Figure S1. Normal development of DP thymocytes in CD1d-EYFP expressing mice.

Thymocytes from 6-9 week old mice were stained with CD4 and CD8 mAbs and percentage of DP thymocytes was assessed by flow cytometry. Surface CD1d expression between CD1d+/+ and CD1d-EYFP/EYFP or CD1d-EYFP/+ is significantly different ($p<0.05$). Comparable development of DP thymocytes was found in CD1d+/+, CD1d-EYFP/EYFP, CD1d-EYFP/+, CD1d+/- and CD1d-/- mice (NS, $p>0.1$).

Figure S2. Increased apoptosis among Vα14 iNKT cells from CD1d-EYFP/EYFP mice.

A. Thymocytes from CD1d+/+ or CD1d-EYFP/EYFP mice were incubated for 30 minutes at 37°C to induce apoptosis. After incubation cells were incubated with Annexin V, mAbs against TCRβ, CD44, NK1.1, ⟨GalCer-loaded CD1d-tetramers and analyzed by flow cytometry. Data shown are representative of at least 3 experiments. The bars represent the mean of at least triplicate values and the brackets indicate SEM. Vα14 iNKT cells from CD1d-EYFP/EYFP mice undergo significantly more apoptosis than CD1d+/+ Vα14 iNKT cells at any age (* $p<0.01$).

B. Representative histogram of AnnexinV staining on mature ($CD44^+NK1.1^+$) Vα14 iNKT cells from 6-9 week old mice. Mature CD1d-EYFP/EYFP Vα14 iNKT cells exhibit significantly more Annexin V staining than CD1d+/+ Vα14 iNKT cells ($p<0.05$).

Figure S3. Decreased production of antigen-induced IL-2 by Vα14 iNKT cells selected on CD1d-EYFP molecules.

Activation of Vα14 iNKT cells *in vitro*. Spleen DCs and liver Vα14 iNKT cells from WT (CD1d+/+) or CD1d-EYFP/EYFP mice were co-cultured in the presence of 100 ng/ml αGalCer (**A**) or 100 ng/ml Gal(α1→2)GalCer (**B**). Results displayed in bar graphs are representative of 2 experiments. Shown are the mean of triplicate values with SEM. Production of IL-2 by CD1d-EYFP/EYFP Vα14 iNKT cells was significantly reduced compared to their CD1d+/+ counterparts (* $p<0.001$). Presentation of Gal(α1→2)GalCer by CD1d-EYFP/EYFP spleen DCs resulted in significantly less IL-2 production by wild-type Vα14 iNKT cells (* $p<0.05$).

Figure S4. Increased surface expression and delay in endocytosis of CD1d-EYFP fusion proteins in T cells, but not in professional APCs.

A. Surface expression of CD1d on DP thymocytes, spleen $CD4^+$ T cells and spleen DCs. DP thymocytes and spleen CD4+ T cells, but not spleen DCs from CD1d-EYFP/EYFP mice express increased surface levels of CD1d compared to wild-type (T cells: $p<0.05$; DCs: NS, $p>0.05$).

B. The extent of internalized CD1d bound by PE-conjugated anti-CD1d antibody in thymocytes, $CD4^+$ T cells and DCs from CD1d+/+, CD1d-EYFP/EYFP and CD1d-/- mice was visualized by flow cytometry. CD1d-EYFP molecules on DP thymocytes exhibit a significantly longer surface retention time than wild-type CD1d molecules, demonstrated within 0.5 hours of endocytosis induction ($p<0.005$). CD1d-EYFP molecules on $CD4^+$ T cells in the periphery exhibit a significantly longer surface retention time than wild-type CD1d molecules ($p<0.05$). Professional APCs in the periphery display less CD1d endocytosis activity than T cells. No difference was found between endocytosis of CD1d-EYFP molecules and CD1d (NS, $p>0.05$). Control thymocytes from CD1d-deficient mice show no CD1d staining at any of the

time points. Histograms shown are representative of 3 independent experiments. Data points in the graph represent the mean of quadruplet values and brackets indicate SEM.

Figure S1

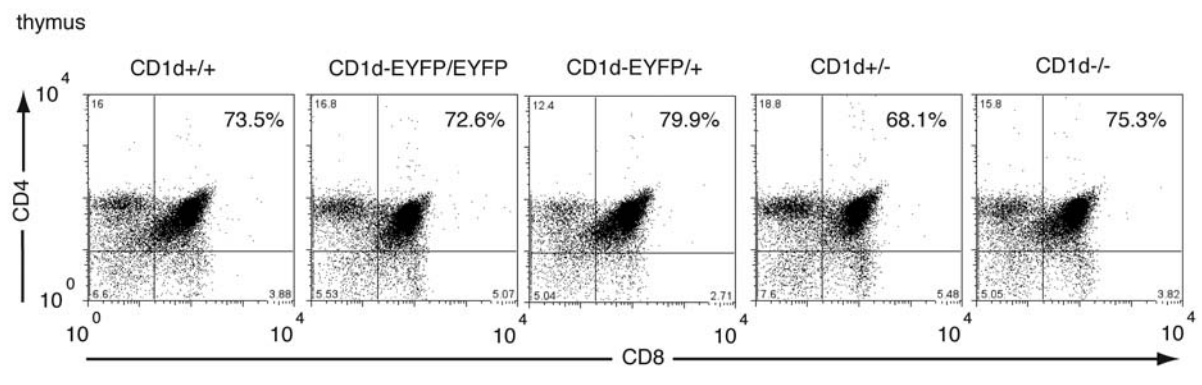

Figure S2

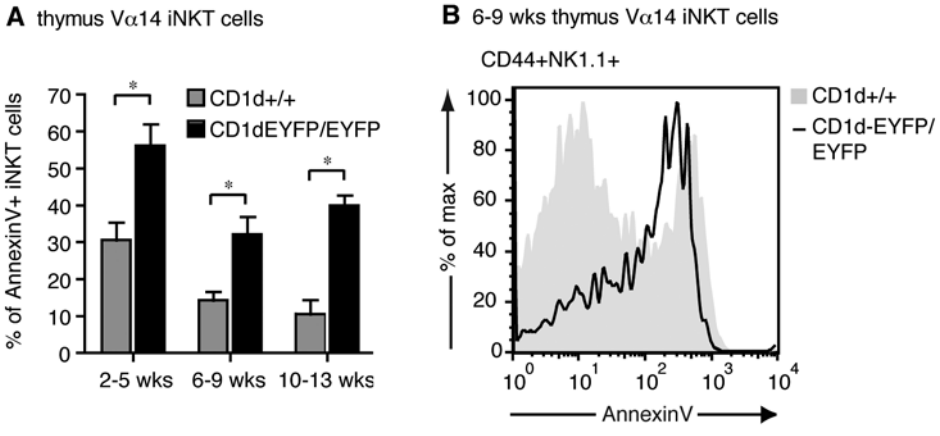

Figure S3

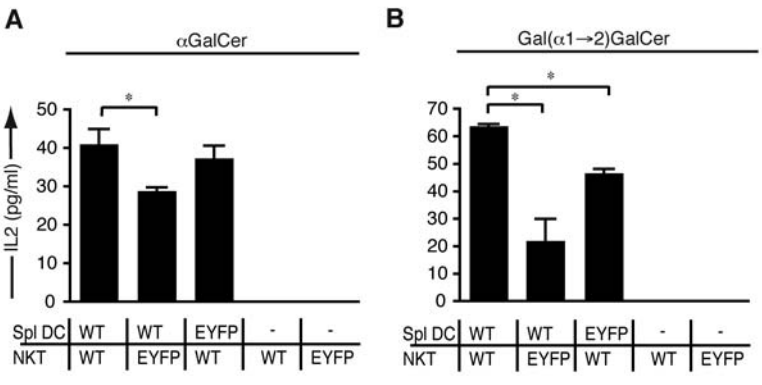

Figure S4