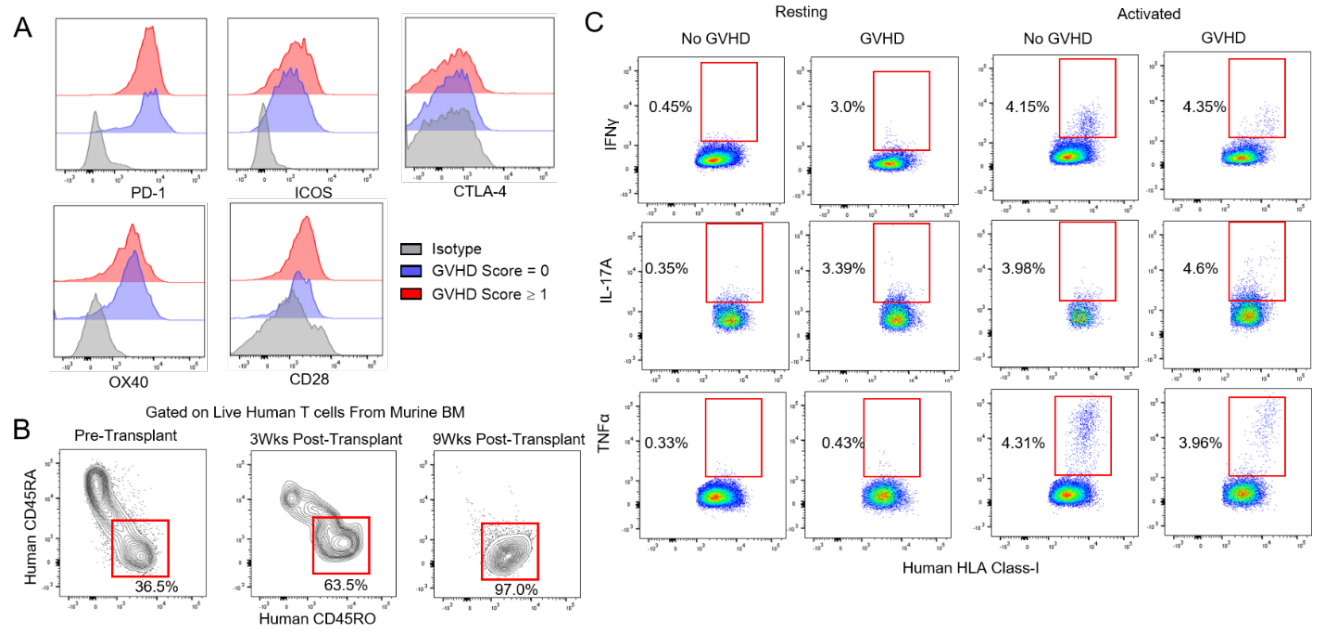


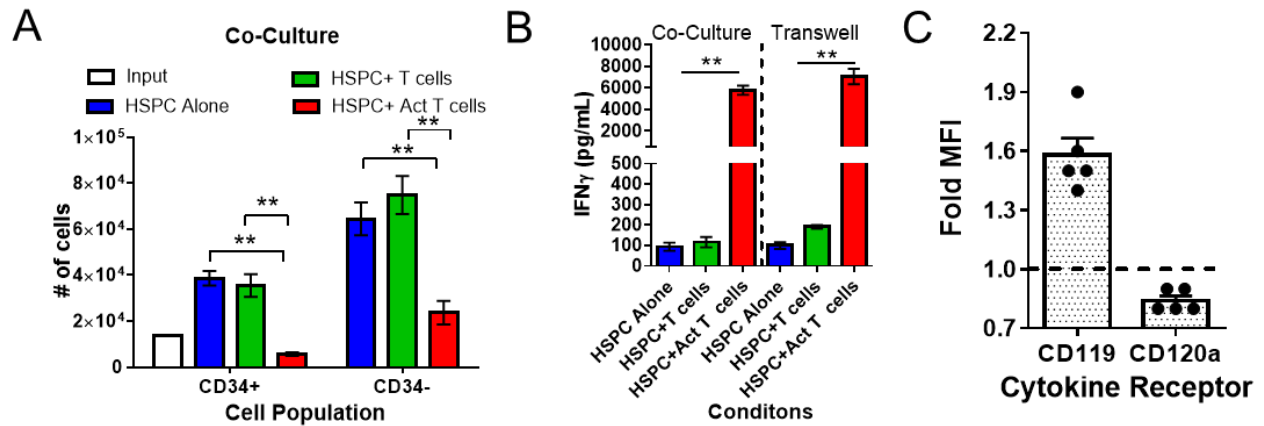
Supplementary Figure 1. Predictive T cell activation metrics do not require graft-APCs.

(A-B) Nonconditioned NBSGW given isolated BM-T cells were separated based on their final GVHD score and quantified for T cell burden in $\sim 150\mu\text{L}$ of blood (time points indicated) (A), murine bone marrow (tibia+femur) (B) and spleen (B). (C-D) Mice were separated as before and analyzed for percentage of blasting T cells in the blood (C) and murine BM and spleen (D). Mice were similarly analyzed for the expression of CD45RO (E). A minimum of 10 T cells events were required to be included in these analyses (C-E).



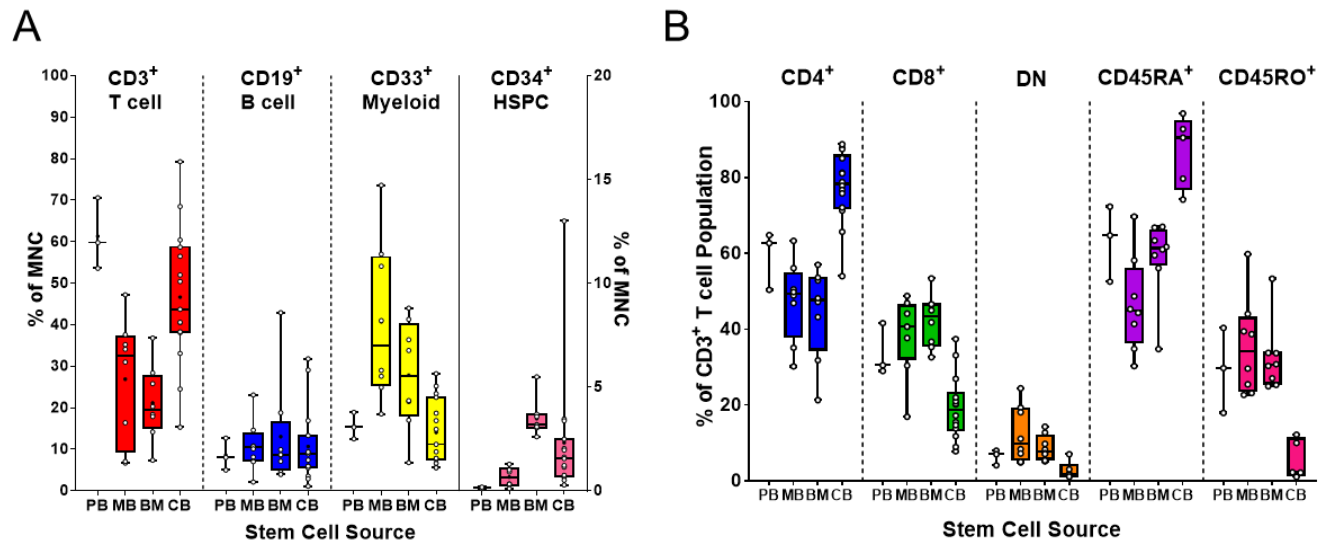
Supplementary Figure 2. Analysis of T cell activation markers and cytokine production.

(A) Representative histograms from splenic human T cells of a non-GVHD and GVHD mouse analyzed for the indicated activation markers. An isotype matched control was run for both non-GVHD and GVHD mice though only one is shown for clarity. (B) Representative flow plots of CD45RA⁺ and CD45RO⁺ T cells in the murine BM at three distinct time points. (C) Representative flow plots of IFN γ , IL-17A and TNF α expression after 6 hours of brefeldin A or PHA/ionomycin treatment.



Supplementary Figure 3. Activated T cell but not resting T cell inhibit BM-HSPC

proliferation. (A-B) Isolated CD34⁺ BM-HSPCs were cultured for 7 days in the presence or absence of autologous T cells in medium containing a hematopoietic expansion cocktail. For “Act T cells”, T cell were activated with α CD3 and α CD28 antibodies. (A) Quantification of total HSPC-derived cell number that either maintained for lost the expression of CD34. Bars represent the mean and SEM of three replicate wells from one of two independent experiments. (B) Supernatants from both co-culture (A) and transwell (Fig 5F) experiments were analyzed for IFN γ production by ELISA. (C) CD34⁺ BM-HSPCs were stained for the expression of IFN γ R1 (CD119) and TNFR1 (CD120a). Median fluorescent intensity (MFI) of each marker was divided by the MFI of an isotype control (Fold MFI). * p < 0.05; ** p < 0.01



Supplementary Figure 4. Cellular frequencies among human clinical graft tissue. (A) The cellular composition of graft tissues mononuclear cell fractions were analyzed for the indicated cell types based on the marker shown. (B) The CD3⁺ T cell fractions were further characterized. All cells were first gated as CD45⁺ and HLA-Class I⁺ prior to gating on cellular markers. Each dot represents one individual donor.