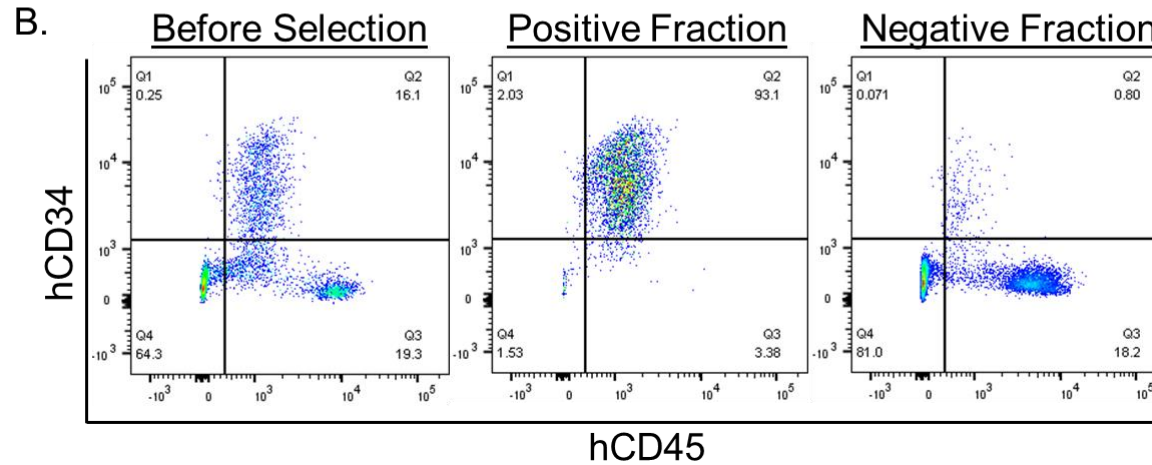
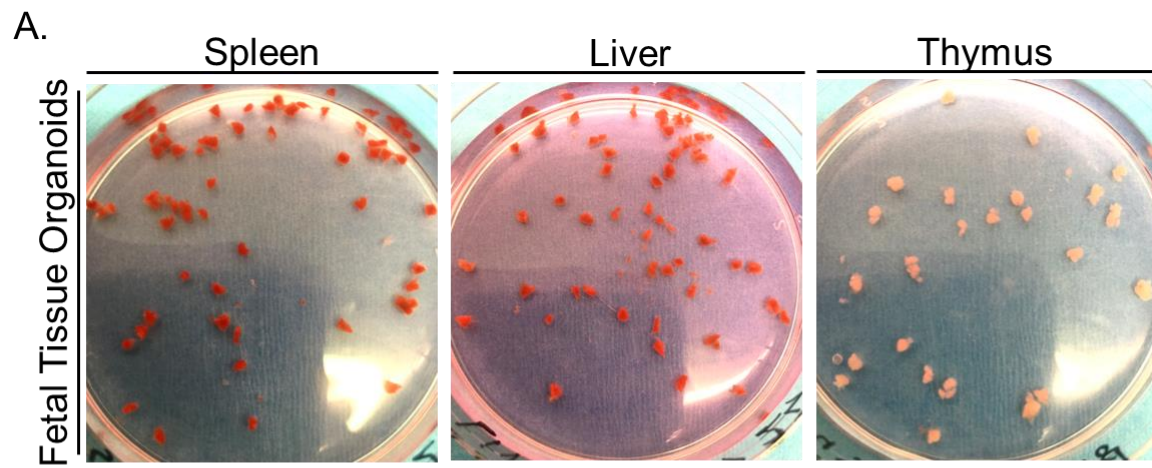
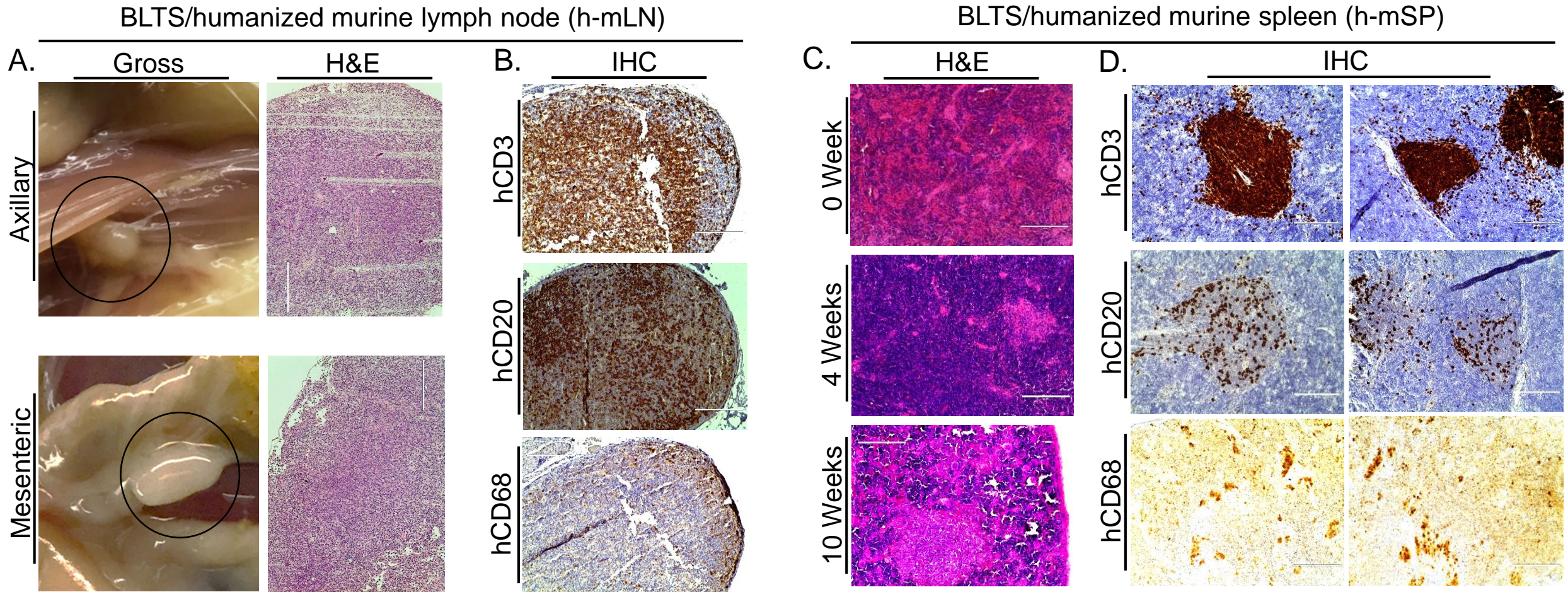


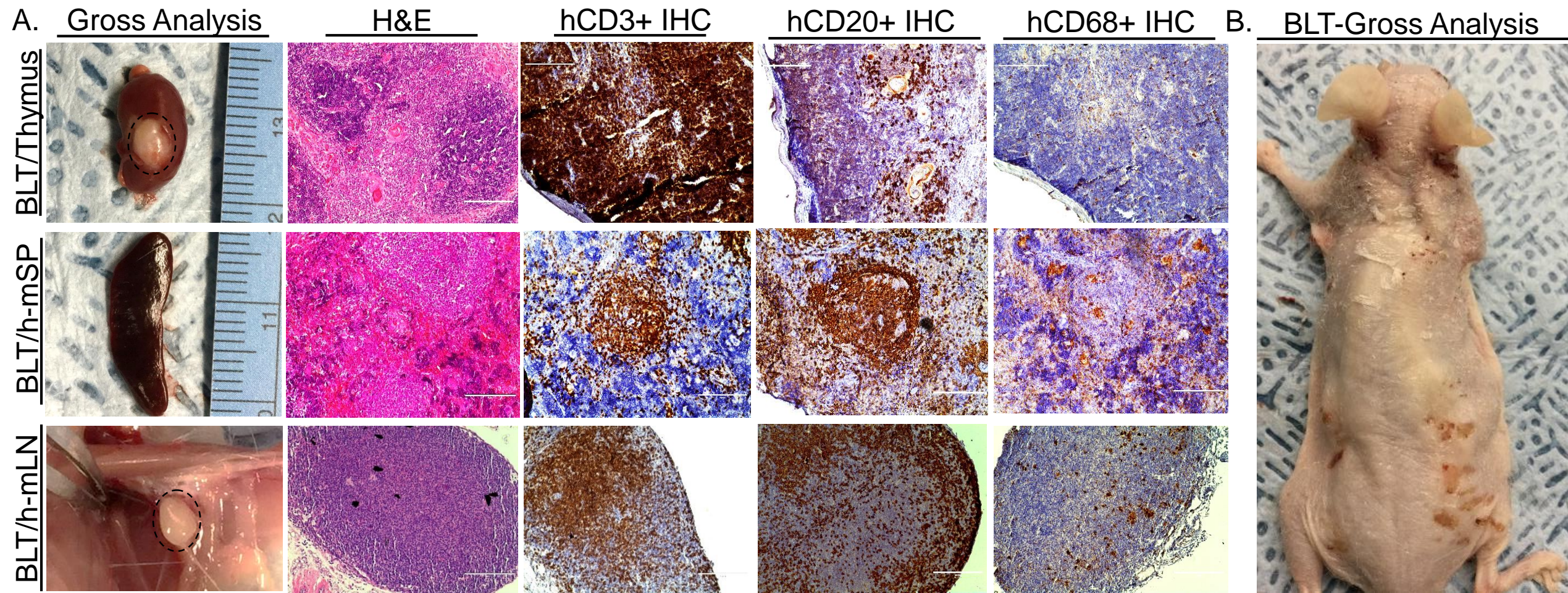
Supplementary Figure 1. Comparative analysis of the microanatomy of human and mouse lymphoid tissues. Human and BALB/c mouse lymphoid tissues were formalin-fixed and paraffin embedded, and slides were stained with hematoxylin and eosin stain. Representative tissue sections are shown from 4 human tissue slides and 4 BALB/c mice. Scale bars: 200 μ m.



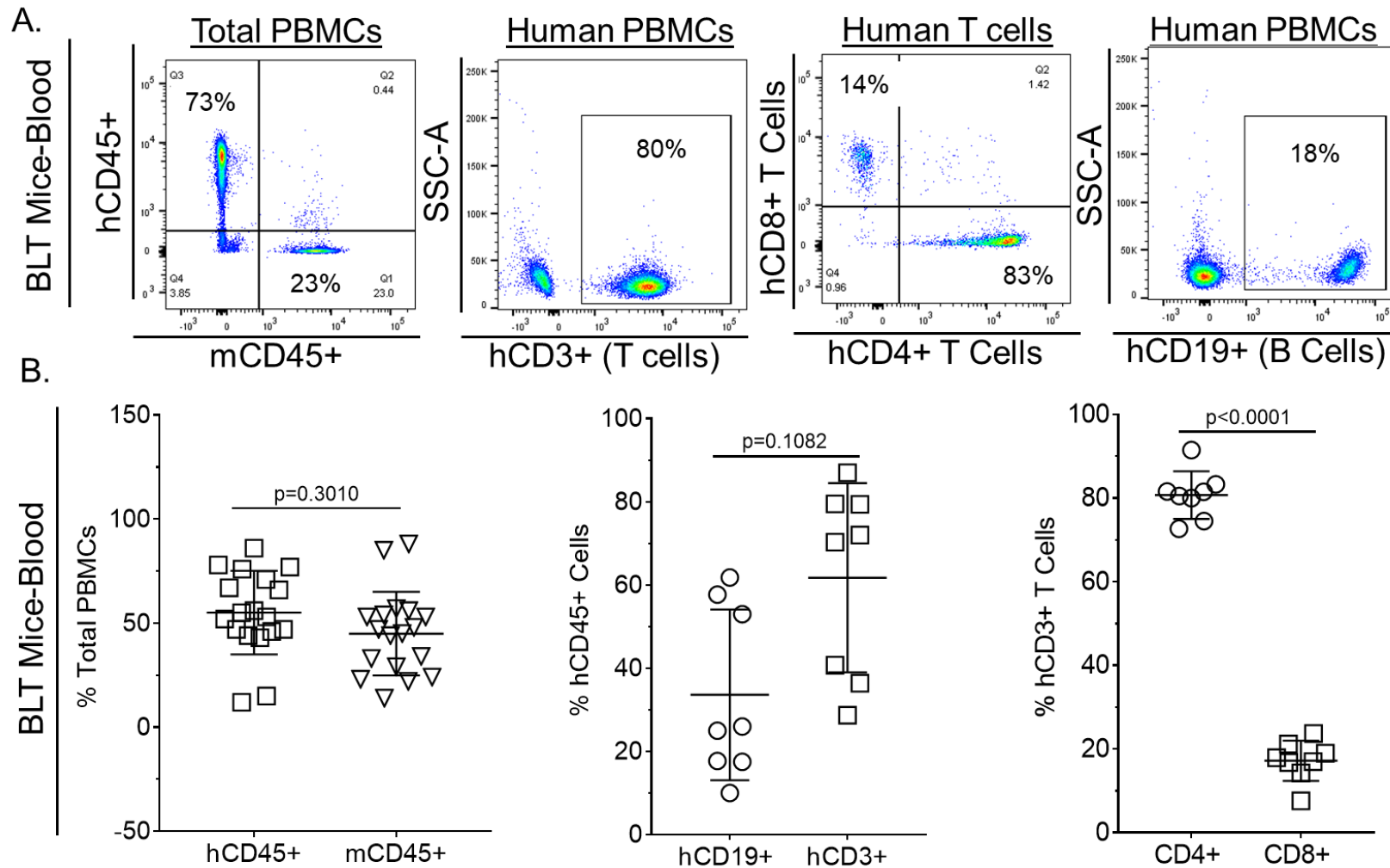
Supplementary Figure 2. Processing of human lymphoid organoids and hematopoietic cells for constructing BLTS-humanized mice. (A) Human fetal organs (spleen, liver and thymus) are processed into 1-1.5 mm² pieces; representative pieces from a portion of respective organs are shown. (B) Human hematopoietic stem cells along with leukocytes (Before Selection) were isolated from the fetal liver and the hematopoietic stem cell fraction (Positive Fraction) was enriched using enriched via immuno-magnetic selection based on human CD34 expression; the leukocyte fraction (Negative Fraction) also confirms enrichment of the hematopoietic stem cell fraction. A representative flow cytometry analysis of human CD34⁺ hematopoietic stem cells enrichment is shown. Data presented are representative of 4 human fetal organs and human CD34⁺ hematopoietic stem cells isolation.



Supplementary Figure 3. Human immune cells development in humanized murine lymphoid tissues in the BLTS-humanized mouse model. (A) Representative gross and histological (hematoxylin and eosin stain – H&E) analysis of humanized murine axillary and mesenteric lymph nodes (h-mLN) in BLTS-humanized mice (n=4 per group) at 16 weeks post-transplantation. (B) Representative human-specific immunohistochemical (IHC; Brown stain - T cells-hCD3+, B Cells-hCD20+, or macrophages-hCD68+) analysis of humanized murine axillary lymph nodes in BLTS-humanized mice (n=4 per group) at 16 weeks post-transplantation. Scale bars: 200 μ m. (C) Representative histological (hematoxylin and eosin stain – H&E) analysis of humanized murine spleen (h-mSP) in BLTS-humanized mice (n=4 per group) at indicated time points post-transplantation. (D) Representative human-specific immunohistochemical (IHC; Brown stain - T cells-hCD3+, B Cells-hCD20+, or macrophages-hCD68+) analysis of humanized murine spleen (h-mSP) in BLTS-humanized mice (n=4 per group) at 10 weeks post-transplantation. Scale bars: 200 μ m.

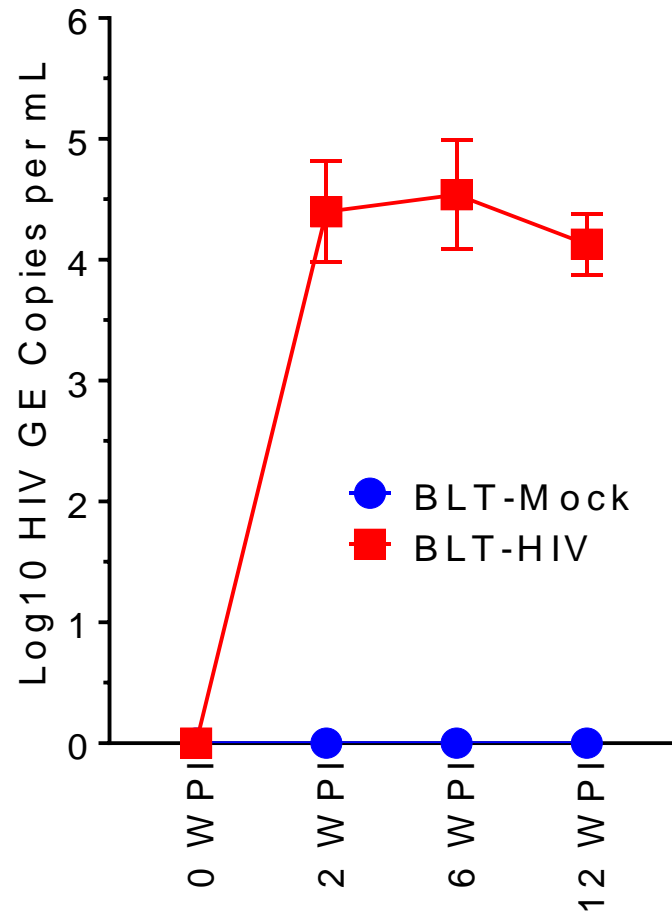


Supplementary Figure 4. Human immune cells development in lymphoid tissues in the BLT-humanized mouse model. (A) Representative gross and histological (hematoxylin and eosin stain – H&E) analysis of human thymus organoid (Thymus), humanized murine spleen (h-mSP), humanized murine lymph node (h-mLN) in BLT-humanized mice (n=3) at 16 weeks post-transplantation; black circles identifies tissues of interest. Representative human-specific immunohistochemical (IHC; Brown stain - T cells-hCD3+, B Cells-hCD20+, or macrophages-hCD68+) analysis of human thymus organoid (Thymus), humanized murine spleen (h-mSP), humanized murine lymph nodes (h-mLN) in BLT-humanized mice (n=3) at 16 weeks post-transplantation. Scale bars: 200 μ m. (B) A representative clinical manifestation (excessive hair loss and wasting syndrome) of GVHD at 24 weeks post-transplantation in the BLT-humanized mouse model (n=4).



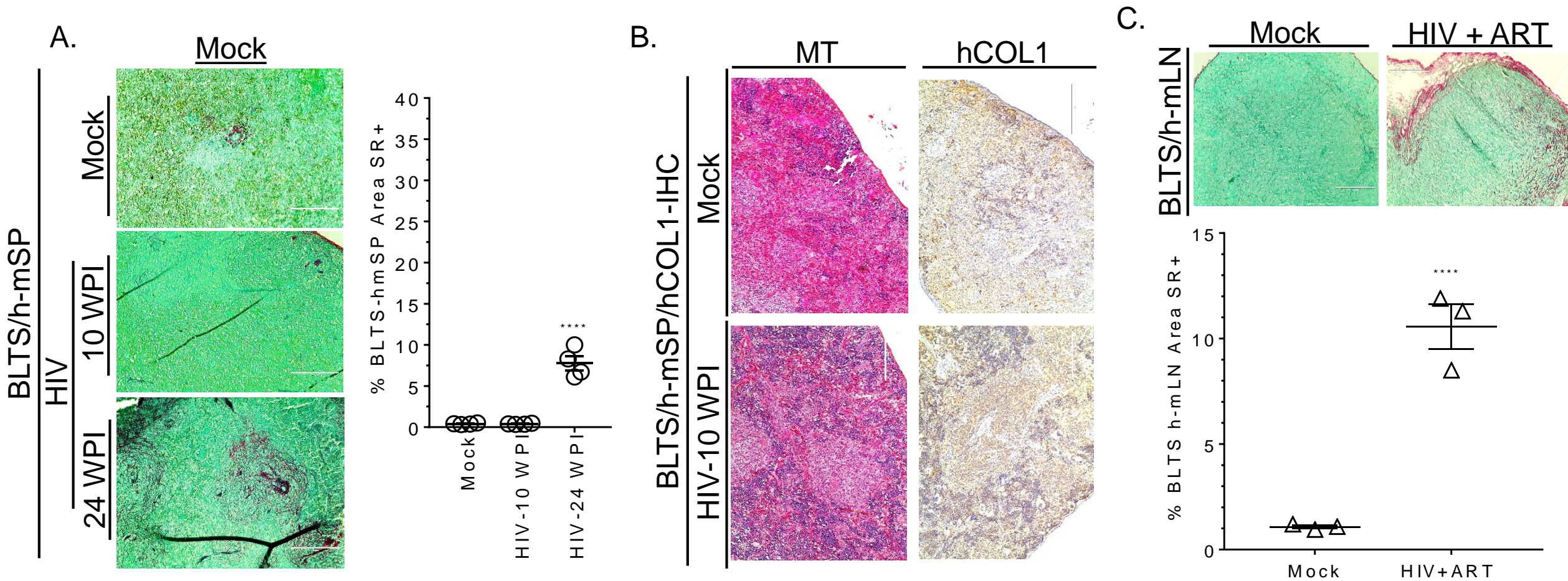
Supplementary Figure 5. Human immune cells development in the peripheral blood in BLT-humanized mice.

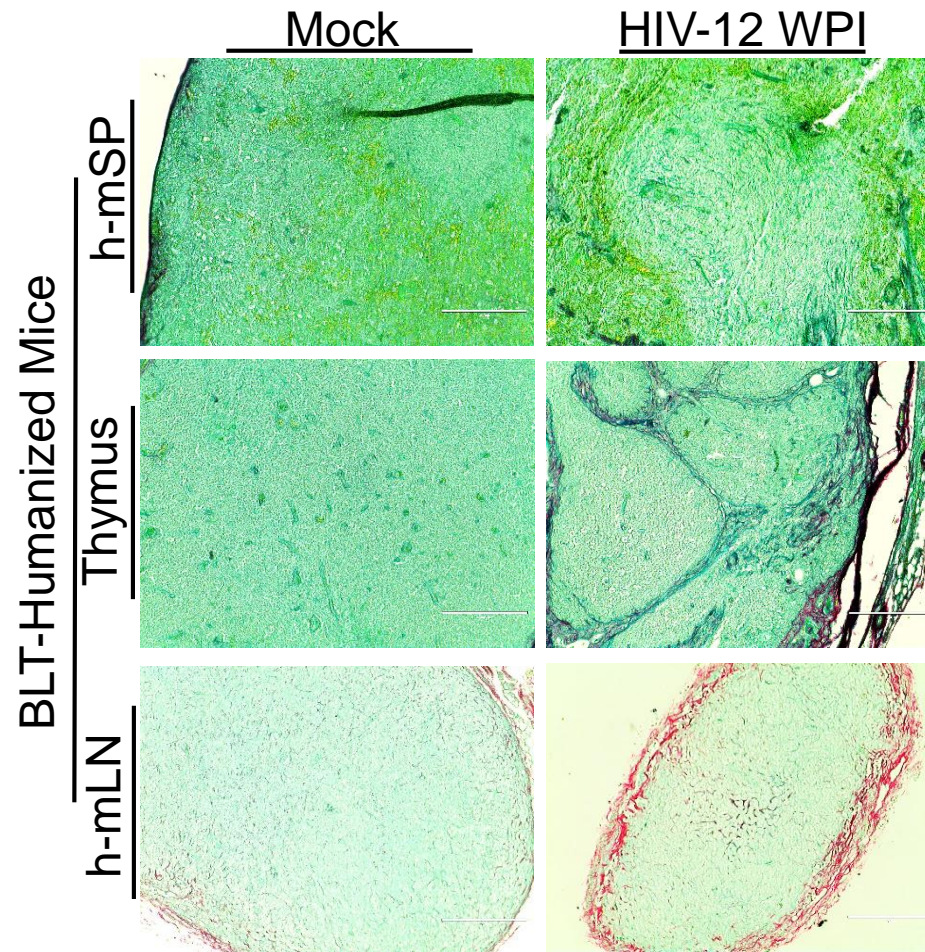
(A) Representative flow cytometry analysis of human immune cell (hCD45+) reconstitution, along with lymphocytes subsets, including T cells (CD3+) and CD4+ and CD8+ T cells subsets and B cells (CD19+) in peripheral blood mononuclear cells (PBMCs) of BLT-humanized mice at 10 weeks post-transplantation. (B) Quantification of human immune cells reconstitution (n=18; 2 independent experiments) and lymphocyte subsets (n=8) in peripheral blood mononuclear cells (PBMCs) of BLT-humanized mice at 10 weeks post-transplantation. Data is presented as mean +/- standard deviation. P values were determined using paired, 2-tailed Student's t test between 2 groups.



Supplementary Figure 6. Analysis of HIV replication kinetics in BLT-humanized mice.

The kinetics of HIV-1 replication (HIV RNA genome copies per mL-HIV GE copies per mL) in the blood (n=4 per group) following inoculation at 1×10^5 IU per mouse in BLT-humanized mice was analyzed using qPCR, with mock as the control group. Data is presented as mean values \pm standard error of mean.





Supplementary Figure 8. Analysis of lymphoid tissue fibrosis in HIV-infected BLT-humanized mice. Chronic HIV infection in BLT-humanized mice was allowed to develop over a period of 12 weeks, at which point human thymus organoid, humanized murine spleen, and humanized murine lymph nodes were analyzed for collagen deposition using Sirius red-fast green stain (red-collagen positive and green-collagen negative), aged-matched mice served as controls. Shown are representative data (n = 4 per group). Scale bars: 200 μ m.