SUPPLEMENTARY MATERIALS AND METHODS

CHO cell transfection and *in vitro* kinase assay

HA-tagged cDNAs of Syk (NM_003177.5), kinase-dead Syk (K402R) and Zap-70 (NM_001079) were cloned into pcDNA3 vectors and stably expressed in chinese hamster ovary cells; for the kinase reaction, anti-HA immunoprecipitates were mixed with recombinant GST-Igβ fusion protein and 1µl γ-ATP-^{32}P (10mCi/ml) for the indicated time points, boiled in reducing loading buffer and separated by PAGE; after fixation, gels were dried and exposed to X-ray films (Amersham) for various time points.

Hep-2 screening assay

Human epithelial (Hep-2) cell substrate slides (Kallestad, Biorad) were permeabilized/blocked (PBS/2%BSA/0.5%Triton-X-100) for 1h in a humidified chamber and subsequently incubated with mouse sera diluted 1:100 in PBS/0.5%BSA/0.05%Triton-X-100; after washing, slides were incubated with anti-IgM or anti-IgG FITC, washed and mounted in mowiol/DABCO; slides were scored in blinded fashion on a Zeiss Axioscope epifluorescence microscope, 10x magnification (NA=0.5).

Blood parameters, platelet glycoprotein expression and stimulation

For the determination of basal blood parameters, heparinized blood was analyzed on a Sysmex KX-21N cell counter (Sysmex Europe); for assessment of glycoprotein expression and platelet count, whole blood samples were incubated with appropriate fluorophore-conjugated monoclonal antibodies and directly analyzed via flow cytometry. For activation studies, blood samples were washed twice with modified Tyrode's-HEPES buffer (134mM NaCl, 0.34mM Na_2HPO_4, 2.9mM KCl, 12mM $NaHCO_3$, 20mM HEPES [pH 7.0], 5mM glucose, 0.35% BSA, 1mM $CaCl_2$) and then activated with the indicated agonists for 15 minutes, stained with fluorophore-labelled antibodies and directly analyzed.

Platelet aggregometry

To determine platelet aggregation, light transmission was measured using washed platelets (2 x 10^8/ml) in the presence of human fibrinogen (70mg/ml, except for thrombin) and the indicated chemicals/antibodies. Transmission was recorded on a Fibrintimer 4 channel aggregometer (APACT Laborgeräte und Analysensysteme) over 10 minutes and was expressed as arbitrary units with transmission through buffer defined as 100% transmission.

Measurement of reactive oxygen species

Prepared PMNs were stained with CM-H_2DCFDA (Invitrogen, 7.5µM) in HBSS [pH 7.4], 20mM Hepes, 1.5mM $CaCl_2$, 1mM $MgCl_2$ for 15 minutes at 37°C; after washing with PBS, cells were rested for additional 15 minutes at 37°C to render the dye responsive to oxidization; baseline fluorescence of triplicate samples (2 x 10^6 cells/ml) was measured in 96 well plates (Biotek Synergy 2, 480/520nm) prior to the addition of the indicated stimulating reagents at 37°C; for assessing FcγR-mediated (rabbit IgG-BSA-H_2DCF) ROS production, PMNs were incubated with Fc OxyBURST® Green Assay Reagent (Invitrogen, 120µg/ml) and the amount of ROS produced is presented as mean fluorescence intensities at the indicated time points; negative controls were kept on ice to subtract background oxidization.

Tissue sectioning, immunofluorescence and PAS staining

For immunofluorescence, kidneys were fixed for 4 hours in PBS/4%PFA, washed in PBS, submerged in Tissue-Tek and snap-frozen (Tissue-Tek); 8µm tissue sections (Microm HM 560) were fixed with ice cold methanol for 10 minutes, washed, blocked (10% heat inactivated goat serum) and stained with anti-IgM-FITC, anti-IgG FITC or anti-C3 (2nd ab: goat anti-rat Alexa 488); after washing, slides were mounted with mowiol/DABCO and analysed via epifluorescence microscopy (20x, NA=0.75); for PAS stainings, fixed samples were embedded over night at 70°C in paraffin (paraplast, Sigma), sectioned (10µm; Microm EC 350-1/2), rehydrated (xylol, 98/96/90/80/70% isopropanol), stained, dehydrated (70/80/90/100% isopropanol, xylol) and mounted with Eukitt; representative images were taken on a Zeiss Axioscope (20x, NA=0.6).

ELISA and Elispot

Mouse sera were obtained at the indicated age, allowed to clot at 37°C for 30 minutes, depleted of cells, snap-frozen and kept at -20°C until use; for measuring total Ig titers, Nunc Maxisorp plates were coated with anti-mouse IgM/IgG1/IgG2a/IgG2b capturing antibodies (1µg/ml each), blocked (PBS/1%BSA) and incubated with serial dilutions of mouse sera; after incubation with secondary AP-conjugated antibodies, p-nitrophenylphosphate (Sigma) was added as substrate and signals were read on a Biotek Synergy 2 Elisa reader at 405nm; Ig titers were determined by correlating linear signal regression curves of samples to Ig standard curves run in parallel; for specific comparative Elisa assays, plates were coated with dsDNA (10µg/ml) or recombinant human insulin (5µg/ml, Huminsulin, Lilly) and titrated signals were correlated from plate to plate by serial dilution of a $Syk^{Zap\text{-}70/Zap\text{-}70}$ serum pool; to

quantitatively measure serum C-peptide levels, a commercially available kit from Millipore was used according to manufacturers instructions (C-peptide 2 Elisa kit). For Elispot analysis, Millipore $Multiscreen_{HTS}$ filter plates were coated with control BSA or insulin; after blocking, sorted splenic $B220^{+}CD19^{+}$ B cells were added in serial dilutions to titrate the amount of spots and were left untreated or stimulated with LPS (20µg/ml) in RPMI 1640, 10% FCS (Gibco), Penicillin/Streptomycin (PAA), L-glutamine (PAA) and 50µM β-mercaptoethanol (Gibco) for 36h at 37°C, 7,5%CO_2; after washing, plates were incubated with anti-IgM-Biotin (Jackson), washed and incubated with Streptavidin-AP (Jackson); spots were visualized by the addition of NBT/BCIP (Sigma), dried and scanned for spot counting using ImageJ.

Serum creatinine, C3, C4 and urea nitrogen (BUN) measurement

Creatinine levels of mice (12 weeks) were photometrically determined using picrinic acid-based Jaffé reactions; C3 and C4 were measured by immunoturbidimetry and BUN values by using urease for enzymatic urea to ammonia and carbonic acid conversion. Ammonia levels corresponding to blood urea were measured photometrically (ammonia/alpha-ketoglutaric acid reaction; 340nm absorbance decrease).

Glucose tolerance test and proteinuria

Mice (n=8/group) were fasted 16 hours prior to measurement of fasting blood glucose levels via tail bleeding (Accuchek AVIVA, Roche); for testing glucose tolerance, 2mg/g body weight of D-glucose was injected intraperitoneally and a small blood sample was collected at time point 0 prior and 15, 30, 60, and 120 minutes post injection. Proteinuria was semi-quantitatively measured by the use of dip sticks (Albustix, Bayer).

SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure 1 Genomic targeting strategy for the Syk locus. (**A**) A human Zap-70 cDNA was inserted in frame to the initiating ATG into the mouse Syk gene, replacing most of exon 2. (**B**) Southern blot analysis of Syk^{wt} (+/+) and $Syk^{+/Zap\text{-}70\ neo}$ (+/t) tail biopsies. (**C**) Genomic PCR of the Syk^{wt} (+), $Syk^{Zap\text{-}70\ neo}$ (t) and $Syk^{Zap\text{-}70\Delta}$ (ki) alleles. All functional experiments in this study were performed with the $Syk^{Zap\text{-}70\Delta}$ allele, which is abbreviated as Syk^{ki} in the manuscript. (**D**) Western blot of total bone marrow from $Syk^{+/+}$, $Syk^{+/Zap\text{-}70}$ (Syk^{ctrl} or ctrl) and $Syk^{Zap\text{-}70/Zap\text{-}70}$ (Syk^{ki} or ki) mice.

Supplementary Figure 2 Increased apoptosis rates of Syk^{ki} bone marrow cells. (**A**) Bone marrow cells were stained with anti-Ly6G/C (Gr1) to analyse myeloid cellularity; numbers in graphs indicate percentages of $Gr1^{int}$ and $Gr1^{hi}$ total bone marrow cells and are representative for 3 independent experimental repeats. (**B**) Hoechst 33342 cell cycle statistics, showing a significantly reduced fraction of proliferating $B220^+CD43^+CD24^+BP1^+$ pre B cells in the bone marrow of Syk^{ki} mice. (**C,D**) AnnexinV / PI staining to distinguish early and late apoptotic cellular fractions detected a weak increase of (**C**) IgM^- and a strong increase of (**D**) IgM^+ apoptotic bone marrow cells. ***$P < 0.001$; n = 3 (**A-D**); error bars indicate means ± S.E.M.; PI, propidium iodide.

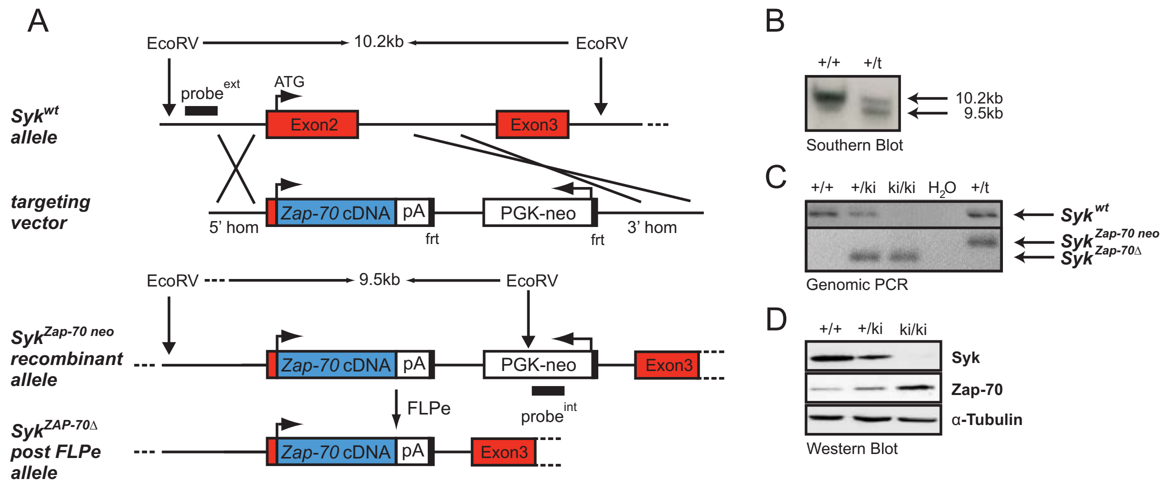
Supplementary Figure 3 Splenic cell counts, BCR internalization, nTreg and activated T cell fractions. (**A**) Total splenic cellularity in Syk^{ctrl} and Syk^{ki} mice. (**B**) Analysis of ($B220^+$) B cell IgM uptake kinetics upon anti-IgM-Biotin stimulation for the indicated time points at 37°C; kinetics indicate that proper Syk kinase activity is dispensable for BCR internalization. (**C**) Representative stainings and statistics of total splenic $CD4^+CD25^+FoxP3^+$ nTregs; numbers in graphs indicate representative nTreg percentages (n = 4). (**D**) Percentage of $CD4^+CD69^+$ and $CD8^+CD69^+$ splenic T cells ($CD3^+$-pre-gated). *$P < 0.05$, ***$P < 0.001$; n = 27 (**A**), 2 (**B**) and 4 (**C,D**) per group; error bars indicate means ± S.E.M.

Supplementary Figure 4 Syk^{ki} thymocytes exhibit a partial arrest at the $CD4^-CD8^-CD25^+CD44^-$ DN3 stage. (**A,D**) Flow cytometric staining and (**C,E**) statistics of developing thymic T cells. (**B**) Total thymic cellularity. *$P < 0.05$; n = 4 (**A-E**) per group; error bars indicate means ± S.E.M; ns, non significant.

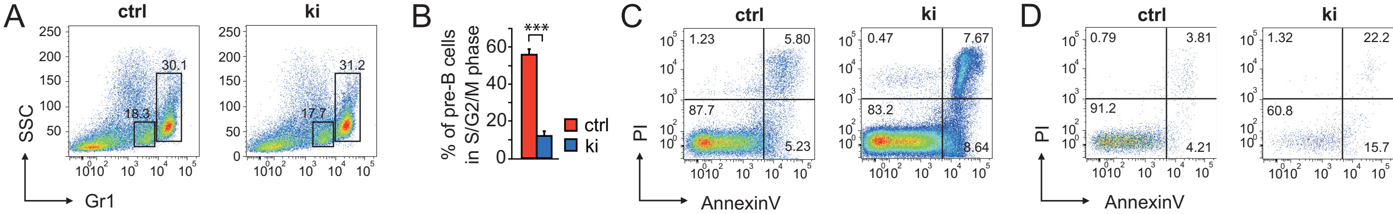
Supplementary Figure 5 *Syk*^{ki} platelets show unaltered glycoprotein expression. (A) Analysis of basal *Syk*^{ctrl} and *Syk*^{ki} blood parameters (heparinized blood, Sysmex KX-21N), platelet counts and size (BD FACS Calibur). **(B)** *Syk*^{ctrl} and *Syk*^{ki} platelets were stained with the indicated fluorophore-conjugated antibodies for 15 minutes and analysed by flow cytometry. **(C)** Syk and Zap-70 protein expression in purified platelets; $*P < 0.05$, n = 8 per group; arb.u., arbitrary units; MFI, mean fluorescence intensity.

Supplementary Figure 6 Serological characterisation of Syk^{ki} mice. (A) Serum C-peptide, **(B)** C3, **(C)** C4, **(D)** creatinine and **(E)** urea nitrogen (BUN) levels of *Syk*^{ctrl} and *Syk*^{ki} mice. **(F)** Representative histological sections of pancreata in young (12w; i, ii) and aged (60w; iii-vi) mice (n = 4 per age and group).

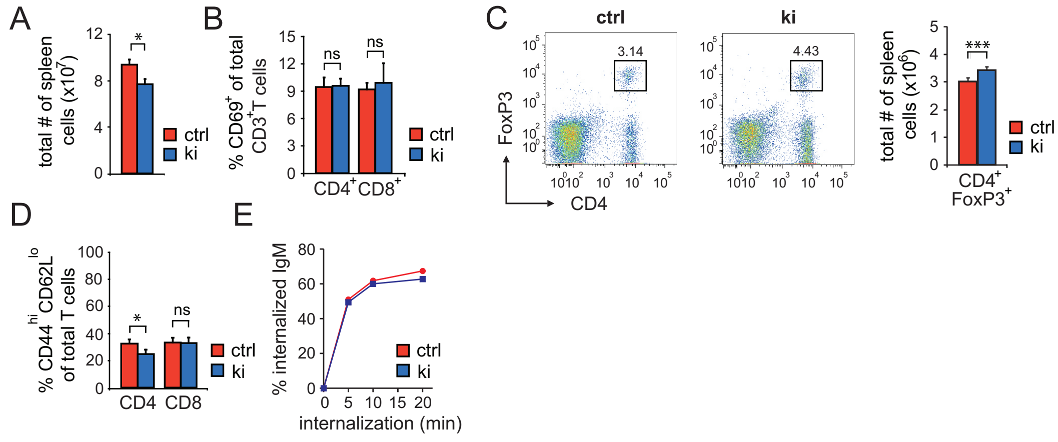
Supplementary Figure 1



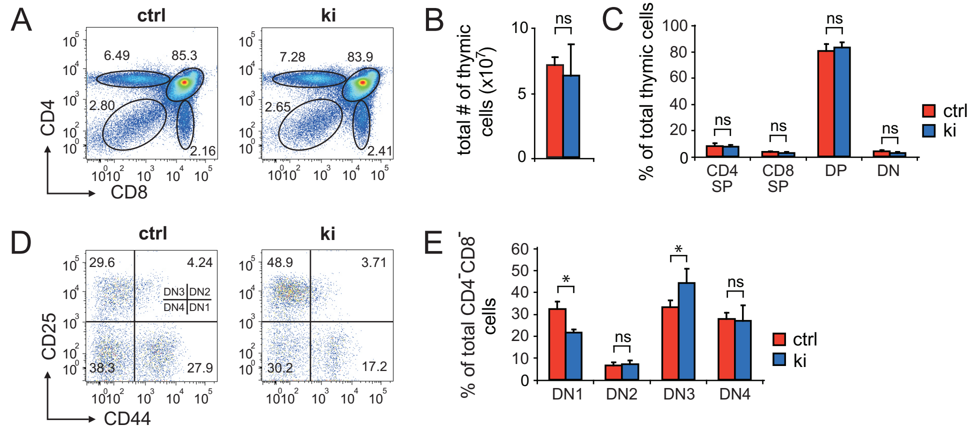
Supplementary Figure 2



Supplementary Figure 3



Supplementary Figure 4

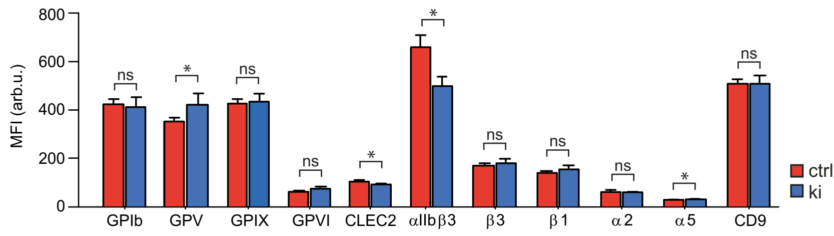


Supplementary Figure 5

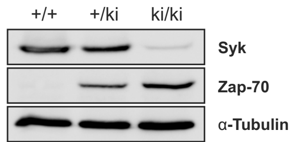
A

parameters	ctrl	ki	sign.
White blood cell count [1/nl]	9.6 ± 3.2	8.4 ± 1.7	ns
Red blood cell count [1/pl]	8.5 ± 1.0	9.2 ± 1.9	ns
Hemoglobin [g/dl]	13.5 ± 1.5	15.0 ± 3.1	ns
Hematocrit [%]	44.1 ± 3.4	48.9 ± 13.0	ns
MCV (mean RBC volume) [fl]	51.9 ± 1.8	52.7 ± 3.2	ns
Platelet count [/nl]	840 ± 181	636 ± 108	ns
Mean platelet volume [fl]	5.3 ± 0.2	5.6 ± 0.5	ns

B



C



Supplementary Figure 6