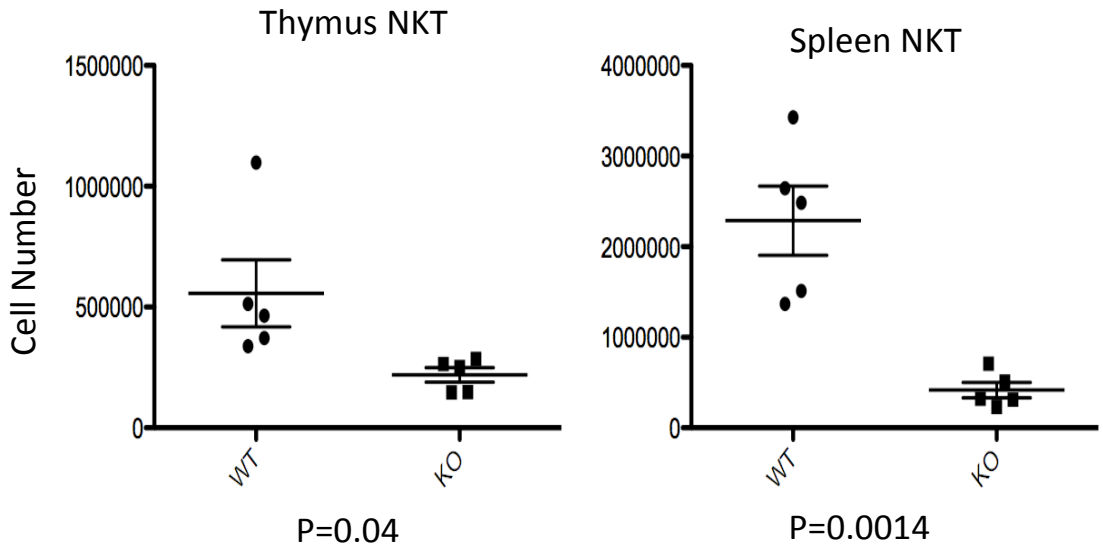
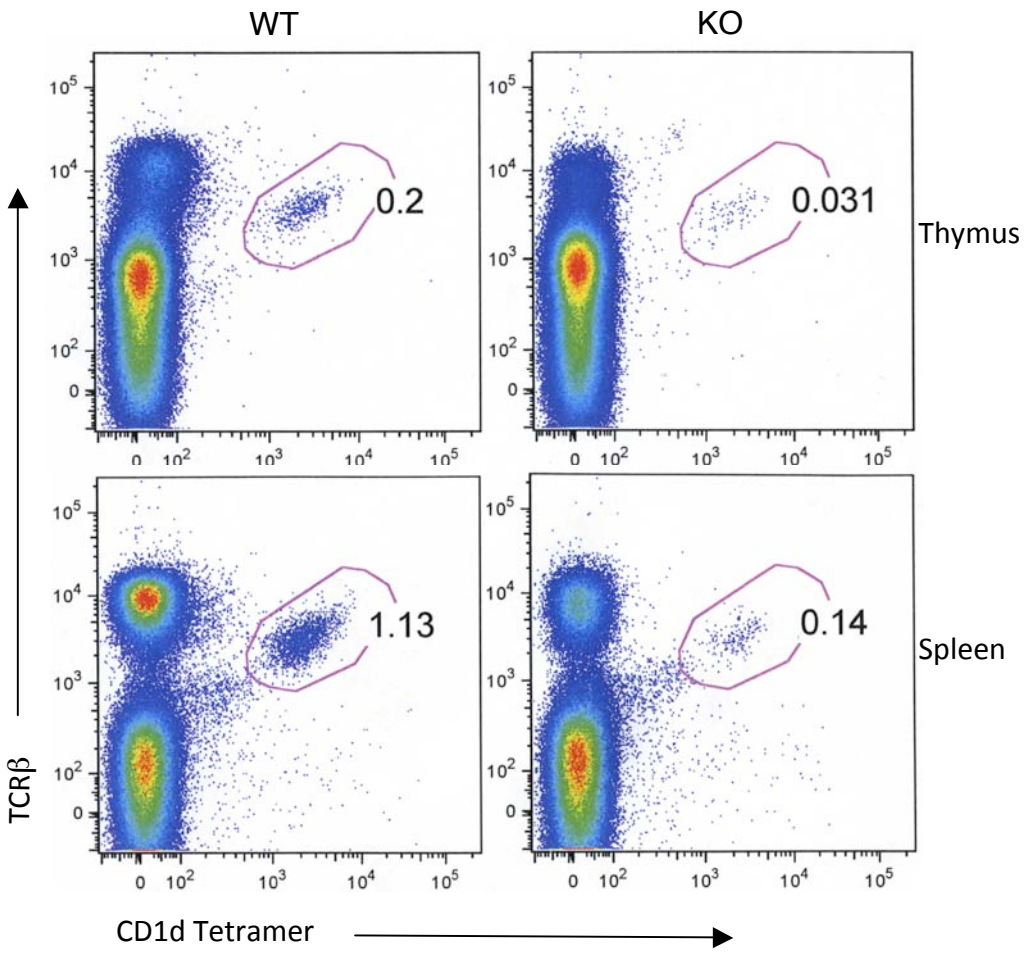
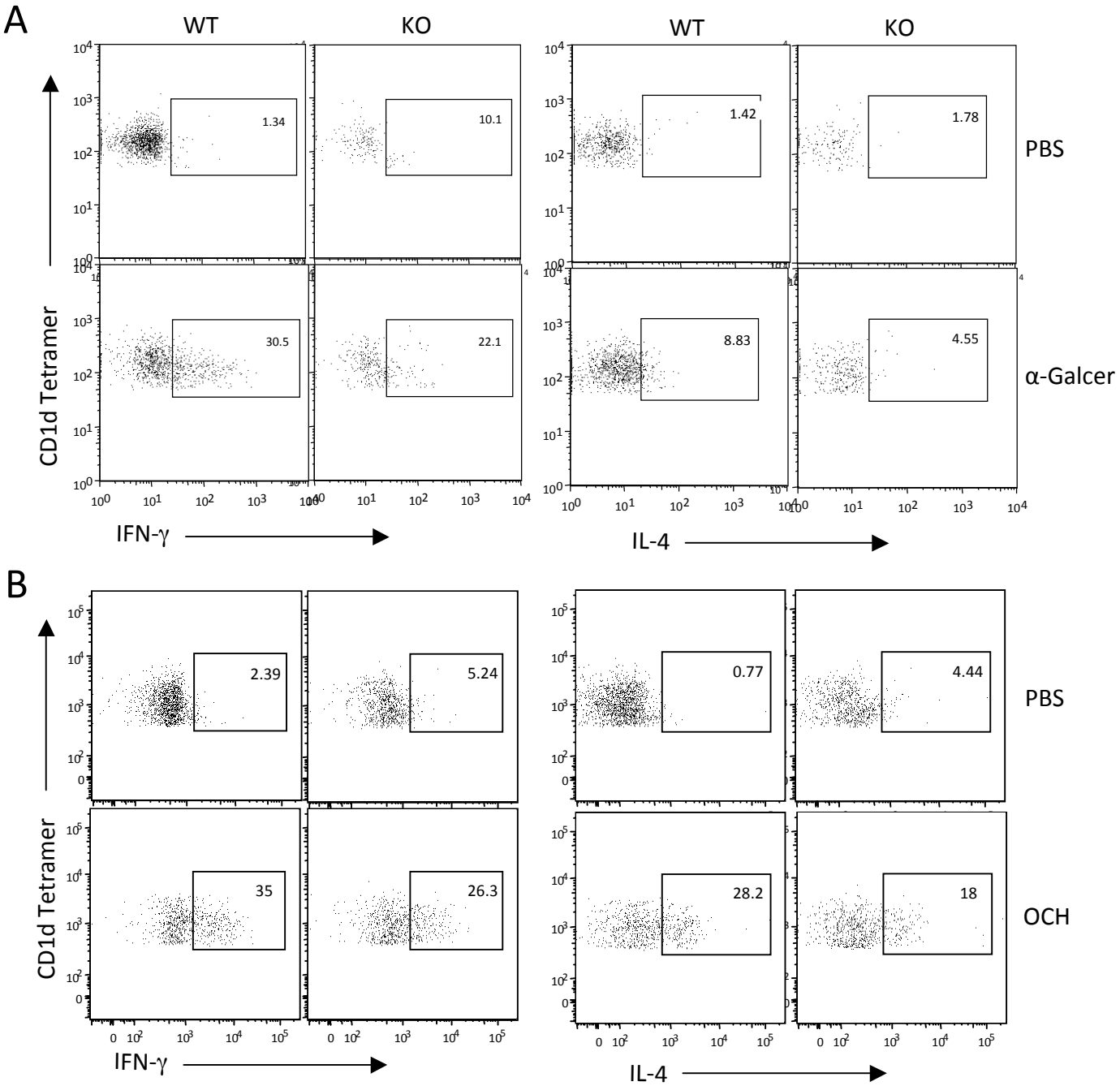


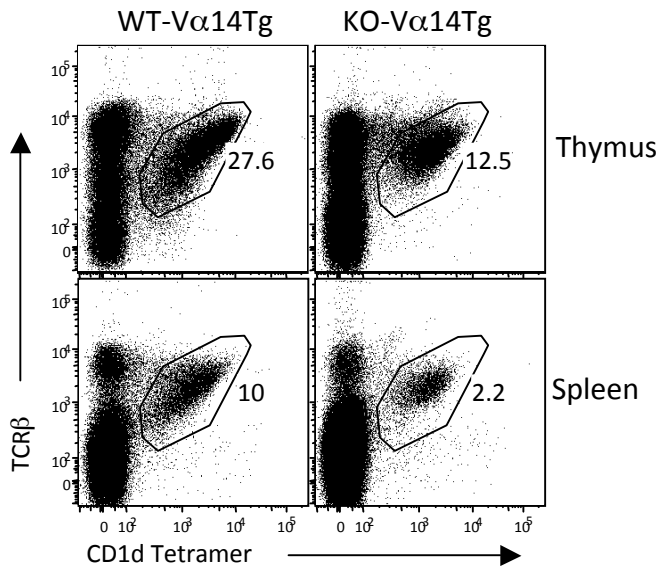
S-Fig. 1. Absolute NKT cell numbers in individual thymuses and spleens of WT and CYLD KO mice (6-8 wk old). Data are presented as mean ± s.d. of 5 WT and 5 CYLD KO mice.



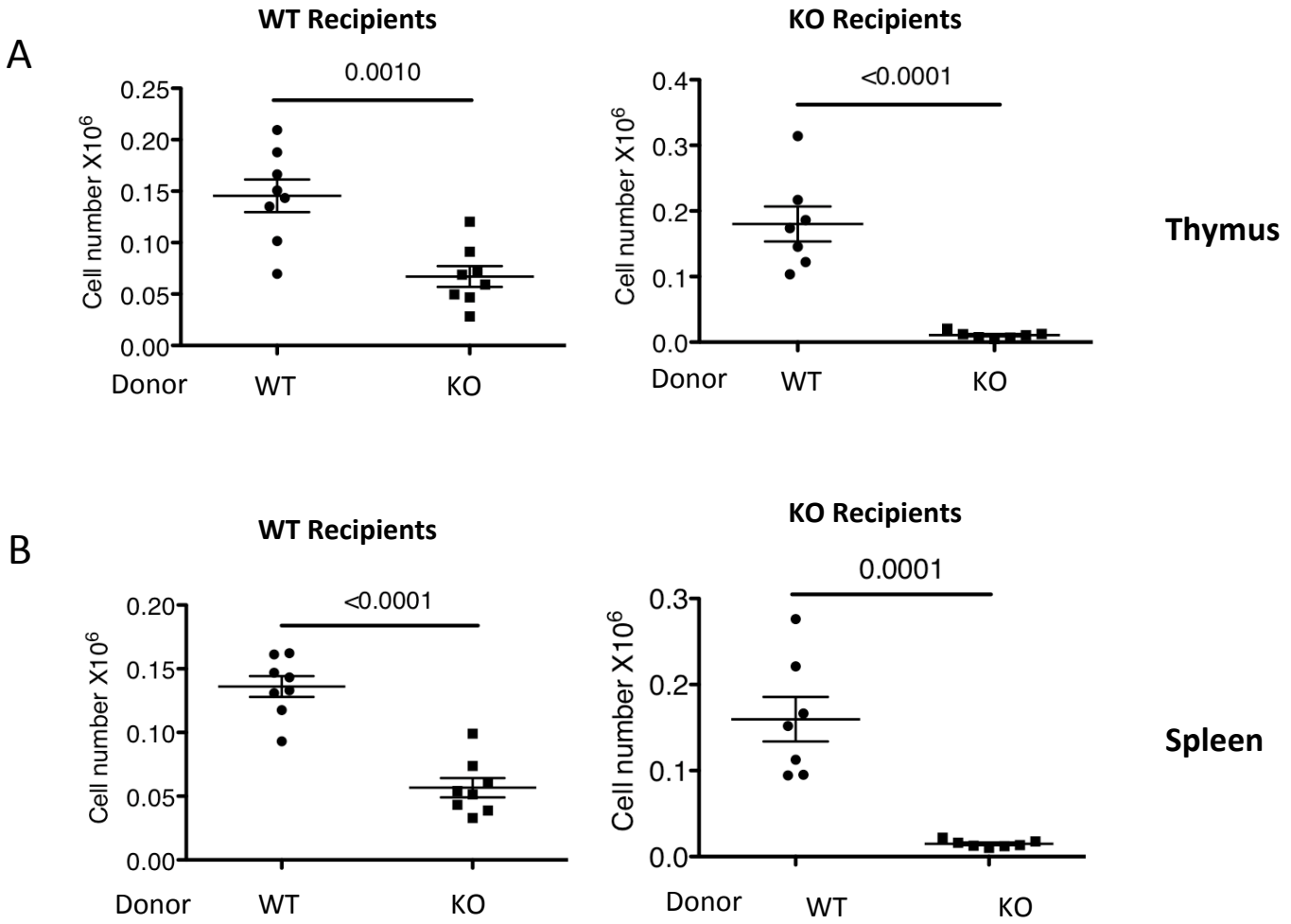
S-Fig. 2. CYLD knockout (KO) mice were backcrossed to C57BL/6 background for 9 generations, and 6 wk old wildtype (WT) or CYLD KO mice were used for preparing thymocytes and splenocytes. Flow cytometry was performed to determine the frequency of NKT cells based on their binding to PBS57-loaded CD1d tetramer and expression of TCRβ.



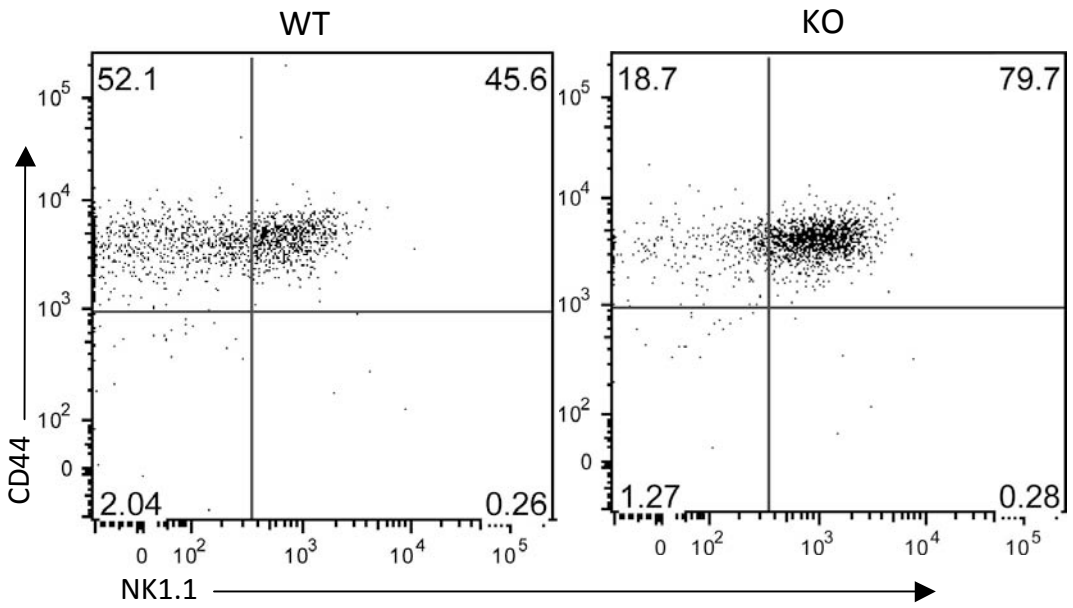
S-Fig. 3. (A) Age- and sex-matched WT and CYLD KO mice were injected (i.v.) with either control PBS or α-Galcer. After 1 hr, splenocytes were subjected to intracellular cytokine staining (ICS) and flow cytometry to detect the percentage of NKT cells producing IL-4 (right panels) and IFN-γ (left panels) (gated on TCRβ$^+$ cells). (B) Age- and sex-matched WT and CYLD KO mice were injected with either PBS or OCH. ICS was performed as described in A. Data are representative of three independent experiments with multiple mice.



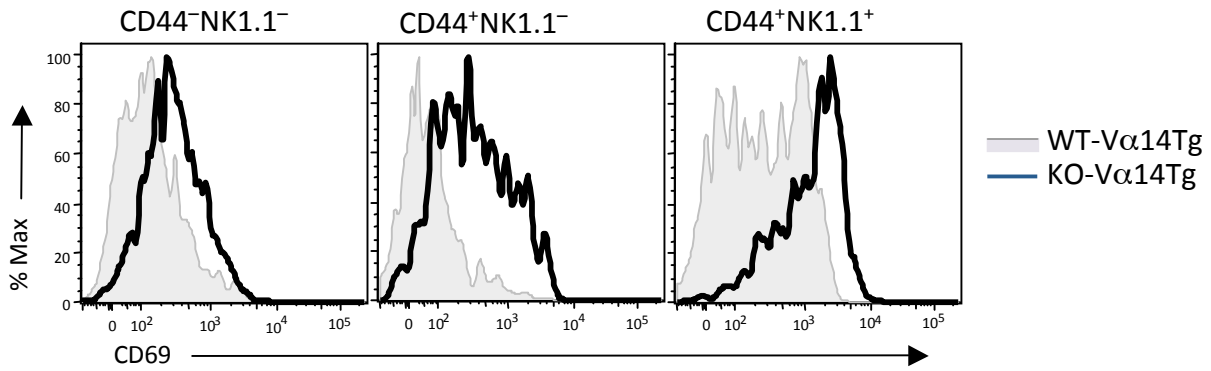
S-Fig. 4. Flow cytometric analysis of NKT cell frequency in the thymocytes and splenocytes of WT and CYLD KO mice crossed with Vα 14Tg mice. Data are representative of 4 independent experiments.



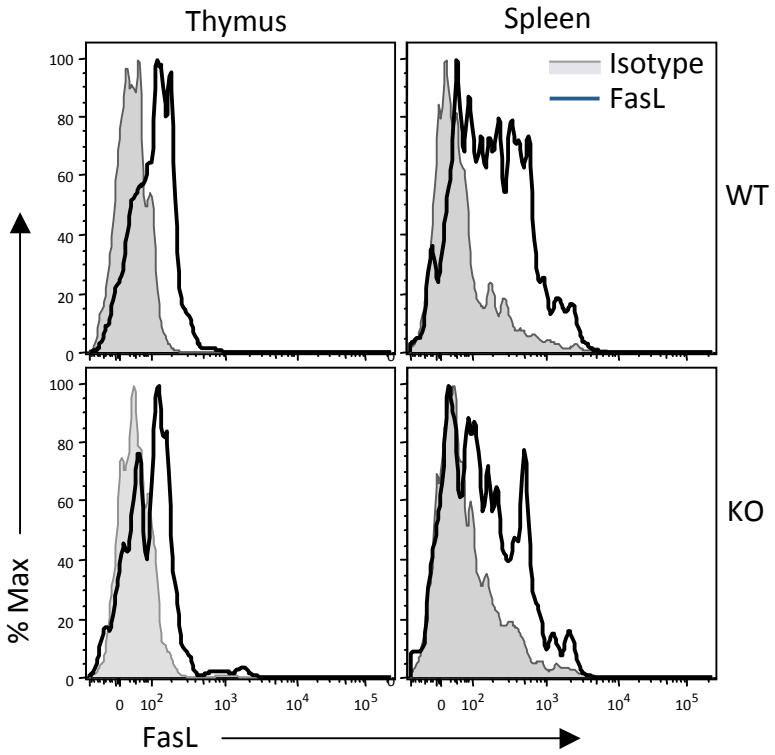
S-Fig. 5. Cell-intrinsic role of CYLD in NKT cell development. CYLD+/+ (WT) and CYLD–/– (KO) recipient mice were γ-irradiated to destroy their hematopoietic cells. The recipient mice were adoptively transferred with bone marrow cells derived from WT (CD45.1+) and KO (CD45.1–) mice (mixed in 1:1 ratio). After 6 weeks, flow cytometry was performed to detect WT ($CD45.1^+$) and KO ($CD45.1^-$) NKT cells in the thymus (A) and spleen (B). Data are presented as mean value of 8 WT and 8 KO recipient mice. P value <0.05 and <0.01 means significance and very significance, respectively.



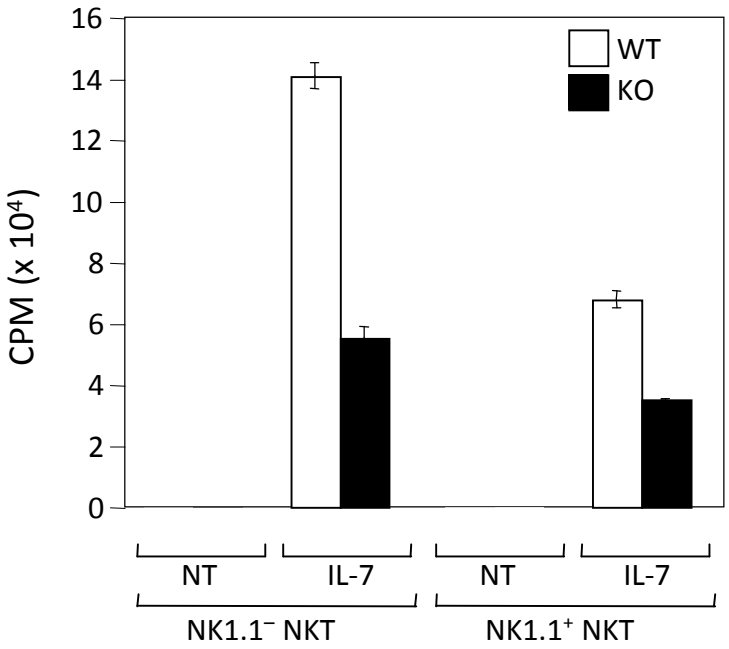
S-Fig. 6. Cells were prepared from the liver of wildtype (WT) and CYLD knockout (KO) mice (6 wk old) and subjected to flow cytometry to determine the frequency of NKT subpopulations based on their surface expression of CD44 and NK1.1.



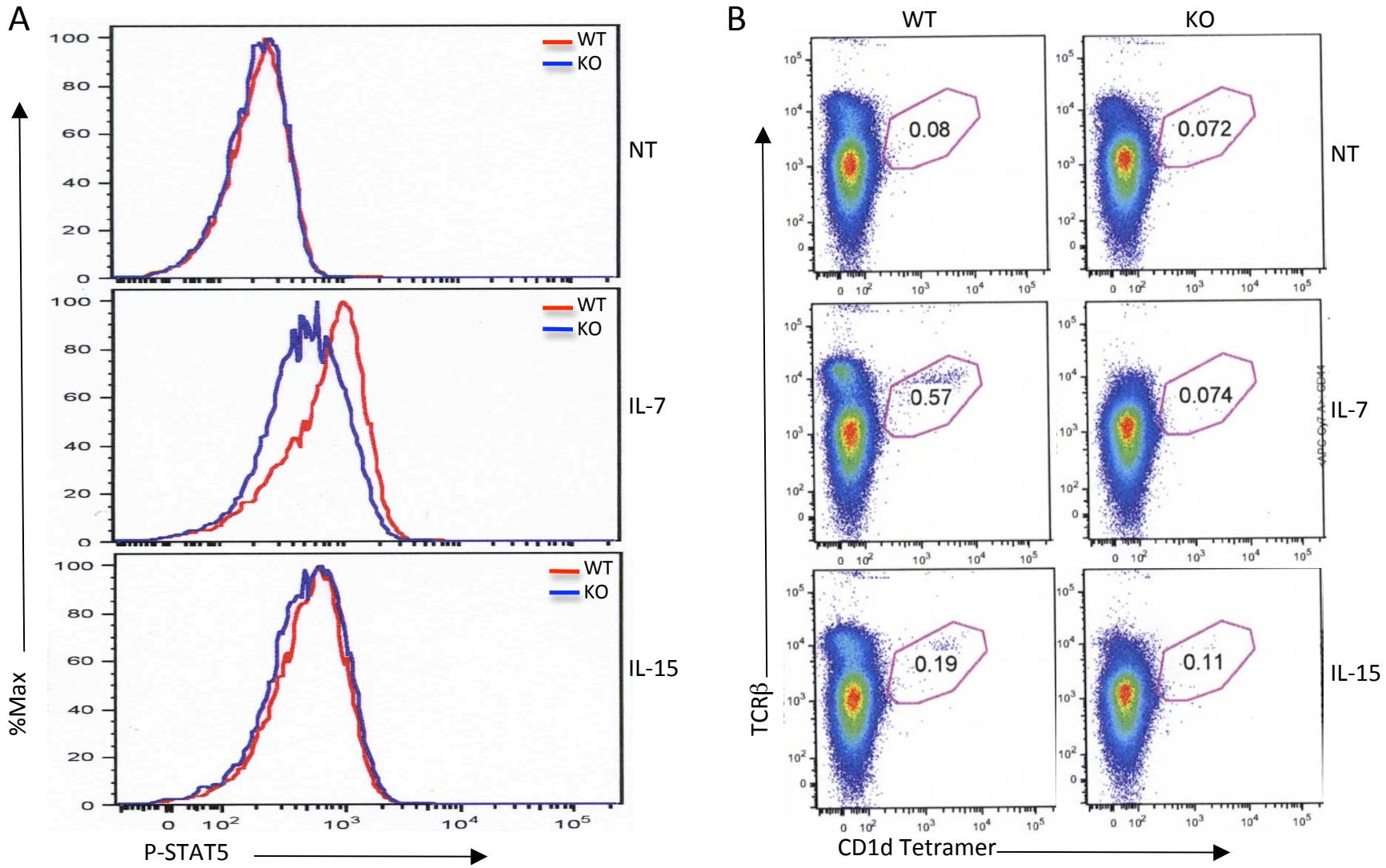
S-Fig. 7. Thymocytes were prepared from wildtype (WT) or CYLD knockout (KO) Vα14 transgenic (Vα14Tg) mice (6 wk old) and subjected to flow cytometry assays to measure CD69 expression on gated NKT subpopulations.



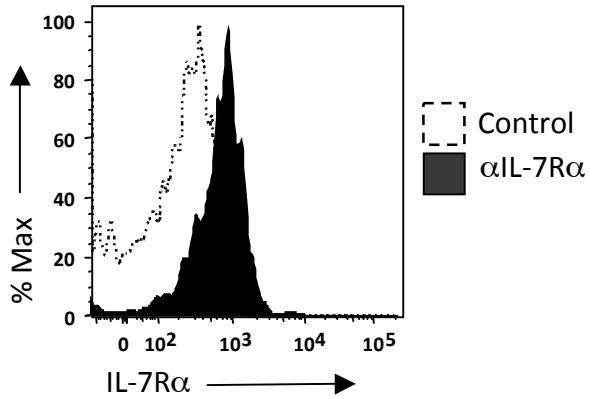
S-Fig. 8. CYLD deficiency does not significantly alter the expression of Fas ligand (FasL). Thymocytes and splenocytes of WT or CYLD KO mice were subjected to flow cytometry assays to detect FasL-expressing populations of NKT cells (gated on TCRβ^+CD1dTetramer$^+$ cells).



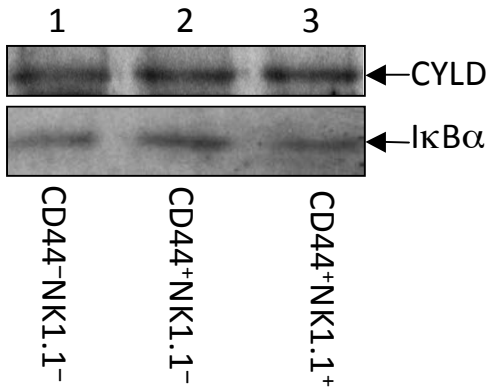
S-Fig. 9. CYLD regulates IL-7-stimulated NKT cell proliferation. Thymocytes were prepared from wildtype (WT) and CYLD knockout (KO) Vα14 transgenic mice (3 WT and 6 KO). NKT cells were first enriched by depleting CD8+ cells (using EasySep CD8+ selection kit, Stemcell Technologies) and then stained with fluorescence-conjugated CD1d-tetramer and anti-NK1.1. The tetramer$^+$NK1.1$^-$ and tetramer$^+$NK1.1$^+$ NKT cell populations were isolated by flow cytometric cell sorting. The isolated cells were incubated in 96 well plate either without (NT) or with IL-7 (50ng/ml) for 5 days and then pulsed for 12 hrs with ^{3}H-thymidine for proliferation assays. Data are presented as mean ± s.d.



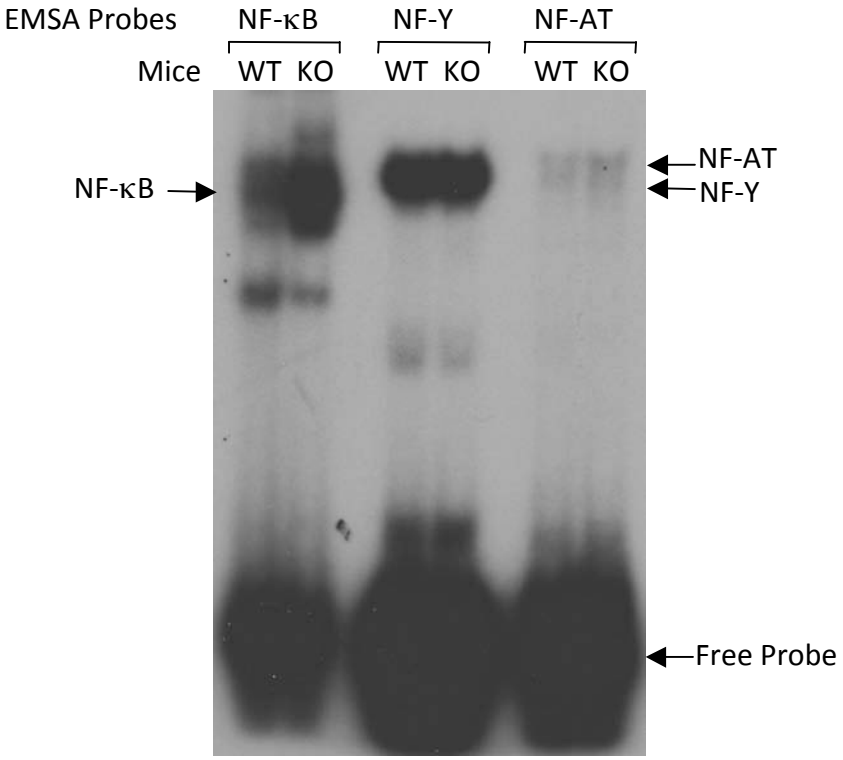
S-Fig. 10. CYLD is dispensable for IL-15-stimulated signaling in NKT cells. (A) Thymocytes from wildtype (WT) or CYLD knockout (KO) Vα14Tg mice were enriched for NKT cells by depleting $CD8^+$ cells using magnetic beads. The cells were incubated for 30 min with either medium (NT), IL-7 (50 ng/ml), or IL-15 (50 ng/ml) and then subjected to intracellular staining of tyrosine-phosphorylated STAT5 (P-STAT5) and NKT surface markers. STAT5 phosphorylation was analyzed in gated NKT cells. (B) NKT-enriched thymocytes derived from WT and CYLD KO mice were incubated *in vitro* with either medium (NT), IL-7 (50 ng/ml), or IL-15 (50 ng/ml) for 48 hr, and the frequency of NKT cells was determined by flow cytometry.



S-Fig. 11. CYLD KO thymocytes were analyzed (by flow cytometry) for IL-7Rα expression on gated NKT cell population.



S-Fig. 12. CYLD expression in different stages of NKT cells. Purified NKT cell subpopulations were subjected to immunoblotting analyses to examine the expression level of CYLD. IκBα was used as a control.



S-Fig. 13. NF-κB, but not NF-AT, is hyper-activated in CYLD knockout (KO) thymocytes. Thymocytes were isolated from wildtype (WT) or CYLD knockout (KO) mice. Nuclear extracts were subjected to EMSA using the indicated probes. The DNA-bound NF-κB, NF-AT, as well as control NF-Y, complexes are indicated.

Mouse	MFI
WT	1144
KO	871
KO-IκBαSR	1049

S-Figure 14. Thymocytes derived WT, CYLD KO, or CYLD KO-IκBαSR mice were depleted of $CD8^+$ cells and subjected to flow cytometry to determine the expression level of IL-7Rα on gated NKT ($TCR\beta^+CD1d$ tetramer$^+$) cells. Data are presented as mean fluorescence index (MFI).