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(54) **Title:** PRIME-BOOST VACCINATION FOR VIRAL INFECTION

(57) **Abstract:** This disclosure relates to methods of inhibiting a viral infection, such as an HIV infection. The methods include administering a prime-boost vaccination to a subject, including a primer vaccine and a booster vaccine. The primer vaccine includes a DNA or adenoviral vector that expresses a viral antigen. The booster vaccine includes a lymphocytic choriomeningitis virus vector that expresses a mismatch of the viral antigen. In several embodiments, the viral antigen is an HIV antigen, such as HIV Env.



PRIME-BOOST VACCINATION FOR VIRAL INFECTION

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Application No.
5 61/489,205, filed May 23, 2011, which is incorporated by reference in its entirety.

FIELD

This disclosure relates to the inhibition of viral infection, such as infection
with human immunodeficiency virus (HIV) or Ebola virus. More specifically, the
10 present disclosure relates to prime-boost vaccination with a mis-matched viral
antigen.

BACKGROUND

Effective vaccination methods for protecting against viral pathogens are
15 gravely needed. For example, over 30 million people are infected with HIV
worldwide, and 2.5 to 3 million new 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 a
vaccine that prevents the spread of HIV infection.

20 Initial attempts at generating neutralizing antibodies by vaccination with
recombinant HIV gp120 protein analogous to some highly effective vaccines have
thus far proved unsuccessful in generating sufficient protective immunity against
HIV. For example, in 2009, a large multicenter, double-blind, placebo-controlled
clinical study revealed that priming immunization with ALVAC-HIV (a canarypox
25 vector vaccine expressing HIV Env, Gag and Pro) followed by AIDSVAX B/E
(recombinant HIV Env gp120 vaccine) boost can reduce the risk of HIV infection
amongst heterosexuals. However, the infection rate was only reduced by 31 percent
and the vaccine only induced short-term protection.

SUMMARY

A novel prime-boost vaccination method for the inhibition of viral infection is provided. The prime-boost vaccination uses heterologous vaccine vectors and has the ability to elicit robust cellular and humoral immune responses. The prime-boost
5 vaccination strategy uses a gene-based viral vector, replication-defective lymphocytic choriomeningitis virus (LCMV) vector in combination with plasmid DNA or adenoviral vectors to express a viral antigen.

The method is useful to inhibit multiple types of viral infection in a subject, including filovirus (such as Ebola virus) infection and lentivirus (such as HIV)
10 infection. In several embodiments, the method includes administering a prime-boost vaccination to the subject, which includes the administration of a primer vaccine, followed by a booster vaccine, to the subject. The primer vaccine includes a recombinant adenoviral vector (rAd) or a DNA vector expressing a viral antigen (such as an HIV antigen). The booster vaccine includes a replication-defective
15 lymphocytic choriomeningitis virus (LCMV) vector expressing a mismatch of the viral antigen.

In some embodiments, one or more open reading frames encoding glycoprotein (GP), nucleoprotein (NP), matrix protein Z and/or RNA-dependent RNA polymerase L are removed or mutated from the genome of the replication-
20 defective LCMV to prevent replication in normal cells but still allow gene expression in LCMV vector-infected cells. In some examples, the second lentivirus antigen is expressed under control of one or more of the LCMV 5' UTR and 3' UTR of the S segment, and 5' UTR and 3' UTR of the L segment.

In several embodiments, the viral antigen is a lentiviral antigen, such as an
25 HIV antigen. For example, the viral antigen can include one or more of HIV-1 Gag protein, HIV-1 Pol protein, HIV-1 Env protein, HIV-1 Tat protein, HIV-1 Reverse Transcriptase (RT) protein, HIV-1 Vif, protein, HIV-1 Vpr protein, HIV-1 Vpu protein, HIV-1 Vpo protein, HIV-1 Integrase protein, HIV-1 Nef protein, and a fusion protein comprising all or part of an HIV-1 Gag protein, HIV-1 Pol protein, or
30 HIV-1 Env protein or an immunogenic fragment thereof. In one example, the viral

antigen is HIV Env protein, or an immogenic fragment thereof, or a modified HIV Env that retains at least one epitope of HIV Env.

The mismatch of the viral antigen includes a primary amino acid sequence that differs from that of the reference viral antigen by at least one amino acid.

5 In one example, the viral antigen is an HIV antigen and the mismatch of the viral antigen is a mismatch of the HIV antigen. For example, in one embodiment, the viral antigen is HIV gp140 and mismatch of the viral antigen is HIV gp145. In another embodiment, the viral antigen is HIVgp140ΔCFI and the mismatch of the viral antigen is HIV gp145ΔCFI. In further embodiments, the viral antigen is
 10 HIVgp140ΔCFIΔV1V2 and the mismatch of the viral antigen is HIV gp145ΔCFIΔV1V2.

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

BRIEF DESCRIPTION OF THE DRAWINGS

FIGs. 1A-1C show that rLCMV vectors are replication defective in cell culture, transduce murine dendritic cells (DCs) and elicit CD8 T cells. (**FIG. 1A**)
 20 LCMV GP expressing 293T/LCMV-GP or wild type 293T cells were infected either with wild type LCMV (LCMV wt) or with rLCMV expressing several HIV and SIV antigens (rLCMV/SIVgag (SEQ ID NO: 212), rLCMV/SIVgp140, rLCMV/SIVgp145 (SEQ ID NO: 214), rLCMV/HIVgp145 (SEQ ID NO: 216)) at an MOI of 0.01 and viral propagation was measured over time as immunofocus
 25 forming units in the supernatant. (**FIG. 1B**) Murine plasmacytoid, myeloid and lymphoid DCs were isolated by MACS sorting from spleens of BALB/c mice and infected with rLCMV/HIVgp145 at a multiplicity of infection (MOI) of 0.01, 0.1, 1 and 10, and infectivity was determined 36 hours post-infection by intracellular staining of LCMV nucleoprotein with monoclonal PE-labeled anti-NP antibody VL-
 30 4 using flow cytometry. (**FIG. 1C**) BALB/c (left graph) and C57BL/6 (right graph) mice were immunized with either rLCMV/HIVgp145 or rLCMV/SIVgag vaccine

vectors, and the antiretroviral CD8 T-cell responses in PBMCs were measured by tetramer staining.

FIGs. 2A-2C show the results of prime-boost regimens using DNA, adenovirus and LCMV vaccine vectors. BALB/c mice were immunized using different combinations of DNA, adenovirus and LCMV encoding either gp140 or gp145 of the HIV envelope gene. The interval between the two immunizations was 4 weeks. Two weeks after the boost immunization cellular immune responses were determined in PBMCs using D^d6433 MHC-I tetramer staining (**FIG. 2A**). Antiretroviral antibody responses were measured by ELISA (HIV gp coated) at two weeks after the boost for the same immunization groups (**FIG. 2B**). The time kinetics of the cellular immune response were monitored by measuring tetramer-specific T cells in PBMCs after immunization (**FIG. 2C**).

FIGs. 3A-3D show the results of rLCMV in combination with alternative adenovector rAd28. PA9 (D^d6433) MHC-I tetramer staining of PBMCs and antibody assessment by ELISA were performed after immunizing BALB/c mice with a prime-boost setting using a four week interval (**FIG. 3A**). Intracellular cytokine staining for IFN- γ and TNF- α was performed with peptide-pulsed splenocytes from the same animals (**FIG. 3B**). T cells were extracted from the gut and CD8 T cells were stained using PA9 (D^d6433) MHC-I tetramers (**FIG. 3C**). BALB/c mice were primed with rAd28 before they were boosted with different doses of rAd5 vector (10^8 and 10^5 focus forming units (FFU)) and then compared with the standard dose (10^5 FFU) used for rLCMV (**FIG. 3D**).

FIGs. 4A-4C show the results of a non-human primate study: vaccination and challenge schema, humoral and cellular immune response. Animals were divided into null and vaccine groups and were immunized at week 0 with rAd5 null or rAd5 SIV Env gp145 at 1×10^{11} viral particles (VP) intramuscularly, and boosted with LCMV null or LCMV SIV Env gp140 at 1×10^8 particle forming units (pfu) through IV, respectively, at Week 8. Animals in the null and vaccine group were challenged weekly from week 14 to week 25 with one AID₅₀ of SIV E660 virus (**FIG. 4A**). Humoral immune responses were measured by ELISA and cellular immune responses in PBMC were measured by ELISpot or intracellular cytokine

staining after stimulation with SIV Env peptide pool (**FIGS. 4B and 4C**). Env-specific cellular immune responses were determined by performing IFN- γ ELISpot (**FIG. 4B**) and intracellular cytokine staining (**FIG. 4C**) assays on PBMC at various time points post-vaccination. Lines are drawn at the mean and P-values were
 5 determined using a paired t-test. Percentage cytokine⁺ represents the frequency of cells making any of the measured cytokines.

FIGS. 5A and 5B show the polyfunctionality and phenotypes of T-cell responses after prime and boost immunization. The frequency of cytokine production after stimulation with a peptide pool for Env was determined by
 10 intracellular cytokine staining for IFN- γ , IL-2, and TNF- α at weeks 3 and 11. The results are shown in bar charts and pie charts. (**FIG. 5A**) CD4⁺ and CD8⁺ T-cell responses are shown separately for each of the 7 functional subsets. (**FIG. 5B**) Analysis of cytokine production based on the expression of CD28 and CD45RA. NL = naïve-like, CM=central memory, EM=effector memory, TE=terminal effector.

FIGS. 6A-6D show the protection of immunized monkeys against acquisition of SIV E660 infection and correlates of protection in vaccinated animals. (**FIG. 6A**) Kaplan-Meier curves for SIV acquisition are shown for the 10 monkeys which were vaccinated using rAd5 encoding SIV *env*, then boosted 8 weeks later with an LCMV vector encoding SIV *env* in comparison with mock (null vector) vaccinated animals.
 20 Weekly challenges were discontinued in infected animals upon detection of SIV viral loads in plasma. The reduction in infection per challenge was 82% while cumulative protection over the course of the study was 62% (p=0.01 by the logrank test). (**FIG. 6B**) For animals that acquired SIV infection during the challenge period independent of whether vaccinated or not, peak plasma SIV viral loads were
 25 recorded each week upon detection. Geometric means with SEMs are plotted for 8 control animals and 3 vaccinated animals that were infected. At week 11, whole blood in EDTA or serum samples were obtained from vaccinated animals. SIV-specific cellular immune responses in PBMC were quantified after *in vitro* stimulation with Env peptide pools and were either analyzed by ELISpot formation
 30 or cytokine production of CD4⁺ and CD8⁺ T cells. (**FIG. 6C**) Cellular immune responses of infected and non-infected vaccinated monkeys were analyzed. (**FIG.**

6D) The serum samples were tested for the presence of SIV env binding antibodies (ELISA), or neutralizing activity against an SIV smE660 Tier 1 clone or smE660 swarm was assessed using TZMbl cells or human PBMCs. The two groups were then compared using statistical analysis.

5 **FIG. 7** shows the results of interval optimization of prime and boost. Time intervals were tested of 2, 4, 6, 8 or 10 weeks between an adenovirus prime and an LCMV boost. Immunogenicity was assessed by IFN- γ production after peptide pool stimulation three weeks after the last immunization.

FIGs. 8A-8B show the intracellular cytokine staining and polyfunctionality
10 of T-cell responses at two weeks after the boost. Spleens were harvested, crushed and then single cell suspensions were pulsed with the HIV envelope peptide pool. (**FIG. 8A**) Intracellular cytokine staining (IFN- γ , TNF- α and IL-2) was performed and respective frequencies for CD4⁺ and CD8⁺ T cells were determined. (**FIG. 8B**) Using SPICE™ software, polyfunctionality of the T cell responses was calculated
15 and displayed.

FIGs. 9-10 are plasmid maps of selected immunogen and vector combinations: VRC 2338 pFL005 LCMV SIV-ENV gp160 (SEQ ID NO: 210); VRC 2335 pFL002 LCMV SIV-gag (SEQ ID NO: 212); VRC 2336 pFL003 LCMV SIV-gp145 mac239 (SEQ ID NO: 214); and VRC 2337 pFL004 LCMV HIV gp145
20 delta CFI Clade B (SEQ ID NO: 216).

FIGs 11A-11B are a set of graphs illustrating that anti-CD4 antibodies were not detected in vaccinated monkeys. (**FIG. 11A**) Purified human CD4 protein was used to coat ELISA plates and anti-CD4 IgG in monkey sera (1:50 dilution) from monkeys in the null or vaccine group at pre-vaccination (Week 0) and post-
25 vaccination (Week 14) were determined. (**FIG. 11B**) HEK 293 cells transfected with plasmid encoding human CD4 were stained with monkey sera and detected with PE-labeled anti-monkey IgG antibodies. There was no significant difference between null and vaccine monkeys at Week 0 or Week 14 ($p < 0.05$).

FIG. 12 illustrates that anti-Ebola virus glycoprotein (GP) (Zaire) specific
30 antibody was detected in the serum of mice immunized with the indicated Ebola virus antigens as a single immunization or a prime-boost immunization. The

following Ebola virus antigens were used for the assays described in this figure: Ebola virus glycoprotein Zaire (Z); Ebola virus glycoprotein Zaire single mutation (smZ); Ebola virus glycoprotein Zaire double mutation (dmZ). The smZ and dmZ viral antigens are mis-match antigens of the Z viral antigen. Groups of five B6D2F1 mice were intramuscularly injected once with LCMV expressing smZ or dmZ at 10^7 FFU/animal as single immunization or as boosting agent following the prime immunization of Ad5 or cAd3 vectors expressing Z protein (10^9 viral particles (VP)/animal). Serum was collected from each animal 14 days after the last immunization, and Z protein specific IgG concentration was measured based on ELISA against Z protein in cell lysate from HEK293 cells expressing the Z protein. The average concentration of anti-Z protein specific antibody within each animal group is indicated. Values are shown as mean of group of five mice; * $p < 0.05$.

FIGs. 13A-13B illustrate that a prime-boost immunization protocol enhances T cell response to the Ebola virus glycoprotein antigen. Groups of five B6D2F1 mice were intramuscularly immunized once with Ad5 or cAd3 expressing Z protein at 10^9 viral particles (VP), or with replication defective LCMV expressing smZ or dmZ at 10^7 FFU. Additional groups of five B6D2F1 mice were intramuscularly injected (primed) once with Ad5 or cAd3 expressing Z protein at 10^9 VP, and three weeks following the prime injection, treated with intramuscularly injection (boost) of LCMV vector expressing smZ or dmZ at 10^7 FFU. The mice were euthanized two weeks following the boost injection. Single cells from the spleen were stimulated with the Z peptide pool for five hours followed by staining with mAbs against surface markers and cytokines, and flow cytometry analysis. **FIG 13A** shows the percentages of cytokine-producing $CD4^+$ cells by flow cytometry: left panel ($INF-\gamma^+$ and $TNG-\alpha^+$); right panel ($INF-\gamma^+$ and $TNG-\alpha^-$). **FIG. 13B** shows the percentages of cytokine-producing $CD8^+$ T cells by flow cytometry: left panel ($INF-\gamma^+$ and $TNG-\alpha^+$); right panel ($INF-\gamma^+$ and $TNG-\alpha^-$); by flow cytometry. Values are shown as mean $\pm$ SE of group of five mice; * $p < 0.05$.

FIG. 14 illustrates plasmid maps of selected immunogen and vector combinations: VRC6632 p195 Ebola GP (Z) (F535R) (SEQ ID NO: 223) is a LCMMV construct encoding Ebola Virus glycoprotein (Zaire) with a F535R amino

acid substitution (smZ) and VRC6633 p195 Ebola GP (Z) (F535R/G536A) (SEQ ID NO: 226) is a LCMMV construct encoding Ebola Virus glycoprotein (Zaire) with F535R and G536A amino acid substitutions (dmZ).

5

SEQUENCE LISTING

The nucleic and amino acid sequences listed in the accompanying sequence listing 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.

10

The Sequence Listing is submitted as an ASCII text file, Annex C/St.25 text file, created on May 22, 2012, 528kb, which is incorporated by reference herein.

In the accompanying sequence listing:

15

SEQ ID NOs: 1-77, 79, 80, 148, 149 and 196 are the amino acid sequences of exemplary HIV antigens.

SEQ ID NO: 78 is the amino acid sequence of the HXB2 core.

SEQ ID NOs: 81-147, 150-161 and 199-208 are exemplary nucleic acid sequences encoding HIV antigens.

20

SEQ ID NO: 162 is the amino acid sequence of an exemplary modified V1/V2 loop.

SEQ ID NO: 163 is the amino acid sequence of an exemplary V1 wild type loop.

SEQ ID NOs: 164-165 are the amino acid sequence of exemplary modified V1 loops.

25

SEQ ID NOs: 166-167 are the amino acid sequence of exemplary modified V2 loops.

SEQ ID NOs: 168-181 are the amino acid sequence of exemplary modified V3 loops.

30

SEQ ID NOs: 182-186 are the amino acid sequence of exemplary modified V4 loops.

SEQ ID NOS: 187-195 are the amino acid sequence of exemplary modified V5 loops.

SEQ ID NO: 197 is the amino acid sequence of an exemplary wild type V5 loop.

5 **SEQ ID NO: 198** is the amino acid sequence of an exemplary wild type V3 loop.

SEQ ID NO: 209 is an exemplary nucleotide sequence encoding SIV-Env gp160, which can be used as an insert sequence in a viral vector.

10 **SEQ ID NO: 210** is the nucleotide sequence of the LCMV vector encoding SIV-Env gp160.

SEQ ID NO: 211 is an exemplary nucleotide sequence encoding SIV-gag, which can be used as an insert sequence in a viral vector.

SEQ ID NO: 212 is the nucleotide sequence of an LCMV vector encoding SIV-gag.

15 **SEQ ID NO: 213** is an exemplary nucleotide sequence encoding SIV-gp145 mac239, which can be used as an insert sequence in a viral vector.

SEQ ID NO: 214 is the nucleotide sequence of an LCMV vector encoding SIV-gp145 mac239.

20 **SEQ ID NO: 215** is an exemplary nucleotide sequence encoding HIV gp145 Δ CFI Clade B, which can be used as an insert sequence in a viral vector.

SEQ ID NO: 216 is the nucleotide sequence of an LCMV vector encoding HIV gp145 Δ CFI Clade B.

SEQ IDNO: 217 is the amino acid sequence of gp120 according to the HXB2 numbering scheme.

25 **SEQ IDNO: 218** is an exemplary nucleic acid sequence encoding Ebola Virus glycoprotein (Zaire) (Z).

SEQ IDNO: 219 is the amino acid sequence of Ebola Virus glycoprotein (Zaire) (Z).

30 **SEQ IDNO: 220** is the sequence of an LCMV construct encoding Ebola Virus glycoprotein (Zaire) (Z).

SEQ IDNO: 221 is an exemplary nucleic acid sequence encoding Ebola Virus glycoprotein (Zaire) with a F535R amino acid substitution (smZ).

SEQ IDNO: 222 is the amino acid sequence of Ebola Virus glycoprotein (Zaire) with a F535R amino acid substitution (smZ).

5 **SEQ IDNO: 223** is the sequence of an LCMV construct encoding Ebola Virus glycoprotein (Zaire) with a F535R amino acid substitution (smZ).

SEQ IDNO: 224 is an exemplary nucleic acid sequence encoding Ebola Virus glycoprotein (Zaire) with F535R and G536A amino acid substitutions (dmZ).

10 **SEQ IDNO: 225** is the amino acid sequence of Ebola Virus glycoprotein (Zaire) with F535R and G536A amino acid substitutions (dmZ).

SEQ IDNO: 226 is the sequence of an LCMV construct encoding Ebola Virus glycoprotein (Zaire) with F535R and G536A amino acid substitutions (dmZ).

DETAILED DESCRIPTION

15 As the human immunodeficiency virus (HIV) pandemic continues to infect millions of people each year, the need for an effective vaccine increases. However the development of such a vaccine has been stymied due to the difficulty in developing therapeutic method and vaccination strategies capable of eliciting a sufficient immune response that adequately inhibits HIV infection. The current
20 disclosure meets these needs.

Disclosed herein, for example, is the surprising discovery of the immunogenicity and protective efficacy of a mis-matched *env* vaccine delivered by heterologous prime-boost vaccination. Recombinant LCMV vectors demonstrate surprising efficacy in alternative prime boost combinations with plasmid DNA and
25 adenoviral vaccine vectors. The assays conducted with these vectors indicate that unmatched *env* alone is sufficient to confer protection against a heterologous virus strain. Furthermore, prime-boost vaccination with an embodiment of the disclosed therapeutic methods stimulates multiple arms of the immune system, including antigen-specific CD4 and CD8 T cells in spleen, PBMCs, gut associated tissue, as
30 well as HIV-1 Env-specific antibodies. The effectiveness of the disclosed therapeutic methods was confirmed *in vivo* using a non-human primate model. An

embodiment of the disclosed therapeutic methods protected against SIV infection in the non-human primate model after repetitive mucosal challenge with an efficacy of 82% percent per exposure and 62% cumulatively. Thus, disclosed herein for the first time is a novel prime-boost vaccination method using heterologous viral vectors
5 expressing a mis-matched Env gene that can prevent lentiviral infection.

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
10 Benjamin Lewin, *Genes V*, published by Oxford University Press, 1994 (ISBN 0-19-854287-9); Kendrew et al. (eds.), *The Encyclopedia of Molecular Biology*, published by Blackwell Science Ltd., 1994 (ISBN 0-632-02182-9); and Robert A. Meyers (ed.), *Molecular Biology and Biotechnology: a Comprehensive Desk Reference*, published by VCH Publishers, Inc., 1995 (ISBN 1-56081-569-8).

15 Unless otherwise explained, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs. The singular terms “a,” “an,” and “the” include plural referents unless context clearly indicates otherwise. Similarly, the word “or” is intended to include “and” unless the context clearly indicates otherwise. The term
20 “comprises” means “includes.” Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of this disclosure, suitable methods and materials are described below. In case of conflict, the present specification, including explanations of terms, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be
25 limiting

To facilitate review of the various embodiments of this disclosure, the following explanations of specific terms are provided:

Adjuvant: A vehicle used to enhance antigenicity; such as a suspension of
30 minerals (alum, aluminum hydroxide, aluminum phosphate) on which antigen is adsorbed; or water-in-oil emulsion in which antigen solution is emulsified in oil

(MF-59, Freund's incomplete adjuvant), sometimes with the inclusion of killed mycobacteria (Freund's complete adjuvant) to further enhance antigenicity (inhibits degradation of antigen and/or causes influx of macrophages). Adjuvants also include immunostimulatory molecules, such as cytokines, costimulatory molecules, and for example, immunostimulatory DNA or RNA molecules, such as CpG oligonucleotides.

An adjuvant is a substance distinct from the antigen for which an immune response is desired. In several embodiments, an adjuvant enhances T cell activation by promoting the innate immune response leading to the accumulation and activation of other leukocytes (accessory cells) at the site of antigen exposure. Thus, adjuvants may enhance accessory cell expression of T cell-activating co-stimulators and cytokines and may also prolong the expression of peptide-MHC complexes on the surface of antigen-presenting cells.

Administration: The introduction of a composition or agent into a subject by a chosen route. Administration can be local or systemic. For example, if the chosen route is intravenous, the composition is administered by introducing the composition into a vein of the subject.

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

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

Animal: A living multicellular vertebrate organism, a category that includes, for example, mammals and birds. A "mammal" includes both human and non-

human mammals, such as mice. The term "subject" includes both human and animal subjects, such as non-human primates.

Antibody: A polypeptide substantially encoded by an immunoglobulin gene or immunoglobulin genes, or fragments thereof, which specifically binds and
5 recognizes an analyte (antigen). Immunoglobulin genes include the kappa, lambda, alpha, gamma, delta, epsilon and mu constant region genes, as well as the myriad immunoglobulin variable region genes.

Antibodies exist, for example as intact immunoglobulins and as a number of well characterized fragments produced by digestion with various peptidases. For
10 instance, Fabs, Fvs, and single-chain Fvs (SCFvs). This includes intact immunoglobulins and the variants and portions of them well known in the art, such as Fab' fragments, F(ab)[']₂ fragments, single chain Fv proteins ("scFv"), and disulfide stabilized Fv proteins ("dsFv"). A scFv protein is a fusion protein in which a light chain variable region of an immunoglobulin and a heavy chain variable region of an
15 immunoglobulin are bound by a linker, while in dsFvs, the chains have been mutated to introduce a disulfide bond to stabilize the association of the chains. The term also includes genetically engineered forms such as chimeric antibodies (such as humanized murine antibodies), heteroconjugate antibodies such as bispecific antibodies). See also, Pierce Catalog and Handbook, 1994-1995 (Pierce Chemical
20 Co., Rockford, IL); Kuby, J., Immunology, 3rd Ed., W.H. Freeman & Co., New York, 1997.

Antigen: A polypeptide that can stimulate the production of antibodies or a T cell response in an animal, including polypeptides that are injected or absorbed into an animal. An antigen reacts with the products of specific humoral or cellular
25 immunity, including those induced by heterologous antigens, such as the disclosed antigens. "Epitope" or "antigenic determinant" refers to the region of an antigen to which B and/or T cells respond. In one embodiment, T cells respond to the epitope, when the epitope is presented in conjunction with an MHC molecule. Epitopes can be formed both from contiguous amino acids or noncontiguous amino acids
30 juxtaposed by tertiary folding of a protein. Epitopes formed from contiguous amino acids are typically retained on exposure to denaturing solvents whereas epitopes

formed by tertiary folding are typically lost on treatment with denaturing solvents. An epitope typically includes at least 3, and more usually, at least 5, about 9, or about 8-10 amino acids in a unique spatial conformation. Methods of determining spatial conformation of epitopes include, for example, x-ray crystallography and
5 nuclear magnetic resonance.

Immunogenic polypeptides and immunogenic peptides are non-limiting examples of antigens. In some examples, antigens include polypeptides derived from a pathogen of interest, such as a virus. An antigen that can stimulate the production of antibodies or a T cell response in a subject to a polypeptide expressed
10 by a virus is a viral antigen. An “HIV antigen” can stimulate the production of antibodies or a T cell response in a subject to a polypeptide expressed by HIV. In some embodiments, an HIV antigen is a polypeptide expressed by HIV, such as gp160, or a fragment thereof, such as gp145, gp140, gp120 or gp41.

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

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

Contacting: Placement in direct physical association; includes both in solid and liquid form.

25 **Control:** A reference standard. In some embodiments, the control is a sample obtained from a healthy patient. In other embodiments, the control is a tissue sample obtained from a patient diagnosed with HIV infection. In still other embodiments, the control is a historical control or standard reference value or range of values (such as a previously tested control sample, such as a group of HIV
30 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
5 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%.

10 **Degenerate variant and conservative variant:** A polynucleotide encoding a polypeptide or an antibody that includes a sequence that is degenerate as a result of the genetic code. For example, a polynucleotide encoding an antigen, such as an HIV protein, 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
15 codon. Therefore, all degenerate nucleotide sequences are included as long as the amino acid sequence encoded by the nucleotide sequence is unchanged. Because of the degeneracy of the genetic code, a large number of functionally identical nucleic acids encode any given polypeptide. For instance, the codons CGU, CGC, CGA, CGG, AGA, and AGG all encode the amino acid arginine. Thus, at every position
20 where an arginine is specified within a protein encoding sequence, the codon can be altered to any of the corresponding codons described without altering the encoded protein. Such nucleic acid variations are "silent variations," which are one species of conservative variations. Each nucleic acid sequence herein that encodes a polypeptide also describes every possible silent variation. One of skill will recognize
25 that each codon in a nucleic acid (except AUG, which is ordinarily the only codon for methionine) can be modified to yield a functionally identical molecule by standard techniques. Accordingly, each "silent variation" of a nucleic acid which encodes a polypeptide is implicit in each described sequence.

Furthermore, one of ordinary skill will recognize that individual
30 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.

Conservative amino acid substitutions providing functionally similar amino acids are well known in the art. The following six groups each contain amino acids that are 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).
- Not all residue positions within a protein will tolerate an otherwise "conservative" substitution. For instance, if an amino acid residue is essential for a function of the protein, even an otherwise conservative substitution may disrupt that activity.

Effective amount: An amount of agent, such as nucleic acid vaccine or other agent that is sufficient to generate a desired response, such as reduce or eliminate a sign or symptom of a condition or disease, such as AIDS. For instance, this can be the amount necessary to inhibit viral replication or to measurably alter outward symptoms of the viral infection, such as increase of T cell counts in the case of an HIV-1 infection. In general, this amount will be sufficient to measurably inhibit virus (for example, HIV) replication or infectivity. When administered to a subject, a dosage will generally be used that will achieve target tissue concentrations (for example, in lymphocytes) that has been shown to achieve in vitro inhibition of viral replication. In some examples, an "effective amount" is one that treats (including prophylaxis) one or more symptoms and/or underlying causes of any of a disorder or disease, for example to treat HIV. In one example, an effective amount is a therapeutically effective amount. In one example, an effective amount is an amount that prevents one or more signs or symptoms of a particular disease or condition from developing, such as one or more signs or symptoms associated with AIDS.

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

Expression Control Sequences: Nucleic acid sequences that regulate the expression of a heterologous nucleic acid sequence to which it is operatively linked. Expression control sequences are operatively linked to a nucleic acid sequence when the expression control sequences control and regulate the transcription and, as appropriate, translation of the nucleic acid sequence. Thus expression control sequences can include appropriate promoters, enhancers, transcription terminators, a start codon (ATG) in front of a protein-encoding gene, splicing signal for introns, maintenance of the correct reading frame of that gene to permit proper translation of mRNA, and stop codons. The term "control sequences" is intended to include, at a minimum, components whose presence can influence expression, and can also include additional components whose presence is advantageous, for example, leader sequences and fusion partner sequences. Expression control sequences can include a promoter. A promoter is a minimal sequence sufficient to direct transcription. Also included are those promoter elements which are sufficient to render promoter-dependent gene expression controllable for cell-type specific, tissue-specific, or inducible by external signals or agents; such elements may be located in the 5' or 3' regions of the gene. Both constitutive and inducible promoters are included (see for example, Bitter et al, Methods in Enzymology 153:516-544, 1987).

A polynucleotide can be inserted into an expression vector, such as a viral vector that contains a promoter sequence, which facilitates the efficient transcription of the inserted genetic sequence.

HIV Envelope protein (Env): The HIV envelope protein is initially synthesized as a longer precursor protein of 845-870 amino acids in size, designated gp160. gp160 forms a homotrimer and undergoes glycosylation within the Golgi apparatus. *In vivo*, it is then cleaved by a cellular protease into gp120 and gp41. gp120 contains most of the external, surface-exposed, domains of the HIV envelope glycoprotein complex, and it is gp120 which binds both to cellular CD4 receptors and to cellular chemokine receptors (such as CCR5). gp41 contains a

transmembrane domain and remains in a trimeric configuration; it interacts with gp120 in a non-covalent manner.

Mature gp120 wildtype polypeptides have about 500 amino acids in the primary sequence. gp120 is heavily N-glycosylated giving rise to an apparent
5 molecular weight of 120 kD. The polypeptide is comprised of five conserved regions (C1-C5) and five regions of high variability (V1-V5). Exemplary sequence of wt gp120 polypeptides are available is GENBANK®, for example accession numbers AAB05604 and AAD12142 (as available on October 16, 2009), incorporated by reference herein. It is understood that there are numerous variation
10 in the sequence of gp120 from what is given in GENBANK®, for example accession numbers AAB05604 and AAD12142, and that these variants are recognized in the art as gp120.

The gp120 core has a molecular structure, which includes two domains: an “inner” domain (which faces gp41) and an “outer” domain (which is mostly exposed
15 on the surface of the oligomeric envelope glycoprotein complex). The two gp120 domains are separated by a “bridging sheet” that is not part of either domain. The gp120 core comprises 25 beta strands, 5 alpha helices, and 10 defined loop segments.

The amino acid sequence of an example of gp41 is set forth in GENBANK®
20 Accession No. CAD20975 (as available on October 16, 2009) which is incorporated by reference herein. It is understood that the sequence of gp41 can vary from that given in GENBANK® Accession No. CAD20975.

The HIV Env antigens disclosed herein can include amino acid substitutions, deletions or insertions, and still retain immunogenic properties, such that they are
25 capable of inducing an immune response to HIV Env in a subject. For example, various deletions of HIV Env are known, such as gp150 (aa 1-752), gp145 (aa 1-704), gp140 (aa 1-680) (amino acid numbering with reference to the HXB2 HIV Env protein). Additionally, deletions of all or part of 1, 2, 3, 4, or all 5 of the variable regions (V1, V2, V3, V4 and V5) can be included in the HIV Env antigen.
30 Reference to an HIV Env protein (or fragment thereof) that refers to “ΔV1” indicates that all or part of the V1 region of the HIV Env protein has been deleted

(similar nomenclature is used for the other variable regions). Further, all or part of functional regions of the HIV Env protein can be deleted or substituted, such as the cleavage site, the fusion peptide heptad repeat 1, heptad repeat 2, or the region internal to the two heptad repeats. Reference to an HIV Env protein (or fragment thereof) that refers to “ Δ CFI” indicates that the HIV Env or fragment thereof, has been modified to lack the cleavage site, the fusion peptide and the region internal to the two heptad repeats. An exemplary HIV Env protein with Δ CFI modification is provided as SEQ ID NO: 215. (See, also Int. Pat. App. No. PCT/US2004/030284, which provides examples of HIV Env protein mutants and is incorporated by reference herein in its entirety; additionally, see Chakrabarti *et al.*, *J. Virol.*, 76: 5357-5368, 2002; and Yang *et al.*, *J. Virol.*, 78: 4029-4036, 2004, each of which is incorporated by reference herein).

The numbering used in the HIV Env derived antigens disclosed herein is relative to the HXB2 numbering scheme as set forth in *Numbering Positions in HIV Relative to HXB2CG* Bette Korber *et al.*, Human Retroviruses and AIDS 1998: A Compilation and Analysis of Nucleic Acid and Amino Acid Sequences. Korber *et al.*, Eds. Theoretical Biology and Biophysics Group, Los Alamos National Laboratory, Los Alamos, NM, which is incorporated by reference herein in its entirety. For example, an exemplary HIV-1 gp160 amino acid sequence (according to the HXB2 numbering scheme) is provided as SEQ ID NO: 217. An exemplary HIV-1 gp120 amino acid sequence is amino acids 1-511 of SEQ ID NO: 217. An exemplary amino acid sequence for gp41 is amino acids 512-856 of SEQ ID NO: 217.

Human Immunodeficiency Virus (HIV): A retrovirus that causes immunosuppression in humans (HIV disease), and leads to a disease complex known as the acquired immunodeficiency syndrome (AIDS). “HIV disease” refers to a well-recognized constellation of signs and symptoms (including the development of opportunistic infections) in persons who are infected by an HIV virus, as determined by antibody or western blot studies. Laboratory findings associated with this disease include a progressive decline in T cells. HIV includes HIV type 1 (HIV-1) and HIV type 2 (HIV-2). Related viruses that are used as

animal models include simian immunodeficiency virus (SIV), and feline immunodeficiency virus (FIV). Treatment of HIV-1 with HAART has been effective in reducing the viral burden and ameliorating the effects of HIV-1 infection in infected individuals.

5 **Highly active anti-retroviral therapy (HAART):** A therapeutic treatment for HIV infection involving administration of multiple anti-retroviral agents (*e.g.*, two, three or four anti-retroviral agents) to an HIV infected individual during a course of treatment. Non-limiting examples of antiretroviral agents include entry inhibitors (*e.g.*, enfuvirtide), CCR5 receptor antagonists (*e.g.*, aplaviroc, vicriviroc, 10 maraviroc), reverse transcriptase inhibitors (*e.g.*, lamivudine, zidovudine, abacavir, tenofovir, emtricitabine, efavirenz), protease inhibitors (*e.g.*, lopivar, ritonavir, raltegravir, darunavir, atazanavir), maturation inhibitors (*e.g.*, alpha interferon, bevirimat and vivecon). One example of a HAART regimen includes treatment with a combination of tenofovir, emtricitabine and efavirenz.

15 **Host cells:** Cells in which a vector, such as a viral vector or DNA vector, can be propagated and its nucleic acid sequences 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 20 when the term “host cell” is used.

Hybridization: Oligonucleotides and their analogs hybridize by hydrogen bonding, which includes Watson-Crick, Hoogsteen or reversed Hoogsteen hydrogen bonding, between complementary bases. Generally, nucleic acid consists of nitrogenous bases that are either pyrimidines (cytosine (C), uracil (U), and thymine 25 (T)) or purines (adenine (A) and guanine (G)). These nitrogenous bases form hydrogen bonds between a pyrimidine and a purine, and the bonding of the pyrimidine to the purine is referred to as “base pairing.” More specifically, A will hydrogen bond to T or U, and G will bond to C. “Complementary” refers to the base pairing that occurs between two distinct nucleic acid sequences or two distinct 30 regions of the same nucleic acid sequence.

“Specifically hybridizable” and “specifically complementary” are terms that indicate a sufficient degree of complementarity such that stable and specific binding occurs between the oligonucleotide (or its analog) and the DNA or RNA target. The oligonucleotide or oligonucleotide analog need not be 100% complementary to its target sequence to be specifically hybridizable. An oligonucleotide or analog is specifically hybridizable when binding of the oligonucleotide or analog to the target DNA or RNA molecule interferes with the normal function of the target DNA or RNA, and there is a sufficient degree of complementarity to avoid non-specific binding of the oligonucleotide or analog to non-target sequences under conditions where specific binding is desired. Such binding is referred to as specific hybridization.

Hybridization conditions resulting in particular degrees of stringency will vary depending upon the nature of the hybridization method of choice and the composition and length of the hybridizing nucleic acid sequences. Generally, the temperature of hybridization and the ionic strength (especially the Na^+ concentration) of the hybridization buffer will determine the stringency of hybridization, though wash times also influence stringency. Calculations regarding hybridization conditions required for attaining particular degrees of stringency are discussed by Sambrook *et al.* (ed.), *Molecular Cloning: A Laboratory Manual*, 2nd ed., vol. 1-3, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989, chapters 9 and 11.

The following is an exemplary set of hybridization conditions and is not limiting:

Very High Stringency (detects sequences that share at least 90% identity)

25	Hybridization:	5x SSC at 65°C for 16 hours
	Wash twice:	2x SSC at room temperature (RT) for 15 minutes each
	Wash twice:	0.5x SSC at 65°C for 20 minutes each

High Stringency (detects sequences that share at least 80% identity)

	Hybridization:	5x-6x SSC at 65°C-70°C for 16-20 hours
30	Wash twice:	2x SSC at RT for 5-20 minutes each
	Wash twice:	1x SSC at 55°C-70°C for 30 minutes each

Low Stringency (detects sequences that share at least 50% identity)

Hybridization: 6x SSC at RT to 55°C for 16-20 hours

Wash at least twice: 2x-3x SSC at RT to 55°C for 20-30 minutes each.

Immune response: A response of a cell of the immune system, such as a B cell, T cell, or monocyte, to a stimulus. An immune response can be a cellular response or a humoral response. In one embodiment, the response is specific for a particular antigen (an “antigen-specific response”). The response can also be a non-specific response such as production of lymphokines. 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 Th1 or a Th2 (subsets of helper T cells) response. In yet another embodiment, the response is a B cell response, and results in the production of specific antibodies.

Immunogenic polypeptide: A polypeptide which comprises an epitope or antigenic determinant such that the polypeptide will bind an MHC molecule and induce an immune response, such as a cytotoxic T lymphocyte (“CTL”) response, and/or a B cell response (for example, antibody production), and/or a T-helper lymphocyte response against the antigen from which the immunogenic polypeptide is derived.

In one embodiment, immunogenic polypeptides are identified using sequence motifs or other methods known in the art. Typically, algorithms are used to determine the “binding threshold” of polypeptides to select those with scores that give them a high probability of binding at a certain affinity and will be immunogenic. The algorithms are based either on the effects on MHC binding of a particular amino acid at a particular position, the effects on antibody binding of a particular amino acid at a particular position, or the effects on binding of a particular substitution in a motif-containing polypeptide. Within the context of an immunogenic polypeptide, a “conserved residue” is one which appears in a significantly higher frequency than would be expected by random distribution at a particular position in a polypeptide. In one embodiment, a conserved residue is one where the MHC structure may provide a contact point with the immunogenic polypeptide.

Immunogenic composition: A composition that includes an immunogenic polypeptide or nucleic acid or viral vector encoding an immunogenic polypeptide that induces a measurable immune response (such as a CTL response or measurable B cell response) against the immunogenic polypeptide. For example, in several
5 embodiments, an immunogenic composition includes a viral vector expressing an immunogenic polypeptide that induces an immune response to an epitope on the immunogenic polypeptide that is also contained on a polypeptide expressed by a viral pathogen, such as HIV. In one example an immunogenic composition includes a nucleic acid encoding an immunogenic polypeptide, such as a nucleic acid vector
10 that can be used to express the polypeptide (and thus be used to elicit an immune response against this polypeptide or an epitope on the polypeptide). In several examples, the immunogenic composition includes one or more adjuvants.

Immunotherapy: A method of evoking an immune response against a virus based on its production of target antigens. Immunotherapy based on cell-mediated
15 immune responses involves generating a cell-mediated response to cells that produce particular antigenic determinants, while immunotherapy based on humoral immune responses involves generating specific antibodies to virus that produce particular antigenic determinants. In several embodiments, immunotherapy includes administration of prime-boost vaccination to a subject.

Inhibiting or treating a disease: Inhibiting the full development of a disease or condition, for example, in a subject who is at risk for a disease such as acquired immunodeficiency syndrome (AIDS). "Treatment" refers to a therapeutic
20 intervention that ameliorates a sign or symptom of a disease or pathological condition after it has begun to develop. The term "ameliorating," with reference to a disease or pathological condition, refers to any observable beneficial effect of the treatment. Inhibiting a disease can include preventing or reducing the risk of the disease, such as preventing or reducing the risk of viral infection. The beneficial effect can be evidenced, for example, by a delayed onset of clinical symptoms of the
25 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
30 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.

5 **Isolated:** An “isolated” biological component (such as a nucleic acid molecule, protein, virus, virus like particle or organelle) has been substantially separated or purified away from other biological components in the cell of the organism in which the component naturally occurs, *e.g.*, other chromosomal and extra-chromosomal DNA and RNA, proteins and/organelles. Nucleic acids and
10 proteins that have been “isolated” include nucleic acids and proteins purified by standard purification methods. The term also embraces nucleic acids and proteins prepared by recombinant expression in a host cell as well as chemically synthesized nucleic acids, such as probes and primers.

Lymphocytic choriomeningitis virus (LCMV): An member of the
15 arenavirus family, LCMV is an envelope-bisegmented negative-strand RNA virus. The two genome segments L and S have approximate sizes of 7.2 and 3.4 kb, respectively. Each segment uses an ambisense strategy to direct the synthesis of two proteins in opposite orientations, separated by an intergenic region. The S RNA contains the nucleoprotein (NP) and the glycoprotein (GP) precursor (GPC) genes,
20 which are encoded in antigenome and genome polarity, respectively. Posttranslational processing of GPC genes produces GP-1 and -2 and has been shown to be mediated by the cellular protease S IP. GP-1 and -2 make up the spikes on the virion envelope and mediate cell entry by interaction with the host cell surface receptor. The L RNA segment codes for the virus RNA-dependent RNA
25 polymerase (L) and a small (11-kDa) RING finger protein (Z) (see, *e.g.*, Pinschewer *et al.*, Proc. Natl. Acad. Sci, 700(13): 7895-7900 (2003)). Recombinant LCMV vectors and Reverse genetic systems for the manipulation of the infectious arenavirus genome are known and described in, for example, Flatz *et al.*, Proc. Natl. Acad. Sci. U.S.A., 103:4663-4668, 2006; Sanchez and de la Torre, Virology
30 350:370, 2006; Pinschewer *et al.*, *supra*, and Flatz *et al.*, Nature Medicine, 16: 339-

345 (2010) and Int. Pat. App. No. PCT/EP2008/010994, all of which are incorporated herein by reference in their entirety.

Lymphocytes: A type of white blood cell that is involved in the immune defenses of the body. There are two main types of lymphocytes: B cells and T cells.

- 5 T cells are white blood cells critical to the immune response. T cells include, but are not limited to, CD4⁺ T cells and CD8⁺ T cells. A CD4⁺ T lymphocyte is an immune cell that carries a marker on its surface known as “cluster of differentiation 4” (CD4). These cells, also known as helper T cells, help orchestrate the immune response, including antibody responses as well as killer T cell responses. CD8⁺ T
- 10 cells carry the “cluster of differentiation 8” (CD8) marker. B cells are white blood cells critical to the antibody response. B cells mature within the bone marrow and leave the marrow expressing an antigen binding antibody on their cell surface.

Mis-matched antigen: An antigen that is similar (but not identical) to a reference antigen (such as a viral antigen), or an immunogenic fragment thereof.

- 15 The primary amino acid sequence of the mis-matched viral antigen differs by at least one amino acid compared to the primary amino acid sequence of the reference viral antigen. For example, the mis-matched antigen can have about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, about 96%, about 97%, about 98% or about 99% sequence identity with the reference antigen. In some embodiments,
- 20 the mis-matched antigen can have up to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or up to 30 amino acid substitutions, or more amino acid substitutions, compared to the reference antigen.

- In several embodiments, a mis-matched antigen is an antigen that is similar (but not identical) to an HIV antigen, such as HIV Env protein, or immunogenic
- 25 fragment thereof. For example, the mis-matched antigen can have about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, about 96%, about 97%, about 98% or about 99% sequence identity with the HIV Env protein, or immunogenic fragment thereof. In some embodiments, the mis-matched antigen can have up to 1,
- 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26,
- 30 27, 28, 29 or up to 30, or more, amino acid substitutions, deletions or insertions, compared to the HIV Env protein, or immunogenic fragment thereof. For example,

in some embodiments, HIV gp140 is a mis-matched antigen of HIV gp145. In additional examples, HIV gp140 Δ CFI Δ V1V2 is a mis-matched antigen of HIV gp145 Δ CFI Δ V1V2.

Nucleotide: "Nucleotide" includes, but is not limited to, a monomer that
5 includes a base linked to a sugar, such as a pyrimidine, purine or synthetic analogs thereof, or a base linked to an amino acid, as in a peptide nucleic acid (PNA). A nucleotide is one monomer in a polynucleotide. A nucleotide sequence refers to the sequence of bases in a polynucleotide.

Nucleic acid: A polymer composed of nucleotide units (ribonucleotides,
10 deoxyribonucleotides, related naturally occurring structural variants, and synthetic non-naturally occurring analogs thereof) linked via phosphodiester bonds, related naturally occurring structural variants, and synthetic non-naturally occurring analogs thereof. Thus, the term includes nucleotide polymers in which the nucleotides and the linkages between them include non-naturally occurring synthetic analogs, such
15 as, for example and without limitation, phosphorothioates, phosphoramidates, methyl phosphonates, chiral-methyl phosphonates, 2-O-methyl ribonucleotides, peptide-nucleic acids (PNAs), and the like. Such polynucleotides can be synthesized, for example, using an automated DNA synthesizer. The term "oligonucleotide" typically refers to short
20 polynucleotides, generally no greater than about 50 nucleotides. It will be understood that when a nucleotide sequence is represented by a DNA sequence (e.g., A, T, G, C), this also includes an RNA sequence (e.g., A, U, G, C) in which "U" replaces "T." "Nucleotide" includes, but is not limited to, a monomer that includes a base linked to a sugar, such as a pyrimidine, purine or synthetic analogs thereof, or a
25 base linked to an amino acid, as in a peptide nucleic acid (PNA).

A nucleotide is one monomer in a polynucleotide. A nucleotide sequence refers to the sequence of bases in a polynucleotide. Conventional notation is used herein to describe nucleotide sequences: the left-hand end of a single-stranded nucleotide sequence is the 5'-end; the left-hand direction of a double-stranded
30 nucleotide sequence is referred to as the 5'-direction. The direction of 5' to 3' addition of nucleotides to nascent RNA transcripts is referred to as the transcription

direction. The DNA strand having the same sequence as an mRNA is referred to as the "coding strand;" sequences on the DNA strand having the same sequence as an mRNA transcribed from that DNA and which are located 5' to the 5'- end of the RNA transcript are referred to as "upstream sequences;" sequences on the DNA strand having the same sequence as the RNA and which are 3' to the 3' end of the coding RNA transcript are referred to as "downstream sequences." "cDNA" refers to a DNA that is complementary or identical to an mRNA, in either single stranded or double stranded form.

"Encoding" refers to the inherent property of specific sequences of nucleotides in a polynucleotide, such as a gene, a cDNA, or an mRNA, to serve as templates for synthesis of other polymers and macromolecules in biological processes having either a defined sequence of nucleotides (for example, rRNA, tRNA and mRNA) or a defined sequence of amino acids and the biological properties resulting therefrom. Thus, a gene encodes a protein if transcription and translation of mRNA produced by that gene produces the protein in a cell or other biological system. Both the coding strand, the nucleotide sequence of which is identical to the rRNA sequence and is usually provided in sequence listings, and non-coding strand, used as the template for transcription, of a gene or cDNA can be referred to as encoding the protein or other product of that gene or cDNA. Unless otherwise specified, a "nucleotide sequence encoding an amino acid sequence" includes all nucleotide sequences that are degenerate versions of each other and that encode the same amino acid sequence. Nucleotide sequences that encode proteins and RNA may include introns. "Recombinant nucleic acid" refers to a nucleic acid having nucleotide sequences that are not naturally joined together. This includes nucleic acid vectors comprising an amplified or assembled nucleic acid, which can be used to transform a suitable host cell. A host cell that comprises the recombinant nucleic acid is referred to as a "recombinant host cell." The gene is then expressed in the recombinant host cell to produce, such as a "recombinant polypeptide."

A recombinant nucleic acid may serve a non-coding function (such as a promoter, origin of replication, ribosome-binding site, etc.) as well.

A first sequence is an "antisense" with respect to a second sequence if a polynucleotide whose sequence is the first sequence specifically hybridizes with a polynucleotide whose sequence is the second sequence. Terms used to describe sequence relationships between two or more nucleotide sequences or amino acid sequences include "reference sequence," "selected from," "comparison window,"
5 "identical," "percentage of sequence identity," "substantially identical," "complementary," and "substantially complementary."

For sequence comparison of nucleic acid sequences and amino acids sequences, typically one sequence acts as a reference sequence, to which test
10 sequences are compared. When using a sequence comparison algorithm, test and reference sequences are entered into a computer, subsequence coordinates are designated, if necessary, and sequence algorithm program parameters are designated. Default program parameters are used. Methods of alignment of sequences for comparison are well known in the art and exemplary methods are
15 given below.

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

Pharmaceutical agent: A chemical compound or composition capable of inducing a desired therapeutic or prophylactic effect when properly administered to
25 a subject or a cell. In some examples a pharmaceutical agent includes one or more of the disclosed polypeptides.

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
30 compositions and formulations suitable for pharmaceutical delivery of the compositions disclosed herein.

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 (such as 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.

Polypeptide: Any chain of amino acids, regardless of length or post-translational modification (such as glycosylation or phosphorylation). “Polypeptide” applies to amino acid polymers to 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. In one embodiment, the polypeptide is a HIV-neutralizing polypeptide. 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 peptide or protein, and is used interchangeably herein to refer to a polymer of amino acid residues.

Polypeptide Modifications: The present disclosure includes mutant polypeptides, as well as synthetic embodiments. In addition, analogues (non-peptide organic molecules), derivatives (chemically functionalized polypeptide molecules obtained starting with the disclosed polypeptide sequences) and variants (homologs) of polypeptides can be utilized in the methods described herein. The polypeptides disclosed herein include a sequence of amino acids that can be either L- and/or D-amino acids, naturally occurring and otherwise.

Peptides can be modified by a variety of chemical techniques to produce derivatives having essentially the same activity as the unmodified polypeptides, and optionally having other desirable properties. For example, carboxylic acid groups of the protein, whether carboxyl-terminal or side chain, may be provided in the form of a salt of a pharmaceutically-acceptable cation or esterified to form a 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 polypeptide, whether amino-terminal or side chain, may be in the form of a pharmaceutically-acceptable acid addition salt, such as the HCl, HBr, acetic, benzoic, toluene sulfonic, maleic, tartaric and other organic salts, or may be modified to C₁-C₁₆ alkyl or dialkyl amino or further converted to an amide.

Hydroxyl groups of the polypeptide side chains can be converted to C₁-C₁₆ alkoxy or to a C₁-C₁₆ ester using well-recognized techniques. Phenyl and phenolic rings of the polypeptide side chains can be substituted with one or more halogen atoms, such as F, Cl, Br or I, or with C₁-C₁₆ alkyl, C₁-C₁₆ alkoxy, carboxylic acids and esters thereof, or amides of such carboxylic acids. Methylene groups of the polypeptide side chains can be extended to homologous C₂-C₄ alkylenes. Thiols can be protected with any one of a number of well-recognized protecting groups, such as acetamide groups. Those skilled in the art will also recognize methods for introducing cyclic structures into the polypeptides of this disclosure to select and provide conformational constraints to the structure that result in enhanced stability. For example, a C- or N-terminal cysteine can be added to the polypeptide, so that when oxidized the polypeptide will contain a disulfide bond, generating a cyclic polypeptide. Other polypeptide cyclizing methods include the formation of thioethers and carboxyl- and amino-terminal amides and esters.

Prime-boost vaccination: An immunotherapy including administration of a first immunogenic composition (the primer vaccine) followed by administration of a second immunogenic composition (the booster vaccine) to a subject to induce an immune response. The primer vaccine and the booster vaccine include a vector (such as a viral vector or DNA vector) expressing the antigen to which the immune

response is directed. The primer vaccine includes a vector expressing a first antigen, and the booster includes a vector expressing a mismatch of the first antigen. In several embodiments, the antigen is a viral antigen, such as an antigen from a lentivirus or a filovirus.

5 In some embodiments, the primer vaccine includes an adenoviral vector expressing a heterologous viral antigen, and the booster vaccine includes a replication deficient LCMV vector expressing a mismatch of the viral antigen. For example, the primer vaccine includes an adenoviral vector expressing an HIV Env protein or fragment thereof (such as HIV gp145) and the booster vaccine includes an
10 replication deficient LCMV vector expressing a mismatch of the HIV Env protein or fragment (such as HIV gp145). In another example, the primer vaccine includes an adenoviral vector expressing an HIV Env protein, or fragment thereof (such as HIV gp145 Δ CFI Δ V1V2) and the booster vaccine includes an LCMV vector expressing a mismatch of the HIV Env protein, or fragment thereof (such as HIV
15 gp140 Δ CFI Δ V1V2).

The booster vaccine is administered to the subject after the primer vaccine; the skilled artisan will understand a suitable time interval between administration of the primer vaccine and the booster vaccine, and examples of such timeframes are disclosed herein.

20 In some embodiments, the primer vaccine, the booster vaccine, or both primer vaccine and the booster vaccine additionally include an adjuvant.

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

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
30 two otherwise separated segments of sequence. This artificial combination is often accomplished by chemical synthesis or, more commonly, by the artificial

manipulation of isolated segments of nucleic acids, *e.g.*, by genetic engineering techniques.

Replication-deficient viral vector: A viral vector that that requires complementation of one or more regions of the viral genome required for replication, as a result of, for example a deficiency in at least one replication-essential gene function. For example, such that the viral vector does not replicate in typical host cells, especially those in a human patient that could be infected by the viral vector in the course of a therapeutic method. A deficiency in a gene, gene function, or genomic region, as used herein, is defined as a deletion of sufficient genetic material of the viral genome to obliterate or impair the function of the gene (e.g., such that the function of the gene product is reduced by at least about 2-fold, 5-fold, 10-fold, 20-fold, 30-fold, or 50-fold) whose nucleic acid sequence was deleted in whole or in part. Deletion of an entire gene region often is not required for disruption of a replication-essential gene function. Examples of replication-deficient viral vectors and systems for their use are known in the art and include; for example replication-deficient LCMV vectors (see, e.g., U.S. Pat. Pub. No. 2010/0297172, incorporated by reference herein in its entirety) and replication deficient adenoviral vectors (see, e.g., Int. Pat. App. Pub. No. WO2000/00628, incorporated by reference herein).

Sample (or biological sample): A biological specimen containing genomic DNA, RNA (including mRNA), protein, or combinations thereof, obtained from a subject. Examples include, but are not limited to, peripheral blood, tissue, cells, urine, saliva, tissue biopsy, fine needle aspirate, surgical specimen, and autopsy material. In one example, a sample includes a HCC tissue biopsy.

Sequence identity/similarity: The identity/similarity between two or more nucleic acid sequences, or two or more amino acid sequences, is expressed in terms of the identity or similarity between the sequences. Sequence identity can be measured in terms of percentage identity; the higher the percentage, the more identical the sequences are. Homologs or orthologs of nucleic acid or amino acid sequences possess a relatively high degree of sequence identity/similarity 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.

10 The NCBI Basic Local Alignment Search Tool (BLAST) (Altschul *et al.*, *J. Mol. Biol.* 215:403-10, 1990) is available from several sources, including the National Center for Biological Information (NCBI, National Library of Medicine, Building 38A, Room 8N805, Bethesda, MD 20894) and on the Internet, for use in connection with the sequence analysis programs blastp, blastn, blastx, tblastn, and
15 tblastx. Blastn is used to compare nucleic acid sequences, while blastp is used to compare amino acid sequences. Additional information can be found at the NCBI web site.

Homologs and variants of a CSF1R protein are typically characterized by possession of at least 50% sequence identity counted over the full length alignment
20 with the amino acid sequence of a native protein using the NCBI Blast 2.0, gapped blastp set to default parameters. For comparisons of amino acid sequences of greater than about 30 amino acids, the Blast 2 sequences function is employed using the default BLOSUM62 matrix set to default parameters, (gap existence cost of 11, and a per residue gap cost of 1). When aligning short peptides (fewer than around 30
25 amino acids), the alignment should be performed using the Blast 2 sequences function, employing the PAM30 matrix set to default parameters (open gap 9, extension gap 1 penalties). Proteins with even greater similarity to the reference sequences will show increasing percentage identities when assessed by this method, such as at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least
30 90%, at least 95%, at least 96%, at least 97%, 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 75% sequence identity over short windows of 10-20 amino acids, and may possess sequence identities of at least 85% or at least 90% at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% depending on their similarity to the reference sequence. Methods for
5 determining sequence identity over such short windows are described at the NCBI website. One of skill in the art will appreciate that these sequence identity ranges are provided for guidance only; it is entirely possible that strongly significant homologs could be obtained that fall outside of the ranges provided.

Once aligned, the number of matches is determined by counting the number
10 of positions where an identical nucleotide or amino acid residue is present in both sequences. The percent sequence identity is determined by dividing the number of matches either by the length of the sequence set forth in the identified sequence, or by an articulated length (such as 100 consecutive nucleotides or amino acid residues from a sequence set forth in an identified sequence), followed by multiplying the
15 resulting value by 100. For example, a nucleic acid sequence that has 1166 matches when aligned with a test sequence having 1554 nucleotides is 75.0 percent identical to the test sequence ($1166 \div 1554 * 100 = 75.0$). The percent sequence identity value is rounded to the nearest tenth. For example, 75.11, 75.12, 75.13, and 75.14 are rounded down to 75.1, while 75.15, 75.16, 75.17, 75.18, and 75.19 are rounded up
20 to 75.2. The length value will always be an integer. One indication that two nucleic acid molecules are closely related is that the two molecules hybridize to each other under stringent conditions.

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

T Cell: A white blood cell critical to the immune response. T cells include, but
30 are not limited to, $CD4^+$ T cells and $CD8^+$ T cells. A $CD4^+$ T lymphocyte is an immune cell that carries a marker on its surface known as "cluster of differentiation 4" (CD4).

These cells, also known as helper T cells, help orchestrate the immune response, including antibody responses as well as killer T cell responses. Th1 and Th2 cells are functional subsets of helper T cells. Th1 cells secrete a set of cytokines, including interferon-gamma, and whose principal function is to stimulate phagocyte-mediated
5 defense against infections, especially related to intracellular microbes. Th2 cells secrete a set of cytokines, including interleukin (IL)-4 and IL-5, and whose principal functions are to stimulate IgE and eosinophil/mast cell-mediated immune reactions and to downregulate Th1 responses.

CD8⁺ T cells carry the “cluster of differentiation 8” (CD8) marker. In one
10 embodiment, a CD8 T cells is a cytotoxic T lymphocytes. In another embodiment, a CD8 cell is a suppressor T cell.

Therapeutic agent: A chemical compound, small molecule, or other composition, such as nucleic acid molecule, capable of inducing a desired therapeutic or prophylactic effect when properly administered to a subject.

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

Therapeutically Effective Amount: An amount of a composition that alone, or together with an additional therapeutic agent(s) (for example an adjuvant) induces the desired response (*e.g.*, inhibition of viral infection, such as HIV infection). In several embodiments, a therapeutically effective amount is the amount
25 necessary to inhibit viral infection, such as HIV infection. When administered to a subject, a dosage will generally be used that will achieve target tissue concentrations that has been shown to achieve a desired *in vitro* effect.

In one example, a desired response is to inhibit or prevent viral infection (such as HIV infection) in a subject (for example to prevent viral infection, such as
30 HIV infection in the subject). Viral infection does not need to be completely inhibited or prevented for the composition to be effective. For example, a

composition can decrease viral infection (such as HIV infection) by a desired amount, for example by at least 10%, at least 20%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, or even at least 100% (prevention of viral infection), as compared to viral infection in the absence of the composition.

A therapeutically effective amount of an composition, can be administered in a single dose, or in several doses, for example daily, during a course of treatment. However, the therapeutically effective amount can depend on the subject being treated, the severity and type of the condition being treated, and the manner of administration.

Vaccine: An immunogenic composition that when administered to a subject, inhibits a disorder or disease, including prevention of the disease or disorder (such as a viral infection), or reduces the risk of the disease or disorder (such as the risk of contracting the viral infection). In one specific, non-limiting embodiment, a vaccine inhibits HIV infection in a subject, by for example, preventing HIV infection in the subject.

Vector: A nucleic acid molecule as introduced into a host cell, thereby producing a transformed host cell. A vector may include nucleic acid sequences that permit it to replicate in a host cell, such as an origin of replication. A vector may also include one or more selectable marker genes and other genetic elements known in the art. Recombinant DNA vectors are vectors having recombinant DNA. Recombinant RNA vectors are vectors having recombinant RNA. A vector can include nucleic acid sequences that permit it to replicate in a host cell, such as an origin of replication. A vector can also include one or more selectable marker genes and other genetic elements known in the art. Viral vectors are recombinant vectors having at least some nucleic acid sequences derived from one or more viruses. Non-limiting examples of vectors include viral vectors such as adenoviral vectors and LCMV vectors.

Virus: Microscopic infectious organism that reproduces inside living cells. A virus consists essentially of a core of a single nucleic acid surrounded by a protein coat, and has the ability to replicate only inside a living cell. "Viral replication" is

the production of additional virus by the occurrence of at least one viral life cycle. A virus may subvert the host cells' normal functions, causing the cell to behave in a manner determined by the virus. For example, a viral infection may result in a cell producing a cytokine, or responding to a cytokine, when the uninfected cell does not normally do so. "Retroviruses" are RNA viruses wherein the viral genome is RNA. When a host cell is infected with a retrovirus, the genomic RNA is reverse transcribed into a DNA intermediate which is integrated very efficiently into the chromosomal DNA of infected cells. The integrated DNA intermediate is referred to as a provirus. The term "lentivirus" is used in its conventional sense to describe a genus of viruses containing reverse transcriptase. The lentiviruses include the "immunodeficiency viruses" which include human immunodeficiency virus (HIV) type 1 and type 2 (HIV-1 and HIV-2), simian immunodeficiency virus (SIV), and feline immunodeficiency virus (FIV).

15 **II. Description of Several Embodiments**

A. Viral Antigens

In several embodiments, the disclosed vectors (such as a DNA or viral vector) include a nucleic acid sequence that encodes a viral antigen. For example, the viral antigen can be an antigen from one of (but not limited to) the following virus families: *Retroviridae* (for example, lentiviruses such as human immunodeficiency virus (HIV), human T-cell leukemia viruses); *Picornaviridae* (for example, poliovirus, hepatitis A virus, enteroviruses, human coxsackie viruses, rhinoviruses, echoviruses, foot-and-mouth disease virus); *Caliciviridae* (such as strains that cause gastroenteritis, including Norwalk virus); *Togaviridae* (for example, alphaviruses (including chikungunya virus, equine encephalitis viruses, Simliki Forest virus, Sindbis virus, Ross River virus, rubella viruses); *Flaviridae* (for example, hepatitis C virus, dengue viruses, yellow fever viruses, West Nile virus, St. Louis encephalitis virus, Japanese encephalitis virus, Powassan virus and other encephalitis viruses); *Coronaviridae* (for example, coronaviruses, severe acute respiratory syndrome (SARS) virus); *Rhabdoviridae* (for example, vesicular stomatitis viruses, rabies viruses); *Filoviridae* (for example, Ebola virus, Marburg

virus); *Paramyxoviridae* (for example, parainfluenza viruses, mumps virus, measles virus, respiratory syncytial virus); *Orthomyxoviridae* (for example, influenza viruses); *Bunyaviridae* (for example, Hantaan viruses, Sin Nombre virus, Rift Valley fever virus, bunya viruses, phleboviruses and Nairo viruses); *Arenaviridae* (such as

5 Lassa fever virus and other hemorrhagic fever viruses, Machupo virus, Junin virus); *Reoviridae* (e.g., reoviruses, orbiviruses, rotaviruses); *Birnaviridae*; *Hepadnaviridae* (hepatitis B virus); *Parvoviridae* (parvoviruses); *Papovaviridae* (papilloma viruses, polyoma viruses, BK-virus); *Adenoviridae* (adenoviruses); *Herpesviridae* (herpes simplex virus (HSV)-1 and HSV-2; cytomegalovirus; Epstein-Barr virus; varicella

10 zoster virus; and other herpes viruses, including HSV-6); *Poxviridae* (variola viruses, vaccinia viruses, pox viruses); and *Iridoviridae* (such as African swine fever virus); *Astroviridae*; and unclassified viruses (for example, the etiological agents of spongiform encephalopathies, the agent of delta hepatitis (thought to be a defective satellite of hepatitis B virus). In several embodiments, the viral antigen can be

15 modified such that it exhibits enhanced immunogenicity in vivo. In several embodiments, the viral antigen is a viral envelope glycoprotein, or immunogenic fragment thereof.

In some embodiments, the viral antigen is an antigen from the *Filoviridae* virus family, such as a Ebola virus or Marburg virus antigen. For example the viral

20 antigen can be an Ebola virus antigen, such as Ebola virus glycoprotein (Z), or immunogenic fragment thereof. For example, the Ebola virus antigen can have about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, about 96%, about 97%, about 98% or about 99% sequence identity with the wild-type Ebola virus glycoprotein (Z). In some embodiments, the Ebola virus antigen can have up

25 to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or up to 30, or more, amino acid substitutions, deletions or insertions, compared to the Ebola virus glycoprotein (Z).

1. HIV Antigens

30 In several embodiments, the disclosed vectors (such as a viral or DNA vector) include a nucleic acid sequence that encodes an HIV antigen. Examples of

suitable HIV antigens include all or part of HIV Gag, Env, Pol, Tat, Reverse Transcriptase (RT), Vif, Vpr, Vpu, Vpo, Integrase, or Nef proteins. Preferably, the HIV antigen comprises all or part (such as a fragment) of an HIV Gag, Env, and/or Pol protein. Suitable Env proteins are known in the art and include, for example, gpl60, gpl20, gp41, gpl45, gpl40. In addition, an HIV antigen can be modified such that it exhibits enhanced immunogenicity in vivo. For example, the antigen can be an Env protein comprising mutations in the cleavage site, fusion peptide, or interhelical coiled-coil domains of the Env protein (ACFI Env proteins) (see, e.g., Cao et al., J. Virol., 71, 9808-9812 (1997), and Yang et al., J. Virol, 78, 4029-4036 (2004)). The antigen also can be a monomeric or trimeric HIV polypeptide (e.g., Env) which has been modified to increase its stability in vivo, such as those described in, e.g., U.S. Patent Application Publication Nos. 2009/0191235 and 2009/0110690. In addition, the HIV antigen can be synthetically generated. Synthetically generated antigen sequences include, for example, consensus HIV antigens, mosaic HIV antigens, and other bioinformatically generated antigens. Examples of HIV antigens are provided herein.

Consensus HIV antigens are generated by comparing the amino acid sequences of a plurality of naturally-occurring HIV antigens to identify common sequences within them and generating a synthetic HIV antigen in which every amino acid is present in a plurality of sequences. Methods for the generation of "consensus" sequences for the HIV-1 Env protein are described in, for example, Weaver et al., J. Virol, 80: 6745-6756 (2006).

"Mosaic HIV sequences" are generated using natural sequences as input to algorithms, such as genetic algorithms, which maximize the diversity of potential T-cell epitopes present in the natural sequences. The genetic algorithm identifies potential T-cell epitopes within the input sequences, generates potential recombinants between the input sequences, and identifies those recombinants which have the greatest diversity of T cell epitopes. Epitopes which occur infrequently may be omitted from the mosaic sequences while those which provide enhanced coverage relative to a sequence lacking that epitope may be incorporated into the mosaic sequence. Methods for generating mosaic sequences are described in, e.g.,

Fischer et al., Nature Medicine, 13(1): 100-106 (2007); and international Patent Application Publications WO 2007/024941 and WO 2010/042817. Other bioinformatic algorithms known in the art can also be employed to generate HIV-1 antigen sequences having enhanced immunogenicity relative to naturally-occurring sequences.

Any clade of HIV is appropriate for antigen selection, including HIV clades A, B, C, D, E, MN, and the like. Thus, it will be appreciated that the following HIV antigens can be used in the embodiments disclosed herein: HIV clade A Gag, Env (such as gp150, gp145, gp140, gp120), and/or Pol; HIV clade B Gag, Env (such as gp150, gp145, gp140, gp120), and/or Pol proteins; HIV clade C Gag, Env (such as gp150, gp145, gp140, gp120), and/or Pol proteins; and HIV clade MN Gag, Env (such as gp150, gp145, gp140, gp120), and/or Pol proteins. While it is preferred that the antigen is a Gag, Env, and/or Pol protein, any HIV protein or portion thereof capable of inducing an immune response in a mammal can be used in connection with the disclosed methods. HIV Gag, Env, and Pol proteins from the different HIV clades (e.g., HIV clades A, B, C, MN, etc.), 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 (2003), HIV Sequence Database ([hiv- web. lanl.gov/content/hiv-db/mainpage.html](http://hiv-web.lanl.gov/content/hiv-db/mainpage.html)), Sambrook et al., Molecular Cloning, a Laboratory Manual, 2d edition, Cold Spring Harbor Press, Cold Spring Harbor, N.Y. (1989), and Ausubel et al., Current Protocols in Molecular Biology, Greene Publishing Associates and John Wiley & Sons, New York, N.Y. (1994)).

It will be appreciated that an entire, intact HIV protein is not required to produce an immune response. Indeed, most antigenic epitopes of HIV proteins are relatively small in size. Thus, fragments (e.g., epitopes or other antigenic fragments) of an HIV protein, such as any of the HIV proteins described herein, can be used as an HIV antigen. Antigenic fragments and epitopes of the HIV Gag, Env, and Pol proteins, as well as nucleic acid sequences encoding such antigenic fragments and epitopes, are known (see, e.g., HIV Immunology and HIV/SIV Vaccine Databases,

Vol. 1 , Division of AIDS, National Institute of Allergy and Infectious Diseases (2003)).

HIV antigens also include fusion proteins and polyproteins. A fusion protein can comprise one or more antigenic HIV protein fragments (e.g., epitopes) fused to one another, or fused to all or part of a different HIV protein or other polypeptide. The fusion protein can comprise all or part of any of the HIV antigens described herein. For example, all or part of an HIV Env protein (such as gp150, gp145, gp140, gp120), can be fused to all or part of the HIV Pol protein, or all or part of HIV Gag protein can be fused to all or part of the HIV Pol protein. Such fusion proteins effectively provide multiple HIV antigens and can be used to generate a more complete immune response against a given HIV pathogen as compared to that generated by a single HIV antigen. Similarly, polyproteins also can provide multiple HIV antigens. Polyproteins useful include those that provide two or more HIV antigens, such as two or more of any of the HIV antigens described herein. Delivery of fusion proteins or polyproteins via adenoviral vector to a mammal allows exposure of an immune system to multiple antigens using a single nucleic acid sequence and, thus, conveniently allows a single composition to provide immunity against multiple HIV antigens or multiple epitopes of a single antigen. Nucleic acid sequences encoding fusion proteins and polyproteins of HIV antigens can be prepared and inserted into vectors using known methods (see, e.g., U.S. Patents 5,130,247 and 5, 130,248, Sambrook et al., supra, and Ausubel et al., supra).

For example, the viral vectors disclosed herein can include a nucleic acid sequence encoding an antigen derived from an HIV glycoprotein, that is useful to induce immunogenic responses in vertebrate animals (such as mammals, for example primates, such as humans) to HIV (for example HIV-1 and HIV-2). In some embodiments, the antigen is an HIV antigen, such as a modified gp120, gp140 or gp145 or an immunogenic fragment thereof. In specific embodiments, the disclosed antigen is HIV-1 gp120 or an immunogenic fragment thereof, for example, the outer domain (OD) of gp120.

In several embodiments the HIV antigens have been substantially resurfaced from the wild type sequence, such that the surface of the antigen has been altered to

focus the immune response to a particular feature, or epitope on the surface of the antigen (for example, HIV Env antigen, such as (such as gp150, gp145, gp140, gp120). In some embodiments, the antigen is a resurfaced HIV Env antigen (such as gp150, gp145, gp140, gp120) in which the one or more of the V1, V2, V3, V4
5 and/or V5 variable loops from gp120 or an immunogenic fragment, thereof such as a gp120 outer domain, are removed or truncated. In some examples, the antigens have been modified to substitute the surface-exposed amino acids located exterior to the target epitope to focus the antigenicity of the antigen to the target epitope. For example, the method can remove non-target epitopes that might interfere with
10 specific binding of an antibody to the target epitope. In some examples, the amino acid substitutions result in the antigen not being bound by antibodies in a polyclonal serum that specifically bind surface-exposed amino acid residues of the wild-type antigen located exterior of the target epitope. In some embodiments, the amino acid substitutions alter antigenicity of the antigen *in vivo* as compared to the wild-type
15 antigen (unsubstituted antigen), but do not introduce additional glycosylation sites as compared to the wild-type antigen. In some embodiments, that antigen is glycosylated. Examples of antigen resurfacing methods are given in PCT Publication No. WO 09/100376, which is specifically incorporated by reference in its entirety.

20 HIV-I 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-I. The disclosed antigens can be derived from any subtype of HIV, such as groups M, N, O, or P or clade A, B, C, D, F, G, H, J or K and the like. The nucleic acid sequence can encode an Env polypeptide from any
25 group or clade of HIV. HIV Env proteins, or fragments thereof (such as gp150, gp145, gp140, gp120) from the different HIV 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
30 Diseases (2003); HIV Sequence Database (hiv-web.lanl.gov/content/hiv-db/mainpage.html); Sambrook et al., Molecular Cloning, a Laboratory Manual, 2d

edition, Cold Spring Harbor Press, Cold Spring Harbor, N. Y. (1989); Ausubel et al., Current Protocols in Molecular Biology, Greene Publishing Associates and John Wiley & Sons, New York, N. Y. (1994)).

In some embodiments, an antigen includes the outer domain of a gp120. In some examples, the outer domain of gp120 includes amino acid residues 252-482 of gp120. In some examples, the outer domain of gp120 includes amino acid residues 213-492 of gp120. In some examples, the outer domain of gp120 includes amino acid residues 213-482 of gp120. In some examples, the outer domain of gp120 includes amino acid residues 252-492 of gp120. In specific embodiments, the antigen is HIV-1 gp120. In other specific embodiments, the antigen is HIV-1 gp140 or gp145.

In some examples, V1/V2 and β 20/ β 21 regions of gp120, gp140 or gp145 are modified to reduce the immunogenicity or at least alter the immunogenicity of those regions. In some examples, the β 20/ β 21 bridging sheet of the gp120, gp140 or gp145 antigen or immunogenic fragment thereof is removed by replacing the amino acid residues between I423 and Y435 with Gly-Gly. In some examples, the β 20/ β 21 bridging sheet of the antigen is removed, for example by replacing residues 422-436 of gp120, gp140 or gp145 or the outer domain of gp120 with Gly-Gly. In some examples, residues 128-194 of gp120 in the V1, V2 loop region are replaced by Gly-Arg-Gly. In some examples, a modified V1/V2 is taken from a core gp120 previously designed that has improved expression yields known as “new 9c” (see International Patent Publication NO. WO 2007/030518, which is incorporated herein by reference), and includes the insertion of VKLTPLAGATSVITQA (SEQ ID NO: 162) between C119 and C205.

In some examples, the wild type V1 loop (residues 131-157 of gp120) of an HIV Env antigen (or immunogenic fragment thereof) includes the amino acid sequence CTDLRNATNTTSSSWETMEKGKNC (SEQ ID NO: 163). In some examples, the V1 loop is replaced with the amino acid sequence CTDLRGSGGGSGGGSEIKNC (SEQ ID NO: 164), which in some examples is designated Δ V1.1.

In some examples, the wild type V2 loop (residues 157-198 of gp120) of an HIV Env antigen (or immunogenic fragment thereof) includes the amino acid sequence CSFNITTSIRDKVQKEYALFYKLDVVPIDNDNTSYRLINC (SEQ ID NO: 165). In some examples, the V2 loop is replaced with the amino acid sequence
 5 CSFNITTSIRDKVQKEYALFYKLDVVPIGGSGGSYRLINC (SEQ ID NO: 166), which in some examples is designated V2.1, or the amino acid sequence CSFNITTSIRDKVQKEYALFYKLDVVPIGSGGGSGSYRLINC (SEQ ID NO: 167), which in some examples is designated V2.2.

In some embodiments, the V3 loop of an HIV Env antigen (or immunogenic
 10 fragment thereof) is mutated and/or truncated as compared to a wild type HIV Env or immunogenic fragment thereof. In some examples, the wild-type V3 loop (residues 296-331) includes the amino acid sequence CSRPNNNTRKSIPMGPGRAFYTGTGQIIGDIRQAHG (SEQ ID NO: 168). In some examples, the V3 loop is shortened by 18 amino acids, for example by removing 9
 15 amino acids from each end of the loop, which in certain examples is designated V3-9,9. In some examples, residues 302-323 of gp120, part of the V3 loop, are replaced with a basic hexapeptide (NTRGRR; SEQ ID NO: 169). In some examples, residues 305-323 of gp120 in the V3 loop are replaced by Gly-Arg-Arg. In some examples, the V3 loop is replaced with the m5 loop, having the amino acid sequence
 20 CSRPNNNTRGRRGSSGGSHC (SEQ ID NO: 170). In some examples, the V3 loop is replaced with the m4 loop, having the amino acid sequence CSRPNNGGSGSGGSSGGSHC (SEQ ID NO: 171). In some examples, the V3 loop is replaced with Gly-Val-Gly. In some examples, the V3 loop is at least partially replaced with Gly-Ser. In some examples, the V3 loop is at least partially
 25 replaced with Gly-Ser-Leu. In some examples, the V3 loop is truncated to an 11-mer. In some examples, the V3 loop is replaced with the amino acid sequence CARPSNNTRGRRGDIRQAYC (SEQ ID NO: 172). In some examples, the V3 loop is replaced with the amino acid sequence CARPSNNTDIRQAYC (SEQ ID NO: 173), which in some examples is designated V3.1. In some examples, the V3
 30 loop is replaced with the amino acid sequence CARPSNNTRQAYC (SEQ ID NO: 174), which in some examples is designated V3.2. In some examples, the V3 loop is

replaced with the amino acid sequence CARPSNNTQYC (SEQ ID NO: 175), which in some examples is designated V3.3. In some examples, the V3 loop is replaced with the amino acid sequence CARGSGSGSYC (SEQ ID NO: 176), which in some examples is designated V3.4. In some examples, the V3 loop is replaced with the amino acid sequence CSRPNNTTRGRRGDIRQAHC (SEQ ID NO: 177), which in some examples is designated V3(GSL). In some examples, the V3 loop is replaced with the amino acid sequence CSRPNNTTRRQAHC (SEQ ID NO: 178), which in some examples is designated V3.2. In some examples, the V3 loop is replaced with the amino acid sequence CSRPNNGGSGQAHC (SEQ ID NO: 179), which in some examples is designated V3.2GS. In some examples, the wild-type V3 loop includes the amino acid sequence CTRPNNTTRKSIHIGPGQAFYATGDIIGDIRQAHC (SEQ ID NO: 205). In some examples, the V3 loop is replaced with the amino acid sequence CTRPNNGGSGSGGSSGGSHC (SEQ ID NO: 180), which in some examples is designated V3.4. In some examples, the V3 loop is replaced with the amino acid sequence CTRPNNTTRGRRGSSGGSHC (SEQ ID NO: 181), which in some examples is designated V3.5. In some examples, the V3 loop is replaced with a 15 mer with a native glycan at the tip. In some examples, the V3 loop is replaced with a 15 mer with a slightly shifted glycan at the tip.

In some embodiments, the V4 loop of an HIV Env antigen (or immunogenic fragment thereof) is mutated and/or truncated as compared to a wild-type HIV Env antigen (or immunogenic fragment thereof). In some examples, the V4 loop is at least partially replaced with Gly-Ser. In some examples, 9 amino acids are removed from the V4 loop, which in some examples is designated V4.2.1. In some examples, the V4 loop is shortened to SIWNNGGSGGGSGGGSDTIT (SEQ ID NO: 182), which in some examples is designated V4.GS. In some examples, the V4 loop is replaced with the V4 loop from strain Ker2018 (a clade A strain), which in some examples is designated V4.Ker A. In some examples, one of the two glycan sites is removed from the V4 loop from strain Ker2018, which in some examples is designated V4.Ker A/ Δ G2, or V4.Ker A/ Δ G1. In some examples, both of the two glycan sites are removed from the V4 loop from strain Ker2018r, which in some examples is designated V4.Ker A/ Δ G2, or V4.Ker A/ Δ G12. In some examples, the

wild-type V4 loop includes the amino acid sequence STWFNSTWSTKGSNNTEGSDTIT (SEQ ID NO: 183). In some examples, the V4 loop is replaced with the amino acid sequence STWFNGSGSGSGGTIT (SEQ ID NO: 184), which in some examples is designated V4.1. In some examples, the V4 loop is replaced with the amino acid sequence STWFNSTWSTKGSNNTEGSDTIT (SEQ ID NO: 185), which in some examples is designated V4.2. In some examples, the V4 loop is replaced with the amino acid sequence STWFQGS GSGSGGTIT (SEQ ID NO: 186), which in some examples is designated V4.3. In some examples, the glycan site at the N terminal end of the V4 is removed, which is designated the V4.7 loop. In some examples, the glycan site at the C terminal end of the V4 is removed, which is designated v4.8. In some examples, the glycan site at both the N and C terminal end is removed, which is designated v4.9. In some examples, the V4 loop is replaced with a Gly-Ser repeat, which is designated V4.x

In some embodiments, the V5 loop of an HIV Env antigen (or immunogenic fragment thereof) is mutated and/or truncated as compared to a wild type HIV Env antigen (or immunogenic fragment thereof). In some examples, the V5 loop is at least partially replaced with Gly-Ser. In some examples, a wild type V5 loop has the amino acid sequence GGNTGNNSRTC (SEQ ID NO: 202). In some examples, the V5 loop is truncated to a 7-mer. In some examples, the V5 loop is truncated to a 5-mer. In some examples, the V5 loop is replaced with the amino acid sequence GGNTNRTC (SEQ ID NO: 187), which in some examples is designated V5.1. In some examples, the V5 loop is replaced with the amino acid sequence GGSGSGTC (SEQ ID NO: 188), which in some examples is designated V5.2. In some examples, the V5 loop is replaced with the amino acid sequence GGSGSTC (SEQ ID NO: 189), which in some examples is designated V5.2. In some examples, the V5 loop is truncated to a 16-mer. In some examples, the V5 loop is truncated to NDSDGNETFR (SEQ ID NO: 190) for example from KDDNSRDGNETFR (SEQ ID NO: 191), which in some examples is designated V5.2.1. In some examples, the V5 loop is replaced with the amino acid sequence SGGSGQETFR (SEQ ID NO: 192), which in some examples is designated V5.2GS. In some examples, the wild-type V5 loop includes the amino acid sequence GGNDNNESEI (SEQ ID NO: 193).

In some examples, the V5 loop is replaced with the amino acid sequence GGGSGSGEI (SEQ ID NO: 194), which in some examples is designated V5.1. In some examples, the V5 loop is replaced with an 8 amino acid Gly-Ser repeat.

In some embodiments, an HIV Env antigen (or immunogenic fragment thereof) includes an outer domain of gp120 including residues 252-482 of gp120. In specific embodiments, the outer domain of gp120, and thus the HIV Env antigen (or immunogenic fragment thereof), includes the sequence set forth as SEQ ID NO: 58, or a variant thereof that retains the outer domain fold of gp120. In some examples, the outer domain of gp120 includes additional mutations. In specific examples, the HIV Env antigen (or immunogenic fragment thereof) including the outer domain of gp120 includes one or more of the following mutations made in the context of SEQ ID NO: 203 (OD 1.0): 273N, T283N, T339N, A341T, 360N, 362N, N363Q, P369N, I371T, 371N, T373N, T373G, 377N, A379T, F383T, N386Q, D392N, R419N, G421N, G424N, A431T, P437T, N465Q, W479N, N280C and G458, K358C and N465C, and/or V255C and M475C. The cysteine residues can be introduced to stabilize the HIV Env antigen (or immunogenic fragment thereof), for example by stabilizing the loops and/or core of the folded polypeptide. When the residue number is not preceded by a residue but is followed by a N it is meant that an asparagine is introduced at that position regardless of the starting residue, for example to insert a glycosylation site at that position.

In specific examples, an HIV Env antigen (or immunogenic fragment thereof) including the outer domain of gp120 includes one or more of the following mutations made in the context of SEQ ID NO: 1 (A2.3_KER2018.11): 273N, T283N, T339N, A341T, 360N, 362N, N363Q, P369N, I371T, 371N, T373N, T373G, 377N, A379T, F383T, N386Q, D392N, R419N, G421N, G424N, A431T, P437T, N465Q, W479N, N280C and G458, K358C and N465C, and/or V255C and M475C. The cysteine residues can be introduced to stabilize the polypeptide, for example by stabilizing the loops and/or core of the folded polypeptide. In specific examples, the HIV Env antigen (or immunogenic fragment thereof) including the outer domain of gp120 includes one or more of the following mutations made in the context of SEQ ID NO: 1: V257N, V272N, N276Q, N276D, N276E, T283V,

A297T, D368R, N362T, E363N, P364S, T373N, S375T, F382T, S388A, A388S, K389D, E398N, N406Q, N410Q, K421T, G422V, V427N, V442N, R444T, N478L, S481T, and/or E482S.

In specific examples, an HIV Env antigen (or immunogenic fragment thereof) including the outer domain of gp120 includes one or more of the following mutations made in the context of SEQ ID NO: 78 (HXB2 core): T26N, K55N, A49S, D91T, T92N, D94T, D98N, M100T V103N, K113N, P118N, T207N, R209T, V257N, V272N, N276D, R421N, I423T, T424N, M426T, I434N, A436T, R487N, V489T, and/or R490N. In specific examples, the HIV Env antigen (or immunogenic fragment thereof) including the outer domain of gp120 includes all of the following mutations made in the context of SEQ ID NO: 74: T26N, V257N, A49S, V103N, V272N. In specific examples, the HIV Env antigen (or immunogenic fragment thereof) including the outer domain of gp120 includes all of the following mutations made in the context of SEQ ID NO: 74: T84N, A107S, K113N, V244N, V427N, and V442N.

In some examples, an HIV Env antigen (or immunogenic fragment thereof) includes the amino acid sequence set forth as anyone of SEQ ID NOs: 74-76. In some examples, the HIV Env antigen (or immunogenic fragment thereof) includes the amino acid sequence set forth as anyone of SEQ ID NOs: 74-76.

In some examples, glycosylation sites are mutationally introduced into the HIV Env antigen (or immunogenic fragment thereof). In some examples, glycosylation sites are introduced at one or more of position 295, 442, 479, 272, 377, 436, 424, 398, 348, and/or 369 of a the HIV Env antigen (or immunogenic fragment thereof; wherein the amino acid numbering refers to position in gp120). In some examples glycosylation sites are introduced positions 295 and 442, which is termed HG.1. In some examples glycosylation sites are introduced positions 295, 442, and 479, which is termed HG.2. In some examples glycosylation sites are introduced positions 295, 442, and 436, which is termed HG.3. In some examples glycosylation sites are introduced positions 295, 442, and 424, which is termed HG.4. In some examples glycosylation sites are introduced positions 295, 442, and 398, which is termed HG.5. In some examples glycosylation sites are introduced

positions 295, 442, and 369, which is termed HG.6. In some examples glycosylation sites are introduced positions 295, 442, 273, 377, and 348, which is termed HG.7. In some examples glycosylation sites are introduced positions 295, 442, 479, 273, 377, 436*, 398, 348, and 369, which is termed HG.8. In some examples glycosylation sites are introduced positions 295, 442, 479, 273, 377, 424*, 398, 348, and 369, which is termed HG.9. In some examples glycosylation sites are introduced positions 295, 442, 479, 273, and 377, which is termed HG.10. In specific examples, the immunogen including the outer domain of gp120 includes one or more of the following mutations V257N, V272N, N276Q, N276D, N276E, T283V, A297T, D368R, N362T, E363N, P364S, T373N, S375T, F382T, S388A, A388S, K389D, E398N, N406Q, N410Q, K421T, G422V, V427N, V442N, R444T, N478L, S481T, and/or E482S. Mutations that are recited outside of the gp120 outer domain are made in the context of a larger HIV immunogen, such as a gp120, gp140 or gp145 immunogen.

In specific examples, an HIV Env antigen (or immunogenic fragment thereof) including the outer domain of gp120 includes one or more of the following mutations V257N, V272N, N276Q, N276D, N276E, T283V, A297T, D368R, N362T, E363N, P364S, T373N, S375T, F382T, S388A, A388S, K389D, E398N, N406Q, N410Q, K421T, G422V, V427N, V442N, R444T, N478L, S481T, and/or E482S. Mutations that are recited outside of the gp120 outer domain are made in the context of a larger HIV Env antigen (or immunogenic fragment thereof), such as a gp120, gp140 or gp145. In specific examples, the HIV Env antigen (or immunogenic fragment thereof) including the outer domain of gp120 includes all of the following mutations N363Q, K358C-N465C, T283N, N386Q, and N465Q. In specific examples, the HIV Env antigen (or immunogenic fragment thereof) including the outer domain of gp120 includes all of the following mutations K358C-N465C, T283N, N386Q, and N465Q. In specific examples, the HIV Env antigen (or immunogenic fragment thereof) including the outer domain of gp120 includes all of the following mutations V255C-M475C, T283N, D392N, T339N, and N465Q. In specific examples, the HIV Env antigen (or immunogenic fragment thereof) including the outer domain of gp120 includes all of the following mutations N363Q,

V255C-M475C, T283N, N465Q, D392N, T339N, A431T, and T373N. In specific examples, the HIV Env antigen (or immunogenic fragment thereof) including the outer domain of gp120 includes all of the following mutations N363Q, V255C-M475C, T283N, N465Q, and R419N. In specific examples, the HIV Env antigen (or immunogenic fragment thereof) including the outer domain of gp120 includes all of the following mutations V255C-M475C, T283N, N465Q, D392N, T339N, G424N, and P437T. In specific examples, the HIV Env antigen (or immunogenic fragment thereof) including the outer domain of gp120 includes all of the following mutations N276D, T283N, A297T, E398N, V442N, R444T, N478L, W479N, S481T, E482S, N362T, E363N, and P364S. In specific examples, the HIV Env antigen (or immunogenic fragment thereof) including the outer domain of gp120 includes all of the following mutations K358C-N465C, T283N, and N386Q. In specific examples, the HIV Env antigen (or immunogenic fragment thereof) including the outer domain of gp120 includes all of the following mutations K358C-N465C, T283N, N386Q, and N465Q. In specific examples, the HIV Env antigen (or immunogenic fragment thereof) including the outer domain of gp120 includes all of the following mutations F383T, and N280C-G458C, In specific examples, the HIV Env antigen (or immunogenic fragment thereof) including the outer domain of gp120 includes all of the following mutations F383T, K358C-N465C, In specific examples, the HIV Env antigen (or immunogenic fragment thereof) including the outer domain of gp120 includes all of the following mutations F383T, V255C-M475C. In specific examples, the HIV Env antigen (or immunogenic fragment thereof) including the outer domain of gp120 includes all of the following mutations D392N, T339N, and A341T, In specific examples, the HIV Env antigen (or immunogenic fragment thereof) including the outer domain of gp120 includes all of the following mutations D392N, T339N, A341T, and 386N, In specific examples, the HIV Env antigen (or immunogenic fragment thereof) including the outer domain of gp120 includes all of the following mutations P369N and I371T. In specific examples, the HIV Env antigen (or immunogenic fragment

thereof) including the outer domain of gp120 includes all of the following mutations 421N, 377N, and 479N. In specific examples, the HIV Env antigen (or immunogenic fragment thereof) including the outer domain of gp120 includes all of the following mutations 421N, 377N, 479N, and 273N. In specific examples, the HIV Env antigen (or immunogenic fragment thereof) including the outer domain of gp120 includes all of the following mutations 424N, 377N, 479N, 273N. In specific examples, the HIV Env antigen (or immunogenic fragment thereof) including the outer domain of gp120 includes all of the following mutations 424N, 377N, and 479N. In specific examples, the HIV Env antigen (or immunogenic fragment thereof) including the outer domain of gp120 includes all of the following mutations 421N and 363N. In specific examples, the HIV Env antigen (or immunogenic fragment thereof) including the outer domain of gp120 includes all of the following mutations 421N, 377N, 479N, and 363N.

Exemplary amino acid sequences of gp120, gp140 or gp145 antigens or immunogenic fragments thereof are provided herein as SEQ ID NOs: 1-77, 79 and 80. In some embodiments, a HIV Env antigen, or an immunogenic fragment thereof, such as gp120, gp140 or gp145 comprises an amino acid sequence that is at least 95% identical to the amino acid sequence set forth as one of SEQ ID NOs: 1-80, such as at least 95% identical, at least 96% identical, at least 97% identical, at least 98% identical, at least 99% identical, or even 100% identical to the amino acid sequence set forth as one of SEQ ID NOs: 1-77, 79 and 80. In some embodiments, the a gp120, gp140 or gp145 antigen or immunogenic fragment thereof consists of the amino acid sequence set forth as one of SEQ ID NOs: 1-77, 79 and 80.

For example, exemplary amino acid sequences of HIV gp120, gp140 or gp145 antigens or immunogenic fragments thereof, include those provided in Int. Pat. App. Nos. PCT/US2004/030284 and PCT/US2011/042434 (each of which is incorporated by reference herein in its entirety) and disclosed herein as SEQ ID NOs: 1-80.

2. Additional embodiments

In some embodiments the antigen is a multimer, such as a multimer of an HIV Env antigen (or immunogenic fragment thereof, such as gp120, gp140, gp145), for example, a dimer, trimer, etc., of HIV-1 gp120, gp140, gp145 or an immunogenic fragment thereof, such as an outer domain of HIV-1 gp120. Several exogenous oligomerization motifs have been successfully used to promote stable trimers of soluble recombinant proteins: the GCN4 leucine 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 fibritin foldon (Miroshnikov *et al.* 1998 *Protein Eng* 11:329-414). Thus, in some examples, the antigen includes one or more of a foldon domain. In specific examples, the foldon domain is a T4 fibritin foldon domain, which adopts a β -propeller conformation, and can fold and trimerize in an autonomous way (Tao *et al.* 1997 *Structure* 5:789-798).

In some embodiments, the antigen includes a ferritin polypeptide or hybrid of different ferritin polypeptides (for example, to induce multimerization). In specific examples, the ferritin polypeptide is *E. coli* ferritin, human light chain ferritin, bullfrog ferritin or a hybrid thereof, such as *E. coli*-human hybrid ferritin, *E. coli*-bullfrog hybrid ferritin, or human-bullfrog hybrid ferritin. Exemplary amino acid sequences of ferritin polypeptides and nucleic acid sequences encoding ferritin polypeptides for use in the antigens can be found in GENBANK®, for example at accession numbers ZP_03085328, ZP_06990637, AAA35832, NP_000137 AAA49532, AAA49525, AAA49524 and AAA49523, which are specifically incorporated by reference herein in their entirety as available June 29, 2010; and in the Protein Data Base (PDB), for example at PDB accession numbers 1eum, 2ffx and 1rcc, which are specifically incorporated by reference herein in their entirety as available June 29, 2010.

Ferritin is the iron ion storage protein ubiquitously found in almost all living organisms, including bacteria, fungi, and higher plants, and animals. It forms an octahedron consisting of 24 subunits of ~20 kDa protein. In some examples, a immunogen has been genetically fused to the amino terminus of engineered ferritin,

such as eumrcc (a hybrid *E coli* and human ferritin), with a Ser-Gly linker. When the constructs have been made in HEK 293 Freestyle cells, the fusion proteins are secreted from the cells and self-assembled into octahedral particles. The particles can be purified by a few different chromatography procedures, *e.g.* Mono Q (anion
5 exchange) followed by size exclusion (SUPEROSE® 6) chromatography.

In some embodiments, an antigen includes a transmembrane domain, for example to anchor the antigen to the surface of a cell. In some examples, the transmembrane domain is a HIV-1 gp41 transmembrane domain. In some examples, the transmembrane domain is a CD4 transmembrane domain. In some specific
10 examples, an immunogen including a HA transmembrane domain includes the amino acid sequence set forth as one of SEQ ID NOs: 56-57.

In some examples an antigen includes a secretion signal sequence, such as human CD5-derived secretion signal sequence or an IL-2 secretion signal sequence at the N-terminus so that the antigen is secreted from a cell, for example to aid in
15 production and purification of the antigen. In some specific examples, an immunogen including a CD5 leader amino acid sequence includes the amino acid sequence set forth as one of SEQ ID NOs: 53-55 and 75-76. In some specific examples, an immunogen including a murine IL-2 amino acid sequence includes the amino acid sequence set forth as one of SEQ ID NOs: 58-70.

20 In some examples, the antigen is a part of a virus-like particle (VLP), such as a CHIKV VLP. In one non-limiting example, the antigen such as a gp120, gp140 or immunogenic fragment thereof, for example a gp120 outer domain (OD), is inserted between Chikungunya virus (CHIKV) E2 205 amino acid (a.a.) and 206 a.a. on CHIKV VLP (strain 37997). Immunogens are typically presented multimerically
25 (240 molecules per CHIKV VLP particle) to immune cells such as B cells and antigen presenting cells. This results in effectively inducing immune responses against the immunogen, in particular, antibody responses. In specific embodiments, the antigen that is part of a CHIKV VLP includes the amino acid sequence set forth as one of SEQ ID NOs: 79-80.

30 In some embodiments, an antigen includes a six-histidine residue tag (for example, to induce oligomerization and/or aid in purification). In some examples the

antigen includes a 3C protease cleavage site, for example so that a 6X His tag or other peptide fragment, such as those described herein can be cleaved from the antigen.

5 3. Mis-matched antigens

Several embodiments include a mis-matched viral antigen, which is antigen that is similar (but not identical) to a reference viral antigen, or an immunogenic fragment thereof. The primary amino acid sequence of the mis-matched viral antigen differs by at least one amino acid compared to the primary amino acid
10 sequence of the reference viral antigen. For example, some embodiments include a viral vector expressing a first viral antigen that is a HIV antigen, such as HIV Env protein, or an immunogenic fragment thereof (e.g., as provided herein), and also include a second viral vector that expresses a mismatch of the HIV antigen.

For example, the mis-matched antigen can have about 70%, about 75%,
15 about 80%, about 85%, about 90%, about 95%, about 96%, about 97%, about 98% or about 99% sequence identity with the reference antigen. In some embodiments, the mis-matched antigen can have up to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or up to 30 amino acid substitutions, or more amino acid substitutions, compared to the reference antigen.

20 In several embodiments, a mis-matched antigen is an antigen that is similar (but not identical) to an HIV antigen, such as HIV Env protein, or immunogenic fragment thereof (such as gp120, gp140, gp145 or gp150). For example, the mis-matched antigen can have about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, about 96%, about 97%, about 98% or about 99% sequence identity
25 with the HIV Env protein, or immunogenic fragment thereof. In some embodiments, the mis-matched antigen can have up to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or up to 30, or more, amino acid substitutions, deletions or insertions, compared to the HIV Env protein, or immunogenic fragment thereof. For example, in some embodiments,
30 HIV gp140 is a mis-matched antigen of HIV gp145. In additional examples, HIV gp140 Δ CFI Δ V1V2 is a mis-matched antigen of HIV gp145 Δ CFI Δ V1V2.

In additional embodiments, a mis-matched antigen is an antigen that is similar (but not identical) to a Ebola virus antigen, such as Ebola virus glycoprotein (Z), or immunogenic fragment thereof. For example, the mis-matched antigen can have about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, about 5 96%, about 97%, about 98% or about 99% sequence identity with the Ebola virus glycoprotein (Z). In some embodiments, the mis-matched antigen can have up to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or up to 30, or more, amino acid substitutions, deletions or insertions, compared to the Ebola virus glycoprotein (Z).

10

B. Polynucleotides Encoding Viral Antigens

Polynucleotides encoding the antigens disclosed herein are provided. These polynucleotides include DNA, cDNA and RNA sequences which encode the antigen.

15 Methods for the manipulation and insertion of the nucleic acids of this disclosure into vectors are well known in the art (see for example, Sambrook *et al.*, *Molecular Cloning, a Laboratory Manual*, 2d edition, Cold Spring Harbor Press, Cold Spring Harbor, N.Y., 1989, and Ausubel *et al.*, *Current Protocols in Molecular Biology*, Greene Publishing Associates and John Wiley & Sons, New York, N.Y., 20 1994).

A nucleic acid encoding an antigen can be cloned or amplified by *in vitro* methods, such as the polymerase chain reaction (PCR), the ligase chain reaction (LCR), the transcription-based amplification system (TAS), the self-sustained sequence replication system (3SR) and the Q β replicase amplification system (QB). 25 For example, a polynucleotide encoding the protein can be isolated by polymerase chain reaction of cDNA using primers based on the DNA sequence of the molecule. A wide variety of cloning and *in vitro* amplification methodologies are well known to persons skilled in the art. PCR methods are described in, for example, U.S. Patent No. 4,683,195; Mullis *et al.*, *Cold Spring Harbor Symp. Quant. Biol.* 51:263, 1987; 30 and Erlich, ed., *PCR Technology*, (Stockton Press, NY, 1989). Polynucleotides also

can be isolated by screening genomic or cDNA libraries with probes selected from the sequences of the desired polynucleotide under stringent hybridization conditions.

The polynucleotides encoding an antigen include a recombinant DNA which is incorporated into a vector into an autonomously replicating plasmid or virus or
5 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 of the invention can be ribonucleotides, deoxyribonucleotides, or modified forms of either nucleotide. The term includes single and double forms of DNA.

DNA sequences encoding the antigen can be expressed *in vitro* by DNA
10 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.

15 Polynucleotide sequences encoding antigens 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,
20 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.

Hosts can include microbial, yeast, insect and mammalian organisms. Methods of expressing DNA sequences having eukaryotic or viral sequences in
25 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). Exemplary cells of use include *Escherichia coli*, *Bacillus subtilis*, *Saccharomyces cerevisiae*, *Salmonella typhimurium*, SF9 cells, C129 cells, 293 cells, *Neurospora*, and immortalized
30 mammalian myeloid and lymphoid cell lines. Techniques for the propagation of mammalian cells in culture are well-known (see, Jakoby and Pastan (eds), 1979,

Cell Culture. Methods in Enzymology, volume 58, Academic Press, Inc., Harcourt
Brace Jovanovich, N.Y.). Examples of commonly 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
5 desirable glycosylation patterns, or other features.

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
10 and subsequently treated by the CaCl_2 method using procedures well known in the
art. Alternatively, MgCl_2 or RbCl can be used. 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
15 microinjection, electroporation, insertion of a plasmid encased in liposomes, or viral
vectors can be used. Eukaryotic cells can also be co-transformed with
polynucleotide sequences encoding an 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)
20 or bovine papilloma virus, to transiently infect or transform eukaryotic cells and
express the protein (see for example, *Eukaryotic Viral Vectors*, Cold Spring Harbor
Laboratory, Gluzman ed., 1982).

In some embodiments, a nucleic acid molecule that encodes an antigen is a
nucleic acid provided herein as any one of SEQ ID NOs: 81-147, 150-161 and 206-
25 215. In some embodiments, a nucleic acid molecule that encodes an antigen
comprises a nucleic acid sequence at least about 95% identical, such as about 95%,
about 96%, about 97%, about 98%, about 99% or even 100% identical to the nucleic
acid sequence according to one of SEQ ID NOs: 81-147, 150-161 and 206-215. In
some embodiments, a nucleic acid molecule that encodes an antigen consists of a
30 nucleic acid sequence according to one of SEQ ID NOs: 81-147, 150-161 and 206-
215. For example, exemplary nucleic acid sequences encoding amino acid

sequences of an HIV Env antigen (or immunogenic fragment thereof, such as HIV gp120, gp140 or gp145), include those provided in Int. Pat. App. No.

PCT/US2011/042434 (incorporated by reference herein in its entirety) and disclosed herein.

5

C. *Viral Vectors*

The nucleic acid molecules encoding viral antigens disclosed herein can be included in a viral vector, for example for expression of the antigen in a host cell, or for immunization of a subject as disclosed herein. In some embodiments, the viral
10 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.

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.*
15 *Virol.*, 73:1533-1536), adenovirus (Berkner, 1992, *Curr. 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-
20 256), 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;
25 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 5,221,879), 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
30 *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).

Baculovirus (Autographa californica multinuclear polyhedrosis virus; AcMNPV)
5 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.). Additional viral vectors are disclosed herein.

1. Lymphocytic Choriomeningitis Virus (LCMV)

10 In several embodiments, the methods and compositions disclosed herein include a LCMV vector that is engineered to contain a genome with the ability to amplify and express its genetic information in infected cells but unable to produce further infectious progeny particles in normal, not genetically engineered cells. These LCMV vectors have been further modified to include additional nucleic acids
15 coding for a viral antigen of interests, for example a viral antigen derived from a lentivirus, such as HIV, such that the antigen of interest is expressed in a host cell.

As disclosed herein the LCMV vectors include a modified genome, in which at least one of the four arenavirus open reading frames glycoprotein (GP), nucleoprotein (NP), matrix protein Z and RNA-dependent RNA polymerase L are
20 removed or mutated to prevent propagation of infectivity in normal cells but still allowing gene expression in such cells.

In some embodiments, nucleic acid sequences encoding an antigenic lentiviral polypeptide are introduced and are transcribed from one or more of four LCMV promoters 5' UTR and 3' UTR of the S segment, and 5' UTR and 3' UTR of
25 the L segment, or from additionally introduced promoters that can be read by the viral RNA-dependent RNA polymerase, by cellular RNA polymerase I, RNA polymerase II or RNA polymerase III, respectively. In some examples, one or more internal ribosome entry sites are introduced in the viral transcript sequence to enhance expression of proteins in an LCMV infected cell. In certain embodiments,
30 the LCMV vector is used to produce a LCMV particle that can be used to infect a host cell. In some examples the LCMV vectors are engineered to contain a genome

with the ability to amplify and express its genetic information in infected cells but unable to produce further infectious progeny particles in normal, not genetically engineered cells (see WO/2009/083210, published July 9, 2009, which is specifically incorporated herein by reference in its entirety.)

5 Replication of LCMV vectors requires genetically engineered cells complementing the replication-deficient vector. Upon infection of a cell, the LCMV vector genome expresses not only LCMV proteins but also additional proteins of interest, for example antigens of interest. LCMV vectors are produced by standard reverse genetic techniques as described for (L. Flatz, A. Bergthaler, J. C. de la Torre,
10 and D. D. Pinschewer, Proc Natl Acad Sci USA 103:4663-4668, 2006; A. B. Sanchez and J. C. de la Torre, Virology 350:370, 2006, each of which is incorporated by reference in its entirety).

 Using LCMV vectors a gene of interest, for example a nucleic acids encoding an HIV antigen, can be introduced into cells of a subject, such as a human
15 subject. By abolishing replication capacity of the LCMV vector, the total number of infected cells is limited by the inoculum administered, and thereby limiting or eliminating the chance of LCMV disease and/or LCMV mediated immunosuppression. Therefore, abolishing replication of LCMV vectors prevents pathogenesis as a result of intentional or accidental transmission of vector particles.

20 Nucleic acids coding for an antigen of interest, such as an HIV antigen, can be introduced in into a LCMV vector. In some examples, the nucleic acids encoding an antigen of interest are introduced into the LCMV vector by replacement or fusion to the open reading frame of glycoprotein GP, the matrix protein Z, the nucleoprotein NP, or the polymerase protein L. In some examples, the nucleic acid
25 encoding the antigen of interest is introduced into the LCMV vector such that it can be transcribed and/or expressed under control of one four LCMV promoters (e.g. 5' UTR and 3' UTR of the S segment, and 5' UTR and 3' UTR of the L segment). In some examples, the nucleic acid encoding the antigen of interest is introduced into the LCMV vector with regulatory elements that can be read by the viral RNA-
30 dependent RNA polymerase, cellular RNA polymerase I, RNA polymerase II or RNA polymerase III, such as duplications of viral promoter sequences that are

naturally found in the viral UTRs, the 28S ribosomal RNA promoter, the beta-actin promoter or the 5S ribosomal RNA promoter, respectively. The proteins or nucleic acids can be transcribed and/or expressed either by themselves or as read-through by fusion to LMCV open reading frames and genes, respectively, and/or in combination
5 with one or more, e.g. two, three or four, internal ribosome entry sites.

Preferred antigens of interest are peptidic or proteinaceous antigens from lentiviral origin, such as from HIV, such as those described above. In specific examples, the antigen is one that is useful for the prevention of infectious disease.

10 2. Adenovirus

In several embodiments, the methods and compositions disclosed herein include an adenoviral vector expresses a viral antigen of interest, such as an antigen from HIV. Adenoviruses are generally associated with benign pathologies in humans, and the 36 kilobase (kb) adenoviral genome has been extensively studied.
15 Adenoviral vectors can be produced in high titers (e.g., about 10^{13} particle forming units (pfu)), and can transfer genetic material to nonreplicating, as well as replicating, cells in contrast with, e.g., retroviral vectors, which only transfer genetic material to replicating cells. The adenoviral genome can be manipulated to carry a large amount of exogenous DNA (up to about 8 kb), and the adenoviral capsid can
20 potentiate the transfer of even longer sequences (Curiel et al., Hum. Gene Ther. , 3, 147-154 (1992)). Additionally, adenoviruses generally do not integrate into the host cell chromosome, but rather are maintained as a linear episome, thus minimizing the likelihood that a recombinant adenovirus will interfere with normal cell function. In addition to being a superior vehicle for transferring genetic material to a wide
25 variety of cell types, adenoviral vectors represent a safe choice for gene transfer, a particular concern for therapeutic applications.

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, avian, canine, ovine, or bovine adenoviruses)
30 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, 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
5 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 serotypes. For
10 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 41), an unclassified serogroup (e.g., serotypes
15 49 and 51), or any other adenoviral serotype. Adenoviral serotypes 1 through 51 are available from the American Type Culture Collection (ATCC, Manassas, VA). In some examples, the adenoviral vector is of human subgroup C, such as serotype 2 or serotype 5. However, non-group C adenoviruses can be used to prepare adenoviral vectors for delivery of gene products, such as lentiviral antigens to host cells. In
20 some examples, adenoviruses used in the construction of non- group C adenoviral gene transfer vectors include Ad35 (group B), Ad26 (group D), and Ad28 (group D). Non-group C adenoviral vectors, methods of producing non- group C adenoviral vectors, and methods of using non- group C adenoviral vectors are disclosed in, for example, U.S. Patent Nos. 5,801,030, 5,837,511, and 5,849,561 and International
25 Patent Application Publication Nos. WO 97/12986 and WO 98/53087 all of which are specifically incorporated herein by reference in their entirety.

In some examples, the adenoviral vector can be replication-competent. For example, the adenoviral vector can have a mutation (e.g., a deletion, an insertion, or a substitution) in the adenoviral genome that does not inhibit viral replication in host
30 cells. The adenoviral vector also can be conditionally replication-competent.

In other examples, the adenoviral vector is replication- deficient in host cells. Deletion of an entire gene region often is not required for disruption of a replication-essential gene function. However, for the purpose of providing sufficient space in the adenoviral genome for one or more transgenes, removal of a majority of a gene
5 region may be desirable. While deletion of genetic material is preferred, mutation of genetic material by addition or substitution also is appropriate for disrupting gene function. Replication-essential gene functions are those gene functions that are required for replication (e.g., propagation) and are encoded by, for example, the adenoviral early regions (e.g., the E1 , E2, and E4 regions), late regions (e.g., the L1-
10 L5 regions), genes involved in viral packaging (e.g., the IVa2 gene), and virus-associated RNAs (e.g., VA-RNA1 and/or VA-RNA-2).

The replication-deficient adenoviral vector desirably requires complementation of at least one replication-essential gene function of one or more regions of the adenoviral genome. In some examples, the adenoviral vector has
15 complementation of at least one gene function of the E1 A region, the E1 B region, or the E4 region of the adenoviral genome required for viral replication (denoted an E1 -deficient or E4-deficient adenoviral vector). In addition to a deficiency in the E1 region, the recombinant adenovirus also can have a mutation in the major late promoter (MLP), as discussed in International Patent Application Publication No.
20 WO 00/00628. In some examples, the adenoviral vector is deficient in at least one replication-essential gene function (desirably all replication-essential gene functions) of the E1 region and at least one gene function of the nonessential E3 region (denoted an E1/E3 -deficient adenoviral vector). With respect to the E1 region, the adenoviral vector can be deficient in part or all of the E1 A region and/or
25 part or all of the E1B region, e.g., in at least one replication-essential gene function of each of the E1 A and E1B regions, thus requiring complementation of the E1 A region and the E1B region of the adenoviral genome for replication. The adenoviral vector also can require complementation of the E4 region of the adenoviral genome for replication, such as through a deficiency in one or more replication- essential
30 gene functions of the E4 region.

When the adenoviral vector is deficient in at least one replication-essential gene function in one region of the adenoviral genome (e.g., an E1- or E1/E3 - deficient adenoviral vector), the adenoviral vector is referred to as "singly replication-deficient." In some examples, a singly replication-deficient adenoviral vector is, for example, a replication-deficient adenoviral vector requiring, at most, 5 complementation of the E1 region of the adenoviral genome, so as to propagate the adenoviral vector (e.g., to form adenoviral vector particles).

The adenoviral vector can be "multiply replication-deficient," meaning that the adenoviral vector is deficient in one or more replication-essential gene functions 10 in each of two or more regions of the adenoviral genome, and requires complementation of those functions for replication. For example, the aforementioned E1- deficient or E1/E3 -deficient adenoviral vector can be further deficient in at least one replication- essential gene function of the E4 region (denoted an E1/E4- or E1/E3/E4-deficient adenoviral vector), and/or the E2 region 15 (denoted an E1/E2- or E1/E2/E3 -deficient adenoviral vector), preferably the E2A region (denoted an E1/E2A- or E1/E2A/E3 -deficient adenoviral vector). An adenoviral vector deleted of the entire E4 region can elicit a lower host immune response.

The adenoviral vector can include an adenoviral genome deficient in one or 20 more replication-essential gene functions of each of the E1 and E4 regions (e.g., the adenoviral vector is an E1/E4-deficient adenoviral vector), preferably with the entire coding region of the E4 region having been deleted from the adenoviral genome. In other words, all the open reading frames (ORFs) of the E4 region have been removed. In some examples, the adenoviral vector is rendered replication-deficient 25 by deletion of all of the E1 region and by deletion of a portion of the E4 region. The E4 region of the adenoviral vector can retain the native E4 promoter, polyadenylation sequence, and/or the right-side inverted terminal repeat (ITR).

The adenoviral vector, when multiply replication-deficient, especially in replication- essential gene functions of the E1 and E4 regions, can include a spacer 30 sequence to provide viral growth in a complementing cell line similar to that achieved by singly replication-deficient adenoviral vectors, particularly an E1 -

deficient adenoviral vector. The spacer sequence can contain any nucleotide sequence or sequences which are of a desired length, such as sequences at least about 15 base pairs (e.g., between about 15 base pairs and about 12,000 base pairs), preferably about 100 base pairs to about 10,000 base pairs, more preferably about 500 base pairs to about 8,000 base pairs, even more preferably about 1,500 base pairs to about 6,000 base pairs, and most preferably about 2,000 to about 3,000 base pairs in length. The spacer sequence can be coding or non-coding and native or non-native with respect to the adenoviral genome, but does not restore the replication-essential function to the deficient region. The spacer can also contain a promoter-variable expression cassette. More preferably, the spacer comprises an additional polyadenylation sequence and/or a passenger gene. Preferably, in the case of a spacer inserted into a region deficient for E4, both the E4 polyadenylation sequence and the E4 promoter of the adenoviral genome or any other (cellular or viral) promoter remain in the vector. The spacer is located between the E4 polyadenylation site and the E4 promoter, or, if the E4 promoter is not present in the vector, the spacer is proximal to the right-side ITR. The spacer can comprise any suitable polyadenylation sequence. Examples of suitable polyadenylation sequences include synthetic optimized sequences, BGH (Bovine Growth Hormone), Polyoma virus, TK (Thymidine Kinase), EBV (Epstein Barr Virus), and the papillomaviruses, including human papillomaviruses and BPV (Bovine Papilloma Virus). Preferably, particularly in the E4 deficient region, the spacer includes an SV40 Polyadenylation sequence. The SV40 polyadenylation sequence allows for higher virus production levels of multiply replication deficient adenoviral vectors. In the absence of a spacer, production of fiber protein and/or viral growth of the multiply replication-deficient adenoviral vector is reduced by comparison to that of a singly replication-deficient adenoviral vector. However, inclusion of the spacer in at least one of the deficient adenoviral regions, preferably the E4 region, can counteract this decrease in fiber protein production and viral growth. Ideally, the spacer comprises the glucuronidase gene. The use of a spacer in an adenoviral vector is further described in, for example, U.S. Patent No. 5,851,806 and International Patent Application Publication No. WO 97/21826.

In some embodiments, the adenoviral vector requires, at most, complementation of replication-essential gene functions of the E1, E2A, and/or E4 regions of the adenoviral genome for replication (e.g., propagation). However, the adenoviral genome can be modified to disrupt one or more replication-essential gene functions as desired by the practitioner, so long as the adenoviral vector remains deficient and can be propagated using, for example, complementing cells and/or exogenous DNA (e.g., helper adenovirus) encoding the disrupted replication-essential gene functions. In this respect, the adenoviral vector can be deficient in replication-essential gene functions of only the early regions of the adenoviral genome, only the late regions of the adenoviral genome, both the early and late regions of the adenoviral genome, or all adenoviral genes (e.g., a high capacity adenovector (HC-Ad); see Morsy et al., Proc. Natl. Acad. Sci. USA, 95: 965-976 (1998); Chen et al., Proc. Natl. Acad. Sci. USA, 94: 1645-1650 (1997); Kochanek et al., Hum. Gene Ther., 10: 2451-2459 (1999)). 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; 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.

By removing all or part of, for example, the E1, E3, and E4 regions of the adenoviral genome, the resulting adenoviral vector is able to accept inserts of exogenous nucleic acid sequences while retaining the ability to be packaged into adenoviral capsids (thereby resulting in adenoviral vector constructs). The inventive nucleic acid molecule can be positioned in the E1 region, the E3 region, or the E4 region of the adenoviral genome. Indeed, the nucleic acid molecule can be inserted anywhere in the adenoviral genome so long as the position does not prevent expression of the nucleic acid sequence or interfere with packaging of the adenoviral vector. The adenoviral vector also can include one or more additional nucleic acid sequences encoding the same or different HIV polypeptide. Each nucleic acid sequence can be operably linked to the same promoter, or to different promoters depending on the expression profile desired by the practitioner, and can be inserted

in the same region of the adenoviral genome (e.g., the E4 region) or in different regions of the adenoviral genome (e.g., one nucleic acid sequence is inserted into the E1 region, and a second nucleic acid sequence is inserted into the E4 region).

Preferred antigens of interest are peptidic or proteinaceous antigens from lentiviral origin, such as from HIV, such as those described above. In specific examples of, the antigen is one that is useful for the prevention of infectious disease.

D. Therapeutic Methods and Pharmaceutical Compositions

Disclosed are methods of treating, inhibiting, and/or preventing viral infection (such as a lentiviral infection) in a subject, for example by inducing an immune response, such as a protective immune response in a subject. The disclosed methods include administering to the subject a prime-boost vaccination including administering a priming vaccine and then, after a period of time has past, administering to the subject a boosting vaccine. The immune response is "primed" upon administration of the priming vaccine, and is "boosted" upon administration of the boosting vaccine. The priming vaccine includes a DNA vector or a viral vector (such as an adenoviral vector) that encodes a heterologous viral antigen (such as an antigen from a lentivirus, such as HIV, an antigen derived from a lentivirus, such as HIV). The boost vaccine includes a lymphocytic choriomeningitis virus (LCMV) vector that encodes a heterologous viral antigen (such as an antigen from a lentivirus, such as HIV, or an antigen derived from a lentivirus, such as HIV). The viral antigen encoded by the boost vaccine is a mismatch of the viral antigen encoded by the prime vaccine. Thus, the viral antigen encoded by the prime vaccine has a different primary amino acid sequence compared to the viral antigen encoded by the boost vaccine. For example, the antigen encoded by the prime vaccine could be HIV Env protein (or an immunogenic fragment thereof), and the mismatch of the viral antigen encoded by the boost vaccine could be the same HIV Env protein (or an immunogenic fragment thereof) having at least one amino acid substitution (or addition or deletion). In one example, the antigen encoded by the prime vaccine is HIV Env protein (or an immunogenic fragment thereof) from a first HIV Clade, and the mismatch of the viral antigen encoded by the boost vaccine is HIV Env protein

(or an immunogenic fragment thereof) from a second HIV Clade, wherein the viral antigen and the mismatch of the viral antigen have a different primary amino acid sequence.

The priming composition is a gene transfer vector that includes a nucleic acid sequence encoding an antigen. Any gene transfer vector can be employed, including viral and non-viral gene transfer vectors. Examples of suitable viral gene transfer vectors include, but are not limited to, retroviral vectors, adeno-associated virus vectors, vaccinia virus vectors, herpes virus vectors, and adenoviral vectors. Examples of suitable non-viral vectors include, but are not limited to, plasmids, liposomes, and molecular conjugates (e.g., transferrin). In specific examples, the priming composition includes a plasmid construct or an adenoviral vector construct.

When the priming composition is an adenoviral vector construct, it can be, for example, an adenoviral vector construct derived from any human or non-human animal. In some embodiments, the priming composition comprises a human adenoviral vector construct (e.g., serotype 5, 26, 28, or 35) or a simian adenoviral vector construct (e.g., cAd3).

The gene transfer vector of the priming composition and the LCMV boosting composition each include at least one nucleic acid sequence encoding an antigen, for example an antigen derived from a lentivirus such as HIV. The antigen encoded by the nucleic acid sequence of the priming composition and/or the boosting composition can be the same as the antigen. Alternatively, the antigen encoded by the nucleic acid sequence of the priming composition and/or the boosting composition can be different. In some embodiments, the gene transfer vector of the priming composition and/or the boosting composition comprises multiple (e.g., two or more) nucleic acid sequences encoding the same antigen. In another embodiment, the gene transfer vector of the priming composition and/or the boosting composition can comprise multiple nucleic acid sequences encoding two or more different antigens, as described herein.

The booster vaccine is administered to the subject after the primer vaccine. Administration of the priming vaccine and the boosting vaccine can be separated by any suitable timeframe. For example, the booster vaccine can be administered at

least 1 week (e.g., 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 7 weeks, 8 weeks, 9 weeks, 10 weeks, 11 weeks, 12 weeks, 13 weeks, 14 weeks, 15 weeks, 16 weeks, 17 weeks, 18 weeks, 19 weeks, 20 weeks, 24 weeks, 28 weeks, 35 weeks, 40 weeks, 50 weeks, or at least 52 weeks, or a range defined by any two of the foregoing values) following administration of the first immunogenic compound. In some embodiments, the booster vaccine can be administered at about 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 7 weeks, 8 weeks, 9 weeks, 10 weeks, 11 weeks, 12 weeks, 13 weeks, 14 weeks, 15 weeks, 16 weeks, 17 weeks, 18 weeks, 19 weeks, 20 weeks, 24 weeks, 28 weeks, 35 weeks, 40 weeks, 50 weeks, or about 52 weeks, or a range defined by any two of the foregoing values, following administration of the first immunogenic compound. More than one dose of priming vaccine and/or boosting vaccine can be provided in any suitable timeframe. The dose of the priming vaccine and boosting vaccine administered to the mammal depends on a number of factors, including the extent of any side-effects, the particular route of administration, and the like.

The methods can include selecting a subject in need of treatment, such as a subject at risk of viral infection, such as HIV infection.

When a viral vector is utilized, it is desirable to provide the recipient with a dosage of each recombinant virus in the composition in the range of from about 10^5 to about 10^{10} plaque forming units/mg mammal, although a lower or higher dose can be administered. The composition of recombinant viral vectors can be introduced into a mammal prior to following any evidence of a viral infection, such as HIV infection. Examples of methods for administering the composition into mammals include, but are not limited to, exposure of cells to the recombinant virus ex vivo, or injection of the composition into the affected tissue or intravenous, subcutaneous, intradermal or intramuscular administration of the virus. Alternatively the recombinant viral vector or combination of recombinant viral vectors may be administered locally by direct injection into the cancerous lesion in a pharmaceutically acceptable carrier. Generally, the quantity of recombinant viral vector, carrying the nucleic acid sequence of one or more viral antigens, such as a HIV antigen to be administered is based on the titer of virus particles. An

exemplary range of the virus to be administered is 10^5 to 10^{11} virus particles per mammal, such as a human.

In one embodiment the recombinant viruses have been constructed to express cytokines (such as TNF- α , IL-6, GM-CSF, and IL-2), and co-stimulatory and
5 accessory molecules (B7-1, B7-2) alone and in a variety of combinations. Simultaneous production of an immunostimulatory molecule and one or more heterologous viral antigens enhances the immune response. Without being bound by theory, dependent upon the specific immunostimulatory molecules, different mechanisms might be responsible for the enhanced immunogenicity: augmentation
10 of help signal (IL-2), recruitment of professional APC (GM-CSF), increase in CTL frequency (IL-2), effect on antigen processing pathway and MHC expression (IFN γ and TNF α) and the like. For example, IL-2, IL-6, interferon, tumor necrosis factor, or a nucleic acid encoding these molecules, can be administered in conjunction with one or more TASA immunogenic polypeptides, or a nucleic acid encoding one or
15 more immunogenic TASA peptides. The co-expression of one or more immunogenic TASA peptides together with at least one immunostimulatory molecule can be effective in an animal model to show anti-tumor effects.

The prime and boost vaccine are typically administered as a pharmaceutically acceptable (e.g., physiologically acceptable) composition, which
20 comprises a carrier, preferably a pharmaceutically carrier (e.g., physiologically acceptable) and the nucleic acid molecule, construct, or vector. The prime and boost vaccines can be administered alone, or in combination with at least one additional immunogenic agent or composition. It will be understood by those of skill in the art that the ability to produce an immune response after exposure to an antigen is a
25 function of complex cellular and humoral processes, and that different subjects have varying capacity to respond to an immunological stimulus. Accordingly, the compositions disclosed herein are capable of eliciting an immune response in an immunocompetent subject, that is a subject that is physiologically capable of responding to an immunological stimulus by the production of a substantially
30 normal immune response, e.g., including the production of antibodies that specifically interact with the immunological stimulus, and/or the production of

functional T-cells (CD4⁺ and/or CD8⁺ T-cells) that bear receptors that specifically interact with the immunological stimulus. It will further be understood that a particular effect of infection with HIV is to render a previously immunocompetent subject immunodeficient. Thus, with respect to the methods disclosed herein, it is
5 generally desirable to administer the compositions to a subject prior to exposure to HIV (that is, prophylactically, e.g., as a vaccine) or therapeutically at a time following exposure to HIV during which the subject is nonetheless capable of developing an immune response to a stimulus, such as an antigenic polypeptide.

Suitable formulations for the compositions include aqueous and non-aqueous
10 solutions, isotonic sterile solutions, which can contain anti-oxidants, buffers, and bacteriostats, and aqueous and non-aqueous sterile suspensions that can include suspending agents, solubilizers, thickening agents, stabilizers, and preservatives. The formulations can be presented in unit-dose or multi-dose sealed containers, such as ampules and vials, and can be stored in a freeze-dried (lyophilized) condition
15 requiring only the addition of the sterile liquid carrier, for example, water, immediately prior to use. Extemporaneous solutions and suspensions can be prepared from sterile powders, granules, and tablets. Preferably, the carrier is a buffered saline solution. The compositions can be formulated to protect the nucleic acid sequence or vector from damage prior to administration. For example, the
20 pharmaceutical composition can be formulated to reduce loss of the nucleic acid or construct on devices used to prepare, store, or administer the composition, such as glassware, syringes, or needles. The composition can be formulated to decrease the light sensitivity and/or temperature sensitivity of the nucleic acid sequence or construct. To this end, the composition preferably comprises a pharmaceutically
25 acceptable liquid carrier, such as, for example, those described above, and a stabilizing agent selected from the group consisting of Polysorbate 80, L-arginine, polyvinylpyrrolidone, trehalose, and combinations thereof. Use of such a composition will extend the shelf life of the nucleic acid sequence or construct, facilitate administration, and increase the efficiency of the inventive method.

30 A composition also can be formulated to enhance transduction efficiency of the nucleic acid molecule or construct. In addition, one of ordinary skill in the art

will appreciate that the composition can comprise other therapeutic or biologically-active agents. For example, factors that control inflammation, such as ibuprofen or steroids, can be part of the composition to reduce swelling and inflammation associated with in vivo administration of the composition. Antibiotics, e.g.,
5 microbicides and fungicides, can be present to treat existing infection and/or reduce the risk of future infection, such as infection associated with gene transfer procedures.

The composition also can be formulated to contain an adjuvant in order to enhance the immunological response. Suitable adjuvants include, but are not limited to, mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, other peptides, oil emulsions, and potentially useful human adjuvants such as *Bacillus Calmette Guerin* (BCG) and *Corynebacterium parvum*. Adjuvants for inclusion in the inventive composition desirably are safe, well tolerated, and effective in humans, such as QS-21, Detox-
15 PC, MPL-SE, MoGM-CSF, TiterMax-G, CRL-1005, GERBU, TERamide, PSC97B, Adjuver, PG-026, GSK-1, GcMAF, B-aletine, MPC-026, Adjuvax, CpG ODN, Betafectin, Alum, and MF59 (as described in, e.g., Kim et al., *Vaccine*, 18: 597 (2000)). Other adjuvants that can be administered to a mammal include lectins, growth factors, cytokines, and lymphokines (e.g., alpha-interferon, gamma-
20 interferon, platelet derived growth factor (PDGF), gCSF, gMCSF, TNF, epidermal growth factor (EGF), IL-1, IL-2, IL-4, IL-6, IL-8, IL-10, and IL-12).

Any route of administration can be used to deliver the composition to the mammal. Indeed, although more than one route can be used to administer the composition, a particular route can provide a more immediate and more effective
25 reaction than another route. In some examples, the composition is administered via intramuscular injection, for example, using a syringe or needleless delivery device. In this respect, this disclosure also provides a syringe or a needleless delivery device comprising the composition. The pharmaceutical composition also can be applied or instilled into body cavities, absorbed through the skin (e.g., via a transdermal patch),
30 inhaled, ingested, topically applied to tissue, or administered parenterally via, for instance, intravenous, peritoneal, or intraarterial administration.

The composition can be administered in or on a device that allows controlled or sustained release, such as a sponge, biocompatible meshwork, mechanical reservoir, or mechanical implant. Implants (see, e.g., U.S. Patent 5,443,505), devices (see, e.g., U.S. Patent 4,863,457), such as an implantable device, e.g., a mechanical
5 reservoir or an implant or a device comprised of a polymeric composition, are particularly useful for administration of the composition. The composition also can be administered in the form of a sustained-release formulation (see, e.g., U.S. Patent 5,378,475) comprising, for example, gel foam, hyaluronic acid, gelatin, chondroitin sulfate, a polyphosphoester, such as bis-2-hydroxyethyl-terephthalate (BHET),
10 and/or a polylactic-glycolic acid.

The dose of the composition administered to the mammal will depend on a number of factors, including the size of a target tissue, the extent of any side-effects, the particular route of administration, and the like. The dose ideally comprises an "effective amount" of the composition, e.g., a dose of composition, which provokes
15 a desired immune response in the mammal. The desired immune response can entail production of antibodies, protection upon subsequent challenge, immune tolerance, immune cell activation, and the like. One dose or multiple doses of the composition can be administered to a mammal to elicit an immune response with desired characteristics, including the production of HIV specific antibodies, or the
20 production of functional T-cells that react with HIV. In certain embodiments, the T-cells may be CD8 T-cells.

It may be advantageous to administer the compositions disclosed herein with other agents such as proteins, peptides, antibodies, and other anti-HIV agents. Examples of such anti-HIV therapeutic agents include nucleoside reverse
25 transcriptase inhibitors, such as abacavir, AZT, didanosine, emtricitabine, lamivudine, stavudine, tenofovir, zalcitabine, zidovudine, and the like, non-nucleoside reverse transcriptase inhibitors, such as delavirdine, efavirenz, nevirapine, protease inhibitors such as amprenavir, atazanavir, indinavir, lopinavir, nelfinavir, osamprenavir, ritonavir, saquinavir, tipranavir, and the like, and fusion protein
30 inhibitors such as enfuvirtide and the like. In certain embodiments, immunogenic compositions are administered concurrently with other anti-HIV therapeutic agents.

In certain embodiments, the immunogenic compositions are administered sequentially with other anti-HIV therapeutic agents, such as before or after the other agent. One of ordinary skill in the art would know that sequential administration can mean immediately following or after an appropriate period of time, such as hours
5 days, weeks, months, or even years later.

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

D. Kits

Kits are also provided. For example, kits for inhibiting viral infection (such
25 as HIV infection) in a subject using the prime-boost vaccination described herein. In several embodiments, the kit includes a first container including a primer vaccine including a recombinant adenoviral vector (rAd) or a DNA vector expressing a HIV antigen as disclosed herein, and a second container including a booster vaccine comprising a replication-defective LCMV vector expressing a mismatch of the HIV
30 antigen. In several embodiments, the primer vaccine and the booster vaccine are formulated for use in a prime-boost vaccination to inhibit HIV in a subject.

In one embodiment, a kit includes instructional materials disclosing means of use for a prime-boost vaccination as described herein. For example, the kit includes instructional materials for use of a primer vaccine comprising a recombinant adenoviral vector (rAd) or a DNA vector expressing a HIV antigen, and a booster vaccine comprising a replication-defective LCMV vector expressing a mismatch of the HIV antigen to inhibit HIV infection in a subject. The instructional materials may be written, in an electronic form (such as a computer diskette or compact disk) or may be visual (such as video files). The kits may also include additional components to facilitate the particular application for which the kit is designed. Thus, for example, the kit may additionally include buffers and other reagents routinely used for the practice of a particular method. Such kits and appropriate contents are well known to those of skill in the art.

EXAMPLES

The following examples are provided to illustrate certain particular features and/or embodiments. These examples should not be construed to limit the disclosure to the particular features or embodiments described.

Example 1

Materials and methods used in Examples 2-8

Animals. 6- to 10-week-old female BALB/c mice were used for mouse studies. 3- to 5-years old male *Macaca mulatta* of Indian origin with an average body weight of 4.8kg were used in the non-human primate (NHP) study.

Vaccine vectors. The rLCMV vectors were generated and titrated as described previously(see Flatz, *et al.*, *Nat Med* 16, 339-345 (2010)). Briefly, the HIV clade B gp145 Δ CFI Δ V1V2 or gp140 Δ CFI Δ V1V2 was inserted into a GP-deleted S segment under the control of a murine pol I promoter, and viral vectors were recovered using a pol I/pol II rescue system as previously described (see Flatz, *et al.*, *Proc Natl Acad Sci U S A* 103, 4663-4668 (2006)). rAd5 vectors are replication-defective E1-, E3-, and E4-deleted human Ads. rAd28 vectors are E1- and E3-deleted replication-defective vectors and all have been previously described

(see Wang, *et al.*, *J Virol* 83, 7166-7175 (2009)). DNA plasmids have been extensively described and used in clinical trials (see e.g. Catanzaro, *et al.*, *Vaccine* 25, 4085-4092 (2007) and Chakrabarti, *et al.*, *J Virol* 76, 5357-5368 (2002)).

Previous experiments with gp140 and gp145 DNA plasmids have revealed
5 comparable immunogenicity. All animals were either injected i.m. with recombinant adenoviral vectors or DNA, and i.v. with rLCMV vectors unless noted.

Intracellular cytokine staining and MHC-tetramer staining. Splenic lymphocytes from individual mice were used for tetramer staining and ICS. A detailed description of the MHC tetramer and ICS stimulation has been published
10 previously (see Honda, *et al.*, *J Immunol* 183, 2425-2434 (2009)). For nonhuman primates, a qualified ICS assay was performed in batch on cryopreserved PBMC or freshly isolated mucosal cells.

Intracellular cytokine staining, MHC-tetramer staining and ELISpot assay. Splenic lymphocytes from individual mice were used for tetramer staining
15 and ICS. A detailed description of the MHC tetramer and ICS stimulation has been published previously (see Honda, *et al.*, *J Immunol* 183, 2425-2434 (2009)). For nonhuman primates, a qualified ICS assay was performed in batch on cryopreserved PBMC. Cryopreserved PBMC were thawed in a 37°C water bath, washed, and resuspended at 1-2 million cells/ml in R10 and rested overnight in a 37°C/5% CO₂
20 incubator. *In vitro* stimulations were performed the following morning. Cells were transferred to a 96 well v-bottom plate at 1 to 3 million cells/well and stimulated with the SIV Env peptide pool (15-mers overlapping by 11 amino acids spanning SIVmac239 Env; provided by the NIH AIDS Research & Reference Reagent Program (NIH-ARRRP), Germantown, MD) in the presence of Brefeldin A at a final
25 concentration of 2 µg/ml and 10 µg/ml, respectively, for 6 hours. Negative controls received an equal amount of DMSO (the peptide diluent) as the peptide-stimulated cells. At the end of the incubation, the plate was placed at 4°C overnight. Staining for cell surface and intracellular molecules was performed the next morning. Cells were surface stained with CD4-QD605 (clone MT477, Invitrogen), CD28-Cy5PE (clone 28.2, BD Biosciences), CD45RA-Cy7PE (clone L48, BD Biosciences), fixed
30 and permeabilized with Cytofix/Cytoperm (BD Biosciences) then intracellularly

stained with CD3-Cy7APC (clone SP34.2, BD Biosciences), IFN- γ -APC (clone B27, BD Biosciences), IL-2-PE (clone MQ1-17H12, BD Biosciences), and TNF- α -FITC (clone Mab11, BD Biosciences). Aqua LIVE/DEAD kit (Invitrogen, Carlsbad, CA) was used to exclude dead cells. All antibodies were titrated to determine the
5 saturating dilution. Samples were acquired on an LSR II flow cytometer and analyzed using FlowJo software (Treestar, Inc., Ashland, OR) and SPICE™ 5.2 software (see, e.g., exon.niaid.nih.gov).

For ELISpot assay, multiscreen 96-well Immobilon-P plates (Millipore) were coated with purified mouse anti-human IFN- γ (BD Pharmingen) at 5 μ g/mL for 2
10 hours at 37°C, washed with 0.1% Tween 20/PBS and blocked with R10 (RPMI, 10% FBS and 1% Penicillin/Streptomycin) for 1 hour. Cells were plated in triplicate, and 2×10^5 cells/well were stimulated with SIV Env peptide pool (15-mers overlapping by 11 amino acids spanning SIVmac239 Env; provided by the NIH AIDS Research & Reference Reagent Program, Germantown, MD) at 1 μ g/ml and
15 incubated for 18 hours at 37°C. Wells were washed with 0.1% Tween 20/PBS 9 times followed by one wash with dd H₂O. Next, wells were incubated with rabbit polyclonal anti-human IFN- γ -Biotin (U-Cytech) at 100 μ g/mL for 2 hours at RT. After 2 hours incubation, wells were washed with 0.1% Tween/PBS six times and incubated with Streptavidin Alkaline Phosphatase diluted at 1:500 (Southern
20 Biotechnology) for 2 hours at RT. Wells were washed five times with 0.1% Tween/PBS and three times with PBS. Wells were then incubated for 10 minutes with nitro-blue tetrazolium chloride/5-bromo-4-chloro-3'-indolyphosphate *p*-toluidine salt chromogen (Pierce) and washed thoroughly with tap water. Plates were allowed to dry overnight and read with an ELISpot reader (Cellular Technology
25 Limited). The number of spot-forming cells (SFC) per million PBMCs was calculated and graphed using SPICE™ 5.2.

ELISA. Levels of HIV gp140B-specific IgG in the sera were assessed using an ELISA described previously (see Ko, *et al.*, *J Virol* 83, 748-756 (2009)). Absorbance at 450 nm was determined by a Spectra Max instrument (Molecular
30 Devices).

SIV challenge. To evaluate infectibility of immunized and control animals, 10 vaccinated *Macaca mulatta* of Indian origin and 10 controls were challenged intra-rectally with SIVsmE660 at the dose of one AID₅₀ (median animal infectious dose) six weeks after the rLCMV boost as recently described with the same virus stock (Letvin, *et al.*, *Sci Transl Med* 3, 81ra36 (2011)).

TRIM5 genotyping. Genomic DNA was isolated from lymphocytes of monkeys using the QIAamp DNA kit (Qiagen) and sequenced for TRIM5 exons as previously described (Letvin, *et al.*, *Sci Transl Med* 3, 81ra36 (2011)).

Statistical Analysis. For all statistical analysis an unpaired two tailed t-test was used with a confidence interval of 95% from Prism 5 for Mac OS X. P-values of less than 0.05 were considered significant and P-values of less than 0.01 highly significant. Presentation and statistical comparison of ICS functional and memory phenotype distributions was performed using SPICE™ version 5.2 (Roederer *et al.*, *Cytometry*, 79A:167-174, 2011).

MACS sorting. The following Miltenyi MACS sorting kits were used according to the manufacturer's instructions: CD4⁺ Dendritic Cell Isolation kit (130-091-262), Plasmacytoid Dendritic Cell Isolation Kit II (130-092-786), CD8⁺ Dendritic Cell Isolation Kit (130-091-169). For CD4⁺ DC and CD8⁺ DC isolation, negative selection was followed by positive selection while for pDC isolation, only negative selection was used. CD4 and CD11c were stained for CD4⁺ DCs, CD8 and CD11c were stained for CD8⁺ DCs and Ly-6c and mPDCA-1 were stained for pDCs. The purity for all three DCs was 88-92%.

Neutralization Assays. Neutralizing antibody responses against SIVsmE660 Env pseudo- viruses were measured with a luciferase-based assay in TZM-bl cells. PBMC-based neutralization assays were performed as described previously (Letvin, *et al.*, *Sci Transl Med* 3, 81ra36 (2011)). PBMC-based neutralization assays were performed as described previously (Letvin, *et al.*, *Sci Transl Med* 3, 81ra36 (2011)).

Example 2

rLCMV targets dendritic cells and efficiently induces Env- and Gag-specific immune responses in mice after a single immunization.

Using a previously described pol-I pol-II based four plasmid transfection system, rLCMV vectors were generated expressing either HIV/SIV *Env* or *Gag* (see Flatz, *et al.*, *Proc Natl Acad Sci U S A*, 103, 4663-4668 (2006)). All of the vectors generated were replication-incompetent and could only be productively grown on
 5 producer cell lines complementing the vector with the LCMV glycoprotein (FIG. 1A), a protein necessary for infectivity of rLCMV vector particles. Dendritic cells (DC) are critical for the priming of both CD4⁺ and CD8⁺ T cells (Pulendran *et al.*, *Curr. Opin. Immunol.*, 20:61-67, 2008). To assess the ability of rLCMV to infect dendritic cells, mouse myeloid, plasmacytoid and lymphoid DCs were
 10 purified from mouse spleens and infected them with rLCMV vectors encoding the truncated HIV envelope *in vitro*. The different DC subsets were transduced at MOIs of 10 with an infection rate from 6 to 10% measured by anti-LCMV NP fluorochrome stained cells 36 hours after infection (FIG. 1B). BALB/c or C57BL/6 mice were then immunized rLCMV vector (2×10^5 IFU) to assess T-cell priming *in*
 15 *vivo*. Both rLCMV vectors (rLCMV/HIVgp145 and rLCMV/SIVgag) induced CD8⁺ T-cell responses as measured by H-2D^d/PA9 or H-2K^b/AL11 tetramer staining respectively 14 days after a single immunization in mice (FIG. 1C).

Example 3

20 DNA or rAd5 priming followed by rLCMV boost induces cellular and humoral immunity

To establish an optimal heterologous prime-boost vector combination, different prime and boost combinations was compared using rLCMV/HIVgp145, rAd5 and plasmid DNA as immunogens. Plasmid DNA priming followed by rAd5
 25 boost was used for comparison since this combination has been evaluated extensively in non-human primate studies and formed the basis for a current clinical trial (see Letvin *et al.*, *Science* 312, 1530-1533 (2006)). This combination has been shown to increase survival in an SIV challenge model in non-human primates and has also proven effective in blocking acquisition in a recent NHP study, though the
 30 relative contributions of Gag vs. Env inserts was not evaluated. When tested in different combinations, it was found that both rLCMV/HIVgp145 and

rAd5/HIVgp140 were comparably immunogenic when used as a boost after plasmid DNA priming. However, when rAd5 priming was followed by rLCMV boosting, significantly more potent T-cell immunity was elicited, particularly in CD8⁺ T cells (**FIG. 2A**). All immunization protocols elicited substantial antibody responses as
5 detected by *Env*-specific ELISA (**FIG. 2B**). rAd5-rLCMV/HIVgp145 immunization elicited CD8⁺ T-cell responses that could be detected for 100 days at a frequency of about 20% of total CD8⁺ T cells (**FIG. 2C**). With this vector combination, a rapid contraction phase was not observed, as previously described for immunization with rAd5-rAd5 (Finn, *et al.*, *J Virol* 83, 12027-12036 (2009)). To determine whether the
10 prime-boost interval could affect immunogenicity, the time of boosting was varied over a range of two to eight weeks. These trials showed that two weeks was not a sufficient prime-boost interval for optimal CD4⁺ and CD8⁺ T-cell responses; after four weeks, an increase in the magnitude of the boost was observed (**FIG. 7**).

The ability of T cells to simultaneously produce a number of effector
15 cytokines upon antigen stimulation is relevant for the protective efficacy of a T-cell response in a murine leishmania model. Therefore the polyfunctionality of rLCMV-induced T cells was analyzed following priming with either DNA or rAd5, and compared it with DNA-rAd5. No significant difference in terms of CD8⁺ and CD4⁺ T-cell polyfunctionality was found among the immunization groups. All three
20 groups displayed similar CD4⁺ and CD8⁺ T-cell intracellular cytokine staining (ICS) patterns, with the majority of cells producing two or three cytokines simultaneously upon stimulation with cognate antigen (**FIGS. 8A and 8B**).

Example 4

25 **Alternative serotype D rAd, rAd28, can also prime for an rLCMV/HIVgp145 boost.**

Although the DNA-rLCMV/HIVgp145 combination was immunogenic, it was unclear whether alternative rAd vectors could substitute for DNA or rAd5 and improve priming. Since D serotype vectors such as rAd26 or rAd28 have been
30 shown to function well for priming *in vivo* (Abbink *et al.*, *J. Virol.*, 81:4654-4663, 2007), it was asked whether they might also prime for the rLCMV boost (Ko *et al.*,

AIDS Vaccine, 6:P309, 2009). No significant differences in CD8⁺ MHC-tetramer-binding T-cell frequencies were elicited by rAd28-rAd5, rAd5-rLCMV and rAd28-rLCMV encoding HIV Env (**FIG. 3A**). Likewise, the three immunization protocols elicited similar levels of Env antibodies by ELISA (**FIG. 3A**). ICS analysis revealed
5 no statistically significant differences in magnitude between rAd5 and rAd28 primed animals (**FIG. 3B**). These prime-boost combinations also elicited similar gut-localized cellular immune responses (**FIG. 3C**).

Example 5

10 **rLCMV/HIVenv is more efficient for boosting on a particle basis compared to rAd5 after a rAd28 prime**

To compare the efficacy of rLCMV for boosting relative to rAd5, mice were first primed with rAd28 and then boosted with equal titers of either rLCMV/HIVgp145 or rAd5 boosting, based on the focus forming units (FFU) used
15 routinely for rLCMV in mice (1×10^5 FFU). The CD8⁺ T-cell immunogenicity data show that the rAd5 boost was less effective on a per particle/FFU basis in eliciting a tetramer response when used at the same low dose as regularly used for rLCMV/HIVenv (**FIG. 3D**). This finding indicates that rLCMV/HIV env boosted more efficiently than rAd5 as a vector.

20

Example 6

Induction of humoral and cellular immune responses in non-human primates following immunization with rAd5/rLCMV

Based on the mouse data, the immunogenicity of the rAd5/rLCMV eight
25 week prime-boost vector combination was determined in primates (**FIG. 4A**). Empty vectors were used for control animals. There was an increase in the magnitude of the cellular immune response three weeks after rLCMV injection (**FIGS. 4B and 4C**). The antibody response measured by ELISA remained unchanged from week 3 to week 8, and there was a 10-fold increase after the boost
30 at week 11 (**FIG. 4B**; $p < 0.001$). Similarly, although antigen-specific ELISpot and cytokine-producing T cells decreased after administration of rAd5 from week three

to week eight, an increase was observed following rLCMV immunization (**FIG. 4C**). There was no significant difference in the polyfunctionality of CD8⁺ or CD4⁺ T cells before and after boosting (weeks 3 and 11, respectively) (**FIGS. 4B, 4C and 5A**). Memory phenotyping of these T cells defined by antibodies to CD28 and CD45RA revealed that the rLCMV/SIVgp140 boosting marginally altered the proportions of central memory, terminal effector and effector memory CD8⁺ T cells (**FIG. 5B**). Before and after boosting, most SIV-specific CD4⁺ T cells were CD28⁺CD45RA⁻, which is characteristic of central memory T cells. For SIV-specific CD8⁺ T cells, a more balanced distribution was observed between central and effector memory T cells (**FIG. 5B**). The percentage of transitional memory T cells also decreased while the effector memory CD8⁺ T cells slightly increased after the boost.

Example 7

15 **Vaccination with rAd5/rLCMV protects non-human primates against acquisition of SIVsm E660 infection**

To determine whether prime-boost vaccination with a mis-matched env could confer protection against lentiviral infection, 12 consecutive weekly intra-rectal challenges of SIVsmE660 were administered to the animals (10 vaccinees and 10 null vaccinated controls) six weeks after the rLCMV boost, with SIV plasma viral load as an endpoint as described for DNA/rAd Gag Pol Env vaccines (Letvin *et al.*, *Sci. Transl. Med.*, 3:81ra36, 2011). Once an animal became infected, no additional challenges were performed, and the rAd5/LCMV Env vaccine was compared to the same vectors without inserts as negative controls. A substantial reduction in acquisition in the SIV *Env* immunized animals was observed (Fig. 6 A; p=0.01 by logrank test). In the vaccinated group, three of the ten monkeys became infected after 99 challenges (3% infection rate per challenge), while in the control null group, eight of the 10 monkeys were infected after 47 challenges (17% infection rate per challenge), indicating a protective efficacy of 82% per challenge. Over the course of twelve exposures, three animals in the vaccinated group and eight in the control arm became infected, indicating a cumulative protective efficacy of 62%. In

contrast, the vaccine had no significant effect on either peak or set-point plasma viremia in infected monkeys, although viral load at weeks 2, 3, and 4 post-infection trended lower in the vaccine group compared to the null group (Fig. 6A and FIG. 6B). In terms of genetic factors that may have affected infectivity in these groups, no animals expressed the previously identified SIV-restrictive MHC alleles including Mamu A*O1, B*08 and B*17, and the restrictive or sensitive TRIM alleles were randomly distributed among groups. They showed no correlation with vaccine-induced protection ($p=0.3$, log-rank). None of the three infected monkeys in the vaccine group had the restrictive TRIM5^{TFP/TFP} alleles, while all of the monkeys with the restrictive TRIM5^{TFP/TFP} alleles in the control group were infected (see Table 1).

Table 1. Monkeys and SIV infection status.

Group ID	Animal #	Body Weight	Trim5 Allele	Week Infected	Peak viremia (RNA copies/ml)
Vaccine	AS72	3.85	RR		
	AZ61	5.5	sR		
	AZ74	5.14	ss	18	5080320
	AV53	4.29	RR		
	BC49	6.06	sR		
	DCA3	4.27	sR	22	2669351
	T4232	5.14	RR		
	7-42	4.95	sR	17	9012954
	7-104	4.31	sR		
	7-152	5.16	sR		
Null	AZ04	4.85	sR	17	1251927
	AZ41	4.86	sR	22	3872290
	AZ60	4.08	sR	15	49553554
	AZ80	4.74	ss	19	229126334

	AZ87	4.11	sR	15	62656944
	AV12	5.81	sR		
	DCJ4	4.3	ss	16	2569070
	T4244	5.16	RR	16	1327513
	7-26	4,74	sR	15	28614920
	7-60	4.88	sR		

In Table 1, Trim5 alleles were typed based on the sequences in the SPRY region, R: restrict allele 1-5: Trim5^{TRP}; s: sensitive allele 6-11: Trim5^Q.

5

Example 8

Identification of an immune correlate of protection

To determine whether the immune responses elicited by vaccination correlated with the protection seen after challenge, the pre-challenge immune responses induced by immunization in monkeys that were infected with monkeys that were not infected in the vaccine group was compared. There were no significant differences in the frequencies of T-cell ELISpot responses or percentages of T cells that produced cytokines after SIV antigen stimulation in the uninfected monkeys compared with the infected ones (Fig. 6C; ELISpot, $p=0.5$; CD4⁺ cell response, $p=1$; CD8⁺ cell response, $p=1$). This was also true for the anti-SIV Env binding IgG ELISA titers or the neutralizing titers against a smE660 tier 1 clone (Fig. 6D; ELISA $p=0.5$; tier 1 clone, $p=0.8$) or against a smE660 swarm assayed in TZM-bl cells (Fig. 6D, $p=0.7$). Strikingly, however, a highly significant difference when the neutralizing activity of sera from vaccinated protected animals was compared to unprotected animals using the SIVsmE660 swarm used for the challenge upon assay in PBMC was found (Fig. 6D; SIVsmE660 swarm, $p=0.02$). Such neutralization differences were not due to anti-human CD4 antibodies, as anti-human CD4 antibodies were not detectable in any of the monkeys (Fig. 11A), and there were no significant differences between the null group and the vaccine group for anti-human 293 expressing human CD4 antibodies (Fig. 11B).

Example 9

rAd5 or cAd3 priming followed by rLCMV boost induces immune response to Ebola virus glycoprotein

5 The example illustrated use of the Adenovirus/LCMV prime-boost vaccination strategy for inducing an immune response to an Ebola virus antigen.

 The following Ebola virus antigens were used for the assays described in this example: Ebola virus glycoprotein Zaire (Z) (SEQ ID NO: 219); Ebola virus
10 glycoprotein Zaire with a F535R amino acid substitution single mutation (smZ) (SEQ ID NO: 222); Ebola virus glycoprotein Zaire with F535R and G536A amino acid substitutions (dmZ) (SEQ ID NO: 225). These are mutations to generate an entry defect glycoprotein, so that the rLCMV would not replicate. Plasmid maps for the LCMV constructs encoding smZ and dmZ are shown in FIG. 14. The smZ and
15 dmZ viral antigens are mis-match antigens of the Z viral antigen, because their primary amino acid sequence differs by at least one residue.

 First, groups of five B6D2F1 mice were intramuscularly injected once with LCMV expressing smZ or dmZ at 10^7 FFU/animal as single immunization or as
20 boosting agent following the prime immunization of Ad5 or cAd3 vectors expressing Z protein (10^9 VP/animal). Serum was collected from each animal 14 days after the last immunization, and Z protein specific IgG concentration was measured based on ELISA against Z protein in cell lysate from HEK293 cells expressing the Z protein. The average concentration of anti-Z protein specific
25 antibody within each animal group is indicated in FIG. 12. The results show that anti-Z protein specific antibody was detected in the serum of mice injected as single immunization or as boosting agent with LCMV expressing the smZ or dmZ viral antigens. Values are shown as mean of group of five mice; * $p < 0.05$.

 Next, groups of five B6D2F1 mice were intramuscularly immunized once
30 with Ad5 or cAd3 expressing Z protein at 10^9 VP, or with LCMV expressing smZ or dmZ at 10^7 FFU. Additional groups of five B6D2F1 mice were intramuscularly

injected (primed) once with Ad5 or cAd3 expressing Z protein at 10^9 VP, and three weeks following the prime injection, treated with intramuscularly injection (boost) of LCMV vector expressing smZ or dmZ at 10^7 FFU. The mice were euthanized two weeks following the boost injection. Single cells from the spleen were

5 stimulated with the Z peptide pool for five hours followed by staining with mAbs against surface markers and cytokines, and then were analyzed by flow cytometry. The results of these assays are presented in FIG. 13A and 13B. FIG 13A shows the percentages of cytokine-producing $CD4^+$ cells by flow cytometry: left panel ($INF-\gamma^+$ and $TNG-\alpha^+$); right panel ($INF-\gamma^+$ and $TNG-\alpha^-$). FIG. 13B shows the percentages of

10 cytokine-producing $CD8^+$ T cells by flow cytometry: left panel ($INF-\gamma^+$ and $TNG-\alpha^+$); right panel ($INF-\gamma^+$ and $TNG-\alpha^-$); by flow cytometry. Values are shown as mean $\pm$ SE of group of five mice; * $p < 0.05$. The results show that T cell responses elicited by single immunization of LCMV vectors at the doses 10^7 FFU were lower than that of Ad5 (10^9 VP), but with strong responses as boosting agent with Ad5 or

15 cAd3 combinations groups.

Example 10

Prime-Boost vaccination for the inhibition of HIV-1 infection

This example describes a particular method that can be used to inhibit HIV

20 in a human subject by administration of a prime-boost vaccination as disclosed herein. Although particular methods, dosages, and modes of administrations are provided, one skilled in the art will appreciate that variations can be made without substantially affecting the treatment.

Based upon the teaching disclosed herein HIV-1 can be treated by

25 administering a therapeutically effective amount of a priming vaccine followed by a boosting vaccine, thereby inhibiting HIV infection in the subject, for example, inhibiting the chances of HIV infection in the subject.

Screening subjects

30 Pre-screening is not required prior to administration of the therapeutic compositions disclosed herein. However, in some examples, a subject that is at risk

of getting an HIV infection is screened, is selected for treatment. For example a subject that has been recently exposed to HIV.

Administration of therapeutic compositions

5 A therapeutically effective amount the prime and boost vaccines described herein are administered to the subject (such as an adult human or a newborn infant at risk for contracting HIV). For example, the prime vaccine can be administered about 1 week, or 2 weeks, or 3 weeks, or 4 weeks, or 5 weeks, or 6 weeks, or 7 weeks, or 8 weeks, or 9 weeks, or 16 weeks, or 20 weeks, or 24 weeks, or 28 weeks,
10 or 32 weeks before administration of the boost vaccine. Additional agents, such as anti-viral agents, can also be administered to the subject simultaneously or prior to or following administration of the disclosed agents. Administration can be achieved by any method known in the art, such as oral administration, inhalation, intravenous, intramuscular, intraperitoneal, or subcutaneous.

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

 In particular examples, if subjects have a minor, mixed or partial response
25 (such as an immune response) to the prime-boost vaccination, they can be re-treated to receive a second prime-boost vaccination.

Assessment

 Following the administration of the prime-boost vaccination, the treated
30 subject can be monitored for HIV infection, including monitoring of CD4+ T cell count, or reductions in one or more clinical symptoms associated with HIV, or for an

immune response, such as the production of neutralizing anti-HIV antibodies. In particular examples, subjects are analyzed one or more times, starting 7 days following treatment. Subjects can be monitored using any method known in the art. For example, biological samples from the subject, including blood, can be obtained
5 and alterations in HIV or CD4+ T cell levels, or concentration of anti-HIV antibodies, can be evaluated.

In view of the many possible embodiments to which the principles of the disclosed invention may be applied, it should be recognized that the illustrated
10 embodiments are only preferred examples of the invention and should not be taken as limiting the scope of the invention. Rather, the scope of the invention is defined by the following claims. We therefore claim as our invention all that comes within the scope and spirit of these claims.

We claim:

1. A method for inhibiting a viral infection in a subject, comprising administering a prime-boost vaccination to the subject, wherein the prime-boost vaccination comprises administering a primer vaccine and a booster vaccine to the
5 subject, wherein:
the primer vaccine comprises a recombinant adenoviral vector (rAd) or a DNA vector expressing a viral antigen; and
the booster vaccine comprises a replication-defective lymphocytic choriomeningitis virus (LCMV) vector expressing a mismatch of the viral antigen,
10 wherein the mismatch enhances an immune response to the viral antigen;
thereby inhibiting the viral infection in the subject
2. The method of claims 1, wherein the viral antigen is a lentivirus antigen.
15
3. The method of claim 2, wherein the lentivirus antigen is a human immunodeficiency virus (HIV) antigen.
4. The method of claim 3, wherein the HIV-1 antigen is HIV-1 Gag
20 protein, HIV-1 Pol protein, HIV-1 Env protein, HIV-1 Tat protein, HIV-1 Reverse Transcriptase (RT) protein, HIV-1 Vif, protein, HIV-1 Vpr protein, HIV-1 Vpu protein, HIV-1 Vpo protein, HIV-1 Integrase protein, HIV-1 Nef protein, and a fusion protein comprising all or part of an HIV-1 Gag protein, HIV-1 Pol protein, or HIV-1 Env protein or an immunogenic fragment thereof.
25
5. The method of claim 4, wherein the HIV antigen comprises HIV Env protein, modified HIV Env protein that retains at least one epitope of HIV Env protein, or an immunogenic fragment of.

6. The method of claim 5, wherein the immunogenic fragment of HIV Env protein comprises gp150, gp145, gp140, gp145, gp120 or gp41, or modified gp150, gp145, gp140, gp145, gp120 or gp41.

5 7. The method of claim 3, wherein the HIV-1 antigen is a mosaic antigen.

8. The method of any one of claims 1-6, wherein the HIV antigen is HIV gp140 and mismatch of the HIV antigen is HIV gp145.

10

9. The method of any one of claims 1-6, wherein the HIV antigen is HIV gp140 Δ CFI and the mismatch of the HIV antigen is HIV gp145 Δ CFI.

10. The method of any one of claims 1-6, wherein the HIV antigen is HIV gp140 Δ CFI Δ V1V2 and the mismatch of the HIV antigen is HIV gp145 Δ CFI Δ V1V2.

15

11. The method of any one of claims 1-10, wherein the HIV-1 antigen is an HIV clade A antigen, HIV clade B antigen, HIV clade C antigen, or an HIV clade MN antigen.

20

12. The method of any one of claims 1-11, wherein the method is a method of inducing an immune response against HIV-1 in a mammal.

25 13. The method of claim 12, wherein the immune response is a CD4+ T cell response.

14. The method of claim 12, wherein the immune response is a CD8+ T cell response.

30

15. The method of claim 1, wherein the viral antigen is a filovirus antigen.

16. The method of claim 15, wherein the filovirus antigen is an Ebola virus antigen.

17. The method of claim 16, wherein the Ebola virus antigen is Ebola virus glycoprotein (GP).

18. The method of any one of claims 15-17, wherein the method is a method of inducing an immune response against Ebola virus in a mammal.

19. The method of claim 18, wherein the immune response is a CD4+ T cell response.

20. The method of claim 18, wherein the immune response is a CD8+ T cell response.

21. The method of any one of claims 1-20, wherein the booster vaccine is administered to the subject about 1 week, or 2 weeks, or 3 weeks, or 4 weeks, or 5 weeks, or 6 weeks, or 7 weeks, or 8 weeks, or 9 weeks, or 16 weeks, or 20 weeks, or 24 weeks, or 28 weeks, or 32 weeks after administration of the primer vaccine.

22. The method of any one of claims 1-21, wherein the primer vaccine, the booster vaccine, or both, further comprise an adjuvant.

23. The method of any one of claims 1-22, wherein the primer vaccine comprises an adenoviral vector expressing the viral antigen.

24. The method of claim 23, wherein the adenoviral vector comprises a human adenoviral vector.

25. The method of claim 24, wherein the adenoviral vector comprises Ad28 and/or Ad5.

5 26. The method of claim 23, wherein the adenoviral vector comprises a non-human adenoviral vector.

27. The method of claim 26, wherein the adenoviral vector comprises cAd3.

10

28. The method of any one of claims 1-27, wherein one or more open reading frames encoding glycoprotein (GP), nucleoprotein (NP), matrix protein Z and/or RNA-dependent RNA polymerase L are removed or mutated from the genome of the replication-defective LCMV vector to prevent replication in normal
15 cells but still allowing gene expression in LCMV vector-infected cells and wherein the mis-matched viral antigen is expressed under control of one or more of the LCMV 5' UTR and 3' UTR of the S segment, and 5' UTR and 3' UTR of the L segment.

20 29. The method of claim 28, wherein the LCMV open reading frame glycoprotein (GP) is removed or mutated.

30. The method of claim 28, wherein the LCMV open reading frame glycoprotein (GP) is removed and replaced by a nucleic acid sequence encoding the
25 mis-matched viral antigen.

31. Use of a prime-boost vaccination to inhibit human immunodeficiency virus (HIV) infection in a subject, wherein the prime-boost vaccination comprises administering a primer vaccine and a booster vaccine to the subject, wherein:
30 the primer vaccine comprises a recombinant adenoviral vector (rAd) or a DNA vector expressing a HIV antigen; and

the booster vaccine comprises a replication-defective lymphocytic choriomeningitis virus (LCMV) vector expressing a mismatch of the HIV antigen; thereby inhibiting the viral infection in the subject

5 32. The use of claim 31, wherein the HIV antigen comprises HIV Env protein, modified HIV Env protein that retains at least one epitope of HIV Env protein, or an immunogenic fragment of.

10 33. The use of any one of claims 32, wherein the HIV antigen is HIV gp140 and mismatch of the HIV antigen is HIV gp145.

34. The use of any one of claims 32, wherein the HIV antigen is HIV gp140ΔCFI and the mismatch of the HIV antigen is HIV gp145ΔCFI.

15 35. The use of any one of claims 32, wherein the HIV antigen is HIV gp140ΔCFIΔV1V2 and the mismatch of the HIV antigen is HIV gp145ΔCFIΔV1V2.

20 36. The use of any one of claims 32-35, wherein the HIV-1 antigen is an HIV clade A antigen, HIV clade B antigen, HIV clade C antigen, or an HIV clade MN antigen.

37. A composition comprising a nucleic acid comprising SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6 or SEQ ID NO: 8.

25 38. The composition of claim 37, further comprising a pharmaceutical carrier.

30 39. A kit for inhibiting human immunodeficiency virus (HIV) in a subject comprising a first container and a second container, wherein the first container comprises a primer vaccine comprising a recombinant adenoviral vector (rAd) or a DNA vector expressing a HIV antigen; and

the second container comprises a booster vaccine comprising a replication-defective lymphocytic choriomeningitis virus (LCMV) vector expressing a mismatch of the HIV antigen;

wherein the primer vaccine and the booster vaccine are formulated for use in
5 a prime-boost vaccination to inhibit HIV in a subject; and
instructions for using the kit.

40. The use of claim 39, wherein the HIV antigen comprises HIV Env protein, modified HIV Env protein that retains at least one epitope of HIV Env
10 protein, or an immunogenic fragment of.

41. The use of any one of claims 40, wherein the HIV antigen is HIV gp140 and mismatch of the HIV antigen is HIV gp145.

15 42. The use of any one of claims 40, wherein the HIV antigen is HIV gp140 Δ CFI and the mismatch of the HIV antigen is HIV gp145 Δ CFI.

43. The use of any one of claims 40, wherein the HIV antigen is HIV gp140 Δ CFI Δ V1V2 and the mismatch of the HIV antigen is HIV gp145 Δ CFI Δ V1V2.
20

FIG. 1A

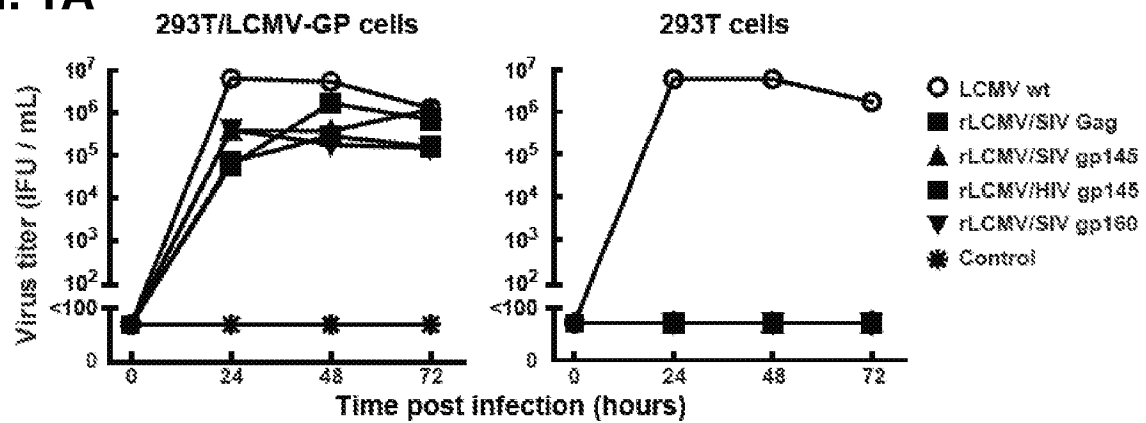


FIG. 1B

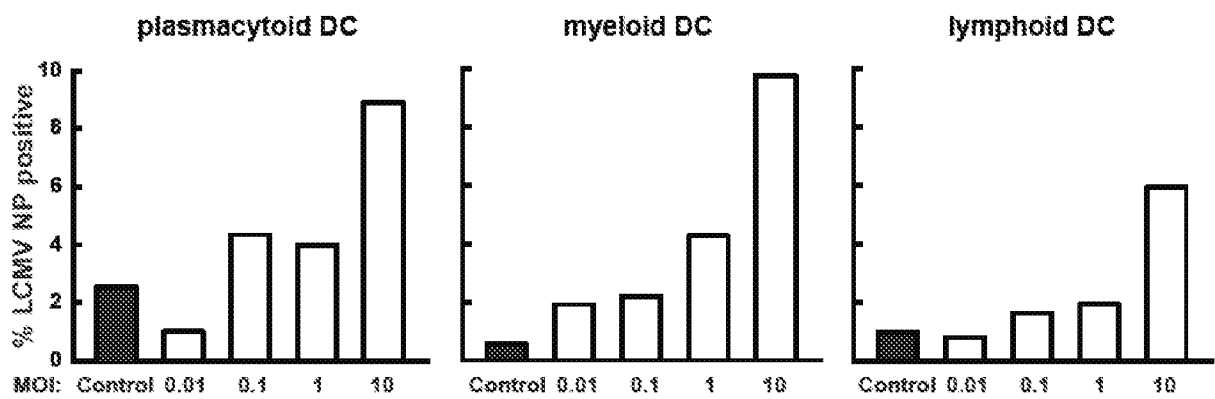


FIG. 1C

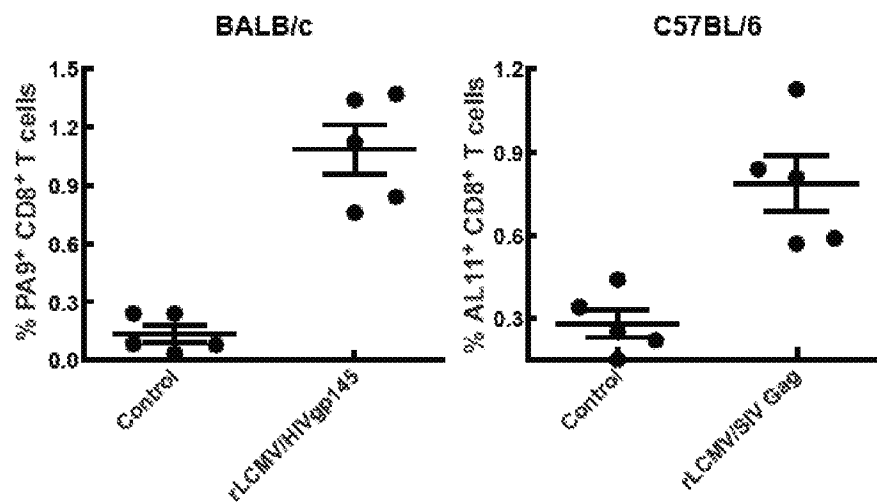


FIG. 2C

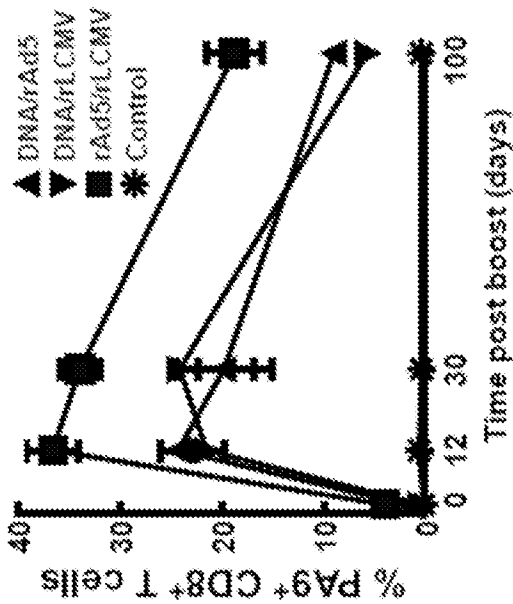


FIG. 2B

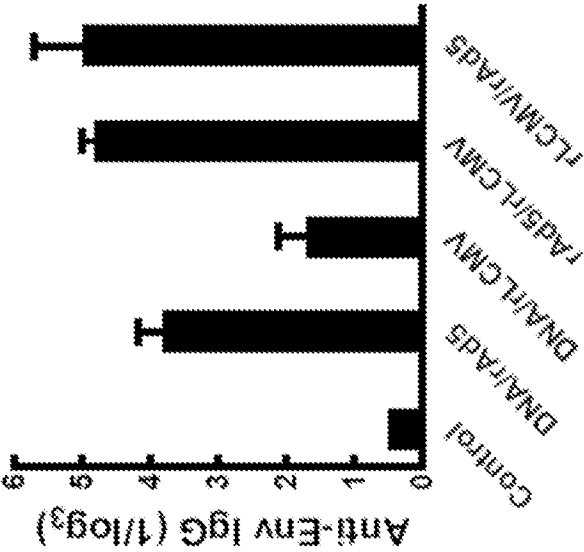


FIG. 2A

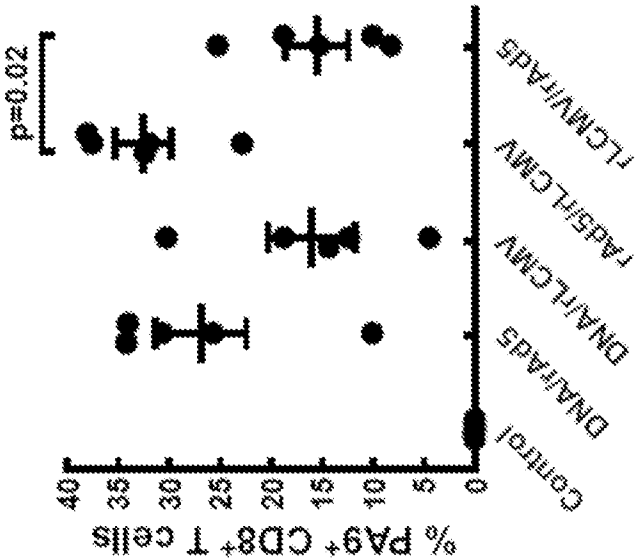


FIG. 3A

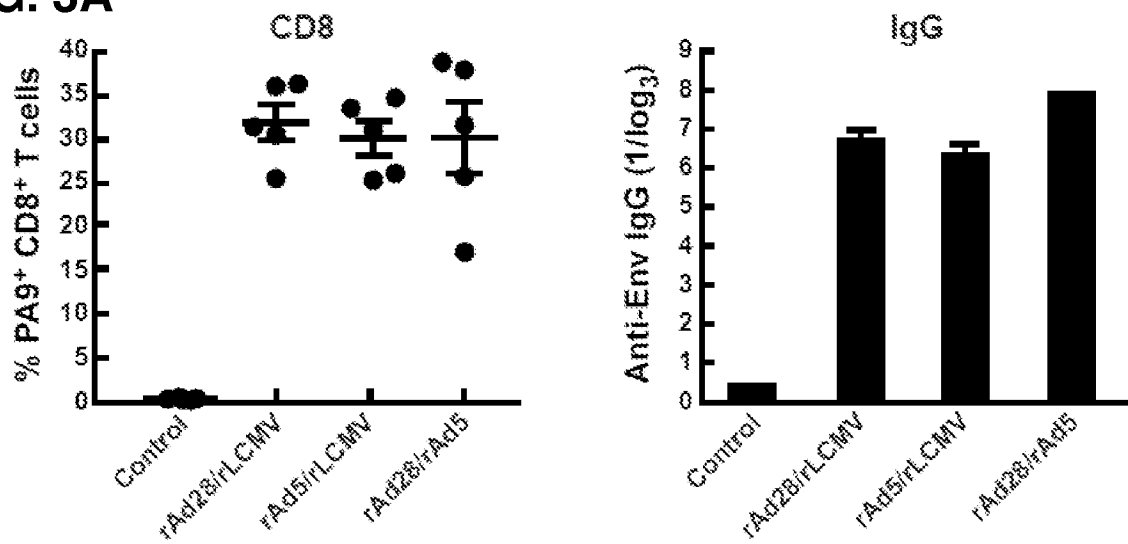


FIG. 3B

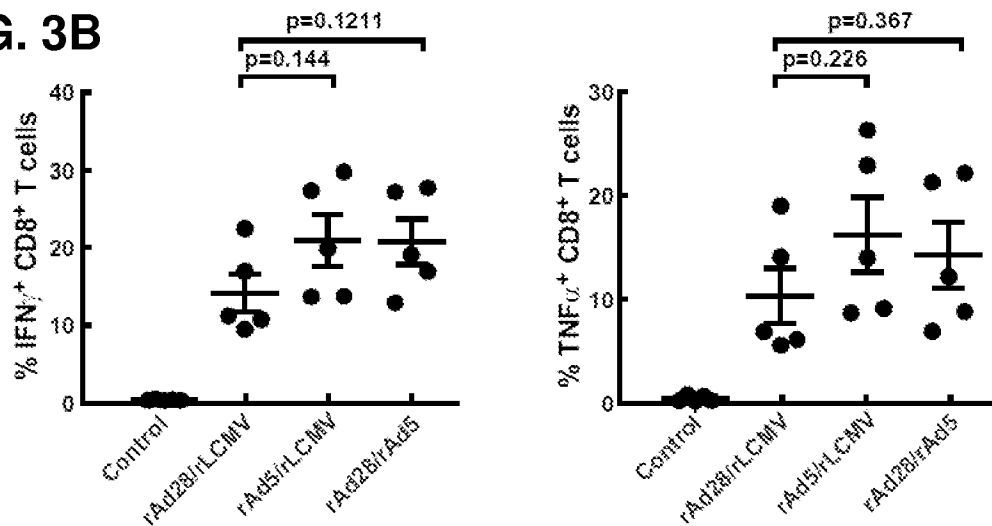


FIG. 3C

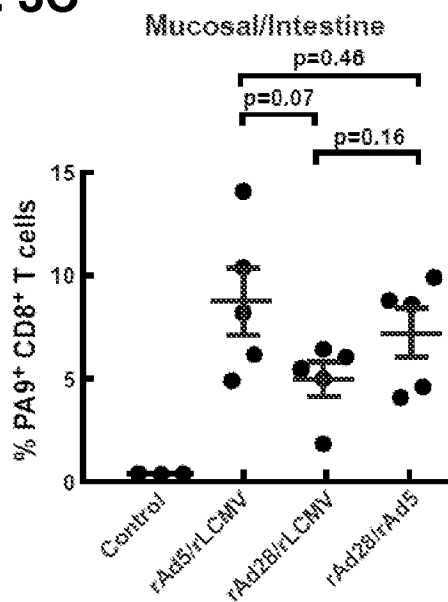
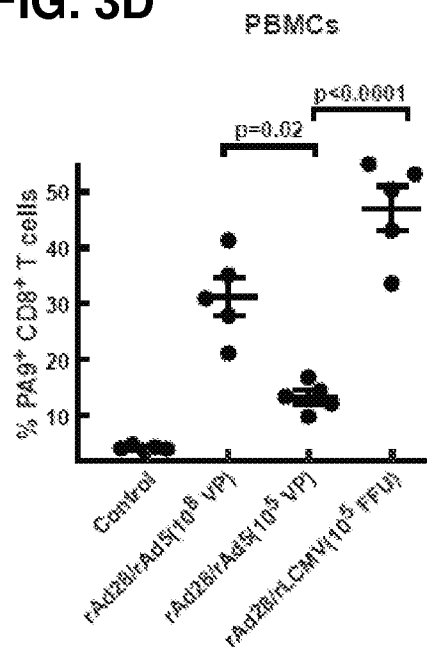


FIG. 3D



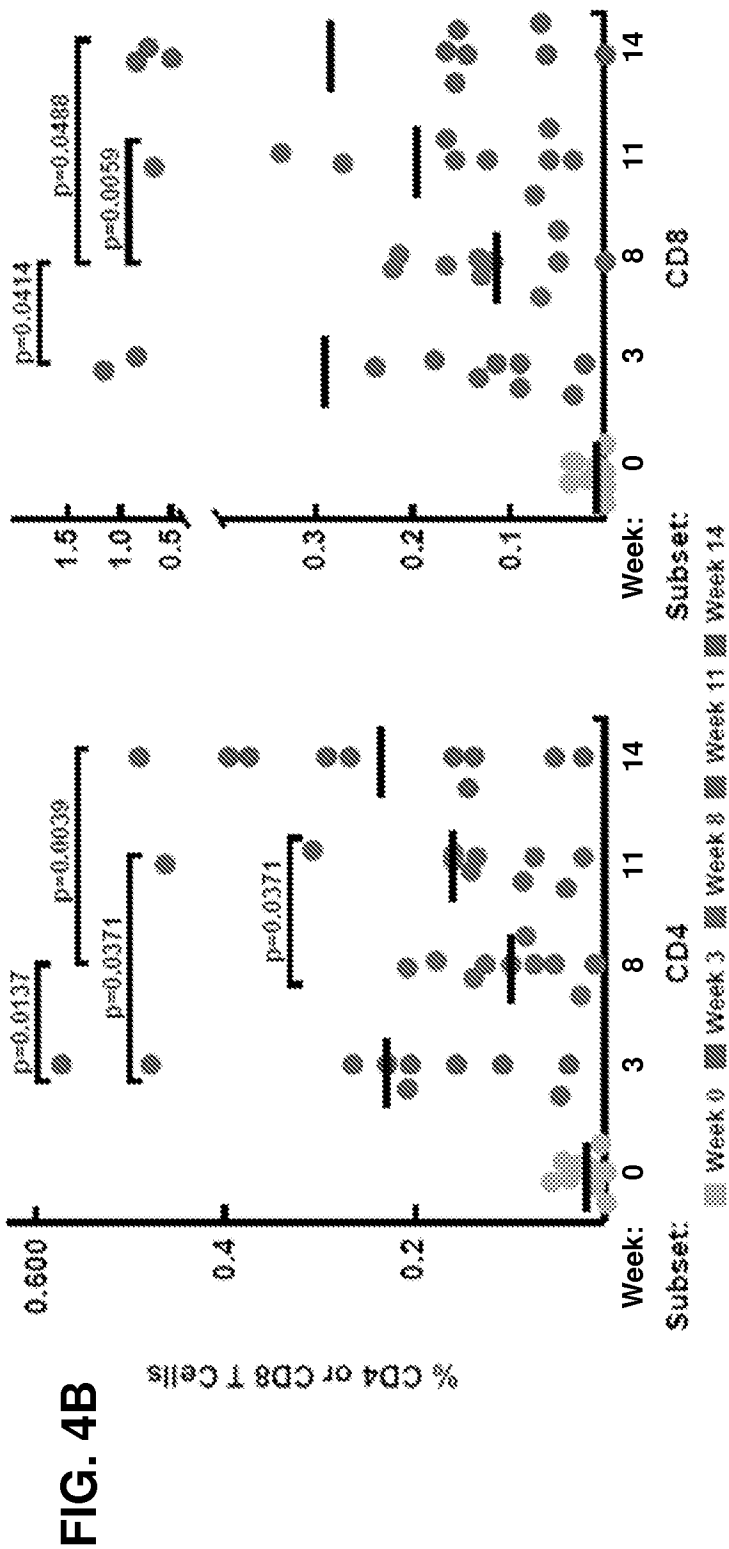
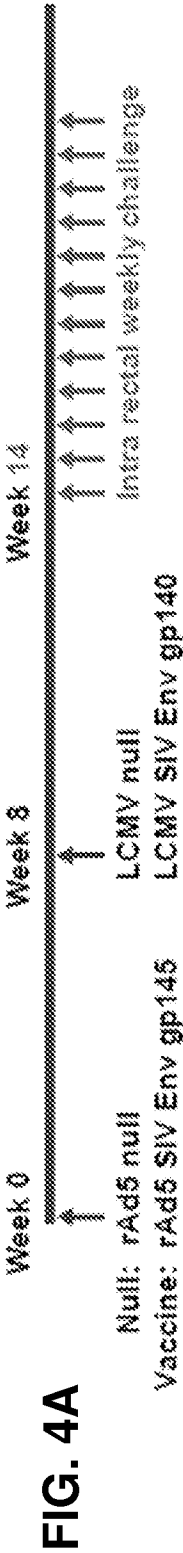


FIG. 4C

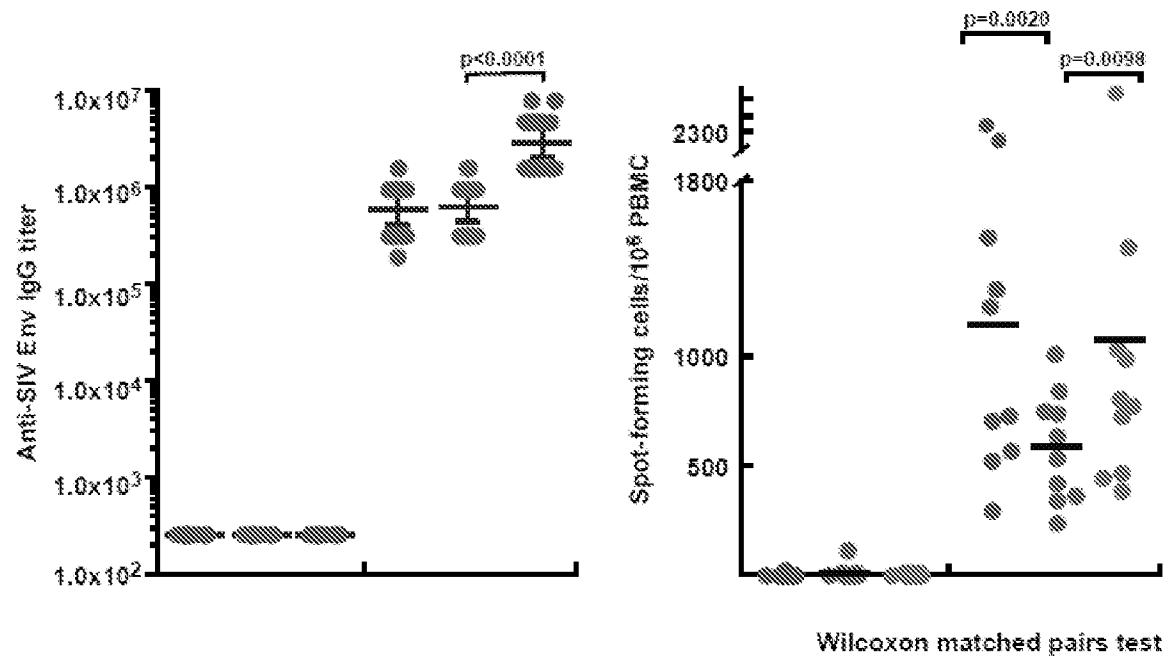


FIG. 5A

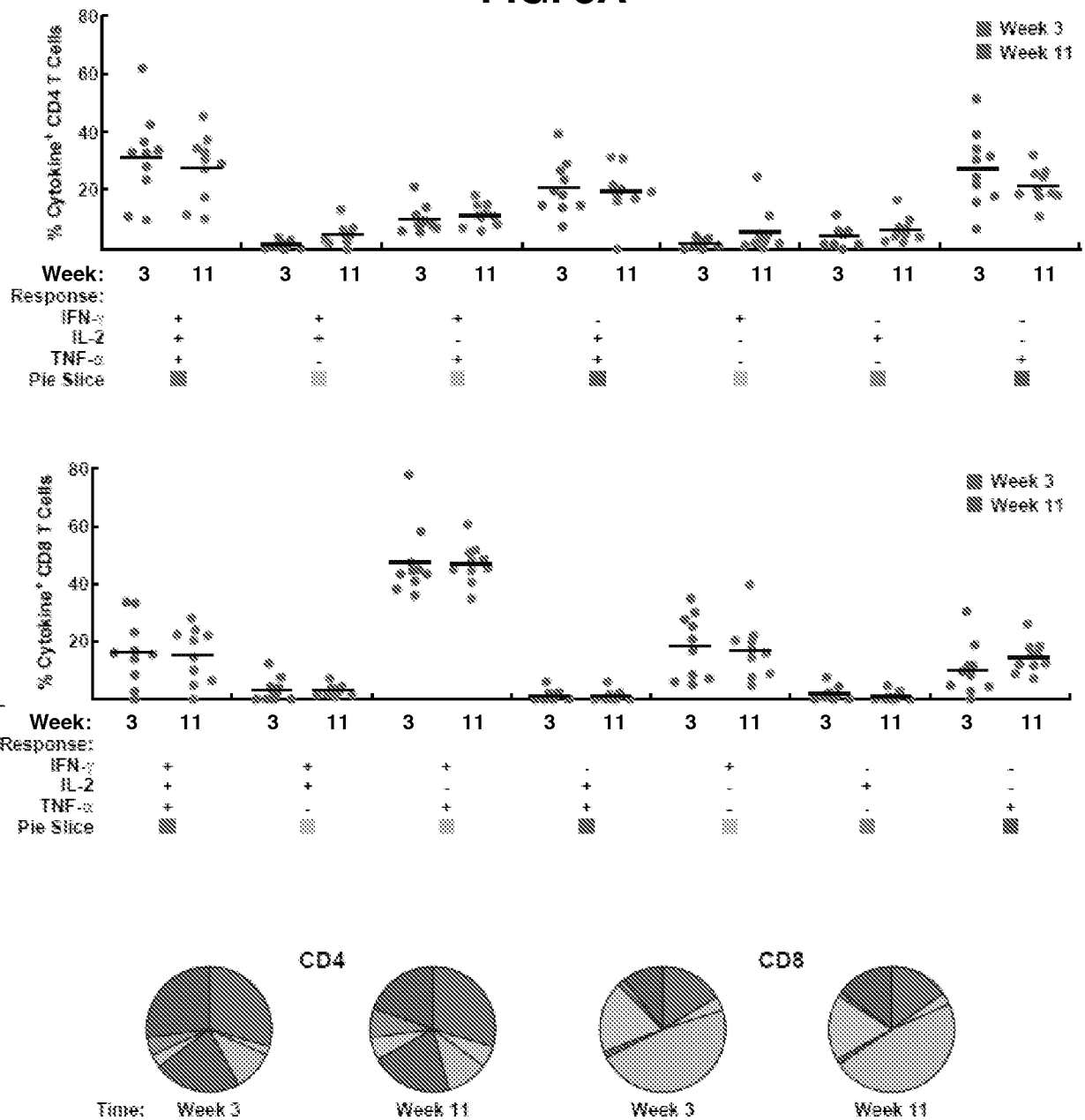


FIG. 5B

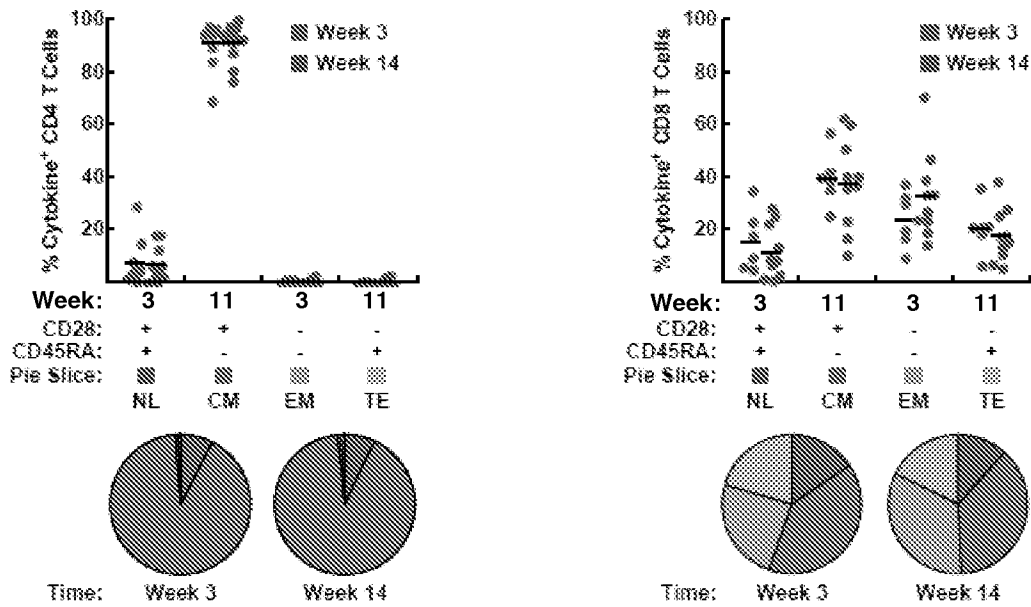


FIG. 6A

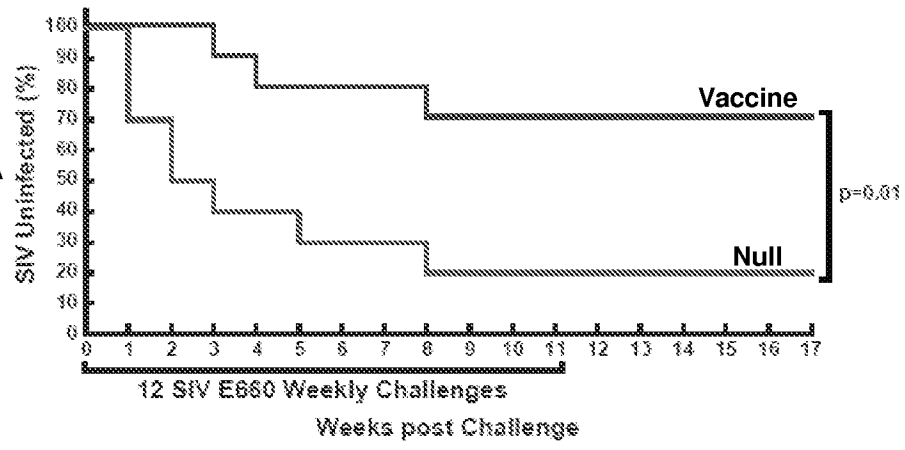


FIG. 6B

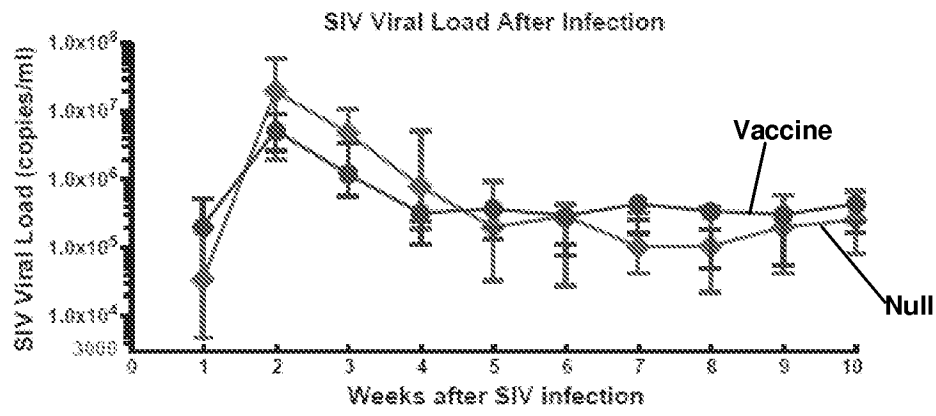


FIG. 6C

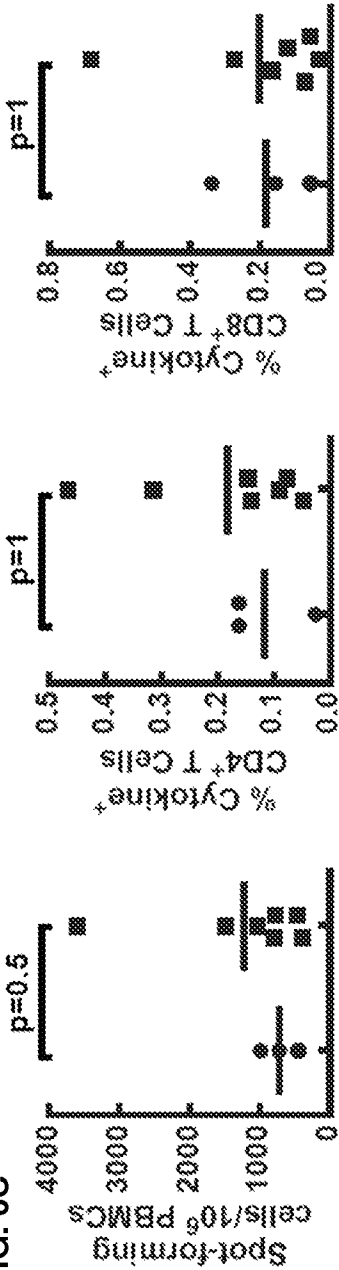


FIG. 6D

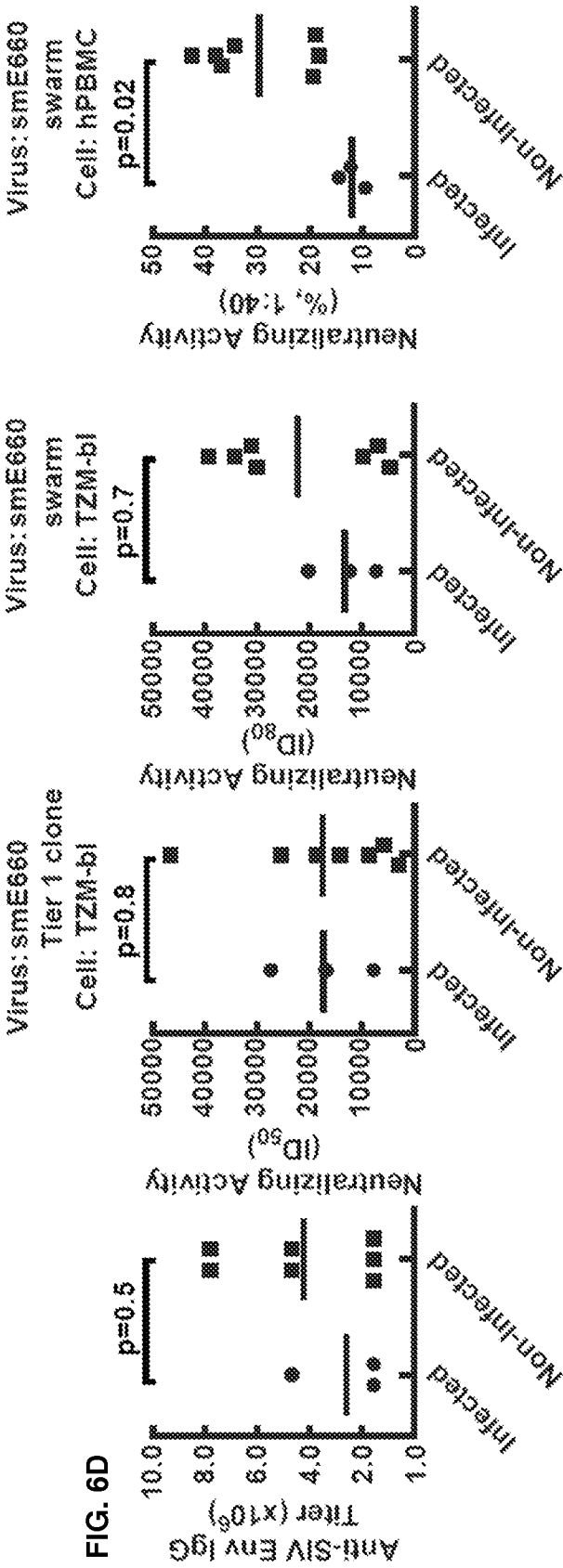


FIG. 7

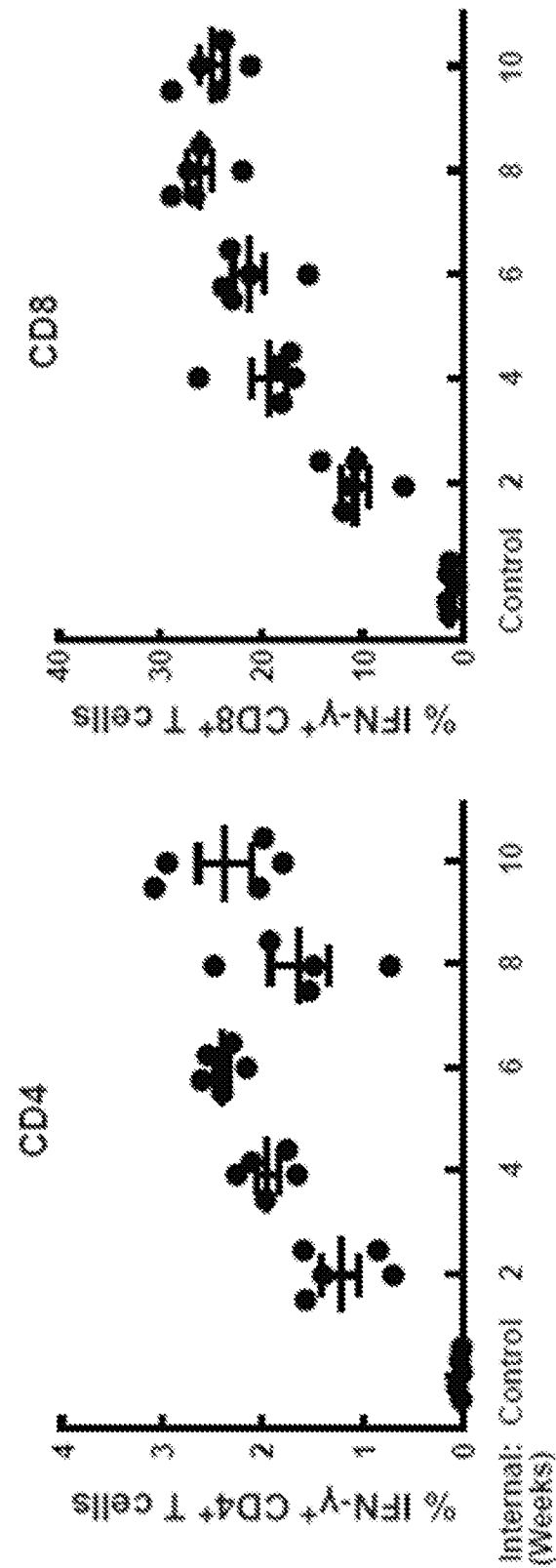


FIG. 8A

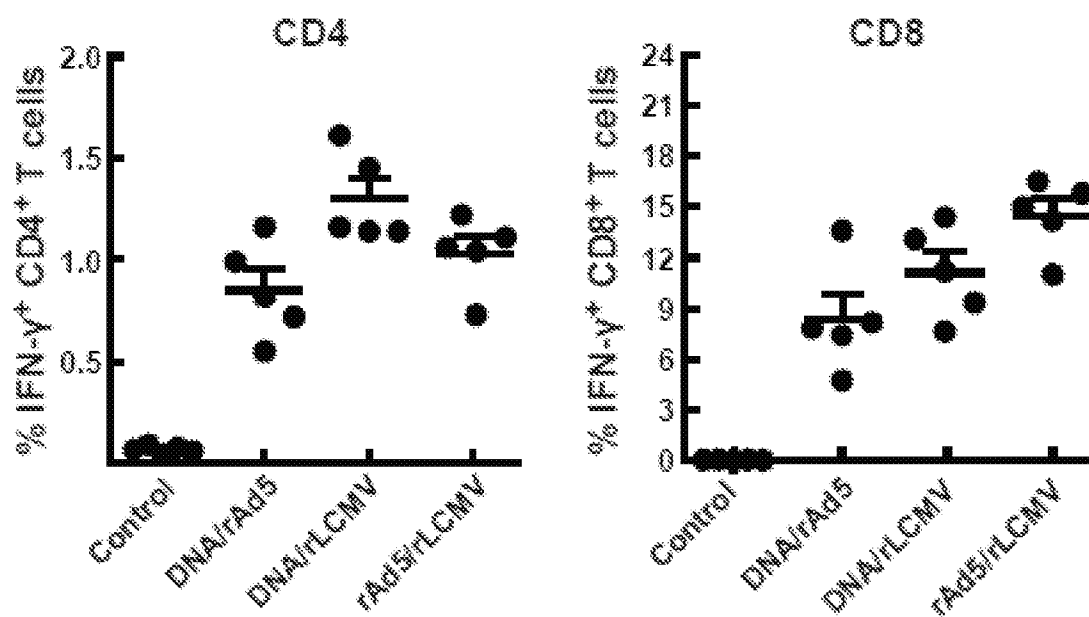


FIG. 8B

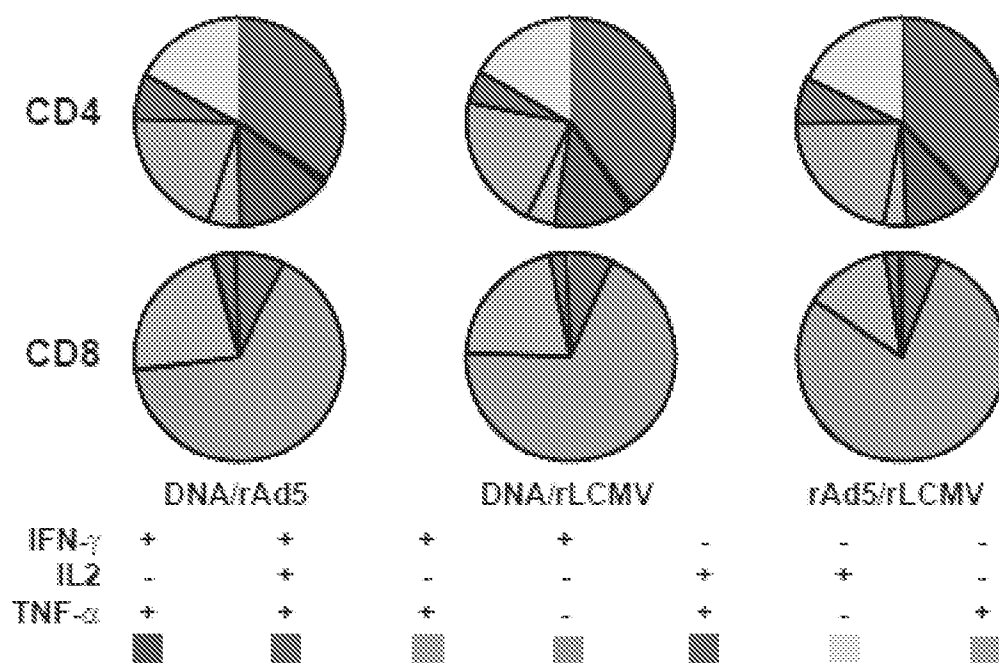


FIG. 9

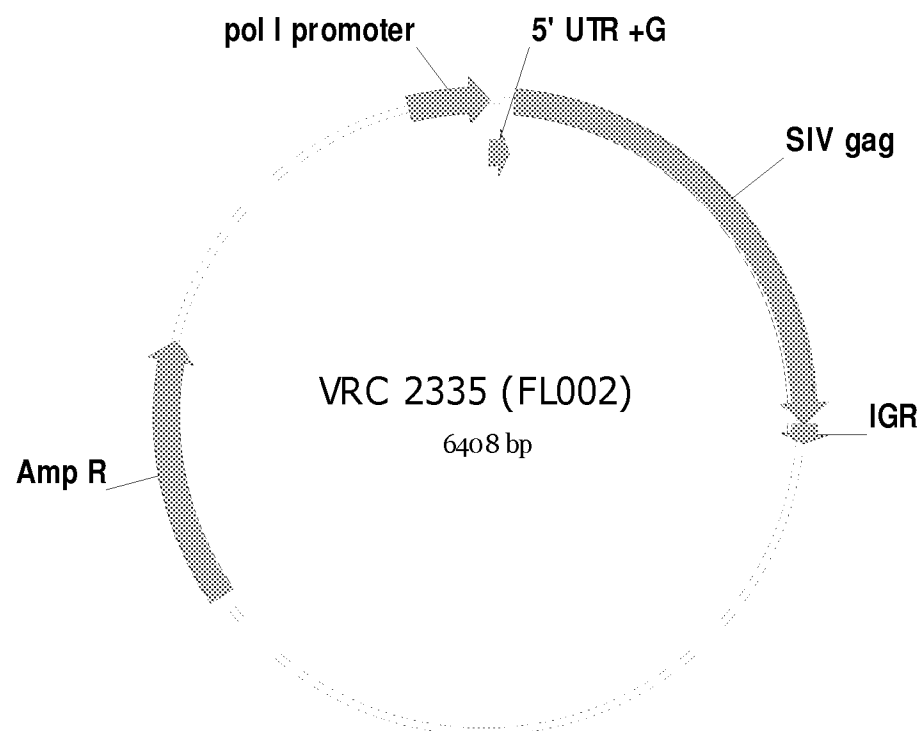
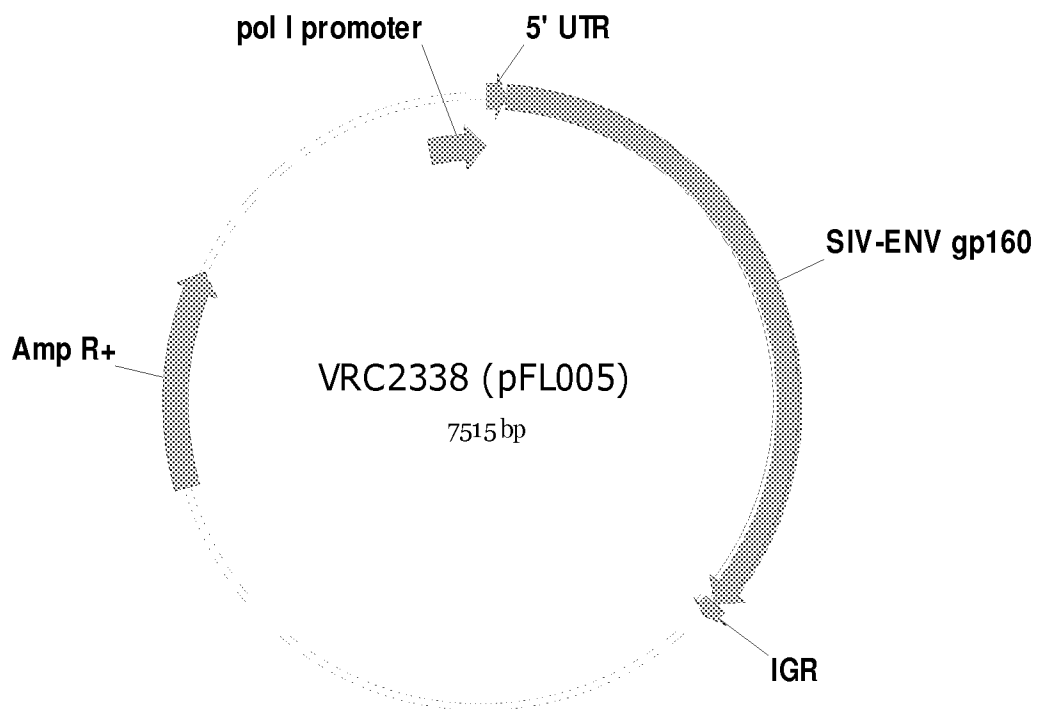


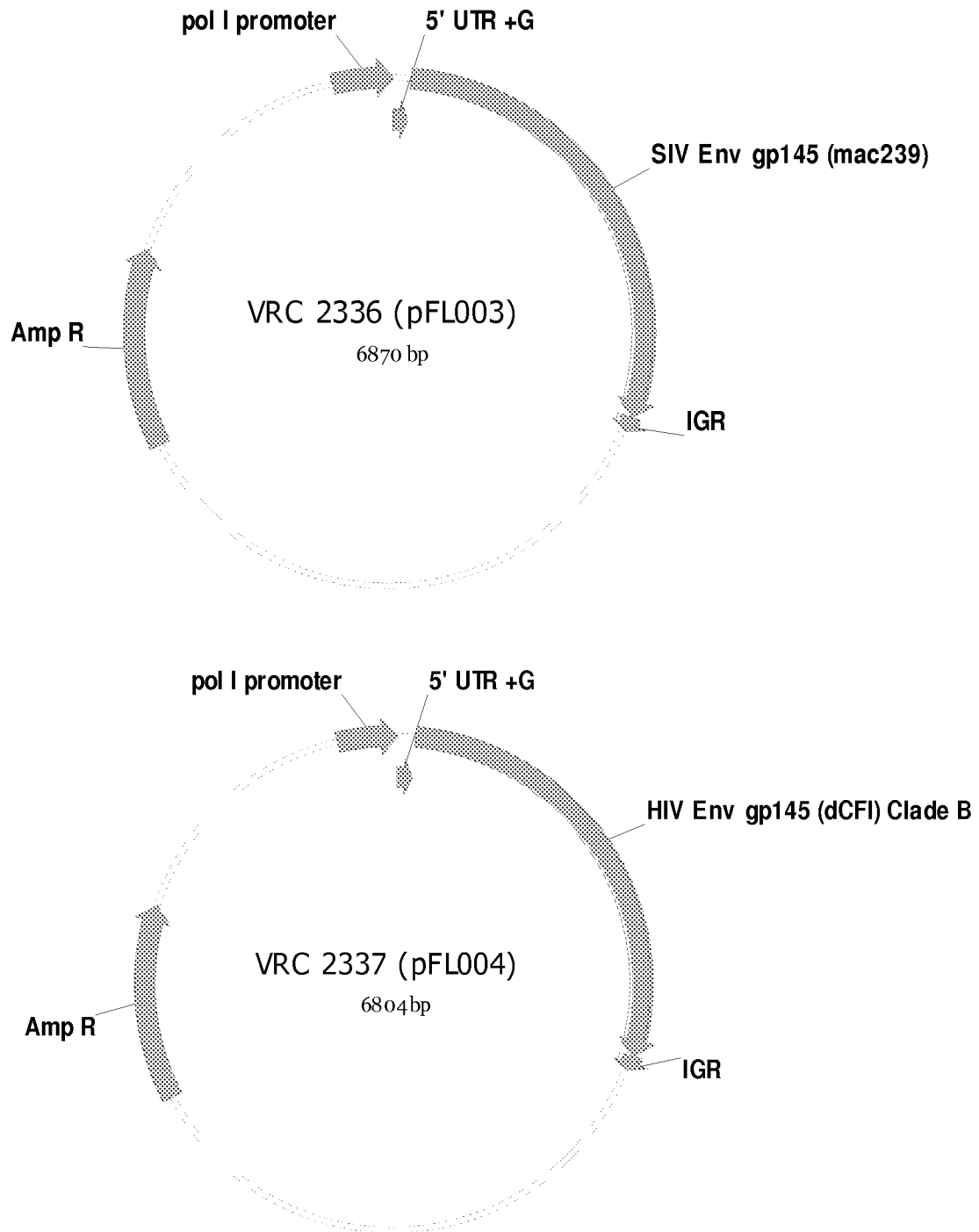
FIG. 10

FIG. 11A

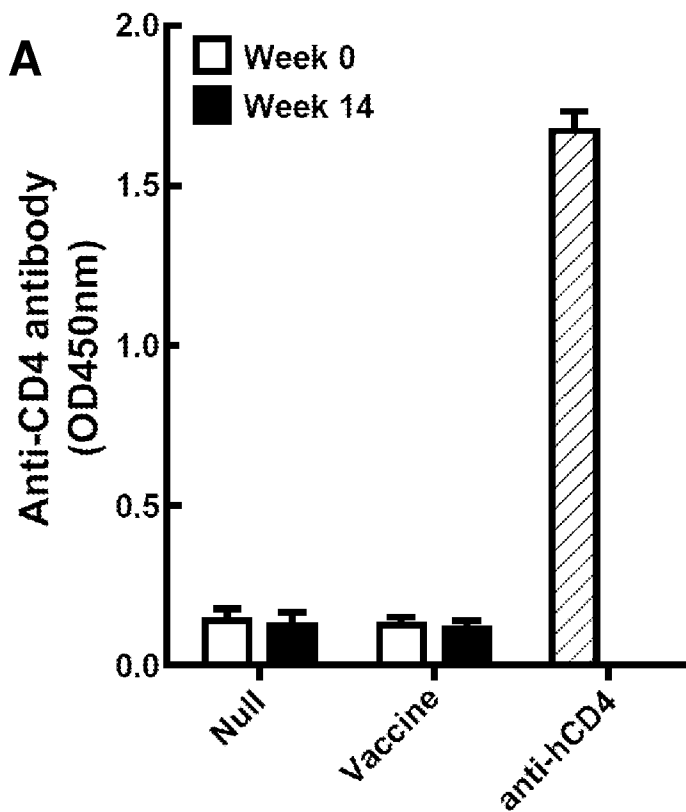


FIG. 11B

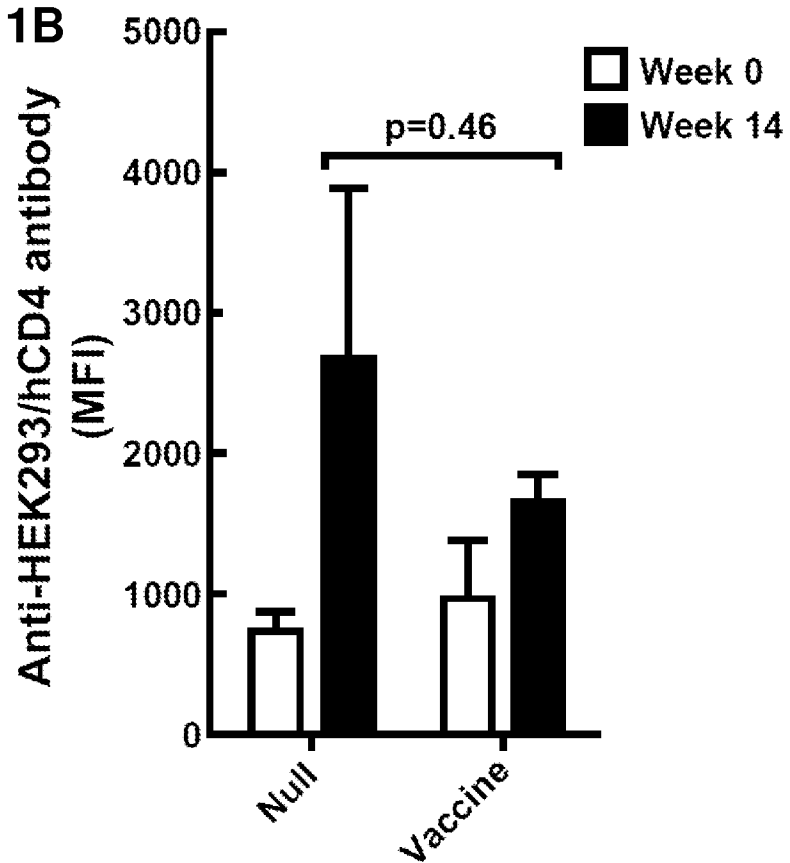


FIG. 12

Ebola virus GP(Zaire)-specific Ab response

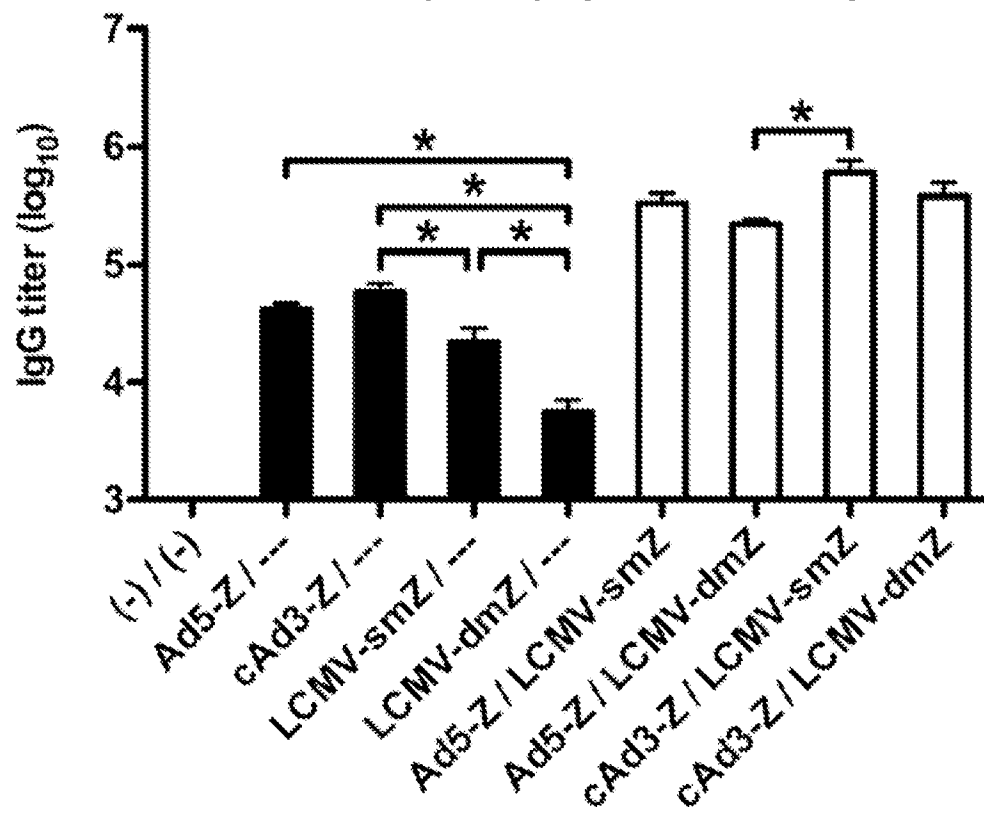


FIG. 13A
CD4⁺ T cell responses

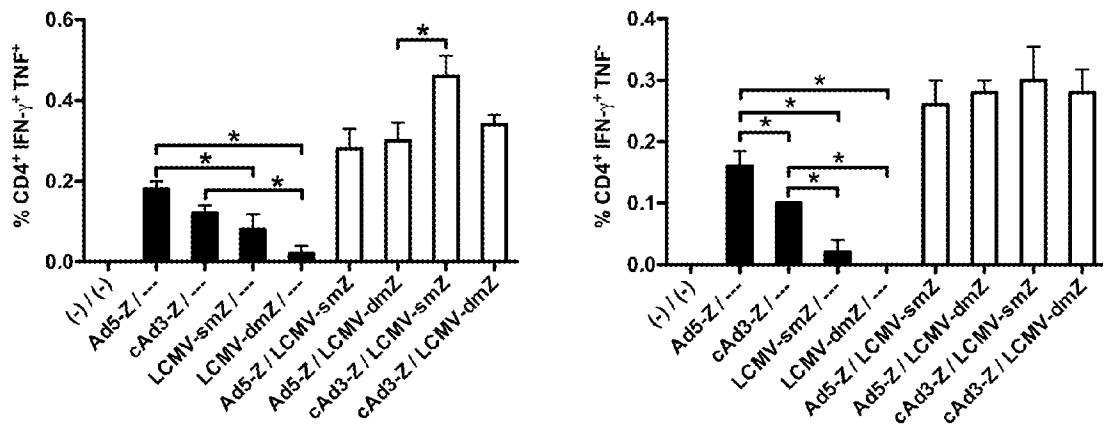


FIG. 13B
CD8⁺ T cell responses

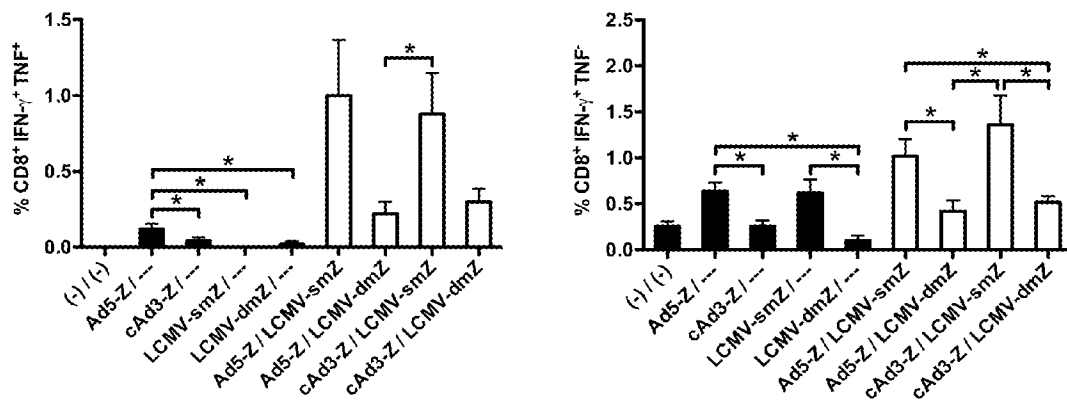
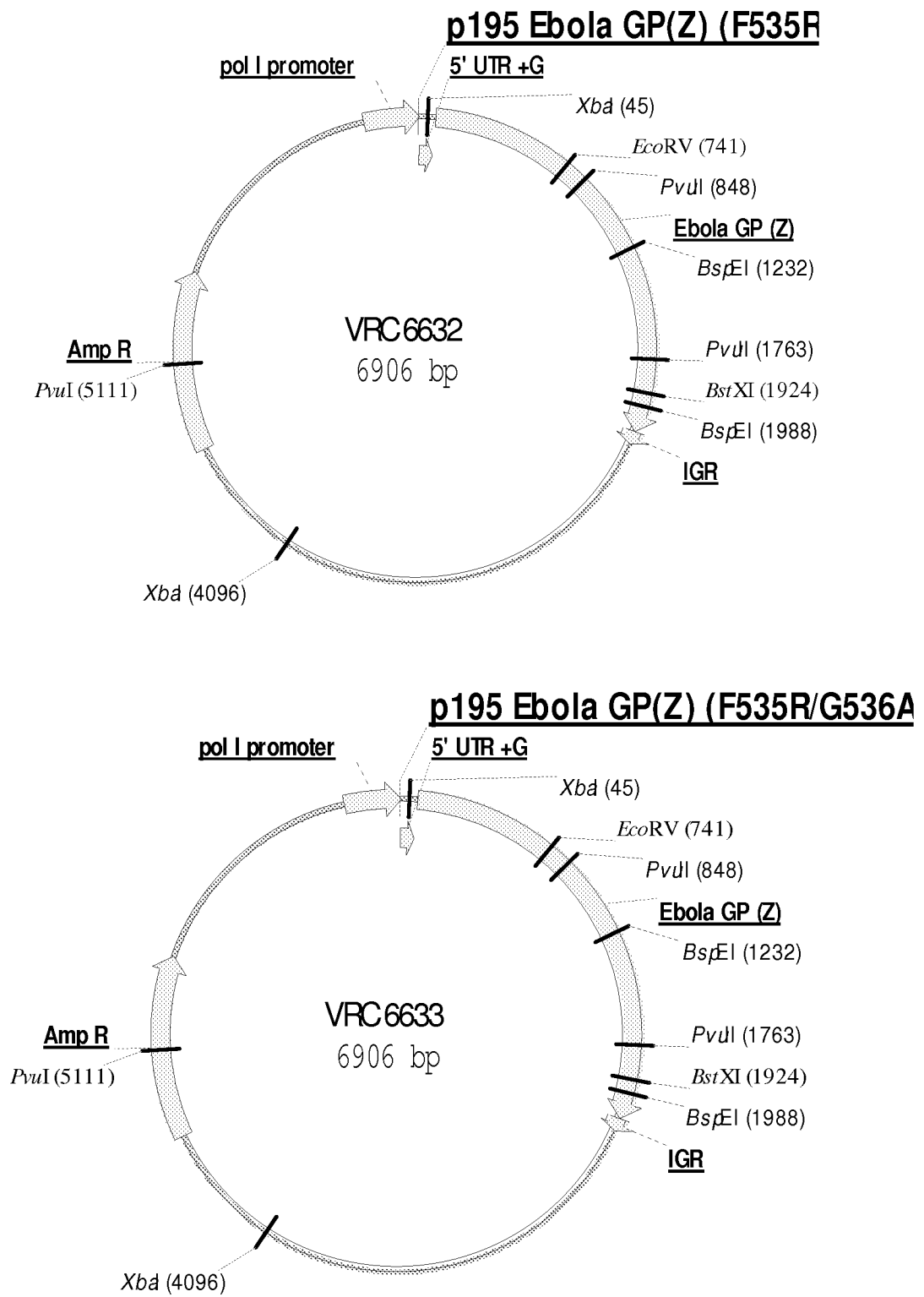


FIG. 14



INTERNATIONAL SEARCH REPORT

International application No
PCT/US2012/039197

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K39/21 A61K39/12
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)
A61K

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, 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	FLATZ L R ET AL: "Replication-defective lymphocytic choriomeningitis virus vectors boost cellular and humoral immunity after DNA or adenovirus vector priming", RETROVIROLOGY, vol. 6, no. Suppl. 3, 2009, XP002681605, & AIDS VACCINE MEETING 2009; PARIS, FRANCE; OCTOBER 19 -22, 2009 right-hand column, paragraph top ----- -/--	1-36, 39-43



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

9 August 2012

Date of mailing of the international search report

12/11/2012

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

Mata Vicente, Teresa

INTERNATIONAL SEARCH REPORT

International application No

PCT/US2012/039197

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	FLATZ LUKAS ET AL: "Single-cell gene-expression profiling reveals qualitatively distinct CD8 T cells elicited by different gene-based vaccines", PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, vol. 108, no. 14, April 2011 (2011-04), pages 5724-5729, XP002681606, ISSN: 0027-8424 page 5724, right-hand column, last paragraph -----	1-36, 39-43
A	WO 2005/027840 A2 (CHIRON CORP [US]; US NAT INST HEALTH [US]; BARNETT SUSAN W [US]; GOMEZ) 31 March 2005 (2005-03-31) page 34, line 14 page 35, line 24 - page 36, line 6 page 51, line 17 page 130, line 8 - line 10 page 132, line 15 - line 20 -----	1-36, 39-43
A	COREY L ET AL: "Cytotoxic T cell and neutralizing antibody responses to human immunodeficiency virus type 1 envelope with a combination vaccine regimen. AIDS Vaccine Evaluation Group.", THE JOURNAL OF INFECTIOUS DISEASES FEB 1998 LNKD- PUBMED:9466515, vol. 177, no. 2, February 1998 (1998-02), pages 301-309, XP002681607, ISSN: 0022-1899 abstract -----	1-36, 39-43

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2012/039197

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

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, the international search was carried out on the basis of:
 - a. (means)

☐	on paper
☒	in electronic form
 - b. (time)

☒	in the international application as filed
☐	together with the international application in electronic form
☐	subsequently to this Authority for the purpose of search
2. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2012/039197

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.:

1-36, 39-43

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.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

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

1. claims: 1-36, 39-43

Method for inhibiting a viral infection in a subject, comprising administering a prime-boost vaccination to the subject, wherein the prime-boost vaccination comprises administering a primer vaccine which comprises a recombinant adenoviral vector (rAd) or a DNA vector expressing a viral antigen and a booster vaccine which comprises a replication-defective lymphocytic choriomeningitis virus (LCMV) vector expressing a mismatch of the viral antigen, and wherein the mismatch enhances an immune response to the viral antigen.

2. claims: 37, 38(partially)

Composition comprising a nucleic acid comprising SEQ ID NO:2.

3. claims: 37, 38(partially)

Composition comprising a nucleic acid comprising SEQ ID NO:4.

4. claims: 37, 38(partially)

Composition comprising a nucleic acid comprising SEQ ID NO:6.

5. claims: 37, 38(partially)

Composition comprising a nucleic acid comprising SEQ ID NO:8.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2012/039197

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2005027840 A2	31-03-2005	CA 2539021 A1	31-03-2005
		EP 1667631 A2	14-06-2006
		US 2007166784 A1	19-07-2007
		WO 2005027840 A2	31-03-2005
