## SUPPLEMENTAL METHODS

# T cell protein tyrosine phosphatase attenuates T cell signaling to maintain tolerance in mice

Florian Wiede[1], Benjamin J. Shields[1], Sock Hui Chew[1], Konstantinos Kyparissoudis[2], Catherine van Vliet[1], Sandra Galic[1], Michel L. Tremblay[3], Sarah M. Russell [4, 5], Dale I. Godfrey[2] and Tony Tiganis[1]

[1]Department of Biochemistry and Molecular Biology, Monash University, Clayton, Victoria 3800, Australia, [2]Department of Microbiology and Immunology, University of Melbourne, Parkville, Victoria 3010, Australia, [3]McGill Cancer Centre and Department of Biochemistry, McGill University, Montreal, Quebec, Canada, [4]Immune Signaling Laboratory, Cancer Immunology, Peter MacCallum Cancer Centre, East Melbourne, Victoria 3002, Australia, and [5]Centre for MicroPhotonics, Swinburne University of Technology, Hawthorn, Vic 3084, Australia.

***Materials***

Mitomycin C and fluorescein isothiocyanate (FITC)-conjugated peanut-agglutinin (PNA) were purchased from Sigma-Aldrich. Recombinant mouse IL-2, human IL-2 and mouse IL-4 were purchased from PeproTech. Mouse α-phospho-STAT5 (Y694) and rabbit α-phospho-STAT6 (Y641), α-STAT5, α-phospho-Pyk2 (Y402), α-phospho-PLCγ1 (Y783) and PLCγ1 were purchased from Cell Signaling; mouse α-p-Tyr (4G10) from Upstate, mouse α-STAT6 (E10) from Santa Cruz and the FoxP3 Staining Buffer Kit from eBioscience. The α-mouse IL-2 (S4B6) was a gift from P. Hodgkin (Walter and Eliza Hall Institute, Melbourne, Australia).

***Reagents for flow cytometry and MACS purification***

The following antibodies and secondary reagents from BD Pharmingen (San Jose, CA) were used for staining: FITC-conjugated or phycoerythrin (PE)-cyanine dye 5 (Cy5)-conjugated α–CD44 (IM7), PE-conjugated or allophycocyanin (APC)- conjugated α–CD62L (MEL-14), APC-cyanin dye 7 (Cy7) conjugated or Alexa Fluor 647-conjugated α–CD8 (53-6.7), Pacific Blue (PB)-conjugated or PE-cyanine dye 7 (Cy7)-conjugated α–CD4 (RM4-5), PE-Cy7-conjugated α–CD69 (H1.2F3), PE-conjugated or PE-Cy7-conjugated α–CD3 (145-2C11), APC-conjugated or FITC-conjugated α–TCR (H57-597), FITC-conjugated α–TCRvα5.1/5.2 (MR9-4), PE-conjugated α–TCRvα2 (B20.1), PE-conjugated α–CD5 (53-7.3), biotinylated α–KLRG1 (2F1), PE-conjugated CD45.2 (104), APC-conjugated or PE-conjugated α–CD25 (PC61), PE-conjugated α–CD127 (SB/199), PE-conjugated α–CD122 (TM-β1), PE-conjugated α-Ly6C/G (Gr-1; RB6-8C5) and PE-Cy7-conjugated or TexasRed®-PE-conjugated Streptavidin. Pacific Blue-conjugated α–CD8 (5H10) from Invitrogen (Invitrogen-Molecular Probes, Carlsbad, CA), PE-conjugated α-Nur77 (12.14) and PE-conjugated α-FoxP3 from eBioscience (San Diego, CA) were also used for staining. The following reagents from Miltenyi (Miltenyi Biotec, Bergisch-Gladbach, Germany) were used

for MACS purification: PE-conjugated α-CD4 (M-T466), PE-conjugated α-CD8 (BW135/80) and α-PE mouse IgG1 MicroBeads®.

***Analysis of CD4+CD25+ regulatory T cell proliferation and suppressor function***

Splenocytes from C57BL/6 mice were FACS-purified for TCRβ negative cells to obtain T cell-depleted accessory cells. Accessory cells were incubated with mitomycin C (50 μg/ml) at 37ºC for 20 min and then washed three times with complete RPMI [RPMI 1640 (SIGMA) supplemented with 10% (v/v) heat inactivated FBS (CSL), 1 x non-essential amino acids (SIGMA), 1 mM sodium pyruvate, 2 mM L-glutamine (Invitrogen), 50 μM β-mercaptoethanol (SIGMA) plus 100 U/ml penicillin and 100 μg/ml streptomycin]. FACS-purified CD4+CD25+ regulatory T cells (1 x $10^5$) from *Ptpn2*$^{lox/lox}$ and *Lck*-Cre;*Ptpn2*$^{lox/lox}$ mice were stained with 5 μM CFSE and incubated with mitomycin C-treated accessory cells (5 x $10^4$) in the presence of IL-2 (3 ng/ml) and α-CD3ε (3 μg/ml) for 48 h and processed for flow cytometric analysis.

For the assessment of regulatory T cell suppressor function FACS-purified CD4+CD25$^{lo}$CD44$^{lo}$ naïve T cells (1 x $10^5$) from C57BL/6 mice were incubated with CD4+CD25+ regulatory T cells (5-25 x $10^3$) from *Ptpn2*$^{lox/lox}$ and *Lck*-Cre;*Ptpn2*$^{lox/lox}$ mice in the presence of mitomycin C-treated accessory cells (5 x $10^4$) and α-CD3ε (1 μg/ml). After 48 h cells were pulsed with 1 μCi [$^3$H]-thymidine (Amersham Biosciences) for 16 h and harvested using a Tomtec Mach IIM Cell Harvester and analyzed on a scintillation counter.

***Immunohistochemistry***

Fluorescence immunohistochemistry was performed on frozen spleen sections. Briefly, spleen samples were embedded in Tissue Tek optimum cutting temperature compound (ProScitech) and frozen on dry ice. 8-μm sections were cut and mounted onto gelatin-coated glass slides, oven dried for 1 h at 60°C and fixed in acetone for 10 min at −20°C. Before staining, sections were re-hydrated with PBS and blocked with 10% FBS. Sections were incubated with PNA-FITC (Sigma-Aldrich) for 30 min at room temperature, washed in PBS and wet mounted for immunofluorescence microscopy using a Zeiss Axioskop 2 mot plus microscope (Zeiss).

**SUPPLEMENTAL DATA**

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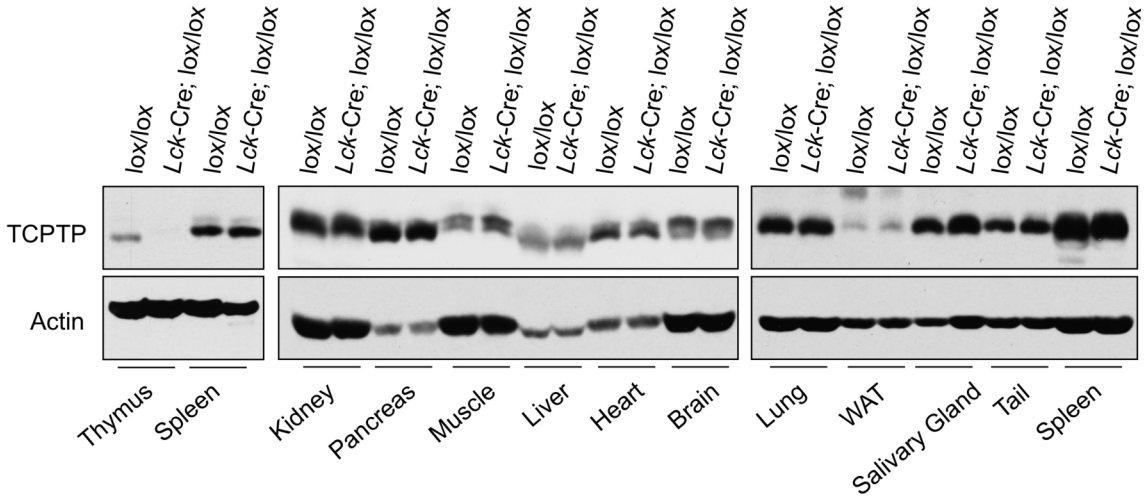
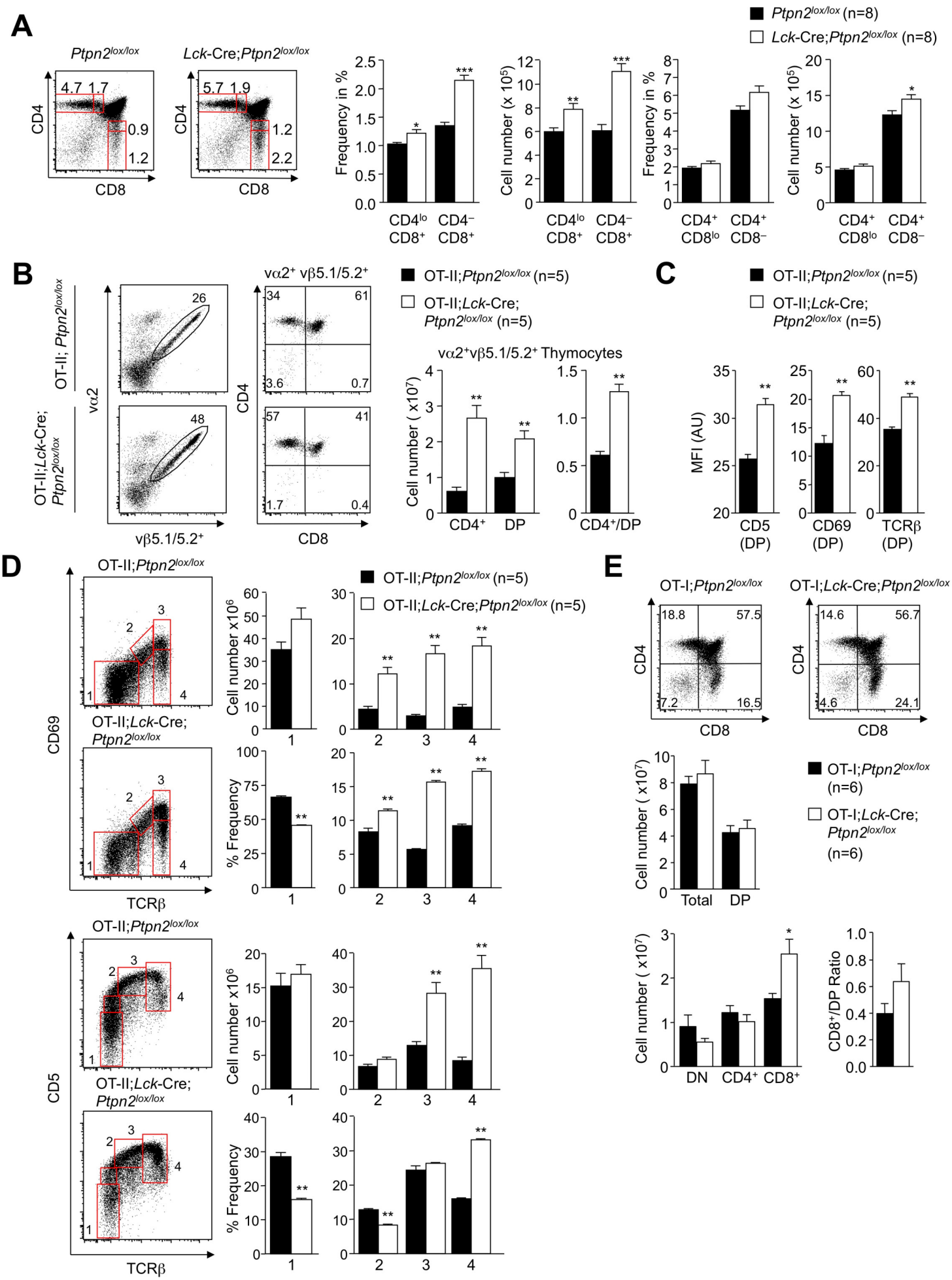



***Supplementary Figure 1. TCPTP expression in Lck*-Cre;*Ptpn2*$^{lox/lox}$ mice.** TCPTP protein expression (6F3) in lymphoid and non-lymphoid tissues from 4 week old *Ptpn2*$^{lox/lox}$ and *Lck*-Cre;*Ptpn2*$^{lox/lox}$ (C57BL/6) mice. These results are representative of at least three independent experiments.

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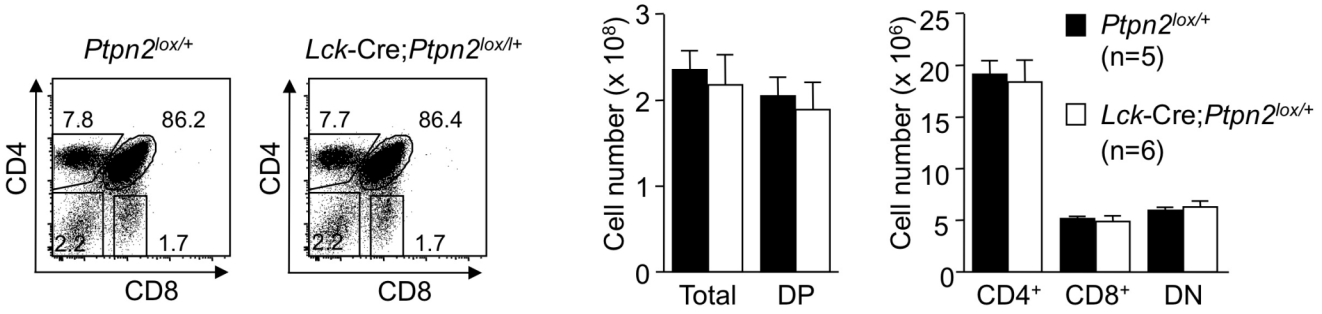
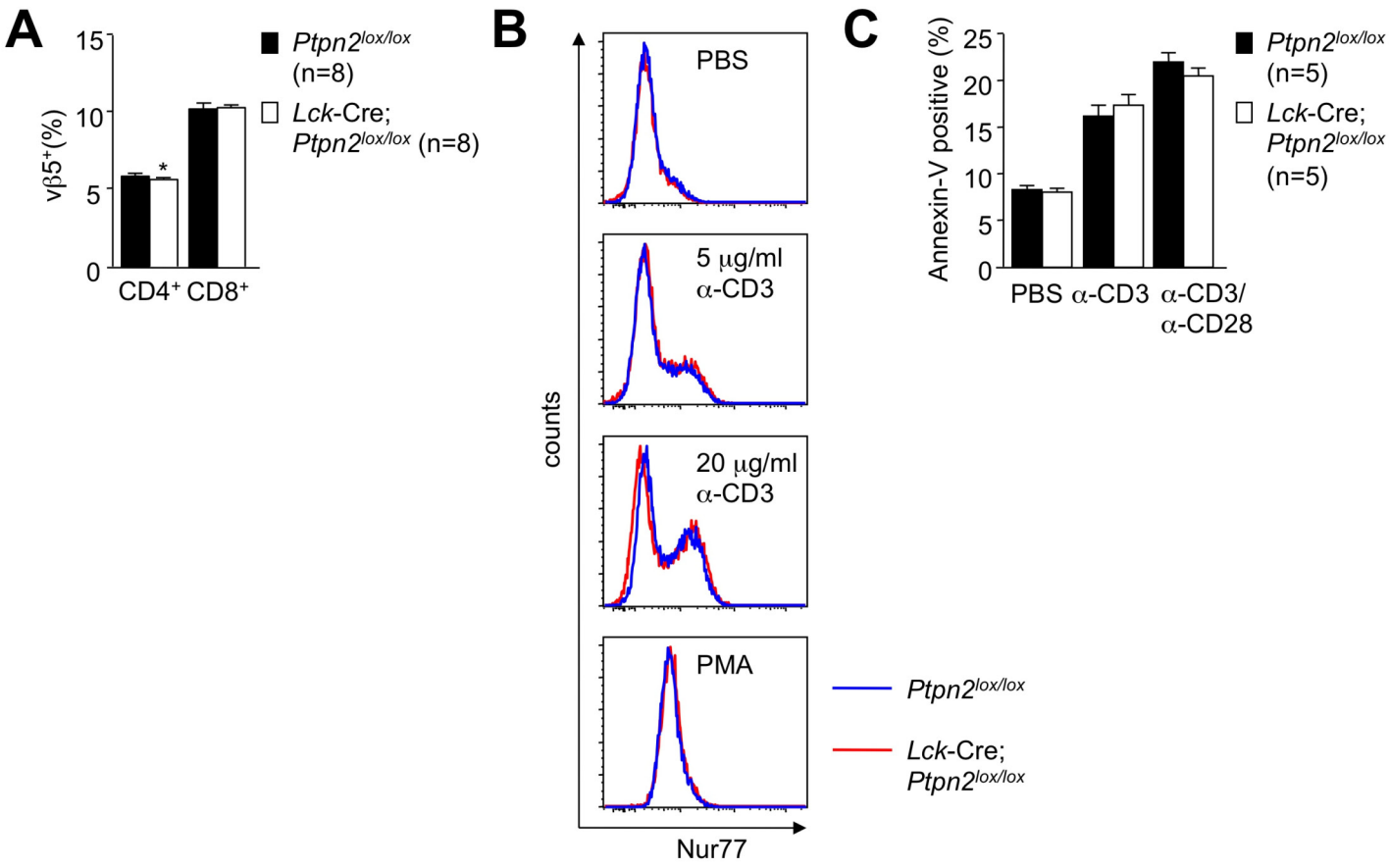
***Supplementary Figure 2. Thymocyte development in Lck-Cre;Ptpn2$^{lox/lox}$ and OT-II;Lck-Cre;Ptpn2$^{lox/lox}$ mice.*** Thymocytes (3 x $10^6$) from (**a**) *Ptpn2*$^{lox/lox}$ and *Lck*-Cre;*Ptpn2*$^{lox/lox}$, (**b-d**) OT-II TCR transgenic *Ptpn2*$^{lox/lox}$ (OT-II;*Ptpn2*$^{lox/lox}$) and *Lck*-Cre;*Ptpn2*$^{lox/lox}$ (OT-II;*Lck*-Cre;*Ptpn2*$^{lox/lox}$) or (**e**) OT-I TCR transgenic *Ptpn2*$^{lox/lox}$ (OT-I;*Ptpn2*$^{lox/lox}$) and *Lck*-Cre;*Ptpn2*$^{lox/lox}$ (OT-I;*Lck*-Cre;*Ptpn2*$^{lox/lox}$) mice were stained with fluorochrome-conjugated antibodies against CD4 and CD8 and either TCRvα2 and TCRvβ5.1/5.2, or TCRβ, CD69 and CD5 and analyzed by flow cytometry. (**a**) Cells were gated for SP and intermediate SP ($CD4^{lo}CD8^{+}$; $CD4^{+}CD8^{lo}$) thymocytes and absolute numbers determined. Representative dot blots and results from three independent experiments are shown. (**b**) TCRvα2 and TCRvβ5.1/5.2 co-expressing cells were gated for DP and CD4+SP (CD4+) thymocytes and absolute numbers and the indicated ratios determined. Representative dot blots and results from two independent experiments are shown. (**c**) CD69, CD5 and TCRβ levels (mean fluorescence intensity; MFI) in OT-II;*Ptpn2*$^{lox/lox}$ versus OT-II;*Lck*-Cre;*Ptpn2*$^{lox/lox}$ DP thymocytes were determined; AU: arbitrary units. Representative results from two independent experiments are shown. **d**) Cells were gated for the different developmental stages (labelled 1-4) according to the expression of the selection markers CD69, CD5 and TCRβ and absolute numbers determined. Representative dot blots and results from two independent experiments are shown. (**e**) Cells were gated for DN, DP, CD4+SP (CD4+) and CD8+SP (CD8+) thymocytes and absolute numbers and the indicated ratios determined. Representative dot blots and results from three independent experiments are shown. Numbers in outlined areas are % of cells in gate. Results in **a-e** are means ± SEM for the indicated number of mice; significance determined using 2-tailed Mann-Whitney U Test; *$p<0.05$, ** $p<0.01$, *** $p<0.001$.

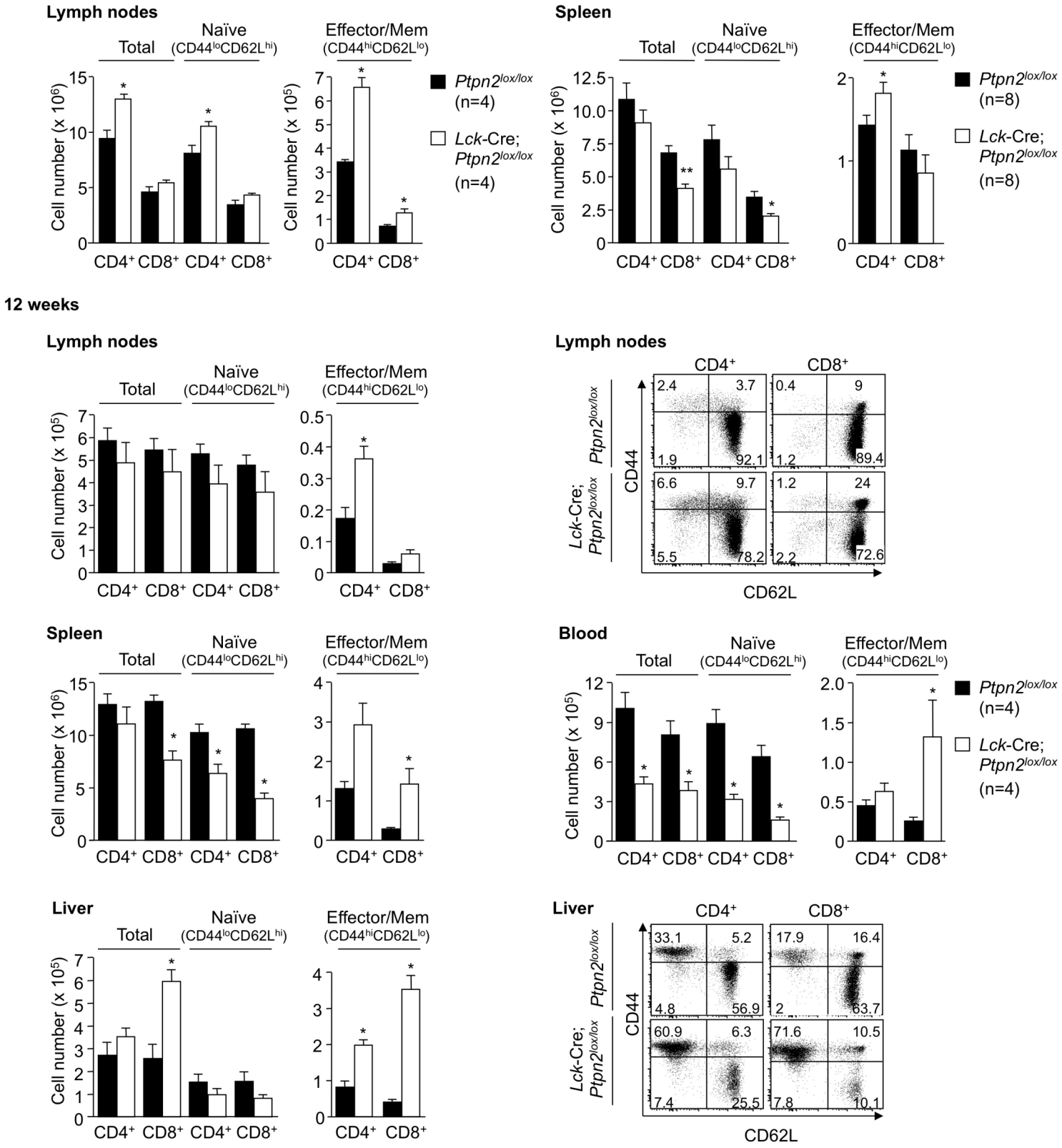



| | CD4⁺/CD8⁺ | CD8⁺/DP | CD4⁺/DP |
|---|---|---|---|
| *Ptpn2*$^{lox/+}$ | 3.70 ± 0.23 | 0.027 ± 0.004 | 0.10 ± 0.014 |
| *Lck*-Cre; *Ptpn2*$^{lox/+}$ | 3.99 ± 0.05 | 0.026 ± 0.002 | 0.11 ± 0.010 |
| *p*-Value | 0.33 | 0.93 | 0.33 |

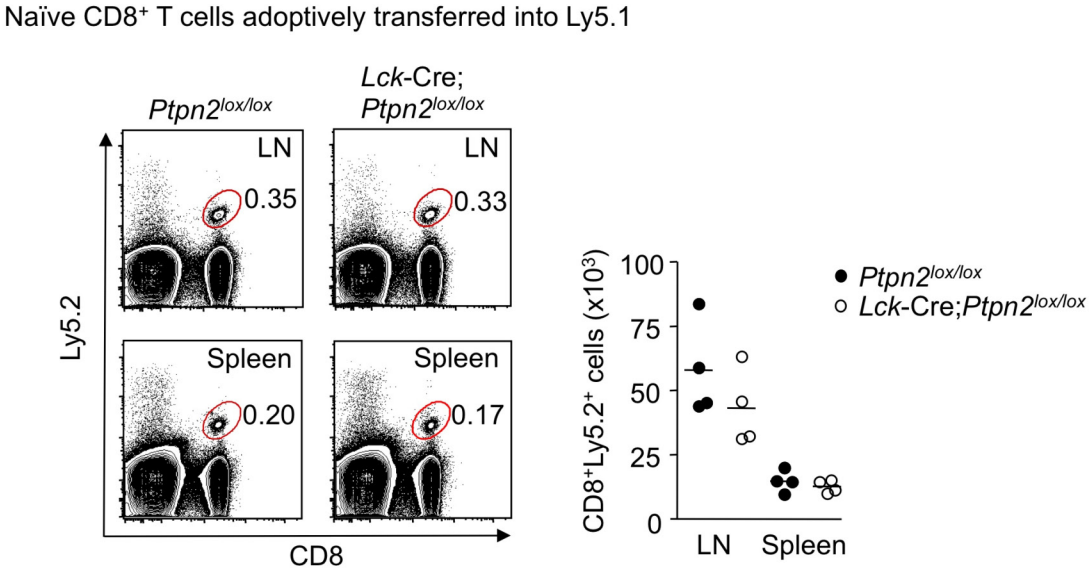
***Supplementary Figure 3. Thymocyte development in Lck-Cre;Ptpn2$^{lox/+}$ heterozygous mice.*** *Ptpn2*$^{lox/+}$ and *Lck*-Cre;*Ptpn2*$^{lox/+}$ thymocytes from 4 week old mice were stained with fluorochrome-conjugated antibodies against CD4 and CD8 and analyzed by flow cytometry. Cells were gated for DN, DP, CD4+SP (CD4+) and CD8+SP (CD8+) thymocytes and absolute numbers and the indicated ratios determined. Numbers are % of cells in gate. Results are means ± SEM for the indicated number of mice and are representative of two independent experiments; p-values determined using 2-tailed Mann-Whitney U Test.



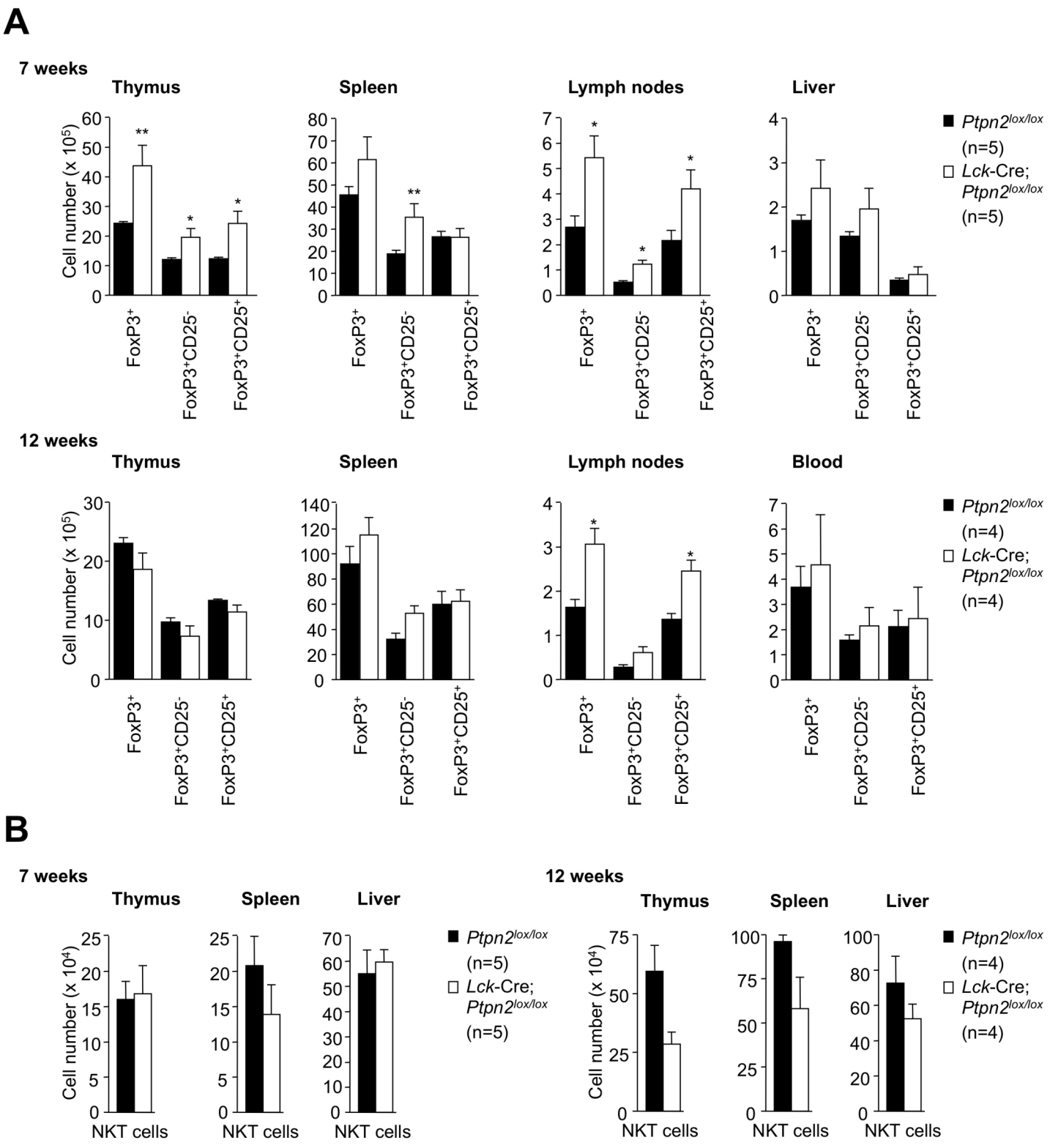

***Supplementary Figure 4. Thymocyte negative selection in Lck-Cre;Ptpn2$^{lox/lox}$ mice.*** (**a**) *Ptpn2*$^{lox/lox}$ and *Lck*-Cre;*Ptpn2*$^{lox/lox}$ thymocytes from 4 week old mice were stained with fluorochrome-conjugated antibodies against CD4, CD8, CD3ε and TCRvβ5.1/2 and analyzed by flow cytometry. Cells were gated for CD4+SP (CD4+) and CD8+SP (CD8+) thymocytes and the percentage of TCRvβ5+CD3ε+ thymocytes determined. Representative results from two independent experiments are shown. (**b**) Total thymocytes from 4 week old *Ptpn2*$^{lox/lox}$ and *Lck*-Cre;*Ptpn2*$^{lox/lox}$ mice were stimulated with plate-bound α-CD3 (5-20 μg/ml) or PMA for 2 h, followed by an incubation with fluorochrome-conjugated antibodies against CD4 and CD8. The cells were then fixed, permeabilized and stained with fluorochrome-conjugated antibodies for Nur77 in DP thymocytes (Foxp3 Staining Buffer kit). Representative profiles from two independent experiments with two mice per genotype per experiment are shown. (**c**) DP thymocytes from 4 week old *Ptpn2*$^{lox/lox}$ and *Lck*-Cre;*Ptpn2*$^{lox/lox}$ mice were purified by FACS and stimulated with plate-bound α-CD3 (10 μg/ml) ± α-CD28 (10 μg/ml) for 32 h. Cells were stained with Annexin V-FITC (Annexin V-FITC Apoptosis Detection Kit I, BD Pharmingen) and propidium iodide and analyzed by flow cytometry. Annexin V positive/propidium iodide negative cells were defined as apoptotic. Representative results (means ± SEM) from two independent experiments are shown. Results in **a** and **c** are means ±SEM; significance determined using 2-tailed Mann-Whitney U Test; *p<0.05.



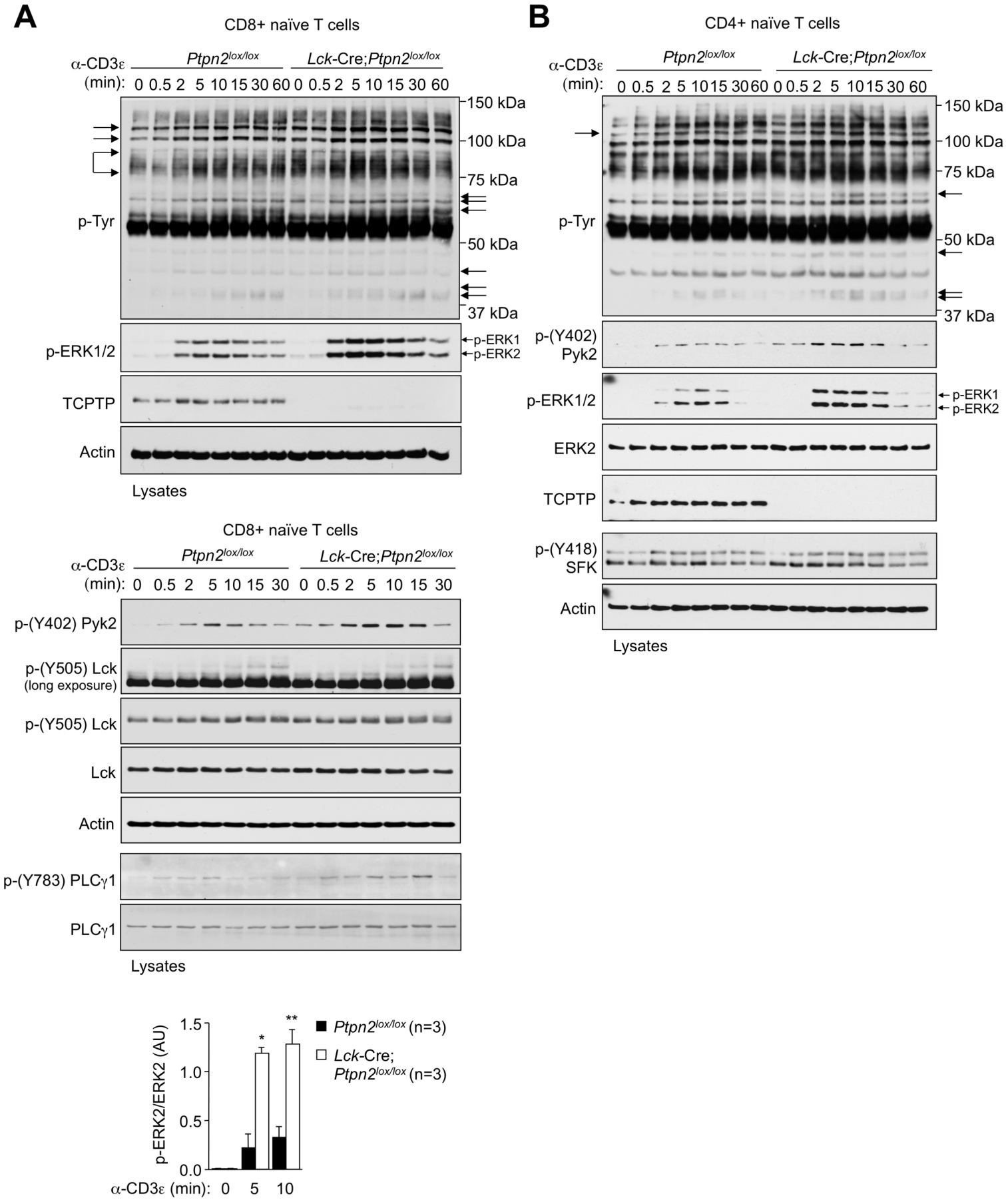

***Supplementary Figure 5. Increased effector/memory T cell subsets in Lck-Cre;Ptpn2$^{lox/lox}$ mice.*** *Ptpn2$^{lox/lox}$* and *Lck*-Cre;*Ptpn2$^{lox/lox}$* lymphocytes (3 x $10^6$) isolated from lymph nodes, spleen, liver or blood of 4 and 12 week old mice were stained with fluorochrome-conjugated antibodies against CD4, CD8, TCRβ, CD44 and CD62L and analyzed by flow cytometry. Absolute numbers of total CD4+TCRβ+ or CD8+TCRβ+ T cells and CD4+ versus CD8+ naïve ($CD44^{lo}CD62L^{hi}$) and effector/memory-like (Effector/Mem; $CD44^{hi}CD62L^{lo}$) T cells were determined. Numbers in outlined areas are % of cells in gate. Representative dot plots and results (means ± SEM) from at least two independent experiments are shown; significance determined using 2-tailed Mann-Whitney U Test; *p<0.05 ** p<0.01.




***Supplementary Figure 6. TCPTP deficiency does not alter CD8+ T cell homing.*** $CD8^+$ naïve LN T cells from *Ptpn2*$^{lox/lox}$ and *Lck*-Cre;*Ptpn2*$^{lox/lox}$ mice were adoptively transferred (2 x $10^6$/recipient) into replete congenic Ly5.1 hosts. At 36 h post-transfer peripheral LN cells and splenocytes were isolated and stained with fluorochrome-conjugated antibodies against CD8 and Ly5.2 and analyzed by flow cytometry; numbers are % of cells in gate. Representative dot blots and quantified results (means for the indicated number of mice) from one experiment are shown.




***Supplementary Figure 7. Regulatory T cells and Natural Killer T cells in Lck-Cre;Ptpn2$^{lox/lox}$ mice.*** *Ptpn2$^{lox/lox}$* and *Lck*-Cre;*Ptpn2$^{lox/lox}$* lymphocytes (3 x $10^6$) isolated from thymus, lymph nodes, spleen, liver or blood of 7 and 12 week old mice were stained with fluorochrome-conjugated antibodies against **a**) CD4, CD25 and FoxP3, or **b**) TCRβ and α-GalCer/CD1d tetramer and analyzed by flow cytometry. Absolute numbers of $CD4^+FoxP3^+$, $CD4^+FoxP3^+CD25^-$ or $CD25^+$ regulatory T cells or α-GalCer/CD1d tetramer+/TCRβ+ Natural Killer T (NKT) cells were determined. Results shown are means ± SEM for the indicated number of mice; significance determined using 2-tailed Mann-Whitney U Test; *p<0.05 ** p<0.01.

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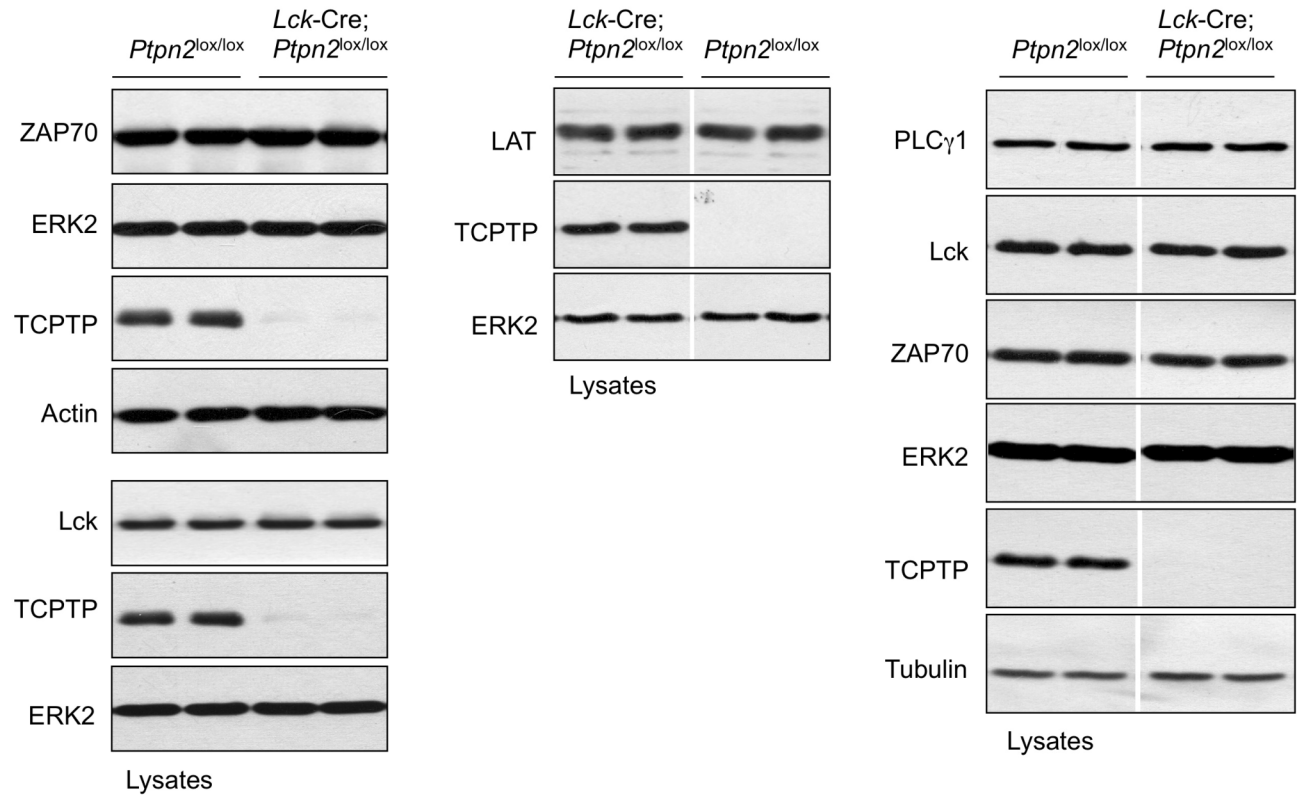
***Supplementary Figure 8. Enhanced TCR signaling in Lck-Cre;Ptpn2$^{lox/lox}$ T cells.*** FACS-purified CD8+ and CD4+ naïve T cells from 4 week old *Ptpn2*$^{lox/lox}$ versus *Lck*-Cre;*Ptpn2*$^{lox/lox}$ mice were left untreated or stimulated with α-CD3ε for the indicated times. Cell lysates were resolved by SDS-PAGE and immunoblotted with antibodies specific for phosphotyrosine (p-Tyr), Y505 phosphorylated Lck [p-Lck (Y505)], phosphorylated and activated SFKs [p-SFK (Y418)], phosphorylated Pyk2 [p-Pyk (Y402)], phosphorylated PLCγ1 [p-PLCγ1 (Y783)] and phosphorylated and activated ERK1/2 (p-ERK1/2) and reprobed for Lck, PLCγ1, ERK2, TCPTP and actin. Results shown are representative of at least three independent experiments. Proteins exhibiting enhanced tyrosine phosphorylation in *Lck*-Cre;*Ptpn2*$^{lox/lox}$ thymocytes and T cells are indicated by arrows. Phosphorylated ERK1 (p-ERK1) and ERK2 (p-ERK2) are highlighted by arrows. p-ERK2 was quantified by densitometric analysis and normalised for ERK2; results are means ± SEM and units are arbitrary (AU). Significance was determined using 2-tailed Student's t-test; * $p < 0.05$, ** $p<0.01$.

**A**




**B**

Thymocytes

| MFI | CD3 on CD4+ | CD3 on CD8+ | CD3 on DP | TCRβ on CD4+ | TCRβ on CD8+ | TCRβ on DP |
|---|---|---|---|---|---|---|
| *Ptpn2*$^{lox/lox}$ | 1373.8±81 | 805.6±32.5 | 26.3±2.3 | 4932.8±22.3 | 3549.8±65.0 | 279.0±4.5 |
| *Lck*-Cre; *Ptpn2*$^{lox/lox}$ | 1338.2±15 | 726.6±20.5 | 30.6±1.5 | 5059.8±21.7 | 3534.2±58.9 | 308.2±7.5 |
| *p*-Value (n=5) | 0.69 | 0.22 | 0.29 | 0.016 | 0.84 | 0.028 |

Naïve splenic T cells

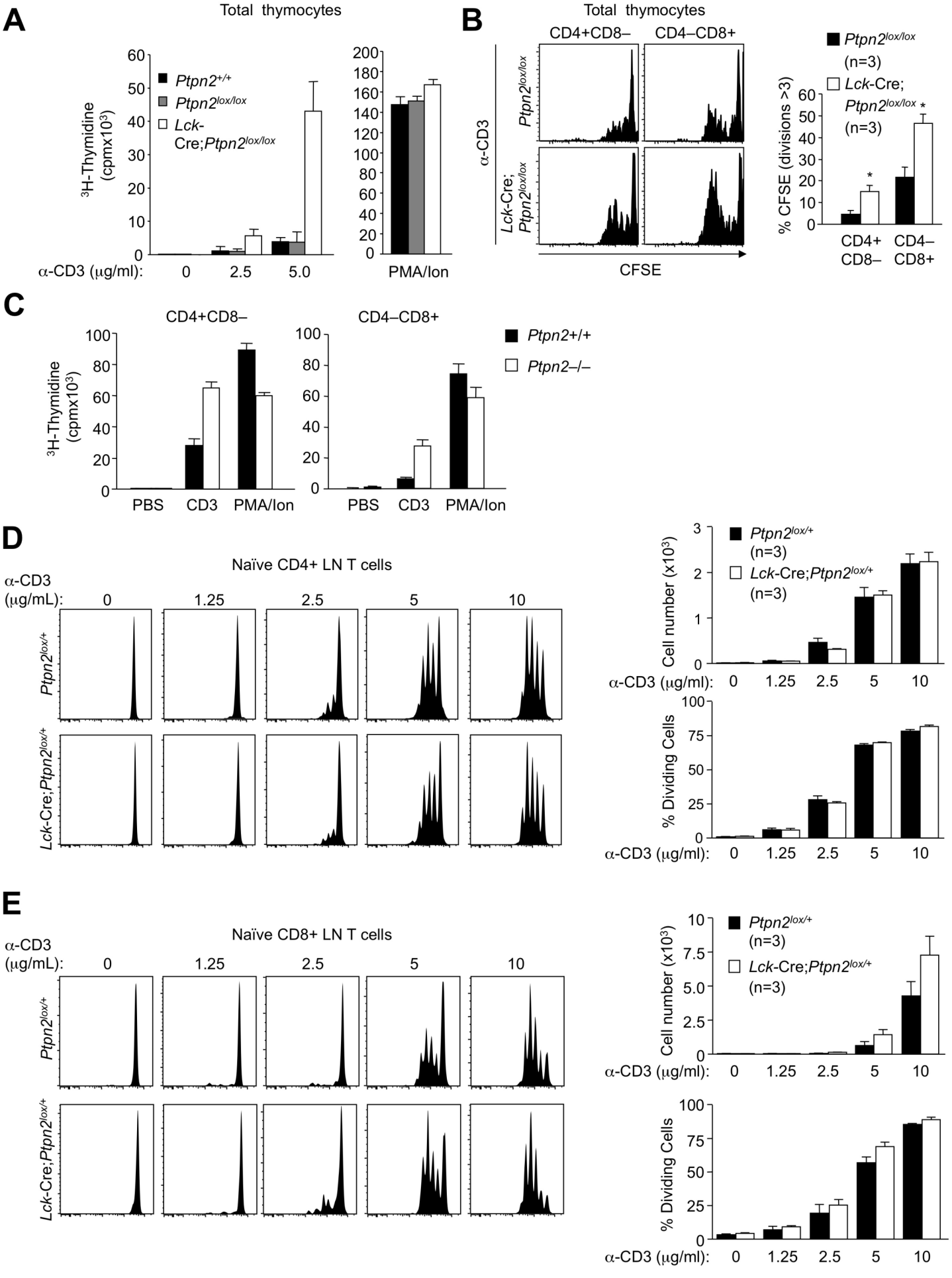
| MFI | CD3 on CD4+ | CD3 on CD8+ | TCRβ on CD4+ | TCRβ on CD8+ |
|---|---|---|---|---|
| *Ptpn2*$^{lox/lox}$ | 3042 ± 53 | 1190 ± 20 | 4776 ± 17 | 3688 ± 12 |
| *Lck*-Cre; *Ptpn2*$^{lox/lox}$ | 3010 ± 48 | 1053 ± 31 | 4591 ± 33 | 3393 ± 56 |
| *p*-Value (n=5) | 1.0 | 0.02 | 0.008 | 0.008 |

Naïve LN T cells

| MFI | CD3 on CD4+ | CD3 on CD8+ | TCRβ on CD4+ | TCRβ on CD8+ |
|---|---|---|---|---|
| *Ptpn2*$^{lox/lox}$ | 3019 ± 34 | 1260 ± 14 | 5119 ± 25 | 3766 ± 18 |
| *Lck*-Cre; *Ptpn2*$^{lox/lox}$ | 3099 ± 53 | 1188 ± 36 | 5005 ± 43 | 3657 ± 41 |
| *p*-Value (n=5) | 0.2 | 0.1 | 0.05 | 0.03 |

***(Refer to figure legend on the next page)***

***Supplementary Figure 9. Cell surface and intracellular signaling protein expression is unaltered by TCPTP deficiency.*** (**a**) Thymocyte and FACS purified CD8+ lymph node T cell lysates from 5 week old *Ptpn2*$^{lox/lox}$ versus *Lck*-Cre;*Ptpn2*$^{lox/lox}$ mice were resolved by SDS-PAGE and immunoblotted as indicated. Results shown are representative of three independent experiments. (**b**) Thymocytes and T cells from 5 week old *Ptpn2*$^{lox/lox}$ versus *Lck*-Cre;*Ptpn2*$^{lox/lox}$ mice were stained with fluorochrome-conjugated antibodies against CD4, CD8, CD3ε, CD44 and TCRβ and analyzed by flow cytometry for CD3ε and TCRβ surface levels. The mean fluorescence intensity (MFI) was determined using FlowJo7 Software. Significance values determined using 2-tailed Mann-Whitney U Test.

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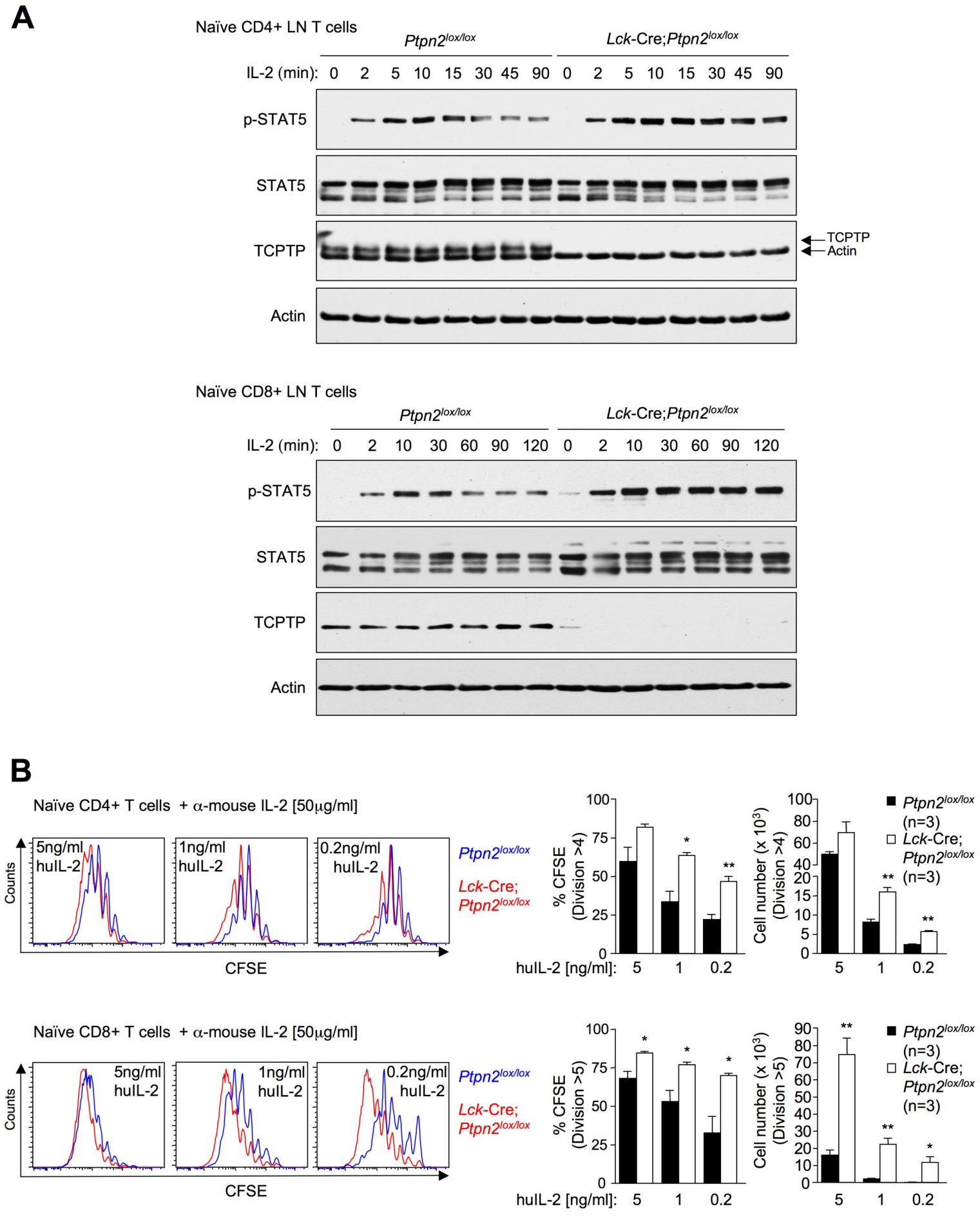
***Supplementary Figure 10. Thymocyte and T cell proliferation in TCPTP-deficient mice.*** (**a**) *Ptpn2*$^{+/+}$, *Ptpn2*$^{lox/lox}$ and *Lck*-Cre;*Ptpn2*$^{lox/lox}$ total thymocytes (2 x $10^5$) from 4 week old mice were stimulated with plate-bound α-CD3ε or PMA (1 ng/ml) plus ionomycin (Ion; 200 ng/ml). After 48 h proliferation was determined by [$^3$H]-thymidine incorporation over 16 h. Results are means ± SD from quadruplicate determinations and are representative of three independent experiments. (**b**) *Ptpn2*$^{lox/lox}$ and *Lck*-Cre;*Ptpn2*$^{lox/lox}$ total thymocytes (3 x $10^5$) from 4 week old mice were stained with 2 μM CFSE and stimulated with plate bound α-CD3ε (5 μg/ml) for 72 h and analyzed by flow cytometry. Shown are representative profiles from two independent experiments and the percentage (mean ± SEM) of cells in division >3 for three mice per genotype in one experiment; significance determined using 2-tailed Student's t-test; * $p < 0.05$. (**c**) *Ptpn2*$^{+/+}$ and *Ptpn2*$^{-/-}$ (BALB/c) FACS-purified CD4+SP and CD8+SP thymocytes from 2 week old mice were stimulated with plate-bound α-CD3ε or PMA (1 ng/ml) plus ionomycin (Ion; 200 ng/ml) and proliferation determined by [$^3$H]-thymidine incorporation. Results shown means ± SD and are representative of two independent experiments. FACS purified (**d**) CD4+ and (**e**) CD8+ naïve (CD44$^{lo}$) LN T cells (1 x $10^5$) from 4 week old *Ptpn2*$^{lox/+}$ and *Lck*-Cre;*Ptpn2*$^{lox/+}$ mice were stained with 2 μM CFSE and stimulated with plate bound α-CD3ε for 72 h. Representative FACS profiles from two independent experiments are shown. Proliferating cells were quantified and means ± SEM from three mice per genotype are shown.

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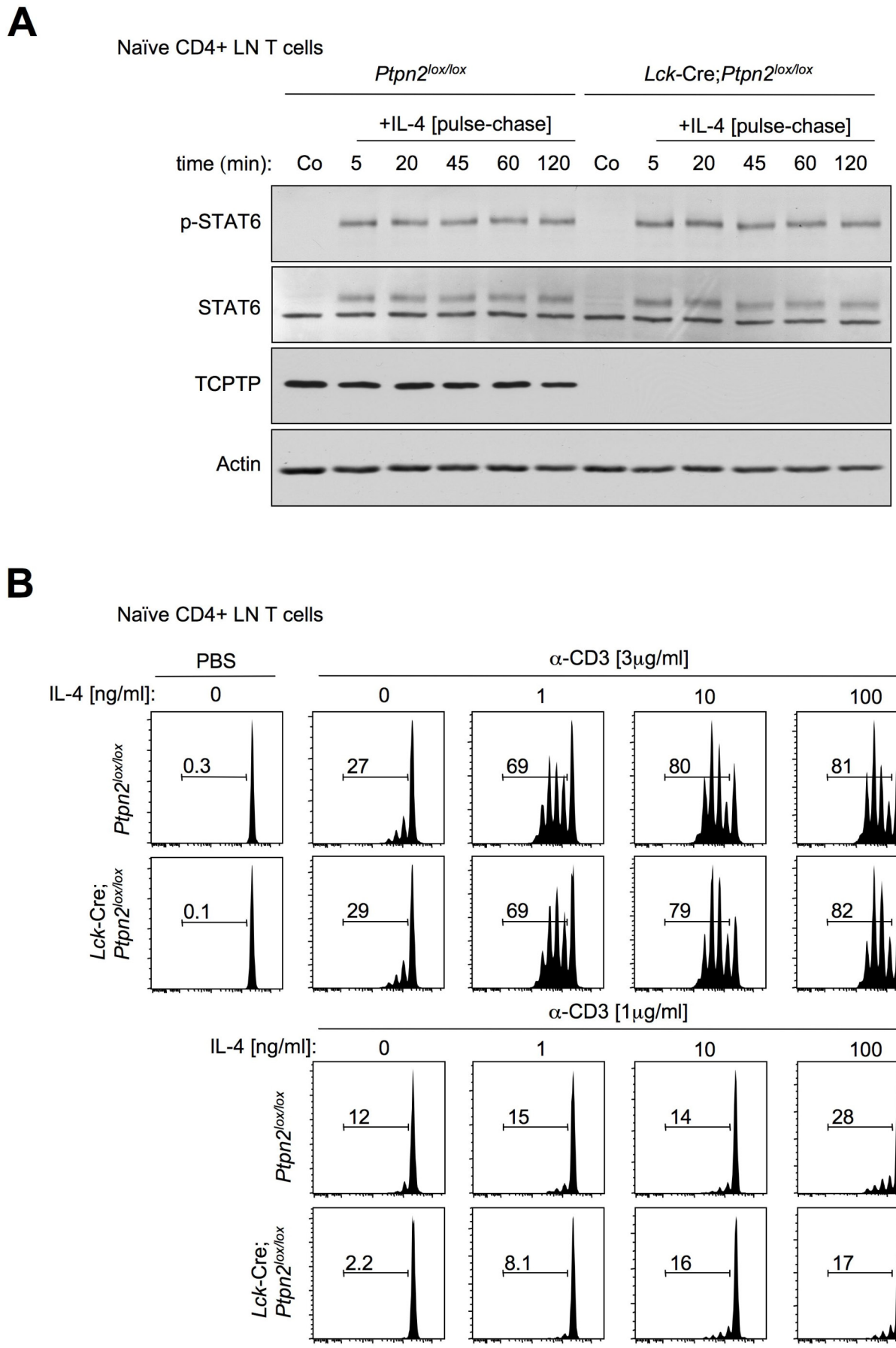
***Supplementary Figure 11. Enhanced IL-2 signaling in Lck-Cre;Ptpn2$^{lox/lox}$ T cells.*** **(a)** FACS-purified CD4+ and CD8+ naïve T cells from 4 week old *Ptpn2*$^{lox/lox}$ versus *Lck*-Cre;*Ptpn2*$^{lox/lox}$ mice were incubated with plate bound α-CD3ε/α-CD28 for 48 h. Cells were removed from the plates, serum starved for 3 h and then stimulated with recombinant 5 ng/ml mouse IL-2 for the indicated times. Cell lysates were resolved by SDS-PAGE and immunoblotted with antibodies specific for p-STAT5 (Y694), actin, TCPTP and total STAT5. Results shown are representative of at least three independent experiments. (**b**) FACS-purified CD4+ and CD8+ naïve T cells from 4 week old *Ptpn2*$^{lox/lox}$ versus *Lck*-Cre;*Ptpn2*$^{lox/lox}$ mice were stained with 5 μM CFSE and incubated with plate bound α-CD3ε/α-CD28 for 48 h and then stimulated with recombinant human IL-2 (huIL-2) for 48 h (without α-CD3ε/α-CD28); 50 μg/ml α-mouse IL-2 was added to the cell culture to neutralize endogenous IL-2 production. Representative FACS profiles from three independent experiments are shown. Proliferating cells were quantified and means ± SEM from three mice per genotype are shown; significance determined using 2-tailed Student's t-test; * $p < 0.05$, ** $p<0.01$.

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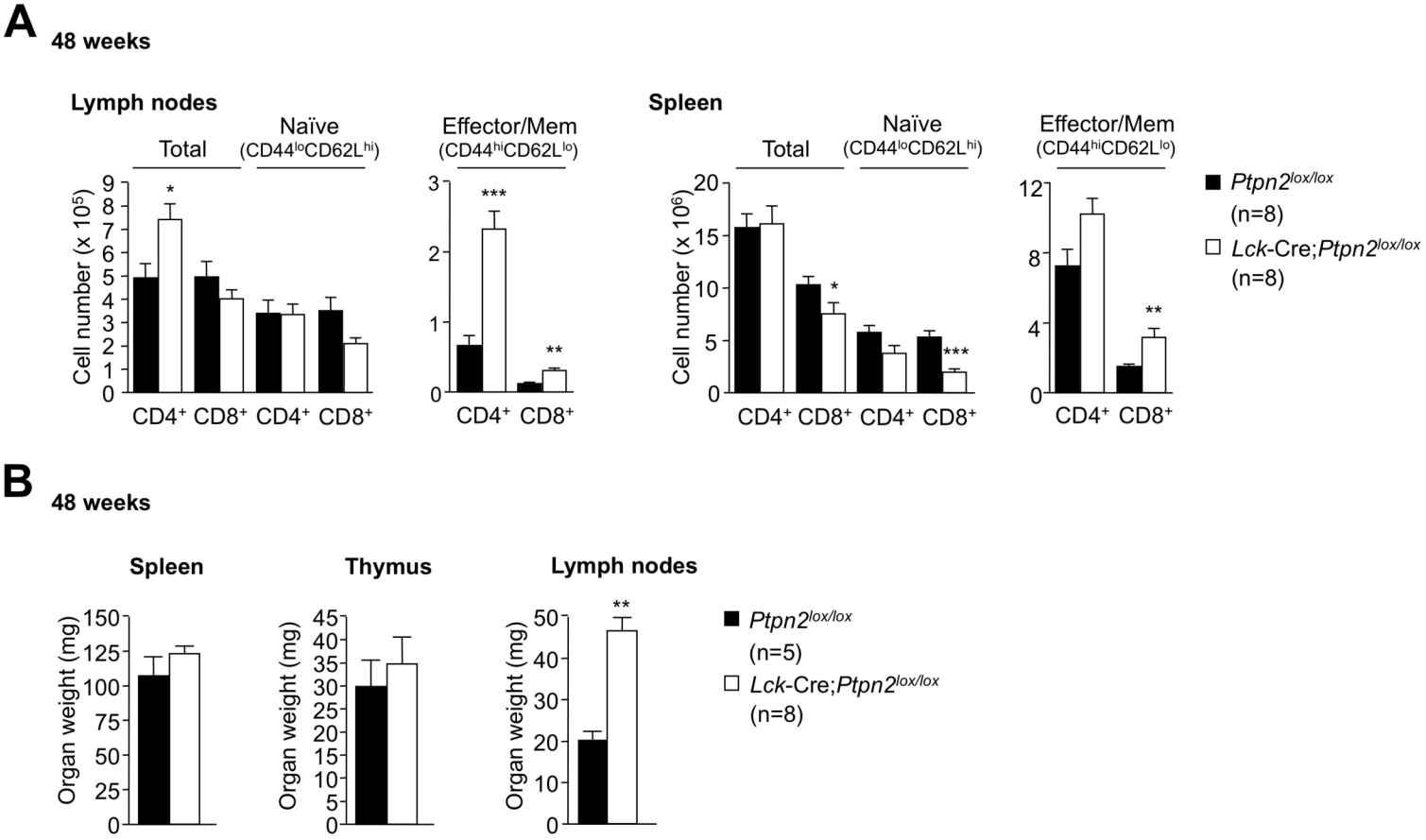
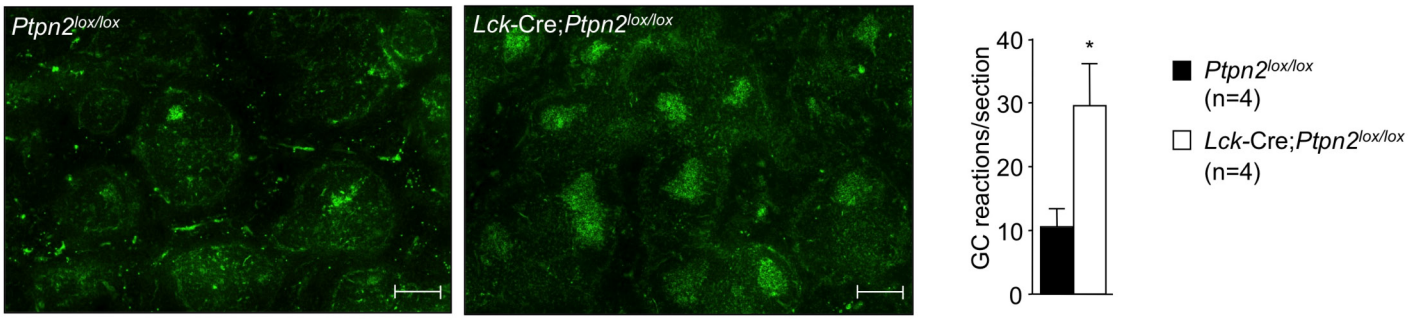
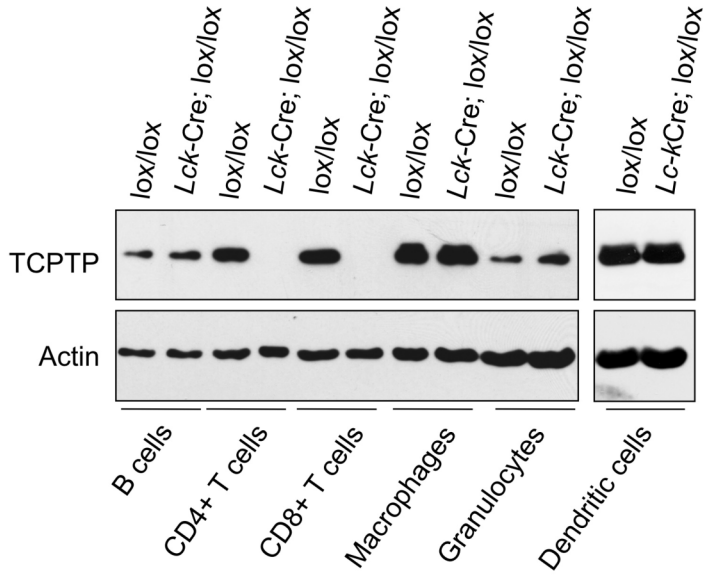
***Supplementary Figure 12. IL-4 signaling is not altered in naïve Lck-Cre;Ptpn2$^{lox/lox}$ T cells.*** **(a)** FACS-purified CD4+ naïve T cells from 4 week old *Ptpn2*$^{lox/lox}$ versus *Lck*-Cre;*Ptpn2*$^{lox/lox}$ mice were pulsed with 10 ng/ml recombinant mouse IL-4 (Peprotech) or left untreated (Co) for 10 min at 37°C, washed with RPMI medium containing 1% (v/v) FBS and incubated at 37°C for the indicated times. Cell lysates were resolved by SDS-PAGE and immunoblotted with antibodies specific for p-STAT6 (Y641), STAT6, TCPTP and actin. Results shown are representative of three independent experiments. (**b**) FACS-purified CD4+ naïve T cells from 4 week old *Ptpn2*$^{lox/lox}$ versus *Lck*-Cre;*Ptpn2*$^{lox/lox}$ mice were stained with 5 μM CFSE and incubated with plate bound α-CD3ε and the indicated concentrations of recombinant mouse IL-4 for 72h. Representative FACS profiles are shown. Numbers in histograms are % of cells that have undergone proliferation. Results shown are representative of two independent experiments.



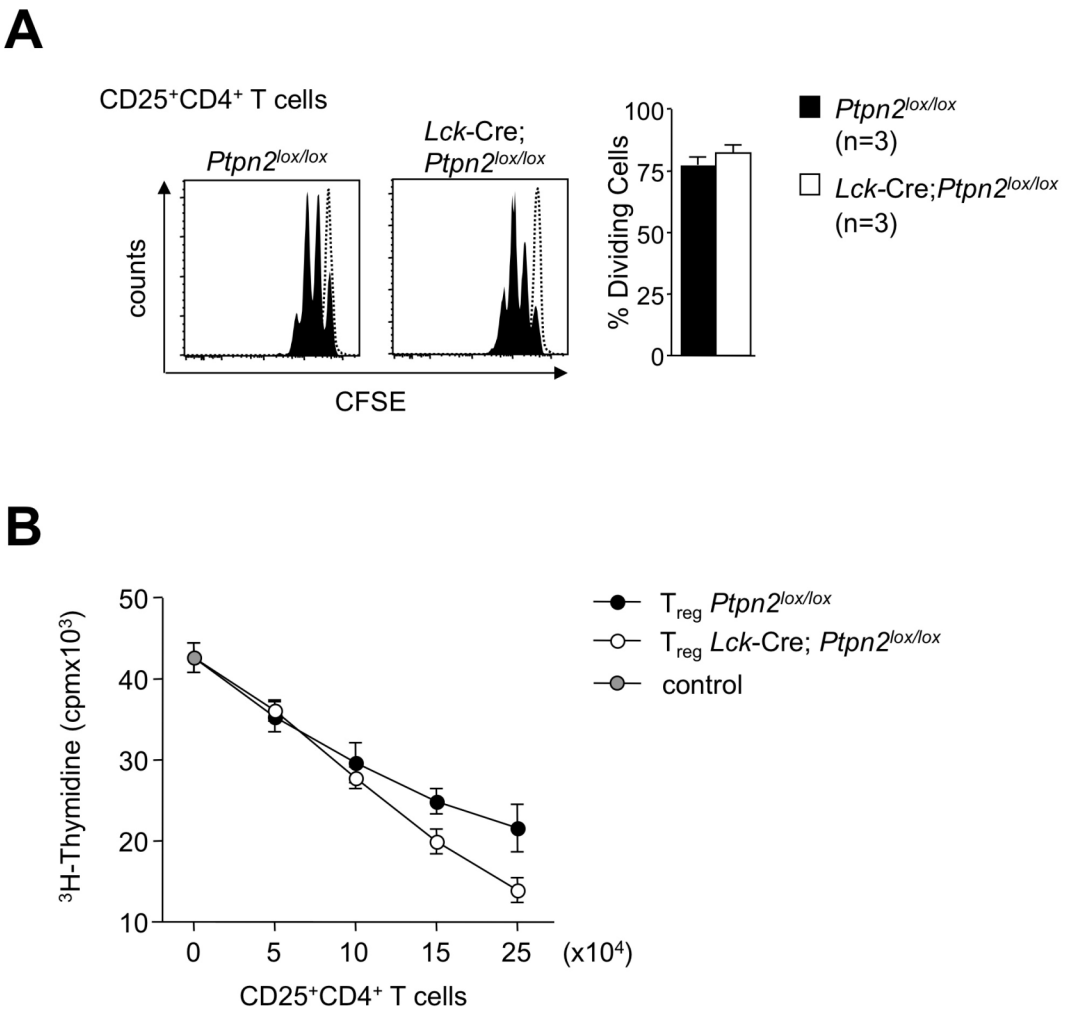

***Supplementary Figure 13. T cell subsets in 48 week old Lck-Cre;Ptpn2$^{lox/lox}$ mice.*** *Ptpn2*$^{lox/lox}$ and *Lck*-Cre;*Ptpn2*$^{lox/lox}$ lymphocytes (3 x $10^6$) isolated from lymph nodes and spleen of 48 week old mice were stained with fluorochrome-conjugated antibodies against CD4, CD8, CD44 and CD62L and analyzed by flow cytometry. (**a**) Absolute numbers of total CD4+ or CD8+ T cells and CD4+ versus CD8+ naïve (CD44$^{lo}$CD62L$^{hi}$) and effector/memory-like (Effector/Mem; CD44$^{hi}$CD62L$^{lo}$) T cells were determined. Quantified data from one experiment with eight mice per genotype is shown. (**b**) The indicated organ weights were determined using an analytical balance. Quantified data for the indicated number of mice from one experiment is shown. Results in **a**-**b** are means ± SEM; significance determined using 2-tailed Mann-Whitney U Test; *p<0.05, ** p<0.01, *** p<0.001.



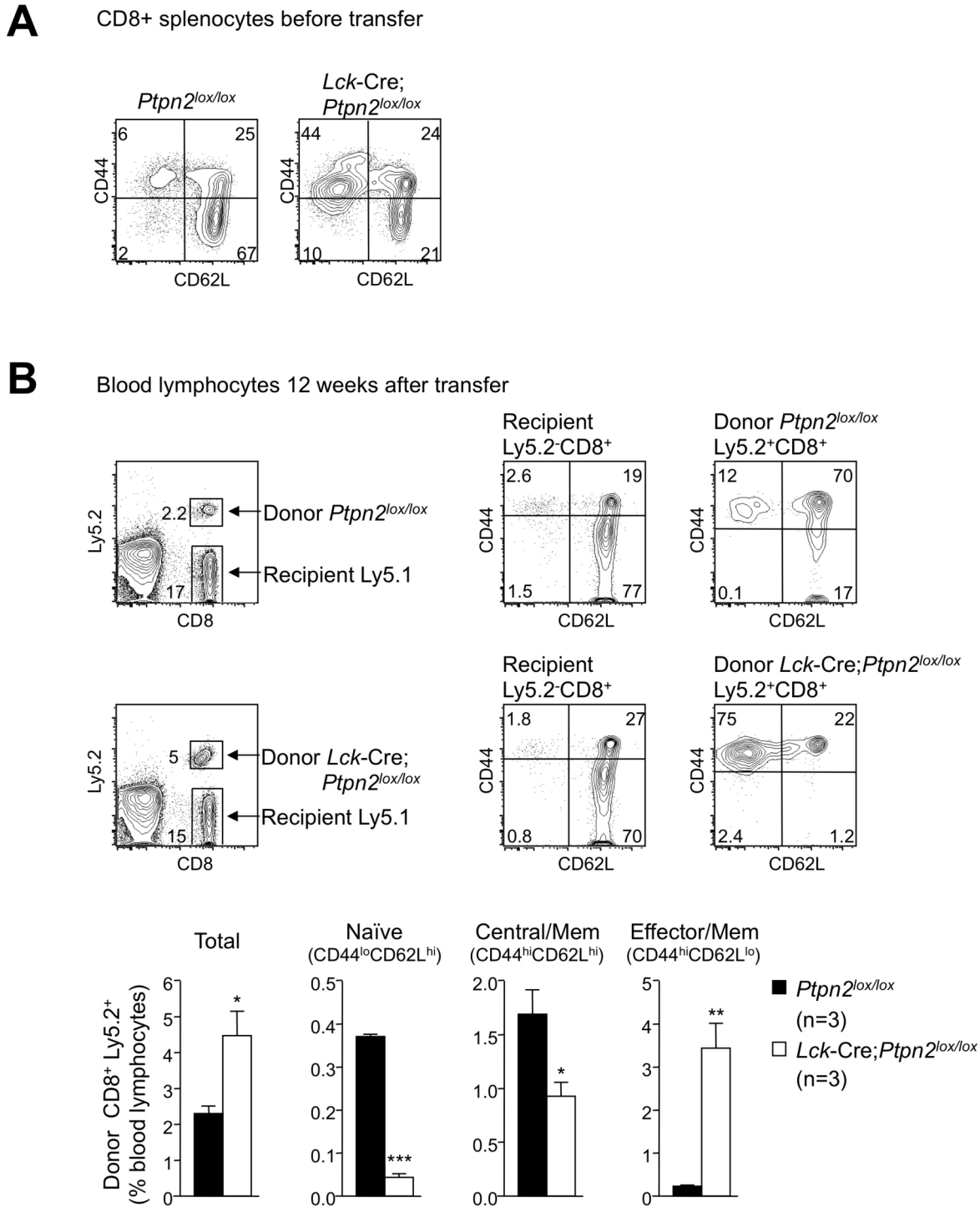

***Supplementary Figure 14. Enhanced germinal centre reaction in 40 week old Lck-Cre;Ptpn2$^{lox/lox}$ mice.*** Cryosections of spleens from 40 week old mice were stained with PNA-FITC and the numbers of germinal centre (GC) reactions per section determined. Representative images and quantified data (means ± SEM) are shown. Scale bars: 200 μM.




***Supplementary Figure 15. TCPTP expression in aged mice.*** FACS-purified lymph node CD4+ and CD8+ T cells, splenic B220+ B cells, $Ly6G^{-/lo}CD11B^{+}$ splenic macrophages and $Ly6G^{hi}CD11B^{+}$ granulocytes and MACS®-purified splenic CD11C+ dendritic cells were isolated from 40 week old *Ptpn2*$^{lox/lox}$ and *Lck*-Cre;*Ptpn2*$^{lox/lox}$ mice, lysed and proteins resolved by SDS-PAGE and immunoblotted as indicated.




***Supplementary Figure 16. Regulatory T cell proliferation and function is not impaired in Lck-Cre;Ptpn2$^{lox/lox}$ mice.*** **(a)** FACS-purified CD4+CD25+ regulatory T cells ($T_{reg}$) from 4 week old *Ptpn2*$^{lox/lox}$ versus *Lck*-Cre;*Ptpn2*$^{lox/lox}$ mice were stained with 5 μM CFSE and incubated with IL-2 (3 ng/ml), α-CD3ε (3 μg/ml) and mitomycin C-treated accessory cells. Representative FACS profiles and quantified results (means ± SEM) from three mice per genotype are shown. Results are representative of two independent experiments. **(b)** FACS-purified CD4+CD25$^{lo}$CD44$^{lo}$ naïve T cells (1 x $10^5$) from 4 week old C57BL/6 mice were incubated with α-CD3ε (1 μg/ml) and mitomycin C-treated accessory cells and either vehicle control, or FACS-purified CD4+CD25+ regulatory T cells (5-25 x $10^4$) from 4 week old *Ptpn2*$^{lox/lox}$ versus *Lck*-Cre;*Ptpn2*$^{lox/lox}$ mice. After 48 h proliferation was determined by [$^3$H]-thymidine incorporation over 16 h. Results are means ± SD from quadruplicate determinations and are representative of three independent experiments.




***Supplementary Figure 17. Adoptive transfer of CD8+ T cells into sub-lethally irradiated congenic hosts.*** (**a**) FACS-purified CD8+ T cells from the spleens of aged *Ptpn2*$^{lox/lox}$ and *Lck*-Cre;*Ptpn2*$^{lox/lox}$ mice were stained with fluorochrome-conjugated antibodies to CD8, CD62L and CD44 and the ratios of naïve (CD44$^{lo}$CD62L$^{hi}$), central memory (Central/Mem; CD44$^{hi}$CD62L$^{hi}$) and effector/memory (Effector/Mem; CD44$^{hi}$CD62L$^{lo}$) T cells determined by FACS. (**b**) FACS purified CD8+ lymphocytes isolated from aged mice were transferred (2 x 10$^6$/recipient) into sub-lethally irradiated (600 Rad) congenic Ly5.1 hosts. 12 weeks post-transfer blood lymphocytes were stained with fluorochrome-conjugated antibodies against CD8, CD45.2, CD62L and CD44. Relative numbers of CD8+CD45.2+ transferred T cells were determined by FACS. Numbers in outlined areas are % of cells in gate. Results are means ± SEM; significance determined using 2-tailed Student's t-test; * $p < 0.05$, ** $p<0.01$.