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(54) Title: CROSS-REACTIVE T CELL EPITOPES OF HIV, SIV, AND FIV FOR VACCINES IN HUMANS AND CATS

(57) Abstract: The subject invention concerns methods and materials for inducing an immune response in an animal or person against an immunodeficiency virus, such as HIV, SIV, or FIV. In one embodiment, a method of the invention comprises administering one or more antigens and/or immunogens to the person or animal wherein the antigen and/or immunogen comprises one or more evolutionarily conserved epitopes of immunodeficiency viruses. In one embodiment, the epitope is one that is conserved between HIV and SIV, or between HIV and FIV. In another embodiment, the epitope is one that is conserved between HIV, SIV, and FIV.

DESCRIPTION

CROSS-REACTIVE T CELL EPITOPES OF HIV, SIV, AND FIV FOR VACCINES IN HUMANS AND CATS

CROSS-REFERENCE TO RELATED APPLICATIONS

The present application is a continuation of U.S. Application Serial No. 14/617,711, filed February 9, 2015, which is a continuation-in-part of International Application No. PCT/US2013/054191, filed August 8, 2013, which claims the benefit of U.S. Provisional Application Serial No. 61/841,122, filed June 28, 2013, U.S. Provisional Application Serial No. 61/684,592, filed August 17, 2012, and U.S. Provisional Application Serial No. 61/681,014, filed August 8, 2012, each of which is hereby incorporated by reference herein in its entirety, including any figures, tables, nucleic acid sequences, amino acid sequences, or drawings.

GOVERNMENT SUPPORT

This invention was made with government support under grant numbers R01-AI65276 and R01-AI30904 awarded by the National Institutes of Health. The government has certain rights in the invention.

BACKGROUND OF THE INVENTION

An effective prophylactic HIV-1 vaccine is needed to eradicate the HIV/AIDS pandemic but designing such a vaccine is a challenge. Despite many advances in vaccine technology and approaches to generate both humoral and cellular immune responses, major phase-II and -III vaccine trials against HIV/AIDS have resulted in only moderate successes. The modest achievement of the phase-III RV144 prime-boost trial in Thailand re-emphasized the importance of generating robust humoral and cellular responses against HIV. While antibody-directed approaches are being pursued by some groups, others are attempting to develop vaccines targeting cell-mediated immunity, since evidence show

CTLs to be important for the control of HIV replication. Phase-I and -IIa multi-epitope vaccine trials have already been conducted with vaccine immunogens consisting of known CTL epitopes conserved across HIV subtypes, but have so far fallen short of inducing robust and consistent anti-HIV CTL responses. Thus, a need remains in the art for an effective vaccine against HIV.

Domestic cats are subject to infection by several retroviruses, including feline leukemia virus (FeLV), feline sarcoma virus (FeSV), endogenous type C oncoronavirus (RD-114), and feline syncytia-forming virus (FeSFV). Of these, FeLV is the most significant pathogen, causing diverse symptoms including lymphoreticular and myeloid neoplasms, anemias, immune-mediated disorders, and an immunodeficiency syndrome that is similar to human acquired immune deficiency syndrome (AIDS). Recently, a particular replication-defective FeLV mutant, designated FeLV-AIDS, has been more particularly associated with immunosuppressive properties.

The discovery of feline T-lymphotropic lentivirus (now designated as feline immunodeficiency virus, FIV) was first reported in Pedersen *et al.* (1987). Characteristics of FIV have been reported in Yamamoto *et al.* (1988a); Yamamoto *et al.* (1988b); and Ackley *et al.* (1990). Seroepidemiologic data have shown that infection by FIV is indigenous to domestic and wild felines throughout the world. A wide variety of symptoms are associated with infection by FIV, including abortion, alopecia, anemia, conjunctivitis, chronic rhinitis, enteritis, gingivitis, hematochezia, neurologic abnormalities, periodontitis, and seborrheic dermatitis. The immunologic hallmark of domestic cats infected with FIV is a chronic and progressive depletion of feline $CD4^+$ peripheral blood lymphocytes, a reduction in the CD4:CD8 cell ratio and, in some cases, an increase in CD8-bearing lymphocytes.

Cloning and sequence analysis of FIV has been reported in Olmsted *et al.* (1989a); Olmsted *et al.* (1989b); and Talbott *et al.* (1989). Hosie and Jarrett (1990) described the serological response of cats infected with FIV. FIV virus subtypes can be classified according to immunotype based on the level of cross-neutralizing antibodies elicited by each strain (Murphy and Kingsbury, 1990). Recently, viruses have been classified into subtypes according to genotype based on nucleotide sequence homology. Although HIV and FIV subtyping is based on genotype (Sodora *et al.*, 1994; Rigby *et al.*, 1993; and Louwagie *et al.*, 1993), little is known about the correlation between the genotype and

immunotype of subtypes. FIV viral isolates have been classified into five FIV subtypes: A, B, C, D, and E (Kakinuma *et al.*, 1995; Yamamoto *et al.*, 2007; Yamamoto *et al.*, 2010). Infectious isolates and infectious molecular clones have been described for all FIV subtypes except for subtypes C and E (Sodora *et al.*, 1994). Subtype C FIV has originally been identified from cellular DNA of cats from Canada (Sodora *et al.*, 1994; Rigby *et al.*, 1993; Kakinuma *et al.*, 1995). Examples of FIV strains identified in the art include (subtype of the strain is shown in parenthesis) Petaluma (A), Dixon (A), UK8 (A), Dutch113 (A), Dutch19K (A), UK2 (A), SwissZ2 (A), Sendai-1 (A), USCAzepy01A (A), USCAhnky11A (A), USCAtt-10A (A), USCAlemy01 (A), USCAsam-01A (A), PPR (A), FranceWo, Netherlands, Bangston (A/B), Aomori-1 (B), Aomori-2 (B), USILbrny03B (B), TM2 (B), Sendai-2 (B), USCKlgri02B (B), Yokohama (B), USMAsboy03B (B), USTXmtex03B (B), USMCglwd03B (B), CABCpbar03C (C), CABCpbar07C (C), CABCpady02C (C), Shizuoka (D), Fukuoka (D), LP3 (E), LP20 (E), and LP24 (E).

The commercial release of an effective HIV-1 vaccine is not imminent even after completion of four major phase IIB-III vaccine trials against HIV/AIDS (Saunders *et al.* (2012)). Our limited understanding about the mechanisms of vaccine protection (Plotkin (2008)) and the identity of the protective viral epitopes (Mothe *et al.* (2011); Koff (2010)) further hampers the development of an effective vaccine. Initial studies focused on antibody-based vaccine designs with an emphasis on generating broadly virus neutralizing antibodies (bNAbs) (Stamatatos (2012)). However, two phase-III vaccine trials using envelope (Env) immunogens failed (Flynn *et al.* (2005); Pitisuttithum *et al.* (2006)). Subsequent focus was placed on the T-cell-based vaccines that generate protective cell-mediated immunity (CMI) against global HIV-1 isolates (Buchbinder *et al.* (2008)). The CMI responses, essential for an effective vaccine, most likely include cytotoxic T lymphocyte (CTL) activities that specifically target HIV-1 infected cells (Ogg *et al.* (1998); Walker *et al.* (1988); Belyakov *et al.* (2012)). Unlike NAb epitopes which reside exclusively on the Env proteins, the selection of specific vaccine epitopes for the development of T-cell-based vaccines is more difficult to achieve. A vast number of CTL-associated epitopes can be found to span the whole length of most HIV proteins (Los Alamos National Laboratory (LANL) database, hiv-web.lanl.gov/content/immunology/maps/ maps.html) (Llano *et al.* (2009)). The goal to

develop T-cell-based vaccines is challenged by the capacity of the virus to evade antiviral immunity through mutation(s) for resistance (Li *et al.* (2011); Leslie *et al.* (2004)).

A recent phase III trial consisting of priming with a *gag-pr-gp41-gp120* canarypox vectored vaccine and boosting with Env gp120 induced both humoral immunity and CMI and conferred a modest overall efficacy (Rerks-Ngarm *et al.* (2009)). However, phase I and II vaccine trials consisting of cross-subtype conserved CTL-associated peptide epitopes have shown minimal CMI responses (Sanou *et al.* (2012a); Hanke *et al.* (2007); Salmon-Ceron *et al.* (2010)). Therefore, a thorough selection of potent anti-HIV T cell-associated epitopes, which are conserved among HIV-1 subtypes and do not mutate without negatively affecting viral fitness (Troyer *et al.* (2009); Goulder *et al.* (2008); Rolland *et al.* (2007)), would be valuable for an effective HIV-1 vaccine. One approach is to select conserved, non-mutable CTL epitopes on essential viral structural proteins or enzymes that also persist on the older subgenuses of the lentivirinae which have survived evolutionary pressure (Yamamoto *et al.* (2010)). Such an approach was successfully used in the development of the initial smallpox vaccines (Jenner (1798)). In line with this strategy, the recognition of conserved epitopes on other lentivirus species has been made by the PBMC from HIV-1 positive (HIV+) humans (Balla-Jhagjhoorsingh *et al.* (1999)), HIV-2 vaccinated and SIV-challenged non-human primates (Walther-Jallow *et al.* (2001)), and HIV-1 p24-vaccinated and FIV-challenged cats (Abbott *et al.* (2011); Coleman *et al.* (2005)).

The viral enzyme, reverse transcriptase (RT), is one of the most conserved viral proteins by possessing the lowest entropy value among the HIV-1 proteins from various subtypes (Yusim *et al.* (2002)) and contains many CTL-associated epitopes (Walker *et al.* (1988)). The RT proteins of HIV-1 and FIV also share the highest degree of identity in their amino acid (aa) sequences (Yamamoto *et al.* (2010)). The current studies were undertaken to identify the conserved CTL-associated epitopes on FIV and HIV-1 RT proteins which are recognized by the PBMC and T cells from HIV+ subjects. The major objective of such studies is to identify evolutionarily-conserved CMI epitopes that may be more resistant to mutation, and thus useful in the development of an effective, T-cell-based HIV-1 vaccine.

BRIEF SUMMARY OF THE INVENTION

The subject invention concerns methods and materials for inducing an immune response in an animal or person against an immunodeficiency virus, such as HIV, SIV, or FIV. In one embodiment, a method of the invention comprises administering one or more antigens and/or immunogens to the person or animal wherein the antigen and/or immunogen comprises one or more evolutionarily conserved epitopes of immunodeficiency viruses. In one embodiment, the epitope is one that is conserved between HIV and SIV, or between HIV and FIV. In another embodiment, the epitope is one that is conserved between HIV, SIV, and FIV. In one embodiment, the epitope is a T-cell epitope. In a specific embodiment, the T-cell epitope is a cytotoxic T lymphocyte (CTL) and T-helper (Th) epitope.

The subject invention also concerns evolutionarily conserved epitopes of immunodeficiency viruses. In one embodiment, the epitope is one that is conserved between HIV and SIV, or is one that is conserved between HIV and FIV. In another embodiment, the epitope is one that is conserved between HIV, SIV, and FIV. In one embodiment, the epitope is a T-cell epitope. In a specific embodiment, the T-cell epitope is a cytotoxic T lymphocyte (CTL) and T-helper (Th) epitope.

The subject invention also concerns antibodies that bind to HIV, SIV, and/or FIV epitopes. In one embodiment, an antibody of the invention binds specifically to an HIV protein, *e.g.*, an HIV p24 protein. In another embodiment, an antibody of the invention binds specifically to an FIV protein, *e.g.*, an HIV p24 protein. In a further embodiment, an antibody of the invention binds specifically to both an HIV and an FIV protein, *i.e.*, the antibody cross-reacts with both an HIV and an FIV protein, such as a p24 protein.

BRIEF DESCRIPTION OF THE DRAWINGS

The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Patent and Trademark Office upon request and payment of the necessary fee.

Figures 1A and 1B. NetCTL-1.2 prediction of HIV, SIV, and FIV CTL epitopes.NetCTL-1.2, which is based on proteosomic C-terminal cleavage, TAP transport efficiency, and epitope binding to MHC class I alleles, was used to predict CTL epitopes

shown by HLA supertypes (cbs.dtu.dk/services/NetCTL/). The total number of predicted epitopes by HLA supertype (Figure 1A): HIV (78), SIV (74), and FIV (85) were tallied after analysis of the full-length integrase sequence from each virus. The predicted CTL epitopes were compared and the conserved epitopes between the viruses were identified based on aa position and same predicted HLA supertype (Figure 1B): HIV-SIV (34), HIV-FIV (25), and HIV-SIV-FIV (17).

Figure 2. Possible Location of Counterpart Epitopes. HIV proteins (A, B) aligned to FIV proteins (A, B) showing four HIV epitopes (h1, h2, h3, h4) and three FIV epitopes (f1, f2, f3) with arrows indicating the location of the direct counterpart (arrow a) and indirect counterpart epitopes (arrows b, and c).

Figures 3A and 3B. Comparison Between FIV RT and HIV-1 RT. CD3+CD4+ and CD3+CD8+ T-cell CFSE-proliferation (n=24) and PBMC IFNγ ELISpot (n=28) responses of HIV+ subjects to overlapping FIV RT (FRT 1-21, top) and HIV-1 (HRT 1-21, bottom) peptide pools. Results are shown as a mean value of % $CFSE_{low}$ or spot forming unit (SFU) per 10^6 PBMC with percentage of responders over total subjects tested (% Responder). Uninfected subjects (n=10) had negligible to no responses. Dark red box represents the highest response while boxes with lighter shades of red showing lesser responses with blue representing minimal to no responses. Thresholds are ≥2% for CFSE-proliferation and ≥70 SFU for ELISpot.

Figures 4A and 4B. Comparison Between FIV p24 and HIV-1 p24. CD3+CD4+ and CD3+CD8+ T-cell CFSE-proliferation (n=24) and PBMC IFNγ ELISpot (n=31) responses to overlapping FIV p24 (Fp 1-17, top) and HIV-1 p24 (Hp 1-18, bottom) peptide pools. Results are shown as a mean value of % $CFSE_{low}$ or spot forming unit/10^6 PBMC with (% Responder). Dark red box represents the highest response while lighter shades of red show lesser responses with blue representing minimal to no response.

Figure 5. Sequence comparison between $HIV\text{-}1_{LAI}$ reverse transcriptase (RT) (441 aa) and FIV_{FC1} RT (445 aa).

Figure 6. Sequence comparison between $HIV\text{-}1_{LAI}$ RT (441 aa) and SIV_{Mm251} (439 aa).

Figure 7. Sequence comparisons between FIV-Pet p24 (223 aa) and SIV-Delta B670 p24 (228 aa), between HIV1 HXB2 p24 (231 aa) and FIV-Pet p24 (223 aa), and between HIV1-HXB2 p24 (231 aa) and SIV-Delta B670 p24 (228 aa).

Figures 8A-8C. Chimera HIVp24/FIV-Shizuoka infected PBMC (batch 10) with MAb reactive to both FIV p24 and HIV-1 p24 (Figure 8A). Chimera HIVp24/FIV-Shizuoka infected PBMC (batch 10) with MAb reactive to FIV gp95 (surface envelope) (Figure 8B). Chimera HIVp24/FIV-Shizuoka infected PBMC (batch 10) with isotype IgG control antibody (Figure 8C). Indirect fluorescent antibody (IFA) analysis of feline PBMC infected with chimera HIVp24/FIV-Shizuoka (subtype-D backbone) virus. Note HIV-1_{UCD1} belongs to HIV-1 subtype B. The culture supernatant from chimera transinfected 293T cells was inoculated into uninfected feline PBMC culture, and then 2-3 weeks later when the cells were dying co-cultured with fresh uninfected feline PBMC for 2-3 weeks before the cells were used for IFA analysis. Murine MAb reactive to both FIV p24 and HIV-1 p24 (Figure 8A) and anti-FIV gp95 (surface envelope) MAb (Figure 8B) in combination with FITC-labeled anti-mouse IgG detect chimera HIVp24/FIV-Shizuoka virus infected PBMC (green fluorescent cell in Figures 8A and 8B). Murine isotype IgG control antibody in combination with FITC-labeled anti-mouse IgG has no fluorescent reactivity (Figure 8C). Thus, the chimera HIVp24/FIV-Shizuoka virus is infectious to feline PBMC.

Figures 9A-9D. Indirect fluorescent antibody (IFA) analysis of feline PBMC infected with chimera HIVp24/FIV-Petaluma (subtype-A backbone) virus. Chimera HIVp24/FIV-Petaluma infected PBMC (batch 14) with MAb reactive to both FIV p24 and HIV-1 p24 (Figure 9A). Chimera HIVp24/FIV-Petaluma infected PBMC (batch 14) with anti-FIV gp95 MAb (Figure 9B). Chimera HIVp24/FIV-Petaluma infected PBMC (batch 14) with anti-FIV gp95 MAb (Figure 9C). Chimera HIVp24/FIV-Petaluma infected PBMC (batch 14) with isotype IgG control antibody (Figure 9D). The culture supernatant from chimera transinfected 293T cells was inoculated into uninfected feline PBMC culture, and then 2-3 weeks later when the cells were dying co-cultured with fresh uninfected feline PBMC for 2-3 weeks before the cells were used for IFA analysis. Murine MAb reactive to both FIV p24 and HIV-1 p24 (Figure 9A) and anti-FIV gp95 (surface envelope) MAb (Figures 9B and 9C) in combination with FITC-labeled anti-mouse IgG detect chimera HIVp24/FIV-Shizuoka virus infected PBMC (green fluorescent cell in Figures 9A, 9B, 9C). Murine isotype IgG control antibody in combination with FITC-labeled anti-mouse IgG has no fluorescent reactivity (Figure 9D). Thus, the chimera HIVp24/FIV-Petaluma virus is infectious to feline PBMC.

Figure 10. Reactivity of MAbs to HIV-1 and FIV p24 proteins in Western blot (WB) with FIV (top) or HIV-1 (bottom) p24 substrate. FIV p24-specific MAbs HL2350 and HL2351 do not cross react to HIV-1 p24 proteins or its degraded proteins in WB and ELISA. HIV-1 p24-specific MAbs HL2309 and HL2310 do not cross react with FIV p24 proteins or its degraded proteins in WB and ELISA.

Figures 11A and 11B. Reactivity of HIV-1+ human subjects: comparison between FIV RT and HIV-1 RT. CD3+CD4+ and CD3+CD8+ T-cell CFSE-proliferation (n=24) and PBMC IFNγ ELISpot (n=28) responses of HIV+ subjects to overlapping FIV RT (FRT 1-21, top) and HIV-1 (HRT 1-21, bottom) peptide pools. Results are shown as a mean value of % $CFSE_{low}$ or spot forming unit (SFU) per 10^6 PBMC with percentage of responders over total subjects tested (% Responder). Uninfected subjects (n=10) had negligible to no responses. Dark red box represents the highest response while boxes with lighter shades of red showing lesser responses with blue representing minimal to no responses. Thresholds are ≥2% for CFSE-proliferation and ≥70 SFU for ELISpot.

Figures 12A and 12B. Reactivity of HIV-1+ human subjects: comparison between FIV p24 and HIV-1 p24. CD3+CD4+ and CD3+CD8+ T-cell CFSE-proliferation (n=24) and PBMC IFNγ ELISpot (n=31) responses to overlapping FIV p24 (Fp 1-17, top) and HIV-1 p24 (Hp 1-18, bottom) peptide pools. Results are shown as a mean value of % $CFSE_{low}$ or spot forming unit/10^6 PBMC with (% Responder). Dark red box represents the highest response while lighter shades of red show lesser responses with blue representing minimal to no responses. HIV p24 sequence is longer than that of FIV. Therefore, the alignment shifts after Fp6, and Fp7 is the counterpart of Hp8 and so on.

Figure 13A. Full aa sequence alignment of HIV-1_{LAI} reverse transcriptase (RT) (441 aa) and FIV_{FC1} RT (445 aa) show 47.7% identity and 70.8% homology. Based on the results in Figures 11A and 11B above, four epitopic regions (HRT3/FRT3, HRT6/FRT6, HRT11/FRT11, HRT13/FRT13) with moderate to high reactivity to FIV RT peptide pool by either IFNγ-ELISpot or CFSE-proliferation were selected for aa sequence analysis using GENESTREAM network server. Red aa sequence represents HRT peptide pool sequence, while blue aa sequence represents FRT peptide pool sequence. The aa sequence with red or blue underline are counterpart section between FIV and HIV RT proteins and are also evaluated for aa identity and homology as shown on the right. GENESTREAM network server (xylian.igh.cnrs.fr/bin/align-guess.cgi):

Pearson, W.R., Wood, T., Zhang, Z., and Miller, W. (1997), Comparision of DNA sequences with protein sequences, Genomics 46: 24-36.

Figure 13B. Full aa sequence alignment of HIV-1_{LAI} RT (441 aa) and SIV_{Mm251} (439 aa) show 59.6% identity and 81.9% homology. Based on the results in Figure 14 below, four epitopic regions (HRT3, HRT6, HRT11, HRT14) with moderate to high reactivity to HIV RT peptide pool by IFNγ-ELISpot were selected for aa sequence analysis using GENESTREAM network server. Red aa sequence represents HRT peptide pool sequence, while blue aa sequence represents FRT peptide pool sequence. These counterpart peptide pool regions of HIV and SIV RT proteins are also evaluated for aa identity and homology as shown on the right. The yellow highlight represents the HIV peptide pool and the macaques (immediately below the counterpart SIV sequence) reacting to the corresponding peptide pool. PBMCs from infected macaques were also tested with shorter peptides of HRT3 which are known to be CTL epitope for HIV+ humans. PBMCs from macaques R395 and R397 reacted to peptide "WRKLVDFRE" (SEQ ID NO:450) (red & counterpart blue underlined) while that of macaque R416 reacted to both overlapping peptide pool HRT3 and individual peptide "KWRKLVDFRELNKR" (SEQ ID NO:166) in green highlight. Furthermore, PBMC of macaque R422 reacted to FIV p24 peptide "NPWNTPVFAIKKK" (SEQ ID NO:61) and its SIV counterpart sequence is shown in aqua highlight. The reason why only R416 responded to overlapping peptide pool while others (R395, R397, R422) only reacted to smaller peptides are technical reasons such as smaller peptides can bind to MHC more readily than the larger peptides (11-16mers) used for overlapping peptide pools and that other peptide(s) in the pool can decrease the responses.

Figures 14A-14B. IFNγ ELISpot Responses to HIV RT (reverse transcriptase) and HIV p24 (core protein) of the Primate PBMCs. Overlapping HIV-1 p24 (Figure 14A) and RT (Figure 14B) peptide pool analyses are shown for nine SIV-infected rhesus macaques and four pre-infection macaques. Frozen PBMC were thawed and plated at the concentration of 1.4 X 10^5 viable cells per mL. Peptides were used at a concentration of 15 μg/mL. Each bar represents an individual primate's response in spot forming units (SFU/10^6 PBMC) after subtraction of 2 times the media control; except for the black and red bars. The black bar represents the average response of the pre-infection responders (Av. n=3) and the red bar represents the average response of all 4 pre-infection samples

(Av. total n=4). Since these cells were frozen for over 5 years, positive responses are values of ≥50 SFU. We believe that fresh (non-cryopreserved) cells will give higher responses to HIV p24 peptide pools (Figure 14A: Hp1-Hp18) and HIV RT peptide pools (Figure 14B: Hr1-Hr21). Various mitogens (Mito.) (concanavalin A, *Staphyloccocal* enterotoxin A, phytohemaglutinin A) were used since these frozen cells did not always respond to mitogen.

Figures 15A and 15B. HIV-1 and FIV p24 sequences and individual peptides in the peptide pools. (SEQ ID NOs:257-262,264-319,321-335,337-370).

Figure 16. FRT3 induces the secretion of multiple cytokines in the PBMC of 3/5 HIV+ individuals tested.

Figures 17A-17F. IFNγ and CD8+ T cell proliferation responses of HIV-infected subjects to HIV and FIV reverse transcriptase (RT) peptide pools. The IFNγ ELISpot (Figures 17A and 17B, n=32; 12 LTS, 12 ST, 8 ART+) and CD3+CD8+ T-cell proliferation (Figures 17C and 17D, n=26; 11 LTS, 7 ST, 8 ART+) responses to overlapping peptide pools of HIV RT (H1-H21; Figures 17A and 17C) and FIV RT (F1-F21; Figures 17B and 17D) are shown. The HIV+ subjects (panel-A insert for Figures 17A-17D) consisted of long-term survivors (LTS) who have had HIV infection for over 10 years without antiretroviral therapy (ART) (LTS; black bar); subjects recently diagnosed with short-term infection without ART (ST; grey bar); and subjects on ART at various duration of infection (ART+, red bar). Each bar represents a positive response by an individual with a threshold of 70 spot forming units (SFU) per 10^6 PBMC for ELISpot or threshold of 3% CFSElow for CD3+CD8+ T-cell proliferation. Cells from each individual were stimulated with T-cell mitogen, phytohemaglutinin A (PHA), as positive control. The HIV- control subjects (n=10) had no responses (data not shown). All responses below the positive threshold are not shown to clearly distinguish those positive values close to the threshold.

The average frequencies of IFNγ and proliferation responders to HIV-1 (Figure 17E) and FIV (Figure 17F) RT peptide pools are derived from Figures 17A-17D and are shown as % responders. The solid bar for each peptide represents an average responder frequency of IFNγ responses, while the grey bar represents of the CD8+ T-cell proliferation responses.

Figures 18A-18B. CD3+CD4+ T-cell proliferation responses to HIV and FIV RT peptide pools. The CD3+CD4+ T-cell proliferation responses to overlapping peptide pools of HIV RT (H1-H21; Figure 18A) and FIV RT (F1-F21; Figure 18B) are shown for HIV+ subjects (n=26; 11 LTS, 7 ST, 8 ART+). The insert in panel Figure 18A shows the bar color codes for both panels as: LTS without ART (LTS; black bar), ST without ART (ST; grey bar), and those on ART (ART+; white bar). Each bar represents a positive response by an individual with a positive threshold of 70 SFU per 10^6 PBMC for ELISpot or 3% $CFSE^{low}$ for CD3+CD4+ T-cell proliferation. None of the HIV- control subjects (n=10) had positive responses (data not shown). Responses below positive thresholds are not shown.

Figures 19A-19B. Persistence of IFNγ and CD8+ T-cell proliferation responses to selected peptide pools. The IFNγ (Figure 19A) and CD8+ T-cell proliferation (Figure 19B) responses of HIV+ subjects who responded first time (t1) and second time (t2, at least 1 year later) are shown for peptide-pool F3 (both analyses), H11 (both analyses), and H6 (IFNγ) or F6 (T-cell proliferation). The total number of HIV+ subjects who participated is 22 subjects (Figure 19A: 9 LTS, 8 ST, 5 ART+) in IFNγ study and 11 subjects (Figure 19B: 3 LTS, 6 ST, 2 ART+) in CD8+ T-cell proliferation study. However, the number of subjects with different clinical status differs among the peptide-pool groups since it is based on the number of responders to the peptide at the first time (t1). In addition, the IFNγ responses to H6 and F3 have a third time point (t3, ≥2 yr). The *p*-value of each peptide-pool group indicates that the results from t1 are statistically different from those from t2 when $p<0.5$. Only CD8+ T-cell proliferation responses to F6 at t1 are statistically different from those at t2. A statistical comparisons between t2 and t3 of H6 (n=5) and F3 (n=5) were $p=0.124$ and $p=0.133$, respectively (*p*-value not shown).

Figures 20A-20B. F3 peptide epitopes recognized by F3 responders. The peptide-pool F3 consists of five overlapping 13-15mer peptides spanning from amino- to carboxyl-terminal (F3-1, F3-2, F3-3, F3-4, and F3-5). IFNγ (Figure 20A; n=10; 5 LTS, 3 ST, 2 ART+) and CD8+ T-cell proliferation (Figure 20B; n=8; 3 LTS, 3 ST, 2 ART+) by cells from HIV-infected F3 responders to each of these peptides are shown along with responses to F3 pool. F3 responders consist of those subjects with long-term HIV-1 infection but not on ART (LTS; black bar); those with short-term infection and not on

ART (ST; grey bar); and those on ART with various duration of infection (ART+; red bar). All responses below positive thresholds are not shown.

Figures 21A-21D. Characterization of CTL-associated epitopes on H6, H11, F3, and F6 pools. ICS analysis for perforin (Perf), granzyme A (GrzA), and granzyme B (GrzB) expression is shown for CD8+ T cells (left column) and CD4+ T cells (right column) from selected HIV+ responders of designated peptide pools (H6, n=8; H11, n=8; F3, n=11; F6, n=6; and PHA, n=12) (Figures 21A-21C). The HIV+ subjects (panel-A insert for Figures 21A-21C) consist of the following individuals: those with long-term infection without ART (LTS; black closed circle); those recently diagnosed, with short-term infection without ART (ST; grey closed circle); and those on ART with various duration of infection (ART+; open circle). The number of each clinical status group is the following for each peptide-pool group: H6 (4 LTS, 2 ST, 2 ART+), H11 (4 LTS, 1 ST, 3 ART+), F3 (5 LTS, 3 ST, 3 ART+), F6 (2 LTS, 2 ST, 2 ART+), and PHA (5 LTS, 3 ST, 4 ART+). Six F3-pool responders (5 LTS, 1 ART+) were tested for Perf (●), GrzA (□), and GrzB (Δ) responses to the five 13-15mer F3 peptides (Figure 21D). Only three subjects were the same as those from above (Figures 21A-21C), but the blood was collected at a different time-point.

Figures 22A-22B. The aa sequence identity between counterpart HIV/FIV peptide pools and between various lentiviruses. The percentage of aa identity (Figure 22A) between the sequences of each HIV (H) peptide pool and its counterpart FIV (F) peptide pool were obtained by alignment of the sequences using ebi.ac.uk/Tools/psa/emboss_needle/. Note that the highest aa identity observed is 68.7% with peptide-pools H4/F4 and the second highest is 66.7% with peptide-pools H3/F3. In Figure 22B, the percentages shown on the right of the diagonal divider represent % aa sequence similarity and those on the left represent % aa sequence identity between the two viruses intersecting the value. The lentivirus strains compared are HIV-1HXB2 (GenBank K03455.1), FIVFC1 (DQ365597.1), SIVMm251 (AAB59906.1), and CAEV (AAG48629.1).

Figures 23A-23C. IFNγ responses to HIV, FIV and SIV p24 peptide pools. The IFNγ ELISpot responses to overlapping peptide pools of HIV p24 (Hp1-Hp18, n=31; Figure 23A), FIV p24 (Fp1-Fp17, n=31; Figure 23B), and SIV p24 (Sp1-Sp17, n=15; Figure 23C) are depicted as spot forming units (SFU) per 10^6 PBMC. The HIV^+ subjects

(Figure 23A insert for Figures 23A and 23B; Figure 23C insert for Figure 23C) consisted of long-term survivors (LTS) without antiretroviral therapy (ART) (LTS; black bar); recently diagnosed subjects (<1 year) with short-term infection without ART (ST; grey bar); and those receiving ART with various duration of infection (ART^+, red bar). Each bar represents a positive response by an individual subject with a threshold of >50 SFU per 10^6 PBMC. Asterisk after the peptide pool(s) represent those with highest frequency of responders. The FIV p24 pool with the highest and the most frequent responses and its counterpart HIV-1 and SIV pools are highlighted in blue.

Figures 24A-24F. Proliferation responses to HIV, FIV and SIV p24 peptide pools. The $CD3^+CD8^+$ T-cell proliferation responses to overlapping peptide pools of HIV p24 (Hp1-Hp18, n=27; Figure 24A), FIV p24 (Fp1-Fp17, n=27; Figure 24B), and SIV p24 (Sp1-Sp17 n=13; Figure 24C) are depicted as % $CFSE^{low}$ for $CD3^+CD8^+$ and $CD3^+CD4^+$ T-cell proliferation. The HIV^+ subjects (Figure 24A insert for Figures 24A and 24B; Figure 24C insert for Figure 24C) consisted of long-term survivors (LTS) without ART (LTS; black bar); recently diagnosed subjects (<1 year) with short-term infection without ART (ST; grey bar); and those on ART with various duration of infection (ART^+, red bar). Each bar represents a positive response by an individual with a threshold of >1% $CFSE^{low}$ for $CD3^+CD8^+$ T-cell proliferation. Asterisk after the peptide pool(s) represents those with highest frequency of responders. The two FIV p24 pools with the highest and the most frequent responses and their counterpart HIV-1 and SIV pools are highlighted either in blue (Hp15/Fp14/Sp14) or red (Hp10/Fp9/Sp9).

Figures 25A-25B. The persistence of IFNγ and T-cell proliferation responses to selected peptide pools. The IFNγ (Figure 25A) and $CD8^+$ T cell proliferation (Figure 25B) responses of HIV^+ subjects who responded at the first sample collection (t1), 2 yr later (t2), and 4 yr later (t3) are shown for peptide pool Fp14 (IFNγ), Hp15 (IFNγ), Hp10 (proliferation), and Fp9 (proliferation). The *p*-value indicates statistical differences between t1 and t2, t2 and t3, and t1 and t3. The threshold for IFNγ and proliferation responses are >50 SFU and >1% $CFSE^{low}$, respectively.

Figures 26A-26C. IFNγ and $CD8^+$ T-cell proliferation responses to Fp9, Fp14, and Hp15 peptide epitopes. Peptide pool Fp14 consists of four overlapping 13-15mer peptides spanning the amino- to carboxyl-terminus (Fp14-1, Fp14-2, Fp14-3, Fp14-4), while pools Hp15 (Hp15-1, Hp15-2, Hp15-3) and Fp9 (Fp9-1, Fp9-2, Fp9-3)

each consist of three overlapping 13-15mer peptides. IFNγ responses to individual Fp14 peptides (Figure 26A; n=9), Hp15 peptides (Figure 26B; n=9) and $CD8^+$ T-cell proliferation responses to individual Fp9 peptides (Figure 26C; n=7) are shown along with responses to their corresponding peptide pools. Only responders to pools Fp14 (Figure 26A), Hp15 (Figure 26B), and Fp9 (Figure 26C) were tested. The responders consisted of long-term survivors (LTS) without ART (LTS; black bar); recently diagnosed subjects (<1 year) with short-term infection without ART (ST; grey bar); and those on ART with various duration of infection (ART^+, red bar). Each bar with (*) in panels A and B are from the same three LTS.

Figures 27A-27F. IFNγ and T-cell proliferation responses to 9-13mers of FIV peptides Fp9-3, Fp14-3, and Fp14-4. Six 9-12mer peptides for the Fp9 pool (Figures 27A, 27C, 27E) and seven 9-13mer peptides for the Fp14 pool (Figures 27B, 27D, 27F) described in Table 13 were tested for their ability to induce $CD8^+$ T-cell proliferation (Figures 27A and 27B), $CD4^+$ T-cell proliferation (Figures 27C and 27D), and IFNγ production (Figures 27E and 27F) and compared to results with the Fp9 and Fp14 peptide pools. 13-15mer peptides Fp9-3, Fp14-3, and Fp14-4; and mitogen (PHA) were included as controls. A total of nine HIV^+ responders to the Fp9 pool (Figures 27A, 27C, 27E) and 10 HIV^+ responders to the Fp14 pool (Figures 27B, 27D, 27F) were tested up to 4 yr after the beginning of the study. Two to four of the responders were lost to follow up. Their proliferation and IFNγ results to the 13-15mer peptides Fp9-3, Fp14-3, and Fp14-4, and are denoted with an (*) for each individual missing.

Figures 28A-28D. Stimulation of cytotoxins by CTL epitopes on Fp9, Fp14, and Hp15 peptide pools, and their individual peptides. The ICS analyses for perforin (Perf, ○), granzyme A (GrzA, □), and granzyme B (GrzB, Δ) are shown for $CD8^+$ T cells (Figures 28A, 28D) and $CD4^+$ T cells (Figures 28B, 28C) from five HIV^+ responders with or without ART. Two LTS/ART^-, one LTS/ART^+, one ST/ART^+, and one ST/ART^- were first evaluated (Figures 28A, 28B). Peptides tested included Fp9, Fp14, and/or Hp15 peptide pools or large 13-15mer peptides (Figures 28A, 28B). Responses from five HIV^+ responders of short-term HIV-infected subjects not on ART (ST/ART^-) were tested using small 9-13mer peptides within Fp9-3, Fp14-1, Fp14-3, Fp14-4, and Sp14-1 (Figures 28C, 28D). Note that Sp14 pool is a single 13mer peptide Sp14-1. Counterpart peptides are shown with blue-dashed box for Fp14-1a/Hp15-1c/Sp14-1c, purple-dashed box for Fp14-

1b/Hp15-1a/Sp14-1b, and red-dashed box for Fp14-3/4f/Hp15-2/3a/Sp14-1a. The peptides tested with one less subject are denoted by (**), while their number of subjects and number of responses are denoted by (*). Each separate symbol color represents one subject, and each color-coded subject is shown with his/her infection status. The threshold for T cells expressing cytotoxin is set at >0.1% $CD4^+$ or $CD8^+$ T cells.

Figure 29. Summary of functional epitope analyses. Results from three functional analyses are summarized according to frequency of HIV^+ (lanes 1-4) or HIV^- (lane 5) responders and are shown as (+) for frequency of >25% responders, (±) for 19-25% responders, (-) for <19% responders, or as (*) for not available. Each lane, abridged in the insert, shows the ability of the peptide pool or peptide to stimulate an IFNγ response (lane 1), $CD8^+$ T-cell proliferation (lane 2), and $CD4^+$ T-cell proliferation (lane 3). Lane 4 denotes the ability to induce cytotoxin(s) in only $CD8^+$ T cells (+) or in both $CD8^+$ and $CD4^+$ T cells (++) by four or more HIV^+ subjects when at least nine subjects tested. In lane 5, the positive result (+) indicates substantial frequency of proliferation responses from HIV^- subjects and may indicate a safety concern; whereas a negative result (-) indicates no substantial HIV^- response to the peptide pool or peptide. Positive response of HIV^- control had $CD8^+$ T-cell proliferation response in 30-42% of HIV^- subjects. The large 13-15mer peptides with the best CMI responses without stimulation in HIV^- subjects are shown with dashed boxes, while the best small peptides are shown with solid boxes. Therefore, Hp15-1, Hp15-2/3a, Fp14-4, Fp14-1b, Fp14-3/4e, and Fp14-3/4f peptides appear to contain the best potential epitopes to target for use as HIV immunogens.

Figures 30A-30F. $CD8^+$ T-cell proliferation (Figures 30A-30C) and IFNγ (Figures 30D-30F) responses to HIV-1, FIV, and SIV p24 peptide pools by HIV^+ subjects (Roff *et al.* 2015). The PBMCs were stimulated with HIV-1 (Hp1-Hp18) (Figures 30A, 30D) and corresponding SIV (Sp1-Sp17) (Figures 30C, 30F) and FIV (Fp1-Fp17) (Figures 30B, 30E) p24 peptide pools at 5 μg/mL peptide pool for proliferation and 6-8 μg pool/well for IFNγ. Positive control cultures were stimulated with T-cell mitogen phytohemaglutinin A (PHA) or concanavalin A (ConA) each at 5 μg/mL. The PBMCs and T cells were from long-term survivors (LTS, black bar), subjects with short-term infection without ART (ST, grey bar), and subjects on ART at various duration of infection (ART^+, red bar). The results are shown after subtraction of the individual media

control and responses to each peptide pool by PBMC or T cells from HIV-negative subjects. The HIV counterpart for FIV and SIV pools is offset by one additional pool starting from Fp7/Sp7 and therefore HIV counterpart for Fp9/Sp9 and Fp14/Sp14 are Hp10 and Hp1, respectively. They are shown with red and blue boxes for Hp10/Fp9/Sp9 and Hp15/Fp14/Sp14, respectively.

Figures 31A and 31B. Stimulation of cytotoxins by CTL epitopes on Fp9, Fp14, and Hp15 peptide pools, and their individual peptides by HIV^+ subjects. The intracellular cytotoxin staining (ICS) analyses for perforin (Perf, ○), granzyme A (GrzA, □), and granzyme B (GrzB, Δ) are shown for $CD8^+$ T cells (Figure 31A) and $CD4^+$ T cells (Figure 31B) from five HIV^+ responders with or without ART. Two LTS/ART^-, one LTS/ART^+, one ST/ART^+, and one ST/ART^- were first evaluated (Figures 31A, 31B). Peptides tested included Fp9, Fp14, and/or Hp15 peptide pools or large 13-15mer peptides (Figures 31A, 31B). Each separate symbol color represents one subject, and each color-coded subject is shown with his/her infection status. The threshold for T cells expressing cytotoxin is set at >0.1% $CD4^+$ or $CD8^+$ T cells. Additional five ST/ART^- subjects are shown in Figure 6 of reference (Roff *et al.* 2015).

Figures 32A and 32B. T-cell proliferation responses to FIV p24 peptide pools by vaccinated cats. $CD8^+CD4^+$ (Figure 32A) and $CD3^+CD8^+$ (Figure 32B) T-cell proliferation responses are shown for prototype FIV-vaccinated cats. The FIV vaccinated cats were semi-inbred cats with blue, dark red, and pink/green bars from different MHC-lineage colonies. The semi-inbred cats with light pink and green bars are from the same MHC lineage. Pools Fp9 and Fp14 are recognized by both HIV^+ subjects and vaccinated cats (*i.e.*, evolutionarily conserved [EC] epitopes) but pools Fp3 and Fp4 are not recognized by HIV^+ subjects (*i.e.*, non-EC epitopes). The IFNγ responses of these cats were either low or below the cut-off threshold of 50 SFU/$1x10^6$ PBMC. The peptides that stimulated the most proliferation responses: non-EC peptide Fp4-3 in pool Fp4 and EC peptide Fp14-1 in pool Fp14 were used in *in vivo* study below (Figure 34).

Figures 33A-33C. IFNγ and T-cell proliferation responses to Fp9 and Fp14 by the FIV-vaccinated cats and FIV-infected cats. The T cells or PBMC from 32 FIV-vaccinated cats were stimulated with either Fp9 or Fp14 and evaluated for proliferation (Figure 33A) or IFNγ (Figure 33B) responses. In addition, the PBMC from 32 FIV-infected cats at 22-52 weeks of infection and 20 HIV-1 p24-immunized cats were tested

for IFNγ responses to the same peptide pools (Figure 33B). Three types of laboratory cats were used in these studies: semi-inbred line 1 (Semi-Inbred 1), semi-inbred line 2 (Semi-Inbred 2), and outbred cats (Outbred) from laboratory cat vendors. Semi-inbred cats have been inbred for 3-5 generations with defined feline leukocyte antigen (FLA) alleles and are divided into two FLA haplotype lines. FIV vaccine consisted of prototype dual-subtype FIV vaccine (Coleman *et al.* 2014). The insert in each panel defines the numbers of each type of cats as well as vaccination (Vac.) or infection (Infect.) status. The number of cat responders used varies between the analyses performed for the individual Fp14 peptides (Figure 33C, left for IFNγ and right for proliferation).

Figure 34. 3 MAPs with 1-2 FIV peptides for Table 17. These peptides stimulate IL-2 and IFNγ responses in T cells from FIV-vaccinated cats. Two different formulations of lipophylic (palmitate C16, PAM) MAP were mixed in FD-1 adjuvant and immunized in prototype vaccine-primed SPF cats. Furin-sensitive sequence (RVKR) (SEQ ID NO:494) was used to link the two peptides (Nakayama 1997; Kotterman and Schaffer 2014). After entering the cell, the peptide chains can enter the endoplasmic reticulum and trans-Golgi network, where the furin processes the furin sensitive sequence into two peptides. Subsequently, these peptides bind to MHC. The peptide-MHC complexes are then transported to the cell surface where they interact with T cells.

Figure 35. FIV-enhancing *versus* inhibitory epitopes on peptides and MAPs used in pilot MAP Study. Statistically significant enhancement (Fp4-3, FRT3-3, MAP1) and inhibition (FRT3-4, MAP2) of *in vitro* FIV infection in the PBMC from naïve cats are shown. Virus + control, FIV-enhancing mitogen (ConA) control, and uninfected cell control had an average RT titer (cpm/mL) of 26,518 (1:500 dilution), 54,634 (1:10000) and 2612 (1:500), respectively. Abbreviation: MAP1 plus MAP2 mix (MAP1+2).

Figure 36A and 36B. FIV/HIV-1 enhancing *versus* inhibitory epitopes. Statistically significant enhancement (Fp4-3 & FRT3-3) and inhibition (Fp9-3 & FRT3-4) of FIV infection of naïve cat PBMC were observed (Figure 36A). Virus positive (+) control, enhancement-positive mitogen (concanavalin A, ConA) control, and uninfected cell control had an average RT titer of 26,518 cpm/mL (1:500 dilution), 54,634 cpm/mL (10,000 dilution), and 2612 cpm/mL (1:500 dilution), respectively (85,000 cpm/mL stock). Four epitope peptides were tested in an *in vivo* efficacy study with prototype vaccine prime and MAP boosts against pathogenic FIV challenge (see MAP section).

Notably, inhibition of HIV-1 infection of normal human PBMC was observed with peptide Fp9-3 (lowest dose, p=0.024) but not with peptide FRT3-4 (Figure 36B) (92,000 cpm/mL stock).

BRIEF DESCRIPTION OF THE SEQUENCES

SEQ ID NOs:1-40 are epitopes contemplated within the scope of the invention.

SEQ ID NOs:41 and 42 are chimeric polynucleotides of the present invention.

SEQ ID NOs:43 and 44 are chimeric polypeptides encoded by a chimeric polynucleotide of the invention.

SEQ ID NOs:45-450 are epitopes contemplated within the scope of the invention.

SEQ ID NOs: 451-591 are epitopes contemplated within the scope of the invention.

DETAILED DESCRIPTION OF THE INVENTION

The subject invention concerns methods and materials for providing an immune response in an animal or person against an immunodeficiency virus, such as HIV, SIV, or FIV. In one embodiment, a method of the invention comprises administering one or more antigens and/or immunogens to the person or animal wherein the antigen or immunogen comprises one or more epitopes evolutionarily conserved between different immunodeficiency viruses. In one embodiment, the epitope is one that is conserved between HIV and SIV, or between HIV and FIV. In another embodiment, the epitope is conserved between FIV and SIV. In another embodiment, the epitope is one that is conserved between HIV, SIV, and FIV. In one embodiment, where a human is administered the antigen and/or immunogen, the antigen or immunogen is from an FIV or HIV, and the epitope is evolutionarily conserved between HIV and FIV. In one embodiment, where the animal is a feline animal, the antigen and/or immunogen is from an HIV or FIV, and the epitope is evolutionarily conserved between HIV and FIV. In one embodiment of a method of the present invention, the epitope is a T-cell epitope. In a specific embodiment, the epitope induces one or more T cell responses, such as release of cytotoxins (*e.g.*, perforin, granzymes, and/or granulysin) and/or cytokines (IFNγ, TNF-α, IL-2, IL-4, IL-5, IL-9, IL-10, IL-13, *etc.*). In a specific embodiment, the T-cell epitope is a cytotoxic T lymphocyte (CTL), polyfunctional T cell epitope, and/or T-helper (Th)

epitope. Antigens and immunogens of the invention can be peptides and/or proteins that comprise one or more evolutionarily conserved epitopes of the invention.

Examples of epitopes contemplated within the scope of the invention include peptides or proteins comprising the amino acid sequence shown in any of SEQ ID NOs:1-40 or in any of SEQ ID NOs:45-591, independently or any possible combination thereof, or in any of the examples, figures or tables of the subject application, or an immunogenic fragment or variant of the amino acid sequence. In a specific embodiment, a peptide or protein of the invention comprises the amino acid sequence shown in any of SEQ ID NOs:10, 21, 22, 23, 61, 62, 63, 64, 65, 163, 164, 165, 166, 167, 176, 177, 178, 179, 214, 215, 216, 217, 218, 288, 301, 303, 304, 359, 361, 431, 432, 438, 442, 443, 453, 459, 460, 466, 479, 488, 492, and/or 493. In one embodiment, a plurality of peptides and/or proteins comprising an epitope of the invention are administered to the person or animal. For example, in one embodiment, two or more peptides or proteins comprising the amino acid sequence of any of SEQ ID NOs:10, 21, 22, 23, 61, 62, 63, 64, 65, 163, 164, 165, 166, 167, 176, 177, 178, 179, 214, 215, 216, 217, 218, 288, 301, 303, 304, 359, 361, 431, 432, 438, 442, 443, 453, 459, 460, 466, 479, 488, 492, and/or 493 are administered. For example, a first peptide comprising SEQ ID NO:61 and a second peptide comprising SEQ ID NO:63 can be administered. In another embodiment, a peptide or protein comprising two or more epitopes of the present invention is administered to the person or animal. In one embodiment, the peptide or protein can comprise two or more epitopes by linking two or more peptide sequences of the invention together, or by having a polynucleotide encode two or more peptide sequences together in a single protein, and expressing the polynucleotide to produce the protein. In one embodiment, a peptide or protein comprising two or more amino acid sequences shown in any of SEQ ID NOs:10, 21, 22, 23, 61, 62, 63, 64, 65, 163, 164, 165, 166, 167, 176, 177, 178, 179, 214, 215, 216, 217, 218, 288, 301, 303, 304, 359, 361, 431, 432, 438, 442, 443, 453, 459, 460, 466, 479, 488, 492, and/or 493 is administered to the person or animal. In a specific embodiment, a peptide or protein comprising the amino acid sequence of SEQ ID NO:63 and/or SEQ ID NO:64 is administered to the person or animal. In yet another embodiment, a peptide or protein utilized in the present invention comprises an amino acid sequence shown in any of SEQ ID NO:10, SEQ ID NO: 21, SEQ ID NO:22, or SEQ ID NO:23. In a further embodiment, a peptide or protein utilized in the present invention comprises an amino

acid sequence shown in any of SEQ ID NOs:176, 177, 178, 179, 214, 215, 216, 217, or 218. In yet a further embodiment, a peptide or protein utilized in the present invention comprises an amino acid sequence shown in any of SEQ ID NO:288, SEQ ID NO:301, SEQ ID NO:303, SEQ ID NO:304, SEQ ID NO:359, SEQ ID NO:361, SEQ ID NO:453, SEQ ID NO:459, SEQ ID NO:460, SEQ ID NO:466, SEQ ID NO:479, SEQ ID NO:488, 492, and/or 493.

In one embodiment, the immune response induced by a method of the present invention is a T cell response, such as a CTL-associated immune response and/or a T helper cell response. In a specific embodiment, the immune response induced by a method of the present invention comprises CD4+ and/or CD8+ T cell responses, and/or gamma interferon (IFNγ) production. In one embodiment, cytotoxins (such as perforin, granzyme A, granzyme B, *etc.*) and/or cytokines (IFNγ, IL-4, IL-5, IL-9, IL-10, IL-13, *etc.*) are produced. In one embodiment, the immune response is a protective immune response that provides protection to the person or animal from infection by an immunodeficiency virus. In a specific embodiment, the immune response provides the person or animal with protection from infection by HIV or FIV. In one embodiment, the person or animal receiving the antigen or immunogen is already infected with an immunodeficiency virus. In another embodiment, the person or animal is not infected with an immunodeficiency virus prior to administration of the antigen or immunogen.

The subject invention also concerns evolutionarily conserved epitopes of immunodeficiency viruses. In one embodiment, the epitope is one that is conserved between HIV and SIV, or between HIV and FIV. In another embodiment, the epitope is one that is conserved between HIV, SIV, and FIV. In one embodiment, the epitope is a T-cell epitope. In a specific embodiment, the T-cell epitope is a cytotoxic T lymphocyte (CTL) epitope, polyfunctional T cell (CD3+CD4+ and CD3+CD8+ T cells that express multiple cytokines, cytotoxins, chemokines, and functional activities such as proliferation) epitope, and/or T-helper (Th) epitope. In one embodiment, the epitopes are from a viral integrase protein. In another embodiment, the epitopes are from a viral reverse transcriptase (RT) protein. In a further embodiment, the epitopes are from a viral core or capsid (p24) protein. Antigens and immunogens of the invention can be peptides and/or proteins that comprise one or more evolutionarily conserved epitopes of the invention. Examples of epitopes contemplated within the scope of the invention include

peptides or proteins comprising the amino acid sequence shown in SEQ ID NOs:1-40 or in any of SEQ ID NOs:45-591, independently or any possible combination thereof, or in any of the examples, figures or tables of the subject application, or an immunogenic fragment or variant of the amino acid sequence. In a specific embodiment, an epitope of the invention comprises a peptide or protein comprising the amino acid sequence shown in any of SEQ ID NOs:10, 21, 22, 23, 61, 62, 63, 64, 65, 163, 164, 165, 166, 167, 176, 177, 178, 179, 214, 215, 216, 217, 218, 288, 301, 303, 304, 359, 361, 431, 432, 438, 442, 443, 453, 459, 460, 466, 479, 488, 492, and/or 493. In another embodiment, an epitope of the invention comprises a peptide or protein comprising two or more amino acid sequences of any of SEQ ID NOs:10, 21, 22, 23, 61, 62, 63, 64, 65, 163, 164, 165, 166, 167, 176, 177, 178, 179, 214, 215, 216, 217, 218, 288, 301, 303, 304, 359, 361, 431, 432, 438, 442, 443, 453, 459, 460, 466, 479, 488, 492, and/or 493. In a specific embodiment, an epitope of the invention comprises a peptide or protein comprising the amino acid sequence of SEQ ID NO:63 and/or SEQ ID NO:64. In yet another embodiment, an epitope of the invention comprises a peptide or protein comprising an amino acid sequence shown in any of SEQ ID NO:10, SEQ ID NO: 21, SEQ ID NO:22, or SEQ ID NO:23. In a further embodiment, an epitope of the invention comprises a peptide or protein comprising an amino acid sequence shown in any of SEQ ID NOs:176, 177, 178, 179, 214, 215, 216, 217, or 218. In yet a further embodiment, an epitope of the invention comprises a peptide or protein comprising an amino acid sequence shown in any of SEQ ID NO:288, SEQ ID NO:301, SEQ ID NO:303, SEQ ID NO:304, SEQ ID NO:359, SEQ ID NO:361, SEQ ID NO:453, SEQ ID NO:459, SEQ ID NO:460, SEQ ID NO:466, SEQ ID NO:479, SEQ ID NO:488, SEQ ID NO:492, and/or SEQ ID NO:493. The subject invention also concerns polynucleotides encoding the amino acid sequence of epitopes of the invention.

The subject invention also concerns vaccines comprising one or more antigens and/or immunogens that comprise or encode evolutionarily conserved epitopes of the present invention. The vaccine or immunogenic compositions of the subject invention also encompass recombinant viral vector-based or polynucleotide constructs that may comprise a nucleic acid encoding a peptide or protein comprising an evolutionarily conserved epitope of the present invention or encoding a chimeric polypeptide of the present invention. Examples of epitopes contemplated within the scope of the invention

include peptides or proteins comprising the amino acid sequence shown in SEQ ID NOs:1-40 or in any of SEQ ID NOs:45-591, independently or any possible combination thereof, or in any of the examples, figures or tables of the subject application, or an immunogenic fragment or variant of the amino acid sequence. In a specific embodiment, a peptide or protein of the invention comprises the amino acid sequence shown in any of SEQ ID NOs:10, 21, 22, 23, 61, 62, 63, 64, 65, 163, 164, 165, 166, 167, 176, 177, 178, 179, 214, 215, 216, 217, 218, 288, 301, 303, 304, 359, 361, 431, 432, 438, 442, 443, 453, 459, 460, 466, 479, 488, 492, and/or 493. In another embodiment, a peptide or protein of the invention can comprise two or more amino acid sequences of any of SEQ ID NOs:10, 21, 22, 23, 61, 62, 63, 64, 65, 163, 164, 165, 166, 167, 176, 177, 178, 179, 214, 215, 216, 217, 218, 288, 301, 303, 304, 359, 361, 431, 432, 438, 442, 443, 453, 459, 460, 466, 479, 488, 492, and/or 493. In a specific embodiment, a peptide or protein comprises the amino acid sequence of SEQ ID NO:63 and/or SEQ ID NO:64. In yet another embodiment, a peptide or protein of the present invention comprises an amino acid sequence shown in any of SEQ ID NO:10, SEQ ID NO: 21, SEQ ID NO:22, or SEQ ID NO:23. In a further embodiment, a peptide or protein of the present invention comprises an amino acid sequence shown in any of SEQ ID NOs:176, 177, 178, 179, 214, 215, 216, 217, or 218. In yet a further embodiment, a peptide or protein utilized in the present invention comprises an amino acid sequence shown in any of SEQ ID NO:288, SEQ ID NO:301, SEQ ID NO:303, SEQ ID NO:304, SEQ ID NO:359, SEQ ID NO:361, SEQ ID NO:453, SEQ ID NO:459, SEQ ID NO:460, SEQ ID NO:466, SEQ ID NO:479, SEQ ID NO:488 SEQ ID NO:492, and/or SEQ ID NO:493. In an exemplified embodiment, a chimera polynucleotide comprises the sequence shown in SEQ ID NO:41 or SEQ ID NO:42. In a further exemplified embodiment, a chimera polypeptide comprises the sequence shown in SEQ ID NO:43 or SEQ ID NO:44. Any suitable viral vector that can be used to prepare a recombinant vector/virus construct is contemplated for use with the subject invention. For example, viral vectors derived from adenovirus, avipox, herpesvirus, vaccinia, canarypox, entomopox, swinepox, West Nile virus and others known in the art can be used with the compositions and methods of the present invention. Recombinant polynucleotide vectors that encode and express components can be constructed using standard genetic engineering techniques known in the art. In addition, the various vaccine compositions described herein can be used separately and in combination with

each other. For example, primary immunizations of an animal may use recombinant vector-based constructs, having single or multiple strain components, followed by secondary boosts with vaccine compositions comprising inactivated virus or inactivated virus-infected cell lines. Other immunization protocols with the vaccine compositions of the invention are apparent to persons skilled in the art and are contemplated within the scope of the present invention.

The subject invention also concerns compositions comprising epitopes and/or chimeric polypeptides of the invention, or polynucleotides encoding them. In one embodiment, a composition of the invention comprises a pharmaceutically or biologically acceptable carrier, diluent, and/or adjuvant.

The subject invention also concerns antibodies, or an antigen binding fragment thereof, that bind to HIV, SIV, and/or FIV epitopes. In one embodiment, an antibody of the invention is a monoclonal antibody. In one embodiment, an antibody of the invention binds specifically to an HIV protein, *e.g.*, an HIV p24 protein. In a specific embodiment, an antibody of the invention is the monoclonal antibody designated as HL2309 (produced by clone 2B3-1F6) or HL2310 (produced by clone 2B3-2A4). In another embodiment, an antibody of the invention binds specifically to an FIV protein, *e.g.*, an HIV p24 protein. In a specific embodiment, an antibody of the invention is the monoclonal antibody designated as HL2350 (produced by clone 8B2-1E1) or HL2351 (produced by clone 8B2-2A1). In a further embodiment, an antibody of the invention binds specifically to both an HIV and an FIV protein, *i.e.*, the antibody cross-reacts with an epitope that is present on both an HIV and an FIV protein, such as a p24 protein. The subject invention also concerns the epitopes recognized by an antibody of the invention. Table 1 shows monoclonal antibodies of the present invention and their reactivity with HIV p24 and FIV p24.

Table 1.

Monoclonal ID	clone number	antigen	isotype/light chain	cross-reactivity**
HL 2309	2B3-1F6	HIV-1 UCD-1 p24	IgG1 / kappa	NO***
HL 2310	2B3-2A4	HIV-1 UCD-1 p24	IgG1 / kappa	NO***
HL 2311	2B4-1B6	HIV-1 UCD-1 p24	IgG1 / kappa	NO***
HL 2312	2B4-1E8	HIV-1 UCD-1 p24	IgG1 / kappa	NO***
HL 2335	4C3	HIV-1 UCD-1 p24	IgG2b / kappa	NO***

Monoclonal ID	clone number	antigen	isotype/light chain	cross-reactivity**
HL 2336	5G2	HIV-1 UCD-1 p24	IgM / kappa	YES
HL 2322	9D6	FIV Petaluma p24	IgG1 / kappa	YES
HL 2323	7A3	FIV Petaluma p24	IgG1 / kappa	NO***
HL 2324	2G12	FIV Petaluma p24	IgG1 / kappa	YES
HL 2348	9A12-2A3	FIV Petaluma p24	IgG1 / kappa	YES
HL 2349	9A12-2C2	FIV Petaluma p24	IgG1 / kappa	YES
HL 2350	8B2-1E1	FIV Petaluma p24	IgG1 / kappa	NO***
HL 2351	8B2-2A1	FIV Petaluma p24	IgG1 / kappa	NO***

* All mouse monoclonal antibodies (MAbs) to HIV-1 p24 are positive by ELISA and Weternblot to HIV-1 p24. Similarly, all MAbs to FIV p24 are positive by ELISA and WB to FIV p24.

**Cross-reactivity denotes reactivity of anti-HIV-1 p24 MAbs to FIV p24 and vice versa.

*** These MAbs differentiate between HIV- p24 and FIV p24 when used in right combinations.

The subject invention also concerns expression constructs comprising one or more polynucleotides of the invention. Expression constructs of the invention will also generally include regulatory elements that are functional in the intended host cell in which the expression construct is to be expressed. Thus, a person of ordinary skill in the art can select regulatory elements for use in, for example, bacterial host cells, yeast host cells, plant host cells, insect host cells, mammalian host cells, and human host cells. Regulatory elements include promoters, transcription termination sequences, translation termination sequences, enhancers, and polyadenylation elements. As used herein, the term “expression construct” refers to a combination of nucleic acid sequences that provides for transcription of an operably linked nucleic acid sequence. As used herein, the term “operably linked” refers to a juxtaposition of the components described wherein the components are in a relationship that permits them to function in their intended manner. In general, operably linked components are in contiguous relation.

An expression construct of the invention can comprise a promoter sequence operably linked to a polynucleotide sequence encoding a peptide of the invention. Promoters can be incorporated into a polynucleotide using standard techniques known in the art. Multiple copies of promoters or multiple promoters can be used in an expression construct of the invention. In a preferred embodiment, a promoter can be positioned

about the same distance from the transcription start site as it is from the transcription start site in its natural genetic environment. Some variation in this distance is permitted without substantial decrease in promoter activity. A transcription start site is typically included in the expression construct.

For expression in animal cells, an expression construct of the invention can comprise suitable promoters that can drive transcription of the polynucleotide sequence. If the cells are mammalian cells, then promoters such as, for example, actin promoter, metallothionein promoter, NF-kappaB promoter, EGR promoter, SRE promoter, IL-2 promoter, NFAT promoter, osteocalcin promoter, SV40 early promoter and SV40 late promoter, Lck promoter, BMP5 promoter, TRP-1 promoter, murine mammary tumor virus long terminal repeat promoter, STAT promoter, or an immunoglobulin promoter can be used in the expression construct.

Expression constructs of the invention may optionally contain a transcription termination sequence, a translation termination sequence, signal peptide sequence, and/or enhancer elements. Transcription termination regions can typically be obtained from the 3' untranslated region of a eukaryotic or viral gene sequence. Transcription termination sequences can be positioned downstream of a coding sequence to provide for efficient termination. Signal peptides are a group of short amino terminal sequences that encode information responsible for the relocation of an operably linked peptide to a wide range of post-translational cellular destinations, ranging from a specific organelle compartment to sites of protein action and the extracellular environment. Targeting a peptide to an intended cellular and/or extracellular destination through the use of operably linked signal peptide sequence is contemplated for use with the immunogens of the invention. Chemical enhancers are cis-acting elements that increase gene transcription and can also be included in the expression construct. Chemical enhancer elements are known in the art, and include, but are not limited to, the cytomegalovirus (CMV) early promoter enhancer element and the SV40 enhancer element. DNA sequences which direct polyadenylation of the mRNA encoded by the structural gene can also be included in the expression construct.

Unique restriction enzyme sites can be included at the 5' and 3' ends of the expression construct to allow for insertion into a polynucleotide vector. As used herein, the term “vector” refers to any genetic element, including for example, plasmids,

cosmids, chromosomes, phage, virus, and the like, which is capable of replication when associated with proper control elements and which can transfer polynucleotide sequences between cells. Vectors contain a nucleotide sequence that permits the vector to replicate in a selected host cell. A number of vectors are available for expression and/or cloning, and include, but are not limited to, pBR322, pUC series, M13 series, and pBLUESCRIPT vectors (Stratagene, La Jolla, CA).

Polynucleotides, vectors, and expression constructs of the invention can be introduced *in vivo* via lipofection (DNA transfection via liposomes prepared from synthetic cationic lipids) (Felgner *et al.*, 1987). Synthetic cationic lipids (LIPOFECTIN, Invitrogen Corp., La Jolla, CA) can be used to prepare liposomes to encapsulate a polynucleotide, vector, or expression construct of the invention. A polynucleotide, vector, or expression construct of the invention can also be introduced as naked DNA using methods known in the art, such as transfection, microinjection, electroporation, calcium phosphate precipitation, and by biolistic methods.

As used herein, the terms "nucleic acid" and "polynucleotide sequence" refer to a deoxyribonucleotide or ribonucleotide polymer in either single- or double-stranded form, and unless otherwise limited, would encompass known analogs of natural nucleotides that can function in a similar manner as naturally-occurring nucleotides. The polynucleotide sequences include both the DNA strand sequence that is transcribed into RNA and the RNA sequence that is translated into protein. The polynucleotide sequences include both full-length sequences as well as shorter sequences derived from the full-length sequences. It is understood that a particular polynucleotide sequence includes the degenerate codons of the native sequence or sequences which may be introduced to provide codon preference in a specific host cell. The polynucleotide sequences falling within the scope of the subject invention further include sequences which specifically hybridize with the exemplified sequences. The polynucleotide includes both the sense and antisense strands as either individual strands or in the duplex.

The methods of the present invention contemplate a primary immunization with an antigen, immunogen, peptide, polypeptide, polynucleotide, and/or composition of the invention. Subsequent or secondary immunizations are also contemplated within the scope of the subject methods. The antigen, immunogen, peptide, polypeptide, polynucleotide, and/or composition used for secondary immunizations can be the same as

or vary from that used for primary immunization. For example, primary immunizations of an animal may use recombinant vector-based HIV, FIV, or SIV constructs, having single or multiple strain components, followed by secondary boosts with compositions comprising HIV-, FIV-, or SIV-infected cell lines, or HIV, FIV, or SIV polypeptides, or cell free HIV or SIV virus, also having single or multiple strain components. Primary immunizations can also use an HIV, FIV, and/or SIV DNA vaccine. In one embodiment, a recombinant vector construct is used for the primary immunization, whereas a protein, or protein plus recombinant vector construct, subunit vaccine composition is used for secondary boosts. Other immunization protocols with the vaccine compositions of the invention are apparent to persons skilled in the art and are contemplated within the scope of the present invention.

The antibodies can be polyclonal or monoclonal in form. The antibodies can be derived from any animal capable of producing antibodies to the epitopes, and include, for example, human, ape, monkey, mouse, rat, goat, sheep, pig, cow, and feline animals. Also contemplated within the scope of the invention are non-human antibodies that have been "humanized" using standard procedures known in the art, such as those described in U.S. Patent Nos. 5,530,101; 5,585,089; 5,693,762; 6,180,370; and 6,407,213.

An antibody that is contemplated for use in the present invention can be in any of a variety of forms, including a whole immunoglobulin, an antibody fragment such as Fv, Fab, and similar fragments, as well as a single chain antibody that includes the variable domain complementarity determining regions (CDR), and similar forms, all of which fall under the broad term "antibody," as used herein.

The term "antibody fragment" refers to a portion of a full-length antibody, generally the antigen binding or variable region. Examples of antibody fragments include Fab, Fab', $F(ab')_2$ and Fv fragments. Papain digestion of antibodies produces two identical antigen binding fragments, called the Fab fragment, each with a single antigen binding site, and a residual "Fc" fragment, so-called for its ability to crystallize readily. Pepsin treatment of an antibody yields an $F(ab')_2$ fragment that has two antigen binding fragments, which are capable of cross-linking antigen, and a residual other fragment (which is termed pFc'). Additional fragments can include diabodies, linear antibodies, single-chain antibody molecules, and multispecific antibodies formed from antibody

fragments. As used herein, "antigen binding fragment" with respect to antibodies, refers to, for example, Fv, F(ab) and $F(ab')_2$ fragments.

Antibody fragments can retain an ability to selectively bind with the antigen or analyte are contemplated within the scope of the invention and include:

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

(2) Fab' is the fragment of an antibody molecule can be obtained by treating whole antibody with pepsin, followed by reduction, to yield an intact light chain and a portion of the heavy chain. Two Fab' fragments are obtained per antibody molecule. Fab' fragments differ from Fab fragments by the addition of a few residues at the carboxyl terminus of the heavy chain CH1 domain including one or more cysteines from the antibody hinge region.

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

(4) Fv is the minimum antibody fragment that contains a complete antigen recognition and binding site. This region consists of a dimer of one heavy and one light chain variable domain in a tight, non-covalent association (V_H-V_L dimer). It is in this configuration that the three CDRs of each variable domain interact to define an antigen-binding site on the surface of the V_H-V_L dimer. Collectively, the six CDRs confer antigen-binding specificity to the antibody. However, even a single variable domain (or half of an Fv comprising only three CDRs specific for an antigen) has the ability to recognize and bind antigen, although at a lower affinity than the entire binding site.

(5) Single chain antibody ("SCA"), defined as a genetically engineered molecule containing the variable region of the light chain (V_L), the variable region of the heavy chain (V_H), linked by a suitable polypeptide linker as a genetically fused single chain molecule. Such single chain antibodies are also referred to as "single-chain Fv" or "sFv" antibody fragments. Generally, the Fv polypeptide further comprises a polypeptide linker between the V_H and V_L domains that enables the sFv to form the desired structure for antigen binding. For a review of sFv fragments, see Pluckthun in The Pharmacology of

Monoclonal Antibodies, vol. 113, Rosenburg and Moore eds. Springer-Verlag, N.Y., pp. 269 315 (1994).

Antibodies within the scope of the invention can be of any isotype, including IgG, IgA, IgE, IgD, and IgM. IgG isotype antibodies can be further subdivided into IgG1, IgG2, IgG3, and IgG4 subtypes. IgA antibodies can be further subdivided into IgA1 and IgA2 subtypes.

Antibodies to be used in the subject invention can be genus or species specific to a target cell. Antibodies of the invention can be prepared using standard techniques known in the art. Antibodies useful in the invention can be polyclonal or monoclonal antibodies. Monoclonal antibodies can be prepared using standard methods known in the art (Kohler *et al.*, 1975).

The subject invention also concerns hybridomas that produce monoclonal antibodies of the present invention.

Peptide and/or polypeptide antigens and immunogens of the present invention can also be provided in the form of a multiple antigenic peptide (MAP) construct, with or without lypophylic attachment to each peptide string. The preparation of MAP constructs has been described in Tam (1988) and Kowalczyk *et al.* (2010). MAP constructs utilize a core matrix of lysine residues onto which multiple copies of an immunogen are synthesized (Posnett *et al.*, 1988). In one embodiment, MAP constructs of the invention can comprise one or more fatty acids attached to the core matrix. The fatty acid can comprise from about 4 to about 48 or more carbon atoms, and can be saturated and/or unsaturated. In a specific embodiment, the fatty acid is palmitic acid (hexadecanoic acid). Multiple MAP constructs, each containing the same or different immunogens, can be prepared and administered in a vaccine composition in accordance with methods of the present invention. In one embodiment, the same or different peptides are linked end to end. The same or different peptides can be linked directly to each other (*i.e.*, without a linker sequence) or they can be linked via a linker moiety such as a short amino acid sequence (*e.g.*, a furin-sensitive linker), examples of which include, but are not limited to, peptides comprising SEQ ID NO:494. In one embodiment, a MAP construct is provided with and/or administered with one or more adjuvants. In one embodiment, a MAP of the invention comprises one or more peptides that comprise the amino acid sequences of one or more of SEQ ID NOs:1-40 or 45-591.

Natural, recombinant or synthetic polypeptides of immunodeficiency viral proteins, and peptide fragments thereof, can also be used as vaccine compositions according to the subject methods. Procedures for preparing FIV, SIV, and HIV polypeptides are well known in the art. For example, FIV, SIV, and HIV polypeptides can be synthesized using solid-phase synthesis methods (Merrifield, 1963). FIV, SIV, and HIV polypeptides can also be produced using recombinant DNA techniques wherein a polynucleotide molecule encoding an FIV, SIV, or HIV protein or peptide is expressed in a host cell, such as bacteria, yeast, or mammalian cell lines, and the expressed protein purified using standard techniques of the art.

According to the methods of the subject invention, the antigenic and immunogenic compositions described herein can be administered to susceptible hosts in an effective amount and manner to induce an immune response and/or protective immunity against subsequent challenge or infection of the host by FIV, SIV, or HIV. The immunogens are typically administered parenterally, by injection, for example, either subcutaneously, intradermally, intraperitoneally, or intramuscularly, or by oral or nasal administration, or any combination of such routes of administration. Usually, the immunogens are administered to a host animal at least two times, with an interval of one or more weeks between each administration. However, other regimens for the initial and booster administrations of the immunogens are contemplated, and may depend on the judgment of the practitioner and the particular host animal being treated.

Antigens and immunogens that can be used in accordance with the present invention can be provided with a pharmaceutically-acceptable carrier or diluent. Compounds and compositions useful in the subject invention can be formulated according to known methods for preparing pharmaceutically useful compositions. Formulations are described in detail in a number of sources which are well known and readily available to those skilled in the art. For example, *Remington's Pharmaceutical Science* by E.W. Martin, Easton Pennsylvania, Mack Publishing Company, 19th ed., 1995, describes formulations which can be used in connection with the subject invention. In general, the compositions of the subject invention will be formulated such that an effective amount of an antigen or immunogen is combined with a suitable carrier in order to facilitate effective administration of the composition. The compositions used in the present methods can also be in a variety of forms. These include, for example, solid, semi-solid,

and liquid dosage forms, such as tablets, pills, powders, liquid solutions or suspension, suppositories, injectable and infusible solutions, and sprays. The preferred form depends on the intended mode of administration and therapeutic application. The compositions also preferably include conventional pharmaceutically acceptable carriers and diluents which are known to those skilled in the art. Examples of carriers or diluents for use with the subject peptidomimetics include, but are not limited to, water, saline, oils including mineral oil, ethanol, dimethyl sulfoxide, gelatin, cyclodextrans, magnesium stearate, dextrose, cellulose, sugars, calcium carbonate, glycerol, alumina, starch, and equivalent carriers and diluents, or mixtures of any of these. Formulations of an immunogen of the invention can also comprise suspension agents, protectants, lubricants, buffers, preservatives, and stabilizers. To provide for the administration of such dosages for the desired therapeutic treatment, pharmaceutical compositions of the invention will advantageously comprise between about 0.1% and 45%, and especially, 1 and 15% by weight of the antigen, antigens, immunogen or immunogens based on the weight of the total composition including carrier or diluent.

The immunogenic compositions of the subject invention can be prepared by procedures well known in the art. For example, the antigens or immunogens are typically prepared as injectables, *e.g.*, liquid solutions or suspensions. The antigens or immunogens are administered in a manner that is compatible with dosage formulation, and in such amount as will be therapeutically effective and immunogenic in the recipient. The optimal dosages and administration patterns for a particular antigen or immunogen formulation can be readily determined by a person skilled in the art.

Virus and cells in an antigenic or immunogenic formulation may be inactivated or attenuated using methods known in the art. The amount of cell-free whole or partial virus in a vaccine dose will usually be in the range from about 0.1 mg to about 5 mg, and more usually being from about 0.2 mg to about 2 mg. The dosage for formulations comprising virus-infected cell lines will usually contain from about 10^6 to about 10^8 cells per dose, and more usually from about 5×10^6 to about 7.5×10^7 cells per dose. The amount of protein or peptide immunogen in a dose for a feline animal can vary from about 0.1 μg to 10000 μg, or about 1 μg to 5000 μg, or about 10 μg to 1000 μg, or about 25 μg to 750 μg, or about 50 μg to 500 μg, or 100 μg to 250 μg, depending upon the size, age, *etc.*, of the animal receiving the dose.

In one embodiment, an antigen or immunogen of the invention is provided with one or more adjuvants that increase the person or animal's immune response against the antigen or immunogen. Antigens and immunogens of the invention can be provided with and/or administered with any suitable adjuvant or adjuvants known in the art. In one embodiment, the adjuvant is one that helps induce a strong cellular immune response. Adjuvants that can be used in the antigen and immunogen formulations of the invention include threonyl muramyl dipeptide (MDP) (Byars *et al.*, 1987), Ribi adjuvant system components (Corixa Corp., Seattle, WA) including the cell wall skeleton (CWS) component, Freund's complete, and Freund's incomplete adjuvants, bacterial lipopolysaccharide (LPS), such as from *E. coli*, or a combination thereof. A variety of other adjuvants suitable for use with the methods and vaccines of the subject invention, such as alum, aluminum hydroxide, and saponin are well known in the art and are contemplated for use with the subject invention. Cytokines (γ-IFN, GM-CSF, CSF, *etc.*) and lymphokines and interleukins (IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8. IL-9, IL-10, IL-11, IL-12, IL-13, IL-14, IL-15, IL-16, IL-17, IL-18, IL-19, IL-20, IL-21, and IL-22) have also been used as adjuvants and/or supplements to vaccine compositions and are contemplated within the scope of the present invention. One or more different cytokines and lymphokines can be included in a composition comprising an antigen or immunogen of the invention. In one embodiment, an antigen or immunogen of the invention is administered to an animal in combination with the lymphokine interleukin-12 (IL-12) in combination with another adjuvant. Also specifically contemplated within the scope of the invention is the use of the lymphokine interleukin-18 (IL-18) as part of an adjuvant composition. In one embodiment, an adjuvant composition used with the subject invention comprises a combination of IL-12 and IL-15, or IL-15 and IL-18, or IL-12 and IL-18, or IL-12, IL-15, and IL-18. The cytokine selected is of a species that has biological activity in the animal receiving the antigen or immunogen. For example, if the animal is a cat, then the cytokine can be a human cytokine or a feline cytokine, *e.g.*, feline IL-12, feline IL-15, feline IL-18, *etc.*

Abbreviations of FIV strains used herein are shown below:

Strain (subtype)	Abbreviation	Strain (subtype)	Abbreviation
Petaluma (A)	FIV_{Pet}	PPR (A)	FIV_{PPR}
Dixon (A)	FIV_{Dix}	FranceWo	FIV_{Fra}

Strain (subtype)	Abbreviation	Strain (subtype)	Abbreviation
UK8 (A)	FIV_{UK8}	Netherlands	FIV_{Net}
Bangston (B)	FIV_{Bang}	USILbrny03B (B)	FIV_{USI03}
Aomori-1 (B)	FIV_{Aom1}	TM2 (B)	FIV_{TM2}
Aomori-2 (B)	FIV_{Aom2}	USCKlgri02B (B)	FIV_{USC02}
FC1 (B)	FIV_{FC1}	Yokohama (B)	FIV_{Yok}
Shizuoka (D)	FIV_{Shi}	USMAsboy03B (B)	FIV_{USMA03}
Dutch113 (A)	FIV_{Dut113}	USTXmtex03B (B)	FIV_{UST03}
Dutch19K (A)	FIV_{Dut19}	USMCglwd03B (B)	FIV_{USMC03}
UK2 (A)	FIV_{UK2}	CABCpbar03C (C)	FIV_{CAB03}
SwissZ2 (A)	FIV_{SwiZ2}	CABCpbar07C (C)	FIV_{CAB07}
Sendai-1 (A)	FIV_{Sen1}	CABCpady02C (C)	FIV_{CAB02}
Sendai-2 (B)	FIV_{Sen2}	Fukuoka (D)	FIV_{Fuku}
USCAzepy01A (A)	FIV		
USCAhnky11A (A)	FIV_{USC11}		
USCAtt-10A (A)	FIV_{USC10}		
USCAlemy01 (A)	FIV		
USCAsam-01A (A)	FIV		

Antigens and immunogens of the invention are typically administered parenterally, by injection, for example, either subcutaneously, intradermally, intraperitoneally, or intramuscularly. Other suitable modes of administration include oral or nasal administration. Usually, the antigens and immunogens are administered to a human or animal at least two times, with an interval of one or more weeks between each administration. However, other regimens for the initial and booster administrations of the antigens and immunogens are contemplated, and may depend on the judgment of the practitioner and the patient being treated.

Antigenic and immunogenic compositions of the subject invention can be prepared by procedures well known in the art. For example, the antigens and immunogens are typically prepared as injectables, *e.g.*, liquid solutions or suspensions. The antigens and immunogens are administered in a manner that is compatible with dosage formulation, and in such amount as will be therapeutically effective and

immunogenic in the recipient. The optimal dosages and administration patterns for a particular antigen and immunogen formulation can be readily determined by a person skilled in the art.

Antigens and immunogens that can be used in accordance with the present invention can be provided with a pharmaceutically-acceptable carrier or diluent. In one embodiment, an antigen or immunogen of the invention is provided with one or more adjuvants that increase the human or animal's immune response against the antigen or immunogen. Antigens and immunogens of the invention can be provided with and/or administered with any suitable adjuvant or adjuvants known in the art.

The antigenic or immunogenic peptides contemplated in the subject invention include the specific peptides exemplified herein as well as equivalent peptides which may be, for example, somewhat longer or shorter than the peptides exemplified herein. For example, using the teachings provided herein, a person skilled in the art could readily make peptides having from 1 to about 15 or more amino acids added to, or 1 to 10 amino acids removed from, either or both ends of the disclosed peptides using standard techniques known in the art. Any added amino acids can be different or the same as the corresponding amino acids of the full-length protein from which the peptide is derived. The skilled artisan, having the benefit of the teachings disclosed in the subject application, could easily determine whether a longer or shorter peptide retained the immunogenic activity of the specific peptides exemplified herein.

Substitution of amino acids other than those specifically exemplified or naturally present in a peptide of the invention are also contemplated within the scope of the present invention. For example, non-natural amino acids can be substituted for the amino acids of a peptide, so long as the peptide having the substituted amino acids retains substantially the same immunogenic activity as the peptide in which amino acids have not been substituted. Examples of non-natural amino acids include, but are not limited to, ornithine, citrulline, hydroxyproline, homoserine, phenylglycine, taurine, iodotyrosine, 2,4-diaminobutyric acid, α-amino isobutyric acid, 4-aminobutyric acid, 2-amino butyric acid, γ-amino butyric acid, ε-amino hexanoic acid, 6-amino hexanoic acid, 2-amino isobutyric acid, 3-amino propionic acid, norleucine, norvaline, sarcosine, homocitrulline, cysteic acid, τ-butylglycine, τ-butylalanine, phenylglycine, cyclohexylalanine, β-alanine, fluoro-amino acids, designer amino acids such as β-methyl amino acids, C-methyl amino

acids, N-methyl amino acids, and amino acid analogues in general. Non-natural amino acids also include amino acids having derivatized side groups. Furthermore, any of the amino acids in the protein can be of the D (dextrorotary) form or L (levorotary) form.

Amino acids can be generally categorized in the following classes: non-polar, uncharged polar, basic, and acidic. Conservative substitutions whereby a peptide of the present invention having an amino acid of one class is replaced with another amino acid of the same class fall within the scope of the subject invention so long as the peptide having the substitution still retains substantially the same immunogenic activity as the peptide that does not have the substitution. Table 2 below provides a listing of examples of amino acids belonging to each class.

Table 2.

Class of Amino Acid	Examples of Amino Acids
Nonpolar	Ala, Val, Leu, Ile, Pro, Met, Phe, Trp
Uncharged Polar	Gly, Ser, Thr, Cys, Tyr, Asn, Gln
Acidic	Asp, Glu
Basic	Lys, Arg, His

Polynucleotides encoding a specifically exemplified peptide or chimeric polypeptide of the invention, or a shorter or longer peptide or chimeric polypeptide, or a peptide having one or more amino acid substitutions in the sequence are contemplated within the scope of the present invention. The subject invention also concerns variants of the polynucleotides of the present invention that encode a peptide of the invention. Variant sequences include those sequences wherein one or more nucleotides of the sequence have been substituted, deleted, and/or inserted. The nucleotides that can be substituted for natural nucleotides of DNA have a base moiety that can include, but is not limited to, inosine, 5-fluorouracil, 5-bromouracil, hypoxanthine, 1-methylguanine, 5-methylcytosine, and tritylated bases. The sugar moiety of the nucleotide in a sequence can also be modified and includes, but is not limited to, arabinose, xylulose, and hexose. In addition, the adenine, cytosine, guanine, thymine, and uracil bases of the nucleotides

can be modified with acetyl, methyl, and/or thio groups. Sequences containing nucleotide substitutions, deletions, and/or insertions can be prepared and tested using standard techniques known in the art.

Fragments and variants of a peptide or a chimeric polypeptide of the present invention can be generated as described herein and tested for the presence of immunogenic activity using standard techniques known in the art.

Polynucleotides, peptides, and chimeric polypeptides contemplated within the scope of the subject invention can also be defined in terms of more particular identity and/or similarity ranges with those sequences of the invention specifically exemplified herein. The sequence identity will typically be greater than 60%, preferably greater than 75%, more preferably greater than 80%, even more preferably greater than 90%, and can be greater than 95%. The identity and/or similarity of a sequence can be 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% as compared to a sequence exemplified herein. Unless otherwise specified, as used herein percent sequence identity and/or similarity of two sequences can be determined using the algorithm of Karlin and Altschul (1990), modified as in Karlin and Altschul (1993). Such an algorithm is incorporated into the NBLAST and XBLAST programs of Altschul *et al.* (1990). BLAST searches can be performed with the NBLAST program, score = 100, wordlength = 12, to obtain sequences with the desired percent sequence identity. To obtain gapped alignments for comparison purposes, Gapped BLAST can be used as described in Altschul *et al.* (1997). When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (NBLAST and XBLAST) can be used. *See* Worldwide Website: ncbi.nlm.nih.gov.

Factors affecting the preferred dosage regimen may include, for example, the age, weight, sex, diet, activity, lung size, and condition of the subject; the route of administration; the efficacy, safety, and duration-of-immunity profiles of the particular vaccine used; whether a delivery system is used; and whether the vaccine is administered as part of a drug and/or vaccine combination. Thus, the dosage actually employed can vary for specific animals, and, therefore, can deviate from the typical dosages set forth above. Determining such dosage adjustments is generally within the skill of those in the art using conventional means. It should further be noted that live attenuated viruses are

generally self-propagating; thus, the specific amount of such a virus administered is not necessarily critical.

It is contemplated that the vaccine may be administered to the patient a single time; or, alternatively, two or more times over days, weeks, months, or years. In some embodiments, the vaccine is administered at least two times. In some such embodiments, for example, the vaccine is administered twice, with the second dose (*e.g.*, the booster) being administered at least about 2 weeks after the first. In some embodiments, the vaccine is administered twice, with the second dose being administered no greater than 8 weeks after the first. In some embodiments, the second dose is administered at from about 2 weeks to about 4 years after the first dose, from about 2 to about 8 weeks after the first dose, or from about 3 to about 4 weeks after the first dose. In some embodiments, the second dose is administered about 4 weeks after the first dose. In the above embodiments, the first and subsequent dosages may vary, such as, for example, in amount and/or form. Often, however, the dosages are the same as to amount and form. When only a single dose is administered, the amount of vaccine in that dose alone generally comprises a therapeutically effective amount of the vaccine. When, however, more than one dose is administered, the amounts of vaccine in those doses together may constitute a therapeutically effective amount.

In some embodiments, the vaccine is administered before the recipient is infected with virus. In such embodiments, the vaccine may, for example, be administered to prevent, reduce the risk of, or delay the onset of one or more (typically two or more) clinical symptoms.

In some embodiments, the vaccine is administered after the recipient is infected with influenza. In such embodiments, the vaccine may, for example, ameliorate, suppress, or eradicate the virus or one or more (typically two or more) clinical symptoms.

It is contemplated that the vaccine may be administered via the feline patient's drinking water and/or food. It is further contemplated that the vaccine may be administered in the form of a treat or toy.

"Parenteral administration" includes subcutaneous injections, submucosal injections, intravenous injections, intramuscular injections, intrasternal injections, transcutaneous injections, and infusion. Injectable preparations *(e.g.,* sterile injectable aqueous or oleaginous suspensions) can be formulated according to the known art using

suitable excipients, such as vehicles, solvents, dispersing, wetting agents, emulsifying agents, and/or suspending agents. These typically include, for example, water, saline, dextrose, glycerol, ethanol, corn oil, cottonseed oil, peanut oil, sesame oil, benzyl alcohol, benzyl alcohol, 1,3-butanediol, Ringer's solution, isotonic sodium chloride solution, bland fixed oils (*e.g.,* synthetic mono- or diglycerides), fatty acids (*e.g.,* oleic acid), dimethyl acetamide, surfactants (*e.g.,* ionic and non-ionic detergents), propylene glycol, and/or polyethylene glycols. Excipients also may include small amounts of other auxiliary substances, such as pH buffering agents.

The vaccine may include one or more excipients that enhance a patient's immune response (which may include an antibody response, cellular response, or both), thereby increasing the effectiveness of the vaccine. Use of such excipients (or "adjuvants") may be particularly beneficial when using an inactivated vaccine. The adjuvant(s) may be a substance that has a direct (*e.g.*, cytokine or Bacillé Calmette-Guerin ("BCG")) or indirect effect (liposomes) on cells of the patient's immune system. Examples of often suitable adjuvants include oils (*e.g.*, mineral oils), metallic salts (*e.g.*, aluminum hydroxide or aluminum phosphate), bacterial components (*e.g.*, bacterial liposaccharides, Freund's adjuvants, and/or MDP), plant components (*e.g.*, Quil A), and/or one or more substances that have a carrier effect (*e.g.*, bentonite, latex particles, liposomes, and/or Quil A, ISCOM). It should be recognized that this invention encompasses both vaccines that comprise an adjuvant(s), as well as vaccines that do not comprise any adjuvant.

It is contemplated that the vaccine may be freeze-dried (or otherwise reduced in liquid volume) for storage, and then reconstituted in a liquid before or at the time of administration. Such reconstitution may be achieved using, for example, vaccine-grade water.

The present invention further comprises kits that are suitable for use in performing the methods described above. The kit comprises a dosage form comprising a vaccine described above. The kit also comprises at least one additional component, and, typically, instructions for using the vaccine with the additional component(s). The additional component(s) may, for example, be one or more additional ingredients (such as, for example, one or more of the excipients discussed above, food, and/or a treat) that can be mixed with the vaccine before or during administration. The additional component(s) may alternatively (or additionally) comprise one or more apparatuses for

administering the vaccine to the patient. Such an apparatus may be, for example, a syringe, inhaler, nebulizer, pipette, forceps, or any medically acceptable delivery vehicle. In some embodiments, the apparatus is suitable for subcutaneous administration of the vaccine. In some embodiments, the apparatus is suitable for intranasal administration of the vaccine.

Other excipients and modes of administration known in the pharmaceutical or biologics arts also may be used.

The subject invention also concerns a method for selecting antigens and/or immunogens for use in a vaccine against an immunodeficiency virus, such as HIV or FIV, wherein the method comprises identifying evolutionarily conserved epitopes of the target protein from two or more immunodeficiency viruses, wherein one or more of the identified epitopes are selected for use as an antigen or immunogen in the vaccine. In one embodiment, one or more overlapping peptides of an FIV protein and a corresponding HIV protein, or an FIV protein and a corresponding SIV protein, or an SIV protein and a corresponding HIV protein are assayed to identify those that are capable of inducing one or more T cell responses (cell mediated immune responses). Cells are contacted with the one or more peptides for a period of time and then assays are conducted to determine if one or more T cell responses was induced. In one embodiment, a response assayed for is IFNγ production. In another embodiment, a response assayed for is induction of T cell proliferation, such as proliferation of $CD4^+$ and/or $CD8^+$ T cells. In another embodiment, a response assayed for is the production and/or expression of cytotoxic T cell-associated molecules (*e.g.*, cytotoxins), such as granzyme A, granzyme B, perforin, and/or CD107a. In one embodiment, a method of the invention comprises testing one or more peptides for induction of IFNγ production by cells (*e.g.*, peripheral blood mononuclear cells (PBMS)) using an enzyme-linked immunosorbent spot (ELISpot) assay for IFNγ. In one embodiment, a method of the invention comprises testing one or more peptides for induction of T cell proliferation using a carboxyfluorescein diacetate succinimide ester (CFSE) proliferation assay. The assays contemplated for determining the induction of a T cell response can provide quantitative and/or qualitative results. In one embodiment, the cells contacted with the one or more peptides are cells from a feline animal. In one embodiment, the feline animal is infected with FIV or has been vaccinated against FIV. In another embodiment, the feline animal has not been infected with FIV or vaccinated

against FIV. In another embodiment, the cells are from a primate or a human. In one embodiment, the primate or human has not been infected with HIV. In another embodiment, the primate or human has been infected with HIV (HIV+). In one embodiment, the HIV+ subject is a long-term survivor (LTS). In another embodiment, the subject is a short-term (ST) survivor. The HIV+ subject can be one that has received antiretroviral therapy (ART) or one that has not received ART.

The subject invention also concerns chimeric polynucleotides and polypeptides that comprise sequences from more than one immunodeficiency virus. In one embodiment, a chimera of the invention comprises sequences of HIV and FIV. In a specific embodiment, a chimera of the invention is a chimeric Gag protein wherein matrix (MA) and nucleocapsid (NC) sequences are from FIV and wherein the core or capsid (CA) (p24) sequences are from an HIV. In an exemplified embodiment, a chimera polynucleotide comprises the sequence shown in SEQ ID NO:41 or SEQ ID NO:42. In a further exemplified embodiment, a chimera polypeptide comprises the sequence shown in SEQ ID NO:43 or SEQ ID NO:44. The subject invention contemplates that HIV proteins can be substituted for corresponding FIV proteins in other chimeric polynucleotides and polypeptides of the invention. For example, HIV pol sequences can be substituted into corresponding FIV pol sequences.

The subject invention also concerns methods for determining whether an animal, such as a feline animal, has been vaccinated against FIV with an FIV vaccine of the present invention, or is infected by FIV or has been infected by FIV. In one embodiment, a biological sample, such as a blood or serum sample, is obtained from a feline animal, and the sample is assayed to determine whether the animal has antibodies that bind specifically to HIV antigens. In a specific embodiment, if an animal is vaccinated with a chimeric polynucleotide or polypeptide of the present invention wherein p24 of FIV is replaced with p24 of HIV, then antibodies specific for the HIV p24 will be present in the animal and can be detected. In one embodiment, a chimera polypeptide comprises the sequence shown in SEQ ID NO:43 or SEQ ID NO:44. If an animal has been infected with FIV, then that animal will not have antibodies that bind to certain HIV p24 epitopes. If an animal has been vaccinated with a chimera polypeptide comprising an HIV protein and an FIV protein, then the animal will have antibodes that bind to HIV. Epitopes of an

HIV protein that are only recognized by HIV antibodies and that are not recognized by FIV antibodies can be used in the subject invention.

All patents, patent applications, provisional applications, and publications referred to or cited herein are incorporated by reference in their entirety, including all figures and tables, to the extent they are not inconsistent with the explicit teachings of this specification.

Following are examples that illustrate procedures for practicing the invention. These examples should not be construed as limiting. All percentages are by weight and all solvent mixture proportions are by volume unless otherwise noted.

Example 1—Selection of HIV immunogens and conserved epitopes

Careful design of vaccine immunogens for protection against a wide number of HIV variants will be required to deal with the large antigenic diversity. Conserved viral antigens, subtype-matched antigens, consensus antigens, variants of single antigens and multiple antigens have all been used alone or in combination (Li *et al.* (2007); Korber *et al.* (2009)). Table 7 shows a few examples for each of the strategies. CTL responses have been shown to preferentially target the conserved regions over the more variable ones (Cao *et al.* (1997)), and these responses have been associated with better HIV disease outcomes or no disease manifestation (Kiepiela *et al.* (*2007)*; Rowland-Jones *et al.* (1998); Johnson *et al.* (1991)).

The most conserved regions of HIV, especially those conserved across subtypes (Korber *et al.* (2009)) or among lentiviruses (Yamamoto *et al.* (2010)), may be the best targets of the immune system for inducing vaccine protection. Some of these regions may be protective and are less likely to mutate because they hold a functional or structural importance to the virus species (possibly to the genus); a mutation would induce impairment to viral fitness (Santra *et al.* (2010); Barouch *et al.* (2010)). This possibility makes the identification of conserved epitopes an important aspect of immunogen selection in vaccine design. One means of including these conserved regions is to construct polyvalent mosaic proteins as vaccine immunogens; thus far, preclinical evaluations of the mosaic vaccine have demonstrated great potential for broad T-cell responses, across subtypes (Korber *et al.* (2009); Smith (2004); Wang *et al.* (2009)).

A method of selecting highly conserved regions is to identify those with the lowest entropy, which is the lowest variability at each aa position. Based on this concept, the most conserved HIV proteins have been shown to be (in order of lowest variability): integrase (IN), core capsid (Gag-p24), reverse transcriptase (RT), and protease (PR) (Table 3) (Yusim *et al.* (2002)). They were followed by Vpr, Vif, matrix (Gag-p17), Nef, Rev, and the surface envelope (SU-Env). Tat and Vpu have the highest variability (Table 3). This observation suggests that the selection of conserved vaccine epitopes should be done first from IN, Gag-p24, RT, and PR.

While Jenner may not have considered functional conservation when developing his smallpox vaccine, he can be considered to have been the first developer of a vaccine that was based on conserved features between two different viral species (Jenner (1798)). In a similar fashion, comparisons with other lentiviruses could help identify highly conserved epitopes that are required for viral function and survival. FIV is a lentivirus that is only distantly related to HIV-1, but may still be relevant to the evolutionary conserved approach of vaccine development because of the shared similarities between the HIV and FIV viruses in terms of aa sequence, structure, and pathogenesis (Yamamoto *et al.* (2007)). A comparison of the aa composition of proteins between HIV-1 and FIV demonstrates the following percentages of identity/homology: RT, 47/72; IN, 37/65; Gag-p24, 32/63; nucleocapsid (Gag-p7), 30/54; PR, 24/48; Gag-p17, 20/50; SU-Env, 19/43; transmembrane envelope (TM-Env) 18/42 (Yamamoto *et al.* (2010)) (Table 3). The three most conserved proteins are also those that have the lowest entropy calculation, as shown in Table 3 (Yusim *et al.* (2002)). Hence, the IN, RT, and Gag-p24 proteins appear to be excellent targets for identifying evolutionary conserved regions that may also contain conserved T-cell epitopes.

Table 3. HIV-1/FIV proteins.

	IN	**Gag-p24**	**RT**	PR	Gag-p17	SU-Env	Ref.
Approximate average entropy scores [a]	0.16	0.18	0.21	0.23	0.45	0.6	†
HIV/FIV protein % aa identity/homology [b]	37/65	32/63	47/72	24/48	20/50	19/43	‡

[a] The average Shannon entropy score is the average value of variability of a given protein at each aa position, calculated by using many aligned sequences. The approximate values shown are derived from the figure of HIV-1 (group M) protein variability from Yusim *et*

al (Yusim *et al.* (2002))), where the proteins are presented from lowest to highest variability. Lower scores represent lower variability and therefore higher aa conservation.
[b] The percentage of aa identity and homology between HIV and FIV proteins are shown, with the three most conserved HIV and FIV proteins bolded.
† (Yusim *et al.* (2002))
‡ (Yamamoto *et al.* (2010))

Example 2—Identification of evolutionary conserved HIV CTL epitopes: Use of FIV proteins

Immunoninformatics has become an integral part in the design of new vaccines with great promise of rapid and effective vaccine discovery (Ardito *et al.* (2011); Moss *et al.* (2011); De Groot *et al.* (2008)). A number of tools and databases are now available online including HLA class-I and -II binding predictions (Los Alamos National Laboratory. HIV molecular immunology database: Best-defined CTL/CD8$^+$ Epitope Summary: (www.hiv.lanl.gov/content/immunology/tables/optimal_ctl_ summary.html); Yongqun *et al.* (2010)), and a number of tools that are useful for the prediction of CTL epitopes (Table 4). In one study performed by our group, NetCTL-1.2 was used to identify CTL epitopes on the integrase sequences of HIV, SIV and FIV (Figure 1A). For the twelve HLA supertypes shown in Figure 1, a large number of CTL epitopes were predicted on each integrase sequence regardless of the virus: HIV with 78 epitopes, SIV with 74 epitopes, and FIV with 85 epitopes. Some of these were conserved between HIV and SIV (34 epitopes), as well as between HIV and FIV (25 epitopes) (Figure 1B). A smaller number (17 epitopes) was conserved among all three viruses, reducing the target epitopes to the expected most evolutionary conserved.

Table 4. CTL-epitope prediction tools.

Name	Website	Developer	Ref.
CTLPred	www.imtech.res.in/raghava/ctlpred/	India	†
NetCTL	www.cbs.dtu.dk/services/NetCTL/	Denmark	‡
NetCTLpan	www.cbs.dtu.dk/services/NetCTLpan/	Denmark	§

† Bhasin *et al.* (2004)
‡ Larsen *et al.* (2007)
§ Stranzl *et al.* (2010)

Thirteen HIV CTL epitopes termed best-defined CTL epitopes have been identified empirically on HIV integrase by different laboratories and compiled on the Los Alamos National Laboratory (LANL) website (Table 5). In this regard, based on observations using SIV and FIV, an evolutionary conserved HIV CTL epitope can be defined as a CTL epitope with a direct or indirect SIV and/or FIV CTL counterpart (Figure 2). Using the direct counterpart approach (Figure 2, arrow a), three of these epitopes are predicted to be CTL epitopes conserved between HIV, SIV, and FIV and one was shown to be an indirect FIV counterpart (Table 6). They share the same HLA binding and CTL supertype predictions (Larsen *et al.* (2007); Lundegaard *et al.* (2008)). The evolutionary conserved (Figure 2, arrow c). An indirect counterpart to an HIV epitope (bolded in Table 6), located upstream on FIV integrase, has higher aa identity and homology than the direct counterpart (Figure 2, arrow b). This indirect FIV counterpart has the same binding alleles and predicted CTL supertype as the HIV epitope.

Table 5. Best defined CTL epitopes on HIV integrase[a].

	Epitope	**Position on HXB2**	**HLA**
1	LPPIVAKEI (SEQ ID NO:1)	28-36	B42
2	THLEGKIIL (SEQ ID NO:2)	66-74	B*1510
3	STTVKAACWW (SEQ ID NO:3)	123-132	B57
4	IQQEFGIPY (SEQ ID NO:4)	135-143	B*1503
5	VRDQAEHL (SEQ ID NO:5)	165-172	Cw18
6	KTAVQMAVF (SEQ ID NO:6)	173-181	B*5701
7	AVFIHNFKRK (SEQ ID NO:7)	179-188	A*0301,A*1101
8	FKRKGGIGGY (SEQ ID NO:8)	185-194	B*1503
9	KRKGGIGGY (SEQ ID NO:9)	186-194	B*2705
10	IIATDIQTK (SEQ ID NO:10)	203-211	A*1101
11	KIQNFRVYY (SEQ ID NO:11)	219-227	A*3002

	Epitope	Position on HXB2	HLA
12	VPRRKAKII (SEQ ID NO:12)	260-268	B42
13	RKAKIIRDY (SEQ ID NO:13)	263-271	B*1503

[a] Adapted from LANL (hiv.lanl.gov/content/immunology/tables/optimal_ctl_summary.html) which was last updated on 2009-08-31. The best defined CTL epitopes or "A list" represent the epitopes whose specific HLA class I allele has been demonstrated with strong certainty and are judged to be at their optimal length.

Table 6. HIV-1 integrase CTL epitopes and direct FIV counterparts.

Allele (supertype)	Virus	Epitopes	Iden.	Hom.	IEDB Prediction: Binding Allele (nM value)	Supertype (Total # of binding alleles)	NetCTL Supertype
B*1510 (B39)	HIV	THLEGKIIL (SEQ ID NO:2)			B*3901(9); B*1501(425)	B39(2)	B39
	SIV	THLEGKIII (SEQ ID NO:14)	78	100	B*3901(44)	B39(1)	B39
	FIV	THFNGKIII (SEQ ID NO:15)	56	78	B*3901(64); B*1501(373)	B39(2)	B39
A*0301 (A3) A*1101 (A3)	HIV	MAVFIHNFK (SEQ ID NO:16)			A*0301 (363); A*1101 (20)	A3(5); A1(1)	A3
	SIV	MAVHCMNFK (SEQ ID NO:17)	67	67	A*0301(174); A*1101(25)	A3(4); A1(1)	A3
	FIV	LALYCLNFK (SEQ ID NO:18)	44	78	A*3001(113); A*1101(55)	A3(3); A1(1)	A3
B42 (B7)	HIV	VPRRKAKII (SEQ ID NO:12)			B*0702(43); B*0801(53)	B7(1); B8(1)	B7; B8
	SIV	VPRRKAKII (SEQ ID NO:12)	100	100	B*0702(43); B*0801(53)	B7(1); B8(1)	B7; B8
	FIV	VPRRHIRRV (SEQ ID NO:19)	44	67	B*0702(20); B*0801(124)	B7(1); B8(1)	B7; B8
*A*1101 (A3)*	*HIV*	*IIATDIQTK* (SEQ ID NO:10)			*A*1101(404); A*6801(204)*	*A3(3)*	*A3*
	SIV	*ILATDIQTT* (SEQ ID NO:21)	*78*	*89*	*A*0250(10)*	*A2(5)*	*None*
	FIV	*QESLRIQDY* (SEQ ID NO:22)	*22*	*33*	*B*4402(88)*	*B44(3)*	*None*
	FIV	***IVAEEIKRK***[d] (SEQ ID NO:23)	*44*	*78*	*A*1101(338); A*6801(206)*	*A3(2)*	*A3*

[a] The HIV epitope sequences are from the LANL list of the best defined CTL epitopes for HIV integrase. The SIV counterpart sequences are derived from LANL SIVmm239 and the FIV counterpart sequences are derived from GenBank (ABD16378) after aa alignment with HXB2 sequence.

[b] The identity (iden.) and homology (hom.) values were obtained using EMBOSS Stretcher - Pairwise Sequence Alignment (www.ebi.ac.uk/Tools/psa/emboss_stretcher/).

[c] MHC binding for HIV, SIV and FIV counterpart epitopes were predicted using the Immune Epitope Database (IEDB) MHC class I binding prediction tool (http://tools.immuneepitope.org/analyze/html/mhc_binding.html). The matching binding alleles are shown along with their binding affinity values (nM) which are derived from the Artificial Neural Network (ANN) analysis, where lower values represent higher binding affinity and potential for $CD8^+$ T-cell activity. The total numbers of binding alleles with affinity below 500 nM are shown in parenthesis next to the supertypes.

[d] HIV epitope with non-matching SIV and FIV (direct counterparts) is in italics and the bolded FIV epitope is an indirect counterpart with matching alleles to the HIV epitope.

The predicted results of SIV sequences can be explained by the high aa identity between HIV and SIV as SIV is more closely related to HIV than FIV. However, despite the relatively lower aa identity between HIV and FIV, FIV counterpart epitopes still appear to be potentially effective HIV antigens (see Table 6), most likely due to the slightly higher aa homology observed between the two viruses. This finding indicates that both SIV and FIV epitopes could induce CTL responses in human PBMCs. Therefore, conserved SIV and FIV integrase peptides can be used as immunogens *in vitro* to compare and identify conserved immune responses generated by the PBMCs of HIV^+ individuals.

Table 7. Phase I and IIa clinical trials of HIV CTL multi-epitope vaccine.

Trial[a]	**Site (# subjects enrolled in the study)**[b]	**Vaccine type (Regimen)**[c]	**Dose /route DNA (mg), MVA (p.f.u.)**[d]	**HIV Antigens (# of CTL epitopes)**[e]	**HIV subtypes involved**[f]	**HLA super-type of CTL epitopes**	**Epitope selection method**	**(%) IFNγ**[g] **responders**	**REF**[h]
IAVI-001	UK (18)	DNA (d: 0,21)	0.1 or .05 mg / i.m.	p24/p17 gene [contains TH epitopes] + 24 CTL epitopes [p24(6),pol(6), nef(8),Env (4)]	A* [A,B,C, D,E,F,G, H][j]	A2 ,A3, A24, B7, B8, B27, B44	Most common HIV subtype in Kenya Conserved epitopes	78%	[129, 130]
IAVI-002	Kenya (18)	DNA (d: 0,21)	0, 0.1 or 0.5 mg / i.m.					15%	[131]
IAVI-003	UK (8)	MVA (d: 0,21)	$5x10^7$ p.f.u. / i.d.					78%	[129]
IAVI-004	Kenya (18)	MVA (mo: 0,1) (mo: 0)	0 or $5x10^7$ p.f.u. / i.d.					25%	[131]
IAVI-011	Switzerland/ UK/SA (81)	MVA (mo: 0,2)	0, $5x10^6$, $5x10^7$ or $2.5x10^8$ p.f.u. / i.d., i.m. or s.c.					6%	[132]
IAVI-005	UK (9)	p-DNA (d: 0,21)[i] b-MVA	0.1 or 0.5 mg / i.m. $5x10^7$ p.f.u. / i.d.					89%	[129]
IAVI-006	UK (119)	p-DNA (mo: 0) b-MVA (mo: 2, 3 or 5,6)	0, 0.5 or 2 mg / i.m. 0 or $5x10^7$ p.f.u. / i.d.					12%	[132]
IAVI-008	Kenya (10)	p-DNA (d: 0,21) b-MVA (mo: 9, 10)	0.5 or 1 mg / i.m. $5x10^7$ p.f.u. / i.d.					10%	[131]
IAVI-009	Uganda (50)	p-DNA(mo: 0,1 or 0) b-MVA (mo: 5,8)	0 or 0.5 mg 1x or 2x/ i.m. 0 or $5x10^7$ p.f.u. / i.d.					15%	[131]
IAVI-010	Kenya/ UK (114)	p-DNA (mo: 0,1) b-MVA (mo: 5,8)	0.5 mg / i.m. 0, $5x10^6$, $5x10^7$ or $2.5x10^8$ p.f.u. / i.d.					3%	[132]
IAVI-016	UK (24)	p-DNA (mo: 0, 1)	0 or 4 mg / i.m.					50%	[133]

Trial[a]	Site (# subjects enrolled in the study)[b]	Vaccine type (Regimen)[c]	Dose /route DNA (mg), MVA (p.f.u.)[d]	HIV Antigens (# of CTL epitopes)[e]	HIV subtypes involved[f]	HLA super-type of CTL epitopes	Epitope selection method	(%) IFNγ[g] responders	REF[h]
		b-MVA (mo: 2 or 0,1)	0 or $2.5x10^8$ p.f.u. / i.d.						
HVTN-048	USA, Bostwana (36)	DNA (mo: 0,1,3,6)	0.5 mg 4x / i.m.	21 CTL epitopes [Gag(4), Pol(8), Vpr(1), Nef(2), Rev(1), Env(5)] + TH epitope (1 pan-DR)	A, B, C, D, AE, AG	A2, A3, B7	Conserved Epitopes	0%	[134]
			2 mg 4x / i.m.					0%	
			4 mg 4x / i.m.				HLA coverage	13%	
HVTN-056	USA (40)	MEP [peptides+adjuvant] (mo: 0,1,3)	1 mg MEP + 50 µg adjuvant 3x / i.m.	4 peptides (55 CTL epitopes): Env-TH/Gag-CTL(5) Gag-TH/GagCTL(19) Env-TH/Nef-CTL(15) Env-TH/Gag-CTL(16)	B*	A1, A2, A3, A24, B7, B8, B27, B58, B62	Epitope clustering on LANL	13%	[135]
	USA (40)	MEP [peptides + adjuvant +GM-CSF] (mo: 0,1,3)	1 mg MEP / 50 µg adjuvant + 50 ug GM-CSF 3x / i.m.					3%	
ANRS VAC18	France (99)	Lipopeptides (mo: 0, 1, 3, 6)	50 µg 4x / i.m.	5 lipopeptides (77 CTL epitopes, containing 7 TH epitopes): [Gag1(9), Gag2(21), Nef1(16), Nef2(21), Pol (10)]		A1, A2, A3, A24, B7, B8, B27, B58, B62	Conserved regions	71%[k]	[136]
			150 µg 4x / i.m.					60%[k]	
			500 µg 4x / i.m.					70%[k]	

[a] All trials are phase I clinical trials except for the bolded trial numbers which are phase IIa (with subjects not at risks of HIV infection); International AIDS Vaccine Initiative (IAVI); HIV Vaccine Trials Network (HVTN); Agence National de Recherche sur le SIDA (ANRS).

[b] United Kingdom (UK); South Africa (SA); United States of America (USA).

[c] Prime (p); boost (b); day (d); month (mo); modified vaccinia Ankara (MVA); multi-epitope peptide (MEP); granulocyte macrophage colony stimulating factor (GM-CSF). [i]Nine of the 18 volunteers from IAVI-001 who were primed with HIVA-DNA agreed to receive a boost 9-14 months later.

[d] Intramuscular immunization (i.m.); intradermal immunization (i.d.); subcutaneous immunization (s.c.).

[e] MHC class I molecules can accommodate CTL epitopes of 8 to 11 aa in length [137]. The p24/p17 represents 73% of the Gag and contains both CTL and T-helper epitopes. The pan-DR T-helper epitope is a 13-mer that binds to all common HLA-DR alleles. Each of the four peptides in the MEP vaccine is made up of both TH and CTL epitopes; T helper (TH).

[f] The HIV subtypes used in the vaccine. *Consensus sequence. [j]The CTL epitopes are present in 50-90% of HIV isolates from the different subtypes.

[g] Percentage of vaccinees with detected IFNγ ELISpot responses to the CTL epitopes. The responses were detected at different time points, before or after the end of the immunization schedule for the IAVI studies; after the last immunization for HVTN 064; and after the 2nd or 3rd vaccination (single time point) for HVTN 056.

[k] Cultured ELISpot assay results.

[h] Reference (REF).

Example 3—Monoclonal Antibodies to HIV-1 and FIV Recombinant p24 Antibodies

Monoclonal antibodies (MAbs) to HIV-1 p24 and FIV p24 were produced by immunizing mice with recombinant HIV-1 p24 and recombinant FIV p24, respectively (Table 8). Two of seven MAbs to HIV-1 p24 (HL2309 and HL2310) were only reactive to HIV-1 p24, while remaining five MAbs were reactive to both HIV-1 and FIV p24 proteins. Two of six MAbs to FIV p24 (HL2350 and HL2351) were only reactive to FIV

p24, while remaining four MAbs were reactive to both HIV-1 and FIV p24 proteins. Based on Western blot (WB) and ELISA results (Fig. 8), the epitopes recognized by HIV-1 p24-specific MAbs HL2309 and HL2310 are specific for HIV-1 and are not likely to be specific for FIV. Hence, such HIV-1-specific epitopes can be used in Western blot or ELISA to detect HIV-1 p24 specific antibodies in chimera HIV-1 p24/FIV backbone vaccine (hereon called chimera HIV/FIV vaccine) immunized cats but such Western blot or ELISA should not react with antibodies from FIV-infected cats since HL2309 and HL2310 epitopes are specific for HIV-1 and not for FIV. Using the same concept, the epitopes recognized by FIV p24-specific MAbs HL2350 and HL2351 are specific for FIV and are not likely to be specific for HIV-1. Therefore, HL2350 and HL2351 epitopes can be used in WB or ELISA to detect antibodies from FIV-infected cats but should not react to antibodies from cats immunized with chimera HIV/FIV vaccine. HL2309, HL2310, HL2350, and HL2351 epitope peptides can be used in WB or ELISA based assays to differentiate chimera HIV/FIV vaccinated cats from FIV-infected cats.

Table 8. ELISA Reactivity of Monoclonal Antibodies to HIV-1_{UCD-1} p24 and FIV_{Pet} p24

Monoclonal ID	clone number	Antigen	isotype/light chain	cross-reactivity**
HL 2309	2B3-1F6	HIV-1 UCD-1 p24	IgG1 / kappa	NO
HL 2310	2B3-2A4	HIV-1 UCD-1 p24	IgG1 / kappa	NO
HL 2311	2B4-1B6	HIV-1 UCD-1 p24	IgG1 / kappa	NO
HL 2312	2B4-1E8	HIV-1 UCD-1 p24	IgG1 / kappa	NO
HL 2335	4C3	HIV-1 UCD-1 p24	IgG2b / kappa	NO
HL 2336	5G2	HIV-1 UCD-1 p24	IgM / kappa	YES
HL 2322	9D6	FIV Petaluma p24	IgG1 / kappa	YES
HL 2323	7A3	FIV Petaluma p24	IgG1 / kappa	NO
HL 2324	2G12	FIV Petaluma p24	IgG1 / kappa	YES
HL 2348	9A12-2A3	FIV Petaluma p24	IgG1 / kappa	YES
HL 2349	9A12-2C2	FIV Petaluma p24	IgG1 / kappa	YES
HL 2350	8B2-1E1	FIV Petaluma p24	IgG1 / kappa	NO

Monoclonal ID	clone number	Antigen	isotype/light chain	cross-reactivity**
HL 2351	8B2-2A1	FIV Petaluma p24	IgG1 / kappa	NO

* All mouse monoclonal antibodies (MAbs) to HIV-1 p24 are positive by ELISA and Western blot to HIV-1 p24. Similarly, all MAbs to FIV p24 are positive by ELISA and WB to FIV p24.

**Cross-reactivity denotes reactivity of anti-HIV-1 p24 MAbs to FIV p24 and vice versa.

Example 4

See Figures 11 and 12. To investigate evolutionarily conserved CTL epitopes using FIV peptides, the individual 11-16-mer peptides in the pools with high responses such as FRT3/HRT3, FRT11/HRT11, FP9/Hp10, and Fp14/Hp15 are being tested with PBMCs from the peptide-pool responders. IFNγ and CD3+CD8+ T-cell proliferation responses to FIV RT peptide FRT3-3 ("KKKSGKWRMLIDFRV" (SEQ ID NO:63), 15mer) are highly conserved among HIV+ subjects and no normal (HIV-negative) individuals responded. Since FRT3 induced perforin response in PBMCs from HIV+ subjects, FRT3-3 should have excellent probability in inducing CTL responses detected by perforin.

HUMAN PBMC ASSAYS

STIMULANTS:

HIV-1 p24 (Hp1-Hp18) & FIV p24 (Fp1-Fp17) peptide pools were 3-4 overlapping peptides of 11-15 aa long per pool, while HIV-1 RT (HRT1-HRT21) & FIV RT (FRT1-FRT21) peptide pools 3-5 overlapping peptides of 11-15 aa_long per pool. These peptides had an overlap of 9 aa spanning the entire length of the proteins.

IFNγ-ELISpot

$1.0x10^5$-$2.0x10^5$ PBMCs were stimulated with peptides (15 μg peptide/well) in ELISpot plates for 18 hours in AIMS V medium (at 10% normal human serum). The spots were counted with an ELISpot reader and adjusted to spot forming units (SFU) per 10^6 cells.

Positive reactivity was defined at ≥70 SFU/10^6 cells after subtracting the background derived from non-specific peptide control or media control, and the average of 3 HIV-1 negative controls (<30 SFU/10^6 cells).

CFSE-PROLIFERATION

$2x10^5$-$5x10^5$ CFSE labeled PBMCs were incubated with 15-20μg of peptides in 600 μL of RPMI media with 10% FBS for 5 days at 37°C (5% CO_2). After harvesting, they were labeled with allophycocyanin (APC), APC-H7, and Pacific Blue labeled monoclonal antibodies (MAb) to human CD3, CD4, and CD8, respectively and analyzed for T-cell proliferation by flow cytometry using BD LSRII (BD Biosciences). The proliferating $CD4^+$ or $CD8^+$ T cell populations were defined from the $CD3^+$ cell population as either $CD3^+CD8^+$ or $CD3^+CD4^+$T cells (mutually exclusive) with low CFSE ($CFSE^{low}$) staining.

INTRACELLULAR STAINING (ICS)

Briefly, $1x10^6$ PBMCs (freshly isolated) are stimulated with 20 μg of peptides for 6 hours in a total volume of 200 μL in a 96-well plate in presence of Golgi transport inhibitor (37°C, 5% CO_2). The cells are processed as previously described (Horton *et al.* (2007)). Cells were stained with the LIVE/DEAD® fixable yellow dye (Invitrogen, Eugene, OR). The monoclonal antibodies fluorochome-conjugated to human cytokines used are: APC-H7 to CD3 (clone SK7); BD Horizon V450 to CD4 (clone RPA-T4); Qdot to CD8 (clone 3B5); PE-cy7 to IFN-γ (clone 4S.B3); APC to IL-2 (clone MQ1-17H12) PerCP to perforin (clone B-D48); Alexa Fl.700 to granzyme-B (clone GB11) and PE to granzyme-A (clone CB9). The flow cytometry data is collected using BD LSRII and analyzed with the FACS DIVA software.

POPULATION OF STUDY

HIV-1 positive adult males and females were evaluated for IFNγ ELISpot (n=28 for p24 & n=31 for RT), $CD3^+CD4^+$ T-cell CSFE-proliferation (n=24), and $CD3^+CD8^+$ T-cell CFSE-proliferation (n=24) responses to overlapping HIV-1 p24, HIV-1 RT, FIV p24, or FIV RT peptide pools. A total of 10 normal healthy (HIV-1 negative) males and

females were used as uninfected control group. All patients have signed an approved IRB consent form.

Figures 14A-14B. IFNγ ELISpot Responses to HIV RT (reverse transcriptase) and HIV p24 (core protein) of the Primate PBMCs.

Overlapping HIV-1 p24 (Figure 14A) and RT (Figure 14B) peptide pool analyses are shown for nine SIV-infected rhesus macaques and four pre-infection macaques. Frozen PBMC were thawed and plated at the concentration of 1.4 X 10^5 viable cells per mL. Peptides were used at a concentration of 15 μg/mL. Each bar represents an individual primate's response in spot forming units (SFU/10^6 PBMC) after subtraction of 2 times the media control; except for the black and red bars. The black bar represents the average response of the pre-infection responders (Av. n=3) and the red bar represents the average response of all 4 pre-infection samples (Av. total n=4). Since these cells were frozen for over 5 years, positive responses are values of ≥50 SFU. We believe that fresh (non-cryopreserved) cells will give higher responses to HIV p24 peptide pools (Figure 14A: Hp1-Hp18) and HIV RT peptide pools (Figure 14B: Hr1-Hr21). Various mitogens (Mito.) (concanavalin A, *Staphyloccocal* enterotoxin A, phytohemaglutinin A) were used since these frozen cells did not always respond to mitogen.

Note that only two infected macaques responded to Hp15 while three pre-infection macaques also responded. Similarly three infected macaques responded to Hr11 (i.e., HRT11) while three pre-infection macaques also responded. These results suggest that the PBMC from uninfected macaques recognize these Hp15 and Hr11. Consequently the epitopes to these peptide pools are cross-reacting with epitopes already present in the uninfected macaques. In contrast three infected macaques are responding to Hr6 and two infected macaques are responding to Hr14 (i.e., HRT14), but the uninfected macaques do not respond to these peptide epitopes, indicating that recognition of these peptide pools are due to SIV infection. The PBMCs from HIV+ subjects respond robustly to Hr6 and Hr14 but those of uninfected subjects did not. Thus, Hr6 (i.e., HRT6) and Hr14 may be conserved between HIV and SIV, and is currently being evaluated for the presence of conserved CTL epitopes.

NOTE that in the text we call individual peptides in the pool according to the order below. In the case of FRT2, individual peptide FRT2-3 is the same as Peptide 8 (VERLELEGKVKRA (SEQ ID NO:51)) or the third one listed under Pool FRT2.

FIV-FC1 RT (12-17-mer) with RNASE in **bold**.

Pool FRT1

1) AQISEKIPIVKVRMK (SEQ ID NO:52)
2) IPIVKVRMKDPTQGPQV (SEQ ID NO:53)
3) KDPTQGPQVKQWPL (SEQ ID NO:54)
4) GPQVKQWPLSNEKI (SEQ ID NO:55)
5) KQWPLSNEKIEAL (SEQ ID NO:56)

Pool FRT2

6) LSNEKIEALTDIVER (SEQ ID NO:57)
7) EALTDIVERLELEGK (SEQ ID NO:58)
8) VERLELEGKVKRA = FRT2-3 (SEQ ID NO:51)
9) ELEGKVKRADPNNPW (SEQ ID NO:59)
10) KRADPNNPWNTPVFA (SEQ ID NO:60)

Pool FRT3

11) NPWNTPVFAIKKK (SEQ ID NO:61)
12) TPVFAIKKKSGKWRM (SEQ ID NO:62)
13) KKKSGKWRMLIDFRV (SEQ ID NO:63)
14) WRMLIDFRVLNKL (SEQ ID NO:64)
15) IDFRVLNKLTDKGA (SEQ ID NO:65)

Pool FRT4

16) LNKLTDKGAEVQLGL (SEQ ID NO:66)
17) KGAEVQLGLPHPAGL (SEQ ID NO:67)
18) LGLPHPAGLKMRKQV (SEQ ID NO:68)
19) AGLKMRKQVTVLDI (SEQ ID NO:69)

Pool FRT5

20) RKQVTVLDIGDAYF (SEQ ID NO:70)
21) VLDIGDAYFTIPL (SEQ ID NO:71)
22) GDAYFTIPLDPDYA (SEQ ID NO:72)
23) TIPLDPDYAPYTAF (SEQ ID NO:73)

Pool FRT6

24) PDYAPYTAFTLPRK (SEQ ID NO:74)
25) YTAFTLPRKNNA (SEQ ID NO:75)
26) FTLPRKNNAGPGRRY (SEQ ID NO:76)
27) NNAGPGRRYVWCSL (SEQ ID NO:77)

Pool FRT7

28) GRRYVWCSLPQGWVL (SEQ ID NO:78)
29) CSLPQGWVLSPLIY (SEQ ID NO:79)
30) GWVLSPLIYQSTL (SEQ ID NO:80)
31) SPLIYQSTLDNIL (SEQ ID NO:81)

Pool FRT8

32) YQSTLDNILQPFIR (SEQ ID NO:82)
33) DNILQPFIRQNPEL (SEQ ID NO:83)

34) PFIRQNPELDIYQYM (SEQ ID NO:84)
35) PELDIYQYMDDIYI (SEQ ID NO:85)
36) YQYMDDIYIGSDLNK (SEQ ID NO:86)

Pool FRT9
37) IYIGSDLNKKEHKQK (SEQ ID NO:87)
38) LNKKEHKQKVEELRK (SEQ ID NO:88)
39) KQKVEELRKLLLWW (SEQ ID NO:89)
40) ELRKLLLWWGFETPEDK (SEQ ID NO:90)
41) WGFETPEDKLQEEPPY (SEQ ID NO:91)

Pool FRT10
42) DKLQEEPPYKWMGY (SEQ ID NO:92)
43) EPPYKWMGYELHPL (SEQ ID NO:93)
44) WMGYELHPLTWSI (SEQ ID NO:94)
45) ELHPLTWSIQQKQL (SEQ ID NO:95)
46) TWSIQQKQLEIPER (SEQ ID NO:96)

Pool FRT11
47) IQQKQLEIPERPTL (SEQ ID NO:97)
48) LEIPERPTLNELQKL (SEQ ID NO:98)
49) PTLNELQKLVGKINW (SEQ ID NO:99)
50) LQKLVGKINWASQTI (SEQ ID NO:100)
51) KINWASQTIPDLSIK (SEQ ID NO:101)

Pool FRT12
52) SQTIPDLSIKELTTM (SEQ ID NO:102)
53) LSIKELTTMMRGDQR (SEQ ID NO:103)
54) TTMMRGDQRLDSIR (SEQ ID NO:104)
55) GDQRLDSIREWTTEA (SEQ ID NO:105)
56) SIREWTTEAKKEVQK (SEQ ID NO:106)

Pool FRT13
57) TEAKKEVQKAKEAI (SEQ ID NO:107)
58) EVQKAKEAIETQAQL (SEQ ID NO:108)
59) EAIETQAQLKYY (SEQ ID NO:109)
60) ETQAQLKYYDPSREL (SEQ ID NO:110)
61) KYYDPSRELYAKLSL (SEQ ID NO:111)

Pool FRT14
62) RELYAKLSLVGPHQI (SEQ ID NO:112)
63) LSLVGPHQICYQVYH (SEQ ID NO:113)
64) HQICYQVYHKNPEHV (SEQ ID NO:114)
65) VYHKNPEHVLWYGKM (SEQ ID NO:115)
66) EHVLWYGKMNRQKKK (SEQ ID NO:116)

Pool FRT15
67) GKMNRQKKKAENTCDI (SEQ ID NO:117)
68) KKAENTCDIALRACY (SEQ ID NO:118)
69) CDIALRACYKIR (SEQ ID NO:119)
70) ALRACYKIREESIIR (SEQ ID NO:120)
71) KIREESIIRIGKEPI (SEQ ID NO:121)

Pool FRT16
72) IIRIGKEPIYEIPA (SEQ ID NO:122)
73) KEPIYEIPASREAW (SEQ ID NO:123)
74) EIPASREAWESNLIR (SEQ ID NO:124)
75) EAWESNLIRSPYLKA (SEQ ID NO:125)
76) LIRSPYLKAPPPEV (SEQ ID NO:126)

Pool FRT17

77) YLKAPPPEVEFIHAA (SEQ ID NO:127)
78) PEVEFIHAALNIKRA (SEQ ID NO:128)
79) HAALNIKRALSMI (SEQ ID NO:129)
80) NIKRALSMIQDTPIL (SEQ ID NO:130)
81) SMIQDTPILGAETWY (SEQ ID NO:131)
82) PILGAETWY**IDGGRK** (SEQ ID NO:132)

Pool **FRT18**

83) TWY**IDGGRKQGKAAR** (SEQ ID NO:133)
84) GRKQGKAARAAYW (SEQ ID NO:134)
85) GKAARAAYWTDTGKW (SEQ ID NO:135)
86) AYWTDTGKWQVMEI (SEQ ID NO:136)
87) TGKWQVMEIEGSNQK (SEQ ID NO:137)

Pool FRT19

88) MEIEGSNQKAEVQAL (SEQ ID NO:138)
89) NQKAEVQALLLALQA (SEQ ID NO:139)
90) VQALLLALQAGPEEM (SEQ ID NO:140)
91) ALQAGPEEMNII (SEQ ID NO:141)
92) AGPEEMNIITDSQYI (SEQ ID NO:142)

Pool FRT20

93) NIITDSQYILNII (SEQ ID NO:143)
94) DSQYILNIITQQPDL (SEQ ID NO:144)
95) NIITQQPDLMEGLW (SEQ ID NO:145)
96) TQQPDLMEGLWQEVL (SEQ ID NO:146)
97) MEGLWQEVLEEMEKK (SEQ ID NO:147)

Pool FRT21

98) EVLEEMEKKIAIFI (SEQ ID NO:148)
99) MEKKIAIFIDWVPGH (SEQ ID NO:149)
100) IFIDWVPGHKGI (SEQ ID NO:150)
101) DWVPGHKGIPGNEEV (SEQ ID NO:151)
102) KGIPGNEEVDKLCQTM (SEQ ID NO:152)

Subtype-B HIV-1-UCD1 RT (11-16-mer) with RNAse in **bold**.

Pool HRT1

1) PISPIETVPVKLK (SEQ ID NO:153)
2) IETVPVKLKPGM (SEQ ID NO:154)
3) VPVKLKPGMDGPKVK (SEQ ID NO:155)
4) PGMDGPKVKQWPL (SEQ ID NO:156)
5) GPKVKQWPLTEEKIK (SEQ ID NO:157)

Pool HRT2

6) WPLTEEKIKALIEI (SEQ ID NO:158)
7) EKIKALIEICTEMEK (SEQ ID NO:159)
8) IEICTEMEKEGKISK (SEQ ID NO:160)
9) MEKEGKISKIGPENPY (SEQ ID NO:161)
10) SKIGPENPYNTPVFA (SEQ ID NO:162)

Pool HRT3

11) NPYNTPVFAIKKK (SEQ ID NO:163)
12) TPVFAIKKKDSTKWR (SEQ ID NO:164)
13) KKKDSTKWRKLVDFR (SEQ ID NO:165)
14) KWRKLVDFRELNKR (SEQ ID NO:166)

15) VDFRELNKRTQDFW (SEQ ID NO:167)

Pool HRT4

16) LNKRTQDFWEVQLGI (SEQ ID NO:168)
17) DFWEVQLGIPHPAGL (SEQ ID NO:169)
18) LGIPHPAGLKKKKSV (SEQ ID NO:170)
19) AGLKKKKSVTVLDV (SEQ ID NO:171)

Pool HRT5

20) KKSVTVLDVGDAYF (SEQ ID NO:172)
21) VLDVGDAYFSVPLDK (SEQ ID NO:173)
22) AYFSVPLDKDFRKY (SEQ ID NO:174)
23) PLDKDFRKYTAFTI (SEQ ID NO:175)

Pool HRT6

24) FRKYTAFTIPSI (SEQ ID NO:176)
25) FTIPSTNNETPGIRY (SEQ ID NO:177)
26) NNETPGIRYQYNVL (SEQ ID NO:178)
27) GIRYQYNVLPQGWK (SEQ ID NO:179)

Pool HRT7

28) YNVLPQGWKGSPAIF (SEQ ID NO:180)
29) GWKGSPAIFQSSMTK (SEQ ID NO:181)
30) AIFQSSMTKILEPFR (SEQ ID NO:182)
31) MTKILEPFRKQNPDI (SEQ ID NO:183)

Pool HRT8

32) PFRKQNPDIVIYQYM (SEQ ID NO:184)
33) PDIVIYQYMDDLYV (SEQ ID NO:185)
34) YQYMDDLYVGSDLEI (SEQ ID NO:186)
35) LYVGSDLEIGQHRTK (SEQ ID NO:187)
36) LEIGQHRTKIEELR (SEQ ID NO:188)

Pool HRT9

37) HRTKIEELRQHLLRW (SEQ ID NO:189)
38) ELRQHLLRWGFTTPDK (SEQ ID NO:190)
39) RWGFTTPDKKHQK (SEQ ID NO:191)
40) TTPDKKHQKEPPFLW (SEQ ID NO:192)
41) HQKEPPFLWMGYELH (SEQ ID NO:193)

Pool HRT10

42) FLWMGYELHPDKWTV (SEQ ID NO:194)
43) ELHPDKWTVQPIML (SEQ ID NO:195)
44) KWTVQPIMLPEKDSW (SEQ ID NO:196)
45) IMLPEKDSWTVNDI (SEQ ID NO:197)
46) KDSWTVNDIQKLVGK (SEQ ID NO:198)

Pool HRT11

47) NDIQKLVGKLNWA (SEQ ID NO:199)
48) KLVGKLNWASQIYA (SEQ ID NO:200)
49) LNWASQIYAGIKVR (SEQ ID NO:201)
50) SQIYAGIKVRQLCKL (SEQ ID NO:202)
51) IKVRQLCKLLRGAKA (SEQ ID NO:203)

Pool HRT12

52) CKLLRGAKALTEVI (SEQ ID NO:204)
53) GAKALTEVIPLTKEA (SEQ ID NO:205)
54) EVIPLTKEAELELA (SEQ ID NO:206)
55) TKEAELELAENREIL (SEQ ID NO:207)

56) ELAENREILKEPVH (SEQ ID NO:208)

Pool HRT13

57) REILKEPVHGVYY (SEQ ID NO:209)
58) KEPVHGVYYDPSKDL (SEQ ID NO:210)
59) VYYDPSKDLIAEIQK (SEQ ID NO:211)
60) KDLIAEIQKQGQGQW (SEQ ID NO:212)
61) IQKQGQGQWTYQIY (SEQ ID NO:213)

Pool HRT14

62) GQGQWTYQIYQEPFK (SEQ ID NO:214)
63) YQIYQEPFKNLKTGK (SEQ ID NO:215)
64) PFKNLKTGKYARMR (SEQ ID NO:216)
65) KTGKYARMRGAH (SEQ ID NO:217)
66) KYARMRGAHTNDVK (SEQ ID NO:218)

Pool HRT15

67) RGAHTNDVKQLTEAV (SEQ ID NO:219)
68) DVKQLTEAVQKIV (SEQ ID NO:220)
69) LTEAVQKIVTESIVI (SEQ ID NO:221)
70) KIVTESIVIWGKTPK (SEQ ID NO:222)
71) IVIWGKTPKFKLPI (SEQ ID NO:223)

Pool HRT16

72) KTPKFKLPIQKETW (SEQ ID NO:224)
73) KLPIQKETWEAWW (SEQ ID NO:225)
74) IQKETWEAWWTEYW (SEQ ID NO:226)
75) WEAWWTEYWQATWI (SEQ ID NO:227)
76) TEYWQATWIPEWELV (SEQ ID NO:228)

Pool HRT17

77) TWIPEWELVNTPPLV (SEQ ID NO:229)
78) ELVNTPPLVKLWYQL (SEQ ID NO:230)
79) PLVKLWYQLEKEPI (SEQ ID NO:231)
80) WYQLEKEPIEGAETF (SEQ ID NO:232)
81) EPIEGAETF**YVDGAA** (SEQ ID NO:233)
82) **ETFYVDGAANRETKL** (SEQ ID NO:234)

Pool HRT18

83) GAANRETKLGKAGYV (SEQ ID NO:235)
84) TKLGKAGYVTNRGR (SEQ ID NO:236)
85) AGYVTNRGRQKVVPL (SEQ ID NO:237)
86) RGRQKVVPLTDA (SEQ ID NO:238)
87) RQKVVPLTDATNQK (SEQ ID NO:239)

Pool HRT19

88) PLTDATNQKTELEAI (SEQ ID NO:240)
89) NQKTELEAIHLAL (SEQ ID NO:241)
90) ELEAIHLALQDSGL (SEQ ID NO:242)
91) HLALQDSGLEVNIV (SEQ ID NO:243)
92) DSGLEVNIVTDSQYA (SEQ ID NO:244)

Pool HRT20

93) NIVTDSQYALGIIQA (SEQ ID NO:245)
94) SQYALGIIQAQPDK (SEQ ID NO:246)
95) GIIQAQPDKSESELV (SEQ ID NO:247)
96) PDKSESELVSQII (SEQ ID NO:248)
97) ESELVSQIIEQLIKK (SEQ ID NO:249)

Pool HRT21

98) SQIIEQLIKKEKVYL (SEQ ID NO:250)
99) LIKKEKVYLAWVPAH (SEQ ID NO:251)
100) VYLAWVPAHKGI (SEQ ID NO:252)
101) AWVPAHKGIGGNEQV (SEQ ID NO:253)
102) KGIGGNEQVDKLV (SEQ ID NO:254)
103) GNEQVDKLVSSGIRK (SEQ ID NO:255)
104) KLVSSGIRKVL (SEQ ID NO:256)

NOTE that in the text we call individual peptides in the pool according to the order below each pool. In the case of Fp3, individual peptide Fp3-3 is the same as Peptide 10 (VQLWFTAFSANL) (SEQ ID NO:257) or the third one listed under Pool Fp3.

FIV p24 Overlapping Peptides (subtype-A FIV-Bangston backbone with subtype-B FIV-FC1 (tubes 47-51))

Pool Fp1

1) PIQTVNGAPQYVAL (SEQ ID NO:258)
2) TVNGAPQYVALDPKM (SEQ ID NO:259)
3) APQYVALDPKMVSIF (SEQ ID NO:260)
4) VALDPKMVSIFMEKA (SEQ ID NO:261)

Pool Fp2

5) PKMVSIFMEKAREGL (SEQ ID NO:262)
6) SIFMEKAREGLGGEEV (SEQ ID NO:263)
7) KAREGLGGEEVQLWF (SEQ ID NO:265)

Pool Fp3

8) GLGGEEVQLWFTAF (SEQ ID NO:266)
9) GEEVQLWFTAFSANL (SEQ ID NO:267)
10) VQLWFTAFSANL =Fp3-3 (SEQ ID NO:257)
11) LWFTAFSANLTPTDM (SEQ ID NO:268)

Pool Fp4

12) AFSANLTPTDMATLI (SEQ ID NO:269)
13) NLTPTDMATLIMAA (SEQ ID NO:270)
14) PTDMATLIMAAPGCA (SEQ ID NO:271)

Pool Fp5

15) ATLIMAAPGCAADK (SEQ ID NO:272)
16) IMAAPGCAADKEIL (SEQ ID NO:273)
17) APGCAADKEILDESL (SEQ ID NO:274)

Pool Fp6

18) AADKEILDESLKQL (SEQ ID NO:275)
19) KEILDESLKQLTAEY (SEQ ID NO:276)
20) DESLKQLTAEYDRTH (SEQ ID NO:277)
21) KQLTAEYDRTHPPDGPR (SEQ ID NO:278)

Pool Fp7

22) YDRTHPPDGPRPLPY (SEQ ID NO:279)
23) HPPDGPRPLPYFTAA (SEQ ID NO:280)
24) GPRPLPYFTAAEIM (SEQ ID NO:281)
25) PLPYFTAAEIMGIGL (SEQ ID NO:282)

Pool Fp8

26) FTAAEIMGIGLTQEQQA (SEQ ID NO:283)
27) MGIGLTQEQQAEARF (SEQ ID NO:284)
28) LTQEQQAEARFAPAR (SEQ ID NO:285)

Pool Fp9

29) EQQAEARFAPARM (SEQ ID NO:286)
30) AEARFAPARMQCRAW (SEQ ID NO:287)
31) FAPARMQCRAWYLEA (SEQ ID NO:288)
Pool Fp10
32) RMQCRAWYLEALGKL (SEQ ID NO:289)
33) RAWYLEALGKLAAIK (SEQ ID NO:290)
34) LEALGKLAAIKAK (SEQ ID NO:291)
Pool Fp11
35) ALGKLAAIKAKSPRA (SEQ ID NO:292)
36) LAAIKAKSPRAVQLR (SEQ ID NO:293)
37) KAKSPRAVQLRQGAK (SEQ ID NO:294)
Pool Fp12
38) PRAVQLRQGAKEDY (SEQ ID NO:295)
39) VQLRQGAKEDYSSFI (SEQ ID NO:296)
40) RQGAKEDYSSFIDRL (SEQ ID NO:297)
Pool Fp13
41) KEDYSSFIDRLFAQI (SEQ ID NO:298)
42) DRLFAQIDQEQNTA (SEQ ID NO:299)
43) FAQIDQEQNTAEVKL (SEQ ID NO:300)

Pool Fp14
44) DQEQNTAEVKLYLK (SEQ ID NO:301)
45) EQNTAEVKLYLKQSL (SEQ ID NO:302)
46) AEVKLYLKQSLSIA (SEQ ID NO:303)
47) KLYLKQSLSIANA (SEQ ID NO:304)
Pool Fp15
48) YLKQSLSIANANPDCK (SEQ ID NO:305)
49) LSIANANPDCKRAM (SEQ ID NO:306)
50) ANANPDCKRAMSHLK (SEQ ID NO:307)
Pool Fp16
51) PDCKRAMSHLKPESTL (SEQ ID NO:308)
52) AMSHLKPESTLEEKL (SEQ ID NO:309)
53) LKPESTLEEKLRA (SEQ ID NO:310)
Pool Fp17
54) PESTLEEKLRACQEV (SEQ ID NO:311)
55) LEEKLRACQEVGSPGY (SEQ ID NO:312)
56) RACQEVGSPGYKMQLL (SEQ ID NO:313)

Consensus Subtype-B HIV-1 p24 Overlapping Peptides

Pool Hp1
1) PIVQNLQGQMVHQAI (SEQ ID NO:314)
2) NLQGQMVHQAISPRT (SEQ ID NO:315)
3) QMVHQAISPRTLNAW (SEQ ID NO:316)
4) QAISPRTLNAWVKVV (SEQ ID NO:317)
Pool Hp2
5) PRTLNAWVKVVEEKA (SEQ ID NO:318)
6) NAWVKVVEEKAFSPE (SEQ ID NO:319)
7) KVVEEKAFSPEVIPM (SEQ ID NO:320)
Pool Hp3
8) EKAFSPEVIPMFSAL (SEQ ID NO:322)
9) SPEVIPMFSALSEGA (SEQ ID NO:323)
10) IPMFSALSEGATPQD (SEQ ID NO:324)
Pool Hp4
11) SALSEGATPQDLNTM (SEQ ID NO:325)
12) EGATPQDLNTMLNTV (SEQ ID NO:326)
13) PQDLNTMLNTVGGHQ (SEQ ID NO:327)
Pool Hp5
14) NTMLNTVGGHQAAMQ (SEQ ID NO:328)
15) NTVGGHQAAMQMLKE (SEQ ID NO:329)
16) GHQAAMQMLKETINE (SEQ ID NO:330)
Pool Hp6
17) AMQMLKETINEEAAE (SEQ ID NO:331)
18) LKETINEEAAEWDRL (SEQ ID NO:332)
19) INEEAAEWDRLHPVH (SEQ ID NO:333)

Pool Hp7
20) AAEWDRLHPVHAGPI (SEQ ID NO:334)
21) DRLHPVHAGPIAPGQ (SEQ ID NO:335)
22) PVHAGPIAPGQMREP (SEQ ID NO:336)
Pool Hp8
23) GPIAPGQMREPRGSD (SEQ ID NO:338)
24) PGQMREPRGSDIAGT (SEQ ID NO:339)
25) REPRGSDIAGTTSTL (SEQ ID NO:340)
Pool Hp9
26) GSDIAGTTSTLQEQI (SEQ ID NO:341)
27) AGTTSTLQEQIGWMT (SEQ ID NO:342)
28) STLQEQIGWMTNNPP (SEQ ID NO:343)
Pool Hp10
29) EQIGWMTNNPPIPVG (SEQ ID NO:344)
30) WMTNNPPIPVGEIYK (SEQ ID NO:345)
31) NPPIPVGEIYKRWII (SEQ ID NO:346)
Pool Hp11
32) PVGEIYKRWIILGLN (SEQ ID NO:347)
33) IYKRWIILGLNKIVR (SEQ ID NO:348)
34) WIILGLNKIVRMYSP (SEQ ID NO:349)
Pool Hp12
35) GLNKIVRMYSPTSIL (SEQ ID NO:350)
36) IVRMYSPTSILDIRQ (SEQ ID NO:351)
37) YSPTSILDIRQGPKE (SEQ ID NO:352)
Pool Hp13
38) SILDIRQGPKEPFRD (SEQ ID NO:353)
39) IRQGPKEPFRDYVDR (SEQ ID NO:354)
40) PKEPFRDYVDRFYKT (SEQ ID NO:355)
Pool Hp14
41) FRDYVDRFYKTLRAE (SEQ ID NO:356)
42) VDRFYKTLRAEQASQ (SEQ ID NO:357)
43) YKTLRAEQASQEVKN (SEQ ID NO:358)
Pool Hp15
44) RAEQASQEVKNWMTE (SEQ ID NO:359)
45) ASQEVKNWMTETLLV (SEQ ID NO:360)
46) VKNWMTETLLVQNAN (SEQ ID NO:361)
Pool Hp16
47) MTETLLVQNANPDCK (SEQ ID NO:362)
48) LLVQNANPDCKTILK (SEQ ID NO:363)
49) NANPDCKTILKALGP (SEQ ID NO:364)
Pool Hp17
50) DCKTILKALGPAATL (SEQ ID NO:365)
51) ILKALGPAATLEEMM (SEQ ID NO:366)
52) LGPAATLEEMMTACQ (SEQ ID NO:367)
Pool Hp18
53) ATLEEMMTACQGVGG (SEQ ID NO:368)
54) EMMTACQGVGGPGHK (SEQ ID NO:369)
55) ACQGVGGPGHKARVL (SEQ ID NO:370)

SIVmm251 RT

SRT1

1) PIAKVEPVKVTLK**R** (SEQ ID NO:495)
2) EPVKVTLKPGKV**GP**K (SEQ ID NO:496)
3) KPGKVGPKLKQW**P**L (SEQ ID NO:497)
4) PKLKQWPLSKEKIVA (SEQ ID NO:498)

SR2

5) LSKEKIVALREICEK (SEQ ID NO:499)
6) ALREICEKMEKDGQL (SEQ ID NO:500)
7) KMEKDGQLEEAPPTNPY (SEQ ID NO:501)
8) EAPPTNPYNTPTFAI (SEQ ID NO:502)

SRT3

9) YNTPTFAIKKKDKNK (SEQ ID NO:503)
10) IKKKDKNKWRMLIDF (SEQ ID NO:504)
11) KWRMLIDFRELNRV (SEQ ID NO:505)
12) DFRELNRVTQDFTEV (SEQ ID NO:506)

SRT4

13) VTQDFTEVQLGIPH (SEQ ID NO:507)
14) EVQLGIPHPAGLAKR (SEQ ID NO:508)
15) HPAGLAKRKRITVL (SEQ ID NO:509)

SRT5

16) KRKRITVLDIGDAYF (SEQ ID NO:510)
17) DAYFSIPLDEEFR (SEQ ID NO:511)
18) SIPLDEEFRQYTAF (SEQ ID NO:512)

SRT6

19) EFRQYTAFTLPSV (SEQ ID NO:513)
20) TAFTLPSVNNAEPGK (SEQ ID NO:514)
21) VNNAEPGKRYIYKVL (SEQ ID NO:515)
22) KRYIYKVLPQGWK (SEQ ID NO:516)

SRT7

23) KVLPQGWKGSPAIFR (SEQ ID NO:517)
24) WKGSPAIFQYTMRHV (SEQ ID NO:518)
25) FQYTMRHVLEPFRKA (SEQ ID NO:519)
26) RHVLEPFRKANPDV (SEQ ID NO:520)

SRT8

27) FRKANPDVTLVQYM (SEQ ID NO:521)
28) VQYMDDILIASDRR (SEQ ID NO:522)
29) DILIASDRTDLEHDR (SEQ ID NO:523)
30) RTDLEHDRVVLQLK (SEQ ID NO:524)

SRT9

31) DLEHDRVVLQLKEL (SEQ ID NO:525)
32) LKELLNSIGFSTPEEK (SEQ ID NO:526)
33) GFSTPEEKFQKDPPF (SEQ ID NO:527)
34) KFQKDPPFQWMGYEL (SEQ ID NO:528)

SRT10

35) FQWMGYELWPTKWKL (SEQ ID NO:529)
36) LWPTKWKLQKIEL (SEQ ID NO:530)
37) WKLQKIELPQRETW (SEQ ID NO:531)
38) ELPQRETWTVNDIQK (SEQ ID NO:532)
39) VNDIQKLVGVLNRR (SEQ ID NO:533)

SRT11

40) VGVLNWAAQIYRRR (SEQ ID NO:534)
41) LNWAAQIYPGIKTKH (SEQ ID NO:535)
42) YPGIKTKHLCRLIR (SEQ ID NO:536)
43) KHLCRLIRGKMTL (SEQ ID NO:537)

SRT12

44) LIRGKMTLTEEVQW (SEQ ID NO:538)
45) TLTEEVQWTEMAEA (SEQ ID NO:539)
46) VQWTEMAEAEYEENK (SEQ ID NO:540)
47) EAEYEENKIILSQER (SEQ ID NO:541)

SRT13

48) KIILSQEQEGCYY (SEQ ID NO:542)
49) SQEQEGCYYQEGKPL (SEQ ID NO:543)
50) YYQEGKPLEATVIK (SEQ ID NO:544)
51) PLEATVIKSQDNQW (SEQ ID NO:545)
52) IKSQDNQWSYKIH (SEQ ID NO:546)

SRT14

53) NQWSYKIHQEDKILK (SEQ ID NO:547)
54) HQEDKILKVGKFAKI (SEQ ID NO:548)
55) KVGKFAKIKNTHTNGV (SEQ ID NO:549)

SRT15

56) KNTHTNGVRLLAHVI (SEQ ID NO:550)
57) RLLAHVIQKIGKEAR (SEQ ID NO:551)
58) KIGKEAIVIWGQR (SEQ ID NO:552)
59) WGQVPKFHLPV (SEQ ID NO:553)

SRT16

60) VPKFHLPVERDVW (SEQ ID NO:554)
61) LPVERDVWEQWWTDY (SEQ ID NO:555)
62) WEQWWTDYWQVTWI (SEQ ID NO:556)
63) DYWQVTWIPEWDFI (SEQ ID NO:557)

SRT17

64) TWIPEWDFISTPPLVR (SEQ ID NO:558)
65) STPPLVRLVFNRR (SEQ ID NO:559)
66) RLVFNLVKDPIEGEETY (SEQ ID NO:560)

SIVmm251 p24

Pool Sp1

1) PVQQIGGNYVHLPLSPR (SEQ ID NO:561)
2) GNYVHLPLSPRTLNA (SEQ ID NO:562)
3) SPRTLNAWVKLIEEKK (SEQ ID NO:563)

Pool Sp2

4) LNAWVKLIEEKKFGA (SEQ ID NO:564)
5) IEEKKFGAEVVPGF (SEQ ID NO:565)

Pool Sp3

6) KKFGAEVVPGFQALSEGR (SEQ ID NO:566)
7) FQALSEGCTPYDIR (SEQ ID NO:567)

Pool Sp4

8) EGCTPYDINQMLNCV (SEQ ID NO:568)
9) YDINQMLNCVGDHQA (SEQ ID NO:569)

Pool Sp5

10) DHQAAMQIIRDIINEEA (SEQ ID NO:570)
11) MQIIRDIINEEAADW (SEQ ID NO:571)
12) INEEAADWDLQHPQPA (SEQ ID NO:572)

Pool Sp6

13) DLQHPQPAPQQGQLR (SEQ ID NO:573)

Pool Sp7

14) APQQGQLREPSGSDI (SEQ ID NO:574)
15) REPSGSDIAGTTSSV (SEQ ID NO:575)

Pool Sp8

16) IAGTTSSVDEQIQWM (SEQ ID NO:576)
17) VDEQIQWMYRQQNPI (SEQ ID NO:577)

Pool Sp9

18) MYRQQNPIPVGNIYR (SEQ ID NO:578)
19) NPIPVGNIYRRWI (SEQ ID NO:579)

Pool Sp10

20) RRWIQLGLQKCVRMY (SEQ ID NO:580)
21) LQKCVRMYNPTNIL (SEQ ID NO:581)

Pool Sp11

22) MYNPTNILDVKQGPK (SEQ ID NO:582)

Pool Sp12

23) LDVKQGPKEPFQSYV (SEQ ID NO:583)
24) KEPFQSYVDRFYKSL (SEQ ID NO:584)

Pool Sp13

25) VDRFYKSLRAEQTDA (SEQ ID NO:585)
26) LRAEQTDAAVKNWM (SEQ ID NO:586)

Pool Sp14

27) TDAAVKNWMTQTL (SEQ ID NO:469)

Pool Sp15

28) WMTQTLLIQNANPDCK (SEQ ID NO:587)
29) IQNANPDCKLVLK (SEQ ID NO:588)

Pool Sp16

30) KGLGVNPTLEEMLTAR (SEQ ID NO:589)

Pool Sp17

31) NPTLEEMLTACQGVGGPGQK (SEQ ID NO:590)
32) GVGGPGQKARLM (SEQ ID NO:591)

PEPTIDES FOR MAPPING MAB EPITOPES

Antibody FIV p24 Peptides (FB=Bangston; FC=FC1; FCS= only “PDCK” changed to FC1

Code	Peptide sequence	(aa-mer)	
FB1)	PIQTVNGAPQYVALDPKMVSIFMEKAREGL	(30)	(SEQ ID NO:371)
FB2)	QYVALDPKMVSIFMEKAREGLGGEEVQL	(28)	(SEQ ID NO:372)
FC2)	EVQLWFTAFSANLTPTDMATLIMAAP	(26)	(SEQ ID NO:373)
FB3)	TLIMAAPGCAADKEILDESLKQLTAEYDR	(29)	(SEQ ID NO:374)
FB4)	SLKQLTAEYDRTHPPDGPRPLPYFTAAEIM	(30)	(SEQ ID NO:375)
FB5)	PLPYFTAAEIMGIGLTQEQQAEARFAPARM	(30)	(SEQ ID NO:376)
FB6)	EQQAEARFAPARMQCRAWYLEALGKLAAIK	(30)	(SEQ ID NO:377)
FB7)	LEALGKLAAIKAKSPRAVQLRQGAKEDY	(28)	(SEQ ID NO:378)
FB8)	VQLRQGAKEDYSSFIDRLFAQIDQEQNTA	(29)	(SEQ ID NO:379)
FB9)	FAQIDQEQNTAEVKLYLKQSLSIANANA	(28)	(SEQ ID NO:380)
FB10)	KQSLSIANANAECKKAMSHLKPESTLEEKL	(30)	(SEQ ID NO:381)
FB11)	LKPESTLEEKLRACQEVGSPGYKMQLL	(28)	(SEQ ID NO:382)
FCS12)	FAQIDQEQNTAEVKLYLKQSLSIANAN**PD**CK	(31)	(SEQ ID NO:383)
FCS13)	LSIANANPDCKRAMSHLKPESTLEEKLRA	(29)	(SEQ ID NO:384)

FIV-Bang p24 (30-mer)

Code	Peptide sequence	(aa-mer)	[Hydrophlicity]	
FBO1)	PIQTVNGAPQYVALDPKMVSIFMEKAREGL	(30)	[-0.02]	(SEQ ID NO:371)
FBO2)	PQYVALDPKMVSIFMEKAREGLGGEEVQ	(28)	[-0.30]	(SEQ ID NO:385)
FBO3)	IFMEKAREGLGGEEVQLWFTAFSANLTPTD	(30)	[-0.12]	(SEQ ID NO:386)

FBO4) KAREGLGGEEVQLWFTAFSANLTPTDMA (28) [-0.20] (SEQ ID NO:387)
FCO5) NLTSTDMATLIMSAPGCAADKEILDETLKQ (30) [-0.03] FC1 (SEQ ID NO:388)
FBO6) TLIMAAPGCAADKEILDESLKQLTAEYDRT (30) [-0.18] (SEQ ID NO:389)
FBO7) AAPGCAADKEILDESLKQLTAEYDRTHPPD (30) [-0.83] (SEQ ID NO:390)
FBO8) CAADKEILDESLKQLTAEYDRTHPPDAPRP (30) [-1.08] (SEQ ID NO:391)
FBO9) SLKQLTAEYDRTHPPDAPRPLPYFTAAEIM (30) [-0.64] (SEQ ID NO:392)
FBO10) RTHPPDAPRPLPYFTAAEIMGIGLTQEQQA (30) [-0.56] (SEQ ID NO:393)
FBO11) LPYFTAAEIMGIGLTQEQQAEARFAPARMQ (30) [-0.11] (SEQ ID NO:394)
FBO12) GIGLTQEQQAEARFAPARMQCRAWYLEALG (30) [-0.33] (SEQ ID NO:395)
FBO13) EARFAPARMQCRAWYLEALGKLAAIKAKSP (30) [-0.16] (SEQ ID NO:396)
FBO14) CRAWYLEALGKLAAIKAKSPRAVQLRQGAK (30) [-0.20] (SEQ ID NO:397)
FBO15) KLAAIKAKSPRAVQLRQGAKEDYSSFIDRL (30) [-0.53] (SEQ ID NO:398)
FBO16) RAVQLRQGAKEDYSSFIDRLFAQIDQEQNT (30) [-0.94] (SEQ ID NO:399)
FBO17) EDYSSFIDRLFAQIDQEQNTAEVKLYLKQS (30) [-0.76] (SEQ ID NO:400)
FBO18) FAQIDQEQNTAEVKLYLKQSLSIANANAEC (30) [-0.37] (SEQ ID NO:401)
FBO19) AEVKLYLKQSLSIANANAECKKAMSHLKPE (30) [-0.39] (SEQ ID NO:402)
FBO20) LSIANANAECKKAMSHLKPESTLEEKLRAC (30) [-0.45] (SEQ ID NO:403)
FBO21) KKAMSHLKPESTLEEKLRACQEVGSPGYKM (30) [-0.92] (SEQ ID NO:404)
FBO22) STLEEKLRACQEVGSPGYKMQLL (23) [-0.44] (SEQ ID NO:405)

HIV-1-UCD1 p24 (22-30-mer)

HB1) PVVQNLQGQMVHQPISPRTLNAWVKVVEEK (30) [-0.34] (SEQ ID NO:406)
HB2) QMVHQPISPRTLNAWVKVVEEKAFSPEVIP (30) [-0.13] (SEQ ID NO:407)
HB3) KVVEEKAFSPEVIPMFTALSEGATPQDLNT (30) [-0.12] (SEQ ID NO:408)
HB4) SPEVIPMFTALSEGATPQDLNTMLNTVGGH (30) [-0.00] (SEQ ID NO:409)
HB5) TALSEGATPQDLNTMLNTVGGHQAAMQMLK (30) [-0.19] (SEQ ID NO:410)
HB6) DLNTMLNTVGGHQAAMQMLKETINEEAAEW (30) [-0.43] (SEQ ID NO:411)
HB7) GHQAAMQMLKETINEEAAEWDRLHPVHAGP (30) [-0.75] (SEQ ID NO:412)
HB8) ETINEEAAEWDRLHPVHAGPIAPDQMREPR (30) [-1.12] (SEQ ID NO:413)
HB9) DRLHPVHAGPIAPDQMREPRGSDIAGITST (30) [-0.64] (SEQ ID NO:414)
HB10) IAPDQMREPRGSDIAGITSTLQEQIGWMTN (30) [-0.56] (SEQ ID NO:415)
HB11) GSDIAGITSTLQEQIGWMTNNPPIPVGEIY (30) [-0.09] (SEQ ID NO:416)
HB12) LQEQIGWMTNNPPIPVGEIYKRWIILGLNK (30) [-0.22] (SEQ ID NO:417)
HB13) MTNNPPIPVGEIYKRWIILGLNKIVRMYSP (30) [-0.02] (SEQ ID NO:418)
HB14) KRWIILGLNKIVRMYSPTSILDIRQGPKEP (30) [-0.31] (SEQ ID NO:419)
HB15) IVRMYSPTSILDIRQGPKEPFRDYVDRFYK (30) [-0.72] (SEQ ID NO:420)
HB16) ETINEEAAEWDRLHPVHAGPIAPDQMREPR (30) [-1.12] (SEQ ID NO:413)
HB17) DRLHPVHAGPIAPDQMREPRGSDIAGITST (30) [-0.64] (SEQ ID NO:414)
HB18) IAPDQMREPRGSDIAGITSTLQEQIGWMTN (30) [-0.56] (SEQ ID NO:415)
HB19) GSDIAGITSTLQEQIGWMTNNPPIPVGEIY (30) [-0.09] (SEQ ID NO:416)
HB20) LQEQIGWMTNNPPIPVGEIYKRWIILGLNK (30) [-0.22] (SEQ ID NO:417)
HB21) MTNNPPIPVGEIYKRWIILGLNKIVRMYSP (30) [-0.02] (SEQ ID NO:418)
HB22) KRWIILGLNKIVRMYSPTSILDIRQGPKEP (30) [-0.31] (SEQ ID NO:419)
HB23) IVRMYSPTSILDIRQGPKEPFRDYVDRFYK (30) [-0.72] (SEQ ID NO:420)
HB24) LDIRQGPKEPFRDYVDRFYKTLRAEQASQD (30) [-1.32] (SEQ ID NO:421)
HB25) FRDYVDRFYKTLRAEQASQDVKNWMTETLL (30) [-0.83] (SEQ ID NO:422)
HB26) TLRAEQASQDVKNWMTETLLVQNANPDCKT (30) [-0.79] (SEQ ID NO:423)
HB27) VKNWMTETLLVQNANPDCKTILKALGPAAT (30) [-0.01] (SEQ ID NO:424)
HB28) VQNANPDCKTILKALGPAATLEEMMTACQG (30) [-0.02] (SEQ ID NO:425)
HB29) TLEEMMTACQGVGGPGHKARVL (22) [-0.04] (SEQ ID NO:426)

MATERIALS AND METHODS FOR EXAMPLES 5-11

Study population. Blood from HIV-1 infected subjects was obtained from the University of California at San Francisco (UCSF), the University of South Florida in Tampa, and the University of Florida Center for HIV/AIDS Research, Education and Service (UF CARES) in Jacksonville. These subjects are distributed into three groups according to the length of infection and the anti-retroviral therapy (ART) status (Table 9). The HIV-infected (HIV+) subjects consist of long-term survivors (LTS) who have been infected for more than 10 years and remain healthy without antiretroviral therapy (LTS/ART-); subjects with short-term infection without ART (ST/ART-) and subjects on ART for various amounts of time (ART+). T-cell counts and HIV-1 RNA levels were performed by clinical laboratories at UCSF Medical Center and UF Shands Medical Center (Gainesville, FL). Bloods from HIV seronegative (HIV-) samples were obtained from LifeSouth Community Blood Centers (Gainesville, FL) or randomly selected volunteers at UF. The blood collections were performed according to the policy and protocol approved by the Institutional Review Boards at UF and UCSF and processed in 2-30 hours after collection.

RT overlapping peptides. Overlapping peptides of subtype-B HIV-1UCD1 and subtype-B FIVFC1 RT proteins and selected peptides for epitope mapping were produced initially by SynPep (Dublin, CA) and later by RS Synthesis LLC (Louisville, KY) with similar findings. Four to five consecutive peptides (11-16 aa long with 8-10 aa overlap) were grouped into 21 pools: H1-H21 for HIV and counterparts F1-F21 for FIV. In addition, 9mer and 15-16mer peptides with modified sequences were also synthesized by RS Synthesis LLC and used for peptide epitope mapping as shown in Table 10.

ELISpot assays. Enzyme-linked immunosorbent spot assays (ELISpot) for IFNγ (R&D Systems, Minneapolis, MN) were performed with AIM V medium containing 5% heat-inactivated (56°C, 30 min) human serum as previously described (Abbott *et al.* (2012)). The PBMC from HIV+ subjects were stimulated with either peptide pool (4-5 consecutive peptides per pool at 5 μg per peptide) or individual peptide (15 μg/well). The peptides were 11-16 aa in length with 8-10 aa overlap. The results were analyzed with an ELISpot reader (MVS Pacific LLC, Minneapolis, MN) and adjusted to spot forming units (SFU) per 10^6 cells, after subtraction of the average medium control for each subject. The PBMC from HIV+ subjects were stimulated with T-cell mitogen, phytohemaglutinin A

(PHA, 5 μg/mL), as positive control. At a positive threshold of 70 SFU, HIV- subjects had no substantial IFNγ responses (>50 SFU) to HIV and FIV peptide pools.

Flow cytometry (FACS) for carboxyfluoresein diacetate succinimide ester (CFSE)-proliferation and intracellular cytotoxin staining (ICS). The CFSE-proliferation analysis was performed on PBMC according to the manufacturer's protocol (Invitrogen, Carlsbad, CA) and processed as previously described (Lichterfeld *et al.* (2004)). Modifications consisted of using 2.0-5.0x10^5 CFSE-labeled cells stimulated for 5 days (37°C, 5% CO^2) with 30 μg/well of total peptides in a pool (15 μg/well for individual peptide, Table 10) or 5 μg/mL PHA in AIM V medium containing 25 μg/mL of gentamycin and 10% heat-inactivated human serum. Subsequently these cells were harvested and labeled with the LIVE/DEAD fixable yellow dye (Invitrogen) and then treated 5 min with anti-CD16/CD32 antibody (Biolegend, San Diego, CA) for blocking non-specific binding before phenotype-specific antibodies. The following antibodies were used for the CFSE-proliferation analysis: anti-CD4 APC, anti-CD3 APC-H7, and anti-CD8 Pacific Blue (BD Biosciences, San Jose, CA).

The ICS analysis (Horton *et al.* (2007)) involved stimulating 0.5-1.0x10^6 freshly isolated PBMC for 6 h with the same peptide stimulant and culture conditions as the proliferation analysis in the presence of 1 μg/mL of Golgi transport inhibitor and monensin followed by labeling with LIVE/DEAD fixable yellow dye and then treatment with anti-CD16/CD32 antibody and T-cell phenotypic antibodies. The cells were subsequently fixed and permeabilized with Cytofix/Cytoperm solution (BD Biosciences) before reaction with anti-cytotoxin antibodies. The antibodies consisted of anti-CD3 APC-H7, anti-CD4 BD Horizon V450, and anti-CD8 FITC followed by anti-GrzB Alexa 700 and anti-GrzA PE (all from BD Biosciences), and anti-perforin PerCP (*Abcam*, Boston, MA).

In both analyses, 1.0-2.0x10^4 cells were fixed in phosphate-buffered saline (PBS) containing 2% paraformaldehyde and analyzed on BD LSRII using FACSDIVA Software (BD Biosciences), with a positive threshold of 3% $CFSE^{low}$ for CFSE-proliferation and 1% T cells expressing cytotoxin for ICS. The final value for each subject was derived after subtraction of the subject's medium control and the average value of peptide-stimulated cells from uninfected control subjects.

Statistics. Paired Student t-test with two-tailed distribution (SigmaPlot version 11.0, San Jose, CA) was used to evaluate the statistical differences between the results from two time points in Figure 19. These results were considered statistically different when $p<0.05$.

Example 5—Screening for IFNγ-inducing epitopes on HIV-1 and FIV RT

As a first step towards identifying the CTL-associated reactive sites on HIV-1 and FIV RT proteins, the PBMC from HIV+ subjects and HIV- subjects were screened by ELISpot analysis for IFNγ responses to overlapping RT peptide pools of HIV-1 and FIV. NK cells, CD3+CD4+ T-helper cells, CD3+CD4+ CTLs, and CD3+CD8+ CTLs generally produce IFNγ responses to viral peptides (Abbas *et al.* (2010); Soghoian *et al.* (2012)). In this study, many HIV-1 pools induced IFNγ responses of high magnitudes above the threshold level (≥70 SFU) with the PBMC from HIV+ subjects (Figure 17A) but none with the PBMC from HIV- subjects (data not shown). Therefore, the viral specificity of the IFNγ responses is associated with HIV-1 infection. The average responder frequency for all 21 pools was 25% (range, 4-54%) (Figure 17E). Of all HIV peptide pools screened, pool H11 induced the highest and the most frequent IFNγ responses.

Compared to the HIV peptide-pool responses, the magnitude and the frequency of the IFNγ responses to the FIV peptide pools were much lower in the PBMC from HIV+ subjects (Figures 17B and 17F). The average responder frequency for all 21 pools was 17% (range, 4-69%) (Figure 17F). A noticeable exception was observed with pool F3 which induced the highest and the most frequent cellular responses among the FIV pools (Figures 17B and 17F). PBMC from only five subjects (SF08, J08, J02, J09, TP01) responded to a counterpart pool H3 (grouped data only, Figure 17A), and remarkably PBMC from four of these subjects responded to both F3 and H3 (grouped data only, Figures 17A and 17B). As expected, the PBMC of HIV- subjects had no IFNγ responses to the FIV pools (data not shown). Overall, HIV pools induced much higher and more frequent IFNγ responses than the FIV counterparts except for pool F3. However, only a few HIV and FIV counterparts were detected by the same individual (4-5 responders detected both: H3/F3, H6/F6, H7/F7, H11/F11, H13/F13).

The immune responses observed were present in all three clinical groups (LTS/ART-, ST/ART-, ART+). In addition these groups were not statistically different in terms of cell counts. However, it is noteworthy that 4 of 5 responders to pool H3 are in the ST group which may indicate that this response has been lost in the LTS and ART+ group, possibly due to HIV infection.

Example 6—Screening for T-cell proliferation epitopes on HIV-1 and FIV RTs

The presence of strong T-cell proliferation responses to HIV antigen(s) has been associated with lower viral load and better disease outcome in HIV+ individuals (McKinnon *et al.* (2012)). In the current studies, CD3+CD4+ T cells (hereon CD4+ T cells) from HIV+ subjects (Figures 18A and 18B) had fewer proliferation responses than CD3+CD8+ T cells (CD8+ T cells) to both HIV and FIV pools (Figures 17C and 17D). The CD4+ T cells from only 5 of 26 HIV+ subjects responded to at least one HIV pool, whereas 9 of 26 HIV+ subjects responded to at least one FIV pool (Figures 18A and 18B). In contrast, the CD8+ T cells from 17 of 26 HIV+ subjects responded to the HIV pools, and 22 of 26 subjects responded to the FIV pools (Figures 17C and 17D).

The most striking result was the high magnitude and the high frequency of CD8+ T-cell proliferation to the FIV pools in comparison to HIV pools (Figures 17C and 17D). Furthermore, the average responder frequency to all FIV pools was 17% (range, 0-54%) (Figure 17F), which is higher than the average of 10% (range, 0-24%) observed with the HIV pools (Figure 17E). Only one of the HIV pools (H11) had a responder frequency of >20% (Figure 17E), while the responder frequencies to the six FIV pools (F3, F6, F7, F11, F15, F21) were >20% (Figure 17E). In addition, only a few HIV and FIV counterparts were detected by the same subject (2-4 responders detected both: H3/F3, H6/F6, H14/F14, H15/F15, H17/F17, H19/F19), but 43% (23 of 53) of the total positive proliferation responses to the HIV-1 pools were also positive to FIV counterparts.

The above results support the view that the CD8+ T-cell proliferative responses to FIV pools are more robust or possibly more intact than those to HIV pools. This finding is clearly opposite from the results of the IFNγ studies where the IFNγ responses were stronger against the HIV pools than the FIV pools (Figures 17A and 17B). These conflicting findings may be partially attributed to the difference in the cell types used (PBMC *versus* CD8+ T cells). Moreover, 12 of 15 responders showing CD8+ T-cell

proliferation to the F3 pool also had robust IFNγ responses to the F3 pool (Figures 17B and 17D). These findings indicate that these subjects recognize peptide epitope(s) that induce both responses.

Example 7—The persistence of IFNγ and proliferation responses to selected HIV and FIV peptide pools

Due to the ability of HIV to quickly escape from immunological pressure (Leslie *et al.* (2004); Troyer *et al.* (2009)), the PBMC from IFNγ responders (Figures 17A and 17B) were retested at least one year later for IFNγ responses to peptide pools H6, H11, and F3. The majority of the individuals tested retained positive IFNγ responses at the second time-point to H6 (8 of 11 responders) and to F3 (11 of 14 responders) but fewer to H11 (5 of 14 responders) (Figures 19A). The cells from a few subjects were retested against H6 and F3 for a third time-point and demonstrated the persistence of the IFNγ responses to the F3 pool (4 of 5) but to a lesser extent to the H6 pool (2 of 4) after 3 years (Figure 19A). More importantly, the persistence of the IFNγ responses to the F3 pool demonstrated the reproducibility of this activity even though no IFNγ responses were observed to the HIV-counterpart H3 by those who were tested after 2 years (Table 10, top for H3-3 peptide).

The CD4+ and CD8+ T-cell proliferation responses did not correlate with the IFNγ responses in general as only a low frequency of CD4+ T responses were observed. However, more CD4+ T-cell responses were observed in the production of cytotoxins. The lack of correlation between IFNγ ELISpot and CD8+ T-cell proliferation responses has been described before, with p24 proteins. In this case, the majority of responses (64%) were IFNγ+/proliferation- and only 30% of the responses were IFNγ+/proliferation+ (Richmond *et al.* (2011)). Furthermore, the use of PBMC in the IFNγ analysis may have contributed to the lack of correlation between IFNγ and T-cell proliferation responses. Cells such as NK cells in PBMC are known to be high producer of IFNγ (Caligiuri (2008)) and could have also given the IFNγ responses.

In addition, the CD8+ T-cell proliferation responses to F3 pool (7 of 9 responders) persisted but not to H11 pool (2 of 5) and F6 pool (1 of 4) (Figure 19B). Overall, these results suggest a major loss of H11-specific IFNγ and proliferation responses with maintenance of reactions to the F3 pool. Due to the strong persistent proliferation and

IFNγ responses to the F3 pool, subsequent studies focused on the F3 peptide pool and its five individual peptide/epitopes.

Example 8—Identifying the peptide epitope(s) on F3 region that induces IFNγ and CD8+ T-cell proliferation responses

The finding that 69% and 58% of the HIV+ subjects responded to pool F3 with IFNγ production and CD8+ T-cell proliferation respectively (Figures 17E and 17F) suggested the potential that the F3 region contains multiple epitopes. The F3 peptide pool has five overlapping peptides of 13-15mers (F3-1, F3-2, F3-3, F3-4, F3-5). All ten F3 responders tested 1-2 years later had IFNγ responses to the F3-3 peptide, and one individual each had low IFNγ responses to individual peptides F3-1 and F3-4 (Figure 20A). Furthermore, 4 of 22 F3 responders had IFNγ responses to the counterpart pool H3 in the first year (Figure 17A), but all three of the H3 responders tested on or after the second year lost IFNγ responses to H3 and had no reactivity to any of the individual 13-15mer H3 peptides (Table 10, top; 0/3 IFNγ response to H3-3). As expected, all ten HIV-control subjects had no IFNγ responses to individual peptides of both pools F3 and H3 (data not shown).

Remarkably, the majority of IFNγ responses observed to pool F3 were specific for the F3-3 peptide, and the highest responder frequency of CD8+ T-cell proliferation was observed with F3-3 (5 of 8) as well; slightly lower reactions were noted with F3-2, F3-4, and F3-5 (3 of 8 each) (Figure 20B). F3-3 is therefore the predominant FIV RT peptide that gives both IFNγ and T-cell proliferation responses.

Example 9—Characterization of CTL-associated activities induced by the F3 pool and F3-3 peptide

One of the most important CMI activities needed to control HIV infection is potent cytotoxicity (Betts *et al.* (1999)). Both CD4+ CTLs and CD8+ CTLs against HIV-1 have been detected in HIV+ subjects (McDermott *et al.* (2012)) and in HIV- individuals immunized with a candidate HIV-1 vaccine (de Souza *et al.* (2012)). Although activities to H6, H11, and F6 pools were demonstrated, our focus was on CTL-associated activities to F3 pool (Figures 21A-21C) and its five individual peptides (Figure 21D). 100% (11 of 11) of the F3 responders expressed at least one cytotoxin (GrzA, GrzB, or perforin) in

their CD4+ or CD8+ T cells similar to the 100% (8 of 8) of H11 responders but higher than the 75% (6 of 8) of H6 responders and 50% (3 of 6) of F6 responders. Hence, CTL-associated epitope(s) present on F3 and H11 were recognized by all the subjects tested. These findings suggest that multiple CTL epitopes may reside on each of these regions.

This study showed that all five individual F3 peptides induced GrzA, GrzB, and/or perforin in the CD4+ and/or CD8+ T cells of at least one or more HIV+ subjects tested (Figure 21D). Based on this finding, different CTL-associated epitopes appear to be present within all five of the individual F3 peptides (data not shown).

Example 10—CMI epitopes at H3-3 and F3-3 are conserved among lentiviruses

According to LANL QuickAlign analysis, the H3 pool makes up a stretch of aa that is highly conserved among lentiviruses as it is identical to 47% of the HIV-1 RTs and 7% of the SIV RTs (hiv.lanl.gov/content/sequence/QUICK_ALIGN/QuickAlign.html). AA sequence analysis of all HIV and FIV counterpart pairs determined that H3/F3 had the second highest aa identity of 66.7% (Figure 22A). Furthermore, aa sequence analysis of the individual 13-15mer peptides shows high aa sequence identity and similarity between HIV and FIV (Figure 22B). The HIV and FIV pair with the highest similarity was shown in order of the highest to the lowest: H3-1/F3-1 (92%), H3-2/F3-2 (81%), H3-3/F3-3 (75%), H3-4/F3-4 (71%), and H3-5/F3-5 (71%). Considerable similarities in sequences were observed when the H3 and F3 13-15mer peptides were compared to SIV and CAEV counterpart sequences. Based on aa sequence similarity, both the H3/F3 peptide-pool regions and their counterpart individual peptides are evolutionarily conserved (Figures 22A and 22B).

Due to the consistently higher CMI responses to F3-3 than to the other four individual F3 peptides (Figure 20), subsequent studies focused on F3-3 and its HIV-counterpart H3-3. According to LANL QuickAlign analysis, H3-3 has an 83% and 35% aa identity with various HIV-1 and SIV sequences, respectively. H3-3 and F3-3 peptides have 69% identity and 75% similarity with two gaps (Figure 22B; Table 10, top). Even with such sequence similarity, IFNγ and CD8+ T-cell proliferation responses greatly differed between these peptides (Table 10).

F3-3 differs from the H3-3 used in the current study (row 1 *versus* row 2, Table 10) by lacking one aa (Asp on position 4 of H3-3) and having four aa differences at the

F3-3 positions 5, 9, 11, and 15. The combination of a D4 deletion and three changes at K10, V12, and E16 of H3-3 with aa identical to F3-3 resulted in IFNγ responses approaching F3-3 (Table 10, F3-3m6). The addition of D4 to F3-3 (16mer) (F3-3m2) also resulted in IFNγ responses approaching F3-3, whereas the removal of V16 from F3-3m2, giving 15mer F3-3m1, caused a major loss in IFNγ responses and also a modest loss in CD8+ T-cell proliferation responses. Furthermore, a single aa change at F3-3 positions 9 (M9→K9; F3-3m5), 11 (I11→V11; F3-3m3), or 15 (V15→E15; CAEV & MVV peptide) caused major losses in both IFNγ and CD8+ T-cell proliferation responses. Note that none of the modifications of H3-3 and the peptides tested in Table 10, induced IFNγ or T-cell proliferation responses in the PBMC or the T cells from HIV- control subjects (data not shown).

Peptide F3-3 has high degrees of aa identity to those of ungulate lentiviruses (93%, caprine arthritis-encephalitis virus [CAEV] and Maedi-Visna virus [MVV]) (Table 10). Thus, the F3-3 sequence is greatly conserved among lentiviruses. In this regard, the ungulate peptide counterpart of F3-3, induced IFNγ responses in the PBMC from 1 of 9 F3-3 responders tested (Table 10, top). The above results demonstrate that the F3-3 sequence contains evolutionarily-conserved epitope(s) that induces persistent CMI responses, including strong CTL-associated activity, even when the responses to the counterpart H3-3 are lost.

Example 11

In these studies, the CMI responses by the HIV+ subjects to FIV and HIV RT peptides or peptide pools resulted in three major observations: First, the CD8+ T-cell proliferation responses to FIV pools were more robust with higher frequency of responders than those induced by the HIV pools (Figures 17E and 17F). This observation was unexpected since higher levels of IFNγ responses were observed with HIV pools than with FIV pools. These proliferation responses to the FIV pools, especially to F3, persisted over a longer time period than those to the HIV pools tested (Figure 19B). Thus, the few aa differences between these viruses may be sufficient for the CD8+ T cells to recognize the F3 but not the H3 peptides. In fact, three aa substitutions in the H3-3 aa sequence (V10→I10; K12→M12; E16→V16) with aa identical to F3-3 led to immunological responses more consistent with that of F3-3 (Table 10). This observation clearly supports

our finding that only a few aa changes can substantially alter the responses to a peptide epitope.

The robust CD8+ T-cell responses by the HIV+ subjects to FIV peptide pools suggest that these peptide regions contain evolutionarily-conserved epitopes. Importantly, 23 of 53 (43%) total positive CD8+ T-cell proliferation responses and 40 of 166 (23%) total positive IFNγ responses to HIV pools were also positive for their counterpart FIV pools (Figure 17). This observation suggests that the T-cell response measured by CD8+T-cell proliferation (43%) was more successful at screening for evolutionarily conserved peptide epitopes than by the IFNγ response (23%).

The second observation was the profound and persistent IFNγ and CD8+ T-cell proliferation responses to pool F3 which had more responders than to any HIV pool (Figure 19). The pool F3 induced IFNγ responses in the PBMC from a large number (69%) of HIV+ subjects and CD8+ T-cell proliferation responses in a substantial number (58%) of these subjects. These results suggest the presence of multiple CD8+ T-cell epitopes in the F3 region. One to three F3 responders had IFNγ or CD8+ T-cell proliferation responses to F3-1 and F3-4, and both peptide epitopes induced CTL-associated activities (Figure 21). In fact, the LANL database shows three CTL-associated epitopes (NTPVFAIKK, NK9 (SEQ ID NO:427); NTPVFAIKKK, NK10 (SEQ ID NO:428); and KLVDFRELNK, KK10 (SEQ ID NO:429)) on the counterpart H3. The NK9 and NK10 sequences are identical between FIV and HIV-1 and are found at the carboxy-end of both 13mer peptides F3-1 and H3-1. F3-1 only differs from H3-1 by having tryptophan (W3) instead of tyrosine (Y3) at position 3. This finding suggests that this single aa difference resulted in the CD8+ T-cell proliferation response to F3-1 but not to H3-1. In the case of KK10, this epitope resides on H3-4 and differs by three aa from its direct counterpart on F3-4 (mLiDFRvLNK (MK10) (SEQ ID NO:430); different aa indicated in lower case).

The third major observation was the robust IFNγ (100%, all ten F3 responders tested) and CD8+ T-cell proliferation (62%, 5 of 8) responses to the 15mer peptide F3-3 (Figure 20). These unusually high frequencies of responders to the F3-3 epitope raised a question as to whether more than one CMI epitope resides on F3-3. In this regard, current studies, using modified epitopes, identified three CMI epitopes on F3-3, which were not previously described in LANL: KKKSGKWRMLIDFRV (KV15) (SEQ ID NO:63),

WRMLIDFRV (WV9) (SEQ ID NO:431), and KWRMLIDFR (KR9) (SEQ ID NO:432) (Table 10, bottom). The largest of these epitopes (F3-3; KV15) induce cytotoxin expression, and thus, one or more of them most likely are CTL-associated epitopes. These epitopes are closely related in sequence and evolution to ungulate lentiviruses (Table 10, top). Therefore, these findings indicate that the F3-3 epitopes are also evolutionarily conserved.

The unique example of pools F3/H3 (Figure 22) highlights the existence of evolutionarily conserved HIV RT epitope region that is less immunogenic than its FIV RT counterpart sequence based on pools F3/H3 and peptide F3-3/H3-3 analyses. The selection pressure against HIV in humans may explain the lack of responses against the HIV sequence; the same pressure may not exist in cats against FIV. The use of FIV approach shows that F3-3 region may be a great target for T-cell responses in an HIV vaccine, since both IFNγ and proliferation responses to peptide F3-3 by HIV+ subjects indicate that they have previously encountered such sequence or its variant. The approach of using FIV to identify conserved regions for an HIV vaccine is a tool that compliments most approaches for developing a T-cell-based vaccine as in mosaic vaccines (Corey *et al.* (2010); Barouch *et al.* (2010); Santra *et al.* (2012)). Computational analyses identify potential conserved epitopes that are later tested for relevant biological activity. These analyses have been used to select conserved HIV/FIV sequences such as the one described for HIV/FIV integrase (Sanou *et al.* (2012a)). The current FIV approach simultaneously compares both HIV/FIV epitope sequences and immunological responses.

This cross-recognition of the F3-3 epitope(s) by the HIV+ subjects demonstrates the polyfunctionality of the T-cell subsets tested. Three patterns with either PBMC or T cells were observed: 1) IFNγ production by PBMC (IFNγ/PBMC), CD8+ T-cell proliferation, and CD4+ or CD8+ (CD4+/CD8+) T-cell cytotoxin expression; 2) IFNγ/PBMC and CD4+/CD8+ T-cell cytotoxin expression; and 3) CD8+ T-cell proliferation and CD4+/CD8+ T-cell cytotoxin expression. These observations are important since polyfunctional T-cell epitopes are likely to be associated with an effective HIV vaccine (McDermott *et al.* (2012); Betts *et al.* (2006)). Although current studies have had minimal focus on CD4+ T-cell responses, 2 of 3 F3 responders showing CD4+ T-cell proliferation also had expressed CD8+ T-cell responses to F3-3 (Figure 17D and Figure 18B). Moreover, the CD4+ T cells from substantial numbers of F3 responders had CTL-

associated cytotoxin activities in response to pool F3 (Figure 21). A vaccine is generally administered to HIV-naïve subjects with normal CD4+ T-cell immunity. Therefore, the importance of the CD4+ T-cell responses to F3-3 should be considered when identifying CTL-associated epitopes for an HIV vaccine. The vaccine epitopes that induce both anti-HIV CD8+ and CD4+ T responses are likely to be needed for effective vaccine protection. These studies using FIV RT peptide pools suggest that evolutionarily-conserved immunologic epitopes could be important for an effective HIV vaccine.

Table 9. Population Characteristics

Group	Subject[a]	Age	Gender	Race	HIV[+b]	CD4/μL	CD8/μL	Viral Load[c]
LTS/ART	J01	25	F	Black	11	699	935	Undetectable
	J10	35	F	Black	12	897	659	Undetectable
	J11	21	F	Black	21	564	829	932
	J14	47	M	Hispanic	25	722	1421	6790
	SF01	42	M	White	16	1021	996	Undetectable
	SF02	44	M	White	12	528	394	Undetectable
	SF03	59	M	White	24	567	1040	5500
	SF08	48	M	White	31	529	920	3401
	SF17[d]	56	M	White	11	374	1037	2000
	SF19	40	M	White	12	292	556	40000
	SF23	39	M	White	10	784	1018	3160
	SF24	50	M	White	11	675	213	Undetectable
ST/ART	TP01	19	F	Hispanic	<1	391	583	13400
	TP02	28	M	White	<1	1280	1375	Undetectable
	J02	50	F	Black	9mo	639	1248	710
	J03	27	F	Black/ Hispanic	8mo	368	1254	25700
	J04	22	M	Black	6mo	537	1907	1740
	J05	32	M	Black	2mo	384	2202	691
	J06	28	M	White	6mo	501	1110	134000
	J07	26	F	Black	4mo	448	1306	5120
	J08	19	F	Black/ Hispanic	9mo	323	932	3040
	J09	27	M	White	2mo	482	882	109168
	J12	26	M	Pacific Islander	5mo	352	688	405000
	J13	41	F	Black	2mo	513	632	22100
ART[+]	SF04	55	M	White	28	540	864	Undetectable
	SF07	65	M	White	25	610	2170	Undetectable
	SF16	50	M	White	8	827	1018	Undetectable
	SF18	38	M	White	4	1082	1298	1500
	SF20	53	M	White	23	76	1172	Undetectable
	SF22	48	M	White	13	1205	517	Undetectable
	J15	56	F	Black	17	291	1101	Undetectable
	J16	48	F	White	21	1250	NA[d]	Undetectable

Group	Subject[a]	Age	Gender	Race	HIV+[b]	CD4/μL	CD8/μL	Viral Load[c]
HIV-	NB1	NA[e]	M	Unknown				
	NB2	NA[e]	F	Unknown				
	NB3	NA[e]	F	Unknown				
	N1	58	F	Asian				
	N2	27	M	Black				
	N3	27	F	Hispanic				
	N4	24	M	Asian				
	N5	36	M	Black				
	N6	19	F	White				
	N7	27	F	Black/ Hispanic				

Table 9 Footnote

[a] HIV+ subjects from UF at Jacksonville (J), UCSF (SF), and University of South Florida at Tampa (TP); normal blood from blood bank (NB); normal blood from UF (N).

[b] Number of years of HIV infection or in months (mo).

[c] Virus load shown as copies/mL; undetectable at either <50 or <75.

[d] Subject started ART during the study.

[e] NA: not available. See Materials and Methods for other abbreviations.

Table 10. Variation of H3-3/F3-3 aa sequences and immunological responses

Virus (Subtype)[a]	9mer or 15mer Sequence[b]	SEQ ID NO:	Average SFU of IFNγ [range] (positive/total)[cd]	% $CD8^+$ T Proliferation [range] (positive/total)[cd]
Evolutionary epitopes:				
HIV-1 H3-3	**KKKdStKWRkLvDFR**	165	0 [0] (0/3)	0 [0] (0/1)
FIV F3-3	**KKK SGKWRMLIDFRV**	63	536 [105-2500] (13/13)	5.4 [0-15] (6/9)
HIV-1 (C)	**KKK StKWRkLvDFRe**	433	7 [0-37] (0/9)	0 [0] (0/5)
HIV-1 (A,B,C,D,01_AE)	**KKn StKWRkLvDFRe**	434	0 [0] (0/9)	0.9 [0-4] (1/5)
SIVcpz-*Pts*	**KKKdStKWRkLvDFRe** [e]	435	0 [0] (0/4)	0 [0] (0/4)
CAEV & MVV	**KKK SGKWRMLIDFRe** [f]	436	28 [0-100] (1/9)	0.2 [0-0.8] (0/5)
Modifications of F3-3: [g]				
HIV-1 H3-3	**KKKdStKWRkLvDFR**	165	0 [0] (0/3)	0 [0] (0/1)
FIV F3-3m1	**KKKdSGKWRMLIDFR**	437	11 [0-45] (0/9)	0.7 [0-3.2] (1/5)
FIV F3-3m2	**KKKdSGKWRMLIDFRV** [e]	438	280 [0-1208] (7/9)	2.0 [0-5.6] (2/5)
FIV F3-3m3	**KKK SGKWRMLvDFRV**	439	7 [0-22] (0/9)	0 [0] (0/5)
FIV F3-3m4	**KKK StKWRkLIDFRV**	440	9 [0-60] (0/9)	0.2 [0-0.9] (0/5)
FIV F3-3m5	**KKK SGKWRkLIDFRV**	441	20 [0-52] (0/9)	0.3 [0-1.6] (0/5)
FIV F3-3m6	**KKK StKWRMLIDFRV**	442	251 [20-1254] (7/9)	7.0 [0-29.1] (2/5)
FIV F3-3	**KKK SGKWRMLIDFRV**	63	536 [105-2500] (13/13)	5.4 [0-15] (6/9)
Epitopes on F3-3: [h]				
FIV F3-3 (KV15)	**KKK SGKWRMLIDFRV**	63	536 [105-2500] (13/13)	5.4 [0-15] (6/9)
FIV 3-3 (WV9)	**WRMLIDFRV**	431	278 [0-1295] (8/11)	1.3 [0-4.2] (1/6)
FIV3-3 (KR9)	**KWRMLIDFR**	432	20 [0-70] (1/9)	0 [0] (0/6)

Table 10 footnotes:

[a] Genbank numbers as follows: HIV-1 H3-3 (K03455.1); FIV F3-3 (DQ365597.1); HIV-1 (C) (FJ595343); HIV-1 (A,B,C,D, 01_AE) (AJ313415, HM035584, HQ012309, HQ586068, HE590997); SIVcpz-*Pts* (ACM63211); CAEV (AAG48629.1); MVV (CAC44543); HERV-K (ABA28284).

[b] Lower case letter aa different from FIV F3-3. Many HIV-1 strains have glutamate (E) immediately after the carboxyl end of H3-3.

[c] Used only responders from Figures 17-19; range of IFNγ responses in SFU [range]; positive responses over total tested (positive/total).

[d] Small total participant numbers due to the use of only the F3 or H3 responders who are still positive during the second or third time-point. Only cells from H3 responders were used to test peptide H3-3; while cells from F3 responders were used to test other peptides.

[e] Only 16mer sequences.

[f] Replacing V15 with E15 in modifications F3-3m3 to F3-3m6 resulted in almost total loss of both IFNγ and proliferation responses.

[g] Six modifications of 15-16mer F3-3 sequences (F3-3m1 to F3-3m6) with aa present on H3-3.

[j] Sequence designation shown in parenthesis with the first and the last aa followed by the number of aa.

FIV RT Peptide-Pool F3
NPWNTPVFAIKKKSGKWRMLIDFRVVLNKLTDKGA (SEQ ID NO:443)
NPYNTPVFAIKKKDSTKWRKLVDFRELNKRTQDFW (SEQ ID NO:23)
HIV RT Peptide-Pool H3

FIV RT peptides for Pool F3:
F3-1: NPWNTPVFAIKKK (13 aa) (SEQ ID NO:61)
F3-2: TPVFAIKKKSGKWRM (15) (SEQ ID NO:62)
F3-3: KKKSGKWRMLIDFRV (15) (SEQ ID NO:63)
F3-4: WRMLIDFRVLNKL (13) (SEQ ID NO:64)
F3-5: IDFRVLNKLTDKGA (14) (SEQ ID NO:65)

HIV-1 RT peptides for Pool H3:
H3-1: NPYNTPVFAIKKK (13) (SEQ ID NO:163)
H3-2: TPVFAIKKKDSTKWR (15) (SEQ ID NO:164)
H3-3: KKKDSTKWRKLVDFR (15) (SEQ ID NO:165)
H3-4: KWRKLVDFRELNKR (14) (SEQ ID NO:166)

H3-5: VDFRELNKRTQDFW (14) (SEQ ID NO:167)

Combine sequence of Pool F6 immediately below:
PDYAPYTAFTLPRKNNAGPGRRYVWCSL (SEQ ID NO:444)
FRKYTAFTIPSTNNETPGIRYQYNVLPQGWK (SEQ ID NO:445)
Combined sequence of Pool H6 immediately above:

Peptides in pool F6
F6-1: PDYAPYTAFTLPRK (14) (SEQ ID NO:74)
F6-2: YTAFTLPRKNNA (12) (SEQ ID NO:75)
F6-3: FTLPRKNNAGPGRRY (15) (SEQ ID NO:76)
F6-4: NNAGPGRRYVWCSL (14) (SEQ ID NO:77)

Peptides in pool H6
H6-1: FRKYTAFTIPSI (12) (SEQ ID NO:176)
H6-2: FTIPSTNNETPGIRY (15) (SEQ ID NO:177)
H6-3: NNETPGIRYQYNVL (14) (SEQ ID NO:178)
H6-4: GIRYQYNVLPQGWK (14) (SEQ ID NO:179)

Combine sequence of Pool F7 immediately below:
GRRYVWCSLPQGWVLSPLIYQSTLDNIL (SEQ ID NO:446)
YNVLPQGWKGSPAIFQSSMTKILEPFRKQNPDI (SEQ ID NO:447)
Combined sequence of Pool H7 immediately above:

Peptides in pool F7
F7-1: GRRYVWCSLPQGWVL (15) (SEQ ID NO:78)
F7-2: CSLPQGWVLSPLIY (14) (SEQ ID NO:79)
F7-3: GWVLSPLIYQSTL (13) (SEQ ID NO:80)
F7-4: SPLIYQSTLDNIL (13) (SEQ ID NO:81)

Peptides in pool H7
H7-1: YNVLPQGWKGSPAIF (15) (SEQ ID NO:180)
H7-2: GWKGSPAIFQSSMTK (15) (SEQ ID NO:181)
H7-3: AIFQSSMTKILEPFR (15) (SEQ ID NO:182)
H7-4: MTKILEPFRKQNPDI (15) (SEQ ID NO:183)

Combine sequence of Pool F15 immediately below:
GKMNRQKKKAENTCDIALRACYKIREESIIRIGKEPI (SEQ ID NO:448)
RGAHTNDVKQLTEAVQKIVTESIVIWGKTPKFKLPI (SEQ ID NO:449)
Combined sequence of Pool H15 immediately above:

Peptides for pool F15
F15-1: GKMNRQKKKAENTCDI (16) (SEQ ID NO:117)
F15-2: KKAENTCDIALRACY (15) (SEQ ID NO:118)
F15-3: CDIALRACYKIR (12) (SEQ ID NO:119)
F15-4: ALRACYKIREESIIR (15) (SEQ ID NO:120)
F15-5: KIREESIIRIGKEPI (15) (SEQ ID NO:121)

Peptides for pool H15
H15-1: RGAHTNDVKQLTEAV (15) (SEQ ID NO:219)
H15-2: DVKQLTEAVQKIV (13) (SEQ ID NO:220)
H15-3: LTEAVQKIVTESIVI (15) (SEQ ID NO:221)
H15-4: KIVTESIVIWGKTPK (15) (SEQ ID NO:222)
H15-5: IVIWGKTPKFKLPI (14) (SEQ ID NO:223)

MATERIALS AND METHODS FOR EXAMPLES 12-17

Study population. The blood samples of HIV^+ subjects were obtained from the University of California at San Francisco (UCSF) and the University of Florida Center for HIV/AIDS Research, Education and Service (UF CARES) in Jacksonville using the protocol approved by the Institutional Review Board at UF. HIV^+ subjects consisted of fourteen long-term survivors (LTS) who are not receiving ART, ten subjects with short-term infection (ST) not receiving ART, and eleven HIV^+ subjects receiving ART. Age, gender, and race as well as the viral and immune status of the HIV^+ subjects used in the current study are outlined in Table 11. The blood samples were processed within 48 hours of collection. T-cell phenotyping and HIV-1 load were performed by the clinical laboratories at the UCSF Medical Center and UF CARES. The samples from twenty-two healthy HIV seronegative (HIV^-) subjects were obtained from LifeSouth Community Blood Centers (Gainesville, FL) or from UF.

ELISpot assays. Human enzyme-linked immunosorbent spot assays (ELISpot) (R&D Systems, Cat# XEL285) which measure IFNγ production were performed (Abbott *et al.* 2011). The positive threshold for human IFNγ responses was >50 spot forming units (SFU)/10^6 cells. The final value for each subject was derived after subtracting the result of each HIV^+ subject with the media control followed by subtraction with the average response of the HIV^- subjects which was rarely more than 10 SFU.

Flow cytometry (FACS) for measuring CFSE-proliferation and intracellular cytokine staining (ICS). Carboxyfluorescein diacetate succinimide ester (CFSE)-proliferation analysis was performed according to the manufacturer's protocol (Invitrogen) and processed as previously described (Lichterfeld *et al.* 2004) using the following modification: 2.5-5.0x10^5 CFSE-labeled PBMC stimulated for 4-5 days (37°C, 5% CO_2) with 15-30 μg of peptides in culture media (AIM V medium, 25 μg/mL gentamycin, and 10% heat-inactivated fetal bovine

serum). The ICS analysis was performed as previously described (Horton *et al.* 2007; Pattacini *et al.* 2012).

The antibodies used for the proliferation analysis consisted of anti-CD4 allophycocyanin (APC) anti-CD3 APC-H7, and anti-CD8 Pacific Blue, and those for ICS were anti-CD3 APC-H7, anti-CD4 BD Horizon V450, anti-CD8 FITC, anti-granzyme B (GrzB) Alexa 700, anti-granzyme A (GrzA) PE (BD Biosciences, Cat# 555349, 560176, 558207, 560345, 555366, 560213, 558904), and anti-perforin PerCP (Abcam, Cat# ab86319). Both analyses were performed with BD LSRII and FACSDIVA^{TM} Software (BD Biosciences), using a positive threshold of >1% $CFSE^{low}$ for CFSE-proliferation except for ICS studies with threshold of >0.1% T cells expressing cytotoxin. The final value for each subject was derived after subtracting the result of each HIV^+ subject with the media control followed by subtraction with the average response of the media-control subtracted HIV^- subjects.

Human leukocyte antigen (HLA) analyses. The affinity of peptide binding to HLA was determined by NetMHC version 3.2 for HLA class-I (cbs.dtu.dk/services/NetMHC/), NetMHCII version 2.2 for HLA class-II (cbs.dtu.dk/services/NetMHCII/), and NetCTL version 1.2 for CTL-associated epitopes (cbs.dtu.dk/services/NetCTL/). The LANL database for $CD8^+$ and $CD4^+$ epitopes are based on the HIV-1 HXB2 sequence and identifies the epitope-interacting HLA allele(s).

Statistical analysis. Statistically significant differences between the results from two time points were calculated using a paired Student t-test with a two-tailed distribution (SigmaPlot version 11.0) and were considered statistically significant when $p<0.05$.

Example 12—Determining conserved cell-mediated immune (CMI) peptides based on IFNγ responses

The PBMC from the 31 HIV^+ subjects developed robust IFNγ responses to the full length HIV-1 p24 peptide pools (Figure 23A, 144 total responses), whereas minimal to no responses were observed with the PBMC from HIV-negative (HIV^-) control subjects (range of 0-15 spot forming unit (SFU)). The highest responder frequencies were observed to human p24 peptide pool 3 (Hp3) (18 of 31; 58%) followed by Hp2, Hp10, and Hp15 (11 of 31 each; 35%). A lower number of subjects responded to Hp7 and Hp14 (10 of 31 each; 32%). In addition, PBMC from the HIV^+ subjects produced IFNγ responses of low

magnitudes and frequencies to all FIV p24 peptide pools (Figure 23B, 53 total responses) except for feline p24 peptide pools (Fp)13 and Fp14 which correspond to HIV sequences within Hp14 and Hp15 peptide pools. The highest responder frequencies were observed to Fp14 (11 of 31; 35%) with lower responder frequency to Fp13 (5 of 31; 16%). These findings suggested that these FIV pools contain potential cross-reactive epitopes to HIV. In addition, overlapping SIV p24 peptide pool (Sp) analysis identified a moderate number of responses to the corresponding SIV peptide pool Sp14 (5 of 15; 33%) (Figure 23C), the counterpart to Fp14 and Hp15. These results suggest that evolutionarily conserved, cross-reactive epitope(s) may reside on Hp15, Fp14, and Sp14.

Example 13—Conserved CMI peptides based on T-cell proliferation responses

The $CD8^+$ T cells of the HIV^+ subjects proliferated more frequently and at higher magnitudes to the HIV p24 peptide pools (Figure 24A) than to the $CD4^+$ T cells (Figure 24D) (36 $CD8^+$ T-cell responses vs. 12 $CD4^+$ T-cell responses). The highest frequency of $CD8^+$ T-cell proliferation responses to HIV p24 was against pools Hp1 (6 of 27; 22%) and Hp10 (8 of 27; 30%) followed by Hp9 (4 of 27; 15%) (Figure 24A). The same analysis performed on T cells from healthy HIV^- subjects indicated that their $CD4^+$ and $CD8^+$ T cells did not significantly recognize HIV p24 pools except for the Hp15 pool with a frequency of 42%. All results are shown after subtraction of the average result of the responders for each peptide pool or peptide. $CD8^+$ T cells from 42% (5 of 12) of the HIV^- subjects had a substantial response to the Hp15 peptide pool. The number of HIV^+ responders to Hp15 is low due to a high average result (28% $CFSE^{low}$) of HIV^- responders to Hp15 that were subtracted from the HIV^+ responses (Figure 24A). Except for the Hp15 pool (the counterpart of the Fp14 pool), the Fp9 pool, the Hp15-3 peptide in Hp15 pool, and the Fp9-3 peptide in Fp9 pool with 25-51% of the value before subtraction, none of the other peptide pools or peptides induced substantial $CD8^+$ or $CD4^+$ T-cell proliferation in HIV^- subjects (0-20% of the value before subtraction).

Twelve HIV^+ subjects had $CD8^+$ T-cell proliferation responses to FIV p24 pools (Figure 24B) compared to only five subjects with $CD4^+$ T-cell proliferation responses to the same peptide pools (Figure 24E). Remarkably, substantial $CD8^+$ T-cell proliferation responses were detected in HIV^+ subjects to Fp9 (8 of 27; 30%) and to Fp14 (4 of 27; 15%) (Figure 24B). A lower responder frequency at a lower magnitude was detected in HIV^-

subjects to Fp9 with a minimal response to Fp14. Figure 24B shows HIV^+ response after subtracting the average of HIV^- responders. When compared with the SIV p24 pools, the Fp14-counterpart Sp14 pool, but not the Fp9-counterpart Sp9 pool, was recognized by $CD8^+$ T cells from the HIV^+ cohorts (Figure 24C). In addition, $CD8^+$ T cells from HIV^+ subjects recognized multiple SIV peptide pools at a frequency of 38-54% (Sp1, Sp2, Sp4, Sp10, Sp14, Sp15) (Figure 24C). However, Sp2, Sp4, Sp10, Sp14, and Sp15 were also recognized by $CD8^+$ T cells from HIV^- subjects at a low frequency of 20-30%. Figure 24C shows the HIV^+ response after subtracting the average of HIV^- responders. Notably, both the Sp1 pool and its counterpart Hp1 pool were recognized by the $CD8^+$ T cells from HIV^+ subjects (Figures 24A and 24C).

Among the FIV peptide pools, the Fp9 pool induced strong $CD8^+$ T-cell proliferation responses but few IFNγ responses, while the Fp14 pool induced both IFNγ and $CD8^+$ T-cell proliferation responses (Figures 23B and 24B). As a result, subsequent studies focused on the Fp14 and Fp9 peptide pools and their HIV counterparts, Hp15 and Hp10, respectively.

Example 14—The persistence of IFNγ responses to Fp14 and $CD8^+$ T-cell proliferation to Fp9

PBMC from 8 of 10 (80%) HIV^+ subjects who initially responded to the Fp14 pool retained the IFNγ response for the duration of the 2-yr study period while 3 of 7 tested continued to respond in the 4th yr (Figure 25A, left graph). Although there was no statistical difference between the IFNγ responses during the 1st yr and the 2nd yr of the seven HIV^+ subjects monitored (t1 and t2, p=0.39), a statistically significant decrease in IFNγ response was detected when the levels were compared between the 2nd yr and 4th yr (t2 and t3, p=0.035) and between the initial time point and the 4th yr (t1 and t3, p=0.014). Similarly, the PBMC in 6 of 8 (75%) initial responders to Hp15, the counterpart for Fp14, remained responsive to Hp15 through the 2nd yr but showed a substantial declining trend in the magnitude of proliferation by the 4th yr (Figure 25A, right graph).

$CD8^+$ T cells from 7 of 9 (78%) initial Fp9 responders retained T-cell proliferation responses to Fp9 during the 2-yr monitoring period (Figure 25B, left graph). Two of the seven subjects responding to the Fp9 pool (SF17, SF19) were treated with ART during or shortly after the 2nd yr time point (Table 11) leaving four subjects on ART and four subjects not on ART by yr 4. Three of 4 subjects on ART (SF17, SF19, SF20) maintained stable or

increased levels of proliferation responses to Fp9, while one subject (SF18) continued to remain non-responsive. This finding suggests that the magnitude of response to Fp9 can improve in subjects (SF19, SF20) undergoing ART. In comparison, only 3 of 5 (60%) initial responders retained activity against Hp10, the counterpart of Fp9, through yr 2 (Figure 25B, right graph). This response declined in magnitude by the 4th yr. A large majority of these responders had either a major decrease or a loss of response to the Hp10 peptide pool, while the response to pools Fp9, Fp14, and Hp15 persisted for at least 2 yr and some for as long as 4 yr. The Fp9 and Hp10 pools have very little sequence similarity (Table 12), and the response to Fp9, but not Hp10, was retained over time (Figure 25B). Therefore, subsequent studies focused on the Fp9, Fp14, and Hp15 peptide pools. Due to the high frequency and magnitude of the response, Fp9 was selected for epitope mapping.

Example 15—Identifying the p24 epitope(s) that induce CMI responses

The HIV Hp15 pool has three well-established $CD8^+$ CTL epitopes described in LANL database that are present within the Hp15-1a, Hp15-1c, and Hp15-2/3a peptides (Table 12). These CTL epitopes have high sequence similarity to FIV Fp14-1b, Fp14-1a, and Fp14-3/4f respectively (Table 12). Furthermore, SIV Sp14-1b and Sp14-1a have sequence similarity to their direct counterparts Hp15-1a/Fp14-1b and Hp15-2/3a/Fp14-3/4f. Hence, these peptide epitopes show moderate to high conservation between species-specific lentiviruses.

Three to four overlapping 13-15mer peptides constitute each of the peptide pools Fp9 (Fp9-1, Fp9-2, Fp9-3), Fp14 (Fp14-1, Fp14-2, Fp14-3, Fp14-4), and Hp15 (Hp15-1, Hp15-2, Hp15-3). Fp9-3 and Hp10-3 have an aa sequence similarity of 29% and identity of 12% with four single aa differences due to gaps (Table 12). This low degree of sequence similarity and identity further supports the concept that epitope(s) on Fp9 are most likely not in the same location as those on Hp10. The analysis of individual 13-15mer peptides in the Fp9 pool indicates that the $CD8^+$ T cells of the Fp9 responders proliferate predominantly in response to Fp9-3 (6 of 7) and to a lesser extent to Fp9-2 (3 of 7) (Figure 26C). Based on this result, the 15mer Fp9-3 peptide was further evaluated to map specific proliferative epitope(s) using shorter (9mer) overlapping peptides.

When compared to Fp9 and Fp10 pools, Fp14 and Hp15 pools have a higher aa sequence similarity (65%) and identity (35%) with one aa difference due to a gap (Table 12).

Based on aa sequence alignment analysis, the approximate counterpart for Hp15-1 and Hp15-2 peptides are Fp14-1 and Fp14-2 peptides respectively, whereas the Hp15-3 peptide contains regions that overlap both Fp14-3 and Fp14-4 peptides. Smaller regions have more similarity between Fp14-1 and Hp15-1 peptides (Table 12, section D) and between Fp14-4 and Hp15-3 peptides (Table 12, section B). PBMC from Fp14 responders had substantial IFNγ responses to peptide Fp14-3 (6 of 9 responders) followed by peptides Fp14-1 and Fp14-4 (both 3 of 9) (Figure 26A). The majority of Hp15 responders had substantial IFNγ responses to Hp15-1 (7 of 9) and fewer responses to Hp15-3 (5 of 9) (Figure 26B). Thus, Fp14-3 and Hp15-1 contain epitopes that induce significant IFNγ responses. These peptides are not counterpart FIV and HIV peptides based on aa sequence analysis and therefore are not expressing common epitope(s). However, PBMC from three LTS responded to both Fp14-3 and its counterpart Hp15-3, indicating a conserved CMI epitope within these peptides (Figures 26A and 26B; bars with *).

When specific epitope analyses of Fp9 and Fp14 regions were performed, two 9mer peptides (Fp9-3c and Fp9-3d) of the Fp9 region, differing by a single aa in carboxyl-end or amino-end, provided the highest frequency of a $CD8^+$ T-cell proliferation response (5/6 of 9) (Table 13) but at a low magnitude (<13% $CFSE^{low}$) (Figure 27). Proliferation responses to Fp9-3c and Fp9-3d peptides were much lower than the levels of proliferation observed with the Fp9 pool (Figure 27). Furthermore, this result is in stark contrast to the high frequency of responders (3 of 6, 50%) and the higher levels (average magnitude of 14% $CFSE^{low}$) of $CD8^+$ T-cell proliferation to the 15mer Fp9-3 peptide (Figure 27A). Thus, the 15mer, but not the 9mer Fp9-3 peptides, appears to contain the epitope that induces the bulk of the proliferation responses of both $CD8^+$ and $CD4^+$ T cells (Figures 27A and 27C).

Similarly specific epitope analysis of the Fp14-3 region with an overlap with the Fp14-2 and Fp14-4 regions determined that a higher frequency of HIV^+ subjects respond to epitopes in Fp14-3 (Fp14-3d) and Fp14-4 (Fp14-3/4f, overlapping both Fp14-3 and Fp14-4) more than in Fp14-2, based on both $CD8^+$ T-cell proliferation and IFNγ responses (Figures 27B and 27F). Since the shorter sequence of 10mer Fp14-3/4f also resides in the larger 13mer Fp14-3d sequence (Table 13), it is still possible that both contain the same epitope (*i.e.*, Fp14-3/4f). This observation is supported by in silico analysis using the HLA algorithm where the predicted HLA A2 supertype is common among all four responders for both Fp14-

3d and Fp14-3/4f (Table 14). Thus, Fp14-3/4f contains the major epitope residing in Fp14-3 and Fp14-4 that is identified by the HLA A2 supertype.

Example 16—Characterization of CTL-associated activity induced by Fp9, Fp14, and Hp15 peptides

In intracellular staining (ICS) analysis for cytotoxins, both $CD8^+$ and $CD4^+$ T cells expressed granzyme B (GrzB) most consistently in response to all three peptide pools tested (Fp9, Fp14, Hp15) (Figure 28A). 40% (2 of 5) HIV^+ subjects had $CD8^+$ T cell GrzB expression to individual peptide pool Fp9, whereas 80% (4 of 5) responded to Fp14, and 80% (4 of 5) to Hp15 (Figure 28A). Notably, GrzB was expressed by both $CD4^+$ and $CD8^+$ T cells from the same individuals although the overall expression magnitude of GzB, but not the frequency, was lower in the $CD4^+$ T cells (Figure 28B). A few of the subjects who did not have GrzB responses did demonstrate either GrzA or perforin expression (Figure 28A). When individual 13-15mer peptides from each pool were examined, $CD8^+$ T cells from all five HIV^+ subjects responded to Fp14-3, Fp14-4, and Hp15-1, whereas up to four HIV^+ subjects responded to Fp9-3 with the production of one or more cytotoxins. Hence, the majority of the Fp9, Fp14, and Hp15 peptides induced expression of one or more cytotoxins.

The short (9-13mer) peptides of Fp9-3, Fp14-3, and Fp14-4 from previous IFNγ and proliferation studies as well as a few additional short peptides (Table 13) were further tested with T cells from short-term HIV-infected subjects not on ART (ST/ART^-) for production of cytotoxins and expression of CD107a (Figures 28C and 28D), which are commonly expressed by CTLs. These short 9-13mer epitopes were produced based on the CTL algorithm of NetCTL 1.2 and HLA algorithm of NetMHC 3.4. The shortest peptides that predicted the highest $CD8^+$ T-cell responder frequency were 9mer peptides Fp9-3c (RMQCRAWYL) (SEQ ID NO:451) and Fp9-3d (ARMQCRAWY) (SEQ ID NO:452), 10mer peptide Fp14-3/4f (KLYLKQSLSI) (SEQ ID NO:453), and 13mer peptide Fp14-3d (AEVKLYLKQSLSI) (SEQ ID NO:454) (Table 13). Peptides Fp9-3, Fp9-3c and Fp9-3d induced very little cytotoxin expression in $CD4^+$ T cells (Figure 28C). Among these peptides, Fp9-3 and Fp9-3d induced more cytotoxins in $CD8^+$ T cells from a slightly larger number of ST/ART^- subjects (Figure 28D). In comparison, Fp14-4 and Fp14-3/4f peptides followed by Fp14-1b and Fp14-3/4e induced a high frequency of cytotoxin expression in both $CD4^+$ and $CD8^+$ T cells from a majority of ST/ART^- subjects (Figures 28C and 28D).

Remarkably, the Fp14-4 and Fp14-3/4f peptides had a higher number of cytotoxin responses and a higher frequency of responders for both $CD4^+$ and $CD8^+$ T cells than their counterparts on HIV (Hp15-3; Hp15-2/3a) and SIV (Sp14-1; Sp14-1a).

When NetCTL and NetMHC predictions were compared to the responders' HLA class-I supertype(s), four responders to peptide Fp9-3c had the predicted responder HLA supertype A2 (Table 14). Three of them also had an additional HLA supertype (A1, A3, B27, or B62) predicted to have a strong binding affinity to Fp9-3c. The same analysis performed on Fp9-3d determined that 3 of 4 responders possessed supertype B44 while one responder had HLA supertype A1. Both supertypes are predicted to have a strong binding affinity to Fp9-3d. Similarly, Fp14-3/4f showed supertype A2 as the common HLA supertype correlating with all four responders. Moreover, three more subjects had additional HLA supertypes (A1, B27, or B58) with strong predicted binding affinity for Fp14-3/4f (Table 14). Hence, the ICS and the combined NetCTL/NetMHC analyses support the presence of $CD8^+$ T-cell epitopes on Fp9-3, Fp14-3, and Fp14-4 peptides.

Example 17

Based on IFNγ ELISpot and CFSE-proliferation analysis, the PBMC and T cells from HIV^+ subjects (Table 11) identified at least two cell-mediated immune (CMI) peptide epitopes in the FIV p24 pools Fp9 and Fp14 that could serve as potential T-cell immunogens (Figures 23 and 24). These peptides were effective in the majority of subjects that had a corresponding in silico predicted HLA and did not induce substantial responses in $CD4^+$ T cells from HIV^- subjects. Peptide pool Fp14 induced robust IFNγ production with a high frequency of responders (32%) and moderate $CD8^+$ T-cell proliferation responses (Figures 23B and 24B). In contrast, peptide pool Fp9 induced robust $CD8^+$ T-cell proliferation with a high frequency of responders (26%) and no IFNγ responses. Most notably, the immune activity induced by the Fp9 and Fp14 peptide pools was highly reproducible and persisted for up to 4 years (Figure 25).

Unexpectedly, SIV p24 pools induced more $CD4^+$ and $CD8^+$ T-cell proliferation responses than the corresponding HIV p24 pools in HIV^+ subjects (Figures 24A and 24C). Moreover, the attenuated $CD4^+$ T-cell proliferation responses to the SIV pools were highly correlative to $CD8^+$ T-cell proliferation responses (Figure 24F). In some cases, these $CD4^+$ T cells could be more sensitive to HIV-1 infection (see below). However, possibly the $CD4^+$ T

cells that are cross-reactive to these peptides persist in HIV^+ subjects and can perhaps be stimulated by these conserved epitopes to enhance the $CD8^+$ T-cell responses against HIV.

In addition to high aa sequence similarity (Table 12), the peptide pools Hp15 and Fp14 induced IFNγ responses, notably, only in PBMC from HIV^+ subjects (Figures 26A and 26B). Furthermore, Fp14-3 and its HIV counterpart, Hp15-3, had a high frequency of IFNγ responders. When small 9-13mer peptides from the Fp14 region were evaluated (Table 13), two overlapping peptides within Fp14-3 and Fp14-4 (Fp14-3d, Fp14-3/4f) showed high $CD8^+$ and $CD4^+$ T-cell proliferation responses, low IFNγ responses in HIV^+ subjects (Figures 27B, 27D, 27F) and elicited no response in HIV^- subjects (average of <10 SFU). Thus, the epitope(s) present in peptide pools Hp15 and Fp14 are specific and likely to be evolutionarily conserved.

Peptide analysis of the Fp9 region gave a high frequency of responders measured by $CD8^+$ T-cell proliferation to peptides Fp9-3c and Fp9-3d but higher $CD8^+$ T-cell proliferation responses to the 15mer peptide Fp9-3 (Table 13, Figures 27A and 27C). One concern regarding the Fp9-3 peptide is its ability to elicit non-specific $CD8^+$ and $CD4^+$ T-cell proliferation (mitogenic effect); mitogenic stimulation can serve as an activation signal and could enhance HIV-1 infection (Spina *et al.* 1997; Stevenson *et al.* 1990). However, Fp9-3 peptide is not a classical T-cell mitogen (compared to PHA and concanvalin A) because it does not induce IFNγ in T cells from either HIV^+ or HIV^- subjects (Figure 27E). IFNγ production in some cases could enhance HIV-1 infection (Yamamoto *et al.* 1986; Roff *et al.* 2014). A percentage of $CD8^+$ T cells from HIV^- subjects proliferated in response to the Fp9 pool and the 15mer Fp9-3 peptide but not to Fp9-3c and Fp9-3d 9mer peptides. These less mitogenic peptides have a strong algorithmic prediction for $CD8^+$ T-cell activity with the most common HLA supertypes A2 and B44 (Table 14) (Marsh *et al.* 2000). Therefore, the less mitogenic Fp9-3c and Fp9-3d peptides are likely better candidates as vaccine immunogens.

In this report, the CTL epitopes Hp15-1c, Hp15-1a, and Hp15-2/3a were further evaluated for cytotoxin expression along with their counterpart in FIV Fp14-1a, Fp14-1b, and Fp14-3/4f, respectively (Figures 28C and 28D). In these studies, the HIV peptides and their FIV counterparts, Hp15-1c/Fp14-1a (blue box), Hp15-1a/Fp14-1b (purple box), and Hp15-2/3a/Fp14-3/4f (red box) had high cytotoxin and CD107a expression. The respective SIV

counterparts Sp14-1c, Sp14-1b, and Sp14-1a had a slightly lower number of responses than either their FIV or HIV-1 counterpart (Figures 28C and 28D).

The CTL epitope within the Hp15-1 peptide described in LANL database is predicted to bind strongly to HLA supertype B44 (Kiepiela *et al.* 2007). The B44 supertype is associated with a lower incidence of HIV disease progression in study subjects in South Africa, Botswana, and Zimbabwe (Leslie *et al.* 2010; Carlson *et al.* 2012). The inducers of $CD8^+$ T-cell proliferation in HIV^- subjects are epitopes within Hp15-3 and, to a lesser extent Hp15-2, but not in the Hp15-1 peptide (Figure 29). Thus, two known CTL epitopes within Hp15-1c and Hp15-1a elicited substantial levels of cytotoxin and CD107a expression but not as high as the well characterized epitope Hp15-2/3a. Hp15-2/3a induced expression in both $CD4^+$ and $CD8^+$ T cells and did not stimulate T cells from HIV^- subjects. As a result, it should be a better candidate for use as a vaccine immunogen.

Among the three peptides in Fp9 pool, the 15mer Fp9-3 had the highest frequency of responders expressing one or more cytotoxins by ICS analysis (Figure 28A and 28D). Based on NetCTL analysis, this peptide can mediate $CD8^+$ T-cell activity by expressing peptide-specific cytotoxin(s) and using multiple HLA supertypes (A2, B7, B8, B27, B62). This finding makes it a strong candidate as a HIV-1 vaccine immunogen (Table 14). Similarly, the epitopes in Fp14-4 and lesser extent in Fp14-3 (Figure 28) had the highest frequency of responders expressing cytotoxin(s) with high binding affinity for supertypes A2 and/or B44 (Table 14). Since the HLA B44 supertype is associated with either control of HIV infection and/or slow progression to AIDS (Tang *et al.* 2011; Zhang *et al.* 2013; Goulder and Walker 2012) and the HLA A2 supertype is associated with low HIV transmission (MacDonald *et al.* 2001a; MacDonald *et al.* 2001b; Liu *et al.* 2003), targeting these supertypes are likely beneficial in the development of an effective vaccine. In addition, a recent study correlated HLA A2 alleles with vaccine efficacy in the RV144 HIV vaccine trial and highlighted the importance of HLA allotypes in developing an effective HIV vaccine (Gartland *et al.* 2014). Notably, the current observations indicate that the cross-reactive peptides Fp9-3, Fp9-3c, Fp9-3d, Fp14-3d, and Fp14-3/4f induce CMI responses in HIV^+ subjects and are predicted to bind with highly prevalent HLA supertypes A2 and/or B44 (Table 14) (Marsh *et al.* 2000; Gonzalez-Galarza *et al.* 2011; Allele Frequency Net Database, allelefrequencies.net, accessed October 2, 2014).

Previously, we described evolutionarily conserved $CD8^+$ T-cell epitopes on the FIV reverse transcriptase (Sanou *et al.* 2013). In the current study, we have identified cross-reactive p24 epitopes that are found in both HIV and FIV peptide sequences. These results support the existence of an evolutionary lineage among essential proteins of inter-species lentiviruses. Being conserved, these sequences are most likely essential for viral fitness, and thus less likely to mutate (Sanou *et al.* 2012b).

In summary, by evaluating IFNγ production, CFSE proliferation, and ICS expression in both HIV^+ and HIV^- subjects (Figure 29), we can conclude that the large 13-15mer peptides, HIV Hp15-1 and FIV Fp14-4, and small 9-10mers Hp15-2/3a, Fp14-1b, Fp14-3/4e, and Fp14-3/4f induce robust CMI responses without mitogenic stimulation. Furthermore, since the Fp9 and Fp14 epitopes possess polyfunctional activity (a combination of IFNγ, T-cell proliferation and/or cytotoxin responses), they also merit consideration as potential immunogens for inclusion in an effective HIV-1 vaccine (de Souza *et al.* 2012; Almeida *et al.* 2007). Selectively targeting these conserved sequences and monitoring non-mitogenic, T-cell specific responses allow the identification of conserved FIV, HIV, and SIV immunogenic peptides that could be included in an HIV vaccine for prophylaxis and immunotherapy.

Table 11. Description of HIV$^+$ Population

Group	Subject [a]	Age	Gender [b]	Race	HIV$^+$ [c]	CD4/μL	CD8/μL	Virus Load [d]
LTS/ART-	SF01	42	M	White	16	1021	996	Undetectable
	SF02	44	M	White	12	528	394	Undetectable
	SF03	59	M	White	24	567	1040	5500
	SF05	49	M	White	22	483	1242	Undetectable
	SF08	48	M	White	31	529	920	3401
	SF17 [ef]	56	M	White	11	374	1037	2000
	SF19 [ef]	40	M	White	12	292	556	40000
	SF21	43	M	White	15	800	1375	3584
	SF23 [f]	39	M	White	10	784	1018	3160
	SF24 [f]	50	M	White	11	675	213	Undetectable
	J01	25	F	Black	11	699	935	Undetectable
	J10	35	F	Black	12	897	659	Undetectable
	J11	21	F	Black	21	564	829	932
	J14	47	M	Hispanic	25	722	1421	6790
ST/ART-	J02 [f]	50	F	Black	9mo	639	1248	710
	J03	27	F	Black/Hispanic	8mo	368	1254	25700
	J04	22	M	Black	6mo	537	1907	1740
	J05	32	M	Black	2mo	384	2202	691
	J06 [f]	28	M	White	6mo	501	1110	134000
	J07	26	F	Black	4mo	448	1306	5120
	J08	19	F	Black/Hispanic	9mo	323	932	3040
	J09 [f]	27	M	White	2mo	482	882	109168
	J12 [f]	26	M	Pacific Islander	5mo	352	688	405000
	J17	51	F	Black	2mo	375	1271	2140
ART+	SF04	55	M	White	28	540	864	Undetectable
	SF07	65	M	White	25	610	2170	Undetectable
	SF13	52	M	White	25	304	372	Undetectable
	SF16	50	M	White	8	827	1018	Undetectable
	SF18 [f]	38	M	White	4	1082	1298	1500
	SF20 [f]	53	M	White	23	76	1172	Undetectable
	SF22	48	M	White	13	1205	517	Undetectable
	J16	48	F	White	21	1250	NA [g]	Undetectable
	J22	36	F	Black	15	391	490	Undetectable
	J23	50	F	Black	16	949	1261	Undetectable
	J24	41	M	Black	23	202	1192	Undetectable

Table 11 Footnotes:

[a] SF prefix, HIV$^+$ subject from the University of California, San Francisco. J prefix, HIV$^+$ subject from the University of Florida at Jacksonville; for definition of subjects, see legend to Figure 23. The results for virus load and CD4/CD8 T-cell counts are from the 1st sample obtained from patients (yr 1).

[b] M, male; F, female.

[c] Duration of known HIV infection (yr).

[d] Virus loads are shown as RNA copies/mL; undetectable ≤75 RNA copies/mL.

[e] HIV^+ subject who was on ART starting at or shortly after yr 2.

[f] Subjects monitored for 4 yr (Figure 25).

[g] NA, not available.

Table 12. Fp9/Fp9-3, Fp14/Fp14-3, and their counterpart aa sequences and CMI responses

Peptide Pool & Individual Peptide [a]	AA Sequence (with gap) [b]	SEQ ID NO.	Compared Sequences [b] *Similarity (Identity) [gap]*
A. Hp10/Hp10-3 vs. Fp9/Fp9-3:			
Hp10 Pool	EQIGWMTNNPPIPVGEIYKRWII	455	Hp10 & Fp9: *36% (16%) [5]*
	** ---:: *. : * :--		
Fp9 Pool	EQQ---AEARFAPARMQCRAWYLEA	456	
Hp10-3	NPPIPVGEIYKRWII	346	Hp10-3 & Fp9-3: *29% (12%) [4]*
	-- *. : * :--		
Fp9-3	FAPARMQCRAWYLEA	288	
B. Hp15/Hp15-3 vs. Fp14/Fp14-3/Fp14-4:			
Hp15 Pool	RAEQASQEVKNWMTETLLVQNAN	457	Hp15 & Fp14: *65% (35%) [1]*
	** : *** ::.::* : **-		
Fp14 Pool	DQEQNTAEVKLYLKQSLSIANA	458	
Hp15-3	VKNWMTETLLVQNAN	361	Hp15-3 & Fp14-3: *53% (18%) [5]*
	--** ::.::* : ---		
Fp14-3	AEVKLYLKQSLSIA	303	
Hp15-3	VKNWMTETLLVQNAN	361	Hp15-3 & Fp14-4: *67% (27%) [2]*
	-* ::.::* : **-		
Fp14-4	KLYLKQSLSIANA	304	
C. 9mer Peptides of Fp14-3/4f vs. Hp15-2/3a vs. Sp14-1c:			
Fp14-3/4f	KLYLKQSLS	459	Hp15-2/3a & Fp14-3/4f: *70% (20%) [2]*
	-* ::.::*-		
Hp15-2/3a	VKNWMTETL	460	Hp15-2/3a & Sp14-1a: *100% (89%) [0]*
	******:**		
Sp14-1a	VKNWMTQTL	461	
D. 10mer Peptides of Fp14-1a/b vs. Hp15-1c/a vs. Sp14-1c/b:			
Fp14-1a	QEQNTAEVKL	462	Hp15-1c & Fp14-1a: *60% (50%) [0]*
	** : ***		
Hp15-1c	AEQASQEVKN	463	Hp15-1c & Sp14-1b: *50% (33%) [4]*
	---*:. ****--		
Sp14-1b [c]	QTDAAVKNWM	464	
Sp14-1c	TDAAVKNWMT	465	
Fp14-1b	QNTAEVKLYL	466	Hp15-1a & Fp14-1b: *70% (40%) [0]*
	* : *** ::		
Hp15-1a	QASQEVKNWM	467	Hp15-1a & Sp14-1b: *80% (60%) [0]*
	*:. *****		
Sp14-1b [c]	QTDAAVKNWM	468	

5

Table 12 Footnotes:

[a] Peptide pools are not hyphenated (*e.g.*, Fp9) while the individual large peptides have a hyphen followed by a number (*e.g.*, Fp9-3) indicating the number of the overlapping individual peptide starting from amino-end.

[b] Alignments denote identical amino acids (aa) as (*), aa with most similarity based on charge, polarity, acid/base, and hydrophilicity/hydrophobicity as (:), those with some similarity as (.), and each gap with a (-). The internal gaps are due to best alignment and the external gaps are due the length of the selected peptide or peptide pool. The percentage of aa sequence similarity and identity was determined from these alignment criteria.

[c] Note that Sp14 pool is a single 13mer peptide Sp14-1 (TDAAVKNWMTQTL) (SEQ ID NO:469). As a result, Sp14-1c, the counterpart SIV Sp14-1 peptide for HIV Hp15-1 peptide, is a 10mer and did not include the first three aa (AEQ). Whereas Sp14-1b is a 10mer with glutamine (Q) added to amino-end and threonine (T) deleted from the carboxyl-end rather than a sequence of Sp14-1c.

Table 13. 9-13mer T-cell epitope mapping of Fp9 and Fp14 sequences using responders to Fp9 or Fp14

Peptide Code [a] (No. of aa) [b]	SEQ ID NO.	Peptide Sequence [a]	$CD8^+$ T-cell Responder Frequency (%) [c]	$CD4^+$ T-cell Responder Frequency (%) [c]
Fp9 Peptide Pool				
Fp9-1 (13)	286	**EQQAEARFAPARM**	1/7 (14)	0/7 (0)
Fp9-1/2a (9)	470	EARFAPARM	5/9 (56)	0/9 (0)
Fp9-2 (15)	287	**AEARFAPARMQCRAW**	3/7 (43)	1/7 (14)
Fp9-2/3b (9)	471	APARMQCRA	3/9 (33)	0/9 (0)
Fp9-3 (15)	288	**FAPARMQCRAWYLEA**	6/7 (86)	1/7 (14)
Fp9-3c (9)	451	RMQCRAWYL	**5/9 (56) [d]**	**4/9 (44) [d]**
Fp9-3d (9)	452	ARMQCRAWY	**6/9 (67) [d]**	**3/9 (33) [d]**
Fp9-3e (10)	472	ARMQCRAWYL	3/9 (33)	1/9 (11)
Fp9-3f (12)	473	APARMQCRAWYL	4/9 (44)	1/9 (11)
Fp14 Peptide Pool				
Fp14-1 (14)	301	**DQEQNTAEVKLYLK**	4/10 (40)	2/10 (20)
Fp14-1a (10)	474	QEQNTAEVKL	0/4 (0) [e]	1/4 (25) [e]
Fp14-1b (10)	466	QNTAEVKLYL	2/4 (50) [e]	1/4 (25) [e]
Fp14-1/2a (9)	476	NTAEVKLYL	4/10 (40)	1/10 (10)
Fp14-2 (15)	302	**EQNTAEVKLYLKQSL**	3/6 (50)	0/6 (0)
Fp14-2/3b (9)	477	AEVKLYLKQ	3/10 (30)	1/10 (10)
Fp14-2/3c (11)	478	AEVKLYLKQSL	9/10 (90)	5/10 (50)
Fp14-3 (14)	303	**AEVKLYLKQSLSIA**	5/6 (83)	0/6 (0)
Fp14-3d (13)	454	AEVKLYLKQSLSI	**10/10 (100) [d]**	**7/10 (70) [d]**
Fp14-3/4e (9)	479	LYLKQSLSI	9/10 (90)	6/10 (60)
Fp14-3/4f (10)	453	KLYLKQSLSI	**10/10 (100) [d]**	**6/10 (60) [d]**
Fp14-3/4g (9)	480	YLKQSLSIA	6/10 (60)	0/10 (0)
Fp14-4 (13)	304	**KLYLKQSLSIANA**	6/6 (100)	1/6 (17)

Table 13 Footnotes:

[a] The 13-15mer peptide designations used in the peptide pools and their corresponding aa sequences are in bold.

[b] Number of amino acids.

[c] The responder frequencies to the large peptides were derived from yr 2 and included only responders to either the Fp9 or Fp14 peptide pool via CFSE proliferation. The responder frequencies to the small 9-13mer peptides were obtained at yr 4 and included only responders to either the Fp9 or Fp14 peptide pool via both proliferation and IFNγ.

[d] The two highest frequency of responders to small peptides by both $CD8^+$ and $CD4^+$ T cells are highlighted in bold.

[e] Proliferation results are from small number of subjects tested (n=4) but ICS results are from five subjects (Figure 28).

Table 14. HLA supertypes of the responders to key short and long peptides

Peptide Code (No. of aa)	NetCTL Prediction [a]	NetMHC Prediction [a]	Supertypes of Responder (HLA-A/B supertypes) [a]	Algorithm and Responder Common Supertype(s) [b]	Responder Frequency in % (magnitude) type [c]
Fp9-3 (15)	A2, B7, B8, B27, B62	A2, B7, B8, B27	SF17 (A1/A2, B7/B7) SF18 (A1/A1, B8/B44) SF19 (A2/A3, B44/B62) SF20 (A2/A2, B44/ B58)	SF17 (**A2**, B7) SF18 (B8) SF19 (**A2**, B62) SF20 (**A2**)	67% (X-high) CD8 Proliferation 83% (high) CD4 Proliferation 14% (medium) IFNγ High Cytotoxins
Fp9-3c (9)	A2, B8, B62	A1, A2, A3, B8, B27	SF17 (A1/A2, B7/B7) SF19 (A2/A3, B44/B62) SF20 (A2/A2, B44/ B58) SF24 (A2,A3, B27/B44)	SF17 (A1, **A2**) SF19 (**A2**, B62) SF20 (**A2**) SF24 (**A2**, A3, B27)	56% (low) CD8 Proliferation 44% (low) CD4 Proliferation 22% (low) IFNγ Extremely Low Cytotoxins
Fp9-3d (9)	B27	A1, A3, B27, B44	SF17 (A1/A2, B7/B7) SF19 (A2/A3, B44/B62) SF20 (A2/A2, B44/ B58) SF24 (A2,A3, B27/B44)	SF17 (A1) SF19 (A3, **B44**) SF20 (**B44**) SF24 (A3, B27, **B44**)	56% (low) CD8 Proliferation 33% (low) CD4 Proliferation 0% (none) IFNγ Moderate Cytotoxins (only CD8)
Fp14-3d (13)	A24, B44	A1, A2, A24, B27, B44	SF17 (A1/A2, B7/B7) SF19 (A2/A3, B44/B62) SF20 (A2/A2, B44/ B58) SF24 (A2,A3, B27/B44)	SF17 (A1, **A2**) SF19 (**A2, B44**) SF20 (**A2**, **B44**) SF24 (**A2**, B27, **B44**)	100% (high) CD8 Proliferation 70% (low) CD4 Proliferation 40% (low) IFNγ Low Cytotoxins (only CD8)
Fp14-3/4f (10)	A24	A1, A2, A24, B27, B58	SF17 (A1/A2, B7/B7) SF19 (A2/A3, B44/B62) SF20 (A2/A2, B44/ B58) SF24 (A2,A3, B27/B44)	SF17 (A1, **A2**) SF19 (**A2**) SF20 (**A2**, B58) SF24 (**A2**, B27)	100% (high) CD8 Proliferation 60% (low) CD4 Proliferation 40% (low) IFNγ High Cytotoxins

Peptide Code (No. of aa)	NetCTL Prediction [a]	NetMHC Prediction [a]	Supertypes of Responder (HLA-A/B supertypes) [a]	Algorithm and Responder Common Supertype(s) [b]	Responder Frequency in % (magnitude) type [c]
Fp14-1 (14)	A1, B39, B58	A1, B44, B62	SF17 (A1/A2, B7/B7) SF18 (A1/A3, B8/B44) SF20 (A2/A2, B44/B58) SF23 (A1/A3, B27/B44)	SF17 (**A1**) SF18 (**A1, B44**) SF20 (**B44**) SF23 (**A1, B44**)	50% (low) CD8 Proliferation 37% (low) CD4 Proliferation 33% (medium) IFNγ High Cytototoxins
Hp15-1 (14)	B58	B44, B58	SF18 (A1/A1, B8/B44) SF19 (A2/A3, B44/B62) SF20 (A2/A2, B44/ B58) SF24 (A2,A3, B27/B44)	SF18 (**B44**) SF19 (**B44**) SF20 (**B44**, B58) SF24 (**B44**)	50% (low) CD8 Proliferation 0% (none) CD4 Proliferation 78% (medium) IFNγ High Cytotoxins

Table 14 Footnotes:

[a] Four subjects who responded to the designated peptide were HLA class I typed, and their HLA alleles were compared to the HLA supertype(s) predicted for the designated peptide using the NetCTL 1.2 and NetMHC 3.2 algorithms. The HLA A and HLA B allotypes for the subjects are shown as HLA supertypes.

[b] The most common supertypes between subjects and the HLA algorithm predictions are shown. The bolded supertype represents the most common supertypes among the subjects.

[c] All results were from responders to either Fp9 or Fp14 peptide pools. The results for individual 9-11mer peptides were derived from Figure 28. Those for 13-15mer peptides (Fp9-1, Fp14-1, Hp15-1) were derived from Figures 5 and/or 7. The average positive values are considered low when the frequency of response is <15% CFSE or <125 SFU, and high when >30 CSFE or >300 SFU. The cytotoxin result is considered high when four or five subjects express one or more cytotoxins in the $CD8^+$ T cells.

Example 18

Selection of conserved FIV and HIV-1 p24 and RT peptide pools and peptides.

In our recent studies, the PBMC and T cells from HIV^+ subjects responded to two FIV p24 peptide-pools Fp9 and Fp14 (Figure 30) (Roff *et al.* 2015) and one FIV RT peptide-pool FRT3 (Sanou *et al.* 2013). These peptide pools were identified by IFNγ ELISpot of PBMC and carboxyfluoresein diacetate succinimide ester (CFSE)-proliferation of $CD3^+CD4^+$ and $CD3^+CD8^+$ T cells. FIV p24-pool Fp14 and RT-pool FRT3 induced both $CD8^+$ T-cell proliferation and IFNγ responses but minimal $CD4^+$ T-cell proliferation. In contrast, pool Fp9 induced predominantly $CD8^+$ T-cell proliferation but minimal $CD4^+$ T-cell proliferation or IFNγ responses. The p24-peptide Fp14-3 of pool Fp14 induced the most IFNγ responses followed by peptide Fp14-1, while the p24-peptide Fp9-3 of pool Fp9 induced $CD8^+$ T-cell proliferation. The majority of the responses to RT-pool FRT3 were specific for peptide FRT3-3. However, intracellular cytokine/cytotoxin staining (ICS) analyses determined that peptides Fp14-3, Fp14-4, and FRT3-4 induced the highest frequency and levels of $CD8^+$ CTL-associated activity followed by peptide Fp9-3, Fp14-1, and FRT3-1 (Fp14 peptides in Figures 31A-31B; FRT3 peptides in (Sanou *et al.* 2013)). The magnitude and frequency of CTL-associated cytotoxin (perforin, granzyme A, granzyme B) responses were much higher and more frequent in $CD8^+$ T cells than $CD4^+$ T cells (Roff *et al.* 2015; Sanou *et al.* 2013). This observation suggests that $CD8^+$ CTLs are induced in responses to FIV peptides Fp9-3, Fp14-3, and Fp14-4, and also to the counterpart HIV-1 peptides Hp15-1 and Hp15-3. Our approach identified the HIV-1 peptides Hp15-1 and Hp15-3 to be the evolutionarily or lentivirally conserved epitopes which induced CTL-associated activity in T cells.

Remarkably, prototype FIV (IWV)-vaccinated cats (Coleman *et al.* 2014) also responded with high magnitude and/or frequency of T-cell proliferation to pools Fp9 and Fp14 (Figures 32A-32B). Hence, Fp9 and Fp14 pools are recognized by both HIV^+ subjects and FIV-vaccinated cats and thus contain EC T-cell epitopes (Figures 30 and 32). Additional FIV-vaccinated semi-inbred and outbred cats were tested for Fp9 and Fp14 peptide pools with similar results as in Figure 30 (Figure 33A). However they induced more IFNγ responses to pools Fp9 and Fp14 than those induced by FIV-infected cats. Individual peptides Fp14-1, Fp14-2, and Fp14-3 induced consistently low levels (*e.g.*, 50-200 SFU considered low levels) of IFNγ responses in FIV-vaccinated cats (Figure 33C,

left), whereas peptide Fp14-4 induced the most $CD8^+$ T-cell proliferation responses followed by peptides Fp14-2 and Fp14-3 (Figure 33C, right). Notably, peptides Fp14-1 and Fp14-2 induced the most $CD4^+$ T-cell proliferation.

Evolutionarily conserved (EC) or lentivirally conserved T-cell epitopes.

Above studies demonstrate that both HIV^+ subjects and FIV-vaccinated cats recognized certain regions of FIV (*e.g.*, Fp9 pool) or of both FIV and HIV-1 (*e.g.*, Fp14 and Hp15 pools; FRT3 and HRT3 pools). Moderate to identical aa sequence similarities/identities are observed between HIV-1 and FIV or SIV at conserved regions (*i.e.*, overlapping peptide pools) of Hp15/Sp14/Fp14 (Table 15) and at conserved epitope FRT3-3 (Table 16). Notably, there is high (100%) similarity and identity (87-96%) within HIV-1 subtypes A-D at the Hp15 region (Table 15). 100% identity is observed among all HIV subtypes at the FRT3-3 epitope (Table 16). Thus, the Hp15/Fp14 region on p24 and HRT3-3/FRT3-3/SRT3-3 epitopes on RT are highly conserved epitopes and considered evolutionarily conserved (EC) epitopes. The high similarity between HIV-1 and SIV strongly suggests the existence of more EC epitopes between these lentiviruses that induce a high $CD8^+$ T-cell responder frequency in PBMC from HIV^+ subjects (HIV-1 pool Hp1 and SIV pool Sp1 Figures 30A, 30C) (Roff *et al.* 2015). Interestingly, our approach has identified additional non-conserved reactive epitopes including pool Fp9, which has minimal identity or similarity to the HIV and SIV counterparts and has the closest similarity to HIV Nef based on Los Alamos National Laboratory (LANL) QuickAlign program (53% similarity, 47% identity, 2 gaps). These additional identified non-conserved sequences including this 13mer Nef counterpart of Fp9 will also be evaluated as candidate peptide epitope for AIDS vaccine.

Sections A and C in Table 15 show the EC epitope selection methods for Hp15 peptides (Section A) and Fp14 peptides (Section C). EC peptides are first screened by IFNγ ELISpot analysis (IFN) and CFSE-based $CD8^+$ (8P) and $CD4^+$ (4P) T-cell proliferation analyses followed by $CD8^+$ T-cell ICS (8C), $CD4^+$ T-cell ICS (4C), and viral enhancing/inhibitory assay, and responses in HIV negative control subjects (NC).

Multiple antigenic peptide (MAP) vaccine study with EC epitope peptides.

Since peptide pools and individual peptides of Fp14 and FRT3 induced CTL-associate cytotoxin expression in T cells from HIV^+ subjects, an *in vivo* study was performed to test whether if vaccination with these EC epitope peptides of FIV p24 and RT can elicit protective immunity against FIV in laboratory cats. Eight semi-inbred cats that were primed 1X with the prototype vaccine and boosted 4X-6X with 200 μg of lipophylic (Pam, palmitate C16)-MAP. The three Pam-MAPs consisted of FIV p24-peptide Fp14-1 alone (MAP1b) or together with the FIV p24-peptide Fp4-3 (MAP1: Fp4-3/furin-sensitive-linker/14-1) and FIV RT peptides FRT3-3 overlapped with FRT3-4 (MAP2) and were administered SC/ID in FD-1 adjuvant with feline IL12 (FeIL12) (Figure 34) (Table 17). Peptide Fp4-3 and peptide-pool Fp4 induced predominantly IFNγ, IL2, and $CD4^+$ T-cell proliferation responses (pool Fp4 in Figures 31A-31B; data not shown for Fp4-3), whereas peptide Fp14-1 induced IFNγ and $CD4^+$ T-cell proliferation responses in the PBMC from FIV-vaccinated cats (Figures 33A-33C). Using the same assay system, FRT3-3 and FRT3-4 induced strong IL2 and $CD8^+$ T-cell proliferation responses but modest IFNγ (FRT3-3) and negligible $CD4^+$ T-cell proliferation responses (data not shown). However in the T cells from HIV^+ subjects, Fp14-1, FRT3-3, and FRT3-4 stimulated IFNγ production, $CD8^+$ T-cell proliferation, and EC-CTL-associated activities (Figures 31A-31B) (Roff *et al.* 2015; Sanou *et al.* 2013), and is therefore an EC T-cell epitope. These peptides were chosen based on their ability to elicit FIV-specific immune responses in our FLA-defined semi-inbred cats. This pilot MAP study showed only moderate IFNγ responses and T-cell proliferation to individual peptide after the 2nd boost (Table 18). No adverse effects were observed throughout the study. More importantly, after 10 CID_{50} of FIV_{FC1} challenge, complete protection in 1 of 4 cats and partial protection in 2 of 4 cats were observed in the 4X MAP1/MAP2 boosted Group 1 with the second lowest amount of Fp4-3 peptide. All non-vaccinated control (Group 4, n=4) and 1X primed control (Group 3, n=3) groups were infected (Table 17). Partial protection consisted of a 3-6 wk delay in FIV detection and 2-log lower viral set point compared to the control group. However, only 1 of 2 cats with partial protection was observed in the 6X MAP1b/MAP1/MAP2 boosted Group 2b with the lowest amount of Fp4-3 peptide. The 6X MAP1/MAP2 boosted counterpart Group 2a with the most Fp4-3 peptide had no protection. Since Group 2b was immunized initially with MAP1b

instead of MAP1 and had one partially protected cat, this suggested that the non-EC peptide Fp4-3 may be blocking protection and instead enhancing infection.

To test the possibility that Fp4-3 peptide may be enhancing FIV infection, we performed an *in vitro* FIV enhancing/inhibitory analysis with all MAP peptides and immunogens. Most remarkably, significant enhancement of FIV infection was observed with peptide Fp4-3 (Fp4-3 vs. Positive Control, $p<0.05$), peptide FRT3-3 and MAP1, whereas peptide FRT3-4 ($p<0.05$) and MAP2 ($p<0.01$) significantly inhibited FIV infection (Figure 35). Since peptide Fp14-1 and MAP1b had no significant *in vitro* effect, peptide Fp4-3 in the MAP1 is most likely the cause of the *in vitro* enhancement observed with MAP1. Another notable observation is that the most significant inhibition was observed with MAP2 which correlates with the strong inhibition observed with FRT3-4 (no difference between FRT3-4 *vs* MAP2, $p=0.184$). Since MAP2 consisted of peptides FRT3-3 and FRT3-4 with a natural overlap (Figure 34), such overlap may have blocked the enhancing activity of peptide FRT3-3 while allowing the inhibitory activity of peptide FRT3-4. Hence, MAP2 was the most protective immunogen followed by MAP1b. Moreover, none of the MAP vaccinated cats had early detection or enhancement in viral load compared to the control cats. Perhaps, the strong immunity generated by the MAP2 has blocked the enhancing activity of the MAP1 when boosted together (Group 1, Table 3). To test this possibility, *in vitro* study is currently undergoing to evaluate whether MAP2 can block FIV enhancing activity of MAP1. Furthermore, more cats will be vaccinated with MAP1b and MAP2 to determine the levels of cytotoxins generated to peptides Fp14-1, FRT3-3 and FRT3-4. Since these peptides have induced CTL-associated cytotoxin expression in T cells from HIV^+ subjects (Figures 31A-31B; Figure 5 in (Sanou *et al.* 2013)), these EC peptides may also mediate their *in vivo* effects by inducing anti-FIV specific CTL activity. The *in vitro* results together with *in vivo* efficacy results further suggest that our *in vitro* tests (enhancing/inhibitory assay; cytotoxin/cytokine analyses) can select epitopes with potent antiviral activities which should decrease the number of protective EC epitopes needed to be tested in *in vivo* studies and more efficiently produce an effective T-cell based vaccine against FIV and HIV-1.

Evaluating the direct viral enhancing or inhibitory effect of Fp9-3 and Hp15 peptides.

Based on our FIV enhancing/inhibitory (E/I) assay, we have determined non-EC peptide Fp4-3 and EC peptide FRT3-3 significantly enhanced *in vitro* FIV infection. However, only peptide Fp4-3 correlated with the *in vitro* enhancement induced by MAP1. Since the *in vitro* E/I assay uses PBMC from naïve specific pathogen free (SPF) cats, it is unlikely that the *in vitro* stimulation of anti-FIV CTLs occurred in the culture and eliminated the infected cells. More likely, these enhancing or inhibitory peptides induced cytokines (enhancement: IFNγ, TNFα, GM-CSF; inhibition: IFNα, IL10), chemokines (inhibition: MIP1α, MIP1β, SDF-1, RANTES), or cellular restriction factors (inhibition: Trim5α, APOBEC3g) that either enhanced or inhibited FIV/HIV-1 infection. The two identified FIV-enhancing epitopes (Fp4-3 and FRT3-3 epitope peptides) (Figure 35) also induced high levels of IFNγ production in the PBMC of FIV-vaccinated cats (data not shown) but moderate levels to FRT3-3 (Sanou *et al.* 2013) and modest levels to Fp4 pool (Figures 30A-30F) (Roff *et al.* 2015) in the PBMC of HIV^+ subjects. IFNγ has been shown to enhance *in vitro* FIV and HIV-1 infections of naïve cat and human PBMCs respectively (Tanabe and Yamamoto 2001; Yamamoto *et al.* 1986). IFNγ is produced during inflammatory responses to HIV-1 infection (Ipp and Zemlin 2013). Thus, high levels of antiviral cellular immune responses are needed while minimizing both non-specific and viral-specific activation of $CD4^+$ T cells which can enhance viral replication (Lane 2010). Removal of the enhancing epitopes on FIV and HIV-1 immunogens will be critical to produce an effective HIV-1 vaccine. To accomplish this goal, we will design a vaccine based on antiviral epitopes directed at inducing viral-specific $CD4^+$ CTLs, $CD8^+$ CTLs and polyfunctional T cells, and identifying viral enhancing epitopes to remove from vaccine candidates.

More importantly, our most recent *in vitro* studies demonstrated that EC epitope FRT3-4 directly inhibited the FIV infection. This observation is completely opposite of the FIV enhancement observed with EC epitope FRT3-3. Notably the enhancing epitope overlaps with the inhibitory epitope. Hence, careful epitope mapping must be performed to remove any enhancing epitopes from FIV vaccine immunogen. Similar to Figure 36A, repeated *in vitro* E/I studies demonstrated that peptides Fp14-3 and Fp14-4 do not directly enhance or inhibit FIV infection. Therefore these EC T-cell peptides especially the Fp14-

4 peptide, which induced strong $CD8^+$ T-cell proliferation in FIV-vaccinated cats (Figures 33A-33C), should be next tested for its ability to induce FIV-specific cytotoxin expression before *in vivo* challenge efficacy study. Its HIV-1 counterpart peptide Hp15-3 induced high levels and frequency of cytotoxin responses in T cells from HIV^+ subjects (Figures 31A-31B) (Roff *et al.* 2015). Similarly, Hp15-1, the HIV counterpart of Fp14-1, induced robust cytotoxin levels and responder frequencies in the $CD8^+$ T cells from HIV^+ subjects. Moreover, Hp15-1 strongly inhibited *in vitro* HIV-1 infection, whereas Hp15-3 had no direct effect on HIV-1 infection. Consequently, EC peptide Hp15-1 followed by peptide Hp15-3 should be included as the HIV-1 vaccine immunogens.

Another remarkable observation is that FIV p24 epitope peptide Fp9-3 inhibited *in vitro* infection of both FIV (Figure 36A) and HIV-1 (Figure 36B) but FRT3-4 inhibited only FIV. However these *in vitro* results indicate that FRT3-4 does not enhance FIV and HIV-1 infections and may be a promising T-cell epitope for vaccine since it induced CTL-associated cytotoxin expression in T cells from HIV^+ subjects (Sanou *et al.* 2013). If it also induces FIV-specific CTL activity in PBMC from FIV-vaccinated cats then this peptide will have direct and indirect modes of anti-FIV activities. In conclusion, we now have EC peptides Fp14-1, FRT3-4, and FRT3-3/3-4 as protective T-cell epitopes against FIV, and EC peptides Hp15-1 and Hp15-3 as protective T-cell epitopes against HIV-1, whereas cross-reactive Fp9-3 peptide may be useful against both FIV and HIV-1. We have also determined that palmitated MAP (Pam-MAP) is an excellent vehicle to deliver the peptide-based vaccine.

Table 15. Sequence conservation in EC p24 epitopes of Hp15/Fp14 pools

A. Subtype-B HIV-1 Hp15 Peptides									
	SEQ ID NO.		**IFN**	**8P**	**4P**	**8C**	**4C**	**E/I**	**NC**
Hp15-1	359	RAEQASQEVKNWMTE	+	+	+	+	+	↓	-
Hp15-2	360	ASQEVKNWMTETLLV	+	-	-	+	+	n	-
Hp15-3	361	VKNWMTETLLVQNAN	+	+	+	+	+	↓	+
B. Subtypes/Strains									
	SEQ ID NO.	**Fp14 Pool Counterparts**	**Compared to UCD1**						
HIV-1 UCD1 (B)	457	RAEQASQEVKNWMTETLLVQNAN *****:****.************	Identity				(Similarity)		
HIV-1 (A)	481	RAEQA**T**QEVK**G**WMTETLLVQNAN **********************	91%				(100%)		
HIV-1 (B)	457	RAEQASQEVKNWMTETLLVQNAN *****:*:******:********	100%				(100%)		
HIV-1 (C)	482	RAEQA**T**Q**D**VKNWMT**D**TLLVQNAN *******:***************	87%				(100%)		
HIV-1 (D)	483	RAEQASQ**D**VKNWMTETLLVQNAN **********:***:********	96%				(100%)		
SIV CPZ	484	RAEQASQEVK**T**WMT**D**TLLVQNAN ****... ******.***:****	91%				(100%)		
SIV Mac251/Mac239	485	RAEQ**TDAA**VKNWMT**Q**TLL**I**QNAN ** : ** ::.::* : ***	74%				(96%)		
FIV (A,B,D) [Fp14]	486	**DQ**EQ**NTA**EVK**LYLKQS**L**SIA**NAN ** : **:::.::* : ***	39%				(70%)		
FIV (C)	487	**DQ**EQ**NTA**EVK**TYLKQS**L**SLA**NAN	39%				(74%)		
C. Subtype-B FIV Fp14 Peptides									
	SEQ ID NO.		**IFN**	**8P**	**4P**	**8C**	**4C**	**E/I**	**NC**
Fp14-1	301	DQEQNTAEVKLYLK	+	+	+	+	+	-	-
Fp14-2	302	EQNTAEVKLYLKQSL	-	+	-	+	+	n	-
Fp14-3	303	AEVKLYLKQSLSIA	+	+	+	+	+	-	-
Fp14-4	488	KLYLKQSLSIANAN	+	+	+	+	+	-	-

Sections A and C show positive (+) or negative (-) response for IFNγ (IFN); $CD8^+$ T-cell proliferation (8P); $CD4^+$ T-cell proliferation (4P); $CD8^+$ T-cell cytotoxins (8C); $CD4^+$ T-cell cytotoxins (4C); or HIV enhancing or inhibitory (E/I) in HIV^+ subjects when compared to those in HIV^- control group (NC). Symbols: inhibitory (↓); identical aa (*); closely (:) or moderately (.) similar aa; not done (n); and **bold aa** residue differs from the one on HIV-1/UCD1.

Table 16. Sequence conservation in EC epitope FRT3-3

Subtypes/Strains	**SEQ ID NO.**	**FRT3-3 Counterparts**	**Compared to UCD1**	
HIV-1 UCD1 (B)	435	**KKKDSTKWRKLVDFRE** **********	Identity	(Similarity)
HIV-1 (A)	435	KKKDSTKWRKLVDFRE **********	100%	(100%)
HIV-1 (B)	435	KKKDSTKWRKLVDFRE **********	100%	(100%)
HIV-1 (C)	435	KKKDSTKWRKLVDFRE **********	100%	(100%)
HIV-1 (D)	435	KKKDSTKWRKLVDFRE **********	100%	(100%)
SIV CPZ	435	KKKDSTKWRKLVDFRE ****..***.*:****	100%	(100%)
SIV MM	489	KKKD**KN**KWR**M**L**I**DFRE *** *:***.*:****	75%	(100%)
FIV (A,C)	490	KKK-S**G**KWR**M**L**I**DFRE *** *:***.*:***	75%	(94%)
FIV (B,D) [FRT3-3]	63	KKK-S**G**KWR**M**L**I**DFR**V**	67%	(87%)

Strong IFNγ and T-cell proliferation responses to FRT3-3 decreased to zero when the following aa's on the FRT3-3 peptide were changed with the corresponding ones on the HRT3-3 peptide: V15→E15, I11→V11, and M9→K9 (highest to lowest decrease) (Sanou *et al.* 2013). The underline shows an epitope which is also detected by HIV^+ subjects.

Table 17. Prototype Dual-Subtype FIV (IWV) Vaccine Prime and MAP Vaccine Boosts

Group # [# of cats]	Prime	Boost-1	Boost-2	Boost-3	Boost-4	Boost-5	Boost-6	Full-to-Partial Protection
1 [4]	1x IWV →	MAP1 →	MAP1+2 →	MAP2 →	MAP1+2	-	-	3 / 4 (1 Full + 2 Partial)
2a [2]	1x IWV →	MAP1 →	MAP1 →	MAP1 →	MAP2 →	MAP2 →	MAP1+2	0 / 2
2b [2]	1x IWV →	MAP1b →	MAP1b →	MAP1b →	MAP2 →	MAP2 →	MAP1+2	1 / 2 (Partial)
3 [3]	1x IWV	-	-	-	-	-	-	0 / 3
4 [4]	PBS	PBS	PBS	PBS	PBS	-	-	0 / 4

Table 18. Prototype Dual-Subtype FIV (IWV) Vaccine Prime and Post-SECOND MAP Boost

Cat ID	**Prime Boost-1 Boost-2**	**IFNγ (SFU)** **Fp4-3 / Fp14-1**	**IL2 (SFU)** **Fp4-3 / Fp14-1**	**Proliferation ($CFSE^{low}$)** $CD4^+$ **T cells**	$CD8^+$ **T cells**
QVW	1x IWV → MAP1 → MAP1	21 / 0 [0]	0 / 0 [9]	0 / **6 [7]**	0 / **5 [6]**
DVD	1x IWV → MAP1 → MAP1	**132** / 37 **[206]**	**95 / 53 [296]**	**3** / 0 [2]	**6** / 0 **[9]**
QVQ	1x IWV → MAP1b → MAP1b	0 / 0 [43]	0 / 12 **[165]**	1 / 1 **[7]**	0 / **6 [7]**
DVB	1x IWV → MAP1b → MAP1b	0 / 0 [0]	0 / **70 [76]**	0 / 2 [1]	0 / **12 [10]**

Peptide Fp4-3 (left), peptide Fp14-1 (middle) and MAP1 [right] were culture stimulants. IL2 and IFNγ ELISpot results of >50 spot forming units (SFU/10^6 PBMC) and CFSE-proliferation of >2 are considered positive (**bolded**). The prime-boost vaccination induced IL2 responses in 3

of 4 cats and IFNγ response in one. Larger responses were induced by MAP1 than individual peptides in culture. These results demonstrate that both peptides in the MAP are recognized by the cats and MAP formulation may be more effective in delivering peptides into cells.

It should be understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and the scope of the appended claims. In addition, any elements or limitations of any invention or embodiment thereof disclosed herein can be combined with any and/or all other elements or limitations (individually or in any combination) or any other invention or embodiment thereof disclosed herein, and all such combinations are contemplated with the scope of the invention without limitation thereto.

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CLAIMS

I claim:

1. A method for inducing an immune response in a person or animal against an immunodeficiency virus, comprising administering one or more antigens and/or immunogens to the person or animal, wherein said one or more antigens and/or immunogens comprise one or more evolutionarily conserved epitopes, wherein said epitopes are conserved between two or more different immunodeficiency viruses, and wherein said epitope comprises the amino acid sequence of any of SEQ ID NOs:451-493 or 495-591.

2. The method according to claim 1, wherein said epitope is a T-cell epitope.

3. The method according to claim 2, wherein said T cell epitope induces one or more T cell responses.

4. The method according to claim 3, wherein said T cell response is release of cytotoxins and/or cytokines.

5. The method according to claim 1, wherein said epitope comprises the amino acid sequence of any of SEQ ID NOs:453, 459, 460, 466, 479, 488, 492, or 493.

6. The method according to claim 1, wherein said antigens and/or immunogens are peptides or proteins, and wherein two or more peptides or proteins comprising the amino acid sequence of any of SEQ ID NOs:453, 459, 460, 466, 479, 488, 492, or 493 are administered to the person or animal.

7. The method according to claim 1, wherein said antigens and/or immunogens are peptides or proteins, and wherein said peptides or proteins comprise two or more amino acid sequences of any of SEQ ID NOs:453, 459, 460, 466, 479, 488, 492, and/or 493.

8. The method according to claim 1, wherein said induced immune response is a CTL-associated immune response.

9. The method according to claim 1, wherein said induced immune response comprises a CD4+ and/or CD8+ T cell response.

10. The method according to claim 1, wherein a person is administered said one or more antigens and/or immunogens that are from an HIV and/or FIV.

11. The method according to claim 1, wherein the animal is a feline animal and is administered said one or more antigens and/or immunogens that are from an FIV and/or HIV.

12. An antibody, or an antigen binding fragment thereof, that binds to a peptide comprising an FIV or HIV epitope, wherein said epitope comprises an amino acid sequence of any of SEQ ID NOs:451-493 or 495-591.

13. The antibody according to claim 12, wherein said antibody is a monoclonal antibody.

14. A composition comprising:

i) one or more epitopes evolutionarily conserved between different immunodeficiency viruses; and/or

ii) one or more polynucleotides that encode said one or more evolutionarily conserved epitopes;

wherein said epitopes comprise an amino acid sequence of any of SEQ ID NOs:451-493 or 495-591.

15. The composition according to claim 14, wherein said epitope comprises the amino acid sequence of any of SEQ ID NOs:443, 453, 459, 460, 466, 479, 488, 492, or 493.

16. The composition according to claim 14, wherein said composition comprises two or more peptides, wherein said two or more peptides comprise, independently, the amino acid sequence of any of SEQ ID NOs:453, 459, 460, 466, 479, 488, 492, or 493.

17. The composition according to claim 14, wherein said composition comprises a peptide or protein that comprises two or more amino acid sequences of any of SEQ ID NOs:453, 459, 460, 466, 479, 488, 492, and/or 493.

18. The composition according to claim 14, wherein said composition further comprises a pharmaceutically acceptable carrier or diluent.

19. A multiple antigenic peptide (MAP) construct, wherein said MAP comprises one or more peptides that comprise one or more amino acid sequences of any of SEQ ID NOs:451-493 or 495-591.

20. The MAP construct according to claim 19, wherein said MAP comprises a core matrix of lysine residues.

21. The MAP construct according to claim 20, wherein said MAP comprises one or more fatty acids attached to said core matrix.

22. The MAP construct according to claim21, wherein said fatty acid is palmitic acid.

23. The MAP construct according to claim 19, wherein the same or different peptides are linked end to end.

24. The MAP construct according to claim 19, wherein the same or different peptides are linked directly to each other.

25. The MAP construct according to claim 19, wherein the same or different peptides are linked via a linker moiety.

26. The MAP construct according to claim25, wherein said linker moiety is a furin-sensitive linker.

27. The MAP construct according to claim 26, wherein said furin-sensitive linker comprises the amino acid sequence of SEQ ID NO:494.

28. A vaccine that comprises a composition of claim 14 or a MAP construct of claim 19.

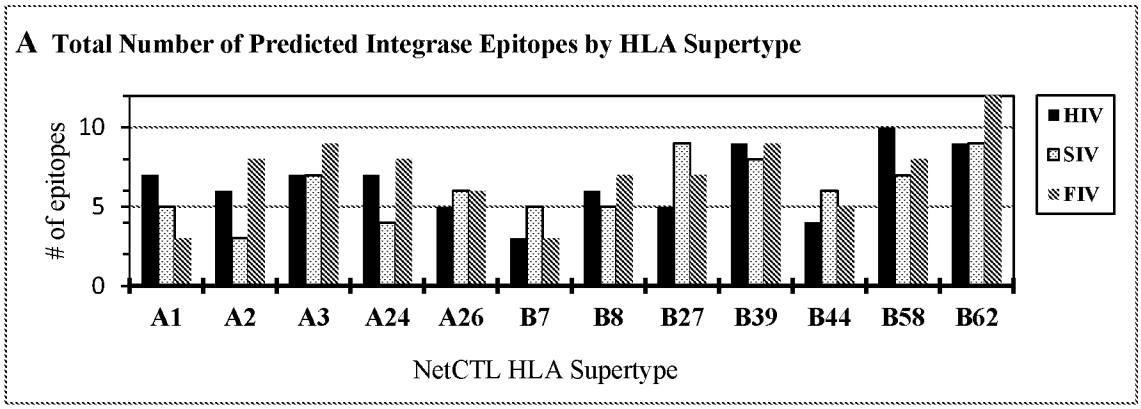


FIG. 1A

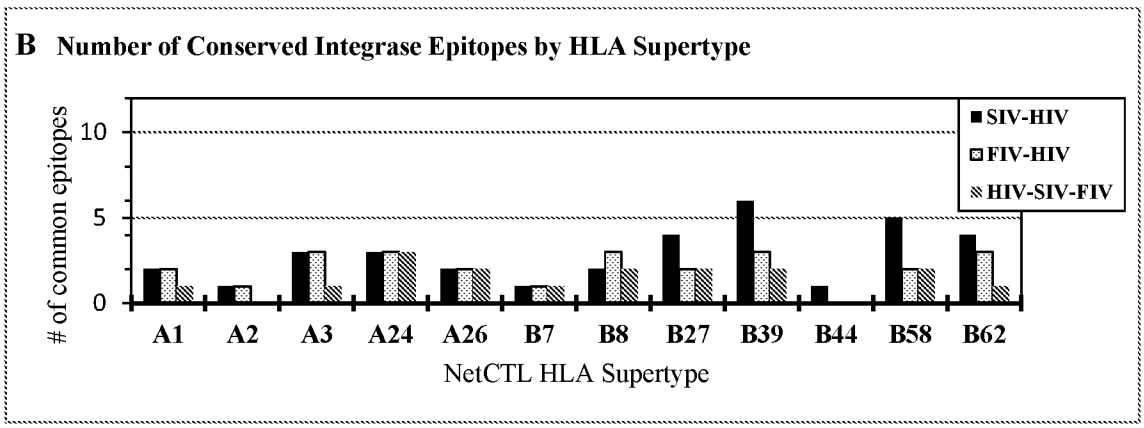


FIG. 1B

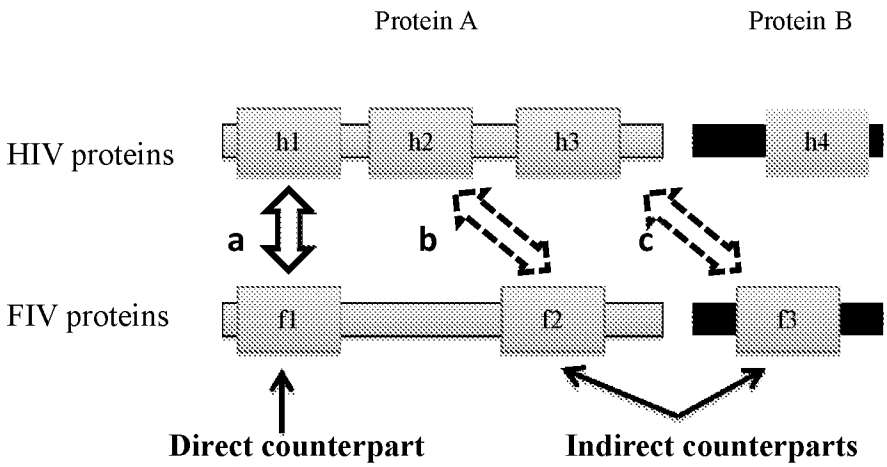


FIG. 2

FM RT POOL	FR11	FR12	FR13	FR14	FR15	FR16	FR17	FR18	FR19	FR110	FR111
CD4+ T Prolif.	2 (8%)	5 (21%)	12 (12%)	3 (4%)	9 (4%)	8 (4%)	12 (4%)	6 (8%)	9 (12%)	4 (12%)	6 (12%)
CD8+ T Prolif.	4 (12%)	4 (21%)	21 (57%)	2 (4%)	3 (8%)	10 (21%)	30 (63%)	6 (21%)	20 (21%)	8 (12%)	5 (29%)
PBMC IFNγ	110 (21%)	75 (7%)	500 (61%)	74 (14%)	204 (21%)	132 (21%)	181 (18%)	117 (21%)	80 (4%)	110 (21%)	86 (18%)
HIV RT POOL	HR11	HR12	HR13	HR14	HR15	HR16	HR17	HR18	HR19	HR110	HR111
CD4+ T Prolif.	16 (12%)	8 (4%)	13 (8%)	6 (8%)	4 (8%)	10 (4%)	52 (8%)	10 (8%)	25 (8%)	8 (4%)	16 (8%)
CD8+ T Prolif.	9 (8%)	8 (4%)	5 (12%)	4 (4%)	4 (12%)	3 (12%)	9 (12%)	15 (12%)	7 (17%)	3 (4%)	20 (17%)
PBMC IFNγ	264 (21%)	108 (29%)	135 (14%)	143 (25%)	184 (39%)	412 (39%)	322 (46%)	127 (21%)	236 (32%)	195 (36%)	880 (57%)

FIG. 3A

FM RT POOL	FR112	FR113	FR114	FR115	FR116	FR117	FR118	FR119	FR120	FR121	PHA
CD4+ T Prolif.	4 (8%)	8 (12%)	8 (8%)	15 (16%)	9 (8%)	9 (8%)	13 (4%)	0 (0%)	11 (8%)	9 (12%)	48 (79%)
CD8+ T Prolif.	3 (8%)	8 (17%)	5 (37%)	13 (63%)	4 (17%)	15 (12%)	7 (8%)	11 (21%)	9 (12%)	8 (33%)	42 (75%)
PBMC IFNγ	115 (11%)	114 (56%)	116 (18%)	140 (11%)	85 (18%)	88 (11%)	160 (11%)	90 (7%)	81 (25%)	70 (18%)	3968 (100%)
HIV RT POOL	HR112	HR113	HR114	HR115	HR116	HR117	HR118	HR119	HR120	HR121	PHA
CD4+ T Prolif.	12 (4%)	26 (12%)	5 (8%)	4 (4%)	2 (4%)	0 (0%)	8 (8%)	12 (4%)	2 (4%)	10 (4%)	48 (79%)
CD8+ T Prolif.	6 (12%)	28 (12%)	5 (17%)	5 (12%)	0 (0%)	6 (17%)	5 (12%)	10 (21%)	9 (0%)	4 (17%)	40 (79%)
PBMC IFNγ	381 (29%)	275 (29%)	230 (39%)	149 (25%)	146 (25%)	95 (18%)	88 (11%)	85 (4%)	149 (14%)	95 (18%)	3968 (100%)

FIG. 3B

FV p24 POOL	Fp1	Fp2	Fp3	Fp4	Fp5	Fp6	Fp7	Fp8	Fp9	Fp10
CD4+ T Prolif.	0 (0%)	0 (0%)	3 (4%)	0 (0%)	26 (4%)	5 (4%)	0 (0%)	3 (4%)	7 (4%)	4 (8%)
CD8+ T Prolif.	20 (12%)	4 (4%)	20 (4%)	0 (0%)	0 (0%)	3 (17%)	10 (4%)	4 (8%)	29 (21%)	41 (8%)
PBMC IFNγ	180 (6%)	70 (3%)	97 (10%)	156 (13%)	121 (6%)	112 (13%)	93 (16%)	73 (10%)	72 (13%)	144 (13%)
HIV p24 POOL	Hp1	Hp2	Hp3	Hp4	Hp5	Hp6	Hp7	Hp8	Hp9	Hp10
CD4+ T Prolif.	7 (4%)	5 (8%)	5 (8%)	5 (4%)	11 (4%)	2 (4%)	0 (0%)	29 (12%)	3 (4%)	5 (12%)
CD8+ T Prolif.	20 (21%)	6 (8%)	0 (0%)	0 (0%)	19 (4%)	2 (12%)	5 (8%)	3 (8%)	22 (12%)	6 (12%)
PBMC IFNγ	262 (29%)	213 (42%)	534 (61%)	212 (23%)	252 (23%)	626 (32%)	721 (39%)	512 (26%)	272(29%)	215 (48%)

FIG. 4A

FV p24 POOL	Fp11	Fp12	Fp13	Fp14	Fp15	Fp16	Fp17		ConA
CD4+ T Prolif.	3 (12%)	0 (0%)	0 (0%)	7 (4%)	0 (0%)	0 (0%)	8 (4%)		45 (79%)
CD8+ T Prolif.	4 (8%)	3 (4%)	0 (0%)	23 (8%)	53 (4%)	0 (0%)	0 (0%)		44 (79%)
PBMC IFNγ	126 (16%)	215 (6%)	107 (16%)	302 (26%)	145 (10%)	85 (3%)	160 (10%)		5031 (100%)
HIV p24 POOL	Hp11	Hp12	Hp13	Hp14	Hp15	Hp16	Hp17	Hp18	PHA
CD4+ T Prolif.	0 (0%)	4 (4%)	0 (0%)	0 (0%)	0 (0%)	5 (4%)	48 (4%)	0 (0%)	45 (79%)
CD8+ T Prolif.	32 (12%)	7 (17%)	0 (0%)	4 (4%)	15 (12%)	5 (12%)	0 (0%)	4 (4%)	44 (79%)
PBMC IFNγ	470 (63%)	283 (36%)	120 (10%)	324 (39%)	218 (32%)	211 (19%)	176 (26%)	109 (16%)	5031 (100%)

FIG. 4B

FIG. 5

47.7% identity and 70.8% homology

```
                10        20        30        40        50         .
HIV-1  P-ISP-IETVPVKLKPGMDGPKVKQWPLTEEKIKALVEICTEMEKEGKISKIGPENPYNT
         ::  :  : :..:   .:::::::::.::::::::.  ..: :::...  :.:::.::
FIV    AQISEKIPIVKVRMKDPTQGPQVKQWPLSNEKIEALTDIVERLELEGKVKRADPNNPWNT
               10        20        30        40        50        60

      60        70        80        90       100       110
HIV-1  PVFAIKKKDSTKWRKLVDFRELNKRTQDFWEVQLGIPHPAGLKKKKSVTVLDVGDAYFSV
       :::::::: : ::: :.::: ::: :.   :::::.:::::::  .:.:::::.::::::..
FIV    PVFAIKKK-SGKWRMLIDFRVLNKLTDKGAEVQLGLPHPAGLKMRKQVTVLDIGDAYFTI
               70        80        90       100       110

      120       130       140       150       160       170
HIV-1  PLDEDFRKYTAFTIPSINNETPGIRYQYNVLPQGWKGSPAIFQSSMTKILEPFRKQNPDI
       ::: :.  ::::::.:  ::  :: :: .  :::::  :: :.:::. .::.:: .:::..
FIV    PLDPDYAPYTAFTLPRKNNAGPGRRYVWCSLPQGWVLSPLIYQSTLDNILQPFIRQNPEL
     120       130       140       150       160       170

      180       190       200       210       220       230
HIV-1  VIYQYMDDLYVGSDLEIGQHRTKIEELRQHLLRWGLTTPDKKHQKEPPFLWMGYELHPDK
        :::::::.:::::.  .:. :.::::. :: ::. ::. : :.::::. ::::::::
FIV    DIYQYMDDIYIGSDLNKKEHKQKVEELRKLLLWWGFETPEDKLQEEPPYKWMGYELHPLT
     180       190       200       210       220       230
```

HRT3 *vs.* FRT3 (red line region)
69% Identity
77% Homology

HRT6 *vs.* FRT6 (red line section)
52% Identity
63% Homology

FIG. 5 (continued)

```
      240         250         260         270         280         290
HIV-1 WTVQP--IVLPEKDSWTVNDIQKLVGKLNWASQIYPGIKVRQLCKLLRGTKALTEVIPLT
      :..:    . .:::.  :.:.:::::::::::::  : .....:  ..::  . :  .   :
FIV   WSIQQKQLEIPERP--TLNELQKLVGKINWASQTIPDLSIKELTTMMRGDQRLDSIREWT
     240        250         260         270         280         290

       300         310         320         330         340          350
HIV-1 EEAELELAENREILKEPVHGVYYDPSKDLIAEIQKQGQGQWTYQIYQE-PFKNLKTGKYA
       ::. :. . .: ..  ..  :::::::: :...  :  :  ::.:.. : . :  ::.
FIV   TEAKKEVQKAKEAIETQAQLKYYDPSRELYAKLSLVGPHQICYQVYHKNPEHVLWYGKMN
      300         310         320         330         340         350

       360         370         380         390         400         410
HIV-1 RTRGAHTNDVKQLTEAVQKITTESIVIWGKTPKFKLPIQKETWETWW--TEYWQATWIPE
      : .    :       .:  ::  :::.  :: : ...: ..:.::.    . : .:   ::
FIV   RQKKKAENTCDIALRACYKIREESIIRIGKEPIYEIPASREAWESNLIRSPYLKAP-PPE
      360         370         380         390         400         410

          420         430         440
HIV-1 WEFVNTPPLVKLWYQLEKE-PIVGAETFY   (SEQ ID NO:45)
       ::...   .:   .. .. ::.::::.:
FIV   VEFIHAALNIKRALSMIQDTPILGAETWY   (SEQ ID NO:46)
       420         430         440
```

HRT11 *vs.* FRT11
(red line section)
57% Identity
87% Homology

HRT13 *vs.* FRT13
(red line section)
32% Identity
72% Homology

GENESTREAM network server (http://xylian.igh.cnrs.fr/bin/align-guess.cgi). *Pearson, W.R., Wood, T., Zhang, Z., and Miller, W. (1997). Comparison of DNA sequences with protein sequences. Genomics 46:24-36.*

Red word sequence = epitope region on both HIV-1 & FIV recognized by HIV+ subjects except for HRT3 where only FIV region recognized.

Green word sequence = counterpart epitope region on FIV

Underlined HIV sequence in green/black = HRT14; Over-lined HIV sequence in black = HRT15

Grey on FIV sequence = Macaque reactive site on FIV

FIG. 6

HIV-1 and SIV RT sequences: 59.6% identity and 81.9% homology

```
                10        20        30        40        50        60
HIV-1   PISPIETVPVKLKPGMDGPKVKQWPLTEEKIKALVEICTEMEKEGKISKIGPENPYNTPV
        ::. .: : : :::: ::::::::::.::: :: ::: .:::.:.. .  : :::::::
SIV     PIAKVEPVKVTLKPGKVGPKLKQWPLSKEKIVALREICEKMEKDGQLEEAPPTNPYNTPT
                10        20        30        40        50        60

                70        80        90       100       110       120
HIV-1   FAIKKKDSTKWRKLVDFRELNKRTQDFWEVQLGIPHPAGLKKKKSVTVLDVGDAYFSVPL
        ::::::::..::: :.:::::::. :::: :::::::::::: :.: .::::.:::::::::
SIV     FAIKKKDKNKWRMLIDFRELNRVTQDFTEVQLGIPHPAGLAKRKRITVLDIGDAYFSIPL
                70        80        90       100       110       120
                  R397,R395; R416; R422 (FIV NPWNTPVFAIKKK peptide)
              130       140       150       160       170       180
HIV-1   DEDFRKYTAFTIPSINNETPGIRYQYNVLPQGWKGSPAIFQSSMTKILEPFRKQNPDIVI
        ::.::.::::::.::.::  :: :: :.:::::::::::::::: .: ..::::::: :::...
SIV     DEEFRQYTAFTLPSVNNAEPGKRYIYKVLPQGWKGSPAIFQYTMRHVLEPFRKANPDVTL
      MamuA02   130       140       150       160 MamuA01 170       180

                190       200       210       220       230       240
HIV-1   YQYMDDLYVGSDLEIGQHRTKIEELRQHLLRWGLTTPDKKHQKEPPFLWMGYELHPDKWT
         :::::. ..::    .:   . .:.. :   :..:::.: ::.::: :::::: : ::
SIV     VQYMDDILIASDRTDLEHDRVVLQLKELLNSIGFSTPEEKFQKDPPFQWMGYELWPTKWK
                190       200       210       220       230       240
```

HRT3 *vs.* SRT3
77% Identity
91% Homology

HRT6 *vs.* SRT6
71% Identity
84% Homology

FIG. 6 (continued)

```
                250       260       270       280       290       300
HIV-1   VQPIVLPEKDSWTVNDIQKLVGKLNWASQIYPGIKVRQLCKLLRGTKALTEVIPLTEEAE
        .: : ::....:::::::::::: ::::.:::::::::::..::.::  .::: .  :: ::
SIV     LQKIELPQRETWTVNDIQKLVGVLNWAAQIYPGIKTKHLCRLIRGKMTLTEEVQWTEMAE
                250       260  R414 270       280       290       300

                310       320       330       340       350       360
HIV-1   LELAENREILKEPVHGVYYDPSKDLIAEIQKQGQGQWTYQIYQEPFKNLKTGKYARTRGA
         :  ::. ::..  .: ::. .: : : . :. ..:::::::::  : ::.::::. ...
SIV     AEYEENKIILSQEQEGCYYQEGKPLEATVIKSQDNQWSYKIHQED-KILKVGKFAKIKNT
        MamuA11 310 MamuA02 320       330       340       350

                370       380       390       400       410       420
HIV-1   HTNDVKQLTEAVQKITTESIVIWGKTPKFKLPIQKETWETWWTEYWQATWIPEWEFVNTP
        ::: :. :....::::  :.:::::.::::::::..:: :::.:::.::::::.:.::
SIV     HTNGVRLLAHVIQKIGKEAIVIWGQVPKFHLPVERDVWEQWWTDYWQVTWIPEWDFISTP
     360       370       380 MamuA01 390       400       410       MamuA01

                430       440
HIV-1   PLVKLWYQLEKEPIVGAETFY  (SEQ ID NO:45)
        ::::: ..: :.::: : :: :
SIV     PLVRLVFNLVKDPIEGEET-Y  (SEQ ID NO:47)
     420       430
```

HRT11 *vs.* SRT11
71% Identity
91% Homology

HRT13 *vs.* SRT13
36% Identity
72% Homology

Red word sequence = epitope region on both HIV-1 & FIV recognized by HIV+ subjects except for HRT3 where only FIV region recognized.
Blue word sequence = counterpart epitope region on SIV
Underlined HIV sequence = HRT14; Over-lined HIV sequence = HRT15
Highlights: **Grey** on HIV-1 sequence = Macaque reactive site on HIV-1
Grey on SIV sequence = Macaque reactive site on FIV
Yellow & Magenta on SIV sequence = Macaque $CD8^+$ CTL epitope with reactive Mamu allele (LANL)
Green & Aqua Blue = Counterpart HIV-1 sequence of known macaque CTL epitope

FIG. 7

FIV-Pet p24 223 aa vs.
SIV-Delta B670 p24 228 aa
scoring matrix: , gap penalties: -12/-2
29.0% identity (full sequences); Global alignment score: 337

```
                10        20        30        40        50        60
FIV    PIQTVNGVPQYVALDPKMVSIFMEKAREGLGGEEVQLWFTAFSANLTPTDMATLIMAAPG
       :.: ..:    .. :.:. .. ...  .:   : ::   : :.: . :: :.  ..  .
SIV    PVQQIGGNYVHLPLSPRTLDAWVKLIEEKKFGAEVVPGFQALSEGCTPYDINQMLNCVGD
               10        20        30        40        50        60

               70        80        90         100       110
FIV    CAADKEILDESLKQLTAEYDRTHPPDAPRP--LPYFTAAEIMGIGLTQEQQAEARF----
         :  .:. . ... .:..:  ::  ::.   :   ....: :   . ..: .  .
SIV    HQAAMQIIRDIINEEAADWDLQHPQPAPQQGQLREPSGSDIAGTTSSVDEQIQWMYRQQN
               70        80        90       100       110       120

           120       130       140       150       160       170
FIV    -APARMQCRAWYLEALGKLAAIKAKSPRAV-QLRQGAKEDYSSFIDRLFAQIDQEQNTAE
         :.    : :    ::    ..  .:  . ...:: :: ..:..:::. ..  ::. :
SIV    PIPVGNIYRRWI--QLGLQRCVRMYNPTNILDVKQGPKEPFQSYVDRFYKSLRAEQTDAA
              130       140       150       160       170

            180       190       200       210       220
FIV    VKLYLKQSLSIANANADCKKAMSHLKPESTLEEKLRACQEIGSPGYKMQLL  (SEQ ID NO:48)
       :: .. :.: : ::: ::: ... :  . :::: : ::: . .:: : .:
SIV    VKNWMTQTLLIQNANPDCKLVLKGLGVNPTLEEMLTACQGVEGPGQKARL-  (SEQ ID NO:49)
     180       190       200       210       220
```

Hp10/Fp9

Hp15 *vs.* Fp14
45.5% Identity
63.6% Homology

FIG. 7 (continued)

HIV-1 HXB2 p24 231 aa vs.
FIV-Pet p24 223 aa
scoring matrix: , gap penalties: -12/-2
31.8% identity (full sequences); Global alignment score: 408

```
                10        20        30        40        50        60
HIV-1  PIVQNIQGQMVHQAISPRTLNAWVKVVEEKAFSPEVIPMFSALSEGATPQDLNTMLNTVG
       :: :...:    . :..:. .. ... ..:    . ::   :.:.: . :: :. :.. ..
FIV    PI-QTVNGVPQYVALDPKMVSIFMEKAREGLGGEEVQLWFTAFSANLTPTDMATLIMAAP
                10        20        30        40        50

                70        80        90       100       110       120
HIV-1  GHQAAMQMLKETINEEAAEWDRVHPVHAGPIAPGQMREPRGSDIAGTTSTLQEQIGWMTN
       :  :  ..: :.... .:::::::    : ::  .    ...: :   : :::   ...
FIV    GCAADKEILDESLKQLTAEYDRTHP----PDAPRPLPYFTAAEIMGIGLT-QEQ---QAE
      60        70        80             90       100            110

               130       140       150       160       170
HIV-1  NPPIPVGEIYKRWIILGLNKI--VRMYSPTSILDIRQGPKEPFRDYVDRFYKTLRAEQAS
           :.    . : . .:.:.  ..  :: .. ..::: :: . ...:.. .  :: .
FIV    ARFAPARMQCRAWYLEALGKLAAIKAKSPRAV-QLRQGAKEDYSSFIDRLFAQIDQEQNT
              120       130       140       150       160       170

      180       190       200       210       220       230
HIV-1  QEVKNWMTETLLVQNANPDCKTILKALGPAATLEEMMTACQGVGGPGHKARVL
        ::: .. ..: . ::: :::  .. : : .:::: . ::: .:.:::.: ..:
FIV    AEVKLYLKQSLSIANANADCKKAMSHLKPESTLEEKLRACQEIGSPGYKMQLL
               180       190       200       210       220
```

(SEQ ID NO:50)

(SEQ ID NO:48)

Hp10/Fp9

Hp15 *vs.* Fp14
36.4% Identity
63.6% Homology

Fp9 = FIV epitope region recognized by HIV+ subjects
Hp15/Fp14 = epitope region on both HIV-1 & FIV recognized by HIV+ subjects
Hp15 = HIV epitope region recognized by SIV+ macaques

FIG. 7 (continued)

HIV1-HXB2 p24 231 aa vs.
SIV-Delta B670 p24 228 aa
66.4% identity full sequences; gap penalties: -12/-2, Global alignment score: 1036

```
                10        20        30        40        50        60
HIV-1   PIVQNIQGQMVHQAISPRTLNAWVKVVEEKAFSPEVIPMFSALSEGATPQDLNTMLNTVG
        : ::.: :.::  .:::::::::::.::: :. :::: :.:::::: :: :.: ::: ::
SIV     P-VQQIGGNYVHLPLSPRTLDAWVKLIEEKKFGAEVVPGFQALSEGCTPYDINQMLNCVG
                10        20        30        40        50

                70        80        90       100       110
HIV-1   GHQAAMQMLKETINEEAAEWDRVHPVHAGPIAPGQMREPRGSDIAGTTSTLQEQIGWM-T
         ::::::::.. :::::::::  :: . .:   ::.::: ::::::::::::.::: ::
SIV     DHQAAMQIIRDIINEEAADWDLQHP-QPAP-QQGQLREPSGSDIAGTTSSVDEQIQWMYR
       60        70        80           90        100       110

     120       130       140       150       160       170
HIV-1   NNPPIPVGEIYKRWIILGLNKIVRMYSPTSILDIRQGPKEPFRDYVDRFYKTLRAEQASQ
        .. :::::.::::: ::::. :::::.::::.::::::::.::::::::::::::..
SIV     QQNPIPVGNIYRRWIQLGLQRCVRMYNPTNILDVKQGPKEPFQSYVDRFYKSLRAEQTDA
        120       130       140       150       160       170

     180       190       200       210       220       230
HIV-1   EVKNWMTETLLVQNANPDCKTILKALGPAATLEEMMTACQGVGGPGHKARVL
         ::::::.:::.:::::::: .::.::   ::::::.:::::: :::.::: :
SIV     AVKNWMTQTLLIQNANPDCKLVLKGLGVNPTLEEMLTACQGVEGPGQKAR-L
        180       190       200       210       220
```

(SEQ ID NO:50)

(SEQ ID NO:49)

Hp10/Sp10

Hp15 *vs.* Fp14
72.7% Identity
90.9% Homology

Key for p24:
Yellow on SIV sequence = recognized macaque CD8+ CTL epitope (LANL)
Green = Counterpart HIV-1 sequence of known macaque CTL epitope
Aqua Blue = Counterpart FIV sequence of known macaque CTL epitope

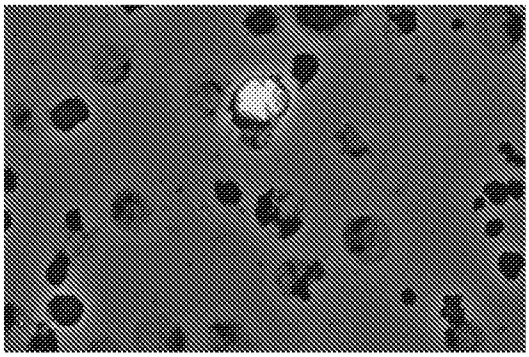


FIG. 8A

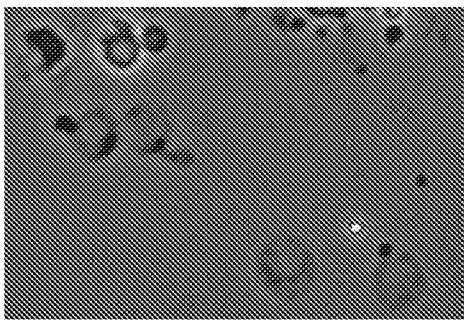


FIG. 8B

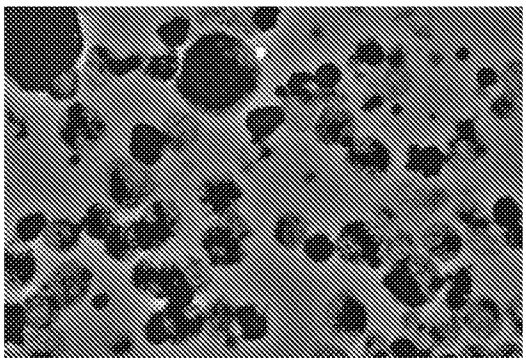


FIG. 8C

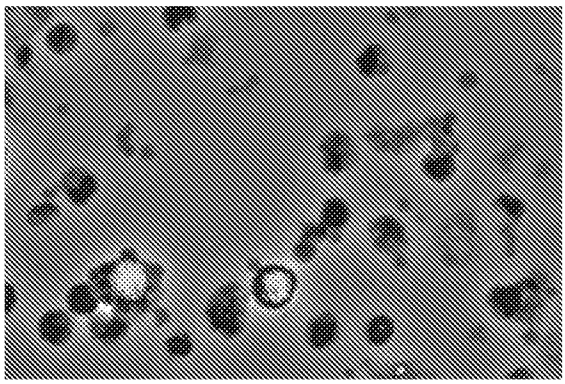


FIG. 9A

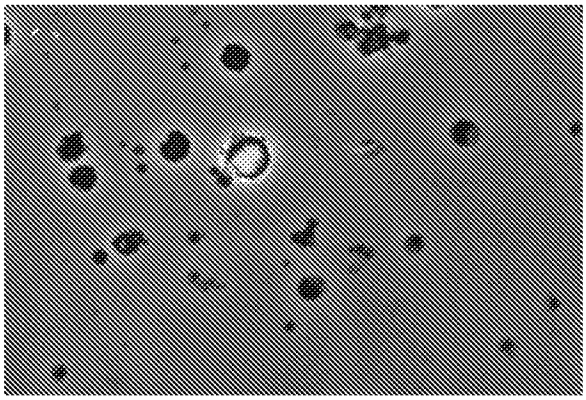


FIG. 9B

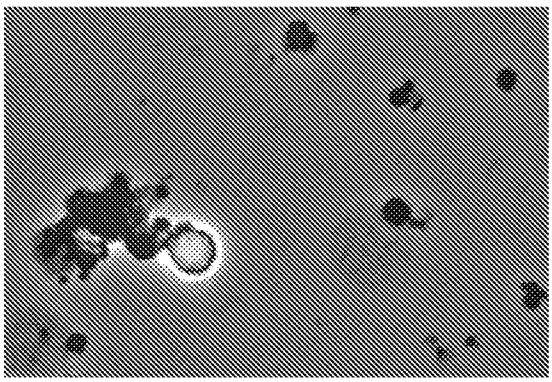


FIG. 9C

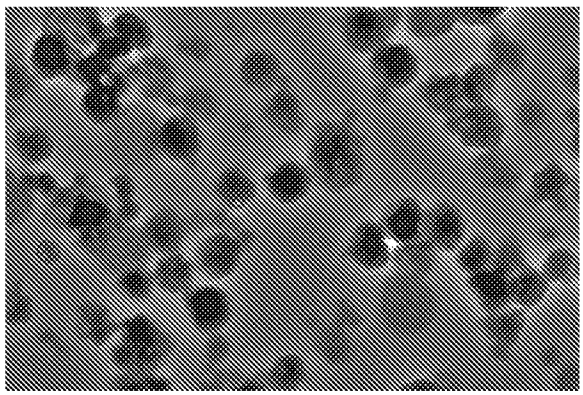


FIG. 9D

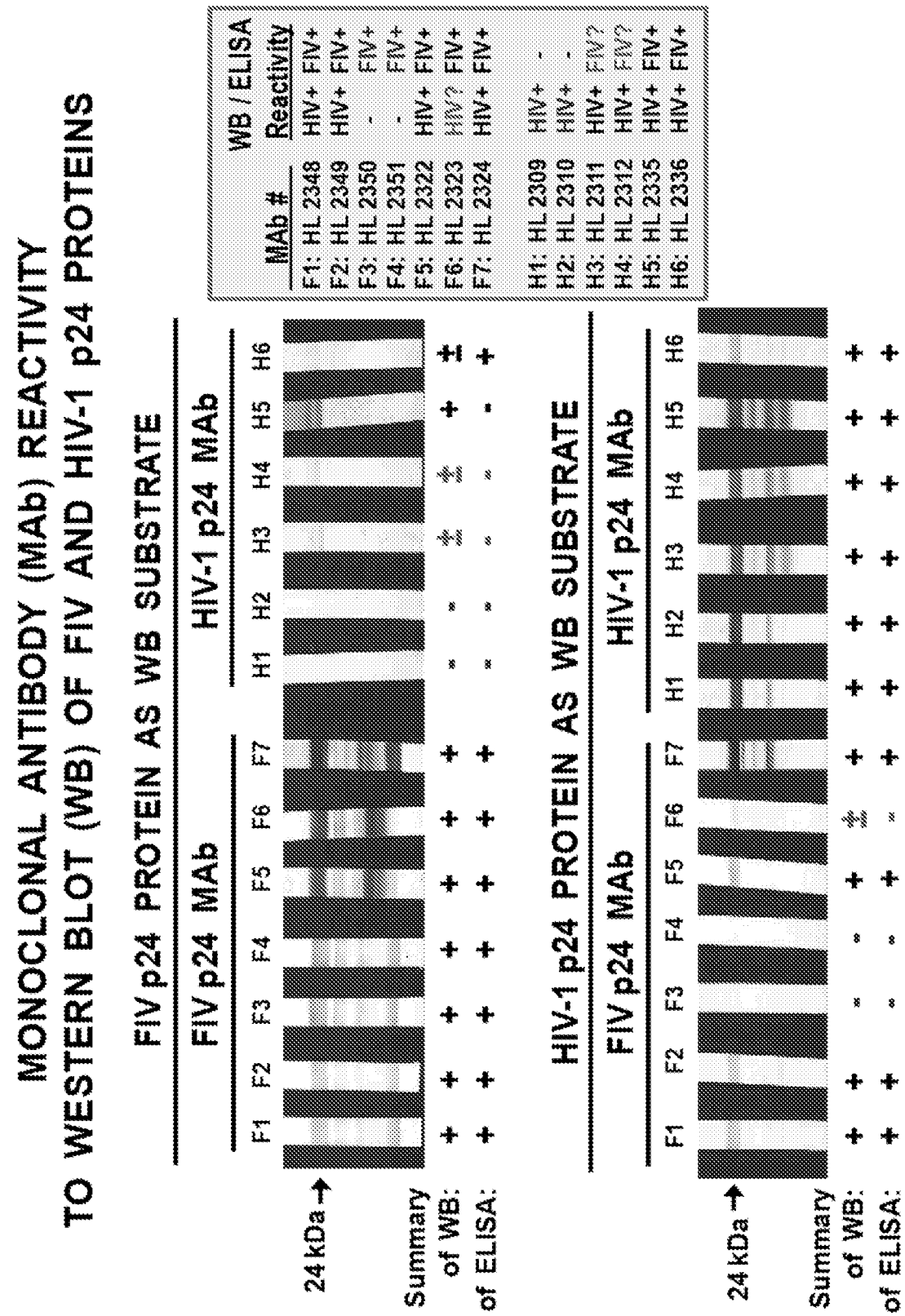

MONOCLONAL ANTIBODY (MAb) REACTIVITY
TO WESTERN BLOT (WB) OF FIV AND HIV-1 p24 PROTEINS
FIV p24 PROTEIN AS WB SUBSTRATE
FIV p24 MAb
HIV-1 p24 MAb
F1 F2 F3 F4 F5 F6 F7 H1 H2 H3 H4 H5 H6
24 kDa
Summary of WB:
of ELISA:
HIV-1 p24 PROTEIN AS WB SUBSTRATE
FIV p24 MAb
HIV-1 p24 MAb
F1 F2 F3 F4 F5 F6 F7 H1 H2 H3 H4 H5 H6
24 kDa
Summary of WB:
of ELISA:
WB / ELISA
MAb # Reactivity
F1: HL 2348 HIV+ FIV+
F2: HL 2349 HIV+ FIV+
F3: HL 2350 - FIV+
F4: HL 2351 - FIV+
F5: HL 2322 HIV+ FIV+
F6: HL 2323 HIV? FIV+
F7: HL 2324 HIV+ FIV+
H1: HL 2309 HIV+ -
H2: HL 2310 HIV+ -
H3: HL 2311 HIV+ FIV?
H4: HL 2312 HIV+ FIV?
H5: HL 2335 HIV+ FIV+
H6: HL 2336 HIV+ FIV+

FIG. 10

FMRT POOL	FRT1	FRT2	FRT3	FRT4	FRT5	FRT6	FRT7	FRT8	FRT9	FRT10	FRT11
CD4+ T Prolif.	2 (8%)	5 (21%)	12 (12%)	3 (4%)	9 (4%)	8 (4%)	12 (4%)	6 (8%)	9 (12%)	4 (12%)	6 (12%)
CD8+ T Prolif.	4 (12%)	4 (21%)	21 (37%)	2 (4%)	3 (6%)	10 (21%)	30 (33%)	6 (21%)	20 (21%)	8 (12%)	5 (23%)
PBMC IFN	110 (21%)	75 (7%)	580 (6%)	74 (14%)	294 (21%)	132 (23%)	181 (16%)	117 (21%)	80 (4%)	110 (21%)	86 (18%)
FMRT POOL	FRT1	FRT2	FRT3	FRT4	FRT5	FRT6	FRT7	FRT8	FRT9	FRT10	FRT11
CD4+ T Prolif.	16 (12%)	8 (4%)	13 (8%)	6 (8%)	4 (6%)	10 (4%)	52 (6%)	10 (8%)	25 (8%)	8 (4%)	16 (8%)
CD8+ T Prolif.	9 (8%)	8 (4%)	5 (12%)	4 (4%)	4 (12%)	3 (12%)	9 (12%)	15 (12%)	7 (17%)	3 (4%)	20 (17%)
PBMC IFN	284 (21%)	108 (29%)	135 (14%)	149 (25%)	184 (39%)	412 (59%)	322 (45%)	127 (21%)	236 (32%)	195 (36%)	880 (57%)

FIG. 11A

FMRT POOL	FRT12	FRT13	FRT14	FRT15	FRT16	FRT17	FRT18	FRT19	FRT20	FRT21	PHA
CD4+ T Prolif.	4 (8%)	8 (12%)	8 (8%)	15 (16%)	9 (8%)	9 (8%)	13 (4%)	0 (0%)	11 (8%)	9 (12%)	48 (79%)
CD8+ T Prolif.	3 (8%)	8 (17%)	5 (37%)	13 (33%)	4 (17%)	15 (12%)	7 (8%)	11 (21%)	9 (12%)	8 (33%)	42 (75%)
PBMC IFN	115 (11%)	124 (36%)	116 (18%)	140 (11%)	85 (18%)	88 (11%)	160 (11%)	90 (7%)	81 (25%)	70 (18%)	3968 (100%)
FMRT POOL	FRT12	FRT13	FRT14	FRT15	FRT16	FRT17	FRT18	FRT19	FRT20	FRT21	PHA
CD4+ T Prolif.	12 (4%)	26 (12%)	5 (8%)	4 (4%)	2 (4%)	0 (0%)	8 (8%)	12 (4%)	2 (4%)	10 (4%)	48 (79%)
CD8+ T Prolif.	6 (12%)	28 (12%)	5 (17%)	5 (12%)	0 (0%)	6 (17%)	5 (12%)	10 (21%)	9 (0%)	4 (17%)	40 (79%)
PBMC IFN	381 (29%)	225 (29%)	230 (59%)	149 (25%)	146 (25%)	95 (18%)	88 (11%)	85 (4%)	149 (14%)	95 (18%)	3968 (100%)

FIG. 11B

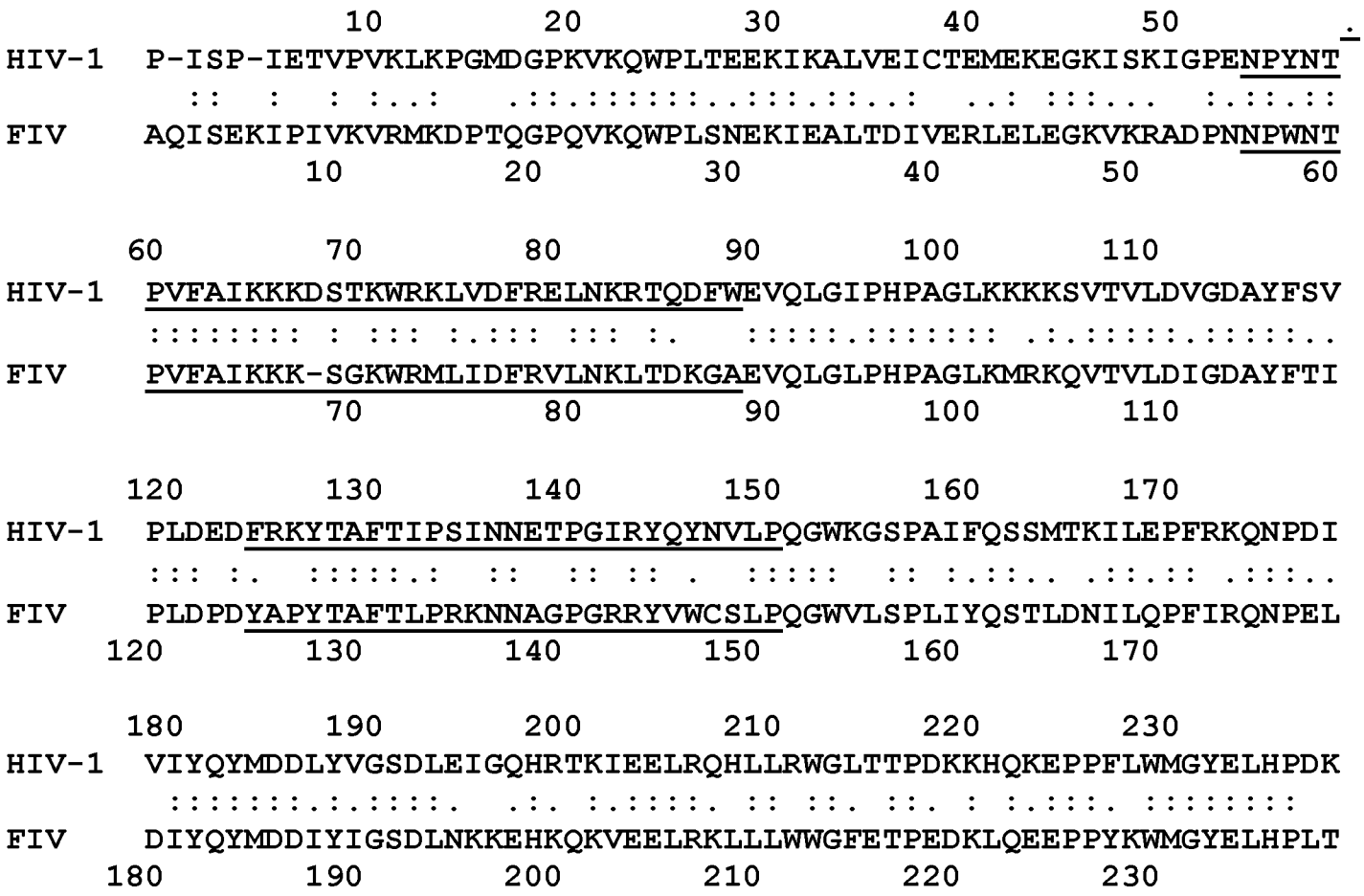
FV p24 POOL	Fp1	Fp2	Fp3	Fp4	Fp5	Fp6		Fp7	Fp8	Fp9
CD4+ T Prolif.	0 (0%)	0 (0%)	3 (4%)	0 (0%)	26 (4%)	5 (4%)	SEQUENCE SHIFT	0 (0%)	3 (4%)	7 (4%)
CD8+ T Prolif.	20 (12%)	4 (4%)	20 (4%)	0 (0%)	0 (0%)	3 (17%)		10 (4%)	4 (6%)	23 (21%)
PBMC IFNγ	180 (6%)	70 (3%)	97 (10%)	156 (13%)	121 (6%)	112 (13%)		93 (16%)	73 (10%)	72 (13%)
FV p24 POOL	Fp1	Fp2	Fp3	Fp4	Fp5	Fp6		Fp7	Fp8	Fp9
CD4+ T Prolif.	7 (4%)	5 (8%)	5 (8%)	5 (4%)	11 (4%)	2 (4%)	0 (0%)	23 (12%)	3 (4%)	5 (12%)
CD8+ T Prolif.	20 (21%)	6 (8%)	0 (0%)	0 (0%)	19 (4%)	2 (12%)	5 (8%)	3 (8%)	22 (12%)	6 (12%)
PBMC IFNγ	252 (29%)	213 (42%)	634 (61%)	212 (29%)	252 (23%)	526 (32%)	721 (39%)	512 (26%)	272 (29%)	215 (48%)

FIG. 12A

FV p24 POOL	Fp10	Fp11	Fp12	Fp13	Fp14	Fp15	Fp16	Fp17	ConA
CD4+ T Prolif.	4 (8%)	3 (12%)	0 (0%)	0 (0%)	7 (4%)	0 (0%)	0 (0%)	8 (4%)	45 (79%)
CD8+ T Prolif.	41 (8%)	4 (8%)	3 (4%)	0 (0%)	23 (8%)	53 (4%)	0 (0%)	0 (0%)	44 (79%)
PBMC IFNγ	144 (13%)	126 (16%)	215 (6%)	107 (16%)	302 (26%)	145 (10%)	85 (3%)	160 (10%)	5031 (100%)
FV p24 POOL	Fp11	Fp12	Fp13	Fp14	Fp15	Fp16	Fp17	Fp18	PHA
CD4+ T Prolif.	0 (0%)	4 (4%)	0 (0%)	0 (0%)	0 (0%)	5 (4%)	48 (4%)	0 (0%)	49 (79%)
CD8+ T Prolif.	32 (12%)	7 (17%)	0 (0%)	4 (4%)	15 (12%)	5 (12%)	0 (0%)	4 (4%)	43 (83%)
PBMC IFNγ	470 (39%)	293 (39%)	120 (10%)	324 (39%)	218 (32%)	211 (19%)	176 (26%)	109 (16%)	4918 (100%)

FIG. 12B

FIG. 13A



HRT3 *vs.* FRT3
(lined section)
69% Identity
77% Homology

HRT6 *vs.* FRT6
(lined section)
52% Identity
63% Homology

FIG. 13A
(continued)

```
      240         250         260         270         280         290
HIV-1 WTVQP--IVLPEKDSWTVNDIQKLVGKLNWASQIYPGIKVRQLCKLLRGTKALTEVIPLT
      :..:   . .:::.   :.:..:::::::::::::  : .....:  ..::: . :  .   :
FIV   WSIQQKQLEIPERP--TLNELQKLVGKINWASQTIPDLSIKELTTMMRGDQRLDSIREWT
     240        250         260         270         280         290

       300         310         320         330         340         350
HIV-1 EEAELELAENREILKEPVHGVYYDPSKDLIAEIQKQGQGQWTYQIYQE-PFKNLKTGKYA
       ::. :. . .: ..  ..  :::::::: :...  :  :  :::.:. : . :  ::.
FIV   TEAKKEVQKAKEAIETQAQLKYYDPSRELYAKLSLVGPHQICYQVYHKNPEHVLWYGKMN
      300         310         320         330         340         350

        360         370         380         390         400         410
HIV-1 RTRGAHTNDVKQLTEAVQKITTESIVIWGKTPKFKLPIQKETWETWW--TEYWQATWIPE
      : .    :      .:  ::  :::.  :: : ...: ..:.::.    . : .:   ::
FIV   RQKKKAENTCDIALRACYKIREESIIRIGKEPIYEIPASREAWESNLIRSPYLKAP-PPE
      360         370         380         390         400         410

          420         430         440
HIV-1 WEFVNTPPLVKLWYQLEKE-PIVGAETFY  (SEQ ID NO:45)
       ::...   .:   .. .. ::.::::.:
FIV   VEFIHAALNIKRALSMIQDTPILGAETWY  (SEQ ID NO:46)
          420         430         440
```

HRT11 *vs.* FRT11
(lined section)
57% Identity
87% Homology

HRT13 vs. FRT13
(lined section)
32% Identity
72% Homology

FIG. 13B

```
                10        20        30        40        50        60
HIV-1  PISPIETVPVKLKPGMDGPKVKQWPLTEEKIKALVEICTEMEKEGKISKIGPENPYNTPV
       ::. .: : : :::: :::.:::::..:::: :: ::: .:::.:.. .  : ::::::.
SIV    PIAKVEPVKVTLKPGKVGPKLKQWPLSKEKIVALREICEKMEKDGQLEEAPPTNPYNTPT
                10        20        30        40        50        60

                70        80        90        100       110       120
HIV-1  FAIKKKDSTKWRKLVDFRELNKRTQDFWEVQLGIPHPAGLKKKKSVTVLDVGDAYFSVPL
       :::::::..::: :.:::::::. :::: :::::::::::::: :.: .:::::.:::::.::
SIV    FAIKKKDKNKWRMLIDFRELNRVTQDFTEVQLGIPHPAGLAKRKRITVLDIGDAYFSIPL
                70        80        90        100       110       120

                     R416      R397,R395; R416; R422 reacted to FIV NPWNTPVFAIKKK peptide
                130       140       150       160       170       180
HIV-1  DEDFRKYTAFTIPSINNETPGIRYQYNVLPQGWKGSPAIFQSSMTKILEPFRKQNPDIVI
       ::.::.:::::.::.:: :: :: :.:::::::::::::: .: ..:::::: :::...
SIV    DEEFRQYTAFTLPSVNNAEPGKRYIYKVLPQGWKGSPAIFQYTMRHVLEPFRKANPDVTL
                130       140       150       160       170       180

                     R397,R425,R426
                190       200       210       220       230       240
HIV-1  YQYMDDLYVGSDLEIGQHRTKIEELRQHLLRWGLTTPDKKHQKEPPFLWMGYELHPDKWT
       :::::. ...:: .:  . .:... :  :...::...: ::.::: :::::: : ::
SIV    VQYMDDILIASDRTDLEHDRVVLQLKELLNSIGFSTPEEKFQKDPPFQWMGYELWPTKWK
                190       200       210       220       230       240
```

HRT3 vs. SRT3
77% Identity
91% Homology

HRT6 vs. SRT6
71% Identity
84% Homology

FIG. 13B
(continued)

```
             250       260       270       280       290       300
HIV-1  VQPIVLPEKDSWTVNDIQKLVGKLNWASQIYPGIKVRQLCKLLRGTKALTEVIPLTEEAE
       .: : ::.....:::::::::::: ::::.:::::::::::..:::.::  .:::: .  :: ::
SIV    LQKIELPQRETWTVNDIQKLVGVLNWAAQIYPGIKTKHLCRLIRGKMTLTEEVQWTEMAE
             250       260       270       280       290       300
                                R414,R425,R426
             310       320       330       340       350       360
HIV-1  LELAENREILKEPVHGVYYDPSKDLIAEIQKQGQGQWTYQIYQEPFKNLKTGKYARTRGA
        :  ::. ::..  .: ::. .: : : . :. ..:::::::::  : ::.::::. ...
SIV    AEYEENKIILSQEQEGCYYQEGKPLEATVIKSQDNQWSYKIHQED-KILKVGKFAKIKNT
            310       320       330       340       350
                                   R397,R426
             370       380       390       400       410       420
HIV-1  HTNDVKQLTEAVQKITTESIVIWGKTPKFKLPIQKETWETWWTEYWQATWIPEWEFVNTP
       ::: :. :.....:::  :.:::::::::::::::..:: :::.:::.:::::::.:..::
SIV    HTNGVRLLAHVIQKIGKEAIVIWGQVPKFHLPVERDVWEQWWTDYWQVTWIPEWDFISTP
     360       370       380       390       400       410

             430       440
HIV-1  PLVKLWYQLEKEPIVGAETFY   (SEQ ID NO:45)
       ::::.: ..: :.::: : :: :
SIV    PLVRLVFNLVKDPIEGEET-Y   (SEQ ID NO:47)
     420       430
```

HRT11 vs. SRT11
71% Identity
91% Homology

HRT14 vs. SRT14
47% Identity
82% Homology

FIG. 14A

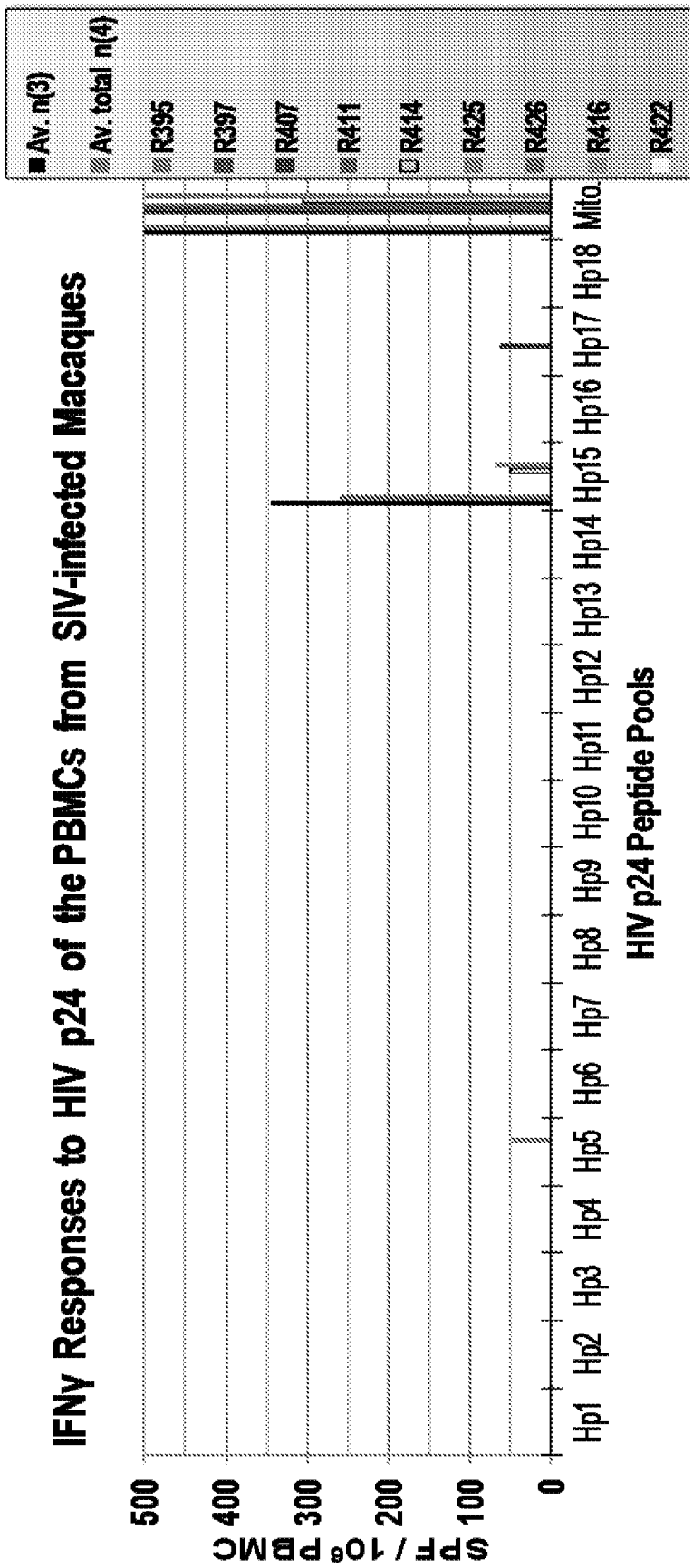

IFNγ Responses to HIV p24 of the PBMCs from SIV-infected Macaques
Av. n(3)
Av. total n(4)
R395
R397
R407
R411
R414
R425
R426
R416
R422
SPF / 10⁶ PBMC
500
400
300
200
100
0
Hp1 Hp2 Hp3 Hp4 Hp5 Hp6 Hp7 Hp8 Hp9 Hp10 Hp11 Hp12 Hp13 Hp14 Hp15 Hp16 Hp17 Hp18 Mito.
HIV p24 Peptide Pools

FIG. 14B

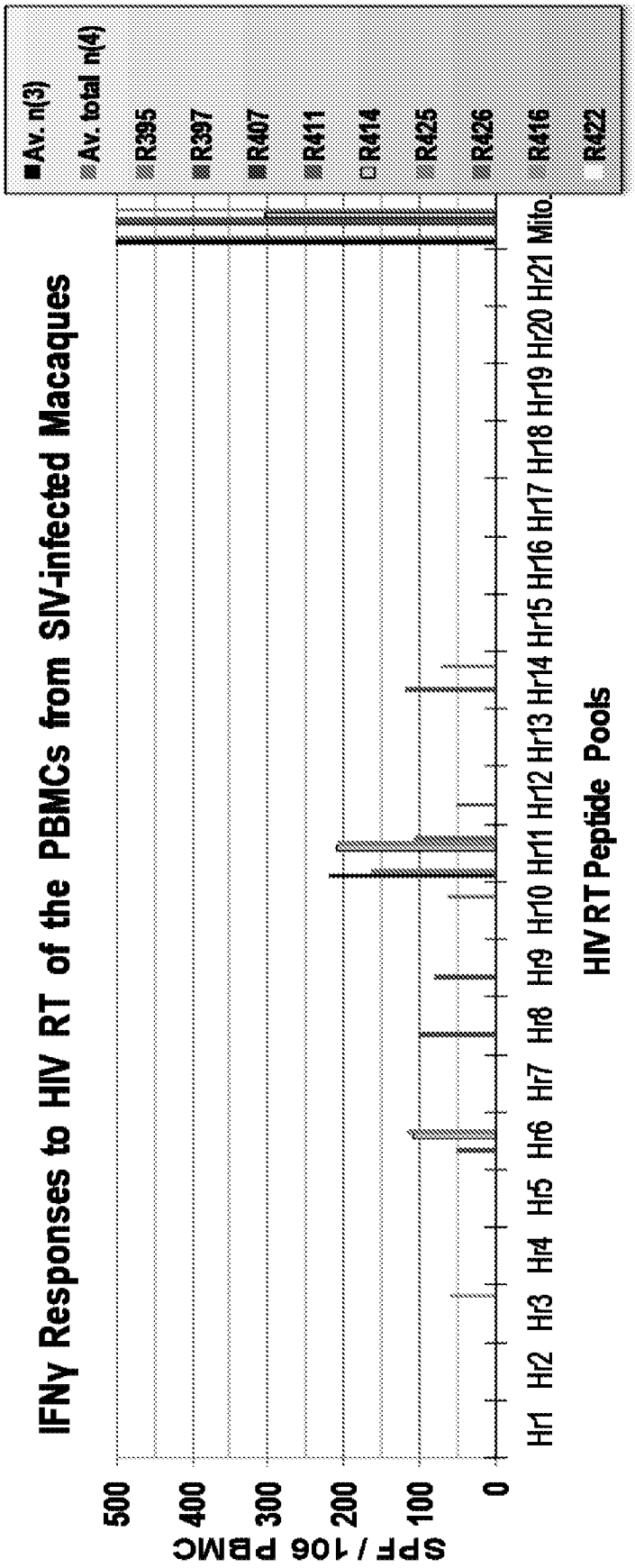

IFNγ Responses to HIV RT of the PBMCs from SIV-infected Macaques
Av. n(3)
Av. total n(4)
R395
R397
R407
R411
R414
R425
R426
R416
R422
SFF / 106 PBMC
500
400
300
200
100
0
Hr1 Hr2 Hr3 Hr4 Hr5 Hr6 Hr7 Hr8 Hr9 Hr10 Hr11 Hr12 Hr13 Hr14 Hr15 Hr16 Hr17 Hr18 Hr19 Hr20 Hr21 Mito.
HIV RT Peptide Pools

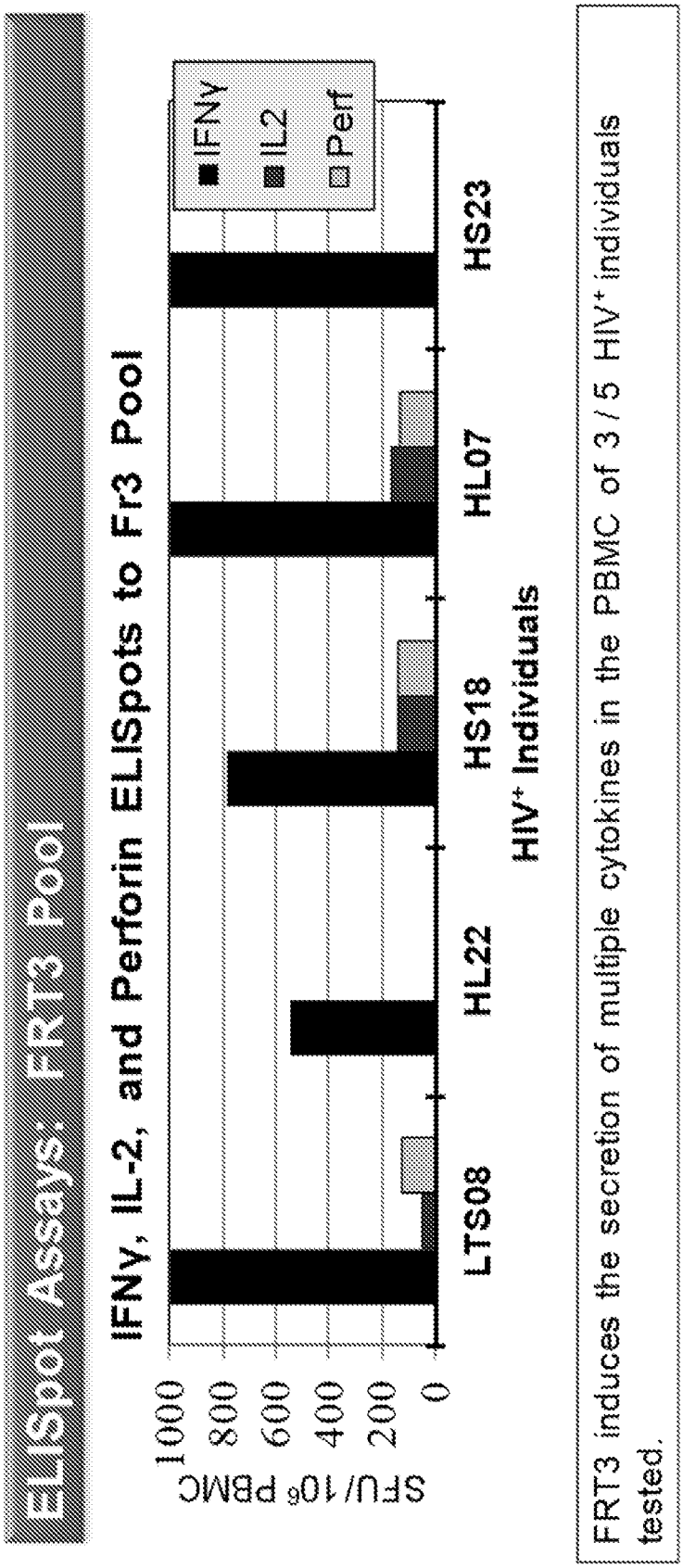
FIG. 15A

HIV-1 AND FIV p24 SEQUENCES AND INDIVIDUAL PEPTIDES IN THE PEPTIDE POOLS
A
p24 aa 1-116
Fp1 VALDPRMVSIIMEKA
Fp2 KAREGLGGEEVQLWF
Fp3 LWETAFSAWLTPTDM
Fp4 PTDMATLIMAAPGCA
Fp5 APGCAADKEILDESL
Fp6 KQLTAEYDRTHP
Fp7 PLPYITAAEIMGIGL
Fp8 LT QEQ
Hp1 NLQGQMVHQAISPRT
Hp2 PRTLNAWVKVVEEKA
Hp3 SPEVIPMFSALSEGA
Hp4 SALSEGATPQDLNTM
Hp5 NTVGGHQAMQMLKE
Hp6 AMQMLKETINEEAAE
Hp7 DRLHPVHAGPIAPGQ
Hp8 MGIGLT QEQ
Hp9 GSDIAGTTSTLQEQI
HIV-1: PIVQNLQGQMVHQAISPRTLNAWVKVVEEKAFSPEVIPMFSALSEGATPQDLNTMLNTVGGHQAAMQMLKETINEEAAEWDRLHPVHAGPIAPGQMREPRGSDIAGTTSTLQEQIG
FIV: PI-QTVNGAPQYVALDPRMVSIIMEKAREGLGGEEVQLWFTAFSAWLTPTDMATLIMAAPGCAADKEILDESLKQLTAEYDRTHP----PDGPRLPYTTAAEIMGIGLT-QEQ--
PIVQNLQGQMVHQAI EKAFSPEVIPMFSAL NTMLNTVGGHQAAMQ AAEWDRLHPVHAGPI
QMVHQAISPRTLNAW IPMFSALSEGATPQD GHQAAMQMLKETINE PVHAGPIAPGQMREPH AGTTSTLQEQIG
QAISPRTLNAWVKVV SALSEGATPQDLNTM AMQMLKETINEEAAE GPIAPGQMREPRGSD STLQEQIG
NAWVKVVEEKAFSPE PQDLNTMLNTVGGHQ LKETINEEAAEWDRL PGQMREPRGSDIAGT EQIG
KVVEEKAFSPEVIPMF INEEAAEWDRLHPVH REPRGSDIAGTTSTL

FIG. 15B

B
p24 aa 117-230
Fp14 KLYLKQSLSIANA
QAEARFAPAR Fp10 LEALGKLAAIKAK Fp12 RQGAKEDYSSFIDRL AEVKLVLKQSLSIA Fp16 LKPESTLEEKLRA
QAEARF RAWYLEALGKLAAIK V QLRQGAKEDYSSFI EQNTAEVKLYLKQSL AMSHLKPESTLEEKL
QA RMQCRAWYLEALGKL PRAV QLRQGAKEDY DQEQNTAEVKLYLK PDCKRAMSHLKPESTL
Fp8 FAPARMQCRAWYLEA Fp11 KAKSPRAV QLRQGAK Fp13 FAQIDQEQNTAEVKL Fp16 ANAMPDCKRAMSHLK Fp17 RACQEVGSPGYRMQLL
AEAKFAPARMQCRAW LAAIKAKSPRAV QLR DRLFAQIDQEQNTA LSIANANPDCKRAM LEEKLRACQEVGSPGY
QAEARFAPARM ALGKLAAIKAKSPRA KEDYSSFIDRLFAQI YLKQSLSIANANPDCK PESTLEEKLRACQEV
-QAEARFAPARMQCRAWYLEALGKLAAIKAKSPRAV-QLRQGAKEDYSSFIDRLFAQIDQEQNTAEVKLYLKQSLSIANANPDCKRAMSHLKPESTLEEKLRACQEVGSPGYRMQLL
117 PVGEIYKRWIILGLN SILDIRQGPKEPFRD RAEQASQEVKNWMTE DCKTILKALGPAATL 230
WMT Hp11 IYKRWIILGLNKIVR Hp13 IRQGPKEPFRDYVDR Hp15 ASQEVKNWMTETLIV Hp17 ILKALGPAATLEMM
WMTNMPP WIILGLNKIVRMYSP PKEPFRDYVDRFYKT VKNWMTETLIVQNAN LGPAATLEMWTACQ
Hp10 WMTNMPPIPVG Hp12 GLNKIVRMYSPTSIL Hp14 IRDYVDRFYKTLRAE Hp16 MTETLLVQNANPDCK Hp18 ATLEMWTACQGVGG
WMTNMPPIPVGEIYK IVRMYSPTSILDIRQ VDRFYKTLRAEQASQ LLVQNANPDCKTILK EMWTACQGVGGPGHK
NPPIPVGEIYKRWII YSPTSILDIRQGPKE YKTLRAEQASQEVKN NANPDCKTILKALGP ACQGVGGPGHKARVL

FIG. 16


ELISpot Assays: FRT3 Pool
IFNγ, IL-2, and Perforin ELISpots to Fr3 Pool
IFNγ
IL2
Perf
SFU/ 10⁶ PBMC
1000
800
600
400
200
0
LTS08
HL22
HS18
HL07
HS23
HIV+ Individuals
FRT3 induces the secretion of multiple cytokines in the PBMC of 3 / 5 HIV+ individuals tested.

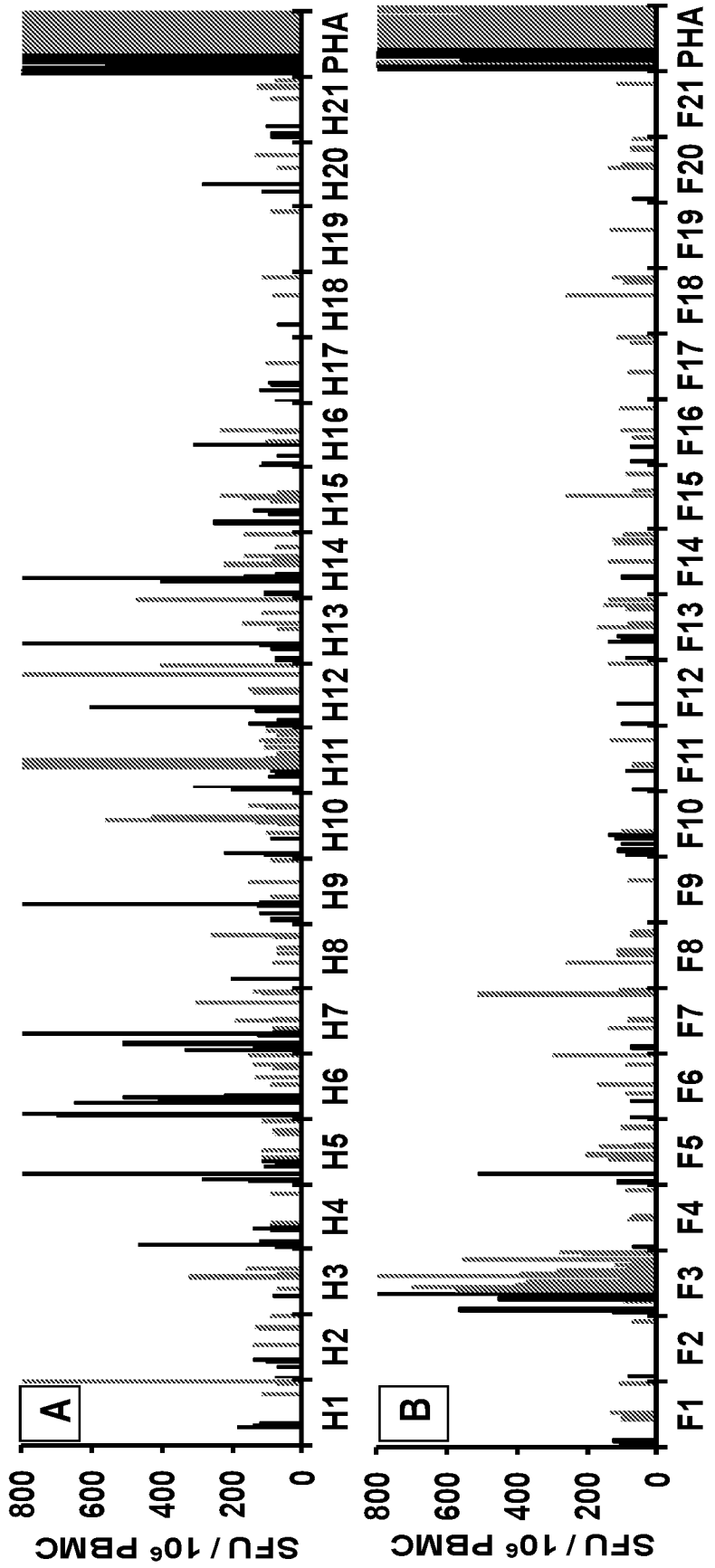


FIG. 17A

FIG. 17B

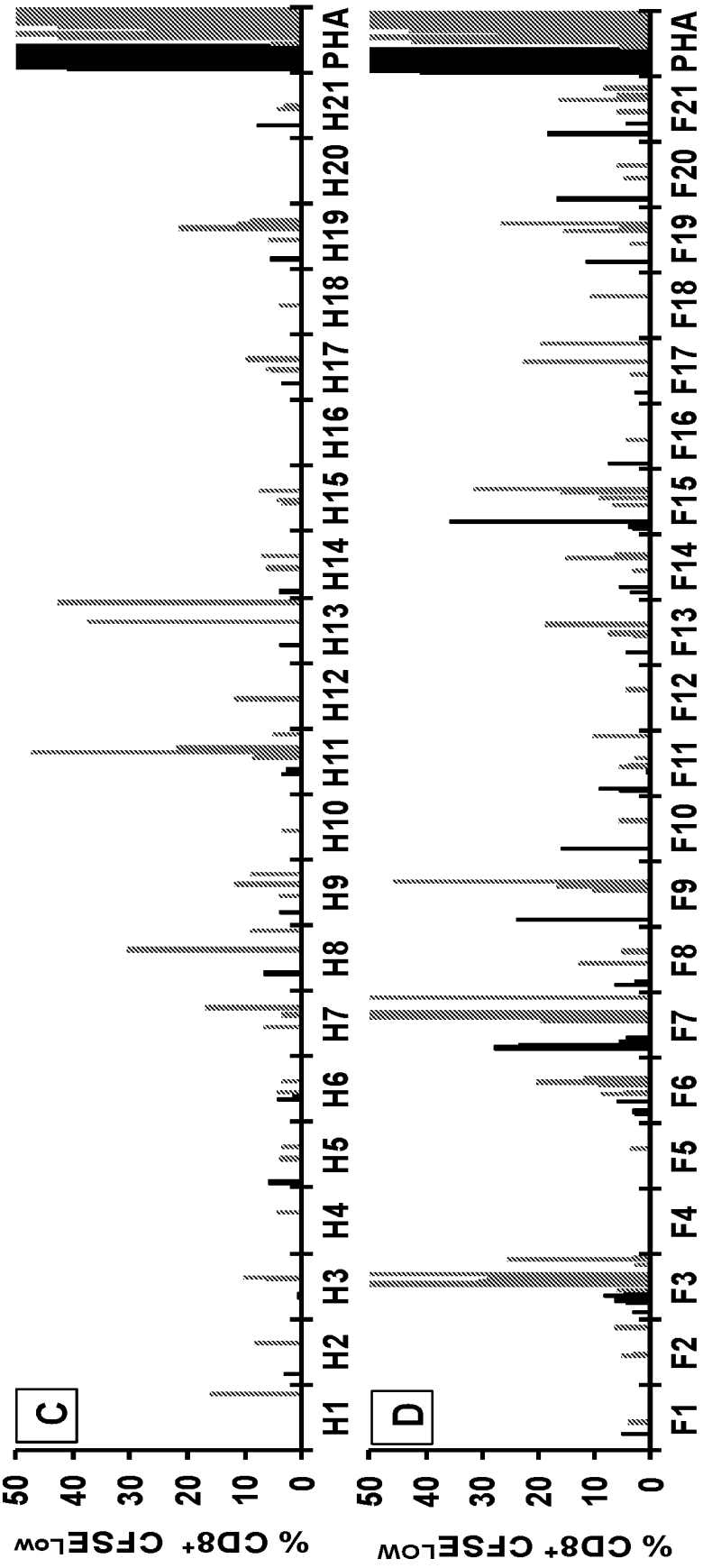


FIG. 17C

FIG. 17D

FIG. 17F

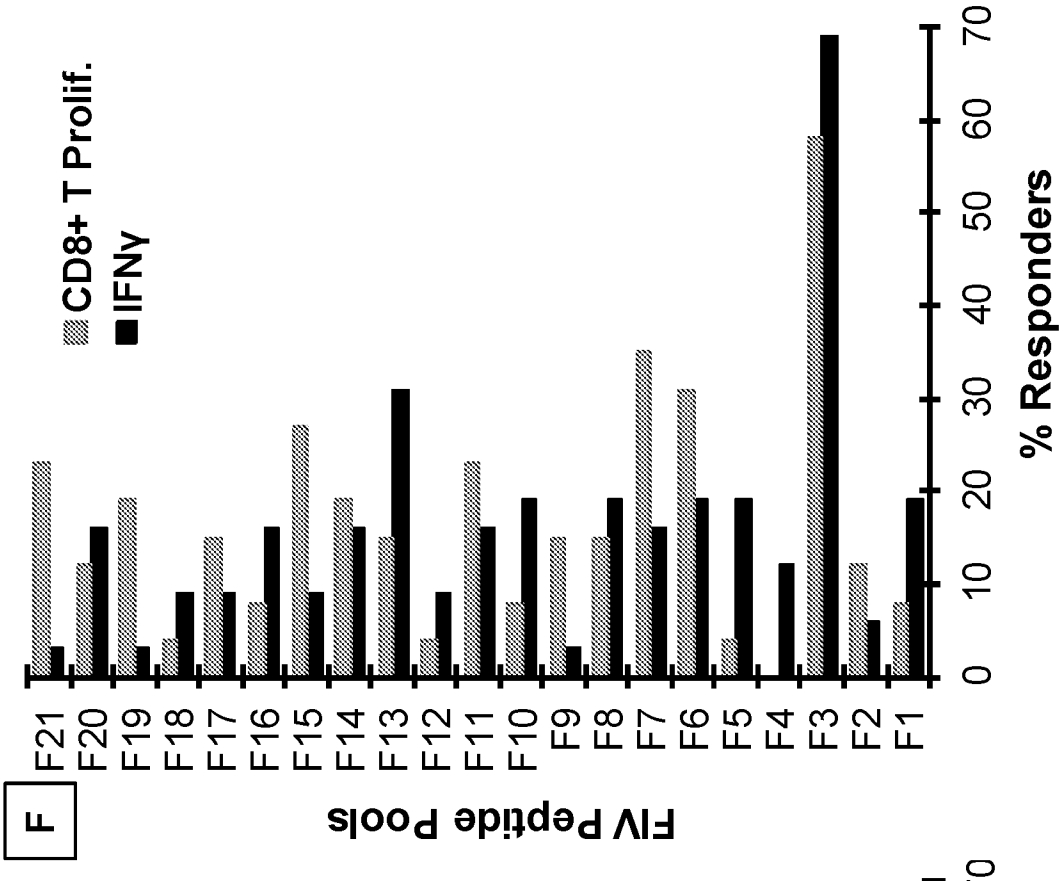
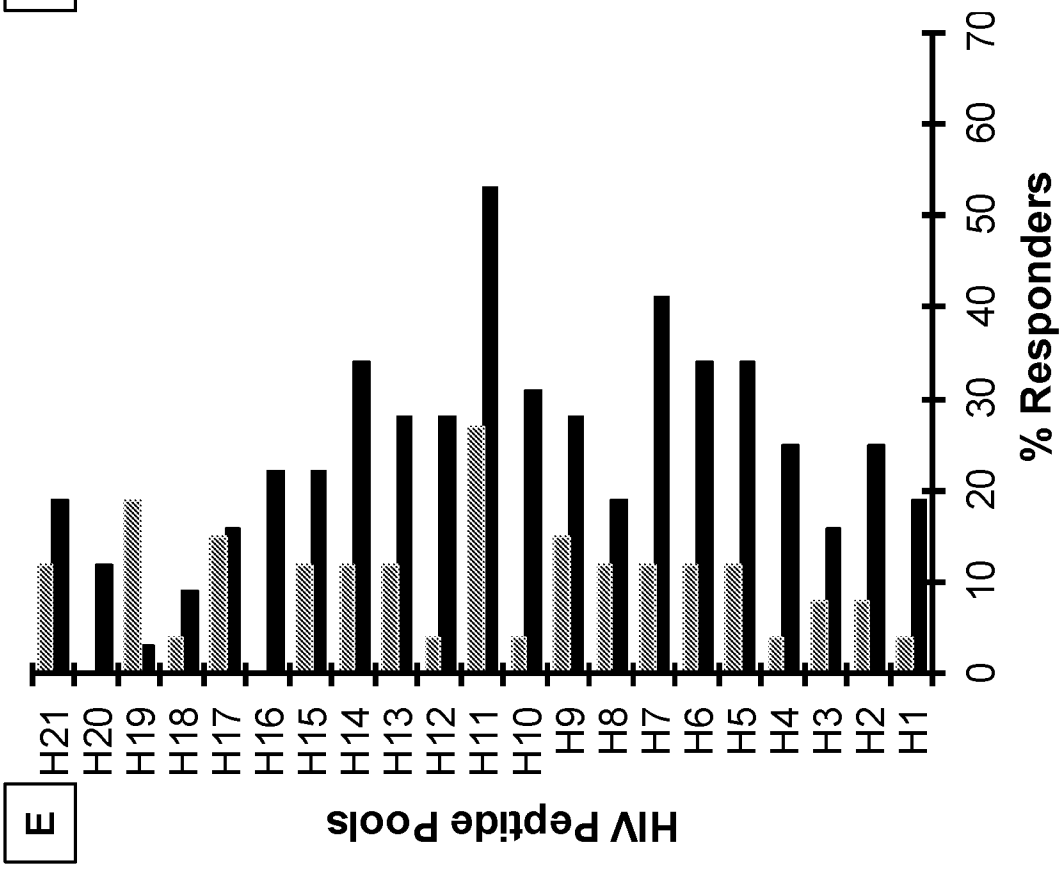


FIG. 17E



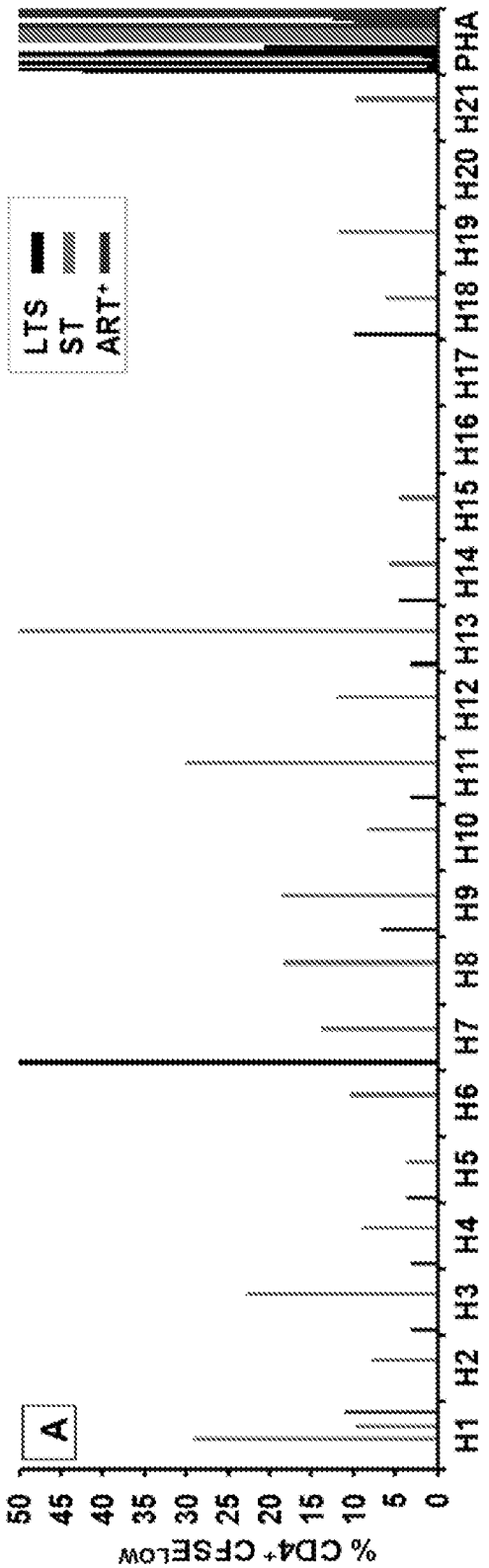


FIG. 18A

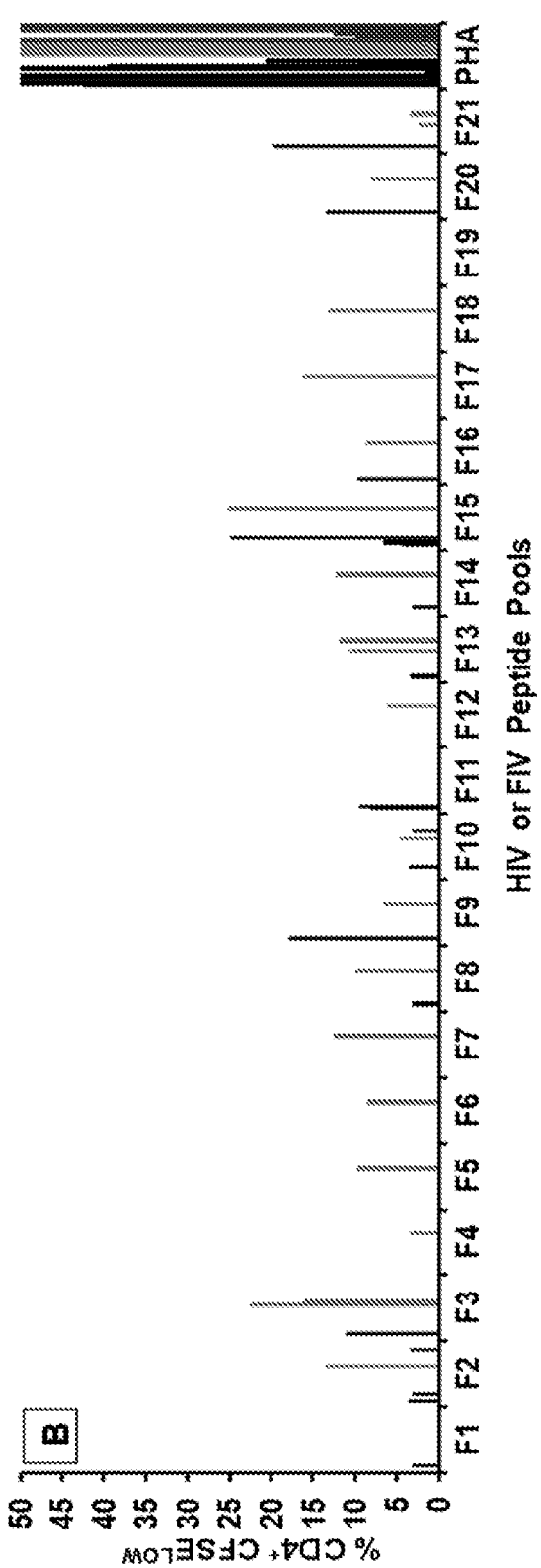


FIG. 18B

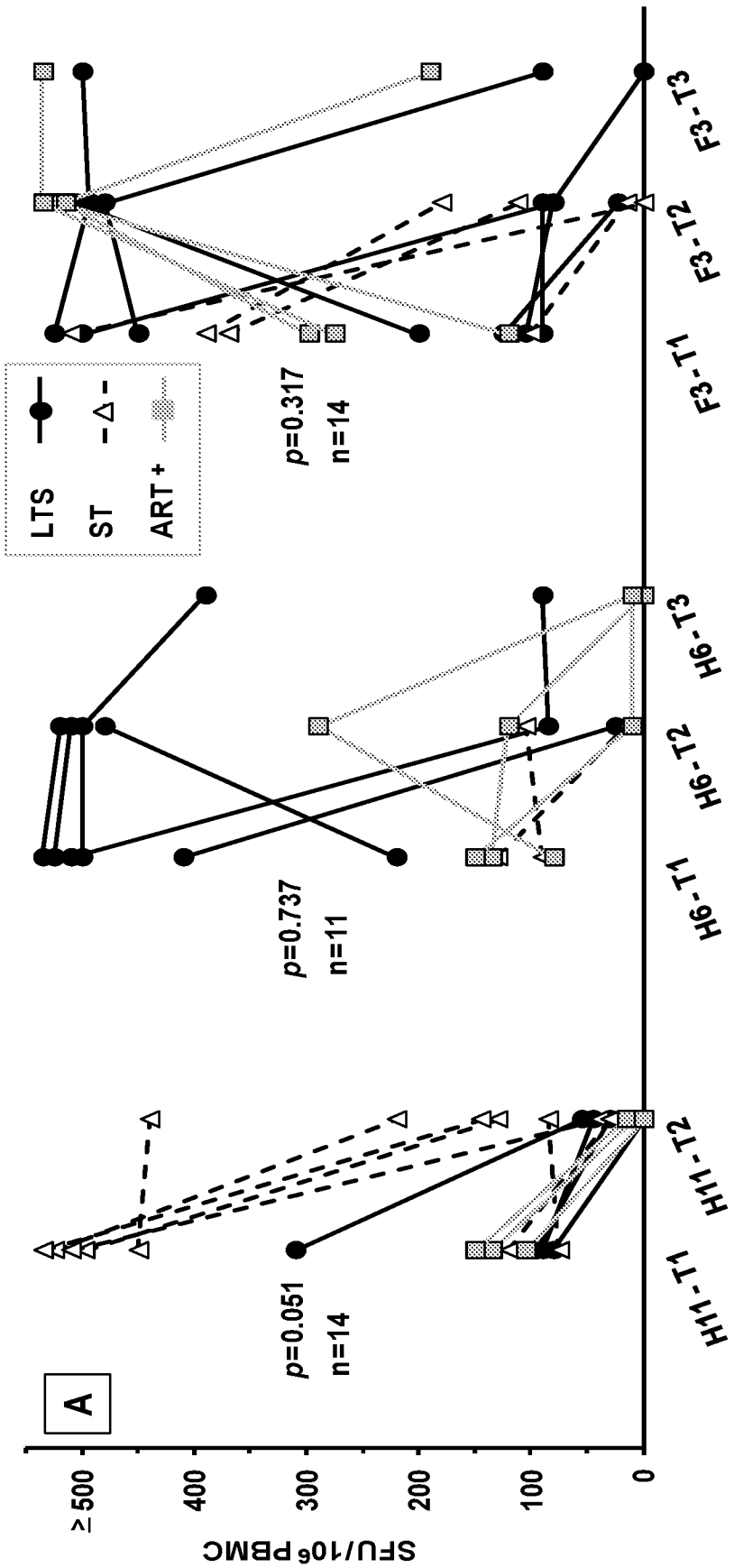

A
SFU/10^6 PBMC
≥ 500
400
300
200
100
0
p=0.051
n=14
p=0.737
n=11
p=0.317
n=14
H11-T1
H11-T2
H6-T1
H6-T2
H6-T3
F3-T1
F3-T2
F3-T3
LTS
ST
ART +

FIG. 19A

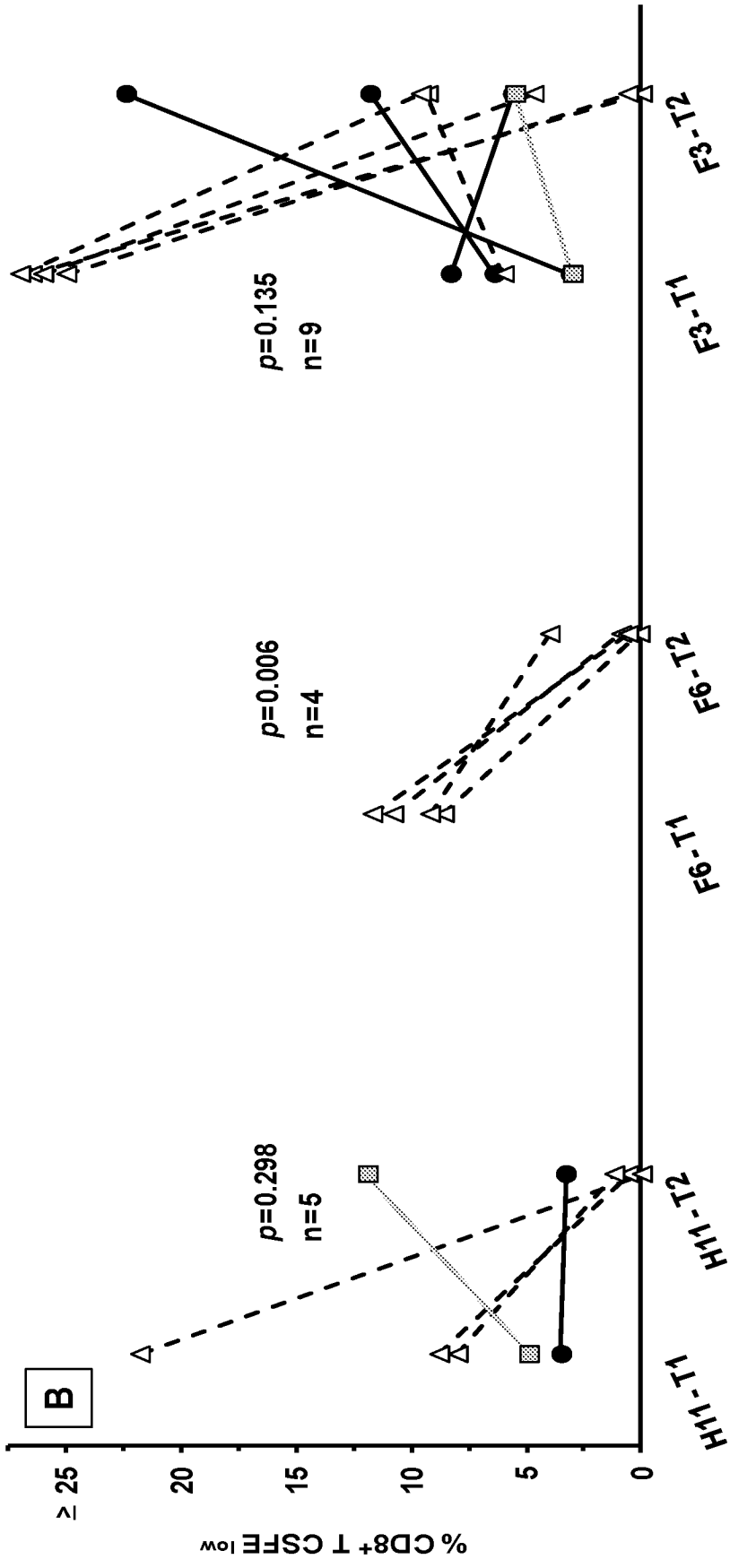

B
% CD8+ T CSFE low
≥ 25
20
15
10
5
0
H11-T1
H11-T2
F6-T1
F6-T2
F3-T1
F3-T2
p=0.298
n=5
p=0.006
n=4
p=0.135
n=9

FIG. 19B

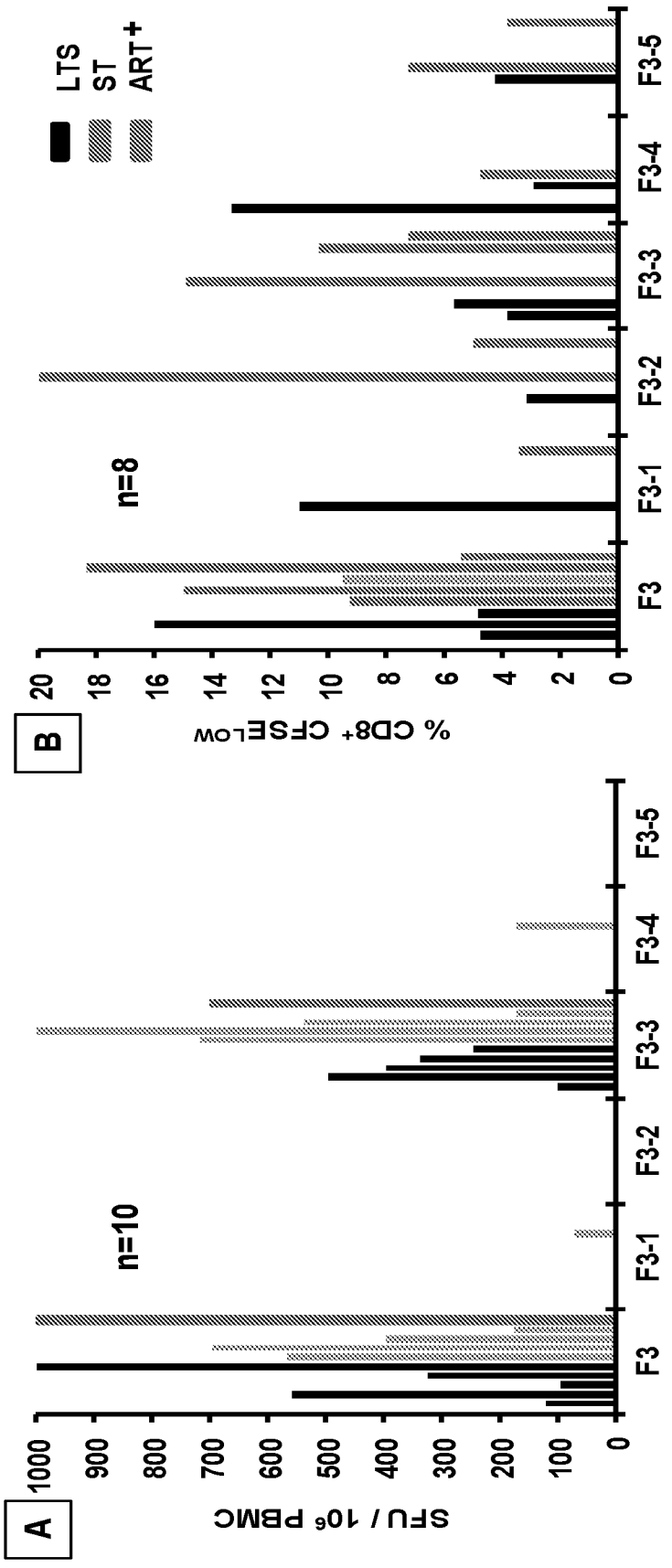


FIG. 20A

FIG. 20B

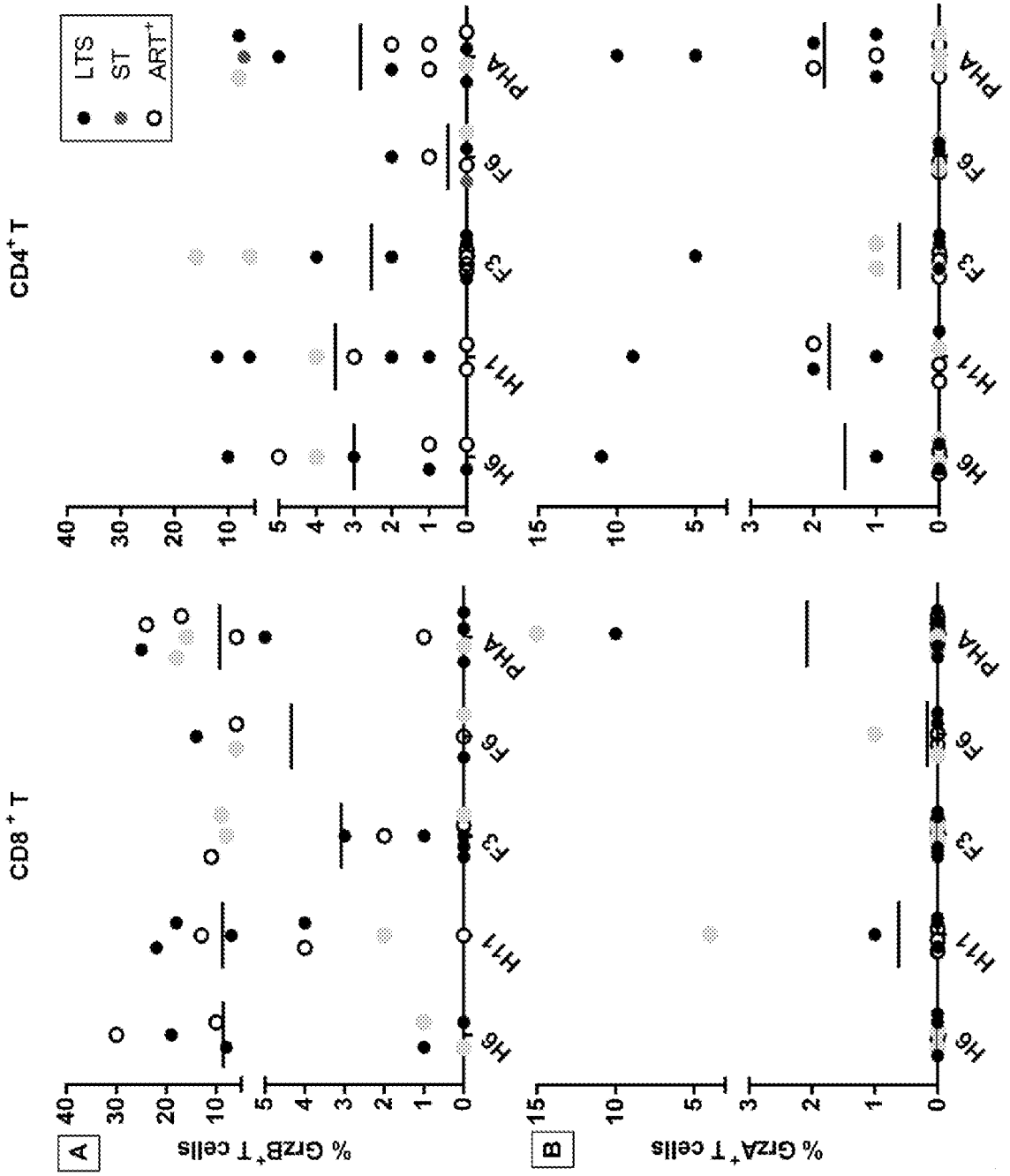


FIG. 21A

FIG. 21B

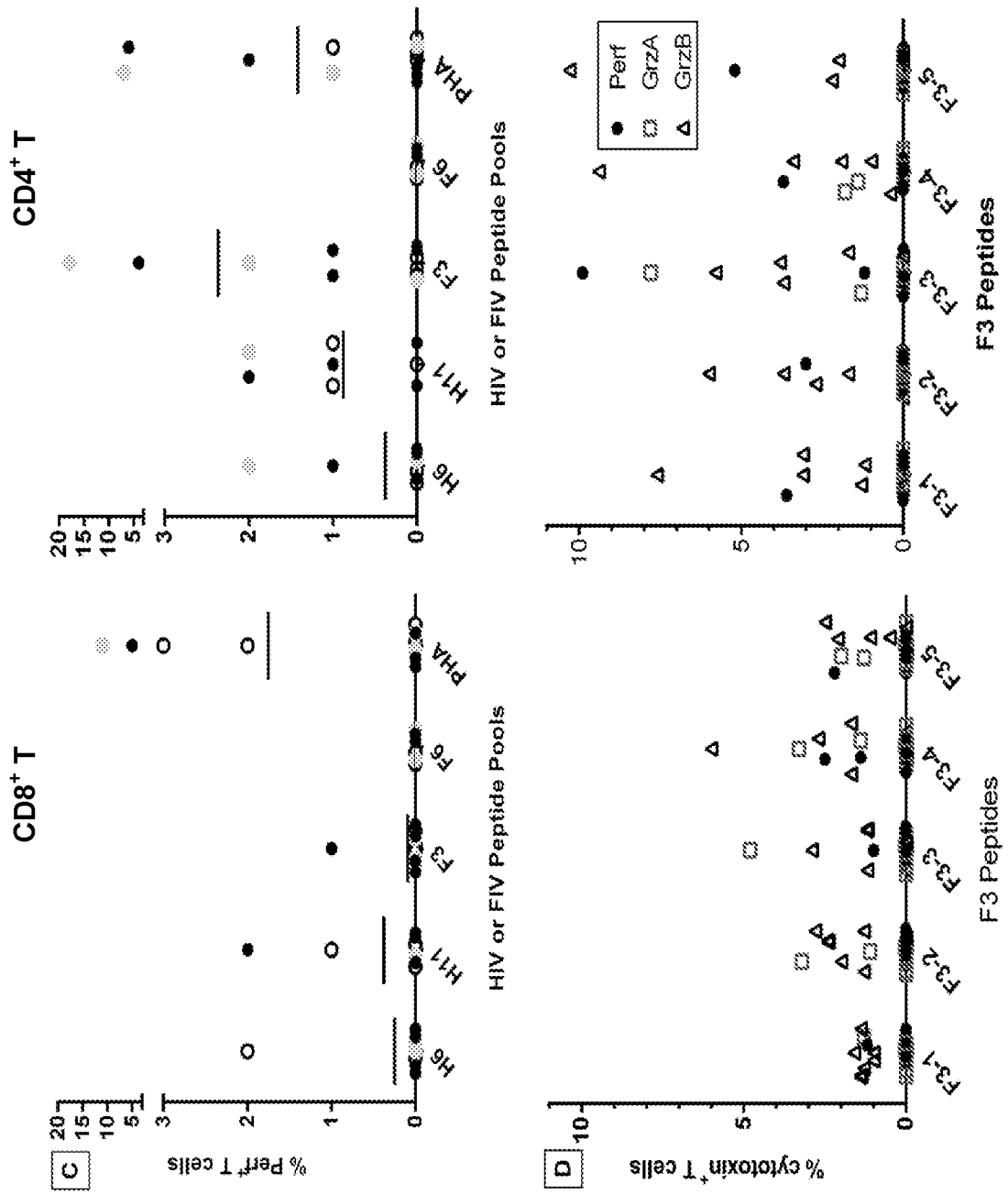


FIG. 21C

FIG. 21D

A

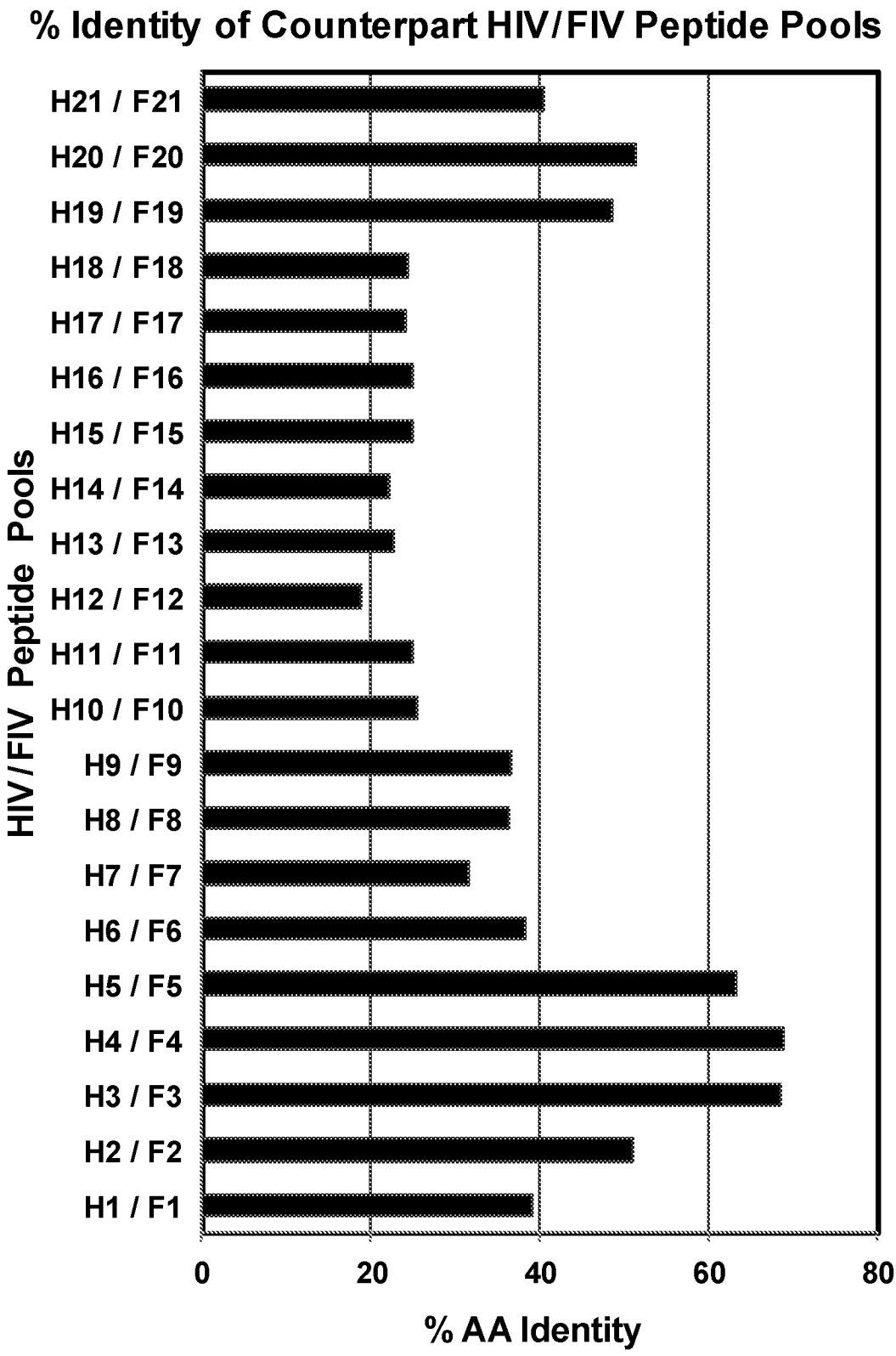

% Identity of Counterpart HIV/FIV Peptide Pools
HIV/FIV Peptide Pools
H21 / F21
H20 / F20
H19 / F19
H18 / F18
H17 / F17
H16 / F16
H15 / F15
H14 / F14
H13 / F13
H12 / F12
H11 / F11
H10 / F10
H9 / F9
H8 / F8
H7 / F7
H6 / F6
H5 / F5
H4 / F4
H3 / F3
H2 / F2
H1 / F1
0
20
40
60
80
% AA Identity

FIG. 22A

B

% AA SEQUENCE IDENTITY		% AA SEQUENCE SIMILARITY			
		HIV-1	FIV	SIV	CAEV
		Peptide 3-1			
Peptide 3-1	HIV-1		92	100	92
	FIV	69		100	92
	SIV	92	85		85
	CAEV	62	69	62	
		Peptide 3-2			
Peptide 3-2	HIV-1		81	100	81
	FIV	81		87	100
	SIV	80	69		87
	CAEV	69	87	69	
		Peptide 3-3			
Peptide 3-3	HIV-1		75	93	75
	FIV	69		87	93
	SIV	75	75		87
	CAEV	69	93	75	
		Peptide 3-4			
Peptide 3-4	HIV-1		71	86	93
	FIV	64		92	85
	SIV	71	77		93
	CAEV	79	85	86	
		Peptide 3-5			
Peptide 3-5	HIV-1		71	86	79
	FIV	50		86	71
	SIV	71	50		86
	CAEV	64	64	64	

FIG. 22B

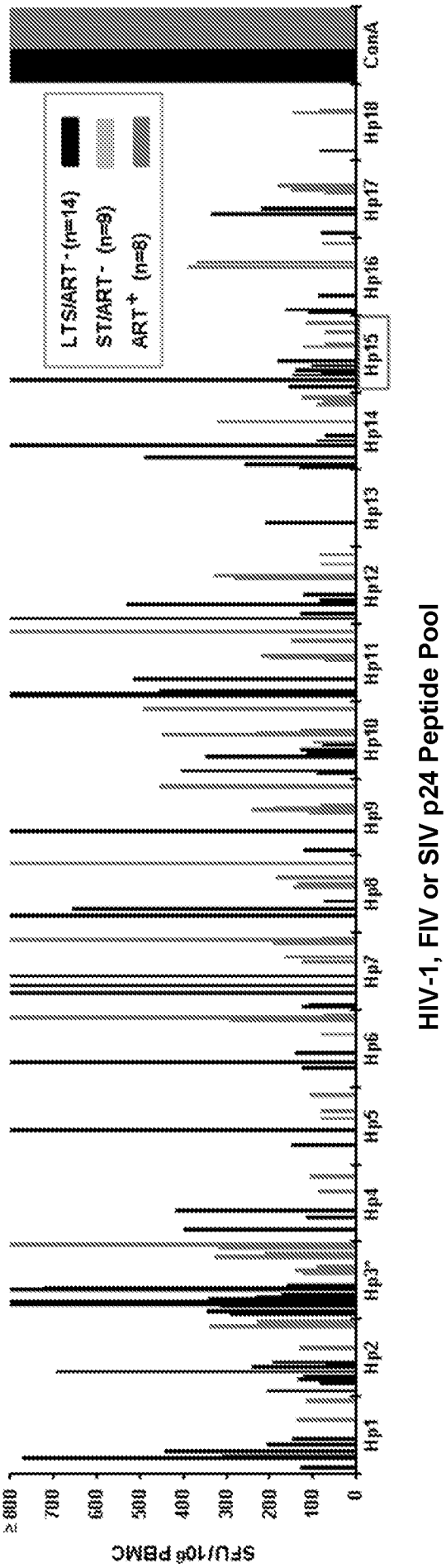

LTS/ART+ (n=14)
STI/ART- (n=9)
ART+ (n=8)
SFU/10e PBMC
≥800
700
600
500
400
300
200
100
0
Hp1
Hp2
Hp3*
Hp4
Hp5
Hp6
Hp7
Hp8
Hp9
Hp10
Hp11
Hp12
Hp13
Hp14
Hp15
Hp16
Hp17
Hp18
ConA
HIV-1, FIV or SIV p24 Peptide Pool

FIG. 23A

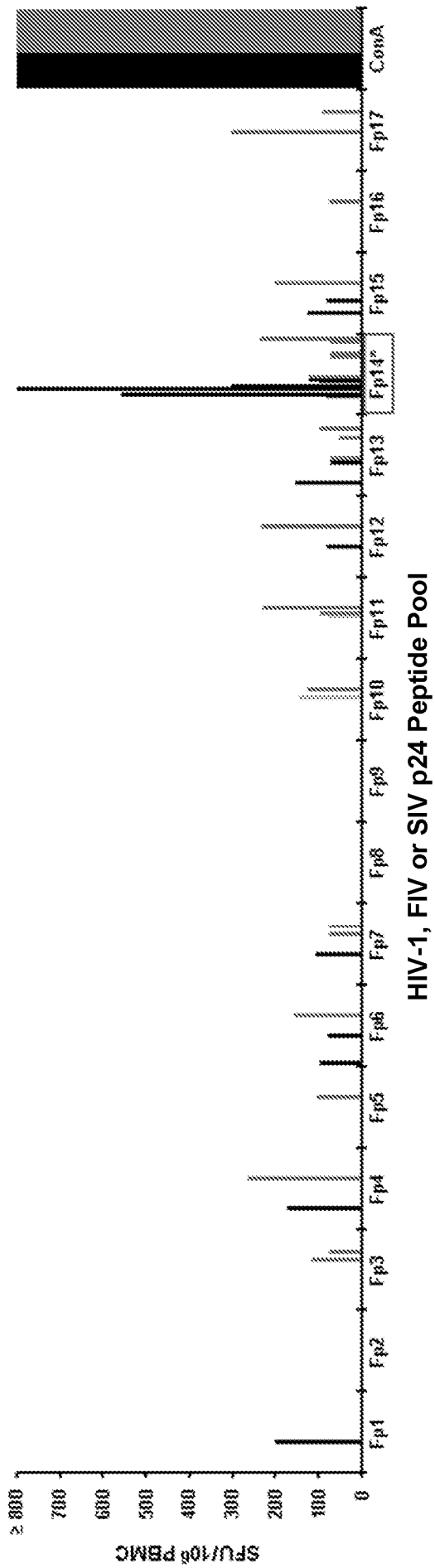


FIG. 23B

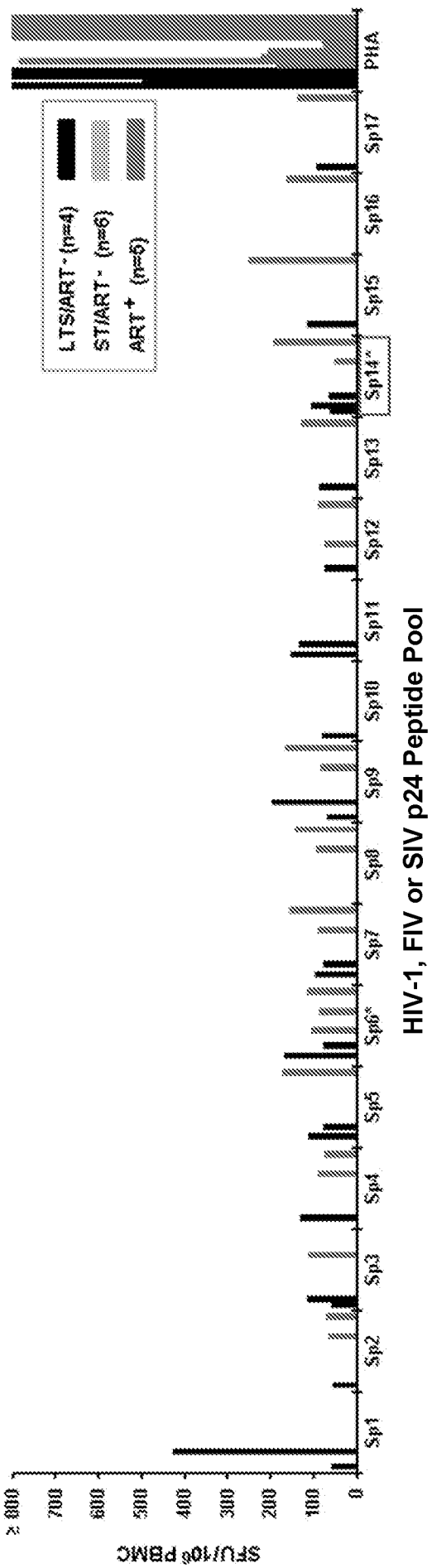


FIG. 23C

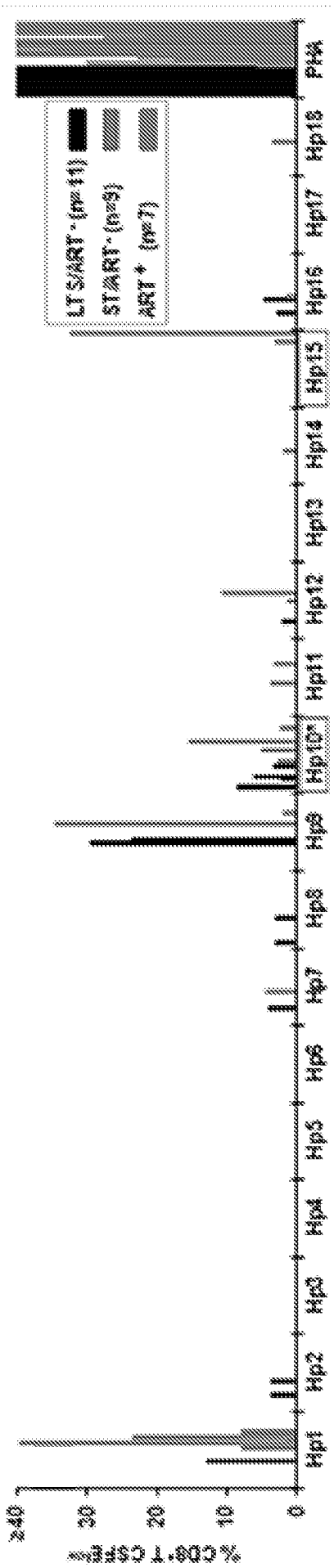


FIG. 24A

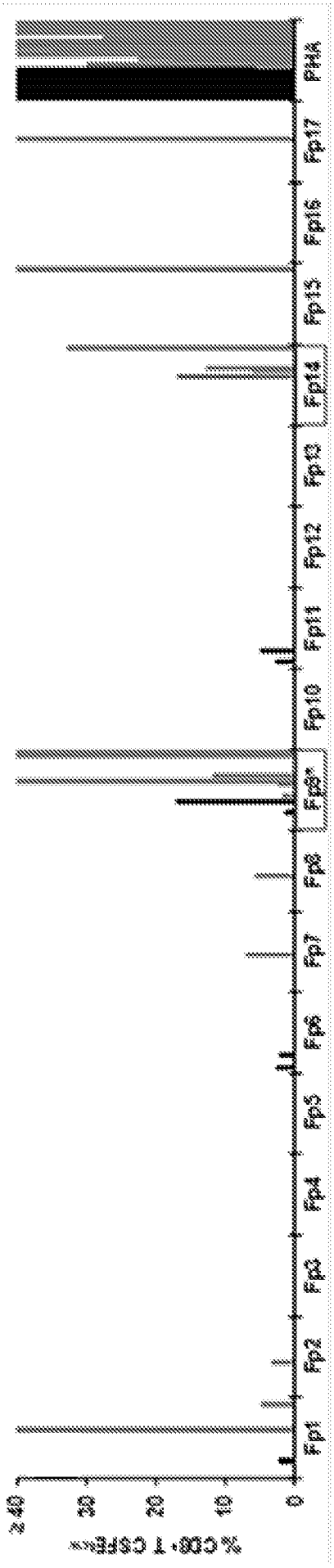


FIG. 24B

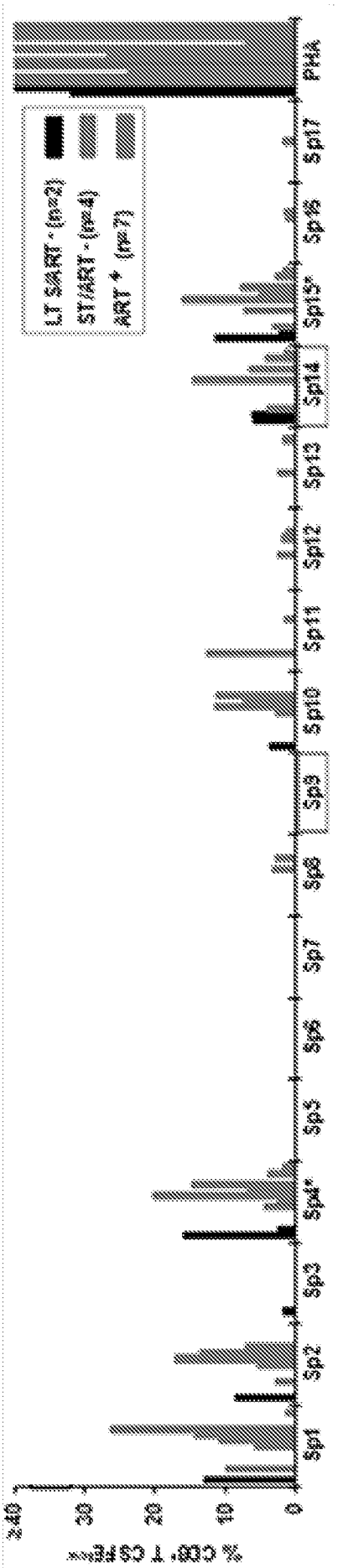


FIG. 24C

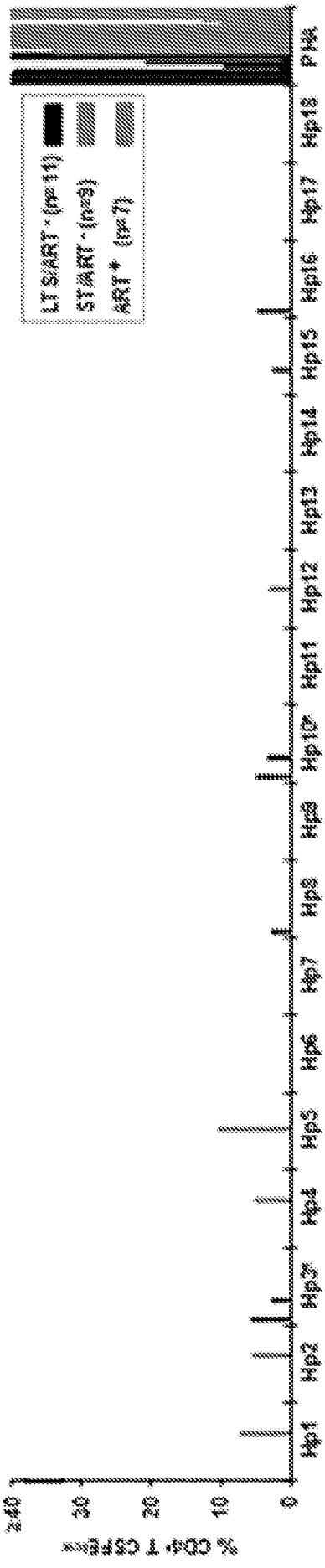


FIG. 24D

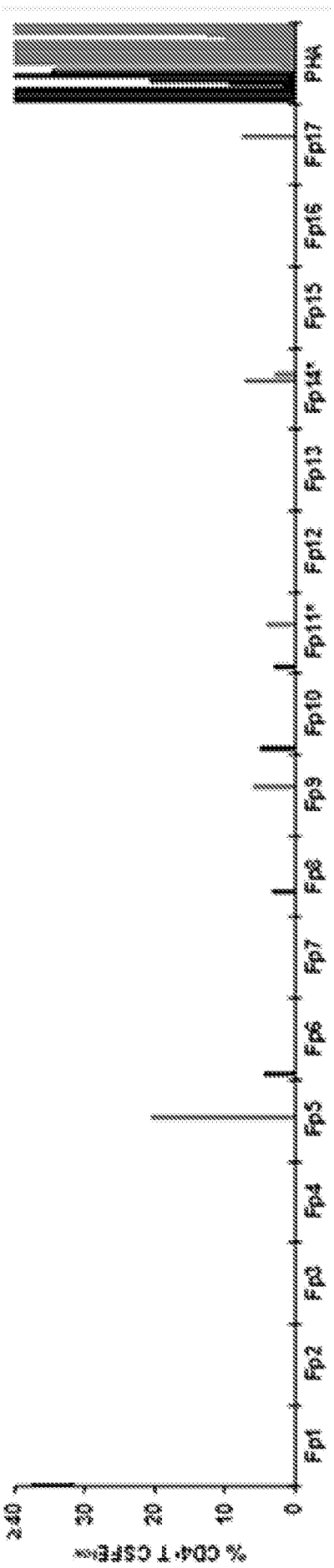


FIG. 24E

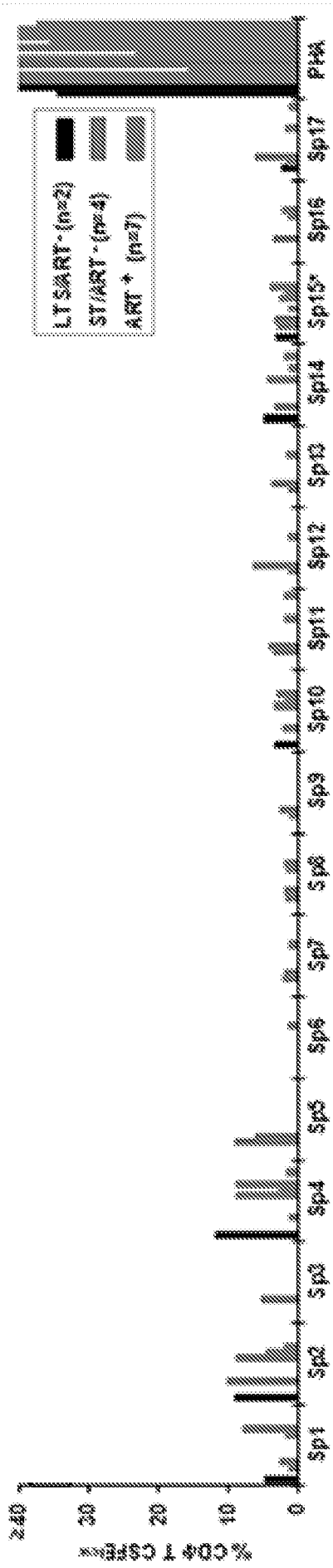


FIG. 24F

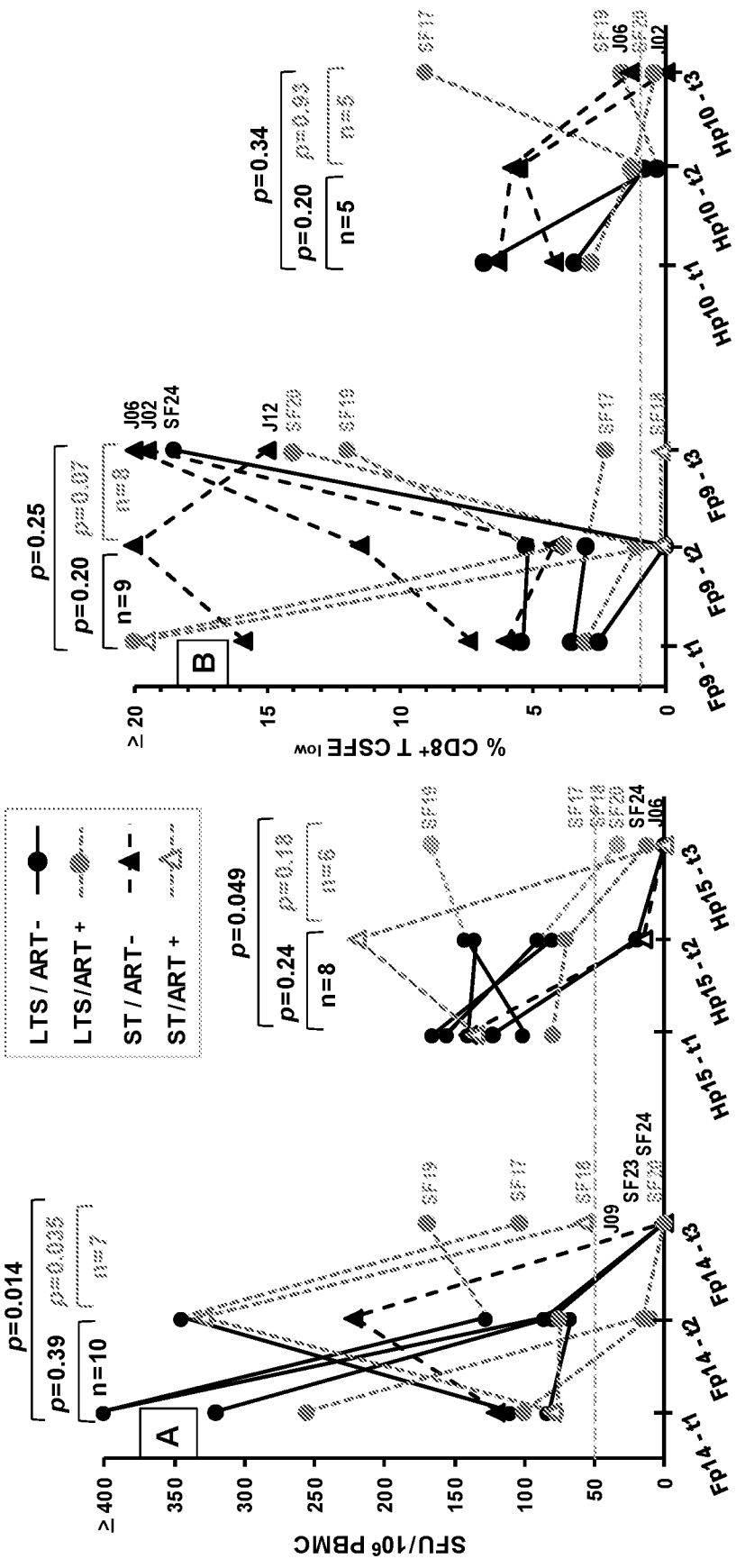


FIG. 25A

FIG. 25B

FIG. 26A

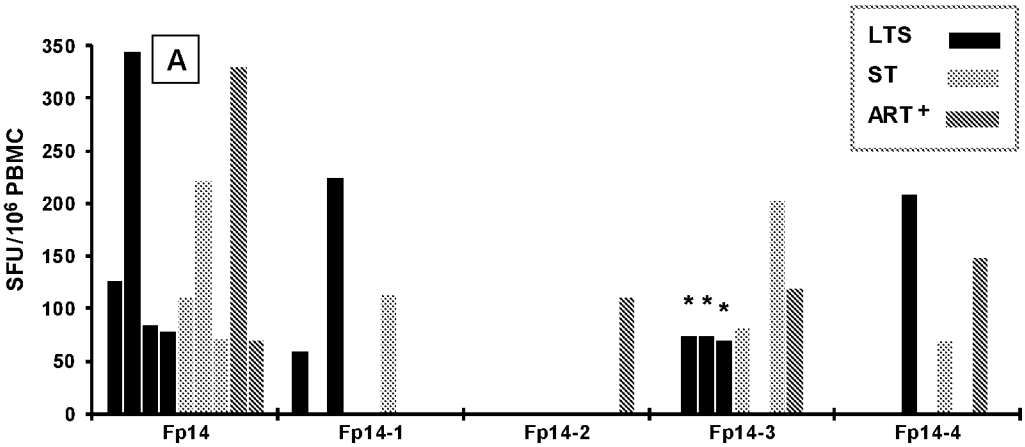


FIG. 26B

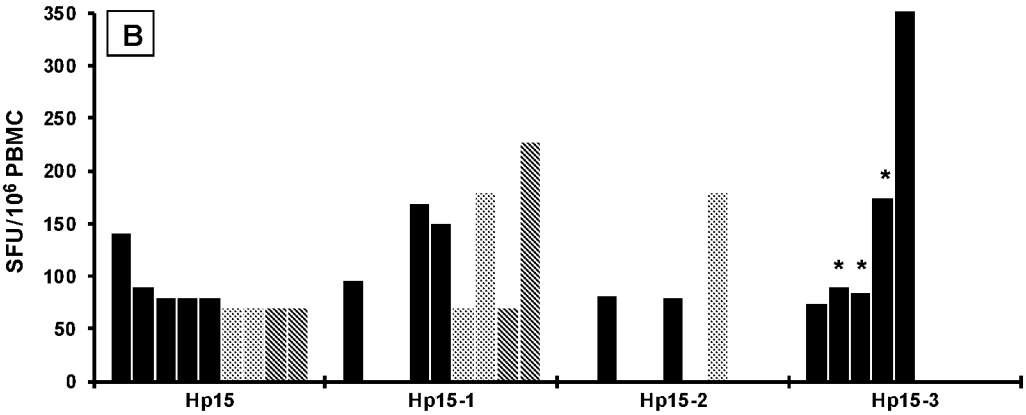
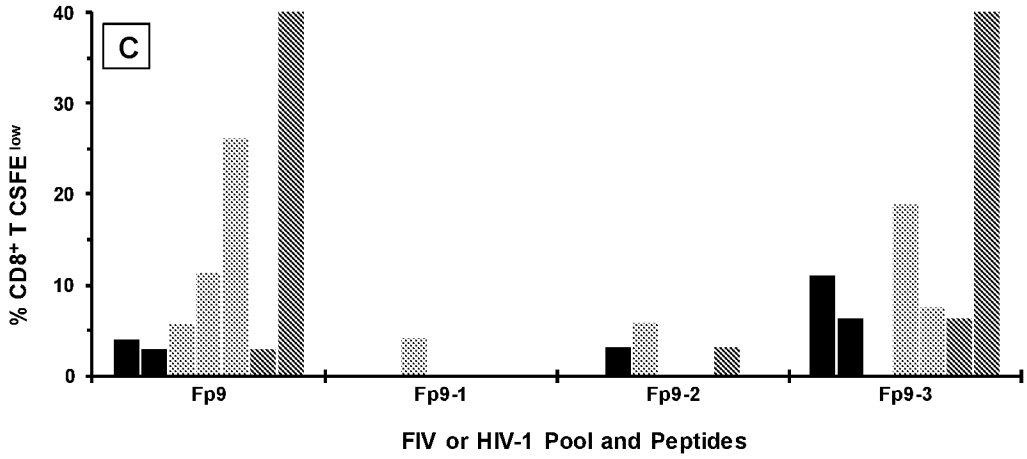


FIG. 26C



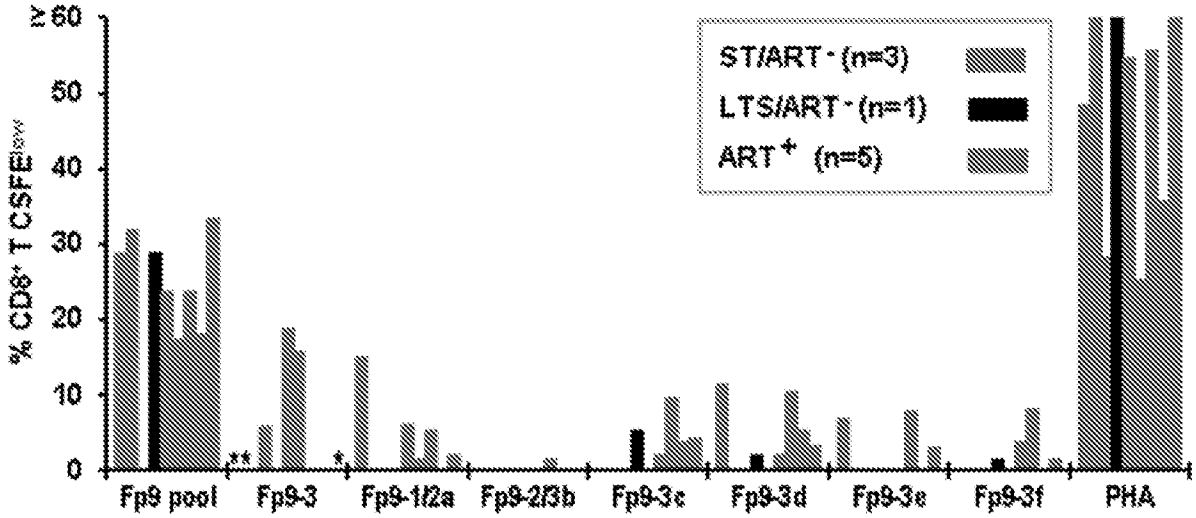


FIG. 27A

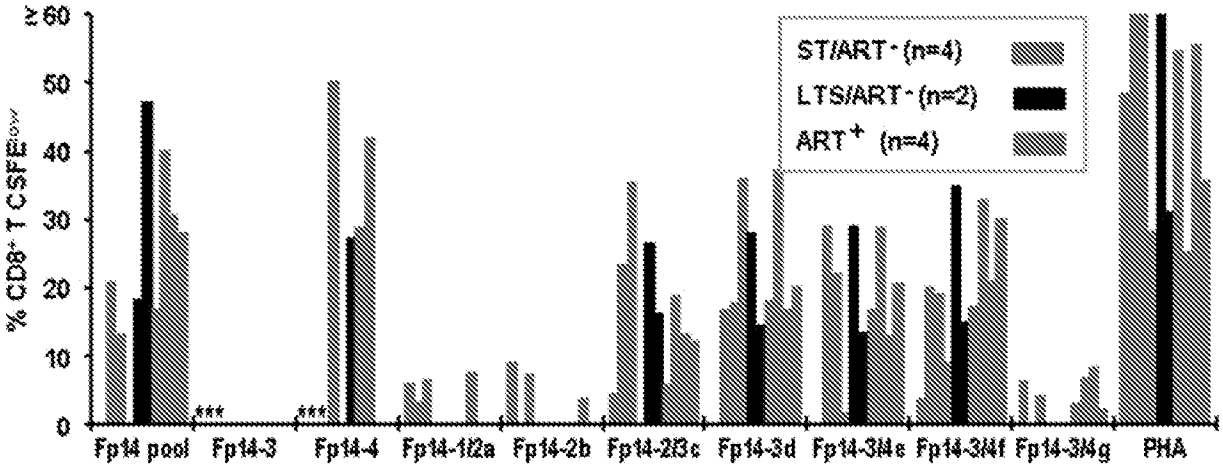


FIG. 27B

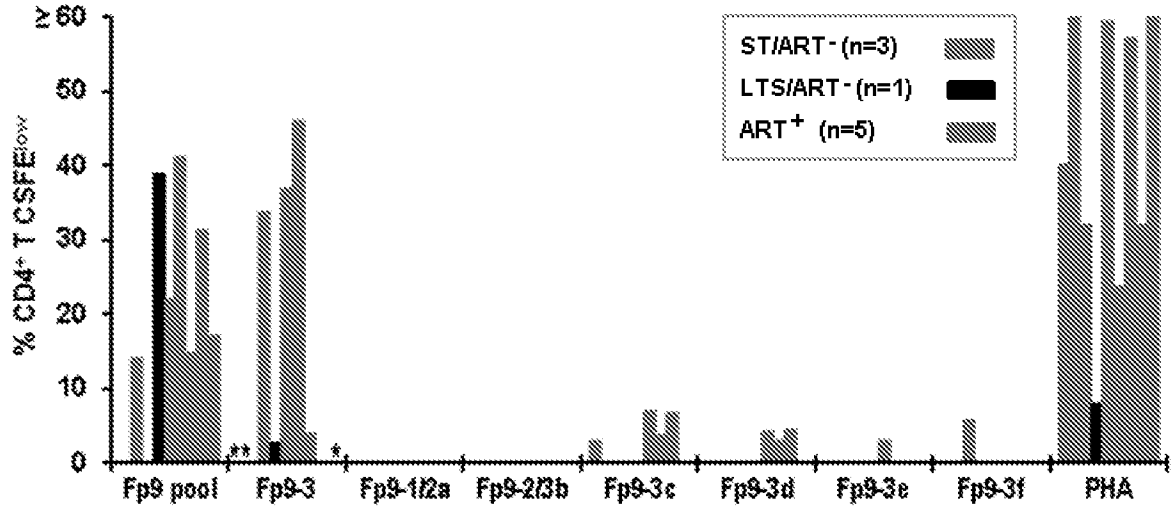


FIG. 27C

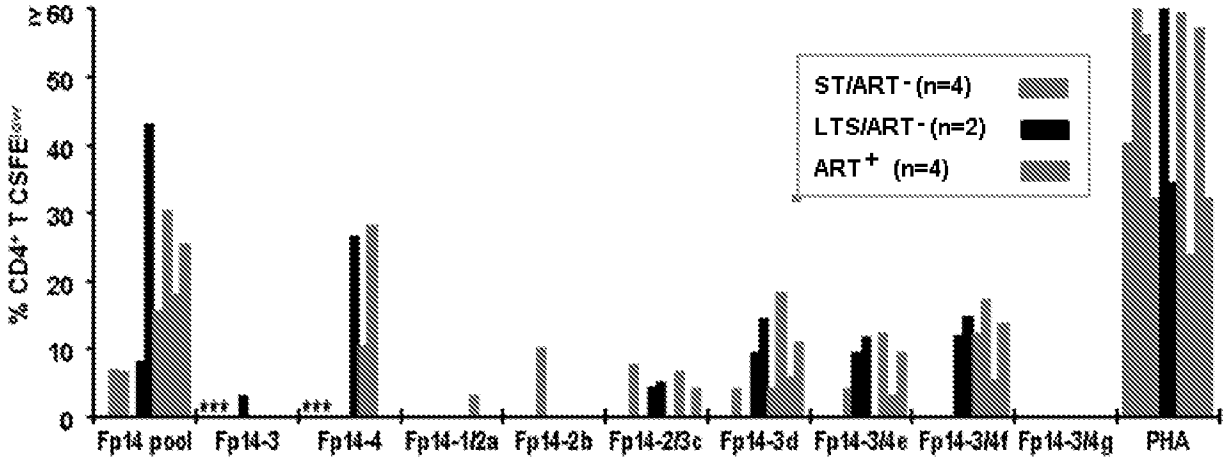


FIG. 27D

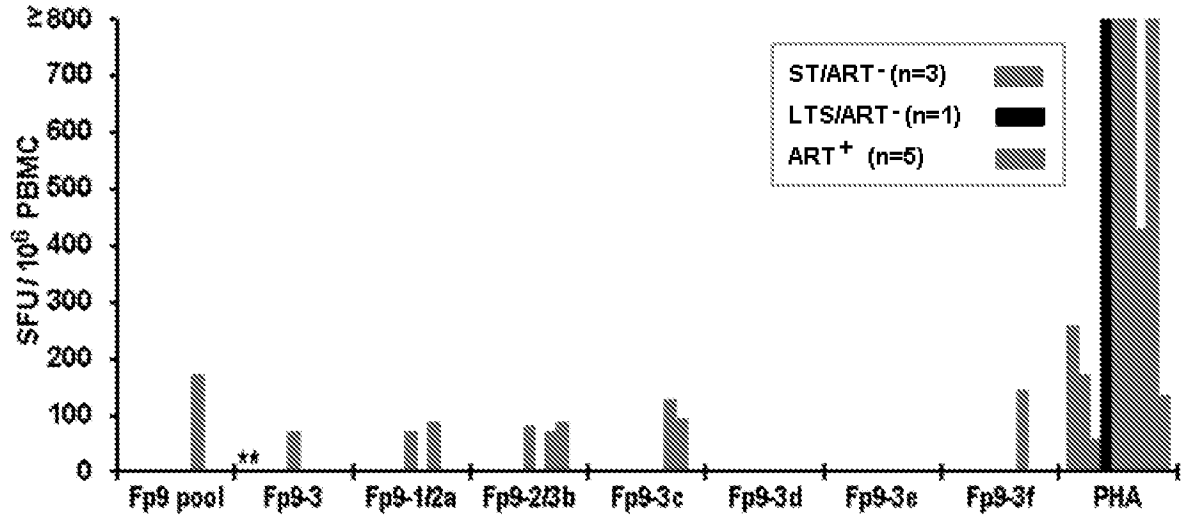


FIG. 27E

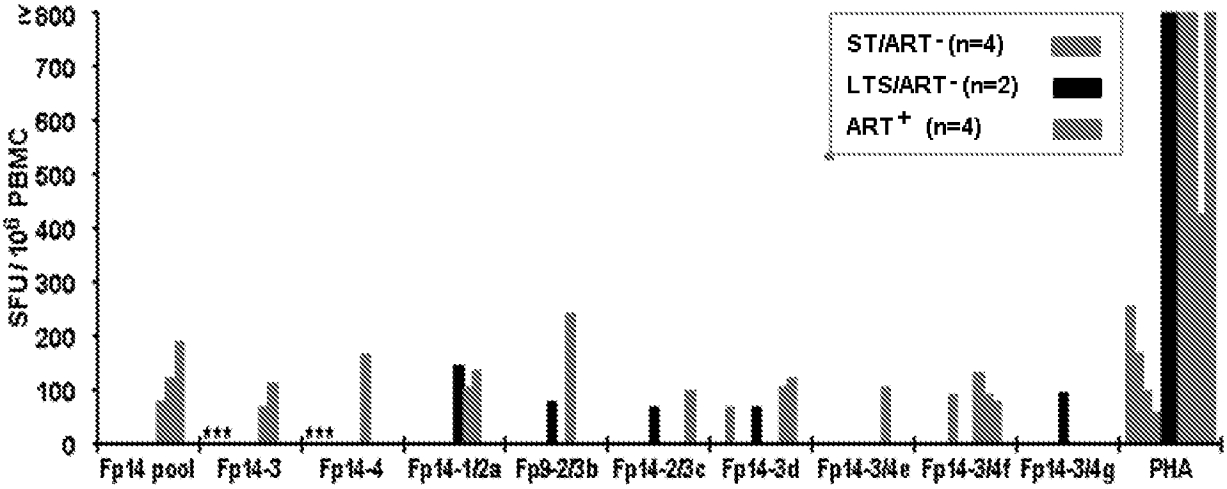


FIG. 27F

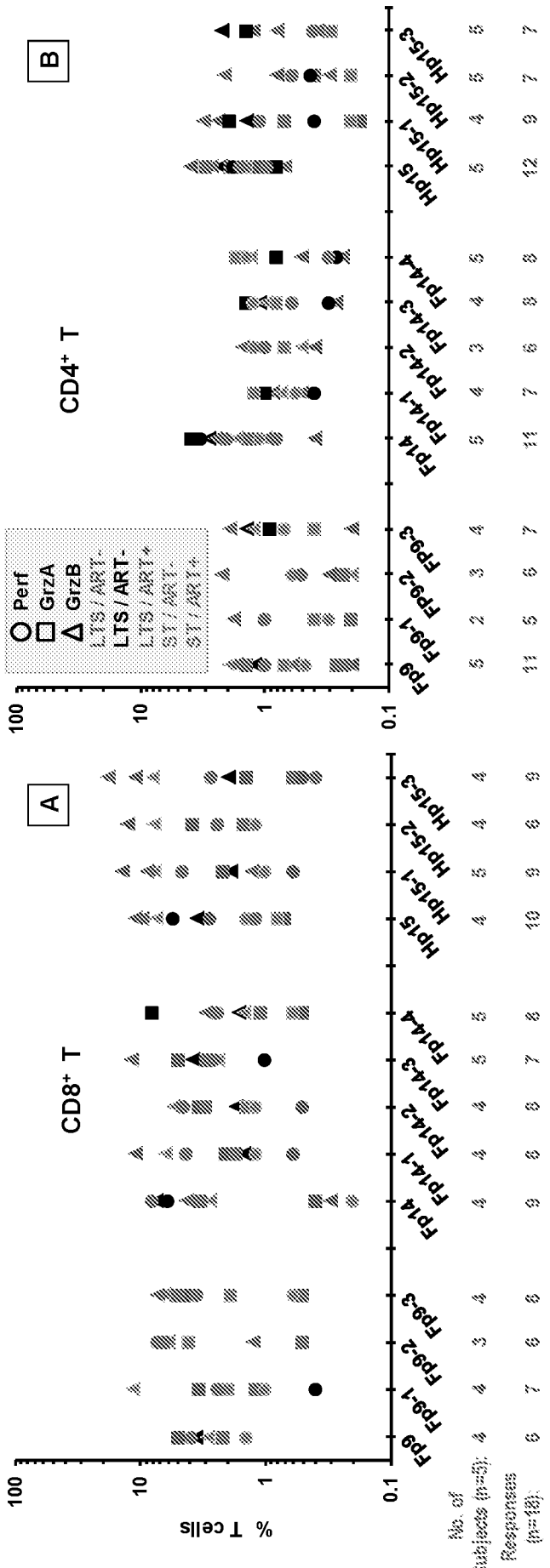


FIG. 28A

FIG. 28B

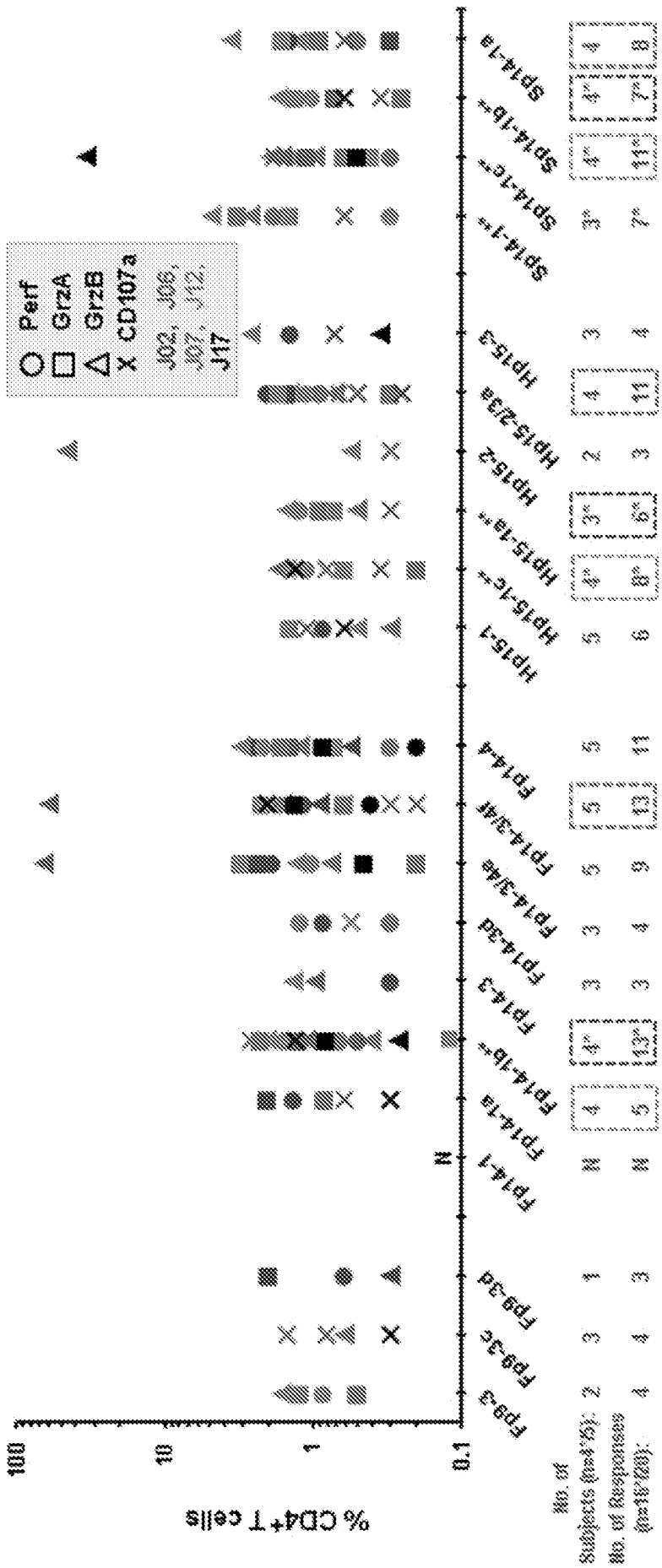

Perf
GrzA
GrzB
CD107a
J02, J06, J07, J12, J17
% CD4+ T cells
100
10
1
0.1
FP9-3
FP9-3c
FP9-3d
FP14-1
FP14-1a
FP14-1b*
FP14-3
FP14-3d
FP14-3Ae
FP14-3Af
FP14-4
HP15-1
HP15-1c*
HP15-1a*
HP15-2
HP15-2/3a
HP15-3
SP14-1*
SP14-1c*
SP14-1b*
SP14-1a
N
No. of Subjects (n=17):
No. of Responses (n=17):

FIG. 28C

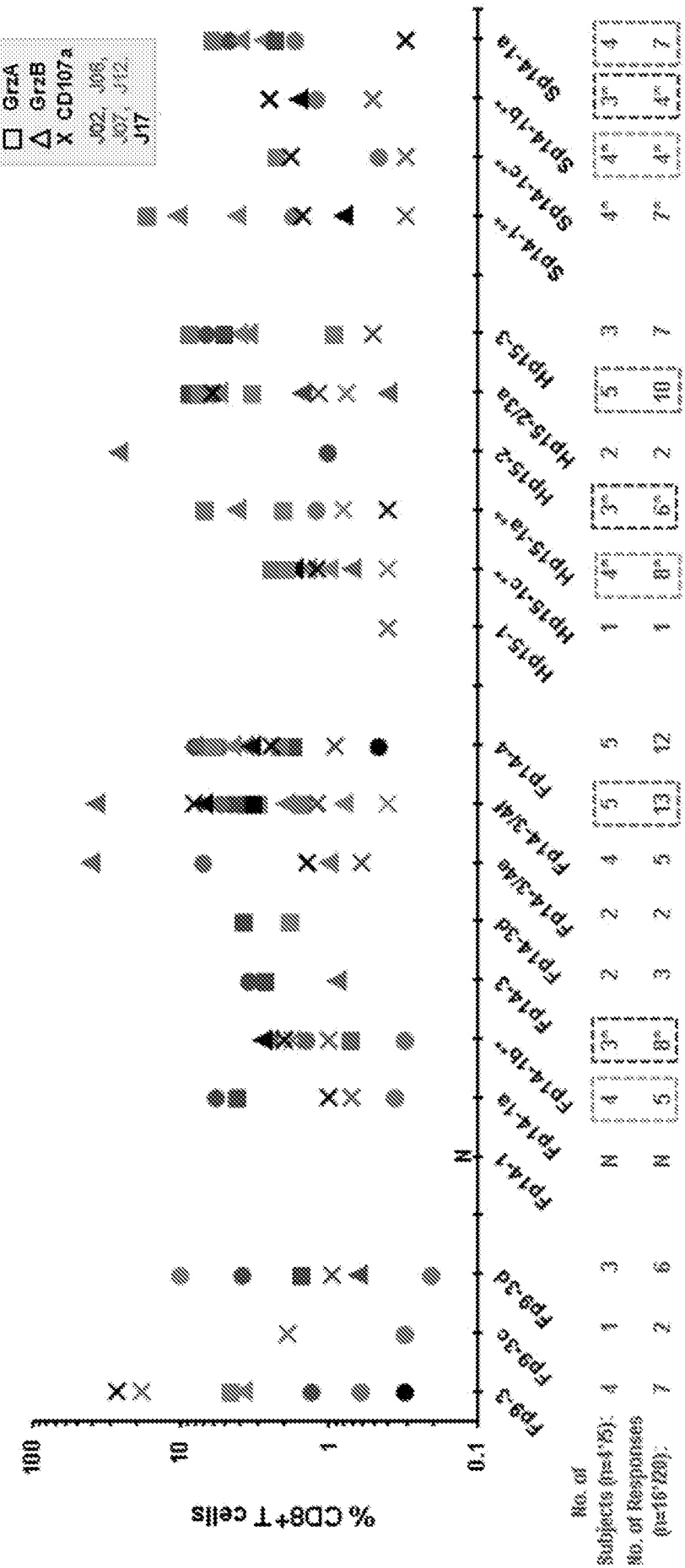

Perf
GrzA
GrzB
CD107a
J02, J08, J07, J12, J17
% CD8+T cells
100
10
1
0.1
N
FP9-3
FP9-3c
FP9-3d
FP14-1
FP14-1a
FP14-1b*
FP14-3
FP14-3d
FP14-3Ja
FP14-3Jd
FP14-4
HP15-1
HP15-1c*
HP15-1a*
HP15-2
HP15-2/3a
HP15-3
SP14-1*
SP14-1c*
SP14-1b*
SP14-1a
No. of Subjects (n=17/6):
No. of Responses (n=16/106):

FIG. 28D

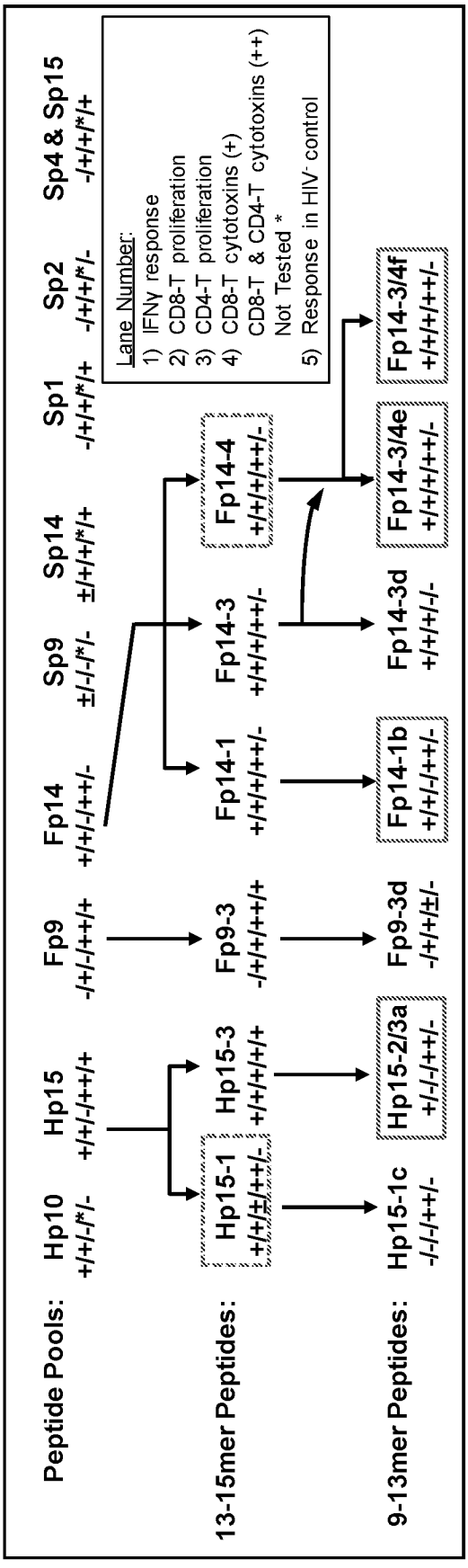
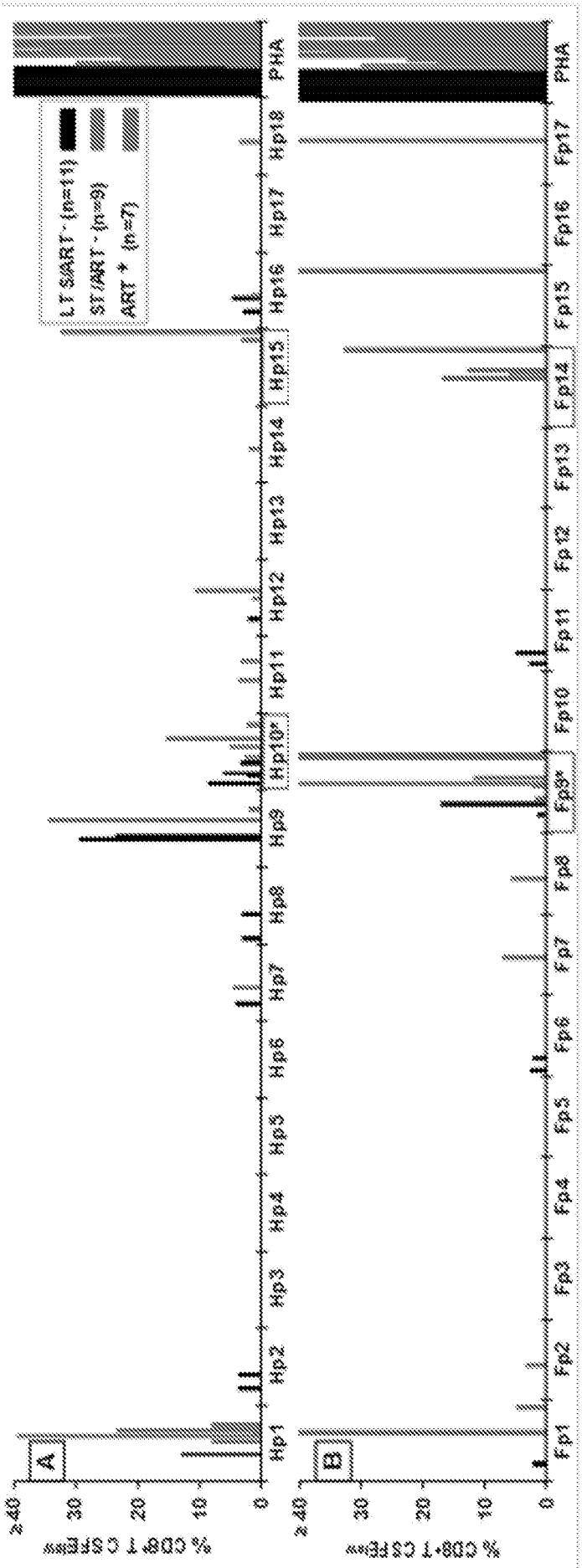

Peptide Pools:
Hp10 +/+/-/*/-
Hp15 +/+/-/++/+
Fp9 -/+/-/++/+
Fp14 +/+/-/++/-
Sp9 ±/-/-/*/-
Sp14 ±/+/+/*/+
Sp1 -/+/+/*/+
Sp2 -/+/+/*/-
Sp4 & Sp15 -/+/+/*/+
13-15mer Peptides:
Hp15-1 +/+/±/++/-
Hp15-3 +/+/+/+/+
Fp9-3 -/+/+/++/+
Fp14-1 +/+/+/++/-
Fp14-3 +/+/+/++/-
Fp14-4 +/+/+/++/-
9-13mer Peptides:
Hp15-1c -/-/-/++/-
Hp15-2/3a +/-/-/++/-
Fp9-3d -/+/+/±/-
Fp14-1b +/+/-/++/-
Fp14-3d +/+/+/-/-
Fp14-3/4e +/+/+/++/-
Fp14-3/4f +/+/+/++/-
Lane Number:
1) IFNγ response
2) CD8-T proliferation
3) CD4-T proliferation
4) CD8-T cytotoxins (+)
CD8-T & CD4-T cytotoxins (++)
Not Tested *
5) Response in HIV- control

FIG. 29



HIV-1, FIV or SIV p24 Peptide Pool

FIG. 30A

FIG. 30B

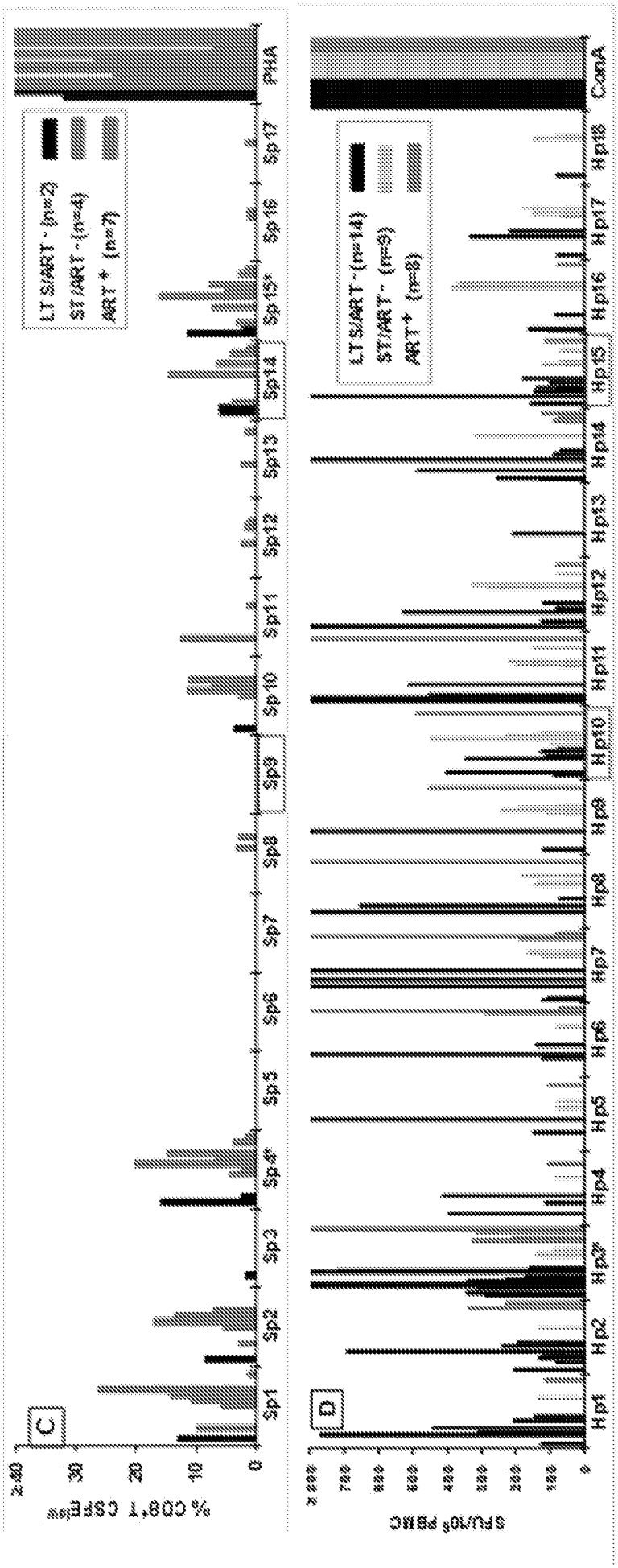


FIG. 30C

FIG. 30D

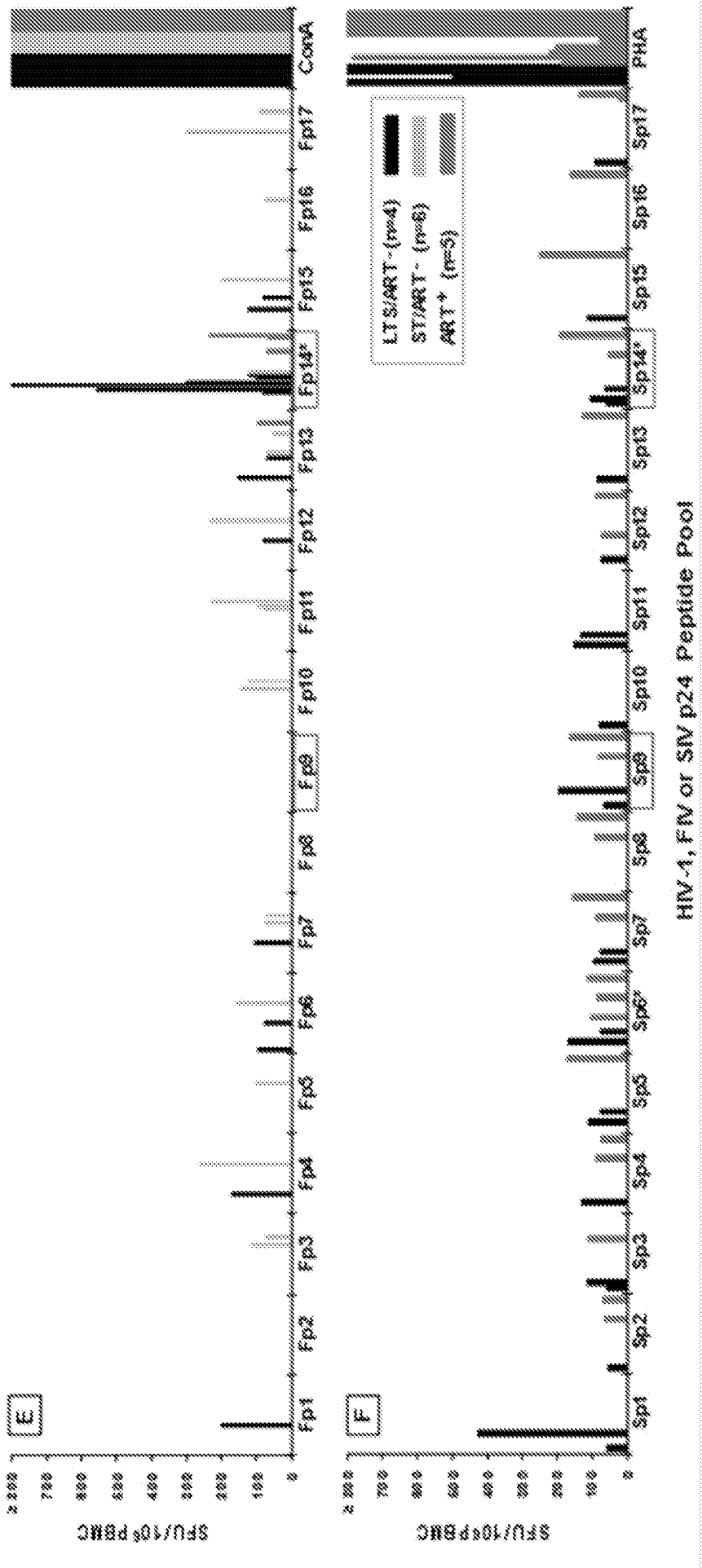


FIG. 30E

FIG. 30F

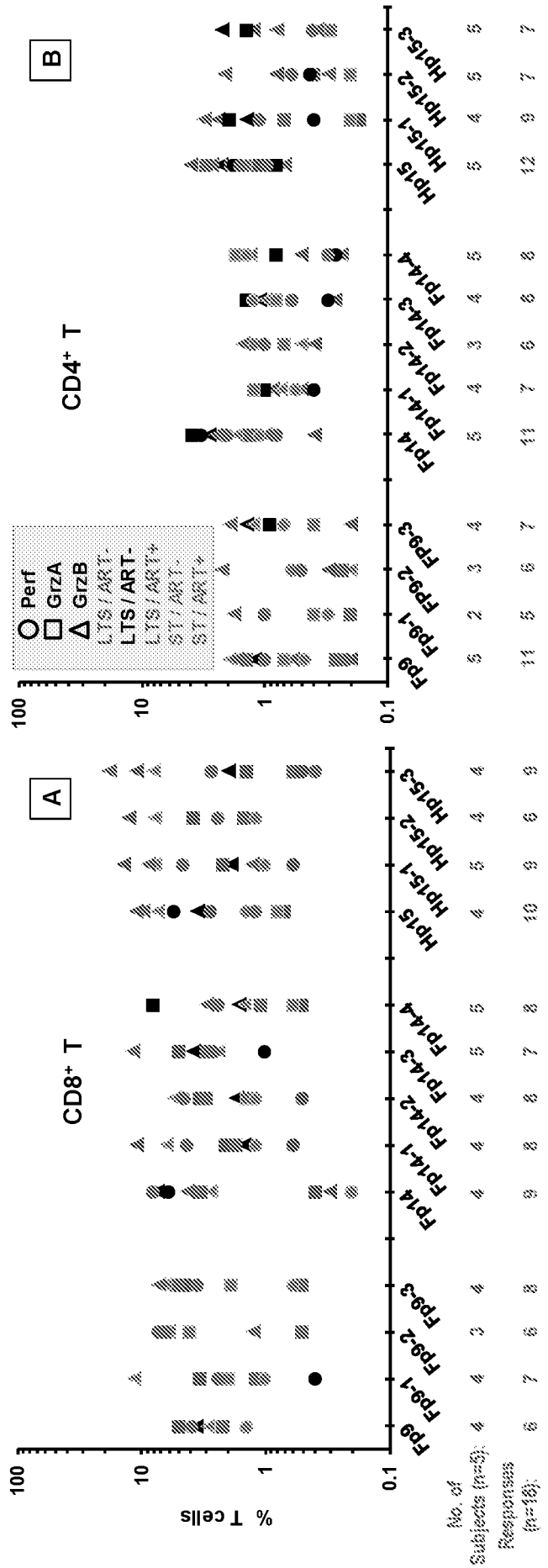


FIG. 31A

FIG. 31B

FIG. 32A

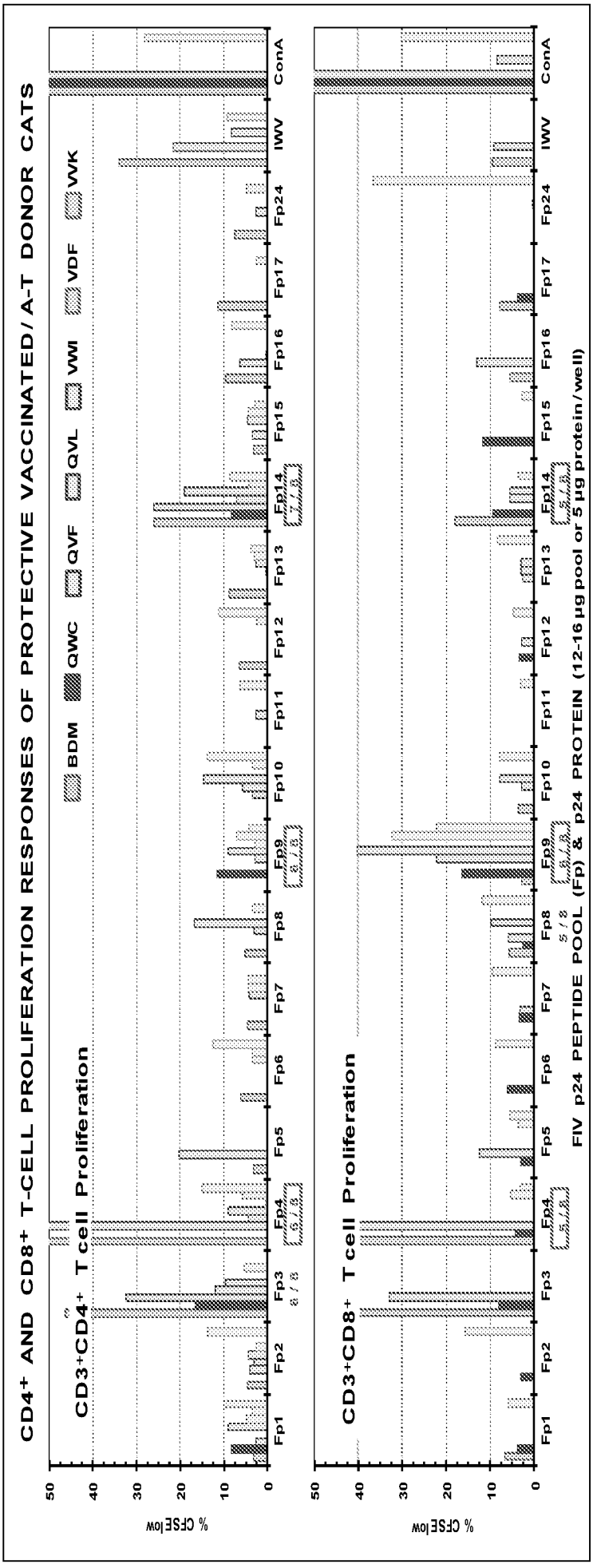


FIG. 32B

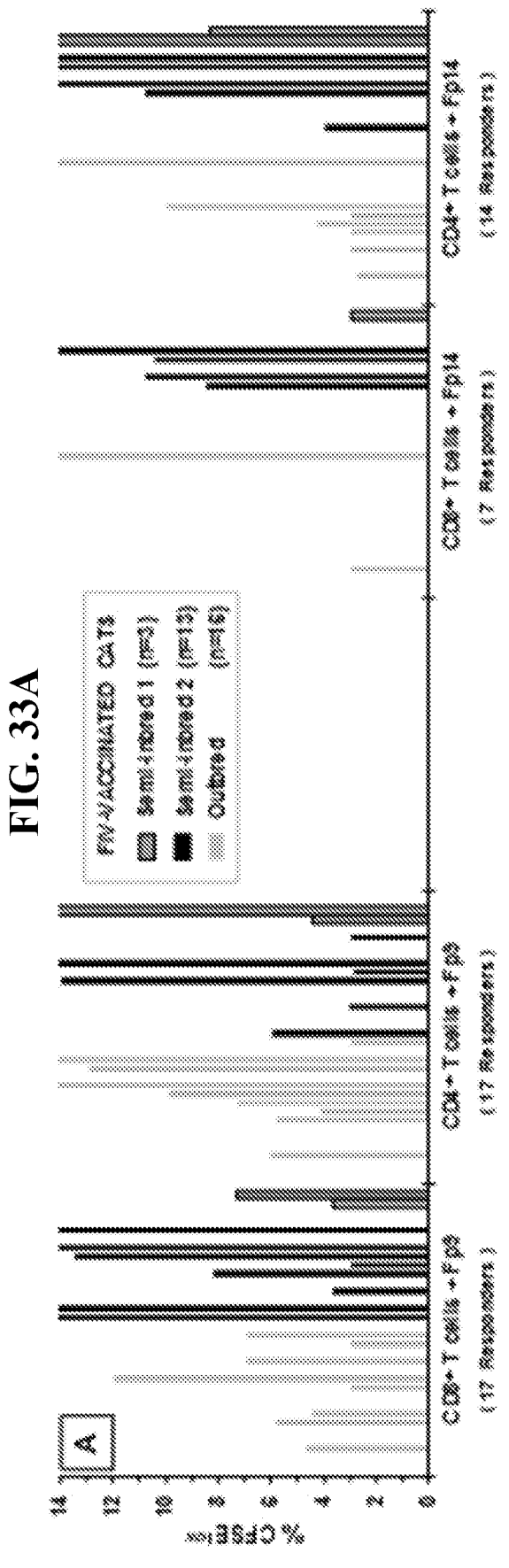


FIG. 33A

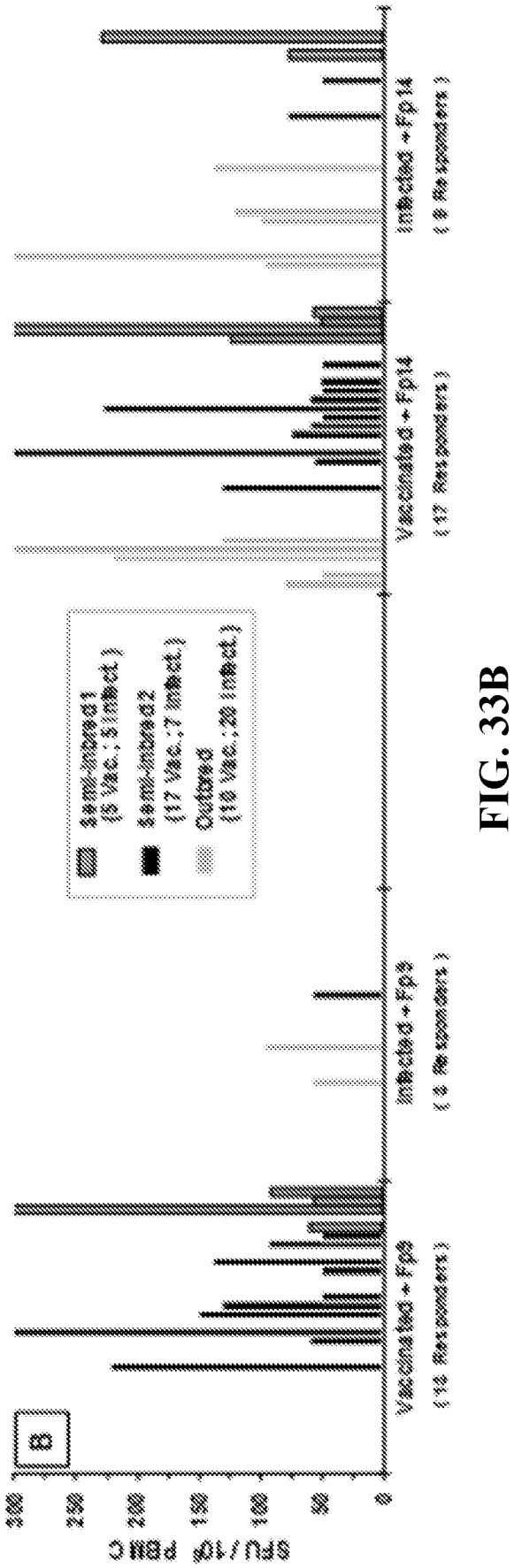


FIG. 33B

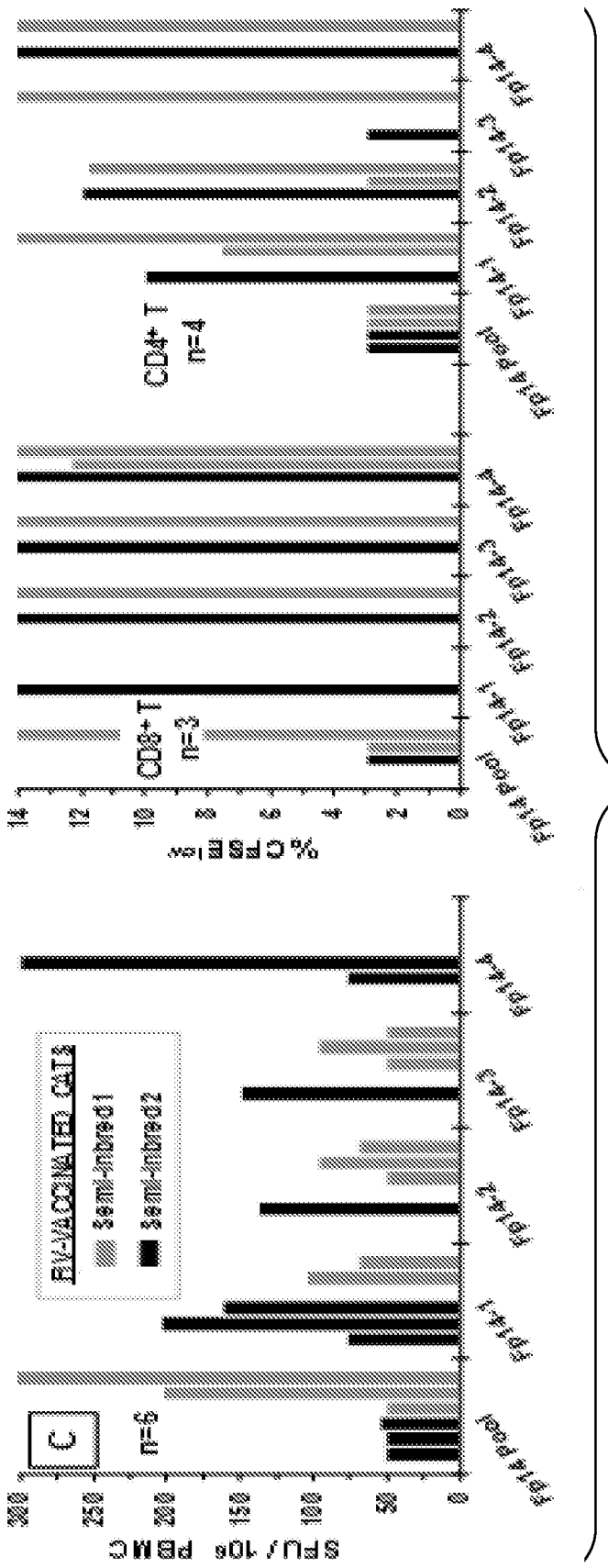

C
n=6
SFU / 10e6 PBMC
250
200
150
100
50
0
FLU-MATRIX CATS
Semi-Ammad1
Semi-Ammad2
CD8+ T
n=3
CD4+ T
n=4
% CFSE-
14
12
10
8
6
4
2
0

FIG. 33C

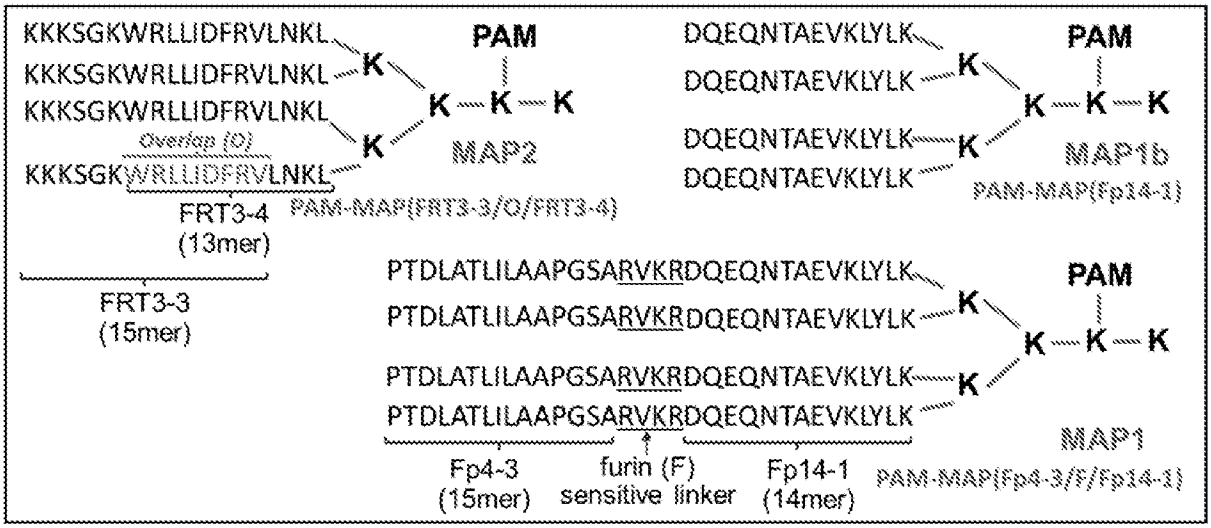

KKKSGKWRLLIDFRVLNKL
KKKSGKWRLLIDFRVLNKL
KKKSGKWRLLIDFRVLNKL
Overlap (O)
KKKSGKWRLLIDFRVLNKL
FRT3-4
(13mer)
FRT3-3
(15mer)
PAM
K
K
K
K
K
MAP2
PAM-MAP(FRT3-3/O/FRT3-4)
DQEQNTAEVKLYLK
DQEQNTAEVKLYLK
DQEQNTAEVKLYLK
DQEQNTAEVKLYLK
PAM
MAP1b
PAM-MAP(Fp14-1)
PTDLATLILAAPGSARVKRDQEQNTAEVKLYLK
PTDLATLILAAPGSARVKRDQEQNTAEVKLYLK
PTDLATLILAAPGSARVKRDQEQNTAEVKLYLK
PTDLATLILAAPGSARVKRDQEQNTAEVKLYLK
PAM
MAP1
Fp4-3
(15mer)
furin (F)
sensitive linker
Fp14-1
(14mer)
PAM-MAP(Fp4-3/F/Fp14-1)

FIG. 34

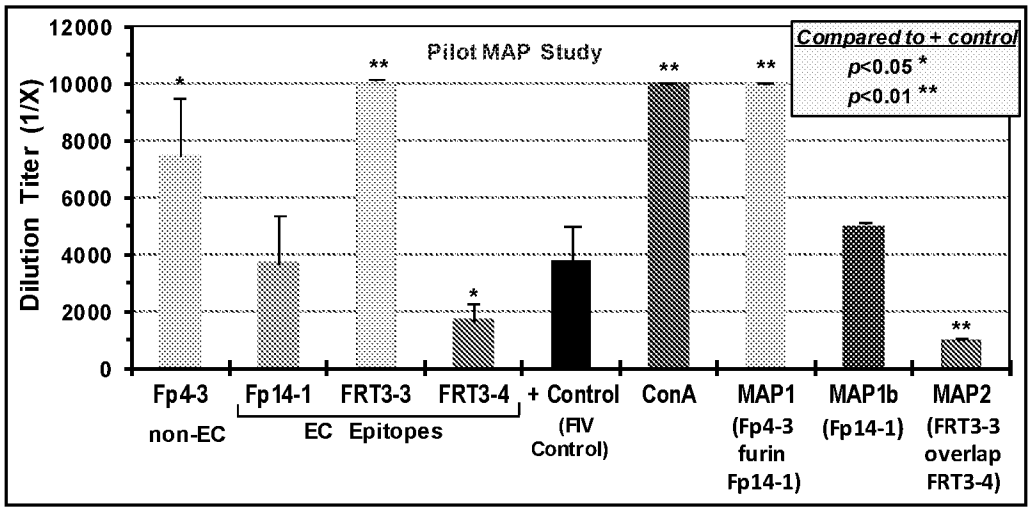

Pilot MAP Study
Dilution Titer (1/X)
12000
10000
8000
6000
4000
2000
0
Compared to + control
p<0.05 *
p<0.01 **
Fp4-3
non-EC
Fp14-1
FRT3-3
FRT3-4
EC Epitopes
+ Control
(FIV
Control)
ConA
MAP1
(Fp4-3
furin
Fp14-1)
MAP1b
(Fp14-1)
MAP2
(FRT3-3
overlap
FRT3-4)

FIG. 35

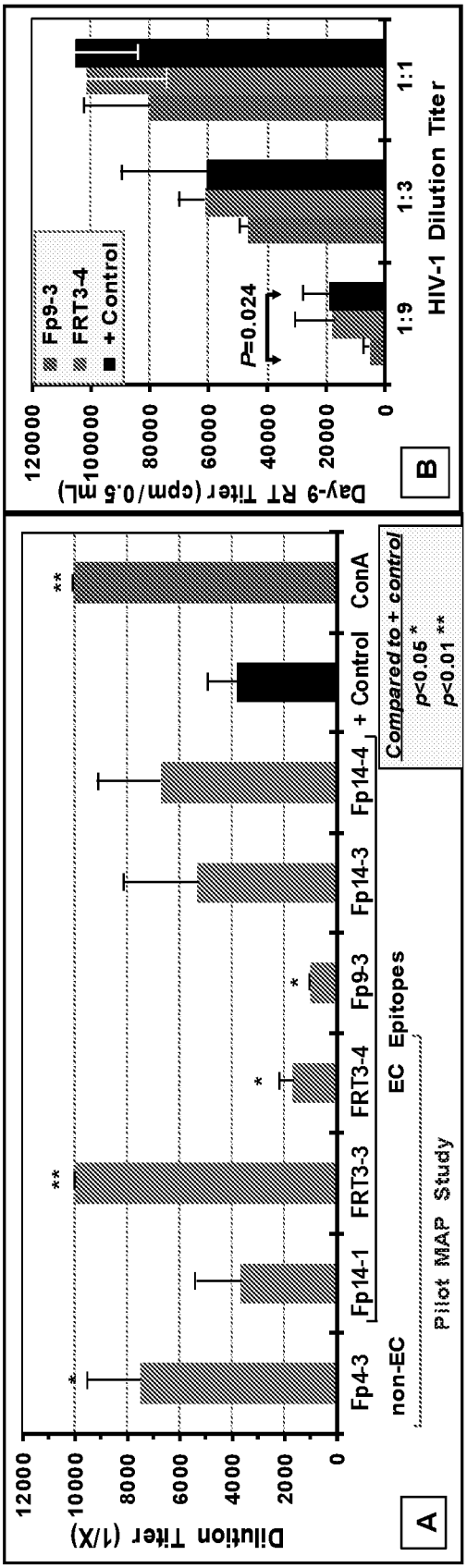


FIG. 36A

FIG. 36B