Supplementary Materials for

RhCMV/SIV tropism modulation programs unconventional CD8$^+$ T cell priming and vaccine efficacy

Scott G. Hansen, Meaghan H. Hancock, Daniel Malouli, Emily E. Marshall, Colette M. Hughes, Kurt T. Randall, David Morrow, Julia C. Ford, Roxanne M. Gilbride, Andrea N. Selseth, Renee Espinosa Trethewy, Lindsey M Bishop, Kelli Oswald, Rebecca Shoemaker, Brian Berkemeier, William J. Bosche, Michael Hull, Lorna Silipino, Michael Nekorchuk, Kathleen Busman-Sahay, Jacob D. Estes, Michael K. Axthelm, Jeremy Smedley, Danica Shao, Paul T. Edlefsen, Jeffrey D. Lifson, Klaus Früh, Jay A. Nelson, and Louis J. Picker

Corresponding author: pickerl@ohsu.edu

The PDF file includes:

Table S2. Summary of restriction-assigned 15mer peptide responses for all study RMs.

Table S3. Epitope density analysis of differentially tropism-restricted (response programmed) 68-1 RhCMV/SIV vectors.

Table S4. List 15mers with sequences outside the MHC-E supertope-focused insert ($\leq$ 4 amino acid overlap) that are recognized by $CD8^+$ T cell responses elicited by the miR-126-restricted 68-1 RhCMV vectors expressing MHC-E supertope-focused SIV inserts.

Table S5. List 15mers with sequences outside the MHC-II supertope-focused insert ($\leq$ 4 amino acid overlap) that are recognized by $CD8^+$ T cell responses elicited by the miR-142-restricted 68-1 RhCMV vectors expressing MHC-II supertope-focused SIV inserts.

Table S6. Cells used for adoptive transfer analysis of replication competent SIV in protected RMs.

Other Supplementary Material for this manuscript includes the following:

Table S7. Raw data file: non-quantitative epitope analysis data (Excel spreadsheet)

Table S8. Raw data file: quantitative immunologic data for all Figures (Excel spreadsheet)

SUPPLEMENTAL MATERIALS AND METHODS

Rhesus macaques. At assignment, all study RMs were free of cercopithicine herpesvirus 1, D-type simian retrovirus, simian T-lymphotrophic virus type 1, and *Mycobacterium tuberculosis*, but all were naturally RhCMV-infected. All study RMs were housed at the Oregon National Primate Research Center (ONPRC) in Animal Biosafety level (ABSL)-2 (vaccine phase) and ABSL-2+ rooms (challenge phase) rooms with autonomously controlled temperature, humidity, and lighting. Study RMs were both single- and pair-cage housed. RMs were only paired with one another during the vaccine phase if they were from the same vaccination group. All RMs were single cage-housed during the challenge phase due to the infectious nature of the study. Regardless of their pairing, all RMs had visual, auditory and olfactory contact with other animals. Single cage-housed RMs received an enhanced enrichment plan that was designed and overseen by NHP behavior specialists. RMs were fed commercially prepared primate chow twice daily and received supplemental fresh fruit or vegetables daily. Fresh, potable water was provided via automatic water systems. Physical exams including body weight and complete blood counts were performed monthly and at scheduled protocol time points. RMs were sedated with ketamine HCl with the addition of Dexmeditomidine and Atipamezole as a reversal agent for some procedures, including subcutaneous vaccine administration, venipuncture, and SIV challenge. At humane or scheduled endpoints, RMs were euthanized with sodium pentobarbital overdose (>50 mg/kg) and exsanguinated via the distal aorta, and tissue collection at necropsy was performed by a certified veterinary pathologist.

Generation, recovery, and characterization of recombinant RhCMV vectors. For RhCMV vector construction, the SW105 *E. coli* strain carrying the 68-1 or 68-1.2 SIV antigen BAC was used to introduce an expression cassette encoding the galactokinase as well as an aminoglycoside O-phosphotransferase gene conferring kanamycin resistance (KanR) flanked by 80bp homology arms to the targeted regions in the Rh108 or Rh156 (IE2) 3' untranslated regions. Correctly recombined clones were identified based on the production of bright pink colonies on MacConkey agar containing kanamycin as an antibiotic selection marker and Sanger sequencing of the inserted region. The galK-KanR cassette was replaced by the insert of interest by homologous recombination and correctly recombined clones were identified through negative selection on 2-deoxy-galactose-containing plates. These constructs were also analyzed by restriction digest and Sanger sequencing of the inserted region. Unintended off-target alterations in the genome sequence were ruled out by next generation sequencing (NGS) of the complete genome using an Illumina MiSeq or iSeq sequencing platform.

For RhCMV vector reconstitution, primary embryonal rhesus fibroblasts (RFs) were maintained in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal bovine serum (FBS) and antibiotics (1× *Pen/Strep;* Gibco) and grown at 37°C in humidified air with 5% CO_2. Depending on the reconstituted construct, viral plaques became visible within 3-5 days. The cells from well-isolated plaques were harvested by trypsin digestion using cloning cylinders, and infected cells were dispersed onto fresh monolayers of RFs. This plaque purification was performed a total of two times and then a single plaque was allowed to proceed to full cytopathic effect at which time cells and supernatants were harvested and stored at -80ºC until final use. Viral stocks were produced on RFs by infecting eight confluent T-175 tissue culture flasks with an approximate MOI of 0.05-0.1. After full CPE was reached within 4-8 days, cells and supernatants were harvested and frozen once overnight at -80°C to release intracellular virus from infected cells. Subsequently the supernatant was clarified by centrifugation in two steps, first at 2,000 x g for 10 minutes at 4°C and secondly at 7,500 x g for 15 minutes, after which the virus was purified through a sorbitol cushion (20% D-sorbitol, 50 mM Tris [pH 7.4], 1 mM $MgCl_2$) by centrifugation at 64,000 x g for 1 h at 4°C in a Beckman SW28 rotor. The pelleted virus was resuspended in complete DMEM, aliquoted and stored at -80°C until use.

Immunologic assays. For flow cytometric intracellular cytokine analysis individual 15-mer peptides or whole viral protein mixes of sequential 15-mer peptides (11 amino acid overlap) spanning the SIV_{mac239} Gag, 5'-Pol, Nef, Rev, Tat, and Vif proteins or individual SIV_{mac239} Gag, Rev, Nef, Tat and 5'-Pol supertope

peptides (**fig. S6**) or SIV-infected versus mock-infected autologous $CD4^+$ T cells were used as stimulating antigens in conjunction with co-stimulatory anti-CD28 (CD28.2, Purified 500 ng/test: eBioscience, Custom Bulk 7014-0289-M050) and anti-CD49d mAb (9F10, Purified 500 ng/test: eBioscience, Custom Bulk 7014-0499-M050). For peptide response assays, mononuclear cells were incubated at 37°C with individual peptides or peptide mixes and antibodies for 1 h, followed by an additional 8 h incubation in the presence of Brefeldin A (5 μg/ml; Sigma-Aldrich). Stimulation in the absence of peptides served as background control. For infected cell recognition assays (see **fig. S10**), bead purified, negatively selected $CD8^+$ T cells ($CD8^+$ T cell Isolation Kit, non-human primate, Miltenyi Biotec, 130-092-143) were combined with autologous $CD4^+$ T cells that were productively infected with SIV_{mac239}. To generate SIV-infected $CD4^+$ T cells, PBMCs were positively selected by bead purification (CD4 MicroBeads, non-human primate, Miltenyi Biotec, 130-091-102) as per manufacturer's instructions. Isolated $CD4^+$ T cells were resuspended in R15, RPMI supplemented with 15% newborn calf serum (Hyclone, SH30401.01), and 100U/mL of IL-2 (Peprotech, 200-02), and then subsequently activated using anti-CD28, anti-CD49d, anti-CD3 (SP34-2, BD Biosciences, 551916), and SEB (Toxin Technology Inc, BT202) in 24-well plates, 2-6 million cells per well, and incubated at 37°C. After 24 h, plates were washed 3 times with R15 then incubated for 3 additional days in R15 and 100U/mL of IL-2 with media changed as needed. Cells were then infected with SIV_{mac239} by spinoculation then incubated at 37°C. After 24 h, SIV-infected cells were combined with autologous negatively selected $CD8^+$ T cells at an effector to target (E:T) ratio of 40:1 prior to standard flow cytometric ICS (plus or minus blocking reagents). Mock-infected autologous $CD4^+$ T cells served as negative controls.

After incubation, stimulated cells were stored at 4°C until staining with combinations of fluorochrome-conjugated monoclonal antibodies including: anti-CD3 (SP34-2: Alexa700; BD Biosciences, Custom Bulk 624040 and Pacific Blue; BD Biosciences, Custom Bulk 624034), anti-CD4 (L200: AmCyan; BD Biosciences, Custom Bulk 658025, BV510; BD Biosciences, Custom Bulk 624340 and BUV395; BD Biosciences, Custom Bulk 624165), anti-CD8α (SK1: PerCP-eFluor710; Life Tech, Custom Bulk CUST04424), anti-TNF-α (MAB11: FITC; BD Biosciences, Custom Bulk 624046 and PE; BD Biosciences, Custom Bulk 624049), anti-IFN-γ (B27: APC; BD Biosciences, Custom Bulk 624078), and anti-CD69 (FN50: PE; eBioscience, Custom Bulk CUST01282 and PE-TexasRed; BD Biosciences, Custom Bulk 624005) and for polycytokine analyses, anti-IL-2 (MQ1-17H12; PE Cy-7; Biolegend), and anti-MIP-1β (D21-1351, BV421; BD Biosciences). For analysis of memory differentiation (central-, transitional-, or effector-memory) of SIV Gag-specific $CD4^+$ and $CD8^+$ T cells, PBMCs were stimulated as described above, except that the CD28 co-stimulatory mAb was used as a fluorochrome conjugate to allow CD28 expression levels to be later assessed by flow cytometry. In these experiments, cells were surface-stained after incubation for lineage markers CD3, CD4, CD8, CD95 and CCR7 (see below for mAb clones) prior to fixation/permeabilization and then intracellularly staining for response markers (CD69, IFN-γ, TNF-α; note that Brefeldin A treatment preserves the pre-stimulation cell-surface expression phenotype of phenotypic markers examined in this study).

Stained samples were analyzed on an LSR-II or FACSymphony A5 flow cytometer (BD Biosciences). Data analysis was performed using FlowJo software (Tree Star). In all analyses, gating on the lymphocyte population was followed by the separation of the $CD3^+$ T cell subset and progressive gating on $CD4^+$ and $CD8^+$ T cell subsets. Ag-responding cells in both $CD4^+$ and $CD8^+$ T cell populations were determined by their intracellular expression of CD69 and either or both of the cytokines IFN-γ and TNF-α (or in polycytokine analyses, expression of CD69 and any combination of the cytokines: IFN-γ, TNF-α, IL-2, MIP-1β). Assay limit of detection was determined as previously described (*61*), with 0.05% after background subtraction being the minimum threshold used in this study. After background subtraction, the raw response frequencies above the assay limit of detection were "memory-corrected" (e.g., % responding out of the memory population), as previously described (*1-3, 61*), using combinations of the following fluorochrome-conjugated mAbs to define the memory versus naïve subsets: CD3 (SP34-2: Alexa700 and PerCP-Cy5.5; BD Biosciences Custom Bulk 624060), CD4 (L200: AmCyan), CD8α (SK-1: PerCP-eFluor710, RPA-T8: APC; BioLegend), TNF-α (MAB11; FITC), IFN-γ (B27; APC), CD69 (FN50; PE),

CD28 (CD28.2; PE/Dazzle 594, BioLegend), CD95 (DX2; PE, BioLegend), CCR7 (15053; Biotin, R&D Systems), streptavidin (Pacific Blue, Life Tech and BV605; BD Biosciences, Custom Bulk 624342), and Ki67 (B56; FITC, BD Biosciences, Custom Bulk 624046). For memory phenotype analysis of SIV Gag-specific T cells, all $CD4^+$ or $CD8^+$ T cells expressing CD69 plus IFN-γ and/or TNF-α were first Boolean OR gated, and then this overall Ag-responding population was subdivided into the memory subsets of interest using surface phenotype (CCR7 versus CD28). Similarly, for polycytokine analysis of SIV Gag-specific T cells, all $CD4^+$ or $CD8^+$ T cells expressing CD69 plus cytokines were Boolean OR gated, and polyfunctionality was delineated with any combination of the 4 cytokines tested (IFN-γ, TNF-α, IL-2, MIP-1β) using the Boolean AND function. Gating strategies for these ICS analyses are illustrated in **fig. S10**.

The MHC restriction type (MHC-Ia, MHC-E, MHC-II) of a 15mer peptide response was determined by pre-incubating isolated mononuclear cell aliquots for 1 h at room temperature (prior to adding peptides or combining effector and target cells and incubating per the standard ICS assay) in the presence (or absence) of each the following specific inhibitors: 1) the pan anti-MHC-I mAb W6/32 (10μg/mL), 2) the MHC-II-blocking mAb G46.6 (10μg/mL), or 3) the MHC-E blocking VL9 peptide (VMAPRTLLL; 20μM) (see **fig. S10**). Stimulated cells were fixed, permeabilized, stained, and analyzed as described above. To be considered MHC-E-restricted, the individual peptide response must have been reduced in magnitude by >50% by both anti-pan MHC-I clone W6/32 and MHC-E-binding peptide VL9, and not blocked by anti-MHC-II. MHC-II-restricted responses were blocked by anti-MHC-II but not pan anti-MHC-I or VL9, and MHC-I-restricted responses were blocked by pan anti-MHC-I only (*4, 7, 8*). Responses that did not meet these inhibition criteria were considered indeterminate. Minimal independent epitope numbers were estimated from the positive responses identified by testing of consecutive 15mer peptides by the following criteria: single positive peptide of same restriction type = 1 independent epitope; 2 adjacent positive peptides of same restriction type = 1 independent epitope; 3 adjacent positive peptides of same restriction type = 2 independent epitopes; 4 adjacent positive peptides of same restriction type = 2 independent epitopes; 5 or 6 adjacent positive peptides of same restriction type = 3 independent epitopes; and 7 or 8 adjacent positive peptides of same restriction type = 4 independent epitopes. Minimal epitope densities were calculated by determining the minimal number of independent epitopes every 100 amino acids of SIV protein sequence analyzed. For analysis of SIV Ag epitope densities outside the ST-focused inserts, peptides with >3 amino acids overlap with these inserts were excluded from consideration.

RhCMV vector immunofluorescence analysis. In brief, 5 μm sections were deparaffinized in xylene and rehydrated through a series of graded ethanols to distilled water. Heat-induced epitope retrieval (HIER) was performed with EDTA buffer (Biocare; CB917M) in a Biocare NxGen Decloaking Chamber at 110°C for 15 min, washed in ddH_2O, and endogenous peroxidases were blocked by incubating slides in 1.5% H_2O_2 in TBS-T for 5 min. Slides were first stained with rabbit anti-CD34 (Sigma; HPA036723) overnight at 1:250 in antibody diluent (1x TBS containing 0.25% casein and 0.05% Tween-20), washed in TBS-T for 5 min, incubated with Rabbit Polink-1 HRP (GBI Labs; D13-110) for 20 min, and developed with Alexa Fluor 488 tyramide (Invitrogen; B40953) at 1:250 for 15 min. Following CD34 stain development, antibody stripping was performed by heating slides at 95°C for 10 min in Cit6 buffer (GBI labs; B05C-100B), then transferred to citraconic anhydride buffer (0.01% + 0.05% Tween-20) heated to 95°C and allowed to cool for 20 min at room temperature and rinsed in ddH_2O. Next, slides were stained with rabbit anti-IBA-1(Biocare; CP290A) at 1:500 in antibody diluent for 1 h, washed in TBS-T for 5 min, incubated with Rabbit Polink-1 HRP (GBI Labs; D13-110) for 20 min, and developed with Alexa Fluor 568 tyramide (Invitrogen; B40956) at 1:500 for 5 min. Following IBA-1 stain development, antibody stripping was performed by heating slides at 95°C for 10 min in Cit6 buffer (GBI labs; B05C-100B), then transferred to citraconic anhydride buffer heated to 95°C and allowed to cool for 20 min at room temperature and rinsed in ddH_2O. Next, slides were stained with mouse monoclonal anti-RhCMV IE2 antibody (clone 11A5.2) at 0.75ug/mL and mouse monoclonal anti-RhCMV pp65b (clone19C12.2; both generated at the VGTI MAb Core) at 0.2ug/mL in antibody diluent for 1 h, washed in TBS-T for 5 min, incubated with Mouse Polink-1 HRP (GBI Labs; D12-110) for 20 min, and developed with Alexa Fluor 647 tyramide (Invitrogen; B40958) at

1:500 for 4 min. Following anti-RhCMV IE2 and pp65b stain development, antibody stripping was performed by heating slides at 95°C for 10 min in Cit6 buffer (GBI labs; B05C-100B), then transferred to DAKO buffer (Agilent; S236784-2) heated to 95°C and allowed to cool for 20 min at room temperature and rinsed in ddH_2O. Finally, slides were stained with anti-CD31 (Novus biologicals; NB100-2284) at 1:500 in antibody diluent, incubated with Rabbit Polink-1 HRP (GBI Labs; D13-110) for 20 min, and developed with CF750 (Biotium; 96052) at 1:1000 for 10 min. All staining was performed at room temperature; slides were counter-stained with 4',6-diamidino-2-phenylindole (DAPI; Invitrogen; D1306) at 0.5μg/mL for 10 min, and were mounted in Prolong Gold Antifade Mountant (Thermofisher; P36930), dried overnight, whole tissue-scanned on an AxioScan.Z1 (Zeiss) using a Plan Apochromat 20× objective (NA = 0.8, FWD = 0.55 mm), and analyzed with Halo software (Indica Labs; v3.2.1851) using the Highplex FL v4.0.4 and FISH Multiplex RNA 2.1 modules.

SUPPLEMENTAL FIGURES

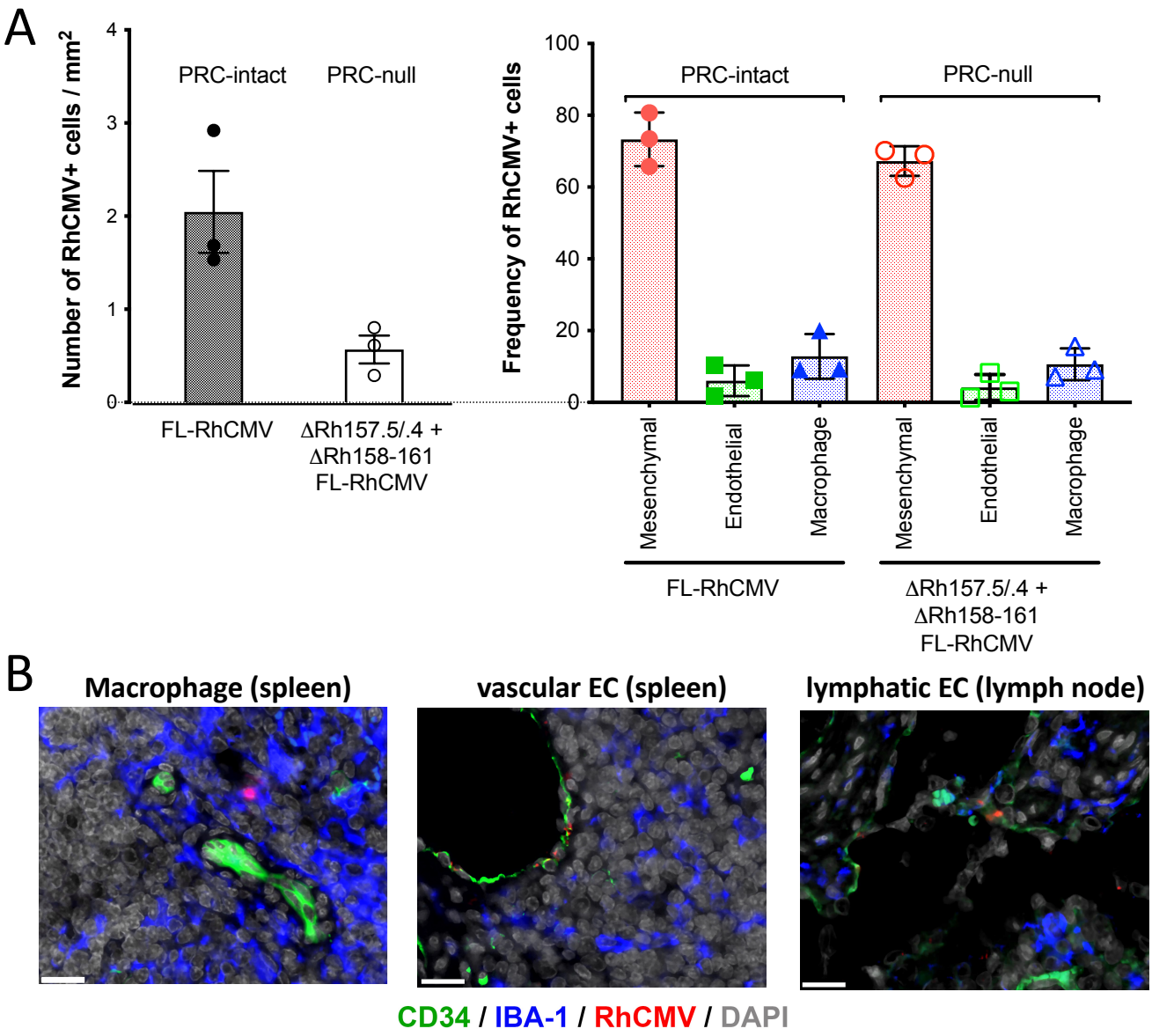


Figure S1. The pentameric receptor complex (PRC) is not required for RhCMV vector infection of macrophages or endothelial cells *in vivo*. Tissues were obtained at necropsy from 6 CMV seronegative RMs 14 days after infection with 10^7 PFU of either full length (wildtype-like) RhCMV (PRC-intact; n = 3) or the same virus with Rh157.5 + Rh157.4 and Rh158-161 deleted (double-deleted; 68-1-like; PRC-null; n = 3) (*18*), and used for multiplex immunofluorescent analysis of RhCMV infection in combination with markers of macrophage, endothelial, and mesenchymal cell lineage (see Methods). (**A**) The total number of RhCMV$^+$ cells (RNAscope) within spleen tissue were greater in RMs infected with the full length (PRC- and Rh158-161-intact) RhCMV compared to the PRC-null and Rh158-161-deleted RhCMV (left panel); however, the proportion of RhCMV$^+$ (IE-1$^+$/pp65b$^+$) mesenchymal (vimentin$^+$), endothelial (CD34$^+$/CD31$^+$) and macrophage (IBA-1$^+$) lineage cells was not different (right panel). (**B**) Multiplex immunofluorescence images documenting double-deleted (PRC-null) RhCMV infection of splenic macrophages (left panel), splenic vascular endothelium (middle panel), and lymph node lymphatic endothelium (right panel). Scale bars = 20um.

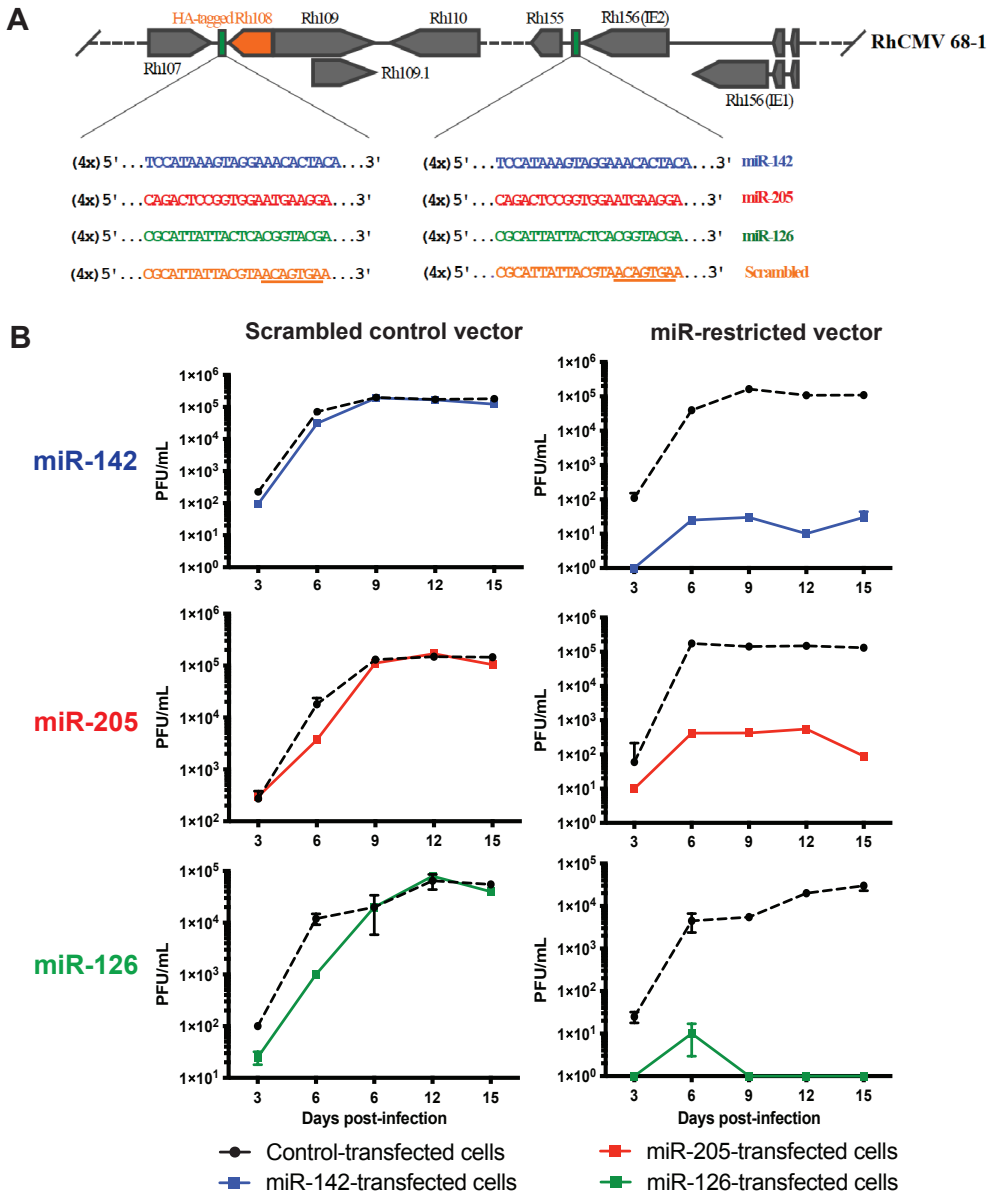


Figure S2. Effect of individual miR-mediated tropism restrictions on 68-1 RhCMV replication in cell culture. (A) Schematic of the miRNA target sites inserted downstream of both *Rh156* and *Rh108* (orthologs of HCMV *UL122*/IE2 and *UL79*), which are essential for viral replication. Blue sequences represent insertion of four miR-142 recognition sites. Red sequences represent insertion of four miR-205 recognition sites. Green sequences represent insertion of four miR-126 recognition sites. A control miR containing vector was designed whereby the seed sequence targeting region from miR-126 was changed to 6 random nucleotides (underlined in the orange sequence) and this non-targeting scrambled control was used in all subsequent experiments. The orange box represents the *Rh108* ORF with a hemagglutinin (HA) epitope tag. **(B)** Growth analysis of the 68-1 scrambled control virus (left) versus 68-1 miRNA-restricted virus (right) in the presence or absence of the indicated miRNA. Primary RFs were transfected with negative control or miRNA mimic and infected 24 hours later with the 68-1-miRNA or control RhCMV vectors at an MOI of 0.01. Cell supernatants were harvested at the indicated time points and titered on primary RFs. Results are representative of two independent experiments.

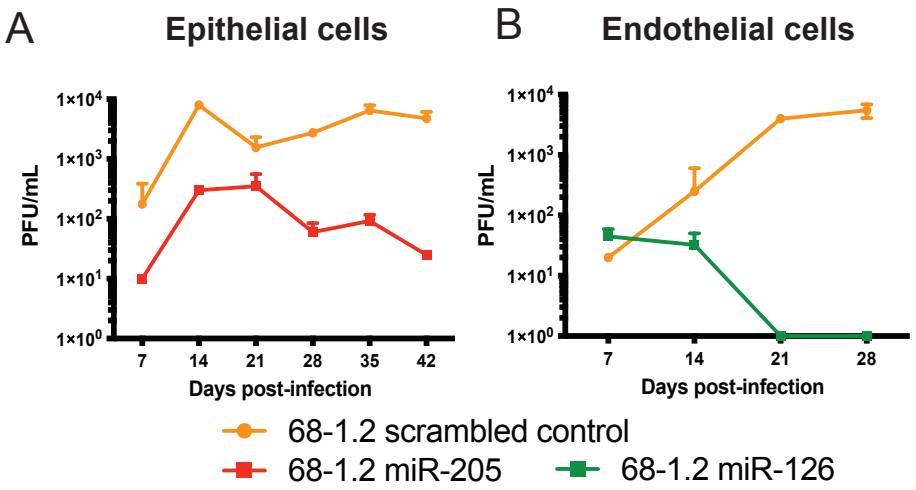


Figure S3. miR-126- and miR-205-mediated tropism restriction of 68-1.2 RhCMV in their corresponding cell types. To test the ability of miR-205 and miR-126-restricted RhCMV vectors to replicate in primary epithelial and endothelial cells, respectively, we incorporated these miR-restriction elements into the pentameric receptor complex-repaired 68-1.2 vector, as the pentameric receptor complex -null 68-1 RhCMV vector does not sufficiently infect these cell types *in vitro* to make these measurements feasible. Thus, primary rhesus epithelial cells (**A**) or endothelial cells **(B)** were infected with 68-1.2-miR-205 or 68-1.2-miR-126 viruses, respectively, or a 68-1.2 scrambled control virus at an MOI of 5. Cell supernatants were harvested at the indicated time points and titered on primary RFs. Results are representative of two independent experiments. The 68-1.2-miR-142 vector was previously validated for growth restriction in primary rhesus macrophages (*4*).

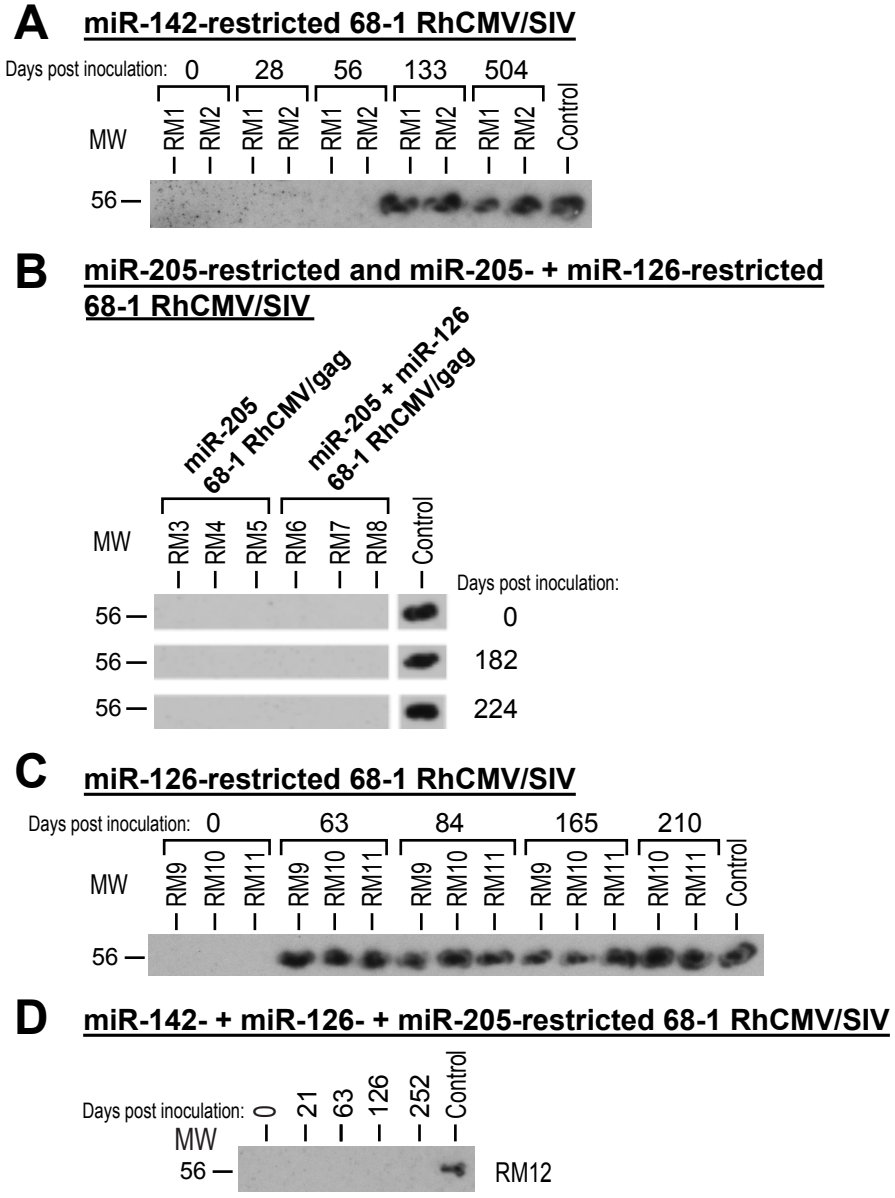


Fig. S4. Effect of miR-mediated tropism restrictions on 68-1 RhCMV/SIVgag vector shedding in urine. (A-D) Urine from RMs vaccinated with the indicated vectors was collected at the designated timepoints post inoculation, centrifuged to enrich for virus and then the pellet was cocultured with rhesus fibroblasts for 42 days or until observation of cytopathic effect. Cells were lysed and examined by Western blot analysis for expression of the SIV Gag insert in each vector (control = analysis of lysate of rhesus fibroblasts infected with a 68-1 RhCMV vector expressing the same insert). Detection of SIV insert expression indicates the presence of shed RhCMV/SIV vector in the urine. The parental 68-1 RhCMV/SIV vector is typically detected in urine by day 70 post vaccination (*24*), and to date, any 68-1-based vector that is capable of being shed is detectable in urine by day 112 (*25*).

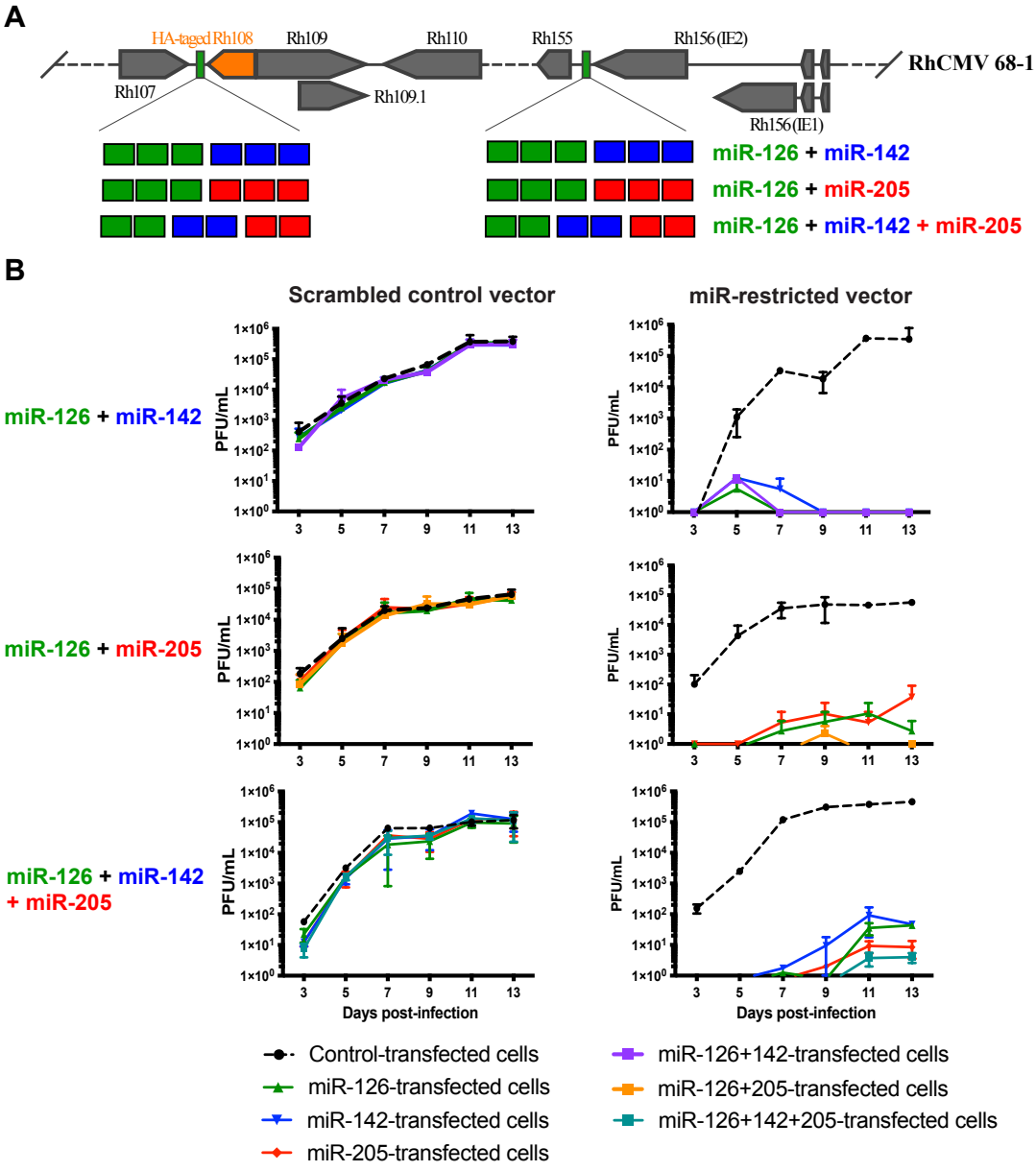
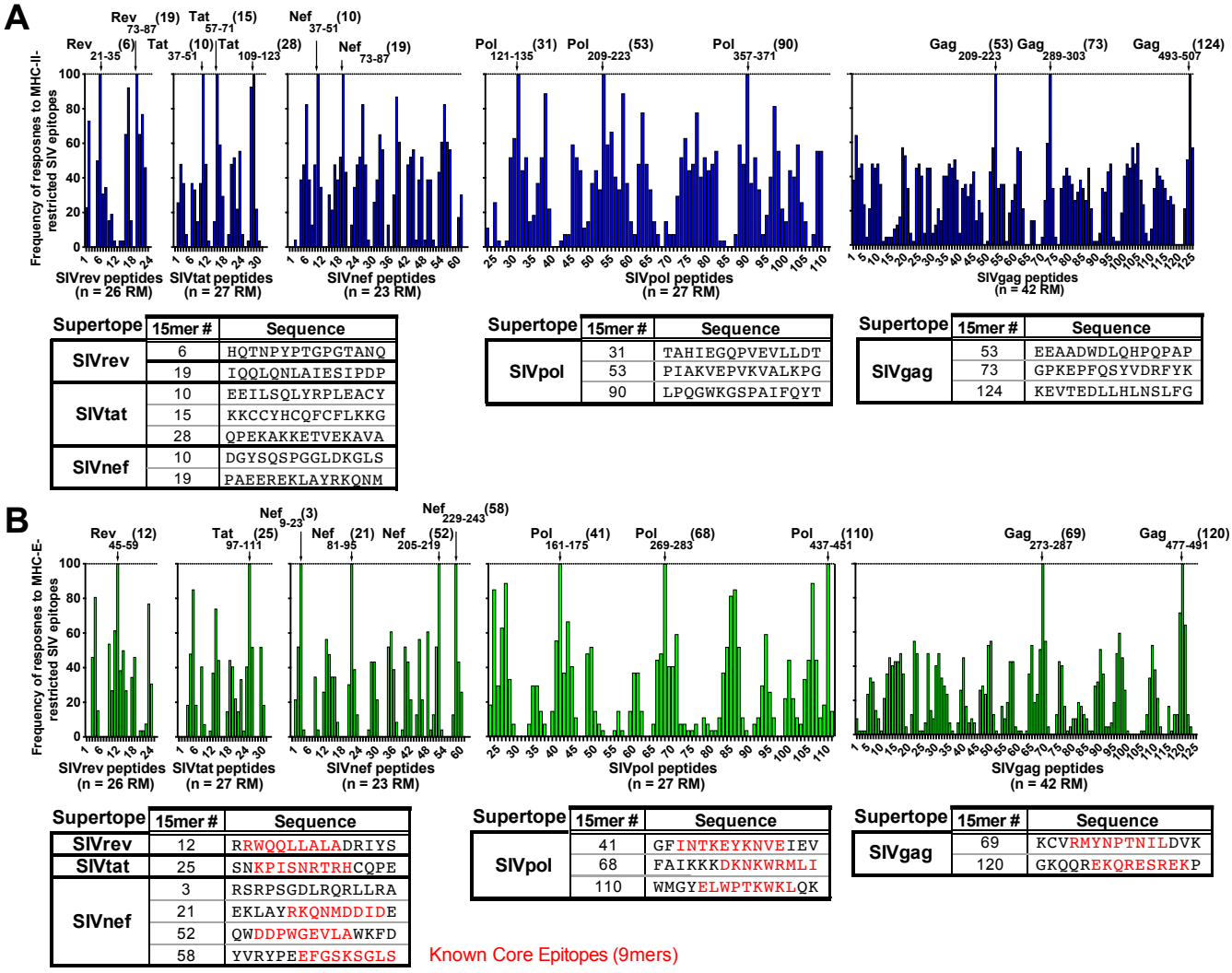


Figure S5. Effect of combination miR-restrictions on 68-1 RhCMV replication in cell culture. (A) Schematic of the miRNA target sites inserted downstream of both *Rh156* and *Rh108*. Green boxes represent one copy of the miR-126 recognition site (see **Fig. S2**). Blue boxes represent one copy of the miR-142 recognition site. Red boxes represent one copy of the miR-205 recognition site. **(B)** Growth analysis of the 68-1 scrambled control virus (left) versus 68-1 miRNA-restricted virus (right) in the presence or absence of the indicated miRNA. Primary RFs were transfected with negative control or miRNA mimic(s) and infected 24 hours later with the 68-1-miRNA or control RhCMV vectors at an MOI of 0.01. Cell supernatants were harvested at the indicated time points and titered on primary RFs. Results are representative of two independent experiments.



Supertope	15mer #	Sequence
SIVrev	6	HQTNPYPTGPGTANQ
	19	IQQLQNLAIESIPDP
SIVtat	10	EEILSQLYRPLEACY
	15	KKCCYHCQFCFLKKG
	28	QPEKAKKETVEKAVA
SIVnef	10	DGYSQSPGGLDKGLS
	19	PAEEREKLAYRKQNM

Supertope	15mer #	Sequence
SIVpol	31	TAHIEGQPVEVLLDT
	53	PIAKVEPVKVALKPG
	90	LPQGWKGSPAIFQYT

Supertope	15mer #	Sequence
SIVgag	53	EEAADWDLQHPQPAP
	73	GPKEPFQSYVDRFYK
	124	KEVTEDLLHLNSLFG

Supertope	15mer #	Sequence
SIVrev	12	RRWQQLLALADRIYS
SIVtat	25	SNKPISNRTRHCQPE
SIVnef	3	RSRPSGDLRQRLLRA
	21	EKLAYRKQNMDDIDE
	52	QWDDPWGEVLAWKFD
	58	YVRYPEEFGSKSGLS

Supertope	15mer #	Sequence
SIVpol	41	GFINTKEYKNVEIEV
	68	FAIKKKDKNKWRMLI
	110	WMGYELWPTKWKLQK

Supertope	15mer #	Sequence
SIVgag	69	KCVRMYNPTNILDVK
	120	GKQQREKQRESREKP

Known Core Epitopes (9mers)

Figure S6. Validation of supertopes (universal epitopes) in the SIV Gag, Retanef and 5'-Pol vector inserts. (A,B) The top panel of each figure shows the frequency of $CD8^+$ T cell recognition of each consecutive 15mer peptide (with 11 amino acid overlap) expressed in the SIV Retanef (Rev/Nef/Tat segments), 5'-Pol, and Gag inserts in RMs vaccinated with the parental 68-1 RhCMV/SIV vector set expressing these inserts (n for each analysis shown). Responses were classified with respect to MHC-II versus MHC-E restriction, as described in **Figure 1**, with the responses classified as MHC-II-restricted and MHC-E-restricted shown in panel **A** (blue) and **B** (green), respectively. The 15mer peptides classified as supertopes in this analysis (100% response frequency) are specifically indicated above each response in the top panels, with the amino sequence of these 15mers shown in bottom panels. For the MHC-E-restricted supertope 15mers (**B**), the core 9mer supertopes are shown in red in the bottom panel where known.

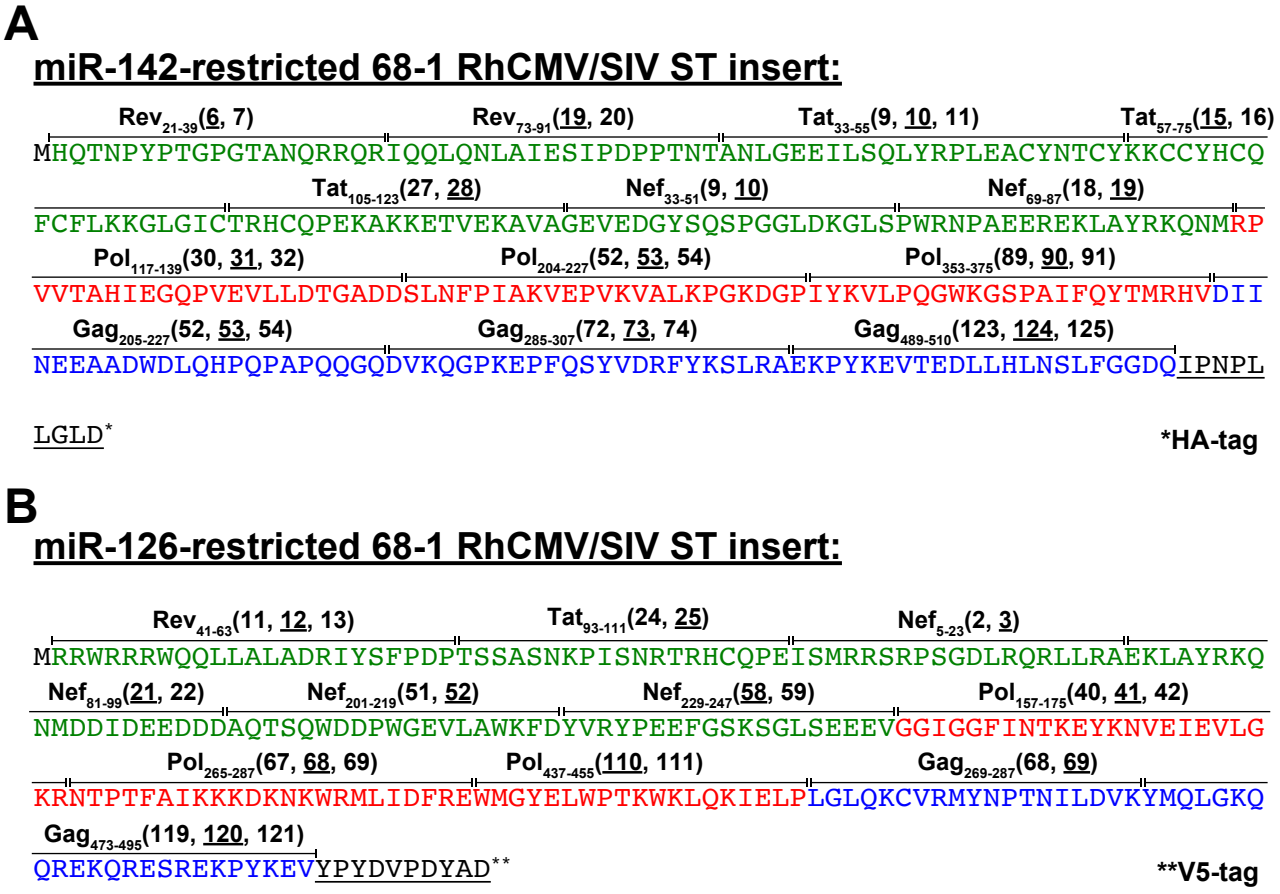


Figure S7. Amino acid sequence of MHC-II and MHC-E supertope-focused vector inserts. (A,B) Based on the 15mer supertope peptides identified in **fig. S6**, we constructed a vector insert comprised of linear array of the sequence encoding these core supertope 15mers and the adjacent 4 amino acids on either one or both sides of the core supertope 15mer for both MHC-II-restricted (**A**) and MHC-E-restricted (**B**) responses. The overlapping 15mer peptides that are completely included in each segment are listed above the sequence with the core supertope 15mers designated by underlining. It should be noted, however, that the 15mers adjacent to the designated 15mers on either side (for example, peptides 67 and 70 for the $Gag_{269-287}$ segment) are partially included in the insert (11 of the 15 amino acids) and could therefore also directly contribute to direct response priming.

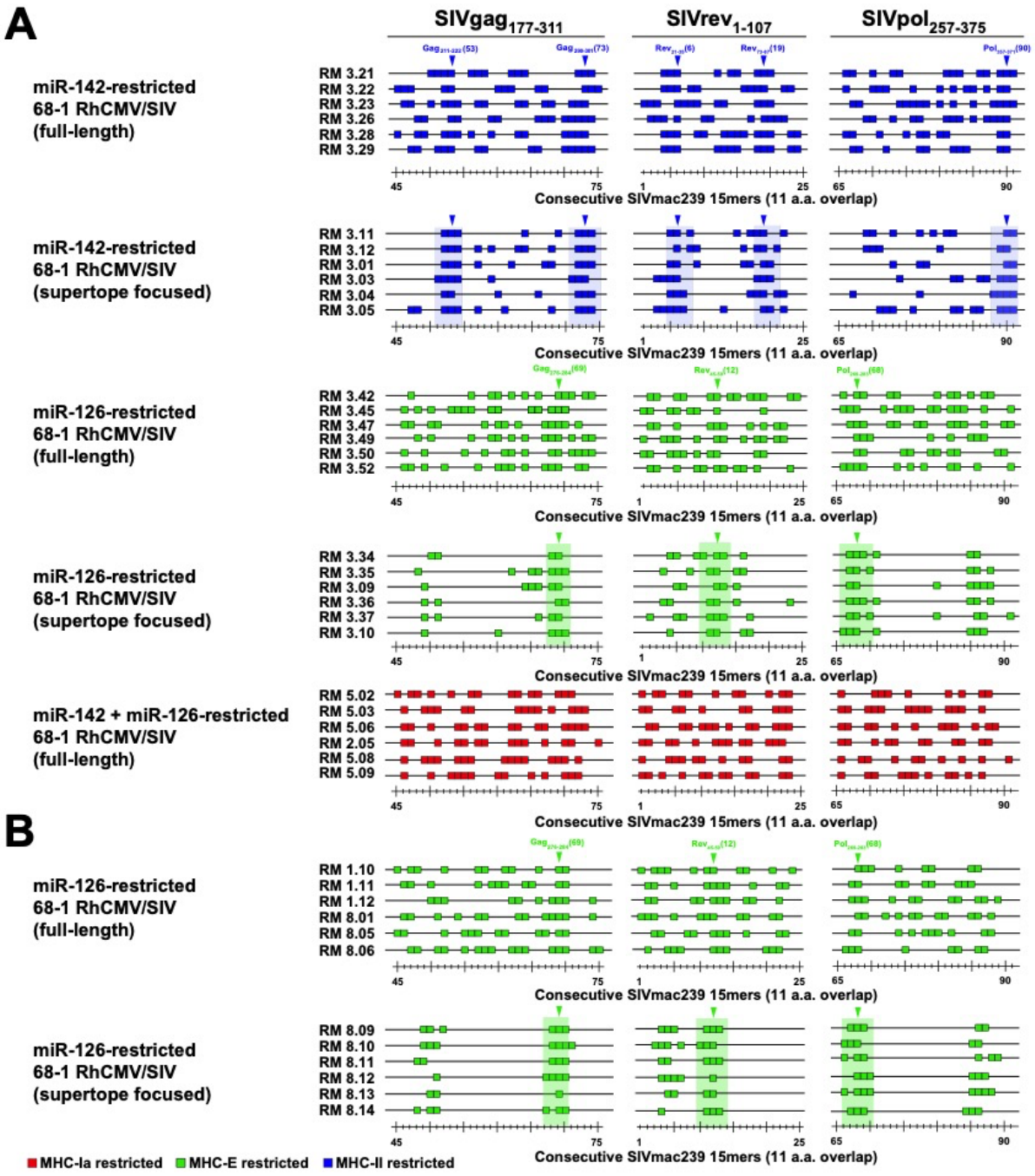


Figure S8. Analysis of MHC restriction patterns of the SIV epitope-specific responses elicited by different miR-restricted 68-1 RhCMV/SIV vaccine vectors used in the challenge studies. (A,B) $CD8^+$ T cell responses to the individual 15mer peptides comprising the indicated segments of the SIV Gag, Rev, and 5'-Pol proteins were analyzed by flow cytometric ICS assay and then restriction-assigned as described in **Fig. 1** for 6 RMs in each vaccine group with the goal of demonstrating that all vectors used in the challenged studies showed the expected epitope recognition patterns in both intra-vaginally (**A**) and intra-rectally (**B**) challenged RMs. The shaded boxes indicate the 15mer peptides included in whole (15/15 amino acids) or in part (11 of 15 amino acids) within the supertope-focused inserts, with core supertope 15mers indicated by arrowheads. Since each examined region contained at least one supertope of each type (MHC-II, MHC-E), this analysis allows visualization of the direct and indirect (cross-reactive) responses to the supertope-focused inserts. A summary of the epitope analyses of all RMs vaccinated with the miR-restricted 68-1 RhCMV/SIV vectors is shown in **table S2.**

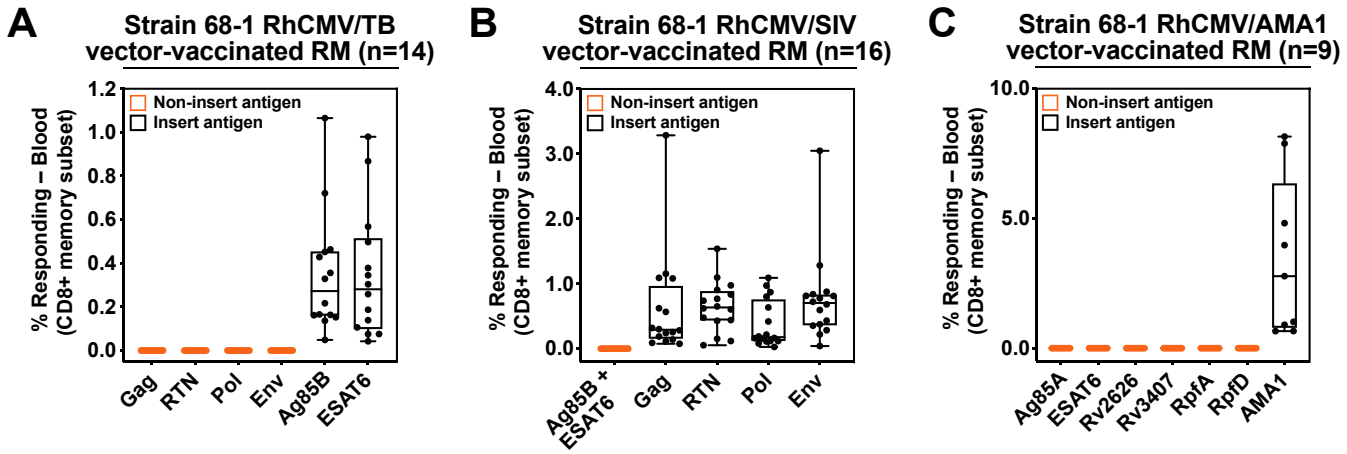


Figure S9. Analysis of cross-reactivity with non-vaccine expressed Ags after vaccination with 68-1 RhCMV vectors. (A-C) $CD8^+$ T cell responses elicited by 68-1 RhCMV expressing *Mycobacterium tuberculosis* (**A**), SIV (**B**), and malaria (**C**) Ag inserts were compared by ICS for their reactivity to consecutive overlapping 15mer peptide mixes comprising the insert Ags or non-related, non-insert Ags. These responses were analyzed after 2 inoculations with the same vectors (homologous prime and boost separated by 12-14 weeks) in plateau phase (>50 weeks post first vaccination). In all RMs, all non-insert responses were below threshold.

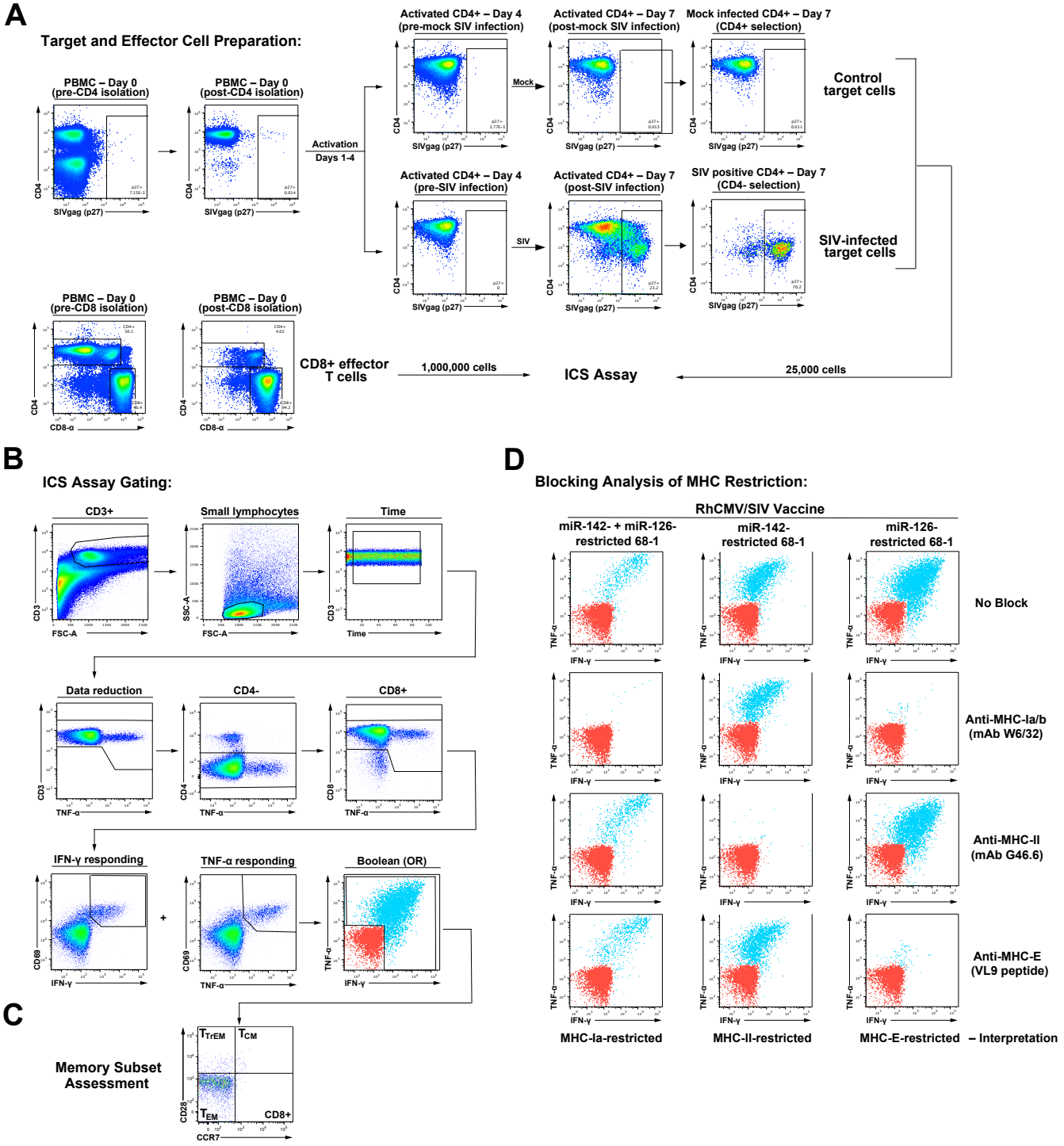


Figure S10. Assay procedure and gating strategy for flow cytometric cytokine production-based SIV-infected cell recognition analysis. (A) Preparation sequence and flow cytometric analysis of autologous control and SIV-infected $CD4^+$ target cells and $CD8^+$ effector cells. (**B**) Gating strategy for delineating $CD8^+$ T cell responses by ICS. (**C**) Memory subset gating of Boolean $CD8^+$ T cell response gate. (**D**) Representative examples of Boolean gated $CD8^+$ T cell response blocking patterns and their interpretation.

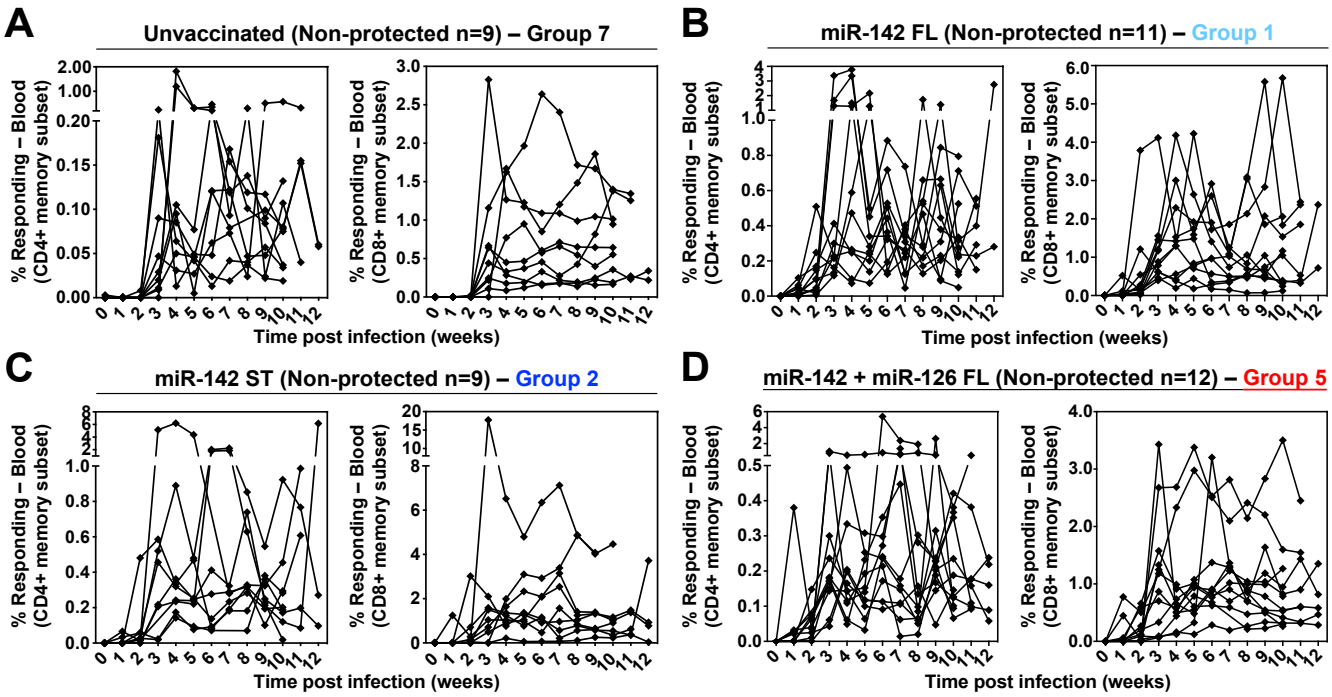


Figure S11. Induction of SIV Vif-specific T cell responses with take of SIV infection after challenge (RM groups 7, 1, 2, 5). The figure shows ICS analysis of $CD4^+$ and $CD8^+$ T cell responses to the SIV Vif 15mer mix before and after SIV challenge for the non-protected RM cohorts from **Fig. 7**. As with the protected RM cohorts shown in **Fig. 7**, all RMs in these cohorts show *de novo* induction of SIV Vif-specific T cell responses concomitant with the take of SIV infection.

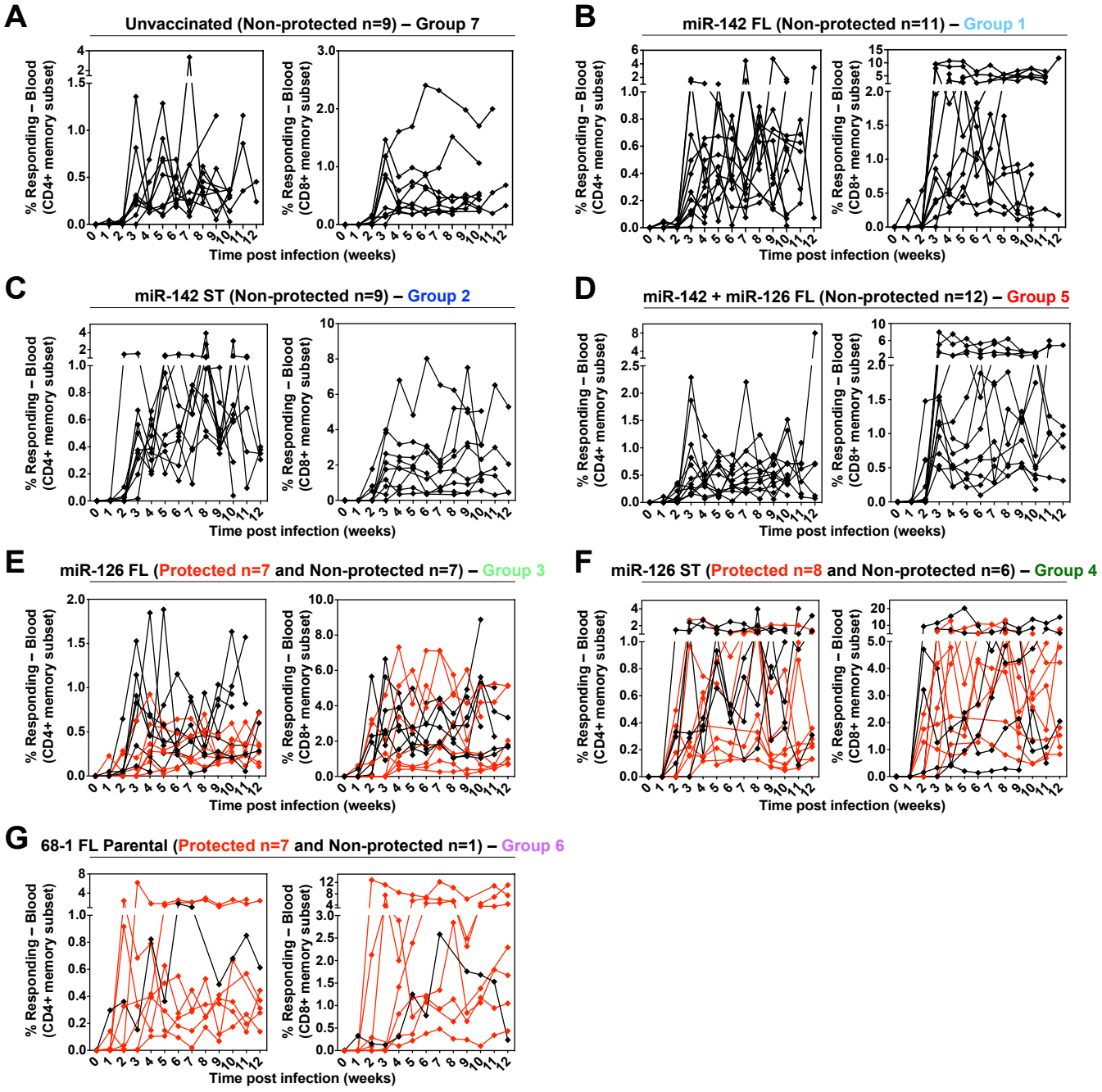


Fig. S12. Induction of SIV Env-specific T cell responses with take of SIV infection after challenge (RM groups 1-7). The figure shows ICS analysis of $CD4^+$ and $CD8^+$ T cell responses to the SIV Env 15mer mix before and after SIV challenge of the same RM cohorts shown in **Fig. 7**. As in **Fig. 7**, protected RMs are shown in red and non-protected RMs in black. Note that prior to initiation of SIV challenge, SIV Env-induced T cell responses were below threshold in all RMs, but above-threshold responses were induced in all RMs post SIV infection take. [FL = full length SIV inserts; ST = supertope-focused SIV inserts]

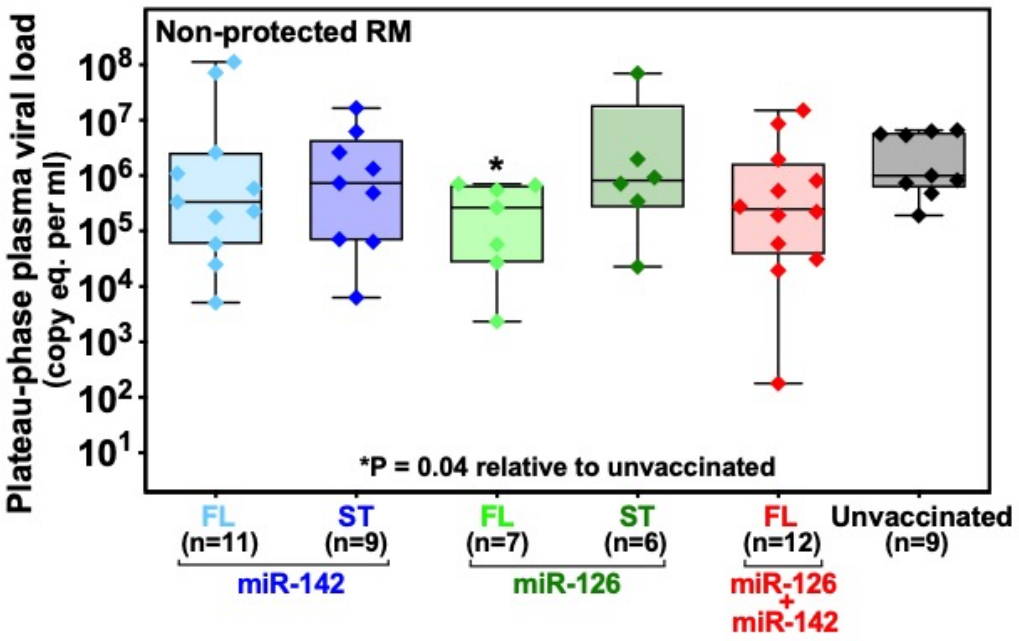


Figure S13. Comparison of viral set points of 68-1 RhCMV/SIV vaccinated, but unprotected, RMs in each vaccine group relative to unvaccinated controls. The figure shows the log mean of plasma viral loads from week 6 to week 10 post infection (defined as plateau phase) for each non-protected (progressively infected) RM in the intravaginal challenge study (**Fig. 7**), grouped by vaccine type. The significance of differences between unvaccinated RMs and the RMs given the various miR-restricted 68-1 RhCMV/SIV vectors was assessed by the Wilcoxon test, with significant P-values after Holm multiplicity adjustment indicated. A 0.58 log10 reduction was noted in the median plateau phase viral loads manifested by the full length insert miR-126 68-1 RhCMV/SIV vaccinated RMs relative to unvaccinated controls.

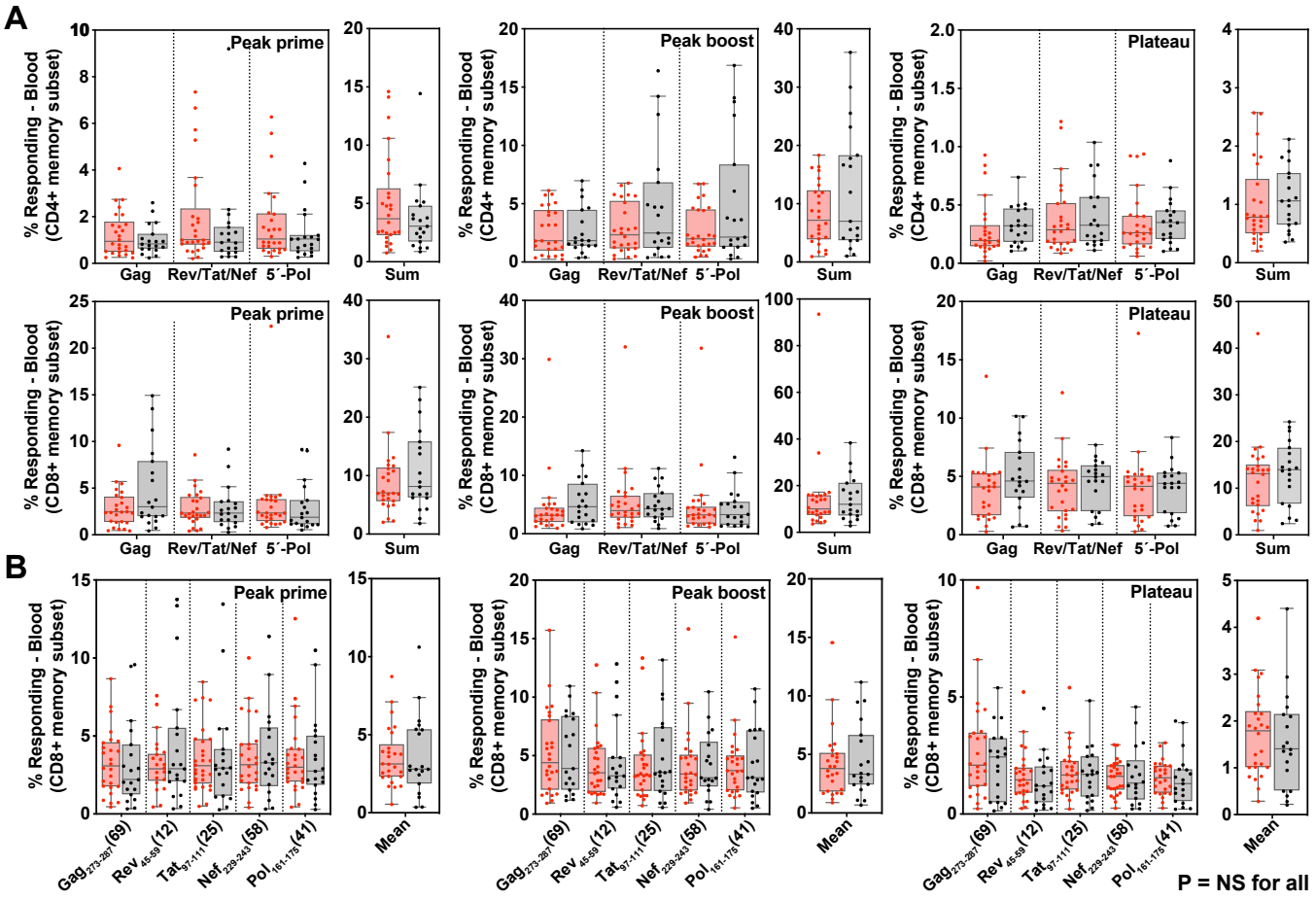


Figure S14. The magnitude of the SIV-specific, MHC-E-restricted CD8$^+$ T cell response in blood at peak and plateau during the vaccine phase does not predict efficacy. (A) Comparison of the peak (both post-prime and post-boost) and plateau phase total- and SIV protein-specific CD8$^+$ T cell response in protected (red) versus non-protected (black/gray) RMs among all miR-126-restricted 68-1 RhCMV/SIV vaccinated RMs with a known challenge outcome in this study, including both the intra-vaginal and intra-rectal challenge cohorts, and RMs vaccinated with vectors expressing either the full length or supertope-focused SIV inserts (n = 26 and n = 19 for protected and non-protected RMs, respectively). (**B**) Same comparisons in the same RMs for the indicated individual MHC-E supertope responses and the mean of these responses in each RM. No significant differences were noted in the magnitude of any of these responses in protected versus non-protected RMs using the Wilcoxon rank-sum test with Holm multiplicity adjustment.

SUPPLEMENTAL TABLES

Rh157.5 (UL128)	Rh157.4 (UL130)	Rh158-161 (UL146/147)	Rh67 (UL40)	MHC restriction of rector-elicited CD8+ T cells	SIV efficacy	RhCMV vector designation
intact	intact	intact	intact	MHC-Ia	not done	wildtype or FL
deleted	deleted	deleted	intact	MHC-II and MHC-E	Yes	68-1 or dd FL
deleted	deleted	intact*	intact	MHC-Ia	not done	ΔRh157.5/157.4
intact	intact	deleted	intact	MHC-Ia	No	68-1.2 or ΔRh158-161
deleted	intact	deleted	intact	MHC-Ia and MHC-II	No	ΔRh157.5 68-1.2
intact	deleted	deleted	intact	MHC-Ia and MHC-II	not done	ΔRh157.4 68-1.2
deleted	deleted	deleted	deleted	MHC-II	No	ΔRh67 68-1

*Expression of any one of Rh158-Rh161 or UL146/UL147 is sufficient for suppression of unconventional restriction (leaving MHC-Ia responses)
"Deleted" = absent or functionally inactivated. Abbreviations: FL - Full length; dd - double-deleted

Table S1. Summary of RhCMV gene products influencing the priming of unconventionally restricted CD8+ T cell responses.

Table S2A

Vector	# of RM	GAG: Total restricton-assigned peptides	GAG: Ia restriction (peptides)	GAG: II restriction (peptides)	GAG: E restriction (peptides)
68-1 RhCMV/SIV	8	281	0	143 (50.9%)	138 (49.1%)
miR-205 68-1 RhCMV/SIV	3	198	0	100 (50.5%)	98 (49.5%)
miR-126 + miR-142 + miR-205 68-1 RhCMV/SIV	1	49	49 (100%)	0	0
miR-126 + miR-205 68-1 RhCMV/SIV	3	140	0	0	140 (100%)
miR-126 + miR-142 68-1 RhCMV/SIV FL	10	334	334 (100%)	0	0
miR-126 68-1 RhCMV/SIV FL	17	375	0	0	375 (100%)
miR-126 68-1 RhCMV/SIV ST	22	181	0	0	181 (100%)
miR-142 68-1 RhCMV/SIV FL	12	287	0	287 (100%)	0
miR-142 68-1 RhCMV/SIV ST	10	132	0	132 (100%)	0

Table S2B

Vector	# of RM	POL: Total restricton-assigned peptides	POL: Ia restriction (peptides)	POL: II restriction (peptides)	POL: E restriction (peptides)
68-1 RhCMV/SIV	5	66	0	31 (47.0%)	35 (53.0%)
miR-205 68-1 RhCMV/SIV	-	-	-	-	-
miR-126 + miR-142 + miR-205 68-1 RhCMV/SIV	-	-	-	-	-
miR-126 + miR-205 68-1 RhCMV/SIV	-	-	-	-	-
miR-126 + miR-142 68-1 RhCMV/SIV FL	9	99	99 (100%)	0	0
miR-126 68-1 RhCMV/SIV FL	16	168	0	0	168 (100%)
miR-126 68-1 RhCMV/SIV ST	22	163	0	0	163 (100%)
miR-142 68-1 RhCMV/SIV FL	9	108	0	108 (100%)	0
miR-142 68-1 RhCMV/SIV ST	10	82	0	82 (100%)	0

Table S2C

Vector	# of RM	REV: Total restricton-assigned peptides	REV: Ia restriction (peptides)	REV: II restriction (peptides)	REV: E restriction (peptides)
68-1 RhCMV/SIV	2	29	0	11 (37.9%)	18 (62.1%)
miR-205 68-1 RhCMV/SIV	-	-	-	-	-
miR-126 + miR-142 + miR-205 68-1 RhCMV/SIV	-	-	-	-	-
miR-126 + miR-205 68-1 RhCMV/SIV	-	-	-	-	-
miR-126 + miR-142 68-1 RhCMV/SIV FL	8	91	91 (100%)	0	0
miR-126 68-1 RhCMV/SIV FL	14	147	0	0	147 (100%)
miR-126 68-1 RhCMV/SIV ST	20	134	0	0	134 (100%)
miR-142 68-1 RhCMV/SIV FL	9	105	0	105 (100%)	0
miR-142 68-1 RhCMV/SIV ST	9	64	0	64 (100%)	0

Table S2D

Vector	# of RM	TOTAL (GAG+POL+REV): Total restricton-assigned peptides	TOTAL (GAG+POL+REV): Ia restriction (peptides)	TOTAL (GAG+POL+REV): II restriction (peptides)	TOTAL (GAG+POL+REV): E restriction (peptides)
68-1 RhCMV/SIV	8	376	0	185 (49.2%)	191 (50.8%)
miR-205 68-1 RhCMV/SIV	3	198	0	100 (50.5%)	98 (49.5%)
miR-126 + miR-142 + miR-205 68-1 RhCMV/SIV	1	49	49 (100%)	0	0
miR-126 + miR-205 68-1 RhCMV/SIV	3	140	0	0	140 (100%)
miR-126 + miR-142 68-1 RhCMV/SIV FL	10	524	524 (100%)	0	0
miR-126 68-1 RhCMV/SIV FL	17	690	0	0	690 (100%)
miR-126 68-1 RhCMV/SIV ST	22	478	0	0	478 (100%)
miR-142 68-1 RhCMV/SIV FL	12	500	0	500 (100%)	0
miR-142 68-1 RhCMV/SIV ST	10	278	0	278 (100%)	0

Table S2. Summary of restriction-assigned 15mer peptide responses for all study RMs.

Table S3A

Vector	# of RM	Total restricton-assigned epitopes	Restriction assignment	Average density of total restriction-assigned epitopes (epitopes/100 a.a. of tested SIV protein sequence)	Reduction of epitope density with ST-focused inserts (from corresponding FL inserts)	Average density of non-insert encoded epitopes (epitopes/100 a.a. of tested non-insert encoded peptides)
			GAG			
68-1 RhCMV/SIV	8	185	II (51.9%), E (48.1%)	8.1	-	-
miR-126 + miR-142 68-1 RhCMV/SIV FL	10	251	Ia (100%)	6.9	-	-
miR-126 68-1 RhCMV/SIV FL	18	241	E (100%)	6.7	-	-
miR-126 68-1 RhCMV/SIV ST	22	122	E (100%)	2.9	57%	1.5
miR-142 68-1 RhCMV/SIV FL	12	184	II (100%)	6.7	-	-
miR-142 68-1 RhCMV/SIV ST	10	96	II (100%)	3.7	45%	2.2

Table S3B

Vector	# of RM	Total restricton-assigned epitopes	Restriction assignment	Average density of total restriction-assigned epitopes (epitopes/100 a.a. of tested SIV protein sequence)	Reduction of epitope density with ST-focused inserts (from corresponding FL inserts)	Average density of non-insert encoded epitopes (epitopes/100 a.a. of tested non-insert encoded peptides)
			POL			
68-1 RhCMV/SIV	5	43	II (44.2%), E (55.8%)	7.5	-	-
miR-126 + miR-142 68-1 RhCMV/SIV FL	9	67	Ia (100%)	6.5	-	-
miR-126 68-1 RhCMV/SIV FL	16	111	E (100%)	6	-	-
miR-126 68-1 RhCMV/SIV ST	22	107	E (100%)	4.2	30%	2.7
miR-142 68-1 RhCMV/SIV FL	9	69	II (100%)	6.7	-	-
miR-142 68-1 RhCMV/SIV ST	10	53	II (100%)	4.6	31%	4.2

Table S3C

Vector	# of RM	Total restricton-assigned epitopes	Restriction assignment	Average density of total restriction-assigned epitopes (epitopes/100 a.a. of tested SIV protein sequence)	Reduction of epitope density with ST-focused inserts (from corresponding FL inserts)	Average density of non-insert encoded epitopes (epitopes/100 a.a. of tested non-insert encoded peptides)
			REV			
68-1 RhCMV/SIV	2	19	II (47.4%), E (52.6%)	8.9	-	-
miR-126 + miR-142 68-1 RhCMV/SIV FL	8	58	Ia (100%)	6.8	-	-
miR-126 68-1 RhCMV/SIV FL	14	95	E (100%)	6.3	-	-
miR-126 68-1 RhCMV/SIV ST	20	83	E (100%)	3.9	38%	2.7
miR-142 68-1 RhCMV/SIV FL	9	64	II (100%)	6.6	-	-
miR-142 68-1 RhCMV/SIV ST	9	42	II (100%)	4.4	33%	1.3

Table S3. Epitope density analysis of differentially tropism-restricted (response programmed) 68-1 RhCMV/SIV vectors.

	MHC-E targeted 15mer	AA Sequence	Number of RM responding	Number of RM Screened
SIVgag	5	EKIRLRPNGKKKYML	2	5
	10	AANELDRFGLAESLL	1	
	12	GLAESLLENKEGCQK	1	
	13	SLLENKEGCQKILSV	1	
	18	PTGSENLKSLYNTVC	1	
	19	ENLKSLYNTVCVIWC	1	
	26	KQIVQRHLVVETGTT	1	
	33	SGRGGNYPVQQIGGN	2	
	38	LSPRTLNAWVKLIEE	1	
	39	TLNAWVKLIEEKKFG	1	
	42	KFGAEVVPGFQALSE	1	
	45	LSEGCTPYDINQMLN	3	22
	47	DINQMLNCVGDHQAA	3	
	48	MLNCVGDHQAAMQII	10	
	49	VGDHQAAMQIIRDII	14	
	50	QAAMQIIRDIINEEA	11	
	51	QIIRDIINEEAADWD	12	
	52	DIINEEAADWDLQHP	1	
	55	QHPQPAPQQGQLREP	1	
	56	PAPQQGQLREPSGSD	1	
	58	REPSGSDIAGTTSSV	2	
	59	GSDIAGTTSSVDEQI	1	
	60	AGTTSSVDEQIQWMY	2	
	61	SSVDEQIQWMYRQQN	1	
	62	EQIQWMYRQQNPIPV	1	
	63	WMYRQQNPIPVGNIY	1	
	64	QQNPIPVGNIYRRWI	2	
	65	IPVGNIYRRWIQLGL	4	
	66	NIYRRWIQLGLQKCV	7	
	67	RWIQLGLQKCVRMYN	8	
	71	TNILDVKQGPKEPFQ	3	
	72	DVKQGPKEPFQSYVD	2	
	73	GPKEPFQSYVDRFYK	2	
	80	WMTQTLLIQNANPDC	1	5
	92	AEALKEALAPVPIPF	1	
	100	GKEGHSARQCRAPRR	1	
	108	DRQAGFLGLGPWGKK	1	
	110	LGPWGKKPRNFPMAQ	2	
	114	HQGLMPTAPPEDPAV	1	

	MHC-E targeted 15mer	AA Sequence	Number of RM responding	Number of RM Screened
SIVpol	66	TNPYNTPTFAIKKKD	10	22
	71	MLIDFRELNRVTQDF	6	
	72	FRELNRVTQDFTEVQ	1	
	76	GIPHPAGLAKRKRIT	3	
	77	PAGLAKRKRITVLDI	1	
	78	AKRKRITVLDIGDAY	1	
	80	LDIGDAYFSIPLDEE	3	
	83	DEEFRQYTAFTLPSV	1	
	84	RQYTAFTLPSVNNAE	4	
	85	AFTLPSVNNAEPGKR	18	
	86	PSVNNAEPGKRYIYK	18	
	87	NAEPGKRYIYKVLPQ	14	
	88	GKRYIYKVLPQGWKG	11	
	89	IYKVLPQGWKGSPAI	4	
	90	LPQGWKGSPAIFQYT	1	
	91	WKGSPAIFQYTMRHV	4	
SIVrev	2	EREEELRKRLRLIHL	1	21
	3	ELRKRLRLIHLLHQT	4	
	4	RLRLIHLLHQTNPYP	11	
	5	IHLLHQTNPYPTGPG	14	
	6	HQTNPYPTGPGTANQ	13	
	7	PYPTGPGTANQRRQR	9	
	8	GPGTANQRRQRKRRW	6	
	9	ANQRRQRKRRWRRRW	4	
	14	LADRIYSFPDPPTDT	2	
	15	IYSFPDPPTDTPLDL	5	
	16	PDPPTDTPLDLAIQQ	9	
	17	TDTPLDLAIQQLQNL	4	
	18	LDLAIQQLQNLAIES	1	
	19	IQQLQNLAIESIPDP	1	
	23	TNTPEALCDPTEDSR	2	
	24	EALCDPTEDSRSPQD	1	

Overlap with sequence in MHC-E supertope-focused insert

Table S4. List 15mers with sequences outside the MHC-E supertope-focused insert (≤ 4 amino acid overlap) that are recognized by CD8$^+$ T cell responses elicited by the miR-126-restricted 68-1 RhCMV vectors expressing MHC-E supertope-focused SIV inserts.

	MHC-E targeted 15mer	AA Sequence	Number of RM responding	Number of RM Screened
SIVgag	1	MGVRNSVLSGKKADE	2	
	2	NSVLSGKKADELEKI	3	
	3	SGKKADELEKIRLRP	1	
	6	LRPNGKKKYMLKHVV	1	
	9	HVVWAANELDRFGLA	1	
	11	LDRFGLAESLLENKE	1	
	21	TVCVIWCIHAEEKVK	1	5
	25	TEEAKQIVQRHLVVE	1	
	34	GNYPVQQIGGNYVHL	1	
	35	VQQIGGNYVHLPLSP	1	
	36	GGNYVHLPLSPRTLN	1	
	41	IEEKKFGAEVVPGFQ	1	
	42	KFGAEVVPGFQALSE	1	
	45	LSEGCTPYDINQMLN	1	
	47	DINQMLNCVGDHQAA	1	
	48	MLNCVGDHQAAMQII	1	
	57	QGQLREPSGSDIAGT	4	
	58	REPSGSDIAGTTSSV	1	
	59	GSDIAGTTSSVDEQI	4	
	60	AGTTSSVDEQIQWMY	3	
	61	SSVDEQIQWMYRQQN	3	10
	62	EQIQWMYRQQNPIPV	3	
	63	WMYRQQNPIPVGNIY	2	
	64	QQNPIPVGNIYRRWI	2	
	66	NIYRRWIQLGLQKCV	1	
	67	RWIQLGLQKCVRMYN	1	
	68	LGLQKCVRMYNPTNI	4	
	69	KCVRMYNPTNILDVK	1	
	78	QTDAAVKNWMTQTLL	2	
	83	PDCKLVLKGLGVNPT	1	
	84	LVLKGLGVNPTLEEM	1	
	90	PGQKARLMAEALKEA	1	
	92	AEALKEALAPVPIPF	2	5
	105	CGKMDHVMAKCPDRQ	1	
	109	GFLGLGPWGKKPRNF	1	
	113	MAQVHQGLMPTAPPE	1	
	116	PPEDPAVDLLKNYMQ	1	
	117	PAVDLLKNYMQLGKQ	2	

	MHC-E targeted 15mer	AA Sequence	Number of RM responding	Number of RM Screened
SIVpol	67	NTPTFAIKKKDKNKW	2	
	68	FAIKKKDKNKWRMLI	1	
	69	KKDKNKWRMLIDFRE	4	
	70	NKWRMLIDFRELNRV	3	
	71	MLIDFRELNRVTQDF	3	
	72	FRELNRVTQDFTEVQ	1	
	73	NRVTQDFTEVQLGIP	6	
	74	QDFTEVQLGIPHPAG	1	
	76	GIPHPAGLAKRKRIT	4	10
	77	PAGLAKRKRITVLDI	6	
	78	AKRKRITVLDIGDAY	2	
	79	RITVLDIGDAYFSIP	3	
	80	LDIGDAYFSIPLDEE	4	
	81	DAYFSIPLDEEFRQY	3	
	82	SIPLDEEFRQYTAFT	4	
	83	DEEFRQYTAFTLPSV	4	
	85	AFTLPSVNNAEPGKR	1	
	86	PSVNNAEPGKRYIYK	3	
SIVrev	3	ELRKRLRLIHLLHQT	2	
	10	RQRKRRWRRRWQQLL	1	
	13	QLLALADRIYSFPDP	1	9
	15	IYSFPDPPTDTPLDL	2	
	16	PDPPTDTPLDLAIQQ	2	

Overlap with sequence in MHC-II supertope-focused insert

Table S5. List 15mers with sequences outside the MHC-II supertope-focused insert (≤ 4 amino acid overlap) that are recognized by CD8$^+$ T cell responses elicited by the miR-142-restricted 68-1 RhCMV vectors expressing MHC-II supertope-focused SIV inserts.

	Tissue	PID	Number of cells (per tissue)	Total number of cells transferred	
RM 3.41	Bone Marrow LN	35-84	1.3×10^7 7.0×10^7	8.3×10^7	Figure 7E
RM 3.42	Bone Marrow LN	28-86	3.4×10^7 6.6×10^7	1.0×10^8	
RM 3.43	LN	28-84	6.0×10^7	6.0×10^7	
RM 3.45	LN	56-84	6.0×10^7	6.0×10^7	
RM 3.46	Bone Marrow LN	35-59	1.0×10^7 9.0×10^7	1.0×10^8	
RM 3.47	Bone Marrow LN	56-84	2.7×10^7 7.3×10^7	1.0×10^8	
RM 3.50	Bone Marrow LN	28	1.1×10^7 7.0×10^7	8.1×10^7	
RM 3.31	Bone Marrow LN	28	1.1×10^7 8.9×10^7	1.0×10^8	Figure 7F
RM 3.32	Bone Marrow LN	35-87	2.5×10^7 7.5×10^7	1.0×10^8	
RM 3.34	Bone Marrow LN	44-85	4.0×10^7 6.0×10^7	1.0×10^8	
RM 3.35	Bone Marrow LN	44-85	1.8×10^7 8.2×10^7	1.0×10^8	
RM 3.36	Bone Marrow LN	28-84	2.3×10^7 7.7×10^7	1.0×10^8	
RM 3.37	Bone Marrow LN	35-84	9.0×10^6 9.1×10^7	1.0×10^8	
RM 3.10	Bone Marrow LN	56-84	6.0×10^6 9.3×10^7	9.9×10^7	
RM 3.38	Bone Marrow LN	28-84	2.3×10^7 7.7×10^7	1.0×10^8	
RM 5.13	Bone Marrow LN	28-83	4.6×10^7 5.4×10^7	1.0×10^8	Figure 7G
RM 5.14	Bone Marrow LN	29-84	3.0×10^6 9.0×10^7	9.3×10^7	
RM 5.15	Bone Marrow LN	38-87	1.9×10^7 8.1×10^7	1.0×10^8	
RM 1.11	Bone Marrow LN	28-56	9.0×10^6 4.4×10^7	5.3×10^7	Figure 8E
RM 8.01	Bone Marrow LN	40-89	3.5×10^7 5.9×10^7	9.4×10^7	
RM 8.02	Bone Marrow LN	28-56	1.0×10^7 5.0×10^7	6.0×10^7	
RM 8.03	Bone Marrow LN	40-84	5.4×10^7 4.6×10^7	1.0×10^8	
RM 8.05	Bone Marrow LN	40-84	1.0×10^7 4.0×10^7	5.0×10^7	
RM 8.06	Bone Marrow LN	40-84	4.0×10^6 3.0×10^7	3.4×10^7	
RM 8.07	Bone Marrow LN	42-56	4.0×10^6 1.7×10^7	2.1×10^7	Figure 8F
RM 8.09	PBMC	35-84	4.0×10^7	4.0×10^7	
RM 8.11	Bone Marrow LN	35-56	2.0×10^6 4.6×10^7	4.8×10^7	
RM 8.12	LN	56	9.5×10^7	9.5×10^7	
RM 8.13	Bone Marrow LN	28-84	2.0×10^6 5.0×10^7	5.2×10^7	

Table S6. Cells used for adoptive transfer analysis of replication competent SIV in protected RMs.