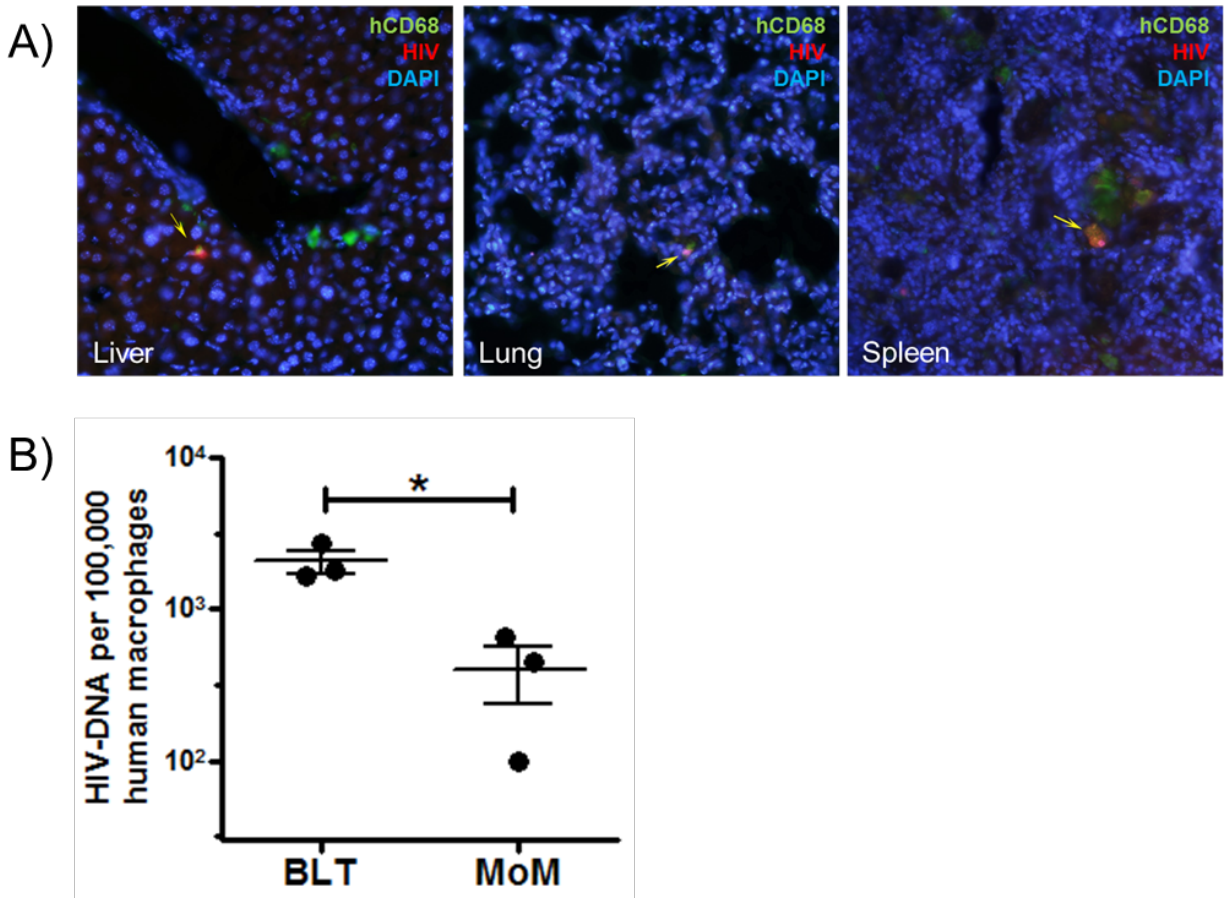
Supplemental Figures



Supplemental Figure 1: Infection of human macrophages in BLT mice. A) In situ hybridization and immunofluorescent analysis of a BLT mouse infected with CH040 4013-env demonstrates the presence of HIV RNA$^+$ (red) in human macrophages (green) in tissue sections from liver, lung and spleen. Cells expressing both hCD68 and HIV RNA are yellow in appearance and are indicated with arrows. Images were taken at 40x magnification. B) HIV-DNA levels were significantly higher in purified human macrophages isolated from BLT mice (n=3) compared to macrophages isolated from MoM (n=3) (p=0.0121). Total mononuclear cells were pooled from the spleen, liver, lung and bone marrow of an individual BLT or MoM infected with HIV-1 CH040. Mouse and human T cells were then depleted using positive magnetic selection. Human macrophages were then further purified using negative magnetic selection essentially as indicated above. Total DNA was then extracted and analyzed for the presence of HIV-DNA using quantitative real time PCR. Bars represent the mean ±SEM and an unpaired t test was used to compare groups of mice.

Supplemental Methods

In situ hybridization analysis of BLT tissues. After de-paraffinizing sections in xylene, and two washes in ethanol, the slides were dried, boiled for 10 minutes in ACD kit Pretreat 2, rinsed twice in water, and treated for 30 minutes at 40°C with Pretreat 3 protease diluted 1:4 in PBS. After rinsing in water twice, the probe is hybridized at 38-40°C for 2hrs, and washed twice in Wash Buffer. Amp 1-FL, Amp2-FL, Amp 3-FL and Amp 4-FL hybridizations to the oligonucleotides in the kit were done at 38-40°C for the times listed in manufacturer's instructions. After blocking with donkey block for 1 hr, slides were incubated overnight with a 1:40 dilution of Invitrogen (Fisher) mouse anti human CD68 clone KP-1 at 4C. After washing in TBS Tween twice, slides were incubated with 1:300 dilution of donkey anti-mouse Alexa 488 (Thermo Fisher). After washing twice in TBS Tween, slides were treated with 1% Sudan black for 5 minutes to reduce auto-fluorescence and stained with ACD (kit) Dapi and coverslipped with Prolong Gold Mount

Tissue macrophage isolation. Positive magnetic selection for mouse cells (StemCell, EasySep Mouse/Human Chimera Isolation Kit, Cat. 19849) and human $CD3^+$ T cells (Miltenyi, CD3 MicroBeads, Cat. 130-050-101) was performed to remove these cells from the total MNC pool. Next, negative magnetic selection for monocytes (Miltenyi, Pan Monocyte Isolation Kit, Cat. 130-096-537) was performed on the human non-CD3 cell population to yield a >90% pure macrophage population.

Analysis of HIV-1 infection. HIV-DNA analysis of tissue macrophages isolated from BLT mice or MoM was performed using a one-step reverse transcriptase real-time PCR assay (ABI custom, TaqMan Assays-by-design) according to the manufacturer's instructions (with primers 5'-CATGTTTTCAGCATTATCAGAAGGA-3' and 5'-TGCTTGATGTCCCCCCACT-3'; assay sensitivity of ~4.5 DNA copies). Genomic DNA from MNCs (1×10^5–2×10^6) from animal tissues was prepared using QIAamp DNA blood mini columns (Qiagen) according to the manufacturer's protocol.

Statistics. All data was graphed and analyzed using GraphPad Prism (version 5.04). Data are represented as mean ±SEM. A comparison of the HIV-DNA copies per 100,000 human macrophages was performed using an unpaired t test. A p-value ≤0.05 was considered statistically significant.