(51) International Patent Classification:
C12N 15/86 (2006.01)      C12N 5/0783 (2010.01)
C12N 15/12 (2006.01)      C12Q 1/68 (2006.01)
C12N 15/33 (2006.01)      A61K 48/00 (2006.01)
C07K 14/705 (2006.01)    A61P 35/00 (2006.01)
C07K 14/725 (2006.01)

(21) International Application Number:
                              PCT/US2016/017373

(22) International Filing Date:
                    10 February 2016 (10.02.2016)

(25) Filing Language:                          English

(26) Publication Language:                     English

(30) Priority Data:
    62/114,203    10 February 2015 (10.02.2015)    US
    62/196,520    24 July 2015 (24.07.2015)        US
    62/220,703    18 September 2015 (18.09.2015)   US

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(81) Designated States (unless otherwise indicated, for every
    kind of national protection available): AE, AG, AL, AM,
    AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY,
    BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM,
    DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT,
    HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR,
    KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG,
    MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM,
    PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC,
    SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN,
    TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

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

Published:
—    with international search report (Art. 21(3))

(54) Title: METHODS AND COMPOSITIONS USEFUL IN GENERATING NON CANONICAL CD8+ T CELL RESPONSES

(57) Abstract: Methods of inducing a CD8+ T cell response to a heterologous antigen in which at least 10% of the CD8+ T cells are
MHC-E restricted are disclosed. The method involves immunizing with a CMV vector that does not express UL128 and UL130 pro-
teins. Also disclosed are recombinant CMV vectors comprising nucleic acids encoding a heterologous protein antigen, a UL40 pro-
tein, and a US28 protein but that do not express an active UL128 and UL130 protein. Also disclosed are recombinant CMV vectors
comprising nucleic acids encoding a heterologous protein antigen, but that do not express an active UL40 protein, UL128 protein,
UL130 protein, and optionally a US28 protein. Also disclosed are recombinant CMV vectors comprising nucleic acids encoding a
heterologous protein antigen, but that do not express an active US28 protein, UL128 protein, UL130 protein, and optionally a UL40
protein.

## TITLE

METHODS AND COMPOSITIONS USEFUL IN GENERATING NON CANONICAL CD8+ T CELL
RESPONSES

## CROSS REFERENCE TO RELATED APPLICATIONS

The present application claims the priority benefit of U.S. Provisional Application No.
62/114,203, filed February 10, 2015; U.S. Provisional Application No. 62/196,520, filed July 24,
2015; and U.S. Provisional Application No. 62/220,703, filed September 18, 2015, each of which
are hereby incorporated by reference in their entirety.

## FIELD

Generally, the field is the use of CMV vectors in immunization. More specifically, the
field is the generation of CD8$^+$ immune responses characterized by non-canonical MHC
restriction.  Still more specifically, the field is the generation of T cells, including CD8$^+$ with
receptors that are restricted by MHC-E.

## ACKNOWLEDGEMENT OF GOVERNMENT SUPPORT

## BACKGROUND

Rhesus Cytomegalovirus (RhCMV) vaccine vectors expressing Simian Immunodeficiency
Virus (SIV) proteins (RhCMV/SIV) provide protection from pathogenic SIV (Hansen, S.G. *et al.*,
*Nat Med* 15, 293 (2009); Hansen, S.G. *et al.*, *Nature* 473, 523 (2011); both of which are
incorporated by reference herein). This protection is fundamentally distinct from other T cell
vaccines in its extreme efficacy and nearly instantaneous onset, with ~50% of vaccinnees
manifesting complete control of viral replication following a profoundly blunted and contracted

acute phase of viremia. Although RhCMV-protected macaques exhibited periodic low-level "blips" of viremia, CD4$^+$ memory T cell depletion was not observed, SIV-specific antibody responses did not develop, and subsequently, over time, viral nucleic acid became barely quantifiable while replication competent virus disappeared from the tissues of protected

5      animals. These events did not occur in spontaneous SIV elite controllers and DNA prime/Ad5 boost vaccinated controllers (Hansen, S.G. *et al.*, *Nature* 502, 100 (2013); incorporated by reference herein). Given the central role of RhCMV-induced CD8$^+$ T cells in mediating this protective effect in RhCMV/SIV-vaccinated macaques, defining the functional properties of these T cells is critical to understanding their mechanistic contribution to RhCMV/SIV vector-

10     induced control of SIV replication. Understanding these properties can in turn lead to new uses for cytomegalovirus (CMV) vaccine vectors expressing heterologous antigens.


**SUMMARY**

Disclosed herein is a method of generating an immune response to at least one

15     heterologous antigen in a subject. The method involves administering to the subject an effective amount of a CMV vector. The CMV vector comprises a first nucleic acid that encodes the at least one heterologous antigen, a second nucleic acid sequence that encodes at least one active UL40 protein, or a homolog or ortholog thereof, and a third nucleic acid sequence that encodes at least one US28 protein, or a homolog or ortholog thereof. The CMV vector does not

20     express an active UL128 protein, or an ortholog thereof, and does not express an active UL130 protein, or an ortholog thereof, and at least 10% of the CD8$^+$ T cells generated by the vector are restricted by MHC-E or a homolog thereof. In some embodiments, the third nucleic acid sequence encodes two through five active US28 proteins, or homologs or orthologs thereof. The heterologous antigen can be any antigen, including a pathogen-specific antigen derived

25     from, for example, human immunodeficiency virus (HIV), simian immunodeficiency virus (SIV), herpes simplex virus, hepatitis B or C virus, papillomavirus, *Plasmodium* parasites, and *Mycobacterium tuberculosis*. In still further examples, the heterologous antigen can be a tumor antigen including, for example, a tumor antigen related to acute myelogenous leukemia,

chronic myelogenous leukemia, myelodysplastic syndrome, acute lymphoblastic leukemia, chronic lymphoblastic leukemia, non-Hodgkin's lymphoma, multiple myeloma, malignant melanoma, breast cancer, lung cancer, ovarian cancer, prostate cancer, pancreatic cancer, colon cancer, renal cell carcinoma (RCC), and germ cell tumors. In still further examples, the heterologous antigen can be a tissue-specific antigen or a host self-antigen including, for example, an antigen derived from the variable region of a T cell receptor (TCR), an antigen derived from the variable region of a B cell receptor, a sperm antigen, or an egg antigen. In still further examples, the vector does not encode (1) an active UL40 protein (or an ortholog thereof) and/or an active US28 protein (or an ortholog thereof), (2) an active UL128 protein (or an ortholog thereof), and (3) an active UL130 protein (or an ortholog thereof), giving rise to MHC-II "supertope" restricted CD8$^+$ T cells but not HLA-E restricted CD8$^+$ T cells.

Also disclosed herein is a human or animal cytomegalovirus vector that includes a first nucleic acid sequence that encodes (1) at least one heterologous protein antigen, (2) a second nucleic acid sequence that encodes at least one active UL40 protein, or a homolog or ortholog thereof, and (3) a third nucleic acid sequence that encodes at least one active US28 protein, or a homolog or ortholog thereof. The vector does not express active UL128 and UL130 proteins, or orthologs thereof. In some embodiments, the third nucleic acid sequence encodes two through five active US28 proteins, or homologs or orthologs thereof.

Also disclosed is a human or animal cytomegalovirus vector that (1) does not express an active UL128 protein (or an ortholog thereof), (2) does not express an active UL130 protein (or an ortholog thereof), and (3) does not express an active UL40 protein (or an ortholog thereof) and/or an active US28 protein (or an ortholog thereof).

Also disclosed herein is a method of generating CD8$^+$ T cells that recognize MHC-E-peptide complexes. This method involves administering to a first subject a CMV vector that encodes (1) at least one heterologous antigen, (2) at least one active UL40 protein (or an ortholog or homolog thereof), and (3) at least one active US28 gene (or an ortholog or homolog thereof), in an amount effective to generate a set of CD8$^+$ T cells that recognize MHC-E/peptide complexes. The CMV vector does not encode active UL128 and UL130 proteins, or orthologs

thereof. In some embodiments, the CMV vector encodes two through five active US28 proteins or orthologs or homologs thereof. The heterologous antigen can be any antigen, including a pathogen-specific antigen, a tumor antigen, a self-antigen, or a tissue-specific antigen. In some embodiments, the self-antigen is an antigen derived from the variable region of a T or B cell receptor. In some embodiments, this method may further comprise identifying a first CD8$^+$ T cell receptor from the set of CD8$^+$ T cells, wherein the first CD8$^+$ TCR recognizes a MHC-E/heterologous antigen-derived peptide complex. In some embodiments, the first CD8$^+$ T cell receptor is identified by DNA or RNA sequencing. In some embodiments, this method may further comprise transfecting one or more T cells isolated from the first subject or a second subject with an expression vector, wherein the expression vector comprises a nucleic acid sequence encoding a second CD8$^+$ T cell receptor and a promoter operably linked to the nucleic acid sequence encoding the second CD8$^+$ T cell receptor, wherein the second CD8$^+$ T cell receptor comprises CDR3α and CDR3β of the first CD8$^+$ T cell receptor, thereby generating one or more transfected CD8$^+$ T cells that recognize a MHC-E/heterologous antigen-derived peptide complex. In some embodiments, this method may further comprise administering the transfected CD8$^+$ T cells to the first or second subject to treat a disease, such as cancer, a pathogenic infection, or an autoimmune disease or disorder. In some embodiments, this method may further comprise administering the transfected CD8$^+$ T cells to the first or second subject to induce an autoimmune response to a self-antigen or a tissue-specific antigen.

Also disclosed is a transfected CD8$^+$ T cell that recognizes MHC-E-peptide complexes prepared by a process comprising the steps of: (1) administering to a first subject a CMV vector in an amount effective to generate a set of CD8$^+$ T cells that recognize MHC-E/peptide complexes, wherein the CMV vector comprises a first nucleic acid sequence encoding at least one heterologous antigen, a second nucleic acid sequence encoding at least one active UL40 protein, or an ortholog or homolog thereof, and a third nucleic acid sequence encoding at least one active US28 protein, or an ortholog or homolog thereof, and wherein the CMV vector does not express active UL128 and UL130 proteins, or orthologs thereof; (2) identifying a first CD8$^+$ T cell receptor from the set of CD8$^+$ T cells, wherein the first CD8$^+$ T cell receptor recognizes a

MHC-E/heterologous antigen-derived peptide complex; (3) isolating one or more CD8⁺ T cells from the first subject or a second subject; and (4) transfecting the one or more CD8⁺ T cells isolated from the first or second subject with an expression vector, wherein the expression vector comprises a nucleic acid sequence encoding a second CD8⁺ T cell receptor and a promoter operably linked to the nucleic acid sequence encoding the second T cell receptor, wherein the second CD8⁺ T cell receptor comprises CDR3α and CDR3β of the first CD8⁺ T cell receptor, thereby creating a transfected T cell that recognizes MHC-E-peptide complexes. The heterologous antigen can be any antigen, including a pathogen-specific antigen or a tumor antigen. In some embodiments, the third nucleic acid sequence of the CMV vector encodes two through five active US28 proteins, or orthologs or homologs thereof. Also disclosed herein are methods of treating a disease, such as cancer, a pathogenic infection, or an autoimmune disease or disorder, the method comprising administering the transfected CD8⁺ T cell that recognizes MHC-E-peptide complexes to the first or second subject.


## BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWINGS

Some of the graphs and plots included herein may be better understood using color, which is not available in a patent application publication. Applicants consider all originally disclosed images and graphs (whether in color or not) part of the original disclosure and reserve the right to present color graphs and plots of the herein described figures in later proceedings.

Figure 1A is a set of flow cytometry plots of peripheral blood mononuclear cells (PBMCs) from a strain 68-1 RhCMV/gag-vaccinated macaque (either Rh22034 or Rh21826). As discussed in Example 1, RhCMV strain 68-1 does not express gene products from the Rh13, Rh60, Rh157.5 and 157.4 (HCMV RL11, UL36, UL128 and UL130, respectively) open reading frames. PBMCs were evaluated for peptide-specific CD8⁺ T cell recognition using flow cytometric intracellular cytokine staining (ICS) to detect IFN-γ and/or TNF-α production (response frequencies of CD8⁺ T cells shown in each quadrant) following incubation with the indicated antigen presenting cells that were pulsed with the peptide shown. The parental, MHC-I negative K562 cells were used as

negative controls and also transfected to express the MHC-I molecule indicated, while autologous B-lymphoblastoid cell lines (BLCL) were used as the positive control.

Figure 1B is a set of flow cytometry plots (left panel) and a bar graph (right panel) of CD8$^+$ T cells in PBMC from a strain 68-1 RhCMV/gag vector-vaccinated macaque (Rh22034 and Rh21826) showing IFN-γ and/or TNF-α production (response frequencies of CD8$^+$ T cells shown in each quadrant) following incubation with antigen presenting cells (autologous BLCL or K562 transfectant expressing only Mamu-E) that were pulsed with Gag$_{273-287}$ (SIVmac239 Gag 15-mer #69). The antigen presenting cells were incubated with the Gag 15-mer indicated along with either no additional peptide (no blocking) or in the presence of the Mamu-E binding peptide Rh67$_{8-16}$ VL9 (Rh67 VL9) or the Mamu-A*002:01 binding peptide Gag$_{71-79}$ GY9 (SIVgag GY9). The right panel is a comparison of peptide blocking conditions on IFN-γ and/or TNF-α production from CD8$^+$ T cells from four strain 68-1 RhCMV/gag vector-vaccinated macaques incubated with autologous BLCL or a Mamu-E transfectant pulsed with Gag$_{273-287}$ (SIVmac239 Gag 15-mer #69). Data are normalized to the response observed with no peptide blocking.

Figure 1C is a set of flow cytometry plots (left panel) and a bar graph (right panel) of CD8$^+$ T cells in PBMC from a strain 68-1 RhCMV/gag vector-vaccinated macaque (Rh22034 and Rh21826) showing IFN-γ and/or TNF-α production (response frequencies of CD8$^+$ T cells shown in each quadrant) following incubation with antigen presenting cells (autologous BLCL or K562 transfectant expressing only Mamu-E) that were pulsed with Gag$_{477-491}$ (SIVmac239 Gag 15-mer #120). The antigen presenting cells were incubated with the Gag 15-mer indicated along with either no additional peptide (no blocking) or in the presence of the Mamu-E binding peptide Rh67$_{8-16}$ VL9 (Rh67 VL9) or the Mamu-A*002:01 binding peptide Gag$_{71-79}$ GY9 (SIVgag GY9). The right panel is a comparison of peptide blocking conditions on IFN-γ and/or TNF-α production from CD8$^+$ T cells from four strain 68-1 RhCMV/gag vector-vaccinated macaques incubated with autologous BLCL or a Mamu-E transfectant pulsed with Gag$_{477-491}$ (SIVmac239 Gag 15-mer #120). Data are normalized to the response observed with no peptide blocking.

Figure 2A is a table illustrating the CD8$^+$ T cell responses to SIVmac239 Gag epitope-mapped using flow cytometric ICS to detect recognition of 125 consecutive 15mer Gag peptides

(with an 11 amino acid overlap) in macaques vaccinated with strain 68-1 RhCMV/gag vectors (n = 6), strain 68-1.2 RhCMV/gag vectors (n = 9), MVA/gag vectors (n = 7), and in SIVmac239 infected macaques (n = 8). As discussed in Example 1, expression of Rh60, Rh157.5, and Rh157.4 (HCMV UL36, UL128, and UL130, respectively) is restored in RhCMV strain 68-1.2.

5    Peptides resulting in above background CD8$^+$ T cell responses were subjected to MHC-I (mAb W6/32), MHC-E (Rh67 VL9), and MHC-II (mAb G46-6) blockade and classified as MHC-I blocked (boxes with white fill), fully MHC-E blocked (boxes with grey fill), partially MHC-E blocked (boxes with horizontal hatch fill), MHC-II blocked (boxes with black fill), or indeterminate (boxes with vertical hatch fill). The minimal number of independent MHC-E blocked epitopes potentially

10   contained within these reactive peptides in each macaque is designated at right (see Methods). Note that macaques 22063 and 22624 were vaccinated with BAC-derived RhCMV/gag while macaques 21826, 22034, 22436, and 22607 were vaccinated with non-BAC derived RhCMVgag(L).

Figure 2B is a set of flow cytometry plots of CD8$^+$ T cells in PBMC from a

15   MamuA1*001:01+ strain 68-1 RhCMV/gag vector-vaccinated macaque showing IFN-γ and/or TNF-α production (response frequencies of CD8$^+$ T cells shown in each quadrant) following incubation with antigen presenting cells (autologous BLCL or K562 transfectant expressing only MamuA1*001:01 or Mamu-E) that were pulsed with the Gag$_{69-83}$ (Gag #18) peptide alone (no blocking), or in the presence of MHC-E-binding Rh67$_{8-16}$ VL9 or Mamu-A*01-binding Gag$_{181-189}$

20   CM9 peptide.

Figure 2C is a set of flow cytometry plots of CD8$^+$ T cells in PBMC from a MamuA1*001:01- strain 68-1 RhCMV/gag vector-vaccinated macaque incubated with antigen presenting cells as described for Figure 2B.

Figure 3A is a set of two plots showing bulk surface MHC-I (measured by mAb W6/32)

25   on the surface of productively SIV-infected (CD4$^-$ Gag p27$^+$) or uninfected (CD4$^+$ Gagp27$^-$) CD4+ T cell targets. Representative flow cytometry plots are shown on the left panel while the right panel depicts the mean fluorescent intensity (MFI) of bulk MHC-I staining in SIV infected versus uninfected CD4$^+$ T cells derived from a total of 16 unrelated rhesus macaques.

Figure 3B is a set of two plots showing MHC-E (measured by mAb 4D12) on the surface of productively SIV-infected (CD4$^-$ Gag p27$^+$) or uninfected (CD4$^+$ Gagp27$^-$) CD4$^+$ T cell targets. Representative flow cytometry plots are shown on the left panel while the right panel depicts the MFI of MHC-E staining in SIV infected versus uninfected CD4$^+$ T cells derived from a total of 16 unrelated rhesus macaques.

Figure 3C is a plot showing the phenotype of MHC-E restricted CD8$^+$ T cells responding to Gag$_{273-287}$ (69) or Gag$_{477-491}$ (120) peptide stimulation. Percentages were calculated by examining the number of IFN-γ and/or TNF-α producing cells expressing each marker.

Figure 4A is a set of representative flow cytometry plots of CD8$^+$ T cells isolated from macaques vaccinated with either strain 68-1 RhCMV/gag, MVA/gag, strain 68-1.2 RhCMV/gag, or infected with SIV, showing IFN-γ and/or TNF-α production from CD8$^+$ T cells following incubation with autologous SIVmac239-infected CD4$^+$ T cells alone (no block), or in the presence of the MHC-II binding Class II-associated invariant chain peptide (CLIP) plus the pan-MHC-I blocking mAb W6/32 (W6/32 + CLIP), or Rh67$_{8-16}$ VL9 plus CLIP (VL9 + CLIP).

Figure 4B is a bar graph of a comparison of the normalized response frequencies for the recognition and blocking experiments represented in Fig. 4A for CD8$^+$ T cells from macaques vaccinated with strain 68-1 RhCMV/gag (n = 5), MVA/gag (n = 6), strain 68-1.2 RhCMV/gag (n = 4), or infected with SIV (n = 6).

Figure 4C is a set of flow cytometry plots illustrating the recognition of SIV-infected cells by CD8$^+$ T cell lines (CL) specific for either the MHC-E restricted Gag$_{477-491}$ Gag #120 epitope (top row) or the Mamu-A*001:01 restricted Gag$_{181-189}$ CM9 epitope (bottom row). CLs were incubated with uninfected or SIV-infected CD4+ T cells (from Rh22607) in the presence of the blocking conditions indicated.

Figure 5 (left panel) shows the percentage of CD8$^+$ T cells in PBMC from a rhesus macaque inoculated with a Rh67 (UL40)-deleted 68-1 RhCMV expressing SIVgag showing IFN-γ and/or TNF-α production following incubation with overlapping peptides corresponding to SIVgag at the indicated time points. The central panel shows that CD8$^+$ T cells in PBMC from the same animal do not respond to the Mamu-E-restricted peptides Gag$_{273-287}$ (Gag69) or Gag$_{477-491}$

(Gag120). The right panel shows the percentage of CD8$^+$ T cells in PBMC from the same animal responding to MHC-II restricted peptides (Gag53 and Gag73). The MHC-II peptides correspond to so-called supertopes, *i.e.* these peptides are presented by many different MHC-II alleles and hence elicit responses in most animals.

5         Figure 6 is a table illustrating the CD8$^+$ T cell responses to SIVmac239 Gag epitope-mapped using flow cytometric ICS to detect recognition of 125 consecutive 15mer Gag peptides (with an 11 amino acid overlap) in macaques vaccinated with strain 68-1 RhCMV/gag vectors (n = 3) lacking Rh67. Peptides resulting in above background CD8$^+$ T cell responses were subjected to MHC-I (mAb W6/32), MHC-E (Rh67 VL9), and MHC-II (mAb G46-6) blockade and classified as

10    MHC-I blocked (boxes with white fill), MHC-E blocked (boxes with grey fill), and MHC-II blocked (boxes with black fill). Note that all peptides are restricted by MHC-II demonstrating the need for Rh67 to elicit HLA-E specific CD8$^+$ T cell responses.

Figure 7A is a set of plots showing surface staining of MHC-II, MHC-Ia, MHC-E, or MHC-F by cell lines transfected with single Mamu-D molecules.

15    Figure 7B is a table showing genotyping of the indicated rhesus macaque (RM) individuals. Individuals were *Mamu-A, -B, and –E* genotyped by Roche/454 pyrosequencing. Grey shading indicates alleles selected for MHC-I transfectant generation. Where multiple alleles are listed, the bolded allele was produced.

Figure 7C is a set of two plots wherein one *MHC-Ia* or *MHC-Ib* allele was transfected into

20    a parental (MHC-I negative) cell line (.221 cells or K562, respectively). Cells were stained with a cross-reactive human MHC-I monoclonal antibody (W6/32) for 15 minutes at room temperature to assess MHC-I expression. Cells were washed once with 1X PBS supplemented with 10% fetal bovine serum, fixed with 2% paraformaldehyde, collected on a LSRII flow cytometer, and analyzed with FlowJo. MHC-I-expressing B-lymphoblastoid cells (BLCL) served as

25    a positive control, while the MHC-I negative parental cell lines were used as a negative control.

Figure 8A is a set of plots showing representative flow data of a restriction assay from Rh22607 for Gag 120.

Figure 8B is a table showing PBMC from the 4 indicated RM (#s 21826, 22436, 22034, and 22607; *Mamu-I* alleles shown in Fig. 7B) were incubated with autologous B lymphoblastoid cells (BLCL), MHC-I-null .221 or K562 cells, or the indicated single Mamu-I transfectants pulsed with the indicated SIVgag peptides and were then analyzed for $CD8^+$ T cell responses by flow cytometric ICS (see Fig. 1). Beginning in the second column, combinations that resulted in $CD8^+$ T cell responses above background (no peptide) are indicated by + signs (grey boxes); combinations that did not result in $CD8^+$ T cell responses above background are indicated by - signs (open boxes). In the first column, *Mamu-I* alleles that are expressed in each RM are indicated in grey boxes; non-expressed alleles are shown in open boxes.

Figure 9 is a set of flow cytometry plots of MHC-I vs. MHC-E blockade studies. Representative flow cytometry plots of $CD8^+$ T cells in PBMC from (left) a strain 68-1 RhCMV/gag vector-vaccinated macaque, or (B) a strain 68-1.2 RhCMV/gag vector-vaccinated macaque showing IFN-γ and/or TNF-α production (response frequencies of $CD8^+$ T cells shown in each quadrant) following incubation with the Gag 15-mer peptide indicated on top and the blocking condition indicated at left.

Figure 10A is a set of flow cytometry plots showing PBMC from strain 68-1 RhCMV/gag vector-vaccinated macaques were stimulated with $Gag_{273-287}$ (SIVmac239 Gag 15-mer #69) and flow cytometric ICS was performed. $CD8^+$ T cells responding to these MHC-E bound Gag peptides were identified via IFN-γ and TNF-α and then compared against the remaining cells in PBMC for expression of the markers indicated. Numbers in black indicate the overall percentage of cells in PBMC that are positive for the marker indicated, while the numbers in gray indicate the percentage of IFN-γ and TNF-α producing cells that are positive.

Figure 10B is a set of flow cytometry plots showing PBMC from strain 68-1 RhCMV/gag vector-vaccinated macaques were stimulated with $Gag_{477-491}$ (SIVmac239 Gag 15-mer #120) and flow cytometric ICS was performed. $CD8^+$ T cells responding to these MHC-E bound Gag peptides were identified via IFN-γ and TNF-α and then compared against the remaining cells in PBMC for expression of the markers indicated. Numbers in black indicate the overall

percentage of cells in PBMC that are positive for the marker indicated, while the numbers in gray indicate the percentage of IFN-γ and TNF-α producing cells that are positive.

Figure 11 collectively shows MHC restriction of strain 68-1 RhCMV/SIVgag-elicited CD8^{+} T cells.

5      Figure 11A is a set of plots showing results from flow cytometric intra-cellular cytokine staining (ICS) analysis of PBMC from a representative strain 68-1 RhCMV/SIVgag-vaccinated macaque (Rh22034; of 4 similarly analyzed). PBMC from vaccinated macaques were stimulated with the indicated 15mer peptide epitopes pulsed onto the surface of the indicated MHC-I transfectants or control cells with CD8^{+} T cell recognition determined by detection of IFN-γ
10     and/or TNF-α production by flow cytometric ICS assay (response frequencies of gated CD8^{+} T cells shown in each quadrant). The parental MHC-I-negative .221 and K562 cells were used as negative controls, while autologous B-lymphoblastoid cells (BLCL) were used as the positive control. The MHC-I molecules tested included both those expressed by Rh22034.

Figure 11B is a set of plots showing results from flow cytometric ICS analysis of
15     additional macaque and human MHC-E molecules not expressed by Rh22034 similar to that of Figure 11A.

Figure 11C is a set of plots showing phenotypic analysis of PBMC from RM treated with the same strain 68-1 RhCMV/SIVgag vector-vaccinated macaque as shown above (representative of 4 similarly analyzed) were stimulated with autologous BLCL pulsed with
20     either SIVgag$_{273-287}$(69) or SIVgag$_{477-491}$(120), and responding CD3^{+} lymphocytes (IFN-γ and TNF-α-producing; gate shown in left plot) were phenotyped by flow cytometric ICS assay with responding cells and non-responding cells within the designated gates indicated in grey and black, respectively, in each plot (and their relative % within the rectangular regions shown in each plot indicated in the same colors).

25     Figure 11D is a set of plots of the results where single MHC-E transfectants were pre-incubated with canonical MHC-E-binding peptide VMAPRTLLL (VL9) or a control non-MHC-E binding peptide (SIVgag GY9) prior to pulsing with the indicated SIVgag 15mer peptide epitope. Flow cytometric ICS assays were conducted as described above using PBMC from strain 68-1

RhCMV/SIVgag-vaccinated macaques, and the following MHC-E transfectants: Mamu-E*02:04 for SIVgag$_{273-287}$(69), SIVgag$_{385-399}$(97), and SIVgag$_{433-447}$(109) and Mamu-E*02:11 for SIVgag$_{257-271}$(65) and SIVgag$_{477-491}$(120).

Figure 12 collectively shows that MHC-E restriction is limited to CD8$^+$ T cell responses elicited by ΔRh157.5/.4 RhCMV vectors.

Figure 12A is a table showing that CD8$^+$ T cell responses to SIVgag were epitope-mapped using flow cytometric ICS to detect recognition of 125 consecutive 15mer gag peptides (with an 11 amino acid overlap) in macaques vaccinated with the indicated SIVgag expressing viral vectors or infected with SIVmac239 itself (n = 6 per group shown). Peptides resulting in above background CD8$^+$ T cell responses are indicated by a box, with the fill of the box designating MHC restriction as determined by blocking with the anti-pan-MHC-I mAb W6-32, the MHC-E blocking peptide VL9 and the MHC-II blocking peptide CLIP. MHC-Ia-, MHC-E-, and MHC-II-restriction was based on >90% response blocking by W6-32 alone (boxes with white fill), W6-32 and VL9 alone (boxes with grey fill), and CLIP alone (boxes with black fill), respectively, with responses not meeting these criteria labeled indeterminate (boxes with vertical hatch fill). The minimal number of independent epitopes in these MHC restriction categories is shown at right for each macaque.

Figure 12B is a table showing CD8$^+$ T cell responses to SIVpol and the *M. tuberculosis* proteins Ag85B, ESAT-6, and RpfA epitope-mapped as described above in macaques vaccinated with strain 68-1 RhCMV vectors expressing these proteins.

Figure 12C is a set of plots (right), another set of plots (middle), and a bar graph (right) showing that analysis of SIV-infected CD4$^+$ cell recognition by CD8$^+$ cells isolated from macaques vaccinated with strain 68-1 RhCMV/gag, MVA/gag, strain 68-1.2 RhCMV/gag vectors, or infected with SIV. The flow profiles at left show IFN-γ and TNF-α production following CD8$^+$ T cell incubation with autologous SIVmac239-infected CD4$^+$ T cells alone (no block), or in the presence of the pan-MHC-I-blocking mAb W6/32 plus the MHC-II-binding CLIP peptide (anti-MHC-I + CLIP), or MHC-E-binding peptide VL9 plus CLIP (VL9 + CLIP). All plots are gated on live, CD3$^+$, CD8$^+$ cells. The bar graph at right shows the results from all studied macaques.

Figure 13A is a plot showing a comparison of the total number of distinct MHC E- (grey) vs. MHC-Ia (black)-restricted SIVgag epitopes recognized by circulating CD8$^+$ T cells in individual macaques vaccinated with strain 68-1 RhCMV/gag vs. conventional viral vectors, the latter including MVA/gag (n = 11), Ad5/gag (n = 3) and electroporated DNA/gag + IL-12 (n = 4), or in macaques with controlled SIVmac239 infection (plasma viral load <10,000 copies/ml; n = 12). The horizontal bars indicate median values.

Figure 13B is a plot showing a comparison of the density (epitope number per 100 amino acids of protein length) of MHC E-restricted epitopes recognized by circulating CD8$^+$ T cells in individual macaques vaccinated with strain 68-1 RhCMV vectors expressing each of the indicated antigens (note: RhCMV IE1 responses were evaluated in CMV naïve macaques administered 68-1 RhCMV/gag). The horizontal bars indicate median values for each group.

Figure 13C is a bar graph of an analysis of the breadth of MHC-E-restricted SIVgag epitope-specific CD8$^+$ T cell responses across 125 overlapping (11 amino acid overlap), consecutive SIVgag 15mer peptides in 42 strain 68-1 RhCMV/gag vector-vaccinated macaques. Note that 109/125 15mers (87%) were recognized by MHC-E-restricted CD8$^+$ T cells in at least 1 macaque.

Figure 13D (left) is a sequence LOGO indicating the frequency of each amino acid in a given position (relative to their background frequency in SIVmac239 Gag; see methods) by the height of the letter, based on 11 optimal, MHC-E-restricted SIVgag 9mer peptide epitopes recognized by CD8+ T cells in strain 68-1 RhCMV vector-vaccinated macaques. The sequence LOGO is colored according to enrichment (letters with grey fill or hatched letters) or underrepresentation (letters with white fill) among 551 peptides eluted from HLA-E in a TAP-deficient setting by Lampen MH *et al.*, Mol Immunol 53, 126-131 (2013); incorporated by reference herein. Amino acids enriched in the 2$^{nd}$ and C-terminal anchor positions among the 551 Lampen *et al.* peptides were rare among our 11 optimal SIVgag peptides (right), while those that were significantly underrepresented were enriched. The percentage of strain 68-1 RhCMV/gag-vaccinated macaques that responded to each optimal peptide is noted as the "Recognition Frequency".

Figure 14 is a plot showing that the $SIVgag_{276\text{-}284}$ and $SIVgag_{482\text{-}490}$ epitopes are recognized by $CD8^+$ T cells in all strain 68-1 RhCMV/gag-vaccinated rhesus macaques. The $CD8^+$ T cell response to the indicated SIVgag 9mer peptides was determined in 120 strain 68-1 RhCMV/gag-vaccinated RM using flow cytometric ICS, using peptide-specific induction of TNF-α and/or IFN-γ within $CD3^+/CD8^+$ T cells as the response read-out. All macaques manifested detectable responses to these supertopic epitopes after background subtraction. The response frequencies shown have been memory-corrected to reflect the frequency of epitope-responding cells with the $CD8^+$, $CD95^{high}$ memory subset. Horizontal bars indicate median values.

Figure 15 collectively shows the validation of transfected cell lines expressing single MHC-I molecules corresponding to MHC-I molecules expressed by 4 strain 68-1 RhCMV/SIVgag-vaccinated macaques.

Figure 15A is a table showing the results where four strain 68-1 RhCMV/SIVgag-vaccinated macaques were Mamu-A, -B, and -E genotyped by Roche/454 pyrosequencing. Grey shading indicates alleles selected for MHC-I transfectant generation. Where multiple alleles are listed, a transfectant expressing the bolded allomorph was produced.

Figure 15B is a set of two plots showing expression of single MHC-I molecules. MHC-Ia or MHC-Ib alleles were transfected into a parental (MHC-I negative) cell line (.221 cells or K562 cells) and stained with pan-MHC-I monoclonal antibody (W6/32). MHC-I-expressing B-lymphoblastoid cells (BLCL) served as a positive control, while the MHC-I-negative parental cell lines were used as negative controls.

Figure 16A and 16B collectively show a comprehensive analysis of the MHC-Ia and MHC-Ib specificity of RhCMV/SIVgag-induced $CD8^+$ T cell response in 4 macaques.

Figure 16A is a set of plots showing representative flow cytometric ICS profiles of MHC restriction analysis of the $SIVgag_{433\text{-}447}$(109) response using PBMC from Rh22034. The TNF-α vs. IFN-γ flow profiles shown were gated on $CD3^+$, $CD8^+$ lymphocytes, with the fraction of cells in each quadrant indicated in the figure.

Figure 16B is a table showing PBMC from the 4 indicated macaques (MHC-typing shown in Fig. 15A) that were incubated with autologous B-lymphoblastoid cells (BLCL), MHC-I-negative .221 or K562 cells, or single MHC-I transfectants pulsed (and washed) with the indicated SIVgag peptides, and were then analyzed for CD8$^+$ T cell responses by flow cytometric ICS. Beginning in the second column, combinations that resulted in CD8$^+$ T cell responses above background (no peptide) are indicated by + signs (grey boxes); combinations that did not result in CD8$^+$ T cell responses above background are indicated by - signs (open boxes). In the first column, MHC-I alleles expressed in each RM are indicated in grey boxes; non-expressed alleles are shown in open boxes (expression of Mamu-F*01:01 unknown).

Figure 17 is a table showing that classical MHC-Ia allomorphs capable of presenting SIVgag peptides to strain 68-1 RhCMV/SIVgag-elicited CD8$^+$ T cells are not the restricting MHC alleles for these T cell responses. A cohort of 20 strain 68-1 RhCMV/SIVgag vector-vaccinated macaques were MHC-typed for the presence of Mamu-A1*001:01 and –A1*002:01 and tested for CD8$^+$ T cell responses specific for SIVgag$_{69-83}$(18), SIVgag$_{129-143}$(33), and SIVgag$_{197-211}$(50). Note that the detection of CD8$^+$ T cells specific for these three epitopes in strain 68-1 RhCMV/gag vector-vaccinated macaques is independent of the presence of Mamu-A1*001:01 or –A1*002:01 in the vaccinated animal.

Figures 18A and 18B collectively show that strain 68-1 RhCMV/SIVgag-elicited CD8$^+$ T cells recognize peptide in the context of both rhesus macaque and human MHC-E molecules.

Figure 18A is a set of plots showing PBMCs from strain 68-1 RhCMV/SIVgag vector-vaccinated macaques [Rh21826: SIVgag$_{89-103}$(23), SIVgag$_{129-143}$(33), SIVgag$_{257-271}$(65), SIVgag$_{473-487}$(119); Rh22034: SIVgag$_{61-75}$(16), SIVgag$_{69-83}$(18), SIVgag$_{271-287}$(69), SIVgag$_{385-399}$(97), SIVgag$_{477-491}$(120); Rh22436: SIVgag$_{197-211}$(30), SIVgag$_{197-211}$(50)] were evaluated for peptide-specific CD8$^+$ T cell recognition using flow cytometric ICS to detect IFN-$\gamma$ and/or TNF-$\alpha$ production (response frequencies of CD8$^+$ T cells shown in each quadrant) following incubation with the indicated Gag 15-mer peptides pulsed (and washed) on the indicated MHC-E transfectants and control antigen presenting cells (see Fig. 11). Note that all 12 MHC-E-restricted 15mer peptide epitopes

can be effectively presented to strain 68-1 RhCMV/SIVgag vector-elicited CD8$^+$ T cells on both Mamu-E allomorphs and on HLA-E.

Figure 18B is an amino acid alignment of the α1 and α2 regions of human and rhesus macaque MHC-E molecules expressed by transfectants represented in Fig. 18A, with the key B and F pocket residues indicated with grey shading. All of the B and F pocket residues interacting with bound peptide are conserved between HLA-E*01:03, Mamu-E*02:04, and Mamu-E*02:11, while substitutions exist in these residues in Mamu-E*02:20, the most disparate of the MHC-E molecules studied here. Despite harboring substitutions in both B and F pocket residues compared to the other allomorphs, Mamu-E*02:20 is able to bind and present the identical peptides.

Figure 19 is a plot showing that strain 68-1 RhCMV/SIVgag-elicited, supertope-specific CD8+ T cells exhibit a conventional CD8αβ$^+$ T cell phenotype. The figure summarizes the phenotypic analysis of MHC-E-restricted CD8$_+$ T cells responding to SIVgag$_{273-287}$(69) or SIVgag$_{477-491}$(120) peptide stimulation in four 68-1 RhCMV/SIVgag-vaccinated macaques (Rh21826, Rh22034, Rh22436, Rh22607). The figure shows the percentages of peptide-responding CD3$^+$ T cells (IFN-γ$^+$ and TNF-α$^+$) that express the designated phenotypes (see flow cytometric profiles in Fig. 11C).

Figure 20A is a set of plots of single MHC-E transfectants pre-incubated with canonical MHC-E-binding peptide VMAPRTLLL (VL9) or control peptide prior to pulsing with the indicated SIVgag 15-mer peptide epitope. Flow cytometric ICS was conducted as described for Fig. 11 using PBMC from strain 68-1 RhCMV/SIVgag-vaccinated macaques: Rh21826 for SIVgag$_{89-103}$(23), SIVgag$_{129-143}$(33), SIVgag$_{197-211}$(50), and SIVgag$_{473-487}$(119) responses; Rh22034 for SIVgag$_{61-75}$(16) and SIVgag$_{69-83}$(18) responses; Rh22436 for the SIVgag$_{117-131}$(30) response. The following MHC-E transfectants were utilized: Mamu-E*02:04 for the SIVgag$_{69-83}$(18) and SIVgag$_{89-103}$(23) responses; Mamu-E*02:11 for the SIVgag$_{61-75}$(16), SIVgag$_{117-131}$(30), SIVgag$_{129-143}$(33), SIVgag$_{197-211}$(50), and SIVgag$_{473-487}$(119) responses. The following control peptides were utilized at a final concentration of 20μM: Mamu-A1*002:01-binding peptide SIVgag$_{71-79}$(GY9) for SIVgag$_{89-103}$(23), SIVgag$_{117-131}$(30), and SIVgag$_{129-143}$(33) responses, and the Mamu-

A1*001:01-binding peptide SIVgag$_{181-189}$(CM9) for the SIVgag$_{69-83}$(18), SIVgag$_{197-211}$(50), and SIVgag$_{473-487}$(119) responses. These data, along with the data in Fig. 11D, indicate that the VL9 peptide efficiently blocks CD8+ T cell recognition of 12 diverse MHC-E-presented 15mer peptide epitopes.

5          Figure 20B is a plot where the indicated antigen-presenting cells were pre-incubated with increasing concentrations of VL9 prior to pulse with the SIVgag$_{477-491}$(120) SIVgag 15-mer or optimal Mamu-A1*001:01-restricted Gag-CM9 or Tat-SL8 peptides. These antigen-presenting cells were then incubated with the indicated effectors for flow cytometric ICS analysis, as described for Fig. 20A. Rh22436 is a 68-1 RhCMV/SIVgag-vaccinated RM, while Rh27002 is SIV-

10      infected. Note that increasing concentrations of VL9 peptide progressively block the ability of MHC-E-expressing antigen-presenting cells to activate SIVgag$_{477-491}$(120)-specific CD8$^+$ T cells from a strain 68-1 RhCMV/gag vector-vaccinated macaque, but have no effect on conventionally MHC-Ia-restricted CD8+ T cells specific for Gag-CM9 or Tat-SL8.

          Figure 21 shows formal truncation analysis for 8 additional MHC-E-restricted 15 peptide

15      epitopes using peptide-specific CD8$^+$ T cell expression of TNF-α and/or IFN-γ by flow cytometric ICS as response readout. CD8$^+$ T cell responses to amino terminal and carboxy terminal truncations of the parent 15mer were initially determined to define optimal peptide length and the amino- and carboxy-termini of the core epitope (top panel, with grey shading indicating the terminal amino acids of the most stimulatory amino- and carboxy-terminal-truncated peptides).

20      The optimal 9mer implied by this truncation approach was then confirmed by analysis of the 7 consecutive 9mers that make up each 15mer (bottom panel). The 9mers shaded in grey in each of the bottom panels represent the optimal epitope for each parent 15mer.

          Figure 22A and 22B collectively show dose response of MHC-E-restricted CD8$^+$ T cells to optimal 9mers pulsed on human and rhesus macaques MHC-E transfectants. Mamu-E*02:04,

25      Mamu-E*02:20 and HLA-E*01:03 transfectants were pulsed with the indicated concentration of the optimal SIVgag 9mer peptide epitopes SIVgag$_{476-484}$, SIVgag$_{259-267}$, SIVgag$_{276-284}$, or SIVgag$_{482-490}$ (see fig. 21), washed, and combined with PBMC from 3-4 68-1 RhCMV/SIVgag-vaccinated

macaques for flow cytometric ICS determination of the frequency of responding $CD8^+$ T cells ($IFN-\gamma^+$ and/or $TNF-\alpha^+$).

Figure 22A is a set of plots showing a representative analysis of the dose response to $SIVgag_{476-484}$ in Rh22607.

Figure 22B is a set of plots showing the dose response (mean $\pm$ SEM response frequencies) for $CD8^+$ T cells responding to $SIVgag_{476-484}$, $SIVgag_{259-267}$, $SIVgag_{276-284}$, $SIVgag_{482-490}$ with response frequencies normalized to the response observed with the transfectant pulsed with 10 $\mu M$ peptide dose.

Figure 23 is a chart of genomic differences between RhCMV vector strains 68-1, 68-1.2 and ΔRh157.4/.5 68-1.2. In low passage isolates of RhCMV, the Rh157.5 (UL128), Rh157.4 (UL130) and Rh157.6 (UL131A) genes are encoded on the $2^{nd}$ strand in reverse orientation. During serial passage in tissue culture, RhCMV 68-1 acquired distinctive fibroblast adaptations. The Rh157.5 (UL128) ORF and most of exon 2 of the Rh157.4 (UL130) ORF were deleted and the adjacent genomic region inverted, resulting in loss of the pentameric receptor complex that mediates viral entry into non-fibroblasts. Fibroblast adaptation of strain 68-1 RhCMV also resulted in insertion of an additional thymidine in the Rh61/Rh60 (UL36) gene, resulting in a frame shift mutation and a premature stop codon. In RhCMV 68-1.2, a functional pentameric complex was restored by insertion of Rh157.5 (UL128) and exon 2 of Rh157.4 (UL130) from RhCMV strain 180.92 into RhCMV 68.1 right after the first exon of Rh157.4 (UL130), and the Rh61/Rh60 (UL36) mutation was reverted to wild type configuration. To ensure that the unconventional MHC restriction of $CD8^+$ T cells elicited by strain 68-1 RhCMV vectors was attributable to the Rh157.5/.4 (UL128/UL130) deletion (and consequent lack of a functional pentameric complex), Rh157.5 (UL128) and Rh157.4 (UL130) were specifically re-deleted from strain 68-1.2 by homologous recombination starting 50 bp upstream of the Rh 157.6 (UL131A) stop codon up to the Rh157.5 (UL128) stop codon, leaving the Rh61/Rh60 (UL36) repair intact. Therefore, phenotypic features shared between this ΔRh157.5/.4 (ΔUL128/UL130) strain 68-1.2 RhCMV vector and the original strain 68-1 vectors that differ from the repaired strain 68-1.2 RhCMV vector would be directly attributable to Rh157.5/.4 (UL128/UL130) deletion.

Figure 24 collectively shows differential utilization of MHC-E vs. MHC-Ia by CD8$^+$ T cells elicited by strain 68-1 vs. strain 68-1.2 RhCMV/gag vectors.

Figure 24A shows representative flow cytometric response profiles (IFN-γ vs. TNF-α on gated CD3$^+$, CD8$^+$ T cells) of MHC-I-dependent, SIVgag epitope-specific CD8$^+$ T cells elicited by the strain 68-1 (Rh157.4/.5-deleted) RhCMV/gag vectors, with and without blocking with the pan anti-MHC-I-blocking mAb W6-32 or the MHC-E-blocking VL9 peptide.

Figure 24B shows representative flow cytometric response profiles (IFN-γ vs. TNF-α on gated CD3$^+$, CD8$^+$ T cells) of MHC-I-dependent, SIVgag epitope-specific CD8$^+$ T cells elicited by the strain 68-1.2 (Rh157.4/.5-intact) RhCMV/gag vectors, with and without blocking with the pan anti-MHC-I-blocking mAb W6-32 or the MHC-E-blocking VL9 peptide (see Fig. 20). Note that the VL9 peptide only blocks all MHC-I-dependent responses elicited by the strain 68-1 RhCMV vector.

Figure 25 is a restriction analysis of epitope-specific CD8$^+$ T cell responses elicited by RhCMV/gag vectors (strains 68.1 and 68-1.2), MVA/gag vector, and by controlled SIV infection. As described for Fig. 12A, CD8$^+$ T cell responses to SIVgag were epitope-mapped using flow cytometric ICS to detect recognition of 125 consecutive 15mer gag peptides (with an 11 amino acid overlap) in additional macaques (over the 6 animals from each group shown in Fig. 12A) vaccinated with the indicated SIVgag expressing viral vectors or infected with SIVmac239 itself (SIVmac239 controller macaques). Peptides resulting in above background CD8$^+$ T cell responses are indicated by a box, with the fill of the box designating MHC restriction, as determined by blocking with the anti-pan-MHC-I mAb W6-32, the MHC-E blocking peptide VL9 and the MHC-II blocking peptide CLIP.  MHC-Ia-, MHC-E-, and MHC-II-restriction was based on >90% response blocking by W6-32 alone (boxes with white fill), W6-32 and VL9 alone (boxes with grey fill), and CLIP alone (boxes with black fill), respectively, with responses not meeting these criteria labeled indeterminate (boxes with vertical hatch fill). The minimal number of independent epitopes in these MHC restriction categories is shown at right for each macaque. Note that all evaluable epitopes recognized by CD8$^+$ T cells from strain 68-1 RhCMV/gag vector-vaccinated macaques were unconventionally restricted, either by MHC-II or MHC-E. In contrast,

all responses elicited by the strain 68-1.2 RhCMV/gag and MVA/gag vectors were conventionally MHC-Ia-restricted. The vast majority of SIVgag epitope-specific CD8$^+$ T cell responses identified in SIV controller macaques were also MHC-Ia-restricted, but 4 of 12 of these animals manifested one epitope-specific response that was unequivocally MHC-II-

5    restricted (4 responses out of 179 total responses = 2.2%), indicating the MHC-II-restricted CD8$^+$ T cells can be identified as a minor component of conventional immune responses to infection.

Figure 26 is a chart showing epitope mapping of CD8$^+$ T cell responses to the RhCMV Immediate Early-1 (IE1) protein in natural (wild type) RhCMV infection and both primary and secondary infection with the strain 68-1 RhCMV/gag vector. CD8$^+$ T cell responses to RhCMV IE1

10   were epitope-mapped using flow cytometric ICS to detect recognition of 137 consecutive 15mer IE1 peptides (with an 11 amino acid overlap) in 1) macaques that were naturally infected with wildtype (colony circulating) RhCMV (top panel), 2) RhCMV naïve macaques inoculated with the strain 68-1 RhCMV/gag vector (middle panel), and 3) naturally wild type RhCMV-infected macaques that were superinfected with the strain 68-1 RhCMV/gag vector (bottom

15   panel). Peptides resulting in above background CD8$^+$ T cell responses are indicated by a box, with the fill of the box designating MHC restriction as determined by blocking with the anti-pan-MHC-I mAb W6-32, the MHC E blocking peptide VL9 and the MHC-II blocking peptide CLIP. MHC-Ia-, MHC-E-, and MHC-II-restriction was based on >90% response blocking by W6-32 alone (boxes with white fill), W6-32 and VL9 alone (boxes with grey fill), and CLIP alone (boxes with

20   black fill), respectively, with responses not meeting these criteria labeled indeterminate (boxes with vertical hatch fill). The minimal number of independent epitopes in these MHC restriction categories is shown at right for each macaque. Note that the IE1 epitope-specific responses in the naturally infected macaques are entirely MHC Ia-restricted, whereas in the macaques solely infected with the strain 68-1 RhCMV/gag vector, these responses are broader and entirely

25   unconventionally restricted (an ~1:1 ratio of MHC-II- and MHC-E-restricted epitopes). The naturally RhCMV-infected macaques that were superinfected with the strain 68-1 RhCMV/gag vectors show the expected admixture of IE1-epitope-specific CD8$^+$ T cells that were conventionally (MHC-Ia) and unconventionally (MHC-II and MHC-E) restricted.

Figure 27A is a validation of the specificity of MHC-E-specific mAb 4D12 in rhesus macaques. Histograms showing surface staining of single MHC-Ia or MHC-Ib transfectants by the pan-MHC-I mAb W6/32 (top row) versus the MHC-E-specific mAb 4D12 (bottom row). Note that all Mamu-Ia and Mamu-E allomorphs were transfected into the murine cell line RMA-S, which expresses human β2-microglubulin. Macaque BLCL were used as a positive control, whereas the parent RMA-S cell line was used as a negative control (light gray histogram). Note the restriction of 4D12 reactivity to the Mamu-E transfectants.

Figure 27B shows the surface expression of total MHC-I as determined by staining with mAb W6/32),

Figure 27C shows the surface expression of total MHC-I, as determined by staining with mAb 4D12, on productively SIV-infected and uninfected $CD4^+$ T cells in the same cultures, with SIV-infected cells recognized by intracellular expression of Gag Ag and CD4 down-regulation ($Gag^+/CD4^{low}$), and uninfected cells recognized by lack of Gag reactivity and high levels of surface CD4 expression ($Gag^-/CD4^{high}$). The left panels show representative flow cytometric histograms. The right panels depict the MFI of total MHC-I or specific MHC-E staining in SIV infected versus uninfected $CD4^+$ T cells derived from a total of 16 unrelated macaques. P values were determined by the paired Student's T test.

Figure 28 is a population-level analysis of MHC-Ia-restricted $CD8^+$ T cell responses to SIVgag. Analysis of the breadth of conventionally MHC-Ia-restricted SIVgag epitope-specific $CD8^+$ T cell responses across 125 overlapping (11 amino acid), consecutive SIVgag 15mer peptides in 30 macaques vaccinated with conventional SIVgag-expression vaccines (11 MVA/gag, 3 Ad5/gag, 4 DNA/gag + IL-12) or infected with SIVmac239 (with plateau-phase viral loads <10,000 copies/ml; n = 12). The asterisk (*) indicates the Gag-45 15mer peptide which includes the Mamu-A1*001:01-restricted immunodominant $SIVgag_{181-189}$(CM9) epitope. Selection of monkeys for this cohort was largely unbiased with respect to MHC-Ia allomorphs, except for preferential selection of Mamu-A1*001:01 (expressed by 19 of the 30 macaques), accounting for the high frequency of monkeys responding to the Gag45 15mer. Except for the Gag45 peptide, with its artificially increased response frequency, the frequency of monkeys

with MHC-I-restricted CD8$^+$ T cells reactive to any of the other Gag 15mers is relatively low (only two 15mers with 40% recognition and none >40%), compared to the MHC-E-restricted CD8$^+$ T cell responses elicited by the strain 68-1 RhCMV/gag vector (19 epitopes with ≥40% recognition frequency, including 2 universal supertopes; Fig. 3C). However, all but one of the 125 consecutive SIVgag 15mers are recognized by MHC-Ia-restricted CD8$^+$ T cells in at least one macaque, and all but 13 SIVgag 15mers are targeted in 2 or more macaques. In contrast, the MHC-E-restricted CD8$^+$ T cells elicited in 42 macaques by the strain 68-1 RhCMV/gag vector failed to recognize 16 of 125 SIVgag 15mers. Thus, while the MHC-E-restricted CD8$^+$ T cell responses elicited by strain 68-1 RhCMV vectors are remarkably broad for a functionally monomorphic restricting element, they are not as broad as responses supported by an entire population of polymorphic MHC-Ia molecules, perhaps accounting for the evolutionary dominance of the MHC-Ia-restricted antigen presentation system.

Figure 29 is a set of three plots. The left panel shows the percentage of CD8$^+$ T cells in PBMC from a rhesus macaque inoculated with a Rh214 to Rh220-deleted 68-1RhCMV expressing SIVgag showing IFN-γ and/or TNF-α production following incubation with overlapping peptides corresponding to SIVgag at the indicated time points. The gene region Rh214 to Rh220 encodes five genes with homology to human cytomegalovirus (HCMV) US28: Rh214, Rh215, Rh216, Rh218, Rh220 (D. Malouli *et al.*, *J Virol* 86, 8959 (2012); incorporated by reference herein). The center panel shows that CD8$^+$ T cells in PBMC from the same animal do not respond to the Mamu-E-restricted peptides Gag$_{273-287}$ (Gag69) or Gag$_{477-491}$ (Gag120). The right panel shows the percentage of CD8$^+$ T cells in PBMC from the same animal responding to MHC-II restricted peptides (Gag53 and Gag73). The MHC-II peptides correspond to so-called supertopes, *i.e.* these peptides are presented by many different MHC-II alleles and hence elicit responses in most animals.

Figure 30 is a table illustrating the CD8$^+$ T cell responses to SIVmac239 Gag epitope-mapped using flow cytometric ICS to detect recognition of 125 consecutive 15mer Gag peptides (with an 11 amino acid overlap) in macaques vaccinated with strain 68-1 RhCMV/gag vectors (n = 3) lacking Rh214-220. Peptides resulting in above background CD8$^+$ T cell responses were

subjected to MHC-I (mAb W6/32), MHC-E (Rh67 VL9), and MHC-II (mAb G46-6) blockade and classified as MHC-I blocked (boxes with white fill), MHC-E blocked (boxes with grey fill), MHC-II blocked (boxes with black fill), or indeterminate (boxes with hatch fill). Note that all peptides are restricted by MHC-II demonstrating the need for Rh214-220 to elicit HLA-E specific CD8$^+$ T

5    cell responses.


## DETAILED DESCRIPTION

The present invention provides novel recombinant CMV vectors including, but not limited to, recombinant CMV vectors comprising nucleic acids encoding at least one

10   heterologous protein antigen, at least one active UL40 protein, and at least one active US28 protein, but that do not express active UL128 and UL130 proteins. The present invention also provides recombinant CMV vectors including, but not limited to, recombinant CMV vectors comprising nucleic acids encoding at least one heterologous antigen, but that do not express (1) an active UL40 protein and/or an active US28 protein, (2) an active UL128 protein, and (3) an

15   active UL130 protein. Methods of using the novel recombinant CMV vectors, such as methods of generating an immune response to at least one heterologous antigen in a subject, methods of generating CD8$^+$ T cells that recognize MHC-E-peptide complexes, and methods of treating disease, are further provided.


20   I.      Definitions

Unless otherwise noted, technical terms are used according to conventional usage. Definitions of common terms in molecular biology may be found in Benjamin Lewin, Genes V, published by Oxford University Press, 1994 (ISBN 0-19-854287-9); Kendrew et al. (eds.), The Encyclopedia of Molecular Biology, published by Blackwell Science Ltd., 1994 (ISBN 0-632-

25   02182-9); and Robert A. Meyers (ed.), Molecular Biology and Biotechnology: a Comprehensive Desk Reference, published by VCR Publishers, Inc., 1995 (ISBN 1-56081-569-8).

All publications, patents, patent applications, internet sites, and accession numbers/database sequences (including both polynucleotide and polypeptide sequences) cited

herein are hereby incorporated by reference in their entirety for all purposes to the same extent as if each individual publication, patent, patent application, internet site, or accession number/database sequence were specifically and individually indicated to be so incorporated by reference.

5        Unless otherwise explained, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs. The singular terms "a," "an," and "the" include plural referents unless context clearly indicates otherwise. Similarly, the word "or" is intended to include "and" unless the context clearly indicates otherwise. It is further to be understood that all base sizes or amino acid sizes,

10      and all molecular weight or molecular mass values, given for nucleic acids or polypeptides are approximate, and are provided for description. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of this disclosure, suitable methods and materials are described below. The term "comprises" means "includes."

In addition, the materials, methods, and examples are illustrative only and not intended to be

15      limiting. In order to facilitate review of the various embodiments of the disclosure, the following explanations of specific terms are provided:

        Antigen: As used herein, the terms "antigen" or "immunogen" are used interchangeably to refer to a substance, typically a protein, which is capable of inducing an immune response in a subject. The term also refers to proteins that are immunologically active in the sense that

20      once administered to a subject (either directly or by administering to the subject a nucleotide sequence or vector that encodes the protein) is able to evoke an immune response of the humoral and/or cellular type directed against that protein.

        Administration: To provide or give a subject an agent, such as a composition comprising an effective amount of an HCMV vector comprising an exogenous antigen by any effective

25      route. Exemplary routes of administration include, but are not limited to, injection (such as subcutaneous, intramuscular, intradermal, intraperitoneal, and intravenous), oral, sublingual, rectal, transdermal, intranasal, vaginal and inhalation routes.

Cancer: A disease or condition in which abnormal cells divide without control and are able to invade other tissues. Cancer cells may spread to other body parts through the blood and lymphatic systems. Cancer is a term for many diseases. There are more than 100 different types of cancer in humans. Most cancers are named after the organ in which they originate. For instance, a cancer that begins in the colon may be called a colon cancer. However, the characteristics of a cancer, especially with regard to the sensitivity of the cancer to therapeutic compounds, are not limited to the organ in which the cancer originates. A cancer cell is any cell derived from any cancer, whether in vitro or in vivo.

Cancer also includes malignant tumors characterized by abnormal or uncontrolled cell growth. Other features often associated with cancer include metastasis, interference with the normal functioning of neighboring cells, release of cytokines or other secretory products at abnormal levels and suppression or aggravation of inflammatory or immunological response, invasion of surrounding or distant tissues or organs, such as lymph nodes, etc.

"Metastatic disease" or "metastasis" refers to cancer cells that have left the original tumor site and migrate to other parts of the body for example via the bloodstream or lymph system. The "pathology" of cancer includes all phenomena that compromise the wellbeing of the subject. This includes, without limitation, abnormal or uncontrollable cell growth, metastasis, interference with the normal functioning of neighboring cells, release of cytokines or other secretory products at abnormal levels, suppression or aggravation of inflammatory or immunological response, neoplasia, premalignancy, malignancy, invasion of surrounding or distant tissues or organs, such as lymph nodes, etc.

Effective amount: As used herein, the term "effective amount" refers to an amount of an agent, such as a CMV vector comprising a heterologous antigen or a transfected CD8+ T cell that recognizes a MHC-E/heterologous antigen-derived peptide complex, that is sufficient to generate a desired response, such as reduce or eliminate a sign or symptom of a condition or disease or induce an immune response to an antigen. In some examples, an "effective amount" is one that treats (including prophylaxis) one or more symptoms and/or underlying causes of any of a disorder or disease. An effective amount can be a therapeutically effective amount,

including an amount that prevents one or more signs or symptoms of a particular disease or condition from developing, such as one or more signs or symptoms associated with infectious disease, cancer, or autoimmune disease.

Mutation: A mutation is any difference in a nucleic acid or polypeptide sequence from a normal, consensus or "wild type" sequence. A mutant is any protein or nucleic acid sequence comprising a mutation. In addition a cell or an organism with a mutation may also be referred to as a mutant.

Some types of coding sequence mutations include point mutations (differences in individual nucleotides or amino acids); silent mutations (differences in nucleotides that do not result in an amino acid changes); deletions (differences in which one or more nucleotides or amino acids are missing, up to and including a deletion of the entire coding sequence of a gene); frameshift mutations (differences in which deletion of a number of nucleotides indivisible by 3 results in an alteration of the amino acid sequence. A mutation that results in a difference in an amino acid may also be called an amino acid substitution mutation. Amino acid substitution mutations may be described by the amino acid change relative to wild type at a particular position in the amino acid sequence.

As used herein, an "inactivating mutation" is any mutation in a viral gene which finally leads to a reduced function or to a complete loss of function of the viral protein.

Nucleotide sequences or nucleic acid sequences: The terms "nucleotide sequences" and "nucleic acid sequences" refer to deoxyribonucleic acid (DNA) or ribonucleic acid (RNA) sequences, including, without limitation, messenger RNA (mRNA), DNA/RNA hybrids, or synthetic nucleic acids. The nucleic acid can be single-stranded, or partially or completely double stranded (duplex). Duplex nucleic acids can be homoduplex or heteroduplex.

Recombinant: A recombinant nucleic acid or polypeptide is one that has a sequence that is not naturally occurring or has a sequence that is made by an artificial combination of two or more otherwise separated segments of sequence, for example a CMV vector comprising a heterologous antigen and/or made replication deficient by the mutation of one or more genes. This artificial combination is often accomplished by chemical synthesis or, more commonly, by

the artificial manipulation of isolated segments of nucleic acids, *e.g.*, by genetic engineering techniques. A recombinant polypeptide can also refer to a polypeptide that has been made using recombinant nucleic acids, including recombinant nucleic acids transferred to a host organism that is not the natural source of the polypeptide (for example, nucleic acids encoding

5      polypeptides that form a CMV vector comprising a heterologous antigen).

Replication-deficient: As used herein, a replication deficient CMV is a virus that once in a host cell, cannot undergo viral replication, or is significantly limited in its ability to replicate its genome and thus produce virions. In other examples, replication-deficient viruses are dissemination-deficient, i.e. they are capable of replicating their genomes, but unable to infect

10     another cell either because virus particles are not released from the infected cell or because non-infectious viral particles are released. In other examples, replication-deficient viruses are spread-deficient, i.e. infectious virus is not secreted from the infected host are therefore the virus is unable to spread from host to host. In some embodiments, a replication-deficient CMV is a CMV comprising a mutation that results in a lack of expression of one or more genes

15     essential for viral replication ("essential genes") or required for optimal replication ("augmenting genes"). CMV essential and augmenting genes have been described in the art (in particular US 2013/0136768, which is incorporated by reference herein) and are disclosed herein.

Pharmaceutically acceptable carriers: The pharmaceutically acceptable carriers of use

20     are conventional. Remington's Pharmaceutical Sciences, by E.W. Martin, Mack Publishing Co., Easton, PA, 19th Edition, 1995, describes compositions and formulations suitable for pharmaceutical delivery of the compositions disclosed herein. In general, the nature of the carrier will depend on the particular mode of administration being employed. For instance, parenteral formulations usually comprise injectable fluids that include pharmaceutically and

25     physiologically acceptable fluids such as water, physiological saline, balanced salt solutions, aqueous dextrose, glycerol or the like as a vehicle. For solid compositions (such as powder, pill, tablet, or capsule forms), conventional non-toxic solid carriers can include, for example, pharmaceutical grades of mannitol, lactose, starch, or magnesium stearate. In addition to

biologically neutral carriers, pharmaceutical compositions to be administered can contain minor amounts of non-toxic auxiliary substances, such as wetting or emulsifying agents, preservatives, and pH buffering agents and the like, for example sodium acetate or sorbitan monolaurate.

Polynucleotide: As used herein, the term "polynucleotide" refers to a polymer of ribonucleic acid (RNA) or deoxyribonucleic acid (DNA). A polynucleotide is made up of four bases; adenine, cytosine, guanine, and thymine/uracil (uracil is used in RNA). A coding sequence from a nucleic acid is indicative of the sequence of the protein encoded by the nucleic acid.

Polypeptide: The terms "protein", "peptide", "polypeptide", and "amino acid sequence" are used interchangeably herein to refer to polymers of amino acid residues of any length. The polymer can be linear or branched, it may comprise modified amino acids or amino acid analogs, and it may be interrupted by chemical moieties other than amino acids. The terms also encompass an amino acid polymer that has been modified naturally or by intervention; for example disulfide bond formation, glycosylation, lipidation, acetylation, phosphorylation, or any other manipulation or modification, such as conjugation with a labeling or bioactive component.

Sequence identity/similarity: The identity/similarity between two or more nucleic acid sequences, or two or more amino acid sequences, is expressed in terms of the identity or similarity between the sequences. Sequence identity can be measured in terms of percentage identity; the higher the percentage, the more identical the sequences are. Sequence similarity can be measured in terms of percentage identity or similarity (which takes into account conservative amino acid substitutions); the higher the percentage, the more similar the sequences are. Polypeptides or protein domains thereof that have a significant amount of sequence identity and also function the same or similarly to one another (for example, proteins that serve the same functions in different species or mutant forms of a protein that do not change the function of the protein or the magnitude thereof) can be called "homologs."

Methods of alignment of sequences for comparison are well known in the art. Various programs and alignment algorithms are described in: Smith & Waterman, *Adv Appl Math* 2,

482 (1981); Needleman & Wunsch, *J Mol Biol* 48, 443 (1970); Pearson & Lipman, *Proc Natl Acad Sci USA* 85, 2444 (1988); Higgins & Sharp, *Gene* 73, 237-244 (1988); Higgins & Sharp, *CABIOS* 5, 151-153 (1989); Corpet *et al.*, *Nuc Acids Res* 16, 10881-10890 (1988); Huang *et al*, *Computer App Biosci* 8, 155-165 (1992); and Pearson *et al*, *Meth Mol Bio* 24, 307-331 (1994). In

5    addition, Altschul *et al.*, J Mol Biol 215, 403-410 (1990), presents a detailed consideration of sequence alignment methods and homology calculations.

The NCBI Basic Local Alignment Search Tool (BLAST) (Altschul *et al.* (1990), *supra*) is available from several sources, including the National Center for Biological Information (NCBI, National Library of Medicine, Building 38A, Room 8N805, Bethesda, MD 20894) and on the

10   Internet, for use in connection with the sequence analysis programs blastp, blastn, blastx, tblastn and tblastx. Additional information can be found at the NCBI web site.

BLASTN is used to compare nucleic acid sequences, while BLASTP is used to compare amino acid sequences. If the two compared sequences share homology, then the designated output file will present those regions of homology as aligned sequences. If the two compared

15   sequences do not share homology, then the designated output file will not present aligned sequences.

Once aligned, the number of matches is determined by counting the number of positions where an identical nucleotide or amino acid residue is presented in both sequences. The percent sequence identity is determined by dividing the number of matches either by the

20   length of the sequence set forth in the identified sequence, or by an articulated length (such as 100 consecutive nucleotides or amino acid residues from a sequence set forth in an identified sequence), followed by multiplying the resulting value by 100. For example, a nucleic acid sequence that has 1166 matches when aligned with a test sequence having 1154 nucleotides is 75.0 percent identical to the test sequence (1166÷1554*100=75.0). The percent sequence

25   identity value is rounded to the nearest tenth. For example, 75.11, 75.12, 75.13, and 75.14 are rounded down to 75.1, while 75.15, 75.16, 75.17, 75.18, and 75.19 are rounded up to 75.2. The length value will always be an integer. In another example, a target sequence containing a 20-nucleotide region that aligns with 20 consecutive nucleotides from an identified sequence as

follows contains a region that shares 75 percent sequence identity to that identified sequence (that is, 15÷20*100=75).

For comparisons of amino acid sequences of greater than about 30 amino acids, the Blast 2 sequences function is employed using the default BLOSUM62 matrix set to default parameters, (gap existence cost of 11, and a per residue gap cost of 1). Homologs are typically characterized by possession of at least 70% sequence identity counted over the full-length alignment with an amino acid sequence using the NCBI Basic Blast 2.0, gapped blastp with databases such as the nr database, swissprot database, and patented sequences database. Queries searched with the blastn program are filtered with DUST (Hancock & Armstrong, *Comput Appl Biosci* 10, 67-70 (1994.) Other programs use SEG. In addition, a manual alignment can be performed. Proteins with even greater similarity will show increasing percentage identities when assessed by this method, such as at least about 75%, 80%, 85%, 90%, 95%, 98%, or 99% sequence identity to a protein.

When aligning short peptides (fewer than around 30 amino acids), the alignment is be performed using the Blast 2 sequences function, employing the PAM30 matrix set to default parameters (open gap 9, extension gap 1 penalties). Proteins with even greater similarity to the reference sequence will show increasing percentage identities when assessed by this method, such as at least about 60%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 99% sequence identity to a protein. When less than the entire sequence is being compared for sequence identity, homologs will typically possess at least 75% sequence identity over short windows of 10-20 amino acids, and can possess sequence identities of at least 85%, 90%, 95% or 98% depending on their identity to the reference sequence. Methods for determining sequence identity over such short windows are described at the NCBI web site.

One indication that two nucleic acid molecules are closely related is that the two molecules hybridize to each other under stringent conditions, as described above. Nucleic acid sequences that do not show a high degree of identity may nevertheless encode identical or similar (conserved) amino acid sequences, due to the degeneracy of the genetic code. Changes in a nucleic acid sequence can be made using this degeneracy to produce multiple nucleic acid

molecules that all encode substantially the same protein. Such homologous nucleic acid sequences can, for example, possess at least about 50%, 60%, 70%, 80%, 90%, 95%, 98%, or 99% sequence identity to a nucleic acid that encodes a protein.

Subject: As used herein, the term "subject" refers to a living multi-cellular vertebrate organisms, a category that includes both human and non-human mammals.

Treatment: As used herein, the term "treatment" refers to an intervention that ameliorates a sign or symptom of a disease or pathological condition. As used herein, the terms "treatment", "treat" and "treating," with reference to a disease, pathological condition or symptom, also refers to any observable beneficial effect of the treatment. The beneficial effect can be evidenced, for example, by a delayed onset of clinical symptoms of the disease in a susceptible subject, a reduction in severity of some or all clinical symptoms of the disease, a slower progression of the disease, a reduction in the number of relapses of the disease, an improvement in the overall health or well-being of the subject, or by other parameters well known in the art that are specific to the particular disease. A prophylactic treatment is a treatment administered to a subject who does not exhibit signs of a disease or exhibits only early signs, for the purpose of decreasing the risk of developing pathology. A therapeutic treatment is a treatment administered to a subject after signs and symptoms of the disease have developed.

II.    Recombinant CMV Vectors and Methods of Using the Same

Disclosed herein are human or animal cytomegalovirus (CMV) vectors capable of repeatedly infecting an organism. The CMV vectors comprise a nucleic acid sequence that encodes a heterologous protein antigen and lack expression of active UL128 and UL130 proteins, or orthologs thereof (homologous genes of CMVs that infect other species). The heterologous antigen can be any antigen, including a pathogen-specific antigen derived from, for example, HIV, SIV, herpes simplex virus, hepatitis B or C virus, papillomavirus, Plasmodium parasites, and *Mycobacterium tuberculosis.* In still further examples, the heterologous antigen can be a tumor antigen including, for example, a tumor antigen related to acute myelogenous leukemia, chronic myelogenous leukemia, myelodysplastic syndrome, acute lymphoblastic

leukemia, chronic lymphoblastic leukemia, non-Hodgkin's lymphoma, multiple myeloma, malignant melanoma, breast cancer, lung cancer, ovarian cancer, prostate cancer, pancreatic cancer, colon cancer, renal cell carcinoma (RCC), and germ cell tumors. In some examples the CMV vectors also lack an active UL40 protein (or an ortholog thereof) and/or an active US28 protein (or an ortholog thereof). In still further examples, the heterologous antigen can be a tissue-specific antigen or a host self-antigen including, for example, an antigen derived from the variable region of a T cell receptor, an antigen derived from the variable region of a B cell receptor, a sperm antigen, or an egg antigen.

In some examples, the vector does not express an active UL128, UL130, US28 or UL40 protein due to the presence of a mutation in the nucleic acid sequence encoding UL128, UL130, or UL40 (or orthologs thereof). The mutation may be any mutation that results in a lack of expression of active UL128, UL130, US28 or UL40 protein. Such mutations can include point mutations, frameshift mutations, deletions of less than all of the sequence that encodes the protein (truncation mutations), or deletions of all of the nucleic acid sequence that encodes the protein, or any other mutations.

In further examples, the vector does not express an active UL128, UL130, US28 or UL40 protein (or an ortholog thereof) due to the presence of a nucleic acid sequence in the vector that comprises an antisense or RNAi sequence (siRNA or miRNA) that inhibits the expression of the UL128, UL130, or UL40 protein (or an ortholog thereof). Mutations and/or antisense and/or RNAi can be used in any combination to generate a CMV vector lacking active UL128, UL130, US28 or UL40 (or an ortholog thereof).

The CMV vector can comprise additional inactivating mutations known in the art to provide different immune responses, such as an inactivating US11 mutation or an inactivating UL82 (pp71) mutation, or any other inactivating mutation. The CMV vector may also comprise at least one inactivating mutations in one or more viral genes encoding viral proteins known in the art to be essential or augmenting for viral dissemination (*i.e.* spread from cell to cell) *in vivo.* Such inactivating mutations may result from point mutations, frameshift mutations, truncation mutations, or a deletion of all of the nucleic acid sequence encoding the viral protein.

Inactivating mutations include any mutation in a viral gene which finally leads to a reduced function or to a complete loss of function of the viral protein.

Also disclosed herein are methods of generating CD8$^+$ T cell responses to heterologous antigens in a subject. The methods involve administering an effective amount of a CMV vector to the subject. In one embodiment, the CMV vector is characterized by having a nucleic acid sequence that encodes at least one heterologous antigen and a nucleic acid sequence that does not express an active UL128 protein (or an ortholog thereof), does not express an active UL130 protein (or an ortholog thereof), and expresses at least one active UL40 protein and at least one active US28 protein. The at least one active UL40 protein and the at least one active US28 protein can be orthologs or homologs of UL40 and US28. The CD8$^+$ T cell response elicited by this vector is characterized by having at least 10% of the CD8$^+$ T cells directed against epitopes presented by MHC-E. In further examples, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 75%, at least 90%, at least 95% or at least 95% of the CD8$^+$ T cells are restricted by MHC-E. In some embodiments, the CMV vector expresses two to five active US28 proteins or orthologs or homologs thereof. In some embodiments, the method further comprises identifying a CD8$^+$ T cell receptor from the CD8$^+$ T cells elicited by the CMV vector, wherein the CD8$^+$ T cell receptor recognizes a MHC-E/heterologous antigen-derived peptide complex. In some embodiments, the CD8$^+$ T cell receptor is identified by RNA or DNA sequencing. In another embodiment, the CMV vector is characterized by having a nucleic acid sequence that does not express active UL128, UL130, and UL40 proteins, and this vector can be used to elicit CD8$^+$ T cells recognizing MC-II supertopes either together with HLA-E-restricted CD8$^+$ T cells (elicited by one or more additional vectors containing intact US28 and UL40) or without HLA-E restricted CD8$^+$ T cells (elicited by one or more additional vectors lacking a functional UL40 or US28 protein). In another embodiment, the CMV vector is characterized by having a nucleic acid sequence that does not express active UL128, UL130, and US28 proteins, and this vector can be used to elicit CD8$^+$ T cells recognizing MC-II supertopes either together with HLA-E-restricted CD8$^+$ T cells (elicited by one or more additional vectors containing intact US28 and UL40) or without HLA-E restricted CD8$^+$ T cells (elicited by one or more additional

vectors lacking a functional UL40 or US28 proteins). In another embodiment, the CMV vector is characterized by having a nucleic acid sequence that does not express active UL128, UL130, US28, and UL40 proteins, and this vector can be used to elicit CD8$^+$ T cells recognizing MC-II supertopes either together with HLA-E-restricted CD8$^+$ T cells (elicited by one or more additional vectors containing intact US28 and UL40) or without HLA-E restricted CD8$^+$ T cells (elicited by one or more additional vectors lacking a functional UL40 or US28 proteins).

Also disclosed herein is a method of generating CD8$^+$ T cells that recognize MHC-E-peptide complexes. This method involves administering to a first subject (or animal) a CMV vector that encodes at least one heterologous antigen and an active UL40 protein, or a homolog or ortholog thereof, to generate a set of CD8$^+$ T cells that recognize MHC-E/peptide complexes. The CMV vector does not encode active UL128 and UL130 proteins, or orthologs thereof, and the heterologous antigen can be any antigen, including a pathogen-specific antigen, a tumor antigen, a tissue-specific antigen, or a host self-antigen. In some embodiments, the host self-antigen is an antigen derived from the variable region of a T cell receptor or a B cell receptor. This method further comprises: identifying a first CD8$^+$ T cell receptor from the set of CD8$^+$ T cells, wherein the first CD8$^+$ T cell receptor recognizes a MHC-E/heterologous antigen-derived peptide complex; and transfecting the one or more CD8$^+$ T cells with an expression vector, wherein the expression vector comprises a nucleic acid sequence encoding a second CD8$^+$ T cell receptor and a promoter operably linked to the nucleic acid sequence encoding the T cell receptor, wherein the second CD8$^+$ T cell receptor comprises CDR3α and CDR3β of the first CD8$^+$ TCR, thereby creating one or more transfected CD8$^+$ T cells that recognize MHC-E-peptide complexes. The one or more CD8$^+$ T cells for transfection with the expression vector may be isolated from the first subject or a second subject. In some embodiments, this method may further comprise administering the one or more transfected T cells to the first or second subject to treat a disease such as cancer, a pathogenic infection, or an autoimmune disease or disorder. In some embodiments, this method may further comprise administering the one or more transfected T cells to the first or second subject to induce an autoimmune response to a tissue-specific antigen or a host self-antigen.

Also disclosed is a transfected CD8$^+$ T cell that recognizes MHC-E-peptide complexes prepared by a process comprising the steps of: (1) administering to a first subject a CMV vector in an amount effective to generate a set of CD8$^+$ T cells that recognize MHC-E/peptide complexes, wherein the CMV vector comprises a first nucleic acid sequence encoding at least one heterologous antigen and further comprises a second nucleic acid sequence encoding an active UL40 protein, and wherein the CMV vector does not express active UL128 and UL130 proteins, or orthologs thereof; (2) identifying a first CD8$^+$ T cell receptor from the set of CD8$^+$ T cells, wherein the first CD8$^+$ T cell receptor recognizes a MHC-E/heterologous antigen-derived peptide complex; (3) isolating one or more CD8$^+$ T cells from the first subject or a second subject; and (4) transfecting the one or more CD8$^+$ T cells isolated from the first or second subject with an expression vector, wherein the expression vector comprises a nucleic acid sequence encoding a second CD8$^+$ T cell receptor and a promoter operably linked to the nucleic acid sequence encoding the second T cell receptor, wherein the second CD8$^+$ T cell receptor comprises CDR3α and CDR3β of the first CD8$^+$ T cell receptor, thereby creating a transfected T cell that recognizes MHC-E-peptide complexes. The heterologous antigen can be any antigen, including a pathogen-specific antigen, tissue-specific antigen, a host self-antigen, or a tumor antigen. In some embodiments, the first CD8$^+$ T cell receptor is identified by RNA or DNA sequencing. Also disclosed herein are methods of treating a disease, such as cancer, a pathogenic infection, or an autoimmune disease or disorder, the method comprising administering the transfected T cell that recognizes MHC-E-peptide complexes to the first or second subject. Also disclosed herein are methods of inducing an autoimmune response to a host self-antigen or tissue-specific antigen, the method comprising administering the transfected T cell that recognizes MHC-E-peptide complexes to the first or second subject.

In further examples, the methods involve administering an effective amount of a second CMV vector, the second CMV vector comprising a nucleic acid sequence that encodes a second heterologous antigen to the subject. This second vector can be any CMV vector, including a CMV vector with an active UL128 protein (or a homolog or ortholog thereof)) and/or an active UL130 protein (or a homolog or ortholog thereof). The second CMV vector can comprise a

second heterologous antigen. The second heterologous antigen can be any heterologous antigen, including a heterologous antigen identical to the heterologous antigen in the first CMV vector. The second CMV vector can be administered at any time relative to the administration of the first CMV vector including before, concurrently with, or after the administration of the first CMV vector. This includes administration of the second vector any number of months, days, hours, minutes or seconds before or after the first vector.

Human or animal CMV vectors, when used as expression vectors, are innately non-pathogenic in the selected subjects such as humans. In some embodiments, the CMV vectors have been modified to render them non-pathogenic (incapable of host-to-host spread) in the selected subjects.

A heterologous antigen can be any protein or fragment thereof that is not derived from CMV, including cancer antigens, pathogen-specific antigens, model antigens (such as lysozyme, keyhole-limpet hemocyanin (KLH), or ovalbumin), tissue-specific antigens, host self-antigens, or any other antigen.

Pathogen-specific antigens can be derived from any human or animal pathogen. The pathogen may be a viral pathogen, a bacterial pathogen, or a parasite, and the antigen may be a protein derived from the viral pathogen, bacterial pathogen, or parasite. The parasite may be an organism or disease caused by an organism. For example, the parasite may be a protozoan organism, a protozoan organism causing a disease, a helminth organism or worm, a disease caused by a helminth organism, an ectoparasite, or a disease caused by an ectoparasite.

The antigen can be a protein derived from cancer. The cancers include, but are not limited to, acute myelogenous leukemia, chronic myelogenous leukemia, myelodysplastic syndrome, acute lymphoblastic leukemia, chronic lymphoblastic leukemia, non-Hodgkin's lymphoma, multiple myeloma, malignant melanoma, breast cancer, lung cancer, ovarian cancer, prostate cancer, pancreatic cancer, colon cancer, renal cell carcinoma (RCC), and germ cell tumors.

The antigen can be a host self-antigen. Host self-antigens include, but are not limited to, antigens derived from the variable region of a T cell receptor or from the variable region of a B

cell receptor. The antigen can be a tissue-specific antigen. Tissue-specific antigens include, but are not limited to, sperm antigens or egg antigens.

The CMV vectors disclosed herein can be used as an immunogenic, immunological or vaccine composition containing the recombinant CMV virus or vector, and a pharmaceutically

5    acceptable carrier or diluent. An immunological composition containing the recombinant CMV virus or vector (or an expression product thereof) elicits an immunological response--local or systemic. The response can, but need not be, protective. An immunogenic composition containing the recombinant CMV virus or vector (or an expression product thereof) likewise elicits a local or systemic immunological response which can, but need not be, protective. A

10   vaccine composition elicits a local or systemic protective response. Accordingly, the terms "immunological composition" and "immunogenic composition" include a "vaccine composition" (as the two former terms can be protective compositions).

The CMV vectors disclosed herein can be used in methods of inducing an immunological response in a subject comprising administering to the subject an immunogenic, immunological

15   or vaccine composition comprising the recombinant CMV virus or vector and a pharmaceutically acceptable carrier or diluent. For purposes of this specification, the term "subject" includes all animals, including non-human primates and humans, while "animal" includes all vertebrate species, except humans; and "vertebrate" includes all vertebrates, including animals (as "animal" is used herein) and humans. And, of course, a subset of "animal"

20   is "mammal", which for purposes of this specification includes all mammals, except humans.

The CMV vectors disclosed herein can be used in therapeutic compositions containing the recombinant CMV virus or vector and a pharmaceutically acceptable carrier or diluent. The CMV vectors disclosed herein can be prepared by inserting DNA comprising a sequence that encodes the heterologous antigen into an essential or non-essential region of the CMV genome.

25   The method can further comprise deleting one or more regions from the CMV genome. The method can comprise *in vivo* recombination. Thus, the method can comprise transfecting a cell with CMV DNA in a cell-compatible medium in the presence of donor DNA comprising the heterologous DNA flanked by DNA sequences homologous with portions of the CMV genome,

whereby the heterologous DNA is introduced into the genome of the CMV, and optionally then recovering CMV modified by the in vivo recombination. The method can also comprise cleaving CMV DNA to obtain cleaved CMV DNA, ligating the heterologous DNA to the cleaved CMV DNA to obtain hybrid CMV-heterologous DNA, transfecting a cell with the hybrid CMV-heterologous

5    DNA, and optionally then recovering CMV modified by the presence of the heterologous DNA. Since in vivo recombination is comprehended, the method accordingly also provides a plasmid comprising donor DNA not naturally occurring in CMV encoding a polypeptide foreign to CMV, the donor DNA is within a segment of CMV DNA that would otherwise be co-linear with an essential or non-essential region of the CMV genome such that DNA from an essential or

10   nonessential region of CMV is flanking the donor DNA. The heterologous DNA can be inserted into CMV to generate the recombinant CMV in any orientation that yields stable integration of that DNA, and expression thereof, when desired.

The DNA encoding the heterologous antigen in the recombinant CMV vector can also include a promoter. The promoter can be from any source such as a herpes virus, including an

15   endogenous CMV promoter, such as a HCMV, RhCMV, murine CMV (MCMV), or other CMV promoter. The promoter can also be a non-viral promoter such as the EF1α promoter. The promoter can be a truncated transcriptionally active promoter which comprises a region transactivated with a transactivating protein provided by the virus and the minimal promoter region of the full-length promoter from which the truncated transcriptionally active promoter is

20   derived. The promoter can be composed of an association of DNA sequences corresponding to the minimal promoter and upstream regulatory sequences. A minimal promoter is composed of the CAP site plus TATA box (minimum sequences for basic level of transcription; unregulated level of transcription); "upstream regulatory sequences" are composed of the upstream element(s) and enhancer sequence(s). Further, the term "truncated" indicates that the full-

25   length promoter is not completely present, *i.e.*, that some portion of the full-length promoter has been removed. And, the truncated promoter can be derived from a herpesvirus such as MCMV or HCMV, e.g., HCMV-IE or MCMV-IE. There can be up to a 40% and even up to a 90% reduction in size, from a full-length promoter, based upon base pairs. The promoter can also be

a modified non-viral promoter. As to HCMV promoters, reference is made to U.S. Pat. Nos. 5,168,062 and 5,385,839. As to transfecting cells with plasmid DNA for expression therefrom, reference is made to Felgner *et al.* (1994), *J. Biol. Chem.* 269, 2550-2561. And, as to direct injection of plasmid DNA as a simple and effective method of vaccination against a variety of

5    infectious diseases reference is made to *Science*, 259:1745-49, 1993. It is therefore within the scope of this invention that the vector can be used by the direct injection of vector DNA.

Also disclosed is an expression cassette that can be inserted into a recombinant virus or plasmid comprising the truncated transcriptionally active promoter. The expression cassette can further include a functional truncated polyadenylation signal; for instance an SV40

10   polyadenylation signal which is truncated, yet functional. Considering that nature provided a larger signal, it is indeed surprising that a truncated polyadenylation signal is functional. A truncated polyadenylation signal addresses the insert size limit problems of recombinant viruses such as CMV. The expression cassette can also include heterologous DNA with respect to the virus or system into which it is inserted; and that DNA can be heterologous DNA as

15   described herein.

As to antigens for use in vaccine or immunological compositions, see also Stedman's Medical Dictionary (24th edition, 1982, *e.g.*, definition of vaccine (for a list of antigens used in vaccine formulations); such antigens or epitopes of interest from those antigens can be used. As to heterologous antigens, one skilled in the art can select a heterologous antigen and the

20   coding DNA therefor from the knowledge of the amino acid and corresponding DNA sequences of the peptide or polypeptide, as well as from the nature of particular amino acids (*e.g.*, size, charge, *etc.*) and the codon dictionary, without undue experimentation.

One method to determine T epitopes of an antigen involves epitope mapping. Overlapping peptides of the heterologous antigen are generated by oligo-peptide synthesis.

25   The individual peptides are then tested for their ability to bind to an antibody elicited by the native protein or to induce T cell or B cell activation. This approach has been particularly useful in mapping T-cell epitopes since the T cell recognizes short linear peptides complexed with MHC molecules.

An immune response to a heterologous antigen is generated, in general, as follows: T cells recognize proteins only when the protein has been cleaved into smaller peptides and is presented in a complex called the "major histocompatibility complex (MHC)" located on another cell's surface. There are two classes of MHC complexes--class I and class II, and each class is made up of many different alleles. Different species, and individual subjects have different types of MHC complex alleles; they are said to have a different MHC type. One type of MHC class I molecule is called MHC-E (HLA-E in humans, Mamu-E in RM, Qa-1b in mice).

It is noted that the DNA comprising the sequence encoding the heterologous antigen can itself include a promoter for driving expression in the CMV vector or the DNA can be limited to the coding DNA of the heterologous antigen. This construct can be placed in such an orientation relative to an endogenous CMV promoter that it is operably linked to the promoter and is thereby expressed. Further, multiple copies of DNA encoding the heterologous antigen or use of a strong or early promoter or early and late promoter, or any combination thereof, can be done so as to amplify or increase expression. Thus, the DNA encoding the heterologous antigen can be suitably positioned with respect to a CMV-endogenous promoter, or those promoters can be translocated to be inserted at another location together with the DNA encoding the heterologous antigen. Nucleic acids encoding more than one heterologous antigen can be packaged in the CMV vector.

Further disclosed are pharmaceutical and other compositions containing the disclosed CMV vectors. Such pharmaceutical and other compositions can be formulated so as to be used in any administration procedure known in the art. Such pharmaceutical compositions can be via a parenteral route (intradermal, intramuscular, subcutaneous, intravenous, or others). The administration can also be via a mucosal route, *e.g.*, oral, nasal, genital, etc.

The disclosed pharmaceutical compositions can be prepared in accordance with standard techniques well known to those skilled in the pharmaceutical arts. Such compositions can be administered in dosages and by techniques well known to those skilled in the medical arts taking into consideration such factors as the breed or species, age, sex, weight, and condition of the particular patient, and the route of administration. The compositions can be

administered alone, or can be co-administered or sequentially administered with other CMV vectors or with other immunological, antigenic or vaccine or therapeutic compositions. Such other compositions can include purified native antigens or epitopes or antigens or epitopes from the expression by a recombinant CMV or another vector system; and are administered

5      taking into account the aforementioned factors.

Examples of compositions include liquid preparations for orifice, *e.g.*, oral, nasal, anal, genital, *e.g.*, vaginal, *etc.*, administration such as suspensions, syrups or elixirs; and, preparations for parenteral, subcutaneous, intradermal, intramuscular or intravenous administration (*e.g.*, injectable administration) such as sterile suspensions or emulsions. In such

10     compositions the recombinant may be in admixture with a suitable carrier, diluent, or excipient such as sterile water, physiological saline, glucose or the like.

Antigenic, immunological or vaccine compositions typically can contain an adjuvant and an amount of the CMV vector or expression product to elicit the desired response. In human applications, alum (aluminum phosphate or aluminum hydroxide) is a typical adjuvant. Saponin

15     and its purified component Quil A, Freund's complete adjuvant and other adjuvants used in research and veterinary applications have toxicities which limit their potential use in human vaccines. Chemically defined preparations such as muramyl dipeptide, monophosphoryl lipid A, phospholipid conjugates such as those described by Goodman-Snitkoff *et al.* J. Immunol. 147:410-415 (1991), encapsulation of the protein within a proteoliposome as described by

20     Miller *et al.*, J. Exp. Med. 176:1739-1744 (1992), and encapsulation of the protein in lipid vesicles such as Novasome lipid vesicles (Micro Vescular Systems, Inc., Nashua, N.H.) can also be used.

The composition may be packaged in a single dosage form for immunization by parenteral (*i.e.*, intramuscular, intradermal or subcutaneous) administration or orifice

25     administration, *e.g.*, perlingual (*e.g.*, oral), intragastric, mucosal including intraoral, intraanal, intravaginal, and the like administration. And again, the effective dosage and route of administration are determined by the nature of the composition, by the nature of the expression product, by expression level if recombinant CMV is directly used, and by known

factors, such as breed or species, age, sex, weight, condition and nature of host, as well as $LD_{50}$ and other screening procedures which are known and do not require undue experimentation. Dosages of expressed product can range from a few to a few hundred micrograms, *e.g.*, 5 to 500 μg. The CMV vector can be administered in any suitable amount to achieve expression at

5    these dosage levels. In nonlimiting examples: CMV vectors can be administered in an amount of at least $10^2$ pfu; thus, CMV vectors can be administered in at least this amount; or in a range from about $10^2$ pfu to about $10^7$ pfu. Other suitable carriers or diluents can be water or a buffered saline, with or without a preservative. The CMV vector can be lyophilized for resuspension at the time of administration or can be in solution. "About" can mean within 1%,

10   5%, 10% or 20% of a defined value.

It should be understood that the proteins and the nucleic acids encoding them of the present invention can differ from the exact sequences illustrated and described herein. Thus, the invention contemplates deletions, additions, truncations, and substitutions to the sequences shown, so long as the sequences function in accordance with the methods of the

15   invention. In this regard, substitutions will generally be conservative in nature, i.e., those substitutions that take place within a family of amino acids. For example, amino acids are generally divided into four families: (1) acidic--aspartate and glutamate; (2) basic--lysine, arginine, histidine; (3) non-polar--alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan; and (4) uncharged polar--glycine, asparagine, glutamine, cysteine,

20   serine threonine, and tyrosine. Phenylalanine, tryptophan, and tyrosine are sometimes classified as aromatic amino acids. It is reasonably predictable that an isolated replacement of leucine with isoleucine or valine, or vice versa; an aspartate with a glutamate or vice versa; a threonine with a serine or vice versa; or a similar conservative replacement of an amino acid with a structurally related amino acid, will not have a major effect on the biological activity.

25   Proteins having substantially the same amino acid sequence as the proteins described but possessing minor amino acid substitutions that do not substantially affect the immunogenicity of the protein are, therefore, within the scope of the disclosure.

The nucleotide sequences of the present invention can be codon optimized, for example the codons can be optimized for use in human cells. For example, any viral or bacterial sequence can be so altered. Many viruses, including HIV and other lentiviruses, use a large number of rare codons and, by altering these codons to correspond to codons commonly used

5       in the desired subject, enhanced expression of the heterologous antigen can be achieved as described in Andre *et al., J. Virol.* 72:1497-1503, 1998.

Nucleotide sequences encoding functionally and/or antigenically equivalent variants and derivatives of the CMV vectors and the glycoproteins included therein are contemplated. These functionally equivalent variants, derivatives, and fragments display the ability to retain

10      antigenic activity. For instance, changes in a DNA sequence that do not change the encoded amino acid sequence, as well as those that result in conservative substitutions of amino acid residues, one or a few amino acid deletions or additions, and substitution of amino acid residues by amino acid analogs are those which will not significantly affect properties of the encoded polypeptide. Conservative amino acid substitutions are glycine/alanine;

15      valine/isoleucine/leucine;        asparagine/glutamine;        aspartic        acid/glutamic        acid; serine/threonine/methionine; lysine/arginine; and phenylalanine/tyrosine/tryptophan. In one embodiment, the variants have at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least

20      96%, at least 97%, at least 98% or at least 99% homology or identity to the antigen, epitope, immunogen, peptide or polypeptide of interest.

Sequence identity or homology is determined by comparing the sequences when aligned so as to maximize overlap and identity while minimizing sequence gaps. In particular, sequence identity may be determined using any of a number of mathematical algorithms. A

25      nonlimiting example of a mathematical algorithm used for comparison of two sequences is the algorithm of Karlin & Altschul, Proc. Natl. Acad. Sci. USA 1990; 87: 2264-2268, modified as in Karlin & Altschul, Proc. Natl. Acad. Sci. USA 1993;90: 5873-5877.

Another example of a mathematical algorithm used for comparison of sequences is the algorithm of Myers & Miller, CABIOS 1988;4: 11-17. Such an algorithm is incorporated into the ALIGN program (version 2.0) which is part of the GCG sequence alignment software package. When utilizing the ALIGN program for comparing amino acid sequences, a PAM120 weight residue table, a gap length penalty of 12, and a gap penalty of 4 can be used. Yet another useful algorithm for identifying regions of local sequence similarity and alignment is the FASTA algorithm as described in Pearson & Lipman, Proc. Natl. Acad. Sci. USA 1988; 85: 2444-2448.

Advantageous for use according to the present invention is the WU-BLAST (Washington University BLAST) version 2.0 software. WU-BLAST version 2.0 executable programs for several UNIX platforms can be downloaded from ftp://blast.wustl.edu/blast/executables. This program is based on WU-BLAST version 1.4, which in turn is based on the public domain NCBI-BLAST version 1.4 (Altschul & Gish, 1996, Local alignment statistics, Doolittle ed., Methods in Enzymology 266: 460-480; Altschul *et al.* (1990), *supra*; Gish & States, 1993; Nature Genetics 3: 266-272; Karlin & Altschul, Proc. Natl. Acad. Sci. USA 90: 5873-5877 (1993); all of which are incorporated by reference herein).

The various recombinant nucleotide sequences and antibodies and/or antigens of the invention are made using standard recombinant DNA and cloning techniques. Such techniques are well known to those of skill in the art. See for example, "Molecular Cloning: A Laboratory Manual", second edition (Sambrook *et al.* 1989).

The nucleotide sequences of the present invention may be inserted into "vectors." The term "vector" is widely used and understood by those of skill in the art, and as used herein the term "vector" is used consistent with its meaning to those of skill in the art. For example, the term "vector" is commonly used by those skilled in the art to refer to a vehicle that allows or facilitates the transfer of nucleic acid molecules from one environment to another or that allows or facilitates the manipulation of a nucleic acid molecule.

Any vector that allows expression of the viruses of the present invention can be used in accordance with the present invention. In certain embodiments, the disclosed viruses can be used in vitro (such as using cell-free expression systems) and/or in cultured cells grown in vitro

in order to produce the encoded heterologous antigen (*e.g.*, pathogen-specific antigens, HIV antigens, tumor antigens, and antibodies) which may then be used for various applications such as in the production of proteinaceous vaccines. For such applications, any vector that allows expression of the virus in vitro and/or in cultured cells may be used.

5        For the disclosed heterologous antigens to be expressed, the protein coding sequence of the heterologous antigen should be "operably linked" to regulatory or nucleic acid control sequences that direct transcription and translation of the protein. As used herein, a coding sequence and a nucleic acid control sequence or promoter are said to be "operably linked" when they are covalently linked in such a way as to place the expression or transcription and/or

10     translation of the coding sequence under the influence or control of the nucleic acid control sequence. The "nucleic acid control sequence" can be any nucleic acid element, such as, but not limited to promoters, enhancers, IRES, introns, and other elements described herein that direct the expression of a nucleic acid sequence or coding sequence that is operably linked thereto. The term "promoter" will be used herein to refer to a group of transcriptional control modules

15     that are clustered around the initiation site for RNA polymerase II and that when operationally linked to the protein coding sequences of the invention lead to the expression of the encoded protein. The expression of the transgenes of the present invention can be under the control of a constitutive promoter or of an inducible promoter, which initiates transcription only when exposed to some particular external stimulus, such as, without limitation, antibiotics such as

20     tetracycline, hormones such as ecdysone, or heavy metals. The promoter can also be specific to a particular cell-type, tissue or organ. Many suitable promoters and enhancers are known in the art, and any such suitable promoter or enhancer may be used for expression of the transgenes of the invention. For example, suitable promoters and/or enhancers can be selected from the Eukaryotic Promoter Database (EPDB).

25     The disclosure relates to a recombinant viral vector expressing a heterologous protein antigen. In some examples, the antigen is an HIV antigen. Advantageously, the HIV antigens include, but are not limited to, the HIV antigens discussed in U.S. Pub. Nos. 2008/0199493 A1 and 2013/0136768 A1, both of which are incorporated by reference herein. HIV, nucleic acid or

immunogenic fragments thereof, may be utilized as an HIV protein antigen. For example, the HIV nucleotides discussed in U.S. Pub. Nos. 2008/0199493 A1 and 2013/0136768 A1 can be used. Any antigen recognized by an HIV antibody can be used as an HIV protein antigen. The protein antigen can also be an SIV antigen. For example, the SIV antigens discussed in U.S. Pub. Nos. 2008/0199493 A1 and 2013/0136768 A1 can be used.

The vectors used in accordance with the present invention can contain a suitable gene regulatory region, such as a promoter or enhancer, such that the antigens of the invention can be expressed.

Expressing antigens of the invention in vivo in a subject, for example in order to generate an immune response against an HIV-1 antigen and/or protective immunity against HIV-1, expression vectors that are suitable for expression on that subject, and that are safe for use in vivo, should be chosen. In some examples, it may be desired to express the antibodies and/or antigens in a laboratory animal, such as for pre-clinical testing of the HIV-1 immunogenic compositions and vaccines of the invention. In other examples, one can express the antigens in human subjects, such as in clinical trials and for actual clinical use of the immunogenic compositions and vaccine of the invention.

The CMV vectors described herein can contain mutations that can prevent host to host spread, thereby rendering the virus unable to infect immunocompromised or other subjects that could face complications as a result of CMV infection. The CMV vectors described herein can also contain mutations that result in the presentation of immunodominant and non-immunodominant epitopes as well as non-canonical MHC restriction. However, mutations in the CMV vectors described herein do not affect the ability of the vector to re-infect a subject that has been previously infected with CMV. Such CMV mutations are described in, for example, US Patent Publications 2013-0136768; 2010-0142823; 2014-0141038; and PCT application publication WO 2014/138209, all of which are incorporated by reference herein.

The disclosed CMV vectors can be administered in vivo, for example where the aim is to produce an immunogenic response, including a CD8$^+$ immune response, including an immune response characterized by a high percentage of the CD8$^+$ T cell response being restricted by

MHC Class II and/or MHC-E (or a homolog or ortholog thereof). For example, in some examples it may be desired to use the disclosed CMV vectors in a laboratory animal, such as rhesus macaques for pre-clinical testing of immunogenic compositions and vaccines using RhCMV. In other examples, it will be desirable to use the disclosed CMV vectors in human subjects, such as
5      in clinical trials and for actual clinical use of the immunogenic compositions using HCMV.

For such *in vivo* applications the disclosed CMV vectors are administered as a component of an immunogenic composition further comprising a pharmaceutically acceptable carrier. The immunogenic compositions of the invention are useful to stimulate an immune response against the heterologous antigen, including a pathogen-specific antigen and may be
10     used as one or more components of a prophylactic or therapeutic vaccine against HIV-1 for the prevention, amelioration or treatment of AIDS. The nucleic acids and vectors of the invention are particularly useful for providing genetic vaccines, i.e. vaccines for delivering the nucleic acids encoding the antigens of the invention to a subject, such as a human, such that the antigens are then expressed in the subject to elicit an immune response.

15     Immunization schedules (or regimens) are well known for animals (including humans) and can be readily determined for the particular subject and immunogenic composition. Hence, the immunogens can be administered one or more times to the subject. Preferably, there is a set time interval between separate administrations of the immunogenic composition. While this interval varies for every subject, typically it ranges from 10 days to several weeks, and is
20     often 2, 4, 6 or 8 weeks. For humans, the interval is typically from 2 to 6 weeks. In a particularly advantageous embodiment of the present invention, the interval is longer, advantageously about 10 weeks, 12 weeks, 14 weeks, 16 weeks, 18 weeks, 20 weeks, 22 weeks, 24 weeks, 26 weeks, 28 weeks, 30 weeks, 32 weeks, 34 weeks, 36 weeks, 38 weeks, 40 weeks, 42 weeks, 44 weeks, 46 weeks, 48 weeks, 50 weeks, 52 weeks, 54 weeks, 56 weeks, 58 weeks, 60 weeks, 62
25     weeks, 64 weeks, 66 weeks, 68 weeks or 70 weeks. The immunization regimes typically have from 1 to 6 administrations of the immunogenic composition, but may have as few as one or two or four. The methods of inducing an immune response can also include administration of an adjuvant with the immunogens. In some instances, annual, biannual or other long interval

(5-10 years) booster immunization can supplement the initial immunization protocol. The present methods also include a variety of prime-boost regimens. In these methods, one or more priming immunizations are followed by one or more boosting immunizations. The actual immunogenic composition can be the same or different for each immunization and the type of immunogenic composition (*e.g.*, containing protein or expression vector), the route, and formulation of the immunogens can also be varied. For example, if an expression vector is used for the priming and boosting steps, it can either be of the same or different type (*e.g.*, DNA or bacterial or viral expression vector). One useful prime-boost regimen provides for two priming immunizations, four weeks apart, followed by two boosting immunizations at 4 and 8 weeks after the last priming immunization. It should also be readily apparent to one of skill in the art that there are several permutations and combinations that are encompassed using the DNA, bacterial and viral expression vectors of the invention to provide priming and boosting regimens. CMV vectors can be used repeatedly while expressing different antigens derived from different pathogens.

## EXAMPLES

The following examples are illustrative of disclosed methods. In light of this disclosure, those of skill in the art will recognize that variations of these examples and other examples of the disclosed method would be possible without undue experimentation.

*Example 1 - Induction of MHC-E Restricted CD8$^+$ T cells by Rhesus Cytomegalovirus Vaccine Vectors lacking UL128 and UL130 but containing UL40 and US28 genes.*

It has been previously demonstrated that RhCMV/SIV vectors drive an alternate SIV-specific CD8$^+$ T cell response that is completely distinct from the canonical responses engendered by conventional vaccine modalities and even from SIV infection itself (Hansen, S.G. *et al., Science* 340, 1237874 (2013), incorporated by reference herein).

While it had been established that the RhCMV/SIV-induced CD8$^+$ T cell response was dominated by the existence of a population of MHC-II restricted CD8$^+$ T cells, the molecule

WO 2016/130693                                                                          PCT/US2016/017373

restricting the remaining CD8⁺ T cells - those which were inhibited by the pan-MHC-I blocking antibody W6/32 - remained unknown.

In particular, administration of 68-1 RhCMV/gag vector elicited MHC-I-restricted CD8⁺ T cells targeting the SIVmac239 Gag$_{273-287}$ (Gag 15-mer #69) and Gag$_{477-491}$ (Gag 15-mer #120) "supertopes" in every RhCMV/gag vector-vaccinated macaque regardless of MHC-Ia expression. This implied the involvement of a functionally conserved "non-classical" (*i.e.* non-polymorphic) MHC-Ib molecule. Described herein is the identification of the restricting MHC-I allele of these CD8⁺ T cells. A panel of MHC-I transfectants expressing either a single "classical" (*i.e.* polymorphic) MHC-Ia or non-classical MHC-Ib allele was developed from a cohort of four strain 68-1 RhCMV/gag-vaccinated macaques mounting strong RhCMV/gag-induced CD8⁺ T cell responses (Fig. 7). Using a previously described MHC restriction assay (Hansen *et al. Science* (2013), *supra*), it was established that CD8⁺ T cells targeting the Gag$_{273-287}$ and Gag$_{477-491}$ supertopes recognize these epitopes in the context of MHC-E (Fig. 1A).

MHC-E (HLA-E in humans, Mamu-E in rhesus macaques, and Qa-1$^{b}$ in mice) is a highly monomorphic, non-classical MHC-Ib molecule expressed in nearly every nucleated cell in the body, with particularly high expression in immune system cells (N. Lee *et al.*, *Proc Natl Acad Sci USA* 95, 5199 (1998) and S. Coupel *et al.*, *Blood* 109, 2806 (2007), both of which are incorporated by reference herein). In contrast to the over 8,500 HLA class I alleles currently identified (J. Robinson *et al.*, *Nucleic Acids Res* 41, D1222 (2013); incorporated by reference herein) only two HLA-E molecules exist, which vary at one amino acid located outside the peptide-binding groove, and are thus likely functionally identical (R. K. Strong *et al.*, *J Biol Chem* 278, 5082 (2003); incorporated by reference herein). This highly monomorphic nature of MHC-E likely explains how every RhCMV/gag-vaccinated macaque is able to target the same Gag MHC-I supertope independent of the MHC-Ia alleles present in each animal.

MHC-E was also identified as the restricting allele for the remaining MHC-I blocked CD8⁺ T cells in RhCMV/gag-vaccinated macaques (Fig. 1A). Although the structure of MHC-E is similar to that of classical MHC-Ia molecules, under normal physiological conditions MHC-E repetitively binds and presents only a single 9-mer peptide derived from the leader sequence of MHC-Ia

molecules for presentation to NK cells. However, under conditions of cellular stress such as during viral infection, MHC-E binds a completely separate set of highly diverse CD8$^+$ T cell epitopes whose binding motif do not match that of the dominant MHC-Ia leader peptides (Lampen *et al.*, *supra* and C.C. Oliviera *et al.*, *J Exp Med* 207, 207 (2010); both of which are incorporated by reference herein). The ability of MHC-E to disengage the leader peptide and subsequently present an alternate peptide repertoire to CD8$^+$ T cells suggests that the alternate MHC-I-restricted CD8$^+$ T cell response is due largely, if not entirely, to presentation by MHC-E.

HLA-E restricted CD8$^+$ T cells have recently been discovered against several human pathogens including CMV (G. Pietra *et al.*, *Proc Natl Acad Sci U S A* 100, 10896 (2003); incorporated by reference herein); EBV (Jorgensen PB *et al.*, *PLoS One* 7, e46120 (2012); incorporated by reference herein); *Salmonella typhi* (R. Salerno-Goncalves, *et al.*, *J Immunol* 173, 5852 (2004); incorporated by reference herein);  and *Mycobacterium tuberculosis* (A. S. Heinzel *et al.*, *J Exp Med* 196, 1473 (2002) and SA Joosten *et al.* PLoS Pathol 6, e1000782 (2010); both of which are incorporated by reference herein). However, no HIV/SIV-specific MHC-E restricted CD8$^+$ T cell response has been reported and no vaccine platform currently exists that induces these non-classically restricted CD8$^+$ T cells against any heterologous antigen.

MHC restriction data from animals was confirmed using MHC "blocking" peptides that bind to specific MHC allomorphs with high affinity, thereby outcompeting other peptides for the binding groove of that MHC molecule. To protect its infected host cell from NK-cell mediated lysis, HCMV encodes the glycoprotein UL40 (the RCMV homolog is Rh67), that contains the exact 9-mer peptide (VMAPRTLLL, Rh67$_{8-16}$ VL9) derived from classical MHC-Ia leader sequences. The VL9 peptide specifically binds the MHC-E peptide binding groove with extremely high affinity (P. Tomasec *et al.*, *Science* 287, 1031 (2000); incorporated by reference herein). Antigen presenting cells were pre-incubated with either the Rh67-derived VL9 peptide to block binding of the Gag$_{273-287}$ and Gag$_{477-491}$ peptides to MHC-E, or with an irrelevant Mamu-A*002:01 (A*02)-binding Gag$_{71-79}$ GY9 peptide. CD8$^+$ T cell recognition of the Gag$_{273-287}$ and Gag$_{477-491}$ supertopes on both autologous BLCL and transfectants expressing a single MHC-E allele was completely inhibited by the presence of the MHC-E high-affinity binding peptide

Rh67$_{8-16}$ VL9, confirming MHC-E as the presenting allele for the MHC-I supertope responses (Figs. 1B and 1C).

The contribution of MHC-E to the overall Gag-specific CD8$^+$ T cell response elicited by RhCMV/gag vectors was compared to that of a conventional Modified Vaccinia Ankara (MVA/gag) vector and native SIV infection. Flow cytometric ICS using blocking monoclonal antibodies (mAbs) specific for MHC-I or MHC-II along with the MHC-E blocking Rh67$_{8-16}$ VL9 peptide was used to assess the restriction of each epitope-specific response found in a cohort of 25 macaques: 6 vaccinated with strain 68-1 RhCMV/gag, 9 with strain 68-1.2 RhCMV/gag, 7 with MVA/gag, and 8 SIV-infected macaques. MHC-E-blocked CD8$^+$ T cell responses were found only in macaques vaccinated with strain 68-1 RhCMV/gag. Furthermore, every MHC-I-blocked response observed in the macaques vaccinated with strain 68-1 RhCMV/gag was presented by MHC-E (Figs. 2A, 8, and 9). No MHC-E restricted CD8$^+$ T cells were observed in macaques vaccinated with strain 68-1.2 RhCMV/gag vector.

The lack of MHC-E restricted CD8$^+$ T cells in strain 68-1.2 RhCMV/gag vector-vaccinated macaques was surprising given the minimal differences between the two CMV strains. During *in vitro* culture on fibroblasts prior to being cloned as a bacterial artificial chromosome (BAC), RhCMV 68-1 lost the ability to express gene products from the Rh13, Rh60, Rh157.5, and Rh157.4 (HCMV RL11, UL36, UL128, and UL130, respectively) open reading frames (D. Malouli *et al.*, J Virol 86, 8959 (2012) and WO 2014/138209; incorporated by reference herein). Of these, expression is restored for Rh60, Rh157.5, and Rh157.4 in the RhCMV 68-1.2 strain (A. E. Lilja, T. Shenk, *Proc Natl Acad Sci U S A* 105, 19950 (2008); incorporated by reference herein), suggesting that the presence of one or a combination of these gene products is sufficient to inhibit priming of CD8$^+$ T cells on MHC-E. Rh60 can be excluded as the gene mediating this inhibitory effect because it is present in the non-BAC derived RhCMV/gag(L) vector (Hansen, S,G, *et al.*, *Science* 328, 102 (2010); incorporated by reference herein) which induces MHC-E restricted CD8$^+$ T cells (Fig. 2A). Thus, the absence of the genes Rh157.5 and Rh157.4 (UL128-130 in HCMV) from CMV is necessary for induction of MHC-E restricted CD8$^+$ T cells.

Incomplete VL9 blocking for certain responses was observed (see Gag 15-mer #18 in Rh22607 in Fig. 2A). Indeed, while every RhCMV/gag-induced, W6/32-blocked CD8$^+$ T cell response recognized peptide in the context of MHC-E, these incompletely VL9-blocked peptides were recognized in the context of classical MHC-Ia alleles, such as Gag$_{69-83}$ (Gag 15-mer #18) presented by Mamu-A*001:01 (A*01) in Rh22607 (Fig. 8B). To more closely understand this dual presentation, peptide blockade studies were performed. These showed that although the presence of the Mamu-A*01-binding Gag$_{181-189}$ CM9 peptide was sufficient to inhibit presentation of Gag$_{69-83}$ on the Mamu-A*01 transfectant, and the presence of the Rh67-derived VL9 peptide inhibited presentation of Gag$_{69-83}$ on the MHC-E transfectant, both peptides were required to completely block presentation of Gag$_{69-83}$ on autologous BLCL from a Mamu-A*01$^+$ macaque (Fig. 2B). In contrast, presentation of the same Gag$_{69-83}$ epitope was fully blocked by the presence of the Rh67$_{8-16}$ VL9 peptide alone on BLCL from a Mamu-A*01- macaque, underscoring MHC-E as the primary restricting allele for these peptides (Fig. 2C). However, given the ability of MHC-E restricted CD8$^+$ T cells to respond to cognate peptide in the context of either MHC-E or a classical MHC-Ia molecule, the TCR of these cells likely directly recognize the MHC-bound peptide itself or in conjunction with a conserved MHC structural motif. Surprisingly, the presence of an MHC allele capable of binding a specific peptide epitope was not sufficient for the generation of a CD8$^+$ T cell response targeting that epitope (Fig. 8), indicating additional layers of immunological regulation in determining the specific set of epitopes targeted in each RhCMV-vaccinated macaque.

Next, it was established that MHC-E restricted CD8$^+$ T cells participate in the immune response against SIV. HIV and SIV evade CD8$^+$ T cell recognition by Nef-mediated down regulation of the classical MHC class I molecules from the cell surface (O. Schwartz, et al., Nat Med 2, 338 (1996); K. L. Collins et al., Nature 391, 397 (1998); both of which are incorporated by reference herein). In contrast, Nef is unable to down regulate HLA-E and its surface expression actually increases with HIV infection (J. Natterman et al., Antivir Ther 10, 95, (2005); incorporated by reference herein). First, the fate of Mamu-E on the surface of productively SIV-infected CD4$^+$ T cells was determined. Using the pan-MHC-I mAb W6/32 and the Mamu-E-

specific mAb 4D12, it was demonstrated that, like HLA-E during HIV infection, Mamu-E surface expression is significantly increased during SIV infection (Figs. 3A and 3B). Therefore, MHC-E restricted CD8$^+$ T cells might be particularly effective since they would be impervious to Nef-mediated down-modulation of their restricting MHC-I molecule. MHC-E interacts with both TCRαβ and CD94/NKG2 complexes, which are expressed on the surface of CD8$^+$ T cells (V.M. Braud *et al.*, Nature 391, 795 (1998); incorporated by reference herein).

In particular, high NKG2C expression is driven by CMV infection, and engagement of the NKG2C receptor triggers activation of NK and T cells interacting with MHC-E (S. Lopez-Verges *et al.*, Proc Natl Acad Sci U S A 108, 14725 (2011) and M. Guma *et al.*, Eur J Immunol 35, 2071 (2005); both of which are incorporated by reference herein). To investigate the possibility that the MHC-E restricted CD8$^+$ T cells in strain 68-1 RhCMV/gag-vaccinated macaques utilize NKG2C receptors to mediate MHC-E induced activation, the surface phenotype of these cells was examined, and little, if any, NKG2A/NKG2C expression (Figs. 3C and 10) was found. Furthermore, the MHC-E restricted CD8$^+$ T cells exhibited a conventional CD3$^+$, CD8αβ$^+$, TCRγδ$^-$, NKG2A/C$^-$ phenotype suggesting that these T cells recognized MHC-E-bound peptides *via* CD8-stabilized TCRαβ interactions.

Next, the ability of MHC-E restricted CD8$^+$ T cells present in 68-1 RhCMV/SIV vaccinated macaques to specifically recognize autologous, SIV-infected CD4$^+$ T cells was compared to that of the classically MHC-I restricted CD8$^+$ T cells found in macaques vaccinated with MVA/gag or strain 68-1.2 RhCMV/gag or infected with SIV. CD8$^+$ T cells isolated from all treated macaques robustly recognized autologous SIV-infected CD4$^+$ T cells, and this recognition was completely blocked by the addition of the pan-MHC-I blocking mAb W6/32 and the MHC-II binding CLIP peptide (Figs. 4A and 4B). In contrast, CD8$^+$ T cell recognition of infected cells was fully restored when the MHC-I blocking mAb W6/32 was replaced by the MHC-E blocking Rh67$_{8-16}$ VL9 peptide in all cases except for CD8$^+$ T cells isolated from strain 68-1 RhCMV/SIV-vaccinated macaques. This suggests that MHC-E restricted CD8$^+$ T cells recognized SIV infected cells.

To more precisely examine if MHC-E bound, SIV-derived epitopes were present on the surface of infected cells a Gag$_{477-491}$ (Gag #120) supertope-specific, MHC-E restricted CD8$^+$ T cell

line was generated. This line was tested for the ability to respond to autologous SIV-infected CD4$^+$ T cells. For comparison, a classically MHC-restricted (Mamu-A*001:01 restricted) Gag$_{181-189}$ CM9 CD8$^+$ T cell line was also assessed. Both Gag-specific CD8$^+$ T cell lines specifically recognized SIV-infected cells, and recognition was blocked when targets were pre-incubated with the pan-MHC-I blocking mAb W6/32. In contrast, only the MHC-E restricted CD8$^+$ T cell line was unable to recognize SIV-infected cells when targets were pre-incubated with the MHC-E binding peptide Rh67$_{8-16}$ VL9 (Fig. 4C). Cumulatively, these data indicate that MHC-E restricted CD8$^+$ T cells specifically recognize SIV-derived peptide epitopes on the surface of infected cells.

Strain 68-1 vectors induce CD8$^+$ T cells that recognize peptide antigen in the context of the non-classical MHC-E molecule. Such CD8$^+$ T cells represent a new cellular immune response for vaccine development and may be particularly effective given the unique immunobiology of MHC-E. In contrast to classical HLA molecules that are down regulated from the surface of HIV-infected cells, HLA-E expression is up regulated, and the increase of MHC-E expression occurs within the first 24 hours of infection at the portal of viral entry (J. Natterman *et al.*, *Antivir Ther* 10, 95 (2005) and L. Shang *et al.*, *J Immunol* 193, 277 (2014); both of which are incorporated by reference herein). Only two functionally identical HLA-E alleles are present in the human population (R. K. Strong *et al.*, *J Biol Chem* 278, 5082 (2003); incorporated by reference herein). Therefore, a vaccine platform specifically inducing MHC-E restricted T cells could result in a truly universal CD8$^+$ T cell vaccine with every vaccinated individual mounting identical T cell responses impervious to HIV Nef-mediated immune evasion. Indeed, as disclosed herein, MHC-E-restricted CD8$^+$ T cells are strongly elicited by strain 68-1 RhCMV vectors, which have shown unparalleled protection against SIV in macaques (Hansen *et al.* (2009), *supra*; Hansen *et al.* (2011), *supra*; Hansen *et al. Nature* (2013), *supra*). Thus, a HIV vaccine that induces responses against both classical as well as non-classical epitopes might provide the necessary breadth of T cell responses required to effectively block viral replication and subsequently blunt viral transmission.

As mentioned above, HCMV encodes the glycoprotein UL40 (the RCMV homolog is Rh67), that contains the 9-mer peptide (VMAPRTLLL, Rh67$_{8-16}$ VL9) binding the MHC-E peptide

binding groove with extremely high affinity (P. Tomasec *et al.*, *Science* 287, 1031 (2000); incorporated by reference herein). Since the *in vitro* data indicated that VL9 competes for binding with antigen-derived peptides the possibility that deletion of Rh67 (UL40) from the genome of RhCMV 68-1 would further increase the frequency of HLA-E restricted CD8$^+$ T cells *in vivo* was considered. To examine this possibility, Rh67 (UL40) was deleted from the 68-1 RhCMV/gag vector. The resulting recombinant virus was inoculated into an animal that was naturally infected with RhCMV. At days 0, 7, 14, 21, 28 and 42 post-inoculation, PBMC were obtained, and the frequency of CD8$^+$ T cells responding to total SIVgag as well as MHC-E was measured by intracellular cytokine staining using specific peptides. As shown in Fig. 5, SIVgag responses to total SIVgag were detectable beginning at day 14 post-inoculation. In addition, CD8$^+$ T cells responded to MHC-II-restricted "supertope" peptides Gag53 and Gag73. Contrary to our expectations however, there was no increase of T cell responses to HLA-E-specific supertopes. In fact, no responses were detected to either HLA-E peptides examined in this experiment (Gag69 and Gag120). This surprising result therefore suggests that vectors lacking UL40 and UL128 and UL130 induce MHC-II restricted CD8$^+$ T cells, including MHC-II restricted supertopes, but not MHC-E-restricted CD8$^+$ T cells. Induction of MHC-E restricted CD8$^+$ T cells thus requires the presence of UL40 and the absence of UL128 and UL130.

To determine whether, in addition to Rh67 (UL40), RhCMV encodes additional genes that are required for the induction of HLA-E and/or MHC-II-restricted T cell responses by RhCMV lacking UL128-130, gene regions that are non-essential for growth *in vitro* were deleted from RhCMV 68-1, and the T cell response upon inoculation of rhesus macaques was monitored. While most deletion mutants did not affect T cell specificities, it was observed that deletion of the gene region Rh214-Rh220 eliminated the ability of RhCMV 68-1 to elicit MHC-E restricted responses, whereas MHC-II restricted CD8$^+$ T cell responses were still observed (Figs. 29 and 30). This result was unexpected since the Rh214-Rh220 region encodes five copies of genes that are homologous to (*i.e.*, homologs of) HCMV US28: Rh214, Rh215, Rh216, Rh218 and Rh220 (also known as RhUS28.4, RhUS28.3, RhUS28.2, RhUS28.1, and RhUS28.5, respectively, M. E. Penfold *et al. J Virol* 77: 10404 (2003) incorporated by reference herein). The previously

predicted open reading frames Rh217 and Rh219 are not considered to represent functional genes based on a series of previously described criteria (D. Malouli *et al., J Virol* 86, 8959 (2012) incorporated by reference herein). HCMV US28 encodes a G-protein coupled receptor that binds to CC-chemokines (J.L. Gao and P. M. Murphy *J Biol Chem* 269: 28539 (1993)) and

5    chemokine binding was confirmed for at least one of the five RhCMV homologues (M. E. Penfold *et al.* J Virol 77: 10404 (2003)). However, a requirement of US28 for the induction of MHC-E restricted T cell responses was unexpected. This surprising result therefore suggests that vectors lacking US28, UL128, and UL130 induce MHC-II restricted CD8$^+$ T cells, including MHC-II restricted supertopes, but not MHC-E-restricted CD8$^+$ T cells. Induction of MHC-E

10    restricted CD8$^+$ T cells thus requires the presence of US28 and UL40, and the absence of UL128 and UL130.


Materials and Methods:

Rhesus macaques: A total of 46 purpose-bred male or female rhesus macaques (RM)

15    (*Macaca mulatta*) of Indian genetic background were used in the experiments reported in this example, including 9 RM vaccinated with strain 68-1 RhCMV/gag,  RM vaccinated with strain 68-1.2 RhCMV/gag, 1 RM inoculated with Rh67-deleted 68-1 RhCMV/gag, 7 RM vaccinated with MVA/gag, 19 unvaccinated RM with SIV infection, and 6 unvaccinated RM naturally infected with colony-circulating strains of RhCMV. All RM were used with the approval of the Oregon

20    National Primate Research Center Institutional Animal Care and Use Committee, under the standards of the US National Institutes of Health Guide for the Care and Use of Laboratory Animals. RM used in these experiments were free of cercopithicine herpesvirus 1, D-type simian retrovirus, and simian T-lymphotrophic virus type 1. Selected RM were MHC-I-genotyped by deep sequencing. Briefly, amplicons of Mamu class I sequences were generated via

25    amplification of cDNA by PCR using high-fidelity Phusion$^{TM}$ polymerase (New England Biolabs) and a pair of universal MHC-I-specific primers with the following thermocycling conditions: 98°C for 3min, (98 °C for 5s, 57 °C for 1s, 72 °C for 20s) for 23 cycles, and 72°C for 5 min. Each PCR primer contained a unique 10 bp Multiplex Identifier (MID) tag along with an adaptor sequence

for 454 Sequencing™ (5'-GCCTCCCTCGCGCCATCAG-MID-GCTACGTGGACGACACG-3'; 5'-GCCTTGCCAGCCCGCTCAG-MID-TCGCTCTGGTTGTAGTAGC-3'). Resulting amplicons span 190 bp of a highly polymorphic region within exon two. The primary cDNA-PCR products were purified using AMPure XP magnetic beads (Beckman Coulter Genomics). Emulsion PCR and

5   pyrosequencing procedures were carried out with Genome Sequencer FLX instruments (Roche/454 Life Sciences) as per the manufacturer's instructions. Data analysis was performed using a Labkey database in conjunction with Geneious-Pro® bioinformatics software (Biomatters Ltd.) for sequence assembly.

RhCMV/SIV Vectors: The construction, characterization, and administration of strain 68-

10  1-derived RhCMV/SIV have been previously described in detail (Hansen *et al.* (2009), *supra*; Hansen *et al.* (2011), *supra*; Hansen *et al. Nature* (2013), *supra*; Hansen *et al. Science* (2013), *supra*; Hansen *et al.* (2010), *supra*). All recombinant viruses used in this study were derived from strain RhCMV 68-1 BAC. Due to tissue culture adaptation, RhCMV 68-1 constructs contain a deletion of ORF 157.5 and most of ORF Rh157.4 encoding homologs of HCMV UL128 and

15  UL130, respectively (Hansen, S.G. *et al., J Virol* 77, 6620 (2003); incorporated by reference herein).

To generate a vector that lacks UL40 expression, ORF Rh67 was deleted from RhCMV 68-1 by BAC recombineering. Briefly, Rh67 was replaced with a FRT-flanked Kanamycin-resistance gene-containing PCR fragment by homologous recombination, followed by excision of the

20  KanR-gene using FLP recombinase. Virus was recovered in rhesus fibroblasts and characterized for antigen expression and loss of Rh67(UL40).

To generate a vector with complete UL128-130 expression, the SIVgag expression cassette was inserted into Rh211 of RhCMV 68-1.2, a recombinant virus in which Rh61/Rh60 (UL36), Rh157.4 (UL130), and Rh157.5 (UL128) had been repaired (A. E. Lilja and T. Shenk, *Proc*

25  *Natl Acad Sci U.S.A.* 105, 19950 (2008); incorporated by reference herein). All of the recombinant viruses were characterized and confirmed by restriction digest, and antigen inserts, including their flanking regions, were sequence verified. Expression of SIV antigens was verified by immunoblot. Additionally, adjacent gene expression was verified by RT-PCR.

Other Vaccines

MVA/gag was constructed by insertion of codon-optimized, full-length SIVmac239 gag gene into the MVA shuttle vector, pLW44, under the control of MH5, an early/late vaccinia promoter, to generate the recombinant plasmid, pJV7. Flanking sequences within pLW44 directed insertion of the recombinant construct into the thymidine kinase locus by homologous recombination. Chicken embryonic fibroblast cells were transfected with pJV7 followed by infection with MVA strain 1974 to generate recombinant virus expressing SIVmac239gag (SIVgag expression confirmed by immunoblot). Recombinant virus was plaque-purified and amplified in large-scale culture. Viral stocks were purified over a 24–40% sucrose gradient followed by pelleting through a 36% sucrose cushion with the pellet then suspended in 1 mM Tris-Cl, pH 9.0. For MVA/gag vaccination, RM were administered $10^8$ plaque-forming units of this vector via intramuscular injection.

Antigens and Antigen-Presenting Cells: Sequential 15-mer peptides (overlapping by 11 amino acids) comprising the SIVgag protein were obtained from the NIH AIDS Reagent Program. Synthesis of specific 9-14-mer peptides within these proteins was performed by Genscript (Piscataway, NJ). All peptides are identified by the position of their inclusive amino acids from the n-terminus (e.g., $Gag_{xx-yy}$). Consecutive 15-mers are also designated by their position starting from the n-terminal 15-mer (e.g., $Gag_{1-15}$ is 15mer #1; $Gag_{5-19}$ is 15mer #2, etc.). Unless otherwise specified, these peptides were used in T cell assays used at 2 μg/ml. Autologous B-lymphoblastoid cell lines (BLCL) were generated by infecting rhesus PBMC with Herpesvirus papio, as described previously (Hansen et al. Science (2013), supra). Mammalian expression vectors for Mamu class I molecules were generated by ligating each allele into pCEP4 KpnI/NotI or HindIII/NotI restriction sites. Plasmids were cloned in DH5α E. coli (Life Technologies, Grand Island, NY), sequence confirmed, and electroporated into MHC-I-negative K562, 721.221, or RMA-S (K. S. Anderson et al., J Immunol 151, 3407 (1993); incorporated by reference herein) cells using Nucleofector II/Kit C (Lonza, Allendale NJ). Transfectants were maintained on drug selection (Hygromycin B) and routinely confirmed for surface expression of MHC-I by staining with pan-MHC-I antibody clone W6/32. Throughout use in T cell assays, mRNA from MHC-I

transfectants was extracted using the AllPrep DNA/RNA Mini Kit (Qiagen), amplified by RT-PCR using primer pairs flanking a highly polymorphic region within exon 2, and sequence confirmed. MHC-I transfectants and BLCL were pulsed with Gag peptide of interest at a final concentration of 10 μM for 90 minutes then washed three times with warm PBS and once with warm R10 to remove unbound peptide before combining with freshly isolated PBMC at an effector:target ratio of 10:1. In order to stabilize Mamu-E surface expression, Mamu-E transfectants were incubated at 27° C for 3 hours prior to use in assays and maintained at 27° C throughout peptide incubation until combined with effectors. Autologous SIV-infected target cells were generated by isolation of $CD4^+$ T cells from PBMC with CD4 microbeads and LS columns (Miltenyi Biotec), activation with a combination of IL-2 (vendor), Staphylococcus enterotoxin B (vendor), and anti-CD3 (NHP Reagent Resource), anti-CD28, and anti-CD49d mAbs (BD Biosciences), and spinoculation with sucrose-purified SIVmac239, followed by 3-4 days of culture. Prior to use in T cell assays, SIV-infected target cells were purified using CD4 microbeads and LS columns (Miltenyi Biotec), as previously described (J. B. Sacha *et al.*, *J Immunol* 178, 2746 (2007); incorporated by reference herein). Infected cell preparations were >95% $CD4^+$ T cells and >50% SIV-infected following enrichment and were used at an effector:target ratio of 40:1 (PBMC and isolated $CD8^+$ T cells) or 8:1 (T cell line effectors). In these experiments, uninfected, activated $CD4^+$ T cells served as negative control APCs (uninfected targets from $SIV^+$ RM were cultured with tenofovir (NIH AIDS Reagent Program, concentration)). To assess bulk MHC-I and MHC-E, SIV-infected $CD4^+$ T cells were generated as described above without post-infection purification and stained for surface MHC-I (clone W6/32), MHC-E (clone 4D12; anti-mouse IgG1 M1-14D12), CD3, CD4 and intracellular SIV Gag p27 capsid.

T Cell Assays: Mononuclear cell preparations for immunologic assays were obtained from blood with Ficoll-Paque (GE Healthcare). Purified $CD8^+$ T cells (>90% pure) were obtained from PBMC using CD8 microbeads and LS columns (Miltenyi Biotec). Epitope-specific T cell lines were prepared by stimulation of PBMC with irradiated, peptide-pulsed BLCL and subsequent culture in media containing IL-2 (vendor), with re-stimulation performed weekly. SIV-specific

CD8^{+} T cell responses were measured by flow cytometric ICS. Briefly, effector T cells (mononuclear cells, isolated CD8^{+} T cells, or T cell lines) were incubated with antigen (peptide, peptide-pulsed APCs, or SIV-infected CD4^{+} T cells) and co-stimulatory monoclonal antibodies (mAbs) to CD28 and CD49d (BD Biosciences) for 1 hour, followed by addition of Brefeldin A

5   (Sigma-Aldrich) for an additional 8 hours. Co-stimulation in the absence of antigen (no peptide, unpulsed APCs, or uninfected targets) served as background control. In restriction assays using MHC-I transfectants as APCs, co-stimulation in the presence of peptide-pulsed MHC-I-negative parental cell lines K562 or 721.221 cells served as additional negative controls. When indicated, mononuclear cells or antigen-presenting cells were pre-incubated for 1 hour with the following

10  blocking reagents: anti-MHC-I mAb (clone W6/32; 10 µg/ml), CLIP peptide (MHC-II-associated invariant chain, amino acids 89-100; 2 µg/ml), MHC-E-binding peptide VL9 (VMAPRTLLL; 20 µM), Mamu-A1*001:01-binding peptide CM9 (CTPYDINQM; 20 µM), or Mamu-A1*002:01-binding peptide GY9 (GSENLKSLY; 20 µM). Stimulated cells were fixed, permeabilized, and stained as previously described (Sacha *et al., The Journal of Immunology,* 178, 2746-2754

15  (2007); incorporated by reference herein) and flow cytometric analysis was performed on an LSR-II instrument (BD Biosciences). Analysis was done using FlowJo software (Tree Star), gating first on small lymphocytes followed by progressive gating on CD3^{+}, then CD4^{-}/CD8a^{+} T cell subsets. Antigen specific response frequencies for resulting CD4^{-}/CD8a^{+} populations were determined from intracellular expression of TNF-α and IFN-γ. For epitope deconvolution

20  experiments, strict response criteria were used to prevent false positives. In these studies, a response to a given 15-mer peptide was considered positive if the frequency of events clustered as CD69^{+}, TNF-α^{+} and IFN-γ^{+} was >0.05%, with background <0.01% in at least 2 independent assays. The classification of individual peptide responses as blocked, shown in Figures 2A and 6, was based on >90% inhibition by blockade relative to the isotype control.

25  Define partial blockade. Responses that did not meet these criteria were considered indeterminate. To be considered MHC-E-restricted by blocking, the individual peptide response must have been (1) blocked by both anti-MHC-I clone W6/32 and MHC-E-binding peptide VL9, and (2) not blocked by CLIP.

Antibodies

The following conjugated Abs were used in these studies: a) from BD Biosciences, L200 (CD4; AmCyan), SP34-2 (CD3; PacBlu), SK1 (CD8a; TruRed, AmCyan), 25723.11 (IFNg; APC, FITC), 6.7 (TNF; APC), b) from Beckman Coulter, L78 (CD69; PE).

5    *Example 2 – Generation of CD8⁺ T cells specific for peptides of interest in the context of MHC-E*

T cell receptors recognizing antigen-derived peptides of interest in the context of classical, polymorphic MHC-Ia molecules can be used to transfect autologous T cells for immunotherapy of disease, such as cancer or infectious disease. A major obstacle to this approach is the MHC-Ia diversity in the human population that limits the use of a given TCR to

10   MHC-Ia matched patients. By generating TCR recognizing antigen-derived peptides of interest (*e.g.*, tumor antigen-derived peptides and pathogen-derived peptides) in the context of non-classical, non-polymorphic MHC-E molecules, MHC-matching becomes obsolete, and the resulting TCR can be used in all patients.

CD8⁺ T cells recognizing MHC-E/peptide complexes are rare in nature, and there is not

15   currently a reliable method to generate such T cells directed against antigens of interest, such as tumor antigens, pathogen-derived antigens, tissue-specific antigens, or host self-antigens. The method described herein is based upon the finding that a rhesus cytomegalovirus (RhCMV) lacking the genes Rh157.5 and Rh157.4 (homologs of HCMV UL128 and UL130) elicits MHC-E-restricted CD8⁺ T cells in rhesus monkeys at a frequency of about 1 peptide epitope per 30-40

20   amino acids of protein sequence. By inserting an antigen of interest into UL128 and 130-deleted RhCMV, CD8⁺ T cells directed against individual peptides presented by MHC-E can be generated. The MHC-E/peptide-recognizing TCRs can be identified by any of a number of methods but generally rely on sequencing the alpha and beta chains either directly by PCR from the cDNA of single cells, clonally expanded single cells, or deep sequencing pools of peptide

25   specific CD8⁺ T cells. Alternatively the sequence may be derived indirectly by expanding the RNA template by first creating a whole transcriptome library for a single cell, clonally expanded single cell, or pool of peptide specific CD8⁺ T cells. Peptide specific variable sequences may be generated by rapid amplification of cDNA ends (RACE) or switching mechanism at 5'end of RNA

template (SMART) protocols performed on the mRNA. PCR anchored in flanking constant regions or similarly from whole transcriptome libraries of single peptide reactive CD8^{+} cells can be sequenced directly or deep sequenced for their respective TCR variable regions. Validated combinations of alpha and beta chains derived from the TCR sequence of individual or pools of

5    peptide reactive CD8^{+} T-cells can further be synthesized or cloned. The resulting TCR constructs can then be transfected into T cells that can in turn be administered to patients as a therapy (*e.g.*, cancer therapy or infectious disease therapy). Methods of cloning and transfecting TCR variable regions are also discussed in Barsov EV *et al.*, PLoS One 6, e23703 (2011), which is incorporated by reference herein.

10   ***Example 3 - Broadly targeted CD8^{+} T cell responses restricted by major histocompatibility complex-E***

Major histocompatibility complex (MHC)-E is a highly conserved, ubiquitously expressed, non-classical, MHC-Ib molecule with limited polymorphism primarily involved in regulation of NK cell reactivity via interaction with NKG2/CD94 receptors. Here, priming of

15   rhesus macaques with Rh157.5/.4 gene-deleted RhCMV vectors uniquely diverts MHC-E function to presentation of highly diverse peptide epitopes to CD8α/β^{+} T cells, approximately 4 distinct epitopes per 100 amino acids, in all tested protein antigens. Since MHC-E is up-regulated on cells infected with HIV/SIV and other persistent viruses to evade NK cell activity, MHC-E-restricted CD8^{+} T cell responses have the potential to exploit pathogen immune evasion

20   adaptations, a capability that might endow these unconventional responses with superior efficacy.

Adaptive cellular immunity against intracellular pathogens is the primary responsibility of CD8^{+} T cells that recognize short (8-10mer) pathogen-derived peptide epitopes presented by highly polymorphic MHC-Ia molecules on the surface of infected cells (Neefjes J *et al.*, Nat Rev

25   Immunol 11, 823 (2011) and Nikolich-Zugich J *et al.*, Microbes Infect 6, 501 (2004); both of which are incorporated by reference herein). MHC-Ia allomorphs vary considerably in their peptide binding properties, and therefore the particular pathogen-derived peptides targeted by pathogen-specific CD8^{+} T cells is largely determined by the peptide binding specificity of the

limited number of MHC-Ia allomorphs expressed by the infected individual (Yewdell JW, Immunity 25, 533 (2006); incorporated by reference herein) Consequently, the epitopes recognized by CD8$^+$ T cells responding to the same pathogen are highly diverse across individuals. This recognition heterogeneity is important, as the nature of epitopes targeted by

5      CD8$^+$ T cell responses can have an enormous influence on the ability of the individual to clear or control various intracellular pathogens, in particular agents like HIV with a high intrinsic capacity for mutational immune escape (Nikolich Zugich (2004), *supra* and Goulder, P.J. and Watkins, D.I. Nat Rev Immunol 8, 619 (2008); incorporated by reference herein). From an evolutionary perspective, this MHC-Ia polymorphism-mediated response diversity allows large

10     populations to survive emerging pathogens because of the high likelihood that at least some members of the population will have MHC-Ia allomorphs that support effective CD8$^+$ T cell responses (Nikolich-Zugich (2004), *supra* and Prugnolle F *et al.*, Curr Biol 15, 1022 (2005); incorporated by reference herein). On the other hand, this biology inevitably results in certain individuals within a population being highly susceptible to a given pathogen, even when

15     vaccinated, which hampers efforts to develop universally effective vaccines based on CD8$^+$ T cell responses (Goulder and Watkins (2008), *supra* and Picker, L.J. *et al.*, Ann Rev Med 63, 95 (2012); incorporated by reference herein)

It was recently reported that SIV-targeted vaccine vectors based on strain 68-1 (fibroblast-adapted) RhCMV strikingly violate the above-described rules of MHC-Ia-restricted

20     CD8$^+$ T cell recognition (Hansen *et al. Science* (2013), *supra*), and offer a potential solution to MHC-Ia-dependent response diversity in CD8$^+$ T cell-targeted vaccination. In rhesus monkeys, RhCMV/SIV vectors provide profound protection against highly pathogenic SIV challenge, resulting in stringent control and ultimate clearance of infection (Hansen *et al.* (2011), *supra* and Hansen *et al. Nature* (2013), *supra*). These vectors elicit SIV-specific CD8$^+$ T cell responses

25     that are entirely non-overlapping with conventional MHC-Ia-restricted CD8$^+$ T cells, despite responding to 3-fold as many epitopes as conventional vaccines expressing the same SIV protein. Part of this lack of epitope overlap was explained by the finding that many of these epitopes were restricted by MHC-II molecules, rather than MHC-Ia, a rare, but not

unprecedented mode of epitope recognition by CD8$^+$ T cells (Hansen *et al. Science* (2013), *supra*). Strain 68-1 RhCMV/SIVgag vectors also elicited CD8$^+$ T cells that recognized multiple MHC-I-dependent epitopes (*e.g.*, responses entirely blocked by anti-MHC-I antibodies) that were common to most, or even all MHC-disparate macaques, an unprecedented degree of

5  cross-recognition for MHC-Ia-restricted CD8+ T cell responses. Indeed, two epitopes in the SIV gag protein (SIVgag$_{276-284}$ and SIVgag$_{482-490}$) were targeted by 42 of 42 strain 68-1 RhCMV/SIVgag vector-immunized monkeys in the previous report (Hansen *et al. Science* (2013), *supra*), and CD8$^+$ T cell responses to these two 9mer epitopes have since been documented in 120 of 120 monkeys inoculated with this vector (Fig. 14).

10     To understand the basis of this unusually universal MHC-I-dependent recognition, 4 strain 68-1 RhCMV/SIVgag vector-vaccinated monkeys were selected for detailed MHC-I restriction analysis. These macaques displayed robust, unconventional MHC-I-dependent CD8$^+$ T cell responses to SIVgag, including responses to the SIVgag$_{276-284}$ and SIVgag$_{482-490}$ supertopes, as well as 10 other commonly recognized responses. The expressed MHC-I genes, both classical

15  MHC-Ia and non-classical MHC-Ib (Wiseman, R.W. *et al.*, Nat Med 15, 1322 (2009); incorporated by reference herein), were sequenced in each monkey, and a panel of MHC-I transfectants singly expressing these MHC-I molecules was constructed individually (Fig. 15). These single MHC-I molecule transfectants were then used in a flow cytometric intra-cellular cytokine staining (ICS) assay to present the epitopic 15mer peptides to the strain 68-1 RhCMV/SIVgag

20  vector-induced CD8$^+$ T cells from these monkeys (using parental MHC-I-negative and autologous B lymphoblastoid cell lines as negative and positive controls, respectively) (Figs. 11A, 11B; and 16). Remarkably, classical MHC-Ia allomorphs were able to present only 3 of the 12 epitopic peptides to these T cells (Mamu-A1*001:01: SIVgag$_{69-83}$(18) and SIVgag$_{197-211}$(50); Mamu-A1*002:01: SIVgag$_{129-143}$(33)), and expression of these allomorphs in monkeys did not

25  track with these epitope-specific CD8$^+$ T cell responses (*e.g.*, many monkeys lacking these allomorphs were still able to recognize these 3 peptides; Fig. 17). However, all 12 epitopic peptides stimulated CD8$^+$ T cells from all monkeys when presented by non-classical MHC-E molecules, and indeed, all peptides were presented by transfectants expressing 3 different

rhesus monkey MHC-E allomorphs (Mamu-E02:04, -E02:11, and -E02:20), independent of whether the responses originated in monkeys that expressed these alleles, as well as by a transfectant expressing a human version of this molecule (HLA-E*01:03) (Figs. 11A, 11B, 16 and 18).

5        MHC-E is known to avidly bind canonical VMAPRTL(LVI)L peptides and other closely related 9mer peptides that are derived from positions 3-11 of MHC-Ia leader sequences for presentation to NKG2A (and to a lesser extent, NKG2C) molecules on NK cells (Lee, N. *et al.*, J Immunol 160, 4951 (1998); Braud, V.M. *et al.*, Nature 391, 795 (1998); Sullivan, L.C. *et al.*, Tissue Antigens 72, 415 (2008); and van Hall, T. *et al.*, Microbes Infect 12, 910 (2010); all of
10     which are incorporated by reference herein). This highly conserved interaction delivers a predominately inhibitory signal to NK cells when cells express normal levels of MHC-Ia. However, upon interference with MHC-Ia biosynthesis by viral infection or neoplastic transformation, this inhibitory signal is reduced, facilitating NK cell activation in response to virally-infected or neoplastic cells (Lodoen, M.B. and Lanier, L.L. Nat Rev Microbiol 3, 59 (2005)
15     and Wieten L *et al.*, Tissue Antigens 84, 523 (2014); both of which are incorporated by reference herein). Although a subset of CD8$^+$ T cells can also express NKG2A and/or NKG2C (Arlettaz L *et al.*, Eur J Immunol 34, 3456 (2004); incorporated by reference herein), phenotypic analysis of MHC-E-dependent, strain 68-1 RhCMV/SIVgag vector-elicited CD8$^+$ T cells revealed the vast majority of responding cells were CD8α/β$^+$, TCR γ/δ$^-$ T cells that lack both NKG2A and
20     NKG2C expression (Figs. 11C and 19). Moreover, pre-incubation of MHC-E transfectants or PBMC with a canonical MHC-E-binding VMAPRTLLL (VL9) peptide prior to specific peptide loading specifically blocked CD8$^+$ T cell recognition of all 12 peptides (Figs. 11D and 20), suggesting that the T cell recognition of these peptides is not mediated by NKG2A/C binding to peptide-loaded MHC-E, but rather reflects MHC-E-restricted epitope presentation to antigen-
25     specific T cells. Indeed, each of the parent 15mers studied could be truncated to an optimal 9mer peptide that was common among different strain 68-1 RhCMV/SIVgag vector-vaccinated monkeys with responses to the parent 15mer (Fig. 21) (Hansen *et al. Science* (2013), *supra*). These optimal 9mers could trigger CD8$^+$ T cells from these monkeys when pulsed on Mamu-E

transfectants at doses less than 1 nM (Fig. 22), functional avidities that are comparable to T cell recognition of classically MHC-Ia-restricted epitopes (O'Connor DH *et al.*, Nat Med 8, 493 (2002); incorporated by reference herein). Taken together, these data strongly suggest the unconventional, MHC-I-dependent CD8$^+$ T cells elicited by strain 68-1 RhCMV/SIVgag vectors are SIVgag-specific CD8$^+$ T cells that are primarily restricted by MHC-E, although in some cases can also recognize their specific peptide on conventional MHC-Ia allomorphs.

MHC-E-restricted CD8$^+$ T cell responses have been previously identified in HCMV, Hepatitis C virus, *Mycobacterium tuberculosis*, and *Salmonella enterica* infections, typically involving epitopes that are structurally related to the canonical MHC-Ia leader sequence peptides, but foreign to the host (Sullivan (2008), *supra*; van Hall (2010), *supra*; Pietra G *et al.*, J Biomed Biotechnol 2010, 907092 (2010); and Caccamo N *et al.*, Eur J Immunol 45, 1069 (2015); all of which are incorporated by reference herein). To determine the extent to which MHC-E restricts responses to SIVgag in different settings, blocking with high affinity MHC-E-binding peptide VL9 (in conjunction with blocking with anti-MHC-II CLIP peptide and anti-MHC I mAb W6/32) was used to restriction-classify all SIVgag epitope-specific CD8$^+$ T cell responses in monkeys vaccinated with strain 68-1 RhCMV/SIVgag vectors (Rh157.5/.4 gene-deleted), strain 68-1.2 RhCMV/SIVgag vectors (Rh157.5/.4-intact), ΔRh157.5/.4 strain 68-1.2 RhCMV/SIVgag vectors (in which the Rh157.5/.4 genes were specifically re-deleted; Fig. 23), and Modified Vaccinia Ankara (MVA)/SIVgag vectors, as well as monkeys infected with SIV itself (Figs. 12, 24 and 25). This analysis revealed that essentially all SIVgag epitope-specific responses in strain 68-1 RhCMV/SIVgag vector- and ΔRh157.5/.4 strain 68-1.2 RhCMV/SIVgag vector-vaccinated monkeys were either >90% blocked by the CLIP peptide or by both the anti-MHC-I mAb W6/32 and the VL9 peptide, demonstrating that the unconventional T cell responses elicited by Rh157.5/.4-deficient RhCMV are effectively entirely of either MHC-II- or MHC-E-restricted CD8$^+$ T cells.

In contrast, all SIVgag-specific CD8$^+$ T cell responses in the MVA/SIVgag vector-vaccinated and the strain 68-1.2 (Rh157.5/.4-expressing) RhCMV/SIVgag vector-vaccinated macaques were blocked by mAb W6/32, but not the VL9 peptide, indicating classical MHC-Ia

restriction. This was also the case for 98% of CD8$^+$ T cell responses in SIV-infected macaques, with the exception of 4 MHC-II-restricted CD8$^+$ T cell responses. The ability of Rh157.5/.4-deficient RhCMV vectors to elicit MHC-E- and MHC-II-restricted CD8$^+$ T cells is not limited to SIVgag-specific responses. Similar mixtures of MHC-E- and MHC-II-restricted, antigen-specific

5      CD8$^+$ T cell responses were observed with strain 68-1 (Rh157.5/.4-deficient) RhCMV vectors encoding SIVpol97-441, *M. tuberculosis* proteins (Ag85B, ESAT6 and RpfA), as well as intrinsic RhCMV proteins such as the Immediate Early-1 (IE1) protein (Figs. 12B and 26).

It has been previously reported that CD8$^+$ T cells elicited by strain 68-1 RhCMV/SIV vectors recognize autologous SIV-infected CD4$^+$ T cells, and that this recognition is partially

10     blocked by the anti-MHC-I mAb W6/32 and by the MHC-II-blocking CLIP peptide (Hansen *et al.* *Science* (2013), *supra*). To determine the contribution of MHC-E restriction to the MHC-I component of this recognition, it was asked whether the high affinity MHC-E-binding VL9 peptide could substitute for mAb W6/32 in blocking these responses. This experiment demonstrated that the combination of the MHC-II-blocking CLIP peptide and either mAb W6/32

15     or the VL9 peptide blocks these responses completely, whereas SIV-infected autologous cell recognition by SIVgag-specific CD8$^+$ T cells elicited by MVA/SIVgag vector- or strain 68-1.2 RhCMV/gag vector-vaccination or SIV infection was insensitive to the CLIP + VL9 peptide combination (Fig. 12C). Taken together, these data confirm that strain 68-1 RhCMV vectors uniquely elicit CD8$^+$ T cell responses that are either MHC-II or MHC-E-restricted, and that this

20     unusual immunobiology is a specific consequence of deletion of the RhCMV Rh157.5/.4 genes, which are orthologs of the HCMV UL128/UL130 genes and encode 2 components of the pentameric receptor complex involved in CMV infection of non-fibroblasts (Lilja AE and Shenk T, Proc Natl Acad Sci U.S.A. 105, 19950 (2008); incorporated by reference herein). Moreover, these data confirm that at least some of the epitopes recognized by these MHC-E-restricted

25     CD8+ T cells are naturally processed and presented by cells infected by SIV, a heterologous (non-CMV) pathogen.

Among 42 strain 68-1 RhCMV/SIVgag vector-vaccinated monkeys, a median of 20 distinct CD8$^+$ T cell-recognized, MHC-E-restricted, SIVgag 15mer epitopes per animal were

identified, a breadth that exceeds the median 11 and 14.5 distinct MHC-1a-restricted SIVgag-specific epitopes identified within SIVgag-specific CD8$^+$ T cell responses elicited by conventional vaccines or SIV infection, respectively (Fig. 13A). The density of MHC-E-restricted epitopes (~4 independent MHC-E-restricted epitopes per 100 amino acids of protein length) is similar among

5 all strain 68-1 RhCMV vector-elicited CD8$^+$ T cell responses, regardless of nature of the antigen analyzed (Fig. 13B). Notably, among the same 42 strain 68-1 RhCMV/SIVgag vector-vaccinated macaques, 109 of the 125 overlapping SIVgag 15mer peptides (87%) were recognized by MHC-E-restricted CD8$^+$ T cells in at least one macaque (Fig. 13C). Although MHC-E has previously been shown to bind a broader array of peptides than the canonical leader sequence peptides

10 (van Hall (2010), *supra* and Lampen *et al.*, *supra*), the extent of epitope diversity and breadth observed is highly surprising, especially given the limited polymorphism of MHC-E and the observation that the presentation of all MHC-E-restricted epitopes tested to date is independent of this limited sequence polymorphism as well as the sequence difference between Mamu-E and HLA-E (Figs. 11B, 18 and 22). These data suggest that MHC-E-mediated

15 epitope presentation (*e.g.,* MHC-E peptide binding) is even more diverse than previously believed. In keeping with this, sequence analysis of 11 optimal MHC-E-restricted SIVgag 9mer epitopes showed only one epitope (the Gag$_{273-287}$ supertope) with a canonical (M at position 2: L at position 9) MHC-E-binding motif, whereas the other 10 optimal epitopes not only lacked this motif, but manifested no statistically significant overlap with previously characterized sets

20 of MHC-E bound peptides (Lampen *et al.*, *supra*) (Fig. 13D). Indeed, the other SIVgag$_{482-490}$ supertope manifested what could be considered an anti-MHC-E peptide-binding motif with lysines at both positions 2 and 9 (Fig. 13D). The molecular mechanisms for loading and binding of epitopic peptides to MHC-E are discussed in Hansen, S.G. *et al.*, "Broadly targeted CD8+ T cell responses restricted by major histocompatibility complex E", Science, published electronically

25 on Jan 21, 2016, which is incorporated by reference herein.

Both HCMV and RhCMV encode proteins with a strategically embedded canonical VL9 peptide within the UL40 and Rh67 genes, respectively (Prod'homme, V. *et al.*, J Immunol 188, 2794 (2012) and Richards, R. *et al.*, J Virol 85, 8766 (2011); both of which are incorporated by

reference herein). The VL9 peptide of UL40 was shown to be loaded onto nascent MHC-E chains by a TAP-independent mechanism, and therefore functions to stabilize and up-regulate MHC-E expression in HCMV-infected cells in the face of virus-mediated TAP inhibition and profound MHC-Ia down-regulation mediated by the HCMV US2-11 gene products (Lodoen & Lanier (2005), *supra* and Prod'homme (2012), *supra*). A similar function is likely for RhCMV Rh67 (Richards (2011), *supra*). MHC-E up-regulation is therefore thought to be a key viral strategy for evading the NK cell response to infected cells that lack MHC-Ia expression. However, this evasion strategy would have the consequence of enhancing MHC-E expression in virally infected cells, increasing the opportunity for loading and presentation of novel peptides to MHC-E-restricted T cells. In this regard, the canonical MHC-E binding VL9 peptide might act as a chaperone that facilitates stable high expression of MHC-E and delivery to an endosomal compartment that would facilitate peptide exchange, analogous to the invariant chain-associated CLIP peptide and MHC-II. Consistent with such a peptide exchange mechanism, MHC-E peptide loading has been directly demonstrated in the *M. tuberculosis* phagolysosome (Grotzke JE *et al.*, PLoS Pathog 5, e1000374 (2009); incorporated by reference herein).

CMV is not the only intracellular pathogen to up-regulate MHC-E expression. Hepatitis C also encodes an MHC-E-binding peptide which up-regulates MHC-E expression (Natterman J *et al.*, Am J Pathol 166, 443 (2005); incorporated by reference herein), and both HIV and SIV up-regulate MHC-E by an uncharacterized mechanism in concert with MHC-Ia down-regulation (Natterman J *et al.*, Antivir Ther 10, 95 (2005); incorporated by reference herein) (Fig. 27). This common adaptation suggests that, for these and likely other intracellular pathogens, the evolutionary pressure to up-regulate MHC-E to counter NK cell responses outweighs the potential risk of increased susceptibility to MHC-E-restricted CD8^{+} T cells, perhaps because MHC-E-restricted CD8^{+} T cells are poorly primed during infection by these agents. The reason MHC-E-restricted CD8^{+} T cell responses are such a minor component of the modern mammalian immune system is unclear, especially given the finding in this report that such responses can be quite diverse and broad (although, arguably, less diverse and broad on a population level than polymorphic MHC-1a; Fig. 28). However, Rh157.5/.4 gene-deleted RhCMV vectors are able to

bypass the intrinsic constraint of MHC-E-restricted CD8$^+$ T cell priming. Although the mechanism by which this bypass is accomplished remains to be elucidated, the ability of these vectors to strongly elicit broad, diverse and MHC-Ia haplotype-independent CD8$^+$ T cell responses offers the opportunity to develop MHC-E-restricted, CD8$^+$ T cell-targeted vaccines

5      that exploit MHC-E up-regulation, an intrinsic vulnerability in the immune-evasion strategies of many highly adapted persistent pathogens. Moreover, because of limited MHC-E polymorphism, an MHC-E-restricted CD8$^+$ T cell response-targeted vaccine would elicit largely similar responses in all or most vaccinees, potentially providing for efficacy in all individuals regardless of MHC genotype. Evolution may have disfavored MHC-E as a primary restricting

10     molecule for CD8$^+$ T cells in modern mammals in lieu of the polymorphic MHC-Ia system, but if HCMV vectors are able to recapitulate in humans the biology of Rh157.5/.4 gene-deleted RhCMV vectors in macaques (or if alternative, non-CMV-based strategies to elicit broadly targeted MHC-E-restricted CD8$^+$ T cell responses can be developed), vaccinologists may be able to resurrect this dormant MHC-E-based adaptive immune system to attack pathogens with

15     novel immune responses that they are not adapted to effectively evade.

Materials and Methods:

       Vaccines: The construction, characterization, and administration of 1) the strain 68-1 RhCMV vectors expressing SIV Gag and 5'-Pol, 2) the strain 68-1.2 RhCMV vector expressing SIV Gag, 3) the MVA and Adenovirus 5 (Ad5) vectors expressing SIV Gag, and 4) the SIV Gag-

20     encoding DNA + IL-12 vaccine have been previously reported (Hansen *et al.* Science (2013), *supra*; Hansen *et al.* (2011), *supra*; Hansen *et al. Nature* (2013), *supra*; and Hansen *et al.* (2009), *supra*). A strain RhCMV 68-1 expressing a fusion protein of the *M. tuberculosis* gene products RpfA, RpfC and RpfD driven by an MCMV IE promoter and inserted into the 5' region of Rh211 was provided by Aeras (Rockville, MD, USA). A Rh157.5 (UL128) − Rh157.4 (UL130) double

25     deletion mutant based on strain 68-1.2 RhCMV/gag was also constructed by homologous recombination. To accomplish this, recombination primers flanking the target region (forward mutagenesis primer 5'-AAAACTATAATCAACAACTCTATACCTTTGTTTTGCTGATGCTA TTGCGT-3' and reverse mutagenesis primer 5'-ATTTTTCGATAAAAAAATCACAGCAAACATACTG

GTTTTACACACTTTAT-3') were designed. Since the Rh157.6 (UL131A) and Rh157.4 (UL130) open reading frames (ORFs) overlap in RhCMV, the deletion was constructed in a fashion that retained the end of the Rh157.6 (UL131A) ORF plus additional 50 bp to ensure expression of the encoded protein. Mini plasmid R6K-kan-F5 was used to amplify a kanamycin resistance cassette

5    flanked by alternative (F5) FRT sites using the forward primer binding site (5'-GAAAAGTGCCACCTGCAGAT-3') and reverse primer binding site (5'-CAGGAACACTTAACGGCTGA-3'), which were added to the 3' end of the mutagenesis primers. E/T homologous recombination in *E. coli* strain SW105 (Warming S *et al.*, Nucleic Acids Res 33, e36 (2005); incorporated by reference herein) was performed as published elsewhere (Muyrers JP *et al.*,

10   Nucleic Acids Res 27, 1555 (1999); incorporated by reference herein). Successful deletion of the targeted ORFs was confirmed by performing polymerase chain reactions on viral DNA and cDNA of infected cells with primers specific for the deleted and neighboring genes. Expression of the SIVmac239gag transgene was confirmed by immunoblot analysis of primary rhesus fibroblasts infected with the ΔRh157.5 (UL128) – Rh157.4 (UL130) 68-1.2 RhCMV/gag vector. See Fig. 23

15   for depiction of genomic differences between strain 68-1, 68-1.2, and ΔRh157.5/Rh157.4 (ΔUL128/UL130) RhCMV vectors.

Rhesus Macaques: A total of 207 purpose-bred male or female rhesus macaques (*Macaca mulatta*) of Indian genetic background were used in the experiments reported in this example, 88 of which were also studied in a previous report (Hansen *et al. Science* (2013),

20   *supra*). These macaques included 159 macaques vaccinated with strain 68-1 RhCMV vectors expressing SIVgag, SIV5'-pol, TB-ESAT-6/Ag85B or TB-RpfA/RpfC/RpfD inserts (62 previously reported); 9 macaques vaccinated with strain 68-1.2 RhCMV/gag vectors (6 previously reported); 4 macaques vaccinated with ΔRh157.5/.4-deleted strain 68-1.2 RhCMV/gag vectors (none previously reported); 11, 3, and 4 macaques vaccinated with SIVgag-expressing MVA/gag,

25   Ad5/gag, and DNA/gag+IL-12 vaccines, respectively (3, all, and all previously reported, respectively); 13 unvaccinated macaques with controlled SIVmac239 infection (plateau phase plasma viral loads < 10,000 copies/ml; 6 previously reported); and 4 unvaccinated macaques naturally infected with ONPRC colony-circulating strains of RhCMV (all previously reported). All

macaques were used with the approval of the Oregon National Primate Research Center Institutional Animal Care and Use Committee, under the standards of the US National Institutes of Health Guide for the Care and Use of Laboratory Animals. Macaques used in these experiments were free of cercopithicine herpesvirus 1, D-type simian retrovirus, and simian T-lymphotrophic virus type 1. Selected macaques were MHC-I genotyped by deep sequencing, as described (Wiseman (2009), *supra*). Briefly, amplicons of Mamu class I sequences were generated via amplification of cDNA by PCR using high-fidelity Phusion™ polymerase (New England Biolabs) and a pair of universal MHC-I-specific primers with the following thermocycling conditions: 98° C for 3 minutes, (98° C for 5 seconds, 57° C for 1 second, 72° C for 20 seconds) for 23 cycles, and 72° C for 5 minutes. Each PCR primer contained a unique 10 bp Multiplex Identifier (MID) tag along with an adaptor sequence for 454 Sequencing™ (5'-GCCTCCCTCGCGCCATCAG-MID-GCTACGTGGACGACACG-3'; 5'-GCCTTGCCAGCCCGCTCAG-MID-TCGCTCTGGTTGTAGTAGC-3'). Resulting amplicons span 190 bp of a highly polymorphic region within exon two. The primary cDNA-PCR products were purified using AMPure XP magnetic beads (Beckman Coulter Genomics). Emulsion PCR and pyrosequencing procedures were carried out with Genome Sequencer FLX instruments (Roche/454 Life Sciences) as per the manufacturer's instructions. Data analysis was performed using a Labkey database in conjunction with Geneious-Pro® bioinformatics software (Biomatters Ltd.) for sequence assembly.

Antigens and Antigen-Presenting Cells: The synthesis of sequential 15-mer peptides (overlapping by 11 amino acids) comprising the SIVgag and pol, RhCMV IE1, and TB Ag85B, ESAT-6, and RpfA proteins, as well as specific 9-14mer peptides within these proteins, was performed by Intavis AG, based on the SIVmac239 Gag and Pol sequence (Genbank Accession #M33262), the strain 68-1 RhCMV IE-1 sequence (Genbank Accession #AY186194), or Erdman strain *M. tuberculosis* Ag85B, ESAT-6, and RpfA sequences (Genbank Accession #s BAL65871.1; BAL68013.1; and BAL64766.1, respectively). All peptides are identified by the position of their inclusive amino acids from the N-terminus (*e.g.*, Gag$_{xx-yy}$). Consecutive 15mers are also designated by their position starting from the N-terminal 15mer (*e.g.*, Gag1-15 (1) is 15mer #1;

Gag5-19 (2) is 15mer #2, *etc.*). Unless otherwise specified, these peptides were used in T cell assays at 2 µg/ml. Autologous B-lymphoblastoid cell lines (BLCL) were generated by infecting rhesus macaque PBMC with Herpesvirus papio, as previously described (Hansen *et al. Science* (2013), *supra*). Mammalian expression vectors for Mamu class I molecules were generated by ligating each allele into pCEP4 KpnI/NotI or HindIII/NotI restriction (Ulbrecht M *et al.*, J Immunol 164, 5019 (2000); incorporated by reference herein) sites. Plasmids were cloned in DH5α *E. coli* (Life Technologies), sequence confirmed, and electroporated into MHC-I-negative K562, 721.221, or RMAS cells (Anderson KS *et al.*, J Immunol 151, 3407 (1993); incorporated by reference herein) using Nucleofector II/Kit C (Lonza). Transfectants were maintained on drug selection (Hygromycin B) and routinely confirmed for surface expression of MHC-I by staining with pan-MHC-I antibody clone W6/32. Throughout use in T cell assays, mRNA from MHC-I transfectants was extracted using the AllPrep DNA/RNA Mini Kit (Qiagen), amplified by RT-PCR using primer pairs flanking a highly polymorphic region within exon 2, and sequence confirmed. MHC-I transfectants and BLCL were pulsed with peptides of interest at a final concentration of 10 µM for 90 minutes then washed three times with warm PBS and once with warm RPMI 1640 media with 10% fetal calf serum to remove unbound peptide before combining with freshly isolated PBMC at an effector:target ratio of 10:1. In order to stabilize Mamu-E surface expression, Mamu-E transfectants were incubated at 27° C for >3 hours prior to use in assays and maintained at 27° C throughout peptide incubation until combined with effectors. Autologous SIV-infected target cells were generated by isolation of CD4$^+$ T cells from PBMC with CD4 microbeads and LS columns (Miltenyi Biotec), activation with a combination of IL-2 (NIH AIDS Reagent Program), Staphylococcus enterotoxin B (Toxin Technologies Inc.), and anti-CD3 (NHP Reagent Resource), anti-CD28, and anti-CD49d mAbs (BD Biosciences), and spinoculation with sucrose-purified SIVmac239, followed by 3-4 days of culture. Prior to use in T cell assays, SIV infected target cells were purified using CD4 microbeads and LS columns (Miltenyi Biotec), as previously described (Sacha JB et al, J Immunol 178, 2746 (2007); incorporated by reference herein). Infected cell preparations were >95% CD4$^+$ T cells and >50% SIV-infected following enrichment and were used at an effector:target ratio of 40:1 (PBMC and isolated CD8+ T cells)

or 8:1 (T cell line effectors). In these experiments, uninfected, activated CD4+ T cells served as negative control APCs (uninfected targets from SIV+ macaques were cultured with 400 μM tenofovir (NIH AIDS Reagent Program)). To assess total MHC-I and MHC-E expression, SIV-infected CD4$^+$ T cells were generated as described above without post-infection purification and

5      stained for surface MHC-I (clone W6/32), MHC-E (clone 4D12; anti-mouse IgG1 clone M1-14D12), CD3, and CD4, followed by intracellular SIV Gag.

T Cell Assays: SIV-, RhCMV-, and TB-specific CD8$^+$ T cell responses were measured in mononuclear cell preparations from blood by flow cytometric ICS, as previously described (Hansen *et al. Science* (2013), *supra*). Briefly, mononuclear cells or isolated CD8$^+$ T cells were

10     incubated with antigen (peptides, peptide-pulsed BLCL or MHC-Ia or MHC-E transfectants, or SIV-infected autologous CD4$^+$ T cells) and the co-stimulatory molecules CD28 and CD49d (BD Biosciences) for 1 hour, followed by addition of Brefeldin A (Sigma-Aldrich) for an additional 8 hours. Co-stimulation without antigen served as the primary background control. The MHC association (MHC-Ia, MHC-E, MHC-II) of a response was determined by pre-incubating isolated

15     mononuclear cells, antigen-presenting cells or SIV-infected CD4$^+$ cells for 1 hour at room temperature (prior to adding peptides or combining effector and target cells and incubating per the standard ICS assay) in the presence of the following blockers: 1) the pan anti-MHC-I mAb W6/32 (10 mg/ml), 2) the MHC-II-blocking CLIP peptide (MHC-II-associated invariant chain, amino acids 89-100; 20μM), and 3) the MHC-E blocking VL9 peptide (VMAPRTLLL; 20 μM), alone

20     or in combination. In some experiments, the Mamu-A1*001:01-binding peptide CM9 (CTPYDINQM; 20 μM), or the Mamu-A1*002:01-binding peptide GY9 (GSENLKSLY; 20 μM) were used as blocking controls. Stimulated cells were fixed, permeabilized and stained as previously described (Hansen *et al. Science* (2013), *supra*), and flow cytometric analysis was performed on an LSR-II instrument (BD Biosciences). Analysis was done using FlowJo software (Tree Star). In

25     all analyses, gating on the light scatter signature of small lymphocytes was followed by progressive gating on the CD3$^+$ population and then the CD4$^-$/CD8$^+$ T cell subset. Antigen-specific response frequencies for CD8$^+$ T cell populations were routinely determined from intracellular expression of CD69 and either or both TNF-α and IFN-γ. For epitope deconvolution

experiments, strict response criteria were used to prevent false positives. In these studies, a response to a given 15mer peptide was considered positive if the frequency of events clustered as $CD69^+$, $TNF-\alpha^+$ and $IFN-\gamma^+$ was >0.05%, with background <0.01% in at least 2 independent assays. The classification of an individual peptide response as blocked was based on >90% inhibition by blockade relative to the isotype control. Responses that did not meet these criteria were considered indeterminate. To be considered MHC-E-restricted by blocking, the individual peptide response must have been blocked by both anti-MHC-I clone W6/32 and MHC-E-binding peptide VL9, and not blocked by the CLIP peptide. Minimal independent epitope numbers were estimated from the positive responses identified by testing of consecutive 15mer peptides by the following criteria: single positive peptide of same restriction type = 1 independent epitope; 2 adjacent positive peptides of same restriction type = 1 independent epitope; 3 adjacent positive peptides of same restriction type = 2 independent epitopes; 4 adjacent positive peptides of same restriction type = 2 independent epitopes; and 5 adjacent positive peptides of same restriction type = 3 independent epitopes.

Antibodies: The following conjugated antibodies were used in these studies: a) from BD Biosciences, L200 (CD4; AmCyan), SP34-2 (CD3; PacBlu), SK1 (CD8a; TruRed, AmCyan), 25723.11 (IFN-γ; APC, FITC), 6.7 (TNF; APC), MAb11 (TNF; Alexa700), b) from Beckman Coulter, L78 (CD69; PE), 2ST8.5H7 (CD8β; PE), Z199 (NKG2A/C or CD159a/c; PE), c) from Biolegend, W6/32 (pan-MHC-I; PE), OKT-4 (CD4; PE-Cy7), B1 (TCRγ/δ; Alexa647), d) from Miltenyi Biotec, M-T466 (CD4; APC), e) from eBiosciences, M1-14D12 (mouse IgG1; PE-Cy7). The following unconjugated antibodies were used in these studies: a) from Advanced BioScience Laboratories, 4324 (SIV Gag p27), b) from LSBio, 4D12 (HLA-E), c) W6/32 (pan-MHC-I). LIVE/DEAD Fixable Yellow Dead Cell Stain (LIFE Technologies) was used to assess cell viability.

Epitope Sequence Analysis: Sequence LOGOs were created using the Los Alamos HIV database tool Analyze Align (http://www.hiv.lanl.gov/content/sequence/ANALYZEALIGN/analyze_align.html), which was based on WebLogo3 {Crooks, 2004 #150}. Statistical enrichment or underrepresentation of amino acids in each position in the 11 optimal MHC-E 9-mer epitopes recognized in the

macaques, and for the 551 HLA-E eluted peptides from TAP-deficient cells published in Lampen *et al.*, *supra* were calculated using the Composition Profiler Tool (http://cprofiler.org/cgi-bin/profiler.cgi) (Vacic V *et al.*, BMC Bioinform 8, 211 (2007); incorporated by reference herein). The amino acid composition of each position in the 11 optimal peptides was compared to the amino acid frequencies found in SIVmac239 Gag (GenBank accession #M33262), the insert strain used for the vaccine. To compare the per position composition of the 11 optimal peptides to previously published peptides eluted from HLA-E in a TAP-deficient setting, the full set of 551 eluted peptides previously published in Lampen *et al.* was used. The peptides in Lampen *et al.* varied in length, between 8 and 13 amino acids; 9 was the most common length. They had used a motif searching algorithm to explore amino acid enrichment and under-representation among 315 9 mers in their eluted set (Fig. 2 in Lampen *et al.*) as position 2 and the C-terminal position was of most interest, regardless of length, a slightly different approach was taken to exploring their published data, and characterized an aligned version of all of their 551 eluted peptides. Gaps were added to maintain the alignment as needed after position 8, to enable a $2^{nd}$ position and aligned C-terminus evaluation including all peptides. Their data was compared for each alignment position to the amino acid frequencies found in natural proteins based on SwissProt 51 (Bairoch A *et al.*, Nucleic Acids Res 33, D154 (2005); incorporated by reference herein).

The sequence LOGO shown in Fig. 13D indicates the frequency of each amino acid in a given position (relative to their background frequency in SIVmac239 Gag) by the height of the letter, based on 11 optimal, MHC-E-restricted SIVgag 9mer peptide epitopes recognized by CD8+ T cells in strain 68-1 RhCMV vector-vaccinated macaques. The sequence LOGO in Fig. 13D is colored according to enrichment (boxes with grey fill or hatched boxes) or underrepresentation (boxes with white fill) among 551 peptides eluted from HLA-E in a TAP-deficient setting by Lampen *et al.* As shown in the right panel of Fig. 13D, amino acids enriched in the $2^{nd}$ and C-terminal anchor positions among the 551 Lampen *et al.* peptides were rare among our 11 optimal SIVgag peptides, while those that were significantly underrepresented were enriched.

It is 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 person skilled in the art and are to be included within the spirit and purview of this application.

5      All publications, patents, patent applications, internet sites, and accession numbers/database sequences including both polynucleotide and polypeptide sequences cited herein are hereby incorporated by reference herein in their entirety for all purposes to the same extent as if each individual publication, patent, patent application, internet site, or accession number/database sequence were specifically and individually indicated to be so

10    incorporated by reference.

## CLAIMS

1.      A cytomegalovirus (CMV) vector comprising:

(1) a first nucleic acid sequence encoding at least one heterologous antigen;

(2) a second nucleic acid sequence encoding at least one active UL40 protein, or an ortholog or homolog thereof; and

(3) a third nucleic acid sequence encoding at least one active US28 protein, or an ortholog or homolog thereof;

wherein the CMV vector does not express an active UL128 protein, or an ortholog thereof, and does not express an active UL130 protein, or an ortholog thereof.

2.      The CMV vector of claim 2, wherein the at least one heterologous antigen comprises a pathogen-specific antigen, a tumor antigen, a tissue-specific antigen, or a host self-antigen.

3.      The CMV vector of claim 2, wherein the host self-antigen is an antigen derived from the variable region of a T cell receptor (TCR) or an antigen derived from the variable region of a B cell receptor.

4.      The CMV vector of claim 2, wherein the pathogen-specific antigen is derived from a pathogen selected from the group consisting of: human immunodeficiency virus, simian immunodeficiency virus, herpes simplex virus, hepatitis B virus, hepatitis C virus, papillomavirus, Plasmodium parasites, and *Mycobacterium tuberculosis.*

5.      The CMV vector of claim 2, wherein the tumor antigen is related to a cancer selected from the group consisting of: acute myelogenous leukemia, chronic myelogenous leukemia, myelodysplastic syndrome, acute lymphoblastic leukemia, chronic lymphoblastic leukemia, non-Hodgkin's lymphoma, multiple myeloma, malignant melanoma, breast cancer, lung cancer, ovarian cancer, prostate cancer, pancreatic cancer, colon cancer, renal cell carcinoma (RCC), and germ cell tumors.

6.      The CMV vector of any one of claims 1-5, wherein the CMV vector does not express an active UL128 or UL130 protein, or orthologs thereof, due to the presence of one or more mutations in the nucleic acid sequence encoding UL128 or UL130, or orthologs thereof.

7.      The CMV vector of claim 6, wherein the one or more mutations in the nucleic acid sequence encoding UL128 or UL130, or orthologs thereof, are selected from the group consisting of: point mutations, frameshift mutations, truncation mutations, and deletion of all of the nucleic acid sequence encoding the protein.

8.      The CMV vector of any one of claims 1-7, wherein the CMV vector further comprises a fourth nucleic acid sequence, wherein the fourth nucleic acid sequence comprises an antisense sequence or an RNAi sequence that inhibits the expression of UL128 or UL130, or orthologs thereof.

9.      The CMV vector of any one of claims 1-8, wherein the CMV vector further comprises at least one inactivating mutation in one or more viral genes encoding viral proteins that are essential, non-essential, or augmenting for growth *in vivo*

10.     The CMV vector of claim 9, wherein the at least one inactivating mutation is selected from the group consisting of: point mutation, frameshift mutation, truncation mutation, and deletion of all of the nucleic acid sequence encoding the viral protein.

11.     The CMV vector of claim 9 or 10, wherein the at least one inactivating mutation is in UL82 (pp71)

12.     The CMV vector of any one of claims 9-11, wherein the at least one inactivating mutation is in US11.

13.     The CMV vector of any one of claims 1-12, wherein the CMV vector is a human CMV (HCMV) or rhesus CMV (RhCMV) vector.

14.     A method of generating an immune response to at least one heterologous antigen in a subject, the method comprising administering to the subject the CMV vector of any one of claims 1-13 in an amount effective to elicit a CD8$^+$ T cell response to the first heterologous antigen in the subject.

15.     The method of claim 14, wherein at least 10% of CD8$^+$ T cells elicited by the CMV vector are restricted by MHC-E, or an ortholog thereof.

16.     The method of claim 15, wherein at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 75%, at least 90%, at least 95%, or at least 95% of the CD8+ T cells elicited by the CMV vector are restricted by MHC-E, or an ortholog thereof.

17.     The method of claim 15 or 16, further comprising identifying a CD8$^+$ TCR from the CD8$^+$ T cells elicited by the CMV vector, wherein the CD8$^+$ TCR recognizes a MHC-E/heterologous antigen-derived peptide complex.

18.     The method of claim 17, wherein the CD8$^+$ TCR is identified by DNA or RNA sequencing.

19.     The method of any one of claims 14-18, wherein the subject has been previously exposed to CMV.

20.     The method of any one of claims 14-19, wherein the subject is a human or nonhuman primate.

21.    The method of any one of claims 14-20, wherein administering the CMV vector comprises subcutaneous, intravenous, intramuscular, intraperitoneal, or oral administration of the CMV vector.

22.    The method of any one of claims 14-21, further comprising administering a second CMV vector to the subject, wherein the second CMV vector comprises a nucleic acid sequence encoding at least one heterologous antigen.

23.    The method of claim 22, wherein the second CMV vector expresses an active UL128 protein.

24.    The method of claim 22 or 23, wherein the second CMV vector expresses an active UL130 protein.

25.    The method of any one of claims 22-24, wherein the at least one heterologous antigen of the second CMV vector is a pathogen-specific antigen, a tumor antigen, a tissue-specific antigen, or a host self-antigen

26.    The method of claim 25, wherein the host self-antigen is an antigen derived from the variable region of a TCR or an antigen derived from the variable region of a B cell receptor.

27.    The method of claim 25, wherein the pathogen-specific antigen of the second CMV vector is derived from a pathogen selected from the group consisting of: human immunodeficiency virus, simian immunodeficiency virus, herpes simplex virus, hepatitis B virus, hepatitis C virus, papillomavirus, Plasmodium parasites, and *Mycobacterium tuberculosis.*

28.    The method of claim 25, wherein the tumor antigen of the second CMV vector is related to a cancer selected from the group consisting of: acute myelogenous leukemia, chronic

myelogenous leukemia, myelodysplastic syndrome, acute lymphoblastic leukemia, chronic lymphoblastic leukemia, non-Hodgkin's lymphoma, multiple myeloma, malignant melanoma, breast cancer, lung cancer, ovarian cancer, prostate cancer, pancreatic cancer, colon cancer, renal cell carcinoma (RCC), and germ cell tumors.

29.     The method of any one of claims 22-24, wherein the at least one heterologous antigen of the first CMV vector and the second CMV vector are the same antigen.

30.     The method of any one of claims 22-29, wherein the second CMV vector is a HCMV or RhCMV vector.

31.     The method of any one of claims 22-30, wherein administering the second CMV vector comprises intravenous, intramuscular, intraperitoneal, or oral administration of the second CMV vector.

32.     The method of any one of claims 22-31, wherein the second CMV vector is administered before, concurrently with, or after the first CMV vector.

33.     A CMV vector comprising a nucleic acid sequence encoding at least one heterologous antigen, wherein the CMV vector:

      (1) does not express an active UL128 protein, or an ortholog thereof;

      (2) does not express an active UL130 protein, or an ortholog thereof; and

      (3) does not express an active protein selected from UL40 or US28, or an ortholog thereof.

34.     The CMV vector of claims 33, wherein the at least one heterologous antigen comprises a pathogen-specific antigen, a tumor antigen, a tissue-specific antigen, or a host self-antigen.

35.    The CMV vector of claim 34, wherein the host self-antigen is an antigen derived from the variable region of a TCR or an antigen derived from the variable region of a B cell receptor.

36.    The CMV vector of claim 34, wherein the pathogen-specific antigen is derived from a pathogen selected from the group consisting of: human immunodeficiency virus, simian immunodeficiency virus, herpes simplex virus, hepatitis B virus, hepatitis C virus, papillomavirus, Plasmodium parasites, and *Mycobacterium tuberculosis.*

37.    The CMV vector of claim 34, wherein the tumor antigen is related to a cancer selected from the group consisting of: acute myelogenous leukemia, chronic myelogenous leukemia, myelodysplastic syndrome, acute lymphoblastic leukemia, chronic lymphoblastic leukemia, non-Hodgkin's lymphoma, multiple myeloma, malignant melanoma, breast cancer, lung cancer, ovarian cancer, prostate cancer, pancreatic cancer, colon cancer, renal cell carcinoma (RCC), and germ cell tumors.

38.    The CMV vector of any one of claims 33-37, wherein the vector does not express an active UL128, UL130, UL40, or US28 protein, or an ortholog thereof, due to the presence of one or more mutations in the nucleic acid sequence encoding UL128, UL130, UL40, or US28, or an ortholog thereof.

39.    The CMV vector of claim 38, wherein the one or more mutations in the nucleic acid sequence encoding UL128, UL130, UL40, or US28 protein, or an ortholog thereof, are selected from the group consisting of: point mutations, frameshift mutations, truncation mutations, and deletion of all of the nucleic acid sequences encoding the protein.

40.    The CMV vector of any one of claims 33-39, further comprising a second nucleic acid sequence, wherein the second nucleic acid sequence comprises an antisense sequence or an

RNAi sequence that inhibits the expression of UL128, UL130, UL40, or US28, or an ortholog thereof.

41.    The CMV vector of any one of claims 33-40, wherein the CMV vector further comprises at least one inactivating mutation in one or more in viral genes encoding viral proteins that are essential, non-essential, or augmenting for growth *in vivo.*

42.    The CMV vector of claim 41, wherein the at least one inactivating mutation is selected from the group consisting of: point mutation, frameshift mutation, truncation mutation, and deletion of all of the nucleic acid sequence encoding the viral protein.

43.    The CMV vector of claim 41 or 42, wherein the at least one inactivating mutation is in UL82 (pp71).

44.    The CMV vector of any one of claims 41-43, wherein the at least one inactivating mutation is in US11.

45.    The CMV vector of any one of claims 33-44, wherein the CMV vector is a human CMV (HCMV) or rhesus CMV (RhCMV) vector.

46.    A method of generating an immune response to at least one heterologous antigen in a subject, the method comprising administering to the subject the CMV vector of any one of claims 33-45 in an amount effective to elicit a CD8$^+$ T cell response to the at least one heterologous antigen in the subject.

47.    The method of claim 46, wherein less than 1% of the CD8$^+$ T cells elicited by the CMV vector are restricted by MHC-E.

48.    The method of claim 46 or 47, wherein more than 10% of CD8$^+$ T cells elicited by the CMV vector are stimulated by peptides presented by single or multiple MHC-II alleles.

49.    The method of any one of claims 46-48, wherein the subject has been previously exposed to CMV.

50.    The method of any one of claims 46-49, wherein the subject is a human or nonhuman primate.

51.    The method of any one of claims 46-50, wherein administering the CMV vector comprises intravenous, intramuscular, intraperitoneal, or oral administration of the first CMV vector.

52.    The method of any one of claims 46-51, further comprising administering a second CMV vector to the subject, wherein the second CMV vector comprises a nucleic acid sequence encoding at least one heterologous antigen.

53.    The method of claim 52, wherein the second CMV vector expresses an active UL128 protein.

54.    The method of claim 52 or 53, wherein the second CMV vector expresses an active UL130 protein.

55.    The method of any one of claims 52-54, wherein the at least one heterologous antigen of the second vector is a pathogen-specific antigen, a tumor antigen, a tissue-specific antigen, or a host self-antigen.

56.     The method of claim 55, wherein the host self-antigen is an antigen derived from the variable region of a TCR or an antigen derived from the variable region of a B cell receptor.

57.     The method of claim 55, wherein the pathogen-specific antigen of the second CMV vector is derived from a pathogen selected from the group consisting of: human immunodeficiency virus, simian immunodeficiency virus, herpes simplex virus, hepatitis B virus, hepatitis C virus, papillomavirus, Plasmodium parasites, and *Mycobacterium tuberculosis*.

58.     The method of claim 55, wherein the tumor antigen of the second CMV vector is related to a cancer selected from the group consisting of: acute myelogenous leukemia, chronic myelogenous leukemia, myelodysplastic syndrome, acute lymphoblastic leukemia, chronic lymphoblastic leukemia, non-Hodgkin's lymphoma, multiple myeloma, malignant melanoma, breast cancer, lung cancer, ovarian cancer, prostate cancer, pancreatic cancer, colon cancer, renal cell carcinoma (RCC), and germ cell tumors.

59.     The method of any one of claims 52-54, wherein the at least one heterologous antigen of the first CMV vector and the second CMV vector are the same antigen.

60.     The method of any one of claims 52-59, wherein the second CMV vector is a HCMV or RhCMV vector.

61.     The method of any one of claims 52-60, wherein administering the second CMV vector comprises intravenous, intramuscular, intraperitoneal, or oral administration of the second CMV vector.

62.     The method of any one of claims 52-61, wherein the second CMV vector is administered before, concurrently with, or after the first CMV vector.

63.     A method of generating CD8$^+$ T cells that recognize MHC-E-peptide complexes, the method comprising:

    (1)    administering to a subject the CMV vector of any one of claims 1-13 in an amount effective to generate a set of CD8$^+$ T cells that recognize MHC-E/peptide complexes;

    (2)    identifying a first CD8$^+$ TCR from the set of CD8$^+$ T cells, wherein the first CD8$^+$ TCR recognizes a MHC-E/heterologous antigen-derived peptide complex;

    (3)    isolating one or more CD8$^+$ T cells from the subject; and

    (4)    transfecting the one or more CD8$^+$ T cells with an expression vector, wherein the expression vector comprises a nucleic acid sequence encoding a second CD8$^+$ TCR and a promoter operably linked to the nucleic acid sequence encoding the second CD8$^+$ TCR, wherein the second CD8$^+$ TCR comprises CDR3α and CDR3β of the first CD8$^+$ TCR, thereby generating one or more transfected CD8$^+$ T cells that recognize a MHC-E/heterologous antigen-derived peptide complex.

64.     The method of claim 63, wherein the first CD8$^+$ TCR is identified by DNA or RNA sequencing.

65.     The method of claim 63 or 64, wherein the second CD8$^+$ TCR comprises CDR1α, CDR2α, CDR3α, CDR1β, CDR2β, and CDR3β of the first CD8$^+$ TCR.

66.     The method of claim 65, wherein the nucleic acid sequence encoding the second CD8$^+$ TCR is identical to the nucleic acid sequence encoding the first CD8$^+$ TCR.

67.     The method of any one of claims 63-66, wherein administering the CMV vector to the subject comprises intravenous, intramuscular, intraperitoneal, or oral administration of the CMV vector to the subject.

68.     The method of any one of claims 63-67, wherein the subject has been previously exposed to CMV.

69.     The method of any one of claims 63-68, wherein the subject is a human or nonhuman primate.

70.     The method of any one of claims 63-69, wherein the at least one heterologous antigen of the CMV vector comprises a tumor antigen.

71.     The method of claim 70, wherein the tumor antigen is related to a cancer selected from the group consisting of: acute myelogenous leukemia, chronic myelogenous leukemia, myelodysplastic syndrome, acute lymphoblastic leukemia, chronic lymphoblastic leukemia, non-Hodgkin's lymphoma, multiple myeloma, malignant melanoma, breast cancer, lung cancer, ovarian cancer, prostate cancer, pancreatic cancer, colon cancer, renal cell carcinoma (RCC), and germ cell tumors.

72.     The method of claim 70 or 71, further comprising administering the transfected CD8$^+$ T cells to the subject to treat cancer.

73.     The method of any one of claims 63-69, wherein the at least one heterologous antigen of the CMV vector comprises a pathogen-specific antigen.

74.     The method of claim 73, wherein the pathogen-specific antigen is derived from a pathogen selected from the group consisting of: human immunodeficiency virus, simian immunodeficiency virus, herpes simplex virus, hepatitis B virus, hepatitis C virus, papillomavirus, Plasmodium parasites, and *Mycobacterium tuberculosis*.

75.     The method of claim 73 or 74, further comprising administering the transfected CD8$^+$ T cells to the subject to treat a pathogenic infection.

76.     The method of any one of claims 63-69, wherein the at least one heterologous antigen of the CMV vector comprises a host self-antigen or a tissue-specific antigen.

77.     The method of claim 76, wherein the host self-antigen is an antigen derived from the variable region of a TCR or an antigen derived from the variable region of a B cell receptor.

78.     The method of claim 76 or 77, further comprising administering the transfected CD8$^+$ T cells to the subject to treat an autoimmune disease or disorder.

79.     The method of claim 76 or 77, further comprising administering the transfected CD8$^+$ T cells to the subject to induce an autoimmune response to the host self-antigen or tissue-specific antigen.

80.     A method of generating CD8$^+$ T cells that recognize MHC-E-peptide complexes, the method comprising:

    (1)     administering to a first subject the CMV vector of any one of claims 1-13 in an amount effective to generate a set of CD8$^+$ T cells that recognize MHC-E/peptide complexes;

    (2)     identifying a first CD8$^+$ TCR from the set of CD8$^+$ T cells, wherein the first CD8$^+$ TCR recognizes a MHC-E/heterologous antigen-derived peptide complex;

    (3)     isolating one or more CD8$^+$ T cells from a second subject; and

    (4)     transfecting the one or more CD8$^+$ T cells with an expression vector, wherein the expression vector comprises a nucleic acid sequence encoding a second CD8$^+$ TCR and a promoter operably linked to the nucleic acid sequence encoding the second CD8$^+$ TCR, wherein the second CD8$^+$ TCR comprises CDR3α and CDR3β of

the first CD8$^+$ TCR, thereby generating one or more transfected CD8$^+$ T cells that recognize a MHC-E/heterologous antigen-derived peptide complex.

81.     The method of claim 80, wherein the first CD8$^+$ TCR is identified by DNA or RNA sequencing.

82.     The method of claim 80 or 81, wherein the first subject is a human or nonhuman primate.

83.     The method of any one of claims 80-82, wherein the second subject is a human or nonhuman primate.

84.     The method of any one of claims 80-83, wherein the first subject is a nonhuman primate and the second subject is a human, and wherein the second CD8$^+$ TCR is a chimeric nonhuman primate-human CD8$^+$ TCR comprising the non-human primate CDR3α and CDR3β of the first CD8$^+$ TCR.

85.     The method of claim 84, wherein the second CD8$^+$ TCR comprises the non-human primate CDR1α, CDR2α, CDR3α, CDR1β, CDR2β, and CDR3β of the first CD8$^+$ TCR.

86.     The method of any one of claims 80-83, wherein the second CD8$^+$ TCR comprises CDR1α, CDR2α, CDR3α, CDR1β, CDR2β, and CDR3β of the first CD8$^+$ TCR.

87.     The method of claim 86, wherein the nucleic acid sequence encoding the second CD8$^+$ TCR is identical to the nucleic acid sequence encoding the first CD8$^+$ TCR.

88.     The method of any one of claims 80-83, wherein the second CD8$^+$ TCR is a chimeric CD8$^+$ TCR.

89.    The method of claim 88, wherein the second CD8$^+$ TCR comprises CDR1α, CDR2α, CDR3α, CDR1β, CDR2β, and CDR3β of the first CD8$^+$ TCR.

90.    The method of any one of claims 80-89, wherein administering the CMV vector to the first subject comprises intravenous, intramuscular, intraperitoneal, or oral administration of the CMV vector to the first subject.

91.    The method of any one of claims 80-90, wherein the first subject has been previously exposed to CMV.

92.    The method of any one of claims 80-91, wherein the at least one heterologous antigen comprises a tumor antigen.

93.    The method of claim 92, wherein the tumor antigen is related to a cancer selected from the group consisting of: acute myelogenous leukemia, chronic myelogenous leukemia, myelodysplastic syndrome, acute lymphoblastic leukemia, chronic lymphoblastic leukemia, non-Hodgkin's lymphoma, multiple myeloma, malignant melanoma, breast cancer, lung cancer, ovarian cancer, prostate cancer, pancreatic cancer, colon cancer, renal cell carcinoma (RCC), and germ cell tumors.

94.    The method of claim 92 or 93, further comprising administering the transfected CD8$^+$ T cells to the second subject to treat cancer.

95.    The method of any one of claims 80-91, wherein the at least one heterologous antigen comprises a pathogen-specific antigen.

96.    The method of claim 95, wherein the pathogen-specific antigen is derived from a pathogen selected from the group consisting of: human immunodeficiency virus, simian immunodeficiency virus, herpes simplex virus, hepatitis B virus, hepatitis C virus, papillomavirus, Plasmodium parasites, and *Mycobacterium tuberculosis.*

97.    The method of claim 95 or 96, further comprising administering the transfected CD8$^+$ T cells to the second subject to treat a pathogenic infection.

98.    The method of any one of claims 80-91, wherein the at least one heterologous antigen comprises a host self-antigen or a tissue-specific antigen.

99.    The method of claim 98, wherein the host self-antigen is an antigen derived from the variable region of a TCR or an antigen derived from the variable region of a B cell receptor.

100.   The method of claim 98 or 99, further comprising administering the transfected CD8$^+$ T cells to the second subject to treat an autoimmune disease or disorder.

101.   The method of claim 98 or 99, further comprising administering the transfected CD8$^+$ T cells to the subject to induce an autoimmune response to the host self-antigen or tissue-specific antigen.

102.   A CD8$^+$ T cell generated by the method of any one of claims 63-101.

103.   The CD8$^+$ T cell of claim 102, wherein the at least one heterologous antigen of the CMV vector comprises a tumor antigen.

104.   The CD8$^+$ T cell of claim 103, wherein the tumor antigen is related to a cancer selected from the group consisting of: acute myelogenous leukemia, chronic myelogenous leukemia,

myelodysplastic syndrome, acute lymphoblastic leukemia, chronic lymphoblastic leukemia, non-Hodgkin's lymphoma, multiple myeloma, malignant melanoma, breast cancer, lung cancer, ovarian cancer, prostate cancer, pancreatic cancer, colon cancer, renal cell carcinoma (RCC), and germ cell tumors.

105. The CD8$^+$ T cell of claim 102, wherein the at least one heterologous antigen of the CMV vector comprises a pathogen-specific antigen.

106. The CD8$^+$ T cell of claim 105, wherein the pathogen-specific antigen is derived from a pathogen selected from the group consisting of: human immunodeficiency virus, simian immunodeficiency virus, herpes simplex virus, hepatitis B virus, hepatitis C virus, papillomavirus, Plasmodium parasites, and *Mycobacterium tuberculosis.*

107. The CD8$^+$ T cell of claim 102, wherein the at least one heterologous antigen of the CMV vector comprises a host self-antigen or a tissue-specific antigen.

108. The CD8$^+$ T cell of claim 107, wherein the host self-antigen is an antigen derived from the variable region of a TCR or an antigen derived from the variable region of a B cell receptor.

109. A method of treating cancer, the method comprising administering the CD8$^+$ T cell of claim 103 or 104 to a subject.

110. A method of treating a pathogenic infection, the method comprising administering the CD8$^+$ T cell of claim 105 or 106 to a subject.

111. A method of treating an autoimmune disease or disorder, the method comprising administering the CD8$^+$ T cell of claim 107 or 108 to a subject.

112.    A method of inducing an autoimmune response to a host self-antigen or tissue-specific antigen, the method comprising administering the CD8$^+$ T cell of claim 107 or 108 to the subject.

# Figure 1A
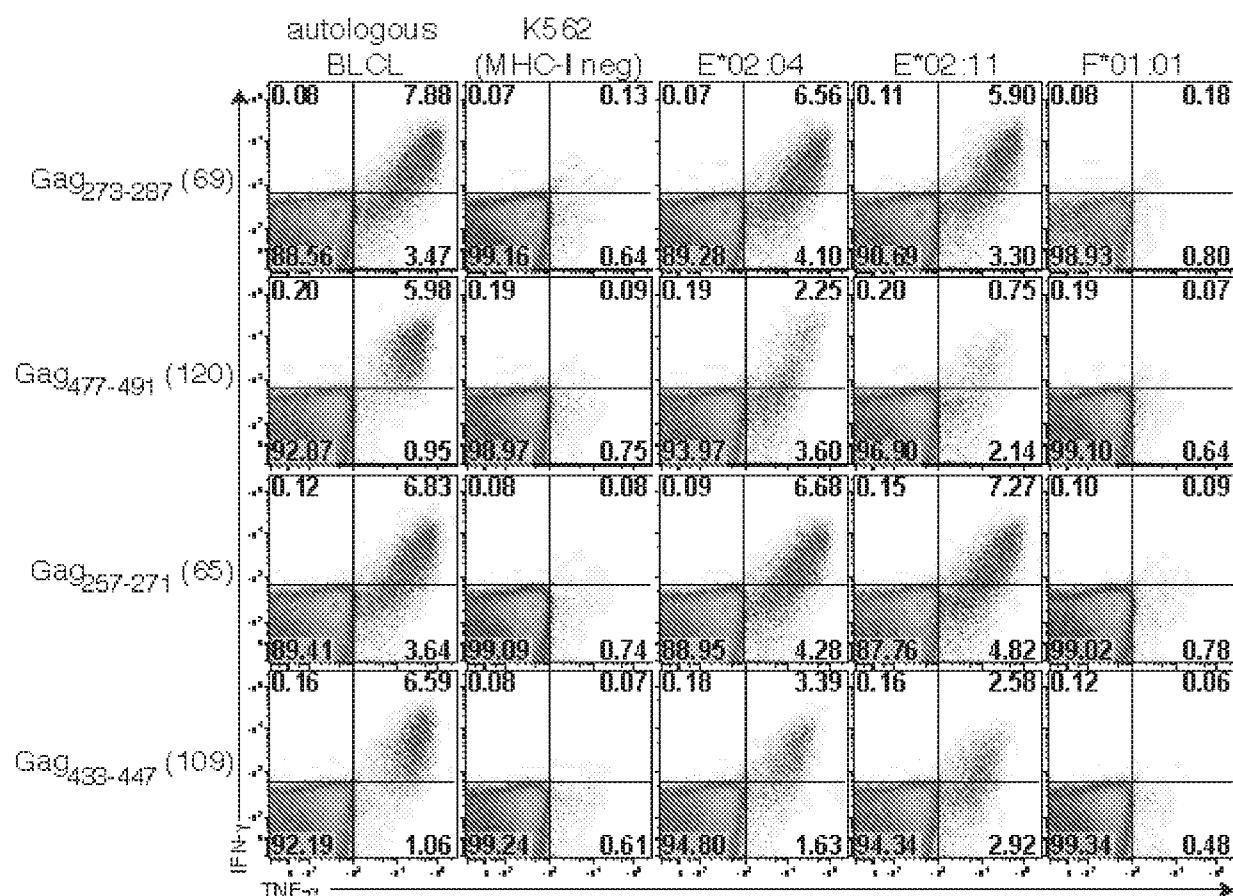
## Figure 1B
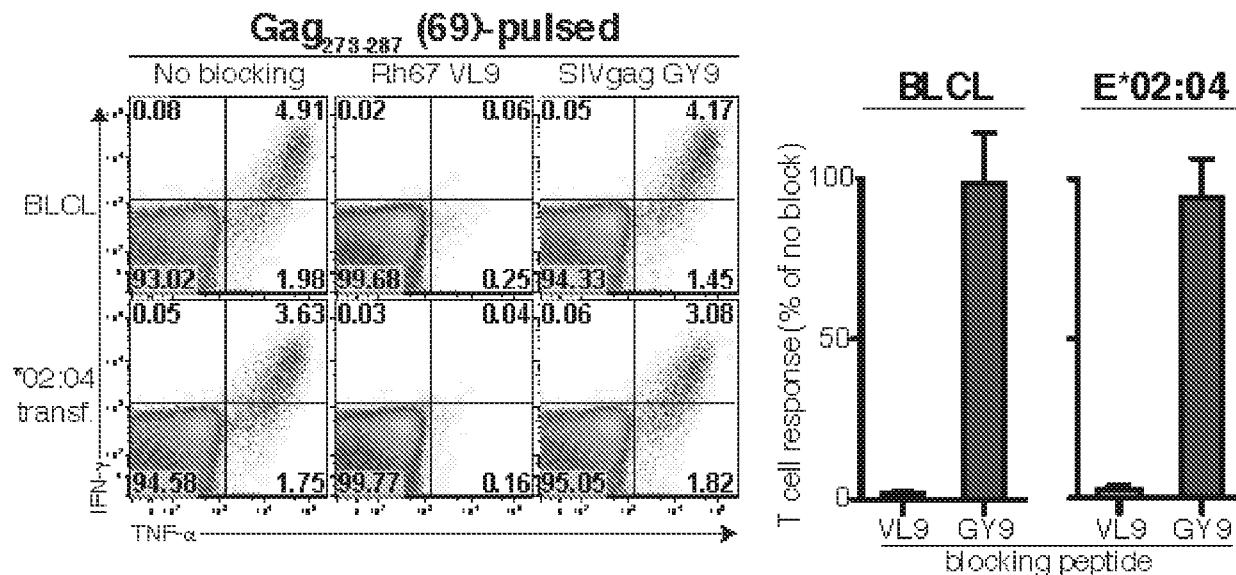


## Figure 1C

Figure 2A

Figure 2B



Gag$_{69-83}$ (18)-pulsed (Rh22607 A*01+)

E*02:04 transf.    IFN-γ

TNF-α

Figure 2C



Gag$_{69-83}$ (18)-pulsed (Rh22034 A*01-)

IFN-γ

TNF-α
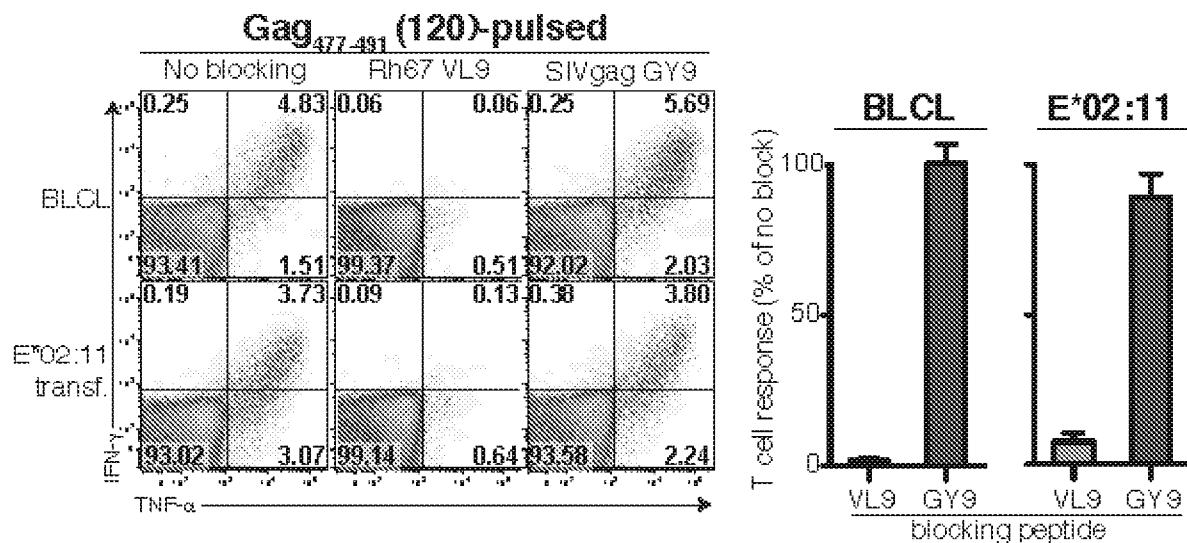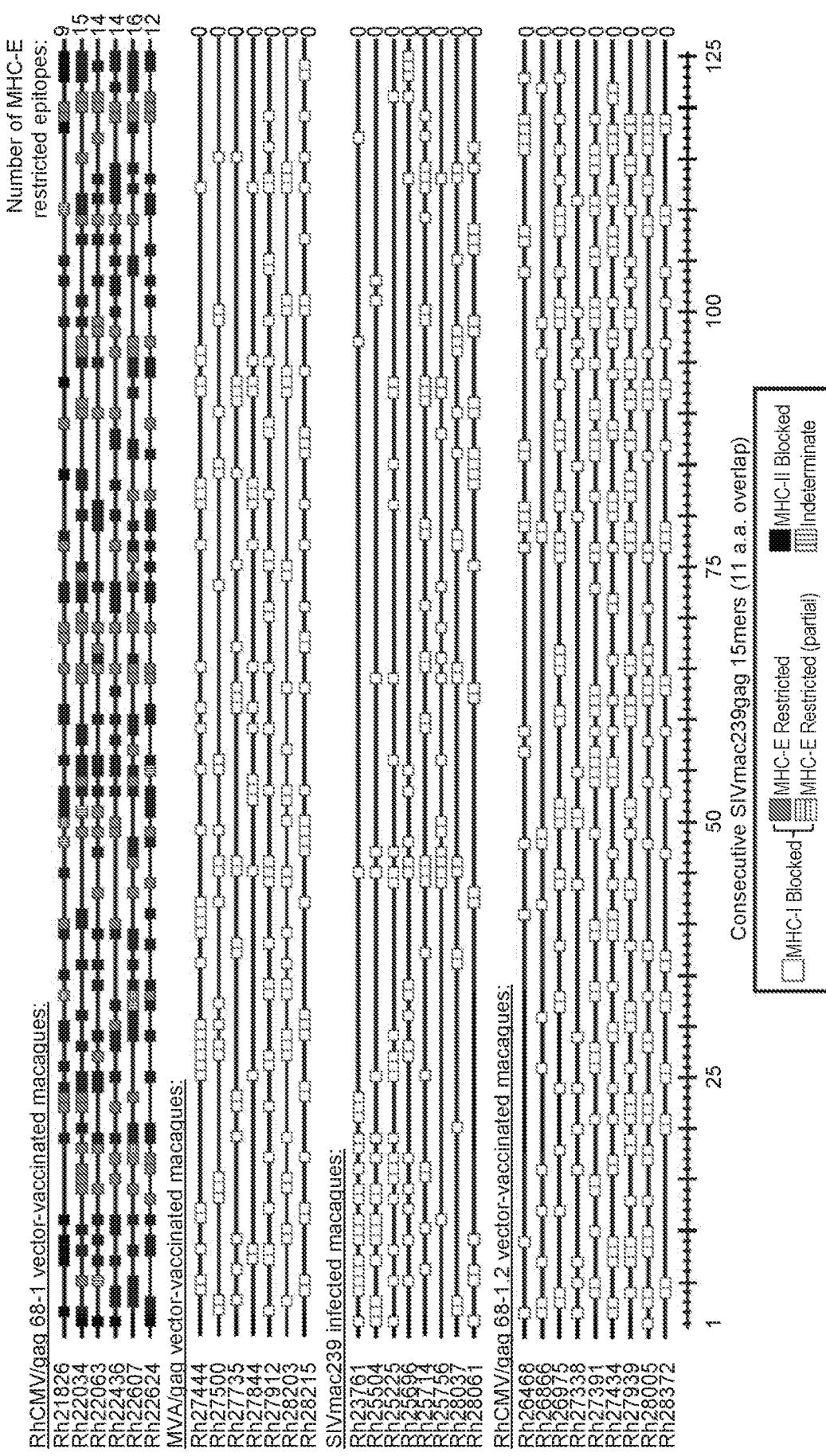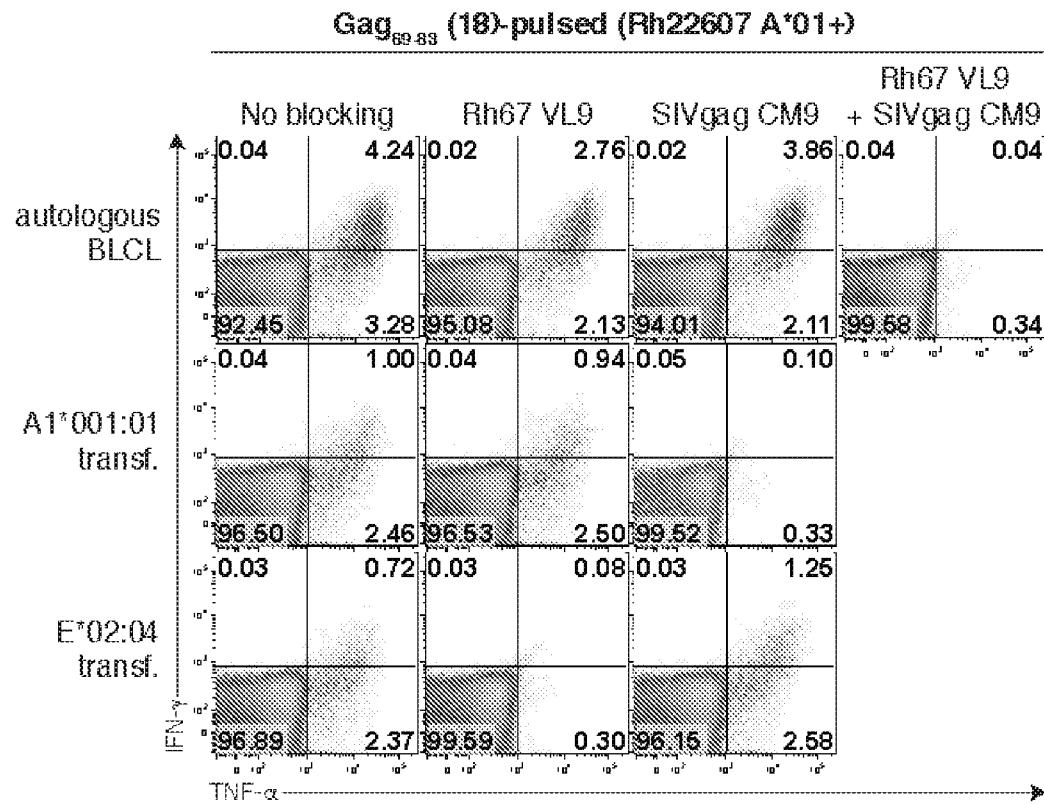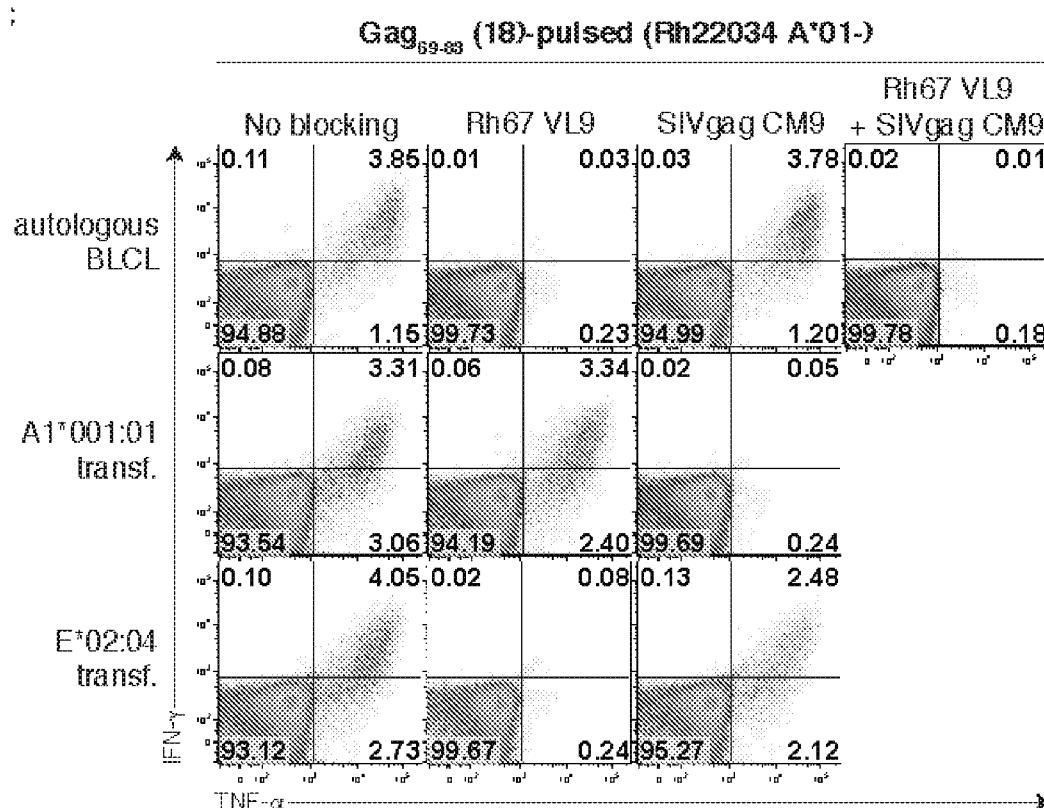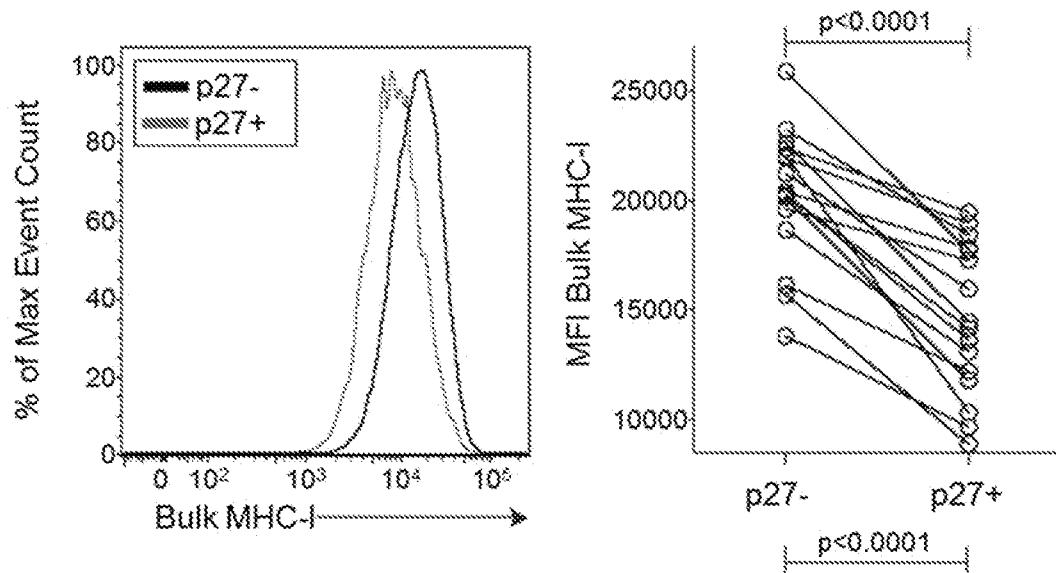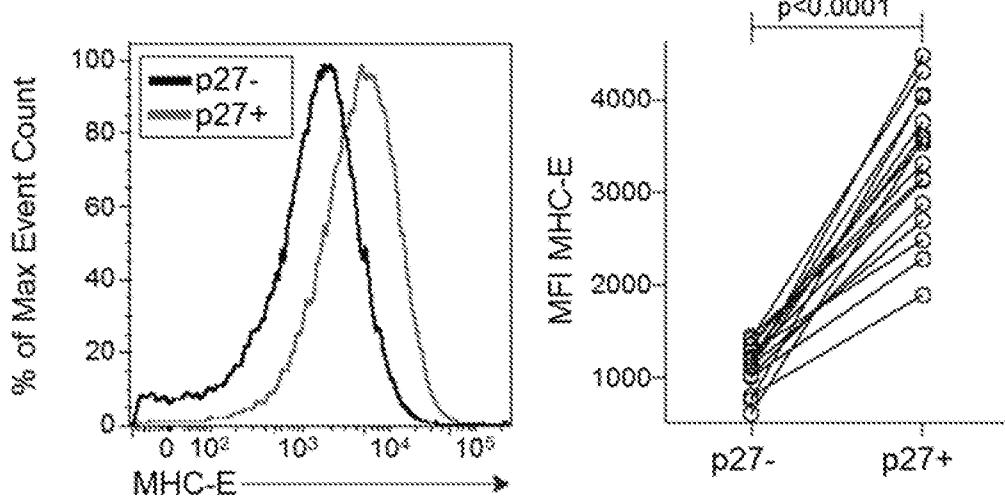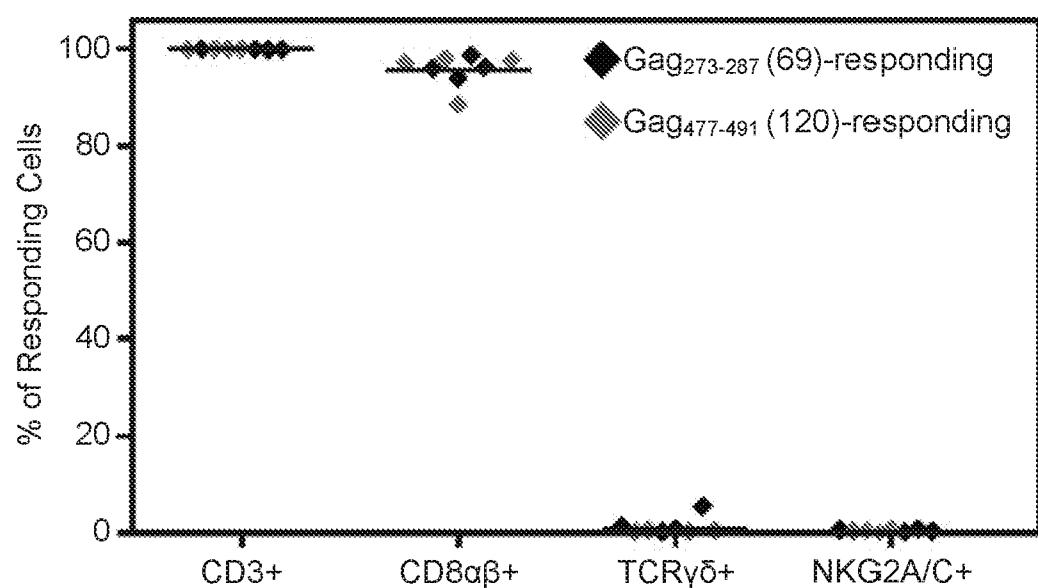
Figure 3A



Figure 3B

Figure 3C

Figure 4A
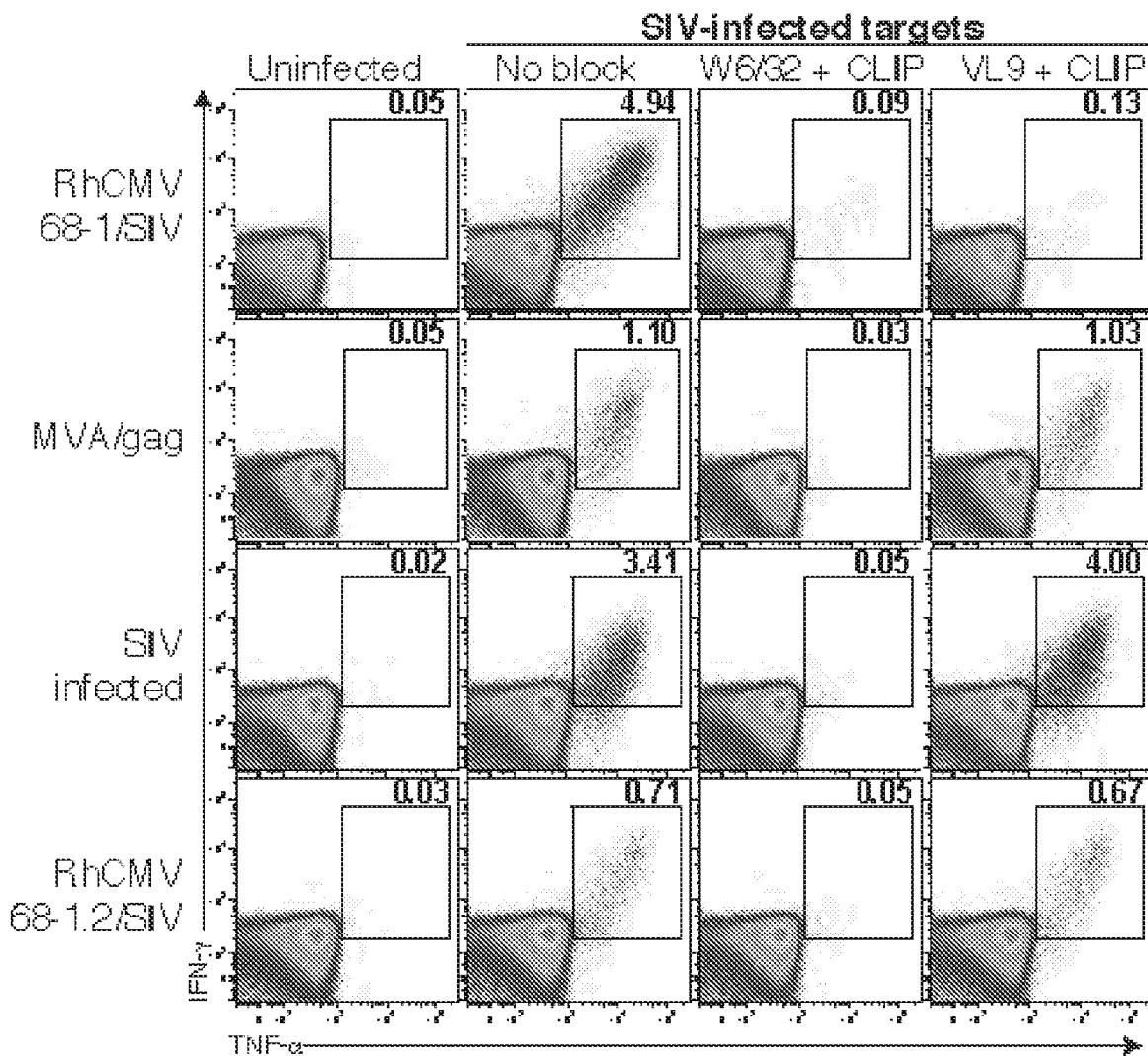


Figure 4B
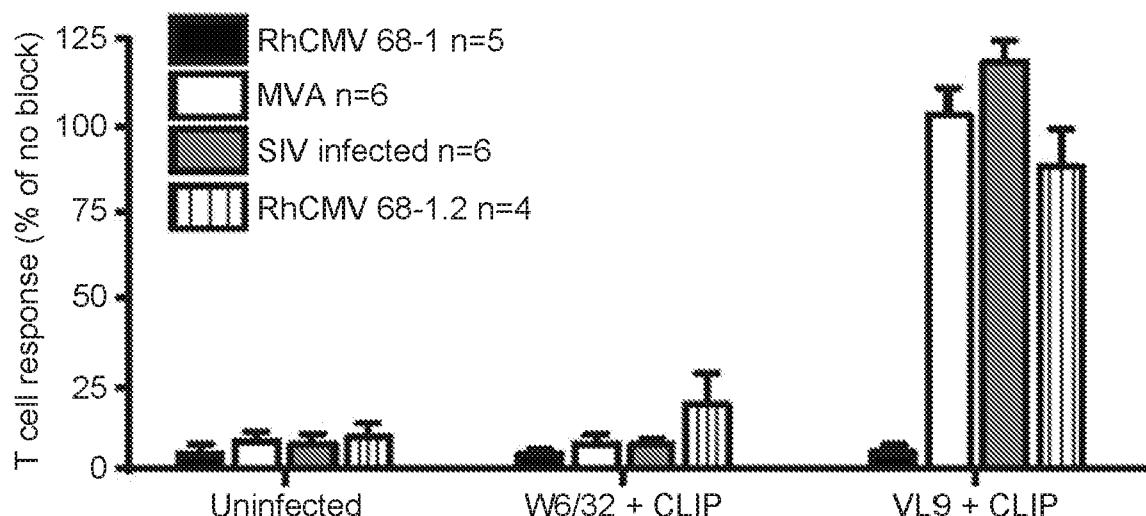
Figure 4C
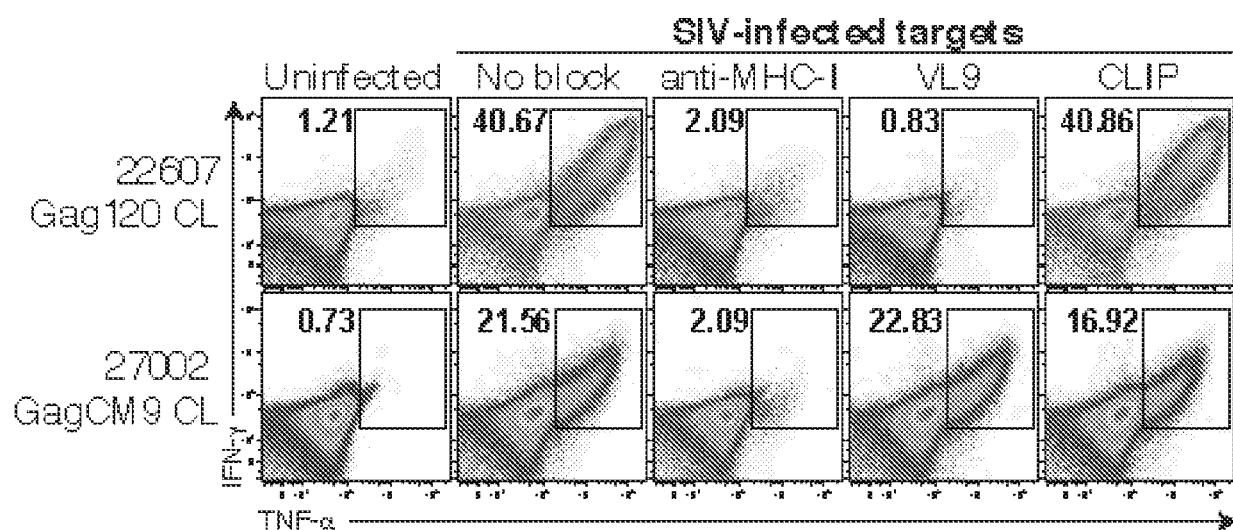
Figure 5

Rh67 deleted; strain 68-1 RhCMV/gag (UL128/UL130 deleted) vaccine:

Figure 6



Consecutive SIVmac239gag 15mers (11 a.a. overlap)

Rh23199

Rh23970

Rh24939

1    25    50    75    100    125

☐ MHC-Ia restricted
▨ MHC-E restricted
■ MCH-II restricted
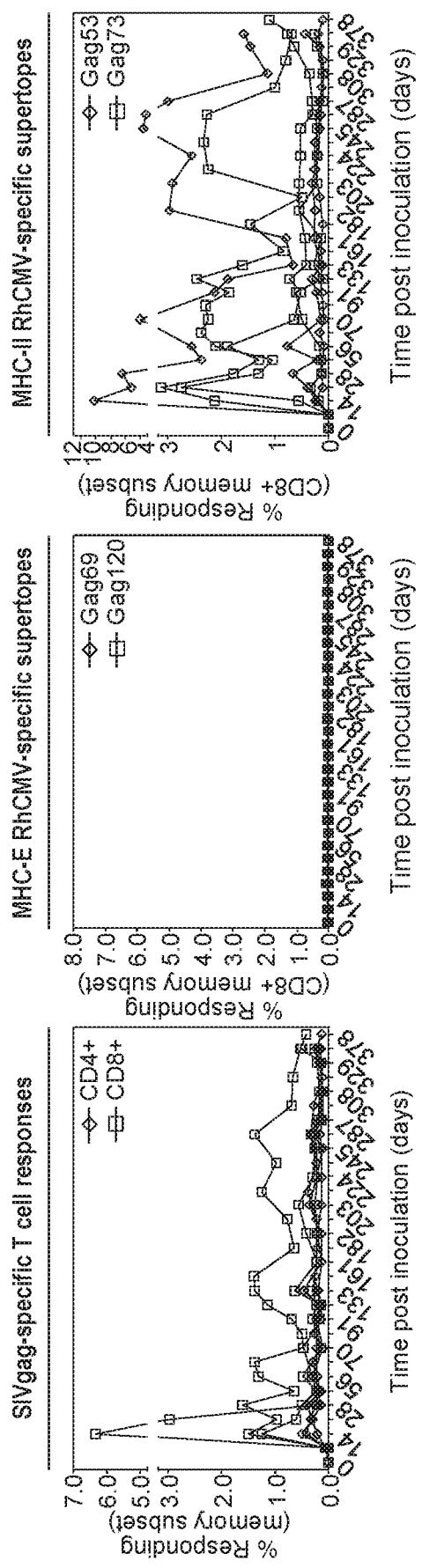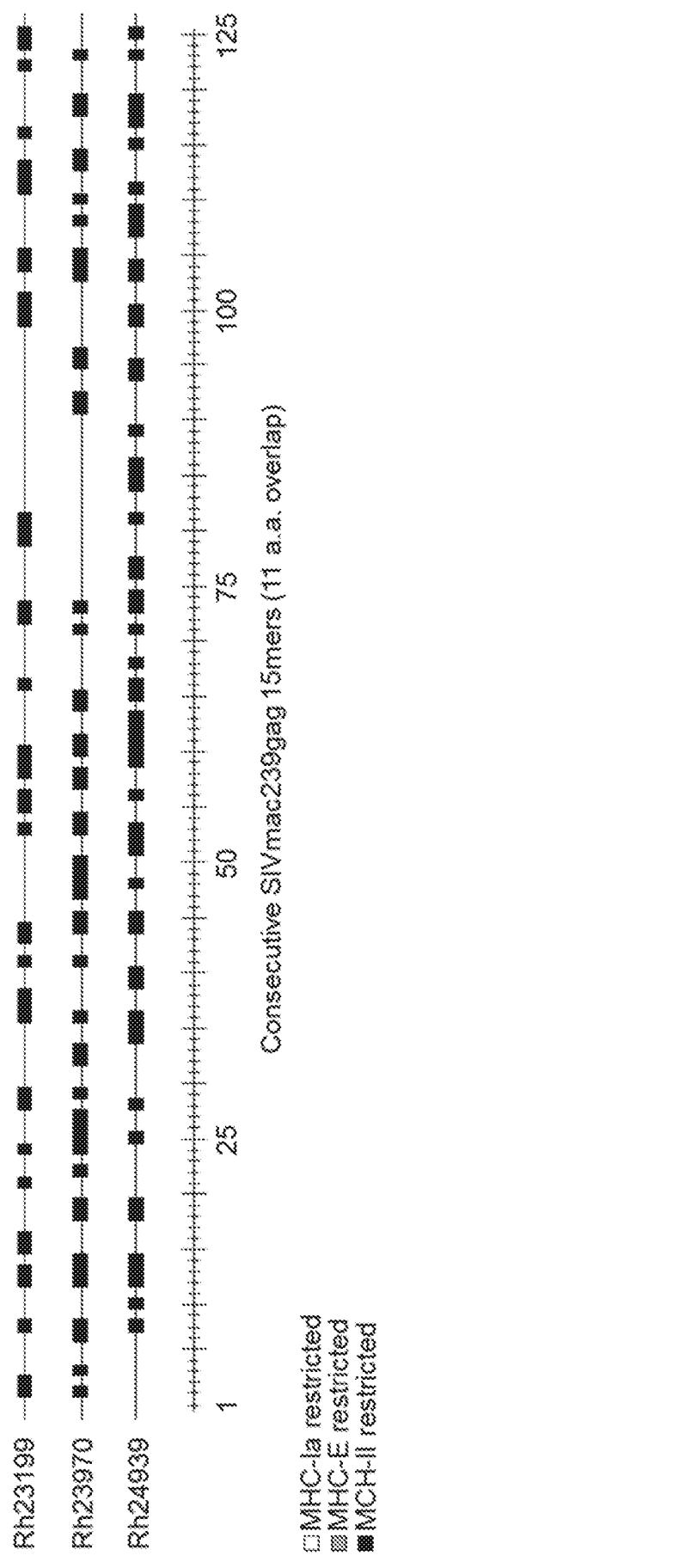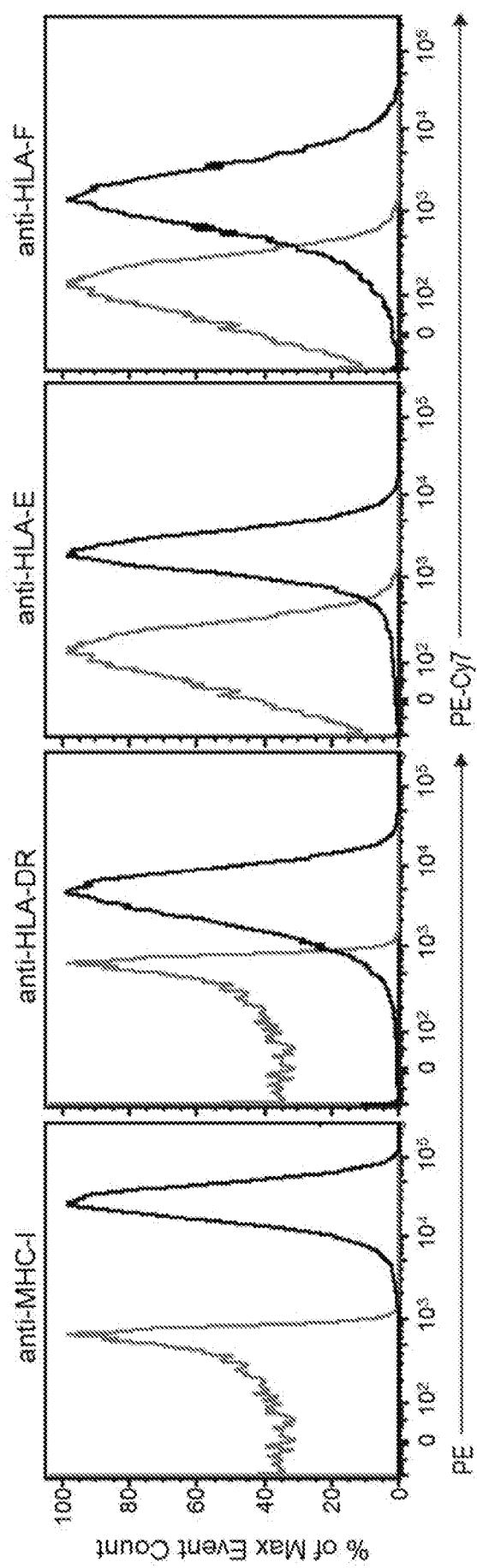
Figure 7A

Figure 7B

### Rhesus macaques

| Mamu Class I Alleles | 21626 | 22034 | 22436 | 22607 |
|---|---|---|---|---|
| A1*001:01 | X | | | X |
| A1*002:01 | X | X | X | X |
| A1*012:01 | | | X | |
| A1*023:01 | | X | | |
| A2*05:11; A2*05:28; A2*05:32:01; A2*05:32:02; A2*05:45 | | | X | |
| A2*05:04:01; A2*05:04:03; A2*05:10; A2*05:14 | | | | X |
| A3*13:02 | X | X | X | X |
| A4*14:03:01; A4*14:09 | | X | | |
| B*001:01:01 | | X | | X |
| B*007:02 | | X | | X |
| B*012:01 | X | | | X |
| B*017:01 | X | | | |
| B*021:01 | | X | | |
| B*022:01 | X | | | |
| B*028:01 | | X | | |
| B*029:01 | X | | | |
| B*030:01 | X | | | |
| B*030:05 | | X | | X |
| B*031:01 | X | | | |
| B*041:01 | | | X | |
| B*046:01:02 | | X | | |
| B*048:01 | | | X | |
| B*055:01 | | | X | |
| B*057:01 | X | | | X |
| B*058:02 | | | X | |
| B*060:02 | X | | | |
| B*061:01 | X | | | |
| B*064:01 | | | X | |
| B*068:03 | | X | | |
| B*072:01; B*072:02; Mm-B*nov121 | | X | | X |
| B*074:01 | X | | | X |
| B*082:02 | | | | X |
| Mm-B*nov037 | X | | | |
| Mm-B*nov113 | | X | | |
| E*02:01:02; E*02:10; E*02:11 | X | | | X |
| E*02:04 | X | X | X | X |
| E*02:09 | X | | | |
| E*02:12:01; E*02:12:02 | | X | | |
| E*02:20 | X | | | X |

Transfectant generated

Figure 7C

Figure 8A
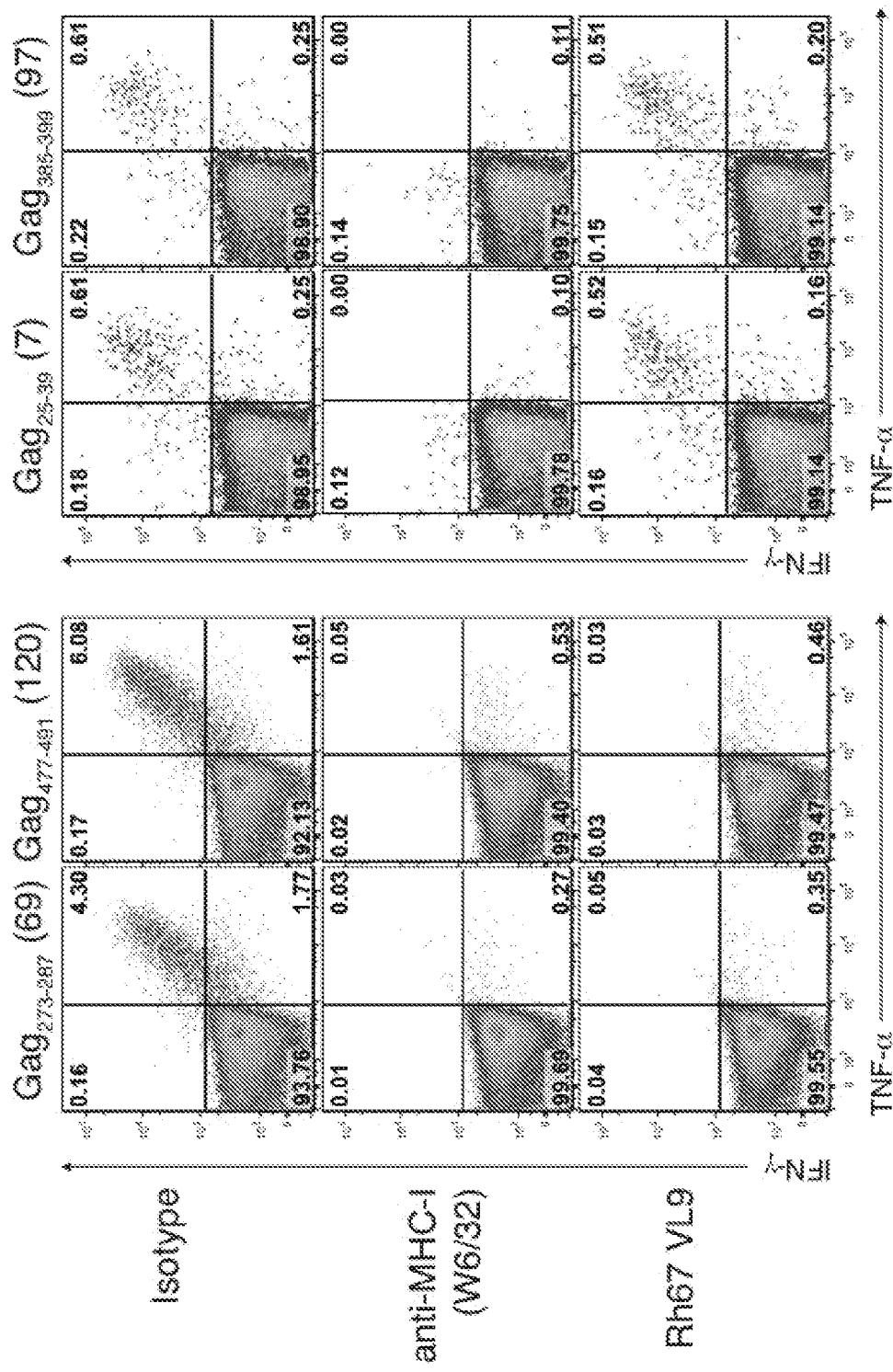
Figure 8B
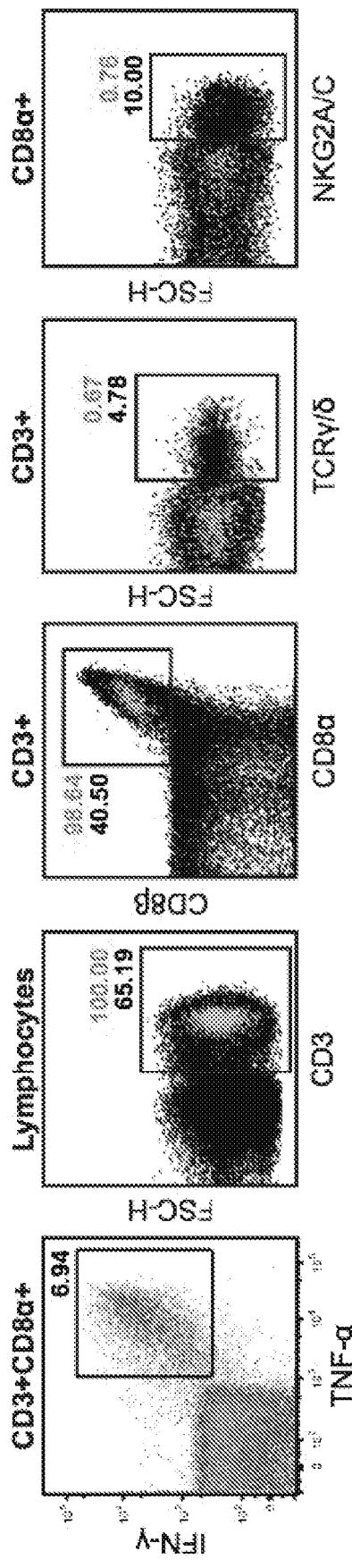
Figure 9

Figure 10A



Figure 10B
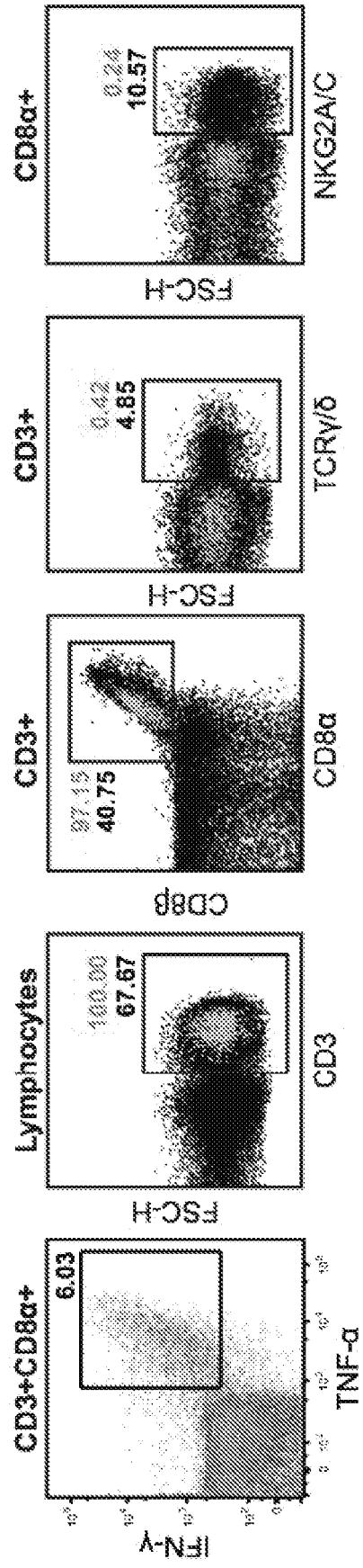
Figure 11A
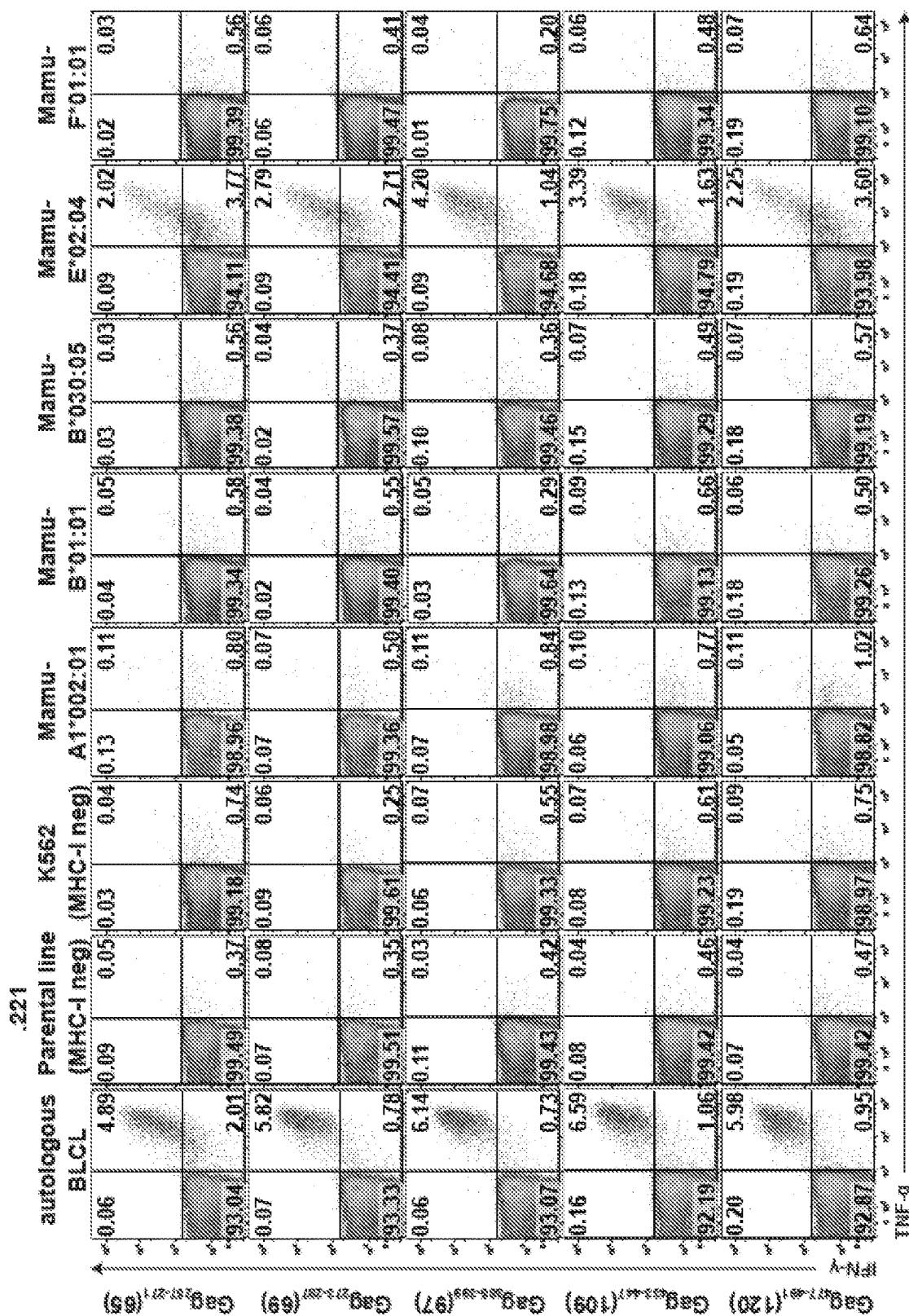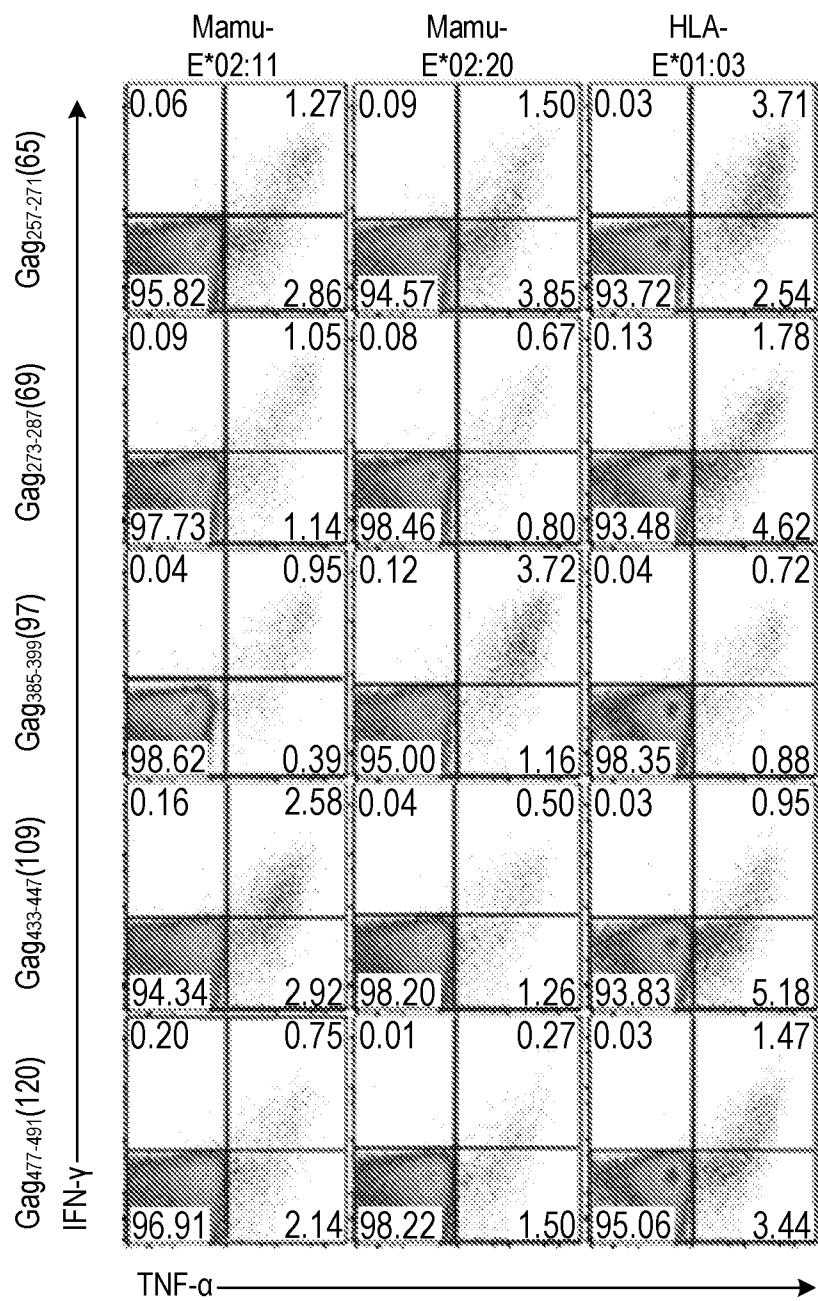
# Figure 11B

## Figure 11C
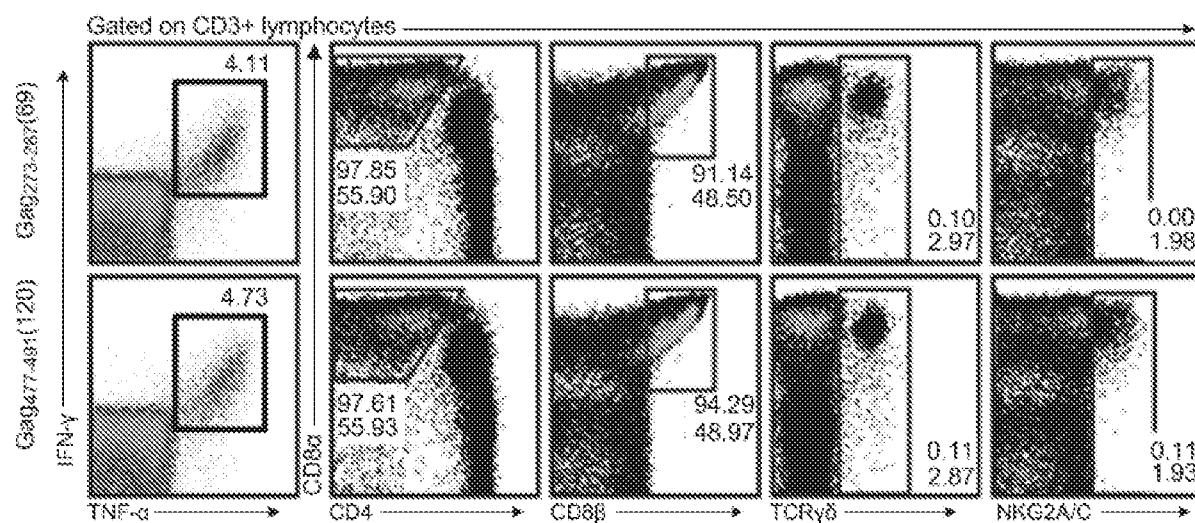


## Figure 11D
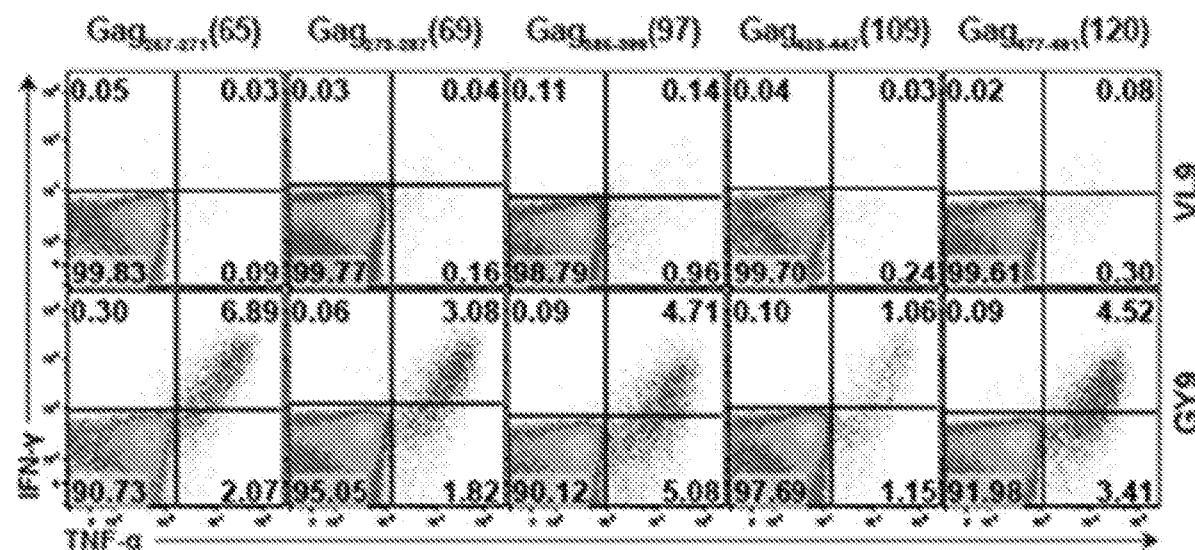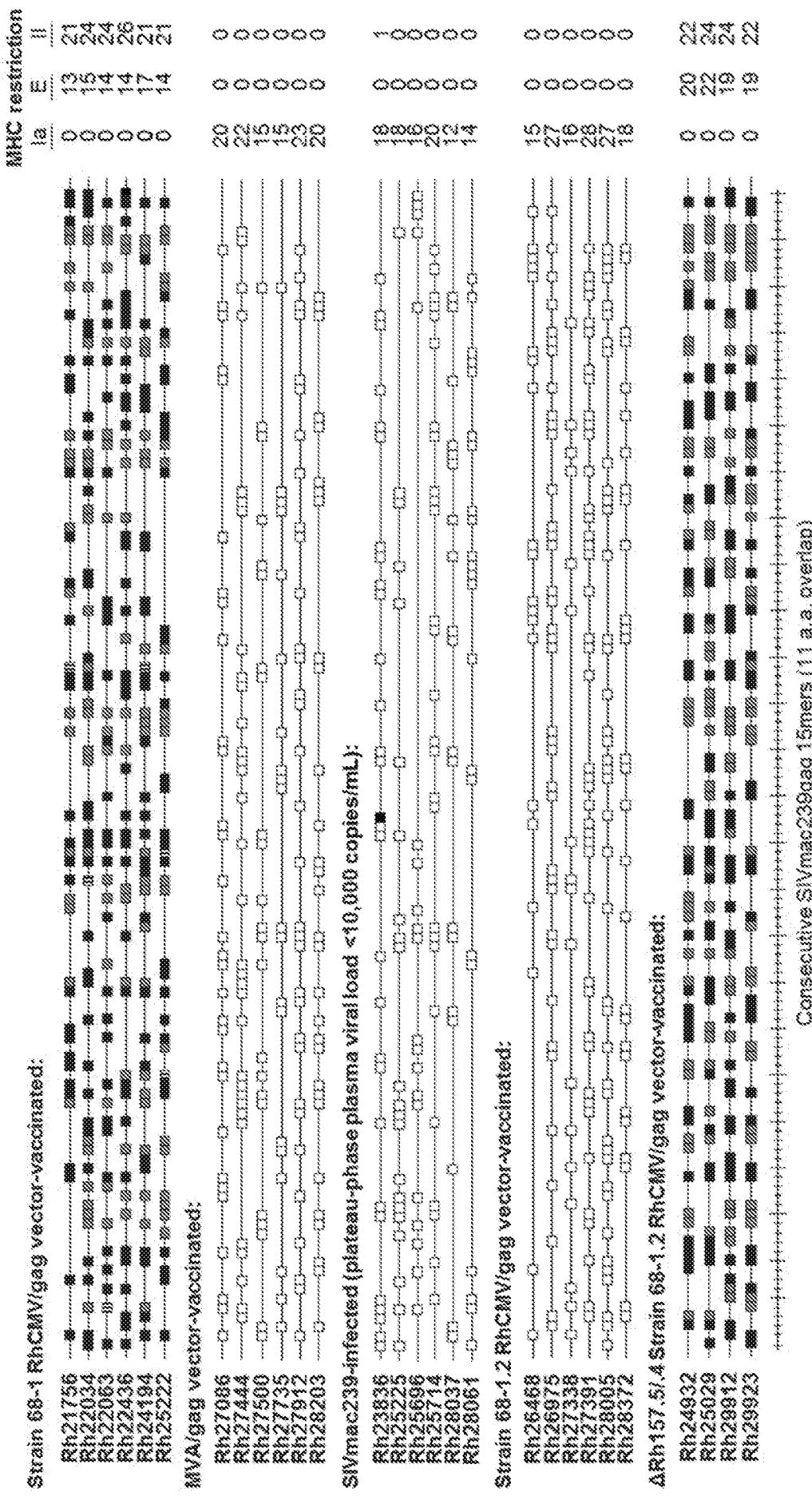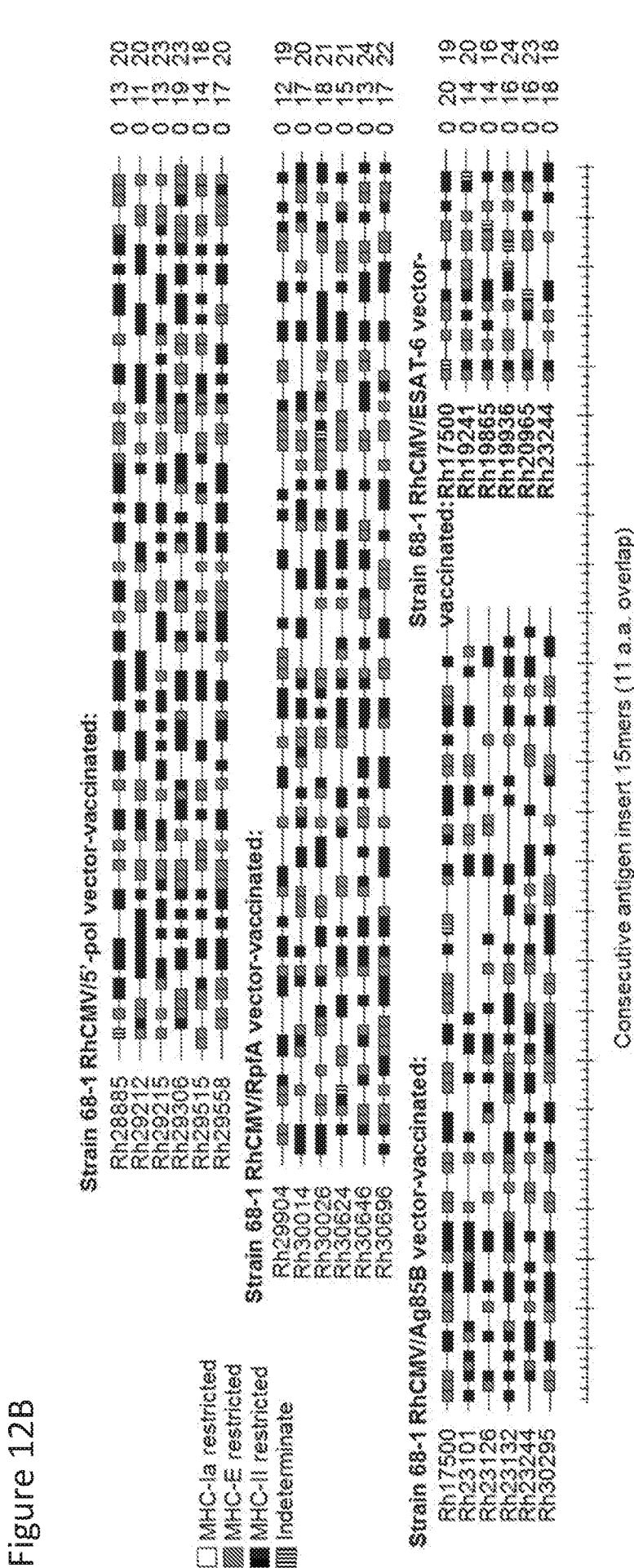
Figure 12A
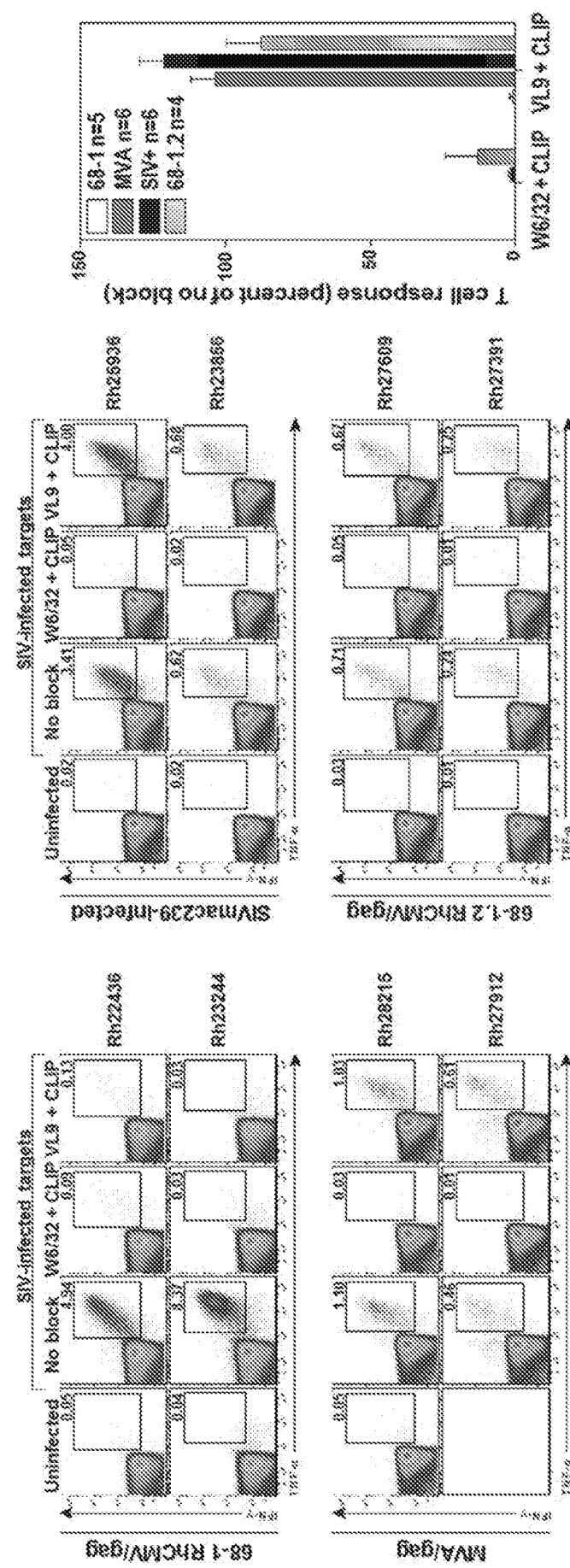
Figure 12B

Figure 12C

Figure 13A
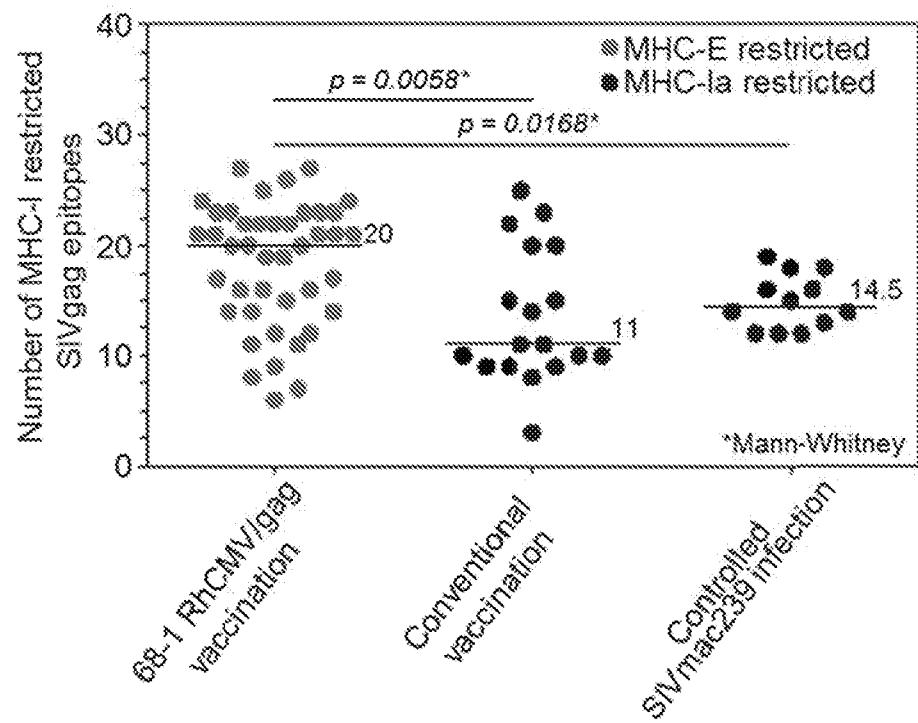


Figure 13B

# Figure 13C

# Figure 13D

| 15mer Peptide | Recognition Frequency** | Optimal Epitopes | | | | | | | | | 9mer Peptide |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Gag61-75 | 35 | A | P | L | V | P | T | G | S | E | Gag65-73 |
| Gag69-83 | 40 | S | E | N | L | K | S | L | Y | N | Gag72-80 |
| Gag89-103 | 50 | E | K | V | K | H | T | E | E | A | Gag92-100 |
| Gag117-131 | 35 | K | T | S | R | P | T | A | P | S | Gag120-128 |
| Gag129-143 | 35 | G | G | N | Y | P | V | Q | Q | I | Gag132-140 |
| Gag197-211 | 45 | Q | I | I | R | D | I | I | N | E | Gag201-209 |
| Gag257-271 | 25 | V | G | N | I | Y | R | R | W | I | Gag259-267 |
| Gag273-287 | 100* | R | M | Y | N | P | T | N | I | L | **Gag276-284** |
| Gag385-399 | 55 | K | P | I | K | C | W | N | C | G | Gag389-397 |
| Gag473-487 | 60 | L | G | K | Q | Q | R | E | K | Q | Gag476-484 |
| Gag477-491 | 100* | E | K | Q | R | E | S | R | E | K | **Gag482-490** |

▨ Enriched
☐ Underrepresented
▥ MHC-E canonical peptide anchors

*Supertopes
**Percent vaccinated animals



Probability (y-axis, 0.0 to 1.0)
Amino acid position (x-axis, 1 to 9)

Figure 14

# Figure 15A

|  | Rhesus macaques | | | |
|---|---|---|---|---|
|  | 21826 | 22034 | 22436 | 22607 |
| A1*001:01 | X |  |  | X |
| A1*002:01 | X | X | X | X |
| A1*012:01 |  |  | X |  |
| A1*023:01 |  | X |  |  |
| A2*05:01; A2*05:11; A2*05:28; A2*05:32:01; A2*05:32:02; A2*05:45 |  |  | X |  |
| A2*05:04:01; A2*05:04:03; A2*05:10; A2*05:14 |  |  |  | X |
| A3*13:02 | X | X | X | X |
| A4*14:03:01; A2*14:09 |  | X |  |  |
| B*001:01:01 |  | X |  | X |
| B*007:02 |  | X |  | X |
| B*012:01 | X |  |  | X |
| B*17:01 | X |  |  |  |
| B*021:01 |  | X |  |  |
| B*022:01 | X |  |  |  |
| B*028:01 |  | X |  |  |
| B*029:01 | X |  |  |  |
| B*030:01 | X |  |  |  |
| B*030:05 |  | X |  | X |
| B*031:01 | X |  |  |  |
| B*041:01 |  |  | X |  |
| B*046:01:02 |  | X |  |  |
| B*048:01 |  |  | X |  |
| B*055:01 |  |  | X |  |
| B*057:01 | X |  |  | X |
| B*058:02 |  |  | X |  |
| B*060:02 | X |  |  |  |
| B*061:01 | X |  |  |  |
| B*064:01 |  |  | X |  |
| B*068:03 |  | X |  |  |
| B*072:01; B*072:02; Mm-B*nov121 |  | X |  | X |
| B*074:01 | X |  |  | X |
| B*082:02 |  |  |  | X |
| Mm-B*nov037 | X |  |  |  |
| Mm-B*nov113 |  | X |  |  |
| E*02:01:02; E*02:10; E*02:11 | X |  |  | X |
| E*02:04 | X | X | X | X |
| E*02:09 | X |  |  |  |
| E*02:12:01; E*02:12:02 |  | X |  |  |
| E*02:20 | X |  |  | X |

Mamu Class I Alleles

▨ Transfectant generated

Figure 15B

Figure 16A

# Figure 16B

| Rh21826 | Gag61-75 (16) | Gag69-83 (18) | Gag89-103 (23) | Gag117-131 (30) | Gag129-143 (33) | Gag197-211 (50) | Gag257-271 (65) | Gag273-287 (69) | Gag385-399 (97) | Gag433-447 (109) | Gag473-487 (119) | Gag477-491 (120) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| BLCL | - | - | + | - | + | + | + | + | - | - | + | + |
| 221 | - | - | - | - | - | - | - | - | - | - | - | - |
| K562 | - | - | - | - | - | - | - | - | - | - | - | - |
| A1*001:01 | - | - | - | - | - | + | - | - | - | - | - | - |
| A1*002:01 | - | - | - | - | + | - | - | - | - | - | - | - |
| A3*13:02 | - | - | - | - | - | - | - | - | - | - | - | - |
| B*029:01 | - | - | - | - | - | - | - | - | - | - | - | - |
| B*030:01 | - | - | - | - | - | - | - | - | - | - | - | - |
| E*02:04 | - | + | + | - | + | + | + | + | - | - | + | + |
| E*02:11 | - | + | + | - | + | + | + | + | - | - | + | + |
| F*01:01 | - | - | - | - | - | - | - | - | - | - | - | - |

| Rh22034 | Gag61-75 (16) | Gag69-83 (18) | Gag89-103 (23) | Gag117-131 (30) | Gag129-143 (33) | Gag197-211 (50) | Gag257-271 (65) | Gag273-287 (69) | Gag385-399 (97) | Gag433-447 (109) | Gag473-487 (119) | Gag477-491 (120) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| BLCL | + | + | + | - | - | - | + | + | + | + | - | + |
| 221 | - | - | - | - | - | - | - | - | - | - | - | - |
| K562 | - | - | - | - | - | - | - | - | - | - | - | - |
| A1*002:01 | - | - | - | - | - | - | - | - | - | - | - | - |
| A3*13:02 | - | - | - | - | - | - | - | - | - | - | - | - |
| B*001:01 | - | - | - | - | - | - | - | - | - | - | - | - |
| B*030:05 | - | - | - | - | - | - | - | - | - | - | - | - |
| E*02:04 | + | + | + | - | - | - | + | + | + | + | - | + |
| E*02:11 | + | + | + | - | - | - | + | + | + | + | - | + |
| F*01:01 | - | - | - | - | - | - | - | - | - | - | - | - |

# Figure 16B (cont.)

| Rh22436 | Gag61-75 (16) | Gag69-83 (18) | Gag89-103 (23) | Gag117-131 (30) | Gag129-143 (33) | Gag197-211 (50) | Gag257-271 (65) | Gag273-287 (69) | Gag385-399 (97) | Gag433-447 (109) | Gag473-487 (119) | Gag477-491 (120) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| BLCL | - | + | - | + | - | + | + | + | - | + | + | + |
| 221 | - | - | - | - | - | - | - | - | - | - | - | - |
| K562 | - | - | - | - | - | - | - | - | - | - | - | - |
| A1*002:01 | - | - | - | - | - | - | - | - | - | - | - | - |
| A3*13:02 | - | - | - | - | - | - | - | - | - | - | - | - |
| B*048:01 | - | - | - | - | - | - | - | - | - | - | - | - |
| E*02:04 | - | - | - | + | - | + | + | + | - | + | + | + |
| E*02:11 | - | - | - | + | - | + | + | + | - | - | - | - |
| F*01:01 | - | - | - | - | - | - | - | - | - | - | - | - |

| Rh22607 | Gag61-75 (16) | Gag69-83 (18) | Gag89-103 (23) | Gag117-131 (30) | Gag129-143 (33) | Gag197-211 (50) | Gag257-271 (65) | Gag273-287 (69) | Gag385-399 (97) | Gag433-447 (109) | Gag473-487 (119) | Gag477-491 (120) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| BLCL | - | + | + | - | - | - | + | + | + | + | + | + |
| 221 | - | - | - | - | - | - | - | - | - | - | - | - |
| K562 | - | - | - | - | - | - | - | - | - | - | - | - |
| A1*001:01 | - | - | - | - | - | - | - | - | - | - | - | - |
| A1*002:01 | - | + | - | - | - | - | - | - | - | - | - | - |
| A3*13:02 | - | - | - | - | + | - | - | - | - | - | - | - |
| B*001:01 | - | - | - | - | - | - | - | - | - | - | - | - |
| B*030:05 | - | - | - | - | - | - | - | - | - | - | - | - |
| E*02:04 | - | + | + | - | + | - | + | + | + | + | + | + |
| E*02:11 | - | + | + | - | + | - | + | + | + | + | + | + |
| F*01:01 | - | - | - | - | - | - | - | - | - | - | - | - |

Figure 17

| Macaque: | A1*001:01 | A1*002:01 | | Gag$_{69-83}$(18) | Gag$_{129-143}$(33) | Gag$_{197-211}$(55) |
|---|---|---|---|---|---|---|
| Rh22607 | + | + | | + | + | - |
| Rh21826 | + | + | | - | + | + |
| Rh25565 | + | - | | + | + | + |
| Rh25545 | + | - | | - | - | - |
| Rh22034 | - | + | | + | - | - |
| Rh28819 | - | + | | + | - | - |
| Rh28808 | - | + | | - | + | + |
| Rh22436 | - | + | | - | - | + |
| Rh24194 | - | + | | - | - | + |
| Rh27517 | - | - | | + | + | - |
| Rh22063 | - | - | | + | - | - |
| Rh27473 | - | - | | + | - | - |
| Rh27715 | - | - | | + | - | - |
| Rh29483 | - | - | | + | - | - |
| Rh25222 | - | - | | - | + | + |
| Rh29208 | - | - | | - | + | + |
| Rh29212 | - | - | | - | + | + |
| Rh22624 | - | - | | - | + | - |
| Rh29482 | - | - | | - | - | + |
| Rh21756 | - | - | | - | - | - |

Figure 18A
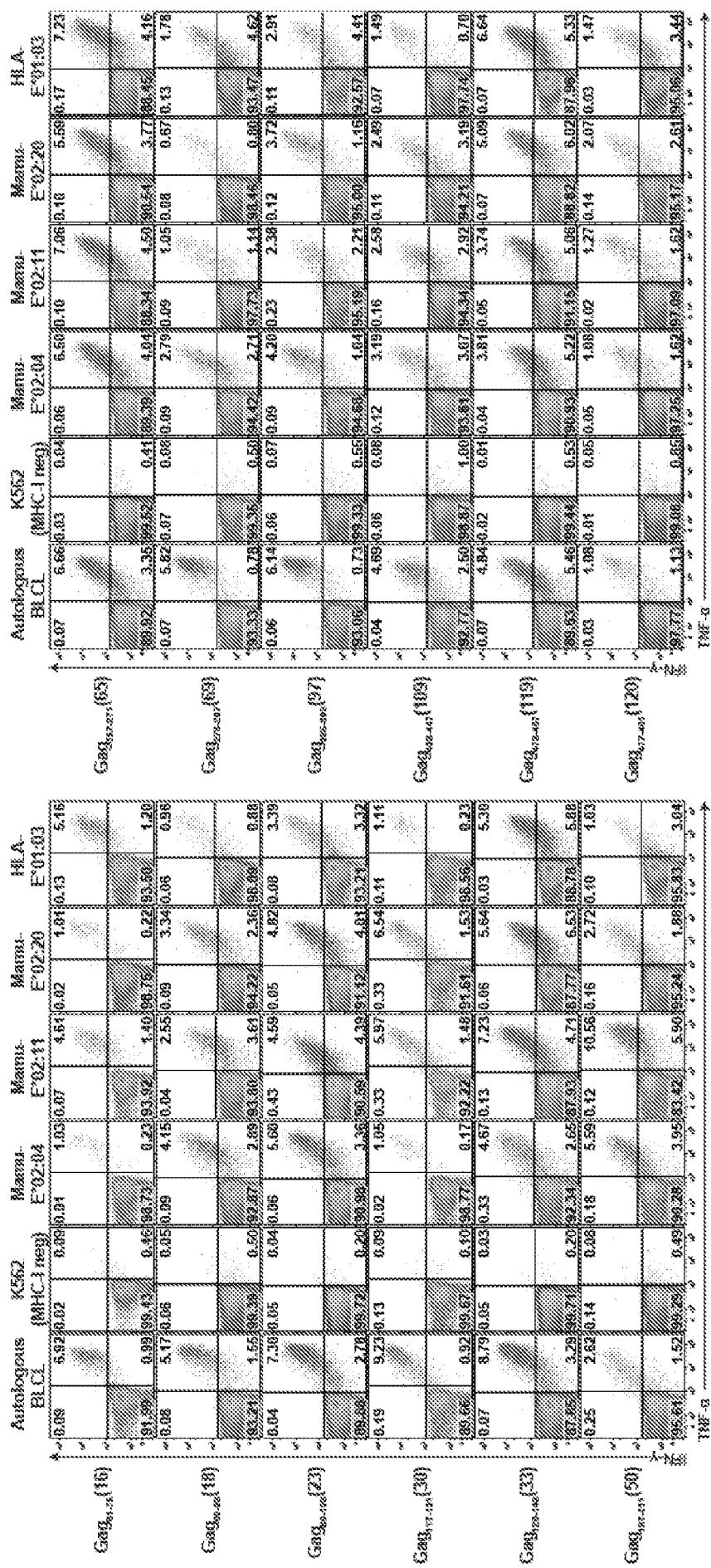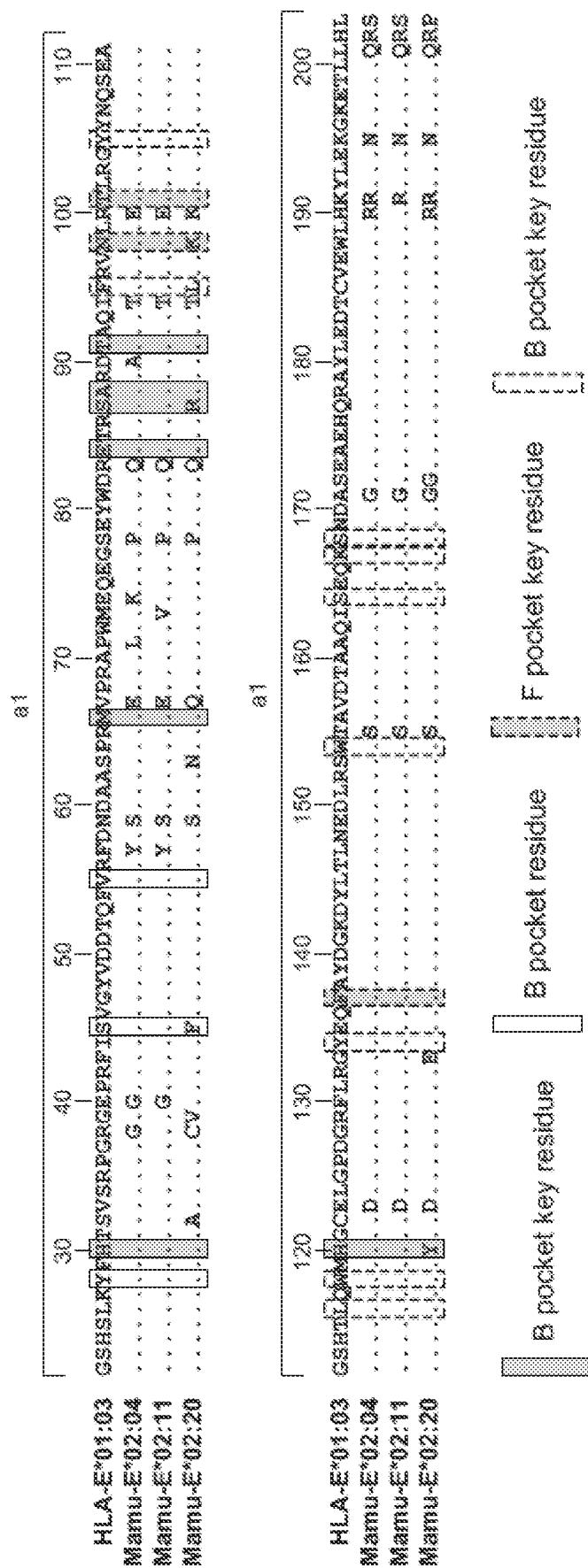
Figure 18B

Figure 19

Figure 20A

## Figure 20B



-○- Rh22436 PBMC/Gag$_{477-491}$(120)-pulsed BLCL

-□- Rh22436 PBMC/Gag$_{477-491}$(120)-pulsed Mamu-E*02:11

-△- Rh27002 PBMC/Gag$_{181-189}$(CM9)-pulsed Mamu-A1*001:01 transf.

-◇- TatSL8 cell line/Tat$_{28-35}$(SL8)-pulsed Mamu-A1*001:01 transf.
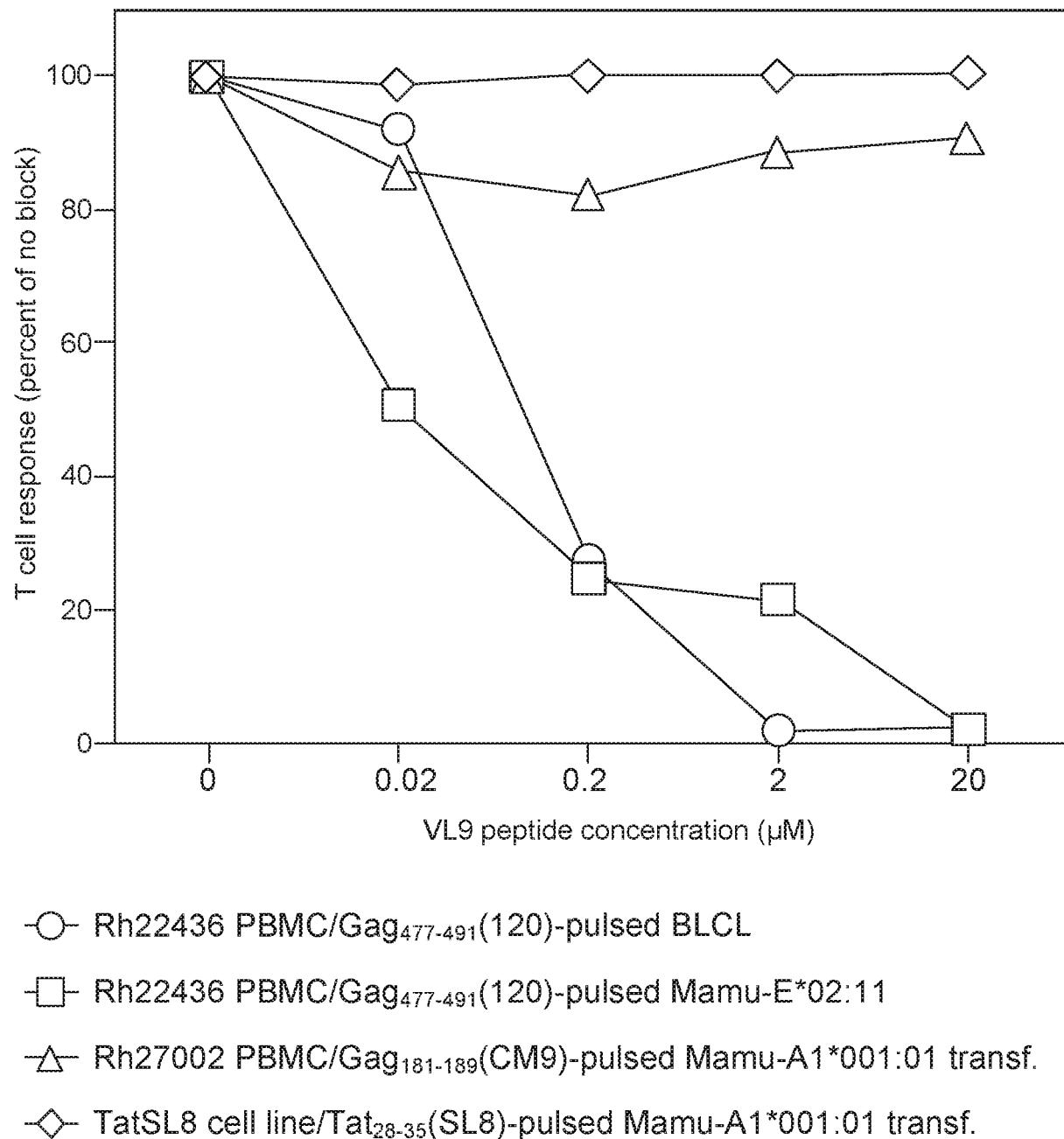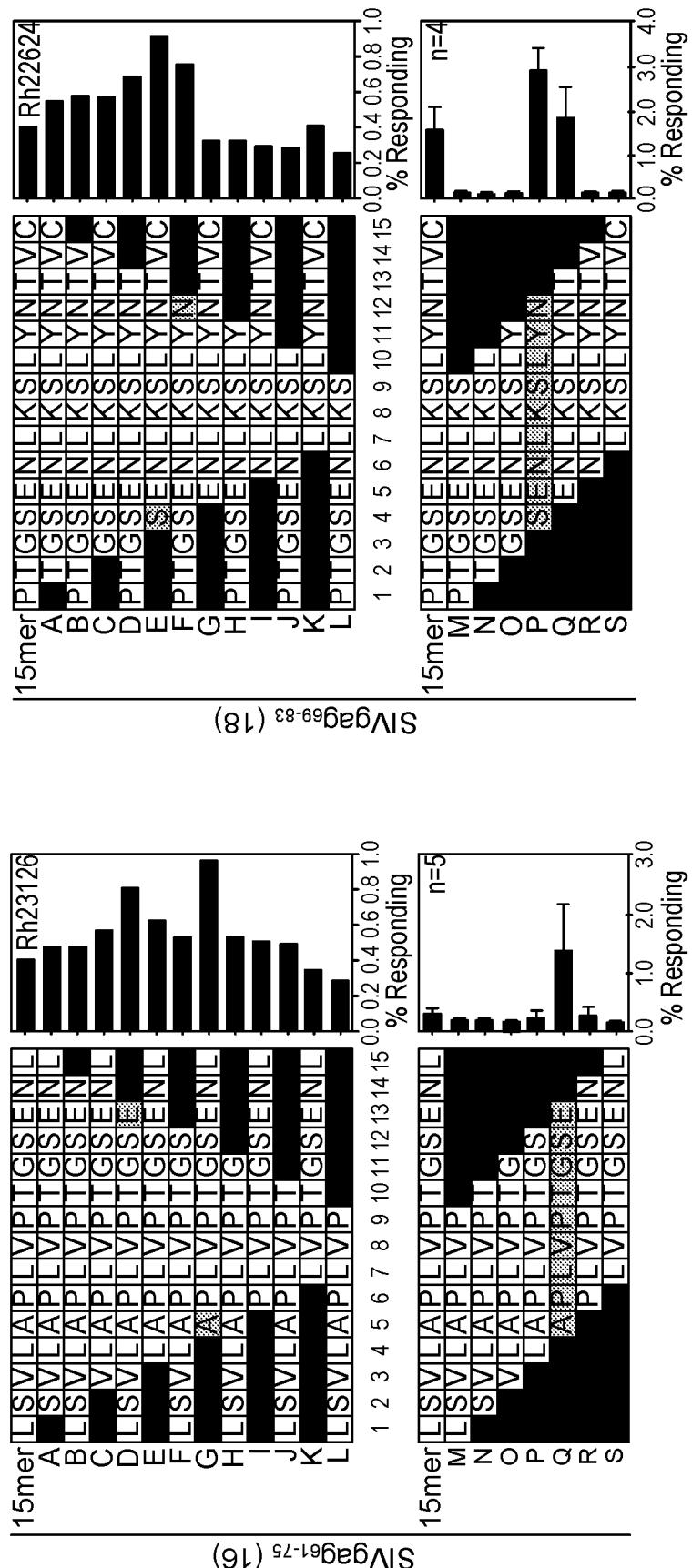
## Figure 21

## Figure 21 (cont.)

Figure 21
(cont.)
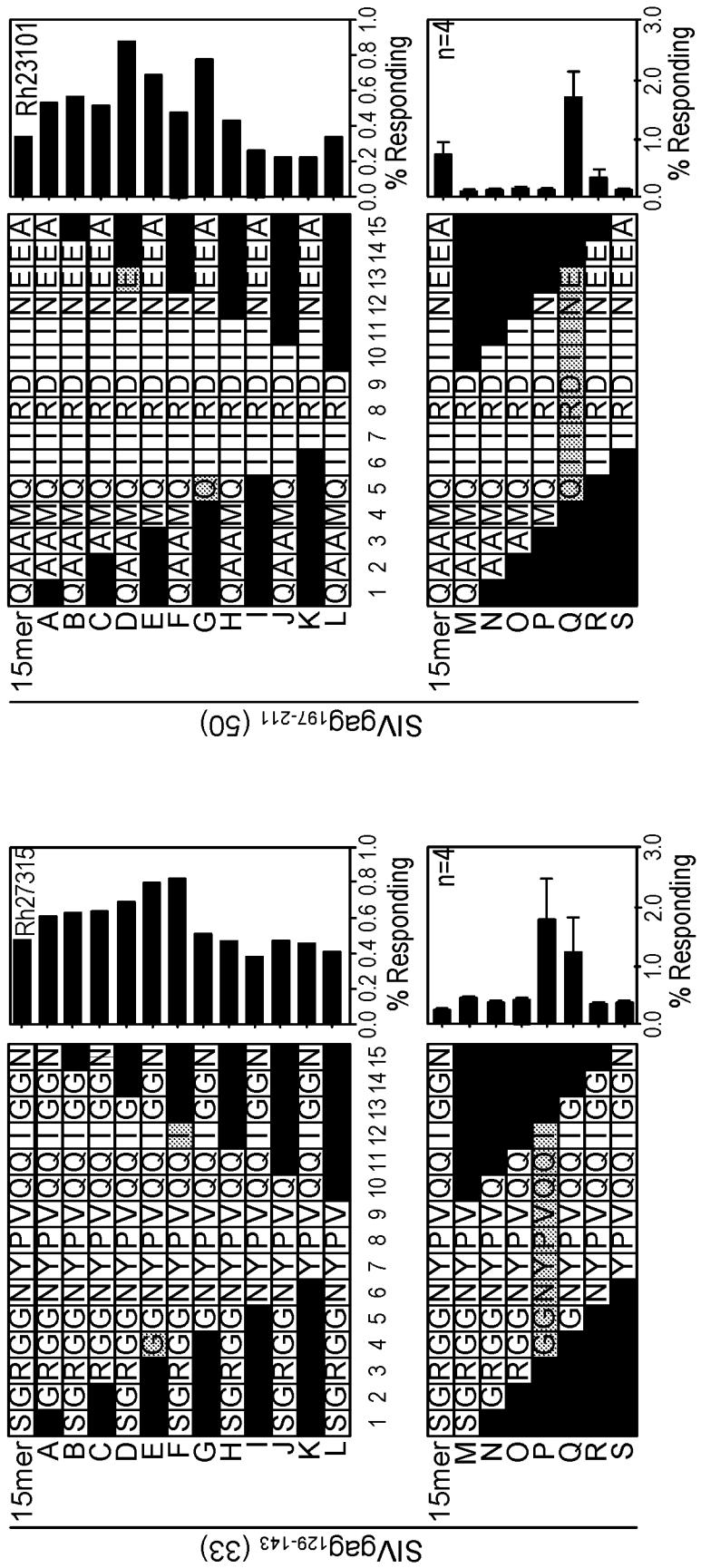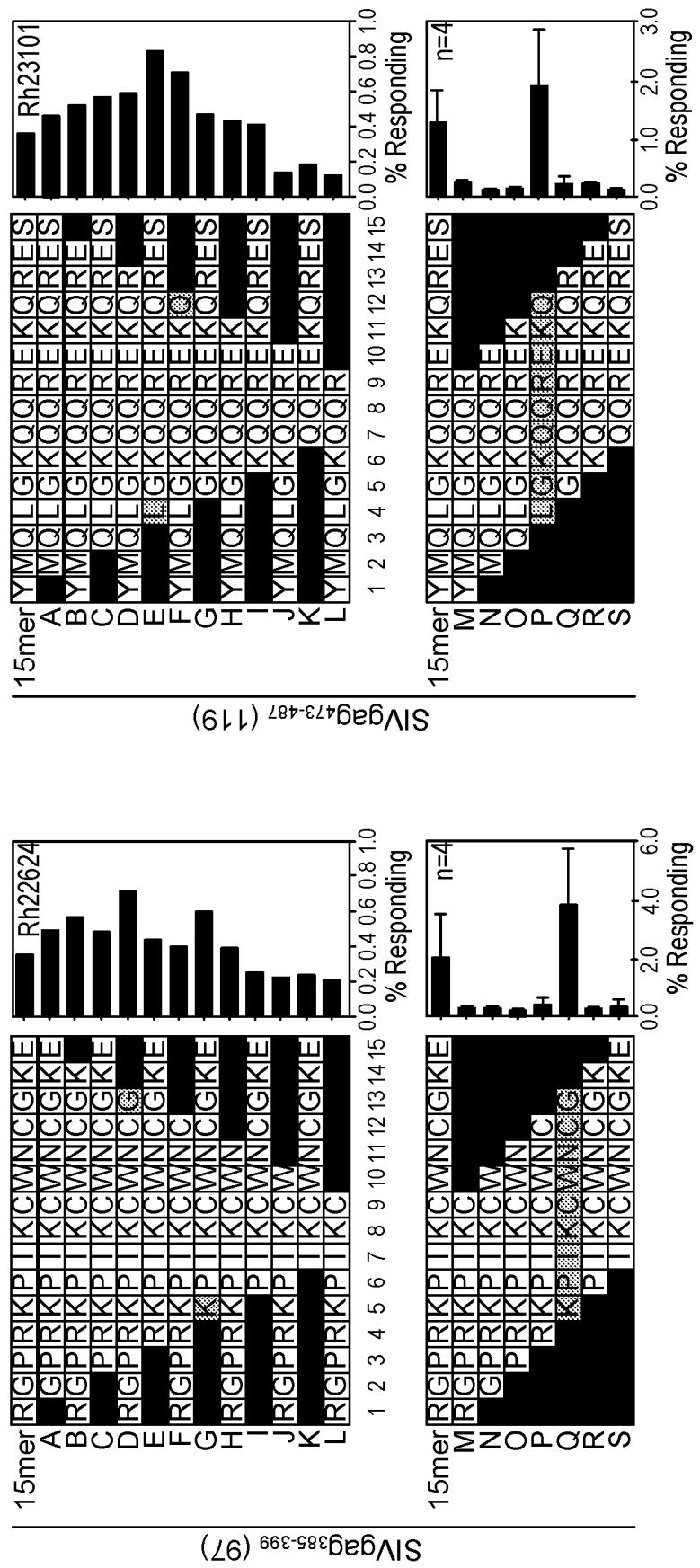
Figure 21
(cont.)

Figure 22A
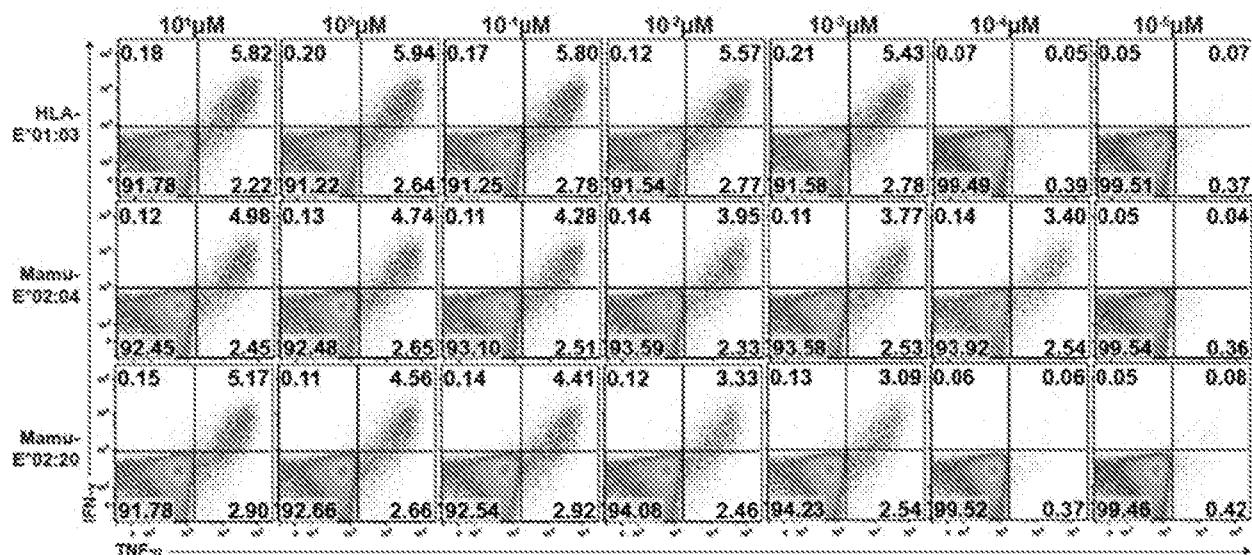


Figure 22B
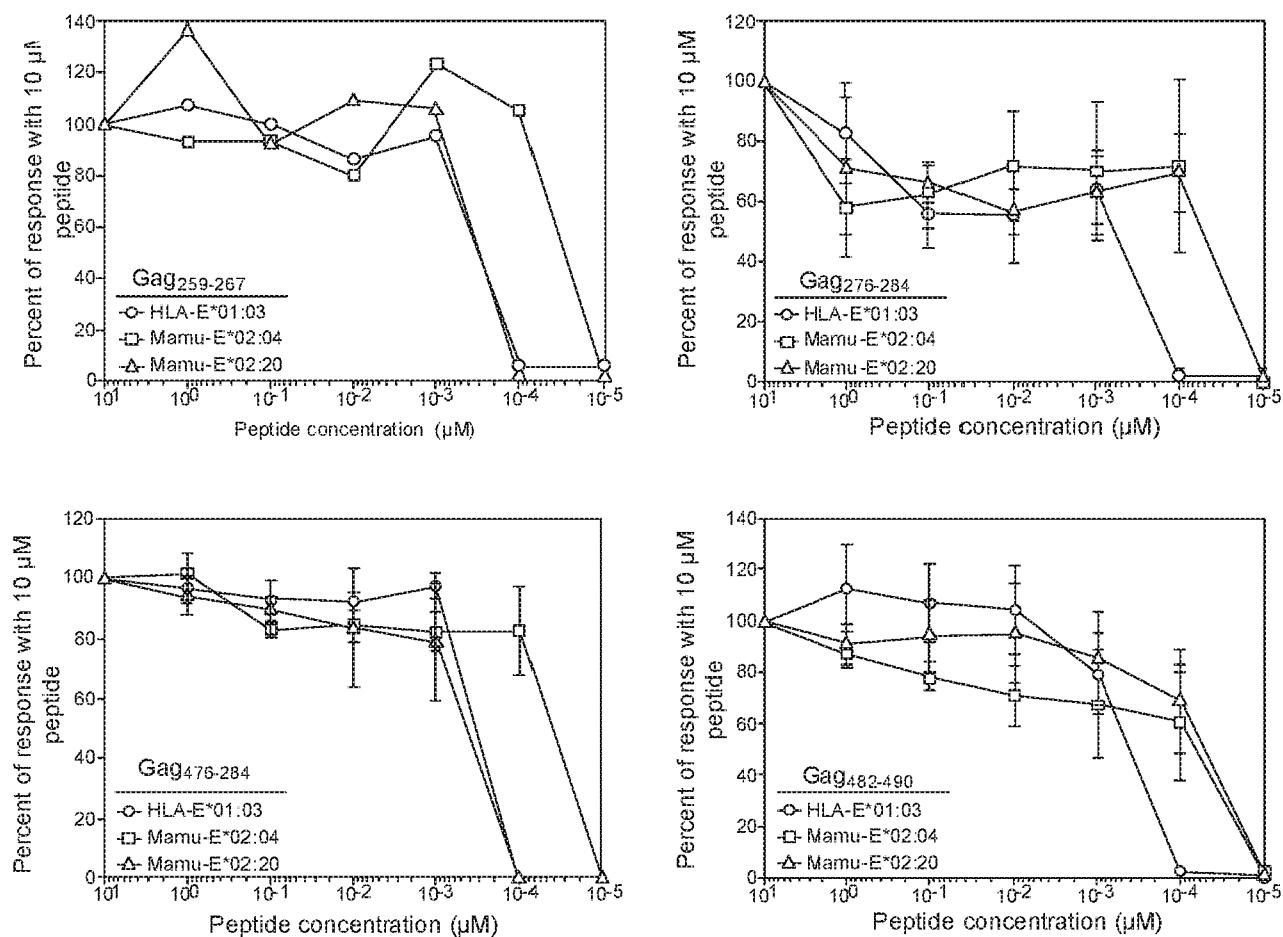
Figure 23
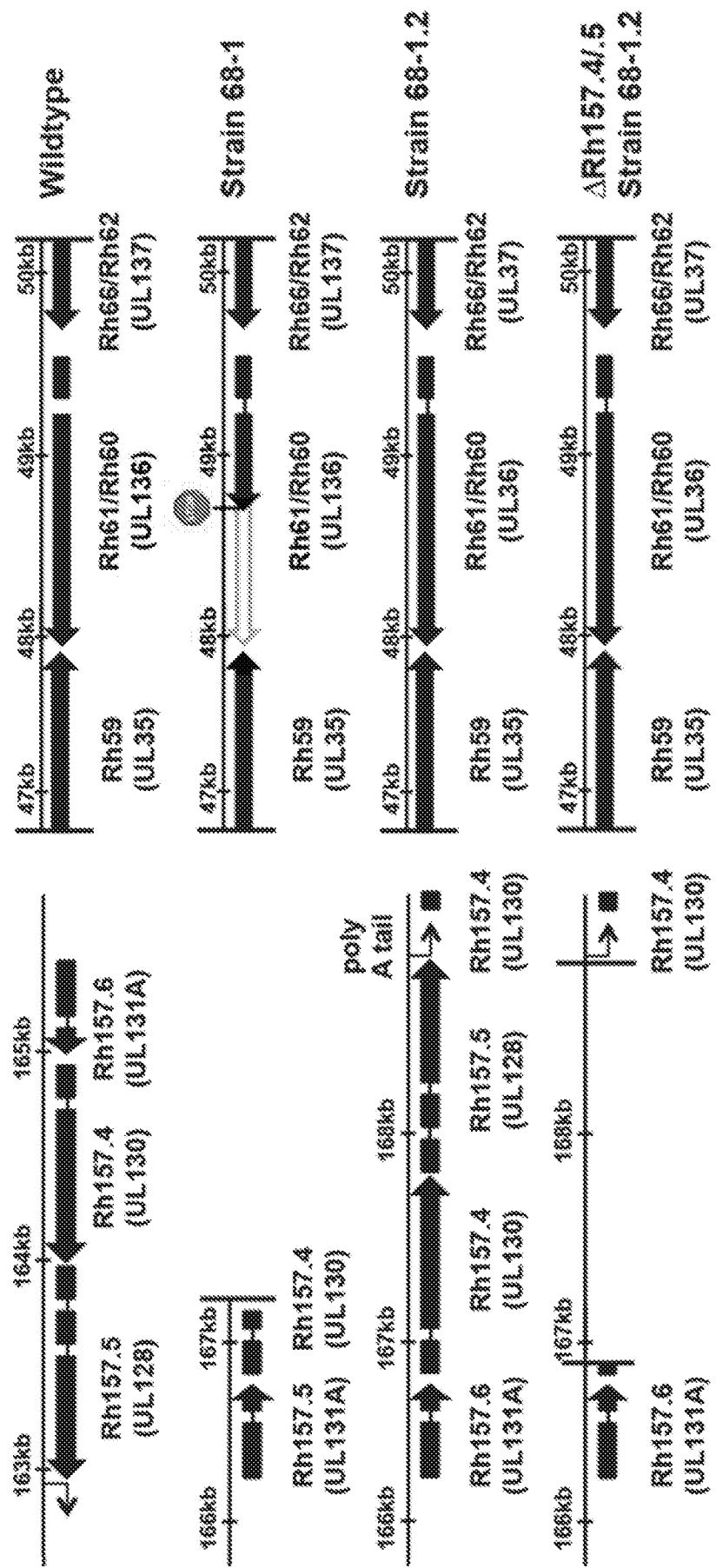
Figure 24A
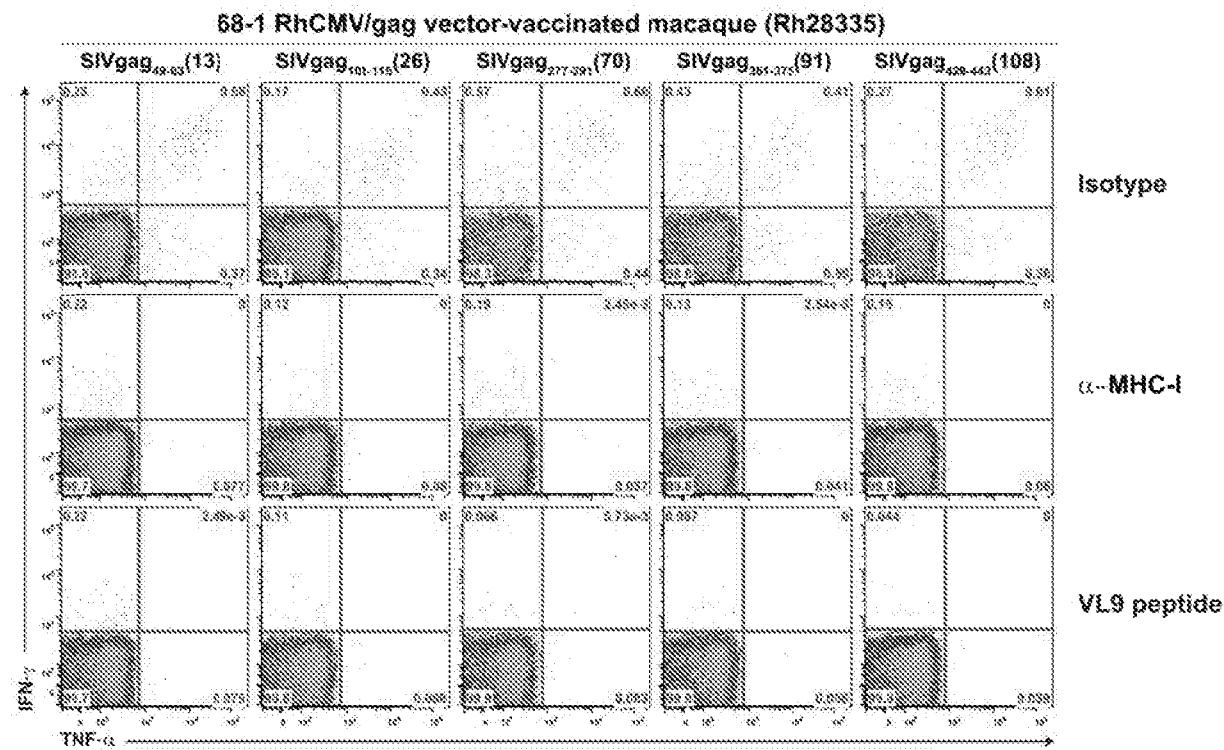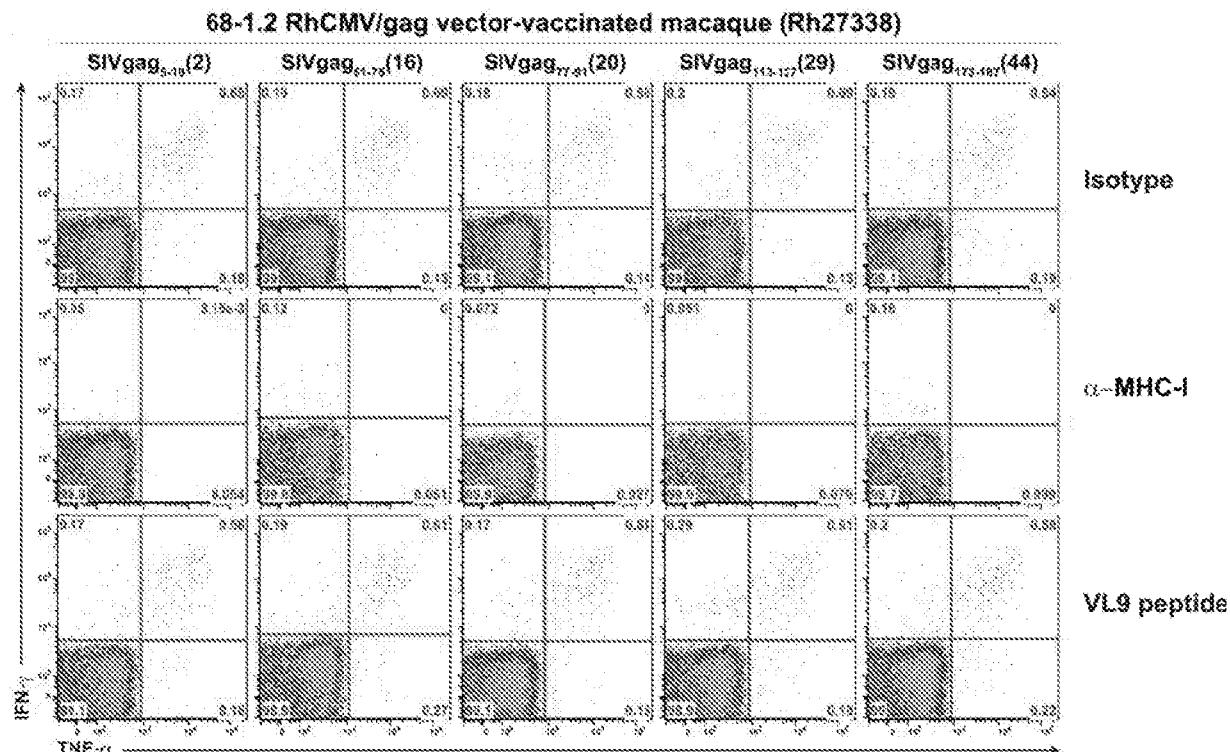


Figure 24B
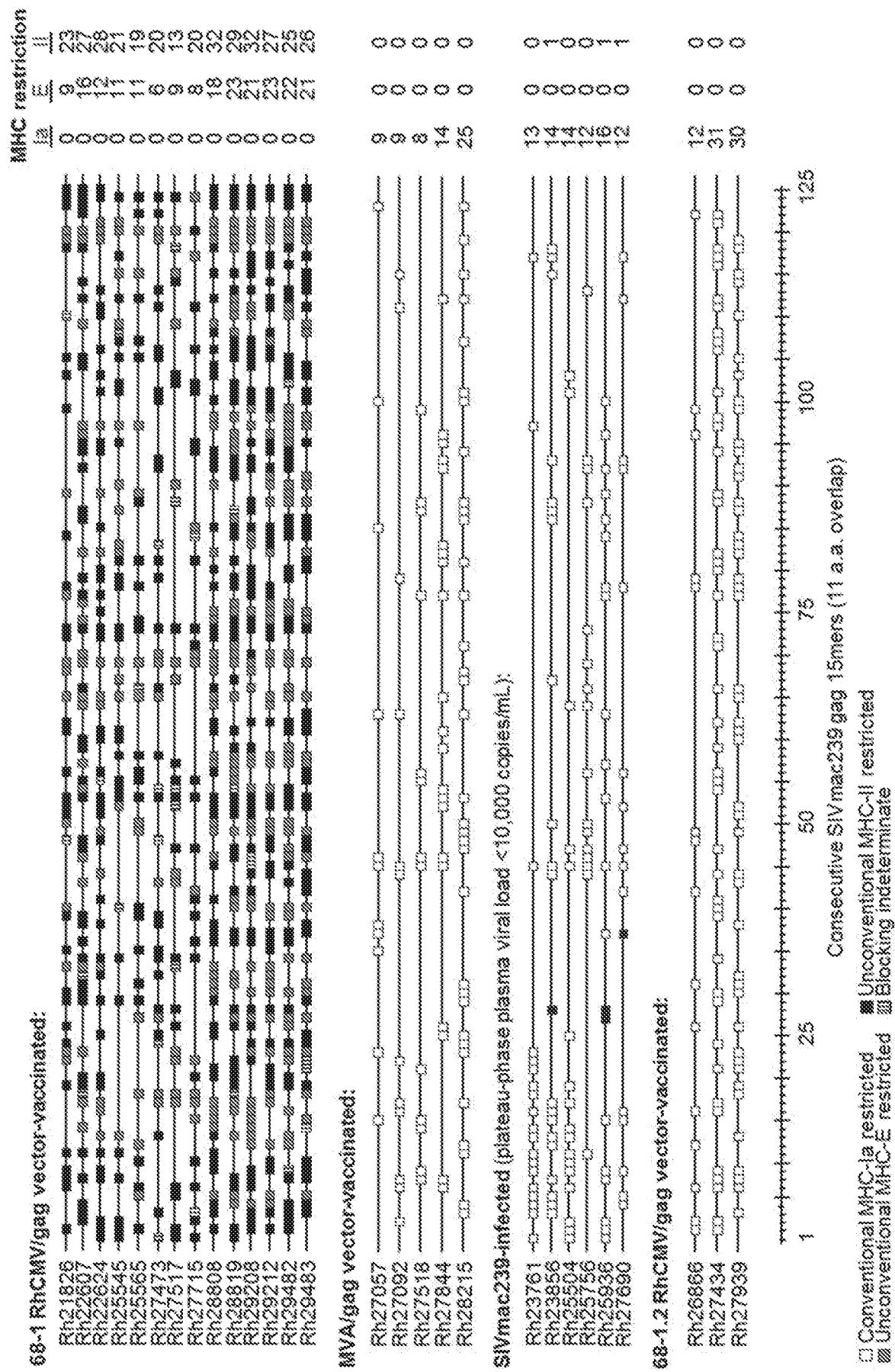
# Figure 25

Figure 26
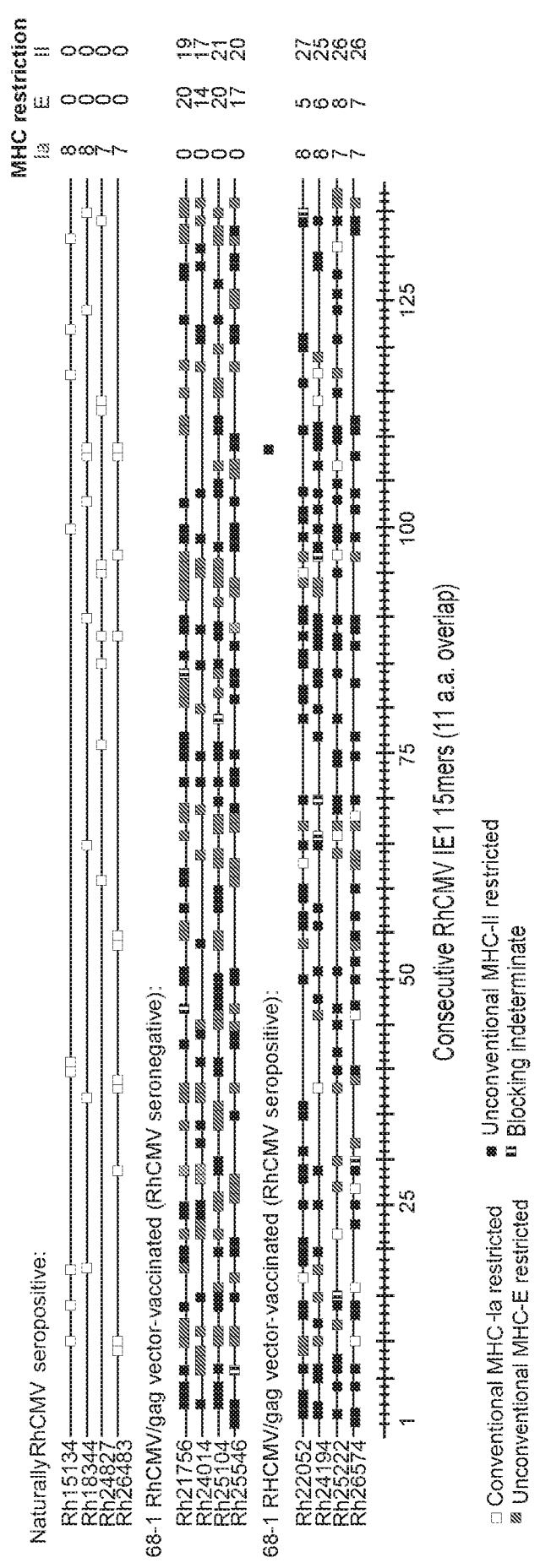
Figure 27A
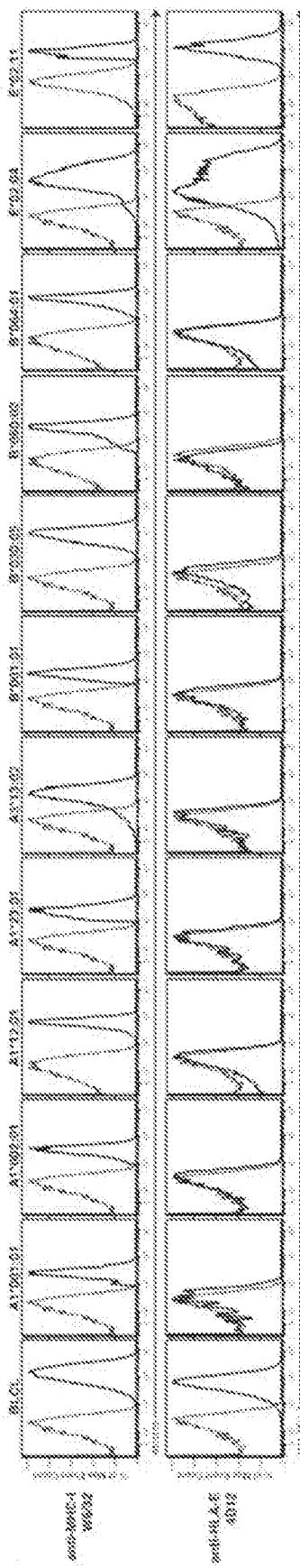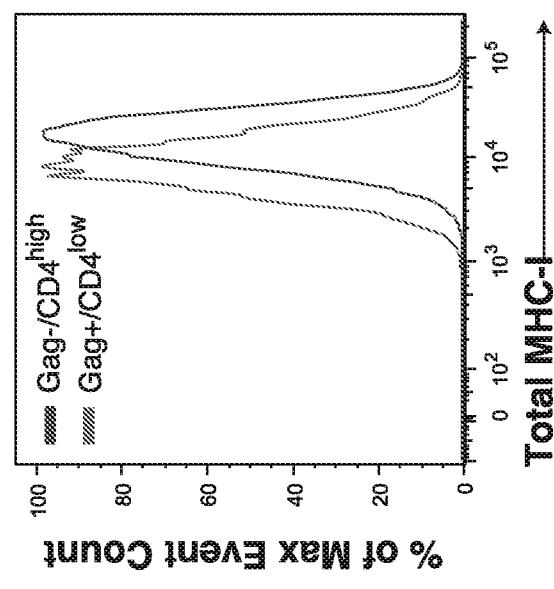


Figure 27C



Figure 27B
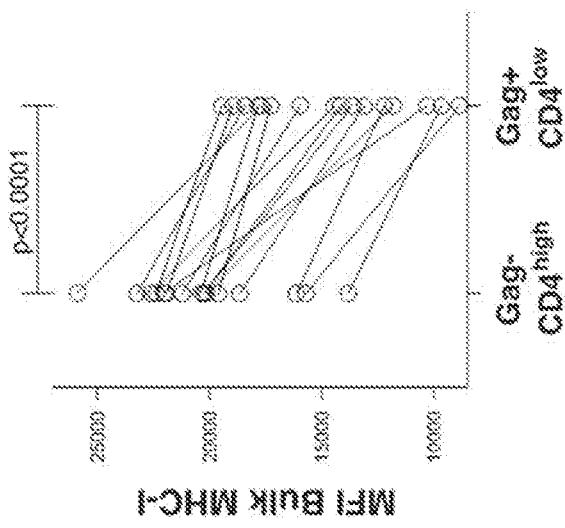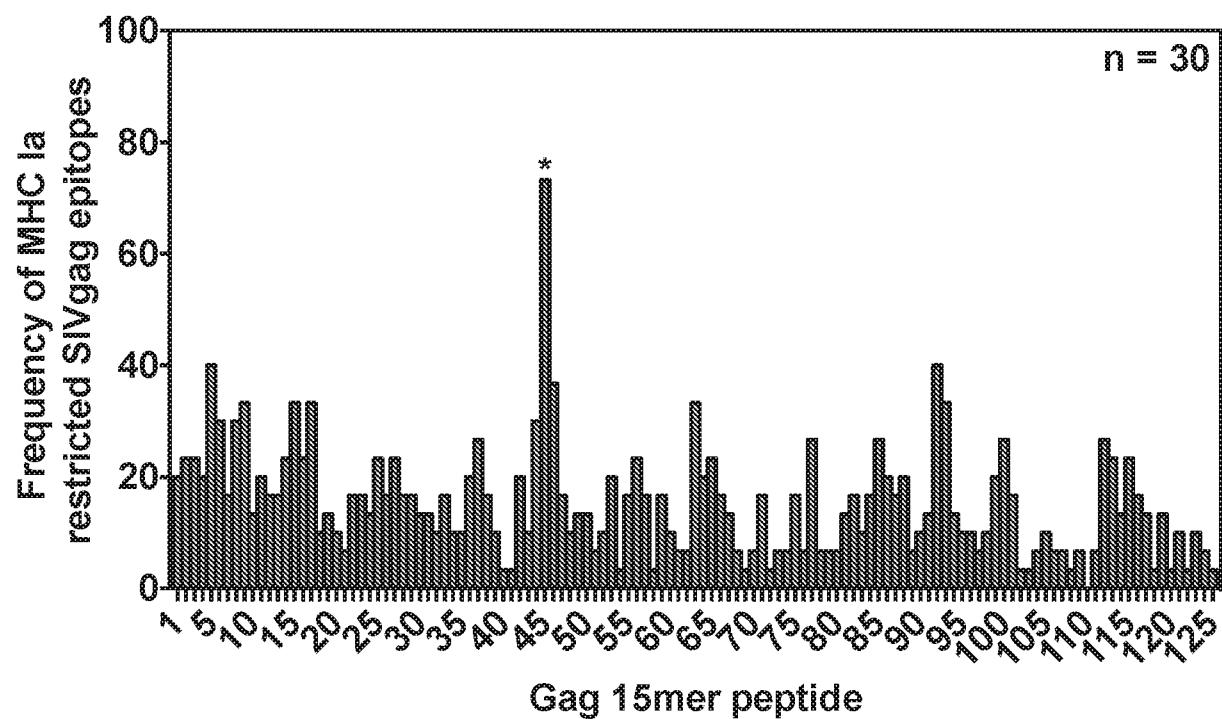
Figure 28

Figure 29
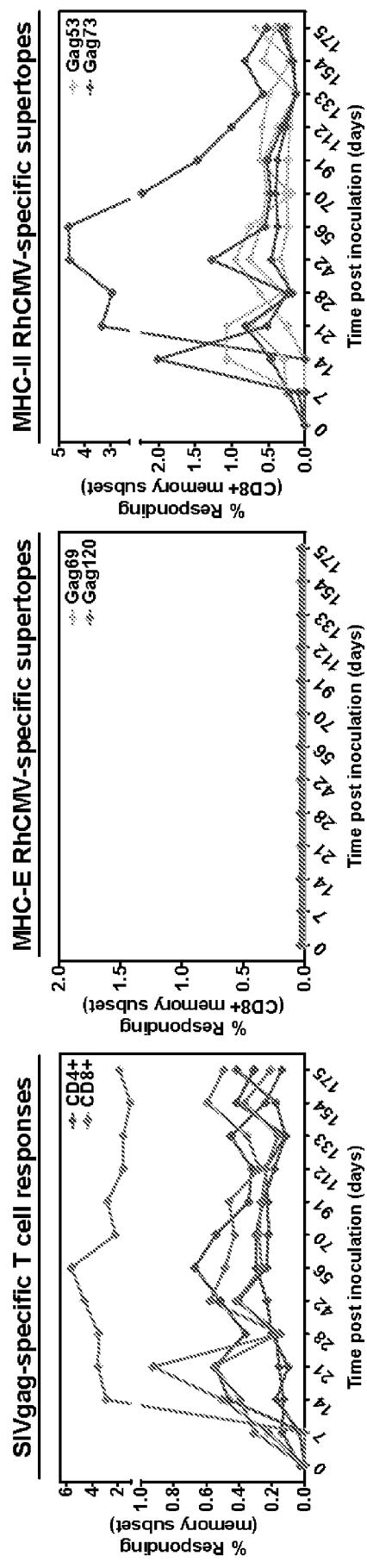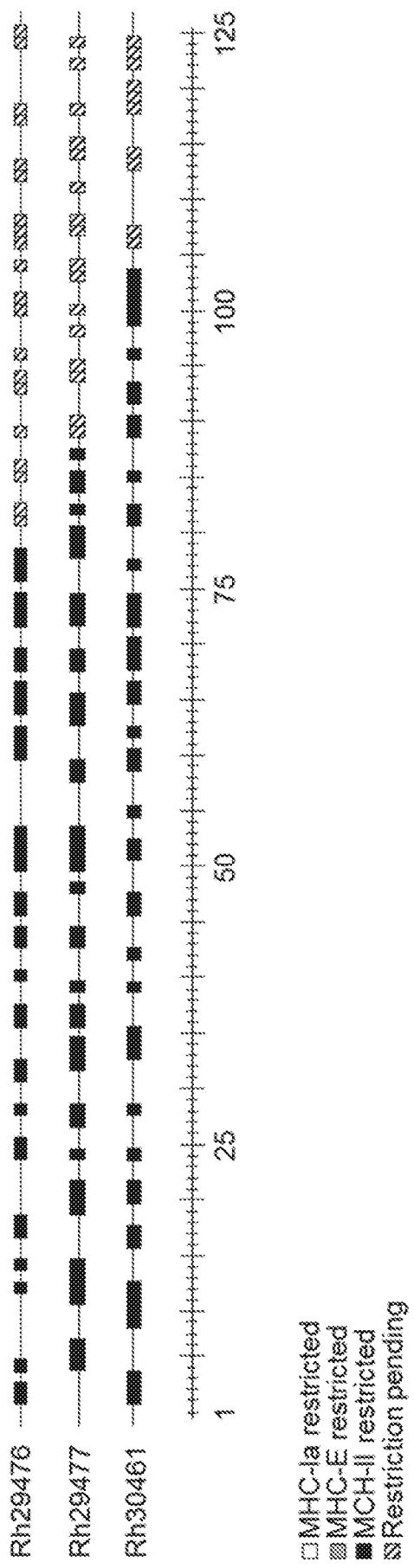
Strain 68-1 ΔRh214-220 (ΔUS28) RhCMV/SIVgag vector-vaccinated macaques:

Figure 30

## A. CLASSIFICATION OF SUBJECT MATTER

**C12N 15/86(2006.01)i, C12N 15/12(2006.01)i, C12N 15/33(2006.01)i, C07K 14/705(2006.01)i, C07K 14/725(2006.01)i, C12N 5/0783(2010.01)i, C12Q 1/68(2006.01)i, A61K 48/00(2006.01)i, A61P 35/00(2006.01)i**

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

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12N 15/86; A61K 39/245; C12N 7/04; C12N 15/12; C12N 7/00; C12N 15/33; A61K 48/00; C12N 15/869; C07K 14/705; C07K 14/725; C12N 5/0783; C12Q 1/68; A61P 35/00

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
Korean utility models and applications for utility models
Japanese utility models and applications for utility models

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
eKOMPASS(KIPO internal) & Keywords:cytomegalovirus, antigen, UL40, US28, UL128, UL130, immune response

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| A | WO 2014-138209 A1 (OREGON HEALTH & SCIENCE UNIVERSITY) 12 September 2014<br>See abstract; claims 1-2. | 1-7,33-39 |
| A | US 2004-0086489 A1 (SCHALL, THOMAS J. et al.) 06 May 2004<br>See abstract; claims 1, 4-5, 12-15 and 24. | 1-7,33-39 |
| A | HANLEY, PATRICK J. et al., 'Controlling cytomegalovirus: helping the immune system take the lead', Viruses, 27 May 2014, Vol. 6, No. 6, pp. 2242-2258<br>See the whole document. | 1-7,33-39 |
| A | WO 2011-143653 A2 (OREGON HEALTH & SCIENCE UNIVERSITY) 17 November 2011<br>See abstract; claims 1-6. | 1-7,33-39 |
| A | US 5720957 A (JONES, THOMAS R. et al.) 24 February 1998<br>See abstract; claims 1-4, 6 and 8-10. | 1-7,33-39 |

☐ Further documents are listed in the continuation of Box C.　　☒ See patent family annex.

| | | | |
|---|---|---|---|
| * | Special categories of cited documents: | "T" | later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention |
| "A" | document defining the general state of the art which is not considered to be of particular relevance | | |
| "E" | earlier application or patent but published on or after the international filing date | "X" | document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone |
| "L" | document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) | "Y" | document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents,such combination being obvious to a person skilled in the art |
| "O" | document referring to an oral disclosure, use, exhibition or other means | | |
| "P" | document published prior to the international filing date but later than the priority date claimed | "&" | document member of the same patent family |

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 23 May 2016 (23.05.2016) | **23 May 2016 (23.05.2016)** |

| Name and mailing address of the ISA/KR | Authorized officer |
|---|---|
| International Application Division<br>Korean Intellectual Property Office<br>189 Cheongsa-ro, Seo-gu, Daejeon, 35208, Republic of Korea | HEO, Joo Hyung |
| Facsimile No.　+82-42-481-8578 | Telephone No.　+82-42-481-8150 |

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

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

1. ☒ Claims Nos.: 14-32,46-101,109-112
   because they relate to subject matter not required to be searched by this Authority, namely:
   Claims 14-32, 46-101 and 109-112 pertain to a method for treatment of the human body by therapy, and thus relate to a subject matter which this International Searching Authority is not required, under PCT Article 17(2)(a)( i ) and PCT Rule 39.1(iv), to search.

2. ☒ Claims Nos.: 10,15-16,18,23,26-28,42,47,53,56-58,64,66,71,74,77,81,85,87,89,93,96,99,103-108
   because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
   Claims 10, 15-16, 18, 23, 26-28, 42, 47, 53, 56-58, 64, 66, 71, 74, 77, 81, 85, 87, 89, 93, 96, 99 and 103-108 are unclear since they are referring to the multiple dependent claims which do not comply with PCT Rule 6.4(a).

3. ☒ Claims Nos.: see extra sheet
   because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

1. ☐ As all required addtional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of any additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

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

- Continuation of Box No. II :

> No. 3.

8-9,11-14,17,19-22,24-25,29-32,40-41,43-46,48-52,54-55,59-63,65,67-70,72-73,75-76,78-80,82-84,86,88,90-92,94-95,97-98,100-102,109-112

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
|---|---|---|---|
| WO 2014-138209 A1 | 12/09/2014 | AU 2014-225886 A1 | 15/10/2015 |
| | | CA 2904001 A1 | 12/09/2014 |
| | | EP 2964769 A1 | 13/01/2016 |
| | | US 2016-0010112 A1 | 14/01/2016 |
| US 2004-0086489 A1 | 06/05/2004 | AU 2002-242085 A1 | 19/08/2002 |
| | | CA 2437201 A1 | 15/08/2002 |
| | | CA 2437201 C | 18/11/2008 |
| | | EP 1364037 A2 | 26/11/2003 |
| | | EP 1364037 A4 | 03/08/2005 |
| | | US 2002-0176870 A1 | 28/11/2002 |
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（54）发明名称
可用于产生非典型CD8+ T细胞应答的方法
和组合物

（57）摘要
    公开了诱导对异源抗原之CD8+T细胞应答的
方法，其中所述CD8+T细胞的至少10％受MHC-E限
制。所述方法包括用不表达UL128和UL130蛋白的
CMV载体进行免疫接种。还公开了包含编码异源
蛋白抗原、UL40蛋白和US28蛋白的核酸但不表达
活性UL128和UL130蛋白的重组CMV载体。还公开
了包含编码异源蛋白抗原的核酸但不表达活性
UL40蛋白、UL128蛋白、UL130蛋白和任选地US28
蛋白的重组CMV载体。还公开了包含编码异源蛋
白抗原的核酸但不表达活性US28蛋白、UL128蛋
白、UL130蛋白和任选地UL40蛋白的重组CMV载
体。

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1.巨细胞病毒(CMV)载体,其包含:

(1)编码至少一种异源抗原的第一核酸序列;

(2)编码至少一种活性UL40蛋白或者其直系同源物或同源物的第二核酸序列;以及

(3)编码至少一种活性US28蛋白或者其直系同源物或同源物的第三核酸序列;

其中所述CMV载体不表达活性UL128蛋白或其直系同源物,并且不表达活性UL130蛋白或其直系同源物。

2.权利要求2所述的CMV载体,其中所述至少一种异源抗原包含病原体特异性抗原、肿瘤抗原、组织特异性抗原或宿主自身抗原。

3.权利要求2所述的CMV载体,其中所述宿主自身抗原是来自于T细胞受体(TCR)之可变区的抗原或来自于B细胞受体之可变区的抗原。

4.权利要求2所述的CMV载体,其中所述病原体特异性抗原来自于选自以下的病原体:人类免疫缺陷病毒、猿猴免疫缺陷病毒、单纯疱疹病毒、乙型肝炎病毒、丙型肝炎病毒、乳头瘤病毒、疟原虫属寄生虫和结核分枝杆菌(Mycobacterium tuberculosis)。

5.权利要求2所述的CMV载体,其中所述肿瘤抗原与选自以下的癌症相关:急性髓细胞性白血病、慢性髓细胞性白血病、骨髓增生异常综合征、急性淋巴细胞白血病、慢性淋巴细胞白血病、非霍奇金淋巴瘤、多发性骨髓瘤、恶性黑素瘤、乳腺癌、肺癌、卵巢癌、前列腺癌、胰腺癌、结肠癌、肾细胞癌(RCC)和生殖细胞肿瘤。

6.权利要求1至5中任一项所述的CMV载体,其中由于在编码UL128或UL130或者其直系同源物的核酸序列中存在一个或更多个突变,所述CMV载体不表达活性UL128或UL130蛋白或者其直系同源物。

7.权利要求6所述的CMV载体,其中编码UL128或UL130或者其直系同源物的核酸序列中的所述一个或更多个突变选自:点突变、移码突变、截短突变和编码所述蛋白之全部核酸序列的缺失。

8.权利要求1至7中任一项所述的CMV载体,其中所述CMV载体还包含第四核酸序列,其中所述第四核酸序列包含抑制UL128或UL130或者其直系同源物表达的反义序列或RNAi序列。

9.权利要求1至8中任一项所述的CMV载体,其中所述CMV载体还包含编码病毒蛋白的一个或更多个病毒基因中的至少一个失活突变,所述病毒蛋白对于体内生长是必需的、非必需的或增强性的。

10.权利要求9所述的CMV载体,其中所述至少一个失活突变选自:点突变、移码突变、截短突变和编码所述病毒蛋白之全部核酸序列的缺失。

11.权利要求9或10所述的CMV载体,其中所述至少一个失活突变在UL82(pp71)中。

12.权利要求9至11中任一项所述的CMV载体,其中所述至少一个失活突变在US11中。

13.权利要求1至12中任一项所述的CMV载体,其中所述CMV载体是人CMV(HCMV)或恒河猴CMV(RhCMV)载体。

14.在对象中产生对至少一种异源抗原之免疫应答的方法,所述方法包括向所述对象施用在所述对象中有效引起对第一异源抗原的CD8⁺T细胞应答之量的权利要求1至13中任一项所述的CMV载体。

15.权利要求14所述的方法,其中由所述CMV载体引起的CD8⁺T细胞的至少10％受MHC-E

或其直系同源物限制。

16.权利要求15所述的方法,其中由所述CMV载体引起的CD8+T细胞的至少20%、至少30%、至少40%、至少50%、至少60%、至少75%、至少90%、至少95%或至少95%受MHC-E或其直系同源物限制。

17.权利要求15或16所述的方法,其还包括鉴定来自由所述CMV载体引起的CD8⁺T细胞的CD8⁺TCR,其中所述CD8⁺TCR识别来自于MHC-E/异源抗原的肽复合物。

18.权利要求17所述的方法,其中通过DNA或RNA测序鉴定所述CD8⁺TCR。

19.权利要求14至18中任一项所述的方法,其中所述对象先前已暴露于CMV。

20.权利要求14至19中任一项所述的方法,其中所述对象是人或非人灵长类。

21.权利要求14至20中任一项所述的方法,其中施用所述CMV载体包括皮下、静脉内、肌内、腹膜内或经口施用所述CMV载体。

22.权利要求14至21中任一项所述的方法,其还包括向所述对象施用第二CMV载体,其中所述第二CMV载体包含编码至少一种异源抗原的核酸序列。

23.权利要求22所述的方法,其中所述第二CMV载体表达活性UL128蛋白。

24.权利要求22或23所述的方法,其中所述第二CMV载体表达活性UL130蛋白。

25.权利要求22至24中任一项所述的方法,其中所述第二CMV载体的所述至少一种异源抗原是病原体特异性抗原、肿瘤抗原、组织特异性抗原或宿主自身抗原。

26.权利要求25所述的方法,其中所述宿主自身抗原是来自于TCR之可变区的抗原或来自于B细胞受体之可变区的抗原。

27.权利要求25所述的方法,其中所述第二CMV载体的所述病原体特异性抗原来自于选自以下的病原体:人类免疫缺陷病毒、猿猴免疫缺陷病毒、单纯疱疹病毒、乙型肝炎病毒、丙型肝炎病毒、乳头瘤病毒、疟原虫属寄生虫和结核分枝杆菌。

28.权利要求25所述的方法,其中所述第二CMV载体的所述肿瘤抗原与选自以下的癌症相关:急性髓细胞性白血病、慢性髓细胞性白血病、骨髓增生异常综合征、急性淋巴细胞白血病、慢性淋巴细胞白血病、非霍奇金淋巴瘤、多发性骨髓瘤、恶性黑素瘤、乳腺癌、肺癌、卵巢癌、前列腺癌、胰腺癌、结肠癌、肾细胞癌(RCC)和生殖细胞肿瘤。

29.权利要求22至24中任一项所述的方法,其中所述第一CMV载体和所述第二CMV载体的所述至少一种异源抗原是相同抗原。

30.权利要求22至29中任一项所述的方法,其中所述第二CMV载体是HCMV或RhCMV载体。

31.权利要求22至30中任一项所述的方法,其中施用所述第二CMV载体包括静脉内、肌内、腹膜内或经口施用所述第二CMV载体。

32.权利要求22至31中任一项所述的方法,其中所述第二CMV载体在所述第一CMV载体之前、与其同时或之后施用。

33.CMV载体,其包含编码至少一种异源抗原的核酸序列,其中所述CMV载体:

(1)不表达活性UL128蛋白或其直系同源物;

(2)不表达活性UL130蛋白或其直系同源物;并且

(3)不表达选自UL40或US28的活性蛋白质或其直系同源物。

34.权利要求33所述的CMV载体,其中所述至少一种异源抗原包含病原体特异性抗原、肿瘤抗原、组织特异性抗原或宿主自身抗原。

35.权利要求34所述的CMV载体,其中所述宿主自身抗原是来自于TCR之可变区的抗原或来自于B细胞受体之可变区的抗原。

36.权利要求34所述的CMV载体,其中所述病原体特异性抗原来自于选自以下的病原体:人类免疫缺陷病毒、猿猴免疫缺陷病毒、单纯疱疹病毒、乙型肝炎病毒、丙型肝炎病毒、乳头瘤病毒、疟原虫属寄生虫和结核分枝杆菌。

37.权利要求34所述的CMV载体,其中所述肿瘤抗原与选自以下的癌症相关:急性髓细胞性白血病、慢性髓细胞性白血病、骨髓增生异常综合征、急性淋巴细胞白血病、慢性淋巴细胞白血病、非霍奇金淋巴瘤、多发性骨髓瘤、恶性黑素瘤、乳腺癌、肺癌、卵巢癌、前列腺癌、胰腺癌、结肠癌、肾细胞癌(RCC)和生殖细胞肿瘤。

38.权利要求33至37中任一项所述的CMV载体,其中由于在编码UL128、UL130、UL40或US28或者其直系同源物的核酸序列中存在一个或更多个突变,所述载体不表达活性UL128、UL130、UL40或US28蛋白或者其直系同源物。

39.权利要求38所述的CMV载体,其中编码UL128、UL130、UL40或US28蛋白或者其直系同源物的核酸序列中的所述一个或更多个突变选自:点突变、移码突变、截短突变和编码所述蛋白之全部核酸序列的缺失。

40.权利要求33至39中任一项所述的CMV载体,其还包含第二核酸序列,其中所述第二核酸序列包含抑制UL128、UL130、UL40或US28或者其直系同源物表达的反义序列或RNAi序列。

41.权利要求33至40中任一项所述的CMV载体,其中所述CMV载体还包含编码病毒蛋白的一个或更多个病毒基因中的至少一个失活突变,所述病毒蛋白对于体内生长是必需的、非必需的或增强性的。

42.权利要求41所述的CMV载体,其中所述至少一个失活突变选自:点突变、移码突变、截短突变和编码所述病毒蛋白之全部核酸序列的缺失。

43.权利要求41或42所述的CMV载体,其中所述至少一个失活突变在UL82(pp71)中。

44.权利要求41至43中任一项所述的CMV载体,其中所述至少一个失活突变在US11中。

45.权利要求33至44中任一项所述的CMV载体,其中所述CMV载体是人CMV(HCMV)或恒河猴CMV(RhCMV)载体。

46.在对象中产生对至少一种异源抗原之免疫应答的方法,所述方法包括向所述对象施用在所述对象中有效引起对所述至少一种异源抗原的CD8$^+$T细胞应答之量的权利要求33至45中任一项所述的CMV载体。

47.权利要求46所述的方法,其中由所述CMV载体引起的CD8$^+$T细胞的少于1%受MHC-E限制。

48.权利要求46或47所述的方法,其中由所述CMV载体引起的CD8$^+$T细胞的多于10%被由单个或多个MHC-II等位基因呈递的肽刺激。

49.权利要求46至48中任一项所述的方法,其中所述对象先前已暴露于CMV。

50.权利要求46至49中任一项所述的方法,其中所述对象是人或非人灵长类。

51.权利要求46至50中任一项所述的方法,其中施用所述CMV载体包括静脉内、肌内、腹膜内或经口施用所述第一CMV载体。

52.权利要求46至51中任一项所述的方法,其还包括向所述对象施用第二CMV载体,其

中所述第二CMV载体包含编码至少一种异源抗原的核酸序列。

53.权利要求52所述的方法,其中所述第二CMV载体表达活性UL128蛋白。

54.权利要求52或53所述的方法,其中所述第二CMV载体表达活性UL130蛋白。

55.权利要求52至54中任一项所述的方法,其中所述第二载体的所述至少一种异源抗原是病原体特异性抗原、肿瘤抗原、组织特异性抗原或宿主自身抗原。

56.权利要求55所述的方法,其中所述宿主自身抗原是来自于TCR之可变区的抗原或来自于B细胞受体之可变区的抗原。

57.权利要求55所述的方法,其中所述第二CMV载体的所述病原体特异性抗原来自于选自以下的病原体:人类免疫缺陷病毒、猿猴免疫缺陷病毒、单纯疱疹病毒、乙型肝炎病毒、丙型肝炎病毒、乳头瘤病毒、疟原虫属寄生虫和结核分枝杆菌。

58.权利要求55所述的方法,其中所述第二CMV载体的所述肿瘤抗原与选自以下的癌症相关:急性髓细胞性白血病、慢性髓细胞性白血病、骨髓增生异常综合征、急性淋巴细胞白血病、慢性淋巴细胞白血病、非霍奇金淋巴瘤、多发性骨髓瘤、恶性黑素瘤、乳腺癌、肺癌、卵巢癌、前列腺癌、胰腺癌、结肠癌、肾细胞癌(RCC)和生殖细胞肿瘤。

59.权利要求52至54中任一项所述的方法,其中所述第一CMV载体和所述第二CMV载体的所述至少一种异源抗原是相同抗原。

60.权利要求52至59中任一项所述的方法,其中所述第二CMV载体是HCMV或RhCMV载体。

61.权利要求52至60中任一项所述的方法,其中施用所述第二CMV载体包括静脉内、肌内、腹膜内或经口施用所述第二CMV载体。

62.权利要求52至61中任一项所述的方法,其中所述第二CMV载体在所述第一CMV载体之前、与其同时或之后施用。

63.产生识别MHC-E-肽复合物之CD8$^+$T细胞的方法,所述方法包括:

(1)向对象施用有效产生识别MHC-E/肽复合物的CD8$^+$T细胞组之量的权利要求1至13中任一项所述的CMV载体;

(2)鉴定来自所述CD8$^+$T细胞组的第一CD8$^+$TCR,其中所述第一CD8$^+$TCR识别来自于MHC-E/异源抗原的肽复合物;

(3)从所述对象中分离一种或更多种CD8$^+$T细胞;以及

(4)用表达载体转染所述一种或更多种CD8$^+$T细胞,其中所述表达载体包含编码第二CD8$^+$TCR的核酸序列和与编码所述第二CD8$^+$TCR的核酸序列可操作地连接的启动子,其中所述第二CD8$^+$TCR包含所述第一CD8$^+$TCR的CDR3α和CDR3β,从而产生识别来自于MHC-E/异源抗原的肽复合物的一种或更多种经转染CD8$^+$T细胞。

64.权利要求63所述的方法,其中通过DNA或RNA测序鉴定所述第一CD8$^+$TCR。

65.权利要求63或64所述的方法,其中所述第二CD8$^+$TCR包含所述第一CD8$^+$TCR的CDR1α、CDR2α、CDR3α、CDR1β、CDR2β和CDR3β。

66.权利要求65所述的方法,其中编码所述第二CD8$^+$TCR的核酸序列与编码所述第一CD8$^+$TCR的核酸序列相同。

67.权利要求63至66中任一项所述的方法,其中向所述对象施用所述CMV载体包括向所述对象静脉内、肌内、腹膜内或经口施用所述CMV载体。

68.权利要求63至67中任一项所述的方法,其中所述对象先前已暴露于CMV。

69.权利要求63至68中任一项所述的方法,其中所述对象是人或非人灵长类。

70.权利要求63至69中任一项所述的方法,其中所述CMV载体的所述至少一种异源抗原包含肿瘤抗原。

71.权利要求70所述的方法,其中所述肿瘤抗原与选自以下的癌症相关:急性髓细胞性白血病、慢性髓细胞性白血病、骨髓增生异常综合征、急性淋巴细胞白血病、慢性淋巴细胞白血病、非霍奇金淋巴瘤、多发性骨髓瘤、恶性黑素瘤、乳腺癌、肺癌、卵巢癌、前列腺癌、胰腺癌、结肠癌、肾细胞癌(RCC)和生殖细胞肿瘤。

72.权利要求70或71所述的方法,其还包括向所述对象施用所述经转染CD8$^+$T细胞以治疗癌症。

73.权利要求63至69中任一项所述的方法,其中所述CMV载体的所述至少一种异源抗原包含病原体特异性抗原。

74.权利要求73所述的方法,其中所述病原体特异性抗原来自于选自以下的病原体:人类免疫缺陷病毒、猿猴免疫缺陷病毒、单纯疱疹病毒、乙型肝炎病毒、丙型肝炎病毒、乳头瘤病毒、疟原虫属寄生虫和结核分枝杆菌。

75.权利要求73或74所述的方法,其还包括向所述对象施用所述经转染CD8$^+$T细胞以治疗病原体感染。

76.权利要求63至69中任一项所述的方法,其中所述CMV载体的所述至少一种异源抗原包含宿主自身抗原或组织特异性抗原。

77.权利要求76所述的方法,其中所述宿主自身抗原是来自于TCR之可变区的抗原或来自于B细胞受体之可变区的抗原。

78.权利要求76或77所述的方法,其还包括向所述对象施用所述经转染CD8$^+$T细胞以治疗自身免疫性疾病或病症。

79.权利要求76或77所述的方法,其还包括向所述对象施用所述经转染CD8$^+$T细胞以诱导对所述宿主自身抗原或组织特异性抗原的自身免疫应答。

80.产生识别MHC-E肽复合物之CD8$^+$T细胞的方法,所述方法包括:

(1)向第一对象施用有效产生识别MHC-E/肽复合物的CD8$^+$T细胞组之量的权利要求1至13中任一项所述的CMV载体;

(2)鉴定来自所述CD8$^+$T细胞组的第一CD8$^+$TCR,其中所述第一CD8$^+$TCR识别来自于MHC-E/异源抗原的肽复合物;

(3)从第二对象中分离一种或更多种CD8$^+$T细胞;以及

(4)用表达载体转染所述一种或更多种CD8$^+$T细胞,其中所述表达载体包含编码第二CD8$^+$TCR的核酸序列和与编码所述第二CD8$^+$TCR的核酸序列可操作地连接的启动子,其中所述第二CD8$^+$TCR包含所述第一CD8$^+$TCR的CDR3α和CDR3β,从而产生识别来自于MHC-E/异源抗原的肽复合物的一种或更多种经转染CD8$^+$T细胞。

81.权利要求80所述的方法,其中通过DNA或RNA测序鉴定所述第一CD8$^+$TCR。

82.权利要求80或81所述的方法,其中所述第一对象是人或非人灵长类。

83.权利要求80至82中任一项所述的方法,其中所述第二对象是人或非人灵长类。

84.权利要求80至83中任一项所述的方法,其中所述第一对象是非人灵长类且所述第二对象是人,并且其中所述第二CD8$^+$TCR是包含所述第一CD8$^+$TCR的非人灵长类CDR3α和CDR3

β的嵌合非人灵长类-人CD8+TCR。

85.权利要求84所述的方法,其中所述第二CD8+TCR包含所述第一CD8+TCR的非人灵长类CDR1α、CDR2α、CDR3α、CDR1β、CDR2β和CDR3β。

86.权利要求80至83中任一项所述的方法,其中所述第二CD8+TCR包含所述第一CD8+TCR的CDR1α、CDR2α、CDR3α、CDR1β、CDR2β和CDR3β。

87.权利要求86所述的方法,其中编码所述第二CD8+TCR的核酸序列与编码所述第一CD8+TCR的核酸序列相同。

88.权利要求80至83中任一项所述的方法,其中所述第二CD8+TCR是嵌合CD8+TCR。

89.权利要求88所述的方法,其中所述第二CD8+TCR包含所述第一CD8+TCR的CDR1α、CDR2α、CDR3α、CDR1β、CDR2β和CDR3β。

90.权利要求80至89中任一项所述的方法,其中向所述第一对象施用所述CMV载体包括向所述第一对象静脉内、肌内、腹膜内或经口施用所述CMV载体。

91.权利要求80至90中任一项所述的方法,其中所述第一对象先前已暴露于CMV。

92.权利要求80至91中任一项所述的方法,其中所述至少一种异源抗原包含肿瘤抗原。

93.权利要求92所述的方法,其中所述肿瘤抗原与选自以下的癌症相关:急性髓细胞性白血病、慢性髓细胞性白血病、骨髓增生异常综合征、急性淋巴细胞白血病、慢性淋巴细胞白血病、非霍奇金淋巴瘤、多发性骨髓瘤、恶性黑素瘤、乳腺癌、肺癌、卵巢癌、前列腺癌、胰腺癌、结肠癌、肾细胞癌(RCC)和生殖细胞肿瘤。

94.权利要求92或93所述的方法,其还包括向所述第二对象施用所述经转染CD8+T细胞以治疗癌症。

95.权利要求80至91中任一项所述的方法,其中所述至少一种异源抗原包含病原体特异性抗原。

96.权利要求95所述的方法,其中所述病原体特异性抗原来自于选自以下的病原体:人类免疫缺陷病毒、猿猴免疫缺陷病毒、单纯疱疹病毒、乙型肝炎病毒、丙型肝炎病毒、乳头瘤病毒、疟原虫属寄生虫和结核分枝杆菌。

97.权利要求95或96所述的方法,其还包括向所述第二对象施用所述经转染CD8+T细胞以治疗病原体感染。

98.权利要求80至91中任一项所述的方法,其中所述至少一种异源抗原包含宿主自身抗原或组织特异性抗原。

99.权利要求98所述的方法,其中所述宿主自身抗原是来自于TCR之可变区的抗原或来自于B细胞受体之可变区的抗原。

100.权利要求98或99所述的方法,其还包括向所述第二对象施用所述经转染CD8+T细胞以治疗自身免疫性疾病或病症。

101.权利要求98或99所述的方法,其还包括向所述对象施用所述经转染CD8+T细胞以诱导对所述宿主自身抗原或组织特异性抗原的自身免疫应答。

102.通过权利要求63至101中任一项所述的方法产生的CD8+T细胞。

103.权利要求102所述的CD8+T细胞,其中所述CMV载体的所述至少一种异源抗原包含肿瘤抗原。

104.权利要求103所述的CD8+T细胞,其中所述肿瘤抗原与选自以下的癌症相关:急性髓

细胞性白血病、慢性髓细胞性白血病、骨髓增生异常综合征、急性淋巴细胞白血病、慢性淋巴细胞白血病、非霍奇金淋巴瘤、多发性骨髓瘤、恶性黑素瘤、乳腺癌、肺癌、卵巢癌、前列腺癌、胰腺癌、结肠癌、肾细胞癌(RCC)和生殖细胞肿瘤。

105.权利要求102所述的CD8$^+$T细胞,其中所述CMV载体的所述至少一种异源抗原包含病原体特异性抗原。

106.权利要求105所述的CD8$^+$T细胞,其中所述病原体特异性抗原来自于选自以下的病原体:人类免疫缺陷病毒、猿猴免疫缺陷病毒、单纯疱疹病毒、乙型肝炎病毒、丙型肝炎病毒、乳头瘤病毒、疟原虫属寄生虫和结核分枝杆菌。

107.权利要求102所述的CD8$^+$T细胞,其中所述CMV载体的所述至少一种异源抗原包含宿主自身抗原或组织特异性抗原。

108.权利要求107所述的CD8$^+$T细胞,其中所述宿主自身抗原是来自于TCR之可变区的抗原或来自于B细胞受体之可变区的抗原。

109.治疗癌症的方法,所述方法包括向对象施用权利要求103或104所述的CD8$^+$T细胞。

110.治疗病原体感染的方法,所述方法包括向对象施用权利要求105或106所述的CD8$^+$T细胞。

111.治疗自身免疫性疾病或病症的方法,所述方法包括向对象施用权利要求107或108所述的CD8$^+$T细胞。

112.诱导对宿主自身抗原或组织特异性抗原之自身免疫应答的方法,所述方法包括向对象施用权利要求107或108所述的CD8$^+$T细胞。

# 可用于产生非典型CD8+ T细胞应答的方法和组合物

[0001]　　相关申请的交叉引用

[0002]　　本申请要求2015年2月10日提交的美国临时申请No.62/114,203、2015年7月24日提交的美国临时申请No.62/196,520、以及2015年9月 18日提交的美国临时申请No.62/220,703的优先权权益,其各自通过引用整体并入本文。

## 技术领域

[0003]　　总体来说,本领域是在免疫接种中使用CMV载体。更具体地,本领域是产生以非典型MHC限制性为特征的CD8$^+$免疫应答。更具体地,本领域是产生具有受MHC-E限制之受体的T细胞,包括CD8$^+$。

[0004]　　政府支持声明

[0005]　　本发明是在由美国国立卫生研究院(National Institutes of Health) 授予的基金号P01 AI094417的美国政府支持下产生的。美国政府对本发明享有一定的权利。

## 背景技术

[0006]　　表达猿猴免疫缺陷病毒(Simian Immunodeficiency Virus,SIV)蛋白的恒河猴巨细胞病毒(RhCMV) 疫苗载体(RhCMV/SIV)提供了针对病原性SIV的防护(Hansen,S.G.等,Nat Med 15,293(2009);Hansen, S.G.等,Nature 473,523(2011);二者均通过引用并入本文)。这种防护与其他T细胞疫苗的根本区别在于其极高的效力以及几乎瞬时起效,约 50％的疫苗接种者(vaccinnee)在显著平缓且缩短的病毒血症急性期后表现出对病毒复制的完全控制。尽管RhCMV保护的恒河猴显示病毒血症的周期性低水平"尖峰(blip)",但是未观察到CD4$^+$记忆T细胞耗竭,未发生SIV特异性抗体应答,并且随后,随着时间的推移病毒核酸变得几乎不可量化,同时能够复制的病毒从受保护动物的组织中消失。这些事件在自发的SIV原种对照者(elite controller)和DNA致敏(prime)/Ad5 加强疫苗接种的(boostvaccinated)对照者中并不发生(Hansen,S.G.等, Nature 502,100(2013)中,通过引用并入本文)。鉴于RhCMV诱导的 CD8$^+$ T细胞在经RhCMV/SIV疫苗接种的恒河猴中介导这种保护作用中的中心作用,定义这些T细胞的功能特性对于理解其对RhCMV/SIV载体诱导之SIV复制控制的机制性贡献至关重要。理解这些性质可进而导致表达异源抗原之巨细胞病毒(CMV)疫苗载体的新用途。

## 发明内容

[0007]　　本文中公开了在对象中产生对至少一种异源抗原之免疫应答的方法。所述方法包括向所述对象施用有效量的CMV载体。所述CMV载体包含编码所述至少一种异源抗原的第一核酸、编码至少一种活性UL40蛋白或者其同源物或直系同源物的第二核酸序列、以及编码至少一种US28 蛋白或者其同源物或直系同源物的第三核酸序列。所述CMV载体不表达活性UL128蛋白或其直系同源物,并且不表达活性UL130蛋白或其直系同源物,并且由所述载体产生的CD8$^+$ T细胞的至少10％受MHC-E或其同源物限制。在一些实施方案中,所述第三核酸

序列编码二至五种活性 US28蛋白或者其同源物或直系同源物。所述异源抗原可以是任何抗原,包括来自于例如以下的病原体特异性抗原:人类免疫缺陷病毒(HIV)、猿猴免疫缺陷病毒(SIV)、单纯疱疹病毒、乙型或丙型肝炎病毒、乳头瘤病毒(papillomavirus)、疟原虫属(Plasmodium)寄生虫和结核分枝杆菌(Mycobacterium tuberculosis)。在另一些实例中,所述异源抗原可以是肿瘤抗原,包括例如与以下相关的肿瘤抗原:急性髓细胞性白血病(acute myelogenous leukemia)、慢性髓细胞性白血病(chronic myelogenous leukemia)、骨髓增生异常综合征(myelodysplastic syndrome)、急性淋巴细胞白血病(acute lymphoblastic leukemia)、慢性淋巴细胞白血病(chronic lymphoblastic leukemia)、非霍奇金淋巴瘤 (non-Hodgkin's lymphoma)、多发性骨髓瘤、恶性黑素瘤、乳腺癌、肺癌、卵巢癌、前列腺癌、胰腺癌、结肠癌、肾细胞癌(renal cell carcinoma,RCC)和生殖细胞肿瘤(germ cell tumor)。在另一些实例中,所述异源抗原可以是组织特异性抗原或宿主自身抗原,包括例如来自于T细胞受体(TCR)之可变区的抗原、来自于B细胞受体之可变区的抗原、精子抗原或卵抗原。在另一些实例中,所述载体不编码(1)活性UL40蛋白(或其直系同源物)和/或活性US28蛋白(或其直系同源物),(2)活性 UL128蛋白(或其直系同源物),以及(3)活性UL130蛋白(或其直系同源物),产生MHC-II"超表位(supertope)"限制性CD8$^+$ T细胞而非 HLA-E限制性CD8$^+$ T细胞。

[0008]　　本文中还公开了人或动物巨细胞病毒载体,其包含编码(1)至少一种异源蛋白抗原的第一核酸序列,(2)编码至少一种活性UL40蛋白或者其同源物或直系同源物的第二核酸序列,以及 (3)编码至少一种活性US28 蛋白或者其同源物或直系同源物的第三核酸序列。所述载体不表达活性 UL128和UL130蛋白或其直系同源物。在一些实施方案中,所述第三核酸序列编码二至五种活性US28蛋白或者其同源物或直系同源物。

[0009]　　还公开了人或动物巨细胞病毒载体,其(1)不表达活性UL128蛋白 (或其直系同源物),(2)不表达活性UL130蛋白 (或其直系同源物),并且(3)不表达活性UL40蛋白(或其直系同源物) 和/或活性US28蛋白(或其直系同源物)。

[0010]　　本文中还公开了产生识别MHC-E-肽复合物之CD8$^+$ T细胞的方法。所述方法包括向第一对象施用有效产生识别MHC-E/肽复合物的CD8$^+$ T 细胞组之量的CMV载体,其编码(1)至少一种异源抗原,(2)至少一种活性UL40蛋白(或其直系同源物或同源物),以及(3)至少一种活性 US28基因(或其直系同源物或同源物)。所述CMV载体不编码活性 UL128和UL130蛋白或其直系同源物。在一些实施方案中,所述CMV 载体编码二至五种活性US28蛋白或者其直系同源物或同源物。所述异源抗原可以是任何抗原,包括病原体特异性抗原、肿瘤抗原、自身抗原或组织特异性抗原。在一些实施方案中,所述自身抗原是来自于T或B细胞受体之可变区的抗原。在一些实施方案中,该方法还可包括鉴定所述CD8$^+$ T细胞组的第一CD8$^+$ T 细胞受体,其中所述第一CD8$^+$ TCR识别来自于 MHC-E/异源抗原的肽复合物。在一些实施方案中,通过DNA或RNA 测序鉴定所述第一CD8$^+$ T细胞受体。在一些实施方案中,该方法还可包括用表达载体转染从第一对象或第二对象分离的一种或更多种T细胞,其中所述表达载体包含编码第二CD8$^+$ T细胞受体的核酸序列和与编码所述第二CD8$^+$ T细胞受体的核酸序列可操作地连接的启动子,其中所述第二CD8$^+$ T细胞受体包含所述第一CD8$^+$ T细胞受体的CDR3α和CDR3β,从而产生识别来自于MHC-E/异源抗原的肽复合物的一种或更多种经转染CD8$^+$ T细胞。在一些实施方案中,该方法还可包括向第一或第二对象施用所述经转染CD8$^+$ T

细胞以治疗疾病,例如癌症、病原体感染 (pathogenic infection)或自身免疫性疾病或病症。在一些实施方案中,该方法还可包括向第一或第二对象施用所述经转染CD8$^+$ T细胞以诱导对宿主自身抗原或组织特异性抗原的自身免疫应答。

[0011]　　还公开了通过包括以下步骤的方法制备的识别MHC-E-肽复合物的经转染CD8$^+$ T细胞:(1)向第一对象施用有效产生识别MHC-E/肽复合物的CD8$^+$ T细胞组之量的CMV载体,其中所述CMV载体包含编码至少一种异源抗原的第一核酸序列、编码至少一种活性UL40蛋白或者其直系同源物或同源物的第二核酸序列、以及编码至少一种活性US28蛋白或者其直系同源物或同源物的第三核酸序列,并且其中所述CMV载体不表达活性UL128和UL130蛋白或其直系同源物;(2) 鉴定所述CD8$^+$ T细胞组的第一CD8$^+$ T细胞受体,其中所述第一CD8$^+$ T细胞受体识别来自于MHC-E/异源抗原的肽复合物;(3) 从所述第一对象或第二对象分离一种或更多种CD8$^+$ T细胞;以及(4)用表达载体转染从所述第一或第二对象分离的所述一种或更多种CD8$^+$ T细胞,其中所述表达载体包含编码第二CD8$^+$ T细胞受体的核酸序列和与编码所述第二CD8$^+$ T细胞受体的核酸序列可操作地连接的启动子,其中所述第二CD8$^+$ T细胞受体包含所述第一CD8$^+$ T细胞受体的CDR3α和CDR3β,从而产生识别MHC-E- 肽复合物的经转染T细胞。所述异源抗原可以是任何抗原,包括病原体特异性抗原或肿瘤抗原。在一些实施方案中,所述CMV载体的第三核酸序列编码二至五种活性US28蛋白或者其直系同源物或其同源物。本文中还公开了治疗疾病(例如癌症、病原体感染或自身免疫性疾病或病症)的方法,所述方法包括向第一或第二对象施用识别MHC-E-肽复合物的经转染CD8$^+$ T细胞。

[0012]　　附图简述

[0013]　　使用彩色可以更好地理解本文中所包括的一些图表(graph)和曲线图(plot),这在专利申请公开中不可用。申请人考虑原始公开内容的所有原始公开的图像和图表(无论是否为彩色)部分,并且保留在以后的诉讼程序(proceedings)中呈现本文中所描述附图的彩色图表和曲线图的权利。

[0014]　　图1A是来自经毒株68-1 RhCMV/gag疫苗接种的恒河猴(Rh22034 或Rh21826)的外周血单个核细胞(peripheral blood mononuclear cell,PBMC)的一组流式细胞术图。如实施例1中所讨论的,RhCMV毒株68-1不表达来自Rh13、Rh60、Rh157.5和157.4(分别为HCMV RL11、UL36、UL128和UL130) 开放阅读框的基因产物。使用流式细胞术细胞内细胞因子染色 (intracellular cytokine staining,ICS)检测在与用所示肽脉冲的指定的抗原呈递细胞一起孵育后的IFN-γ 和/或TNF-α产生(每个象限中所示CD8$^+$ T细胞的应答频率) 来评价PBMC的肽特异性CD8$^+$ T细胞识别。亲本MHC-I阴性K562细胞用作阴性对照并且还被转染以表达指定的MHC-I分子,而自体B淋巴母细胞样细胞系 (B-lymphoblastoid cellline,BLCL)用作阳性对照。

[0015]　　图1B是来自经毒株68-1 RhCMV/gag载体疫苗接种的恒河猴 (Rh22034和Rh21826)的PBMC中CD8$^+$ T细胞的一组流式细胞术图 (左图)和柱状图(右图),示出了在与用经Gag$_{273-287}$(SIVmac239Gag 15聚体#69)脉冲的抗原呈递细胞(自体BLCL或仅表达Mamu-E的K562 转染子)一起孵育后的IFN-γ 和/或TNF-α产生(每个象限中所示CD8$^+$ T 细胞的应答频率)。在没有额外肽(无阻断)或者在Mamu-E结合肽 Rh67$_{8-16}$VL9(Rh67VL9)或Mamu-A＊002:01结合肽Gag$_{71-79}$GY9 (SIVgag GY9)的存在下,将抗原呈递细胞与指定的Gag 15聚体(Gag 15-met)一起孵育。右图是肽阻断条件对CD8$^+$ T细胞的IFN-γ 和/或 TNF-α产生的比

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较,所述CD8$^+$T细胞来自与经Gag$_{273-287}$(SJVmac239Gag 15聚体#69)脉冲的自体BLCL或Mamu-E转染子一起孵育的四只经毒株68-1 RhCMV/gag载体疫苗接种的恒河猴。数据相对于不用肽阻断所观察到的应答进行归一化。

[0016] 图1C是来自经毒株68-1 RhCMV/gag载体疫苗接种的恒河猴 (Rh22034和Rh21826)的PBMC中CD8$^+$T细胞的一组流式细胞术图 (左图)和柱状图(右图),示出了在与用经Gag$_{477-491}$(SIVmac239Gag 15聚体#120)脉冲的抗原呈递细胞(自体BLCL或仅表达Mamu-E的K562转染子)一起孵育后的IFN-γ和/或TNF-α产生(每个象限中所示 CD8$^+$T细胞的应答频率)。在没有额外肽(无阻断)或者在Mamu-E结合肽Rh67$_{8-16}$VL9(Rh67VL9)或Mamu-A＊002:01结合肽Gag$_{71-79}$GY9 (SIVgag GY9)的存在下,将抗原呈递细胞与指定的Gag 15聚体一起孵育。右图是肽阻断条件对CD8$^+$T细胞的IFN-γ和/或TNF-α产生的比较,所述CD8$^+$T细胞来自与经Gag$_{477-491}$(SIVmac239Gag 15聚体#120)脉冲的自体BLCL或Mamu-E转染子一起孵育的四只经毒株68-1 RhCMV/gag载体疫苗接种的恒河猴。数据相对于不用肽阻断所观察到的应答进行归一化。

[0017] 图2A是说明了以下的表:在用毒株68-1 RhCMV/gag载体(n=6)、毒株68-1.2 RhCMV/gag载体(n=9)、MVA/gag载体(n=7)疫苗接种的恒河猴中以及在SIVmac239感染的恒河猴(n=8)中,使用流式细胞术 ICS检测125个连续的15聚体Gag肽(具有11个氨基酸重叠)的识别来对CD8$^+$T细胞对SIVmac239Gag的应答进行表位作图 (epitope-mapped)。如实施例1所讨论的,RhCMV毒株68-1.2中恢复了Rh60、Rh157.5和Rh157.4(分别为HCMV UL36、UL128和UL130) 的表达。使导致高于背景CD8$^+$T细胞应答的肽进行MHC-I(mAb W6/32)、MHC-E(Rh67VL9)和MHC-II(mAb G46-6)阻断,并分类为MHC-I阻断(白色填充框)、完全MHC-E阻断(灰色填充框)、部分 MHC-E阻断(水平阴影线(hatch)填充框)、MHC-II阻断(黑色填充框)或不确定(垂直阴影线填充框)。右侧指出了每只恒河猴中潜在包含在这些反应性肽中的独立MHC-E阻断表位的最小数目(参见方法)。注意,恒河猴22063和22624用来自于BAC的RhCMV/gag疫苗接种,而恒河猴21826、22034、22436和22607用来自于非BAC的RhCMVgag(L)疫苗接种。

[0018] 图2B是来自经MamuA1＊001:01+毒株68-1 RhCMV/gag载体疫苗接种的恒河猴的PBMC中CD8$^+$T细胞的一组流式细胞术图,示出了在与用Gag$_{69-83}$(Gag#18)肽单独脉冲(无阻断)或者在MHC-E结合Rh67$_{8-16}$ VL9或Mamu-A＊01结合Gag$_{181-189}$CM9肽的存在下与抗原呈递细胞(自体BLCL或仅表达MamuA1＊001:01或Mamu-E的K562转染子)一起孵育后的IFN-γ和/或TNF-α产生(每个象限中所示CD8$^+$T细胞的应答频率)。

[0019] 图2C是来自经与如图2B所述的抗原呈递细胞一起孵育的 MamuA1＊001:01-毒株68-1 RhCMV/gag载体疫苗接种的恒河猴的PBMC 中CD8$^+$T细胞的一组流式细胞术图。

[0020] 图3A是一组两幅图,示出了生产性SIV感染(CD4$^-$Gag p27$^+$)或未感染(CD4$^+$Gagp27$^-$)的CD4$^+$T细胞靶标的表面上的本体(bulk)表面MHC-I(通过mAb W6/32测量)。在左图上示出了代表性流式细胞术图,而右图示出了来自总共16只不相关恒河猴的SIV感染的与未感染的 CD4$^+$ T细胞中本体MHC-I染色的平均荧光强度(mean fluorescent intensity,MFI)。

[0021] 图3B是一组两幅图,示出了生产性SIV感染(CD4$^-$Gag p27$^+$)或未感染(CD4$^+$Gagp27-)的CD4$^+$ T细胞靶标的表面上的MHC-E(通过 mAb 4D12测量)。在左图上示出了代表

性流式细胞术图,而右图示出了来自总共16只不相关恒河猴的SIV感染的与未感染的CD4$^+$ T细胞中 MHC-E染色的MFI。

[0022]　　图3C是示出了响应于Gag$_{273-287}$(69)或Gag$_{477-491}$(120)肽刺激的 MHC-E限制性CD8$^+$ T细胞的表型的图。通过检查表达每种标志物的 IFN-γ 和/或TNF-α产生细胞的数目来计算百分比。

[0023]　　图4A是分离自用毒株68-1 RhCMV/gag、MVA/gag、毒株68-1.2 RhCMV/gag疫苗接种的或者用SIV感染的恒河猴的CD8$^+$ T细胞的一组代表性流式细胞术图,示出了在与单独的经自体SIVmac239感染的CD4$^+$ T细胞(无阻断)或者在MHC-II结合II类相关恒定链肽(MHC-II binding Class II-associated invariant chain peptide,CLIP)加泛MHC-I阻断mAb W6/32(W6/32+CLIP)或Rh67$_{8-16}$VL9加CLIP(VL9+CLIP)的存在下孵育后CD8$^+$ T细胞的IFN-γ 和/或TNF-α产生。

[0024]　　图4B是对于来自用毒株68-1 RhCMV/gag(n=5)、MVA/gag(n=6)、毒株68-1.2 RhCMV/gag(n=4)疫苗接种或用SIV感染(n=6)的恒河猴的CD8$^+$ T细胞之图4A所示的识别和阻断实验的归一化应答频率进行比较的柱状图。

[0025]　　图4C是一组流式细胞术图,说明了MHC-E限制性Gag$_{477-491}$Gag #120表位(上排)或Mamu-A＊001:01限制性Gag$_{181-189}$CM9表位(下排)特异的CD8$^+$ T细胞系(CL)对SIV感染细胞的识别。在指出的阻断条件的存在下将CL与未感染或SIV感染的CD4+ T细胞(来自 Rh22607)一起孵育。

[0026]　　图5(左图)示出了来自用表达SIVgag的Rh67(UL40)缺失的68-1 RhCMV接种的恒河猴的PBMC中CD8$^+$ T细胞的百分比,示出了在指定时间点与对应于SIVgag的重叠肽一起孵育后的IFN-γ 和/或TNF-α产生。中间的图示出了来自相同动物的PBMC中的CD8$^+$ T细胞不响应于 Mamu-E限制性肽Gag$_{273-287}$(Gag69)或Gag$_{477-491}$(Gag120)。右图示出了来自响应于MHC-II限制性肽 (Gag53和Gag73)的同一动物的PBMC 中CD8$^+$ T细胞的百分比。MHC-II肽对应于所谓的超表位,即这些肽被许多不同的MHC-II等位基因呈递,并因此在大多数动物中引起应答。

[0027]　　图6是说明了以下的表:在用缺少Rh67的毒株68-1 RhCMV/gag载体(n=3)疫苗接种的恒河猴中,使用流式细胞术ICS检测125个连续的 15聚体Gag肽(具有11个氨基酸重叠)的识别来对CD8$^+$ T细胞对 SIVmac239Gag的应答进行表位作图。对导致高于背景CD8$^+$ T细胞应答的肽进行MHC-I(mAb W6/32)、MHC-E(Rh67VL9)和MHC-II(mAb G46-6)阻断,并分类为MHC-I阻断(白色填充框)、MHC-E阻断(灰色填充框)和MHC-II阻断(黑色填充框)。注意,所有肽都受MHC-II 限制,表明需要Rh67来引起HLA-E特异性CD8$^+$ T细胞应答。

[0028]　　图7A是示出了通过用单个Mamu-D分子转染的细胞系的MHC-II、MHC-Ia、MHC-E或MHC-F表面染色的一组图。

[0029]　　图7B是示出了指定的恒河猴(rhesus macaque,RM)个体的基因分型的表。将个体通过Roche/454焦磷酸测序进行Mamu-A、Mamu-B和 Mamu-E基因分型。灰色阴影表示选择用于MHC-I转染子产生的等位基因。如果列出多个等位基因,则产生粗体表示的等位基因。

[0030]　　图7C是一组两幅图,其中将一个MHC-Ia或MHC-Ib等位基因转染到亲本(MHC-I阴性)细胞系(分别为.221细胞或K562)中。将细胞用交叉反应性人MHC-I单克隆抗体(W6/32)在室温下染色15分钟以评估 MHC-I表达。将细胞用补充有10％胎牛血清的1×PBS洗涤1次,

用2%多聚甲醛固定,收集在LSRII流式细胞仪上,并用FlowJo进行分析。表达MHC-I的B淋巴母细胞样细胞(BLCL)用作阳性对照,而MHC-I 阴性亲本细胞系用作阴性对照。

[0031]　　　图8A是示出了来自Rh22607的Gag 120的限制性测定的代表性流式数据(flow data)的一组图。

[0032]　　　图8B是示出了以下的表:来自4只指定的RM(#s 21826、22436、22034和22607;图7B中所示Mamu-I等位基因)的PBMC与用指定的 SIVgag肽脉冲的自体B淋巴母细胞样细胞系(BLCL)、MHC-I-null.221 或K562细胞或者指定的单一Mamu-I转染子一起孵育,并随后通过流式细胞术ICS分析CD8$^+$T细胞应答(参见图1)。从第二列开始,导致高于背景(无肽)的CD8$^+$T细胞应答的组合用+符号(灰色框) 表示;不导致高于背景的CD8$^+$ T细胞应答的组合用-符号(空白框)表示。在第一列中,在每只RM中表达的Mamu-I等位基因以灰色框显示;未表达的等位基因以空白框显示。

[0033]　　　图9是MHC-I与MHC-E阻断研究的一组流式细胞术图。来自(左) 经毒株68-1 RhCMV/gag载体疫苗接种的恒河猴或(B)经毒株68-1.2 RhCMV/gag载体疫苗接种的恒河猴的PBMC中CD8$^+$ T细胞的代表性流式细胞术图,示出了在与上方指出的Gag 15聚体肽一起并且在左侧指出的阻断条件下孵育后的IFN-γ 和/或TNF-α生产(每个象限中所示CD8$^+$ T 细胞的应答频率)。

[0034]　　　图10A是一组流式细胞术图,示出了用Gag$_{273-287}$(SIVmac239 Gag 15 聚体#69)刺激来自经毒株68-1RhCMV/gag载体疫苗接种的恒河猴的 PBMC并且进行流式细胞术ICS。通过IFN-γ和TNF-α鉴定响应于这些 MHC-E结合的Gag肽的CD8$^+$ T细胞,并随后与PBMC中的其余细胞对于指定标志物的表达进行比较。黑色数字表示PBMC中对于指定标志物为阳性的细胞的总体百分比,而灰色数字表示阳性的IFN-γ 和TNF-α产生细胞的百分比。

[0035]　　　图10B是一组流式细胞术图,示出了用Gag$_{477-491}$(SIVmac239Gag 15 聚体#120)刺激来自经毒株68-1RhCMV/gag载体疫苗接种的恒河猴的 PBMC并且进行流式细胞术ICS。通过IFN-γ和TNF-α鉴定应答于这些 MHC-E结合的Gag肽的CD8$^+$ T细胞,并随后与PBMC中的其余细胞对于指定标志物的表达进行比较。黑色数字表示PBMC中对于指定标志物为阳性的细胞的总体百分比,而灰色数字表示阳性的IFN-γ和TNF-α产生细胞的百分比。

[0036]　　　图11共同示出了毒株68-1 RhCMV/SIVgag引起的CD8$^+$T细胞的 MHC限制性。

[0037]　　　图11A是示出了来自经代表性毒株68-1 RhCMV/SIVgag疫苗接种的恒河猴(四只类似地分析的Rh22034)的PBMC的流式细胞术细胞内细胞因子染色(ICS)分析之结果的一组图。用脉冲到指定的MHC-I转染子或对照细胞的表面上的指定的15聚体肽表位刺激来自经疫苗接种恒河猴的PBMC,通过用流式细胞术ICS测定检测IFN-γ 和/或TNF-α产生来确定CD8$^+$ T细胞识别(每个象限中所示设门CD8$^+$ T细胞的应答频率)。亲本MHC-I阴性.221和K562细胞用作阴性对照,而自体B淋巴母细胞样细胞(BLCL)用作阳性对照。所测试的MHC-I分子包括二者由Rh22034 表达的那些。

[0038]　　　图11B是示出了来自类似于图11A那些不由Rh22034表达的另外的恒河猴和人MHC-E分子的流式细胞术ICS分析之结果的一组图。

[0039]　　　图11C是示出了将如上所述用相同的毒株68-1 RhCMV/SIVgag载体疫苗接种的恒河猴(4只类似地分析的代表)处理的RM的PBMC之表型分析的一组图,所述PBMC用SIVgag$_{273-287}$(69)或SIVgag$_{477-491}$(120) 脉冲的自体BLCL刺激,并且通过流式细胞术ICS测定

对应答性CD3<sup>+</sup>淋巴细胞(IFN-γ和TNF-α产生性的;左图中示出门(gate))进行表型分析,在每个图中,指定门内的应答细胞和非应答细胞分别以灰色和黑色表示(每个图中所示矩形区域内其相对％以相同颜色指示)。

[0040]　　图11D是以下结果的一组图,其中将单一MHC-E转染子与典型 MHC-E结合肽VMAPRTLLL(VL9)或对照非MHC-E结合肽(SIVgag GY9)一起预孵育,然后用指定的SIVgag 15聚体肽表位进行脉冲。使用来自经毒株68-1 RhCMV/SIVgag疫苗接种的恒河猴的PBMC以及以下 MHC-E转染子如上所述进行流式细胞术ICS测定:对于SIVgag$_{273-287}$(69)、SIVgag$_{385-399}$(97)和SIVgag$_{433-447}$(109)为Mamu-E＊02:04,对于SIVgag$_{257-271}$(65)和SIVgag$_{477-491}$(120)为Mamu-E＊02:011。

[0041]　　图12共同示出了MHC-E限制性受限于由ΔRh157.5/.4 RhCMV载体引起的CD8<sup>+</sup> T细胞应答。

[0042]　　图12A是示出了以下的表:在用指定的SIVgag表达病毒载体疫苗接种或者用SIVmac239本身感染的恒河猴(所示每组n＝6)中,使用流式细胞术ICS检测125个连续的15聚体Gag肽(具有11个氨基酸重叠) 的识别来对SIVgag的CD8<sup>+</sup> T细胞应答进行表位作图。导致高于背景 CD8<sup>+</sup> T细胞应答的肽用框表示,其中框的填充指示通过用抗泛MHC-I mAb W6-32、MHC E阻断肽VL9和MHC-II阻断肽CLIP阻断确定的 MHC限制性。MHC-Ia限制性、MHC-E限制性和MHC-II限制性为基于＞90％的应答分别被单独W6-32(白色填充框)、单独W6-32和VL9 (灰色填充框)以及单独CLIP(黑色填充框)所阻断,不符合这些标准的应答标记为不确定(垂直阴影线填充框)。在右侧示出了对于每只恒河猴,这些MHC限制性类别中独立表位的最小数目。

[0043]　　图12B是示出了如上所述在用表达这些蛋白质的毒株68-1 RhCMV 载体疫苗接种的恒河猴中对于SIVpol和结核分枝杆菌蛋白Ag85B、ESAT-6和RpfA的CD8<sup>+</sup> T细胞应答进行表位作图的表。

[0044]　　图12C是一组图(右)、另一组图(中)和柱状图(右),示出了分离自用毒株68-1 RhCMV/gag、MVA/gag、毒株68-1.2 RhCMV/gag载体疫苗接种或者用SIV感染的恒河猴的CD8<sup>+</sup>细胞对SIV感染的CD4<sup>+</sup>细胞识别的分析。左侧的流式图示出了在CD8<sup>+</sup> T细胞与自体SIVmac239感染的CD4<sup>+</sup> T细胞单独(无阻断)或者在泛MHC-I阻断mAb W6/32加MHC-II结合CLIP肽 (抗MHC-I+CLIP)或MHC-E结合肽VL9加 CLIP(VL9+CLIP)的存在下孵育后的IFN-y和TNF-α产生。所有图在活的CD3<sup>+</sup>、CD8<sup>+</sup>细胞上进行设门。右侧的柱状图示出了来自所有研究的恒河猴的结果。

[0045]　　图13A是示出了在用毒株68-1 RhCMV/gag与常规病毒载体(后者包括MVA/gag(n＝11)、Ad5/gag(n＝3)和经电穿孔的DNA/gag+IL-12 (n＝4))疫苗接种的个体恒河猴中,或者在具有受控的SIVmac239感染的恒河猴(血浆病毒载量＜10,000拷贝/ml;n＝12)中,由循环CD8<sup>+</sup> T 细胞识别的不同MHC E(灰色)和MHC-Ia(黑色)限制性SIVgag表位的总数的比较的图。水平条表示中位值。

[0046]　　图13B是示出了在用表达每种指定的抗原的毒株68-1 RhCMV/gag 载体疫苗接种的个体恒河猴(注意,在施用RhCMV/gag的CMV未感染( naïve )恒河猴中评估RhCMV IE1应答)中,由循环CD8<sup>+</sup> T细胞识别的MHC E限制性表位的密度(每100个氨基酸的蛋白质长度的表位数) 的比较的图。水平条表示每组的中位值。

[0047]　　图13C是分析42只经毒株68-1 RhCMV/gag载体疫苗接种的恒河猴中125个重叠的(11个氨基酸重叠)、连续的SIVgag15聚体肽的MHC-E 限制性SIVgag表位特异性CD8⁺ T细胞应答的宽度的柱状图。注意，109/12515聚体(87%)在至少1只恒河猴中被MHC-E限制性CD8⁺ T 细胞识别。

[0048]　　图13D(左)是基于在经毒株68-1 RhCMV载体疫苗接种的恒河猴中由CD8⁺ T细胞识别的11种最佳MHC-E限制性SIVgag 9聚体肽表位，通过字母高度指示给定位置处每种氨基酸的频率(相对于其在 SIVmac239Gag中的背景频率，参见方法)的序列LOGO。序列LOGO 根据Lampen MH等，53，126-131 (2013) (其通过引用并入本文)在TAP 缺陷背景中从HLA-E洗脱的551种肽中的富集(灰色填充字母或阴影线字母)或代表性不足(underrepresentation) (白色填充字母)进行着色。551种Lampen等的肽中在第2位和C末端锚定位置富集的氨基酸是我们的11种最佳SIVgag肽中罕见的(右图)，而显著代表性不足的那些被富集。响应于每种最佳肽的毒株68-1 RhCMV/gag疫苗接种的恒河猴的百分比被记作"识别频率"。

[0049]　　图14是示出在所有经毒株68-1 RhCMV/gag疫苗接种的恒河猴中 SIVgag$_{276-284}$和SIVgag$_{482-490}$表位被CD8⁺ T细胞识别的图。在120只经毒株68-1 RhCMV/gag疫苗接种的RM中，使用流式细胞术ICS确定对指定的SIVgag 9聚体肽的CD8⁺ T细胞应答，使用CD3⁺/CD8⁺ T细胞中 TNF-α和/或IFN-γ的肽特异性诱导作为应答读出(read-out)。所有的恒河猴在背景减除之后表现出对这些超表位的可检出的应答。所示的应答频率已经记忆校正以反映具有CD8⁺、CD95$^{高}$记忆亚群的表位应答细胞的频率。水平条表示中位值。

[0050]　　图15共同示出了表达单一MHC-I分子的经转染的细胞系的验证，所述MHC-I分子对应于由4只经毒株68-1 RhCMV/SIVgag疫苗接种的恒河猴表达的MHC-I分子。

[0051]　　图15A是示出了以下结果的表，其中通过Roche/454焦磷酸测序对4 只经毒株68-1 RhCMV/SIVgag疫苗接种的恒河猴进行Mamu-A、Mamu-B和Mamu-E基因分型。灰色阴影指示选择用于MHC-I转染子产生的等位基因。当列出多个等位基因时，产生了表达粗体所示同质异型体(allomorph)的转染子。

[0052]　　图15B是一组两幅图，示出了单一MHC-I分子的表达。将MHC-Ia 或MHC-Ib等位基因转染到亲本(MHC-I阴性)细胞系(.221细胞或 K562细胞)中，并用泛MHC-I单克隆抗体(W6/32)染色。表达MHC-I 的B淋巴母细胞样细胞(BLCL)用作阳性对照，而MHC-I阴性亲本细胞系用作阴性对照。

[0053]　　图16A和16B共同示出了对4只恒河猴中RhCMV/SIVgag诱导的 CD8⁺ T细胞应答的MHC-Ia和MHC-Ib特异性的综合分析。

[0054]　　图16A是示出使用来自Rh22034的PBMC之SIVgag$_{433-447}$(109)应答的MHC限制性分析的代表性流式细胞术ICS图的一组图。所示的 TNF-α与IFN-γ的流式图在CD3⁺、CD8⁺淋巴细胞上进行设门，每个象限中的细胞分数如图所示。

[0055]　　图16B是示出了以下的表，来自4只指定的恒河猴(MHC分型示于图15A中)的PBMC，所述PBMC与用指定的SIVgag肽脉冲(并洗涤) 的自体B淋巴母细胞样细胞(BLCL)、MHC-I-阴性.221或K562细胞或者单一Mamu-I转染子一起孵育，并随后通过流式细胞术ICS分析CD8⁺ T 细胞应答。从第二列开始，导致高于背景(无肽)CD8⁺ T细胞应答的组合用+符号(灰色框)表示；不导致高于背景的CD8⁺ T细胞应答的组合用-符号(空白框)表示。在第一列中，在每只RM中表达的MHC-I等位基因以灰色框表示；未表达的等位基因以空白框显示(Mamu-F＊

01:01 的表达未知)。

[0056]　　图17是示出以下的表:能够将SIVgag肽呈递至毒株68-1 RhCMV/SIVgag引起的CD8$^+$T细胞的经典MHC-Ia同质异型体不是这些T细胞应答的限制性MHC等位基因。将一组20只经毒株68-1 RhCMV/SIVgag载体疫苗接种的恒河猴对于Mamu-A1＊001:01和 Mamu-A1＊002:01的存在进行MHC分型,并且测试SIVgag$_{69-83}$(18)、SIVgag129-143(33)和SIVgag197-211(50)特异性的CD8$^+$ T细胞应答。注意,经毒株68-1 RhCMV/gag载体疫苗接种的恒河猴中对这三种表位特异的CD8$^+$ T细胞的检测不依赖于经疫苗接种动物中Mamu-A1＊001:01或Mamu-A1＊002:01的存在。

[0057]　　图18A和18B共同示出,毒株68-1 RhCMV/SIVgag引起的CD8$^+$ T 细胞识别恒河猴和人MHC-E分子两种背景下的肽。

[0058]　　图18A是示出了以下的一组图:使用流式细胞术ICS检测在与指定的MHC-E转染子和对照抗原呈递细胞上脉冲(并洗涤)的指定的Gag 15 聚体肽一起孵育后的IFN-γ 和/或TNF-α产生(每个象限中所示CD8$^+$ T 细胞的应答频率)来评估来自经毒株68-1 RhCMV/SIVgag载体疫苗接种的恒河猴[Rh21826:SIVgag$_{89-103}$(23)、SIVgag$_{129-143}$(33)、SIVgag$_{257-271}$(65)、SIVgag$_{473-487}$(119);Rh22034:SIVgag$_{61-75}$(16)、SIVgag$_{69-83}$(18)、 SIVgag$_{271-287}$(69)、SIVgag$_{385-399}$(97)、SIVgag$_{477-491}$(120);Rh22436：SIVgag$_{197-211}$(30)、SIVgag$_{197-211}$(50)]的PBMC的肽特异性CD8$^+$ T细胞识别(参见图11)。注意,所有12个MHC-E限制性15聚体肽表位可有效地呈递至Mamu-E同质异型体和HLA-E二者上的毒株68-1 RhCMV/SIVgag载体引起的CD8$^+$ T细胞。

[0059]　　图18B是由图18A中所示的转染子所表达的人和恒河猴MHC-E分子的α1和α2区域的氨基酸比对,其中关键的B和F口袋残基用灰色阴影表示。与结合肽相互作用的所有B和F口袋残基在HLA-E＊01:03、Mamu-E＊02:04和Mamu-E＊02:11之间是保守的,而Mamu-E＊02:20的这些残基中存在替换,这是本文研究的MHC-E分子最大区别。虽然与其他同质异型体相比,在B和F口袋残基中存在替换,但是Mamu-E＊02:20 能够结合并呈递相同的肽。

[0060]　　图19是示出了以下的图,毒株68-1 RhCMV/SIVgag引起的超表位特异性CD8+ T细胞显示出常规CD8a$^+$ T细胞表型。该图总结了4只经 68-1 RhCMV/SIVgag疫苗接种的恒河猴(Rh21826、Rh22034、Rh22436、Rh22607)中响应于SIVgag$_{273-287}$(69)或SIVgag$_{477-491}$(120)肽刺激的 MHC-E限制性CD8+ T细胞的表型分析。该图示出了表达指定表型的肽响应性CD3$^+$ T细胞(IFN-γ$^+$和TNF-α$^+$)的百分比(参见图11C中的流式细胞图)。

[0061]　　图20A是在用指定的SIVgag 15聚体肽表位脉冲之前,与典型 MHC-E-结合肽VMAPRTLLL(VL9)或对照肽一起预孵育的单一 MHC-E转染子的一组图。使用来自经毒株68-1 RhCMV/SIVgag疫苗接种的恒河猴(对于SIVgag$_{89-103}$(23)、SIVgag$_{129-143}$(33)、SIVgag$_{197-211}$(50) 和SIVgag$_{473-487}$(119)应答为Rh21826;对于SIVgag$_{61-75}$(16)和SIVgag$_{69-83}$ (18)应答为Rh22034;对于SIVgag$_{117-131}$(30)应答为Rh22436)的PBMC 如图11所述进行流式细胞术ICS。使用以下MHC-E转染子:对于 SIVgag$_{69-83}$(18)和SIVgag$_{89-103}$(23)应答为Mamu-E＊02:04;对于 SIVgag$_{61-75}$(16)、SIVgag$_{117-131}$(30)、SIVgag$_{129-143}$(33)、SIVgag$_{197-211}$ (50)和SIVgag$_{473-487}$(119)应答为Mamu-E＊02:11)。使用20μM终浓度的以下对照肽:对于SIVgag$_{89-103}$(23)、SIVgag$_{117-131}$(30)和SIVgag$_{129-143}$ (33)应答为Mamu-A1＊002:01结合肽SIVgag$_{71-79}$(GY9),且对于 SIVgag$_{69-83}$(18)、SIVgag$_{197-211}$(50)和SIVgag$_{473-487}$(119)应答为 Mamu-A1＊001:01结

合肽SIVgag$_{181-189}$(CM9)。这些数据与图11D中的数据一起表明VL9肽有效阻断12种不同MHC-E呈递的15聚体肽表位的CD8$^+$T细胞识别。

[0062] 图20B是这样的图,其中指定的抗原呈递细胞与升高浓度的VL9一起预孵育,然后用SIVgag$_{477-491}$(120)SIVgag 15聚体或最佳 Mamu-A1＊001:01限制性Gag-CM9或Tat-SL8脉冲。然后将这些抗原呈递细胞与指定的效应物一起孵育用于如图20A所述的流式细胞术ICS分析。Rh22436是经68-1 RhCMV/SIVgag疫苗接种的RM,而Rh27002是 SIV感染的。注意,升高浓度的VL9肽逐渐阻断表达MHC-E的抗原呈递细胞活化来自经毒株68-1 RhCMV/gag载体疫苗接种的恒河猴的 SIVgag$_{477-491}$(120)特异性CD8$^+$ T细胞的能力,但对Gag-CM9或Tat-SL8特异性的常规MHC-Ia限制性CD8$^+$ T细胞没有影响。

[0063] 图21示出了使用通过流式细胞术ICS的TNF-α和/或IFN-γ的肽特异性CD8$^+$T细胞表达作为应答读出的8个另外的MHC-E限制性15肽表位的形式截短分析(formal truncation analysis)。首先确定对亲本15 聚体的氨基末端和羧基末端截短物的CD8$^+$ T细胞应答,以确定核心表位的最佳肽长度以及氨基和羧基末端(上图,灰色阴影表示最高刺激性的氨基和羧基末端截短肽的末端氨基酸)。然后,通过分析组成每个15聚体的 7个连续的9聚体来确认这种截短方法所暗示的最佳9聚体(下图)。每个下图中灰色阴影的9聚体表示每个亲本15聚体的最佳表位。

[0064] 图22A和22B共同示出MHC-E限制性CD8$^+$ T细胞对人和恒河猴 MHC-E转染子上脉冲的最佳9聚体的剂量响应。将Mamu-E＊02:04、Mamu-E＊02:20和HLA-E＊01:03转染子用指定浓度的最佳SIVgag 9聚体肽表位SIVgag$_{476-484}$、SIVgag$_{259-267}$、SIVgag$_{276-284}$或SIVgag$_{482-490}$(参见图 4)脉冲,洗涤,并且与来自3-4只经68-1 RhCMV/SIVgag疫苗接种的恒河猴的PBMC组合用于流式细胞术ICS确定应答性CD8$^+$ T细胞(IFN-γ$^+$和/或TNF-α$^+$)的频率。

[0065] 图22A是示出了Rh22607中对SIYgag$_{476-484}$的剂量响应的代表性分析的一组图。

[0066] 图22B是示出了响应于SIVgag$_{476-484}$、SIVgag$_{259-267}$、SIVgag$_{276-284}$、 SIVgag$_{482-490}$的CD8$^+$ T细胞的剂量响应(平均值±SEM应答频率)的一组图,其应答频率相对于在用10μM肽剂量脉冲的转染子中观察到的响应进行归一化。

[0067] 图23是RhCMV载体毒株68-1、68-1.2和ΔRh157.4/.568-1.2之间的基因组差异图。在RhCMV的低传代分离物中,Rh157.5(UL128)、Rh157.4(UL130)和Rh157.6(UL131A)基因以相反取向编码在第2链上。在组织培养中连续传代期间,RhCMV 68-1获得独特的成纤维细胞适应性。Rh157.5(UL128)ORF以及Rh157.4(UL130)ORF外显子2的大部分缺失并且相邻的基因组区域反转,导致介导病毒进入非成纤维细胞的五聚体受体复合物的丧失。毒株68-1 RhCMV的成纤维细胞适应也导致在Rh61/Rh60(UL36)基因中插入另外的胸苷,这导致移码突变和提前终止密码子(premature stop codon)。在RhCMV 68-1.2中,通过将来自RhCMV毒株180.92的Rh157.5(UL128)和Rh157.4(UL130)外显子2插入到RhCMV 68.1的Rh157.4(UL130)第一外显子之后,恢复了功能性五聚体复合物,并且Rh61/Rh60(UL36)突变恢复为野生型构型。为了确保由毒株68-1 RhCMV载体引起的CD8$^+$ T细胞的非常规MHC限制性可归因于Rh157.5/.4(UL128/UL130)缺失(以及因此缺少功能性五聚体复合物),通过从Rh157.6(UL131A)终止密码子上游50bp开始直到Rh157.5(UL128)终止密码子同源重组使Rh157.5(UL128)和Rh157.4 (UL130)从毒株68-1.2中特异性重新缺失,留下Rh61/Rh60(UL36) 修复未受损。因此,这种ΔRh157.5/.4(ΔUL128/UL130)毒株68-1.2 RhCMV载体与不同于经修复

毒株68-1.2 RhCMV载体的原始毒株68-1 载体之间共有的表型特征可直接归因于Rh157.5/.4(UL128/UL130)缺失。

[0068]　　　图24共同示出了由毒株68-1与毒株68-1.2 RhCMV/gag载体引起的 CD8$^+$T细胞对MHC-E与MHC-Ia的差别利用。

[0069]　　　图24A示出了在具有和不具有泛抗-MHC-I阻断mAb W6-32或 MHC-E阻断VL9肽的情况下,由毒株68-1(Rh157.4/.5缺失)RhCMV/gag 载体引起的MHC-I依赖性、SIVgag表位特异性CD8$^+$T细胞的代表性流式细胞术应答图(在设门的CD3$^+$、CD8$^+$T细胞上的IFN-γ与TNF-α)。

[0070]　　　图24B示出了在具有和不具有用泛抗-MHC-I阻断mAb W6-32或 MHC-E阻断VL9肽阻断的情况下,由毒株68-1.2(Rh157.4/.5-未受损) RhCMV/gag载体引起的MHC-I依赖性、SIVgag表位特异性CD8$^+$T细胞的代表性流式细胞术应答图(在设门的CD3$^+$、CD8$^+$T细胞上的IFN-γ与TNF-α)。注意,VL9肽仅阻断由毒株68-1 RhCMV载体引起的所有 MHC-I依赖性应答。

[0071]　　　图25是由RhCMV/gag载体(毒株68.1和68-1.2)、MVA/gag载体和受控的SIV感染引起的表位特异性CD8$^+$T细胞应答的限制性分析。如图12A所述,在用指定的SIVgag表达病毒载体疫苗接种或者用 SIVmac239本身感染(SIVmac239对照者恒河猴)的另外的恒河猴(在来自图12A所示每组的6只动物)中,使用流式细胞术ICS检测125个连续15聚体gag肽(具有11个氨基酸重叠)的识别来对SIVgag的CD8$^+$T细胞应答进行表位作图。导致高于背景CD8$^+$T细胞应答的肽用框表示,其中框的填充指示通过用抗泛MHC-I mAb W6-32、MHC-E阻断肽VL9和MHC-II阻断肽CLIP阻断确定的MHC限制性。MHC-Ia限制性、MHC-E限制性和MHC-II限制性分别为基于>90%的应答被单独W6-32 (白色填充框)、单独W6-32和VL9(灰色填充框)以及单独CLIP(黑色填充框)所阻断,不符合这些标准的应答标记为不确定(垂直阴影线填充框)。对于每只恒河猴,在右侧示出了这些MHC限制性类别中独立表位的最小数目。注意,被来自经毒株68-1 RhCMV/gag载体疫苗接种的恒河猴的CD8$^+$T细胞识别的所有可评价表位受MHC-II或MHC-E非常规限制性。相比之下,毒株68-1.2 RhCMV/gag和MVA/gag载体引起的所有应答是常规MHC-Ia限制性的。在SIV对照者恒河猴中鉴定的绝大多数SIVgag表位特异性CD8$^+$T细胞应答也是MHC-Ia限制性的,但是这 12只动物中的4只表现出明确为MHC-II限制性的一个表位特异性应答 (179个总应答中的4个应答=2.2%),表明MHC-II限制性CD8$^+$T细胞可鉴定为对于感染的常规免疫应答的次要组分。

[0072]　　　图26是示出了天然(野生型)RhCMV感染以及用毒株68-1 RhCMV/gag载体初次和二次感染中,对RhCMV立即早期1(IE1)蛋白的CD8$^+$T细胞应答的表位作图的图。在1)用野生型(克隆循环) RhCMV天然感染的恒河猴(上图),2)用毒株68-1 RhCMV/gag载体疫苗接种的RhCMV未感染恒河猴(中图),和3)用毒株68-1 RhCMV/gag 载体超感染(superinfected)的天然野生型RhCMV感染的恒河猴(下图) 中,使用流式细胞术ICS检测137个连续15聚体IE1肽(具有11个氨基酸重叠)的识别来对RhCMV IE1的CD8$^+$T细胞应答进行表位作图。导致高于背景CD8$^+$T细胞应答的肽由框表示,其中框的填充指示通过用抗泛MHC-I mAb W6-32、MHC E阻断肽VL9和MHC-II阻断肽CLIP 阻断所确定的MHC限制性。MHC-Ia限制性、MHC-E限制性和MHC-II 限制性为基于>90%的应答分别被单独W6-32(白色填充框)、单独W6-32 和VL9(灰色填充框)以及单独CLIP(黑色填充框)所阻断,不符合这些标准的应答标记为不确

定(垂直阴影线填充框)。对于每只恒河猴,在右侧示出了这些MHC限制性类别中独立表位的最小数目。注意,天然感染的恒河猴中的IE1表位特异性应答完全是MHC Ia限制性的,而在仅用毒株68-1 RhCMV/gag载体感染的恒河猴中,这些应答更宽并且完全是非常规限制性的(约1:1比例的MHC-II限制性表位和MHC-E限制性表位)。用毒株68-1 RhCMV/gag载体超感染的天然RhCMV感染的恒河猴显示出常规(MHC-Ia)和非常规(MHC-II和/或MHC-E)限制性的IE1- 表位特异性CD8$^+$ T细胞的预期混合物。

[0073]　　　图27A是恒河猴中MHC-E特异性mAb 4D12的特异性的验证。直方图示出了通过泛MHC-I mAb W6/32(上排)与MHC-E特异性mAb 4D12(下排)的单一MHC-Ia或MHC-Ib转染子的表面染色。注意,将所有Mamu-Ia和Mamu-E同质异型体转染到表达人β2-微球蛋白的鼠细胞系RMA-S中。恒河猴BLCL用作阳性对照,而亲本RMA-S细胞系用作阴性对照(浅灰色直方图)。注意,4D12对Mamu-E转染子的反应性的限制性。

[0074]　　　图27B示出了通过用mAb W6/32染色确定的总MHC-I的表面表达。

[0075]　　　图27C示出了在相同培养物中的生产性SIV感染的和未感染的CD4$^+$ T细胞上如通过用mAb 4D12染色确定的总MHC-I的表面表达,其中SIV 感染的细胞通过Gag Ag的细胞内表达和CD4下调(Gag$^+$/CD4$^{低}$)识别,而未感染的细胞通过缺少Gag反应性和高水平的表面CD4表达 (Gag$^-$/CD4$^{高}$)识别。左图示出了代表性流式细胞术直方图。右图描述了来自总共16只不相干恒河猴的SIV感染的CD4$^+$ T细胞与未感染的CD4$^+$ T细胞中总MHC-I或特异性MHC-E染色的MFI。通过配对T检验确定 P值。

[0076]　　　图28是对SIVgag的MHC-Ia限制性CD8$^+$ T细胞应答的群体水平分析。常规MHC-Ia限制性SIVgag表位特异性CD8$^+$ T细胞应答的宽度的分析跨越用常规SIVgag表达疫苗(11个MVA/gag、3个Ad5/gag、4 个DNA/gag+IL-12)疫苗接种的或者用SIVmac239感染(平台期病毒载量<10,000拷贝/ml;n=12)的30只恒河猴中的125个重叠(11个氨基酸)、连续SIVgag 15聚体肽。星号(＊)表示包含Mamu-A1＊001:01限制性免疫显性SIVgag$_{181-189}$(CM9)表位的Gag-4515聚体肽。对于MHC-Ia 同质异型体,用于该组的猴的选择很大程度上无偏倚,除了优先选择 Mamu-A1＊001:01(30只恒河猴中的19只表达),其占响应于Gag4515 聚体的猴的高频率。除了具有人工提高的应答频率的Gag45肽,与由毒株68-1 RhCMV/gag载体引起的MHC-E限制性CD8$^+$ T细胞应答(19个表位具有≥40%识别频率,包括2个通用超表位) 相比,具有与任何其他 Gag 15聚体反应的MHC-I限制性CD8$^+$ T细胞的猴的频率相当低(仅2 个15聚体,具有40%识别,且没有一个>40%)(图3C)。然而,除了一个以外,全部125个连续的SIVgag 15聚体在至少一只恒河猴中被 MHC-Ia限制性CD8$^+$ T细胞识别,并且除了13个以外,全部SIVgag 15 聚体在2只或更多只恒河猴中被靶向。相比之下,42只恒河猴中通过毒株68-1 RhCMV/gag载体引起的MHC-E限制性CD8$^+$ T细胞无法识别125 个SIVgag 15聚体中的16个。因此,尽管由毒株68-1 RhCMV载体引起的MHC-E限制性CD8$^+$ T细胞应答对于功能性单型限制性元件显著广泛,但它们并不像整个多型性MHC-Ia分子群体支持的应答那样广泛,可能是由于MHC-Ia限制性抗原呈递系统的进化优势。

[0077]　　　图29是一组三幅图。左图示出了用Rh214至Rh220缺失的表达SIVgag的68-1 RhCMV接种的恒河猴的PBMC中CD8$^+$ T细胞的百分比,示出了在指定时间点与对应于SIVgag的重叠肽一起孵育后的IFN-γ 和/ 或TNF-α产生。基因区域Rh214至Rh220编码与人巨细胞病毒(HCMV) US28具有同源性的5个基因:Rh214、Rh215、Rh216、Rh218、Rh220 (D.Maloulideng,J

Virol 86,8959(2012);其通过引用并入本文)。中间的图示出来自相同动物的PBMC中的CD8$^+$ T细胞不响应于Mamu-E限制性肽Gag$_{273-287}$(Gag69)或Gag$_{477-491}$(Gag120)。右图示出来自相同动物的PBMC中响应于MHC-II限制性肽(Gag53和Gag73)的CD8$^+$ T 细胞的百分比。MHC-II肽对应于所谓的超表位,即这些肽被许多不同的 MHC-II等位基因呈递,并因此在大多数动物中引起应答。

[0078]　　图30是说明了以下的表:在用缺少Rh214-222的毒株68-1 RhCMV/gag载体(n＝3)疫苗接种的恒河猴中,使用流式细胞术ICS检测125个连续的15聚体Gag肽(具有11个氨基酸重叠)的识别来对CD8$^+$ T细胞对SIVmac239Gag的应答进行表位作图。对导致高于背景CD8$^+$ T细胞应答的肽进行MHC-I(mAb W6/32)、MHC-E(Rh67VL9)和MHC-II (mAb G46-6)阻断,并分类为MHC-I阻断(白色填充框)、MHC-E阻断(灰色填充框)、MHC-II阻断(黑色填充框)或不确定(阴影线填充框)。注意,所有肽都受MHC-II限制,这表明需要Rh214-222来引起 HLA-E特异性CD8$^+$ T细胞应答。

[0079]　　发明详述

[0080]　　本发明提供了新的重组CMV载体,其包括但不限于包含编码至少一种异源蛋白抗原、至少一种活性UL40蛋白和至少一种US28蛋白的核酸但不表达活性UL128和UL130蛋白的重组CMV载体。本发明还提供了重组CMV载体,其包括但不限于包含编码至少一种异源抗原的核酸但不表达(1)活性UL40蛋白和/或活性US28蛋白、(2)活性UL128蛋白和 (3)活性UL130蛋白的重组CMV载体。还提供了使用新的重组CMV 载体的方法,例如在对象中产生对至少一种异源抗原之免疫应答的方法、产生识别MHC-E-肽复合物之CD8$^+$ T细胞的方法、以及治疗疾病的方法。

[0081]　　I.定义

[0082]　　除非另有说明,否则按照常规用法使用技术术语。分子生物学中常用术语的定义可见于:Benjamin Lewin,Genes V,Oxford University Press 出版,1994(ISBN 0-19-854287-9);Kendrew等.(编辑),The Encyclopedia of Molecular Biology,Blackwell Science Ltd.出版,1994(ISBN 0-632-02182-9);以及Robert A.Meyers(编辑),Molecular Biology and Biotechnology:a Comprehensive Desk Reference,VCR Publishers,Inc. 出版,1995(ISBN 1-56081-569-8)。

[0083]　　本文中引用的所有出版物、专利、专利申请、互联网网站和登录号/ 数据库序列(包括多核苷酸和多肽序列二者)均通过引用整体并入本文用于所有目的,其程度如同每个单独的出版物、专利、专利申请、互联网网站或登录号/数据库序列被具体且单独地指明如此通过引用并入。

[0084]　　除非另有说明,否则本文中使用的所有技术和科学术语具有与本公开内容所属领域的普通技术人员通常理解的相同的含义。除非上下文另外明确指出,否则没有数量词修饰的名词表示一个/种或更多个/种。类似地,除非上下文另外明确指出,否则词语“或”旨在包括“和”。还应理解,对于核酸或多肽给出的所有碱基大小或氨基酸大小以及所有分子量或分子质量值是近似值,并且提供用于描述。尽管与本文中所述相似或等同的方法和材料可用于本公开内容的实践或测试中,但是以下描述了合适的方法和材料。术语“包含”意指“包括”。此外,材料、方法和实例仅是举例说明性的并不旨在限制。为了便于审阅本公开内容的多个实施方案,提供了具体术语的以下说明:

[0085]    抗原：本文中使用的术语"抗原"或"免疫原"可互换使用，是指能够在对象中诱导免疫应答的物质，通常为蛋白质。该术语还指以下意义上的免疫活性蛋白质：一旦施用于对象(直接地或通过向对象施用编码所述蛋白质的核苷酸序列或载体)就能够引起通常针对所述蛋白质的体液和/或细胞类型的免疫应答。

[0086]    施用：通过任何有效途径向对象提供或给予药剂，例如包含有效量的含有异源抗原的HCMV载体的组合物。示例性施用途径包括但不限于注射(例如皮下、肌内、皮内、腹膜内和静脉内)、经口、舌下、经直肠、透皮、鼻内、经阴道和吸入途径。

[0087]    癌症：其中异常细胞不受控制地分裂并且能够侵袭其他组织的疾病或病症。癌细胞可通过血液和淋巴系统扩散到其他身体部位。癌症是用于许多疾病的术语。人有超过100种不同类型的癌症。大多数癌症以其来源的器官命名。例如，在结肠中开始的癌症可称为结肠癌。然而，癌症的特征(特别是关于癌症对治疗性化合物的敏感性的特征)不限于癌症来源的器官。癌细胞是来自于任何癌症的任何细胞，无论是体外还是体内。

[0088]    癌症还包括以异常或不受控制的细胞生长为特征的恶性肿瘤。经常与癌症相关的其他特征包括转移，干扰相邻细胞的正常功能，以异常水平释放细胞因子或其他分泌产物，以及抑制或加剧炎性或免疫应答，侵入周围或远端组织或器官(例如淋巴节)等。

[0089]    "转移性疾病"或"转移"是指已经离开初始肿瘤部位并例如通过血流或淋巴系统迁移至身体其他部位的癌细胞。癌症的"病理状况 (pathology)"包括损害对象健康的所有现象。这包括但不限于异常或不可控制的细胞生长、转移、干扰相邻细胞的正常功能，以异常水平释放细胞因子或其他分泌产物，抑制或加剧炎性或免疫应答，瘤形成(neoplasia)，癌前病变(premalignancy)，恶性肿瘤，侵入周围或远端组织或器官(例如淋巴节)等。

[0090]    有效量：本文中使用的术语"有效量"是指药剂(例如包含异源抗原的CMV载体或识别来自于MHC-E/异源抗原的肽复合物的经转染CD8+ T细胞)的量，其足以产生期望的应答，例如减轻或消除病症或疾病的体征或症状，或者诱导对抗原的免疫应答。在一些实例中，"有效量"是治疗 (包括预防)任何障碍或疾病的一种或更多种症状和/或潜在病因的量。有效量可以是治疗有效量，其包括防止特定疾病或病症的一种或更多种体征或症状(例如与感染性疾病、癌症或自身免疫病相关的一种或更多种体征或症状)发生的量。

[0091]    突变：突变是与正常、共有或"野生型"序列在核酸或多肽序列中的任何差异。突变体是包含突变的任何蛋白质或核酸序列。此外，具有突变的细胞或生物体也可称为突变体。

[0092]    一些类型的编码序列突变包括点突变(个别核苷酸或氨基酸的差异)；沉默突变(不导致氨基酸变化的核苷酸差异)；缺失(其中一个或更多个核苷酸或氨基酸丢失直至并包括基因的整个编码序列缺失的差异)；移码突变(不可被3整除的多个核苷酸缺失导致氨基酸序列改变的差异)。导致氨基酸差异的突变也可称为氨基酸替换突变。氨基酸替换突变可通过相对于氨基酸序列中特定位置处的野生型的氨基酸变化来描述。

[0093]    本文中使用的"失活突变"是病毒基因中的任何突变，其最终导致病毒蛋白质的功能降低或功能完全丧失。

[0094]    核苷酸序列或核酸序列：术语"核苷酸序列"和"核酸序列"是指脱氧核糖核酸(DNA)或核糖核酸(RNA)序列，包括但不限于信使RNA (mRNA)、DNA/RNA杂交体或合成核酸。核酸可以是单链或者部分或完全双链(双链体)。双链体核酸可以是同源双链体或异源双链体。

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[0095]　　重组:重组核酸或多肽是具有不是天然存在的序列或者具有通过将序列的两个或更多个区段人工组合(否则其将是分开的)制备的序列的那些,例如包含异源抗原和/或通过一个或更多个基因的突变使复制缺陷的 CMV载体。这种人工组合通常通过化学合成或更常见地通过例如通过基因工程技术对核酸的分离区段进行人工操作来完成。重组多肽还可以指使用重组核酸(包括转移到不是多肽的天然来源的宿主生物体的重组核酸 (例如,编码形成包含异源抗原的CMV载体的多肽的核酸))制备的多肽。

[0096]　　复制缺陷:本文中使用的复制缺陷CMV是一旦在宿主细胞中,不经历病毒复制或者复制其基因组并因此产生病毒粒子的能力显著受限的病毒。在另一些实例中,复制缺陷病毒是传播缺陷的 (dissemination-deficient),即它们能够复制其基因组,但不能感染另一细胞,因为病毒颗粒不从受感染细胞释放或者因为释放非感染性病毒颗粒。在另一些实例中,复制缺陷病毒是扩散缺陷的(spread-deficient),即感染性病毒不从受感染的宿主分泌,因此病毒不能在宿主之间扩散。在一些实施方案中,复制缺陷CMV是包含导致缺少病毒复制所必需的一个或更多个基因("必需基因")或最佳复制所需的一个或更多个基因("增强基因")之表达的突变的CMV。本领域中已经描述了CMV必需基因和增强基因(特别是US 2013/0136768,其通过引用并入本文)并且在本文中公开。

[0097]　　可药用载体:可药用载体的使用是常规的。Remington's Pharmaceutical Sciences,E.W.Martin,Mack Publishing Co.,Easton,PA,第19版,1995描述了适合于本文中公开的组合物的药物递送的组合物和制剂。一般来说,载体的性质将取决于所采用的具体施用模式。例如,肠胃外制剂通常包含可注射流体作为载剂,所述可注射流体包括药学和生理学上可接受的流体,例如水、生理盐水、平衡盐溶液、葡萄糖水溶液、甘油等。对于固体组合物(例如散剂、丸剂、片剂或胶囊剂形式),常规无毒固体载体可包括例如药用级甘露醇、乳糖、淀粉或硬脂酸镁。除生物中性载体之外,待施用的药物组合物可包含少量的无毒辅助物质,例如润湿剂或乳化剂、防腐剂和pH缓冲剂等,例如乙酸钠或脱水山梨糖醇单月桂酸酯。

[0098]　　多核苷酸:本文中使用的术语"多核苷酸"是指核糖核酸(RNA)或脱氧核糖核酸(DNA)的聚合物。多核苷酸由四种碱基构成:腺嘌呤、胞嘧啶、鸟嘌呤和胸腺嘧啶/尿嘧啶(尿嘧啶用于RNA)。来自核酸的编码序列指示由核酸编码的蛋白质的序列。

[0099]　　多肽:术语"蛋白质"、"肽"、"多肽"和"氨基酸序列"在本文中可互换使用,是指任何长度的氨基酸残基的聚合物。聚合物可以是直链或支链的,其可包含经修饰的氨基酸或氨基酸类似物,并且其可被氨基酸以外的化学部分中断。该术语还涵盖已经自然地或通过干预修饰的氨基酸聚合物:例如二硫键形成、糖基化、脂化、乙酰化、磷酸化或任何其他操作或修饰(例如与标记或生物活性组分缀合)。

[0100]　　序列同一性/相似性:两个或更多个核酸序列或者两个或更多个氨基酸序列之间的同一性/相似性以序列之间的同一性或相似性表示。序列同一性可以用同一性百分比来测量;百分比越高,序列越一致。序列相似性可以用同一性或相似性百分比(其考虑保守氨基酸替换)来测量;百分比越高,序列越相似。具有显著量的序列同一性并且彼此功能也相同或相似的多肽或其蛋白质结构域(例如,在不同物种中具有相同功能的蛋白质,或者不改变蛋白质的功能或其大小的蛋白质的突变体形式)可称为"同源物"。

[0101]　　用于比较的序列比对方法是本领域中公知的。多种程序和比对算法描述于:

Smith&Waterman,Adv Appl Math 2,482(1981);Needleman& Wunsch,J Mol Biol 48,443 (1970;Pearson&Lipman,Proc NatlAcad Sci USA 85,2444(1988);Higgins&Sharp,Gene 73,237-244(1988);Higgins &Sharp,CABIOS 5,151-153(1989);Corpet等,Nuc Acids Res 16, 10881-10890(1988);Huang等,Computer App Biosci 8,155-165(1992);以及Pearson 等,Meth Mol Bio 24,307-331(1994)。此外,Altschul等,J Mol Biol 215,403-410(1990) 提出了序列比对方法和同源性计算的详细考虑。

[0102]　　NCBI局部序列比对检索基本工具(Basic Local Alignment Search Tool,BLAST) (Altschul等,(1990),同上)可从许多来源获得,包括国家生物信息中心(NCBI,National Library of Medicine,Building 38A, Room 8N805,Bethesda,MD 20894)以及互联网上, 用于与序列分析程序blastp、blastn、blastx、tblastn和tblastx结合使用。更多信息可以 在NCBI 网站找到。

[0103]　　BLASTN用于比较核酸序列,而BLASTP用于比较氨基酸序列。如果两个所比较的序 列具有同源性,则指定的输出文档将这些同源区域显示为比对的序列。如果两个所比较的 序列不具有同源性,则指定的输出文档将不显示比对的序列。

[0104]　　一经比对,通过对在两个序列中存在相同核苷酸或氨基酸残基的位置的数目进行 计数来确定匹配数。百分比序列同一性通过将匹配数除以所鉴定序列中所列出的序列的长 度或计算的长度(例如来自所鉴定序列中所列出的序列的100个连续核苷酸或氨基酸残 基),然后将所得值乘以100 来计算。例如,当与具有1154个核苷酸的测试序列进行比对时, 具有1166 个匹配的核酸序列与测试序列具有75.0％的同一性 (1166÷1554＊100＝ 75.0)。百分比序列同一性值舍入到最接近的十分位。例如,75.11、75.12、75.13和75.14被 舍入到75.1,而75.15、75.16、75.17、75.18和75.19被进入到75.2。长度值将始终为整数。 在另一个实例中,包含20个核苷酸区域的靶序列与来自鉴定序列的20个连续核苷酸如下排 列,它包含的区域与鉴定序列具有75％序列同一性(即15÷20＊100＝75)。

[0105]　　对于大于约30个氨基酸的氨基酸序列的比较,采用使用设置为默认参数(间隙存 在代价为11,且每个残基间隔代价为1)的默认BLOSUM62 矩阵的Blast 2序列函数。同源物 的通常特征在于使用NCBI Basic Blast 2.0用氨基酸序列的计数全长比对具有至少70％ 的序列同一性,用数据库 (例如nr数据库、swissprot数据库和专利序列数据库)进行有间 隙的 blastp。用DUST(Hancock&Armstrong,Comput Appl Biosci 10,67-70 (1994))过滤 用blastn程序搜索到的查询。其他程序使用SEG。此外,可以进行手动比对。具有甚至更大相 似性的蛋白质当通过该方法评估时会显示出提高的同一性百分比,例如与蛋白质具有至少 约75％、80％、85％、90％、95％、98％或99％的序列同一性。

[0106]　　当比对短肽(少于约30个氨基酸)时,采用设置为默认参数(开放间隙9,延伸间隙1 罚分)的PAM30矩阵使用Blast 2序列函数进行比对。与参考序列具有甚至更大相似性的蛋 白质当通过该方法评估时会显示出提高的百分比同一性,例如与蛋白质具有至少约60％、 70％、75％、80％、85％、90％、95％、98％或99％的序列同一性。当对小于完整序列进行序 列同一性比较时,同源物通常在10-20个氨基酸的短窗口上具有至少75％的序列同一性,并 且根据它们与参考序列的同一性可具有至少85％、90％、95％或98％的序列同一性。在 NCBI网站上描述了在这样的短窗口上确定序列同一性的方法。

[0107]　　两个核酸分子密切相关的一个指示是这两个分子在如上所述的严格条件下彼此

杂交。尽管如此,由于遗传密码的简并性,不显示高度同一性的核酸序列可编码相同或相似(保守的)氨基酸序列。可使用这种简并性来进行核酸序列的变化以产生全部编码基本上相同蛋白质的多个核酸分子。这样的同源核酸序列可例如与编码蛋白质的核酸具有至少约50％、60％、70％、80％、90％、95％、98％或99％的序列同一性。

[0108]　　对象:本文中使用的术语"对象"是指活的多细胞脊椎动物生物体(包括人和非人哺乳动物的一大类)。

[0109]　　治疗:本文中使用的术语"治疗"是指改善疾病或病理状况的体征或症状的干预。提及疾病、病理状况或症状本文中使用的术语"治疗"("treatment"、"treat"和"treating")也指治疗的任何可观察到的有益效果。有益效果可通过例如以下证明:通过易感对象中疾病的临床症状的延迟发作,疾病的一些或全部临床症状的严重性降低,疾病的较慢进展,疾病的复发次数降低,对象的整体健康或健康状态的改善,或者本领域中公知的特定疾病特异性的其他参数。预防性治疗是向不显示疾病的体征或者只显示早期体征的对象施用的治疗,其目的是降低发生病理状况的风险。治疗性治疗是在疾病的体征和症状发生之后向对象施用的治疗。

[0110]　　II.重组CMV载体及其使用方法

[0111]　　本文中公开了能够重复感染生物体的人或动物巨细胞病毒(CMV) 载体。所述CMV载体包含编码异源蛋白抗原的核酸序列,并且缺少活性 UL128和UL130蛋白或其直系同源物(感染其他物种的CMV的同源基因)的表达。异源抗原可以是任何抗原,包括来自于例如HIV、SIV、单纯疱疹病毒、乙型或丙型肝炎病毒、乳头瘤病毒、疟原虫属寄生虫和结核分枝杆菌的病原体特异性抗原。在另一些实例中,异源抗原可以是肿瘤抗原,包括例如与急性髓细胞性白血病、慢性髓细胞性白血病、骨髓增生异常综合征、急性淋巴细胞白血病、慢性淋巴细胞白血病、非霍奇金淋巴瘤、多发性骨髓瘤、恶性黑素瘤、乳腺癌、肺癌、卵巢癌、前列腺癌、胰腺癌、结肠癌、肾细胞癌(RCC)和生殖细胞肿瘤相关的肿瘤抗原。在一些实例中,CMV载体还缺少活性UL40蛋白(或其直系同源物)和/或活性 US28蛋白(或其直系同源物)。在另一些实施例中,异源抗原可以是组织特异性抗原或宿主自身抗原,包括例如来自于T细胞受体之可变区的抗原、来自于B细胞受体之可变区的抗原、精子抗原或卵抗原。

[0112]　　在一些实例中,由于在编码UL128、UL130或UL40(或其直系同源物)的核酸序列中存在突变,所述载体不表达活性UL128、UL130、US28 或UL40蛋白。突变可以是导致缺少活性UL128、UL130、US28或UL40 蛋白表达的任何突变。这样的突变可包括点突变、移码突变、少于编码蛋白质的全部序列的缺失(截短突变)、或编码蛋白质之全部核酸序列的缺失、或任何其他突变。

[0113]　　在另一些实例中,由于载体中存在包含抑制UL128、UL130或UL40 蛋白(或其直系同源物)表达的反义或RNAi序列(siRNA或miRNA) 的核酸序列,载体不表达活性UL128、UL130、US28或UL40蛋白(或其直系同源物)。可以以任何组合使用突变和/或反义和/或RNAi以产生缺少活性UL128、UL130、US28或UL40(或其直系同源物)的CMV载体。

[0114]　　CMV载体可包含本领域中已知提供不同免疫应答的另外的失活突变,例如失活US11突变或失活UL82(pp71)突变,或任何其他失活突变。CMV载体还可包含编码本领域中已知对于体内病毒传播(即从细胞扩散到细胞)必需的或增强体内病毒传播的病毒蛋白质的一种或更多种病毒基因中的至少一个失活突变。这样的失活突变可以由点突变、移码突变、

截短突变或编码病毒蛋白之全部核酸序列的缺失引起。失活突变包括病毒基因中最终导致病毒蛋白质的功能降低或功能完全丧失的任何突变。

[0115]　　本文中还公开了在对象中产生对异源抗原之CD8⁺T细胞应答的方法。所述方法包括向对象施用有效量的CMV载体。在一个实施方案中，CMV载体的特征在于具有编码至少一种异源抗原的核酸序列，以及不表达活性UL128蛋白（或其直系同源物）、不表达活性UL130蛋白（或其直系同源物）、并且表达至少一种活性UL40蛋白和至少一种活性US28 蛋白的核酸序列。所述至少一种活性UL40蛋白和至少一种活性US28蛋白可以是UL40和US28的直系同源物或同源物。由这种载体引起的CD8⁺T细胞应答的特征在于具有至少10％的针对由MHC-E呈递的表位的 CD8⁺T细胞。在另一些实例中，至少20％、至少30％、至少40％、至少50％、至少60％、至少75％、至少90％、至少95％或至少95％的CD8⁺T细胞受MHC-E限制。在一些实施方案中，CMV载体表达二至五种活性US28蛋白或者其直系同源物或同源物。在一些实施方案中，所述方法还包括鉴定来自由CMV载体引起的CD8⁺T细胞的CD8⁺ T细胞受体，其中所述CD8⁺T细胞受体识别来自于MHC.E/异源抗原的肽复合物。在一些实施方案中，通过RNA或DNA测序鉴定CD8⁺T细胞受体。在另一个实施方案中，CMV载体的特征在于具有不表达活性UL128、UL130和 UL40蛋白的核酸序列，并且这种载体可用于与HLA-E限制性CD8⁺T 细胞（由包含完整US28和UL40的一种或更多种另外的载体引起）一起，或在没有HLA-E限制性CD8⁺T细胞（由缺少功能性UL40或US28蛋白的一种或更多种另外的载体引起）的情况下引起识别MC-II超表位的 CD8⁺T细胞。在另一个实施方案中，CMV载体的特征在于具有不表达活性UL128、UL130和UL28蛋白的核酸序列，并且这种载体可用于与 HLA-E限制性CD8⁺T细胞（由包含完整US28和UL40的一种或更多种另外的载体引起）一起，或者在没有HLA-E限制性CD8⁺T细胞（由缺少功能性UL40或US28蛋白的一种或更多种另外的载体引起）的情况下引起识别MC-II超表位的CD8⁺T细胞。在另一个实施方案中，CMV载体的特征在于具有不表达活性UL128、UL130、UL28和UL40蛋白的核酸序列，并且这种载体可用于与HLA-E限制性CD8⁺T细胞（由包含完整US28和UL40的一种或更多种另外的载体引起）一起，或者在没有 HLA-E限制性CD8⁺T细胞（由缺少功能性UL40或US28蛋白的一种或更多种另外的载体引起）的情况下引起识别MC-II超表位的CD8⁺T细胞。

[0116]　　本文中还公开了产生识别MHC-E-肽复合物之CD8⁺T细胞的方法。该方法包括向第一对象（或动物）施用编码至少一种异源抗原和活性UL40 蛋白或者其同源物或直系同源物的CMV载体，以产生识别MHC-E/肽复合物的CD8⁺T细胞组。所述CMV载体不编码活性UL128和UL130蛋白或其直系同源物，并且所述异源抗原可以是任何抗原，包括病原体特异性抗原、肿瘤抗原、组织特异性抗原或宿主自身抗原。在一些实施方案中，宿主自身抗原是来自于T细胞受体或B细胞受体之可变区的抗原。该方法还包括：鉴定CD8⁺T细胞组的第一CD8⁺T细胞受体，其中所述第一 CD8⁺T细胞受体识别来自于MHC-E/异源抗原的肽复合物；并用表达载体转染所述一种或更多种CD8⁺T细胞，其中所述表达载体包含编码第二 CD8⁺T细胞受体的核酸序列和与编码T细胞受体的核酸序列可操作地连接的启动子，其中所述第二CD8⁺T细胞受体包含第一CD8⁺TCR的 CDR3α和CDR3β，从而产生识别MHC-E肽复合物的一种或更多种经转染CD8⁺T细胞。用于用表达载体转染的一种或更多种CD8⁺T细胞可以从第一对象或第二对象分离。在一些实施方案中，该方法还可包括向第一或第二对象施用所述一种或更多种经转染T细胞以治疗疾病（例如癌症、病原体感染或自身免疫性疾病或病症）。在一些实

施方案中,该方法还可包括向第一或第二对象施用所述一种或更多种经转染 T 细胞以诱导
对组织特异性抗原或宿主自身抗原的自身免疫应答。

[0117]　　还公开了通过包括以下步骤的方法制备的识别 MHC-E-肽复合物的经转染 CD8⁺ T
细胞:(1)向第一对象施用有效产生识别 MHC-E/肽复合物的 CD8⁺ T 细胞组之量的 CMV 载体,
其中所述 CMV 载体包含编码至少一种异源抗原的第一核酸序列,并且还包含编码活性 UL40
蛋白的第二核酸序列,并且其中所述 CMV 载体不表达活性 UL128 和 UL130 蛋白或其直系同源
物;(2)鉴定所述 CD8⁺ T 细胞组的第一 CD8⁺ T 细胞受体,其中所述第一 CD8⁺ T 细胞受体识别
来自于 MHC-E/异源抗原的肽复合物;　(3)从所述第一对象或第二对象分离一种或更多种
CD8⁺ T 细胞;以及　(4)用表达载体转染从第一或第二对象分离的所述一种或更多种 CD8⁺ T
细胞,其中所述表达载体包含编码第二 CD8⁺ T 细胞受体的核酸序列和与编码第二 T 细胞受
体的核酸序列可操作地连接的启动子,其中第二 CD8⁺ T 细胞受体包含第一 CD8⁺ T 细胞受体
的 CDR3α 和 CDR3β,从而产生识别 MHC-E-肽复合物的经转染 T 细胞。所述异源抗原可以是任何
抗原,包括病原体特异性抗原、组织特异性抗原、宿主自身抗原或肿瘤抗原。在一些实施方
案中,通过 RNA 或 DNA 测序鉴定第一 CD8⁺ T 细胞受体。本文中还公开了治疗疾病(例如癌症、
病原体感染或者自身免疫性疾病或病症)的方法,所述方法包括向第一或第二对象施用识
别 MHC-E 肽复合物的经转染 T 细胞。本文中还公开了诱导对宿主自身抗原或组织特异性抗原
的自身免疫应答的方法,所述方法包括向第一或第二对象施用识别 MHC-E 肽复合物的经转
染 T 细胞。

[0118]　　在另一些实例中,所述方法涉及向对象施用有效量的第二 CMV 载体,所述第二 CMV
载体包含编码第二异源抗原的核酸序列。该第二载体可以是任何 CMV 载体,包括具有活性
UL128 蛋白(或其同源物或直系同源物))和/或活性 UL130 蛋白(或其同源物或直系同源物)
的 CMV 载体。所述第二 CMV 载体可包含第二异源抗原。所述第二异源抗原可以是任何异源抗
原,包括与第一 CMV 载体中的异源抗原相同的异源抗原。所述第二 CMV 载体可以相对于第一
CMV 载体的施用在任何时间施用,包括在施用第一 CMV 载体之前、与其同时或之后。这包括在
第一个载体之前或之后的任何的月数、天数、小时数、分钟数或秒数施用第二载体。

[0119]　　当用作表达载体时,人或动物 CMV 载体在所选对象(例如人)中是天生非病原性的。
在一些实施方案中,已经修饰了 CMV 载体以使其在所选对象中非病原性(不能在宿主之间扩
散)。

[0120]　　异源抗原可以是不是来自于 CMV 的任何蛋白质或其片段,包括癌抗原、病原体特异
性抗原、模型抗原(例如溶菌酶、匙孔戚血蓝蛋白　(keyhole-limpet hemocyanin,KLH)或卵
清蛋白)、组织特异性抗原、宿主自身抗原或任何其他抗原。

[0121]　　病原体特异性抗原可以来自于任何人或动物病原体。病原体可以是病毒病原体、
细菌病原体或寄生虫,并且抗原可以是来自于病毒病原体、细菌病原体或寄生虫的蛋白质。
寄生虫可以是生物体或由生物体引起的疾病。例如,寄生虫可以是原生动物生物体、引起疾
病的原生动物生物体、蠕虫(helminth)生物体或蠕虫(worm)、由蠕虫生物体引起的疾病、体
外寄生虫(ectoparasite)或由体外寄生虫引起的疾病。

[0122]　　抗原可以是来自于癌症的蛋白质。癌症包括但不限于急性髓细胞性白血病、慢性
髓细胞性白血病、骨髓增生异常综合征、急性淋巴细胞白血病、慢性淋巴细胞白血病、非霍
奇金淋巴瘤、多发性骨髓瘤、恶性黑素瘤、乳腺癌、肺癌、卵巢癌、前列腺癌、胰腺癌、结肠癌、

肾细胞癌(RCC) 和生殖细胞肿瘤。

[0123]　　抗原可以是宿主自身抗原。宿主自身抗原包括但不限于来自于T细胞受体之可变区或来自于B细胞受体之可变区的抗原。抗原可以是组织特异性抗原。组织特异性抗原包括但不限于精子抗原或卵抗原。

[0124]　　本文中公开的CMV载体可用作包含重组CMV病毒或载体以及可药用载体或稀释剂的免疫原性、免疫或疫苗组合物。包含重组CMV病毒或载体(或其表达产物)的免疫组合物引起局部或全身性免疫应答。应答可以是但不一定是保护性的。包含重组CMV病毒或载体(或其表达产物) 的免疫原性组合物同样引起局部或全身性免疫应答,其可以是但不一定是保护性的。疫苗组合物引起局部或全身性保护性应答。因此,术语"免疫组合物"和"免疫原性组合物"包括"疫苗组合物"(因为前两个术语可以是保护性组合物)。

[0125]　　本文中公开的CMV载体可用于在对象中诱导免疫应答的方法中,所述方法包括向对象施用包含重组CMV病毒或载体和可药用载体或稀释剂的免疫原性、免疫或疫苗组合物。为了本说明书的目的,术语"对象"包括所有动物,包括非人灵长类和人,而"动物"包括人以外的所有脊椎动物物种;并且"脊椎动物"包括所有脊椎动物,包括动物(如本文中使用的"动物") 和人。此外,当然,"动物"的一个子集是"哺乳动物",为了本说明书的目的,其包括除人之外的所有哺乳动物。

[0126]　　本文中公开的CMV载体可用在包含重组CMV病毒或载体和可药用载体或稀释剂的治疗组合物中。本文中公开的CMV载体可以通过将包含编码异源抗原的序列的DNA插入CMV基因组的必需或非必需区域来制备。所述方法还可包括从CMV基因组中缺失一个或更多个区域。所述方法可包括体内重组。因此,所述方法可包括在供体DNA的存在下在细胞相容性培养基中用CMV DNA转染细胞,所述供体DNA包含侧翼为与 CMV基因组的一部分同源的DNA序列的异源DNA,从而将异源DNA 引入到CMV的基因组中,并且任选地然后回收通过体内重组修饰的 CMV。所述方法还可包括切割CMV DNA以获得经切割的CMV DNA,将异源DNA连接至经切割的CMV DNA以获得杂交CMV-异源DNA,用杂交CMV-异源DNA转染细胞,以及任选地然后回收通过异源DNA 的存在而修饰的CMV。由于包含了体内重组,因此所述方法还提供了包含编码CMV外源多肽的非天然存在于CMV中的供体DNA的质粒,所述供体DNA在CMV DNA的区段内,否则所述区段将与CMV基因组的必需或非必需区域共线,使得来自CMV必需或非必需区域的DNA在供体DNA的侧翼。可将异源DNA插入到CMV中以产生任何取向的重组 CMV,其在期望时产生该DNA的稳定整合及其表达。

[0127]　　在重组CMV载体中编码异源抗原的DNA也可包含启动子。启动子可以来自任何来源,例如疱疹病毒,包括内源CMV启动子,如HCMV、RhCMV、鼠CMV(MCMV)或其他CMV启动子。启动子也可以是非病毒启动子,例如EF1α启动子。启动子可以是截短的转录活性启动子,其包含由病毒提供的反式激活蛋白反式激活的区域和截短的转录活性启动子所来源的全长启动子的最小启动子区。启动子可以由对应于最小启动子的DNA序列和上游调节序列的缔合构成。最小启动子由CAP位点加 TATA框(基本转录水平的最小序列;不受调节的转录水平)构成;"上游调节序列"由上游元件和增强子序列构成。此外,术语"截短的"表示全长启动子不完全存在,即全长启动子的某些部分已被除去。并且,截短的启动子可以来自于例如MCMV或HCMV的疱疹病毒,例如HCMV-IE 或MCMV-IE。基于碱基对,大小可从全长启动子降低达到至多40％,甚至至多90％。启动子也可以是经修饰的非病毒启动子。关于HCMV启动子,参考

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美国专利No.5,168,062和5,385,839。关于用质粒DNA转染细胞用于由其进行表达,参考
Felgner等(1994),J.Biol.Chem.269,2550-2561。而且,关于直接注射质粒DNA作为简单且
有效的针对多种传染病接种疫苗的方法,参考Science,259:1745-49,1993。因此,可通过直
接注射载体DNA来使用载体在本发明的范围内。

[0128]　　还公开了可插入重组病毒或质粒的表达盒,所述表达盒包含截短的转录活性启动
子。表达盒还可包含功能性截短的多聚腺苷酸化信号;例如截短的但是仍有功能性的SV40
聚腺苷酸化信号。考虑到本质上提供了更大信号,确实出人意料的是截短的聚腺苷酸化信
号是功能性的。截短的多聚腺苷酸化信号解决重组病毒(例如CMV)的插入大小限制问题。表
达盒还可包含相对于其所插入的病毒或系统异源的DNA;并且所述DNA 可以是本文中所述
的异源DNA。

[0129]　　关于用于疫苗或免疫组合物的抗原,还参见Stedman's Medical Dictionary(第
24版,1982,例如,疫苗的定义(疫苗制剂中使用的抗原的列表);可使用来自这些抗原的目
标抗原或表位。关于异源抗原,本领域技术人员可根据肽或多肽的氨基酸以及相应DNA序列
的知识以及特定氨基酸的性质(例如,大小、电荷等)和密码子词典来选择异源抗原及其编
码DNA,而无需过度的实验。

[0130]　　确定抗原的T表位的一种方法涉及表位作图。通过寡肽合成产生异源抗原的重叠
肽。然后测试各肽与由天然蛋白质引起的抗体结合或诱导T 细胞或B细胞活化的能力。这种
方法在T细胞表位作图时特别有用,因为T细胞识别与MHC分子复合的短线性肽。

[0131]　　一般来说,如下产生对异源抗原的免疫应答:只有当蛋白质被切割成较小肽并且
在位于另一细胞表面的"主要组织相容性复合物(MHC)"的复合物中呈递时,T细胞才识别蛋
白质。有两类MHC复合物-I类和 II类,且每类由许多不同等位基因组成。不同物种和个体对
象具有不同类型的MHC复合物等位基因;称其具有不同的MHC类型。一类MHC I类分子称为
MHC-E(人中为HLA-E,RM中为Mamu-E,小鼠中为 Qa-1b)。

[0132]　　应注意,包含编码异源抗原之序列的DNA本身可包含用于在CMV 载体中驱动表达
的启动子,或者所述DNA可以限于异源抗原的编码DNA。该构建体可以相对于内源性CMV启动
子以这样的取向放置,使其可操作地与启动子连接并由此表达。此外,可以进行编码异源抗
原的DNA的多个拷贝或使用强或早期启动子或者早期和晚期启动子或其任何组合以增强或
提高表达。因此,编码异源抗原的DNA可以相对于CMV 内源性启动子适当定位,或者那些启
动子可以与编码异源抗原的DNA一起被移位以被插入到另一位置。编码多于一种异源抗原
的核酸可以包装在 CMV载体中。

[0133]　　还公开了包含所公开的CMV载体的药物和其他组合物。可以配制这样的药物和其
他组合物以便以本领域已知的任何施用方法使用。这样的药物组合物可以通过肠胃外途径
(皮内、肌内、皮下、静脉内等)。施用也可以通过黏膜途径,例如经口、经鼻、经生殖器等。

[0134]　　所公开的药物组合物可以根据药学领域技术人员公知的标准技术来制备。这样的
组合物可以以医学领域技术人员公知的剂量和技术施用,考虑例如特定患者的品种或物
种、年龄、性别、体重和病症以及施用途径等因素。组合物可以单独施用,或者可与其他CMV
载体或其他免疫、抗原性或疫苗或治疗组合物共施用或依次施用。这样的其他组合物可包
含纯化的天然抗原或表位或者由重组CMV或其他载体系统表达的抗原或表位,并且在考虑
上述因素的情况下施用。

[0135] 组合物的实例包括用于孔口(例如,经口、经鼻、经肛门、经生殖器(例经如阴道)等)施用的液体制剂,例如混悬剂、糖浆剂或酏剂;以及用于胃肠外、皮下、皮内、肌内或静脉内施用(例如,注射施用)的制剂,例如无菌混悬剂或乳剂。在这样的组合物中,重组体可以与合适的载体、稀释剂或赋形剂(例如无菌水、生理盐水、葡萄糖等)混合。

[0136] 抗原性、免疫或疫苗组合物通常可包含佐剂和一定量的CMV载体或表达产物以引起期望的应答。在人类应用中,明矾(alum)(磷酸铝或氢氧化铝)是典型的佐剂。皂苷及其纯化组分Quil A、Freund完全佐剂以及用于研究和兽医应用的其他佐剂具有毒性,这限制了其在人疫苗中的潜在用途。也可使用化学上定义的制剂,例如胞壁酰二肽、单磷酰脂质A、磷脂缀合物,例如由Goodman-Snitkoff等,J.Immunol.147:410-415(1991) 所述的那些,蛋白质包封在蛋白脂质体内,如Miller等,J.Exp.Med. 176:1739-1744(1992)所述,以及蛋白质包封在脂质囊泡例如Novasome 脂质囊泡中(Micro Vescular Systems,Inc.,Nashua,N.H.)。

[0137] 组合物可以包装在单一剂型中,用于通过肠胃外(即,肌内、皮内或皮下)施用或孔口施用(例如经舌(perlingual)(例如,经口)、胃内、黏膜(包括口腔内、肛门内、阴道内等))施用。此外,有效剂量和施用途径由组合物的性质、由表达产物的性质、如果直接使用重组CMV则由表达水平,以及由已知因素(例如宿主的品种或物种、年龄、性别、体重、病症和性质,以及$LD_{50}$和已知的并且不需要过度实验的其他筛选程序) 确定。表达产物的剂量可以从几到几百微克变化,例如5至500μg。CMV 载体可以以任何合适的量施用以在这些剂量水平下实现表达。在一些非限制性实例中:CMV载体可以以至少$10^{2}$pfu的量施用;因此,CMV载体可以以至少该量施用;或在约$10^{2}$pfu至约$10^{7}$pfu范围内。其他合适的载体或稀释剂可以是具有或没有防腐剂的水或缓冲盐水。CMV载体可以是冻干的,用于在施用时重悬,或可以在溶液中。"约"可以意指在限定值的 1%、5%、10%或20%内。

[0138] 应理解,本发明的蛋白质及其编码核酸可以不同于本文中所示和所述的精确序列。因此,本发明考虑所示序列的缺失、添加、截短和替换,只要序列根据本发明的方法起作用即可。在这方面,替换将通常在性质上是保守的,即在氨基酸家族内发生的那些替换。例如,氨基酸通常分为四个家族:(1) 酸性-天冬氨酸和谷氨酸;(2) 碱性-赖氨酸、精氨酸、组氨酸;(3) 非极性-丙氨酸、缬氨酸、亮氨酸、异亮氨酸、脯氨酸、苯丙氨酸、甲硫氨酸、色氨酸;以及 (4) 不带电荷极性的-甘氨酸、天冬酰胺、谷氨酰胺、半胱氨酸、丝氨酸、苏氨酸和酪氨酸。苯丙氨酸、色氨酸和酪氨酸有时被分类为芳香族氨基酸。可以合理地预期,在以下情况下不会对生物活性产生重大影响:用异亮氨酸或缬氨酸独立地替换亮氨酸,或反之亦然;用谷氨酸替换天冬氨酸,或反之亦然;用丝氨酸替换苏氨酸,或反之亦然;或用结构相关氨基酸进行氨基酸的类似保守替换。因此,与所述蛋白质具有基本相同的氨基酸序列,但是不具有实质上影响蛋白质免疫原性的小的氨基酸替换的蛋白质也在本公开内容的范围内。

[0139] 本发明的核苷酸序列可以进行密码子优化,例如密码子可被优化用于人细胞。例如,可如此改变任何病毒或细菌序列。许多病毒(包括HIV 和其他慢病毒)使用大量的稀有密码子,并且通过将这些密码子改变以使其符合期望对象中常用的密码子,可以实现异源抗原的增强表达,如 Andre等.J.Virol.72:1497-1503,1998中所述。

[0140] 考虑编码CMV载体和包含在其中的糖蛋白的功能和/或抗原性等同变体和衍生物的核酸序列。这些功能等同变体、衍生物和片段显示出保留抗原活性的能力。例如,不改变

所编码氨基酸序列的DNA序列的变化以及导致氨基酸残基的保守替换、一个或几个氨基酸缺失或添加、以及氨基酸残基被氨基酸类似物替换将不会显著影响所编码多肽的性质。保守氨基酸替换为甘氨酸/丙氨酸;缬氨酸/异亮氨酸/亮氨酸;天冬酰胺/谷氨酰胺;天冬氨酸/谷氨酸;丝氨酸/苏氨酸/甲硫氨酸;赖氨酸/精氨酸;以及苯丙氨酸/酪氨酸/色氨酸。在一个实施方案中,变体与目标抗原、表位、免疫原、肽或多肽具有至少50%、至少55%、至少60%、至少65%、至少 70%、至少75%、至少80%、至少85%、至少86%、至少87%、至少88%、至少89%、至少90%、至少91%、至少92%、至少93%、至少 94%、至少95%、至少96%、至少97%、至少98%或至少99%的同源性或同一性。

[0141]　　　可通过比对时比较序列来确定序列同一性或同源性,以使重叠和同一性最大化,同时使序列空位最小化。特别地,可以使用许多数学算法中的任一种来确定序列同一性。用于比较两个序列的数学算法的非限制性实例是Karlin&Altschul,Proc.Natl.Acad.Sci.USA 1990;87:2264-2268 的算法,在Karlin&Altschul,Proc.Natl.Acad.Sci.USA 1993;90：5873-5877中进行了改进。

[0142]　　　用于比较序列的数学算法的另一个实例是Myers&Miller,CABIOS 1988;4:11-17的算法。将这样的算法并入作为GCG序列比对软件包一部分的ALGER程序(2.0版本)中。当使用ALIGN程序比较氨基酸序列时,可以使用PAM120权重残基表,空位长度罚分为12,且空位罚分为 4。用于鉴定局部序列相似性和比对的区域的另一种可用算法是如 Pearson&Lipman,Proc.Natl.Acad.Sci.USA 1988;85:2444-2448中描述的FASTA算法。

[0143]　　　根据本发明有利地使用的是WU-BLAST(Washington University BLAST)2.0版软件。可从ftp://blast.wustl.edu/blast/executables下载适用于多个UNIX平台的WU-BLAST 2.0版可执行程序。该程序基于 WU-BLAST 1.4版本,后者进而又基于公共领域NCBI-BLAST 1.4版 (Altschul&Gish,1996,Local alignment statistics,Doolittle编辑,Methods in Enzymology 266:460-480;Altschul等.(1990),同上;Gish& States,1993;Nature Genetics 3:266-272;Karlin&Altschul,Proc.Natl. Acad.Sci.USA 90:5873-5877(1993);所有这些均通过引用并入本文)。

[0144]　　　使用标准重组DNA和克隆技术制备本发明的多种重组核苷酸序列和抗体和/或抗原。这样的技术是本领域技术人员公知的。参见例如"Molecular Cloning:A Laboratory Manual",第二版(Sambrook等. 1989)。

[0145]　　　本发明的核苷酸序列可以插入"载体"中。术语"载体"是本领域技术人员广泛使用和理解的,并且如本文中所使用的术语"载体"与本领域技术人员所理解的含义一致使用。例如,本领域技术人员通常使用术语"载体"用于指允许或促进将核酸分子从一个环境转移到另一个环境或者允许或促进核酸分子操作的载剂。

[0146]　　　可以根据本发明使用允许表达本发明的病毒的任何载体。在某些实施方案中,所公开的病毒可以体外使用(例如使用无细胞表达系统)和/ 或在体外生长的培养细胞中以产生编码的异源抗原(例如,病原体特异性抗原、HIV抗原、肿瘤抗原和抗体),其然后可用于多种应用,例如生产蛋白质疫苗。对于这样的应用,可以使用允许在体外和/或培养的细胞中表达病毒的任何载体。

[0147]　　　对于待表达的所公开异源抗原,异源抗原的蛋白质编码序列应该与指导蛋白质的转录和翻译的调节或核酸控制序列"可操作地连接"。如本文中使用的,当编码序列和核酸

控制序列或启动子以一定方式共价连接，使得编码序列的表达或转录和/或翻译处于核酸控制序列的影响下或控制下时，认为其是"可操作地连接"。"核酸控制序列"可以是任何核酸元件，例如但不限于启动子、增强子、IRES、内含子以及指导与其可操作地连接的核酸序列或编码序列之表达的本文中所述的其他元件。术语"启动子"在本文中将用于指围绕RNA聚合酶II的起始位点聚集的一组转录控制模块，并且当其与本发明的蛋白质编码序列可操作地连接时，导致所编码蛋白质的表达。本发明转基因的表达可以在组成型启动子或诱导型启动子的控制下，后者仅在暴露于某些特定的外部刺激(例如但不限于抗生素如四环素，激素如蜕皮素，或重金属)时才启动转录。启动子也可以是特定细胞类型、组织或器官特异性的。许多合适的启动子和增强子是本领域已知的，并且任何这样的合适的启动子或增强子可用于表达本发明的转基因。例如，合适的启动子和/或增强子可选自真核启动子数据库(Eukaryotic Promoter Database,EPDB)。

[0148]　　本公开内容涉及表达异源蛋白抗原的重组病毒载体。在一些实例中，抗原是HIV抗原。有利地，HIV抗原包括但不限于在美国公开No．2008/0199493 A1和2013/0136768A1中讨论的HIV抗原，其二者均通过引用并入本文。HIV、核酸或其免疫原性片段可用作HIV蛋白质抗原。例如，可以使用美国公开No.2008/0199493 A1和2013/0136768 A1中讨论的HIV核苷酸。HIV抗体识别的任何抗原也可用作HIV蛋白质抗原。蛋白质抗原也可以是SIV抗原。例如，可以使用美国公开No.2008/0199493 A1和2013/0136768 A1中讨论的SIV抗原。

[0149]　　根据本发明使用的载体可包含合适的基因调节区(例如启动子或增强子)，使得可表达本发明的抗原。

[0150]　　在对象体内表达本发明的抗原，例如以便产生针对HIV-1抗原的免疫应答和/或针对HIV-1的保护性免疫，应选择适合于在对象中表达并且对于体内使用安全的表达载体。在一些实例中，可期望在实验动物中表达抗体和/或抗原，例如用于本发明的HIV-1免疫原性组合物和疫苗的临床前测试。在另一些实例中，可以在人对象中表达抗原，例如在本发明的免疫原性组合物和疫苗的临床试验和实际临床应用中。

[0151]　　本文中描述的CMV载体可包含可防止宿主间扩散的突变，从而使得病毒不能感染免疫受损的或其他由于CMV感染而可能面临并发症的对象。本文中所述的CMV载体还可包含导致存在免疫显性和非免疫显性表位以及非典型MHC限制性的突变。然而，本文中所述的CMV载体中的突变不影响载体重新感染先前已被CMV感染的对象的能力。这样的 CMV突变描述于例如美国专利公开2013-0136768、2010-0142823、2014-0141038、以及PCT申请公开WO2014/138209中，其全部均通过引用并入本文。

[0152]　　所公开的CMV载体可以在体内施用，例如其目的是产生免疫原性应答，包括CD8$^+$免疫应答，包括特征在于高百分比的CD8$^+$ T细胞应答被 MHC II类和/或MHC-E(或其同源物或直系同源物)限制的免疫应答。例如，在一些实例中，可能期望在实验室动物(例如恒河猴)中使用所公开的CMV载体，用于使用RhCMV的免疫原性组合物和疫苗的临床前测试。在另一些实例中，期望在人对象中使用所公开的CMV载体，例如在使用HCMV的免疫原性组合物的临床试验中和用于实际临床应用。

[0153]　　对于这样的体内应用，所公开的CMV载体作为还包含可药用载体的免疫原性组合物的组分施用。本发明的免疫原性组合物可用于刺激针对异源抗原(包括病原体特异性抗原)的免疫应答，并可用作针对HIV-1的预防性或治疗性疫苗的一种或更多种组分以用于预

防、改善或治疗AIDS。本发明的核酸和载体特别地可用于提供基因疫苗,即,用于将编码本发明抗原的核酸递送至对象(例如人)的疫苗,使得抗原然后在对象中表达以引起免疫应答。

[0154]　　用于动物(包括人)的免疫接种安排(或方案)是已知的,并且对于特定对象和免疫原性组合物可以容易地确定。因此,免疫原可以向对象施用一次或更多次。优选地,在免疫原性组合物的分开施用之间存在设定的时间间隔。尽管该间隔对于每个对象而言是不同的,但通常为10天至数周,并且通常为2、4、6或8周。对于人,间隔通常为2至6周。在本发明的一个特别有利的实施方案中,间隔更长,有利地为约10周、12周、14周、16周、18周、20周、22周、24周、26周、28周、30周、32周、34周、36周、38周、40周、42周、44周、46周、48周、50周、52周、54周、56周、58周、60周、62周、64周、66周、68周或70周。免疫接种方案通常具有免疫原性组合物的1至6次施用,但可以具有少至1 或2或4次。诱导免疫应答的方法还可包括施用与免疫原一起的佐剂。在一些情况下,每年、每两年或其他长时间间隔(5至10年)的加强免疫接种可以补充初始免疫方案。本发明的方法还包括多次致敏-加强方案。在这些方法中,一次或更多次致敏免疫接种接着一次或更多次加强免疫接种。每次免疫接种的实际免疫原性组合物可以相同或不同,免疫原性组合物的类型(例如,含有蛋白质或表达载体)、途径和免疫原的制剂也可以变化。例如,如果表达载体用于致敏和加强步骤,则其可以是相同或不同类型(例如,DNA或者细菌或病毒表达载体)。一种可用的致敏-加强方案提供间隔4周的两次致敏免疫接种,接着是在最后一次致敏免疫接种后的第4周和第8周进行两次加强免疫接种。对于本领域技术人员来说还很明显的是,在使用本发明的DNA、细菌和病毒表达载体来提供致敏和加强方案中涵盖多种排列和组合。可重复使用CMV载体,同时表达来自于不同病原体的不同抗原。

**实施例**

[0155]　　以下实施例举例说明了所公开的方法。根据本公开内容,本领域技术人员将认识到,所公开方法的这些实施例和其他实施例的变化将是可能的,而无需过度的实验。

[0156]　　实施例1.通过缺少UL128和UL130但含有UL40和US28基因的恒河猴巨细胞病毒疫苗载体诱导MHC-E限制性CD8$^+$ T细胞。

[0157]　　先前已经证明,RhCMV/SIV载体驱动替代的SIV特异性CD8$^+$ T细胞应答,其完全不同于常规疫苗形式产生的典型应答,且甚至不同于SIV 感染本身(Hansen,S.G.等,Science 340,1237874(2013),其通过引用并入本文)。

[0158]　　尽管已经确定了RhCMV/SIV诱导的CD8$^+$ T细胞应答以MHC-II 限制性CD8$^+$ T细胞群体的存在为主,但限制其余CD8$^+$ T细胞(被泛 MHC-I阻断抗体W6/32抑制的那些)的分子仍然是未知的。

[0159]　　特别地,施用68-1 RhCMV/gag载体在经每种RhCMV/gag载体疫苗接种的恒河猴中引起靶向SIVmac239Gag$_{273-287}$(Gag 15聚体#69)和 Gag$_{477-491}$(Gag 15聚体#120)"超表位"的MHC-I限制性CD8$^+$ T细胞,而不管MHC-Ia的表达。这意味着功能保守的"非经典"(即非多态)MHC-Ib分子的参与。本文中描述了这些CD8$^+$ T细胞的限制性MHC-I 等位基因的鉴定。由具有强的RhCMV/gag诱导的CD8$^+$ T细胞应答的4 只经毒株68-1 RhCMV/gag疫苗接种的恒河猴的组群中开发表达单一"经典"(即多态)MHC-Ia或非经典MHC-Ib等位基因的MHC-I转染子组

(图7)。使用先前描述的MHC限制性测定(Hansen等.Science(2013),同上),确定了靶向 Gag273-287和Gag477-491超表位的CD8$^+$T细胞在MHC-E 的情况下识别这些表位(图1A)。

[0160]　　MHC-E(人中的HLA-E、恒河猴中的Mamu-E、以及小鼠中的Qa-1$^b$) 是在体内几乎每个有核细胞中表达的高度单型、非经典MHC-Ib分子,其在免疫系统细胞中特别地高表达 (N.Lee等,Proc Natl Acad Sci USA 95, 5199(1998)和S.Coupel等,Blood 109,2806 (2007),二者都通过引用并入本文)。与目前鉴定的超过8,500个HLA I类等位基因 (J.Robinson等, Nucleic Acids Res 41,D1222(2013);其通过引用并入本文) 相反,仅存在两个HLA-E分子,其在位于肽结合槽外部的一个氨基酸处变化,并因此可能是功能上相同的(R.K.Strong等,J Biol Chem 278,5082(2003);其通过引用并入本文)。MHC-E的这种高度单型性质可能解释了每个经 RhCMV/gag免疫接种的恒河猴如何能够独立于每个动物中存在的 MHC-Ia等位基因而靶向相同的Gag MHC-I超表位。

[0161]　　MHC-E也被鉴定为在经RhCMV/gag疫苗接种的恒河猴中其余 MHC-I阻断的CD8$^+$ T细胞的限制性等位基因(图1A)。虽然MHC-E的结构与经典MHC-Ia分子的结构相似,但是在正常的生理条件下,MHC-E 仅重复地结合并呈递来自于MHC-Ia分子的前导序列的单个9聚体肽用于呈递至NK细胞。然而,在细胞应激的条件下,例如在病毒感染期间,MHC-E结合一组完全独立的高度多样化的CD8$^+$ T细胞表位,其结合基序与主要MHC-Ia前导肽的结合基序不匹配(Lampen等,同上,和C.C. Oliviera等,J E.xp Med 207,207(2010);二者均通过引用并入本文)。MHC-E脱离前导肽并随后将替代肽库(repertoire)呈递至CD8$^+$T细胞的能力表明,替代的MHC-I限制性CD8$^+$T细胞应答主要(如果不是全部的话)由于通过MHC-E的呈递。

[0162]　　最近已经发现HLA-E限制性CD8$^+$T细胞针对多种人病原体,包括 CMV(G.Pietra 等,Proc Natl Acad Sci USA 100,10896(2003);其通过引用并入本文);EBV(Jorgensen PB等,PLoS One 7,e46120(2012);其通过引用并入本文);伤寒沙门氏菌(Salmonella typhi)(R. Salerno-Goncalves等,J Immunol 173,5852(2004);其通过引用并入本文);以及结核分枝杆菌(Mycobacteriumtuberculosis)(A.S.Heinzel等, J Exp Med 196,1473 (2002)和SA Joosten等.PLoS Pathol 6,el000782 (2010);二者均通过引用并入本文)。然而,没有报道HIV/SIV特异性 MHC-E限制性CD8$^+$ T细胞应答,并且目前不存在诱导针对任何异源抗原的这些非经典限制性CD8$^+$ T细胞的疫苗平台。

[0163]　　使用MHC"阻断"肽确认了来自动物的MHC限制性数据,所述 MHC"阻断"肽以高亲和力与特异性MHC同质异型体结合,因此对于该 MHC分子的结合槽胜过其他肽。为了保护其感染的宿主细胞免受NK细胞介导的裂解,HCMV编码糖蛋白UL40(RCMV同源物为Rh67),其包含来自于经典MHC-Ia前导序列的精确的9聚体肽(VMAPRTLLL, Rh67$_{8-16}$VL9)。VL9肽以极高的亲和力特异性地结合MHC-E肽结合槽 (P.Tomasec等,Science 287,1031(2000);其通过引用并入本文)。将抗原呈递细胞与来自于Rh67的VL9肽预孵育以阻断Gag273-287和Gag477-491肽与MHC-E的结合,或与不相关的Mamu-A＊002:01(A＊02)结合的 Gag71-79GY9肽的结合。MHC-E高亲和力结合肽Rh67$_{8-16}$VL9的存在完全抑制了表达单个MHC-E等位基因的自体BLCL和转染子二者上的 Gag273-287和Gag477-491超表位的CD8$^+$T细胞识别,这证实了MHC-E作为 MHC-I超表位应答的呈递等位基因(图1B和1C)。

[0164]　　将MHC-E对由RhCMV/gag载体引起的总体Gag特异性CD8$^+$T细胞应答的贡献与常规改良牛痘安卡拉(Modified Vaccinia Ankara) (MVA/gag)载体和天然SIV感染进行了比

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较。将使用MHC-I或MHC-II 特异性的阻断单克隆抗体 (mAb) 以及阻断Rh67$_{8-16}$VL9肽的MHC-E的流式细胞术ICS来评估在25只恒河猴组群中 (6只用毒株68-1 RhCMV/gag免疫接种、9只用毒株68-1.2 RhCMV/gag免疫接种、7只用 MVA/gag免疫接种、以及8只经SIV感染的恒河猴) 发现的每个表位特异性应答的限制性。仅在用毒株68-1 RhCMV/gag免疫接种的恒河猴中发现MHC-E阻断的CD8$^+$ T细胞应答。此外,在用毒株68-1 RhCMV/gag 免疫接种的恒河猴中观察到的每个MHC-I阻断应答由MHC-E呈递 (图 2A、8和9)。在用毒株68-1.2 RhCMV/gag载体免疫接种的恒河猴中没有观察到MHC-E限制性CD8$^+$ T细胞。

[0165] 考虑到两种CMV毒株之间的最小差异,经毒株68-1.2 RhCMV/gag 载体疫苗接种的恒河猴中缺少MHC-E限制性CD8$^+$ T细胞是出人意料的。在作为细菌人工染色体 (BAC) 克隆之前在成纤维细胞上体外培养期间,RhCMV 68-1失去了表达来自Rh13、Rh60、Rh157.5和Rh157.4 (分别为HCMV RL11、UL36、UL128和UL130) 开放阅读框的基因产物的能力 (D.Malouli等,J Virol 86,8959 (2012) 和WO 2014/138209;其通过引用并入本文)。其中,在RhCMV 68-1.2毒株中Rh60、Rh157.5和 Rh157.4的表达恢复 (A.E.Lilja,T.Shenk,Proc Natl Acad Sci U S A 105, 19950 (2008);其通过引用并入本文),表明这些基因产物中的一种或组合的存在足以抑制MHC-E上CD8$^+$ T细胞的致敏。可以排除Rh60作为介导这种抑制作用的基因,因为其存在于来自于非BAC的RhCMV/gag (L) 载体中 (Hansen,S,G等,Science 328,102 (2010);其通过引用并入本文),其诱导MHC-E限制性CD8$^+$ T细胞 (图2A)。因此,CMV中不存在基因Rh157.5和Rh157.4 (HCMV中为UL128-130) 对于诱导MHC-E限制性CD8$^+$ T细胞是必需的。

[0166] 观察到某些应答的不完全的VL9阻断 (参见图2A中Rh22607中的 Gag 15聚体#18)。实际上,虽然每个RhCMV/gag诱导的、W6/32阻断的CD8$^+$ T细胞应答在MHC-E的情况下识别肽,但是在经典MHC-Ia等位基因的情况下识别了这些不完全VL9-阻断的肽,例如在Rh22607中由 Mamu-A＊001:01 (A＊01) 呈递的Gag$_{69-83}$ (Gag 15聚体#18) (图8B)。为了更加接近地理解这种双重呈递,进行了肽阻断研究。这些表明,尽管 Mamu-A＊01结合的Gag$_{181-189}$CM9肽的存在足以抑制Mamu-A＊01转染子上Gag$_{69-83}$的呈递,并且来自于Rh67的VL9肽的存在抑制MHC-E转染子上Gag$_{69-83}$的呈递,但是需要这两种肽来完全阻断来自Mamu-A＊01$^+$恒河猴的自体BLCL上Gag$_{69-83}$的呈递 (图2B)。相比之下,相同的Gag$_{69-83}$表位的呈递被来自Mamu-A＊01恒河猴的BLCL上单独Rh67$_{8-16}$VL9肽的存在完全阻断,强调了MHC-E作为这些肽的主要限制性等位基因 (图 2C)。然而,鉴于MHC-E限制性CD8$^+$ T细胞在MHC-E或经典MHC-Ia 分子的情况下响应于同源肽的能力,这些细胞的TCR可能直接识别MHC 结合肽本身或与保守的MHC结构基序结合。出乎意料地,能够结合特异性肽表位的MHC等位基因的存在不足以产生靶向所述表位的CD8$^+$ T细胞应答 (图8),这指示在确定每个经RhCMV免疫接种的恒河猴中靶向的特异性表位组中存在另外的免疫调节层。

[0167] 接下来,确定了MHC-E限制性CD8$^+$ T细胞参与针对SIV的免疫应答。HIV和SIV通过Nef介导的来自细胞表面的经典MHC I类分子的下调来逃避CD8$^+$ T细胞识别 (O.Schwartz等,Nat Med2,338 (1996);K.L. Collins等,Nature 391,397 (1998);二者均通过引用并入本文)。相比之下, Nef不能下调HLA-E,且其表面表达实际上随HIV感染而提高 (J. Natterman等,Antivir Ther 10,95, (2005);其通过引用并入本文)。首先,确定了生产性SIV感染的CD4$^+$ T细胞表面上Mamu-E的命运。使用泛 MHC-I mAb W6/32和Mamu-E特异性mAb

4D12,证明了与HIV感染期间的HLA-E相似,SIV感染期间Mamu-E表面表达显著提高(图3A 和 3B)。因此,MHC-E限制性CD8$^+$T细胞可能是特别有效的,因为它们不受Nef介导的其限制性 MHC-I分子下调的影响。MHC-E与CD8$^+$ T 细胞表面上表达的TCRαβ和CD94/NKG2复合物二者 相互作用(V.M. Brahd等,Nature 391,795(1998);其通过引用并入本文)。

[0168]　　特别地,高NKG2C表达由CMV感染驱动,且NKG2C受体的参与触发与MHC-E相互作用 的NK和T细胞的活化(S.Lopez-Verges等, Proc Natl Acad Sci U S A 108,14725(2011) 和M.Guma等,Eur J Immunol 35,2071(2005);二者均通过引用并入本文)。为了研究在经毒 株68-1 RhCMV/gag疫苗接种的恒河猴中MHC-E限制性CD8$^+$T细胞利用NKG2C受体以介导 MHC-E诱导的活化的可能性,检查这些细胞的表面表型,并且发现了很少(如果有的话) NKG2A/NKG2C表达(图3C 和10)。此外,MHC-E限制性CD8$^+$T细胞表现出常规CD3$^+$、CD8αβ$^+$、 TCR γ δ$^-$、NKG2A/C$^-$表型,表明这些T细胞通过CD8稳定化TCRαβ相互作用识别MHC-E结合的肽。

[0169]　　接下来,将经68-1 RhCMV/SIV免疫接种的恒河猴中存在的MHC-E 限制性CD8$^+$T细 胞特异性识别自体、SIV感染的CD4$^+$ T细胞的能力与用MVA/gag或毒株68-1.2 RhCMV/gag免 疫接种或用SIV感染的恒河猴中发现的经典MHC-I限制性CD8$^+$T细胞的能力进行比较。从所 有经处理的恒河猴中分离的CD8$^+$T细胞强烈识别自体SIV感染的CD4$^+$ T细胞,并且这种识别 通过添加泛MHC-I阻断mAb W6/32和MHC-II结合CLIP 肽被完全阻断(图4A和4B)。相比之下, 在除了从经毒株68-1 RhCMV/SIV免疫接种的恒河猴中分离的CD8$^+$T细胞以外,当MHC-E 阻 断Rh67$_{8-16}$VL9肽替换MHC-I阻断mAb W6/32时,经感染细胞的CD8$^+$T细胞识别完全恢复。这表 明MHC-E限制性CD8$^+$T细胞识别SIV感染的细胞。

[0170]　　为了更准确地检查MHC-E结合的、来自于SIV的表位是否存在于经感染细胞的表 面,产生Gag$_{477-491}$(Gag#120)超表位特异性、MHC-E 限制性CD8$^+$T细胞系。测试该系响应于自 体SIV感染的CD4$^+$T细胞的能力。为了比较,还评估了经典MHC限制性(Mamu-A＊001:01限制 性) Gag$_{181-189}$CM9CD8$^+$T细胞系。这两种Gag特异性CD8$^+$T细胞系均特异性识别SIV感染的细 胞,并且当靶标与泛MHC-I阻断mAb W6/32预孵育时,识别被阻断。相比之下,当靶标与MHC-E 结合肽Rh67$_{8-16}$VL9 预孵育时,仅MHC-E限制性CD8$^+$ T细胞系不能识别SIV感染的细胞(图 4C)。总之,这些数据表明MHC-E限制性CD8$^+$ T细胞特异性识别经感染细胞表面的来自于SIV 的肽表位。

[0171]　　毒株68-1载体诱导在非经典MHC-E分子情况下识别肽抗原的CD8$^+$T细胞。这样的 CD8$^+$T细胞代表了用于疫苗开发的新的细胞免疫应答,并且考虑到MHC-E的独特的免疫生 物学,其可以是特别有效。与从HIV 感染的细胞表面下调的经典HLA分子相比,HLA-E表达上 调,并且 MHC-E表达的提高发生在病毒入口处感染的前24小时内(J.Natterman 等, Antivir Ther 10,95(2005)和L.Shang等,J Immunol 193,277(2014);二者均通过引用并 入本文)。在人群中仅存在两种功能相同的HLA-E等位基因(R.K.Strong等,J Biol Chem 278,5082(2003);其通过引用并入本文)。因此,特异性诱导MHC-E限制性T细胞的疫苗平台 可导致真正通用的CD8$^+$T细胞疫苗,每个经疫苗接种的个体具有不受HIV Nef介导的免疫 逃避影响的相同T细胞应答。事实上,如本文中所公开的,MHC-E 限制性CD8$^+$T细胞由毒株 68-1 RhCMV载体强烈引起,其在恒河猴中显示出针对SIV的无与伦比的保护(Hansen等 (2009),同上;Hansen等. (2011),同上;Hansen等.Nature(2013),同上)。因此,诱导针对经 典和非经典表位二者之应答的HIV疫苗可提供有效阻断病毒复制以及随后钝化病毒传播所

需的必要广度的T细胞应答。

[0172]　　如上所述，HCMV编码包含以极高亲和力结合MHC-E肽结合槽的 9聚体肽(VMAPRTLLL，Rh67$_{8-16}$VL9)的糖蛋白UL40(RCMV同源物为Rh67)(P.Tomasec等，Science 287,1031(2000)；其通过引用并入本文)。由于体外数据表明VL9竞争与来自抗原的肽结合，所以考虑了使 Rh67(UL40)从RhCMV 68-1的基因组中缺失将进一步提高体内HLA-E 限制性CD8$^+$T细胞之频率的可能性。为了检查这种可能性，使Rh67 (UL40)从68-1 RhCMV/gag载体中缺失。将所得重组病毒接种到天然感染有RhCMV的动物中。在接种后第0、7、14、21、28和42天，获得PBMC，并通过使用特异性肽的细胞内细胞因子染色来测量应答于总 SIVgag以及MHC-E的CD8$^+$T细胞的频率。如图5所示，在接种后第 14天开始可检测到对总SIVgag的SIVgag应答。此外，CD8$^+$T细胞响应于MHC-II限制性"超表位"肽Gag53和Gag73。然而，与我们的预期相反，对于HLA-E特异性超表位的T细胞应答没有提高。事实上，未检测到对于该实验中检查的任一HLA-E肽(Gag69和Gag120)的应答。因此，该出人意料的结果表明，缺少UL40和UL128和UL130的载体诱导 MHC-II限制性CD8$^+$T细胞，包括MHC-II限制性超表位，但不诱导 MHC-E限制性CD8$^+$T细胞。因此，MHC-E限制性CD8$^+$T细胞的诱导需要存在UL40，并且不存在UL128和UL130。

[0173]　　为了确定除了Rh67(UL40)之外，RhCMV是否还编码对于通过缺少UL128-130的RhCMV诱导HLA-E和/或MHC-II限制性T细胞应答所需的其他基因，使对于体外生长非必需的基因区域从RhCMV 68-1中缺失，并且监测恒河猴接种后的T细胞应答。虽然大多数缺失突变体不影响T细胞特异性，但是观察到基因区域Rh214-Rh220的缺失消除了 RhCMV 68-1引起MHC-E限制性应答的能力，但仍然观察到MHC-II 限制性CD8$^+$T细胞应答(图29和30)。由于Rh214-Rh220区域编码与 HCMV US28同源(即，其同源物)的5个拷贝的基因:Rh214、Rh215、Rh216、Rh218和Rh220(也分别称为RhUS28.4、RhUS28.3、RhUS28.2、RhUS28.1和RhUS28.5，M.E.Penfold等.J Virol 77:10404(2003)，其通过引用并入本文)，该结果是出乎意料的。基于一系列先前描述的标准，认为先前预测的开放阅读框Rh217和Rh219并不代表功能性基因(D. Malouli等,J Virol 86,8959(2012)，其通过引用并入本文)。HCMV US28 编码结合CC-趋化因子的G蛋白偶联受体(J.L Gao和P.M.Murphy J Biol Chem 269:28539(1993))，并证实了五种RhCMV同源物中至少一种的趋化因子结合(M.E.Penfold等.J Virol 77:10404(2003))。然而，未预料到对于诱导MHC-E限制性T细胞应答需要US28。因此，该出人意料的结果表明，缺少US28、UL128和UL130的载体诱导MHC-II限制性CD8$^+$T细胞，包括MHC-II限制性超表位，但不诱导MHC-E限制性 CD8$^+$T细胞。因此，MHC-E限制性CD8$^+$T细胞的诱导需要存在US28 和UL40，并且不存在UL128和UL130。

[0174]　　材料和方法:

[0175]　　恒河猴:在本实施例中报道的实验中，使用总计46只专用饲养 (purpose-bred)的印度遗传背景的雄性或雌性恒河猴(RM)(Macaca mulatto)，其中包括9只用毒株68-1 RhCMV/gag疫苗接种的RM，用毒株68-1.2 RhCMV/gag疫苗接种的RM，1只用Rh67缺失的68-1 RhCMV/gag接种的RM，7只用MVA/gag疫苗接种的RM，19只具有 SIV感染的未疫苗接种的RM，以及6只天然感染有RhCMV的集落循环毒株(colony-circulating strain)的未疫苗免疫接种的RM。所有RM经俄勒冈国家灵长类研究中心机构动物护理和使用委员会(Oregon National Primate Research Center Institutional Animal Care and Use Committee)

的批准,并按照美国国家卫生研究院实验动物护理和使用指南(US National Institutes of Health Guide for the Care and Use of Laboratory Animals)的标准使用。在这些实验中使用的RM没有1型猕猴疱疹病毒(cercopithicine herpesvirus 1)、D型猿猴逆转录病毒和1型猿猴T淋巴细胞病毒。将选择的RM通过深度测序进行MHC-I基因分型。简言之,通过使用高保真Phusion™聚合酶(New England Biolabs)和一对通用MHC-I特异性引物在以下热循环条件下通过PCR扩增cDNA来产生Mamu I类序列的扩增子:98℃3分钟,(98℃5秒,57℃1秒,72℃20秒)23个循环,以及72℃5分钟。每个PCR引物包含独特的 10bp多重标识符(Multiplex Identifier,MID)标签以及用于454 Sequencing™的衔接子序列 (5′-GCCTCCCTCGCGCCATCAG-MID-GCTACGTGGACGACACG-3′;5′- GCCTTGCCAGCCCGCTCAG-MID-TCGCTCTGGTTGTAGTAGC-3′)。所得的扩增子跨越外显子2中190bp的高度多态性区域。使用AMPure XP磁珠(Beckman Coulter Genomics)纯化初级cDNA-PCR产物。根据制造商的说明书,用Genome Sequencer FLX仪器(Roche/454Life Sciences)进行乳化PCR(emulsion PCR)和焦磷酸测序程序。使用Labkey 数据库并结合**Geneious-Pro**®生物信息学软件(Biomatters Ltd.)进行数据分析以进行序列组装。

[0176] RhCMV/SIV载体:先前已经详细描述了来自于毒株68-1的 RhCMV/SIV的构建、表征和施用(Hansen等.(2009),同上;Hansen等. (2011),同上;Hansen等.Nature(2013),同上;Hansen等.Science(2013),同上;Hansen等.(2010),同上)。本研究中使用的所有重组病毒均来自于毒株RhCMV 68-1BAC。由于组织培养适应,RhCMV 68-1构建体含有分别编码HCMV UL128和UL130的同源物的ORF 157.5和大部分ORF Rh157.4的缺失(Hansen,S.G.等,J Virol 77,6620(2003);其通过引用并入本文)。

[0177] 为了产生缺少UL40表达的载体,通过BAC重组使ORF Rh67从 RhCMV 68-1中缺失。简言之,通过同源重组用含有FRT-侧翼的卡那霉素抗性基因的PCR片段代替Rh67,然后使用FLP重组酶切除KanR基因。在恒河猴成纤维细胞中回收病毒,并表征抗原表达和Rh67(UL40)损失。

[0178] 为了产生具有完整UL128-130表达的载体,将SIVgag表达盒插入到 RhCMV 68-1.2的Rh211中,这是其中Rh61/Rh60(UL36)、Rh157.4 (UL130)和Rh157.5(UL128)已被修复的重组病毒(A.E.Lilja和T. Shenk,Proc Natl Acad Sci U.S.A.105,19950(2008);其通过引用并入本文)。通过限制性消化表征和证实所有的重组病毒,并对抗原插入片段(包括其侧翼区)进行序列验证。通过免疫印迹证实SIV抗原的表达。另外,通过RT-PCR验证临近基因表达。

[0179] 其他疫苗

[0180] 通过在MH5(一种早期/晚期痘苗启动子)的控制下将密码子优化的全长SIVmac239gag基因插入MVA穿梭载体pLW44中来构建MVA/gag,以产生重组质粒pJV7。pLW44内的侧翼序列通过同源重组将重组构建体定向插入胸苷激酶基因座。用pJV7转染鸡胚胎成纤维细胞,然后用MVA 毒株1974感染以产生表达SIVmac239gag的重组病毒(通过免疫印迹证实SIVgag表达)。对重组病毒进行噬斑纯化(plaque-purified),并在大规模培养中扩增。将病毒原液通过24-40%蔗糖梯度纯化,然后通过36%蔗糖垫沉淀,然后将沉淀悬浮于pH 9.0的1mM Tris-Cl中。对于MVA/gag 疫苗接种,通过肌内注射向RM施用$10^8$噬斑形成单位的这种载体。

[0181]　　抗原和抗原呈递细胞：从NIH AIDS Reagent Program获得包含 SIVgag蛋白的连续15聚体肽(重叠11个氨基酸)。通过Genscript (Piscataway,NJ)进行这些蛋白质中特异性9-14聚体肽的合成。所有肽通过其包含的氨基酸从n末端的位置来标识(例如,Gag$_{xx-yy}$)。连续的 15聚体也由其从n末端15聚体开始的位置命名(例如,Gag$_{1-15}$是15mer #1；Gag$_{5-19}$是15mer#2等) 来指定。除非另有说明,否则这些肽以2μg/ml 用于T细胞测定。如先前所述(Hansen等.Science(2013),同上),通过用狒狒疱疹病毒(Herpesvirus papio)感染恒河猴PBMC来产生自体B 淋巴母细胞样细胞系(BLCL)。通过将每个等位基因连接到pCEP4 KpnI/NotI或HindIII/NotI限制性位点来产生Mamu I类分子的哺乳动物表达载体。将质粒克隆到DH5α大肠杆菌(Life Technologies,Grand Island， NY)中,确认序列,并使用Nucleofector II/Kit C(Lonza,Allendale NJ) 电穿孔到MHC-I阴性K562、721.221或RMA-S中(K.S.Anderson等,J Immunol 151,3407(1993)；其通过引用并入本文)细胞。将转染子维持药物选择(潮霉素B),并通过用泛MHC-I抗体克隆W6/32染色来常规地证实MHC-I的表面表达。在T细胞测定的整个使用中,使用AllPrep DNA/RNA Mini Kit(Qiagen)提取来自MHC-I转染子的mRNA,使用在外显子2内的高度多态性区域侧翼的引物对通过RT-PCR扩增,并确认序列。将MHC-I转染子和BLCL用终浓度为10μM的目标Gag肽脉冲90分钟,然后用温热PBS洗涤三次,并且用温热的R10洗涤一次以除去未结合的肽,然后与新鲜分离的PBMC以10:1的效应物:靶标比例组合。为了稳定Mamu-E表面表达,将Mamu-E转染子在用于测定前在 27℃下孵育3小时,并在肽孵育期间保持在27℃,直到与效应物组合。如下产生自体SIV感染的靶细胞:用CD4微珠和LS柱(Miltenyi Biotec) 从PBMC中分离CD4$^+$ T细胞,用IL-2(供应商)、葡萄球菌肠毒素B(供应商)以及抗CD3(NHP Reagent Resource)、抗CD28和抗CD49d mAb (BD Biosciences)的组合活化,用蔗糖纯化的SIVmac239进行离心接种 (spinoculation),然后培养3至4天。在用于T细胞测定之前,如先前所述使用CD4微珠和LS柱(Miltenyi Biotec)纯化SIV感染的靶细胞(J．B.Sacha等,J Immunol 178,2746(2007)；其通过引用并入本文)。感染的细胞制备物在富集后为＞95％的CD4$^+$ T细胞和＞50％的SIV感染,并以 40:1(PBMC和分离的CD8$^+$ T细胞)或8:1(T细胞系效应物)的效应物:靶标比例使用。在这些实验中,未感染的活化的CD4$^+$ T细胞用作阴性对照APC(来自SIV$^+$RM的未感染的靶标用替诺福韦(NIH AIDS Reagent Program,浓缩) 培养。为了评估本体MHC-I和MHC-E,如上所述产生SIV感染的CD4$^+$ T细胞但是不进行感染后纯化,并对表面 MHC-I(克隆W6/32)、MHC-E(克隆4D12;抗小鼠IgG1 M1-14D12)、CD3、CD4和细胞内SIV Gag p27衣壳进行染色。

[0182]　　T细胞测定:用Ficoll-Paque(GE Healthcare)从血液获得用于免疫测定的单个核细胞制备物。使用CD8微珠和LS柱(Miltenyi Biotec)从 PBMC获得纯化的CD8$^+$ T细胞(＞90％纯度)。通过用经辐照、经肽脉冲的BLCL刺激PBMC并随后在含有IL-2(供应商)的培养基中培养,每周进行重复刺激来制备表位特异性T细胞系。通过流式细胞术ICS测量SIV特异性CD8$^+$ T细胞应答。简言之,将效应T细胞(单个核细胞、分离的CD8$^+$ T细胞或T细胞系)与抗原(肽、经肽脉冲的APC或经SIV 感染的CD4$^+$ T细胞)和CD28和CD49d的共刺激单克隆抗体(mAb)(BD Biosciences)孵育1小时,然后添加布雷菲德菌素A(Brefeldin A) (Sigma-Aldrich)再孵育8小时。不存在抗原(无肽、未脉冲的APC或未感染的靶标)的共刺激作为背景对照。在使用MHC-I转染子作为APC 的限制性测定中,在经肽脉冲的MHC-I阴性亲本细胞系K562或721.221 细胞的存在下的共刺激作为另外的阴性对照。当指明时,将单个核细胞

或抗原呈递细胞与以下阻断试剂一起预孵育1小时：抗MHC-I mAb(克隆 W6/32；10μg/ml)，CLIP肽(MHC-II相关恒定链，氨基酸89-100；2 μg/ml)，MHC-E结合肽VL9(VMAPRTLLL；20μM)，Mamu-A1＊001：01 结合肽CM9(CTPYDINQM；20μM) 或Mamu-A1＊002：01结合肽GY9(GSENLKSLY；20μM)。如先前所述将经刺激的细胞固定、透化并染色(Sacha等，The Journal of Immunology，178，2746-2754(2007)；其通过引用并入本文)，并且在LSR-II仪(BD Biosciences)上进行流式细胞术分析。使用FlowJo软件(Tree Star)进行分析，首先在小淋巴细胞上设门，然后在CD3$^+$、随后CD4$^-$/CD8a$^+$ T细胞亚群上进行递进设门 (progressive gating)。由TNF-α和IFN-γ的细胞内表达确定所得 CD4$^-$/CD8a$^+$群的抗原特异性应答频率。对于表位解卷积(deconvolution) 实验，使用严格的应答标准来防止假阳性。在这些研究中，如果在至少2 个独立测定中，聚集为CD69$^+$、TNF-α$^+$和IFN-γ$^+$的事件频率＞0.05％，背景＜0.01，则认为对于给定的15聚体肽的应答为阳性的。图2A和6所示，将单个肽应答分类为被阻断是基于相对于同种型对照通过阻断＞90％的抑制。定义部分阻断。认为不符合这些标准的应答是不确定的。单个肽应答必须已经(1)被抗MHC-I克隆W6/32和MHC-E结合肽VL9二者所阻断，并且(2)不被CLIP阻断，则认为被阻断MHC-E限制。

[0183]　　抗体

[0184]　　以下缀合的Ab用于这些研究：a)来自BD Biosciences，L200(CD4；AmCyan)、SP34-2(CD3；PacBlu)、SK1(CD8a；TruRed，AmCyan)、 25723.11(IFNg；APC，FITC)、6.7(TNF；APC)，b)来自Beckman Coulter，L78(CD69；PE)。

[0185]　　实施例2-在MHC-E情况下目标肽特异性CD8$^+$ T细胞的产生

[0186]　　在经典的多态性MHC-Ia分子情况下识别来自于抗原的目标肽的T 细胞受体可用于转染自体T细胞，以用于疾病(例如癌症或感染性疾病) 的免疫治疗。这种方法的主要障碍是人群中MHC-Ia的多样性，其将给定TCR的使用限制于MHC-Ia匹配的患者。通过产生在非经典、非多态性MHC-E分子情况下识别来自于抗原的目标肽(例如，来自于肿瘤抗原的肽和来自于病原体的肽)，MHC匹配变得过时，并且所得到TCR可用于所有患者。

[0187]　　在自然界中识别MHC-E/肽复合物的CD8$^+$ T细胞很稀少，并且目前还没有可靠的方法来产生针对目标抗原(例如肿瘤抗原、来自于病原体的抗原、组织特异性抗原或宿主自身抗原) 的此类T细胞。本文中描述的方法基于以下发现：缺少基因Rh157.5和Rh157.4(HCMV UL128和 UL130的同源物)的恒河猴巨细胞病毒(RhCMV)以蛋白质序列的每 30至40个氨基酸约1个肽表位的频率在恒河猴中引起MHC-E-限制性 CD8$^+$ T细胞。通过将目标抗原插入到UL128和UL130缺失的RhCMV 中，可以产生针对由MHC-E呈递的各肽的CD8$^+$ T细胞。识别MHC-E/ 肽的TCR可以通过许多方法中的任何一种来鉴定，但是通常依赖于通过 PCR从单细胞、克隆扩增的单细胞的cDNA对α和β链直接测序，或者 CD8$^+$ T细胞特异性肽库的深度测序。或者，可通过首先产生单细胞、克隆扩增的单细胞或CD8$^+$ T细胞特异性的肽库的全转录组文库来通过扩增 RNA模板间接产生所述序列。可以通过cDNA末端的快速扩增(rapid amplification of cDNA end，RACE) 或者在mRNA上进行的RNA模板 5′端的切换机制 (switching mechanism at 5′end of RNA template，SMART)方案来产生可变序列特异性的肽。锚定在侧翼恒定区域或类似地来自单个肽反应性CD8$^+$细胞的全转录组文库的PCR，可以对其各自的 TCR可变区直接测序或深度测序。可以进一步合成或克隆来自单个肽反应CD8$^+$ T细胞或肽反应CD8$^+$ T细胞库的TCR序列的α和β链的经验证组合。然后将所得的TCR构

建体转染到T细胞中,所述T细胞进而可作为治疗(例如,癌症治疗或感染性疾病治疗)施用于患者。克隆和转染 TCR可变区的方法还在Barsov EV等,PLoS One 6,e23703(2011)中讨论,其通过引用并入本文。

[0188]　　　实施例3-受主要组织相容性复合物E限制的广泛靶向的CD8⁺T细胞应答

[0189]　　　主要组织相容性复合物(MHC)-E是具有有限的多态性的高度保守、普遍存在的非经典MHC-Ib分子,其主要通过与NKG2/CD94受体相互作用来参与NK细胞反应性的调节。在这里,在所有测试的蛋白质抗原中,用Rh157.5/.4基因缺失的RhCMV载体致敏恒河猴独特地转移了MHC-E 功能,以将高度多样性肽表位呈递至CD8α/β⁺ T细胞,每100个氨基酸约4个不同的表位。由于MHC-E在HIV/SIV和其他持续性病毒感染的细胞上被上调以逃避NK细胞活性,MHC-E限制性CD8⁺ T细胞应答具有利用病原体免疫逃避适应的可能性,可能会使这些非常规应答非常有效的能力。

[0190]　　　针对细胞内病原体的适应性细胞免疫是识别被感染细胞表面上高度多态性MHC-Ia分子呈递的来自于短(8-10聚体)病原体之肽表位的CD8⁺ T细胞的主要责任(Neefjes J等,Nat Rev Immunol 11,823(2011)和 Nikolich-Zugich J等,Microbes Infect 6,501(2004);二者均通过引用并入本文)。MHC-Ia同质异型体在其肽结合性质方面差异很大,并因此由病原体特异性CD8⁺ T细胞靶向的特定的来自于病原体的肽主要由被感染个体表达的有限数目的MHC-Ia同质异型体的肽结合特异性决定 (Yewdell JW,Immunity 25,533(2006);其通过引用并入本文)。因此,被响应于相同病原体的CD8⁺ T细胞识别的表位在个体之间是高度多样的。这种识别异质性是重要的,因为由CD8⁺ T细胞应答靶向的表位的性质对于个体清除或控制多种细胞内病原体的能力可具有巨大的影响,特别是具有突变免疫逃避的高内在能力的病原体(如HIV)(Nikolich Zugich (2004),同上,以及Goulder,P.J.和Watkins,D.I.Nat Rev Immunol 8,619 (2008),其通过引用并入本文)。从进化的观点看,这种MHC-Ia多态性介导的应答多样性允许大群体幸免于在新出现的病原体,因为有很大可能性至少有一些群体成员具有支持有效的CD8⁺ T细胞应答的MHC-Ia同质异型体(Nikolich-Zugich(2004),同上和Prugnolle F等,Curr Biol 15,1022(2005);其通过引用并入本文)。另一方面,这种生物学不可避免地导致群体中的某些个体对给定的病原体高度敏感,即使当接种疫苗,这阻碍了基于CD8⁺ T细胞应答开发普遍有效的疫苗的努力(Goulder和 Watkins(2008),同上,以及Picker,LJ等,Ann Rev Med 63,95(2012);其通过引用并入本文)。

[0191]　　　最近报道,基于毒株68-1(成纤维细胞适应的)RhCMV的SIV靶向疫苗载体显著违背了MHC-Ia限制性CD8⁺ T细胞识别的上述原则 (Hansen等,Science(2013),同上),并提供了在CD8⁺ T细胞靶向疫苗接种中MHC-Ia依赖性应答多样性的潜在解决方案。在恒河猴中,RhCMV/SIV载体对高度致病性SIV攻击提供了强烈的保护,导致了严格控制和最终清除感染(Hansen等(2011),同上,以及Hansen等Nature (2013),同上)。尽管响应于表达相同SIV蛋白的常规疫苗3倍多的表位,但这些载体引起与常规MHC-Ia限制性CD8⁺ T细胞完全不重叠的 SIV特异性CD8⁺ T细胞应答。这种缺少表位重叠一部分由以下发现解释:许多这些表位受MHC-II分子而非MHC-Ia的限制,后者是CD8⁺ T细胞的罕见但并非前所未有的表位识别模式(Hansen等,Science(2013),同上)。毒株68-1 RhCMV/SIVgag载体还引起识别多种MHC-I依赖性表位的CD8⁺ T细胞(例如,被抗MHC-I抗体完全阻断的应答),所述表位是大部分或其

至所有MHC不同的恒河猴中常见的,MHC-Ia限制性CD8$^+$ T 细胞应答的前所未有的交叉识别程度。事实上,在先前的报道(Hansen 等,Science(2013),同上)中,42只经毒株68-1 RhCMV/SIVgag载体免疫接种的猴中的42只靶向了SIV gag蛋白中的两个表位(SIVgag$_{276-284}$和SIVgag$_{482-490}$),并且对于这两种9聚体表位的CD8$^+$应答已经在用这种载体接种的120只猴中的120只中有记录(图14)。

[0192]　　为了理解这种非常普遍的MHC-I依赖性识别的基础,选择4只经毒株68-1 RhCMV/SIVgag载体疫苗接种的猴进行详细的MHC-I限制性分析。这些恒河猴显示出对SIVgag的稳健、非常规的MHC-I依赖性CD8$^+$ T细胞应答,包括对SIVgag$_{276-284}$和SIVgag$_{482-490}$超表位的应答以及10种其他常见应答。在每只猴子中对表达的MHC-I基因(经典MHC-Ia和非经典MHC-Ib二者)(Wiseman,R.W.等,Nat Med 15,1322(2009);其通过引用并入本文)进行测序,分别构建单一表达这些MHC-I分子的MHC-I转染子组(图15)。然后将这些单一MHC-I分子转染子用于流式细胞术细胞内细胞因子染色(ICS)测定,以将表位15聚体肽呈递给来自这些猴的68-1 RhCMV/SIVgag载体诱导的CD8$^+$ T细胞(分别使用亲本MHC-I阴性和自体B淋巴母细胞样细胞系作为阴性和阳性对照)(图 11A、11B和16)。显著地,经典MHC-Ia同质异型体能够仅向这些T细胞呈递12种表位肽中的3种(Mamu-A1＊001:01:SIVgag$_{69-83}$(18)和 SIVgag$_{197-211}$(50);Mamu-A1＊002:01:SIVgag$_{129-143}$(33)),并且猴中这些同质异型体的表达不追踪这些表位特异性CD8$^+$T细胞应答(例如,许多缺少这些同质异型体的猴仍然能够识别这3种肽;图17)。然而,当由非经典MHC-E分子呈递时,所有12种表位肽刺激来自所有猴的CD8$^+$ T细胞,并且实际上,所有肽被表达3种不同恒河猴MHC-E同质异型体的转染子(Mamu-E02:04、Mamu-E02:11和Mamu-E02:20)呈递,而不论应答是源于表达这些等位基因的猴以及表达人形式的该分子的转染子 (HLA-E＊01:03)(图11A、11B、16和18)。

[0193]　　已知MHC-E强烈地结合典型VMAPRTL(LVI)L肽和来自于 MHC-Ia前导序列的位置3-11的其他紧密相关的9聚体肽,用于呈递至 NK细胞上的NKG2A(和在较少程度上为NKG2C)分子(Lee,N.等,J Immunol 160,4951(1998);Braud,V.M.等,Nature 391,795(1998);Sullivan,L.C.等,Tissue Antigens 72,415(2008);以及van Hall,T.等, Microbes Infect 12,910(2010);其全部通过引用并入本文)。当细胞表达正常水平的MHC-Ia时,这种高度保守的相互作用向NK细胞递送主要抑制性信号。然而,在通过病毒感染或肿瘤转化干扰MHC-Ia生物合成后,这种抑制信号降低,促使NK细胞响应于病毒感染的细胞或肿瘤细胞而活化(Lodoen,M.B.和Lanier,L.L Nat Rev Microbiol 3,59(2005),以及Wieten L等,Tissue Antigens 84,523(2014);二者均通过引用并入本文)。尽管CD8$^+$细胞的亚群也可表达NKG2A和/或NKG2C(Arlettaz L等,Eur J Immunol 34,3456(2004);其通过引用并入本文),MHC-E依赖性毒株68-1 RhCMV/SIVgag载体引起的CD8$^+$ T细胞的表型分析显示,绝大多数应答细胞是缺少NKG2A和NKG2C二者表达的CD8α/β$^+$、TCR γ/δ$^-$T细胞(图11C和19)。此外,在特异性肽加载之前,MHC-E转染子或PBMC与典型MHC-E结合的VMAPRTLLL(VL9) 肽的预孵育特异性阻断所有12种肽的CD8$^+$ T细胞识别(图11D和20),这表明这些肽的T细胞识别不是由与加载肽的MHC-E结合的NKG2A/C介导的,而是反映MHC-E限制性表位呈递至抗原特异性T细胞。实际上,所研究的每一种亲本15聚体可被截短至相对于亲本15聚体在经不同毒株68-1 RhCMV/SIVgag载体疫苗接种的猴中常见的最佳9聚体肽(图21) (Hansen等,Science(2013),同上)。当以小于1nM的剂量对Mamu-E 转染子脉冲时,这些最佳9聚体可以从这些猴

中触发CD8<sup>+</sup> T细胞(图22),其功能性活性与经典MHC-Ia限制性表位的T细胞识别相当(O'Connor DH等,Nat Med 8,493(2002);其通过引用并入本文)。总之,这些数据强烈地表明,由毒株68-1 RhCMV/SIVgag载体引起的非常规的MHC-I 依赖性CD8<sup>+</sup> T细胞是主要受MHC-E限制的SIVgag特异性CD8<sup>+</sup> T细胞,但是在某些情况下也可识别常规MHC-Ia同质异型体上的其特异性肽。

[0194]    先前已经在HCMV、丙型肝炎病毒、结核分枝杆菌和肠沙门菌 (Salmonella enterica)感染中鉴定了MHC-E限制性CD8<sup>+</sup> T细胞应答,所述应答通常涉及与典型MHC-Ia前导序列肽结构相关但是对于宿主是外源的表位(Sullivan(2008),同上;van Hall(2010),同上;Pietra G等, J Biomed Biotechnol 2010,907092(2010);以及Caccamo N等,Eur J Immunol 45,1069(2015);其均通过引用并入本文)。为了确定在不同环境中MHC-E限制对SIVgag的应答的程度,使用高亲和力MHC-E结合肽VL9的阻断(与抗MHC-II CLlP肽和抗MHC I mAb W6/32的阻断结合)对用毒株68-1 RhCMV/SIVgag载体(Rh157.5/.4基因缺失)、毒株68-1.2 RhCMV/SIVgag载体(Rh157.5/.4-未受损)、ΔRh157.5/.4毒株68-1.2 RhCMV/SIVgag载体(其中Rh157.5/.4基因被特异性重新缺失;图23) 和改良牛痘安卡拉(MVA)/SIVgag载体疫苗接种的猴以及用SIV本身感染的猴(图12、24和25)中所有SIVgag表位特异性CD8<sup>+</sup> T细胞应答进行限制性分类。该分析显示,经毒株68-1 RhCMV/SIVgag载体和ΔRh157.5/.4毒株68-1.2 RhCMV/SIVgag载体疫苗接种的猴中基本上所有 SIVgag表位特异性应答被CLIP肽或者被抗MHC-I mAb W6/32和VL9 肽二者阻断>90%,证明由Rh157.5/.4缺陷的RhCMV引起的非常规T 细胞应答有效地完全为MHC-II或MHC-E限制性CD8<sup>+</sup> T细胞。

[0195]    相比之下,经MVA/SIVgag载体疫苗接种的和毒株68-1.2(表达 Rh157.5/.4)RhCMV/SIVgag载体疫苗接种的恒河猴中的SIVgag特异性 CD8<sup>+</sup> T细胞应答被mAb W6/32而非VL9肽所阻断,表明经典的MHC-Ia 限制性。对于经SIV感染的恒河猴中98%的CD8<sup>+</sup> T细胞应答也是如此,4种MHC-II限制性CD8<sup>+</sup> T细胞应答除外。Rh157.5/.4缺陷的RhCMV 载体引起MHC-E和MHC-II限制性CD8<sup>+</sup> T细胞的能力不限于SIVgag 特异性应答。利用编码SIVpo197-441、结核分枝杆菌蛋白质(Ag85B、ESAT6和RpfA)以及内在的RhCMV蛋白(例如立即早期1(IE1)蛋白)的毒株68-1(Rh157.5/.4缺陷)RhCMV载体观察到了MHC-E和 MHC-I限制性抗原特异性CD8<sup>+</sup> T细胞应答的类似混合物(图12B和26)。

[0196]    先前已经报道,由毒株68-1 RhCMV/SIV载体引起的CD8<sup>+</sup> T细胞识别自体SIV感染的CD4<sup>+</sup> T细胞,并且该识别被抗MHC-I mAb W6/32和 MHC-II阻断CLIP肽部分地阻断(Hansen等,Science(2013),同上)。为了确定MHC-E限制性对该识别的MHC-I组分的贡献,想知道高亲和力MHC-E结合VL9肽是否可代替mAb W6/32阻断这些应答。该实验表明,MHC-II阻断CLIP肽和mAb W6/32或VL9肽的组合完全阻断了这些应答,而通过由MVA/SIVgag载体或毒株68-1.2 RhCMV/gag载体疫苗接种或SIV感染引起的SIVgag特异性CD8<sup>+</sup> T细胞的经SIV感染的自体细胞识别对CLIP+VL9肽组合不敏感(图12)。总之,这些数据证实了毒株68-1 RhCMV载体独特地引起了MHC-II或MHC-E限制性的CD8<sup>+</sup> T细胞应答,并且这种不常见免疫生物学是RhCMV Rh157.5/.4基因缺失的特异性结果,所述基因是HCMV UL128/UL130基因的直系同源物并且编码参与非成纤维细胞的CMV感染的五聚体受体复合物的2种成分 (Lilja AE和Shenk T,Proc Natl Acad Sci U.S.A.105,19950(2008);其通过引用并入本文)。此外,这些数据证实,由这些MHC-E限制性CD8<sup>+</sup> T 细胞识别的至少一些表位天然地由被SIV

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(异源(非CMV)病原体)感染的细胞加工和呈递。

[0197]　　在42只经毒株68-1 RhCMV/SIVgag载体疫苗接种的猴中,确定了每只动物20个不同的CD8⁺ T细胞识别的MHC-E限制性SIVgag 15聚体表位的中位值,其宽度超过了分别通过常规疫苗或SIV感染引起的 SIVgag特异性CD8⁺ T细胞应答中确定的中位值11和14.5个不同的 MHC-1a限制性SlVgag特异性表位(图13A)。在所有毒株68-1 RhCMV 载体引起的CD8⁺ T细胞应答中,MHC-E限制性表位的密度(每100个氨基酸的蛋白质长度约4个独立的MHC-E限制性表位) 类似,而不论所分析的抗原的性质(图13B)。特别地,在相同的42只经68-1 RhCMV/SIVgag载体疫苗接种的恒河猴中,125个重叠的SIVgag 15聚体肽中的109个(87%)被至少一只恒河猴中的MHC-E限制性CD8⁺ T细胞识别(图13C)。尽管先前已经表明MHC-E比典型前导序列肽结合更广泛的肽(van Hall(2010),同上,以及Lampen等,同上),但是所观察到的表位多样性的程度和宽度非常出人意料,尤其是考虑到MHC-E的有限多态性以及观察到迄今测试的所有MHC-E限制性表位的呈递独立于这种有限的序列多态性以及Mamu-E与HLA-E之间的序列差异(图 11B、18和22)。这些数据表明,MHC-E介导的表位呈递(例如,MHC-E 肽结合)比以前所认为的甚至更加多样化。与此一致,11种最佳MHC-E 限制性SIVgag 9聚体表位的序列分析显示仅一个表位(Gag$_{273-287}$超表位) 具有典型(位置2处M:位置9处L)的MHC-E结合基序,而其余10 种最佳表位不仅缺少该基序,而且与以前表征的MHC-E结合肽组(Lampen等,同上)没有统计学显著的重叠(图13D)。实际上,另一个SIVgag$_{482-490}$超表位显示出被认为是抗MHC-E肽结合基序,在位置2 和9两处具有赖氨酸(图13D)。表位肽加载和结合MHC-E的分子机制在以下中讨论:Hansen,S.G.等,"Broadly targeted CD8+ T cell responses restricted by major histocompatibility complex E",Science,2016年1月21日电子公开,其通过引用并入本文。

[0198]　　HCMV和RhCMV二者分别编码具有在UL40和Rh67基因内策略性嵌入的典型VL9肽的蛋白质(Prod'homme,V.等,J Immunol 188,2794 (2012)和Richards,R.等,J Virol 85,8766(2011);二者均通过引用并入本文)。显示UL40的VL9肽通过TAP非依赖性机制加载于新生的MHC-E 链上,并因此在病毒介导的TAP抑制和HCMV US2-11基因产物介导的突出的MHC-Ia下调的情况下,发挥稳定并且上调经HCMV感染的细胞中的MHC-E表达的作用(Lodoen&Lanier(2005),同上,以及 Prod'homme(2012),同上)。RhCMV Rh67也具有类似的功能(Richards (2011),同上)。因此,认为MHC-E上调是逃避NK细胞对缺少MHC-Ia 表达之感染细胞的应答的关键病毒策略。然而,这种逃避策略将具有增强病毒感染的细胞中MHC-E表达的结果,这提高了向MHC-E限制性T 细胞加载和呈递新肽的机会。在这方面,典型MHC-E结合VL9肽可作为促进MHC-E的稳定高表达和递送至可促进肽交换的内体区室的分子伴侣(chaperone),类似于恒定链相关的CLIP肽和MHC-II。与这种肽交换机制一致,已经在结核分枝杆菌吞噬溶酶体中直接证明了MHC-E肽加载(Grotzke JE等,PLoS Pathog 5,el000374 (2009);其通过引用并入本文)。

[0199]　　CMV不是唯一上调MHC-E表达的细胞内病原体。丙型肝炎也编码上调MHC-E表达的MHC-E结合肽(Natterman J等,Am J Pathol 166,443(2005);其通过引用并入本文),并且HIV和SIV二者通过与MHC-Ia 下调一致的未表征机制上调MHC-E(Natterman J等,Antivir Ther 10,95(2005);其通过引用并入本文)(图27)。这种常见的适应性表明,对于这些和可能的其他细胞内病原体,上调MHC-E以抵抗NK细胞应答的进化压力超过了对MHC-E限制性

CD8$^+$ T细胞提高的敏感性的潜在风险，可能是因为MHC-E限制性CD8$^+$ T细胞在这些病原体感染期间致敏较差。MHC-E限制性CD8$^+$ T细胞应答是现在哺乳动物免疫系统的如此小成分的原因不清楚，尤其是鉴于本报道中发现这种应答可能非常多样且广泛（尽管在群体水平上多样性和广泛性可能低于多态性MHC-Ia；图 28）。然而，Rh157.5/.4基因缺失的RhCMV载体能够绕过MHC-E限制性CD8$^+$ T细胞致敏的内在约束。尽管实现这种绕过的机制仍有待阐明，但这些载体强烈地引起广泛、多样且MHC-Ia单体型依赖性CD8$^+$ T细胞应答的能力为开发利用MHC-E上调的MHC-E限制性CD8$^+$ T细胞靶向疫苗提供了机会，这是许多高度适应性持久性病原体的免疫逃避策略的固有弱点。此外，由于有限的MHC-E多态性，MHC-E限制性CD8$^+$ T细胞应答靶向疫苗将在所有或大多数疫苗接种者中引起大致类似的应答，潜在地为所有个体提供效力，而不管MHC基因型如何。进化可能具有不利的MHC-E作为现代哺乳动物中CD8$^+$ T细胞的主要限制性分子代替多态性MHC-Ia系统，但是如果HCMV载体能够在人中概括恒河猴中 Rh157.5/.4基因缺失的HCMV载体的生物学（或者如果作为替代地，可以开发基于非CMV的策略来引起广泛靶向的MHC-E限制性CD8$^+$ T细胞应答），疫苗学家可以能够复活这种休眠的基于MHC-E的适应性免疫系统以利用病原体尚未适应于有效逃避的新免疫应答攻击病原体。

[0200]　材料和方法：

[0201]　疫苗：1) 表达SIV Gag和5′-Pol的毒株68-1 RhCMV载体，2) 表达SIV Gag的毒株68-1.2 RhCMV载体，3) 表达SIV Gag的MVA和腺病毒5(Ad5) 载体，以及4) SIV Gag编码DNA+IL-12疫苗的构建、表征和施用先前已经报道（Hansen等，Science(2013)，同上；Hansen 等(2011)，同上；Hansen等Nature(2013)，同上；以及Hansen等(2009)，同上）。表达由MCMV IE启动子驱动并插入在Rh211的5′区域的结核分枝杆菌基因产物RpfA、RpfC和RpfD的融合蛋白的毒株RhCMV 68-1 由Aeras(Rockville,MD,USA)提供。还通过同源重组构建了基于毒株68-1.2 RhCMV/gag的Rh157.5(UL128)-Re157(UL130) 双缺失突变体。为了实现这一目的，设计靶区域侧翼的重组引物（正向诱变引物 5′-AAAACTATAATCAACAACTCTATACCTTTGTTTTGCTGATGCTA TTGCGT-3′和反向诱变引物5′-ATTTTTCGATAAAAAAATCACAGCAAACATACTG GTTTTACACACTTTAT-3′）。由于在RhCMV中Rh157.6(UL131A) 和Rh157.4 (UL130) 开放阅读框(ORF) 重叠，因此以保留Rh157.6(UL131A) ORF的末端加另外50bp的方式构建缺失以确保所编码蛋白质的表达。使用正向引物结合位点(5′-GAAAAGTGCCACCTGCAGAT-3′) 和反向引物结合位点(5′-CAGGAACACTTAACGGCTGA-3′)，将小型质粒R6K-kan-F5用于扩增侧翼为替代的(F5)FRT位点（其将添加到诱变引物的3′末端）的卡那霉素抗性盒。如别处公布的(Muyrers JP等,Nucleic Acids Res 27，1555(1999)；其通过引用并入本文)，进行大肠杆菌菌株SW105内的E/T 同源重组(Warming S等,Nucleic Acids Res 33,e36(2005)；其通过引用并入本文)。通过用缺失基因和相邻基因特异性的引物对病毒DNA和被感染细胞的cDNA进行聚合酶链反应来证实靶ORF的成功缺失。通过用 ΔRh157.5(UL128)-Rh57.4(UL130)68-1.2 RhCMV/gag载体感染的原代恒河猴成纤维细胞的免疫印迹分析证实SIVmac239gag转基因的表达。参见图23，描绘了毒株68-1、68-1.2和ΔRh157.5/Rh157.4(ΔUL128/UL130) RhCMV载体之间的基因组差异。

[0202]　恒河猴：在本实施例中报道的实验中，使用总计207只专用饲养的印度遗传背景的雄性或雌性恒河猴(Macaca mulatto)，其中88只还在先前的报道中进行了研究(Hansen等，

Science(2013)，同上)。这些恒河猴包括：用表达SIVgag、SIV5′-pol、TB-ESAT-6/Ag85B或TB-RpfA/RpfC/RpfD插入片段的毒株68-1 RhCMV载体疫苗接种的159 只恒河猴(62只先前有报道)；用毒株68-1.2 RhCMV/gag载体疫苗接种的9只恒河猴(6只先前有报道)；用Δ Rh157.5/.4缺失的毒株68-1.2 RhCMV/gag载体疫苗接种的4只恒河猴(先前没有报道)；分别用表达 SIVgag的MVA/gag、Ad5/gag和DNA/gag+IL-12疫苗接种的11、3和 4只恒河猴(分别是3只、全部和全部先前有报道)；具有受控的SIVmac239 感染的13只未疫苗接种的恒河猴(平台期血浆病毒载量＜10,000拷贝/ml；6只先前有报道)；以及用RhCMV的ONCMC集落循环毒株自然感染的 4只未疫苗接种的恒河猴(全部在前期有报道)。所有恒河猴经俄勒冈国家灵长类研究中心机构动物护理和使用委员会的批准，并按照美国国家卫生研究院实验动物护理和使用指南的标准使用。在这些实验中使用的恒河猴没有1型猕猴疱疹病毒、D型猿猴逆转录病毒和1型猿猴T淋巴细胞病毒。如所述(Wiseman(2009)，同上)，将选择的恒河猴通过深度测序进行MHC-I基因分型。简言之，通过使用高保真Phusion™聚合酶(New England Biolabs)和一对通用MHC-I特异性引物在以下热循环条件下通过PCR扩增cDNA来产生Mamu I类序列的扩增子：98℃3分钟，(98℃ 5秒，57℃1秒，72℃20秒)23个循环，以及72℃5分钟。每个PCR 引物包含独特的10bp多重标识符(MID)标签以及用于454Sequencing™的衔接子序列 (5′- GCCTCCCTCGCGCCATCAG-MID-GCTACGTGGACGACACG-3′；5′-GCCTTGCCAGCCCGCTCAG-MID- TCGCTCTGGTTGTAGTAGC-3′)。产生的扩增子跨越外显子2中190bp的高度多态性区域。使用AMPure XP磁珠(Beckman Coulter Genomics)纯化初级cDNA-PCR产物。根据制造商的说明书，用Genome Sequencer FLX仪器(Roche/454Life Sciences)进行乳化PCR和焦磷酸测序程序。使用Labkey数据库并结合**Geneious-Pro**®生物信息学软件(Biomatters Ltd.)进行数据分析以进行序列组装。

[0203]　　抗原和抗原呈递细胞：基于SIVmac239Gag和Pol序列(Genbank 登录号#M33262)、毒株68-1 RhCMV IE-1序列(Genbank登录号 #AY186194)或Erdman毒株结核分枝杆菌Ag85B、ESAT-6和RpfA序列(分别为Genbank登录号#BAL65871.1；BAL68013；和BAL64766.1)，通过Intavis AG进行这些蛋白质中包含SIVgag和pol、RhCMV IEI和 TB Ag85B、ESAT-6和RpfA蛋白的连续15聚体肽(重叠11个氨基酸) 以及特异性9-14聚体肽的合成。所有肽通过其包含的氨基酸从N末端的位置来标识(例如，Gag$_{xx-yy}$)。连续的15聚体也由其从N末端15聚体开始的位置命名(例如，Gag1-15(1)是15聚体#1；Gag5-19(2)是15聚体#2 等)来指定。除非另有说明，否则这些肽以2μg/ml用于T细胞测定。如先前所述(Hansen等.Science(2013)，同上)，通过用狒狒疱疹病毒感染恒河猴PBMC来产生自体B淋巴母细胞样细胞系(BLCL)。通过将每个等位基因连接到pCEP4KpnI/NotI或HindIII/NotI限制性(Ulbrecht M 等，J Immunol 164,5019(2000)；其通过引用并入本文)位点来产生Mamu I类分子的哺乳动物表达载体。将质粒克隆到DH5α大肠杆菌(E.coli)(Life Technologies)中，确认序列，并使用Nucleofector II/Kit C(Lonza)电穿孔至MHC-I阴性K562、721.221或RMA-S细胞中(Anderson KS等，J Immunol151,3407(1993)；其通过引用并入本文)。将转染子维持药物选择(潮霉素B)，并通过用泛MHC-I抗体克隆W6/32染色来常规地证实MHC-I的表面表达。在T细胞测定中的整个使用中，使用AIIPrep DNA/RNA Mini Kit(Qiagen)提取来自MHC-I转染子的mRNA，使用在外显子2内的高度多态性区域侧翼的引物对通过RT-PCR扩增，并确认序列。将MHC-I转染子和BLCL用终浓度为10μM的目标肽脉冲90 分钟，然后用温热PBS洗涤三

次,并且用具有10％胎牛血清的温热RPMI 1640培养基洗涤一次,以除去未结合的肽,然后与新鲜分离的PBMC以 10:1的效应物:靶标比例组合。为了稳定Mamu-E表面表达,将Mamu-E转染子在用于测定前在27℃下孵育3小时,并在整个肽孵育期间保持在 27℃,直到与效应物组合。如下产生自体SIV感染的靶细胞:用CD4微珠和LS柱(Miltenyi Biotec)从PBMC中分离CD4⁺ T细胞,用IL-2(NIH AIDS Reagent Program)、葡萄球菌肠毒素B(Toxin Technologies Inc.) 以及抗CD3(NHP Reagent Resource)、抗CD28和抗CD49d mAb(BD Biosciences)的组合激活,并用蔗糖纯化的SIVmac239进行离心接种,然后培养3至4天。在用于T细胞测定之前,如先前所述使用CD4微珠和LS柱(Miltenyi Biotec)纯化SIV感染的靶细胞(Sacha JB等,J Immunol 178,2746(2007);其通过引用并入本文)。被感染的细胞制备物在富集后为＞95％的CD4⁺ T细胞和＞50％的SIV感染,并以40:1(PBMC 和分离的CD8+ T细胞)或8:1(T细胞系效应物)的效应物:靶标比例使用。在这些实验中,未感染的活化的CD4⁺ T细胞用作阴性对照APC(来自SIV+RM的未感染的靶标用替诺福韦(NIHAIDS Reagent Program) 培养。为了评估总MHC-I和MHC-E表达,如上所述产生SIV感染的 CD4⁺ T细胞但不进行感染后纯化,并对表面MHC-I(克隆W6/32), MHC-E(克隆4D12;抗小鼠IgG1克隆M1-14D12)、CD3和CD4,随后细胞内SIV Gag进行染色。

[0204] 　　T细胞测定:如先前所述(Hansen等,Science(2013),同上),通过流式细胞术ICS测量来自血液的单个核细胞制备物中的SIV-、RhCMV- 和TB-特异性CD8⁺ T细胞应答。简言之,将单个核细胞或分离的CD8⁺ T 细胞与抗原(肽、经肽脉冲的BLCL或MHC-Ia或MHC-E转染子或SIV 感染的自体CD4⁺ T细胞)和共刺激分子CD28和CD49d(BD Biosciences) 一起孵育1小时,然后添加布雷菲德菌素A(Sigma-Aldrich)再孵育8 小时。没有抗原的共刺激作为主要背景对照。通过在以下阻断剂的存在下将分离的单个核细胞、抗原呈递细胞或SIV感染的CD4⁺细胞在室温下预孵育1小时(在添加肽或者将效应物和靶细胞组合和根据标准ICS测定孵育之前)来确定应答的MHC缔合(MHC-Ia、MHC-E、MHC-II):1) 泛抗MHC-I mAb W6/32 (10mg/m1),2)MHC-II阻断CLIP肽(MHC-II 相关恒定链,第89-100位氨基酸;20μM),和3)MHC-E阻断VL9肽 (VMAPRTLLL;20μM),单独或组合。在一些实验中,使用 Mamu-A1＊001:01结合肽CM9(CTPYDI NQM;20μM)或 Mamu-A1＊002:01结合肽GY9(GSEN LKSLY;20μM) 作为阻断对照。如先前所述(Hansen等Science(2013),同上)将经刺激的细胞固定、透化并染色,并在LSR-II仪器(BD Biosciences)上进行流式细胞术分析。使用FlowJo软件(Tree Star)进行分析。在所有分析中,在小淋巴细胞的光散射特征设门后之后在CD3⁺群体和随后的CD4⁺/CD8⁺ T细胞亚群上进行递进设门。由CD69以及TNF-α和IFN-γ 任一或二者的细胞内表达常规确定CD8⁺ T细胞群的抗原特异性应答频率。对于表位解卷积实验,使用严格的应答标准来防止假阳性。在这些研究中,如果在至少2个独立测定中,聚集为CD69⁺、TNF-α⁺和IFN-γ⁺的事件的频率＞0.05％,背景＜0.01,则认为对于给定的15聚体肽的应答为阳性的。将单个肽应答分类为被阻断的是基于相对于同种型对照阻断＞90％的抑制。认为不符合这些标准的应答是不确定的。单个肽应答必须被抗MHC-I克隆W6/32和 MHC-E结合肽VL9二者所阻断,并且不被CLIP阻断,则被认为是被阻断MHC-E限制。依据以下标准从通过测试连续15聚体肽鉴定的阳性应答中评估最小独立表位数:相同限制性类型的单一阳性肽＝1个独立表位;相同限制性类型的2个相邻阳性肽＝1个独立表位;相同限制性类型的3 个相邻阳性肽＝2个独立表位;相同限制性类型的4个相邻阳性肽＝2个独立表位;以及相同限制性类型的5个相

邻阳性肽＝3 个独立表位。

[0205]　　抗体：这些研究中使用了以下缀合的抗体：a)来自 BD Biosciences，L200(CD4；AmCyan)、SP34-2(CD3；PacBlu)、SK1(CD8a；TruRed，AmCyan)、25723.11(IFN-γ；APC、FITC)、6.7(TNF；APC)、MAb11 (TNF；Alexa700)，b)来自 Beckman Coulter，L78(CD69；PE)、2ST8.5H7 (CD8β；PE)、z199(NKG2A/C 或 CD159a/c；PE)，c)来自 Biolegend，W6/32(泛 MHC-I，PE)、OKT-4(CD4；PE-Cy7)、B1(TCRγ/δ；Alexa647)，d)来自 Miltenyi Biotec，M-T466(CD4；APC)，e)来自 eBiosciences，M1-14D12(小鼠 IgG1；PE-Cy7)。在这些研究中使用以下未缀合的抗体：a)来自 Advanced Bioscience Laboratories，4324(SIV Gag p27)，b)来自 LSBio，4D12(HLA-E)，c)W6/32(泛 MHC-I)。LIVE/DEAD 可固定的黄色死细胞染色(LIFE Technologies)用于评估细胞生存力。

[0206]　　表位序列分析：使用 Los Alamos HIV 数据库工具 Analyze Align (http://www.hiv.lanl.gov/content/sequence/ANALYZEALIGN/analyze_al ign.html)产生序列 LOGO，所述工具基于 WebLogo3{Crooks，2004#150}。使用 Composition Profiler Tool (http://cprofiler.org/cgi-bin/profiler.cgi) (Vacic V 等，BMC Bioinform 8，211 (2007)；其通过引用并入本文)计算在恒河猴中识别的 11 种最佳 MHC-E 9 聚体表位的以及来自 Lampen 等 (同上)公开的 TAP 缺陷细胞的 551 种 HLA-E 洗脱肽的每个位置中氨基酸的统计学富集和代表性不足。将 11 种最佳肽中每个位置的氨基酸组成与用于疫苗的插入毒株 SIVmac239Gag(GenBank 登录号#M33262) 中发现的氨基酸频率进行比较。为了将 11 种最佳肽的每个位置组成与先前公开的在 TAP 缺陷环境中从 HLA-E 洗脱的肽进行比较，使用 Lampen 等先前公开的全组 551 种洗脱肽。Lampen 等中的肽的长度不等，为 8 至 13 个氨基酸；9 是最常见的长度。他们已使用基序搜索算法来探索其洗脱组中的 315 种 9 聚体的氨基酸富集和代表性不足 (Lampen 等中图 2)，因为不论长度如何，位置 2 和 C 末端位置是最感兴趣的，采用略微不同的方法来探索其已公开的数据，并表征了其全部 551 种洗脱肽的比对形式。在位置 8 之后根据需要添加空位以维持比对，以实现包括所有肽在内的第二位置和经比对 C 端的评估。基于 SwissProt 51，对于每个比对位置将其数据与在天然蛋白质中发现的氨基酸频率进行比较 (Bairoch A 等，Nucleic Acids Res 33，D154 (2005)；其通过引用并入本文)。

[0207]　　基于经毒株 68-1 RhCMV 载体疫苗接种的恒河猴中被 CD8+ T 细胞识别的 11 种最佳 MHC-E 限制性 SIVgag 9 聚体肽表位，图 13D 中所示的序列 LOGO 通过字母高度指出了给定位置中每种氨基酸的频率 (相对于其在 SIVmac239Gag 中的背景频率)。根据在 Lampen 等的 TAP 缺陷环境中从 HLA-E 洗脱的 551 种肽的富集 (灰色填充框或阴影线框)或代表性不足 (白色填充框)，对图 13D 中的序列 LOGO 进行着色。如图 13D 的右图所示，551 种 Lampen 等的肽中在第 2 位和 C 末端锚定位置中富集的氨基酸在我们的 11 种最佳 SIVgag 肽中是罕见的，而显著代表性不足的那些被富集。

[0208]　　应理解，本文中描述的实施例和实施方案仅用于举例说明的目的，且本领域技术人员将提出鉴于它们的多种修改和改变，并且包括在本申请的精神和范围内。

[0209]　　本文中引用的所有出版物、专利、专利申请、互联网网站和登录号/ 数据库序列(包括多核苷酸和多肽序列二者)均通过引用整体并入本文用于所有目的，其程度如同每个单独的出版物、专利、专利申请、互联网网站或登录号/数据库序列被具体且单独地指出如此通过引用并入本文。
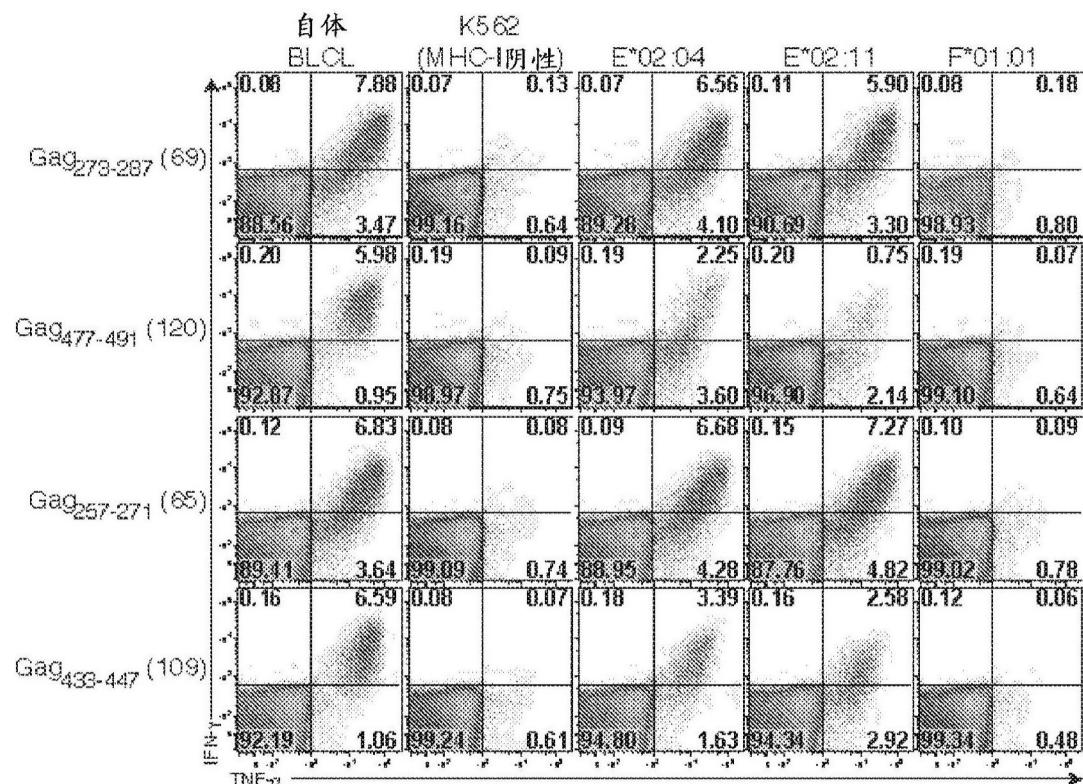
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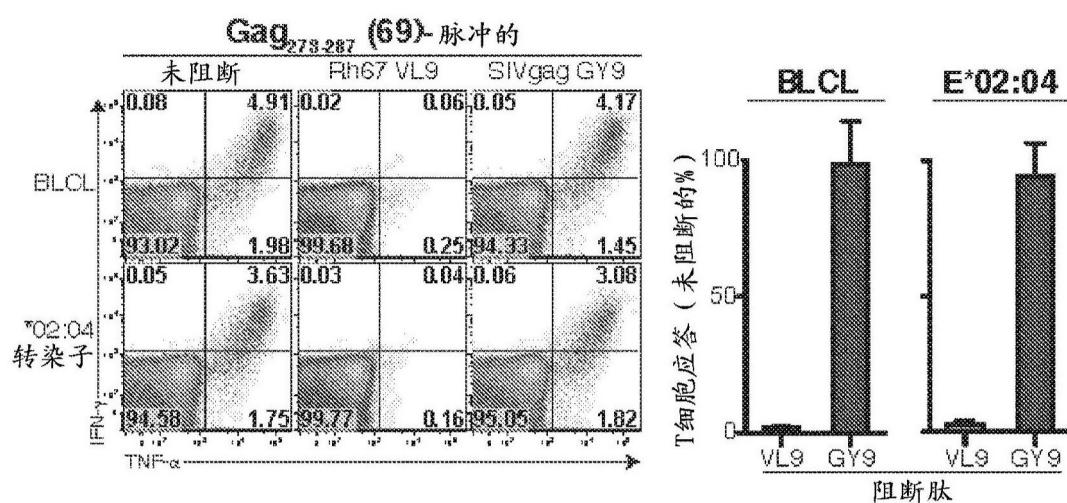


图1B
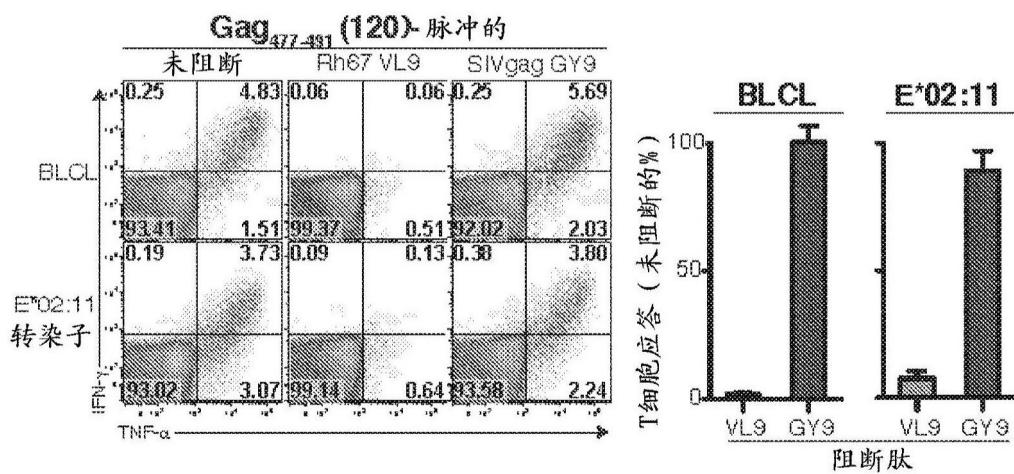
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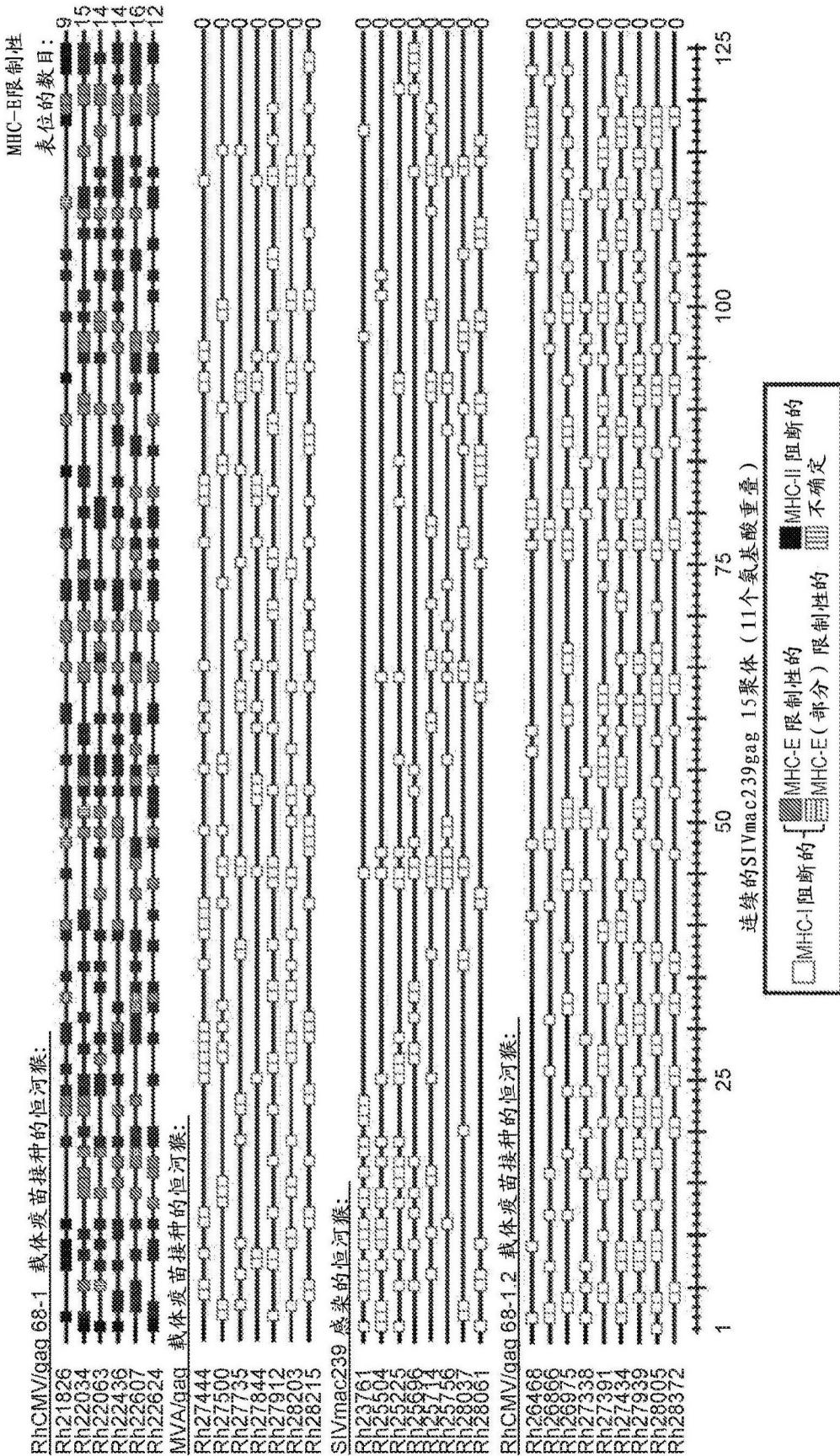
图2A
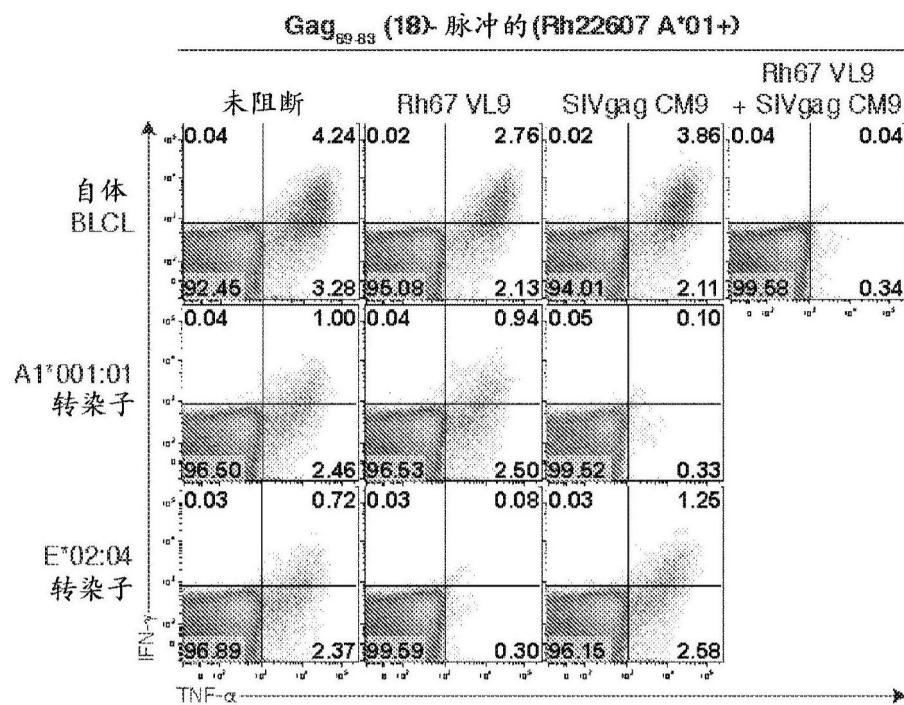
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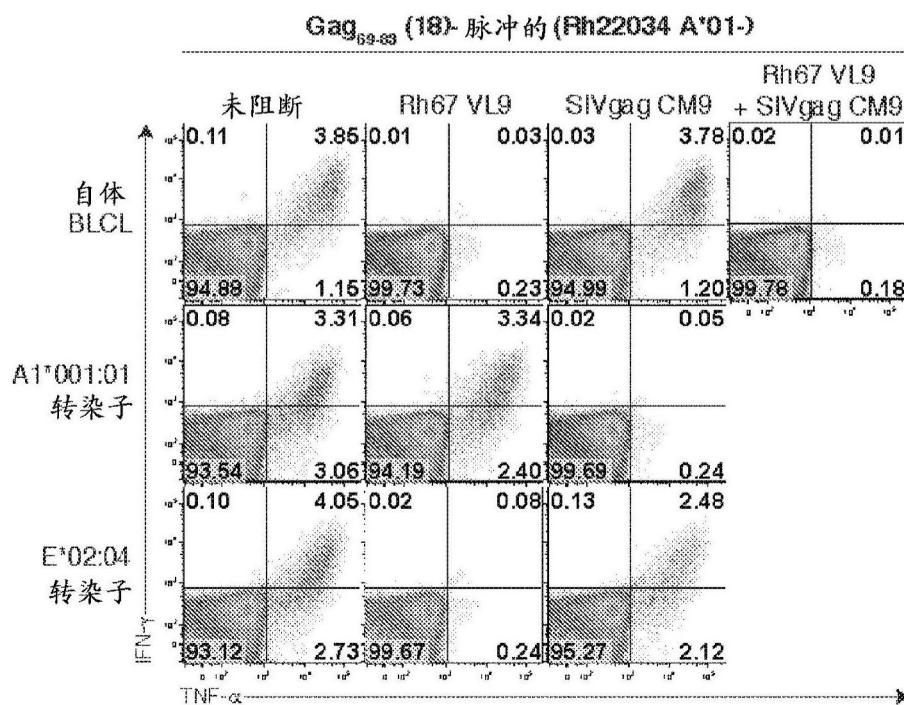


图2C
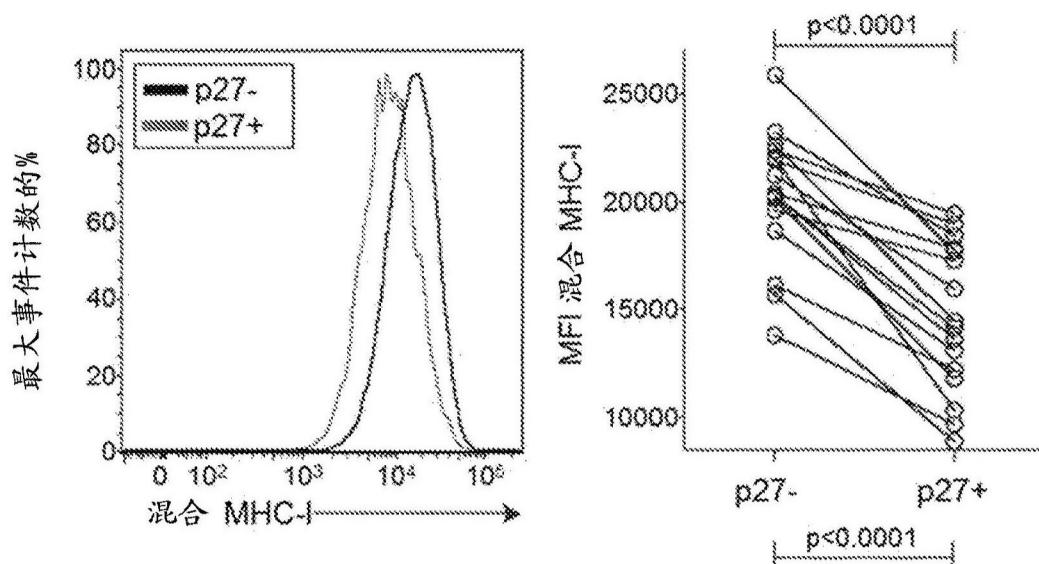
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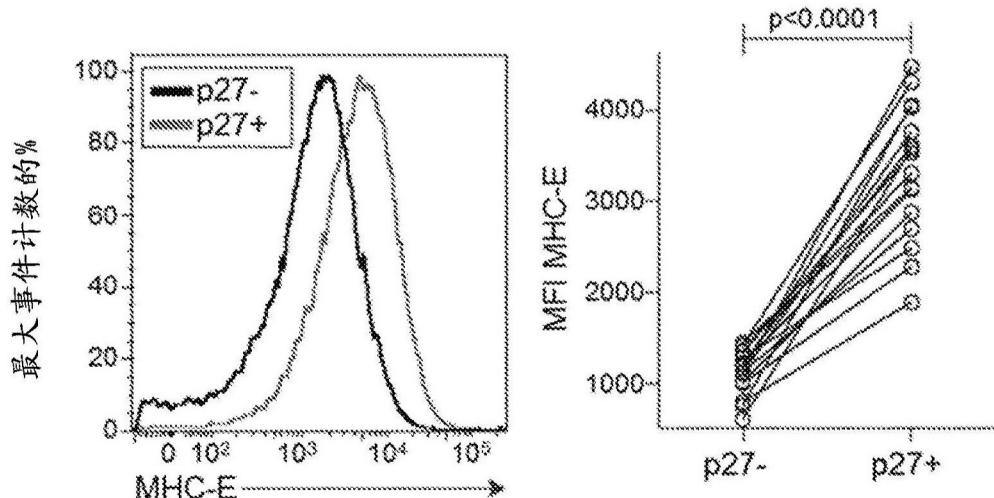


图3B
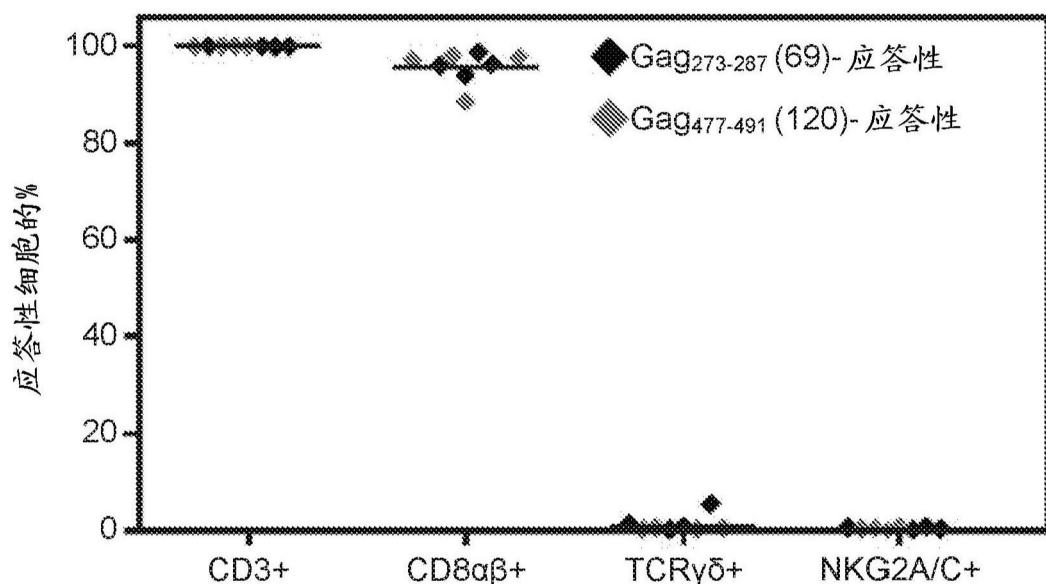
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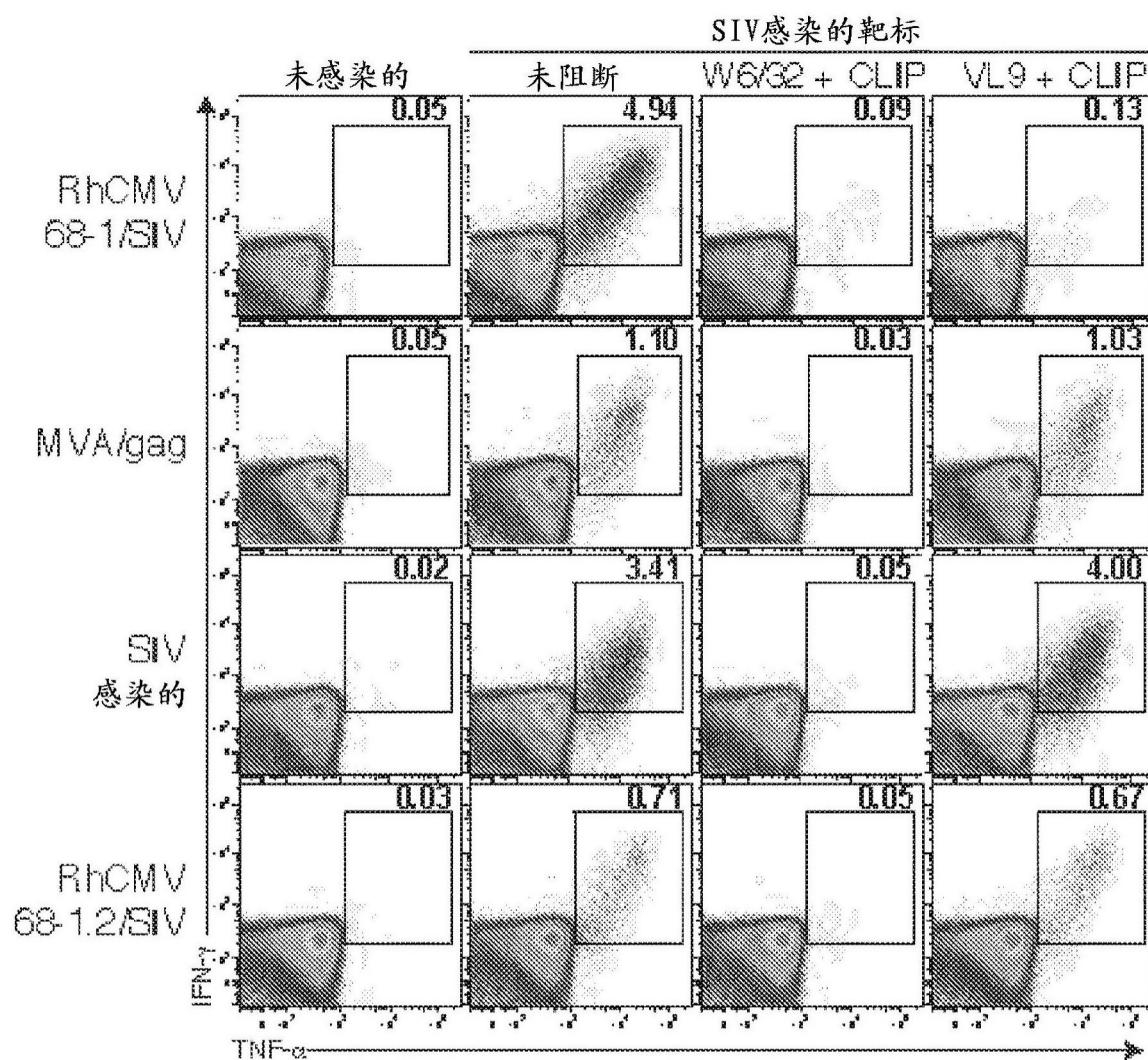
图4A
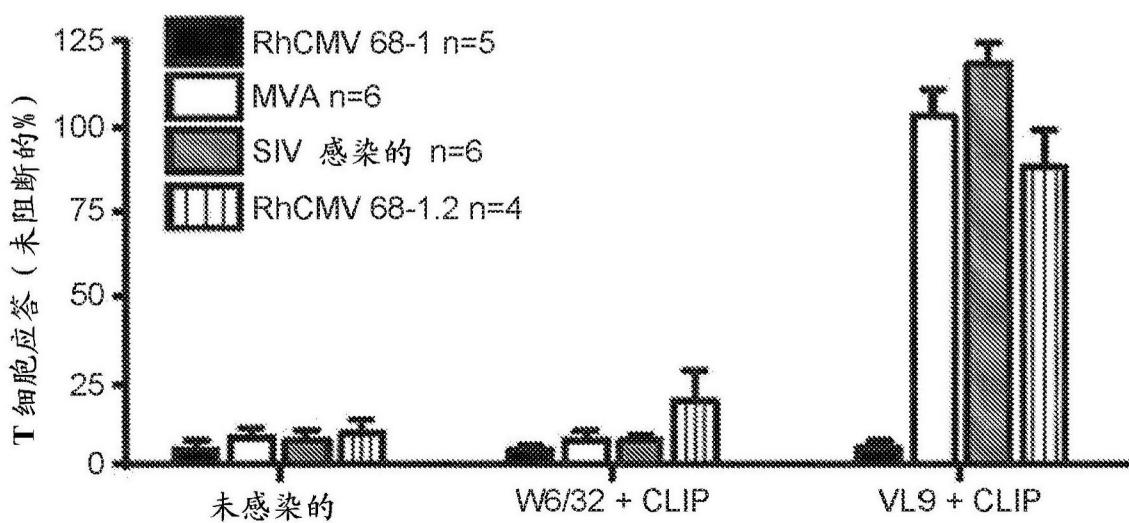


图4B
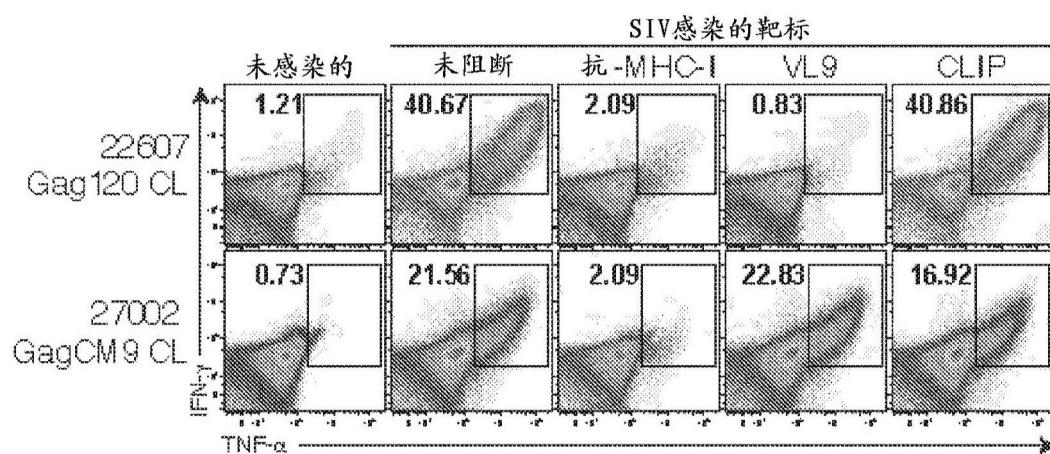
图4C

Rh67缺失；毒株68-1 RhCMV/gag（UL128/UL130缺失）疫苗：



图5

图6

图7A

| Mamu I类等位基因 | 恒河猴 | | | |
|---|---|---|---|---|
| | 21626 | 22034 | 22436 | 22607 |
| A1*001:01 | X | | | X |
| A1*002:01 | X | | X | X |
| A1*012:01 | | | X | |
| A1*023:01 | | X | | |
| A2*05:11; A2*05:28; A2*05:32:01; A2*05:32:02; A2*05:45 | | | X | |
| A2*05:04:01; A2*05:04:03; A2*05:10; A2*05:14 | | | | X |
| A3*13:02 | X | X | X | X |
| A4*14:03:01; A4*14:09 | | X | | |
| B*001:01:01 | | X | | X |
| B*007:02 | | X | | X |
| B*012:01 | X | | | X |
| B*017:01 | X | | | |
| B*021:01 | | X | | |
| B*022:01 | X | | | |
| B*028:01 | | X | | |
| B*029:01 | X | | | |
| B*030:01 | X | | | |
| B*030:05 | | X | | X |
| B*031:01 | X | | | |
| B*041:01 | | | X | |
| B*046:01:02 | | X | | |
| B*048:01 | | | X | |
| B*055:01 | | | X | |
| B*057:01 | X | | | X |
| B*058:02 | | | X | |
| B*060:02 | X | | | |
| B*061:01 | X | | | |
| B*064:01 | | | X | |
| B*068:03 | | X | | |
| B*072:01; B*072:02; Mm-B*nov121 | | X | | X |
| B*074:01 | X | | | X |
| B*082:02 | | | | X |
| Mm-B*nov037 | X | | | |
| Mm-B*nov113 | | X | | |
| E*02:01:02; E*02:10; E*02:11 | X | | | X |
| E*02:04 | X | X | X | X |
| E*02:09 | X | | | |
| E*02:12:01; E*02:12:02 | | X | | |
| E*02:20 | X | | | X |

▨ 产生的转染子

图7B

60

图7C

图8A

图8B

图9

图10A

图10B
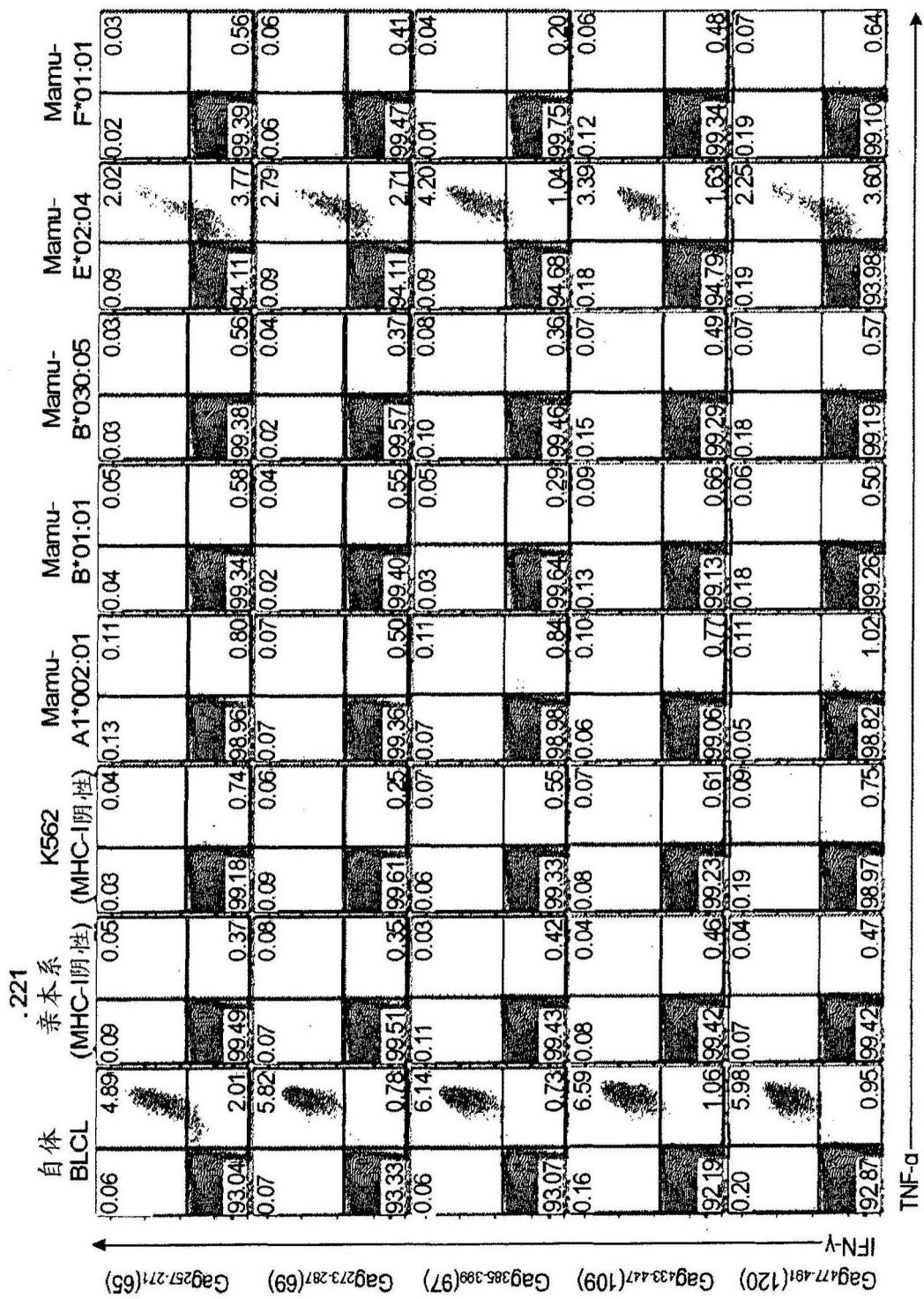
图11A

图11B

图11C

图11D

图12A

图12B

图12C

图13A



图13B

图13C

| 15聚体肽 | 识别频率** | 最佳表位 | 9聚体肽 |
|---|---|---|---|
| Gag61-75 | 35 | A P L V P T G S E | Gag65-73 |
| Gag69-83 | 40 | S E N L K S L Y N | Gag72-80 |
| Gag89-103 | 50 | E K V K H T E E A | Gag92-100 |
| Gag117-131 | 35 | K T S R P T A P S | Gag120-128 |
| Gag129-143 | 35 | G G N Y P V Q Q I | Gag132-140 |
| Gag197-211 | 45 | Q I I R D I I N E | Gag201-209 |
| Gag257-271 | 25 | V G N I Y R W I | Gag259-267 |
| Gag273-287 | 100* | R M Y N P T N I L | **Gag276-284** |
| Gag385-399 | 55 | K P I K C W N C G | Gag389-397 |
| Gag473-487 | 60 | L G K Q Q R E K Q | Gag476-484 |
| Gag477-491 | 100* | E K Q R E S R E K | **Gag482-490** |

* 超表位
** 疫苗接种的动物百分比

富集的
二代表性不足的
MHC-E 典型肽锚定

氨基酸位置

频率

图13D

图14

恒河猴

| Mamu I类等位基因 | 21826 | 22034 | 22436 | 22607 |
|---|---|---|---|---|
| A1*001:01 | X |  |  | X |
| A1*002:01 | X | X | X | X |
| A1*012:01 |  |  | X |  |
| A1*023:01 |  | X |  |  |
| A2*05:01;A2*05:11;A2*05:28;A2*05:32:01;A2*05:32:02;A2*05:45 |  |  | X |  |
| A2*05:04:01;A2*05:04:03;A2*05:10;A2*05:14 |  |  |  | X |
| A3*13:02 | X | X | X | X |
| A4*14:03:01;A2*14:09 |  | X |  |  |
| B*001:01:01 |  | X |  | X |
| B*007:02 |  | X |  | X |
| B*012:01 | X |  |  | X |
| B*17:01 | X |  |  |  |
| B*021:01 |  | X |  |  |
| B*022:01 | X |  |  |  |
| B*028:01 |  | X |  |  |
| B*029:01 | X |  |  |  |
| B*030:01 | X |  |  |  |
| B*030:05 |  | X |  | X |
| B*031:01 | X |  |  |  |
| B*041:01 |  |  | X |  |
| B*046:01:02 |  | X |  |  |
| B*048:01 |  |  | X |  |
| B*055:01 |  |  | X |  |
| B*057:01 | X |  |  | X |
| B*058:02 |  |  | X |  |
| B*060:02 | X |  |  |  |
| B*061:01 | X |  |  |  |
| B*064:01 |  |  | X |  |
| B*068:03 |  | X |  |  |
| B*072:01;B*072:02;Mm-B*nov121 |  | X |  | X |
| B*074:01 | X |  |  | X |
| B*082:02 |  |  |  | X |
| Mm-B*nov037 | X |  |  |  |
| Mm-B*nov113 |  | X |  |  |
| E*02:01:02;E*02:10;E*02:11 | X |  |  | X |
| E*02:04 | X | X | X | X |
| E*02:09 | X |  |  |  |
| E*02:12:01;E*02:12:02 |  | X |  |  |
| E*02:20 | X |  |  | X |

▨ 产生的转染子

图15A

图15B

图16A

| Rh21826 | Gag$_{61-75}$ (16) | Gag$_{69-83}$ (18) | Gag$_{89-103}$ (23) | Gag$_{117-131}$ (30) | Gag$_{129-143}$ (33) | Gag$_{197-211}$ (50) | Gag$_{257-271}$ (65) | Gag$_{273-287}$ (69) | Gag$_{385-399}$ (97) | Gag$_{433-447}$ (109) | Gag$_{473-487}$ (119) | Gag$_{477-491}$ (120) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| BLCL | - | - | + | - | + | + | + | + | - | - | + | + |
| 221 | - | - | - | - | - | - | - | - | - | - | - | - |
| K562 | - | - | - | - | - | - | - | - | - | - | - | - |
| A1*001:01 | - | - | - | - | - | + | - | - | - | - | - | - |
| A1*002:01 | - | - | - | - | + | - | - | - | - | - | - | - |
| A3*13:02 | - | - | - | - | - | - | - | - | - | - | - | - |
| B*029:01 | - | - | - | - | - | - | - | - | - | - | - | - |
| B*030:01 | - | - | - | - | - | - | - | - | - | - | - | - |
| E*02:04 | - | - | + | - | - | + | + | + | - | - | + | + |
| E*02:11 | - | - | + | - | + | + | + | + | - | - | + | + |
| F*01:01 | - | - | - | - | - | - | - | - | - | - | - | - |

| Rh22034 | Gag$_{61-75}$ (16) | Gag$_{69-83}$ (18) | Gag$_{89-103}$ (23) | Gag$_{117-131}$ (30) | Gag$_{129-143}$ (33) | Gag$_{197-211}$ (50) | Gag$_{257-271}$ (65) | Gag$_{273-287}$ (69) | Gag$_{385-399}$ (97) | Gag$_{433-447}$ (109) | Gag$_{473-487}$ (119) | Gag$_{477-491}$ (120) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| BLCL | + | + | + | - | - | - | + | + | + | + | - | + |
| 221 | - | - | - | - | - | - | - | - | - | - | - | - |
| K562 | - | - | - | - | - | - | - | - | - | - | - | - |
| A1*002:01 | - | - | - | - | - | - | - | - | - | - | - | - |
| A3*13:02 | - | - | - | - | - | - | - | - | - | - | - | - |
| B*001:01 | - | - | - | - | - | - | - | - | - | - | - | - |
| B*030:05 | - | - | - | - | - | - | - | - | - | - | - | - |
| E*02:04 | + | + | + | - | - | - | + | + | + | + | - | + |
| E*02:11 | + | + | + | - | - | - | + | + | + | + | - | + |
| F*01:01 | - | - | - | - | - | - | - | - | - | - | - | - |

图16B

81

| Rh22436 | Gag61-75 (16) | Gag69-83 (18) | Gag89-103 (23) | Gag117-131 (30) | Gag129-143 (33) | Gag197-211 (50) | Gag257-271 (65) | Gag273-287 (69) | Gag385-399 (97) | Gag433-447 (109) | Gag473-487 (119) | Gag477-491 (120) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| BLCL | - | - | - | + | - | + | + | + | - | + | + | + |
| 221 | - | - | - | - | - | - | - | - | - | - | - | - |
| K562 | - | - | - | - | - | - | - | - | - | - | - | - |
| A1*002:01 | - | - | - | - | - | - | - | - | - | - | - | - |
| A3*13:02 | - | - | - | - | - | - | - | - | - | - | - | - |
| B*048:01 | - | - | - | - | - | - | - | - | - | - | - | - |
| E*02:04 | - | - | - | + | - | + | + | + | - | + | + | + |
| E*02:11 | - | - | - | + | - | + | + | + | - | - | - | - |
| F*01:01 | - | - | - | - | - | - | - | - | - | - | - | - |

| Rh22607 | Gag61-75 (16) | Gag69-83 (18) | Gag89-103 (23) | Gag117-131 (30) | Gag129-143 (33) | Gag197-211 (50) | Gag257-271 (65) | Gag273-287 (69) | Gag385-399 (97) | Gag433-447 (109) | Gag473-487 (119) | Gag477-491 (120) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| BLCL | - | + | + | - | + | - | + | + | + | + | + | + |
| 221 | - | - | - | - | - | - | - | - | - | - | - | - |
| K562 | - | - | - | - | - | - | - | - | - | - | - | - |
| A1*001:01 | - | + | - | - | - | - | - | - | - | - | - | - |
| A1*002:01 | - | - | - | - | + | - | - | - | - | - | - | - |
| A3*13:02 | - | - | - | - | - | - | - | - | - | - | - | - |
| B*001:01 | - | - | - | - | - | - | - | - | - | - | - | - |
| B*030:05 | - | - | - | - | - | - | - | - | - | - | - | - |
| E*02:04 | - | + | + | - | + | - | + | + | + | + | + | + |
| E*02:11 | - | + | + | - | + | - | + | + | + | + | + | + |
| F*01:01 | - | - | - | - | - | - | - | - | - | - | - | - |

图16B(续)

| 恒河猴: | A1*001:01 | A1*002:01 | | Gag$_{69-83}$(18) | Gag$_{129-143}$(33) | Gag$_{197-211}$(55) |
|---|---|---|---|---|---|---|
| Rh22607 | + | + | | + | + | - |
| Rh21826 | + | + | | - | + | + |
| Rh25565 | + | - | | + | + | + |
| Rh25545 | + | - | | - | - | - |
| Rh22034 | - | + | | + | - | - |
| Rh28819 | - | + | | + | - | - |
| Rh28808 | - | + | | - | + | + |
| Rh22436 | - | + | | - | - | + |
| Rh24194 | - | + | | - | - | + |
| Rh27517 | - | - | | + | + | - |
| Rh22063 | - | - | | + | - | - |
| Rh27473 | - | - | | + | - | - |
| Rh27715 | - | - | | + | - | - |
| Rh29483 | - | - | | + | - | - |
| Rh25222 | - | - | | - | + | + |
| Rh29208 | - | - | | - | + | + |
| Rh29212 | - | - | | - | + | + |
| Rh22624 | - | - | | - | + | - |
| Rh29482 | - | - | | - | - | + |
| Rh21756 | - | - | | - | - | - |

图17

图18A

HLA-E*01:03
Mamu-E*02:04
Mamu-E*02:11
Mamu-E*02:20
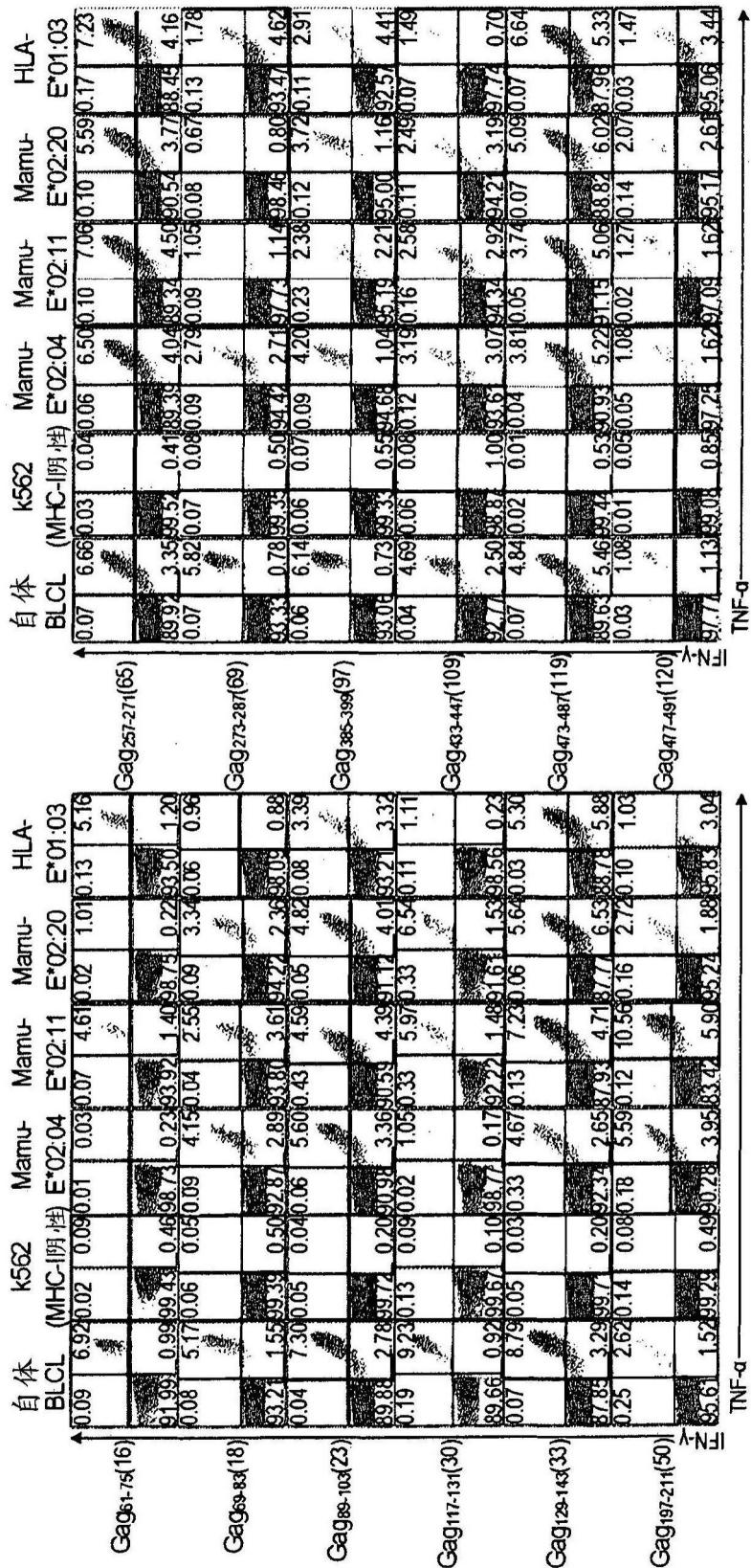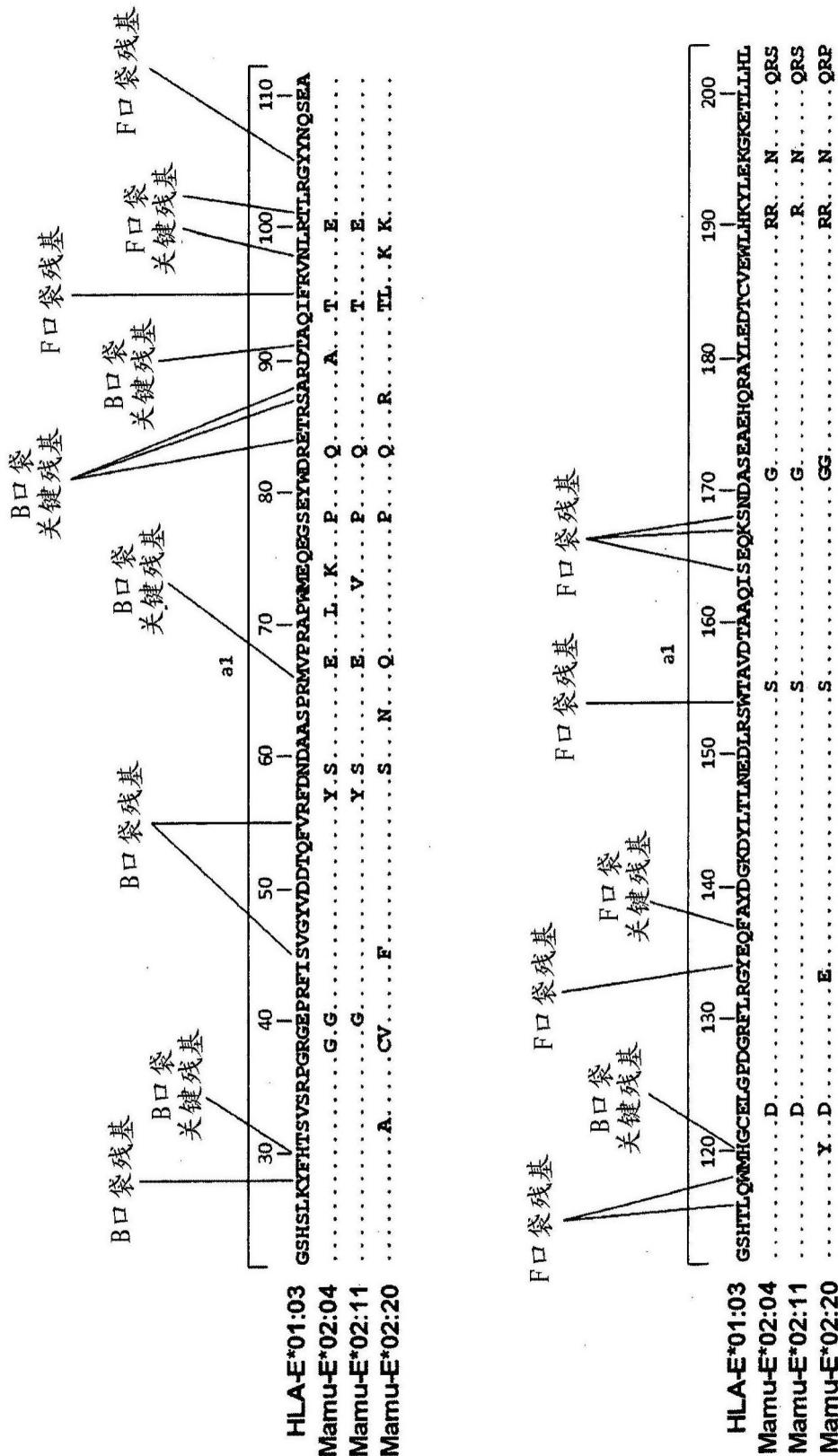
图18B
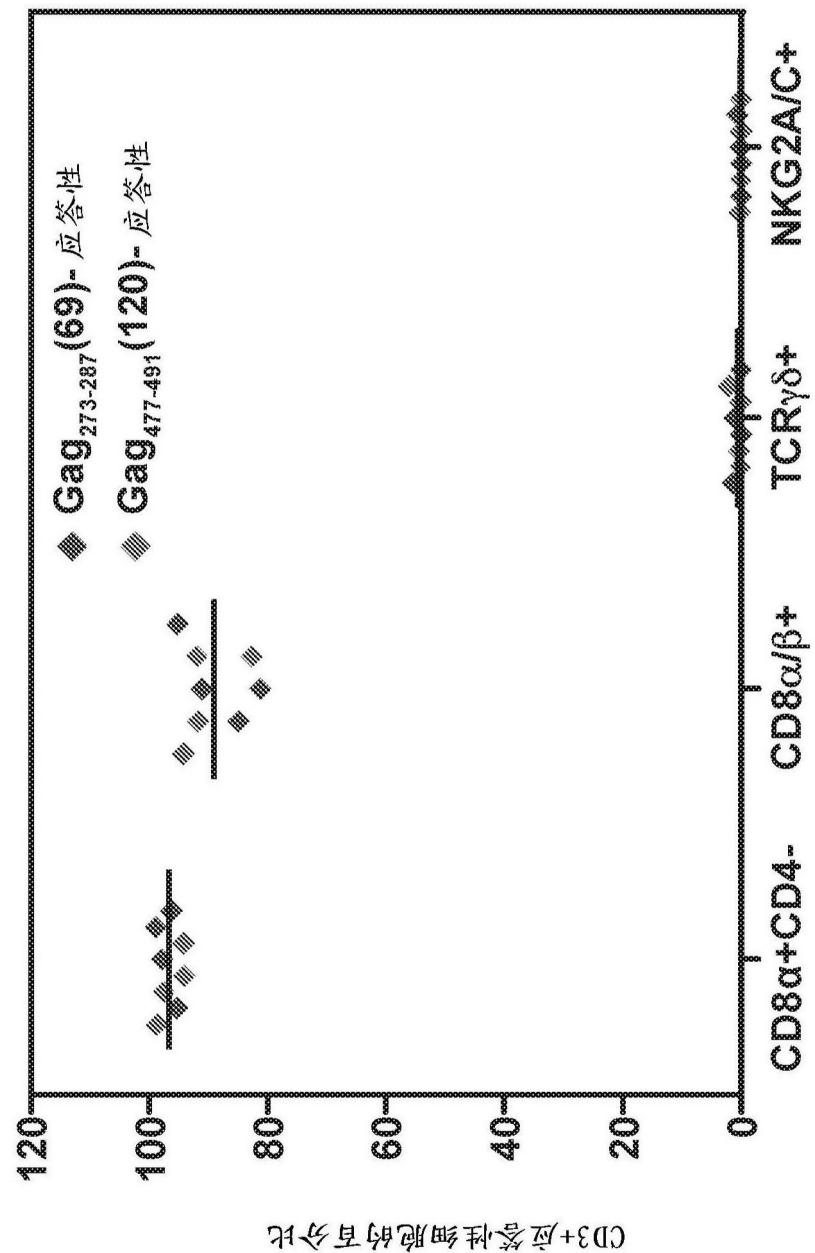
图19
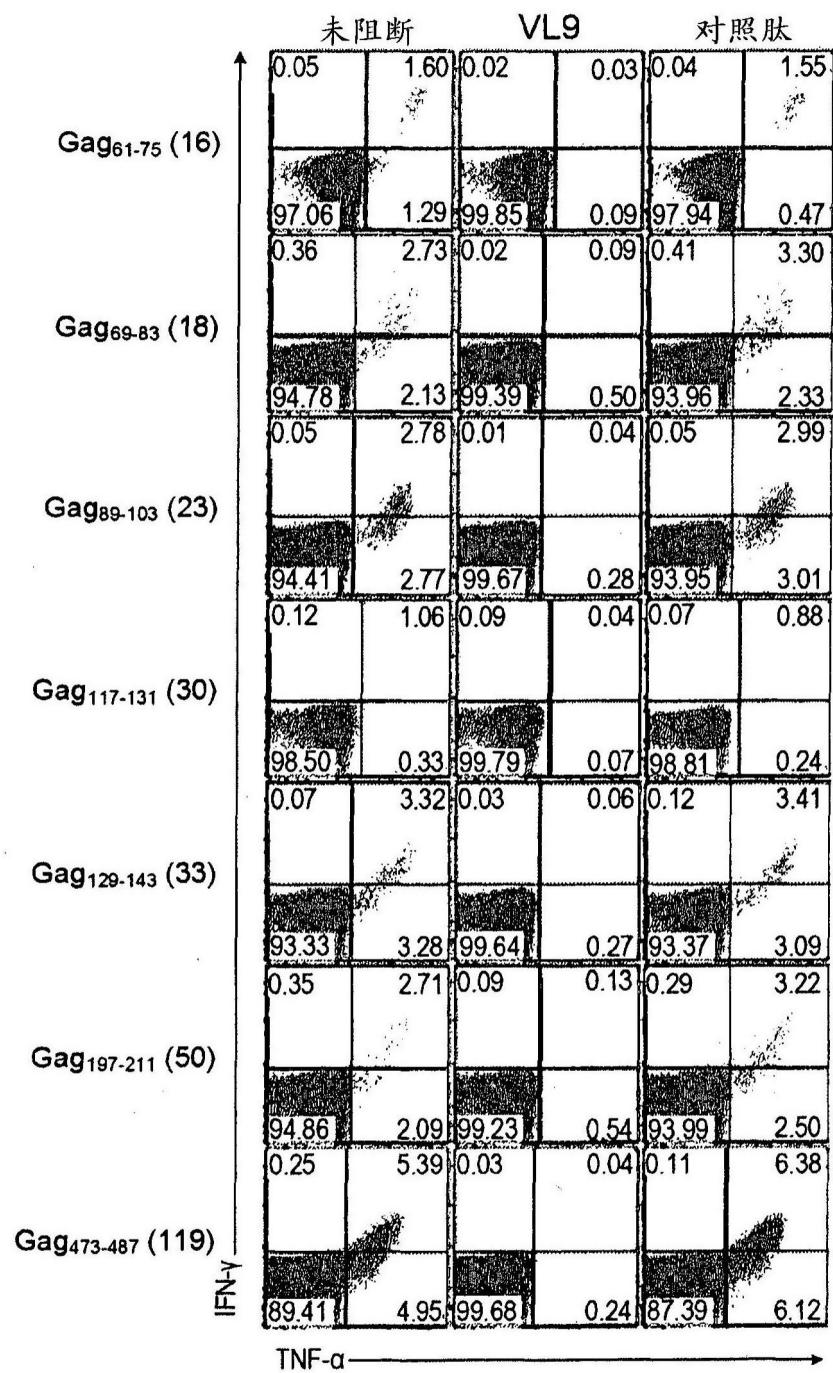
图20A
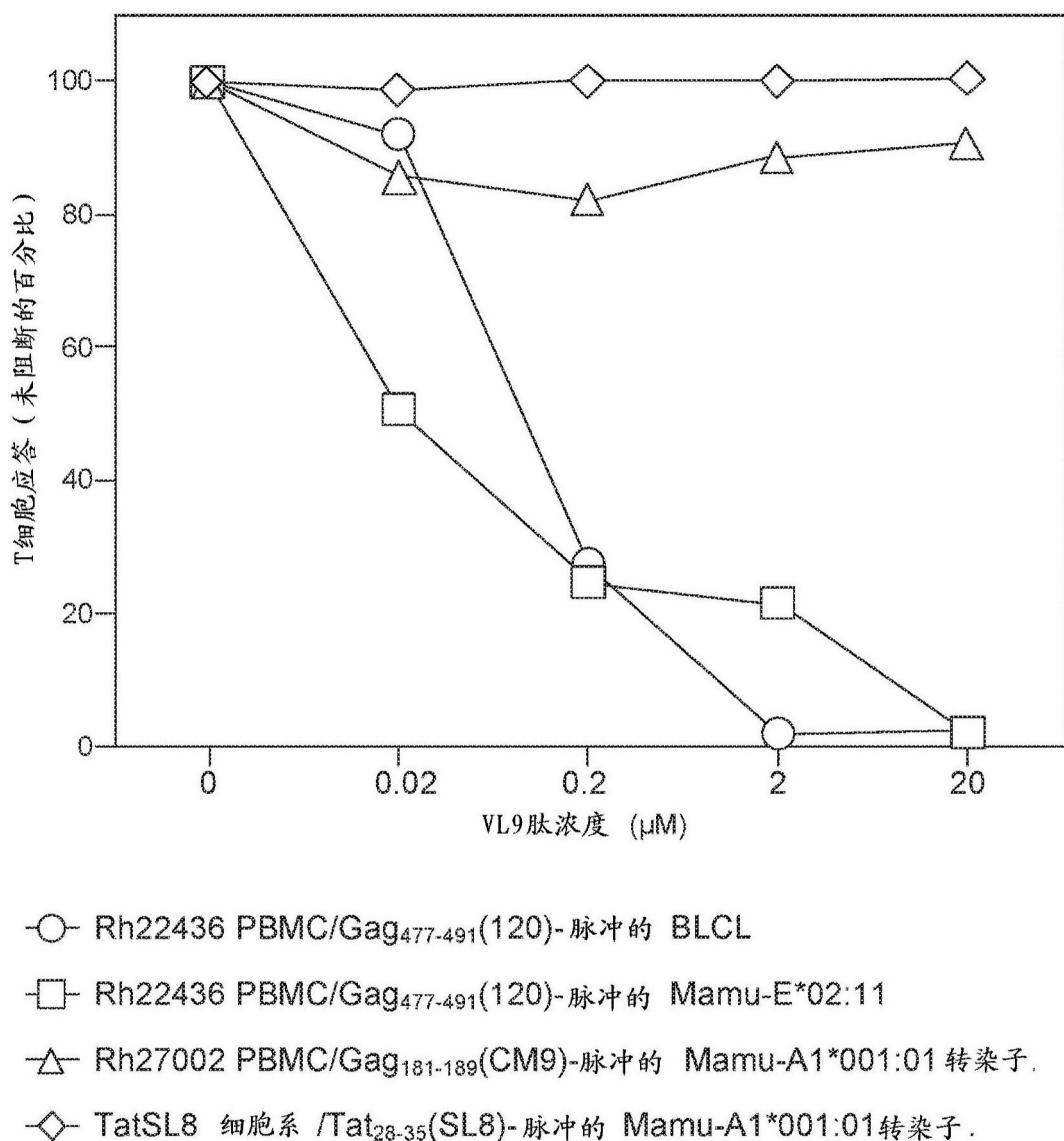
图20B
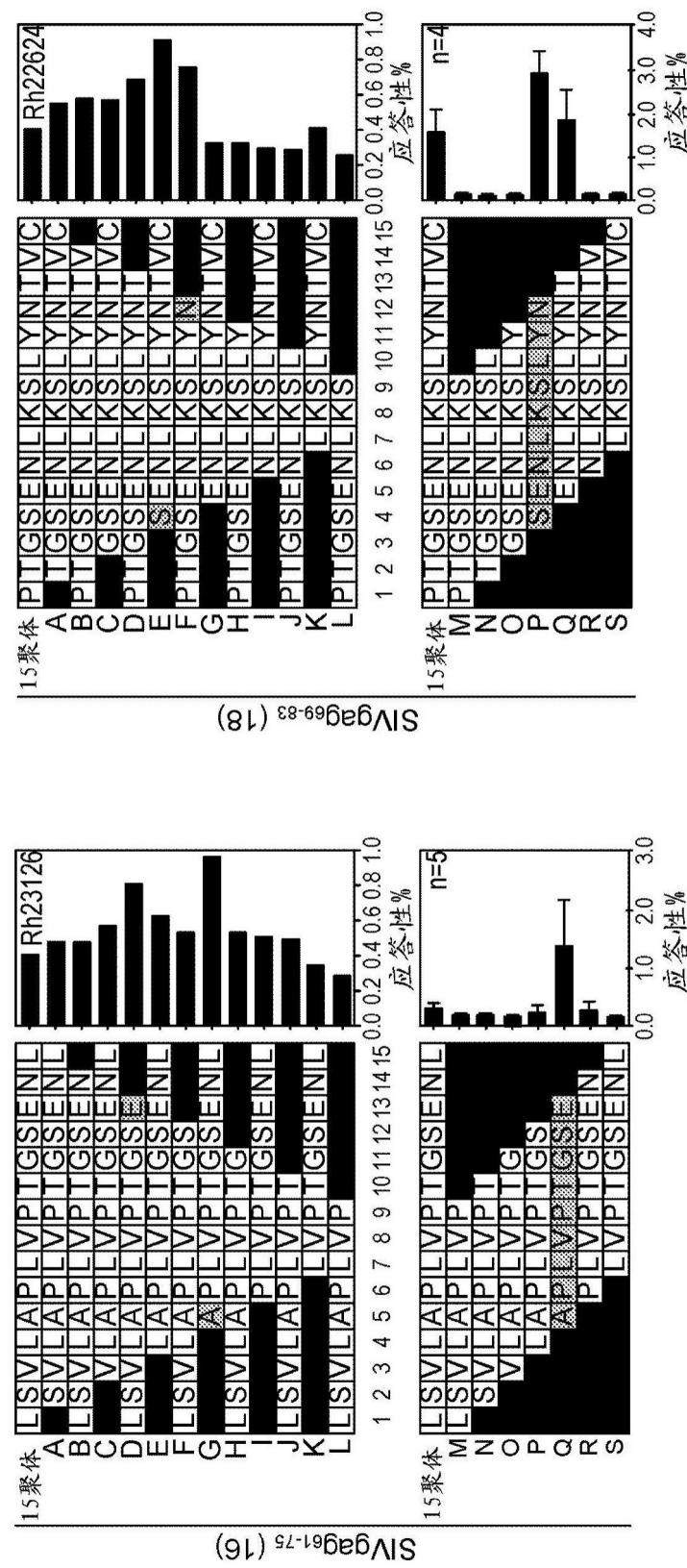
图21
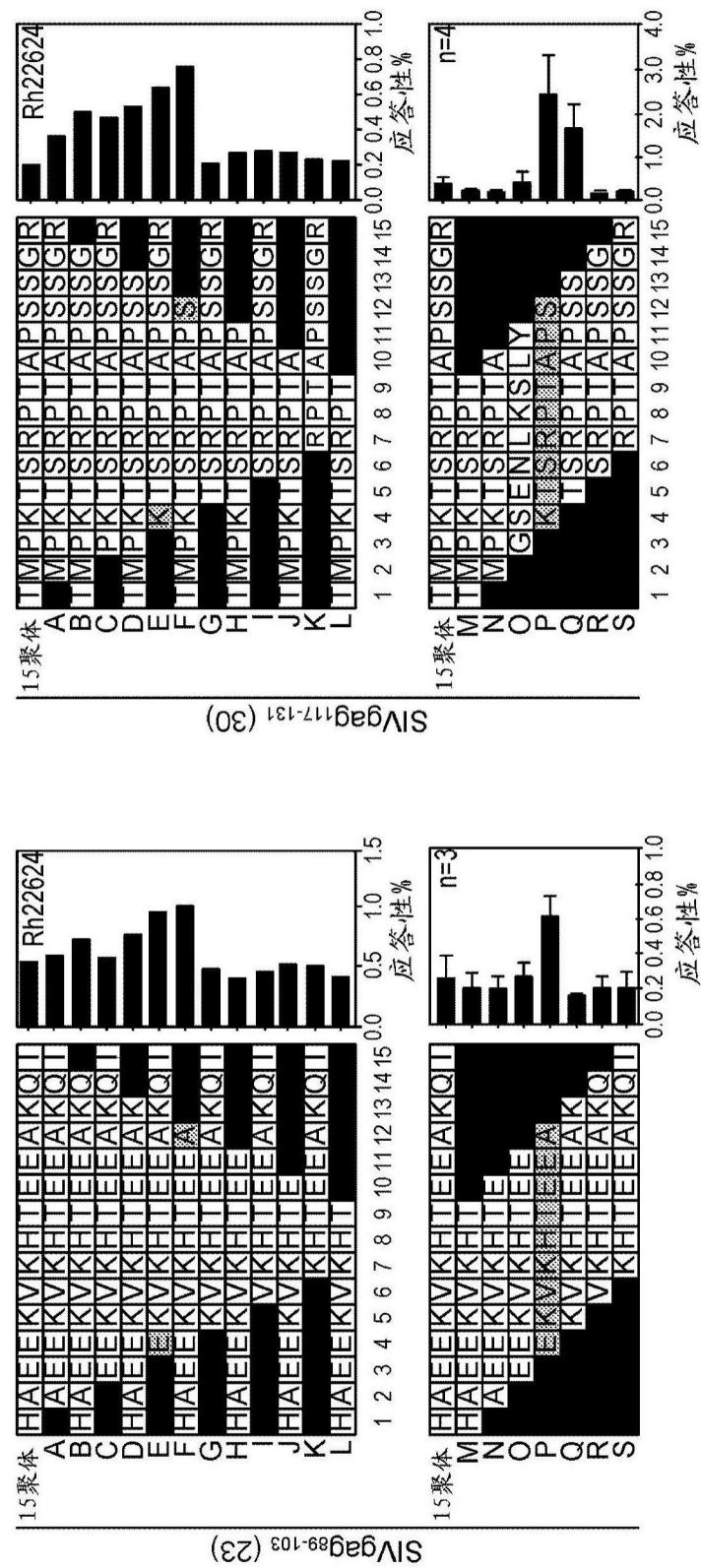
图21(续)

图21(续)

图21(续)

图22A



图22B

图23

**68-1 RhCMV/gag 载体疫苗接种的恒河猴 (RH28335)**

| SIVgag$_{49-63}$(13) | SIVgag$_{101-115}$(26) | SIVgag$_{277-291}$(70) | SIVgag$_{361-375}$(91) | SIVgag$_{429-443}$(108) | |
|---|---|---|---|---|---|



图24A

**68-1.2 RhCMV/gag 载体疫苗接种的恒河猴 (RH27338)**

| SIVgag$_{5-19}$(2) | SIVgag$_{61-75}$(16) | SIVgag$_{77-91}$(20) | SIVgag$_{113-127}$(20) | SIVgag$_{173-187}$(44) | |
|---|---|---|---|---|---|



图24B

95
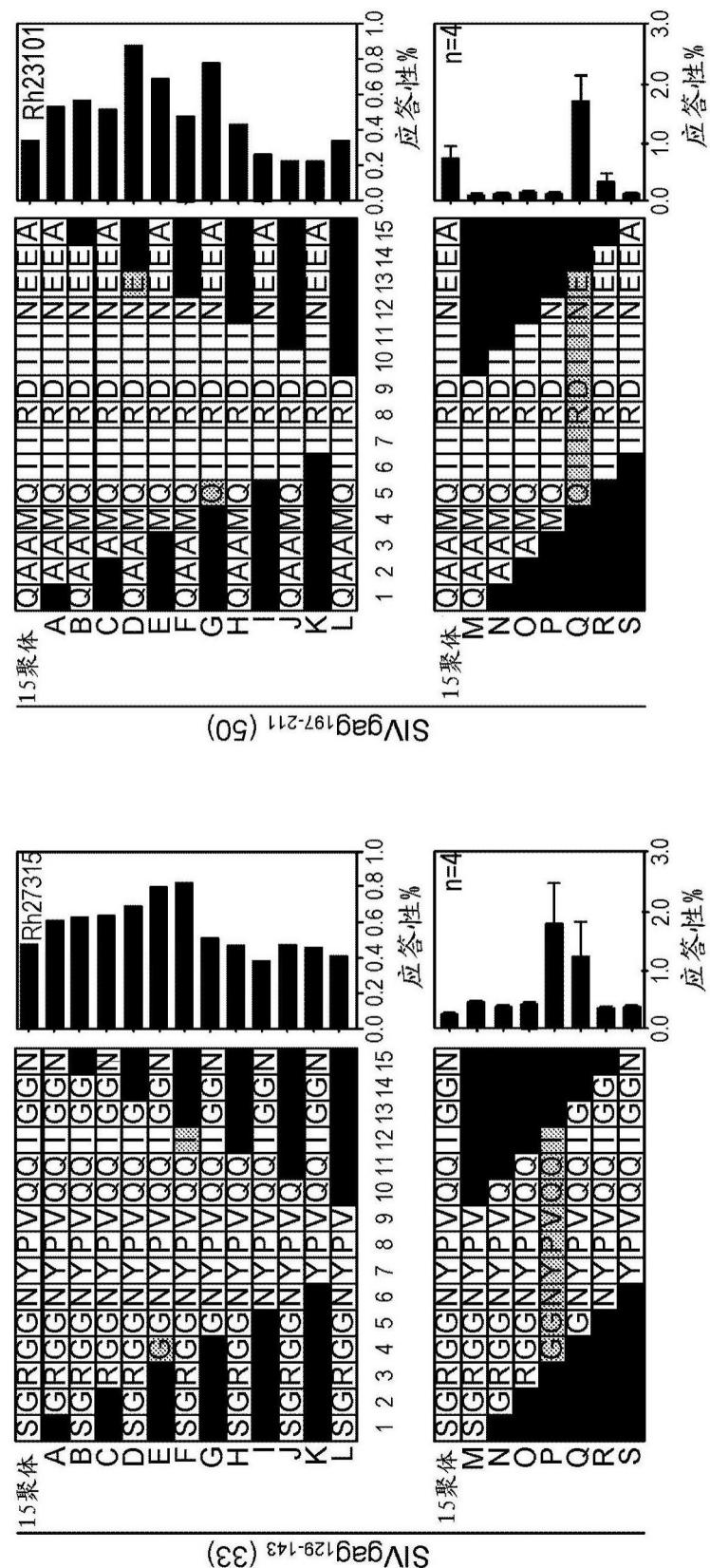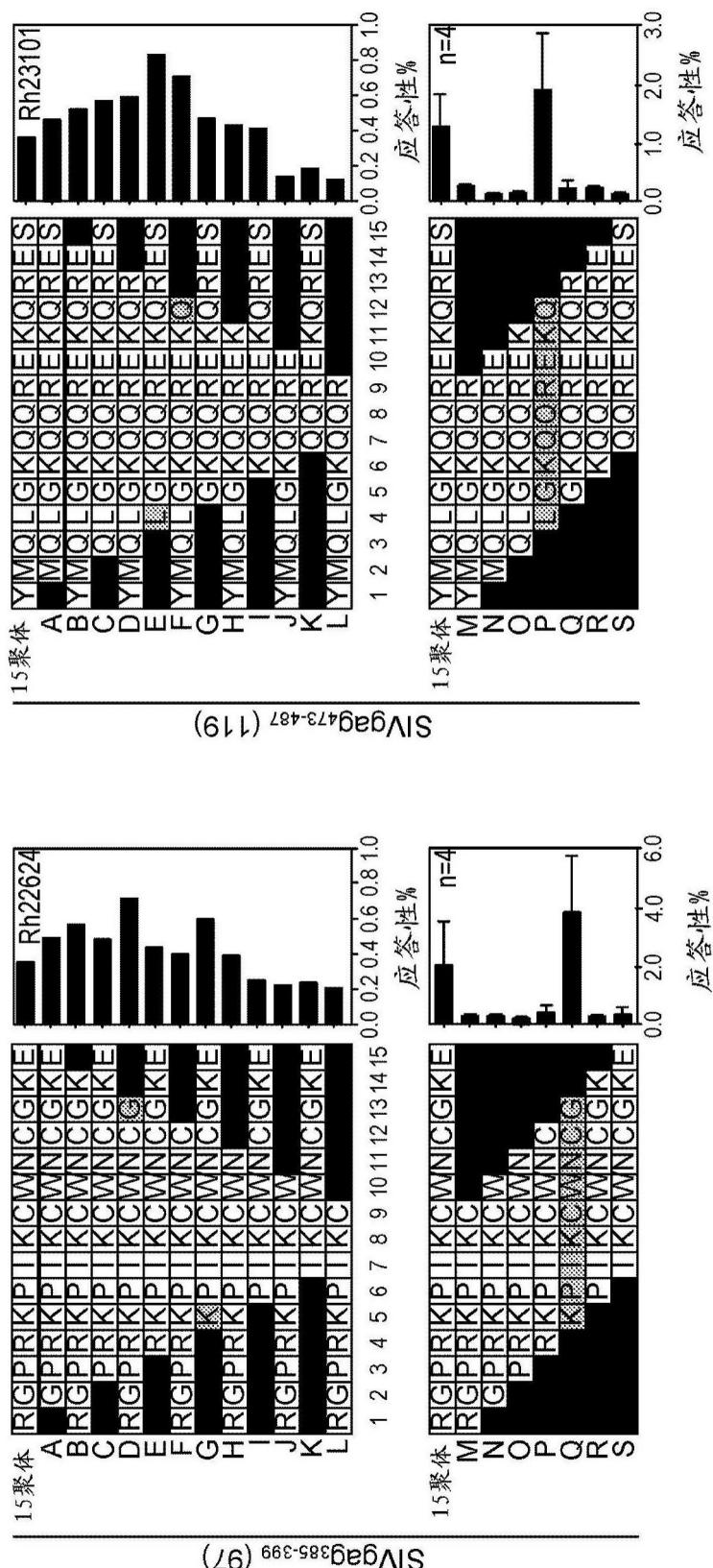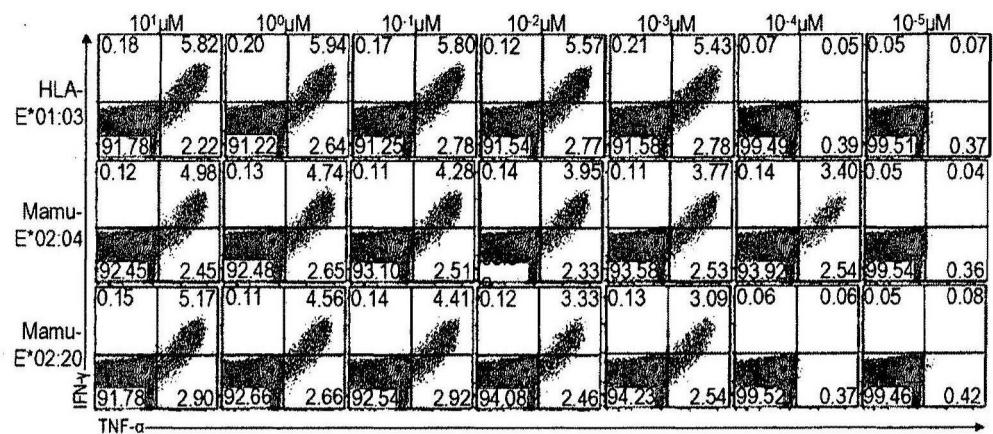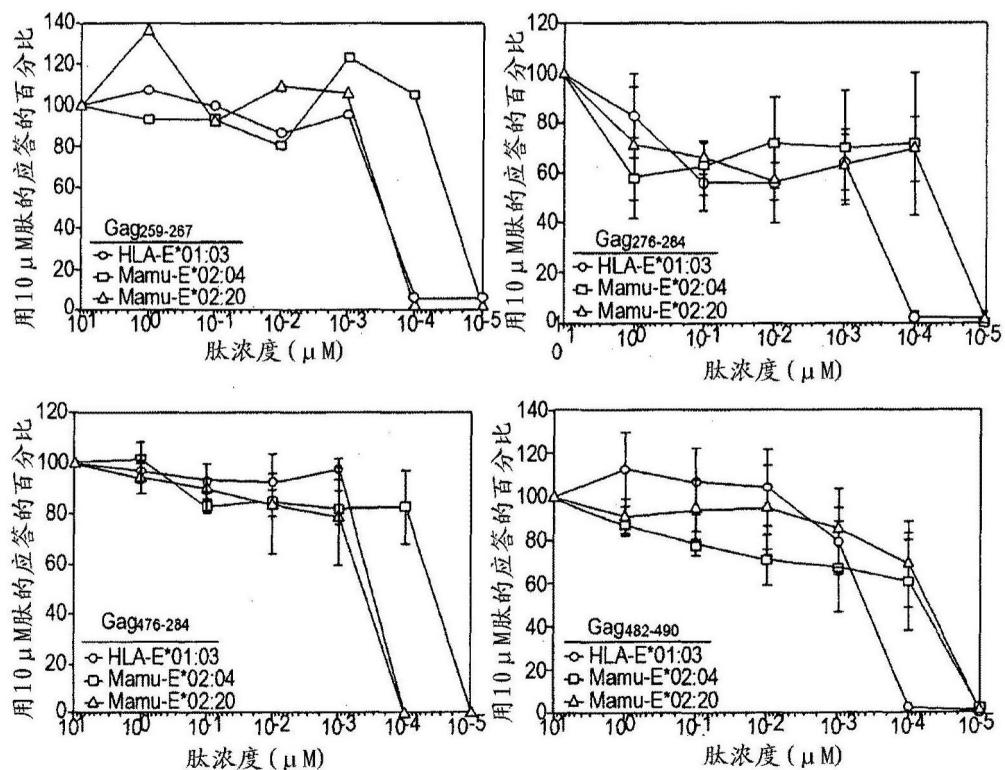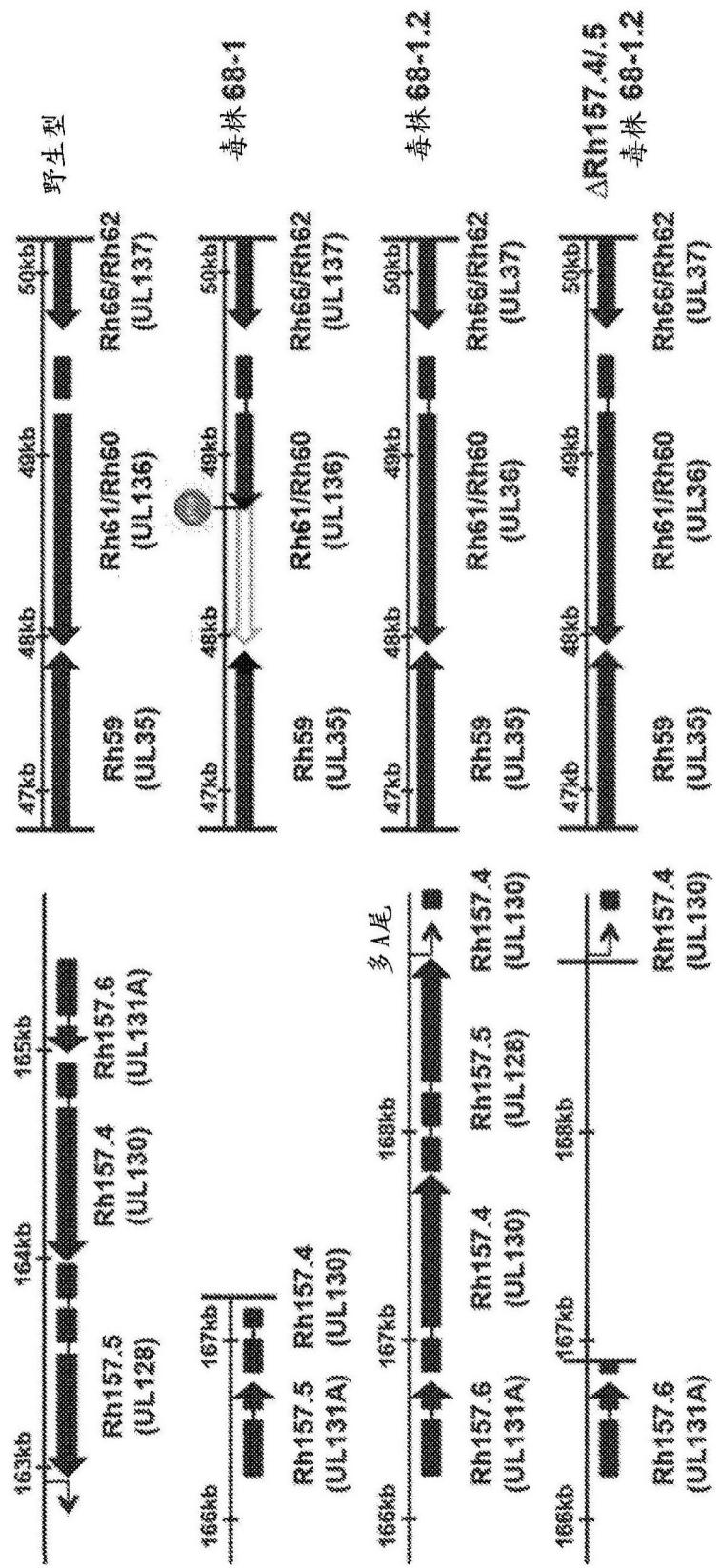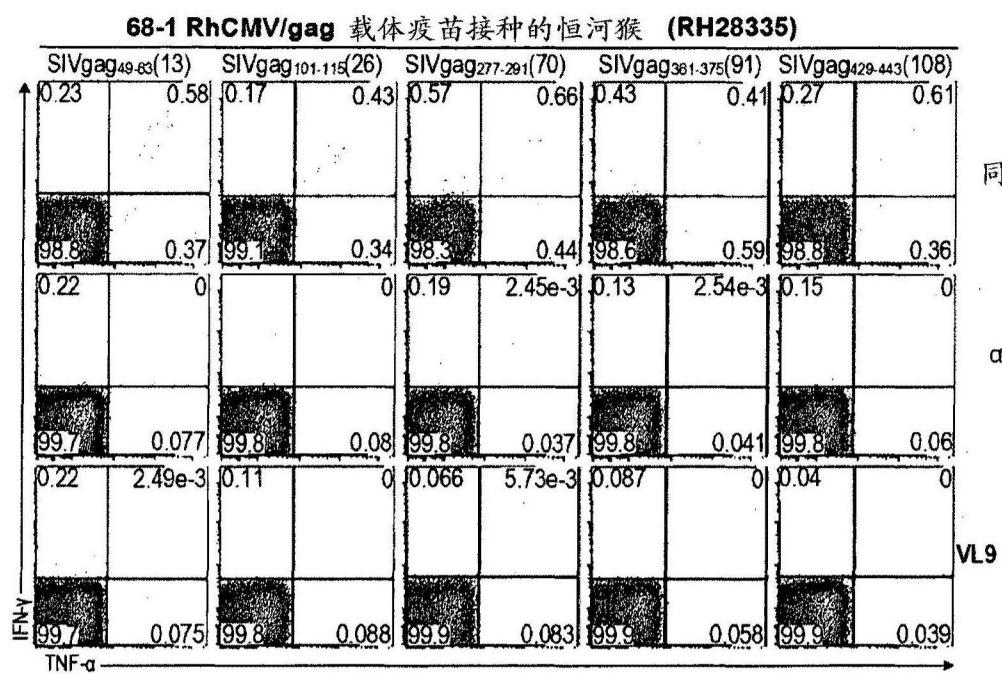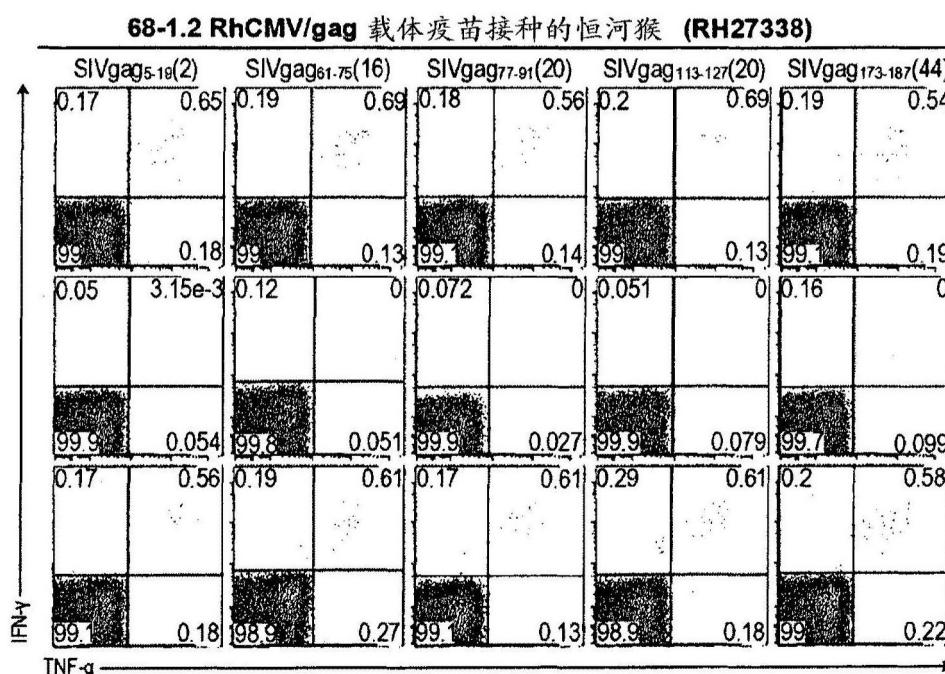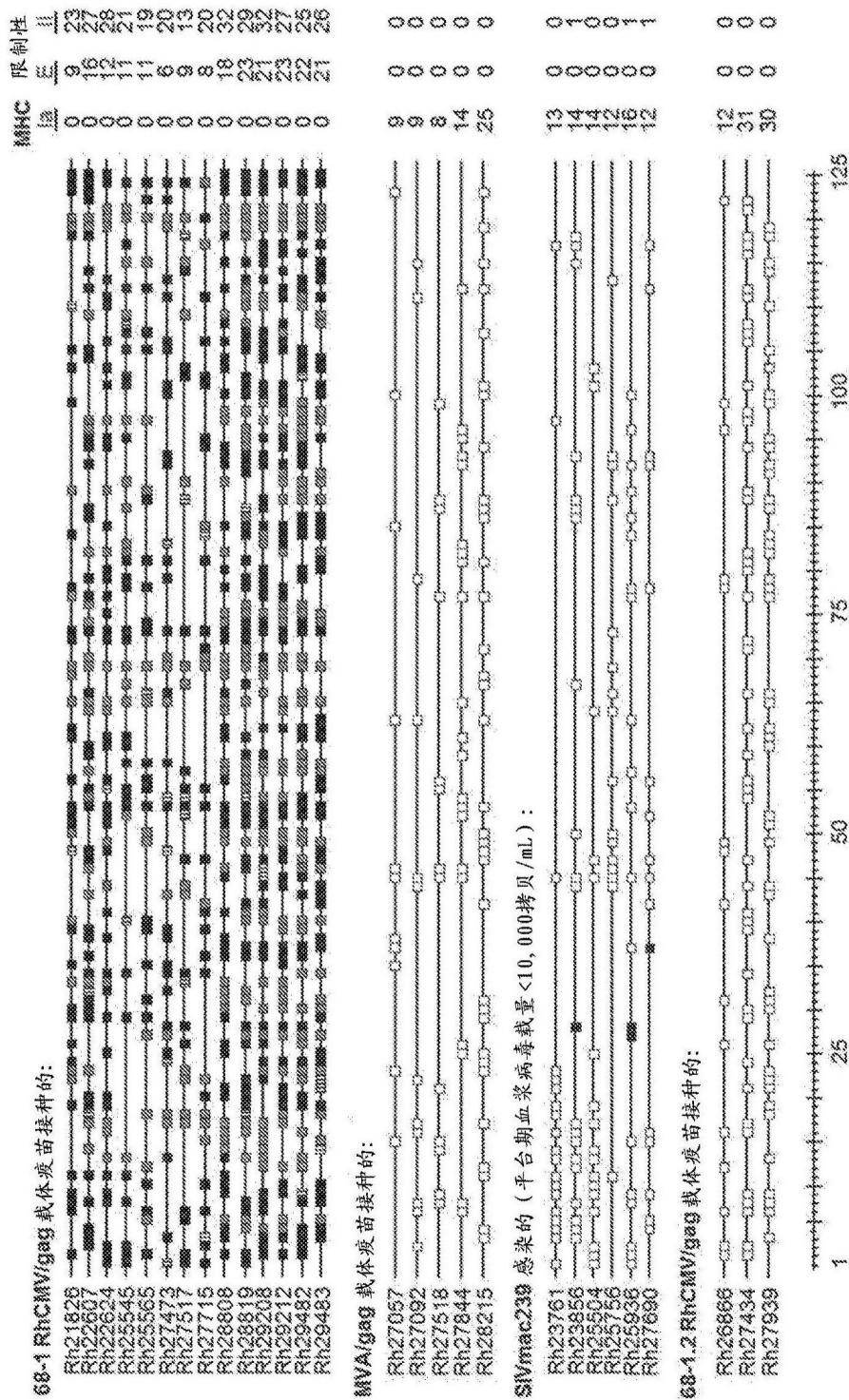
图25
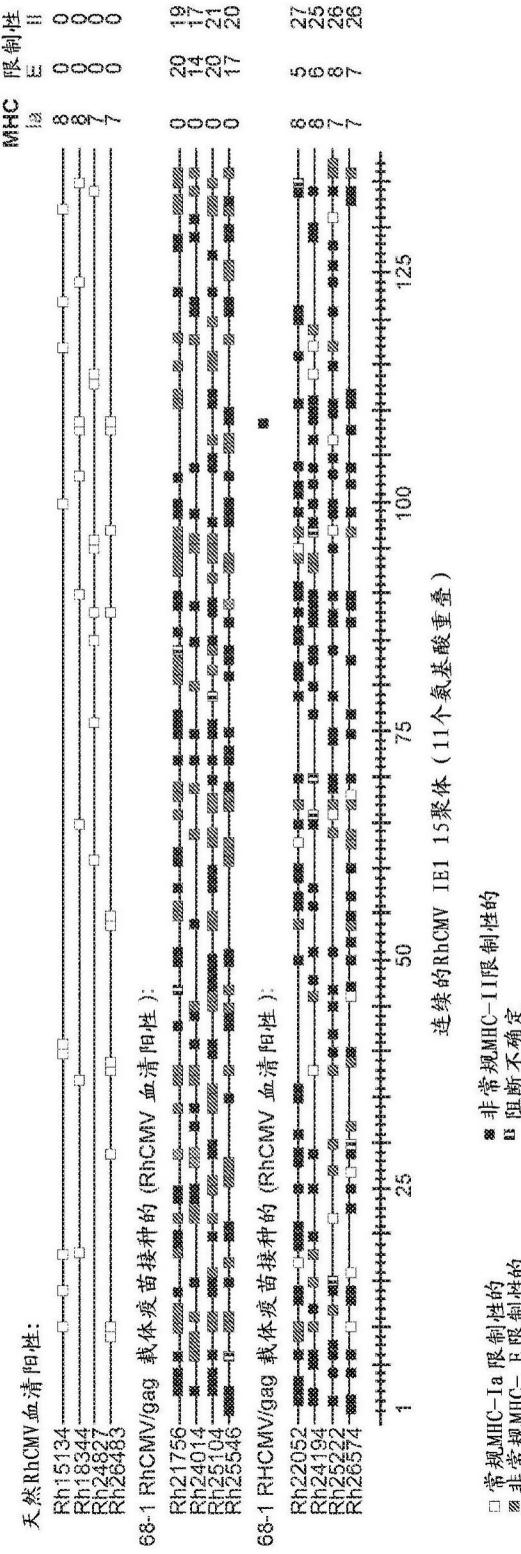
图26
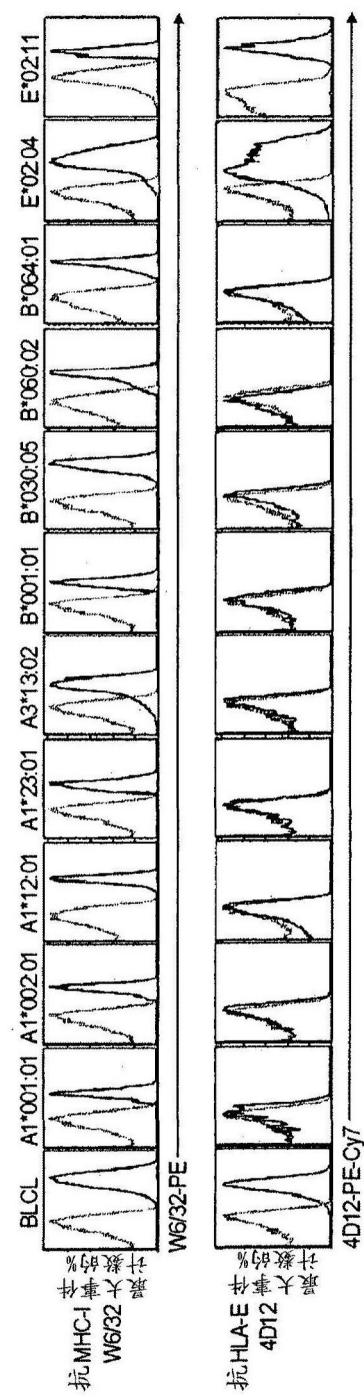
图27A

图27B



图27C

图28

图29

图30