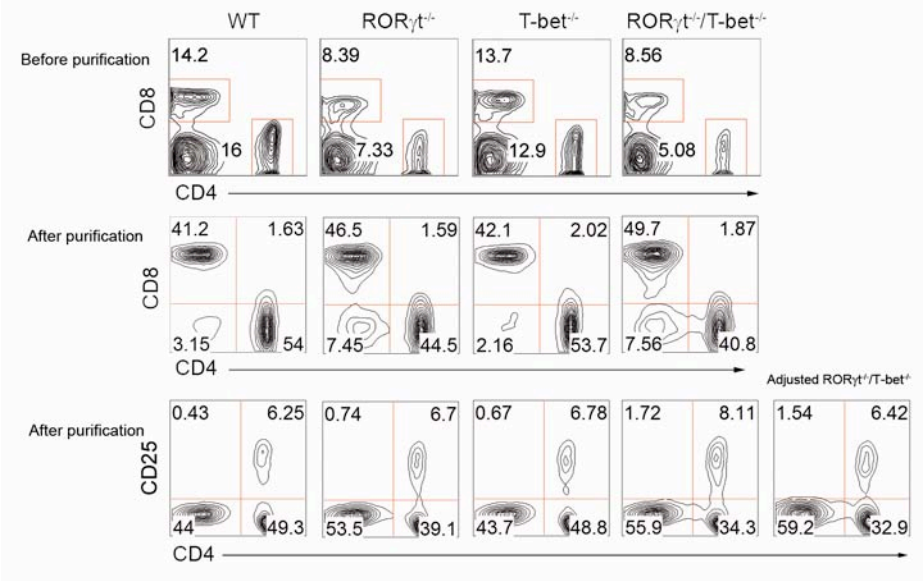

Figure S1. Phenotype and composition of T cells used for BMT from WT, T-bet$^{-/-}$, RORγt$^{-/-}$ or RORγt$^{-/-}$/T-bet$^{-/-}$ mice. Spleens were harvested from WT, T-bet$^{-/-}$, RORγt$^{-/-}$ or RORγt$^{-/-}$/T-bet$^{-/-}$ age- and sex-matched mice. CD4 and CD8 expression before (upper panel) and after (middle panel) T cell purification and CD4 versus CD25 expression (lower panel) are shown in gated live cells. The adjusted RORγt$^{-/-}$/T-bet$^{-/-}$ T cells were the cells used form BMT and it is the mixture of purified non-Tregs CD4^{+}CD25^{-} T cells and the purified RORγt$^{-/-}$/T-bet$^{-/-}$ T cells to obtain the same ratio of Tregs as the other three strains. The data shown are from pooled spleens from each strain and represent the phenotypes in all the experiments presented in current report.

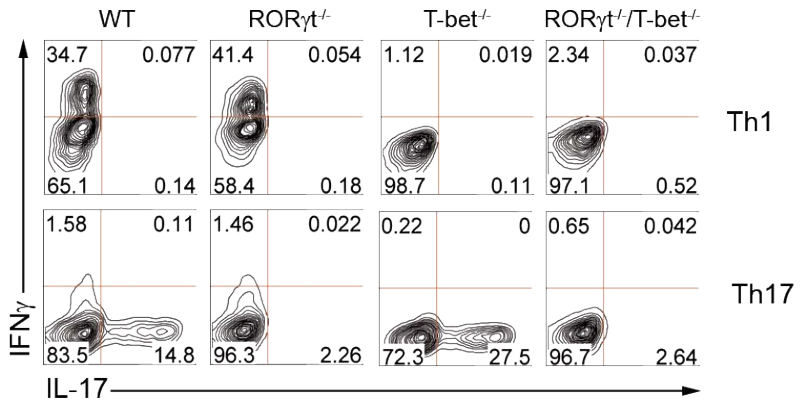

Figure S2. In vitro differentiation of WT, T-bet$^{-/-}$, RORγt$^{-/-}$ or RORγt$^{-/-}$/T-bet$^{-/-}$ CD4^{+} T cells. Th1 and Th17 cells were generated from WT, T-bet$^{-/-}$, RORγt$^{-/-}$ or T-bet$^{-/-}$/RORγt$^{-/-}$ CD4^{+} T cells *in vitro* as described in "*Materials and Methods*". Expression levels of intracellular IFN-γ and IL-17 on live CD4^{+} cells from WT, T-bet$^{-/-}$, RORγt$^{-/-}$ or RORγt$^{-/-}$/ T-bet$^{-/-}$ mice are shown for Th1 (upper panel) and Th17 cells (lower panel) 4 days after polarization. One mouse each strain was used, and the experiment was repeated 2 times.

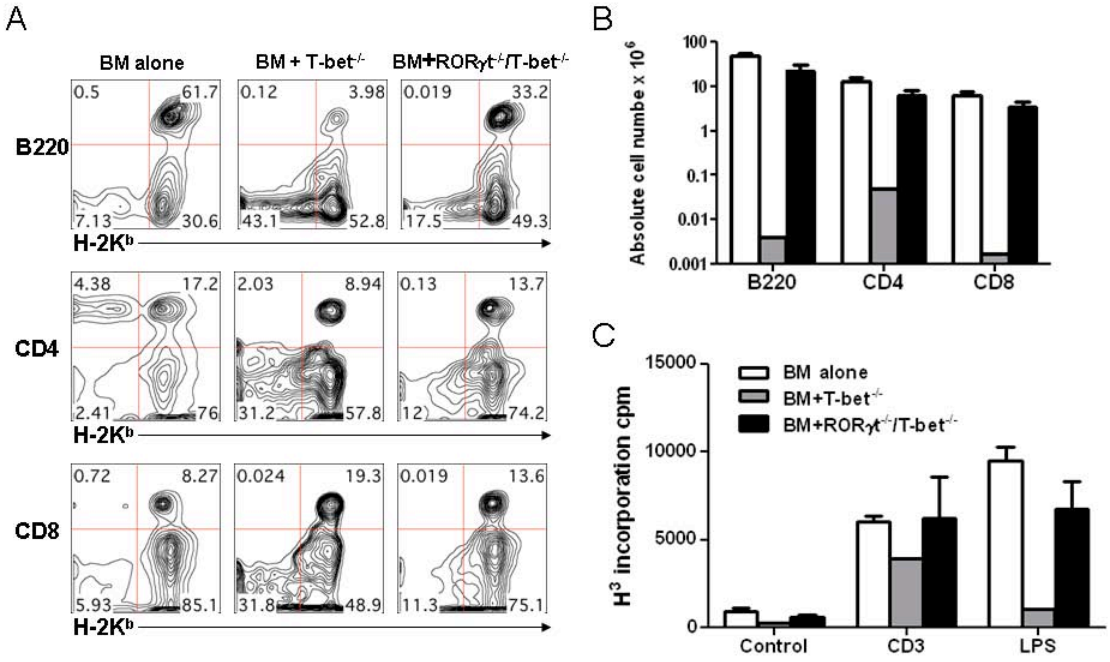

Figure S3. Donor-derived B- and T-cell reconstitution after BMT. Lethally irradiated BALB/c mice (n = 10 per group) were transplanted with 5 x 10^6 TCD-BM alone or with 2 x 10^6 purified T cells from WT, T-bet$^{-/-}$, RORγt$^{-/-}$ or RORγt$^{-/-}$/T-bet$^{-/-}$ mice. Upon completion of the experiment on day 80, phenotypes of the spleen cells from survived recipients were analyzed. (A) FACS plots show B-cell (B220) and T-cell (CD4 and CD8) reconstitution from representative recipients transplanted with BM alone, BM plus T-bet$^{-/-}$, or BM plus RORγt$^{-/-}$/T-bet$^{-/-}$ T cells. (B) Absolute numbers of donor-derived T and B cells (H2K^{b+}) in survived recipients with donor graft indicated. (C) Recipient splenocytes were stimulated with anti-CD3 or LPS to measure T- and B-cell function using ^{3}H-thymidine incorporation assay. Four mice in each group except one in T-bet$^{-/-}$ group were shown, and the experiment was repeated 2 times.

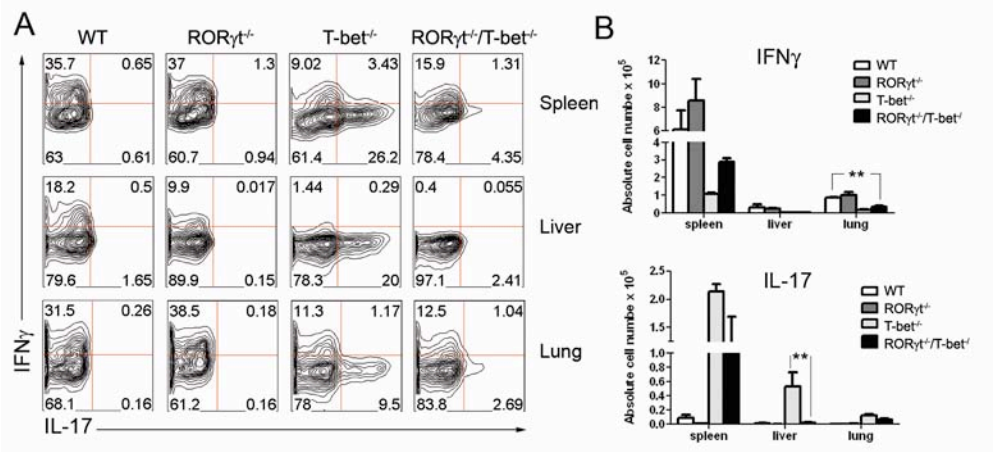

Figure S4. Blockade of T-bet and RORγt signaling results in a significant reduction in Tc1 and Tc17 cells. Lethally irradiated (800 cGy) BALB/c mice were transplanted with purified B6 WT, T-bet$^{-/-}$, RORγt$^{-/-}$ or RORγt$^{-/-}$/T-bet$^{-/-}$ T cells. Recipient mice were humanely killed 5 days after transplantation. (**A**) Representative plot depicts the percentage of IL-17– and/or IFN-γ–secreting cells in recipient spleen, liver and lung in gated H-2K^{b+}CD8^{+} donor T cells. (**B**) Absolute numbers of IFN-γ- and/or IL-17–secreting cells from spleen, liver and lung in the gated H-2K^{b+} CD8^{+} donor T cells. Data shows one representative mouse of 3 mice per group and one of 3 replicate experiments. **P < 0.01.

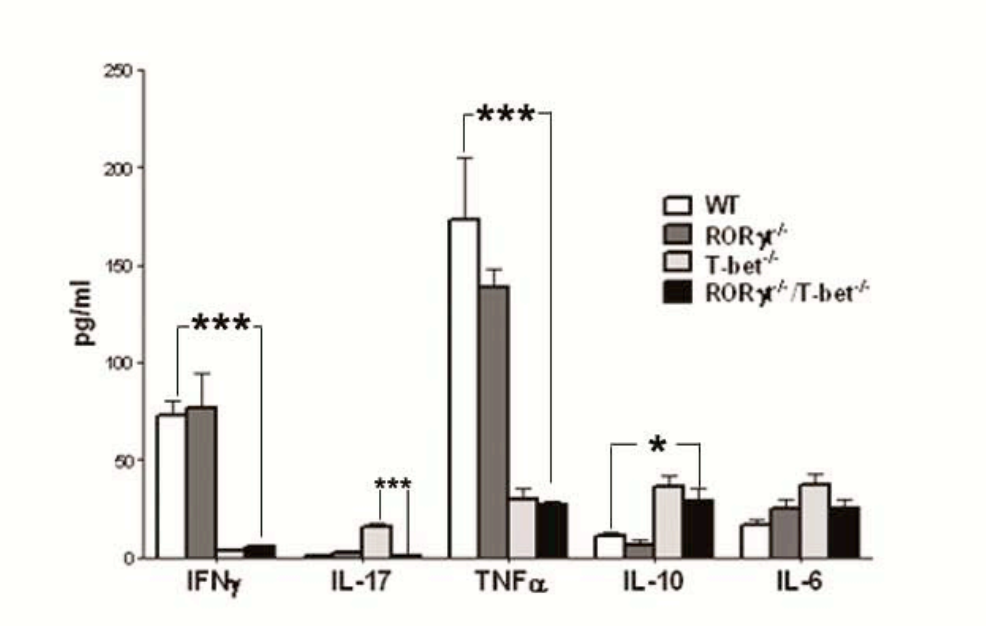

Figure S5. Serum cytokine profile in recipients that are transplanted with WT T-bet$^{-/-}$, RORγt$^{-/-}$ or RORγt$^{-/-}$/T-bet$^{-/-}$ donor T cells. Lethally irradiated BALB/c mice were transplanted with TCD-BM plus 2 x 10^6 purified B6 WT, T-bet$^{-/-}$, RORγt$^{-/-}$ or RORγt$^{-/-}$/ T-bet$^{-/-}$ CD4$^+$ and CD8$^+$ donor T cells. Indicated cytokines were measured in recipient serum on day 14 after BMT as described in *"Materials and Methods."* The data are presented as means ± 1SD of 5 to 6 mice per group, and represent 1 of 3 replicate experiments. Asterisk indicates statistical significance; *P < 0.05 and ***P < 0.001.

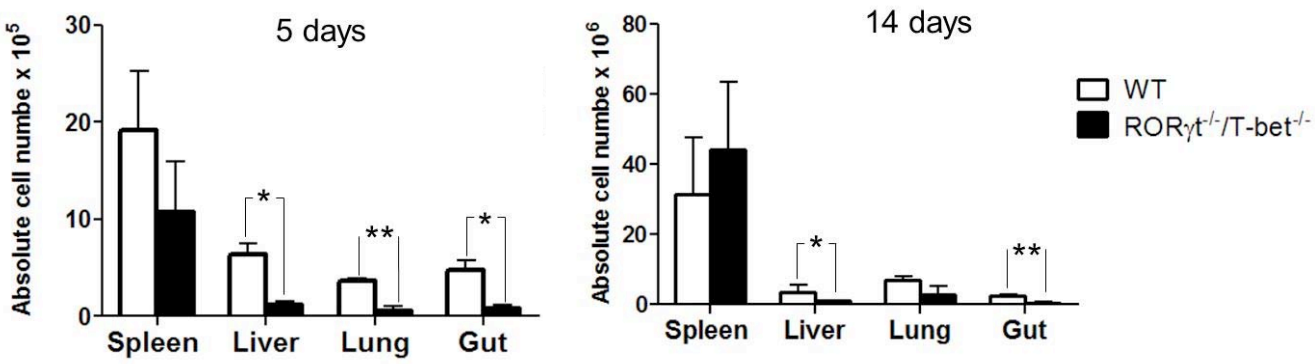

Figure S6. Effects of RORγt/T-bet on donor T-cell infiltration in GVHD target organs. Lethally irradiated BALB/c mice were transplanted with 3 x 10^6 purified B6 WT or RORγt$^{-/-}$/T-bet$^{-/-}$ T cells in the absence of TCD-BM (day 5) or with 2 x 10^6 purified B6 WT or RORγt$^{-/-}$/T-bet$^{-/-}$ T cells in the presence of TCD-BM (day 14). Mononuclear cells were isolated from recipient spleen, liver, lung and gut, and subjected for FACS analysis. The absolute number of donor cells (H2K^{b+}) in each organ is shown as average ± 1SD from 3-4 mice per group.