Supplementary Materials for

Cytomegalovirus Vectors Violate CD8+ T Cell Epitope Recognition Paradigms

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This PDF file includes:

Supplemental Figures

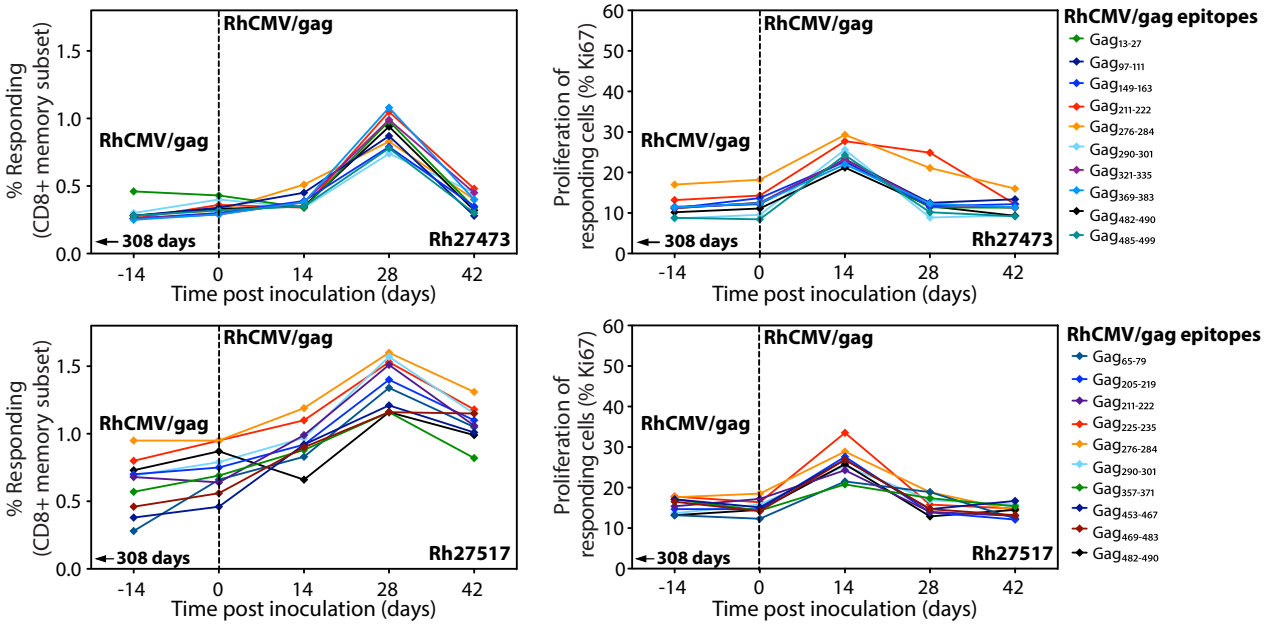

Fig. S1. RhCMV/gag vector-elicited CD8+ T cells respond to homologous boosting with an increase in peripheral blood response frequencies and induction of proliferation. The CD8+ T cell response to individual SIVgag 15mer peptides was determined in two strain 68-1 RhCMV/gag-vaccinated RM (Rh27473 and Rh27517) using flow cytometric ICS (as described in Fig. 1A). The frequency of the top 10 of these epitope-specific responses in blood and the proliferative status of the responding cells (as measured by Ki-67 expression on the TNF-α + and/or IFN-γ + cells) were followed after re-administration of the same RhCMV/gag (308 days post initial vaccination).

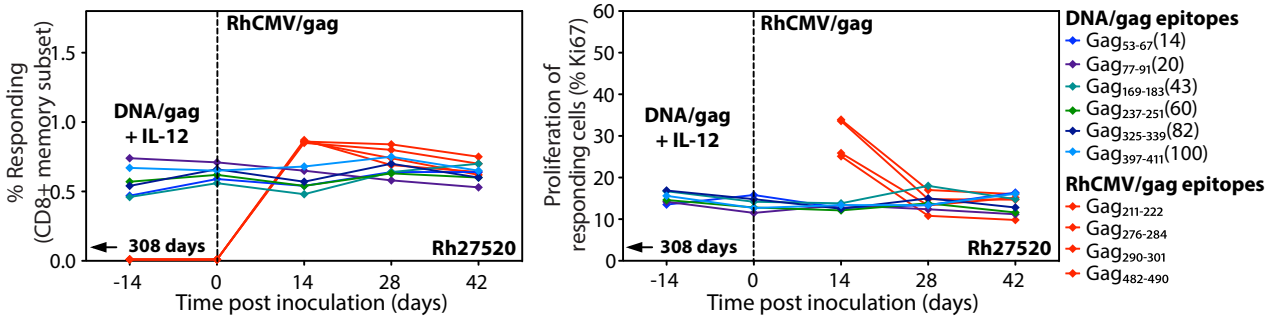

Fig. S2. RhCMV/gag vectors do not boost gag-specific CD8+ T cell responses primed with electroporated DNA/gag + IL-12 vaccination. The CD8+ T cell response to individual SIVgag 15mer peptides was determined in a second RM (Rh27520) vaccinated by electroporation of a DNA/gag + IL-12 plasmid using flow cytometric ICS (results from the other RM is shown in Fig. 1E). The frequency of the top 6 epitope-specific responses in blood and the proliferative status of the peptide-responding cells (as measured by Ki-67 expression on the TNF-α + and/or IFN-γ + cells) were followed after boosting with strain 68-1 RhCMV/gag (308 days post initial vaccination). This figure also shows induction of 4 SIVgag 15mer-specific CD8+ T cell responses after RhCMV/gag vaccination that were not detectable in the pre-existing SIVgag-specific responses elicited by the DNA/gag + IL-12 vaccine.

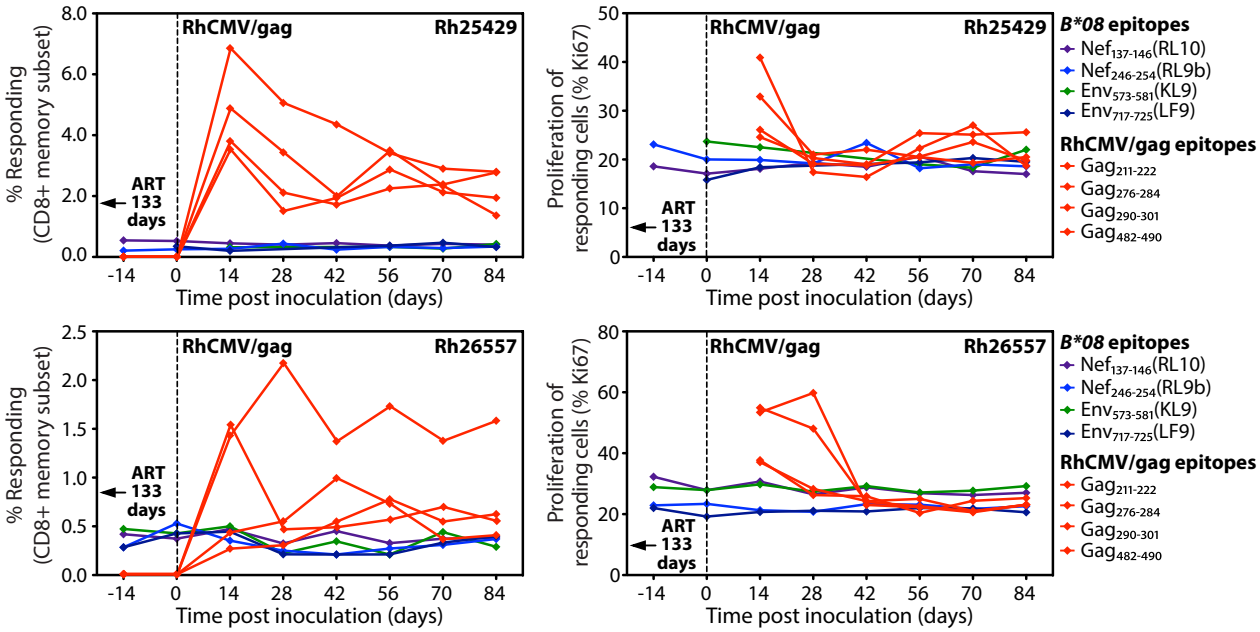

Fig. S3. RhCMV/gag vectors do not boost canonical gag-specific CD8+ T cell responses in SIV+ RM on fully suppressive anti-retroviral therapy (ART). Two SIVmac251-infected *Mamu-B*08*+ RM (Rh25429 and Rh26557; plasma viral loads = 53,000 copies/ml and 6,700 copies/ml, respectively), were treated with combination anti-retroviral therapy consisting of 2 reverse transcriptase inhibitors (20mg/day Tenofovir, 50mg/day Emtricitabine), an integrase inhibitor (2.5 mg/day Dolutegravir) and protease inhibitor (1200 mg/day Darunavir boosted with 200mg/day Ritonavir) at 288 and 260 days post-SIV infection. Plasma viral loads were suppressed below the level of detection (30 copies/ml) within 2 weeks of ART initiation and maintained at undetectable levels for the duration of the experiment. The magnitude of CD8+ T cell responses to the Mamu-*B*08*-restricted canonical epitopes $Nef_{137\text{-}146}$ (RL10), $Nef_{246\text{-}254}$ (RL9b), $Env_{573\text{-}581}$ (KL9), and $Env_{717\text{-}725}$ (LF9) was determined, as well as the proliferative status of the epitope-responding cells (as measured by Ki-67 expression on the TNF-α + and/or IFN-γ + cells), using flow cytometric ICS, and then these parameters were followed after vaccination of these 2 RM with strain 68-1 RhCMV/gag (133 days after the initiation of ART). This figure also shows induction of 4 SIVgag 15mer-specific CD8+ T cell responses after RhCMV/gag vaccination that were not detectable in the pre-existing SIVgag-specific responses elicited by SIV infection.

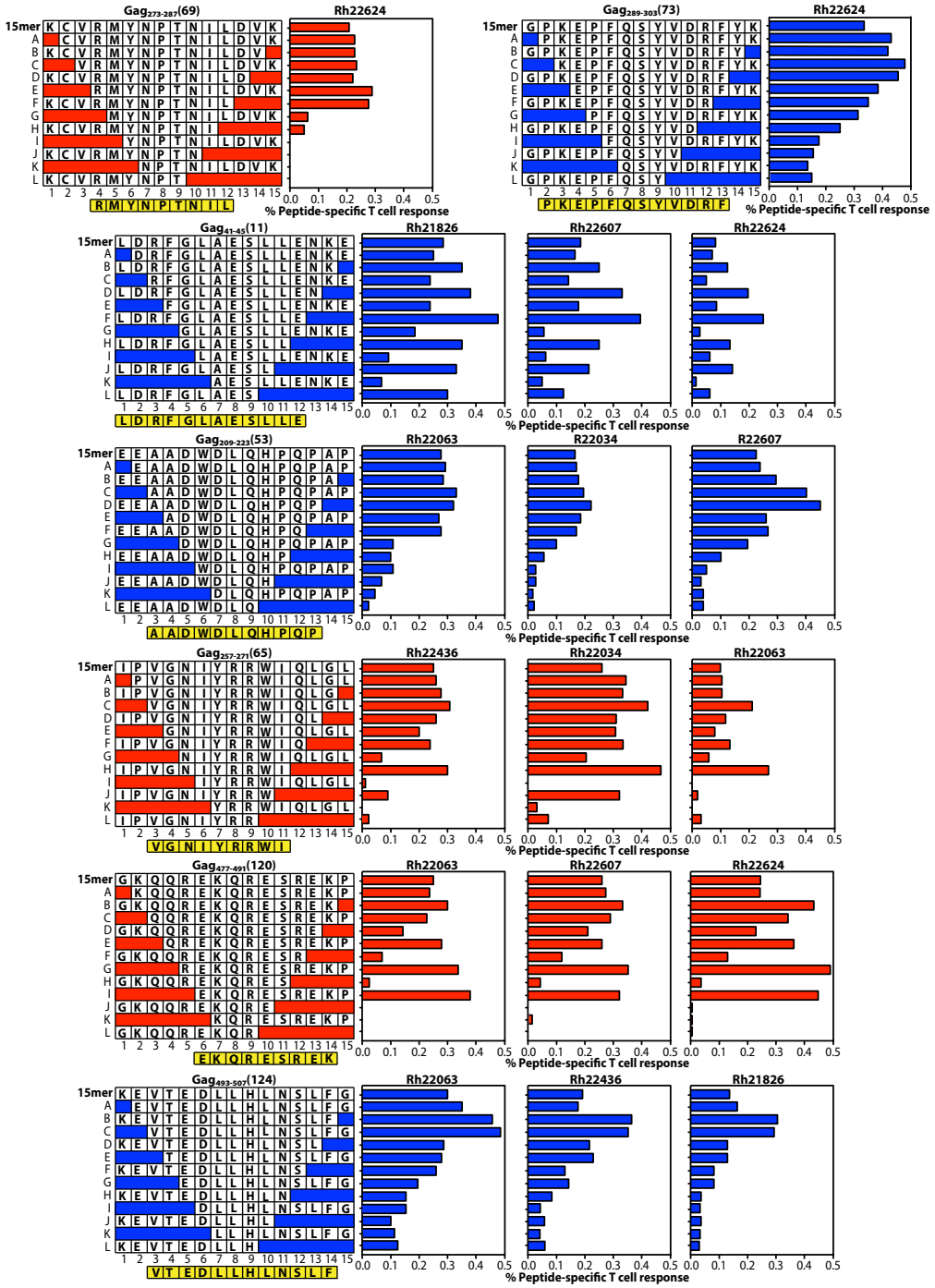

Fig. S4. Truncation analysis of SIVgag 15mers targeted commonly by RhCMV/gag vector-elicited CD8+ T cells. The core epitopes of selected SIVgag 15mer peptides, designated by amino acid position (and the # of the consecutive 15mer from the amino terminus), targeted by CD8+ T cells from strain 68-1 RhCMV/gag-vaccinated RM were determined by flow cytometric ICS analysis of CD8+ T cell responses to the indicated truncated peptides. The figure shows analysis of 3 different RM per peptide, except for 15mer #s 69 and 73, for which 2 RM each are shown in Fig. 2B. The amino terminal and carboxy terminal truncations showing the highest response above that of the parent 15mer define the core epitopes (yellow). Note the striking similarity in the response pattern to each of these truncated peptides between the 3 RM tested, consistent with the same epitope accounting for the response to the parent 15mer in all animals.

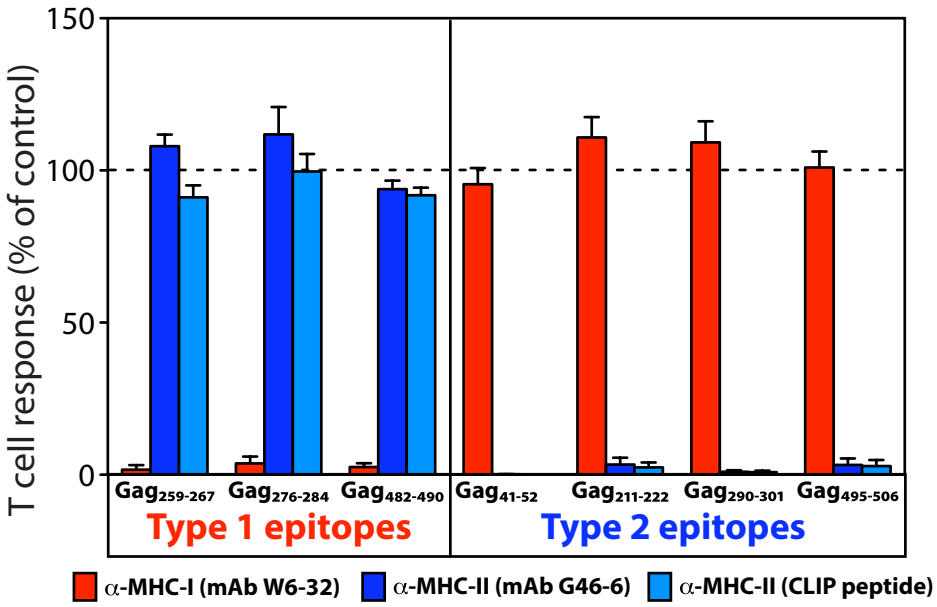

Fig. S5. The recognition of SIVgag supertopes by RhCMV/gag vector-elicited CD8+ T cells can be inhibited with either MHC-I or MHC-II blockade. PBMC from strain 68-1 RhCMV/gag-vaccinated RM (n = 8 for $Gag_{211-222}$, $Gag_{276-284}$, $Gag_{290-301}$, $Gag_{482-490}$, $Gag_{495-506}$; n = 5 for Gag_{41-52}, $Gag_{259-267}$) were stimulated with the designated SIVgag core epitopes (classified as Type 1 vs. Type 2 by the length of the core epitope; see Fig. 2B and fig. S4) in the presence of irrelevant isotype control mAbs (IgG_1 – clone X40 + IgG_{2a} – clone X39; 10μg/ml each), an anti-MHC-I mAb (W6-32; 10μg/ml), an anti-MHC-II mAb (G46-6; 10μg/ml), or the CLIP peptide (MHC-II-associated invariant chain, amino acids 89-100; 2μg/ml). The response frequencies were normalized to the response frequencies in the isotype control-treated cultures and the mean ± SEM of these normalized response frequencies are shown for each treatment. Note that the responses to the 3 epitopes classified as Type 1 were only blocked with the anti-MHC-I mAb and the 4 epitopes classified as Type 2 were only blocked with the anti-MHC-II mAb and the CLIP peptide.

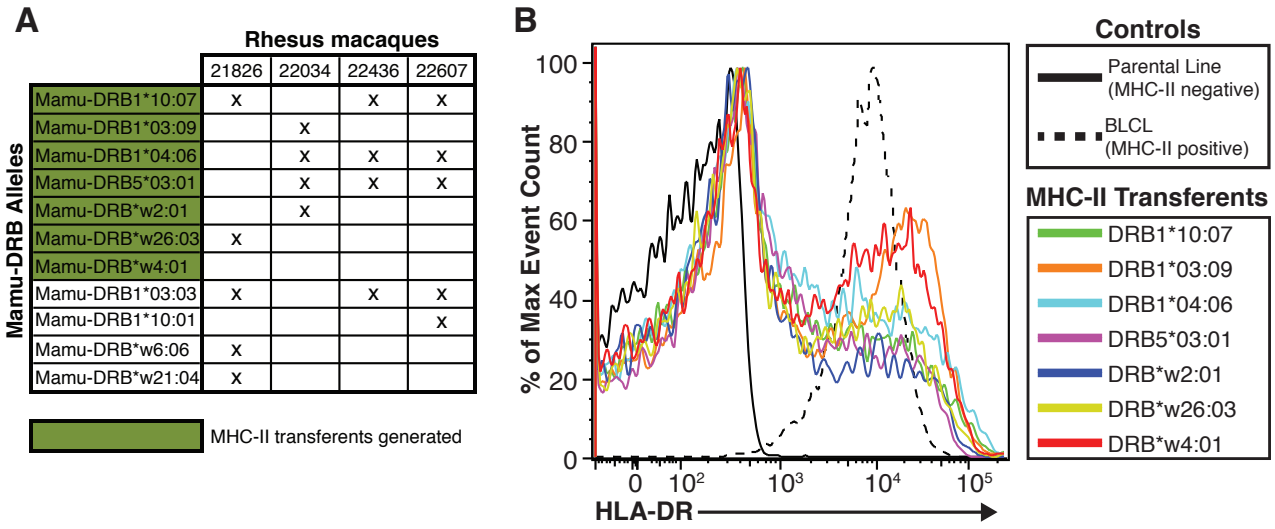

Fig. S6. Validation of transfected cell lines expressing single Mamu-DR molecules corresponding to MHC-II allomorphs expressed by 4 strain 68-1 RhCMV/gag vector-vaccinated RM. (**A**) RM #s 21826, 22034, 22436, and 22607 were *Mamu-DRB*-genotyped by Roche/454 pyrosequencing. Green highlight indicates alleles selected for MHC-II transfectant generation. (**B**) One *Mamu-DRB* allele and its paired *Mamu-DRA* allele were transfected into a parental (MHC-II-negative) cell line (RM3 cells). Cells were stained with a cross-reactive human HLA-DR-PE monoclonal antibody for 15 minutes at room temperature to assess Mamu-DR expression. Cells were washed once with 1X PBS supplemented with 10% fetal bovine serum, fixed with 2% paraformaldehyde, collected on an LSR-II flow cytometer, and analyzed with FlowJo. MHC-II-expressing B-lymphoblastoid cells (BLCL) served as a positive control, while the MHC-II-negative parental cell line (RM3) was used as a negative control. Note that the intensity of Mamu-DR expression on the surface of the single *Mamu-DRA*/B transfectants was as high or higher than that of the BLCL.

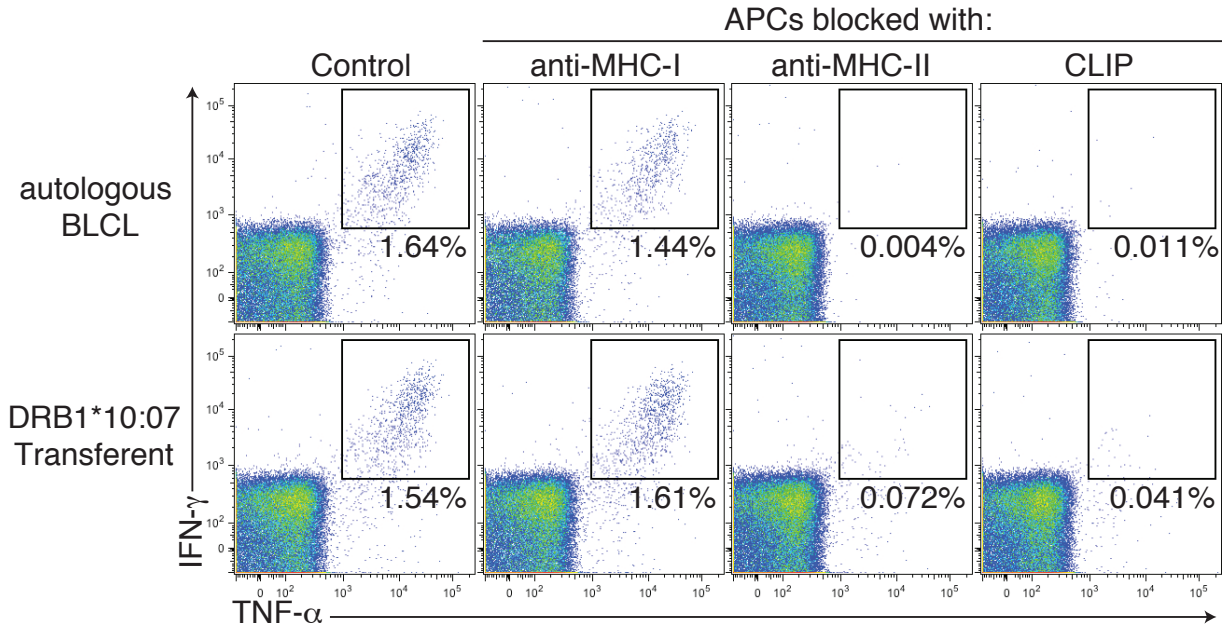

Fig. S7. Inhibition of an MHC-II transfectant-mediated CD8+ T cell response with MHC-II, but not MHC-I, blockade. Autologous B-lymphoblastoid cells (BLCL) and the DRB1*10:07 expressing transfectant were pulsed with the $Gag_{289\text{-}303}$ 15mer peptide (15mer #73) in the presence of irrelevant isotype control mAbs (IgG_1 – clone X40 + IgG_{2a} – clone X39; 10μg/ml each), an anti-MHC-I mAb (W6/32; 10μg/ml), an anti-MHC-II mAb (G46-6; 10μg/ml), or the CLIP peptide (MHC-II-associated invariant chain, amino acids 89-100; 2μg/ml). Note that these responses are only inhibited with MHC-II blockade.

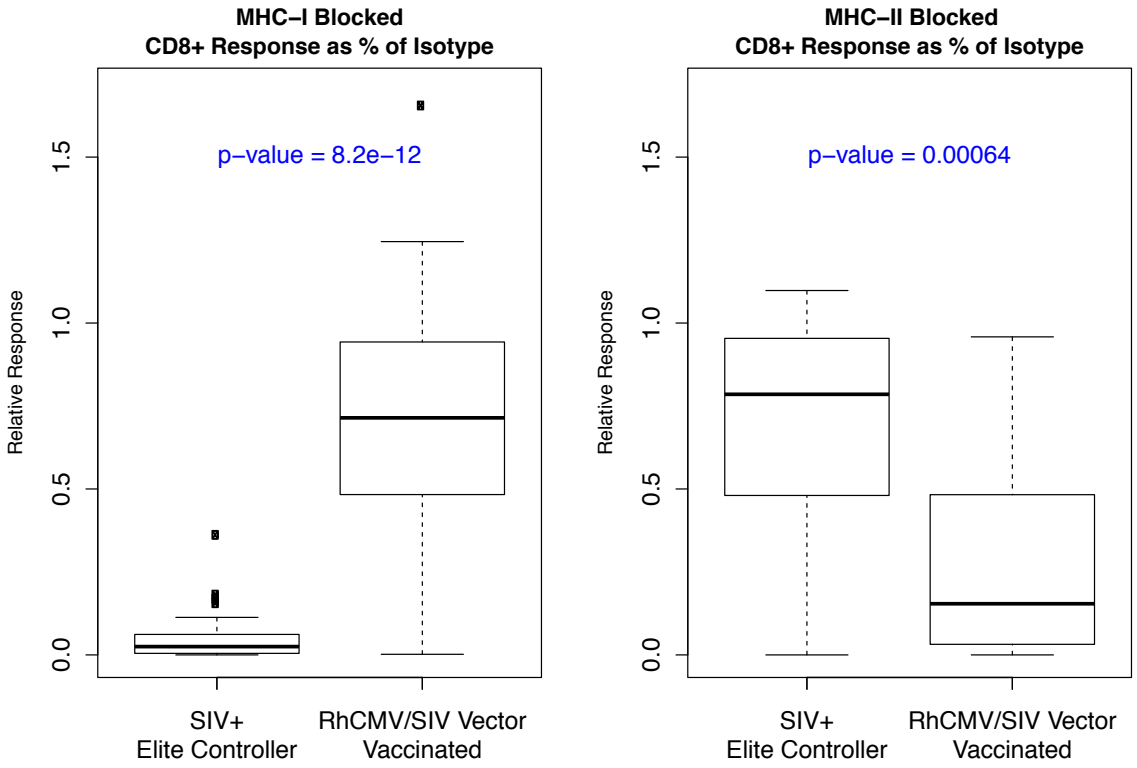

Fig. S8. Statistical analysis of the MHC association of AT-2 SIV-specific CD8+ T cell responses in SIV+ elite controllers vs. RhCMV/SIV vector-vaccinated RM. The boxplots illustrate the comparison of MHC-I-blocked (and MHC-II-blocked) CD8+ T cell responses between elite SIV controllers and strain 68-1 RhCMV/SIV-vaccinated RM. MHC-I-blocked (and MHC-II-blocked) responses at all AT-2 SIV dilutions, represented as fractions of the isotype response at that dilution, are combined across dilutions. The depicted p-values, from two-tailed Wilcoxon rank-sum tests, indicate that the differences for both MHC-I-blocked and MHC-II-blocked CD8+ T cell response fractions are statistically significant.

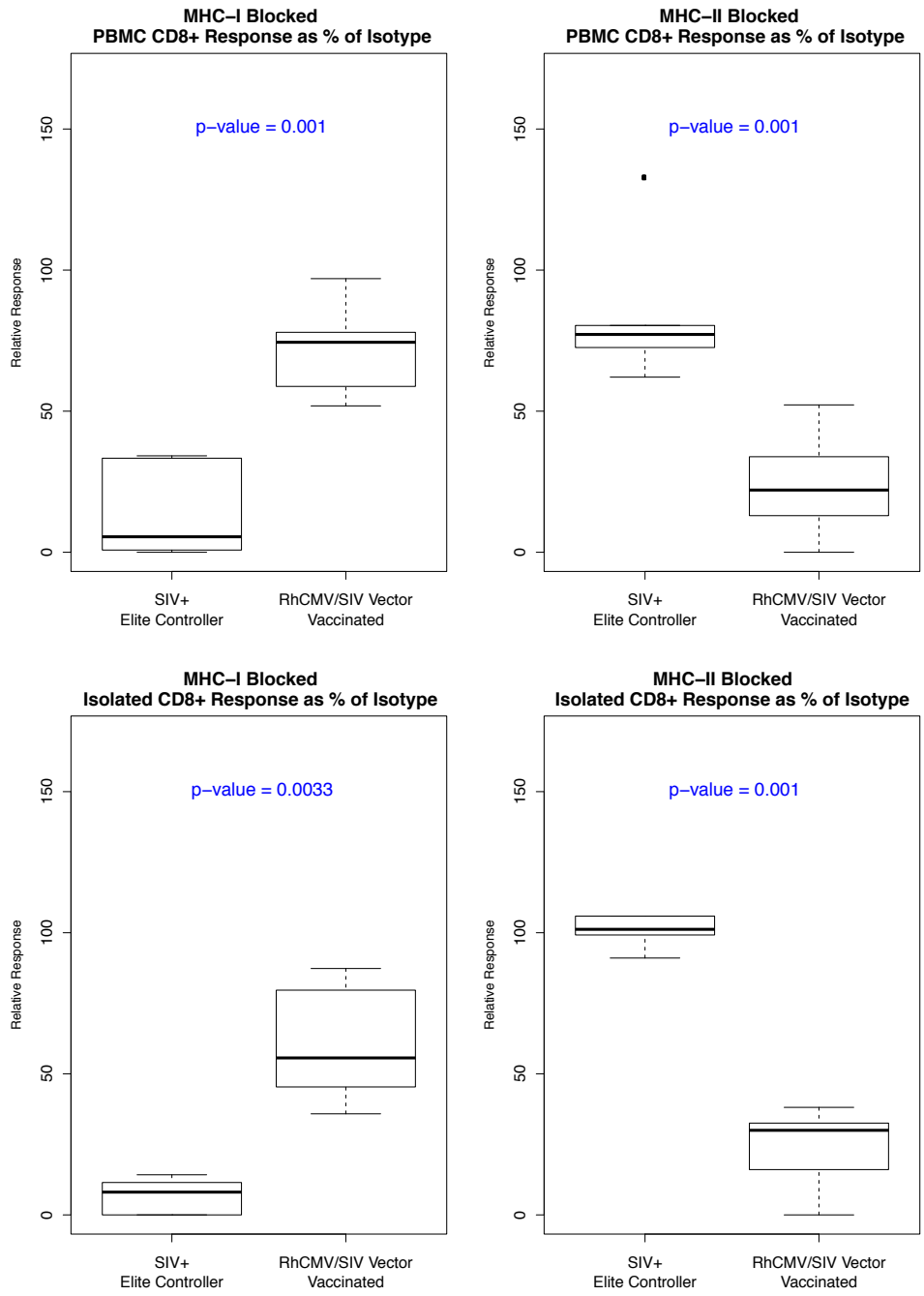

Fig. S9. Statistical analysis of the MHC association of SIV-infected cell-specific CD8+ T cell responses in SIV+ elite controllers vs. RhCMV/SIV vector-vaccinated RM. The boxplots illustrate the comparison of MHC-I-blocked (and MHC-II-blocked) CD8+ T cell responses between elite SIV controllers and strain 68-1 RhCMV/SIV-vaccinated RM. MHC-I-blocked (and MHC-II-blocked) are represented as fractions of the isotype response. The depicted p-values, from two-tailed Wilcoxon rank-sum tests, indicate that the differences for both MHC-I-blocked and MHC-II-blocked CD8+ T cell response fractions are statistically significant.

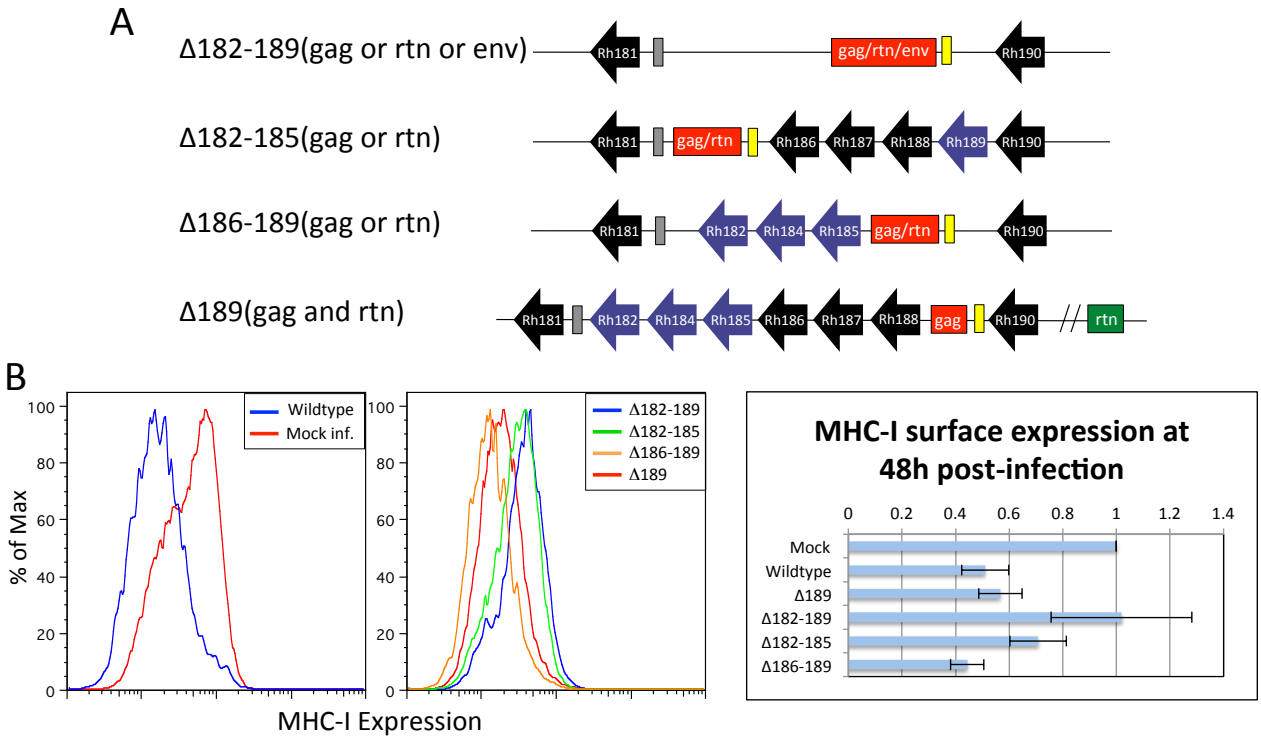

Fig. S10. Effect of Rh182-189 gene expression on the ability of RhCMV to down-modulate surface expression of MHC-1 in infected fibroblasts. (**A**) The diagram illustrates the gene modifications made in strain 68-1 RhCMV to determine the role of the Rh182-189 genes (US2-11 orthologues) in the unconventional epitope targeting of SIV-specific CD8+ T cells elicited by RhCMV/SIV vectors. For the vectors lacking the whole Rh182-189 region or the Rh182-185 and Rh186-189 subregions, these regions were replaced with either SIVgag- or SIVrev/tat/nef (rtn)-coding cassettes (using the EF1α promoter) or SIVenv (using the HCMV gH promoter). For the vector lacking Rh189 alone, a strain 68-1 RhCMV/rtn vector (with EF1α–driven RTN coding cassette at a different place in the RhCMV genome) was modified by replacement of the Rh189 coding region with the SIVgag coding region, such that SIVgag expression is driven by the Rh189 promoter (creating a dual gag/rtn-expressing vector). (**B**) Flow cytometry was used to compare the level of MHC-I expression on rhesus fibroblasts infected with these deletant vectors, compared to the strain 68-1 wildtype vector and mock infection, 48 hours after infection. Infected cells were stained on the cell surface with an Alexafluor647-conjugated, anti-MHC-I mAb (w6/32) and then fixed and permeabilized and stained with an in-house produced RhCMV-specific mAb (biotinylated and then visualized with streptavidin-Cy7). MHC-I levels were analyzed on an LSR-II flow cytometer. Flow cytometric profiles of MHC-I expression on RhCMV antigen-expressing fibroblasts from a representative experiment are shown at left. The bar graph at right shows the average mean fluorescent intensity of MHC-I staining normalized to mock infection (± SEM) from 4 independent experiments. Note that deletion of the entire Rh182-189 region counters all RhCMV-mediated MHC-I down-regulation, and deletion of the Rh182-185 region has a partial effect. However, deletion of Rh186-189 and of Rh189 alone (both lacking only one known MHC-I down-modulator – Rh189/US11) has little to no effect, with cell surface of MHC-I overlapping that of the wildtype strain 68-1 RhCMV.

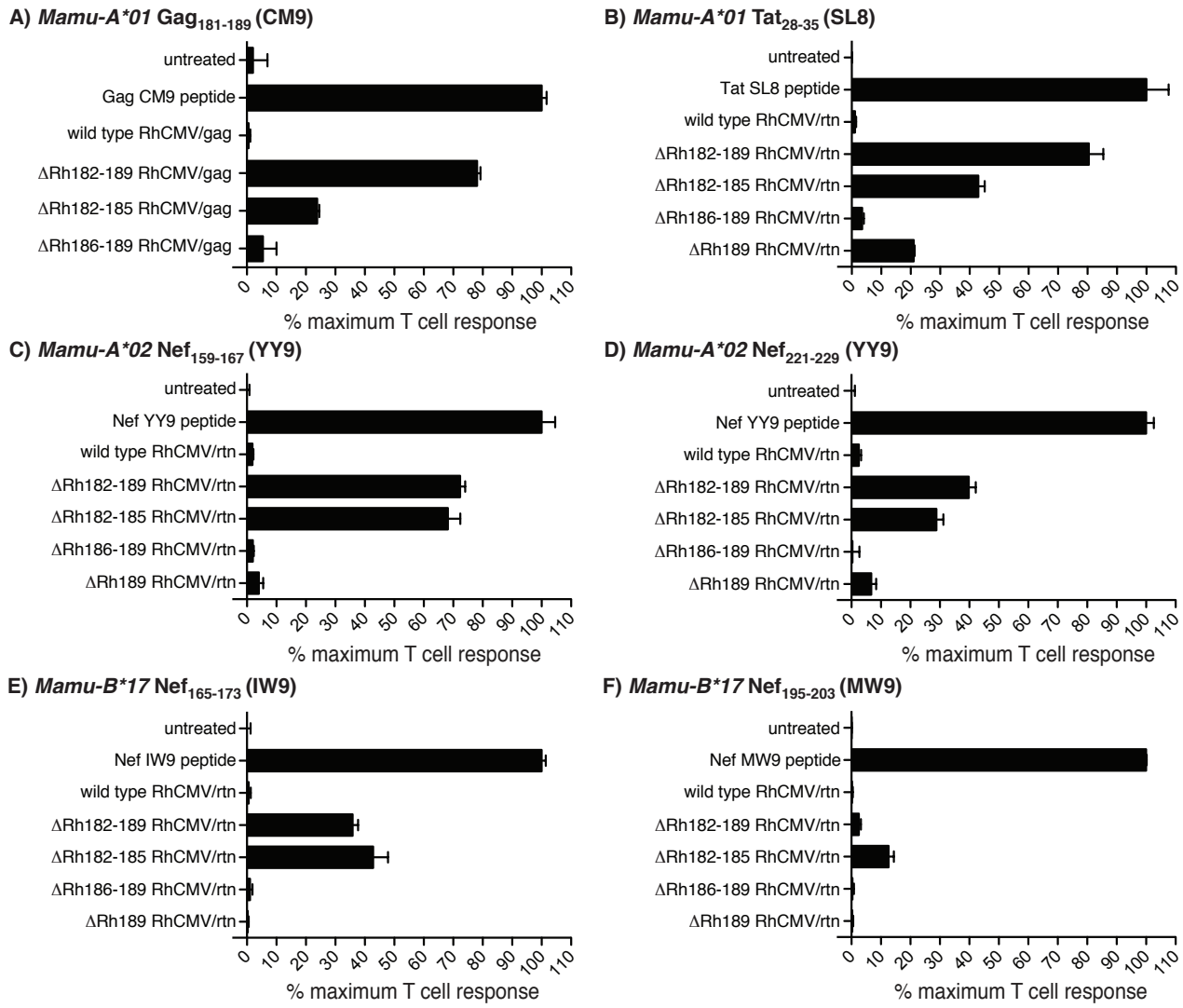

Fig. S11. Effect of Rh182-189 gene expression on the ability of RhCMV/gag- or RhCMV/rtn-infected fibroblasts to present canonical epitopes to epitope-specific T cell lines. Rhesus fibroblasts expressing the appropriate MHC-I molecule (Mamu-A*01, Mamu-A*02, or Mamu-B*17) were infected with each of the indicated strain 68-1-derived RhCMV/gag or /rtn vectors (see fig. S10) at a multiplicity of infection of 10. After 24 hours of incubation, the frequency of infected cells in each culture was determined by intracellular staining (using either SIVgag- or RhCMV-specific mAbs), and the fibroblasts were co-cultured with CD8+ T cell lines in an IFN-γ ELISPOT assay at a 1:1 ratio of infected fibroblasts and T cells. The CD8+ T cell lines used were selected for their specificity for the following SIVmac239-derived epitopes: A) $Gag_{181-189}$ CM9, B) Tat_{28-35} SL8, C) $Nef_{159-167}$ YY9, D) $Nef_{221-229}$ YY9, E) $Nef_{165-173}$ IW9, or F) $Nef_{159-203}$ MW9. Untreated fibroblasts and peptide-pulsed fibroblasts were used as the negative and positive controls, respectively.

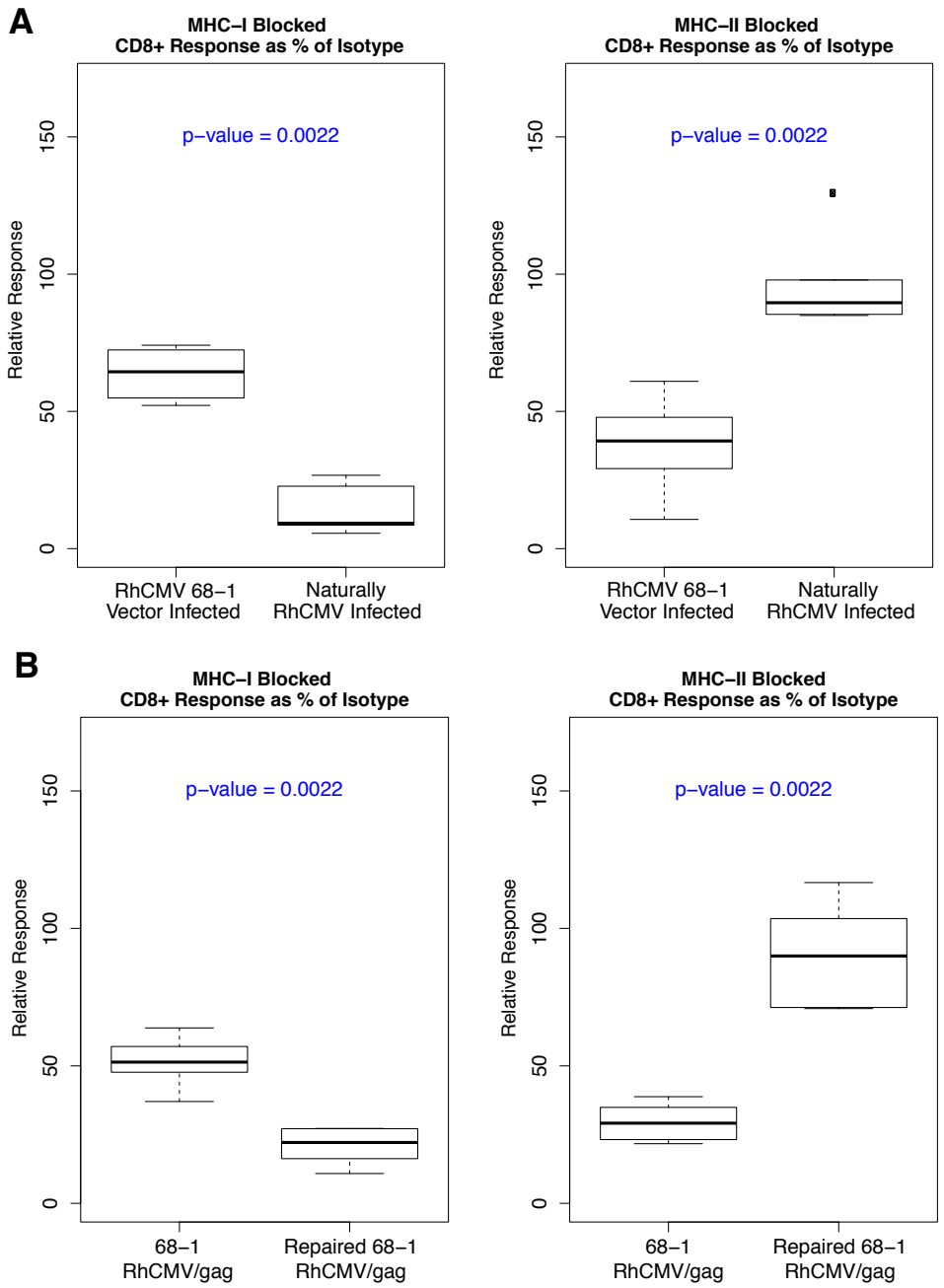

Fig. S12. Statistical analysis of the MHC association of IE-1- and SIVgag-specific CD8+ T cell responses elicited by RhCMV vectors/viruses with and without Rh157.4-.6 (UL128-131). (A) The boxplots illustrate the comparison of MHC-I- and MHC-II-blocked CD8+ T cell responses to total RhCMV IE-1 15mer peptides between naturally RhCMV-infected RM and RM vaccinated with strain 68-1 RhCMV vectors (see Fig. 8B). (**B**) The boxplots illustrate the comparison of MHC-I- and MHC-II-blocked CD8+ T cell responses to total SIVgag 15mer peptides between RM vaccinated with the original strain 68-1 RhCMV/gag vector and the Rh157.4-.6 (UL128-131)-repaired RhCMV/gag vector (see Fig. 8E). In both sets of analyses, the MHC-I-blocked (and MHC-II-blocked) are represented as fractions of the isotype response. The depicted p-values, from two-tailed Wilcoxon rank-sum tests, indicate that the differences for both MHC-I-blocked and MHC-II-blocked CD8+ T cell response fractions are statistically significant in both A and B.

Supplemental Tables

A*01 Epitopes

Peptide Name	*Sequence*
$Gag_{149-157}$(LW9)	LSPRTLNAW
$Gag_{170-177}$(VL8)	VVPGFQAL
$Gag_{181-189}$(CM9)	CTPYDINQM
$Gag_{254-262}$(QI9)	QNPIPVGNI
$Gag_{340-349}$(VT10)	VNPTLEEMLT
$Gag_{372-379}$(LF8)	LAPVPIPF
$Gag_{372-380}$(LA9)	LAPVPIPFA
Tat_{28-35}(SL8)	STPESANL
$Env_{235-243}$(CL9)	CAPPGYALL
$Env_{622-630}$(TL9)	TVPWPNASL
$Pol_{143-152}$(LV10)	LGPHYTPKIV
$Pol_{359-368}$(GM10)	GSPAIFQYTM
$Pol_{621-629}$(SV9)	STPPLVRLV

A*02 Epitopes

Peptide Name	*Sequence*
Gag_{71-79}(GY9)	GSENLKSLY
Nef_{20-28}(LY9)	LLRARGETY
$Nef_{159-167}$(YY9)	YTSGPGIRY
$Nef_{221-229}$(YY9)	YTYEAYVRY
$Env_{296-304}$(RY9)	RTIISLNKY
$Env_{788-795}$(RY8)	RTLLSRVY
$Pol_{324-332}$(FF9)	FSIPLDEEF

B*08 Epitopes

Peptide Name	*Sequence*
$Gag_{263-271}$(YL9)	YRRWIQLGL
Rev_{12-20}(KL9)	KRLRLIHLL
Rev_{44-51}(RL8)	RRRWQQLL
Nef_{8-16}(RL9a)	RRSRPSGDL
$Nef_{137-146}$(RL10)	RRHRILDIYL
$Nef_{245-253}$(RL9c)	RRRLTARGL
$Nef_{246-254}$(RL9b)	RRLTARGLL
$Env_{524-532}$(KF9)	KRGVFVLGF
$Env_{573-581}$(KL9)	KRQQELLRL
$Env_{717-725}$(LF9)	LRQGYRPVF
$Env_{868-876}$(RL9)	RRIRQGLEL

B*17 Epitopes

Peptide Name	*Sequence*
$Gag_{407-415}$(RW9)	RAPRRQGCW
$Nef_{165-173}$(IW9)	IRYPKTFGW
$Nef_{195-203}$(MW9)	MHPAQTSQW
$Env_{830-838}$(FW9)	FHEAVQAVW

Table S1. List of canonical epitopes associated with conventional SIVgag-specific CD8+ T cell responses in RM expressing the *Mamu-A*01, -A*02, -B*08,* and *-B*17* MHC-I alleles. The table shows the canonical epitopes restricted by the indicated *Mamu* alleles analyzed in this study.

A*01 Epitopes																		
RM#	***Mamu***	**Gag**	**RTN**	**Env**	**Pol**	**$Gag_{149-157}$**	**$Gag_{170-177}$**	**$Gag_{181-189}$**	**$Gag_{254-262}$**	**$Gag_{340-349}$**	**$Gag_{372-379}$**	**$Gag_{372-380}$**	**Tat_{28-35}**	**$Env_{235-243}$**	**$Env_{622-630}$**	**$Pol_{143-152}$**	**$Pol_{359-368}$**	**$Pol_{621-629}$**
Rh24075	*A*01*	0.26	0.14	0.19	0.11	BT	BT	BT	BT	BT	BT	BT	BT	BT	BT	BT	BT	BT
Rh24102	*A*01*	0.59	0.54	0.34	0.42	BT	BT	BT	BT	BT	BT	BT	BT	BT	BT	BT	BT	BT
Rh24115	*A*01*	1.14	0.58	0.54	1.60	BT	BT	BT	BT	BT	BT	BT	BT	BT	BT	BT	BT	BT
Rh24179	*A*01*	0.33	0.38	0.29	0.39	BT	BT	BT	BT	BT	BT	BT	BT	BT	BT	BT	BT	BT
Rh24250	*A*01*	0.43	0.25	0.73	0.46	BT	BT	BT	BT	BT	BT	BT	BT	BT	BT	BT	BT	BT
Rh24272	*A*01*	0.31	0.24	0.23	0.44	BT	BT	BT	BT	BT	BT	BT	BT	BT	BT	BT	BT	BT
Rh24438	*A*01*	0.94	0.90	0.62	0.58	BT	BT	BT	BT	BT	BT	BT	BT	BT	BT	BT	BT	BT
Rh24513	*A*01*	0.26	0.68	0.39	0.59	BT	BT	BT	BT	BT	BT	BT	BT	BT	BT	BT	BT	BT
Rh25565	*A*01*	0.60				BT	BT	BT	BT	BT	BT	BT						
Rh25937	*A*01*	0.42	0.35	0.38	0.45	BT	BT	BT	BT	BT	BT	BT	BT	BT	BT	BT	BT	BT
Rh26060	*A*01*	0.59	0.64	0.66	0.52	BT	BT	BT	BT	BT	BT	BT	BT	BT	BT	BT	BT	BT
Rh26908	*A*01*	3.65	3.23	2.84	3.91	BT	BT	BT	BT	BT	BT	BT	BT	BT	BT	BT	BT	BT
Rh27677	*A*01*	8.30	5.63	0.60	7.10	BT	BT	BT	BT	BT	BT	BT	BT	BT	BT	BT	BT	BT
Rh27686	*A*01*	1.62	1.16	1.10	1.20	BT	BT	BT	BT	BT	BT	BT	BT	BT	BT	BT	BT	BT
Rh28401	*A*01*	0.71	0.67	0.71	0.74	BT	BT	BT	BT	BT	BT	BT	BT	BT	BT	BT	BT	BT
Rh25545	*A*01/B*08*	0.33				BT	BT	BT	BT	BT	BT	BT						
Rh26994	*A*01/A*02*	2.89	2.15	2.84	2.68	BT	BT	BT	BT	BT	BT	BT	BT	BT	BT	BT	BT	BT
Rh27000	*A*01/A*02*	0.58	0.27	0.36	0.58	BT	BT	BT	BT	BT	BT	BT	BT	BT	BT	BT	BT	BT
Rh21756	*A*01/B*17*	0.48				BT	BT	BT	BT	BT	BT	BT						
Rh26407	*A*01/B*17*	1.85	0.53	1.97	2.80	BT	BT	BT	BT	BT	BT	BT	BT	BT	BT	BT	BT	BT
Rh27881	*A*01/B*17*	1.17	1.05	1.30	1.25	BT	BT	BT	BT	BT	BT	BT	BT	BT	BT	BT	BT	BT

B*08 Epitopes																
RM#	***Mamu***	**Gag**	**RTN**	**Env**	**Pol**	**$Gag_{263-271}$**	**Rev_{12-20}**	**Rev_{44-51}**	**Nef_{8-16}**	**$Nef_{137-146}$**	**$Nef_{245-253}$**	**$Nef_{246-254}$**	**$Env_{524-532}$**	**$Env_{573-581}$**	**$Env_{717-725}$**	**$Env_{868-876}$**
Rh22642	*B*08*	0.79	1.12	1.04		BT	BT	BT	BT	BT	BT	BT	BT	BT	BT	BT
Rh27031	*B*08*	1.49	1.14	1.30	3.63	BT	BT	BT	BT	BT	BT	BT	BT	BT	BT	BT
Rh27080	*B*08*	0.40	0.19	0.79	0.79	BT	BT	BT	BT	BT	BT	BT	BT	BT	BT	BT
Rh25545	*A*01/B*08*	0.33				BT										

A*02 Epitopes											
RM#	***Mamu***	**Gag**	**RTN**	**Env**	**Pol**	**Gag_{71-79}**	**Nef_{20-28}**	**$Nef_{159-167}$**	**$Env_{296-304}$**	**$Env_{788-795}$**	**$Pol_{324-332}$**
Rh25998	*A*02*	1.86	0.93	1.27	1.84	BT	BT	BT	BT	BT	BT
Rh26422	*A*02*	0.67	0.54	0.63	1.18	BT	BT	BT	BT	BT	BT
Rh26548	*A*02*	3.55	4.84	6.24	5.52	BT	BT	BT	BT	BT	BT
Rh26998	*A*02*	2.03	2.29	3.75	1.30	BT	BT	BT	BT	BT	BT
Rh27211	*A*02*	3.14	1.67	3.32	1.29	BT	BT	BT	BT	BT	BT
Rh27331	*A*02*	0.67	0.41	0.57	0.35	BT	BT	BT	BT	BT	BT
Rh27472	*A*02*	4.75	1.67	4.42	1.14	BT	BT	BT	BT	BT	BT
Rh27526	*A*02*	0.75	0.50	0.67	0.55	BT	BT	BT	BT	BT	BT
Rh27834	*A*02*	4.25	1.09	2.86	0.45	BT	BT	BT	BT	BT	BT
Rh28148	*A*02*	2.63	1.74	3.52	1.53	BT	BT	BT	BT	BT	BT
Rh26994	*A*01/A*02*	2.89	2.15	2.84	2.68	BT	BT	BT	BT	BT	BT
Rh27000	*A*01/A*02*	0.58	0.27	0.36	0.58	BT	BT	BT	BT	BT	BT
Rh27352	*A*02/B*17*	2.99	1.31	2.21	1.14	BT	BT	BT	BT	BT	BT

B*17 Epitopes									
RM#	***Mamu***	**Gag**	**RTN**	**Env**	**Pol**	**$Gag_{407-415}$**	**$Nef_{165-173}$**	**$Nef_{195-203}$**	**$Env_{830-838}$**
Rh26103	*B*17*	1.43	1.35	1.68	1.83	BT	BT	BT	BT
Rh27087	*B*17*	0.92	0.90	1.05	0.92	BT	BT	BT	BT
Rh27158	*B*17*	1.52	1.60	1.64	1.62	BT	BT	BT	BT
Rh21756	*A*01/B*17*	0.48				BT			
Rh26407	*A*01/B*17*	1.85	0.53	1.97	2.80	BT	BT	BT	BT
Rh27881	*A*01/B*17*	1.17	1.05	1.30	1.25	BT	BT	BT	BT
Rh27352	*A*02/B*17*	2.99	1.31	2.21	1.14	BT	BT	BT	BT

Table S2. Comprehensive analysis of CD8+ T cell responses to canonical epitopes in RM vaccinated with RhCMV/SIV vectors and expressing one or more of the *Mamu-A*01, A*02, B*08, and B*17* MHC-I alleles. The table shows all of the RhCMV/SIV-vaccinated RM in which CD8+ T cell responses to canonical epitopes restricted by the indicated *Mamu* alleles were assayed by flow cytometric ICS. No above-background response was identified to any of these canonical epitopes in any "wildtype" strain 68-1 RhCMV/SIV vector-vaccinated RM that expressed the appropriate restricting *Mamu* allele. BT = Below Threshold

Rh21826	Gag_{29-43} (8)	Gag_{73-87} (19)	$Gag_{141-155}$ (36)	$Gag_{209-223}$ (53)	$Gag_{221-235}$ (56)	$Gag_{273-287}$ (69)	$Gag_{289-303}$ (73)	$Gag_{377-391}$ (95)	$Gag_{493-507}$ (124)
BLCL	+	+	-	+	+	+	+	-	+
RM3	-	-	-	-	-	-	-	-	-
DRB1*10:07	+	+	-	-	+	-	+	-	+
DRB1*03:09	+	+	-	+	-	-	-	-	-
DRB1*04:06	-	-	-	-	-	-	-	-	-
DRB5*03:01	+	-	-	-	+	-	+	-	+
DRB*w2:01	-	-	-	+	-	-	-	-	-
DRB*w26:03	-	+	-	+	+	-	-	-	-
DRB*w4:01	-	+	-	-	+	-	-	-	-

Rh22436	Gag_{29-43} (8)	Gag_{73-87} (19)	$Gag_{141-155}$ (36)	$Gag_{209-223}$ (53)	$Gag_{221-235}$ (56)	$Gag_{273-287}$ (69)	$Gag_{289-303}$ (73)	$Gag_{377-391}$ (95)	$Gag_{493-507}$ (124)
BLCL	+	+	+	+	+	+	+	+	+
RM3	-	-	-	-	-	-	-	-	-
DRB1*10:07	+	+	-	-	+	-	+	+	+
DRB1*03:09	+	+	+	+	-	-	-	+	-
DRB1*04:06	-	-	+	-	-	-	-	+	-
DRB5*03:01	+	-	+	-	+	-	+	+	+
DRB*w2:01	-	-	-	+	-	-	-	-	-
DRB*w26:03	-	+	-	+	+	-	-	-	-
DRB*w4:01	-	+	+	-	+	-	-	-	-

Rh22034	Gag_{29-43} (8)	Gag_{73-87} (19)	$Gag_{141-155}$ (36)	$Gag_{209-223}$ (53)	$Gag_{221-235}$ (56)	$Gag_{273-287}$ (69)	$Gag_{289-303}$ (73)	$Gag_{377-391}$ (95)	$Gag_{493-507}$ (124)
BLCL	+	-	+	+	+	+	+	+	+
RM3	-	-	-	-	-	-	-	-	-
DRB1*1007	+	-	-	-	+	-	+	+	+
DRB1*03:09	+	-	+	+	-	-	-	+	-
DRB1*04:06	-	-	+	-	-	-	-	+	-
DRB5*03:01	+	-	+	-	+	-	+	+	+
DRB*w2:01	-	-	-	+	-	-	-	-	-
DRB*w26:03	-	-	-	+	+	-	-	-	-
DRB*w4:01	-	-	+	-	+	-	-	-	-

Rh22607	Gag_{29-43} (8)	Gag_{73-87} (19)	$Gag_{141-155}$ (36)	$Gag_{209-223}$ (53)	$Gag_{221-235}$ (56)	$Gag_{273-287}$ (69)	$Gag_{289-303}$ (73)	$Gag_{377-391}$ (95)	$Gag_{493-507}$ (124)
BLCL	+	+	-	+	+	+	+	+	+
RM3	-	-	-	-	-	-	-	-	-
DRB1*10:07	+	+	-	-	+	-	+	+	+
DRB1*03:09	+	+	-	+	-	-	-	+	-
DRB1*04:06	-	-	-	-	-	-	-	+	-
DRB5*03:01	+	-	-	-	+	-	+	+	+
DRB*w2:01	-	-	-	+	-	-	-	-	-
DRB*w26:03	-	+	-	+	+	-	-	-	-
DRB*w4:01	-	+	-	-	+	-	-	-	-

Table S3. Comprehensive analysis of the *Mamu* DR allomorph specificity of RhCMV/gag-elicited MHC-II-restricted, CD8+ T cell responses in 4 RM. PBMC from the 4 indicated RM (see also fig. S6) were incubated with autologous B-lymphoblastoid cells (BLCL), MHC-II-null RM3, or the indicated single Mamu-DR allomorph transfectants pulsed with the indicated SIVgag peptides and were then analyzed for CD8+ T cell responses by flow cytometric ICS (see Fig. 4). Combinations that resulted in CD8+ T cell responses above background (no peptide) are indicated by + signs (blue boxes); combinations that did not result in CD8+ T cell responses above background are indicated by - signs (open boxes). *Mamu-DR* alleles that are expressed in each RM are indicated in red; non-expressed alleles are shown in white. Note that CD8+ T cells recognizing a given peptide in the context of an autologous (expressed) Mamu-DR molecule can also respond to that peptide in the context of an allogeneic Mamu-DR molecule, as long as that molecule is also capable of presenting that particular peptide in RM expressing that allele.