Supporting Information

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SI Materials and Methods

Flow Cytometry. Thymi and spleens were mashed into single-cell suspensions and erythrocytes were lysed. Liver cell suspensions were purified over a Percoll gradient (GE Healthcare). Briefly, liver cells were resuspended in a 20% Percoll solution and underlaid with 70% and 40% Percoll solutions. Hepatic mononuclear cells were isolated by centrifugation at 900 × *g* for 1 h at room temperature, collected at the 70% to 40% interface, and stained for FACS analysis. Cells were blocked with 10 μg of Rat IgG antibody (Jackson ImmunoResearch) in PBS/5% FCS for 15 min on ice. Cells were then washed once with PBS/5% FCS and stained with fluorescently labeled antibodies (clones are indicated in parentheses) against B220 (RA3-6B2), CD1d (1B1), CD4 (L3T4), CD8α (53-6.7), CD24 (M1/69), CD44 (IM7), CD45.2 (104), CD69 (H1.2F3), IL-2Rβ (TM-b1), IL-4 (BVD6-24G2), IFN-γ (XMG1.2), Mac-1 (M1/70), NK1.1 (PK136), TCRβ (H57-597), T-bet (eBio4B10), GATA-3 (L50-823), Bcl-x_L (H5), and PLZF (D-9) purchased from eBioscience, BD Biosciences, Caltag, or Santa Cruz Biotechnology. Unloaded and PBS-57–loaded CD1d tetramers were provided by the National Institutes of Health Tetramer Facility. For intracellular staining, cells were first stained for surface markers, washed, and then fixed and permeabilized following manufacturer's instructions (Foxp3 Staining Buffer Set; eBioscience). Intracellular cytokine staining was performed using BD Cytofix/Cytoperm solutions (BD Biosciences). Flow cytometric analysis and cell sorting were performed using a BD FACS LSRII or Aria (Beckton Dickinson). Results were analyzed using FlowJo software (TreeStar).

Quantitative RT-PCR. Total RNA was extracted with TRIzol solution (Invitrogen) and reverse transcription was carried out using the SuperScript-II-kit (Invitrogen). Quantitative RT-PCR was performed on the 7500 Fast Real-time PCR System of Applied Biosystems using the Roche probe library or nonspecific product detection (SYBR Green). Primer sequences and probe numbers for the genes analyzed using the Roche probe library are listed in the table below. Primer sequences for the genes analyzed by SYBR Green are from D'Cruz et al. (1).

Genes	Primer sequence (5′ to 3′)	Probe#
Vα14-F	GGA TGA CAC TGC CAC CTA CAT	#75
Jα18-R	GCT GAG TCC CAG CTC CAA	
Cα-F	ATG GAT TCC AAG AGC AAT GG	#1
Cα-R	GCA TCA CAG GGA ACG TCT G	
Med1-F (cDNA)	GGA CCT TTC TAA AAT GGC TAT TAT GT	#97
Med1-R (cDNA)	CGG GGT GAG ATA ACC AAC AC	
Med1-F (gDNA)	ATA TAT GTG TGG GGG CCA GA	#27
Med1-R (gDNA)	TGC CTC AAG GGA CTA AAG CA	
GATA-3-F	GAC TCT TCC CAC CCA GCA	#3
GATA-3-R	CCC CGC AGT TCA CAC ACT	
T-bet-F	GAA AGG CAG AAG GCA GCA T	#72
T-bet-R	GAG CTT TAG CTT CCC AAA TGA A	
RORγt-F	GGT GAC CAG CTA CCA GAG GA	#22
RORγt-R	AGC TCC ATG AAG CCT GAA AG	
c-Myb-F	TGT CAA CAG AGA ACG AGC TGA	#40
c-Myb-R	GCT GCA AGT GTG GTT CTG TG	
HEB-F	TGG GAA AAC GAG ACC AAC A	#48
HEB-R	CTC CAC GCT CAT CCA TAC CT	
Id2-F	GAC AGA ACC AGG CGT CCA	#89
Id2-R	AGC TCA GAA GGG AAT TCA GAT G	
VDR-F	TGG ACA TTG GCA TGA TGA A	#69
VDR-R	GGC CTC AGA CTG TCC TTC AA	
ZAP70-F	CAG ACC GAC GGC AAG TTC	#68
ZAP70-R	CAT AGA CCA GGG ACA GTG CAT	
$S1P_1$-F	CGG TGT AGA CCC AGA GTC CT	#66
$S1P_1$-R	AGC TTT TCC TTG GCT GGA G	
Itk-F	GGA AAA AGC TTG TGT CAT CCA	#71
Itk-R	CCA AAG TCG GAC ACC TTG AT	
Bcl-2-F	GTA CCT GAA CCG GCA TCT G	#75
Bcl-2-F	GGG GCC ATA TAG TTC CAC AA	
Bcl-x_L-F	CCT TGG ATC CAG GAG AAC G	#66
Bcl-xL-F	CAG GAA CCA GCG GTT GAA	

Generation of Bone-Marrow Chimeras. Recipient CD45.1$^+$ congenic mice were subjected to 950 rads irradiation 1 d before transfer. Bone-marrow cells were harvested from the tibiae and femurs of donor mice. 1×10^7 cells were injected intravenously into the recipient mice. The resulting chimeras were analyzed 10 to 12 wk after bone marrow reconstitution.

In Vivo α-GalCer Stimulation. Two micrograms of α-GalCer in 100 μL PBS was intravenously injected into mice. Mice were bled at 2 and 5 h after injection. Blood was allowed to clot and the serum was separated from the clots. IFN-γ and IL-4 production were measured by ELISA following the manufacturer's instructions (ELISA Ready-SET-Go; eBioscience). For the secondary activation of B cells, T cells, and macrophages, splenocytes were collected 5 h after injection and stained with antibodies against CD69, B220, CD8α, and Mac-1.

BrdU-Labeling Assay. Mice were injected intraperitoneally with 1 mg BrdU. Twenty-four hours later, thymi were isolated and mashed into single-cell suspensions. Cells were first stained for cell surface markers. After fixation and permeabilization using BD Cytofix/Cytoperm solutions (BD Biosciences), cells were treated with 300 μg/mL DNase for 1 h at 37 °C. BrdU incorporation was detected using a FITC labeled anti-BrdU antibody (eBioscience).

Annexin V Staining. Freshly isolated total thymocytes were cultured in complete RPMI medium in vitro. Samples were taken at various time points and stained for cell surface markers. Next, 1×10^6 cells were resuspended in 1 mL Annexin V binding buffer. Then, 100 μL of resulting cell suspension was incubated with 5 μL APC-labeled Annexin V (eBioscience) for 15 min at room temperature in the dark. Next, 400 μL Annexin V binding buffer was added and the cells were analyzed by flow cytometry within 1 h.

Antigen Presentation Assay. For the antigen presentation assay, 1×10^6 thymocytes isolated from Med1$^{+/-}$ and Med1$^{-/-}$ mice were incubated with 5×10^4 NKT cell hybridoma DN32.D3 (kindly provided by A. Bendelac, University of Chicago, Chicago, IL)

with or without α-GalCer (100 ng/mL), as previously described (2, 3). Supernatants were collected 24 h later and IL-2 production was assayed by ELISA according to the manufacturer's instructions (eBioscience).

1. D'Cruz LM, Knell J, Fujimoto JK, Goldrath AW (2010) An essential role for the transcription factor HEB in thymocyte survival, Tcra rearrangement and the development of natural killer T cells. *Nat Immunol* 11:240–249.
2. Egawa T, et al. (2005) Genetic evidence supporting selection of the Valpha14i NKT cell lineage from double-positive thymocyte precursors. *Immunity* 22:705–716.
3. Lazarevic V, et al. (2009) The gene encoding early growth response 2, a target of the transcription factor NFAT, is required for the development and maturation of natural killer T cells. *Nat Immunol* 10:306–313.

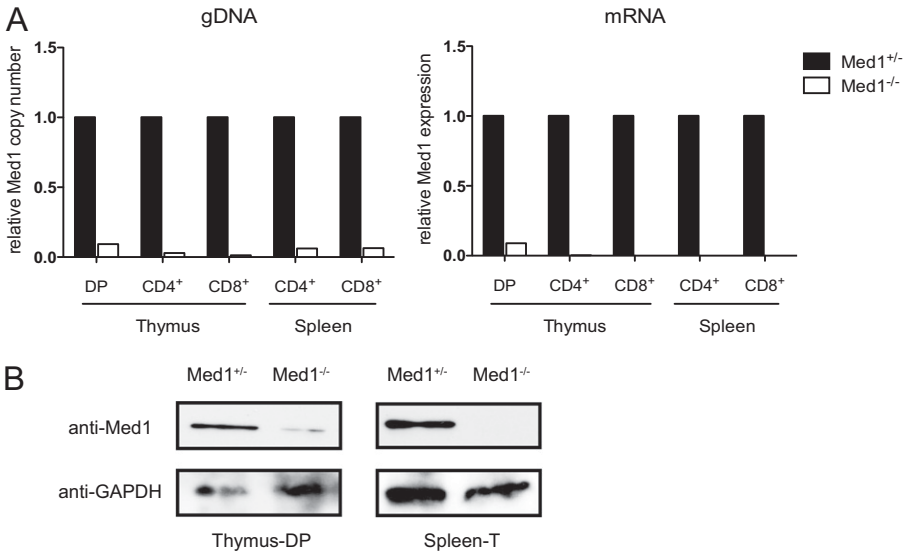

Fig. S1. Quantitative real-time PCR and Western blot analysis of Med1 level in T-cell lineage. (*A*) Med1 level by sorted thymocyte and splenocyte subpopulations was determined by qRT-RCR at both gDNA (*Left*, normalized to a control genomic sequence: −106 kb upstream of the GATA-1 locus) and mRNA (*Right*, normalized to the expression of TATA binding protein: TBP) level. (*B*) Western blot was performed on sorted $CD4^+CD8^+$ double-positive (DP) thymocytes and $TCR\beta^+$ splenic T cells using Med1-specific antibody; GAPDH was used as a loading control.

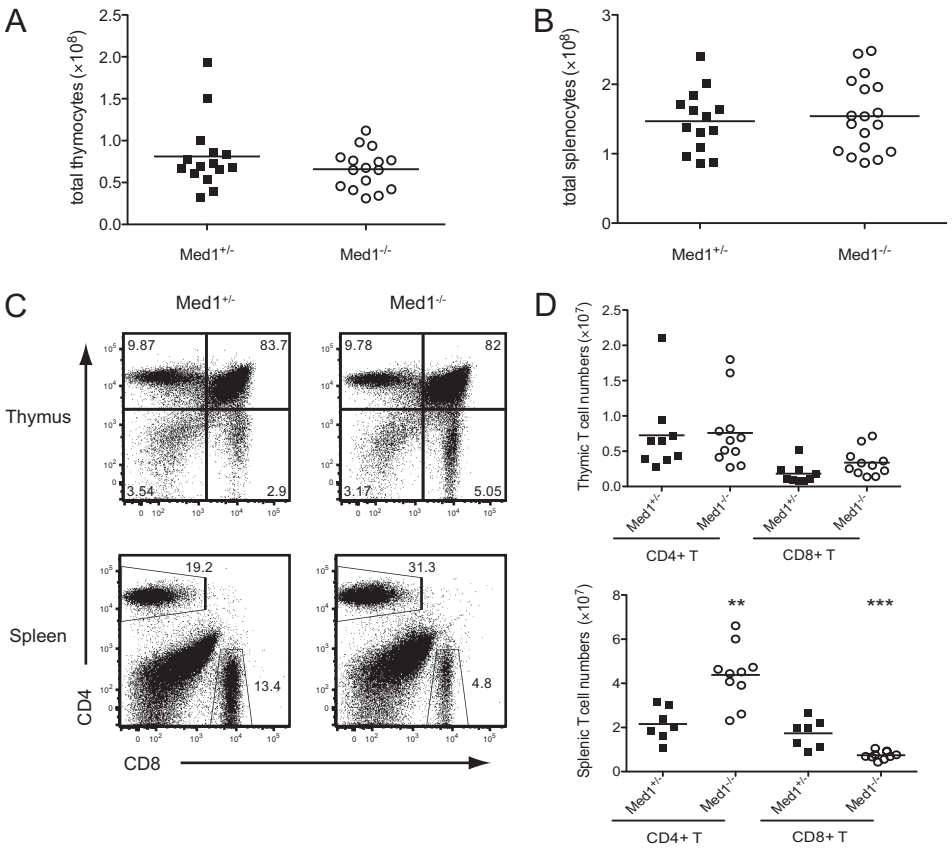

Fig. S2. Analysis of thymic and splenic total cellularity and conventional T-cell compartments. Total thymocyte (*A*) and splenocyte (*B*) numbers are comparable in Med1$^{+/-}$ and Med1$^{-/-}$ mice. (*C*) Representative flow cytometric plots of thymic (*Upper*) and splenic (*Lower*) conventional CD4$^+$ and CD8$^+$ T cells in Med1$^{+/-}$ or Med1$^{-/-}$ mice. The percentages are indicated in each plot. (*D*) Statistical analysis of thymic (*Upper*) and splenic (*Lower*) conventional CD4$^+$ and CD8$^+$ T cells. Data are representative of at least five independent experiments. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ (unpaired two-tailed *t* test).

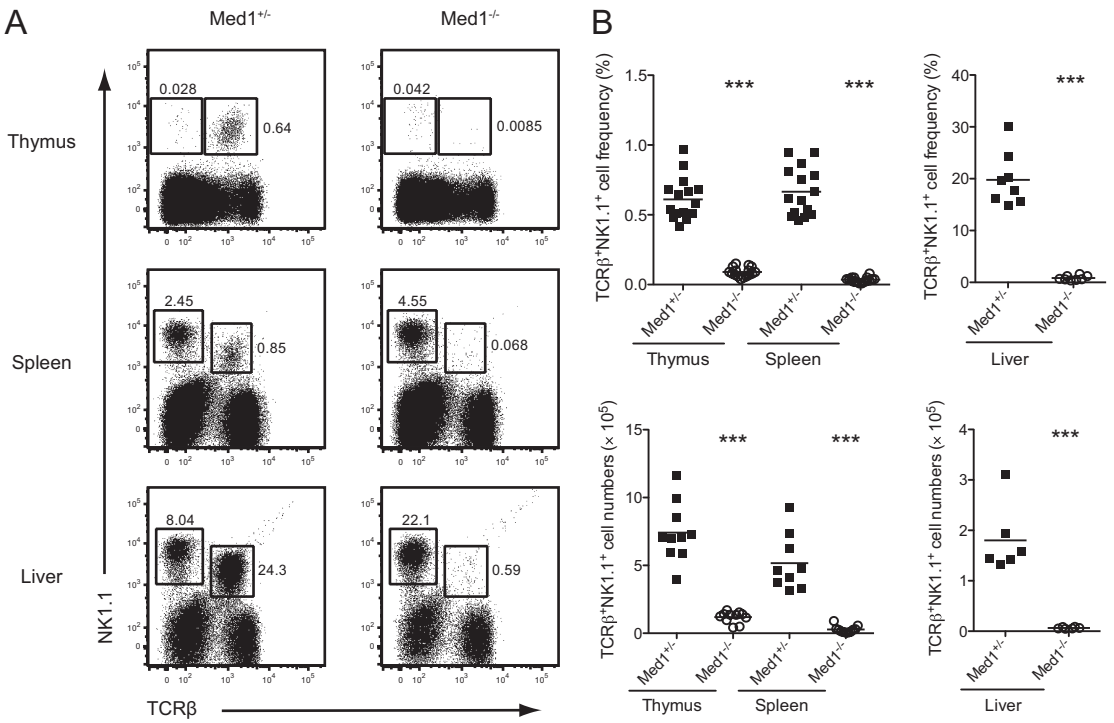

Fig. S3. TCRβ$^+$ NK1.1$^+$ cells are significantly reduced in the absence of Med1. (*A*) Representative dot plots depicting the staining of thymocytes, splenocytes, and liver mononuclear cells from Med1$^{+/-}$ or Med1$^{-/-}$ mice with anti-TCRβ and anti-NK1.1 antibodies. The percentages of TCRβ$^+$NK1.1$^+$ cells are indicated in each plot. (*B*) Statistical analysis of the frequency (*Upper*) and absolute cell numbers (*Lower*) of TCRβ$^+$NK1.1$^+$ cells in the thymus, spleen, and liver mononuclear cells from Med1$^{+/-}$ or Med1$^{-/-}$ mice. Numbers represent the mean of at least six mice per group. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ (unpaired two-tailed *t* test).

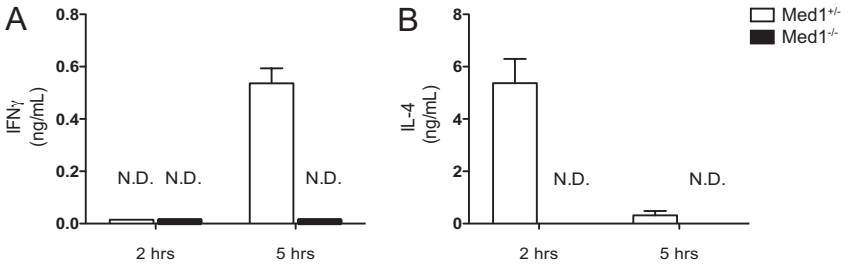

Fig. S4. Med1$^{-/-}$ invariant NKT (iNKT) cells have impaired cytokine production in response to α-GalCer stimulation. Med1$^{+/-}$ and Med1$^{-/-}$ mice were injected with 2 μg α-GalCer intravenously. Serum was collected at 2 and 5 h after injection for detection of IFN-γ (*A*) and IL-4 (*B*) production by ELISA. N.D., not detectable.

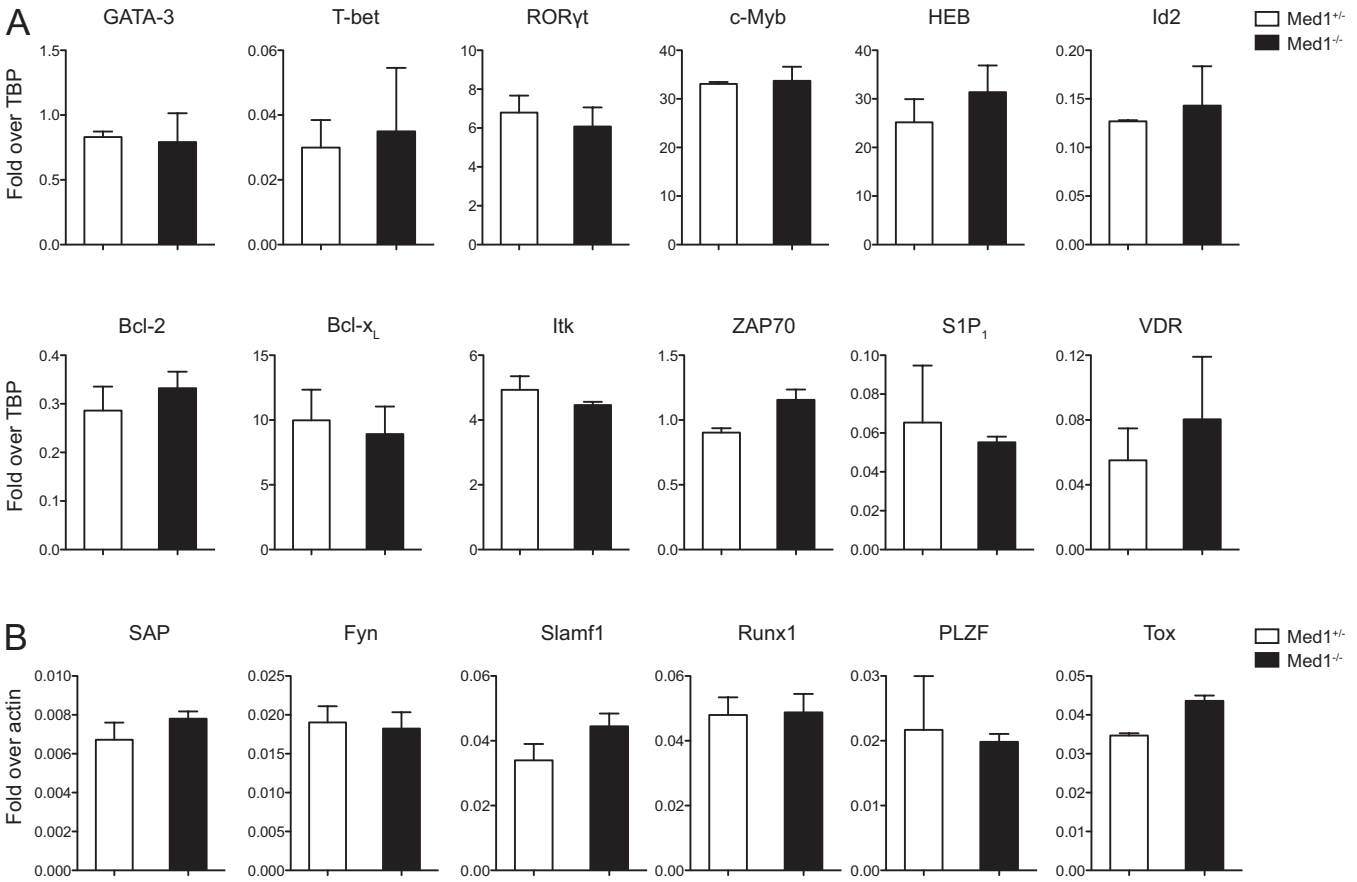

Fig. S5. Analysis of a subset of genes on sorted $CD4^+CD8^+$ double-positive (DP) thymocytes from $Med1^{+/-}$ or $Med1^{-/-}$ mice by quantitative real-time PCR. (*A*) The expression of GATA-3, T-bet, RORγt, c-Myb, HEB, Id2, Bcl-2, Bcl-x_L, Itk, ZAP70, $S1P_1$, and vitamin D receptor (VDR) was analyzed using the Roche probe library (normalized to the expression of TATA binding protein: TBP). (*B*) The expression of SAP, Fyn, Slamf1, Runx1, PLZF, and Tox was analyzed with the nonspecific product detection SYBR Green (normalized to the expression of β-actin). Results are shown as mean ± SEM from two independent experiments.

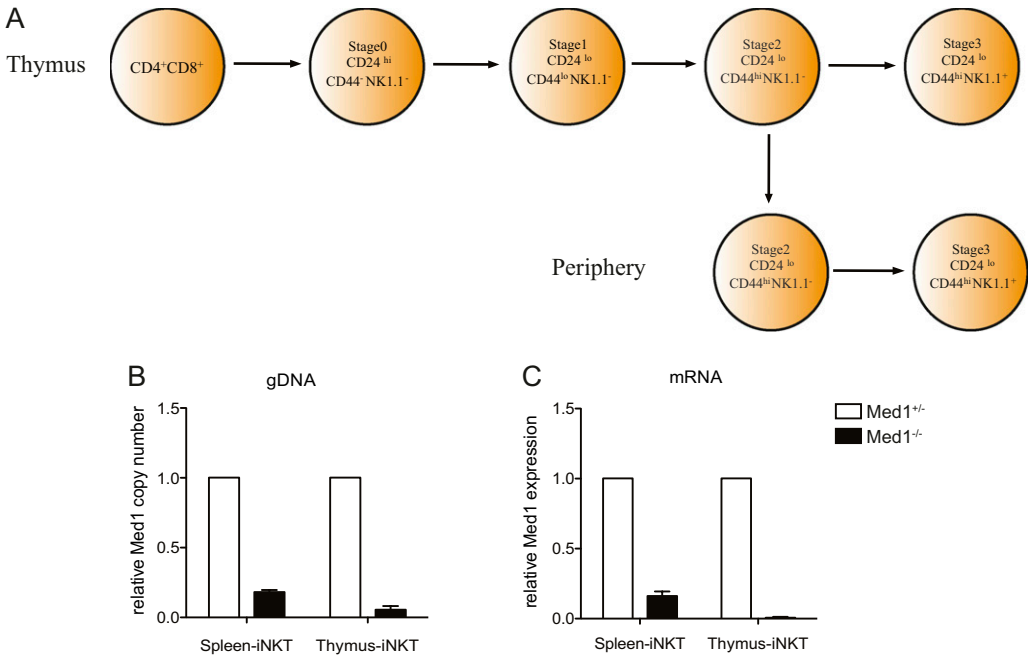

Fig. S6. (*A*) Schematic description of iNKT cell development and maturation: iNKT cells are derived from $CD4^+CD8^+$ DP thymocytes and progress through four distinct developmental stages, from stages 0 to 3, defined by the expression of CD24, CD44, and NK1.1. Stage 2 cells can emigrate from the thymus and mature in the periphery. (*B* and *C*) Med1 was efficiently deleted in splenic and thymic iNKT cells in $Med1^{-/-}$ mice: CD1d-tetramer$^+$ iNKT cells from $Med1^{+/-}$ or $Med1^{-/-}$ mice were sorted and Med1 deletion efficiency was analyzed at both gDNA (normalized to a control genomic sequence: −106 kb upstream of the GATA-1 locus) and mRNA (normalized to the expression of TBP) level by quantitative RT-PCR. Results are shown as mean ± SEM from three independent experiments.

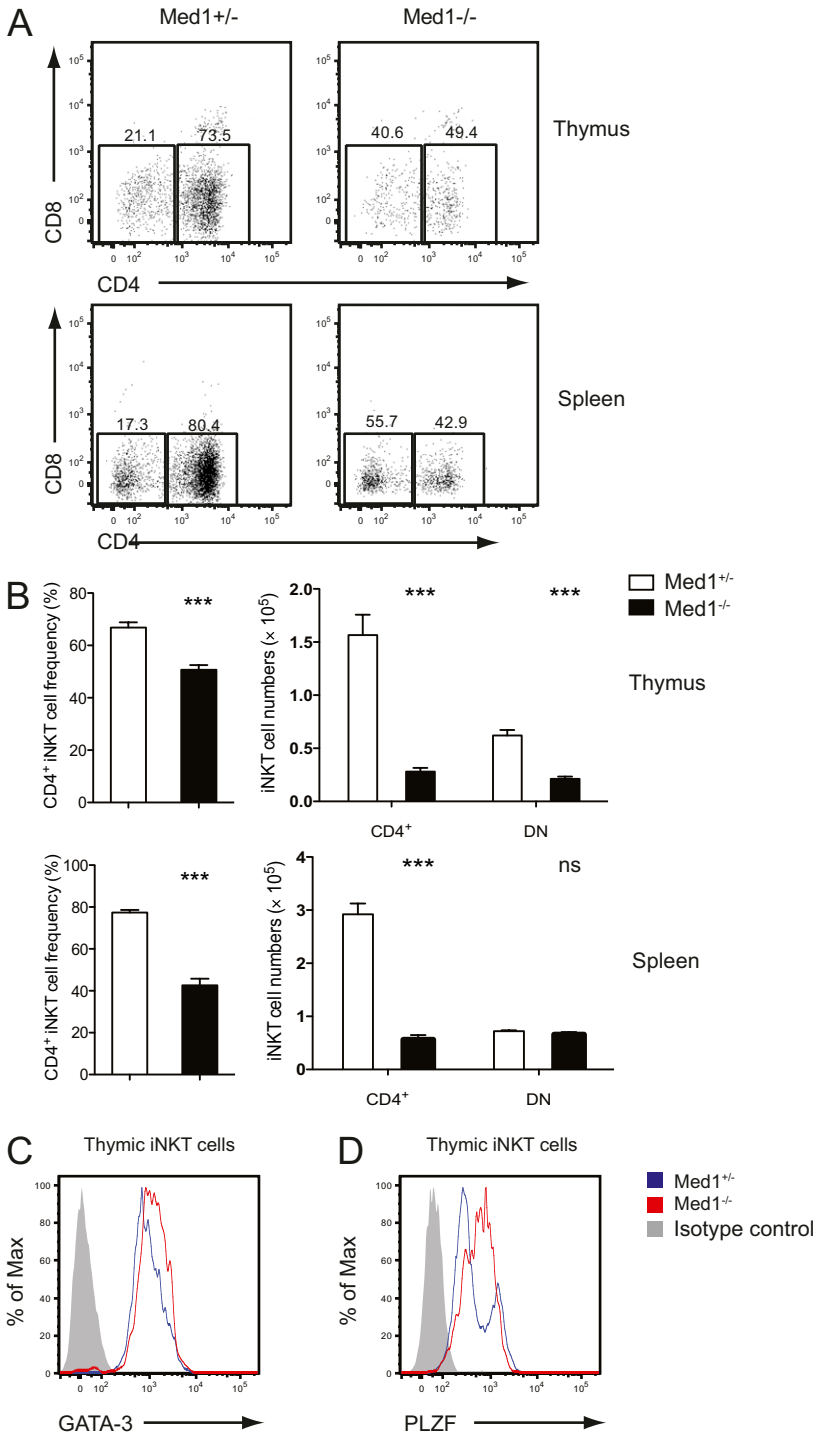

Fig. S7. Analysis of the subset composition of iNKT cells in the absence of Med1. (*A*) Expression of CD4 and CD8α by thymic (*Upper*) and splenic (*Lower*) CD1d-tetramer$^+$ iNKT cells from Med1$^{+/-}$ or Med1$^{-/-}$ mice were analyzed by flow cytometry to define the CD4$^+$ and DN (double-negative) subsets. (*B*) Statistical analysis of the percentage (*Left*) and absolute numbers (*Right*) of CD4$^+$ and DN subsets of thymic and splenic CD1d-tetramer$^+$ iNKT cells. Six mice of each genotype were analyzed. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ns: not significant (unpaired two-tailed *t* test). (*C* and *D*) The expression of GATA-3 and PLZF by thymic CD1d-tetramer$^+$ iNKT cells from Med1$^{+/-}$ or Med1$^{-/-}$ mice was analyzed by intracellular FACS staining (blue: Med1$^{+/-}$ mice; red: Med1$^{-/-}$ mice; gray solid: isotype control). Data are representative of two independent experiments.

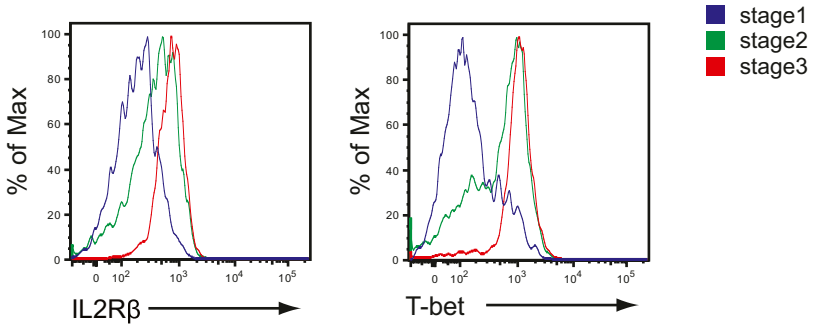

Fig. S8. Expression pattern of IL-2Rβ and T-bet in thymic iNKT cells from Med1$^{+/-}$ mice at different developmental stages. The expression of IL-2Rβ (*Left*) and T-bet (*Right*) in thymic CD1d-tetramer^{+} iNKT cells is up-regulated when the cells progress from stage1 to stage 3 (blue: stage 1 cells; green: stage 2 cells; red: stage 3 cells). Data are representative of five independent experiments.

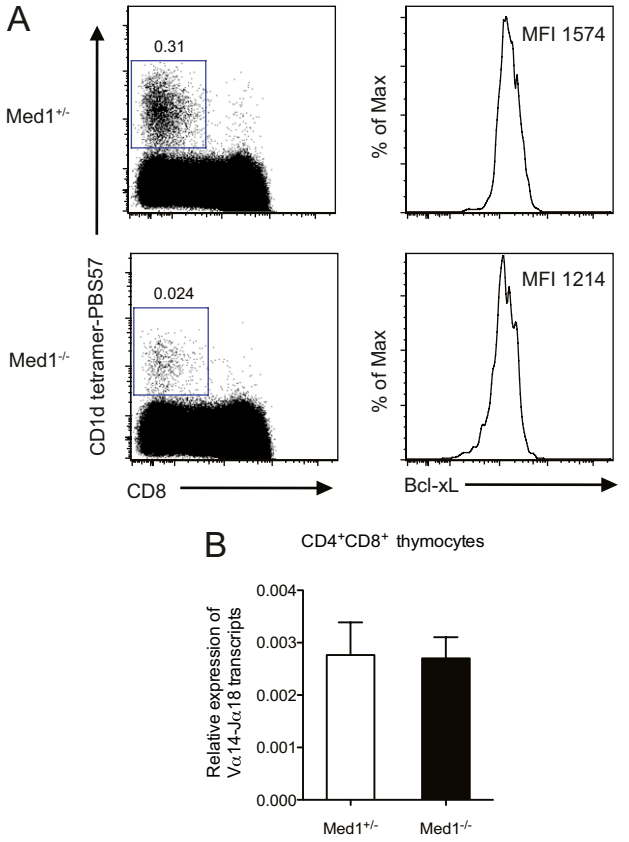

Fig. S9. (*A*) Analysis of Bcl-x_L expression by thymic iNKT cells: The expression of Bcl-x_L by thymic CD1d-tetramer^{+} iNKT cells from Med1$^{+/-}$ (*Upper*) or Med1$^{-/-}$ mice (*Lower*) was analyzed by intracellular FACS staining. The geometric mean fluorescent intensity (MFI) was shown. Data are representative of two independent experiments. (*B*) Quantitative real-time PCR analysis of Vα14-Jα18 transcripts in sorted CD4^{+}CD8^{+} DP thymocytes from Med1$^{+/-}$ or Med1$^{-/-}$ mice. Level of Vα14-Jα18 transcripts was normalized to the quantity of Cα transcripts. Results are shown as mean ± SEM from three independent experiments.