Fig S1

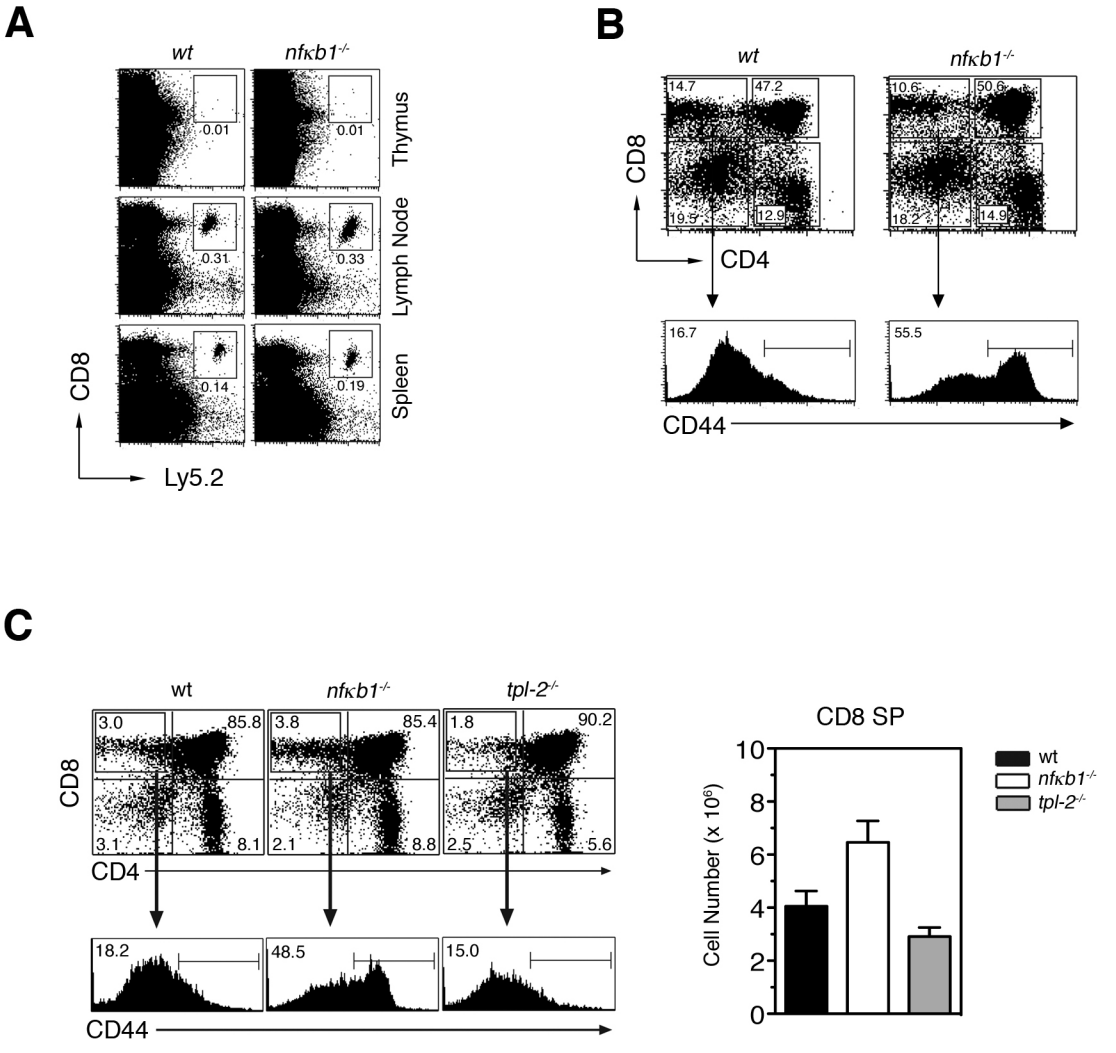

A
wt
nfκb1-/-
0.01
0.01
Thymus
0.31
0.33
Lymph Node
0.14
0.19
Spleen
CD8
Ly5.2
B
wt
nfκb1-/-
14.7
47.2
10.6
50.6
19.5
12.9
18.2
14.9
CD8
CD4
16.7
55.5
CD44
C
wt
nfκb1-/-
tpl-2-/-
3.0
85.8
3.8
85.4
1.8
90.2
3.1
8.1
2.1
8.8
2.5
5.6
CD8
CD4
18.2
48.5
15.0
CD44
CD8 SP
Cell Number (x 10⁶)
10
8
6
4
2
0
wt
nfκb1-/-
tpl-2-/-

Fig S2

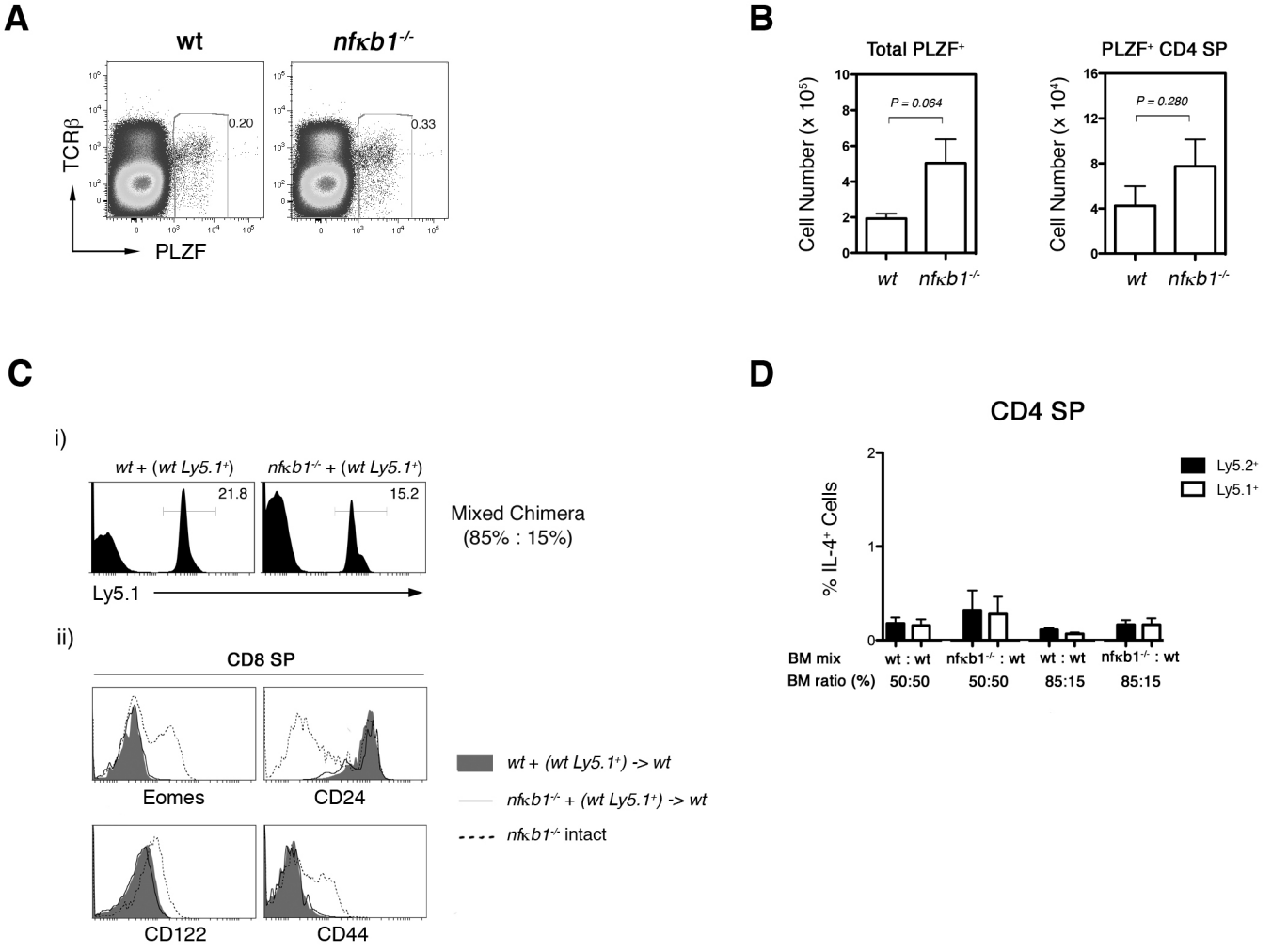
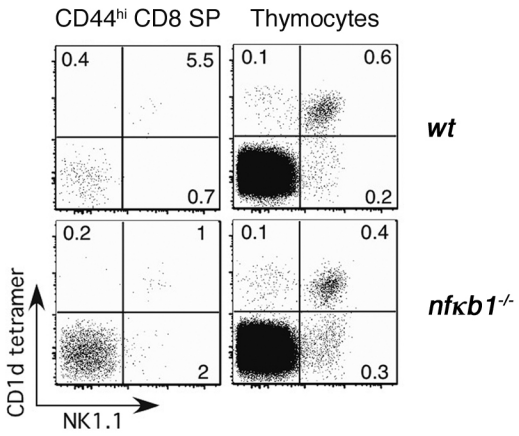
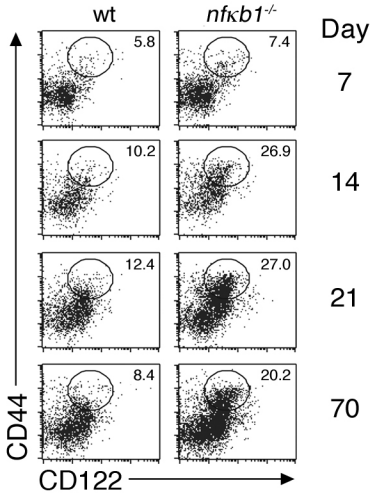

A
wt
nfκb1-/-
TCRβ
PLZF
0.20
0.33
B
Total PLZF+
PLZF+ CD4 SP
Cell Number (x 10^5)
Cell Number (x 10^4)
P = 0.064
P = 0.280
wt
nfκb1-/-
C
i)
wt + (wt Ly5.1+)
nfκb1-/- + (wt Ly5.1+)
21.8
15.2
Ly5.1
Mixed Chimera
(85% : 15%)
ii)
CD8 SP
Eomes
CD24
CD122
CD44
wt + (wt Ly5.1+) -> wt
nfκb1-/- + (wt Ly5.1+) -> wt
nfκb1-/- intact
D
CD4 SP
% IL-4+ Cells
Ly5.2+
Ly5.1+
BM mix
BM ratio (%)
wt : wt
nfκb1-/- : wt
wt : wt
nfκb1-/- : wt
50:50
50:50
85:15
85:15

Fig S3

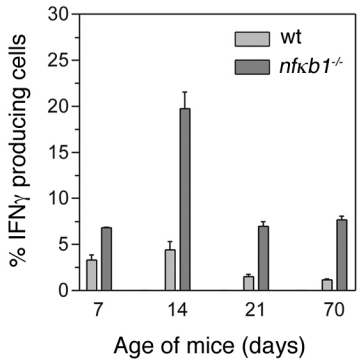
A


CD44hi CD8 SP
Thymocytes
0.4
5.5
0.7
0.1
0.6
0.2
wt
0.2
1
2
0.1
0.4
0.3
nfκb1-/-
CD1d tetramer
NK1.1

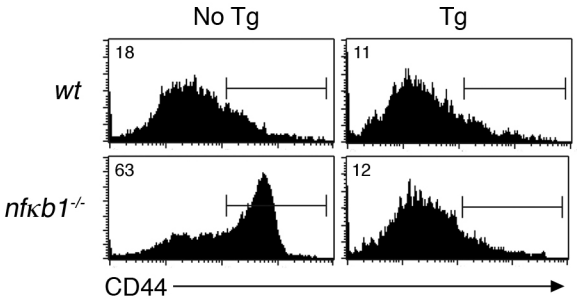
B


wt
nfκb1-/-
Day
5.8
7.4
7
10.2
26.9
14
12.4
27.0
21
8.4
20.2
70
CD44
CD122

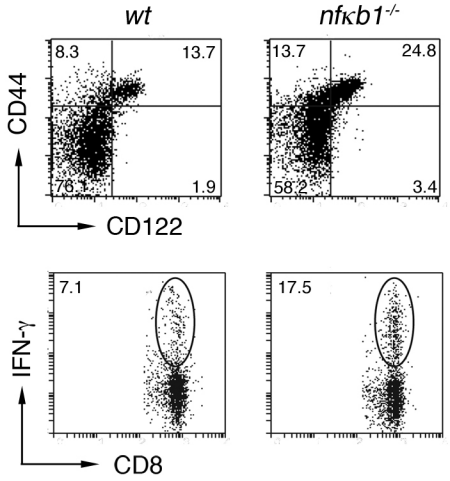
C


% IFNγ producing cells
wt
nfκb1-/-
30
25
20
15
10
5
0
7
14
21
70
Age of mice (days)

D


No Tg
Tg
wt
18
11
nfκb1-/-
63
12
CD44

E


wt
nfκb1-/-
CD44
8.3
13.7
76.1
1.9
13.7
24.8
58.2
3.4
CD122
IFN-γ
7.1
17.5
CD8

SUPPLEMENTARY INFORMATION

Materials and Methods

The following antibodies were used for flow cytometric analysis: anti-CD4-PE, - PerCP-Cy5.5, -FITC; anti-CD8α-APC, -PE, -FITC; anti-CD8β-PE; anti-CD44-FITC, -APC; anti-CD122-PE; anti-CD24 (HSA) -FITC; anti-TCRβ-PE; anti-CD69-FITC; anti-CD25-PE; anti-CD62L-FITC; anti-NK1.1-APC; anti-Ly5.1-APC; anti-Ly5.2-APC; anti-IFN-γ-PE; anti-Vα2-PE; anti-Vβ5-FITC; anti-CD11c-FITC; anti-CD45R-APC; anti-CD172a-PE (all from BD Pharmingen); anti-CD5-biotin (eBioscience); anti-Vβ3; anti-Vβ4; anti-Vβ5; anti-Vβ6; anti-Vβ7; anti-Vβ8.2; anti-Vβ9; anti-Vβ10; anti-Vβ11; anti-HYTCR (T3.70); CD1d tetramer loaded with α-galactosylceramide (all in house biotin Abs). Biotinylated antibodies were detected by incubation with streptavidin-PE or -PerCP-Cy5.5 (BD Pharmingen).

Adoptive transfer of CD8SP thymocytes

As per the Miltenyi Biotec protocol, *wt* and *nfκb1*$^{-/-}$ thymocytes were incubated with anti-CD4-biotin to deplete CD4SP and DP thymocytes. Cells were captured with anti-biotin microbeads and flow through cell fraction collected and stained with anti-CD4 and anti-CD8 MoAbs for FACS sorting (Mo-Flo; Cytomation). CD8SP cells sorted to high purity (>97%) were adoptively transferred (1-2 x10^6) into Ly5.1$^+$ mice. After 7 days, the presence of Ly5.2$^+$CD8SP cells was assessed in the spleens, lymph nodes and thymi of recipient mice using flow cytometry.

Foetal thymic organ culture (FTOC)

Embryonic day 15 thymic lobes were harvested and incubated on membrane filters supported by gelfoam in 24-well tissue-culture plates. Cultures were maintained for 12 days in RPMI medium (supplemented with 10% FCS, 2-mercaptoethanol, penicillin/streptomycin, HEPES, glutamine and sodium pyruvate) and then analysed by flow cytometry.

Table S1: Primer Sequences for RT-PCR*

	Forward	**Reverse**
Hprt probe #95	tcctcctcagaccgctttt	cctggttcatcatcgctaatc
Eomes probe #51	acactggctcccactggat	tgcagagactgcaacactatca
Bcl-6 probe #107	ccccacagcatacagagatg	ttgcagaagaaggtcccatt
Tbx21 probe#106	agggggcttccaacaatg	agacgtgtgtgttagaagcactg
Gzmb probe#66	ctggcctccaggacaaag	ataaggaagcccccacatatc
Prf1 probe#42	aatatcaataacgactggcgtgt	catgtttgcctctggccta

*Primers were designed using the Universal ProbeLibrary assay design centre;
All qRT-PCR reactions were prepared in 10uL with final concentrations of 1x Light Cycler 480 Probes Master, 200nM forward and reverse primers, and 100nM Universal ProbeLibrary probe, using the following cycling conditions: 95ºC for 10 minutes, 45 cycles of 95ºC (10 sec) and 60ºC (30 sec), 40ºC 1 min to cool.

Supplementary Figure Legends:

SFigure 1. Intra-thymic development of CD8 SP thymocytes with a memory phenotype

(**A**) Adoptive transfer of CD8SP thymocytes from *wt* or *nfκb1*$^{-/-}$ mice (Ly5.2^{+}) into congenic *wt* mice (Ly5.1^{+}). Thymus, lymph nodes and spleen were harvested and processed 7 days post-transfer. Cells were stained for CD8 and Ly5.2 and donor-derived CD8 T cells analysed by flow cytometry for each tissue (shown as percent cells in the

gated region). Data are representative of two experiments with two recipient mice per group. (**B**) E15 *wt* and *nfκb1*$^{-/-}$ thymic lobes were cultured by FTOC. Staining for CD4, CD8 and CD44 cell surface markers was performed on day 12. Expression of CD44 is shown for CD8SP thymocytes. Data are representative of 3 experiments with at least 4 embryos per genotype analysed. (**C**) The absence of p50NF-κB1 promotes the development of memory-like CD8 thymocytes. CD4 and CD8 expression by *wt*, *nfκb1*$^{-/-}$ and *tpl-2*$^{-/-}$ thymocytes with values representing percent cells in each quadrant. CD44 expression by CD8SP thymocytes. Absolute numbers (Mean ± SEM) of CD8SP thymocytes for each genotype. Data are representative of 3 experiments (n=4 mice per genotype).

SFigure 2. Similar levels of IL-4-producing and PLZF^{+} thymocytes in wt and *nfκb1*$^{-/-}$ mice **(A)** *Wt* and *nfκb1*$^{-/-}$ thymocytes were stained for PLZF and cell surface TCRβ expression. Values represent percentages of cells in the gated region. Data in A are representative of 4 mice per genotype examined in 4 separate experiments **(B)** Absolute numbers (Mean ± SEM) of PLZF^{+} and PLZF^{+}CD4SP thymocytes in *wt* and *nfκb1*$^{-/-}$ mice. Data are derived from 4 mice per genotype examined in 4 experiments. *P*-values were determined by an unpaired two-tailed Student's *t* test. **(C)** Expression of Eomes, CD122, CD44 and CD24 by *wt* (Ly5.1^{+}) CD8SP thymocytes isolated from wt + (*wt* Ly5.1^{+}) (shaded histograms) or *nfκb1*$^{-/-}$ + (*wt* Ly5.1^{+}) (black lines) chimera mice. Memory phenotype of *nfκb1*$^{-/-}$ CD8SP cells from *nfκb1*$^{-/-}$ mice (hatched lines) served as concurrent positive control. Ly5.1^{+} staining (i) was performed to distinguish *wt* (Ly5.1^{+}) hematopoietic cells in chimeras. Data are representative of 3 different chimera cohorts

(n>6 mice per group). BM chimeras were established by engrafting *wt* (Ly5.2^{+}) hosts with a mix (85:15) of *nfκb1*$^{-/-}$ and *wt* (Ly5.1^{+}) hematopoietic cells. Hosts were euthanized and examined 5-7 weeks post-transplant. (**D**) CD4 T cells (TCRβ^{+}CD4^{+}CD8^{-}) were assessed for IL-4 expression. Thymocytes were isolated from equal (50:50) or unequal (85:15) chimeras (see x-axis), and stimulated *in vitro* with PMA and ionomycin for 2 h. Analysis for IL-4 levels was performed by flow cytometry on Ly5.2^{+} and Ly5.1^{+} cells. Data in C and D are representative of 2 chimera cohorts with at least 4 mice per group examined.

SFigure 3 (**A**) CD1d tetramer staining of *wt* and *nfκb1*$^{-/-}$ CD44^{hi}CD8SP thymocytes. CD1d tetramer and NK1.1 expression gated on CD44^{hi}CD8^{+} T cells or total thymocytes from *wt* and *nfκb1*$^{-/-}$ mice. Data are representative of 2 experiments (5 mice per genotype). (**B**) Expression of CD44 and CD122 gated on CD8SP thymocytes from *wt* and *nfκb1*$^{-/-}$ mice at specific time points (days). n>3 mice per genotype for each time point. (**C**) Percentage of IFN-γ producing CD8SP cells from *wt* or *nfκb1*$^{-/-}$ mice after 5 h *in vitro* stimulation with PMA plus ionomycin. Values were derived from IFN-γ versus CD8 dot plots (n>3 mice per genotype for each time point; Mean ± SEM). (**D**) Expression of CD44 by transgenic (Tg) or non-transgenic (No Tg) CD8SP thymocytes. Thymocytes from OT-I^{Tg+} and OT-I^{Tg-} mice were stained for CD4, CD8 and CD44. Values indicate percent CD44^{hi} cells. Data are representative of four mice per genotype from 2 experiments. (**E**) CD44 and CD122 expression by splenic CD8^{+} T cells. Splenocytes stimulated in culture with anti-CD3/anti-CD28 Abs for 5 h were examined for IFN-γ and CD8 expression. Data are from 6 mice per genotype and representative of 3 experiments.