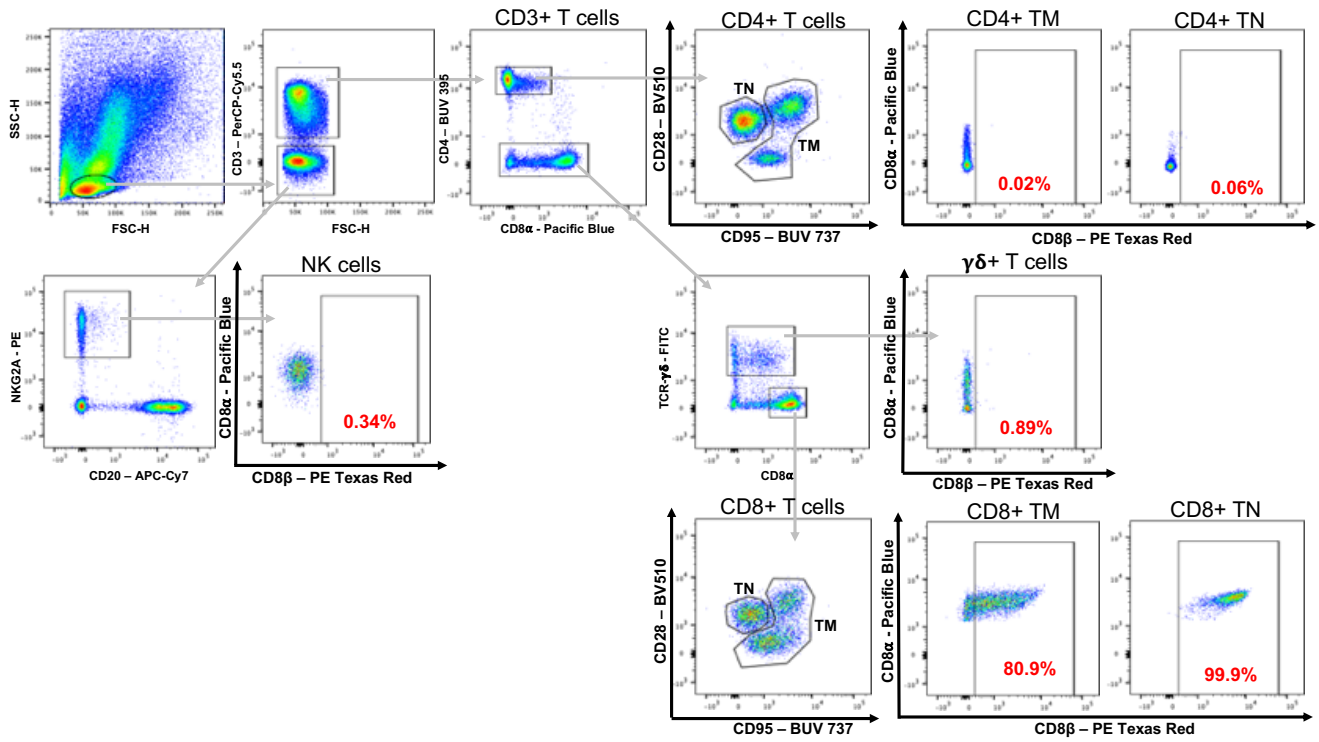
Supplemental Materials

$CD8^+$ T cells fail to limit SIV reactivation following ART withdrawal until after viral amplification

Afam A. Okoye, Derick D. Duell, Yoshinori Fukazawa, Benjamin Varco-Merth, Alejandra Marenco, Hannah Behrens, Morgan Chaunzwa, Andrea N. Selseth, Roxanne M. Gilbride, Jason Shao, Paul T. Edlefsen, Romas Geleziunas, Mykola Pinkevych, Miles P. Davenport, Kathleen Busman-Sahay, Michael Nekorchuk, Haesun Park, Jeremy Smedley, Michael K. Axthelm, Jacob D. Estes, Scott G. Hansen, Brandon F. Keele, Jeffery D. Lifson, and Louis J. Picker

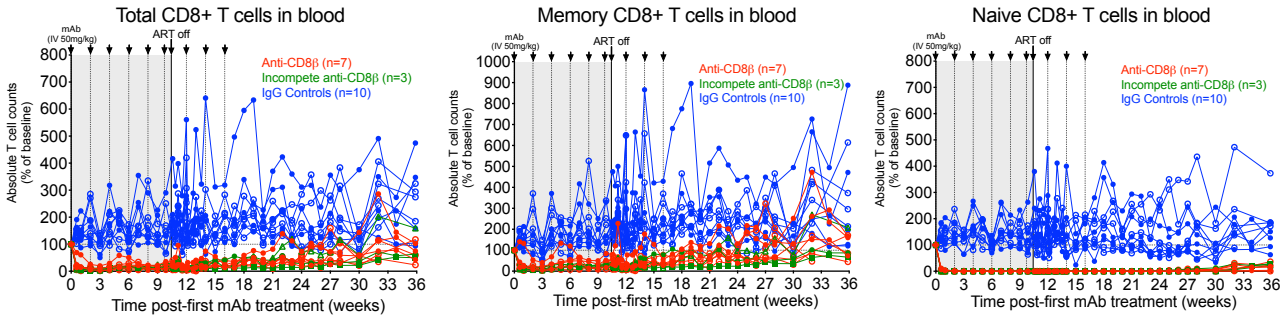
Corresponding author: Louis J. Picker, M.D., Vaccine and Gene Therapy Institute, Oregon Health & Science University – West Campus. 505 NW 185th Ave., Beaverton, OR 97006. Phone: (503) 418-2720. FAX: (503) 418-2719. Email: pickerl@ohsu.edu



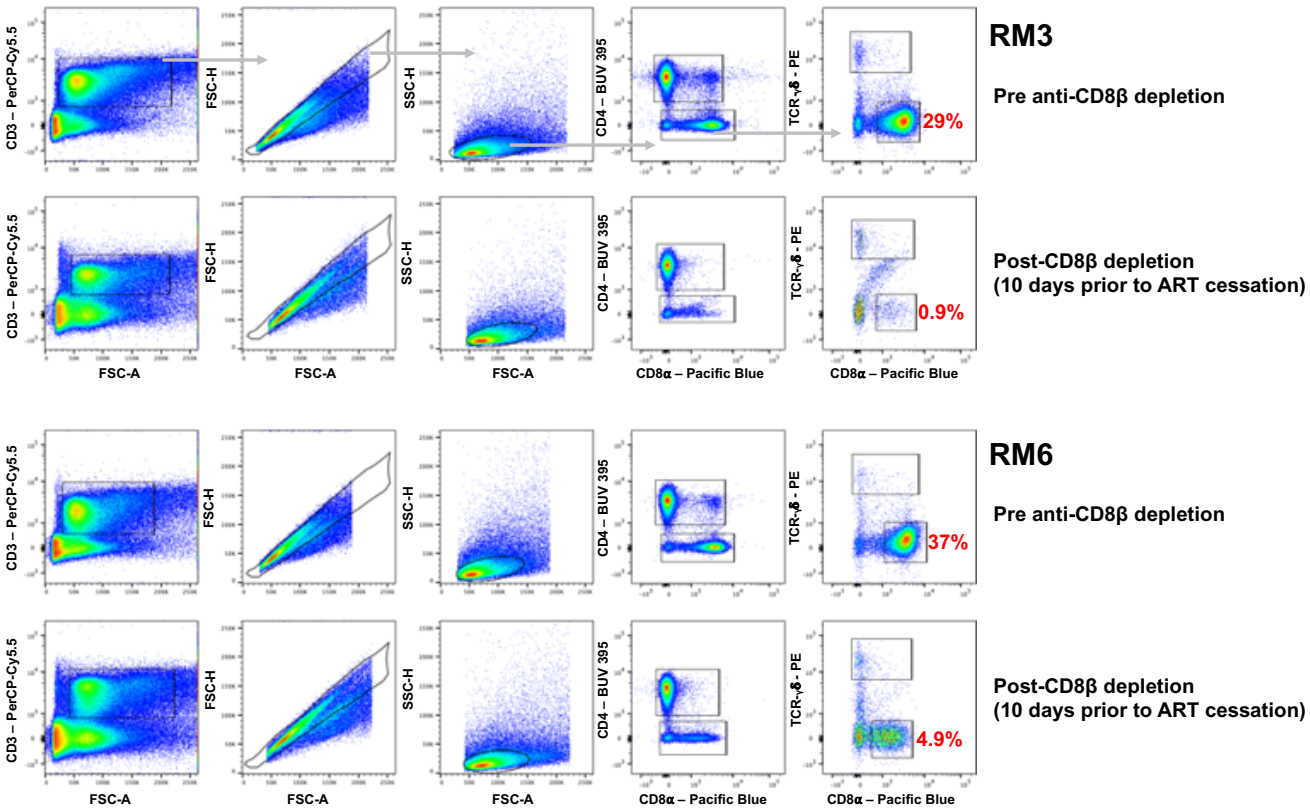
Supplementary Figure 1: Representative flow cytometric analysis showing CD8α vs. CD8β expression on $CD4^+$ and $CD8^+$ memory (T_M) and naïve (T_N) T cells, NK cells and TCR-$\gamma\delta^+$ T cells in RM peripheral blood. Note that 1) NK cells and a proportion of both TCR-$\gamma\delta^+$ T cells and $CD4^+$ T cells (predominantly in the memory subset) express CD8α in the absence of CD8β (e.g., as CD8αα homodimers) and 2) $CD8\alpha^+$ T_M cells include a mixture of $CD8\beta^+$ and $CD8\beta^-$ cells (the latter predominantly non-classical, innate-type $CD8\alpha\alpha^+$ T cells), whereas $CD8\alpha^+$ T_N cells uniformly express CD8β.

RM#	Sex	Age at time of SIV infection (Years)	MHC	Day of ART (dpi)	Time on ART (Days)	mAb treatment group
RM1	Male	4	A*01	12	278	Anti-CD8β
RM2	Male	4	A*01	12	278	Anti-CD8β
RM3	Male	4	A*01	12	278	Anti-CD8β
RM4	Female	4	A*01	12	278	Anti-CD8β
RM5	Male	4	A*01	12	278	Anti-CD8β
RM6	Female	7	B*08	12	278	Anti-CD8β
RM7	Female	6	B*08	12	278	Anti-CD8β
RM8	Male	4	B*08	12	278	Anti-CD8β
RM9	Female	4	B*08	12	278	Anti-CD8β
RM10	Male	3	B*08	12	278	Anti-CD8β
RM11	Male	4	A*01	12	278	Control IgG
RM12	Male	4	A*01	12	278	Control IgG
RM13	Female	4	A*01	12	278	Control IgG
RM14	Male	4	A*01	12	278	Control IgG
RM15	Female	3	A*01	12	278	Control IgG
RM16	Female	6	B*08	12	278	Control IgG
RM17	Male	4	B*08	12	278	Control IgG
RM18	Female	4	B*08	12	278	Control IgG
RM19	Female	3	B*08	12	278	Control IgG
RM20	Male	3	B*08	12	278	Control IgG

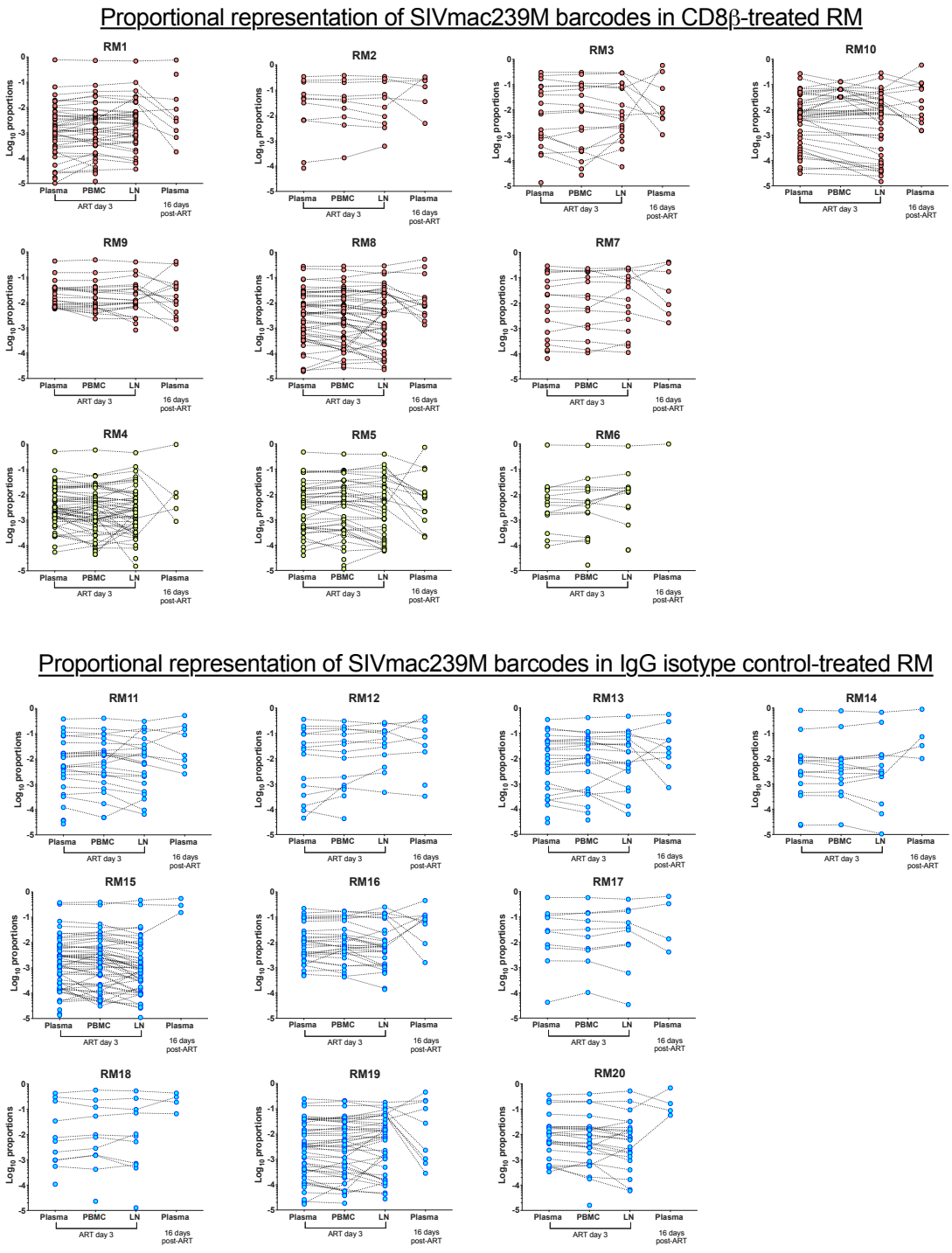
Supplementary Table 1: Characteristics of anti-CD8β mAb-treated and IgG isotype control-treated RMs. The table shows the sex and age of RMs at time of SIV infection, MHC-I alleles, day of ART initiation, and time on ART.



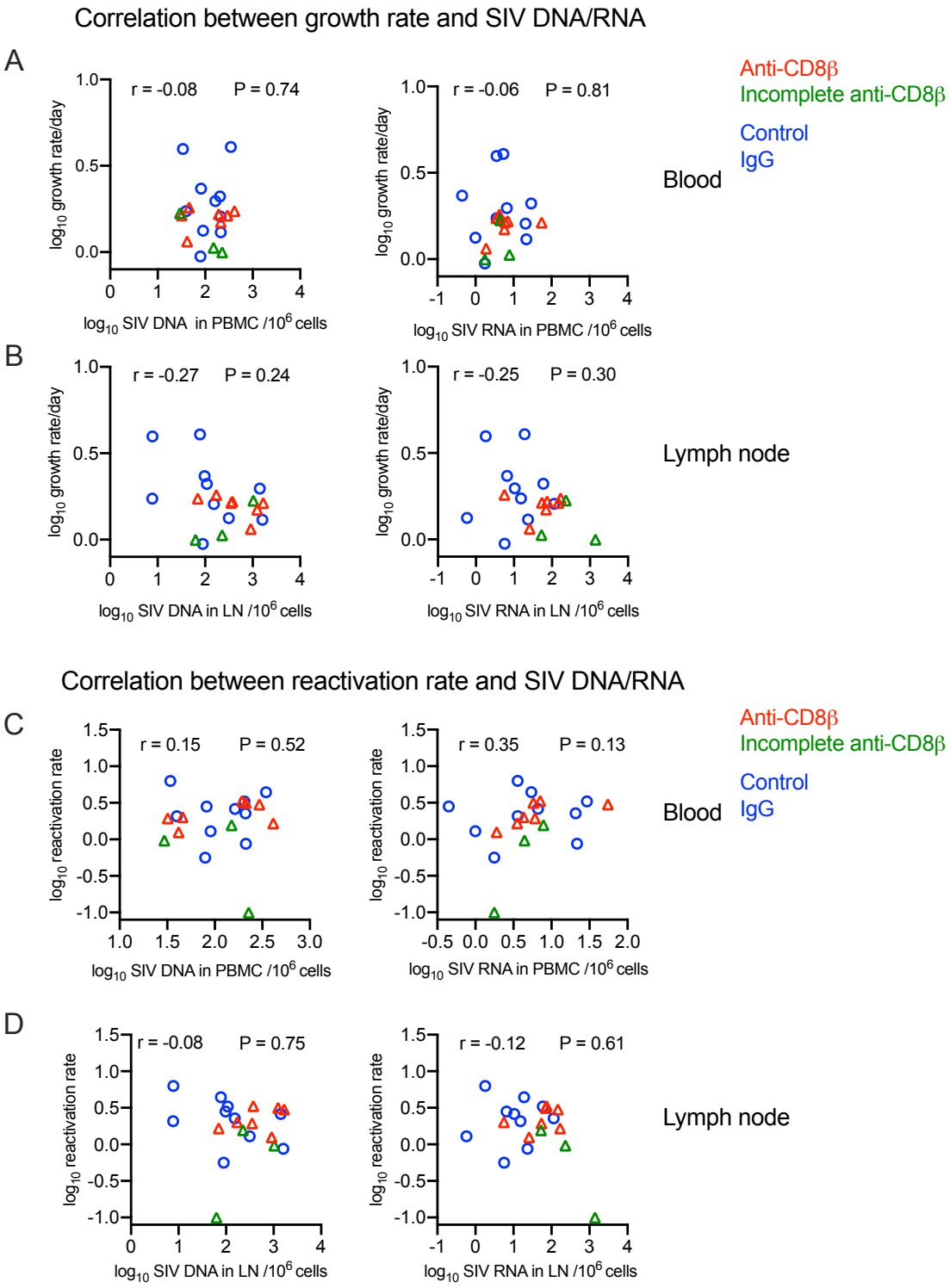
Supplementary Figure 2: Percent (%) change from baseline in the absolute counts of total, memory, and naïve $CD8^+$ T cells in peripheral blood of individual monkeys following anti-CD8β (red; n=10) vs. IgG isotype control (blue; n=10) mAb treatment. Arrows indicate anti-CD8β or IgG control mAb administration and time of ART cessation. RMs with full $CD8^+$ T_M depletion in tissues are shown in red (n=7), RMs with incomplete $CD8^+$ T_M depletion in tissues are shown in green (n=3) and IgG isotype controls are shown in blue (n=10).



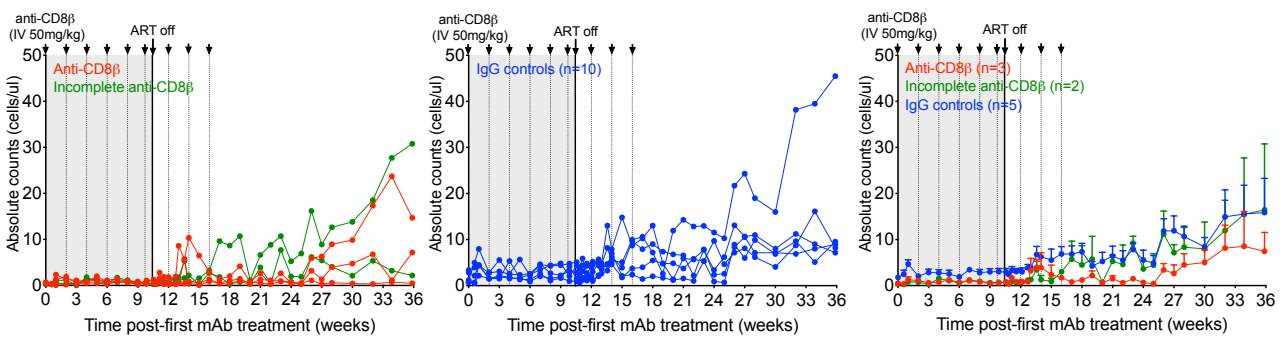
Supplementary Figure 3: Representative flow cytometric analysis of $CD8^+$ T cells in LN showing maximal ("complete") vs. incomplete depletion. The profiles show the strategy used to delineate $CD8^+$ T cells by gating first on $CD3^+$ small lymphocytes and then excluding $CD4^+$ T cells, leaving visualization of TCR-γδ vs. CD8α expression (note that CD8β expression is blocked after in vivo administration of the anti-CD8β mAb). The values in red represent the fraction of $CD8\alpha^+$ T cells of total $CD3^+$ T cells in LN (far right panels). Excess residual $CD8^+$ T cells are found in the post-depletion profiles of RM 6(reflecting incomplete depletion), as compared to RM3, (reflecting maximal depletion in our study).



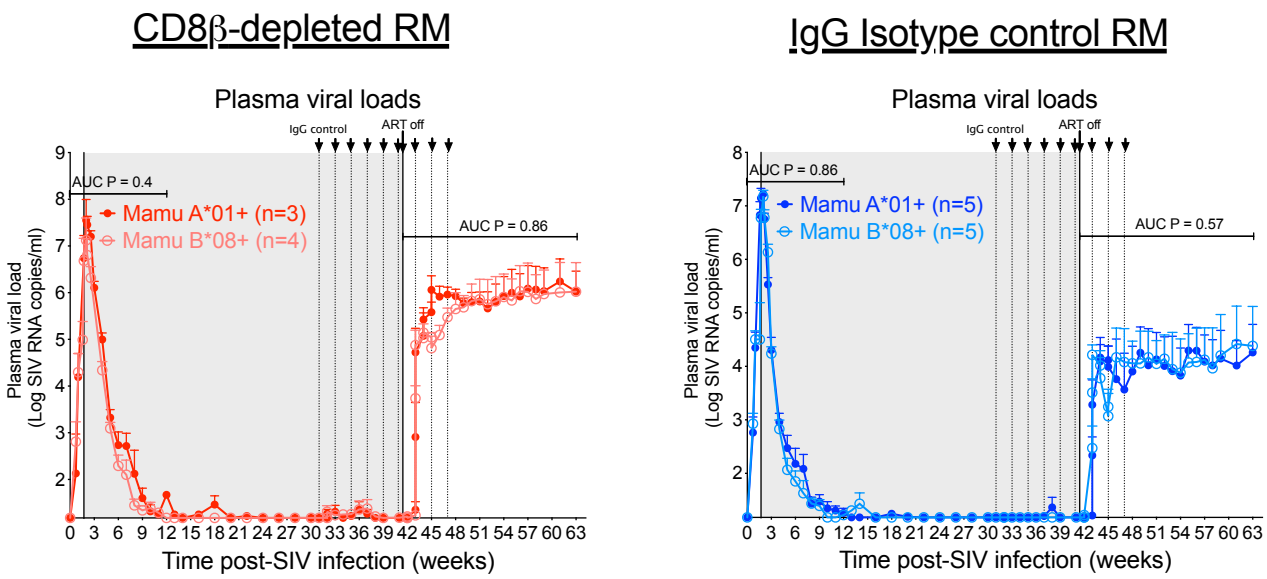
Supplementary Figure 4: Barcode frequencies at peak viral replication during primary SIVmac239M infection and post-rebound. The frequency of barcode clonotypes identified by high throughput sequencing of plasma, PBMC, and LN after 3 days of ART and in plasma 16 days post-ART cessation in anti-CD8β mAb-treated RM and IgG isotype-treated controls. RM with full $CD8^+$ T cell depletion are show in red (n=7), RM with incomplete depletion are shown in green (n=3), and control RM are shown in blue. Each dot represents a unique barcode and the linked dots indicate the same barcode across the different tissues. Note that the dynamics of virus rebound following ART cessation and the relative abundance of each clonotype sequence detected in plasma during rebound were used to calculate the reactivation rate.



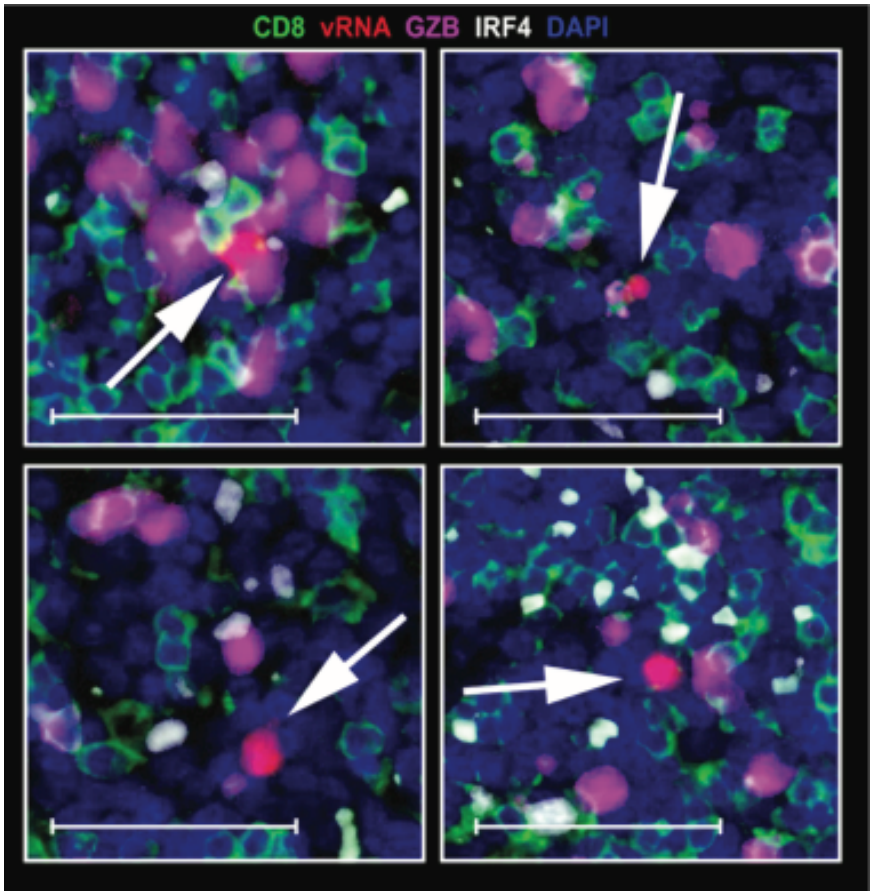
Supplementary Figure 5: A, B: Scatterplots of post-ART growth rates vs. levels of cell-associated SIV DNA and RNA in (A) blood and (B) LN 10 days prior to ART release. **C, D:** Scatterplots of post-ART reactivation rates and levels of cell associated SIV DNA and RNA in (C) blood and (D) LN 10 days prior to ART release. All panels show Spearman rank correlation coefficient *r* with unadjusted p-values testing for association between paired samples. Note the lack of correlation between pre-ART release measures of residual virus and post-ART rebound dynamics.



Supplementary Figure 6: Quantification of Gag-CM9^{+}, CD8^{+} T cell counts in blood of *Mamu* A*01^{+} RMs with effective CD8^{+} T cell depletion (red; n=3), incomplete CD8^{+} T cell depletion (green; n=2), and IgG isotype controls (blue; n=5).



Supplementary Figure 7: Comparison of pvl dynamics in *Mamu* A*01^{+} vs. *Mamu* B*08^{+} RM. Mean (SEM) pvl profiles of RM with full anti-CD8β-depletion (left panel) and IgG isotype control RM (right panel) stratified by MHC type (*Mamu* A*01; open circle vs. *Mamu* B*08; closed circle) following SIV infection, ART initiation, mAb administration, and ART cessation. The WRS test was used to determine the significance of differences in AUC between these *Mamu* allomorph-defined groups following SIV infection and ART administration from weeks 0 – 12 and following ART cessation from weeks 41 – 62 (P values shown).



Supplementary Figure 8: Imaging of the CD8$^+$ T cell intercept of SIV-infection 16 days post ART release. Immunofluorescent images showing CD8α^+ lymphocytes (green) intercepting rebounding SIV infection (vRNA$^+$ cells; red; arrows) in LN sections 16 days post-ART release in a control (non-CD8-depleted) RM. IRF4 expression (white) indicates TCR-mediated activation. Granzyme B (GZB; magenta) delineates cytotoxic effector differentiation. Scale bar = 50 μm.