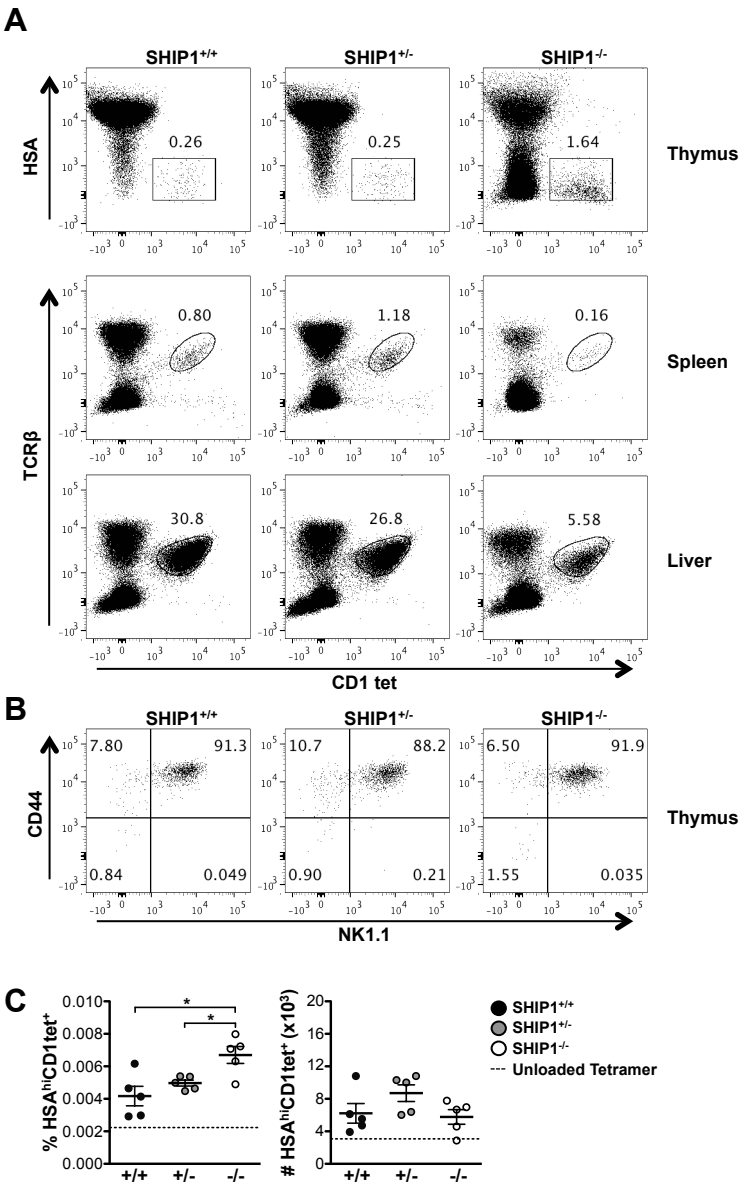
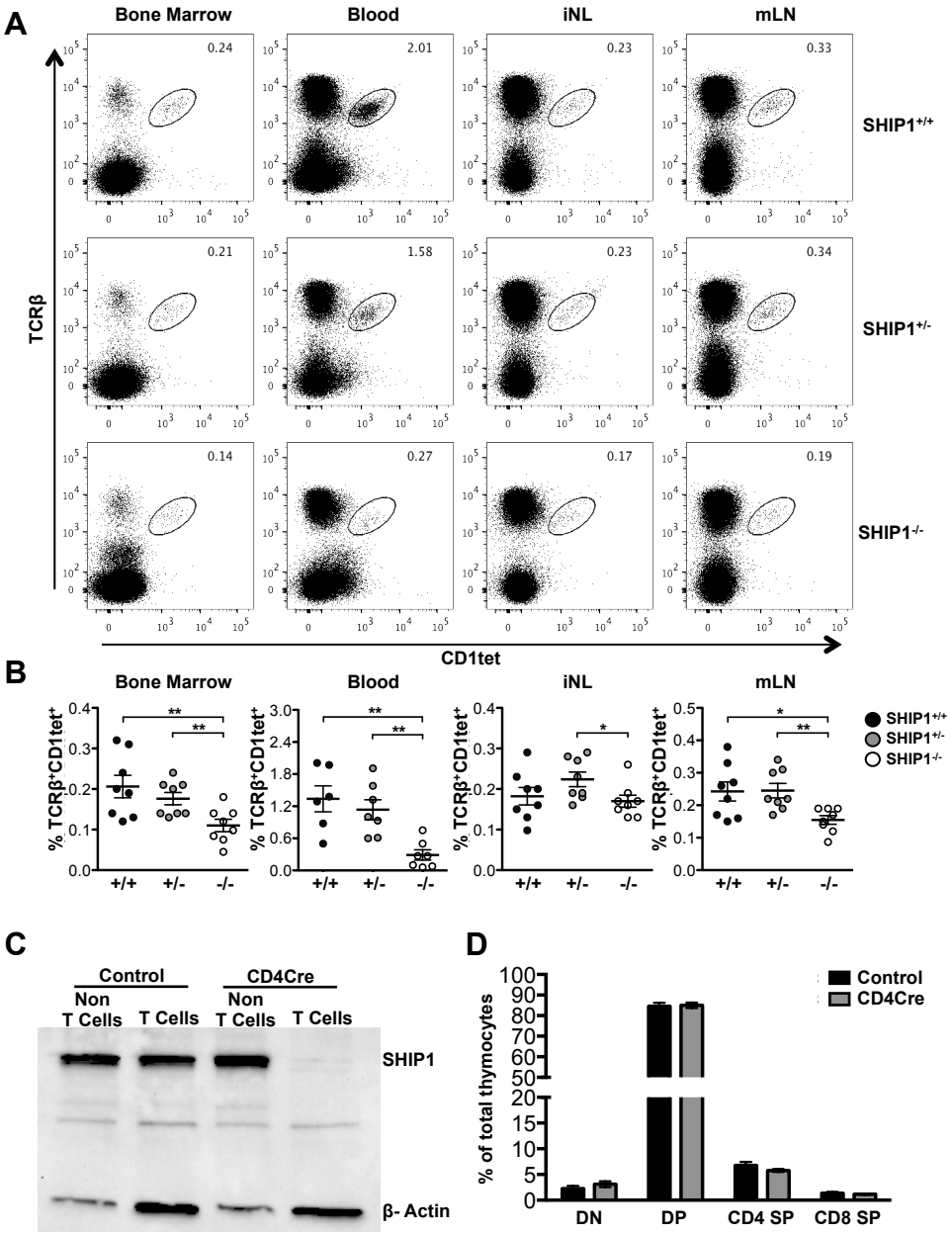
Sup. Fig. 1



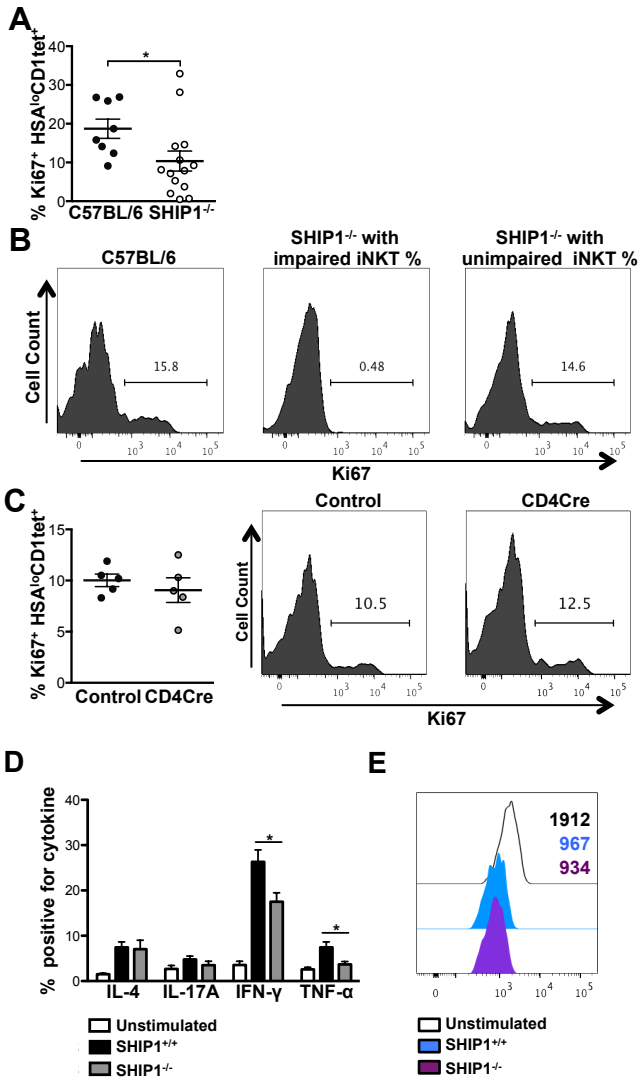
Supplementary Figure 1. Loss of thymic and peripheral iNKT cells in SHIP1$^{-/-}$ mice. (A) Representative staining of iNKT cell populations in the thymus, spleen, and liver from SHIP1$^{+/+}$, SHIP1$^{+/-}$, and SHIP1$^{-/-}$ mice. (B) Representative staining of thymic iNKT cell maturation: CD44^{lo}NK1.1^{-} (Stage I), CD44^{hi}NK1.1^{-} (Stage II), and CD44^{hi}NK1.1^{+} (Stage III). iNKT cells are defined as HSA^{lo}CD1tet^{+} in the thymus and TCRβ $^{+}$CD1tet^{+} in the spleen and liver within the lymphoid gate. (C) Frequency and absolute number of thymic Stage 0 iNKT cells from SHIP1$^{+/+}$, SHIP1$^{+/-}$, and SHIP1$^{-/-}$ mice. Data are pooled from 2 independent experiments (n=5). Black circles: SHIP1$^{+/+}$, gray circles: SHIP1$^{+/-}$, white circles: SHIP1$^{-/-}$ mice, dotted line: unloaded CD1 tetramer control. Error bars indicate SEM.

Sup. Fig. 2



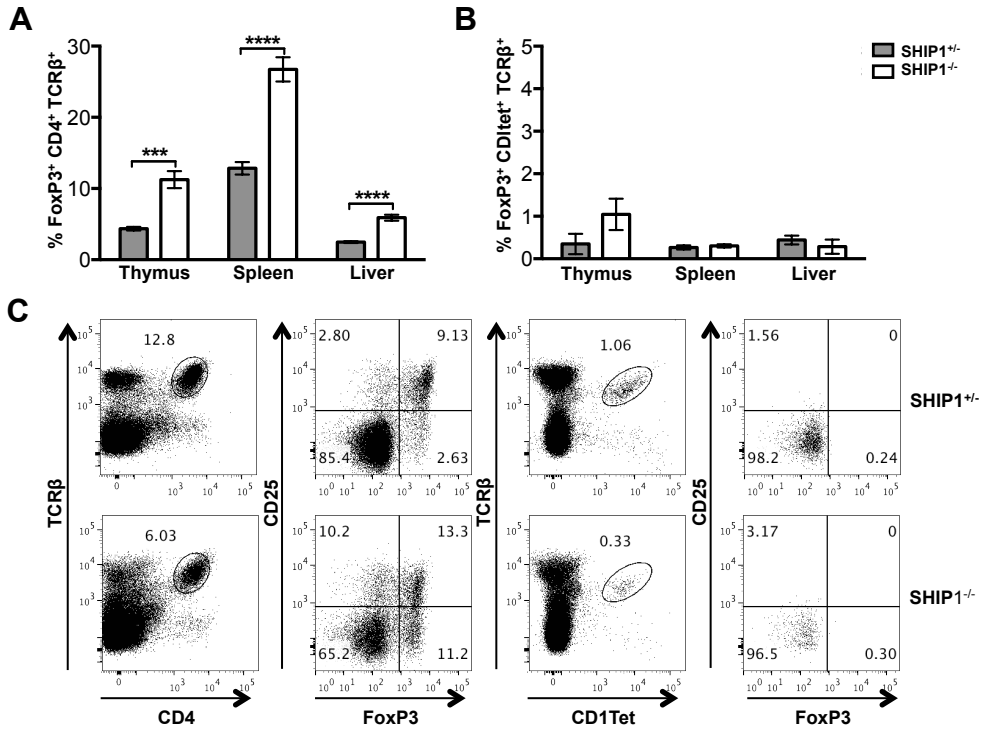
Supplementary Figure 2. Loss of thymic and peripheral iNKT cells in SHIP1$^{-/-}$ mice, but normal T cell populations in CD4CreSHIP1$^{fl/fl}$ mice. (A) Representative staining of iNKT cell populations in the bone marrow, blood, inguinal lymph nodes (iLN), and mesenteric lymph nodes (mLN) from SHIP1$^{+/+}$, SHIP1$^{+/-}$, and SHIP1$^{-/-}$ mice. (B) Frequency of iNKT cells in each indicated organ from SHIP1$^{+/+}$, SHIP1$^{+/-}$, and SHIP1$^{-/-}$ mice. Data are pooled from 3 independent experiments (n=6-8). (C) SHIP1 protein expression from splenocytes was determined using Western blot. T cells (TCRβ^{+}CD4^{+}) and Non T cells (TCRβ^{-}CD4^{-}) were sorted from CD4Cre mice and littermate Controls. Cell lysate was blotted with α-SHIP1 and β-actin antibodies. (D) Frequencies of thymic lymphocyte populations that are DN (CD4^{-} CD8^{-}), DP (CD4^{+}CD8^{+}), CD4 SP, and CD8 SP from CD4Cre mice (n=10) and littermate controls (n=10). Data are pooled from 3 independent experiments and each dot is representative of 1 mouse. iNKT cells are defined as TCRβ^{+}CD1tet^{+} within the lymphoid gate. Black circles: SHIP1$^{+/+}$, gray circles: SHIP1$^{+/-}$, and white circles: SHIP1$^{-/-}$ mice. Black bars: Control mice and grey bars: CD4Cre. Error bars indicate SEM.

Sup. Fig. 3



Supplementary Figure 3A-C. SHIP1 deficiency influences the proliferative capacity and cytokine production of thymic iNKT cells from SHIP1$^{-/-}$ mice. Intracellular Ki67 expression was used to determine steady state proliferative capacity of thymic iNKT cells. (A) Frequencies of Ki67^{+} thymic iNKT cells from C57BL/6 (n=8) and SHIP1$^{-/-}$ mice (n=14). Data are representative of 4 independent experiments. (B) Representative staining of Ki67^{+} thymic iNKT cells from C57BL/6 control mice and SHIP1$^{-/-}$ mice with impaired iNKT cell frequency and normal iNKT cell frequency. (C) Frequencies of Ki67^{+} thymic iNKT cells from CD4Cre mice (n=5) and littermate controls (n=5) and representative staining. Data are representative of 2 independent experiments. iNKT cells are defined as HSA^{lo}CD1tet^{+} in the thymus within the lymphoid gate. Error bars indicate SEM. (D) Cytokine production of thymic iNKT cells from SHIP1$^{+/+}$ (n=6) and SHIP1$^{-/-}$ (n=6) mice following 2.5 hours of PMA and Ionomycin stimulation, compared to unstimulated controls (n=4). Data are pooled from two independent experiments. (E) Representative TCRβ MFI of thymic iNKT cells following PMA and Ionomycin stimulation from SHIP1$^{+/+}$ and SHIP1$^{-/-}$ mice and unstimulated control. Numbers in upper right corner indicate MFI for each histogram. iNKT cells are defined as TCRβ^{+}CD1tet^{+} within the lymphoid gate. Black bars: SHIP1$^{+/+}$, gray bars: SHIP1$^{-/-}$, and white bars: unstimulated control. Error bars indicate SEM.

Sup. Fig. 4



Supplementary Figure 4. Increased Foxp3 expression of conventional T cells, but not iNKT cells, in SHIP1$^{-/-}$ animals. (A) Regulatory T cells in the thymus, spleen, and liver of SHIP1$^{+/-}$ and SHIP1$^{-/-}$ mice. (B) Frequency of Foxp3^{+} iNKT cells in the thymus, spleen, and liver of SHIP1$^{+/-}$ and SHIP1$^{-/-}$ mice. (C) Representative staining of Foxp3 and CD25 expression of splenic CD4^{+} T cell and iNKT cell populations in SHIP1$^{+/-}$ (n=7) and SHIP1$^{-/-}$ mice (n=7). Data are representative of 3 independent experiments. Regulatory T cells are defined as Foxp3^{+}CD4^{+}TCRβ^{+} and iNKT cells are defined as TCRβ $^{+}$CD1tet^{+} within the lymphoid gate. Error bars indicate SEM.