Supporting Information

Carnathan et al. 10.1073/pnas.1407466112

SI Materials and Methods

Vectors. The recombinant VV containing SIVGag/Tat was created as follows: The pSC11 vector, which contains the lacZ gene, was used as a shuttle vector to transfer SIV_{mac239} Gag and Tat genes. For rescue of rVV, CV-1 cells were transfected with the insert containing pSC11 vector and then were infected a few hours later with 0.1 pfu of VV strain Copenhagen. Cells were harvested 2 d later and were frozen and thawed three times. A fraction of the supernatant was used to infect TK cells, which were treated with bromodeoxyuridine to suppress the propagation of wild-type VV. Cells were overlaid with agarose and were stained 2 d later with β-galactosidase. Blue plaques were selected, and virus was isolated by freeze-thawing and subcloned four times until no more clear plaques indicating contamination with wild-type virus were detected. Recombinant VV was expanded on TK cells and purified by centrifugation through a sucrose gradient. Viral titers (in plaque-forming units) were determined upon infection of TK-cell monolayers in six-well plates with serial dilutions of purified virus. Two hours after infection, cells were overlaid with agarose. The next day a second overlay, containing X-Gal, was added. Blue plaques were counted under an inverted microscope to determine plaque-forming units per milliliter. As a further quality control, recombinant batches were checked for contamination with nonrecombinant viruses, for LPS contamination, and for expression of transgene product by Western blots.

Vaccination Protocol. Blood was collected at weeks −5, 1, 2, 4, 12, 16, 17, 18, 20, 28, 32, 33, 34, 36, and 44 from the first immunization, and RBs were collected at weeks 2, 16, 17, 18, 20, 32, 33, 34, and 44 from the first immunization (Fig. 1). No RBs were obtained before immunization, for logistical reasons. No RBs were collected before challenge in the unvaccinated, control animals. Because of inclement weather, a few macaques (one from DNA/EP-AdC6-AdC7, two from DNA/EP-VV-AdC6, and two from DNA/EP-VV-AdC7) were sampled on week 3 rather than week 2, and a few other macaques (one from DNA/EP-AdC6-AdC7, one from VV-AdC6-AdC7, one from DNA/EP-VV-AdC6, one from DNA/EP-VV-AdC7, and one from AdHu5-AdHu5-AdHu5) were sampled on week 19 rather than week 18 (Figs. 2 and 3).

Immunophenotyping and Flow Cytometry. Predetermined optimal concentrations of the following antibodies were used: anti-CD8 QDot705 (clone 3B5), anti-CD14 QDot655 (cloneH147), anti-CD20 (clone TuK4) (LIVE/DEAD Aqua Blue Dead Cell Stain Kit; Invitrogen); anti–CD4 APC-Cy7 (clone OKT4) (BioLegend); anti-CCR5 PE (clone 3A9), anti-CD95 PE-Cy5 (clone DX2), anti-CD3 Pacific Blue (clone SP34-2), and anti–Ki-67 Alexa700 (clone B56) (BD Biosciences); and anti-CD28 ECD (clone 28.2) (Beckman Coulter).

Multifunctional Assessment of SIV-Specific T-Cell Responses. Anti-CD107a FITC (clone H4A3; BD Biosciences) was added at the start of all stimulation periods. The mixture of antibodies for surface staining included Aqua Blue Viability Dye (Invitrogen), CD8 QDot705 (clone 3B5; Invitrogen), CD14 QDot655 (cloneTuK4; Invitrogen), CD20 QDot655 (clone H147; Invitrogen), CD4 APC-Cy7 (clone OKT-4; BD Biosciences), CD95 PE-Cy5 (clone DX2; BD Biosciences), and CD28 ECD (clone CD28.2; Beckman Coulter). The mixture for intracellular staining included CD3 Pacific Blue (clone SP34-2; BD Biosciences), IFN-γ APC (cloneB27; BD Biosciences), IL-2 PE (clone rat MQ1-17H12; BD Biosciences), and TNF-α Alexa700 (clone MAb11; BD Biosciences). Flow cytometric acquisition and analysis of samples was performed on at least 100,000 lymphocytes on an LSRII flow cytometer driven by the DiVa software package (BD Biosciences). Analyses of the acquired data were performed using FlowJo software (Tree Star, Inc.) and SPICE (National Institute of Allergy and Infectious Diseases).

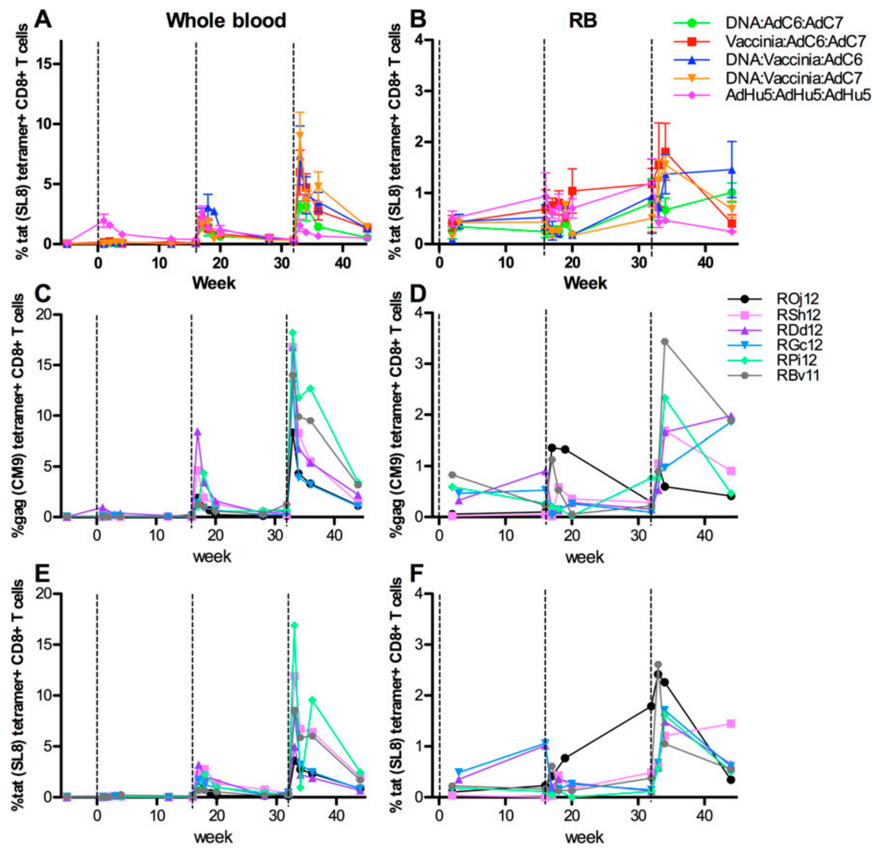

Fig. S1. Vaccination regimens induce SIVGag-specific CD8+ T cells. The percent of CD8+ T cells binding Tat-SL8 (*A*, *B*, *E*, and *F*) or Gag-CM9 (*C* and *D*) Mamu-A*01 tetramers at various time points postimmunization in peripheral blood (*A*, *C*, and *E*) and rectal mucosa (*B*, *D*, and *F*) are shown. (*A* and *B*) The average percent of tetramer-binding CD8+ T cells for each immunization group. (*C*–*F*) The percent of tetramer-binding CD8+ T cells for each animal belonging to the DNA/EP-VV-AdC7 immunization group. Black dashed lines at 0, 16, and 32 wk indicate immunization time points. Error bars indicate the SEM for each vaccination group. The color scheme in *A* and *B* is as follows: DNA/EP-AdC6-AdC7, green; VV-AdC6-AdC7, red; DNA/EP-VV-AdC6, blue; DNA/EP-VV-AdC7, orange; and AdHu5-AdHu5-AdHu5, pink. The color scheme in *C*–*F* is as follows: ROj12, black; RSh12, pink; RDd12, purple; RGc12, blue; RPi12, green; and RBv11, gray.

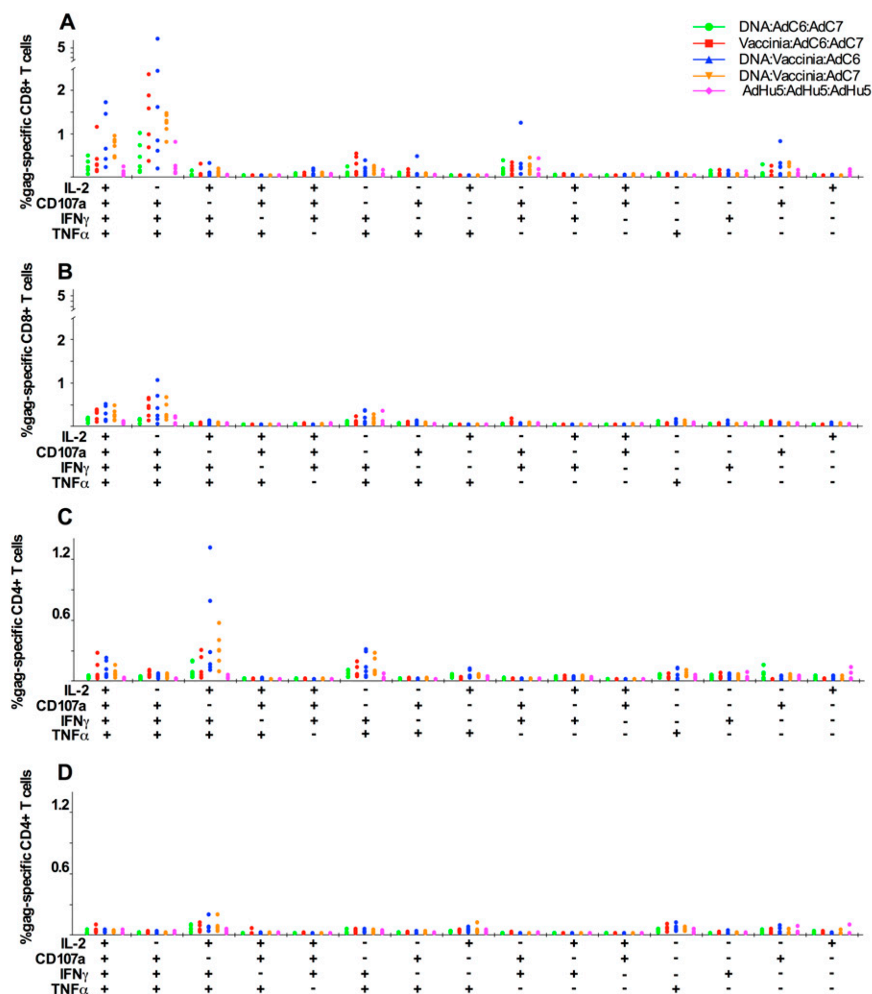

Fig. S2. Vaccination regimens induce polyfunctional SIVGag-specific $CD8^+$ and $CD4^+$ T cells. SIV-specific $CD8^+$ (*A* and *B*) and $CD4^+$ (*C* and *D*) T-cell responses measured by intracellular cytokine and CD107a staining after stimulation of cryopreserved PBMCs with SIV_{mac239} Gag peptides were examined in all groups of animals 1 wk after the final immunization (*A* and *C*) and 12 wk after the final immunization (*B* and *D*). Responsive cells are considered to be cytokine producing and/or CD107a expressing after background subtraction. The color scheme is as follows: DNA/EP-AdC6-AdC7, green; VV-AdC6-AdC7, red; DNA/EP-VV-AdC6, blue; DNA/EP-VV-AdC7, orange; and AdHu5-AdHu5-AdHu5, pink.

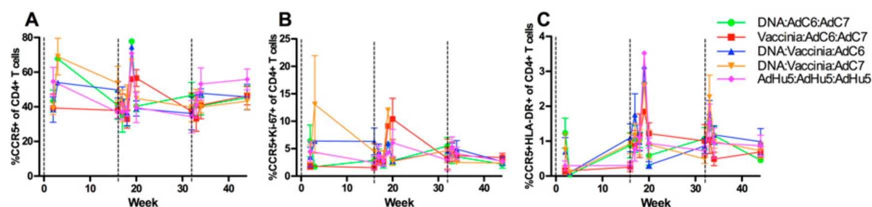

Fig. S3. Level of activated and proliferating $CCR5^+CD4^+$ T cells after immunization in rectal mucosa. Average levels of $CCR5^+$ (*A*), $CCR5^+Ki\text{-}67^+$ (*B*), and $CCR5^+$ $HLA\text{-}DR^+$ (*C*) T cells measured as a percentage of total $CD4^+$ T cells in rectal mucosa in vaccinated RMs. Black dashed lines at 0, 16, and 32 wk indicate immunization time points. Error bars represent SEM. The color scheme is as follows: DNA/EP-AdC6-AdC7, green; VV-AdC6-AdC7, red; DNA/EP-VV-AdC6, blue; DNA/EP-VV-AdC7, orange; and AdHu5-AdHu5-AdHu5, pink.

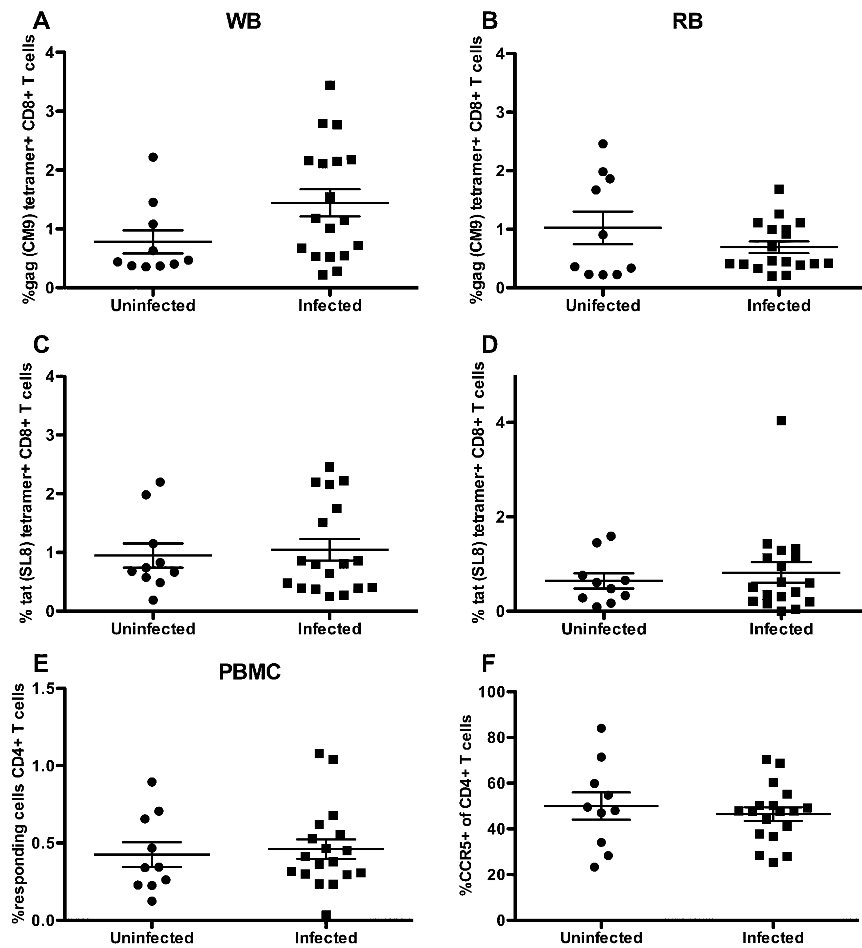

Fig. S4. SIV acquisition is not associated with higher levels of SIV-specific CD8$^+$ T cells as measured by tetramer staining in blood or rectal mucosa (*A*–*D*), or SIV-specific CD4$^+$ T cells in blood (*E*), or total levels of CD4$^+$CCR5$^+$ T cells in the rectal mucosa before challenge (*F*). (*A*–*D*) Comparison of the percent of SIVGag tetramer-specific CD8$^+$ T cells (*A* and *B*) or SIVTat tetramer-specific CD8$^+$ T cells (*C* and *D*) in whole blood (*A* and *C*) or rectal mucosa (*B* and *D*) before challenge in immunized macaques that remained uninfected (circles) and those that acquired SIV infection (squares). (*E*) Comparison of SIVGag peptide-responsive CD4$^+$ T cells in immunized macaques that remained uninfected (circles) and those that acquired SIV infection (squares). Responsive cells are considered to be cytokine producing and/or CD107a expressing after background subtraction. (*F*) Comparison of the percent of CD4$^+$CCR5$^+$ T cells in rectal mucosa 12 wk after the final immunization and before challenge in immunized macaques that remained uninfected (circles) and those that acquired SIV infection (squares). The Mann–Whitney *u* test was used to determine differences between uninfected and infected groups.

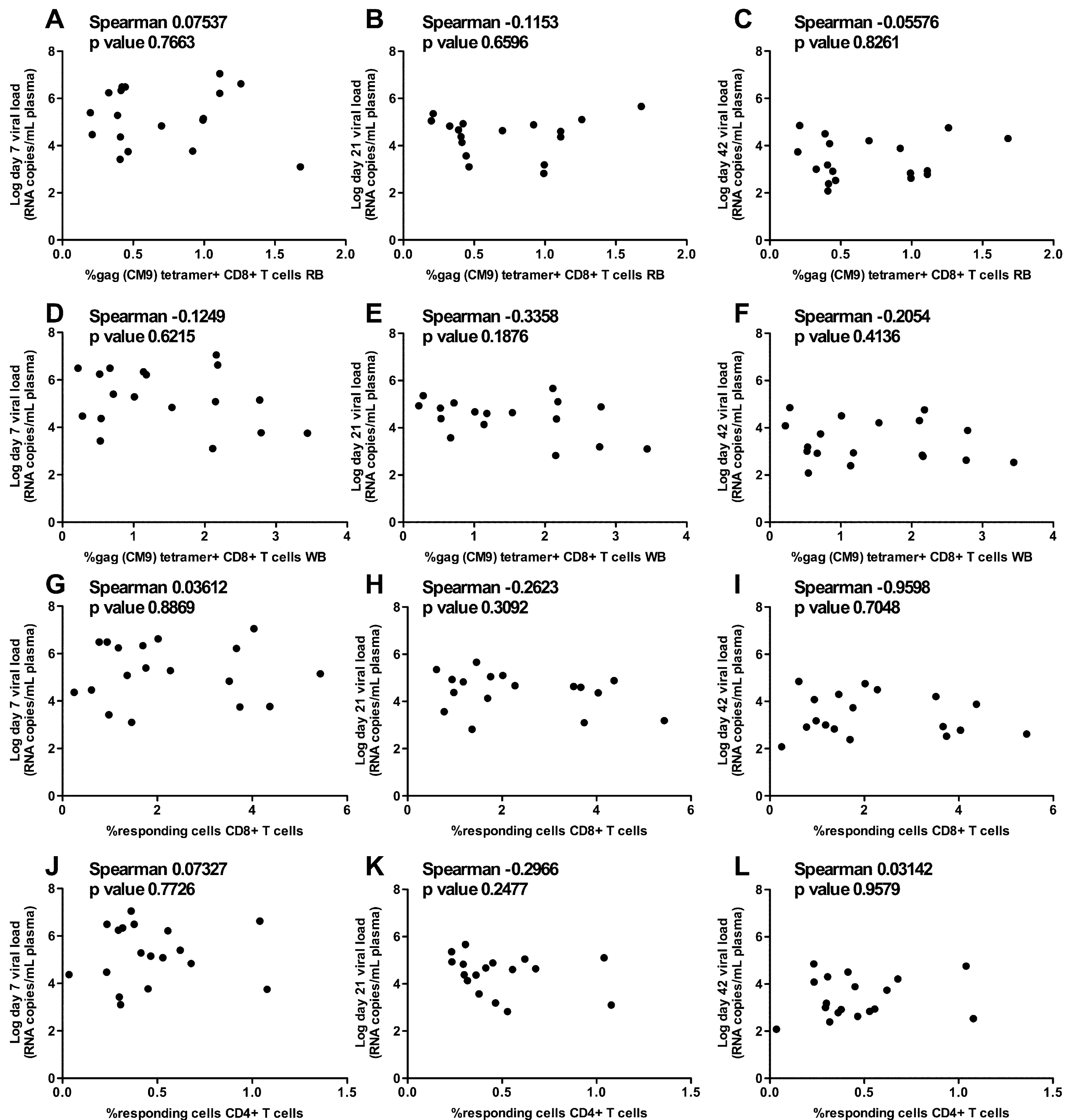

Fig. S5. Viral replication does not correlate with the levels of SIV-specific $CD8^+$ or $CD4^+$ T cells. The percentage of SIVGag tetramer-specific cells $CD8^+$ T cells in rectal mucosa (*A*–*C*), Gag tetramer-specific $CD8^+$ T cells in whole blood (*D*–*F*), Gag peptide-responsive $CD8^+$ T cells (G–*I*), and Gag peptide-responsive $CD4^+$ T cells (*J*–*L*) before challenge did not correlate with viral load at day 7 (*A*, *D*, *G*, and *J*), day 21 (*B*, *E*, *H*, and *K*), or day 42 (*C*, *F*, *I*, and *L*) in the immunized RMs experiencing infection. Spearman's correlation was used to determine the significance of all correlations.

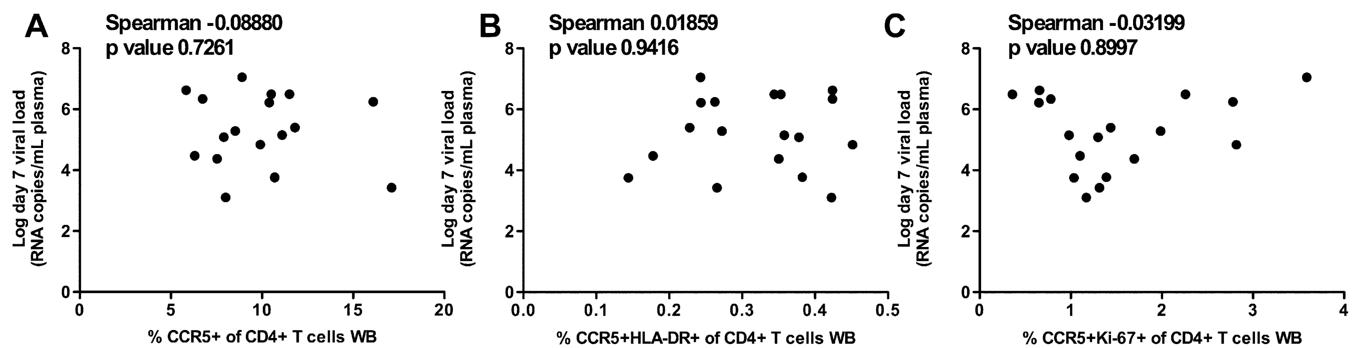

Fig. S6. Early virus replication is not associated with the level of target CD4$^+$ T cells in whole blood before challenge. The percentage of total CD4$^+$CCR5$^+$ (*A*), CD4$^+$CCR5$^+$HLA-DR$^+$ (*B*), and CD4$^+$CCR5$^+$Ki-67$^+$ (*C*) T cells in whole blood before challenge does not correlate with day 7 viral load in the immunized RMs experiencing infection. Spearman's correlation was used to determine the significance of all associations.

Fig. S7. Correlation between virus replication at days 7, 21, and 42 postinfection and the level of target CD4$^+$ T cells in the rectal mucosa before challenge. The percentage of total CD4$^+$CCR5$^+$ (*A*, *C*, and *F*), CD4$^+$CCR5$^+$HLA-DR$^+$ (*B*, *D*, and *G*), and CD4$^+$CCR5$^+$Ki-67$^+$ (*E* and *H*) in rectal mucosa before challenge is plotted against the levels of virus replication at day 7 (*A* and *B*), day 21 (*C*–*E*), and day 42 (*F*–*H*) postinfection. Spearman's correlation was used to determine the significance of all associations.