Supplemental Experimental Procedures

Cell-associated SIVgag DNA

DNA was extracted from sorted peripheral and lymph node $CD4^+$ T cells and $CD4^+$ T cell memory subsets using the Blood DNA Mini Kit (QIAGEN). Quantification of SIV_{mac} *gag* DNA was performed as previously described on the extracted cell-associated DNA by quantitative PCR using the 5′ nuclease (TaqMan) assay with an ABI7500 system (PerkinElmer Life Sciences) (Chahroudi et al., 2014). The sequence of the forward primer for SIVmac *gag* was 5′-GCAGAGGAGGAAATTACCCAGTAC-3′; the reverse primer sequence was 5′-CAATTTTACCCAGGCATTTAATGTT-3′; and the probe sequence was 5′-6 FAM-TGTCCACCTGCCATTAAGCCCGA-TAMRA-3′. For cell number quantification, quantitative PCR was performed simultaneously for monkey albumin gene copy number.

Single genome analysis of circulating SIV

Viral RNA was extracted from plasma samples collected at Day 10, Day 56, Day 79 or Day 93, and various time points between 1-6 weeks post CD8 depletion, using either the QIAamp RNA isolation kit or the QIAGEN Ultrasens Virus kit (QIAGEN) following manufacturer's instructions. Reverse transcription of viral RNA into cDNA was performed using the Superscript III kit (Invitrogen) with SIVsm/macEnvR1 5′-TGTAATAAATCCCTTCCAGTCCCCCC-3'. Single genome amplification by nested PCR was performed on each cDNA using a limiting dilution that yielded one-third or less positive amplicons (Burton et al., 2015; Smith et al., 2016). First-round PCR was performed in a 15 µl total volume using Phusion Hotstart II high-fidelity DNA polymerase (Thermo Scientific) with the following primers: SIVsm/macEnvF1 5′-CCTCCCCCTCCAGGACTAGC-3', and SIVsm/macEnvR1 5′-TGTAATAAATCCCTTCCAGTCCCCCC-3′. Cycling conditions were as follows: 94°C for 2 min; 35 cycles of 94°C for 15 sec, 55°C for 30 s, and 68°C for 4 min; 68°C for 10 min; and 4°C hold. Second-round PCR was performed with the same enzyme in a 10 µl volume using 1 µl of the first round PCR product with SIVmacEnvF2 5′-TATAATAGACATGGAGACACCCTTGAGGGAGC-3′ and SIVsmEnvR2 5′-ATGAGACATRTCTATTGCCAATTTGTA-3′. Cycling conditions were 94°C for 2 min; 45 cycles of 94°C for 15 s, 55°C for 30 s, and 68°C for 4 min; 68°C for 10 min; and 4°C hold. Positive bands were gel purified via QIAquick Gel Extraction Kit (QIAGEN). Purified samples were sequenced with the following primers: SIVmac251seqF1 5'-GGGATATGTTATGAGCAGTCACG-3'; SIVmac251seqF2 5'-ATCCAAGAGTCTTGTGACAAGC-3'; SIVmac251seqF3 5'-AAGAGAGGGAGACCTCACG-3'; SIVmac251seqF4 5'-AGGCCAGTGTTCTCTTCC-3'; SIVmac251seqR1 5'-CTTGTTCCAAGCCTGTGC-3'; SIVmac251seqR2 5'-CCTCTGCAATTTGTCCACATG-3'; SIVmac251seqR3 5'-TCCAAGAAGTCAACCTTTCGC-3'; SIVmac251seqR4 5'-AGCTGGGTTTCTCCATGG-3'. Sequencher v5 was used to generate nucleotide sequence contigs, and sequences with no ORF or evidence of mixed peaks were omitted from the analysis. Geneious v6.1.7 was used to translate nucleotide sequences and create alignments. Highlighter plots were generated with Highlighter for Amino Acids v1.3.4 (www.hiv.lanl.gov/content/sequence/HIGHLIGHT/highlighter_top.html) (Keele et al., 2008). SGA derived Env amino acid sequences have been submitted under Genbank accession numbers xxxx-xxxx.

Deep sequencing of cell-associated SIV-DNA

Deep-sequencing of two amplicons covering nucleotides 6679-7103 and 8748-9196 of the wildtype SIVmac239 genome (NCBI accession#: M33262.1) and corresponding to Env amino acids 35-158 and 724-845 were amplified from peripheral blood cell DNA by nested PCR as previously described (Vanderford et al., 2011). Primers were designed using each animal's SGA plasma viral RNA sequences to avoid potential mismatches. For the pre-ART amplicons, two different primer combinations were compared to rule out PCR or primer-induced bias in genotype frequencies in our PCR protocol. Bands of the correct size were gel purified and libraries were generated from 10 ng of amplicon DNA using the Kapa HyperPlus DNA kit. The amplicons were end-repaired, A-tailed and single index barcodes and sequencing primers were added according to the manufacturer protocol. Libraries were assessed by Bioanalyzer capillary electrophoresis, quantified, pooled and sequenced on an Illumina MiSeq system as 300 bp paired-end reactions. Each sample was sequenced to an approximate depth of 1 million reads. Sequence reads were extracted from the Illumina MiSeq run and samples were identified by barcoded adaptors. After adaptors were trimmed, paired-end sequence reads were trimmed for quality and length using Trimmomatic (Bolger et al., 2014) using the recommended parameters in paired-end mode. The inner nested PCR primers were identified near the beginning of both reads and trimmed. Reads were then aligned to the original SIVmac239 genomic sequence using BLAT (Kent, 2002). Reads were included in the analysis only if they (i) overlapped by more than 10 nucleotides, (ii) contained both inner nested primers prior to trimming, (iii) contained no ambiguous base calls, and (iv) uniquely

aligned to the wildtype SIVmac239 genome without insertions or deletions. To account for the low level of sequencing-induced mutations in the pool of reads, baseline amino acid mutation frequencies for each position in the reconstructed amplicons were estimated at likely invariant sites. A conservative cutoff amino acid mutation frequency of 0.01 was chosen under which a particular site was considered invariant within each sample. The frequency of reads identical to the original SIVmac239 genomic sequence was then calculated allowing for mutations only at variant sites. Similar results were obtained if all sites were allowed to vary in the analysis. All sequence analysis was performed Python v3.4, except where noted.

Supplemental References

Bolger, A.M., Lohse, M., and Usadel, B. (2014). Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics *30*, 2114-2120.

Burton, S.L., Kilgore, K.M., Smith, S.A., Reddy, S., Hunter, E., Robinson, H.L., Silvestri, G., Amara, R.R., and Derdeyn, C.A. (2015). Breakthrough of SIV strain smE660 challenge in SIV strain mac239-vaccinated rhesus macaques despite potent autologous neutralizing antibody responses. Proceedings of the National Academy of Sciences of the United States of America *112*, 10780-10785.

Chahroudi, A., Cartwright, E., Lee, S.T., Mavigner, M., Carnathan, D.G., Lawson, B., Carnathan, P.M., Hashempoor, T., Murphy, M.K., Meeker, T.*, et al.* (2014). Target cell availability, rather than breast milk factors, dictates mother-to-infant transmission of SIV in sooty mangabeys and rhesus macaques. PLoS pathogens *10*, e1003958.

Keele, B.F., Giorgi, E.E., Salazar-Gonzalez, J.F., Decker, J.M., Pham, K.T., Salazar, M.G., Sun, C., Grayson, T., Wang, S., Li, H.*, et al.* (2008). Identification and characterization of transmitted and early founder virus envelopes in primary HIV-1 infection. Proceedings of the National Academy of Sciences of the United States of America *105*, 7552-7557.

Kent, W.J. (2002). BLAT--the BLAST-like alignment tool. Genome Res *12*, 656-664.

Smith, S.A., Kilgore, K.M., Kasturi, S.P., Pulendran, B., Hunter, E., Amara, R.R., and Derdeyn, C.A. (2016). Signatures in Simian Immunodeficiency Virus SIVsmE660 Envelope gp120 Are Associated with Mucosal Transmission but Not Vaccination Breakthrough in Rhesus Macaques. Journal of virology *90*, 1880-1887.

Vanderford, T.H., Bleckwehl, C., Engram, J.C., Dunham, R.M., Klatt, N.R., Feinberg, M.B., Garber, D.A., Betts, M.R., and Silvestri, G. (2011). Viral CTL escape mutants are generated in lymph nodes and subsequently become fixed in plasma and rectal mucosa during acute SIV infection of macaques. PLoS pathogens *7*, e1002048.

Supplemental Figures and Tables

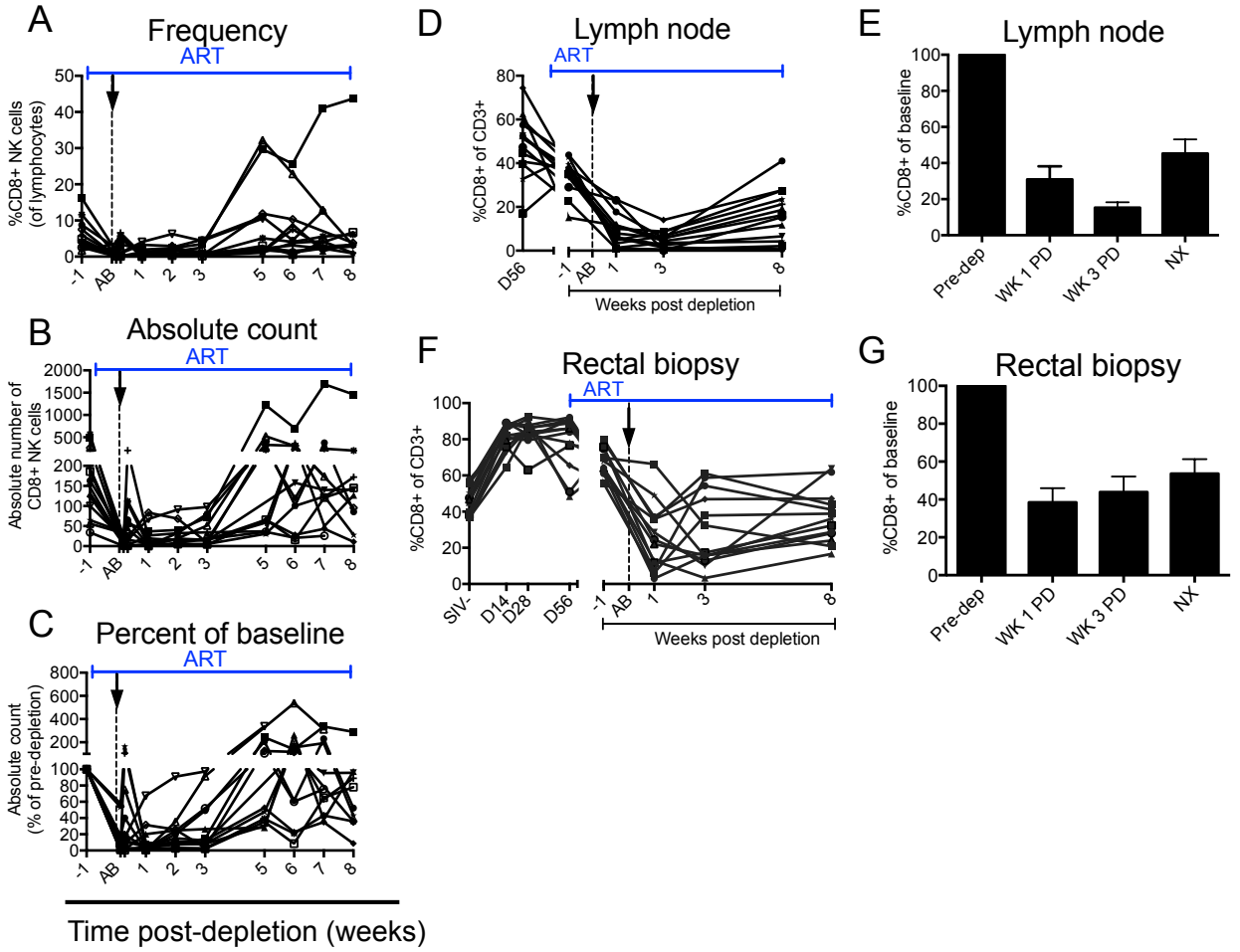

Figure S1. Related to Figure 1**.** MT-807R1 administration also depletes CD8α+ NK cells. Cells were gated on lymphocytes, then CD3-CD20-. (A) Frequency of CD8α+ NK cells as a proportion of total lymphocytes and (B) absolute count of CD8α+ NK cells in PBMC pre- and post-MT-807R1 administration. (C) Absolute count of CD8+ NK cells as a proportion of pre-depletion CD8α+ NK cell counts. $CD8^+$ T cells in lymph nodes as a percentage of (D) $CD3^+$ lymphocytes and (E) percentage of pre-depletion frequencies ("baseline"). $CD8^+$ T cells in rectal biopsies as a percentage of (F) $CD3^+$ lymphocytes and (G) percentage of pre-depletion frequencies ("baseline"). Gap in x-axis represents 8-32 weeks of continuous ART. Black arrow and dotted vertical line indicate MT-807R1 administration. NX=necropsy. Data shown is for 13 SIV-infected ART treated rhesus macaques. (E, G) Bars drawn at the mean, error bars represent standard error of mean (SEM).

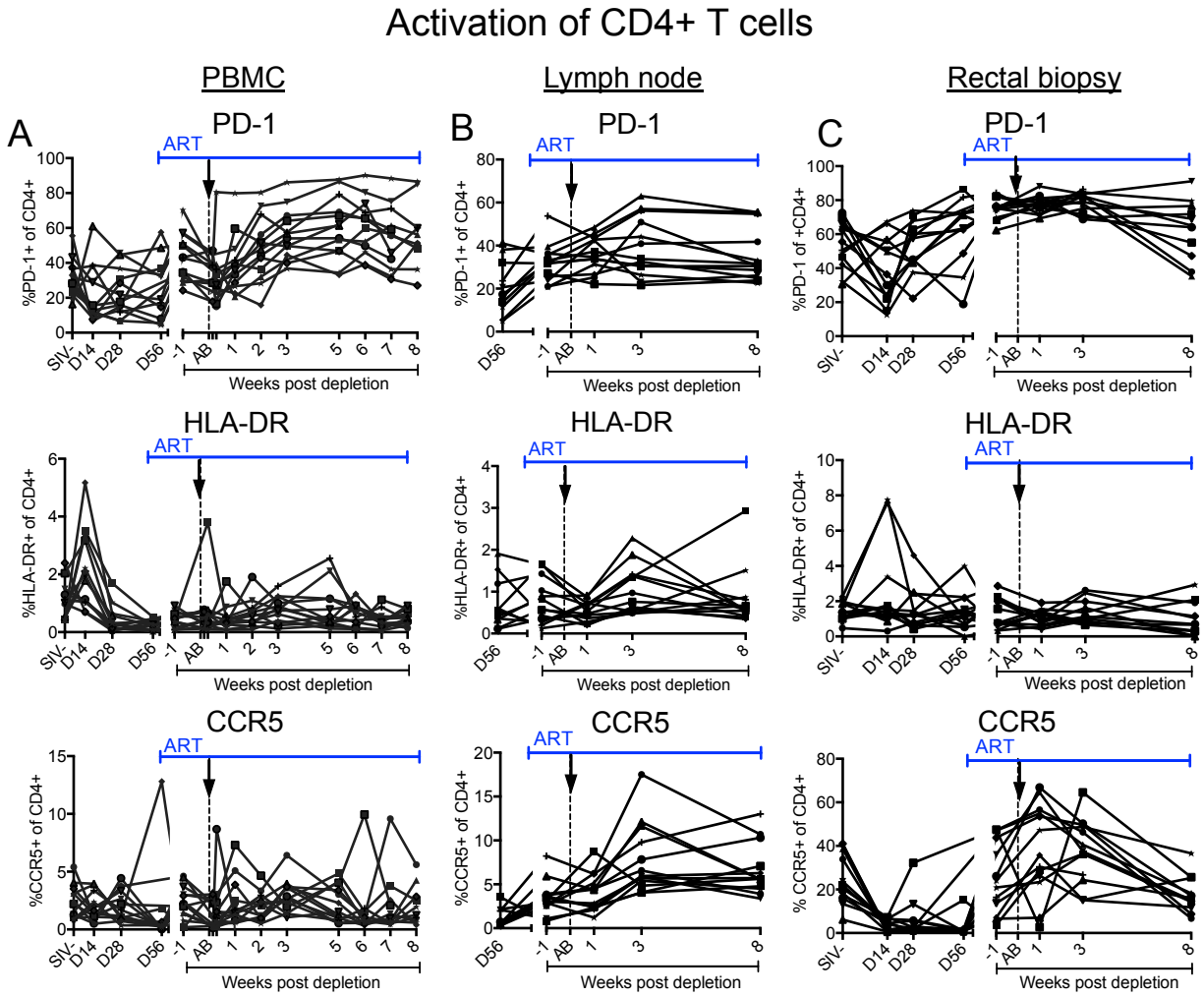

Figure S2. Related to Figure 1. Administration of anti-CD8 antibody MT-807R1 to SIV-infected ART treated RM results in variable increases in CD4+ T cell activation. Expression of activation markers PD-1, HLA-DR, and CCR5 on CD4+ T cells in (A) PBMC, (B) Lymph Node and (C) Rectal Biopsy. Gap in x-axis represents 8-32 weeks of continuous ART. Data shown is for 13 SIV-infected ART treated RM. Black arrow and dotted vertical line indicate MT-807R1 administration.

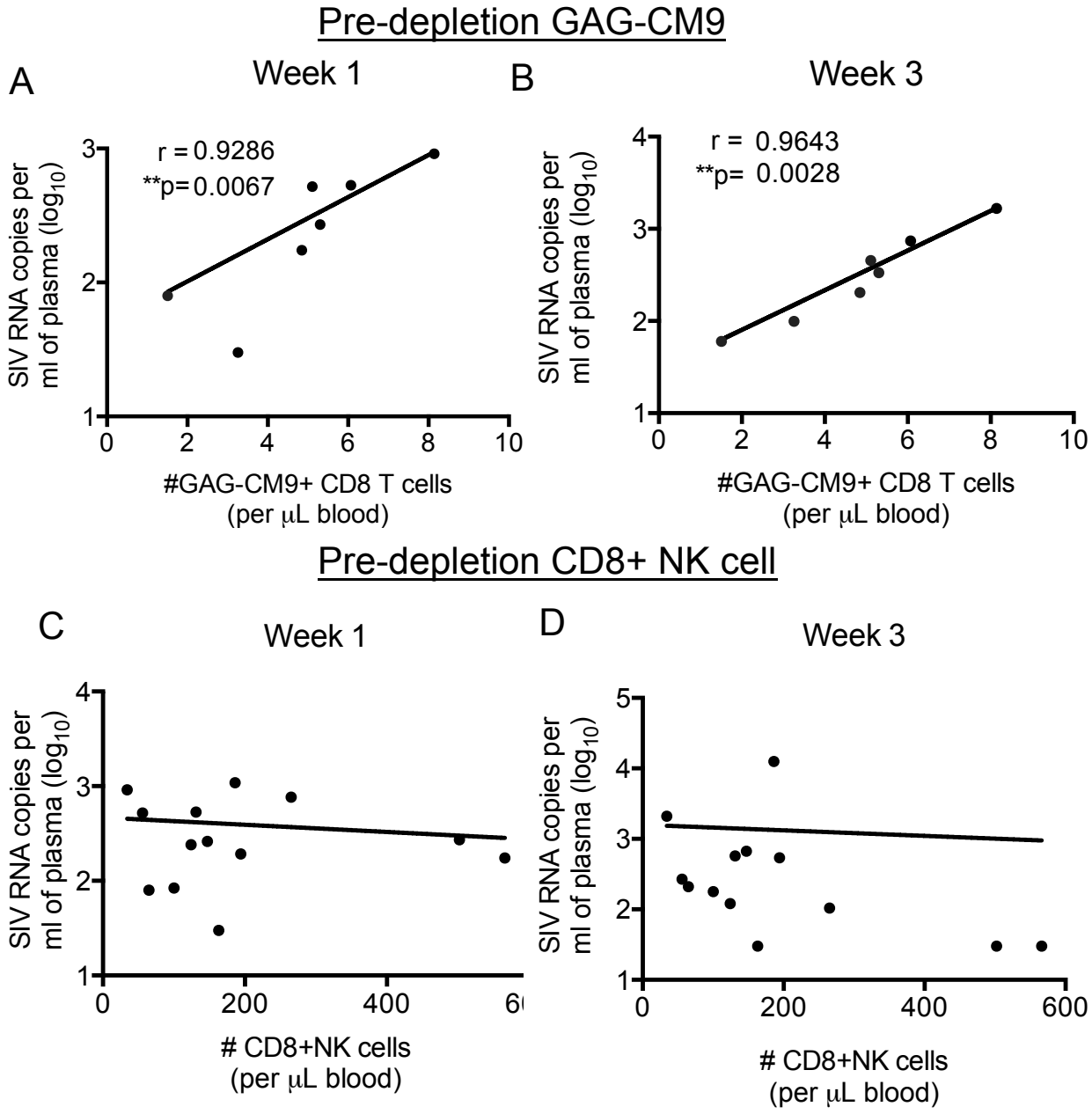

Figure S3. Related to Figure 2. Number of pre-depletion SIV-specific CD8+ T cells predicts post depletion viral loads. Panels A-B: Presence of a significant direct correlation of number of GAG-CM9 specific CD8+ T cells in PBMCs pre-depletion with plasma viral load at (A) week 1 and (B) week 3 post-depletion. Data shown in (A) and (B) is for 7 SIV-infected ART treated *Mamu-A*01* rhesus macques. Panels C-D: Lack of correlation of number of NK cells in PBMCs pre-depletion with plasma viral load at (C) week 1 and (D) week 3 post-depletion. Data shown in (C) and (D) is for 13 SIV-infected ART treated rhesus macaques. Spearman rank correlation, two tailed.

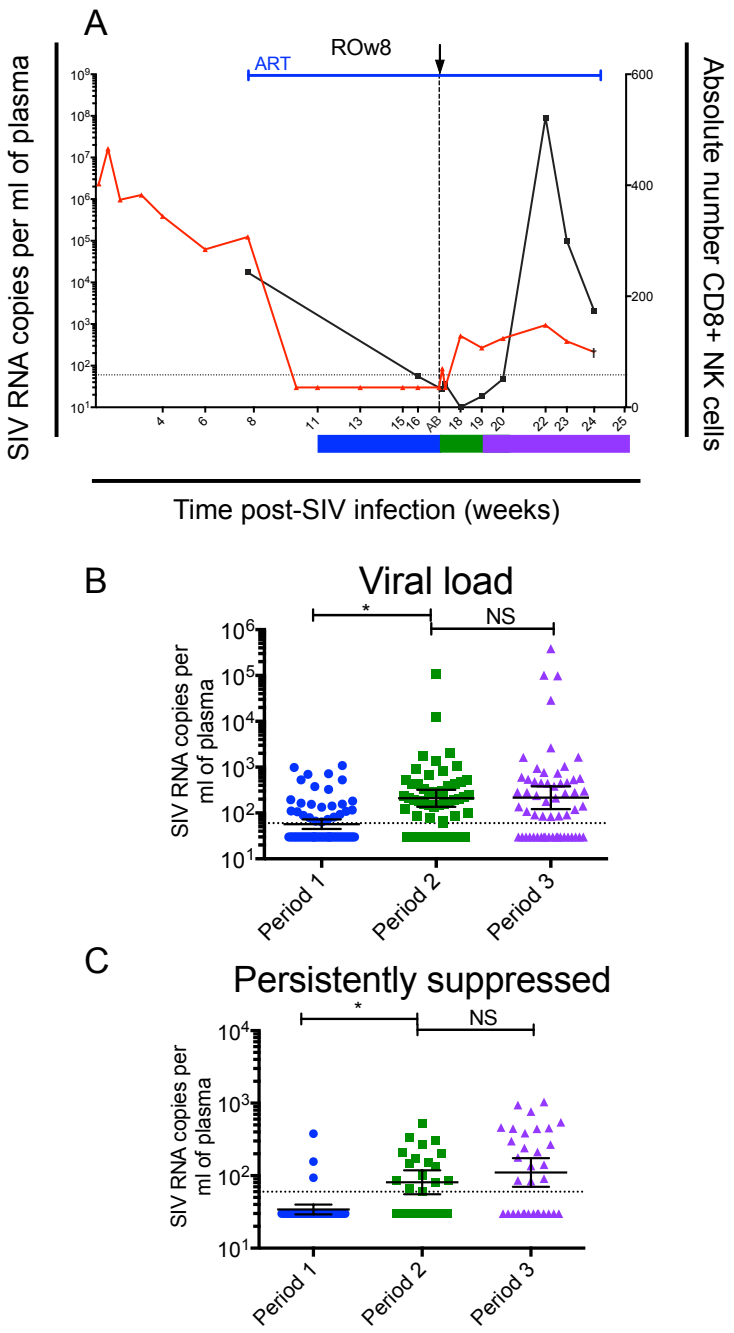

Figure S4. Related to Figure 2. NK cell reconstitution does not coincide with viral load decline in ART-treated SIV-infected RMs. (A) Viral load (red line) and CD8+ NK cell counts (black line) of a representative SIV-infected RM on ART. Black arrow and dotted line indicates anti-CD8 MT-807R1 administration. (B) Geometric mean viremia for each statistical period (NK cell) for all 13 animals. Bars show the geometric mean with 95% CI, *p<0.05. (C) Geometric mean viremia for each NK cell statistical period for 7 persistently suppressed animals. Bars shown at the geometric mean with 95% CI. Dashed horizontal line represents limit of detection of standard viral load assay (60 copies/ml). Statistical analysis (B, C) was conducted using a linear, mixed effects model, *p<0.05.

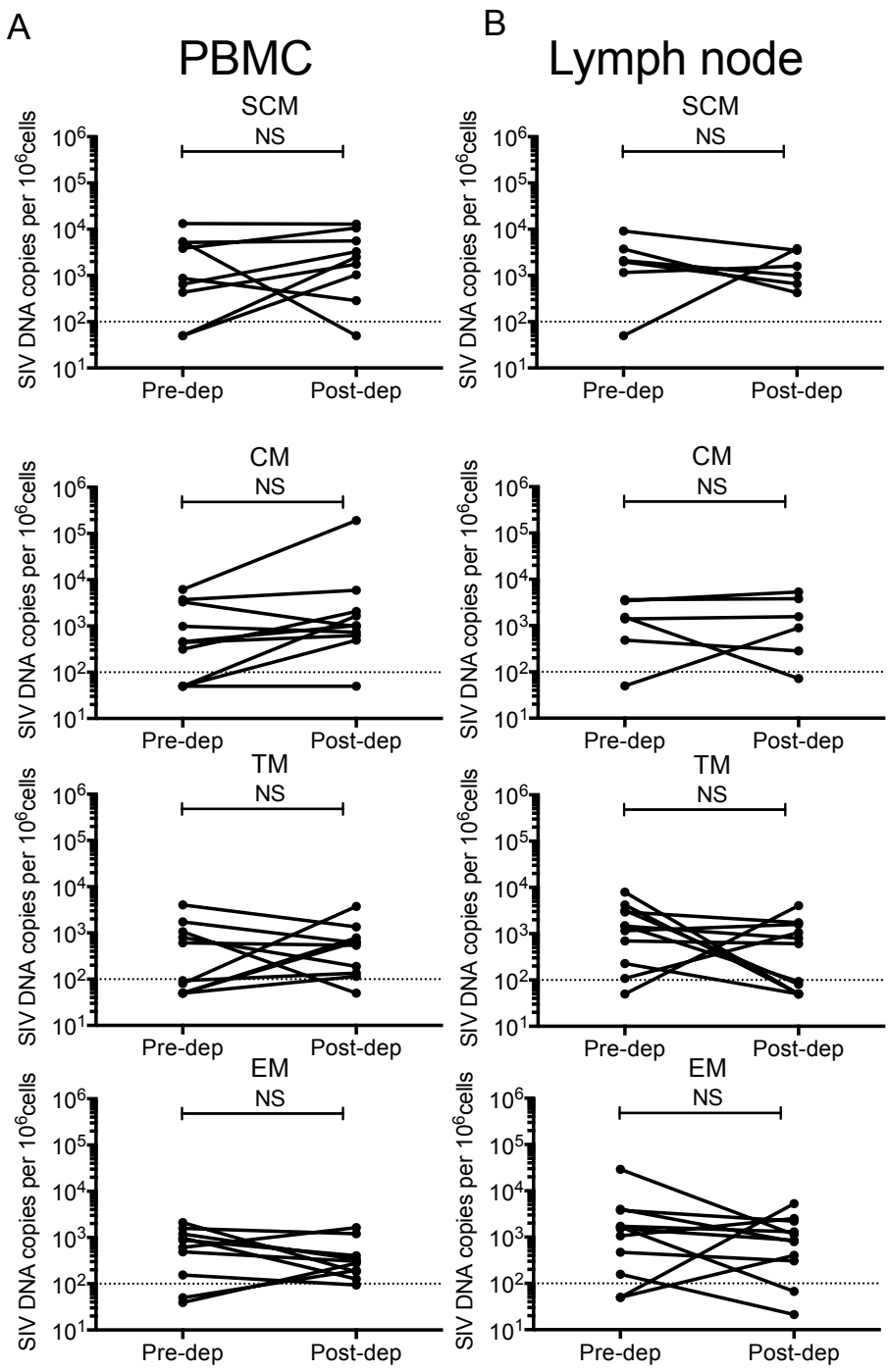

Figure S5. Related to Figure 5. No significant changes in total cell-associated SIV-DNA in sorted memory CD4+ T cell subsets of SIV-infected ART-treated RM before and after CD8 depletion. Fraction of SIV-infected sorted T_{SCM}, T_{CM}, T_{TM} and T_{EM} (as defined in Methods) in (A) PBMC (data shown for all animals with >10,000 sorted cells (T_{SCM}=6, T_{CM}=6, T_{TM}=11, T_{EM}=11) and (B) lymph node (data shown for all animals with >10,000 sorted cells (T_{SCM}=6, T_{CM}=6, T_{TM}=11, T_{EM}=11). Dashed horizontal line represents limit of detection of SIV DNA assay (100 copies/10^6 cells). Statistical analysis was conducted using the Wilcoxon matched-pairs signed rank test.

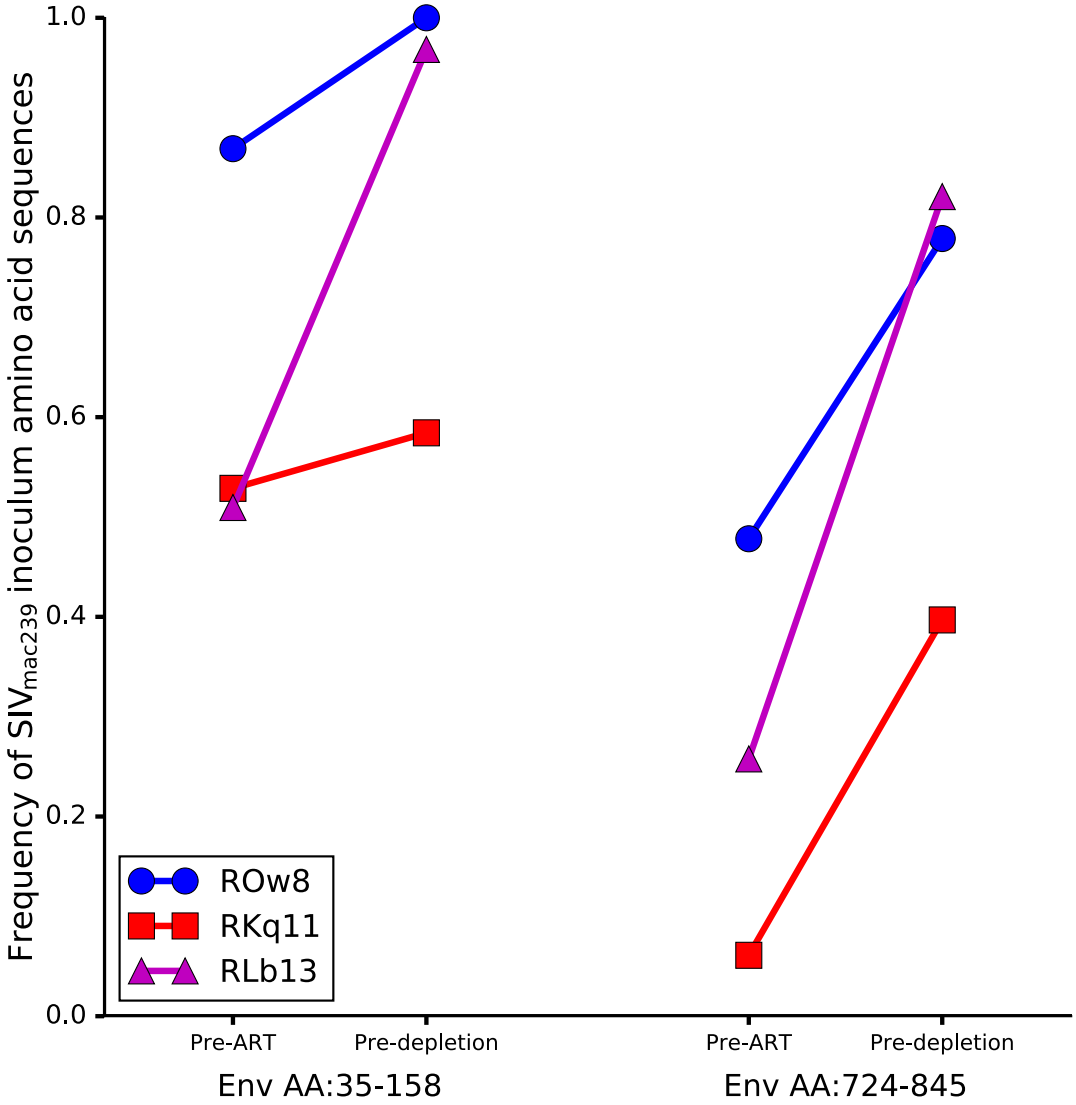

Figure S6. Related to Figure 6. ART adminstration results in an increase in the frequency of virus bearing the SIVmac239 inoculum amino acid sequence. Two amplicons covering Env amino acids 35-158 and 724-845 were amplified from peripheral blood cell DNA and sequenced via Illumina MiSeq to verify the presence of amino acid mutations identified in the circulating plasma virus. The frequency of the wildtype sequence was estimated for each sample prior to ART administration and just prior to CD8 depletion.

Number of animals	ART Regimen	Duration (post-ART initiation)
3	20 mg/kg PMPA s.c., 30 mg/kg FTC s.c., 100 mg Raltegravir and 400 mg Darunavir orally b.i.d.	From 0 to 16 weeks
5	20 mg/kg PMPA s.c., 30 mg/kg FTC s.c., 100 mg Raltegravir and 400 mg Darunavir orally b.i.d. followed by 20 mg/kg PMPA s.c., 30 mg/kg FTC s.c., 150 mg Raltegravir and 600 mg Darunavir orally b.i.d.	From 0 to 8 weeks From 8 to 24 weeks
5	20 mg/kg PMPA s.c., 30 mg/kg FTC s.c., 100 mg Raltegravir and 400 mg Darunavir orally b.i.d. followed by 20 mg/kg PMPA s.c., 30 mg/kg FTC s.c., 150 mg Raltegravir and 600 mg Darunavir orally b.i.d. followed by 20 mg/kg PMPA s.c., 30 mg/kg FTC s.c., 150 mg Raltegravir and 800 mg Darunavir orally b.i.d.	From 0 to 8 weeks From 8 to 24 weeks From 24 to 40 weeks

Table S1. Related to Figure 1**.** Antiretroviral drug regimen and length of treatment for SIV-infected ART-treated RM. All 13 animals were treated for at least 16 weeks. After 8 weeks of continuous treatment, 10 of the animals were given increased doses of Raltegravir and Darunavir. After another 16 weeks of increased Raltegravir and Darunavir, 5 animals received a further increased dose of Darunavir.

Magnitude of SIV suppression during ART

	Pre-ART (copies/ml of plasma)	Post-ART (Pre CD8 Depl.) (copies/ml of plasma)	% Suppression
RGb13	433	Below 3	100
RLb13	233,000	7	99.992
RVy10	2,940,000	10	99.998
ROw8	124,000	15	99.985
RKq11	599,000	25	99.993
RBv13	1,580,000	65	99.994
RWj14	496,000	90	99.979
RSj14	921,000	80	99.989
RDh10	3,400,000	90	99.997
RLc10	1,940,000	160	99.985
RAz12	2,270,000	300	99.982
RYf14	5,870,000	330	99.991

Table S2**.** Related to Figure 1 and 2. Magnitude of virus suppression during ART, prior to CD8+ lymphocyte depletion is >99.9% in all animals where at least 1 undetectable time point was achieved. Animals in red were “persistently suppressed”, animals in black were “intermittently suppressed.”

Code	Period 1	Period 2	Period 3
RGb13	6 weeks	3 weeks	4 weeks
RLb13	6 weeks	3 weeks	4 weeks
RVy10	6 weeks	3 weeks	1 week
RKq11	6 weeks	7 weeks	1 week
RBv13	6 weeks	3 weeks	4 weeks
RWj14	6 weeks	3 weeks	4 weeks
RSj14	6 weeks	3 weeks	4 weeks
RYf14	6 weeks	6 weeks	2 weeks
RAz12	6 weeks	5 weeks	3 weeks
RLc10	6 weeks	7 weeks	n/a
ROw8	6 weeks	7 weeks	n/a
RDh10	6 weeks	8 weeks	n/a
ROn13	6 weeks	8 weeks	n/a

Table S3. Related to Figure 2. Length of the periods 1-3 used for statistical analysis as described in Figure 2 for each SIV-infected ART-treated RM. Period 1 is pre-CD8 depletion (blue), Period 2 is post-CD8 depletion, pre-CD8 T cell recovery (green), and Period 3 is during CD8 T cell recovery (purple). CD8+ T-cell reconstitution above 20% of baseline (i.e., pre-depletion) levels was not acheived in four animals (RLc10, ROw8, RDh10, and ROn13).

Subset	Time Point	Tissue	p
Total CD4	WK 1 Post Depletion	PBMC	NS
CD95+ Memory	WK 1 Post Depletion	PBMC	NS
T_{SCM}	WK 1 Post Depletion	PBMC	NS
T_{CM}	WK 1 Post Depletion	PBMC	NS
T_{TM}	WK 1 Post Depletion	PBMC	NS
T_{EM}	WK 1 Post Depletion	PBMC	NS
Total CD4	WK 1 Post Depletion	Lymph node	NS
CD95+ Memory	WK 1 Post Depletion	Lymph Node	NS
T_{SCM}	WK 1 Post Depletion	Lymph Node	NS
T_{CM}	WK 1 Post Depletion	Lymph Node	NS
T_{TM}	WK 1 Post Depletion	Lymph Node	NS
T_{EM}	WK 1 Post Depletion	Lymph Node	NS
Total CD4	WK 1 Post Depletion	Rectal Biopsy	NS
CD95+ Memory	WK 1 Post Depletion	Rectal Biopsy	NS
Total CD4	WK 3 Post Depletion	PBMC	NS
CD95+ Memory	WK 3 Post Depletion	PBMC	NS
T_{SCM}	WK 3 Post Depletion	PBMC	NS
T_{CM}	WK 3 Post Depletion	PBMC	NS
T_{TM}	WK 3 Post Depletion	PBMC	NS
T_{EM}	WK 3 Post Depletion	PBMC	NS
Total CD4	WK 3 Post Depletion	Lymph node	NS
CD95+ Memory	WK 3 Post Depletion	Lymph Node	NS
T_{SCM}	WK 3 Post Depletion	Lymph Node	NS
T_{CM}	WK 3 Post Depletion	Lymph Node	NS
T_{TM}	WK 3 Post Depletion	Lymph Node	NS
T_{EM}	WK 3 Post Depletion	Lymph Node	NS
Total CD4	WK 3 Post Depletion	Rectal Biopsy	NS
CD95+ Memory	WK 3 Post Depletion	Rectal Biopsy	NS

Table S4. Related to Figure 5. No correlation between the frequency of proliferating (Ki67+) CD4+ T-cells and plasma viral load at weeks 1 and 3 post-CD8 depletion. Spearman rank correlation.

	Time Point (Post Depletion)	Tissue	r, p
CCR5+CD4+	Day 1	PBMC	NS
	Day 2	PBMC	NS
	Week 1	PBMC	NS
	Week 1	Lymph Node	NS
	Week 3	Lymph Node	NS
	Week 1	Rectal Biopsy	NS
	Week 3	Rectal Biopsy	NS
PD-1+CD4+	Day 1	PBMC	r=0.6599, p=0.0120
	Day 2	PBMC	r=0.5144, p=0.0120
	Week 1	PBMC	NS
	Week 1	Lymph Node	NS
	Week 3	Lymph Node	NS
	Week 1	Rectal Biopsy	NS
	Week 3	Rectal Biopsy	NS
HLA-DR+CD4+	Day 1	PBMC	r=0.6308, p=0.0163
	Day 2	PBMC	NS
	Week 1	PBMC	NS
	Week 1	Lymph Node	NS
	Week 3	Lymph Node	NS
	Week 1	Rectal Biopsy	r=0.6099, p=0.0151
	Week 3	Rectal Biopsy	NS

Table S5. Related to Figure 5. Correlation between PD-1+CD4+ and HLA-DR+CD4+ T cells in PBMC and RB and plasma viral load at early time points (Day 1, Day 2, and Week 1) post-depletion. Spearman rank correlation.