



(51) International Patent Classification:

C12N 15/10 (2006.01)

(21) International Application Number:

PCT/US2019/057268

(22) International Filing Date:

21 October 2019 (21.10.2019)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

62/748,905

22 October 2018 (22.10.2018)

US

(71) Applicants (for all designated States except US): THE

UNITED STATES OF AMERICA, AS REPRESENTED BY THE SECRETARY, DEPARTMENT OF HEALTH AND HUMAN SERVICES [US/US]; National Institutes of Health, Office of Technology Transfer, 6011 Executive Boulevard, Suite 325, MSC 7660, Bethesda, Maryland 20892-7660 (US). NEW YORK UNIVERSITY [US/US]; 70 Washington Square South, New York, New York 10012 (US).

(72) Inventors; and

(71) Applicants (for US only): FRANCHINI, Genoveffa [US/US]; NIH/CCR, 41 Center Drive, Bethesda, Maryland 20892 (US). CARDOZO, Timothy [US/US]; New York University School of Medicine, 180 Varick St., New York, New York 10014 (US). BECERRA-FLORES, Manuel [US/US]; New York University School of Medicine, 550 First Avenue, Medical Science Building, New York, New York 10016 (US). SILVA DE CASTRO, Isabela [US/US]; NIH/CCR, 41 Center Drive, Bethesda, Maryland 20892 (US). GORINI, Giacomo [US/US]; NIH/CCR, 41 Center Drive, Bethesda, Maryland 20892 (US). BISSA, Massimiliano [US/US]; NIH/CCR, 41 Center Drive, Bethesda, Maryland 20892 (US).

(74) Agent: SCOTT, Gregory K.; Klarquist Sparkman, LLP, One World Trade Center, Suite 1600, 121 SW Salmon Street, Portland, Oregon 97204 (US).

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ,

OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every

kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

- as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))
- as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))

Published:

- with international search report (Art. 21(3))
- before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))
- with sequence listing part of description (Rule 5.2(a))

(54) Title: RECOMBINANT GP120 PROTEIN WITH V1-LOOP DELETION

(57) Abstract: Embodiments of recombinant HIV-1 gp120 proteins that contain a V1 deletion are disclosed. Also provided are gp140, gp145, and gp160 proteins containing the V1 deletion, as well as HIV-1 Env ectodomain trimers containing protomers containing the V1 deletion. Nucleic acid molecules encoding these proteins are also provided. In several embodiments, the disclosed recombinant HIV-1 proteins and/or nucleic acid molecules can be used to generate an immune response to HIV-1 in a subject, for example, to treat or prevent an HIV-1 infection in the subject.

RECOMBINANT GP120 PROTEIN WITH V1-LOOP DELETION**CROSS REFERENCE TO RELATED APPLICATIONS**

This application claims priority to U.S. Provisional Application No. 62/748,905, filed
5 October 22, 2018, which is incorporated by reference in its entirety.

FIELD

This disclosure relates to recombinant Human immunodeficiency virus type 1 (HIV-1)
gp120 proteins and HIV-1 Envelope (Env) ectodomain trimers including the recombinant gp120
10 proteins for treatment and inhibition of HIV-1 infection and disease.

**STATEMENT REGARDING FEDERALLY SPONSORED
RESEARCH AND DEVELOPMENT**

This invention was made with Government Support under project number ZIA BC
15 011126 awarded by the National Institutes of Health, National Cancer Institute. The
Government has certain rights in this invention.

BACKGROUND

Millions of people are infected with HIV-1 worldwide, and 2.5 to 3 million new
20 infections have been estimated to occur yearly. Although effective antiretroviral therapies are
available, millions succumb to AIDS every year, especially in sub-Saharan Africa, underscoring
the need to develop measures to prevent the spread of this disease.

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
25 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-1 envelope spike, which is a target for
neutralizing antibodies.

It is believed that immunization with an effective immunogen based on HIV-1 Env can
30 elicit a neutralizing response, which may be protective against HIV-1 infection. However,
despite extensive effort, a need remains for agents capable of such action.

SUMMARY

This disclosure provides recombinant HIV-1 gp120 proteins that include a novel V1
35 domain deletion that unmask epitopes targeted by protective immune responses, and which are

shown to elicit a surprisingly effective immune response for viral inhibition in a primate model. The recombinant gp120 proteins and related embodiments, such as HIV-1 Env ectodomain trimers containing the recombinant gp120 proteins, can be used to elicit an immune response in a subject that inhibits HIV-1 infection.

5 In some embodiments, a recombinant gp120 protein comprising a deletion of HIV-1 Env residues 137-152 according to the HXBc2 numbering system is provided. The recombinant gp120 protein elicits an immune response that inhibits HIV-1 infection in a subject. In some embodiments, the recombinant gp120 protein comprises or consists of HIV-1 Env residues 31-507 containing the deletion of residues 137-152 (HXBc2 numbering). In some embodiments, 10 the recombinant gp120 protein comprises or consists the amino acid sequence set forth as any one of SEQ ID NOs: 1-3, or an amino acid sequence at least 90% identical thereto.

In some embodiments, the recombinant gp120 protein is included in a recombinant gp140 protein, a recombinant gp145 protein, or a recombinant gp160 protein.

In some embodiments, a recombinant HIV-1 Env ectodomain trimer is provided that 15 comprises protomers comprising the recombinant gp120 protein and a gp41 ectodomain. The recombinant HIV-1 Env ectodomain trimer elicits an immune response that inhibits HIV-1 infection in a subject. In some embodiments, the recombinant gp120 protein in the protomer comprises or consists of HIV-1 Env residues 31-507 containing the deletion of residues 137-152 (HXBc2 numbering), and the gp41 ectodomain in the protomer comprises or consists of HIV-1 20 Env residues 512-664 (HXBc2 numbering). In some embodiments, the protomers of the HIV-1 Env ectodomain trimer comprise or consist of the amino acid sequence set forth as any one of SEQ ID NOs: 4-5 and 66, or an amino acid sequence at least 90% identical thereto.

In some embodiments, a recombinant V1V2 domain of a gp120 protein is provided that comprises a deletion of HIV-1 Env residues 137-152 according to the HXBc2 numbering 25 system. In some embodiments, the recombinant V1V2 domain of the gp120 protein comprises or consists of HIV-1 Env residues 126-196 or 119-205 with the deletion of residues 137-152 (HXBc2 numbering). In some embodiments, the recombinant V1V2 domain comprises or consists of the amino acid sequence set forth as SEQ ID NO: 8 or an amino acid sequence at least 90% identical thereto. In some embodiments, the recombinant V1V2 domain can be fused 30 to a scaffold protein, such as a gp70 protein, a typhoid toxin protein, and an antibody Fc domain

Nucleic acid molecules encoding the disclosed recombinant gp120, gp140, gp160, or HIV-1 Env ectodomain trimer, or V1V2 domain are also provided. In some embodiments, the nucleic acid molecule can encode a precursor protein of a gp120-gp41 protomer of a disclosed recombinant HIV-1 Env trimer. Expression vectors (such as an inactivated or attenuated viral 35 vector) including the nucleic acid molecules are also provided.

Immunogenic compositions including one or more of the disclosed recombinant gp120, gp140, gp160, or HIV-1 Env ectodomain trimer or V1V2 domain are also provided. The composition may be contained in a unit dosage form. The composition can further include an adjuvant.

5 Methods of eliciting an immune response to HIV-1 envelope protein in a subject are disclosed, as are methods of treating, inhibiting or preventing an HIV-1 infection in a subject. In such methods a subject, such as a human subject, is administered an effective amount of a disclosed recombinant gp120, gp140, gp160, or HIV-1 Env ectodomain trimer or V1V2 domain to elicit the immune response. The subject can be, for example, a human subject at risk of or
10 having an HIV-1 infection.

In additional embodiments, a method for prognosis of an immune response to HIV-1 in a subject is provided. The method comprises contacting a biological sample from a subject with one or more peptides comprising or consisting of the amino acid sequence of HIV Env residues 141-154 (V1a), HIV Env residues 157-173 (V2b), or HIV Env residues 166-180 (V2c)
15 according to the HXBc2 numbering system, and detecting specific binding activity of antibodies in the biological sample to the one or more peptides. Detecting specific binding activity of antibodies in the biological sample to the V2b peptide or to the V2c peptide identifies the immune response to HIV-1 in the subject as an immune response that inhibits HIV-1 infection. Detecting specific binding activity of antibodies in the biological sample to the V1a peptide
20 identifies the immune response to HIV-1 in the subject as an immune response that does not inhibit HIV-1 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.

25 BRIEF DESCRIPTION OF THE FIGURES

FIGs. 1A-1K. (FIGs. 1A and 1B) Correlation of SIV DNA level in rectal mucosa obtained 2 weeks after infection in vaccinated macaques with serum recognition (detected by ELISA, optical density) of peptide 27 and 29 respectively. (FIG. 1C) Schematic representation
30 of the V1 and V2 SIV_{mac251} loops. The amino acids shown are not a linear sequence. (FIG. 1D) Serum level (detected by ELISA, optical density) of antibodies against peptide 23 and 24 in animals vaccinated with protective and non-protective vaccines following immunization (week 27). (FIG. 1E) Correlation of antibodies to V1 and time of SIV_{mac251} acquisition. (FIG. 1F-1G) Inhibition of binding to gp120 SIVsmE660/V1/V2 scaffold using combinations of the mAbs
35 NCI09 or NCI05 as probe and as competitors together with ITS101 and ITS41. (FIG. 1H)

Competition of increasing amount (0, 2, 5, 5, 10, and 20 $\mu\text{g/ml}$ of ITS41) with binding of NCI09 to SIV_{mac251} infected CD4⁺ cells. (FIG. 1I) Binding to human $\alpha 4\beta 7$ of native SIV_{mac251} M766 gp120 and of deglycosylated SIV_{mac251} M766 gp120 alone, in presence of non-relevant mAb ELN3 at 2.5 $\mu\text{g/ml}$ or NCI09 at 1.25 $\mu\text{g/ml}$. (FIG. 1J) ITS41-mediated inhibition of gp120 binding to $\alpha 4\beta 7$ at the concentrations tested in the competition experiment presented in FIG. 1K. (FIG. 1K) NCI09-mediated inhibition of binding of $\alpha 4\beta 7$ to deglycosylated SIV_{mac251} gp120 preincubated with serial dilutions of competitor ITS41 NCI09-mediated 81% inhibitory activity is calculated as a ratio of percent binding of native gp120 to $\alpha 4\beta 7$: percent binding of deglycosylated gp120 to $\alpha 4\beta 7$ in presence of NCI09.

FIGs. 2A-2N. (FIG. 2A and 2B) Spatial relationship of the V1 and V2 loops. (FIG. 2C) Modeling of the gp120 ΔV1 α -helix V2 (in blue). (FIG. 2D) Modeling of the gp120 ΔV1gp β -strand. (FIGs. 2E-2J) Western blot of the purified gp120_{WT}, gp120 ΔV1 and gp120 ΔV1gp with the α -V1 monoclonal antibodies NCI06 and ITS41, the polyclonal α -gp120 rabbit serum and the α -V2 mAbs NCI05 and NCI09. ELISA binding of the α -V2 mAbs MCI05 and NCI09 and the α -V1 mAb NCI06 to (FIG. 22) gp120_{WT}, (FIG. 2I) the gp120 ΔV1 and (FIG. 2M) the gp120 ΔV1gp . (FIG. 2N) Binding of simian soluble CD4 to the gp120_{WT}, the gp120 ΔV1 , the gp120 ΔV1gp and the recombinant protein IGF-1 as control.

FIGs. 3A-3N. (FIG. 3A) Schematic representation of the study design. Each vaccinated group included 14 young male macaques and the control group consisted of 18 naïve young male macaques. All animals were exposed beginning at week 17 to weekly low doses of SIV_{mac251} by the intrarectal route. Risk of SIV_{mac251} acquisition in animals immunized with WT (FIG. 3B), ΔV1 (FIG. 3C), or ΔV1gp (FIG. 3D) envelope immunogens. (FIG. 3E) Titers of antibody response cyclic V2. F: Amino acid sequence of the Diagnostic Peptide that recognize the second V2 $\alpha 4\beta 7$ site DP2 $\alpha 4\beta 7$ of SIV_{mac251} and SIV_{SME543} and of the epitopes recognized by NCI09 and NI05. (FIG. 3F) sequences of DP2a4b7₂₅₁ (SEQ ID NO: 22), DP2a4b7_{E543} (SEQ ID NO: 23), NCI09 (SEQ ID NO: 18), and NCI05 (SEQ ID NO: 35). Binding of the DP2 $\alpha 4\beta 7₂₅₁ and DP2 $\alpha 4\beta 7_{E543} to (FIG. 3G) NCI05 and (FIG. 3H) NCI09. Binding of sera from the immunized groups to (FIG. 3I) DP2 $\alpha 4\beta 7₂₅₁ or (FIG. 3J) DP2 $\alpha 4\beta 7_{E543}. (FIG. 3K) correlation of the serum immune response to DP2 $\alpha 4\beta 7_{E543} and the time of SIV_{mac251} acquisition C. Serum neutralizing antibodies to the Tier 2 SIV_{mac251CS.41} pseudovirus at week 17 were higher in the ΔV1 envelope immunogens group (FIG. 3M) and correlated with faster acquisition of SIV_{mac251} (FIG. 3N). Inhibition of V2 binding to $\alpha 4\beta 7$ by the sera of immunized animals (1:200 dilution) in an adhesion assay.$$$$$

FIGs. 4A and 4B. (FIG. 4A) Vaccine efficacy in the 78 animals cohort. Acquisition curves in animal vaccinated with ALVAC-SIV/gp120/alum (27 animals), ALVAC-SIV/gp120/MF9 (27 animals), DNA/ALVAC-SIV/gp120/alum (12 animals) and Ad26 ALVAC-SIV/gp120/alum (12 animals) compared to controls (53 animals). (FIG. 4B) Average per exposure risk of SIV_{mac251} acquisition (VE).

FIGs. 5A-5H. (FIG. 5A) V2 overlapping peptides encompassing the sequence of SIV_{mac251} K6W. A portion of the V2 sequence (NETSSCIAQNNCTGLEQEQMISCKFTMTGLKRDKTKEYNETWYSTDLVCEQGNSTD, SEQ ID NO: 36) is shown, as are Peptides 25 (SEQ ID NO: 37), Peptide 26 (SEQ ID NO: 38), Peptide 27 (SEQ ID NO: 39), Peptide 28 (SEQ ID NO: 40), and Peptide 29 (SEQ ID NO: 41). (FIG. 5B) Simplified schematic representation of the V1 (black) and V2 (red) loops. The amino acids shown are not a linear sequence. (FIG. 5C) Average serum antibody levels at week 27 to peptide 25-29 in vaccinees. (FIG. 5D-5H) Correlation between serum response at week 27 to each peptide and virus acquisition in vaccinees.

FIGs. 6A-6D. (FIG. 6A) V2 overlapping peptides encompassing the sequence of SIV_{mac251} K6W. A portion of the V1 sequence (NKSETDRWGLTKSSTTITTAAPTSAPVSEKIDMVNETSS, SEQ ID NO: 42) is shown, as are Peptide 15 (SEQ ID NO: 43), Peptide 16 (SEQ ID NO: 44), Peptide 17 (SEQ ID NO: 45), Peptide 18 (SEQ ID NO: 46), Peptide 19 (SEQ ID NO: 47), Peptide 20 (SEQ ID NO: 48), Peptide 21 (SEQ ID NO: 49), Peptide 22 (SEQ ID NO: 50), Peptide 23 (SEQ ID NO: 51), and Peptide 24 (SEQ ID NO: 52). (FIG. 6B) Simplified schematic representation of the V1 (black) and V2 (red) loops. The amino acids shown are not a linear sequence. (FIG. 6C) Average serum antibody levels at week 27 to peptide 15-24 in animal vaccinated with protective or non-protective vaccine. (FIG. 6D) No difference in the correlation with SIV_{mac251} acquisition with high or low (above or below average) serum response (week 27) to V1 peptides in animal immunized with protective vaccine.

FIGs. 7A-7D. (FIG. 7A) Serum antibody level to SIV gp120 in animals immunized and challenged protected against the first SIV_{mac251} challenge exposure (week 28) and subsequently immunized 9 times with ALVAC-SIV/gp120/alum. The titers of antibodies thereafter remain stable at 10⁴ up to 4 years. (FIG. 7B) Vaccine efficacy following the second exposure to SIV_{mac251}. Animal 770 remained uninfected. (FIG. 7C) Staining strategy on PBMCs to identify B cells from animal P770 positive for either or both the 1J08 SIV_{smE543} and the SIV_{smE543} V1V2 scaffolds. (FIG. 7D) Retrospective color identification of B-cells that produced the mAbs NCI04, NCI06, NCI05 and NCI09 isolated by cloning in animal 770.

FIGs. 8A-8D. (FIG. 8A) Summary of binding in ELISA of monoclonal antibodies NCI04, NCI06, NCI05, and NCI09. SEQ ID NOs: 20, 53, 54, 55, and 56 are shown in the table. (FIG. 8B) NCI09 binding to SIV_{mac251} infected CD4⁺ cells. (FIG. 8C) Overlapping peptides used to compete NCI09 binding to cyclic V2 in (FIG. 8D). In FIG. 8C, the sequences of Peptide p41 (SEQ ID NO: 57), Peptide p42 (SEQ ID NO: 58), Peptide p43 (SEQ ID NO: 59), Peptide p44 (SEQ ID NO: 60), Peptide p45 (SEQ ID NO: 61), Peptide p46 (SEQ ID NO: 62), Peptide p47 (SEQ ID NO: 63), and Peptide p48 (SEQ ID NO: 64) are shown.

FIGs. 9A-9E. (FIG. 9A) NCI05 binding to SIV_{mac251} infected CD4⁺ cells. (FIGs. 9B-9D) K_A and K_D of NCI05 and NCI09 binding to SIV_{mac251} cyclic V2. (FIG. 9E) Competition of NCI05 binding with the overlapping peptide depicted in FIG. 8C.

FIG. 10A-10C. (FIG. 10A and 10B) Overall structure of NCI09 in complex with a SIV_{mac251} linear peptide. The peptide sequence (KFTMTGLKRDKTKEYN, SEQ ID NO: 18) is that of consensus SIV_{mac251}, in complex with Fab of antibody NCI09. The N-terminal 4 residues were not ordered in the structure. The NCI09 heavy and light chains are displayed as yellow and blue ribbons, respectively. (FIG. 10C) Side chains in peptide making most of the contact with NCI09 are shown in red space filling spheres with sizes proportional to the area of contact with NCI09: these key contacts, and therefore the NCI09-targeted epitope may be precisely represented by the sequence motif RxKxxEY (SEQ ID NO: 68).

FIGs. 11A-11D (FIG. 11A) α -V1 and α -V2 monoclonal antibodies neutralizing activity against Tier 1 and Tier 2 SIV_{mac251} and SIV_{smE660} pseudoviruses. >50% neutralization. (FIG. 11B) Dose dependent inhibition of α 4 β 7 binding of mildly deglycosylated SIV_{M766} (adhesion assay) by NCI09. (FIG. 11C) Inhibition of α 4 β 7 binding of mildly deglycosylated SIV_{M766} (adhesion assay) by sera of immunized (p770, p842, p888, p897) or naïve animals (MAE, MGM, MT3). The mAb 2B4 serves as positive control. (FIG. 11D) Titration of the inhibitory α 4 β 7 binding activity of the sera from two of the immunized macaques presented in Fig 4 and S4a.

FIGs. 12A-12D. (FIG. 12A) ELISA profiles of anti-V1 ITS41 probe competed by NCI09, ITS41, ITS01, and BSA. (FIG. 12B) ELISA profiles of anti-V1 ITS41 probe competed by NCI05, ITS41, ITS01, and BSA. (FIG. 12C) ELISA profiles of anti-V1 NCI06 probe competed by NCI09, ITS01, NCI06, and BSA. (FIG. 12D) ELISA profiles of anti-V2 NCI09 probe competed by NCI06, NCI09, ITS01 and BSA.

FIGs. 13A-13B. (FIG. 13A) ELISA titers of serum antibodies against SIV_{mac251/766} five weeks post immunizations. (FIG. 13B) ELISA peptide array on the entire gp120 with sera collected five weeks post immunization.

FIG. 14A and 14B. (FIG. 14A) Ribbon diagram showing a superimposed models of the SIVmac251 gp120 with the 137-152 Δ V1 deletion (red) and HIV-1 A244 gp120 with the 137-152 Δ V1 deletion (green) that illustrates the identical conformation of each mutated gp120. The structure shown is gp120 of the HIV-1 Env trimer in the prefusion mature closed conformation.

(FIG. 14B) Binding values (OD) of macaque serum from macaque sera from an animal vaccinated with a protective vaccine compared to A244 HIV V2b probe.

FIG. 15. ELISA binding of the V1/V2 specific antibody PG9 and the V2 specific antibody CH58 to gp120 from HIV-1 strain A244 with (Δ V1) and without (WT) deletion of V1 residues 137-152 (HXBc2 numbering), or SIV gp120 with the corresponding V1 deletion. The sequence of the A244 gp120 Δ V1 is provided as SEQ ID NO: 1. The gp120 proteins were produced in 293 cells or CHO cells.

SEQUENCES

The nucleic and amino acid sequences listed herein 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" (~84 kb), which was created on October 9, 2019 which is incorporated by reference herein.

DETAILED DESCRIPTION

A major obstacle to the development of a protective HIV-1 vaccine is the antigenic variation of the viral envelope protein, which varies epitopes that could be targeted by the human or other host immune system from strain to strain and also conceals conserved epitopes via glycosylation and conformational masking. This remarkable variation and plasticity of the viral envelope spike underlies the belated and inconsistent appearance of protective and/or broadly neutralizing antibodies in HIV-infected individuals, as well as the failure of experimental vaccines to elicit such antibodies.

Variable region 1 and Variable Region 2 (V1/V2) of the gp120 component of the viral spike are believed to both harbor key epitopes that could be targeted by the host immune system to reduce the risk of viral acquisition and contribute greatly to the antigenic variation and conformational masking that facilitates evasion of host antibody responses, including but not limited to neutralizing antibody responses. Localized to a membrane-distal, apical "cap," which holds the spike in a neutralization-resistant conformation, V1/V2 is not essential for host cell entry, but removal in its entirety renders the virus sensitive to antibody-mediated neutralization.

The ~50-90 residues that comprise V1/V2 contain two of the most sequence-variable portions of the virus, and one in ten residues of V1/V2 are *N*-glycosylated. Despite the diversity and glycosylation of V1/V2, a number of broadly neutralizing and non-neutralizing, cross-reactive human antibodies have been identified that target this region. As discussed in the examples, the majority of these antibodies share specificity for the V2 portion of the V1V2 domain. However, despite extensive effort, immunogens embodying intact V1V2 have proven ineffective at eliciting a V2-based immune response that is protective against HIV-1 infection.

In the current disclosure, the V1 was assessed as responsible for conformational masking of the key epitopes in V2 targeted by the host immune system to reduce the risk of viral acquisition. Structure-guided design was used to identify deletions of V1 residues that exposes V2 epitopes. Surprisingly, immunogens containing a particular V1 deletion (HXBc2 residues 137-152) elicited higher responses to V2 (suggesting that V2 is masked in the presence of V1) than the corresponding wild type immunogen (V1 replete) or immunogens with deletion of different V1 residues. In addition, the V1-deleted immunogen (HXBc2 residues 137-152) exhibited increased binding to soluble CD4 and V2 antibodies relative to the corresponding wild-type control and other V1-deleted immunogens, suggesting that these areas are more exposed in the V1-deleted immunogens. Even more surprisingly, the V1-deletion yielded an immunogen that elicits a protective immune response in animal models. These results are particularly unexpected in view of prior observations that V1-deleted HIV-1 immunogens fail to elicit a V2-directed antibody response.

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.” 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, particularly suitable methods and materials are

described herein. 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:

5 **Adjuvant:** A component of an immunogenic composition used to enhance antigenicity. In some embodiments, an adjuvant can include a suspension of minerals (alum, aluminum hydroxide, or phosphate) on which antigen is adsorbed; or water-in-oil emulsion, for example, in which antigen solution is emulsified in mineral oil (Freund incomplete adjuvant), sometimes with the inclusion of killed mycobacteria (Freund's complete adjuvant) to further enhance
10 antigenicity (inhibits degradation of antigen and/or causes influx of macrophages). In some embodiments, the adjuvant used in a disclosed immunogenic composition is a combination of lecithin and carbomer homopolymer (such as the ADJUPLEX™ adjuvant available from Advanced BioAdjuvants, LLC, see also Wegmann, Clin Vaccine Immunol, 22(9): 1004-1012, 2015). Additional adjuvants for use in the disclosed immunogenic compositions include the
15 QS21 purified plant extract, Matrix M, AS01, MF59, and ALFQ adjuvants. Immunostimulatory oligonucleotides (such as those including a CpG motif) can also be used as adjuvants. Adjuvants include biological molecules (a “biological adjuvant”), such as costimulatory molecules. Exemplary adjuvants include IL-2, RANTES, GM-CSF, TNF- α , IFN- γ , G-CSF, LFA-3, CD72, B7-1, B7-2, OX-40L, 4-1BBL and toll-like receptor (TLR) agonists, such as
20 TLR-9 agonists. The person of ordinary skill in the art is familiar with adjuvants (see, *e.g.*, Singh (ed.) Vaccine Adjuvants and Delivery Systems. Wiley-Interscience, 2007). Adjuvants can be used in combination with the disclosed immunogens.

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
25 composition (such as a composition including a disclosed immunogen) 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.

30 **Amino acid substitution:** The replacement of one amino acid in a polypeptide with a different amino acid. In some examples, an amino acid in a polypeptide is substituted with an amino acid from a homologous polypeptide, for example, an amino acid in a recombinant Clade A HIV-1 Env polypeptide can be substituted with the corresponding amino acid from a Clade B HIV-1 Env polypeptide.

Antibody: An immunoglobulin, antigen-binding fragment, or derivative thereof, that specifically binds and recognizes an analyte (antigen), such as 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 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). Light and heavy 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 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.

Biological sample: A sample of biological material obtained from a subject. Biological samples include all clinical samples useful for detection of disease or infection (*e.g.*, HIV infection) in subjects. Appropriate samples include any conventional biological samples, including clinical samples obtained from a human or veterinary subject. Exemplary samples include, without limitation, cells, cell lysates, blood smears, cytocentrifuge preparations, cytology smears, bodily fluids (*e.g.*, blood, plasma, serum, saliva, sputum, urine, bronchoalveolar lavage, semen, cerebrospinal fluid (CSF), etc.), tissue biopsies or autopsies, fine-needle aspirates, and/or tissue sections. In a particular example, a biological sample is obtained from a subject having, suspected of having or at risk of having HIV infection.

Carrier: An immunogenic molecule to which an antigen (such as gp120) can be linked. When linked to a carrier, the antigen may become more immunogenic. Carriers are chosen to increase the immunogenicity of the antigen and/or to elicit antibodies against the carrier which are diagnostically, analytically, and/or therapeutically beneficial. Useful carriers include polymeric carriers, which can be natural (for example, proteins from bacteria or viruses), semi-synthetic or synthetic materials containing one or more functional groups to which a reactant moiety can be attached.

Conservative variants: “Conservative” amino acid substitutions are those substitutions that do not substantially affect or decrease a function of a protein, such as the ability of the protein to elicit an immune response when administered to a subject. The term conservative variation also includes the use of a substituted amino acid in place of an unsubstituted parent amino acid. Furthermore, individual substitutions, deletions or additions which alter, add or delete a single amino acid or a small percentage of amino acids (for instance less than 5%, in some embodiments less than 1%) in an encoded sequence are conservative variations where the alterations result in the substitution of an amino acid with a chemically similar amino acid.

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 recombinant Env protein, such as the ability to elicit an immune response when administered to a subject. 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.

Contacting: Placement in direct physical association; includes both in solid and liquid form. 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 contact another polypeptide, such as an antibody. Contacting also includes administration, such as administration of a disclosed antigen to a subject by a chosen route.

Control: A reference standard. In some embodiments, the control is a negative control sample obtained from a healthy patient. In other embodiments, the control is a positive control sample obtained from a patient diagnosed with HIV-1 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-1 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%, at least about 500%, or greater than 500%.

Degenerate variant: In the context of the present disclosure, a “degenerate variant” refers to a polynucleotide encoding a polypeptide (such as a disclosed immunogen) that includes a sequence that is degenerate as a result of the genetic code. There are 20 natural amino acids, most of which are specified by more than one codon. Therefore, all degenerate nucleotide sequences encoding a peptide are included as long as the amino acid sequence of the peptide 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 a V1a, V2b, or V2c peptide as disclosed herein, 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, fluorescent proteins, chemiluminescent agents, enzymatic linkages, radioactive isotopes and heavy metals or compounds (for example super paramagnetic iron oxide nanocrystals for detection by MRI). In some embodiments, the detectable marker a radiolabeled amino acid incorporated into the peptide, or attachment of the peptide to 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). Any suitable method of labeling peptides and may be used. Examples of labels for peptides include, but are not limited to: 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, 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. Detection can include a physical readout, such as fluorescence or a reaction output, or the results of a PCR assay.

Diagnosis: The process of identifying a disease by its signs, symptoms and results of various tests. The conclusion reached through that process is also called “a diagnosis.” Forms of testing commonly performed include blood tests, medical imaging, urinalysis, and biopsy.

Effective amount: An amount of agent, such as an immunogen, that is sufficient to elicit a desired response, such as an immune response in a subject. It is understood that to obtain a protective immune response against an antigen of interest can require multiple administrations of a disclosed immunogen, and/or administration of a disclosed immunogen as the “prime” in a prime boost protocol wherein the boost immunogen can be different from the prime immunogen. Accordingly, an effective amount of a disclosed immunogen can be the amount of the immunogen sufficient to elicit a priming immune response in a subject that can be subsequently boosted with the same or a different immunogen to elicit a protective immune response.

In one example, a desired response is to inhibit or reduce or prevent HIV-1 infection. The HIV-1 infection does not need to be completely eliminated or reduced or prevented for the method to be effective. For example, administration of an effective amount of the agent can decrease the HIV-1 infection (for example, as measured by infection of cells, or by number or percentage of subjects infected by HIV-1) by a desired amount, for example by 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 or prevention of detectable HIV-1 infection), as compared to a suitable control.

Epitope-Scaffold Protein: A chimeric protein that includes an epitope sequence fused to a heterologous “acceptor” scaffold protein. Design of the epitope-scaffold is performed, for example, computationally in a manner that preserves the native structure and conformation of the epitope when it is fused onto the heterologous scaffold protein. Several embodiments include an epitope scaffold protein with a recombinant V1V2 domain included on a heterologous scaffold protein. When linked to the heterologous scaffold, the recombinant V1V2 domain a conformation similar to that of the recombinant V1V2 domain in the context of the HIV-1 Env ectodomain trimer.

Expression: Transcription or translation of a nucleic acid sequence. For example, a gene is expressed when its DNA is transcribed into an RNA or RNA fragment, which in some

examples is processed to become mRNA. A gene may also be expressed when its mRNA is translated into an amino acid sequence, such as a protein or a protein fragment. In a particular example, a heterologous gene is expressed when it is transcribed into an RNA. In another example, a heterologous gene is expressed when its RNA is translated into an amino acid sequence. The term “expression” is used herein to denote either transcription or translation. Regulation of expression can include controls on transcription, translation, RNA transport and processing, degradation of intermediary molecules such as mRNA, or through activation, inactivation, compartmentalization or degradation of specific protein molecules after they are produced.

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 (ATG) in front of a protein-encoding gene, splicing signals 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. Non-limiting examples of expression vectors include 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.

Heterologous: A heterologous polypeptide or polynucleotide refers to a polypeptide or polynucleotide derived from a different source or species.

Human Immunodeficiency Virus Type 1 (HIV-1): A retrovirus that causes immunosuppression in humans (HIV-1 disease), and leads to a disease complex known as the acquired immunodeficiency syndrome (AIDS). “HIV-1 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-1 virus, as determined by antibody or western blot studies. Laboratory findings associated with this disease include a progressive decline in T cells. 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 HIV-1 Envelope protein and inhibiting HIV-1 function. 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 PG9 and VRC01.

HIV-1 envelope protein (Env): The HIV-1 Env protein is initially synthesized as a precursor protein of 845-870 amino acids in size. Individual precursor 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 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 HIV-1 Env ectodomain comprises the gp120 protein (approximately HIV-1 Env positions 31-511) and the gp41 ectodomain (approximately HIV-1 Env positions 512-664). An HIV-1 Env ectodomain trimer comprises a protein complex of three HIV-1 Env ectodomains. As used
 5 herein "HIV-1 Env ectodomain trimer" includes both soluble trimers (that is, trimers without gp41 transmembrane domain or cytoplasmic tail) and membrane anchored trimers (for example, trimers including a full-length gp41).

Mature gp120 includes approximately 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
 10 cellular CD4 receptors and to cellular chemokine receptors (such as CCR5). The mature gp120 wild-type polypeptide is heavily N-glycosylated, giving rise to an apparent molecular weight of 120 kD. Native gp120 includes five conserved regions (C1-C5) and five regions of high variability (V1-V5).

Variable region 1 and Variable Region 2 (V1/V2 domain) of gp120 include ~50-90
 15 residues which contain two of the most variable portions of HIV-1 (the V1 loop and the V2 loop), and one in ten residues of the V1/V2 domain are N-glycosylated. Despite the diversity and glycosylation of the V1/V2 domain, a number of broadly neutralizing human antibodies have been identified that target this region, including the somatically related antibodies PG9 and PG16 (Walker *et al.*, *Science*, 326:285-289, 2009). In certain examples the V1/V2 domain
 20 includes gp120 position 126-196.

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.

A standardized numbering scheme for HIV-1 Env proteins (the HXBc2 numbering 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 *et al.*, Eds. Theoretical Biology and Biophysics Group, Los Alamos National Laboratory, Los Alamos, NM, which is incorporated by reference herein in its entirety.
 25 For reference, the amino acid sequence of HIV-1 Env of HXB2 is set forth as SEQ ID NO: 10 (GENBANK® GI:1906382, incorporated by reference herein).

HXBc2 (Clade B, SEQ ID NO: 10):

MRVKEKYQHLWRWGWRGTMLLGLMLICSATEKLWVTVYYGVPVWKEATTTLCASDAKAYDTEVHNWATHACVPT
 35 DPNPQEVVLNVNVTENFNMWKNDMVEQMHEDIISLWDQSLKPCVKLTPLCVSLKCTDLKNDTNTNSSSGRMIMEKGEI
 KNCSFNISTSIIRGKVQKEYAFFYKLDIIPIDNDTTSYKLTSCNTSVITQACPKVSFEPIPIHYCAPAGFAILKCNK
 TFNGTGPTNVSTVQCTHGIRPVVSTQLLLNGSLAEEVVIRSVNFTDNAKTIIVQLNTSVEINCTRPNNNTRKRIR
 IQRGPGRFVVTIGKIGNMRQAHCNISRAKWNNTLQKIASKLREQFGNNKTIIFKQSSGGDPEIVTHSFNCGGEFFYC
 NSTQLFNSTWFNSTWSTEGSNNTGSDTITLPCRIKQIINMWQKVGKAMYAPPISGQIRCSSNITGLLLTRDGGNSN

NESEIFRPGGGDMRDNRSELYKYKVVKIEPLGVAPTAKARRVVQREKRAVGIGALFLGFLGAAGSTMGAASMTLTV
 QARQLLSGIVQQNNLLRAIEAQQHLLQLTVWGIKQLQARILAVERYLKDQQLLGIWGC SGKLICTTAVPWNASWSN
 KSLEQIWNHTTWMEWDREINNYTSLIHSLIEESQNQQEKNEQELLELDKWASLWNWFNITNWLWYIKL FIMIVGGLV
 GLRIVFAVLSIVNRVRQGYSPLSFQTHLPTPRGPDREPIEGEEGERDRDRSIRLVNGSLALIWDLLRSLCLFSYHR
 LRDLLLIIVTRIVELLGRRGWEALKYWWNLLQYWSQELKNSAVSLLNATAI AVEGTDRVIEVVQGACRAIRHIPRI
 RQGLERILL

HIV-1 gp140: A recombinant HIV Env polypeptide including gp120 and the gp41 ectodomain, 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 gp145: A recombinant HIV Env polypeptide including gp120, the gp41 ectodomain, and the gp41 transmembrane domain. HIV-1 gp145 polypeptides can trimerize to form a membrane-anchored HIV-1 Env ectodomain trimers.

HIV-1 gp160: A recombinant HIV Env polypeptide including gp120 and the entire gp41 protein (ectodomain, transmembrane domain, and cytosolic tail).

Host cells: Cells in which a vector can be propagated and its DNA expressed. 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. However, such progeny are included when the term “host cell” is used.

Immunogenic conjugate: A composition composed of at least two heterologous molecules (such as an HIV-1 Env trimer and a carrier, such as a protein carrier) linked together that stimulates or elicits an immune response to a molecule in the conjugate in a vertebrate. In some embodiments where the conjugate include a viral antigen, the immune response is protective in that it enables the vertebrate animal to better resist infection from the virus from which the antigen is derived.

Immune response: A response of a cell of the immune system, such as a B cell, T cell, or monocyte, to a stimulus, such as a vaccination or an infection. In one embodiment, the response is specific for a particular antigen (an “antigen-specific response”). In one embodiment, an immune response is a T cell response, such as a CD4⁺ response or a CD8⁺ response. In another embodiment, the response is a B cell response, and results in the production of specific antibodies. “Priming an immune response” refers to treatment of a subject with a “prime” immunogen to induce an immune response that is subsequently “boosted” with a boost immunogen. Together, the prime and boost immunizations produce the desired immune response in the subject. “Enhancing an immune response” refers to co-administration of an adjuvant and an immunogenic agent, wherein the adjuvant increases the desired immune response to the immunogenic agent compared to administration of the immunogenic agent to the subject in the absence of the adjuvant.

Immunogen: A protein or a portion thereof that is capable of inducing an immune response in a mammal, such as a mammal infected or at risk of infection with a pathogen.

Immunogenic composition: A composition comprising a disclosed immunogen, or a nucleic acid molecule or vector encoding a disclosed immunogen, that elicits a measurable CTL response against the immunogen, or elicits a measurable B cell response (such as production of antibodies) against the immunogen, when administered to a subject. It further refers to isolated nucleic acids encoding an immunogen, such as a nucleic acid that can be used to express the immunogen (and thus be used to elicit an immune response against this immunogen). For *in vivo* use, the immunogenic composition will typically include the protein or nucleic acid molecule in a pharmaceutically acceptable carrier and may also include other agents, such as an adjuvant.

Isolated: An “isolated” biological component has been substantially separated or purified away from other biological components, such as other biological components in which the component naturally occurs, such as other chromosomal and extrachromosomal DNA, RNA, and proteins. Proteins, peptides, nucleic acids, and viruses that have been “isolated” include those purified by standard purification methods. Isolated does not require absolute purity, and can include protein, peptide, nucleic acid, or virus molecules that are at least 50% isolated, such as at least 75%, 80%, 90%, 95%, 98%, 99%, or even 99.9% isolated.

Linked: The term “linked” means joined together, either directly or indirectly. For example, a first moiety may be covalently or noncovalently (e.g., electrostatically) linked to a second moiety. This includes, but is not limited to, covalently bonding one molecule to another molecule, noncovalently bonding one molecule to another (e.g. electrostatically bonding), non-covalently bonding one molecule to another molecule by hydrogen bonding, non-covalently bonding one molecule to another molecule by van der Waals forces, and any and all combinations of such couplings. Indirect attachment is possible, such as by using a “linker”. In several embodiments, linked components are associated in a chemical or physical manner so that the components are not freely dispersible from one another, at least until contacting a cell, such as an immune cell.

Linker: One or more molecules or groups of atoms positioned between two moieties. Typically, linkers are bifunctional, *i.e.*, the linker includes a functional group at each end, wherein the functional groups are used to couple the linker to the two moieties. The two functional groups may be the same, *i.e.*, a homobifunctional linker, or different, *i.e.*, a heterobifunctional linker. In several embodiments, a peptide linker can be used to link the C-terminus of a first protein to the N-terminus of a second protein. Non-limiting examples of peptide linkers include glycine-serine peptide linkers, which are typically not more than 10

amino acids in length. Typically, such linkage is accomplished using molecular biology techniques to genetically manipulate DNA encoding the first polypeptide linked to the second polypeptide by the peptide linker.

Native protein, sequence, or disulfide bond: A polypeptide, sequence or disulfide bond that has not been modified, for example, by selective mutation. For example, selective mutation to focus the antigenicity of the antigen to a target epitope, or to introduce a disulfide bond into a protein that does not occur in the native protein. Native protein or native sequence are also referred to as wild-type protein or wild-type sequence. A non-native disulfide bond is a disulfide bond that is not present in a native protein, for example, a disulfide bond that forms in a protein due to introduction of one or more cysteine residues into the protein by genetic engineering.

Nucleic acid molecule: A polymeric form of nucleotides, which may include both sense and anti-sense strands of RNA, cDNA, genomic DNA, and synthetic forms and mixed polymers of the above. A nucleotide refers to a ribonucleotide, deoxynucleotide or a modified form of either type of nucleotide. The term “nucleic acid molecule” as used herein is synonymous with “nucleic acid” and “polynucleotide.” A nucleic acid molecule is usually at least 10 bases in length, unless otherwise specified. The term includes single- and double-stranded forms of DNA. A polynucleotide may include either or both naturally occurring and modified nucleotides linked together by naturally occurring and/or non-naturally occurring nucleotide linkages. “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.

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 is operably linked to a coding sequence if the promoter affects the transcription or expression of the coding sequence. Generally, operably linked nucleic acid sequences are contiguous and, where necessary to join two protein-coding regions, in the same reading frame.

Peptide: A polymer in which the monomers are amino acid residues that are joined together through amide bonds. The amino acids included in a peptide may be subject to post-translational modification (e.g., glycosylation or phosphorylation). In some embodiments, a peptide can be between 10 and 30 amino acids in length, such as from 10 to 20 amino acids in

length. In several embodiments, a polypeptide or peptide is at most 50 amino acids in length, such as at most 40, at most 30, or at most 20 amino acids in length. Peptides for use in the method embodiments disclosed herein can be linked to heterologous moieties, such as tags and labels.

5 Peptides include analogs (non-peptide organic molecules), derivatives (chemically functionalized peptide molecules obtained starting with the disclosed peptide sequences) and variants (homologs) that can be utilized in the methods described herein.

 Peptides can be modified by a variety of chemical techniques to produce derivatives having essentially the same activity as the unmodified peptides, and optionally having other
10 desirable properties. For example, carboxylic acid groups of the peptide, whether carboxyl-terminal or side chain, can be provided in the form of a salt of a pharmaceutically-acceptable cation or esterified to form a C₁-C₁₆ ester, or converted to an amide of formula NR₁R₂ wherein R₁ and R₂ are each independently H or C₁-C₁₆ 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
15 chain, can 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 can be modified to C₁-C₁₆ alkyl or dialkyl amino or further converted to an amide for the incorporation of certain functionalities of linkage of ligand molecules, such as an adjuvant.

 Hydroxyl groups of the peptide side chains may be converted to C₁-C₁₆ alkoxy or to a
20 C₁-C₁₆ ester using well-recognized techniques to introduce hydrophobic characteristics to the peptide. Alternatively, the hydroxyl groups may be sulfated or phosphorylated to introduce negative charge and increase water solubility. Phenyl and phenolic rings of the peptide side chains may be substituted with one or more halogen atoms, such as fluorine, chlorine, bromine or iodine, or with C₁-C₁₆ alkyl, C₁-C₁₆ alkoxy, carboxylic acids and esters thereof, or amides of
25 such carboxylic acids. Methylene groups of the peptide side chains can be extended to homologous C₂-C₄ alkenes. Thiols can be protected with any one of a number of well-recognized protecting groups, such as acetamide groups. Thiols may be reacted with maleimides or disulfides. Those skilled in the art will also recognize methods for introducing cyclic structures into the peptides of this invention to select and provide conformational constraints to
30 the structure that result in enhanced stability.

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 immunogens.

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 or otherwise contained in a unit dosage form containing one or more measured doses of the composition suitable to elicit the desired anti-HIV-1 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, or lyophilized for subsequent solubilization and administration or in a solid or controlled release dosage.

Polypeptide: Any chain of amino acids, regardless of length or post-translational modification (*e.g.*, glycosylation or phosphorylation). “Polypeptide” applies to amino acid polymers including naturally occurring amino acid polymers and non-naturally occurring amino acid polymer as well as in which one or more amino acid residue is a non-natural amino acid, for example, an artificial chemical mimetic of a corresponding naturally occurring amino acid. 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. “Polypeptide” is used interchangeably with protein, and is used herein to refer to a polymer of amino acid residues.

Prime-boost immunization: An immunotherapy including administration of multiple immunogens over a period of time to elicit the desired immune response.

Prognosis of an immune response to HIV-1 in a subject: A prediction of the likelihood that an immune response in a subject will (or will not) inhibit HIV-1 infection in the subject. For example, the prediction can include determining the likelihood that an immune response in a subject will (or will not) prevent HIV-1 infection in the subject. In some embodiments, the prediction includes determining the likelihood that an immune response in a subject will (or will not) inhibit signs or symptoms of HIV-1 in a subject already infected with HIV-1, such as full development of HIV-1 in the subject, a delayed onset of clinical symptoms

of the HIV-1 infection, a reduction in severity of some or all clinical symptoms of the HIV-1 infection, a slower progression of the HIV-1 disease (such as a slower progression to AIDS).

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, for example, the artificial manipulation of isolated segments of nucleic acids, for example, using genetic engineering techniques. A recombinant protein 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. In several embodiments, a recombinant protein is 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.

RV144 Trial: A phase III clinical trial of a prime-boost HIV-1 vaccine that was carried out in Thailand. The immunization protocol consisted of four injections of ALVAC HIV (vCP1521) followed by two injections of AIDSVAX B/E. ALVAC HIV (vCP1521) is a canarypox vector genetically engineered to express HIV-1 Gag and Pro (subtype B LAI strain) and CRF01_AE (subtype E) HIV-1 gp120 (92TH023) linked to the transmembrane anchoring portion of gp41 (LAI). AIDSVAX B/E is a bivalent HIV gp120 envelope glycoprotein vaccine containing a subtype E envelope from the HIV-1 strain A244 (CM244) and a subtype B envelope from the HIV-1 MN each produced in Chinese hamster ovary cell lines. The envelope glycoproteins, 300 µg of each, were co-formulated with 600 µg of alum adjuvant. The RV144 trial, ALVAC HIV (vCP1521), and AIDSVAX B/E are described in Rerks-Ngarm et al. (New Eng J Med. 361 (23): 2209–2220, 2009, incorporated by reference herein). In some embodiments, the Env ectodomain encoding portion of ALVAC HIV (vCP1521) and the gp120 proteins of AIDSVAX B/E can be modified to encode or contain the V1 deletion provided herein (deletion of residues 137-152 according to HXBc2 numbering) and administered to a subject using the rv144 prime-boost protocol (or any other suitable protocol).

Sensitivity and specificity: Statistical measurements of the performance of a binary classification test. Sensitivity measures the proportion of actual positives which are correctly identified (*e.g.*, the percentage of samples that are identified as including nucleic acid from a particular virus). Specificity measures the proportion of negatives which are correctly identified (*e.g.*, the percentage of samples that are identified as not including nucleic acid from a particular virus).

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; the higher the percentage, the more similar the two sequences are. Homologs, orthologs, 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 & Waterman, *Adv. Appl. Math.* 2:482, 1981; Needleman & Wunsch, *J. Mol. Biol.* 48:443, 1970; Pearson & Lipman, *Proc. Natl. Acad. Sci. USA* 85:2444, 1988; Higgins & Sharp, *Gene*, 73:237-44, 1988; Higgins & Sharp, *CABIOS* 5:151-3, 1989; Corpet *et al.*, *Nuc. Acids Res.* 16:10881-90, 1988; Huang *et al.* *Computer Appls. In the Biosciences* 8, 155-65, 1992; and Pearson *et al.*, *Meth. Mol. Bio.* 24:307-31, 1994. Altschul *et al.*, *J. Mol. Biol.* 215:403-10, 1990, presents a detailed consideration of sequence alignment methods and homology calculations.

Variants of 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.

As used herein, reference to “at least 90% identity” (or similar language) refers to “at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or even 100% identity” to a specified reference sequence.

Signal Peptide: A short amino acid sequence (*e.g.*, approximately 18-30 amino acids in length) that directs newly synthesized secretory or membrane proteins to and through membranes (for example, the endoplasmic reticulum membrane). Signal peptides are typically located at the N-terminus of a polypeptide and are removed by signal peptidases after the polypeptide has crossed the membrane. Signal peptide sequences typically contain three common structural features: an N-terminal polar basic region (n-region), a hydrophobic core, and a hydrophilic c-region). Exemplary signal peptide sequences are set forth as residues 1-11 of SEQ ID NOs: 10, 6, and 7.

Specifically bind: When referring to the formation of an antibody:antigen protein complex, or a protein:protein complex, refers to a binding reaction which determines the presence of a target protein, peptide, or polysaccharide (for example, a glycoprotein), in the presence of a heterogeneous population of proteins and other biologics. Thus, under designated conditions, a particular antibody or protein binds preferentially to a particular target protein, peptide or polysaccharide (such as an antigen present on the surface of a pathogen, for example, gp120) 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 standard methods. A first protein or antibody specifically binds to a target protein when the interaction has a K_D of less than 10^{-7} Molar, such as less than 10^{-8} Molar, less than 10^{-9} , or even less than 10^{-10} Molar.

Subject: Living multicellular vertebrate organisms, a category that includes human and non-human mammals. In an example, a subject is a human. 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.

Transmembrane domain: An amino acid sequence that inserts into a lipid bilayer, such as the lipid bilayer of a cell or virus or virus-like particle. A transmembrane domain can be used to anchor an antigen to a membrane.

Treating or inhibiting HIV-1: Inhibiting the full development of HIV-1 in a subject who is at risk for or has an HIV-1 infection or acquired immunodeficiency syndrome (AIDS).

“Treatment” refers to a therapeutic intervention that ameliorates a sign or symptom of HIV-1 infection in an infected subject. 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.

Inhibiting HIV-1 in an uninfected subject refers to a reduction in infection rate or likelihood of infection. In this context, the term “reduces” is a relative term. An immunogenic composition that induces an immune response that inhibits HIV-1, can, but does not necessarily completely, inhibit HIV-1 infection of a subject (or group of subjects), 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% of (that is to 10% or less than) the infection or response in the absence of the agent, or in comparison to a reference agent.

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.

Vaccine: A pharmaceutical composition that elicits a prophylactic or therapeutic immune response in a subject. In some cases, the immune response is a protective immune response. Typically, a vaccine elicits an antigen-specific immune response to an antigen of a pathogen, for example a viral pathogen, or to a cellular constituent correlated with a pathological condition. A vaccine may include a polynucleotide (such as a nucleic acid encoding a disclosed antigen), a peptide or polypeptide (such as a disclosed antigen), a virus, a cell or one or more cellular constituents. In one specific, non-limiting example, a vaccine reduces the severity of the symptoms associated with HIV-1 infection and/or decreases the viral load compared to a control. In another non-limiting example, a vaccine reduces HIV-1 infection compared to a control.

Vector: An entity containing a DNA or RNA molecule bearing a promoter(s) that is operationally linked to the coding sequence of an immunogenic protein of interest and can express the coding sequence. Non-limiting examples include a naked or packaged (lipid and/or protein) DNA, a naked or packaged RNA, a subcomponent of a virus or bacterium or other microorganism that may be replication-incompetent, or a virus or bacterium or other microorganism that may be replication-competent. A vector is sometimes referred to as a construct. 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. Viral vectors are recombinant nucleic acid vectors having at least some nucleic acid sequences derived from one or more viruses.

A non-limiting example of a DNA-based expression vector is pCDNA3.1, which can include includes a mammalian expression enhancer and promoter (such as a CMV promoter). Non-limiting examples of viral vectors include adeno-associated virus (AAV) vectors as well as Poxvirus vector (e.g., Vaccinia, MVA, avian Pox, or Adenovirus).

Virus-like particle (VLP): A non-replicating, viral shell, derived from any of several viruses. VLPs are generally composed of one or more viral proteins, such as, but not limited to, those proteins referred to as capsid, coat, shell, surface and/or envelope proteins, or particle-forming polypeptides derived from these proteins. VLPs can form spontaneously upon recombinant expression of the protein in an appropriate expression system. The presence of

VLPs following recombinant expression of viral proteins can be detected using conventional techniques, such as by electron microscopy, biophysical characterization, and the like. Further, VLPs can be isolated by known techniques, *e.g.*, density gradient centrifugation and identified by characteristic density banding. See, for example, Baker *et al.* (1991) *Biophys. J.* 60:1445-1456; and Hagensee *et al.* (1994) *J. Virol.* 68:4503-4505; Vincente, *J Invertebr Pathol.*, 2011; Schneider-Ohrum and Ross, *Curr. Top. Microbiol. Immunol.*, 354: 53073, 2012).

II. Immunogens

Embodiments of immunogens comprising a recombinant gp120 protein that is modified to expose V2 epitopes are provided herein. The modification comprises deletion of HXBc2 residues 137-152 from the gp120 protein, which, as discussed in the examples, exposes V2 epitopes and is shown to produce a protective immune response in an animal model. Additionally provided are isolated V1V2 domain proteins that contain the V1 deletion, as well as HIV-1 Env trimers containing the recombinant gp120 protein with the V1 deletion.

A. Recombinant gp120 and HIV-1 Env proteins containing same

Isolated immunogens are disclosed that include a recombinant gp120 protein that is modified to include a deletion of V1 residues 137-152 according to the HXBc2 numbering system. As described herein, deletion of these V1 residues exposes V2 epitopes on the gp120 protein, and immunogens including this modification are shown to elicit a protective immune response that targets the V2 epitopes. Also provided are HIV-1 Env ectodomain trimers comprising protomers including the deletion of V1 residues 137-152, as well as gp140 proteins, gp145 proteins, and gp160 proteins including this deletion.

In several embodiments, the recombinant gp120, gp140, gp145, gp160, or the protomers of the recombinant HIV-1 Env ectodomain trimer specifically bind to an antibody that targets the V2 portion of the V1V2 domain such as the human monoclonal antibody CH58 and/or CH59. The determination of specific binding may readily be made by using or adapting routine procedures, such as ELISA, immunocompetition, surface plasmon resonance, or other immunosorbant assays (described in many standard texts, including Harlow and Lane, *Using Antibodies: A Laboratory Manual*, CSHL, New York, 1999).

HIV-1 can be classified into four groups: the “major” group M, the “outlier” group O, group N, and group P. Within group M, there are several genetically distinct clades (or subtypes) of HIV-1. The disclosed recombinant HIV-1 Env proteins can be derived from any type of HIV, such as groups M, N, O, or P, or clade, such as clade A, B, C, D, F, G, H, J, or K, and the like. HIV-1 Env proteins from the different HIV-1 clades, as well as nucleic acid

sequences encoding such proteins and methods for the manipulation and insertion of such nucleic acid sequences into vectors, are known (see, *e.g.*, HIV Sequence Compendium, Division of AIDS, National Institute of Allergy and Infectious Diseases (2013); HIV Sequence Database (hiv-web.lanl.gov/content/hiv-db/mainpage.html); 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). Exemplary native HIV-1 Env protein sequences are available in the HIV Sequence Database (hiv-web.lanl.gov/content/hiv-db/mainpage.html).

In some embodiments, any of the recombinant gp120, gp140, gp145, gp160, or the protomers of the recombinant HIV-1 Env ectodomain trimer can include the corresponding amino acid sequence from a native HIV-1 Env protein, for example, from genetic subtype A-F as available in the HIV Sequence Database (hiv-web.lanl.gov/content/hiv-db/mainpage.html), or an amino acid sequence at least 90% (such as at least 95%, at least 96%, at least 97%, at least 98% or at least 99%) identical thereto that has been modified to include a deletion of HXBc2 residues 137-152.

In some embodiments, the recombinant gp120 protein comprises or consists essentially of the amino acid sequence set forth as any one of:

SEQ ID NO: 1. gp120 of HIV-1 Env circulating recombinant form AE, strain A244 (GenBank: KU562843.1, incorporated by reference herein in its entirety) with V1 137-152 deletion.

Deletion boundaries are underlined.

NLWKWGTLILGLVLIICSASDNLWVTVYYGVPVWRDADTTLFCASDAKAQETEAHNVWATHACVPTDPNPQELHLENV
TENFNMWKNMVEQMVEDVISLWDQSLKPCVKLTPLCVTLNCTNANLEVRNCSFNMTTELDRDKKQKVHALFYKLDIV
PIEDNTSSSKYRLINCNTSVIKQACPKISFDPIPIHYCTPAGYAILKCNDKNFNGTGPKKNVSSVQCTHGKIPAVST
QLLLNGSLAEEEEIIIRSENLTNNAKTIIIVHLNKSVEINCTRPSNNTRTSINIGPGQVFYRTGDIIGDIRKAYCEING
AKWNEVLKKVTEKLKEHFNNKTIIIFQPPSGGDLEITMHHFNCRGEFFYCNTTRLFNNTCMENETMEGCNGTIIILPCK
IKQIINMWQRAGQAMYAPPISGRINCVSNITGILLTRDGGNLNTNETFRPGGNIKDNWRSELYKYKVQIEPLGIA
PTRAKRRVVEREKR

SEQ ID NO: 2. gp120 of HIV-1 Env clade B strain MN (GenBank: AAL66251.1, incorporated by reference herein in its entirety) with V1 137-152 deletion. Deletion boundaries are underlined.

HWGWGTMLLGLLMICSATEKLWVTVYYGVPVWKEATTTLFCASDAKAYDTEVHNWATHACVPTDPNPQEVQLVNV
TEDFNMWKNMVEQMVEDIIISLWDQSLKPCVKLTPLCVTLNCTDLRNEKNCSFNITTSIRDQMKEYALLYKLDIV
AIDKNTSYRLISCNTSVITQACPKVSFEPIPIHYCAPAGFAILKCNDKNFTGKGPKKNVSTVQCTHGIRPVVSTQL
LLNGSLAEEVEVIRSENFTNNAKTIIIVHLNESVQINCTRPYNNRRTRIHIHPGRAFYTTKNIKGTIRQAHCTISSAK
WNDTLRQIVSKLKEQFKNKTIVFKQSSGGDPEIVMHSFNCGGEFFYCNTSSLFNSTWNGNNTWNNTTGSNSNITLQC
KIKQIINMWQEVGKAMYAPPIEGQIRCSSNITGLLLLTRDGGNDTDTNNTETIFRPGGDMRDNRSELYKYKVVTIEP
LGVAPTAKRRVVQREKR

SEQ ID NO: 3. gp120 of HIV-1 Env clade C strain 96ZM651 (GenBank: AAK30970.1, incorporated by reference herein in its entirety) with V1 137-152 deletion. Deletion boundaries are underlined.

RWWTWGILGFWMIMICNVWGNLWVTVYYGVPVWKEAKTTLFCASDAKSYEKEVHNWATHACVPTDPNPQEIVLGNV
 TENFNMWKNMVDQMHEDIISLWDQSLKPCVKLTPLCVTLNCTEVNNDMKNCSFNITTELKDKKKNVYALFYKLDIV
 SLNETDDSETGNSSKYRLINCNTSALTQACPKVSFDPIPIHYCAPAGYAILKCNNKTFTNGTGPCHNVSTVQCTHGI
 KPVVSTQLLLNGSLAEEGIIIRSENLTNNVKTIIVHLNRSIEIVCVRPNNNTRQSIIRIGPGQTFYATGDIIGDIRQA
 HCNISRTNWTKTLEVRNKLREHFNPKNITFKPSSGGDLEITTHSFNCRGEFFYCNTSGLFSINYTENNTDGTPTIL
 PCRIQIINMWQEVGRAMYAPPIEGNIACKSDITGLLLVRDGGSTNDSTNNTEIFRPAGGDMRDNRSELYKYKV
 EIKPLGIAPTEAKRRVVEREK

In some embodiments, the recombinant gp120 protein comprises or consists essentially
 of an amino acid sequence at least 90% (such as at least 95%, at least 96%, at least 97%, at least
 98% or at least 99%) identical to any one of SEQ ID NOs: 1-3 that comprises the V1 deletion of
 residues 137-152 (HXBc2 numbering).

In some embodiments, the immunogen comprises a gp160 or a HIV-1 Env trimer
 comprising the recombinant gp120 protein with the deletion of residues 137-152 (HXBc2
 numbering. In some embodiments, the gp160 or the protomers of the HIV-1 Env trimer
 comprise or consist essentially of the amino acid sequence set forth as any one of:

SEQ ID NO: 4. HIV-1 A244 Env with V1 137-152 deletion and without signal peptide.

Deletion boundaries are underlined.

NLWKWGTLILGLVIIICASADNLWVTVYYGVPVWRDADTTLFCASDAKAQETEAHNWATHACVPTDPNPQELHLENV
 TENFNMWKNMVEQMVEDVISLWDQSLKPCVKLTPLCVTLNCTNANLEVRNCSFNMTTELKDKKQKVHALFYKLDIV
 PIEDNTSSSKYRLINCNTSVIKQACPKISFDPIPIHYCTPAGYAILKCNDKNFTNGTGPCKNVSSVQCTHGIKPAVST
 QLLLNGSLAEEIIIRSENLTNNAKTIIVHLNKSVEINCTRPSNNTRTSINIGPGQVFYRTGDIIGDIRKAYCEING
 AKWNEVLKKVTEKLKEHFNNKTIIIFQPPSSGGDLEITMHHFNCRGEFFYCNTTRLFNNTCMENETMEGCNGTIIILPCK
 IKQIINMWQRAQAMYPISGRINCVSNITGILLTRDGGLNNTNETFRPGGGNIKDNWRSELYKYKVQIEPLGIA
 PTRAKRRVVEREKRAVGIGAMIFGFLGAAGSTMGAASITTLTVQARQLLSGIVQQQSNLLRAIEAQQHLLQLTVWGIK
 QLQARVLAVERYLKDQKLLGLWGCSGKIICCTAVPWNSTWSNRSFEEIWNMTWIEWEREISNYTNQIYEILTQSQN
 QQDRNEKDLELDKWLWKFDTNWLWYIKIFIMIVGGGLIGLRIIFAVLSIVNRVRQGYSPSLQIPTHHQREPD
 RPERIEEGGGEQGRDKSVRLVSGFLALTWDDLRLSLCLFSYHRLRDFISIAARTVELLGHSSSLKGLRRGWEGLKYLGN
 LILYWGQELKISAILLNATAIAVAGWTDREVIAQGAWRALHIPPRIHQGLERTLL

SEQ ID NO: 5. HIV-1 MN Env with V1 137-152 deletion and without signal peptide. Deletion
 boundaries are underlined.

HWGWGTMLLGLLMICSAATEKLWVTVYYGVPVWKEATTLFCASDAKAYDTEVHNWATHACVPTDPNPQEVQLVNV
 TEDFNMWKNMVEQMHEDIISLWDQSLKPCVKLTPLCVTLNCTDLRNEMKNCSFNITTSIRDKMQKEYALLYKLDIV
 AIDKNTSYRLISCNTSVITQACPKVSFEPIPIHYCAPAGFAILKCNDKNFTGKGPKNVSTVQCTHGIRPVVSTQL
 LLNGSLAEEVVIRSENFNNAKTIIVHLNESVQINCTRPYNRRTRIHIHGPGRAFYTTKNIKGTIRQAHTISSAK
 WNDTLRQIVSKLKEQFKNTIVFKQSSGGDPEIVMHSFNCGGEFFYCNTSSLFNSTWGNNTWNNTTGSNSNITLQC
 KIKQIINMWQEVGKAMYAPPIEGQIRCSSNITGLLLTRDGGNDTDTNTEIFRPGGGDMRDNRSELYKYKVVTIEP
 LGVAPTAKRRVVQREKRAAIGALFLGFLGAAGSTMGAASMLTVQARQLLSGIVQQQNLLRAIEAQQHMLQLTVW
 GIKQLQARVLAVERYLRDQQLGIWGCSGKLICTTVPWNASWSNKSQEDIWNNMTWMQWEREIDNYTSTIYELLEK
 SQNQEQEKNEQELLELDKWLWNFDTNWLWYIKIFIMIVGGGLIGLRIVFAVLSIVNRVRQGYSPSLQTRPPVPR
 GPDRPEGTEEEGGERDRDTSGRILDGFLAIWVDLRSLLLFSYHRLRDLLLIAARIVELLGRRGWEILKYWWNLLQY
 WSQELKNSAVSLLNATAVAVAEGTDRVIEVLQAGRAILHIPTRIRQGLERALL

SEQ ID NO: 66. HIV-1 96ZM651 Env with V1 137-152 deletion and without signal peptide.

Deletion boundaries are underlined.

RWWTWGILGFWMIMICNVWGNLWVTVYYGVPVWKEAKTTLFCASDAKSYEKEVHNWATHACVPTDPNPQEIVLGNV
 TENFNMWKNMVDQMHEDIISLWDQSLKPCVKLTPLCVTLNCTEVNNDMKNCSFNITTELKDKKKNVYALFYKLDIV
 SLNETDDSETGNSSKYRLINCNTSALTQACPKVSFDPIPIHYCAPAGYAILKCNNKTFTNGTGPCHNVSTVQCTHGI
 KPVVSTQLLLNGSLAEEGIIIRSENLTNNVKTIIVHLNRSIEIVCVRPNNNTRQSIIRIGPGQTFYATGDIIGDIRQA

HCNISRTNWTKTLEVRNKLREHFNPKNITFKPSSGGDLEITTHSFNCRGEFFYCNTSGLFSINYTENNTDGTPTIL
 PCRIHQIINMWQEVGRAMYAPPIEGNIACKSDITGLLLVRDGGSTNDSTNNNTEIFRPAGGDMRDNRSELYKYKVV
 EIKPLGIAPTEAKRRVVEREKRAVIGAVFLGFLGAAGSTMGAASITLTAQARQVLSGIVQQQSNLLRAIEAQHLL
 5 QLTWVGIKQLQTRVLAIERYLKDQQLLGLWGCSGKLICTTAVPWNISWSNKSCTDIWDNMTWMQWDREISNYNTIY
 RLLEDSSQSQEQNEKDLLALDSWNNLWNWFDITKWLWYIKIFIMIVGGLIGLRIIFAVLSIVNRVRQGYSPLSFQTL
 IPNPREDPRPGRIEEEGGEQDKERSVRLVSGFLALAWDDLRSICLFSYHRLRDFILVTARAVELLRRSSSLKGLQRGW
 EALKYLGSLVQYWGLELKKSAISLLDTIAIAVAEGTDRIELIQGICRAIRNVPRRIRQGFETALL

In some embodiments, the recombinant gp160 or the protomers of the HIV-1 Env trimer
 10 comprise or consist essentially of an amino acid sequence at least 90% (such as at least 95%, at
 least 96%, at least 97%, at least 98% or at least 99%) identical to any one of SEQ ID NOs: 4-5
 and 66 that comprises the V1 deletion of residues 137-152 (HXBc2 numbering).

The full-length sequences of A244 Env and HIV-1 Env clade B with the V1 137-152
 deletion are provided as:

15 SEQ ID NO: 6. HIV-1 A244 Env with the V1 137-152 deletion. Deletion boundaries are
 underlined.

MRVKETQMTWPNLWKWGTLILGLVIIICASDNLWVTVYYGVPVWRDADTTLFCASDAKAQETEAHNVWATHACVPTD
 PNPQELHLENVTENFNMWKNMVEQM~~QEDVISLWDQSLKPCVKLTPLCVTLNCTNANLEVRNCSFNMTTEL~~RDKKQK
 20 VHALFYKLDIVPIEDNTSSSKYRLINCNTSVIKQACPKISFDPIPIHYCTPAGYAILKCNCKNFNGTGPCKNVSSVQ
 CTHGIKPAVSTQLLNGSLAEEIIIRSENLTNNAKTIIIVHLNKSVEINCTRPNNTRTSINIGPGQVFYRTGDIIG
 DIRKAYCEINGAKWNEVLKVKTEKLKEHFNNKTIIFQPPSSGGDLEITMHHFNCRGEFFYCNTTRLFNNTCMENETME
 GCNGTIIILPCKIKQIINMWQRAQAMYPPIISGRINCVSNITGILLTRDGGNNNTNETFRPGGGNIKDNWRSELYKY
 KVVQIEPLGIAPTRAKRRVVEREKRAVIGAMIFGFLGAAGSTMGAASITLTVQARQLLSGIVQQQSNLLRAIEAQQ
 25 HLLQLTVWVGIKQLQARVLAVERYLKDQKLLGLWGCSGKIICTTAVPWNSTWSNRSFEEIWNMTWIEWEREISNYTN
 QIYEILTQSQNQDRNEKDLLELDKWLKWF~~FDITNWLWYIKIFIMIVGGLIGLRIIFAVLSIVNRVRQGYSP~~LSL
 QIPTHHQREPDRPERIEEGGGEQGRDKSVRLVSGFLALTWDDLRSICLFSYHRLRDFISIAARTVELLGHSSSLKGLR
 RGWEGLKYLGNLILYWQELKISAILLNATAIAVAGWTDRIEVAQGAWRALHIPRRIRQGLERTLL

SEQ ID NO: 7. HIV-1 MN Env with the V1 137-152 deletion. Deletion boundaries are
 30 underlined.

MRVKGIRRNRYQHWGWGTMLLGLLMICSA~~TEKLWVTVYYGVPVWKEATTT~~FCASDAKAYDTEVHNWATHACVPTD
 PNPQEVQLVNVTE~~DFNMWKNMVEQMHEDIISLWDQSLKPCVKLTPLCVTLNCTDLRNE~~MKNCSFNITTSIRDKMQK
 EYALLYKLDIVAIKDNTSYRLISCNTSVITQACPKVSFEPIPIHYCAPAGFAILKCNCKNFTGKGPCKNVSTVQCT
 35 HGIRPVVSTQLLNGSLAEEEVVIRSENFTNNAKTIIIVHLNESVQINCTRPYNNRRTRIHI~~GPGRAFYTT~~KNIKGTI
 RQAHCTISSAKWNTDLRQIVSKLKEQFKNKTIVFKQSSGGDPEIVMHSFNCGGEFFYCNTSSLFNSTWNGNNTWNNT
 TGSNSNITLQCKIKQIINMWQEVGKAMYAPPIEQIRCSSNITGLLLTRDGGNDTDTNNTTEIFRPGGGDMRDNRSE
 LYKYKVVTIEPLGVAPTAKRRVVRQEKRAAIGALFLGFLGAAGSTMGAASMMTLTVQARQLLSGIVQQQNNLLRAIE
 AQQHMLQLTVWVGIKQLQARVLAVERYLRDQQLLGIWGCSGKLICTTVPWNASWSNKSQEDIWNMTWMQWEREIDN
 40 YTSTIYELLEKSQ~~NQOEKNEQELLELDKWLKWF~~FDITNWLWYIKIFIMIVGGLIGLRIIFAVLSIVNRVRQGYSP
 LSLQTRPPVPRGPDREGE~~TEEEGGERDRTSGRLVDGFLAI~~IWVDLRSLLLSYHRLRDL~~LLIAARIVELL~~GRRGWE
 ILKYWWNLLQYWSQELKNSAVSLLNATAVAVAGWTDRIEVLQ~~RAGRAILHIPTRIRQGLERALL~~

SEQ ID NO: 67. HIV-1 96ZM651 Env strain with V1 137-152 deletion. Deletion boundaries
 are underlined.

MRVREILRNWQRWWTWGILGFWMLMICNVWGNLWVTVYYGVPVWKEAKTTTLFCASDAKS~~YEKEVHNWATHACVPTD~~
 PNPQEI~~VLGNVTENFNMWKNMVDQMHEDIISLWDQSLKPCVKLTPLCVTLNCTE~~VN~~VD~~MKNCSFNITTELKDKKKN
 45 VYALFYKLDIVSLNETDDSETGNSSKYRLINCNTSALTQACPKVSFDPIPIHYCAPAGYAILKCNKKT~~FNGTG~~PCH
 NVSTVQCTHGIKPVVSTQLLNGSLAEEGIIIRSENLTNNVKTIIIVHLNRSIEIVCVRPNNNTRQSI~~RIGPGQT~~FYA
 TGDIIIGDIRQAHCNISRTNWTKTLEVRNKLREHFNPKNITFKPSSGGDLEITTHSFNCRGEFFYCNTSGLFSINYT
 50 ENNTDGTPTILPCRIRQIINMWQEVGRAMYAPPIEGNIACKSDITGLLLVRDGGSTNDSTNNNTEIFRPAGGDMRDN
 WRSELYKYKVVEIKPLGIAPTEAKRRVVEREKRAVIGAVFLGFLGAAGSTMGAASITLTAQARQVLSGIVQQQSNL
 LRAIEAQQHLLQLTVWVGIKQLQTRVLAIERYLKDQQLLGLWGCSGKLICTTAVPWNISWSNKSCTDIWDNMTWMQWD

REISNYTNTIYRLLEDSQSQQEQNEKDLLALDSWNNLWNWFDITKWLWYIKIFIMIVGGLIGLRIFAVLSIVNRVR
 QGYSPLSFQTLIPNPREDPRPGRIEEEGGEQDKERSVRLVSGFLALAWDDLRLCLFSYHRLRDFILVTARAVELLR
 RSSLKGLQRGWEALKYLGSLVQYWGLELKKSAISLLDTIAIAVAEGTDRIIELIQGICRAIRNVPRRIRQGFETALL

5 In some embodiments, the recombinant gp120, gp140, gp145, gp160, or the protomers of the recombinant HIV-1 Env ectodomain trimer can further include a non-natural disulfide bond between HIV-1 Env positions 201 and 433. For example, the non-natural disulfide bond can be introduced by including cysteine substitutions at positions 201 and 433 (*e.g.*, I201C and A433C substitutions). The presence of the non-natural disulfide bond between residues 201 and 433
 10 contributes to the stabilization of the HIV-1 Env protein in its prefusion mature closed conformation.

In some embodiments, the protomers of the recombinant HIV-1 Env ectodomain trimer can include gp120-gp41 ectodomain protomers further including the “SOSIP” substitutions, which include a non-natural disulfide bond between cysteine residues introduced at HIV-1 Env
 15 positions 501 and 605 (for example, by A501C and T605C substitutions), and a proline residue introduced at HIV-1 Env positions 559 (for example, by an I559P substitution). The presence of the non-natural disulfide bond between positions 501 and 605 and the proline residue at position 559 contributes to the stabilization of the HIV-1 Env ectodomain in the prefusion mature closed conformation. In several embodiments, the protomers of the recombinant HIV-1 Env
 20 ectodomain trimer can further include a non-natural disulfide bond between HIV-1 Env positions 201 and 433 (*e.g.*, by introduction of I201C and A433C substitutions) and the HIV-1 Env ectodomain trimer can further included the SOSIP mutations.

In some embodiments, the recombinant gp120, gp140, gp145, gp160, or the protomers of the recombinant HIV-1 Env ectodomain trimer can further include an N-linked glycosylation
 25 site at HIV-1 Env position 332 (if not already present on the ectodomain). For example, by T332N substitution in the case of BG505-based immunogens. The presence of the glycosylation site at N332 allows for binding by 2G12 antibody.

In some embodiments, the recombinant gp120, gp140, gp145, gp160, or the protomers of the recombinant HIV-1 Env ectodomain trimer can include a lysine residue at HIV-1 Env
 30 position 168 (if not already present on the ectodomain). For example, the lysine residue can be added by amino acid substitution (such as an E168K substitution in the case of the JR-FL based immunogens). The presence of the lysine residue at position 168 allows for binding of particular broadly neutralizing antibodies to the V1V2 loops of gp120.

In some embodiments, the protomers of the recombinant HIV-1 Env ectodomain trimer
 35 can further include mutations to add an N-linked glycan sequon at position 504, position 661, or

positions 504 and 661, to increase glycosylation of the membrane proximal region of the ectodomain.

Native HIV-1 Env sequences include a furin cleavage site between positions 508 and 512 (HXBc2 numbering), that separates gp120 and gp41. Any of the disclosed recombinant gp160 proteins and HIV-1 Env ectodomains can further include an enhanced cleavage site between gp120 and gp41 proteins. The enhanced cleavage site can include, for example, substitution of six arginine residues for the four residues of the native cleavage site (*e.g.*, REKR, SEQ ID NO: 11) to RRRRRR (SEQ ID NO: 12). It will be understood that protease cleavage of the furin or enhanced cleavage site separating gp120 and gp41 can remove a few amino acids from either end of the cleavage site.

The recombinant HIV-1 Env ectodomain trimer includes a protein complex of gp120-gp41 ectodomain protomers. The gp120-gp41 ectodomain protomer can include separate gp120 and gp41 polypeptide chains, or can include gp120 and gp41 polypeptide chains that are linked (*e.g.*, by a peptide linker) to form a single polypeptide chain (*e.g.*, a “single chain”). In several embodiments, the recombinant HIV-1 Env ectodomain trimer is membrane anchored and can include a trimeric complex of recombinant HIV-1 Env ectodomains that are linked to a transmembrane domain (*e.g.*, a gp145 protein including a gp120 protein and a gp41 ectodomain and transmembrane domain).

In several embodiments, the N-terminal residue of the recombinant gp120 protein is one of HIV-1 Env positions 1-35, and the C-terminal residue of the recombinant gp120 protein is one of HIV-1 Env positions 503-511. In some embodiments, the N-terminal residue of the recombinant gp120 protein is HIV-1 Env position 31 and the C-terminal residue of the recombinant gp120 protein is HIV-1 Env position 511 or position 507. In some embodiments, the recombinant gp120 protein comprises or consists of HIV-1 Env positions 31-507 (HXBc2 numbering).

The purified proteins provided herein typically do not include a signal peptide (for example, the purified recombinant gp120 protein typically does not include HIV-1 Env positions 1-30), as the signal peptide is proteolytically cleaved during cellular processing.

In embodiments including a soluble recombinant HIV-1 Env ectodomain, the gp41 ectodomain is not linked to a transmembrane domain or other membrane anchor. However, in embodiments including a membrane anchored recombinant HIV-1 Env ectodomain trimer the gp41 ectodomain can be linked to a transmembrane domain (such as, but not limited to, an HIV-1 Env transmembrane domain).

In some embodiments, the HIV-1 Env ectodomain trimer includes the recombinant gp120 protein and the gp41 ectodomain, wherein the N-terminal residue of the recombinant

gp120 protein is HIV-1 Env position 31; the C-terminal residue of the recombinant gp120 protein is HIV-1 Env position 507 or 511; the N-terminal residue of the gp41 ectodomain is HIV-1 Env position 512; and the C-terminal residue of the gp41 ectodomain is HIV-1 Env position 664. In some embodiments, the HIV-1 Env ectodomain trimer includes the

5 recombinant gp120 protein and the gp41 ectodomain, wherein the N-terminal residue of the recombinant gp120 protein is HIV-1 Env position 31; the C-terminal residue of the recombinant gp120 protein is HIV-1 Env position 507; the N-terminal residue of the gp41 ectodomain is HIV-1 Env position 512; and the C-terminal residue of the gp41 ectodomain is HIV-1 Env position 664. In some embodiments, the C-terminal residue of the recombinant HIV-1 Env
10 ectodomain is position 683 (the entire ectodomain, terminating just before the transmembrane domain). In additional embodiments, the C-terminal residue of the recombinant HIV-1 Env ectodomain is position 707 (the entire ectodomain, terminating just after the transmembrane domain).

In view of the conservation and breadth of knowledge of HIV-1 Env sequences, the
15 person of ordinary skill in the art can easily identify corresponding HIV-1 Env amino acid positions between different HIV-1 Env strains and subtypes. The HXBc2 numbering system has been developed to assist comparison between different HIV-1 amino acid and nucleic acid sequences. The numbering of amino acid substitutions disclosed herein is made according to the HXBc2 numbering system, unless context indicates otherwise.

20 It is understood in the art that some variations can be made in the amino acid sequence of a protein without affecting the activity of the protein. Such variations include insertion of amino acid residues, deletions of amino acid residues, and substitutions of amino acid residues. These variations in sequence can be naturally occurring variations or they can be engineered through the use of genetic engineering technique known to those skilled in the art. Examples of such
25 techniques are found in 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, 2013, both of which are incorporated herein by reference in their entirety.

The recombinant gp120, gp140, gp145, gp160, or the protomers of the recombinant
30 HIV-1 Env ectodomain trimer can be derivatized or linked to another molecule (such as another peptide or protein). In general, the derivatization is such that the binding of antibodies that bind to the V2 domain (or of the V2b or V2c peptides disclosed herein) is not affected adversely by the derivatization or labeling. In some embodiments, the recombinant gp120, gp140, gp145, gp160, or the protomers of the recombinant HIV-1 Env ectodomain trimer can be functionally

linked (by chemical coupling, genetic fusion, noncovalent association or otherwise) to one or more other molecular entities, such as an antibody or protein or detection tag.

Membrane anchored embodiments

5 In some embodiments, the HIV-1 Env ectodomain trimer including the recombinant gp120 protein can be a membrane anchored HIV-1 Env ectodomain trimer, for example, the HIV-1 Env ectodomains in the trimer can each be linked to a transmembrane domain. The transmembrane domain can be linked to any portion of the HIV-1 Env ectodomain, as long as the presence of the transmembrane domain does not disrupt the structure of the HIV-1 Env
10 ectodomain, or its ability to induce an immune response to HIV-1. In non-limiting examples, the transmembrane domain can be linked to the N- or C-terminal residue of a gp120 polypeptide, or the C-terminal residue of a gp41 ectodomain included in the HIV-1 Env ectodomain. One or more peptide linkers (such as a gly-ser linker, for example, a 10 amino acid glycine-serine peptide linker, such as a peptide linker comprising the amino acid sequence set
15 forth as SEQ ID NO: 13 (GGSGGGGSGG) can be used to link the transmembrane domain and the gp120 or gp41 protein. In some embodiments a native HIV-1 Env MPER sequence can be used to link the transmembrane domain and the gp120 or gp41 protein.

Non-limiting examples of transmembrane domains for use with the disclosed embodiments include the BG505 TM domain (KIFIMIVGGLIGLRIVFAVLSVIHRVR, SEQ ID
20 NO: 14), the Influenza A Hemagglutinin TM domain (ILAIYSTVASSLVLLVSLGAISF, SEQ ID NO: 15), and the Influenza A Neuraminidase TM domain (IITIGSICMVVGIIISLILQIGNIISIWVS, SEQ ID NO: 16).

The recombinant HIV-1 Env ectodomain linked to the transmembrane domain can include any of the mutations provided herein (or combinations thereof) as long as the
25 recombinant HIV-1 Env ectodomain linked to the transmembrane domain retains the desired properties.

Linkage to a Trimerization Domain

In several embodiments, the HIV-1 Env ectodomain trimer including the recombinant
30 gp120 protein can be linked to a trimerization domain, for example, the C-terminus of the gp41 ectodomains included in the HIV-1 Env ectodomain trimer can be linked to the trimerization domain. The trimerization domain can promote trimerization of the three protomers of the recombinant HIV-1 Env protein. Non-limiting examples of exogenous multimerization domains that promote stable trimers of soluble recombinant proteins include: the GCN4 leucine
35 zipper (Harbury *et al.* 1993 *Science* 262:1401-1407), the trimerization motif from the lung

surfactant protein (Hoppe *et al.* 1994 *FEBS Lett* 344:191-195), collagen (McAlinden *et al.* 2003 *J Biol Chem* 278:42200-42207), and the phage T4 fibrin Foldon (Miroshnikov *et al.* 1998 *Protein Eng* 11:329-414), any of which can be linked to the recombinant HIV-1 Env ectodomain (e.g., by linkage to the C-terminus of the gp41 polypeptide to promote trimerization of the recombinant HIV-1 protein.

In some examples, the recombinant HIV-1 Env ectodomain can be linked to a T4 fibrin Foldon domain, for example, the recombinant HIV-1 Env ectodomain can include a gp41 polypeptide with a Foldon domain linked to its C-terminus. In specific examples, the T4 fibrin Foldon domain can include the amino acid sequence GYIPEAPRDGQAYVRKDGWVLLSTF (SEQ ID NO: 17), which adopts a β -propeller conformation, and can fold and trimerize in an autonomous way (Tao *et al.* 1997 *Structure* 5:789-798).

Typically, the heterologous trimerization domain is positioned C-terminal to the gp41 protein. Optionally, the heterologous trimerization is connected to the recombinant HIV-1 Env ectodomain via a linker, such as an amino acid linker. Exemplary linkers include Gly or Gly-Ser linkers, such as SEQ ID NO: 13 (GGSGGGGSGG). Some embodiments include a protease cleavage site for removing the trimerization domain from the HIV-1 polypeptide, such as, but not limited to, a thrombin site between the recombinant HIV-1 Env ectodomain and the trimerization domain.

Carrier molecules

In some embodiments, a disclosed the recombinant gp120, gp140, gp145, gp160, or recombinant HIV-1 Env ectodomain trimer can be linked to a carrier protein by a linker (such as a peptide linker) or can be directly linked to the carrier protein (for example, by conjugation, or synthesis as a fusion protein) too form an immunogenic conjugate.

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. One skilled in the art will recognize, for an immunogenic conjugate from two or more constituents, each of the constituents will contain the necessary reactive groups. Representative combinations of such groups are amino with carboxyl to form amide linkages or carboxy with hydroxyl to form ester linkages or amino with alkyl halides to form alkylamino linkages or thiols with thiols to form disulfides or thiols with maleimides or alkylhalides to form thioethers. Hydroxyl, carboxyl, amino and other functionalities, where not present may be introduced by known methods. Likewise, as those skilled in the art will recognize, a wide variety of linking groups may be employed. In some cases, the linking group can be designed to be either hydrophilic or hydrophobic in order to enhance the desired binding characteristics of the HIV-1 Env protein

and the carrier. The covalent linkages should be stable relative to the solution conditions under which the conjugate is subjected.

In some embodiments, 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 some embodiments, the recombinant gp120, gp140, gp145, gp160, or the protomers of the recombinant HIV-1 Env ectodomain trimer, the linker, and the carrier can be encoded as a single fusion polypeptide such that the recombinant gp120, gp140, gp145, gp160, or the protomers of the recombinant HIV-1 Env ectodomain trimer and the carrier are joined by peptide bonds.

The procedure for attaching a molecule to a polypeptide varies according to the chemical structure of the molecule. Polypeptides typically contain a variety of functional groups; for example, carboxylic acid (COOH), free amine (-NH₂) or sulfhydryl (-SH) groups, which are available for reaction with a suitable functional group on a polypeptide. Alternatively, the polypeptide is derivatized to expose or attach additional reactive functional groups. The derivatization may involve attachment of any of a number of linker molecules such as those available from Pierce Chemical Company, Rockford, IL.

It can be advantageous to produce conjugates in which more than one recombinant gp120, gp140, gp145, gp160, or HIV-1 Env ectodomain trimer is conjugated to a single carrier protein. In several embodiments, the conjugation of multiple recombinant gp120, gp140, gp145, gp160, or HIV-1 Env ectodomain trimers to a single carrier protein is possible because the carrier protein has multiple lysine or cysteine side-chains that can serve as sites of attachment.

Examples of suitable carriers are those that can increase the immunogenicity of the conjugate and/or elicit antibodies against the carrier which are diagnostically, analytically, and/or therapeutically beneficial. Useful carriers include polymeric carriers, which can be natural, recombinantly produced, semi-synthetic or synthetic materials containing one or more amino groups, such as those present in a lysine amino acid residue present in the carrier, to which a reactant moiety can be attached. Carriers that fulfill these criteria are generally known in the art (see, for example, Fattom *et al.*, *Infect. Immun.* 58:2309-12, 1990; Devi *et al.*, *PNAS* 88:7175-79, 1991; Szu *et al.*, *Infect. Immun.* 59:4555-61, 1991; Szu *et al.*, *J. Exp. Med.* 166:1510-24, 1987; and Pavliakova *et al.*, *Infect. Immun.* 68:2161-66, 2000). A carrier can be useful even if the antibody that it elicits is not of benefit by itself.

Specific, non-limiting examples of suitable polypeptide carriers include, but are not limited to, natural, semi-synthetic or synthetic polypeptides or proteins from bacteria or viruses. In one embodiment, bacterial products for use as carriers include bacterial toxins. Bacterial toxins include bacterial products that mediate toxic effects, inflammatory responses, stress,

shock, chronic sequelae, or mortality in a susceptible host. Specific, non-limiting examples of bacterial toxins include, but are not limited to: *B. anthracis* PA (for example, as encoded by bases 143779 to 146073 of GENBANK® Accession No. NC 007322); *B. anthracis* LF (for example, as encoded by the complement of bases 149357 to 151786 of GENBANK® Accession No. NC 007322); bacterial toxins and toxoids, such as tetanus toxin/toxoid (for example, as described in U.S. Patent Nos. 5,601,826 and 6,696,065); diphtheria toxin/toxoid (for example, as described in U.S. Patent Nos. 4,709,017 and 6,696,065), such as tetanus toxin heavy chain C fragment; *P. aeruginosa* exotoxin/toxoid (for example, as described in U.S. Patent Nos. 4,428,931, 4,488,991 and 5,602,095); pertussis toxin/toxoid (for example, as described in U.S. Patent Nos. 4,997,915, 6,399,076 and 6,696,065); and *C. perfringens* exotoxin/toxoid (for example, as described in U.S. Patent Nos. 5,817,317 and 6,403,094) *C. difficile* toxin B or A, or analogs or mimetics of and combinations of two or more thereof. Viral proteins, such as hepatitis B surface antigen (for example, as described in U.S. Patent Nos. 5,151,023 and 6,013,264) and core antigen (for example, as described in U.S. Patent Nos. 4,547,367 and 4,547,368) can also be used as carriers, as well as proteins from higher organisms such as keyhole limpet hemocyanin (KLH), horseshoe crab hemocyanin, Concholepas Concholepas Hemocyanin (CCH), Ovalbumin (OVA), edestin, mammalian serum albumins (such as bovine serum albumin), and mammalian immunoglobulins. In some examples, the carrier is bovine serum albumin.

In some embodiments, the carrier is selected from one of: Keyhole Limpet Hemocyanin (KLH), tetanus toxoid, tetanus toxin heavy chain C fragment, diphtheria toxoid, diphtheria toxin variant CRM197, or H influenza protein D (HiD). CRM197 is a genetically detoxified form of diphtheria toxin; a single mutation at position 52, substituting glutamic acid for glycine, causes the ADP-ribosyltransferase activity of the native diphtheria toxin to be lost. For description of protein carriers for vaccines, see Pichichero, Protein carriers of conjugate vaccines: characteristics, development, and clinical trials, Hum Vaccin Immunother., 9: 2505-2523, 2013, which is incorporated by reference herein in its entirety).

Following conjugation of the recombinant gp120, gp140, gp145, gp160, or HIV-1 Env ectodomain trimer to the carrier protein, the conjugate can be purified by a variety of techniques well known to one of skill in the art. The conjugates can be purified away from unconjugated material by any number of standard techniques including, for example, size exclusion chromatography, density gradient centrifugation, hydrophobic interaction chromatography, or ammonium sulfate fractionation. See, for example, Anderson *et al.*, *J. Immunol.* 137:1181-86, 1986 and Jennings & Lugowski, *J. Immunol.* 127:1011-18, 1981. The compositions and purity of the conjugates can be determined by GLC-MS and MALDI-TOF spectrometry, for example.

In several embodiments, the disclosed immunogenic conjugates can be formulated into immunogenic composition (such as vaccines), for example by the addition of a pharmaceutically acceptable carrier and/or adjuvant.

5 **B. Recombinant V1V2 domain and Epitope Scaffold proteins**

In additional embodiments, a recombinant V1V2 domain that comprises the deletion of HIV-1 Env residues 137-152 (HXBc2 numbering) is provided as an isolated protein. The recombinant V1V2 domain elicits an immune response to HIV-1.

The minimal residues of the V1V2 domain are typically understood to be set by the
10 disulfide bridge between cysteine-119 and cysteine-205 of HIV-1 Env (HXBc2 numbering). In some embodiments, the recombinant V1V2 domain comprises or consists essentially of residues 119-205 with the 137-152 V1 deletion (HXBc2 numbering). However, the N- and C- terminal residues of the recombinant V1V2 domain can be any HIV-1 Env position that maintains the structure of the recombinant V1V2 domain with the V1 deletion as described in the examples.
15 In some embodiments, the recombinant V1V2 domain comprises or consists essentially of residues 126-196 with the 137-152 V1 deletion (HXBc2 numbering).

In some embodiments, the recombinant V1V2 domain can be included on an epitope scaffold protein. The recombinant V1V2 domain with the V1 deletion may be scaffolded onto other proteins using a variety of start and stop points, including but not limited to those noted
20 above.

In some embodiments, the scaffold protein is a gp70 protein. For example, the epitope-scaffold protein including a recombinant V1V2 from the clade B CaseA2 strain on the gp70 scaffold comprises or consists of the amino acid sequence set forth as:

SEQ ID NO: 8, gp70-ΔV1-V1/V2 CaseA2

25 QVYNITWEVTNGDRETVAISGNHPLWTWVPVLTPLDCLMLALSGPPHWGLEYPAPYSSPPGPPCCSGSSGSSAGCSR
DCDEPLTSLTPRCNTAWNRLKLDQVTHKSSEGFYVCPGSHRPREAKSCGGPDSFYCASWGCETTGRVYWKPSSWDY
ITVDNNLTTSQAVQVCKDNKWCNPLAIQFTNAGKQVTSWTTGHYWGLRLYVSGRDPGLTFGIRLRYQNLGPRVPIGP
NPVLADQLSLPRPNPLPKPAKSPPASVKLTPLCVTLNCIDLRLNEIKNCSEFNITTSIRDKVQKEYALFYKLDIVPIDN
30 PKNSTNYRLISCNTSVITQA

However, any protein with a beta-hairpin joining point superimposable on the original disulfide bridge of the V1V2 domain of the HIV-1 Env protein in native prefusion closed conformation may be a scaffold for the recombinant V1V2 domain. Non-limiting examples include typhoid toxin, and antibody Fc domains (PMID: 27707920).

35 In additional embodiments, the epitope-scaffold protein is any one of the V1V2 scaffolds disclosed in PCT Pub. No. 2013/039792, which is incorporated by reference herein in its entirety.

Suitable methods for identifying and selecting appropriate scaffolds are available and include (but are not limited to) superposition-, grafting-, and de novo-based methods disclosed herein and known to the person of ordinary skill in the art. For example, methods for superposition, grafting and de novo design of epitope-scaffolds are disclosed in U.S. Patent Application Publication No. 2010/0068217, incorporated by reference herein in its entirety.

"Superposition" epitope-scaffolds are based on scaffold proteins having an exposed segment with similar conformation as the target epitope--the backbone atoms in this "superposition-region" can be structurally superposed onto the target epitope with minimal root mean square deviation (RMSD) of their coordinates. Suitable scaffolds are identified by computationally searching through a library of protein crystal structures; epitope-scaffolds are designed by putting the epitope residues in the superposition region and making additional mutations on the surrounding surface of the scaffold to prevent clash or other interactions with the antibody.

"Grafting" epitope-scaffolds utilize scaffold proteins that can accommodate replacement of an exposed segment with the crystallized conformation of the target epitope. For each suitable scaffold identified by computationally searching through all protein crystal structures, an exposed segment is replaced by the target epitope and the surrounding sidechains are redesigned (mutated) to accommodate and stabilize the inserted epitope. Finally, as with superposition epitope-scaffolds, mutations are made on the surface of the scaffold and outside the epitope, to prevent clash or other interactions with the antibody. Grafting scaffolds require that the replaced segment and inserted epitope have similar translation and rotation transformations between their N- and C-termini, and that the surrounding peptide backbone does not clash with the inserted epitope. One difference between grafting and superposition is that grafting attempts to mimic the epitope conformation exactly, whereas superposition allows for small structural deviations.

"De novo" epitope-scaffolds are computationally designed from scratch to optimally present the crystallized conformation of the epitope. This method is based on computational design of a novel fold (Kuhlman, B. et al. 2003 Science 302:1364-1368). The de novo allows design of immunogens that are both minimal in size, so they do not present unwanted epitopes, and also highly stable against thermal or chemical denaturation.

In several embodiments, the native scaffold protein (without epitope insertion) is not a viral envelope protein. In additional embodiments, the scaffold protein is not an HIV protein. In still further embodiments, the scaffold protein is not a viral protein.

C. *Polynucleotides and Expression*

Polynucleotides encoding a disclosed immunogen are also provided. These polynucleotides include DNA, cDNA and RNA sequences which encode the antigen. One of skill in the art can readily use the genetic code to construct a variety of functionally equivalent nucleic acids, such as nucleic acids which differ in sequence but which encode the same protein sequence, or encode a conjugate or fusion protein including the nucleic acid sequence.

For example, in some embodiments, the polynucleotide encodes a V1 deleted HIV-1 Env sequence such as any one of SEQ ID NOs: 4-7 and 66-67; for example, the polynucleotide comprises the DNA sequence set forth as:

10 A244 gp160 V1 deleted (SEQ ID NO: 9)

```

15 ATGAGAGTGAAGGAGACACAGATGAATTGGCCAAACTTGTGGAAATGGGGGACTTTGATCCTTGGGTTGGTGATAAT
TTGTAGTGCCTCAGACAACTTGTGGGTTACAGTTTATTATGGGGTTCTGTGTGGAGAGATGCAGATACCACCCTAT
TTTGTGCATCAGATGCCAAAGCACATGAGACAGAAGTGCACAATGTCTGGGCCACACATGCCTGTGTACCCACAGAC
20 CCCAACCCACAAGAAATAGACCTGGAAAATGTAACAGAAAATTTTAACATGTGGAAAAATAACATGGTAGAGCAGAT
GCAGGAGGATGTAATCAGTTTATGGGATCAAAGTCTAAAGCCATGTGTAAAGTTAACTCCTCTCTGCGTTACTTTAC
ATTGTACTAATGCTAATTTGGAAGTAAGAACTGTTCTTTTAATATGACCACAGAACTAAGAGATAAGAAGCAGAAG
GTCCATGCACTTTTTTATAAGCTTGATATAGTACCAATTGAAGATAATAACGATAATAGTAAGTATAGGTTAATAAAA
TTGTAATACTTCAGTCATTAAGCAGGCTTGTCCAAAGATATCCTTTGATCCAATTCCTATACATTATTGTACTCCAG
25 CTGGTTATGCGATTTTAAAGTGTAATGATAAGAAATTTCAATGGGACAGGGCCATGTAAAAACGTCAGCTCAGTACAA
TGCACACATGGAATTAAGCCAGTGGTATCAACTCAATTGCTGTTAAATGGCAGTCTAGCAGAAGAAGAGATAATAAT
CAGATCTGAAGATCTCACAAACAATGCCAAAACCATAATAGTGCACCTTAATAAATCTGTAGTAATCAATTGTACCA
GACCTCCAAACAATACAAGAACAAGTATAACTATAGGACCAGGACAAAGTATTCTATAGAACAGGAGACATAATAGGA
GATATAAGAAAAGCATATTGTGAGATTAATGGAACAGAATGGAATAAGCTTTAAAAACAGGTAACCTGAAAAGTTAAA
30 AGAGCACTTTAATAATAAGCCCAATAATCTTTCAACCACCTCAGGAGGAGATCTAGAAAATTACAATGCATCATTTTA
ATTGTAGAGGAGAATTTTCTATTGCAATACAACACGACTGTTTAATAATACTTGCCATAGCAAATGGAACCATAGAG
GGGTGTAATGGCAATATCACACTTCCATGCAAGATAAAAACAAATTATAAACATGTGGCAGGGAGCAGGACAAGCAAT
GTATGCTCCTCCCATCAGTGGAAACAATTAATTGTGTATCAAAATATTACAGGAATACTATTGACAAGAGATGGTGGTG
CTACTAATAATACGAATAACGAGACCTTCAGACCTGGAGGAGGAAAATATAAAGGACAATTGGAGAAATGAATTATAT
35 AAATATAAAGTAGTACAAATTGAACCACTAGGAGCAGCACCCACCAGGGCAAAGAGAAGAGTGGTGGAGAGAGAAAA
AAGAGCAGTGGGAATAGGAGCTATGATCTTTGGGTTCTTAGGAGCAGCAGGAAGCACTATGGGCGCGGCGTCAATAA
CGCTGACGGTACAGGCCAGACAATTATTGTCTGGTATAGTGCAACAGCAAAGCAATTTGCTGAGGGCTATAGAGGCG
CAGCAGCATCTGTTGCAACTCACAGTCTGGGGCATTAACAGCTCCAGGCAAGAGTCCTGGCTGTGGAAAGATACCT
AAAGGATCAAAAGTTCTAGGACTTTGGGGCTGCTCTGAAAAATCATCTGCACCACTGCAGTGCCCTGGAACCTCA
40 CTTGGAGTAATAAATCTCTTGAAGAGATTTGGAACAACATGACATGGATAGAATGGGAGAGAGAAAATTAGCAATTAC
ACAAACCAATATATGAGATACTTACAAAATCGCAGGACCAGCAGGACAGGAATGAAAAGGATTTGTTAGAATTGGA
TAAATGGGCAAGTCTGTGGACTTGGTTTGACATAACAAATTTGGCTGTGGTATATAAAAAATTTTATAATGATAGTGG
GAGGTTTAATAGGATTAAGAATAATTTTGTCTGTGCTTTCTATAGTGAATAGAGTTAGGCAGGGATACTCACCTTTG
TCTTTCCAGACCCCTTGCCATCATCAGAGGGAACCCGACAGACCCGAAAGAATCGAAGAAGAAGGTGGCGAGCAAGG
45 CAGAGACAGATCCGTGCGATTAGTGAGCGGATTCTTAGCTCTTGCATGGGACGATCTACGGAGCCTGTGCCCTCTTCA
GCTACCACCGCTTGAGAGACTTCATCTTGATTGCAGCGAGGACTGTGGAACTTCTGGGACGCAGCAGTCTCAAGGGA
CTGAGACGGGGGTGGGAAGGCCTCAAATATCTGGGAATCTTCTGTTATATTGGGGTCAGGAACATAAAATTAGTGC
TATTTCTTTGCTTGATGCTACAGCAATAGCAGTAGCGGGGTGGACAGATAGGGTTATAGAAGTAGCACAAAGGAGCTT
GGAAAGCCATTCTCCACATACCTAGAAGAATCAGACAGGGCTTAGAAAGGGCTTTGCAATAA

```

In several embodiments, the nucleic acid molecule encodes a precursor of a protomer of a disclosed HIV-1 Env trimer, that, when expressed in cells under appropriate conditions, forms HIV-1 Env trimers and is processed into the mature form of the HIV-1 Env protein.

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, 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.

The polynucleotides encoding a disclosed immunogen can include 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 (such as a cDNA) independent of other sequences. The nucleotides can be ribonucleotides, deoxyribonucleotides, or modified forms of either nucleotide. The term includes single and double forms of DNA.

Polynucleotide sequences encoding a disclosed immunogen can be operatively linked to expression control sequences. An expression control sequence operatively linked to a coding sequence is ligated such that expression of the coding sequence is achieved under conditions compatible with the expression control sequences. The expression control sequences include, but are not limited to, 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.

DNA sequences encoding the disclosed immunogen 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.

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. Non-limiting examples of suitable host cells include bacteria, archaea, insect, fungi (for example, yeast), plant, and animal cells (for example, mammalian cells, such as human).

5 Exemplary cells of use include *Escherichia coli*, *Bacillus subtilis*, *Saccharomyces cerevisiae*, *Salmonella typhimurium*, SF9 cells, C129 cells, 293 cells, *Neurospora*, and immortalized mammalian myeloid and lymphoid cell lines. Techniques for the propagation of mammalian cells in culture are well-known (see, *e.g.*, Helgason and Miller (Eds.), 2012, Basic Cell Culture Protocols (Methods in Molecular Biology), 4th Ed., Humana Press). Examples of commonly
10 used mammalian host cell lines are VERO and HeLa cells, CHO cells, and WI38, BHK, and COS cell lines, although cell lines may be used, such as cells designed to provide higher expression, desirable glycosylation patterns, or other features. In some embodiments, the host cells include HEK293 cells or derivatives thereof, such as GnTI^{-/-} cells (ATCC® No. CRL-3022), or HEK-293F cells.

15 Transformation of a host cell with recombinant DNA can be carried out by conventional techniques as are well known to those skilled in the art. Where the host is prokaryotic, such as, but not limited to, *E. coli*, competent cells which are capable of DNA uptake can be prepared from cells harvested after exponential growth phase and subsequently treated by the CaCl₂ method using procedures well known in the art. Alternatively, MgCl₂ or RbCl can be used.
20 Transformation can also be performed after forming a protoplast of the host cell if desired, or by electroporation.

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 viral vectors can be used.
25 Eukaryotic cells can also be co-transformed with polynucleotide sequences encoding a disclosed antigen, 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
30 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.

In one non-limiting example, a disclosed immunogen is expressed using the pVRC8400 vector (described in Barouch *et al.*, *J. Virol*, 79, 8828-8834, 2005, which is incorporated by
35 reference herein).

Modifications can be made to a nucleic acid encoding a disclosed immunogen 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.

D. Viral Vectors

A nucleic acid molecule encoding a disclosed immunogen (*e.g.*, a recombinant gp120 protein or a HIV-1 Env ectodomain trimer comprising the recombinant gp120 protein) can be included in a viral vector, for example, for expression of the immunogen in a host cell, or for immunization of a subject as disclosed herein. In some embodiments, the viral vectors are administered to a subject as part of a prime-boost vaccination. In several embodiments, the viral vectors are included in a vaccine, such as a primer vaccine or a booster vaccine for use in a prime-boost vaccination.

In several examples, the viral vector can be replication-competent. For example, the viral vector can have a mutation in the viral genome that does not inhibit viral replication in host cells. The viral vector also can be conditionally replication-competent. In other examples, the viral vector is replication-deficient in host cells.

A number of viral vectors have been constructed, that can be used to express the disclosed antigens, including polyoma, *i.e.*, SV40 (Madzak *et al.*, 1992, *J. Gen. Virol.*, 73:1533-1536), adenovirus (Berkner, 1992, *Cur. Top. Microbiol. Immunol.*, 158:39-6; Berliner *et al.*, 1988, *Bio Techniques*, 6:616-629; Gorziglia *et al.*, 1992, *J. Virol.*, 66:4407-4412; Quantin *et al.*, 1992, *Proc. Natl. Acad. Sci. USA*, 89:2581-2584; Rosenfeld *et al.*, 1992, *Cell*, 68:143-155; Wilkinson *et al.*, 1992, *Nucl. Acids Res.*, 20:2233-2239; Stratford-Perricaudet *et al.*, 1990, *Hum. Gene Ther.*, 1:241-256), vaccinia virus (Mackett *et al.*, 1992, *Biotechnology*, 24:495-499), adeno-associated virus (Muzyczka, 1992, *Curr. Top. Microbiol. Immunol.*, 158:91-123; On *et al.*, 1990, *Gene*, 89:279-282), herpes viruses including HSV and EBV (Margolskee, 1992, *Curr. Top. Microbiol. Immunol.*, 158:67-90; Johnson *et al.*, 1992, *J. Virol.*, 66:2952-2965; Fink *et al.*, 1992, *Hum. Gene Ther.*, 3:11-19; Breakfield *et al.*, 1987, *Mol. Neurobiol.*, 1:337-371; Fresse *et al.*, 1990, *Biochem. Pharmacol.*, 40:2189-2199), Sindbis viruses (H. Herweijer *et al.*, 1995, *Human Gene Therapy* 6:1161-1167; U.S. Pat. Nos. 5,091,309 and 5,2217,879), alphaviruses (S. Schlesinger, 1993, *Trends Biotechnol.* 11:18-22; I. Frolov *et al.*, 1996, *Proc. Natl. Acad. Sci. USA* 93:11371-11377) and retroviruses of avian (Brandyopadhyay *et al.*, 1984, *Mol. Cell Biol.*,

4:749-754; Petropoulos *et al.*, 1992, *J. Virol.*, 66:3391-3397), murine (Miller, 1992, *Curr. Top. Microbiol. Immunol.*, 158:1-24; Miller *et al.*, 1985, *Mol. Cell Biol.*, 5:431-437; Sorge *et al.*, 1984, *Mol. Cell Biol.*, 4:1730-1737; Mann *et al.*, 1985, *J. Virol.*, 54:401-407), and human origin (Page *et al.*, 1990, *J. Virol.*, 64:5370-5276; Buchschalcher *et al.*, 1992, *J. Virol.*, 66:2731-2739).

- 5 Baculovirus (Autographa californica multinuclear polyhedrosis virus; AcMNPV) vectors are also known in the art, and may be obtained from commercial sources (such as PharMingen, San Diego, Calif.; Protein Sciences Corp., Meriden, Conn.; Stratagene, La Jolla, Calif.).

In several embodiments, the viral vector can include an adenoviral vector that expresses a disclosed recombinant HIV-1 Env ectodomain or immunogenic fragment thereof. Adenovirus from various origins, subtypes, or mixture of subtypes can be used as the source of the viral genome for the adenoviral vector. Non-human adenovirus (*e.g.*, simian, chimpanzee, gorilla, avian, canine, ovine, or bovine adenoviruses) can be used to generate the adenoviral vector. For example, a simian adenovirus can be used as the source of the viral genome of the adenoviral vector. A simian adenovirus can be of serotype 1, 3, 7, 11, 16, 18, 19, 20, 27, 33, 38, 39, 48, 49, 10 50, or any other simian adenoviral serotype. A simian adenovirus can be referred to by using any suitable abbreviation known in the art, such as, for example, SV, SAdV, SAV or sAV. In some examples, a simian adenoviral vector is a simian adenoviral vector of serotype 3, 7, 11, 16, 18, 19, 20, 27, 33, 38, or 39. In one example, a chimpanzee serotype C Ad3 vector is used (see, *e.g.*, Peruzzi *et al.*, *Vaccine*, 27:1293-1300, 2009). Human adenovirus can be used as the source of the viral genome for the adenoviral vector. Human adenovirus can be of various subgroups or 20 serotypes. For instance, an adenovirus can be of subgroup A (*e.g.*, serotypes 12, 18, and 31), subgroup B (*e.g.*, serotypes 3, 7, 11, 14, 16, 21, 34, 35, and 50), subgroup C (*e.g.*, serotypes 1, 2, 5, and 6), subgroup D (*e.g.*, serotypes 8, 9, 10, 13, 15, 17, 19, 20, 22, 23, 24, 25, 26, 27, 28, 29, 30, 32, 33, 36-39, and 42-48), subgroup E (*e.g.*, serotype 4), subgroup F (*e.g.*, serotypes 40 and 25 41), an unclassified serogroup (*e.g.*, serotypes 49 and 51), or any other adenoviral serotype. Replication competent and deficient adenoviral vectors (including singly and multiply replication deficient adenoviral vectors) can be used with the disclosed embodiments. Examples of replication-deficient adenoviral vectors, including multiply replication-deficient adenoviral vectors, are disclosed in U.S. Patent Nos. 5,837,511; 5,851,806; 5,994,106; 6,127,175; 30 6,482,616; and 7,195,896, and International Patent Application Nos. WO 94/28152, WO 95/02697, WO 95/16772, WO 95/34671, WO 96/22378, WO 97/12986, WO 97/21826, and WO 03/022311.

E. Virus-Like Particles

In some embodiments, a virus-like particle (VLP) is provided that includes a disclosed immunogen (e.g., a recombinant HIV-1 Env ectodomain or immunogenic fragment thereof). VLPs lack the viral components that are required for virus replication and thus represent a highly attenuated, replication-incompetent form of a virus. However, the VLP can display a polypeptide (e.g., a recombinant HIV-1 Env protein) that is analogous to that expressed on infectious virus particles and should be equally capable of eliciting an immune response to HIV when administered to a subject. Virus like particles and methods of their production are known, and viral proteins from several viruses are known to form VLPs, including human papillomavirus, HIV (Kang *et al.*, Biol. Chem. 380: 353-64 (1999)), Semliki-Forest virus (Notka *et al.*, Biol. Chem. 380: 341-52 (1999)), human polyomavirus (Goldmann *et al.*, J. Virol. 73: 4465-9 (1999)), rotavirus (Jiang *et al.*, Vaccine 17: 1005-13 (1999)), parvovirus (Casal, Biotechnology and Applied Biochemistry, Vol 29, Part 2, pp 141-150 (1999)), canine parvovirus (Hurtado *et al.*, J. Virol. 70: 5422-9 (1996)), hepatitis E virus (Li *et al.*, J. Virol. 71: 7207-13 (1997)), and Newcastle disease virus. The formation of such VLPs can be detected by any suitable technique. Examples of suitable techniques known in the art for detection of VLPs in a medium include, e.g., electron microscopy techniques, dynamic light scattering (DLS), selective chromatographic separation (e.g., ion exchange, hydrophobic interaction, and/or size exclusion chromatographic separation of the VLPs) and density gradient centrifugation.

The virus like particle can include any of the recombinant gp120 proteins or recombinant HIV-1 Env ectodomain trimers or an immunogenic fragments thereof, that are disclosed herein.

III. Immunogenic Compositions

Immunogenic compositions comprising a disclosed immunogen and a pharmaceutically acceptable carrier are also provided. Such compositions can be administered to subjects by a variety of administration modes, for example, intramuscular, subcutaneous, intravenous, intra-arterial, intra-articular, intraperitoneal, or parenteral routes. Methods for preparing administrable compositions are described in more detail in such publications as *Remingtons Pharmaceutical Sciences*, 19th Ed., Mack Publishing Company, Easton, Pennsylvania, 1995.

Thus, an immunogen described herein can be formulated with pharmaceutically acceptable carriers to help retain biological activity while also promoting increased stability during storage within an acceptable temperature range. Potential carriers include, but are not limited to, physiologically balanced culture medium, phosphate buffer saline solution, water, emulsions (e.g., oil/water or water/oil emulsions), various types of wetting agents, cryoprotective additives or stabilizers such as proteins, peptides or hydrolysates (e.g., albumin,

gelatin), sugars (e.g., sucrose, lactose, sorbitol), amino acids (e.g., sodium glutamate), or other protective agents. The resulting aqueous solutions may be packaged for use as is or lyophilized. Lyophilized preparations are combined with a sterile solution prior to administration for either single or multiple dosing.

5 Formulated compositions, especially liquid formulations, may contain a bacteriostat to prevent or minimize degradation during storage, including but not limited to effective concentrations (usually $\leq 1\%$ w/v) of benzyl alcohol, phenol, m-cresol, chlorobutanol, methylparaben, and/or propylparaben. A bacteriostat may be contraindicated for some patients; therefore, a lyophilized formulation may be reconstituted in a solution either containing or not
10 containing such a component.

The pharmaceutical compositions of the disclosure can contain as pharmaceutically acceptable vehicles substances as required to approximate physiological conditions, such as pH adjusting and buffering agents, tonicity adjusting agents, wetting agents and the like, for example, sodium acetate, sodium lactate, sodium chloride, potassium chloride, calcium chloride,
15 sorbitan monolaurate, and triethanolamine oleate.

The pharmaceutical composition may optionally include an adjuvant to enhance an immune response of the host. Suitable adjuvants are, for example, toll-like receptor agonists, alum, AlPO₄, alhydrogel, Lipid-A and derivatives or variants thereof, oil-emulsions, saponins, neutral liposomes, liposomes containing the vaccine and cytokines, non-ionic block copolymers,
20 and chemokines. Non-ionic block polymers containing polyoxyethylene (POE) and polyxypropylene (POP), such as POE-POP-POE block copolymers, MPL™ (3-O-deacylated monophosphoryl lipid A; Corixa, Hamilton, IN) and IL-12 (Genetics Institute, Cambridge, MA), may be used as an adjuvant (Newman et al., 1998, *Critical Reviews in Therapeutic Drug Carrier Systems* 15:89-142). These adjuvants have the advantage in that they help to stimulate the
25 immune system in a non-specific way, thus enhancing the immune response to a pharmaceutical product.

In some embodiments, the composition can be provided as a sterile composition. The pharmaceutical composition typically contains an effective amount of a disclosed immunogen and can be prepared by conventional techniques. Typically, the amount of immunogen in each
30 dose of the immunogenic composition is selected as an amount which elicits an immune response without significant, adverse side effects. In some embodiments, the composition can be provided in unit dosage form for use to elicit an immune response in a subject, for example, to prevent HIV-1 infection in the subject. A unit dosage form contains a suitable single preselected dosage for administration to a subject, or suitable marked or measured multiples of

two or more preselected unit dosages, and/or a metering mechanism for administering the unit dose or multiples thereof. In other embodiments, the composition further includes an adjuvant.

IV. Methods of Inducing an Immune Response

5 The disclosed immunogens (*e.g.*, a recombinant gp120 protein comprising a V1-deletion or HIV-1 Env trimer containing the recombinant gp120 protein), polynucleotides and vectors encoding the disclosed immunogens, and compositions including same, can be used in methods of inducing an immune response to HIV-1 to treat or inhibit (including prevent) an HIV-1 infection.

10 When inhibiting or treating HIV-1 infection, the methods can be used either to avoid infection in an HIV-1 seronegative subject (*e.g.*, by inducing an immune response that protects against HIV-1 infection), or to treat existing infection in an HIV-1 seropositive subject. The HIV-1 seropositive subject may or may not carry a diagnosis of AIDS. Hence in some embodiments the methods involve selecting a subject at risk for contracting HIV-1 infection, or
15 a subject at risk of developing AIDS (such as a subject with HIV-1 infection), and administering a disclosed immunogen to the subject to elicit an immune response to HIV-1 in the subject.

To identify subjects for prophylaxis or treatment according to the methods of the disclosure, accepted screening methods are employed to determine risk factors associated with a targeted or suspected disease or condition, or to determine the status of an existing disease or
20 condition in a subject. These screening methods include, for example, conventional work-ups to determine environmental, familial, occupational, and other such risk factors that may be associated with the targeted or suspected disease or condition, as well as diagnostic methods, such as various ELISA and other immunoassay methods to detect and/or characterize HIV-1 infection. These and other routine methods allow the clinician to select patients in need of
25 therapy using the methods and pharmaceutical compositions of the disclosure. In accordance with these methods and principles, a composition can be administered according to the teachings herein, or other conventional methods, as an independent prophylaxis or treatment program, or as a follow-up, adjunct or coordinate treatment regimen to other treatments.

The disclosed immunogens can be used in coordinate (or prime-boost) immunization
30 protocols or combinatorial formulations. In certain embodiments, novel combinatorial immunogenic compositions and coordinate immunization protocols employ separate immunogens or formulations, each directed toward eliciting an anti-HIV-1 immune response, such as an immune response to HIV-1 Env protein. Separate immunogenic compositions that elicit the anti-HIV-1 immune response can be combined in a polyvalent immunogenic

composition administered to a subject in a single immunization step, or they can be administered separately (in monovalent immunogenic compositions) in a coordinate immunization protocol.

In one embodiment, a suitable immunization regimen includes at least two separate inoculations with one or more immunogenic compositions including a disclosed immunogen, with a second inoculation being administered more than about two, about three to eight, or about four, weeks following the first inoculation. A third inoculation can be administered several months after the second inoculation, and in specific embodiments, more than about five months after the first inoculation, more than about six months to about two years after the first inoculation, or about eight months to about one year after the first inoculation. Periodic inoculations beyond the third are also desirable to enhance the subject's "immune memory." The adequacy of the vaccination parameters chosen, *e.g.*, formulation, dose, regimen and the like, can be determined by taking aliquots of serum from the subject and assaying antibody titers during the course of the immunization program. Alternatively, the T cell populations can be monitored by conventional methods. In addition, the clinical condition of the subject can be monitored for the desired effect, *e.g.*, prevention of HIV-1 infection or progression to AIDS, improvement in disease state (*e.g.*, reduction in viral load), or reduction in transmission frequency to an uninfected partner. If such monitoring indicates that vaccination is sub-optimal, the subject can be boosted with an additional dose of immunogenic composition, and the vaccination parameters can be modified in a fashion expected to potentiate the immune response. Thus, for example, a dose of a disclosed immunogen can be increased or the route of administration can be changed.

It is contemplated that there can be several boosts, and that each boost can be a different immunogen. It is also contemplated in some examples that the boost may be the same immunogen as another boost, or the prime.

The prime and the boost can be administered as a single dose or multiple doses, for example, two doses, three doses, four doses, five doses, six doses or more can be administered to a subject over days, weeks or months. Multiple boosts can also be given, such one to five, or more. Different dosages can be used in a series of sequential inoculations. For example, a relatively large dose in a primary inoculation and then a boost with relatively smaller doses. The immune response against the selected antigenic surface can be elicited by one or more inoculations of a subject.

In several embodiments, a disclosed immunogen can be administered to the subject simultaneously with the administration of an adjuvant. In other embodiments, the immunogen can be administered to the subject after the administration of an adjuvant and within a sufficient amount of time to elicit the immune response.

Determination of effective dosages in this context is typically based on animal model studies followed up by human clinical trials and is guided by administration protocols that significantly reduce the occurrence or severity of targeted disease symptoms or conditions in the subject, or that elicit a desired response in the subject (such as a neutralizing immune response).

5 Suitable models in this regard include, for example, murine, rat, porcine, feline, ferret, non-human primate, and other accepted animal model subjects known in the art. Alternatively, effective dosages can be determined using *in vitro* models (for example, immunologic and histopathologic assays). Using such models, only ordinary calculations and adjustments are required to determine an appropriate concentration and dose to administer an effective amount
10 of the composition (for example, amounts that are effective to elicit a desired immune response or alleviate one or more symptoms of a targeted disease). In alternative embodiments, an effective amount or effective dose of the composition may simply inhibit or enhance one or more selected biological activities correlated with a disease or condition, as set forth herein, for either therapeutic or diagnostic purposes.

15 Dosage can be varied by the attending clinician to maintain a desired concentration at a target site (for example, systemic circulation). Higher or lower concentrations can be selected based on the mode of delivery, for example, trans-epidermal, rectal, oral, pulmonary, or intranasal delivery versus intravenous or subcutaneous delivery. The actual dosage of disclosed immunogen will vary according to factors such as the disease indication and particular status of
20 the subject (for example, the subject's age, size, fitness, extent of symptoms, susceptibility factors, and the like), time and route of administration, other drugs or treatments being administered concurrently, as well as the specific pharmacology of the composition for eliciting the desired activity or biological response in the subject. Dosage regimens can be adjusted to provide an optimum prophylactic or therapeutic response.

25 A non-limiting range for an effective amount of the disclosed immunogen within the methods and immunogenic compositions of the disclosure is about 0.0001 mg/kg body weight to about 10 mg/kg body weight, such as about 0.01 mg/kg, about 0.02 mg/kg, about 0.03 mg/kg, about 0.04 mg/kg, about 0.05 mg/kg, about 0.06 mg/kg, about 0.07 mg/kg, about 0.08 mg/kg, about 0.09 mg/kg, about 0.1 mg/kg, about 0.2 mg/kg, about 0.3 mg/kg, about 0.4 mg/kg, about
30 0.5 mg/kg, about 0.6 mg/kg, about 0.7 mg/kg, about 0.8 mg/kg, about 0.9 mg/kg, about 1 mg/kg, about 1.5 mg/kg, about 2 mg/kg, about 2.5 mg/kg, about 3 mg/kg, about 4 mg/kg, about 5 mg/kg, or about 10 mg/kg, for example, 0.01 mg/kg to about 1 mg/kg body weight, about 0.05 mg/kg to about 5 mg/kg body weight, about 0.2 mg/kg to about 2 mg/kg body weight, or about 1.0 mg/kg to about 10 mg/kg body weight. In some embodiments, the dosage includes a set

amount of a disclosed immunogen such as from about 1-300 µg, for example, a dosage of about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 30, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250, or about 300 µg.

The dosage and number of doses will depend on the setting, for example, in an adult or anyone primed by prior HIV-1 infection or immunization, a single dose may be a sufficient booster. In naïve subjects, in some examples, at least two doses would be given, for example, at least three doses. In some embodiments, an annual boost is given, for example, along with an annual influenza vaccination.

HIV-1 infection does not need to be completely inhibited for the methods to be effective. For example, elicitation of an immune response to HIV-1 with one or more of the disclosed immunogens can reduce or inhibit HIV-1 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 or prevention of detectable HIV-1 infected cells), as compared to HIV-1 infection in the absence of the therapeutic agent. In additional examples, HIV-1 replication can be reduced or inhibited by the disclosed methods. HIV-1 replication does not need to be completely eliminated for the method to be effective. For example, the immune response elicited using one or more of the disclosed immunogens can reduce HIV-1 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 or prevention of detectable HIV-1 replication), as compared to HIV-1 replication in the absence of the immune response.

To successfully reproduce itself, HIV-1 must convert its RNA genome to DNA, which is then imported into the host cell's nucleus and inserted into the host genome through the action of HIV-1 integrase. Because HIV-1's primary cellular target, CD4+ T-Cells, can function as the memory cells of the immune system, integrated HIV-1 can remain dormant for the duration of these cells' lifetime. Memory T-Cells may survive for many years and possibly for decades. This latent HIV-1 reservoir can be measured by co-culturing CD4+ T-Cells from infected patients with CD4+ T-Cells from uninfected donors and measuring HIV-1 protein or RNA (See, e.g., Archin et al., *AIDS*, **22**:1131–1135, 2008). In some embodiments, the provided methods of treating or inhibiting HIV-1 infection include reduction or elimination of the latent reservoir of HIV-1 infected cells in a subject. For example, a reduction of 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-1) of the latent reservoir of HIV-1 infected cells in a subject, as compared to the latent reservoir of HIV-1 infected cells in a subject in the absence of the treatment with one or more of the provided immunogens.

Following immunization of a subject, serum can be collected from the subject at appropriate time points, frozen, and stored for neutralization testing. Methods to assay for neutralization activity, and include, but are not limited to, plaque reduction neutralization (PRNT) assays, microneutralization assays, flow cytometry based assays, single-cycle infection assays (e.g., as described in Martin *et al.* (2003) *Nature Biotechnology* 21:71-76), and pseudovirus neutralization assays (e.g., as described in Georgiev *et al.* (*Science*, 340, 751-756, 2013), Seaman *et al.* (*J. Virol.*, 84, 1439-1452, 2005), and Mascola *et al.* (*J. Virol.*, 79, 10103-10107, 2005), each of which is incorporated by reference herein in its entirety. In some embodiments, the serum neutralization activity can be assayed using a panel of HIV-1 pseudoviruses as described in Georgiev *et al.*, *Science*, 340, 751-756, 2013 or Seaman *et al.* *J. Virol.*, 84, 1439-1452, 2005. Briefly, pseudovirus stocks are prepared by co-transfection of 293T cells with an HIV-1 Env-deficient backbone and an expression plasmid encoding the Env gene of interest. The serum to be assayed is diluted in Dulbecco's modified Eagle medium-10% FCS (Gibco) and mixed with pseudovirus. After 30 min, 10,000 TZM-bl cells are added, and the plates are incubated for 48 hours. Assays are developed with a luciferase assay system (Promega, Madison, WI), and the relative light units (RLU) are read on a luminometer (Perkin-Elmer, Waltham, MA). To account for background, a cutoff of $ID_{50} \geq 40$ can be used as a criterion for the presence of serum neutralization activity against a given pseudovirus.

In some embodiments, administration of an effective amount of one or more of the disclosed immunogens to a subject (e.g., by a prime-boost administration of a DNA vector encoding a disclosed immunogen followed by a protein boost) elicits a neutralizing immune response in the subject, wherein serum from the subject neutralizes, with an $ID_{50} \geq 40$, at least 10% (such as at least 15%, at least 20%, at least 30%, at least 40%, at least 50%, or at least 70%) of pseudoviruses is a panel of pseudoviruses including the HIV-1 Env proteins listed in Table S5 or Table S6 of Georgiev *et al.* (*Science*, 340, 751-756, 2013), or Table 1 of Seaman *et al.* (*J. Virol.*, 84, 1439-1452, 2005).

One approach to administration of nucleic acids is direct immunization with plasmid DNA, such as with a mammalian expression plasmid. Immunization by nucleic acid constructs is taught, for example, in U.S. Patent No. 5,643,578 (which describes methods of immunizing vertebrates by introducing DNA encoding a desired antigen to elicit a cell-mediated or a humoral response), and U.S. Patent No. 5,593,972 and U.S. Patent No. 5,817,637 (which describe operably linking a nucleic acid sequence encoding an antigen to regulatory sequences enabling expression). U.S. Patent No. 5,880,103 describes several methods of delivery of nucleic acids encoding immunogenic peptides or other antigens to an organism. The methods include liposomal delivery of the nucleic acids (or of the synthetic peptides themselves), and

immune-stimulating constructs, or ISCOMSTM, negatively charged cage-like structures of 30-40 nm in size formed spontaneously on mixing cholesterol and Quil ATM (saponin). Protective immunity has been generated in a variety of experimental models of infection, including toxoplasmosis and Epstein-Barr virus-induced tumors, using ISCOMSTM as the delivery vehicle for antigens (Mowat and Donachie, *Immunol. Today* 12:383, 1991). Doses of antigen as low as 1 µg encapsulated in ISCOMSTM have been found to produce Class I mediated CTL responses (Takahashi *et al.*, *Nature* 344:873, 1990).

In some embodiments, a plasmid DNA vaccine is used to express a disclosed immunogen in a subject. For example, a nucleic acid molecule encoding a disclosed immunogen can be administered to a subject to elicit an immune response to HIV-1 gp120. In some embodiments, the nucleic acid molecule can be included on a plasmid vector for DNA immunization, such as the pVRC8400 vector (described in Barouch *et al.*, *J. Virol.*, 79, 8828-8834, 2005, which is incorporated by reference herein).

In another approach to using nucleic acids for immunization, a disclosed immunogen (such as a protomer of a HIV-1 Env ectodomain trimer) can be expressed by attenuated viral hosts or vectors or bacterial vectors. Recombinant vaccinia virus, adeno-associated virus (AAV), herpes virus, retrovirus, cytomegalo virus or other viral vectors can be used to express the peptide or protein, thereby eliciting a CTL response. For example, vaccinia vectors and methods useful in immunization protocols are described in U.S. Patent No. 4,722,848. BCG (Bacillus Calmette Guerin) provides another vector for expression of the peptides (see Stover, *Nature* 351:456-460, 1991).

In one embodiment, a nucleic acid encoding a disclosed immunogen (such as a protomer of a HIV-1 Env ectodomain trimer) 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 HELIOSTM Gene Gun. The nucleic acids can be "naked," consisting of plasmids under control of a strong promoter. 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).

In some embodiments, an immunization protocol that mirrors the "rv144" trial is used with the immunogens provided herein. As discussed in Rerks-Ngarm *et al.* (New Eng J Med. 361 (23): 2209-2220, 2009, incorporated by reference herein) rv144 was a phase III trial of a prime-boost HIV-1 vaccine consisting of four injections of ALVAC HIV (vCP1521) followed by two injections of AIDSVAX B/E. ALVAC HIV (vCP1521) is a canarypox vector containing HIV-1 env, gag, and pol genes, and AIDSVAX B/E is a genetically engineered form of gp120.

The env gene of ALVAC HIV (vCP1521) and the AIDSVAX B/E gp120 can be modified to encode or contain the V1 deletion provided herein (deletion of residues 137-152 according to HXBc2 numbering) and administered to a subject using the rv144 prime-boost protocol (or any other suitable protocol).

5 In some embodiments, a modified form of the “rv144” immunization protocol can be used. For example, there can be additional (or fewer) prime or boost administrations, and the initial prime can be a DNA based immunization including a plasmid vector encoding HIV-1 Env with or without the V1 deletion as disclosed herein.

10 **V. Prognosis of an immune response to HIV-1**

Also provided herein is a method of interrogating an immune response to HIV-1 in a subject (such as a human subject) to predict if the immune response is or is not likely to inhibit HIV-1 infection in the subject. In some embodiments, the method provides a prognosis of an immune response to HIV-1 in a subject. In some embodiments, the method comprises

15 contacting a biological sample from a subject with one or more peptides comprising or consisting of the amino acid sequence of HIV Env residues 141-154 (V1a), HIV Env residues 157-173 (V2b), or HIV Env residues 166-180 (V2c) according to the HXBc2 numbering system, and detecting specific binding activity of antibodies in the biological sample to the one or more peptides. As described in the examples, subjects who received an HIV-1 vaccine and

20 subsequently exhibit antibody binding activity for the V2b or V2c peptide are less likely to become infected with HIV-1 subsequent to viral challenge relative to unvaccinated controls. Further, subjects who received an HIV-1 vaccine and subsequently exhibit antibody binding activity for the V1a peptide are more likely to become infected with HIV-1 subsequent to viral challenge relative to unvaccinated controls. Accordingly, detecting specific binding activity of

25 antibodies in the biological sample to the V2b peptide or to the V2c peptide identifies the biological sample as originating from a subject with an immune response that inhibits HIV-1 infection, and detecting specific binding activity of antibodies in the biological sample to the V1a peptide identifies the biological sample as originating from a subject with an immune response that does not inhibit HIV-1 infection,

30 The V1a, V2b, and V2c peptides for use in the disclosed methods can include any HIV-1 Env peptide sequence containing the relevant HXBc2 residues, that is residues 141-154 for the V1a peptide, residues 157-173 for the V2b, and residues 166-180 for the V2c peptide. Exemplary peptide sequences for use in the disclosed methods are provided in the following table.

Table 1. Exemplary V1a, V2b, and V2c peptide sequences

Exemplary V1a peptide sequences (Hxhc2 numbering 127-140)			
SEQ ID NO	Sequence	HIV Strain (isolate)	Subtype
26	VT LHCTNANLTNAN	90TH_CM235	CRF01_AE
27	VT LNCTDLRNTTNT	84US_MNp	B.MN
28	VT LNCTEVNVTRNV	96ZM651 gp120-TM	C
Exemplary V2b peptide sequences (Hxhc2 numbering 157-173)			
SEQ ID NO	Sequence	HIV Strain (isolate)	Subtype
29	CSFNMTTEL RDKKQKVH	90TH_CM235	CRF01_AE
30	CSFNITTSIRDKMQKEY	84US_MNp	B.MN
31	CSFNITTELKDKKKNVY	96ZM651 gp120-TM	C
Exemplary V2c peptide sequences (Hxhc2 numbering 167-180)			
SEQ ID NO	Sequence	HIV Strain (isolate)	Subtype
32	DKKQKVHALFYKLD	90TH_CM235	CRF01_AE
33	DKMQKEYALLYKLD	84US_MNp	B.MN
34	DKKKNVYALFYKLD	96ZM651 gp120-TM	C

The peptides for use in the disclosed methods comprise, consist essentially of, or consist of the V1a, V2b, or V2c sequence. In some embodiments, the V1a, V2b, and/or V2c peptide used in the assay is a cocktail of V1a, V2b, or V2c peptides from different HIV-1 strains. The V1a, V2b, and V2c peptides can be from HIV-1 Env from any HIV-1 strain, for example as listed in the Los Alamos HIV sequence database (hiv.lanl.gov).

In some embodiments, the peptide comprises, consists essentially of, or consists of the V1a, V2b, or V2c sequence and further comprises additional heterologous amino acids, such as a peptide tag. The peptides for use in the disclosed methods can be any suitable length of amino acids. In some embodiments, the peptides are no more than 50 amino acids in length, such as no more than 40 amino acids in length, or no more than 30 amino acids in length, or no more than 20 amino acids in length. In some embodiments, the peptides for use in the disclosed embodiments are from 10 to 30 amino acids in length, such as from 10 to 20 amino acids in length.

The biological sample can be any sample from a subject containing antibodies, 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. In some embodiments, the biological sample is obtained from a subject prior to assessment using the methods disclosed herein.

In one embodiment, the peptide is labeled with a detectable marker. Non-limiting
5 examples of suitable labels are known and include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, magnetic agents, and radioactive materials.

In some embodiments, a subject is selected for assessment using the method of prognosis of the immune response to HIV-1. Suitable subjects include, for example, individuals exposed to HIV-1 virus, or who have an HIV-1 infection, as well as subjects with or with an HIV-1
10 infection who have been administered an HIV-1 vaccine. For example, a biological sample from a subject with a known HIV-1 infection can be tested for specific binding activity to the V1a, V2b, and V2c peptides as described above to determine if the subject has produced an immune response to HIV-1 that is or is not likely to inhibit the HIV-1 infection. In another example, a biological sample from a subject without an HIV-1 infection who has been
15 administered an HIV-1 vaccine can be tested for specific binding activity to the V1a, V2b, and V2c peptides as described above to determine if the vaccination elicited production of an immune response to HIV-1 in the subject that is or is not likely to inhibit a subsequent HIV-1 infection. In another example, a biological sample from a subject with an HIV-1 infection who has been administered an HIV-1 vaccine can be tested for specific binding activity to the V1a,
20 V2b, and V2c peptides as described above to determine if the vaccination elicited production of an immune response to HIV-1 in the subject that is or is not likely to inhibit the HIV-1 infection.

In several embodiments, detection of specific binding activity for the V2b or V2c peptides indicates that the immune response to HIV-1 in the subject is likely to inhibit HIV-1 infection, for example, the immune response is likely to prevent or reduce subsequent infection
25 of the subject with HIV-1, or is likely to inhibit progression of HIV-1 disease in a subject already infected with HIV-1. Detecting specific binding activity for the V2b or V2c peptides includes, for example, detecting a positive signal in an appropriate assay as well as detecting an increase in binding activity relative to a suitable control.

In some embodiments, detection of specific binding activity for the V2b or V2c peptides
30 indicates that the immune response to HIV-1 in the subject has a good prognosis.

The good prognosis can refer to any positive clinical outcome, such as, but not limited to, an increase in likelihood of survival (such as overall survival or AIDS-free survival), an increase in the time of survival (*e.g.*, more than 5 years, more than one year, or more than two months), absence or reduction of HIV-1 replication, likelihood of benefit of the subject to
35 therapy (*e.g.*, HAART therapy), an increase in response to therapy (*e.g.*, HAART therapy), or

the like. The relative “goodness” of a prognosis, in various examples, may be in comparison to historical measure of other subjects with the same or similar infection, or similar presentation of symptoms of HIV-1 infection, for example.

In several embodiments, detection of specific binding activity for the V1a peptide indicates that the immune response to HIV-1 in the subject is not likely to inhibit HIV-1 infection, for example, the immune response is not likely to prevent or reduce subsequent infection of the subject with HIV-1, or is not likely to inhibit progression of HIV-1 disease in a subject already infected with HIV-1. Detecting specific binding activity for the V1a peptide includes, for example, detecting a positive signal in an appropriate assay as well as detecting an increase in binding activity relative to a suitable control.

In some embodiments, detection of specific binding activity for the V1a peptide indicates that the immune response to HIV-1 in the subject has a poor prognosis.

The poor prognosis can refer to any negative clinical outcome, such as, but not limited to, a decrease in likelihood of survival (such as overall survival or AIDS-free survival), a decrease in the time of survival (*e.g.*, less than 5 years, less than one year, or less than two months), presence or increase in HIV-1 replication, an increase in the severity of disease, resistance to therapy (*e.g.*, HAART therapy), an decrease in response to therapy (*e.g.*, HAART therapy), or the like. The relative “poorness” of a prognosis, in various examples, may be in comparison to historical measure of other subjects with the same or similar infection, or similar presentation of symptoms of HIV-1 infection, for example.

Antibodies in the biological sample specific for the V1a, V2b, and/or V2c peptides can be detected by any suitable assay, including, but not limited to, ELISA, immunoprecipitation, generic binding to solid supports, surface plasmon resonance. In some embodiments, antibodies in the biological sample specific for the V1a, V2b, and V2c peptides can be detected by any suitable immunoassay one of a number of immunoassay, such as those presented in Harlow and Lane, *Using Antibodies: A Laboratory Manual*, CSHL, New York, 1999). In some embodiments, a standard immunoassay format (such as ELISA, Western blot, or RIA assay) can be used to measure antibody levels. Immunohistochemical techniques can also be utilized for antibody detection and quantification, for example using formalin-fixed, paraffin embedded (FFPE) slides coupled with an automated slide stainer. General guidance regarding such techniques can be found in Bancroft et al. (*Theory and Practice of Histological Techniques*, 8th ed., Elsevier, 2018) and Ausubel *et al.* (In *Current Protocols in Molecular Biology*, John Wiley & Sons, New York, through supplement 104, 2013).

For the purposes of quantitating the disclosed proteins, a sample that includes antibodies can be used. Quantitation of antibodies can be achieved by immunoassay. In some

embodiments, a level of specific binding activity for the V1a, V2b, and/or V2c peptides can be assessed in the sample and optionally in a corresponding control sample, such as a sample from a subject known to have a protective or non-protective immune response to HIV-1 or other control (such as a standard value or reference value). A significant increase or decrease in the amount can be evaluated using statistical methods known in the art.

In some embodiments, the method of detection can include contacting a cell or sample, with the V1a, V2b, and/or V2c peptide or conjugate thereof (*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 peptide).

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

Unmasking the α -helix conformation of V2 by V1 deletion augments

HIV-1 Vaccine Efficacy

ALVAC-gp120/alum HIV vaccine candidates decrease the risk of virus acquisition in both humans and macaques. Antibodies to the envelope variable V2 region 2 (V2) is the primary correlate of risk in both species. This example shows that serum antibodies to the envelope variable region 1 (V1) from macaques vaccinated with these vaccine modalities interfere *in vitro* with binding of V2-specific antibodies. Furthermore, the α -V1 antibody levels in vaccinated macaques correlated with an increased risk of SIV_{mac251} acquisition. Accordingly, V1-deleted envelope immunogens elicited higher titers of antibodies to V2 in macaques.

Strikingly, however, only the V1-deleted immunogen engineered to maintain a V2 α -helix conformation was associated with a decreased risk of SIV_{mac251} acquisition correlating with serum levels of antibodies to a V2 α -helix diagnostic peptide. These data confirm V2 as a viral vulnerability site and support the development and testing of V1-deleted HIV immunogens in humans.

The HIV recombinant Canarypox-derived vector (ALVAC) in combination with two gp120-envelope proteins formulated in alum afforded limited but significant efficacy (31.2%) in the RV144 HIV vaccine trial (Rerks-Ngarm et al., N Engl J Med, 361, 2209-2220, 2009). This vaccine regimen induced high titers of binding antibodies to the HIV-1 envelope proteins and envelope-specific CD4⁺ T cells in nearly all vaccines and negligible CD8⁺ T cell responses (Rerks-Ngarm et al., N Engl J Med, 361, 2209-2220, 2009). The primary correlates of risk of

HIV acquisition were the titers of serum IgG to the gp70-V1/V2 scaffold (Haynes et al., *N Engl J Med*, 366, 1275-1286, 2012) and to linear V2 peptides (Zolla-Pazner et al., *PLoS One*, 9, e87572, 2014; Gottardo et al., *PLoS One*, 8, e75665, 2013). Sieve analysis demonstrated genetic markers of immunologic pressure at positions 169 and 181 in the more conserved

5 carboxyl-terminus region of V2 (Rolland et al., *Nature*, 490, 417-420, 2012), corresponding to sites comprising, or allosterically influencing, gp120 binding to the $\alpha 4\beta 7$ integrin receptor. In the macaque model, vaccination with a similar SIV-based vaccine platform also significantly decreased the risk of virus acquisition (44% efficacy) following mucosal exposure of immunized macaques to repeated low doses of SIV_{mac251} (Pegu et al., *J Virol*, 87, 1708-1719, 2013; Gordon

10 et al., *J Immunol*, 193, 6172-6183, 2014; Klionsky et al., *Autophagy*, 12, 1-222, 2016). In this example, linear peptide arrays encompassing the entire gp120 of SIV_{K6W} were used to characterize the serum antibody response to V1 and V2 in a cohort of 78 vaccinated macaques immunized with ALVAC-SIV/gp120 based vaccines whereby the alum adjuvant was substituted with the more immunogenic MF59 (Vaccari et al., *Nat Med*, 22, 762-770, 2016) or the ALVAC-

15 SIV prime was substituted with either the DNA-SIV or Ad26-SIVprime (Vaccari et al., *Nat Med*, 24, 847-856, 2018). The efficacy of these vaccine regimens was evaluated as the average risk of virus acquisition following intrarectal exposure to low repeated doses of the identical SIV_{mac251} and ranged between 9% and 52%. For simplicity, these vaccine regimens are referred to as protective (39 animals; vaccine efficacy ranges from 44 to 52%; $p = 0. <0.05$) or non-

20 protective (39 animals, vaccine efficacy range 9 to 13%, $p > 0.05$) when compared to controls (**FIGs. 4A-4B**) (Vaccari et al., *Nat Med*, 22, 762-770, 2016; Vaccari et al., *Nat Med*, 24, 847-856, 2018).

It was shown previously that mucosal antibody levels to conformational cyclic V2 correlated with decreased SIV_{mac251} acquisition in animals immunized with protective vaccines

25 (Vaccari et al., *Nat Med*, 24, 847-856, 2018). Here it is shown that the levels of serum antibodies to all linear peptides encompassing V2 (**FIGs. 5A-5B**) did not differ between the animals immunized with protective and non protective vaccines (**FIG. 5C**), and none of the individual linear V2 peptides correlated with SIV_{mac251} acquisition (**FIG. 5D**). The levels of serum recognition of the V2 peptides 27 and 29 (hereafter referred as to V2b and V2c),

30 however, was associated with a decreased amount of SIV DNA in the mucosa of the vaccinated animals that became infected following immunization (**FIG. 1A-1B**). Notably, V2b and V2c (**FIG. 1C**) correspond to the allosteric and actual binding sites of gp120 to the $\alpha 4\beta 7$ integrin respectively. Thus, antibodies to specific V2 epitopes may play a role not only in preventing acquisition but also in post-infection control of local virus spreading.

Analyses of serum reactivity to overlapping V1 peptides 15-24 (**FIGs. 6A-6B**) revealed no differences between the immunized groups (**FIG. 6C**), nor a correlation with SIV_{mac251} acquisition in animal immunized with protective vaccines (**FIG. 6D**). However, in animals immunized with non-protective vaccines, we found a significantly higher response to peptides 23 and 24 encompassing amino acids IAQNNCTGLEQEQM (SEQ ID NO: 19, designated as V1a), than those immunized with protective vaccines and this response, was associated with an increased risk of SIV_{mac251} acquisition (**FIG. 1D-1E**). V1a is directly N-terminal to V2b and both V1a and V2b are part of a continuous, exposed area at the very apex of the envelope trimer, while V1a also is superficial to, directly buries and contacts V2c (**FIG. 2A-2B**). Thus, the three biochemical sites found to influence risk of SIV_{mac251} acquisition are consistent with the crystallographic 3D structural architecture of the V1V2 domain in the viral envelope, with immunologic RV144 correlates and with $\alpha 4\beta 7$ r functional sites. This suggests that V1a interferes with host recognition of vulnerable V2b and V2c sites.

To assess this conclusion monoclonal antibodies recognizing V1a and V2b and V2c were cloned from the B-cells of an animal, P770, that was vaccinated with ALVAC-SIV/gp120/alum (Vaccari et al., Nat Med, 22, 762-770, 2016), resisted 10 SIV_{mac251} challenges, was subsequently immunized and challenged again with 10 additional SIV_{mac251} challenges years later and remained uninfected (**FIGs. 7A and 7B**). Memory B cells were identified and sorted from animal P770 that stained with either the 1J08 SIV_{smE543} V1/V2 scaffold alone or in combination with the 1J08 SIV_{mac251} V1/V2 scaffold and accounted for 0.78% of the memory cells and 0.13% of the total B cells in blood (**FIG. 7C and 7D**). two α -V2 mAbs, NCI05 and NCI09, were isolated that recognized both SIV_{mac251} and SIV_{smE543} gp120 and their respective 1J08 V1/V2 scaffolds (**FIG. 8A**). NCI09 recognized the V2 linear peptide TGLKRDKTKEY (SEQ ID NO: 53), corresponding to the center of V2b, the SIV_{mac251} and SIV_{smE543} cyclic V2 peptides, native gp120 on the surface of SIV_{mac251} infected cells (**FIG. 8B**), and its target epitope was confirmed by peptides competition assay (**FIG. 8C-8D**) as well as by crystallography (**FIG. 1**). NCI05 also bound to native gp120 on the surface of SIV_{mac251} infected cells (**FIG. 9A**) and its binding to cyclic V2 was competed by peptides 43 and 44 corresponding loosely to V2c (**FIG. 9E**). Additionally, two α -V1 antibodies, NCI04 and NCI06, were characterized from animal P770, both of which recognize the V1 RCNKSETDRWGLTK (SEQ ID NO: 20) region that is N terminal to V1a (**FIG. 8A**). None of the NCI mAbs demonstrated potent neutralization properties as tested against a panel of Tier 1 and Tier 2 SIV_{mac251} and SIV_{smE660} (**FIG. 11A**). NCI09 inhibited mildly deglycosylated SIV gp120 binding $\alpha 4\beta 7$ in a dose-dependent manner in a cell adhesion assay (**FIG. 11B**), but NCI05 did not (data not shown), consistent with the exposure and burial of the two targets, respectively. This inhibitory activity was also observed

in the sera of animal P770 as well in other immunized animals' sera and tittered to a 60% activity at a 1:3,200 serum dilution (**FIG. 11D**).

Next, *in vitro* binding competition assays were performed using soluble SIV gp120 and mAb ITS41, which recognizes V1a and was isolated from a vaccinated macaques that was not protected from SIV infection (**FIG. 8A**). ITS41 inhibited the binding to soluble gp120 of both the mAb NCI09 and NCI05 when pre-bound to soluble gp120 (**FIGs. 1F and 1G**). Conversely, binding of mAb NCI09 or NCI05 to gp120 was not affected by the later addition of ITS-41 demonstrating asymmetric competition (**FIGs. 12A and 12B**), which is consistent with proximal interference of V1a with the V2 sites. The α -V1 mAbs NCI04 and NCI06 did not interfere with NCI05 or NCI09 binding to gp120 (**FIGs. 12C and 12D**), likely because their common target epitope is distant from V1a. The NCI09 binding to the surface of SIV_{mac251} infected cells was also inhibited in a dose dependent manner by pre-treatment of cells with ITS41 (**FIG. 1H**). NCI09 at a 1.25 μ g/ml concentration inhibits by 80% gp120 binding to α 4 β 7 (**FIG. 1I**) and by titrating in increasing amount of ITS41 (**FIG. 1J**), it was found that ITS41 reversed the inhibitory activity of NCI09 (**FIG. 1K**). Taken together these data suggest that ITS41 interferes with V2b and V2c binding by NCI09 and NCI05 either via steric hindrance or allosteric competition, which is consistent with the 3D structural locations of these sites on the SIV envelope trimer (**FIGs. 2A and 2B**).

To test this hypothesis *in vivo* and investigate more directly the role of V1a and V2 in vaccine efficacy, structure-based design was used to delete V1 from models of the SIV trimer while preserving V2 folded conformations. The V1 origin and insertion (stem) to the holo V1/V2 domain connects the A and B β -strands (McLellan et al., Nature, 480, 336-343, 2011). The gp120 Δ V1 was engineered by truncating V1 at its stem and energy minimized it using the Biased-Probability Monte Carlo (BPMC) conformational search as previously described (Abagyan et al., J Mol Biol, 235, 983-1002, 1994; Cardozo et al., Proteins, 23, 403-414, 1995), to determine that the conformational rearrangement in V2 resulted in stable, low energy α -helix at its core (**FIG. 2C**). A prior study has shown that an alternative α -helical conformation to that inferred in most Env crystallography structures may be the target of protective Abs in the RV144 trial (Aiyegbo et al., PLoS One, 12, e0170530, 2017), which suggests that the V1 loop may enforce a particular, probably β -strand conformation, perhaps as a way of masking the V2 sites of vulnerability. However, since a β -strand is observed for this segment in the HIV trimer crystallographically, we could not rule out the relevance of the β -strand conformation. Accordingly, gp120 Δ V1_{gpg} was engineered as control by inserting the Gly-Pro-Gly β -turn at the

excision point, which should minimally perturb the crystallographically-evident V1/V2 Greek key β -sheet fold (**FIG. 2D**).

The two M766 (SIV_{mac251})-based gp120 immunogens deleted in V1 (gp120 Δ V1 and gp120 Δ V1gpg) were engineered and expressed in CHO cells together with the wild type gp120 (gp120_{WT}) (**FIG. 2E**). Both purified monomeric gp120 Δ V1 and gp120 Δ V1gpg were not recognized in western blot by the anti V1 antibodies NCI06, and ITS41, as expected (**FIGs. 2F-2G**) but reacted to a polyclonal anti SIV gp120 rabbit serum (**FIG. 2H**) as well to the mAb NCI05 and NCI09 (**FIGs. 2I-2J**). Both gp120 Δ V1 and gp120 Δ V1gpg proteins bound better than the gp120_{WT} in ELISA to both NCI05 and NCI09 mAbs and to the simian CD4 molecule indicative that V1 deletion increased V2 and CD4 accessibility. matching SIV_{mac251}M766 -based gp160 DNA Δ V1 and gp160 Δ V1gpg constructs were also engineered and tested in a study in macaques with a short and simpler immunization regimen than the previously established 52% vaccine efficacy baseline DNA/ALVAC/gp120 protocol with the intent to amplifying differences in the vaccination outcomes. Three groups of 14 macaques were each vaccinated with two inoculations of SIV gp160_{WT}, or gp160 Δ V1 or gp160 Δ V1gpg together with SIVp57 Gag DNA to produce pseudo-virions at 0 and 4 weeks, followed by one boost at week 8 with ALVAC-SIV (that expressed the gp120 wild type and an additional boost at week 12 with ALVAC-SIV together with the gp120 _{WT}, or gp120 Δ V1 or gp120 Δ V1gpg in alum (**FIG. 3A**).

Vaccinated macaques together with an additional unimmunized control group of 18 macaques were exposed weekly to a total of 11 low doses of SIV_{mac251} by the intrarectal route beginning at five weeks after the last immunization (week 17). Strikingly a significant decrease in the risk of SIV_{mac251} acquisition was observed only in the group of macaques immunized with the gp160 DNA Δ V1 and the gp120 protein immunogens engineered to maintain predominantly the α -helix V2 conformation (**FIG. 3C**) but not with the WT or the Δ V1gpg envelope immunogens (**FIGs. 3B-3D**). Analyses of serum antibody titers to the SIV₇₆₆gp120 envelope protein demonstrated no significant differences in the three immunized groups (**FIG. 13A**) and, as expected, the sera of animals vaccinated with the Δ V1 and Δ V1gpg envelope immunogens did not recognize overlapping peptides in the V1 region (**FIG. 13B**). Importantly the antibody titers to the SIV_{mac251} cyclic V2 peptide were higher in both groups immunized with the Δ V1 or Δ V1gpg than the wild type envelope immunogens (**FIG. 3E**). Prior results indicated that an HIV peptide correlating with protection in the RV144 study adopted an α -helical conformation. Therefore, diagnostic peptides were designed that included the second binding site of V2 to α 4 β 7, encompassing the amino acid sequence of SIV_{mac251}, DKTKKEYNETWYSTD (SEQ ID NO: 22, designated as DP2 α 4 β 7₂₅₁) and of SIV_{SME543} DKKIEYNETWYSRD (SEQ ID

NO: 23, designated as DP2 α 4 β 7_{E543}) (**FIG. 3F**). As expected, both DP2 α 4 β 7₂₅₁ and DP2 α 4 β 7_{E543} bound NCI05 (**FIG. 3G**) but not NCI09 (**FIG. 3H**). The sera of macaques immunized with the Δ V1 or Δ V1gpg immunogens had significantly higher reactivity to DP2 α 4 β 7₂₅₁ than those immunized with WT (**FIG. 3I**). In contrast, reactivity to the DP2 α 4 β 7_{E543} was significantly higher only in the Δ V1 group (**FIG. 3J**) and interestingly this response inversely correlated with the risk of SIV_{mac251} acquisition, despite its difference in amino acid sequence from the challenge virus (**FIG. 3K**). Analysis of the ability of the sera from the immunized macaques to inhibit the binding of cyclic V2 to α 4 β 7 revealed that it was highest in the group immunized with the Δ V1gpg envelope immunogens and this activity did not correlate with SIV_{mac251} acquisition (**FIG. 3L**). Animals immunized with the Δ V1 also exhibited the highest-level neutralizing antibodies against the Tier 2 SIV_{mac251CS.41} (**FIG. 3M**), surprisingly, however, this response was associated with an increased risk of virus acquisition (**FIG. 3N**).

Discussion

Viruses, including the Western Equine Encephalitis, Polio, Hepatitis C, Influenza viruses, and SARS-Coronavirus (Sautto et al., *Antiviral Res.*, 96, 82-89, 2012; To et al., *Clin Vaccine Immunol.*, 19:1012-1018, 2012; Zhong et al., *Biochem Biophys Res Commun*, 390:1056-1060, 2009; Tripp et al., *J Virol Methods*, 128:21-28, 2005; Dulbecco et al., *Virology*, 2, 162-205, 1956; Nicasio et al., *Viruses*, 4, 1731-1752, 2012) use several strategies to escape the host B-cell immune responses to viral surface proteins. These include antibody interference mediated by non-protective antibodies inhibiting immunoglobulin binding to distant, protective epitopes. The molecular mechanisms underlying antibody interference include steric hindrance that directly inhibits antibody access to the epitope or allosteric inhibition when antibody binding induce conformational changes that alter distant epitopes recognition (Klionsky et al., *Autophagy*, 12, 1-222, 2016; Sautto et al., *Antiviral Res*, 96, 82-89, 2012). Interference of antibodies to the HIV gp41 has been observed (Verrier et al. *J Virol*, 75, 9177-9186, 2001), but little is known about interfering antibodies that target apical gp120 domains and in particular the V1/V2 gp120 domains that constituted the correlate of risk of HIV acquisition in RV144. By using protein engineering and vaccine efficacy, measured as the risk of SIV acquisition as a read out, data presented in this example shows that SIV uses a similar mechanism for V1 to interfere with antibodies to V2. These results show that V1 has evolved in SIV, and likely HIV, to protect at least two viral vulnerability sites, the V2c by steric hindrance and the V2b by

allosteric inhibition, possibly by creating a V1/V2 domain in a Greek-key β -sheet fold that presents non-protective epitopes to the host immune system.

Materials and Methods:

Animals studies: All animals used in the study were colony-bred rhesus macaques (*Macaca mulatta*), obtained from either Covance Research Products (Alice, TX) or Morgan Island. The animals were housed and handled in accordance with the standards of the Association for the Assessment and Accreditation of Laboratory Animal Care International.

The first cohort of animals consisted of a total of 78 vaccinated animals and 53 controls and the vaccine immunogens are previously reported (Vaccari et al., Nat Med, 22, 762-770, 2016; Vaccari et al., Nat Med, 24, 847-856, 2018). As a source for the molecular cloning of monoclonal antibodies we used the PBMCs of animal P770, a colony-bred Rhesus macaque (*Macaca mulatta*) included in the study described in (Klionsky et al., Autophagy, 12, 1-222, 2016). Briefly, P770 was immunized at weeks 0, 4, 12, and 24 with intramuscular inoculations of 10^8 plaque-forming units (PFU) of ALVAC (vCP2432) expressing SIV genes gag-pro and gp120TM (Sanofi Pasteur). The sequence of the SIV genes was that of M766r, a mucosally transmitted founder variant of SIV_{mac251}. At weeks 12 and 24, the animal was administered in the thigh opposite as that of vector immunization a protein boost of 200 μ g each of monomeric SIV_{mac251}-M766 gp120-gD and SIV_{smE660} gp120-gD CG7V both formulated in alum. Four weeks after the final immunization, the animal underwent a challenge phase of 10 low-dose intrarectal 120 TCID₅₀ SIV_{mac251} administrations and resulted uninfected. At week 53, P770 underwent a second round of 9 immunizations (referred to in the text as *hyperimmunizations*) administered every five weeks up to week 93. At week 131, the animal was challenged weekly for ten weeks using 120 TCID₅₀ of the same SIV_{mac251} challenge stock used at week 28 and resulted (**FIG. 7A**).

The second cohort of animal included 3 groups of 14 animals each that were vaccinated intramuscularly with SIVp57Gag DNA (1mg) together with either SIVgp160_{WT}, or Δ V1 or Δ V1_{gpg} at week 0,1. At week 8 and 12 all animals received an intramuscular immunization of 10^8 pfu of ALVAC-SIV (vCP 23.). At week 12 animals received also on the contralateral thigh either SIVgp120_{WT}, or Δ V1 or Δ V1_{gpg}, all formulated in alum. At five weeks after the last immunization (week 17) all vaccinated animals, together with another group of 18 naïve animals as controls, were exposed to one weekly dose of SIV_{mac251} (1:200 dilution; TCID₅₀) for a total of 11 weeks.

Cloning of monoclonal antibodies from animal the vaccinated protected animal 770:

The protein scaffold 1J08, which was previously demonstrated to present the SIV Env V1V2

domain in the conformation naturally found on the native V1V2 protomer basing on stable expression, clash score and solvent accessibility, was used to identify V1V2-specific B cell clones and produced as described in (Vaccari et al., Nat Med, 22, 762-770, 2016). The expression vector pVRC8400 encoding the C-terminal His-tagged, avi-tagged 1J08-scaffolded SIV_{mac251}-M766r or SIV_{smE543} V1V2 sequences (GenScript) was used to transfect 293Freestyle (293F) cells with 293fectin transfection reagent (Invitrogen) following the company's instructions. 6 days post-transfection, cell culture supernatants were harvested and filtered through 0.22 µm filter and supplemented with protease inhibitor tablets (Roche). The constructs were passed through a Ni-Sepharose excel affinity media (GE Healthcare) and further purified with size exclusion chromatography (SEC) on a HiLoad 16/600 200 pg Superdex column (GE Healthcare).

The mAbs NCI05 and NCI09 were cloned from the hyperimmunized protected Rhesus macaque P770 following the methods described in (Vaccari et al., Nat Med, 22, 762-770, 2016). Briefly, frozen P770 PBMCs from week 85 (two weeks after the 7th hyperimmunization) were thawed and stained to allow the identification of CD20+, CD3-, CD4-, CD8-, CD14-, IgG+, IgM- memory B cells. After staining, the cells were washed twice with PBS and resuspended in 200 µl of PBS containing 1J08 SIV_{mac251}-M766 V1V2 conjugated to APC and 1J08 SIV_{smE543} V1V2 conjugated to PE and incubated in the dark for 15 minutes at room temperature. The cells were then washed in PBS, analyzed and sorted with a modified 3-laser FACS Aria cell sorter using the FACSDiva software (BD Biosciences). Cells that resulted positive for binding to SIV_{smE543}/V1V2 only or SIV_{smE543} and SIV_{mac251}/V1V2 were singularly sorted into well of 96-well plates containing lysis solution. Flow cytometric data was analyzed with FlowJo 9.7.5.

Total RNA was reverse transcribed in each well, and rhesus immunoglobulin heavy (H), light kappa (Lκ) and light lambda (Lλ) chains variable domain genes amplified by nested PCR. Positive amplification products as analyzed on 2% agarose gel (Embi-Tec) were sequenced, and those that were identified as carrying Igγ and IgLκ or IgLλ sequences were re-amplified with sequence-specific primers carrying unique restriction sites using the first-round nested PCR products as template. Resulting PCR products were run on a 1% agarose gel, purified with QIAGEN Gel Extraction Kit (QIAGEN) and eluted with 25µl of nuclease-free water (Quality Biological). Purified PCR products were then digested and ligated into rhesus Igγ, IgLκ and IgLλ expression vectors containing a multiple cloning site upstream of the rhesus Igγ, Igκ or Igλ constant regions. Full-length IgG were expressed as by co-transfecting 293F cells with equal amounts of paired heavy and light chain plasmids then purified using Protein A Sepharose beads (GE Healthcare) according to the manufacturer's instructions.

Monoclonal antibody binding and competition assays: The ITS41 mAbs were previously isolated from a SIV_{smE660}-infected rhesus macaque (Mason *et al.*, *PLoS Pathog* 12, e1005537 (2016). ITS41.01 bind to a V1 epitope 1 as previously reported (Gottardo *et al.*, *PLoS One* 8, e75665, 2013). The monoclonal NCI04, NCI06, NCI05, and NCI09 antibodies were generated as described in the present example. Binding of SIV-specific mAbs to viral proteins or synthetic peptides was measured by enzyme-linked immunosorbent assay (ELISA). Plates were coated overnight at 4°C with 50µl, 100 ng/well of antigen in PBS, then blocked with 300µl/well of 1% PBS-BSA for 1 hour at 37°C. When cyclic V2 (cV2) was tested, plates were coated at 4°C overnight with 200 ng/well of streptavidin (Sigma-Aldrich) in bicarbonate buffer, pH 9.6, then incubated with biotinylated cV2 peptide (produced by JPT Peptide Technologies and kindly provided by Dr. Rao, Military HIV Research Program) for 1 h at 37°C and blocked with 0.5% milk in 1× PBS, 0.1% Tween 20, pH 7.4 overnight at 4°C. Coated, blocked plates were incubated with 40µl/well of serial dilutions of mAbs in 1% PBS-BSA for 1 hour at 37°C. 40µl/well of a polyclonal preparation of Horseradish peroxidase-conjugated goat anti-monkey IgG antibody (Abcam) at 1:30,000 incubated for 1 hour at 37°C. Plates were washed between each step with 0.05% Tween 20 in PBS. Plates were developed using either 3,3',5,5'-tetramethylbenzidine (TMB) (Thermo Scientific) and read at 450 nm. When testing binding to linear peptides, cyclic V2 or 1J08 V1V2 scaffolds, a ratio of the molecular weights of these constructs to the native glycoprotein monomer was calculated to obtain coating with the same number of epitopes/well. Competition assays of anti-V2 mAbs were performed by enzyme-linked immunosorbent assay (ELISA) as described in Mason 2016 (PLoS Pathog, 12, e1005537, 2016) and Sautto 2012 Sautto et al., *Antiviral Res*, 96, 82-89, 2012). Briefly, plates were coated with 100ng/well of purified proteins SIVmac251-M766/gp120 (Advanced BioScience Laboratories, Inc.), SIV_{smE660} 1J08 V1V2 scaffold (Mason et al., *PLoS Pathog*, 12, e1005537, 2016, Fazi et al., *J Biol Chem*, 277, 5290-5298, 2002) and blocked with 1% PBS/BSA. Serial dilutions of unbiotinylated competitor mAb in 1% PBS-BSA were then added to the wells for 15 mins prior to addition of biotinylated probe mAbs at a concentration to yield ~50% saturating OD450. After incubation with streptavidin-HRP (KPL) for 1 hr at 37°C, signal was developed through incubation with 3,3',5,5' tetramethylbenzidine (TMB) substrate (Thermo Fisher Scientific) and Optical density (OD) read at 450 nm. Two negative (1%PBS/BSA or serial dilutions of anti-CD4bs ITS01) and one positive (serial dilutions of unbiotinylated probe mAb) control of competition were included in each assay.

Neutralization activity of monoclonal antibodies. SIV pseudoviruses were produced as previously described (Tassaneetrithep et al., *PLoS One*, 9, e108446, 2014). Briefly, a luciferase reporter plasmid containing essential HIV genes was used in combination with a plasmid

encoding for SIV gp160 to yield pseudoviruses exposing SIV Env on their surface. Plasmids encoding SIV gp160, clones SIV_{smE660.CP3C}, SIV_{smE660.CR54}, SIV_{mac251.H9} and SIV_{mac251.30} were kindly provided by David Montefiori. Single-round infection of TZM-bl was detected quantitatively in relative light units (RLU). Virus neutralization was measured as the 50% inhibitory concentration of mAb necessary to cause a 50% reduction in RLU as compared to virus control wells after subtraction of background RLU.

Adhesion assay and peptide arrays: A static adhesion assay was used to characterize the interaction between gp120 and $\alpha_4\beta_7$ based on a previously described method developed by Peachman and colleagues in which RPMI8866 cells, which express $\alpha_4\beta_7$ on the cell surface, were allowed to adhere to the recombinant Env proteins, V1/V2 scaffolds, or synthetic V2 cyclic peptides (FIG. 4A). The $\alpha_4\beta_7$ -expressing RPMI8866 cell line was derived from a human B cell lymphoma, and expresses $\alpha_4\beta_7$, but no detectable CD4 or CCR5. Cells were grown in media containing retinoic acid, which increased levels of both expression and clustering of $\alpha_4\beta_7$ (FIG. 5B). In some assays, anti-integrin and anti-gp120 mAbs or sera were included as adhesion inhibitors. This cell-based assay measured adhesion between two multivalent surfaces. For the serum peptide arrays: sera were collected at 1 week after the last immunization for linear mapping, diluted to 1:20, and added to plates coated with peptides encompassing the entire SIV_{K6W} gp120 amino acid sequence as previously described (Pegu et al., J Virol, 87, 1708-1719, 2013).

Antibody binding measured by surface plasmon resonance. To characterize the interaction between gp120 and $\alpha_4\beta_7$ a novel surface-plasmon resonance (SPR) based assay was developed that utilized dextran surfaces coated with recombinant envelope (Env) proteins, V1/V2 scaffolds, or synthetic V2 cyclic peptides. The analyte that we reacted with these surfaces was a recombinant soluble $\alpha_4\beta_7$ heterodimer in which the carboxy-terminal transmembrane and cytoplasmic tail domains of both chains were removed and replaced by short peptides that function as an “ α_4 chain acid- β_7 chain base coiled-coil clasp” (Nishiuchi et al., Matrix Biol, 25, 189-197, 2006). This acid-base clasp was joined by a disulfide bond that served to stabilize the heterodimer. In one iteration of this assay, short linear peptides derived from V2 we employed as competitive inhibitors.

Structural Analysis. The variable region of the NCI09 heavy chain was synthesized and cloned into a pVRC8400 vector containing an HRV3C cleavage site in the hinge region as previously described (McLellan Nature 2011). Heavy and light chain plasmids were co-expressed in 1 liter of Expi293F cells. IgG was purified from the supernatant through binding to a protein A Plus Agarose (Pierce) column and eluting with IgG Binding Buffer (Thermo Fisher). Antibodies were buffer-exchanged to PBS and then 10 mg of IgG was cleaved with HRV3C

protease. The digested IgG was then passed over a 2 ml protein A Plus column to remove the Fc fragment. The Fab was further purified over a Superdex 200 gel filtration column in buffer containing 5 mM HEPES 7.5, 50 mM NaCl, and 0.02% NaN₃. To form NCI09-V2 peptide complexes, 5 mg of purified fab at a concentration of 2 mg/ml was incubated at room

5 temperature for 30 minutes with a five-fold molar excess of SIV V2 peptide, synthesized by GenScript, and the complex was then concentrated down to 10 mg/ml using 10,000 MWCO Ultra centrifugal filter units (EMD Millipore). Antibody-peptide complexes were then screened against 576 crystallization conditions using a Mosquito crystallization robot mixing 0.1 µl of protein complex with 0.1 µl of the crystallization screening reservoir. Larger crystals were then

10 grown by the vapor diffusion method in a sitting drop at 20°C by mixing 1 µl of protein complex with 1 µl of reservoir solution (22% (w/v) PEG 4000, 0.1 M Na Acetate pH 4.6). Crystals were flash frozen in liquid nitrogen supplemented with 20% ethylene glycol as a cryoprotectant. Data were collected at 1.00Å using the SER-CAT beamline ID-22 of the Advanced Photon Source, Argonne National Laboratory. Diffraction data were processed with HKL2000 (HKL

15 Research). A molecular replacement solution was obtained with Phenix (phenix-online.org) contained one Fab molecule per asymmetric unit in space group P2₁2₁2₁. Model building was carried out using COOT software (mrc-lmb.cam.ac.uk/personal/pemsley/coot/), and was refined with Phenix. Final data collection and refinement statistics are shown in Table S1. The Ramachandran plot determined by Molprobit (molprobit.biochem.duke.edu) shows 98.2% of

20 all residues in favored regions and 100% of all residues in allowed regions for the complex structure.

α-helix peptide design. Peptide specific for the epitopes in the region near the α4β7 receptor site in the V2 loop of SIVmac251 and SIVmac543/E660, but distinct from the epitope targeted by NCI09 were designed by *ab initio* (computational chemistry) folding of

25 overlapping fragments of amino acid length 5 to 17 from that region from position 167 in the V2 loop to position 184. Initial folding was performed as previously described using a method verified by NMR (Abagyan et al., J mol. Biol., 235(3):983-1002, 1994; Aiyegbo et al., PLoS one.;12(1):e0170530, 2017; Totrov et al., Biopolymers, 60(2):124-33, 2001). Optimal characteristics were a) an alpha-helical lowest energy conformation and b) helical stability,

30 assessed by the energy spectrum of the folding. The optimal fragment from SIVmac543/E660 was 14 amino acids in length with sequence DKKIEYNETWYSRD (SEQ ID NO: 24) and the equivalent fragment from 251, DKTKEYNETWYSTD (SEQ ID NO: 25), which also had optimal alpha-helical structure, was also used. Peptides were synthesized commercially (Genewiz Inc) with an N-terminal biotin attached. ELISA assays were performed as previously

described (Almond Adv Virol., 2012:803535, 2012; Cardozo et al., Vaccine, 32(39):4916-24, 2014).

Statistical analysis. Fisher Exact Test was used for all pairwise comparisons. Wilcoxon rank sum and Kruskal-Wallis tests was used to compare populations of continuous data for groups of 2 and ≥ 3 , respectively. ANOVA was used to determine the significance of titers or the reduction in viral load over time in well controlled animals, if applicable. Threshold: 2-sided alpha-level of 0.05, with Bonferroni correction made for multiple comparisons.

Challenge exposure of vaccinated macaques: The numbers of macaques were chosen based on the assumption that the probability of infection in each naïve control is 30% at each challenge exposure. In this case, each comparison of the 12 animals in one vaccinated group versus the 18 in the control group will have approximately 48% power if the vaccine has 50% efficacy, 70% power if it has 60% efficacy, and 90% power if it reaches the anticipated 70% efficacy.

Evaluation of immune responses: Distributions of serum IgG log-transformed titers to gp70-V1/V2 proteins typically result in standard deviations of 0.19. If one vaccine regimen has an expected mean log titer 0.12 (0.63 SD) greater than the other two, with 12 animals in each group, and if the log titers are normally distributed, the best regimen has 84% probability of being superior. The actual empirical distributions were negatively skewed, increasing the probability of the superior regimen to 90%.

Example 2

V1-deleted HIV-1 Env proteins for human immunization

The results presented in Example 1 show that immunization with the identified gp120 V1-deletion in the context of the DNA/ALVAC/gp120/platform in an SIV model reduces SIV_{mac251} acquisition to a greater degree than the prior best performing regimen in a stringent, highly translational macaque model. This example illustrates HIV-1 Env immunogens containing the V1 deletion identified in Example 1 for use in humans.

A large number of reports suggest that the SIV and HIV envelopes are architecturally identical (e.g., Julien et al., Science, 342, 1477-1483, 2013; Chuang et al., J Virol, 91, e02268-16, 2017), therefore V1-deletion in an HIV envelope is expected to achieve the same bioactivity as observed in the SIV model.

Of the diverse HIV strains available, the A244 HIV gp120 protein was selected for three reasons:

- 1) A244 protein was used in the RV144 study;

2) mAbs CH58 and CH59 from a protected individual in the RV144 study targeting V2b/c were specific for A244 (Liao et al., *Immunity*, 38(1):176-86, 2013);

3) molecular modeling shows high similarity between the SIV gp120 used in Example 1 and A244 gp120 V2 regions (**FIG. 14A**).

Although the V1 loop is shorter in A244 than SIV_{mac251}, the disulfide bridge at positions 131 and 157 (Hxhc2 numbering) that is both the origin and insertion of V1 and therefore defines it, is conserved in nearly all HIV and SIV strains. Therefore, the V1-deleted V2 domain and the rest of gp120 in A244 and SIV_{mac251} or SIV_{SM/E660} are architecturally identical, because the V1-defining disulfide bridge normalizes any dramatic structural changes transmitted to the rest of the protein by V1 deletion between 131 and 157. Deletion of the V1 loop of A244 is believed to unmask V2b and V2c in similar manner as in the SIV_{mac251} construct, since it connects directly to V2b and lies directly over V2c. In support of this concept, the bioequivalence of this envelope region of the A244 and SIV_{mac251} was established by a preliminary probe bearing the HIV equivalents of the NCI05 and NCI09 V2 target sites. It was found that macaque sera from an animal vaccinated with a protective vaccine (that used alum as an adjuvant for the protein boost, Vaccari *et al.*, Adjuvant-dependent innate and adaptive immune signatures of risk of SIV_{mac251} acquisition. *Nat Med* **22**, 762-770, 2016) recognized an HIV equivalent V2c peptide better than the sera of animals immunized with a non-protective vaccine (that used MF59 as an adjuvant in the protein boost) (**FIG. 14B**). These data support a structural and immunological equivalence of V1-deletion and the key V2 sites in SIV and HIV-1 A244. Accordingly, the probability is high that A244 V1V2 architecture and antigenicity of the V1V2 domain is identical to SIV_{mac251} and SIV_{SM E660} despite different sequence, and all that is needed to produce an A244 HIV-1 Δ V1 immunogen with equivalent antigenicity and immunogenicity to SIV_{mac251M766} Δ V1 is fine-tuning by the same protein engineering techniques used to successfully design SIV_{mac251M766} Δ V1.

To show that the V1-deleted HIV A244 will recapitulate the antigenicity of the Δ V1-deleted SIV envelope immunogens, gp120 from the A244 strain was modified with the 137-152 deletion and expressed in 293 or CHO cells as assessed for PG9 and CH58 binding by ELISA (**FIG. 15**). The sequence of the V1-deleted A244 gp120 is provided as SEQ ID NO: 1. The

PG9 antibody specifically binds to a conformational epitope of the gp120 V1/V2 domain, whereas CH58 antibody is specific for a conformational epitope of the V2 domain. The ELISA binding data shows that the Δ V1 modification disrupted PG9 binding to both A244 and SIV gp120, but had no effect on CH58 binding to these proteins. Thus, A244 gp120 with the 137-152 deletion recapitulated the antigenicity of the Δ V1-deleted SIV envelope.

Exemplary sequences are provided as follows:

- A244 gp120 with V1 137-152 deletion: SEQ ID NO: 1
MN gp120 with V1 137-152 deletion: SEQ ID NO: 2
96ZM651 gp120 with V1 137-152 deletion: SEQ ID NO: 3
5 A244 Env with Δ V1 137-152 deletion and without signal peptide: SEQ ID NO: 4
MN Env with Δ V1 137-152 deletion and without signal peptide: SEQ ID NO: 5
96ZM651 Env with Δ V1 137-152 deletion and without signal peptide: SEQ ID NO: 66
Full-length A244 Env with the Δ V1 137-152 deletion: SEQ ID NO: 6
Full-length MN Env with Δ V1 137-152 deletion: SEQ ID NO: 7
10 Full-length 96ZM651 Env with Δ V1 137-152 deletion SEQ ID NO: 67.

- 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
15 below.

It is claimed:

1. A recombinant gp120 protein comprising a deletion of HIV-1 Env residues 137-152 according to the HXBc2 numbering system, wherein the recombinant gp120 protein elicits an immune response to HIV-1.
2. The recombinant gp120 protein of claim 1, comprising or consisting of HIV-1 Env residues 31-507, with the deletion of residues 137-152, according to the HXBc2 numbering system.
3. The recombinant gp120 protein of claim 1 or claim 2, comprising or consisting of an amino acid sequence at least 90% identical to any one of SEQ ID NOs: 1-3.
4. The recombinant gp120 protein of claim 3, comprising or consisting of the amino acid sequence set forth as any one of SEQ ID NOs: 1-3.
5. A recombinant gp140 protein, a recombinant gp145 protein, or a recombinant gp160 protein comprising the recombinant gp120 protein of any one of the prior claims, wherein the recombinant gp140 protein, the recombinant gp145 protein, or the recombinant gp160 protein elicits an immune response to HIV-1.
6. A recombinant HIV-1 Env ectodomain trimer, comprising protomers comprising the recombinant gp120 protein of any one of the prior claims and a gp41 ectodomain, wherein the HIV-1 Env ectodomain trimer elicits an immune response to HIV-1.
7. The recombinant HIV-1 Env ectodomain trimer of claim 6, wherein the recombinant gp120 protein comprises or consists of HIV-1 Env residues 31-507, with the deletion of residues 137-152, according to the HXBc2 numbering system, and the gp41 ectodomain comprises or consists of HIV-1 Env residues 512-664 according to the HXBc2 numbering system.
8. The recombinant HIV-1 Env ectodomain trimer of claim 6 or claim 7, wherein the protomers of the HIV-1 Env ectodomain trimer comprise or consist of an amino acid sequence at least 90% identical to any one of SEQ ID NOs: 4-5 and 66.

9. The recombinant HIV-1 Env ectodomain trimer of claim 8, wherein the protomers of the HIV-1 Env ectodomain trimer comprise or consist of the amino acid sequence set forth as any one of SEQ ID NOs: 4-5 and 66.
10. The recombinant HIV-1 Env ectodomain trimer of any one of claims 6-9, wherein the protomers of the recombinant HIV-1 Env ectodomain trimer comprise a C-terminal fusion to a trimerization domain by a peptide linker, or directly fused to the trimerization domain.
11. The recombinant HIV-1 Env ectodomain trimer of any one of claims 6-10, wherein the recombinant HIV-1 Env ectodomain trimer is soluble.
12. The recombinant HIV-1 Env ectodomain trimer of any one of claims 6-10, wherein the protomers of the recombinant HIV-1 Env ectodomain trimer are each fused c-terminally to a transmembrane domain.
13. A recombinant V1V2 domain of a gp120 protein comprising a deletion of HIV-1 Env residues 137-152 according to the HXBc2 numbering system, wherein the recombinant V1V2 domain elicits an immune response to HIV-1.
14. The recombinant V1V2 domain of claim 13, comprising or consisting of HIV-1 Env residues 126-196 with the deletion of residues 137-152 according to the HXBc2 numbering system.
15. The recombinant V1V2 domain of claim 13, comprising or consisting of HIV-1 Env residues 119-205 with the deletion of residues 137-152 according to the HXBc2 numbering system.
16. The recombinant V1V2 domain of claim 13 or claim 14, comprising or consisting of an amino acid sequence at least 90% identical to SEQ ID NO: 8.
17. The recombinant V1V2 domain of claim 16, comprising or consisting of the amino acid sequence set forth as SEQ ID NO: 8.

18. The recombinant V1V2 domain of any one of claims 13-17, fused to a scaffold protein.

19. The recombinant V1V2 domain of claim 18, wherein the scaffold protein is selected from any one of a gp70 protein, a typhoid toxin protein, and an antibody Fc domain

20. The recombinant gp120 protein, the recombinant gp140 protein, the recombinant gp145 protein, the recombinant gp160 protein, the recombinant HIV-1 Env ectodomain trimer, or the recombinant V1V2 domain of any one or the prior claims, further comprising one or more additional amino acid substitutions or deletions.

21. The recombinant gp120 protein, the recombinant gp140 protein, the recombinant gp145 protein, the recombinant gp160 protein, the recombinant HIV-1 Env ectodomain trimer, or the recombinant V1V2 domain of any one or the prior claims, wherein the immune response inhibits HIV-1 infection.

22. An immunogenic conjugate comprising the recombinant gp120 protein, the recombinant gp140 protein, the recombinant gp145 protein, the recombinant gp160 protein, the recombinant HIV-1 Env ectodomain trimer, or the recombinant V1V2 domain of any one or the prior claims conjugated to a heterologous carrier.

23. A virus-like particle comprising the recombinant gp120 protein, the recombinant gp140 protein, the recombinant gp145 protein, the recombinant gp160 protein, the recombinant HIV-1 Env ectodomain trimer, or the recombinant V1V2 domain of any one of claims 1-21.

24. A nucleic acid molecule encoding the recombinant gp120 protein, the recombinant gp140 protein, the recombinant gp145 protein, the recombinant gp160 protein, a protomer of the recombinant HIV-1 Env ectodomain trimer, or the recombinant V1V2 domain of any one of claims 1-21.

25. The nucleic acid molecule of claim 26, encoding a precursor of the recombinant gp120 protein, the recombinant gp140 protein, the recombinant gp145 protein, the recombinant gp160 protein, the protomer of the recombinant HIV-1 Env ectodomain trimer, or the recombinant V1V2 domain.

26. A vector comprising the nucleic acid molecule of claim 24 or claim 25.
27. The vector of claim 26, wherein the vector is a viral vector.
28. An immunogenic composition for use to elicit an immune response to HIV-1 in a subject, comprising the recombinant gp120 protein, the recombinant gp140 protein, the recombinant gp145 protein, the recombinant gp160 protein, the recombinant HIV-1 Env ectodomain trimer, the recombinant V1V2 domain, the immunogenic conjugate, the virus-like particle, the nucleic acid molecule, or the vector of any one of the prior claims, and a pharmaceutically acceptable carrier.
29. The immunogenic composition of claim 28, further comprising an adjuvant.
30. A method for eliciting an immune response to HIV-1 in a subject, comprising administering to the subject an effective amount of the recombinant gp120 protein, the recombinant gp140 protein, the recombinant gp145 protein, the recombinant gp160 protein, the recombinant HIV-1 Env ectodomain trimer, the recombinant V1V2 domain, the immunogenic conjugate, the virus-like particle, the nucleic acid molecule, the vector, or the immunogenic composition of any one of the prior claims to elicit the immune response.
31. The method of claim 30, wherein the immune response treats or inhibits HIV-1 infection in the subject.
32. A method of producing a recombinant gp120 protein, a recombinant gp140 protein, a recombinant gp145 protein, a recombinant gp160 protein, a recombinant HIV-1 Env ectodomain trimer, or a recombinant V1V2 domain, comprising:
expressing the nucleic acid molecule of any one of claims 24-25 in a host cell; and
purifying the recombinant gp120 protein, the recombinant gp140 protein, the recombinant gp145 protein, the recombinant gp160 protein, the recombinant HIV-1 Env ectodomain trimer, or the recombinant V1V2 domain.
33. Use of the recombinant gp120 protein, the recombinant gp140 protein, the recombinant gp145 protein, the recombinant gp160 protein, the recombinant HIV-1 Env ectodomain trimer, the recombinant V1V2 domain, the immunogenic conjugate, the virus-like

particle, the nucleic acid molecule, the vector, or the immunogenic composition of any of claims 1-29 to elicit an immune response to a HIV-1 Env in a subject.

34. A method for prognosis of an immune response to HIV-1 in a subject, comprising:

contacting a biological sample from a subject with one or more peptides comprising or consisting of the amino acid sequence of HIV Env residues 141-154 (V1a), HIV Env residues 157-173 (V2b), or HIV Env residues 166-180 (V2c) according to the HXBc2 numbering system; and

detecting specific binding activity of antibodies in the biological sample to the one or more peptides; and wherein:

detecting specific binding activity of antibodies in the biological sample to the V2b peptide or to the V2c peptide identifies the immune response to HIV-1 in the subject as an immune response that inhibits HIV-1 infection; and

detecting specific binding activity of antibodies in the biological sample to the V1a peptide identifies the immune response to HIV-1 in the subject as an immune response that does not inhibit HIV-1 infection.

35. The method of claim 34, wherein the peptide is no more than 30 amino acids in length.

36. The method of claim 34 or claim 35, wherein the immune response that inhibits HIV-1 infection protects against HIV-1 infection in an uninfected subject.

37. The method of any one of claims 34-36, wherein the subject has received an HIV-1 vaccine and does not have an HIV-1 infection.

38. The method of claim 34 or claim 35, wherein the immune response that inhibits HIV-1 infection inhibits HIV-1 disease in an infected subject

39. The method of any one of claims 34-38, further comprising obtaining the biological sample from the subject.

FIG. 1A

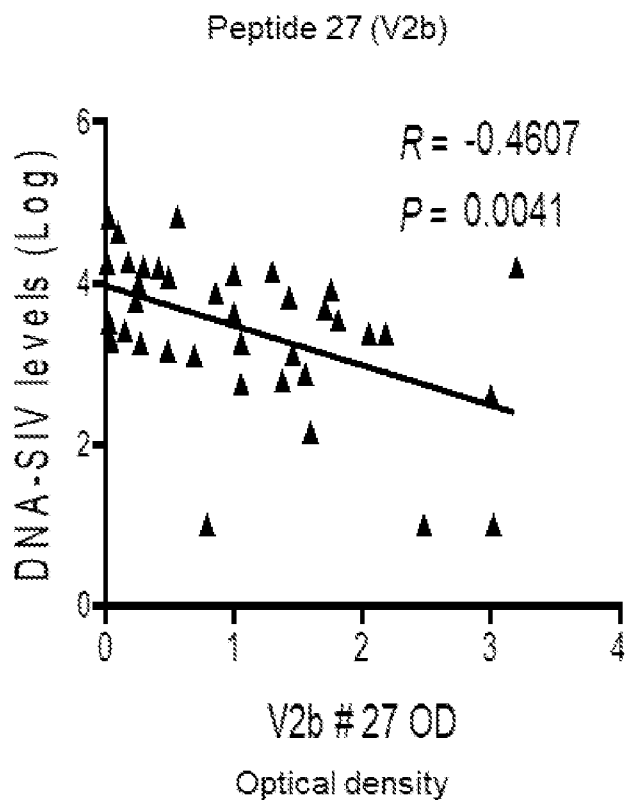


FIG. 1B

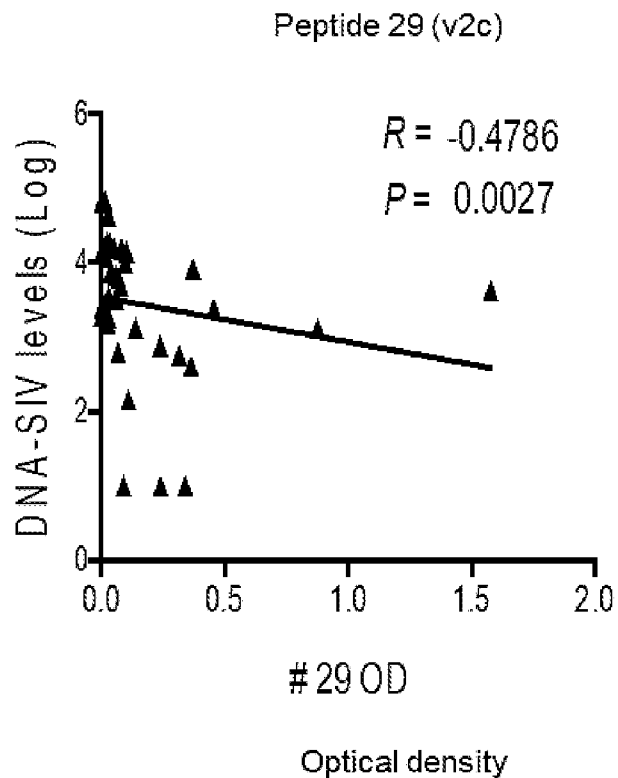


FIG. 1C

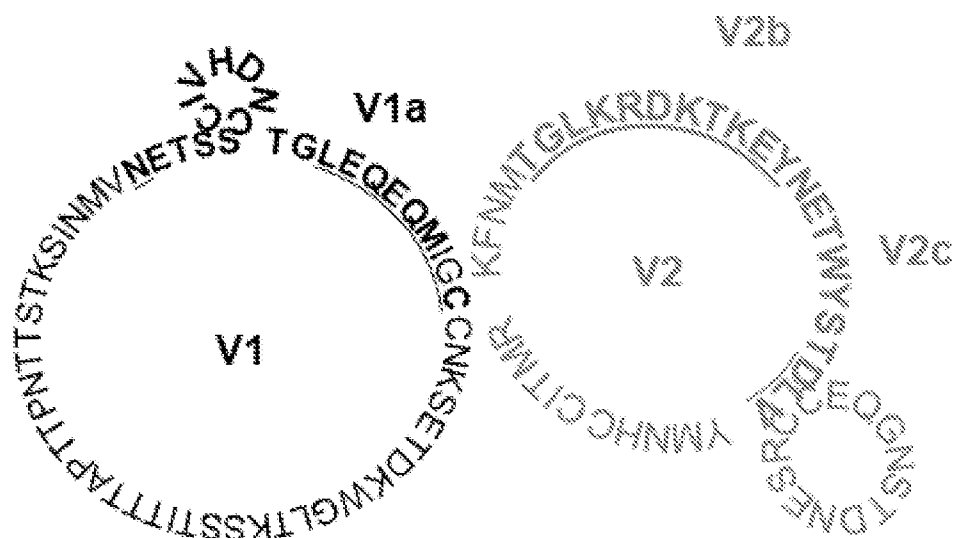


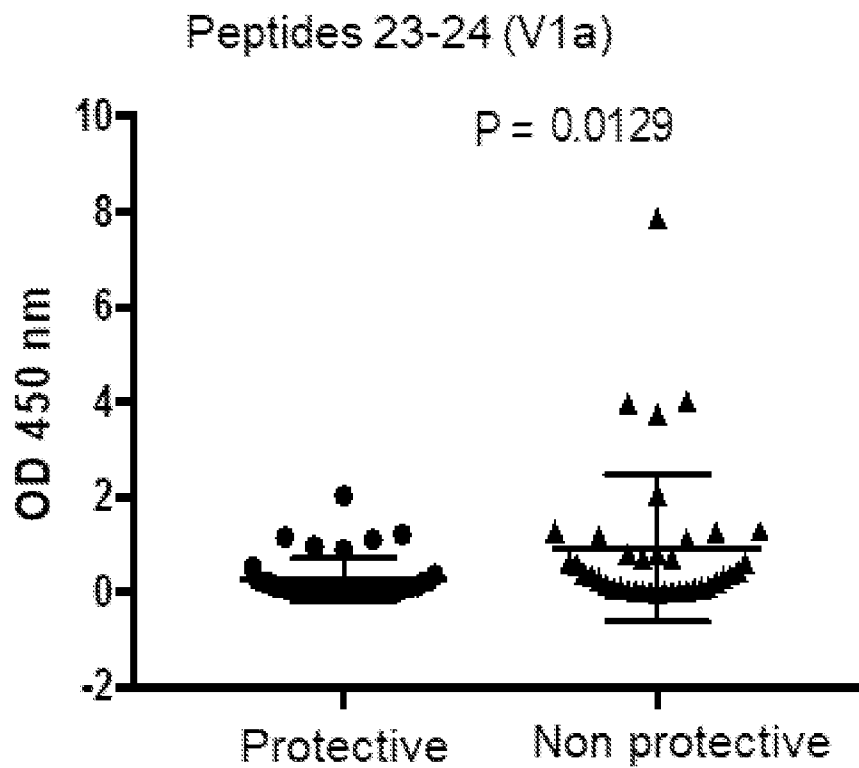
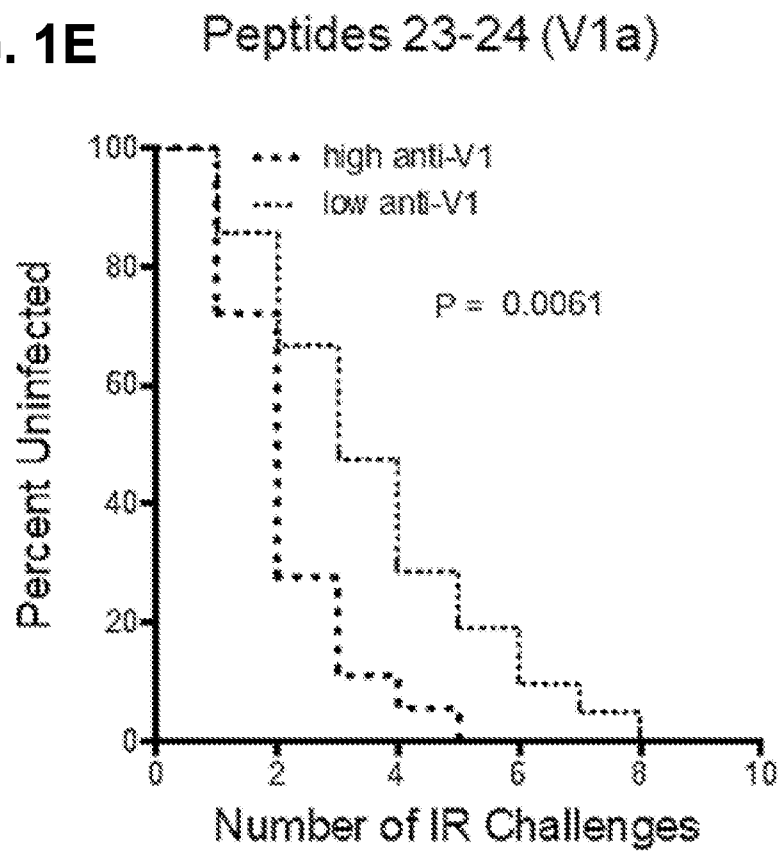
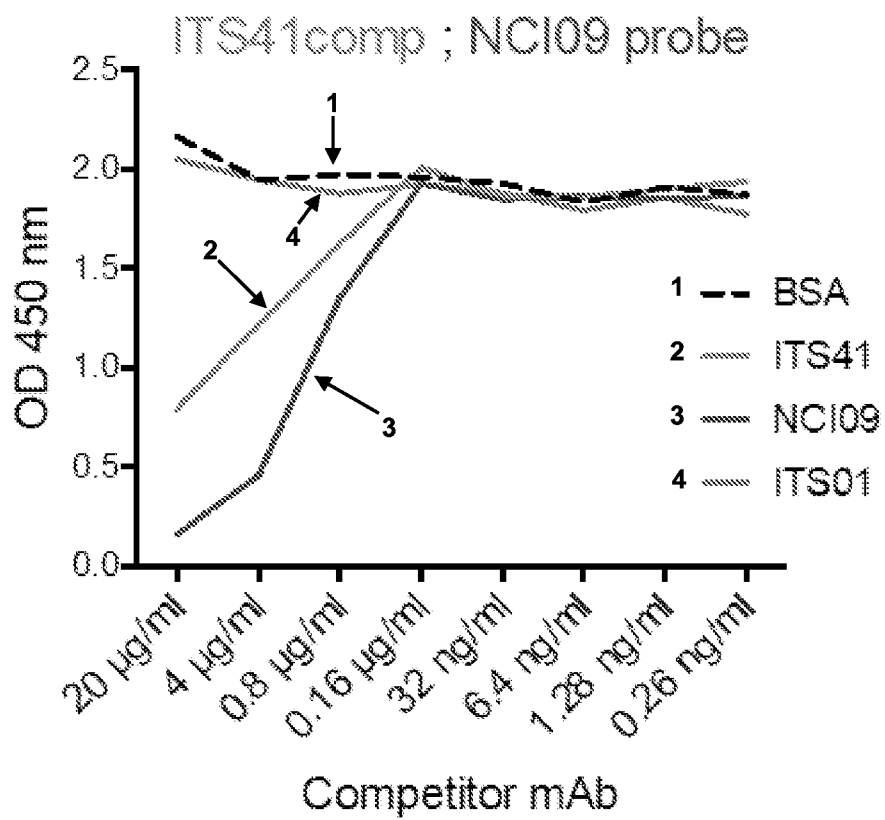
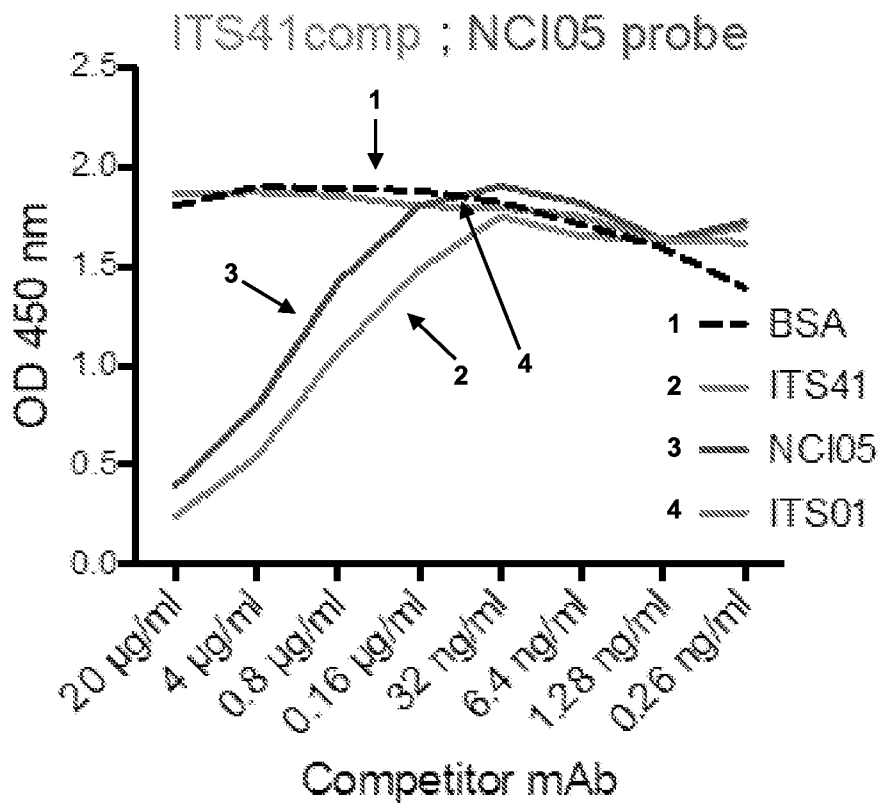
FIG. 1D**FIG. 1E**

FIG. 1F**FIG. 1G**

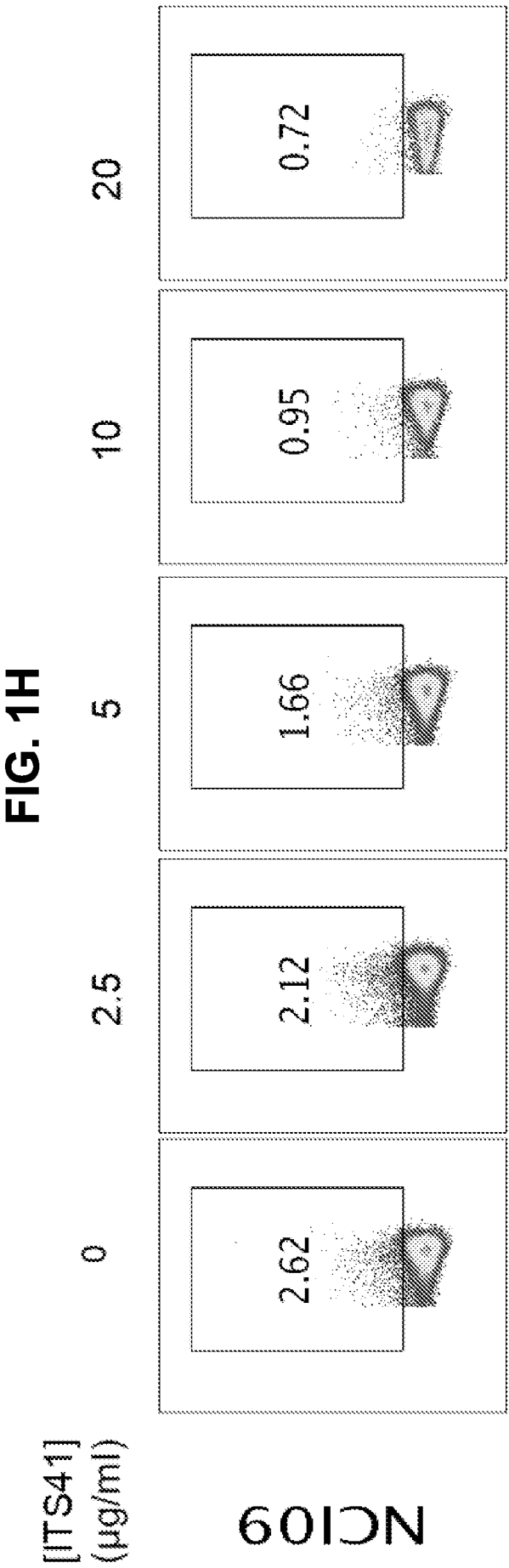


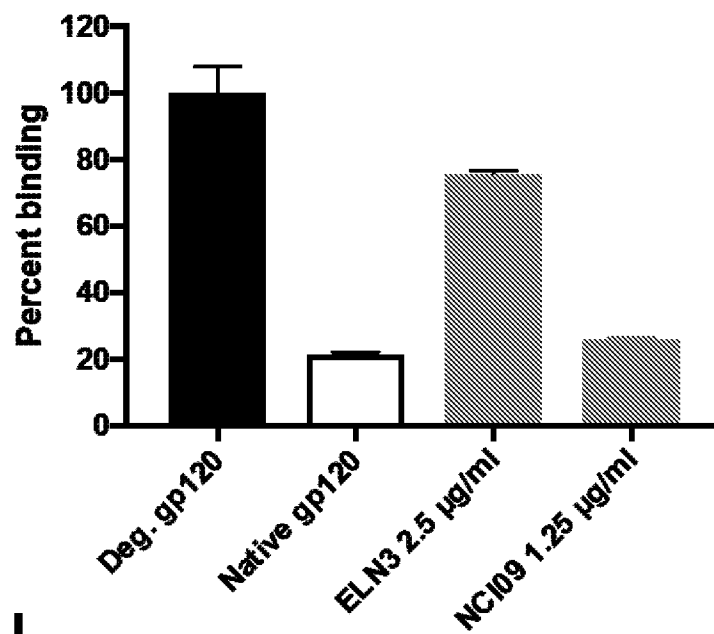
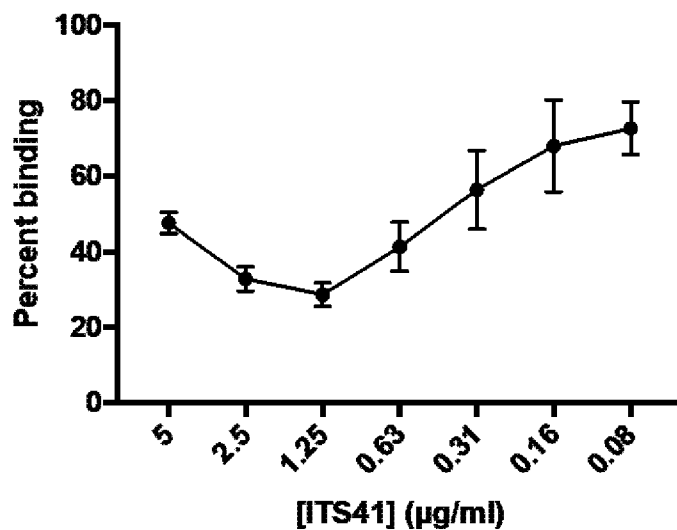
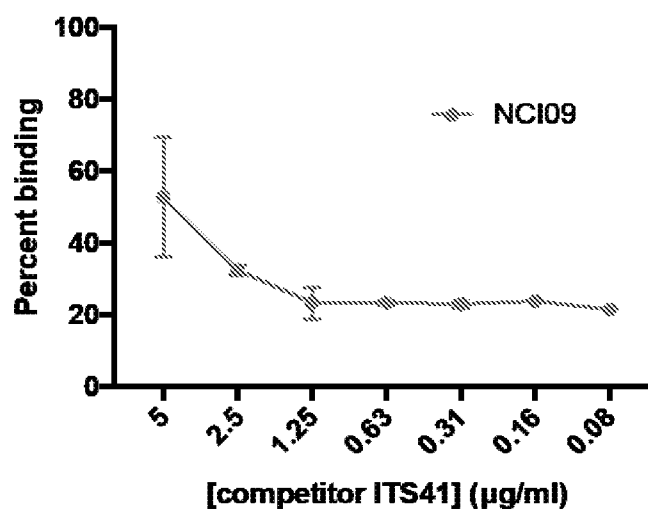
FIG. 1I**FIG. 1J****FIG. 1K**

FIG. 2A

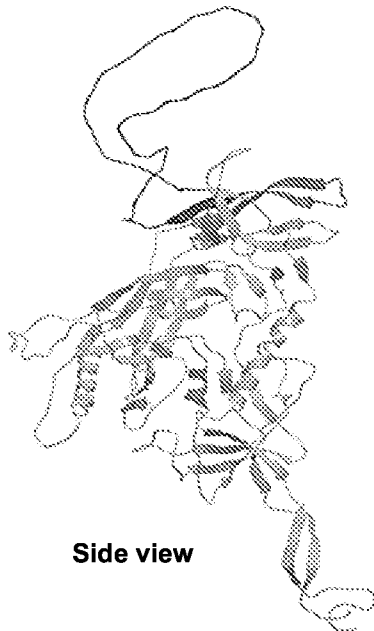


FIG. 2B

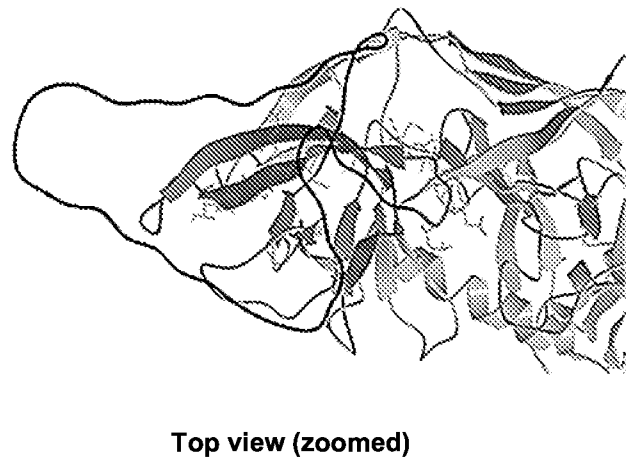


FIG. 2C

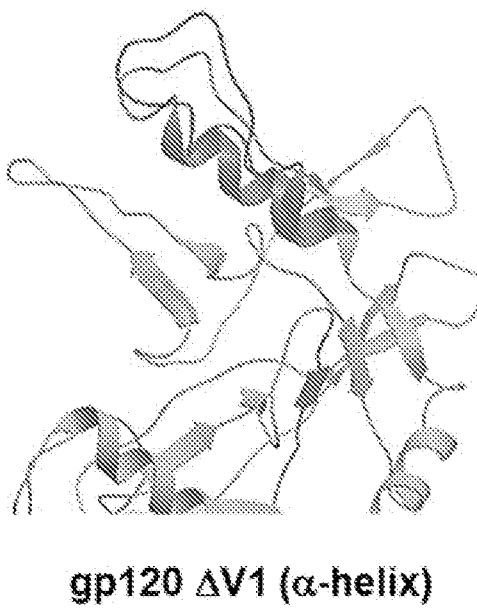
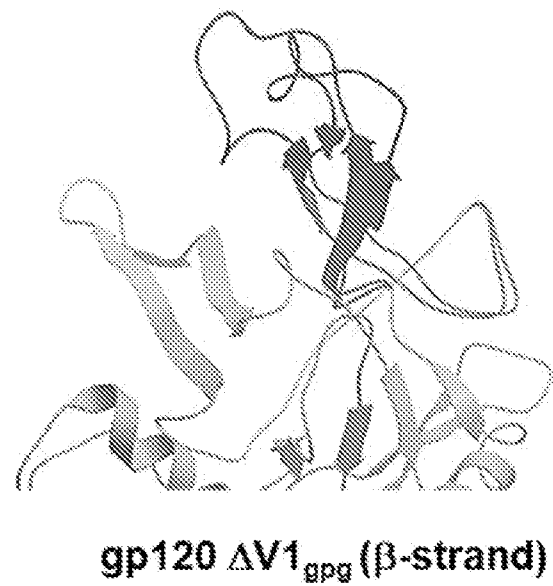


FIG. 2D



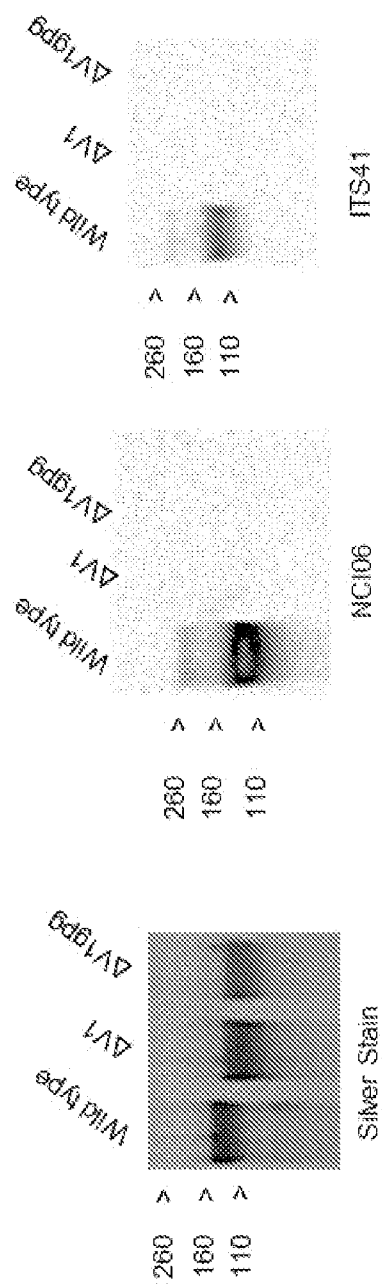


FIG. 2G

FIG. 2F

FIG. 2E

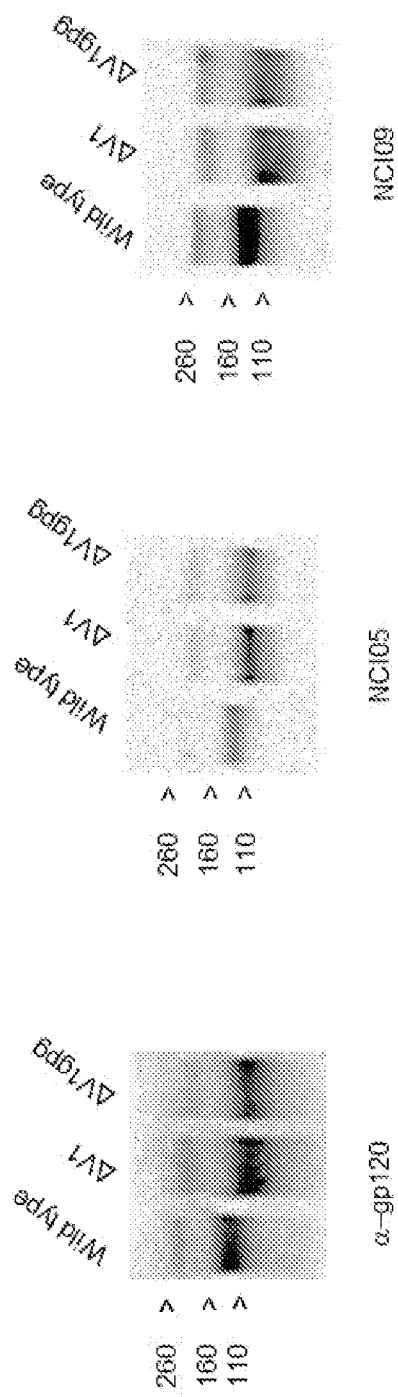


FIG. 2J

FIG. 2I

FIG. 2H

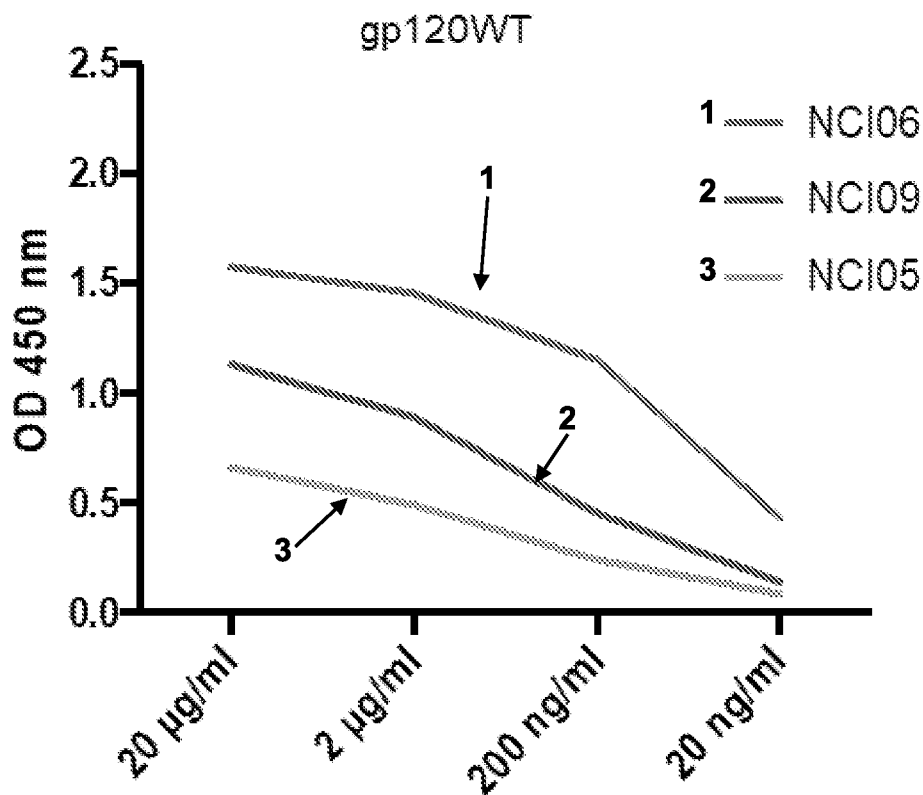
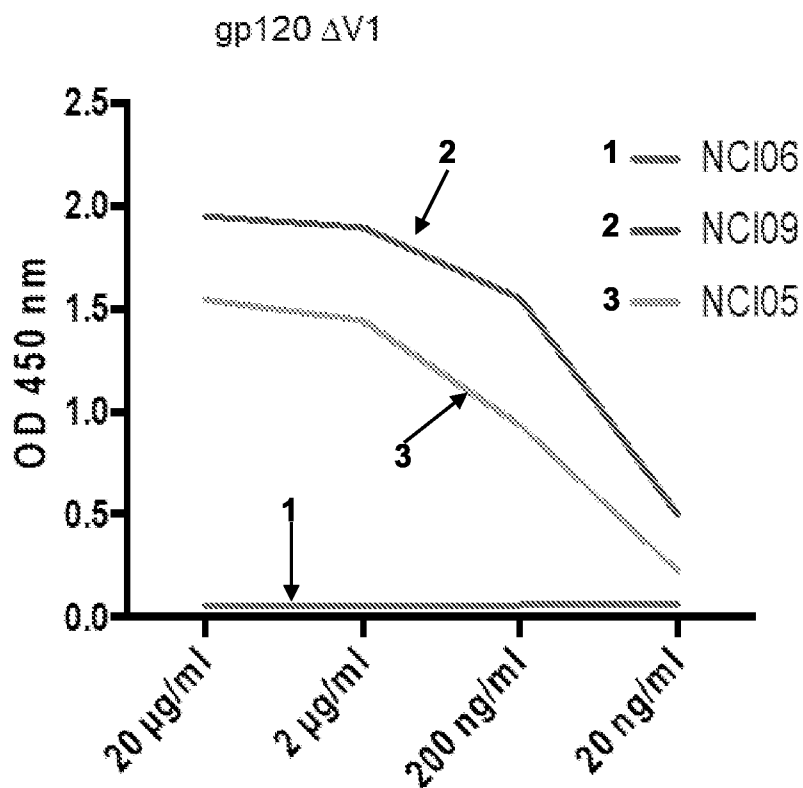
FIG. 2K**FIG. 2L**

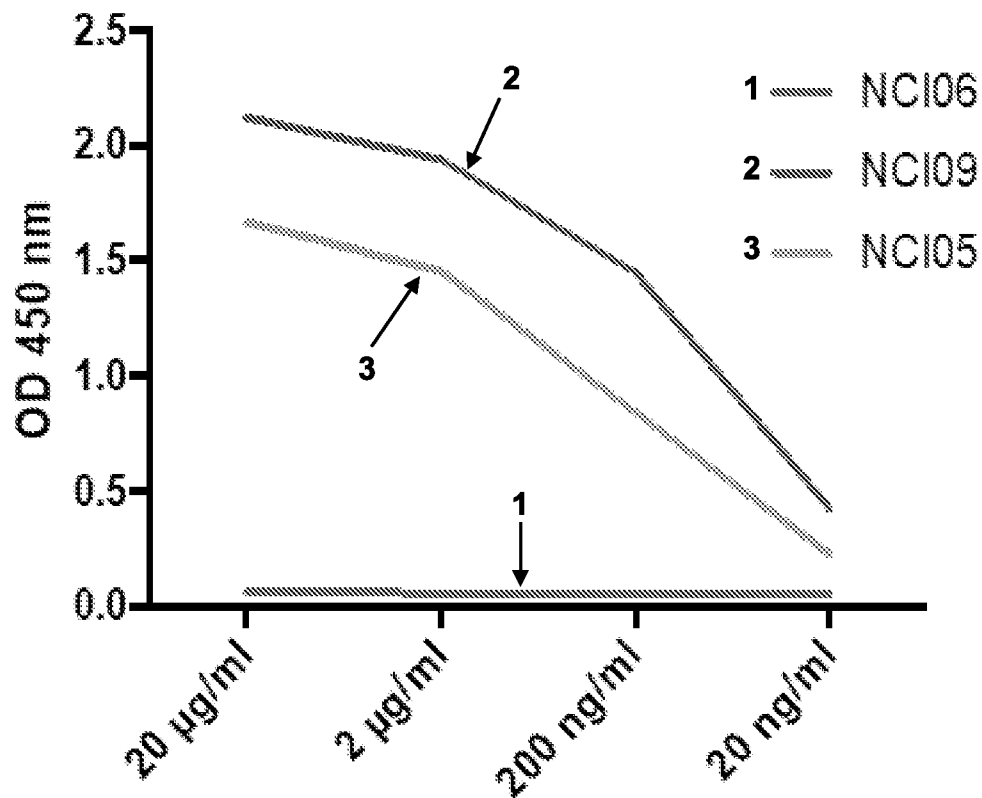
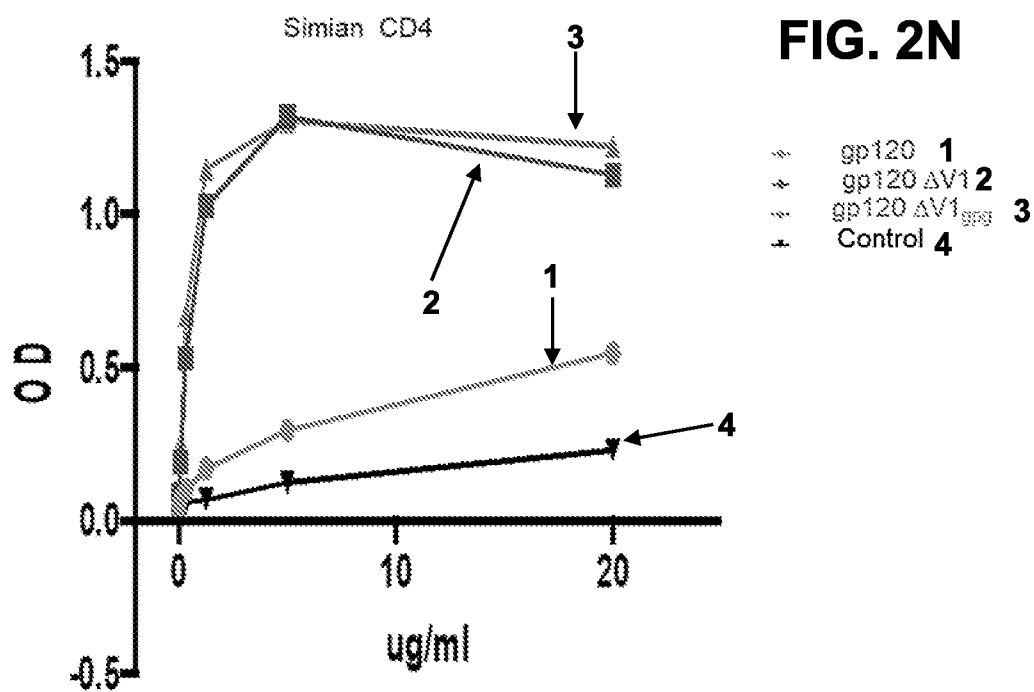
FIG. 2Mgp120 $\Delta V1_{\text{gpg}}$ **FIG. 2N**

FIG. 3A

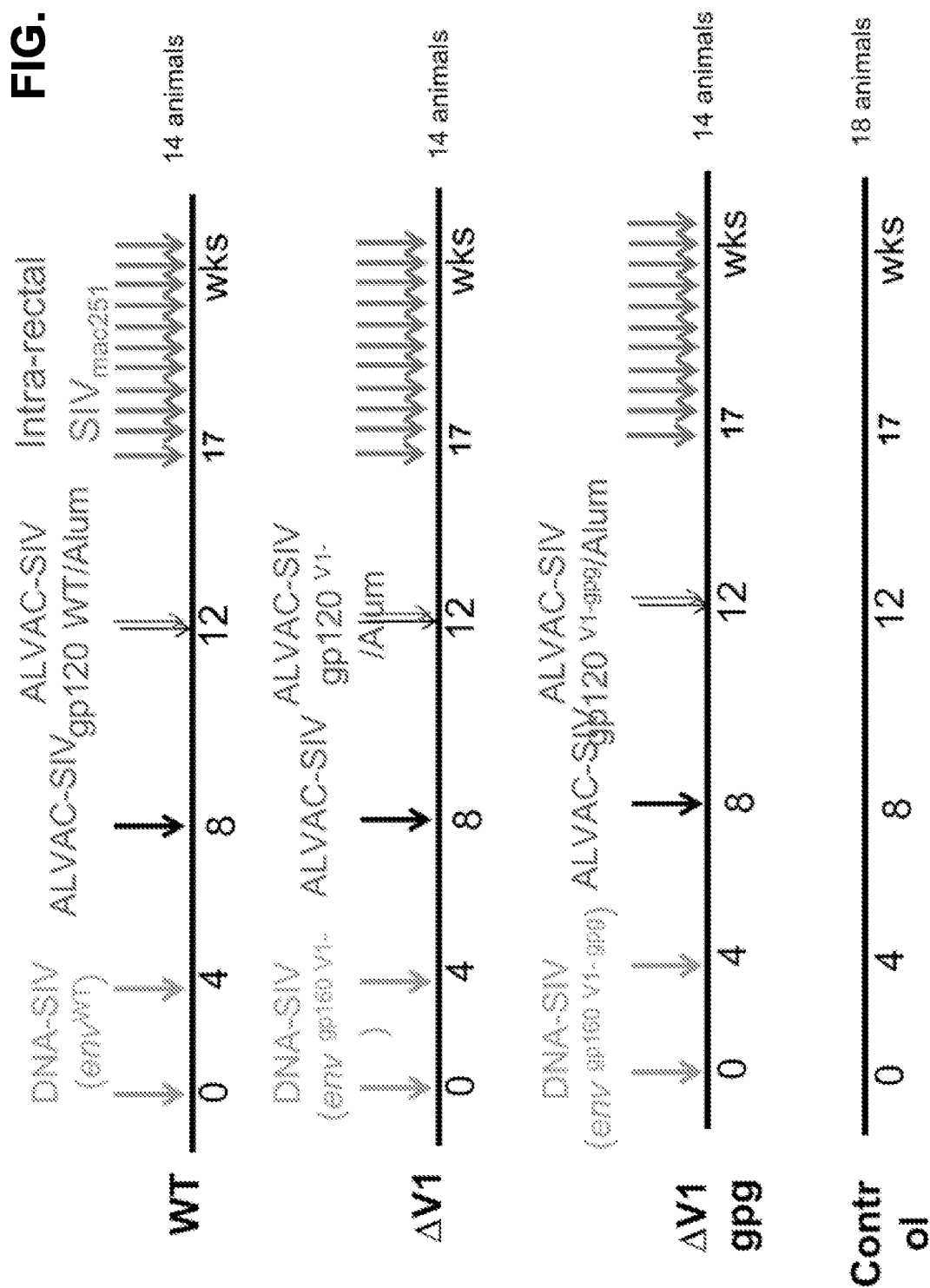
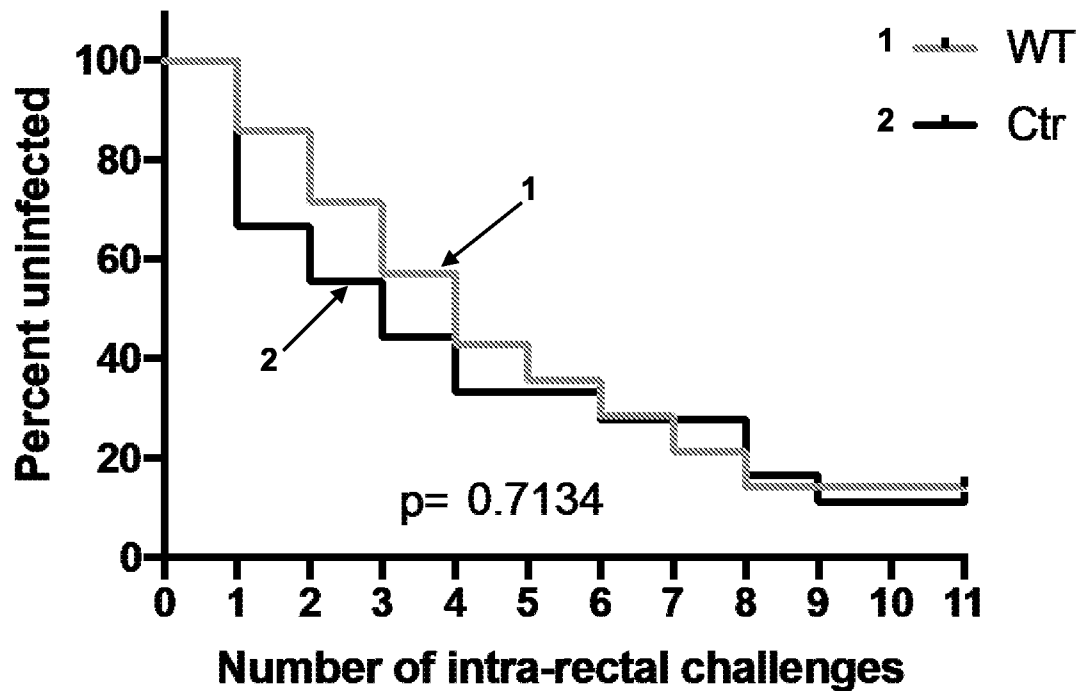
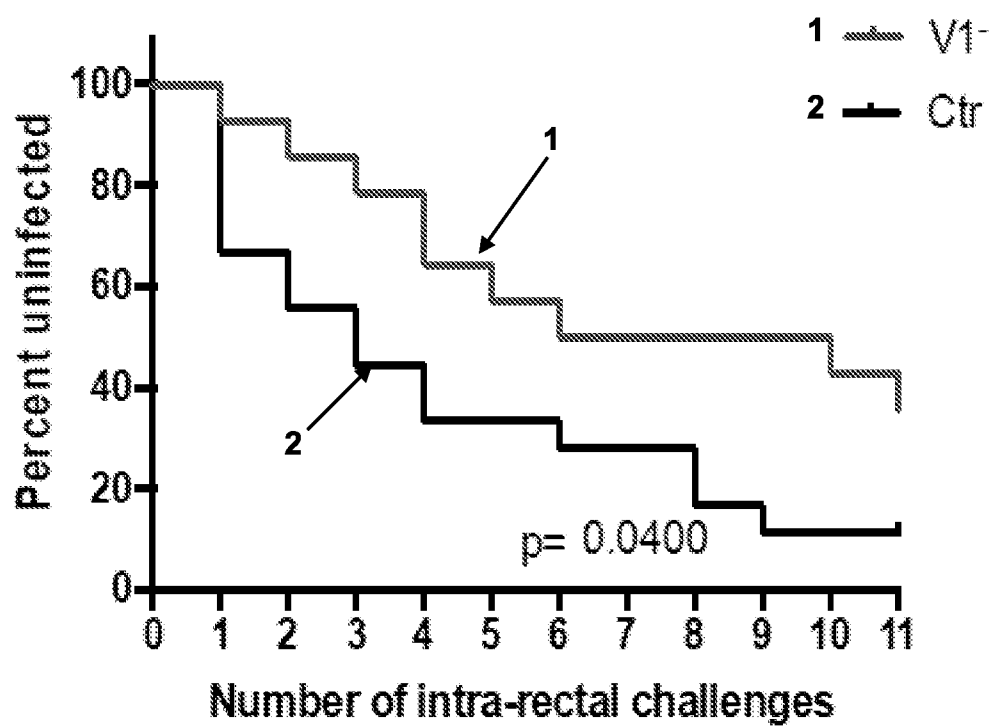


FIG. 3B**WT X Ctr****FIG. 3C****V1⁻ X Ctr**

1 - - - V1-gpg
2 — Ctr

Percent uninfected

100
80
60
40
20
0

0 1 2 3 4 5 6 7 8 9 10 11

Number of intra-rectal challenges

$p = 0.6468$

FIG.3E

Titers of IgG
SIVmac251 short cyclic V2 peptide - biot
(wk17 - basal)

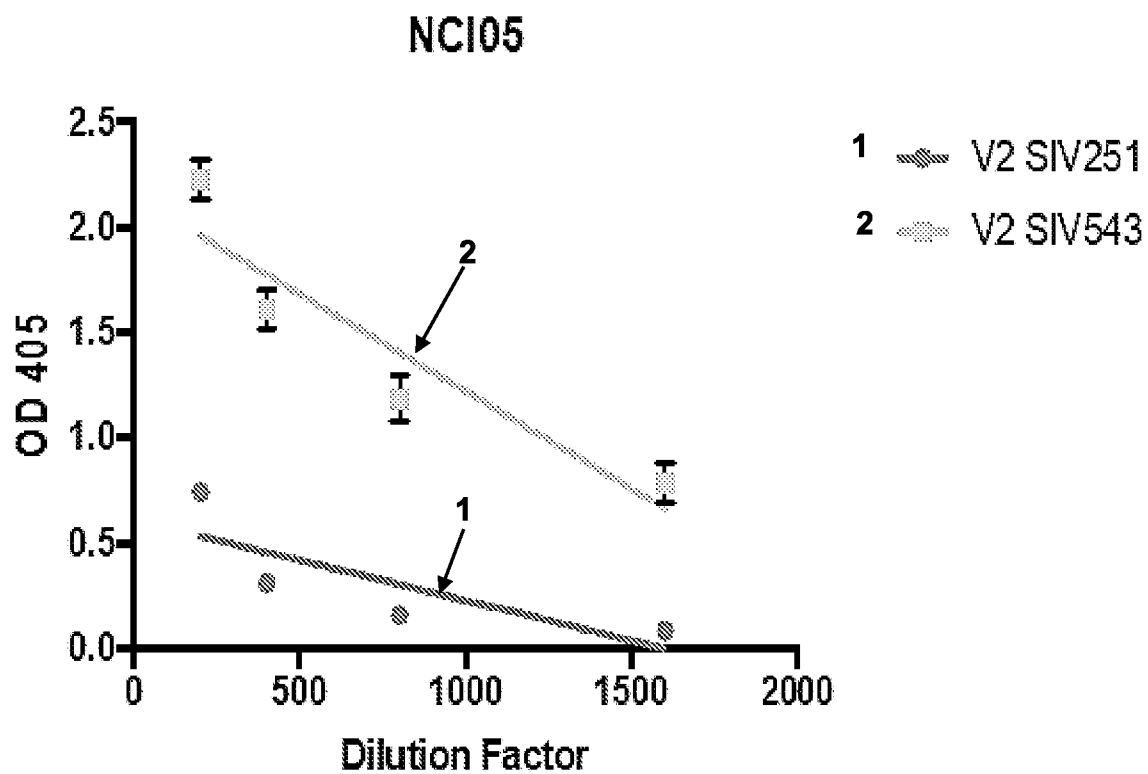
The figure is a scatter plot with error bars representing the mean and standard deviation. The y-axis is labeled 'Titers of IgG' and 'SIVmac251 short cyclic V2 peptide - biot (wk17 - basal)', indicating it's a log scale from 10² to 10⁶. The x-axis has three categories: WT (represented by circles), V1- (represented by squares), and V1- gp9 gp (represented by triangles). Statistical significance is indicated by horizontal brackets above the groups: p = 0.0021 between WT and V1-, and p = 0.0046 between V1- and V1- gp9 gp.

Group	Approximate Titers (Mean ± SD)
WT	~8 × 10 ⁴ ± 2 × 10 ⁴
V1-	~2 × 10 ⁵ ± 5 × 10 ⁴
V1- gp9 gp	~2 × 10 ⁵ ± 3 × 10 ⁵

FIG. 3F

DP2 α 4 β 7 ₂₅₁		DKTK	EYNETWYSTD	SIV _{mac251}
DP2 α 4 β 7 ₂₅₄₃		DKKI	EYNETWYSRD	SIV ₂₅₄₃
NCI09	KFTMTGLKR	DKTK	EYN	
NCI05	R	DKKK	EYNETWYSAD	

FIG. 3G



NCI09

FIG. 3H

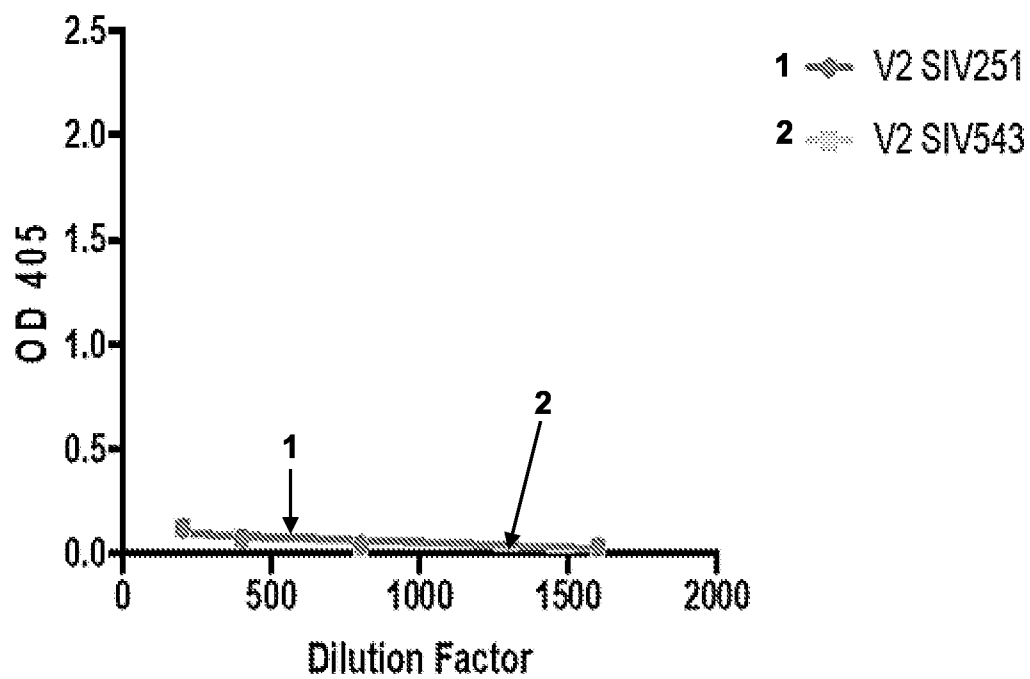
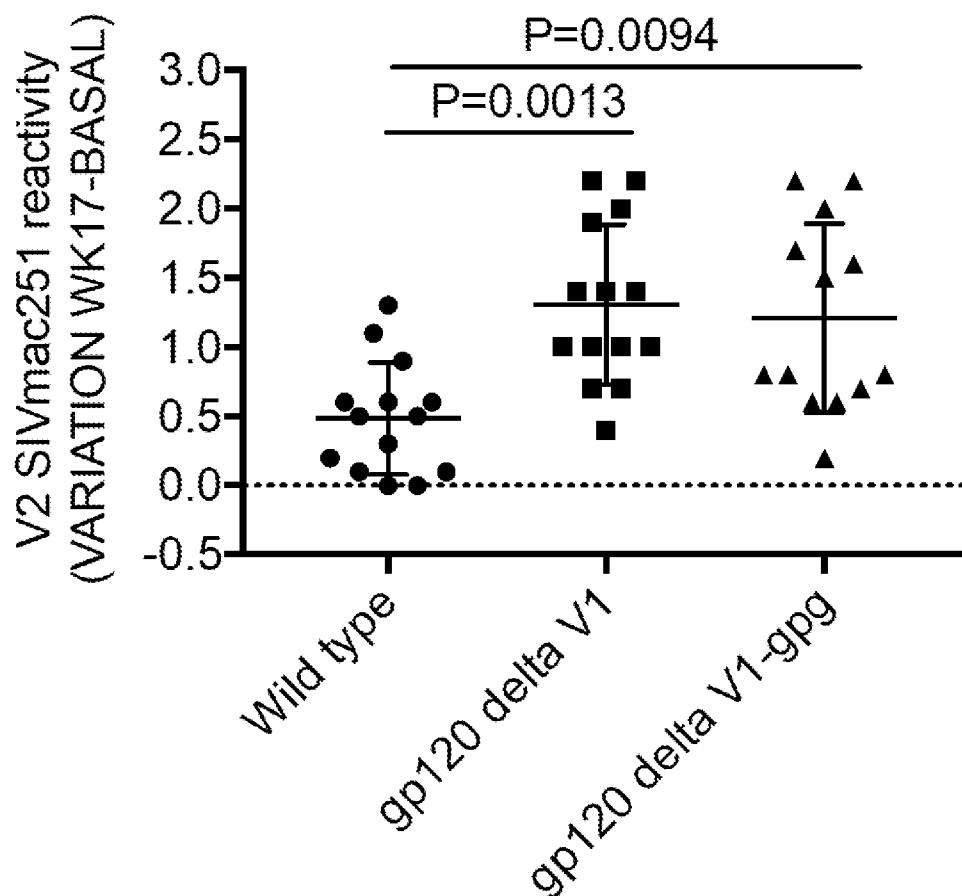


FIG. 3I



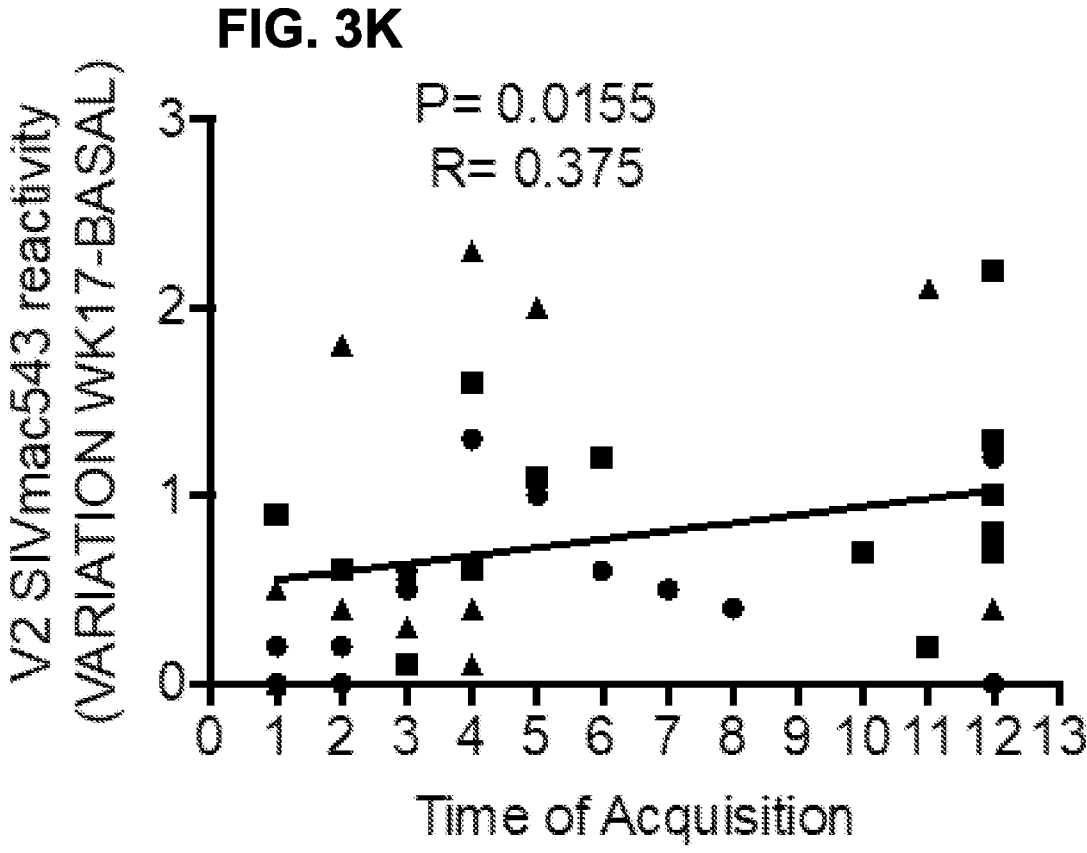
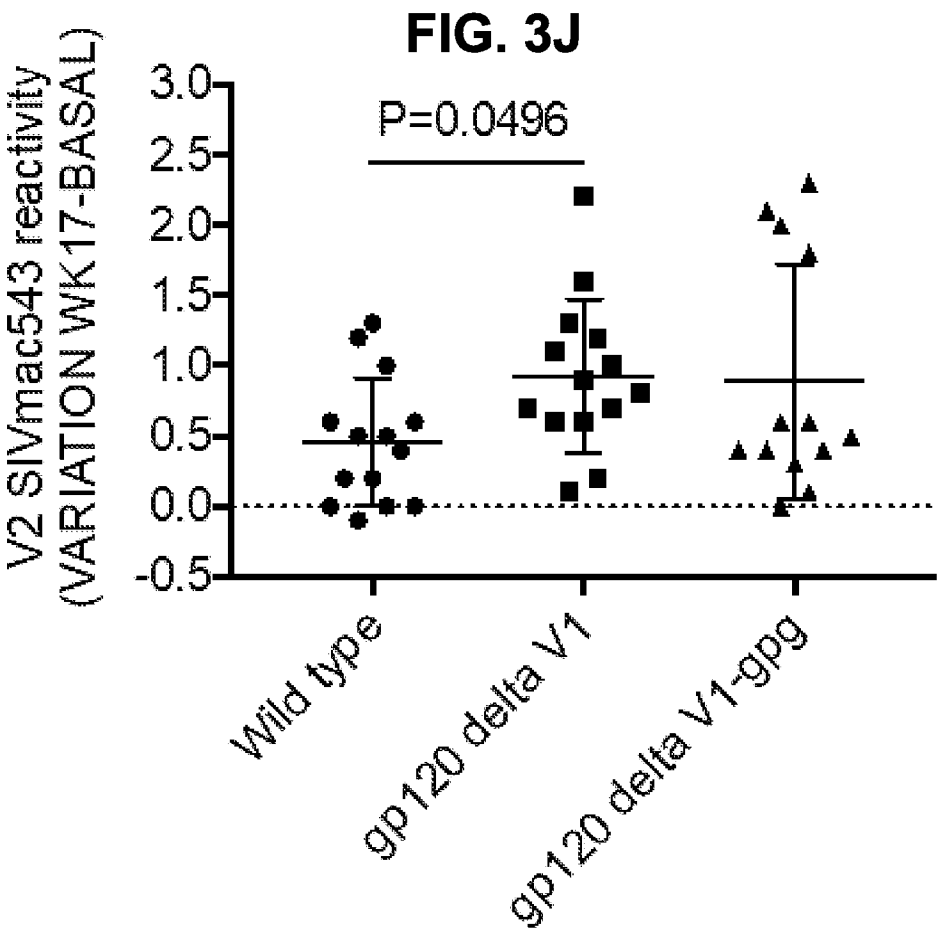


FIG. 3L

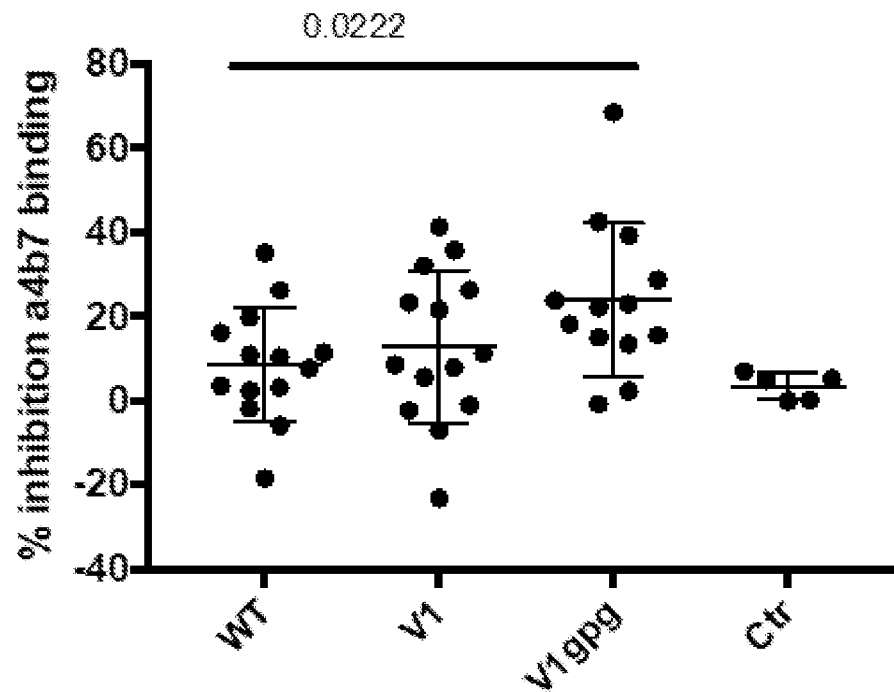


FIG. 3M

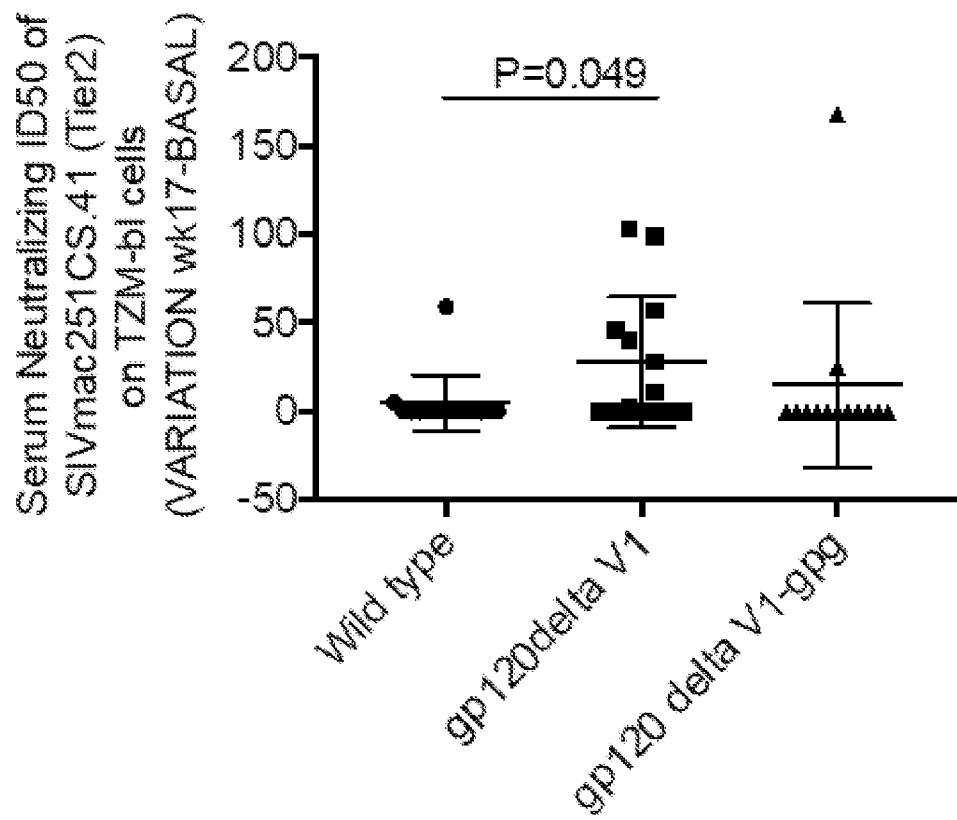


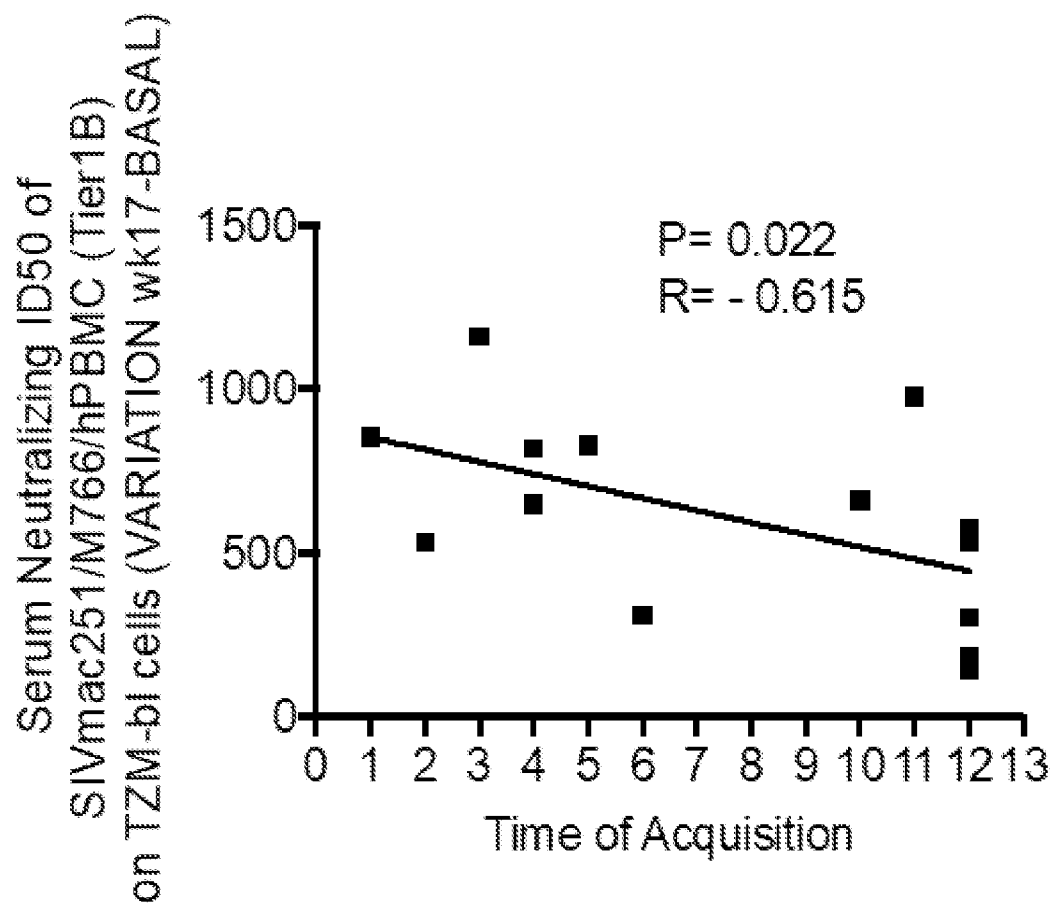
FIG. 3N

FIG. 4A

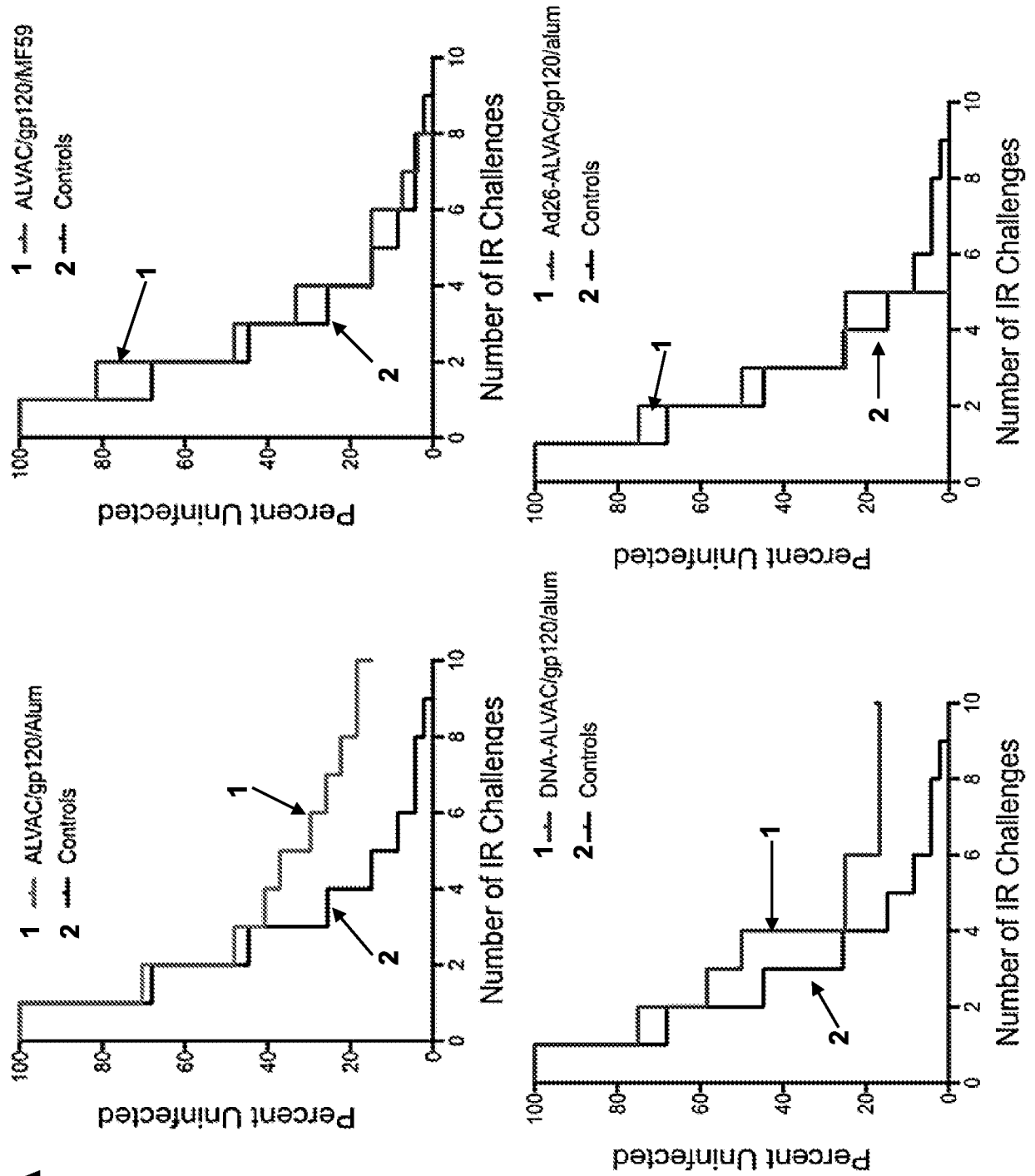


FIG. 4B

Group	Relative VE	P=
ALVAC-SIV+ gp120 alum	44%	0.025
DNA-SIV + ALVAC-SIV + gp120 alum	52%	0.029
ALVAC-SIV+ gp120 MF59	9%	ns
Ad26 + ALVAC-SIV + gp120 alum	13%	ns

FIG. 5A**V2 OVERLAPPING PEPTIDES**

	N-ter	NETSSCIAQNNCTGLEQEQMISCKFTMTGLKRDKTKEYNETWYSTDLVCEQGNSTD C-OOH
Peptide 25		TGLEQEQMISCKFTMTGLKR
Peptide 26		QMISCKFTMTGLKRDKTKEY
Peptide 27		FTMTGLKRDKTKEYNETWYS
Peptide 28		KRDKTKEYNETWYSTDLVCE
Peptide 29		EYNETWYSTDLVCEQGNSTD

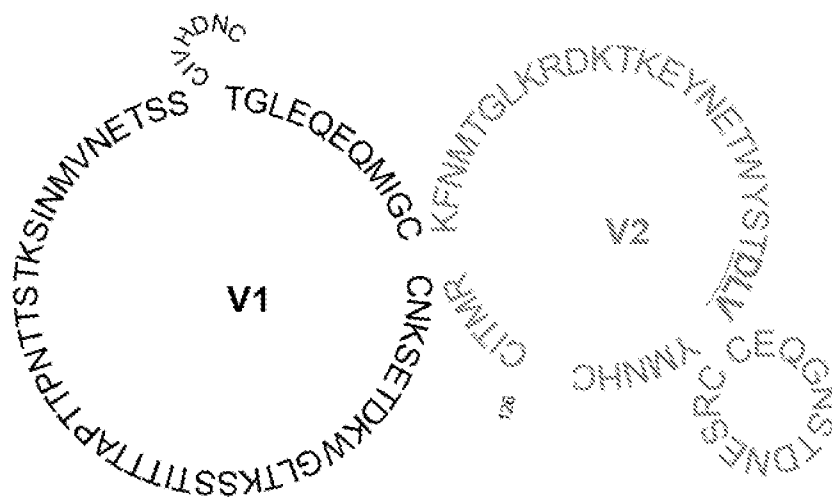
FIG. 5B

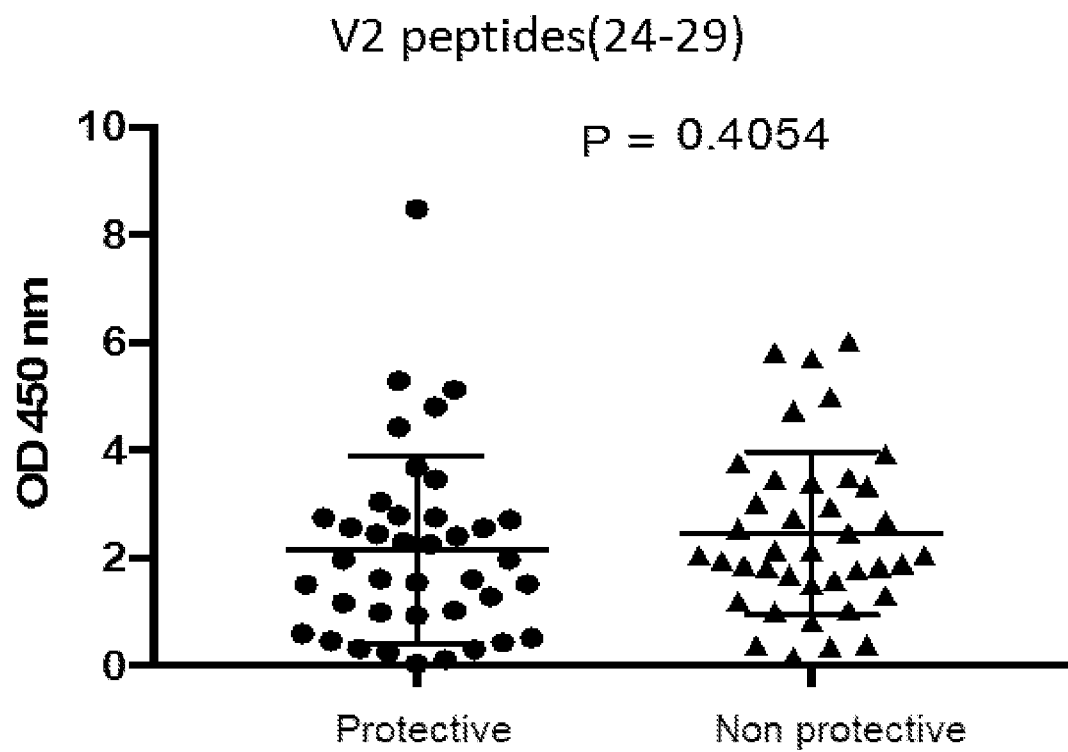
FIG. 5C

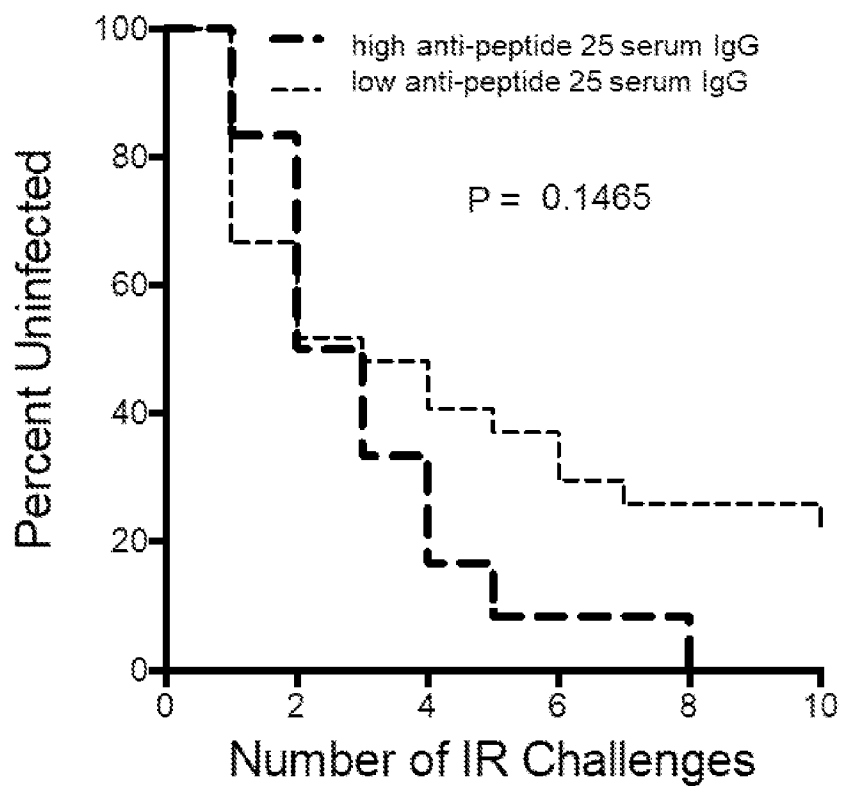
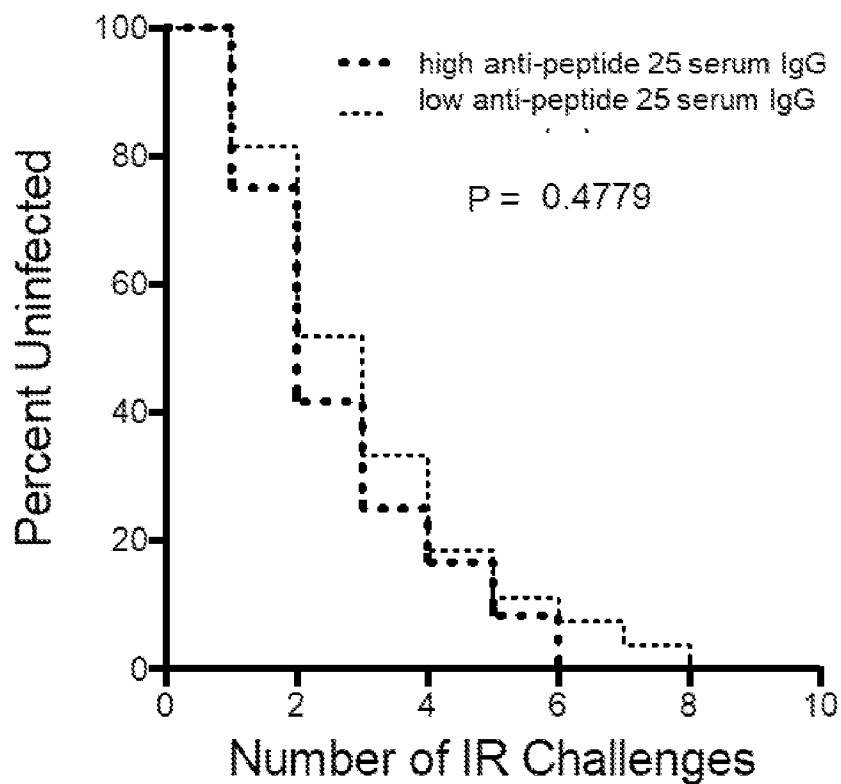
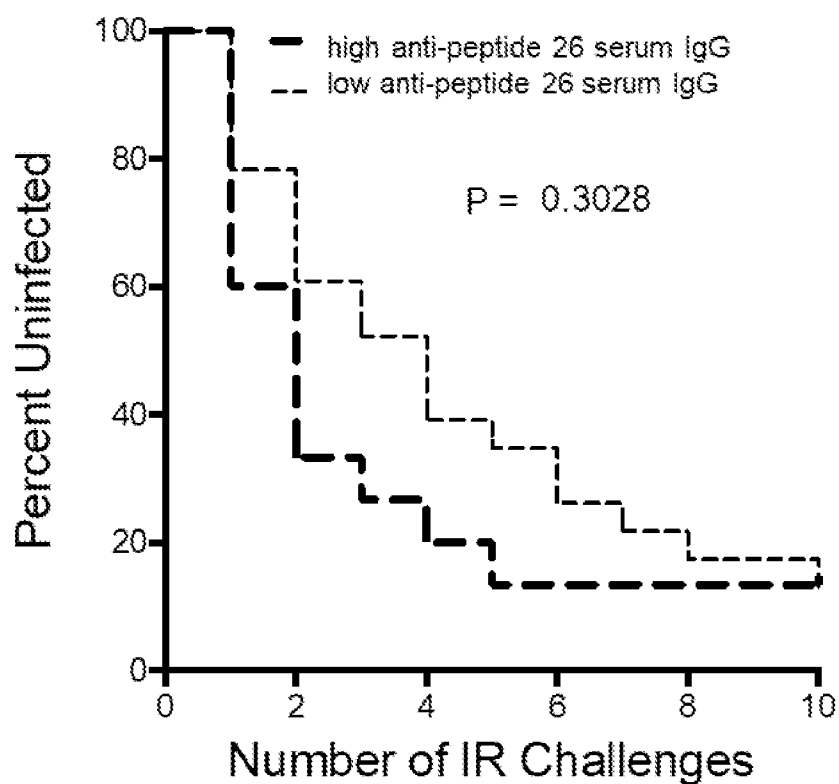
FIG. 5D **PROTECTIVE VACCINES****NON PROTECTIVE VACCINES**

FIG. 5E PROTECTIVE VACCINES



NON PROTECTIVE VACCINES

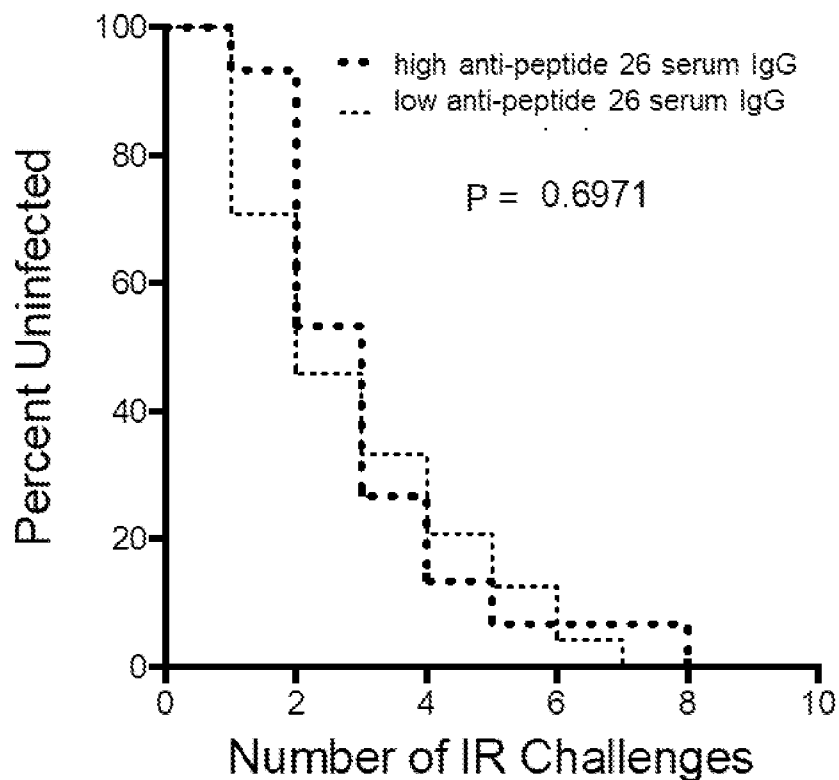
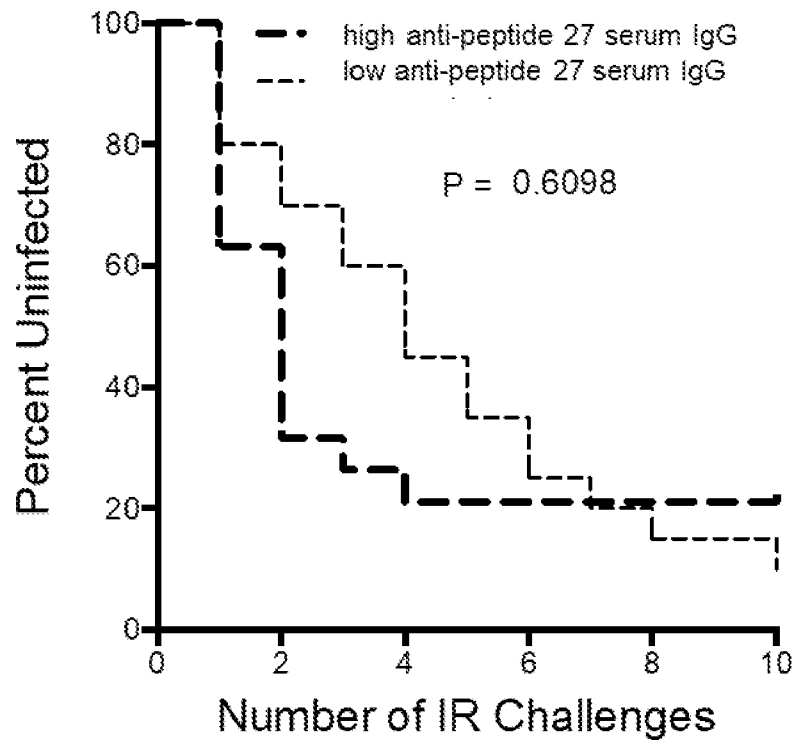


FIG. 5F PROTECTIVE VACCINES



NON PROTECTIVE VACCINES

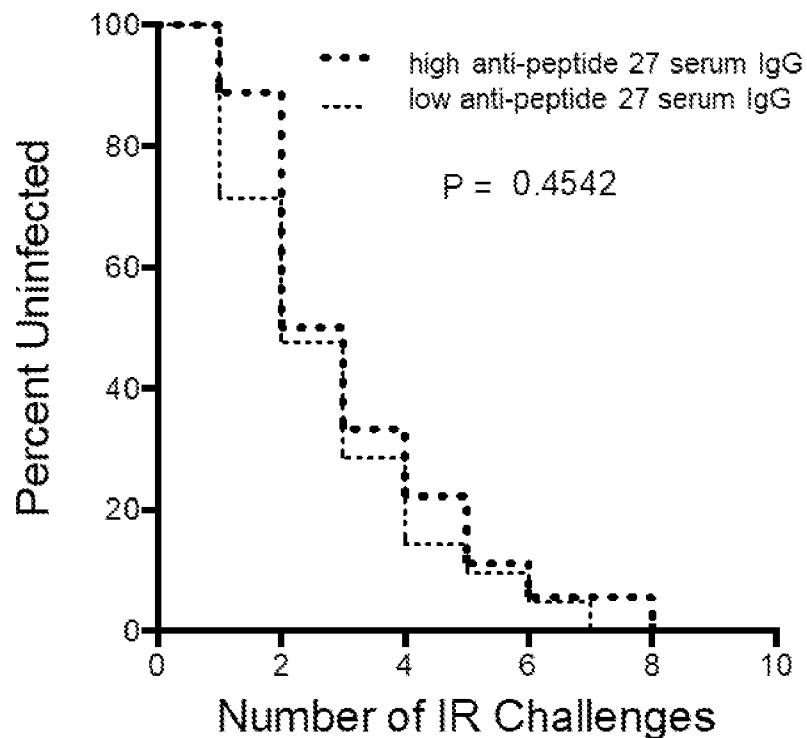


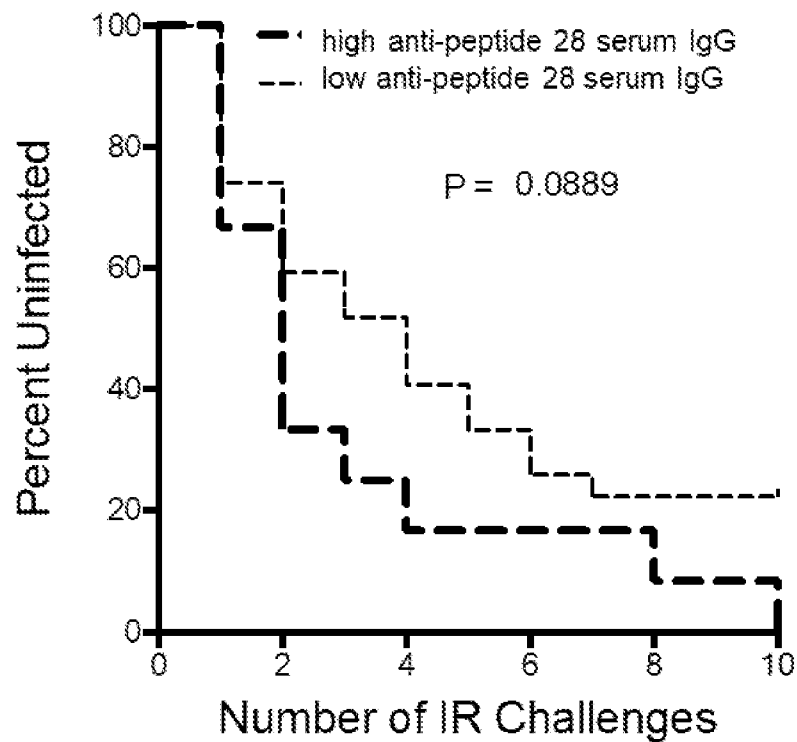
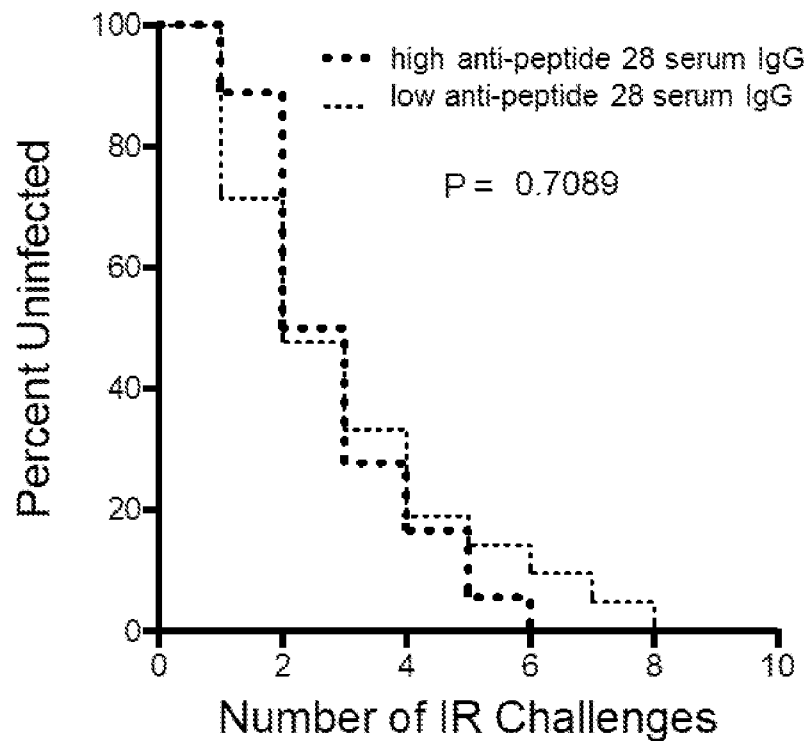
FIG. 5G **PROTECTIVE VACCINES****NON PROTECTIVE VACCINES**

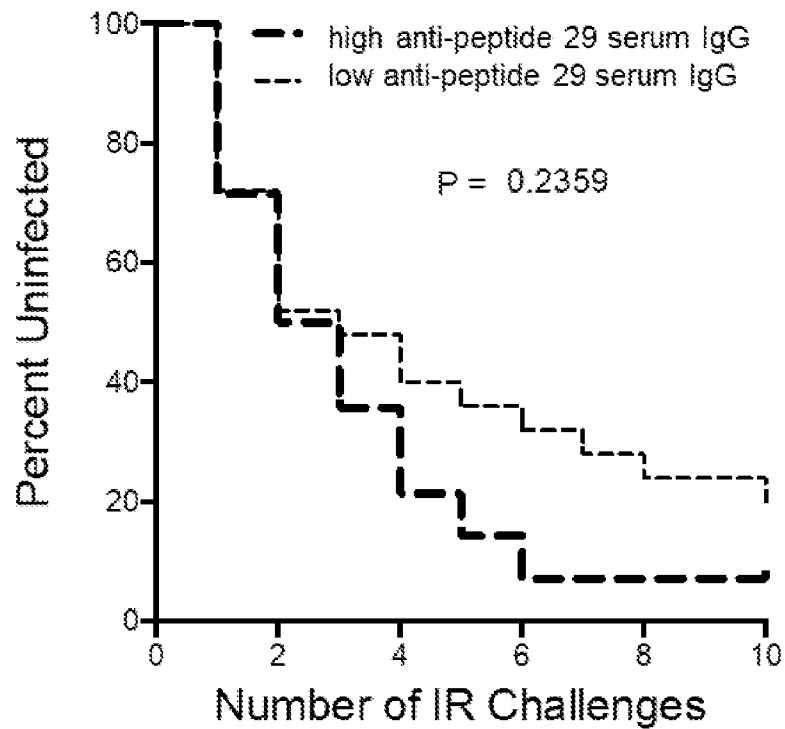
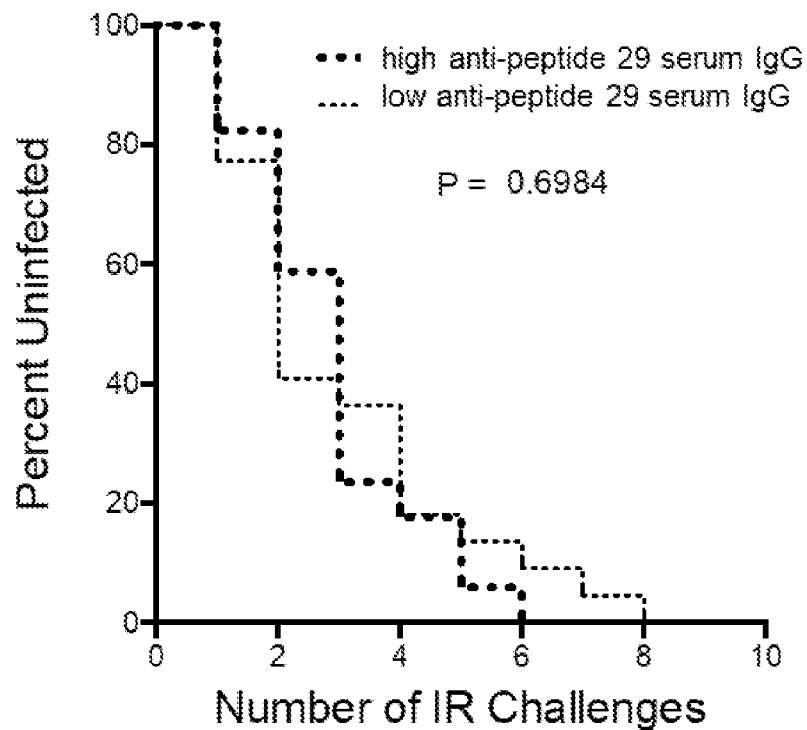
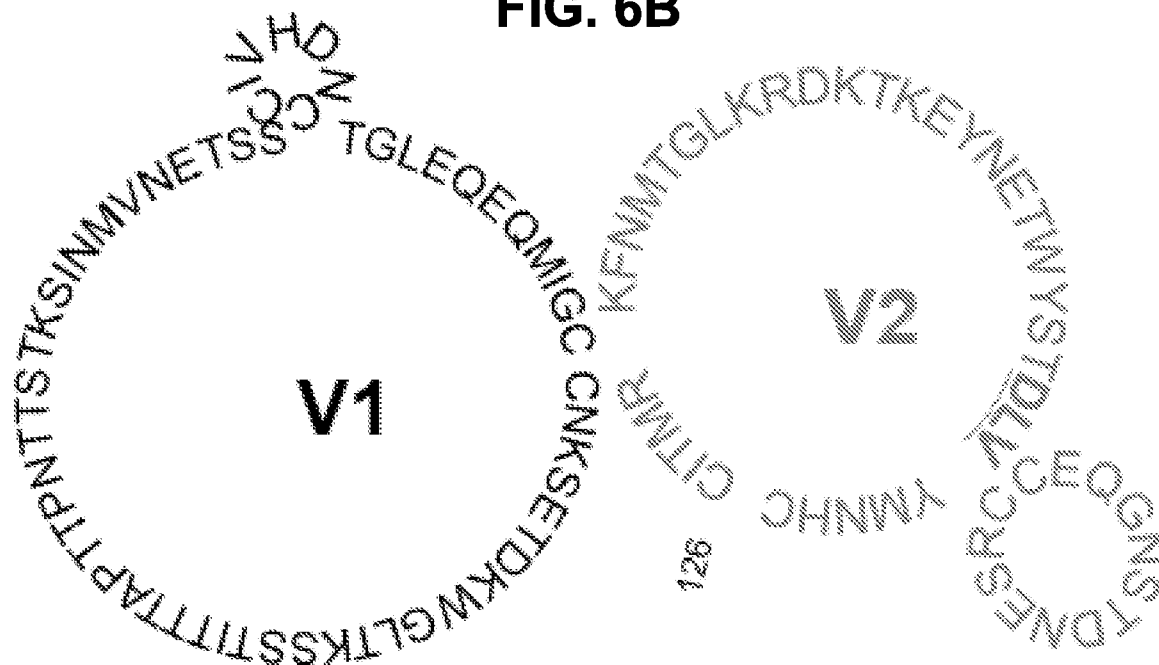
FIG. 5H**PROTECTIVE VACCINES****NON PROTECTIVE VACCINES**

FIG. 6A**V1 OVERLAPPING PEPTIDES**

	NH ₂ -NKSETDRWGLTKSSTTITTAAPTSAPVSEKIDMVNETSS-COOH
Peptide 15	PCVKLSPLOITMRCNKSETD
Peptide 16	PLCITMRCNKSETDRWGLTK
Peptide 17	RCNKSETDRWGLTKSSTTIT
Peptide 18	TDRWGLTKSSTTITTAAPTS
Peptide 19	TKSSTTITTAAPTSAPVSEK
Peptide 20	ITTAAPTSAPVSEKIDMVNE
Peptide 21	TSAPVSEKIDMVNETSSCIA
Peptide 22	EKIDMVNETSSCIAQNNCTG
Peptide 23	NETSSCIAQNNCTGLEQEQM
Peptide 24	IAQNNCTGLEQEQMISCKFT

FIG. 6B

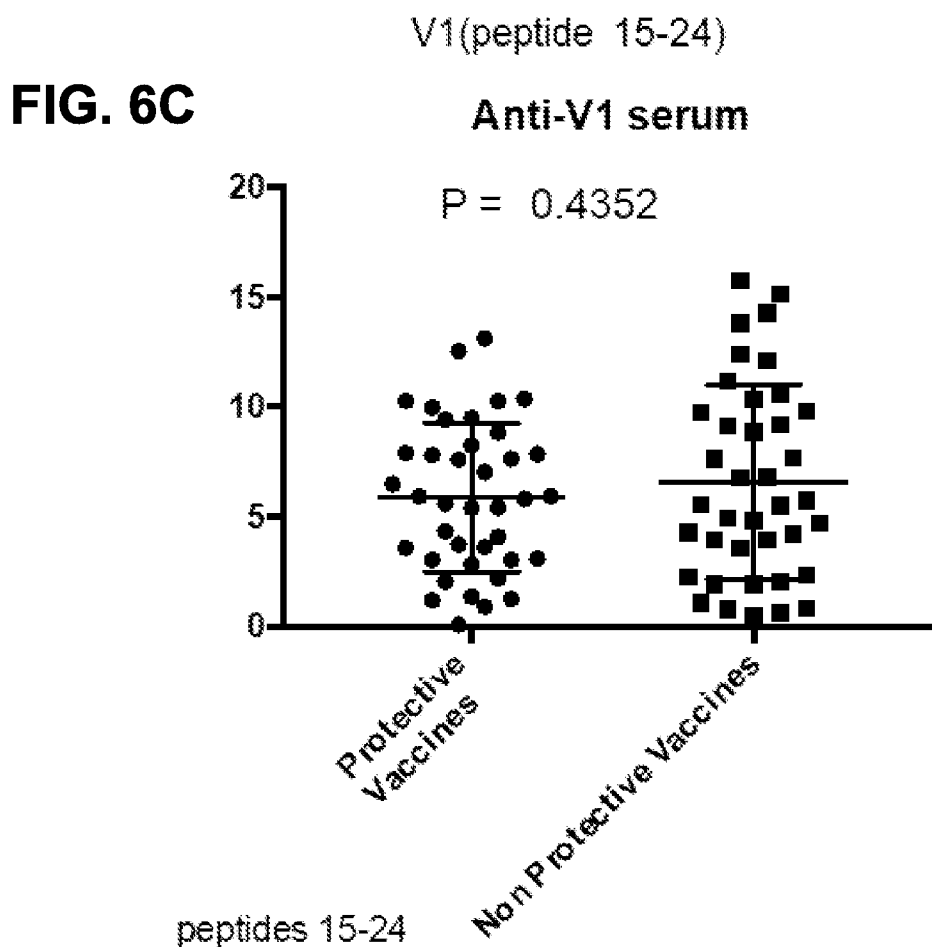


FIG. 6D **PROTECTIVE VACCINES**

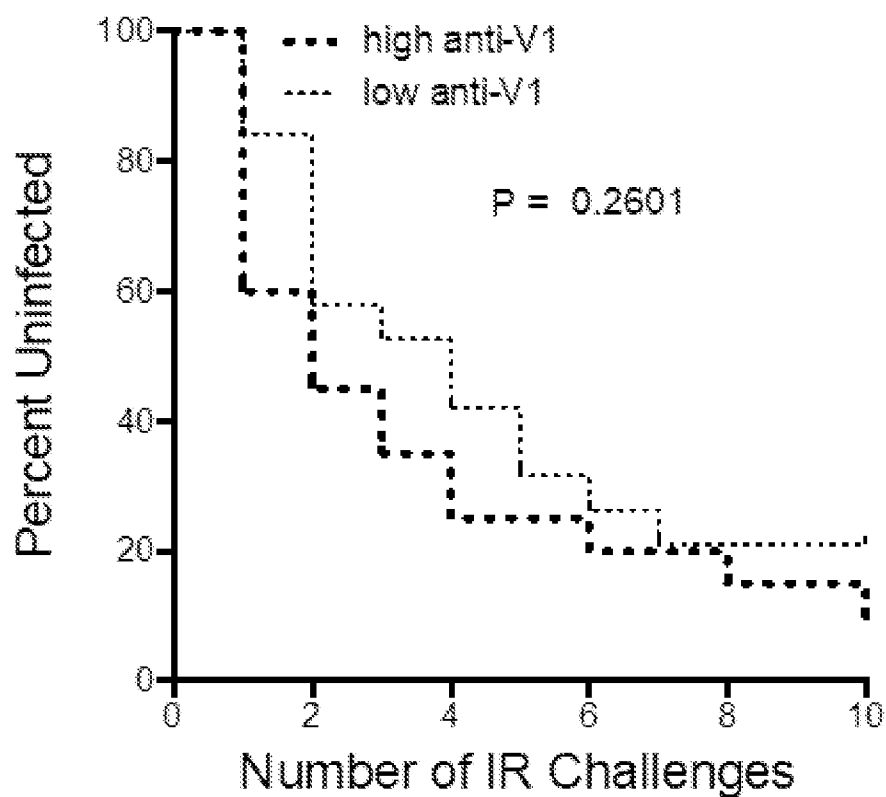


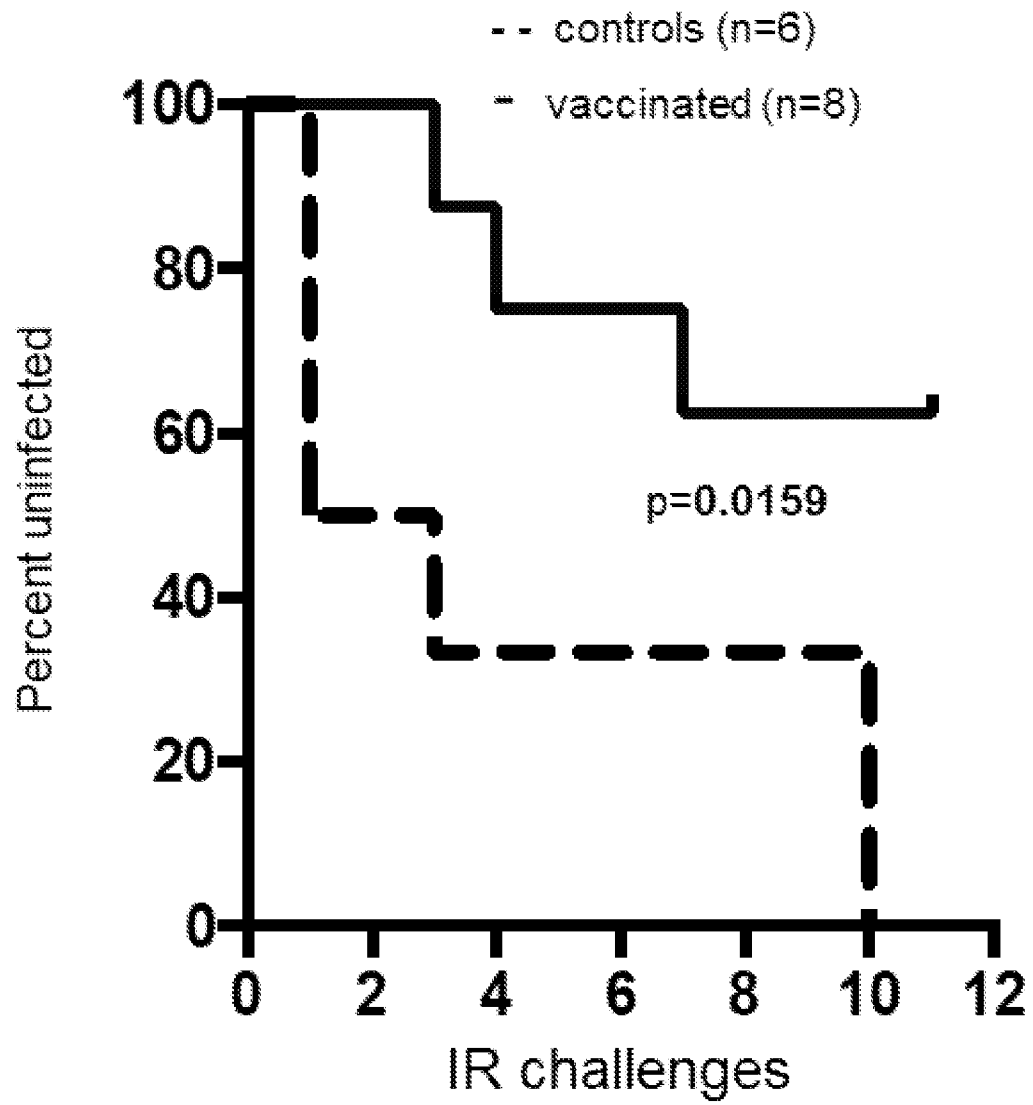
FIG. 7B

FIG. 7C

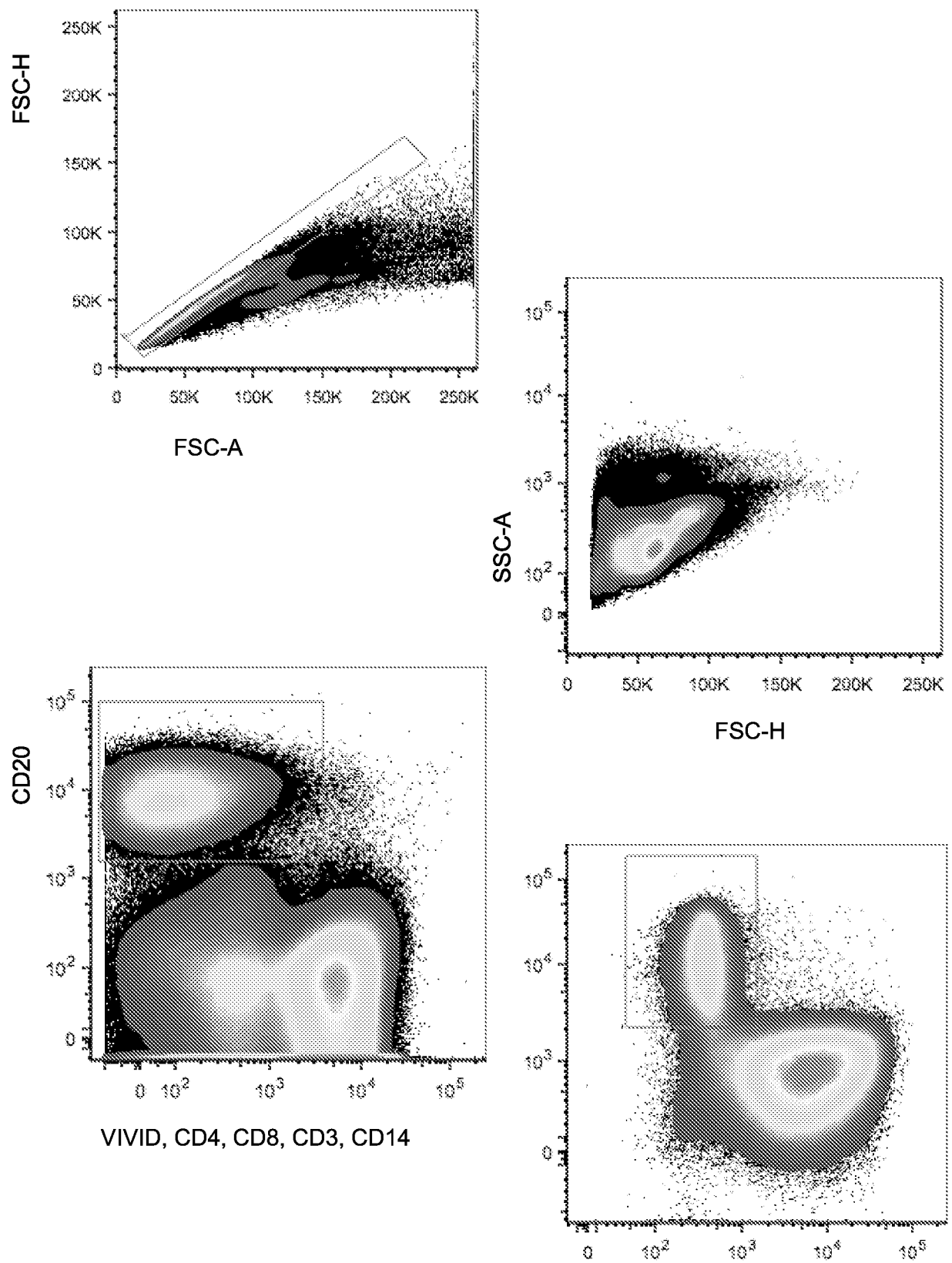


FIG. 7D

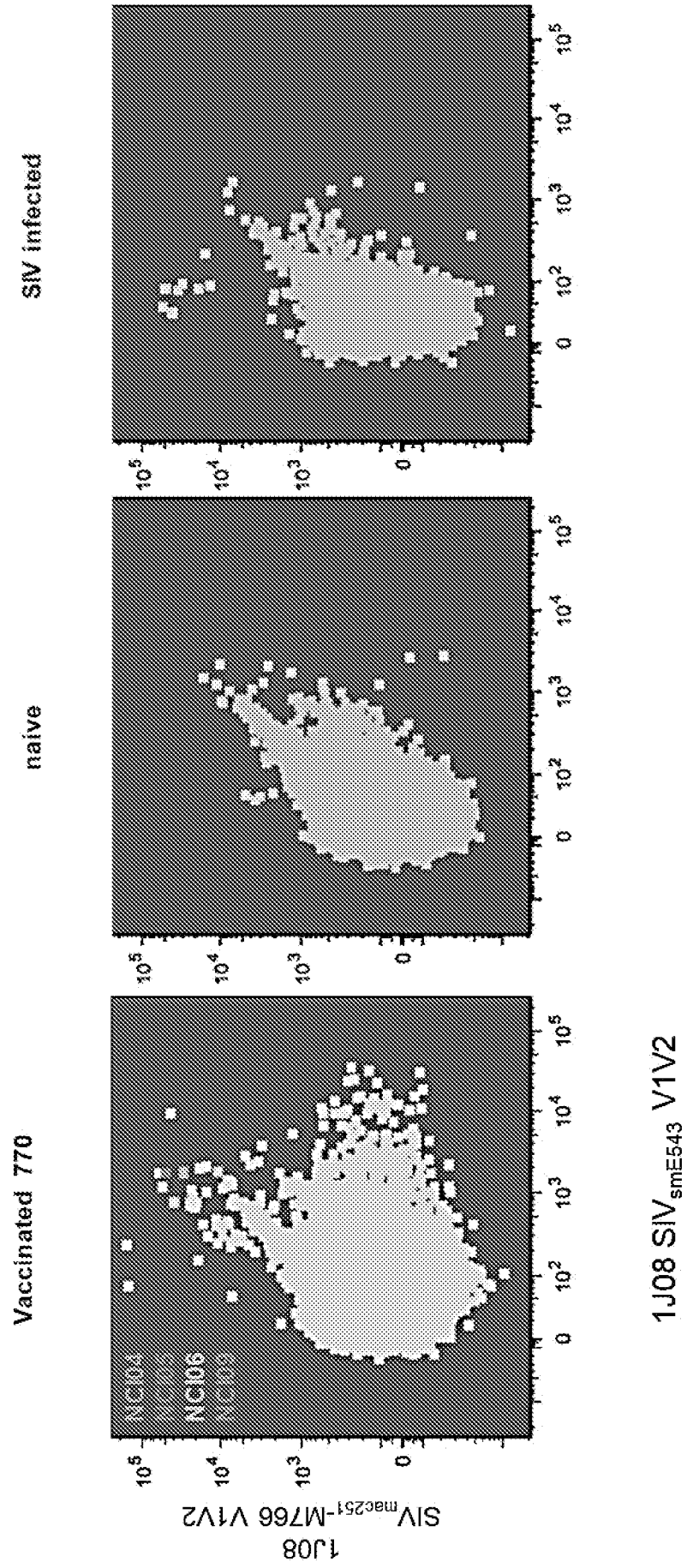


FIG. 8A

Monoclonal Antibody Binding

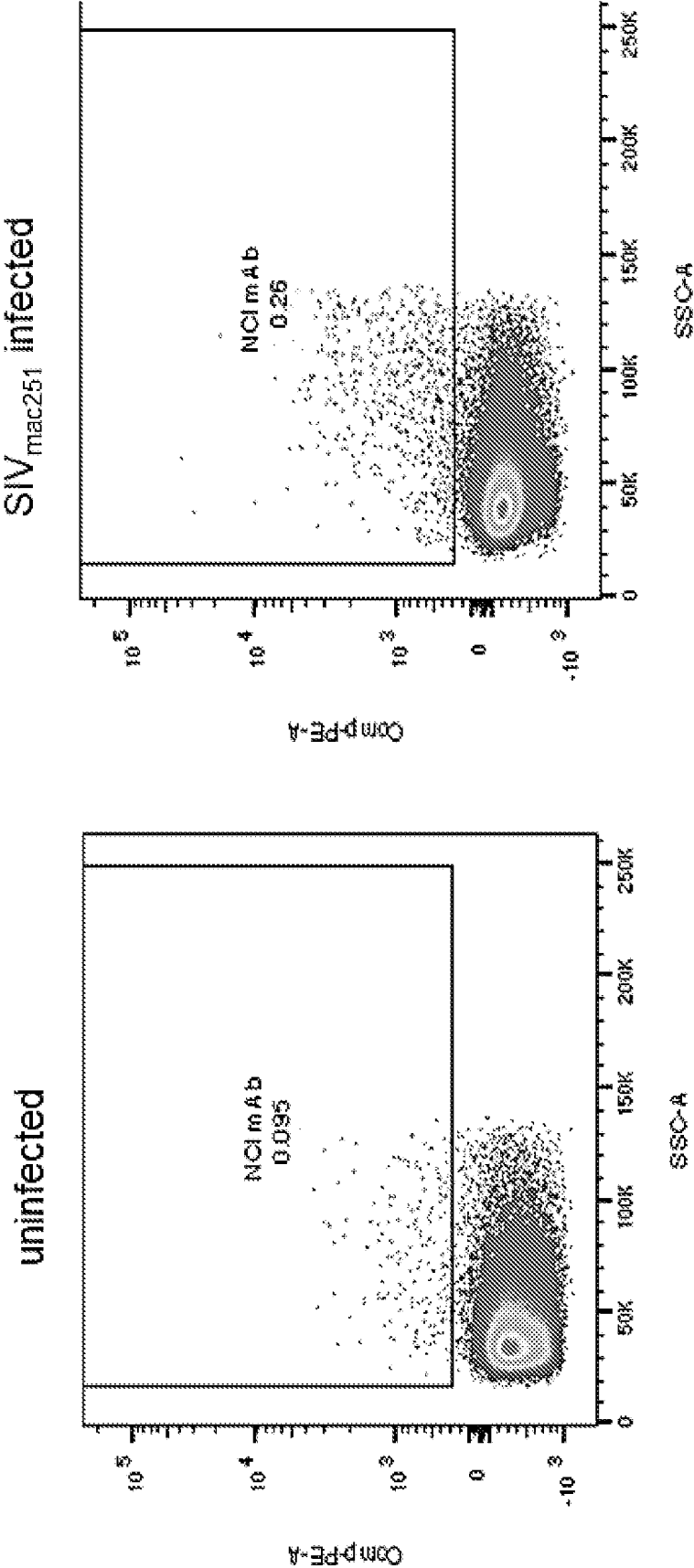
	NCI05	NCI09	NCI04	NCI06	ITS09	ITS41
SIVmac251 M766 gp120	++	++	++	++	++	++
SIVsmE660.CR54 gp140	++	++	-	++	++	-
SIVsmE543 1J08 V1V2	++	++	-	++	ND	ND
SIVmac251 1J08 V1V2	++	++	++	++	ND	ND
SIVmac239 1J08 V1V2	++	++	+	++	ND	ND
SIVsmE543 cV2	++	++	-	-	ND	ND
SIVmac251 cV2	~†	++	-	-	ND	ND
V1 peptide)	-	-	PLCITMRCNKSE TDRWGLTK (SEQ ID NO: 54)	RCNKSETDRWGLTK (SEQ ID NO: 20)	ND	ND
V2 peptide	-	TGLKRDKTKEY (SEQ ID NO: 53)	-	-	TGLKRDKKKEY (SEQ ID NO: 55)	EQQMISCKFN MTGL (SEQ ID NO: 56)

++
+
-

$OD_{450} \geq 2$
 $1 \leq OD_{450} < 2$
 $OD_{450} < 0.5$

† borderline $0.5 \leq OD_{450} < 1$

FIG. 8B



NCI09

FIG. 8C**SIV_{mac239} Peptides**

p41-- EQEQMISCKFNMTGL
 p42-- MISCKFNMTGLKRDK
 p43-- KFNMTGLKRDKKKEY
 p44-- TGLKRDKKKEYNETW
 p45-- RDKKKEYNETWYSAD
 p46-- KEYNETWYSADLVCE
 p47-- ETWYSADLVCEQGNN
 p48-- SADLVCEQGNNTGNE

FIG. 8D

cV2 binding inhibition (NCI09)

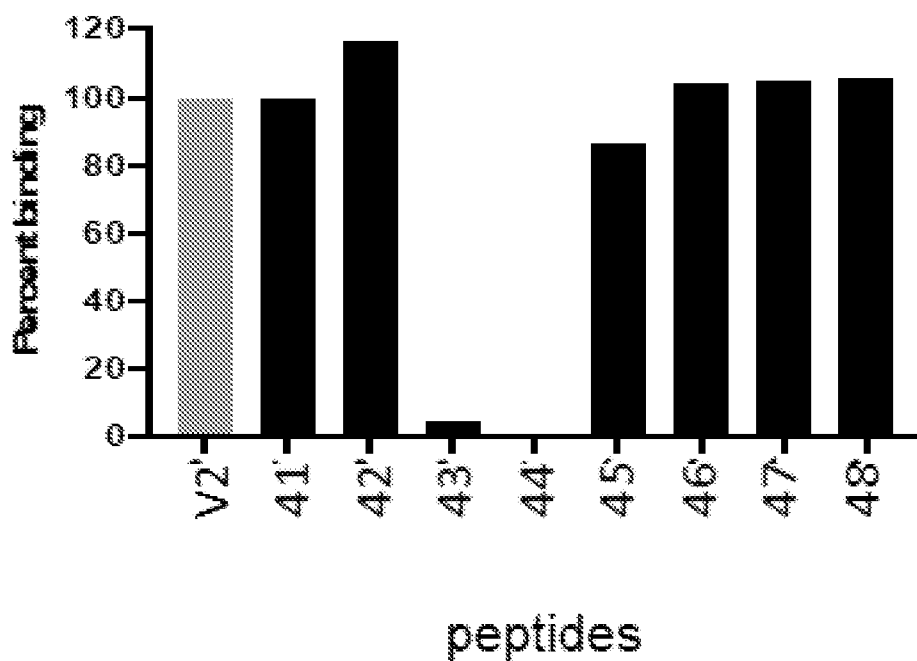


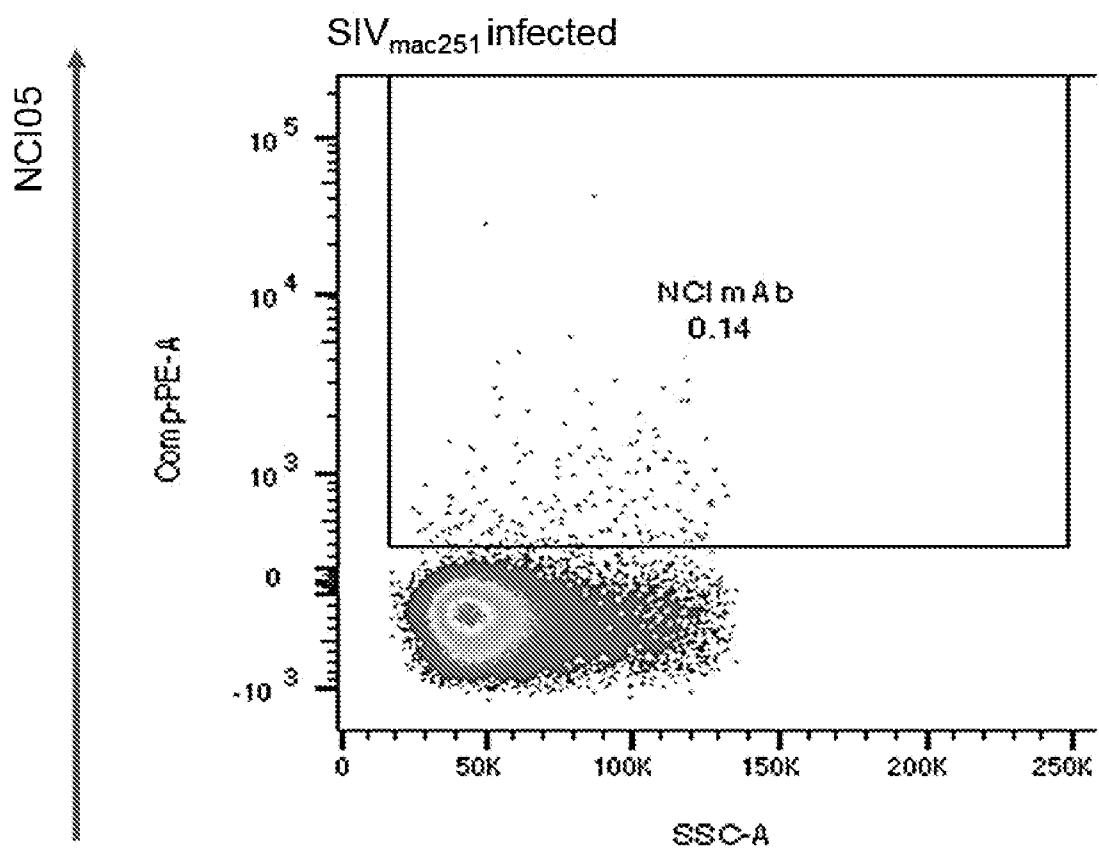
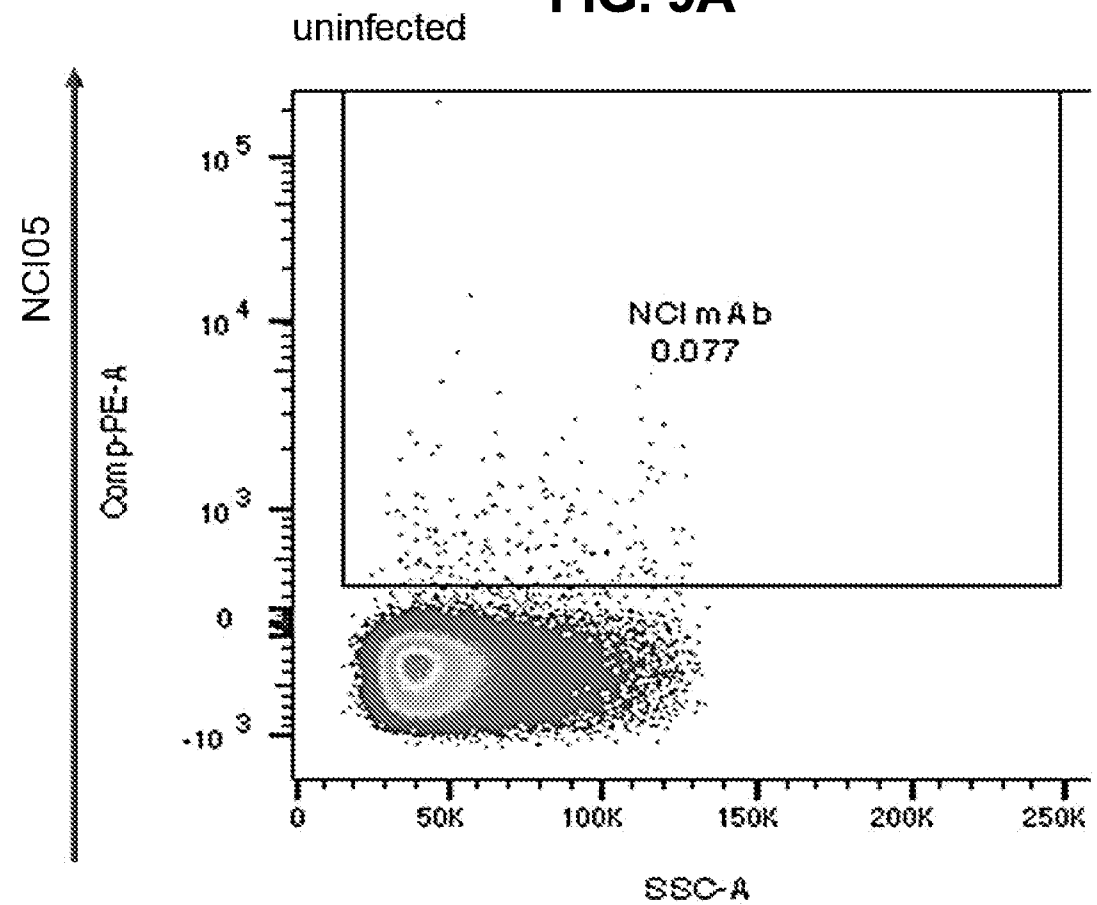
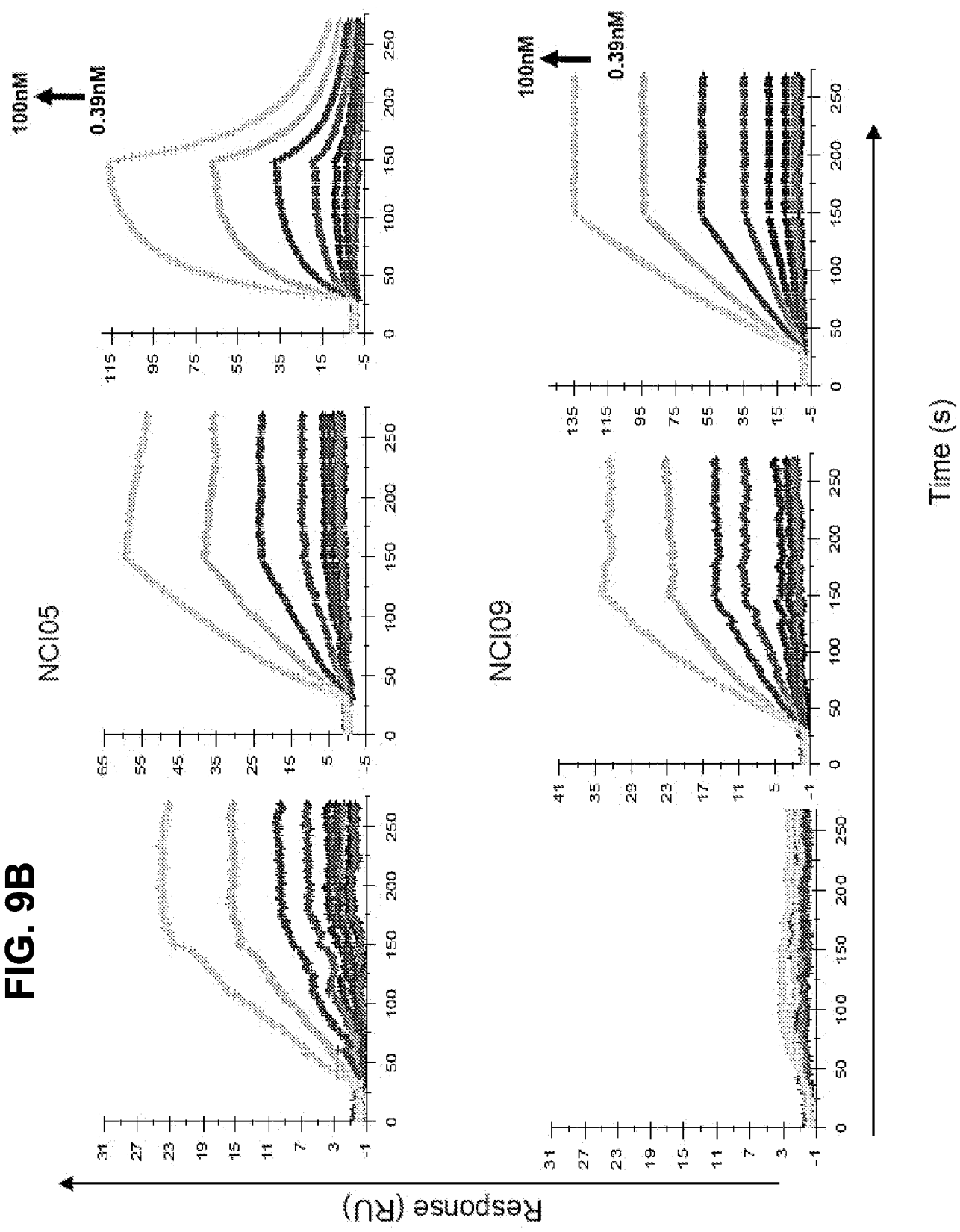
FIG. 9A

FIG. 9B

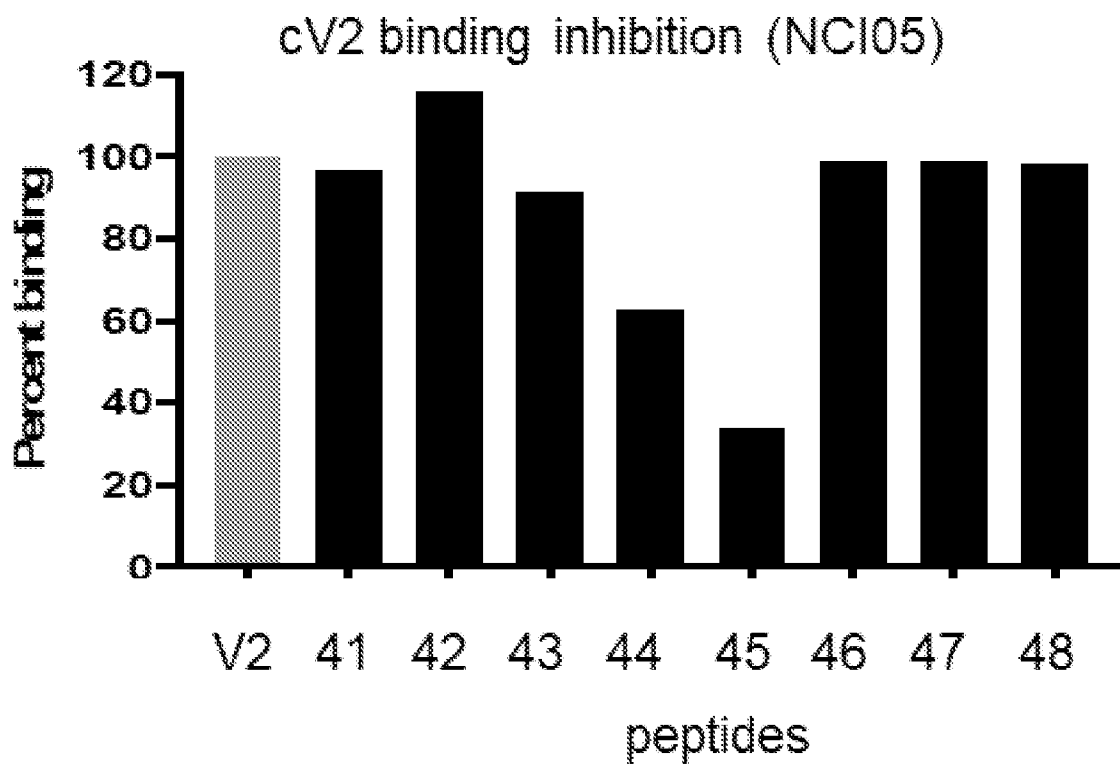
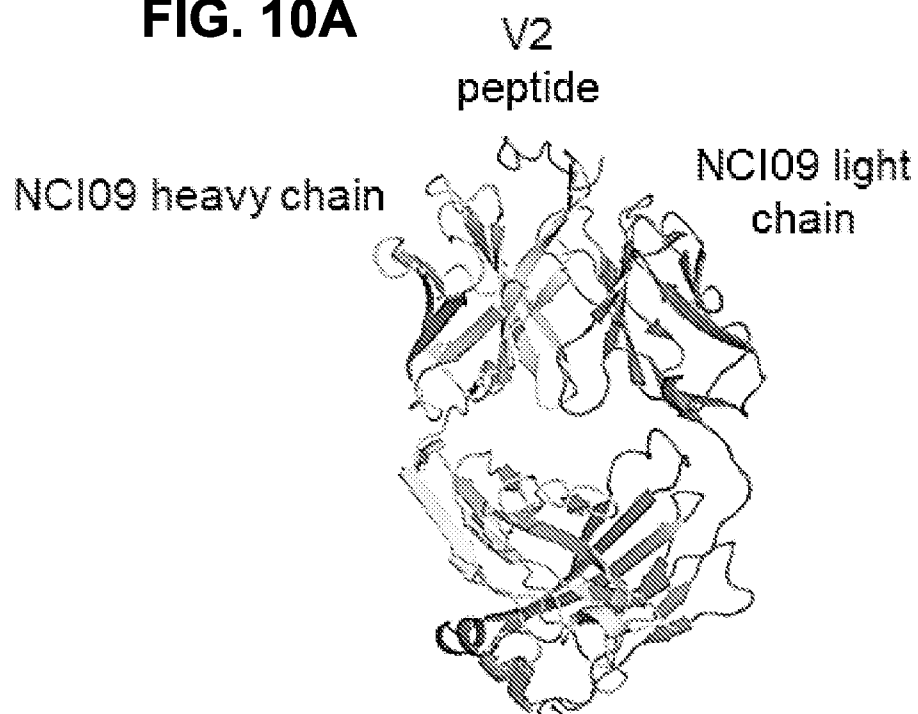


	NCI05			NCI09	
	KA	KD	KA	KD	
SIVmac251-M766 gp120	1.78E+08	5.60E-09	8.19E+09	1.22E-10	
SIVsmE660.CR54 gp140	9.60E+10	1.04E-11	N/A	N/A	
SIVmac251-M766 cV2	5.12E+06	1.97E-07	9.13E+11	1.10E-12	

FIG. 9C

	ITS41		NCI05		NCI09	
	KA	KD	KA	KD	KA	KD
SIVmac251-M766 gp120	3.90E+04	2.57E-05	1.78E+08	5.60E-09	8.19E+09	1.22E-10
SIVsmE660.CR54 gp140	N/A	N/A	9.60E+10	1.04E-11	N/A	N/A
SIVmac251-M766 cV2	1.06E+08	9.46E-09	5.12E+06	1.97E-07	9.13E+11	1.10E-12

FIG. 9D

FIG. 9E**FIG. 10A**

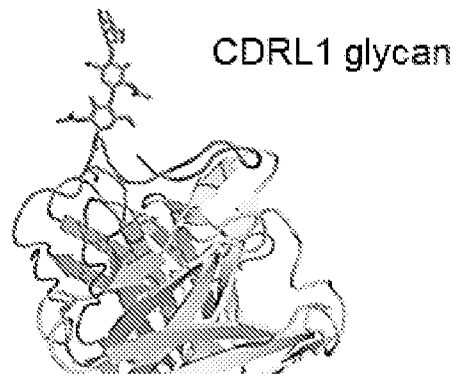
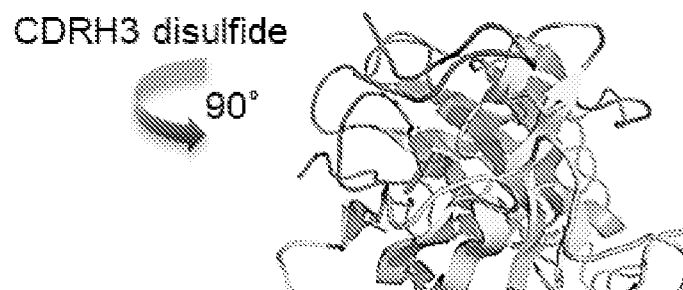
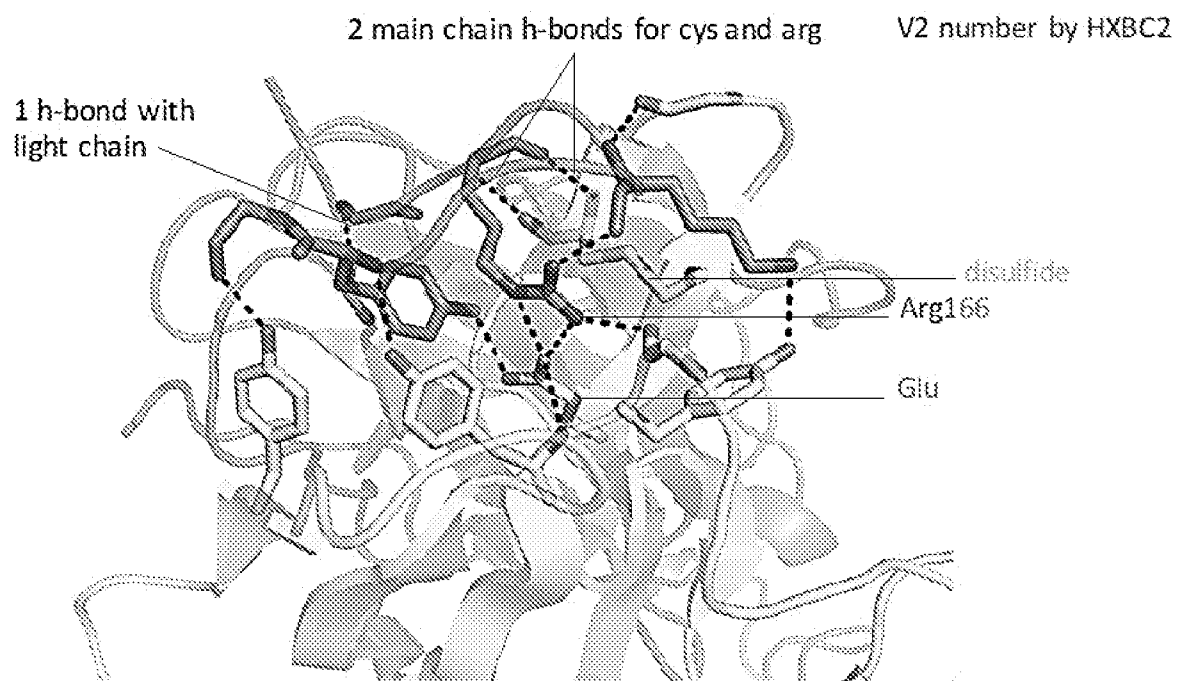
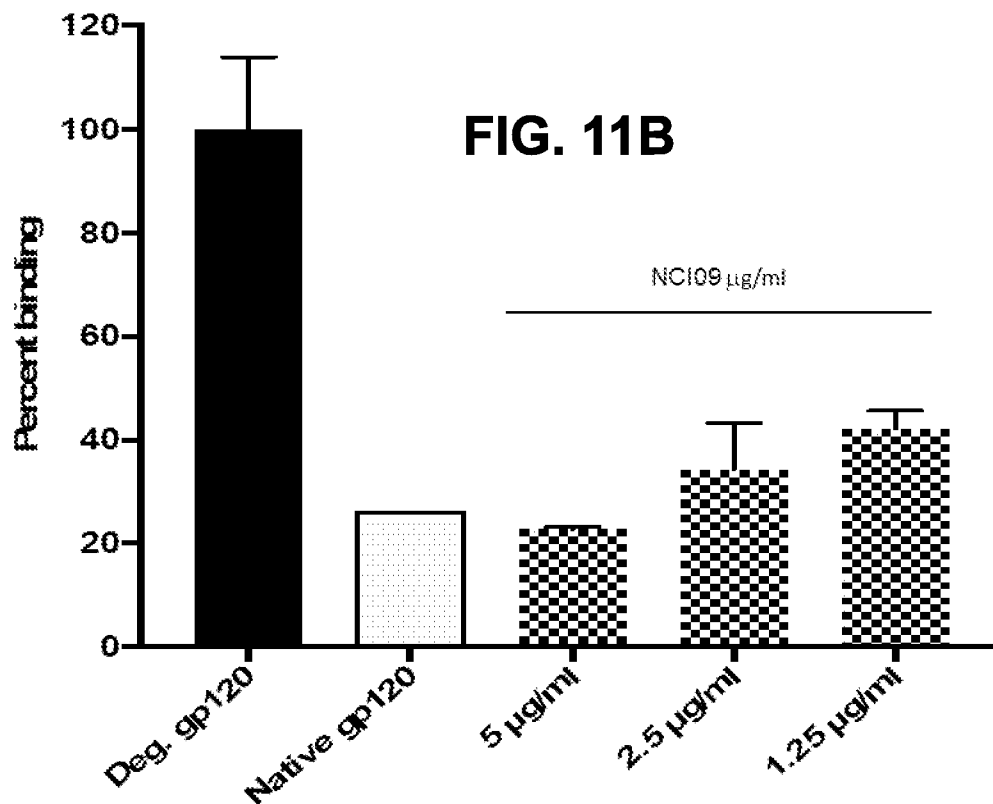
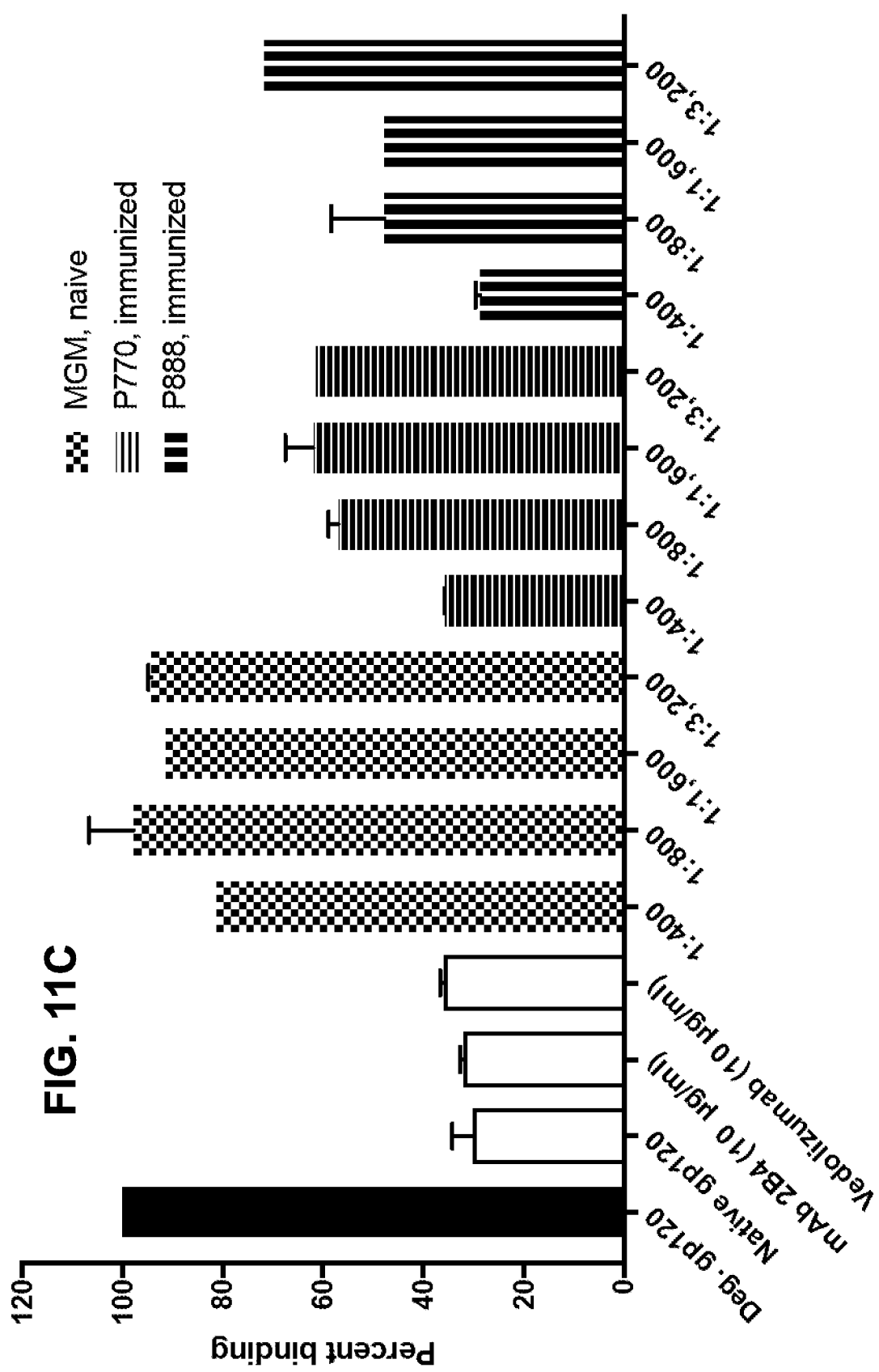
**FIG. 10B****FIG. 10C**

FIG. 11A**Monoclonal Antibody Neutralization**

	NCI05	NCI09	NCI04	NCI06
SIVsmE660.CP3C (Tier 1)	4.449 *	>50 [†]	>50 [†]	>50 [†]
SIVsmE660.CR54 (Tier 2)	>50 [†]	>50 [†]	>50 [†]	>50 [†]
SIVmac251.H9 (Tier 1)	>50	0.412 **	>50	0.024 ***
SIVmac251.30 (Tier 2)	>50 [†]	>50 [†]	>50 [†]	>50 [†]

* 1 - 9.99 $\mu\text{g/ml}$ ** 0.01 - 0.099 $\mu\text{g/ml}$ *** 0.1 - 0.99 $\mu\text{g/ml}$ [†] curve plateaued below 50%



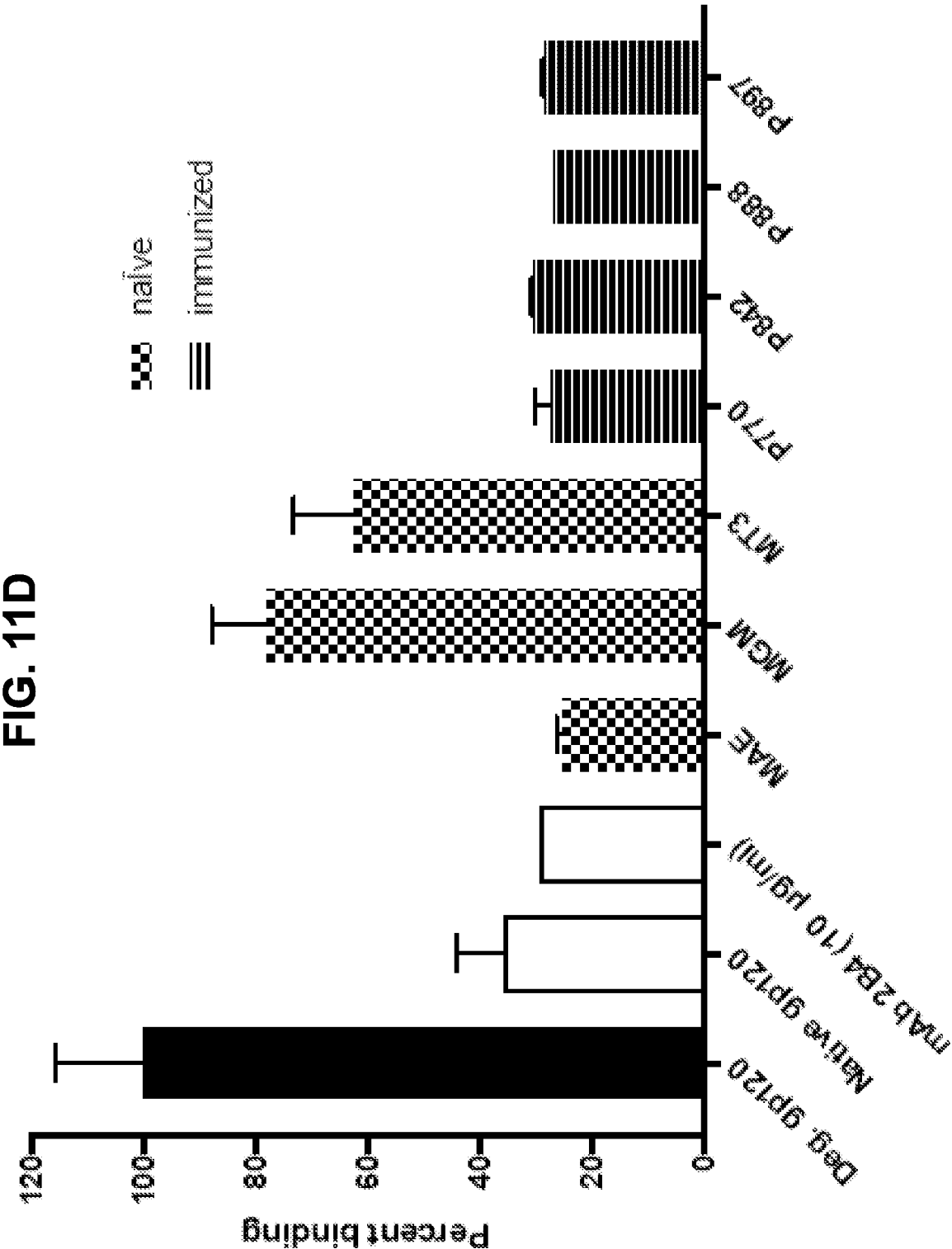
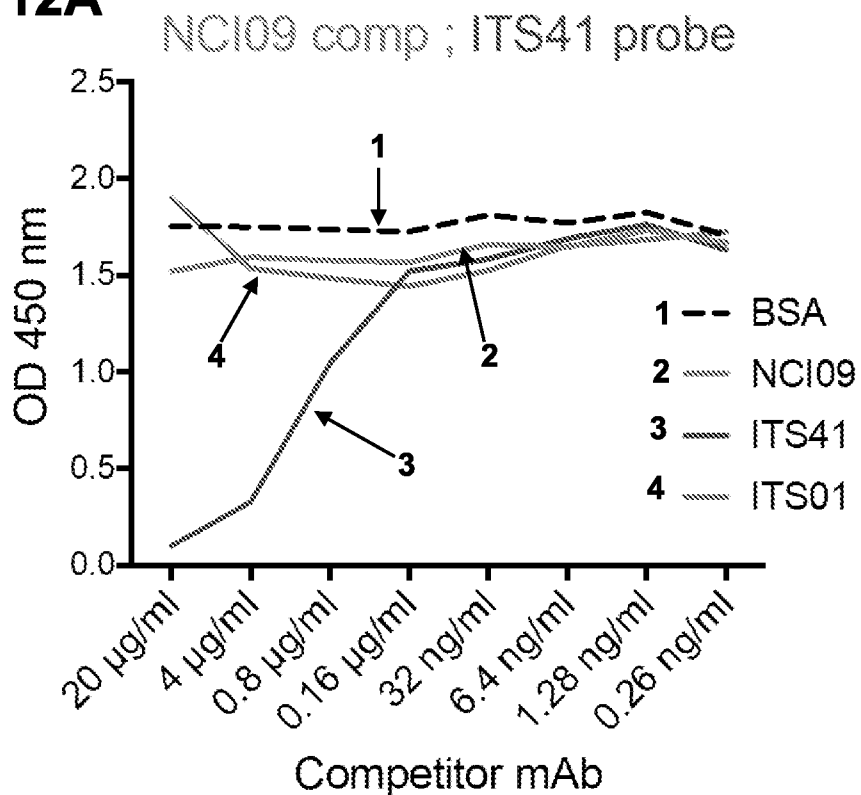
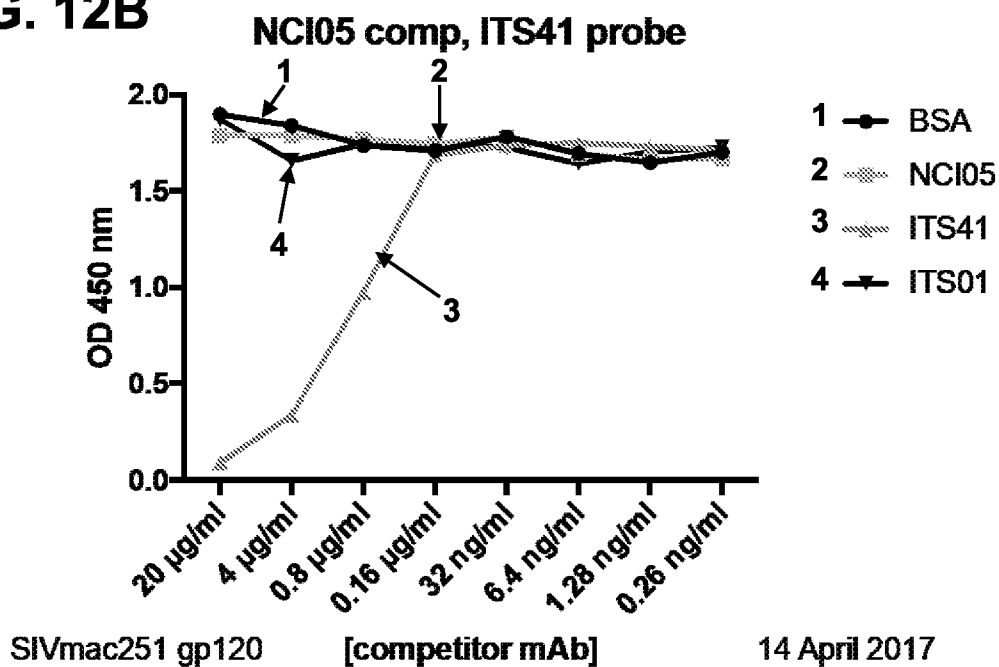


FIG. 12A**FIG. 12B**

14 April 2017

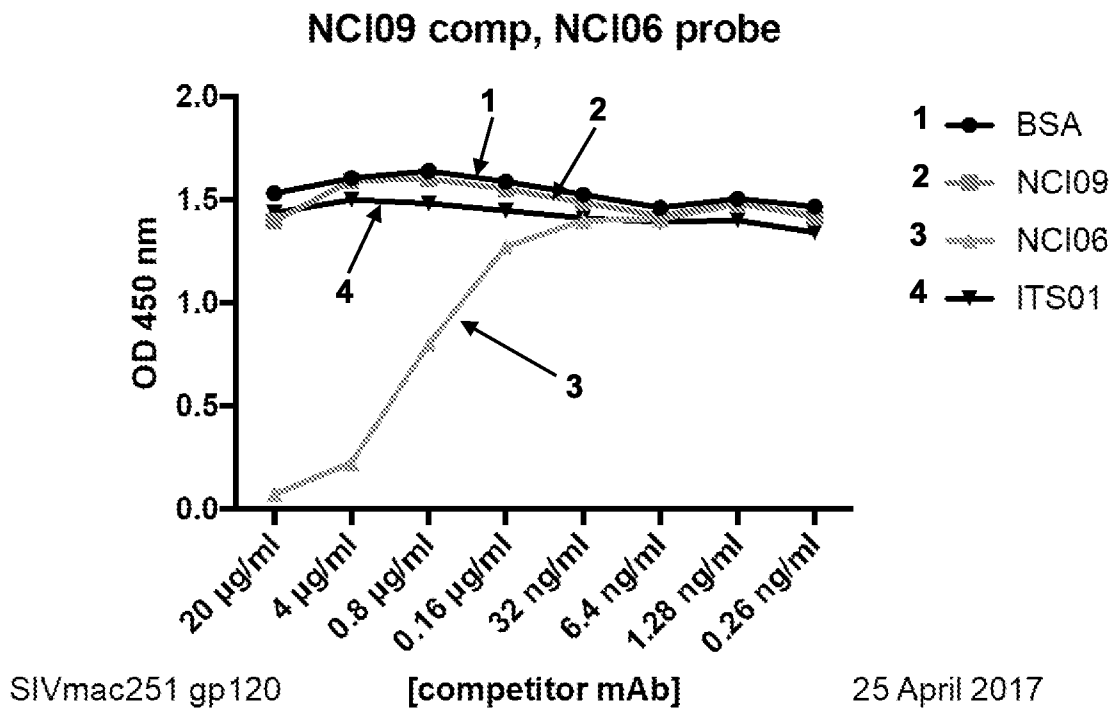
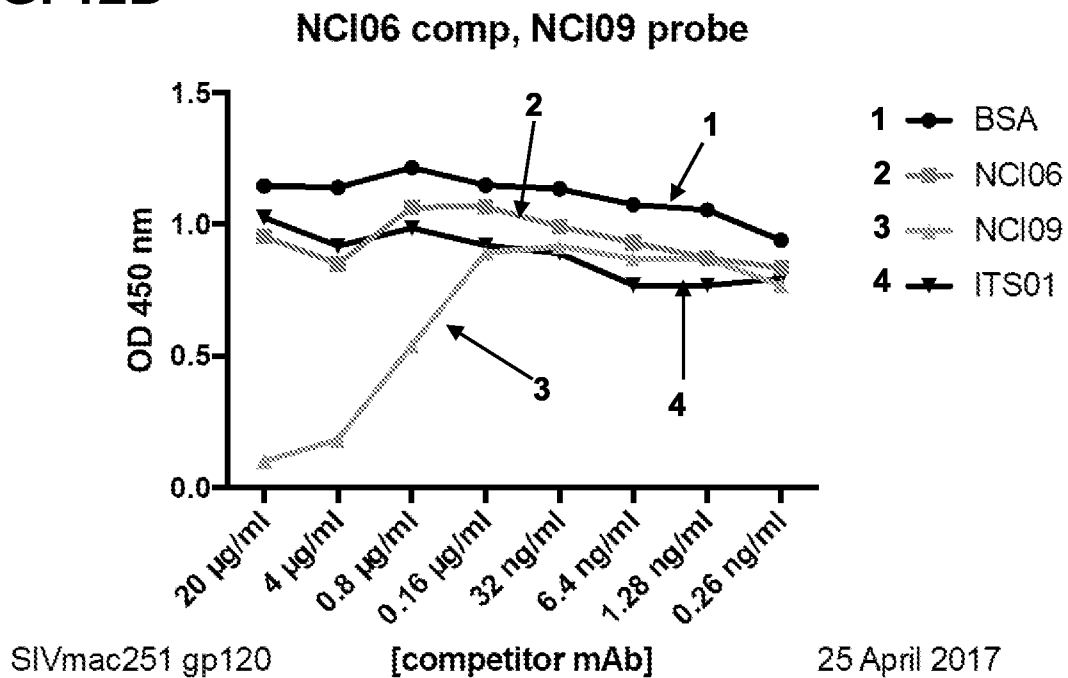
FIG. 12C**FIG. 12D**

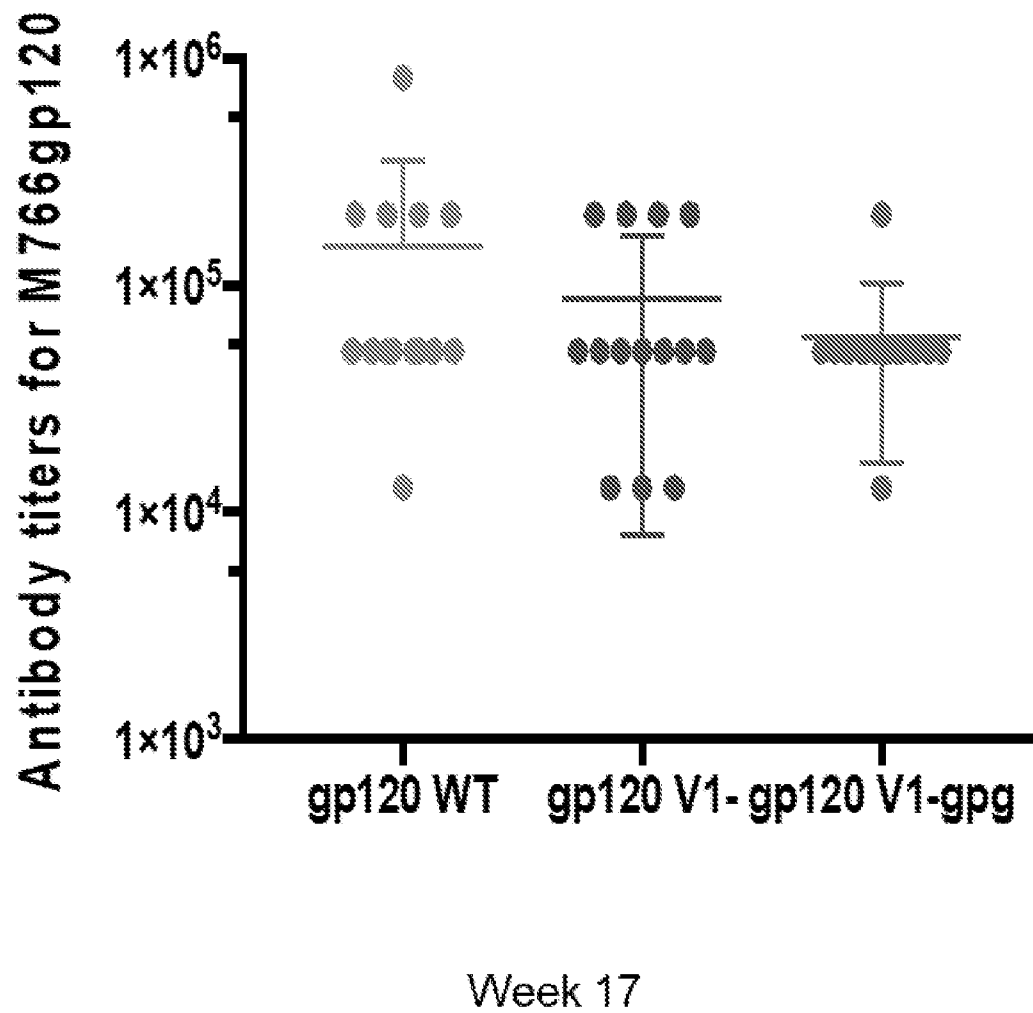
FIG. 13A

FIG. 13B

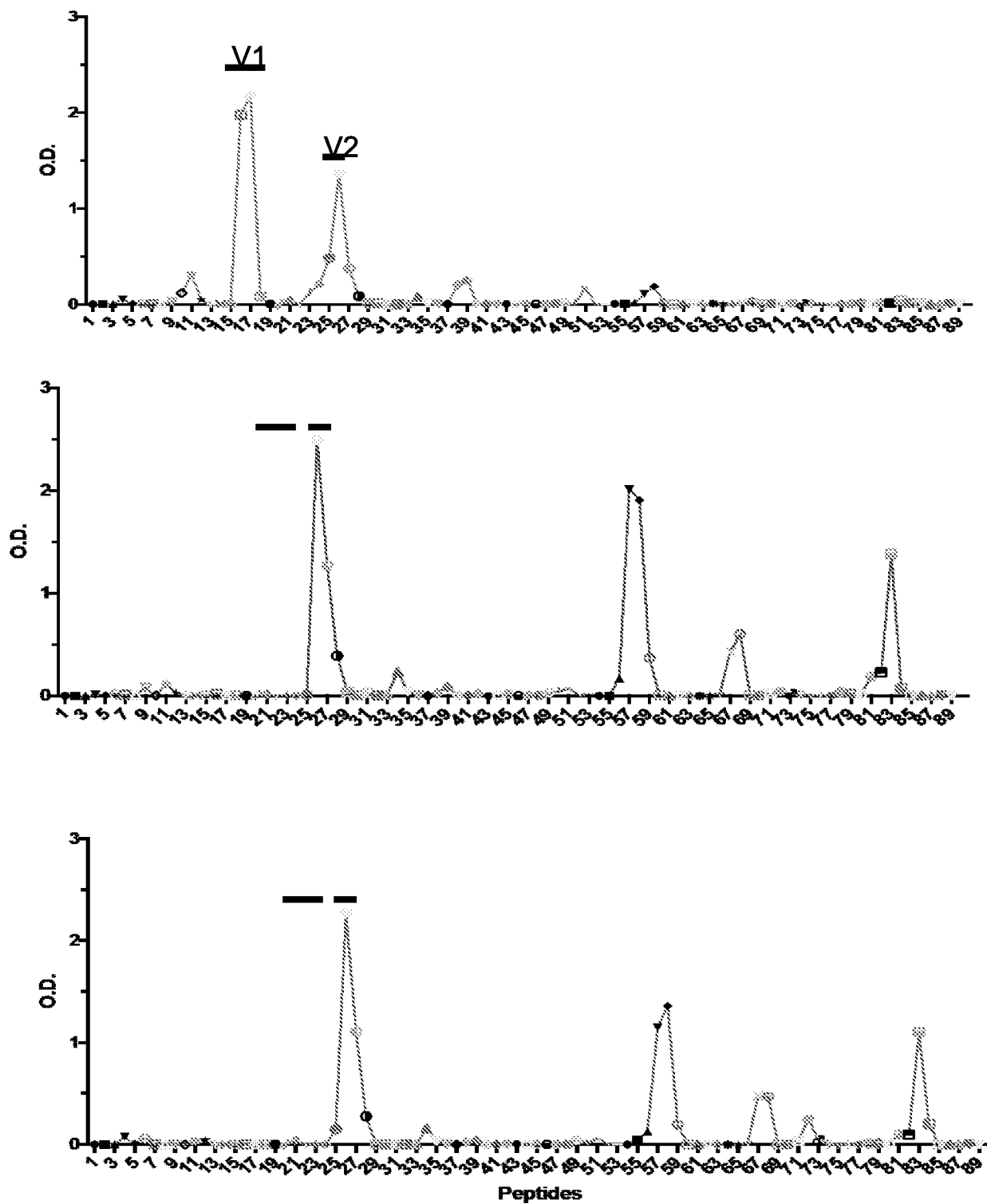


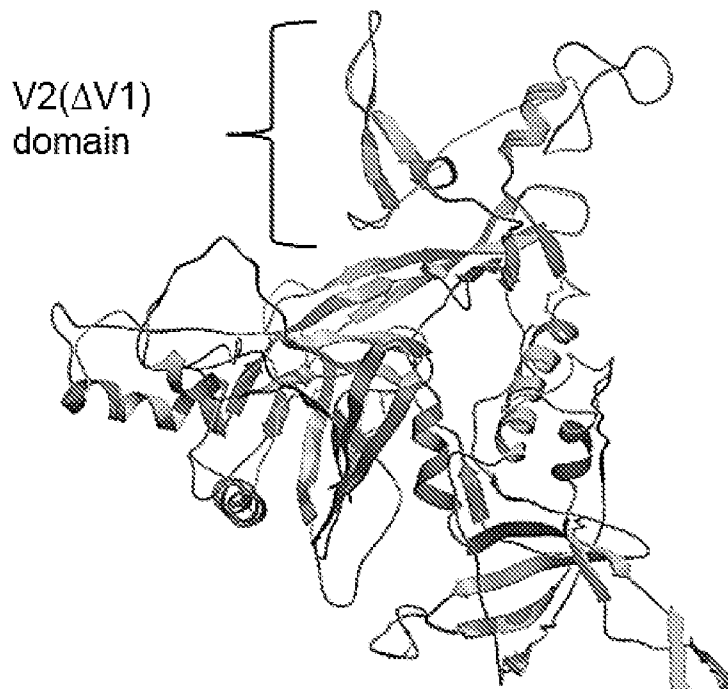
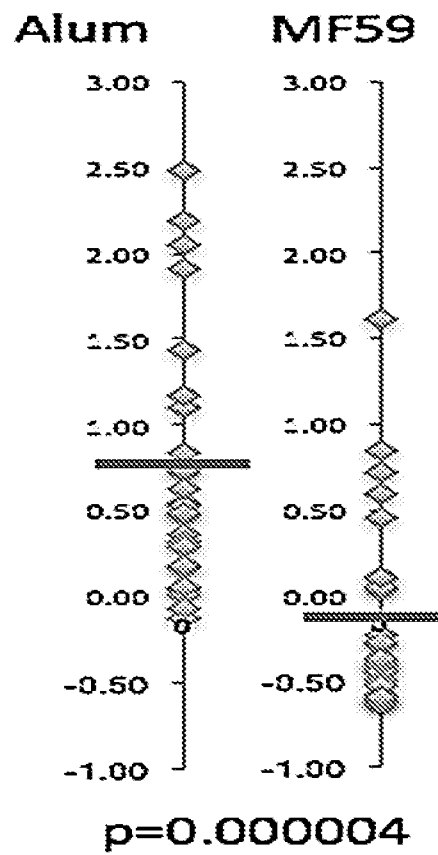
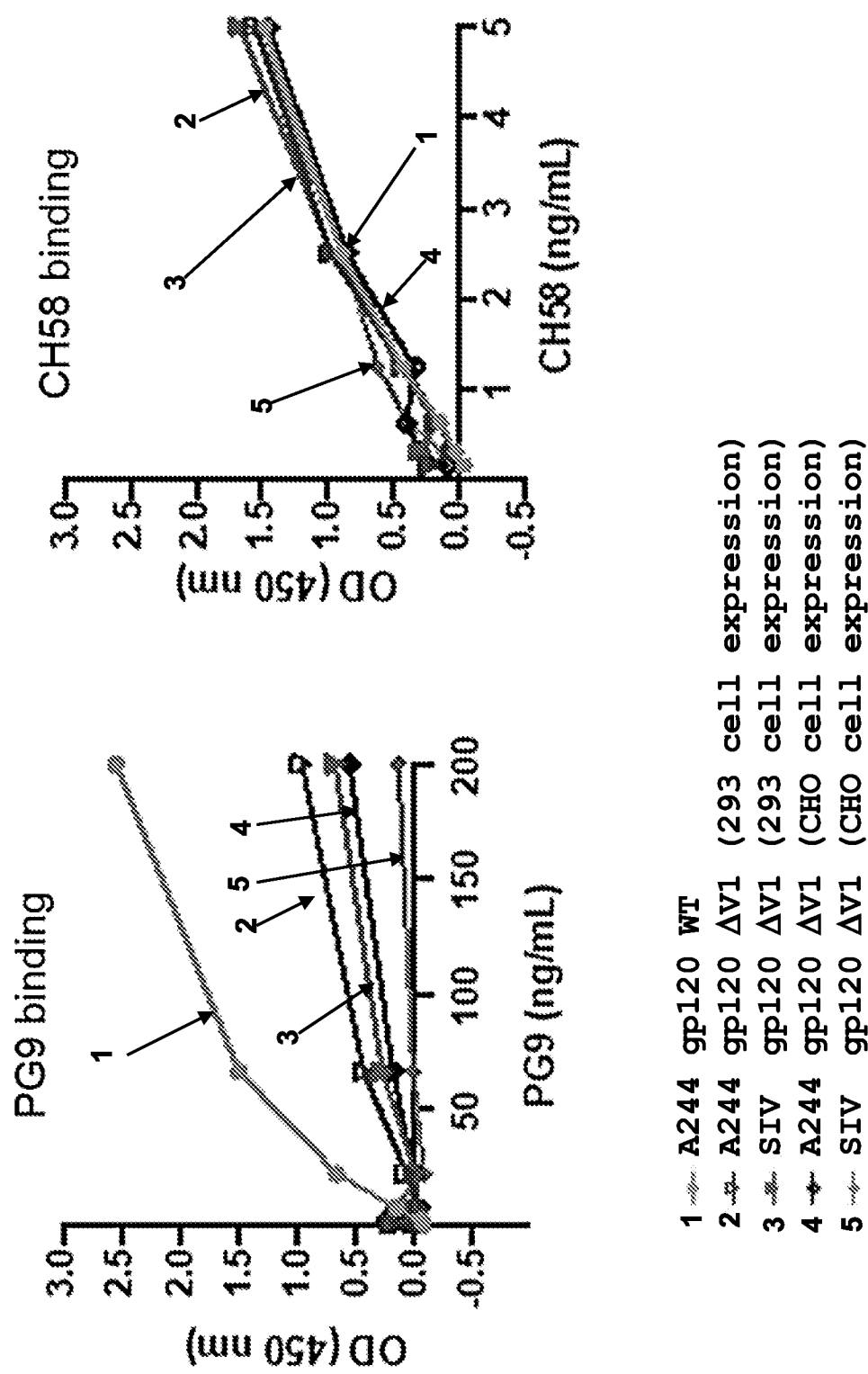
FIG. 14A**FIG. 14B**

FIG. 15



INTERNATIONAL SEARCH REPORT

International application No
PCT/US2019/057268

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12N15/10
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)
C12N C40B

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, BIOSIS, Sequence Search, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	LI YANPENG ET AL: "The V1 region of gp120 is preferentially selected during SIV/HIV transmission and is indispensable for envelope function and virus infection", VIROLOGICA SINICA, SPRINGER, DE, vol. 31, no. 3, 19 April 2016 (2016-04-19), pages 207-218, XP035991869, ISSN: 1674-0769, DOI: 10.1007/S12250-016-3725-5 [retrieved on 2016-04-19] abstract Materials and Methods ----- -/-	1-33



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

16 December 2019

Date of mailing of the international search report

16/03/2020

Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040,
Fax: (+31-70) 340-3016

Authorized officer

Keller, Yves

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2019/057268

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2004/053100 A2 (SCRIPPS RESEARCH INST [US]; BURTON DENNIS R [US] ET AL.) 24 June 2004 (2004-06-24) abstract paragraph [0013] - paragraph [0018] table 1 -----	1-33
X	EP 1 433 851 A2 (CHIRON CORP [US]) 30 June 2004 (2004-06-30) page 3 abstract page 39 figure 52 claims 3,4,40-60 -----	1-33
X	TINGTING YUAN ET AL: "HIV-1 Envelope Glycoprotein Variable Loops Are Indispensable for Envelope Structural Integrity and Virus Entry", PLOS ONE, vol. 8, no. 8, 1 August 2013 (2013-08-01), page e69789, XP055323451, DOI: 10.1371/journal.pone.0069789 the whole document -----	1-33
X	S. BASMACIOGULLARI ET AL: "Identification of Conserved and Variable Structures in the Human Immunodeficiency Virus gp120 Glycoprotein of Importance for CXCR4 Binding", JOURNAL OF VIROLOGY., vol. 76, no. 21, 1 November 2002 (2002-11-01), pages 10791-10800, XP055652796, US ISSN: 0022-538X, DOI: 10.1128/JVI.76.21.10791-10800.2002 the whole document -----	1-11, 20-33
T	J.-P. JULIEN ET AL: "Crystal Structure of a Soluble Cleaved HIV-1 Envelope Trimer", SCIENCE, vol. 342, no. 6165, 20 December 2013 (2013-12-20), pages 1477-1483, XP055292533, ISSN: 0036-8075, DOI: 10.1126/science.1245625 the whole document ----- -/--	

INTERNATIONAL SEARCH REPORT

International application No

PCT/US2019/057268

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	GZYL J ET AL: "Effect of partial and complete variable loop deletions of the human immunodeficiency virus type 1 envelope glycoprotein on the breadth of gp160-specific immune responses", VIROLOGY, ELSEVIER, AMSTERDAM, NL, vol. 318, no. 2, 20 January 2004 (2004-01-20), pages 493-506, XP004490187, ISSN: 0042-6822, DOI: 10.1016/J.VIROL.2003.10.009 the whole document -----	5,6, 8-11, 20-33

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2019/057268

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-33

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2019/057268

Patent document cited in search report		Publication date		Patent family member(s)		Publication date	
WO 2004053100	A2	24-06-2004	AU	2003300870	A1	30-06-2004	
			WO	2004053100	A2	24-06-2004	

EP 1433851	A2	30-06-2004	NONE				

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-33

A HIV-1 gp protein having a deletion in the V1 loop and use of said protein for the manufacture of a vaccine and treatment of AIDS.

2. claims: 34-39

Use of a peptide for from a GP protein of HIV-1 for the prognosis of an immune response to HIV-1
