_8 is G or D), and SEQ ID NO: 19 (GLLRDGX_1STWLPX_1L, wherein X_1 is S or P and X_2 is H or Y), respectively. In some embodiments, the antibody includes a light chain variable region including a LCDR1, a LCDR2, and/or a LCDR3 including the amino acid sequence of SEQ ID NO: 20 (TGP X_1SX_2CCSHKSIS, wherein X_1 is S or N and X_2 is V or A), SEQ ID NO: 21 (ED$X_1X_2X_3X_4X_5$, wherein X_1 is S or N, X_2 is E or K, X_3 is R or K, X_4 is S, F, or A, and X_5 is W, S, or P), and SEQ ID NO: 22 (CSYTHNSGCV), respectively. In some embodiments, the antibody includes a heavy chain variable region including a HCDR1, a HCDR2, and/or a HCDR3 including the amino acid sequences of SEQ ID NO: 17, SEQ ID NO: 18, and SEQ ID NO: 19, respectively, and further includes a light chain variable region including a LCDR1, a LCDR2, and/or a LCDR3 including the amino acid sequences of SEQ ID NO: 20, SEQ ID NO: 21, and SEQ ID NO: 22, respectively.

In some embodiments, the antibody includes a heavy chain variable region including a HCDR1, a HCDR2, and a HCDR3 including amino acid sequences at least 90% (such as at least 95%, 96%, 97%, 98%, or 99%) identical to amino acids 31-35, 50-66, and/or 107-120, respectively, of one of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, or 15. In some embodiments, the antibody includes a light chain variable region including a LCDR1, a LCDR2, and a LCDR3 including amino acid sequences at least 90% (such as at least 95%, 96%, 97%, 98%, or 99%) identical to amino acids amino acids 23-36, 50-66, and/or 91-100, respectively, of one of SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, or 16.

In additional embodiments, the antibody includes a heavy chain variable region including a HCDR1, a HCDR2, and a HCDR3 including amino acid sequences at least 90% (such as at least 95%, 96%, 97%, 98%, or 99%) identical to amino acids 31-35, 50-66, and/or 107-120, respectively, of SEQ ID NO: 1, and a light chain variable region including a LCDR1, a LCDR2, and a LCDR3 including amino acid sequences at least 90% (such as at least 95%, 96%, 97%, 98%, or 99%) identical to amino acids amino acids 23-36, 50-66, and/or 91-100, respectively, of SEQ ID NO: 2. In additional embodiments,

the antibody includes (a) a heavy chain variable region including a HCDR1, a HCDR2, and a HCDR3 and (b) a light chain variable region including a LCDR1, a LCDR2, and a LCDR3, each including CDR amino acid sequences at least 90% (such as at least 95%, 96%, 97%, 98%, or 99%) identical to (a) amino acids 31-35, 50-66, and/or 107-120, respectively, and (b) amino acids 23-36, 50-66, and/or 91-100, respectively, of one of SEQ ID NOs: 1 and 2, SEQ ID NOs: 3 and 4, SEQ ID NO: 5 and 6, SEQ ID NOs: 7 and 8, SEQ ID NOs: 9 and 10, SEQ ID NOs: 11 and 12, SEQ ID NOs: 13 and 14, or SEQ ID NOs: 15 and 16.

1. Additional Description of Antibodies and Antigen Binding Fragments

In some embodiments, the antibody can include an eight amino acid insertion between kabat positions 72 and 73 of framework region 3. For example the insertion can include the amino acid sequence set forth as residues 74-81 of one of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, or 15. In one non-limiting embodiment, the insertion can include the amino acid sequence set forth as residues 74-81 of SEQ ID NO: 1 (TEVPVTSF).

In some embodiments, the antibody includes a heavy chain variable region including an amino acid sequence at least 80%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence set forth as one of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, or SEQ ID NO: 15. In more embodiments, the antibody includes a light chain variable region including an amino acid sequence at least 80%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence set forth as one of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, or SEQ ID NO: 16.

In additional embodiments, the antibody includes a heavy chain variable region and a light chain variable region, which include amino acid sequences that are independently at least 80% (such as at least 90%, 95%, 96%, 97%, 98%, or 99%) identical to the amino acid sequences set forth as one of SEQ ID NOs: 1 and 2, respectively, SEQ ID NOs: 3 and 4, respectively, SEQ ID NOs: 5 and 6, respectively, SEQ ID NOs: 7 and 8, respectively, SEQ ID NOs: 9 and 10, respectively, SEQ ID NOs: 11 and 12, respectively, SEQ ID NOs: 13 and 14, respectively, or SEQ ID NOs: 15 and 16, respectively.

In additional embodiments, the antibody includes a heavy chain variable region including the amino acid sequence set forth as one of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, or SEQ ID NO: 15. In more embodiments, the antibody includes a light chain variable region including the amino acid sequence set forth as one of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, or SEQ ID NO: 16. Variable light chain (V_L) and variable heavy chain (V_H) segments of the disclosed antibodies can be "mixed and matched", in which different pairs of the V_L and V_H segments are combined and screened for binding to HIV-1 Env protein to select V_L/V_H pair combinations of interest.

In some embodiments, the antibody includes heavy and light chain variable regions including the amino acid sequences set forth as one of SEQ ID NOs: 1 and 2, respectively, SEQ ID NOs: 3 and 4, respectively, SEQ ID NOs: 5 and 6, respectively, SEQ ID NOs: 7 and 8, respectively, SEQ ID NOs: 9

and 10, respectively, SEQ ID NOs: 11 and 12, respectively, SEQ ID NOs: 13 and 14, respectively, or SEQ ID NOs: 15 and 16, respectively.

In some embodiments, antibody means for specifically binding an epitope on an HIV-1 Env trimer, wherein the epitope comprises HIV-1 Env residues 87-92 and 617-633, and wherein residue 88 is glycosylated with an N-linked glycan, are provided. Exemplary antibody means for such embodiments include the 35O22, 10J4, 10M6, 13I10, 2N5, 4O20, 7B9, and 7K3 monoclonal antibodies, and/or an antibody including the heavy and light chain CDRs (e.g., as set forth in Tables 2 and 3) of one of the 35O22, 10J4, 10M6, 13I10, 2N5, 4O20, 7B9, and 7K3 monoclonal antibodies, and/or an antibody including a heavy and light chain variable region comprising one of: SEQ ID NOs: 1 and 2, respectfully, SEQ ID NOs: 3 and 4, respectfully, SEQ ID NOs: 5 and 6, respectfully, SEQ ID NOs: 7 and 8, respectfully, SEQ ID NOs: 9 and 10, respectfully, SEQ ID NOs: 11 and 12, respectfully, SEQ ID NOs: 13 and 14 respectfully, or SEQ ID NOs: 15 and 16, respectfully.

The antibody or antigen binding fragment can be a human antibody or fragment thereof. Chimeric antibodies are also provided. The antibody or antigen binding fragment can include any suitable framework region, such as (but not limited to) a human framework region. Human framework regions, and mutations that can be made in a human antibody framework regions, are known in the art (see, for example, in U.S. Patent No. 5,585,089, which is incorporated herein by reference). Further, the 35O22, 10J4, 10M6, 13I10, 2N5, 4O20, 7B9, and 7K3 antibodies are human antibodies, therefore, any of the antibodies or antigen binding fragments can include one or more framework regions of the 35O22, 10J4, 10M6, 13I10, 2N5, 4O20, 7B9, and 7K3 as disclosed herein. Alternatively, a heterologous framework region, such as, but not limited to a mouse framework region, can be included in the heavy or light chain of the antibodies. (See, for example, Jones *et al.*, *Nature* 321:522, 1986; Riechmann *et al.*, *Nature* 332:323, 1988; Verhoeyen *et al.*, *Science* 239:1534, 1988; Carter *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 89:4285, 1992; Sandhu, *Crit. Rev. Biotech.*12:437, 1992; and Singer *et al.*, *J. Immunol.*150:2844, 1993.)

The antibody can be of any isotype. The antibody can be, for example, an IgM or an IgG antibody, such as IgG_1, IgG_2, IgG_3, or IgG_4. The class of an antibody that specifically binds HIV-1 Env can be switched with another. In one aspect, a nucleic acid molecule encoding V_L or V_H is isolated using methods well-known in the art, such that it does not include any nucleic acid sequences encoding the constant region of the light or heavy chain, respectively. A nucleic acid molecule encoding V_L or V_H is then operatively linked to a nucleic acid sequence encoding a C_L or C_H from a different class of immunoglobulin molecule. This can be achieved using a vector or nucleic acid molecule that comprises a C_L or C_H chain, as known in the art. For example, an antibody that specifically binds HIV-1 Env, that was originally IgM may be class switched to an IgG. Class switching can be used to convert one IgG subclass to another, such as from IgG_1 to IgG_2, IgG_3, or IgG_4.

In some examples, the disclosed antibodies are oligomers of antibodies, such as dimers, trimers, tetramers, pentamers, hexamers, septamers, octomers and so on.

(a) Binding affinity

In several embodiments, the antibody or antigen binding fragment can specifically bind HIV-1 Env protein with an affinity (e.g., measured by K_d) of no more than 1.0×10^{-8} M, no more than 5.0×10^{-8} M, no more than 1.0×10^{-9} M, no more than 5.0×10^{-9} M, no more than 1.0×10^{-10} M, no more than 5.0×10^{-10} M, or no more than 1.0×10^{-11} M. K_d can be measured, for example, by a radiolabeled antigen binding assay (RIA) performed with the Fab version of an antibody of interest and its antigen using known methods. In one assay, solution binding affinity of Fabs for antigen is measured by equilibrating Fab with a minimal concentration of (^{125}I)-labeled antigen in the presence of a titration series of unlabeled antigen, then capturing bound antigen with an anti-Fab antibody-coated plate (see, e.g., Chen et al., *J. Mol. Biol.* 293:865-881 (1999)). To establish conditions for the assay, MICROTITER® multi-well plates (Thermo Scientific) are coated overnight with 5 µg/ml of a capturing anti-Fab antibody (Cappel Labs) in 50 mM sodium carbonate (pH 9.6), and subsequently blocked with 2% (w/v) bovine serum albumin in PBS for two to five hours at room temperature (approximately 23° C.). In a non-adsorbent plate (Nunc #269620), 100 µM or 26 pM [^{125}I]-antigen are mixed with serial dilutions of a Fab of interest (e.g., consistent with assessment of the anti-VEGF antibody, Fab-12, in Presta et al., *Cancer Res.* 57:4593-4599 (1997)). The Fab of interest is then incubated overnight; however, the incubation may continue for a longer period (e.g., about 65 hours) to ensure that equilibrium is reached. Thereafter, the mixtures are transferred to the capture plate for incubation at room temperature (e.g., for one hour). The solution is then removed and the plate washed eight times with 0.1% polysorbate 20 (TWEEN-20®) in PBS. When the plates have dried, 150 µl/well of scintillant (MICROSCINT-20™; Packard) is added, and the plates are counted on a TOPCOUNT™ gamma counter (Packard) for ten minutes. Concentrations of each Fab that give less than or equal to 20% of maximal binding are chosen for use in competitive binding assays.

In another assay, K_d can be measured using surface plasmon resonance assays using a BIACORE®-2000 or a BIACORE®-3000 (BIAcore, Inc., Piscataway, N.J.) at 25° C with immobilized antigen CM5 chips at ~10 response units (RU). Briefly, carboxymethylated dextran biosensor chips (CM5, BIACORE®, Inc.) are activated with N-ethyl-N′-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS) according to the supplier's instructions. Antigen is diluted with 10 mM sodium acetate, pH 4.8, to 5 µg/ml (~0.2 µM) before injection at a flow rate of 5 l/minute to achieve approximately 10 response units (RU) of coupled protein. Following the injection of antigen, 1 M ethanolamine is injected to block unreacted groups. For kinetics measurements, two-fold serial dilutions of Fab (0.78 nM to 500 nM) are injected in PBS with 0.05% polysorbate 20 (TWEEN-20™) surfactant (PBST) at 25° C at a flow rate of approximately 25 l/min. Association rates (k_{on}) and dissociation rates (k_{off}) are calculated using a simple one-to-one Langmuir binding model (BIACORE® Evaluation Software version 3.2) by simultaneously fitting the association and dissociation sensorgrams. The equilibrium dissociation constant (K_D) is calculated as the ratio k_{off}/k_{on}. See, e.g., Chen et al., *J. Mol. Biol.* 293:865-881 (1999). If the on-rate exceeds 106 M^{-1} s^{-1} by the surface plasmon resonance assay above, then the on-rate can be determined by using a fluorescent quenching technique that measures the

increase or decrease in fluorescence emission intensity (excitation=295 nm; emission=340 nm, 16 nm band-pass) at 25° C. of a 20 nM anti-antigen antibody (Fab form) in PBS, pH 7.2, in the presence of increasing concentrations of antigen as measured in a spectrometer, such as a stop-flow equipped spectrophometer (Aviv Instruments) or a 8000-series SLM-AMINCO™ spectrophotometer (ThermoSpectronic) with a stirred cuvette.

(b) Neutralization

In some embodiments, the antibody or antigen binding fragment can also be distinguished by neutralization breadth. In some embodiments, the antibody or antigen binding fragment can neutralize at least 70% (such as at least 75%, at least 80%, at least 85%, or least 90%,) of the HIV-1 isolates listed in FIG. 15 or FIG. 17 with an IC_{50} of less than 50 μg/ml. The HIV-1 isolated listed in FIG. 15 include Clade A isolates (KER2018, RWO20.2, Q168.a2, Q769.d22, Q769.h5), Clade B isolates (JRFL.JB, BaL.01, YU2.DG, PVO.04, TRO.11, CAAN.A2, TRJO.58, THRO.18, BG1168.1, 6101.1), and Clade C isolates (ZA012.29, DU156.12, DU422.01, ZM106.9, ZM55.28a). The person of ordinary skill in the art if familiar with methods of measuring neutralization breadth and potency, for example such methods include the single-round HIV-1 Env-pseudoviruses infection of TZM-bl cells (see, e.g., Li *et al., J Virol* 79, 10108-10125, 2005, incorporated by reference herein; see also, PCT Pub. No. WO2011/038290, incorporated by reference herein).

(c) Multispecific antibodies

In some embodiments, the antibody or antigen binding fragment is included on a multispecific antibody, such as a bi-specific antibody. Such multispecific antibodies can be produced by known methods, such as crosslinking two or more antibodies, antigen binding fragments (such as scFvs) of the same type or of different types. Exemplary methods of making multispecific antibodies include those described in PCT Pub. No. WO2013/163427, which is incorporated by reference herein in its entirety. Suitable crosslinkers include those that are heterobifunctional, having two distinctly reactive groups separated by an appropriate spacer (such as m-maleimidobenzoyl-N-hydroxysuccinimide ester) or homobifunctional (such as disuccinimidyl suberate). Such linkers are available from Pierce Chemical Company, Rockford, Ill.

In some embodiments, the antibody or antigen binding fragment is included on a bispecific antibody that that specifically binds to HIV-1 Env protein and further specifically binds to CD3. Examples of CD3 binding domains that can be included on the bispecific antibody or antigen binding fragment are known and include those disclosed in PCT Pub. No. WO2013/163427, which is incorporated by reference herein in its entirety.

Various types of multi-specific antibodies are known. Bispecific single chain antibodies can be encoded by a single nucleic acid molecule. Examples of bispecific single chain antibodies, as well as methods of constructing such antibodies are known in the art (see, *e.g.*, U.S. Pat. Nos. 8,076,459, 8,017,748, 8,007,796, 7,919,089, 7,820,166, 7,635,472, 7,575,923, 7,435,549, 7,332,168, 7,323,440,

7,235,641, 7,229,760, 7,112,324, 6,723,538, incorporated by reference herein). Additional examples of bispecific single chain antibodies can be found in PCT application No. WO 99/54440; Mack, *J. Immunol.*, 158:3965-3970, 1997; Mack, *PNAS*, 92:7021-7025, 1995; Kufer, *Cancer Immunol. Immunother.*, 45:193-197, 1997; Loffler, *Blood*, 95:2098-2103, 2000; and Bruhl, *J. Immunol.*, 166:2420-2426, 2001. Production of bispecific Fab-scFv ("bibody") molecules are described, for example, in Schoonjans et al. (J. Immunol. 165:7050-57, 2000) and Willems et al. (J Chromatogr B Analyt Technol Biomed Life Sci. 786:161-76, 2003). For bibodies, a scFv molecule can be fused to one of the VL-CL (L) or VH-CH1 chains, *e.g.*, to produce a bibody one scFv is fused to the C-term of a Fab chain.

(d) Fragments

Antigen binding fragments are encompassed by the present disclosure, such as Fab, $F(ab')_2$, and Fv which include a heavy chain and light chain variable region and specifically bind HIV-1 Env protein. These antibody fragments retain the ability to selectively bind with the antigen and are "antigen-binding" fragments. These fragments include:

(1) Fab, the fragment which contains a monovalent antigen-binding fragment of an antibody molecule, can be produced by digestion of whole antibody with the enzyme papain to yield an intact light chain and a portion of one heavy chain;

(2) Fab', the fragment of an antibody molecule can be obtained by treating whole antibody with pepsin, followed by reduction, to yield an intact light chain and a portion of the heavy chain; two Fab' fragments are obtained per antibody molecule;

(3) $(Fab')_2$, the fragment of the antibody that can be obtained by treating whole antibody with the enzyme pepsin without subsequent reduction; $F(ab')_2$ is a dimer of two Fab' fragments held together by two disulfide bonds;

(4) Fv, a genetically engineered fragment containing the variable region of the light chain and the variable region of the heavy chain expressed as two chains; and

(5) Single chain antibody (such as scFv), defined as a genetically engineered molecule containing the variable region of the light chain, the variable region of the heavy chain, linked by a suitable polypeptide linker as a genetically fused single chain molecule. A scFv is a fusion protein in which a light chain variable region of an immunoglobulin and a heavy chain variable region of an immunoglobulin are bound by a linker (see, e.g., Ahmad et al., Clin. Dev. Immunol., 2012, doi:10.1155/2012/980250; Marbry, IDrugs, 13:543-549, 2010). The intramolecular orientation of the V_H-domain and the V_L-domain in a scFv, is not decisive for the provided antibodies (*e.g.*, for the provided multispecific antibodies). Thus, scFvs with both possible arrangements (V_H-domain-linker domain-V_L-domain; V_L-domain-linker domain-V_H-domain) may be used.

(6) A dimer of a single chain antibody ($scFV_2$), defined as a dimer of a scFV. This has also been termed a "miniantibody."

Methods of making these fragments are known in the art (see for example, Harlow and Lane, *Antibodies: A Laboratory Manual*, 2nd, Cold Spring Harbor Laboratory, New York, 2013).

In a further group of embodiments, the antibody binding fragment can be an Fv antibody, which is typically about 25 kDa and contain a complete antigen-binding site with three CDRs per each heavy chain and each light chain. To produce F_V antibodies, the V_H and the V_L can be expressed from two individual nucleic acid constructs in a host cell.

If the V_H and the V_L are expressed non-contiguously, the chains of the Fv antibody are typically held together by noncovalent interactions. However, these chains tend to dissociate upon dilution, so methods have been developed to crosslink the chains through glutaraldehyde, intermolecular disulfides, or a peptide linker. Thus, in one example, the Fv can be a disulfide stabilized Fv (dsFv), wherein the heavy chain variable region and the light chain variable region are chemically linked by disulfide bonds.

In an additional example, the Fv fragments comprise V_H and V_L chains connected by a peptide linker. These single-chain antigen binding proteins (scFv) are prepared by constructing a nucleic acid molecule encoding the V_H and V_L domains connected by an oligonucleotide. The nucleic acid molecule is inserted into an expression vector, which is subsequently introduced into a host cell such as a mammalian cell. The recombinant host cells synthesize a single polypeptide chain with a linker peptide bridging the two V domains. Methods for producing scFvs are known in the art (see Whitlow *et al.*, *Methods: a Companion to Methods in Enzymology*, Vol. 2, page 97, 1991; Bird *et al.*, *Science* 242:423, 1988; U.S. Patent No. 4,946,778; Pack *et al.*, *Bio/Technology* 11:1271, 1993; Ahmad *et al.*, *Clin. Dev. Immunol.*, 2012, doi:10.1155/2012/980250; Marbry, *IDrugs*, 13:543-549, 2010). Dimers of a single chain antibody ($scFV_2$), are also contemplated.

Antigen binding fragments can be prepared by proteolytic hydrolysis of the antibody or by expression in a host cell (such as an *E. coli* cell) of DNA encoding the fragment. Antigen binding fragments can also be obtained by pepsin or papain digestion of whole antibodies by conventional methods. For example, antigen binding fragments can be produced by enzymatic cleavage of antibodies with pepsin to provide a 5S fragment denoted $F(ab')_2$. This fragment can be further cleaved using a thiol reducing agent, and optionally a blocking group for the sulfhydryl groups resulting from cleavage of disulfide linkages, to produce 3.5S Fab' monovalent fragments. Alternatively, an enzymatic cleavage using pepsin produces two monovalent Fab' fragments and an Fc fragment directly (see U.S. Patent No. 4,036,945 and U.S. Patent No. 4,331,647, and references contained therein; Nisonhoff *et al.*, *Arch. Biochem. Biophys.* 89:230, 1960; Porter, *Biochem. J.* 73:119, 1959; Edelman *et al.*, *Methods in Enzymology*, Vol. 1, page 422, Academic Press, 1967; and Coligan *et al.* at sections 2.8.1-2.8.10 and 2.10.1-2.10.4).

Other methods of cleaving antibodies, such as separation of heavy chains to form monovalent light-heavy chain fragments, further cleavage of fragments, or other enzymatic, chemical, or genetic techniques may also be used, so long as the fragments bind to the antigen that is recognized by the intact antibody.

Antigen binding single V_H domains, called domain antibodies (dAb), have also been identified from a library of murine V_H genes amplified from genomic DNA of immunized mice (Ward *et al. Nature 341*:544-546, 1989). Human single immunoglobulin variable domain polypeptides capable of binding

antigen with high affinity have also been described (see, for example, PCT Publication Nos. WO 2005/035572 and WO 2003/002609). The CDRs disclosed herein can also be included in a dAb.

In some embodiments, one or more of the heavy and/or light chain complementarity determining regions (CDRs) from a disclosed antibody (such as the 35O22, 10J4, 10M6, 13I10, 2N5, 4O20, 7B9, and 7K3 antibody) is expressed on the surface of another protein, such as a scaffold protein. The expression of domains of antibodies on the surface of a scaffolding protein are known in the art (see *e.g.* Liu *et al., J. Virology* 85(17): 8467-8476, 2011). Such expression creates a chimeric protein that retains the binding for HIV-1 Env. In some specific embodiments, one or more of the heavy chain CDRs is grafted onto a scaffold protein, such as one or more of heavy chain CDR1, CDR2, and/or CDR3. One or more CDRs can also be included in a diabody or another type of single chain antibody molecule.

(e) Additional antibodies that bind to the 35O22 epitope on HIV-1 Env protein

Also included are antibodies that bind to the same epitope on HIV-1 Env protein to which the 35O22 antibody binds. Antibodies that bind to such an epitope can be identified based on their ability to cross-compete (for example, to competitively inhibit the binding of, in a statistically significant manner) with the 35O22 antibodies provided herein in HIV-1 Env protein binding assays (such as those described in the Examples). An antibody "competes" for binding when the competing antibody inhibits HIV-1 Env protein binding of the 35O22 antibody by more than 50%, in the presence of competing antibody concentrations higher than 10^6 x K_D of the competing antibody. In a certain embodiment, the antibody that binds to the same epitope on HIV-1 Env protein as the 35O22 antibody is a human monoclonal antibody. Such human monoclonal antibodies can be prepared and isolated as described herein.

Human antibodies that bind to the same epitope on HIV-1 Env protein to which the 35O22 antibody binds can be produced using various techniques known in the art. Human antibodies are described generally in van Dijk and van de Winkel, *Curr. Opin. Pharmacol.* 5: 368-74 (2001) and Lonberg, *Curr. Opin. Immunol.* 20:450-459 (2008). Such antibodies may be prepared, for example, by administering an immunogen to a transgenic animal that has been modified to produce intact human antibodies or intact antibodies with human variable regions in response to antigenic challenge. Such animals typically contain all or a portion of the human immunoglobulin loci, which replace the endogenous immunoglobulin loci, or which are present extrachromosomally or integrated randomly into the animal's chromosomes. In such transgenic mice, the endogenous immunoglobulin loci have generally been inactivated. For review of methods for obtaining human antibodies from transgenic animals, see Lonberg, *Nat. Biotech.* 23:1117-1125 (2005). See also, e.g., U.S. Pat. Nos. 6,075,181 and 6,150,584 describing XENOMOUSE™ technology; U.S. Pat. No. 5,770,429 describing HUMAB® technology; U.S. Pat. No. 7,041,870 describing K-M MOUSE® technology, and U.S. Patent Application Publication No. US 2007/0061900, describing VELOCIMOUSE® technology). Human variable regions from intact antibodies generated by such animals may be further modified, e.g., by combining with a different human constant region.

Human antibodies that bind to the same epitope on HIV-1 Env protein to which the 35O22 antibody binds can also be made by hybridoma-based methods. Human myeloma and mouse-human heteromyeloma cell lines for the production of human monoclonal antibodies have been described. (See, e.g., Kozbor *J. Immunol.,* 133: 3001 (1984); Brodeur et al., *Monoclonal Antibody Production Techniques and Applications*, pp. 51-63 (Marcel Dekker, Inc., New York, 1987); and Boerner et al., *J. Immunol.,* 147: 86 (1991).) Human antibodies generated via human B-cell hybridoma technology are also described in Li et al., *Proc. Natl. Acad. Sci. USA,* 103:3557-3562 (2006). Additional methods include those described, for example, in U.S. Pat. No. 7,189,826 (describing production of monoclonal human IgM antibodies from hybridoma cell lines) and Ni, *Xiandai Mianyixue,* 26(4):265-268 (2006) (describing human-human hybridomas). Human hybridoma technology (Trioma technology) is also described in Vollmers and Brandlein, *Histology and Histopathology,* 20(3):927-937 (2005) and Vollmers and Brandlein, *Methods and Findings in Experimental and Clinical Pharmacology,* 27(3): 185-91 (2005). Human antibodies may also be generated by isolating Fv clone variable domain sequences selected from human-derived phage display libraries. Such variable domain sequences may then be combined with a desired human constant domain.

Antibodies and antigen binding fragments that specification bind to the same epitope on HIV-1 Env protein as 35O22 invention can also be isolated by screening combinatorial libraries for antibodies with the desired binding characteristics. For example, a variety of methods are known in the art for generating phage display libraries and screening such libraries for antibodies possessing the desired binding characteristics. Such methods are reviewed, e.g., in Hoogenboom et al. in *Methods in Molecular Biology* 178:1-37 (O'Brien et al., ed., Human Press, Totowa, N.J., 2001) and further described, e.g., in the McCafferty et al., *Nature* 348:552-554; Clackson et al., *Nature* 352: 624-628 (1991); Marks et al., *J. Mol. Biol.* 222: 581-597 (1992); Marks and Bradbury, in *Methods in Molecular Biology* 248:161-175 (Lo, ed., Human Press, Totowa, N.J., 2003); Sidhu et al., *J. Mol. Biol.* 338(2): 299-310 (2004); Lee et al., *J. Mol. Biol.* 340(5): 1073-1093 (2004); Fellouse, *Proc. Natl. Acad. Sci. USA* 101(34): 12467-12472 (2004); and Lee et al., *J. Immunol. Methods* 284(1-2): 119-132 (2004).

In certain phage display methods, repertoires of VH and VL genes are separately cloned by polymerase chain reaction (PCR) and recombined randomly in phage libraries, which can then be screened for antigen-binding phage as described in Winter et al., *Ann. Rev. Immunol.,* 12: 433-455 (1994). Phage typically display antibody fragments, either as single-chain Fv (scFv) fragments or as Fab fragments. Libraries from immunized sources provide high-affinity antibodies to the immunogen without the requirement of constructing hybridomas. Alternatively, the naive repertoire can be cloned (e.g., from human) to provide a single source of antibodies to a wide range of non-self and also self antigens without any immunization as described by Griffiths et al., *EMBO J,* 12: 725-734 (1993). Finally, naive libraries can also be made synthetically by cloning unrearranged V-gene segments from stem cells, and using PCR primers containing random sequence to encode the highly variable CDR3 regions and to accomplish rearrangement in vitro, as described by Hoogenboom and Winter, *J. Mol. Biol.,* 227: 381-388 (1992). Patent publications describing human antibody phage libraries include, for example: U.S. Pat.

No. 5,750,373, and US Patent Publication Nos. 2005/0079574, 2005/0119455, 2005/0266000, 2007/0117126, 2007/0160598, 2007/0237764, 2007/0292936, and 2009/0002360.

(f) Variants

In certain embodiments, amino acid sequence variants of the antibodies provided herein are contemplated. For example, it may be desirable to improve the binding affinity and/or other biological properties of the antibody. Amino acid sequence variants of an antibody may be prepared by introducing appropriate modifications into the nucleotide sequence encoding the antibody, or by peptide synthesis. Such modifications include, for example, deletions from, and/or insertions into and/or substitutions of residues within the amino acid sequences of the antibody. Any combination of deletion, insertion, and substitution can be made to arrive at the final construct, provided that the final construct possesses the desired characteristics, e.g., antigen-binding.

In certain embodiments, antibody variants having one or more amino acid substitutions are provided. Sites of interest for substitutional mutagenesis include the CDRs and the framework regions. Amino acid substitutions may be introduced into an antibody of interest and the products screened for a desired activity, e.g., retained/improved antigen binding, decreased immunogenicity, or improved ADCC or CDC.

The variants typically retain amino acid residues necessary for correct folding and stabilizing between the V_H and the V_L regions, and will retain the charge characteristics of the residues in order to preserve the low pI and low toxicity of the molecules. Amino acid substitutions can be made in the V_H and the V_L regions to increase yield.

In some embodiments, the heavy chain of the antibody includes up to 10 (such as up to 1, up to 2, up to 3, up to 4, up to 5, up to 6, up to 7, up to 8, or up to 9) amino acid substitutions (such as conservative amino acid substitutions) compared to the amino acid sequence set forth as one of SEQ ID NOs : 1, 3, 5, 7, 9, 11, 13, or 15. In some embodiments, the light chain of the antibody includes up to 10 (such as up to 1, up to 2, up to 3, up to 4, up to 5, up to 6, up to 7, up to 8, or up to 9) amino acid substitutions (such as conservative amino acid substitutions) compared to the amino acid sequence set forth as one of SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, or 16.

In some embodiments, the antibody or antigen binding fragment can include up to 10 (such as up to 1, up to 2, up to 3, up to 4, up to 5, up to 6, up to 7, up to 8, or up to 9) amino acid substitutions (such as conservative amino acid substitutions) in the framework regions of the heavy chain of the antibody, or the light chain of the antibody, or the heavy and light chains of the antibody, compared to a known framework region, or compared to the framework regions of the 35O22 antibody, and maintain the specific binding activity for HIV-1 Env protein.

In certain embodiments, substitutions, insertions, or deletions may occur within one or more CDRs so long as such alterations do not substantially reduce the ability of the antibody to bind antigen. For example, conservative alterations (e.g., conservative substitutions as provided herein) that do not substantially reduce binding affinity may be made in CDRs. In certain embodiments of the variant VH

and VL sequences provided above, each CDR either is unaltered, or contains no more than one, two or three amino acid substitutions.

To increase binding affinity of the antibody, the V_L and V_H segments can be randomly mutated, such as within H-CDR3 region or the L-CDR3 region, in a process analogous to the *in vivo* somatic mutation process responsible for affinity maturation of antibodies during a natural immune response. Thus *in vitro* affinity maturation can be accomplished by amplifying V_H and V_L regions using PCR primers complementary to the H-CDR3 or L-CDR3, respectively. In this process, the primers have been "spiked" with a random mixture of the four nucleotide bases at certain positions such that the resultant PCR products encode V_H and V_L segments into which random mutations have been introduced into the V_H and/or V_L CDR3 regions. These randomly mutated V_H and V_L segments can be tested to determine the binding affinity for HIV-1 Env protein. In particular examples, the V_H amino acid sequence that is mutated is one of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, or 15. In other examples, the V_L amino acid sequence that is mutated is SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, or 16. Methods of in vitro affinity maturation are known (see, e.g., Chowdhury, *Methods Mol. Biol.* 207:179-196 (2008)), and Hoogenboom et al. in *Methods in Molecular Biology* 178:1-37 (O'Brien et al., ed., Human Press, Totowa, N.J., (2001).)

A useful method for identification of residues or regions of an antibody that may be targeted for mutagenesis is called "alanine scanning mutagenesis" as described by Cunningham and Wells (1989) *Science,* 244:1081-1085. In this method, a residue or group of target residues (e.g., charged residues such as arg, asp, his, lys, and glu) are identified and replaced by a neutral or negatively charged amino acid (e.g., alanine or polyalanine) to determine whether the interaction of the antibody with antigen is affected. Further substitutions may be introduced at the amino acid locations demonstrating functional sensitivity to the initial substitutions. Alternatively, or additionally, a crystal structure of an antigen-antibody complex is used to identify contact points between the antibody and antigen. Such contact residues and neighboring residues may be targeted or eliminated as candidates for substitution. Variants may be screened to determine whether they contain the desired properties.

In certain embodiments, an antibody or antigen binding fragment is altered to increase or decrease the extent to which the antibody or antigen binding fragment is glycosylated. Addition or deletion of glycosylation sites may be conveniently accomplished by altering the amino acid sequence such that one or more glycosylation sites is created or removed.

Where the antibody comprises an Fc region, the carbohydrate attached thereto may be altered. Native antibodies produced by mammalian cells typically comprise a branched, biantennary oligosaccharide that is generally attached by an N-linkage to Asn297 of the CH_2 domain of the Fc region. See, e.g., Wright et al. *TIBTECH* 15:26-32 (1997). The oligosaccharide may include various carbohydrates, e.g., mannose, N-acetyl glucosamine (GlcNAc), galactose, and sialic acid, as well as a fucose attached to a GlcNAc in the "stem" of the biantennary oligosaccharide structure. In some embodiments, modifications of the oligosaccharide in an antibody may be made in order to create antibody variants with certain improved properties.

In one embodiment, antibody variants are provided having a carbohydrate structure that lacks fucose attached (directly or indirectly) to an Fc region. For example, the amount of fucose in such antibody may be from 1% to 80%, from 1% to 65%, from 5% to 65% or from 20% to 40%. The amount of fucose is determined by calculating the average amount of fucose within the sugar chain at Asn297, relative to the sum of all glycostructures attached to Asn 297 (e.g. complex, hybrid and high mannose structures) as measured by MALDI-TOF mass spectrometry, as described in WO 2008/077546, for example. Asn297 refers to the asparagine residue located at about position 297 in the Fc region; however, Asn297 may also be located about ±3 amino acids upstream or downstream of position 297, i.e., between positions 294 and 300, due to minor sequence variations in antibodies. Such fucosylation variants may have improved ADCC function. See, e.g., US Patent Publication Nos. US 2003/0157108 (Presta, L.); US 2004/0093621 (Kyowa Hakko Kogyo Co., Ltd). Examples of publications related to "defucosylated" or "fucose-deficient" antibody variants include: US 2003/0157108; WO 2000/61739; WO 2001/29246; US 2003/0115614; US 2002/0164328; US 2004/0093621; US 2004/0132140; US 2004/0110704; US 2004/0110282; US 2004/0109865; WO 2003/085119; WO 2003/084570; WO 2005/035586; WO 2005/035778; WO2005/053742; WO2002/031140; Okazaki et al. *J. Mol. Biol.* 336:1239-1249 (2004); Yamane-Ohnuki et al. *Biotech. Bioeng.* 87: 614 (2004). Examples of cell lines capable of producing defucosylated antibodies include Lec 13 CHO cells deficient in protein fucosylation (Ripka et al. *Arch. Biochem. Biophys.* 249:533-545 (1986); US Pat Appl No US 2003/0157108 A1, Presta, L; and WO 2004/056312 A1, Adams et al., especially at Example 11), and knockout cell lines, such as alpha-1,6-fucosyltransferase gene, FUT8, knockout CHO cells (see, e.g., Yamane-Ohnuki et al. *Biotech. Bioeng.* 87: 614 (2004); Kanda, Y. et al., *Biotechnol. Bioeng.*, 94(4):680-688 (2006); and WO2003/085107).

Antibodies variants are further provided with bisected oligosaccharides, e.g., in which a biantennary oligosaccharide attached to the Fc region of the antibody is bisected by GlcNAc. Such antibody variants may have reduced fucosylation and/or improved ADCC function. Examples of such antibody variants are described, e.g., in WO 2003/011878 (Jean-Mairet et al.); U.S. Pat. No. 6,602,684 (Umana et al.); and US 2005/0123546 (Umana et al.). Antibody variants with at least one galactose residue in the oligosaccharide attached to the Fc region are also provided. Such antibody variants may have improved CDC function. Such antibody variants are described, e.g., in WO 1997/30087 (Patel et al.); WO 1998/58964 (Raju, S.); and WO 1999/22764 (Raju, S.).

In several embodiments, the constant region of the antibody includes one or more amino acid substitutions to optimize *in vivo* half-life of the antibody. The serum half-life of IgG Abs is regulated by the neonatal Fc receptor (FcRn). Thus, in several embodiments, the antibody includes an amino acid substitution that increases binding to the FcRn. Several such substitutions are known to the person of ordinary skill in the art, such as substitutions at IgG constant regions T250Q and M428L (see, e.g., Hinton *et al., J Immunol.,* 176:346-356, 2006); M428L and N434S (the "LS" mutation, see, e.g., Zalevsky, *et al., Nature Biotechnology, 28:157-159,* 2010); N434A (see, e.g., Petkova *et al., Int. Immunol.*, 18:1759-1769, 2006); T307A, E380A, and N434A (see, e.g., Petkova *et al., Int. Immunol.*,

18:1759-1769, 2006); and M252Y, S254T, and T256E (see, e.g., Dall'Acqua *et al., J. Biol. Chem.*, 281:23514-23524, 2006). The disclosed antibodies and antigen binding fragments can be linked to a Fc polypeptide including any of the substitutions listed above, for example, the Fc polypeptide can include the M428L and N434S substitutions.

In some embodiments, the constant region of the antibody includes one of more amino acid substitutions to optimize Antibody-dependent cell-mediated cytotoxicity (ADCC). ADCC is mediated primarily through a set of closely related Fcγ receptors. In some embodiments, the antibody includes one or more amino acid substitutions that increase binding to FcγRIIIa. Several such substitutions are known to the person of ordinary skill in the art, such as substitutions at IgG constant regions S239D and I332E (see, e.g., Lazar *et al., Proc. Natl., Acad. Sci. U.S.A.*, 103:4005-4010, 2006); and S239D, A330L, and I332E (see, e.g., Lazar *et al., Proc. Natl., Acad. Sci. U.S.A.*, 103:4005-4010, 2006).

Combinations of the above substitutions are also included, to generate an IgG constant region with increased binding to FcRn and FcγRIIIa. The combinations increase antibody half-life and ADCC. For example, such combination include antibodies with the following amino acid substitution in the Fc region:

(1) S239D/I332E and T250Q/M428L;

(2) S239D/I332E and M428L/N434S;

(3) S239D/I332E and N434A;

(4) S239D/I332E and T307A/E380A/N434A;

(5) S239D/I332E and M252Y / S254T/T256E;

(6) S239D/A330L/I332E and T250Q/M428L;

(7) S239D/A330L/I332E and M428L/N434S;

(8) S239D/A330L/I332E and N434A;

(9) S239D/A330L/I332E and T307A/E380A/N434A; or

(10) S239D/A330L/I332E and M252Y/S254T/T256E.

In some examples, the antibodies, or an antigen binding fragment thereof is modified such that it is directly cytotoxic to infected cells, or uses natural defenses such as complement, antibody dependent cellular cytotoxicity (ADCC), or phagocytosis by macrophages.

In certain embodiments, an antibody provided herein may be further modified to contain additional nonproteinaceous moieties that are known in the art and readily available. The moieties suitable for derivatization of the antibody include but are not limited to water soluble polymers. Non-limiting examples of water soluble polymers include, but are not limited to, polyethylene glycol (PEG), copolymers of ethylene glycol/propylene glycol, carboxymethylcellulose, dextran, polyvinyl alcohol, polyvinyl pyrrolidone, poly-1,3-dioxolane, poly-1,3,6-trioxane, ethylene/maleic anhydride copolymer, polyaminoacids (either homopolymers or random copolymers), and dextran or poly(n-vinyl pyrrolidone)polyethylene glycol, propropylene glycol homopolymers, prolypropylene oxide/ethylene oxide co-polymers, polyoxyethylated polyols (e.g., glycerol), polyvinyl alcohol, and mixtures thereof. Polyethylene glycol propionaldehyde may have advantages in manufacturing due to its stability in water.

The polymer may be of any molecular weight, and may be branched or unbranched. The number of polymers attached to the antibody may vary, and if more than one polymer are attached, they can be the same or different molecules. In general, the number and/or type of polymers used for derivatization can be determined based on considerations including, but not limited to, the particular properties or functions of the antibody to be improved, whether the antibody derivative will be used in a therapy under defined conditions, etc.

The antibody or antigen binding fragment can be derivatized or linked to another molecule (such as another peptide or protein). In general, the antibody or antigen binding fragment is derivatized such that the binding to HIV-1 Env is not affected adversely by the derivatization or labeling. For example, the antibody or antigen binding fragment can be functionally linked (by chemical coupling, genetic fusion, noncovalent association or otherwise) to one or more other molecular entities, such as another antibody (for example, a bi-specific antibody or a diabody), a detectable marker, an effector molecule, or a protein or peptide that can mediate association of the antibody or antibody portion with another molecule (such as a streptavidin core region or a polyhistidine tag).

B. Conjugates

Monoclonal antibodies and antigen binding fragments that specifically bind to an epitope on HIV-1 Env can be conjugated to an agent, such as an effector molecule or detectable marker, using any number of means known to those of skill in the art. Both covalent and noncovalent attachment means may be used. Conjugates include, but are not limited to, molecules in which there is a covalent linkage of an effector molecule or a detectable marker to an antibody or antigen binding fragment that specifically binds HIV-1 Env. One of skill in the art will appreciate that various effector molecules and detectable markers can be used, including (but not limited to) toxins and radioactive agents such as ^{125}I, ^{32}P, ^{14}C, ^{3}H and ^{35}S and other labels, target moieties and ligands, etc. The choice of a particular effector molecule or detectable marker depends on the particular target molecule or cell, and the desired biological effect.

The choice of a particular effector molecule or detectable marker depends on the particular target molecule or cell, and the desired biological effect. Thus, for example, the effector molecule can be a cytotoxin that is used to bring about the death of a particular target cell (such as a HIV-1 infected cell). In other embodiments, the effector molecule can be a cytokine, such as IL-15; conjugates including the cytokine can be used, e.g., to stimulate immune calls locally.

Effector molecules and detectable markers can be linked to an antibody or antigen binding fragment of interest using any number of means known to those of skill in the art. Both covalent and non-covalent attachment means may be used. The procedure for attaching an effector molecule or detectable marker to an antibody or antigen binding fragment varies according to the chemical structure of the effector. Polypeptides typically contain a variety of functional groups; such as carboxylic acid (COOH), free amine (-NH_2) or sulfhydryl (-SH) groups, which are available for reaction with a suitable functional group on a polypeptide to result in the binding of the effector molecule or detectable marker.

Alternatively, the antibody or antigen binding fragment is derivatized to expose or attach additional reactive functional groups. The derivatization may involve attachment of any of a number of known linker molecules such as those available from Pierce Chemical Company, Rockford, IL. The linker can be any molecule used to join the antibody or antigen binding fragment to the effector molecule or detectable marker. The linker is capable of forming covalent bonds to both the antibody or antigen binding fragment and to the effector molecule or detectable marker. Suitable linkers are well known to those of skill in the art and include, but are not limited to, straight or branched-chain carbon linkers, heterocyclic carbon linkers, or peptide linkers. Where the antibody or antigen binding fragment and the effector molecule or detectable marker are polypeptides, the linkers may be joined to the constituent amino acids through their side groups (such as through a disulfide linkage to cysteine) or to the alpha carbon amino and carboxyl groups of the terminal amino acids.

In several embodiments, the linker can include a spacer element, which, when present, increases the size of the linker such that the distance between the effector molecule or the detectable marker and the antibody or antigen binding fragment is increased. Exemplary spacers are known to the person of ordinary skill, and include those listed in U.S. Pat. Nos. 7,964,5667, 498,298, 6,884,869, 6,323,315, 6,239,104, 6,034,065, 5,780,588, 5,665,860, 5,663,149, 5,635,483, 5,599,902, 5,554,725, 5,530,097, 5,521,284, 5,504,191, 5,410,024, 5,138,036, 5,076,973, 4,986,988, 4,978,744, 4,879,278, 4,816,444, and 4,486,414, as well as U.S. Pat. Pub. Nos. 20110212088 and 20110070248, each of which is incorporated by reference in its entirety.

In some embodiments, the linker is cleavable under intracellular conditions, such that cleavage of the linker releases the effector molecule or detectable marker from the antibody or antigen binding fragment in the intracellular environment. In yet other embodiments, the linker is not cleavable and the effector molecule or detectable marker is released, for example, by antibody degradation. In some embodiments, the linker is cleavable by a cleaving agent that is present in the intracellular environment (for example, within a lysosome or endosome or caveolea). The linker can be, for example, a peptide linker that is cleaved by an intracellular peptidase or protease enzyme, including, but not limited to, a lysosomal or endosomal protease. In some embodiments, the peptide linker is at least two amino acids long or at least three amino acids long. However, the linker can be 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15 amino acids long, such as 1-2, 1-3, 2-5, 3-10, 3-15, 1-5, 1-10, 1-15, amino acids long. Proteases can include cathepsins B and D and plasmin, all of which are known to hydrolyze dipeptide drug derivatives resulting in the release of active drug inside target cells (see, for example, Dubowchik and Walker, 1999, Pharm. Therapeutics 83:67-123). For example, a peptide linker that is cleavable by the thiol-dependent protease cathepsin-B, can be used (for example, a Phenylalanine -Leucine or a Glycine- Phenylalanine -Leucine-Glycine linker). Other examples of such linkers are described, for example, in U.S. Pat. No. 6,214,345, incorporated herein by reference. In a specific embodiment, the peptide linker cleavable by an intracellular protease is a Valine-Citruline linker or a Phenylalanine-Lysine linker (see, for example, U.S. Pat. No. 6,214,345, which describes the synthesis of doxorubicin with the Valine-Citruline linker).

In other embodiments, the cleavable linker is pH-sensitive, i.e., sensitive to hydrolysis at certain pH values. Typically, the pH-sensitive linker is hydrolyzable under acidic conditions. For example, an acid-labile linker that is hydrolyzable in the lysosome (for example, a hydrazone, semicarbazone, thiosemicarbazone, cis-aconitic amide, orthoester, acetal, ketal, or the like) can be used. (See, for example, U.S. Pat. Nos. 5,122,368; 5,824,805; 5,622,929; Dubowchik and Walker, 1999, Pharm. Therapeutics 83:67-123; Neville et al., 1989, Biol. Chem. 264:14653-14661.) Such linkers are relatively stable under neutral pH conditions, such as those in the blood, but are unstable at below pH 5.5 or 5.0, the approximate pH of the lysosome. In certain embodiments, the hydrolyzable linker is a thioether linker (such as, for example, a thioether attached to the therapeutic agent via an acylhydrazone bond (see, for example, U.S. Pat. No. 5,622,929).

In yet other embodiments, the linker is cleavable under reducing conditions (for example, a disulfide linker). A variety of disulfide linkers are known in the art, including, for example, those that can be formed using SATA (N-succinimidyl-S-acetylthioacetate), SPDP (N-succinimidyl-3-(2-pyridyldithio)propionate), SPDB (N-succinimidyl-3-(2-pyridyldithio)butyrate) and SMPT (N-succinimidyl-oxycarbonyl-alpha-methyl-alpha-(2-pyridyl-dithio)toluene)- , SPDB and SMPT. (See, for example, Thorpe et al., 1987, Cancer Res. 47:5924-5931; Wawrzynczak et al., In Immunoconjugates: Antibody Conjugates in Radioimagery and Therapy of Cancer (C. W. Vogel ed., Oxford U. Press, 1987); Phillips et al., Cancer Res. 68:92809290, 2008). See also U.S. Pat. No. 4,880,935.)

In yet other specific embodiments, the linker is a malonate linker (Johnson et al., 1995, Anticancer Res. 15:1387-93), a maleimidobenzoyl linker (Lau et al., 1995, Bioorg-Med-Chem. 3(10):1299-1304), or a 3'-N-amide analog (Lau et al., 1995, Bioorg-Med-Chem. 3(10):1305-12).

In several embodiments, the linker is resistant to cleavage in an extracellular environment. Whether or not a linker is resistant to cleavage in an extracellular environment can be determined, for example, by incubating the conjugate containing the linker of interest with plasma for a predetermined time period (for example, 2, 4, 8, 16, or 24 hours) and then quantitating the amount of free effector molecule or detectable marker present in the plasma. A variety of exemplary linkers that can be used in conjugates are described in WO 2004010957, U.S. Publication No. 2006/0074008, U.S. Publication No. 20050238649, and U.S. Publication No. 2006/0024317, each of which is incorporated by reference herein in its entirety.

In view of the large number of methods that have been reported for attaching a variety of radiodiagnostic compounds, radiotherapeutic compounds, labels (such as enzymes or fluorescent molecules), toxins, and other agents to antibodies one skilled in the art will be able to determine a suitable method for attaching a given agent to an antibody or antigen binding fragment or other polypeptide. For example, the antibody or antigen binding fragment can be conjugated with effector molecules such as small molecular weight drugs such as Monomethyl Auristatin E (MMAE), Monomethyl Auristatin F (MMAF), maytansine, maytansine derivatives, including the derivative of maytansine known as DM1 (also known as mertansine), or other agents to make an antibody drug conjugate (ADC). In several embodiments, conjugates of an antibody or antigen binding fragment and

one or more small molecule toxins, such as a calicheamicin, maytansinoids, dolastatins, auristatins, a trichothecene, and CC1065, and the derivatives of these toxins that have toxin activity, are provided.

The antibody or antigen binding fragment can be conjugated with a detectable marker; for example, a detectable marker capable of detection by ELISA, spectrophotometry, flow cytometry, microscopy or diagnostic imaging techniques (such as computed tomography (CT), computed axial tomography (CAT) scans, magnetic resonance imaging (MRI), nuclear magnetic resonance imaging NMRI), magnetic resonance tomography (MTR), ultrasound, fiberoptic examination, and laparoscopic examination). Specific, non-limiting examples of detectable markers include fluorophores, chemiluminescent agents, enzymatic linkages, radioactive isotopes and heavy metals or compounds (for example super paramagnetic iron oxide nanocrystals for detection by MRI). For example, useful detectable markers include fluorescent compounds, including fluorescein, fluorescein isothiocyanate, rhodamine, 5-dimethylamine-1-napthalenesulfonyl chloride, phycoerythrin, lanthanide phosphors and the like. Bioluminescent markers are also of use, such as luciferase, Green fluorescent protein (GFP), Yellow fluorescent protein (YFP). An antibody or antigen binding fragment can also be conjugated with enzymes that are useful for detection, such as horseradish peroxidase, β- galactosidase, luciferase, alkaline phosphatase, glucose oxidase and the like. When an antibody or antigen binding fragment is conjugated with a detectable enzyme, it can be detected by adding additional reagents that the enzyme uses to produce a reaction product that can be discerned. For example, when the agent horseradish peroxidase is present the addition of hydrogen peroxide and diaminobenzidine leads to a colored reaction product, which is visually detectable. An antibody or antigen binding fragment may also be conjugated with biotin, and detected through indirect measurement of avidin or streptavidin binding. It should be noted that the avidin itself can be conjugated with an enzyme or a fluorescent label.

The antibody or antigen binding fragment can be conjugated with a paramagnetic agent, such as gadolinium. Paramagnetic agents such as superparamagnetic iron oxide are also of use as labels. Antibodies can also be conjugated with lanthanides (such as europium and dysprosium), and manganese. An antibody or antigen binding fragment may also be labeled with a predetermined polypeptide epitopes recognized by a secondary reporter (such as leucine zipper pair sequences, binding sites for secondary antibodies, metal binding domains, epitope tags).

The antibody or antigen binding fragment can also be conjugated with a radiolabeled amino acid. The radiolabel may be used for both diagnostic and therapeutic purposes. For instance, the radiolabel may be used to detect HIV-1 Env and HIV-1 Env expressing cells by x-ray, emission spectra, or other diagnostic techniques. Examples of labels for polypeptides include, but are not limited to, the following radioisotopes or radionucleotides: ^{3}H, ^{14}C, ^{15}N, ^{35}S, ^{90}Y, ^{99}Tc, ^{111}In, ^{125}I, ^{131}I.

Means of detecting such detectable markers are well known to those of skill in the art. Thus, for example, radiolabels may be detected using photographic film or scintillation counters, fluorescent markers may be detected using a photodetector to detect emitted illumination. Enzymatic labels are typically detected by providing the enzyme with a substrate and detecting the reaction product produced

by the action of the enzyme on the substrate, and colorimetric labels are detected by simply visualizing the colored label.

An antibody or antigen binding fragment can also be derivatized with a chemical group such as polyethylene glycol (PEG), a methyl or ethyl group, or a carbohydrate group. These groups may be useful to improve the biological characteristics of the antibody or antigen binding fragment, such as to increase serum half-life or to increase tissue binding.

The average number of effector molecule or detectable marker moieties per antibody or antigen binding fragment in a conjugate can range, for example, from 1 to 20 moieties per antibody or antigen binding fragment. In certain embodiments, the average number of effector molecule or detectable marker moieties per antibody or antigen binding fragment in a conjugate range from about 1 to about 2, from about 1 to about 3, about 1 to about 8; from about 2 to about 6; from about 3 to about 5; or from about 3 to about 4. The loading (for example, effector molecule/antibody ratio) of an conjugate may be controlled in different ways, for example, by: (i) limiting the molar excess of effector molecule-linker intermediate or linker reagent relative to antibody, (ii) limiting the conjugation reaction time or temperature, (iii) partial or limiting reductive conditions for cysteine thiol modification, (iv) engineering by recombinant techniques the amino acid sequence of the antibody such that the number and position of cysteine residues is modified for control of the number or position of linker-effector molecule attachments.

C. Chimeric Antigen Receptors (CARs)

Also disclosed herein are chimeric antigen receptor (CARs) that are artificially constructed chimeric proteins including an extracellular antigen binding domain (e.g., single chain variable fragment (scFv)) that specifically binds to HIV-1 Env, linked to a transmembrane domain, linked to one or more intracellular T-cell signaling domains. Characteristics of the disclosed CARs include their ability to redirect T-cell specificity and reactivity towards HIV-1 Env expressing cells in a non-MHC-restricted manner. The non-MHC-restricted HIV-1 Env recognition gives T cells expressing a disclosed CAR the ability to recognize antigen independent of antigen processing.

The intracellular T cell signaling domains can include, for example, a T cell receptor signaling domain, a T cell costimulatory signaling domain, or both. The T cell receptor signaling domain refers to a portion of the CAR comprising the intracellular domain of a T cell receptor, such as the intracellular portion of the CD3 zeta protein. The costimulatory signaling domain refers to a portion of the CAR comprising the intracellular domain of a costimulatory molecule, which is a cell surface molecule other than an antigen receptor or their ligands that are required for an efficient response of lymphocytes to antigen.

1. Extracellular Region

Several embodiments provide a CAR including an antigen binding domain that specifically binds to HIV-1 Env as disclosed herein (see, e.g., section II.A). For example, the antigen binding domain can

be a scFv including the heavy chain variable region and the light chain variable region of any of the antibodies or antigen binding fragments thereof disclosed in section II.A.

In some embodiment, the antigen binding domain can include a heavy chain variable region and a light chain variable region including the HCDR1, HCDR2, and HCDR3, and LCDR1, LCDR2, and LCDR3 of the of the heavy and light chain variable regions, respectively, of one of the 35O22, 10J4, 10M6, 13I10, 2N5, 4O20, 7B9, and 7K3 antibodies (e.g., as set forth in Table 2 or Table 3).

In some embodiments, the antigen binding domain includes a heavy chain variable region and a light chain variable region including the amino acid sequences set forth as SEQ ID NOs: 1 and 2, respectively; SEQ ID NOs: 3 and 4, respectively; SEQ ID NOs: 5 and 6, respectively; SEQ ID NOs: 7 and 8, respectively, SEQ ID NOs: 9 and 10, respectively; SEQ ID NOs: 11 and 12, respectively; SEQ ID NOs: 13 and 14, respectively; or SEQ ID NOs: 15 and 16, respectively.

In several embodiments, the antigen binding domain can be a scFv. In some embodiments, the scFv includes a heavy chain variable region and a light chain variable region joined by a peptide linker, such as a linker including the amino acid sequence set forth as GGGGSGGGGSGGGGS (SEQ ID NO: 23).

The CAR can include a signal peptide sequence, e.g., N-terminal to the antigen binding domain. The signal peptide sequence may comprise any suitable signal peptide sequence. In an embodiment, the signal peptide sequence is a human granulocyte-macrophage colony-stimulating factor (GM-CSF) receptor sequence, such as an amino acid sequence including or consisting of LLVTSLLLCELPHPAFLLIPDT SEQ ID NO: 24. While the signal peptide sequence may facilitate expression of the CAR on the surface of the cell, the presence of the signal peptide sequence in an expressed CAR is not necessary in order for the CAR to function. Upon expression of the CAR on the cell surface, the signal peptide sequence may be cleaved off of the CAR. Accordingly, in some embodiments, the CAR lacks a signal peptide sequence.

Between the antigen binding domain and the transmembrane domain of the CAR, there may be a spacer domain, which includes a polypeptide sequence. The spacer domain may comprise up to 300 amino acids, preferably 10 to 100 amino acids and most preferably 25 to 50 amino acids. In some embodiments, the spacer domain can include an immunoglobulin domain, such as a human immunoglobulin sequence. In an embodiment, the immunoglobulin domain comprises an immunoglobulin CH2 and CH3 immunoglobulin G (IgGl) domain sequence (CH2CH3). In this regard, the spacer domain can include an immunoglobulin domain comprising or consisting of the amino acid sequence set forth as SEQ ID NO: 25:

EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVH
NAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSR
DELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSC
SVMHEALHNHYTQKSLSLSPGKKDPK

Without being bound to a particular theory, it is believed that the CH2CH3 domain extends the antigen binding domain of the CAR away from the membrane of CAR-expressing cells and may more accurately mimic the size and domain structure of a native TCR.

2. Transmembrane Domain

With respect to the transmembrane domain, the CAR can be designed to comprise a transmembrane domain that is fused to the extracellular domain of the CAR. In one embodiment, the transmembrane domain that naturally is associated with one of the domains in the CAR is used.

The transmembrane domain may be derived either from a natural or from a synthetic source. Where the source is natural, the domain may be derived from any membrane-bound or transmembrane protein. Exemplary transmembrane domains for use in the disclosed CARs can include at least the transmembrane region(s) of) the alpha, beta or zeta chain of the T-cell receptor, CD28, CD3 epsilon, CD45, CD4, CD5, CDS, CD9, CD 16, CD22, CD33, CD37, CD64, CD80, CD86, CD 134, CD137, CD154. Alternatively the transmembrane domain may be synthetic, in which case it will comprise predominantly hydrophobic residues such as leucine and valine. In several embodiments, a triplet of phenylalanine, tryptophan and valine will be found at each end of a synthetic transmembrane domain.

Optionally, a short oligo- or polypeptide linker, preferably between 2 and 10 amino acids in length may form the linkage between the transmembrane domain and the intracellular T cell signaling domain and/or T cell costimulatory domain of the CAR. A exemplary linker sequence includes one or more glycine-serine doublets.

In some embodiments, the transmembrane domain comprises the transmembrane domain of a T cell receptor, such as a CD8 transmembrane domain. Thus, the CAR can include a CD8 transmembrane domain including or consisting of SEQ ID NO: 26:

TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYC

In another embodiment, the transmembrane domain comprises the transmembrane domain of a T cell costimulatory molecule, such as CD137 or CD28. Thus, the CAR can include a CD28 transmembrane domain including or consisting of SEQ ID NO: 27:

IEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPLFPGPSKPFWVLVVVGGVLACYSLLVTVAFIIFWVR

3. Intracellular Region

The intracellular region of the CAR includes one or more intracellular T cell signaling domains responsible for activation of at least one of the normal effector functions of a T cell in which the CAR is expressed or placed in. Exemplary T cell signaling domains are provided herein, and are known to the person of ordinary skill in the art.

While an entire intracellular T cell signaling domain can be employed in a CAR, in many cases it is not necessary to use the entire chain. To the extent that a truncated portion of the intracellular T cell signaling domain is used, such truncated portion may be used in place of the intact chain as long as it transduces the relevant T cell effector function signal.

Examples of intracellular T cell signaling domains for use in the CAR include the cytoplasmic sequences of the T cell receptor (TCR) and co-stimulatory molecules that act in concert to initiate signal

transduction following antigen receptor engagement, as well as any derivative or variant of these sequences and any synthetic sequence that has the same functional capability.

T cell receptor signaling domains regulate primary activation of the T cell receptor complex either in a stimulatory way, or in an inhibitory way. The disclosed CARs can include primary cytoplasmic signaling sequences that act in a stimulatory manner, which may contain signaling motifs that are known as immunoreceptor tyrosine-based activation motifs or ITAMs. Examples of ITAM containing primary cytoplasmic signaling sequences that can be included in a disclosed CAR include include those from CD3 zeta, FcR gamma, FcR beta, CD3 gamma , CD3 delta , CD3 epsilon, CDS, CD22, CD79a, CD79b, and CD66d proteins. In several embodiments, the cytoplasmic signaling molecule in the CAR includes an intracellular T cell signaling domain from CD3 zeta.

The intracellular region of the CAR can include the ITAM containing primary cytoplasmic signaling domain (such as CD3-zeta) by itself or combined with any other desired cytoplasmic domain(s) useful in the context of a CAR. For example, the cytoplasmic domain of the CAR can include a CD3 zeta chain portion and an intracellular costimulatory signaling domain. The costimulatory signaling domain refers to a portion of the CAR comprising the intracellular domain of a costimulatory molecule. A costimulatory molecule is a cell surface molecule other than an antigen receptor or their ligands that is required for an efficient response of lymphocytes to an antigen. Examples of such molecules include CD27, CD28, 4-1BB (CD137), OX40 (CD134), CD30, CD40, PD-1, ICOS, lymphocyte function-associated antigen 1 (LFA-1), CD2, CD7, LIGHT, NKG2C, and B7-H3. An additional example of a signaling domain that can be included in a disclosed CARs is a Tumor necrosis factor receptor superfamily member 18 (TNFRSF18; also known as glucocorticoid-induced TNFR-related protein, GITR) signaling domain.

In some embodiments, the CAR can include a CD3 zeta signaling domain, a CD8 signaling domain, a CD28 signaling domain, a CD137 signaling domain or a combination of two or more thereof. In one embodiment, the cytoplasmic domain includes the signaling domain of CD3-zeta and the signaling domain of CD28. In another embodiment, the cytoplasmic domain includes the signaling domain of CD3 zeta and the signaling domain of CD137. In yet another embodiment, the cytoplasmic domain includes the signaling domain of CD3-zeta and the signaling domain of CD28 and CD137. The order of the one or more T cell signaling domains on the CAR can be varied as needed by the person of ordinary skill in the art.

Exemplary amino acid sequences for such T cell signaling domains are provided. For example, the CD3 zeta signaling domain can include or consist of the amino acid sequence set forth as SEQ ID NO: 28 (RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNEL QKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR), the CD8 signaling domain can include or consist of the amino acid sequence set forth as SEQ ID NO: 29 (FVPVFLPAKPTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTC GVLLLSLVITLYCNHRNR), the CD28 signaling domain can include or consist of the amino acid sequence set forth as SEQ ID NO: 30

(SKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRS), the CD137 signaling domain can include or consist of the amino acid sequences set forth as SEQ ID NO: 31 (KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL) or SEQ ID NO: 32 (RFSVVKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL).

The cytoplasmic signaling sequences within the cytoplasmic signaling portion of the CAR of the invention may be linked to each other in a random or specified order. Optionally, a short polypeptide linker, preferably between 2 and 10 amino acids in length may form the linkage. A glycine-serine doublet provides a particularly suitable linker. Further, between the signaling domain and the transmembrane domain of the CAR, there may be a spacer domain, which includes a polypeptide sequence. The spacer domain may comprise up to 300 amino acids, preferably 10 to 100 amino acids and most preferably 25 to 50 amino acids.

4. Additional Description of CARs

Also provided are functional portions of the CARs described herein. The term "functional portion" when used in reference to a CAR refers to any part or fragment of the CAR, which part or fragment retains the biological activity of the CAR of which it is a part (the parent CAR). Functional portions encompass, for example, those parts of a CAR that retain the ability to recognize target cells, or detect, treat, or prevent a disease, to a similar extent, the same extent, or to a higher extent, as the parent CAR. In reference to the parent CAR, the functional portion can comprise, for instance, about 10%, 25%, 30%, 50%, 68%, 80%, 90%, 95%, or more, of the parent CAR.

The CAR or functional portion thereof, can include additional amino acids at the amino or carboxy terminus, or at both termini, which additional amino acids are not found in the amino acid sequence of the parent CAR. Desirably, the additional amino acids do not interfere with the biological function of the CAR or functional portion, e.g., recognize target cells, detect cancer, treat or prevent cancer, etc. More desirably, the additional amino acids enhance the biological activity, as compared to the biological activity of the parent CAR.

Also provided are functional variants of the CARs described herein, which have substantial or significant sequence identity or similarity to a parent CAR, which functional variant retains the biological activity of the CAR of which it is a variant. Functional variants encompass, for example, those variants of the CAR described herein (the parent CAR) that retain the ability to recognize target cells to a similar extent, the same extent, or to a higher extent, as the parent CAR. In reference to the parent CAR, the functional variant can, for instance, be at least about 30%, about 50%, about 75%, about 80%, about 85%, about 90%, about 91 %, about 92%, about 93%, about 94%, about 95%, about 96%), about 97%, about 98%, about 99% or more identical in amino acid sequence to the parent CAR.

A functional variant can, for example, comprise the amino acid sequence of the parent CAR with at least one conservative amino acid substitution. Alternatively or additionally, the functional variants can comprise the amino acid sequence of the parent CAR with at least one non-conservative amino acid substitution. In this case, it is preferable for the non-conservative amino acid substitution to not interfere

with or inhibit the biological activity of the functional variant. The non-conservative amino acid substitution may enhance the biological activity of the functional variant, such that the biological activity of the functional variant is increased as compared to the parent CAR.

The CARs (including functional portions and functional variants) can be of any length, i.e., can comprise any number of amino acids, provided that the CARs (or functional portions or functional variants thereof) retain their biological activity, e.g., the ability to specifically bind to antigen, detect diseased cells in a mammal, or treat or prevent disease in a mammal, etc. For example, the CAR can be about 50 to about 5000 amino acids long, such as 50, 70, 75, 100, 125, 150, 175, 200, 300, 400, 500, 600, 700, 800, 900, 1000 or more amino acids in length.

The CARs (including functional portions and functional variants of the invention) can comprise synthetic amino acids in place of one or more naturally-occurring amino acids. Such synthetic amino acids are known in the art, and include, for example, aminocyclohexane carboxylic acid, norleucine, a-amino n-decanoic acid, homoserine, S-acetylaminomethyl-cysteine, trans-3- and trans-4-hydroxyproline, 4- aminophenylalanine, 4- nitrophenylalanine, 4-chlorophenylalanine, 4-carboxyphenylalanine, β-phenylserine β-hydroxyphenylalanine, phenylglycine, α -naphthylalanine, cyclohexylalanine, cyclohexylglycine, indoline-2-carboxylic acid, 1 ,2,3,4- tetrahydroisoquinoline-3-carboxylic acid, aminomalonic acid, aminomalonic acid monoamide, N'-benzyl-N'-methyl-lysine, N',N'-dibenzyl-lysine, 6-hydroxylysine, ornithine, α-aminocyclopentane carboxylic acid, α-aminocyclohexane carboxylic acid, oc- aminocycloheptane carboxylic acid, -(2-amino-2-norbornane)-carboxylic acid, γ-diaminobutyric acid, α,β-diaminopropionic acid, homophenylalanine, and α-tert-butylglycine.

The CARs (including functional portions and functional variants) can be glycosylated, amidated, carboxylated, phosphorylated, esterified, N-acylated, cyclized via, e.g., a disulfide bridge, or converted into an acid addition salt and/or optionally dimerized or polymerized, or conjugated.

Methods of generating chimeric antigen receptors, T cells including such receptors, and their use (e.g., for treatment of cancer) are known in the art and further described herein (see, e.g., Brentjens et al., 2010, Molecular Therapy, 18:4, 666-668; Morgan et al., 2010, Molecular Therapy, published online February 23, 2010, pages 1 -9; Till et al., 2008, Blood, 1 12:2261 -2271; Park *et al.*, *Trends Biotechnol.*, 29:550-557, 2011; Grupp et al., N Engl J Med., 368:1509-1518, 2013; Han et al., J. Hematol Oncol., 6:47, 2013; PCT Pub. WO2012/079000, WO2013/126726; and U.S. Pub. 2012/0213783, each of which is incorporated by reference herein in its entirety.) For example, a nucleic acid molecule encoding a disclosed chimeric antigen binding receptor can be included in an expression vector (such as a lentiviral vector) for expression in a host cell, such as a T cell, to make the disclosed CAR. In some embodiments, methods of using the chimeric antigen receptor include isolating T cells from a subject, transforming the T cells with an expression vector (such as a lentiviral vector) encoding the chimeric antigen receptor, and administering the engineered T cells expressing the chimeric antigen receptor to the subject for treatment, for example for treatment of a tumor in the subject.

D. Polynucleotides and Expression

Nucleic acids molecules (for example, cDNA molecules) encoding the amino acid sequences of antibodies, antibody binding fragments, and conjugates that specifically bind HIV-1 Env are provided. Nucleic acids encoding these molecules can readily be produced by one of skill in the art, using the amino acid sequences provided herein (such as the CDR sequences and V_H and V_L sequences), sequences available in the art (such as framework or constant region sequences), and the genetic code. In several embodiments, a nucleic acid molecules can encode the V_H, the V_L, or both the V_H and V_L (for example in a bicistronic expression vector) of a disclosed antibody or antigen binding fragment. In several embodiments, the nucleic acid molecules can be expressed in a host cell (such as a mammalian cell) to produce a disclosed antibody or antigen binding fragment.

Nucleic acid sequences encoding the of antibodies, antibody binding fragments, conjugates, and CARs that specifically bind HIV-1 Env can be prepared by any suitable method including, for example, cloning of appropriate sequences or by direct chemical synthesis by methods such as the phosphotriester method of Narang *et al., Meth. Enzymol.* 68:90-99, 1979; the phosphodiester method of Brown *et al.*, *Meth. Enzymol.* 68:109-151, 1979; the diethylphosphoramidite method of Beaucage *et al., Tetra. Lett.* 22:1859-1862, 1981; the solid phase phosphoramidite triester method described by Beaucage & Caruthers, *Tetra. Letts.* 22(20):1859-1862, 1981, for example, using an automated synthesizer as described in, for example, Needham-VanDevanter *et al.*, *Nucl. Acids Res.* 12:6159-6168, 1984; and, the solid support method of U.S. Patent No. 4,458,066. Chemical synthesis produces a single stranded oligonucleotide. This can be converted into double stranded DNA by hybridization with a complementary sequence or by polymerization with a DNA polymerase using the single strand as a template. One of skill would recognize that while chemical synthesis of DNA is generally limited to sequences of about 100 bases, longer sequences may be obtained by the ligation of shorter sequences.

Exemplary nucleic acids can be prepared by cloning techniques. Examples of appropriate cloning and sequencing techniques, and instructions sufficient to direct persons of skill through many cloning exercises are known (see, e.g, Sambrook *et al.* (Molecular Cloning: A Laboratory Manual, 4th ed, Cold Spring Harbor, New York, 2012) and Ausubel *et al.* (In Current Protocols in Molecular Biology, John Wiley & Sons, New York, through supplement 104, 2013). Product information from manufacturers of biological reagents and experimental equipment also provide useful information. Such manufacturers include the SIGMA Chemical Company (Saint Louis, MO), R&D Systems (Minneapolis, MN), Pharmacia Amersham (Piscataway, NJ), CLONTECH Laboratories, Inc. (Palo Alto, CA), Chem Genes Corp., Aldrich Chemical Company (Milwaukee, WI), Glen Research, Inc., GIBCO BRL Life Technologies, Inc. (Gaithersburg, MD), Fluka Chemica-Biochemika Analytika (Fluka Chemie AG, Buchs, Switzerland), Invitrogen (Carlsbad, CA), and Applied Biosystems (Foster City, CA), as well as many other commercial sources known to one of skill.

Nucleic acids can also be prepared by amplification methods. Amplification methods include polymerase chain reaction (PCR), the ligase chain reaction (LCR), the transcription-based amplification

system (TAS), the self-sustained sequence replication system (3SR). A wide variety of cloning methods, host cells, and *in vitro* amplification methodologies are well known to persons of skill.

In some embodiments, the nucleic acid molecule encodes a CAR as provided herein for expression in a T cell to generate a chimeric antigen receptor T cell. The nucleic acid molecule encoding the chimeric antigen binding receptor can be included in a vector (such as a lentiviral vector) for expression in a host cell, such as a T cell. Exemplary cells include a T cell, a Natural Killer (NK) cell, a cytotoxic T lymphocyte (CTL), and a regulatory T cell. Methods of generating nucleic acid molecules encoding chimeric antigen receptors and T cells including such receptors are known in the art (see, e.g., Brentjens et al., 2010, Molecular Therapy, 18:4, 666-668; Morgan et al., 2010, Molecular Therapy, published online February 23, 2010, pages 1 -9; Till et al., 2008, Blood, 1 12:2261 -2271; Park *et al.*, *Trends Biotechnol.*, 29:550-557, 2011; Grupp et al., N Engl J Med., 368:1509-1518, 2013; Han et al., J. Hematol Oncol., 6:47, 2013; PCT Pub. WO2012/079000, WO2013/126726; and U.S. Pub. 2012/0213783, each of which is incorporated by reference herein in its entirety.)

The nucleic acid molecules can be expressed in a recombinantly engineered cell such as bacteria, plant, yeast, insect and mammalian cells. The antibodies, antigen binding fragments, and conjugates can be expressed as individual V_H and/or V_L chain (linked to an effector molecule or detectable marker as needed), or can be expressed as a fusion protein. Methods of expressing and purifying antibodies and antigen binding fragments are known and further described herein (see, e.g., Al-Rubeai (ed), *Antibody Expression and Production*, Springer Press, 2011). An immunoadhesin can also be expressed. Thus, in some examples, nucleic acids encoding a V_H and V_L, and immunoadhesin are provided. The nucleic acid sequences can optionally encode a leader sequence.

To create a scFv the V_H- and V_L-encoding DNA fragments can be operatively linked to another fragment encoding a flexible linker, e.g., encoding the amino acid sequence $(Gly_4\text{-}Ser)_3$, such that the V_H and V_L sequences can be expressed as a contiguous single-chain protein, with the V_L and V_H domains joined by the flexible linker (see, *e.g.,* Bird *et al., Science* 242:423-426, 1988; Huston *et al., Proc. Natl. Acad. Sci. USA* 85:5879-5883, 1988; McCafferty *et al., Nature* 348:552-554, 1990; Kontermann and Dubel (Ed), Antibody Engineering, Vols. 1-2, 2nd Ed., Springer Press, 2010; Harlow and Lane, *Antibodies: A Laboratory Manual*, 2nd, Cold Spring Harbor Laboratory, New York, 2013,). Optionally, a cleavage site can be included in a linker, such as a furin cleavage site.

The nucleic acid encoding a V_H and/or the V_L optionally can encode an Fc domain (immunoadhesin). The Fc domain can be an IgA, IgM or IgG Fc domain. The Fc domain can be an optimized Fc domain, as described in U.S. Published Patent Application No. 20100/093979, incorporated herein by reference. In one example, the immunoadhesin is an IgG_1 Fc.

The single chain antibody may be monovalent, if only a single V_H and V_L are used, bivalent, if two V_H and V_L are used, or polyvalent, if more than two V_H and V_L are used. Bispecific or polyvalent antibodies may be generated that bind specifically to HIV-1 Env and another antigen, such as, but not limited to CD3. The encoded V_H and V_L optionally can include a furin cleavage site between the V_H and V_L domains.

Those of skill in the art are knowledgeable in the numerous expression systems available for expression of proteins including *E. coli*, other bacterial hosts, yeast, and various higher eukaryotic cells such as the COS, CHO, HeLa and myeloma cell lines.

One or more DNA sequences encoding the antibodies, antibody binding fragments, conjugates, and CARs can be expressed *in vitro* by DNA transfer into a suitable host cell. The cell may be prokaryotic or eukaryotic. The term also includes any progeny of the subject host cell. It is understood that all progeny may not be identical to the parental cell since there may be mutations that occur during replication. Methods of stable transfer, meaning that the foreign DNA is continuously maintained in the host, are known in the art. Hybridomas expressing the antibodies of interest are also encompassed by this disclosure.

The expression of nucleic acids encoding the antibodies or antigen binding fragments described herein can be achieved by operably linking the DNA or cDNA to a promoter (which is either constitutive or inducible), followed by incorporation into an expression cassette. The promoter can be any promoter of interest, including a cytomegalovirus promoter and a human T cell lymphotrophic virus promoter (HTLV)-1. Optionally, an enhancer, such as a cytomegalovirus enhancer, is included in the construct. The cassettes can be suitable for replication and integration in either prokaryotes or eukaryotes. Typical expression cassettes contain specific sequences useful for regulation of the expression of the DNA encoding the protein. For example, the expression cassettes can include appropriate promoters, enhancers, transcription and translation terminators, initiation sequences, a start codon (*i.e.*, ATG) in front of a protein-encoding gene, splicing signal for introns, sequences for the maintenance of the correct reading frame of that gene to permit proper translation of mRNA, and stop codons. The vector can encode a selectable marker, such as a marker encoding drug resistance (for example, ampicillin or tetracycline resistance).

To obtain high level expression of a cloned gene, it is desirable to construct expression cassettes which contain, at the minimum, a strong promoter to direct transcription, a ribosome binding site for translational initiation (internal ribosomal binding sequences), and a transcription/translation terminator. For *E. coli*, this includes a promoter such as the T7, trp, lac, or lambda promoters, a ribosome binding site, and preferably a transcription termination signal. For eukaryotic cells, the control sequences can include a promoter and/or an enhancer derived from, for example, an immunoglobulin gene, HTLV, SV40 or cytomegalovirus, and a polyadenylation sequence, and can further include splice donor and/or acceptor sequences (for example, CMV and/or HTLV splice acceptor and donor sequences). The cassettes can be transferred into the chosen host cell by well-known methods such as transformation or electroporation for *E. coli* and calcium phosphate treatment, electroporation or lipofection for mammalian cells. Cells transformed by the cassettes can be selected by resistance to antibiotics conferred by genes contained in the cassettes, such as the amp, gpt, neo and hyg genes.

The polynucleotide sequences encoding the antibody, or antigen binding fragment or conjugate thereof can be inserted into an expression vector including, but not limited to a plasmid, virus or other vehicle that can be manipulated to allow insertion or incorporation of sequences and can be expressed in

either prokaryotes or eukaryotes. Hosts can include microbial, yeast, insect and mammalian organisms. Methods of expressing DNA sequences having eukaryotic or viral sequences in prokaryotes are well known in the art. Biologically functional viral and plasmid DNA vectors capable of expression and replication in a host are known in the art.

When the host is a eukaryote, such methods of transfection of DNA as calcium phosphate coprecipitates, conventional mechanical procedures such as microinjection, electroporation, insertion of a plasmid encased in liposomes, or virus vectors may be used. Eukaryotic cells can also be cotransformed with polynucleotide sequences encoding the antibody, labeled antibody, or HIV-1 Env binding fragment thereof, and a second foreign DNA molecule encoding a selectable phenotype, such as the herpes simplex thymidine kinase gene. Another method is to use a eukaryotic viral vector, such as simian virus 40 (SV40) or bovine papilloma virus, to transiently infect or transform eukaryotic cells and express the protein (see for example, Viral Expression Vectors, Springer press, Muzyczka ed., 2011). One of skill in the art can readily use an expression systems such as plasmids and vectors of use in producing proteins in cells including higher eukaryotic cells such as the COS, CHO, HeLa and myeloma cell lines.

For purposes of producing a recombinant CAR, the host cell may be a mammalian cell. The host cell may be a human cell. In some embodiments, the host cell may be a peripheral blood lymphocyte (PBL) or a peripheral blood mononuclear cell (PBMC), or a T cell. The T cell can be any T cell, such as a cultured T cell, e.g., a primary T cell, or a T cell from a cultured T cell line, e.g., Jurkat, SupTl, etc., or a T cell obtained from a mammal. If obtained from a mammal, the T cell can be obtained from numerous sources, including but not limited to blood, bone marrow, lymph node, the thymus, or other tissues or fluids. T cells can also be enriched for or purified. The T cell may be a human T cell. The T cell may be a T cell isolated from a human. The T cell can be any type of T cell and can be of any developmental stage, including but not limited to, $CD4^+/CD8^+$ double positive T cells, $CD4^+$ helper T cells, e.g., Th_1 and Th_2 cells, $CD8^+$ T cells (e.g., cytotoxic T cells), tumor infiltrating cells, memory T cells, naive T cells, and the like. The T cell may be a $CD8^+$ T cell or a $CD4^+$ T cell.

Also provided is a population of cells comprising at least one host cell described herein. The population of cells can be a heterogeneous population comprising the host cell comprising any of the recombinant expression vectors described, in addition to at least one other cell, e.g., a host cell (e.g., a T cell), which does not comprise any of the recombinant expression vectors, or a cell other than a T cell, e.g., a B cell, a macrophage, a neutrophil, an erythrocyte, a hepatocyte, an endothelial cell, an epithelial cell, a muscle cell, a brain cell, etc. Alternatively, the population of cells can be a substantially homogeneous population, in which the population comprises mainly host cells (e.g., consisting essentially of) comprising the recombinant expression vector. The population also can be a clonal population of cells, in which all cells of the population are clones of a single host cell comprising a recombinant expression vector, such that all cells of the population comprise the recombinant expression vector. In one embodiment of the invention, the population of cells is a clonal population comprising host cells comprising a recombinant expression vector as described herein

Modifications can be made to a nucleic acid encoding a polypeptide described herein without diminishing its biological activity. Some modifications can be made to facilitate the cloning, expression, or incorporation of the targeting molecule into a fusion protein. Such modifications are well known to those of skill in the art and include, for example, termination codons, a methionine added at the amino terminus to provide an initiation, site, additional amino acids placed on either terminus to create conveniently located restriction sites, or additional amino acids (such as poly His) to aid in purification steps. In addition to recombinant methods, the immunoconjugates, effector moieties, and antibodies of the present disclosure can also be constructed in whole or in part using standard peptide synthesis well known in the art.

Once expressed, the antibodies, antigen binding fragments, and conjugates can be purified according to standard procedures in the art, including ammonium sulfate precipitation, affinity columns, column chromatography, and the like (see, generally, Simpson ed., Basic methods in Protein Purification and Analysis: A laboratory Manual, Cold Harbor Press, 2008). The antibodies, antigen binding fragment, and conjugates need not be 100% pure. Once purified, partially or to homogeneity as desired, if to be used therapeutically, the polypeptides should be substantially free of endotoxin.

Methods for expression of the antibodies, antigen binding fragments, and conjugates, and/or refolding to an appropriate active form, from mammalian cells, and bacteria such as *E. coli* have been described and are well-known and are applicable to the antibodies disclosed herein. See, e.g., Harlow and Lane, *Antibodies: A Laboratory Manual*, 2nd, Cold Spring Harbor Laboratory, New York, 2013, Simpson ed., Basic methods in Protein Purification and Analysis: A laboratory Manual, Cold Harbor Press, 2008, and Ward *et al.*, *Nature* 341:544, 1989.

Often, functional heterologous proteins from *E. coli* or other bacteria are isolated from inclusion bodies and require solubilization using strong denaturants, and subsequent refolding. During the solubilization step, as is well known in the art, a reducing agent must be present to separate disulfide bonds. An exemplary buffer with a reducing agent is: 0.1 M Tris pH 8, 6 M guanidine, 2 mM EDTA, 0.3 M DTE (dithioerythritol). Reoxidation of the disulfide bonds can occur in the presence of low molecular weight thiol reagents in reduced and oxidized form, as described in Saxena *et al.*, *Biochemistry* 9: 5015-5021, 1970.

In addition to recombinant methods, the antibodies, antigen binding fragments, and/or conjugates can also be constructed in whole or in part using standard peptide synthesis. Solid phase synthesis of the polypeptides can be accomplished by attaching the C-terminal amino acid of the sequence to an insoluble support followed by sequential addition of the remaining amino acids in the sequence. Techniques for solid phase synthesis are described by Barany & Merrifield, *The Peptides: Analysis, Synthesis, Biology. Vol. 2: Special Methods in Peptide Synthesis, Part A.* pp. 3-284; Merrifield *et al.*, *J. Am. Chem. Soc.* 85:2149-2156, 1963, and Stewart *et al., Solid Phase Peptide Synthesis, 2nd ed.*, Pierce Chem. Co., Rockford, Ill., 1984. Proteins of greater length may be synthesized by condensation of the amino and carboxyl termini of shorter fragments. Methods of forming peptide bonds by activation of a carboxyl

terminal end (such as by the use of the coupling reagent N, N'-dicylohexylcarbodimide) are well known in the art

E. Methods and Composition

1. Therapeutic methods

Methods are disclosed herein for the prevention or treatment of an HIV infection, such as an HIV-1 infection. Prevention can include inhibition of infection with HIV-1. The methods include contacting a cell with a therapeutically effective amount of a disclosed antibody, antigen binding fragment, conjugate, CAR or T cell expressing a CAR that specifically binds HIV-1 Env, or a nucleic acid encoding such an antibody, antigen binding fragment, conjugate, or CAR. The method can also include administering to a subject a therapeutically effective amount of a disclosed antibody, antigen binding fragment, conjugate, CAR or T cell expressing a CAR that specifically binds HIV-1 Env, or a nucleic acid encoding such an antibody, antigen binding fragment, conjugate, or CAR, to a subject. In some examples, the antibody, antigen binding fragment, conjugate, CAR, T cell expressing a CAR, or nucleic acid molecule, can be used pre-exposure (for example, to prevent or inhibit HIV infection). In some examples, the antibody, antigen binding fragment, conjugate, CAR, T cell expressing a CAR, or nucleic acid molecule, can be used in post-exposure prophylaxis. In some examples, the antibody, antigen binding fragment, conjugate, or nucleic acid molecule, can be used to eliminate or reduce the viral reservoir of HIV-1 in a subject. For example a therapeutically effective amount of an antibody, antigen binding fragment, conjugate, CAR, T cell expressing a CAR, or nucleic acid molecule, can be administered to a subject with HIV-1, such as a subject being treated with anti-viral therapy. In some examples the antibody, antigen binding fragment, conjugate, or nucleic acid molecule is modified such that it is directly cytotoxic to infected cells (e.g., by conjugation to a toxin), or uses natural defenses such as complement, antibody dependent cellular cytotoxicity (ADCC), or phagocytosis by macrophages.

HIV infection does not need to be completely eliminated for the method to be effective. For example, a method can decrease HIV infection by a desired amount, for example by at least 10%, at least 20%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, or even at least 100% (elimination of detectable HIV infected cells), as compared to HIV infection in the absence of the treatment. In some embodiments, the cell is also contacted with a therapeutically effective amount of an additional agent, such as anti-viral agent. The cell can be *in vivo* or *in vitro*. The methods can include administration of one on more additional agents known in the art. In additional embodiments, HIV replication can be reduced or inhibited by similar methods. HIV replication does not need to be completely eliminated for the method to be effective. For example, a method can decrease HIV replication by a desired amount, for example by at least 10%, at least 20%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, or even at least 100% (elimination of detectable HIV), as compared to HIV replication in the absence of the treatment.

Methods to assay for neutralization activity include, but are not limited to, a single-cycle infection assay as described in Martin *et al.* (2003) *Nature Biotechnology* 21:71-76. In this assay, the

level of viral activity is measured via a selectable marker whose activity is reflective of the amount of viable virus in the sample, and the IC_{50} is determined. In other assays, acute infection can be monitored in the PM1 cell line or in primary cells (normal PBMC). In this assay, the level of viral activity can be monitored by determining the p24 concentrations using ELISA. See, for example, Martin *et al.* (2003) *Nature Biotechnology* 21:71-76.

In one embodiment, administration of a disclosed antibody, antigen binding fragment, conjugate, CAR, T cell expressing a CAR, or nucleic acid molecule, results in a reduction in the establishment of HIV infection and/or reducing subsequent HIV disease progression in a subject. A reduction in the establishment of HIV infection and/or a reduction in subsequent HIV disease progression encompass any statistically significant reduction in HIV activity. In some embodiments, methods are disclosed for treating a subject with an HIV-1 infection. These methods include administering to the subject a therapeutically effective amount of a disclosed antibody, antigen binding fragment, conjugate, CAR, T cell expressing a CAR, or nucleic acid molecule, thereby preventing or treating the HIV-1 infection.

Studies have shown that the rate of HIV transmission from mother to infant is reduced significantly when zidovudine is administered to HIV-infected women during pregnancy and delivery and to the offspring after birth (Connor *et al.*, 1994 *Pediatr Infect Dis J* 14: 536-541). Several studies of mother-to-infant transmission of HIV have demonstrated a correlation between the maternal virus load at delivery and risk of HIV transmission to the child. The present disclosure provides antibodies, antigen binding fragments, conjugates, CAR, T cell expressing a CAR, and nucleic acid molecule that are of use in decreasing HIV-transmission from mother to infant. Thus, in some examples, a therapeutically effective amount of a HIV-1 Env-specific antibody or antigen binding fragment thereof or nucleic acid encoding such antibodies or antibody antigen binding fragments, is administered in order to prevent transmission of HIV, or decrease the risk of transmission of HIV, from a mother to an infant. In some examples, a therapeutically effective amount of the antibody, or an antibody binding fragment or nucleic acid encoding such antibodies or antigen binding fragment, is administered to mother and/or to the child at childbirth. In other examples, a therapeutically effective amount of the antibody, antigen binding fragment, or nucleic acid encoding the antibody or antigen binding fragment is administered to the mother and/or infant prior to breast feeding in order to prevent viral transmission to the infant or decrease the risk of viral transmission to the infant. In some embodiments, both a therapeutically effective amount of the antibody, antigen binding fragment, or nucleic acid encoding the antibody or antigen binding fragment and a therapeutically effective amount of another agent, such as zidovudine, is administered to the mother and/or infant.

For any application, the antibody, antigen binding fragment, conjugate, CAR, T cell expressing a CAR, or nucleic acid molecule can be combined with anti-retroviral therapy. Antiretroviral drugs are broadly classified by the phase of the retrovirus life-cycle that the drug inhibits. The disclosed antibodies can be administered in conjunction with nucleoside analog reverse-transcriptase inhibitors (such as zidovudine, didanosine, zalcitabine, stavudine, lamivudine, abacavir, emtricitabine, entecavir, and apricitabine), nucleotide reverse transcriptase inhibitors (such as tenofovir and adefovir), non-nucleoside

reverse transcriptase inhibitors (such as efavirenz, nevirapine, delavirdine, etravirine, and rilpivirine), protease inhibitors (such as saquinavir, ritonavir, indinavir, nelfinavir, amprenavir, lopinavir, fosamprenavir, atazanavir, tipranavir, and darunavir), entry or fusion inhibitors (such as maraviroc and enfuvirtide), maturation inhibitors, (such as bevirimat and vivecon), or a broad spectrum inhibitors, such as natural antivirals. In some examples, a disclosed antibody or active fragment thereof or nucleic acids encoding such is administered in conjunction with IL-15, or conjugated to IL-15.

Studies in have shown that cocktails of HIV-1 neutralizing antibodies that target different epitopes of HIV-1 Env can treat macaques chronically infected with SHIV (Shingai et al., Nature, 503, 277-280, 2013; and Barouch et al., Nature, 503, 224-228, 2013). Accordingly, in some examples, a subject is further administered one or more additional antibodies that bind HIV Env (e.g, that bind to gp120 or gp41), and that can neutralize NIH-1 infection. The additional antibodies can be administrated before, during, or after administration of the novel antibodies disclosed herein (e.g., the 35O22 antibody). In some embodiments, the additional antibody can be an antibody that specifically binds to an epitope on HIV Env such as the CD4 binding site (e.g., b12, 3BNC117, VRC01 or VRC07 antibody), the membrane-proximal external region (e.g., 10E8 antibody), the V1/V2 domain (e.g., PG9 antibody), or the V3 loop (e.g., 10-1074 or PGT128 antibody). Antibodies that specifically bind to these regions and neutralizing HIV-1 infection are known to the person of ordinary skill in the art. Non-limiting examples can be found, for example, in PCT Pub. No. WO 2011/038290, WO/2013/086533, WO/2013/090644, WO/2012/158948, which are incorporated herein by reference in their entirety.

In some embodiments, a subject is administered a nucleic acid molecule encoding a disclosed antibody or antigen binding fragment, to provide *in vivo* production of a therapeutically effective amount of the antibody or antigen binding fragment, for example using the cellular machinery of the subject. Methods of administration of nucleic acid constructs to express a polypeptide from the construct in a subject are well known in the art and taught, for example, in U.S. Patent No. 5,643,578, and U.S. Patent No. 5,593,972 and U.S. Patent No. 5,817,637. U.S. Patent No. 5,880,103 describes several methods of delivery of nucleic acids encoding to an organism. The methods can include liposomal delivery of the nucleic acids. Such methods can be applied to the production of an antibody, or antibody binding fragments thereof, by one of ordinary skill in the art.

2. Dosages

A therapeutically effective amount of a HIV-1 Env-specific antibody, antigen binding fragment, conjugate, CAR, T cell expressing a CAR, or nucleic acid molecule encoding such molecules, will depend upon the severity of the disease and/or infection and the general state of the patient's health. A therapeutically effective amount is that which provides either subjective relief of a symptom(s) or an objectively identifiable improvement as noted by the clinician or other qualified observer. The HIV-1 Env-specific antibody, antigen binding fragment, conjugate, CAR, T cell expressing a CAR, or nucleic acid molecule encoding such molecules, can be administered in conjunction with another therapeutic agent, either simultaneously or sequentially.

Single or multiple administrations of a composition including a disclosed HIV-1 Env-specific antibody, antigen binding fragment, conjugate, CAR, T cell expressing a CAR, or nucleic acid molecule encoding such molecules, can be administered depending on the dosage and frequency as required and tolerated by the patient. Compositions including the HIV-1 Env-specific antibody, antigen binding fragment, conjugate, CAR, T cell expressing a CAR, or nucleic acid molecule encoding such molecules, should provide a sufficient quantity of at least one of the HIV-1 Env-specific antibody, antigen binding fragment, conjugate, CAR, T cell expressing a CAR, or nucleic acid molecule encoding such molecules to effectively treat the patient. The dosage can be administered once, but may be applied periodically until either a therapeutic result is achieved or until side effects warrant discontinuation of therapy. In one example, a dose of the antibody or antigen binding fragment is infused for thirty minutes every other day. In this example, about one to about ten doses can be administered, such as three or six doses can be administered every other day. In a further example, a continuous infusion is administered for about five to about ten days. The subject can be treated at regular intervals, such as monthly, until a desired therapeutic result is achieved. Generally, the dose is sufficient to treat or ameliorate symptoms or signs of disease without producing unacceptable toxicity to the patient.

Data obtained from cell culture assays and animal studies can be used to formulate a range of dosage for use in humans. The dosage normally lies within a range of circulating concentrations that include the ED_{50}, with little or minimal toxicity. The dosage can vary within this range depending upon the dosage form employed and the route of administration utilized. The therapeutically effective dose can be determined from cell culture assays and animal studies.

In certain embodiments, the antibody or antigen binding fragment that specifically binds HIV-1 Env, or conjugate thereof, or a nucleic acid molecule or vector encoding such a molecule, can be administered at a dose in the range of from about 0.001 to about 1000 mg/kg, such as from about 1 to about 100 mg/kg, such as about 5-50 mg/kg, about 25-75 mg/kg, or about 40-60 mg/kg. In some embodiments, the dosage can be administered at about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 150, 200, or 300 mg/kg, or other dose deemed appropriate by the treating physician. Further, the doses described herein can be administered according to the dosing frequency or frequency of administration described herein, including without limitation daily, every other day, 2 or 3 times per week, weekly, every 2 weeks, every 3 weeks, monthly, etc. Additional treatments, including additional courses of therapy with a disclosed agent can be performed as needed.

In methods involving administration of a nucleic acid molecule encoding a disclosed antibody, antigen binding fragment or conjugate, typically, the DNA is injected into muscle, although it can also be injected directly into other sites. Dosages for injection are usually around 0.5 µg/kg to about 50 mg/kg, and typically are about 0.005 mg/kg to about 5 mg/kg (see, *e.g.*, U.S. Patent No. 5,589,466).

3. Modes of Administration

An HIV-1 Env-specific antibody, antigen binding fragment, conjugate, CAR, T cell expressing a CAR, or nucleic acid molecule encoding such molecules, or a composition including such molecules, as

well as additional agents, can be administered to subjects in various ways, including local and systemic administration, such as, e.g., by injection subcutaneously, intravenously, intra-arterially, intraperitoneally, intramuscularly, intradermally, or intrathecally. In an embodiment, a therapeutic agent is administered by a single subcutaneous, intravenous, intra-arterial, intraperitoneal, intramuscular, intradermal or intrathecal injection once a day. The therapeutic agent can also be administered by direct injection at or near the site of disease.

The therapeutic agent may also be administered orally in the form of microspheres, microcapsules, liposomes (uncharged or charged (e.g., cationic)), polymeric microparticles (e.g., polyamides, polylactide, polyglycolide, poly(lactide-glycolide)), microemulsions, and the like.

A further method of administration is by osmotic pump (e.g., an Alzet pump) or mini-pump (e.g., an Alzet mini-osmotic pump), which allows for controlled, continuous and/or slow-release delivery of the therapeutic agent or pharmaceutical composition over a pre-determined period. The osmotic pump or mini-pump can be implanted subcutaneously, or near a target site.

It will be apparent to one skilled in the art that the therapeutic agent or compositions thereof can also be administered by other modes. Determination of the most effective mode of administration of the therapeutic agent or compositions thereof is within the skill of the skilled artisan. The therapeutic agent can be administered as pharmaceutical formulations suitable for, e.g., oral (including buccal and sub-lingual), rectal, nasal, topical, pulmonary, vaginal or parenteral (including intramuscular, intraarterial, intrathecal, subcutaneous and intravenous) administration, or in a form suitable for administration by inhalation or insufflation. Depending on the intended mode of administration, the pharmaceutical formulations can be in the form of solid, semi-solid or liquid dosage forms, such as tablets, suppositories, pills, capsules, powders, liquids, suspensions, emulsions, creams, ointments, lotions, and the like. The formulations can be provided in unit dosage form suitable for single administration of a precise dosage. The formulations comprise an effective amount of a therapeutic agent, and one or more pharmaceutically acceptable excipients, carriers and/or diluents, and optionally one or more other biologically active agents.

One approach to administration of nucleic acids is direct administration with plasmid DNA, such as with a mammalian expression plasmid. The nucleotide sequence encoding the disclosed antibody, or antibody binding fragments thereof, can be placed under the control of a promoter to increase expression.

In another approach to using nucleic acids, a disclosed antibody, or antibody binding fragments thereof can also be expressed by attenuated viral hosts or vectors or bacterial vectors. Recombinant vaccinia virus, adeno-associated virus (AAV), herpes virus, retrovirus, cytomegalovirus or other viral vectors can be used to express the antibody. For example, vaccinia vectors and methods useful protocols are described in U.S. Patent No. 4,722,848. BCG (Bacillus Calmette Guerin) provides another vector for expression of the disclosed antibodies (see Stover, *Nature* 351:456-460, 1991).

In one embodiment, a nucleic acid encoding a disclosed antibody, or antibody binding fragments thereof, is introduced directly into cells. For example, the nucleic acid can be loaded onto gold

microspheres by standard methods and introduced into the skin by a device such as Bio-Rad's HELIOS™ Gene Gun. The nucleic acids can be "naked," consisting of plasmids under control of a strong promoter.

4. Composition

Compositions are provided that include one or more of the HIV-1 Env-specific antibody, antigen binding fragment, conjugate, CAR, T cell expressing a CAR, or nucleic acid molecule encoding such molecules, that are disclosed herein in a carrier. The compositions are useful, for example, for example, for the treatment or detection of an HIV-1 infection. The compositions can be prepared in unit dosage forms for administration to a subject. The amount and timing of administration are at the discretion of the treating physician to achieve the desired purposes. The HIV-1 Env-specific antibody, antigen binding fragment, conjugate, CAR, T cell expressing a CAR, or nucleic acid molecule encoding such molecules can be formulated for systemic or local administration. In one example, the HIV-1 Env-specific antibody, antigen binding fragment, conjugate, CAR, T cell expressing a CAR, or nucleic acid molecule encoding such molecules, is formulated for parenteral administration, such as intravenous administration.

In some embodiments, the compositions comprise an antibody, antigen binding fragment, or conjugate thereof, in at least about 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% purity. In certain embodiments, the compositions contain less than about 10%, 5%, 4%, 3%, 2%, 1% or 0.5% of macromolecular contaminants, such as other mammalian (e.g., human) proteins.

The compositions for administration can include a solution of the HIV-1 Env-specific antibody, antigen binding fragment, conjugate, CAR, T cell expressing a CAR, or nucleic acid molecule encoding such molecules, dissolved in a pharmaceutically acceptable carrier, such as an aqueous carrier. A variety of aqueous carriers can be used, for example, buffered saline and the like. These solutions are sterile and generally free of undesirable matter. These compositions may be sterilized by conventional, well known sterilization techniques. The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions such as pH adjusting and buffering agents, toxicity adjusting agents and the like, for example, sodium acetate, sodium chloride, potassium chloride, calcium chloride, sodium lactate and the like. The concentration of antibody in these formulations can vary widely, and will be selected primarily based on fluid volumes, viscosities, body weight and the like in accordance with the particular mode of administration selected and the subject's needs.

A typical composition for intravenous administration includes about 0.01 to about 30 mg/kg of antibody or antigen binding fragment or conjugate per subject per day (or the corresponding dose of a conjugate including the antibody or antigen binding fragment). Actual methods for preparing administrable compositions will be known or apparent to those skilled in the art and are described in more detail in such publications as *Remington's Pharmaceutical Science, 19th ed.*, Mack Publishing Company, Easton, PA (1995). In some embodiments, the composition can be a liquid formulation including one or more antibodies, antigen binding fragments (such as an antibody or antigen binding fragment that specifically binds to HIV-1 Env), in a concentration range from about 0.1 mg/ml to about 20 mg/ml, or from about 0.5 mg/ml to about 20 mg/ml, or from about 1 mg/ml to about 20 mg/ml, or

from about 0.1 mg/ml to about 10 mg/ml, or from about 0.5 mg/ml to about 10 mg/ml, or from about 1 mg/ml to about 10 mg/ml.

Antibodies, or an antigen binding fragment thereof or a conjugate or a nucleic acid encoding such molecules, can be provided in lyophilized form and rehydrated with sterile water before administration, although they are also provided in sterile solutions of known concentration. The antibody solution, or an antigen binding fragment or a nucleic acid encoding such antibodies or antibody binding fragments, can then be added to an infusion bag containing 0.9% sodium chloride, USP, and typically administered at a dosage of from 0.5 to 15 mg/kg of body weight. Considerable experience is available in the art in the administration of antibody drugs, which have been marketed in the U.S. since the approval of RITUXAN® in 1997. Antibodies, antigen binding fragments, conjugates, or a nucleic acid encoding such molecules, can be administered by slow infusion, rather than in an intravenous push or bolus. In one example, a higher loading dose is administered, with subsequent, maintenance doses being administered at a lower level. For example, an initial loading dose of 4 mg/kg may be infused over a period of some 90 minutes, followed by weekly maintenance doses for 4-8 weeks of 2 mg/kg infused over a 30 minute period if the previous dose was well tolerated.

Controlled-release parenteral formulations can be made as implants, oily injections, or as particulate systems. For a broad overview of protein delivery systems see, Banga, A.J., *Therapeutic Peptides and Proteins: Formulation, Processing, and Delivery Systems*, Technomic Publishing Company, Inc., Lancaster, PA, (1995). Particulate systems include microspheres, microparticles, microcapsules, nanocapsules, nanospheres, and nanoparticles. Microcapsules contain the therapeutic protein, such as a cytotoxin or a drug, as a central core. In microspheres the therapeutic is dispersed throughout the particle. Particles, microspheres, and microcapsules smaller than about 1 µm are generally referred to as nanoparticles, nanospheres, and nanocapsules, respectively. Capillaries have a diameter of approximately 5 µm so that only nanoparticles are administered intravenously. Microparticles are typically around 100 µm in diameter and are administered subcutaneously or intramuscularly. See, for example, Kreuter, J., *Colloidal Drug Delivery Systems*, J. Kreuter, ed., Marcel Dekker, Inc., New York, NY, pp. 219-342 (1994); and Tice & Tabibi, *Treatise on Controlled Drug Delivery*, A. Kydonieus, ed., Marcel Dekker, Inc. New York, NY, pp. 315-339, (1992).

Polymers can be used for ion-controlled release of the antibody compositions disclosed herein. Various degradable and nondegradable polymeric matrices for use in controlled drug delivery are known in the art (Langer, *Accounts Chem. Res.* 26:537-542, 1993). For example, the block copolymer, polaxamer 407, exists as a viscous yet mobile liquid at low temperatures but forms a semisolid gel at body temperature. It has been shown to be an effective vehicle for formulation and sustained delivery of recombinant interleukin-2 and urease (Johnston *et al., Pharm. Res.* 9:425-434, 1992; and Pec *et al., J. Parent. Sci. Tech.* 44(2):58-65, 1990). Alternatively, hydroxyapatite has been used as a microcarrier for controlled release of proteins (Ijntema *et al., Int. J. Pharm.*112:215-224, 1994). In yet another aspect, liposomes are used for controlled release as well as drug targeting of the lipid-capsulated drug (Betageri *et al., Liposome Drug Delivery Systems*, Technomic Publishing Co., Inc., Lancaster, PA (1993)).

Numerous additional systems for controlled delivery of therapeutic proteins are known (see U.S. Patent No. 5,055,303; U.S. Patent No. 5,188,837; U.S. Patent No. 4,235,871; U.S. Patent No. 4,501,728; U.S. Patent No. 4,837,028; U.S. Patent No. 4,957,735; U.S. Patent No. 5,019,369; U.S. Patent No. 5,055,303; U.S. Patent No. 5,514,670; U.S. Patent No. 5,413,797; U.S. Patent No. 5,268,164; U.S. Patent No. 5,004,697; U.S. Patent No. 4,902,505; U.S. Patent No. 5,506,206; U.S. Patent No. 5,271,961; U.S. Patent No. 5,254,342 and U.S. Patent No. 5,534,496).

5. Methods of detection and diagnosis

Methods are also provided for the detection of the expression of HIV-1 Env *in vitro* or *in vivo*. In one example, expression of HIV-1 Env is detected in a biological sample, and can be used to detect HIV-1 infection as the presence of HIV-1 in a sample. The sample can be any sample, including, but not limited to, tissue from biopsies, autopsies and pathology specimens. Biological samples also include sections of tissues, for example, frozen sections taken for histological purposes. Biological samples further include body fluids, such as blood, serum, plasma, sputum, spinal fluid or urine. The method of detection can include contacting a cell or sample, or administering to a subject, an antibody or antigen binding fragment that specifically binds to HIV-1 Env, or conjugate there of (e.g. a conjugate including a detectable marker) under conditions sufficient to form an immune complex, and detecting the immune complex (e.g., by detecting a detectable marker conjugated to the antibody or antigen binding fragment.

In several embodiments, a method is provided for detecting AIDS and/or an HIV-1 infection in a subject. The disclosure provides a method for detecting HIV-1 in a biological sample, wherein the method includes contacting a biological sample from a subject with a disclosed antibody or antigen binding fragment under conditions sufficient for formation of an immune complex, and detecting the immune complex, to detect the HIV-1 Env in the biological sample. In one example, the detection of HIV-1 Env in the sample indicates that the subject has an HIV-1 infection. In another example, the detection of HIV-1 Env in the sample indicates that the subject has AIDS. In another example, detection of HIV-1 Env in the sample confirms a diagnosis of AIDS and/or an HIV-1 infection in the subject.

In some embodiments, the disclosed antibodies or antigen binding fragments thereof are used to test vaccines. For example to test if a vaccine composition including HIV-1 Env assumes a conformation including the 35O22 epitope. Thus provided herein is a method for testing a vaccine, wherein the method includes contacting a sample containing the vaccine, such as a HIV-1 Env immunogen, with a disclosed antibody or antigen binding fragment under conditions sufficient for formation of an immune complex, and detecting the immune complex, to detect the vaccine with an HIV-1 Env protein including the 35O22 epitope in the sample. In one example, the detection of the immune complex in the sample indicates that vaccine component, such as a HIV-1 Env immunogen assumes a conformation capable of binding the antibody or antigen binding fragment.

In one embodiment, the antibody or antigen binding fragment is directly labeled with a detectable marker. In another embodiment, the antibody that binds HIV-1 Env (the first antibody) is unlabeled and a second antibody or other molecule that can bind the antibody that binds the first antibody

is utilized for detection. As is well known to one of skill in the art, a second antibody is chosen that is able to specifically bind the specific species and class of the first antibody. For example, if the first antibody is a human IgG, then the secondary antibody may be an anti-human-IgG. Other molecules that can bind to antibodies include, without limitation, Protein A and Protein G, both of which are available commercially.

Suitable labels for the antibody, antigen binding fragment or secondary antibody are described above, and include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, magnetic agents and radioactive materials. Non-limiting examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, beta-galactosidase, or acetylcholinesterase. Non-limiting examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin. Non-limiting examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin. A non-limiting exemplary luminescent material is luminol; a non-limiting exemplary a magnetic agent is gadolinium, and non-limiting exemplary radioactive labels include ^{125}I, ^{131}I, ^{35}S or ^{3}H.

F. Kits

Kits are also provided. For example, kits for treating a subject with an HIV-1 infection, or for detecting HIV-1 Env in a sample or in a subject. The kits will typically include a disclosed HIV-1 Env-specific antibody, antigen binding fragment, conjugate, CAR, T cell expressing a CAR, or nucleic acid molecule encoding such molecules, or compositions including such molecules. More than one of the disclosed HIV-1 Env-specific antibody, antigen binding fragment, conjugate, CAR, T cell expressing a CAR, or nucleic acid molecule encoding such molecules, or compositions including such molecules can be included in the kit.

In one embodiment, the kit is a diagnostic kit and includes an immunoassay. Although the details of the immunoassays may vary with the particular format employed, the method of detecting HIV-1 Env in a biological sample generally includes the steps of contacting the biological sample with an antibody which specifically reacts, under conditions sufficient to form an immune complex, to HIV-1 Env. The antibody is allowed to specifically bind under immunologically reactive conditions to form an immune complex, and the presence of the immune complex (bound antibody) is detected directly or indirectly.

The kit can include a container and a label or package insert on or associated with the container. Suitable containers include, for example, bottles, vials, syringes, etc. The containers may be formed from a variety of materials such as glass or plastic. The container typically holds a composition including one or more of the disclosed antibodies, antigen binding fragments, conjugates, nucleic acid molecules, or compositions. In several embodiments the container may have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). A label or package insert indicates that the composition is used for treating the particular condition.

The label or package insert typically will further include instructions for use of the antibodies, antigen binding fragments, conjugates, nucleic acid molecules, or compositions included in the kit. The package insert typically includes instructions customarily included in commercial packages of therapeutic products that contain information about the indications, usage, dosage, administration, contraindications and/or warnings concerning the use of such therapeutic products. The instructional materials may be written, in an electronic form (such as a computer diskette or compact disk) or may be visual (such as video files). The kits may also include additional components to facilitate the particular application for which the kit is designed. Thus, for example, the kit may additionally contain means of detecting a label (such as enzyme substrates for enzymatic labels, filter sets to detect fluorescent labels, appropriate secondary labels such as a secondary antibody, or the like). The kits may additionally include buffers and other reagents routinely used for the practice of a particular method. Such kits and appropriate contents are well known to those of skill in the art.

EXAMPLES

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

Example 1

Broad and potent HIV-1 neutralization by a human antibody that binds the gp41-120 interface

This examples illustrates the isolation and characterization of the 35O22 antibody, and several somatic variants thereof. 35O22 antibody is a broad and extremely potent HIV-specific mAb, which binds novel HIV-1 envelope glycoprotein (Env) epitope. 35O22 neutralized 62% of 181 pseudoviruses with an IC_{50}<50 μg/ml. The median IC_{50} of neutralized viruses was 0.033 μg/ml, among the most potent thus far described. 35O22 did not bind monomeric forms of Env tested, but did bind the trimeric BG505 SOSIP.664. Mutagenesis and a reconstruction by negative-stain electron microscopy of the Fab in complex with trimer revealed it to bind a conserved epitope, which stretched across gp120 and gp41. The specificity of 35O22 represents a novel site of vulnerability on HIV Env, which serum analysis indicates to be commonly elicited by natural infection.

Induction of a potent neutralizing antibody response capable of recognizing highly diverse isolates of HIV-1 is among the very most important goals of HIV vaccine research. This represents a considerable challenge given the extraordinary antigenic variability of the Env surface glycoprotein. However, approximately 20% of the HIV-infected population does develop a humoral immune response capable of recognizing highly diverse strains. In the past several years improved patient cohorts, HIV-specific B cell isolation, and IgG cloning techniques have permitted extraordinary progress in isolation of broadly neutralizing monoclonal antibodies (bNabs) from these individuals. Thus far, these primarily fall into four categories based upon the position of their epitopes on the Env protein, a trimer of gp120 and gp41 heterodimers that is the target of neutralizing antibodies. These sites include the CD4-binding site on gp120 (of which VRC01 is an example), the glycan-containing regions of V1V2 on gp120 (of which

PG9 and PG16 are examples), the V3 region centered on the N332 glycan of gp120 (of which PGT121 is an example) and the membrane-proximal external region (MPER) on gp41 (of which 10E8 is an example). It remains unclear to what extent these four categories represent the prevalent and immunodominant sites of Env vulnerability through which broad neutralizing responses are mediated, or whether additional specificities exist.

This example describes the isolation of a broad and potently neutralizing HIV-specific mAb, 35O22, that binds a novel epitope. The neutralizing activity of 35O22 is highly complementary to the activities of other known bNabs. Mutagenesis, crystallography and EM were used to define the Env site targeted by 35O22. The results indicate that 35O22 neutralization occurs by a novel mode of trimer recognition along a conserved face on contiguous areas of gp41 and gp120.

To further understand the specificities that underlie broadly neutralizing antibody responses a technique to identify human mAbs of interest from peripheral blood B cells without prior knowledge of the target specificity was applied (Huang *et al., Nat Protoc* 8, 1907-1915, (2013)). IgG^+ B cells of a donor (N152), with broad and potent neutralizing serum and from whom recently described 10E8 antibody was cloned (Huang *et al., Nature* 491, 406-412, 2012), were sorted and expanded. The supernatants of B cell microcultures were screened for neutralizing activity and IgG genes from positive wells were cloned and re-expressed. In addition to the 10E8 antibody, 8 clonal family variants of an additional antibody with neutralization activity were found, among which the 35O22 antibody was the most potent and broad (FIG. 15). This antibody was derived from *IGHV-1-18*02-* and *IGLV-2-14*02* germline genes, and was highly somatically mutated in variable genes of both heavy chain (35%) and λ light chain (24%) compared to germline. The 35O22 antibody possessed a heavy-chain complementarity-determining 3 region (CDR H3) composed of 14 amino acids (FIG. 1, FIGs. 16A-16D) and an insertion of 8 amino acids in framework 3 (FR3). High levels of somatic mutation and FR3 insertions are features of other HIV-specific bNabs. Autoreactivity or polyreactivity are properties of several HIV-specific antibodies that could limit their use in therapies or prophylaxis. However, 35O22 bound Hep-2 epithelial cells only modestly (FIG. 5A) and did not bind a panel of autoantigens (FIGS. 5B and 5C). Against a large panel of pseudoviruses, 35O22 neutralized 62% of 181 isolates with an IC_{50}<50 μg/ml (FIG. 1, FIGS. 17A-17D). In numerous cases where the IC_{50} of 10E8 was >1 μg, that of 35O22 was 100 to 1000-fold lower (FIG. 15, FIGS. 17A-17D), indicating their activities were highly complementary. It is likely that 35O22-like antibodies account for much of the breadth and potency of the N152 patient serum against clades A and B (FIGS. 17A-17D), whereas 10E8-like antibodies may account for much of the breadth against clade C isolates. Overall, the median IC_{50} of 35O22 for sensitive viruses was 0.033 μg/ml, which is among the most potent thus far described (FIG. 1).

The neutralizing spectrum of 35O22 was then compared to those of other bNabs. The IC_{50} of 35O22 against a panel of diverse isolates did not correlate with those of the bNabs VRC01, 10E8, PG9, and PGT121 (FIG. 6A). In addition, a neutralization-based clustering analysis revealed that 35O22 clustered separately from other bNabs (FIG. 6B). Further, 35O22 did not compete with other known bNabs when bound to virus-like particles (VLPs) (FIG. 6C). Its neutralization of many pseudoviruses did

not exceed 80% even at high concentrations (FIG. 7A).and its potency increased when pseudoviruses were produced in the presence of the glycosidase inhibitors NB-DNJ or kifunensine, consistent with recognition of high mannose (FIG. 7B) (Doores and Burton, D. R., *J Virol* 84, 10510-10521, (2010)). However, neutralization was unaffected by mutation of N-linked glycosylation sites critical for binding of known bNabs (FIG. 8C). Taken together these data suggested that 35O22 binds glycans, but its specificity differed from all previously characterized bNabs.

Mutation of four predicted sites of *N*-linked glycosylation on HIV_{JRCSF} Env diminished neutralization potency: N88A, N230A, N241A, and N625A (FIG. 2A, FIGS. 18A and 18B). This result suggested that 35O22 recognized elements of both gp120 and gp41, a property that may be consistent with several very recently isolated antibodies (Scharf *et al., Cell reports*, (2014); Falkowska *et al., Immunity* 40, 657-668, (2014); Blattner *et al., Immunity* 40, 669-680, (2014); Zhang *et al., PLoS ONE* 7, e44241, (2012)). When mutations were introduced in the 5 residues on either side of these four sites, the V89A, T90A, K227A, T232A, and S243A mutations each diminished neutralization (FIGS. 18A and 18B). With the exception of V89A and K227A, it is likely the impact of each of these mutations was to disrupt the Asn-X-Ser/Thr glycotransferase sequon. There was no impact of T627A suggesting a glycan may not be present at 625 and 35O22 may make a protein contact at this position. Overall, similar results were obtained using replication competent HIV_{LAI} viruses (FIG. 19). Examination of the sequences of resistant pseudoviruses within clade C did not reveal a clear pattern of variation at each of the positions found to affect 35O22 neutralization or within the glycosylation sequon. It is therefore possible that the resistance of clade C viruses is mediated by other factors such as variations in glycosylation pattern or conformation.

35O22 did not bind to a panel of soluble recombinant Env proteins (FIGS. 9A-9C) suggesting these do not have the appropriate conformation or glycosylation for binding. However, the 35O22 antibody did bind a recently described stabilized, cleaved, soluble trimer, BG505 SOSIP.664 (FIG. 2B) (Sanders *et al., PLoS Pathog* 9, e1003618, (2013)), Despite a plateau in neutralization below 50% (FIG. 7A) and lacking glycans at positions 230 and 241, binding to BG505 SOSIP.664 trimer had numerous characteristics consistent with its activity against the HIV_{JRCSF} pseudovirus. 35O22 binding was increased to trimer produced in cells treated with kifunensine or cells deficient in glycan processing, and diminished by mutations at positions 88 and 625 (FIG. 2C). 35O22 did bind to BG505 SOSIP trimer lacking the furin cleavage site (BG505 SOSIP.SEKS). In surface plasmon resonance (SPR) experiments, 35O22 also bound to immobilized BG505 SOSIP.664 with high affinity (K_d=5.6nM) (FIG. 2D). Binding was markedly lower to the gp120-41_{ECTO} protomer and no binding was detected to the gp120 monomer (FIG. 2E). It bound the uncleaved BG505 SOSIP.SEKS but no binding was observed to the uncleaved form lacking the SOSIP mutations (FIG. 2F). These observations, combined with the lack of binding of 35O22 to all other soluble forms of Env tested, suggested this antibody requires a trimeric structure for binding its epitope on gp120 and gp41 (Ringe *et al., PNAS,* 110, 18256-18261, (2013)).

To provide an atomic-level understanding of the structure of the 35O22 antibody, the Fab of 35O22 was crystallized. Crystals were obtained that diffracted to 1.55Å resolution (FIG. 20). Overall the

structure of 35O22 Fab revealed a relatively flat antigen-combining site, lacking long protruding loops, and flanked by the complementarity-determining region 1 on the light chain (CDRL1) and the 8 amino acid insertion in FR3 of the heavy chain (FIG. 3A). The surface of the antigen combining site was heavily altered by somatic mutation and two pairs of cysteines introduced by somatic mutation in CDR L1 and L3 formed disulphide bonds (FIG. 3A, FIG. 10A).

Next, the structure of the antibody-antigen complex was determined. The ability of 35O22 to bind the BG505 SOSIP.664 trimer permitted imaging of the antibody-antigen complex by negative stain electron microscopy (EM). The reconstruction of these images showed that three 35O22 Fabs bound to the trimers at sites close to the predicted viral membrane (FIG. 3B, FIG. 10B). Superposition of the docked negative stain of the soluble BG505 SOSIP.664 with 35O22 Fab onto the BaL EM tomogram of the viral spike (FIG. 10B) suggested the viral membrane to be in close contact to the 35O22 Fab light chain. Tyr68 and Trp69 in the light chain and FR3 tyrosines at residues 65 and 72 form potential surfaces of membrane association. The 35O22 heavy chain was in close proximity to the four sites observed to contribute to the 35O22 epitope in mutagenesis experiments (N88, N230, N241 and N625). The CDR H3 was predicted to interact with N625 and CDRH2 with N88. The 8 amino acid insertion in the framework 3 heavy chain is located close to residues 88-90 on gp120. Reversion of this insertion to germline markedly diminished neutralization against most pseudoviruses in the panel (FIG. 21). 35O22 binds a surface on the Env spike that is distinct from two other antibodies, 8ANC195 and PGT151, reported to bind gp120 and gp14 (FIG. 11) (Scharf *et al., Cell reports*, (2014); Falkowska *et al., Immunity* 40, 657-668, (2014); Blattner *et al., Immunity* 40, 669-680, (2014)).

Analysis of the 35O22 site of vulnerability (FIGS. 12A-12C) indicated it was highly conserved. The glycans predicted at positions 88, 241, and 625 were found to be among the most highly conserved N-linked glycosylation sequons of 4265 HIV-1 sequences in the Los Alamos database (FIG. 22). Despite this high level of conservation, analysis of the Env gene of the patient's plasma virus showed that the predicted amino acid sequence varied at the critical 35O22 contacts. In addition to the previously published 10E8 escape mutations, an N230Q is predicted in one sequence, N241D in half of the sequences, and an N624D and N625Q in all sequences (FIGs. 13A-13C). When these mutations were introduced into HIV_{JRCSF} pseudoviruses there was a drop in neutralization with the greatest effect caused by the N625Q mutation found in all of the plasma sequences (FIG. 13D). These data suggest that the autologous virus has escaped neutralization by 35O22.

To gain insight into the prevalence of the 35O22 specificity, the 35O22 neutralization fingerprint was added to 0 previously identified footprints (FIGS. 12D and 12E) (Georgiev *et al., Science* 340, 751-756, (2013)). Notably, 13 of the sera (38%) showed significant 35O22-neutralization signals (>0.2). This level of prevalence was substantially higher than for the V1V2-directed response (typified by the PG9 antibody) or that of the 8ANC195 antibody. However, it was lower than the prevalence of responses to the MPER (50% prevalence), the CD4-binding site (56% prevalence), or the V3 glycan site (82% prevalence). The neutralizing activity of sera was also measured against HIV_{JRCSF} pseudoviruses bearing N88A, N230A, N241A, or N625A mutations (FIG. 14). These mutations caused a greater than 5-fold

increase in ID_{50} in more than half of donors, with a high level of concordance between the impact of each of these mutations within a given serum. These results suggested that 35O22 is unlikely to be the product of a unique B cell repertoire or infecting virus, but rather arises commonly among patients that develop HIV-specific neutralizing antibodies.

To better understand the mechanism of 35O22's activity, the timing of its binding and neutralization during virus fusion was examined. Given the proximity of the 35O22 epitope to the membrane, it was important to perform these experiments in the context of Env expressed on cells or virions. In these settings, MPER-specific antibodies have limited access to the native trimer and bind best after the conformational changes induced by CD4 attachment (Pancera and Wyatt, R., *Virology* 332, 145-156, (2005)). Binding of 35O22 to Env expressed on the cell surface was low and similar to the MPER antibody 2F5 (FIG. 4A). 35O22 binding to VLPs was weak compared to that of VRC01 but similar to that of 10E8 (FIG. 4B). 35O22 binding was slightly inhibited by sCD4 binding, suggesting the 35O22 epitope is not induced by sCD4 under these experimental conditions. 35O22 neutralization was partially eliminated by washing of pseudovirions prior to infection, a result consistent with limited access to Env on free virions (FIG. 4C) (Pancera and Wyatt, R., *Virology* 332, 145-156, (2005)). However, if 35O22 was incubated with VLPs, permitted to bind to target cells, and then after 2 hours the cells were washed, there was little impact on neutralization similar to all other antibodies except the MPER 10E8 antibody (FIG. 4D). Similar to 2G12 and 10E8, 35O22 activity was relatively high in the post-CD4 format, consistent with prior work showing that virus-sCD4 complexes are more sensitive to neutralization than virus alone ((Crooks *et al., Human antibodies* 14, 101-113, (2005)). In a post-CD4/CCR5 assay, only 10E8 neutralized, consistent with prior observations (FIG. 4D) (Crooks *et al., Human antibodies* 14, 101-113, (2005)). Taken together with the structural data, these results suggest that in the context of a lipid membrane, 35O22 binds Env poorly prior to CD4 attachment. However, following trimer attachment to CD4, 35O22 binds to an early intermediate that exposes the 35O22 epitope possibly by raising the Env spike within the viral membrane (FIG. 4E).

The novel binding site of the 35O22 antibody and the spectrum of activity against HIV strains indicate it can complement other antibodies used in passive immunotherapy or prophylaxis for HIV-1 infection. In addition, the antibody is extremely potent, suggesting its activity *in vivo* may therefore persist even at low concentrations.

METHODS

Study patients. The plasma and peripheral blood mononuclear cells (PBMCs) were selected from HIV-1-infected patients enrolled in the National Institute of Health under a clinical protocol (ClinicalTrials.gov identifier NCT00029445) approved by the Investigational Review Board in the National Institute of Allergy and Infectious Diseases (NIAID-IRB). All participants signed informed consent approved by the NIAID-IRB. The criteria for enrolment were as follows: having a detectable viral load, a stable CD4 T-cell count above 400 cells μl^{-1}, being diagnosed with HIV infection for at least 4 years, and off antiretroviral treatment for at least 5 years. Donor N152 was selected for B-cell sorting

and antibody generation because his serum neutralizing activity is among the most potent and broad in the cohort. At the time of leukapheresis, he had been infected with HIV-1 for 20 years, with CD4 T-cell counts of 325 cells μl^{-1}, plasma HIV-1 RNA values of 3,811 copies ml^{-1} and was not on antiretroviral treatment.

Memory B-cell staining, sorting and antibody cloning. Staining and single-cell sorting of memory B cells were performed following a detailed protocol recently published (Huang *et al., Nat Protoc* 8, 1907-1915, (2013)). Briefly, a total of 140,000 $CD19^+IgA^-IgD^-IgM^-$ memory B cells were sorted and re-suspended in medium with IL-2, IL-21 and irradiated 3T3-msCD40L feeder cells, and seeded into 384-well microtitre plates at a density of 4 cells per well. After 13 days of incubation, supernatants from each well were screened for neutralization activity using a high-throughput micro-neutralization assay against HIV-$1_{MN.3}$ and HIV-$1_{Bal.26}$. From the wells that scored positive in both the HIV-$1_{MN.3}$ and HIV-$1_{Bal.26}$ neutralization assay the variable region of the heavy chain and the light chain of the immunoglobulin gene were amplified by RT–PCR and re-expressed as described previously (Tiller *et al., J Immunological methods* 329, 112-124, (2008)). The full-length IgG was purified using a recombinant protein-A column (GE Healthcare).

Generation of pseudoviruses and VLPs. HIV-1 Env pseudoviruses were generated by co-transfection of 293T cells with pSG3 delta Env backbone and a second plasmid that expressed HIV-1 Env at a ratio of 2:1. 72 h after transfection, supernatants containing pseudoviruses were harvested and frozen at −80°C until further use. Glycosidase-inhibitors were added to the cells at the time of transfection at flowing concentration, 25 μM kifunensine, 500 μM NB-DNJ and 20 μM swainsonine. JRCSF mutants were produced by altering the JRCSF Env plasmid using QuikChange Lightning mutagenesis according to the manufacturer's protocol (Agilent). VLPs for ELISA assays were produced by transient transfection of 293T cells with a pCAGGS Env-expressing plasmid and the subgenomic plasmid pNL4-3.Luc.R-E- as previously described (Tong et al., *J Virol* 86, 3574-3587, (2012)).

Neutralization assays. Neutralization activity of monoclonal antibodies or serum was measured using single-round HIV-1 Env-pseudovirus infection of TZM-bl cells as described previously (Li *et al., J Virol* 79, 10108-10125, (2005)). HIV-1 Env pseudoviruses were generated by co-transfection of 293T cells with an Env-deficient backbone (pSG3ΔEnv) and an expression plasmid for the Env gene of interest. HIV_{LAI} viruses were generated by transfection of a single plasmid containing the entire viral genome. At 72 h after transfection, supernatants containing pseudovirus were harvested and frozen at −80 °C until further use. In the neutralization assay, heat-inactivated patient serum or monoclonal antibody was serially diluted five fold with Dulbecco's modified Eagle medium–10% FCS (Gibco), and 10 μl was incubated with 40 μl of pseudovirus in a 96-well plate at 37 °C for 30 min. TZM-bl cells were then added and plates were incubated for 48h. Assays were then developed with a luciferase assay system (Promega,), and the relative light units (RLU) were read on a luminometer (Perkin Elmer). Washing of pseudovirions to determine access by antibody to the trimer on free virus was performed as described previously Chakrabarti *et al., J Virol* 85, 8217-8226, (2011)). Neutralization in various formats to determine the timing and mechanism of neutralization were performed as described previously (Crooks

et al., Human antibodies 14, 101-113, (2005); Binley *et al., J Virol* 77, 5678-5684, (2003)). Briefly, in the standard "leave in" format monoclonal antibodies were mixed with VLPs 1h prior to infection of CF2.CD4.CCR5 cells with no subsequent washing. Alternatively, in a "cell wash" format, virus and antibody were incubated with target cells for 2h, followed by a wash to remove any unattached virus. In the post-CD4 format, VLPs were premixed with 3 μg/ml of sCD4 for 15 min., then incubated with antibody for 1h before adding to cells that lack CD4 but express CCR5 (CF2.syn.CCR5 cells). In the post-CD4/CCR5 format, SOS VLPs were permitted to attach to cells for 2h, unbound VLPs were washed away and graded concentrations of antibodies were added before infection was activated with DTT.

Prediction of prevalence of 35O22-like antibodies in patient serum. An antibody neutralization fingerprint, the pattern with which an antibody neutralizes a panel of diverse viral strains, was used to delineate the structural epitope recognized by that antibody (Georgiev *et al., Science* 340, 751-756, (2013); Chuang *et al., J Virol* 87, 10047-10058, (2013)). For each serum, neutralization on a panel of 21 HIV-1 strains was represented as a combination of the neutralization fingerprints for a reference set of antibody specificities targeting the other four major sites of Env vulnerability as well as the 35O22 fingerprint, to obtain an estimate of the relative contribution of each of these specificities to neutralization by the given serum. The neutralization behavior of sera was deconvoluted from a cohort of 34 donors, each with neutralization breadths of greater than 50%.

Binding assays. HIV_{YU2} gp160 extracellular domain (gp140) foldon trimer, gp120 and gp41 monomers were produced as described previously (Pancera *et al., J Virol* 79, 9954-9969, (2005)). HIV_{BaL} (clade B), HIV_{CM235} (CRF01_AE), HIV_{CN54} (clade C), $HIV_{96ZM651}$ (clade C), $HIV_{93TH975}$ (clade E) gp120 proteins; and the HIV_{UG37} (clade A), HIV_{CN54} (clade C), HIV_{UG21} (clade D) and HIV_{BR29} (clade F) gp140 monomers were obtained through the NIH AIDS Research and Reagent Program. For ELISA assays each antigen (2 μg ml^{-1}) was coated on 96-well plates overnight at 4 °C. Plates were blocked with BLOTTO buffer (PBS, 1% FBS, 5% non-fat milk) for 1 h at room temperature, followed by incubation with antibody serially diluted in disruption buffer (PBS, 5% FBS, 2% BSA, 1% Tween-20) for 1 h at room temperature. 1:10,000 dilution of horseradish peroxidase (HRP)-conjugated goat anti-human IgG antibody was added for 1 h at room temperature. Plates were washed between each step with 0.2% Tween 20 in PBS. Plates were developed using 3,3′,5,5′-tetramethylbenzidine (TMB) (Sigma) and read at 450 nm. ELISA assays using the BG505 SOSIP.664 trimer and mutants were performed as described previously (Sanders *et al., PLoS Pathog* 9, e1003618, (2013); Ringe *et al., PNAS,* 110, 18256-18261, (2013)). Surface plasmon resonance experiments were performed at 25°C on a Biacore 3000 instrument (GE Healthcare) using D7324 or anti-Histidine capture. For the SPR experiment presented in FIG. 2D an anti-histidine capture was used (R_L= ~500 RU) and in FIGS. 2E and 2F a D7324 capture was used (R_L = ~150 RU for trimer and protomer; 130 RU for gp120 in 2e and R_L = ~500 RU in 2f). ELISA assays using VLPs were performed as previously described (Crooks *et al., Human antibodies* 14, 101-113, (2005); Tong et al., *J Virol* 86, 3574-3587, (2012)).

Autoreactivity assays. Reactivity to HIV-1 negative human epithelial (HEp-2) cells was determined by indirect immunofluorescence on slides using Evans Blue as a counterstain and FITC-

conjugated goat anti-human IgG (Zeus Scientific) (Haynes *et al., Science* 308, 1906-1908, (2005)). Slides were photographed on a Nikon Optiphot fluorescence microscope. Kodachrome slides were taken of each monoclonal antibody binding to HEp-2 cells at a 10-s exposure, and the slides scanned into digital format. The Luminex AtheNA Multi-Lyte ANA test (Wampole Laboratories) was used to test for monoclonal antibody reactivity to SSA/Ro, SS-B/La, Sm, ribonucleoprotein (RNP), Jo-1, double-stranded DNA, centromere B, and histone and was performed as per the manufacturer's specifications and as previously described (Haynes *et al., Science* 308, 1906-1908, (2005)). Monoclonal antibody concentrations assayed were 50, 25, 12.5 and 6.25 μg ml^{-1}. 10 μl of each concentration were incubated with the luminex fluorescent beads and the test performed per the manufacturer's specifications.

Structure determination and analysis. The antigen-binding fragment of 35O22 (Fab) was prepared using HRV3C digestion, as previously described (McLellan *et al., Nature* 480, 336-343, (2011)). HRV3C was introduced in the hinge region of the heavy chain plasmid DNA. Both light and heavy chain plasmids were cotransfected in 293F as described previously. The antibody, 35O22 with HRV3C IgG was purified over protein A, cleaved with HRV3C and the flow-through collected and run on to a size-exclusion chromatography (S200). Purified 35O22 Fab set up for 20°C vapour diffusion sitting-drop crystallizations on the Honeybee 963 robot. A total of 576 initial conditions adapted from the commercially available Hampton (Hampton Research), Precipitant Synergy (Emerald Biosystems) and Wizard (Emerald Biosystems) crystallization screens were set up and imaged using the Rockimager (Formulatrix), followed by hand optimization of crystal hits. Crystals were grown in 15% isopropanol, 25% PEG 3350, 0.2 M Ammonium citrate pH 4.5 diffracted to 1.55 Å resolution in a cryoprotectant composed of mother liquor supplemented with15% 2*R*-3*R*-butanediol. After mounting the crystals on a loop, they were flash cooled and data were collected at 1.00 Å wavelength at SER CAT ID-22 beamlines (APS) and processed using HKL-2000 (Otwinowski and Minor, *Macromolecular Crystallography, Pt A* 276, 307-326, (1997)). Structures were solved through molecular replacement with Phaser (McCoy *et al., J Appl Crystallogr* 40, 658-674, (2007); Winn *et al., Acta Crystallogr D Biol Crystallogr* 67, 235-242, (2011)), using a previously obtained free structure of VRC01 germline as a search model. Structure solution identified one Fab per asymmetric unit in a $P4_12_12$ lattice. Refinement of the structure was undertaken with Phenix, with iterative model building using Coot (Emsley and Cowtan, *Acta Crystallogr D Biol Crystallogr* 60, 2126-2132, (2004)). Refinement resulted in an R_{cryst} value of 16.65% (R_{free}=18.22%). The structure was validated with MolProbity (Davis *et al., Nucleic Acids Res* 35, W375-383, (2007)), yielding 98.1% and 100 % of residues falling within most favoured Ramachandran regions and allowed Ramachandran regions, respectively. All graphics were prepared with Pymol (PyMOL Molecular Graphics System).

Electron microscopy and image processing. Negative stain EM grids were prepared as previously stated (Kong *et al., Nat Struct Mol Biol* 20, 796-803, (2013)). Data were collected using a FEI Tecnai T12 electron microscope operating at 120 keV, with an electron dose of ~30 $e^-/Å^2$ and a magnification of 52,000× that resulted in a pixel size of 2.05 Å at the specimen plane. Images were acquired with a Tietz TemCam-F416 CMOS camera using a nominal defocus range of 800 to 1000 nm

using the Leginon interface (ref). Image processing was carried out as described previously (Thornburg *et al., The Journal of clinical investigation* 123, 4405-4409, (2013)). The final reconstruction was performed using 4746 unbinned particles, refining for 40 iterations with C3 symmetry applied. The resulting density was ~19 Å resolution at a Fourier shell correlation (FSC) cut-off of 0.5. Fab fitting was carried out using the Fit function in Chimera, using 0.0115 contour level for the map. The Fab orientation where the heavy chain is towards gp120 (as shown in FIG. 3B) was chosen on the basis of the two correlation coefficients (0.95)(471 of 7058 atoms outside contour). vs. 0.91 (1012 of 7058 atoms outside contour). In the alternate orientation (light chain towards gp120), the FR3 insertion did not fit within the density. Dominant sites of vulnerability to neutralizing antibody elicited during chronic infection in FIGS. 12A-12E is shown in the context of an EM tomogram from the BAL viral spike (Liu et al., *Nature* 455, 109-113, (2008)).

EXAMPLE 2

Additional details concerning the 35O22 epitope

This example provides additional details concerning the 35O22 epitope on HIV-1 Env in the context of the HIV-1 strain BG505. The ectodomain of the HIV-1 Env protein from the BG505 strain was mutated to include the SOSIP and T332N mutations, and a c-terminal residue at position 664 (HXB2 numbering; termed "BG505 SOSIP.664"). The three dimensional structure of a trimeric form of the BG505.SOSIP.664 protein specifically bound by 35O22 antibody and PGT122 antibody (which targets the V3 domain) was solved. This structure provides additional information concerning the 35O22 epitope (see FIGs. 23A-23E).

In the context of the BG505 SOSIP.664/35O22 crystal structure, the 35O22 antibody makes at least minimal contacts with the following HIV-1 Env residues: 87-92, 238, 240, 527-529, 532, 617-618, 620-621, 624-627, 629-630, and 633. The majority of the 35O22 contacts are with an N-linked glycan moiety attached to an asparagine residue at position 88.

Example 2

Antibodies specific to HIV-1 Env for detecting HIV-1 in a sample or a subject

This example describes the use of HIV-1 monoclonal neutralizing antibodies specific to HIV-1 Env for the detection of HIV-1 in a sample or a subject. This example further describes the use of these antibodies to confirm the diagnosis of HIV-1 in a subject.

A biological sample, such as a blood sample, is obtained from the patient diagnosed with, undergoing screening for, or suspected of having an HIV-1 infection. A blood sample taken from a patient who is not infected is used as a control, although a standard result can also be used as a control. An ELISA is performed to detect the presence of HIV-1 in the blood sample. Proteins present in the blood samples (the patient sample and control sample) are immobilized on a solid support, such as a 96-well plate, according to methods well known in the art (see, for example, Robinson *et al.*, *Lancet* 362:1612-1616, 2003, incorporated herein by reference). Following immobilization, HIV-1 monoclonal

neutralizing antibodies specific to HIV-1 Env that are directly labeled with a fluorescent marker are applied to the protein-immobilized plate. The plate is washed in an appropriate buffer, such as PBS, to remove any unbound antibody and to minimize non-specific binding of antibody. Fluorescence can be detected using a fluorometric plate reader according to standard methods. An increase in fluorescence intensity of the patient sample, relative to the control sample, indicates the HIV-1 Env antibody specifically bound proteins from the blood sample, thus detecting the presence of HIV-1 protein in the sample. Detection of HIV-1 protein in the patient sample indicates the patient has HIV-1, or confirms diagnosis of HIV-1 in the subject.

Example 3

HIV-1 monoclonal neutralizing antibodies specific for HIV-1 Env for the treatment of HIV-1

This example describes a particular method that can be used to treat HIV in a human subject by administration of one or more HIV-1 Env-specific human neutralizing mAbs. Although particular methods, dosages, and modes of administrations are provided, one skilled in the art will appreciate that variations can be made without substantially affecting the treatment.

Based upon the teaching disclosed herein, HIV-1 can be treated by administering a therapeutically effective amount of one or more of the neutralizing mAbs described herein, thereby reducing or eliminating HIV infection.

Screening subjects

In particular examples, the subject is first screened to determine if they have an HIV infection. Examples of methods that can be used to screen for HIV infection include a combination of measuring a subject's CD4+ T cell count and the level of HIV in serum blood levels. Additional methods using the HIV-1 Env-specific mAbs described herein can also be used to screen for HIV.

In some examples, HIV testing consists of initial screening with an enzyme-linked immunosorbent assay (ELISA) to detect antibodies to HIV, such as to HIV-1. Specimens with a nonreactive result from the initial ELISA are considered HIV-negative unless new exposure to an infected partner or partner of unknown HIV status has occurred. Specimens with a reactive ELISA result are retested in duplicate. If the result of either duplicate test is reactive, the specimen is reported as repeatedly reactive and undergoes confirmatory testing with a more specific supplemental test (*e.g.*, Western blot or an immunofluorescence assay (IFA)). Specimens that are repeatedly reactive by ELISA and positive by IFA or reactive by Western blot are considered HIV-positive and indicative of HIV infection. Specimens that are repeatedly ELISA-reactive occasionally provide an indeterminate Western blot result, which may be either an incomplete antibody response to HIV in an infected person, or nonspecific reactions in an uninfected person. IFA can be used to confirm infection in these ambiguous cases. In some instances, a second specimen will be collected more than a month later and retested for subjects with indeterminate Western blot results. In additional examples, nucleic acid testing (*e.g.*, viral RNA or proviral DNA amplification method) can also help diagnosis in certain situations.

The detection of HIV in a subject's blood is indicative that the subject is infected with HIV and is a candidate for receiving the therapeutic compositions disclosed herein. Moreover, detection of a CD4+ T cell count below 350 per microliter, such as 200 cells per microliter, is also indicative that the subject is likely to have an HIV infection.

Pre-screening is not required prior to administration of the therapeutic compositions disclosed herein

Pre-treatment of subjects

In particular examples, the subject is treated prior to administration of a therapeutic agent that includes one or more antiretroviral therapies known to those of skill in the art. However, such pre-treatment is not always required, and can be determined by a skilled clinician.

Administration of therapeutic compositions

Following subject selection, a therapeutically effective dose of a HIV-1 Env-specific neutralizing mAb described herein is administered to the subject (such as an adult human or a newborn infant either at risk for contracting HIV or known to be infected with HIV). Additional agents, such as anti-viral agents, can also be administered to the subject simultaneously or prior to or following administration of the disclosed agents. Administration can be achieved by any method known in the art, such as oral administration, inhalation, intravenous, intramuscular, intraperitoneal, or subcutaneous.

The amount of the composition administered to prevent, reduce, inhibit, and/or treat HIV or a condition associated with it depends on the subject being treated, the severity of the disorder, and the manner of administration of the therapeutic composition. Ideally, a therapeutically effective amount of an agent is the amount sufficient to prevent, reduce, and/or inhibit, and/or treat the condition (*e.g.*, HIV) in a subject without causing a substantial cytotoxic effect in the subject. An effective amount can be readily determined by one skilled in the art, for example using routine trials establishing dose response curves. As such, these compositions may be formulated with an inert diluent or with a pharmaceutically acceptable carrier.

In one specific example, antibodies are administered at 5 mg per kg every two weeks or 10 mg per kg every two weeks depending upon the particular stage of HIV. In an example, the antibodies are administered continuously. In another example, antibodies or antibody fragments are administered at 50 µg per kg given twice a week for 2 to 3 weeks.

Administration of the therapeutic compositions can be taken long term (for example over a period of months or years).

Assessment

Following the administration of one or more therapies, subjects with HIV can be monitored for reductions in HIV levels, increases in a subject's CD4+ T cell count, or reductions in one or more clinical symptoms associated with HIV. In particular examples, subjects are analyzed one or more times, starting

7 days following treatment. Subjects can be monitored using any method known in the art. For example, biological samples from the subject, including blood, can be obtained and alterations in HIV or CD4+ T cell levels evaluated.

Additional treatments

In particular examples, if subjects are stable or have a minor, mixed or partial response to treatment, they can be re-treated after re-evaluation with the same schedule and preparation of agents that they previously received for the desired amount of time, including the duration of a subject's lifetime. A partial response is a reduction, such as at least a 10%, at least 20%, at least 30%, at least 40%, at least 50%, or at least 70% in HIV infection, HIV replication or combination thereof. A partial response may also be an increase in CD4+ T cell count such as at least 350 T cells per microliter.

It will be apparent that the precise details of the methods or compositions described may be varied or modified without departing from the spirit of the described embodiments. We claim all such modifications and variations that fall within the scope and spirit of the claims below.

We claim:

1. An isolated monoclonal antibody or antigen binding fragment thereof that specifically binds to an epitope on an HIV-1 Envelope protein, comprising a heavy chain variable region comprising a HCDR1, a HCDR2, and a HCDR3, and a light chain variable region comprising a LCDR1, a LCDR2, and a LCDR3, wherein:

the HCDR1, the HCDR2, and the HCDR3 comprise the consensus sequences set forth as SEQ ID NO: 17, SEQ ID NO: 18, and SEQ ID NO: 19, respectively;

the LCDR1, the LCDR2, and the LCDR3 comprise the consensus sequences set forth as SEQ ID NO: 20, SEQ ID NO: 21, and SEQ ID NO: 22, respectively; and

the monoclonal antibody or antigen binding fragment neutralizes HIV-1 infection.

2. The antibody or antigen binding fragment of claim 1, comprising:

a heavy chain variable region comprising a HCDR1, a HCDR2, and a HCDR3 of the heavy chain variable region set forth as SEQ ID NO: 1;

a light chain variable region, comprising a LCDR1, a LCDR2, and a LCDR3 of the light chain variable region set forth as SEQ ID NO: 2; or

a heavy chain variable region comprising a HCDR1, a HCDR2, and a HCDR3 of the heavy chain variable region set forth as SEQ ID NO: 1, and a light chain variable region, comprising a LCDR1, a LCDR2, and a LCDR3 of the light chain variable region set forth as SEQ ID NO: 2.

3. The antibody or antigen binding fragment of claim 1 or claim 2, wherein:

the HCDR1, HCDR2, and HCDR3, comprise amino acids 31-35, 50-66, and 107-120 of SEQ ID NO: 1, respectively;

the LCDR1, LCDR2, and LCDR3, comprise amino acids 23-36, 50-66, and 91-100 of SEQ ID NO: 2, respectively; or

the HCDR1, HCDR2, and HCDR3, comprise amino acids 31-35, 50-66, and 107-120 of SEQ ID NO: 1, respectively, and the LCDR1, LCDR2, and LCDR3, comprise amino acids 23-36, 50-66, and 91-100 of SEQ ID NO: 2, respectively.

4. The antibody or antigen binding fragment of any of claims 1-3, further comprising an eight amino acid insertion between kabat positions 72 and 73 of a framework region 3, wherein the insertion comprises the amino acid sequence set forth as residues 74-81 of one of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, or 15.

5. The antibody or antigen binding fragment of claim 4, wherein the insertion comprises the amino acid sequence set forth as residues 74-81 of SEQ ID NO: 1.

6. The antibody or antigen binding fragment of any of the claims 1-5, wherein:

the heavy chain variable region comprises an amino acid sequence at least 80% identical to SEQ ID NO: 1;

the light chain variable region comprises an amino acid sequence at least 80% identical to SEQ ID NO: 2; or

the heavy chain variable region comprises an amino acid sequence at least 80% identical to SEQ ID NO: 1, and the light chain variable region comprises an amino acid sequence at least 80% identical to SEQ ID NO: 2.

7. The antibody or antigen binding fragment of any of claims 1-6, wherein:

the heavy chain variable region comprises the amino acid sequence set forth as SEQ ID NO: 1;

the light chain variable region comprising the amino acid sequence set forth as SEQ ID NO: 2; or

the heavy and light chain variable regions comprise the amino acid sequences set forth as SEQ ID NO: 1 and SEQ ID NO: 2, respectively.

8. The antibody or antigen binding fragment of any of claims 1-7, further comprising up to 10 conservative amino acid substitutions in the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2, and the LCDR3.

9. An isolated monoclonal antibody or antigen binding fragment thereof that specifically binds to an epitope on an HIV-1 Envelope protein comprising or consisting of HIV-1 Envelope protein residues 87-92 and 617-633, wherein residue 88 is glycosylated with an N-linked glycan; and wherein

the monoclonal antibody or antigen binding fragment neutralizes HIV-1 infection.

10. The antibody or antigen binding fragment of claim 9, wherein the epitope comprises or consists of HIV-1 Env residues 87-92, 227-243, and 617-633, wherein residue 88 is glycosylated with an N-linked glycan.

11. The antibody or antigen binding fragment of claim 9 or claim 10, wherein the epitope further comprises an N-linked glycan moiety on one or more of HIV-1 Envelope protein residues N230 or N241.

12. Antibody means for specifically binding an epitope on an HIV-1 Env trimer, wherein the epitope comprises HIV-1 Env residues 87-92 and 617-633, and wherein residue 88 is glycosylated with an N-linked glycan.

13. The antibody or antigen binding fragment of any of the preceding claims, comprising a human framework region.

14. The antibody of any of the preceding claims, wherein the antibody is an IgG, IgM or IgA.

15. The antigen binding fragment of any of claims 1-14.

16. The antigen binding fragment of claim 15, wherein the antigen binding fragment is a Fv, Fab, $F(ab')_2$, scFV or a $scFV_2$ fragment.

17. The antibody or antigen binding fragment of any of the preceding claims, wherein the antibody or antigen binding fragment neutralizes at least 80% of the HIV isolates shown in FIG. 3 with an inhibitory concentration (IC50) of <50 μg/ml.

18. A bispecific antibody comprising the isolated human monoclonal antibody or antigen binding fragment of any of the preceding claims.

19. The bispecific antibody of claim 18, wherein the antibody specifically binds to HIV-1 Envelope protein and to CD3.

20. The antibody or antigen binding fragment of any of claims 1-19, linked to an effector molecule or a detectable marker

21. The antibody or antigen binding fragment of claim 20, wherein the detectable marker is a fluorescent, enzymatic, or radioactive marker.

22. An isolated nucleic acid molecule encoding the antibody or antigen binding fragment of any of claims 1-21.

23. The nucleic acid molecule of claim 22, encoding a chimeric antigen receptor comprising an extracellular domain comprising the antigen binding fragment.

24. The nucleic acid molecule of claim 22 or claim 23, operably linked to a promoter.

25. An expression vector comprising the nucleic acid molecule of any of claims 22-24.

26. An isolated host cell transformed with the nucleic acid molecule or expression vector of any of claims 22-25.

27. The host cell of claim 26, wherein the host cell is a T cell.

28. A pharmaceutical composition for use in treating or preventing an HIV-1 infection, comprising:

a therapeutically effective amount of the antibody, antigen binding fragment, nucleic acid molecule, expression vector, or host cell of any of claims 1-27; and

a pharmaceutically acceptable carrier.

29. The pharmaceutical composition of any of claim 28, wherein the composition is sterile.

30. The pharmaceutical composition of claim 28 or claim 29, wherein the composition is in unit dosage form or a multiple thereof.

31. A method of detecting an HIV-1 infection in a subject, comprising:

contacting a biological sample from the subject with the antibody or antigen binding fragment of any of claims 1-20 under conditions sufficient to form an immune complex; and

detecting the presence of the immune complex on the sample, wherein the presence of the immune complex on the sample indicates that the subject has the HIV-1 infection.

32. A method of preventing or treating an HIV-1 infection in a subject, comprising administering to the subject a therapeutically effective amount of the antibody, antigen binding fragment, nucleic acid molecule, expression vector, host cell, or pharmaceutical composition of any of claims 1-30, thereby preventing or treating the HIV-1 infection.

33. The method of claim 32, wherein the subject has AIDS.

34. The method of claim 32 or claim 33, further comprising administering to the subject an additional anti-viral agent.

35. The method of any of claims 32-34, further comprising administering to the subject one or more additional antibodies, antigen binding fragments, or nucleic acids encoding such antibodies or antigen binding fragments, wherein the additional antibodies or antigen binding fragments specifically bind to HIV-1 gp120 or gp41 and neutralize HIV-1 infection.

36. The method of any of claims 32-35, further comprising measuring HIV-1 viral titer in the subject.

37. A kit comprising the antibody, antigen binding fragment, nucleic acid molecule, expression vector, host cell, or pharmaceutical composition of any of claims 1-30, and instructions for using the kit.

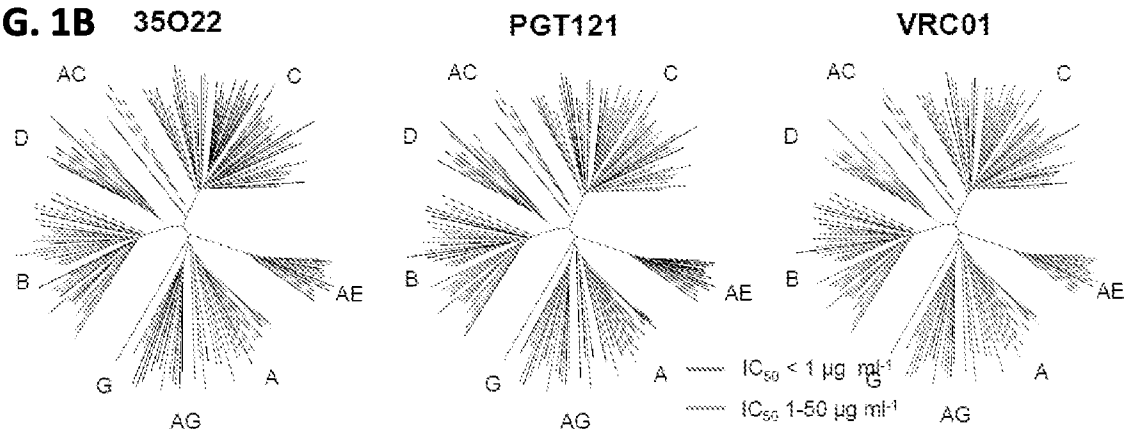
38. Use of the antibody, antigen binding fragment, nucleic acid molecule, expression vector, host cell, or pharmaceutical composition of any of claims 1-30 to inhibit or prevent HIV-1 infection in a subject.

FIG. 1A

35O22 Heavy Chain				
IGHV[a]	IGHD	IGHJ	CDR3 length (amino acid)	VH mutation frequency (nt)
1-18*02	5-24*01	4*02 or 4*03	16	96/276 (35%)

35O22 Light Chain			
IGLV	IGLJ	CDR3 length (amino acid)	VL mutation frequency (nt)
2-14*02, or 2-23*01, or 2-23*02, or 2-23*03	1*01	10	71/288 (24%)

FIG. 1B



	35O22	10E8	4E10	VRC01	NIH4 5-46	3BNC 117	PG9	PG16	PGT 121	PGT 128
No. of viruses	181	180	180	177	180	180	177	177	177	177
IC_{50} <50 µg ml^{-1}	62%	98%	98%	89%	85%	84%	78%	73%	64%	63%
IC_{50} <1 µg ml^{-1}	49%	72%	37%	75%	76%	77%	65%	59%	51%	50%
Geometric Mean IC_{50}*	0.056	0.22	1.3	0.250	0.138	0.09	0.11	0.06	0.05	0.07
Median IC_{50}	0.033	0.35	1.94	0.248	0.141	0.07	0.09	0.02	0.022	0.027

FIG. 2A

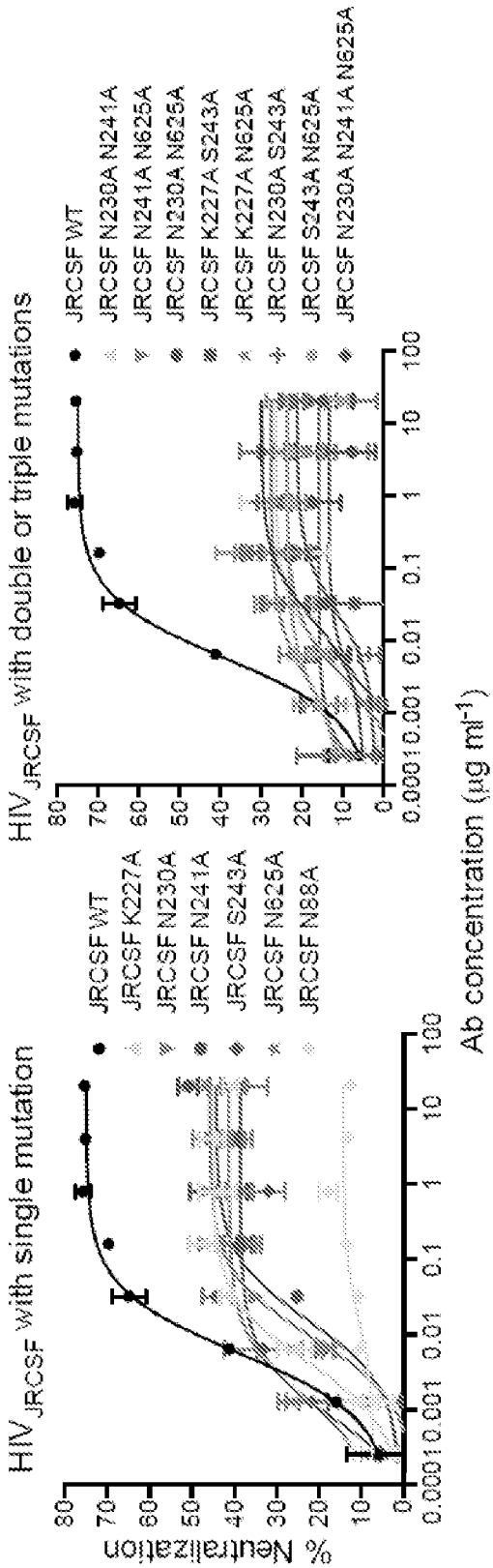
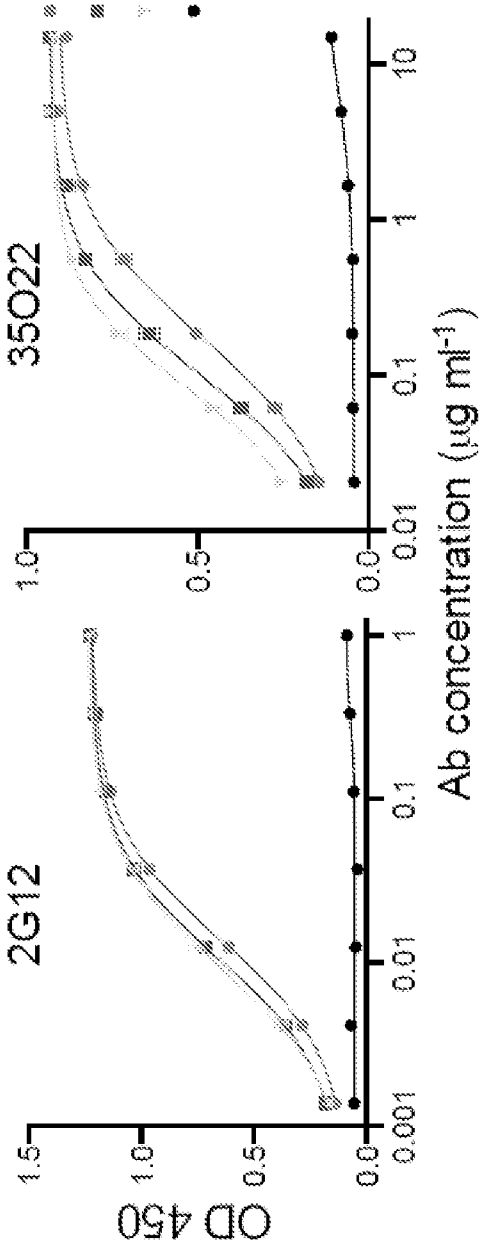

HIV JRCSF with single mutation
HIV JRCSF with double or triple mutations
% Neutralization
Ab concentration (μg ml-1)
JRCSF WT
JRCSF K227A
JRCSF N230A
JRCSF N241A
JRCSF S243A
JRCSF N625A
JRCSF N88A
JRCSF N230A N241A
JRCSF N241A N625A
JRCSF N230A N625A
JRCSF K227A S243A
JRCSF K227A N625A
JRCSF N230A S243A
JRCSF S243A N625A
JRCSF N230A N241A N625A

FIG. 2B


2G12
35O22
OD 450
Ab concentration (μg ml-1)
BG505 SOSIP.664
BG505 SOSIP.664 + kif
BG505 SOSIP.664 293S
mock

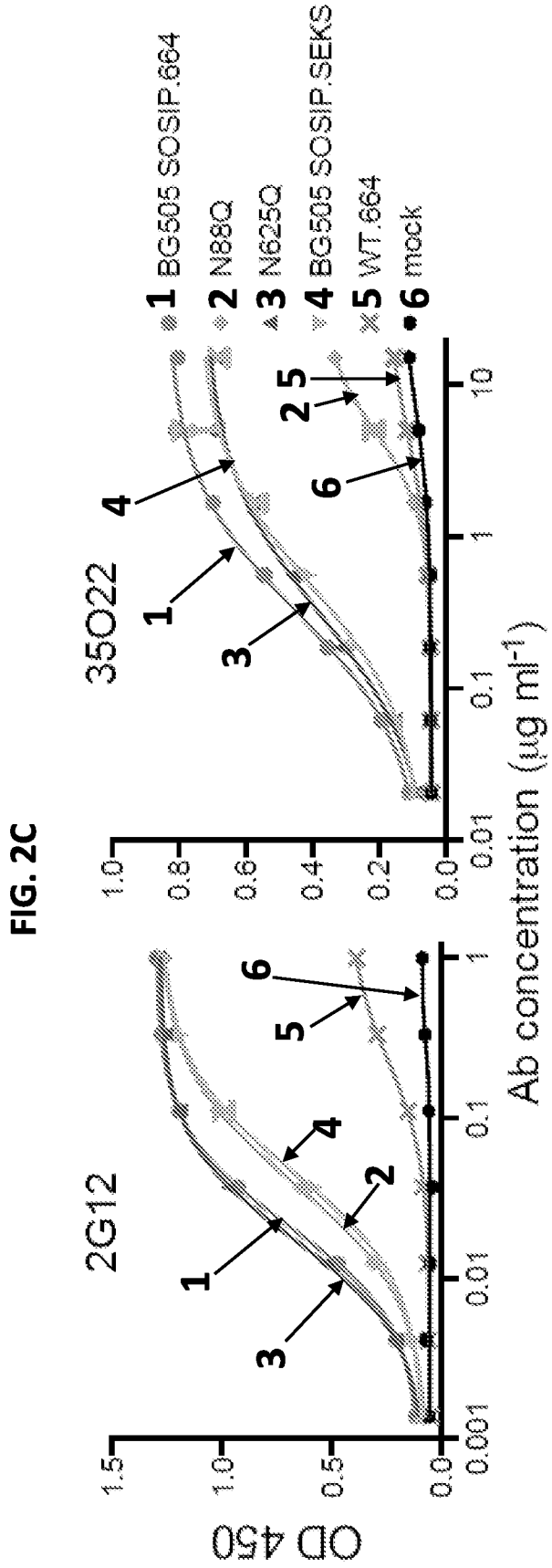

1 BG505 SOSIP.664
2 N88Q
3 N625Q
4 BG505 SOSIP.SEKS
5 WT.664
6 mock
35O22
2G12
OD 450
Ab concentration (µg ml-1)
1.5
1.0
0.5
0.0
0.8
0.6
0.4
0.2
0.001
0.01
0.1
1
10

FIG. 2C

FIG. 2D

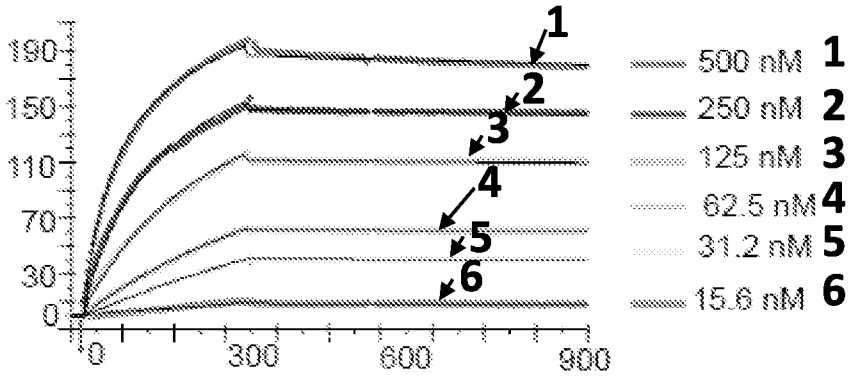


FIG. 2E

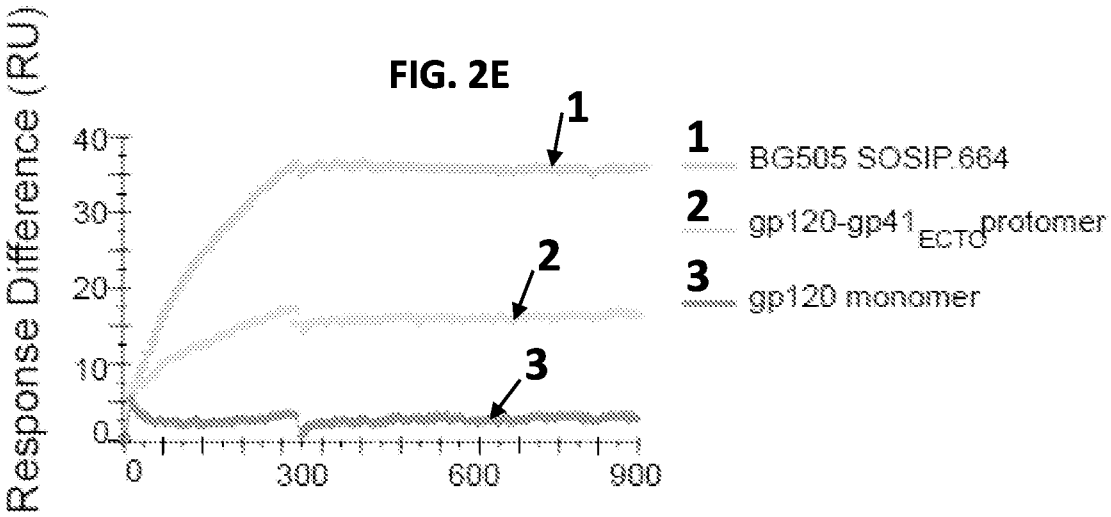


FIG. 2F

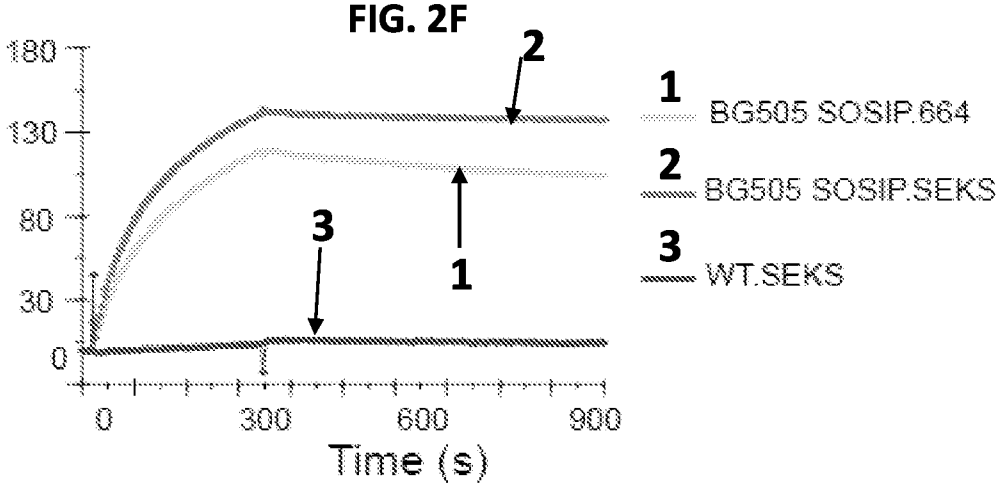


FIG. 3A

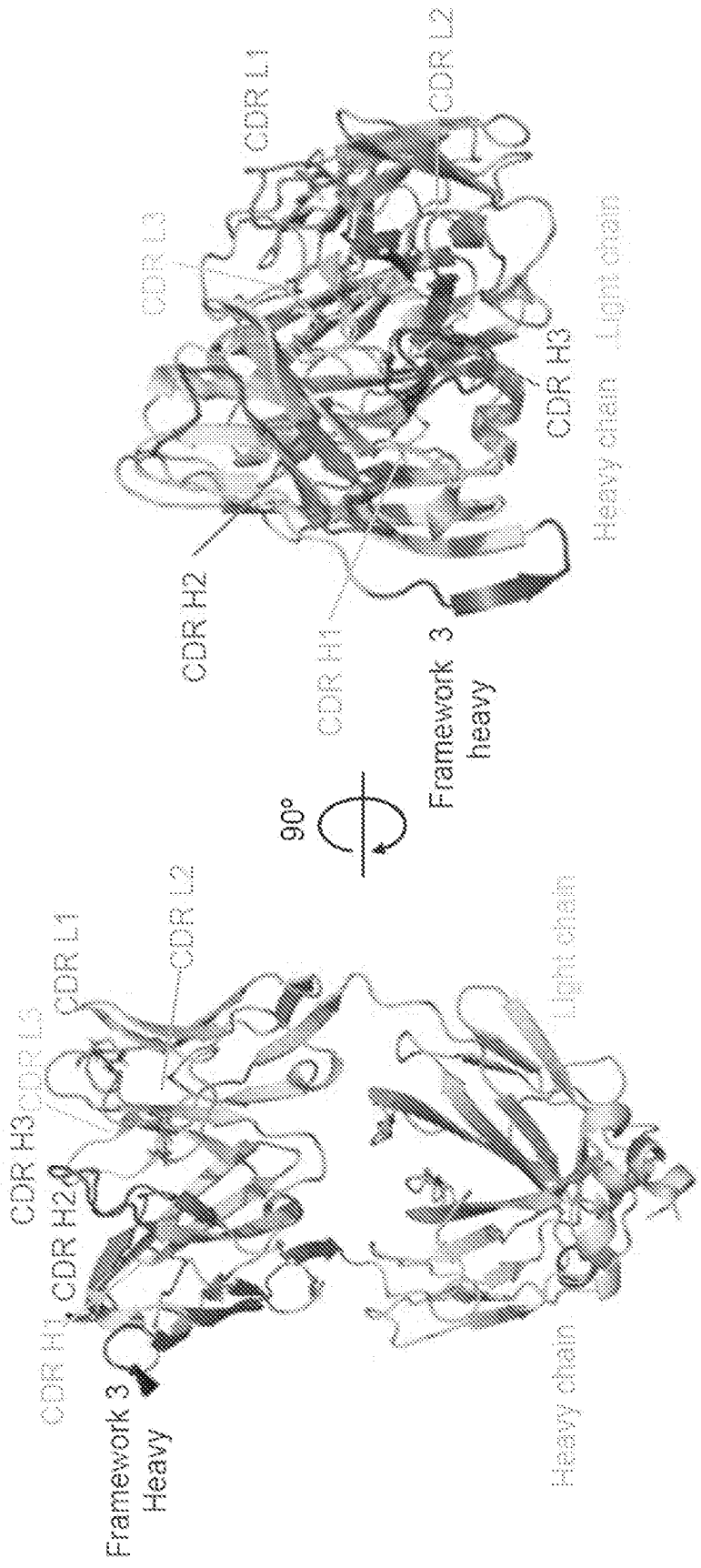

CDR H3
CDR L3
CDR L1
CDR L2
CDR H2
CDR H1
Framework 3
Heavy
Heavy chain
Light chain
90°
CDR L3
CDR L1
CDR L2
CDR H2
CDR H1
CDR H3
Framework 3
heavy
Heavy chain
Light chain

FIG. 3B

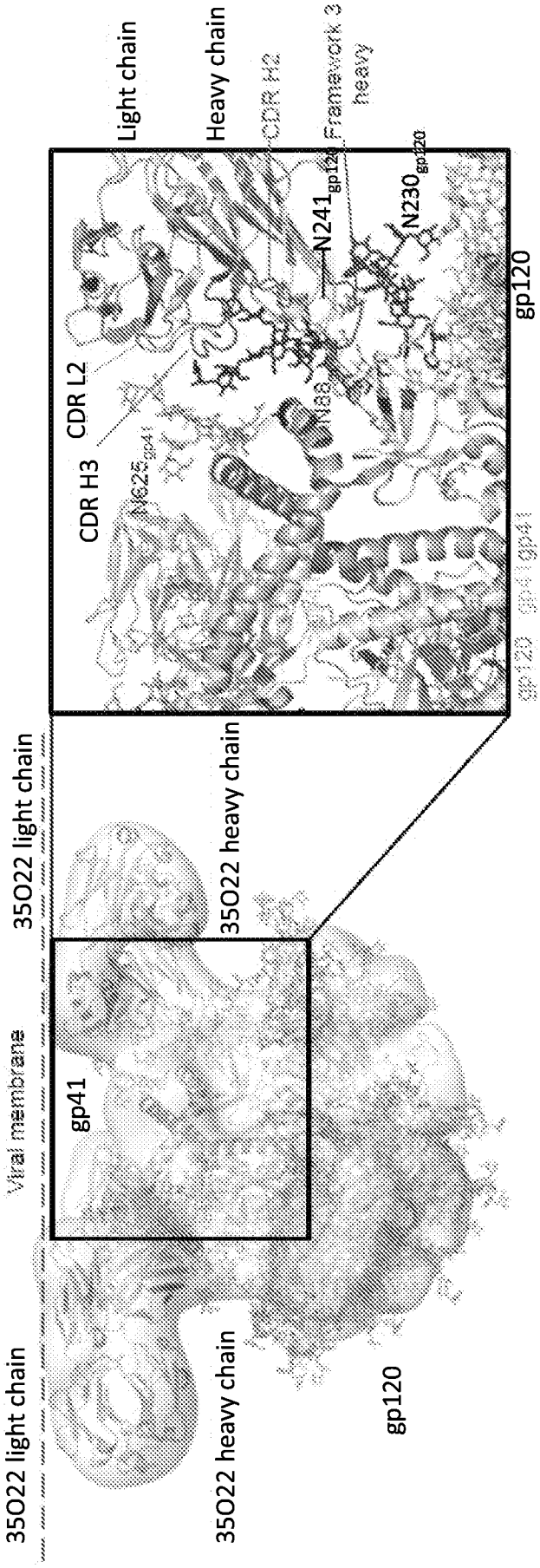

Light chain
Heavy chain
CDR H2
Framework 3 heavy
N241gp120
N230gp120
gp120
CDR L2
CDR H3
N625gp41
N88
gp120 gp41 gp41
35O22 light chain
35O22 heavy chain
Viral membrane
gp41
35O22 light chain
35O22 heavy chain
gp120

FIG. 4A

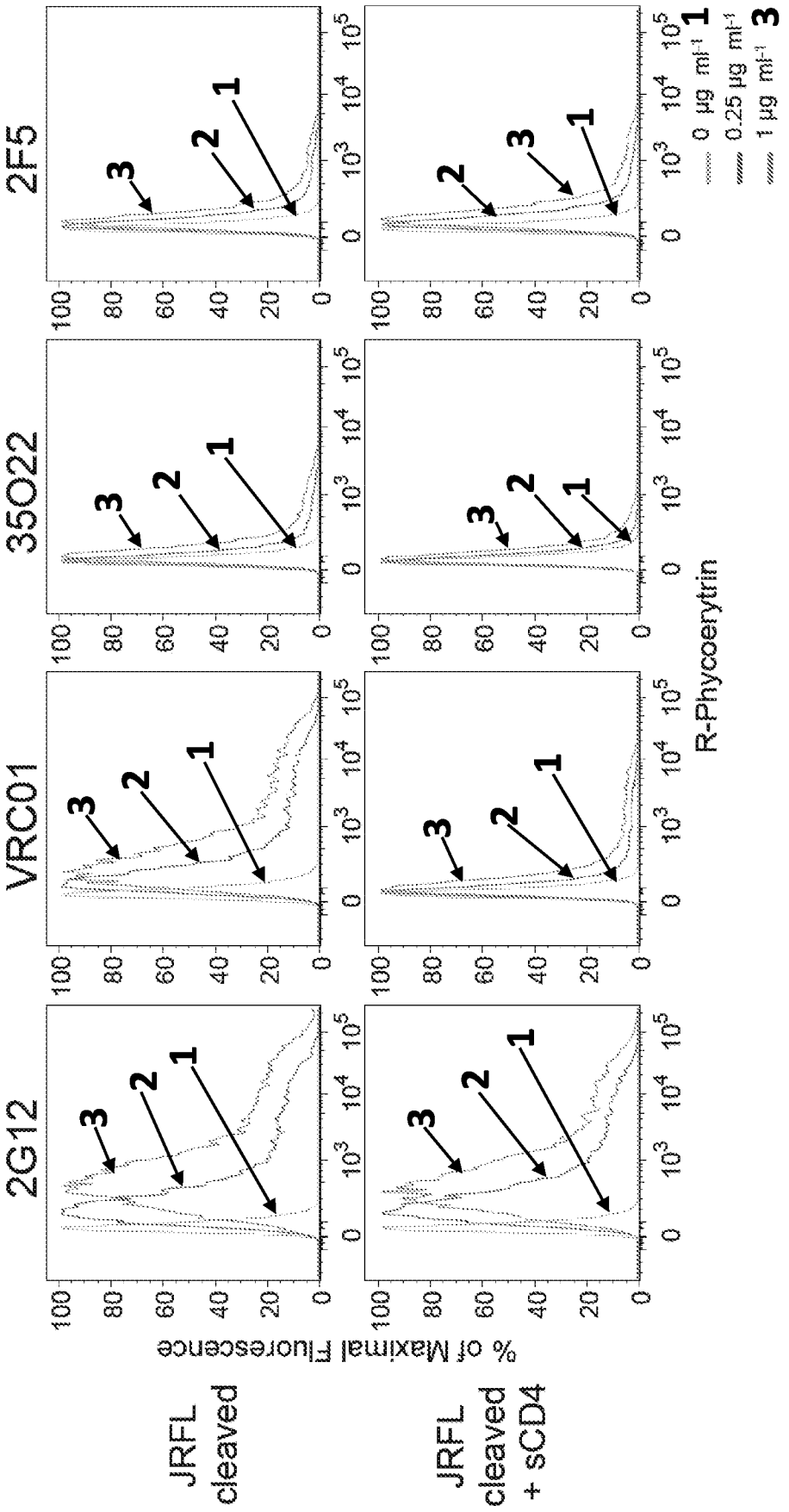

2G12
VRC01
35O22
2F5
JRFL cleaved
JRFL cleaved + sCD4
% of Maximal Fluorescence
R-Phycoerytrin
0 μg ml-1 1
0.25 μg ml-1 2
1 μg ml-1 3

FIG. 4B

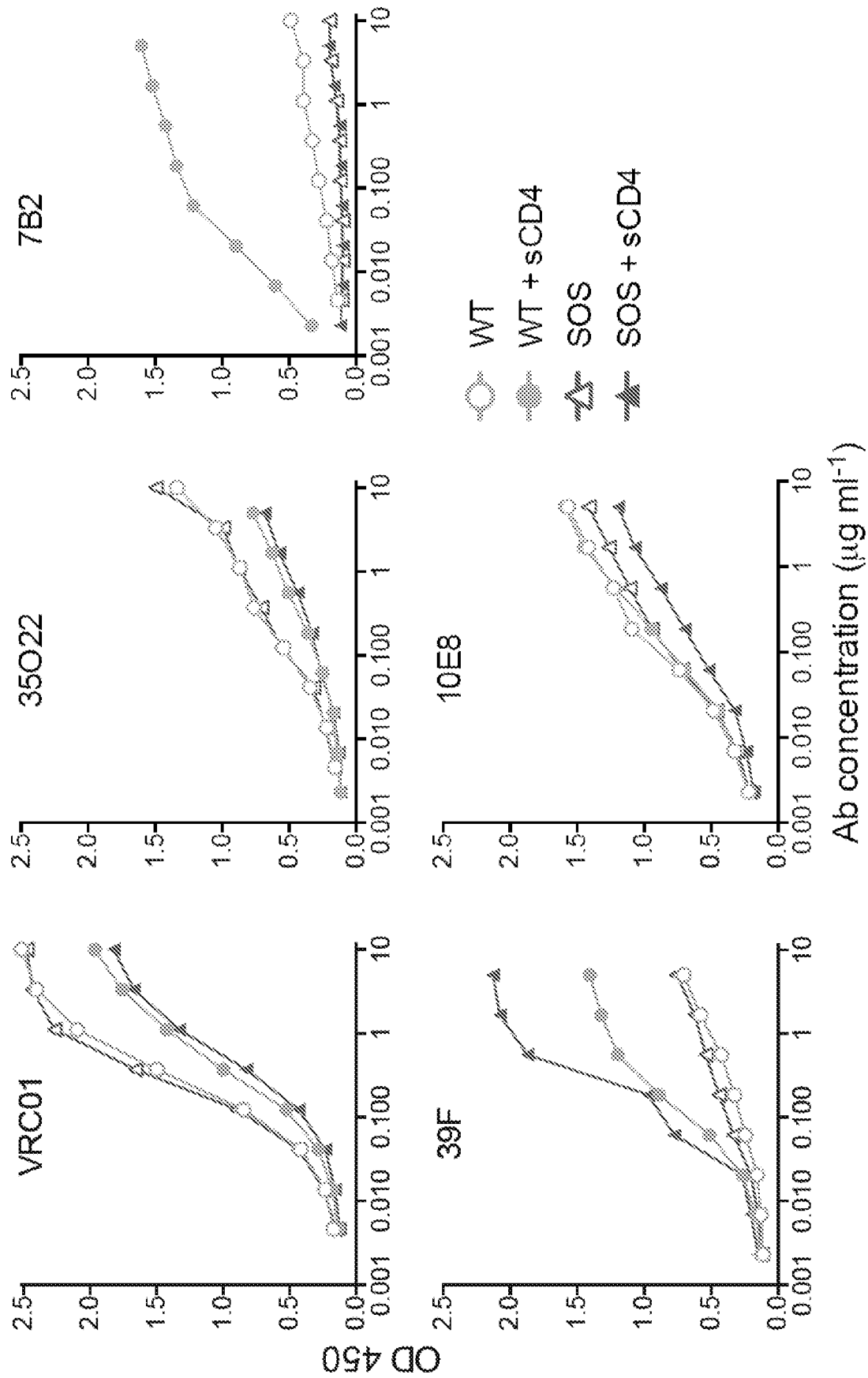

VRC01
35O22
7B2
39F
10E8
WT
WT + sCD4
SOS
SOS + sCD4
OD 450
Ab concentration (μg ml-1)
0.0
0.5
1.0
1.5
2.0
2.5
0.001
0.010
0.100
1
10

FIG. 4C

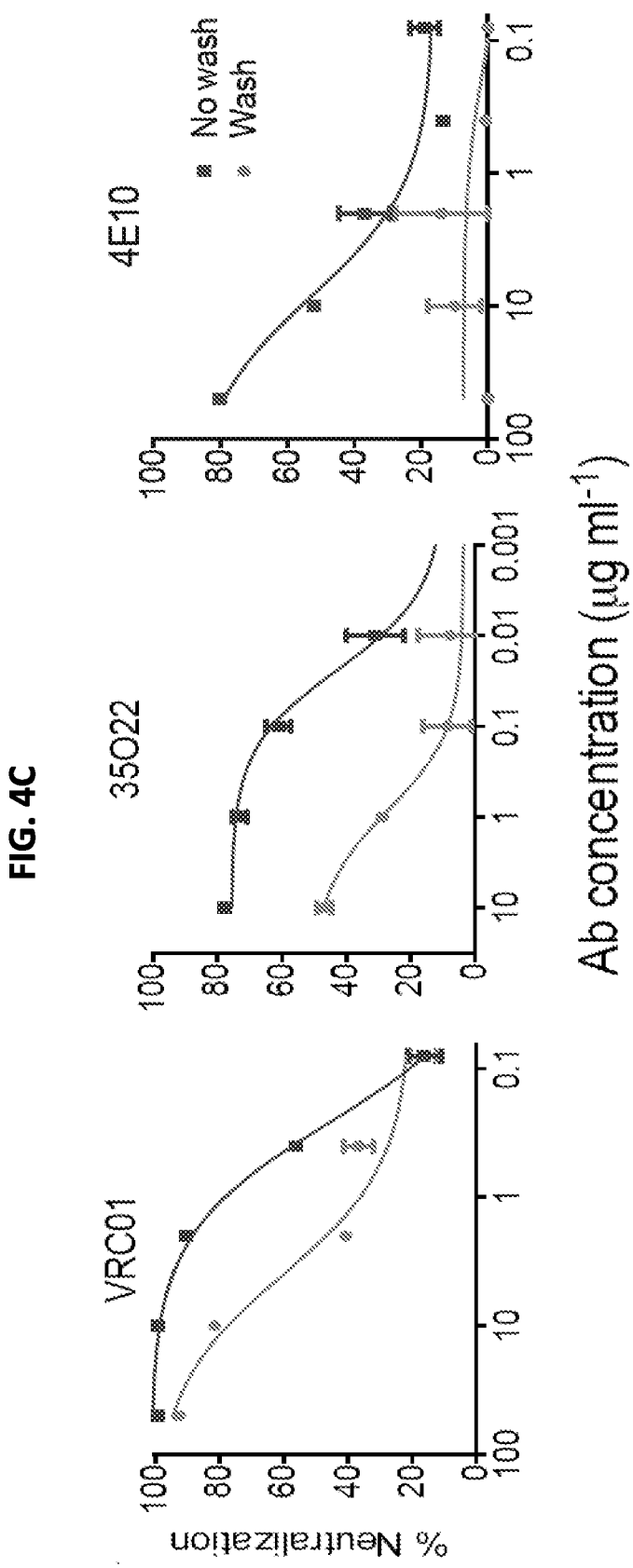

VRC01
3502 2
4E10
No wash
Wash
% Neutralization
Ab concentration (μg ml-1)
0
20
40
60
80
100
0.001
0.01
0.1
1
10
100

FIG. 4D

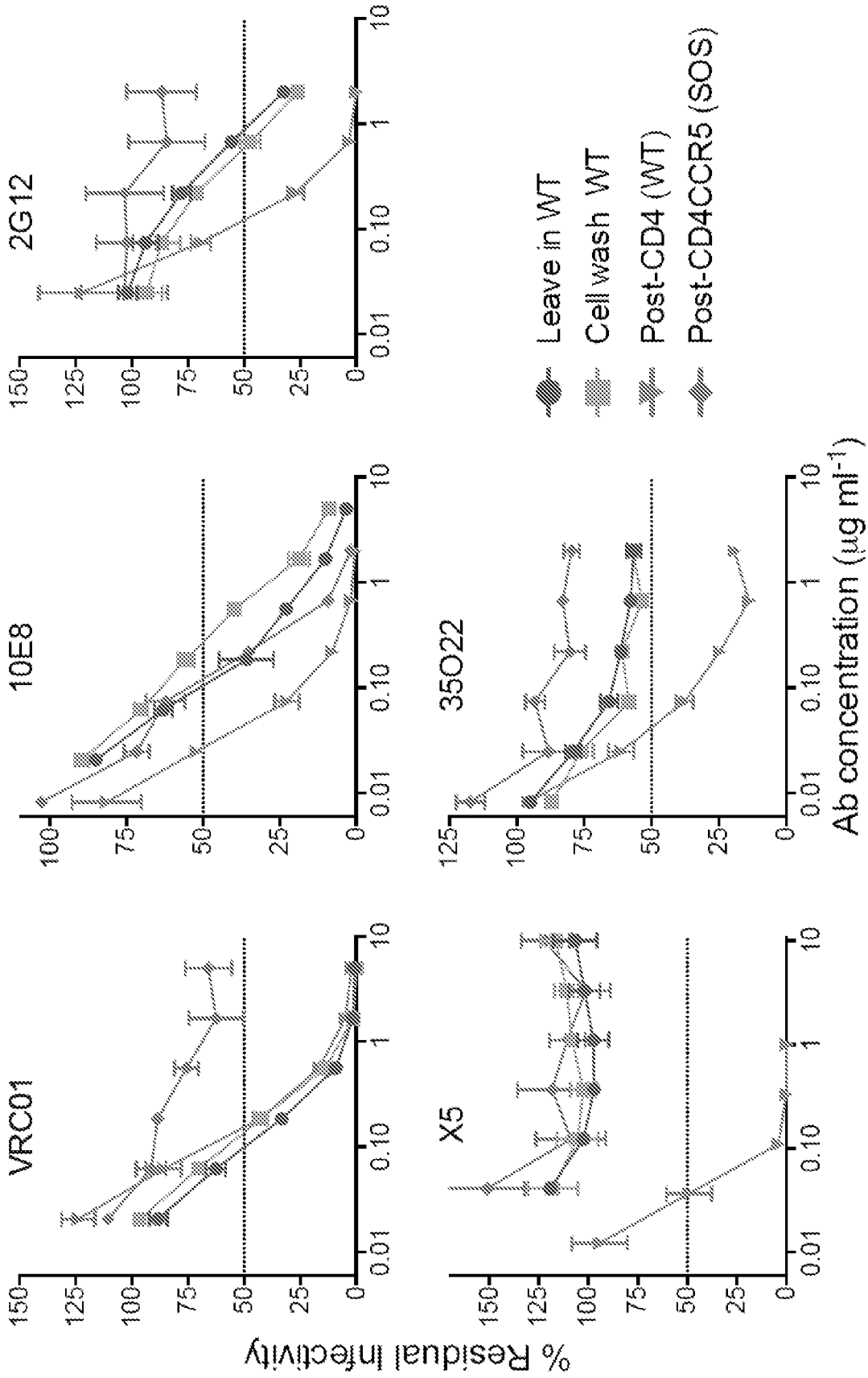

VRC01
10E8
2G12
X5
35O22
% Residual Infectivity
Ab concentration (μg ml-1)
Leave in WT
Cell wash WT
Post-CD4 (WT)
Post-CD4CCR5 (SOS)

FIG. 4E

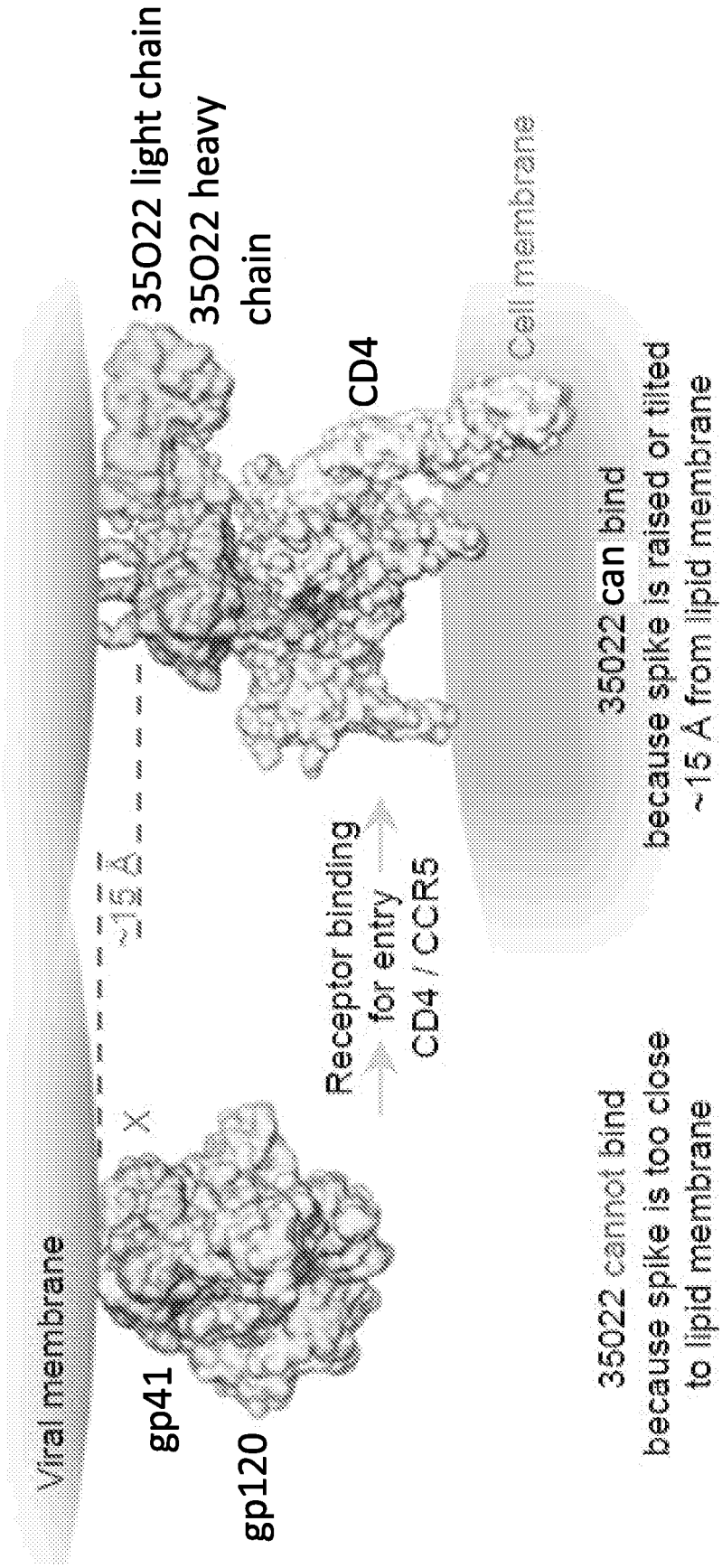

35O22 light chain
35O22 heavy chain
CD4
Cell membrane
Viral membrane
~16 Å
X
gp41
gp120
Receptor binding for entry
CD4 / CCR5
35O22 can bind because spike is raised or tilted ~15 Å from lipid membrane
35O22 cannot bind because spike is too close to lipid membrane

FIG. 5A

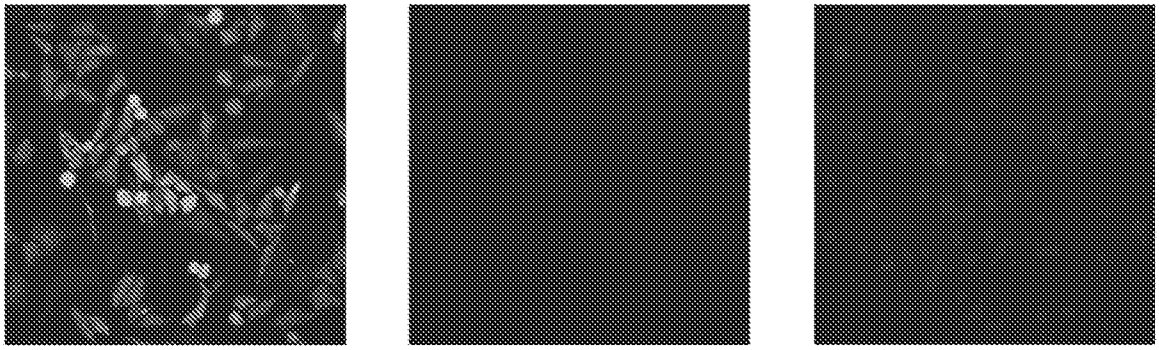


FIG. 5B

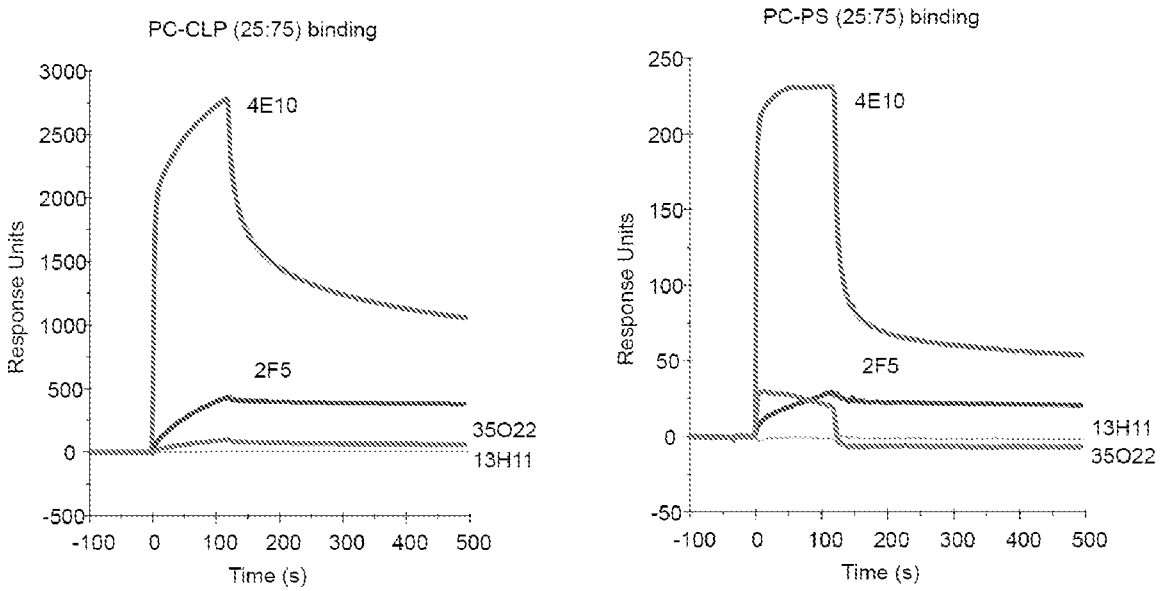


FIG. 5C

Sample ID	SSA	SSB	Sm	RNP	Scl 70	Jo 1	dsDNA	Cent B	Histone
Neg Control	-	-	-	-	-	-	-	-	-
Pos Control 1								273	
Pos Control 2	521	856				336	1036		409
Pos Control 3			658	340	322				
synagis	3	6	5	5	3	1	9	2	3
4E10	90	206	24	12	7	196	14	11	46
35O22	8	8	7	6	3	47	0	2	7

FIG. 6A

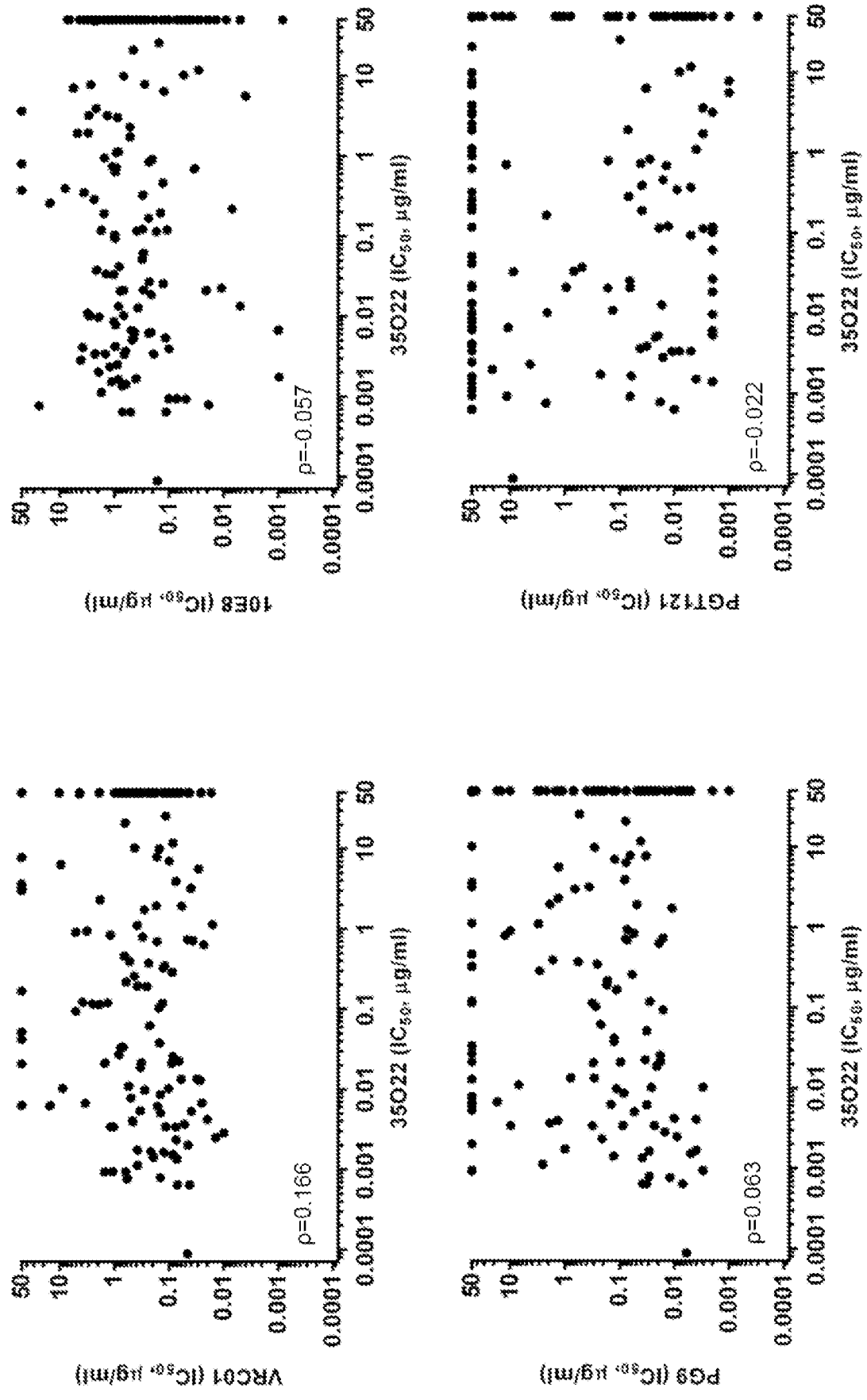

10E8 (IC50, µg/ml)
35O22 (IC50, µg/ml)
ρ=-0.057
PGT121 (IC50, µg/ml)
35O22 (IC50, µg/ml)
ρ=-0.022
VRC01 (IC50, µg/ml)
35O22 (IC50, µg/ml)
ρ=0.166
PG9 (IC50, µg/ml)
35O22 (IC50, µg/ml)
ρ=0.063
50
10
1
0.1
0.01
0.001
0.0001

FIG. 6C

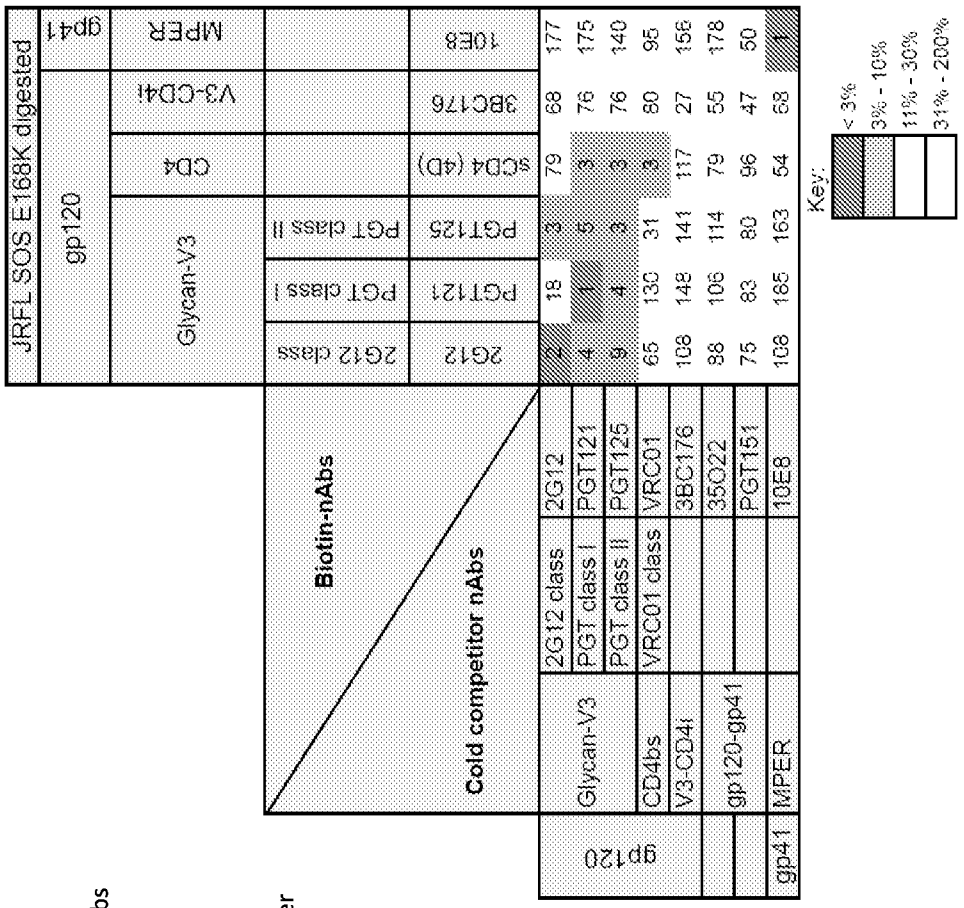

JRFL SOS E168K digested
gp41
gp120
MPER
V3-CD4i
CD4
Glycan-V3
2G12 class
PGT class I
PGT class II
2G12
PGT121
PGT125
10E8
3BC176
sCD4 (4D)
Biotin-nAbs
Cold competitor nAbs
gp120
gp41
Glycan-V3
CD4bs
V3-CD4i
gp120-gp41
MPER
35O22
Key:
< 3%
3% - 10%
11% - 30%
31% - 200%

FIG. 6B

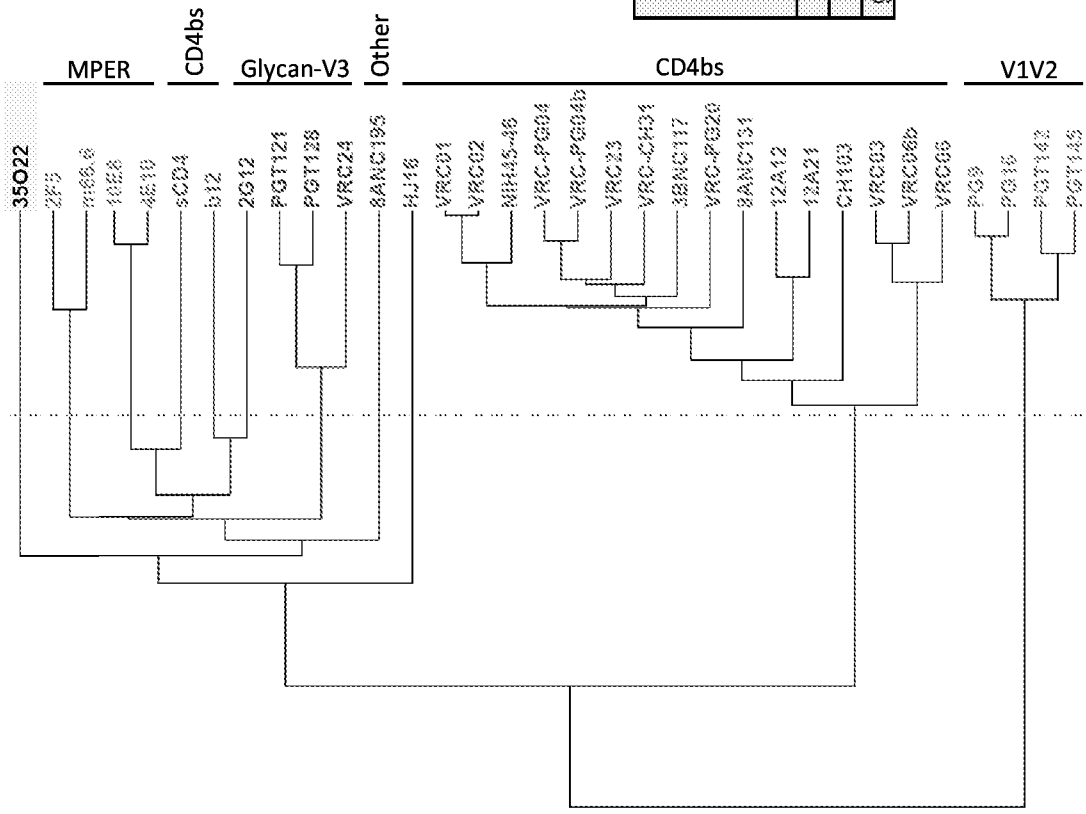

MPER
CD4bs
Glycan-V3
Other
CD4bs
V1V2
35O22
2F5
m66.6
10E8
4E10
sCD4
b12
2G12
PGT121
PGT128
VRC24
8ANC195
HJ16
VRC01
VRC02
NIH45-46
VRC-PG04
VRC-PG04b
VRC23
VRC-CH31
3BNC117
VRC-PG20
8ANC131
12A12
12A21
CH103
VRC03
VRC06b
VRC05
PG9
PG16
PGT142
PGT145

FIG. 7A

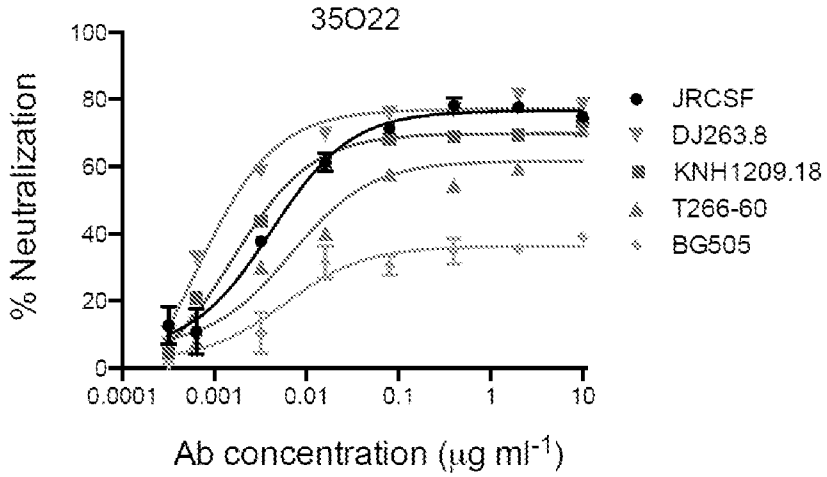
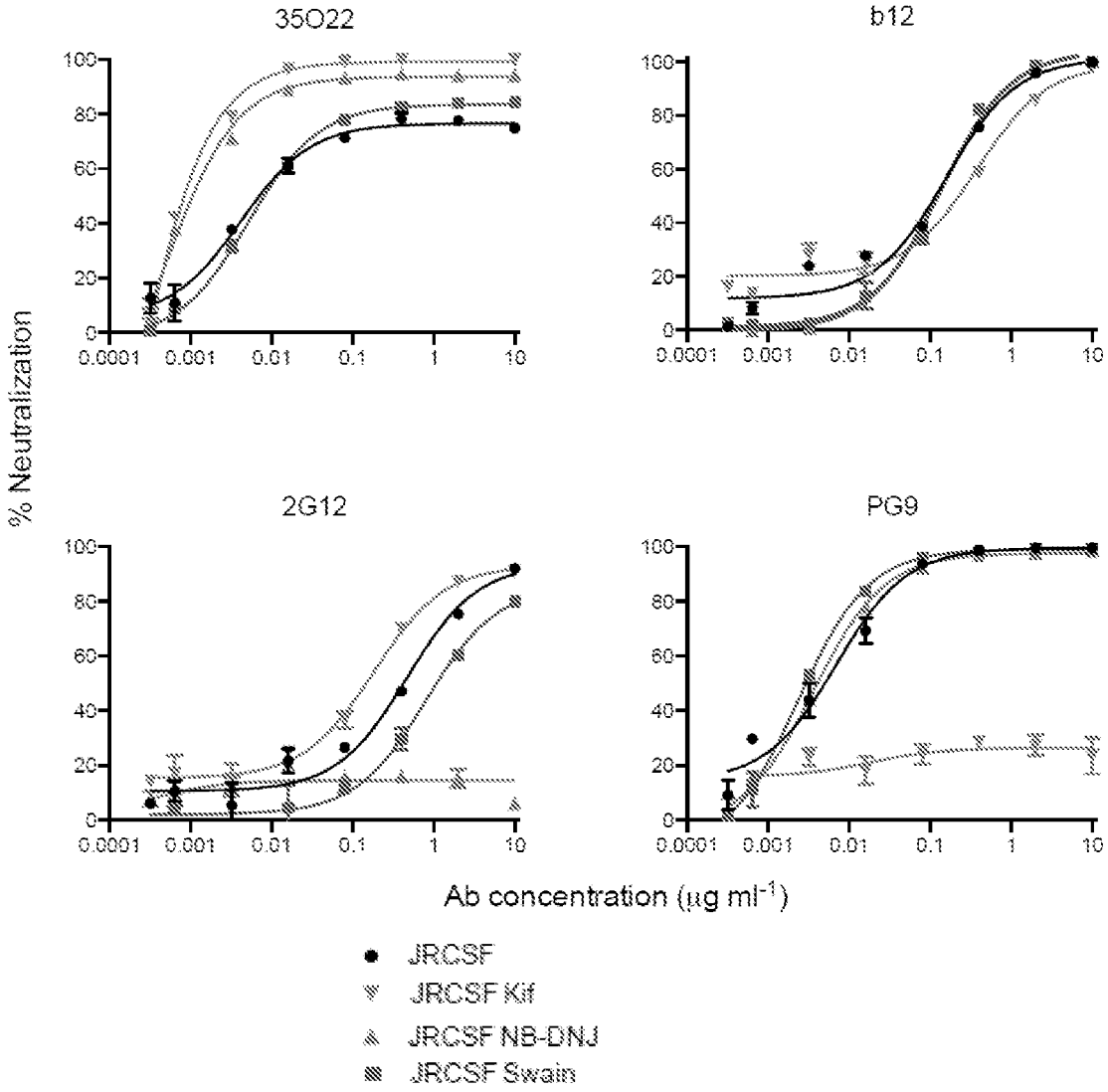

35O22
% Neutralization
100
80
60
40
20
0
0.0001
0.001
0.01
0.1
1
10
Ab concentration (μg ml-1)
JRCSF
DJ263.8
KNH1209.18
T266-60
BG505

FIG. 7B


35O22
b12
2G12
PG9
% Neutralization
Ab concentration (μg ml-1)
JRCSF
JRCSF Kif
JRCSF NB-DNJ
JRCSF Swain

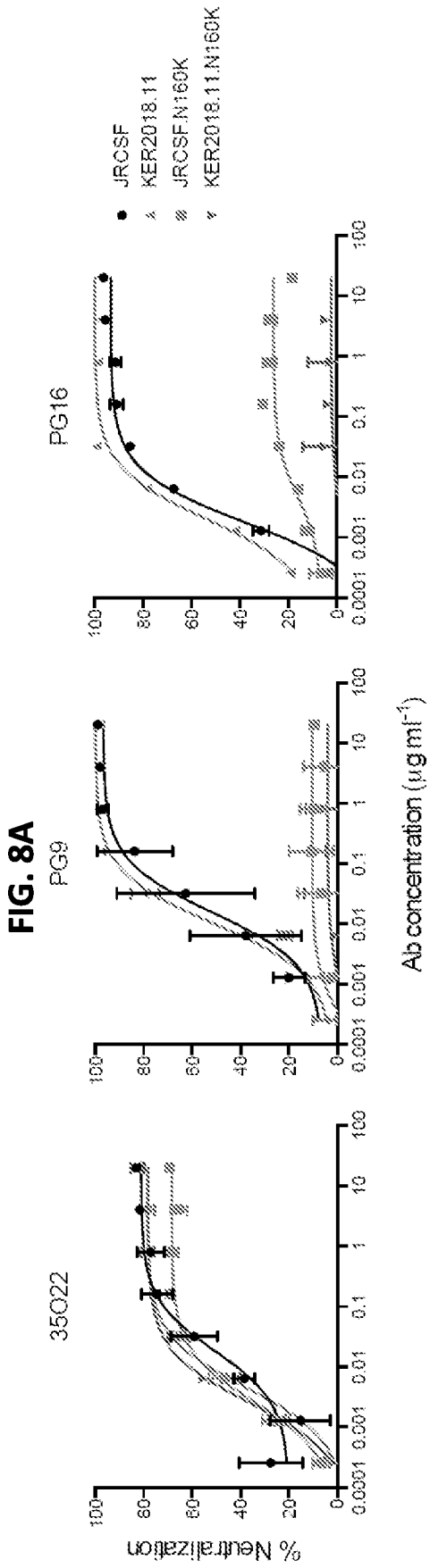

JRCSF
KER2018.11
JRCSF.N160K
KER2018.11.N160K
PG16
PG9
35O22
% Neutralization
Ab concentration (µg ml-1)

FIG. 8A

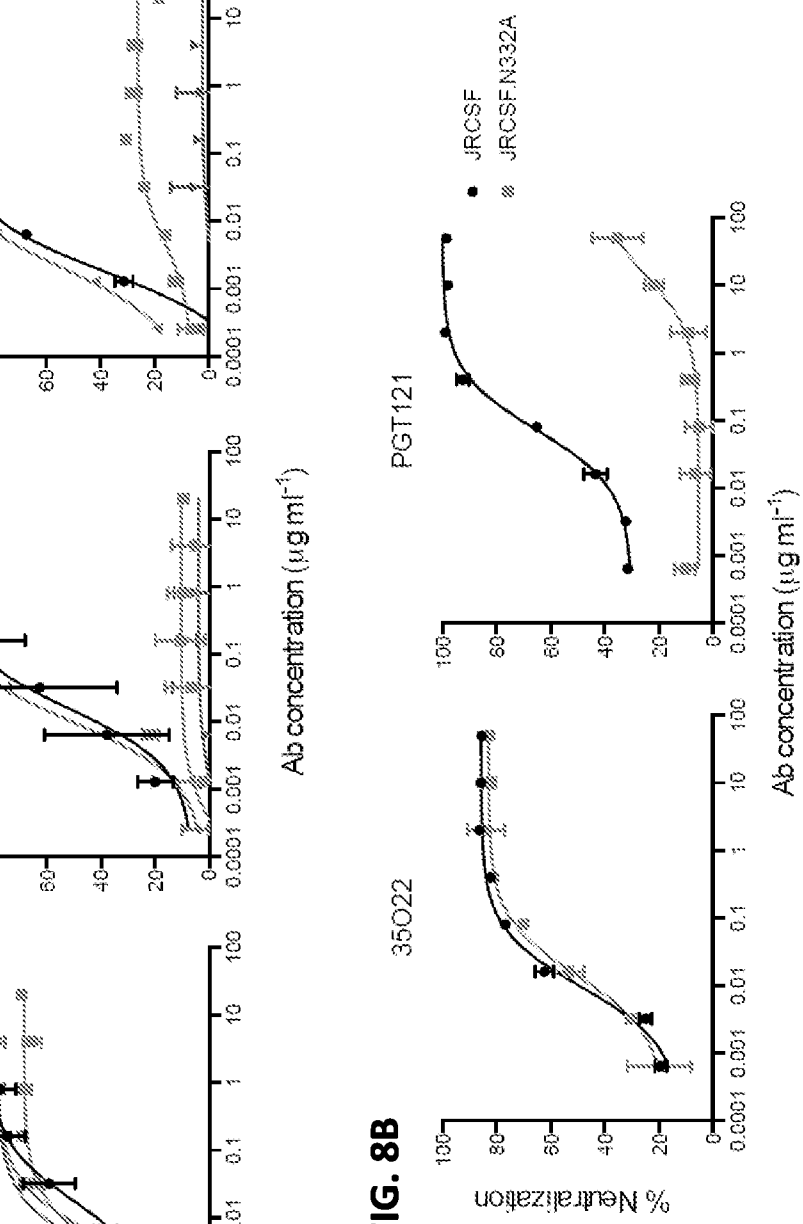

JRCSF
JRCSF.N332A
PGT121
35O22
% Neutralization
Ab concentration (µg ml-1)

FIG. 8B

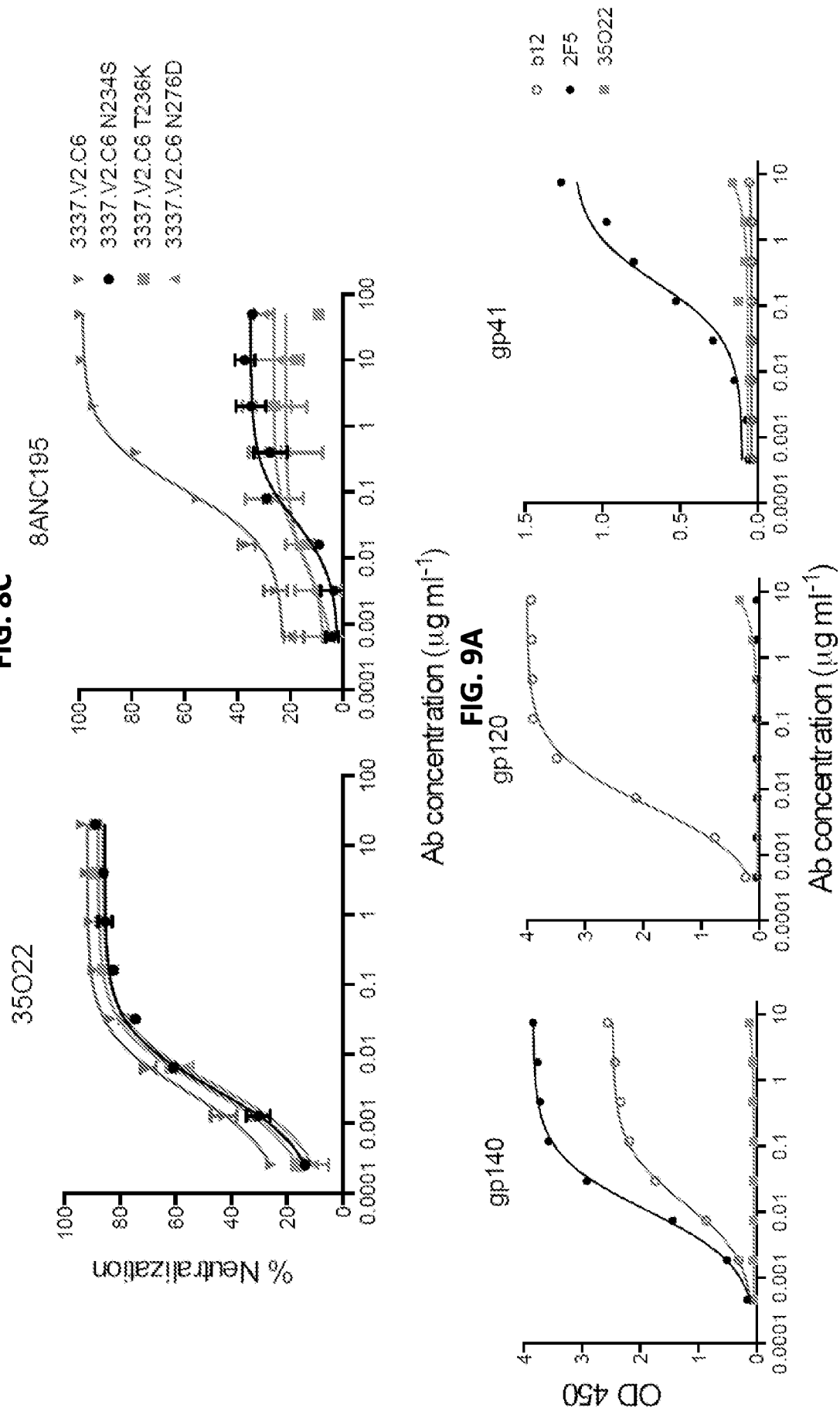


FIG. 8C
35O22
8ANC195
% Neutralization
3337.V2.C6
3337.V2.C6 N234S
3337.V2.C6 T236K
3337.V2.C6 N276D
FIG. 9A
gp140
gp120
gp41
OD 450
Ab concentration (μg ml-1)
b12
2F5
35O22

FIG. 9B

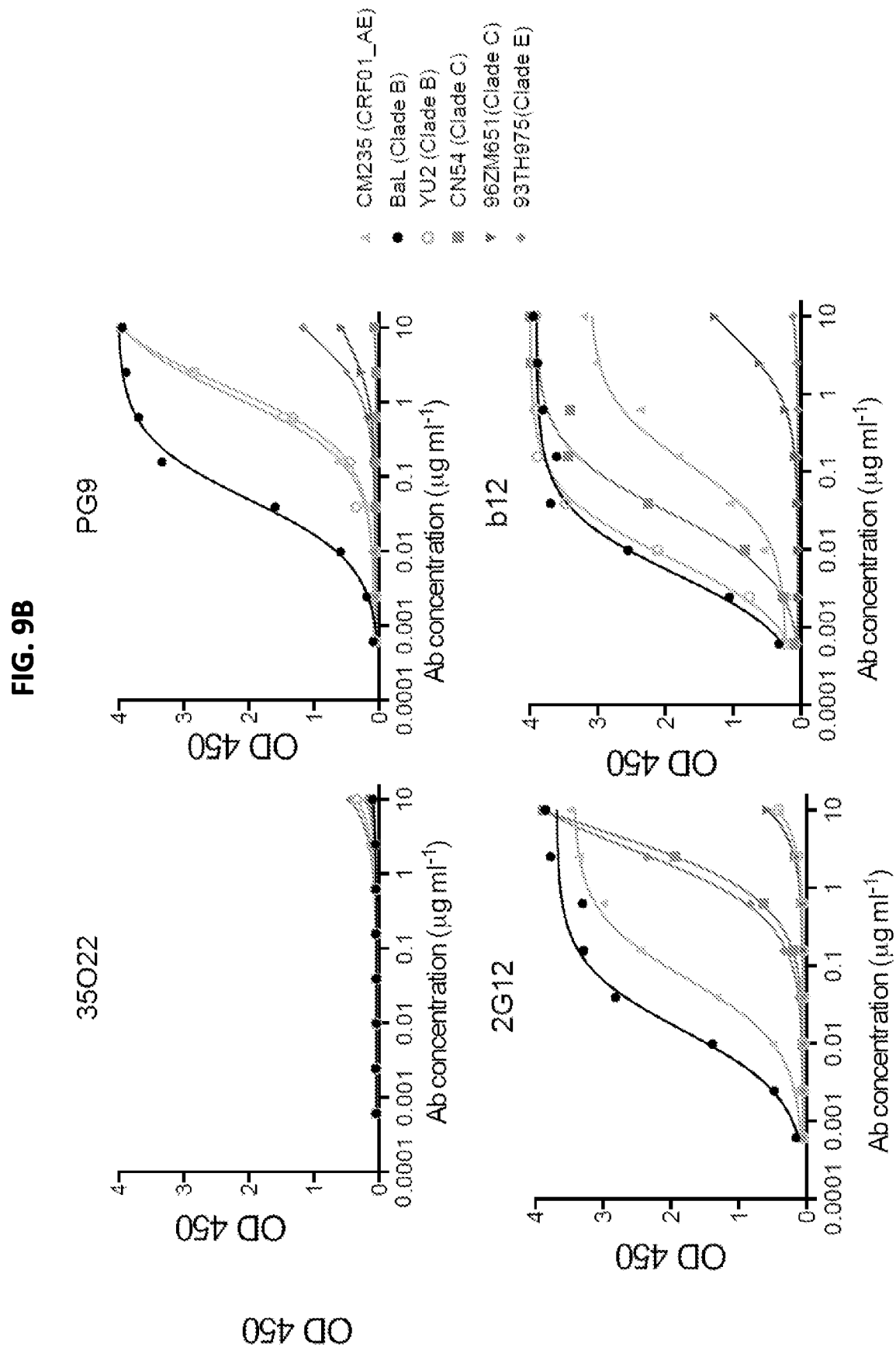

CM235 (CRF01_AE)
BaL (Clade B)
YU2 (Clade B)
CN54 (Clade C)
96ZM651(Clade C)
93TH975(Clade E)
PG9
b12
35O22
2G12
OD 450
Ab concentration (µg ml-1)
0.0001
0.001
0.01
0.1
1
10
0
1
2
3
4

FIG. 9C

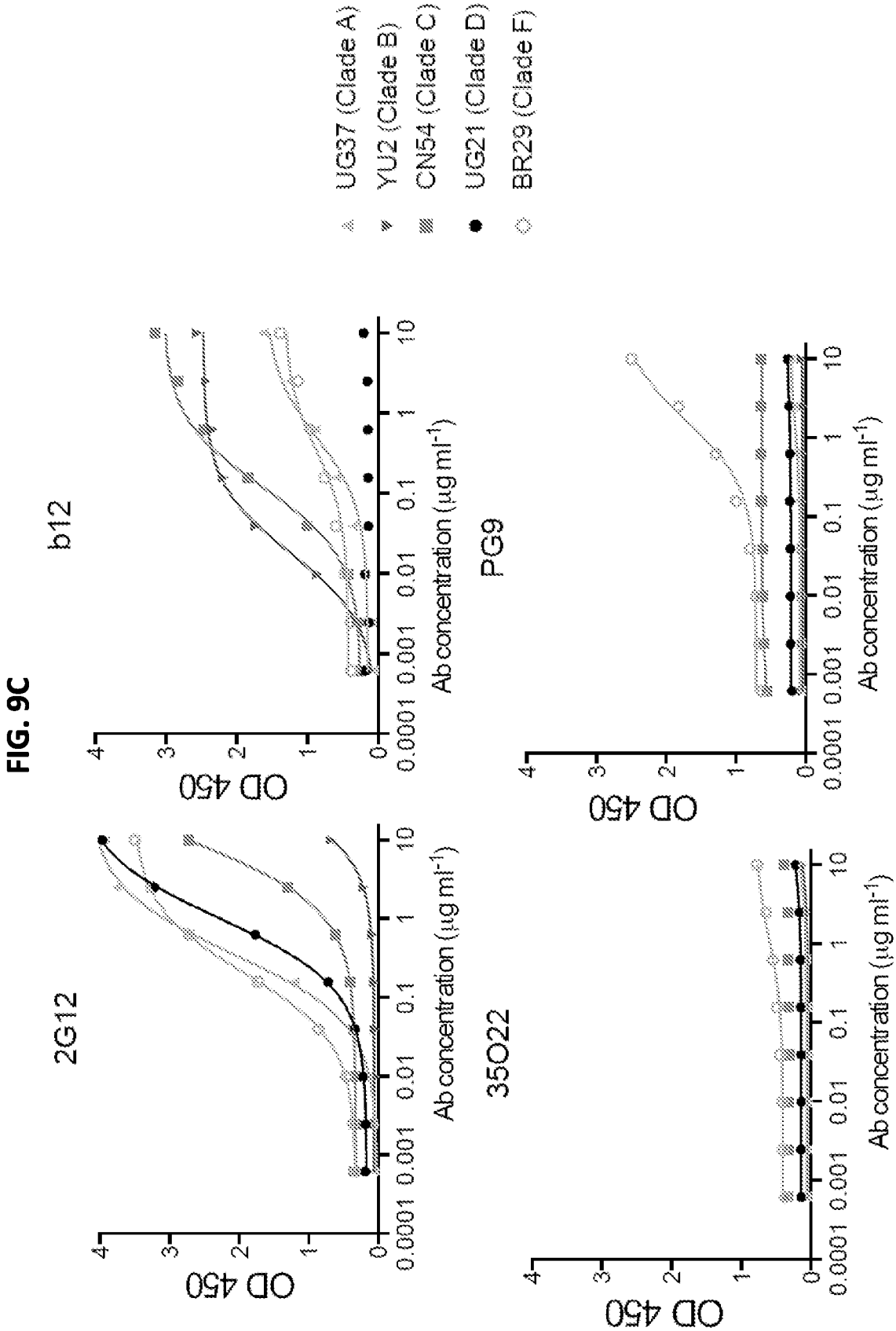

UG37 (Clade A)
YU2 (Clade B)
CN54 (Clade C)
UG21 (Clade D)
BR29 (Clade F)
b12
2G12
PG9
35O22
OD 450
Ab concentration (µg ml-1)
0.0001
0.001
0.01
0.1
1
10
0
1
2
3
4

FIG. 10A

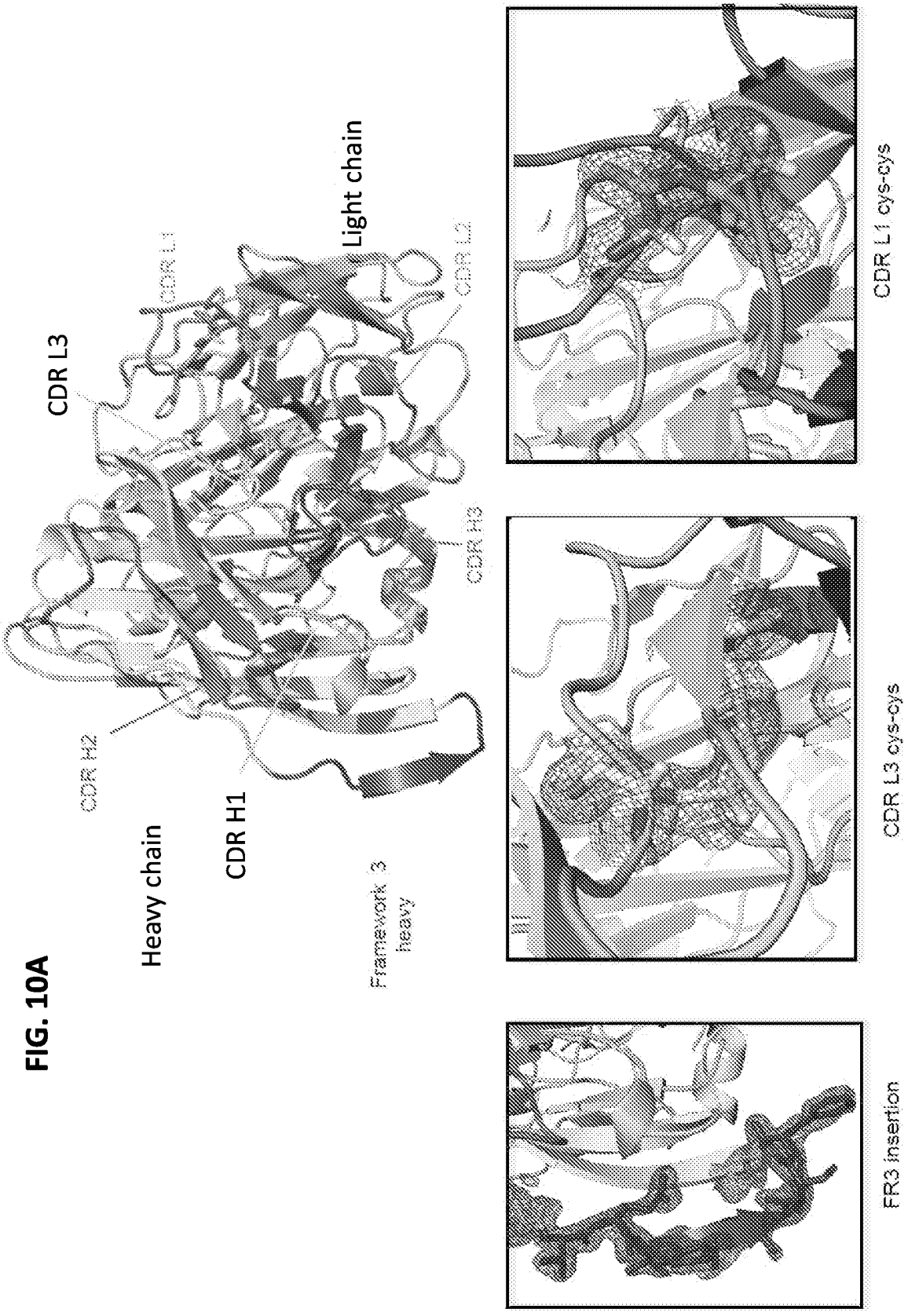

CDR L3
CDR L1
Light chain
CDR L2
CDR H2
CDR H3
Heavy chain
CDR H1
Framework 3 heavy
FR3 insertion
CDR L3 cys-cys
CDR L1 cys-cys

FIG. 10B

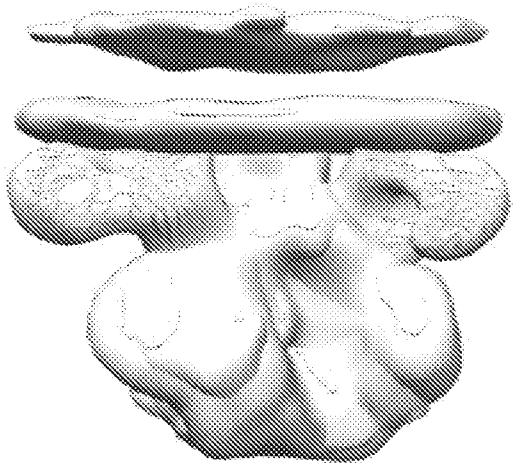


FIG. 11

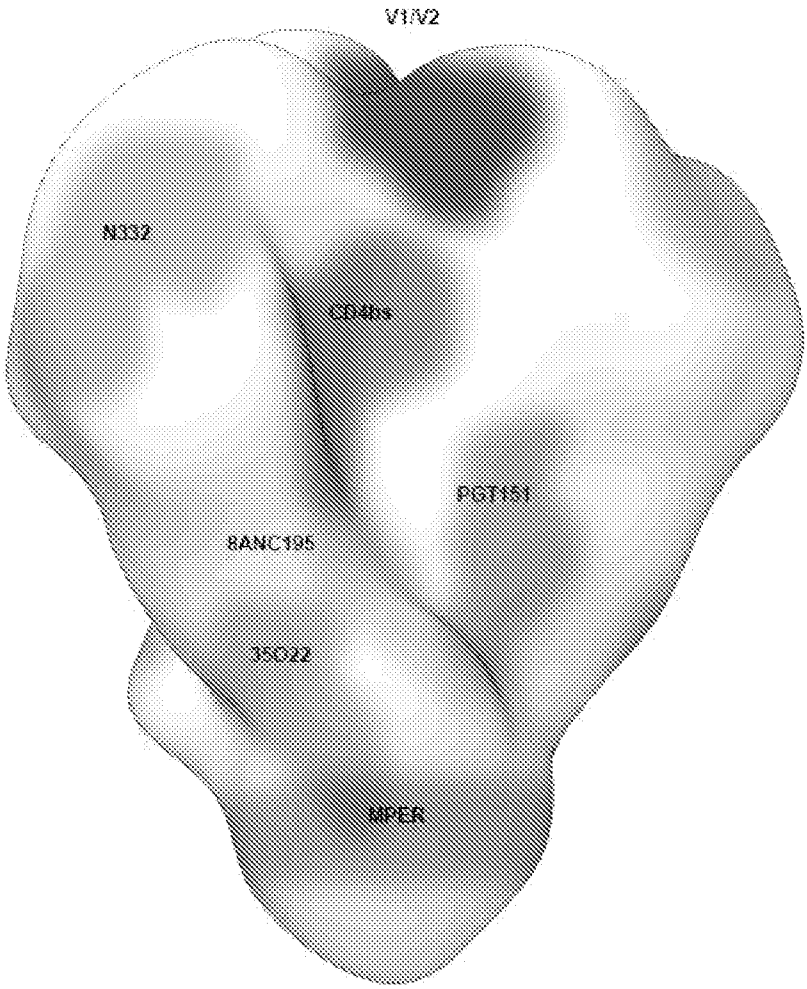


FIG. 12A

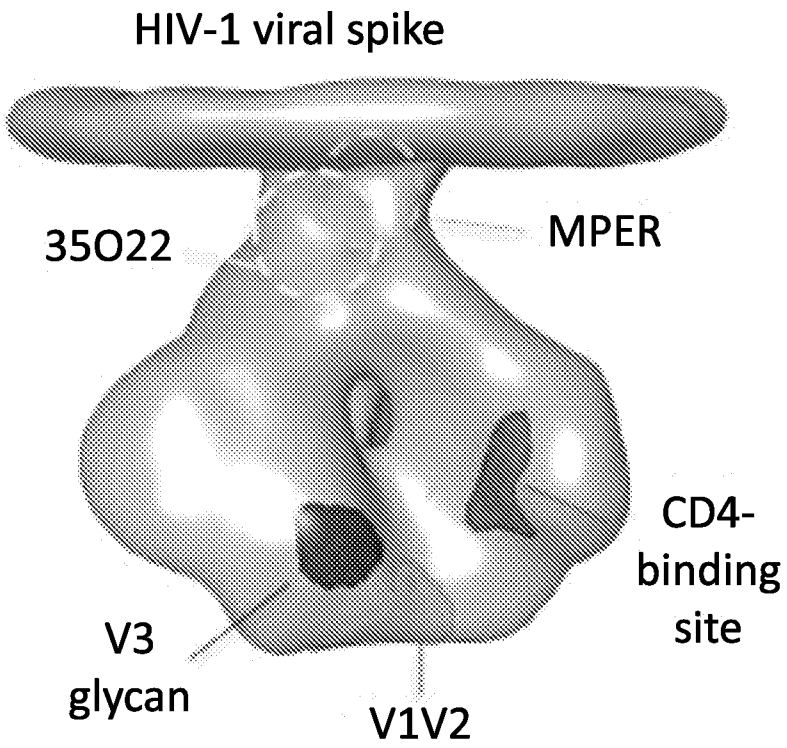


FIG. 12C

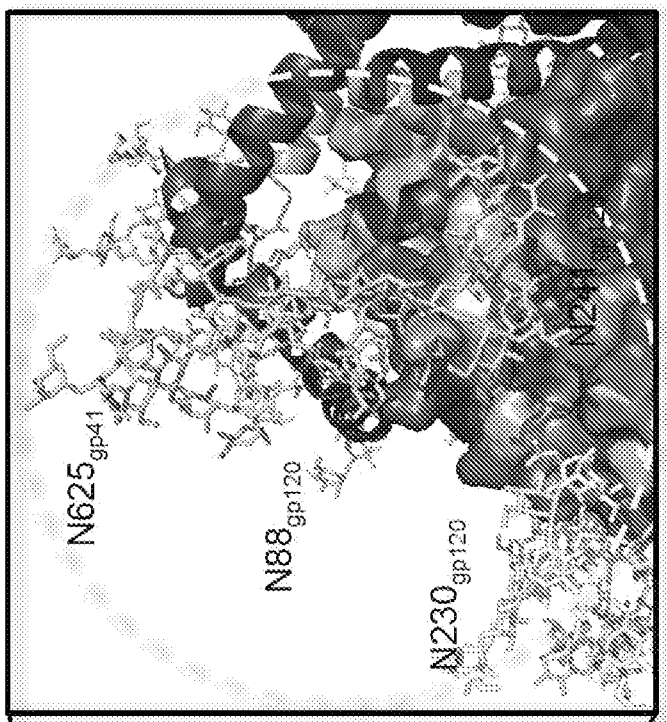


FIG. 12B

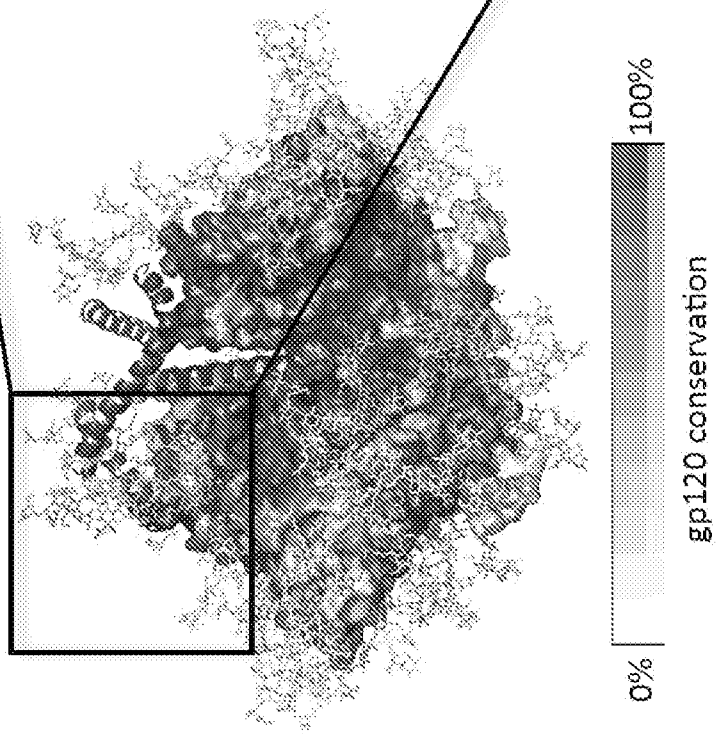


FIG. 12D

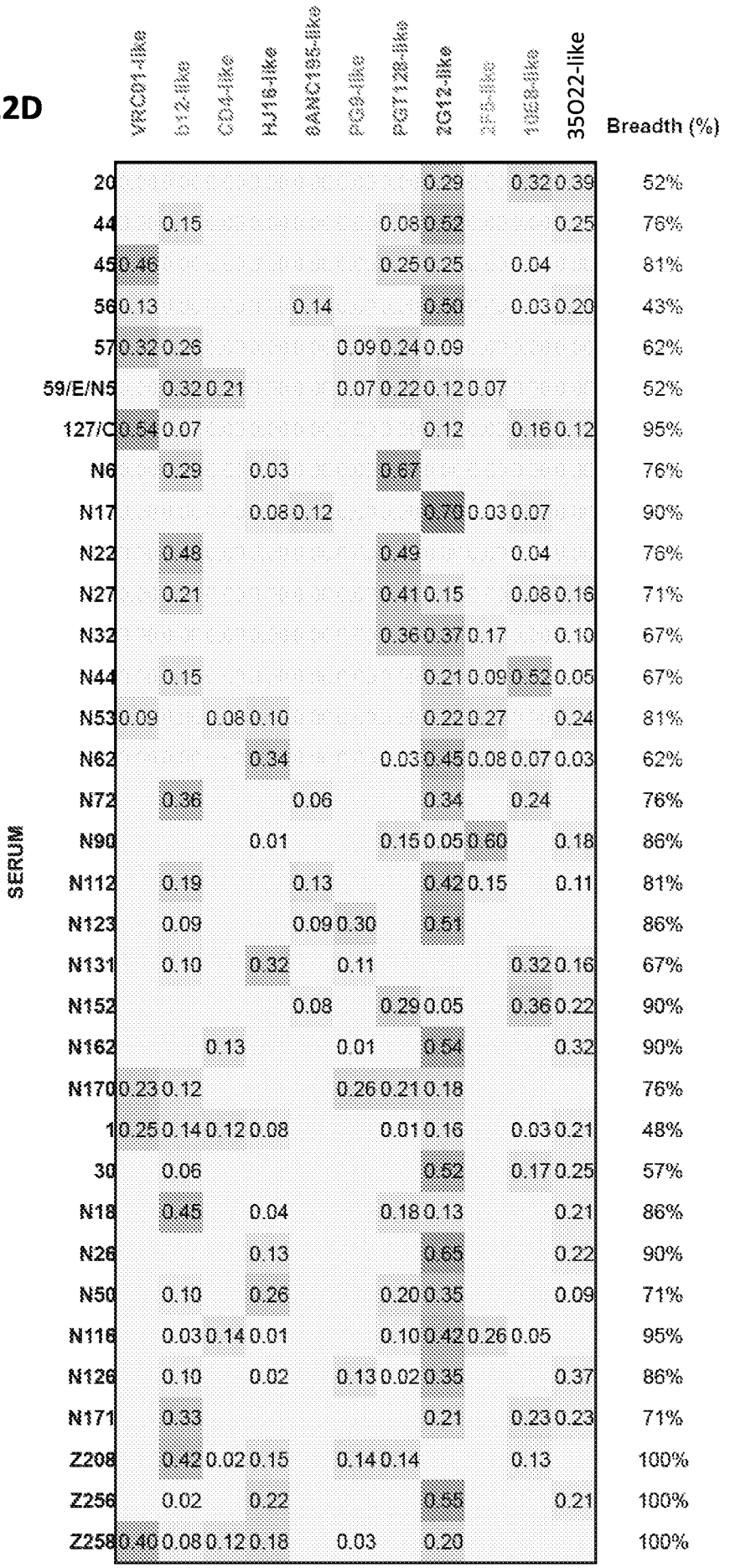

VRC01-like
b12-like
CD4-like
HJ16-like
8ANC195-like
PG9-like
PGT128-like
2G12-like
ZF5-like
10E8-like
35O22-like
Breadth (%)
SERUM
20 0.29 0.32 0.39 52%
44 0.15 0.08 0.52 0.25 76%
45 0.46 0.25 0.25 0.04 81%
56 0.13 0.14 0.50 0.03 0.20 43%
57 0.32 0.26 0.09 0.24 0.09 62%
59/E/N5 0.32 0.21 0.07 0.22 0.12 0.07 52%
127/C 0.54 0.07 0.12 0.16 0.12 95%
N6 0.29 0.03 0.67 76%
N17 0.08 0.12 0.70 0.03 0.07 90%
N22 0.48 0.49 0.04 76%
N27 0.21 0.41 0.15 0.08 0.16 71%
N32 0.36 0.37 0.17 0.10 67%
N44 0.15 0.21 0.09 0.52 0.05 67%
N53 0.09 0.08 0.10 0.22 0.27 0.24 81%
N62 0.34 0.03 0.45 0.08 0.07 0.03 62%
N72 0.36 0.06 0.34 0.24 76%
N90 0.01 0.15 0.05 0.60 0.18 86%
N112 0.19 0.13 0.42 0.15 0.11 81%
N123 0.09 0.09 0.30 0.51 86%
N131 0.10 0.32 0.11 0.32 0.16 67%
N152 0.08 0.29 0.05 0.36 0.22 90%
N162 0.13 0.01 0.54 0.32 90%
N170 0.23 0.12 0.26 0.21 0.18 76%
1 0.25 0.14 0.12 0.08 0.01 0.16 0.03 0.21 48%
30 0.06 0.52 0.17 0.25 57%
N18 0.45 0.04 0.18 0.13 0.21 86%
N26 0.13 0.65 0.22 90%
N50 0.10 0.26 0.20 0.35 0.09 71%
N116 0.03 0.14 0.01 0.10 0.42 0.26 0.05 95%
N126 0.10 0.02 0.13 0.02 0.35 0.37 86%
N171 0.33 0.21 0.23 0.23 71%
Z208 0.42 0.02 0.15 0.14 0.14 0.13 100%
Z256 0.02 0.22 0.55 0.21 100%
Z258 0.40 0.08 0.12 0.18 0.03 0.20 100%

FIG. 12E

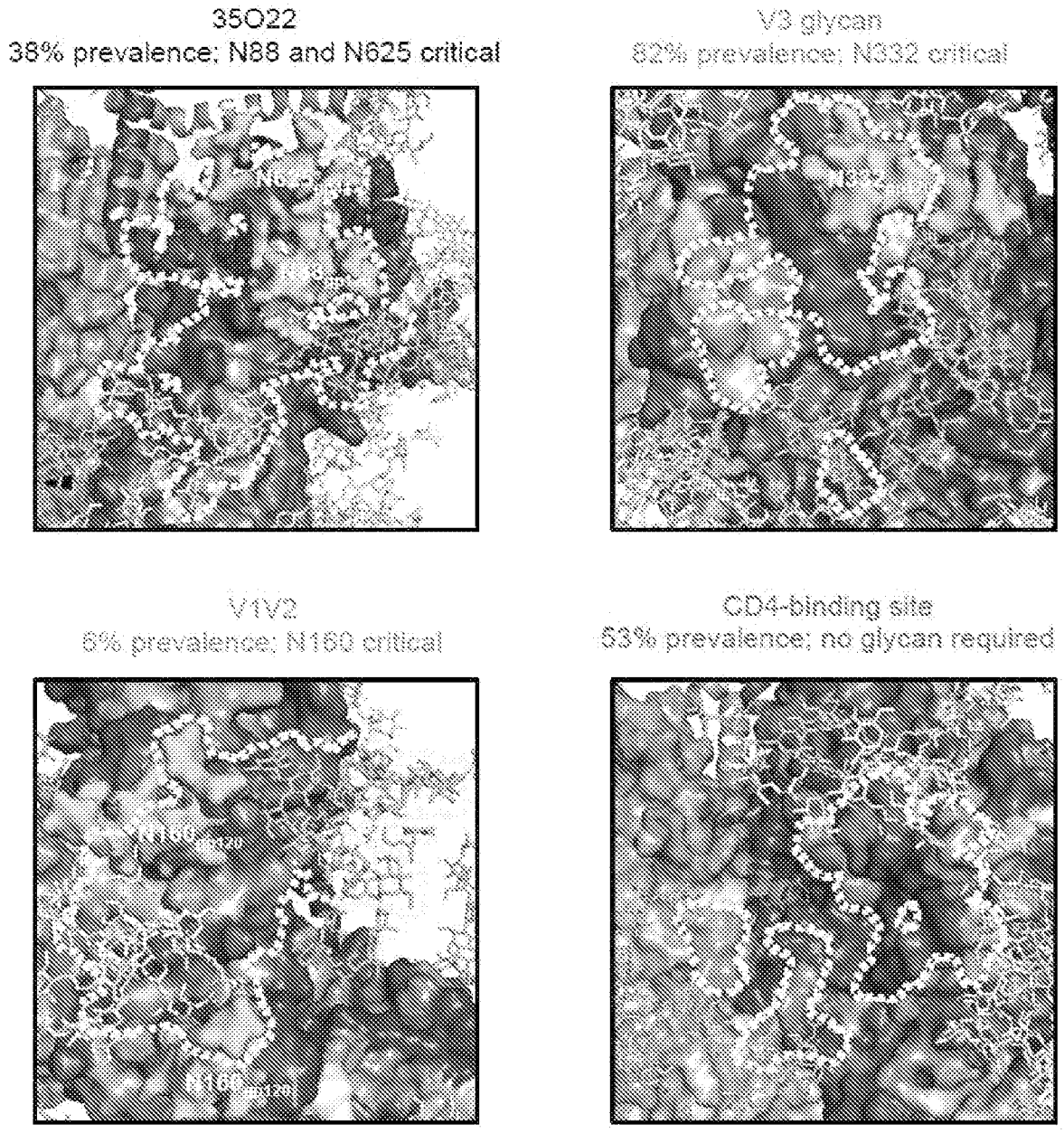

35O22
38% prevalence; N88 and N625 critical
V3 glycan
82% prevalence; N332 critical
V1V2
6% prevalence; N160 critical
CD4-binding site
53% prevalence; no glycan required

FIG. 13A

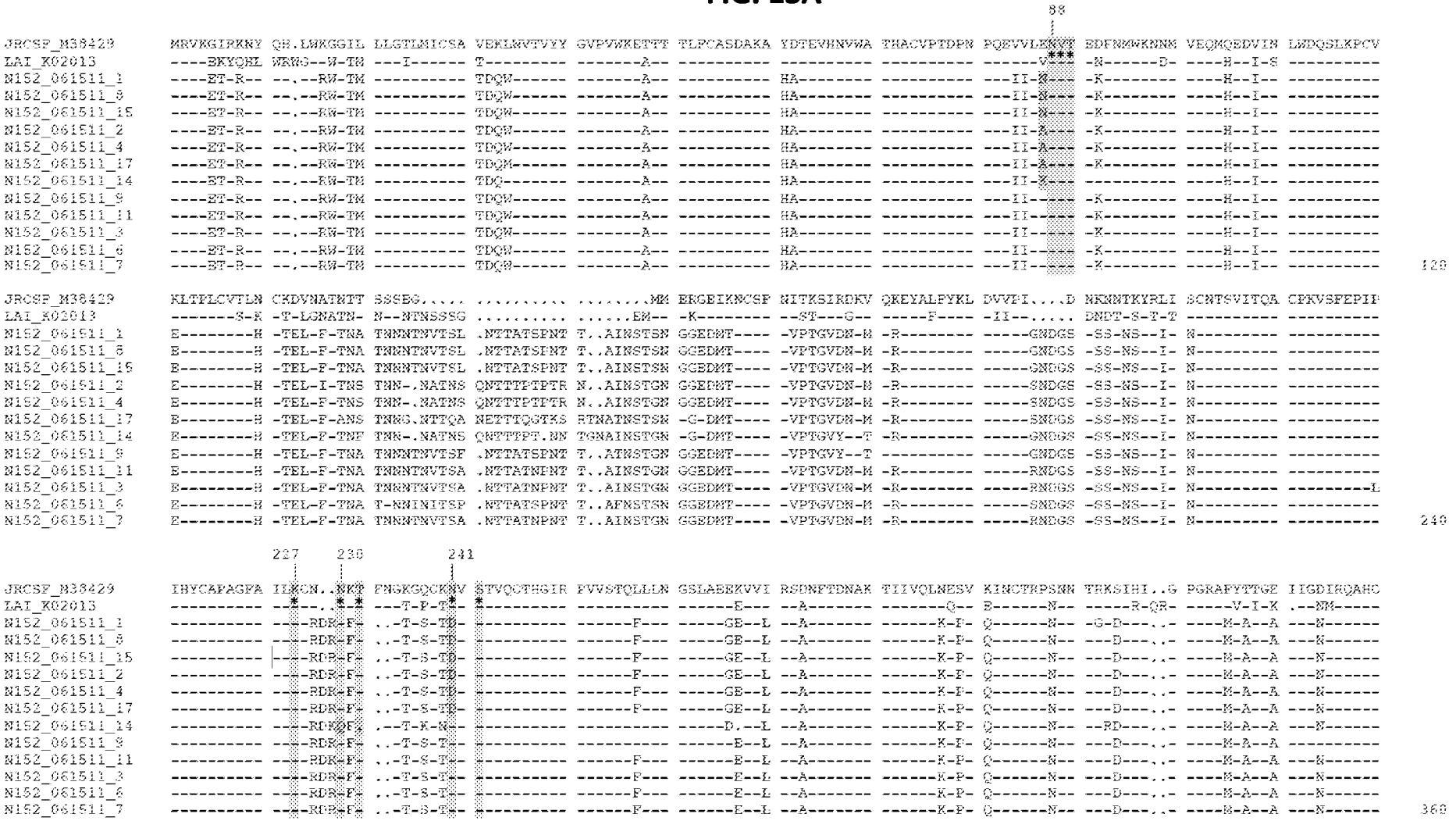

88
227
230
241
JRCSF_M38429
LAI_K02013
N152_061511_1
N152_061511_8
N152_061511_15
N152_061511_2
N152_061511_4
N152_061511_17
N152_061511_14
N152_061511_9
N152_061511_11
N152_061511_3
N152_061511_6
N152_061511_7
120
240
360

FIG. 13B

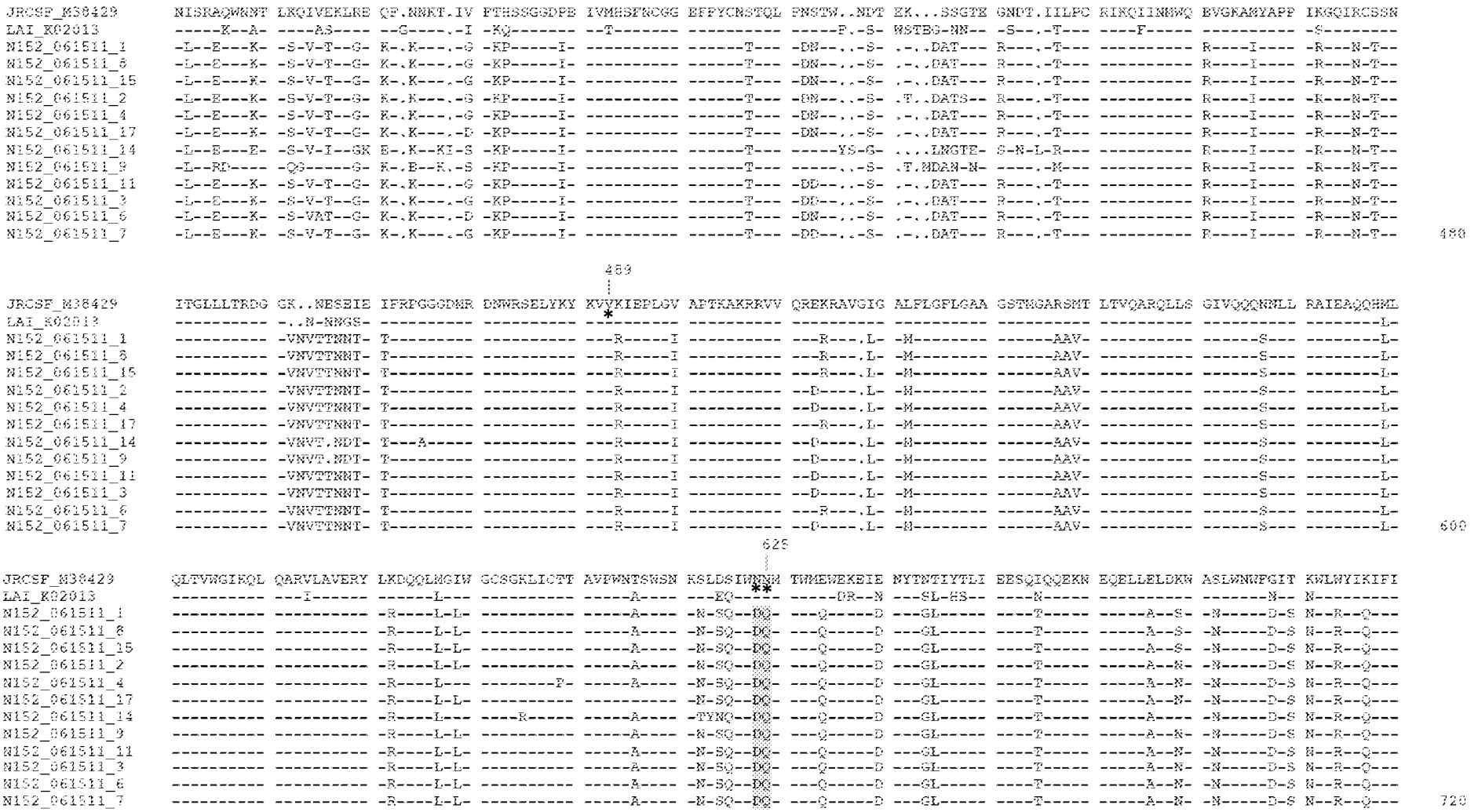

JRCSF_M38429
LAI_K02013
N152_061511_1
N152_061511_8
N152_061511_15
N152_061511_2
N152_061511_4
N152_061511_17
N152_061511_14
N152_061511_9
N152_061511_11
N152_061511_3
N152_061511_6
N152_061511_7
480
489
600
625
720

FIG. 13C

```
JRCSF_M38429      MIVGGLIGLR IVFSVLSIVN RVRQGYSPLS FQTLLPATRG PDRPEGIEEE GGERDRDRSG QLVNGFLALI WVDLRSLFLF SYHRLRDLLL TVTRIVELLG RRGWEILKYW WNLLQYWSQE
LAI_K02013        ------V--- ---A------ ---------- ---H--TP-- ---------- ----------I R----S---- -D-----C-- ---------- I---------- -----A---- ----------
N152_061511_1     ---------- ---L---V-- ---------- L--RF--P-- ------T--- ----------N ---D------ -------C-- -------F-- I---------- -----T---- ----------
N152_061511_8     ---------- ---L---V-- ---------L L--RF--P-- ------T--- ----------N ---D------ -------C-- -------F-- I---------- -----T---- ----------
N152_061511_15    ---------- ---L---V-- ---------- L--RF--P-- ------T--- ----------N R--D------ -------C-- -------F-- I----L---- -----T---- ----------
N152_061511_2     ---------- ---L---V-- ---------- L--RF--P-- ------T--- ---------- R--D------ -------C-- -------F-- I------I-- -----T---- ----------
N152_061511_4     ---------- ---L---V-- ---------- L--RF--P-- ------T--- ---------- R--D------ -------C-- -------F-- I----L---- -----T---- ----------
N152_061511_17    ---------- ---L---V-- ---------- L--RF--P-- ------T--- ---------- R--D------ -------C-- -------F-- I----L---- -----T---- ----------
N152_061511_14    ---------- ---L---V-- ---------- L--RF--P-- ------T--- ----------N ---D------ -------C-- -------F-- I---V----- -----T---- ----------
N152_061511_9     ---------- ---L---V-- ---------- L--RF--P-- ------T--- ----------N ---D------ -------C-- -------F-- I----L---- -----T---- ----------
N152_061511_11    ---------- ---L---V-- ---------- L--RF--PK- ------T--- ---------- R--D------ -------C-- -------F-- I----L-I-- -----T---- ----------
N152_061511_3     ---------- ---L---V-- ---------- L--RF--P-- ------T--- ---------- R--D------ -------C-- -------F-- I----L-I-- -----T---- ----------
N152_061511_6     ---------- ---L---V-- ---------- L--RF--P-- ------T--- ----------N ---D------ -------C-- -------F-- I---------- -----T---- ----------
N152_061511_7     ---------- ---L---V-- ---------- L--RF--P-- ------T--- ---------- R--D------ -------C-- -------F-- I----L-I-- -----T---- ---------- 840

JRCSF_M38429      LKNSAVSLLN ATAIAVAEGT DRIIEVVQRV YRAILHIPTR IRQGLERALL *
LAI_K02013        ---------- ---------- --V-----GA C---R---R- -------I-- *
N152_061511_1     -QK-----F- S--------- --V------A C--------- ---------Q *
N152_061511_8     -QK-----F- S--------- --V------A C--------- ---------Q *
N152_061511_15    -QK-----F- S--------- --V------A C--------- ---------Q *
N152_061511_2     -QK-----F- S--------- --V------A C--------- ---------Q *
N152_061511_4     --K-----F- S--------- ---------A C--------- ---------Q *
N152_061511_17    -RK--I--F- S--------- --V---I--T C--------- ---------Q *
N152_061511_14    -QK-----F- S--------- --V------A C--------- ---------Q *
N152_061511_9     -QK-----F- S--------- --V------A C--------- ---------Q *
N152_061511_11    -QK-----F- S--------- --V------A C--------- ---------Q *
N152_061511_3     -QK-----F- S--------- --V------A C--------- ---------Q *
N152_061511_6     -QK-----F- S--------- ---------A C--------- ---------Q *
N152_061511_7     -QK-----F- S--------- --V------A C--------- ---------Q *      890
```

FIG. 13D

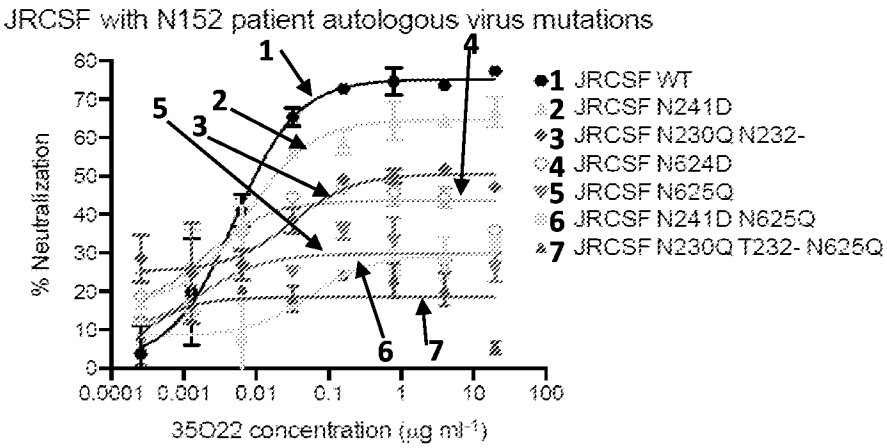


FIG. 14

	ID50					Fold change*			
Sera ID	WT	N88A	N230A	N241A	N625A	N88A	N230A	N241A	N625A
20	6864	7396	5295	5261	5781	0.9	1.3	1.3	1.2
44	6095	2824	1177	2632	776	2.2	5.2	2.3	7.9
45	4019	2422	2094	1678	3265	1.7	1.9	2.4	1.2
56	655	1124	2017	89	1901	0.6	0.3	7.4	0.3
57	7586	626	4981	393	355	12.1	1.5	19.3	21.4
127/C	5217	820	1829	1082	789	6.4	2.9	4.8	6.6
N6	5966	214	437	436	679	27.9	13.7	13.7	8.8
N17	62354	1121	743	2279	1653	55.6	83.9	27.4	37.7
N22	9735	932	3017	1591	3523	10.4	3.2	6.1	2.8
N32	3131	539	1238	2538	686	5.8	2.5	1.2	4.6
N44	5439	1532	2110	2622	2003	3.6	2.6	2.1	2.7
N53	6690	963	1048	352	1044	6.9	6.4	19.0	6.4
N72	6722	1484	1058	199	2482	4.5	6.4	33.8	2.7
N90	4275	3853	1479	1018	10576	1.1	2.9	4.2	0.4
N112	5903	2344	967	706	6071	2.5	6.1	8.4	1.0
N123	21392	1194	1217	771	824	17.9	17.6	27.7	26.0
N131	18777	106	402	163	275	177.1	46.7	115.2	68.3
N152	36636	6677	28758	4339	4279	5.5	1.3	8.4	8.6
N162	17492	558	4078	718	1739	31.3	4.3	24.4	10.1
N170	16472	1342	6564	990	448	12.3	2.5	16.6	36.8
1	3485	1605	343	197	256	2.2	10.2	17.7	13.6
30	14255	5620	3314	13125	6221	2.5	4.3	1.1	2.3
N26	19628	1853	2443	1951	3917	10.6	8.0	10.1	5.0
N18	60183	580	1202	480	656	103.8	50.1	125.4	91.7
N116	586	2504	2176	1948	616	0.2	0.3	0.3	1.0
N27	1088	1446	4656	838	790	0.8	0.2	1.3	1.4
N62	907	536	462	429	263	1.7	2.0	2.1	3.4
N126	2502	1132	1146	3554	1807	2.2	2.2	0.7	1.4
N171	12329	5967	3125	1948	1904	2.1	3.9	6.3	6.5
Z208	21501	9337	4338	8740	1815	2.3	5.0	2.5	11.8
Z256	11467	1584	1881	2055	1240	7.2	6.1	5.6	9.2
Z258	31647	3154	2018	2094	4114	10.0	15.7	15.1	7.7
mAb			**IC50**				**Fold change†**		
10E8	0.078	0.0098	0.01984	0.04513	0.01757	0.13	0.3	0.58	0.23
PGT121	0.01508	0.02161	0.02053	0.02399	0.02431	1.43	1.4	1.59	1.61
PG9	0.00692	0.00216	0.0022	0.00189	0.00238	0.31	0.3	0.27	0.34
35O22	0.0106	>20	>20	>20	>20	>1886	>1886	>1886	>1886

FIG. 15A

Neutralization by 35O22 and its variants against an 8-isolate Env-pseudovirus mini-panel.								
	Antibody Variants							
VirusID	**35O22**	**7B9**	**4O20**	**10J4**	**7K3**	**13I10**	**2N5**	**10M6**
Q168.a2	0.009	3.7	>20	>20	3.28	>20	>20	>20
RW020.2	0.010	0.853	0.041	>20	>20	>20	>20	>20
Q769.h55	0.004	0.593	>20	>20	>20	>20	>20	>20
JRFL.JB	0.013	1.36	0.073	3.93	>20	>20	0.098	>20
THRO.18	0.908	>20	>20	>20	>20	>20	>20	>20
CAAN.A2	0.003	0.212	0.007	0.006	0.004	0.005	>20	0.003
ZAO12.29	>20	>20	>20	>20	>20	13.8	>20	>20
DU156.12	>20	>20	>20	>20	>20	>20	>20	>20

FIG. 15B

Neutralization Profile of patient N152 serum and monoclonal antibodies							
Clade	**VirusID**	**Serum[a]**	**Monoclonal antibody**				
		N152	**35O22**	**10E8**	**VRC01**	**PG9**	**PGT121**
A	KER2018	397	0.004	2.71	0.070	0.0009	>50
	RWO20.2	1341	0.010	1.30	0.303	0.103	0.002
	Q168.a2	509	0.009	0.768	0.140	0.106	>50
	Q769.d22	1834	0.002	1.11	0.015	0.007	>50
	Q769.h5	836	0.004	2.74	0.014	0.002	>50
B	JRFL.JB	860	0.013	0.247	0.033	>50	0.017
	BaL.01	1590	0.003	0.715	0.102	0.052	0.011
	YU2.DG	279	0.287	0.895	0.055	3.69	0.068
	PVO.04	359	0.011	1.78	0.386	6.24	0.132
	TRO.11	2773	>50	0.026	0.343	41.5	0.006
	CAAN.A2	1364	0.003	1.37	1.06	13.0	0.005
	TRJO.58	1231	0.002	0.881	0.079	0.246	4.31
	THRO.18	351	0.908	0.048	4.42	15.0	>50
	BG1168.1	167	>50	0.136	0.449	>50	>50
	6101.1	1372	>50	0.009	0.104	>50	0.002
C	ZA012.29	39	>50	0.827	0.250	27.0	0.005
	DU156.12	2021	11.7	0.007	0.082	0.023	0.005
	DU422.01	325	0.021	1.06	>50	0.303	0.164
	ZM106.9	256	0.372	>50	0.248	0.639	0.005
	ZM55.28a	85	1.93	2.31	0.144	0.571	0.070
Control	SIVmac251.SG3	5	>50	>50	>50	>50	>50
Geo. Mean IC_{50} ($\mu g\ ml^{-1}$)			0.031	0.417	0.149	0.355	0.020
Median IC_{50} ($\mu g\ ml^{-1}$)			0.010	0.881	0.140	0.303	0.009

[a]The data for N152 shows the ID50 of serum against each virus. [b]The data for the monoclonal antibodies shows the IC50.

FIG. 16A

35O22 and its variants V_H (Kabat Numbering)

```
             1       10        20        30          40         5052A   57 60
             |........|.........|.........|  .....  ....|........  |.||....|..|.....
Heavy Chain  ___________FR1_________________ _CDR1 _____FR2______ ___CDR2__________
IGHV1-18*02  QVQLVQSGAEVKKPGASVKVSCKASGYTFT  SYGIS  WVRQAPGQGLEWMG  WISAYNGNTNYAQKLQG
       10J4  QGQLVQSGGELKKPGASVKISCKTSGYRFS  FYHIN  WIRQLVGRGPEWMG  WISPYNGGTNLAPELRG
       10M6  QGQLVQSGGELKRPGASVKISCKTSGYRFS  SYHIN  WIRQVIGRGPEWMG  WISPYSGGTNLAPEFRG
      13I10  QGQLVQSGGELKKPGASVKISCKTSGYRFS  FYHIN  WIRQVVGRGPEWMG  WISPYNGGTNLAPEFRG
        7K3  QGQLVQSGGELKKPGASVKISCKTSGYRFS  FYHIN  WIRQVSGRGPEWMG  WISPYSGDTNLAPDFRG
        2N5  QGQLVQSGAELKKPGASVKISCKTSGYKFS  FFHIN  WIRQTAGRGPEWLG  WISPYSGDKNYAPAFQD
       4O20  QGQLVQSGAELKKPGGSVKISCKTSGYRFN  FYHIN  WIRQTAGRGPEWMG  WISPYSGDKNLAPAFQD
        7B9  QGQLVQSGAELKKPGDSVKISCKTSGYRFN  FYHIN  WIRQTAGRGPEWMG  WISPYSGDKNLAPAFQD
      35O22  QGQLVQSGAELKKPGASVKISCKTSGYRFN  FYHIN  WIRQTAGRGPEWMG  WISPYSGDKNLAPAFQD

                7072ABCDEFGH      8082ABC      90         100ABCDEF 103    110
             ....|.|||||||||.......|.||||.......|....  .....|||||||..  |......|...
Heavy Chain  ____________FR3__________________________  _____CDR3_____  _FR4(HJ4*02)
IGHV1-18*02  RVTMTTD--------TSTSTAYMELRSLRSDDTAVYYCAR     RDGYNYYFDY     WGQGTLVTVSS
       10J4  RLVLTTEREVVDTMTLSTGTAHMELRNLRSDDTGIYFCAK  GLLRDGSSTWLPHL  WGQGTLLTVSS
       10M6  RLVLTTEREVVDTMTLSTGTAHMELRNLKSDDTGIYFCAK  GLLRDGSSTWLPHL  WGQGTLLTVSS
      13I10  RLVLTTEREVVDTMTMSTGTAHMELRNLKSDDTGLYFCAK  GLLRDGPSTWLPHL  WGQGTLLTVSS
        7K3  RVVLTTDREVVDTMTMSTGTAHMELRNLKSDDTGLYFCAK  GLLRDGSSTWLPHL  WGQGTLLTVSS
        2N5  RVIMTTDKEVPVTSFTSTGTAYLEIRSLKPDDTGIYFCAR  GLLRDGSSTWLPYL  WGQGTLLTVSS
       4O20  RVIMTTDKEVPVTAFTSTGTAYMEIRNLKFDDTGIYFCAK  GLLRDGSSTWLPYL  WGQGTLLTVSS
        7B9  RVIMTTDNEVPVTAFTSTGTAYMEIRNLRFDDTGIYFCAK  GLLRDGSSTWLPYL  WGQGTLLTVSS
      35O22  RVIMTTDTEVPVTSFTSTGAAYMEIRNLKFDDTGTYFCAK  GLLRDGSSTWLPYL  WGQGTLLTVSS
```

FIG. 16B

35O22 and its variants V_L (Kabat Numbering)

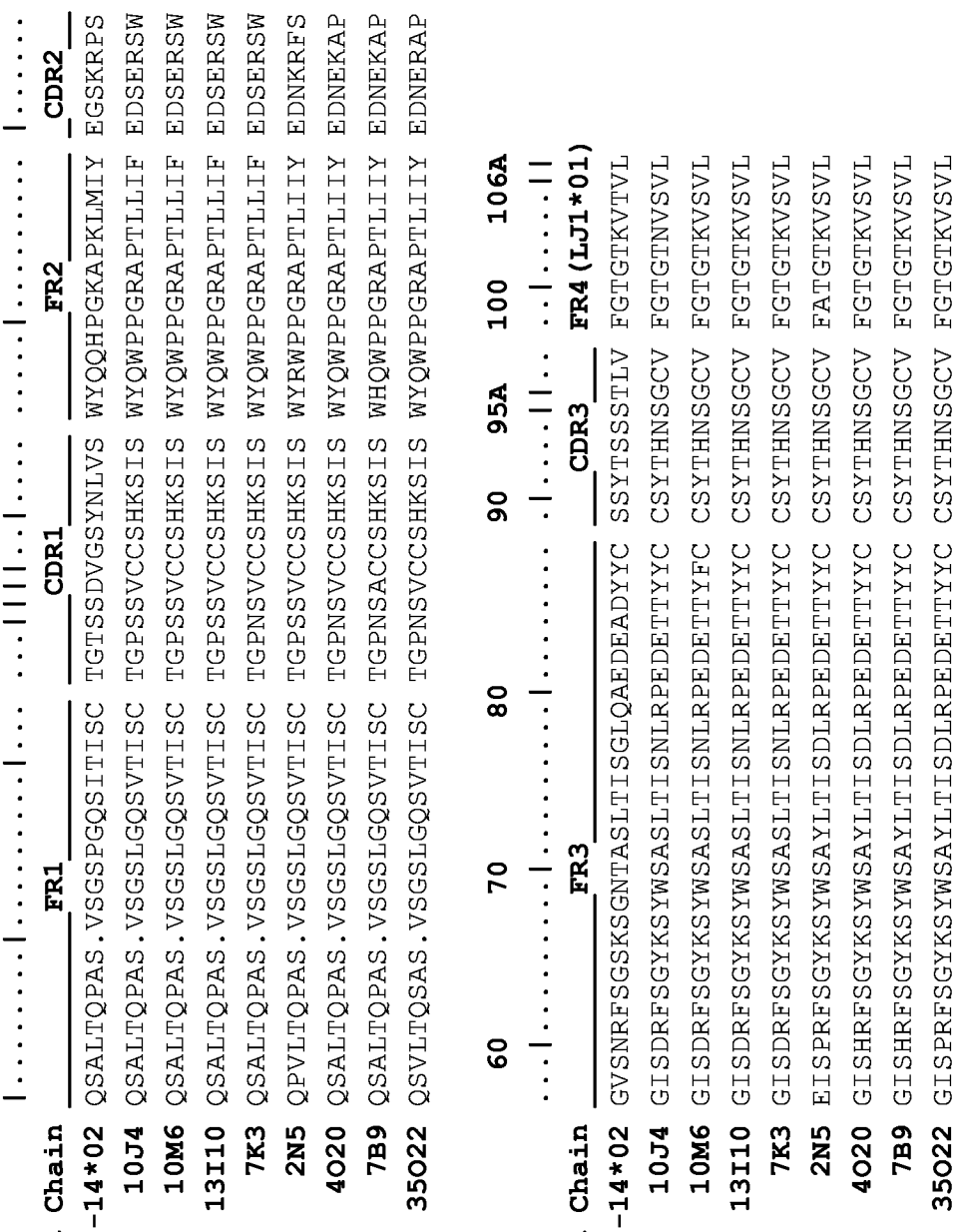


FIG. 16C

35O22 and its variants V_H (IMGT Numbering)

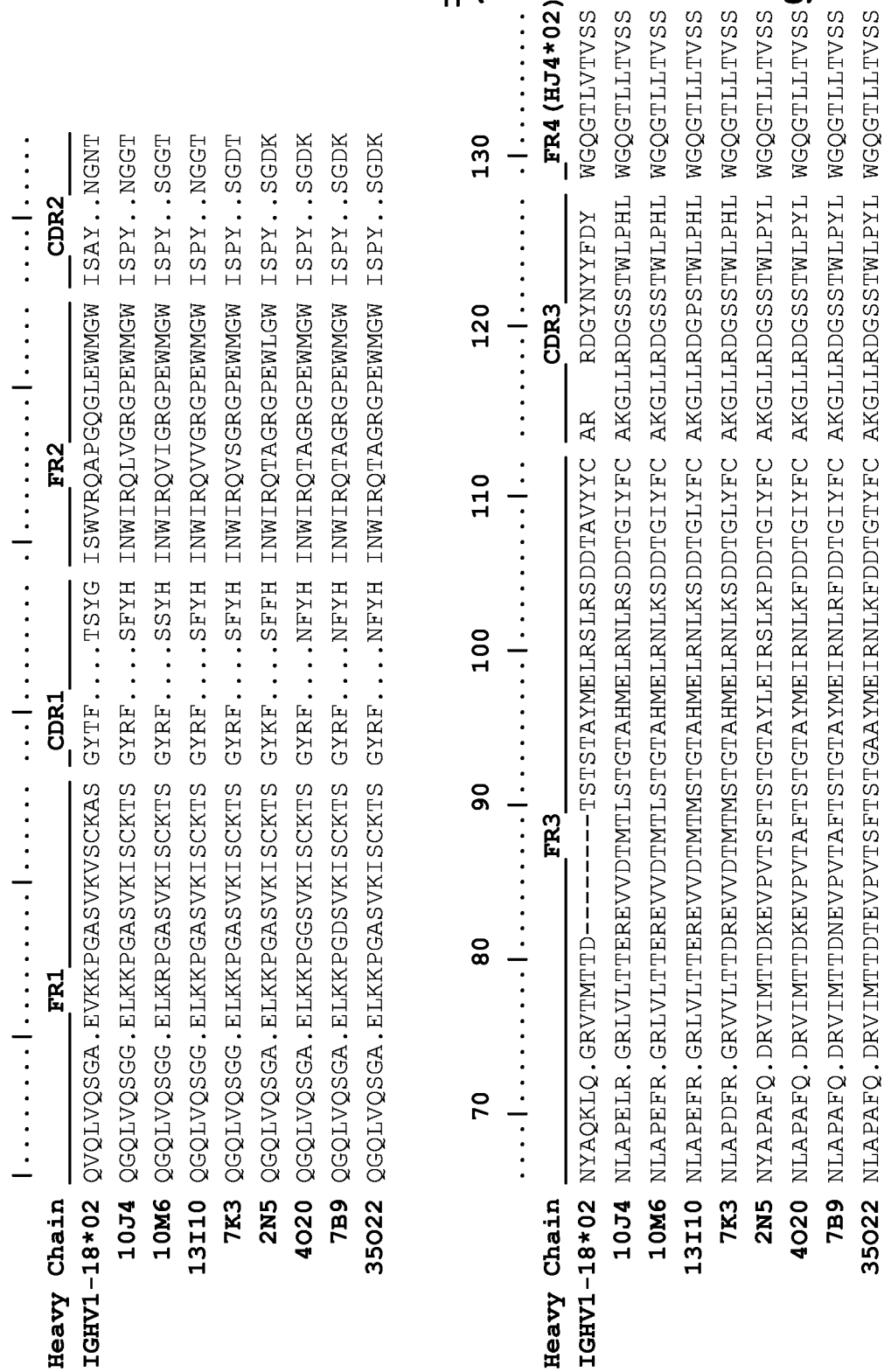


FIG. 16D

35O22 and its variants V_L (IMGT Numbering)

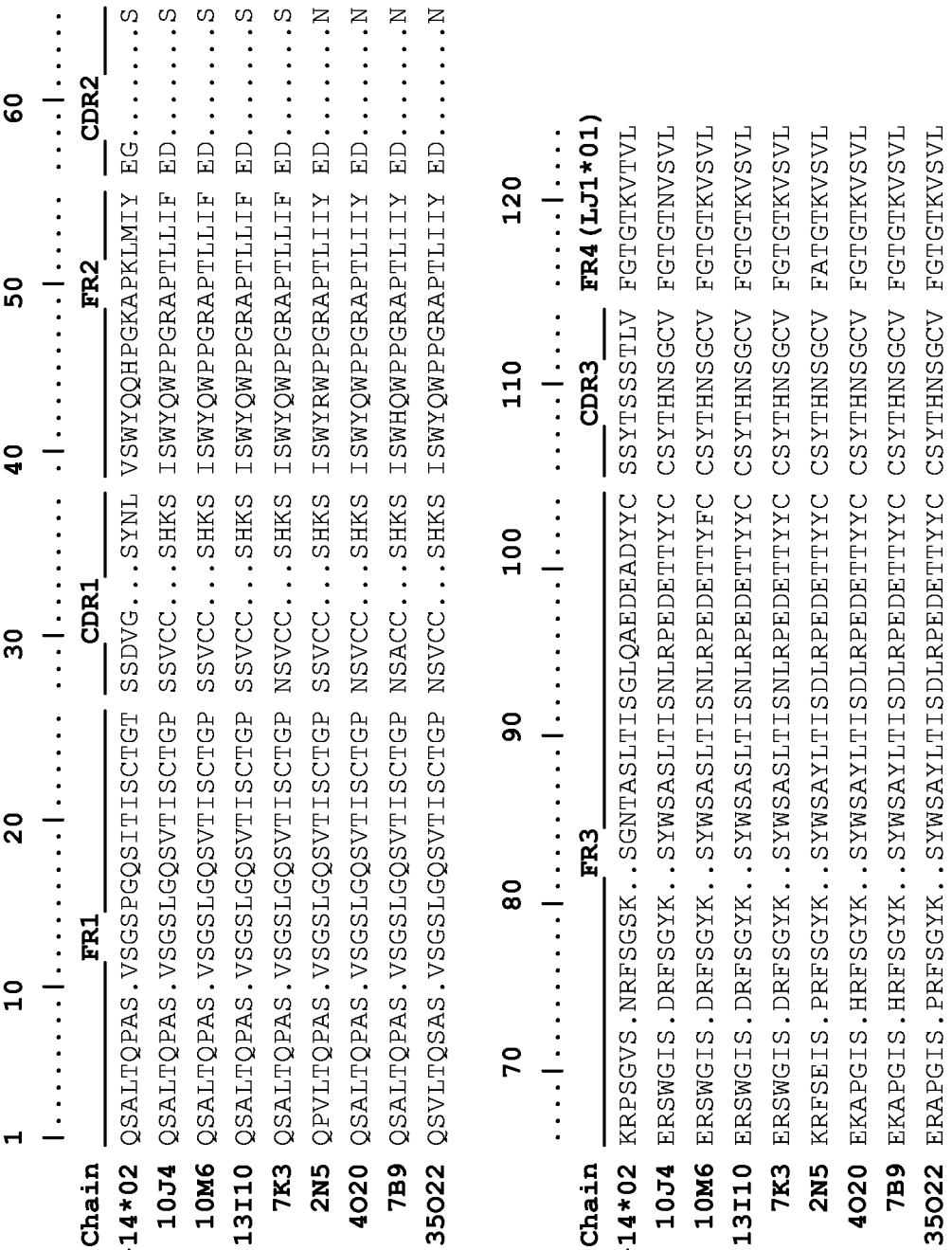


FIG. 17A

		ID50	IC50 (μg ml^{-1})									
Virus ID	Clade	N152 Serum	35O22	10E8	4E10	VRC01	NIH 45-46	3BNC 117	PG9	PG16	PGT 121	PGT 128
0260.v5.c36	A	3906	0.393	8.96	31.7	0.529	0.397	0.200	2.18	2.10	0.039	0.062
0330.v4.c3	A	9263	0.736	1.27	6.86	0.064	0.049	0.013	0.018	0.006	0.041	1.09
0439.v5.c1	A	4111	0.118	0.924	8.24	0.052	0.185	0.215	>50	>50	>50	6.41
3365.v2.c20	A	9810	0.780	2.45	1.38		0.038	0.011			0.059	>50
3415.v1.c1	A	12934	6.99	6.61	11.5	0.092	0.082	0.094	0.149	0.036	>50	0.018
3718.v3.c11	A	2167	>50	1.63	8.55	0.218	0.871	>50	0.050	0.019	1.40	>50
398-F1_F6_20	A	11290	>50	0.942	0.784	0.058	0.157	0.071	>50	>50	0.002	0.002
BB201.B42	A	470	1.73	0.231	2.19	0.343	0.303	3.35	0.014	0.003	0.003	0.004
BB539.2B13	A	1947	3.91	1.00	0.712	0.094	0.022	0.033	0.106	0.012	>50	>50
BI369.9A	A	6422	0.003	0.135	1.36	0.149	0.043	0.020	0.029	0.007	0.008	0.030
BS208.B1	A	9293	>50	0.127	0.618	0.029	0.006	0.002	0.031	0.004	>50	>50
KER2008.12	A	8198	0.0008	>50	1.28	0.563	0.567	0.248	0.017	0.006	2.22	14.7
KER2018.11	A	397	0.004	2.71	8.46	0.070	0.828	0.417	0.0009	0.0006	>50	>50
KNH1209.18	A	2457	0.103	0.422	1.63	0.087	0.246	0.040	0.367	0.678	0.002	0.006
MB201.A1	A	4508	7.85	0.230	2.21	0.237	0.165	0.464	0.024	0.001	0.005	0.016
MB539.2B7	A	726	0.257	8.03	21.7	0.544	0.402	0.087	0.058	0.025	>50	3.57
MI369.A5	A	2259	0.005	0.617	2.88	0.162	0.074	0.033	0.058	0.011	0.022	0.020
MS208.A1	A	14167	>50	0.254	1.56	0.147	0.090	0.019	0.071	0.047	>50	>50
Q23.17	A	5898	0.002	0.350	0.132	0.086	0.106	0.017	0.007	0.002	0.004	0.013
Q259.17	A	953	1.92	3.22	10.2	0.051	0.046	0.017	0.045	0.028	>50	>50
Q461.e2	A	4282	0.001	1.62	5.53	0.410	0.212	0.069	3.01	4.11		
Q769.d22	A	1834	0.002	1.11	1.73	0.015	0.013	0.007	0.007	0.010	>50	>50
Q769.h5	A	836	0.004	2.74	1.17	0.014	0.019	0.006	0.002	0.002	>50	>50
Q842.d12	A	7407	0.003	4.53	8.99	0.006	0.015	0.002	0.005	0.001	0.016	0.026
QH209.14M.A2	A	1490	1.13	1.45	5.93	0.024	0.011	0.008	>50	>50	>50	>50
RW020.2	A	1341	0.010	1.30	8.21	0.303	0.144	0.020	0.103	0.070	0.002	0.008
UG037.8	A	2490	0.026	0.039	0.504	0.035	0.056	0.020	0.021	0.0008	0.065	0.018
3301.V1.C24	AC	9904	0.348	2.81	8.64	0.084	0.055	0.046	0.281	0.023	0.009	0.174
3589.V1.C4	AC	143	0.010		1.94	0.073	0.351	0.061	0.025	0.728	>50	0.028
6540.v4.c1	AC	3626	7.73	2.62	14.3	>50	>50	>50	0.035	0.017	>50	3.13
6545.V3.C13	AC	5118	0.722	1.61	6.07		>50	>50				
6545.V4.C1	AC	3797	4.92	1.54	12.5	>50	>50	>50	0.095	0.068	>50	>50
0815.V3.C3	ACD	465	0.005	0.190	1.66	0.036	0.055	0.018	>50	>50	0.020	0.036
6095.V1.C10	ACD	7918	>50	0.0006	0.004	0.464	0.601	0.096	0.242	0.023	37.3	>50
3468.V1.C12	AD	12304	0.004	0.366	0.964	0.040	0.104	0.073	2.09	2.38	0.042	>50
Q168.a2	AD	509	0.009	0.768	18.7	0.140	0.138	0.050	0.106	0.031	>50	>50
620345.c1	AE	187	>50	0.748	0.469	>50	>50	>50	0.393	>50	>50	>50
C1080.c3	AE	8632	0.0009	0.038	0.598	1.50	0.539	0.096	0.004	0.001	>50	0.117
C2101.c1	AE	12603	0.002	0.671	2.45	0.097	2.38	0.064	0.026	0.009	>50	0.010
C3347.c11	AE	32066	0.022	0.016	0.161	0.037	0.055	0.029	0.038	0.006	>50	0.004
C4118.09	AE	25357	0.0006	0.498	5.26	0.110	0.035	0.019	0.037	0.021	>50	>50
CNE3	AE	70.5	0.939	1.36	2.23	3.56	1.99	0.125	0.079	0.173	>50	>50
CNE5	AE	38.3	>50	1.13	2.99	0.228	0.329	0.386	0.023	0.023	>50	0.096
CNE55	AE	22242	0.195	0.044	0.901	0.292	0.294	0.147	0.146	1.37	>50	>50
CNE56	AE	1145	>50	0.024	0.207	0.442	0.483	0.075	>50	>50	>50	25.0
CNE59	AE	3223	>50	0.001	0.042	0.516	0.102	0.043	0.091	0.113	>50	>50

FIG. 17B

		ID50	IC50 (μg ml^{-1})									
Virus ID	Clade	N152 Serum	35O22	10E8	4E10	VRC01	NIH 45-46	3BNC 117	PG9	PG16	PGT 121	PGT 128
M02138	AE	15153	0.217	0.002	0.151	0.742	0.270	0.154	0.122	0.022	>50	>50
R1166.c1	AE	6893	2.29	0.550	2.69	1.77	1.73	0.230	1.55	0.587	>50	>50
R2184.c4	AE	1474	0.013	0.679	3.79	0.052	0.065	0.035	0.204	0.280	>50	4.84
R3265.c6	AE	35.5	>50	5.10	15.6	0.731	0.019	0.020	1.30	0.036	>50	>50
TH966.8	AE	2803	>50	0.012	0.079	0.331	0.094	0.056	0.042	0.008	>50	0.006
TH976.17	AE	3259	0.325	0.339	0.213	0.066	0.116	0.025	>50	>50	>50	>50
235-47	AG	457	>50	0.077	0.802	0.049	0.250	0.022	0.322	0.246	0.110	>50
242-14	AG	6609	>50	1.35	>50	>50	>50	>50	0.025	0.023	>50	>50
263-8	AG	3500	>50	0.029	0.319	0.119	0.196	0.047	0.353	1.31	1.23	0.488
269-12	AG	95876	>50	0.049	0.133	0.163	9.79	0.151	1.52	0.281	0.164	0.019
271-11	AG	2733	0.711	0.623	3.11	0.052	0.215	0.007	0.097	0.102	11.7	>50
928-28	AG	5652	>50	0.022	0.009	0.378	0.164	0.155	0.060	0.023	31.0	>50
DJ263.8	AG	5021	0.021	0.010	0.081	0.072	0.018	0.025	0.100	0.048	0.064	20.0
T250-4	AG	24732	>50	0.658	0.361	>50	>50	>50	0.0006	0.0006	0.001	0.003
T251-18	AG	6192	0.007	0.132	9.42	3.58	1.36	0.203	>50	10.5	10.8	>50
T253-11	AG	243	>50	0.863	0.755	0.265	0.268	0.116	0.127	4.44	>50	18.9
T255-34	AG	687	0.021	0.153	0.023	0.252	0.779	0.051	0.015	0.005	>50	>50
T257-31	AG	89.7	>50	0.224	2.00	1.68	0.579	0.181	0.020	0.003	>50	>50
T266-60	AG	14457	0.795	>50	4.74	0.353	0.219	0.032	24.0	>50	0.160	0.014
T278-50	AG	2342	3.00	0.362	3.17	>50	>50	>50	0.913	1.13	>50	0.050
T280-5	AG	2740	3.18	2.88	0.726	0.017	0.036	0.019	0.379	0.233	0.002	0.005
T33-7	AG	1731	0.632	0.715	2.89	0.023	0.074	0.007	0.023	0.023	>50	>50
3988.25	B	1332	0.119	0.053	1.37	2.10	0.358	>50	0.010	0.002	0.002	0.007
5768.04	B	140	0.192	2.01	15.0	0.099	0.074	0.201	0.073	0.020	0.039	4.50
6101.1	B	1372	>50	0.0009	0.381	0.104	0.016	0.022	>50	>50	0.002	0.004
6535.3	B	7906	0.114	0.054	0.458	2.16	0.067	0.262	0.465	>50	0.003	0.015
7165.18	B	9575	>50	0.036	0.895	>50	>50	6.54	>50	>50	0.019	0.013
89.6.DG	B	1943	0.459	0.040	1.02	0.460	0.274	0.109	>50	>50	0.016	0.010
AC10.29	B	13018	0.833	0.048	0.408	1.43	0.364	6.05	0.078	0.023	0.028	0.009
ADA.DG	B	15877	>50	0.016	0.656	0.424	0.112	0.086	0.342	0.023	0.002	0.017
Bal.01	B	1590	0.003	0.715	6.74	0.102	0.017	0.012	0.052	8.00	0.011	0.300
BaL.26	B	5407	0.0006	0.264	2.70	0.047	0.013	0.006	0.034	0.136	0.010	0.021
BG1168.01	B	167	>50	0.136	3.34	0.449	1.32	0.179	>50	>50	>50	>50
BL01.DG	B	1044	0.006	0.136	4.68	>50	>50	>50	>50	>50	>50	>50
BR07.DG	B	4626	0.0009	0.031	2.01	1.67	0.362	0.135	>50	>50	0.064	2.28
BX08.16	B	74924	0.019	0.099	0.469	0.281	0.366	0.102	0.024	0.062	0.002	0.006
CAAN.A2	B	1364	0.003	1.37	8.49	1.06	0.205	0.673	13.0	7.43	0.005	0.102
CNE10	B	8422	>50	0.013	0.307	0.776	0.173	0.050	0.243	9.17	0.005	0.008
CNE12	B	2475	0.027	0.117	1.11	0.785	0.516	0.085	>50	>50	0.002	0.017
CNE14	B	7920	0.005	0.080	2.52	0.389	0.091	0.024	>50	>50	0.002	0.014
CNE4	B	13863	0.0009	0.015	0.056	0.871	0.238	0.113	>50	>50	11.5	3.15
CNE57	B	1687	10.2	0.010	0.156	0.535	0.182	0.066	>50	>50	0.008	0.009
HO86.8	B	1372	0.051	0.104	0.344	>50	>50	>50	0.014	0.001	>50	>50
HT593.1	B	7037	>50	0.016	0.397	0.438	0.099	0.229	0.271	0.153	>50	>50
HXB2.DG	B	6443	0.013	0.002	0.043	0.040	0.009	0.037	0.553	>50	>50	11.0
JRCSF.JB	B	4641	0.002	0.065	4.12	0.234	0.060	0.028	0.003	0.002	0.061	0.012

FIG. 17C

		ID50	IC50 (μg ml^{-1})									
Virus ID	Clade	N152 Serum	35O22	10E8	4E10	VRC01	NIH 45-46	3BNC 117	PG9	PG16	PGT 121	PGT 128
JRFL.JB	B	860	0.013	0.247	5.37	0.033	0.009	<0.003	>50	>50	0.017	0.011
MN.3	B	48721	0.007	0.0006	0.016	0.033	0.004	>50	>50	>50	>50	>50
PVO.04	B	359	0.011	1.78	1.99	0.386	0.148	0.074	6.24	19.8	0.132	0.010
QH0515.01	B	7984	0.033	1.78	2.00	0.523	0.689	0.175	>50	>50	8.70	>50
QH0692.42	B	6528	0.021	0.358	1.60	1.16	0.991	0.275	>50	>50	0.940	0.046
REJO.67	B	9758	0.0001	0.093	0.167	0.045	0.011	0.039	0.005	0.005	8.87	>50
RHPA.7	B	1939	>50	0.640	13.3	0.047	0.022	0.019	>50	1.32	0.014	0.029
SC422.8	B	3070	25.5	0.196	1.49	0.132	0.031	0.049	0.535	1.20	0.098	2.41
SF162.LS	B	17317	>50	0.107	0.412	0.237	0.025	0.019	>50	>50	0.004	0.004
SS1196.01	B		0.062	0.193	0.686	0.276	0.047	0.038	0.293	0.069	0.002	0.011
THRO.18	B	351	0.908	0.048	1.77	4.42	1.88	2.80	15.0	0.975	>50	>50
TRJO.58	B	1231	0.002	0.881	8.75	0.079	0.029	0.062	0.246	0.393	4.31	0.014
TRO.11	B	2773	>50	0.026	0.599	0.343	1.04	0.033	41.5	4.86	0.006	0.017
WITO.33	B	133	>50	0.247	1.20	0.112	0.037	0.030	0.023	0.023	0.787	>50
YU2.DG	B	279	0.287	0.895	16.0	0.055	0.031	0.029	3.69	0.041	0.068	0.058
CH038.12	BC	4565	1.10	0.543	4.57	0.379	0.183	>50	0.500	49.0	0.004	0.004
CH070.1	BC	3504	>50	1.13	21.4	18.7	>50	7.89	0.006	0.002	0.003	0.027
CH117.4	BC	8831	0.0006	0.146	0.343	0.059	0.022	0.663	0.008	0.005	>50	>50
CH181.12	BC	4947	>50	0.287	5.45	0.540	0.189	0.124	0.008	0.002	0.007	0.018
CNE15	BC	29955	>50	0.802	3.36	0.080	0.052	>50	0.023	0.023	19.0	>50
CNE40	BC	43426	0.002	0.0006	0.003	0.425	0.320	0.116	1.16	49.0	0.224	>50
CNE7	BC	82869	0.004	0.034	0.128	0.540	0.147	>50	1.66	0.393	0.032	0.058
286.36	C		0.001	1.05	0.988	0.103	0.953	0.067	0.071	0.005	0.002	0.014
288.38	C	1949	>50	0.580	0.456	1.52	0.128	0.063	3.14	0.186	0.006	0.019
0013095-2.11	C	1930	>50	0.003	0.078	0.142	0.019	0.208	0.023	0.023	>50	>50
001428-2.42	C	2688	>50	0.750	9.10	0.023	0.009	0.010	0.023	0.023	0.023	0.039
0077_V1.C16	C	1658	0.003	1.19	0.886	1.04	0.308	>50	0.091	0.023	>50	>50
00836-2.5	C	265	>50	0.492	1.43	0.128	0.015	>50	49.0	>50	31.8	>50
0921.V2.C14	C	46	>50	0.773	5.63		0.181	0.243			>50	>50
16055-2.3	C	7672	>50	0.571	4.12	0.105	0.068	3.24	0.014	0.005	1.02	>50
16845-2.22	C	11099	>50	0.001	0.459	2.41	3.19	29.6	2.38	27.8	9.41	0.233
16936-2.21	C	8448	0.001	0.166	1.79	0.109	0.023	0.059	>50	>50	0.003	0.030
25710-2.43	C	9868	>50	0.013	0.376	0.545	0.202	0.100	0.038	0.023	0.014	0.021
25711-2.4	C	4093	>50	0.448	6.24	0.712	0.718	>50	1.50	0.037	0.010	0.019
25925-2.22	C	3217	>50	0.287	2.23	0.559	0.141	0.136	0.023	0.023	0.024	0.014
26191-2.48	C	35.2	>50	1.07	4.20	0.195	0.115	0.043	0.142	1.95	0.150	0.025
3168.V4.C10	C	7844	0.038	1.23	2.63	0.131	0.178	0.110	0.162	0.037	0.485	>50
3637.V5.C3	C	777	47.9	1.66	3.51	4.09	1.77	>50	>50	>50	>50	>50
3873.V1.C24	C	1353	>50	3.81	3.05	0.954	0.141	6.97	>50	12.2	0.015	0.015
6322.V4.C1	C	8201	3.19	1.80	7.95	>50	>50	>50	>50	>50	>50	>50
6471.V1.C16	C	3099	>50	4.62	23.1	>50	>50	>50	>50	>50	>50	>50
6631.V3.C10	C	49.4	>50	0.643	>50	>50	>50	>50	>50	>50	>50	1.99
6644.V2.C33	C	2654	0.0008	0.002	0.781	0.164	0.071	0.033	0.033	35.3	0.018	0.100
6785.V5.C14	C	17458	>50	0.435	1.96	0.332	0.311	0.195	0.023	0.023	0.019	0.019
6838.V1.C35	C	719	>50	0.207	1.66		1.52	0.281			0.119	>50
96ZM651.02	C	7423	>50	0.004	0.113	0.525	0.416	0.443	>50	>50	0.009	0.131

FIG. 17D

		ID50	IC50 (μg ml^{-1})									
Virus ID	Clade	N152 Serum	35O22	10E8	4E10	VRC01	NIH 45-46	3BNC 117	PG9	PG16	PGT 121	PGT 128
BR025.9	C	7102	0.006	0.143	3.73	0.271	0.067	>50	0.044	0.009	0.002	0.004
CAP210.E8	C	33972	0.042	0.267	4.00	>50	>50	8.16	0.087	0.023	>50	>50
CAP244.D3	C	2859	20.9	0.178	1.98	0.857	0.277	0.073	0.088	0.023	>50	>50
CAP45.G3	C	1909	0.010	0.527	2.36	9.47	>50	0.589	0.023	0.023	2.08	>50
CNE30	C	3150	>50	0.424	1.73	0.927	0.624	0.291	>50	>50	0.061	0.853
CNE31	C	88.2	>50	1.14	3.12	0.962	0.253	9.95	13.5	2.51	0.789	14.9
CNE53	C	14560	>50	0.185	0.032	0.108	0.035	0.051	0.147	>50	0.022	0.034
CNE58	C	368	>50	0.346	0.870	0.124	1.04	0.389	0.023	0.023	>50	7.84
DU123.06	C	12736	6.33	0.089	0.343	13.6	>50	0.183	0.091	0.023	0.033	0.072
DU151.02	C	4533	0.094	0.353	2.97	7.70	>50	>50	0.023	0.023	0.005	0.012
DU156.12	C	2021	11.7	0.007	0.023	0.082	0.029	0.035	0.023	0.023	0.005	0.024
DU172.17	C	2564	>50	0.008	0.023	>50	>50	0.289	0.262	0.030	0.104	0.044
DU422.01	C	325	0.021	1.06	1.65	>50	>50	>50	0.303	0.023	0.164	0.175
MW965.26	C	42805	5.60	0.001	0.025	0.038	0.024	0.005	1.99	0.961	0.011	0.315
SO18.18	C	2603	>50	0.422	8.79	0.071	0.028	0.032	0.061	0.023	0.002	0.056
TV1.29	C	241	>50	0.353	1.95	>50	>50	>50	0.008	0.002	0.118	0.077
TZA125.17	C	11592	>50	0.405	0.431	>50	>50	>50	0.231	0.024	9.96	1.20
TZBD.02	C	409	>50	1.67	2.32	0.072	0.011	45.9	0.266	0.025	0.005	>50
ZA012.29	C	39	>50	0.827	5.80	0.250	0.098	0.063	27.0	0.631	0.005	0.028
ZM106.9	C	256	0.372	>50	16.1	0.248	0.058	0.082	0.639	1.10	0.005	0.024
ZM109.4	C	1856	>50	0.273	0.708	0.134	0.046	0.041	0.106	4.93	13.7	>50
ZM135.10a	C		>50	0.338	0.034	1.28	0.280	0.067	>50	>50	1.50	30.0
ZM176.66	C	551	>50	0.184	0.669	0.038	0.096	>50	0.007	0.002	13.8	0.021
ZM197.7	C	784	>50	0.046	0.425	0.624	0.314	0.398	0.414	0.650	>50	>50
ZM214.15	C	6130	0.034	1.69	1.45	0.881	0.132	0.088	>50	>50	0.682	1.44
ZM215.8	C	2557	0.690	0.017	0.641	0.276	0.015	0.010	0.023	>50	0.014	0.039
ZM233.6	C	955	0.007	0.103	2.06	4.25	5.44	0.202	0.023	0.023		
ZM249.1	C	896	0.001	0.787	2.75	0.082	0.038	0.039	0.033	0.073	>50	10.7
ZM53.12	C	7255	>50	2.56	4.52	0.839	0.205	0.212	0.041	0.023	0.002	>50
ZM55.28a	C	133	1.93	2.31	8.79	0.144	0.085	0.040	0.571	>50	0.070	0.029
3326.V4.C3	CD	111	>50	1.18	1.93	0.073	4.27	48.1	0.023	0.023	>50	>50
3337.V2.C6	CD	22394	0.002	1.23	1.91	0.063	0.034	0.008	>50	>50	21.1	0.006
3817.v2.c59	CD		>50	0.549	4.78	>50	>50	0.216	0.007	0.006	>50	0.022
231965.c1	D	7990	>50	8.03	21.7	0.487	0.060	0.035	1.51	4.72	>50	>50
247-23	D	9426	0.006	0.368	2.37	24.2	47.8	0.028	0.195	>50	>50	>50
3016.v5.c45	D	6403	9.85	0.312	4.80	0.111	>50	0.648	0.286	>50	>50	>50
57128.vrc15	D	11880	0.167	0.203	1.93	>50	>50	0.432	0.104	0.162	2.16	0.069
6405.v4.c34	D	19040	0.115	0.325	3.92	2.63	0.731	0.171	>50	>50	0.019	6.86
A03349M1.vrc4a	D	15192	0.122	0.292	3.12	4.66	>50	0.512	>50	>50	0.013	0.018
NKU3006.ec1	D	12585	0.008	0.588	5.83	0.506	0.615	0.089	>50	>50	>50	45.0
UG021.16	D	7703	>50	0.022	0.196	0.266	1.48	0.410	>50	>50		
UG024.2	D	11754	>50	0.013	0.091	0.106	>50	0.057	3.94	>50	>50	>50
X2088.c9	G	8786	3.62	>50	>50	>50	>50	>50	>50	>50	0.003	>50
SIVmac251.30.SG3	N/A	<5	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
SVA.MLV	N/A	<5	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50

FIG. 18A

	Domain	Virus ID	IC_{50} (μg ml^{-1})[a]	Ratio
		WT	0.0106	NA
gp120	C1	E83A	0.0210	1.98
		V84A	0.0606	5.71
		V85A	0.0436	4.12
		L86A	0.1562	14.74
		E87A	0.0107	1.01
		N88A	>20	>1886
		V89A	>20	>1886
		T90A	>20	>1886
		E91A	0.0136	1.29
		D92A	0.0126	1.19
		F93A	No infection	NA
	V1	N134A	0.0073	0.69
		N141A	0.0049	0.46
		N156A	0.0046	0.44
	V2	N160K	0.0365	3.44
		N186A	0.0071	0.67
	C2	N197A	0.0018	0.17
		L226A	0.0143	1.35
		K227A	>20	>1886
		C228A	0.0115	1.08
		N229A	0.0127	1.20
		N230A	>20	>1886
		K231A	0.1303	12.29
		T232A	0.3346	31.57
		F233A	0.0176	1.66
		N234A	0.0138	1.30
		G235A	0.0078	0.74
		K236A	0.0239	2.25
		K237A	0.0139	1.31
		Q238A	0.0407	3.84
		C239A	No infection	NA
		K240A	0.0025	0.24
		N241A	>20	>1886
		V242A	0.0224	2.11
		S243A	>20	>1886
		T244A	0.0275	2.60
		V245A	0.0155	1.46
		Q246A	0.0121	1.14
		N262A	0.0068	0.64
		N276A	0.0088	0.83
		N280A	0.0027	0.26
		N289A	0.0152	1.44
		N295A	0.0229	2.16
	V3	N301A	0.0140	1.32

FIG. 18B

		N302A	0.0153	1.44
	C3	N332A	0.0065	0.61
		N339A	0.0347	3.27
		N355A	0.0969	9.14
	V4	N392A	0.0089	0.84
		N396A	0.0106	1.00
		N411A	0.0117	1.11
	C4	N425A	0.0063	0.60
		N448A	0.0185	1.75
		N461A	0.0238	2.24
		N478A	0.0189	1.78
gp41	NHR	N553A	0.0128	1.21
		N554A	0.0079	0.75
		N611A	0.0158	1.49
		N616A	0.0144	1.36
	CHR	D620A	0.0076	0.72
		S621A	0.0242	2.29
		I622A	0.0050	0.47
		W623A	0.0844	7.96
		N624A	0.0568	5.36
		N625A	>20	>1886
		M626A	0.0154	1.45
		T627A	0.0034	0.32
		W628A	0.0145	1.37
		M629A	0.1015	9.58
		E630A	0.0366	3.45
		N637A	0.0147	1.39
		N656A	0.0237	2.24
	TR	N671A	0.0390	3.68

Double/Triple Mutants

Virus ID	IC_{50} (μg ml^{-1})[a]	Ratio
N230A N241A	>20	>1886
N241A N625A	>20	>1886
N230A N625A	>20	>1886
K227A N230A	>20	>1886
K227A N241A	>20	>1886
K227A S243A	>20	>1886
K227A N625A	>20	>1886
N230A S243A	>20	>1886
N241A S243A	>20	>1886
S243A N625A	>20	>1886
N230A N241A N625A	>20	>1886

[a]IC_{50} values >30-fold compared to HIV wild-type virus are highlighted in yellow.

FIG. 19

	Domain	Virus ID	IC_{50} (μg ml^{-1})[a]	Ratio
		WT	0.0141	NA
gp120	C1	Y40F	0.0143	1.02
		L52V	0.0074	0.52
		F53V	0.0035	0.25
		N88A	>1	>91
		V89A	0.4041	28.69
	C2	T232Y	0.1901	13.50
		N234A	0.0197	1.40
		N241A	0.1480	10.51
		T244V	0.3240	23.00
	C5	V488A	0.0053	0.38
		V489A	0.3527	25.04
		K490T	0.0203	1.44
		K490F	0.0142	1.01
		T499A	0.0730	5.19
		K500A	0.0315	2.23
		A501T	0.0075	0.53
		K502T	0.0359	2.55
		R504T	0.0533	3.78
		V505A	0.0091	0.65
		V505K	0.0200	1.42
		V506A	0.0191	1.35
		V506K	0.0370	2.63
gp41	NHR	K588A	0.0091	0.65
		K588N	0.0064	0.46
		K588F	0.0067	0.47
		K601Q	0.0099	0.70
		K601A	0.0893	6.34
		A607N	0.0130	0.92
		N611D	0.0080	0.57
		N611A	0.0018	0.13
		N616Q	0.0046	0.32
		N624Q	0.5010	35.57
	CHR	N637Q	0.0048	0.34
		N637A	0.0019	0.14
		E647A	0.0017	0.12

[a] IC_{50} values >10-fold compared to HIV wild-type virus are highlighted in yellow.

FIG. 20

	35O22 Fab
Data collection	
Space group	$P4_12_12$
Cell dimensions	
a, *b*, *c* (Å)	57.2, 57.2, 267.7
a, b, g (°)	90.0, 90.0, 90.0
Resolution (Å)	50-1.55 (1.58-1.55)*
R_{sym} or R_{merge}	11.1 (48.6)
I / s*I*	20.3 (2.9)
Completeness (%)	98.0 (92.8)
Redundancy	11.2 (5.0)
Molecules/ASU	1
Refinement	
Resolution (Å)	39.1-1.55
No. reflections	64,474
R_{work} / R_{free}	0.1665/0.1822
No. atoms	6,866
Protein	
Ligand/ion	0
Water	449
B-factors	
Protein	32.75
Ligand/ion	0
Water	38.59
R.m.s. deviations	
Bond lengths (Å)	0.005
Bond angles (°)	1.035
PDB ID	4TOY

*Values in parentheses are for highest-resolution shell.
The dataset was collected from a single crystal.

FIG. 21

Clade	Virus ID	Monoclonal antibody (concentration in μg ml^{-1})		Fold change (IC_{50} of 35O22 d8aa / IC_{50} of 35O22)
		35O22	35O22 d8aa	
A	KER2018	0.004	>20	>4933
	RWO20.2	0.010	1.110	>114
	Q168.a2	0.009	>20	>2317
	Q769.d22	0.002	>20	>8029
	Q769.h5	0.004	>20	>4765
B	JRFL.JB	0.013	0.060	4.7
	BaL.01	0.003	0.023	6.8
	YU2.DG	0.287	>20	>70
	PVO.04	0.011	0.400	36.5
	TRO.11	>50	>20	NA
	CAAN.A2	0.003	0.482	142.1
	TRJO.58	0.002	0.005	2.2
	THRO.18	0.908	>20	>22
	BG1168.1	>50	>20	NA
	6101.1	>50	>20	NA
C	ZA012.29	>50	>20	NA
	DU156.12	11.700	>20	>2
	DU422.01	0.021	>20	>971
	ZM106.9	0.372	>20	>54
	ZM55.28a	1.930	>20	>10
Control	SIVmac251.SG3	>50	>50	>50
Geometric Mean IC_{50} (μg ml^{-1})		0.031	0.107	3.5
Median IC_{50} (μg ml^{-1})		0.010	0.230	22.0

FIG. 22

Residue number		% Glycan Conservation	Residue number		% Glycan Conservation	Residue number		% Glycan Conservation
Alignment	HXB2		Alignment	HXB2		Alignment	HXB2	
44	29	3.8	272	-	6.75	586	395	1.48
78	49	9.85	273	-	3.82	587	396	25.21
125	88	98.53	274	-	1.55	588	397	9.94
170	130	60.56	311	-	1.24	589	398	37.91
172	132	1.13	312	-	2.42	590	399	8.32
173	133	16.69	313	-	4.97	591	400	7.34
174	134	3.45	314	-	7.2	592	401	8.25
175	135	26.35	315	-	11.04	593	402	20.49
176	136	15.92	316	-	13.6	594	403	11.37
177	137	24.76	317	187	32.45	595	404	12.4
178	138	16.3	318	188	24.45	596	405	8.68
179	139	15.45	327	197	97.84	597	406	5.72
180	140	18.5	366	229	1.69	598	407	5.16
181	141	15.83	367	230	32.68	599	408	4.2
182	142	13.79	369	232	3.02	600	-	1.62
183	-	9.33	371	234	79.7	613	-	1.66
184	-	5.63	380	241	97	614	409	3.05
185	-	4.38	404	262	99.41	615	410	9.99
186	-	3.12	411	268	1.17	616	411	45.96
187	-	1.97	424	276	94.7	617	412	1.24
188	-	1.29	440	289	69.36	618	413	24.01
189	-	1.08	446	293	5.84	667	442	26.89
215	-	1.27	450	295	60.12	669	444	6.57
216	-	1.9	456	301	92.13	675	446	7.22
217	-	3.17	498	332	71.61	677	448	86.4
218	-	4.1	500	334	22.06	693	460	14.82
219	-	7.62	510	337	4.31	694	461	26.68
220	143	11.44	512	339	65.79	695	462	31.82
221	144	10.88	517	344	2.91	696	463	20.28
222	145	12.8	530	354	9.05	697	-	3.87
223	146	5.39	539	355	4.78	698	-	1.62
224	147	9.8	540	356	73.62	733	-	1.83
225	148	4.45	542	358	4.99	734	-	28.14
226	149	12.71	544	360	3.42	736	465	27.15
227	150	1.81	546	362	35.19	919	611	98.41
234	156	95.85	553	363	8.3	937	616	91.07
238	160	91.3	577	386	86.96	939	618	4.48
269	185	7.76	583	392	78.8	949	625	95.99
270	186	15.76	584	393	2.06	962	637	96.2
271	-	5.89	585	394	4.64	1003	674	13.22

The conservation (in percent) of N-linked glycosylation sequons at HIV-1 gp140 residues is based on analysis of 4265 HIV-1 sequences (hiv.lanl.gov/content/sequence/NEWALIGN/align.html). Shown are gp140 residue positions where an N-linked glycosylation sequon is present in more than 1% of the sequences

FIG. 23A

a. 35O22 heavy chain (chain D) and light chain (chain E) interface with gp41 (chain B)

Hydrogen bonds							
	gp41	Dist.[Å]	35O22		gp41	Dist.[Å]	35O22
1	B:ASN625[ND2]	3.68	D:LEU96[O]	3	B:ASP624[O]	3.04	D:ARG98[N]
2	B:ASN625[OD1]	2.44	D:TYR32[OH]	4	B:ASP624[O]	3.20	D:ASP99[N]

List of heavy chain-gp41 interface residues (the residues on each row are not matched interactive partners)									
gp41	HSD*	ASA	BSA	ΔiG	35O22	HSD	ASA	BSA	ΔiG
B:GLY527		69.79	18.98|||	-0.13	D:GLN1		192.89	29.65||	-0.35
B:SER528		36.71	4.59||	0.07	D:PHE31		63.18	12.09||	0.19
B:THR529		40.42	37.31||||||||	0.24	D:TYR32	H	53.73	25.90||||	-0.26
B:ALA532		46.96	3.52|	0.06	D:PHE72H		169.27	95.44|||||	1.53
B:ARG617		120.66	26.65|||	-0.70	D:LEU96	H	56.05	5.65||	-0.06
B:SER620		73.21	21.28|||	-0.18	D:LEU97		90.59	68.55|||||||	1.10
B:GLU621		86.31	8.52|	0.14	D:ARG98	H	153.80	71.96||||	-0.38
B:ASP624	H	98.64	66.75|||||	-0.11	D:ASP99	H	118.53	47.77||||	0.22
B:ASN625	H	112.81	73.74|||||	-0.54	D:GLY100		52.83	5.97||	0.10
B:MET626		7.75	2.28|||	0.02					
B:THR627		29.84	21.28||||||	0.24					
B:LEU629		134.49	18.20||	0.21					
B:GLN630		99.10	36.41||||	0.35					

List of light chain-gp41 interface residues (the residues on each row are not matched interactive partners)									
gp41	HSD	ASA	BSA	ΔiG	35O22	HSD	ASA	BSA	ΔiG
B:LYS633		159.78	17.24||	-0.05	E:TYR49		74.12	32.70||||	-0.26
B:ARG617		120.66	2.47|	-0.09	E:GLU50		85.57	7.34|	-0.10
B:ASN618		90.29	18.66|||	-0.16	E:GLU53		71.21	16.28|||	-0.02
B:SER620		73.21	36.85|||||	0.27	E:ARG54		115.05	11.48|	-0.13
B:GLU621		86.31	58.39||||||	-0.27	E:PRO56		129.25	36.15|||	0.58

FIG. 23B

b. 35O22 interface with gp41 glycans (chain B)

Light chain residues interacting with gp41 glycan NAG1618									
35O22	HSD	ASA	BSA	ΔiG	gp41	HSD	ASA	BSA	ΔiG
E:TYR49		74.12	2.84|	-0.03	B:NAG1618		364.26	155.97||||	-2.95
E:ASN52		101.39	14.60||	-0.07					
E:GLU53		71.21	40.20|||||	0.28					
E:ARG54		115.05	60.92|||||	-1.06					
E:ALA55		8.17	3.50||||	-0.03					
E:PRO56		129.25	1.01|	0.02					
E:ILE58		36.63	3.56|	-0.04					

c. 35O22 heavy chain (chain D) and light chain (chain E) interface with gp120 (chain G)

Hydrogen bonds							
	gp120	Dist.[Å]	35O22		gp120	Dist.[Å]	35O22
1	G:GLU87[OE2]	3.47	D:TYR53[OH]	4	G:THR90[O]	2.67	D:SER72G[OG]
2	G:ASN88[O]	3.52	D:ARG28[NH1]	5	G:THR90[OG1]	3.19	D:ARG28[NH1]
3	G:ASN88[O]	3.57	D:ARG28[NH2]	6	G:GLU92[OE2]	3.66	D:SER72G[N]

List of heavy chain-gp120 interface residues (the residues on each row are not matched interactive partners)									
gp120	HSD*	ASA	BSA	ΔiG	35O22	HSD	ASA	BSA	ΔiG
G:GLU87	H	119.82	30.03|||	-0.04	D:ARG28	H	61.56	41.29|||||	-0.86
G:ASN88	H	153.77	76.82|||||	-0.45	D:ASN30		36.89	0.67|	0.01
G:VAL89		37.55	0.16|	0.00	D:PHE31		63.18	29.42||||	0.47
G:THR90	H	84.18	78.15||||||||	0.06	D:TYR53	H	174.22	57.23||||	0.43
G:GLU91		30.92	6.18||	-0.07	D:GLU72B		31.31	3.07|	-0.05
G:GLU92	H	120.15	37.29||||	-0.20	D:PRO72D		100.81	68.72||||||	0.94
G:PRO238		84.68	52.14||||||	0.79	D:VAL72E		95.12	16.09||	-0.14
G:PRO240		94.84	25.90|||	0.41	D:THR72F		81.85	25.64||||	0.19
					D:SER72G	H	84.96	55.39||||||	0.12
					D:ARG98		153.80	14.44|	-0.15

*. D: Disulfide bond, H: Hydrogen bond, S: Salt bridge.
ASA: Accessible Surface Area, Å², BSA: Buried Surface Area, Å², ΔiG: Solvation energy effect, kcal/mol, \|\|\|\|: Buried area percentage, one bar per 10%.

FIG. 23C

35O22 heavy chain (chain D) and light chain (chain E) interface with gp120 glycans (chain G)

Hydrogen bonds								
	35O22	Dist.[Å]	gp120		35O22	Dist.[Å]	gp120	
1	D:ARG98[NH1]	3.15	G:NAG1088[O5]	12	E:HIS93[ND1]	3.70	G:BMA1090[O4]	
2	D:ARG98[NH1]	3.20	G:NAG1088[O6]	13	E:ASN94[ND2]	2.90	G:MAN1092[O5]	
3	D:HIS33[NE2]	3.19	G:NAG1089[O3]	14	E:ASN94[ND2]	2.98	G:MAN1092[O6]	
4	D:THR100C[OG1]	3.79	G:NAG1089[O3]					
5	D:SER52[OG]	2.81	G:NAG1089[O7]					
6	D:ASP99[OD2]	2.69	G:NAG1089[O6]					
7	D:THR100C[OG1]	3.64	G:BMA1090[O2					
8	D:GLY100[N]	3.81	G:MAN1091[O6]					
9	D:ASP99[OD2]	2.89	G:MAN1091[O6]					
10	D:ASN58[ND2]	2.75	G:MAN1093[O5]					
11	D:LYS57[O]	2.83	G:MAN1093[O6]					

FIG. 23D

Heavy chain residues interacting with gp120 glycans (interactive partners are shaded)									
35O22	HSD*	ASA	BSA	ΔiG	gp120	HSD	ASA	BSA	ΔiG
D:ASN30		36.89	3.80|	-0.04	G:NAG1088	H	361.66	152.80||||	-2.90
D:PHE31		63.18	14.73|||	0.21					
D:SER52		14.76	4.10||	-0.05					
D:TYR53		174.22	56.84||||	0.60					
D:ARG98	H	153.80	34.95|||	0.09					
D:ASN30		36.89	3.30|	-0.04	G:NAG1089	H	351.65	208.97|||||	-3.87
D:PHE31		63.18	6.93||	0.03					
D:TYR32		53.73	2.88|	0.04					
D:HIS33	H	31.99	27.65|||||||	-0.34					
D:SER52	H	14.76	10.65||||||	-0.10					
D:PRO52A		21.95	0.17|	0.00					
D:TYR53		174.22	15.13|	0.24					
D:SER54		79.25	0.49|	-0.01					
D:LEU97		90.59	0.34|	-0.00					
D:ARG98		153.80	29.55||	0.07					
D:ASP99	H	118.53	28.74|||	-0.23					
D:GLY100		52.83	1.75|	-0.02					
D:THR100C	H	23.46	9.80||||	0.07					
D:HIS33		31.99	0.87|	-0.03	G:BMA1090	H	290.20	66.73|||	-1.33
D:TRP50		35.25	6.06||	0.10					
D:ASP56		82.09	5.52|	-0.03					
D:ARG98		153.80	2.90|	-0.03					
D:ASP99		118.53	8.40|	-0.05					
D:GLY100		52.83	16.78||||	-0.19					
D:SER100A		115.04	3.65|	0.06					
D:THR100C	H	23.46	13.65|||||	0.07					
D:TRP100D		96.11	0.78|	0.01					
D:ASP99	H	118.53	22.92||	-0.26	G:MAN1091	H	291.06	83.96|||	-1.30
D:GLY100	H	52.83	24.33||||	-0.02					
D:SER100A		115.04	21.29||	0.32					
D:TRP50		35.25	13.76||||	0.22	G:MAN1092		291.10	71.20|||	-1.09
D:ASP56		82.09	35.67|||||	-0.24					
D:LYS57		97.21	1.59|	-0.02					
D:ASN58		70.22	15.87|||	-0.07					
D:TRP50		35.25	1.77|	0.03	G:MAN1093	H	291.76	65.49|||	-0.59
D:ASP56		82.09	6.71|	-0.08					
D:LYS57	H	97.21	14.49||	-0.17					
D:ASN58	H	70.22	37.81|||||	-0.30					
D:GLN64		112.58	0.14|	-0.00					
D:SER54		79.25	9.66||	0.15	G:MAN1094		291.56	44.25||	-1.07
D:GLY55		40.15	0.00	0.00					
D:ASP56		82.09	30.40|||	0.18					

* D: Disulfide bond, H: Hydrogen bond, S: Salt bridge.
ASA: Accessible Surface Area, Å², BSA: Buried Surface Area, Å², ΔiG: Solvation energy effect, kcal/mol. |||: Buried area percentage, one bar per 10%.

FIG. 23E

35O22	HSD*	ASA	BSA	ΔiG	gp120	HSD	ASA	BSA	ΔiG
D:PRO72D		100.81	2.52|	-0.03	G:NAG1234		364.49	2.64|	-0.03
D:VAL72C		88.44	0.84|	0.01	G:NAG1235		364.33	52.98||	-1.74
D:PRO72D		100.81	12.83||	-0.15					
D:VAL72E		95.12	21.29|||	0.10					

Light chain residues interacting with gp120 glycans (interactive partners are shaded)									
35O22	HSD	ASA	BSA	ΔiG	gp120	HSD	ASA	BSA	ΔiG
E:HIS93	H	185.68	48.93|||	0.03	G:BMA1090	H	290.20	51.57||	-0.37
E:ASN94		129.52	10.53|	-0.04					
E:HIS93		185.68	46.93|||	0.38	G:MAN1091		291.06	49.91||	-1.43
E:HIS93		185.68	8.20|	-0.08	G:MAN1092	H	291.10	52.50||	-0.66
E:ASN94	H	129.52	50.11||||	-0.48					
E:ASN94		129.52	43.33||||	-0.57	G:MAN1093		291.76	44.67||	-1.35
E:SER95		60.35	0.34|	0.01					

*. D: Disulfide bond, H: Hydrogen bond, S: Salt bridge.
ASA: Accessible Surface Area, Å², BSA: Buried Surface Area, Å², ΔiG: Solvation energy effect, kcal/mol, |||: Buried area percentage, one bar per 10%.

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2015/010180

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K16/10 G01N33/569
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07K G01N

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data, BIOSIS, CHEM ABS Data, Sequence Search, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2012/158948 A1 (UNIV ROCKEFELLER [US]; SCHEID JOHANNES [US]; NUSSENZWEIG MICHEL [US];) 22 November 2012 (2012-11-22) cited in the application figures 3A and 3B -----	1-38
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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
2 April 2015	10/04/2015

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INTERNATIONAL SEARCH REPORT

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INTERNATIONAL SEARCH REPORT

International application No
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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

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