Supporting Information

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Fig. S1. $Id2^{+/+}$ and $Id2^{+/-}$ mice generate equivalent NKT cell populations. (A) Percentage of NK1.1⁺TCR β^+ and CD1d tetramer⁺ NKT cells in the liver, thymus, spleen, and bone marrow of 6-week-old $Id2^{+/+}$ and $Id2^{+/-}$ mice. (B) Absolute cell numbers of NK1.1⁺ TCR β^+ or CD1d tetramer⁺ NKT cells for indicated tissues. (C) Percentage of α GalCer-loaded CD1d tetramer⁺ NKT cells for indicated developmental subsets. Hepatic and thymic NKT cells were gated on expression of TCR β and α GalCer-loaded CD1d tetramer, then examined for expression of NK1.1 and CD44. (D) Mean fluorescence of CXCL16-Fc binding to NK1.1⁺ TCR β^+ NKT cells in the liver, thymus, spleen, and bone marrow. All data shown are the average (± SEM), $n = 3 Id2^{WT}$ and $3 Id2^{+, *}$, P < 0.05, **, P < 0.005, ***, P < 0.0005. Statistical significance determined using unpaired two-tailed *t*-test.



Fig. 52. Normal frequency and numbers of naive T cell populations in $Id2^{KO}$ -reconstituted chimeras. (A) Representative flow cytometry plots of CD4⁺ and CD8⁺ expression by lymphocytes for chimeras reconstituted with $Id2^{KO}$ or $Id2^{WT}$ donor cells for indicated tissues. Average (± SEM) frequency (*B*) and total cell numbers (*C*) for CD4⁺ and CD8⁺ conventional T cells, n = 3 pairs of $Id2^{KO}$ and $Id2^{+}$ chimeras.

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Fig. S3. Expression of CXCR6, bcl-2 and bcl-X_L by conventional $Id2^{KO}$ and $Id2^{WT}$ T cells. Expression of (A) CXCR6, (B) bcl-2, and (C) bcl-X_L determined by flow cytometry. Conventional T cells from samples shown in Fig. 5 were identified by expression of congenic marker and were NK1.1⁻TCR β^+ . Isotype controls dashed lines; $Id2^{KO}$ unfilled; $Id2^+$ shaded. Flow cytometry data are representative of at least four pairs of $Id2^{KO}$ and $Id2^+$ chimeras.

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Fig. 54. Less dramatic defect by CXCR6-deficient NKT cells. (A) Representative flow cytometry plots identifying NKT cells by NK1.1 and TCR β expression in liver of CXCR6^{WT} or CXCR6^{KO} mice. (B) Representative histograms of intracellular staining for bcl-2 or bcl-X_L by NK1.1⁺TCR β ⁺ NKT cells as gated in (A). Isotype controls dashed lines; Id2^{KO} unfilled; Id2⁺ shaded. Flow cytometry data are representative of three CXCR6^{WT} or CXCR6^{KO} pairs.

A Thymus



Fig. S5. Analysis of NKT cell populations in thymus and spleen of chimeras reconstituted with $Id2^{WT}$ or $Id2^{KO}$ bim-deficient donor cells. Analysis of lymphocytes recovered from $Id2^{+}bim^{+/-}$, $Id2^{+}bim^{+/-}$, $Id2^{+}bim^{-/-}$, or $Id2^{KO}$ bim^{-/-} fetal liver chimeras. (*A*) Representative flow cytometry plots identifying NKT cells by TCR β and CD1d tetramer staining of thymus. (*B*) Average percentage (\pm SEM) of indicated populations from (*A*). (*C*) Representative flow cytometry plots identifying NKT cells by TCR β and CD1d tetramer staining of spleen. (*D*) Average percentage (\pm SEM) of indicated populations from (*C*). Data are representative of three independent experiments with n = 3-5 per group. Statistical significance determined using unpaired two-tailed *t*-test where ns >0.05.