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**(54) SOLUBLE PD-1 VARIANTS, FUSION CONSTRUCTS, AND USES THEREOF**

LÖSLICHE PD-1-VARIANTEN, FUSIONS-KONSTRUKTE UND VERWENDUNGEN DAVON  
 VARIANTS SOLUBLES DE PD-1, PRODUITS D'ASSEMBLAGE PAR FUSION ET LEURS  
 UTILISATIONS

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**EP 2 638 061 B1**

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**Description**

## BACKGROUND OF THE INVENTION

5 **[0001]** Programmed death 1 (PD-1), expressed primarily on T cells, is a receptor for B7-H1 molecule (also known as programmed death ligand 1 (PD-L1)) and B7-DC molecule (also known as programmed death ligand 2 (PD-L2)). PD-L1 is expressed on many different cell types, whereas PD-L2 is expressed only on antigen-presenting cells such as B cells, dendritic cells and macrophages.

10 **[0002]** The PD-1/PD-L pathway, which transmits negative signals to immune cells, plays a critical role in the modulation of immune responses during infection and cancer. The interaction of PD-1 with PD-L1/L2 inhibits T cell function during HIV infection. A recent study suggested that the blockade of PD-1 during chronic simian immunodeficiency virus (SIV) infection by anti PD-1 antibody resulted in enhanced B cell responses as well as rapid expansion and restoration of SIV-specific polyfunctional CD8 T cells. Other studies suggested that the blockade of the PD-1/PD-L pathway facilitates the restoration of humoral and cell-mediated immune responses during LCMV and HBV infection.

15 **[0003]** Human immunodeficiency virus type I (HIV-1) has contributed to an estimated 25 million deaths since it was first recognized in 1981. Currently, over 33 million people worldwide are living with the virus. One of the existing HIV vaccine compositions, obtained by fusing HIV-1 p24 to DEC-205 antibody, enhances CD4 T cell immune responses and cytokine release. In addition, this vaccine composition confers protection against vaccinia-gag viral challenge. However, this HIV vaccine composition does not improve Th1 CD8 T cell response. Thus, improved HIV-1 vaccine compositions that enhance host immunity and protect against HIV infection are needed. As will be clear from the disclosure that follows, these and other benefits are provided by the present invention.

20 **[0004]** WO2010/027828 provides information on the use of PD-1 receptor antagonist compositions to overcome the inhibition of an immune response via the PD-1/PD-L pathway enhancing thereby the immune response to cancer. The PD-1 variants of the present invention providing alternative means to enhance antigen specific immune response are not described in this document.

## BRIEF SUMMARY OF THE INVENTION

30 **[0005]** The subject invention provides soluble PD-1 (sPD-1) proteins and nucleic acids, and therapeutic compositions comprising sPD-1 proteins and nucleic acids, for enhancing immunity of a subject. In one embodiment, the sPD-1 proteins, nucleic acids, and compositions are formulated as a vaccine composition.

35 **[0006]** One aspect of the subject invention provides sPD-1 protein variants. In an embodiment, the sPD-1 protein variant is mspd1-14del, which has an amino acid sequence comprising SEQ ID NO: 11. In an embodiment, the sPD-1 protein variant is mspd1-322mu, which has an amino acid sequence comprising SEQ ID NO: 15. In an embodiment, the sPD-1 protein variant is hspd1-14del, which was found in healthy Chinese people. The hspd1-14del variant has an amino acid sequence comprising SEQ ID NO: 25.

40 **[0007]** Another aspect of the invention provides nucleic acid molecules that encode the sPD-1 proteins of the subject invention. In an embodiment, the nucleic acid molecule encodes mspd1-14del, and has a sequence comprising SEQ ID NO: 12. In an embodiment, the nucleic acid molecule encodes mspd1-322mu, and has a sequence comprising SEQ ID NO: 16. In an embodiment, the nucleic acid molecule encodes hspd1-14del, and has a sequence comprising SEQ ID NO: 26.

45 **[0008]** In addition, the subject invention provides sPD-1 fusion proteins. In specific embodiments, the subject sPD-1 fusion protein comprises SEQ ID NO: 13, SEQ ID NO: 17, or SEQ ID NO: 27. The subject invention also provides sPD-1 fusion nucleic acid molecules. In specific embodiments, the subject sPD-1 fusion DNA comprises SEQ ID NO: 14, SEQ ID NO: 18, or SEQ ID NO: 28.

50 **[0009]** The present specification provides also information on soluble PD-1 proteins, nucleic acid molecules encoding said proteins, fusion proteins thereof and vaccine compositions containing said proteins corresponding to SEQ ID NO: 19, SEQ ID NO: 23 SEQ ID NO: 20. SEQ ID NO: 24, SEQ ID NO: 1 and SEQ ID NO: 21. However, this subject matter and subject matter relating thereto is not encompassed by the scope of the present claims of the invention but is contained for information purposes only.

55 **[0010]** Another aspect of the subject invention provides methods for the prevention and/or treatment of pathogenic infection, cancer or tumor, and other diseases in which induction of antigen-specific protective immunity would be beneficial. Advantageously, the methods of the subject invention enhance host humoral and cell-mediated immunity. The method comprises administering to a subject in need of such treatment an effective amount of a fusion protein or fusion nucleic acid molecule of the subject invention. In a preferred embodiment, the subject method can be used in the prevention and treatment of HIV or other pathogen infection. In addition, the methods can be used in the prevention and/or treatment of tumor or cancer.

**[0011]** The subject invention further provides for therapeutic or pharmaceutical compositions. In an embodiment, the

composition comprises a therapeutically effective amount of a protein and/or nucleic acid molecule of the subject invention and, optionally, a pharmaceutically acceptable carrier. In a preferred embodiment, the therapeutic composition is a vaccine composition.

5 BRIEF DESCRIPTION OF THE DRAWINGS

[0012]

10 **Figure 1A** shows alignment of amino acid sequences of mspd1-p24-Fc, mspd1-IgVΔ-p24-Fc and p24-Fc fusion proteins. **Figure 1B** shows Western blot analysis of mspd1-p24-Fc, mspd1-IgVΔ-p24-Fc and p24-Fc. Proteins are detected by anti-rabbit Fc antibody.

**Figure 2** shows the binding ability of msPD1-p24-Fc fusion proteins to sPD-1 ligands. **(A)** shows the binding ability of mspd1-p24-Fc, mspd1-IgVΔ-p24-Fc and p24-Fc to mouse PD-L1, respectively. **(B)** shows the binding ability of mspd1-p24-Fc, mspd1-IgVΔ-p24-Fc and p24-Fc to mouse PD-L2, respectively.

15 **Figure 3** shows that wild-type sPD1 DNA elicits humoral and cell-mediated immune responses against HIV p24. **(A)** shows serum levels of anti-p24 IgG1 and IgG2a antibodies in mice immunized with p24-Fc, mspd1-p24-Fc and mspd1-IgVΔ-p24-Fc fusion DNA, respectively. Bars represent the average values of three samples ( $\pm$  standard deviations). **(B)** shows the number of IFN- $\gamma$ -secreting splenocytes specific for p24 epitope gagAI (AMQMLKDTI) for CD8 T cells. Bars represent the average values of three samples ( $\pm$  standard deviations). **(C)** shows images of splenocytes isolated of immunized mice. To analyze p24-specific immune response, splenocytes were stained with H2d-Kd-AMQMLKDTI-PE tetramer for CD8 T cell population analysis. **(D)** shows the number of IFN- $\gamma$ -secreting splenocytes specific for p24 epitope gag26 (TSNPPIPVGDIIYKRWIILGL) for CD4 T cells. Bars represent the average values of three samples ( $\pm$  standard deviations). Data represent three experiments on the same batch of immunized mice.

25 **Figure 4** shows that immunization with wild-type msPD1 fusion DNA protects mice against viral infection. Balb/c mice immunized with p24-Fc, mspd1-p24-Fc or mspd1-IgVΔ-p24-Fc were challenged with  $4 \times 10^7$  PFU of vaccinia VTT-HIV-gagpol intranasally three weeks after the last immunization. The mice were sacrificed 3 days after viral challenge. Viral titers in lungs were evaluated by plaque-forming assay in Vero cells. Bars represent the average values of five samples ( $\pm$  standard deviations).

30 **Figure 5** shows that targeting DCs using sPD-1-p24-fc induces enhanced p24-specific antibody and T cell responses. **(A)** Expression of PD-L1 and PD-L2 on purified CD11c<sup>+</sup> BM-DCs isolated from Balb/c mice were confirmed by flow cytometric analysis using anti-mouse PD-L1 or L2 antibodies (solid line, not shaded). Cells stained with isotype antibody control are shown as shaded histogram. **(B)** BM-DCs treated with purified msPD-1-p24-fc and msPD1-IgVΔ-p24-fc proteins to examine binding. Proteins bound to DCs were detected by flow cytometry using an anti-rabbit Fc-FITC antibody (solid line, not shaded) in parallel to DCs without treatment of proteins as negative control (shaded).  $2 \times 10^6$  DCs treated with  $20 \mu\text{g}$  of msPD-1-p24-fc or msPD1-IgVΔ-p24-fc proteins were introduced to Balb/c mouse by tail vein injection once every three weeks for a total experimental duration of six weeks. Mice that received untreated CD11c<sup>+</sup> DCs served as control. **(C)** Mice sera were collected and analyzed for the presence of IgG1 and IgG2a antibodies specific against HIV-1 p24 by ELISA. **(D)** IFN- $\gamma$  producing CD8<sup>+</sup> and CD4<sup>+</sup> cells were measured by ELISpot assay in mice splenocytes stimulated using specific peptides gagAI and gag26, respectively. H2-Kd-AMQMLKDTI-PE tetramer staining was performed on isolated splenocytes and analyzed by flow cytometry as a column graph of data from groups of immunized mice. Bars represent the mean values of two replicate mice with standard error depicted by error bars. Data are representative of two independent immunization experiments. \*P<0.05.

45 **Figure 6** shows that hspd1-p24-Fc elicits humoral and cell-mediated immune responses against HIV-1 p24. **(A)** shows that hsPD-1-p24-Fc binds to mouse PD-L1. **(B)** shows that hsPD-1-p24-Fc binds to mouse PD-L2. **(C)** shows high sera levels of anti-p24 IgG1 and IgG2a antibodies in mice immunized with hspd1-p24-Fc, when compared to mice immunized with p24-Fc. **(D)** shows the numbers of IFN- $\gamma$ -secreting splenocytes specific for p24 epitope gagAI (AMQMLKDTI) for CD8 T cells and the numbers of IFN- $\gamma$ -secreting splenocytes specific for p24 epitope gag26 (TSNPPIPVGDIIYKRWIILGL) for CD4 T cells. Bars represent the average values of three samples ( $\pm$  standard deviations).

**Figure 7A** shows alignment of amino acid sequences of mspd1-p24-Fc, mspd1-14del-p24-Fc, mspd1-322mu-p24-Fc, and p24-fc fusion proteins. **Figure 7B** shows Western blot analysis of mspd1-14del-p24-Fc, mspd1-322mu-p24-Fc, and p24-Fc. Proteins are detected by anti-rabbit Fc antibody.

55 **Figure 8** shows the binding ability of mspd1-14del-p24-Fc, mspd1-322mu-p24-Fc, and p24-fc fusion proteins to sPD-1 ligands, respectively. **(A)** shows the binding ability of mspd1-14del-p24-Fc, mspd1-322mu-p24-Fc, and p24-Fc fusion proteins to PD-L1, respectively. **(B)** shows the binding ability of mspd1-14del-p24-Fc, mspd1-322mu-p24-Fc, and p24-Fc fusion proteins to PD-L2, respectively.

**Figure 9** shows that variant sPD1 DNA elicits humoral and cell-mediated immune responses against HIV p24. **(A)** shows serum levels of anti-p24 IgG1 and IgG2a antibodies in mice immunized with mspd1-14del-p24-Fc, mspd1-322mu-p24-Fc, and p24-Fc fusion DNA, respectively. Bars represent the average values of three samples ( $\pm$  standard deviations). **(B)** shows the number of IFN- $\gamma$ -secreting splenocytes specific for p24 epitope gagAl (AMQM-LKDTI) for CD8 T cells. Bars represent the average values of three samples ( $\pm$  standard deviations). **(C)** shows images of splenocytes isolated of immunized mice. To analyze p24-specific immune response, splenocytes were stained with H2d-K<sup>d</sup>-AMQMLKDTI-PE tetramer for CD8 T cell population analysis. **(D)** shows the number of IFN- $\gamma$ -secreting splenocytes specific for p24 epitope gag26 (TSNPPIPVGDYIKRWIILGL) for CD4 T cells. Bars represent the average values of three samples ( $\pm$  standard deviations). Data represent three experiments on the same batch of immunized mice.

**Figure 10** shows that immunization with variant msPD1 fusion DNA protects mice against viral infection. Balb/c mice immunized with mspd1-14del-p24-Fc, mspd1-322mu-p24-Fc, and p24-Fc fusion DNA were challenged with  $4 \times 10^7$  PFU of vaccinia VTT-HIV-gagpol intranasally three weeks after the last immunization. The mice were sacrificed 3 days after viral challenge. Viral titers in lungs were evaluated by plaque-forming assay in Vero cells. Bars represent the average values of five samples ( $\pm$  standard deviations).

**Figure 11** shows that hspd1-14del-p24-Fc elicits humoral and cell-mediated immune responses against HIV-1 p24. **(A)** shows that hspd1-14del-p24-Fc does not bind to mouse PD-L1. **(B)** shows that hspd1-14del-p24-Fc does not bind to mouse PD-L2. **(C)** shows high sera levels of anti-p24 IgG1 and IgG2a antibodies in mice immunized with hspd1-14del-p24-Fc, when compared to mice immunized with p24-Fc. **(D)** shows the numbers of IFN- $\gamma$ -secreting splenocytes specific for p24 epitope gagAl (AMQMLKDTI) for CD8 T cells and the numbers of IFN- $\gamma$ -secreting splenocytes specific for p24 epitope gag26 (TSNPPIPVGDYIKRWIILGL) for CD4 T cells. Bars represent the average values of three samples ( $\pm$  standard deviations).

**Figure 12** shows the structures of various clones useful according to the subject invention.

**Figure 13** shows the induction of potent p24-specific immune responses by sPD-1-p24-fc vaccination. **(A)** Schematic representation of constructs encompassing the soluble form of PD-1 or with two amino acid deletions essential for binding with PD-L1/L2 (slgV-PD-1), p24 and rabbit Fc under the CMV promoter. Rabbit Fc was used as a tag for purification purposes. **(B)** Mouse immunization schedule is depicted. Balb/c mice were immunized with sPD-1-p24-fc, slgV-PD-1-p24-fc and p24-fc at week 0, 3 and 6 at a low dose of 20 $\mu$ g or a high dose of 100 $\mu$ g i.m. with EP. Mice that received PBS only served as a negative control. Mice sera and splenocytes were collected two weeks after the final immunization for analysis of antibody and T cell responses, respectively. **(C)** Detection of specific IgG1 and IgG2a antibodies against HIV-1 Gag p24 by ELISA two weeks post immunization. **(D)** Number of IFN- $\gamma$ -secreting CD8<sup>+</sup> and **(E)** CD4<sup>+</sup> T cells measured by ELISpot in specific response to HIV-1 Gag p24 epitopes gagAl and gag26, respectively. **(F)** IFN- $\gamma$ <sup>+</sup> secreting cells in response to stimulation using three different peptide pools derived from 59 peptides that spans the whole HIV-1 Gag p24. **(G)** Representative H2-Kd-AMQMLKDTI-PE tetramer staining of CD8<sup>+</sup> T cell population is shown in flow cytometric plots or data amalgamated into a column graph **(H)**. Data are representative of three independent immunization experiments. \*P<0.05, \*\*P<0.01, \*\*\*P<0.001.

**Figure 14** shows polyfunctionality of sPD1-p24-fc induced T cells. Balb/c mice were immunized with sPD1-p24-fc and slgV-PD1-p24-fc at a dose of 100 $\mu$ g i.m./EP. Mice that received PBS alone served as control. Splenocytes were collected and analyzed by flow cytometry following intracellular staining using antibodies against IFN- $\gamma$ <sup>+</sup>, TNF- $\alpha$ , and IL-2. **(A)** Scatter plots indicating CD8<sup>+</sup> or CD4<sup>+</sup> T cells positive for IFN- $\gamma$ <sup>+</sup> and **(B)** TNF- $\alpha$ . **(C)** Column graphs depicting single, double or triple positive CD8<sup>+</sup> or **(D)** CD4<sup>+</sup> T cells for the cytokines IFN- $\gamma$ <sup>+</sup>, TNF- $\alpha$ , and IL-2. **(E)** Pie chart analysis representing subpopulations of total cytokine secreting CD8<sup>+</sup> or CD4<sup>+</sup> T cells positive for combinations of IFN- $\gamma$ , TNF- $\alpha$ , and IL-2. Columns represent the mean values of three replicate mice with standard error as error bars. Data are representative of two independent immunization experiments. \*P<0.05, \*\*P<0.01.

**Figure 15** shows that vaccination with sPD-1-p24-fc induces specific long lasting and protective immunity. Sera and splenocytes derived from mice 30 weeks after immunization was isolated and examined for antibody and CD8<sup>+</sup> and CD4<sup>+</sup> T cell responses. **(A)** Specific IgG1 and IgG2a antibodies against HIV-1 Gag p24 detected by ELISA. ELISpot assays using specific HIV-1 Gag p24 epitope for **(B)** CD8<sup>+</sup> T cells and **(C)** CD4<sup>+</sup> T cells was performed to test the ability of T cells to produce IFN- $\gamma$ . Mice previously immunized with a dose of 100p.g DNA vaccines were challenged with  $2 \times 10^5$  PFUs of virulent WRGagpol three weeks post immunization to examine immune protection. Each group contained up to 5 mice. **(D)** Immunized mice were weighed daily for eight days after vaccinia challenge. **(E)** Virus titers in the lungs of immunized mice were evaluated by plaque formation on Vero cell monolayers.

**Figure 16** shows expression and binding characteristics of DNA vaccine constructs. **(A)** DNA vaccines encoding sPD-1, the mutated form slgV-PD-1, p24 and fc were tested for protein expression by Western blotting. Lower sized band represents p24-fc, while the higher sized band represents sPD-1-p24-fc or slgV-PD-1-p24-fc. **(B)** 293T cells were transiently transfected with PD-L1 or PD-L2 expression vectors, and the binding profiles of recombinant proteins were examined. Flow cytometric signals were obtained by treating the cells with purified proteins from the constructs followed by detection using anti-rabbit Fc-FITC antibody. Controls included transfected 293T cells stained with anti-

rabbit Fc-FITC antibody (negative, shaded) or anti-mouse PD-L1 or L2 antibodies (positive, solid line, not shaded). **Figure 17** shows that targeting dendritic cells (DCs) using sPD-1-p24-fc induces enhanced p24-specific antibody and T cell responses. **(A)** Expression of PD-L1 and PD-L2 on purified CD11c<sup>+</sup> BM-DCs isolated from Balb/c mice were confirmed by flow cytometric analysis using anti-mouse PD-L1 or L2 antibodies (solid line, not shaded). Cells stained with isotype antibody control are shown as shaded histogram. **(B)** BM-DCs treated with purified sPD-1-p24-fc and slgV-PD-1-p24-fc proteins to examine binding. Proteins bound to DCs were detected by flow cytometry using an anti-rabbit Fc-FITC antibody (solid line, not shaded) in parallel to DCs without treatment of proteins as negative control (shaded).  $2 \times 10^6$  DCs treated with 20 $\mu$ g of sPD-1-p24-fc or slgV-PD-1-p24-fc proteins were introduced to Balb/c mouse by tail vein injection once every three weeks for a total experimental duration of six weeks. Mice that received untreated CD11c<sup>+</sup> DCs served as control. **(C)** Mice sera were collected and analyzed for the presence of IgG1 and IgG2a antibodies specific against HIV-1 p24 by ELISA. **(D)** IFN- $\gamma$  producing CD8<sup>+</sup> and CD4<sup>+</sup> cells were measured by ELISpot assay in mice splenocytes stimulated using specific peptides gagA1 and gag26, respectively. H2-Kd-AMQMLKDTI-PE tetramer staining was performed on isolated splenocytes and analyzed by flow cytometry as a column graph of data from groups of immunized mice **(F)**. Bars represent the mean values of two replicate mice with standard error depicted by error bars. Data are representative of two independent immunization experiments. \*P<0.05.

**Figure 18** characterizes sPD-1-p24-fc DNA vaccination. CD8<sup>+</sup> T cell ELISpot assay of immunization strategy by i.m. **(A)** without electroporation (EP), **(B)** with purified p24-fc and/or sPD-1-fc with EP, or **(c)** using DNA vaccines without rabbit Fc tag with EP. All data points represent the mean  $\pm$  standard error as error bars. \*P<0.05.

**Figure 19** shows that human sPD-1-p24-fc elicits similar p24-specific immunity in mice. **(A)** Binding profiles of hu-sPD-1-p24-fc protein to murine PD-1 ligands transiently expressed on 293T cells. Flow cytometric signals were obtained by treating the cells with hu-sPD-1-p24-fc protein followed by anti-rabbit Fc-FITC antibody for detection. Controls included transfected 293T cells stained with anti-rabbit Fc-FITC antibody (negative, shaded) or anti-mouse PD-L1- or L2- FITC antibodies (positive, solid line, not shaded). Balb/c mice were immunized with hu-sPD-1- p24-fc and p24-fc at a dose of 20 $\mu$ g i.m./EP, or received PBS only serving as a negative control. **(B)** Detection of specific IgG1 and IgG2a antibodies against HIV-1 Gag p24 by ELISA two weeks post immunization in mice sera. **(C)** Frequencies of IFN- $\gamma$ -secreting CD8<sup>+</sup> and CD4<sup>+</sup> T cells in mice splenocytes measured by ELISpot assay in specific response to HIV-1 Gag p24 epitopes specific for CD4<sup>+</sup> and CD8<sup>+</sup> T cells, respectively. Columns represent the mean values of three replicate mice with standard error as error bars. Data are representative of two independent immunization experiments. \*P<0.05, \*\*P<0.01, \*\*\*P<0.001.

#### BRIEF DESCRIPTION OF THE SEQUENCES

##### [0013]

**SEQ ID NO: 1** is an amino acid sequence of the wild-type soluble extracellular domain of mouse PD-1 (mouse spd1).  
**SEQ ID NO: 2** is a nucleic acid sequence of the wild-type mouse spd1 DNA.  
**SEQ ID NO: 3** is an amino acid sequence of HIV p24 useful according to the subject invention.  
**SEQ ID NO: 4** is a nucleic acid sequence of HIV p24 DNA useful according to the subject invention.  
**SEQ ID NO: 5** is an amino acid sequence of rabbit Fc domain useful to the subject invention.  
**SEQ ID NO: 6** is a nucleic acid sequence of rabbit Fc DNA useful to the subject invention.  
**SEQ ID NO: 7** is an amino acid sequence of mspd1-IgV $\Delta$ .  
**SEQ ID NO: 8** is a nucleic acid sequence of mspd1-IgV $\Delta$  DNA.  
**SEQ ID NO: 9** is an amino acid sequence of mspd1-IgV $\Delta$ -p24-Fc fusion protein.  
**SEQ ID NO: 10** is a nucleic acid sequence of mspd1-IgV $\Delta$ -p24-Fc fusion DNA.  
**SEQ ID NO: 11** is an amino acid sequence of mspd1-14del.  
**SEQ ID NO: 12** is a nucleic acid sequence of mspd1-14del DNA.  
**SEQ ID NO: 13** is an amino acid sequence of mspd1-14del-p24-Fc fusion protein.  
**SEQ ID NO: 14** is a nucleic acid sequence of mspd1-14del-p24-Fc fusion DNA.  
**SEQ ID NO: 15** is an amino acid sequence of mspd1-322mu.  
**SEQ ID NO: 16** is a nucleic acid sequence of mspd1-322mu DNA.  
**SEQ ID NO: 17** is an amino acid sequence of mspd1-322mu-p24-Fc fusion protein.  
**SEQ ID NO: 18** is a nucleic acid sequence of mspd1-322mu-p24-Fc fusion DNA.  
**SEQ ID NO: 19** is an amino acid sequence of mspd1-p24-Fc fusion protein.  
**SEQ ID NO: 20** is a nucleic acid sequence of mspd1-p24-Fc fusion DNA.  
**SEQ ID NO: 21** is an amino acid sequence of the wild-type soluble extracellular domain of human PD-1 (human spd1).  
**SEQ ID NO: 22** is a nucleic acid sequence of the wild-type human spd1 DNA.  
**SEQ ID NO: 23** is an amino acid sequence of hspd1-p24-Fc fusion protein.

**SEQ ID NO: 24** is a nucleic acid sequence of hspd1-p24-Fc fusion DNA.

**SEQ ID NO: 25** is an amino acid sequence of hspd1-14del.

**SEQ ID NO: 26** is a nucleic acid sequence of hspd1-14del DNA.

**SEQ ID NO: 27** is an amino acid sequence of hspd1-14del-p24-Fc fusion protein.

5 **SEQ ID NO: 28** is a nucleic acid sequence of hspd1-14del-p24-Fc fusion DNA.

**SEQ ID NO: 29** is an amino acid sequence of a linker sequence useful according to the subject invention.

**SEQ ID NO: 30** is an amino acid sequence of a linker sequence useful according to the subject invention.

**SEQ ID NO: 31** is an amino acid sequence of a linker sequence useful according to the subject invention.

**SEQ ID NO: 32** is an amino acid sequence of a linker sequence useful according to the subject invention.

10 **SEQ ID NO: 33** is an amino acid sequence of a linker sequence useful according to the subject invention.

**SEQ ID NO: 34** is an amino acid sequence of a linker sequence useful according to the subject invention.

**SEQ ID NO: 35** is an amino acid sequence of a linker sequence useful according to the subject invention.

**SEQ ID NO: 36** is an amino acid sequence of a linker sequence useful according to the subject invention.

**SEQ ID NO: 37** is an amino acid sequence of a linker sequence useful according to the subject invention.

15 **SEQ ID NO: 38** is an amino acid sequence of a linker sequence useful according to the subject invention.

**SEQ ID NO: 39** is an amino acid sequence useful according the subject invention.

**SEQ ID NO: 40** is an amino acid sequence useful according the subject invention.

#### DETAILED DISCLOSURE OF THE INVENTION

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**[0014]** The subject invention provides soluble PD-1 (sPD-1) proteins and nucleic acids, and therapeutic compositions comprising soluble PD-1 proteins and nucleic acids, useful for inducing antigen-specific protective immunity against infection and cancer. In one embodiment, the subject sPD-1 proteins, nucleic acids, and compositions are formulated as a vaccine composition. In an embodiment, the subject invention provides novel fusion proteins mspd1-p24Fc, mspd1-14del-p24Fc, mspd1-322mu-p24Fc, and hspd1-14del-p24Fc, and nucleic acid molecules encoding these fusion proteins.

25

**[0015]** The subject invention is based on the findings that the immune regulatory PD-1/PD-L pathway down-regulates HIV-1-specific CD8<sup>+</sup> T cells responses. The present inventors discovered a natural variant of PD-1 present in healthy people that does not interact with either PD-L1 or PD-L2 (the ligands of PD-1). In addition, a point mutation, which is essential for PD-1 and its ligands interaction, is discovered.

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**[0016]** In one embodiment, the subject invention provides a novel DNA vaccine design that mimics the binding of programmed death-1 (PD-1) to its ligands expressed on dendritic cells (DCs) for functional activation, by fusing soluble PD-1 with an antigen of interest. Intramuscular immunization via electroporation (EP) of the fusion DNA vaccine elicited robust anti-Gag antibody titers in mice, with both IgG1 (Th2) and IgG2a (Th1) responses detected. High frequencies of Gag-specific, broadly reactive and polyfunctional T cells, especially CD8<sup>+</sup> T cells were elicited following immunization. These responses were dose-dependent, long lasting and conferred protection against intranasal challenge with virulent vaccinia-Gag virus. Specifically, mspd-p24fc, mspd1-14del-p24fc and mspd1-322mu-p24fc enhance HIV-1 Gag-specific immune responses, as determined by the number of IFN- $\gamma$  expressed CD4 and CD8 T cells using Elispot assays. Thus, soluble PD-1-based DNA/EP vaccination of the subject invention offers an easy, repeatable and effective way to induce durable and protective CD8<sup>+</sup> T cell immunity, which has important implications for vaccine development and gene therapy.

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**[0017]** In one embodiment, the mspd1-14del protein variant is obtained by deleting amino acids 26-39 of the wild-type mspd1 (Amino acids 26-39 are the first 14 amino acids encoded by the second exon of the wild-type mouse PD-1 gene. These 14 amino acids of mspd1 have the same sequence as the first 14 amino acids encoded by the second exon of the human hspd1-14del homologue). The mspd1-322mu protein variant is obtained by changing amino acid residue 108 of the wild-type PD-1 protein from Met to Val. The hspd1-14del variant, which is derived from a natural isoform of human PD-1, has a deletion of amino acids 26-39 of the wild-type hspd1 (encoded by the first part of the second exon of the wild-type human PD-1 gene).

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**[0018]** The mspd1-p24Fc fusion protein binds to PD-1 ligands PD-L1 and PD-L2, and the binding of PD-1 to PD-L can be blocked by anti-PD-L1/L2 antibodies. It is postulated that the binding of mspd1-p24Fc fusion protein inhibits the PD-1/PD-L pathway, which transmits negative signals to immune cells. In comparison, none of mspd1-14del-p24Fc, mspd1-322mu-p24Fc, and hspd1-14del-p24Fc fusion proteins binds to PD-L1 or PD-L2. This indicates that amino acid residues 26-39 encoded by DNA in exon 2 of spd1 and amino acid residue 108 Met of mspd1 are important for PD-L binding.

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**[0019]** Advantageously, the administration of mspd1-p24Fc, mspd1-14del-p24Fc, mspd1-322mu-p24Fc, and hspd1-14del-p24Fc fusion proteins, or fusion DNA thereof, enhanced HIV-1 Gag-specific immune responses. As shown in Figures 3-6 and 8-11, administration of mspd1-p24Fc, mspd1-14del-p24Fc, mspd1-322mu-p24Fc, and hspd1-14del-p24Fc DNA significantly increased anti-p24 IgG1 (Th2) and IgG2a (Th1) antibody titers. In addition, the administration of mspd1-p24Fc, mspd1-14del-p24Fc, mspd1-322mu-p24Fc, and hspd1-14del-p24Fc DNA also significantly increased the number of IFN- $\gamma$ -expressing CD4 and CD8 T cells in mice. Specifically, mice immunized mspd1-p24Fc, mspd1-14del-

p24Fc, or mspd1-322mu-p24Fc DNA had significantly reduced titers of challenge virus upon vaccinia virus-gagpol (VTT-gagpol) challenges.

**[0020]** In comparison, mspd1-IgV $\Delta$ -p24-Fc, which is obtained by deleting amino acids 89-90 of the mouse PD-1 protein, does not bind to PD-1 ligands PD-L1 and PD-L2. In addition, the administration of mspd1-IgV $\Delta$ -p24-Fc DNA does not enhance humoral or cell-mediated immunity in mice. Further, the administration of mspd1-IgV $\Delta$ -p24-Fc DNA does not reduce HIV viral titers upon vaccinia virus-gagpol (MVTT-gagpol) challenges.

#### PD-1 Variants and Fusion Constructs

**[0021]** A first aspect of the subject invention provides sPD-1 protein variants. In one embodiment, the sPD-1 protein variant is obtained by deleting amino acid residues 26-39 of a wild-type sPD-1 protein. The wild-type sPD-1 protein is preferably of mammalian origin (such as a wild-type mouse, rabbit, non-human primates, or pig PD-1 protein), more preferably, of human origin.

**[0022]** In an embodiment, the sPD-1 protein variant is mspd1-14del, which has an amino acid sequence comprising SEQ ID NO: 11. In an embodiment, the sPD-1 protein variant is mspd1-322mu, which has an amino acid sequence comprising SEQ ID NO: 15. In an embodiment, the sPD-1 protein variant is hspd1-14del, which has an amino acid sequence comprising SEQ ID NO: 25.

**[0023]** In certain embodiments, the subject invention encompasses PD-1 protein variants that are homologous to mspd1-14del (SEQ ID NO: 11), mspd1-322mu (SEQ ID NO: 15), or hspd1-14del (SEQ ID NO: 25). In an embodiment, the sPD-1 protein variant has an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99%, or 99.5% identical to SEQ ID NO: 11. In an embodiment, the sPD-1 protein variant has an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99%, or 99.5% identical to SEQ ID NO: 15. In an embodiment, the sPD-1 protein variant has an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99%, or 99.5% identical to SEQ ID NO: 25. In an embodiment, the PD-1 protein variant does not comprise SEQ ID NO: 7.

**[0024]** A second aspect of the subject invention provides nucleic acid molecules that encode the sPD-1 proteins of the subject invention. The nucleic acid molecules encompass DNA molecules (e.g. genomic DNA and cDNA) and RNA molecules. In addition, the subject nucleic acid molecules may be single-stranded or double-stranded.

**[0025]** In one embodiment, the nucleic acid molecule encodes a sPD-1 protein, which is obtained by deleting amino acid residues 26-39 of a wild-type sPD-1 protein (such as a wild-type human, mouse, or rabbit sPD-1 protein). In an embodiment, the nucleic acid molecule encodes mspd1-14del, and has a sequence comprising SEQ ID NO: 12. In an embodiment, the subject nucleic acid molecule encodes mspd1-322mu, and has a sequence comprising SEQ ID NO: 16. In an embodiment, the subject nucleic acid molecule encodes hspd1-14del, and has a sequence comprising SEQ ID NO: 26.

**[0026]** In certain embodiments, the subject invention encompasses nucleic acid molecules that are homologous to nucleic acids encoding mspd1-14del, mspd1-322mu, or hspd1-14del. In an embodiment, the nucleic acid molecule has a sequence that is at least about 95%, 96%, 97%, 98%, 99%, or 99.5% identical to SEQ ID NO: 12, SEQ ID NO: 16, or SEQ ID NO: 26. In an embodiment, the sPD-1 nucleic acid molecule does not comprise SEQ ID NO: 8.

**[0027]** A third aspect of the invention provides PD-1 fusion proteins. In one embodiment, the subject invention provides PD-1 fusion proteins, comprising a sPD-1 protein fragment fused with an antigenic protein fragment. In a further embodiment, the sPD-1 fusion protein comprises a Fc domain. In one embodiment, the soluble PD-1 protein is linked to the antigen via a linker sequence. In an alternative embodiment, the PD-1 fusion protein comprises a PD-1 protein fused with a Fc domain, optionally via a linker sequence.

**[0028]** In an embodiment not part of the invention, the sPD-1 fusion protein comprises the wild-type mouse soluble PD-1 protein (mspd1), which has an amino acid sequence comprising SEQ ID NO: 1. In an embodiment not part of the invention, the sPD-1 fusion protein comprises the wild-type human sPD-1 protein (hspd1), which has an amino acid sequence comprising SEQ ID NO: 21. In an embodiment, the sPD-1 fusion protein is a variant mouse sPD-1 protein mspd1-14del, which has an amino acid sequence comprising SEQ ID NO: 11. In an embodiment, the sPD-1 fusion protein is a variant mouse sPD-1 protein mspd1-322mu, which has an amino acid sequence comprising SEQ ID NO: 15. In an embodiment, the sPD-1 protein is a variant human sPD-1 protein (hspd1-14del), and has an amino acid sequence comprising SEQ ID NO: 25.

**[0029]** The antigenic protein fragment can be derived from an immunogenic fragment of viral, bacterial, fungal, or other microbial pathogens including, but not limited to, human immunodeficiency virus (HIV), HSV including HSV-1 and HSV-2, KSHV, HPV including HPV-6, HPV-11, HPV-16, and HPV-18, respiratory syncytial virus, rhinovirus, hepatitis viruses including hepatitis A virus, hepatitis B virus, hepatitis C virus, hepatitis D virus, hepatitis E virus, hepatitis F virus, and hepatitis G virus, oncoviruses, human T-lymphotropic virus Type I (HTLV-1), influenza, bovine leukemia virus (BLV), Epstein-Barr virus, pertussis, polio, measles, mumps, rubella, smallpox, zoster, anthrax, tetanus, rotavirus, rabies, chickenpox, meningococcus, diphtheria, anapapillomavirus, anthrax, plague, encephalitis, pneumococcus, pneumonia, typhus, typhoid fever, streptococcus, staphylococcus, neisseria, lyme disease, cholera, E. coli, shigella, leishmania, leprosy,

cytomegalovirus (CMV), respiratory syncytial virus, parainfluenza, adenovirus, varicella, flavivirus, dengue toxoplasmosis, coccidiomycosis, schistosomiasis, Mycobacteria tuberculosis, and malaria. The antigenic protein fragment can also be derived from tumor or cancer cells. In one embodiment, the soluble PD-1, its variants, and fusion proteins thereof serve as molecular or protein adjuvants to enhance immune response. Additionally, nucleic acid molecules encoding the soluble PD-1, its variants, and fusion proteins thereof can also be administered to a subject to enhance immune response.

**[0030]** In an embodiment, the antigenic protein fragment is derived from an immunogenic fragment of an HIV protein domain including, but not limited to, p24, gag, pol, nef, tat, rev, gp120, and gp41. In an embodiment, the antigen protein is derived from HIV p24. In a specific embodiment, the antigen protein comprises SEQ ID NO: 3. In a further embodiment, the sPD-1 fusion protein further comprises a Fc domain. In an embodiment, the sPD-1 fusion protein comprises a rabbit Fc domain for protein purification purpose.

**[0031]** The term "Fc domain" encompasses the full length and fragments of native human and animal Fc and Fc variant molecules and sequences, including for example, IgG, IgM, IgD, IgE, IgA and subtypes such as for example IgG1, IgG2, IgG3, IgG4, IgA1, and IgA2. As with Fc variants and native Fc's, the term "Fc domain" includes molecules in monomeric or multimeric form, whether digested from whole antibody or produced by other means.

**[0032]** In an embodiment, the antigenic protein fragment is derived from a tumor antigen.

**[0033]** The term "Fc variant" refers to a molecule or sequence that is modified from a native Fc but still comprises a binding site for the salvage receptor. Fc domains include molecules having two or more polypeptide chains associated covalently, noncovalently, or by both covalent and non-covalent interactions. IgG molecules typically form dimers; IgM, pentamers; IgD, dimers; and IgA, monomers, dimers, trimers, or tetramers. Multimers may be formed by exploiting the sequence and resulting activity of the native Ig source of the Fc or by derivatizing (as defined below) such a native Fc.

**[0034]** The Fc domain within the scope of the invention can be of antibodies 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. In a specific embodiment, the Fc domain is IgG1.

**[0035]** In a further embodiment, the sPD-1 fusion protein of the subject invention comprises a linker sequence that links the soluble PD-1 domain to the antigen. In addition, the Fc domain can also be linked to the fusion protein via a linker sequence. Linker sequence is typically a peptide chain. The length of the peptide may be, for example, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 40, 50 or more amino acid residues, but typically is between 5 and 25 residues.

**[0036]** Depending upon the length and side chain composition, a linker may have, but need not have, greater than average flexibility. Flexibility can be calculated using algorithms known in the art. In an embodiment, the linker sequence is SEQ ID NO: 29. Examples of useful linkers include, but are not limited to, 9Gly (SEQ ID NO: 30), 9Glu (SEQ ID NO: 31), 9Ser (SEQ ID NO: 32), 5GlyCys2ProCys (SEQ ID NO: 33), 4Gly3Ser (SEQ ID NO: 34), Ser Cys Val Pro Leu Met Arg Cys Gly Gly Cys Cys Asn (SEQ ID NO: 35), Pro Ser Cys Val Pro Leu Met Arg Cys Gly Gly Cys Cys Asn (SEQ ID NO: 36), Gly Asp Leu Ile Tyr Arg Asn Gln Lys (SEQ ID NO: 37), and 9 GlyProSerCysValProLeuMetArgCysGlyGlyCysCysAsn (SEQ ID NO: 38).

**[0037]** In a specific embodiment, the subject sPD-1 fusion protein comprises SEQ ID NO: 13. In another specific embodiment, the subject sPD-1 fusion protein comprises SEQ ID NO: 17. In another specific embodiment, the subject sPD-1 fusion protein comprises SEQ ID NO: 27.

**[0038]** In addition, the subject invention provides sPD-1 fusion nucleic acid constructs, comprising a nucleic acid molecule encoding the subject sPD-1 fusion protein. In one embodiment, the sPD-1 fusion construct comprises a nucleic acid molecule encoding a sPD-1 protein fused with a nucleic acid encoding a protein antigen. In a further embodiment, the PD-1 fusion construct comprises an Fc DNA. In one embodiment, the soluble PD-1 DNA is linked to the antigen DNA via a linker sequence. Optionally, the Fc DNA is linked to the sPD-1-antigen DNA via a linker DNA sequence.

**[0039]** The antigenic nucleic acid molecule of the subject invention encodes immunogenic fragments of viral, bacterial, fungal, or other microbial pathogens including, but not limited to, human immunodeficiency virus (HIV), HSV including HSV-1 and HSV-2, KSHV, HPV including HPV-6, HPV-11, HPV-16, and HPV-18, respiratory syncytial virus, rhinovirus, hepatitis viruses including hepatitis A virus, hepatitis B virus, hepatitis C virus, hepatitis D virus, hepatitis E virus, hepatitis F virus, and hepatitis G virus, oncoviruses, human T-lymphotropic virus Type I (HTLV-1), influenza, bovine leukemia virus (BLV), Epstein-Barr virus, pertussis, polio, measles, mumps, rubella, smallpox, zoster, anthrax, tetanus, rotavirus, rabies, chickenpox, meningococcus, diphtheria, anapapillomavirus, anthrax, plague, encephalitis, pneumococcus, pneumonia, typhus, typhoid fever, streptococcus, staphylococcus, neisseria, lyme disease, cholera, E. coli, shigella, leishmania, leprosy, cytomegalovirus (CMV), respiratory syncytial virus, parainfluenza, adenovirus, varicella, flavivirus, dengue toxoplasmosis, coccidiomycosis, schistosomiasis, Mycobacteria tuberculosis, and malaria.

**[0040]** In an embodiment, the antigenic nucleic acid molecule encodes a tumor antigen.

**[0041]** In an embodiment, the fusion nucleic acid molecule comprises the wild-type mouse PD-1 (mspd1) DNA (SEQ ID NO: 2). In an embodiment, the fusion nucleic acid molecule comprises the wild-type human PD-1 (hspd1) DNA (SEQ ID NO: 22). In an embodiment, the fusion nucleic acid molecule comprises a variant mouse PD-1 DNA that has a



sequence of SEQ ID NO: 12 or SEQ ID NO: 16. In an embodiment, the fusion nucleic acid molecule comprises a variant human PD-1 DNA that has a sequence of SEQ ID NO: 26.

**[0042]** In a specific embodiment, the subject PD-1 fusion DNA comprises SEQ ID NO: 14. In another specific embodiment, the subject PD-1 fusion DNA comprises SEQ ID NO: 18. In another specific embodiment, the subject PD-1 fusion DNA comprises SEQ ID NO: 28.

**[0043]** In certain embodiments, the PD-1 protein or nucleic acid of the subject invention is typically substantially free of other components, such as other biological molecules, proteins or peptides, nucleic acids, lipids and carbohydrates. The term "substantially free of," as used herein, encompasses preparations of the subject invention having less than about 20%, 10% and preferably less than 5% (by dry weight) contaminating factors (such as biological molecules, proteins or peptides, nucleic acids, lipids and carbohydrates and other cellular components).

**[0044]** If desired, the subject proteins and nucleic acid molecules can be modified by any suitable process. Strategies for protein optimization are sometimes carried out using random mutagenesis. In these cases positions are chosen randomly, or amino acid changes are made using simplistic rules. For example all residues may be mutated to alanine, referred to as alanine scanning. In addition, substitution of amino acids other than those specifically exemplified or naturally present in a fusion protein of the invention are also within the scope of the subject invention. For example, non-natural amino acids can be substituted for the amino acids of the fusion protein, so long as the fusion protein having the substituted amino acids retains substantially the same functional activity as the fusion protein in which amino acids have not been substituted.

**[0045]** Examples of non-natural amino acids include, but are not limited to, ornithine, citrulline, hydroxyproline, homo-serine, phenylglycine, taurine, iodotyrosine, 2,4-diaminobutyric acid,  $\alpha$ -amino isobutyric acid, 4-aminobutyric acid, 2-amino butyric acid,  $\gamma$ -amino butyric acid,  $\epsilon$ -amino hexanoic acid, 6-amino hexanoic acid, 2-amino isobutyric acid, 3-amino propionic acid, norleucine, norvaline, sarcosine, homocitrulline, cysteic acid,  $\tau$ -butylglycine,  $\tau$ -butylalanine, phenylglycine, cyclohexylalanine,  $\beta$ -alanine, fluoro-amino acids, designer amino acids such as  $\beta$ -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.

**[0046]** The subject invention also concerns variants of nucleic acid molecules that encode functional fusion proteins of the invention. Variant sequences include those sequences wherein one or more nucleotides of the sequence have been substituted, deleted, and/or inserted.

**[0047]** 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 tri-ylated 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.

**[0048]** 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 NCBI/NIH website.

**[0049]** The subject invention also contemplates those nucleic acid molecules having sequences which are sufficiently homologous with the nucleic acid sequences exemplified herein so as to permit hybridization with that sequence under standard stringent conditions and standard methods (Maniatis et al., 1982). As used herein, "stringent" conditions for hybridization refers to conditions wherein hybridization is typically carried out overnight at 20-25 C below the melting temperature ( $T_m$ ) of the DNA hybrid in 6x SSPE, 5x Denhardt's solution, 0.1% SDS, 0.1 mg/ml denatured DNA. The melting temperature,  $T_m$ , is described by the following formula (Beltz et al., 1983):

$$T_m = 81.5 C + 16.6 \log[Na^+] + 0.41(\%G+C) - 0.61(\% \text{ formamide}) - 600/\text{length of duplex in base pairs.}$$

**[0050]** Washes are typically carried out as follows:

- (1) Twice at room temperature for 15 minutes in 1x SSPE, 0.1% SDS (low stringency wash).
- (2) Once at  $T_m - 20$  C for 15 minutes in 0.2x SSPE, 0.1% SDS (moderate stringency wash).

**[0051]** Further, the subject invention provides expression constructs comprising PD-1 nucleic acid molecules or fusion constructs thereof. Expression constructs of the invention generally include regulatory elements that are functional in the intended host cell in which the expression construct is to be expressed. Regulatory elements include promoters, transcription termination sequences, translation termination sequences, enhancers, and polyadenylation elements.

5 **[0052]** An expression construct of the invention can comprise a promoter sequence operably linked to a nucleic acid sequence encoding a peptide of the invention. 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  
10 in the expression construct.

**[0053]** For expression in animal cells, an expression construct of the invention can comprise suitable promoters that can drive transcription of the polynucleotide sequence. For mammalian cells, suitable promoters include such as, for example, Pcmv, 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  
15 promoter, and TRP-1 promoter.

#### Protection against Pathogenic Infection and Cancer

20 **[0054]** Another aspect of the subject invention provides methods for the prevention and/or treatment of pathogenic infection and/or cancer. Advantageously, the methods of the subject invention induce antigen-specific humoral and cell-mediated immunity. In one embodiment, the method comprises administering, to a subject in need of such treatment, an effective amount of a fusion protein or fusion nucleic acid molecule of the subject invention.

**[0055]** In an embodiment, the subject invention provides a method of inducing protective immunity against pathogenic infection and/or cancer. In a specific embodiment, the method comprises administering a composition comprising a fusion nucleic acid molecule, wherein the fusion nucleic acid molecule comprises a nucleic acid encoding an antigen of interest; a sPD-1 nucleic acid encoding a wild-type soluble PD1 protein, a nucleic acid encoding a spd1-14del protein of the invention, or a nucleic acid encoding a spd1-322 del protein of the invention; and, optionally, a nucleic acid encoding Fc domain and a linker nucleic acid sequence that links the sPD-1 nucleic acid and the antigen nucleic acid. In one  
25 embodiment, the composition is administered by intramuscular injection via electroporation (EP).

30 **[0056]** In another specific embodiment, the method comprises administering a composition comprising a fusion protein, wherein the fusion protein comprises an antigen of interest; a soluble PD-1 protein selected from a wild-type soluble PD1 protein, a spd1-14del protein of the invention, or a spd1-322 del protein of the invention; and, optionally, a Fc domain and a linker sequence that links the sPD-1 protein and the antigen protein.

35 **[0057]** The methods can be used for prevention and/or treatment of infection and other diseases where induction of antigen-specific humoral and cell-mediated immunity is beneficial. In a specific embodiment, the subject invention can be used in the prevention and/or treatment of tumor or cancer.

**[0058]** The term "treatment" or any grammatical variation thereof (*e.g.*, treat, treating, and treatment *etc.*), as used herein, includes but is not limited to, ameliorating or alleviating a symptom of a disease or condition, reducing, suppressing, inhibiting, lessening, or affecting the progression, severity, and/or scope of a condition.

40 **[0059]** The term "prevention" or any grammatical variation thereof (*e.g.*, prevent, preventing, and prevention *etc.*), as used herein, includes but is not limited to, delaying the onset of symptoms, preventing relapse to a disease, decreasing the number or frequency of relapse episodes, increasing latency between symptomatic episodes, or a combination thereof. Prevention, as used herein, does not require complete inhibition or elimination of symptoms. The term "effective amount," as used herein, refers to an amount that is capable of treating or ameliorating a disease or condition or otherwise  
45 capable of producing an intended therapeutic effect.

**[0060]** The term "subject," as used herein, describes an organism, including mammals such as primates, to which treatment with the compositions according to the subject invention can be provided. Mammalian species that can benefit from the disclosed methods of treatment include, but are not limited to, apes, chimpanzees, orangutans, humans, monkeys; and other animals such as dogs, cats, horses, cattle, pigs, sheep, goats, chickens, mice, rats, guinea pigs, and hamsters.  
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**[0061]** In certain embodiments, in case of prevention of pathogenic infection or cancer, the sPD-1-based composition of the invention is administered to a subject that does not suffer from the pathogenic infection or cancer type to be prevented, or a subject that does not exhibit symptoms of the pathogenic infection or cancer type to be prevented.

55 **[0062]** In one embodiment, the subject invention can be used in the prevention and/or treatment of infection by viral, bacterial, fungal, or other microbial pathogens including, but not limited to, human immunodeficiency virus (HIV), HSV including HSV-1 and HSV-2, KSHV, HPV including HPV-6, HPV-11, HPV-16, and HPV-18, respiratory syncytial virus, rhinovirus, hepatitis viruses including hepatitis A virus, hepatitis B virus, hepatitis C virus, hepatitis D virus, hepatitis E virus, hepatitis F virus, and hepatitis G virus, oncoviruses, human T-lymphotropic virus Type I (HTLV-1), influenza, bovine

leukemia virus (BLV), Epstein-Barr virus, pertussis, polio, measles, mumps, rubella, smallpox, zoster, anthrax, tetanus, rotavirus, rabies, chickenpox, meningococcus, diphtheria, anapapillomavirus, anthrax, plague, encephalitis, pneumococcus, pneumonia, typhus, typhoid fever, streptococcus, staphylococcus, neisseria, lyme disease, cholera, *E. coli*, shigella, leishmania, leprosy, cytomegalovirus (CMV), respiratory syncytial virus, parainfluenza, adenovirus, varicella, flavivirus, dengue toxoplasmosis, coccidiomycosis, schistosomiasis, *Mycobacteria tuberculosis*, and malaria.

**[0063]** In a specific embodiment, the subject invention can be used to prevent and/or treat HIV infection. In certain embodiments, the method comprises administering to a subject in need of such treatment an effective amount of a fusion protein, comprising an amino acid sequence selected from SEQ ID NOs: 13, 17, and 27. In specific embodiments, the subject method comprises administering to a subject in need of such treatment an effective amount of a fusion DNA, comprising a nucleic acid sequence selected from SEQ ID NOs: 14, 18, and 28.

**[0064]** In addition, the methods can be used in the treatment or amelioration of diseases where enhanced humoral and cell-mediated immunity is beneficial. In an embodiment, the subject invention can be used in the treatment or amelioration of tumor or cancer. In specific embodiments, sPD-1 proteins are useful for the treatment or amelioration of tumor or cancer.

**[0065]** In one embodiment, the sPD-1 protein useful for the treatment or amelioration of tumor comprises an antigenic fragment derived from cancer or tumor cells. Soluble PD-1 proteins useful for the treatment or amelioration of tumor or cancer also include, for example, mspdl-14del (SEQ ID NO: 11), mspd1-322mu (SEQ ID NO: 15), hspd1-14del (SEQ ID NO: 25), or fusion proteins thereof. Additionally or alternatively, the PD-1 protein useful for the treatment or amelioration of tumor or cancer comprises an amino acid sequence that is at least 95%, 96%, 97%, 98%, 99%, or 99.5% identical to mspdl-14del (SEQ ID NO: 11), mspd1-322mu (SEQ ID NO: 15), hspd1-14del (SEQ ID NO: 25), or fusion proteins thereof.

**[0066]** In specific embodiments, sPD-1 nucleic acid molecules useful for the treatment or amelioration of tumor or cancer include, for example, the wild-type mspd-1 DNA (SEQ ID NO: 2), the wild-type hspd1 DNA (SEQ ID NO: 22), mspd1-14del DNA (SEQ ID NO: 12), mspd1-322mu DNA (SEQ ID NO: 16), hspd1-14del DNA (SEQ ID NO: 26), or fusion DNA thereof. Additionally or alternatively, the sPD-1 nucleic acid molecule useful for the treatment or amelioration of tumor or cancer comprises a sequence that is at least 90%, 95%, 96%, 97%, 98%, 99%, or 99.5% identical to the wild-type mspd-1 DNA (SEQ ID NO: 2), the wild-type hspd1 DNA (SEQ ID NO: 22), mspd1-14del DNA (SEQ ID NO: 12), mspd1-322mu DNA (SEQ ID NO: 16), hspd1-14del DNA (SEQ ID NO: 26), or fusion DNA thereof.

#### Therapeutic Compositions and Routes of Administration

**[0067]** The subject invention further provides for therapeutic or pharmaceutical compositions. In an embodiment, the composition comprises a therapeutically effective amount of a protein and/or nucleic acid molecule of the subject invention and, optionally, a pharmaceutically acceptable carrier.

**[0068]** In one embodiment, the proteins and/or nucleic acid molecules are formulated into a vaccine composition for administration to subjects having certain risks of pathogenic infection. A vaccine composition is an antigenic preparation that comprises one or more immunogenic antigens used to produce active immunity to a disease. In addition, the compositions of the subject invention can be administered to a subject with existing infection, and provides for customized vaccine schedules and compositions to prevent or minimize worsening of the diseases.

**[0069]** The subject invention contemplates therapeutic compositions useful for practicing the therapeutic methods described herein. The therapeutic composition can be any form of pharmaceutical format, including injectable formulations such as liquid and lyophilized injections.

**[0070]** In a specific embodiment, a therapeutically effective amount of a protein and/or nucleic acid molecule of the subject invention is typically an amount such that when administered in a physiologically tolerable composition is sufficient to achieve a plasma concentration of from about 0.01 microgram ( $\mu\text{g}$ ) per milliliter (mL) to about 200  $\mu\text{g}/\text{mL}$ . Stated differently, the dosage can vary from about 0.1 mg/kg to about 300 mg/kg, preferably from about 0.2 mg/kg to about 200 mg/kg, most preferably from about 0.5 mg/kg to about 20 mg/kg, in one or more dose administrations daily, for one or several days.

**[0071]** Suitable non-toxic pharmaceutically acceptable carriers for use with the agent will be apparent to those skilled in the art of pharmaceutical formulation. See, for example, Remington's Pharmaceutical Sciences, seventeenth edition, ed. Alfonso R. Gennaro, Mack Publishing Company, Easton, Pa. (1985). Suitable carriers include ethanol, dimethyl sulfoxide, glycerol, silica, alumina, starch, sorbitol, inositol, xylitol, D-xylose, mannitol, powdered cellulose, microcrystalline cellulose, talc, colloidal silicon dioxide, calcium carbonate, magnesium carbonate, calcium phosphate, calcium aluminium silicate, aluminium hydroxide, sodium starch phosphate, lecithin, and equivalent carriers and diluents. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions.

**[0072]** Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene glycol, water, ethanol, and the like. The therapeutic composition, if desired, can also contain minor amounts of wetting or

emulsifying agents, or pH buffering agents. In addition, if desired, the composition can contain minor amounts of auxiliary substances such as wetting or emulsifying agents, pH buffering agents and the like which enhance the effectiveness of the active ingredient.

**[0073]** The amount of active ingredient that may be combined with the carrier materials to produce a single dosage form will vary, depending such as the type of the condition and the subject to be treated. In general, a therapeutic composition contains from about 5% to about 95% active ingredient (w/w). More specifically, a therapeutic composition contains from about 20% (w/w) to about 80%, or about 30% to about 70%, active ingredient (w/w).

**[0074]** The preparation of a pharmacological composition that contains active ingredients dissolved or dispersed therein is well understood in the art and need not be limited based on formulation. Typically such compositions are prepared as injectables either as liquid solutions or suspensions; however, solid forms suitable for solution, or suspensions, in liquid prior to use also can be prepared. The preparation also can be emulsified.

**[0075]** The therapeutic composition of the subject invention can include pharmaceutically acceptable salts of the components therein. Pharmaceutically acceptable salts include the acid addition salts (formed with the free amino groups of a polypeptide) that are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, tartaric, mandelic and the like. Salts formed with the free carboxyl groups also can be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, 2-ethylamino ethanol, histidine, procaine and the like.

**[0076]** As used herein, the terms "pharmaceutically acceptable", "physiologically tolerable" and grammatical variations thereof, as they refer to compositions, carriers, diluents and reagents, are used interchangeably and represent that the materials are capable of administration to or upon a mammal.

**[0077]** The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients, e.g., compound, carrier suitable for administration.

**[0078]** The compositions of the subject invention can be administered to the subject being treated by standard routes, including oral, inhalation, or parenteral administration including intravenous, subcutaneous, topical, transdermal, intradermal, transmucosal, intraperitoneal, intramuscular, intracapsular, intraorbital, intracardiac, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal, epidural and intrasternal injection, infusion, and electroporation, as well as co-administration as a component of any medical device or object to be inserted (temporarily or permanently) into a subject.

**[0079]** In a preferred embodiment, the microparticles of the subject invention can be formulated for parenteral administration. The preparation of an aqueous composition that contains one or more agents, such as a protein or nucleic acid molecule of the subject invention, will be known to those of skill in the art in light of the present disclosure. Typically, such compositions can be prepared as injectables, either as liquid solutions or suspensions; solid forms suitable for using to prepare solutions or suspensions upon the addition of a liquid prior to injection can also be prepared; and the preparations can also be emulsified.

**[0080]** The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions; formulations including sesame oil, peanut oil or aqueous propylene glycol; and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In all cases the form must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms, such as bacteria and fungi.

**[0081]** Sterile injectable solutions are prepared by incorporating the active ingredients in the required amount in the appropriate solvent followed by filtered sterilization. Generally, dispersions are prepared by incorporating the various sterilized active ingredients into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying techniques, which yield a powder of the active ingredient, plus any additional desired ingredient from a previously sterile-filtered solution thereof.

**[0082]** In addition, the nucleic acid molecules and compositions of the subject invention can *delivered in vivo* into a host cell by methods known in the art. In one embodiment, the nucleic acid molecules and compositions of the subject invention can be introduced *in vivo* via a viral vector such as adeno-associated virus (AAV), herpes simplex virus (HSV), retrovirus, papillomavirus, adenovirus, and Epstein-Barr virus (EBV). In addition, the nucleic acid molecules and compositions of the subject invention can also 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 the nucleic acid molecules of the invention. The nucleic acid molecules of the subject invention can also be introduced *in vivo* as naked DNA using methods known in the art, such as transfection, microinjection, electroporation, calcium phosphate precipitation, and by biolistic methods.

## EXAMPLES

**[0083]** Following are examples that illustrate embodiments 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- Construction of mouse sPD-1 vaccine candidates

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**[0084]** This Example illustrates the construction of mouse sPD-1-p24 fusion constructs. To construct mouse sPD-1-p24-Fc construct, PVAX vector that carries the wild-type msPD-1 gene and p24 gene was fused with rabbit Fc DNA. The vector and the p24-Fc DNA were linked by a linker encoding GGGSGGG (SEQ ID NO: 29). The transcription is under the control of promoter Pcmv.

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**[0085]** Mouse sPD1 protein variant mspd1-IgV $\Delta$  was obtained by deleting amino acids 89-90 of the mouse PD-1 protein, which forms the C'D loop of the IgV domain and is essential for PD-1 and PD-L1/L2 interaction. PVAX vector carrying mspd1-IgV $\Delta$  was linked with p24-rabbit Fc DNA via a linker encoding GGGSGGG (SEQ ID NO: 29) linker sequence. In addition, p24-Fc fusion construct was obtained by linking the PVAX vector carrying p24 with rabbit Fc DNA. The transcription is under the control of promoter Pcmv.

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**[0086]** Figure 1A shows alignment of amino acid sequences of msPd1-p24-Fc, mspd1-IgV $\Delta$ -p24-Fc, and p24-fc fusion proteins. Figure 1B shows Western blot results of various fusion constructs useful according to the subject invention. Briefly, 293T cells were transfected with various fusion constructs using polyethylenimine (PEI) and the supernatants were collected 72 hours post transfection. Proteins were detected by anti-rabbit Fc antibody. Figure 1B shows that msPd1-p24-Fc and mspd1-IgV $\Delta$ -p24-Fc are about 72KD in size, while p24-Fc is about 50KD in size. The results also show that msPd1-p24-Fc, mspd1-IgV $\Delta$ -p24-Fc, and p24-Fc fusion proteins are soluble.

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#### EXAMPLE 2- BINDING ABILITY OF MOUSE SPD-1 FUSION PROTEIN TO SPD-1 LIGANDS

**[0087]** This Example shows the binding ability of msPD-1 fusion proteins to mouse sPD-1 ligands. Briefly, 293T cells were transfected with PD-L (PD-L1 and PD-L2). The binding of sPD-1 proteins to PD-1 ligands was detected by FITC-anti rabbit Fc antibody using flow cytometer, and the results were analyzed by flowJo.

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**[0088]** The results, as shown in Figures 2A-B, reveal that mspd1-p24-Fc binds to mouse PD-1 ligands PD-L1 and PD-L2. In contrast, the variant mspd1-IgV $\Delta$  fusion protein does not bind to mouse PD-1 ligands. In addition, p24-Fc does not affect the interaction between PD-1 and PD-L1/L2.

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#### EXAMPLE 3 - INDUCTION OF HUMORAL AND CELL-MEDIATED IMMUNE RESPONSES BY WILD-TYPE MOUSE SPD1 VACCINE

**[0089]** This Example shows that the wild-type msPD1-p24-Fc potently induces humoral and cell-mediated immune responses. Briefly, Balb/c mice were primed at week 0 and boosted at week 3 and week 6 with 20 $\mu$ g/mice DNA vectors encoding msPd1-p24-Fc, mspd1-IgV $\Delta$ -p24-Fc, or p24-Fc via intramuscular electroporation. Mice received PBS served as controls.

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**[0090]** Two weeks after the last immunization, mice sera were collected and contacted with HIV-1 p24 viral proteins. The levels of anti-p24 IgG1 and IgG2a antibodies were measured by ELISA. The level of anti-p24 antibody in control samples is not shown because the absorbance readouts of these samples fell below the cutoff values for determining antibody titers. The anti-p24 antibody endpoint titer is defined as the reciprocal of the highest dilution of a test sample that produces a reading of at least two-fold greater than that of the control sample with the same dilution. The results show that mice immunized with mspd1-p24-Fc had high IgG1 and IgG2a titers, when compared to mice immunized with p24-Fc or mspd1-IgV $\Delta$ -p24-Fc.

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**[0091]** To examine p24-specific immune responses, the number of IFN- $\gamma$ -secreting splenocytes specific for p24 epitope gagAI (AMQMLKDTI) (SEQ ID NO: 39) for CD8 T cells and the number of splenocytes specific for p24 epitope gag26 (TSNPPIPVGDIIYKRWIIILGL) (SEQ ID NO: 40) for CD4 T cells was determined using ELISpot assay. In addition, splenocytes isolated from immunized vaccinated mice were subject to H2d-Kd- AMQMLKDTI-PE tetramer staining, and CD8 T cell and CD4 T cell population was analyzed.

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**[0092]** The results show that mice immunized with mspd1-p24-Fc had high anti-p24 antibody titers (Figure 3A) and high number of IFN- $\gamma$ -secreting splenocytes (Figures 3B and 3D), when compared to mice immunized with p24-Fc or mspd1-IgV $\Delta$ -p24-Fc. Splenocytes isolated from mice immunized with mspd1-p24-Fc contained about five-fold higher H2d-Kd-AMQMLKDTI tetramer-positive cells (Figures 3C), when compared to mice immunized with p24-Fc or mspd1-IgV $\Delta$ -p24-Fc.

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## EXAMPLE 4 - REDUCTION OF VTT-HIV-GAGPOL TITERS IN MICE IMMUNIZED WITH WILD-TYPE MOUSE SPD FUSION PROTEIN

**[0093]** This Example shows that immunization with the wide-type msPD1 fusion protein protects against viral infection. Briefly, Balb/c mice were primed at week 0 and boosted at week 3 and week 6 with 20 $\mu$ g/mice DNA encoding msPd1-p24-Fc, mspd1-IgV $\Delta$ -p24-Fc, or p24-Fc via intramuscular electroporation. Mice received PBS served as controls. Three weeks after immunization, mice were challenged with 4 $\times$ 10<sup>7</sup>PFU vaccinia VTT-HIV-gagpol intranasally. The mice were sacrificed 3 days after viral challenge and viral titers in the lungs were evaluated by plaque assay. The results show that mice immunized with mspd1-p24-Fc exhibited significantly reduced VTT-HIV-gagpol titers upon viral challenge (Figure 4).

## EXAMPLE 5 - INDUCTION OF HUMORAL AND CELL-MEDIATED IMMUNE RESPONSES BY WILD-TYPE HUMAN SPD1 VACCINE

**[0094]** Human sPD-1-p24-Fc was constructed by fusing PVAX vector carrying hsPD-1-p24 with rabbit Fc DNA. The vector and the p24-Fc DNA were linked by a linker encoding GGGSGGG (SEQ ID NO: 29). The transcription is under the control of promoter Pcmv.

**[0095]** To analyze the binding ability of hsPD-1-p24-Fc to SPD-1 ligands, 293T cells were transfected with mouse PD-L1 and PD-L2, respectively. The binding of sPD-1 proteins to PD-1 ligands was detected by mouse sPD-1-Fc proteins and FITC-anti rabbit Fc antibody using flow cytometer, and the results were analyzed by flowJo. The results, as shown in Figures 6A and 6B, reveal that hspd1-p24-Fc fusion protein binds to PD-1 ligands.

**[0096]** To examine the induction of immune responses by hsPD-1-p24-Fc, Balb/c mice were primed at week 0 and boosted at week 3 and week 6 with 20 $\mu$ g/mice DNA encoding hsPD1-p24-Fc or p24-Fc via intramuscular electroporation. Mice received PBS served as controls.

**[0097]** Two weeks after the last immunization, mice sera were collected. The levels of anti-p24 IgG1 and IgG2a antibodies were measured by ELISA. The levels of anti-p24 antibody in control samples is not shown because the absorbance readouts of these samples fell below the cutoff values for determining antibody titers. The anti-p24 antibody endpoint titer is defined as the reciprocal of the highest dilution of a test sample that produces a reading of at least two-fold greater than that of the control sample with the same dilution. The results, as shown in Figure 6C, reveal that mice immunized with hspd1-p24-Fc had high IgG1 and IgG2a titers, when compared to mice immunized with p24-Fc.

**[0098]** To examine p24-specific immune response, the number of IFN- $\gamma$ -secreting splenocytes specific for p24 epitope gagAI (AMQMLKDTI) (SEQ ID NO: 39) for CD8 T cells and the number of IFN- $\gamma$ -secreting splenocytes specific for p24 epitope gag26 (TSNPPIPVGDIYKRWILGL) (SEQ ID NO: 40) for CD4 T cells was determined by ELISpot assay. Bars represent the average values of three samples ( $\pm$  standard deviations). The results, as shown in Figure 6D, reveal that wild-type hsPD1 (hspd1-p24-Fc) binds to mouse PD-L1 and PD-L2, and potently elicits humoral and cell-mediated immune responses.

**[0099]** Figure 5 shows that targeting dendritic cells using sPD-1-p24-fc induces enhanced p24-specific antibody and T cell responses.

## EXAMPLE 6 - CONSTRUCTION OF MOUSE SPD-1 VARIANT VACCINE CANDIDATES

**[0100]** This Example illustrates the construction of variant msPD-1 vaccine candidates. Mouse sPD1 variants, mspd1-14del and mspd1-322mu, were constructed (Figure 7A). The mspd1-14del variant is obtained by deleting amino acids 26-39 of the wild-type mspd1 (encoded by the first part of the second exon of the wild-type mouse PD-1 gene). The mspd1-322mu variant is obtained by changing amino acid residue 108 of the wild-type mouse PD-1 protein from Met to Val.

**[0101]** Mouse sPD-1 fusion constructs were obtained by fusing PVAX vector carrying mspd1 variant-p24 with rabbit Fc DNA. The PVAX vector and the p24-Fc DNA were linked by a linker encoding GGGSGGG (SEQ ID NO: 29). The transcription is under the control of promoter Pcmv.

**[0102]** Figure 7A shows alignment of amino acid sequences of msPd1-p24-Fc, mspd1-14del-p24-Fc, mspd1-322mu-p24-Fc, and p24-Fc fusion proteins. Figure 7B shows Western blot results of various fusion proteins useful according to the subject invention. Briefly, 293T cells were transfected with various fusion constructs using polyethylenimine (PEI) and the supernatants were collected 72 hours post transfection. Proteins were detected by anti-rabbit Fc antibody. Figure 7B shows that mspd1-14del-p24-Fc, and mspd1-322mu-p24-Fc are about 72KD in size, while p24-Fc is about 50KD in size. The results also show that mspd1-14del-p24-Fc, and mspd1-322mu-p24-Fc fusion proteins are soluble.

## EXAMPLE 7 - BINDING ABILITY OF MOUSE SPD-1 VARIANT FUSION PROTEINS TO SPD-1 LIGANDS

**[0103]** This Example shows that msPD-1 variant fusion proteins, mspd1-14del-p24-Fc and mspd1-322mu-p24-Fc, do not bind to mouse sPD-1 ligands PD-L1 and PD-L2 (Figure 8). Briefly, 293T cells were transfected with PD-L (PD-L1

## EP 2 638 061 B1

and PD-L2). The binding of sPD-1 proteins to PD-1 ligands was detected by mouse sPD-1-p24-Fc proteins and FITC-anti rabbit Fc antibody using flow cytometer, and the results were analyzed by flowJo.

EXAMPLE 8 - induction of Humoral and cell-mediated immune responses by variant mouse sPD1 vaccines

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**[0104]** This Example shows that msPD1 variants potently elicit humoral and cell-mediated immune responses. Briefly, Balb/c mice were primed at week 0 and boosted at week 3 and week 6 with 20 $\mu$ g/mice DNA vectors encoding mspd1-14del-p24-Fc, mspd1-322mu-p24-Fc, or p24-Fc via intramuscular electroporation. Mice received PBS served as controls.

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**[0105]** Two weeks after the last immunization, mice sera were collected. The levels of anti-p24 IgG1 and IgG2a antibodies were measured by ELISA. The level of anti-p24 antibody in control samples is not shown because the absorbance readouts of these samples fell below the cutoff values for determining antibody titers. The anti-p24 antibody endpoint titer is defined as the reciprocal of the highest dilution of a test sample that produces a reading of at least two-fold greater than that of the control sample with the same dilution. The results show that mice immunized with mspd1-14del-p24-Fc or mspd1-322mu-p24-Fc had high IgG1 and IgG2a titers, when compared to mice immunized with p24-Fc.

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**[0106]** To examine p24-specific immune response, the number of IFN- $\gamma$ -secreting splenocytes specific for p24 epitope gagAI (AMQMLKDTI) (SEQ ID NO: 39) for CD8 T cells and the number of IFN- $\gamma$ -secreting splenocytes specific for p24 epitope gag26 (TSNPPIPVGDIYKRWILGL) (SEQ ID NO: 40) for CD4 T cells was determined by ELISpot assay.

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**[0107]** The results show that mice immunized with mspd1-14del-p24-Fc or mspd1-322mu-p24-Fc had high anti-p24 antibody titers (Figure 9A) and high number of IFN- $\gamma$ -secreting splenocytes (Figures 9B and 9D). Splenocytes isolated from mice immunized with mspd1-14del-p24-Fc or mspd1-322mu-p24-Fc contained higher H2d-Kd-AMQMLKDTI tetramer-positive cells (Figures 9C), when compared to mice immunized with p24-Fc.

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EXAMPLE 9 - REDUCTION OF VTT-HIV-GAGPOL TITERS IN MICE IMMUNIZED WITH VARIANT MOUSE SPD1 FUSION PROTEINS

**[0108]** This Example shows that immunization with variant mspd1-14del-p24-Fc or mspd1-322mu-p24-Fc protects against HIV infection. Briefly, Balb/c mice were primed at week 0 and boosted at week 3 and week 6 with 20 $\mu$ g/mice DNA encoding mspd1-14del-p24-Fc or mspd1-322mu-p24-Fc via intramuscular electroporation. Three weeks after immunization, mice were challenged with  $4 \times 10^7$  PFU of VTT-HIV-gagpol intranasally. Mice received PBS served as controls. The mice were sacrificed 3 days after viral challenge and viral titers in lungs were evaluated by plaque assay. The results show that mice immunized with mspd1-14del-p24-Fc or mspd1-322mu-p24-Fc exhibited significantly reduced VTT-HIV-gagpol titers upon viral challenge (Figure 10).

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EXAMPLE 10 - INDUCTION OF HUMORAL AND CELL-MEDIATED IMMUNE RESPONSES BY VARIANT HUMAN SPD1 VACCINE

**[0109]** In this Example, variant hsPD1 construct, hsPD1-14del-p24-Fc, was constructed. To analyze the binding ability of hsPD1-14del-p24-Fc protein to mouse PD-L1 and PD-L2, 293T cells were transfected with PD-L1 and PD-L2, respectively. The binding of hsPD1-14del-p24-Fc protein to PD-1 were detected by FITC-anti rabbit Fc antibody using flow cytometer, and the results were analyzed by flowJo. The results, as shown in Figures 11A and 11B, reveal that hspd1-p24-Fc fusion protein binds to PD-1 ligands.

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**[0110]** To examine the induction of immune responses by hsPD1-14del-p24-Fc, Balb/c mice were primed at week 0 and boosted at week 3 and week 6 with 20 $\mu$ g/mice DNA encoding hsPD1-14del-p24-Fc or p24-Fc via intramuscular electroporation. Mice received PBS served as controls.

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**[0111]** Two weeks after the last immunization, mice sera were collected. The levels of anti-p24 IgG1 and IgG2a antibodies were determined by ELISA. The levels of anti-p24 antibody in control samples are not shown because the absorbance readouts of these samples fell below the cutoff values for determining antibody titers. The anti-p24 antibody endpoint titer is defined as the reciprocal of the highest dilution of a test sample that produces a reading of at least two-fold greater than that of the control sample with the same dilution.

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**[0112]** To examine p24-specific immune response, the number of IFN- $\gamma$ -secreting splenocytes specific for p24 epitope gagAI (AMQMLKDTI) (SEQ ID NO: 39) for CD8 T cells and the number of IFN- $\gamma$ -secreting splenocytes specific for p24 epitope gag26 (TSNPPIPVGDIYKRWILGL) (SEQ ID NO: 40) for CD4 T cells was determined by ELISpot assay. Although hspd1-14del-p24-Fc protein does not bind to PD-1, the results show that hspd1-14del-p24-Fc significantly enhanced humoral and cell-mediated immune responses upon HIV viral challenge (Figures 11C and 11D).

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EXAMPLE 11 - ANTIGEN TARGETING TO DENDRITIC CELLS BY SPD-1-BASED VACCINE AMPLIFIES CD8<sup>+</sup> T CELL IMMUNITY

5 **[0113]** This Example shows that sPD-1-based vaccine improves CD8<sup>+</sup> T cell immunity by targeting vaccine antigens to dendritic cells (DCs), while blocking the negative effects of the PD-1/PD-L pathway on T cell function simultaneously.

**[0114]** HIV-1 Gag p24 was chosen as a test antigen because it has been commonly used in other DC targeting strategies as a model immunogen<sup>7,8</sup>. Three DNA vaccines, p24-fc, sPD-1-p24-fc, and slgV-PD-1-p24-fc, were designed (Figure 13a and 16a). slgV-PD-1-p24-fc differs from sPD-1-p24-fc by two essential amino acids in the functional IgV domain of sPD-1, rendering it unable to bind PD-1 ligands<sup>14</sup>.

10 **[0115]** The results show that PD-L1 and PD-L2 interact with recombinant sPD-1-p24-fc protein, but do not interact with slgV-PD-1-p24-fc or p24-fc proteins (Figure 16b).

**[0116]** In addition, Balb/c mice bone marrow (BM) derived CD11c<sup>+</sup> DCs that expresses PD-L1 and PD-L2 (Figure 17a) binds to sPD-1-p24-fc, but does not bind to slgV-PD-1-p24-fc (Figure 17b).

15 **[0117]** 2x10<sup>6</sup>BM-DCs were pulsed with 20μg sPD-1-p24-fc or control proteins, and infused back into Balb/c mice via the tail vein in accordance to a standard immunization schedule<sup>15,16</sup> (Figure 13b). Compared to slgV-PD-1-p24-fc, sPD-1-p24-fc-pulsed BM-DCs elicited higher levels of anti-p24 IgG1 (Th2) and IgG2a (Th1) antibody responses (p<0.05; Figure 17c). Increased levels of p24-specific CD8<sup>+</sup> T cell immunity was also evident as determined by IFN-γ ELISpot (Figure 17d) and H2-Kd-AMQMLKDTI (Gag-AI) tetramer assays<sup>17,18</sup> (Figure 17e). The results show that the sPD-1-based protein vaccine induced p24 specific CD8<sup>+</sup> T cell immunity by targeting dendritic cells.

20 **[0118]** The results also show that sPD-1-p24-fc can be used as a DNA vaccine against infection. The present inventions have previously shown that intramuscular (i.m.)EP enhances the immunogenicity of DNA vaccines consistently<sup>13,19,20</sup>.

**[0119]** In this Example, i.m. sPD-1-p24-fc/EP vaccination was conducted, using a vaccine dose of 20μg or 100μg (Figure 13b). The results show that sPD-1-p24-fc/EP elicited significantly higher levels of IgG1 (4-fold; p<0.01) and IgG2a (8-fold; p<0.01) antibody responses, when compared to the slgV-PD-1-p24-fc/EP control (Fig. 13c), in addition to potent and dose-dependent anti-Gag CD8<sup>+</sup> (p<0.001) and CD4<sup>+</sup> (p<0.05) T cell responses as determined by IFN-γ<sup>+</sup> ELISpots (Figures. 13d and 13e). Specifically, approximately 700 and 1600 ELISpots/10<sup>6</sup> splenocytes were found against the CD8<sup>+</sup>-specific Gag-AI epitope at the doses 20μg and 100μg, respectively. This greatly contrasts with the 200-300 ELISpots/10<sup>6</sup> splenocytes against the same epitope elicited by 1mg/i.m. ADVAX (a codon-optimized HIV DNA vaccine) or 10<sup>6</sup> TCID50/i.m. ADMVA (a vaccinia MVA-vectored HIV-1 vaccine) as previously described by the present inventors

30 **[0120]** The p24-specific T cell immunity was not confined to the single Gag-AI epitope. Approximately 800-1000 ELISpots/10<sup>6</sup> splenocytes was reactive to each of the three nonoverlapping peptide pools spanning the entire p24 protein, indicating a broad breadth in anti-Gag T cell responses following vaccination with sPD-1-p24-fc/EP (Figure 13f). Additionally, over 12.7% and 22% of CD8<sup>+</sup> T cells were positive for H2-Kd-Gag-AI tetramer binding in the 20μg and 100μg sPD-1-p24-fc/EP dose groups respectively, which is significantly higher than that of the slgV-PD-1-p24-fc/EP group (p<0.05, Figure 13g and 13h), and is comparable to those observed in Balb/c mice using a heterologous prime-boost protocol with two live vectors, L. monocytogenes and Ad5<sup>18</sup>.

35 **[0121]** In addition, this Example investigates the ability of p24-specific T cell populations to secrete IFN-γ, TNF-α and IL-2 in response to antigen stimulation. Compared to slgV-PD-1-p24-fc/EP, sPD-1-p24-fc/EP elicited substantially higher frequencies of p24-specific CD8<sup>+</sup> T cells producing IFN-γ (47.1%) and TNF-α (23.6%), and elevated frequencies of p24-specific CD4<sup>+</sup> T cells producing IFN-γ (6.8%) and TNF-α (3.6%) (Figure 14a and 14b). The results show that the proportion of effector-producing CD8<sup>+</sup> and CD4<sup>+</sup> T cell populations was similar in the order of IFN-γ<sup>+</sup>/TNF-α<sup>+</sup> > IFN-γ<sup>+</sup> > IFN-γ<sup>+</sup>/TNF-α<sup>+</sup>/IL-2<sup>+</sup> (Fig. 14c and 14d). Upon analyzing total cytokine-producing p24-specific CD8<sup>+</sup> T cells, high frequency of cells secreting IFN-γ (42.2%), IFN-γ/TNF-α (44.8%) and IFN-γ/TNF-α/IL-2 (9.4%) are indicative of enhanced vaccine potency (Fig. 14e).

45 **[0122]** To characterize sPD-1-p24-fc/EP vaccination and investigate its underlying mechanism(s) of immune induction, additional experiments were performed. Specifically, this Example compared sPD-1-p24-fc DNA vaccination with or without EP at the 20μg dose. Without EP, sPD-1-p24-fc induced 10-fold less IFN-γ<sup>+</sup>-secreting CD8<sup>+</sup> T cells than sPD-1-p24-fc/EP (Figure 18a and 13d), likely due to the omission of EP's effective recruitment of DCs to the site<sup>3</sup>. In addition, the lack of statistical difference between sPD-1-p24-fc and slgV-PD-1-p24-fc induced CD8<sup>+</sup> T cells when delivered without EP (Figure 18a) indicates that sPD-1 alone does not have a strong adjuvant effect.

50 **[0123]** In another experiment, mice were co-immunized with a mixture of 20μg of sPD-1-fc and p24-fc by i.m./EP, and no statistical difference between these two groups in their IFN-γ<sup>+</sup>/CD8<sup>+</sup> T cell response was found (Figure 18b), indicating that *de novo* synthesis of sPD-1-fc alone was insufficient to potentiate immunogenicity. This shows the importance of DC-targeting via fusion of the antigen to sPD-1.

55 **[0124]** To exclude a role of rabbit-Fc in enhancing p24-specific immunity, the rabbit Fc fragment was removed from sPD-1-p24-fc and p24-fc to generate sPD-1-p24 and p24 DNA vaccines for immunization. In corroboration to sPD-1-p24-fc/EP, sPD-1-p24/EP induced significantly higher levels of IFN-γ<sup>+</sup>/CD8<sup>+</sup> T cell response than p24/EP (Figure 18c).



Also, there was no statistical difference between sPD-1-p24-fc/EP and sPD-1-p24/EP in their ability to induce p24-specific IFN- $\gamma$ /CD8<sup>+</sup> T cell responses (Figure 13b and 18c).

**[0125]** In another experiment, a human (hu-)sPD-1-p24-fc vaccine for comparative study, as it is known that hu-sPD-1 cross-reacts with murine PD-L1 and PD-L223 (Figure 19a). The results show that hu-sPD-1-p24-fc/EP induced significantly greater levels of p24-specific IFN- $\gamma$ /CD8<sup>+</sup> T cell and antibody responses, when compared to p24-fc/EP in Balb/c mice (Figures 19b and 19c). Anti-human PD-1 responses were also induced due to the sequence divergence from murine PD-1, which may account for the difference between murine sPD-1-p24-fc/EP and hu-sPD-1-p24-fc/EP in the observed immunogenicity profile (i.e. p24-specific CD4<sup>+</sup> T cell response was weak in mice immunized with hu-sPD-1-p24-fc/EP) (Figure 13e and Figure 19c).

**[0126]** To determine whether sPD-1-p24-fc/EP elicited long-lived p24-specific memory T cell responses, groups of mice 7.5 months were sacrificed after the third immunization with 20 $\mu$ g DNA vaccine. Besides persistent anti-p24 IgG1 and IgG2a antibody responses (Fig. 15a), p24-specific CD8<sup>+</sup> ( $p < 0.05$ ) and CD4<sup>+</sup> ( $p < 0.05$ ) memory T cell responses were sustained in mice immunized with sPD-1-p24-fc/EP compared with controls (Fig 15b and 15c).

**[0127]** To investigate if cellular immunity elicited by sPD-1-p24-fc/EP leads to protection, Balb/c mice immunized with DNA vaccines at a dose of 100 $\mu$ g (Fig. 13b) were challenged intranasally with  $2 \times 10^5$  PFUs of a virulent strain of vaccinia modified to express HIV-1 gag and pol (WRgagpol). Eight days post-challenge, a significant reduction in virus titers in the lungs was observed in mice vaccinated with sPD-1-p24-fc/EP compared to controls ( $p < 0.01$ ; Fig. 15d). Mice immunized with the placebo or slgV-PD-1-p24-fc/EP showed >25% body weight loss within eight days after virus inoculation in contrast to mice immunized with sPD-1-p24-fc/EP that survived the challenge with <7% body weight loss (Fig. 15e). Since there were no anti-vaccinia neutralizing antibodies involved, the results indicated that p24-specific T cell immunity induced by sPD-1-p24-fc/EP provided significant protection against mucosal challenge by a virulent virus.

**[0128]** To summarize, this Example demonstrates that targeting of HIV-1 p24 to DCs via sPD-1 as a DNA vaccine enhanced the magnitude, breadth and polyfunctionality of specific CD8<sup>+</sup> T cell immunity. The sPD-1-based DNA vaccine can be used for inducing protective and long-lasting CD8<sup>+</sup> T cell immunity against pathogenic infections including HIV-1, tuberculosis, and malaria.

## MATERIALS AND METHODS

### *Construction of sPD-1-based vaccine and controls.*

**[0129]** Three DNA vaccines, sPD-1-p24-fc, slgV-PD-1-p24-fc, and p24-fc, were constructed in the background of pVAX1 (Fig. 13a). The coding sequence for the extracellular domain of murine PD-1 (sPD-1) was obtained by nested PCR from mouse cDNA 10,26, and the HIV-1 p24 fragment was amplified from a primary isolate HIV-102HNSq4 of a Chinese patient without codon-optimization<sup>27</sup>. To increase the flexibility of the fusion protein, a linker was applied between the sPD-1 and HIV-1 p24 gene.

**[0130]** A mutant form of sPD-1 (slgV-PD-1) was also cloned following the same strategy as wild type PD-1. slgV-PD1 does not react with PD-1 ligands<sup>4,28</sup> due to a two essential amino acid (position 89-90) in-frame deletion in the IgV domain. Plasmid expressing HIV-1 p24 alone served as a control.

**[0131]** All of the plasmids contained a rabbit Fc tag to facilitate protein purification and characterization. DNA transfection into (HEK-)293T cell was performed using Polyethylenimine (PEI), and protein expression was detected by Western blotting assay using anti-rabbit Fc antibody.

**[0132]** Recombinant proteins were purified from the transfected cell supernatants by affinity chromatography using Protein G Sepharose (Invitrogen), and protein concentration was measured by Micro BCA Protein Assay Kit (Thermo Scientific).

### *Binding characteristics of sPD-1 fusion proteins*

**[0133]**  $10^6$  293T cells transiently expressing PD-L1 and PD-L2 were incubated with 2 $\mu$ g of purified sPD-1-p24-fc, slgV-PD-1-p24-fc or p24-fc fusion protein. Goat anti-rabbit IgG (H+L)-FITC (Invitrogen) was used to capture the positive cells. Transfected 293T cells stained by FITC-rat anti-mouse PD-L1 or PD-L2 antibodies (eBioscience) and FITC-rat IgG1 isotype served as positive and negative controls, respectively. Data was acquired on FACSCalibur instrument (BD Biosciences) and analyzed using BD CellQuest software.

### *Mouse immunization*

**[0134]** All animal experiments were approved by the Committee on the Use of Live Animals in Teaching and Research of the University of Hong Kong. 5-8 weeks old female Balb/c mice were bred under standard pathogen-free conditions in the Laboratory Animal Unit, University of Hong Kong. Mice were housed in cages under standard conditions with

## EP 2 638 061 B1

regulated temperature and humidity, fed with pelleted food and tap water, and cared for according to the criteria outlined in the Guide for the Care and Use of Laboratory Animal.

**[0135]** The immunization procedure was similar to the previous protocols described in <sup>15,16</sup> (Fig. 13b). Mice received three DNA immunizations by intramuscular (i.m.) injection with or without EP given every three weeks at a dose of 20 $\mu$ g or 100 $\mu$ g per mouse. Two weeks after the final immunization, mice were sacrificed, and sera and spleen cells (splenocytes) were collected for immune response analysis.

### *Enzyme-linked immunosorbent assay (ELISA)*

**[0136]** Specific antibody responses were assessed by ELISA as previously described<sup>15,16</sup>. Briefly, high affinity, protein-binding ELISA plates (BD Biosciences) were coated with HIV-1 p24 protein (Abcam). Serial diluted sera were then added and antibodies detected by goat-radish peroxidase (HRP)-labeled anti-mouse IgG1 or IgG2a antibody (Sigma). Relative antibody titer was expressed as the reciprocal highest dilution of samples producing at least two-fold greater optical density readout over that of the control serum sample at the same dilution.

### *Evaluation of HIV-1 Gag p24-specific T cell responses*

**[0137]** IFN- $\gamma$ -producing T cells were evaluated by an ELISpot assay (Millipore) as previously described<sup>15,16</sup>. 2 $\mu$ g/ml of HIV-1 p24 peptide or peptide pools (at a final concentration of 2 $\mu$ g/ml for each peptide, donated by NIH) were used to stimulate splenocytes *in vitro*. Peptide pool consisting of 59-members of Gag p24 libraries were divided into 3 pools of 19-20 peptides that span from amino acids 1-87 (pool 1), 77-167 (pool 2) and 157-231 (pool 3). Peptide gagAI (AMQMLKDTI) is specific for CD8<sup>+</sup> T cells, whereas peptide gag26 (TSNPPIPVGDIYKRWIILGL) is specific for CD4<sup>+</sup> T cells<sup>15,16</sup>.

**[0138]** Cells stimulated by 500ng/ml PMA plus 1  $\mu$ g/ml calcium ionocycin or left in media only served as positive and negative controls, respectively. Cells were stimulated at 37°C, 5% CO<sub>2</sub>, and 100% humidity for 20h. Spots were identified by an immunospot reader and image analyzer (Thermo Scientific).

**[0139]** For intracellular cytokine staining (ICS), splenocytes were stimulated with HIV-1 p24 peptide pool (2 $\mu$ g/ml for each peptide) in the presence of co-stimulatory anti-CD28 antibody (2 $\mu$ g/ml, eBioscience) for 20-24h at 37°C. 10 $\mu$ g/ml Brefeldin A (BFA; Sigma) was added for the last 5h to accumulate intracellular cytokines. Cells were washed and incubated with 2.4G2 mAb for 15min at 4°C to block Fc $\gamma$ . After surface staining with anti-mouse CD3-APC/cy7, CD4-PE/cy5, CD8-Percp/cy5.5 antibodies (eBioscience), cells were permeabilized in 100 $\mu$ l Fixation/Permeabilization solution (BD Biosciences) for 20min at 4°C, washed with Perm/Wash™ buffer (BD), and then stained intracellularly with anti-IFN- $\gamma$ -PE, anti-IL-2-PE/cy7, anti-TNF- $\alpha$ -FITC (eBioscience). Tetramer positive CD8<sup>+</sup> T cell population was evaluated using phycoerythrin (PE)-conjugated major histocompatibility complex (MHC) class I tetramer H2d-Kd-AMQMLKDTI (Beckman Coulter). Flow cytometric data were acquired and analyzed on a BD Arial III flow cytometer (BD Biosciences).

### *Mouse immunization of antigen pulsed-dendritic cells*

**[0140]** Bone marrow DCs (BM- DCs) from Balb/c mice were enriched by Dynabeads Mouse DC Enrichment Kit (Invitrogen). Two million CD11c<sup>+</sup> BM-DCs were co-cultured with 20 $\mu$ g of purified sPD-1-p24-fc or sIgV-PD-1-p24-fc proteins for 1h at 4°C. Cells were then washed extensively with PBS and transduced into mice via tail vein injection. Untreated DCs alone served as control. Immunization procedure and immune responses analysis were the same as described above.

### *Vaccinia viral challenges*

**[0141]** Immunized mice were challenged intranasally with 2x10<sup>5</sup> PFUs vaccinia strain Western Reserve (WR) virus modified to express HIV-1 gag and pol genes. Animal body weight was monitored daily. Groups of animals were also sacrificed 8 days post challenge to measure viral titers in their lungs. Lung homogenate were prepared by physical disruption, and virus titers in the lungs were determined by a plaque-forming assay on monolayer Vero cells and monitored for cytopathic effect.

### *Statistical analysis*

**[0142]** All statistical analyses were performed using the paired one- tailed Student's t test. P values less than 0.05 were considered statistically significant. Data were presented as mean values  $\pm$  the standard error of at least three independent experiments.

**[0143]** All references, including publications, patent applications and patents, cited herein are hereby incorporated by

reference to the same extent as if each reference was individually and specifically indicated to be incorporated by reference and was set forth in its entirety herein.

**[0144]** The terms "a" and "an" and "the" and similar referents as used in the context of describing the invention are to be construed to cover both the singular and the plural, unless otherwise indicated herein or clearly contradicted by context.

**[0145]** Recitation of ranges of values herein are merely intended to serve as a shorthand method of referring individually to each separate value falling within the range, unless otherwise indicated herein, and each separate value is incorporated into the specification as if it were individually recited herein. Unless otherwise stated, all exact values provided herein are representative of corresponding approximate values (e.g., all exact exemplary values provided with respect to a particular factor or measurement can be considered to also provide a corresponding approximate measurement, modified by "about," where appropriate).

**[0146]** The use of any and all examples, or exemplary language (e.g., "such as") provided herein, is intended merely to better illuminate the invention and does not pose a limitation on the scope of the invention unless otherwise indicated. No language in the specification should be construed as indicating any element is essential to the practice of the invention unless as much is explicitly stated.

**[0147]** The description herein of any aspect or embodiment of the invention using terms such as "comprising", "having", "including" or "containing" with reference to an element or elements is intended to provide support for a similar aspect or embodiment of the invention that "consists of", "consists essentially of", or "substantially comprises" that particular element or elements, unless otherwise stated or clearly contradicted by context (e.g., a composition described herein as comprising a particular element should be understood as also describing a composition consisting of that element, unless otherwise stated or clearly contradicted by context).

**[0148]** 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.

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 25 Ser Asp Gly Ser Tyr Phe Leu Tyr Ser Lys Leu Ser Val Pro Thr Ser  
 245 250 255  
 Glu Trp Gln Arg Gly Asp Val Phe Thr Cys Ser Val Met His Glu Ala  
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EP 2 638 061 B1

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|    | ctgggaggct caaacgacat attcaacaac ttcacagtgt ccttctggtt gcgggttccc | 120 |
| 5  | aaggtctctg ctagccacct cgaacaatac ctggaggcca ccaacaccaa agtggacaag | 180 |
|    | accgttgccg cctcgacatg cagcaagccc atgtgccac cccctgaact cctgggggga  | 240 |
|    | ccgtctgtct tcatcttccc cccaaaaccc aaggacaccc tcatgatctc acgcaccccc | 300 |
| 10 | gaggtcacat gcgtgggtgt ggacgtgagc caggatgacc ccgaggtgca gttcacatgg | 360 |
|    | tacataaaca acgagcaggt gcgcaccgcc cggccgccgc tacgggagca gcagttcaac | 420 |
|    | agcacgatcc gcgtggtcag caccctcccc atcgcgcacc aggactggct gaggggcaag | 480 |
| 15 | gagttcaagt gcaaagtcca caacaaggca ctcccggccc ccatcgagaa aaccatctcc | 540 |
|    | aaagccagag ggcagcccct ggagccgaag gtctacacca tgggccctcc ccgggaggag | 600 |
| 20 | ctgagcagca ggtcggtcag cctgacctgc atgatcaacg gcttctacc ttccgacatc  | 660 |
|    | tcggtggagt gggagaagaa cgggaaggca gaggacaact acaagaccac gccgaccgtg | 720 |
| 25 | ctggacagcg acggctccta cttcctctac agcaagctct cagtgccac gagtgagtgg  | 780 |
|    | cagcggggcg acgtcttcac ctgctccgtg atgcacgagg ccttgacaaa ccactacacg | 840 |
|    | cagaagtcca tctcccactc tcctggtaaa taatctagag                       | 880 |
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|    | <212> PRT   |     |
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EP 2 638 061 B1

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 5 Leu Ser Trp Gln Ser Gly Trp Leu Leu Glu Val Pro Asn Gly Pro Trp  
 10 Arg Ser Leu Thr Phe Tyr Pro Ala Trp Leu Thr Val Ser Glu Gly Ala  
 15 Asn Ala Thr Phe Thr Cys Ser Leu Ser Asn Trp Ser Glu Asp Leu Met  
 20 Leu Asn Trp Asn Arg Leu Ser Pro Ser Asn Gln Thr Glu Lys Gln Ala  
 25 Ala Phe Cys Asn Gly Leu Ser Gln Gln Asp Ala Arg Phe Gln Ile Ile  
 30 Gln Leu Pro Asn Arg His Asp Phe His Met Asn Ile Leu Asp Thr Arg  
 35 Arg Asn Asp Ser Gly Ile Tyr Leu Cys Gly Ala Ile Ser Leu His Pro  
 40 Lys Ala Lys Ile Glu Glu Ser Pro Gly Ala Glu Leu Val Val Thr Glu  
 45 Arg Ile Leu Glu Thr Ser Thr Arg Tyr Pro Ser Pro Ser Pro Lys Pro  
 50 Glu Gly Arg Phe Gln Pro Glu Phe Arg Gly Gly Gly Ser Gly Gly Gly  
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EP 2 638 061 B1

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 5 tggctcacag tgctcagaggg agcaaatgcc accttcacct gcagcttgtc caactggctcg 180  
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 10 aggcatgact tccacatgaa catccttgac acacggcgca atgacagtgg catctacctc 360  
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 15 gtggtaacag agagaatcct ggagacctca acaagatc ccagcccctc gcccaaacca 480  
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 20 25 30  
 35 Arg Ser Leu Thr Phe Tyr Pro Ala Trp Leu Thr Val Ser Glu Gly Ala  
 35 40 45  
 40 Asn Ala Thr Phe Thr Cys Ser Leu Ser Asn Trp Ser Glu Asp Leu Met  
 50 55 60  
 45 Leu Asn Trp Asn Arg Leu Ser Pro Ser Asn Gln Thr Glu Lys Gln Ala  
 65 70 75 80  
 Ala Phe Cys Asn Gly Leu Ser Gln Gln Asp Ala Arg Phe Gln Ile Ile  
 50  
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EP 2 638 061 B1

|    |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |  |
|----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|--|
|    |     |     |     |     | 85  |     |     |     |     | 90  |     |     |     |     | 95  |     |  |
| 5  | Gln | Leu | Pro | Asn | Arg | His | Asp | Phe | His | Met | Asn | Ile | Leu | Asp | Thr | Arg |  |
|    |     |     |     | 100 |     |     |     |     | 105 |     |     |     |     | 110 |     |     |  |
| 10 | Arg | Asn | Asp | Ser | Gly | Ile | Tyr | Leu | Cys | Gly | Ala | Ile | Ser | Leu | His | Pro |  |
|    |     |     | 115 |     |     |     |     | 120 |     |     |     |     | 125 |     |     |     |  |
| 15 | Lys | Ala | Lys | Ile | Glu | Glu | Ser | Pro | Gly | Ala | Glu | Leu | Val | Val | Thr | Glu |  |
|    |     | 130 |     |     |     |     | 135 |     |     |     |     | 140 |     |     |     |     |  |
| 20 | Arg | Ile | Leu | Glu | Thr | Ser | Thr | Arg | Tyr | Pro | Ser | Pro | Ser | Pro | Lys | Pro |  |
|    | 145 |     |     |     |     | 150 |     |     |     |     | 155 |     |     |     |     | 160 |  |
| 25 | Glu | Gly | Arg | Phe | Gln | Pro | Glu | Phe | Arg | Gly | Gly | Gly | Ser | Gly | Gly | Gly |  |
|    |     |     |     | 165 |     |     |     |     |     | 170 |     |     |     |     | 175 |     |  |
| 30 | Pro | Ile | Val | Gln | Asn | Leu | Gln | Gly | Gln | Met | Val | His | Gln | Pro | Ile | Ser |  |
|    |     |     |     | 180 |     |     |     |     | 185 |     |     |     |     | 190 |     |     |  |
| 35 | Pro | Arg | Thr | Leu | Asn | Ala | Trp | Val | Lys | Val | Ile | Glu | Glu | Lys | Ala | Phe |  |
|    |     |     | 195 |     |     |     |     | 200 |     |     |     |     |     | 205 |     |     |  |
| 40 | Ser | Pro | Glu | Val | Ile | Pro | Met | Phe | Ser | Ala | Leu | Ser | Glu | Gly | Ala | Thr |  |
|    |     | 210 |     |     |     |     | 215 |     |     |     |     | 220 |     |     |     |     |  |
| 45 | Pro | Gln | Asp | Leu | Asn | Thr | Met | Leu | Asn | Thr | Val | Gly | Gly | His | Gln | Ala |  |
|    | 225 |     |     |     | 230 |     |     |     |     |     | 235 |     |     |     |     | 240 |  |
| 50 | Ala | Met | Gln | Met | Leu | Lys | Glu | Thr | Ile | Asn | Glu | Glu | Ala | Ala | Glu | Trp |  |
|    |     |     |     | 245 |     |     |     |     |     | 250 |     |     |     |     | 255 |     |  |
| 55 | Asp | Arg | Leu | His | Pro | Val | Gln | Ala | Gly | Pro | Val | Ala | Pro | Gly | Gln | Met |  |
|    |     |     | 260 |     |     |     |     |     | 265 |     |     |     |     | 270 |     |     |  |
| 60 | Arg | Glu | Pro | Arg | Gly | Ser | Asp | Ile | Ala | Gly | Thr | Thr | Ser | Asn | Leu | Gln |  |
|    |     | 275 |     |     |     |     |     | 280 |     |     |     |     | 285 |     |     |     |  |
| 65 | Glu | Gln | Ile | Gly | Trp | Met | Thr | Asn | Asn | Pro | Pro | Ile | Pro | Val | Gly | Glu |  |
|    |     | 290 |     |     |     | 295 |     |     |     |     |     | 300 |     |     |     |     |  |
| 70 | Ile | Tyr | Lys | Arg | Trp | Ile | Ile | Leu | Gly | Leu | Asn | Lys | Ile | Val | Arg | Met |  |
|    | 305 |     |     |     |     | 310 |     |     |     |     | 315 |     |     |     |     | 320 |  |
| 75 | Tyr | Ser | Pro | Thr | Ser | Ile | Leu | Asp | Ile | Arg | Gln | Gly | Pro | Lys | Glu | Pro |  |
|    |     |     |     | 325 |     |     |     |     |     | 330 |     |     |     |     | 335 |     |  |

EP 2 638 061 B1

Phe Arg Asp Tyr Val Asp Arg Phe Tyr Lys Thr Leu Arg Ala Glu Gln  
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5 Ala Ser Gln Glu Val Lys Asn Trp Met Thr Glu Thr Leu Leu Val Gln  
 355 360 365

10 Asn Ser Asn Pro Asp Cys Lys Thr Ile Leu Lys Ala Leu Gly Pro Ala  
 370 375 380

15 Ala Thr Leu Glu Glu Met Met Thr Ala Cys Gln Gly Val Gly Gly Pro  
 385 390 395 400

20 Gly His Lys Ala Arg Val Leu Met Gln Tyr Ile Lys Ala Asn Ser Lys  
 405 410 415

25 Phe Ile Gly Ile Thr Glu Leu Lys Lys Leu Gly Gly Ser Asn Asp Ile  
 420 425 430

30 Phe Asn Asn Phe Thr Val Ser Phe Trp Leu Arg Val Pro Lys Val Ser  
 435 440 445

35 Ala Ser His Leu Glu Gln Tyr Leu Glu Ala Thr Asn Thr Lys Val Asp  
 450 455 460

40 Lys Thr Val Ala Pro Ser Thr Cys Ser Lys Pro Met Cys Pro Pro Pro  
 465 470 475 480

45 Glu Leu Leu Gly Gly Pro Ser Val Phe Ile Phe Pro Pro Lys Pro Lys  
 485 490 495

50 Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val  
 500 505 510

55 Asp Val Ser Gln Asp Asp Pro Glu Val Gln Phe Thr Trp Tyr Ile Asn  
 515 520 525

60 Asn Glu Gln Val Arg Thr Ala Arg Pro Pro Leu Arg Glu Gln Gln Phe  
 530 535 540

65 Asn Ser Thr Ile Arg Val Val Ser Thr Leu Pro Ile Ala His Gln Asp  
 545 550 555 560

70 Trp Leu Arg Gly Lys Glu Phe Lys Cys Lys Val His Asn Lys Ala Leu  
 565 570 575

75 Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Arg Gly Gln Pro Leu  
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EP 2 638 061 B1

Glu Pro Lys Val Tyr Thr Met Gly Pro Pro Arg Glu Glu Leu Ser Ser  
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 5 Arg Ser Val Ser Leu Thr Cys Met Ile Asn Gly Phe Tyr Pro Ser Asp  
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 10 Ile Ser Val Glu Trp Glu Lys Asn Gly Lys Ala Glu Asp Asn Tyr Lys  
 625 630 635 640  
 15 Thr Thr Pro Thr Val Leu Asp Ser Asp Gly Ser Tyr Phe Leu Tyr Ser  
 645 650 655  
 Lys Leu Ser Val Pro Thr Ser Glu Trp Gln Arg Gly Asp Val Phe Thr  
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 10 aggcattgact tccacatgaa catccttgac acacggcgca atgacagtgg catctacctc 360  
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EP 2 638 061 B1

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 10 tggatgacag aaaccttggt ggtccaaaat tcgaaccag attgtaagac tattttaaaa 1140  
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 10 Gly Ala Asn Ala Thr Phe Thr Cys Ser Leu Ser Asn Trp Ser Glu Asp  
 15 Leu Met Leu Asn Trp Asn Arg Leu Ser Pro Ser Asn Gln Thr Glu Lys  
 20 Gln Ala Ala Phe Cys Asn Gly Leu Ser Gln Pro Val Gln Asp Ala Arg  
 25 Phe Gln Ile Ile Gln Leu Pro Asn Arg His Asp Phe His Met Asn Ile  
 30 Ser Leu His Pro Lys Ala Lys Ile Glu Glu Ser Pro Gly Ala Glu Leu  
 35 Val Val Thr Glu Arg Ile Leu Glu Thr Ser Thr Arg Tyr Pro Ser Pro  
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EP 2 638 061 B1

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5 agcttgtcca actggtcggg gatccttatg ctgaactgga accgcctgag tcccagcaac 180  
cagactgaaa aacaggccgc cttctgtaat ggtttgagcc aaccctcca ggatgcccgc 240  
ttccagatca tacagctgcc caacaggcat gacttcaca tgaacatcct tgacacacgg 300  
10 cgcaatgaca gtggcatcta cctctgtggg gccatctccc tgcaccccaa ggcaaaaatc 360  
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EP 2 638 061 B1

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20 25 30

10 Gly Ala Asn Ala Thr Phe Thr Cys Ser Leu Ser Asn Trp Ser Glu Asp  
35 40 45

15 Leu Met Leu Asn Trp Asn Arg Leu Ser Pro Ser Asn Gln Thr Glu Lys  
50 55 60

Gln Ala Ala Phe Cys Asn Gly Leu Ser Gln Pro Val Gln Asp Ala Arg  
65 70 75 80

20 Phe Gln Ile Ile Gln Leu Pro Asn Arg His Asp Phe His Met Asn Ile  
85 90 95

25 Leu Asp Thr Arg Arg Asn Asp Ser Gly Ile Tyr Leu Cys Gly Ala Ile  
100 105 110

Ser Leu His Pro Lys Ala Lys Ile Glu Glu Ser Pro Gly Ala Glu Leu  
115 120 125

30 Val Val Thr Glu Arg Ile Leu Glu Thr Ser Thr Arg Tyr Pro Ser Pro  
130 135 140

35 Ser Pro Lys Pro Glu Gly Arg Phe Gln Pro Glu Phe Arg Gly Gly Gly  
145 150 155 160

40 Ser Gly Gly Gly Pro Ile Val Gln Asn Leu Gln Gly Gln Met Val His  
165 170 175

Gln Pro Ile Ser Pro Arg Thr Leu Asn Ala Trp Val Lys Val Ile Glu  
180 185 190

45 Glu Lys Ala Phe Ser Pro Glu Val Ile Pro Met Phe Ser Ala Leu Ser

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EP 2 638 061 B1

|    | 195 |     | 200 |     |     |     |     |     |     |     |     |     | 205 |     |     |     |  |
|----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|--|
| 5  | Glu | Gly | Ala | Thr | Pro | Gln | Asp | Leu | Asn | Thr | Met | Leu | Asn | Thr | Val | Gly |  |
|    |     | 210 |     |     |     |     | 215 |     |     |     |     |     | 220 |     |     |     |  |
| 10 | Gly | His | Gln | Ala | Ala | Met | Gln | Met | Leu | Lys | Glu | Thr | Ile | Asn | Glu | Glu |  |
|    | 225 |     |     |     |     | 230 |     |     |     |     | 235 |     |     |     |     | 240 |  |
| 15 | Ala | Ala | Glu | Trp | Asp | Arg | Leu | His | Pro | Val | Gln | Ala | Gly | Pro | Val | Ala |  |
|    |     |     |     |     | 245 |     |     |     |     | 250 |     |     |     |     | 255 |     |  |
| 20 | Pro | Gly | Gln | Met | Arg | Glu | Pro | Arg | Gly | Ser | Asp | Ile | Ala | Gly | Thr | Thr |  |
|    |     |     |     | 260 |     |     |     |     | 265 |     |     |     |     |     | 270 |     |  |
| 25 | Ser | Asn | Leu | Gln | Glu | Gln | Ile | Gly | Trp | Met | Thr | Asn | Asn | Pro | Pro | Ile |  |
|    |     |     | 275 |     |     |     |     | 280 |     |     |     |     |     | 285 |     |     |  |
| 30 | Pro | Val | Gly | Glu | Ile | Tyr | Lys | Arg | Trp | Ile | Ile | Leu | Gly | Leu | Asn | Lys |  |
|    |     | 290 |     |     |     |     | 295 |     |     |     |     | 300 |     |     |     |     |  |
| 35 | Ile | Val | Arg | Met | Tyr | Ser | Pro | Thr | Ser | Ile | Leu | Asp | Ile | Arg | Gln | Gly |  |
|    | 305 |     |     |     |     | 310 |     |     |     |     | 315 |     |     |     |     | 320 |  |
| 40 | Pro | Lys | Glu | Pro | Phe | Arg | Asp | Tyr | Val | Asp | Arg | Phe | Tyr | Lys | Thr | Leu |  |
|    |     |     |     |     | 325 |     |     |     |     | 330 |     |     |     |     | 335 |     |  |
| 45 | Arg | Ala | Glu | Gln | Ala | Ser | Gln | Glu | Val | Lys | Asn | Trp | Met | Thr | Glu | Thr |  |
|    |     |     |     | 340 |     |     |     |     | 345 |     |     |     |     | 350 |     |     |  |
| 50 | Leu | Leu | Val | Gln | Asn | Ser | Asn | Pro | Asp | Cys | Lys | Thr | Ile | Leu | Lys | Ala |  |
|    |     |     | 355 |     |     |     |     | 360 |     |     |     |     |     | 365 |     |     |  |
| 55 | Leu | Gly | Pro | Ala | Ala | Thr | Leu | Glu | Glu | Met | Met | Thr | Ala | Cys | Gln | Gly |  |
|    |     | 370 |     |     |     |     | 375 |     |     |     |     | 380 |     |     |     |     |  |
| 60 | Val | Gly | Gly | Pro | Gly | His | Lys | Ala | Arg | Val | Leu | Met | Gln | Tyr | Ile | Lys |  |
|    | 385 |     |     |     |     | 390 |     |     |     |     | 395 |     |     |     |     | 400 |  |
| 65 | Ala | Asn | Ser | Lys | Phe | Ile | Gly | Ile | Thr | Glu | Leu | Lys | Lys | Leu | Gly | Gly |  |
|    |     |     |     |     | 405 |     |     |     |     | 410 |     |     |     |     | 415 |     |  |
| 70 | Ser | Asn | Asp | Ile | Phe | Asn | Asn | Phe | Thr | Val | Ser | Phe | Trp | Leu | Arg | Val |  |
|    |     |     |     | 420 |     |     |     |     | 425 |     |     |     |     | 430 |     |     |  |
| 75 | Pro | Lys | Val | Ser | Ala | Ser | His | Leu | Glu | Gln | Tyr | Leu | Glu | Ala | Thr | Asn |  |
|    |     |     | 435 |     |     |     |     | 440 |     |     |     |     | 445 |     |     |     |  |

EP 2 638 061 B1

Thr Lys Val Asp Lys Thr Val Ala Pro Ser Thr Cys Ser Lys Pro Met  
 450 455 460

5 Cys Pro Pro Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Ile Phe Pro  
 465 470 475 480

10 Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr  
 485 490 495

15 Cys Val Val Val Asp Val Ser Gln Asp Asp Pro Glu Val Gln Phe Thr  
 500 505 510

20 Trp Tyr Ile Asn Asn Glu Gln Val Arg Thr Ala Arg Pro Pro Leu Arg  
 515 520 525

25 Glu Gln Gln Phe Asn Ser Thr Ile Arg Val Val Ser Thr Leu Pro Ile  
 530 535 540

30 Ala His Gln Asp Trp Leu Arg Gly Lys Glu Phe Lys Cys Lys Val His  
 545 550 555 560

35 Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Arg  
 565 570 575

40 Gly Gln Pro Leu Glu Pro Lys Val Tyr Thr Met Gly Pro Pro Arg Glu  
 580 585 590

45 Glu Leu Ser Ser Arg Ser Val Ser Leu Thr Cys Met Ile Asn Gly Phe  
 595 600 605

50 Tyr Pro Ser Asp Ile Ser Val Glu Trp Glu Lys Asn Gly Lys Ala Glu  
 610 615 620

55 Asp Asn Tyr Lys Thr Thr Pro Thr Val Leu Asp Ser Asp Gly Ser Tyr  
 625 630 635 640

60 Phe Leu Tyr Ser Lys Leu Ser Val Pro Thr Ser Glu Trp Gln Arg Gly  
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65 Asp Val Phe Thr Cys Ser Val Met His Glu Ala Leu His Asn His Tyr  
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70 Thr Gln Lys Ser Ile Ser His Ser Pro Gly Lys  
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**EP 2 638 061 B1**

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5 <223> mspd1-14del-p24-Fc

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EP 2 638 061 B1

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 5 agcttgtcca actggtcgga ggatcttatg ctgaactgga accgcctgag tcccagcaac 180  
 cagactgaaa aacaggccgc cttctgtaat ggtttgagcc aaccctcca ggatgcccgc 240  
 ttccagatca tacagctgcc caacaggcat gacttcaca tgaacatcct tgacacacgg 300  
 10 cgcaatgaca gtggcatcta cctctgtggg gccatctccc tgcaccccaa ggcaaaaatc 360  
 gaggagagcc ctggagcaga gctcgtggta acagagagaa tcctggagac ctcaacaaga 420  
 15 tatcccagcc cctcgcccaa accagaaggc cggtttcaac cggaatccg ggggtggtggt 480  
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 tcacctagaa ctttaaatgc atgggtaaaa gtaatagaag agaaggcttt tagtccagaa 600  
 20 gtaataccca tgttttcagc attatcagaa ggagccaccc cacaagattt aaacacctatg 660  
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 gaagctgcag aatgggatag attgcatcca gtgcaggcag ggccagttgc accaggccag 780  
 25 atgagagaac caaggggaag tgacatagca ggaactacta gtaatcttca ggagcaaata 840  
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 30 ctgggggttaa ataaaatagt aagaatgtat agccctacca gcattctgga cataagacaa 960  
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EP 2 638 061 B1

cagcccctgg agccgaaggt ctacaccatg ggcctcccc gggaggagct gagcagcagg 1800  
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 30  
 Arg Ser Leu Thr Phe Tyr Pro Ala Trp Leu Thr Val Ser Glu Gly Ala  
 35 40 45  
 35  
 Asn Ala Thr Phe Thr Cys Ser Leu Ser Asn Trp Ser Glu Asp Leu Met  
 50 55 60  
 40  
 Leu Asn Trp Asn Arg Leu Ser Pro Ser Asn Gln Thr Glu Lys Gln Ala  
 65 70 75 80  
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 Ala Phe Cys Asn Gly Leu Ser Gln Pro Val Gln Asp Ala Arg Phe Gln  
 85 90 95  
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 Ile Ile Gln Leu Pro Asn Arg His Asp Phe His Val Asn Ile Leu Asp  
 100 105 110  
 50  
 Thr Arg Arg Asn Asp Ser Gly Ile Tyr Leu Cys Gly Ala Ile Ser Leu  
 115 120 125  
 55  
 His Pro Lys Ala Lys Ile Glu Glu Ser Pro Gly Ala Glu Leu Val Val  
 130 135 140  
 55  
 Thr Glu Arg Ile Leu Glu Thr Ser Thr Arg Tyr Pro Ser Pro Ser Pro  
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EP 2 638 061 B1

Lys Pro Glu Gly Arg Phe Gln Pro Glu Phe Arg Gly Gly Gly Ser Gly  
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5 Gly Gly

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 30 gagctcgtgg taacagagag aatcctggag acctcaacaa gatatcccag ccctcgccc 480  
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 50 Leu Ser Trp Gln Ser Gly Trp Leu Leu Glu Val Pro Asn Gly Pro Trp  
 20 25 30  
 Arg Ser Leu Thr Phe Tyr Pro Ala Trp Leu Thr Val Ser Glu Gly Ala  
 35 40 45  
 55 Asn Ala Thr Phe Thr Cys Ser Leu Ser Asn Trp Ser Glu Asp Leu Met  
 50 55 60

EP 2 638 061 B1

Leu Asn Trp Asn Arg Leu Ser Pro Ser Asn Gln Thr Glu Lys Gln Ala  
 65 70 75 80  
 Ala Phe Cys Asn Gly Leu Ser Gln Pro Val Gln Asp Ala Arg Phe Gln  
 5 85 90 95  
 Ile Ile Gln Leu Pro Asn Arg His Asp Phe His Val Asn Ile Leu Asp  
 10 100 105 110  
 Thr Arg Arg Asn Asp Ser Gly Ile Tyr Leu Cys Gly Ala Ile Ser Leu  
 115 120 125  
 His Pro Lys Ala Lys Ile Glu Glu Ser Pro Gly Ala Glu Leu Val Val  
 130 135 140  
 Thr Glu Arg Ile Leu Glu Thr Ser Thr Arg Tyr Pro Ser Pro Ser Pro  
 145 150 155 160  
 Lys Pro Glu Gly Arg Phe Gln Pro Glu Phe Arg Gly Gly Gly Ser Gly  
 165 170 175  
 Gly Gly Pro Ile Val Gln Asn Leu Gln Gly Gln Met Val His Gln Pro  
 180 185 190  
 Ile Ser Pro Arg Thr Leu Asn Ala Trp Val Lys Val Ile Glu Glu Lys  
 195 200 205  
 Ala Phe Ser Pro Glu Val Ile Pro Met Phe Ser Ala Leu Ser Glu Gly  
 210 215 220  
 Ala Thr Pro Gln Asp Leu Asn Thr Met Leu Asn Thr Val Gly Gly His  
 225 230 235 240  
 Gln Ala Ala Met Gln Met Leu Lys Glu Thr Ile Asn Glu Glu Ala Ala  
 245 250 255  
 Glu Trp Asp Arg Leu His Pro Val Gln Ala Gly Pro Val Ala Pro Gly  
 260 265 270  
 Gln Met Arg Glu Pro Arg Gly Ser Asp Ile Ala Gly Thr Thr Ser Asn  
 275 280 285  
 Leu Gln Glu Gln Ile Gly Trp Met Thr Asn Asn Pro Pro Ile Pro Val  
 290 295 300  
 Gly Glu Ile Tyr Lys Arg Trp Ile Ile Leu Gly Leu Asn Lys Ile Val  
 305 310 315 320

EP 2 638 061 B1

Arg Met Tyr Ser Pro Thr Ser Ile Leu Asp Ile Arg Gln Gly Pro Lys  
 325 330 335

5 Glu Pro Phe Arg Asp Tyr Val Asp Arg Phe Tyr Lys Thr Leu Arg Ala  
 340 345 350

10 Glu Gln Ala Ser Gln Glu Val Lys Asn Trp Met Thr Glu Thr Leu Leu  
 355 360 365

15 Val Gln Asn Ser Asn Pro Asp Cys Lys Thr Ile Leu Lys Ala Leu Gly  
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20 Pro Ala Ala Thr Leu Glu Glu Met Met Thr Ala Cys Gln Gly Val Gly  
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25 Gly Pro Gly His Lys Ala Arg Val Leu Met Gln Tyr Ile Lys Ala Asn  
 405 410 415

30 Ser Lys Phe Ile Gly Ile Thr Glu Leu Lys Lys Leu Gly Gly Ser Asn  
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35 Asp Ile Phe Asn Asn Phe Thr Val Ser Phe Trp Leu Arg Val Pro Lys  
 435 440 445

40 Val Ser Ala Ser His Leu Glu Gln Tyr Leu Glu Ala Thr Asn Thr Lys  
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45 Val Asp Lys Thr Val Ala Pro Ser Thr Cys Ser Lys Pro Met Cys Pro  
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50 Pro Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Ile Phe Pro Pro Lys  
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55 Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val  
 500 505 510

60 Val Val Asp Val Ser Gln Asp Asp Pro Glu Val Gln Phe Thr Trp Tyr  
 515 520 525

65 Ile Asn Asn Glu Gln Val Arg Thr Ala Arg Pro Pro Leu Arg Glu Gln  
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70 Gln Phe Asn Ser Thr Ile Arg Val Val Ser Thr Leu Pro Ile Ala His  
 545 550 555 560

75 Gln Asp Trp Leu Arg Gly Lys Glu Phe Lys Cys Lys Val His Asn Lys  
 565 570 575

EP 2 638 061 B1

Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Arg Gly Gln  
 580 585 590

5 Pro Leu Glu Pro Lys Val Tyr Thr Met Gly Pro Pro Arg Glu Glu Leu  
 595 600 605

10 Ser Ser Arg Ser Val Ser Leu Thr Cys Met Ile Asn Gly Phe Tyr Pro  
 610 615 620

15 Ser Asp Ile Ser Val Glu Trp Glu Lys Asn Gly Lys Ala Glu Asp Asn  
 625 630 635 640

Tyr Lys Thr Thr Pro Thr Val Leu Asp Ser Asp Gly Ser Tyr Phe Leu  
 645 650 655

20 Tyr Ser Lys Leu Ser Val Pro Thr Ser Glu Trp Gln Arg Gly Asp Val  
 660 665 670

25 Phe Thr Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln  
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 gaggatctta tgctgaactg gaaccgcctg agtcccagca accagactga aaaacaggcc 240  
 gccttctgta atggtttgag ccaaccctgc caggatgccc gcttccagat catacagctg 300  
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 gagctcgtgg taacagagag aatcctggag acctcaacaa gatatcccag cccctcgccc 480  
 55 aaaccagaag gccggtttca accggaattc cgggggtggtg gtggttcagg aggaggacct 540  
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EP 2 638 061 B1

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|    | gcattatcag aaggagccac cccacaagat ttaaaccacca tgctaaacac agtgggggga | 720  |
| 5  | catcaagcag ccatgcaaat gttaaaagaa accatcaatg aggaagctgc agaatgggat  | 780  |
|    | agattgcac cagtgcaggc agggccagtt gcaccaggcc agatgagaga accaagggga   | 840  |
|    | agtgacatag caggaactac tagtaatctt caggagcaaa taggatggat gacaaataat  | 900  |
| 10 | ccacctatcc cagtaggaga aatctataaa agatggataa tcctgggggtt aaataaaata | 960  |
|    | gtaagaatgt atagccctac cagcattctg gacataagac aaggacccaaa ggaacccttt | 1020 |
| 15 | agagactatg tagaccgggt ctataaaact ctaagagccg agcaagcttc acaagaggta  | 1080 |
|    | aaaaattgga tgacagaaac cttgttggtc caaaattcga acccagattg taagactatt  | 1140 |
|    | ttaaaagcat tgggaccagc agctacacta gaagaaatga tgacagcatg tcagggagtg  | 1200 |
| 20 | gggggacctg gccataaagc aagagttttg atcctgatgc agtacatcaa ggccaacagt  | 1260 |
|    | aagttcatcg gaatcaccga gcttaagaag ctggggaggct caaacgacat attcaacaac | 1320 |
|    | ttcacagtgt ccttctgggt gggggtccc aaggtctctg ctagccacct cgaacaatac   | 1380 |
| 25 | ctggaggcca ccaacaccaa agtggacaag accgttgccg cctcgacatg cagcaagccc  | 1440 |
|    | atgtgcccac cccctgaact cctgggggga ccgtctgtct tcatcttccc cccaaaaccc  | 1500 |
| 30 | aaggacaccc tcatgatctc acgcaccccc gaggtcacat gcgtggtggt ggacgtgagc  | 1560 |
|    | caggatgacc ccgaggtgca gttcacatgg tacataaaca acgagcaggt gcgcaccgcc  | 1620 |
|    | cggccgccgc tacgggagca gcagttcaac agcacgatcc gcgtggtcag caccctcccc  | 1680 |
| 35 | atcgcgcacc aggactggct gaggggcaag gagttcaagt gcaaagtcca caacaaggca  | 1740 |
|    | ctccccgccc ccatcgagaa aaccatctcc aaagccagag ggcagcccct ggagccgaag  | 1800 |
|    | gtctacacca tgggccctcc ccgggaggag ctgagcagca ggtcggtcag cctgacctgc  | 1860 |
| 40 | atgatcaacg gcttctaccc ttccgacatc tcggtggagt gggagaagaa cgggaaggca  | 1920 |
|    | gaggacaact acaagaccac gccgaccgtg ctggacagcg acggctccta cttcctctac  | 1980 |
| 45 | agcaagctct cagtgccac gagtgagtgg cagcggggcg acgtcttcac ctgctccgtg   | 2040 |
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EP 2 638 061 B1

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 10 Arg Ser Leu Thr Phe Tyr Pro Ala Trp Leu Thr Val Ser Glu Gly Ala  
 35 40 45  
 15 Asn Ala Thr Phe Thr Cys Ser Leu Ser Asn Trp Ser Glu Asp Leu Met  
 50 55 60  
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 65 70 75 80  
 25 Ala Phe Cys Asn Gly Leu Ser Gln Pro Val Gln Asp Ala Arg Phe Gln  
 85 90 95  
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 100 105 110  
 35 Thr Arg Arg Asn Asp Ser Gly Ile Tyr Leu Cys Gly Ala Ile Ser Leu  
 115 120 125  
 40 His Pro Lys Ala Lys Ile Glu Glu Ser Pro Gly Ala Glu Leu Val Val  
 130 135 140  
 45 Thr Glu Arg Ile Leu Glu Thr Ser Thr Arg Tyr Pro Ser Pro Ser Pro  
 145 150 155 160  
 50 Lys Pro Glu Gly Arg Phe Gln Pro Glu Phe Arg Gly Gly Gly Ser Gly  
 165 170 175  
 55 Gly Gly Pro Ile Val Gln Asn Leu Gln Gly Gln Met Val His Gln Pro  
 180 185 190  
 60 Ile Ser Pro Arg Thr Leu Asn Ala Trp Val Lys Val Ile Glu Glu Lys  
 195 200 205  
 65 Ala Phe Ser Pro Glu Val Ile Pro Met Phe Ser Ala Leu Ser Glu Gly  
 210 215 220  
 70 Ala Thr Pro Gln Asp Leu Asn Thr Met Leu Asn Thr Val Gly Gly His  
 225 230 235 240  
 75 Gln Ala Ala Met Gln Met Leu Lys Glu Thr Ile Asn Glu Glu Ala Ala  
 245 250 255

EP 2 638 061 B1

Glu Trp Asp Arg Leu His Pro Val Gln Ala Gly Pro Val Ala Pro Gly  
 260 265 270

5  
 Gln Met Arg Glu Pro Arg Gly Ser Asp Ile Ala Gly Thr Thr Ser Asn  
 275 280 285

10  
 Leu Gln Glu Gln Ile Gly Trp Met Thr Asn Asn Pro Pro Ile Pro Val  
 290 295 300

15  
 Gly Glu Ile Tyr Lys Arg Trp Ile Ile Leu Gly Leu Asn Lys Ile Val  
 305 310 315 320

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 325 330 335

25  
 Glu Pro Phe Arg Asp Tyr Val Asp Arg Phe Tyr Lys Thr Leu Arg Ala  
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 Val Gln Asn Ser Asn Pro Asp Cys Lys Thr Ile Leu Lys Ala Leu Gly  
 370 375 380

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 Pro Ala Ala Thr Leu Glu Glu Met Met Thr Ala Cys Gln Gly Val Gly  
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 Gly Pro Gly His Lys Ala Arg Val Leu Met Gln Tyr Ile Lys Ala Asn  
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50  
 Ser Lys Phe Ile Gly Ile Thr Glu Leu Lys Lys Leu Gly Gly Ser Asn  
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55  
 Asp Ile Phe Asn Asn Phe Thr Val Ser Phe Trp Leu Arg Val Pro Lys  
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60  
 Val Ser Ala Ser His Leu Glu Gln Tyr Leu Glu Ala Thr Asn Thr Lys  
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65  
 Val Asp Lys Thr Val Ala Pro Ser Thr Cys Ser Lys Pro Met Cys Pro  
 465 470 475 480

70  
 Pro Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Ile Phe Pro Pro Lys  
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75  
 Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val  
 500 505 510



EP 2 638 061 B1

|    |             |                     |            |            |            |            |     |     |     |     |     |     |     |     |     |     |  |
|----|-------------|---------------------|------------|------------|------------|------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|--|
|    | Val         | Val                 | Asp        | Val        | Ser        | Gln        | Asp | Asp | Pro | Glu | Val | Gln | Phe | Thr | Trp | Tyr |  |
|    |             |                     | 515        |            |            |            |     | 520 |     |     |     |     | 525 |     |     |     |  |
| 5  | Ile         | Asn                 | Asn        | Glu        | Gln        | Val        | Arg | Thr | Ala | Arg | Pro | Pro | Leu | Arg | Glu | Gln |  |
|    |             | 530                 |            |            |            |            | 535 |     |     |     |     | 540 |     |     |     |     |  |
| 10 | Gln         | Phe                 | Asn        | Ser        | Thr        | Ile        | Arg | Val | Val | Ser | Thr | Leu | Pro | Ile | Ala | His |  |
|    | 545         |                     |            |            |            | 550        |     |     |     |     | 555 |     |     |     |     | 560 |  |
| 15 | Gln         | Asp                 | Trp        | Leu        | Arg        | Gly        | Lys | Glu | Phe | Lys | Cys | Lys | Val | His | Asn | Lys |  |
|    |             |                     |            |            | 565        |            |     |     |     | 570 |     |     |     |     | 575 |     |  |
| 20 | Ala         | Leu                 | Pro        | Ala        | Pro        | Ile        | Glu | Lys | Thr | Ile | Ser | Lys | Ala | Arg | Gly | Gln |  |
|    |             |                     |            | 580        |            |            |     |     | 585 |     |     |     |     | 590 |     |     |  |
| 25 | Pro         | Leu                 | Glu        | Pro        | Lys        | Val        | Tyr | Thr | Met | Gly | Pro | Pro | Arg | Glu | Glu | Leu |  |
|    |             |                     | 595        |            |            |            |     | 600 |     |     |     |     | 605 |     |     |     |  |
| 30 | Ser         | Ser                 | Arg        | Ser        | Val        | Ser        | Leu | Thr | Cys | Met | Ile | Asn | Gly | Phe | Tyr | Pro |  |
|    |             | 610                 |            |            |            |            | 615 |     |     |     |     | 620 |     |     |     |     |  |
| 35 | Ser         | Asp                 | Ile        | Ser        | Val        | Glu        | Trp | Glu | Lys | Asn | Gly | Lys | Ala | Glu | Asp | Asn |  |
|    | 625         |                     |            |            |            | 630        |     |     |     |     | 635 |     |     |     |     | 640 |  |
| 40 | Tyr         | Lys                 | Thr        | Thr        | Pro        | Thr        | Val | Leu | Asp | Ser | Asp | Gly | Ser | Tyr | Phe | Leu |  |
|    |             |                     |            |            | 645        |            |     |     |     | 650 |     |     |     |     | 655 |     |  |
| 45 | Tyr         | Ser                 | Lys        | Leu        | Ser        | Val        | Pro | Thr | Ser | Glu | Trp | Gln | Arg | Gly | Asp | Val |  |
|    |             |                     |            | 660        |            |            |     |     | 665 |     |     |     |     | 670 |     |     |  |
| 50 | Phe         | Thr                 | Cys        | Ser        | Val        | Met        | His | Glu | Ala | Leu | His | Asn | His | Tyr | Thr | Gln |  |
|    |             |                     | 675        |            |            |            |     | 680 |     |     |     |     | 685 |     |     |     |  |
| 55 | Lys         | Ser                 | Ile        | Ser        | His        | Ser        | Pro | Gly | Lys |     |     |     |     |     |     |     |  |
|    |             | 690                 |            |            |            |            | 695 |     |     |     |     |     |     |     |     |     |  |
| 60 | <210>       | 20                  |            |            |            |            |     |     |     |     |     |     |     |     |     |     |  |
|    | <211>       | 2110                |            |            |            |            |     |     |     |     |     |     |     |     |     |     |  |
|    | <212>       | DNA                 |            |            |            |            |     |     |     |     |     |     |     |     |     |     |  |
|    | <213>       | Artificial Sequence |            |            |            |            |     |     |     |     |     |     |     |     |     |     |  |
| 65 | <220>       |                     |            |            |            |            |     |     |     |     |     |     |     |     |     |     |  |
|    | <223>       | mspd1-p24-Fc        |            |            |            |            |     |     |     |     |     |     |     |     |     |     |  |
| 70 | <400>       | 20                  |            |            |            |            |     |     |     |     |     |     |     |     |     |     |  |
| 75 | atgtgggtcc  | ggcagggtacc         | ctggtcattc | acttgggctg | tgctgcagtt | gagctggcaa |     |     |     |     |     |     |     |     |     | 60  |  |
| 80 | tcaggggtggc | ttctagaggt          | ccccaatggg | ccttggaggt | ccctcacctt | ctaccagcc  |     |     |     |     |     |     |     |     |     | 120 |  |

EP 2 638 061 B1

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 5 gccttctgta atggtttgag ccaacccgtc caggatgccc gcttccagat catacagctg 300  
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EP 2 638 061 B1

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Leu Gly Trp Arg Pro Gly Trp Phe Leu Asp Ser Pro Asp Arg Pro Trp  
 20 25 30

25

Asn Pro Pro Thr Phe Ser Pro Ala Leu Leu Val Val Thr Glu Gly Asp  
 35 40 45

30

Asn Ala Thr Phe Thr Cys Ser Phe Ser Asn Thr Ser Glu Ser Phe Val  
 50 55 60

35

Leu Asn Trp Tyr Arg Met Ser Pro Ser Asn Gln Thr Asp Lys Leu Ala  
 65 70 75 80

40

Ala Phe Pro Glu Asp Arg Ser Gln Pro Gly Gln Asp Cys Arg Phe Arg  
 85 90 95

Val Thr Gln Leu Pro Asn Gly Arg Asp Phe His Met Ser Val Val Arg  
 100 105 110

45

Ala Arg Arg Asn Asp Ser Gly Thr Tyr Leu Cys Gly Ala Ile Ser Leu  
 115 120 125

Ala Pro Lys Ala Gln Ile Lys Glu Ser Leu Arg Ala Glu Leu Arg Val  
 130 135 140

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Thr Glu Arg Arg Ala Glu Val Pro Thr Ala His Pro Ser Pro Ser Pro  
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Arg Pro Ala Gly Gln Pro Glu Phe Arg Gly Gly Gly Ser Gly Gly Gly  
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EP 2 638 061 B1

<212> DNA  
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cccaacgggc gtgacttcca catgagcgtg gtcagggccc ggcgcaatga cagcggcacc 360  
20 tacctctgtg gggccatctc cctggccccc aagacgcaga tcaaagagag cctgcgggca 420  
gagctcaggg tgacagagag aagggcagaa gtgccacag cccaccccag cccctcacc 480  
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35 <400> 23

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EP 2 638 061 B1

Met Gln Ile Pro Gln Ala Pro Trp Pro Val Val Trp Ala Val Leu Gln  
 1 5 10 15

5 Leu Gly Trp Arg Pro Gly Trp Phe Leu Asp Ser Pro Asp Arg Pro Trp  
 20 25 30

10 Asn Pro Pro Thr Phe Ser Pro Ala Leu Leu Val Val Thr Glu Gly Asp  
 35 40 45

15 Asn Ala Thr Phe Thr Cys Ser Phe Ser Asn Thr Ser Glu Ser Phe Val  
 50 55 60

Leu Asn Trp Tyr Arg Met Ser Pro Ser Asn Gln Thr Asp Lys Leu Ala  
 65 70 75 80

20 Ala Phe Pro Glu Asp Arg Ser Gln Pro Gly Gln Asp Cys Arg Phe Arg  
 85 90 95

25 Val Thr Gln Leu Pro Asn Gly Arg Asp Phe His Met Ser Val Val Arg  
 100 105 110

30

35

40

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55

EP 2 638 061 B1

Ala Arg Arg Asn Asp Ser Gly Thr Tyr Leu Cys Gly Ala Ile Ser Leu  
115 120 125

5 Ala Pro Lys Ala Gln Ile Lys Glu Ser Leu Arg Ala Glu Leu Arg Val  
130 135 140

10 Thr Glu Arg Arg Ala Glu Val Pro Thr Ala His Pro Ser Pro Ser Pro  
145 150 155 160

Arg Pro Ala Gly Gln Pro Glu Phe Arg Gly Gly Gly Ser Gly Gly Gly  
165 170 175

15 Pro Ile Val Gln Asn Leu Gln Gly Gln Met Val His Gln Pro Ile Ser  
180 185 190

20 Pro Arg Thr Leu Asn Ala Trp Val Lys Val Ile Glu Glu Lys Ala Phe  
195 200 205

25 Ser Pro Glu Val Ile Pro Met Phe Ser Ala Leu Ser Glu Gly Ala Thr  
210 215 220

30 Pro Gln Asp Leu Asn Thr Met Leu Asn Thr Val Gly Gly His Gln Ala  
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Ala Met Gln Met Leu Lys Glu Thr Ile Asn Glu Glu Ala Ala Glu Trp  
245 250 255

35 Asp Arg Leu His Pro Val Gln Ala Gly Pro Val Ala Pro Gly Gln Met  
260 265 270

40 Arg Glu Pro Arg Gly Ser Asp Ile Ala Gly Thr Thr Ser Asn Leu Gln  
275 280 285

Glu Gln Ile Gly Trp Met Thr Asn Asn Pro Pro Ile Pro Val Gly Glu  
290 295 300

45 Ile Tyr Lys Arg Trp Ile Ile Leu Gly Leu Asn Lys Ile Val Arg Met  
305 310 315 320

50 Tyr Ser Pro Thr Ser Ile Leu Asp Ile Arg Gln Gly Pro Lys Glu Pro  
325 330 335

Phe Arg Asp Tyr Val Asp Arg Phe Tyr Lys Thr Leu Arg Ala Glu Gln  
340 345 350

55 Ala Ser Gln Glu Val Lys Asn Trp Met Thr Glu Thr Leu Leu Val Gln  
355 360 365

EP 2 638 061 B1

Asn Ser Asn Pro Asp Cys Lys Thr Ile Leu Lys Ala Leu Gly Pro Ala  
 370 375 380

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 Ala Thr Leu Glu Glu Met Met Thr Ala Cys Gln Gly Val Gly Gly Pro  
 385 390 395 400

10  
 Gly His Lys Ala Arg Val Leu Met Gln Tyr Ile Lys Ala Asn Ser Lys  
 405 410 415

15  
 Phe Ile Gly Ile Thr Glu Leu Lys Lys Leu Gly Gly Ser Asn Asp Ile  
 420 425 430

20  
 Phe Asn Asn Phe Thr Val Ser Phe Trp Leu Arg Val Pro Lys Val Ser  
 435 440 445

25  
 Ala Ser His Leu Glu Gln Tyr Leu Glu Ala Thr Asn Thr Lys Val Asp  
 450 455 460

30  
 Lys Thr Val Ala Pro Ser Thr Cys Ser Lys Pro Met Cys Pro Pro Pro  
 465 470 475 480

35  
 Glu Leu Leu Gly Gly Pro Ser Val Phe Ile Phe Pro Pro Lys Pro Lys  
 485 490 495

40  
 Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val  
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45  
 Asp Val Ser Gln Asp Asp Pro Glu Val Gln Phe Thr Trp Tyr Ile Asn  
 515 520 525

50  
 Asn Glu Gln Val Arg Thr Ala Arg Pro Pro Leu Arg Glu Gln Gln Phe  
 530 535 540

55  
 Asn Ser Thr Ile Arg Val Val Ser Thr Leu Pro Ile Ala His Gln Asp  
 545 550 555 560

60  
 Trp Leu Arg Gly Lys Glu Phe Lys Cys Lys Val His Asn Lys Ala Leu  
 565 570 575

65  
 Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Arg Gly Gln Pro Leu  
 580 585 590

70  
 Glu Pro Lys Val Tyr Thr Met Gly Pro Pro Arg Glu Glu Leu Ser Ser  
 595 600 605

75  
 Arg Ser Val Ser Leu Thr Cys Met Ile Asn Gly Phe Tyr Pro Ser Asp  
 610 615 620

EP 2 638 061 B1

Ile Ser Val Glu Trp Glu Lys Asn Gly Lys Ala Glu Asp Asn Tyr Lys  
625 630 635 640

5 Thr Thr Pro Thr Val Leu Asp Ser Asp Gly Ser Tyr Phe Leu Tyr Ser  
645 650 655

10 Lys Leu Ser Val Pro Thr Ser Glu Trp Gln Arg Gly Asp Val Phe Thr  
660 665 670

Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser  
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15 Ile Ser His Ser Pro Gly Lys  
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atcccagtag gagaaatcta taaaagatgg ataatcctgg ggttaaataa aatagtaaga 960



EP 2 638 061 B1

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55 Gly Asp Asn Ala Thr Phe Thr Cys Ser Phe Ser Asn Thr Ser Glu Ser  
 35 40 45

EP 2 638 061 B1

Phe Val Leu Asn Trp Tyr Arg Met Ser Pro Ser Asn Gln Thr Asp Lys  
50 55 60

5 Leu Ala Ala Phe Pro Glu Asp Arg Ser Gln Pro Gly Gln Asp Cys Arg  
65 70 75 80

10 Phe Arg Val Thr Gln Leu Pro Asn Gly Arg Asp Phe His Met Ser Val  
85 90 95

15 Val Arg Ala Arg Arg Asn Asp Ser Gly Thr Tyr Leu Cys Gly Ala Ile  
100 105 110

20 Ser Leu Ala Pro Lys Ala Gln Ile Lys Glu Ser Leu Arg Ala Glu Leu  
115 120 125

25 Arg Val Thr Glu Arg Arg Ala Glu Val Pro Thr Ala His Pro Ser Pro  
130 135 140

30 Ser Pro Arg Pro Ala Gly Gln Pro Glu Phe Arg Gly Gly Gly Ser Gly  
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30 <210> 26  
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<212> PRT  
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40 <400> 26

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5 Cys Gly Cys Cys Cys Thr Gly Gly Cys Cys Ala Gly Thr Cys Gly Thr  
20 25 30

10 Cys Thr Gly Gly Gly Cys Gly Gly Thr Gly Cys Thr Ala Cys Ala Ala  
35 40 45

Cys Thr Gly Gly Gly Cys Thr Gly Gly Cys Gly Gly Cys Cys Ala Gly  
50 55 60

15 Gly Ala Thr Gly Gly Thr Thr Cys Thr Thr Ala Gly Cys Cys Cys Thr  
65 70 75 80

20 Gly Cys Thr Cys Gly Thr Gly Gly Thr Gly Ala Cys Cys Gly Ala Ala

25

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EP 2 638 061 B1

|    |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
|    |     |     |     |     | 85  |     |     |     |     | 90  |     |     |     |     | 95  |     |
| 5  | Gly | Gly | Gly | Gly | Ala | Cys | Ala | Ala | Cys | Gly | Cys | Cys | Ala | Cys | Cys | Thr |
|    |     |     |     | 100 |     |     |     |     | 105 |     |     |     |     | 110 |     |     |
| 10 | Thr | Cys | Ala | Cys | Cys | Thr | Gly | Cys | Ala | Gly | Cys | Thr | Thr | Cys | Thr | Cys |
|    |     |     | 115 |     |     |     |     | 120 |     |     |     |     | 125 |     |     |     |
| 15 | Cys | Ala | Ala | Cys | Ala | Cys | Ala | Thr | Cys | Gly | Gly | Ala | Gly | Ala | Gly | Cys |
|    |     | 130 |     |     |     |     | 135 |     |     |     |     | 140 |     |     |     |     |
| 20 | Thr | Thr | Cys | Gly | Thr | Gly | Cys | Thr | Ala | Ala | Ala | Cys | Thr | Gly | Gly | Thr |
|    | 145 |     |     |     |     | 150 |     |     |     |     | 155 |     |     |     |     | 160 |
| 25 | Ala | Cys | Cys | Gly | Cys | Ala | Thr | Gly | Ala | Gly | Cys | Cys | Cys | Cys | Ala | Gly |
|    |     |     |     | 165 |     |     |     |     |     | 170 |     |     |     |     | 175 |     |
| 30 | Cys | Ala | Ala | Cys | Cys | Ala | Gly | Ala | Cys | Gly | Gly | Ala | Cys | Ala | Ala | Gly |
|    |     |     | 180 |     |     |     |     | 185 |     |     |     |     | 190 |     |     |     |
| 35 | Cys | Thr | Gly | Gly | Cys | Cys | Gly | Cys | Cys | Thr | Thr | Cys | Cys | Cys | Cys | Gly |
|    |     |     | 195 |     |     |     |     | 200 |     |     |     |     | 205 |     |     |     |
| 40 | Ala | Gly | Gly | Ala | Cys | Cys | Gly | Cys | Ala | Gly | Cys | Cys | Ala | Gly | Cys | Cys |
|    |     | 210 |     |     |     |     | 215 |     |     |     |     | 220 |     |     |     |     |
| 45 | Cys | Gly | Gly | Cys | Cys | Ala | Gly | Gly | Ala | Cys | Thr | Gly | Cys | Cys | Gly | Cys |
|    | 225 |     |     |     |     | 230 |     |     |     | 235 |     |     |     |     |     | 240 |
| 50 | Thr | Thr | Cys | Cys | Gly | Thr | Gly | Thr | Cys | Ala | Cys | Ala | Cys | Ala | Ala | Cys |
|    |     |     |     | 245 |     |     |     |     | 250 |     |     |     |     |     | 255 |     |
| 55 | Thr | Gly | Cys | Cys | Cys | Ala | Ala | Cys | Gly | Gly | Gly | Cys | Gly | Thr | Gly | Ala |
|    |     |     | 260 |     |     |     |     |     | 265 |     |     |     |     | 270 |     |     |
| 60 | Cys | Thr | Thr | Cys | Cys | Ala | Cys | Ala | Thr | Gly | Ala | Gly | Cys | Gly | Thr | Gly |
|    |     |     | 275 |     |     |     | 280 |     |     |     |     |     | 285 |     |     |     |
| 65 | Gly | Thr | Cys | Ala | Gly | Gly | Gly | Cys | Cys | Cys | Gly | Gly | Cys | Gly | Cys | Ala |
|    | 290 |     |     |     |     | 295 |     |     |     |     |     | 300 |     |     |     |     |
| 70 | Ala | Thr | Gly | Ala | Cys | Ala | Gly | Cys | Gly | Gly | Cys | Ala | Cys | Cys | Thr | Ala |
|    | 305 |     |     |     |     | 310 |     |     |     |     | 315 |     |     |     |     | 320 |
| 75 | Cys | Cys | Thr | Cys | Thr | Gly | Thr | Gly | Gly | Gly | Gly | Cys | Cys | Ala | Thr | Cys |
|    |     |     |     | 325 |     |     |     |     |     | 330 |     |     |     |     | 335 |     |

EP 2 638 061 B1

Thr Cys Cys Cys Thr Gly Gly Cys Cys Cys Cys Cys Ala Ala Gly Ala  
 340 345 350

5 Cys Gly Cys Ala Gly Ala Thr Cys Ala Ala Ala Gly Ala Gly Ala Gly  
 355 360 365

10 Cys Cys Thr Gly Cys Gly Gly Gly Cys Ala Gly Ala Gly Cys Thr Cys  
 370 375 380

15 Ala Gly Gly Gly Thr Gly Ala Cys Ala Gly Ala Gly Ala Gly Ala Ala  
 385 390 395 400

Gly Gly Gly Cys Ala Gly Ala Ala Gly Thr Gly Cys Cys Cys Ala Cys  
 405 410 415

20 Ala Gly Cys Cys Cys Ala Cys Cys Cys Cys Ala Gly Cys Cys Cys Cys  
 420 425 430

25 Thr Cys Ala Cys Cys Cys Ala Gly Gly Cys Cys Ala Gly Cys Cys Gly  
 435 440 445

Gly Cys Cys Ala Gly Cys Cys Gly Gly Ala Ala Thr Thr Cys Cys Gly  
 450 455 460

30 Gly Gly Gly Thr Gly Gly Thr Gly Gly Thr Gly Gly Thr Thr Cys Ala  
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35 Gly Gly Ala Gly Gly Ala Gly Gly Ala  
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<210> 27  
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 40 <212> PRT  
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45 <400> 27

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EP 2 638 061 B1

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5 Leu Gly Trp Arg Pro Gly Trp Phe Leu Ala Leu Leu Val Val Thr Glu  
20 25 30

10 Gly Asp Asn Ala Thr Phe Thr Cys Ser Phe Ser Asn Thr Ser Glu Ser  
35 40 45

Phe Val Leu Asn Trp Tyr Arg Met Ser Pro Ser Asn Gln Thr Asp Lys

15

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EP 2 638 061 B1

|    |           |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |     |  |  |
|----|-----------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|-----|--|--|
|    | 50        |            | 55         |            | 60         |            |            |            |            |            |            |            |            |            |            |            |     |  |  |
| 5  | Leu<br>65 | Ala        | Ala        | Phe        | Pro        | Glu<br>70  | Asp        | Arg        | Ser        | Gln        | Pro<br>75  | Gly        | Gln        | Asp        | Cys        | Arg<br>80  |     |  |  |
|    |           | Phe        | Arg        | Val        | Thr        | Gln<br>85  | Leu        | Pro        | Asn        | Gly        | Arg<br>90  | Asp        | Phe        | His        | Met        | Ser<br>95  | Val |  |  |
| 10 |           |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |     |  |  |
|    | Val       | Arg        | Ala        | Arg        | Arg        | Asn        | Asp        | Ser        | Gly<br>105 | Thr        | Tyr        | Leu        | Cys        | Gly<br>110 | Ala        | Ile        |     |  |  |
| 15 |           |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |     |  |  |
|    | Ser       | Leu        | Ala<br>115 | Pro        | Lys        | Ala        | Gln        | Ile<br>120 | Lys        | Glu        | Ser        | Leu        | Arg<br>125 | Ala        | Glu        | Leu        |     |  |  |
| 20 |           |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |     |  |  |
|    | Arg       | Val<br>130 | Thr        | Glu        | Arg        | Arg        | Ala<br>135 | Glu        | Val        | Pro        | Thr        | Ala<br>140 | His        | Pro        | Ser        | Pro        |     |  |  |
| 25 |           |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |     |  |  |
|    | Ser       | Pro        | Arg        | Pro        | Ala        | Gly<br>150 | Gln        | Pro        | Glu        | Phe        | Arg<br>155 | Gly        | Gly        | Gly        | Ser        | Gly<br>160 |     |  |  |
| 30 |           |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |     |  |  |
|    | Gly       | Gly        | Pro        | Ile        | Val<br>165 | Gln        | Asn        | Leu        | Gln        | Gly<br>170 | Gln        | Met        | Val        | His        | Gln<br>175 | Pro        |     |  |  |
| 35 |           |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |     |  |  |
|    | Ile       | Ser        | Pro        | Arg        | Thr        | Leu        | Asn        | Ala        | Trp<br>185 | Val        | Lys        | Val        | Ile        | Glu<br>190 | Glu        | Lys        |     |  |  |
| 40 |           |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |     |  |  |
|    | Ala       | Phe        | Ser<br>195 | Pro        | Glu        | Val        | Ile        | Pro<br>200 | Met        | Phe        | Ser        | Ala        | Leu<br>205 | Ser        | Glu        | Gly        |     |  |  |
| 45 |           |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |     |  |  |
|    | Ala       | Thr<br>210 | Pro        | Gln        | Asp        | Leu        | Asn<br>215 | Thr        | Met        | Leu        | Asn        | Thr<br>220 | Val        | Gly        | Gly        | His        |     |  |  |
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|    | Gln       | Ala        | Ala        | Met        | Gln        | Met<br>230 | Leu        | Lys        | Glu        | Thr        | Ile<br>235 | Asn        | Glu        | Glu        | Ala        | Ala<br>240 |     |  |  |
| 55 |           |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |     |  |  |
|    | Glu       | Trp        | Asp        | Arg        | Leu        | His<br>245 | Pro        | Val        | Gln        | Ala<br>250 | Gly        | Pro        | Val        | Ala        | Pro<br>255 | Gly        |     |  |  |
| 60 |           |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |     |  |  |
|    | Gln       | Met        | Arg        | Glu<br>260 | Pro        | Arg        | Gly        | Ser        | Asp<br>265 | Ile        | Ala        | Gly        | Thr        | Thr<br>270 | Ser        | Asn        |     |  |  |
| 65 |           |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |     |  |  |
|    | Leu       | Gln        | Glu        | Gln        | Ile        | Gly        | Trp<br>280 | Met        | Thr        | Asn        | Asn        | Pro        | Pro<br>285 | Ile        | Pro        | Val        |     |  |  |
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EP 2 638 061 B1

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EP 2 638 061 B1

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EP 2 638 061 B1

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EP 2 638 061 B1

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EP 2 638 061 B1

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EP 2 638 061 B1

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**Claims**

- 55 1. A soluble PD-1 protein comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 11, SEQ ID NO: 15, and SEQ ID NO: 25.
2. A PD-1 nucleic acid molecule encoding a soluble PD-1 protein of claim 1.

## EP 2 638 061 B1

3. A soluble PD-1 fusion protein, comprising a soluble PD-1 protein fragment and an antigenic protein fragment, wherein the soluble PD-1 protein fragment comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 11, SEQ ID NO: 15, and SEQ ID NO: 25.
- 5 4. The soluble PD-1 fusion protein of claim 3, wherein the antigenic protein fragment is from an immunogenic protein fragment of a viral, bacterial, or fungal pathogen, or cancer or tumor cells.
5. The PD-1 fusion protein of claim 4, the pathogen is selected from human immunodeficiency virus (HIV), HSV, respiratory syncytial virus, rhinovirus, hepatitis A virus, hepatitis B virus, hepatitis C virus, hepatitis D virus, hepatitis E virus, hepatitis F virus, hepatitis G virus, oncoviruses, human T-lymphotropic virus Type I (HTLV-1), influenza, bovine leukemia virus (BLV), Epstein-Barr virus, pertussis, polio, measles, mumps, rubella, smallpox, zoster, anthrax, tetanus, rotavirus, rabies, chickenpox, meningococcus, diphtheria, anapapillomavirus, anthrax, plague, encephalitis, pneumococcus, pneumonia, typhus, typhoid fever, streptococcus, staphylococcus, neisseria, lyme disease, cholera, *E. coli*, shigella, leishmania, leprosy, cytomegalovirus (CMV), respiratory syncytial virus, parainfluenza, adenovirus, varicella, flavivirus, dengue toxoplasmosis, coccidiomycosis, schistosomiasis, *Mycobacteria tuberculosis*, or malaria.
- 10 6. The PD-1 fusion protein of claim 4, the antigenic protein fragment is from HIV p24.
7. The PD-1 fusion protein of claim 3, further comprising an Fc domain and which optionally further comprises a linker sequence, wherein the linker sequence links the soluble PD-1 domain and the antigen.
- 20 8. The PD-1 fusion protein of claim 3, comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 13, 17 and 27.
- 25 9. A sPD-1 fusion nucleic acid molecule encoding a PD-1 fusion protein of any one of claims 3-8.
10. The sPD-1 fusion nucleic acid molecule of claim 9, wherein the nucleic acid molecule comprises a sequence selected from the group consisting of SEQ ID NOs: 14, 18 and 28.
- 30 11. A vaccine composition comprising the sPD-1 fusion nucleic acid molecule of claim 9 or 10.
12. An effective amount of a fusion nucleic acid of claim 9 or a fusion protein of claim 3 for use in a method for preventing or treating pathogenic infection and/or tumor or cancer.
- 35 13. The fusion nucleic acid or the fusion protein for use in a method of claim 12, wherein the pathogenic infection is caused by a pathogen selected from human immunodeficiency virus (HIV), HSV, respiratory syncytial virus, rhinovirus, hepatitis A virus, hepatitis B virus, hepatitis C virus, hepatitis D virus, hepatitis E virus, hepatitis F virus, hepatitis G virus, oncoviruses, human T-lymphotropic virus Type I (HTLV-1), influenza, bovine leukemia virus (BLV), Epstein-Barr virus, pertussis, polio, measles, mumps, rubella, smallpox, zoster, anthrax, tetanus, rotavirus, rabies, chickenpox, meningococcus, diphtheria, anapapillomavirus, anthrax, plague, encephalitis, pneumococcus, pneumonia, typhus, typhoid fever, streptococcus, staphylococcus, neisseria, lyme disease, cholera, *E. coli*, shigella, leishmania, leprosy, cytomegalovirus (CMV), respiratory syncytial virus, parainfluenza, adenovirus, varicella, flavivirus, dengue toxoplasmosis, coccidiomycosis, schistosomiasis, *Mycobacteria tuberculosis*, or malaria.
- 40 14. The fusion nucleic acid or the fusion protein for use in a method of claim 13, wherein the fusion nucleic acid comprises an antigenic nucleic acid fragment encoding HIV p24 and the pathogenic infection is HIV infection, preferably wherein the fusion nucleic acid comprises a sequence selected from the group consisting of SEQ ID NOs: 14, 18 and 28.
- 45 15. The fusion nucleic acid or the fusion protein for use in a method of claim 12, wherein the fusion nucleic acid is delivered by injection, preferably wherein the fusion nucleic acid is delivered via electroporation.
- 50

### Patentansprüche

- 55 1. Lösliches PD-1-Protein, enthaltend eine Aminosäuresequenz, die ausgewählt ist aus der Gruppe bestehend aus SEQ. ID NR. 11, SEQ. ID NR. 15, und SEQ. ID NR. 25.
2. PD-1-Nukleinsäuremolekül, das ein lösliches PD-1-Protein nach Anspruch 1 kodiert.

## EP 2 638 061 B1

3. Lösliches PD-1-Fusionsprotein, enthaltend ein lösliches PD-1-Proteinfragment und ein antigenes Proteinfragment, wobei das lösliche PD-1-Proteinfragment eine Aminosäuresequenz enthält, die ausgewählt ist aus der Gruppe bestehend aus SEQ. ID NR. 11, SEQ. ID NR. 15, und SEQ. ID NR. 25.
- 5 4. Lösliches PD-1-Fusionsprotein nach Anspruch 3, wobei das antigenes Proteinfragment von einem immunogenen Proteinfragment eines viralen, bakteriellen oder fungalen Pathogens, oder von Krebs oder Tumorzellen stammt.
5. PD-1-Fusionsprotein nach Anspruch 4, wobei das Pathogen ausgewählt ist aus Humanem Immundefizienz-Virus (HIV), HSV, respiratorischem Syncytial-Virus, Rhinovirus, Hepatitis-A-Virus, Hepatitis-B-Virus, Hepatitis-C-Virus, 10 Hepatitis-D-Virus, Hepatitis-E-Virus, Hepatitis-F-Virus, Hepatitis-G-Virus, Onkoviren, humanem T-Lymphotrop-Virus Typ I (HTLV-1), Influenza, Bovinem Leukämievirus (BLV), Epstein-Barr-Virus, Keuchhusten, Polio, Masern, Mumps, Röteln, Pocken, Zoster, Anthrax, Tetanus, Rotavirus, Tollwut, Windpocken, Meningokokken, Diphtherie, Anpapillomavirus, Anthrax, Pest, Enzephalitis, Pneumokokken, Pneumonie, Typhus, Thyphusfieber, Streptokokken, Staphylokokken, Neisseria, Borreliose, Cholera, E. coli, Shigella, Leishmania, Lepra, Cytomegalie-Virus (CMV), 15 respiratorischem Syncytial-Virus, Parainfluenza, Adenovirus, Windpocken, Flavivirus, Dengue-Toxoplasmose, Kokzidiomykose, Schistosomiasis, Mycobacterium Tuberculosis, oder Malaria.
6. PD-1-Fusionsprotein nach Anspruch 4, wobei das antigenes Proteinfragment von HIV p24 stammt.
- 20 7. PD-1-Fusionsprotein nach Anspruch 3, ferner enthaltend eine Fc-Domäne und optional ferner enthaltend eine Linkersequenz, wobei die Linkersequenz die lösliche PD-1-Domäne und das Antigen verbindet.
8. PD-1-Fusionsprotein nach Anspruch 3, enthaltend eine Aminosäuresequenz ausgewählt aus der Gruppe bestehend aus den SEQ. ID NR. 13, 17 und 27.
- 25 9. sPD-1-Fusionsnukleinsäuremolekül, das ein PD-1-Fusionsprotein nach einem der Ansprüche 3 bis 8 kodiert.
10. sPD-1-Fusionsnukleinsäuremolekül nach Anspruch 9, wobei das Nukleinsäuremolekül eine Sequenz enthält, die ausgewählt ist aus der Gruppe bestehend aus den SEQ. ID NR. 14, 18 und 28.
- 30 11. Impfstoffzusammensetzung, die das sPD-1-Fusionsnukleinsäuremolekül nach Anspruch 9 oder 10 enthält.
12. Wirksame Menge einer Fusionsnukleinsäure nach Anspruch 9 oder eines Fusionsproteins nach Anspruch 3 zur Verwendung in einem Verfahren zur Prävention oder Behandlung einer pathogenen Infektion und/oder eines Tumors oder von Krebs.
- 35 13. Fusionsnukleinsäure oder das Fusionsprotein zur Verwendung in einem Verfahren nach Anspruch 12, wobei die pathogene Infektion durch ein Pathogen verursacht wird, ausgewählt aus Humanem Immundefizienz-Virus (HIV), HSV, respiratorischem Syncytial-Virus, Rhinovirus, Hepatitis-A-Virus, Hepatitis-B-Virus, Hepatitis-C-Virus, Hepatitis-D-Virus, Hepatitis-E-Virus, Hepatitis-F-Virus, Hepatitis-G-Virus, Onkoviren, humanem T-Lymphotrop-Virus Typ I (HTLV-1), Influenza, Bovinem Leukämievirus (BLV), Epstein-Barr-Virus, Keuchhusten, Polio, Masern, Mumps, Röteln, Pocken, Zoster, Anthrax, Tetanus, Rotavirus, Tollwut, Windpocken, Meningokokken, Diphtherie, Anpapillomavirus, Anthrax, Pest, Enzephalitis, Pneumokokken, Pneumonie, Typhus, Thyphusfieber, Streptokokken, Staphylokokken, Neisseria, Borreliose, Cholera, E. coli, Shigella, Leishmania, Lepra, Cytomegalie-Virus (CMV), 40 respiratorischem Syncytial-Virus, Parainfluenza, Adenovirus, Windpocken, Flavivirus, Dengue-Toxoplasmose, Kokzidiomykose, Schistosomiasis, Mycobacterium Tuberculosis, oder Malaria.
- 45 14. Fusionsnukleinsäure oder das Fusionsprotein zur Verwendung in einem Verfahren nach Anspruch 13, wobei die Fusionsnukleinsäure ein antigenes Nukleinsäurefragment enthält, das HIV-p24 kodiert, und die pathogene Infektion eine HIV-Infektion ist, bevorzugt wobei die Fusionsnukleinsäure eine Sequenz enthält, die ausgewählt ist aus der Gruppe bestehend aus den SEQ. ID NR. 14, 18 und 28.
- 50 15. Fusionsnukleinsäure oder das Fusionsprotein zur Verwendung in einem Verfahren nach Anspruch 12, wobei die Fusionsnukleinsäure durch Injektion verabreicht wird, wobei die Fusionsnukleinsäure bevorzugt über Elektroporation verabreicht wird.
- 55



**Revendications**

1. Protéine PD-1 soluble comprenant une séquence d'acides aminés choisie dans le groupe consistant en SEQ ID NO:11, SEQ ID NO:15 et SEQ ID NO:25.
2. Molécule d'acide nucléique de PD-1, codant pour une protéine PD-1 soluble de la revendication 1.
3. Protéine de fusion de PD-1 soluble, comprenant un fragment de protéine PD-1 soluble et un fragment de protéine antigénique, le fragment de protéine PD-1 soluble comprenant une séquence d'acides aminés choisie dans le groupe consistant en SEQ ID NO:11, SEQ ID NO:15 et SEQ ID NO:25.
4. Protéine de fusion de PD-1 soluble selon la revendication 3, dans laquelle le fragment de protéine antigénique provient d'un fragment de protéine immunogène d'un pathogène viral, bactérien ou fongique, ou de cellules cancéreuses ou tumorales.
5. Protéine de fusion de PD-1 selon la revendication 4, le pathogène étant choisi parmi le virus de l'immunodéficience humaine (VIH), le HSV, le virus syncytial respiratoire, le rhinovirus, le virus de l'hépatite A, le virus de l'hépatite B, le virus de l'hépatite C, le virus de l'hépatite D, le virus de l'hépatite E, le virus de l'hépatite F, le virus de l'hépatite G, les oncovirus, le virus T-lymphotrope humain de type I (HTLV-1), la grippe, le virus de la leucémie bovine (BLV), le virus Epstein-Barr, la coqueluche, la polio, la rougeole, les oreillons, la rubéole, la varicelle, le zona, le charbon, le tétanos, le rotavirus, la rage, la varicelle, meningococcus, la diphtérie, l'anapapillomavirus, le charbon, la peste, l'encéphalite, pneumococcus, la pneumonie, la typhus, la fièvre typhoïde, streptococcus, staphylococcus, neisseria, la maladie de Lyme, le choléra, E. coli, shigella, leishmania, la lèpre, le cytomégalovirus (CMV), le virus syncytial respiratoire, le parainfluenza, l'adénovirus, la varicelle, les flavivirus, la toxoplasmose de la dengue, la coccidiomycose, la schistosomiase, la tuberculose à Mycobacteria, ou le paludisme.
6. Protéine de fusion de PD-1 selon la revendication 4, le fragment de protéine antigénique provenant du p24 du VIH.
7. Protéine de fusion de PD-1 selon la revendication 3, comprenant en outre un domaine Fc, et comprenant en outre en option une séquence de liaison, la séquence de liaison liant le domaine PD-1 soluble et l'antigène.
8. Protéine de fusion de PD-1 selon la revendication 3, comprenant une séquence d'acides aminés choisie dans le groupe consistant en SEQ ID NO:13, 17 et 27.
9. Molécule d'acide nucléique de fusion de sPD-1, codant pour une protéine de fusion de PD-1 selon l'une quelconque des revendications 3-8.
10. Molécule d'acide nucléique de fusion de sPD-1 selon la revendication 9, la molécule d'acide nucléique comprenant une séquence choisie dans le groupe consistant en SEQ ID NO:14, 18 et 28.
11. Composition de vaccin comprenant la molécule d'acide nucléique de fusion de sPD-1 de la revendication 9 ou 10.
12. Quantité efficace d'un acide nucléique de fusion de la revendication 9 ou d'une protéine de fusion de la revendication 3 pour utilisation dans un procédé pour la prévention ou le traitement d'une infection pathogénique et/ou d'une tumeur ou d'un cancer.
13. Acide nucléique de fusion ou protéine de fusion pour utilisation dans un procédé selon la revendication 12, dans lequel l'infection pathogénique est provoquée par un pathogène choisi parmi le virus de l'immunodéficience humaine (VIH), le HSV, le virus syncytial respiratoire, le rhinovirus, le virus de l'hépatite A, le virus de l'hépatite B, le virus de l'hépatite C, le virus de l'hépatite D, le virus de l'hépatite E, le virus de l'hépatite F, le virus de l'hépatite G, les oncovirus, le virus T-lymphotrope humain de type I (HTLV-1), la grippe, le virus de la leucémie bovine (BLV), le virus Epstein-Barr, la coqueluche, la polio, la rougeole, les oreillons, la rubéole, la varicelle, le zona, le charbon, le tétanos, le rotavirus, la rage, la varicelle, meningococcus, la diphtérie, l'anapapillomavirus, le charbon, la peste, l'encéphalite, pneumococcus, la pneumonie, la typhus, la fièvre typhoïde, streptococcus, staphylococcus, neisseria, la maladie de Lyme, le choléra, E. coli, shigella, leishmania, la lèpre, le cytomégalovirus (CMV), le virus syncytial respiratoire, le parainfluenza, l'adénovirus, la varicelle, les flavivirus, la toxoplasmose de la dengue, la coccidiomycose, la schistosomiase, la tuberculose à Mycobacteria, ou le paludisme.

## EP 2 638 061 B1

14. Acide nucléique de fusion ou protéine de fusion pour utilisation dans un procédé selon la revendication 13, l'acide nucléique de fusion comprenant un fragment d'acide nucléique antigénique codant pour le p24 du VIH, et l'infection pathogénique étant une infection à VIH, de préférence l'acide nucléique de fusion comprenant une séquence choisie dans le groupe consistant en SEQ ID NO:14, 18 et 28.

5

15. Acide nucléique de fusion ou protéine de fusion pour utilisation dans un procédé selon la revendication 12, l'acide nucléique de fusion étant administré par injection, l'acide nucléique étant de préférence administré par électroporation.

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..... 10 ..... 20 ..... 30 ..... 40 ..... 50 ..... 60 ..... 70 ..... 80 ..... 90 ..... 100 .....
mspdl-p24-fc MWVRQVWSEFTWAVTQLSWQSCMLLEVENGPWRSLTFYPAWLTVSEGANATFTCSLSNWSDDLMLNWNRLSPNQTEKQAFPCNLSQPVDARFQIICL
mspdl-IgVA-p24-fc .....
p24-fc .....

..... 110 ..... 120 ..... 130 ..... 140 ..... 150 ..... 160 ..... 170 ..... 180 ..... 190 ..... 200 .....
mspdl-p24-fc PNRHDFHNIIDTRRNDSCILCCGAISSLHPKAKIESPCAEVLVYTERILETSTRYSPSPPEGRFQPEFRGGCGGPIVQNLQCCQMVHQPIISPRTLNA
mspdl-IgVA-p24-fc .....
p24-fc .....

..... 210 ..... 220 ..... 230 ..... 240 ..... 250 ..... 260 ..... 270 ..... 280 ..... 290 ..... 300 .....
mspdl-p24-fc WVKVIEKAFSPEVIEPMFSALSEGATPQDNLNTVLTNGVGHQAAMQMLKETTINBEAAEWDRLHPVQAGVYAPCGMREPRGSDLAGTTSNLQEQIGMMATNP
mspdl-IgVA-p24-fc .....
p24-fc .....

..... 310 ..... 320 ..... 330 ..... 340 ..... 350 ..... 360 ..... 370 ..... 380 ..... 390 ..... 400 .....
mspdl-p24-fc PIPVGEIYKRWIILGLENKIVRMYSPTSLIDIQCPKEPRFDVDRFYKTLRAEQASQEVKNWMTETLLVONSNDCKTILKALGPAATLEEMMTACQGVG
mspdl-IgVA-p24-fc .....
p24-fc .....

..... 410 ..... 420 ..... 430 ..... 440 ..... 450 ..... 460 ..... 470 ..... 480 ..... 490 ..... 500 .....
mspdl-p24-fc GPGHKARVLMQYIKRNSKEFIGITELKKGSSNDIENFTVGFALVVPKVSASHLEQYLEAINTKVDKTVAPSTGSKPMCPPELLGSPSVTFEPKPKDT
mspdl-IgVA-p24-fc .....
p24-fc .....

..... 510 ..... 520 ..... 530 ..... 540 ..... 550 ..... 560 ..... 570 ..... 580 ..... 590 ..... 600 .....
mspdl-p24-fc LMSRTPVTCVVVDVSQDDPEVQFTWYINNEQVRTARPPREQQFNSTRVSTLPIAQDMLRCKEFCCKVHNKALPAPLEKTKSKARQQPLEPKVYT
mspdl-IgVA-p24-fc .....
p24-fc .....

..... 610 ..... 620 ..... 630 ..... 640 ..... 650 ..... 660 ..... 670 ..... 680 ..... 690 .....
mspdl-p24-fc MGPPRELSRSVSLTCMINGFYPGDISVWEKNGKAEDNYKTTETVLDSDGSFFLSKLSYPTSEWQRCVFTCSVMHEALHNHTQKSIHSECK
mspdl-IgVA-p24-fc .....
p24-fc .....

```

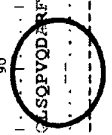


FIG. 1A

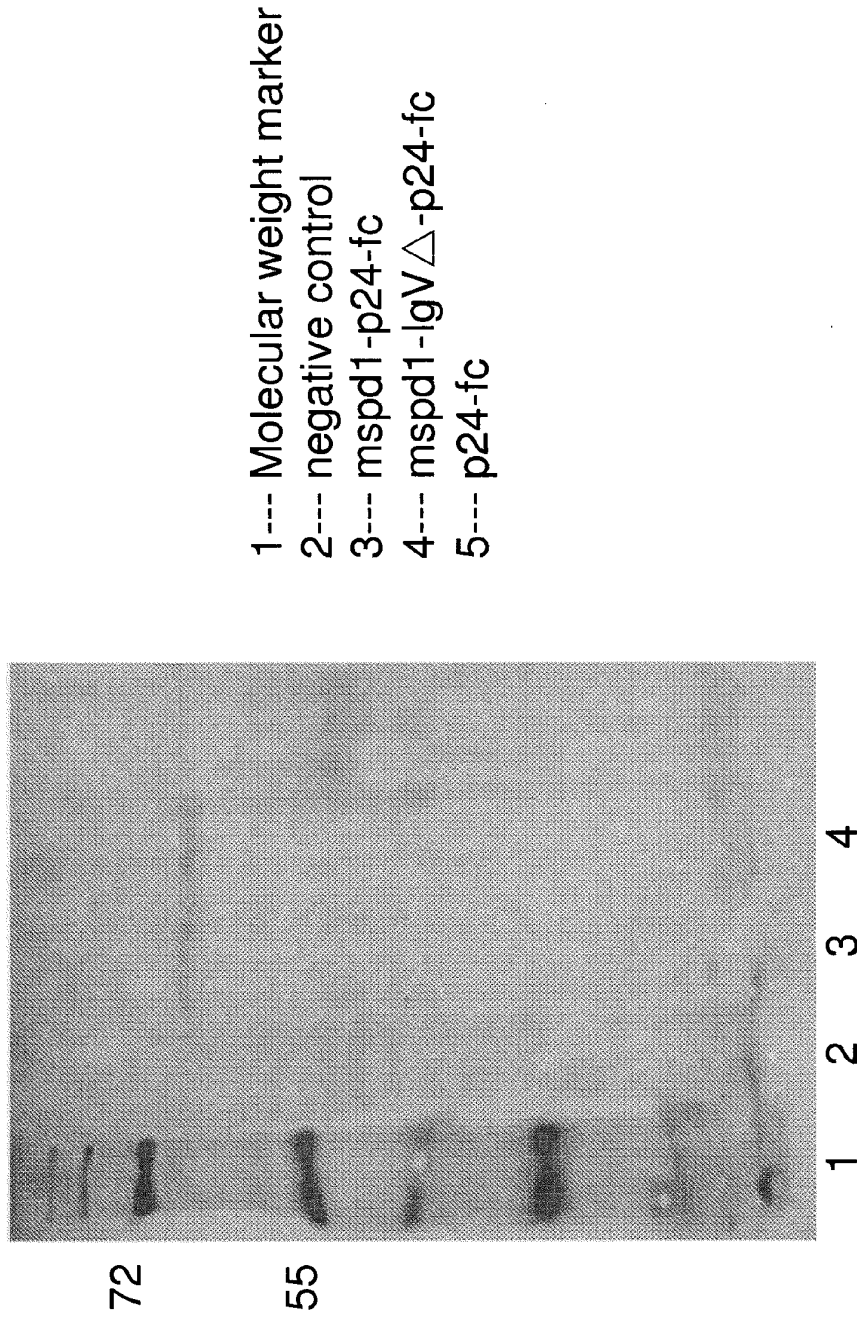


FIG. 1B

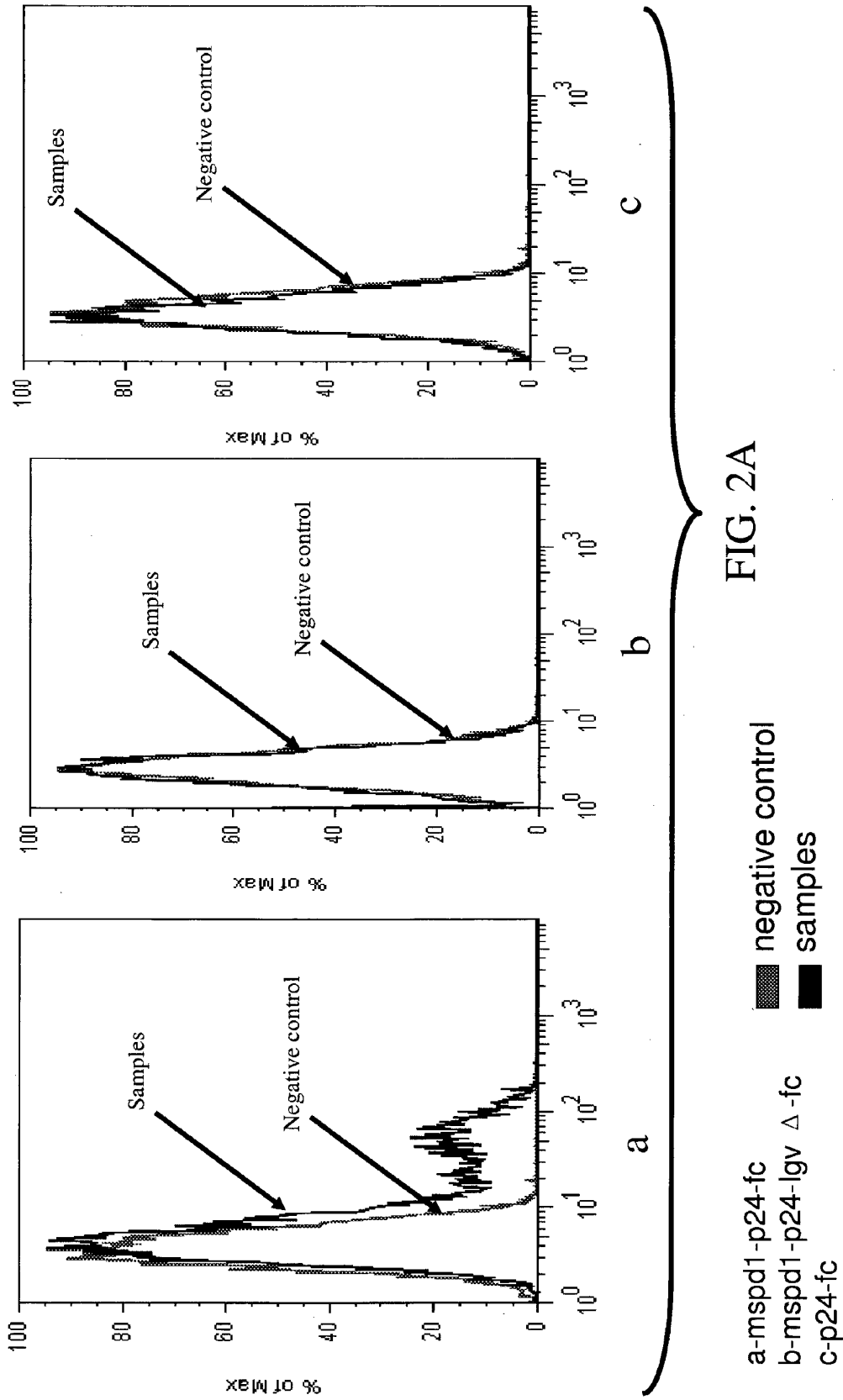


FIG. 2A

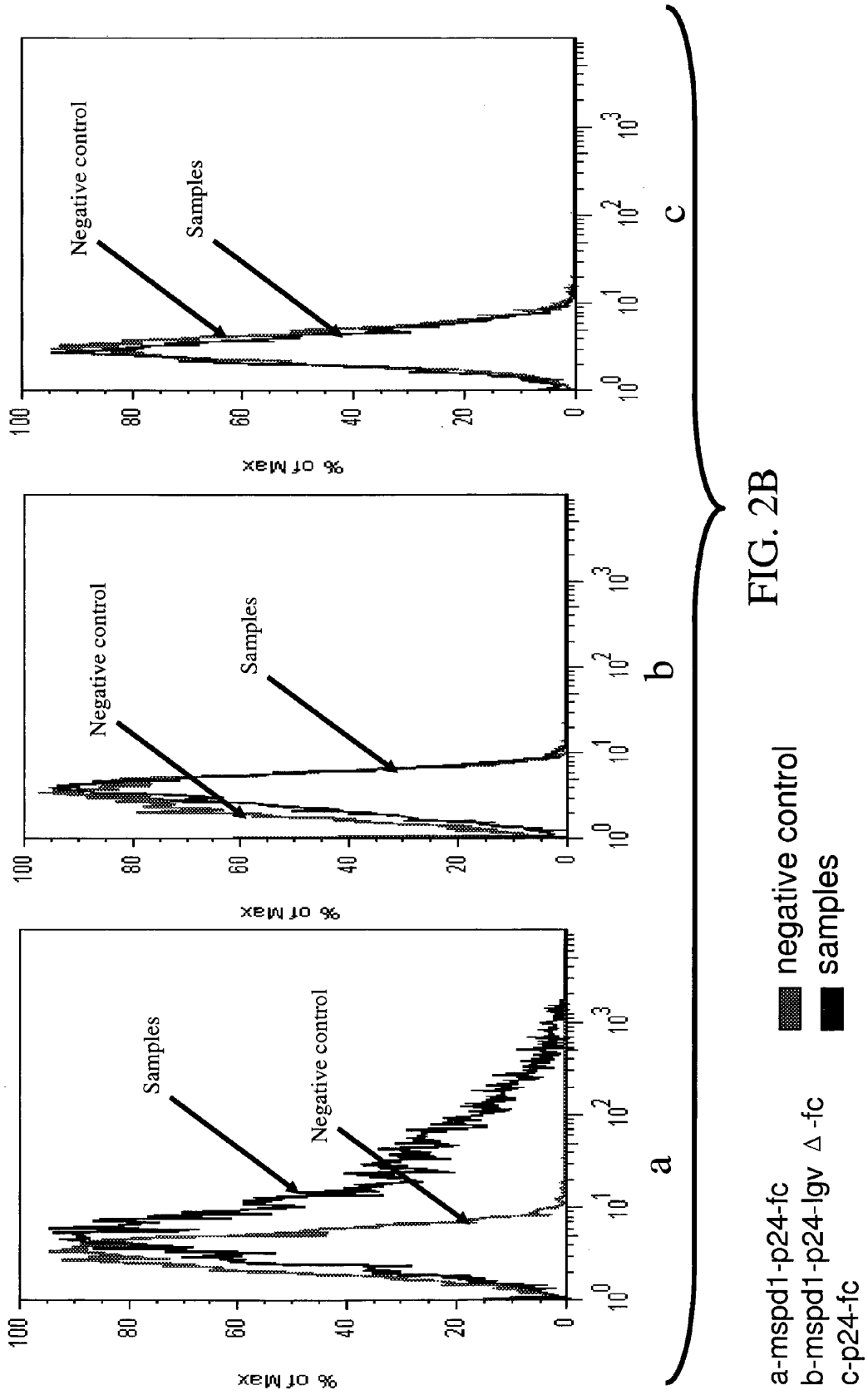


FIG. 2B

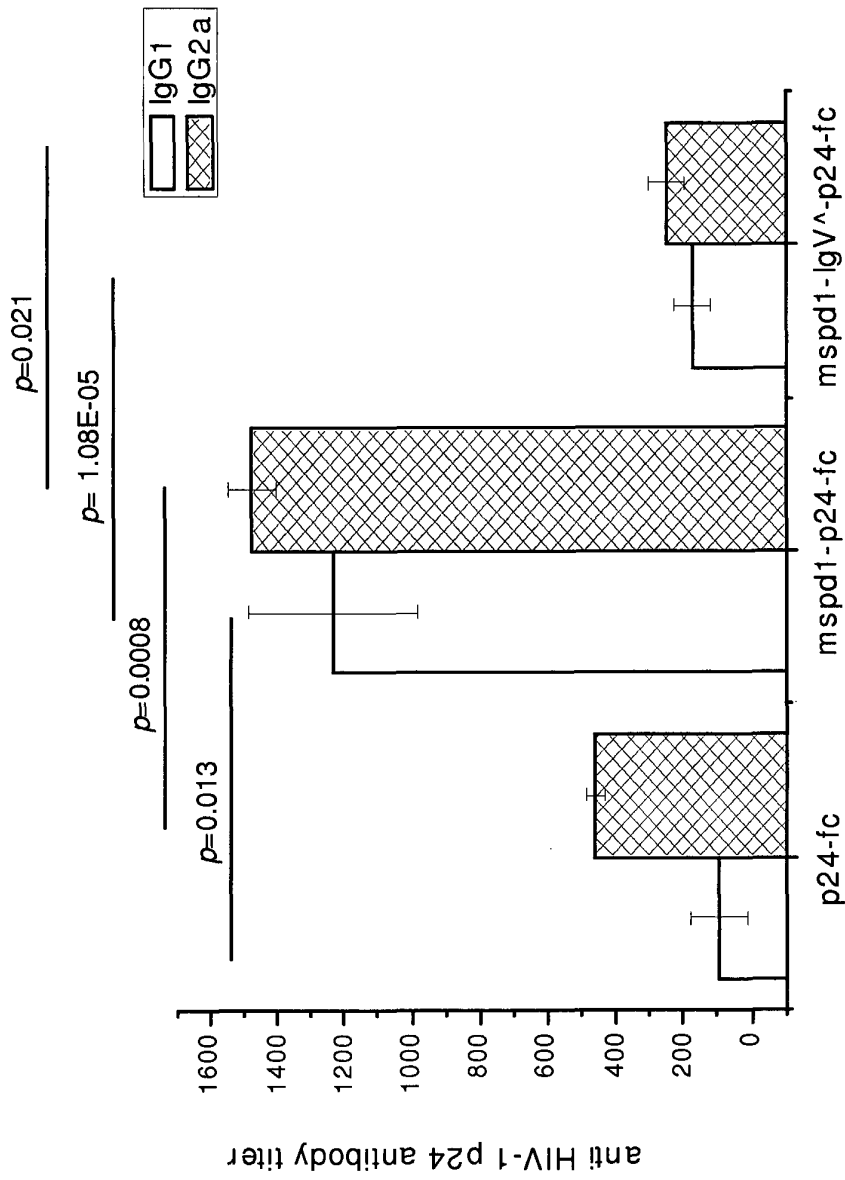


FIG. 3A

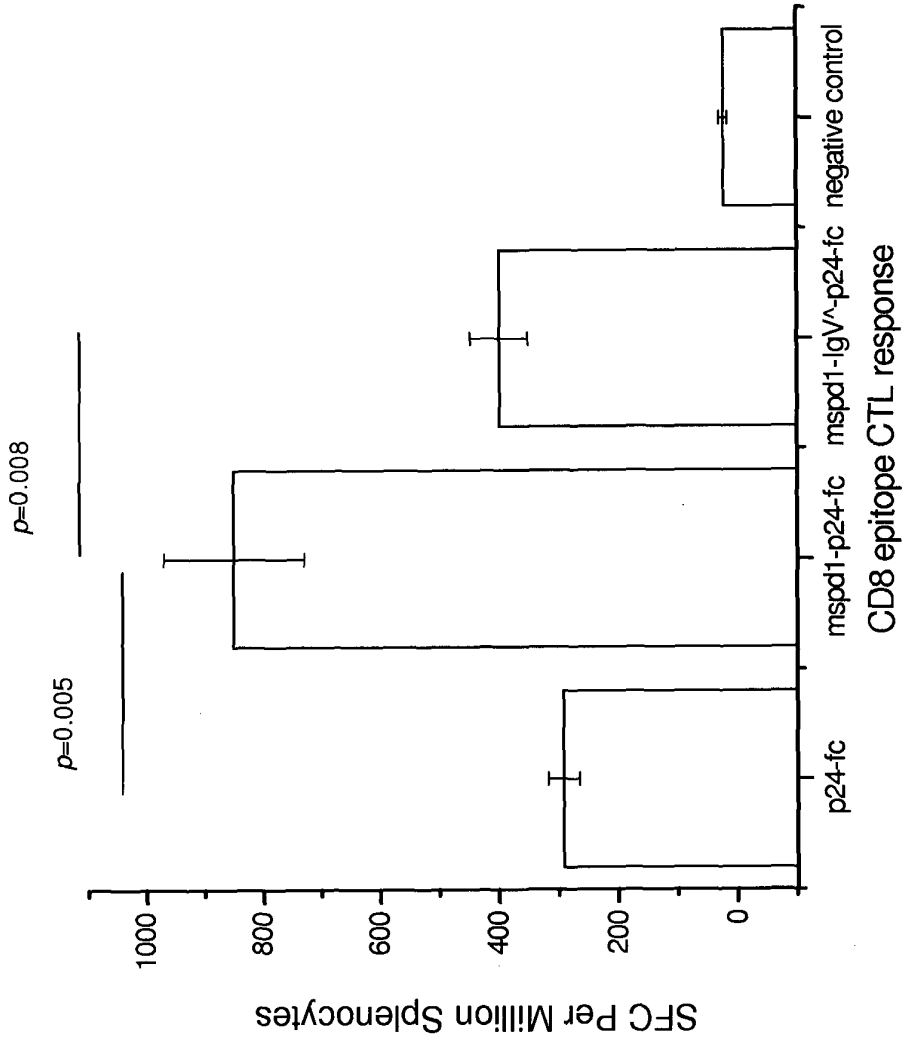
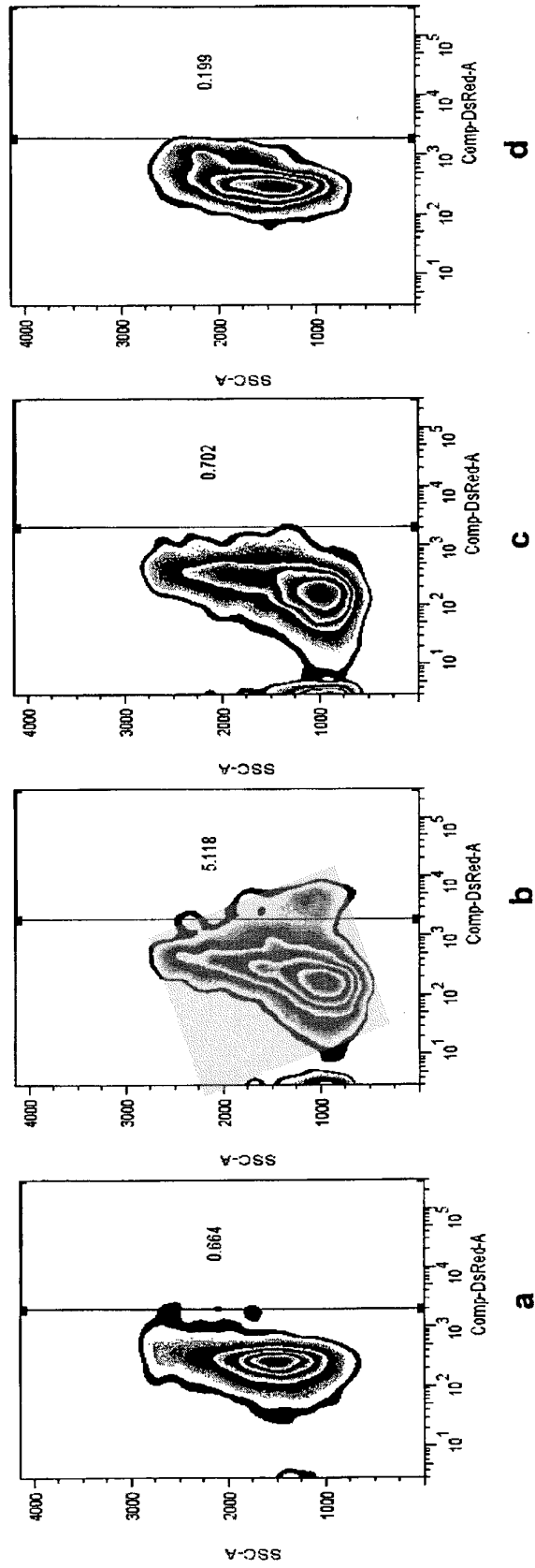


FIG. 3B





a-p24-fc; b-mspd1-p24-fc; c-mspd1- $\Delta$ -p24-fc; d-PBS

FIG. 3C

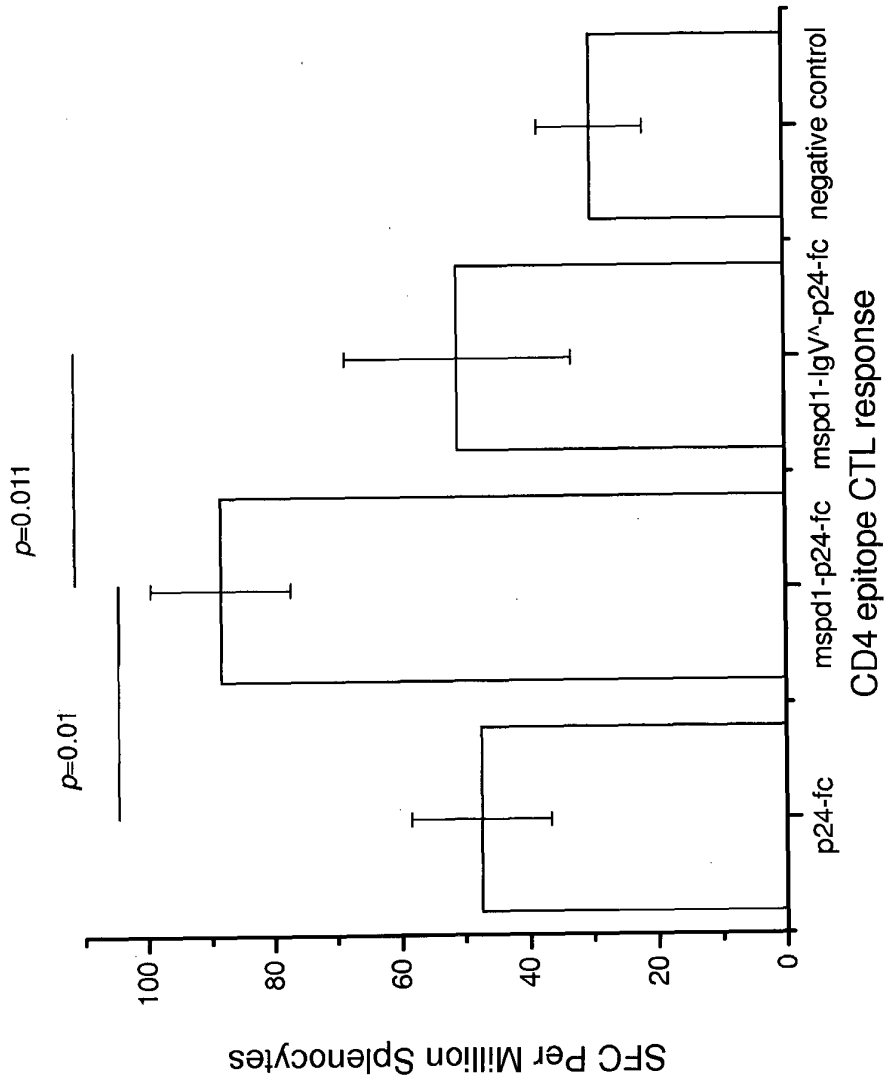


FIG. 3D

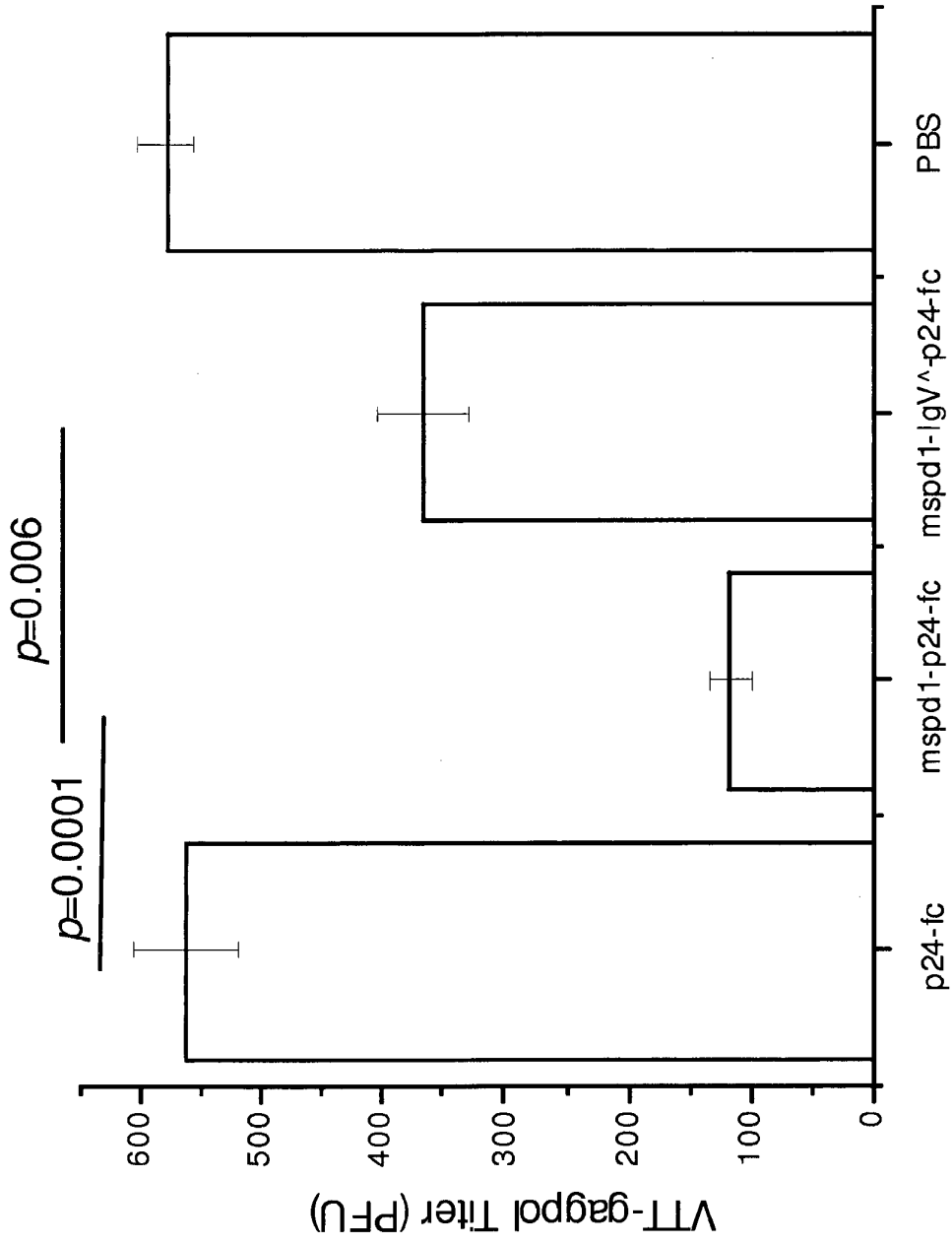
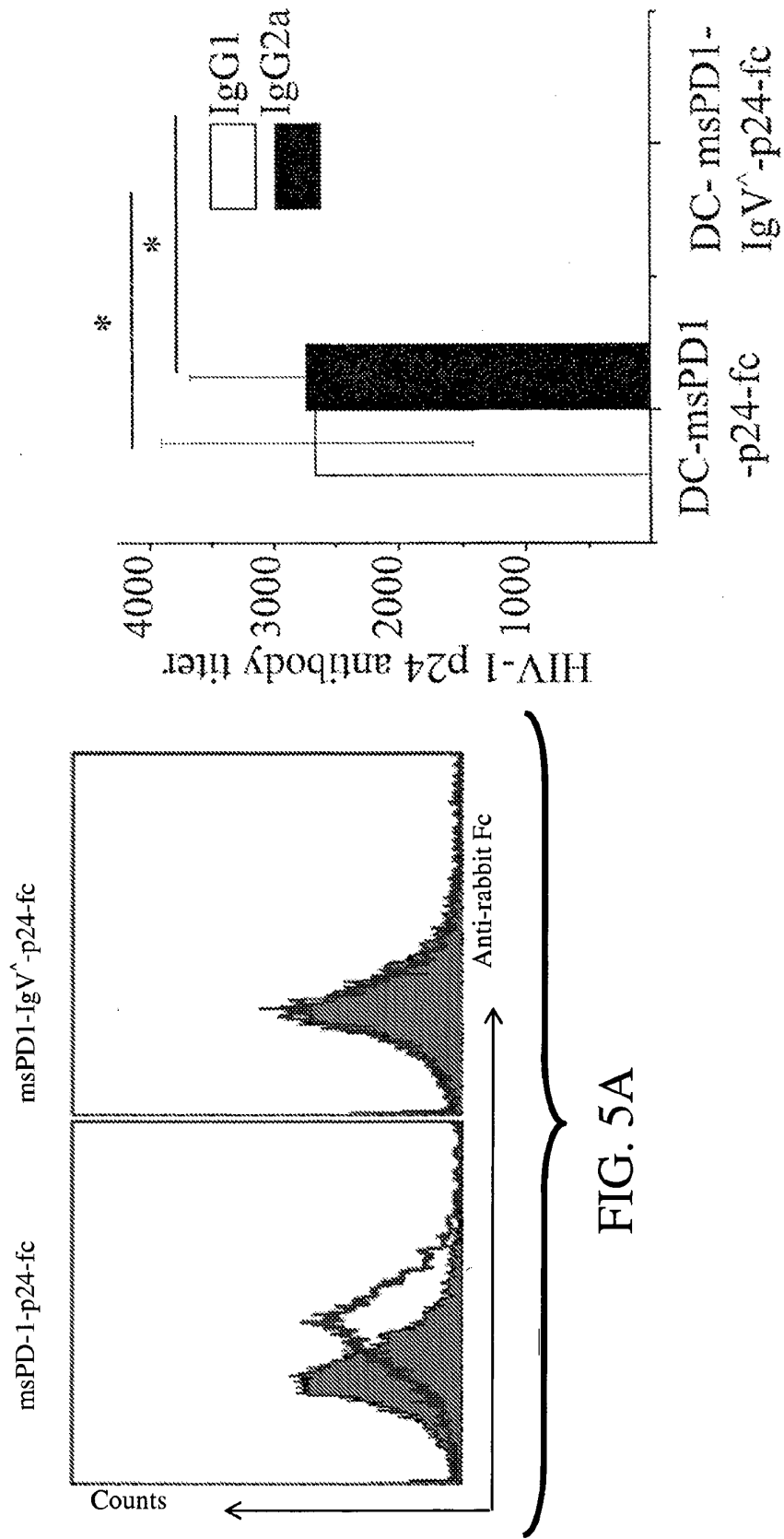


FIG. 4



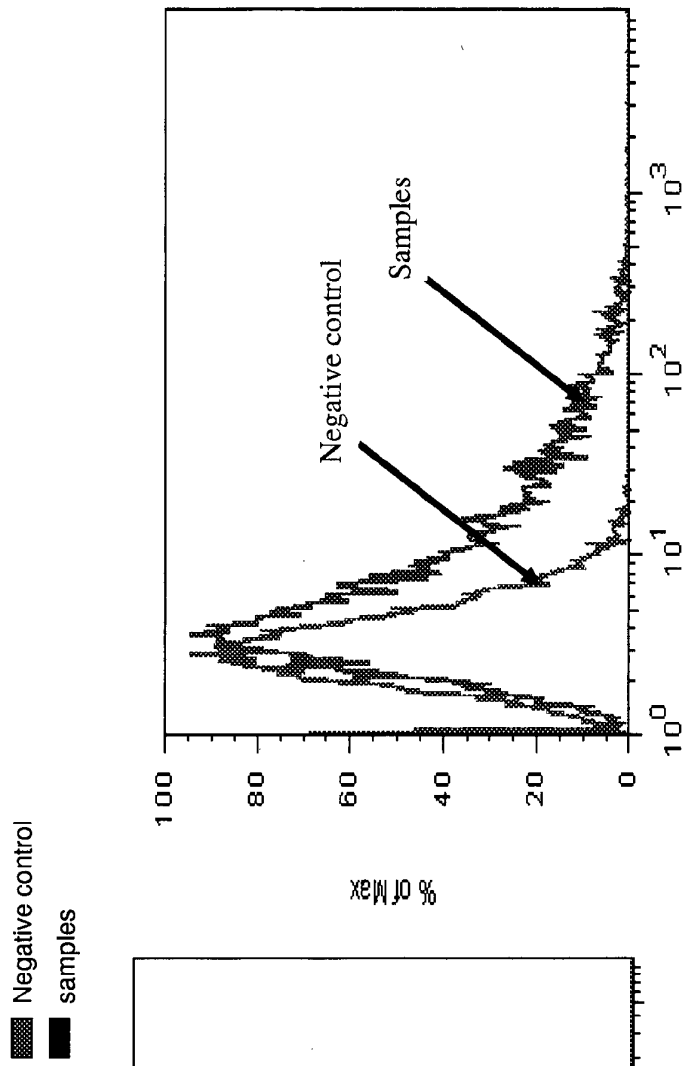


FIG. 6B

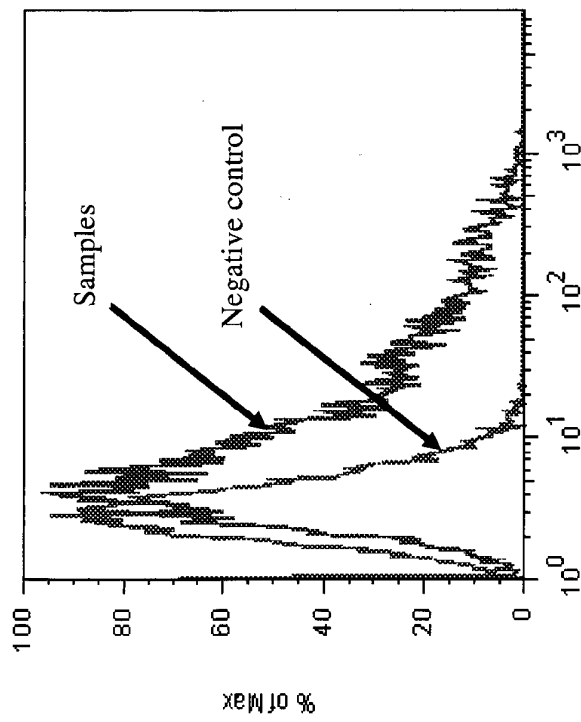


FIG. 6A

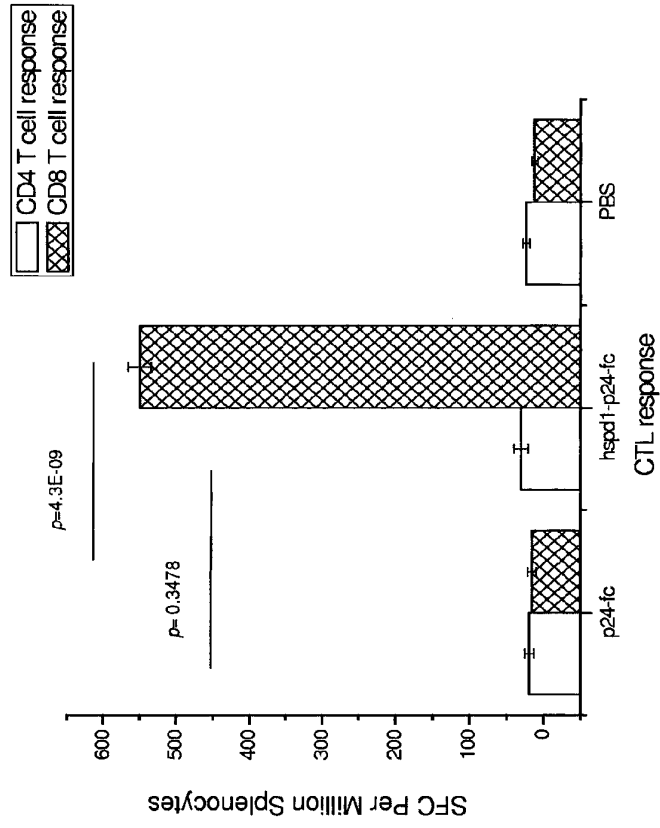


FIG. 6D

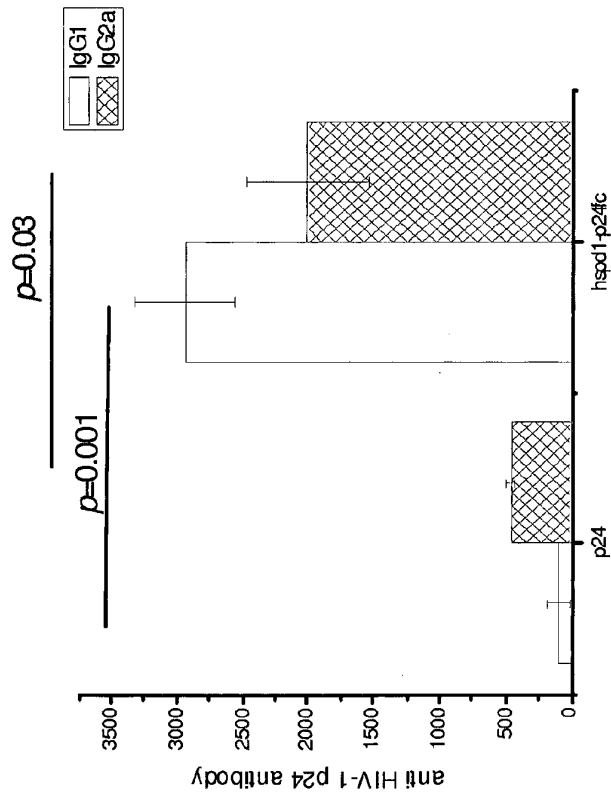


FIG. 6C

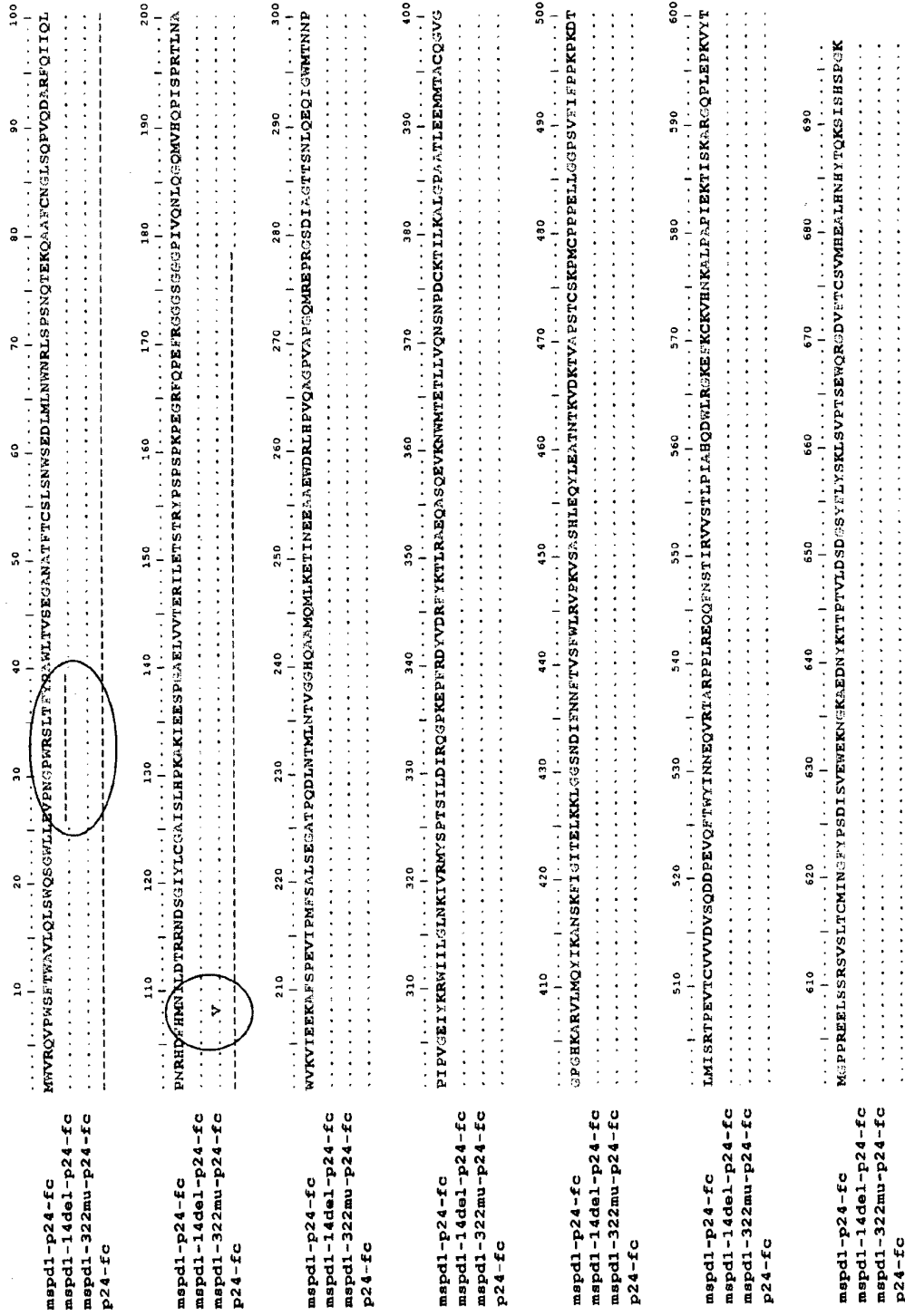


FIG. 7A

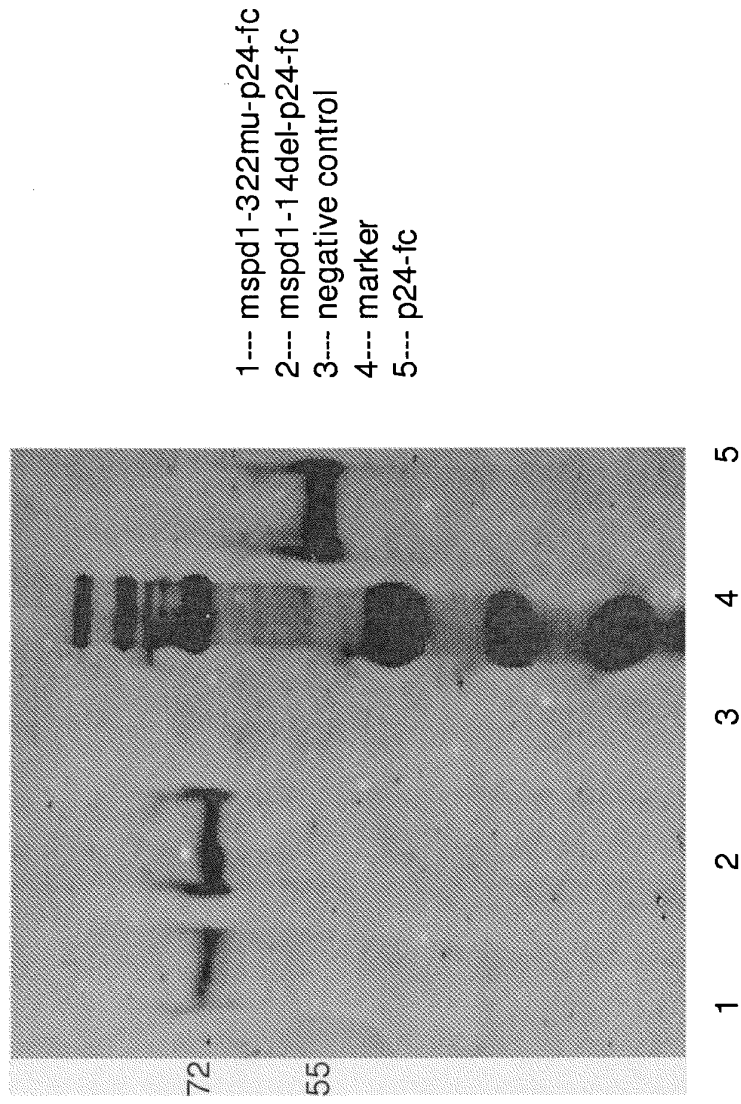
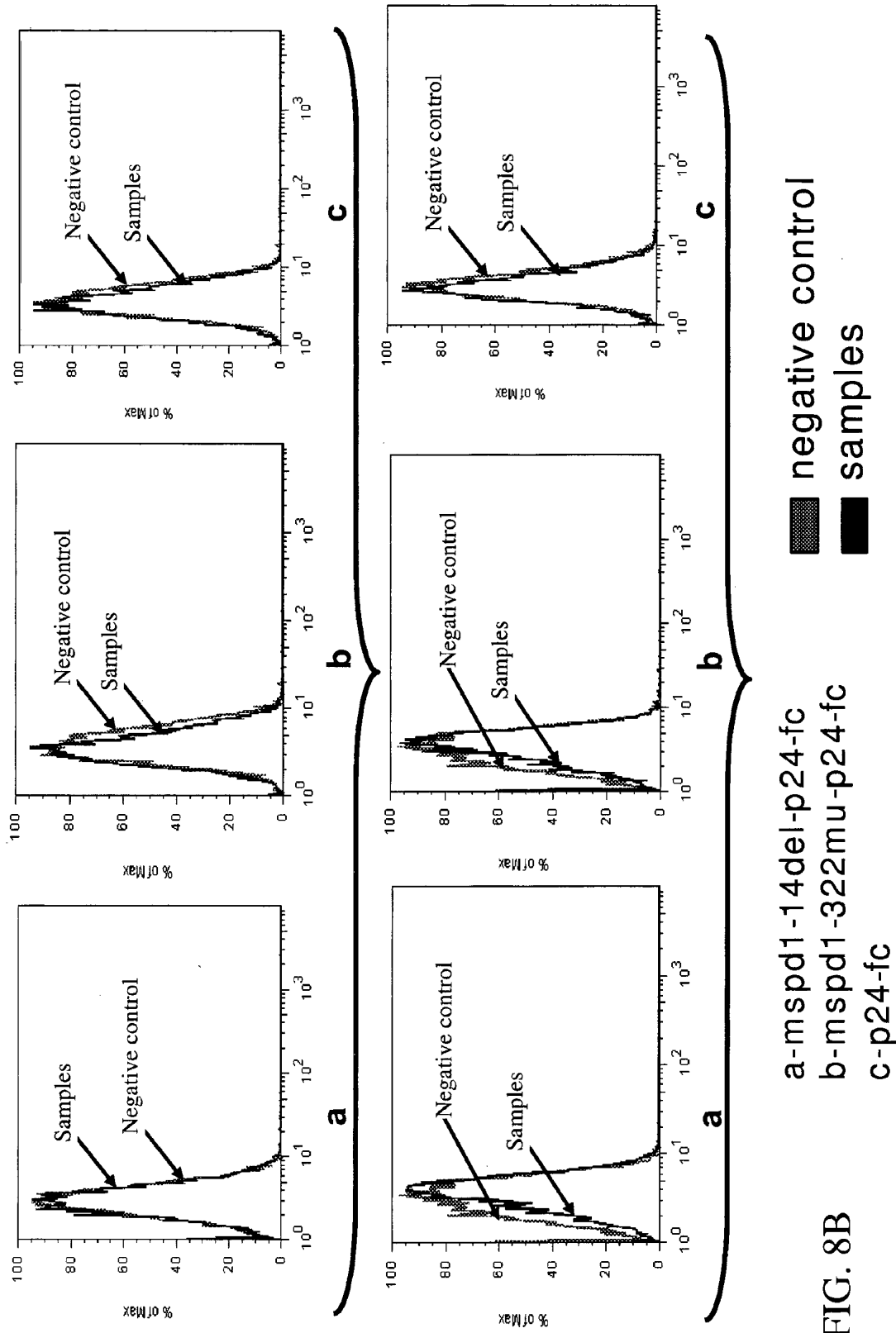


FIG. 7B





a- mspd1-14del-p24-fc  
b- mspd1-322mu-p24-fc  
c- p24-fc

■ negative control  
■ samples

FIG. 8B

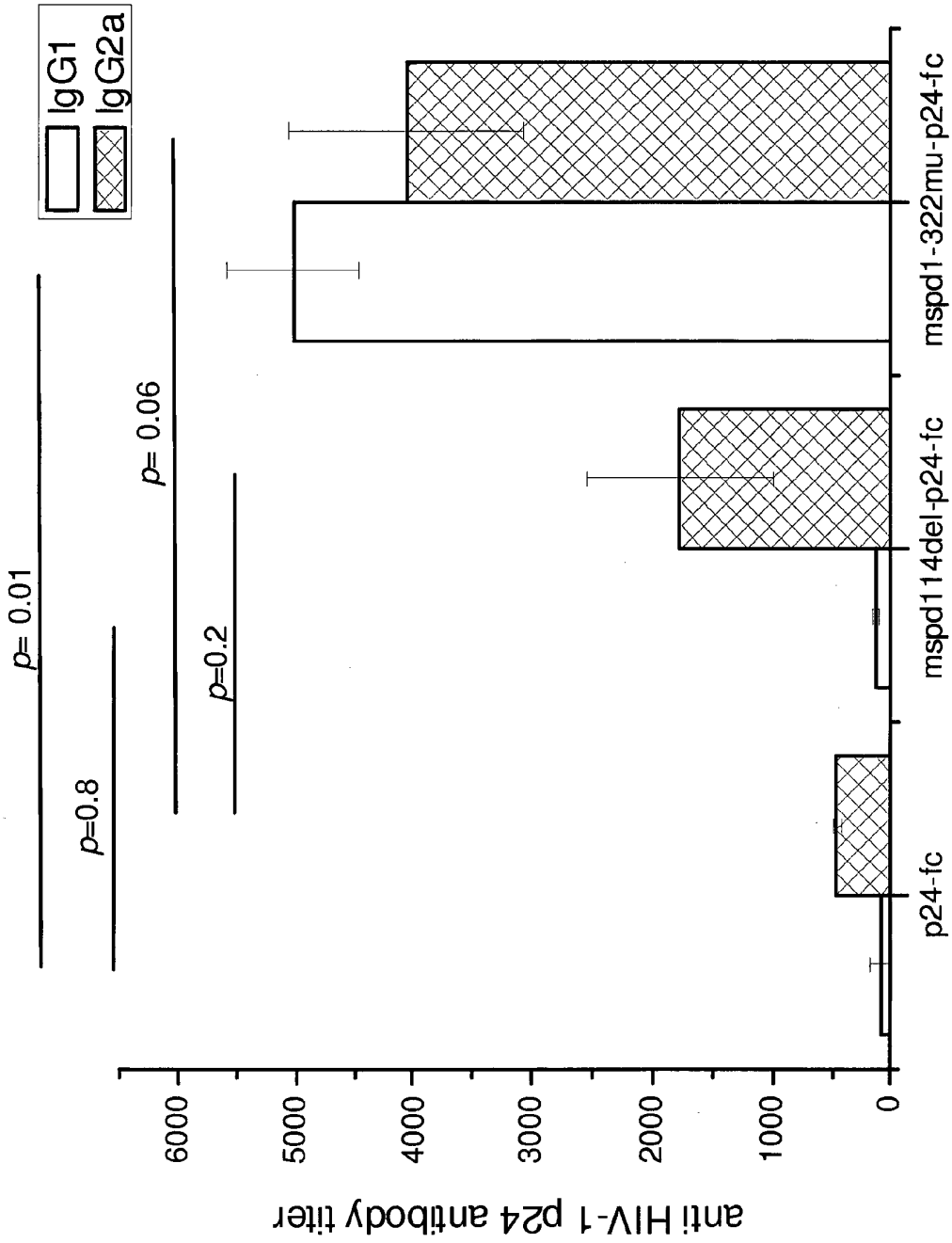


FIG. 9A

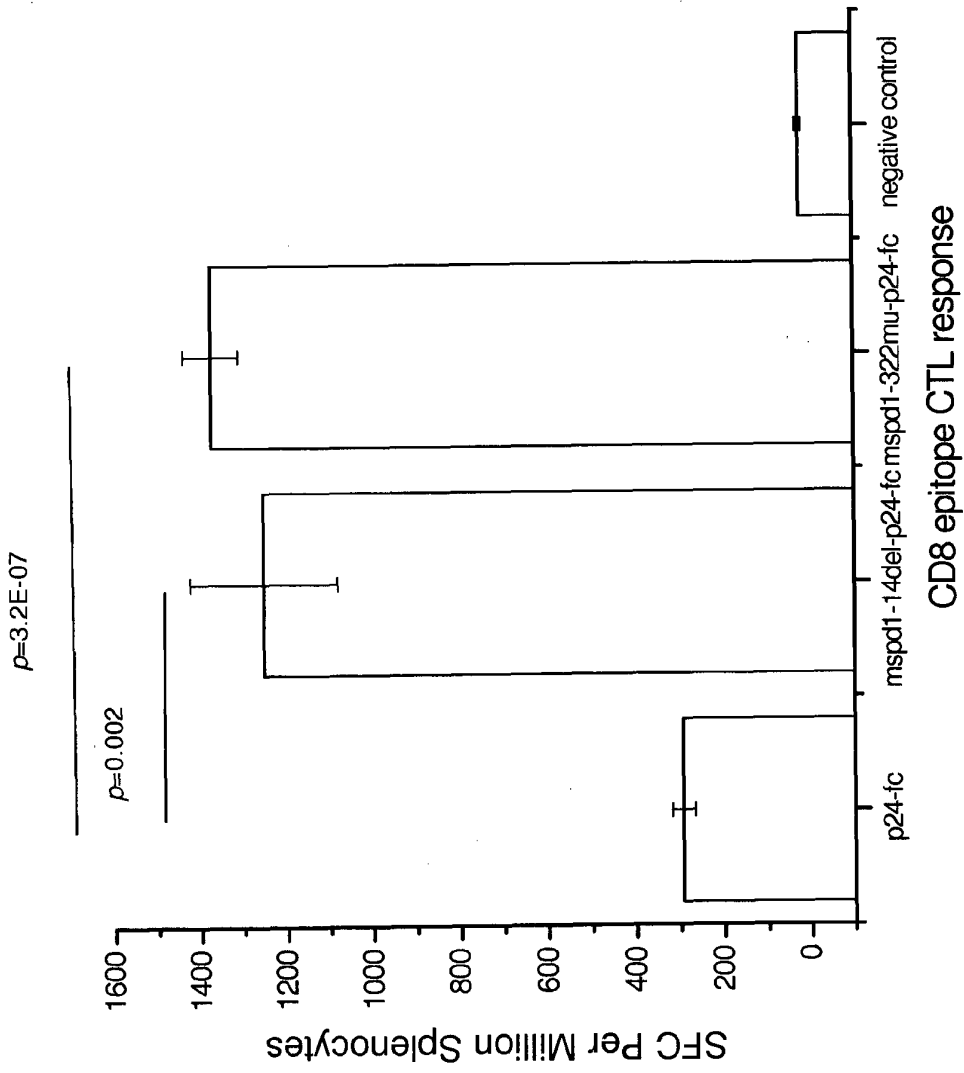


FIG. 9B

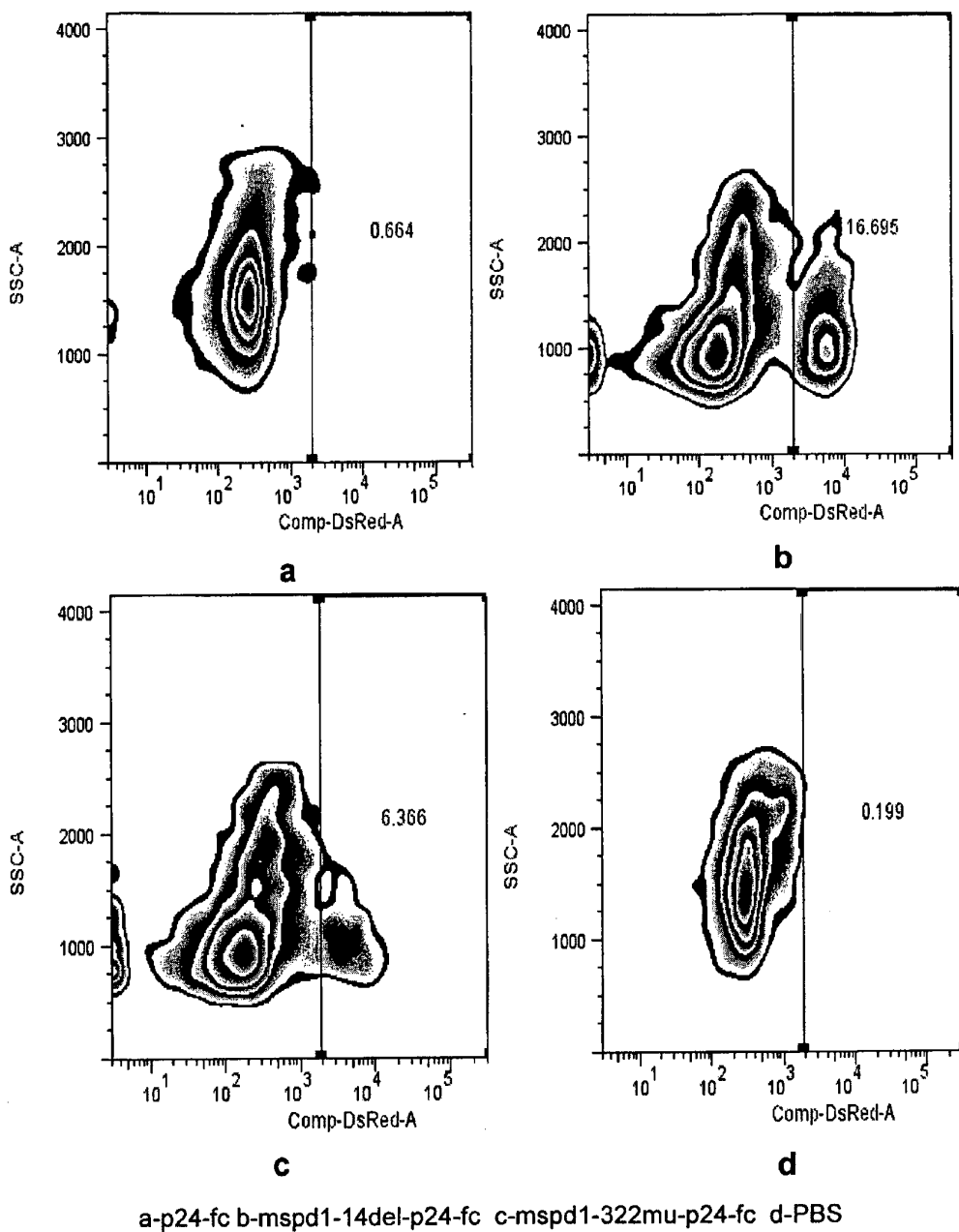


FIG. 9C

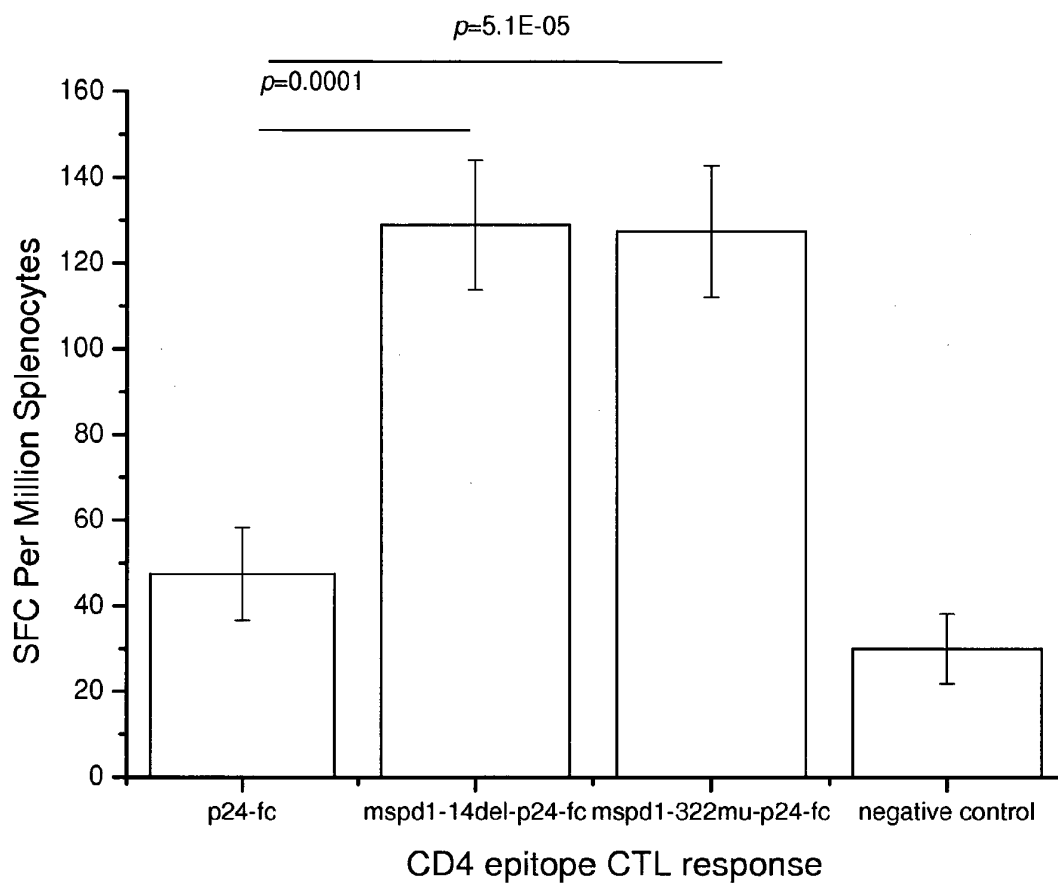


FIG. 9D

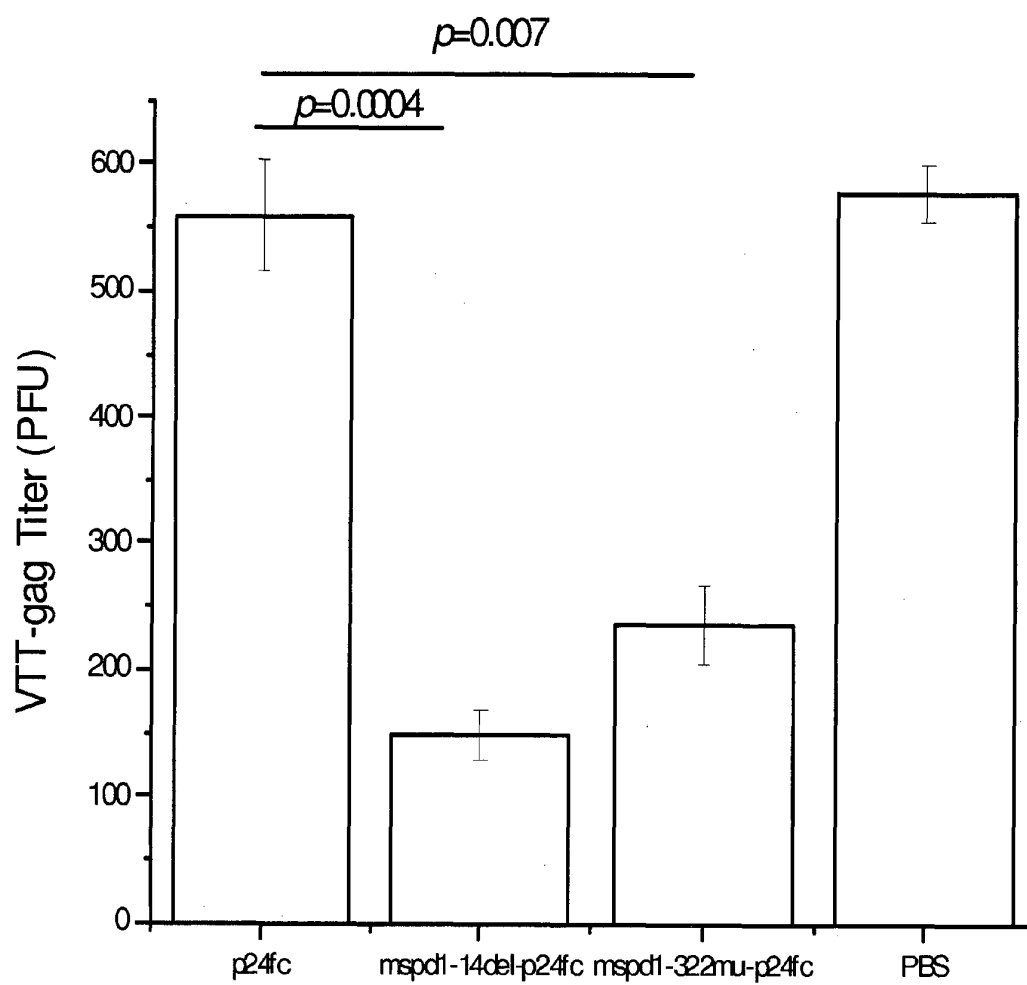


FIG. 10

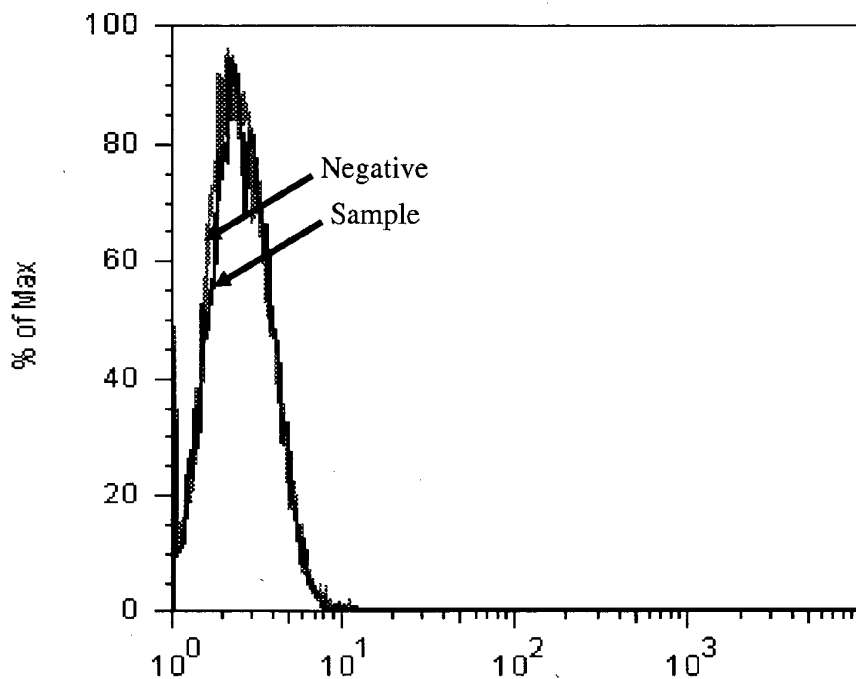


FIG. 11A

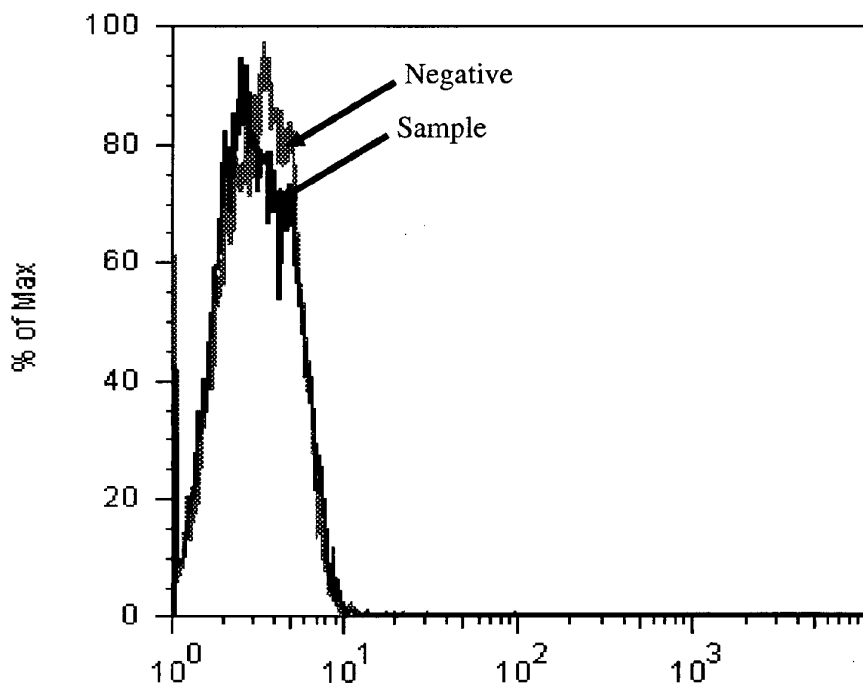


FIG. 11B

■ negative  
■ sample

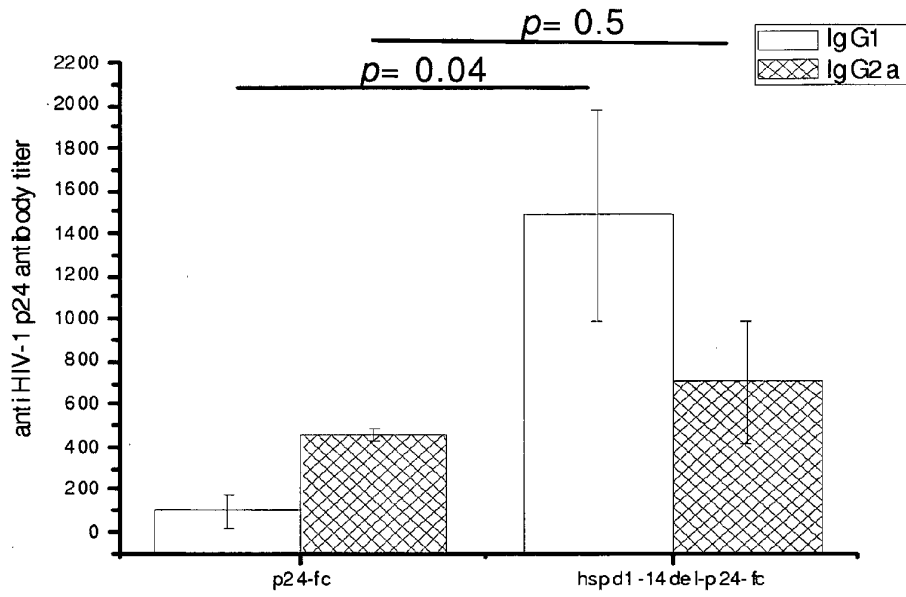


FIG. 11C

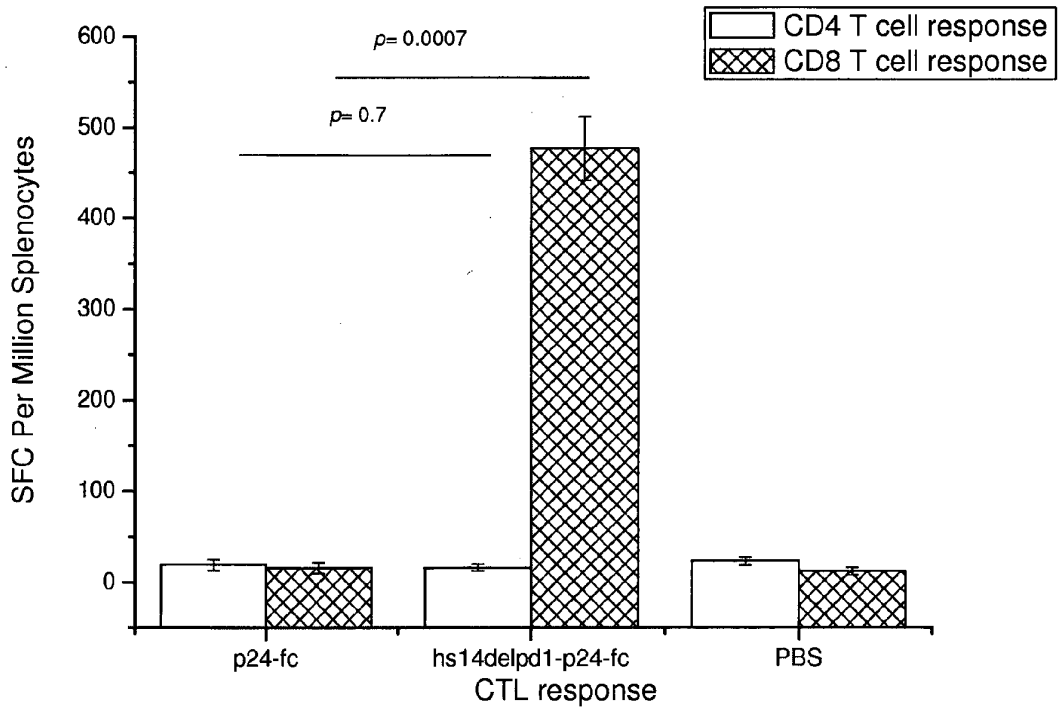
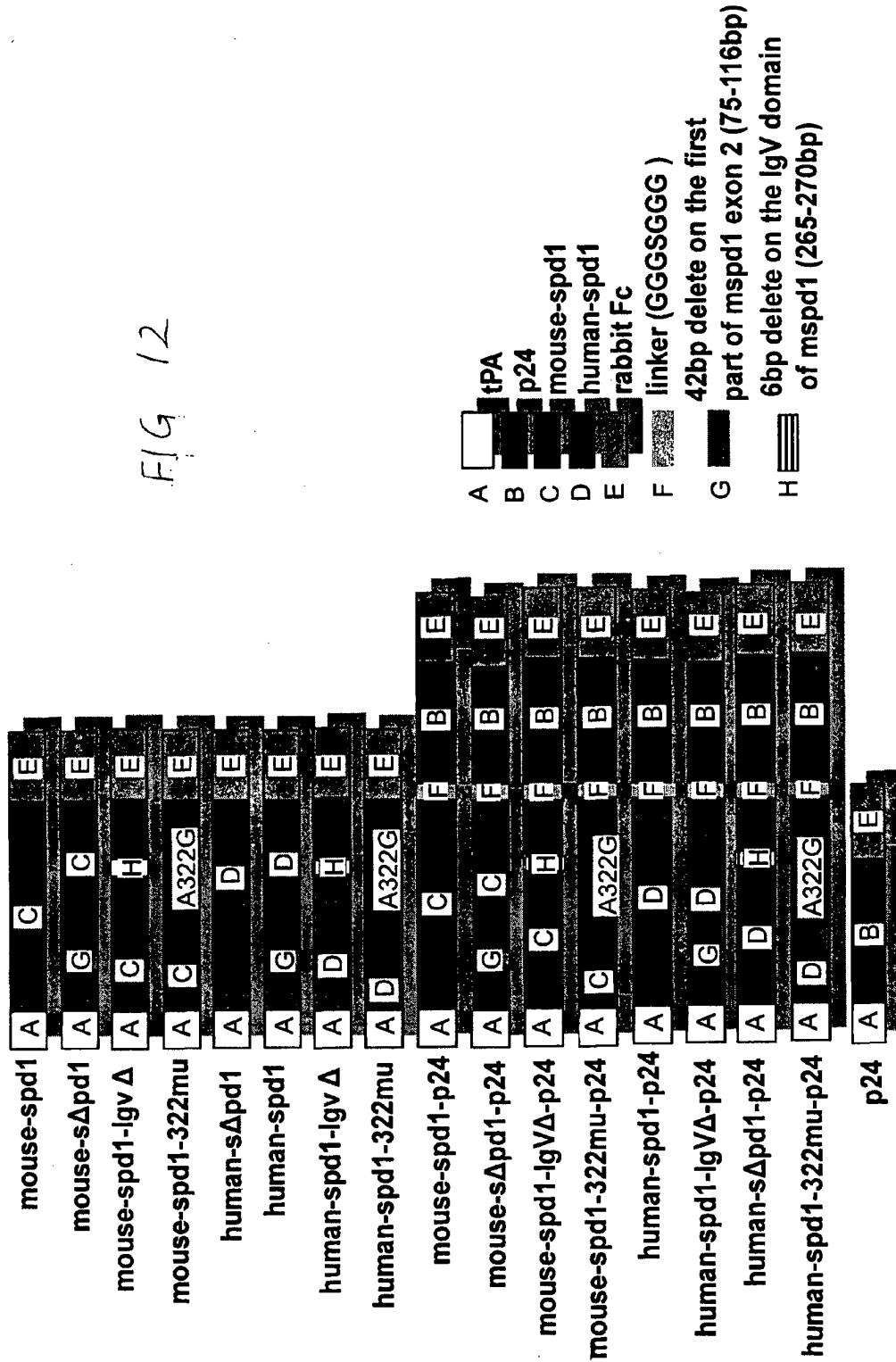


FIG. 11D



FIG 12



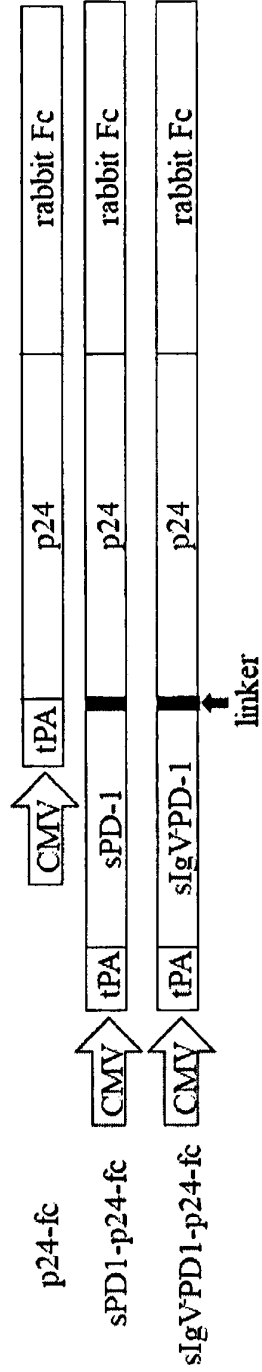


FIG. 13A



FIG. 13B

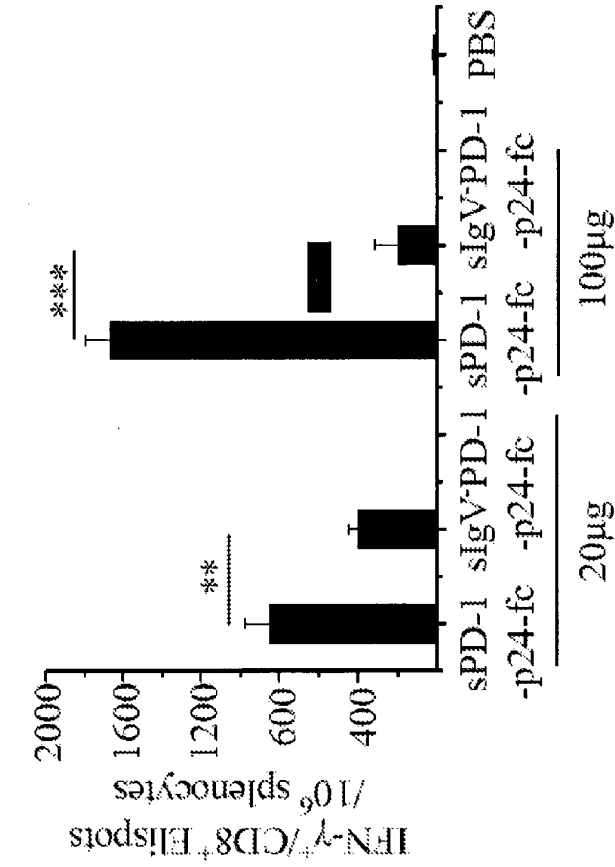


FIG. 13D

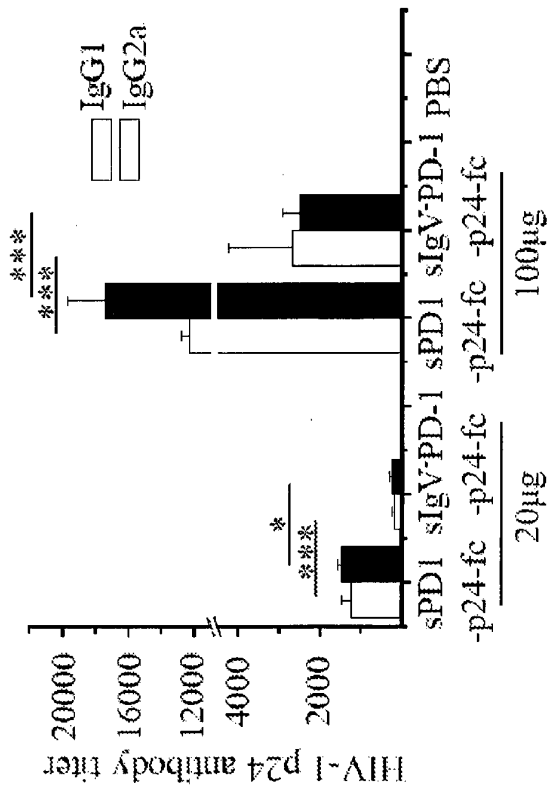


FIG. 13C

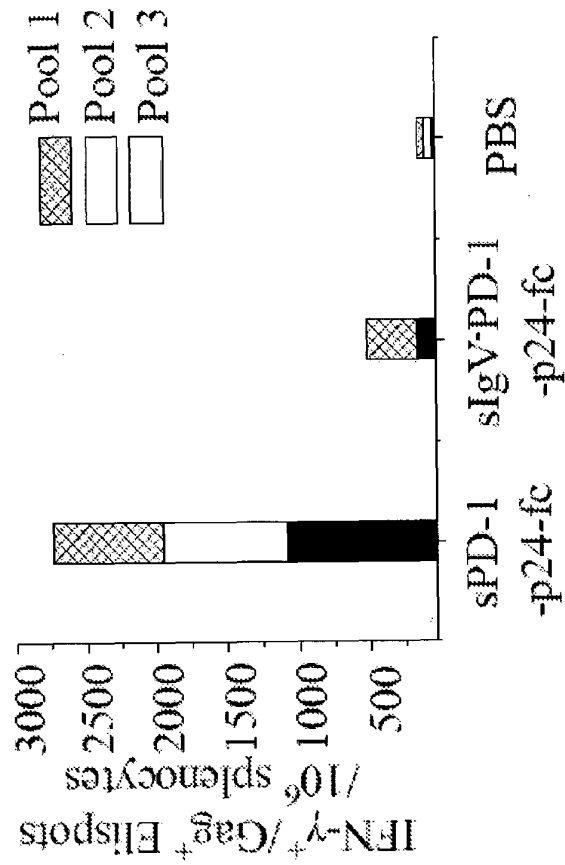


FIG. 13F

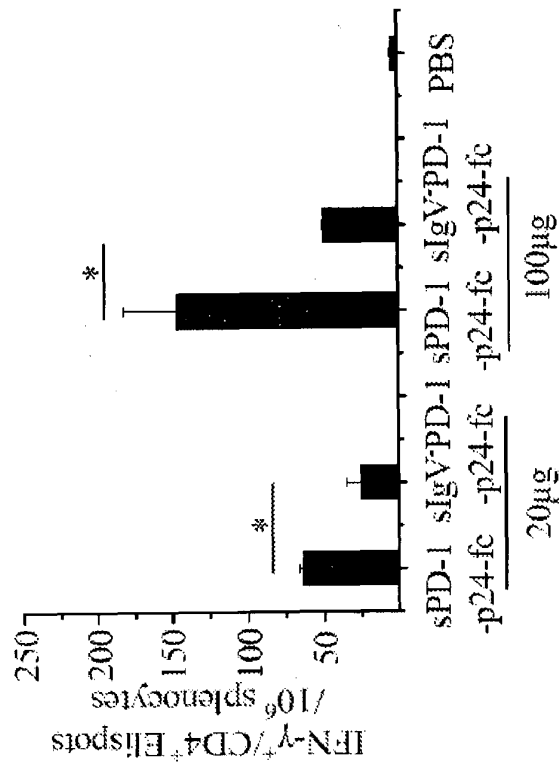


FIG. 13E

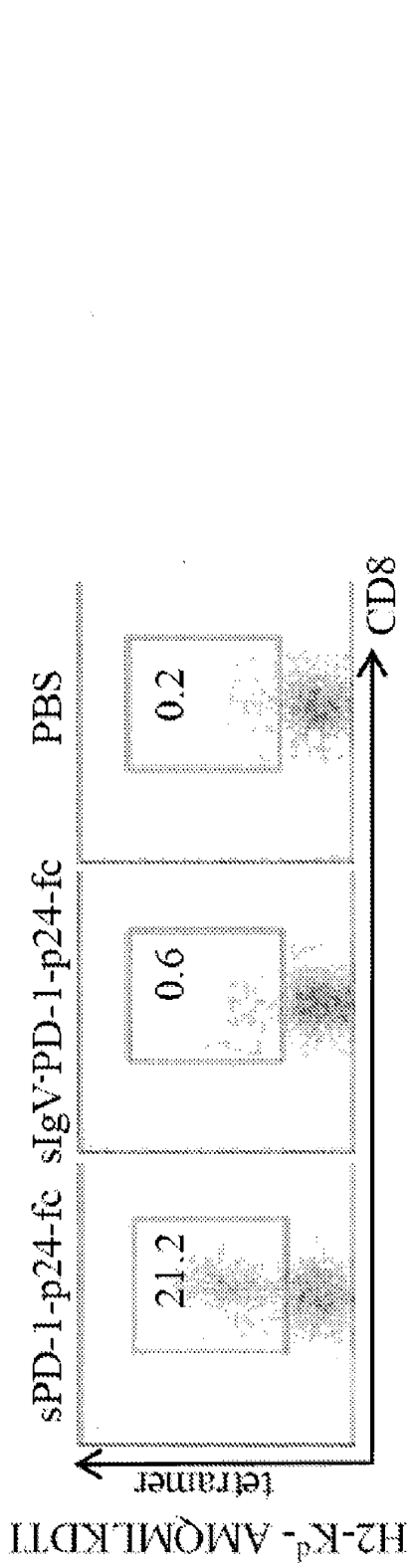


FIG. 13G

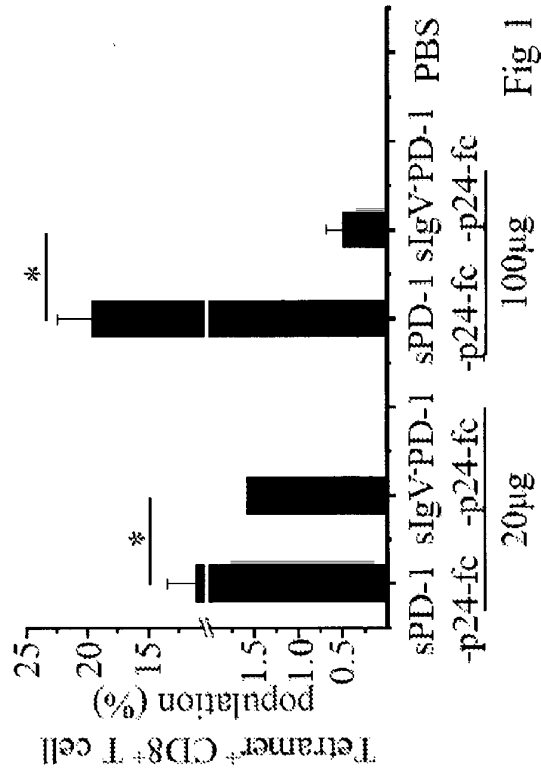


FIG. 13H

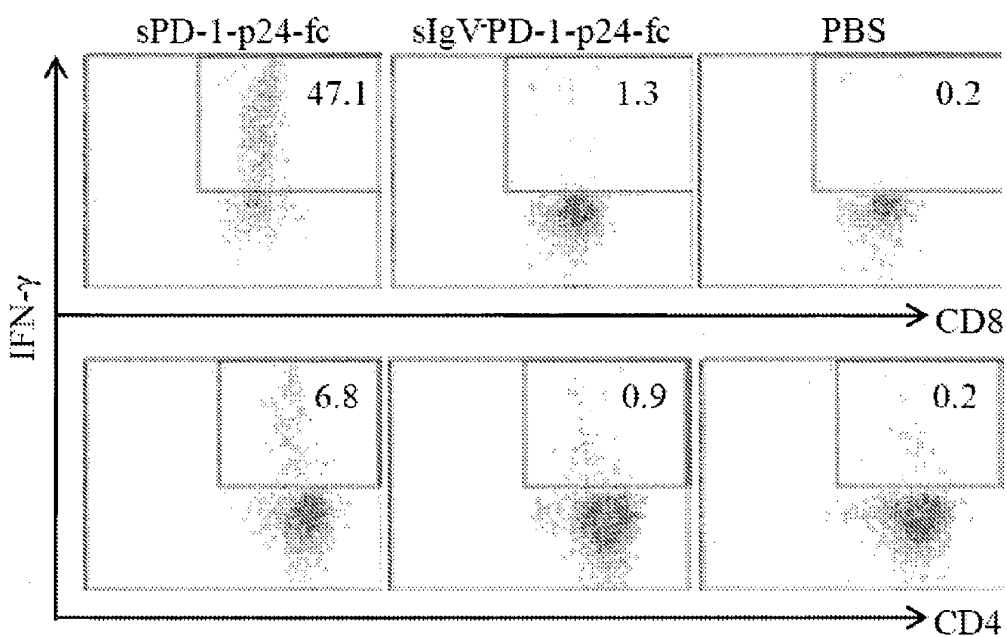


FIG. 14A

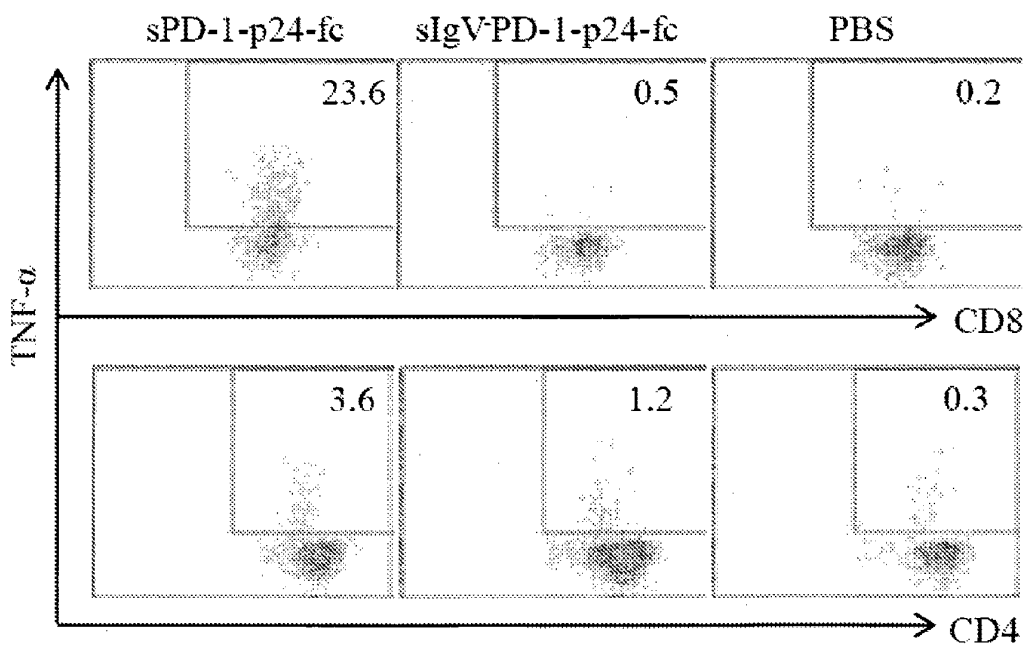


FIG. 14B

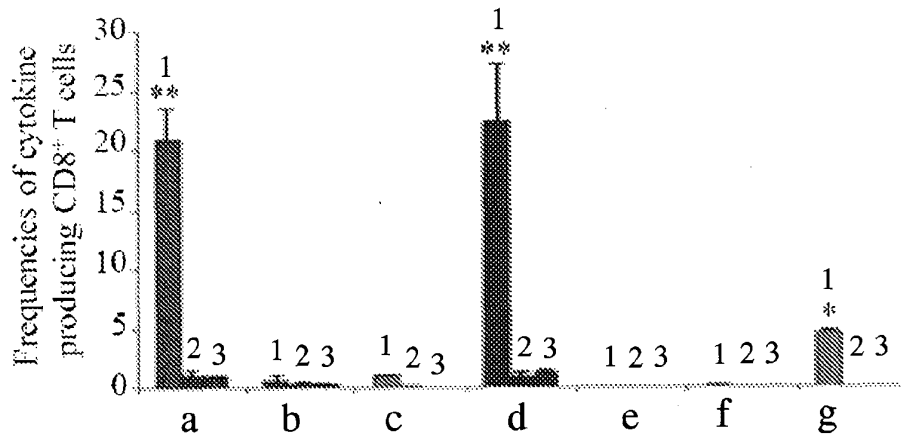


FIG. 14C

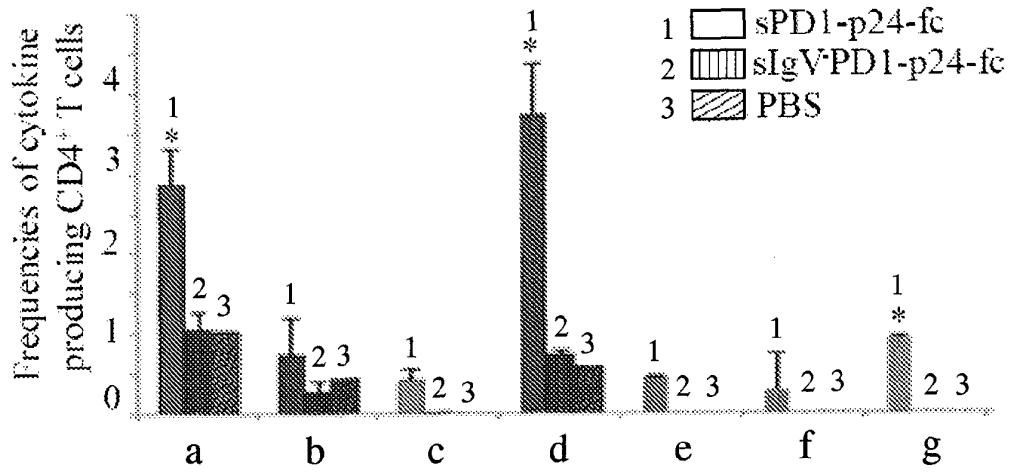


FIG. 14D

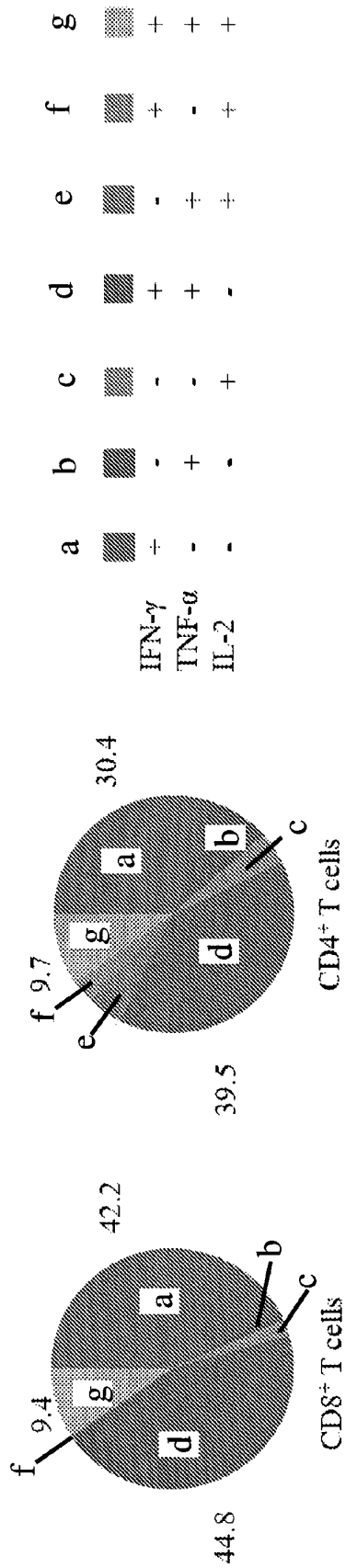


FIG. 14E



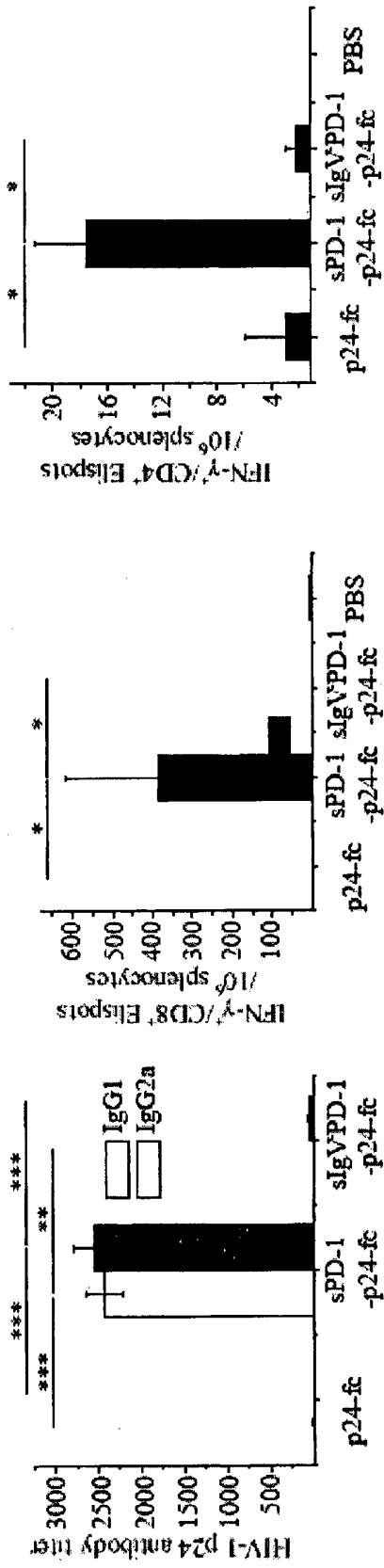


FIG. 15A

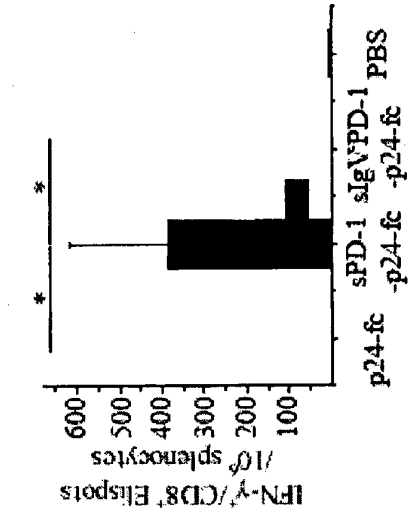


FIG. 15B

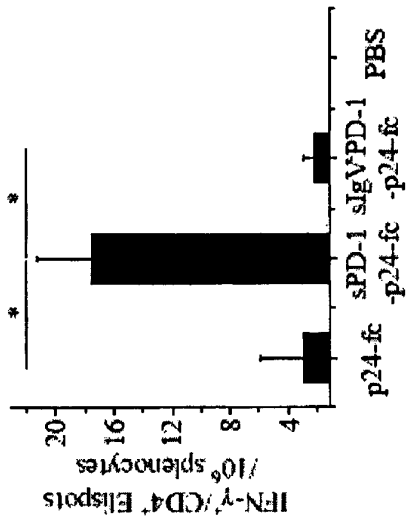


FIG. 15C

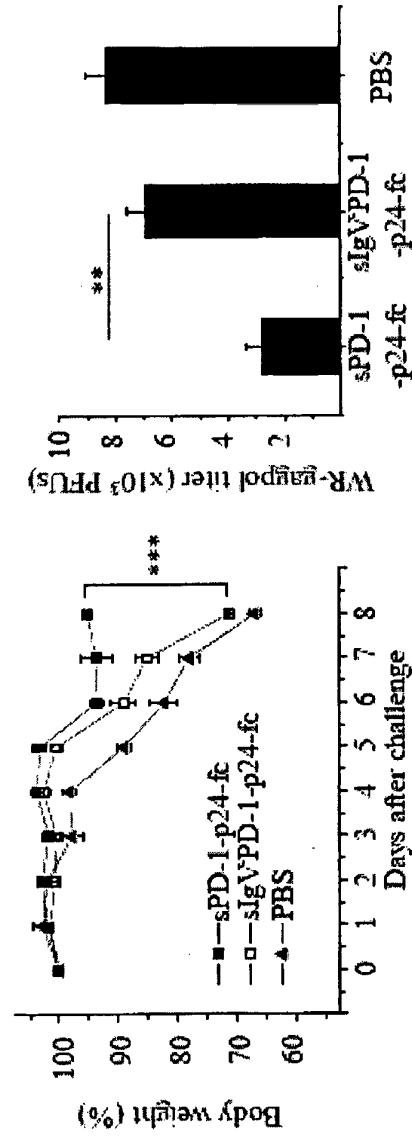


FIG. 15D

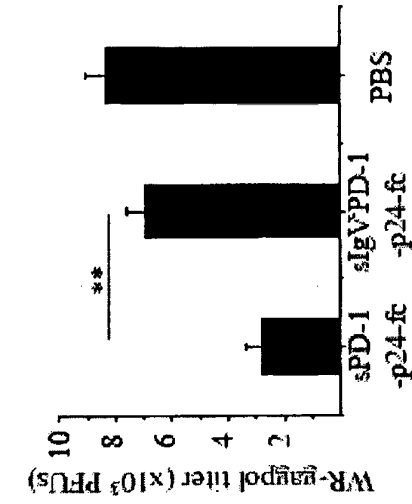


FIG. 15E

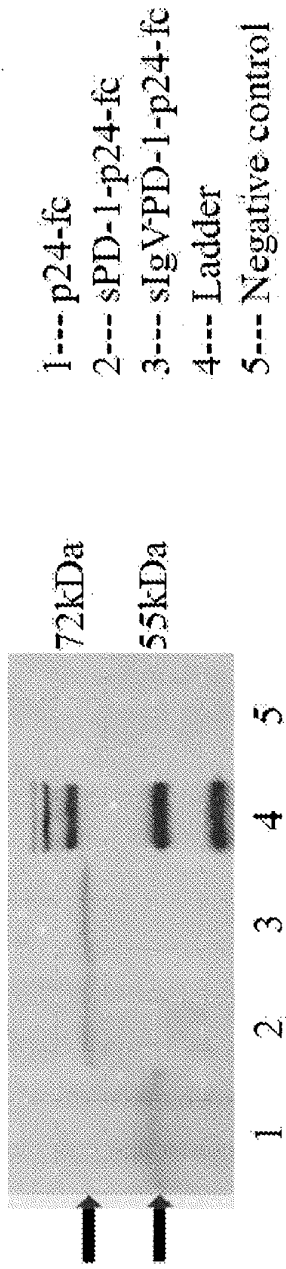


FIG. 16A

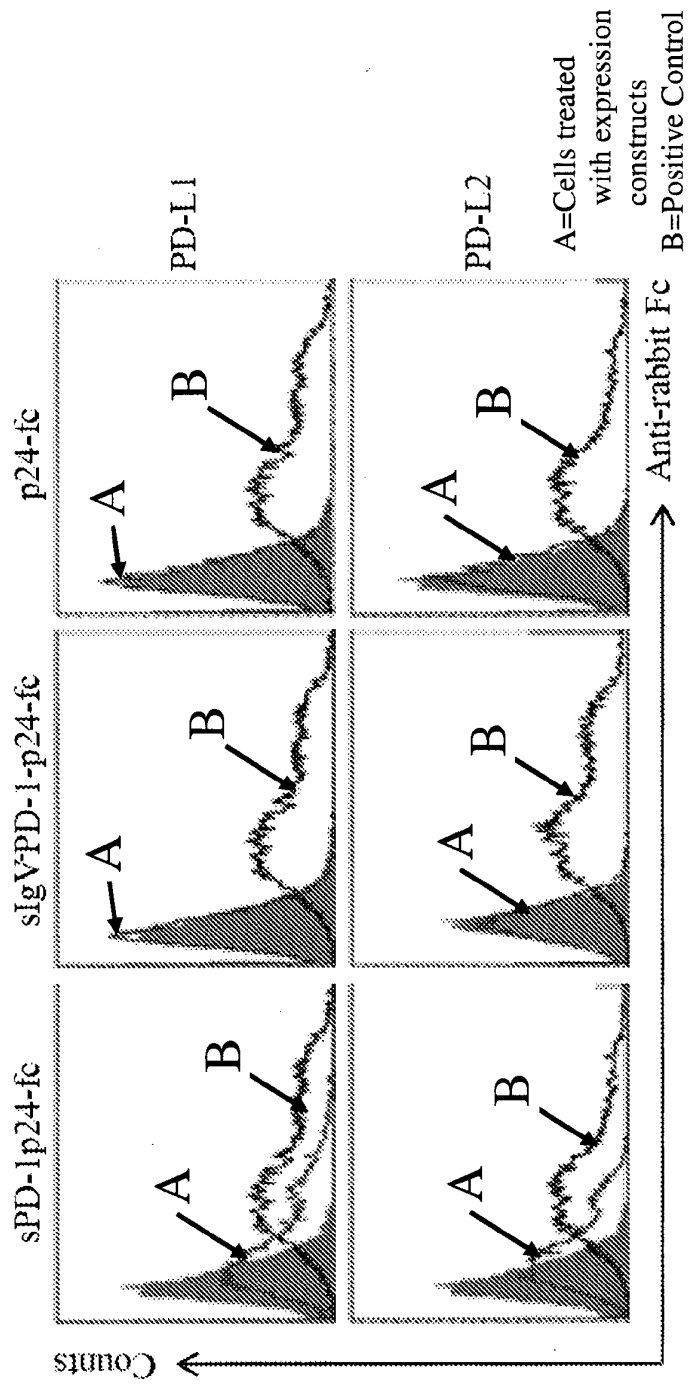


FIG. 16B

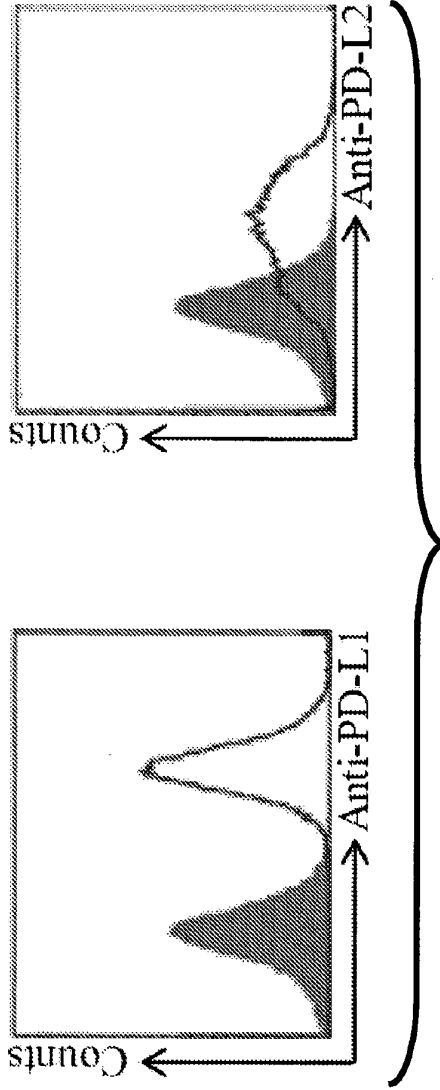


FIG. 17A

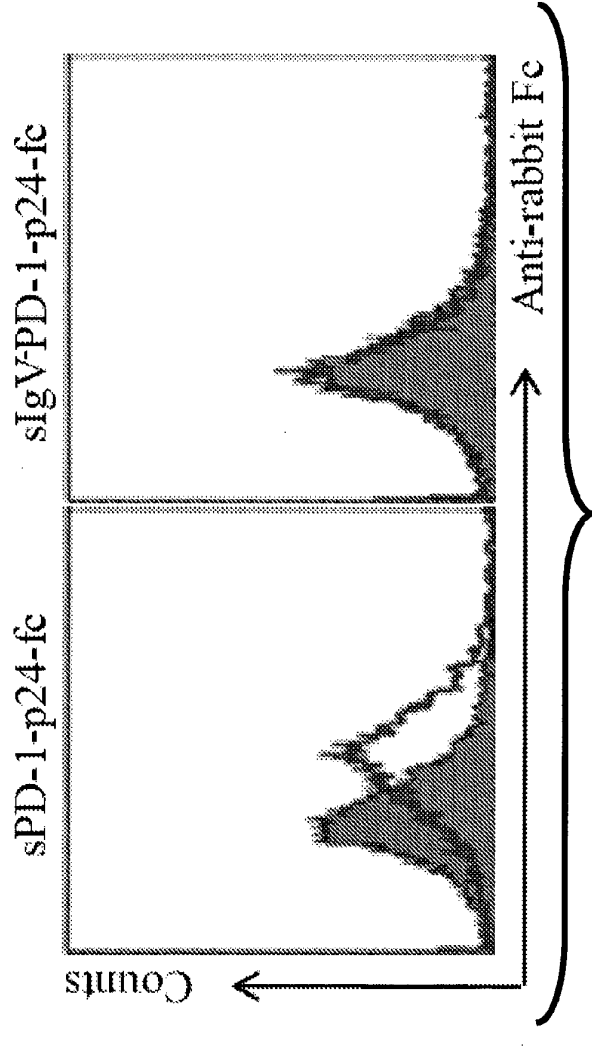


FIG. 17B

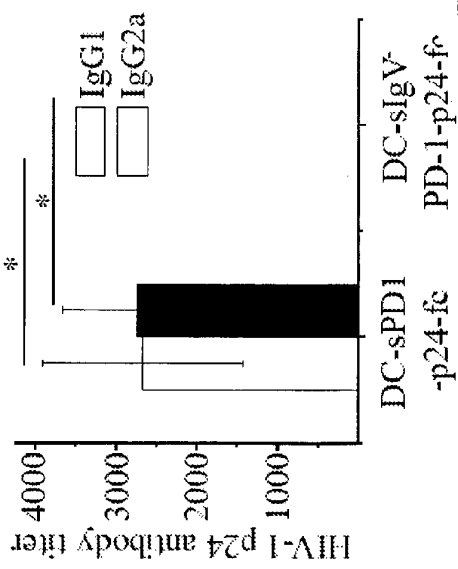


FIG. 17C

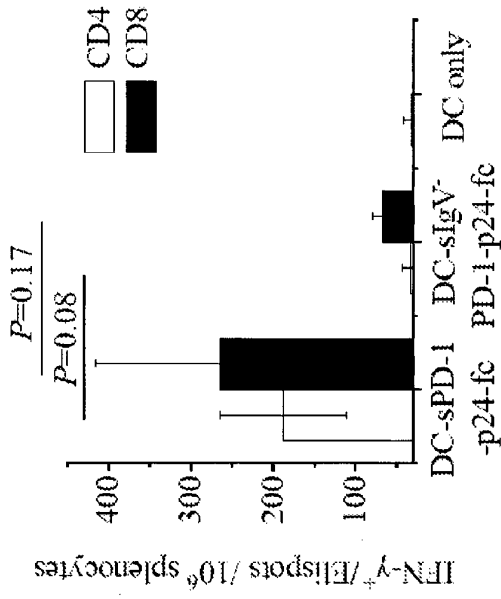


FIG. 17D

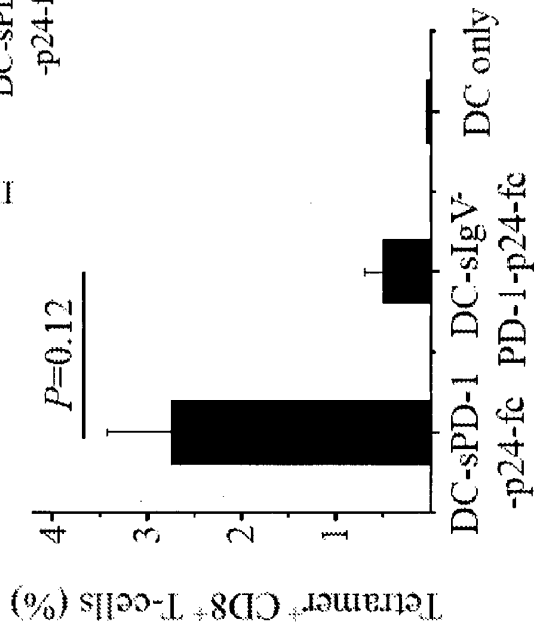


FIG. 17E

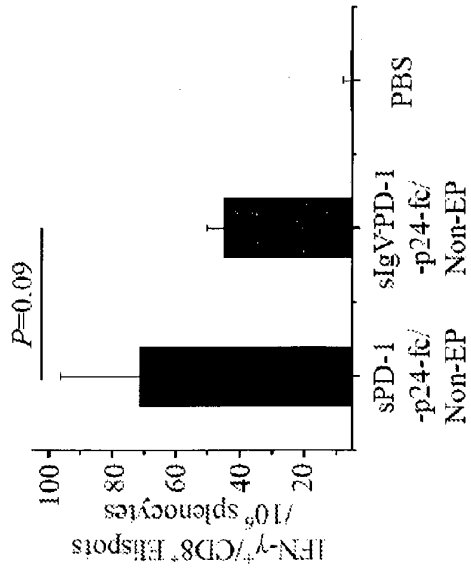


FIG. 18A

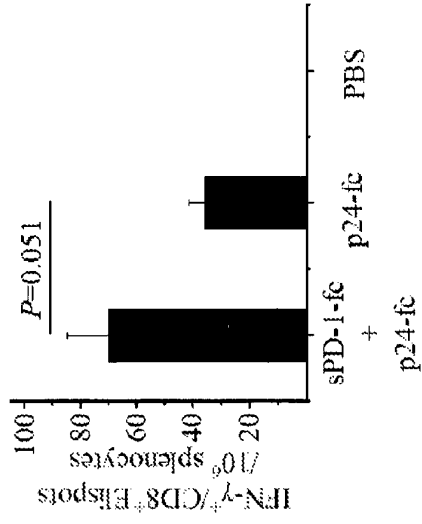


FIG. 18B

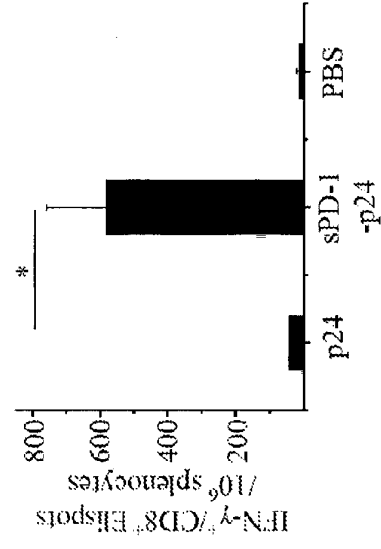


FIG. 18C

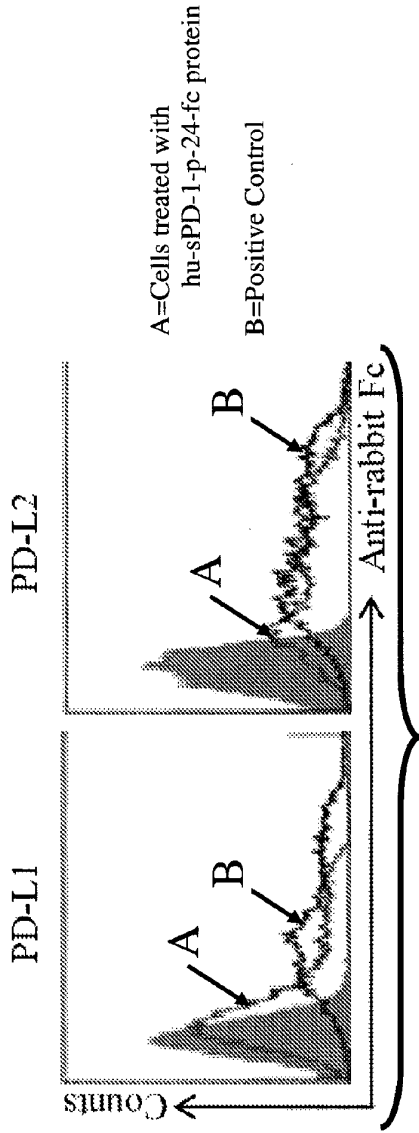


FIG. 19A

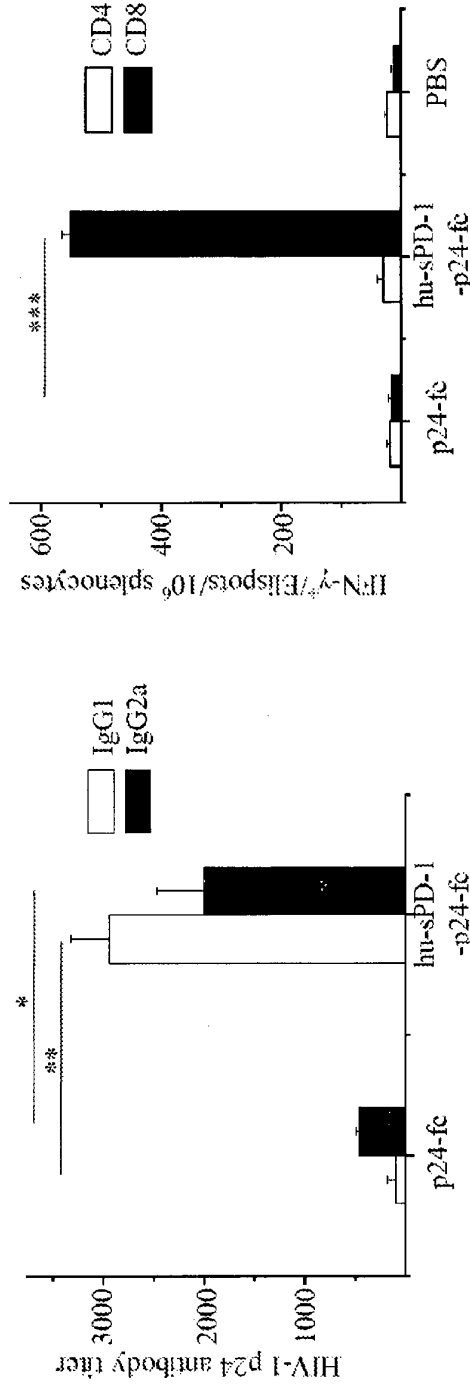


FIG. 19B

FIG. 19C

## REFERENCES CITED IN THE DESCRIPTION

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