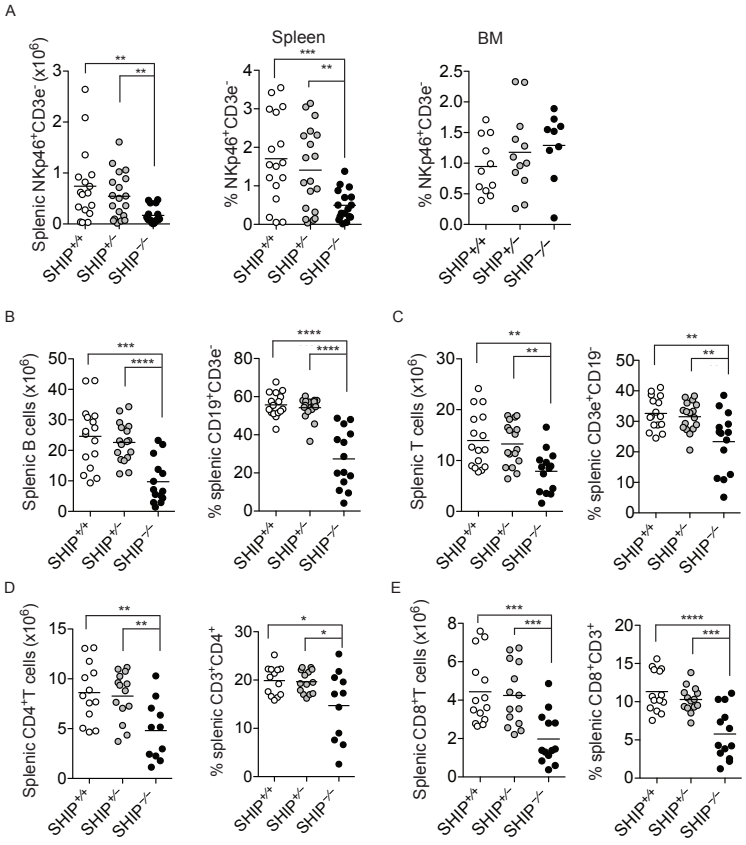
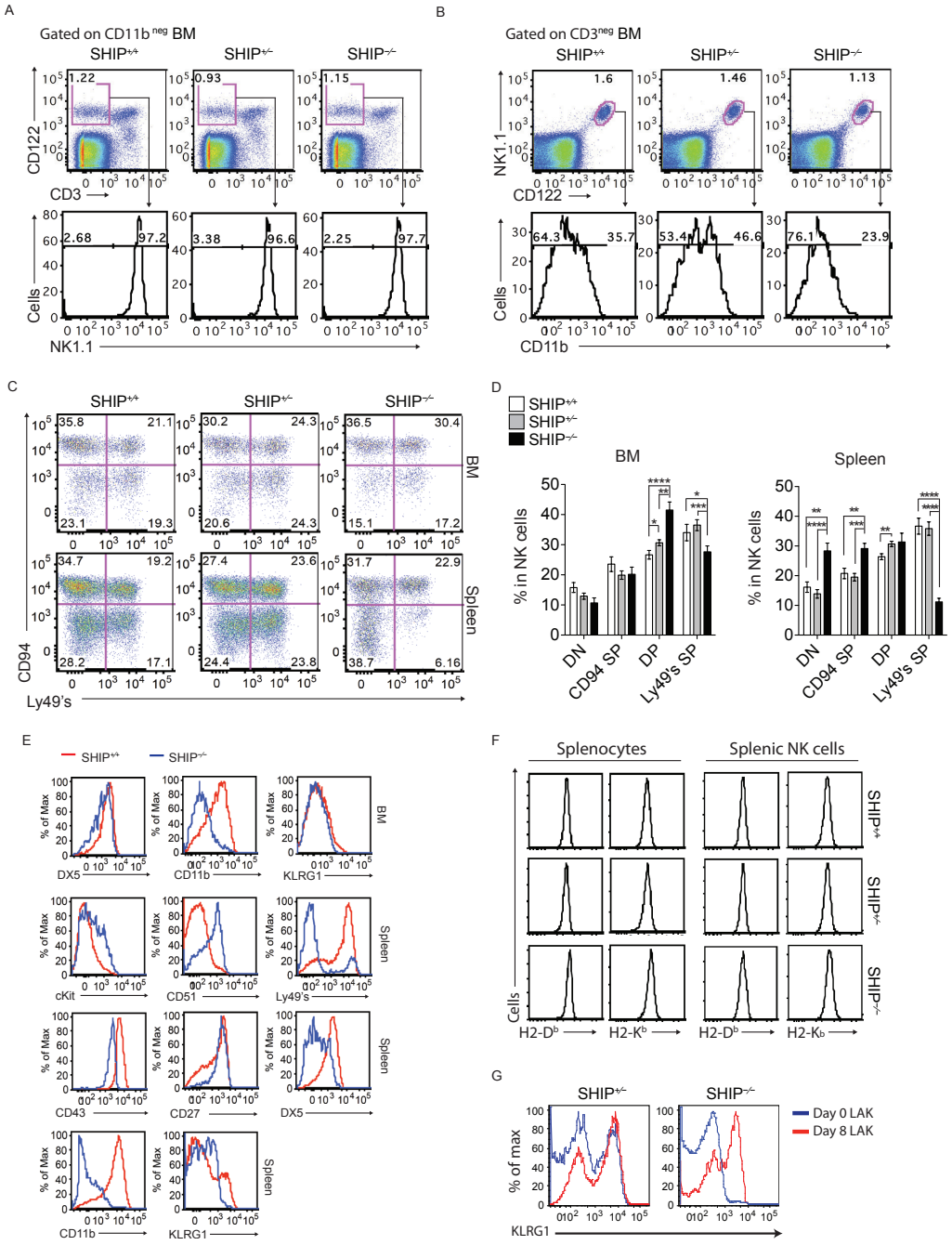
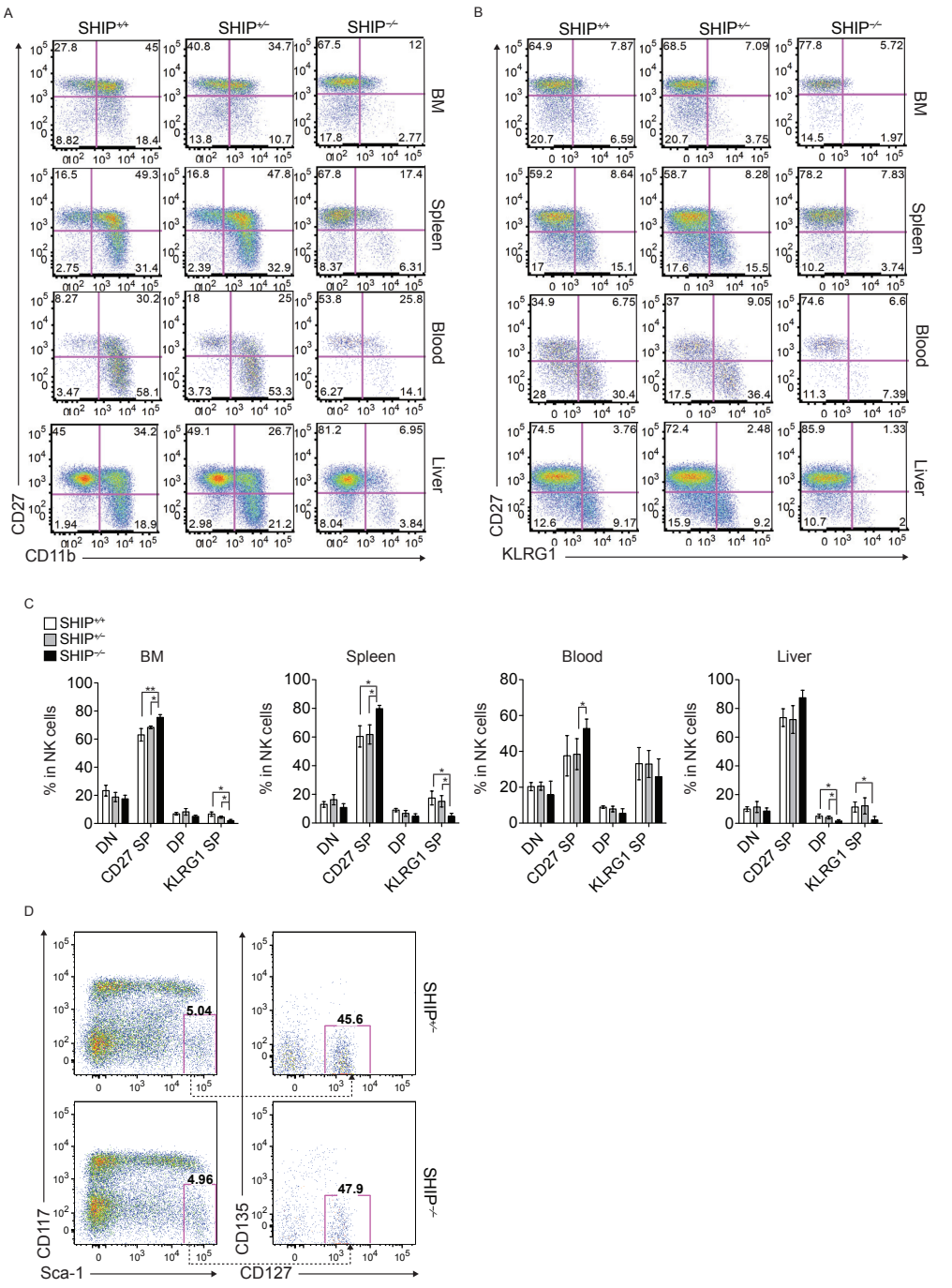


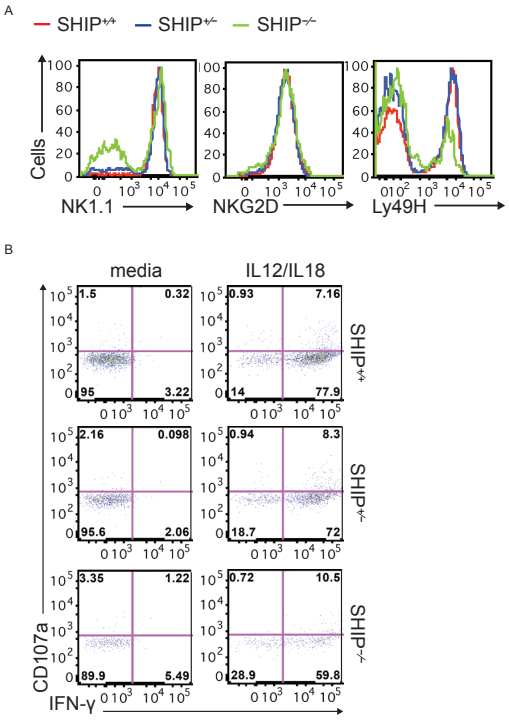
Supplementary Figure 1. Loss of peripheral B and T cells in SHIP$^{-/-}$ mice. (A) Total cell numbers and percentages of NK cell (NKp46^{+}CD3^{-} in the lymphocyte gate) populations in the indicated organs from SHIP$^{+/+}$ (white circles), SHIP$^{+/-}$ (grey circles), and SHIP$^{-/-}$ (black circles) mice. Data are pooled from at least three independent experiments and each dot represents data obtained from one mouse; horizontal lines indicate the mean. (B) Total cell numbers and percentages of splenic B cell (CD19^{+}CD3^{-} in the lymphocyte gate) and (C) T cell (CD3^{+}CD19^{-} in the lymphocyte gate) populations as well as (D-E) T cell subpopulations (CD4^{+} T cells: CD4^{+}CD3^{+} and CD8^{+} T cells: CD8^{+}CD3^{+}) from SHIP$^{+/+}$ (white circles), SHIP$^{+/-}$ (grey circles), and SHIP$^{-/-}$ (black circles) mice. Data are pooled from at least five independent experiments and each dot represents data obtained from one mouse; horizontal lines indicate the mean.



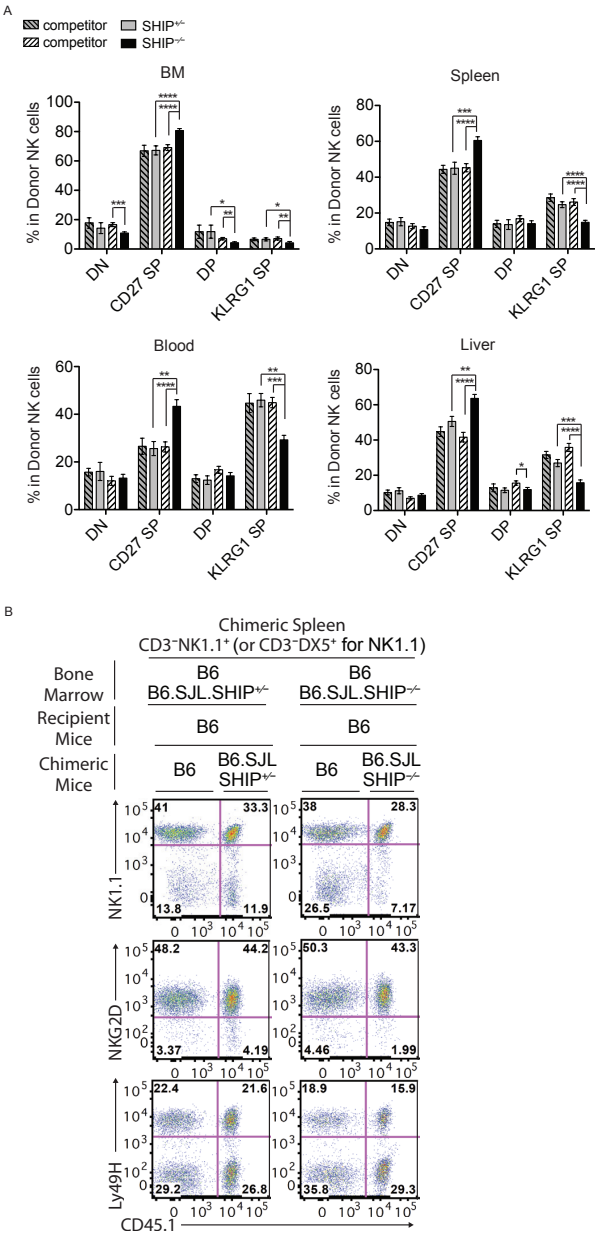
Supplementary Figure 2. Altered NK cell development in SHIP$^{-/-}$ mice. (A&B) Flow cytometry of developing NK cells in bone marrow. Data are representative of six independent experiments. (A) NK1.1 expression on CD122^{+}CD3^{-} populations after gating on CD11b^{-} cells to access NKPs and iNK in bone marrow. (B) CD11b expression on CD122^{+}NK1.1^{+} populations after gating on CD3^{-} cells to access mNK in bone marrow. (C) Flow cytometry of CD94 vs Ly49's expression in NK cells of the indicated organs. Data are representative of at least six independent experiments. (D) Percent in NK cells (NK1.1^{+}CD3^{-} in the lymphocyte gate) expressing DN (CD94^{-} Ly49's $^{-}$), CD94 SP (Ly49's $^{-}$CD94^{+}), DP (CD94^{+} Ly49's $^{+}$), and Ly49's SP (CD94^{-}Ly49's^{+}) populations in the indicated organs from SHIP$^{+/+}$ (white bar), SHIP$^{+/-}$ (grey bar), and SHIP$^{-/-}$ (black bar) mice. Data are pooled from at least six independent experiments. Error bars indicate SEM. (E) Representative expression of a variety of markers on NK cells from SHIP$^{-/-}$ and littermate controls. (F) Flow cytometry of Class I molecules (K^{b} and D^{b}) in total splenocytes (on left) or splenic NK cells (on right) from SHIP$^{+/+}$, SHIP$^{+/-}$, and SHIP$^{-/-}$ mice. Data are representative of at least three independent experiments. (G) KLRG1 is induced on LAK cells from SHIP$^{-/-}$ mice.



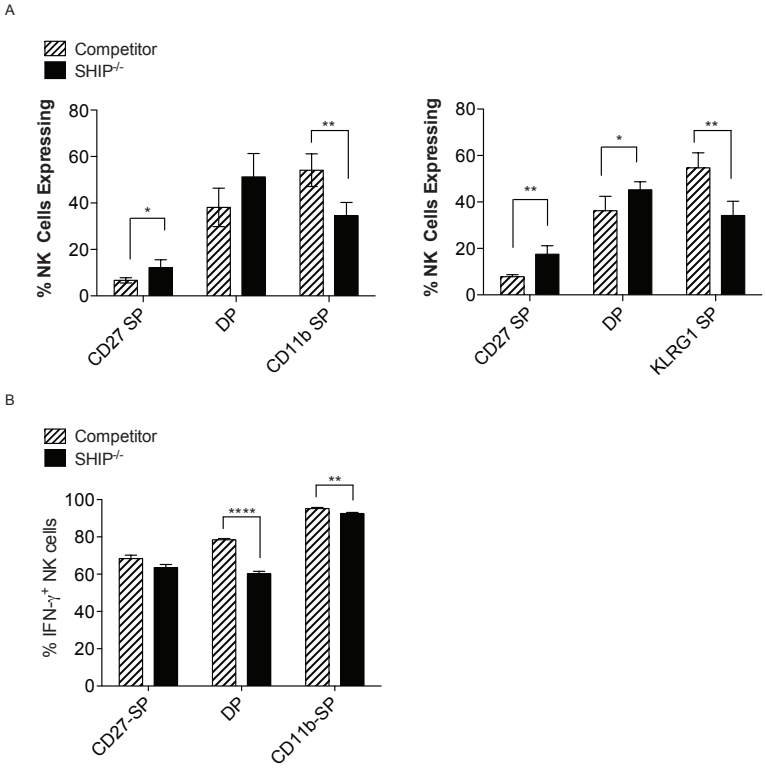
Supplementary Figure 3. Altered NK cell maturation in SHIP$^{-/-}$ mice. (A&B) Flow cytometry of (A) CD27 vs CD11b or (B) CD27 vs KLRG1 expression in NK cells of the indicated organs from SHIP$^{+/+}$, SHIP$^{+/-}$, and SHIP$^{-/-}$ mice. Data are representative of at least eight independent experiments. (C) Percent in NK cells (NK1.1^{+}CD3^{-} in the lymphocyte gate) expressing DN (CD27^{-}KLRG1^{-}), CD27 SP (KLRG1^{-}CD27^{+}), DP (CD27^{+}KLRG1^{+}), and KLRG1 SP (CD27^{-}KLRG1^{+}) populations in the indicated organs as seen in (B). Data are representative of at least eight independent experiments. Data represents means ± SD. D) Representative staining of bone marrow PrePro NK cells (Lineage^{-}, Sca-1^{+}, CD117^{-}, CD135^{-}, CD127^{+}) from SHIP$^{-/-}$ and littermate controls.



Supplementary Figure 4. (A) Expression of NK1.1, NKG2D, and Ly49H. Flow cytometry of NK1.1 (accessed after gating on $DX5^{+}CD3^{-}$ in the lymphocyte gate), NKG2D, and Ly49H (accessed after gating on $NK1.1^{+}CD3^{-}$ in the lymphocyte gate) on $SHIP^{+/+}$ (red histogram), $SHIP^{+/-}$ (blue histogram), and $SHIP^{-/-}$ mice (green histogram). Data are representative of at least three independent experiments. **(B) Representative Intracellular IFN-γ staining in NK cell populations** ($NK1.1^{+}CD3^{-}$ in the lymphocyte gate) from total splenocytes incubated with a mix of IL-12 and IL-18 for six hours.



Supplementary Figure 5. Expression of maturation markers in SHIP-deficient chimera and mixed chimera. (A) Percent in NK cells ($NK1.1^+CD3^-$ in the lymphocyte gate) expressing DN ($CD27^-KLRG1^-$), CD27 SP ($KLRG1^-CD27^+$), DP ($CD27^+KLRG1^+$), and KLRG1 SP ($CD27^-KLRG1^+$) populations in the indicated organs after gating on donor cells ($CD45.1^+$ for $SHIP^{+/-}$ or $SHIP^{-/-}$; $CD45.1^-$ for competitor). Data are pooled from at least five independent experiments. Error bars indicate SEM. (B) Flow cytometry of NK1.1 (accessed after gating on $DX5^+CD3^-$ in the lymphocyte gate), NKG2D, and Ly49H (accessed after gating on $NK1.1^+CD3^-$ in the lymphocyte gate) on spleen donor cells from $SHIP^{+/-}$ or $SHIP^{-/-}$ ($CD45.1^+$) and competitor ($CD45.1^-$). Data are representative of at least three independent experiments.



Supplementary Figure 6.(A) Expression of CD27 vs. CD11b/KLRG1 in *Rag2*$^{-/-}$*x Il2rγ*$^{-/-}$ mice adoptively transferred with splenocytes derived from mixed bone marrow chimeras. Percent in NK cells (NK1.1^{+}CD3^{-} in the lymphocyte gate) expressing CD27 SP (CD11b^{-}CD27^{+} or KLRG1^{-}CD27^{+}), DP (CD27^{+}CD11b^{+} or CD27^{+}KLRG1^{+}), and CD11b SP (CD27^{-}CD11b^{+}) or KLRG1 SP (CD27^{-}KLRG1^{+}) populations in the spleen after gating on donor cells (CD45.1^{+} for SHIP$^{-/-}$; CD45.1^{-} for competitor). Representative of three different experiments. Error bars indicate SEM. **B) The impaired cytokine receptor mediated response from SHIP-1 deficient NK cells is cell intrinsic.** Intracellular IFN-γ in NK cell populations from total splenocytes incubated with IL-12/IL-18 for six hours are accessed after gating on donor cells (CD45.1^{+} for SHIP$^{-/-}$; CD45.1^{-} for competitor) and the different stages of NK cell differentiation. Error bars indicate SEM.