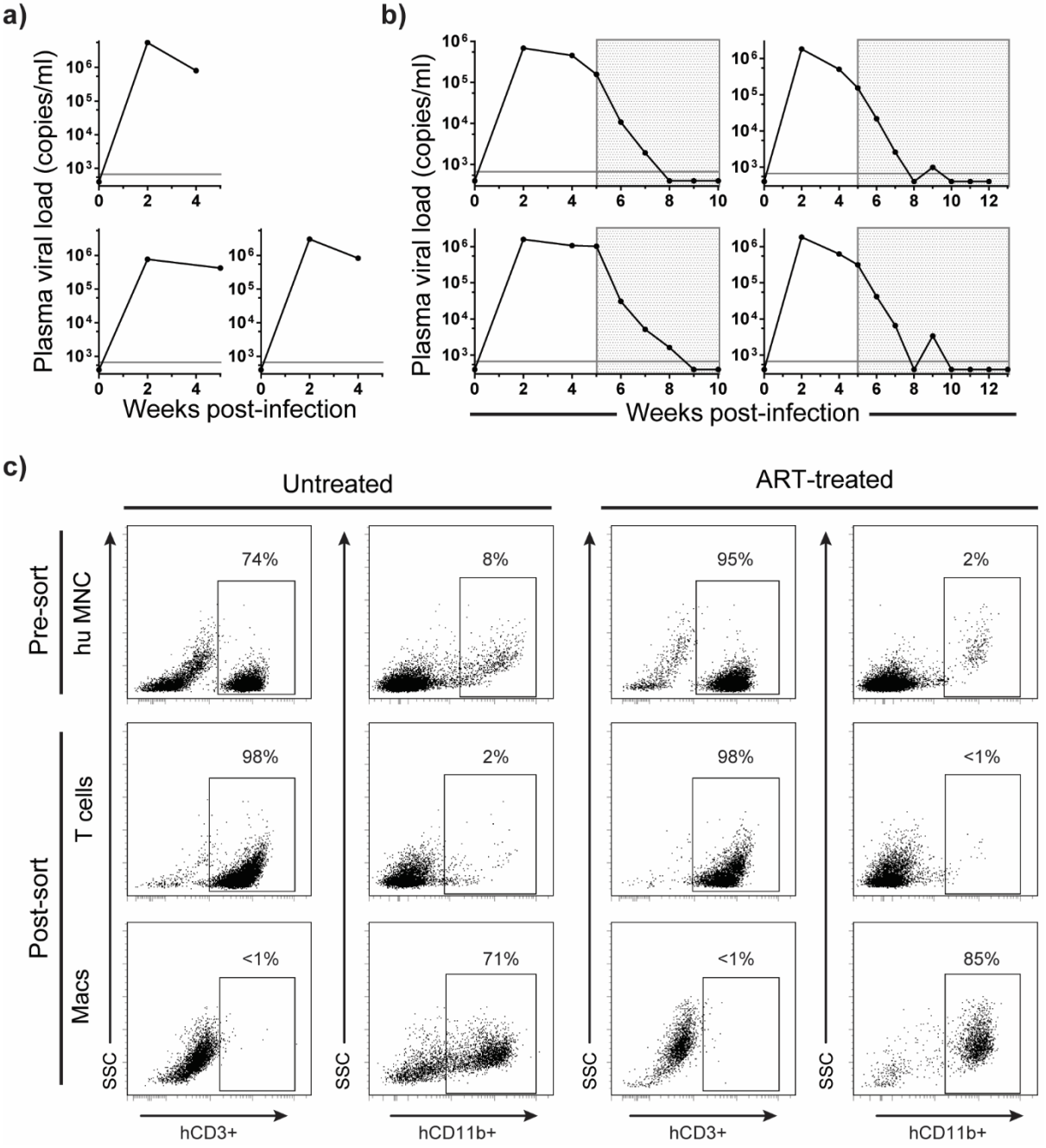
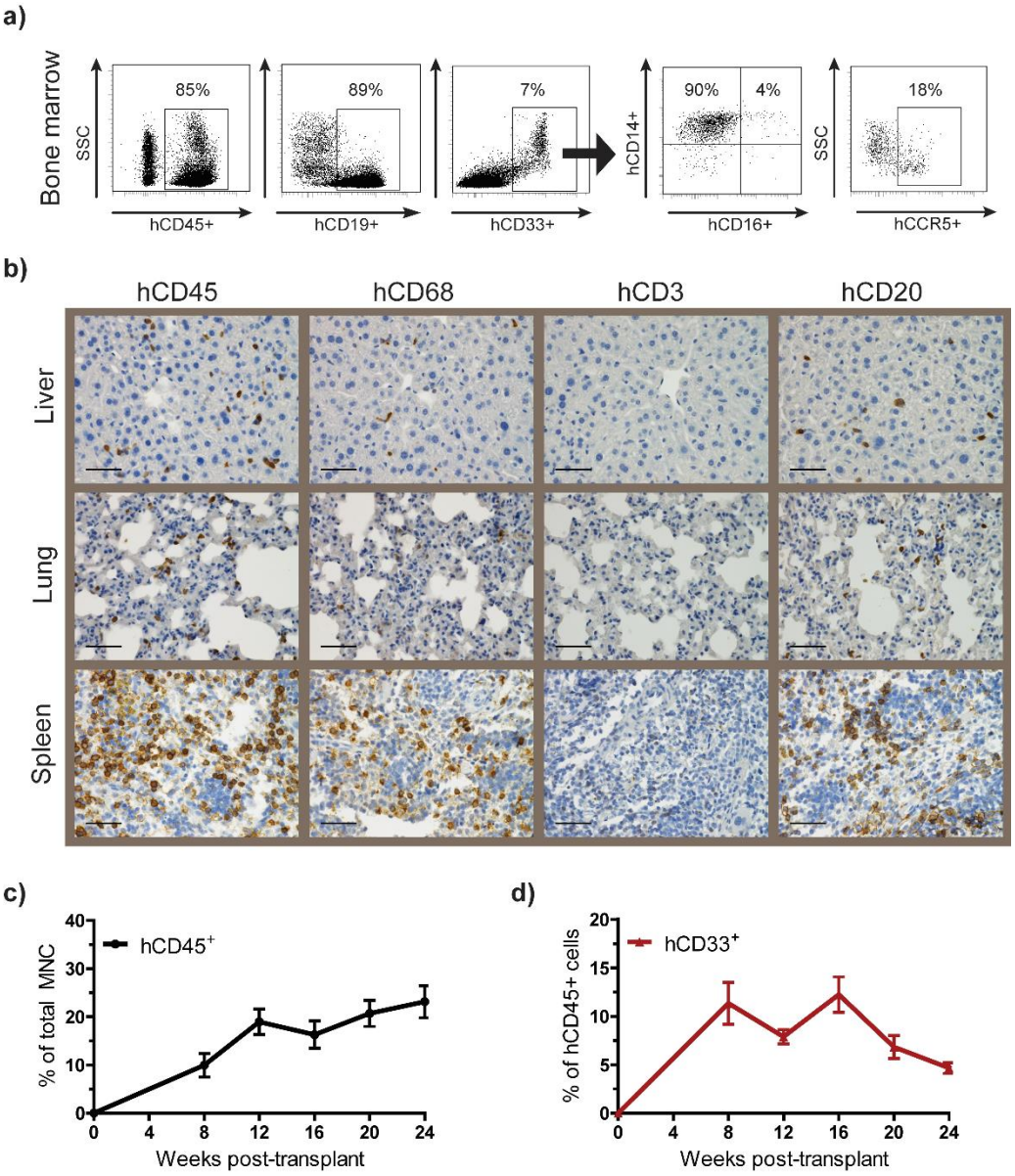
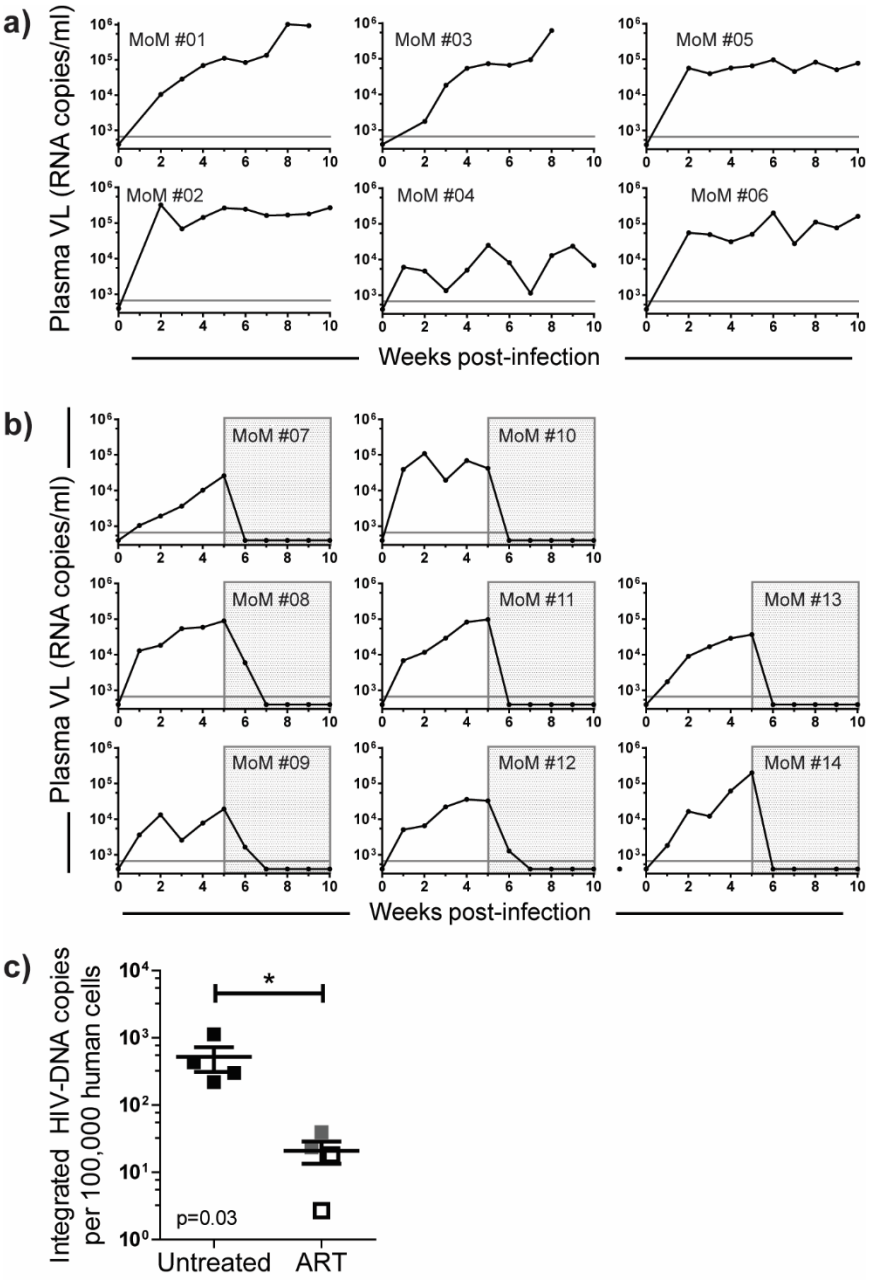
**Supplementary Figures**



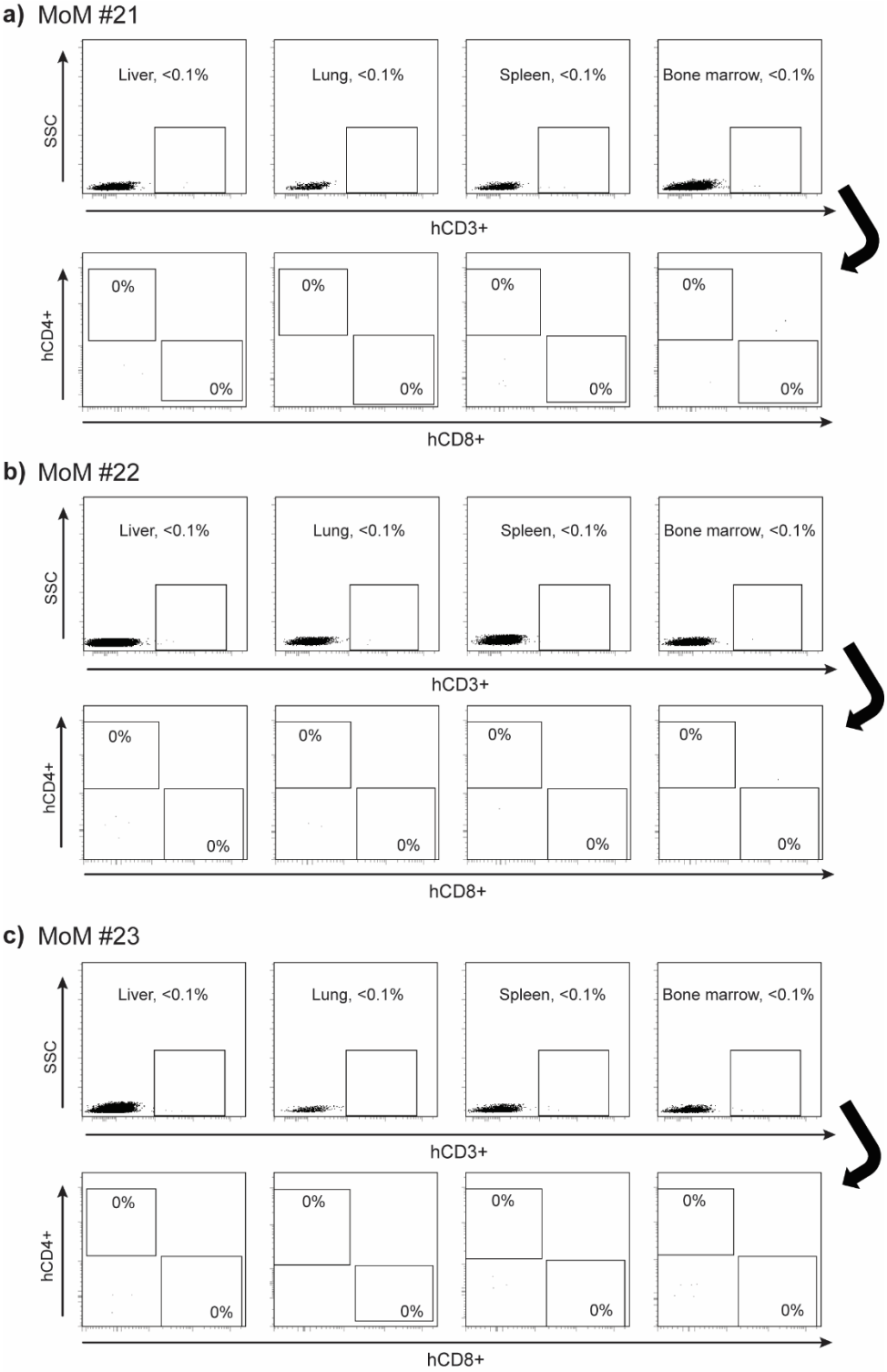

**Supplemental Figure 1**: **Characterization of T cell and macrophage populations purified from the tissues of untreated and ART-treated BLT mice.** The plasma VL was monitored over time in untreated (a, n=3) and ART-treated (b, n=4) BLT mice used for the cell-sorting and subsequent HIV-DNA/RNA analysis in Figure 1e and 1f. c) Representative flow cytometric analysis for the presence of human T cells (CD3+) and macrophages (CD11b+) prior to and after T cell and macrophage isolation from an untreated and an ART-treated BLT mouse. Note that in the T cell fraction there were less than 1% macrophages and in the macrophage fraction there were less than 1% T cells, attesting to the purity of each fraction.



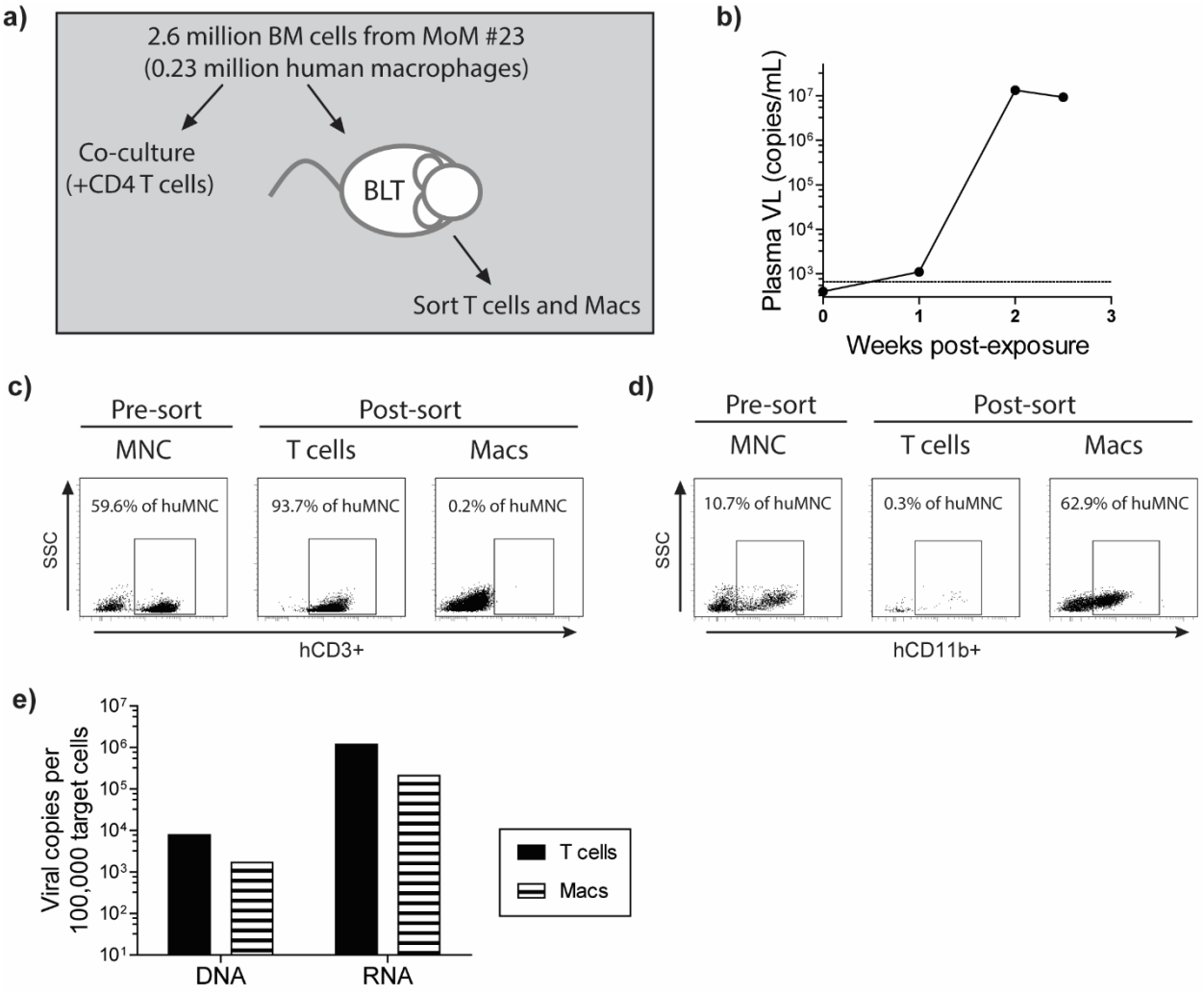

**Supplemental Figure 2: Human myeloid cells are present in the peripheral blood and tissues of MoM.** a) Flow cytometric analysis of cells isolated from the bone marrow of a MoM demonstrates the presence of human hematopoietic cells (hCD45+), human B cells (hCD19+), and human myeloid cells (hCD33+). Human myeloid cells in the bone marrow were further characterized by their expression of hCD14, hCD16 and CCR5. Representative flow plots are shown for a mouse 24 weeks post stem cell transplant. b) Immunohistochemical analysis of the liver, lung, and spleen of a MoM demonstrates the presence of human hematopoietic cells (CD45+), including human macrophages (CD68+) and B cells (CD20+) and the absence of human T cells (CD3+). Scale bar (50 um) is shown in each image. Representative images are shown for a mouse 15 weeks post stem cell transplant. Longitudinal analysis of the peripheral blood of MoM (n=18) demonstrates the presence of human cells (hCD45+) (c) and human myeloid cells (hCD33+) (d).




**Supplemental Figure 3: ART rapidly suppresses HIV levels in HIV-infected MoM.** a) Sustained replication of HIV in MoM as determined by VL analysis over time (n=6). b) ART (indicated by gray box) decreased plasma viremia below the limit of detection in HIV-infected MoM (n=8). c) A portion of samples where HIV-DNA was detectable (Fig. 2d) were analyzed for the presence of integrated HIV-DNA (each group represents two liver, one lung, and one bone marrow sample from either treated or untreated animals shown in Fig. 2d). Undetectable samples are indicated by an empty black box shown at the limit of detection for that sample (dependent on the number of cells available for analysis). Viral DNA levels were normalized per 100,000 human cells and compared between treated and untreated mice (p=0.03). A log-rank test was used to account for censoring due to variation in the limits of detection. Horizontal lines in (c) represent mean ± s.e.m.




**Supplemental Figure 4: T cells are absent from tissues of "Rebound" MoM.** a-c) Flow cytometric analysis of the tissues of MoM where viral rebound was observed following ART-interruption demonstrated a lack of human T cells. Gating scheme: singlets → live cells → hCD45+→lymphocytes. Small number of hCD3+ events lacked expression of hCD4 or hCD8 (shown as second row in panel for each animal).




**Supplemental Figure 5: Replication competent virus is present in "Rebound" MoM.** a) Experimental design for in vivo and in vitro outgrowth evaluation of virus present in "Rebound" MoM #23 bone marrow cells. b) Plasma viremia was monitored over time in exposed BLT mouse from (a). Purity of MNC, T cells and Macs fractions were assessed using flow cytometric analysis of magnetically sorted populations. Percentage of T cells (c) (gating scheme: singlets→live→hCD45+→hCD11b-/hCD19-) and macrophages (d) (gating scheme: singlets→live→hCD45+→hCD3-/hCD19-) was monitored pre- and post-sort. Percentages are reported out of the total human MNC population. e) Cell-associated vDNA and vRNA analysis was performed on the sorted cells from (c-d) and are reported per 100,000 target cells.

**Supplementary Tables**

**Table S1: Characterization of HIV-infected humanized mice used for study.**

| Figure | Hu type | Total (n) | % hCD45 (range) | Sex | Virus(n) | ART treat-ment | Length of ART (wk) | Time to rebound (wk) |
|---|---|---|---|---|---|---|---|---|
| 1a-dashed gray line | BLT | 5 | 60 (37-78) | 4F, 1M | CH040(2) 4013env(3) | N | | |
| 1a-solid black line | | 13 | 49 (34-77) | 8F, 5M | CH040(10) 4013env(3) | Y | 5-10 | |
| 1e/f-circles | | 3 | 42 (32-56) | 3F | CH040(3) | N | | |
| 1e/f-diamonds | | 4 | 58 (34-77) | 4F | CH040(4) | Y | 5-8 | |
| 1g | | 5 | 46 (34-56) | 1F, 4M | CH040(2) 4013env(3) | Y | 5-10 | 1-2 |
| 2a-dashed gray line | MoM | 6 | 26 (10-63) | 3F, 3M | CH040(3) 4013env(3) | N | | |
| 2a-solid black line | | 8 | 23 (16-38) | 7F, 1M | CH040(5) 4013env(3) | Y | 5 | |
| 2d-f, untreated | | 8 | 25 (11-53) | 3F, 5M | CH040(5) 4013env(3) | N | | |
| 2d-f, ART | | 6 | 31 (13-49) | 1F, 5M | CH040(3) 4013env(3) | Y | 1-3 | |
| 3a,c-e No rebound | MoM | 6 | 34 (8-63) | 6F | CH040(6) | Y | 3-5 | not observed |
| 3b-e Rebound | | 3 | 20 (12-26) | 1F, 2M | CH040(2) 4013env(1) | Y | 5 | 7 |

**Table S1:** A summary of the mice used in each figure and subfigure. The average humanization level in the peripheral blood (% hCD45) is shown for each group followed by the range in humanization. For each experimental group, the number and sex of mice (F=female, M=male) and the virus utilized for exposures is indicated. Treatment status (Y=ART-treated, N=untreated), the range of length of treatment and the time to viral rebound post-ART cessation in weeks (wk) is shown for each relevant group.

**Table S2: Characteristics of adoptive transfer recipient mouse.**

| Mouse type | Human % | Sex | Inoculum | vDNA | | | | | | vRNA | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Spln | Liv | Lung | BM | T cells | Macs | Spln | Liv | Lung | BM | T cells | Macs |
| BLT | 84 | F | MoM #23 BM cells | + | + | + | + | + | + | + | + | + | + | + | + |

**Table S2:** HIV-DNA/RNA analysis was performed on cells isolated from BLT mouse exposed to "Rebound" MoM #23 bone marrow (BM) cells 17 days post-exposure. Samples where viral cell-associated DNA or RNA were detected are indicated by a "+". Quantification of the vDNA and vRNA levels in the sorted populations are shown in Supplemental Figure 5.