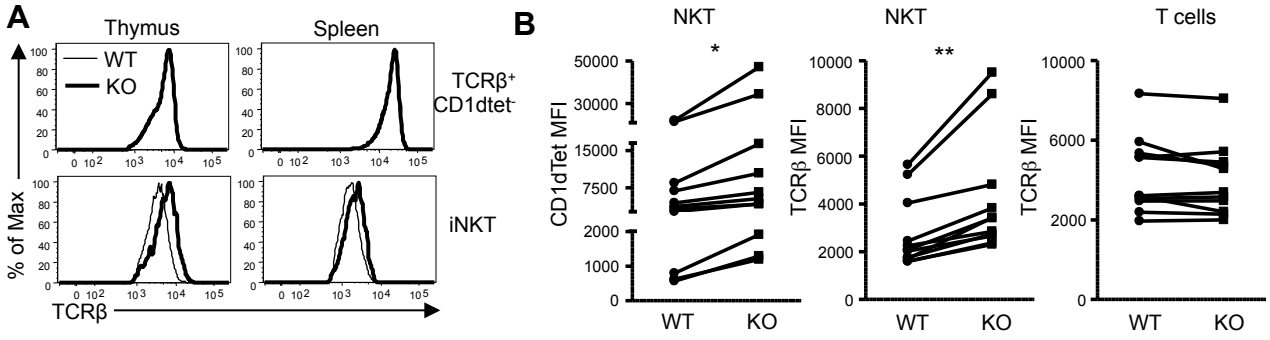


Figure S1. Elevated TCR expression in TSC1KO *i*NKT cells. (A) Overlaid histograms represent CD1dTet and TCRβ expression in WT and TSC1KO *i*NKT cells and TCRβ$^+$CD1dTet$^-$ T cells. **(B)** Mean fluorescence intensity (MFI) of CD1dTet and TCRβ in thymic *i*NKT cells and TCRβ$^+$CD1dTet$^-$ T cells. Each dot and square represents a pair of WT and TSC1KO mice. *, P<0.05 and **, P<0.01 determined by pairwise Student *t*-test.

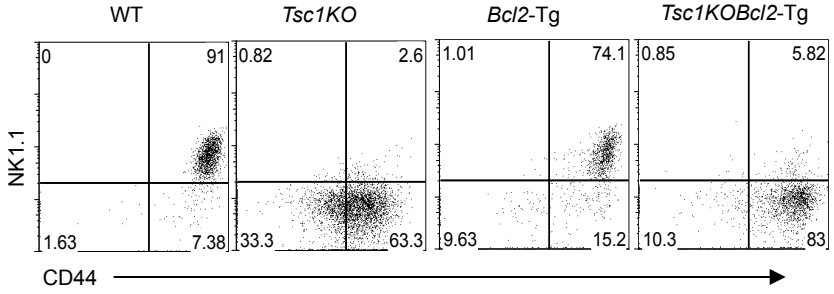


Figure S2. Overexpression of Bcl-2 did not restore *i*NKT cell terminal maturation in TSC1KO mice. Dot plots show CD44 and NK1.1 expression on thymic *i*NKT cells from WT, TSC1KO, Bcl2 transgenic (Bcl2-Tg), and TSC1KO-Bcl2-Tg mice. Data shown represent three experiments.

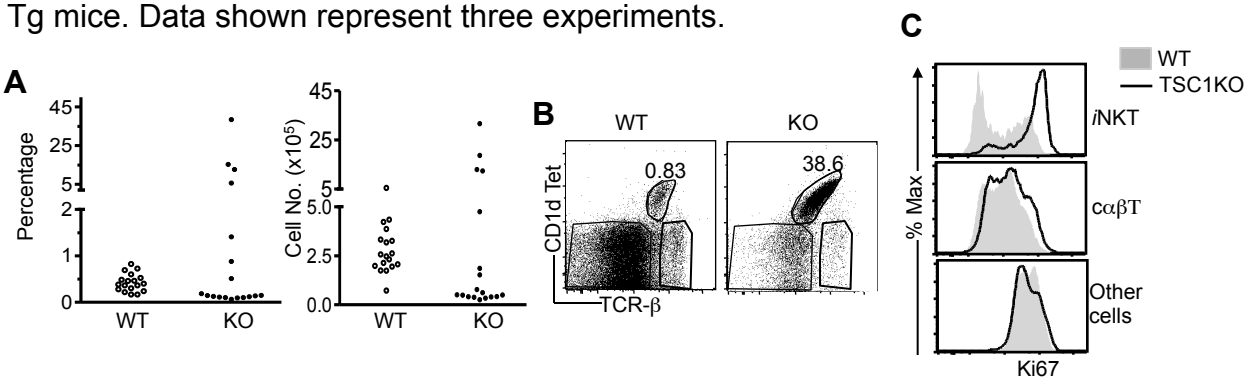


Figure S3. Development of 'tumor' like *i*NKT cells in the thymus of TSC1KO mice. (A) Percentages and numbers of thymic *i*NKT cells. Each dot or cycle represent one mouse. Data shown are calculated from mice of two to four months of age from multiple experiments. (**B**) TCRβ and CD1dTet staining of WT and TSC1KO thymocytes. **(C)** Ki67 staining in the indicated populations. TCRβ represents TCRβ$^+$CD1dTet$^-$. Others represent CD1dTet$^-$TCRβ$^-$ thymocytes.

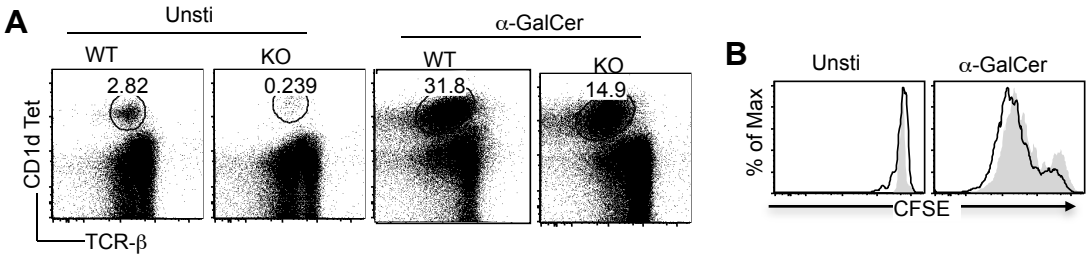
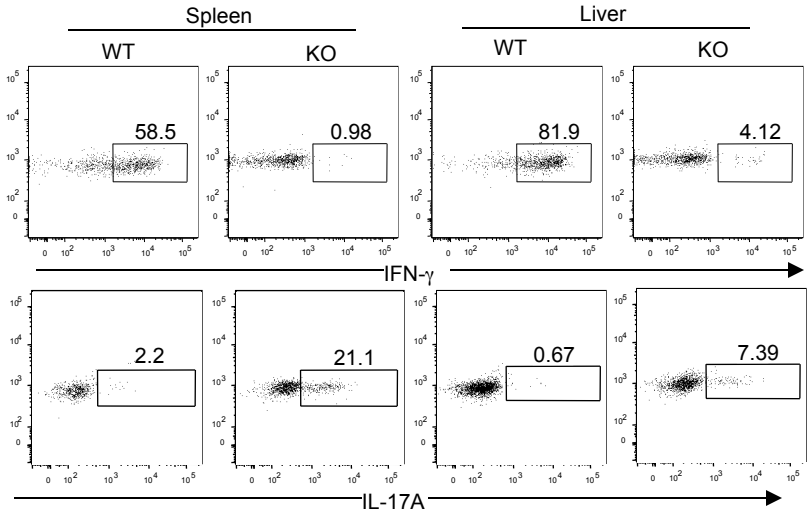


Figure S4. Effects of TSC1 deficiency on iNKT cell proliferation. WT and TSC1KO thymocytes were labeled with CFSE, left unstimulated or stimulated with α-GalCer for 72 hours. Cells were stained with CD1dTet, TCRβ. **A.** Dotplots show CD1dTet and TCRβ staining in live gated cells. **B.** Overlaid histograms show CFSE intensity in gated WT and TSC1KO *i*NKT-cells.



Supplemental Figure S5. Effects of TSC1 deficiency on *i*NKT cell cytokine production i*n vivo* following α-GalCer injection. *TSC1$^{f/f}$* and *TSC1$^{f/f}$-CD4Cre* mice were first intraperitoneally injected with 150 μg brefeldin A (BFA, Sigma), followed by intravenous injection with 2 μg of α-GalCer 90 minutes later. Two hours after α-GalCer injection, spleens and livers were harvested for *i*NKT cell staining and intracellular staining of IFNγ and IL-17A. Data shown represent two experiments.

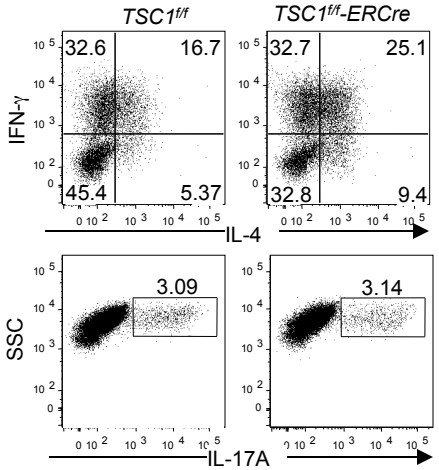


Figure S6. Effects of acute deletion of TSC1 on mature *i*NKT cells. *TSC1$^{f/f}$* and *TSC1$^{f/f}$-ERCre* mice were intraperitoneally injected with 1.5 μg tamoxifen on days 1, 2, and 5. Thymocytes from the mice were harvested on day 8. Following magnetic bead enrichment, *i*NKT cells were stimulated with PMA and ionomycin in the presence of GolgiPlug at 37°C for 5 hours, cell surface-stained for *i*NKT cells and intracellularly stained for IFNγ, IL-4, and IL-17A. Dot plots show IFNγ, IL-4, and IL-17A expression in gated *i*NKT cells. Data shown are representative of three experiments.

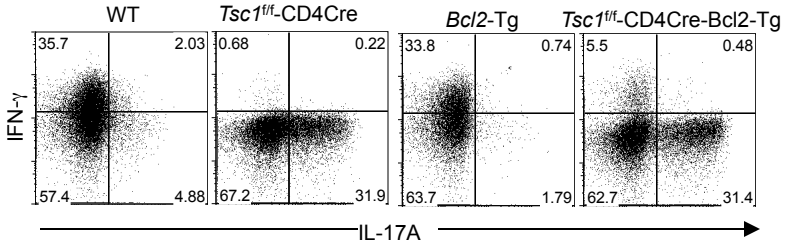


Figure S7. Overexpression of Bcl-2 is not able to revert the *i*NKT-17 predominance over *i*NKT-1 caused by TSC1 deficiency. Dot plots show IL-17A and IFNγ staining in gated thymic *i*NKT cells from WT, TSC1KO (CD4Cre), Bcl2 transgenic (Bcl2-Tg), and TSC1KO-Bcl2-Tg mice following P + I stimulation for 5 hours. Data shown represent three experiments.

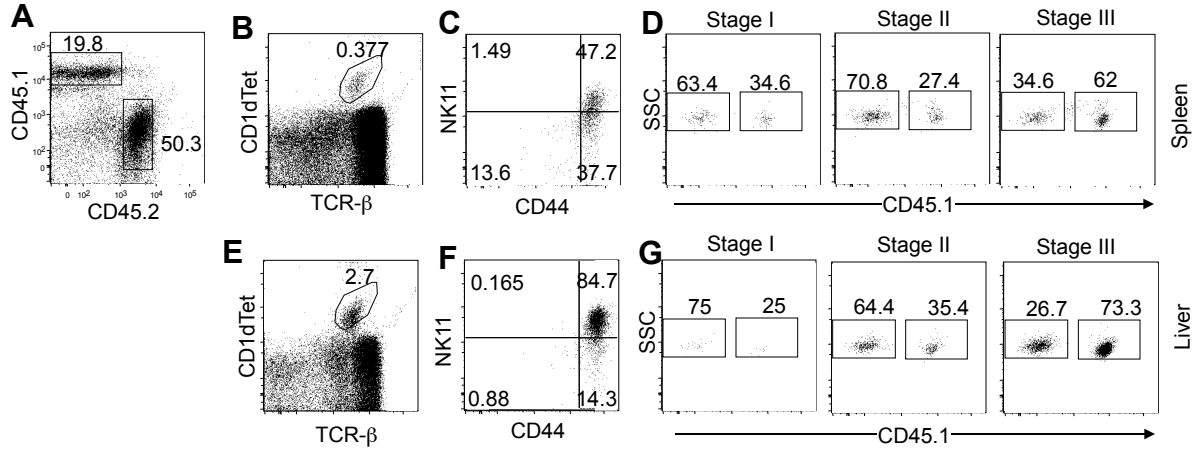


Figure S8. Cell intrinsic mechanisms caused *i*NKT-cell developmental defect. Sublethally irradiated *TCR*$\alpha^{-/-}$ mice were *i.v*. injected with WT (CD45.1) and TSC1KO (CD45.2) BM cells at a 1:2.5 ratio. Splenocytes and liver MNCs from chimeric mice were analyzed eight weeks later. **A.** CD45.1 and CD45.2 staining of WT and TSC1KO BM mixture before injection. **B,E**. TCRβ and CD1dTet staining of Lin^- splenocytes and liver MNCs. **C,F**. CD44 and NK1.1 staining of gated splenic (B) and liver (E) *i*NKT-cells. **D,G**. CD45.1 staining of indicated splenic (C) and liver (F) *i*NKT subsets. Data shown represent three experiments.

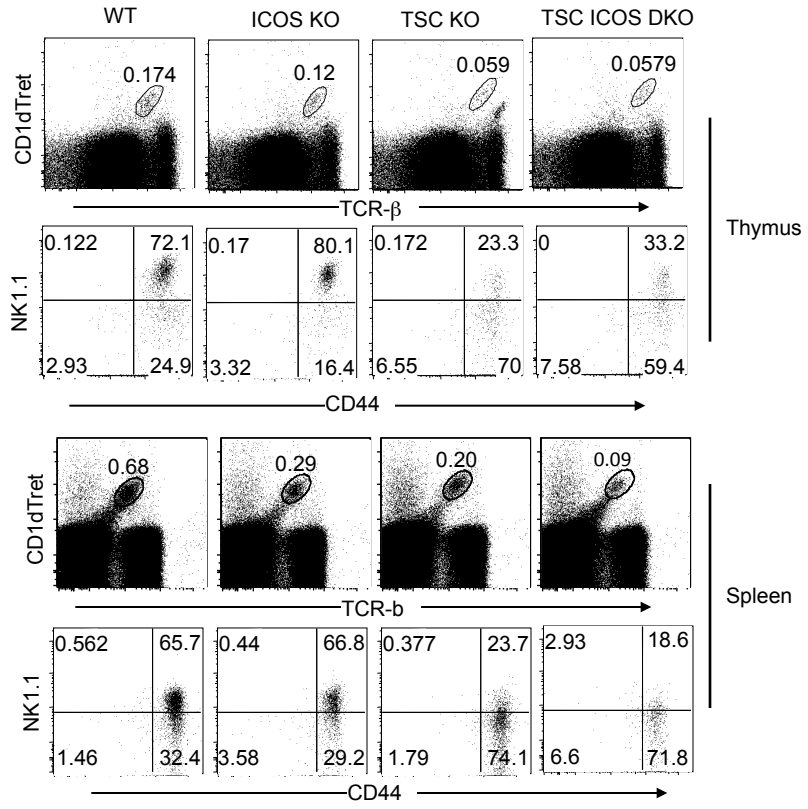


Figure S9. Ablation of ICOS in TSC1-deficient mice does not rescue iNKT terminal maturation defect. Dot plots show TCRβ and CD1dTet staining (top panels) of thymocytes and splenocytes from mice of the indicated genotypes. Bottom panels show CD44 and NK1.1 staining in gated *i*NKT cells.

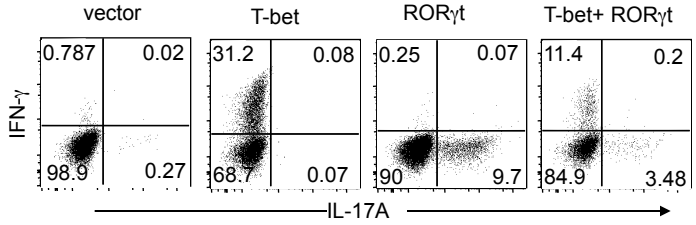


Figure S10. Competition between T-bet and RORγt to direct IFNγ and IL-17 expression. The *i*NKT hybridoma 3C3 were retrovirally transduced with Thy1.1 and GFP, Thy1.1 plus RORγ1, GFP plus T-bet, or both RORγt and T-bet. Transduced cells were stimulated with PMA plus ionomycin for 5 hours in the presence of Golgiplug™ followed by intracellular staining for IFNγ and IL-17A. Dot plots show IFNγ and IL-17A expression in GFP$^+$, Thy1.1$^+$, or GFP$^+$Thy1.1$^+$ cells.