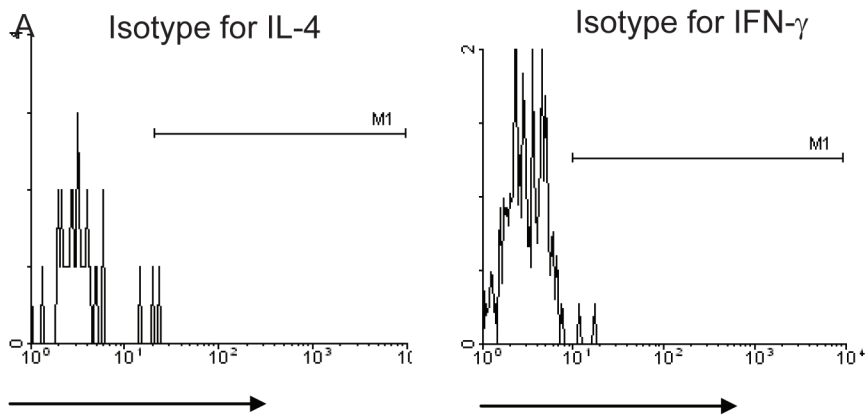
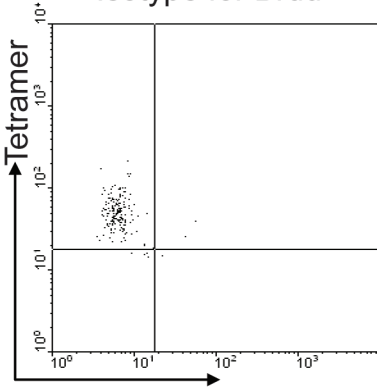


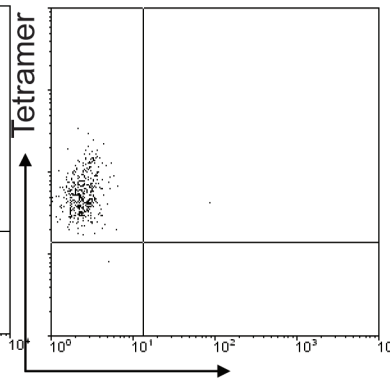
SI. 1



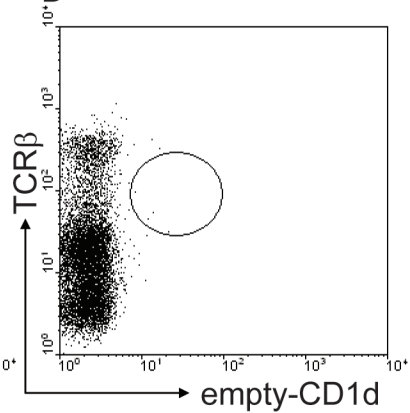
B Isotype for Brdu



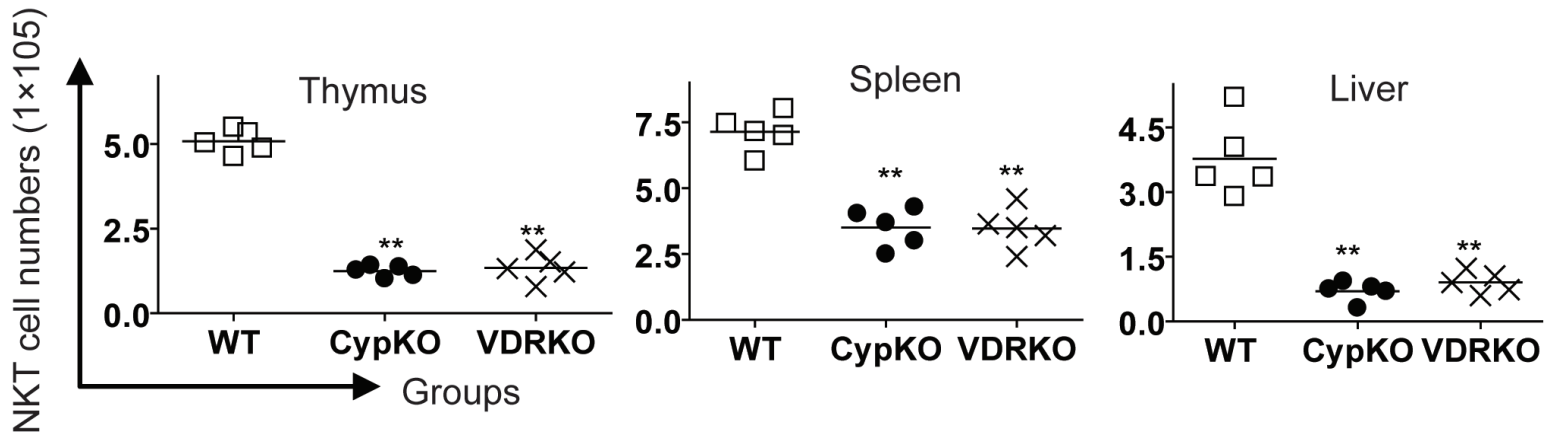
C Negative control
for annexin V



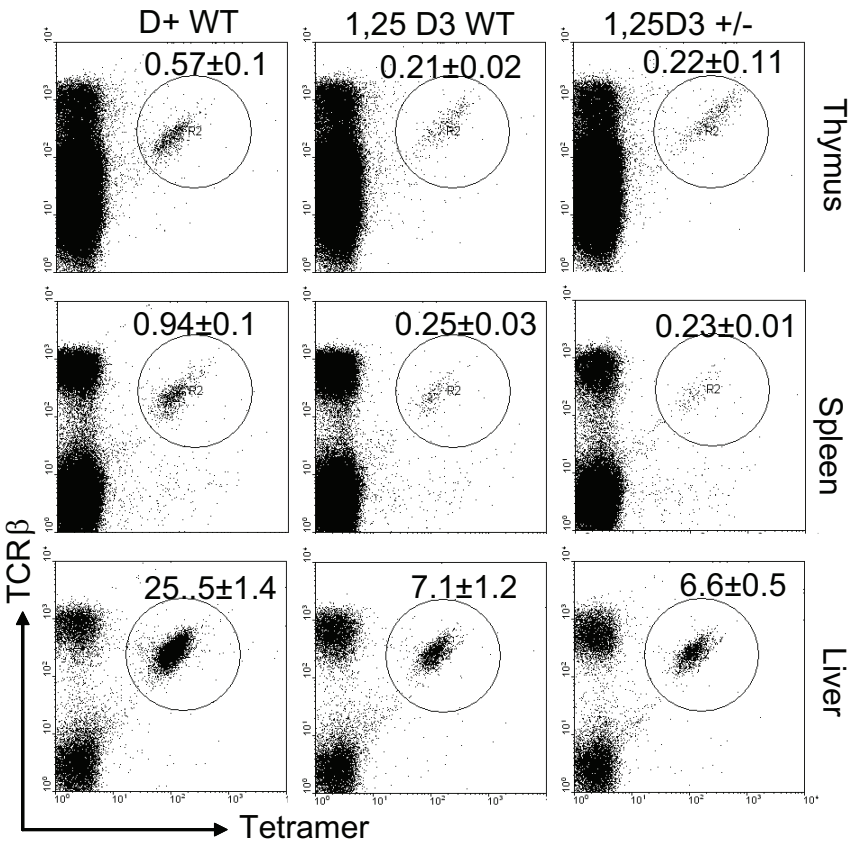
D



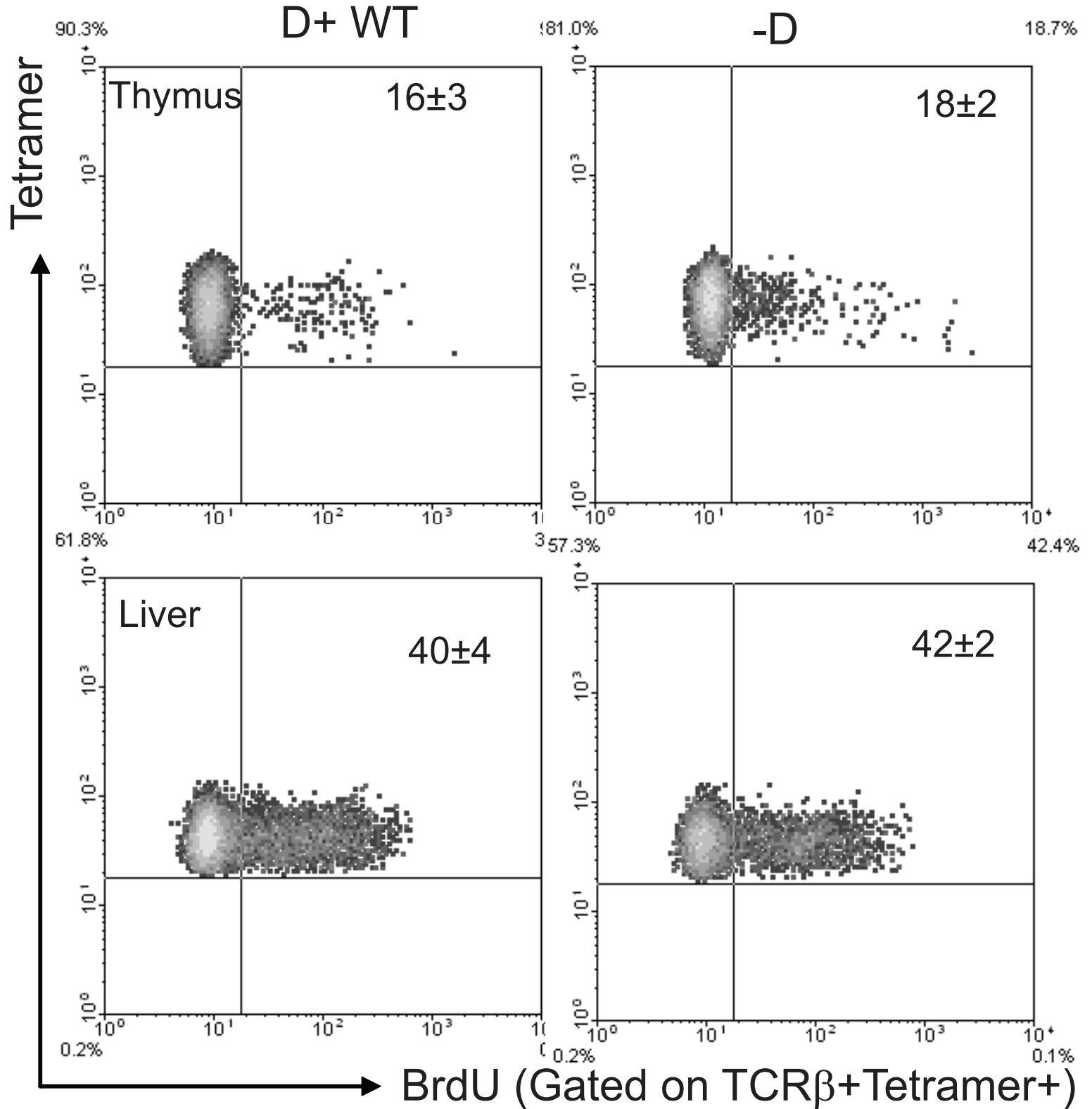
SI. 2



SI 3



SI 4



Supplementary information

Supplementary figure 1. Negative control staining for flow cytometry. (A) TCR β^+ and α GalCer-CD1d tetramer $^+$ cells are gated on. Histograms show isotype control for IL-4 (left panel) and IFN- γ (right panel). (B) Histogram showing tetramer positive isotype control staining for BrdU. (C) Histogram shows negative control staining for annexin V gated on tetramer positive cells. (D) Dot plot showing TCR β^+ and empty CD1d control staining.

Supplementary figure 2. Reduced numbers of iNKT cells in 1,25D3 deficient mice. Total number of iNKT cells in the thymus, liver and spleen of WT, Cyp27B1 KO, and VDR KO mice (n=5). Line is the mean for the group. **, $p < 0.001$

Supplementary figure 3. Exposure to 1,25D3 at embryonic day 13 fails to rescue iNKT cell development. Breeders were fed D- diets and switched to 25ng/mouse/ day of 1,25D3 at embryonic day 13. Values are mean \pm SEM of n=5 mice per group. Percentage of iNKT cells in 1,25D3WT and 1,25D3 +/-ko is significantly lower than values from D+WT mice ($p < 0.05$).

Supplementary figure 4. Vitamin D status does not affect homeostatic proliferation of iNKT cells. BrdU incorporation by iNKT cells from D+ WT and D- mice. Mice were injected ip with 100 μ l of 10 mg/ml BrdU dissolved in PBS at the onset of experiments. At the same time, mice were fed water that contained 0.8mg/ml BrdU/5% glucose mixture and the water was changed daily. 9d later thymocytes, and mononuclear cells from the liver were analyzed for BrdU incorporation using a BrdU flow kit and the

manufacturers instructions (BD Pharmingen). Dot plots showing percentage of BrdU positive iNKT cells in D+ WT and D- controls. Isotype control staining for BrdU is shown in supplementary Fig.1. Data show one representative of 8 mice per group. Values are mean \pm SEM. Numbers indicate percentage of BrdU positive iNKT cells.