

(12) STANDARD PATENT
(19) AUSTRALIAN PATENT OFFICE

(11) Application No. AU 2016355468 B2

(54) Title
CMV vectors comprising microRNA recognition elements

(51) International Patent Classification(s)
C12N 15/86 (2006.01) **A61K 39/04** (2006.01)
A61K 39/00 (2006.01) **A61K 39/12** (2006.01)
A61K 39/015 (2006.01) **A61K 48/00** (2006.01)

(21) Application No: **2016355468** (22) Date of Filing: **2016.11.20**

(87) WIPO No: **WO17/087921**

(30) Priority Data

(31) Number	(32) Date	(33) Country
62/258,393	2015.11.20	US
62/365,259	2016.07.21	US

(43) Publication Date: **2017.05.26**

(44) Accepted Journal Date: **2022.12.15**

(71) Applicant(s)
Oregon Health & Science University

(72) Inventor(s)
Nelson, Jay;Hansen, Scott;Hancock, Meaghan H.;Picker, Louis;Frueh, Klaus

(74) Agent / Attorney
Spruson & Ferguson, GPO Box 3898, Sydney, NSW, 2001, AU

(56) Related Art
US 2013/0136768 A1

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization

International Bureau



WIPO | PCT



(10) International Publication Number

WO 2017/087921 A1

(43) International Publication Date

26 May 2017 (26.05.2017)

(51) International Patent Classification:

C12N 15/86 (2006.01) *A61K 39/04* (2006.01)
A61K 39/00 (2006.01) *A61K 39/12* (2006.01)
A61K 39/015 (2006.01) *A61K 48/00* (2006.01)

(72) **Inventors:** **NELSON, Jay**; Office of Technology Transfer and Business, Development 0690 SW Bancroft Street, Mail Code L106TT, Portland, OR 97239 (US). **HANSEN, Scott**; Office of Technology Transfer and Business, Development 0690 SW Bancroft Street, Mail Code L106TT, Portland, OR 97239 (US). **HANCOCK, Meaghan, H.**; Office of Technology Transfer and Business, Development 0690 SW Bancroft Street, Mail Code L106TT, Portland, OR 97239 (US). **PICKER, Louis**; Office of Technology Transfer and Business, Development 0690 SW Bancroft Street, Mail Code L106TT, Portland, OR 97239 (US). **FREUH, Klaus**; Office of Technology Transfer and Business, Development 0690 SW Bancroft Street, Mail Code L106TT, Portland, OR 97239 (US).

(21) International Application Number:

PCT/US2016/062973

(22) International Filing Date:

20 November 2016 (20.11.2016)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

62/258,393 20 November 2015 (20.11.2015) US
62/365,259 21 July 2016 (21.07.2016) US

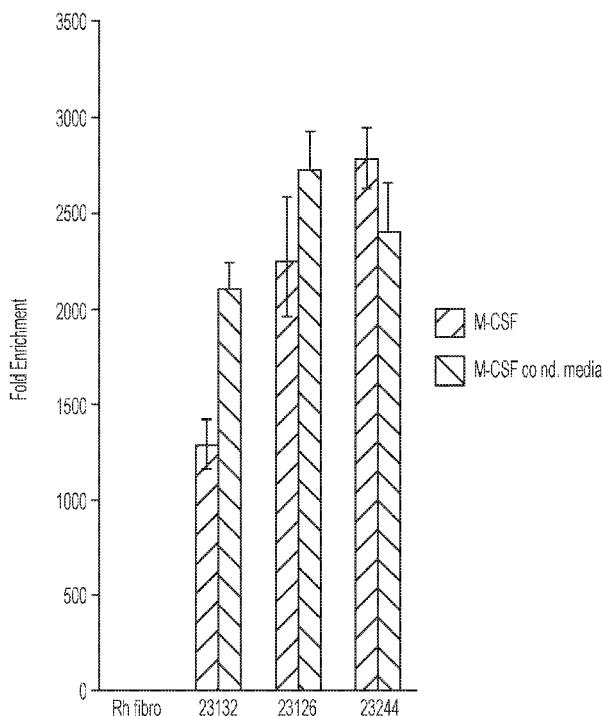
(74) **Agents:** **BUNKER, Gillian, L.** et al.; Schwabe, Williamson & Wyatt, P.C., Pacwest Center, 1211 SW 5th Avenue, Suite 1900, Portland, OR 97204 (US).

(71) **Applicant:** **OREGON HEALTH & SCIENCE UNIVERSITY** [US/US]; Office of Technology Transfer and Business, Development 0690 SW Bancroft Street, Mail Code L106TT, Portland, OR 97239 (US).

(81) **Designated States** (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY,

[Continued on next page]

(54) Title: CMV VECTORS COMPRISING MICRORNA RECOGNITION ELEMENTS



(57) **Abstract:** Disclosed herein are recombinant CMV vectors comprising heterologous antigens and microRNA recognition elements to silence expression of CMV genes in the presence of microRNA derived from myeloid cells, an active UL128 protein and an active UL130 protein. Also disclosed are recombinant CMV vectors comprising heterologous antigens and microRNA recognition elements to silence expression of CMV genes in the presence of microRNA derived from myeloid cells, an inactive UL128 protein and an inactive UL130 protein. Also disclosed are methods of generating an unconventional immune response using these vectors. Such an immune response is characterized by generation of a CD8+ T cell response that is predominantly restricted by MHC-II.

Figure 1



BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) **Designated States** (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH,

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

Published:

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

CMV VECTORS COMPRISING MICRORNA RECOGNITION ELEMENTS

Cross-Reference to Related Applications

[0001] This application is based upon and claims the benefit of priority from U.S. Provisional Application No. 62/258,393, filed on November 20, 2015, entitled CMV VECTORS COMPRISING MICRORNA RECOGNITION ELEMENTS, and U.S. Provisional Application No. 62/365,259, filed on July 21, 2016, entitled CMV VECTORS COMPRISING MICRORNA RECOGNITION ELEMENTS, the disclosures of which are incorporated herein by reference in their entirety.

Technical Field

[0002] Embodiments relate to the use of CMV vectors in immunization, and more specifically, the generation of modified versions of CMV vectors that elicit unconventional immune responses upon immunization with heterologous antigens.

Acknowledgement of Government Support

[0003] This invention was created with the support of the United States government under the terms of grant number P01 AI094417, awarded by the National Institutes of Health. The United States government has certain rights in this invention.

Background

[0004] Rhesus Cytomegalovirus vaccine vectors expressing Simian Immunodeficiency Virus proteins (RhCMV/SIV) provide protection from pathogenic SIV (Hansen SG *et al.*, *Nat Med* 15, 293 (2009); Hansen SG *et al.*, *Nat* 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 vaccinees 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 animals. These events did not occur in spontaneous SIV elite controllers and DNA prime/Ad5 boost vaccinated controllers (Hansen SG 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-induced control of SIV replication. Understanding these properties may in turn lead to new uses for cytomegalovirus vaccine vectors expressing heterologous antigens.

[0005] CMVs may establish latency and reactivate in cells of the myeloid lineage, and macrophages play a central role in viral dissemination. In addition, dendritic cells are essential antigen presenting cell *in vivo*. CMV vectors that express heterologous antigens specifically constructed that they fail to replicate in such cells may be used as new vaccine candidates eliciting a modified, unconventional immune profile.

Summary

[0006] Disclosed herein are cytomegalovirus (CMV) vectors comprising a first nucleic acid sequence that encodes at least one heterologous antigen; and a second nucleic acid sequence comprising a microRNA recognition element (MRE) that silences expression in the presence of a microRNA that is expressed by a cell of the myeloid lineage. The microRNA recognition element is operably linked to a CMV gene that is essential or augmenting for CMV growth *in vivo*. The vector also includes a nucleic acid sequence encoding an active UL128 protein (or ortholog thereof) and a nucleic acid sequence encoding an active UL130 protein (or ortholog thereof). In some embodiments, the vector will lack an active UL128 protein and an active UL130 protein.

[0007] Also disclosed herein are methods of generating an immune response to at least one heterologous antigen in a subject. The method involves administering to the subject a CMV vector of the type disclosed herein in an amount effective to elicit a CD8+ T cell response to the heterologous antigen. The immune response

may be characterized by at least 10% of the CD8+ T cells being restricted by Class II MHC and by fewer than 10% of the cells being restricted by MHC-E.

Brief Description of the Drawings

[0008] Embodiments will be readily understood by the following detailed description in conjunction with the accompanying drawings. Embodiments are illustrated by way of example and not by way of limitation in the figures of the accompanying drawings.

[0009] **Figure 1** is a bar graph showing miR-142-3p expression in CD14+ PBMCs from rhesus macaques differentiated using M-CSF or M-CSF conditioned media, in accordance with various embodiments;

[0010] **Figure 2** is a bar graph showing miR-142-3p expression in the indicated cell types and tissues isolated from rhesus macaques; in accordance with various embodiments;

[0011] **Figures 3A, 3B, 3C, and 3D** collectively show the analysis of the recombinant RhCMV 68-1 RTN Rh156/Rh108 miR-142 3p mutant virus generated via galK recombination, in accordance with various embodiments;

[0012] **Figure 3A** is an image of a gel showing the results where PCR using primers flanking the insertion sites demonstrate the insertion of the galK expression cassette and its replacement with the miR-142 3p target sites; in accordance with various embodiments;

[0013] **Figure 3B** is an image of a gel showing XmaI digestion of BAC DNA indicating maintenance of an intact genome; in accordance with various embodiments;

[0014] **Figure 3C** is an image of a gel showing BAC genomic DNA was isolated and electroporated into rhesus fibroblasts. Virus was plaque-purified, and viral DNA isolated at each of three passages and analyzed by PCR for maintenance of the miR-142 3p target site insertions. In addition, these PCR products were sequenced, and no mutations were detected, in accordance with various embodiments;

[0015] **Figure 3D** is an image of a gel showing gag and HA expression from the construct, in accordance with various embodiments;

[0016] **Figure 4** is a set of two plots each showing a multi-step growth curve using 68-1 RTN Rh156/Rh108 miR-142 3p virus. Telomerized rhesus fibroblasts were transfected with negative control miRNA or a miR-142 3p RNA mimic. 24 hours after transfection, cells were infected with WT or the 68-1 RTN Rh156/Rh108 miR-142 3p viruses at an MOI of 0.01. Cells and supernatant were harvested at the indicated times and titered on rhesus fibroblasts, in accordance with various embodiments;

[0017] **Figure 5** is a bar graph of CD14+ cells isolated from rhesus macaque PBMCs using magnetic cell sorting. Cells were cultured in the presence of M-CSF-conditioned media for 7 days to allow for differentiation into macrophages. Cells were infected with WT 68-1 RTN or the 68-1 RTN Rh156/Rh108 miR-142 3p viruses at an MOI of 10. Viral DNA was harvested at the indicated timepoints and analyzed for Rh87 DNA levels by qPCR, in accordance with various embodiments;

[0018] **Figure 6** is a set of two plots showing peripheral T cells responses to the SIV RTN antigen in animals infected with RhCMV-68-1-Rh156, 108 miR-142-SIVrtn, in accordance with various embodiments;

[0019] **Figure 7** is a set of three plots of CD8+ T cell responses in animals infected with 68-1.2 RhCMV-Rh156, 108 miR142/SIVgag, in accordance with various embodiments;

[0020] **Figure 8** is an epitope MHC-restriction map of animals of the indicated genotypes infected with the indicated vectors, in accordance with various embodimentnts;

[0021] **Figure 9** is a set of two plots showing a growth curve using 68-1.2 RTN Rh156/Rh108 miR-142 3p virus. The procedure was otherwise performed as described for **Figure 4**, in accordancw with various embodiments;

[0022] **Figure 10** is a schematic of the generation of RhCMV Rh156/Rh108 miR-142 3p mutant virus via galK recombination, in accordance with various embodiments;

[0023] **Figures 11A, 11B, and 11C** collectively show the analysis of the recombinant RhCMV 68-1.2 GAG Rh156/Rh108 miR-142 3p mutant virus generated via galK recombination, in accordance with various embodiments;

[0024] **Figure 11A** is an image of a gel showing the results wherein PCR using primers flanking the insertion sites demonstrate the insertion of the galK

expression cassette and its replacement with the miR-142 3p target sites. In addition, BAC genomic DNA was isolated and electroporated into rhesus fibroblasts. Virus was plaque-purified and viral DNA isolated at each of three passages (P1, P2 and P3) and analyzed by PCR for maintenance of the miR-142 3p target site insertions. These PCR products were sequenced and no mutations were detected, in accordance with various embodiments;

[0025] **Figure 11B** is an image of a gel showing XmaI digestion of RhCMV 68-1.2 GAG Rh156/Rh108 miR-142 3p BAC DNA indicating maintenance of an intact genome, in accordance with various embodiments;

[0026] **Figure 11C** is an image of a gel showing GAG expression throughout 3 passages of RhCMV 68-1.2 GAG Rh156/Rh108 miR-142 3p in rhesus fibroblasts, in accordance with various embodiments;

[0027] **Figure 12** is a set of three plots of T cell responses in animals infected with 68-1 RhCMV-Rh156, 108 miR142/SIVgag, in accordance with various embodiments;

[0028] **Figure 13** is an epitope MHC-restriction map of an animal infected with 68-1 GAG Rh156/Rh108 miR-142 3p or four animals infected with 68-1 GAG, in accordance with various embodiments; and

[0029] **Figure 14** is a set of three plots of CD8+ T cell responses in three animals infected with 68-1.2 RhCMV-Rh156, 108 miR142/SIVgag and an epitope MHC-restriction map of these animals, in accordance with various embodiments.

Detailed Description of Disclosed Embodiments

[0030] In the following detailed description, reference is made to the accompanying drawings which form a part hereof, and in which are shown by way of illustration embodiments that may be practiced. It is to be understood that other embodiments may be utilized and structural or logical changes may be made without departing from the scope. Therefore, the following detailed description is not to be taken in a limiting sense, and the scope of embodiments is defined by the appended claims and their equivalents.

[0031] Various operations may be described as multiple discrete operations in turn, in a manner that may be helpful in understanding embodiments; however, the

order of description should not be construed to imply that these operations are order dependent.

[0032] The description may use perspective-based descriptions such as up/down, back/front, and top/bottom. Such descriptions are merely used to facilitate the discussion and are not intended to restrict the application of disclosed embodiments.

[0033] The terms “coupled” and “connected,” along with their derivatives, may be used. It should be understood that these terms are not intended as synonyms for each other. Rather, in particular embodiments, “connected” may be used to indicate that two or more elements are in direct physical or electrical contact with each other. “Coupled” may mean that two or more elements are in direct physical or electrical contact. However, “coupled” may also mean that two or more elements are not in direct contact with each other, but yet still cooperate or interact with each other.

[0034] For the purposes of the description, a phrase in the form “A/B” or in the form “A and/or B” means (A), (B), or (A and B). For the purposes of the description, a phrase in the form “at least one of A, B, and C” means (A), (B), (C), (A and B), (A and C), (B and C), or (A, B and C). For the purposes of the description, a phrase in the form “(A)B” means (B) or (AB) that is, A is an optional element.

[0035] The description may use the terms “embodiment” or “embodiments,” which may each refer to one or more of the same or different embodiments. Furthermore, the terms “comprising,” “including,” “having,” and the like, as used with respect to embodiments, are synonymous.

[0036] Embodiments herein provide novel, recombinant CMV vectors including but not limited to recombinant CMV vectors comprising a nucleic acid encoding at least one heterologous protein antigen, and at least one microRNA recognition element specific for a microRNA expressed by a cell of myeloid lineage that is operably linked to a CMV gene that is essential or augmenting for growth *in vivo*. Methods of using the novel, recombinant CMV vectors, such as methods of generating an immune response to the heterologous antigen in the subject are further disclosed.

I. Terms

[0037] Unless otherwise noted, technical terms are used according to conventional usage. 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. Although methods and materials similar or equivalent to those described herein may be used in the practice or testing of this disclosure, suitable methods and materials are described below. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting. In order to facilitate review of the various embodiments of the disclosure, the following explanations of specific terms are provided:

[0038] 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 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.

[0039] Administration: As used herein, the term “administration” means to provide or give a subject an agent, such as a composition comprising an effective amount of a CMV vector comprising an exogenous antigen by any effective 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.

[0040] 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 may 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.

[0041] **MicroRNA:** As used herein, the term “microRNA” refers to a major class of biomolecules involved in control of gene expression. For example, in human heart, liver or brain, miRNAs play a role in tissue specification or cell lineage decisions. In addition, miRNAs influence a variety of processes, including early development, cell proliferation and cell death, and apoptosis and fat metabolism. The large number of miRNA genes, the diverse expression patterns, and the abundance of potential miRNA targets suggest that miRNAs may be a significant source of genetic diversity.

[0042] A mature miRNA is typically an 18-25 nucleotide non-coding RNA that regulates expression of an mRNA including sequences complementary to the miRNA. These small RNA molecules are known to control gene expression by regulating the stability and/or translation of mRNAs. For example, miRNAs bind to the 3' UTR of target mRNAs and suppress translation. MiRNAs may also bind to target mRNAs and mediate gene silencing through the RNAi pathway. MiRNAs may also regulate gene expression by causing chromatin condensation.

[0043] A miRNA silences translation of one or more specific mRNA molecules by binding to a miRNA recognition element (MRE,) which is defined as any sequence that directly base pairs with and interacts with the miRNA somewhere on the mRNA transcript. Often, the MRE is present in the 3' untranslated region (UTR) of the mRNA, but it may also be present in the coding sequence or in the 5' UTR. MREs are not necessarily perfect complements to miRNAs, usually having only a few bases of complementarity to the miRNA and often containing one or more mismatches within those bases of complementarity. The MRE may be any sequence capable of being bound by a miRNA sufficiently that the translation of a gene to which the MRE is operably linked (such as a CMV gene that is essential or augmenting for growth *in vivo*) is repressed by a miRNA silencing mechanism such as the RISC.

[0044] **Mutation:** As used herein, the term “mutation” refers to 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.

[0045] 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.

[0046] **Nucleotide sequences or nucleic acid sequences:** As used herein 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 may be single-stranded, or partially or completely double stranded (duplex). Duplex nucleic acids may be homoduplex or heteroduplex.

[0047] **Operably Linked:** As the term "operably linked" is used herein, a first nucleic acid sequence is operably linked with a second nucleic acid sequence when the first nucleic acid sequence is placed in such a way that it has an effect upon the second nucleic acid sequence. For instance, a MRE is operably linked to a coding sequence that it silences if binding of the miRNA to the MRE silences the expression of the coding sequence. Operably linked DNA sequences may be contiguous, or they may operate at a distance.

[0048] **Promoter:** As used herein, the term "promoter" may refer to any of a number of nucleic acid control sequences that directs transcription of a nucleic acid. Typically, a eukaryotic promoter includes necessary nucleic acid sequences near the start site of transcription, such as, in the case of a polymerase II type promoter, a TATA element or any other specific DNA sequence that is recognized by one or more transcription factors. Expression by a promoter may be further modulated by enhancer or repressor elements. Numerous examples of promoters are available and well known to those of ordinary skill in the art. A nucleic acid comprising a

promoter operably linked to a nucleic acid sequence that codes for a particular polypeptide may be termed an expression vector.

[0049] **Recombinant:** As used herein, the term "recombinant" with reference to a nucleic acid or polypeptide refers to 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 addition of a miRNA response element operably linked to a CMV gene that is essential or augmenting for growth *in vivo*. 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 may 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 polypeptides that form a CMV vector comprising a heterologous antigen).

[0050] **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 may be dissemination-deficient, e.g., they are capable of replicating their genomes, but unable to infect 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, e.g. 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 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.

[0051] **Pharmaceutically acceptable carriers:** As used herein, a "pharmaceutically acceptable carrier" of use is 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 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 may include, for example, pharmaceutical grades of mannitol, lactose, starch, or magnesium stearate. In addition to biologically neutral carriers, pharmaceutical compositions to be administered may 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.

[0052] 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.

[0053] 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 may 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.

[0054] Sequence identity/similarity: As used herein, 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 may be measured in terms of percentage identity; the higher the percentage, the more identical the sequences are. Sequence similarity may 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) may be called "homologs."

[0055] 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 addition, Altschul et al, *J Mol Biol* 215, 403-410 (1990), presents a detailed consideration of sequence alignment methods and homology calculations.

[0056] 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 Internet, for use in connection with the sequence analysis programs blastp, blastn, blastx, tblastn and tblastx. Additional information may be found at the NCBI web site.

[0057] 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 sequences do not share homology, then the designated output file will not present aligned sequences.

[0058] 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 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 ($1166/1554 \times 100 = 75.0$). The percent sequence identity value is rounded to the nearest tenth. For example, 75.11, 75.12, 75.13, and 75.14 are rounded down to 75.1, while 75.15, 75.16, 75.17, 75.18, and 75.19 are rounded up 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, $15/20 \times 100 = 75$).

[0059] 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 may 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.

[0060] When aligning short peptides (fewer than around 30 amino acids), the alignment is 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 may 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.

[0061] 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 may be made using this degeneracy to produce multiple nucleic acid molecules that all encode substantially the same protein. Such homologous nucleic acid sequences may, 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.

[0062] **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.

[0063] **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 refer to any observable beneficial effect of the treatment. The beneficial effect may 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

[0064] Disclosed herein are human or animal CMV vectors capable of repeatedly infecting an organism. The CMV vectors comprise a nucleic acid sequence that encodes a heterologous protein antigen and further comprise a nucleic acid sequence that serves as a miRNA response element (MRE) that silences expression in the presence of a miRNA expressed by myeloid cells. Examples of such miRNAs expressed by myeloid cells include miR-142-3p, miR-223, miR-27a, miR-652, miR-155, miR-146a, miR-132, miR-21, and/or miR-125 (Gilicze AB *et al*, *Biomed Res Int* 2014, 870267 (2014); incorporated by reference herein) The MRE is operably linked to a CMV gene that is essential or augmenting

for CMV growth *in vivo*. Examples of such genes include I-E2, and UL79, or orthologs thereof. One, two, three or more CMV genes may each be operably linked to one, two, three or more MREs in the vector. The vector also encodes an active UL128 gene and an active UL130 gene. Alternatively, the vectors may lack an active UL128 and an active UL130 gene.

[0065] The MRE may be any miRNA recognition element that silences expression in the presence of a miRNA expressed by myeloid cells. Such an MRE may be the exact complement of a miRNA. Alternatively, other sequences may be used as MREs for a given miRNA. For example, MREs may be predicted from sequences. In one example, the miRNA may be searched on the website microRNA.org (www.microrna.org). In turn, a list of mRNA targets of the miRNA will be listed. For example, the following page:

<http://www.microrna.org/microrna/getTargets.do?matureName=hsa-miR-142-3p&organism=9606>, last accessed 06 Oct 2015, will list putative mRNA targets of miR-142-3p. For each listed target on the page, 'alignment details' may be accessed and putative MREs accessed.

[0066] One of ordinary skill in the art may select a validated, putative, or mutated MRE sequence from the literature that would be predicted to induce silencing in the presence of a miRNA expressed in a myeloid cell such as a macrophage. One example involves the above referenced website. The person of ordinary skill in the art may then obtain an expression construct whereby a reporter gene (such as a fluorescent protein, enzyme, or other reporter gene) has expression driven by a promoter such as a constitutively active promoter or cell specific promoter. The MRE sequence may then be introduced into the expression construct. The expression construct may be transfected into an appropriate cell, and the cell transfected with the miRNA of interest. A lack of expression of the reporter gene indicates that the MRE silences gene expression in the presence of the miRNA.

[0067] The heterologous antigen may be any antigen, including a pathogen specific antigen derived from, for example, human immunodeficiency virus, simian immunodeficiency virus, herpes simplex virus type 1, herpes simplex virus type 2, hepatitis B virus, hepatitis C virus, papillomavirus, Plasmodium parasites, *Clostridium tetani*, and *Mycobacterium tuberculosis*. In still further examples, the heterologous antigen may 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, anal or rectal cancer, renal cell carcinoma (RCC), and germ cell tumors. In still further examples, the heterologous antigen may 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.

[0068] In some examples, the vector does not express an active UL82 (pp71), UL79, US11, or other CMV protein due to the presence of a mutation in the nucleic acid sequence encoding UL82 (pp71), UL79, US11, or other CMV proteins, including homologs thereof or orthologs thereof (homologous genes of CMVs that infect other species). In some embodiments, the vector does not express an active UL82 (pp71) protein or an active UL79 protein, or orthologs thereof. In some embodiments, the vector does not express an active UL82 (pp71) protein or an active US11 protein, or orthologs thereof. In some embodiments, the vector does not express an active UL79 protein or an active US11 protein, or orthologs thereof. In some embodiments, the vector does not express an active UL79 protein, an active UL82 (pp71) protein, or an active US11 protein, or orthologs thereof. The mutation may be any mutation that results in a lack of expression of the active protein. Such mutations may 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.

[0069] 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. The CMV vector has a nucleic acid sequence that encodes at least one heterologous antigen and a nucleic acid sequence that serves as a miRNA response element (MRE) for a miRNA that is expressed by myeloid cells. The MRE is operably linked to a CMV gene that is essential or augmenting for CMV growth *in vivo*.

[0070] In some examples, the CMV vectors also contain nucleic acid sequences that encode an active UL128 protein and an active UL130 protein. These

vectors may be used to elicit a CD8+ T cell response that is characterized by having at least 10% of the CD8+ T cells directed against epitopes presented by Class II MHC. In some embodiments, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, or at least 75%, of the CD8+ T cells are restricted by Class II MHC. This is an unpredictable result because previous studies showed that Class II MHC restricted CD8+ T cells were generated in CMV lacking UL128 and UL130 (Hansen SG *et al*, *Science* 340, 1237874 (2013); incorporated by reference herein). Less than 10% of the CD8+ T cells are restricted by MHC-E.

[0071] In some examples, the CMV vectors do not express an active UL128 and UL130 gene due to the presence of a mutation in the nucleic acid sequence encoding UL128 and UL130 and/or other CMV proteins including homologs thereof or orthologs thereof (homologous genes of CMVs that infect other species). The mutation may be any mutation that results in a lack of expression of the active protein. Such mutations may 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. These vectors may be used to elicit a CD8+ T cell response that is characterized by having less than 10% of the CD8+ T cells restricted by MHC-I and at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, or at least 75%, of the CD8+ T cells are restricted by Class II MHC. These vectors may also be used to elicit CD8+ T cell responses to MHC-II supertopes, e.g., peptides presented by disparate MHC-II molecules to CD8+ T cells. Supertopes are thus recognized by individuals that do not share the same MHC-II alleles.

[0072] 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 may be any CMV vector. The second heterologous antigen may be any heterologous antigen, including a heterologous antigen identical to the heterologous antigen in the first CMV vector. The second CMV vector may 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.

[0073] 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 by limiting intrahost and host-to-host spread in the selected subjects.

[0074] A heterologous antigen may be any protein or fragment thereof that is not derived from CMV, including cancer antigens, pathogen specific antigens, model antigens (such as lysozyme KLH, or ovalbumin), tissue-specific antigens, host self-antigens, or any other antigen.

[0075] Pathogen specific antigens may 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.

[0076] The antigen may be a protein derived from cancer. These 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.

[0077] The antigen may 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 may be a tissue-specific antigen. Tissue-specific antigens include, but are not limited to, sperm antigens or egg antigens.

[0078] The CMV vectors disclosed herein may be used as an immunogenic, immunological, or vaccine composition containing the recombinant CMV virus or vector and a pharmaceutically 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 may, 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 may, but need not be, protective. A 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 may be protective compositions).

[0079] The CMV vectors disclosed herein may be used in methods of inducing an immunological response in a subject comprising administering to the subject an immunogenic, immunological, 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" is "mammal", which for purposes of this specification includes all mammals, except humans.

[0080] The CMV vectors disclosed herein may be used in therapeutic compositions containing the recombinant CMV virus or vector and a pharmaceutically acceptable carrier or diluent. The CMV vectors disclosed herein may be prepared by inserting DNA comprising a sequence that encodes the heterologous antigen into an essential or non-essential region of the CMV genome. The method may further comprise deleting one or more regions from the CMV genome. The method may comprise *in vivo* recombination. Thus, the method may 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.

[0081] The method may 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 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 nonessential region of CMV is flanking the donor DNA. The heterologous DNA may be inserted into CMV to generate the recombinant CMV in any orientation that yields stable integration of that DNA, and expression thereof, when desired.

[0082] The DNA encoding the heterologous antigen in the recombinant CMV vector may also include a promoter. The promoter may be from any source such as a herpes virus, including an endogenous cytomegalovirus (CMV) promoter, such as a human CMV (HCMV), rhesus macaque CMV (RhCMV), murine CMV (MCMV), or other CMV promoter. The promoter may also be a non-viral promoter such as the EF1 α promoter. The promoter may 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 derived. The promoter may 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-length promoter is not completely present, i.e., that some portion of the full-length promoter has been removed. And, the truncated promoter may be derived from a herpesvirus such as MCMV or HCMV, e.g., HCMV-IE or MCMV-IE. There may be up to a 40% and even up to a 90% reduction in size, from a full-length promoter, based upon base pairs.

[0083] The promoter may 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 infectious diseases reference is made to *Science*, 259:1745-49, 1993. It is therefore within the scope of this disclosure that the vector may be used by the direct injection of vector DNA.

[0084] Also disclosed is an expression cassette that may be inserted into a recombinant virus or plasmid comprising the truncated transcriptionally active promoter. The expression cassette may further include a functional truncated polyadenylation signal; for instance an SV40 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 may also include heterologous DNA with respect to the virus or system into which it is inserted; and that DNA may be heterologous DNA as described herein.

[0085] 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 may be used. As to heterologous antigens, one of ordinary skill in the art may select a heterologous antigen and the 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.

[0086] One method to determine T epitopes of an antigen involves epitope mapping. Overlapping peptides of the heterologous antigen are generated by oligo-peptide synthesis. 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.

[0087] 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.

[0088] It is noted that the DNA comprising the sequence encoding the heterologous antigen may itself include a promoter for driving expression in the CMV

vector or the DNA may be limited to the coding DNA of the heterologous antigen. This construct may 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, may be done so as to amplify or increase expression. Thus, the DNA encoding the heterologous antigen may be suitably positioned with respect to a CMV-endogenous promoter, or those promoters may be translocated to be inserted at another location together with the DNA encoding the heterologous antigen. Nucleic acids encoding more than one heterologous antigen may be packaged in the CMV vector.

[0089] Further, disclosed are pharmaceutical and other compositions containing the disclosed CMV vectors. Such pharmaceutical and other compositions may be formulated so as to be used in any administration procedure known in the art. Such pharmaceutical compositions may be via a parenteral route (intradermal, intramuscular, subcutaneous, intravenous, or others). The administration may also be via a mucosal route, e.g., oral, nasal, genital, etc.

[0090] The disclosed pharmaceutical compositions may be prepared in accordance with standard techniques well known to those of ordinary skill in the pharmaceutical arts. Such compositions may be administered in dosages and by techniques well known to those of ordinary skill 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 may be administered alone, or may be co-administered or sequentially administered with other CMV vectors or with other immunological, antigenic or vaccine or therapeutic compositions. Such other compositions may include purified native antigens or epitopes or antigens or epitopes from the expression by a recombinant CMV or another vector system; and are administered taking into account the aforementioned factors.

[0091] 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 compositions the recombinant may be in

admixture with a suitable carrier, diluent, or excipient such as sterile water, physiological saline, glucose or the like.

[0092] Antigenic, immunological, or vaccine compositions typically may 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 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 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.) may also be used.

[0093] The composition may be packaged in a single dosage form for immunization by parenteral (e.g., intramuscular, intradermal or subcutaneous) administration or orifice 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 LD₅₀ and other screening procedures which are known and do not require undue experimentation. Dosages of expressed product may range from a few to a few hundred micrograms, e.g., 5 to 500 µg. The CMV vector may be administered in any suitable amount to achieve expression at these dosage levels. In nonlimiting examples: CMV vectors may be administered in an amount of at least 10² pfu; thus, CMV vectors may be administered in at least this amount; or in a range from about 10² pfu to about 10⁷ pfu. Other suitable carriers or diluents may be water or a buffered saline, with or without a preservative. The CMV vector may be lyophilized for resuspension at the time of administration or may be in solution. "About" may mean within 1%, 5%, 10% or 20% of a defined value.

[0094] It should be understood that the proteins and the nucleic acids encoding them of the present disclosure may differ from the exact sequences illustrated and described herein. Thus, the disclosure contemplates deletions, additions, truncations, and substitutions to the sequences shown, so long as the sequences function in accordance with the methods of the disclosure. 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, 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. 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.

[0095] The nucleotide sequences of the present disclosure may be codon optimized, for example the codons may be optimized for use in human cells. For example, any viral or bacterial sequence may 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 in the desired subject, enhanced expression of the heterologous antigen may be achieved as described in Andre *et al.*, *J. Virol.* 72:1497-1503, 1998.

[0096] 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 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; valine/soleucine/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 96%, at least 97%, at least 98% or at least 99% homology or identity to the antigen, epitope, immunogen, peptide or polypeptide of interest.

[0097] 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 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.

[0098] 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 may 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.

[0099] Advantageous for use according to the present disclosure is the WU-BLAST (Washington University BLAST) version 2.0 software. WU-BLAST version 2.0 executable programs for several UNIX platforms may 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., *Journal of Molecular Biology* 1990; 215:

403-410; Gish & States, 1993; *Nature Genetics* 3: 266-272; Karlin & Altschul, 1993; *Proc. Natl. Acad. Sci. USA* 90: 5873-5877; all of which are incorporated by reference herein).

[00100] The various recombinant nucleotide sequences and antibodies and/or antigens of the disclosure are made using standard recombinant DNA and cloning techniques. Such techniques are well known to those of ordinary skill in the art. See for example, "*Molecular Cloning: A Laboratory Manual*", second edition (Sambrook et al. 1989).

[00101] The nucleotide sequences of the present disclosure may be inserted into "vectors." The term "vector" is widely used and understood by those of ordinary skill in the art, and as used herein the term "vector" is used consistent with its meaning to those of ordinary skill in the art. For example, the term "vector" is commonly used by those of ordinary skill 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.

[00102] Any vector that allows expression of the viruses of the present disclosure may be used in accordance with the present disclosure. In certain embodiments, the disclosed viruses may 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.

[00103] 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 translation of the coding sequence under the influence or control of the nucleic acid control sequence. The "nucleic acid control sequence" may be any nucleic acid element, such as, but not limited to promoters, enhancers, IRES, introns, miRNA recognition elements (MREs) 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 that are clustered around the initiation site for RNA polymerase II and that when operationally linked to the protein coding sequences of the disclosure lead to the expression of the encoded protein. The expression of the transgenes of the present disclosure may 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 tetracycline, hormones such as ecdysone, or heavy metals. The promoter may 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 disclosure. For example, suitable promoters and/or enhancers may be selected from the Eukaryotic Promoter Database (EPDB).

[00104] 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 may be used. Any antigen recognized by an HIV antibody may be used as an HIV protein antigen. The protein antigen may also be an SIV antigen. For example, the SIV antigens discussed in U.S. Pub. Nos. 2008/0199493 A1 and 2013/0136768 A1 may be used.

[00105] The vectors used in accordance with the present disclosure may contain a suitable gene regulatory region, such as a promoter or enhancer, such that the antigens may be expressed.

[00106] Expressing antigens of the disclosure *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 disclosure. In other examples, one may express the antigens in human

subjects, such as in clinical trials and for actual clinical use of the immunogenic compositions and vaccine of the disclosure.

[00107] The CMV vectors described herein may contain mutations that may prevent intrahost and host to host spread, thereby rendering the virus unable to spread in or infect immunocompromised or other subjects that could face complications as a result of CMV infection. The CMV vectors described herein may also contain mutations that result in the presentation of immunodominant and nonimmunodominant epitopes as well as unconventional MHC restriction. However, mutations in the CMV vectors described herein do not affect the ability of the vector to reinfect 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.

[00108] The disclosed CMV vectors may 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 (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 in clinical trials and for actual clinical use of the immunogenic compositions using HCMV.

[00109] 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 disclosure are useful to stimulate an immune response against the heterologous antigen, including a pathogen specific antigen and may be 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 disclosure are particularly useful for providing genetic vaccines, i.e. vaccines for delivering the nucleic acids encoding the antigens of the disclosure to a subject, such as a human, such that the antigens are then expressed in the subject to elicit an immune response.

[00110] Immunization schedules (or regimens) are well known for animals (including humans) and may be readily determined for the particular subject and immunogenic composition. Hence, the immunogens may 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 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 disclosure, 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 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 may also include administration of an adjuvant with the immunogens. In some instances, annual, biannual or other long interval (5-10 years) booster immunization may 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 may 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 may also be varied. For example, if an expression vector is used for the priming and boosting steps, it may 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 ordinary skill in the art that there are several permutations and combinations that are encompassed using the DNA, bacterial and viral expression vectors of the disclosure to provide priming and boosting regimens. CMV vectors may be used repeatedly while expressing different antigens derived from different pathogens.

EXAMPLES

[00111] *Example 1 – CMV Vectors Comprising MicroRNA Recognition Elements (MRE)*

[00112] In order to limit the ability of the RhCMV-based vectors to replicate in myeloid lineage cells, CMV was engineered to express the myeloid-specific miRNA miR-142 3p. It was hypothesized that insertion of miR-142 3p target sites into the 3' UTRs of essential RhCMV genes would result in an inhibition of viral replication due to degradation of the targeted viral mRNAs in cells that express the miRNA.

[00113] The miR-142 3p miRNA is highly expressed in rhesus macaque macrophages derived from the peripheral blood of three animals (Figure 1). Whole blood was fractionated, and CD14-positive cells were isolated using magnetic cell sorting with CD14 beads. CD14-positive cells were plated in RPMI with M-CSF or in a 1:1 mix of RPMI and M-CSF-conditioned RPMI. After 7 days, the CD14-positive cells adhered to the tissue culture plate and differentiated into more mature macrophages. These cells were harvested in Trizol and RNA was isolated using standard procedures. RNA from rhesus fibroblasts was also isolated and qRT-PCR for miR-142 3p was performed on all samples. These data demonstrate that rhesus macaque myeloid lineage cells express much higher levels of miR-142 3p compared to rhesus fibroblasts and provides a rationale for using this miRNA to target essential RhCMV transcripts.

[00114] In order to better understand the expression of miR-142 3p in other cell types RNA was isolated from a variety of homogenized fetal and adult tissues using Trizol (Figure 2). qRT-PCR for miR-142-3p was performed using a miRNA mimic as a standard control. The highest expression of miR-142-3p (~10⁷ copies/10 ng of RNA) was detected in tissues that normally contain large numbers of myeloid cells, including fetal spleen, lymphocytes and liver, as well as in the adult lung. Thus miR-142-3p expression is detectable in a variety of tissues. However it was unclear if the miRNA arose from resident or circulating myeloid-lineage cells or is expressed from other cell types in the tissue. These data demonstrate that limiting replication of the vaccine vector in myeloid-lineage cells may prevent the dissemination of the vector to a wide variety of tissues.

[00115] Since the above data demonstrates that miR-142-3p is highly expressed in rhesus macaque myeloid-lineage cells, vaccine vector tropism was

altered by using miR-142-3p as a host restriction factor. RhCMV 68-1 (UL128/UL130-deleted) and 68-1.2 (UL128/UL130-intact) viruses containing miR-142-3p target sites within the 3' UTRs of Rh156 (an essential immediate early gene homologous to HCMV IE2) and Rh108 (an essential early gene homologous to HCMV UL79) were generated. These vectors additionally expressed a heterologous antigen derived from SIV, e.g., SIVgag or SIV retanef, by inserting this antigen under control of the EF1a promoter into the gene Rh211.

[00116] In cells in which miR-142-3p levels are high, this virus will be severely limited for growth as miR-142-3p will bind to the 3' UTRs of Rh156 and Rh108 and degrade the mRNAs thus severely inhibiting viral replication. Using galK-mediated BAC recombination the galK selection cassette was inserted within the 3' UTR of Rh156 and then replaced with an artificial cassette containing 4 copies of the miR-142-3p binding site separated by 8 random nucleotides. A second series of recombinations was then performed, thereby inserting the galK cassette into the 3' UTR of Rh108 and replacing this with an artificial cassette as above (Figure 10, 3A, 11A). Successful recombinations were confirmed using PCR with primers designed to regions flanking the insertion site and subsequent sequencing of the PCR products. The integrity of the BAC genome was assessed using XmaI digestion of purified BAC DNA followed by electrophoresis on agarose gels (Figure 3B, 11B). Intact BAC DNA was subsequently electroporated into primary rhesus fibroblasts and individual viral plaques were isolated and propagated for a total of three passages. Viral DNA was isolated from supernatants at each stage and assessed for maintenance of the miR-142-3p cassette by PCR using primers flanking the site and sequencing of the PCR products (Figure 3C, 11A).

[00117] The 68-1 and 68-1.2 Rh156/Rh108 virus produced from the above BAC recombinations was then analyzed for growth in the presence of miR-142-3p using a multi-step growth curve (Figures 4, 9). Rhesus fibroblasts were transfected using Lipofectamine 2000® with either negative control miRNA or a miR-142-3p mimic. Two days after transfection, cells were infected with either wild type 68-1 or the 68-1 Rh156/Rh108 miR-142-3p mutant at an MOI of 0.01 (Figure 4). Alternatively, two days after transfection, cells were infected with either wild type 68-1.2 or the 68-1.2 Rh156/Rh108 miR-142-3p mutant at an MOI of 0.01 (Figure 9). Cell pellets and supernatants were harvested at the indicated times and titered on

primary rhesus fibroblasts. The presence of miR-142-3p mimic did not have any effect on the growth of WT virus. Importantly, the 68-1 Rh156/Rh108 miR-142-3p and 68-1.2 Rh156/Rh108 miR-142-3p mutant viruses did not show any growth defect in the presence of negative control miRNA but were severely inhibited for growth in the presence of miR-142-3p. For example, the presence of miR-142-3p reduced the viral titer of the 68-1 Rh156/Rh108 miR-142-3p mutant virus by up to 4 logs but had no effect on the viral titer of the wild type 68-1 virus (Figure 4). Therefore, the virus with miR-142-3p target sites engineered into the 3' UTRs of two essential genes was severely limited for growth in the presence of the miRNA *in vitro*, indicating that this virus would be unable to replicate in cells of the myeloid lineage *in vivo*, where miR-142-3p levels are naturally high.

[00118] The ability of the 68-1 Rh156/Rh108 miR-142-3p virus to replicate in *ex vivo*-derived rhesus macrophages was tested (Figure 5). CD14+ cells from rhesus peripheral blood were isolated and differentiated as described above and infected with WT virus or the 68-1 Rh156/Rh108 miR-142-3p virus at an MOI of 10. Viral DNA levels were assessed using qPCR for Rh87 and GAPDH (as a control) at 8 hours as well as 5 and 10 days post-infection. While viral DNA levels increased over time in the cells infected with WT virus, little increase in viral DNA was detected in cells infected with the mutant virus. These data indicate that the miR-142-3p target sites inserted into the viral genome limit viral replication in rhesus macrophages and is further evidence that miR-142-3p acts as a strong host restriction factor in this cell type.

[00119] The immunogenicity of the miR-142-3p-restricted viruses was assessed *in vivo*. Initially, two adult animals were infected with the 68-1 RhCMV Rh156/Rh108 miR-142-3p/SIVrtn virus (which also contains the RTN transgene (a fusion of SIV rev, tat and nef) under control of the EF1 α promoter) and peripheral blood CD4+ and CD8+ memory T cells were isolated and analyzed for responses to overlapping RTN peptides (Figure 6). Both animals elicited robust, long-term CD4 and CD8 T cell responses to the transgene, indicating that overall immunogenicity of the vaccine vector is not compromised by the introduction of miR-142-3p target sites.

[00120] Peripheral T cell responses in animals infected with a 68-1.2 RhCMV Rh156/Rh108 miR-142-3p/SIVgag virus (which also contains the GAG transgene under control of the EF1 α promoter) were then assessed. As seen in Figure 7 and

Figure 14, this vaccine vector also elicits robust and long-term CD4+ and CD8+ T cell responses to the HIV gag protein encoded within the vaccine vector. It has been previously demonstrated that vaccine vectors lacking UL128 and UL130 (such as 68-1) elicit unconventional MHC-E and MHC-II restricted CD8 T cell responses, including so-called 'supertope' responses that are detectable in all infected animals. In vectors comprising intact UL128 and UL130 (such as 68-1.2) only conventional MHC-I restricted CD8 T cells are detected. **Figures 7 and 14** demonstrate that both MHC-E and MHC-II restricted 'supertopes' are absent in animals infected with the 68-1.2 Rh156/Rh108 miR-142-3p virus.

[00121] Unexpectedly however, through further epitope mapping the T cell responses (**Figures 8 and 14**) it was determined that predominantly unconventional MHC-II restricted T cell responses were elicited by the 68-1.2 Rh156/Rh108 miR-142-3p/SIVgag virus, with relatively few MHC-Ia responses detected. By altering the myeloid cell tropism of 68-1.2 (UL128 and UL130-intact) the response was switched from conventional MHC-Ia to unconventional MHC-II restricted T cell responses, whereas both MHC-E responses and MHC-II 'supertope' responses are lacking. These data demonstrate an alternative method for eliciting the unconventional MHC-II restricted CD8 T cells through preventing viral replication in myeloid lineage cells. In addition, since macrophages play an important role in viral dissemination *in vivo*, using a miR-142 3p-based vector may improve vaccine safety.

[00122] Peripheral T cell responses were also assessed in animals infected with a 68-1 RhCMV Rh156/Rh108 miR-142-3p/SIVgag virus (which also contains the GAG transgene under control of the EF1 α promoter). As seen in **Figure 12**, this vaccine vector also elicits robust and long-term CD4+ and CD8+ T cell responses to the HIV gag protein encoded within the vaccine vector. It has been previously demonstrated that 68-1-based vectors lacking UL128 and UL130 elicit unconventional MHC-E and MHC-II restricted CD8 T cell responses, including so-called 'supertope' responses that are detectable in all infected animals. However, **Figure 12** demonstrates that whereas the 68-1 RhCMV Rh156/Rh108 miR-142-3p virus retained its ability to elicit supertope responses to MHC-II, this vector failed to elicit supertope responses restricted by MHC-E. Furthermore, through further epitope mapping the T cell responses (**Figure 13**) it was determined that the 68-1 RhCMV Rh156/Rh108 miR-142-3p vector exclusively elicited MHC-II restricted

CD8+ T cell responses without any MHC-1-restricted or MHC-E-restricted CD8+ T cells. Thus, this vector elicits only MHC-11 restricted CD8+ T cells. Since CD4+ T cells elicited by RhCMV are always restricted by MHC-11 these data demonstrate that by introducing miR-142-3p targeting sites into vectors that lack UL 128 and UL 130 it is possible to generate a vaccine that elicits only MHC-11 restricted responses, including MHC-11 supertope responses that are shared among many different individuals.

[00123] Although certain embodiments have been illustrated and described herein, it will be appreciated by those of ordinary skill in the art that a wide variety of alternate and/or equivalent embodiments or implementations calculated to achieve the same purposes may be substituted for the embodiments shown and described without departing from the scope. Those with ordinary skill in the art will readily appreciate that embodiments may be implemented in a very wide variety of ways. This application is intended to cover any adaptations or variations of the embodiments discussed herein. Therefore, it is manifestly intended that embodiments be limited only by the claims and the equivalents thereof.

[00124] Definitions of the specific embodiments of the invention as claimed herein follow.

[00125] In a first aspect, the invention relates to a cytomegalovirus (CMV) vector comprising:

- (a) a first nucleic acid sequence encoding at least one heterologous antigen;
- (b) a second nucleic acid sequence comprising two or more microRNA recognition elements (MRE) operably linked to each of I-E2, or an ortholog thereof, and UL79, or an ortholog thereof, and wherein the MRE is complementary to a microRNA that is expressed by a cell of myelod lineage and silences expression in the presence of miR-142-3p; and
- (c) a third nucleic acid sequence that encodes an active UL128 protein or ortholog thereof; and a fourth nucleic acid sequence that encodes an active UL130 protein or ortholog thereof.

[00126] In a second aspect, the invention relates to a method of generating an immune response to the at least one heterologous antigen in a subject, the method comprising administering to the subject the CMV vector of the first aspect in an amount effective to elicit a CD8+ T cell response to the at least one heterologous antigen in the subject.

[00127] In a third aspect, the invention relates to use of a CMV vector of the first aspect in the manufacture of a medicament for generating an immune response to the at least one heterologous antigen in a subject.

[00128] The term “comprise” and variants of the term such as “comprises” or “comprising” are used herein to denote the inclusion of a stated integer or stated integers but not to exclude any

other integer or any other integers, unless in the context or usage an exclusive interpretation of the term is required.

[00129] Any reference to any prior art in this specification is not, and should not be taken as an acknowledgement or any form of suggestion that the prior art forms part of the common general knowledge.

Claims

What is claimed is:

1. A cytomegalovirus (CMV) vector comprising:
 - (a) a first nucleic acid sequence encoding at least one heterologous antigen;
 - (b) a second nucleic acid sequence comprising two or more microRNA recognition elements (MRE) operably linked to each of I-E2, or an ortholog thereof, and UL79, or an ortholog thereof, and wherein the MRE is complementary to a microRNA that is expressed by a cell of myelod lineage and silences expression in the presence of miR-142-3p; and
 - (c) a third nucleic acid sequence that encodes an active UL128 protein or ortholog thereof; and a fourth nucleic acid sequence that encodes an active UL130 protein or ortholog thereof.
2. The CMV vector of claim 1, 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 at least one heterologous antigen is:
 - (a) 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;
 - (b) an antigen 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*; or
 - (c) an antigen 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.
4. The CMV vector of any one of claims 1-3, wherein the CMV vector does not express one or more of an active UL82 (pp71) protein or an active US11 or an ortholog thereof.
5. The CMV vector of any one of claims 1-4, wherein the CMV vector is a human CMV (HCMV) or rhesus CMV (RhCMV) vector.
6. A method of generating an immune response to the at least one heterologous antigen in a subject, the method comprising administering to the subject the CMV vector of any one of claims 1-5 in an amount effective to elicit a CD8+ T cell response to the at least one heterologous antigen in the subject.

7. Use of a CMV vector of any one of claims 1-5 in the manufacture of a medicament for generating an immune response to the at least one heterologous antigen in a subject.

8. The use of claim 7, wherein the subject is administered the CMV vector in an amount effective to elicit a CD8+ T cell response to the at least one heterologous antigen.

9. The method of claim 6 or the use of claim 8, wherein at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60% or at least 75% of the CD8+ T cells are restricted by Class II MHC or an ortholog thereof.

10. The method of claim 6 or claim 9, or the use of any one of claims 7-9, wherein the subject has been previously exposed to CMV.

11. The method of any one of claims 6, 9, and 10, or the use of any one of claims 7-10, wherein the subject is a human or nonhuman primate.

12. The method of any one of claims 6 and 9-11, or the use of any one of claims 7-11, wherein administration of the CMV vector comprises subcutaneous, intravenous, intramuscular, intraperitoneal, or oral administration of the CMV vector.

13. The method of any one of claims 6 and 9-12, or the use of any one of claims 7-13, wherein,

(i) the infectious disease pathogen is 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*; or

(ii) the cancer is 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.

1/14

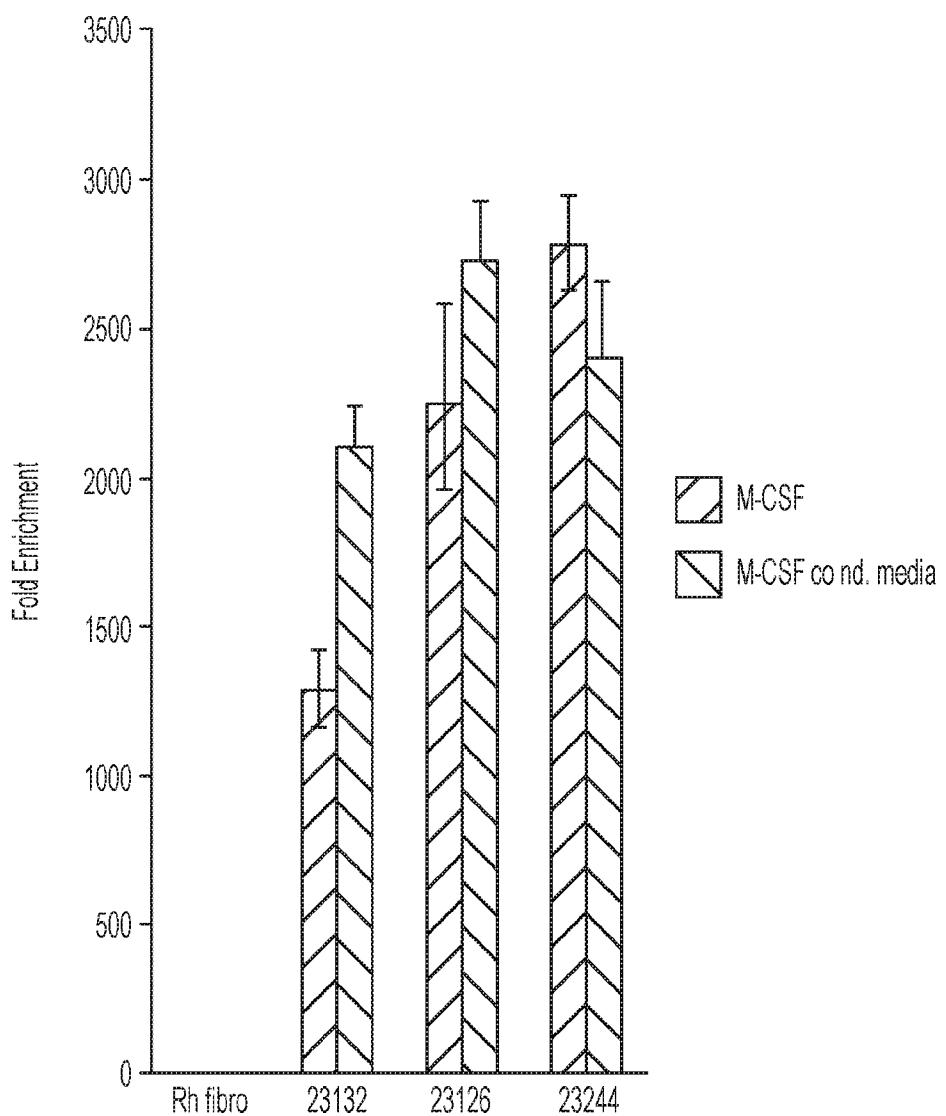


Figure 1

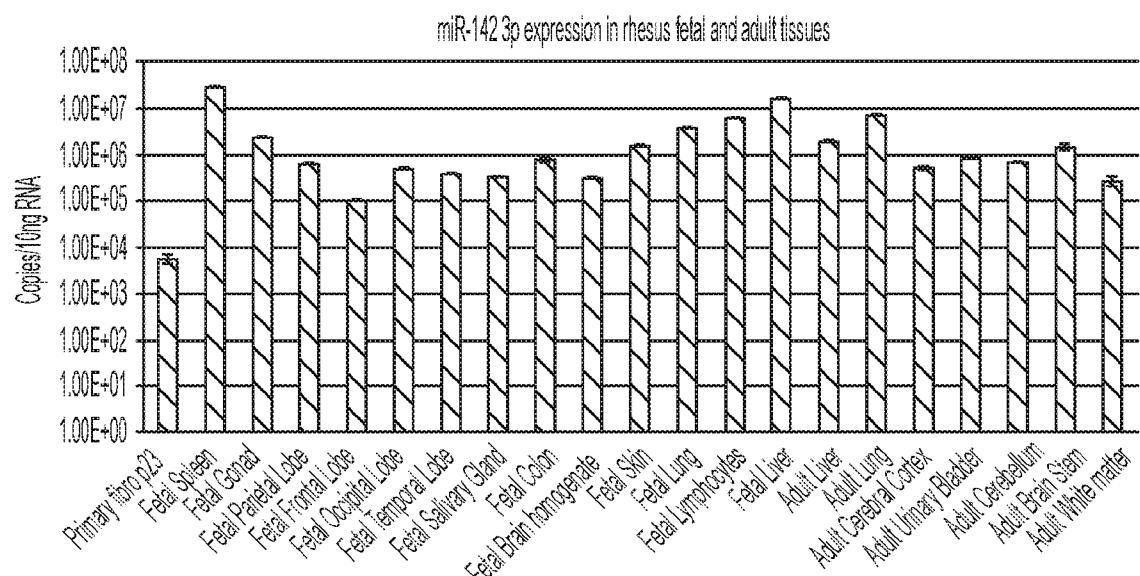


Figure 2

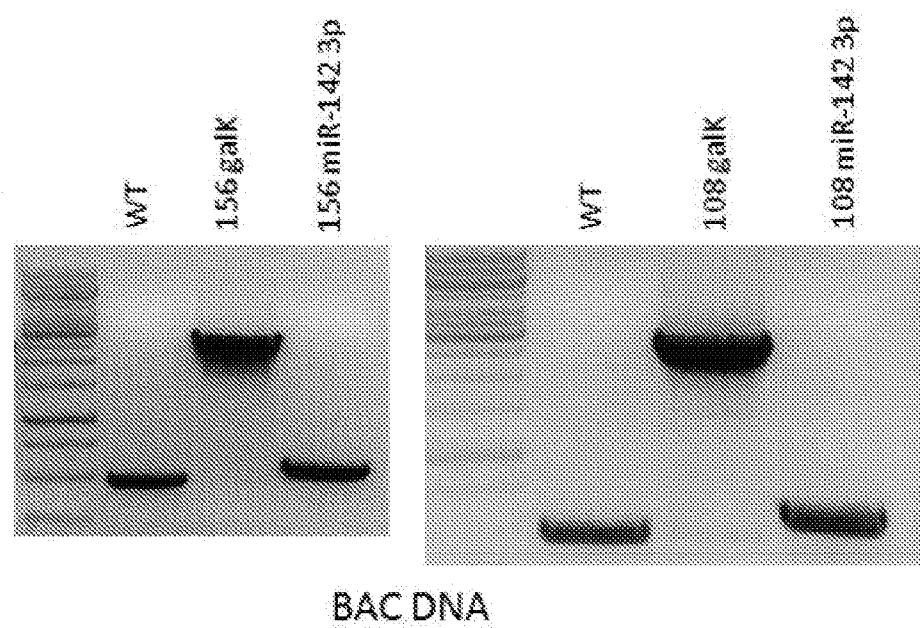


Figure 3A

3/14

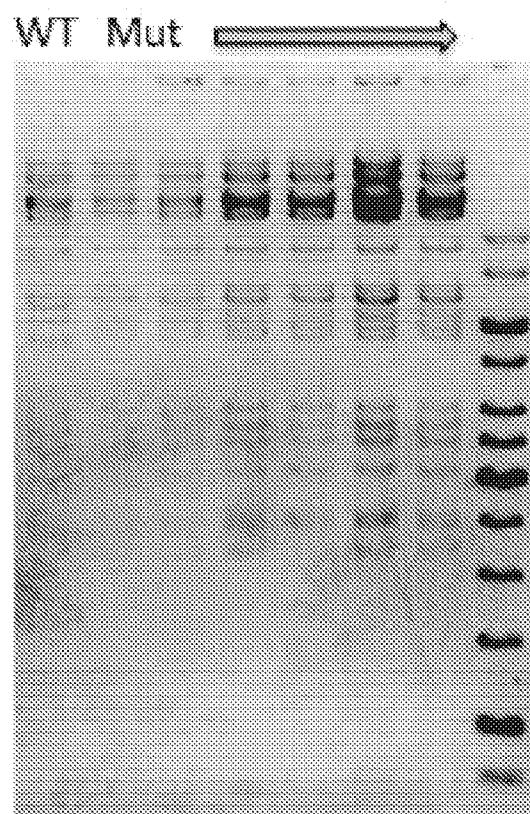


Figure 3B

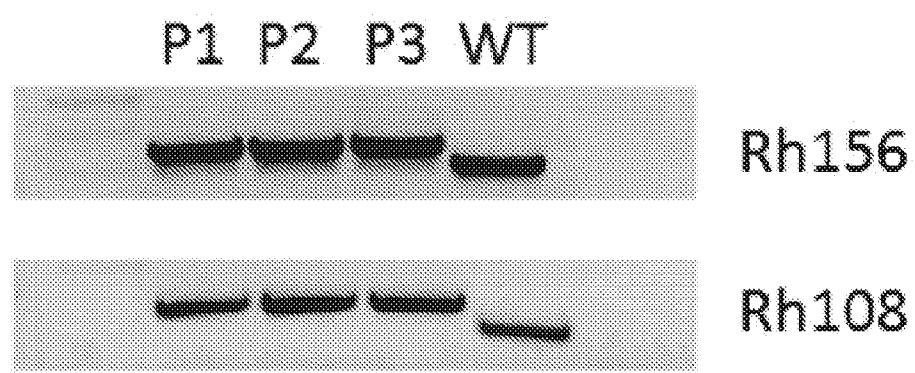


Figure 3C

4/14

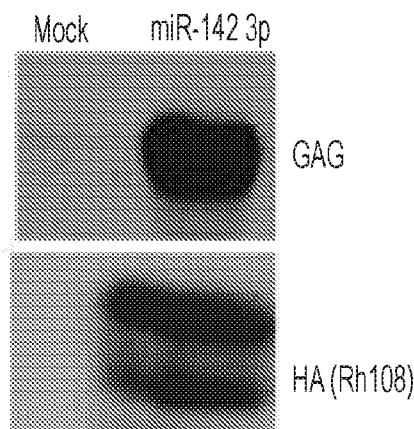


Figure 3D

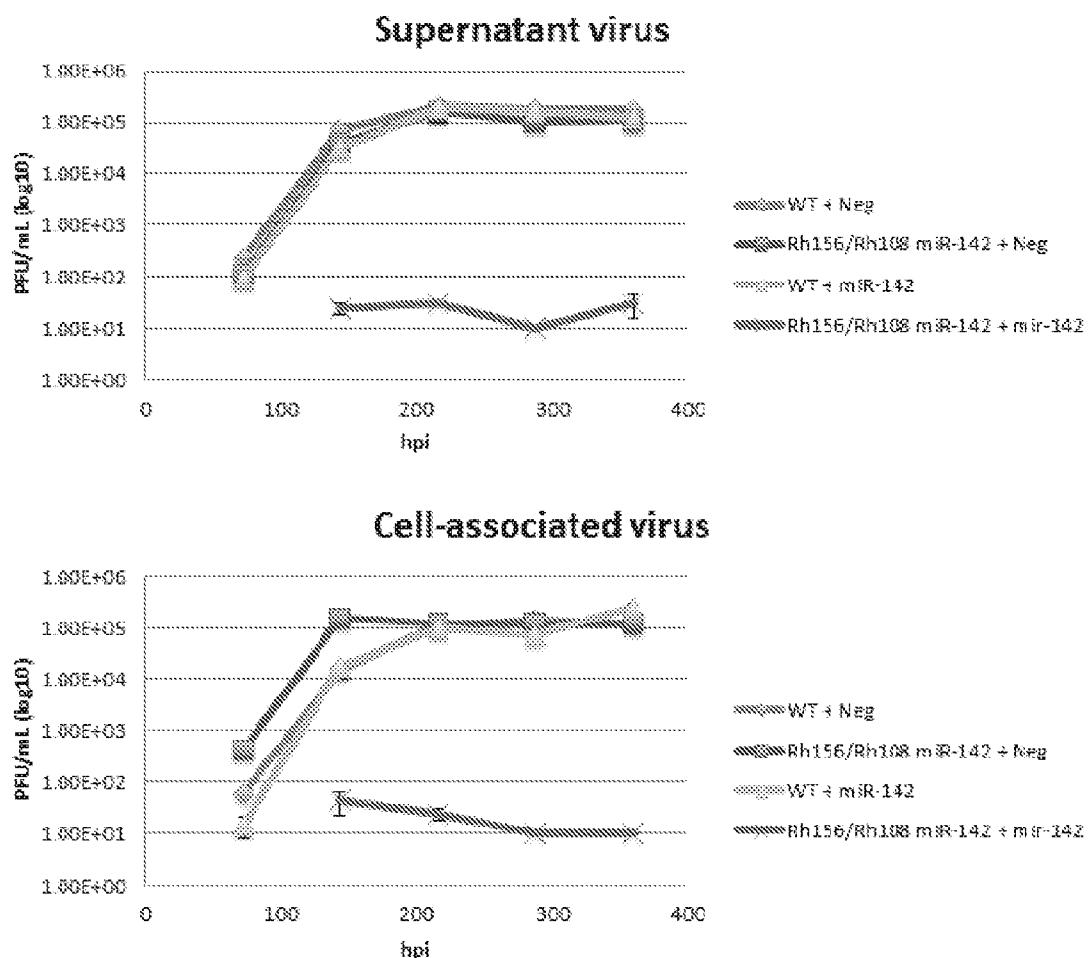


Figure 4

5/14

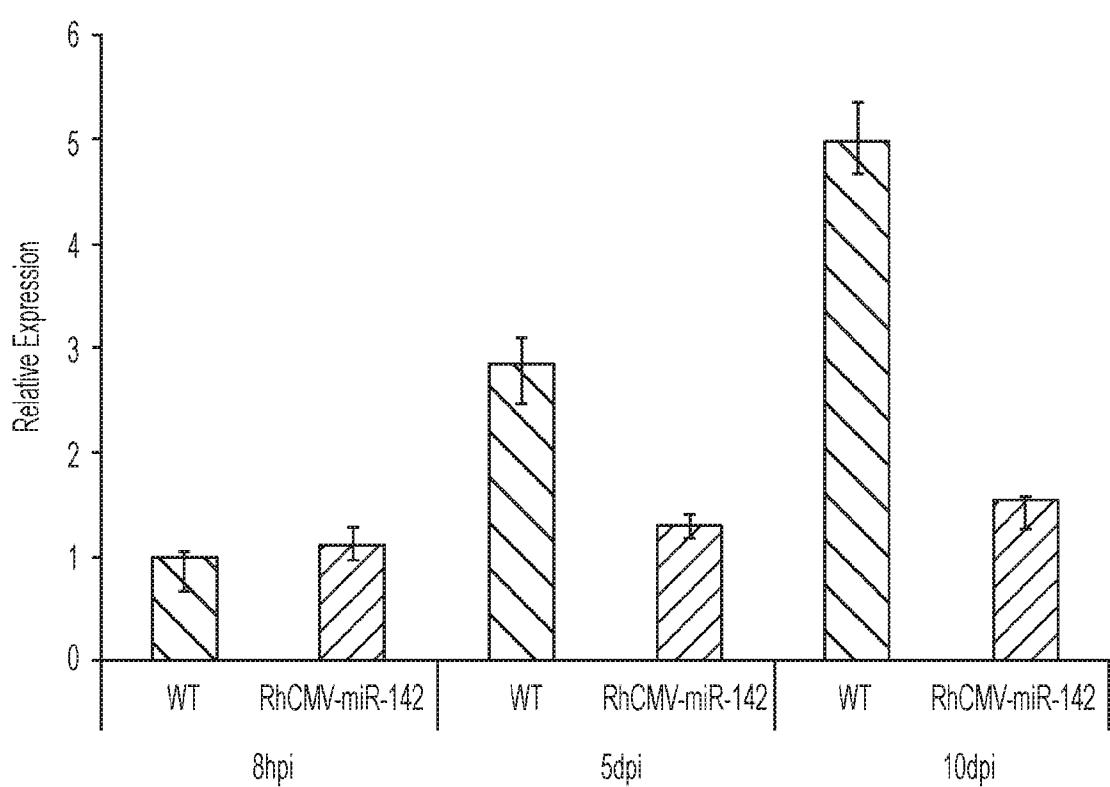


Figure 5

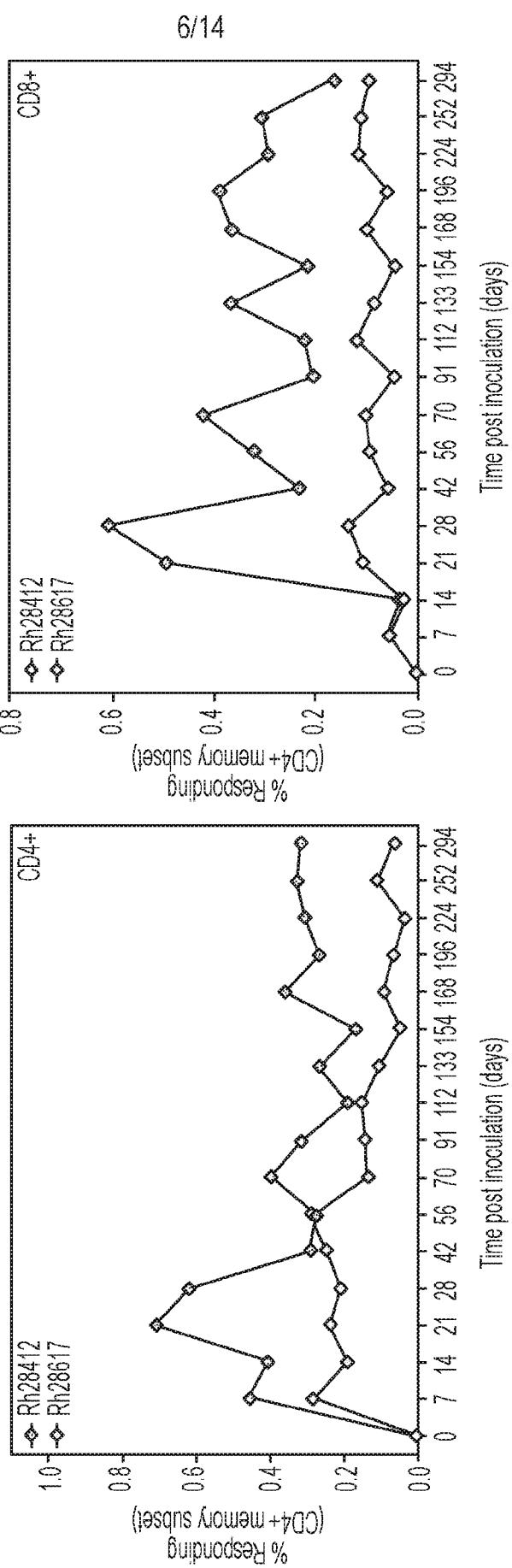
RhCMV-684-Rh156, 108 miR142-SIVth

Figure 6

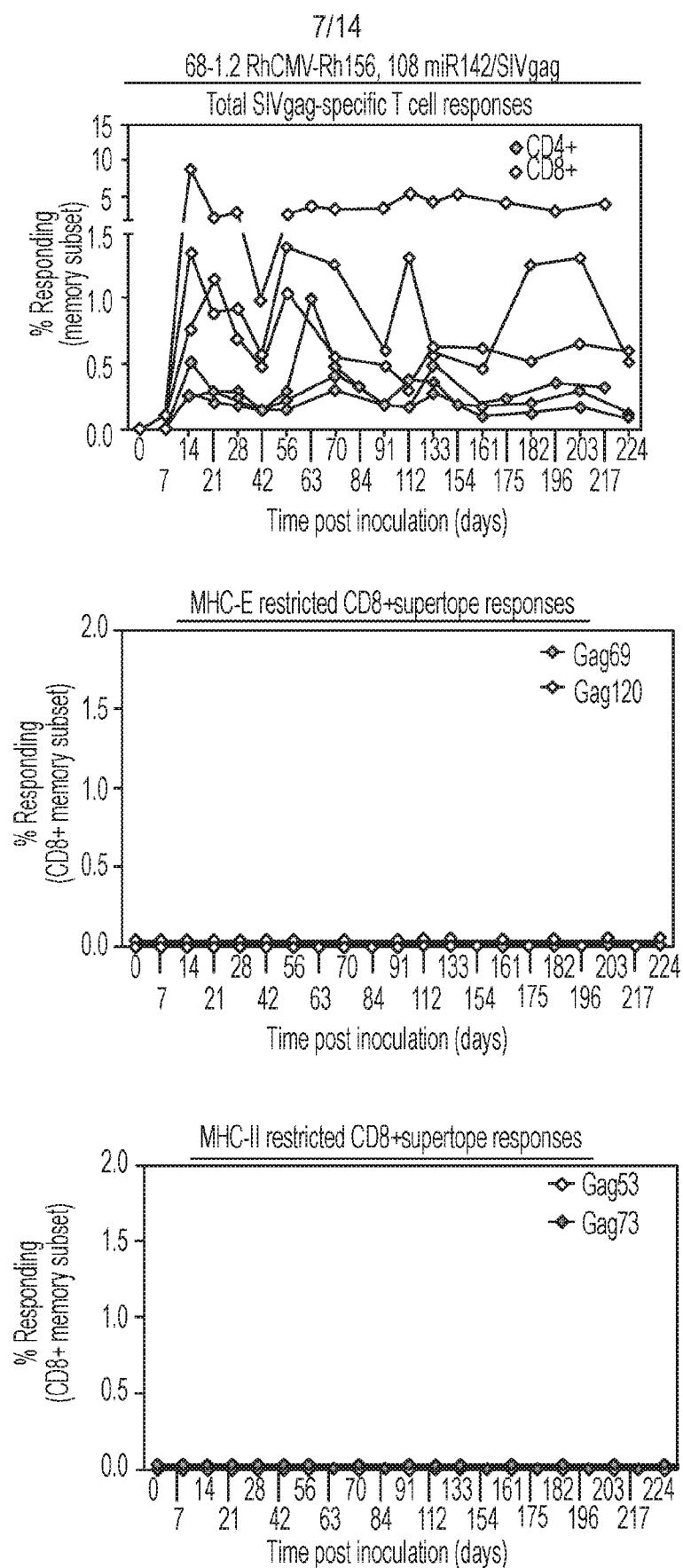


Figure 7

8/14

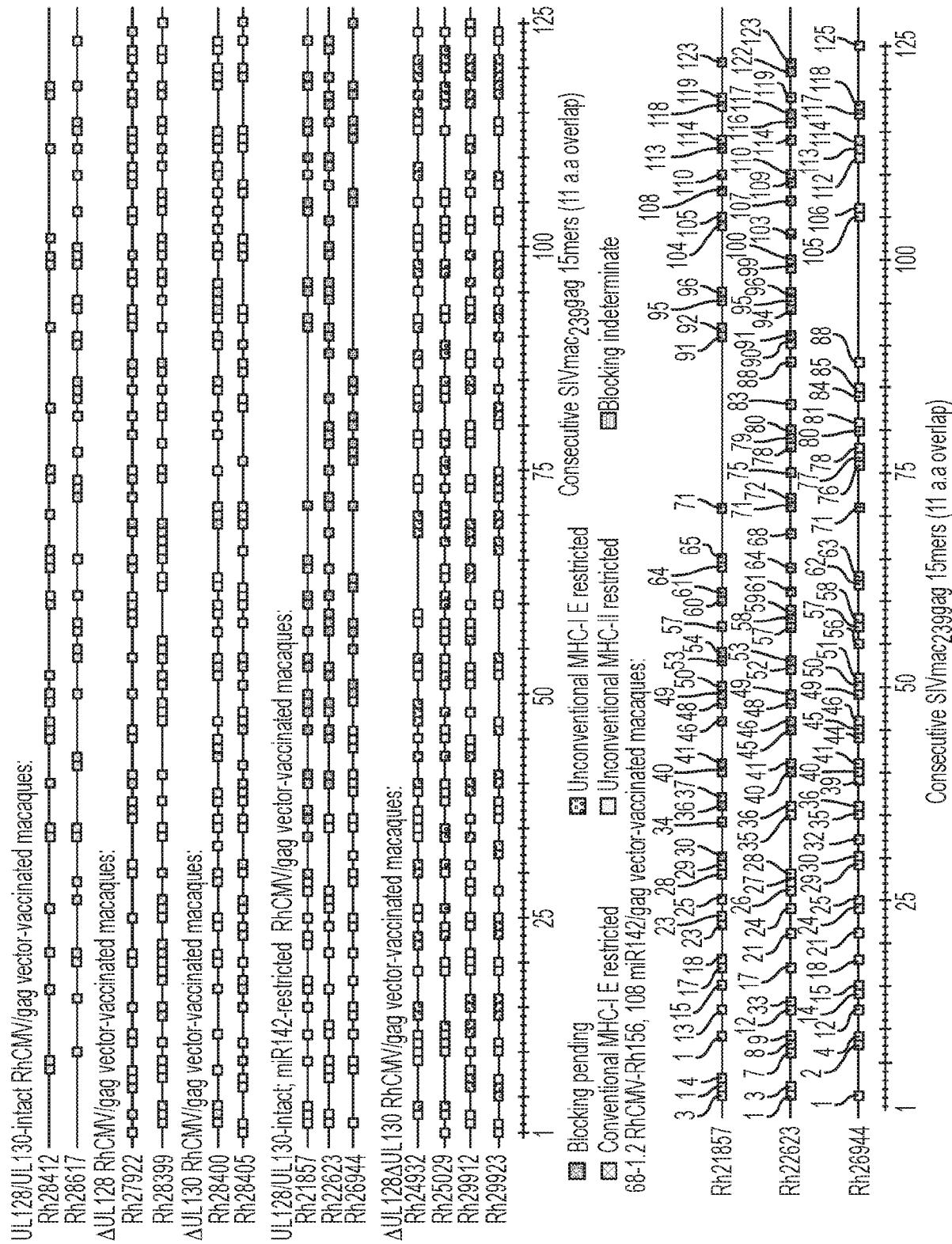


Figure 8

9/14

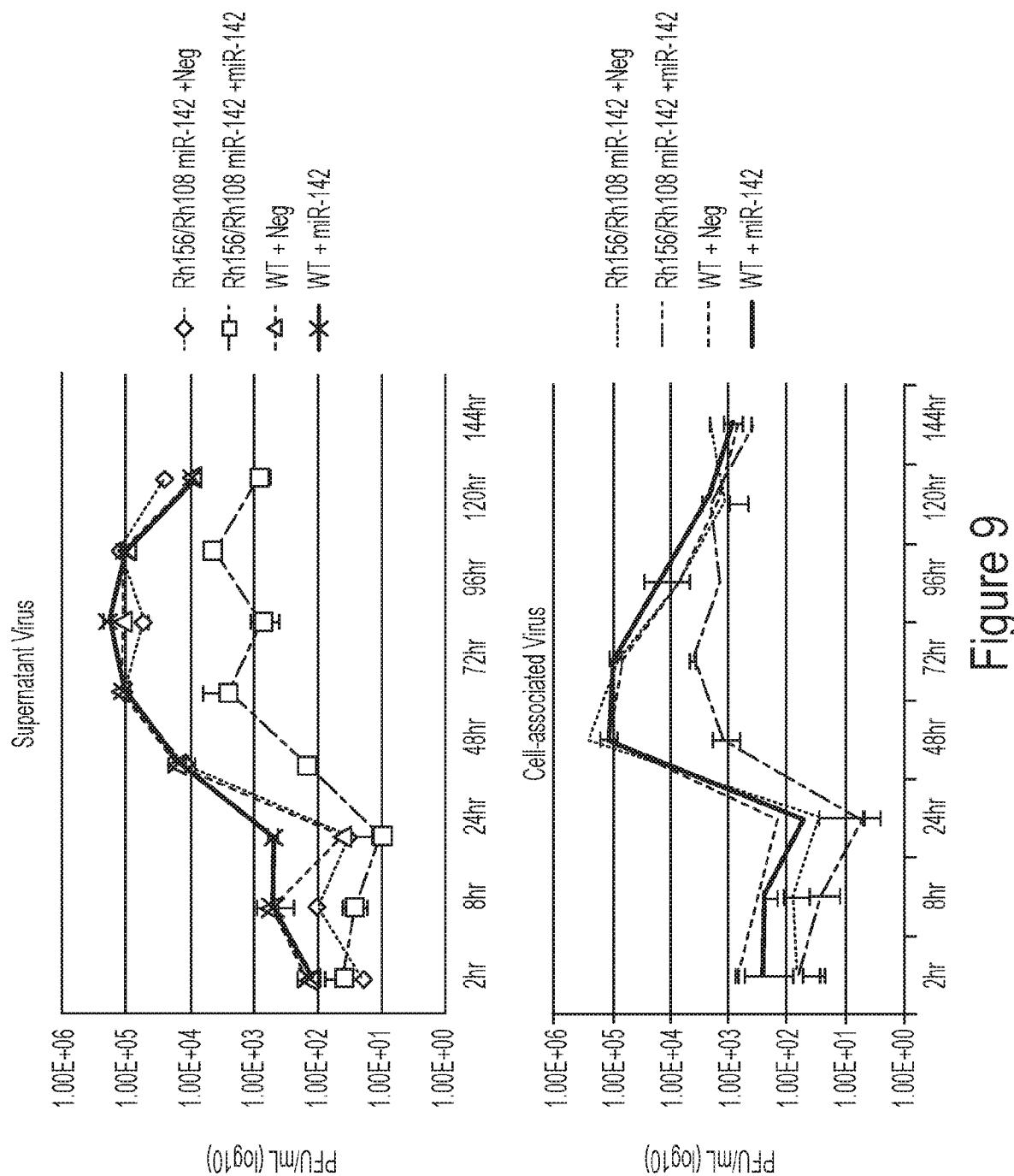


Figure 9

10/14

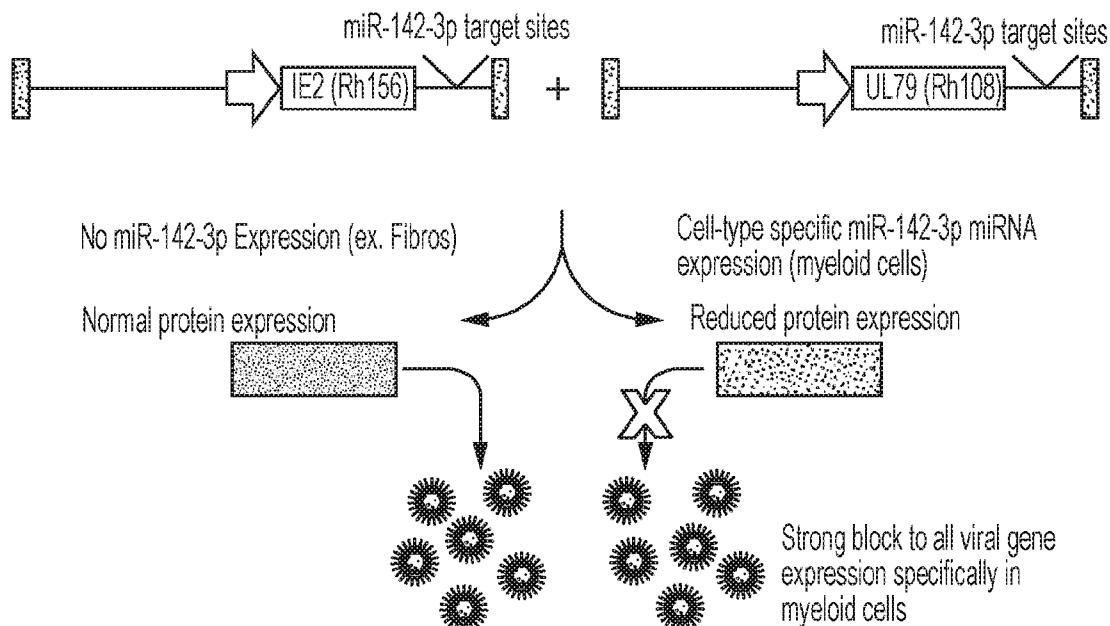
miRNA-targeted tropism recombinants: *Blocking viral growth in myeloid cells using miR-142-3p*

Figure 10

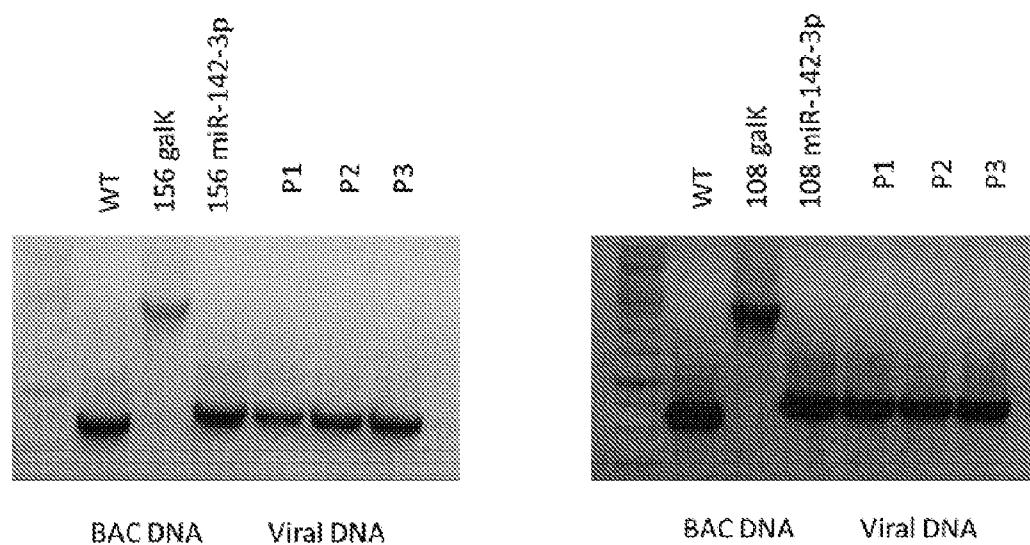


Figure 11A

11/14

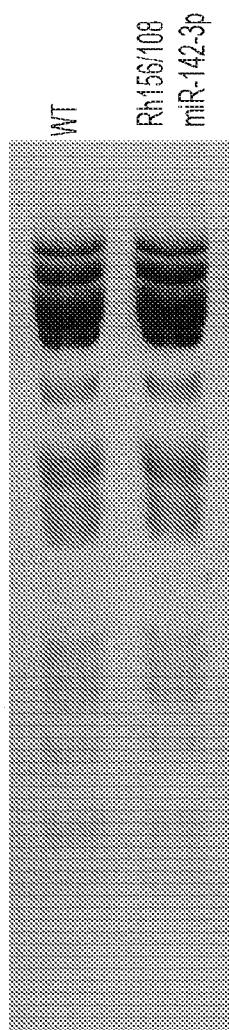


Figure 11B

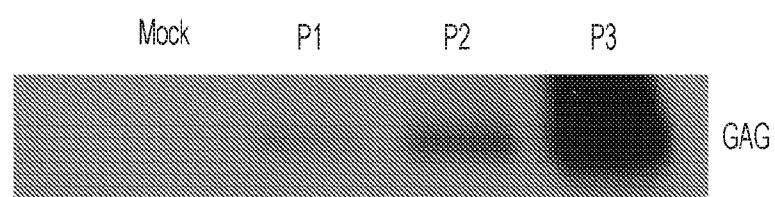


Figure 11C

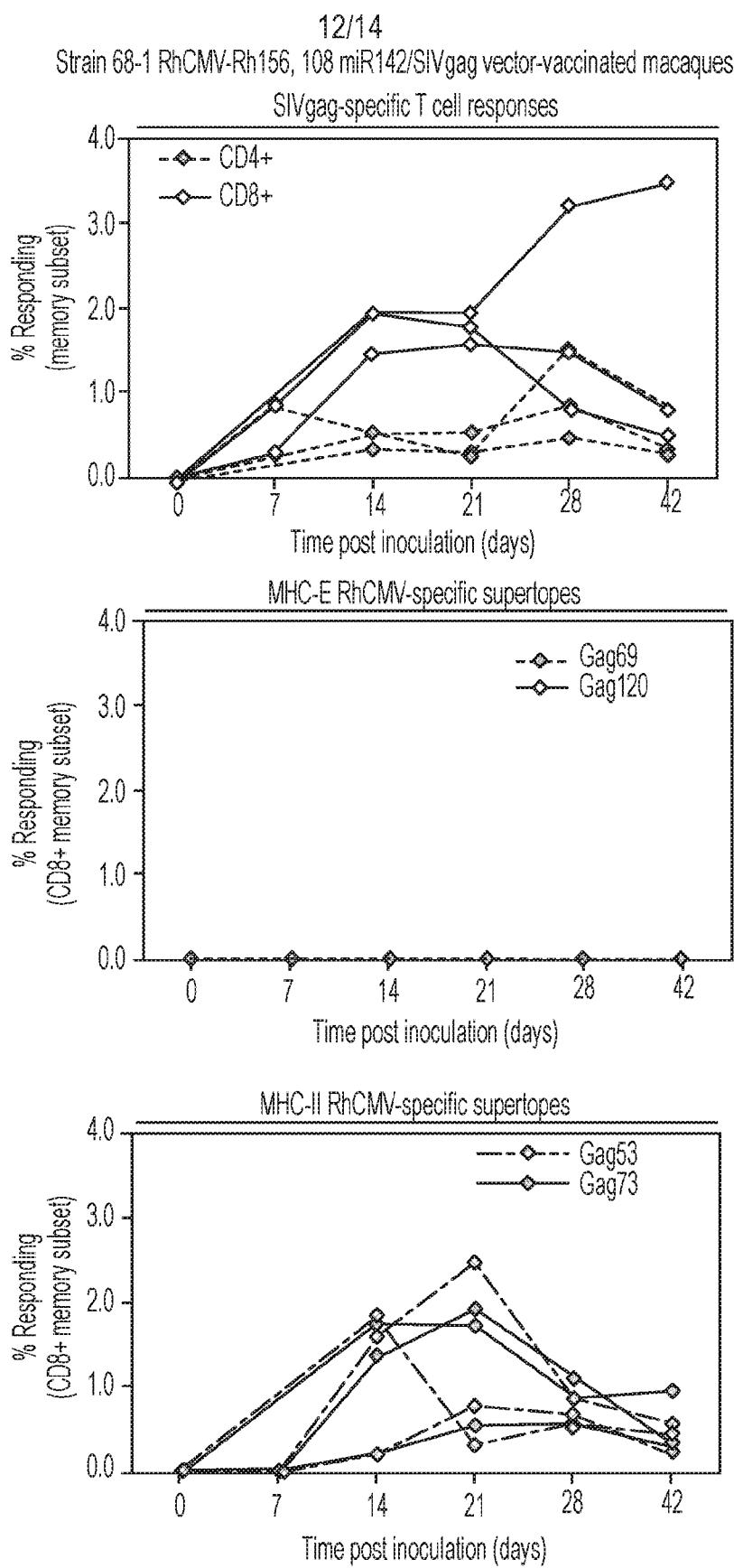


Figure 12

13/14

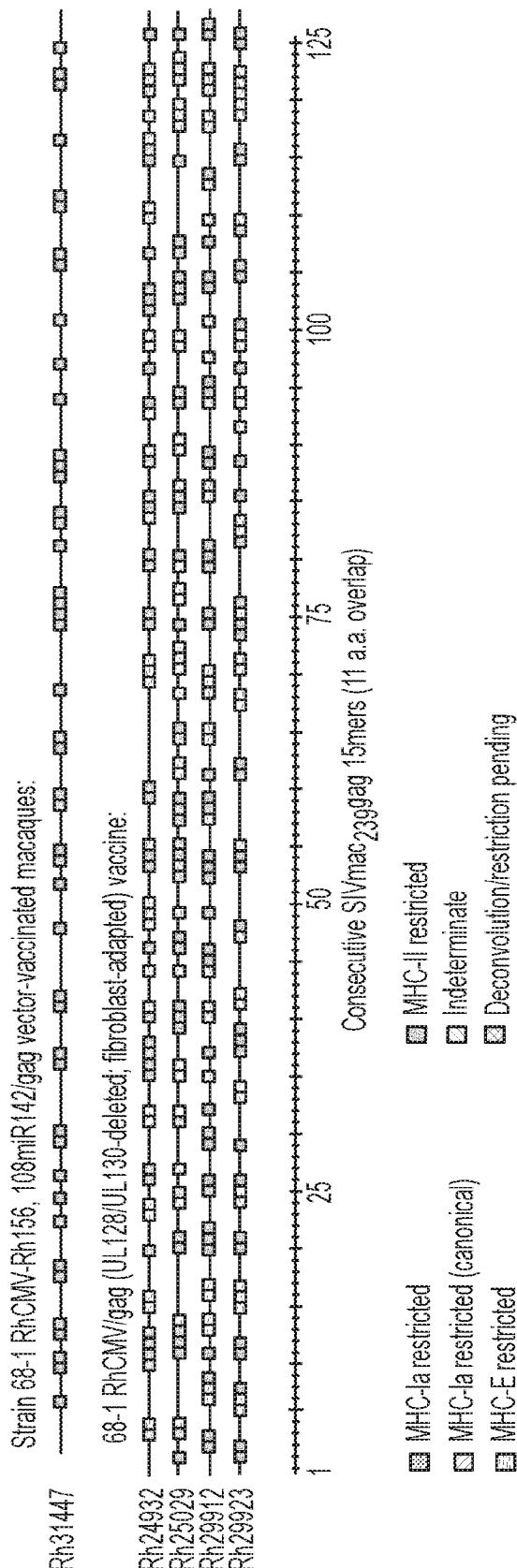


Figure 13

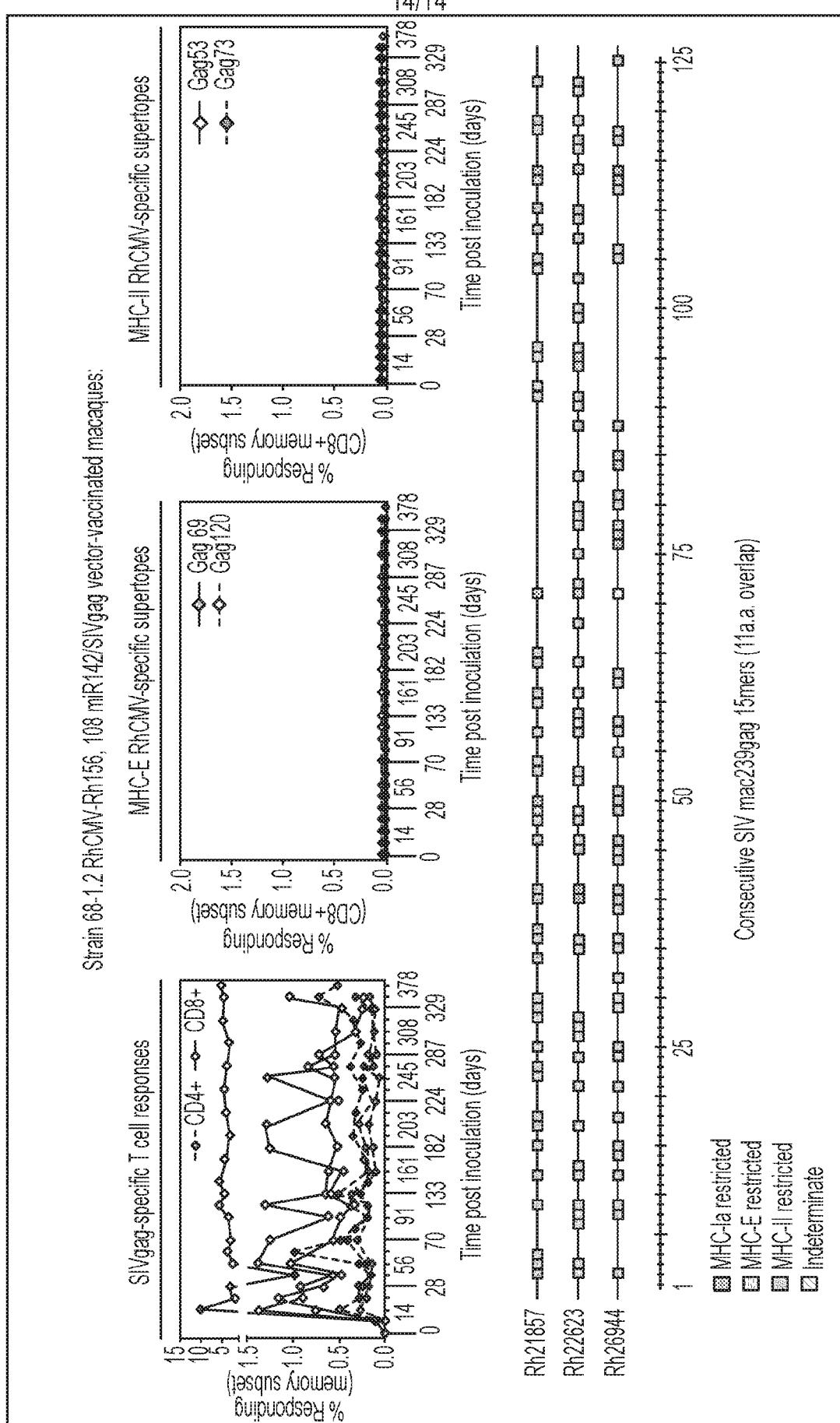


Figure 14