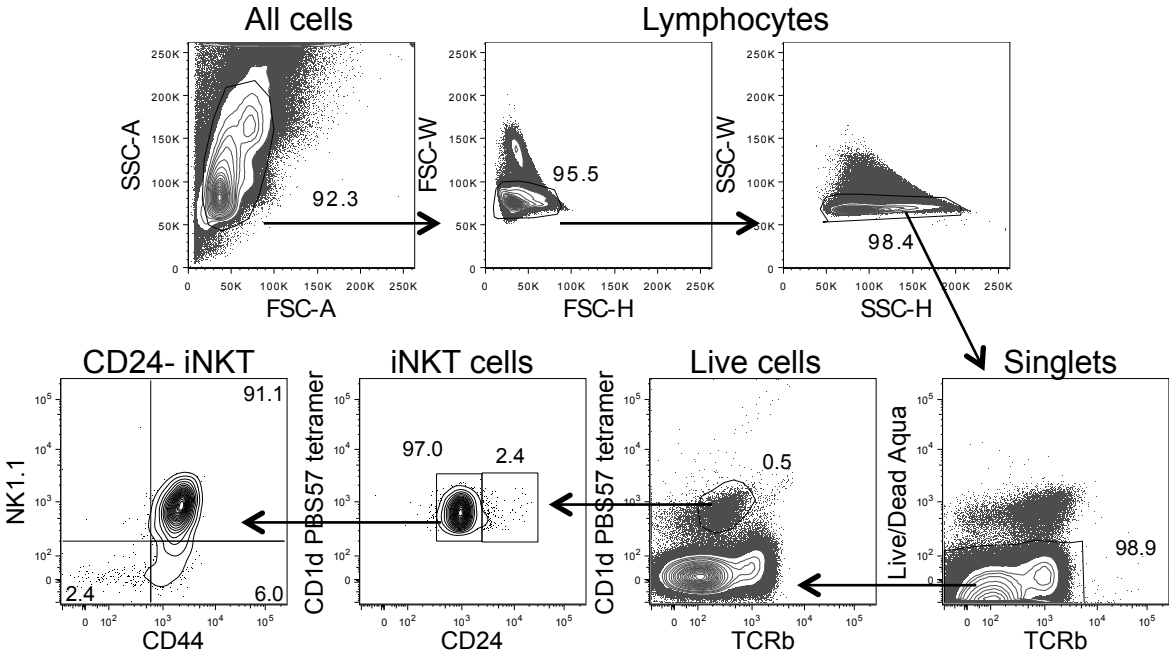
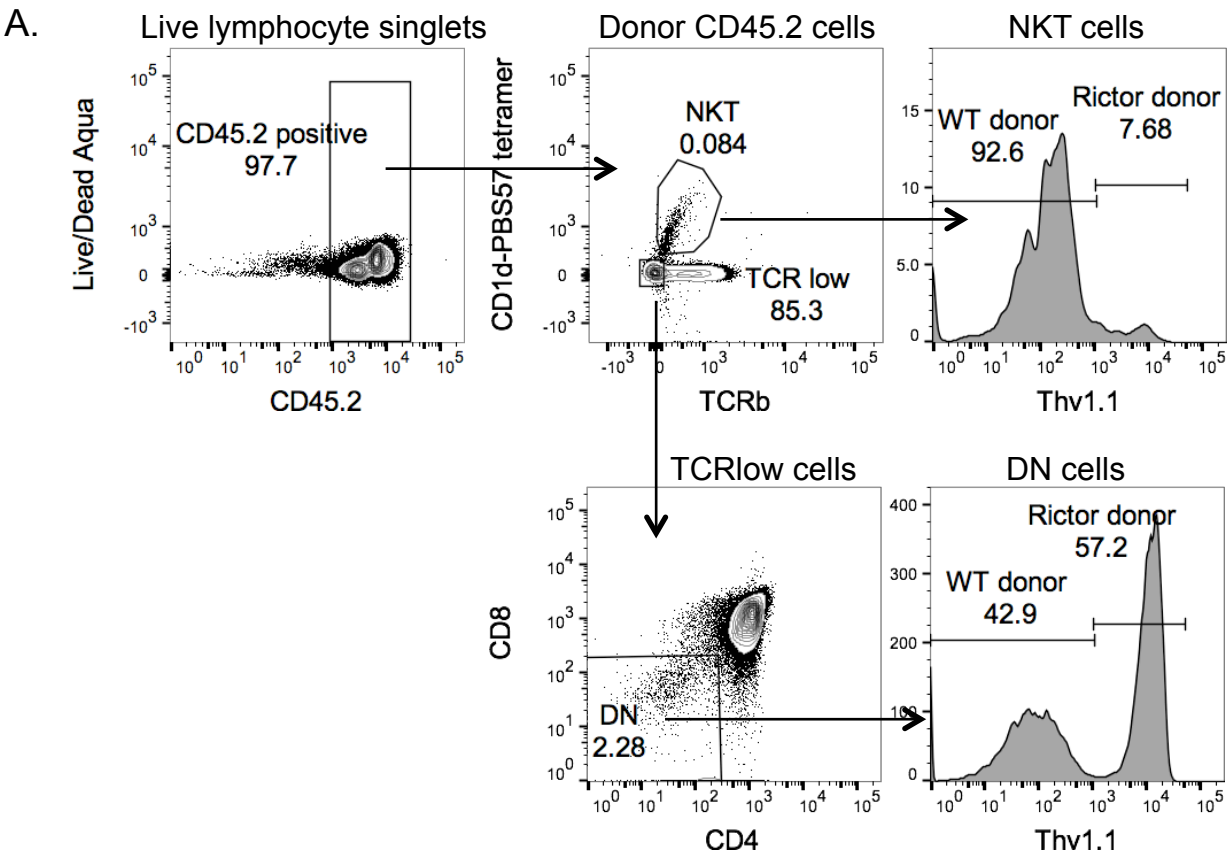
Supporting Information

Supporting Figure 1



Supporting Figure 1. Gating strategy for identification of mature iNKT cells. Lymphocytes were selected based on forward versus side scatter then doublets were gated out through two subsequent gating steps as shown. Live cells were selected based on Live/Dead negative cells. iNKT cells were identified using TCRβ versus CD1d-PBS57 tetramer. Mature iNKT cells were identified as $CD24^{-}$. This strategy is used throughout the manuscript unless noted.

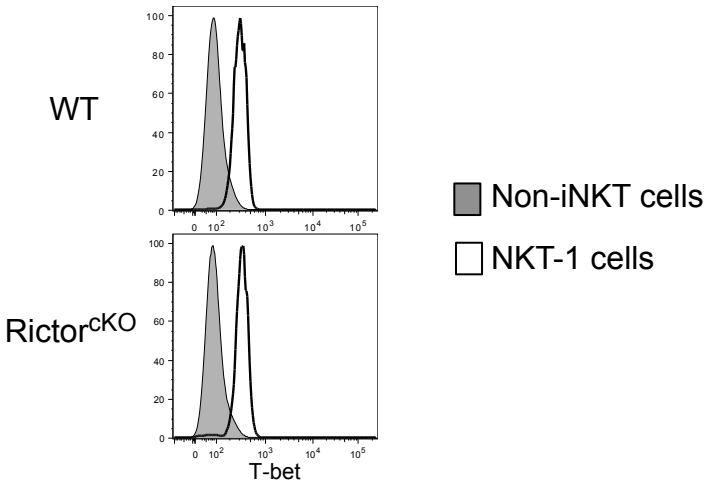
Supporting Figure 2



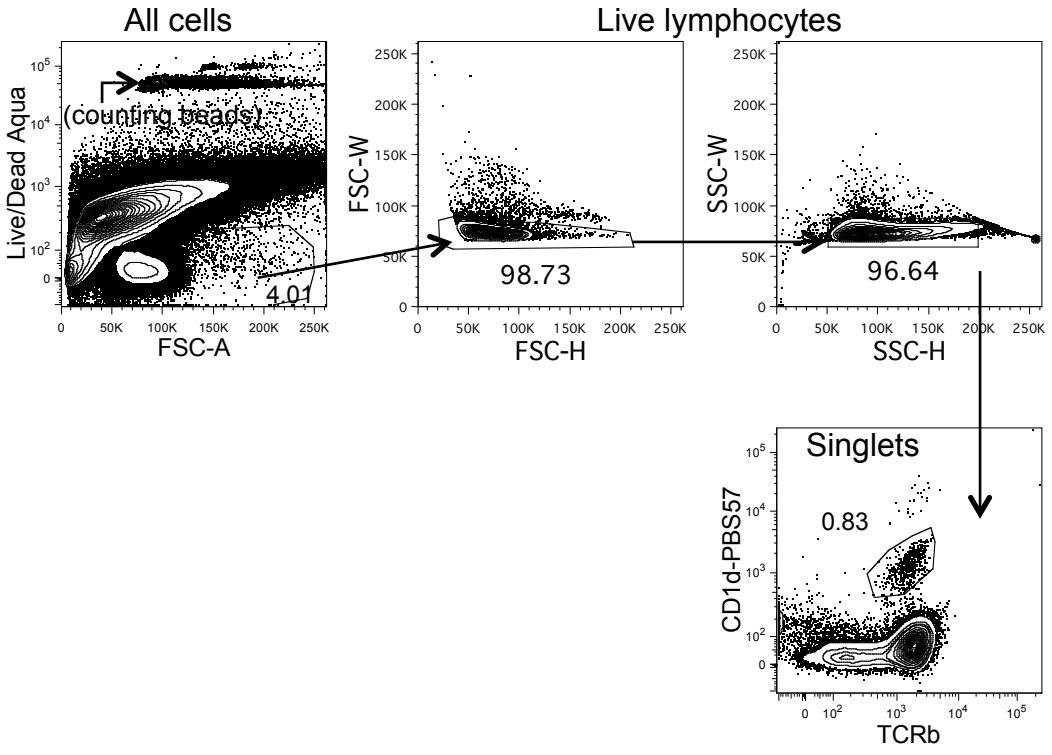
Supporting Figure 2. Defective iNKT cell development in Rictor^{cKO} mice is iNKT cell intrinsic. **A)** Gating strategy for bone marrow chimeras. Live, singlet lymphocytes were gated as described in Supporting Figure 1. iNKT cells were identified using TCRβ versus CD1d-PBS57 tetramer. Donor lymphocytes were identified by CD45.2^{+}. iNKT cells and TCRβ^{low} cells were gated. The frequency of WT versus Rictor^{cKO} derived cells was determined by Thy1.1^{+} staining. DN thymocytes were further selected among TCRβ^{low} cells before assessment of Thy1.1 staining. **B)** Graph represents the average percentages (± S.D.) of WT and Rictor^{cKO} derived cells within the iNKT and its DN precursor population. Data from 1 of 2 independent experiments is shown (n=3 mice per genotype).

Supporting Figure 3

A.

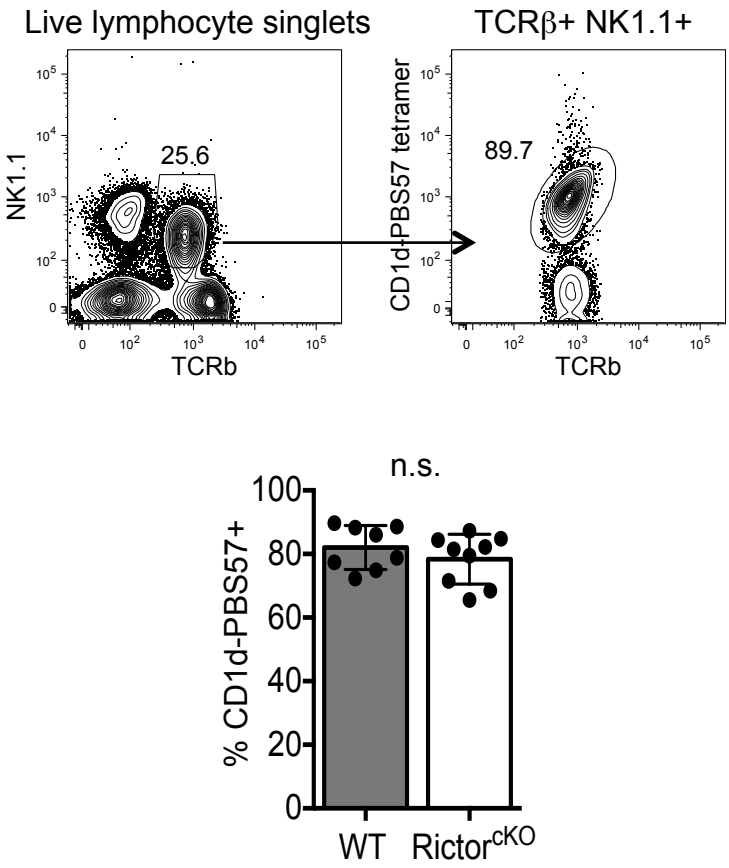


B.



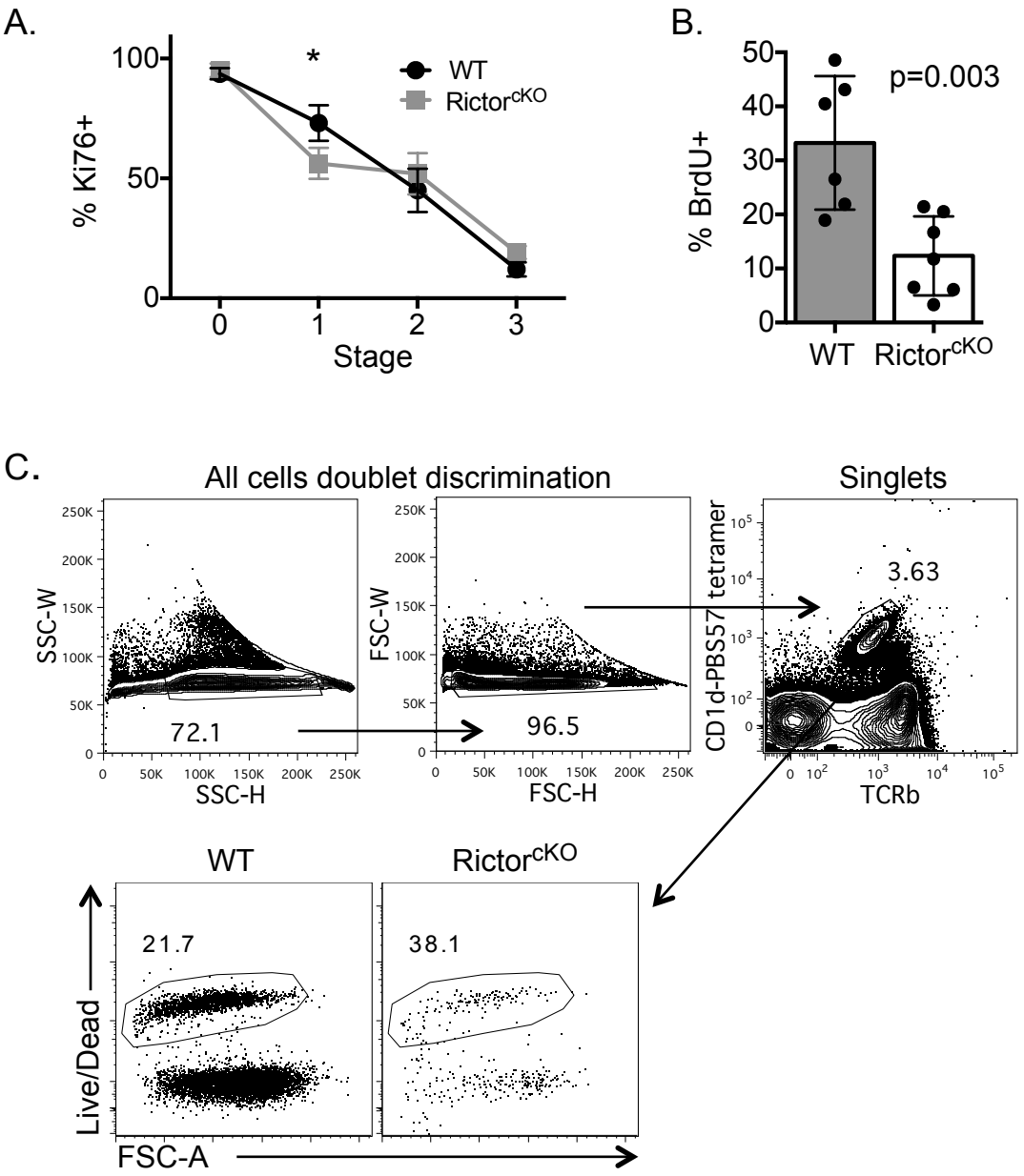
Supporting Figure 3. A) T-bet expression in Rictor^{cKO} iNKT cell is normal. Representative plot of 3 independent experiments, 3 mice per genotype. **B)** Supporting gating strategy for Figure 2C. Division of iNKT cells. iNKT cells were selected from Live singlets.

Supporting Figure 4



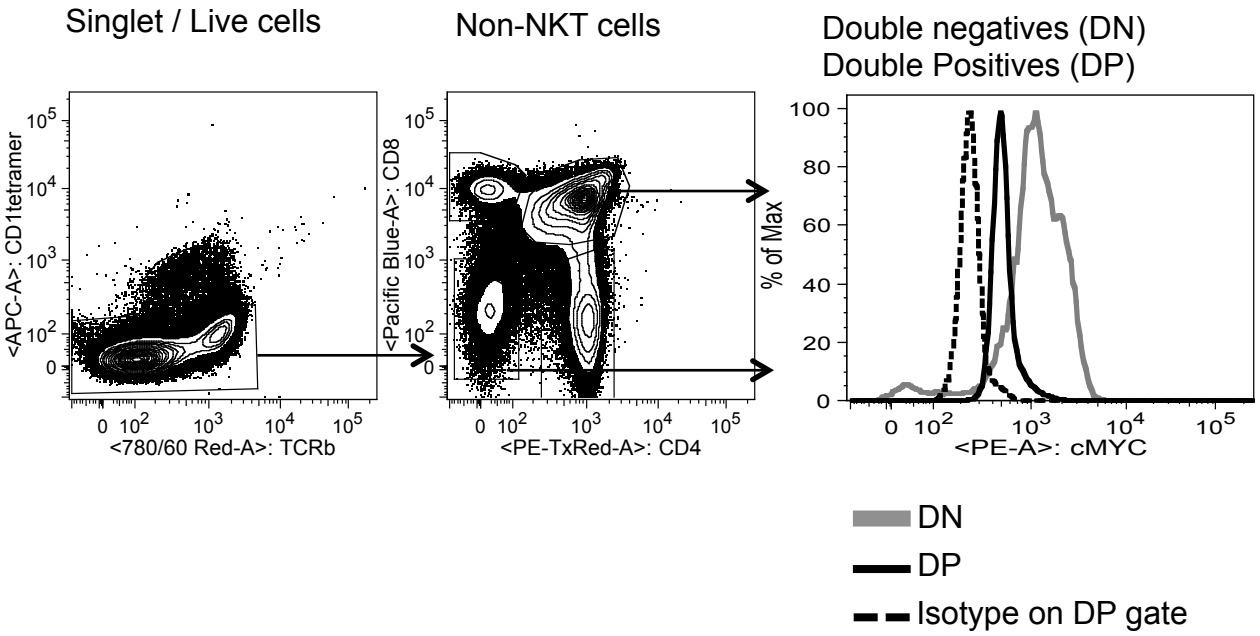
Supporting Figure 4. Graph shows percent of liver NK1.1^{+} TCRb^{+} cells that stain with CD1d-PBS57 tetramer. Compiled from 7 independent experiments with n=8-9 mice per genotype, unpaired t-test mean ± S.D. Live lymphocytes were selected and doublets excluded as indicated previously. NK1.1+TCRβ+ cells were selected as shown in first flow plot. CD1d-PBS57+ cells were gated as iNKT cells.

Supporting Figure 5



Supporting Figure 5. A. The frequency of Ki67+ thymic iNKT cells was determined for stages 0-3. Data are compiled from 3 experiments and analyzed by matched two-way ANOVA followed by Bonferroni post-test. * indicates a significant interaction between stage 1 and genotype. Mean ± SEM is shown. **B.** The frequency of BrdU+ Stage 1 iNKT cells. Data compiled from 2 independent experiments; unpaired t-test mean± S.D. **C.** Supporting gating strategy for Figure 6C. NKT cell enriched thymocytes were cultured overnight. To assess iNKT cell death, all cells were first subjected to doublet discrimination. iNKT cells were identified among singlets followed by Live/Dead determination as shown.

Supporting Figure 6



Supporting Figure 6. Myc expression in WT thymocytes. Live lymphocytes were selected and doublets excluded as indicated previously. CD1d-PBS57- cells were selected then ploted CD4 versus CD8. Histogram shows Myc expression in DN and DP cells. Dotted line indicates isotype control staining on DP cells.