SUPPLEMENTARY DATA

T cell-specific PTPN2-deficiency in NOD mice accelerates the development of type 1 diabetes and autoimmune co-morbidities.

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Generation of Lck-Cre;Ppn2$^{fl/fl}$.NOD mice

Lck-Cre;*Ptpn2*$^{fl/fl}$.C57BL/6J mice (1; 2) were backcrossed onto the NOD/Lt genetic background (3) for 11 generations. DNA samples were extracted from tail biopsies and genotyped by standard PCR using oligos specific for the *Ptpn2* floxed allele (*forward primer: 5' GAA TTC CAG GAC AGC CAA GG 3'; reverse primer: 5' CTG CTC TTA AAG GGG ATC AGG 3'*) and the Cre transgene (*forward primer: 5' ATG TCC AAT TTA CTG ACC 3'; reverse primer: 5' CGC CGC ATA ACC AGTGAA AC* 3'). Amplified products were visualized by gel electrophoresis to distinguish the *Ptpn2* floxed allele (size = 1300 bp) and *wild-type* allele (1100 bp) or the *Lck*-Cre transgene (350 bp). A genome-wide screen was performed by the Australian Genome Research Facility using the iPLEX GOLD chemistry and the Sequenom MassArray spectrometer for SNP genotyping. Data was analyzed using the GeneChip Targeted Genotyping System Software. 11th generation backcrossed NOD mice heterozygous for the *Ptpn2* floxed allele and positive for the *Lck*-Cre transgene were of the NOD genotype across the whole genome except for those markers encompassing the C57BL/6-derived *Ptpn2* floxed allele (chromosome 18) and the C57BL/6-derived *Lck*-Cre transgene (chromosome 15).

Flow cytometry

The following antibodies from BD Biosciences, eBioscience or BioLegend were used for flow cytometry: Phycoerythrin (PE) or peridinin-chlorophyll cyanine 5.5 (PerCP-Cy5.5)- conjugated CD3 (145-2C11); PerCP-Cy5.5 or phycoerythrin-cyanine 7 (PE-Cy7)-conjugated CD4 (RM4-5); Pacific Blue-conjugated (PB) or allophycocyanin-cyanine 7 (APC-Cy7)- conjugated CD8 (53-6.7); PE or APC-Cy7-conjugated CD25 (PC61); Fluorescein isothiocyanate (FITC) or V450-conjugated CD44 (IM7); APC-Cy7-conjugated CD45 (30-F11); APC or APC-Cy7-conjugated CD45R (B220; RA3-6B2); biotin-conjugated CD49d (9C10; MFR4.B); PE-cyanine 5 (PE-Cy5)-conjugated TCR-δ (GL3); PE-Cy7 or APC- conjugated CD62L (MEL-14); PE-conjugated CD127 (SB/199); biotin-conjugated CD185 (CXCR5; 2G8); PE-Cy7-conjugated CD279 (PD-1, RMP1-14); APC-conjugated KLRG1 (2F1), PE or FITC-conjugated GL-7 (GL-7); PE-Cy7 or PE-conjugated IFNg (XMG1.2); PE or V421-conjugated IL-17A (TC11-18H10.1) FITC-conjugated IL-4 (11B11), V450- conjugated FoxP3 (clone MF23). APC- or PE-Cy7-conjugated streptavidin were used to detect cells stained with biotinylated antibodies.

Histological assessment of pancreas and salivary glands infiltrating lymphocytes

To detect CD45R$^+$ (B220$^+$) B cells and CD3$^+$ T cells, sections were deparaffinized and rehydrated. Antigen retrieval was performed in citrate acid buffer (pH 6.0) at 95°C for 20 min. Sections were blocked with 20% (v/v) normal goat serum in 0.1 M phosphate buffer and 0.2% (v/v) Triton X-100 for 1

h at room temperature. Sections were incubated overnight at 4°C with α-CD3ε (1:500; RAM 34 clone, 14-0341, eBioscience) or a-CD45R (1:400; RA3-6B2, BD Biosciences). $CD45R^+$ ($B220^+$) B cells and $CD3^+$ T cells were visualized using rabbit or rat IgG VECTORSTAIN ABC Elite and DAB (3,3'-diaminobenzidine) Peroxidase Substrate Kits (Vector Laboratories) and counterstained with hematoxylin. Sections were visualized on a Zeiss Axioskop 2 mot plus microscope (Carl Zeiss) and Aperio imaging software.

Isolation of intraepithelial and lamina propria lymphocytes

For the isolation of intraepithelial (IEL) and lamina propria (4) lymphocytes colons excised from 5 week old prediabetic *Lck*-Cre*;Ptpn2*$^{fl/fl}$.NOD and *Ptpn2*$^{fl/fl}$.NOD littermate control mice was cut into 0.5-1 cm pieces and incubated twice with Ca^{2+} and Mg^{2+}-free HBSS supplemented with 5% (v/v) FBS, 2 mM EDTA, 0.15 mg/ml DTT (dithiothreitol) and 10mM HEPES shaking at 250 rpm for 15 min at 37°C to isolate intraepithelial lymphocytes. Intestine pieces were further digested in Ca^{2+} and Mg^{2+}-containing HBSS supplemented with 5% (v/v) FBS, 1.5 mg/ml Collagenase D, 0.02 mg/ml Dnase I shaking at 250 rpm for 1h at 37 °C. Lymphocytes from digested colon pieces were enriched using a two-layer Percoll gradient at 40 and 80% (v/v) in DMEM supplemented with 10% (v/v) FBS.

Antigen-induced arthritis (AIA)

8-12 week-old C57BL/6J male mice were immunized subcutaneously with 100 µl mBSA (1 mg/ml; Sigma-Aldrich) emulsified in an equal volume of Complete Freund's Adjuvant (CFA). Mice also received 160 ng of heat-inactivated *Bordetella pertussis* toxin as a single intraperitoneal injection (Sigma-Aldrich). One week later, mice received an identical subcutaneous booster immunization of methylated BSA (mBSA) in CFA. 21 days after the initial immunization, inflammatory arthritis was induced by intraarticular administration of 10 µl mBSA (10 mg/ml) into the right knee joint. Arthritis development by measuring knee joint diameters using a POCO 2T micrometer (Kroeplin).

Histological assessment of joint inflammation

Knee joints were fixed in 10% (v/v) neutral buffered formal saline (Sigma-Aldrich) and decalcified in 10% (v/v) formic acid at 4°C before embedding in paraffin. Parasagittal serial sections (7 µm) were stained with haematoxylin (VWR International), fast green and safranin O (both from Sigma-Aldrich) for histological evaluation of joint pathology. Two observers blinded to the experimental groups scored the sections for sub-synovial inflammation (0 = normal to 5 = ablation of adipose tissue due to leukocyte infiltrate), synovial exudate (0 = normal to 3 = substantial number of cells with large fibrin deposits), synovial hyperplasia (0 = normal with 1-3 cells thick to 3 = over 3 layers thick with overgrowth onto joint surfaces with evidence of cartilage/bone erosion), and cartilage/bone erosion (0 = normal to 3 = destruction of a significant part of the bone). Joint histopathology in pre-diabetic 5 week old *Lck*-Cre*;Ptpn2*$^{fl/fl}$ .NOD and *Ptpn2*$^{fl/fl}$.NOD littermate control mice was compared to C57BL/6J mice with antigen-induced arthritis.

In vitro generation of T_{H1}, T_{reg} and T_{H17} cells

FACS-purified naive $CD4^+CD44^{lo}CD62L^{hi}CD25^{lQ}$ lymph node T cells ($5x10^4$) isolated from 6-8 week old *Lck*-Cre*;Ptpn2*$^{fl/fl}$ and *Ptpn2*$^{fl/fl}$ littermate control mice were stimulated with plate-bound α-CD3ε (1 µg/ml) in the presence of soluble α-CD28 (10 µg/ml) in complete T cell medium overnight in 96-well round bottom plates. For the generation of T_{H1} cells 10 µg/ml soluble α-IL-4 (clone BVD4-1D11, WEHI) was added and for the generation of T_{H17} cells 10 µg/ml soluble α-IL-4 (clone BVD4-1D11,

WEHI) and 10 μg/ml soluble α-IFNγ (clone XMG1.2, WEHI) were added. Cells were harvested and transferred to new 96-well round bottom plates and incubated with IL-2 (10 ng/ml) alone or various concentrations of IL-12 to generate T_{H1} cells or TGFβ (5 ng/ml) to generate T_{reg} cells. To generate $T_{H1}7$ cells, cells were incubated in IL-6 (50 ng/ml) and TGFβ (1 ng/ml) in the absence of IL-2. Cells were harvested at day 4 and 5 and processed for flow cytometry.

Treg suppression assay

FACS-purified naive $CD4^+CD25^{lo}$ responder T cells ($5x10^4$) were labelled with CTV (Cell Tracker Violet, Molecular Probes) and cultured for 72 h with X-ray irradiated (4,000 rad) splenocytes ($5x10^4$) and 1 μg/ml α-CD3ε (145-2C11) in the presence of the indicated ratio of FACS-purified $CD4^+CD25^{hi}$ suppressor T cells.

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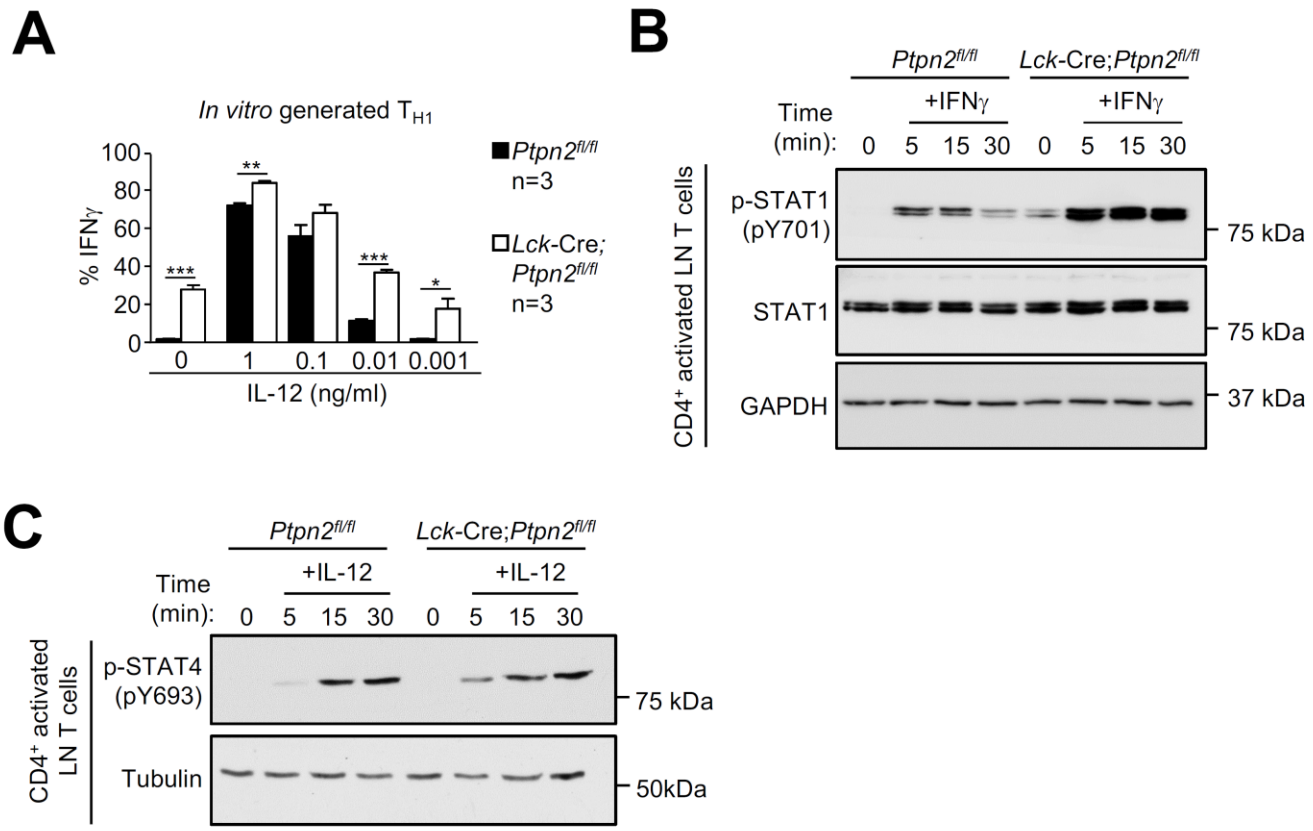
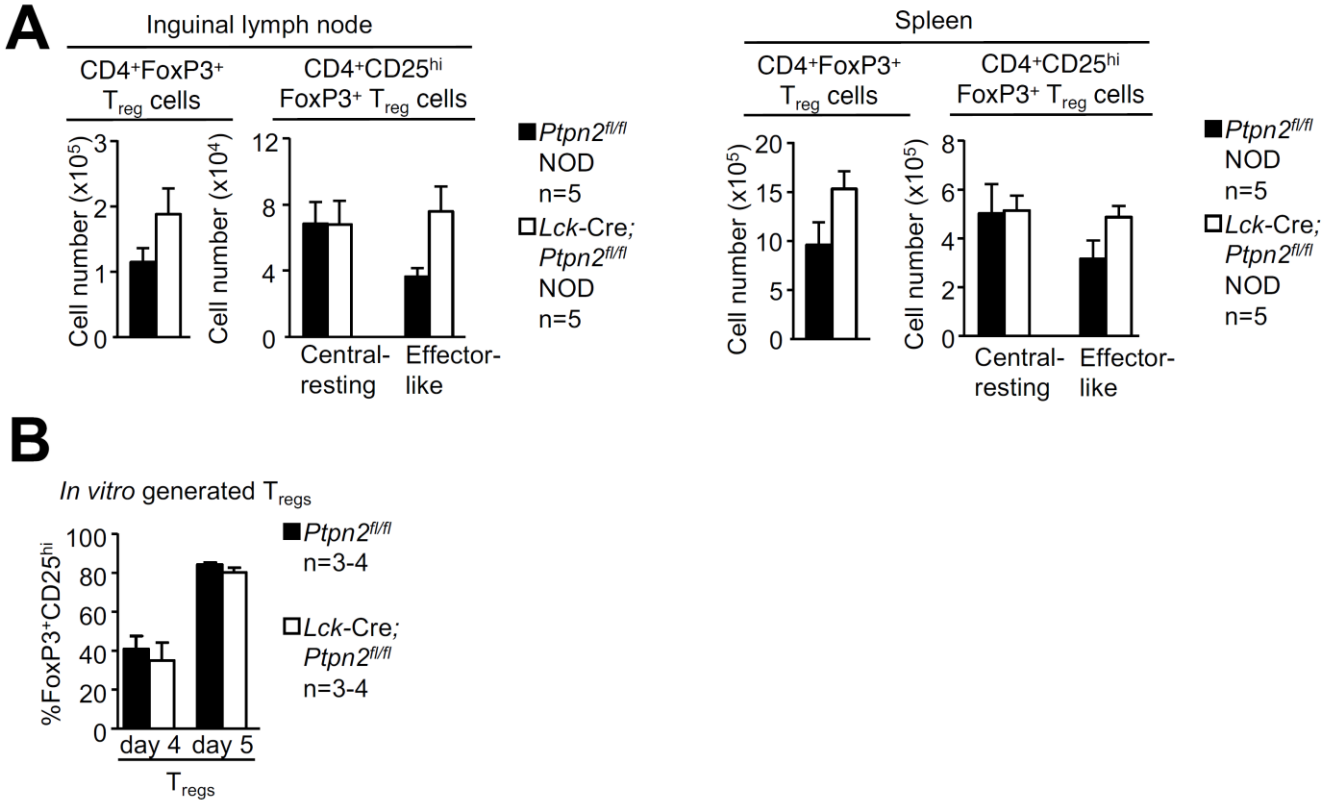
Supplementary Figure 1. ***PTPN2 deficiency enhances the in vitro generation of T_{H1} cells.*** **a)** FACS-purified naive $CD4^+CD44^{lo}CD62L^{hi}CD25^{lo}$ lymph node T cells from *Ptpn2*$^{fl/fl}$ and *Lck*-Cre;*Ptpn2*$^{fl/fl}$ mice were cultured under T_{H1} polarising conditions in the presence of IL-2 (10 ng/ml) and various concentrations of IL-12. At day 4 cells were harvested and stained for intracellular IFNγ and the percentage of $IFN\gamma^+$ T_{H1} cells was determined by flow cytometry. **b-c)** FACS-purified naive $CD4^+$ $CD44^{lo}CD62L^{hi}CD25^{lo}$ lymph node T cells from *Ptpn2*$^{fl/fl}$ and *Lck*-Cre;*Ptpn2*$^{fl/fl}$ mice were incubated with plate-bound α-CD3ε (5 µg/ml)/CD28 (5 µg/ml) for 48 h and then stimulated with **b)** 50 U/ml IFNγ or **c)** 10 ng/ml IL-12 for the indicated times and processed for immunoblotting. Quantified results are means ± SEM for the indicated number of mice and are representative of two independent experiments. Significance was determined using Student's T-test; *p < 0.05, **p < 0.01, ***p < 0.001.



SUPPLEMENTARY DATA

Supplementary Figure 2. ***Regulatory T cell development in vivo and ex vivo.*** **a)** Inguinal lymph node cells and splenocytes from 5 week old female prediabetic *Ptpn2*$^{fl/fl}$.NOD and *Lck*- Cre*;Ptpn2*$^{fl/fl}$.NOD mice were stained for CD4, CD25, CD62L, CD44 and intracellular FoxP3 and the numbers of central-resting ($CD44^{hi}CD62L^{hi}$), and effector-like ($CD44^{hi}CD62L^{lo}$) T_{reg} cells were quantified by flow cytometry. **b)** FACS-purified naive $CD4+CD44^{lo}CD62L^{hi}CD25^{lo}$ lymph node T cells were cultured under T_{reg} polarising conditions in the presence of IL-2 (10 ng/ml) and TGFb (5 ng/ml). At day 4 and 5 cells were harvested and stained for CD25 and intracellular FoxP3 and the percentage of $FoxP3^{+}CD25^{hi}$ T_{regs} were determined by flow cytometry. Quantified results are means ± SEM for the indicated number of mice and are representative of two independent experiments.



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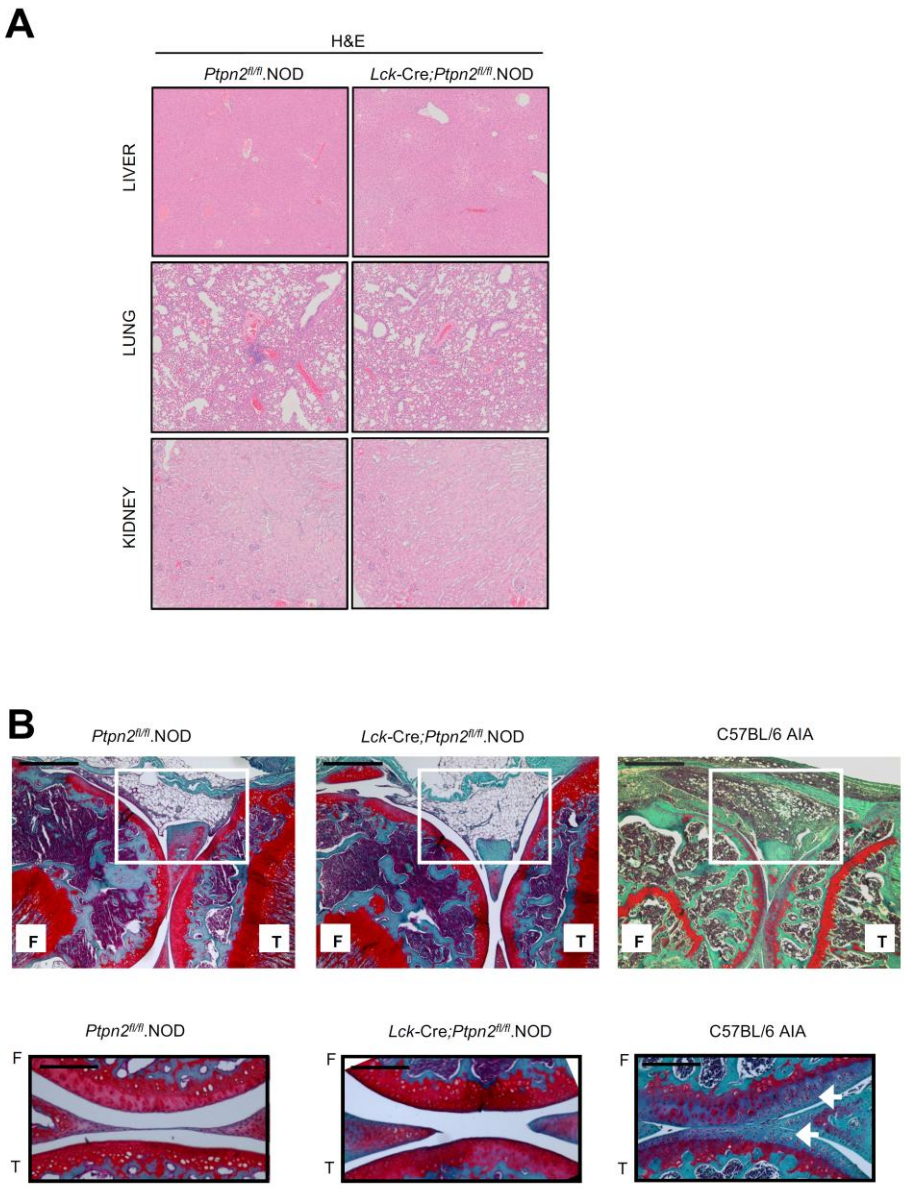
Supplementary Figure 3. ***Histological assessment of liver, lung, kidney and knee-joints.*** **a)** Liver, lung and kidneys from 5 week old female prediabetic *Ptpn2*$^{fl/fl}$.NOD and *Lck*- Cre;*Ptpn2*$^{fl/fl}$.NOD mice were fixed in formalin and processed for histological assessment (hematoxylin and eosin: H&E). **b)** Knee joints from 5 week old female prediabetic *Ptpn2*$^{fl/fl}$.NOD and *Lck*-Cre;*Ptpn2*$^{fl/fl}$.NOD mice fixed in formalin and decalcified in formic acid. Parasagittal serial sections were processed for histological assessment (H&E, fast green and safranin O). Joint histopathology in *Lck*-Cre;*Ptpn2*$^{fl/fl}$ and *Ptpn2*$^{fl/fl}$ mice was compared to C57Bl/6 mice with antigen-induced arthritis (AIA).



SUPPLEMENTARY DATA

Supplementary Figure 4. ***Colon resident intraepithelial and lamina propria γδ-T cells in Lck-Cre;Ptpn2$^{fl/fl}$.NOD mice.*** **a)** FACS-purified naive CD4+CD44^{lo}CD62L^{hi}CD25^{lo} lymph node T cells from *Ptpn2$^{fl/fl}$* and *Lck*-Cre*;Ptpn2$^{fl/fl}$* mice were cultured under T_{H1} polarising conditions in the presence of IL-6 (50 ng/ml) and TGFβ (1 ng/ml). At day 4 cells were harvested and stained for intracellular IL-17A and the percentage of IL-17A$^+$ $T_{H1}7$ cells was determined by flow cytometry. **b)** Lymphocytes from were isolated from the colon of 5 week old female prediabetic *Ptpn2$^{fl/fl}$.**NOD*** and *Lck*-Cre*;Ptpn2$^{fl/fl}$*.NOD mice and intraepithelial versus lamina propria lymphocytes were stained for CD3 and TCR-δ and analysed by flow cytometry. Representative contour-plots are shown. Quantified results are means ± SEM for the indicated number of mice and are representative of two independent