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Supplemental Information

Tracking HIV Rebound following Latency Reversal Using Barcoded HIV

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Supplemental figures

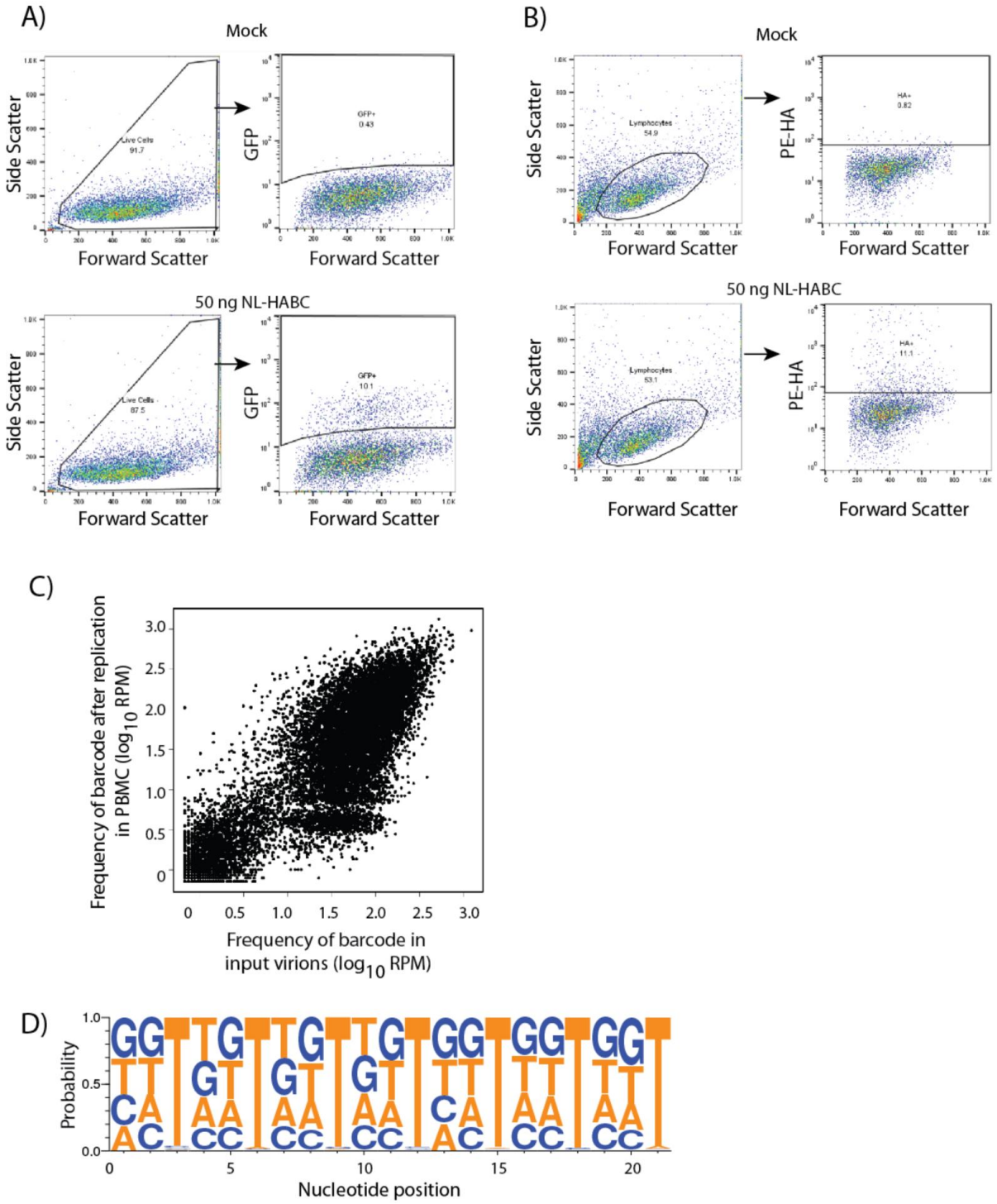

Figure S1. Example flow cytometry profile/gating strategy and additional sequence analysis. Related to Fig 2. A) GHOST cell infections. GHOST cells encode GFP under the control of an HIV promoter and express GFP when newly infected by HIV (after expression of the Tat gene). Consequently, they are used as indicator cell lines for HIV titrations. Upper panels show mock-infected control cells and lower panels show cells exposed to 50 ng of barcoded NL-HABC virus at 48 h post-infection. **B)** Human PBMC mock infections (upper panel) or NL-HABC infections (lower panel). Percentages of gated cells shown. **C)** Scatter plot showing comparison between barcode occurrence in virions before and after replication for 3 days in primary human PBMC. Spearman correlation rho = 0.74. **D)** Plot showing frequency of different nucleotides within barcode sequence after passage in PBMC, indicating no strong bias towards any particular nucleotide in varied bases (the height of each letter represents the probability of occurrence).

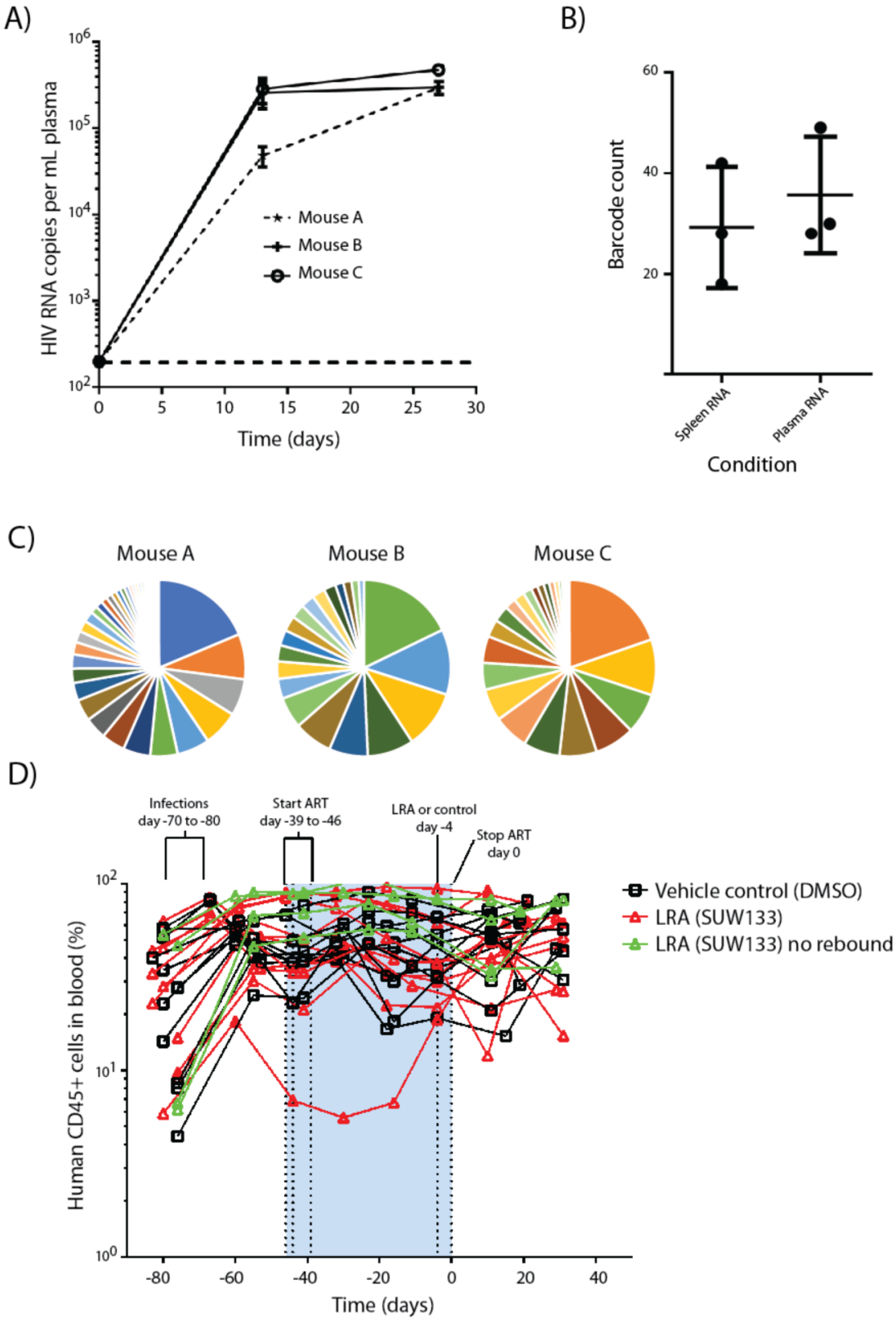

Figure S2. In vivo analysis in humanized BLT mice. Related to Figs 3 and 4. A) Plasma viral loads showing virus replication after primary infection in 3 mice. **B)** Barcode count of individual viral sequences in plasma and spleen at the time of necropsy in these 3 animals (week 4 post-infection). Error bars represent mean+/-SEM. **C)** Pie charts showing barcode frequency distribution in mice A-C with each color representing a unique barcoded sequence present in plasma. **D)** Humanization in mice 1-26 (Fig 3) over time. Percentages of human CD45+ cells were analyzed by flow cytometry. See table S1 for statistical comparisons between groups before and after LRA administration.

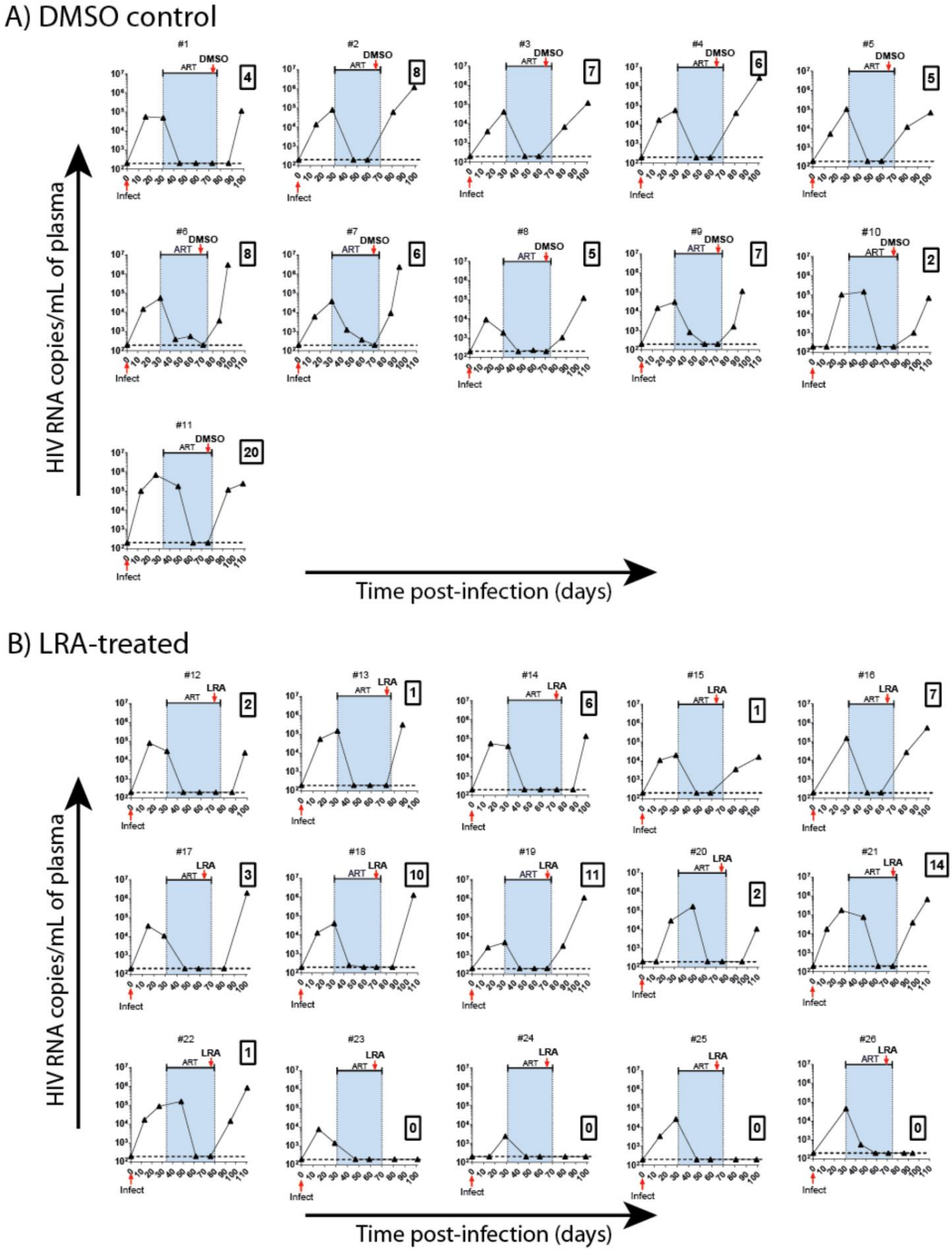

Figure S3. Viral loads and ex vivo outgrowth assays. Related to Figs 3 and 4. A) Viral loads of individual control (DMSO) treated animals **B)** Viral loads of LRA-treated animals. Plasma barcodes identified in each animal indicated within boxes.

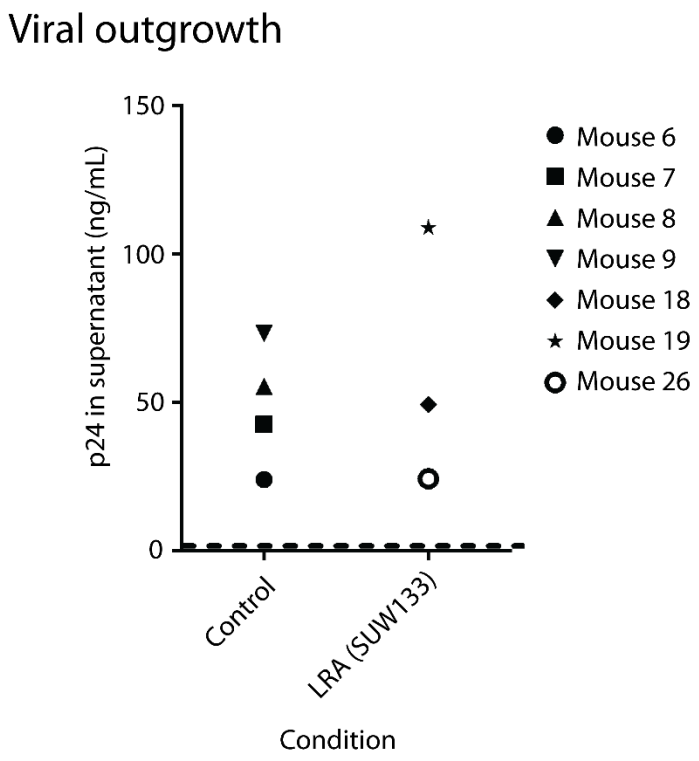

Figure S4. Ex vivo viral outgrowth from spleen. Related to Figs 3 and 4. Splenocytes were costimulated and then incubated with CEM cells to support viral outgrowth from infected tissue. The concentration of HIV p24 in culture supernatant following 14-15d of culture is shown. Notably, cells from non-rebounding mouse #26 (open circle) did produce replicating virus under these conditions, indicating that LRA treatment delayed rebound but did not entirely eliminate viral reservoirs in this animal.

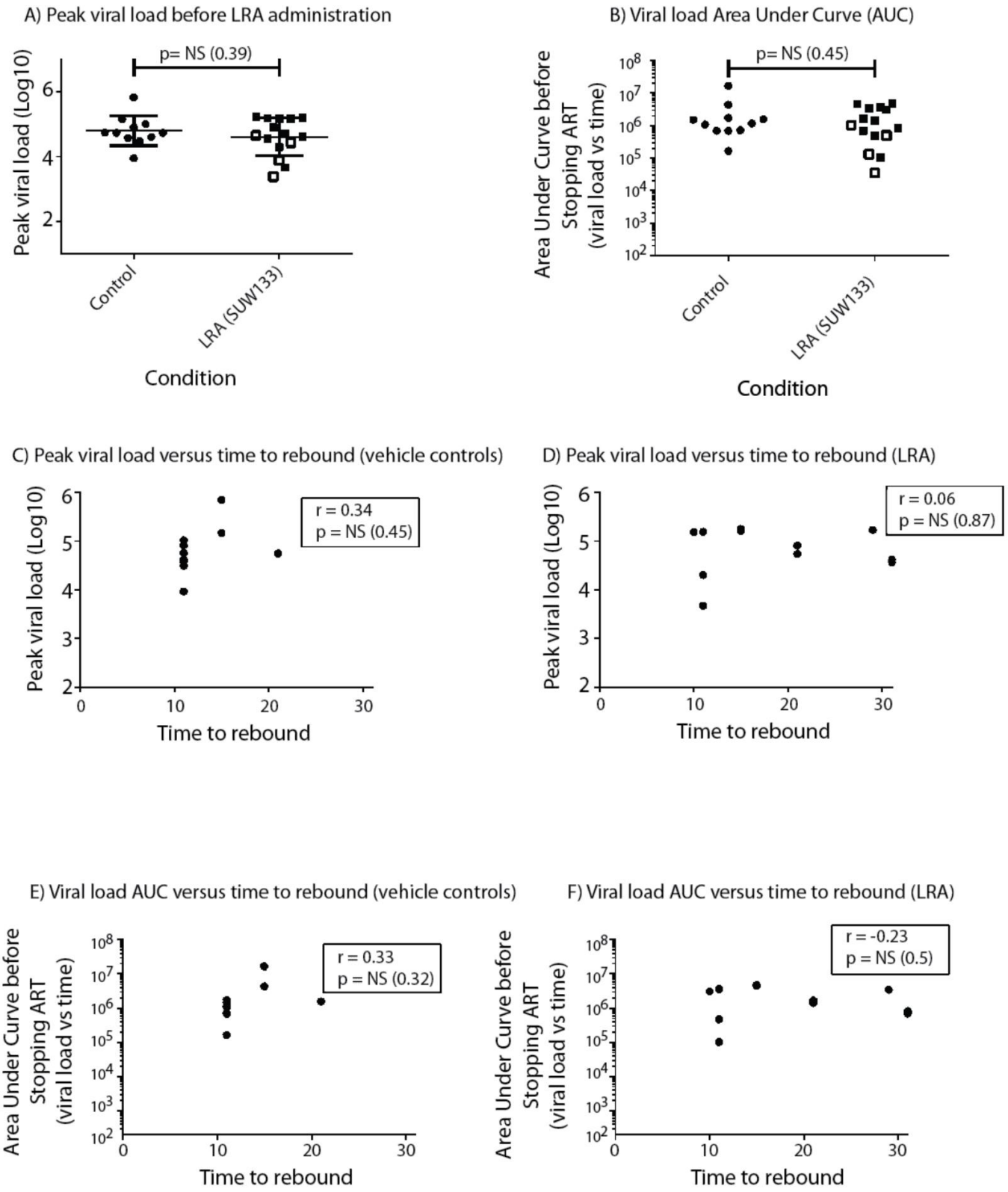

Fig S5. Peak viral load and viral load vs time Area Under Curve (AUC) comparisons. Related to Figs 3 and 4. A) Peak viral load before LRA administration in each animal group. **B)** AUC before stopping ART in each animal group (viral load versus time). **C)** Peak viral load versus time to rebound (control-treated animals). **D)** Peak viral load versus time to rebound (LRA-treated animals). **E)** Viral load AUC versus time to rebound (control-treated animals). **F)** Viral load AUC versus time to rebound (LRA-treated animals). Open squares in panels A and B represent mice non-rebounding mice. A 2-sided equal variance t test was used for comparisons in panels A and B, and Pearson r (2 tailed) for panels C-F. Four non-rebounding mice are not included in panels D and F as no x value is available. Other datapoints are shown but in some cases overlap.

Percentage human CD45+ cells in peripheral blood	**p value**
Before LRA administration: Control group vs LRA group	0.786108
After LRA administration: Control group vs LRA group	0.923628
Control group before vs after Control group after LRA administration	0.896905
LRA group before vs after LRA administration	0.949451

Table S1. In vivo humanization levels are not significantly affected by LRA administration. Related to Figs 3 and 4. P values for comparisons between the closest timepoint before and after LRA administration are shown (2-sided equal variance unpaired t test).

Oligonucleotide sequence	Name
agagcttctagagagtcgcgNNTNNTNNTNNTNNTNNTNNT**ATG**ggcagagcgatggtg	Primer 1
cgcctattctgctatgtcgacaccc	Primer 2
gaagcagagctagaactggcaga	Primer 3
ggactggttttatagacatcactatg	Primer 4
cgcctattctgctatgtcgacaccc	Primer 5
ccccagaagaccaagggc	Primer 6
ctgcgtgggtaggagcag	Primer 7
gccttgccagcacgctcacagnnnnnnnnnnnntaggagcagtgccagaagc	Primer 8
ctgacagaggacaggtggaacaagc	Primer 9
gccttgccagcacgctcacag	Primer 10
aagggccacagagggagc	Primer 11
gccttgccagcacgctcacag	Primer 12

Table S2. Oligonucleotide Primer Sequences. Related to STAR methods.