## **Supporting Information**

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**Fig. S1.** Invariant natural killer T (iNKT) cells in Tie2-Cre transgenic and Dicer<sup>fl/fl</sup> mice. Thymic, splenic, and hepatic iNKT cells from Dicer<sup>fl/fl</sup>Tie-2Cre<sup>+</sup>, Dicer<sup>fl/fl</sup>Tie-2Cre<sup>-</sup>, Dicer<sup>wt/wt</sup>Tie2Cre<sup>+</sup>, and Dicer<sup>wt/wt</sup>Tie2Cre<sup>-</sup> littermates were identified as TCRbeta<sup>+</sup>Tetramer<sup>+</sup> cells. In the spleen and liver, iNKT cells were gated on the B220<sup>lo</sup> spleen and liver cells. There were no significant differences among Dicer<sup>fl/fl</sup>Tie-2Cre<sup>-</sup>, Dicer<sup>wt/wt</sup>Tie-2Cre<sup>+</sup>, and dicer<sup>wt/wt</sup>Tie2Cre<sup>-</sup> mice.



**Fig. S2.** Defective thymic *i*NKT cell development in Dicer<sup>fl/fl</sup>Tie-2Cre<sup>+</sup> mouse. TCRbeta<sup>+</sup>Tetramer<sup>+</sup> thymic *i*NKT cells were analyzed for the expression of CD24 (HSA), CD4, and CD8 in Dicer<sup>fl/fl</sup>Tie-2Cre<sup>+</sup> mice and WT littermate control.

DNAS



**Fig. S3.** The *i*NKT cell homeostasis in the spleen from Dicer deletion mice. CD8-depleted thymocytes (up to 4% of recovered thymocytes were Tetramer<sup>+</sup>*i*/NKT cells) from CD45.1-congenic mice were transferred to irradiated 6 week-old *Dicer<sup>fi/fi</sup>Tie2cre*<sup>+</sup> or littermate control mice (2 to 3 mice per group). The CD45.1<sup>+</sup> lymphocytes from the spleen were analyzed for *i*NKT cells at day 5 after transferring. The percentages of CD4<sup>+</sup> *i*NKT cells were further analyzed in the spleen on the gated iNKT cells.